Qualität, Stabilität und Verfügbarkeit von Oxytocin-Ampullen und Misoprostol-Tabletten in Malawi und Ruanda

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vorgelegt von

Christiane Nhomsai Hagen, geb. Zeller

aus Mutare (Zimbabwe)

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Inhalt

Für meine Tochter und alle Mütter und deren Töchter auf dieser Welt.

Erklärung

Ich erkläre hiermit, dass ich die zur Promotion eingereichte Arbeit mit dem Titel

„Qualität, Stabilität und Verfügbarkeit von Oxytocin-Ampullen und Misoprostol-Tabletten in Malawi und Ruanda“

selbständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und Zitate als solche gekennzeichnet habe.

Ich erkläre, dass die Richtlinien zur Sicherung guter wissenschaftlicher Praxis der Universität Tübingen (Beschluss des Senats vom 25.5.2000) beachtet wurden. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

Tübingen, den

Nhomsai Hagen

Abkürzungen

AMTSL: Active Management of 3rd Stage of Labour

CMST: Central Medical Stores Trust

COMREC: College of Medicine Research and Ethics Committee

EML: Essential Medicines List

GIZ: Gesellschaft für Internationale Zusammenarbeit GmbH

HPLC: High performance liquid chromatography

HPMC: Hydroxypropylmethylcellulose

ICH: International Council of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use

LMIC: Low- and middle-income country

MEDQUARG: Medicine Quality Assessment Reporting Guidelines

MEML: Malawi Essential Medicines List

MHRA: Medicines and Healthcare products Regulatory Agency, U.K.

MKT: Mean kinetic temperature

MSTG: Malawi Standard Treatment Guidelines

Ph. Int.: International Pharmacopoeia

PMPB: Pharmacy, Medicines and Poisons Board

PPH: Post-partum haemorrhage

RH: Relative humidity

RSD: Relative standard deviation

SDG: Sustainable Development Goal

SOP: Standard operating procedure

SRA: Stringent Regulatory Authority

STG: Standard Treatment Guideline

UN: United Nations

UNCoLSC: UN Commission on Life-Saving Commodities for Women and Children

USP: United States Pharmacopeia

WHO: World Health Organization

WHO-PQ: WHO-prequalified product

Zusammenfassung

Nachgeburtsblutungen sind die Hauptursache für Müttersterblichkeit in Ländern mit niedrigem und mittlerem Einkommen. Oxytocin und Misoprostol werden zur Prävention und Behandlung von Nachgeburtsblutungen eingesetzt. Beide Medikamente sind jedoch chemisch instabil und empfindlich gegenüber Umwelteinflüssen. Laut Studien ist die Prävalenz von minderwertigen Oxytocin- und Misoprostol-Präparaten vor allem in Ländern mit niedrigem und mittlerem Einkommen oft hoch, für Malawi und Ruanda lagen bislang jedoch keine Daten vor. Im Rahmen dieser Doktorarbeit wurde daher die Qualität von Oxytocin-Ampullen und Misoprostol-Tabletten untersucht, die in randomisiert ausgewählten Gesundheitseinrichtungen und Apotheken, sowie von Großhändlern in Malawi und Ruanda gesammelt wurden. Die Lagertemperatur von Oxytocin und Misoprostol wurde mithilfe von Temperaturloggern ermittelt. In Malawi wurden zudem auch Daten über die Verfügbarkeit von Oxytocin und Misoprostol, sowie das Wissen des Gesundheitspersonals über die Lageranforderungen und die Anwendung der beiden Medikamente mittels eines Fragebogens erhoben. Zusätzlich wurde die Stabilität von Oxytocin-Ampullen und Misoprostol-Tabletten, die von Großhändlern in Malawi, Ruanda und Europa gekauft wurden, durch beschleunigte Stabilitätsstudien gemäß ICH/WHO-Richtlinien untersucht. Oxytocin-Proben wurden auf Identität, Gehalt und pH-Wert nach United States Pharmacopoeia 40 untersucht. Misoprostol-Proben wurden auf Identität, Gehalt, Wirkstofffreisetzung und verwandte Substanzen gemäß der International Pharmacopoeia 2017 analysiert.

In Malawi war die Verfügbarkeit von Oxytocin ausgezeichnet, jedoch entsprachen die Lagerbedingungen in den Gesundheitseinrichtungen oft nicht den Anforderungen. Sieben von 65 Oxytocin-Proben verfehlten die Anforderungen des Arzneibuchs knapp und enthielten nur 82,2 - 86,8% des angegebenen Oxytocin-Gehalts. Fünf von 30 Misoprostol-Proben waren extrem minderwertig und enthielten nur 12,7 - 30,2% der deklarierten Menge an Misoprostol. Das extrem minderwertige Misoprostol-Präparat wurde den nationalen Behörden und der WHO gemeldet, was in Malawi zu einem sofortigen Rückruf des Präparates führte. Der in Großbritannien ansässige Zwischenhändler dieses Präparates schloss kurz darauf sein Unternehmen.

In Ruanda entsprachen die Lagerbedingungen größtenteils den Anforderungen. Von den 57 Oxytocin-Proben enthielten alle neun Proben derselben Charge eines

chinesischen Herstellers zu viel Oxytocin (117,2 - 121,5% des deklarierten Gehalts), während eine andere Charge desselben Herstellers extreme Gehaltsschwankungen des Konservierungsmittels Benzylalkohol aufwies. Von den 25 Misoprostol Proben waren auch hier alle 10 Proben von zwei indischen Herstellern extrem minderwertig mit 42,5 - 48,7% des deklarierten Gehalts. Auch hier wurden die minderwertigen Misoprostol-Präparate von den Rwandischen Behörden landesweit zurückgerufen.

Die Ergebnisse der durchgeführten Stabilitätsuntersuchungen bestätigen im Fall von Oxytocin die auch von der WHO befürwortete Politik "Buy Quality Oxytocin, Keep It Cool".
Außerdem zeigte das Konservierungsmittel Chlorobutanol bei 40 °C und in forcierten Abbaustudien bei 80 °C eine bemerkenswerte stabilisierende Wirkung auf Oxytocin. Im Falle von Misoprostol konnte die Notwendigkeit einer intakten Primärverpackung für die Stabilität von Misoprostol gezeigt werden.

Sowohl in Malawi als auch in Ruanda war die Oxytocin-Qualität besser als in früheren Studien aus anderen LMICs berichtet. In beiden Ländern wurden extrem minderwertige Misoprostol-Tabletten gefunden, die ein ernsthaftes Risiko für die Gesundheit der Mütter darstellen. Dies zeigt, dass weitere Anstrengungen zur Qualitätssicherung bei der Arzneimittelbeschaffung und -registrierung sowie zur Überwachung nach dem Inverkehrbringen erforderlich sind.

Summary

Postpartum haemorrhage (PPH) is the leading cause of maternal mortality in low- and middle-income countries (LMICs). Oxytocin and misoprostol are used for the prevention and treatment of PPH. However, both medicines are chemically unstable and sensitive to environmental conditions. Previous studies reported a high prevalence of substandard oxytocin and misoprostol preparations in LMICs but no information was available for Malawi and Rwanda. Therefore, the quality of oxytocin injections and misoprostol tablets in randomly selected health facilities and pharmacies and from wholesalers in Malawi and Rwanda was determined. Temperature loggers were used to record the storage temperature of oxytocics. In Malawi, the availability of oxytocics, as well as the knowledge of health workers on storage requirements and use of oxytocics was also assessed. Additionally, the stability of oxytocin injections and misoprostol tablets purchased from wholesalers and medical stores in Malawi, Rwanda and Europe was investigated by accelerated stability testing according to ICH/WHO guidelines. Oxytocin samples were analysed for identity, assay (= quantity of oxytocin) and for pH value according to United States Pharmacopeia 40. Misoprostol samples were analysed for identity, assay, dissolution and related substances according to the International Pharmacopoeia 2017.

In Malawi, all visited hospitals and health centers had oxytocin available, however, storage conditions at the health facilities often did not meet the requirements. Out of 65 oxytocin samples, seven showed moderate deviations from specification, containing 82.2 – 86.8% of the declared amount of oxytocin. Out of 30 misoprostol samples, five showed extreme deviations, containing only 12.7 – 30.2% of the declared amount. The extremely substandard misoprostol was reported to the national authorities and to WHO, leading to an immediate recall of the respective brand in Malawi. The UK-based distributor of this brand closed its business shortly thereafter.

In Rwanda, storage requirements were followed correctly with few minor deviations. From the 57 oxytocin samples, all nine samples of one batch of a Chinese manufacturer showed an excessive content of oxytocin (range 117.2 - 121.5% of declared content), while another batch of the same manufacturer showed extreme variations of the concentration of the preservative benzyl alcohol. While 15 out of the 25 misoprostol samples passed, all 10 samples of two brands from India failed with

extreme deviations, containing only 42.4 - 48.7% of the stated amount of misoprostol. Similar to Malawi, the Rwandan authorities issued a recall of these two brands.

The results of the stability studies of oxytocin samples support the policy “Buy Quality Oxytocin, Keep It Cool”, which is also advocated by WHO.
Additionally, at 40 °C, and in forced degradation studies at 80 °C, chlorobutanol showed a remarkable stabilizing effect on oxytocin which may deserve further investigation.
For misoprostol tablets, the present stability studies have shown the importance of intact primary packaging to ensure the stability of misoprostol.

Both in Malawi and Rwanda, oxytocin quality was better than reported in previous studies in other LMICs. However, in both countries, extremely substandard misoprostol tablets were found, representing a serious risk to maternal health. This shows the need for continued efforts for quality assurance in medicine procurement and registration, as well as for post- marketing surveillance.

Publikationen und Präsentationen

Akzeptierte Publikationen

“Quality, availability and storage conditions of oxytocin and misoprostol in Malawi”

Hagen, N.; Khuluza, F.; Heide, L.

BMC Pregnancy and Childbirth. 2020; 20(1):184

“Stability of oxytocin preparations circulating in Malawi and Rwanda, carrying different storage recommendations. Stabilizing effect of chlorobutanol”

Hagen, N.; Bizimana, T.; Kayumba, P.C.; Khuluza, F.; Heide, L.

American Journal of Tropical Medicine & Hygiene, 2020; 103(5); pp.2129-2141

“Stability of misoprostol tablets collected in Malawi and Rwanda. Importance of intact primary packaging”

Hagen, N.; Bizimana, T.; Kayumba, P.C.; Khuluza, F.; Heide, L.

PloSOne, 2020; 15(9):e0238628.

“Quality of oxytocin and misoprostol in health facilities of Rwanda”

Bizimana, T.; Hagen, N.; Gnegel, G.; Kayumba, P.C.; Heide,L.

PloSOne, 2021; 16(1):e0245054.

Weitere Veröffentlichungen

„Apothekerin untersucht Arzneimittelqualität in Malawi“

Hagen, N.; Heide, L.

Pharmazeutische Zeitung, 44. Ausgabe, 31.10.2019

„Minderwertige Arzneimittel im Visier“

Hagen, N.; Heide, L.

Deutsche Apotheker Zeitung, 43. Ausgabe, 24.10.2019

Mündliche Präsentationen

“Quality, availability and knowledge of rational use of oxytocics in Malawi”
Kurs “Pharmacy in Global Health“, Pharmazeutisches Institut, Universität Tübingen, Oktober 2017

“The identification of substandard misoprostol tablets leads to product recall: Improving patient safety through collaboration of academic research and national authorities in Malawi”
Medicine Quality and Public Health Conference, University of Oxford, UK, September 2018

“Quality and stability of misoprostol and oxytocin preparations in Africa”
German and Japanese perspectives on global substandard & falsified medicines Mini-Symposium, Medi-Quality Security Institute, Kanazawa University, Japan, März 2019

“The importance of oxytocin and misoprostol in the prevention and treatment of post-partum haemorrhage”
Policy Dissemination workshop „Ensuring the quality of oxytocic medications for the prevention and treatment of post-partum haemorrhage in Malawi“, Ufulu Gardens, Lilongwe, Malawi, September 2019

“Oxytocin and misoprostol in Malawi: Pharmaceutical analysis”
Policy Dissemination workshop „Ensuring the quality of oxytocic medications for the prevention and treatment of post-partum haemorrhage in Malawi“, Ufulu Gardens, Lilongwe, Malawi, September 2019

“Quality of oxytocic medication in Malawi for prevention and treatment of post-partum haemorrhage”
Pharmaceutical Society of Malawi (PHASOM) Southern Region Conference, College of Medicine, Blantyre, Malawi, September 2019

“Quality of oxytocin and misoprostol preparations in Malawi”
Obstetrics & Gynaecology Department Queen Elizabeth Central Hospital, Blantyre, Malawi, September 2019

“Quality of oxytocic medication in Malawi for prevention and treatment of post-partum haemorrhage”

College of Medicine Research Talk, University of Malawi, Blantyre, Malawi, September 2019

“Quality of oxytocin and misoprostol in Malawi and how to ensure the quality of these medicines”

Training workshops zu minderwertigen und gefälschten Arzneimitteln, Pharmakovigilanzsystem, Lagermanagement und Qualität und Verfügbarkeit von Oxytocin und Misoprostol in Malawi in:
Blantyre District, College of Medicine, Blantyre, Malawi, September 2019
Chikwawa District, Chikwawa District Hospital, Malawi, September 2019
Neno District, Neno District Hospital, Malawi, September 2019
Ntcheu District, Ntcheu District Health Office; Malawi, September 2019

„Medikamentöse Versorgung in Entwicklungsländern, Katastrophengebieten und bei Großschadenslagen“

Sommerakademie Katastrophenmedizin und Humanitäre Hilfe, Universität Ulm, September 2019

Erklärung der Eigenanteile

“Quality, availability and storage conditions of oxytocin and misoprostol in Malawi”

Hagen, N; Khuluza, F.; Heide, L.;

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Autorenanteile:

- **Nhomsai Hagen**
    - Planung der Studie
    - Etablierung der analytischen Methoden
    - Training und Koordination der Sample Collectors
    - Sammeln der Proben und Daten
    - Dokumentation und Auswertung der gesammelten Proben und Daten
    - Planung, Durchführung und Auswertung der Analytik
    - Schreiben des Manuskripts
- Felix Khuluza
    - Feedback zur Studienplanung
    - Training und Koordination der Sample Collectors
    - Sammeln der Proben und Daten
    - Korrekturlesen des Manuskripts
- Lutz Heide
    - Planung und Initiierung der Studie
    - Betreuung des Projekts
    - Entscheidend an Auswertung der Daten und Diskussion der Ergebnisse beteiligt
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- Thomas Bizimana
    - Sammeln der Proben in Ruanda
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    - Betreuung des Projekts in Ruanda
    - Korrekturlesen des Manuskripts
- Felix Khuluza
    - Sammeln der Proben in Malawi
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    - Schreiben des Manuskripts
- **Nhomsai Hagen**
    - Planung der Studie
    - Etablierung der analytischen Methoden
    - Auswertung der Analytik
    - Korrekturlesen des Manuskripts
- Gesa Gnegel
    - Planung, Durchführung und Auswertung der Benzylalkohol-Konzentrationsbestimmung
    - Korrekturlesen des Manuskripts
- P. Claver Kayumba
    - Betreuung des Projekts in Ruanda
    - Korrekturlesen des Manuskripts
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Einleitung

Nachgeburtsblutungen sind die Hauptursache für Müttersterblichkeit in Ländern mit niedrigem Einkommen. Sie sind definiert als 500ml Blutverlust oder mehr innerhalb von 24 Stunden nach der Geburt (1). Die häufigste Ursache von Nachgeburtsblutungen ist ein atonischer Uterus – die Gebärmutter zieht sich nach der Geburt des Kindes nicht mehr zusammen. Dies kann mit Oxytokika wie dem Nonapeptid Oxytocin als erste Wahl, oder dem Prostaglandin-Analogon Misoprostol als zweite Wahl behandelt werden (Abbildung 1).

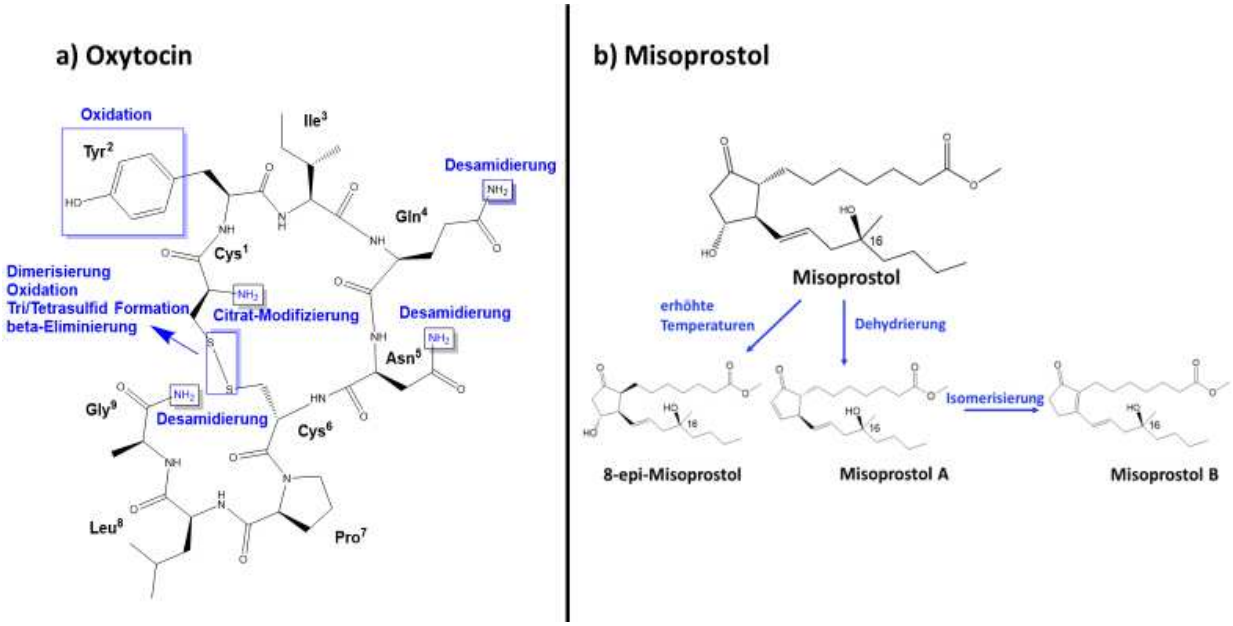

Abbildung 1: Strukturformeln von Oxytocin (modifiziert nach (2)) und Misoprostol mit deren typischen Zersetzungsmechanismen. Kommerzielles Misoprostol ist ein Gemisch aus der abgebildeten Struktur, ihrem Epimer an C-16 und den Enantiomeren beider Verbindungen. Entsprechende Stereoisomere werden für die Zersetzungsprodukte von Misoprostol gefunden. *Modifiziert nach: Hagen et al. (3)*

Vor allem in Ländern mit geringem oder mittlerem Einkommen (low- and middle-income countries, LMICs) werden Oxytocin und Misoprostol regulär auch zur Prophylaxe von Nachgeburtsblutungen im Rahmen der „aktiven Leitung der Nachgeburtsperiode“ (engl. „active management of the third stage of labour“, AMTSL) eingesetzt (1). Beide bewirken durch Bindung an Rezeptoren im Myometrium (Oxytocin an Oxytocin-Rezeptoren (4), Misoprostol an Prostaglandin-Rezeptoren (5)) eine Gebärmutterkontraktion (1). Sowohl Oxytocin als auch Misoprostol werden von der UN-Kommission für lebensrettende Güter für Frauen und Kinder (The United Nations Commission on Life Saving Commodities for Women’s

and Children's Health, UNCoLSC) unter den 13 lebensrettenden Gütern für Frauen und Kinder aufgeführt (6). Für Oxytocin und Misoprostol wurde geschätzt, dass in fünf Jahren ca. 15.000 Mütterleben gerettet werden könnten, wenn die häufig schlechte Qualität dieser Arzneimittel verbessert werden könnte, ihre Aufnahme in die nationalen Listen unentbehrlicher Arzneimittel sichergestellt und ihr flächendeckender und bezahlbarer Zugang erreicht werden könnten, so der Bericht von 2012 (7).
Die Senkung der weltweiten Müttersterblichkeit auf weniger als 70 pro 100.000 Lebendgeburten ist auch eines der Ziele der Vereinten Nationen (UN) für nachhaltige Entwicklung (Sustainable Development Goals, SDGs) (8). Bis zur Erreichung dieses Ziels ist es noch ein langer Weg, insbesondere in LMICs: Die Müttersterblichkeitsrate in Malawi ist eine der höchsten der Welt, mit schätzungsweise 439 Todesfällen pro 100.000 Lebendgeburten gemäß der Malawi Demographic and Health Survey 2015-16 (9), in Ruanda waren es 248 Todesfälle pro 100.000 Lebendgeburten im Jahr 2017 (10). In der fünften Ausgabe der malawischen Standard-Therapieleitlinien (Malawi Standards Treatment Guidelines, MSTG) von 2015, die auch die Liste essentieller Arzneimittel von Malawi (Malawi Essential Medicines List, (MEML)) beinhaltet, werden Oxytocin und Misoprostol als „lebenswichtige" Oxytokika zur Behandlung von Nachgeburtsblutungen aufgeführt (11). Oxytocin und Misoprostol sind auch in der aktuellen nationalen Liste essentieller Arzneimittel für Erwachsene von Ruanda gelistet (12).

Beide Medikamente reagieren allerdings sehr empfindlich auf Umwelteinflüsse (13-17). Die typischen Zersetzungsmechanismen von Oxytocin sind in Abbildung 1 dargestellt. Die Zersetzung von Oxytocin folgt einer Kinetik (pseudo-) erster Ordnung und ist pH-Wert– und temperaturabhängig. Dabei kommt es zum Beispiel je nach pH-Wert zu Desamidierungen und an der Disulfid-Brücke können sich Tri- und Tetrasulfide, sowie Dimere bilden (14). Bei pH 4,5 sind wässrige Oxytocin-Lösungen am stabilsten (14), weshalb die Arzneibücher für Oxytocin-Ampullen einen pH-Wert in diesem Bereich vorschreiben – laut der United States Pharmacopeia (USP), sowie der Internationalen Pharmacopeia (Ph. Int.) muss der pH-Wert im Bereich pH 3,0 - 5,0 liegen (18, 19). Wegen der Temperaturempfindlichkeit müssen die meisten Oxytocin-Produkte laut Packungsbeilage bei 2 - 8 °C gelagert werden, was insbesondere in ländlichen Gebieten in LMICs eine Herausforderung darstellen kann (20-23). Da Misoprostol-Tabletten bei Raumtemperatur gelagert und oral verabreicht

werden können, bieten sie eine Alternative zu Oxytocin-Ampullen – vor allem an Orten, an denen keine geeigneten Lagerbedingungen für Oxytocin gewährleistet werden können, oder an denen kein geschultes Personal für die parenterale Verabreichung von Medikamenten zur Verfügung steht (24). Misoprostol wird auch zur Prophylaxe und Behandlung von Geschwüren verwendet, die durch nicht-steroidal entzündungshemmende Medikamente (NSAIDs) ausgelöst werden, sowie zur Geburtseinleitung und für Abtreibungen (24-26). Wegen letzterer Indikation ist der Einsatz aus Angst vor Missbrauch in vielen Ländern eingeschränkt.
Auch die Instabilität von Misoprostol ist schon seit Jahrzehnten bekannt (27). Die wichtigste Zersetzungsreaktion ist die Dehydrierung, die zu Misoprostol A führt (Abb. 1). Toledo-Vasquez et al. (28) beschrieben, dass diese Reaktion in wässrigen Lösungen einer Kinetik erster Ordnung folgt und bei 60 °C und pH 7,66 zu einer Zersetzung von Misoprostol mit einer Halbwertszeit von nur 8,8 Stunden führt. Auf die Dehydrierung folgt die wesentlich langsamere Isomerisierung zum resonanzstabilisierten Misoprostol B (Abb. 1) (27, 28). Die Epimerisierung von Misoprostol an C-8 ist ein weiterer Zersetzungsmechanismus, der bei höheren Temperaturen auftritt (27).
Reines Misoprostol wird bei 55°C innerhalb von zwei Wochen zu 50% abgebaut (28). Eine Dispersion von Misoprostol in Hydroxypropylmethylcellulose (HPMC) ist deutlich stabiler, was die Verarbeitung zu Tabletten ermöglicht (17, 27-29). Misoprostol-HPMC-Dispersionen müssen jedoch sorgfältig vor Feuchtigkeit geschützt werden, da die Zersetzungsgeschwindigkeit von Misoprostol stark ansteigt, wenn der Wassergehalt der Dispersion 2% übersteigt (29), und Tabletten, die Misoprostol-HPMC-Dispersionen enthalten, nachweislich sehr schnell Wasser absorbieren, wenn sie ungeschützt gelagert werden (17). Wichtig ist die Erkenntnis von Hall (16), dass Kunststoff-Aluminium-Blister, die häufig als Primärverpackung von Tabletten verwendet werden, für die Gewährleistung der Stabilität von Misoprostol-Tabletten völlig unzureichend sind. Diese Studie untersuchte 215 Misoprostol-Proben, die in Asien, Afrika und Lateinamerika gesammelt wurden. Dabei wiesen alle in Kunststoff-Aluminium-Blistern verpackten Proben, die zum Analysenzeitpunkt ein Jahr oder älter waren, einen Misoprostol-Gehalt auf, der unter dem von der Ph. Int. festgelegten Grenzwert (90% der deklarierten Menge) lag. Beidseitige Aluminium-Blister boten einen besseren Schutz, aber ein Jahr oder länger nach der Herstellung wiesen selbst von diesen Tabletten 28% einen Misoprostol-Gehalt unter dem von der

Ph. Int festgelegten Grenzwert auf. Insgesamt waren 45% der 215 untersuchten Proben minderwertig (16).
Die für Oxytocin und Misoprostol bekannten Zersetzungsprodukte sind lediglich inaktiv, aber nicht toxisch (17, 30). Wenn sie jedoch ihre Wirksamkeit verlieren, z.B. durch unsachgemäße Lagerung, kann dies zu höheren Mortalitätsraten aufgrund von Nachgeburtsblutungen führen.

Mehrere Studien haben gezeigt, dass die Qualität von Oxytocin, insbesondere in Ländern mit niedrigem und mittlerem Einkommen (LMIC), oft schlecht ist (13, 22, 23, 31-33). Laut einem Review aus dem Jahr 2016, der 8 Studien mit insgesamt 559 Oxytocin-Proben aus 15 Ländern beinhaltete, wurden 57,5% der in Afrika gesammelten Proben als minderwertig eingestuft (31). Eine 2018 von Anyakora et al. veröffentlichte, in Nigeria durchgeführte Studie ergab, dass sogar 74,2% der analysierten Oxytocin-Ampullen außerhalb der Spezifikation lagen (23).

Die Qualität von Misoprostol-Präparaten, die in LMICs zirkulieren, hat in der wissenschaftlichen Literatur bisher viel weniger Aufmerksamkeit erhalten als die Qualität von Oxytocin. Neben der oben erwähnten Studie von Hall (16) haben außer unserer Arbeitsgruppe bisher nur die ebenfalls schon erwähnte Arbeitsgruppe von Anyakora et al. (23) nebst Oxytocin-Ampullen auch Misoprostol-Tabletten untersucht und ihre Ergebnisse veröffentlicht: 33,7% von 166 Misoprostol-Proben, die in Gesundheitseinrichtungen in Nigeria gesammelt wurden, entsprachen nicht den Spezifikationen der Internationalen Phamacopoeia für den Misoprostol-Gehalt. Leider gaben die Autoren nicht an, in welchem Ausmaß diese Proben vom deklarierten Gehalt abwichen.

Schlechte Qualität von Oxytocin-Ampullen und Misoprostol-Tabletten kann auf schlechte Herstellung (z.B. ungeeignete Formulierung, ungünstige Umweltbedingungen während des Herstellungsprozesses oder inadäquate Primärverpackung), auf schlechte Lager- und Transportbedingungen oder auf eine Kombination dieser Faktoren zurückgeführt werden.

Wie schon erwähnt, müssen die meisten der derzeit auf dem Markt befindlichen Oxytocin-Produkte gemäß der Herstellerangaben als Kühlware bei 2 - 8 °C gelagert werden. Mittlerweile sind aber auch mehrere Oxytocin-Präparate im Umlauf, die laut Hersteller bei Raumtemperatur gelagert werden können. Nach einem Bericht der

Reproductive Health Supplies Commission gibt es fast 300 verschiedene Oxytocin-Produkte von mindestens 100 Herstellern mit unterschiedlichen Angaben zur Lagerung und für viele Produkte ist nicht bekannt, ob es Stabilitätsdaten gibt, die die Kennzeichnung bezüglich Lagerung dieser Präparate rechtfertigen (34). Ähnliche Bedenken wurden von der WHO in einem Bericht über die Qualität von Arzneimitteln für die Gesundheit von Mutter und Kind geäußert, der zu dem Schluss kam: „Es wäre sinnvoll, zu überprüfen, inwieweit die Herstellerangaben für höhere Lagertemperaturen auf zuverlässigen Stabilitätsstudien beruhen“ (35). In einer umfassenden Literaturübersicht über die Stabilitäts- und Lagerspezifikationen von Oxytocin durch das "Promoting the Quality of Medicine Program" der United States Pharmacopeia wurde ebenfalls gefordert, systematische Stabilitätsstudien gemäß den ICH-Richtlinien durchzuführen, um die Angaben für Lagerung und Laufzeit von Oxytocin-Ampullen neu zu bewerten (36).

Die Stabilität von pharmazeutischen Produkten muss von den Herstellern untersucht und dokumentiert werden, nationale Arzneimittelbehörden bestätigen diese Daten in der Regel nicht zusätzlich. Ländern mit sogenannten Stringent Regulatory Authorities (SRAs) wird in der Regel vertraut, dass sie vollständige und korrekte Stabilitätsprüfungen durch die Hersteller gewährleisten. SRAs sind nationale Arzneimittel-Aufsichtsbehörden, die Mitglieder, Beobachter oder assoziierte Mitglieder des International Council of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) sind. Derzeit sind dies die nationalen Aufsichtsbehörden der EU-Mitgliedstaaten, der USA, Japans, der Schweiz, Kanadas, Australiens, Islands, Liechtensteins und Norwegens (37). Darüber hinaus werden im Rahmen des WHO-Programms zur Präqualifikation von Arzneimitteln fertige pharmazeutische Produkte für bestimmte Indikationen, einschließlich der reproduktiven und mütterlichen Gesundheit, auf der Grundlage der von den Herstellern vorgelegten Informationen und auf der Grundlage von Inspektionen der entsprechenden Herstellungsstätten präqualifiziert (38, 39). Viele Medikamente in LMICs sind jedoch weder in einem Land mit einer SRA hergestellt, noch von der WHO präqualifiziert. Nationale Zulassungsbehörden für LMICs haben oft nur begrenzte Kapazitäten für eine unabhängige Bewertung und Analyse (40, 41) und müssen sich bei der Registrierung von Arzneimitteln auf die Daten der Hersteller verlassen. Unabhängige wissenschaftliche Bestätigungen der

Herstellerangaben, z.B. zur Hitzestabilität von Oxytocin-Präparaten, sind daher wünschenswert.

Die ICH hat Richtlinien für die Stabilitätsprüfung von pharmazeutischen Produkten (Q1A-Q1F) formuliert (42). Die Prüfung der Stabilität eines Produkts über seine gesamte Laufzeit ist sehr zeitaufwendig, daher sind beschleunigte Stabilitätsstudien über einen kürzeren Zeitraum, aber bei höherer Temperatur und relativer Luftfeuchtigkeit (RH) zulässig und werden häufig zur Vorhersage der Haltbarkeit und der Lageranforderungen verwendet. Die genauen Bedingungen für beschleunigte Stabilitätsstudien hängen von der beabsichtigten Lagerungsempfehlung sowie von der Klimazone des Landes ab, in dem das Medikament registriert werden soll, wie auch von ICH (43) und den Richtlinien der WHO (37) vorgegeben. Malawi wird derzeit der Klimazone II (subtropisch und mediterran) zugeordnet, Ruanda der Zone IVa (heiß und feucht) (44).
Forcierte Abbaustudien (45) werden bei noch höheren Temperaturen durchgeführt. Sie wurden in dieser Arbeit verwendet, um den Einfluss von Stabilisierungsmitteln auf den Oxytocin-Abbau zu untersuchen.

Aber nicht nur die gute Qualität von Oxytocin und Misoprostol ist für die Senkung der Müttersterblichkeit von entscheidender Bedeutung; ebenso wichtig ist deren Verfügbarkeit und das Wissen des Gesundheitspersonals über den rationellen Einsatz von Oxytocin und Misoprostol, z.B. wann und wie die Medikamente verabreicht werden. Laut Weltgesundheitsorganisation (WHO) ist eine der wichtigsten Interventionen zur Förderung eines rationellen Einsatzes die Anwendung klinischer Leitlinien (46).

Zielsetzung

Daten über die Verfügbarkeit von Oxytocin und Misoprostol in Malawi wurden kürzlich veröffentlicht (47). Bislang gab es jedoch in der wissenschaftlichen Literatur keine Daten über die Qualität von Oxytocin und Misoprostol in Malawi, ebenso wenig über die Lagerungsbedingungen dieser Medikamente, noch über die Kenntnisse des Gesundheitspersonals über die Lagerungsanforderungen und die rationelle Anwendung von Oxytocin und Misoprostol in Malawi.

Auch für Ruanda gab es keine Daten zu der Qualität der im Land verfügbaren Oxytocin- und Misoprostol-Präparate.

Die vorliegende Arbeit zielt darauf ab, diese Lücke zu schließen, indem Oxytocin- und Misoprostol-Proben an verschiedenen Punkten der Versorgungskette (vom Großhändler bis zum Kreissaal) und in verschiedenen Gesundheitseinrichtungen in vier Distrikten Malawis und sechs Distrikten Ruandas gesammelt und ihre Qualität nach den Akzeptanzkriterien der United States Pharmacopeia bzw. der Internationalen Pharmacopoeia analysiert wurden. Durch das Sammeln der Proben von unterschiedlichen Stellen entlang der Versorgungskette sollte untersucht werden, ob sich die Qualität der Medikamente verschlechtert, was auf Zersetzung aufgrund langer/inadäquater Lagerung deuten würde:

Darüber hinaus wurden die Verfügbarkeit und die Lagerungsbedingungen von Oxytocin und Misoprostol in Malawi untersucht, sowie das Wissen des Gesundheitspersonals über die Lagerungsanforderungen und die rationelle Anwendung dieser Medikamente mit Hilfe eines Fragebogens.

Ein weiteres Ziel dieser Doktorarbeit war es, die Stabilität ausgewählter Oxytocin- und Misoprostol-Präparate aus Malawi, Ruanda und Europa mithilfe beschleunigter Stabilitätsstudien gemäß den ICH-Richtlinien zu untersuchen.

Zusätzlich wurde untersucht, wie sich die Stabilität von Misoprostol-Tabletten verändert, wenn die Primärverpackung beschädigt wurde.

Mithilfe forcierter Abbaustudien (45) bei 80 °C wurde der Einfluss von Hilfsstoffen wie Puffern und dem weit verbreiteten Konservierungsmittel Chlorobutanol auf die Stabilität von Oxytocin untersucht.

Ergebnisse

Design der Studie in Malawi

Das Studienprotokoll wurde auf Grundlage der MEDQUARG-Richtlinien (48) und der WHO-Leitlinien für die Durchführung von Studien über die Qualität von Arzneimitteln (49) erstellt.

Die Durchführung der Studie wurden von der Forschungs- und Ethikkommission der Medizinischen Hochschule in Malawi (College of Medicine Research and Ethics Committee, COMREC, Aktenzeichen S.07/27/2215), sowie der Malawischen Aufsichtsbehörde (Pharmacy, Medicines and Poisons Board, PMPB) genehmigt.

Die Studie wurde in vier Distrikten Malawis durchgeführt: Blantyre als urbaner Distrikt mit gemäßigtem Klima, Ntcheu als ländlicher Distrikt mit gemäßigtem Klima, Chikwawa als ländlicher Distrikt mit heißem Klima und Neno als ländlicher Distrikt, der sowohl Gebiete mit heißem als auch mit gemäßigtem Klima aufweist. Oxytocin- und Misoprostol-Proben wurden in den Distrikt- oder Zentralkrankenhäusern, sowie in randomisiert ausgewählten öffentlichen und kirchlichen Gesundheitszentren, Privatkliniken, Apotheken und Drugstores gesammelt.

Zusätzlich wurden Oxytocin- und Misoprostol-Proben auch vom nationalen Großhändler (Central Medical Stores Trust, CMST) und den 12 wichtigsten pharmazeutischen Großhändlern in Malawi angefragt.

Insgesamt wurden Oxytocin- und Misoprostol-Proben von 62 Gesundheitseinrichtungen, Großhändlern, Apotheken und Drugstores angefragt. In 45 dieser Einrichtungen konnten Oxytokika gesammelt werden, die Lage dieser 45 Einrichtungen ist in Abbildung 2 dargestellt. In den meisten Gesundheitseinrichtungen wurden Oxytokika sowohl im Medikamentenlager als auch im Kreissaal aufbewahrt, wenn möglich wurden Proben von beiden Stellen entnommen.

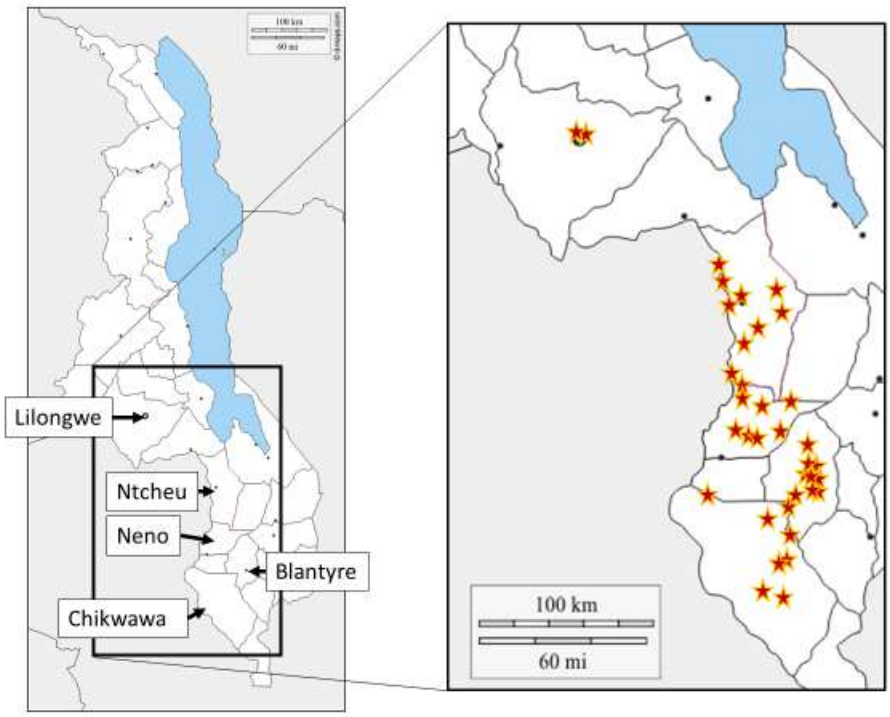

Abbildung 2: Karte mit den 45 Einrichtungen, in denen Oxytocin und Misoprostol gesammelt wurde. Gesammelt wurde in den 4 Distrikten, sowie von Großhändlern aus Blantyre and Lilongwe. *Aus: Hagen et al. (3)*

Das Sammeln der Proben fand zwischen September 2017 (Pilotstudie im Distrikt Ntcheu) und August 2018 (Distrikte Blantyre, Chikwawa und Neno) statt. Alle Einrichtungen in einem Distrikt wurden innerhalb einer Woche besucht. Die Erlaubnis zum Sammeln wurde zuvor beim zuständigen District Health Officer eingeholt. Die Besuche fanden jedoch ohne vorherige Benachrichtigung der jeweiligen Einrichtung statt, um Verfälschungen der Ergebnisse durch Vorbereitungen der Einrichtung zu verhindern.

In Krankenhäusern, Gesundheitszentren und privaten Apotheken wurde offen gesammelt, das heißt die Einrichtungen wurden über den Zweck des Besuchs informiert, und es wurde die Zustimmung zur Teilnahme an der Studie eingeholt. In diesen Einrichtungen wurden auch Temperaturlogger platziert, und Interviews mit Hilfe eines Fragebogens durchgeführt. Im Falle von Großhändlern und Drugstores wurde ein Mystery-Shopper-Ansatz angewandt, die Identität der Probensammelnden und der Zweck des Sammelns wurden also nicht preisgegeben. Daher wurden dort weder Temperaturlogger platziert noch Interviews durchgeführt.

Wenn verschiedene Marken (Original- oder Generikapräparate) oder Chargen verfügbar waren, wurde jede Marke und jede Charge als separate Probe gesammelt. Für jede Probe wurden, wenn möglich, 10 Ampullen Oxytocin bzw. 50 Tabletten

Misoprostol gesammelt, mindestens jedoch drei Ampullen Oxytocin oder sechs Tabletten Misoprostol pro Probe.
Die Proben wurden per Flugzeug in einer Kühltasche nach Deutschland transportiert (< 24 Stunden Transportzeit) und am Pharmazeutischen Institut der Universität Tübingen in einem Kühlschrank oder in einem klimatisierten Raum (< 25 °C) bis zur Analyse gelagert.
Alle Proben wurden an der Universität Tübingen untersucht. Zuerst wurden die Umverpackungen und Beipackzettel auf sichtbare Mängel (z.B. Rechtschreibfehler, manipulierte Verfalls- und Chargendaten, intakte Primärverpackung) begutachtet. Die Oxytocin-Proben wurden anschließend auf Identität, Gehalt und pH-Wert nach den Methoden der United States Pharmacopeia 2017 (USP 40) analysiert. Misoprostol Tabletten wurden auf Identität, Gehalt und Wirkstofffreisetzung nach den Methoden der Internationalen Pharmacopoeia 2017 untersucht. Wenn es Abweichungen im Gehalt gab, wurde zusätzlich der Test auf Abbauprodukte durchgeführt. Untersuchungsergebnisse zwischen 80 - 90% und 110 - 120% des deklarierten Gehalts wurden als mäßige Abweichungen betrachtet, Gehalte von weniger als 80% oder mehr als 120% wurden als extreme Abweichungen eingestuft (50). Gemäß der Definition der WHO wurden Produkte, die ihre Identität, Zusammensetzung oder Herkunft absichtlich falsch darstellen, als gefälscht betrachtet (51).

Verfügbarkeit von Oxytocin und Misoprostol in Malawi

Gemäß der Malawi Essential Medicines List (MEML) 2015 sollte Oxytocin in Gesundheitszentren, Distriktkrankenhäusern und Zentralkrankenhäusern verfügbar sein (11). Tatsächlich war in jedem der 27 besuchten Krankenhäuser und Gesundheitszentren (sowohl in öffentlichen als auch in kirchlich-betriebenen) Oxytocin verfügbar. Auch drei von vier Privatkliniken hatten Oxytocin vorrätig; die eine, die Oxytocin nicht vorrätig hatte, bot keine routinemäßige Geburtshilfe an, hatte jedoch Misoprostol für Abtreibungen auf Lager. Im Gegensatz dazu hatte keine der Apotheken Oxytocin vorrätig.

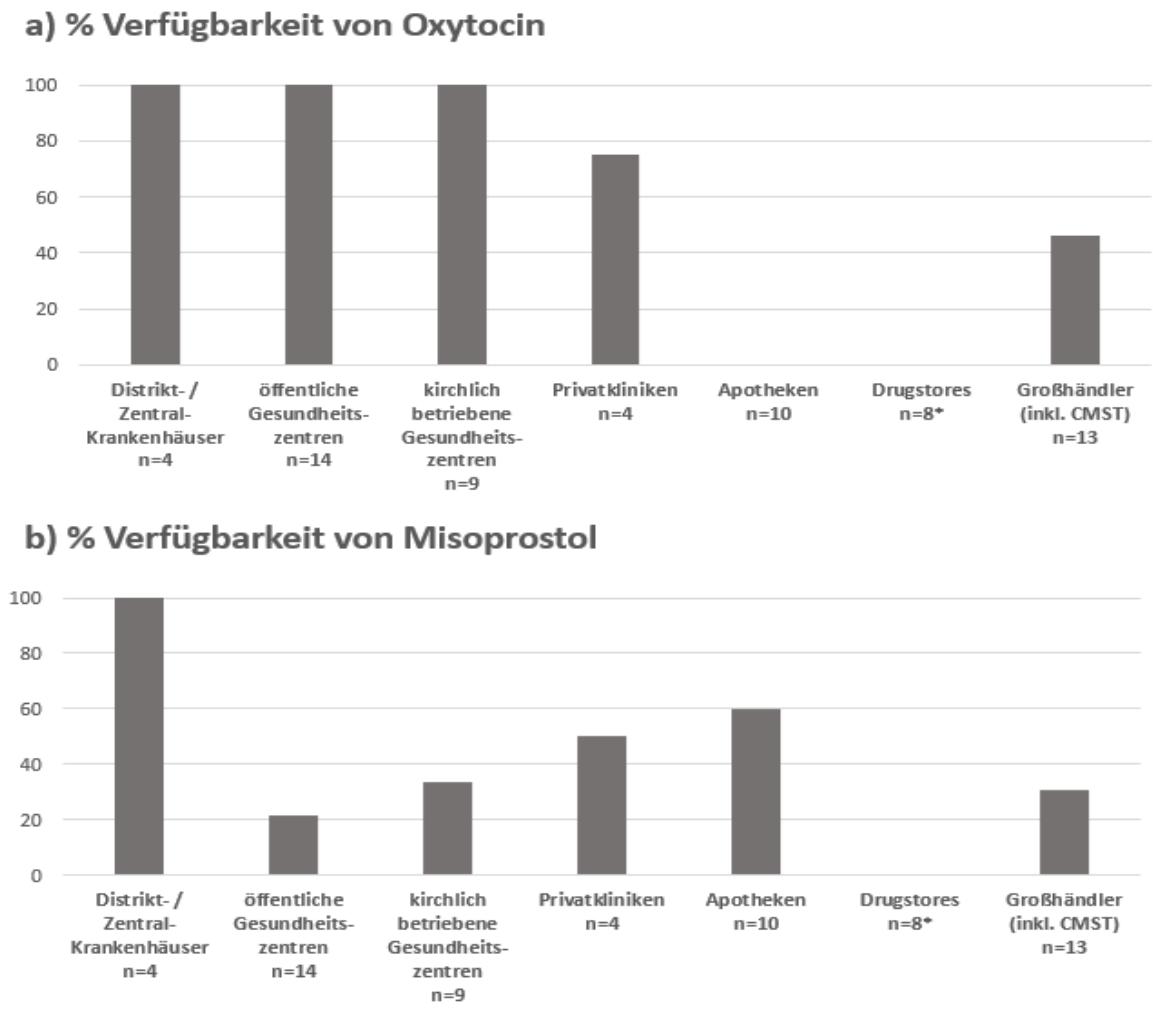

Abbildung 3: Verfügbarkeit von Oxytocin und Misoprostol in den besuchten Einrichtungen. CMST= Central medical stores trust. *Drugstores ist es nicht erlaubt, Oxytocin oder Misoprostol zu verkaufen, deshalb wurde dort eine 0%-Verfügbarkeit erwartet. *Modifiziert nach: Hagen et al. (3)*

Laut der MEML 2015 sollte Misoprostol auf Distrikt- und Zentralkrankenhausebene verfügbar sein (11), jedoch nicht in Gesundheitszentren, es sei denn, diese verfügen über spezielle klinische Expertise. Tatsächlich war Misoprostol in allen vier untersuchten Krankenhäusern verfügbar, jedoch nur in wenigen der Gesundheitszentren und Privatkliniken. Misoprostol war auch in sechs der 10 besuchten Apotheken erhältlich.

In Malawi sind Drugstores lizenzierte private Verkaufsstellen, die keinen Apotheker benötigen, sondern von einem pharmazeutisch-technischen Assistenten, einer Krankenschwester oder einem “clinical officer“ betrieben werden können (52). Sie dürfen eine begrenzte Anzahl gängiger Arzneimittel verkaufen, jedoch keine rezeptpflichtigen oder apothekenpflichtigen Arzneimittel und daher auch kein Oxytocin oder Misoprostol. Nichtsdestotrotz wurden auch Drugstores mit

einbezogen, da nach Angaben unserer lokalen Partner einige Drugstores Misoprostol zur Verwendung bei Schwangerschaftsabbrüchen illegal vertreiben. Alle acht Drugstores, die von den Testkäufern besucht wurden, gaben jedoch an, kein Oxytocin oder Misoprostol zu verkaufen.

Lagerbedingungen für Oxytocin und Misoprostol in Malawi

Um die tatsächliche Lagertemperatur zu bestimmen, wurden an allen Orten, an denen Misoprostol und Oxytocin in den Einrichtungen gelagert wurde (sowohl im Kreissaal als auch gegebenenfalls im Lagerraum) Temperaturdatenlogger aufgestellt, die die Temperatur etwa vier Monate lang aufzeichneten. Zur Auswertung wurde die mittlere kinetische Temperatur (MKT) herangezogen. Die MKT unterscheidet sich von dem arithmetischen Mittel der aufgezeichneten Temperaturen, indem Temperaturschwankungen besonders berücksichtigt werden. Sie wird häufig verwendet, um die Lagerbedingungen von Medikamenten zu beschreiben (53).

Die aufgezeichnete MKT von Loggern, die außerhalb des Kühlschranks platziert wurden, lag zwischen 21,4 und 31,0 °C (Median: 26,2 °C). Erwartungsgemäß war Chikwawa der heißeste Distrikt mit einem Median der MKT von 28,1 °C. Die höchste Einzeltemperaturmessung (40,1 °C) wurde ebenfalls in einer Einrichtung in Chikwawa gemessen. Aber auch für fünf der 17 in Kühlschränken platzierten Logger wurden MKTs zwischen 10,6 und 18,3 °C aufgezeichnet. Drei davon wurden in Kreißsälen aufgezeichnet, die täglich Stromausfälle meldeten.

Interviews mit Gesundheitspersonal

Anhand eines Fragebogens wurden Interviews mit den Personen geführt, die in den jeweiligen Einrichtungen für die Lagerung und/oder Verabreichung von Oxytokika verantwortlich sind. In diesen Interviews gaben nur 24% der für den Medikamentenlagerraum verantwortlichen Personen und 32% der für die Verabreichung von Oxytokika in den Kreißsälen verantwortlichen Personen an, jemals eine Schulung zur Lagerung, Verteilung und Handhabung von kühlpflichtigen Medikamenten erhalten zu haben. Von 61 befragten Personen berichteten sieben, dass sie jemals in ihrer beruflichen Praxis unwirksames Oxytocin (n=5) oder unwirksames Misoprostol (n=2) beobachtet hätten. Allerdings gaben nur zwei dieser sieben Personen an, dass sie die Behörden (oder die Lieferanten) darüber informiert hätten. Standardbehandlungsleitlinien (STGs) für Oxytocin oder Misoprostol waren in

23 (74%) der 31 besuchten Gesundheitseinrichtungen und in 66% der besuchten Kreißsäle verfügbar.

Chemische Analyse von Oxytocin-Proben

Insgesamt wurden 65 Oxytocin-Proben im Laufe dieser Studie gesammelt (siehe Abb. 4). Die visuelle Inspektion zeigte eine überraschend hohe Anzahl von Rechtschreibfehlern in der Packungsbeilage der in China hergestellten Oxytocin-Marke, gab aber ansonsten keinen Hinweis auf Qualitätsmängel oder Fälschungen. Der Gehalt der Oxytocin-Proben wurde mittels HPLC bestimmt, außerdem wurde der pH-Wert gemessen. Gemäß USP 40 müssen Oxytocin-Ampullen zwischen 90 und 110% der deklarierten Menge Oxytocin enthalten und einen pH-Wert zwischen 3,0 und 5,0 aufweisen.

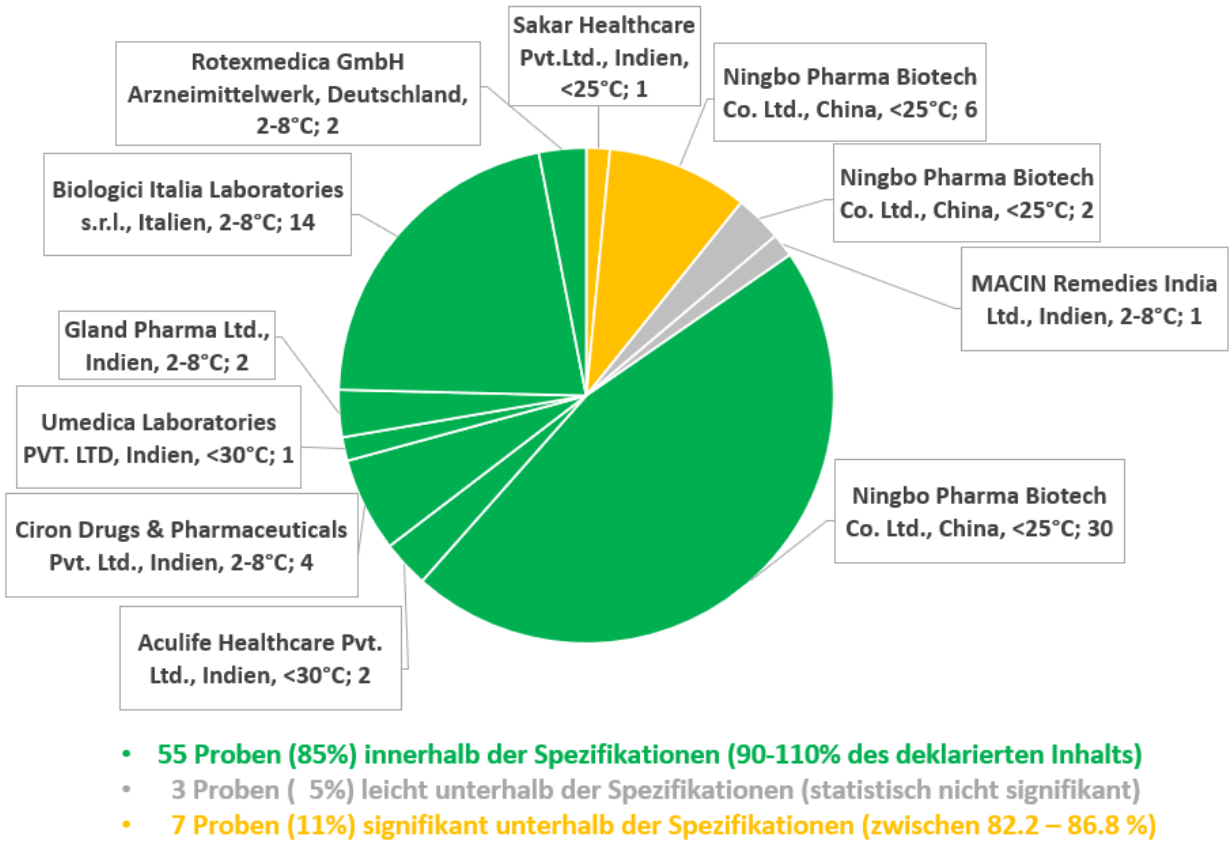

Abbildung 4: Gehaltsergebnisse der untersuchten Oxytocin-Proben inklusive Hersteller, Herstellerland, Lagerbedingungen laut Etikett und Anzahl der Proben. *Modifiziert nach: Hagen et al. (3)*

Der pH-Wert lag bei allen Proben innerhalb der Spezifikation. 55 der 65 Proben (85%) entsprachen auch der Spezifikation für den Oxytocin-Gehalt (Abb. 4). Drei Proben enthielten knapp unter 90% der deklarierten Menge Oxytocin, aber ihre

Abweichungen von der 90%-Schwelle waren angesichts der Standardabweichung der Messung statistisch nicht signifikant. Sieben Proben (11%) wiesen Oxytocin-Gehalte auf, die signifikant unter 90% des angegebenen Gehalts lagen (Bereich 82,2 - 86,8%). Eine der sieben minderwertigen Proben stammte von einem indischen Hersteller, während die anderen sechs aus einer einzigen Charge des chinesischen Herstellers stammten, bei dem bereits Rechtschreibfehler in der Packungsbeilage festgestellt worden waren. Alle Proben von zwei weiteren Chargen desselben chinesischen Herstellers lagen innerhalb der Spezifikation, darunter eine Charge mit einer kürzeren Restlaufzeit als die minderwertigen Proben.
Das Alter der Proben zum Zeitpunkt der Analyse variierte zwischen drei und 34 Monaten. Es gab keine Korrelation zwischen dem Oxytocin-Gehalt und dem Alter der Proben ($r2 = 0,00024$; $p = 0,90$). Der mittlere Gehalt der bei Großhändlern gesammelten Proben betrug 94,9%, während der mittlere Gehalt der Proben aus den Lagerräumen von Gesundheitseinrichtungen 96,0% und aus Kreißsälen 94,7% betrug. Es wurden also keine relevanten Unterschiede zwischen diesen Gruppen beobachtet.
Keine der 23 Proben, die vom Hersteller für die Lagerung bei 2 – 8 °C deklariert waren, waren minderwertig. Dahingegen fielen sieben der 39 Proben, die für die Lagerung bei < 25 °C gekennzeichnet waren, durch die Qualitätsprüfung und zeigten einen zu geringen Oxytocin-Gehalt. Nur drei Proben waren für die Lagerung bei < 30 °C deklariert; keine dieser Proben fiel durch die Qualitätsprüfung.

Chemische Analyse der Misoprostol-Proben

Im Rahmen dieser Studie wurden 30 Misoprostol-Proben gesammelt (Abb. 5). Bei der visuellen Inspektion fiel die ungeeignete Primärverpackung bei zwei Marken auf – eine Marke war in Aluminium-Kunststoff-Blistern (1 Charge, 3 Proben) verpackt, die andere sogar in einfachen Plastikschraubdosen zu 100 Tabletten pro Packung (1 Charge, 2 Proben). Ansonsten gab es keinen Hinweis auf Qualitätsmängel oder Fälschungen. Der Gehalt der Misoprostol-Proben sowie die Wirkstofffreisetzung wurde mittels HPLC nach den Verfahren der Ph. Int. 2017 bestimmt. Gemäß der Ph. Int. müssen Misoprostol-Tabletten zwischen 90 - 110% des deklarierten Gehalts enthalten, und mindestens 80% des Wirkstoffs müssen unter den im Arzneibuch beschriebenen Bedingungen innerhalb von 30 Minuten freigesetzt werden.

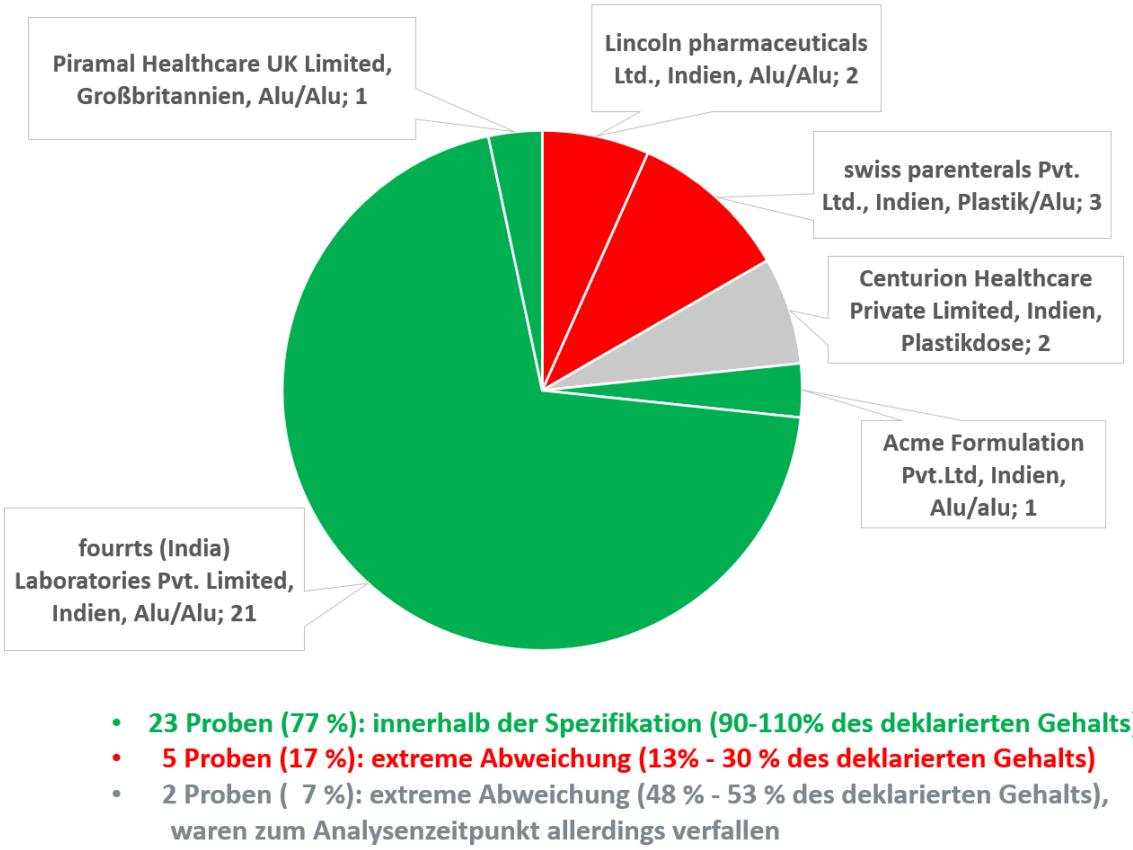

Abbildung 5: Gehaltsergebnisse der untersuchten Misoprostol-Proben inklusive Hersteller, Herstellerland, Art der Primärverpackung und Anzahl der Proben. *Modifiziert nach: Hagen et al. (3)*

23 der 30 Proben (77%) entsprachen der Spezifikation für den Misoprostol-Gehalt, diese 23 erfüllten auch die Spezifikation für die Wirkstofffreisetzung. Wohlgemerkt waren alle konformen Proben in Aluminium-Aluminium-Blister verpackt. Demgegenüber erwiesen sich sowohl die beiden in Plastikdosen verpackten Proben als auch alle drei in Kunststoff-Aluminium-Blistern verpackten Proben als extrem minderwertig, allerdings auch alle Proben einer korrekt in Aluminium-Aluminium-Blistern verpackten Marke.

Die in Plastikdosen verpackte Marke enthielt nur 53,0% (als versiegelte, ungeöffnete Dose gesammelt) und 48,8% (bereits angebrochene, geöffnete Dose zum Sammelzeitpunkt) des deklarierten Misoprostol-Gehalts. Insbesondere die Tabletten in der geöffneten Dose zeigten ebenfalls eine schlechte Wirkstofffreisetzung (34,3% des deklarierten Gehalts) mit einer extrem hohen relativen Standardabweichung (RSD) zwischen den einzelnen getesteten Tabletten (RSD= 37,08% bei sechs getesteten Tabletten). Dies deutet darauf hin, dass die Qualität der Tabletten durch die (ungleiche) Einwirkung von Feuchtigkeit stark beeinträchtigt worden war. Da

diese Tabletten zum Zeitpunkt der Analyse verfallen waren, stellen diese Ergebnisse keinen endgültigen Beweis für eine schlechte Qualität vor dem Verfallsdatum dar, obwohl es sehr wahrscheinlich ist, dass sie bereits vor Erreichen des Verfallsdatums außerhalb der Spezifikation lagen.
Wie bereits erwähnt, lag eine Marke (Lincoln Pharmaceuticals Ltd., Indien), die in Aluminium-Aluminium-Blistern verpackt war, dennoch extrem außerhalb der Spezifikation und enthielt nur 29,7 oder 30,2% der deklarierten Misoprostolmenge. Die Marke mit dem geringsten Misoprostol-Gehalt, die im Rahmen dieser Studie entdeckt wurde, wurde in Indien von Swiss Parenterals PVT, Ltd. hergestellt. Sie war in Kunststoff-Aluminium-Blister verpackt. Die HPLC-Analyse ergab einen Misoprostol-Gehalt von nur 12,7 - 13,4% der deklarierten Menge, und es wurde festgestellt, dass sich nur 7,9 - 9,5% des deklarierten Gehalts freisetzten. Die Prüfung der extrem minderwertigen Misoprostol-Präparate auf verwandte Substanzen zeigte die in Abb. 1 genannten typischen Zersetzungsprodukte.

Produktrückruf in Malawi und Schließung des verantwortlichen Zwischenhändlers in Großbritannien

Die extrem minderwertigen Misoprostol-Präparate von Lincoln Pharmaceuticals Ltd. und swiss parenterals Pvt. Ltd. wurden während der Pilotstudie im September 2017 entdeckt. Wie im Studienprotokoll vorgesehen, wurden PMPB, CMST und die WHO unverzüglich über diesen Befund informiert. PMPB veranlasste daraufhin einen Produktrückruf, und CMST stellte die Lieferung des minderwertigen Misoprostols ein. Im anschließenden Hauptteil dieser Studie (August 2018), wurden keine weiteren Proben der gemeldeten minderwertigen-Misoprostol-Präparate entdeckt, was darauf hindeutet, dass der Rückruf wirksam war.
Das Misoprostol-Präparat von swiss parenterals Pvt. Ltd war mit "Distributed by Premiumway International, www.premiumway.co.uk" gekennzeichnet. Wir berichteten über diesen Fall auch im elektronischen Newsletter der Initiative "e-drug" (54). Im August 2018 wies der Moderator dieses Newsletters darauf hin, dass der Vertreiber Premiumway International laut der Website der Companies House (https://beta.companieshouse.gov.uk/) der britischen Regierung an derselben Adresse ansässig sei und von denselben Personen geleitet werde wie die Firma Unimed International Ltd, die minderwertiges Propofol an die Regierung Sambias vertrieben hatte (55, 56). Daraufhin bat die WHO uns, eine Probe der von

Premiumway International vertriebenen Misoprostol-Tabletten an die britische Arzneimittelbehörde (MHRA) zu schicken, die unsere Analysenergebnisse bestätigte. Die genauen Maßnahmen, die die MHRA in der Folge ergriff, wurden der Öffentlichkeit nicht bekannt gegeben, aber wie auf der Website der Companies House veröffentlicht wurde, lösten sich sowohl Premiumway International als auch Unimed International Ltd am 28. Januar 2019 freiwillig auf.

Studie in Ruanda

Basierend auf dem Studienprotokoll für die Studie in Malawi, ist unter meiner Mitbetreuung eine Dissertation eines ruandischen Doktoranden zu Oxytocin- und Misoprostolqualität in Ruanda entstanden.
Thomas Bizimana hat 57 Oxytocin- und 25 Misoprostol-Proben von 40 randomisiert ausgewählten Gesundheitseinrichtungen und 6 Großhändlern in Ruanda gesammelt. Die Daten der platzierten Temperaturlogger zeigten, dass die notwendigen Lagerungsbedingungen größtenteils eingehalten wurden. Die Oxytocin-Proben bestanden aus sieben verschiedenen Chargen von vier Herstellern. Alle 24 Proben der drei europäischen Hersteller waren innerhalb der Spezifikationen der USP. Demgegenüber enthielten alle neun Proben aus derselben Charge eines chinesischen Herstellers zu viel Oxytocin (117,2 - 121,5% des deklarierten Gehalts) und eine andere Charge desselben Herstellers enthielt schwankende Konzentrationen des Konservierungsmittels Benzylalkohol. Die 25 Misoprostol-Proben bestanden aus 10 Chargen von sechs Herstellern, wovon 15 Proben von guter Qualität waren. Allerdings wurden, ebenso wie in Malawi, zwei extrem minderwertige Misoprostol-Präparate gefunden, von denen alle 10 Proben zu wenig Misoprostol enthielten (42,5 - 48,7% des deklarierten Gehalts). Beide Präparate stammten aus Indien und waren nicht WHO-präqualifiziert. Auch hier wurde die nationale Aufsichtsbehörde benachrichtigt, was auch hier zu einem Rückruf der minderwertigen Produkte führte.

Stabilitätsuntersuchungen von Misoprostol-Tabletten

Bei der Studienplanung wurden die ICH-Leitlinien, sowie die Leitlinien zur Stabilitätstestung pharmazeutischer Produkte der WHO berücksichtigt (37, 43).
Von allen Marken und Chargen von Misoprostol-Tabletten, die im Zeitraum Februar-März 2018 bei pharmazeutischen Großhändlern in Malawi und Ruanda erhältlich waren, wurden 250 Tabletten pro Probe gekauft. Am Pharmazeutischen Institut der

Universität Tübingen wurden alle Proben auf deren Gehalt vorgetestet. Alle Proben, die zu diesem Zeitpunkt innerhalb der Spezifikationen der Internationalen Pharmacopoeia lagen, wurden in die Stabilitätsuntersuchungen aufgenommen. Zum Vergleich wurde das Originalpräparat (Cytotec®) über die Apotheke des Universitätsklinikums Tübingen bezogen.
Letztendlich wurden, wie in Tabelle 1 dargestellt, drei Marken (insgesamt fünf Chargen) in die Stabilitätsprüfung einbezogen. Das Originalpräparat war in Großbritannien hergestellt worden, d.h. in einem Land mit einer SRA. Eine weitere Marke repräsentierte ein von der WHO präqualifiziertes Produkt, das in Indien hergestellt wurde. Dessen Laufzeit endete innerhalb des Untersuchungszeitraums, da es aber in der Vortestung noch innerhalb der Spezifikation lag, wurde es dennoch in die Stabilitätsstudie mit aufgenommen. Die dritte Marke war nicht WHO-präqualifiziert und war ebenfalls in Indien hergestellt worden.

Gesammelt in:	**Hersteller** (und Präparatename)	**Herstellerland**	**Herstellungs/ Verallsdaten**	**Charge**	**Angegebene Laufzeit**	**Angegebene Lagerbedingungen**	**Primär-verpackung**	**Angegebene Zusammensetzung**	**Prä-qualifikations Status**
Malawi	**Fourrts (India) Laboratories Pvt. Limited** (KONTRAC 200)	Indien	Jun 17/ Mai 19	E0571	2 Jahre	Unter 30°C an einem trockenen Ort. Vor Licht geschützt	Alu/Alu Blister	Nicht angegeben	keiner
			Feb 17/ Jan 19	D2205					
Ruanda	**Acme Formulation Pvt. Ltd.** (Ace Miso)	Indien	Sep 16/ Aug 18	ACE160963	2 Jahre	Unter 30°C vor Licht geschützt	Alu/Alu Blister	Misoprostol als HPMC dispersion (1%), excipients q.s.	WHO-PQ
Ruanda	**Piramal Healthcare UK Limited** (Cytotec®)	Großbritannien	Jul 17[1]/ Jun 20	B17173	3 Jahre[1]	Nicht angegeben	Alu/Alu Blister	Misoprostol-HPMC Dispersion (1%), mikrokristalline Cellulose, Natrium-Carboxymethylstärke, Hydriertes Rizinusöl	SRA
Deutschland			Feb 17[1]/ Jan 20	B16131		Unter 30°C vor Feuchtigkeit geschützt		mikrokristalline Cellulose, HPMC, Natrium-Carboxymethylstärke, Hydriertes Rizinusöl	

Tabelle 1: Untersuchte Misoprostol-Proben. Alle Proben enthalten Misoprostol in der Stärke 200 µg/Tablette.
HPMC: Hydroxypropylmethylcellulose. WHO-PQ: Von der WHO präqualifiziertes Produkt. SRA: hergestellt in einem Land mit stringenter Aufsichtsbehörde („stringent regulatory authority"). [1] diese Informationen waren nicht auf der Verpackung angegeben, sondern stammen aus Internet-Datenbanken (25, 26).
Modifiziert nach: Hagen et al. (57)

Die Proben wurden in ihrer Original-Primärverpackung sechs Monate lang (April-Oktober 2018) in Klimaschränken der Alpha-Pharma-Service GmbH, Heilbronn, Deutschland, gelagert. Die Bedingungen wurden in Übereinstimmung mit den ICH- und WHO-Richtlinien für Stabilitätsprüfungen von pharmazeutischen Produkten gewählt (26, 31). Die beschleunigte Stabilitätsprüfung wurde bei 40 °C +/- 2 °C und 75% +/- 5% relativer Luftfeuchtigkeit (RH) durchgeführt. Proben, die bei 25 °C +/- 2 °C und 60% +/- 5% RH, d.h. unter den ICH-Bedingungen für Langzeittests von ungekühlten Produkten, Klimazone II, gelagert wurden, wurden zum Vergleich untersucht. Nach 0, 1, 2, 3 und 6 Monaten wurden von jeder Probe 20 Tabletten aus beiden Klimaschränken entnommen und an der Universität Tübingen auf Gehalt, Wirkstofffreisetzung und verwandte Substanzen nach der Monographie von Misoprostol Tabletten der Internationalen Pharmacopoeia analysiert.
Wie in Abb. 4a zu sehen ist, blieben sowohl die Chargen des Originalpräparates als auch die Charge des von der WHO präqualifizierten Produkts während der gesamten sechsmonatigen Testphase bei 40 °C und 75% RH in den Spezifikationen, d.h. ihr Misoprostol-Gehalt blieb im Bereich von 90 - 110% der deklarierten Menge. Von den beiden Chargen des Produkts, die in einem Nicht-SRA-Land hergestellt und nicht von der WHO präqualifiziert worden waren, blieb jedoch nur eine Charge innerhalb der Spezifikationen, während die andere Charge einen Endgehalt von 86,2% der deklarierten Menge aufwies, welcher außerhalb der Spezifikationen liegt. Die Abnahme des Misoprostol-Gehalts dieser Probe betrug 14,5% über 6 Monate, was signifikant mehr ist als die Abnahme, die bei den Originalpräparaten und bei den von der WHO präqualifizierten Proben beobachtet wurde (Abnahme über 6 Monate: 8,9%, 7,4% bzw. 8,3%; alle $p < 0,0001$), und auch signifikant mehr als bei der anderen untersuchten Charge desselben Herstellers (7,5% Abnahme über 6 Monate; $p < 0,0001$). Entgegen den Erwartungen war die minderwertige Charge diejenige mit der längeren verbleibenden Haltbarkeitsdauer (Tabelle 1), was darauf hindeutet, dass ihre unterschiedliche Stabilität nicht auf das Alter der Probe zurückzuführen war, sondern möglicherweise auf Unterschiede zwischen den einzelnen Chargen bei der Herstellung dieses Produkts oder auf unterschiedliche Lagerbedingungen der beiden Chargen vor dem Kauf der Proben.

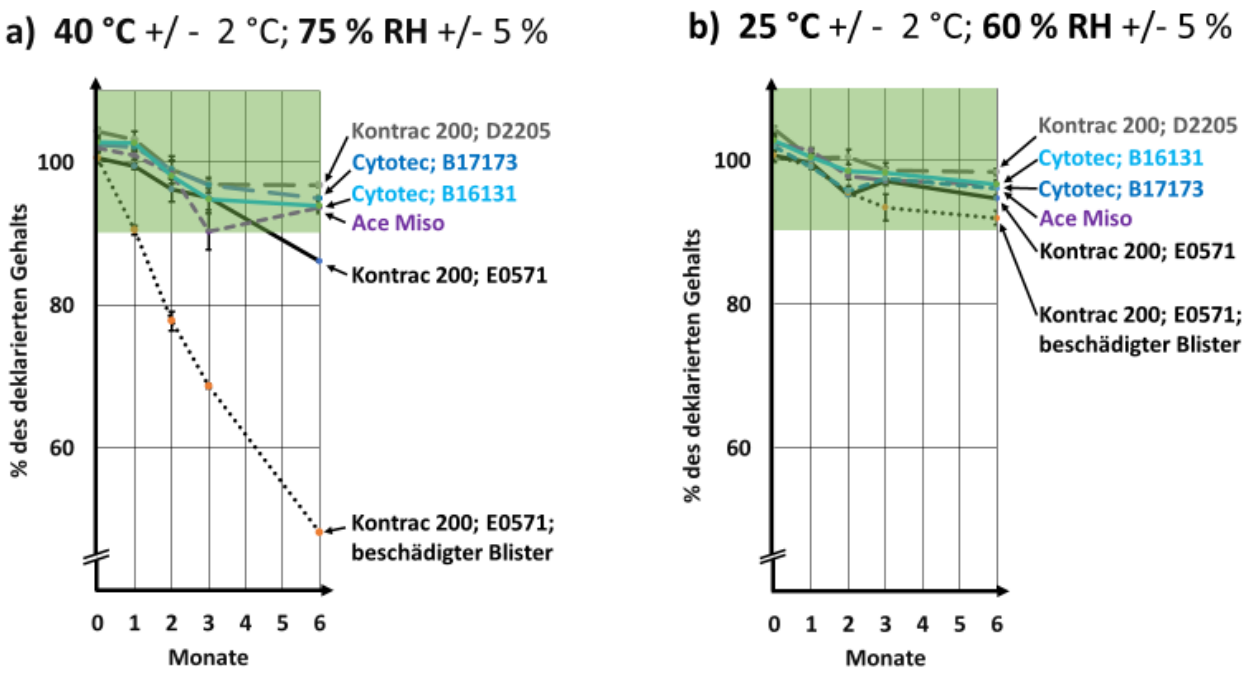

Abbildung 4: Veränderung des Misoprostol-Gehalts über 6 Monate. Fehlerbalken zeigen die Standardabweichung an. Die Internationale Pharmakopeia verlangt einen Misoprostol-Gehalt zwischen 90 - 110% der deklarierten Menge. Dieser Bereich ist in der Abbildung markiert. *Modifiziert nach: Hagen et al. (57)*

Auswirkung von beschädigten Blistern

Alle untersuchten Produkte waren korrekt in doppelseitigen Aluminiumblistern verpackt. Um die Bedeutung einer intakten Primärverpackung für die Stabilität der Misoprostol-Tabletten zu untersuchen, wurden die Blisterstreifen einer Probe absichtlich beschädigt, indem in jede Alveole der Blister ein einzelnes Loch von ca. 1 mm Durchmesser gestochen wurde. Die Probe in diesen punktierten Blister wurde parallel zu den Proben mit den intakten Blistern untersucht. Die Beschädigung der Primärverpackung hatte erwartungsgemäß einen starken negativen Einfluss auf die Stabilität (Abb. 4a): Bereits nach zwei Monaten bei 40 °C und 75% RH war der Misoprostol-Gehalt außerhalb der Spezifikationen, und nach sechs Monaten betrug die Restmenge an Misoprostol nur noch 48,2% des deklarierten Gehalts. Die Prüfung auf verwandte Substanzen zeigte deutlich die Abnahme des Misoprostol-Gehalts und die damit einhergehende Zunahme der typischen Zersetzungsprodukte von Misoprostol, d.h. Misoprostol A und in geringerem Maße auch Misoprostol B und 8-epi-Misoprostol (Abb. 5).

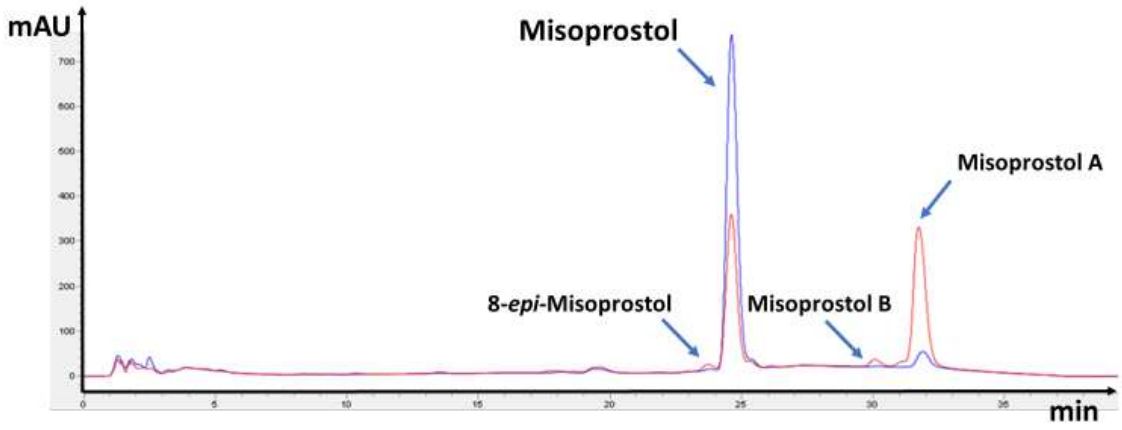

Abbildung 5: HPLC Chromatogramme nach Testung auf verwandte Substanzen von Misoprostol. Rot: beschädigte Blister nach 6-monatiger Lagerung bei 40 °C und 75% RH. Blau: intakte Blister nach 6-monatiger Lagerung bei 25 °C und 60% RH. Verwendet wurden jeweils KONTRAC 200 Tabletten von Fourrts (Indien), Charge E0571 (siehe Tabelle 1)**.** *Aus: Hagen et al. (57)*

Wie aus Abb. 4b hervorgeht, blieben die Testergebnisse aller fünf untersuchten Chargen unter Langzeitbedingungen bei 25 °C und 60% RH während der sechsmonatigen Testphase innerhalb der Spezifikationen. Selbst die Probe mit den durchstochenen Blistern blieb innerhalb der Spezifikationen, obwohl sie nach sechs Monaten von allen untersuchten Proben den niedrigsten Misoprostol-Gehalt aufwies (Abb. 4b).

Die Wirkstofffreisetzung der Proben mit intakten Blistern wurde bei beiden Bedingungen kaum beeinträchtigt – bei allen Proben wurden mehr als die laut Internationalen Pharmacopoeia geforderten 80% des deklarierten Wirkstoffes freigesetzt. Während bei 25 °C und 60% RH auch die Probe mit den beschädigten Blistern innerhalb der Spezifikation blieb, wurden nach sechs Monaten bei 40 °C und 75% RH nur 42,4% der deklarierten Misoprostolmenge freigesetzt, somit weit außerhalb der Spezifikation. Der gemessene Gehalt dieser Probe lag bei 48,2% bei diesen Konditionen.

Stabilitätsuntersuchungen von Oxytocin-Ampullen

Auch hier wurden bei der Studienplanung die ICH-Leitlinien, sowie die Leitlinien zur Stabilitätstestung pharmazeutischer Produkte der WHO berücksichtigt (37, 43).

Das Sammeln der Oxytocin-Proben verlief analog zu dem Sammeln der Misoprostol-Proben für die Stabilitätsuntersuchungen. Im Falle von Oxytocin wurden 100 Ampullen pro Probe gekauft. Auch hier wurden alle Proben auf deren Gehalt vorgetestet – alle Proben, die zu diesem Zeitpunkt innerhalb der Spezifikationen der

USP 40 waren, wurden in die Stabilitätsuntersuchungen einbezogen. Zum Vergleich wurden zwei Präparate über die Apotheke des Universitätsklinikums Tübingen, sowie vom internationalen Großhändler Imres B.V. aus den Niederlanden bezogen.
Auch die Lagerung in den Klimaschränken und Probeentnahmezeitpunkte erfolgte analog zu den Misoprostol-Proben – allerdings wurden die Oxytocin-Proben in vier verschiedenen Klimaschränken gelagert (siehe Tabelle 2).

Temperatur	Relative Feuchtigkeit[a]	ICH / WHO[b] Bedingungen für Stabilitätsuntersuchungen von:	
		Kühlware	Keine Kühlware
5°C +/- 3°C	n.def.	Langzeit	-
25°C +/- 2°C	**60%** +/- 5%	beschleunigt	Langzeit, Zone II
30 °C +/- 2°C	**65%** +/- 5%	beschleunigt[b]	Langzeit[a], Zone IVa, intermediat
40°C +/- 2°C	**75%** +/- 5%	-	beschleunigt

Tabelle 2: Bedingungen für Langzeit- und beschleunigte Stabilitätsprüfungen. Gemäß ICH/WHO (37, 43) sollen Langzeittests die vorgeschlagene Haltbarkeitsdauer oder ein Minimum von 12 Monaten zum Zeitpunkt der Markteinführung abdecken. Stabilitätsprüfungen unter beschleunigten oder intermediären Bedingungen sollten mindestens sechs Monate abdecken. n.def., nicht definiert. [a] Die Klimaschränke entsprachen ebenfalls den Spezifikationen für die relative Luftfeuchtigkeit der WHO-Richtlinien, obwohl dies für die untersuchten Oxytocin-Präparate nicht relevant ist, da die Ampullen als feuchtigkeitsundurchlässig gelten (37). [b] Zusätzlich zu den in den ICH-Richtlinien aufgeführten Bedingungen führen die WHO-Richtlinien auch strengere Bedingungen für Produkte auf, die in den Klimazonen III-IV vermarktet werden sollen. Malawi wird der Klimazone II, Ruanda der Klimazone IVa zugeordnet (44). *Modifiziert nach: Hagen et. al (58)*

Letztendlich wurden acht Marken (insgesamt 11 Chargen) in die Stabilitätsprüfung einbezogen. Vier dieser Marken waren in Belgien, Deutschland oder Italien hergestellt worden, d.h. in Ländern mit einer stringenten Aufsichtsbehörde (SRA) (37). Eine weitere Marke wurde in Lettland hergestellt und stellte ein von der WHO präqualifiziertes Produkt dar (59). Die anderen drei Marken stammten aus Indien und China, d.h. aus Ländern ohne stringente Aufsichtsbehörde. Sechs der acht Marken waren für die Lagerung bei 2 - 8 °C gekennzeichnet, während zwei Marken, die in China und Indien hergestellt wurden, für die Lagerung "unter 25 °C" bzw. "nicht über 30 °C" gekennzeichnet waren (Tabelle 3). Die deklarierte Haltbarkeit der verschiedenen Produkte variierte zwischen zwei und vier Jahren, und alle Proben blieben während der gesamten Dauer der Studie innerhalb ihrer Haltbarkeitsdauer.

Tabelle 3 zeigt auch die deklarierten (oder detektierten) Hilfsstoffe in den untersuchten Präparaten. Für eine optimale Stabilität von Oxytocin schreibt USP 40 vor, dass der pH-Wert zwischen 3,0 und 5,0 liegen muss. Dies wird in der Regel durch den Zusatz von z.B. Acetat- oder Citratpuffern erreicht (2, 60-62). Für die von der WHO präqualifizierte Zubereitung sowie für die vier Zubereitungen, die in Ländern mit einer stringenten Aufsichtsbehörde hergestellt wurden, wurde das Vorhandensein solcher Puffersubstanzen korrekt deklariert. Für das Sterop-Präparat wurde zusätzlich das Vorhandensein von 5 mg/ml des Konservierungsmittels Chlorobutanol deklariert.
Im Gegensatz dazu wurden bei den drei Präparaten aus China und Indien außer Wasser für Injektionszwecke keine Hilfsstoffe auf der Verpackung angegeben (beim Ciron-Präparat wurde nicht einmal das Wasser angegeben). Nichtsdestotrotz lag ihr pH-Wert im korrekten Bereich von 3,0 - 5,0 was auch während der Stabilitätsprüfung so blieb- Dies könnte auf das Vorhandensein von nicht deklarierten Puffersystemen hindeuten. Darüber hinaus zeigte die HPLC-Analyse das (nicht deklarierte) Vorhandensein von 1,5 mg/ml und 5,0 mg/ml Chlorobutanol in den Präparaten von Umedica bzw. Ciron. Chlorobutanol lässt sich in der HPLC-Analyse leicht nachweisen.

Gesammelt in:	Hersteller (und Präparatename, wenn nicht unter dem INN-Name vertrieben wurde)	Hersteller-land	Verfalls datum	Chargen-nummer	Laufzeit	Lagerung laut Etikett	Deklarierte (oder detektierte) Hilfsstoffe								Präqualifikations-Status
							Aqua ad inj.	Chlorobutanol	Natriumacetat	Eisessig	Natriumhydroxid	Kochsalz	Zitronensäure	Andere	
Malawi	Ningbo Pharma Biotech Co., Ltd (WW-Oxy 10)	China	Aug 19	160802	3 Jahre	unter 25°C	+							+[a]	keinen
			Jan 19	160183											
	Umedica Laboratories PVT. LTD	Indien	Jan 20	JA802	2 Jahre	Nicht über 30°C	+	1.5 mg/ml[b]						+[a]	keinen
	Ciron Drugs & Pharmaceuticals Pvt. Ltd.	Indien	Aug 19	7EA01228	2 Jahre	2 - 8°C	+[c]	5 mg/ml[b]							keinen
	Biologici Italia Laboratories S.r.l.	Italien	Mar 19	UF602ON	3 Jahre[d]	2 - 8°C	+		+	+	+				SRA
Ruanda	Laboratoires STEROP	Belgien	Aug 19	160269	3 Jahre	2 - 8°C	+	5 mg/ml				+	+		SRA
	Laboratoires STEROP (Steroxine 10 IU/1ml) [e]		Jan 19	160042											
	Rotexmedica GmbH Arzneimittelwerk	Deutschland	Sep 20	70779A	3 Jahre	2 - 8°C	+		+	+		+			SRA
	AS GRINDEKS	Lettland	Nov 20	37711116	4 Jahre	2 - 8°C	+		+	+	+	+			WHO-PQ
Europa	AS GRINDEKS		Sep 21	38910917											
	Hexal AG	Deutschland	Jun 20	HC0075	3 Jahre[f]	2 - 8°C	+			+		+			SRA

Tabelle 3: Untersuchte Oxytocin-Proben. Alle Proben sind 1ml-Ampullen mit der Stärke 10 IE/ml. WHO-PQ, von der WHO präqualifiziertes Produkt. SRA, hergestellt in einem Land mit strenger Aufsichtsbehörde.

[a] HPLC-Analyse zeigte zusätzliche Peaks, die auf zusätzliche, nicht identifizierte Inhaltsstoffe hindeuteten. [b] Chlorobutanol nicht deklariert, aber mittels HPLC-Analyse nachgewiesen. [c] Wasser für Injektionszwecke nicht explizit deklariert. [d] Herstellungsdatum nicht auf der Verpackung angegeben, Informationen stammen von den Websites der MHRA (http://www.mhra.gov.uk) und der HPRA (http://www.hpra.ie/). [e] Ein Marken- und ein Nichtmarken-Generikum der Laboratoires STEROP wurden in Ruanda gefunden, mit identischer Zusammensetzung. [f] Herstellungsdatum nicht auf der Verpackung angegeben, Information stammt vom Hersteller. *Modifiziert nach: Hagen et. al (58)*

Die Bewertungskriterien für Stabilitätsprüfungen sind in den erwähnten WHO-Richtlinien (37) ausführlich beschrieben, können aber wie folgt zusammengefasst werden: Ob eine Probe die beschleunigten Stabilitätsprüfungen besteht, wird auf der Grundlage von zwei Kriterien beurteilt: a) Über sechs Monate muss die Probe innerhalb der Spezifikation bleiben (bei Oxytocin: Wirkstoffgehalt zwischen 90 und 110% der deklarierten Menge und ein pH-Wert zwischen 3,0 und 5,0); b) innerhalb von sechs Monaten darf keine signifikante Veränderung des Wirkstoffgehalts auftreten, d.h. keine Veränderung des ursprünglichen Wirkstoffgehalts um 5% oder mehr (37, 43).

Die Temperaturbedingungen, denen die Oxytocin-Präparate bei beschleunigten Stabilitätsprüfungen standhalten müssen, hängen mit den Lagerungsempfehlungen zusammen, die der Hersteller auf dem Etikett angibt (37): Produkte, die als Kühlware (2 - 8 °C) gekennzeichnet sind, müssen ihre Stabilität für sechs Monate bei 25 °C oder 30 °C nachweisen, und die Entscheidung für eine dieser beiden Temperaturen sollte "auf einer risikobasierten Bewertung beruhen". Produkte, die mit "nicht über 25 °C lagern" gekennzeichnet sind, wie z.B. die Oxytocin-Zubereitung von Ningbo (Tabelle 3), müssen für sechs Monate bei 30 °C stabil bleiben ("intermediäre Bedingung", falls sie bei 40 °C durchfallen). Und Produkte, die mit "nicht über 30 °C lagern" gekennzeichnet sind, wie z.B. das Oxytocin-Präparat von Umedica (Tabelle 3), müssen ihre Stabilität für sechs Monate bei 40 °C nachweisen.

Das von der WHO präqualifizierte Präparat sowie die vier Präparate, die in Ländern mit einer SRA hergestellt wurden, bestanden die Stabilitätsprüfung und weisen Oxytocin-Gehalte zwischen 97,4% und 109,3% der angegebenen Menge nach sechs Monaten bei 30 °C auf. Es gab auch keine signifikante Veränderung des Wirkstoffgehalts und der pH-Wert blieb nach 6-monatiger Lagerung bei 30 °C zwischen 3 - 5.

Bei dem Präparat von Ciron wurde nach sechs Monaten bei 30 °C ein Oxytocin-Gehalt von 89,3% der deklarierten Menge bestimmt. Angesichts der relativen Standardabweichung dieser Messung (RSD = 1,55%) ist diese Abweichung von den Arzneibuch-Grenzwerten (90 - 110%) statistisch nicht signifikant (p= 0,459), weshalb dieses Präparat nicht als „durchgefallen“ eingestuft wurde. Die Veränderung des Wirkstoffgehalts dieser Zubereitung und ihr pH-Wert lagen innerhalb der zulässigen Grenzen.

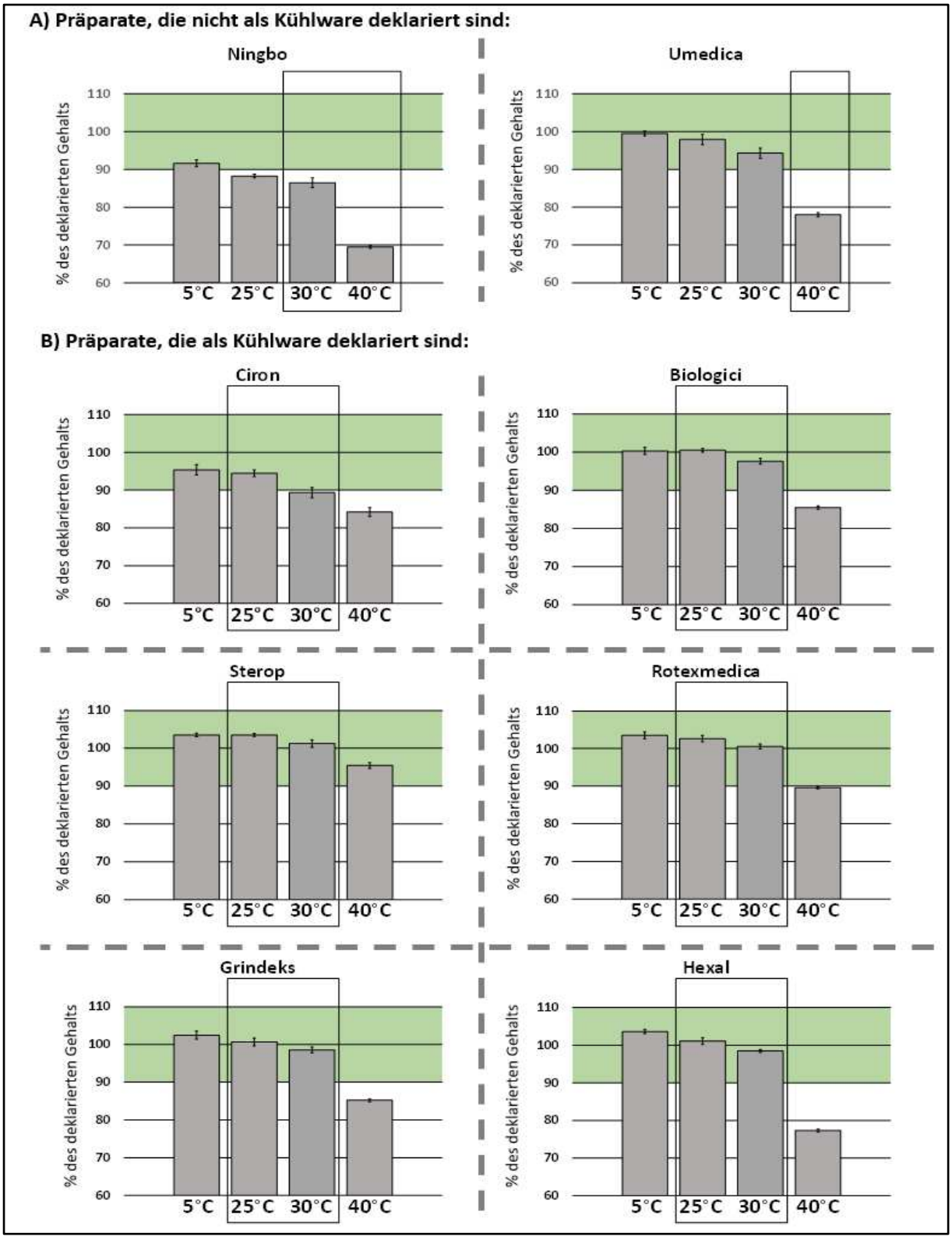

Abbildung 6: Oxytocin-Gehalt nach 6-monatiger Lagerung bei verschiedenen Temperaturen. Die vollständigen Namen der Hersteller und weitere Einzelheiten zu den untersuchten Präparaten sind in Tabelle 3 aufgeführt. Die hier gezeigten Ergebnisse wurden mit der Ningbo-Charge N°160802, der Sterop-Charge N° 160269 und der Grindeks-Charge N° 37711116 erzielt. Fehlerbalken zeigen die Standardabweichung an**.** *Modifiziert nach: Hagen et. al (58)*

Ein anderes Bild ergab sich für die Präparate von Ningbo und Umedica, d.h. für die beiden Präparate, die von ihren Herstellern als nicht kühlpflichtig gekennzeichnet wurden. Wie aus Abb. 6 sofort ersichtlich ist, war ihre Stabilität bei 30 °C und 40 °C nicht besser, sondern geringer als die der als Kühlware gekennzeichneten Präparate. Das Produkt von Umedica war für die Lagerung mit "nicht über 30 °C" gekennzeichnet, so dass es nach den WHO-Richtlinien sechs Monate lang bei 40 °C innerhalb der Spezifikationen bleiben musste. Mit einem endgültigen Oxytocin-Gehalt von nur 78,0% der deklarierten Menge war es jedoch weit außerhalb des in den Pharmakopöen angegebenen Bereichs von 90 - 110%. Darüber hinaus zeigte es einen 21%igen Verlust seines Oxytocin-Gehalts über sechs Monate bei 40 °C, was die zulässige Veränderung von 5% deutlich übersteigt. Das Präparat von Ningbo war für die Lagerung "unter 25 °C" gekennzeichnet. Beide untersuchten Chargen scheiterten eindeutig an der beschleunigten Stabilitätsprüfung bei 40 °C (endgültiger Oxytocin-Gehalt 69,5% bzw. 74,6%) und mussten daher nach WHO-Richtlinien bei "intermediaten Bedingungen" von 30 °C getestet werden. Nach sechs Monaten bei 30 °C enthielten die zwei Chargen 86,5% und 90,7%, wobei die Änderung des Oxytocin-Gehalts von Ersterer nur 3% betrug und damit noch innerhalb der zulässigen Grenze von 5% lag.

Die unterschiedliche Stabilität der untersuchten Präparate bei erhöhter Temperatur ist in Abb. 7 deutlich sichtbar, die den zeitlichen Verlauf des Verlustes an Wirkstoffgehalt über sechs Monate bei 40 °C zeigt. Diese Temperatur ist eine extremere Bedingung als für die Stabilitätsprüfung von kühlpflichtigen Präparaten empfohlen wird. Daher sind die in Abb. 7 dargestellten Daten für die meisten Präparate nicht direkt relevant für die Entscheidung ob das Produkt die Stabilitätsprüfung besteht, aber dennoch interessant im Hinblick auf die stabilisierende Wirkung bestimmter Hilfsstoffe. Die auffälligste Beobachtung aus diesen Daten ist, dass der Oxytocin-Gehalt bei den beiden Produkten am stabilsten war, die 5 mg/ml des Konservierungsmittels Chlorobutanol enthielten. In deutlichem Kontrast zu den anderen untersuchten Produkten zeigten diese beiden Präparate jedoch deutliche Veränderungen ihrer pH-Werte bei 40 °C und erreichten einen Endwert von 2,8 im Falle des Sterop-Produkts und 3,0 im Falle des Ciron-Produkts.

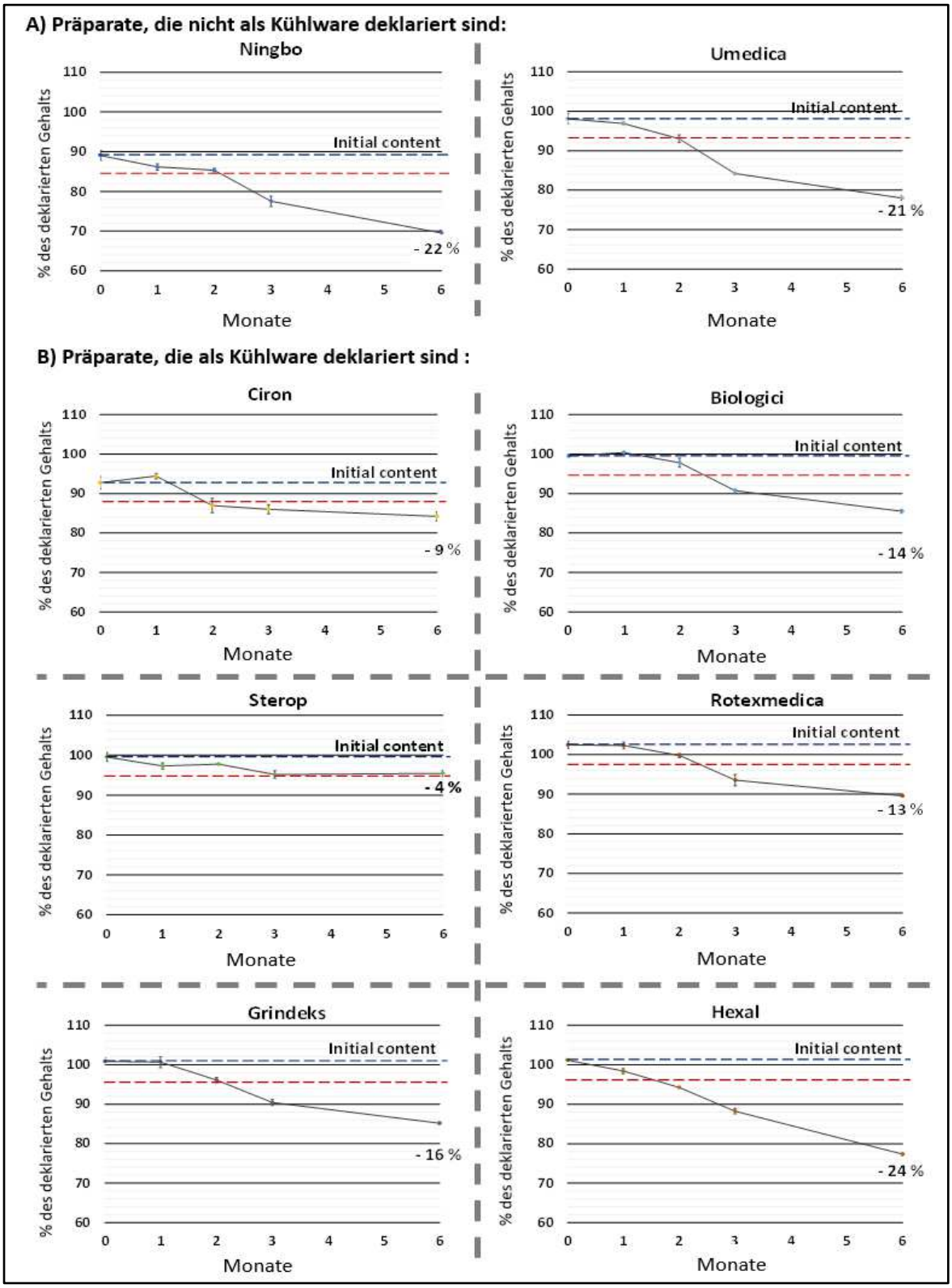

Abbildung 7: Änderung des Oxytocin-Gehalts über 6 Monate bei 40°C. Die hier gezeigten Ergebnisse wurden mit den gleichen Chargen erzielt, wie in Abb. 6 dargestellt. Der Anfangsgehalt und der Anfangsgehalt minus 5% sind als gestrichelte Linien dargestellt. Fehlerbalken zeigen die Standardabweichung an. Der endgültige Gehaltsverlust nach 6 Monaten wurde relativ zum Anfangsgehalt berechnet. *Modifiziert nach: Hagen et. al (58)*

Für drei der untersuchten Oxytocin-Marken wurden je zwei verschiedene Chargen mit unterschiedlichen Herstellungs- und Verfallsdaten gesammelt.
Die für die beiden verschiedenen Chargen derselben Marke gemessenen Gehaltsverluste waren in allen drei Fällen sehr ähnlich, was darauf hindeutet, dass die beobachteten Unterschiede in der Stabilität der verschiedenen Marken in erster Linie auf Unterschiede in der Herstellung und der pharmazeutischen Formulierung zurückzuführen sind und nicht auf Unterschiede im Alter oder in den Lagerbedingungen der untersuchten Produkte vor dem Kauf der Proben.

Forcierte Abbaustudien bei 80 °C für 5 Tage

Um den Einfluss der Zusatzstoffe auf die Stabilität von Oxytocin weiter zu untersuchen, wurden forcierte Abbaustudien bei 80 °C für fünf Tage mit den verschiedenen Präparaten durchgeführt (45).
Für zwei der Präparate (Umedica und Biologici; Tabelle 3) reichte die Anzahl der verbleibenden Ampullen für dieses Experiment nicht aus, für die anderen sechs Präparate sind die Ergebnisse in Abb. 8a dargestellt. In bemerkenswerter Ähnlichkeit mit den Ergebnissen bei 40 °C (Abb. 7) wiesen die vier Präparate von Rotexmedica, Grindeks, Ningbo und Hexal in dieser Reihenfolge eine abnehmende Stabilität auf, mit Oxytocin-Verlusten zwischen 55,9% und 67,5% innerhalb von fünf Tagen. In komplettem Gegensatz zu den bei 40 °C erzielten Ergebnissen zeigten jedoch die beiden Präparate von Ciron und Sterop, d.h. die Präparate mit 5 mg/ml Chlorobutanol, die höchsten Oxytocin-Verluste nach fünf Tagen bei 80 °C (Verlust von 75,2% bzw. 82,4%). Umgekehrt wiesen diese beiden Präparate nach einem Tag bei 80 °C immer noch deutlich höhere Oxytocin-Gehalte auf als die anderen vier (Abb. 8a).

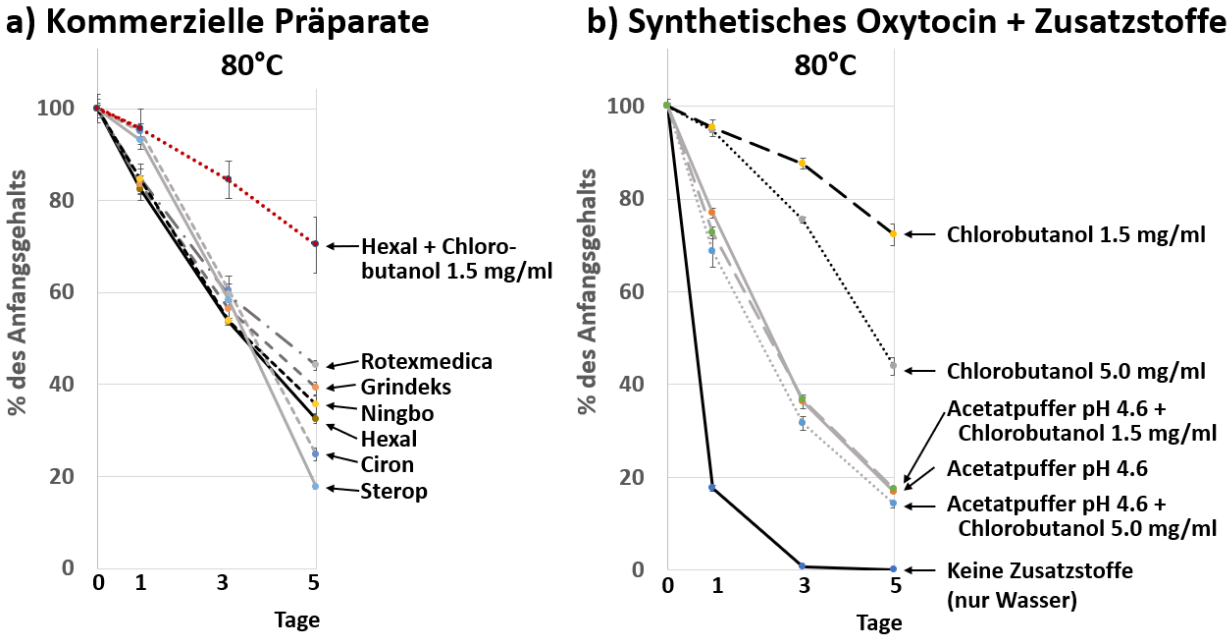

Abbildung 8: a) Forcierte Abbaustudien mit kommerziellen Oxytocin-Formulierungen. Einzelheiten zu den untersuchten Zubereitungen sind in Tabelle 3 aufgeführt.
b) Forcierte Abbaustudien mit Oxytocin-Lösungen von Oxytocin 10 IE/ml in Gegenwart verschiedener Zusatzstoffe. *Modifiziert nach: Hagen et. al (58)*

Es ist bekannt, dass Chlorobutanol bei erhöhter Temperatur unter Bildung von Salzsäure und anderen sauren Reaktionsprodukten hydrolysiert wird (63). Dies dürfte die bereits bei 40 °C beobachtete Änderung des pH-Wertes der Zubereitungen mit 5 mg/ml Chlorobutanol erklären. Wie zu erwarten, war dieser Effekt bei 80 °C noch ausgeprägter: beide Präparate zeigten nach fünf Tagen einen pH-Wert von 2,0 - weit entfernt von dem Stabilitätsoptimum von Oxytocin bei pH 4,5 (14).
Um die Wirkung von Hilfsstoffen auf die Oxytocinstabilität weiter zu untersuchen, wurden forcierte Abbaustudien mit reinem synthetischem Oxytocin in der Konzentration von 10 IE/ml in Wasser mit und ohne Zusatz von Acetatpuffer pH 4,6 und/oder Chlorobutanol in Konzentrationen von 1,5 mg/ml bzw. 5 mg/ml durchgeführt. In reinem Wasser wurde das Oxytocin vollständig abgebaut und fiel innerhalb von fünf Tagen unter die Nachweisgrenze (Abb. 8b). Dieser Abbau erfolgte wesentlich schneller als bei allen untersuchten kommerziellen Präparaten (Abb. 8a), was ein weiterer Hinweis darauf ist, dass alle kommerziellen Präparate Puffer- oder Stabilisierungsmittel enthalten, auch wenn dies auf dem Etikett nicht angegeben ist. Acetatpuffer hatte eine stabilisierende Wirkung auf die Oxytocinlösung (83,1% Oxytocin-Verlust innerhalb von fünf Tagen). Bemerkenswert ist, dass die Zugabe von 1,5 mg/ml Chlorobutanol eine noch stärkere stabilisierende Wirkung hatte: nur 27,7%

der Oxytocin-Menge gingen über 5 Tage bei 80 °C verloren; der gemessene pH-Wert nach 5 Tagen lag bei 2,8. Der Zusatz von Chlorobutanol in einer Konzentration von 5 mg/ml war für die Stabilisierung weniger wirksam (56,1% Oxytocin-Verlust) - hier wurde auch ein End-pH-Wert von 2,2 bestimmt, was weit unter dem Stabilitätsoptimum von 4,5 liegt.

In einem letzten Experiment wurde der kommerziellen Oxytocin-Zubereitung von Hexal Chlorobutanol zugesetzt, bevor es fünf Tage lang bei 80 °C gelagert wurde. Wie in Abb. 8a gezeigt, erhöhte die Zugabe von 1,5 mg/ml die Stabilität von Oxytocin unter diesen Bedingungen stark (29,6% Oxytocin-Verlust, verglichen mit 67,5% in Abwesenheit von Chlorobutanol; End-pH 2,8). Die Zugabe von 5 mg/ml Chlorobutanol zum Hexal-Präparat war für die Stabilisierung weniger wirksam (Oxytocin-Verlust 68,4%), was mit einer beobachteten drastischen Änderung des pH-Wertes (End-pH 2,0) übereinstimmt. Diese Ergebnisse sind ähnlich denen, die mit dem synthetischen Oxytocin beobachtet wurden (Abb. 8b).

Die HPLC-Analysen der bei den forcierten Abbaustudien entnommenen Proben zeigten nicht nur einen quantitativen Effekt von Chlorobutanol auf die Geschwindigkeit des Oxytocinabbaus, sondern auch einen auffälligen qualitativen Effekt (Abb. 9). In Abwesenheit von Chlorobutanol traten gleichzeitig mit der Abnahme des Oxytocin-Peaks (Retentionszeit 8,2 min) mehrere Peaks von Zersetzungsprodukten mit höherer Retentionszeit (15,1 - 19,3 min) auf (Abb. 9a). Dies ist vergleichbar mit den Beobachtungen von Avanti et al.,(2) die diese Zersetzungsprodukte als Oxytocin-trisulfide und -tetrasulfide sowie als verschiedene Disulfid-gebundene Oxytocindimere identifizierten, von denen einige an einer oder mehreren der drei amidierten Carboxylgruppen des Oxytocins desamidiert wurden (Abb. 1). Bemerkenswert ist, dass in der vorliegenden Studie diese Zersetzungsprodukte bei Zusatz von 1,5 mg/ml Chlorobutanol nicht beobachtet wurden (Abb. 9b) - dieselbe Beobachtung wurde für 5 mg/ml Chlorobutanol sowie für die Zubereitungen von Ciron und Sterop gemacht, die auch jeweils 5 mg/ml Chlorobutanol enthielten. Im Gegensatz dazu wiesen alle untersuchten Lösungen, die kein Chlorobutanol enthielten, Zersetzungsprodukte im Bereich von 15,1 - 19,3 min auf.

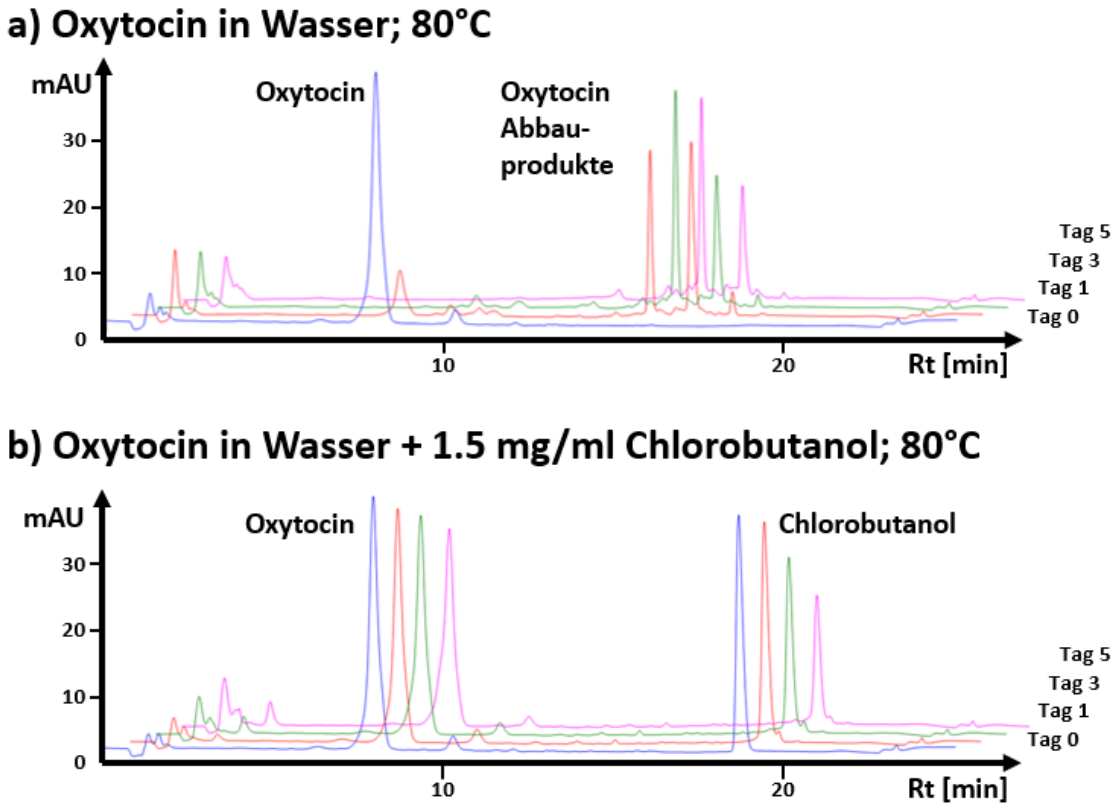

Abbildung 9: HPLC-Chromatogramme von Oxytocin 10 IU/ml in Wasser unter forciertem Abbau bei 80 °C, ohne (a) und mit Chlorobutanol 1,5 mg/ml (b). *Modifiziert nach: Hagen et. al (58)*

Weder in den beschleunigten Stabilitätsstudien noch in den forcierten Abbaustudien wurden bei den untersuchten Produkten sichtbare Qualitätsmängel wie Partikel, Ausfällungen oder Farbveränderungen beobachtet.

Diskussion

Die vorliegende Arbeit zeigte eine gute Verfügbarkeit von Oxytokika, insbesondere von Oxytocin, in den Gesundheitseinrichtungen Malawis. Dies ist ein wichtiger Beitrag zur Erreichung der SDGs, insbesondere zur Senkung der weltweiten Müttersterblichkeit.

Andererseits offenbarte sie weit verbreitete Probleme bei der Einhaltung der korrekten Lagertemperaturen der Medikamente. Dennoch war der Anteil der minderwertigen Proben in Malawi mit 11% der untersuchten Oxytocin-Proben und 17% der untersuchten Misoprostol-Proben weit unter dem Anteil, der in anderen Ländern (6, 13, 16, 22, 23, 31-33) ermittelt wurde. Der bereits erwähnte Review von Torloni et al. aus dem Jahr 2016 (31) wurde 2020 überarbeitet und veröffentlicht (64). Zu den 2016 untersuchten acht Studien über Oxytocin-Qualität kamen sechs weitere

hinzu, darunter auch unsere Malawi-Studie (3). Von den insgesamt 979 Oxytocin-Proben waren 39,7% minderwertig.
Die in Malawi gefundenen Misoprostol-Proben wiesen allerdings extreme Abweichungen auf (mit nur 12,7 - 30,2% der deklarierten Menge des Wirkstoffs), was ein ernsthaftes Risiko für die Patientensicherheit in der mütterlichen Gesundheitsversorgung darstellt. Ähnliches wurde auch in Ruanda beobachtet. Bemerkenswert ist, dass sowohl nationale als auch internationale Behörden schnell und effektiv auf diesen Befund reagiert haben.

Viele derzeit erhältliche Oxytocin-Präparate sind für die Lagerung bei 2 - 8 °C gekennzeichnet. Genau wie frühere Studien in anderen Ländern (23, 65) hat diese Arbeit gezeigt, dass diese Lagertemperatur in mehreren Gesundheitseinrichtungen in Malawi nicht zuverlässig eingehalten werden kann. Gründe dafür waren fehlende Kühlschränke, häufige Stromausfälle und das Fehlen von Notstromgeneratoren oder Sonnenkollektoren in vielen Einrichtungen. In dieser Situation erscheint es verlockend, die kühlpflichtigen Oxytocin-Marken durch Präparate zu ersetzen, die für die Lagerung bei höheren Temperaturen gekennzeichnet sind, oder sogar durch Misoprostol-Tabletten, die keine Kühlung benötigen. Die Ergebnisse dieser Arbeit legen jedoch nahe, dass diese Strategie keine einfache und zuverlässige Lösung des Problems bietet. Insbesondere wurde festgestellt, dass alle gesammelten Oxytocin-Proben, die für die Lagerung bei 2 - 8 °C gekennzeichnet waren, innerhalb der Spezifikationen lagen, selbst wenn sie in der Gesundheitseinrichtung falsch gelagert wurden. In scharfem Kontrast dazu entsprachen 18% der Oxytocin-Präparate, die für die Lagerung bei < 25 °C etikettiert waren, nicht den Spezifikationen.
Die Stabilitätsuntersuchungen haben diese Beobachtung bestätigt: Von den acht untersuchten Oxytocin-Marken haben zwei die beschleunigten Stabilitätsstudien nicht bestanden – beides Präparate, die nicht als Kühlware deklariert waren.
Im Laufe unserer Stabilitätsuntersuchungen veröffentlichten Nguyen et al.(66) ebenfalls Ergebnisse von Stabilitätsstudien von fünf Oxytocin-Produkten. Drei dieser Produkte, die für die Lagerung bei 2 - 8 °C gekennzeichnet sind, wurden von uns ebenfalls untersucht. Die von Nguyen et al. nach 1-, 2- und 3-monatiger Lagerung bei 30 °C und 40 °C erzielten Ergebnisse stimmen hervorragend mit den Unseren überein.
Nguyen et al. untersuchten auch eine europäische Oxytocin-Marke, die für die Lagerung bei < 25 °C etikettiert war, sowie ein argentinisches Produkt, das früher für

die Lagerung bei < 25 °C etikettiert war, aber kurz vor ihrer Studie für die Kühllagerung umetikettiert worden war. Sie berichteten, dass diese beiden Präparate bei 30 °C und 40 °C ein sehr ähnliches Stabilitätsprofil aufwiesen wie die drei als Kühlware etikettierten Produkte. Erst nach vier Monaten bei 40 °C und insbesondere beim forcierten Abbau bei 50 °C zeigte das europäische Produkt, das für die Lagerung bei < 25 °C etikettiert worden war, viel höhere Oxytocin-Verluste als die anderen, was mit einem starken Abfall des pH-Wertes einherging. Dieses Präparat enthielt 5 mg/ml Chlorobutanol. Die Autoren kamen zu dem Schluss, dass von den getesteten Produkten diejenigen, die für die Lagerung bei < 25 °C vorgesehen waren, keinen Stabilitätsvorteil gegenüber den als Kühlware etikettierten Produkten boten.

Die drei Präparate, die unsere beschleunigten Stabilitätsuntersuchungen nicht oder nur sehr knapp bestanden haben, waren alle in Ländern ohne SRA hergestellt worden. Keines der Präparate war WHO-präqualifiziert. Demgegenüber waren alle fünf eindeutig bestandenen Präparate in Ländern mit SRA hergestellt worden oder WHO-präqualifiziert. Ähnliches wurde bei den Stabilitätsuntersuchungen von Misoprostol-Präparaten beobachtet. Diese Beobachtung kann sicherlich nicht für alle Hersteller und Medikamente aus Ländern ohne SRA übertragen werden, macht aber deutlich, wie wichtig eine sorgfältige Lieferantenauswahl bei der Arzneimittelbeschaffung ist.

Die Ergebnisse der vorliegenden Arbeit unterstützen die Maxime "Buy Quality Oxytocin, Keep It Cool", die von der Reproductive Health Supplies Coalition und anderen internationalen Interessengruppen vertreten wird (36, 67-70).

Eine Möglichkeit, gute Qualität zu gewährleisten, besteht darin, die Beschaffung auf von der WHO präqualifizierte Arzneimittel (39, 59) und auf Arzneimittel zu beschränken, die in Ländern mit einer strengen Aufsichtsbehörde (SRA) hergestellt werden (37). Dies gilt nicht nur für Oxytocin-Produkte, sondern insbesondere auch für Misoprostol-Präparate.

Bei Misoprostol-Präparaten sollten nur jene mit einer geeigneten Primärverpackung, z.B. Alu-Alu-Blister, beschafft und verwendet werden und das Gesundheitspersonal und die Endnutzer sollten über die Wichtigkeit der intakten Primärverpackung geschult werden.

Versuche, hitzestabile Formulierungen von Oxytocin zu entwickeln, wurden in der Literatur ausführlich beschrieben (2, 14, 60-62, 71), wobei der Schwerpunkt auf der

Einbeziehung bestimmter Puffer und zweiwertiger Metallionen lag. Die auffällige Wirkung von Chlorobutanol, sowohl auf die Geschwindigkeit der Oxytocinzersetzung (Abb. 8) als auch auf die Art der gebildeten Zersetzungsprodukte (Abb. 9) ist in der Literatur bisher jedoch nicht näher beschrieben worden. Diese stabilisierende Wirkung wurde nur sehr kurz von Liu et al. erwähnt (72) und eine Molekulardynamik-Computersimulation von Xu et al. (73, 74) legte nahe, dass Chlorobutanol die Anzahl der Wasserstoffbrückenbindungen zwischen Oxytocin und Wasser reduzieren und die Aggregation von Oxytocinmolekülen verhindern kann.

Die Beobachtung, dass Chlorobutanol-haltige Lösungen bei forcierten Abbaustudien (ab 50 °C) zu einer Absenkung des pH-Wertes und vermutlich daraus resultierend zu einer Abnahme der Oxytocin-Stabilität führen, mag bei den im Alltag üblichen Lagertemperaturen von Oxytocin möglicherweise nicht relevant sein.

Weiterführende Untersuchungen zur stabilisierenden Rolle des Chlorobutanols gegenüber Oxytocin könnten letztendlich zu vielversprechenden tatsächlich hitzestabilen Oxytocin-Formulierungen führen.

Angesichts des Mangels an konsistenten Beweisen dafür, dass die Produkte, die momentan für die ungekühlte Lagerung gekennzeichnet sind, eine bessere Temperaturstabilität aufweisen als solche, die für die Lagerung bei 2 - 8 °C gekennzeichnet sind, und darüber hinaus angesichts der eindeutigen Hinweise darauf, dass die Lagerungsempfehlungen der Hersteller von "unter 25 °C" und sogar von "unter 30 °C" in vielen Einrichtungen in LMICs nicht zuverlässig eingehalten werden können (3), sollte die Beschaffung von Oxytocin-Ampullen auf Marken beschränkt werden, die für die gekühlte Lagerung gekennzeichnet sind, und nationale Arzneimittelbehörden sollten in Erwägung ziehen, Oxytocin-Produkte nur mit dieser Lagerungsempfehlung zu registrieren (67, 68). Dies würde auch dazu beitragen, eine Verwirrung des Personals in Gesundheitseinrichtungen durch unterschiedliche Lagerungsempfehlungen für verschiedene Oxytocin-Marken zu vermeiden (3, 20, 75, 76).

In LMICs ist es jedoch möglicherweise nicht immer möglich, kurzfristige Expositionen von Oxytocin bei Umgebungstemperaturen zu vermeiden. In diesem Zusammenhang könnten die in Abb. 6 dargestellten Daten von Interesse sein, die zeigen, dass alle Produkte, die für die Lagerung bei 2 - 8 °C gekennzeichnet sind, in der Stabilitätsstudie bei 25 °C bemerkenswert stabil waren. Selbst bei 30 °C wurden über den untersuchten Zeitraum nur sehr moderate Verluste des Wirkstoffgehalts

beobachtet. Dies deutet darauf hin, dass eine versehentliche Lagerung solcher Oxytocin-Präparate außerhalb der Kühlkette für begrenzte Zeiträume möglicherweise nicht die sofortige Entsorgung der Produkte erforderlich macht, was auch mit den Informationen übereinstimmt, die z.B. in der Packungsbeilage des Grindeks-Produktes (Tabelle 3) enthalten sind, wo es heißt, dass es "bei bis zu 30 °C für drei Monate gelagert werden kann, dann aber entsorgt werden muss".
Eine hitzestabile Formulierung von Carbetocin (77) , einem Oxytocin-Analogon, das bereits seit längerem zur Prophylaxe von Nachgeburtsblutungen nach Kaiserschnitt eingesetzt wird und nun auch seine Wirksamkeit als Prophylaxe von Nachgeburtsblutungen nach natürlicher Geburt bewiesen hat (78), wurde erst kürzlich in die WHO-Liste der unentbehrlichen Arzneimittel aufgenommen (24). Wenn die Ankündigung seines Herstellers in die Praxis umgesetzt wird, dass der Preis dieses Produkts mit dem von Oxytocin in LMICs vergleichbar gemacht wird (79), kann dieses Arzneimittel zu einer weiteren wertvollen Option werden, um eine qualitativ hochwertige Medikation bei Nachgeburtsblutungen auch in Einrichtungen sicherzustellen, in denen eine Lagerung bei 2 - 8 °C nicht gewährleistet werden kann.

Bis dahin haben die Ergebnisse dieser Arbeit gezeigt, dass weitere Anstrengungen zur Qualitätssicherung bei der Arzneimittelbeschaffung und -registrierung sowie zur Überwachung nach dem Inverkehrbringen im Rahmen eines funktionierenden Pharmakovigilanzsystems erforderlich sind.

Dem wurde versucht, Rechnung zu tragen, indem bei einer Reise nach Malawi im September 2019 die Ergebnisse dieser Arbeit den Behörden und Institutionen, die im Bereich der Müttergesundheit tätig sind, vorgestellt wurden. Zusätzlich wurden Trainingsworkshops für das Personal der Gesundheitseinrichtungen in den vier Distrikten zu den Themen Arzneimittelqualität, Lagermanagement und Pharmakovigilanz, in Kooperation mit dem Pharmakovigilanz-Zentrum Malawis, angeboten.

Der Fall der extrem minderwertigen Misoprostol-Tabletten macht auch deutlich, wie wichtig die sofortige Meldung minderwertiger Medikamente an nationale und internationale Behörden, sowie in Foren wie „e-drug“ für eine weitere Verbesserung der Patientensicherheit nicht nur innerhalb eines Landes, sondern auch weltweit ist.

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Appendix

BMC Pregnancy and Childbirth

RESEARCH ARTICLE Open Access

Quality, availability and storage conditions of oxytocin and misoprostol in Malawi

Nhomsai Hagen[1], Felix Khuluza[2] and Lutz Heide[1*]

Abstract

Background: Postpartum haemorrhage (PPH) is the leading cause of maternal mortality in low- and middle-income countries (LMICs). Oxytocin and misoprostol are used for the prevention and treatment of PPH. However, both medicines are chemically unstable and sensitive to environmental conditions. Previous studies reported a high prevalence of substandard oxytocin and misoprostol preparations in LMICs.

Methods: In randomly selected health facilities of four districts of Malawi, the availability of oxytocin and misoprostol was determined, and the knowledge of health workers on storage requirements and use of oxytocics was assessed. Temperature loggers were used to record the storage temperature of oxytocics. Samples of oxytocin injections and misoprostol tablets were collected from the health facilities and from wholesalers. Oxytocin samples were analysed for identity, assay (= quantity of oxytocin) and for pH value according to United States Pharmacopeia 40. Misoprostol samples were analysed for identity, assay, dissolution and related substances according to the International Pharmacopeia 2017.

Results: All visited hospitals and health centers had oxytocin available. At non-refrigerated storage sites, the recorded mean kinetic temperature exceeded the oxytocic's storage temperature stated on the labels in 42% of the sites. At refrigerated storage sites, the required temperature of 2–8 °C was exceeded in 33% of the sites. Out of 65 oxytocin samples, 7 (11%) showed moderate deviations from specification, containing 82.2–86.8% of the declared amount of oxytocin. Out of 30 misoprostol samples, 5 (17%) showed extreme deviations, containing only 12.7–30.2% of the declared amount. The extremely substandard misoprostol was reported to the national authorities and to WHO, leading to an immediate recall of the respective brand in Malawi. The UK-based distributor of this brand closed its business shortly thereafter.

Conclusion: Availability of oxytocin was excellent in Malawi, and its quality was better than reported in previous studies in other LMICs. However, storage conditions at the health facilities often did not meet the requirements. Extremely substandard misoprostol tablets were found, representing a serious risk to maternal health. This shows the need for continued efforts for quality assurance in medicine procurement and registration, as well as for post-marketing surveillance.

Keywords: Oxytocin, Misoprostol, Post-partum haemorrhage, Medicine quality, Substandard medicine, Falsified medicine, Medicine storage, Medicine analysis, Rational use, Malawi

* Correspondence: heide@uni-tuebingen.de
[1]Pharmaceutical Institute, Eberhard Karls University Tuebingen, Tuebingen, Germany
Full list of author information is available at the end of the article

Background

Post-partum haemorrhage (PPH) is the leading cause of maternal mortality in low-income countries. It is defined as blood loss of 500 ml or more within 24 h after birth [1]. The most common cause of PPH is an atonic uterus. This condition can be prevented and treated with oxytocics like the nonapeptide oxytocin or the prostaglandin analogue misoprostol (Fig. 1). Their mode of action is uterine contraction [1]. Both oxytocin and misoprostol are listed among 13 life-saving commodities for women and children by the UN Commission on Life-Saving Commodities for Women and Children (UNCoLSC) [3]. For oxytocin and misoprostol, it was estimated that 15,000 maternal lives could be saved over 5 years if common barriers like poor quality of these medicines, or lack of their inclusion into national essential medicines lists, could be overcome and" equitable access" could be achieved, according to the commissioners´ report of 2012 [4].

Reducing global maternal mortality to less than 70 per 100,000 live births is one of the Sustainable Development Goals (SDGs) of the United Nations (UN) [5]. There is still a long way to go to achieve this goal, especially in low-and-middle income countries (LMICs): The maternal mortality ratio in Malawi is one of the highest in the world, with an estimated 439 maternal deaths per 100,000 live births according to the Malawi Demographic and Health Survey 2015–16 [6]. One important intervention to reduce the maternal mortality ratio is the prevention and treatment of PPH with oxytocics. In the fifth edition of the Malawian Standard Treatment Guidelines (MSTG) including the Malawi Essential Medicines List (MEML) of 2015, oxytocin and misoprostol are listed as "vital" oxytocics to treat PPH [7]. Unfortunately, these medicines are very sensitive to environmental conditions like high temperatures (oxytocin, [8–10]) and humidity (misoprostol, [11, 12]). Most oxytocin products should be stored at 2–8 °C according to the labelling, and this can be challenging, especially in rural areas in LMICs [13–16]. Since misoprostol tablets can be stored at room temperature and can be administered orally, they offer an alternative to oxytocin injections in places where appropriate storage conditions for oxytocin cannot be ensured, or where no trained staff is available to administer medicines parenterally [17]. However, misoprostol tablets degrade when exposed to humidity (Fig. 1) and must be packed in aluminium-aluminium blisters in order to avoid degradation [11, 12, 18]. Misoprostol is also used in gynaecology and obstetrics for induction of labour and for abortions [17]. Due to the latter, its use is often restricted for fear of misuse.

The degradation products known for oxytocin or misoprostol are inactive but not toxic [12, 19]. However, when oxytocics lose their potency e.g. due to inappropriate storage, this might result in higher mortality rates of PPH.

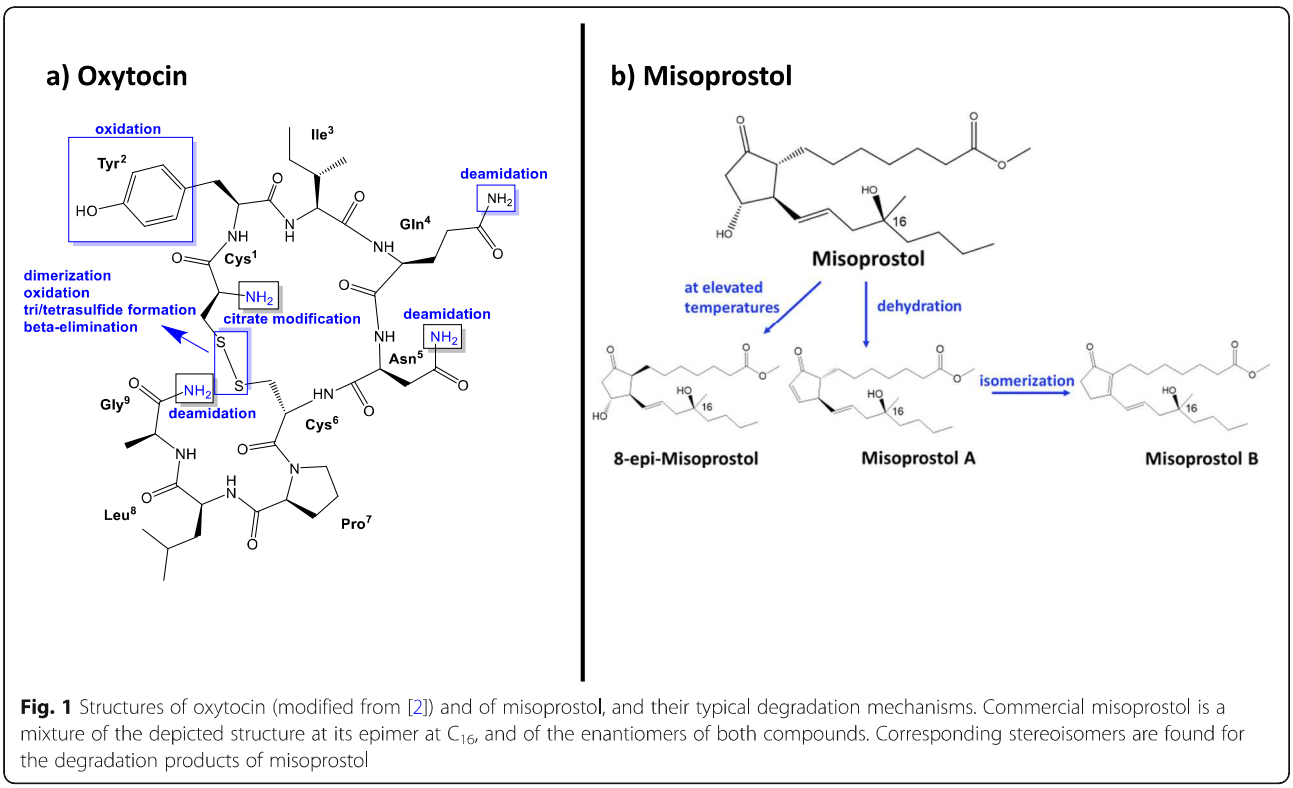

Fig. 1 Structures of oxytocin (modified from [2]) and of misoprostol, and their typical degradation mechanisms. Commercial misoprostol is a mixture of the depicted structure at its epimer at C_{16}, and of the enantiomers of both compounds. Corresponding stereoisomers are found for the degradation products of misoprostol

Several previous studies have shown that the quality of oxytocics, especially in low and middle-income countries (LMIC), is often poor [8, 11, 15, 16, 20–22]. According to a review from 2016, which included 8 studies with 559 oxytocin samples from 15 countries, 57.5% of the samples collected in Africa were reported to be substandard [20]. Similarly, 45% out of 215 misoprostol samples collected in 15 low- and middle-income countries were substandard according to a paper published in WHO Drug Information in 2016 [11]. A recently published study conducted in Nigeria reported even 74.2% of analysed oxytocin injections to be out of specification, as well as 33.7% of analysed misoprostol tablets [16]. Poor quality of oxytocin vials and misoprostol tablets can result from poor manufacturing (e.g. inappropriate formulation, environmental conditions, or poor primary packaging), from poor storage and transportation conditions, or from a combination of these factors.

But not only availability and good quality of oxytocics are essential for lowering maternal mortality; health workers´ knowledge of rational use of oxytocics, e.g. when and how to administer oxytocics, is as important. According to the World Health Organisation (WHO), one of the key interventions to promote rational use is the use of clinical guidelines [23].

A paper including data on the availability of oxytocin and misoprostol in Malawi has recently been published [24]. However, so far there are no data in the scientific literature about the quality of oxytocin and misoprostol in Malawi, and neither on the storage conditions of these medicines, or on health workers' knowledge of storage requirements and of rational use of oxytocics.

The present study aimed to close this gap by collecting oxytocin and misoprostol samples at different points of the supply chain and in different health facilities in four districts of Malawi, and by investigating their quality according to the acceptance criteria of the United States Pharmacopeia and the International Pharmacopeia. Furthermore, availability and storage conditions of oxytocics were investigated, and health workers' knowledge of storage requirements and rational use of these medicines was examined by using a questionnaire (see below).

Methods

The study protocol was developed based on MEDQUARG guidelines [25] and on the WHO guidelines on the conduct of surveys of the quality of medicines [26].

Ethical approval

Ethical clearance to conduct this study has been granted by College of Medicine Research and Ethics Committee in Malawi (COMREC, Reference No. P.07/27/2215). We also obtained support letters from the Malawian medicine regulatory authority (Pharmacy, Medicines and Poisons Board, PMPB), as well as from the responsible district health officers. Additionally, approval by German authorities to import medicine samples for analysis was obtained.

Written informed consent to participate in this study was obtained from all interviewed persons (see Consent Form in Additional File 2).

Sample size calculation

The sample size was calculated using the Cochran formula $n_0 = Z^2pq/e^2$ [27] with Z = 1.96 at 95% confidence level, p = 57.5% estimated proportion of substandard oxytocin samples [20], q = 1-p, and e = 10% margin of error. This resulted in a minimum of 94 samples.

Selection of sampling sites

This study was conducted in four districts in central and southern Malawi: Blantyre, Chikwawa, Neno and Ntcheu. Blantyre represents an urban district with moderate climate, Ntcheu a rural district with moderate climate, Chikwawa a rural district with hot climate, and Neno a rural district which has areas with both hot and moderate climate. A list of all health facilities in these four districts was obtained from the government-operated "Central Medical Stores Trusts" (CMST) and from PMPB. This list included 4 district or central hospitals, 61 public health centers, 28 faith-based health centers, 103 private clinics (most of them in Blantyre), 26 licensed pharmacies (all of them in Blantyre) and 149 drug stores (most of them in Blantyre). For the present study, the district or central hospital of each district was selected. Furthermore, for each district two facilities of each of the other five types were selected randomly if available, using the RAND function of Microsoft Excel. However, the very unequal distribution of facilities in the four districts required an adjustment of the numbers of selected facilities: licensed pharmacies existed only in Blantyre district, and therefore eight pharmacies (and no drug stores) were randomly selected in this district, plus public and faith-based health centers, private clinics and the central hospital. In Chikwawa and Ntcheu districts, the missing pharmacies, drug stores and private clinics were compensated for by inclusion of more public and faith-based health centers, aiming at a total number of 11 health facilities for each of these two districts. For the small Neno district, only 8 facilities in total were listed by CMST and PMPB, therefor all of these were included.

In the course of the study visits, eight of the selected facilities were found to be out of operation, did not offer maternity services, or refused to collaborate. These facilities were excluded from this study and were replaced by the geographically nearest facility of the same type, and if that facility could not be included either, by the

second nearest one. If both attempts were unsuccessful, no further attempt was made to replace that facility. Also if facilities were found to be out of stock for oxytocics, sampling was attempted in the geographically nearest facility of the same type, and if necessary in the second nearest one, as described above.

In addition to health facilities, oxytocics were also requested from CMST and from the 12 most important pharmaceutical wholesalers in Malawi; five of these reported that they did not stock oxytocics.

In total, oxytocics were requested from 62 health facilities and drug outlets, and these are listed in Additional File 1. Oxytocics could be collected from 45 of these facilities, and a map with the location of these 45 sampling sites is shown in Fig. 2. At most health facilities, oxytocics were stored both at the pharmacy (storage room) and at the maternity ward, and in each facility, sampling was done from both sites when possible, as listed in Additional File 1.

Collection of samples

Sampling was conducted between September 2017 (pilot study in Ntcheu district) and August 2018 (Blantyre, Chikwawa and Neno districts). All facilities in one district were sampled within 1 week. Permission to sample was obtained from the responsible district health officer prior to sampling, but facilities were visited without prior notice to the respective facility to avoid bias which might have arisen if the site prepared for our visit.

Sample collectors were the investigators F.K. and N.H., and 3rd year pharmacy students of the College of Medicine, University of Malawi (Blantyre) who had been trained prior to sampling. In hospitals, health centers and private pharmacies, sampling was done by an overt approach, i.e. facilities were informed about the purpose of the visit, and consent to participate in the study was obtained (see Additional file 2). In these facilities, temperature loggers were placed, and interviews were

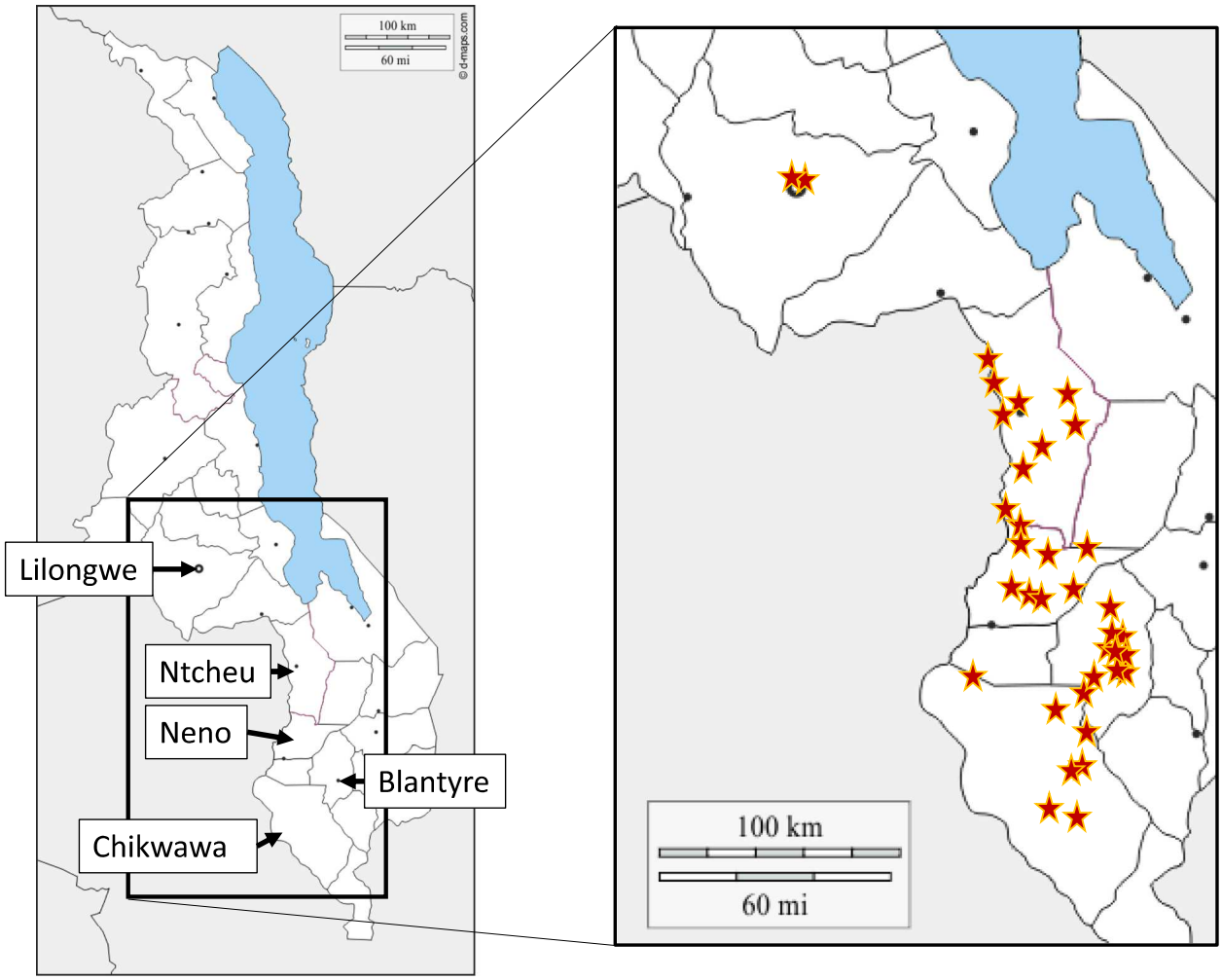

Fig. 2 Map of the locations of the 45 sites from which oxytocics were collected. Medicines were collected from the indicated four districts, and from wholesalers in Blantyre city and in Malawi's capital Lilongwe. Modified from: https://d-maps.com/carte.php?num_car=24744&lang=en

conducted using a questionnaire (see below). In the case of wholesalers and drug stores, a mystery shopper approach was used, and neither placement of temperature loggers nor interviews were carried out.

If different brands (original, generic or branded) or batches of oxytocics were available at a sampling site, each brand and each batch was collected as a separate sample. For each sample, 10 vials of oxytocin and 50 tablets of misoprostol were collected if possible. If only a smaller amount was available, this smaller amount was collected, but not less than three vials of oxytocin or six tablets of misoprostol per sample. Additional File 1 lists the number of samples, and the size of these samples, collected from each sampling site.

In health facilities, replacements for the sampled medicines were offered by the sample collectors in order to avoid that stock-outs would result from this study. The replacement medicines were obtained from CMST and local wholesalers prior to the sampling visits. If the visited facilities preferred, the sampled medicines were paid for, and this was obviously the rule in case of private pharmacies.

Questionnaire

A short interview on the health worker's knowledge about storage requirements, and about rational use of oxytocin and misoprostol, was conducted at all health facilities, as well as in those private pharmacies which stocked oxytocics. The questionnaire used for these interviews is shown in Additional File 2. It was written in English which is the language used in the training of all health workers in Malawi. However, the native sample collectors were free to use the local language (Chichewa), and this was the language used in most of the interviews. The sample collectors filled the questionnaire on-site based on the responses of the health workers. As shown in Additional File 1, the questionnaire was applied in 31 health facilities (hospitals, health centers and private clinics) as well as in 6 private pharmacies. In 24 of the 31 health facilities, the interview was carried out both in the storage room (pharmacy) and in the maternity ward, with the person responsible for storage or for administration of oxytocics at the respective site. In four further health facilities, the interview was carried out only at the maternity ward, and in three facilities, only at the storage room. The interview was therefore conducted with personnel from 28 maternity wards, 27 storage rooms (pharmacies) of health facilities, and 6 private pharmacy shops. In total 61 questionnaires were filled.

Recording of storage temperatures

Temperature data loggers (Tempmate S1 or M1 by imec Messtechnik GmbH, Heilbronn, Germany) were placed at all sites where misoprostol and oxytocin were routinely stored in the visited facilities, i.e. on storage shelves and/or within the refrigerators, both at the maternity ward and in the storage room (pharmacy) if applicable (see Additional File 1). They were fixed in close proximity to the medicines with adhesive tape. The loggers recorded the temperature automatically every 10 min from the time of placement (August or September) to the time of recollection by the study personnel (December; median recording time 130 days). Recorded data was downloaded from the loggers at College of Medicine in Malawi and analysed at Tuebingen University in Germany. The mean kinetic temperature (MKT) was calculated by the imec Messtechnik software.

Transport and storage of samples

Each sample was labelled with a unique code number using pre-printed adhesive labels, and placed in zip-locked plastic bags at the time of collection. All samples were transported from the collection site to the Pharmacy Department, College of Medicine, Blantyre, in the vehicles of the investigators within the same day; samples labelled for storage at 2–8 °C were transported in a 12 V plug-in refrigerator. They were subsequently stored in the Pharmacy Department in an air-conditioned room (< 25 °C) or in a refrigerator according to the storage requirement stated on the label. Samples were hand-carried to Germany via airplane in an insulating bag (< 24 h transport time) and stored at the Pharmaceutical Institute of Tübingen University in a refrigerator or in an air-conditioned room (< 25 °C) until analysis. In order to document the transport and storage conditions of the samples, temperature loggers were placed with the samples from the day of collection until the day of analysis.

Sample analysis

All samples were first inspected visually by N.H. at Tübingen University. Oxytocin injections were analysed according to the United States Pharmacopoeia (USP 40, Oxytocin injections) for identity, assay and pH value. The assay was carried out by High Performance Liquid Chromatography (HPLC, Agilent Infinity 1260 II with binary pump, variable wavelength detector, refrigerated autosampler and integrated column compartment; Agilent Technologies, Santa Clara, CA, USA) with mobile phase A (0.1 M NaH_2PO_4 buffer) and mobile phase B (acetonitrile: H_20 1:1 V/V) using the following gradient: 0 min, 30% B; 10 min, 40% B; 17.5 min, 65% B; 20.5 min, 65% B; 23.5 min, 30% B; 26 min, 30% B. Flow rate was 1.5 ml/min, the injection volume was 70 µl, the column with guard was Reprospher 100 (12.5 cm × 4.6 mm, 5 µm C18; Dr. Maisch GmbH, Tübingen, Germany), and detection was set at 220 nm. From each sample, three vials were analysed independently. USP Reference Standard

(batch N° F3K133) was obtained from Merck KGaA (Darmstadt, Germany). Solvents were HPLC grade.

Misoprostol tablets were tested according to the International Pharmacopeia 2017 (Misoprostol tablets) for identity, assay and dissolution, using the HPLC system mentioned above and a mixture of acetonitrile and water (45:55 V/V) as isocratic mobile phase, with a flow rate of 1.5 ml/min, a ReproSil-XR 120 colunm (C18, 5 µm, 150 mm × 4.6 mm; Dr. Maisch GmbH, Tübingen, Germany), injection volume 100 µl (assay) or 250 µl (dissolution), and UV detection at 200 nm. For assay, 5 tablets each were dissolved in 50 ml mobile phase in two independent experiments, and each of the two solutions was analysed twice by HPLC, resulting in four measurements for each sample. If less than 10 tablets were available for the respective sample, the assay was conducted by dissolving 3–5 individual tablets independently in 10 ml mobile phase each and analysing by HPLC, in order to have tablets left for retesting. Misoprostol Ph. Eur. reference standard (batch N° 3.0) was obtained from EDQM (European Directorate for the Quality of Medicines) Strasbourg. If the chromatogram of the assay showed additional peaks, the sample was also tested for related substances according to Kahsay et al. [28].

Dissolution was tested using a dissolution tester PT-WS 610 (Pharma Test Apparatebau AG, Hainburg, Germany). For each sample, six tablets were investigated independently as described in the misoprostol tablets monograph of the International Pharmacopeia, using 500 ml of water R as dissolution medium. A paddle apparatus with 50 rpm was used, and samples were drawn after 30 min through an in-line filter. Dissolution tests were only performed if at least 30 tablets were available for the respective sample.

For both oxytocin and misoprostol assay, and for misoprostol dissolution, 5-point calibration curves were prepared to assure linearity. Assay methods were validated according to USP 40 for system suitability, linearity and precision. Sample analysis was conducted at the Pharmaceutical Institute of Tübingen University, Germany, unblinded to packaging. All samples were within their shelf life at time of analysis, with the exception of one oxytocin sample and two misoprostol samples. Classification as within specification or out of specification was based for oxytocin injections on the specifications of USP 40 for assay and pH value, and for misoprostol tablets based on the specifications of International Pharmacopeia 2017 for assay and dissolution. Assay results between 80 and 90% and 110–120% of the declared content were considered as moderate deviations, contents of less than 80% or more than 120% were considered as extreme deviations [29]. As per definition of WHO, products that deliberately/fraudulently misrepresent their identity, composition or source were considered falsified [30].

Registration status of medicine brands

PMPB was contacted to enquire the registration status of the medicines collected. If they were registered, the PMPB registration number was requested.

Statistical analysis

Statistical evaluation was done using JMP 14.2 (SAS GmbH, Heidelberg, Germany). Confidence intervals were calculated using descriptive distribution analysis, significance of differences between storage sites were calculated using one-way analysis of variance (ANOVA), and correlation between age of samples and content was calculated using bivariate analysis. Means and relative standard deviations were calculated using Microsoft Excel 2016 (Microsoft Corporation, Redmond, Washington, USA).

Information of national authorities and stakeholders

Following the study protocol and WHO guidelines [26], the national medicine regulatory authority of Malawi (PMPB) and the WHO Rapid Alert System were informed immediately about confirmed out-of-specification results representing a serious health risk. After completion of the study and data analysis, the survey results were presented to PMPB, CMST, the Ministry of Health and national and international stakeholders during a meeting in Lilongwe, Malawi, on Sept. 4th, 2019. Additionally, the findings were presented to health workers in the four study districts Ntcheu, Blantyre, Chikwawa and Neno on Sept. 5th, 10th, 11th and 12th, 2019, respectively, including appropriate trainings in cooperation with the Malawi National Pharmacovigilance Centre.

Results

Availability of oxytocics

Figure 3 shows the availability of oxytocin and misoprostol in the different health facilities and drug outlets included into this study. According to the Malawi Essential Medicines List (MEML) 2015, oxytocin should be available in health centers, district hospital and central hospitals [7]. Indeed, each of the 27 visited hospitals and health centers (both public and faith-based) had oxytocin available. Also, three out of four private clinics had oxytocin in stock; the one not stocking oxytocin did not offer routine delivery services, but did stock misoprostol for abortions. In contrast, none of the private licensed pharmacies had oxytocin in stock.

According to MEML 2015, misoprostol should be available at district and central hospital level [7], but not at health centers unless these have specialised clinical expertise. Indeed, misoprostol was available at all four investigated hospitals, but only in few of the health

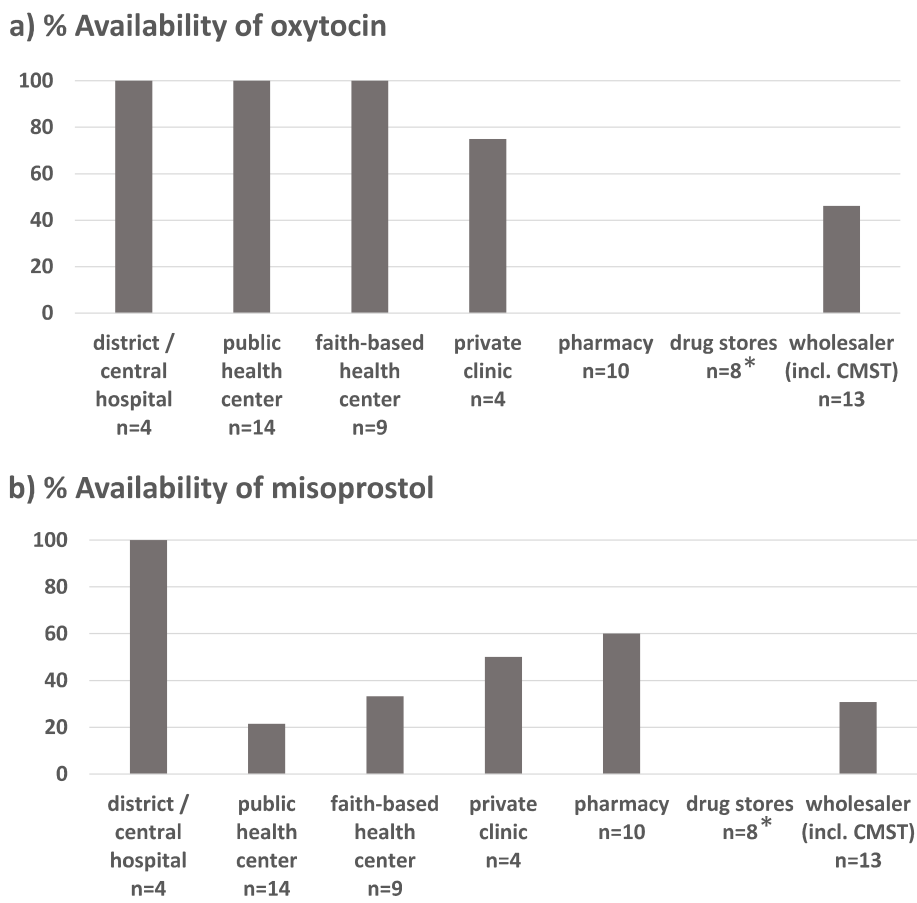

Fig. 3 Availability of oxytocics at visited facilities. n = number of facilities. CMST = Central Medical Stores Trust. * Drug stores are not allowed to stock oxytocin and misoprostol, therefore 0% availability was expected in these facilities

centers and private clinics. Misoprostol was also available at 6 of the 10 investigated private pharmacy shops.

Two hospitals, one faith-based health center and one private clinic reported to have ergometrine in stock, but none of the public health centers and none of the private pharmacies. Ergometrine maleate is still listed as an oxytocic in the MEML 2015, but is increasingly considered as obsolete.

In the interviews, it was enquired whether stock-outs of oxytocin and misoprostol had been experienced in the last 6 months. In case of public and faith-based health facilities and private clinics, this information was verified by inspecting the stock cards for the respective medicines. 73% of these facilities had not experienced oxytocin stock-outs in the last 6 months, and the rest reported less than 1 month of stock-out time. Out of the 12 health facilities and 6 private pharmacies which had misoprostol in stock at the time of the visit, 11 (61%) reported no stock-outs of misoprostol in the last 6 months, but 3 (17%) reported stock-out times of more than 1 month. Detailed results on the reported stock-out times are shown in Additional File 3.

In public and faith-based health facilities as well as in private clinics, also the amount of oxytocin in stock at the time of the visit was checked, and the consumption over the last 6 months was calculated from the stock cards; this information was available in 29 of the 31 included health facilities. The amount of oxytocin in stock was usually sufficient for 1.4 months (= median value; range: 0.1–25 months; mean: 4.0 months).

In Malawi, drug stores are licensed private outlets which do not require a pharmacist but a pharmacy technician, nurse, or clinical officer on their premises [31]. They are allowed to dispense a limited number of common medicines, but not prescription or pharmacist-only medicines, and therefore not oxytocin or misoprostol. Nevertheless, we decided to include also drug stores, because according to local contact persons some drug stores may illegally stock misoprostol for use in abortions. However, all of the eight drug stores visited by the mystery shoppers in this study stated that they do not sell oxytocin or misoprostol.

Storage conditions for oxytocics

According to the manufacturers' information stated on the labels, out of the six misoprostol brands collected in the course of this study, two brands (representing four samples) needed to be stored below 25 °C, three brands (representing 24 samples) below 30 °C, and one brand (representing two samples) showed no information about the storage temperature on the label.

Out of the nine brands of oxytocin collected, five brands (representing 23 samples) were labeled for storage at 2–8 °C, two brands (representing 39 samples) were labeled for storage below 25 °C, and two brands (representing three samples) were labeled for storage below 30 °C (see Tables 1 and 2 and Additional File 1). Therefore, for correct storage of 35% of the oxytocin samples a refrigerator was required. Out of the 31 health facilities (hospitals, health centers and private clinics), one public health center and two faith-based health centers stated to have no functioning refrigerator. While two of these three facilities correctly used oxytocin brands which, according to the label, did not require refrigerated storage, one was found to stock a brand which required storage in a refrigerator (Additional File 1, facility no. 17).

All of the six licenced pharmacies where the interview was conducted stated to have a functioning refrigerator.

All refrigerators were operated with electricity. In 41% of the visited facilities the health worker stated that there were power failures every day. Only 51% of the facilities reported to have generators or solar panels as potential back-ups in case of power failures, while 27% stated that there were no measures taken to maintain appropriate storage conditions in the event of power failures.

Out of the 17 oxytocin samples labelled for storage at 2–8 °C and collected at health facilities, 13 had been correctly stored in a refrigerator, while four had been stored at ambient temperature, in violation of correct storage procedures. These four samples had been collected in public health centers in Blantyre, Chikwawa and Ntcheu. At one of these sites, the responsible person answered to the question how oxytocin should be stored "at room temperature", indicating a lack of knowledge of correct storage procedures. Notably, at most facilities there was only one stock card for oxytocin, regardless of the presence of different oxytocin brands with different storage requirements and therefore different storage locations.

Out of the 36 oxytocin samples labelled for storage at < 25 °C or < 30 °C and collected at health facilities, 29 had indeed been stored at ambient temperature. The other 7 had been stored in the refrigerator, which is no problem for oxytocin stability and therefor no violation of correct storage procedures.

To determine actual storage temperature, temperature data loggers were placed at all sites where misoprostol and oxytocin was stored in the facilities (both at the maternity ward and in the storage room if applicable), and temperature was recorded for approximately 4 months (see Methods). Additional File 1 shows the mean kinetic temperature (MKT) recorded at each site. MKT, rather than the simple arithmetic mean of the recorded temperatures, is the relevant measurement for the stability of pharmaceuticals [34].

The MKT recorded at non-refrigerated storage sites ranged from 21.4 to 31.0 °C (median: 26.2 °C). As expected, Chikwawa was the hottest district with a median MKT of 28.1 °C. The highest single temperature measurement (40.1 °C) was also recorded in a facility in Chikwawa.

35 of the oxytocin samples collected in health facilities (hospitals, health centers and private clinics) were labelled for storage below 25 °C. However, this temperature was exceeded in 16 of the places where these samples had been collected from, and the median MKT in these 16 places was 27.3 °C.

Only three oxytocin samples collected in the course of this study were labelled for storage below 30 °C (one collected in a private clinic, two from wholesalers). However, even this temperature was exceeded in 4 of the facilities visited in this study (median MKT in these four places = 30.2 °C; see Additional File 1).

17 of the oxytocin samples collected in health facilities were labelled for storage at 2–8 °C. As mentioned above, four of these samples were incorrectly stored outside the refrigerator. But also for five of the respective storage sites inside refrigerators, MKTs between 10.6–18.3 °C were recorded (see Additional File 1). Three of these were recorded at maternity wards who reported that there were power failures every day.

Interviews with health workers

Using a questionnaire, interviews were conducted with the persons responsible for storage and /or administration of oxytocics in the respective facilities (see Methods). In these interviews, only 24% of the persons responsible for the storage room (pharmacy), and 32% of the persons responsible for administration of oxytocics in the maternity wards, reported to have received a training on storage, distribution and handling of cold chain medications. Out of 61 interviewed persons, 7 reported that they had observed ineffective oxytocin ($n = 5$) or ineffective misoprostol ($n = 2$) in their professional practice. Notably, only 2 of these 7 stated that they had notified the authorities (or the suppliers) about this.

Standard Treatment Guidelines (STGs) for oxytocin or misoprostol were available at 23 (74%) out of the 31 visited health facilities, and at 66% of the visited maternity wards. At maternity wards, all interviewed health workers

Table 1 List of all oxytocin samples collected in the course of this study. Assay values which are out of specification are marked by "!". RSD = relative standard deviation; HC = Health Center; CMST = Central Medical Stores Trust

Stated storage requirements	Origin	Brand name and stated manufacturer	Registered by PMPB[1]	Batch No.	Manf. / Expiry Date	Facility No.[2]	Facility type	Site in facility	Mean assay (% of declared content)	RSD Assay	Mean pH value	RSD pH value	Age of samples (months)[3]
2-8 °C	Germany	OXYTOCIN 10; Rotexmedica GmbH Arzneimittelwerk	no	60670	Dec 16/ Dec 19	5	public HC	maternity ward	99.9	0.56%	4.2	0.12%	22
						7	public HC	maternity ward	100.9	0.45%	4.2	0.42%	22
	India	OXYTOCIN INJECTION BP; Ciron Drugs & Pharmaceuticals Pvt. Ltd.	yes PMPB/PL388/62	6EA03254	Oct 16/ Sep 18	56	wholesaler	-	101.5	0.36%	4.3	2.14%	15
				6EB04194	Jul 16/ Jun 18	52	wholesaler	-	105.9	0.42%	4.1	0.82%	18
				7EA01228	Sep 17/ Aug 19	52	wholesaler	-	92.7	1.69%	4.5	2.38%	8
						56	wholesaler	-	94.9	0.51%	4.4	0.16%	8
		Oxytocin 10 IU INJECTION BP; Gland Pharma Ltd.	no	EE618X	Nov 16/ Oct 18	3	district hospital	maternity ward	101.7	0.80%	4.4	2.76%	23
						24	faith-based HC	maternity ward	100.5	1.20%	4.2	1.08%	23
		OXYKOP; MACIN Remedies India Ltd.	no	OT1641	Nov 16/ Oct 18	54	wholesaler	-	87.4[5]	4.27%	4.0	0.18%	18
	Italy	OXYTOCIN 10 IU/ml; Biologici Italia Laboratories s.r.l.	no	UF508ON	Apr15[4]/ Mar 18	26	faith-based HC	storage room	103.8	1.90%	3.7	0.41%	33
				UF601ON	Apr16[4]/ Mar 19	17	public HC	storage room	100.8	0.60%	3.8	2.45%	21
				UF602ON	Apr16[4]/ Mar 19	3	district hospital	maternity ward	98.6	0.26%	3.9	0.49%	30
						3	district hospital	storage room	97.7	1.41%	3.9	0.48%	30
						6	public HC	maternity ward	98.1	1.47%	3.9	0.68%	30
						14	public HC	storage room	100.0	0.42%	3.9	0.50%	30
						53	wholesaler	-	101.8	1.31%	3.7	0.50%	21
				UF603ON	Apr16[4]/ Mar 19	4	district hospital	maternity ward	102.2	1.30%	3.7	0.53%	21
						4	district hospital	storage room	104.3	0.36%	3.8	1.50%	21
						25	faith-based HC	storage room	103.1	1.67%	3.8	2.43%	21
				UF701ON	Nov17[4] /Oct20	2	district hospital	storage room	99.2	0.68%	3.9	0.35%	10
						9	public HC	maternity ward	98.9	0.18%	3.9	1.40%	10
				UF702ON	Nov17[4] /Oct20	2	district hospital	maternity ward	99.1	0.49%	3.9	0.73%	10
						9	public HC	storage room	99.0	1.11%	3.9	0.35%	10
below 25 °C	China	WW-Oxy 10; Ningbo Pharma Biotech Co. Ltd.	Yes PMPB/PL381/16	160183	Jan 16/ Jan 19	1	central hospital	maternity ward	98.3	0.75%	4.3	0.46%	34
						1	central hospital	maternity ward	96.9	0.42%	4.2	0.38%	34
						1	central hospital	storage room	95.4	0.49%	4.4	0.67%	34
						8	public HC	maternity ward	94.9	1.72%	4.1	0.62%	33
						8	public HC	storage room	92.9	1.94%	4.1	0.49%	33
						10	public HC	maternity ward	92.6	1.94%	4.1	0.80%	33
						11	public HC	maternity ward	92.5	1.67%	4.1	1.12%	33
						12	public HC	maternity ward	93.5	2.34%	4.1	1.27%	33
						13	public HC	maternity ward	95.2	0.95%	4.1	0.66%	33
						13	public HC	storage room	96.5	0.52%	4.1	1.02%	33
						14	public HC	maternity ward	96.0	0.49%	4.1	0.32%	33

Table 1 List of all oxytocin samples collected in the course of this study. Assay values which are out of specification are marked by "!". RSD = relative standard deviation; HC = Health Center; CMST = Central Medical Stores Trust *(Continued)*

						15	public HC	maternity ward	91.3	1.09%	4.0	1.09%	12
						15	public HC	storage room	90.7	1.13%	3.9	1.38%	12
						16	public HC	maternity ward	91.4	1.31%	3.9	1.50%	12
						16	public HC	storage room	91.5	0.98%	4.0	1.05%	12
						17	public HC	maternity ward	93.1	1.82%	3.9	1.32%	12
						18	public HC	storage room	90.9	1.05%	4.0	1.92%	12
						20	faith-based HC	maternity ward	96.7	1.45%	4.2	0.44%	33
						20	faith-based HC	storage room	98.7	1.50%	4.2	0.49%	33
						22	faith-based HC	maternity ward	91.7	1.91%	4.1	0.87%	33
						22	faith-based HC	storage room	95.9	0.74%	4.2	0.57%	33
						23	faith-based HC	maternity ward	94.5	0.86%	4.1	0.71%	33
						23	faith-based HC	storage room	95.4	1.29%	4.1	0.70%	33
						24	faith-based HC	maternity ward	92.4	0.64%	4.2	2.22%	33
						25	faith-based HC	storage room	92.5	0.32%	3.9	0.34%	12
						27	faith-based HC	maternity ward	92.3	0.85%	3.9	0.86%	12
						29	private clinic	storage room	100.4	0.08%	4.1	0.43%	33
						53	CMST	-	92.0	1.13%	3.9	0.43%	24
						53	CMST	-	94.9	0.87%	4.1	0.88%	28
				160802	Aug 16/ Aug 19	19	faith-based HC	maternity ward	86.8 !	1.33%	4.0	0.48%	26
						19	faith-based HC	storage room	86.2 !	1.39%	4.1	0.77%	26
						21	faith-based HC	maternity ward	89.2[5]	2.85%	4.2	0.98%	26
						21	faith-based HC	maternity ward	86.4 !	1.44%	4.0	1.15%	26
						21	faith-based HC	storage room	85.2 !	1.29%	4.0	1.10%	26
						28	private clinic	storage room	85.4 !	0.25%	4.0	0.76%	26
						30	private clinic	maternity ward	85.4 !	0.98%	4.0	0.79%	26
						55	wholesaler	-	89.0[5]	1.41%	3.9	0.63%	21
				170402	Apr 17/ Apr 20	1	central hospital	storage room	102.0	1.23%	4.5	0.31%	19
	India	OXYCIN-10; Sakar Healthcare Pvt.Ltd.	no	I16043	May16/ Apr 18	50	wholesaler	-	82.2 !	1.83%	3.8	0.81%	20
below 30 °C	India	OXYNIR; Aculife Healthcare Pvt.Ltd	no	5E60239	May16/ Apr 18	28	private clinic	maternity ward	90.1	1.67%	4.0	0.22%	29
						51	wholesaler	-	98.6	3.45%	3.9	0.41%	20
		OXYTOCIN INJECTION BP 10 IU/ml; Umedica Laboratories PVT. LTD	yes PMPB/PL155/7; POM-30/06/14	JA802	Feb 18/ Jan 20	56	wholesaler	-	98.1	1.29%	4.2	1.24%	3

[1] *PMPB = Pharmacy, Medicine and Poisons Board of Malawi; for registered medicines, the PMPB registration number is given;* [2] *facility number as listed in* Additional File 1; [3] *at time of analysis;* [4] *manufacturing date not stated on packaging; information from the websites of MHRA (http://www.mhra.gov.uk) and HPRA (http://www.hpra.ie/);* [5] *assay value out of specification, but deviation from the 90% threshold not statistically significant considering the standard deviation of the measurement*

correctly stated that oxytocin for prevention or treatment of PPH is given "always after delivery of the child". Further results from the interviews are summarized in Additional File 3.

Overview of collected oxytocin samples

Table 1 lists the 65 oxytocin samples collected in the course of this study. As depicted in Fig. 4, the two most frequently encountered preparations were a branded

generic preparation from China labelled for storage at < 25 °C (38 samples, three batches), and a generic preparation from Italy labelled for storage at 2–8 °C (14 samples, six batches). Both were distributed by the government-operated Central Medical Stores Trust (CMST) to the public (and partly also faith-based) health facilities. The remaining 13 samples represented 7 different brands (nine batches) from India and from Germany. Most of these were collected outside of the government health facilities.

The total shelf life of the samples declared by the manufacturer varied from 2 to 4 years. Only a single oxytocin sample was expired at time of collection; it was found in the maternity ward of a private clinic.

Out of the 9 brands collected, only 3 were registered by the Pharmacy, Medicines and Poisons Board (PMPB) of Malawi, 6 were not (Table 1).

Chemical analysis of oxytocin samples

Visual inspection showed a surprisingly high number of spelling errors in the leaflet of the oxytocin brand produced in China, but otherwise gave no indication of quality defects or falsification. The content of the oxytocin samples was determined by HPLC and the pH value was measured, following the procedures of USP 40 (see Methods). According to USP 40, oxytocin injections have to contain between 90 and 110% of declared amount of oxytocin, and to show a pH value between 3.0 and 5.0.

Table 1 shows the pH and assay values determined for all samples. The pH value was found to be within specification for all samples. 55 of the 65 samples (85%) also complied with the specification for the oxytocin content (Fig. 4). Three samples showed assay values slightly below 90%, but their deviations from the 90% threshold were not statistically significant considering the standard deviation of the measurement. Seven samples (11%) showed oxytocin contents which were significantly lower than 90% of the declared content (range 82.2–86.8%). One of the seven failed samples was from an Indian manufacturer, while the other six derived from a single batch produced by the Chinese manufacturer whose preparations had already been noted for spelling errors in the leaflet. Two other batches from that same Chinese manufacturer were found to be within specification, including a batch with a shorter remaining shelf-life than the failed samples.

The age of the samples at time of analysis (i.e. the time elapsed after their manufacturing date) varied from three to 34 months. As shown in Fig. 5, there was no correlation between oxytocin content and age of samples ($r^2 = 0.00024$; $p = 0.90$). The mean content of samples collected from wholesalers was 94.9%, while the mean content of samples from the storage rooms (pharmacies) of health facilities was 96.0%, and from maternity wards 94.7%. Therefore, no relevant differences were observed between these groups.

As mentioned above, four samples of oxytocin labelled for storage at 2–8 °C had been found to be incorrectly stored at ambient temperatures. Nevertheless, all of these were within specification (98.1–100.8% of declared content). Seven samples labelled for storage below 25 or below 30 °C had been found to be stored in the refrigerator. Nevertheless, two of these seven samples were out of specifications.

None of the 23 samples labelled by the manufacturer for storage at 2–8 °C was found to be out of specification. In notable contrast, seven of the 39 samples labelled for storage < 25 °C failed quality testing, showing insufficient oxytocin content. Only three samples labelled for storage < 30 °C were found in this study, and none of these failed quality testing.

Notably, all 32 oxytocin samples collected from government health facilities were within specifications. Of the seven failed samples, four had been collected in faith-based health centers, two in private clinics, and one from a wholesaler.

Overview of collected misoprostol samples

Table 2 lists the 30 misoprostol samples collected in the course of this study. As depicted in Fig. 6, all but one of these samples were manufactured in India. The most frequently encountered brand was a branded generic preparation (21 samples, 5 batches) correctly packaged in aluminium-aluminium blisters and distributed by CMST, as well as by a private wholesaler. Three further brands were also correctly packaged in aluminium-aluminium blisters: one originator brand from the UK (one sample) and two branded generics from India (3 samples, 2 batches); one of these latter ones represented a WHO-prequalified product (see Table 2). Another branded generic from India (3 samples, 1 batch) was incorrectly packaged in aluminium-plastic blisters; it was collected both at CMST and in a district hospital. One further generic preparation from India (2 samples, 1 batch) was even packaged in screw-cap PVC bottles containing 100 tablets, offering no protection of the individual tablets against humidity once the bottle is opened. Such packaging is grossly inadequate for misoprostol tablets. This brand was found in the pharmacy of the central hospital. The two samples of this brand (one sealed bottle and one already opened bottle) had just expired in the month before collection, and had correctly been quarantined by the facility. In view of the highly unusual packaging, it was decided to collect and analyse them nevertheless.

None of the other 28 samples was expired. The total shelf life declared by the manufacturers varied from two to 3 years.

Table 2 List of misoprostol samples. Assay and dissolution values which are out of specification are marked by "!". RSD = relative standard deviation; HC = Health Center; CMST = Central Medical Stores Trust

Primary packaging	Origin	Brand name and stated manufacturer	Registered by PMPB[1]	Batch No.	Manf. / Expiry Date	Facility No.[2]	Facility type	Site in facility	Mean assay (% of declared content)	RSD Assay	Mean dissolution (% of declared content)	RSD Dissolution	Age of samples (months)[3]
Alu/Alu Blister	UK	Cytotec© 200; Piramal Healthcare UK Limited	no	BNB13411	Jan 16[4] /Dec 18	14	public HC	storage room	97.4	1.47%	-[5]	-[5]	32
	India	MISOCLEAR; Acme Formulation Pvt.Ltd[6]	yes PMPB/PL394/1	MCL180101	Jan 18/ Dec 19	57	wholesaler	-	102.9	0.84%	101.2	3.64%	4
		KONTRAC 200; fourrts (India) Laboratories Pvt. Limited	yes PMPB/PL299/22	D1233	Oct 16/ Sep 18	23	faith-based HC	storage room	98.1	1.46%	97.7	2.78%	23
				D1270	Oct 16/ Sep 18	23	faith-based HC	storage room	91.9	6.93%	-[5]	-[5]	23
						52	wholesaler	-	95.2	0.30%	93.0	6.12%	15
				D2205	Feb 17/ Jan 19	1	central hospital	maternity ward	94.9	1.01%	93.0	5.42%	20
						1	central hospital	storage room	94.0	0.21%	90.9	2.26%	20
						2	district hospital	storage room	98.4	0.17%	95.4	2.03%	19
						3	district hospital	storage room	98.4	0.37%	97.4	2.09%	19
						53	CMST	-	104.3	0.43%	103.2	1.97%	14
				E0571	Jun 17/ May 19	20	faith-based HC	maternity ward	95.1	0.72%	-[5]	-[5]	15
						28	private clinic	storage room	96.4	0.86%	94.8	3.42%	15
						32	pharmacy	-	96.8	0.26%	91.5	5.26%	15
						33	pharmacy	-	93.1	0.92%	89.7	3.23%	15
						36	pharmacy	-	97.0	0.40%	94.1	5.05%	15
						52	wholesaler	-	100.7	0.82%	99.2	0.98%	10
				E2291	Jan 18/ Dec 19	7	public HC	storage room	94.0	1.97%	93.6	3.45%	8
						21	faith-based HC	storage room	92.7	0.38%	94.0	4.73%	8
						31	private clinic	storage room	96.1	1.95%	89.9	4.96%	8
						34	pharmacy	-	97.1	0.88%	93.6	4.89%	8
						35	pharmacy	-	94.5	0.79%	94.6	3.84%	8
						36	pharmacy	-	94.2	2.60%	89.9	4.78%	8
						37	pharmacy	-	93.7	0.80%	92.9	5.16%	8
		L-Pill 200; Lincoln pharmaceuticals Ltd.	no	IS6001	Feb 18/ Jan 18	16	public HC	maternity ward	30.2!	5.24%	-[5]	-[5]	23
						55	wholesaler	-	29.7!	4.58%	21.3!	7.45%	23
PVC-Bottle with 100 tablets		Misoprostol Tablets 200mcg; Centurion Healthcare Private Limited	no	M-0001	Sep 15/ Aug 17	1	central hospital	storage room	48.8[7]	2.66%	34.3	37.08%	29
						1	central hospital	storage room	53.0[8]	0.29%	43.1	17.89%	29
Plastic/ Alu Blister		Misoprostol Tablets 200mcg; swiss parenterals Pvt. Ltd.	no[9]	178	Jun 16/ May 19	4	district hospital	storage room	13.2!	0.99%	9.2!	9.72%	19
						4	district hospital	maternity ward	13.4!	1.78%	9.5!	13.57%	19
						53	CMST	-	12.7!	1.44%	7.9!	15.13%	19

[1] *PMPB = Pharmacy, Medicine and Poisons Board of Malawi; for registered medicines, the PMPB registration number is given;* [2] *facility number as listed in* Additional File 1; [3] *at time of analysis;* [4] *manufacturing date not stated on packaging; information from the websites of HPRA (www.hpra.ie) and emc (www.medicines.org.uk/emc);* [5] *no dissolution testing due to small sample size;* [6] *WHO-prequalified finished pharmaceutical product* [32, 33]; [7] *collected in Sep 17 as open PVC-bottle, analysed in Feb 18;* [8] *collected in Sep 17 as sealed PVC-bottle, analysed in Feb 18;* [9] *incomplete information; manufacturer may have won a tender with CMST and may therefore have undergone accelerated registration*

Out of the six brands collected, only two were registered by PMPB in Malawi, four were not (Table 2).

Chemical analysis of misoprostol samples

Visual inspection showed the above-mentioned shortcomings of the packaging of several samples, but otherwise gave no indication of quality defects or falsification. The content of the misoprostol samples, as well as dissolution of the active pharmaceutical ingredient (API), was determined by HPLC following the procedures of the International Pharmacopeia 2017 (see Methods). According to the International Pharmacopeia, misoprostol tablets have to contain between 90 and 110% of the declared content, and at least 80% of the API must dissolve within 30 min under the conditions described in the pharmacopeia.

Table 2 shows the assay and dissolution values determined for all samples. 23 of the 30 samples (77%) complied with the specification for the misoprostol content (Fig. 6), and all of these also complied with the specification for dissolution. Notably, all compliant samples were packaged in aluminium-aluminium blisters.

In contrast, both samples of the brand packaged in PVC bottles and all three samples of the brand packaged in plastic-aluminium blisters were found to be extremely out of specification, but also one brand packaged correctly in aluminium-aluminium blisters (Table 2).

The brand packaged in PVC bottles contained only 53.0% (sealed bottle) and 48.8% (opened bottle) of the declared misoprostol content. Especially the tablets in the opened bottle also showed poor dissolution (34.3% of the declared content), with an extremely high standard deviation between the individual tested tablets (Table 2). This suggests that the quality of the tablets had been severely affected by (unequal) exposure to humidity. Since these tablets were expired at the time of analysis, these results do not represent definitive proof of poor quality before expiry, though it appears very likely that they had been already out of specification before reaching their expiry date.

As mentioned, one brand (by Lincoln Pharmaceuticals Ltd., India) which was packaged in aluminium-aluminium blisters was nevertheless extremely out of specification (Table 2), containing only 29.7 or 30.2% of the declared misoprostol amount.

The worst brand discovered in the course of this study was manufactured in India by Swiss Parenterals PVT, Ltd. It was packaged in plastic-aluminium blisters. HPLC

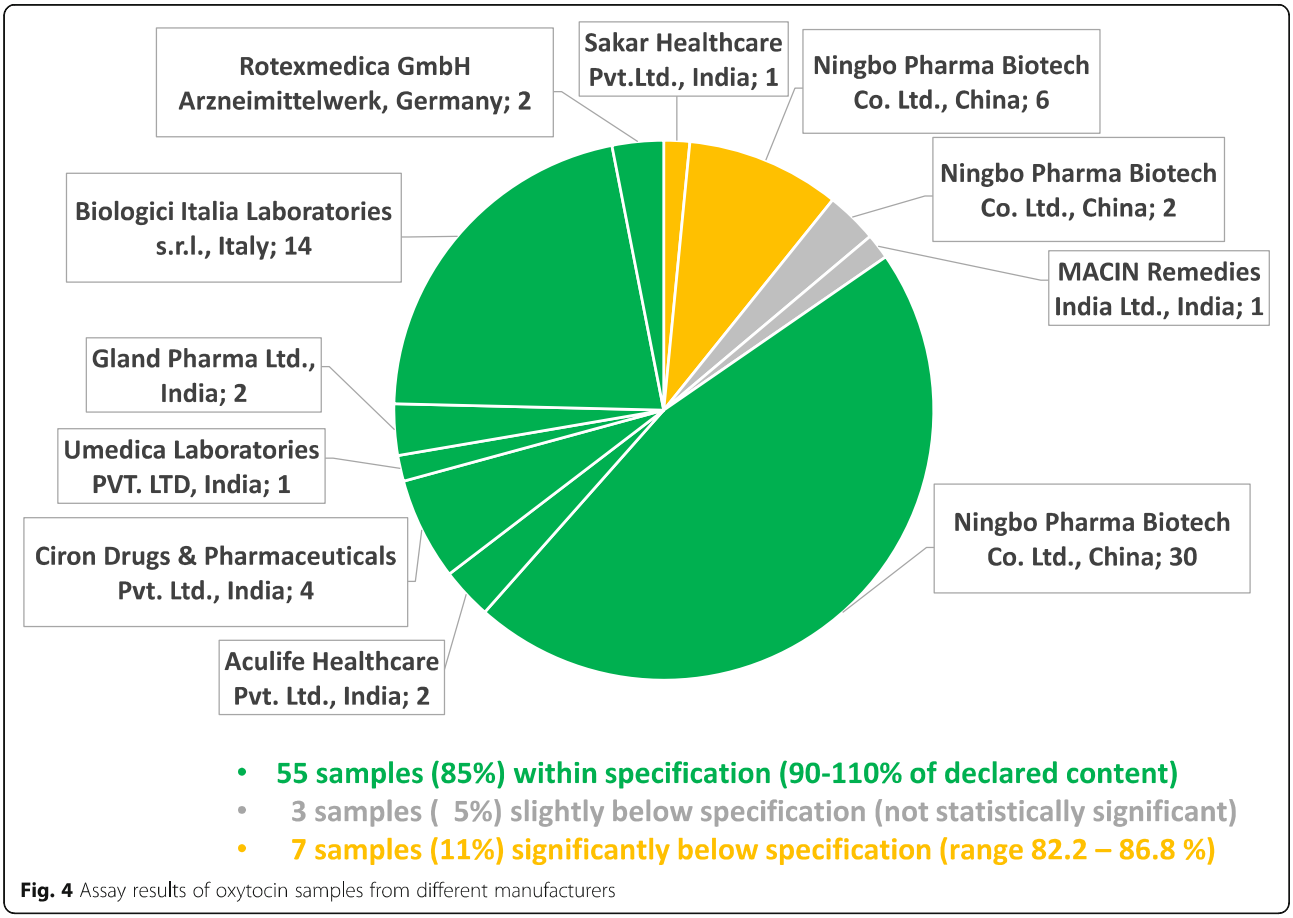

Fig. 4 Assay results of oxytocin samples from different manufacturers

analysis revealed a misoprostol content of only 12.7–13.4% of the declared amount, and only 7.9–9.5% of the declared content was found to dissolve. Testing of the extremely substandard misoprostol preparations for related substances (Additional File 4) showed the typical degradation products named in Fig. 1. Obviously, it cannot be decided whether all of the degradation occurred after manufacturing of the tablets, or whether the tablets were manufactured using an already partially degraded API.

The age of the misoprostol samples at time of analysis was between four and 32 months.

Product recall in Malawi, and closure of the responsible distributor in the United Kingdom

The extremely substandard misoprostol preparations by Lincoln Pharmaceuticals Ltd. and Swiss Parenterals PVT Ltd. were discovered in the first part of this study, by sample collection in September 2017. As foreseen in the study protocol, PMPB, CMST and WHO were informed immediately about this finding. PMPB thereupon issued a product recall, and CMST discontinued supplying the substandard misoprostol. In the subsequent main part of this study, with sample collection in August 2018, no further substandard misoprostol samples were discovered, indicating that the recall had been effective.

The misoprostol preparation by Swiss Parenterals PVT Ltd. was labelled as "Distributed by Premiumway International. U.K. www.premiumway.co.uk". Our finding of the poor quality of this preparation was also reported in the electronic newsletter of the initiative "e-drug" [35]. In August 2018 it was pointed out by the moderator of that newsletter that, according to the UK government website Companies House (https://beta.companieshouse.gov.uk/), the distributor Premiumway International was located at the same address, and managed by the same directors, as the company Unimed International Ltd. who had been reported to distribute poor-quality propofol (an anaesthetic medicine) to the government of Zambia [36, 37]. In the ensuing correspondence, WHO asked the authors of this paper to send a sample of the misoprostol tablets distributed by Premiumway International to the British medicine regulatory authority (MHRA) for confirmatory analysis. The precise actions taken subsequently by MHRA have not been released to the public, but as published on the UK government website Companies House, both Premiumway International and Unimed International Ltd. went into voluntary liquidation on 28. January 2019.

Discussion

The present study showed good availability of oxytocics, especially of oxytocin, in the health facilities of Malawi. On the other hand, it revealed widespread problems with the maintenance of correct medicine storage temperatures. Nevertheless, the majority of the investigated

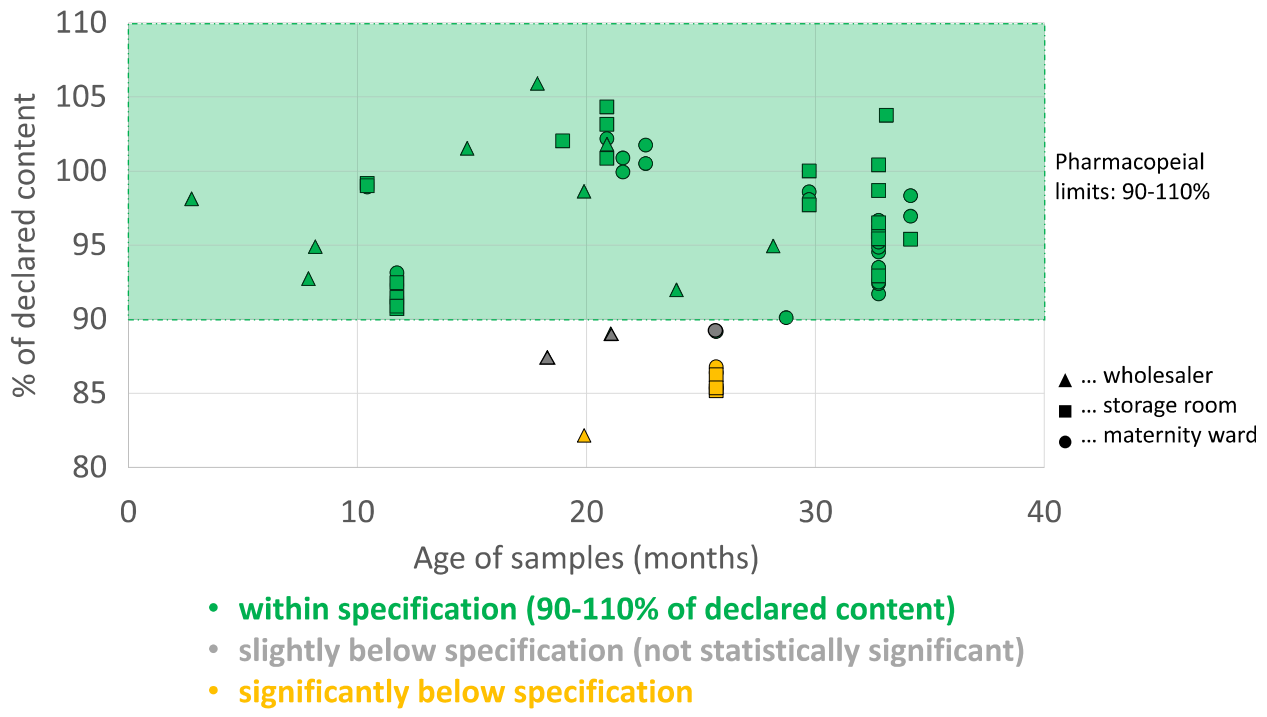

Fig. 5 Oxytocin content in relation to age and collection site of the samples ($n = 65$). The age of the sample denotes the time between manufacturing date and date of analysis in months

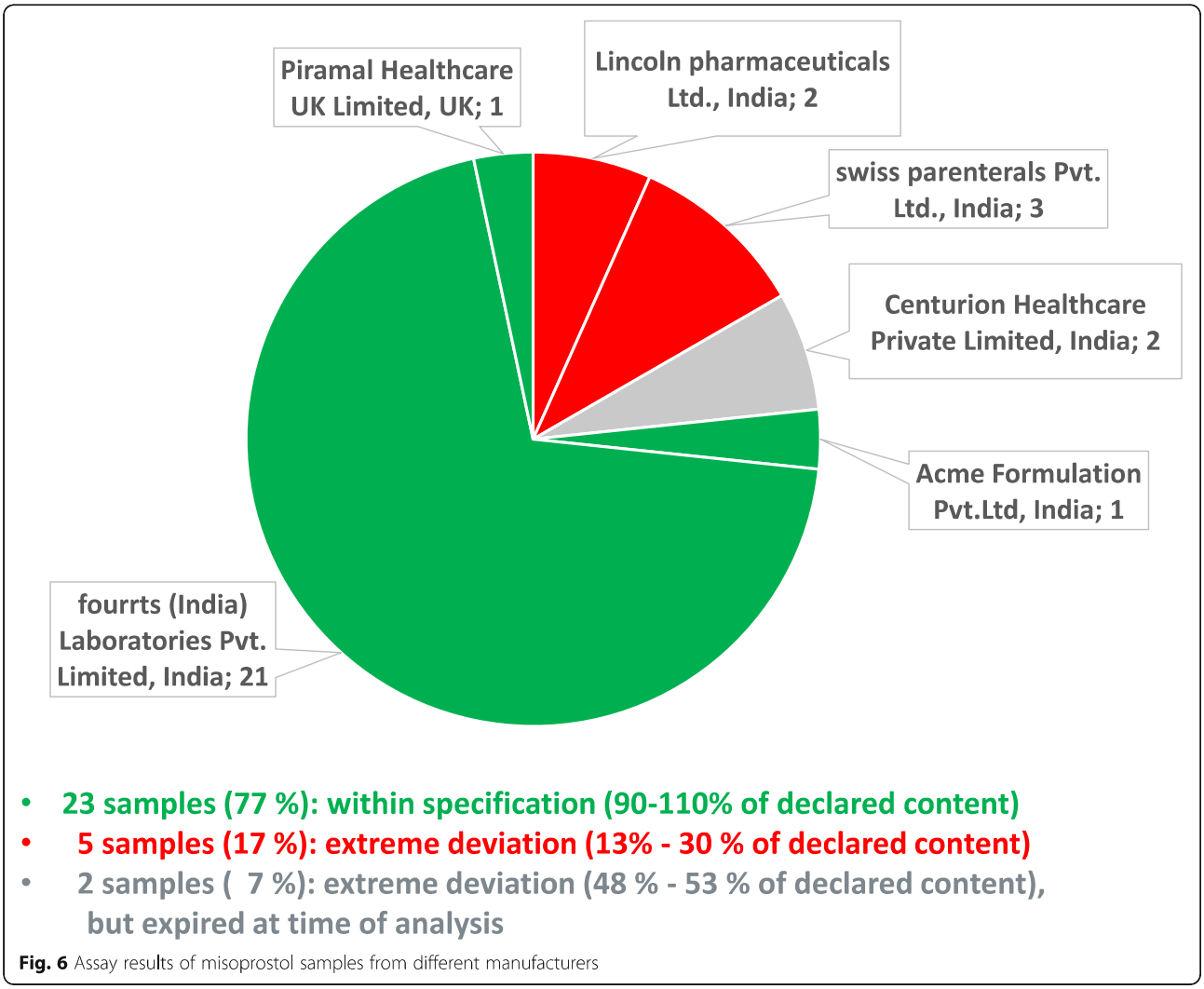

Fig. 6 Assay results of misoprostol samples from different manufacturers

oxytocics showed good quality. However, 11% of the oxytocin samples showed moderate deviations from specification (containing 82.2–86.8% of the declared amount of the API), and 17% of the misoprostol samples even showed extreme deviations (containing only 12.7–30.2% of the declared amount of API). The latter finding represents a serious risk to patient safety in maternal health care. Notably, both national and international authorities reacted swiftly and correctly to this finding.

The excellent oxytocin availability in the investigated government and faith-based health facilities is an important contribution to the attainment of the SDGs, especially to the reduction of global maternal mortality. Our finding is consistent with data from the Malawi Service Provision Assessment 2013–14, according to which 95% of the facilities (hospitals, health centers, clinics, dispensaries, health posts) which offered delivery services had oxytocin available [38].

Nearly half of the oxytocin and misoprostol samples collected in this study were labelled for storage below 25 °C. Using temperature loggers which automatically recorded the storage temperatures over approximately 4 months, our study clearly proved that this storage requirement cannot be complied with in many health facilities in Malawi. Improvements in the construction of storage rooms may reduce this problem, but air conditioning is most likely not an economically and practically feasible solution in rural health facilities of a low-income country such as Malawi. One feasible solution may be the procurement of medicines which have been proven to be stable at storage temperatures of up to 30 °C, as recommended by WHO for very hot countries (climatic zones III and IVA/IVB) but currently not for Malawi [39]. We recorded mean kinetic temperatures even higher than 30 °C in four health facilities in Malawi (Additional File 1). However, the 30 °C threshold was only exceeded by small margins (measured MKTs: 30.1, 30.2, 30.2 and 31.0 °C), and the period of our measurements (August/September until December) included the hottest season in Malawi.

Many currently available oxytocin preparations are labelled for storage at 2–8 °C. Just as previous studies in other countries [16, 40], our investigation showed that this storage temperature could not be reliably maintained in several health facilities in Malawi. Reasons for this included lack of refrigerators, frequent power failures, and lack of back-up generators or solar panels in many facilities. In this situation, it appears tempting to replace the oxytocin brands requiring storage at 2–8 °C by preparations labelled for storage at higher temperatures, or even by misoprostol tablets which do not require refrigeration. Unfortunately, our study suggests that this strategy does not offer a straightforward and reliable solution of the problem. Notably, all collected oxytocin samples labelled for storage at 2–8 °C were found to be within specifications, even when incorrectly stored in the health facility. In sharp contrast, 18% of the oxytocin preparations labelled for storage below 25 °C were found to be out of specification. A heat stable formulation of carbetocin, an oxytocin analogue, has just recently been added to the 2019 Model List of Essential Medicines of the World Health Organisation (WHO) and may become an alternative to other oxytocics [41]. However, it is not yet included into the Malawi Essential Medicines List, and we did not encounter any carbetocin samples in the course of our study.

In this study, only a small number of different oxytocin brands was found and investigated, therefore a generalization of the present results to the global situation may not be possible. But clearly, the present study shows an urgent need to reconfirm the stability claims of oxytocin preparations labelled for room temperature storage. Furthermore, it shows the importance of the procurement of good quality medicines from reliable manufacturers. This applies not only to oxytocin but even more to misoprostol, of which at least two extremely substandard brands were found in circulation in Malawi.

One option to ensure good quality is to restrict procurement to WHO-prequalified medicines [32, 33] and medicines produced in countries with a stringent regulatory authority (SRA) [42]. Of the 95 oxytocin and misoprostol samples collected in the present study, one (Misoclear®) represented a WHO-prequalified product and 17 were manufactured in countries with an SRA. Notably, none of these 18 samples was out of specification. On the other hand, also 5 brands of oxytocin and misoprostol manufactured in countries without an SRA, and not prequalified by WHO, comprised no samples which were out of specification. This demonstrates the importance of supplier prequalification in medicine procurement. The Medical Abortion Commodities Database (www.medab.org) has listed misoprostol products likely to be of good quality, as well as information on their availability. Of the six misoprostol preparations investigated in this study, two are listed in this database, and indeed these were found to be of good quality (i.e. within compendial specifications).

As mentioned above, the total number of different oxytocic preparations encountered in this study was small (9 oxytocin brands, 6 misoprostol brands). This may be a reflection of the small market size of Malawi and the resulting reluctance of manufacturers and international distributors to engage in medicine sales in this country. Unfortunately, this reluctance may severely restrict the possibilities to procure affordable, good-quality medicines. In spite of the above-mentioned problems, 11% of oxytocin samples investigated in this study were out of specification, much less than the 57.5% out-of-specification rate of oxytocin samples collected in Africa reported in a review of studies from 15 LMICs [20], and also than the 74.2% reported from a study in Nigeria [16]. In the latter study, all oxytocin samples were tested for identity, assay, pH value, sterility, and fill volume; all failing samples failed due to the assay values, while no samples failed in any of the other criteria [16].

All oxytocin samples collected in government health facilities in Malawi were of good quality, and the Malawi public health services deserve praise for this achievement, despite the observed problems with the quality of some misoprostol preparations.

The seven failing oxytocin samples showed API contents between 82.2 and 86.8% of the declared content. Following the terminology of an authoritative WHO study on medicine quality [29], we classified these as “moderate deviations” and marked them in Fig. 4 in yellow colour. Even moderate deviations from medicine specifications are not acceptable and need to be ruled out by appropriate measures. Nevertheless, the risk to patient safety posed by these oxytocin preparations is probably limited. However, this situation is clearly different for the five extremely substandard misoprostol preparations identified in this study, which are marked in red in Fig. 6. They contained only 12.7–30.2% of the declared content of misoprostol, and treatment failures will result from such extremely substandard medicines.

Misoprostol is an exceptionally instable API, and misoprostol tablets must therefore be produced competently using appropriate stabilizing agents. To improve misoprostol stability, a 1% dispersion of the API in hydroxypropylmethylcellulose (HPMC) is usually used [18, 43, 44]. Furthermore, a recent publication in WHO Drug Information [11] clearly demonstrated the supreme importance of aluminium-aluminium blisters for packaging of misoprostol tablets and for their protection from humidity. However, our finding of a misoprostol brand using plastic-aluminium blisters, and even of a brand using a PVC bottle with no individual blistering of

the tablets, shows that this information is not yet sufficiently applied in misoprostol manufacturing and procurement practice.

Conclusions

The availability of oxytocin in the four investigated districts of Malawi was found to be very good, and its quality was notably better than reported in previous studies carried out in other LMICs. However, storage conditions for oxytocin and misoprostol at the health facilities often did not meet the requirements stated by the manufacturers on the labels. The observed occurrence of substandard oxytocics apparently resulted both from shortcomings in the manufacturing process, including inappropriate formulation and packaging, and from deterioration during storage, accelerated by inappropriate storage conditions. Yet, this small study could not supply evidence at which part of the supply chain deterioration primarily occurs. Extremely substandard misoprostol tablets were found which represented a serious risk to maternal health. This shows the need for continued efforts for quality assurance in medicine procurement and registration, as well as for post-marketing surveillance within a functioning pharmacovigilance system. The case of the extremely substandard misoprostol tablets also highlights the importance of the immediate reporting of substandard medicines to national authorities, international stakeholder and the medical community, for a further improvement of patient safety within the country and worldwide.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12884-020-2810-9.

Additional file 1. List of included health facilities, pharmacies and wholesalers, with sizes of collected misoprostol and oxytocin samples, and storage conditions.

Additional file 2. Questionnaire and consent form.

Additional file 3. Results from Interviews.

Additional file 4. Related substances chromatogram of Misoprostol Tablets 200mcg by swiss parenterals Pvt. Ltd.

Abbreviations

ANOVA: Analysis of variance; API: Active pharmaceutical ingredient; CMST: Central Medical Stores Trust; COMREC: College of Medicine Research and Ethics Committee; GIZ: Gesellschaft für Internationale Zusammenarbeit GmbH; HPLC: High performance liquid chromatography; HPMC: Hydroxypropylmethylcellulose; LMIC: Low- and middle-income country; MEDQUARG: Medicine Quality Assessment Reporting Guidelines; MEML: Malawi Essential Medicines List; MHRA: Medicines and Healthcare products Regulatory Agency, U.K; MKT: Mean kinetic temperature; MSTG: Malawi Standard Treatment Guidelines; PMPB: Pharmacy, Medicines and Poisons Board; PPH: Post-partum haemorrhage; RSD: Relative standard deviation; SDG: Sustainable Development Goal; SOP: Standard operating procedure; SRA: Stringent Regulatory Authority; STG: Standard Treatment Guideline; UN: United Nations; UNCoLSC: UN Commission on Life-Saving Commodities for Women and Children; USAID: United States Agency for International Development; USP: United States Pharmacopoeia; WHO: World Health Organization

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Authors´ contributions

N.H, L.H. and F.K. designed the study. N.H. and F.K. collected medicines and data. N.H. carried out chemical analysis and evaluated the data. N.H. wrote the first draft of the manuscript, L.H. revised the manuscript. All authors read and approved the final manuscript.

Authors' information

N.H. carried out this study as part of her PhD research at the Pharmaceutical Institute, Tübingen University. F.K. is a senior lecturer at the Pharmacy Department, College of Medicine, University of Malawi. L.H. is professor at the Pharmaceutical Institute, Tübingen University.

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Availability of data and materials

The datasets used and/or analysed during the current study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Ethical approval was obtained from College of Medicine Research and Ethics Committee (COMREC; Reference No. P.07/27/2215) of the University of Malawi. Written informed consent to participate in this study was obtained from all interviewed persons (see Consent Form in Additional File 2).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Pharmaceutical Institute, Eberhard Karls University Tuebingen, Tuebingen, Germany. [2]Pharmacy Department, College of Medicine, University of Malawi, Blantyre, Malawi.

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Publisher's Note

Facility number	Type of facility	District	Sampling site	N° of collected misoprostol tablets per sample	Misoprostol storage conditions stated on label	N° of collected oxytocin vials per sample	Oxytocin storage conditions stated on label	Oxytocin storage conditions in health facility	Mean kinetic room temperature measured over 3 months (°C)	Mean kinetic refrigerator temperature measured over 3 months (°C)	Questionnaire completed? (yes/no)
1	central hospital	Blantyre	maternity ward	40	< 30 °C	5 5	< 25°C < 25°C	RT RT	24.9	n.rf.s.	yes
			storage room	96 100 50	not stated not stated < 30 °C	5 5	< 25°C < 25°C	RT RT	24.5[a]; 22.6[b]	n.rf.s.	yes
2	district hospital	Chikwawa	maternity ward	-		10	2-8°C	refrigerator	n.rt.s.	14.4 !	yes
			storage room	30	< 30°C	10	2-8°C	refrigerator	28.1	13.2[c]	yes
3		Neno	maternity ward	-		10 10	2-8°C 2-8°C	refrigerator refrigerator	n.rt.s.	11.9 !	yes
			storage room	50	< 30°C	10	2-8°C	refrigerator	21.9	2.8	yes
4		Ntcheu	maternity ward	38	< 25 °C	10	2-8°C	refrigerator	26.5 !	3.6	yes
			storage room	50	< 25 °C	10	2-8°C	refrigerator	21.5	3.6	yes
5	public health center	Blantyre	maternity ward	-		10	2-8°C	refrigerator	n.rt.s.	4.5	yes
6		Blantyre	maternity ward	-		8	2-8°C	RT !	26.2	n.rf.s.	yes
7		Blantyre	maternity ward	-		10	2-8°C	refrigerator	23.3	not measured	yes
			storage room	50	< 30°C	-	-	-	23.6	n.rf.s.	yes
8		Chikwawa	maternity ward	-		10	< 25°C	refrigerator!	n.rt.s.	18.3 !	yes
			storage room	-		10	< 25°C	RT	23.7	n.rf.s.	yes
9		Chikwawa	maternity ward	-		9	2-8°C	RT !	not measured	n.rf.s.	yes
			storage room	-		10	2-8°C	RT !	29.2	n.rf.s.	yes
10		Chikwawa	maternity ward	-		6	< 25°C	RT	28.3 !	n.rf.s.	yes
			storage room	-		-	-	-	27.7[a]; 27.9[b]	n.rf.s.	yes
11		Neno	maternity ward	-		10	< 25°C	RT	27.4 !	n.rf.s.	yes
12		Neno	maternity ward	-		10	< 25°C	refrigerator!	n.rt.s.	5.3	yes
13		Neno	maternity ward	-		10	< 25°C	RT	30.2 !	n.rf.s.	yes

			storage room	-		10	< 25°C	RT	28.2 !	n.rf.s.	yes
14		Neno	maternity ward	-		10	< 25°C	refrigerator!	n.rt.s.	missing logger	yes
			storage room	10	< 30 °C	10	2-8°C	refrigerator	26.2	6	yes
15		Ntcheu	storage room	-		10	< 25°C	RT	24.2	n.rf.s.	yes
			maternity ward	-		10	< 25°C	RT	27.4 !	n.rf.s.	yes
16		Ntcheu	maternity ward	10	< 30 °C	4	< 25°C	RT	28 !	n.rf.s.	yes
			storage room	-		4	< 25°C	RT	not measured	n.rf.s.	yes
17		Ntcheu	maternity ward	-		10	< 25°C	RT	22.8	n.rf.s.	yes
			storage room	-		10	2-8 °C	RT !	23	n.rf.s.	yes
18		Ntcheu	storage room	-		10	< 25°C	RT	23.1	n.rf.s.	yes
19	faith-based health center	Blantyre	maternity ward	-		10	< 25°C	RT	24.7	n.rf.s.	no
			storage room	-		10	< 25°C	RT	25.4 !	n.rf.s.	yes
20		Blantyre	maternity ward	13	< 30 °C	10	< 25°C	RT	26.8[a]; 26.2[b] !	n.rf.s.	yes
			storage room	-		10	< 25°C	RT	26.6 !	n.rf.s.	yes
21		Chikwawa	maternity ward	-		10 3	< 25°C < 25°C	refrigerator! RT	26.5 !	11.8[c]	yes
			storage room	19	< 30 °C	10	< 25°C	refrigerator!	30.2 !	13.8 !	yes
22		Neno	maternity ward	-		10	< 25°C	RT	25.5 !	n.rf.s.	yes
			storage room	-		10	< 25°C	RT	26.8 !	n.rf.s.	yes
23		Neno	maternity ward	-		10	< 25°C	RT	28.8 !	n.rf.s.	yes
			storage room	20 30	< 30 °C < 30 °C	10	< 25°C	RT	29.1[a];30.1[b] !	n.rf.s.	yes
24		Neno	maternity ward	-		10 10	2-8°C < 25°C	refrigerator RT	21.4	4.9	yes
			storage room	-		-	-	-	n.rt.s.	n.rf.s.	yes
25		Ntcheu	storage room	-		10 10	2-8°C < 25°C	refrigerator RT	24	4	yes
			maternity ward	-		-	-	-	n.rt.s	n.rf.s.	yes
26		Ntcheu	storage room	-		10	2-8°C	refrigerator	n.rt.s.	4.3	yes
			maternity ward	-		-	-	-	23.5	n.rf.s.	yes

27		Ntcheu	maternity ward	-		10	< 25°C	RT	27.2 !	n.rf.s.	yes
			storage room	-		-	-	-	28.3	n.rf.s.	yes
28	private clinic	Blantyre	maternity ward	-		10	< 30°C	refrigerator!	n.rt.s.	10.6 !	yes
			storage room	60	< 30 °C	10	< 25°C	RT	26.4 !	n.rf.s.	yes
29		Blantyre	maternity ward	-		-	-	-	n.rt.s.	n.rf.s.	yes
			storage room	-		10	< 25°C	RT	12.4[d]	n.rt.s.	yes
30		Chikwawa	maternity ward	-		9	< 25°C	refrigerator!	n.rt.s.	5.5	yes
			storage room	-		-	-	-	n.rt.s.	n.rf.s.	yes
31		Chikwawa	storage room	60	< 30 °C	-	-	-	31 !	n.rf.s.	yes
32	private pharmacy	Blantyre	storage room	23	< 30 °C	-	-	-	24.6	n.rf.s.	yes
33		Blantyre	storage room	50	< 30 °C	-	-	-	24	n.rf.s.	yes
34		Blantyre	storage room	30	< 30 °C	-	-	-	25.4	n.rf.s.	yes
35		Blantyre	storage room	30	< 30 °C	-	-	-	26.5	n.rf.s.	yes
36		Blantyre	storage room	36 20	< 30 °C < 30 °C	-	-	-	not measured	n.rf.s.	yes
37		Blantyre	storage room	50	< 30 °C	-	-	-	25.9	n.rf.s.	yes
38		Blantyre	-	-		-	-	-	n.rt.s.	n.rf.s.	no
39		Blantyre	-	-		-	-	-	n.rt.s.	n.rf.s.	no
40		Blantyre	-	-		-	-	-	n.rt.s.	n.rf.s.	no
41		Blantyre	-	-		-	-	-	n.rt.s.	n.rf.s.	no
42	drug store	Chikwawa	-	-		-	-	At drug stores, samples were collected by mystery shopper approach, therefor no information on actual storage conditions, no temperature loggers placed, and no questionnaires completed			
43		Chikwawa	-	-		-	-				
44		Chikwawa	-	-		-	-				
45		Chikwawa	-	-		-	-				
46		Chikwawa	-	-		-	-				
47		Chikwawa	-	-		-	-				
48		Neno	-	-		-	-				
49		Ntcheu	-	-		-	-				
50	wholesaler & CMST	Blantyre	storage room	-		30	< 25 °C	At wholesalers, samples were collected by mystery shopper approach, therefor no information on actual storage conditions, no temperature loggers placed,			
51		Blantyre	storage room	-		30	< 30 °C				
52		Blantyre	storage room	60 570[e]	< 30 °C < 30 °C	30 100[e]	2-8 °C 2-8 °C				

53		Blantyre (CMST)	storage room	400[e] 300[e]	< 25 °C < 30 °C	80[e] 80[e] 100[e]	2-8 °C < 25 °C < 25 °C	and no questionnaires completed
54		Blantyre	storage room	-		50	2-8 °C	
55		Blantyre	storage room	60	< 30 °C	100[e]	< 25 °C	
56		Lilongwe	storage room	-		30 100[e] 100[e]	2-8 °C 2-8 °C < 30 °C	
57		Lilongwe	storage room	300[e]	< 25 °C	-		
58		Blantyre	-	-		-		
59		Blantyre	-	-		-		
60		Blantyre	-	-		-		
61		Blantyre	-	-		-		
62		Blantyre	-	-		-		

Additional File 1: List of included health facilities, pharmacies and wholesalers, with sizes of collected misoprostol and oxytocin samples, and storage conditions. The cases where actual storage conditions are in conflict with storage conditions stated on the label are highlighted by exclamation marks.

RT = room temperature. n.rf.s. = no refrigerated storage of oxytocics; n.rt.s. = no room temperature storage of oxytocics. CMST = Central Medical Stores Trust.

[a] temperature logger placed at misoprostol storage place; [b] temperature logger placed at oxytocin storage place (if different)

[c] according to information by health facility personnel, logger has not been kept consistently in refrigerator, therefore excluded from further calculations.

[d] recorded temperature indicates storage of temperature logger in refrigerator for at least part of the time, therefore excluded from further calculations. [e] higher number of tablets / vials purchased as replacement samples / for additional stability testing.

Appendix 7 Quality, Availability and Knowledge of Rational Use and Storage Requirements of Oxytocics in Malawi

SAMPLE SITE & DRUG PURCHASE RECORD

Name of survey site: _____ Date of visit: _____

Contact in case of follow-up questions: Name: _____ Mobile number: _____

Description of location (pharmacy /maternity ward): _____ CHAM facility? YES □ NO □ Functioning fridge available? YES □ NO □

Photo taken: YES □ NO □ measured room temperature at facility (+time of measurement): _____

Temperature loggers placed? YES □ NO □ Serial number and location of placed temperature loggers: _____

Drug samples collected	Desired quantity	Obtained quantity	QOM reference number	Reason for not obtaining the desired quantity: - not available at all (NA) - out of stock (OOS) - not available in sufficient amount (NASA) - other (please describe!)	Price per tabs/vial in MWK (if applicable)	Stock on hand (in tabs/vials)	Monthly consumption (in tabs/vials, based on last six months)	Stockout time in last 6 months (= total number of days when this medicine was not available)	SOP for oxytocic storage available? (yes/no) If yes, please take picture / copy	STGs for oxytocics available? (yes/no) If yes, please take picture / copy	Storage conditions: 1) refrigerated (r) /not refrigerated (nr) 2) in original package (op)/out of original package (nop) 3) protected from light (pr) /not protected from light (npr)	1) Thermometer kept with oxytocic medicines (yes/no) 2) Temperature recorded daily (yes/no) If yes, please take picture
						If possible, please take picture / copy of stock book!						
Misoprostol 0,2mg tab.	50										(r) (nr) (op) (nop) (pr) (npr)	1) yes no 2) yes no
Oxytocin 10 IU vial	10										(r) (nr) (op) (nop) (pr) (npr)	1) yes no 2) yes no

Arrival dates and origin (eg CMST, donation) of last two orders: _____

Expected arrival date and origin of next order: _____

What do you do with expired samples? _____

Comments: _____

If samples taken from bulk / without original package, please take picture of original package / bulk container (showing label, batch number, expiry date, manufacturing date, name / adress of manufacturer)!

Collected samples were replaced: YES □ NO □ Collected samples were paid for: YES □ (Attach receipt!) NO □

Name of sampling person: _____ Signature: _____

Name of accompanying person: _____ Signature: _____

Name of person responsible for health facility: _____ Signature: _____

Appendix 8 Quality, Availability and Knowledge of Rational Use and Storage Requirements of Oxytocics in Malawi

Name of survey site: _____ Date of visit: _____

QUESTIONNAIRE FOR KNOWLEDGE OF RATIONAL USE AND STORAGE REQUIREMENTS OF OXYTOCICS:

1. Profession / training level of person responsible of oxytocics /who administers oxytocics: □ Nurse □ Pharmaceutical technician □ Pharmacist
   □ other:_____
2. How long has he/she been doing this work? □ less than 1 year □ 1-3 years □ 4-7 years □ 7-12 years □ more than 12 years
3. Has he/she ever attended training on storage, distribution and handling procedures of cold chain medicines? □ yes □ no
   If yes, how many times has he/she attended such a course within the last three years? □ once □ twice □ thrice □ more than thrice □none within last three years
4. How should oxytocin be stored? □ depending on manufacturer □ at room temperature □ in a fridge
5. How should misoprostol be stored? Multiple answers possible! □ at a dry place □ at room temperature □ in a fridge □ in aluminium blisters

*Oxytocin, that is used right now (manufacturer, declared storage conditions):*_____

Other oxytocin products (+ its storage conditions) that have been used in the last 12 months: _____

Misoprostol, that is used right now (manufacturer, declared storage conditions): _____

Other Misoprostol products that have been used in the last 12 months:_____

6. Have you ever experienced ineffective oxytocics? Misoprostol: □ yes □ no Oxytocin: □ yes □ no
   If yes, what brand?_____
   What actions have been taken? Multiple answers possible!
   □ notify authorities (DHO/PMPB) □ notifiy supplier (CMST, Wholesaler) □ nothing □ buy different brand
7. Do you usually also have (methyl-)ergometrine on stock? □ yes □ no
8. What time do you switch off the fridge in the facility? □ evening □ over the weekend □ never switched off

9. How often do you have power black-outs (approx.)? □ less than once a month □ 1-3 times a month □ once a week □ 1-3 times a week □ daily

10. How do you maintain appropriate storage condition in the event of power failure? □ gas □ solar □ no measures

11. Do you have an automated functional generator system in case of power failure? □ yes □no

For maternity wards / health centers / health posts only:

Number of deliveries in last 6 months:_____ Number of reported cases of PPH in last 6 months:_____

1. Have the numbers of deliveries in the last 6 months significantly increased or decreased? □ yes (increased: □ decreased: □) □ no
   If yes, reasons? Multiple answers possible!
   □ availability of infrastructure (e.g. power / water)
   □ availability of medical staff
   □ availability of medicines / medical devices
   □ lack of family planning
   □other:_____

2. When do you give oxytocin to prevent / treat PPH? □ a) always after delivery of child □ b) always before delivery of child □ c) only when woman is bleeding

3. When do you give misoprostol to prevent / treat PPH? multiple answers possible!
   □ no misoprostol on stock □ if oxytocin is not available □ if oxytocin is not working □ if home delivery is planned/most likely
   □ other:_____

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM (COPY FOR INVESTIGATOR)

Title of the research project: A survey on quality, availability and knowledge of rational use and storage requirements of oxytocics in Malawi.

Principal investigator:
Felix Khuluza, Pharmacy Department, College of Medicine, University of Malawi
Private Bag 360, Chichiri, Blantyre 3, Malawi
Tel. Secretariat +265 (0) 187 4107; www.medcol.mw
Mobile: +265 (0)999 289874
Email: fkhuluza@medcol.mw or fkhuluza@cartafrica.org

Nhomsai Hagen, Pharmaceutical Institute, University of Tuebingen, Auf der Morgenstelle 8, 72076 Tuebingen, Germany; Tel. Secretariat: +49 (0)7071 2972460; www.uni-tuebingen.de
Email: Nhomsai.hagen@uni-tuebingen.de

You are being invited to take part in the survey as titled above. Please take some time to read the information presented here, which will explain the details of this survey. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the College of Medicine Research and Ethics Committee and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, Malawi Guidelines for Good Clinical Practice.

What is this research study all about?

- *This study will be conducted in various districts in Malawi*
- *The aim of the study is to investigate quality, availability and knowledge of rational use and storage requirements of oxytocics at different points of care and different points of the supply chain in Malawi*

Why have you been invited to participate?

- *You have been asked to participate in this study because you are involved in distribution and / or administration of oxytocics and your knowledge and experience in this area will help in collecting vital information for this study.*

What will your responsibilities be?

- *Your responsibility is to provide samples of oxytocics and all information that you know on the questions asked, as honestly and as openly as you can.*

- *To investigate storage conditions of oxytocics, we will place single-use temperature loggers where you store oxytocin and misoprostol, which will be re-collected after three months. Please ensure, that these loggers are not moved during these three months.*

Will you benefit from taking part in this research?

- *The benefits of participating in this study is that the data will help policy makers on possible ways of improving quality and availability of oxytocics in the country as such you will not directly benefit.*

Are there any risks involved in your taking part in this research?

- *There are no risks in taking part in this research and all participants' names and places will be kept confidential, nor will they be published.*

If you do not agree to take part, what alternatives do you have?

- *You are free to decline to take part in this study. Nothing will happen to you if you decide to decline. You can also decide to decline parts of the study (e.g. placing temperature loggers)*

Who will have access to the records of the data?

- *The data will be kept confidential. Only the investigator in this study will have access to the data. When the data is published, we will not use names or any other information that may lead to readers identifying you.*

- *Results will be presented to you via a letter to your DHO, before being made available to any other party or to the public or being published in reputable journals.*

Will you be paid to take part in this study and are there any costs involved?

- *No you will not be paid to take part in the study. There will be no costs involved for you, if you do take part. The samples you provide will be replaced or paid for.*

Is there anything else that you should know or do?

- *You can contact PI at tel: 0999289874 if you have any further queries or encounter any problems.*

- *You can contact the Secretariat of College of Medicine Research and Ethics Committee at* 0111871 911 *if you have any concerns or complaints that have not been adequately addressed by the study staff.*

- *You will receive a copy of this information and consent form for your own records.*

Declaration by participant

By signing below, I ..... agree to take part in a research study entitled *A Survey on quality, availability and knowledge of rational use and storage requirements of oxytocics in Malawi.*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurized to take part.
- I may choose to leave the study at any time and will not be penalized or prejudiced in any way.

Signed at (*place*) ..... on (*date*) ..... 2018.

.....

Signature of participant

Declaration by investigator

I *(name)* ..... declare that:

- I explained the information in this document to .....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above and that his/her participation is voluntary
- I did not use an interpreter.

Signed at (*place*) ..... on (*date*) ..... 2018.

.....

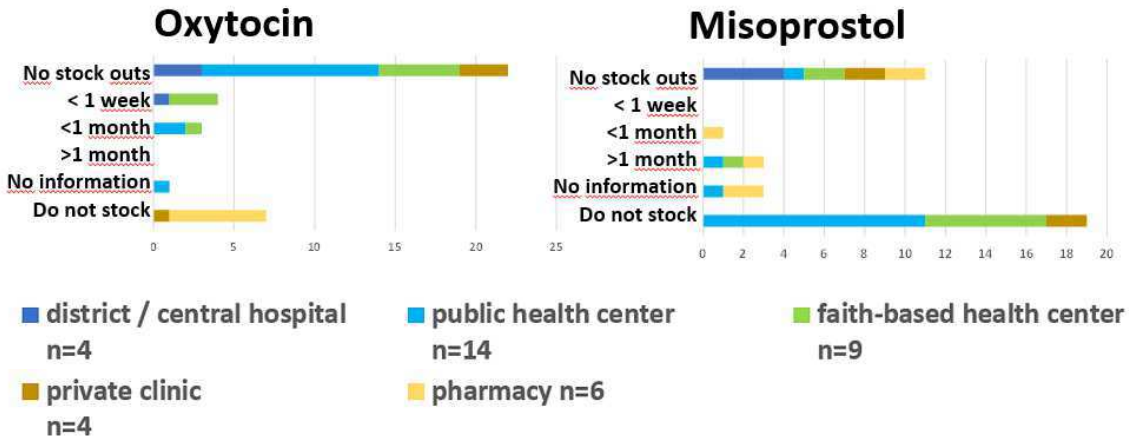
Signature of investigator

Further queries should be addressed to:

The Chairperson
College of Medicine Research and Ethics Committee (COMREC)
Private Bag 360, Chichiri, Blantyre 3
Tel: + 265 (0) 1871 911

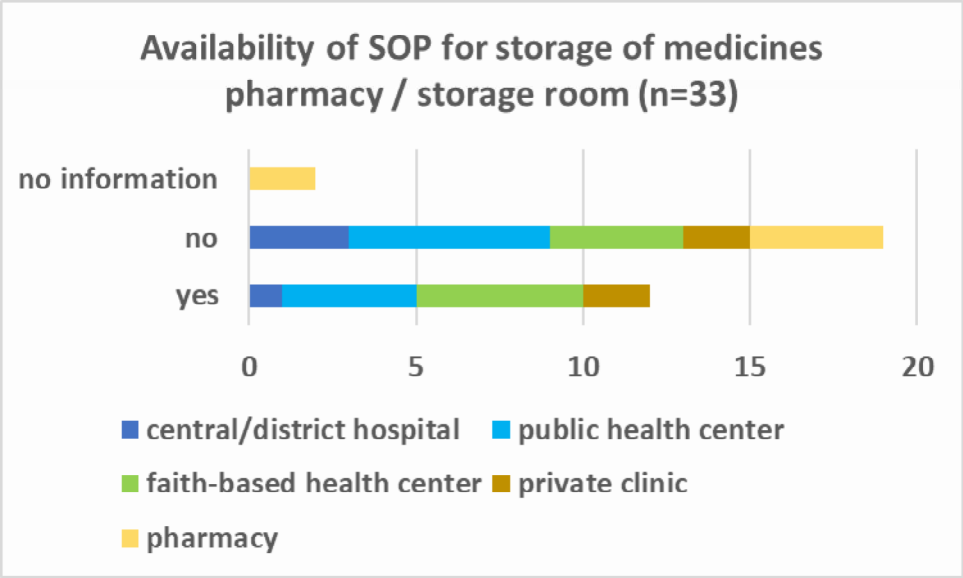
Additional File 3: Results from Interviews

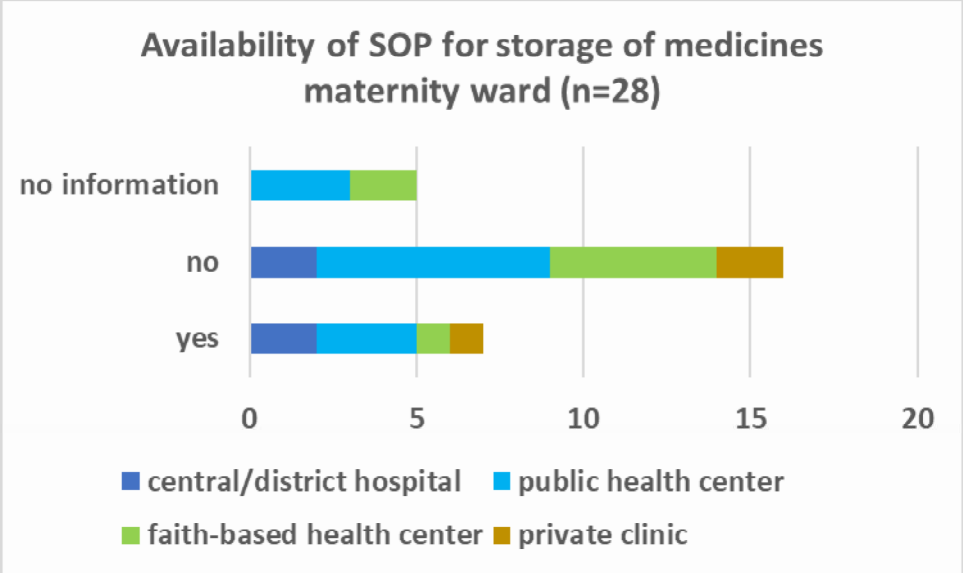
1) Stockout times of oxytocin and misoprostol at health facilities in the last 6 months



In case of public and faith-based health facilities and private clinics, this information was verified by inspecting the stock cards for the respective medicines. n= number of facilities.

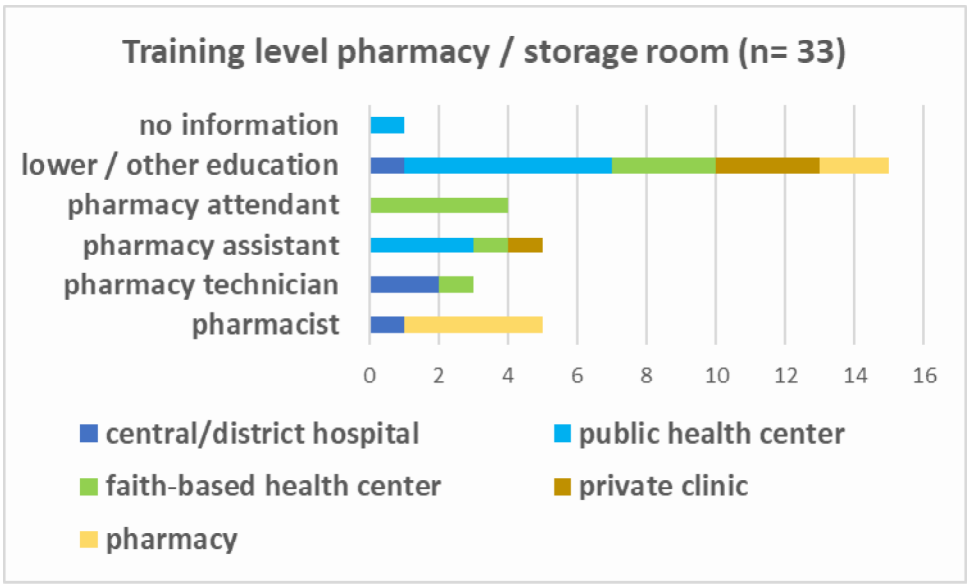
2) Availability of Standard Operating Procedures (SOPs) for (oxytocic) storage

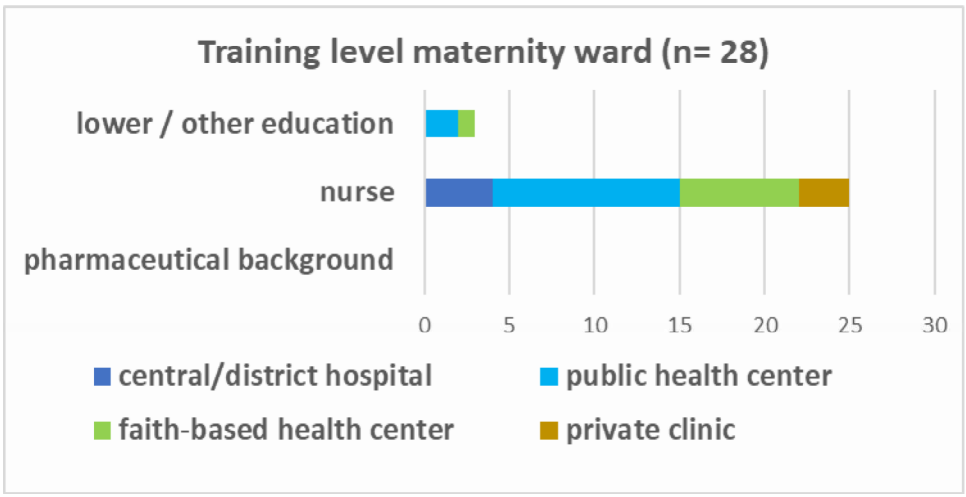




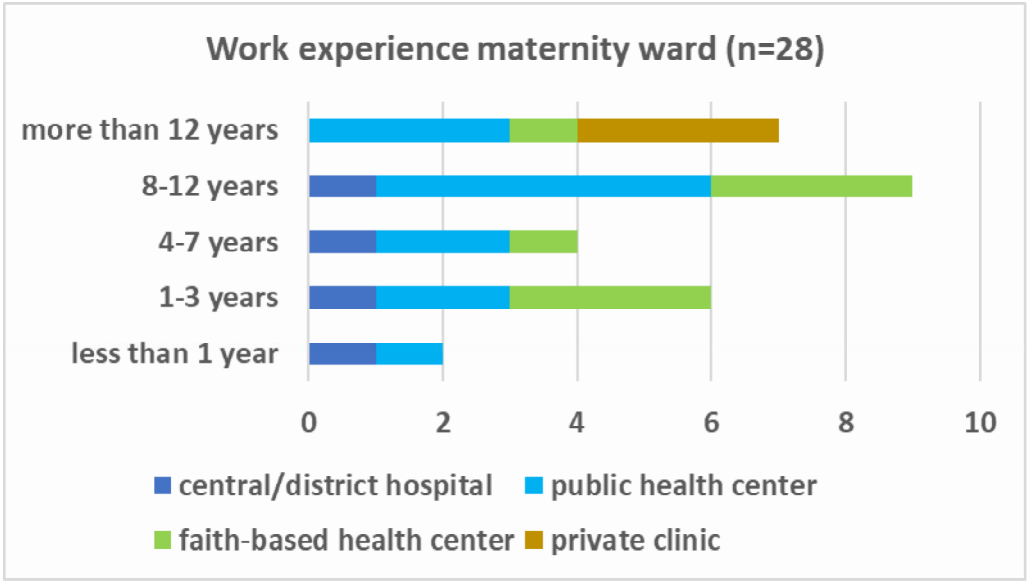
Specific SOPs just for the storage of oxytocin or misoprostol were not available at any health facility /pharmacy.

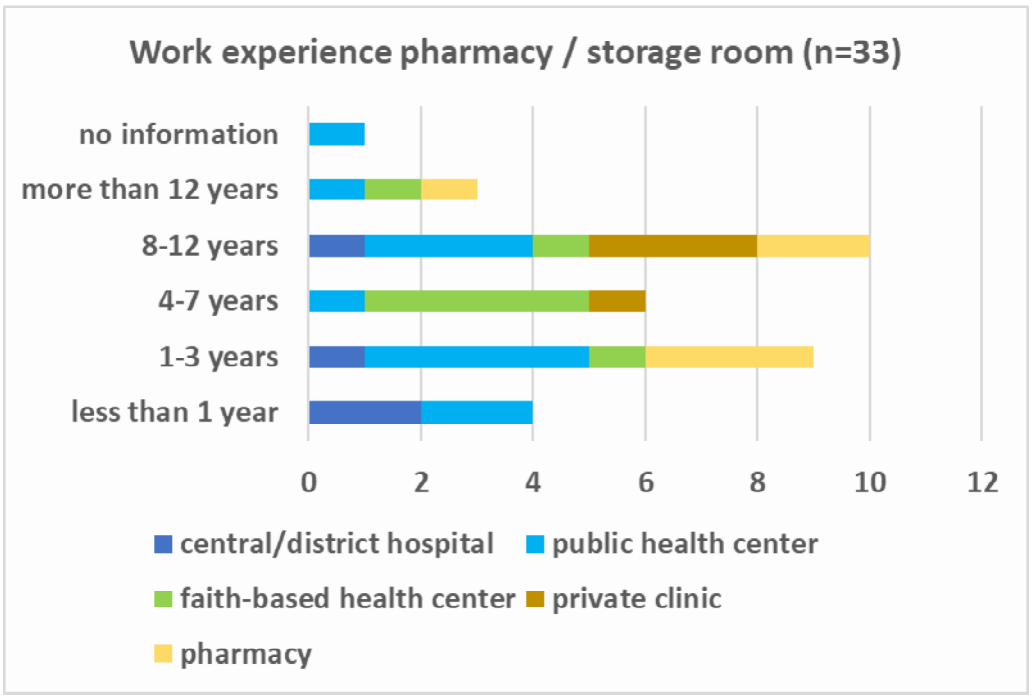
3) Profession / training level of person responsible for oxytocics / who administers oxytocics



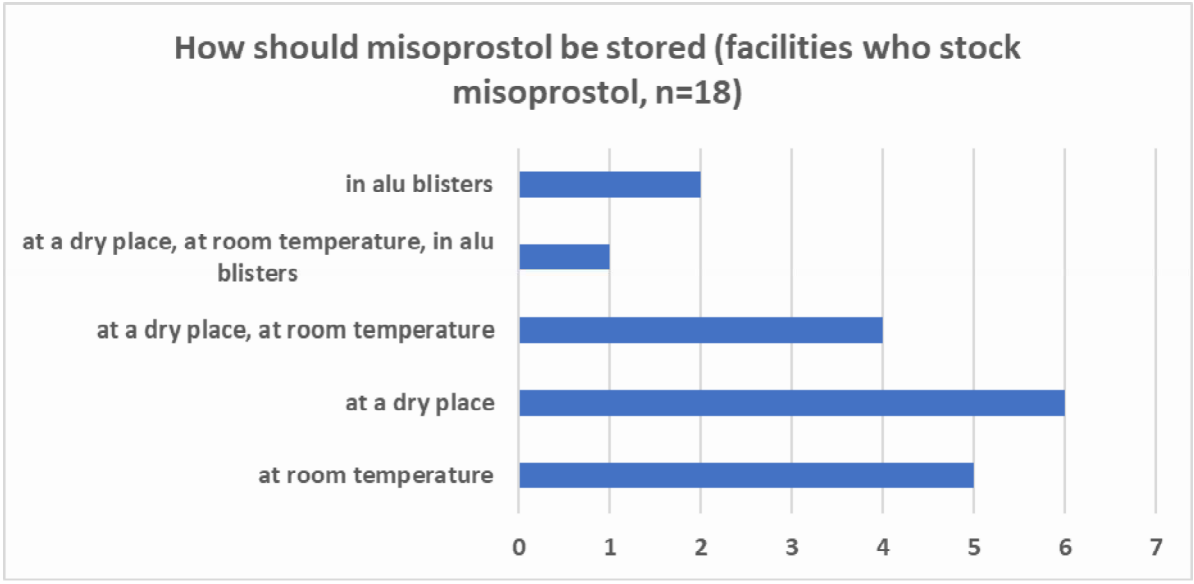


4) Work experience of person responsible for oxytocics / who administers oxytocics





5) Storage of misoprostol

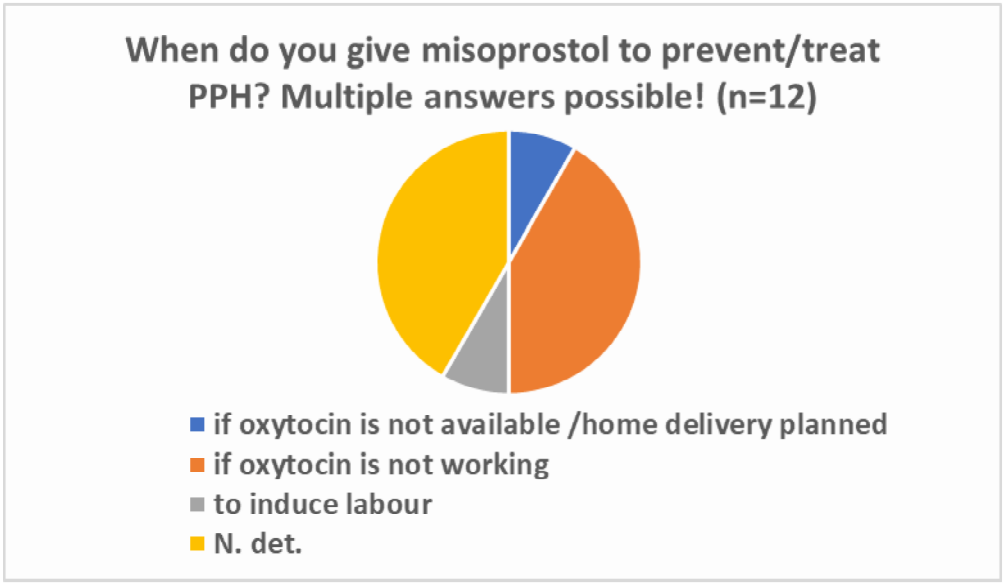


Question as multiple choice. The importance of aluminium /aluminium blisters as primary packaging is not well known (only 3 out of 18 ticked “in aluminium / aluminium blisters”); n= 18 (12 health facilites + 6 pharmacies)

6) At what time do you switch off the fridge in the facility?

One health worker from a public health center ticked “in the evening” on the question “at what time do you switch of the fridge in the facility?”. One health worker from a faith-based health center ticked “over the weekend” when asked this question. All other health worker ticked “never switched off”.

7) When do you give misoprostol do prevent / treat PPH? Multiple answers possible



Answers of all 12 health facilities, who stock misoprostol. N.det.: not determined.

8) Numbers of deliveries at health facilities, and PPH rates

Type of facility	Facility number	number of deliveries in the last 6 months	% reported PPH/deliveries last 6 months	have N° of deliveries increased /decreased?
central hospital	1	6768	N.det.	decreased
district hospital	2	2130	3.38	NA (person new at facility)
	3	780	1.58	increased
	4	3847	0.94	increased
public health center	5	260	0.38	N.det.
	6	N.det.	N.det.	N.det.
	7	1455	1.24	increased
	8	1680	0.36	increased
	9	520	N.det.	increased
	10	203	0.99	increased
	11	30	33.3	varies with months
	12	318	3.1	increased
	13	141	0	N.det.
	14	620	N.det.	increased
	15	330	3.3	increased
	16	540	1.1	N.det.
	17	59	3.39	varies with months
	18	N.det.	N.det.	N.det.
faith-based health center	19	N.det.	N.det.	N.det.
	20	1293	1.47	increased
	21	1800	1.3	increased
	22	78	3.85	decreased
	23	220	0.9	decreased
	24	290	6.21	decreased
	25	23	0	increased
	26	32	6.25	increased
	27	241	0.83	increased
private clinic	28	15	0	neither
	29	7	0	decreased
	30	92	0	N.det.
	31	no delivery services		

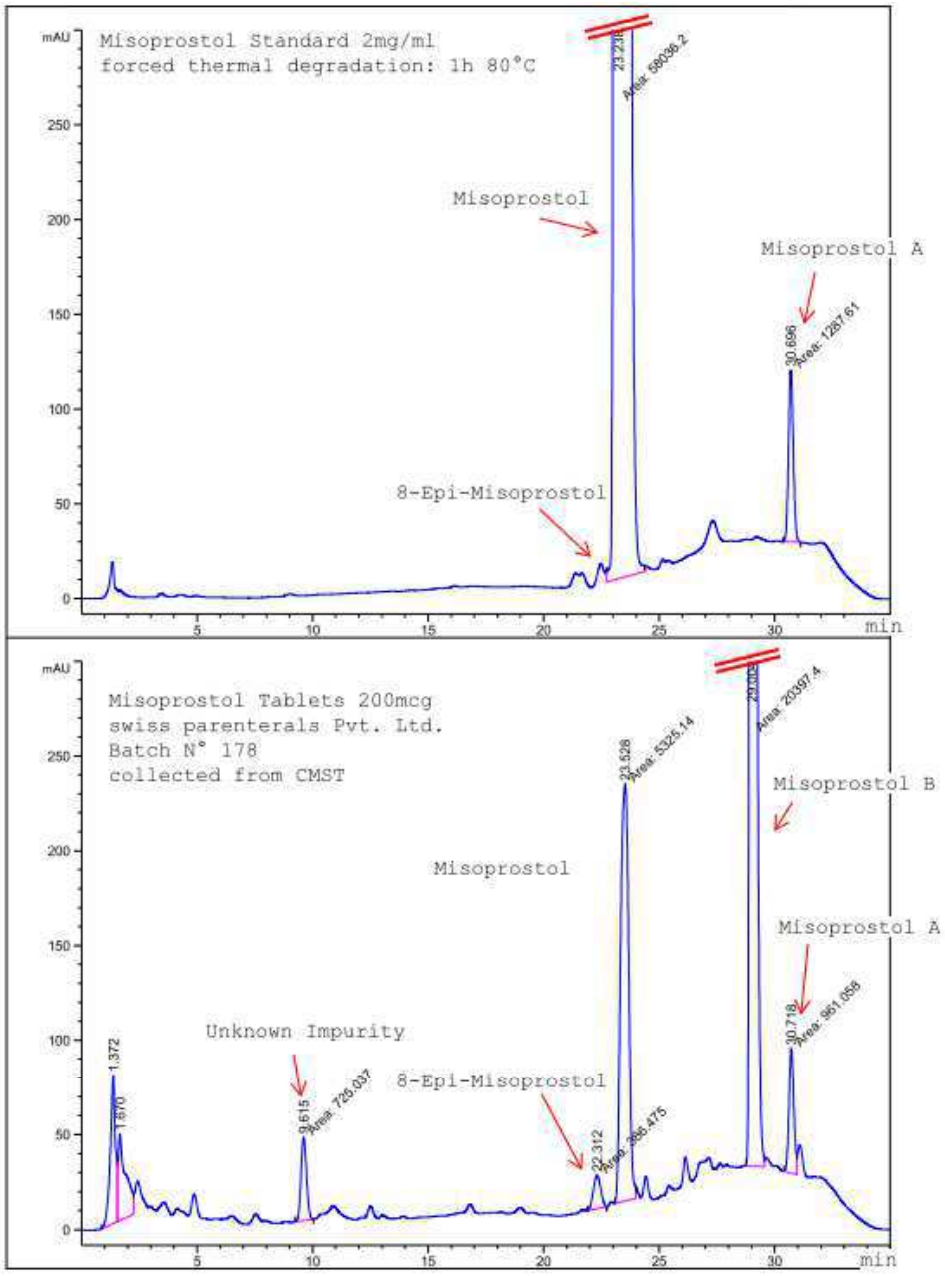
N.det: not determined. PPH: Post-partum haemorrhage

As reasons for **increased** numbers of deliveries were mentioned:

- Lack of familiy planning (n=8)
- Increased teenage pregnancies (n=2)
- People prefer delivering in health center rather than at home (n=3)
- Closing of nearby health center (n=1)

As reasons for **decreased** numbers of deliveries were mentioned:

- Successful family planning campaign (n=2)
- Women are referred to district hospitals due to birth complications (n=1)
- Expensive, people prefer public health center (n=1, private clinic)
- NA (n=1)



Additional File 4: Related substances chromatogram of Misoprostol Tablets 200mcg by swiss parenterals Pvt. Ltd.

Chromatographic conditions according to Kahsay et al. 2015 (Ref. 28):
HPLC: Agilent Infinity 1260 II
Column: Dr. Maisch ReproSil-XR 120 C18, 5µm, 150mm x 4,6 mm with guard
Mobile phase A: ACN-H_20-MeOH, 28:69:3 (v/v/v); mobile phase B: ACN-H_20-MeOH, 47:50:3 (v/v/v)
Gradient (time [min]/%B): 0/0, 5/0 to 15/35, 20/35 to 25/95, 30/95 to 32/0, 35/0
Flow rate: 1.5ml/min; UV detection: 200nm
Column oven: 35°C. Samples in refrigerated autosampler at 4°C
Injection volumes: 200 µl (sample, 0.4mg/ml), 50 µl (reference, 2mg/ml)

Misoprostol Ph.Eur. reference standard (batch N° 3.0) was dissolved in ACN-H_20 45:55 (v/v) at a concentration of 2mg/ml. For forced thermal degradation, this solution was placed for 1h in an oven at 80 °C.

Sample solution was prepared as described in Kahsay et. al., using 3 tablets per sample.

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Stability of Oxytocin Preparations in Malawi and Rwanda: Stabilizing Effect of Chlorobutanol

Nhomsai Hagen,[1] Thomas Bizimana,[2] Pierre Claver Kayumba,[3] Felix Khuluza,[4] and Lutz Heide[1]*

[1]*Pharmaceutical Institute, Eberhard Karls University Tübingen, Tübingen, Germany;* [2]*School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda;* [3]*East African Community Regional Centre of Excellence for Vaccines, Immunizations and Health Supply Chain Management (EAC RCE-VIHSCM), University of Rwanda, Kigali, Rwanda;* [4]*Pharmacy Department, College of Medicine, University of Malawi, Blantyre, Malawi*

Abstract. Oxytocin is used for the prevention and treatment of postpartum hemorrhage, the leading cause of maternal mortality in low- and middle-income countries. Because of the high instability of oxytocin, most products are labeled for storage at 2–8°C. Some other products are on the market which are labeled for non-refrigerated storage, but independent evaluations of their stability hardly exist. In the present study, seven brands (nine batches) of oxytocin were purchased from wholesalers and medical stores in Malawi and Rwanda and investigated by accelerated stability testing according to the ICH/WHO guidelines. Two oxytocin brands approved by a stringent regulatory authority (SRA) or by the WHO Prequalification of Medicines program and purchased in Europe were used as comparison. All investigated brands which were either produced in countries with SRAs, or were WHO-prequalified products, were labeled for storage at 2–8°C, and all of them passed stability testing with very good results. Even exposure to 25°C or 30°C for several months hardly affected their oxytocin content. However, two other investigated brands were labeled for non-refrigerated storage, and both of them had been produced in countries without SRAs. These two preparations showed not higher but lower stability than the brands labeled for storage at 2–8°C, and, for both of them, noncompliance with pharmacopoeial specifications was found after accelerated stability testing. At 40°C, and in forced degradation studies at 80°C, chlorobutanol showed a remarkable stabilizing effect on oxytocin, which may deserve further investigation. The results of the present study support the policy "Buy Quality Oxytocin, Keep It Cool."

INTRODUCTION

Oxytocin is listed in the essential medicines list of the WHO[1] and is used for the prevention and treatment of postpartum hemorrhage (PPH).[2] Postpartum hemorrhage is the leading cause of maternal mortality in low-income countries.[2] One of the sustainable development goals of the United Nations is "to reduce the global maternal mortality ratio to less than 70 per 100,000 live births by 2030."[3] To achieve this, prevention and treatment of PPH with oxytocin is an essential step.

Several previous studies have shown that the quality of oxytocin, especially in low- and middle-income countries (LMICs), is often poor.[4–13] This may result from poor manufacturing (e.g., inappropriate formulation or packaging), from poor storage and/or transportation conditions, or from a combination of these factors. The nonapeptide oxytocin is known to be very sensitive to high temperatures.[4,14–17] The typical degradation mechanisms are shown in Figure 1. If oxytocin is not stored properly, then it may lose its potency, which can result in higher mortality rates of PPH.

Most of the currently marketed oxytocin products have to be stored at 2–8°C according to the labeling information. Maintaining this storage temperature can present a challenge, especially in rural health facilities in LMICs.[6,9,13,18,19] Several oxytocin preparations are now on the market which are labeled for non-refrigerated storage. However, according to a report of the Reproductive Health Supplies Commission, there are nearly 300 different oxytocin products, offered by at least 100 manufacturers, and there is considerable confusion about the storage and labeling requirements for these.[20] Similar concerns were raised by the WHO in a report on the quality of medicines for maternal and child health, which concluded for oxytocin injections: "It would be useful to verify to which extent manufacturers' instructions for higher storage temperatures were based on reliable stability studies."[11] The Promoting the Quality of Medicine Program of the U.S. Pharmacopeia stated a need to conduct "systematic stability studies [. . .] in accordance with the ICH guidelines [. . .], to reassess the specifications for storage and shelf life of oxytocin injections."[21] It was with these recommendations in mind that we decided to investigate the stability of selected oxytocin preparation according to the ICH guidelines (see Methods).

The stability of pharmaceutical products must be investigated and documented by the manufacturers, and National Medicines Regulatory Authorities (NMRAs) usually do not confirm these data independently. Countries with the so-called stringent regulatory authorities (SRAs) are usually trusted to ensure complete and correct stability testing by the manufacturers. Stringent regulatory authorities are NMRAs who are members, observers, or associates of the International Council of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and currently comprise the national regulatory authorities of the EU member states, the United States, Japan, Switzerland, Canada, Australia, Iceland, Liechtenstein, and Norway.[22] In addition, the WHO prequalification of medicines program (now called "WHO Prequalification Team: medicines") prequalifies finished pharmaceutical products for certain indications, including reproductive and maternal health, based on information submitted by manufacturers and on inspections of the corresponding manufacturing sites.[23,24]

However, many medicines circulating in LMICs are neither approved by a stringent regulatory authority (SRA) nor WHO prequalified. National regulatory authorities of LMICs often have only limited capacity for independent evaluation and analysis[25,26] and need to rely on manufacturers' data when registering medical products. Independent scientific confirmations of manufacturers' claims, for example, on heat-stability of oxytocin preparations, are therefore important.

* Address correspondence to Lutz Heide, Pharmaceutical Institute, Eberhard Karls University Tübingen, Auf der Morgenstelle 8, Tübingen 72076, Germany. E-mail: heide@uni-tuebingen.de

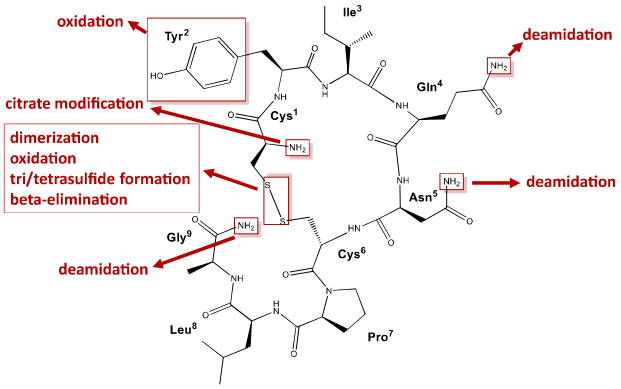

FIGURE 1. Structure of oxytocin and its typical degradation mechanisms. Modified from Avanti et al.[39] This figure appears in color at www.ajtmh.org.

The ICH has formulated guidelines for stability testing of pharmaceutical products (Q1A-Q1F).[27] Testing a product's stability over its entire shelf life is very time consuming; therefore, accelerated stability studies for a shorter time, but at higher temperature and relative humidity (RH), are permissible and are frequently used for the prediction of shelf life and storage requirements. The precise conditions for accelerated stability studies depend on the intended storage recommendation, as well as on the climatic zone of the country where the medicine will be registered, as specified by the ICH[28] and by the WHO[22] guidelines. Malawi is currently assigned to WHO climatic zone II (subtropical and Mediterranean) and Rwanda to zone IVa (hot and humid).[29]

The so-called forced degradation studies[30] are carried out at even higher temperatures. They were used in the current study to investigate the influence of excipients such as buffers and the widely used bacteriostatic agent chlorobutanol on the stability of oxytocin.

MATERIALS AND METHODS

Study design and ethical approval. This study followed the WHO guidelines on the conduct of surveys of the quality of medicines[31] and the MEDQUARG guidelines,[32] where applicable. Ethical clearance to conduct this study was received from the College of Medicine Research and Ethics Committee in Malawi (Reference no. P.07/27/2215). Approval was also granted by the Malawi National Regulatory Agency (Pharmacy, Medicines and Poisons Board [PMPB]), as well as by the Ministry of Health, Rwanda (Reference no. 20/1361/DGPHFIS/2018).

Sample collection. In Malawi and Rwanda, all brands of oxytocin available during the time of sample collection (February–March 2018) at government and faith-based medical stores (i.e., at the Central Medical Stores Trust in Malawi, the Rwanda Biomedical Center/Medical Production, Procurement and Distribution Division of Rwanda, and the Bureau des Formations Médicales Agréées du Rwanda in Rwanda) and from all pharmaceutical wholesalers located in Blantyre and Lilongwe (Malawi) and in Kigali (Rwanda) were purchased by local researchers (F. K. and T. B.). For comparison, additional samples were purchased from the pharmacy of the university hospital in Tübingen, Germany, and from an international wholesaler (Imres B.V., Lelystad, The Netherlands). A mystery shopper approach was used when ordering from commercial wholesalers. An overt approach was used when samples were collected in person from government or faith-based medical stores. For each brand, 100 vials of oxytocin were purchased. If different batches of a certain brand were available, then samples from each batch were purchased. After purchase, samples were stored according to the manufacturers' specifications, and temperature loggers were kept with all samples until their placement in stability chambers. Samples were hand-carried by the investigators from Malawi or Rwanda to Germany via airplane, with traveling time of less than 24 hours. At the Pharmaceutical Institute of Tübingen University, samples were pretested for assay and pH according to the U.S. Pharmacopeia. All samples that were within specifications at the time of pretesting were included into the stability study.

Storage in stability chambers. Samples were stored in four different stability chambers (Table 1) for 6 months (April–October 2018) at Alpha-Pharma-Service GmbH, Heilbronn, Germany. Conditions were chosen in accordance with the ICH and WHO guidelines for stability testing of pharmaceutical products.[22,28] At different time points (months 0, 1, 2, 3, and 6), samples were withdrawn from the chambers and analyzed at the Pharmaceutical Institute at Tübingen University, Germany.

Sample analysis. At the Pharmaceutical Institute of Tübingen University, Germany, samples were visually inspected, and tested for identity, assay, and pH value according to the monograph of the U.S. Pharmacopoeia (USP 40, oxytocin injections). Methods were validated according to USP 40. Analysis was conducted via high-performance liquid chromatography (HPLC, Agilent Infinity 1260 II with a binary pump, a variable wavelength detector, a refrigerated autosampler, and an integrated column compartment; Agilent Technologies, Santa Clara, CA). Solvents were HPLC grade.

For analysis, a gradient system of mobile phase A (0.1 M NaH_2PO_4 buffer) and mobile phase B (ACN: H_20 1:1 V/V) with

TABLE 1
Conditions for accelerated stability testing and controls

		ICH/WHO* stability testing conditions	
Temperature (°C)	Relative humidity (%)	For refrigerated products	For non-refrigerated products
5 ± 3	n. def.	Long term	–
25 ± 2	60 ± 5	Accelerated	Long term, zone II
30 ± 2	65 ± 5	Accelerated, more severe*	Long term*, zone IVa, intermediate
40 ± 2	75 ± 5	–	Accelerated

n. def. = not defined. According to the ICH/WHO,[22,28] long-term testing should cover the proposed shelf life or a minimum of 12 months at the time of submission. Stability testing under accelerated or intermediate conditions should cover a minimum of 6 months.
*In addition to the conditions listed in the ICH guidelines, the WHO guidelines list also more severe conditions for products that are intended to be marketed in climatic zones III–IV. Malawi is assigned to climatic zone II and Rwanda to climatic zone IVa.[29]

the following gradient was used: 0 minutes, 30% B; 10 minutes, 40% B; 17.5 minutes, 65% B; 20.5 minutes, 65% B; 23.5 minutes, 30% B; 26 minutes, 30% B; flow rate, 1.5 mL/minute; injection volume, 70 µL; column with guard from Dr. Maisch GmbH, Ammerbuch, Germany (Reprospher 100: 12.5 cm × 4.6 mm, 5 µm C18); and detection at 220 nm. The U.S. Pharmacopoeia Reference Standard (batch N° F3K133) was obtained from Merck KGaA, Darmstadt, Germany. pH values were tested with a pH-combination microelectrode (N 6000 BNC) from SI Analytics GmbH, Mainz, Germany. From each sample, three vials were analyzed.

Five-point calibration curves were prepared at each time point (i.e., months 0, 1, 2, 3, and 6) to assure linearity. Intermediate precision[33] was calculated from the data of the calibration curves of the reference standards, which showed relative SDs (RSDs) of less than 2.1% for the reference solution corresponding to 100% of the declared content (Supplemental Table S3).

Forced degradation study. Both the ICH and WHO guidelines mention forced degradation studies (sometimes referred to as stress testing); however, they do not fix specific conditions under which forced degradation studies should be performed. In the present study, the conditions described by Blessy et al.[30] in 2013 were used. The commercial preparations of oxytocin were investigated in their original glass vials, and the commercial preparation of oxytocin from Hexal with added chlorobutanol (5 and 1.5 mg/mL) was investigated in tightly sealed flasks. Furthermore, solutions prepared from solid synthetic oxytocin (Sigma-Aldrich/Merck, Taufkirchen, Germany; Batch N° 103H05241; purity ≥ 97% by HPLC), in a concentration of 10 IU/mL in either distilled water or 0.2 M sodium acetate buffer pH 4.6, both either with or without addition of chlorobutanol (1.5 or 5 mg/mL), were prepared and investigated. All samples were stored for 5 days at 80°C. After 24, 72, and 120 hours, one sample of each formulation was removed from the heat chamber and stored at 4°C until analysis. For each formulation, a sample stored at 4°C was used as control. Analysis was carried out as described previously.

Registration status of medicine brands. The PMPB of Malawi was contacted to inquire the registration status of the medicines collected in Malawi. For the preparations by Ningbo Pharma Biotech Co. Ltd., Ningbo, Zhejiang, China, Umedica Laboratories Pvt. Ltd., Mumbai, India, and Ciron Drugs & Pharmaceuticals Pvt. Ltd., Mumbai, India, it was confirmed that they were registered, but registration could not be confirmed for the product by Biologici Italia Laboratories S.r.l., Masate, Italy. The Rwanda Food and Drug Authority (RFDA) was contacted to inquire the registration status of the medicines collected in Rwanda. However, the RFDA's process of full registration of medicines had not yet been in effect at the time of sample collection. It could not be confirmed which of the collected preparations were preregistered according to the previous procedures.

The preparations of Biologici Italia Laboratorie S.r.l., of Rotexmedica GmbH, Trittau, Germany and of AS Grindeks, Riga, Latvia, were also registered in European countries, as was the preparation of Hexal AG, Holzkirchen, Germany. However, no oxytocin preparation by Laboratoires Sterop, Brussels, Belgium, was found in the medicine registers of the European Heads of Medicines Agencies (https://mri.cts-mrp.eu/Human/) and in the national medicines register of Belgium (https://banquededonneesmedicaments.afmps-fagg.be/#/query/human/), indicating that the Sterop preparations collected in Rwanda are manufactured for export only.

Statistical analysis. Statistical evaluation was carried out using JMP 14.2 (SAS GmbH, Heidelberg, Germany). Significance of differences between active pharmaceutical ingredient (API) content and pH values at different conditions and months were calculated using uni- and multivariate analysis of variance and Student's *t*-test.

Information of national authorities and stakeholders. This article was shared with the PMPB of Malawi, with the RFDA, and with the WHO Rapid Alert System. In addition, the results were presented to the PMPB, the Malawi Central Medical Stores Trust, the Ministry of Health and national and international stakeholders during a meeting in Lilongwe, Malawi, on September 4, 2019. First results of this study were also presented in a lecture by L. H. during a visit to the RFDA on December 4, 2018.

RESULTS

Overview of investigated oxytocin samples. Nine brands of oxytocin, that is, five brands in Malawi and four in Rwanda, were collected. Two of the purchased brands had to be excluded from the subsequent investigation: oxytocin inj. 5 IU/mL from MACIN Remedies Ltd., Moga, India, collected in Malawi, was not available in a sufficient amount for stability testing. Oxytocin inj. 10 IU/mL from Jiangxi Xierkangtai Pharmaceutical Co. Ltd., Pingxiang, Jiangxi, China, collected in Rwanda, showed a large peak of an undeclared substance (later identified as the commonly used preservative benzyl alcohol[34]) in HPLC analysis, which precluded precise quantitative analysis of oxytocin because of lack of baseline separation of the closely adjacent HPLC peaks of benzyl alcohol and oxytocin. Therefore, seven brands collected in Malawi and Rwanda were included into the subsequent stability testing. For two of these brands, two different batches were offered at the time of collection, and, in each case, both batches were collected and investigated. As a comparison, two further oxytocin preparations were purchased in Europe: one preparation which is commonly used in Germany, purchased through the pharmacy of the University Hospital Tübingen, and one of the two oxytocin preparations which had been prequalified by the WHO at the time of sample collection; the latter preparation was purchased from the manufacturer in Latvia via Imres B.V. because it was not registered in Germany. A different batch of the latter brand had also been purchased in Rwanda.

Therefore, as shown in Table 2, eight brands (total 11 batches) were included into the stability testing. Four of these brands had been produced in Belgium, Germany, or Italy, that is, in countries with an SRA.[22] As mentioned, one further brand was produced in Latvia and represented a WHO-prequalified product.[35] The other three brands were from India and China, that is, from countries without an SRA. Six of the eight brands were labeled for storage at 2–8°C, whereas two brands, produced in China and India, were labeled for storage "below 25°C" and "not exceeding 30°C," respectively (Table 2). The declared shelf life of the different products varied between 2 and 4 years, and all samples remained within their shelf life during the entire duration of the study. Pretesting for assay and pH showed that 10 of the 11 samples were clearly within USP 40 specifications at the beginning of the stability study. One sample (Ningbo batch no. 160802) showed an assay value of 89.0%. Given the RSD of this measurement (RSD =

TABLE 2
Investigated oxytocin samples

Collected in	Stated manufacturer (and brand name, if not marketed under INN)	Country of manufacture	Expiry date	Batch number	Stated shelf life (years)	Stated storage requirements (°C)	Stated (or detected) excipients								Prequalification status
							Water for inj.	Chlorobutanol (mg/mL)	Sodium acetate	Acetic acid	Sodium hydroxide	Sodium chloride	Citric acid	Others	
Malawi	Ningbo Pharma Biotech Co., Ltd. (WW-Oxy 10)	China	August 19	160802	3	Less than 25	+	–	–	–	–	–	–	+*	None
			January 19	160183											
	Umedica Laboratories Pvt. Ltd.	India	January 20	JA802	2	Not exceeding 30	+	1.5†	–	–	–	–	–	+*	None
	Ciron Drugs & Pharmaceuticals Pvt. Ltd.	India	August 19	7EA01228	2	2–8	+‡	5†	–	–	–	–	–	–	None
	Biologici Italia Laboratories S.r.l.	Italy	March 19	UF602ON	3§	2–8	+	–	+	+	+	–	–	–	SRA
Rwanda	Laboratoires STEROP	Belgium	August 19	160269	3	2–8	+	5	–	–	–	+	+	–	SRA
	Laboratoires STEROP (Steroxine 10 IU/1 mL)‖		January 19	160042											
	Rotexmedica GmbH Arzneimittelwerk	Germany	September 20	70779A	3	2–8	+	–	+	+	–	+	–	–	SRA
	AS Grindeks	Latvia	November 20	37711116	4	2–8	+	–	+	+	+	+	–	–	WHO-PQ
Europe	AS Grindeks		September 21	38910917											
	Hexal AG	Germany	June 20	HC0075	3¶	2–8	+	–	–	+	–	+	–	–	SRA

WHO-PQ = WHO-prequalified product; SRA = produced in a country with stringent regulatory authority. All samples represented 1 mL vials with a stated content of 10 IU oxytocin/mL.
* High-performance liquid chromatography (HPLC) analysis showed additional peaks, indicating additional, unidentified ingredients.
† Chlorobutanol not declared but detected in HPLC analysis.
‡ Water for inj. not explicitly declared.
§ Manufacturing date not stated on the packaging; information received from the websites of the British Medicines and Healthcare products Regulatory Agency (http://www.mhra.gov.uk) and the Irish Health Products Regulatory Authority (http://www.hpra.ie/).
‖ One branded and one unbranded generic oxytocin product of Laboratoires STEROP were found in Rwanda, with identical composition.
¶ Manufacturing date not stated on the packaging but received from the manufacturer.

1.41%; Supplemental Table S1), this deviation from the pharmacopoeial limits (90–110%) was not statistically significant ($P = 0.236$); therefore, this preparation was not classified as being out of specifications.

Table 2 also shows the stated (or detected) excipients in the investigated preparations. For optimal stability of oxytocin, USP 40 specifies that the pH value must be between 3.0 and 5.0. This is usually achieved by the inclusion of, for example, acetate or citrate buffers.[36–39] For the WHO-prequalified preparation, and for the four preparations produced in countries with an SRA, the presence of such buffering substances was correctly declared on the packaging. According to the label information, four of these five preparations also contained sodium chloride (usually included for tonicity). For the Sterop preparation, in addition, the presence of 5 mg/mL of the bacteriostatic agent chlorobutanol was declared.

In clear contrast, for the three preparations from China and India, no excipients were stated on their packaging beyond water for injection (for the Ciron preparation, not even water was stated). Nevertheless, their pH value was found to be in the correct range of 3.0–5.0, and remained so during stability testing, which may indicate the presence of undeclared buffering agents. Furthermore, HPLC analysis showed the (undeclared) presence of 1.5 and 5.0 mg/mL chlorobutanol in the preparations by Umedica and Ciron, respectively. Chlorobutanol can be readily detected in the HPLC analysis.

Accelerated stability studies of oxytocin. All eight brands (11 batches) listed in Table 2 were subjected to accelerated stability testing according to the current WHO guidelines for stability testing of finished pharmaceutical products.[22] Stability chambers used, operated by a commercial company specialized in pharmaceutical stability testing, also complied with the specifications for RH of the WHO guidelines, although this is not relevant for the investigated oxytocin preparations which were packaged as aqueous solutions in sealed glass vials which are considered to be moisture impermeable.[22]

The evaluation criteria for stability testing are described in full detail in the mentioned WHO guidelines,[22] but may be summarized as follows: pass/fail decisions of accelerated stability testing are made on the basis of two criteria: 1) for 6 months, the sample must remain within specification, that is, in the present study, oxytocin samples still had to show an API content between 90% and 110% of the declared amount (as specified by the USP, International Pharmacopeia, and British Pharmacopeia), and a pH value between 3.0 and 5.0; 2) no significant change of the API content must occur within 6 months, that is, no change of the initial content of the API by 5% or more.[22,28]

Obviously, the temperature conditions which the oxytocin preparations must be able to withstand in accelerated stability testing are related to the storage recommendations which the manufacturer states on the label[22]: products labeled for refrigerated storage (2–8°C) must demonstrate their stability for 6 months at 25°C or 30°C, and the decision for either of these two temperatures should be "based on a risk-based evaluation." As described in the following text, in the present study, results obtained at 25°C and at 30°C were similar. Products labeled as "do not store above 25°C," such as the oxytocin preparation by Ningbo (Table 2), must demonstrate their stability for 6 months at 30°C ("intermediate" condition, tested in case they failed at 40°C). And products labeled as "do not store above 30°C," such as the oxytocin preparation by Umedica (Table 2), must demonstrate their stability for 6 months at 40°C.

Figure 2 shows the oxytocin content of all eight brands listed in Table 2 after 6 months of storage at 5°C, 25°C, 30°C, or 40°C. Clearly, the WHO-prequalified preparation as well as the four preparations produced in countries with an SRA passed this stability test, showing oxytocin contents between 97.4% and 109.3% of the declared amount after 6 months at 30°C. Supplemental Table S1 lists the individual assay values determined for each preparation at each time interval (0, 1, 2, 3, and 6 months); none of the mentioned five brands showed a significant change of the API content over 6 months at 30°C; that is, all passed the stability testing also by this criterion. Supplemental Table S2 shows all pH values determined in this study. In all five mentioned preparations, the pH value remained well within specifications, that is, within the 3.0–5.0 range at 30°C.

For the preparation by Ciron, an API content of 89.3% of the declared amount was determined after 6 months at 30°C. Given the RSD of this measurement (RSD = 1.55%, Supplemental Table S1), this deviation from the pharmacopoeial limits (90–110%) is not statistically significant ($P = 0.459$); therefore, this preparation was not classified as failing stability testing. The change of the API content of this preparation, and its pH value, was within the permitted limits.

A different picture emerged for the preparations by Ningbo and Umedica, that is, for the two preparations in this study which were labeled by their manufacturers as not requiring refrigerated storage. As immediately obvious from Figure 2, their stability at 30°C and 40°C was not better but rather lower than that of the preparations labeled for refrigerated storage. The product by Umedica was labeled for storage "not exceeding 30°C" and, by WHO guidelines, therefore had to remain within specifications for 6 months at 40°C. It clearly failed this test, showing a final API content of only 78.0% of the declared amount, far outside of the 90–110% range specified by the pharmacopoeias. Furthermore, it showed a 21% loss of its API content over 6 months at 40°C, grossly exceeding the permitted 5% change. The preparation by Ningbo was labeled for storage "below 25°C." Clearly, both investigated batches failed accelerated stability testing at 40°C (final API content 69.5% and 74.6%, respectively; Figures 2 and 3 and Supplemental Figure S1) and, by WHO guidelines, therefore had to be tested at "intermediate conditions" of 30°C. After 6 months at 30°C, the API content of batch 160802 was found to be 86.5% of the declared amount and was therefore out of specification, even though the change of the API content was 3% and therefore within the permitted limit of 5%. Batch 160183 showed an API content of 90.7% after 6 months at 30°C and therefore remained in specifications.

The different stability of the investigated preparations under temperature stress conditions is clearly visible in Figure 3, which depicts the time course of the loss of the API content over 6 months at 40°C. This temperature is a more extreme condition than recommended for stability testing of preparations labeled for storage at 2–8°C; therefore, the data depicted in Figure 3 are not directly relevant for pass/fail decisions for most of the preparations, but are still interesting in terms of the stabilizing effect of certain excipients. The most striking observation from these data is that the highest stability of oxytocin was shown by the two products which contained 5 mg/mL of the bacteriostatic agent chlorobutanol. However, in clear contrast to the other investigated products, these two preparations showed marked changes of their pH values,

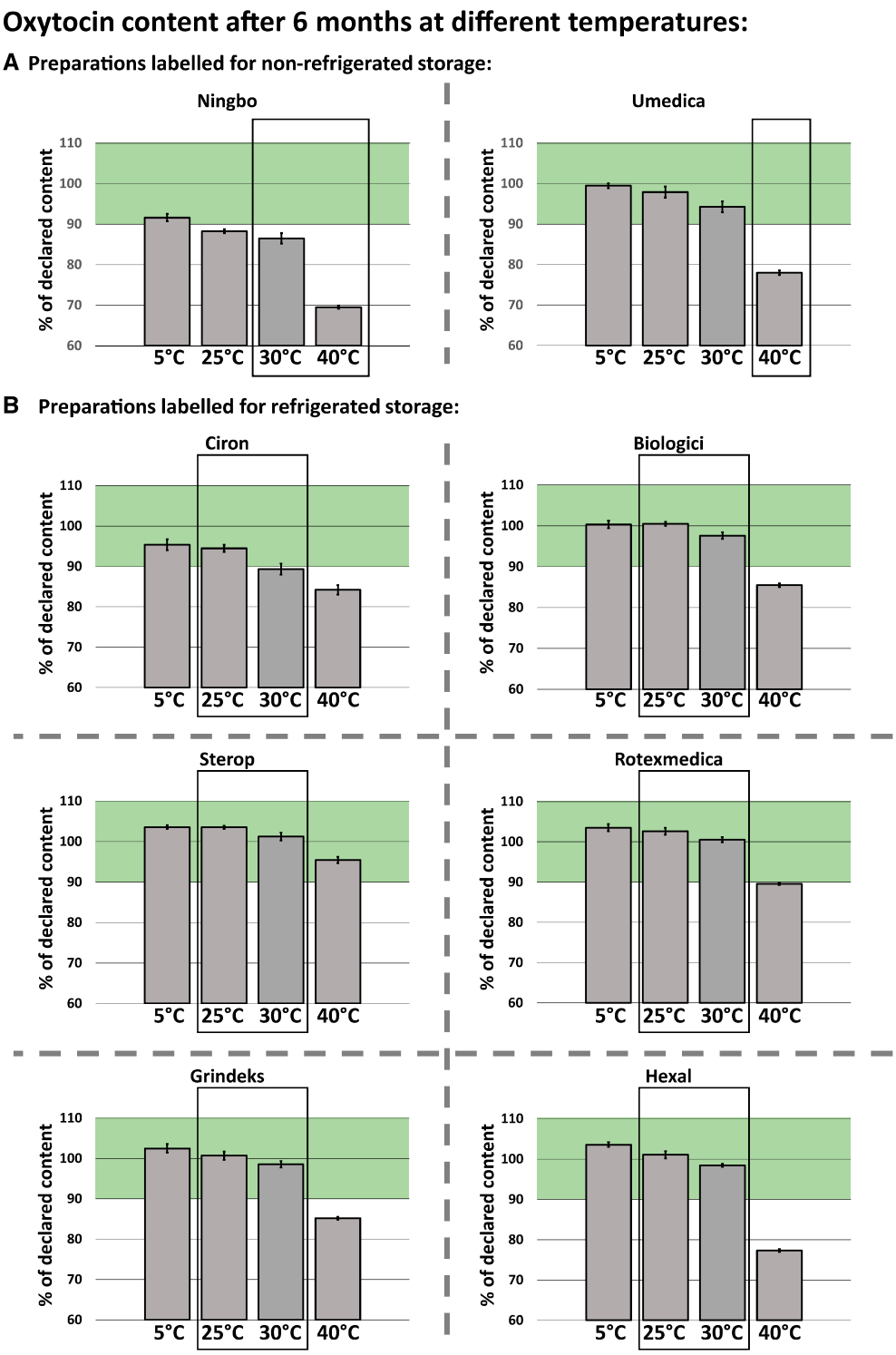

FIGURE 2. Accelerated stability testing of commercial oxytocin preparations: oxytocin content after 6 months at different temperatures. Full names of the manufacturers, and further details of the investigated preparations, are listed in Table 2. Results shown here were obtained with Ningbo batch N° 160802, Sterop batch N° 160269, and Grindeks batch N° 37711116 (see Table 2). The U.S. Pharmacopoeia specifies that the oxytocin content must be between 90% and 110% of the declared amount. This range is marked in the diagrams. Error bars show SD. This figure appears in color at www.ajtmh.org.

reaching a final value of 2.8 in case of the Sterop product and 3.0 in case of the Ciron product (Supplemental Table S2).

As mentioned earlier, for three of the investigated oxytocin brands, two different batches had been collected, with different manufacturing and expiry dates. Supplemental Figure S1 shows the results of the stability testing for these additional three batches, depicted in the same way as in Figures 2 and 3. Notably, API losses measured for the two

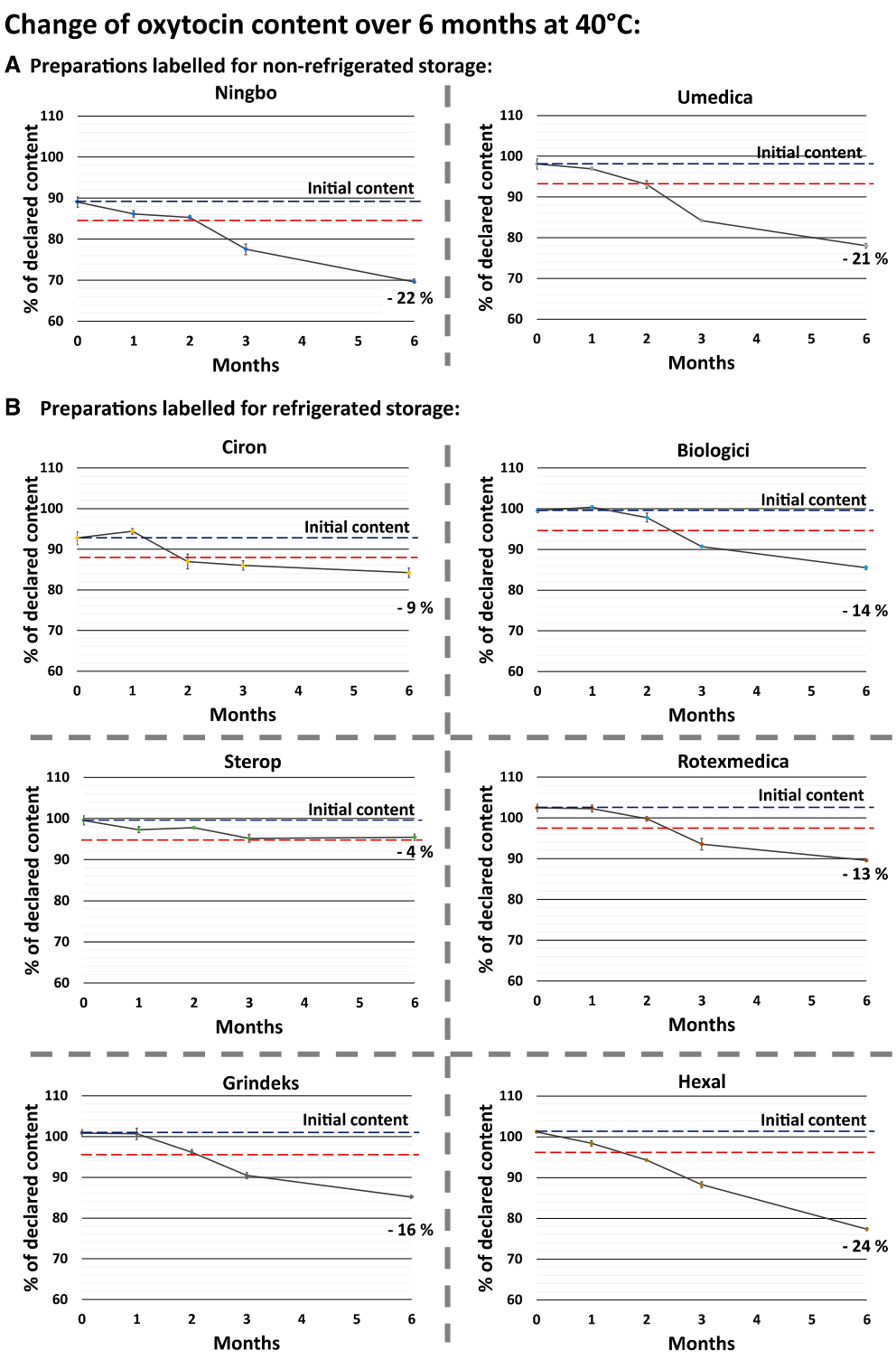

FIGURE 3. Accelerated stability testing of commercial oxytocin preparations: change of oxytocin content over 6 months at 40°C. Full names of the manufacturers, and further details of the preparations, are listed in Table 2. Results shown here were obtained with the same batches as shown in Fig. 2. In stability testing, a "significant change" of the API content is defined as a 5% change of the initial content.[22,28] Initial content and initial content minus 5% are shown as dashed lines. Error bars show SD. Final loss of content after 6 months is calculated relative to the initial content. This figure appears in color at www.ajtmh.org.

different batches of the same brand were very similar in all three cases, indicating that the observed differences in the stability of different brands were primarily because of differences in manufacturing and pharmaceutical formulation, not to differences in age or in pre-sampling storage conditions of the investigated products. Individual measurements for all investigated batches are shown in Supplemental Tables S1 and S2.

Thermal forced degradation studies of oxytocin. The markedly different stability of the investigated preparations in accelerated stability testing (Figures 2 and 3) stimulated our interest to additionally conduct a forced degradation study at higher temperature, that is, at 80°C for 5 days.[30] For two of the preparations (Umedica and Biologici; Table 2), the number of remaining vials was insufficient for this experiment, but for the other six brands, the results are shown in Figure 4A. In remarkable similarity to the results at 40°C (Figure 3), the four preparations by Rotexmedica, Grindeks, Ningbo, and Hexal showed decreasing stability in that order, with API losses between 55.9% and 67.5% within 5 days. However, in sharp contrast to the results obtained at 40°C, the highest losses in the API content after 5 days at 80°C were shown by the two preparations by Ciron and Sterop, that is, the preparations containing 5 mg/mL chlorobutanol (API losses of 75.2% and 82.4%, respectively). Conversely, after 1 day, at 80°C, these two preparations still had clearly higher API contents than the other four (Figure 4A).

Chlorobutanol is known to undergo hydrolysis under elevated temperature, forming hydrochloric acid and other acidic reaction products.[40] This is likely to explain the change of pH of the preparations containing 5 mg/mL chlorobutanol which had been observed already at 40°C (Supplemental Table S2). As may be expected, this effect was even more pronounced at 80°C, with both preparations showing a pH of 2.0 after 5 days, far out of the stability optimum of oxytocin at pH 4.5.[14]

To further investigate the effect of excipients on oxytocin stability, pure synthetic oxytocin in the solid form was purchased from Sigma-Aldrich/Merck and dissolved to a concentration of 10 IU/mL in water, with and without the addition of acetate buffer pH 4.6 (200 mM, i.e., as approximately isotonic solution) and/or chlorobutanol in concentrations of 1.5 mg/mL (8.5 mM) or 5 mg/mL (28 mM). These solutions were subjected to forced degradation at 80°C, and the result is shown in Figure 4B. In pure water, oxytocin degraded completely, falling below the limit of detection within 5 days. This degradation is much more rapid than that observed for any of the investigated commercial preparations (Figure 4A), and that is a further indication that all commercial preparations may have contained buffering or stabilizing agents, even if not declared on the label. Inclusion of acetate buffer had a stabilizing effect on oxytocin solution (83.1% API loss within 5 days). Notably, inclusion of 1.5 mg/mL chlorobutanol had an even stronger stabilizing effect: only 27.7% of the API amount was lost over 5 days at 80°C; the final pH value was determined as 2.8. Inclusion of chlorobutanol in a concentration of 5 mg/mL was less effective for stabilization (56.1% API loss), and, in this case, the final pH value was found to be 2.2, that is, more unfavorable for oxytocin stability.

The stabilizing effects of acetate buffer and chlorobutanol were not additive: in acetate buffer without chlorobutanol, oxytocin degraded with similar velocity as in acetate buffer with added chlorobutanol (1.5 or 5 mg/mL). In the latter experiments, the pH values of the solutions after 5 days at 80°C were 4.6 and 4.5, respectively, indicating that the buffer capacity of 200 mM acetate buffer pH 4.6 was sufficient to compensate for the effect of any acidic hydrolysis products of chlorobutanol.

In a final experiment, chlorobutanol was added to the commercial oxytocin preparation of Hexal, and stability was investigated for 5 days at 80°C. As shown in Figure 4A, addition of 1.5 mg/mL greatly increased the stability of oxytocin under these conditions (29.6% API loss, compared with 67.5% in the absence of chlorobutanol; final pH 2.8). The addition of 5 mg/mL chlorobutanol to the Hexal preparation was less effective for stabilization (API loss 68.4%; Supplemental Table S4), consistent with an observed drastic change of the pH value (final pH 2.0; Supplemental Table S5). These results are similar to the ones observed with the synthetic oxytocin from Sigma-Aldrich/Merck (Figure 4B).

The HPLC analyses of the samples taken during forced degradation studies showed not only a quantitative effect of chlorobutanol on the velocity of oxytocin degradation but also a striking qualitative effect (Figure 5). In the absence of chlorobutanol, concomitantly to the decrease in the oxytocin peak (retention time 8.2 minutes), several peaks of degradation products with higher retention time (15.1–19.3 minutes) appeared (Figure 5A). This is similar to the HPLC observations of Avanti et al.,[39] who identified these degradation products as

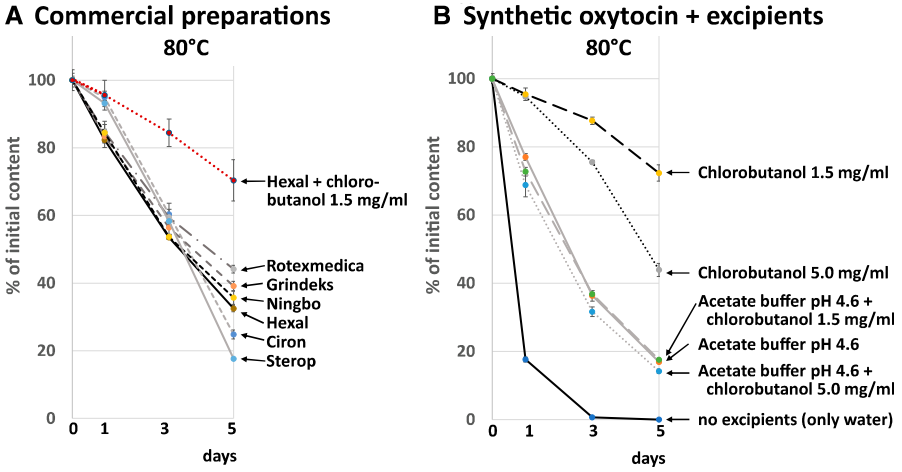

FIGURE 4. (**A**) Forced thermal degradation studies of commercial oxytocin formulations. Details of the investigated preparations are given in Table 2 and in Supplemental Tables S4 and S5. (**B**) Forced thermal degradation studies of solutions of oxytocin (Sigma-Aldrich/Merck; 10 IU/mL) in the presence of different excipients. This figure appears in color at www.ajtmh.org.

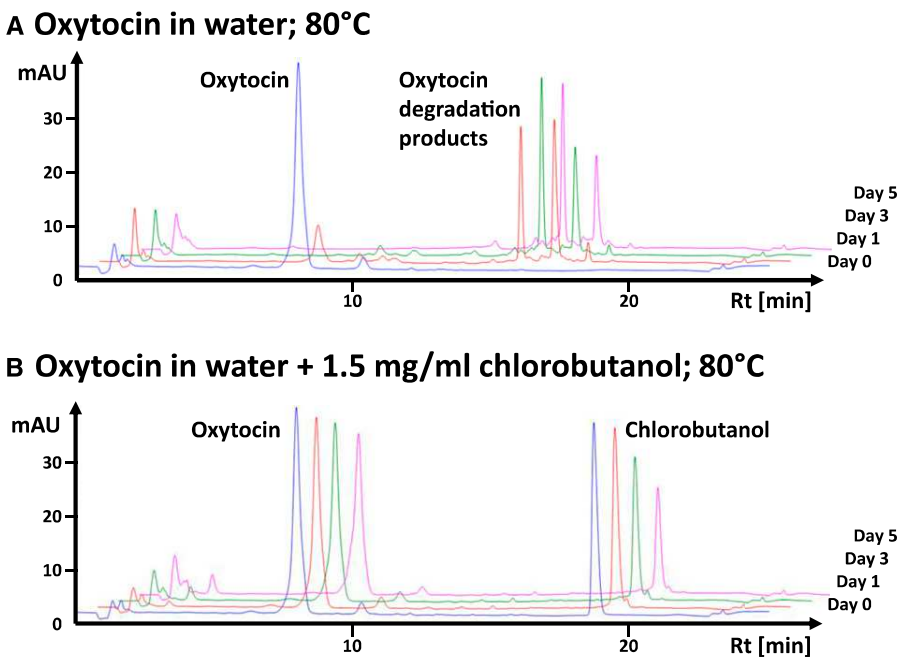

FIGURE 5. High-performance liquid chromatography chromatograms of oxytocin (Sigma-Aldrich/Merck; 10 IU/mL) in water under forced degradation at 80°C, in the absence and presence of chlorobutanol 1.5 mg/mL. This figure appears in color at www.ajtmh.org.

oxytocin trisulfides and tetrasulfides, and as various disulfide-linked oxytocin dimers, some of these showing deamidation of one or more of three amidated carboxyl groups of oxytocin (Figure 1). Notably, in the present study, these decomposition products were not observed in water containing 1.5 mg/mL chlorobutanol (Figure 5B), and the same observation was made for 5 mg/mL chlorobutanol, as well as for the preparations of Ciron and Sterop which each contained 5 mg/mL chlorobutanol. By contrast, all investigated solutions not containing chlorobutanol showed degradation products in the 15.1- to 19.3-minute range.

Neither in the accelerated stability studies nor in the forced degradation studies, visible quality defects such as particles, precipitations, or color changes were observed in any of the investigated products.

DISCUSSION

An encouraging result from the present study is that six of the eight investigated oxytocin brands passed the accelerated stability testing conducted according to the WHO/ICH.[22,28] This is even more encouraging as the products were not purchased ex-factory, but from the supply chains in Malawi and Rwanda (and in case of the Hexal product in Germany). Therefore, the extended storage before testing, possibly even at less than optimal storage conditions, had not affected the quality and stability of these products in regard to the tested criteria.

However, two other samples were found not to comply with specifications after accelerated stability testing, and these were the two brands labeled for non-refrigerated storage. The Umedica product carried a storage recommendation "not exceeding 30°C" and therefore had to be tested at 40°C. It clearly failed at this temperature (Figures 2 and 3), and only complied with the stability testing at 30°C, which is appropriate for products labeled for refrigerated storage. By contrast, batch 160802 of the Ningbo product even failed assay testing after 6 months at 30°C, and at 40°C, it showed the poorest result of all eight tested brands. The initial API content (at month 0) of this Ningbo batch was 89.0% of the declared amount. Although it may be argued that the low initial content could have been caused by inappropriate storage before sample collection, it is noteworthy that also two oxytocin samples stating Ningbo as the manufacturer name had been investigated in a survey conducted by the WHO, and both samples failed assay testing because of the insufficient API content.[11] In the present study, a second batch of Ningbo showed an initial API content of 94.9% of the stated amount. It also failed testing at 40°C, although it passed the test at 30°C.

While this study was in progress, Nguyen et al.[17] published an investigation of the stability of five oxytocin products purchased ex-factory. Three of these products, labeled for storage at 2–8°C, have also been investigated in the present study, that is, the products by Grindeks, Biologici, and Rotexmedica. The results obtained by Nguyen et al. after 1, 2, and 3 months of storage at 30°C and 40°C are in excellent agreement with those of our study. Nguyen et al. did not test for the full period of 6 months; therefore, results at that time point cannot be compared, but there is no evidence pointing at any relevant differences. Nguyen et al. also investigated one European oxytocin brand labeled for storage at ≤ 25°C, as well as one Argentinian product which had formerly been labeled for storage at ≤ 25°C but had been relabeled for refrigerated storage just before their study. They reported that, at 30°C and 40°C, these two preparations exhibited very similar stability profiles as the three products labeled for refrigerated storage. Only after 4 months at 40°C, and especially in a forced degradation study at 50°C, the European product labeled for

storage at ≤ 25°C showed much higher API losses than the others, concomitant with a sharp decrease in the pH value. That preparation contained 5 mg/mL chlorobutanol. The authors concluded that, of the products tested, those designated for storage at ≤ 25°C provided no stability benefit over those labeled for refrigerated storage.

The present study investigated six oxytocin brands labeled for storage at 2–8°C, as well as two brands labeled for storage at ≤ 25°C and at ≤ 30°C. The two latter products had been manufactured in China and India, respectively. The data of the present study are therefore complementary to the data provided by Nguyen et al. in regard to the range of tested products. Our study results give further support to the conclusion that products designated for storage at ≤ 25°C do not provide stability benefits over those labeled for refrigerated storage. In addition, our study shows that some of the former products may even fail the relevant WHO/ICH specifications for the stability of finished pharmaceutical products.

Five of the eight oxytocin brands investigated in this study passed stability testing with excellent results, that is, with final API contents not more than 2.6% below the amount stated on the label after 6 months at 30°C (Supplemental Table S1). Notably, all these five brands were WHO prequalified and/or produced in countries with SRAs. By contrast, of the three preparations produced in India and China, that is, in countries without SRAs, two showed noncompliance with pharmacopoeial specifications after accelerated stability testing, and one passed testing with just borderline results. These poor stability outcomes must not be generalized to all manufacturers and medicines from non-SRA countries. There are certainly manufacturers (usually larger companies), for example, in India and China, whose products are as good as generic medicines produced in countries with SRAs.[41] However, there are also companies (usually smaller ones) in these two countries whose products show a large rate of substandard medicines.[42] It should be both an ethical and an economical interest of the authorities in India and China to address and eliminate this problem.

For the brands which were WHO prequalified and/or produced in countries with SRAs, excipients were correctly declared. By contrast, for the three preparations without WHO prequalification and produced in countries without SRAs, the excipients were not declared correctly, which is a violation of registration requirements in most countries.

The results of the present study strongly support the maxim "Buy Quality Oxytocin, Keep It Cool" which is advocated by the Reproductive Health Supplies Coalition and other international stakeholders.[21,43–46] Given the lack of consistent evidence that products labeled for non-refrigerated storage show better temperature stability than those labeled for storage at 2–8°C, and furthermore given the clear evidence that manufacturers' storage recommendations of "below 25°C" and even of "below 30°C" cannot be reliably complied with in many facilities in LMICs,[13] procurement of oxytocin injections should be limited to brands labeled for refrigerated storage, and national medicines regulatory agencies should consider to only register oxytocin products with this storage recommendation.[43,44] This will also help to avoid confusion of the personnel in supply chains and health facilities by different storage recommendations for different oxytocin brands.[13,18,47,48] All efforts should be made to maintain the cold chain for oxytocin from the manufacturer up to the patient. The WHO/UNICEF recommendation to allow storage of oxytocin in the ubiquitous cold chain facilities of the Expanded Program on Immunization should be implemented as widely as possible for this purpose.[49]

Given the current shortcomings of the cold chain facilities in LMICs, however, it may still not be possible to avoid short-term exposures of oxytocin to ambient temperatures. In this context, the data depicted in Figure 2 may be of interest, showing that all products labeled for storage at 2–8°C were remarkably stable at 25°C in our stability study. Even at 30°C, only very moderate losses of the API content were observed over the investigated period. This indicates that accidental storage of such oxytocin preparations outside of the cold chain for limited time periods may not necessitate the immediate disposal of the products, and this is consistent with the information given, for example, in the leaflet of the Grindeks product (Table 2), stating that it "may be stored up to 30°C for 3 months, but must then be discarded."

According to Hawe et al.,[14] degradation of oxytocin in a 10-IU/mL solution of pH 4.5 has an activation energy of 128 ± 3.8 kJ/mol and follows (pseudo-) first-order kinetics. Using the Arrhenius equation,[14] we calculated that a temperature increase just from 25°C to 30°C would increase the degradation reaction rate by a factor of 2.3. Thakral et al.[21] extrapolated rate constants for oxytocin degradation at 25–40°C from experimentally obtained rate constants at higher temperatures, under the assumption that the mechanism of reaction remains unchanged. Thereby, they calculated the time required for a 10% loss of oxytocin content at temperatures of 25°C, 30°C, and 40°C as 183 days, 82 days, and 17 days, respectively, at pH 4.5. Figures 2 and 3 of the present study clearly show that the degradation rates observed experimentally at these temperatures for commercial oxytocin products were much lower, indicating different reaction mechanisms at lower temperatures and/or the presence of stabilizing agents.

Attempts to devise more heat-stable formulations of oxytocin have been described extensively in the literature,[14,36–39,50] mostly focusing on the inclusion of certain buffers and divalent metal ions. However, to the best of our knowledge, the striking effect of chlorobutanol, a bacteriostatic agent widely used as a preservative in pharmaceuticals,[40] both on the velocity on oxytocin degradation (Figure 4) and on the type of degradation products formed (Figure 5) has not been described in the literature in any detail up to now. This stabilizing effect has only been very briefly mentioned in an abstract by Liu et al.,[51] and a molecular dynamics computer simulation by Xu et al.[52,53] suggested that chlorobutanol can reduce the number of hydrogen bonds between oxytocin and water and prevent oxytocin molecules from aggregating. Evidence has been presented that divalent metal ions may lead to a change of the conformation of oxytocin in certain buffers, thereby shielding the intramolecular disulfide bond (Figure 1) and diminishing degradation reactions which involve that site of the molecule.[36–39] It is tempting to speculate that chlorobutanol may have a similar effect on oxytocin. Possibly, 200 mM acetate buffer may prevent this conformational change, explaining the lack of a stabilizing effect of chlorobutanol observed in this buffer (Figure 4B).

Chlorobutanol itself degrades at higher temperatures, as clearly visible in Figure 5 which shows 47.5% degradation of chlorobutanol in 5 days at 80°C under the used conditions.

The degradation leads to acidic degradation products[40] and lowers the pH value, which then may strongly deviate from the oxytocin stability optimum (pH 4.5), especially when a high concentration of chlorobutanol (i.e., 5 mg/mL) is used in weakly buffered solutions. The detrimental effect of the progressive lowering of the pH value would then compete with the stabilizing effect of chlorobutanol on oxytocin, and this may offer a plausible explanation for the observation depicted in Figure 4A (Ciron and Sterop products) that chlorobutanol 5 mg/mL first increased and later on lowered oxytocin stability at 80°C. The lowering of the pH and the resulting decrease in oxytocin stability was also observed in the forced degradation experiment by Nguyen et al.,[17] similar to our results. This effect may, however, not be relevant at actual storage temperatures of oxytocin because the hydrolysis of chlorobutanol only occurs at higher temperatures.

It may well be worthwhile to investigate the mechanism of the stabilization of oxytocin by chlorobutanol using appropriate methods, especially liquid chromatography-mass spectrometry (LC-MS) analysis for the identification of the degradation products[39] and nuclear magnetic resonance (NMR) spectroscopy to investigate conformational changes of oxytocin.[36] However, such investigations exceed the scope of the present study.

A heat-stable formulation of carbetocin,[54] an oxytocin analogue which is already widely used for the prevention of PPH after caesarean delivery, has very recently been added to the WHO Essential Medicines List for use in PPH.[1] If the announcement of its manufacturer is put into practice that the price of this product will be made comparable to that of oxytocin in LMICs,[55] then this medicine may become a further valuable option to ensure good-quality medication against PPH also in facilities where storage at 2–8°C cannot be ensured.

Limitations of this study. As mentioned earlier, in this study, oxytocin samples were investigated which had been purchased from wholesalers and medical stores in Malawi and Rwanda, that is, preparations which reflect the quality and stability of medicines circulating in these countries. It has to be considered that these samples had already been stored for extended periods after manufacturing, and that the conditions of this storage are not known. Samples were not purchased from retail outlets to minimize the influence of varying conditions during prior storage on the study results. Although the observed differences in the stability of the investigated samples are valid and important, the data provided here do not provide definitive proof that certain products did not comply with their stability requirements already at the time of manufacture.

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Authors' addresses: Nhomsai Hagen and Lutz Heide, Pharmaceutical Institute, Eberhard Karls University Tübingen, Tübingen, Germany, E-mails: nhomsai.hagen@uni-tuebingen.de and heide@uni-tuebingen.de. Thomas Bizimana, Department of Pharmacy, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda, E-mail: bizimanathomas@gmail.com. P. Claver Kayumba, East African Community Regional Centre of Excellence for Vaccines, Immunizations and Health Supply Chain Management (EAC RCE-VIHSCM), University of Rwanda, Kigali, Rwanda, E-mail: pclaver.kayumba@gmail.com. Felix Khuluza, Pharmacy Department, College of Medicine, University of Malawi, Blantyre, Malawi, E-mail: fkhuluza@medcol.mw.

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SUPPLEMENTARY INFORMATION

Figure S1: Accelerated stability testing of commercial oxytocin preparations: results of additional batches

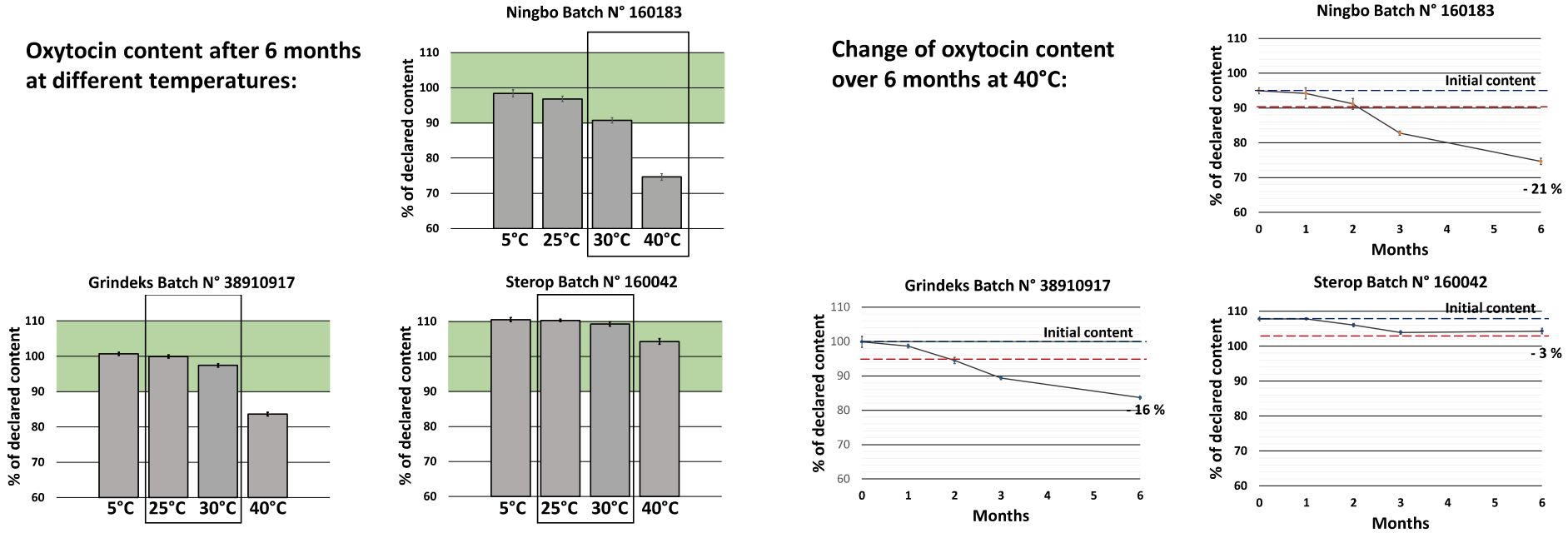

Table S1: Accelerated stability testing of commercial oxytocin preparations: change of oxytocin content over 6 months at different temperatures

		Oxytocin mean content (% of declared content)									
Condition	**Sample**	**Month 0**	**RSD**	**Month 1**	**RSD**	**Month 2**	**RSD**	**Month 3**	**RSD**	**Month 6**	**RSD**
Accelerated Ambient ICH 40 +/- 2° 75% RH +/- 5°	**Ningbo 160802**	89.0	1.41%	86.1	0.90%	85.3	0.48%	77.5	1.65%	69.5	1.05%
	Ningbo 160183	94.9	0.87%	94.2	1.69%	91.2	1.74%	82.8	0.76%	74.6	1.23%
	Umedica	98.1	1.29%	96.9	0.28%	93.0	1.06%	84.2	0.30%	78.0	0.70%
	Ciron	92.7	1.69%	94.4	0.70%	86.9	2.10%	86.0	1.34%	84.2	1.38%
	Biologici	99.5	0.53%	100.4	0.22%	97.8	1.15%	90.7	0.26%	85.5	0.54%
	Sterop 160269	99.6	1.11%	97.3	0.77%	97.8	0.19%	95.2	1.01%	95.4	0.80%
	Sterop 160042	107.8	0.62%	107.8	0.12%	106.1	0.45%	103.9	0.52%	104.3	0.77%
	Rotexmedica	102.4	0.89%	102.3	0.80%	99.8	0.53%	93.5	1.55%	89.6	0.29%
	AS Grindeks 37711116	100.9	0.82%	100.7	1.37%	96.1	0.66%	90.4	0.83%	85.2	0.42%
	AS Grindeks 38910917	99.9	1.65%	98.7	0.54%	94.5	0.99%	89.3	0.47%	83.6	0.59%
	Hexal	101.2	0.15%	98.4	0.69%	94.2	0.05%	88.2	0.82%	77.3	0.46%
Zone IVa; Accelerated Refrigerated ICH more severe conditions 30° +/- 2° 65% RH +/- 5%	**Ningbo 160802**	89.0	1.41%	90.3	0.87%	87.5	0.43%	86.1	0.64%	86.5	0.60%
	Ningbo 160183	94.9	0.87%	97.5	1.29%	98.0	1.78%	93.6	1.23%	90.7	0.80%
	Umedica	98.1	1.29%	100.0	0.47%	99.1	0.22%	93.1	0.39%	94.3	1.45%
	Ciron	92.7	1.69%	94.1	0.52%	92.5	1.70%	92.5	0.63%	89.3	1.55%
	Biologici	99.5	0.53%	102.4	0.38%	102.2	0.96%	95.8	0.52%	97.6	0.81%
	Sterop 160269	99.6	1.11%	99.6	0.11%	98.8	0.03%	97.0	0.21%	101.3	0.95%
	Sterop 160042	107.8	0.62%	108.7	0.32%	108.0	0.48%	104.9	0.08%	109.3	0.55%
	Rotexmedica	102.4	0.89%	104.2	0.16%	103.8	0.74%	99.6	0.45%	100.5	0.64%
	AS Grindeks 37711116	100.9	0.82%	101.9	0.57%	101.4	0.65%	97.8	0.89%	98.6	0.79%
	AS Grindeks 38910917	99.9	1.65%	101.0	0.54%	99.3	0.47%	95.6	0.46%	97.4	0.48%
	Hexal	101.2	0.15%	101.2	0.70%	100.5	0.40%	98.3	0.82%	98.4	0.38%

Zone II; Accelerated Refrigerated ICH 25° +/- 2° 60% RH +/- 5%	**Ningbo 160802**	89.0	1.41%	88.4	0.37%	90.2	1.06%	86.0	1.52%	88.2	0.44%
	Ningbo 160183	94.9	0.87%	100.2	1.35%	97.7	1.47%	96.9	0.75%	96.8	0.84%
	Umedica	98.1	1.29%	101.1	0.37%	100.3	0.37%	95.1	0.44%	97.9	1.40%
	Ciron	92.7	1.69%	94.3	1.63%	95.1	1.17%	92.8	1.20%	94.5	0.93%
	Biologici	99.5	0.53%	102.7	0.46%	102.8	0.96%	98.0	0.20%	100.5	0.48%
	Sterop 160269	99.6	1.11%	100.0	0.37%	98.5	0.33%	97.4	0.86%	103.5	0.35%
	Sterop 160042	107.8	0.62%	107.4	0.28%	108.1	0.39%	105.4	0.33%	110.3	0.32%
	Rotexmedica	102.4	0.89%	104.4	0.56%	104.0	0.69%	100.4	0.98%	102.6	0.83%
	AS Grindeks 37711116	100.9	0.82%	102.3	0.27%	102.0	0.93%	98.7	1.19%	100.7	0.99%
	AS Grindeks 38910917	99.9	1.65%	101.1	0.30%	100.0	1.24%	96.6	0.85%	99.9	0.44%
	Hexal	101.2	0.15%	101.7	0.87%	101.3	0.35%	99.5	0.90%	101.1	0.90%

Control long term testing conditions, refrigerated 5° +/- 3°	**Ningbo 160802**	89.0	1.41%	88.8	0.17%	90.9	1.12%	90.2	0.10%	91.6	1.45%
	Ningbo 160183	94.9	0.87%	97.4	0.08%	97.5	1.50%	96.0	1.67%	98.4	1.06%
	Umedica	98.1	1.29%	100.5	0.63%	100.7	0.39%	95.7	0.92%	99.5	0.65%
	Ciron	92.7	1.69%	97.5	0.74%	94.6	1.66%	94.4	0.40%	95.3	1.44%
	Biologici	99.5	0.53%	102.3	0.76%	103.6	0.16%	97.4	1.29%	100.3	0.91%
	Sterop 160269	99.6	1.11%	100.0	0.25%	99.4	0.42%	98.1	0.48%	103.6	0.46%
	Sterop 160042	107.8	0.62%	109.1	0.20%	108.5	0.29%	105.6	0.48%	110.5	0.53%
	Rotexmedica	102.4	0.89%	104.7	0.46%	105.5	0.83%	100.8	0.19%	103.5	0.87%
	AS Grindeks 37711116	100.9	0.82%	102.3	0.55%	103.4	0.75%	99.7	0.77%	102.5	1.06%
	AS Grindeks 38910917	99.9	1.65%	101.1	0.39%	101.6	0.45%	99.2	1.02%	100.7	0.48%
	Hexal	101.2	0.15%	101.9	0.92%	102.4	0.67%	100.4	0.55%	103.6	0.55%

RSD: relative standard deviation. ICH: International Conference on Harmonization. RH: relative humidity. Oxytocin content calculated as the mean of the results of three individual vials per sample.

Table S2: Accelerated stability testing of commercial oxytocin preparations: change of pH value over 6 months at different temperatures

		Oxytocin mean pH-values									
Condition	**Sample**	**Month 0**	**RSD**	**Month 1**	**RSD**	**Month 2**	**RSD**	**Month 3**	**RSD**	**Month 6**	**RSD**
Accelerated Ambient ICH 40 +/- 2° 75% RH +/- 5°	**Ningbo 160802**	3.9	0.63%	3.9	1.33%	4.3	1.28%	3.9	0.82%	4.2	1.04%
	Ningbo 160183	4.1	0.88%	4.0	0.37%	4.4	1.10%	4.0	0.65%	4.3	0.57%
	Umedica	4.2	1.24%	4.1	0.40%	4.5	1.62%	4.1	0.30%	4.3	0.74%
	Ciron	4.5	2.38%	3.5	1.69%	3.7	2.94%	3.1	1.16%	3.0	2.18%
	Biologici	3.8	0.95%	3.8	0.46%	4.1	2.29%	3.8	0.99%	4.0	0.45%
	Sterop 160269	4.0	0.57%	3.3	1.19%	3.5	2.82%	3.0	1.49%	2.8	1.35%
	Sterop 160042	3.8	0.22%	3.3	1.02%	3.5	2.37%	2.9	1.28%	2.8	1.45%
	Rotexmedica	4.0	0.47%	3.9	0.47%	4.3	1.90%	3.9	0.31%	4.1	0.30%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.44%	4.4	1.93%	4.0	0.32%	4.2	0.32%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.33%	4.4	1.69%	4.0	0.29%	4.2	0.33%
	Hexal	4.2	0.13%	4.1	1.20%	4.6	1.75%	4.3	0.46%	4.5	1.48%
Zone IVa; Accelerated Refrigerated ICH more severe conditions 30° +/- 2° 65% RH +/- 5%	**Ningbo 160802**	3.9	0.63%	3.9	0.77%	4.3	1.58%	3.9	1.15%	4.1	0.50%
	Ningbo 160183	4.1	0.88%	4.0	0.62%	4.4	1.42%	4.0	0.52%	4.2	0.38%
	Umedica	4.2	1.24%	4.1	0.43%	4.5	1.46%	4.1	1.79%	4.3	0.32%
	Ciron	4.5	2.38%	4.0	0.89%	4.4	1.74%	3.9	1.38%	3.8	1.45%
	Biologici	3.8	0.95%	3.8	0.47%	4.2	1.52%	3.8	0.57%	4.0	0.38%
	Sterop 160269	4.0	0.57%	3.7	0.79%	4.0	1.58%	3.6	0.63%	3.5	0.67%
	Sterop 160042	3.8	0.22%	3.6	0.79%	3.9	1.50%	3.4	0.75%	3.5	0.76%
	Rotexmedica	4.0	0.47%	3.9	0.85%	4.3	1.40%	3.9	0.44%	4.1	0.36%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.53%	4.4	1.24%	4.0	0.37%	4.2	0.28%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.56%	4.4	1.22%	4.0	0.41%	4.2	0.29%
	Hexal	4.2	0.13%	4.1	1.11%	4.6	1.39%	4.2	0.36%	4.4	0.69%

Zone II; Accelerated Refrigerated ICH 25° +/ 2° 60% RH +/ 5%	**Ningbo 160802**	3.9	0.63%	3.9	0.54%	4.3	1.28%	3.9	1.03%	4.1	0.43%
	Ningbo 160183	4.1	0.88%	4.0	0.66%	4.4	1.95%	4.0	0.56%	4.2	0.85%
	Umedica	4.2	1.24%	4.2	1.67%	4.5	1.61%	4.1	0.47%	4.3	0.60%
	Ciron	4.5	2.38%	4.2	3.03%	4.5	2.22%	4.1	0.53%	4.3	5.76%
	Biologici	3.8	0.95%	3.8	0.74%	4.2	2.07%	3.8	0.49%	4.0	0.57%
	Sterop 160269	4.0	0.57%	3.8	0.80%	4.2	1.24%	3.8	0.55%	3.8	0.65%
	Sterop 160042	3.8	0.22%	3.6	1.11%	4.0	1.55%	3.6	0.87%	3.7	0.58%
	Rotexmedica	4.0	0.47%	3.9	0.70%	4.3	1.54%	3.9	0.25%	4.1	0.30%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.48%	4.4	1.57%	4.0	0.26%	4.2	0.67%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.46%	4.4	1.49%	4.0	0.26%	4.2	0.29%
	Hexal	4.2	0.13%	4.0	1.70%	4.6	1.59%	4.1	0.58%	4.4	0.40%
Control long term testing conditions, refrigerated 5° +/ 3°	**Ningbo 160802**	3.9	0.63%	3.9	1.10%	4.3	1.99%	3.9	1.52%	4.1	0.81%
	Ningbo 160183	4.1	0.88%	4.1	0.36%	4.4	1.13%	4.0	0.54%	4.2	0.74%
	Umedica	4.2	1.24%	4.1	0.71%	4.5	1.09%	4.1	0.33%	4.3	0.34%
	Ciron	4.5	2.38%	4.2	1.40%	4.8	3.84%	4.2	0.24%	4.4	1.28%
	Biologici	3.8	0.95%	3.8	0.47%	4.2	1.73%	3.8	0.57%	4.0	0.55%
	Sterop 160269	4.0	0.57%	3.8	1.01%	4.3	1.49%	3.9	0.88%	4.0	0.44%
	Sterop 160042	3.8	0.22%	3.7	0.40%	4.1	1.76%	3.7	0.66%	3.9	0.56%
	Rotexmedica	4.0	0.47%	3.9	0.52%	4.3	1.64%	3.9	0.26%	4.1	0.39%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.37%	4.4	1.42%	4.0	0.46%	4.2	0.42%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.42%	4.5	1.64%	4.0	0.41%	4.2	0.43%
	Hexal	4.2	0.13%	4.1	0.24%	4.5	1.60%	4.1	0.59%	4.3	0.59%

RSD: relative standard deviation. ICH: International Conference on Harmonization. RH: relative humidity. pH values calculated as the mean of the results of three individual vials per sample, each vial tested twice, yielding 6 measurements per sample.

Table S3: Determination of intermediate precision[1] of oxytocin assay, using data from five-point calibration curves

	Mean AUC (mAU*s) Month 0	*RSD*	Mean AUC (mAU*s) Month 1	*RSD*	Mean AUC (mAU*s) Month 2	*RSD*	Mean AUC (mAU*s) Month 3	*RSD*	Mean AUC (mAU*s) Month 6	*RSD*	**Mean AUC (mAU*s) all months**	**RSD**
USP Reference 11.5 IU/ml	795.37	*1.25%*	809.72	*0.09%*	818.59	*0.43%*	807.35	*0.23%*	770.90	*0.62%*	**800.39**	**2.31%**
USP Reference 10 IU/ml	697.92	*1.31%*	699.32	*0.24%*	708.83	*0.37%*	699.52	*0.29%*	670.62	*0.79%*	**695.24**	**2.08%**
USP Reference 7 IU/ml	478.30	*0.67%*	483.76	*0.86%*	495.49	*0.08%*	485.75	*1.02%*	468.38	*0.87%*	**482.33**	**2.07%**
USP Reference 5 IU/ml	333.61	*1.52%*	342.19	*0.28%*	344.07	*0.24%*	344.91	*1.73%*	322.33	*1.19%*	**337.42**	**2.83%**
USP Reference 2 IU/ml	123.83	*3.24%*	131.64	*0.92%*	130.95	*1.01%*	140.46	*2.71%*	120.89	*1.10%*	**129.56**	**5.89%**

AUC: area under the curve. RSD: relative standard deviation. IU: international units. Each mean AUC value was calculated from on three HPLC measurements, but each mean AUC value of USP Reference 10 IU/ml from five HPLC measurements.

[1]**Reference:** ICH, 2005. Validation of analytical procedures: Text and methodology Q2(R1). Available from: https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf.

Table S4: Forced thermal degradation studies of solutions of oxytocin (Sigma-Aldrich/Merck; 10 IU/ml) in the presence of different excipients, and of commercial oxytocin formulations: change of oxytocin content over 5 days at 80°C

	Oxytocin content (% of initial content)							
	Day 0	***RSD***	**Day 1**	***RSD***	**Day 3**	***RSD***	**Day 5**	***RSD***
10 IU/ml synthetic oxytocin in destilled water	100.0	*0.44%*	17.6	*4.00%*	0.7	*16.98%*	0.0	*0.00%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6	100.0	*0.04%*	77.0	*1.25%*	36.3	*4.21%*	16.9	*1.29%*
10 IU/ml synthetic oxytocin in destilled water containing 5mg/ml chlorobutanol	100.0	*0.00%*	94.7	*0.34%*	75.5	*0.72%*	43.9	*4.36%*
10 IU/ml synthetic oxytocin in destilled water containing 1.5mg/ml chlorobutanol	100.0	*0.75%*	95.4	*1.89%*	87.6	*1.27%*	72.3	*3.34%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 5mg/ml chlorobutanol	100.0	*0.36%*	68.7	*4.93%*	31.6	*4.45%*	14.2	*4.90%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 1.5mg/ml chlorobutanol	100.0	*1.46%*	72.7	*1.75%*	36.7	*1.47%*	17.5	*2.79%*
Hexal (batch HWZ7694[a])	100.0	*0.04%*	82.3	*2.72%*	53.5	*0.98%*	32.5	*2.86%*
Hexal (batch HWZ7694 [a]) containing 5mg/ml chlorobutanol	100.0	*0.76%*	93.9	*2.23%*	64.8	*6.51%*	31.6	*11.46%*
Hexal (batch HWZ7694 [a]) containing 1.5mg/ml chlorobutanol	100.0	*2.01%*	95.6	*4.57%*	84.4	*4.83%*	70.4	*8.68%*
Ningbo 160183	100.0	*3.07%*	84.5	*2.91%*	53.7	*1.37%*	35.7	*5.51%*
Ciron	100.0	*0.16%*	94.9	*1.89%*	60.3	*5.32%*	24.8	*5.19%*
Sterop 160269	100.0	*0.70%*	93.1	*0.97%*	58.3	*0.31%*	17.6	*1.91%*
Rotexmedica	100.0	*0.68%*	84.7	*3.70%*	59.4	*4.10%*	44.1	*2.58%*
AS Grindeks 38910917	100.0	*1.22%*	83.4	*2.45%*	56.4	*3.67%*	39.1	*3.47%*

IU: international unit. RSD relative standard deviation.

[a] Batch HWZ7694 had identical packaging information as batch HC0075 listed in Table 2, but expiry date June 2021.

Table S5: Forced thermal degradation studies of solutions of oxytocin (Sigma-Aldrich/Merck; 10 IU/ml) in the presence of different excipients, and of commercial oxytocin formulations: change of pH values over 5 days at 80°C

	pH values							
	Day 0	***RSD***	**Day 1**	***RSD***	**Day 3**	***RSD***	**Day 5**	***RSD***
10 IU/ml synthetic oxytocin in destilled water	NA	*NA*	NA	*NA*	NA	*NA*	NA	*NA*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6	4.7	*0.15%*	NA	*NA*	NA	*NA*	NA	*NA*
10 IU/ml synthetic oxytocin in destilled water containing 5mg/ml chlorobutanol	5.6	*2.65%*	2.9	*0.24%*	2.4	*0.30%*	2.2	*0.65%*
10 IU/ml synthetic oxytocin in destilled water containing 1.5mg/ml chlorobutanol	6.4	*2.33%*	3.9	*0.18%*	3.1	*0.23%*	2.8	*2.00%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 5mg/ml chlorobutanol	4.6	*0.00%*	4.7	*0.30%*	4.5	*0.16%*	4.5	*0.16%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 1.5mg/ml chlorobutanol	4.7	*0.60%*	4.6	*0.15%*	4.6	*0.15%*	4.6	*0.30%*
Hexal (batch HWZ7694 [a])	4.2	*0.13%*	4.2	*0.50%*	4.6	*0.15%*	4.3	*0.16%*
Hexal (batch HWZ7694 [a]) containing 5mg/ml chlorobutanol	4.6	*0.76%*	NA	*NA*	2.3	*0.31%*	2.0	*0.35%*
Hexal (batch HWZ7694 [a]) containing 1.5mg/ml chlorobutanol	4.6	*0.77%*	3.8	*0.75%*	2.9	*0.24%*	2.8	*0.25%*
Ningbo 160183	4.1	*0.88%*	4.0	*0.18%*	4.4	*1.13%*	4.1	*1.38%*
Ciron	4.5	*2.38%*	2.7	*0.26%*	2.6	*1.66%*	2.0	*0.70%*
Sterop 160269	4.0	*0.57%*	2.7	*0.53%*	2.5	*1.14%*	2.0	*0.36%*
Rotexmedica	4.0	*0.47%*	3.9	*0.00%*	4.2	*1.17%*	3.9	*0.18%*
AS Grindeks 38910917	4.1	*0.46%*	4.0	*0.35%*	4.3	*0.82%*	4.0	*0.18%*

IU: international unit. RSD: relative standard deviation. NA: no data available.

[a] Batch HWZ7694 had identical packaging information as batch HC0075 listed in Table 2, but expiry date June 2021.

PLOS ONE

RESEARCH ARTICLE

Stability of misoprostol tablets collected in Malawi and Rwanda: Importance of intact primary packaging

Nhomsai Hagen[1], Thomas Bizimana[2], P. Claver Kayumba[3], Felix Khuluza[4], Lutz Heide[1]*

1 Pharmaceutical Institute, Eberhard Karls University Tubingen, Tubingen, Germany, **2** School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda, **3** East African Community Regional Centre of Excellence for Vaccines, Immunizations and Health Supply Chain Management (EAC RCE-VIHSCM), University of Rwanda, Kigali, Rwanda, **4** Pharmacy Department, College of Medicine, University of Malawi, Blantyre, Malawi

* heide@uni-tuebingen.de

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Abstract

Misoprostol is listed in the WHO essential medicines list and can be used for induction of labour, for prevention and treatment of post-partum haemorrhage, and for abortions. The compound is unstable, and substandard misoprostol preparations have been detected in low- and middle-income countries. We now investigated the stability of misoprostol tablets according to the international guidelines for stability testing of pharmaceutical products. Three brands (four batches) of misoprostol tablets were collected in Malawi and Rwanda: the originator product, a WHO-prequalified product, and a generic product without WHO prequalification. A further batch of the originator product was collected in Germany. To investigate the effect of damage to the primary packaging, additional blister strips of one sample were intentionally damaged with a needle and investigated in parallel. Samples were placed in stability chambers for six months at 40˚C/75% relative humidity (RH) and at 25˚C/60% RH. After 0, 1, 2, 3 and 6 months, misoprostol content was determined according to the International Pharmacopeia. At 40˚C/75% RH, all samples showed a decline of misoprostol content, but four of the batches still remained within the pharmacopeial specifications, while one of the two batches of the generic product without WHO-prequalification showed a final content of 86.2% which is out of specifications. Damage to the primary packaging greatly decreased stability, resulting in a final content of only 48.2% of the declared misoprostol amount. At 25˚C/60% RH all samples remained in specifications for six months, even the sample with the damaged blister. Dissolution of misoprostol remained in specifications of the pharmacopoeia for six months for all batches, except for the sample with damaged blisters stored at 40˚C/75% RH. This study confirms that the stability of misoprostol tablets must be ensured by intact, good-quality primary packaging. Careful supplier qualification is required in the procurement process.

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Introduction

Misoprostol (Fig 1), a prostaglandin analogue, is listed in the essential medicines list of the World Health Organization (WHO) [1] as a uterotonic and can be used for prevention and treatment of post-partum haemorrhage (PPH). PPH is the leading cause of maternal mortality in low-income countries. It is defined as a blood loss of 500 ml or more within 24 hours after giving birth [2]. Oxytocin, the medicine of first choice for prevention and treatment of PPH, is very sensitive to environmental conditions. It degrades at high temperatures and usually has to be stored at 2–8 ˚C [3–7]. This storage requirement can be challenging, especially in rural areas of low- and middle-income countries (LMICs) where infrastructure is poor and ambient temperatures are often high [8–12]. Indeed, several previous studies have shown that the quality of oxytocin, especially in LMICs, is often poor [3, 10–17].

Misoprostol is available in form of tablets which can be administered orally and which do not require refrigerated storage. Therefore, misoprostol tablets appear to offer an attractive alternative to oxytocin injections in the prophylaxis and treatment of PPH in places where no trained staff is available to administer medicines parenterally, or where appropriate storage conditions for oxytocin cannot be ensured [1, 18, 19]. Misoprostol is also used for the induction of labour, for treatment and prevention of ulcers induced by non-steroidal anti-inflammatory drugs (NSAIDs) and for abortions [1, 20]. Concerns about its latter use have motivated some countries to restrict its availability.

The instability of misoprostol has been known since decades [21]. The most important degradation reaction is dehydration, leading to misoprostol A (Fig 1). Toledo-Vasquez et al. [22] described that in aqueous solutions, this reaction follows first-order kinetics, and at 60˚C and pH 7.66 results in a degradation of misoprostol with a half-life of only 8.8 hours. Dehydration is followed by the much slower isomerization to the resonance-stabilized misoprostol B [21,

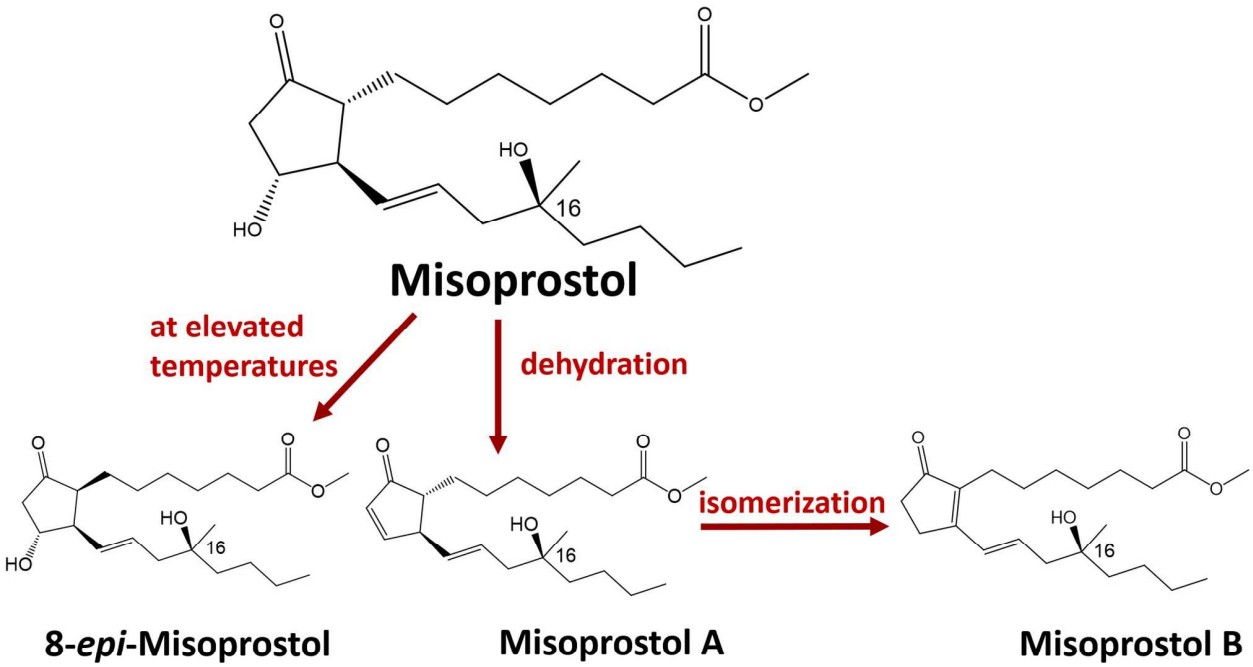

Fig 1. Structure of misoprostol and its typical degradation mechanisms. Commercial misoprostol is a mixture of the depicted structure at its epimer at C-16, as well as the enantiomers of both compounds. Corresponding stereoisomers are found for the degradation products.

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22] (Fig 1). Epimerization of misoprostol at C-8 is a further degradation mechanism occurring at higher temperatures [21].

Pure misoprostol shows 50% degradation within two weeks at 55˚C [22]. Fortunately, a dispersion of misoprostol in hydroxypropyl methylcellulose (HPMC) is much more stable, allowing the formulation of finished pharmaceutical products [21–24]. However, these misoprostol-HPMC dispersions have to be carefully protected from humidity, since the degradation velocity of misoprostol increases sharply when the water content of the dispersion exceeds 2% [24], and tablets containing misoprostol-HPMC dispersions have been shown to absorb water very quickly when stored without protection [23]. Importantly, Hall [25] demonstrated that plastic-aluminium blisters which are frequently used for the packaging of pharmaceutical products are grossly inadequate for ensuring the stability of misoprostol tablets. This study investigated 215 samples of misoprostol tablets of different age after manufacture, collected in Asia, Africa and Latin America. When one year or more had elapsed after manufacture, all samples packaged in plastic-aluminium blisters showed a misoprostol content below the limit specified by the International Pharmacopeia (90% of the declared amount). Double-sided aluminium blisters provided better protection, but one year or more after manufacture, even of those 28% were reported to show misoprostol contents below the pharmacopeial limit [25].

The quality of misoprostol preparations circulating in LMICs has received much less attention in the scientific literature than the quality of oxytocin. Besides the above-mentioned study by Hall [25], only two reports have been published: Anyakora et al. [11] investigated 166 samples of misoprostol tablets collected in health facilities in Nigeria. 33.7% of these were reported to fail the specifications of the International Pharmacopeia for misoprostol content, but the report did not specify how far these samples deviated from declared content. Our group recently investigated the quality of oxytocin and misoprostol samples collected in health facilities and drug outlets in Malawi. Out of the 30 misoprostol samples, five (17%) showed extreme deviations, containing only 12.7–30.2% of the declared content [12]. In the scientific literature, no systematic stability study of finished pharmaceutical products of misoprostol has been published so far.

During the registration of medicines, National Medicines Regulatory Authorities (NMRAs) examine the stability data provided by the manufacturers, usually they do not carry out independent experiments to confirm these data. The NMRAs of the EU member states, USA, Japan, Switzerland, Canada, Australia, Iceland, Liechtenstein and Norway are currently considered as "Stringent Regulatory Authorities" (SRAs) [26], and medicines produced under supervision of an SRA are usually regarded with trust in regard to their manufacturers' specifications for quality and stability. Similar trust is given to medicines which have received "WHO Prequalification" status, based on information submitted by manufacturers to WHO and on inspections of the respective manufacturing sites by experts mandated by WHO [27, 28]. Among the medicine types which are currently eligible for WHO prequalification status are medicines for reproductive and maternal health, including oxytocin injections and misoprostol tablets. However, only a small part of the medicines circulating in LMICs are approved by a SRA or WHO-prequalified [29]. In the present study, we investigated different batches of three misoprostol preparations: one preparation which was produced in a country with an SRA, another one which was a WHO-prequalified product, and one preparation which was produced in a non-SRA country and was not WHO-prequalified.

Rules for stability testing of pharmaceutical products have been devised by the International Council of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), most importantly guidelines Q1A-Q1F [30]. Obviously, the aim of testing is to confirm the stability of the product over the entire shelf-life, but testing durations of several years are difficult from an economic viewpoint. Therefore, so-called accelerated stability

studies are permissible. They are carried out for shorter time periods but at higher temperatures and relative humidities. These studies allow to predict the shelf life of the products under given storage conditions, and their results are used to decide about the label statements regarding shelf life and storage requirements for the respective products. Guidelines by ICH [31] and by WHO [26] specify the precise conditions for accelerated stability studies. Obviously, these conditions are related to the climatic zone of the country where the medicine will be registered. Malawi is currently assigned to WHO climatic zone II (subtropical and Mediterranean), and Rwanda to zone IVa (hot and humid) [32].

In the present study, accelerated stability testing of misoprostol tablets collected in Malawi and Rwanda was carried out according to the ICH guidelines for stability testing of pharmaceutical products [31].

Materials and methods

Study design and ethical clearance

This study was designed observing the recommendations contained in the WHO guidelines on the conduct of surveys of the quality of medicines [33], the MEDQUARG guidelines [34], and the ICH and WHO guidelines for stability testing of pharmaceutical products [26, 31]. The College of Medicine Research and Ethics Committee (COMREC) in Malawi gave ethical clearance to conduct this study (Reference No. P.07/27/2215), as well as the Ministry of Health, Rwanda (Reference No. 20/1361/DGPHFIS/2018). Approval was also granted by the Malawi National Regulatory Agency (Pharmacy, Medicines and Poisons Board, PMPB).

Sample collection

The brands of misoprostol tablets available at government medical stores and pharmaceutical wholesalers in Malawi and Rwanda during the time of sample collection (February-March 2018) were purchased by local researchers (F.K. and T.B.). If different batches of a certain brand were available, samples from each batch were purchased. From each brand and batch, 250 tablets were purchased. Samples were not collected from individual health facilities or private pharmacies, to minimize the influence of possibly incorrect storage conditions on the results of this study. Also, these facilities usually do not stock sufficient amounts of misoprostol tablets for stability testing. After purchase, samples were stored according to the manufacturers' specifications. Temperature data loggers were kept with the samples until these were placed into the stability chambers for testing. The investigators hand-carried the samples from Malawi or Rwanda to Germany via airplane; this transport required less than 24 hours. Samples were pre-tested for their misoprostol content according to International Pharmacopeia. Those samples which were within specifications at the time of pretesting were included into the stability study.

Storage in stability chambers

The samples were stored in their original primary packaging in stability chambers of Alpha-Pharma-Service GmbH, Heilbronn, Germany, for six months (April-October 2018). Conditions were chosen in accordance to ICH and WHO guidelines for stability testing of pharmaceutical products [26, 31]. Accelerated stability testing was carried out at 40˚C +/- 2˚C and 75% +/- 5% relative humidity (RH). Samples stored at 25˚C +/- 2˚C and 60% +/- 5% RH, i.e. at the ICH conditions for long-term testing of non-refrigerated products, climatic zone II, were investigated for comparison. After 0, 1, 2, 3, and 6 months, 20 tablets of each sample were

removed from both chambers and analysed at Tuebingen University, Germany (by N.H. and T.B.).

Sample analysis

Samples were visually inspected and subsequently tested for identity, assay and dissolution following the procedures described in the monograph for misoprostol tablets of the International Pharmacopeia 2017. Prior to the experiments, the methods were validated according to the International Pharmacopeia. Misoprostol was identified and quantified by high performance liquid chromatography (HPLC), using an Agilent Infinity 1260 II with binary pump and variable wavelength detector (Agilent Technologies, Santa Clara, CA, USA), with acetonitrile/water (45:55 V/V) as mobile phase, flow rate 1.5 ml/min, column ReproSil-XR 120 C18, 5 μm, 150 mm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany), and UV detection at 200 nm. The injection volume was 100 μl for identity and assay, or 250 μl for dissolution. For identity and assay, five tablets were dissolved in 50 ml mobile phase; two independent experiments were carried out, and of the resulting solutions two aliquots each were analysed by HPLC, yielding four measurements for each sample. The HPLC autosampler was set to 4 ˚C to avoid misoprostol degradation. Samples showing additional peaks were also tested for related substances according to Kahsay et al. [35], using freshly prepared solutions from three tablets per sample and performing the tests without delay.

Dissolution was investigated with a dissolution tester PT-WS 610 (Pharma Test Apparatebau AG, Hainburg, Germany). Six tablets per sample were tested separately as described in the monograph for misoprostol tablets in the International Pharmacopeia, with 500 ml of water R as dissolution medium, a temperature of 37˚C, and a rotating paddle with 50 revolutions per minute. Samples were withdrawn after 30 min through an in-line filter. Misoprostol reference standard Ph. Eur. (batch N˚ 3.0) was obtained from the European Directorate for the Quality of Medicines (EDQM), Strasbourg, France.

At each time point during the stability testing, 5-point calibration curves were prepared to assure linearity. Intermediate precision [36] was calculated from the data of the calibration curves of the reference standards, (see S2 Table).

Statistical analysis

Statistical evaluation was done using JMP 14.2 (SAS GmbH, Heidelberg, Germany). Significance levels of differences were calculated using uni- and multivariate analysis of variance (ANOVA) and student´s t-test. Differences were considered significant when $p < 0.05$.

Results

Overview of investigated misoprostol samples

In this study, misoprostol samples were investigated which were offered by government medical stores and private wholesalers in Malawi and Rwanda, i.e. preparations which reflect the actual quality and stability of medicines distributed in these two African countries. In February and March 2018, the local investigators (F.K. and T.B.) enquired about the available brands and batches of misoprostol tablets. Samples were then purchased from private wholesalers using a mystery shopper approach (i.e. ordering with the help of a licensed pharmacy shop), and from government medical stores in an overt approach. If different batches of a certain brand were available, samples from each batch were purchased. In Malawi, certain brands of misoprostol tablets had recently been withdrawn from the market [12], and only one single brand was offered for sale at the time of sample collection, in form of two different batches;

Table 1. Investigated misoprostol samples. All samples represented tablets with a stated content of 200 μg/ tablet.

Collected in:	Collected at:	Stated manufacturer (and brand name)	Country of manufacture	Mfg. date/ Exp. date	Batch number	Stated shelf life	Stated storage requirements	Primary packaging	Stated excipients / formulation	Prequalification status
Malawi	wholesaler	**Fourrts (India) Laboratories Pvt. Limited** (KONTRAC 200)	India	Jun 17/ May 19	E0571	2 years	Below 30˚C in a dry place. Protect from light	alu/alu blister	none declared	none
	Central Medical Stores Trust			Feb 17/ Jan 19	D2205					
Rwanda	government district pharmacy	**Acme Formulation Pvt. Ltd.** (Ace Miso)	India	Sep 16/ Aug 18	ACE160963	2 years	Below 30˚C protected from light	alu/alu blister	misoprostol as HPMC dispersion (1%), excipients q.s.	WHO-PQ
	wholesaler	**Piramal Healthcare UK Limited** (Cytotec)	UK	Jul 17[1]/ Jun 20	B17173	3 years[1]	none	alu/alu blister	misoprostol-HPMC dispersion (1%), cellulose (microcrystalline), sodium carboxymethyl-amidon, hydrogenated castor oil	SRA
Germany	pharmacy of Tuebingen university hospital			Feb 17[1]/ Jan 20	B16131		Below 30˚C protected from humidity		cellulose (microcrystalline), HPMC, sodium carboxymethyl-amidon, hydrogenated castor oil	

HPMC: hydroxypropyl methylcellulose. WHO-PQ: WHO-prequalified product. SRA: produced in a country with stringent regulatory authority.

[1] information not stated on the packaging, but obtained from internet data bases [20, 37].

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both batches were purchased. In Rwanda, three brands were offered at the time of sample collection (only a single batch of each brand), and each brand was purchased. However, one of these brands showed an insufficient API content already in pre-testing, and had to be excluded from the subsequent investigation. The Rwanda Food and Drug Authority (RFDA) was alerted about this substandard brand. Further investigations initiated by RFDA confirmed our findings, and effective from February 2019, RFDA recalled the substandard brand from the Rwandan pharmaceutical market. As a comparison, the originator brand (Cytotec®) was purchased through the pharmacy of the University Hospital Tuebingen. This is the brand most commonly used in Germany. A different batch of this originator brand had also been purchased in Rwanda.

Therefore, as shown in Table 1, three brands (total 5 batches) were included into the stability testing. The originator brand had been produced in the UK, i.e. in a country with an SRA. One further brand represented a WHO-prequalified product, manufactured in India. The third brand was not WHO-prequalified, and had also been produced in India.

Surprisingly, packaging and leaflet of the originator brand collected in Rwanda did not state any storage requirements, while packaging and leaflet of the same brand collected in Germany stated that storage below 30˚C was required. The same storage temperature was stated by the manufacturers of the two other brands. The stated requirements for protection from light and humidity were somewhat different between the brands (Table 1). All samples had double-sided aluminium blisters as primary packaging. Pre-testing for content of misoprostol showed that these samples were within the specifications of the International Pharmacopoeia at the beginning of the stability study.

The three manufacturers were contacted and were requested to confirm the authenticity of the samples. The two manufacturers from India confirmed that label information and appearance of the samples conformed to their products. The latest response from Pfizer (the marketing authorization holder)/Piramal was that they were still investigating this matter.The declared shelf life of the investigated brands was either two or three years. While four of the five investigated batches remained within their shelf life during the entire duration of the study, the WHO-prequalified product by Acme expired during the stability testing. Nevertheless, it was found to remain within specifications during the entire testing period, even after storage for six months at 40 ˚C and 75% RH (see below).

Table 1 also shows the stated excipients for the investigated preparations. Notably, for the product Kontrac 200® which was neither SRA-approved nor WHO-prequalified, no excipients were declared at all.

Accelerated stability study

According to the current ICH/WHO guidelines for stability testing of finished pharmaceutical products [26, 31], accelerated stability testing of medicines which are labelled for storage below 30 ˚C has to be performed at 40 ˚C and 75% RH for six months. All five collected batches of misoprostol tablets were tested under these conditions. The results are depicted in Fig 2a, and the exact misoprostol amounts determined at each time point, together with the standard deviation of the measurements, are listed in S1 Table. As visible in Fig 2a, both batches of the originator brand as well as the batch of the WHO-prequalified product remained in specifications over the entire six months testing period, i.e. their misoprostol content remained in the range of 90–110% of the declared amount. However, of the two batches of the product which had been produced in a non-SRA country and which had not been

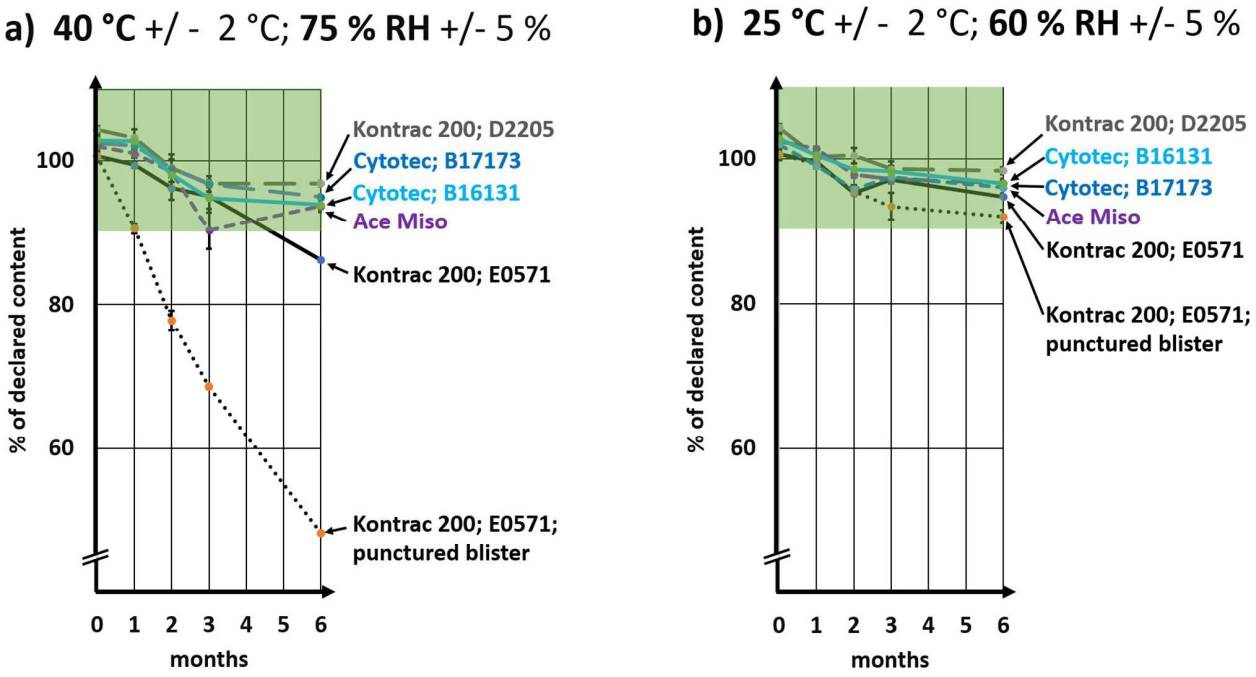

Fig 2. Change of misoprostol content in tablets store for 6 months a) at 40˚C and 75% relative humidity (RH) and b) at 25˚C and 60% RH. Error bars show standard deviation. The International Pharmacopeia requires a content of misoprostol between 90–110% of the declared amount. This range is marked in the figure.

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WHO-prequalified, only one batch remained in specifications, while the other batch showed a final content of 86.2% of the declared amount, which is out of specifications. The decrease of the misoprostol content of this sample was 14.5% over 6 months, which is significantly more that the decrease observed in the originator and the WHO-prequalified samples (decrease over 6 months: 8.9%, 7.4% and 8.3%, respectively; all $p < 0.0001$), and also significantly more than in the other investigated batch from the same manufacturer (7.5% decrease over 6 months; $p < 0.0001$). Contrary to expectations, the failing batch was the one with the longer remaining shelf life (Table 1), indicating that its different stability was not due to the age of the sample, but possibly due to batch-to-batch differences in the manufacturing of this product, or due to different storage conditions of the two batches prior to sample collection.

Effect of damaged blisters

All investigated products were correctly packaged in double-sided aluminium blisters. In order to investigate the importance of the intactness of the primary packaging for the stability of the misoprostol tablets, the blister strips of one sample were intentionally damaged by puncturing a single hole of approximately 1 mm diameter into each alveolus of the blisters (S1 Fig), allowing access of air and humidity to the tablets. The sample in these punctured blisters was investigated in parallel to the samples with the intact blisters. As expected, damage to the primary packaging had a strong detrimental influence on stability (Fig 2a and S1 Table): already after two months at 40 ˚C and 75% RH, the misoprostol content was out of specifications, and after six months the remaining amount of misoprostol was as low as 48.2% of the declared content. HPLC analysis of this sample for related substances according to Kahsay et al. [35] clearly showed the decrease of the misoprostol content and the concomitant increase of the typical degradation products of misoprostol, i.e. misoprostol A and, to a smaller extent, misoprostol B and 8-*epi*-misoprostol (Fig 3).

Stability at long-term storage conditions

Parallel to the accelerated stability testing at 40˚C and 75% RH, a control experiment was conducted at 25 ˚C and 60% RH, which represents the long-term storage condition for non-

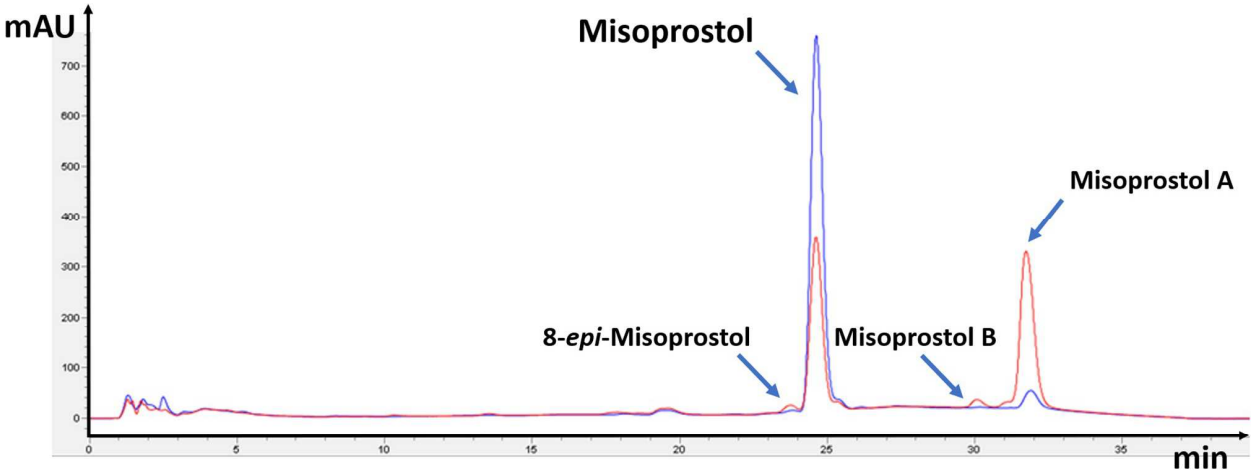

Fig 3. HPLC analysis of misoprostol tablets stored in damaged blisters at 40 ˚C and 75% RH for six months (red line). KONTRAC 200 tablets of Fourrts (India), batch E0571 (see Table 1) were used for this experiment. Tablets of the same batch stored in intact blisters at 25 ˚C and 60% RH for six months are shown as comparison (blue line). HPLC analysis for related substances was carried out according to Kahsay et al. [35].

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refrigerated products in climatic zone II [26, 31]. As shown in Fig 2b and in S1 Table, the assay results of all five investigated batches remained in specifications under these conditions for the six months testing period. Even the sample with the punctured blisters remained in specifications, though it showed the lowest final misoprostol content of all investigated samples (Fig 2b).

Also at 25 ˚C and 60% RH, a decline of the misoprostol content over six month was visible (Fig 2b). Assuming that misoprostol degradation followed first-order kinetics [22], i.e. with a linear relationship between the logarithm of the content and time, we calculated from the measurements depicted in Fig 2b the expected misoprostol content at the time point two years after the date of manufacture upon continued storage at 25 ˚C and 60% RH. This calculation predicted that four of the investigated batches would show contents between 90.2 and 96.6% of the declared amount, i.e. would remain in specifications for assay during that time period. In contrast, the batch which had failed assay testing after accelerated stability testing (KONTRAC 200, batch E0571) was predicted to show a content of 87.3% at the time of its expiry date, which would be out of specifications. Results of such extrapolations need to be evaluated with care, but still they indicate that the differences in stability observed at 25˚C were consistent with those observed at 40˚C.

Dissolution testing

All collected batches, as well as the sample with the intentionally punctured blisters, were also tested for dissolution of the active pharmaceutical ingredient (API). The International Pharmacopoeia requires that at least 80% of the declared amount of misoprostol dissolves under the defined conditions (see Methods). As shown in Table 2, all five investigated batches perfectly complied with this requirement, showing nearly complete dissolution of the API. Storage in intact blisters for six months at 40˚C and 75% RH resulted only in moderate decreases of the dissolution values (Table 2). These mainly reflected the loss of the API content described above, while the dissolved percentage of the API showed only small changes over six months (Table 2, right columns). All five batches remained within specifications, but the batch of the non-SRA product which had failed assay testing after storage under these conditions showed a

Table 2. Dissolution testing results of misoprostol tablets stored at two different conditions, 0 and 6 months.

		Dissolution (% of declared content)				Dissolution (% of assay determined at the respective month [see S1 Table])	
Storage condition	**Sample**	**Month 0**	**RSD**	**Month 6**	**RSD**	**Month 0**	**Month 6**
40˚C +/ - 2˚C	Kontrac 200, batch E0571	99.2	0.98%	81.3	2.81%	98.6	94.3
	Kontrac 200, batch D2205	100.4	5.76%	95.1	1.72%	96.3	98.2
	Ace Miso	99.7	2.59%	90.8	1.57%	97.7	96.9
75% RH +/- 5%	Cytotec, batch B16131	104.0	3.03%	92.3	1.86%	101.1	98.3
	Cytotec, batch B17173	99.9	2.82%	91.0	2.26%	97.5	95.8
	Kontrac 200, batch E0571; punctured blister	99.2	0.98%	42.4	3.87%	98.6	87.9
25˚C +/- 2˚C	Kontrac 200, batch E0571	see above		90.6	2.93%	see above	95.6
	Kontrac 200, batch D2205			93.1	2.21%		94.6
	Ace Miso			93.0	2.93%		96.8
60% RH +/- 5%	Cytotec, batch B16131			93.1	1.97%		96.3
	Cytotec, batch B17173			94.6	1.88%		97.9
	Kontrac 200, batch E0571; punctured blister			89.7	2.46%		97.5

RSD: relative standard deviation. RH: relative humidity.

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final dissolution value of 81.3%, passing the 80% threshold of the pharmacopoeia only by a narrow margin.

The sample with the punctured blisters clearly failed dissolution specifications after six months at 40˚C and 75% RH, showing 42.4% dissolution of the declared API amount. Assay testing had shown a final API content of 48.2% of the declared amount. Therefore, storage in damaged blisters at these conditions strongly reduced the API content, and to some extent also reduced the dissolved percentage of the remaining API (Table 2).

After storage at 25˚C and 60% RH, all tested samples, including the one with the punctured blisters, still complied with the specifications of the pharmacopeia for dissolution of misoprostol (Table 2).

Discussion

The results of the present study confirm that the limited stability of misoprostol represents a problem for safeguarding the quality of misoprostol tablets especially in countries with hot and humid climates. This problem can be reasonably managed by appropriate packaging and by professional formulation of the tablets. The two investigated batches of the originator product, as well as the WHO-prequalified product, were found to remain within the specifications of the International Pharmacopoeia over six months of accelerated stability testing at 40˚C and 75% RH, despite the fact that already 11, 16 and 19 months had elapsed since their date of manufacture when they entered stability testing, respectively. The WHO-prequalified product even exceeded its expiry date 2 months before the end of the stability test, and still conformed to specifications both in assay and in dissolution at the end of the six-months testing period. This may be seen as a further example that WHO-prequalification is a reliable assurance of the good quality of medicines. However, of the two tested batches of a product without WHO-prequalification and produced in a country without stringent regulatory authority (SRA), one batch was clearly out of specifications at the end of the accelerated stability testing. The other batch of this manufacturer showed much less degradation during accelerated stability testing. The clearly different results between the two batches may raise doubts about the batch-to-batch consistency in the manufacturing of this product.

In this study, misoprostol tablets were included in the stability test only if they were within specifications at the beginning of the test. One brand of misoprostol tablets collected in Rwanda (packaged in double-sided aluminium blisters; manufactured in a country without SRA; not WHO-prequalified) had to be excluded due to extreme deviations from specifications in the assay. Furthermore, Hall [25], Anyakora et al. [11] and Hagen et al. [12] have reported insufficient misoprostol contents in many misoprostol preparations collected in LMICs. Therefore, quality and/or stability problems are certainly not a rare observation in misoprostol tablets circulating in LMICs.

The observation that several batches investigated in this study remained in specifications during accelerated stability testing does not prove the absence of stability problems. The current ICH/WHO guidelines [26, 31] require not only that the investigated preparations remain in specifications over the six-months testing period, but also that the content of the API does not change by more than 5%. None of the five investigated batches complied with this criterion in our accelerated stability test. This result has to be interpreted with care, since the products had been stored for extended periods of time, at unknown conditions, before the stability test was conducted. Therefore, the present results do not prove that the preparations failed their stability requirements at the time when they left the manufacturing company. Nevertheless, the data reported in the present study confirm that the stability of misoprostol tablets is problematic. This is also stated in the assessment report of the WHO prequalification programme

for the misoprostol tablets produced by Acme (Table 1): "The product is chemically not very stable; the data show an increase of degradation with time at accelerated and long term storage conditions, though within justified limits" [38].

In the present study, a decrease of the misoprostol content was observed both at 40˚C and 75% RH and at 25˚C and 60% RH (Fig 2). An investigation of the storage conditions of misoprostol tablets in health facilities in Malawi had shown mean kinetic temperatures at the different storage sites ranging from 21.4˚C to 31.0˚C [12]. In view of these observations, a reduction of the stated shelf-life of the originator brand (Table 1) from three to two years may be advisable, especially for those batches which are exported to countries with hot and humid climates. Also the omission of a storage requirement on the packaging of the originator product marketed in Rwanda is surprising and should be corrected by the manufacturer.

Manufacturers are aware of the stability problems of misoprostol. They frequently manufacture tablets with an initial content of more than 100% of the stated amount, to ensure that the misoprostol content of their preparations does not fall below the pharmacopeial limit of 90% of the declared amount within their shelf-life. This was observed in the present study: the content of all preparations at the beginning of the stability testing exceeded 100% of the stated amount, despite the time which had elapsed since manufacture. The data reported by Hall [25] showed that out of 215 samples of misoprostol tablets which were investigated at an age between zero and three years after their date of manufacture, approximately 43% contained between 100–110% of the stated content, and 5% even exceeded the pharmacopeial limit of 110% at the time of analysis. It has to be expected that the prevalence of overdosed preparations would be even higher if all of them had been investigated shortly after their date of manufacture. Also for the preparations investigated in the present study, extrapolation of the data shown in Fig 2b indicates that their misoprostol content at the time of manufacture may have exceeded the pharmacopeial limit of 110%. However, as mentioned above, results of such extrapolations need to be interpreted with care.

It is encouraging that all brands of misoprostol tablets distributed by wholesalers and government stores in Malawi and Rwanda at the time of sample collection were packaged in double-sided aluminium blisters. This may be a consequence of the report by Hall [25] that plastic-aluminium blisters are grossly inadequate to ensure stability of misoprostol tablets. According to the information listed on the website of the WHO prequalification of medicines programme [39], all three WHO-prequalified brands of misoprostol tablets are packaged in double-sided aluminium blisters for protection of the tablet against moisture, and are produced from 1:100 misoprostol dispersion in HPMC [38].

Both the originator product [40, 41] and at least one generic preparation produced in India [12, 25] are also marketed in screw-cap plastic bottles containing 60 or 100 tablets. While such bottles in principle offer good protection if also a desiccant in sufficient amount is included in the packaging [42], their use in LMICs is not advisable since proper closure of the bottle may not always be ensured and thereby access of humidity may severely affect the quality of the tablets. A preparation packaged in such bottles found in Malawi contained only 48.8% of the misoprostol amount declared on the label 29 months after the date of manufacture [12].

For misoprostol tablets packaged in double-sided aluminium blisters, the intactness of the blister is of principal importance for the stability. This was reported by Berard et al. [23] and has been clearly confirmed in the present study. If the blister was damaged, we observed more than 50% loss of misoprostol within 6 months at 40˚C and 75% RH. The velocity of the degradation was strongly dependent on the environmental conditions: at 25˚C and 60% RH, only 8.7% loss occurred within 6 months. 1.5% loss was observed in the first month under these conditions. This is somewhat different from the time pattern reported by Berard et al. [23], who had investigated another brand of misoprostol tablets for a period of one month after

complete removal from their blister packs. At 25˚C and 60% RH, these authors reported a loss of misoprostol content of approximately 10% within the first week, followed (somewhat surprisingly) by a loss of only about 0.5% per week in the following three weeks.

The results of our study show that unprotected storage of misoprostol tablets for extended periods at high temperature and humidity is extremely detrimental, while unprotected storage for one month at 25˚C and 60% RH only had a small effect on the misoprostol content. Nevertheless, in view of the instability of misoprostol, storage outside of intact blisters should be avoided, even for short periods.

The key stability problem of misoprostol tablets is the degradation of the API, especially in the absence of proper protection from humidity. In contrast, dissolution of misoprostol was hardly affected during accelerated stability testing in this study, and even unprotected storage did not affect dissolution of the remaining amount of misoprostol very strongly (see Table 2).

Limitations of this study

This study investigated only those brands and batches of misoprostol tablets which were available at government medical stores and pharmaceutical wholesalers in Malawi and Rwanda during the time of sample collection, and may not be representative for other brands or batches. Furthermore, while this approach to sample collection provides a realistic picture of the stability of the products which were distributed in these countries at that time, it does no give a precise picture of the condition of the samples at the time when they left the manufacturing companies. Differences in the results obtained for different brands and batches may be influenced by the different age of samples at time of analysis, and by their different storage condition prior to collection.

Conclusions

The stability of misoprostol tablets is problematic and must be ensured by intact, good-quality double-sided aluminium blisters and by appropriate manufacturing using HPMC as stabilizing agent. Misoprostol tablets of very different quality and stability are on the market, and careful supplier qualification is required in the procurement process. In doubt, procurement should be restricted to WHO-prequalified products, and/or products manufactured under supervision of a stringent regulatory authority. In the prevention and treatment of PPH, misoprostol tablets offer the advantage over oxytocin injections that they do not need parenteral administration. However, the notion that misoprostol tablets present fewer stability problems than oxytocin injections may be misleading, especially in hot and humid climates.

Supporting information

S1 Fig. Intentionally punctured blister of misoprostol tablets (Kontrac 200, batch E0571).
Needle punctures are highlighted by arrows.
(DOCX)

S1 Table. Assay testing results of misoprostol tablets stored at two different conditions over 6 months.
(DOCX)

S2 Table. Intermediate precision of misoprostol assay and dissolution.
(DOCX)

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Author Contributions

Conceptualization: Nhomsai Hagen, Thomas Bizimana, P. Claver Kayumba, Felix Khuluza, Lutz Heide.

Data curation: Nhomsai Hagen.

Formal analysis: Nhomsai Hagen, Thomas Bizimana.

Funding acquisition: Lutz Heide.

Investigation: Nhomsai Hagen, Thomas Bizimana, Felix Khuluza.

Methodology: Nhomsai Hagen, Thomas Bizimana.

Project administration: P. Claver Kayumba, Felix Khuluza, Lutz Heide.

Supervision: P. Claver Kayumba, Lutz Heide.

Validation: Nhomsai Hagen.

Visualization: Nhomsai Hagen, Lutz Heide.

Writing – original draft: Nhomsai Hagen.

Writing – review & editing: Thomas Bizimana, P. Claver Kayumba, Felix Khuluza, Lutz Heide.

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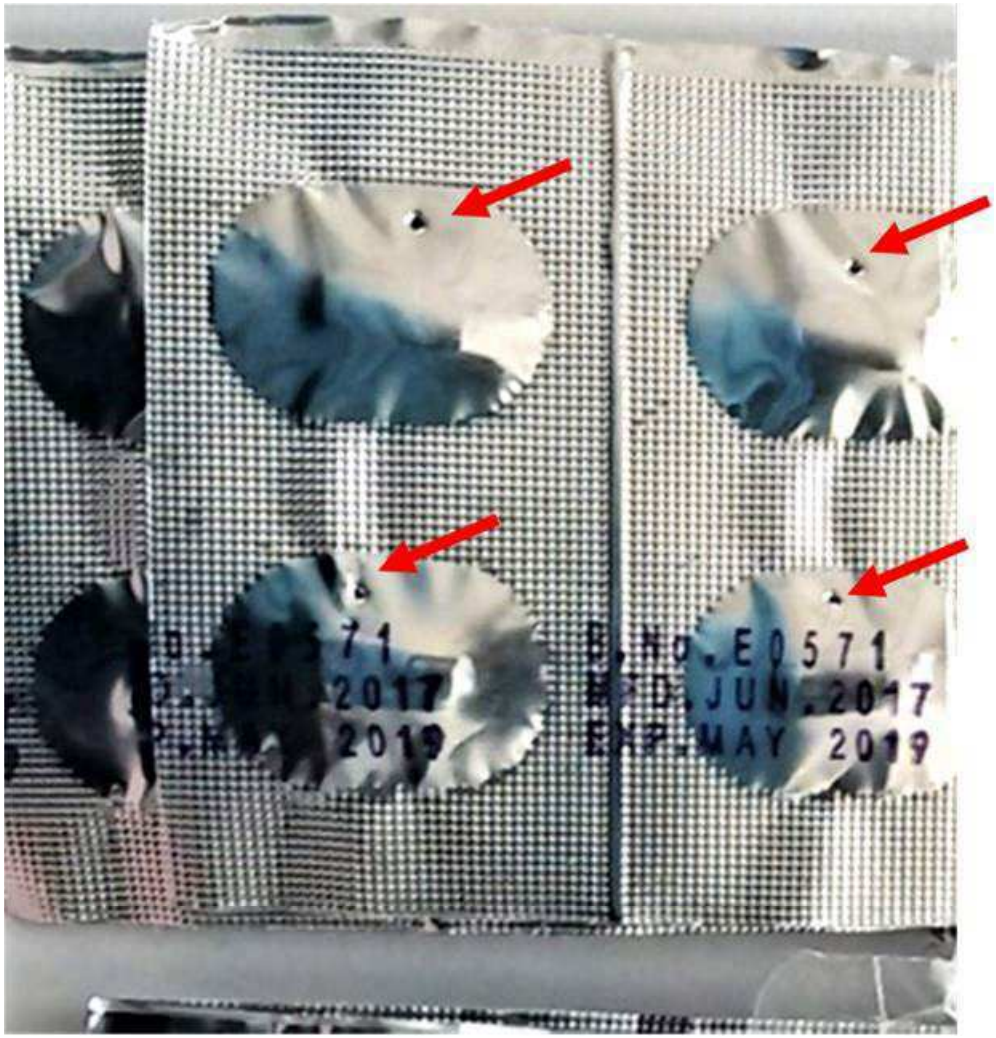

S1 Fig: Intentionally punctured blister of misoprostol tablets (Kontrac 200, batch E0571). Needle punctures are highlighted by arrows.

S1 Table: Assay testing results of misoprostol tablets stored at two different conditions over 6 months

		Misoprostol assay (% of declared content)									
Storage condition	**Sample**	**Month 0**	**RSD**	**Month 1**	**RSD**	**Month 2**	**RSD**	**Month 3**	**RSD**	**Month 6**	**RSD**
40°C +/- 2°C **75% RH** +/- 5%	**Kontrac 200, batch E0571**	100.7	0.82%	99.4	0.42%	96.2	1.71%	95.0	1.85%	86.2	0.15%
	Kontrac 200, batch D2205	104.3	0.43%	103.2	0.14%	99.0	1.22%	96.8	0.50%	96.8	0.42%
	Ace Miso	102.0	0.44%	101.0	0.58%	98.4	0.60%	90.3	2.81%	93.7	0.85%
	Cytotec, batch B16131	102.8	0.41%	102.7	1.56%	98.1	0.42%	94.8	2.07%	93.9	0.72%
	Cytotec, batch B17173	102.4	1.13%	102.1	0.20%	99.0	1.91%	96.8	1.12%	94.9	0.34%
	Kontrac 200, batch E0571; punctured blister	100.7	0.82%	90.5	0.69%	77.8	1.75%	68.6	0.47%	48.2	0.24%
25°C +/- 2°C **60% RH** +/- 5%	**Kontrac 200, batch E0571**	see above		99.6	1.28%	95.2	1.12%	97.1	1.32%	94.8	0.20%
	Kontrac 200, batch D2205			100.3	1.16%	100.4	1.03%	98.6	1.09%	98.4	0.48%
	Ace Miso			99.0	0.21%	95.8	0.62%	97.5	0.93%	96.0	0.87%
	Cytotec, batch B16131			100.5	0.37%	98.6	0.17%	98.3	0.77%	96.6	0.62%
	Cytotec, batch B17173			101.4	0.29%	97.8	0.39%	97.2	0.27%	96.6	0.14%
	Kontrac 200, batch E0571; punctured blister			99.2	0.49%	95.5	0.45%	93.4	2.01%	92.0	1.04%

RSD: relative standard deviation. RH: relative humidity.

S2 Table: Intermediate precision of misoprostol assay and dissolution

Intermediate Precision Calibration Curve Assay Misoprostol												
Reference concentration (µg/ml)	Mean AUC (mAU*s) Month 0	*RSD*	Mean AUC (mAU*s) Month 1	*RSD*	Mean AUC (mAU*s) Month 2	*RSD*	Mean AUC (mAU*s) Month 3	*RSD*	Mean AUC (mAU*s) Month 6	*RSD*	**Mean AUC (mAU*s) all months**	**RSD**
25	1706.08	*0.76%*	1669.21	*0.21%*	1582.35	*1.45%*	1597.30	*0.41%*	1656.25	*0.13%*	**1642.24**	**3.14%**
20	1308.22	*0.64%*	1292.34	*0.58%*	1299.18	*0.34%*	1315.76	*0.36%*	1306.89	*0.49%*	**1304.48**	**0.69%**
15	1044.85	*0.50%*	975.97	*0.47%*	975.27	*0.52%*	1006.14	*0.25%*	981.14	*0.60%*	**996.67**	**2.98%**
10	708.98	*0.24%*	651.95	*0.70%*	700.70	*0.75%*	671.94	*1.07%*	645.61	*0.29%*	**675.84**	**4.20%**
5	353.04	*1.06%*	325.19	*0.62%*	377.79	*1.35%*	372.71	*0.53%*	372.04	*0.30%*	**360.15**	**6.02%**

Intermediate Precision Calibration Curve Dissolution Misoprostol												
Reference concentration (µg/ml)	Mean AUC (mAU*s) Month 0	*RSD*	Mean AUC (mAU*s) Month 1	*RSD*	Mean AUC (mAU*s) Month 2	*RSD*	Mean AUC (mAU*s) Month 3	*RSD*	Mean AUC (mAU*s) Month 6	*RSD*	**Mean AUC (mAU*s) all months**	**RSD**
0.6	94.37	*3.16%*	88.62	*1.34%*	92.06	*0.15%*	91.01	*4.57%*	89.74	*1.72%*	**91.52**	**2.61%**
0.4	61.13	*4.35%*	57.88	*1.12%*	60.58	*2.55%*	59.28	*0.97%*	60.26	*1.73%*	**60.13**	**1.46%**
0.32	49.90	*9.32%*	45.88	*1.17%*	48.36	*2.33%*	45.60	*1.77%*	47.13	*2.72%*	**47.43**	**4.34%**
0.24	34.61	*5.22%*	33.66	*3.49%*	34.07	*4.63%*	33.59	*1.40%*	36.97	*0.55%*	**33.98**	**1.37%**
0.1	11.28	*9.05%*	13.40	*5.32%*	12.80	*4.30%*	13.23	*2.08%*	15.01	*4.07%*	**12.68**	**7.61%**

AUC: area under the curve. RSD: relative standard deviation. Mean AUC calculated based on five measurements for reference concentration 20 µg/ml, and three measurements for all other reference concentrations.

PLOS ONE

RESEARCH ARTICLE

Quality of oxytocin and misoprostol in health facilities of Rwanda

Thomas Bizimana[1], Nhomsai Hagen[2], Gesa Gnegel[2], Pierre Claver Kayumba[3†], Lutz Heide[2]*

1 Department of Pharmacy, School of Medicine and Pharmacy, College of Medicine and Health Sciences (CMHS), University of Rwanda, Kigali, Rwanda, **2** Pharmaceutical Institute, Eberhard Karls University Tübingen, Tübingen, Germany, **3** East African Community Regional Centre of Excellence for Vaccines, Immunizations and Health Supply Chain Management (EAC RCE-VIHSCM), University of Rwanda, Kigali, Rwanda

† Deceased.
* heide@uni-tuebingen.de

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Abstract

Sustainable Development Goal 3.1 calls for a reduction of the maternal mortality ratio to less than 70 per 100,000 live births by 2030. The most important cause of maternal mortality is post-partum haemorrhage (PPH). Oxytocin injections and misoprostol tablets are medicines of first choice for the management of PPH in low- and middle-income countries (LMICs). Unfortunately, both substances are chemically unstable, and previous studies have revealed serious quality problems of these medicines in LMICs. The present study is the first report on their quality in Rwanda. From 40 randomly selected health facilities (hospitals, health centers, retail pharmacies and private clinics) in different parts of Rwanda, as well as from six wholesalers and government stores, oxytocin injections and misoprostol tablets were collected. Oxytocin storage temperatures in the health facilities were monitored for six months using temperature data loggers, and found to correctly follow the storage requirements stated by the manufacturers (2–8˚C, or room temperature) with few minor deviations. Oxytocin injections (57 samples, representing seven batches of four brands) were tested for their oxytocin content and pH value according to the United States Pharmacopeia. Twenty-four samples from three European manufacturers passed all tests. However, all nine samples of one batch of a Chinese manufacturer showed an excessive content of oxytocin (range 117.2–121.5% of the declared amount). Another batch of the same manufacturer showed extreme variations of the concentration of the preservative benzyl alcohol. Misoprostol tablets (25 samples, representing ten batches of six brands) were tested for content and dissolution according to the International Pharmacopoeia. Fifteen samples passed, but all 10 samples of two brands from India failed with extreme deviations, containing only 42.5–48.7% of the stated amount of misoprostol. In conclusion, oxytocin quality in Rwanda was better than reported from other African countries. However, two extremely substandard brands of misoprostol tablets were found. The Rwandan authorities reacted quickly and efficiently, and recalled these substandard medicines from the market. For oxytocin and misoprostol, with their well-known problems of quality and stability, procurement should possibly

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be restricted to medicines which are WHO-prequalified or which have been manufactured in countries with stringent regulatory authorities.

Introduction

In the year 2017, an estimated number of 295,000 women around the world died due to complications of pregnancy and childbirth [1]. The highest maternal mortality ratio is observed in the sub-Saharan region of Africa [1, 2]. Post-partum hemorrhage (PPH) is the most important cause of maternal mortality, and 50% of all cases of PPH worldwide occur in Africa [3]. Rwanda, a low-income country in sub-Saharan Africa [4], has successfully lowered its maternal mortality ratio from 1160 down to 248 per 100,000 live births in the years from 2000 to 2017 [1]. Efforts are made by the government of Rwanda to further reduce the maternal mortality ratio to less than 70 per 100,000 live births by 2030, in accordance with the Sustainable Development Goal (SDG) 3.1 [5, 6]. To achieve this target, oxytocic medicines which are used to treat and prevent PPH are of principal importance. Oxytocin injections and misoprostol tablets are among the medicines of first choice for the prevention and treatment of PPH [3, 7]. They are included as oxytocics (uterotonics) in the WHO model list of essential medicines [8] and in the Rwanda National List of Essential Medicines for adults [9]. Also, they have been included in the list of 13 life-saving items prepared by the United Nations Commission on Life-Saving Commodities for Women and Children (UNCoLSC) [10], as the only medicines for the management of PPH.

The use of substandard and falsified medicines has been shown to result in serious public health problems [11]. Especially in LMICs, the quality of medicines often fails to meet the pharmacopeial specifications, and this has far-reaching adverse consequences for patients, families, national health systems and the economy [11, 12]. The use of substandard oxytocin or misoprostol preparations in the management of PPH may lead to therapeutic failure in the treatment of excessive bleeding, and even to the death of the patient [13, 14]. Avoiding such preventable deaths is one of the key measures required to reach SDG 3.1.

Unfortunately, oxytocics are sensitive to environmental conditions. Oxytocin itself is a peptide hormone containing nine amino acids with an intramolecular disulfide bridge (Fig 1) [15]. It is highly sensitive to elevated temperatures and may degrade quickly when inappropriately stored, especially in tropical climates [16, 17]. The World Health Organization (WHO) recommends to store all preparations of oxytocin in the refrigerator, i.e. between 2˚C and 8˚C [16]. However, some commercial oxytocin preparations carry recommendations for non-refrigerated storage [18, 19]. Oxytocin stability furthermore depends on the pH value, with an optimum stability at pH 4.5 [20]. Both the United States Pharmacopeia (USP) and the International Pharmacopeia (Ph. Int.) demand that oxytocin injections must have a pH value between 3.0 and 5.0 [19].

A systematic review published by Torloni et al. in 2016 [13] listed eight studies on the quality of oxytocin conducted in LMICs. In a subsequent systematic review published by the same authors in 2020 [14], the number of included oxytocin quality studies had increased to 14. Overall, 39.7% of the oxytocin samples investigated in all these studies had been reported to fail quality testing. An insufficient content of the active pharmaceutical ingredient (API) represented by far the most frequently found deficiency. The overall percentage of failing samples had been 31.4% for studies conducted in the time period of 2000–2011 (n = 363 samples), but had increased to 44.4% for studies conducted from the year 2012 onwards (n = 611 samples) [14]. This indicates that in the last two decades, the quality problems of oxytocin have increased rather than decreased.

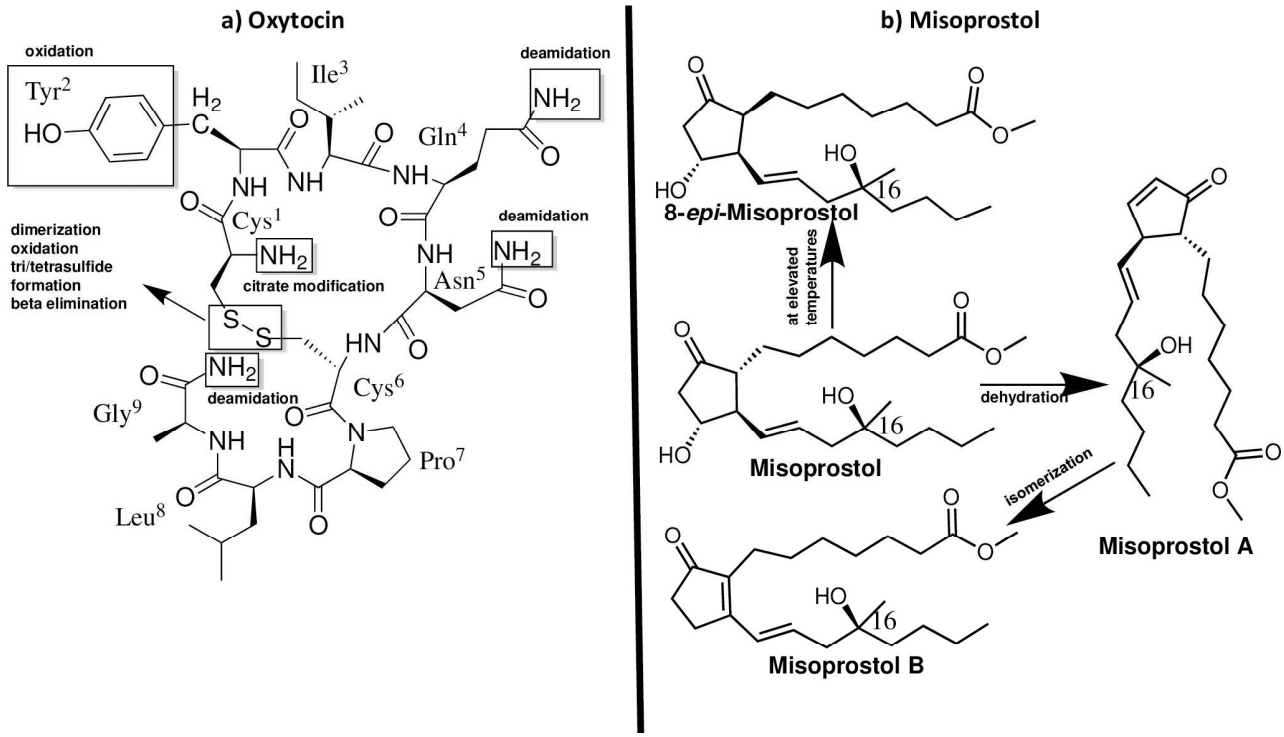

Fig 1. Structures of oxytocin (modified from [21]) and misoprostol, and their typical degradation mechanisms. Commercial misoprostol is a mixture, containing the depicted structure, its epimer at C16, and the enantiomers of both compounds. Likewise, the degradation products of misoprostol contain the corresponding stereoisomers.

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The percentage of oxytocin samples reported to fail quality testing varies notably between different studies. Anyakora et al. [22] reported that 74.2% of the 159 oxytocin samples collected in Nigeria failed quality testing. Similarly, Stanton et al. [23] reported a failure rate of 73.9% upon investigation of 46 oxytocin samples from Ghana. On the other hand, Hagen et al. [24] showed that only 11% out of 65 oxytocin samples collected in Malawi failed testing, and only with moderate deviations from the pharmacopeial specifications. Another study conducted in Ethiopia reported a failure rate of only 4% within 45 oxytocin samples [25]. In all these studies, the failure rate resulted nearly exclusively from an incorrect API amount determined in the samples [14]. Therefore, the different failure rates reported are not due to different parameters being tested in the different studies.

Misoprostol (Fig 1) is an analog of prostaglandin E1. Commercial preparations contain a mixture of both its epimers at C-16, and their enantiomers [26]. Misoprostol is a viscous oil at room temperature and is extremely unstable in the presence of water [27]. Both the raw material and the finished products have to be carefully protected from humidity [27, 28]. In finished pharmaceutical products, misoprostol must be stabilized in form of a 1% dispersion in hydroxypropyl methylcellulose (HPMC) [29], since in the absence of HPMC misoprostol quickly undergoes dehydration, isomerization and epimerization reactions (Fig 1), resulting in a loss of activity. A study by Hall [28] on 215 misoprostol samples, collected in 15 LMICs, reported an incorrect API content in 45% of the samples, and a decomposition of misoprostol in those samples which were packaged in plastic-aluminium blisters. Therefore, it has been strongly recommended that misoprostol tablets should be packaged in double-sided aluminium blisters to protect them from moisture [28]. Storage of misoprostol tablets outside the blisters exposes

them to moisture and has been shown to quickly decrease the amount of the active ingredient, and also to reduce hardness and increase friability of the tablets [27]. In spite of these well-documented problems, misoprostol quality in LMICs has received much less attention than oxytocin quality. The above-mentioned review published by Torloni et al. [14] lists, besides the study by Hall, only two further studies which investigated the quality of misoprostol tablets: Anyakora et al. [22] reported that 56 (33.7%) out of 166 misoprostol samples collected in Nigeria failed quality testing due to incorrect API content, but the study did not state the exact amount of API detected in the samples. Hagen et al. [24] reported that 5 (17%) out of 30 misoprostol samples from Malawi failed pharmacopeial specifications, notably all five with extreme deviations since they contained only 12.7–30.2% of the declared amount.

So far, no data on the quality of oxytocin injections and misoprostol tablets in Rwanda have been published, although the above-mentioned findings from other LMICs indicate that the presence of substandard preparations is likely. Therefore, in the present study samples of oxytocin injections and misoprostol tablets were collected from randomly selected government, faith-based and private health facilities and drug outlets, as well as from government medical stores and private wholesalers in Rwanda, and were investigated for their quality according to the United States Pharmacopeia (USP) and the International Pharmacopeia (Ph. Int.), respectively. In parallel to this study, an evaluation of the availability and prices of essential medicines in health facilities of Rwanda, also beyond oxytocin and misoprostol, has been carried out, and the results have been published elsewhere [30].

Methods

Study design and ethical approval

The study protocol and the methods for collection and investigation of the samples were designed following the MEDQUARG guidelines [31] and the WHO Guidelines on the Conduct of Surveys of the Quality of Medicines [32]. Ethical approval to conduct this study was obtained from the College of Medicine and Health Sciences Institutional Review Board (CMHS-IRB) of the University of Rwanda with approval notice No. 026 /CMHS IRB/2018. An authorization to access health facilities and to conduct this study was kindly granted by the Ministry of Health, Rwanda (reference No. 20/1361/DGPHFIS/2018). In fulfilment of the requirements for sample transfer from Rwanda to Germany, a Material Transfer Agreement (MTA) was signed between investigators and the Rwandan Ministry of Health. Consent to import medicine samples for analysis was also obtained from German authorities (Regierungspräsidium Tübingen, Leitstelle Arzneimittelüberwachung).

Selection of sampling sites

Samples of oxytocin and misoprostol were collected in Kigali city and in five districts representing the provinces of Rwanda, i.e. Bugesera district (Eastern Province), Karongi district (Western Province), Musanze district (Northern Province), and Muhanga and Kamonyi districts (Southern Province). Government, faith-based and private facilities were included, i.e. government district hospitals and health centers, faith-based district hospitals and health centers, private retail pharmacies and private clinics/hospitals. A list of these health facilities in Kigali city and in the five selected districts was obtained from the Ministry of Health, comprising altogether 13 district hospitals (government or faith-based), 77 government health centers, 44 faith-based health centers, 36 private clinics, and 234 private retail pharmacies. For each of the five districts and for Kigali city, two hospitals and two health facilities of each of the other categories (government health centers; faith-based health centers; private retail pharmacies; private clinics) were randomly selected using the RAND function of Microsoft Excel.

However, in each of the two districts Muhanga and Kamonyi only a single district hospital existed; these were included into the study. In Musanze district, no district hospital existed, but a government referral hospital, and this was included. Therefore, a total of 57 health facilities and private retail pharmacies were selected. In the course of the study visits, it turned out that one of the selected district hospitals was a specialized orthopedic hospital and did not stock oxytocin or misoprostol. Also, 11 of the 12 selected private clinics stated that they did not store these medicines, and 5 of the 12 retail pharmacies had none of these two medicines available. Therefore, oxytocin and/or misoprostol could be collected from 40 health facilities and retail pharmacies, and these are listed in S1 Table.

In addition to health facilities and retail pharmacies, oxytocics were also collected from the government central medical store (Medical Procurement and Production Division [MPPD]), from two government district pharmacies and from three large private wholesalers. Therefore, samples were collected from a total of 46 different facilities. Fig 2 shows the location of the facilities on a map of Rwanda.

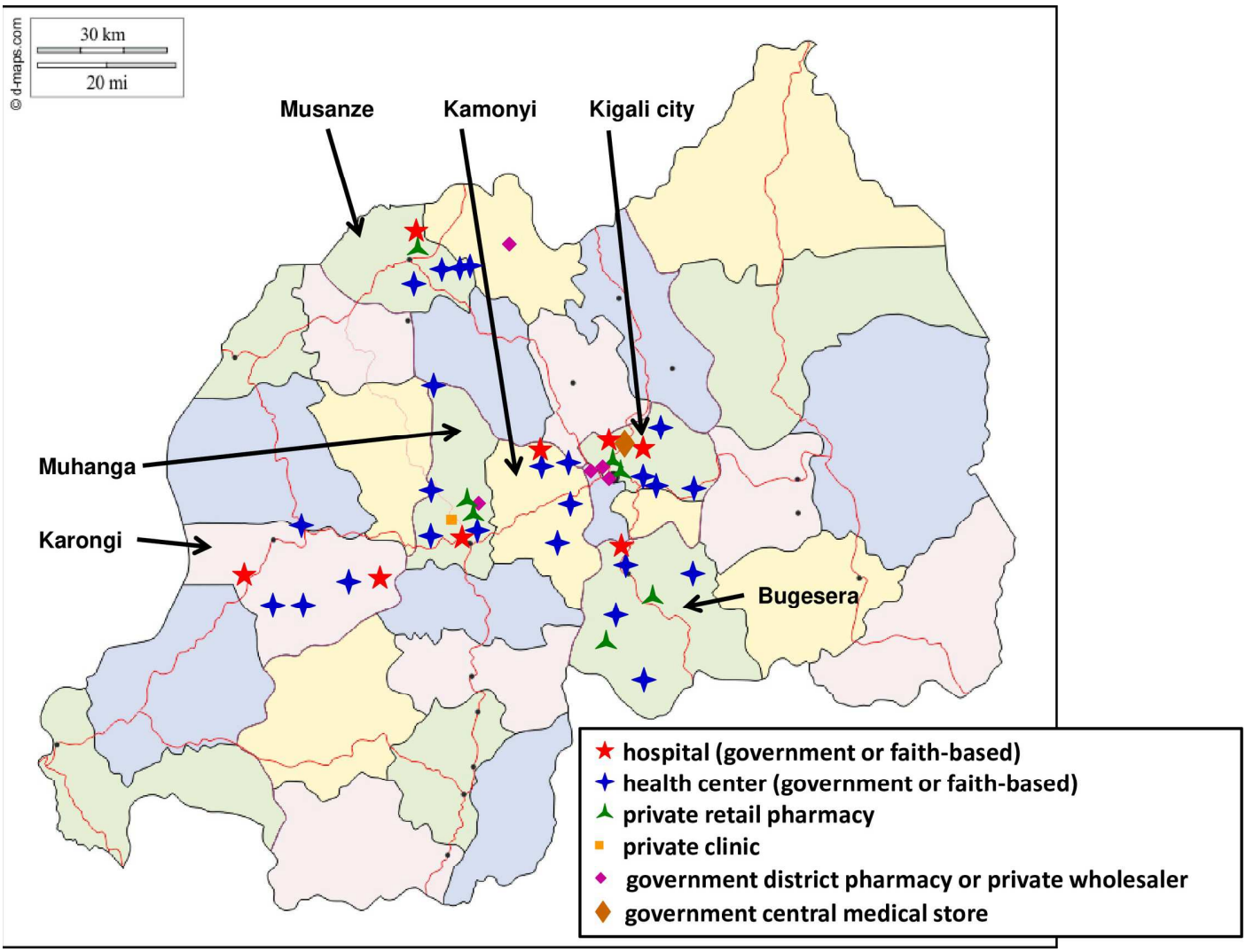

Fig 2. Map of the location of the 46 facilities from which oxytocics were collected. Reprinted (with modifications) from https://d-maps.com/conditions.php?lang=en under a CC BY license, with permission from d-maps.com, original copyright 2007–2020.

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In health facilities which stored oxytocics both in their pharmacy stores and in their maternity wards, samples were collected from both sites if these medicines were available there. Thereby in total samples were collected from 44 storage rooms and 20 maternity wards, as listed in S1 Table.

Sample collection

Sampling was done by the investigator T.B. in March 2018 for the pilot study in Muhanga district, and in September-October 2018 for the main study in the other districts and Kigali city. At each sampling site, from every available brand and batch, 10 vials of oxytocin and 50 tablets of misoprostol were collected if available. A minimum amount of 5 vials of oxytocin and 10 tablets of misoprostol was collected.

When sampling from government and faith-based health facilities, the collected vials and tablets were replaced with medicines which had been purchased by the investigator from the government central medical store or two government district pharmacies, in order to avoid causing stock-outs in the health facilities by the sampling. Samples collected from private facilities (retail pharmacies, private clinics and wholesalers) were paid for in cash. In health facilities and retail pharmacies, samples were collected by an overt approach, i.e. the investigator informed the staff about the purpose of the visit, and the "Sample Site & Drug Purchase Records" shown in S1 Fig were filled and signed by the investigator and by the responsible person at the health facility. Samples from private wholesalers were obtained through a local retail pharmacy using a mystery shopper approach, and no "Sample Site & Drug Purchase Records" were prepared for these purchases.

Upon sample collection, each sample was immediately labeled with a unique sample code. All samples of oxytocin were transported to a central collection site in Rwanda in a 12 V plug-in refrigerator, and subsequently stored between 2˚C and 8˚C until shipment to Germany. Misoprostol tablets were transported and stored at a temperature not exceeding 25˚C. Samples were hand-carried to Tübingen University, Germany, by the investigator T. B. on commercial passenger flights in May 2018 (for the pilot study) and in November 2018 (for the main study). They were placed into appropriate storage conditions at Tübingen University within 24 hours after departure from Rwanda. A temperature data logger was kept with the samples at all times to record the temperature during transport and intermediate storage.

Assessment of oxytocin storage temperatures

Oxytocin storage temperatures were recorded using temperature data loggers (Tempmate M1 by imec Messtechnik GmbH, Heilbronn, Germany). These were placed by the investigator at the storage sites of oxytocin at the time of sample collection, and they automatically recorded the temperature every 10 minutes. Private wholesalers were contacted by a mystery shopper approach, and therefore no temperature data logger could be placed. Temperature data loggers were recollected by the investigator after six months, and the mean kinetic temperature (MKT) was calculated by the imec Messtechnik software.

Registration status of collected medicines

To enquire the registration status of the medicines collected in Rwanda, the Rwanda Food and Drug Authority (RFDA) was contacted. However, the full registration process of medicines by RFDA had not yet been in effect at the time of sample collection. It could not be established which of the collected preparations had been pre-registered according to the previous procedures.

Packaging examination

The information stated on the packaging and in the package inserts of the samples were examined visually for the presence of irregularities or inconsistencies, such as spelling mistakes, unusual batch numbers, unexpected or modified manufacturing or expiry dates, or signs of repacking. The individual dosage units (oxytocin vials and misoprostol tablets) were inspected for visible deficiencies, like color changes, suspended particles within vials, etc. In addition, for misoprostol tablets the material of the primary packaging was recorded, as this is important for misoprostol stability [28].

HPLC analysis

Oxytocin injections were analyzed for identity, assay and pH value following the respective monograph of the United States Pharmacopoeia 40 (USP 40). The assay was performed using High Performance Liquid Chromatography (HPLC; Agilent Infinity 1260 II with binary pump, refrigerated autosampler, integrated column compartment and variable wavelength detector; Agilent Technologies, Santa Clara, CA, USA). A Reprospher column 100 C18, 5 μm; 12.5 cm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany) was used with a column temperature of 21˚C. A linear gradient of mobile phase A (0.1 M aqueous NaH_2PO_4 buffer) and mobile phase B (acetonitrile/water 1:1) was used: 0 min, 30% B; 10 min: 40% B; 17.5 min: 65% B; 20.5 min, 65% B; 23.5 min, 30% B; 26 min, 30% B. Flow rate: 1.5 ml/min. Detection: 220 nm. Injection volume: 70 μl. The diluent used to prepare standard solutions was prepared by dissolving 500 mg of chlorobutanol in 0.5 ml glacial acetic acid, adding 500 mg of ethyl alcohol, 110 mg of sodium acetate and filling up to 100 ml with bi-distilled water. Analysis was performed for three different vials per sample, each injected twice, yielding six measurements per sample. Oxytocin USP Reference Standard purchased from Merck KGaA, Darmstadt, Germany, was used for comparison. The pH value was measured twice for each vial, testing three vials from each sample of oxytocin, and the average value was calculated.

The concentration of the preservative benzyl alcohol, which was only contained in the oxytocin samples manufactured by Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China, was determined using a method modified from Rego and Nelson [33]. The analysis was carried out using HPLC (Agilent 1200 Series with a diode array detector; Agilent Technologies, Santa Clara, CA, USA), with the same column as described above. The mobile phase was composed of 20% acetonitrile and 80% water. Flow rate: 1.5 mL/min. Detection: 254 nm. Injection volume: 50 μl. Samples were diluted 1:30 with bi-distilled water prior to injection, except for sample QOR04 which was injected without dilution. Analysis was performed for at least two different vials per sample, each injected twice. Benzyl alcohol pharmaceutical secondary standard (SIGMA-ALDRICH, St. Louis, MO, USA; Lot LRAC1678; certified purity of 99.98%) was used as reference material.

Misoprostol tablets were analyzed following the respective monograph of the International Pharmacopoeia 2017 (Ph. Int. 2017) for identity, assay and dissolution testing. The Agilent Infinity 1260 II HPLC instrument described above was used with a stainless-steel column packed with ReproSil-XR 120 C18, 5 μm, 150 mm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany) and a guard column containing the same material. The column oven was kept at 35.0˚C. A premixed mobile phase composed of acetonitrile and bi-distilled water in a ratio of 45:55 was used, at a flow rate of 1.5 ml/min in isocratic mode. The injection volume was 100 μl for assay and 250 μl for dissolution testing. HPLC vials were kept in a cooled autosampler at 4˚C until sample injection to avoid degradation. Detection was carried out by UV at 200 nm. Sample and standard solutions were freshly prepared using the mobile phase as diluent. For misoprostol assay, two separate determinations were carried out for each sample. For each determination, five tablets were placed into 50 ml of the mobile phase. In the case of three of

the collected samples, the number of available tablets was insufficient for this procedure, and in these cases for each of the two determinations one tablet was placed into 10 ml of the mobile phase. Misoprostol was dissolved from the tablets using an ultrasonic bath, and ice was added to the bath to avoid degradation by heat. The solution was filtered through Rotilabo PTFE 0.20 μm filters (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and injected into the HPLC, with three injections of each of the two solutions, yielding six measurements per sample. European Pharmacopoeia Reference Standard (batch N˚ 3.0) from the European Directorate for the Quality of Medicines (EDQM) was used for comparison.

Misoprostol tablets were analyzed for related substances according to Ph. Int., using the same HPLC instrumentation and the same column as described above for the assay. The mobile phases consisted of acetonitrile, water and methanol in the ratio 28:69:3 (mobile phase A) and 47:50:3 (mobile phase B), and were used in the gradient mode described in Ph. Int., at a flow rate of 1.5 ml/min and a column temperature of 35˚C. The injection volume was 200 μl. Detection was carried out by UV at 200 nm. Sample solutions were prepared as described in Ph. Int. For comparison, a misoprostol standard solution 20 μg/ml in mobile phase A, and the same standard solution heated for 1 hour at 80˚C, were used. Each of the sample and standard solutions was injected twice. The different degradation products of misoprostol (Fig 1) were identified as described by Ph.Int., based on their relative retention times compared to misoprostol.

Dissolution testing was conducted according to Ph. Int. using a dissolution tester PT-WS 610 (Pharma Test Apparatebau AG, Hainburg, Germany). Into each of the six dissolution vessels filled with 500 ml of de-ionized water, one tablet was placed. The test was conducted at 37 ± 0.5˚C with a paddle rotational speed of 50 revolutions per minute. Samples were withdrawn after 30 min through an in-line filter, and 250 μl of these solutions were injected into the Infinity 1260 II HPLC system described above.

The system suitability for the method for oxytocin assay was verified according to USP 40, and for the methods for misoprostol assay and dissolution according to Ph. Int.

For the determination of the benzyl alcohol concentration, linearity and precision of the applied method were validated according to the International Council for Harmonization (ICH) guideline Q2(R1) [34]. Relative standard deviation of the measurements (repeatability) was 0.2%.

Sample analysis was conducted unblinded to packaging. All oxytocin and misoprostol samples were analyzed before reaching their expiry dates.

Mass spectrometric analysis

Gas chromatography-mass spectrometry (GC-MS) was carried out using a Hewlett Packard/Agilent HP6890 GC system coupled with a HP5973 mass selective detector. The injector temperature was 280˚C. An Agilent HP-5ms Ultra Inert (5%-phenyl)-methylpolysiloxane column 30 m x 0.25 mm with a film thickness 0.25 μm was used. The temperature gradient was 40 to 320˚C with 10˚C/min, followed by 10 min 320˚C isothermal. Helium was used as carrier gas with a flow rate of 1.2 ml/min. Electron impact ionization (EI) was carried out with 70 eV, and a single quadrupole analyzer was used.

HPLC-MS/MS analysis was conducted on a Thermo Scientific UltiMate 3000 HPLC-System coupled with an ESI-TOF Bruker maXis 4G (Bruker Daltonics, Bremen, Germany) in the positive mode.

Definitions for substandard and falsified medicines

Samples were classified as within specification or out of specification (= substandard) based on the criteria of USP 40 for assay and pH value in case of oxytocin injections, and based on the criteria of the Ph. Int. 2017 for assay and dissolution in case of misoprostol tablets. According

to these pharmacopoeias, both oxytocin injections and misoprostol tablets must contain not less than 90.0% and not more than 110.0% of the declared amount of the active pharmaceutical ingredient (API). For oxytocin vials, the pH value must be between 3.0 and 5.0. For dissolution testing of misoprostol tablets, the amount in solution must not be less than 80% (Q) of the amount declared on the label.

Following the terminology introduced by earlier studies of WHO, assay results deviating more than 20% from the declared API content, and dissolution results falling more than 25% below the pharmacopeial Q value, were considered as extreme deviations [35, 36]. Lesser deviations from pharmacopeial specifications were considered as moderate deviations. As per definition of WHO [11], products that deliberately/fraudulently misrepresent their identity, composition or source were considered falsified.

Data analysis

Excel (Microsoft Office Professional Plus 2019) was used to calculate means, medians and percentiles, and relative standard deviations (RSD). Figures of the distribution of the assay test results for oxytocin injections and misoprostol tablets, and the dissolution test results for misoprostol tablets, were generated using the statistical software JMP 14.2 (SAS GmbH, Heidelberg, Germany).

Information of national authorities and stakeholders

Following the request stated in the permission No 20/1361/DGPHFIS/2018 from the Ministry of Health of Rwanda to conduct the present study, the authors have submitted on December 2, 2018, an alert letter to the Rwandan authorities about two extremely substandard brands of misoprostol tablets found to circulate in Rwanda (see Results section). Furthermore, this manuscript was shared with the Rwandan Food and Drug Authority (RFDA) and with the WHO Rapid Alert System.

Results

Overview of sampling sites

As shown in Table 1, oxytocics could be collected from 40 of the 57 randomly selected health facilities and retail pharmacies, as well as from three government medical stores and three private wholesalers (see Methods section). In health facilities, samples were collected both from medicine storage rooms and from maternity wards if available.

Notably, oxytocin injections were available in every visited government and faith-based health facility, i.e. both in hospitals and health centers, consistent with the recommendations of the Rwanda National List of Essential Medicines (REML) [9]. According to the REML, misoprostol tablets are expected to be available as oxytocics in hospitals but not in health centers. Indeed, all eight hospitals but only three of the 24 visited health centers, had misoprostol tablets available in a sufficient amount for sampling.

A total of 12 retail pharmacies had been randomly selected and visited, but only seven had misoprostol tablets available, and none stored oxytocin injections. Of the 12 randomly selected private clinics, most stated that they do not offer maternity services, and oxytocin could be collected only from a single private clinic.

A detailed list of all sampling sites, with the numbers of oxytocin vials and misoprostol tablets collected at each site, is shown in S1 Table.

Overview of collected oxytocin samples

A total of 57 oxytocin samples were collected. All of these were packaged in vials of 1 ml, with a concentration of 10 IU/ml. As shown in Table 2, the 57 samples represented only four

Table 1. Overview of sampling sites for oxytocin injections and misoprostol tablets.

Category of health facility	Number of facilities	Sites in facility	Number of sites	Number of oxytocin samples collected	Number of misoprostol samples collected
Government hospitals	3	storage rooms	3	3	4*
		maternity wards	2	2	1
Faith-based hospitals	5	storage rooms	5	5	5
		maternity wards	5	5	2
Government health centers	12	storage rooms	11	11	2
		maternity wards	6	6	0
Faith-based health centers	12	storage rooms	11	11	1
		maternity wards	7	7	0
Private clinics	1	storage rooms	1	1	0
Retail pharmacies	7	storage rooms	7	0	7
Government central medical store	1	storage rooms	1	2*	0
Government district pharmacies	2	storage rooms	2	1	2
Private wholesalers	3	storage rooms	3	3	1
Total	**46**		**64**	**57**	**25**

* Two brands collected in one sampling site.

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different brands and a total of seven different batches. In addition to the facilities listed in Table 1, three further private pharmaceutical wholesalers in Kigali were contacted, as well as the procurement organization for faith-based health facilities, i.e. the Bureau de Formations

Table 2. List of oxytocin brands and batches collected in this study.

Brand name and stated manufacturer	WHO Pre-Qualified/ SRA	Stated storage temperature requirement	Batch N°	Manufacture/ Expiry Date	Number of samples
Oxytocin injection	-	room temperature [a]	1606573	Jun 16 / Jun 19	24
Jiangxi Xierkangtai Pharmaceutical Co. Ltd			1604521	Apr 16 / Apr 19	9
China					
Steroxine 10 IU/1 ml [b]	SRA	2–8˚C	160042	Feb 16 / Jan 19	1
Laboratoires Sterop					
Belgium			160269	Sep 16 / Aug 19	1
Oxytocin 10 IU/ml	WHO-PQ	2–8˚C	37611016	Oct 16 / Oct 20	4
AS Grindeks					
Latvia [c]			37711116	Nov 16 / Nov 20	1
Oxytocin 10	SRA	2–8˚C	70779A	Sep 17 / Sep 20	17
Rotexmedica GmbH Arzneimittelwerk					
Germany					

WHO-PQ = WHO-prequalified medicine; SRA = manufactured in a country with stringent regulatory authority.

[a] Storage requirement stated on packaging: "Store in a cool dry place, away from light"; storage requirement stated on the package insert: "Store in a dark place at room temperature, protect from light."

[b] Batch 160269 was labeled with the unbranded generic name "Oxytocin 10 IU/1 ml", all other information was identical as in batch 160042.

[c] Marketing authorization holder: Peckforton Pharmaceuticals Ltd., United Kingdom.

https://doi.org/10.1371/journal.pone.0245054.t002

Médicales Agréés du Rwanda (BUFMAR), but none of these had any other oxytocin brands or batches in stock than those shown in Table 2. Therefore, the preparations listed in Table 2 appear to represent most (or all) oxytocin batches which were in circulation in Rwanda at the time of sample collection.

The most frequently encountered preparation, representing 33 samples, was stated to be manufactured by Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China. On its secondary packaging, the storage requirement was stated as "Store in a cool dry place, away from light", while in the package insert, the storage requirement showed a slightly different wording: "Store in a dark place at room temperature, protect from light."

The three other brands (Table 2) were stated to be manufactured in European countries with stringent regulatory authorities (SRAs) [37], and all of them were labeled for refrigerated storage (i.e. storage at 2–8˚C). The product manufactured by AS Grindeks, Latvia (marketing authorization holder: Peckforton Pharmaceuticals Ltd., UK) was a WHO-prequalified medicine [38, 39].

The stated shelf life of three out of the four brands was three years, and four years in case of the preparation by Grindeks. The package inserts of the preparations by Sterop (Belgium), Grindeks (Latvia) and Rotexmedica GmbH Arzneimittelwerk (Germany) stated the excipients, i.e. sodium chloride, different buffering agents, water for injection, and in case of the Sterop preparation also chlorobutanol, a preservative and stabilizing agent [40]. In contrast, no excipients were stated for the product by Jiangxi Xierkangtai.

The preparations by Jiangxi Xierkangtai (China), by Rotexmedica (Germany) and by Grindeks (Latvia) were all found in both government and faith-based facilities. In contrast, the preparation by Sterop (Belgium) was only found in two private pharmaceutical wholesalers at the time of sample collection. No expired samples of oxytocin were found to be in circulation.

Oxytocin storage conditions

Out of 57 samples of oxytocin, 33 were labeled for room temperature storage, and 24 for refrigerated storage. The actual storage place in the facilities could be inspected at 52 of the 56 sampling sites; it could not be inspected at the pharmaceutical wholesalers which were contacted by a mystery shopper approach. Notably, in all inspected sites the actual storage place (i.e. inside or outside the refrigerator) correctly corresponded to the storage recommendation of the manufacturer.

Temperature data loggers were placed at the storage places of oxytocin and recollected after six months. In five cases, the loggers failed to record data or got lost in the facility, but from 47 oxytocin storage sites temperature recordings were obtained. These comprised 18 refrigerated and 29 non-refrigerated storage places. The results recorded at the individual sites are listed in S1 Table.

In 13 out of the 18 refrigerated oxytocin storage sites, the recorded mean kinetic temperature (MKT) ranged from 4.3˚C to 7.3˚C, compliant with the manufacturers' storage requirement of 2–8˚C. In one site, the recorded storage temperature was too low (MKT = -1.3˚C). At this site, except for short temperature spikes (possibly due to opening of the refrigerator), the recorded temperature was constantly around -2˚C. Though this indicates an incorrect temperature setting of the refrigerator, it is unlikely to have caused freezing of the preparation, due to the presence of excipients in the oxytocin vials. In three sites, the recorded MKTs in the refrigerators were slightly too high (8.5˚C, 9.1˚C and 10.5˚C, respectively). And in one further site (a maternity ward of a faith-based hospital), oxytocin was not stored in a refrigerator but in a cool box, reportedly only for immediate use and for not more than 24 hours. Indeed, the temperature data logger at that site recorded alternating periods of cold temperature and room

temperature. The MKT in this cool box over the entire recording period resulted as 15.8˚C, but the storage temperature at the times of oxytocin storage may well have been correct.

In most of the other storage sites, the temperatures were largely constant over the entire recording period. Only in one case larger fluctuations (between +15˚C and -2˚C) of longer duration were observed. Five sites showed few very brief periods of temperatures above 8˚C which may have been due to occasional power failures, as also mentioned by the staff of these facilities.

At the 29 non-refrigerated oxytocin storage sites, the median value of the recorded MKTs was 23.5˚C (range 19.8–26.3˚C), reflecting the temperate climate of Rwanda, most of which is situated at an altitude of approximately 1500 m.

Packaging examination and visual inspection of oxytocin samples

Packaging examination revealed no irregularities except for a few minor mistakes in the use of upper and lower case letters on the packaging and in the package inserts of the product stated to be manufactured by Jiangxi Xierkangtai (China). However, one sampling site (private clinic) stored the vials of oxytocin not in their original secondary packaging but in a box labeled as dexamethasone. Visual inspection showed no deficiencies like color changes or suspended particles.

Chemical analysis of oxytocin samples

The presence of oxytocin could be confirmed for all samples, therefore neither packaging examination nor chemical analysis indicated the presence of any falsified oxytocin samples. Also the pH value which is important for oxytocin stability was within USP specifications (3.0–5.0) for all samples (S2 Table). Fig 3 shows the distribution of the assay results for the investigated brands and batches. Notably, none of the 57 samples showed an insufficient content of oxytocin.

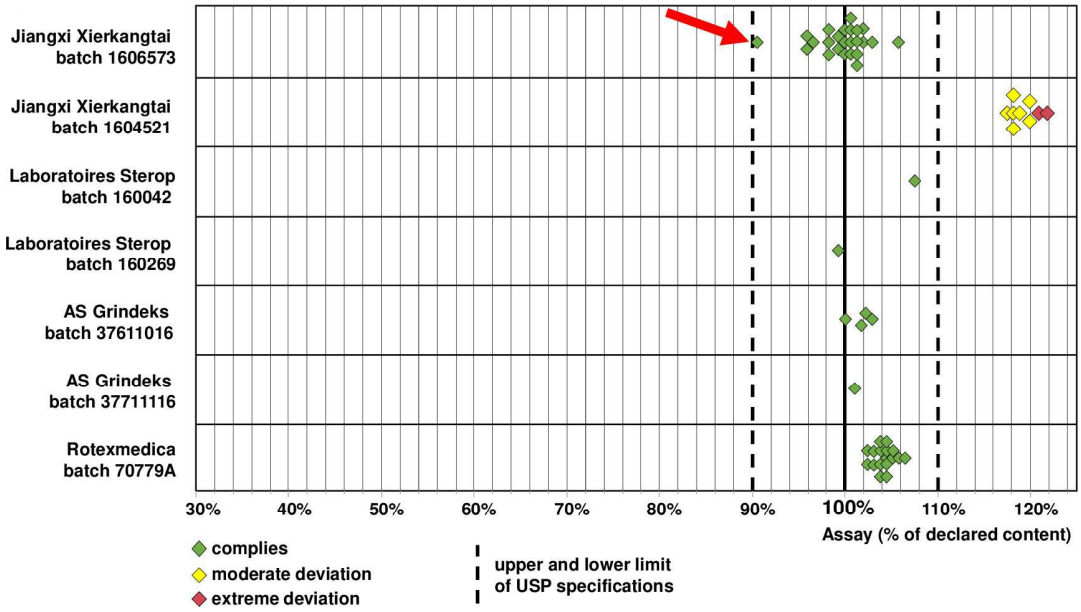

Fig 3. Content of oxytocin determined in each of the investigated samples. The arrow marks a sample with deviating content of the active pharmaceutical ingredient oxytocin and of the preservative benzyl alcohol (see main text and Fig 4).

https://doi.org/10.1371/journal.pone.0245054.g003

The 17 samples of Rotexmedica (Germany) all belonged to one single batch and showed a median content of 103.8% of the declared amount. These samples had been collected at different points of the supply chain (government central medical store; hospitals; government and faith-based health centers), but nevertheless showed a very uniform API content (range 102.0–105.9% of the declared amount). Likewise, the samples of AS Grindeks (Latvia) showed a very uniform content (range 99.9–102.8% of the declared amount). The two samples of Sterop (Belgium), belonging to different batches, contained 99.6 and 107.8% of the declared amount.

In sharp contrast, all nine samples of batch no. 1604521, stated to be manufactured by Jiangxi Xierkangtai (China) were found to deviate from USP specifications (90–110% of the declared amount) by containing too high amounts of oxytocin (median 118.0%). For two of these samples, the obtained assay result even exceeded the declared content by more than 20%, which represents an extreme deviation following the definition used in previous studies of WHO [35, 36].

In the other batch by Jiangxi Xierkangtai (batch no. 1606573), 23 of the 24 samples ranged in their content from 95.6 to 105.5% of the declared amount, well inside the content range specified by USP. However, for one sample of this batch, collected from a private wholesaler (sample no. QOR04; collected from facility no. 44 in S2 Table), the oxytocin content determined for three investigated vials was 89.7%, 90.4% and 91.0% of the declared amount, respectively, therefore on the borderline of USP specifications.

Fig 3 shows that the oxytocin content varied between different brands and batches. The results of the chemical analysis of each oxytocin sample, and the age of the samples at the time of analysis, are shown in S2 Table. In case of Jiangxi Xierkangtai batch no. 1606573, samples which were collected during the pilot study in March 2018 were 24 months old at the time of analysis. These did not show a higher content than those samples which were collected in the main study in September and October 2018 and were 30 months old at time of analysis. This, and the other data in in S2 Table, provide no evidence that differences in the age of the samples were important for the observed differences in oxytocin content.

Unexpectedly, the HPLC assay of the samples stating Jiangxi Xierkangtai as manufacturer showed a very large peak of an unknown substance eluting approximately two minutes earlier than oxytocin (Fig 4). Neither the packaging nor the package insert gave any information on the identity of this substance. An enquiry was sent to the three e-mail addresses given on the website of the stated manufacturer, but remained unanswered. Therefore, the identity of the unknown substance was investigated by mass spectrometry. GC-MS analysis showed the following mass and fragmentation: m/z 108 (M^+; 92%), 107 (67%), 91 (17%), 79 (100%), 77 (63%), 65 (7%), 51 (12%), 39 (8%). Comparison to the database of the National Institute of Standards and Technologies, Gaithersburg, MD, USA (NIST; http://webbook.nist.gov) showed that these data were identical to those of benzyl alcohol, a commonly used preservative in parenteral pharmaceutical preparations [41]. Subsequently, the identity of the unknown substance was confirmed by both GC-MS and HPLC-MS investigation in comparison to authentic benzyl alcohol, showing identical retention times and mass spectrometric fragmentations. The concentration of benzyl alcohol was determined as 0.9% in nearly all samples.

However, the oxytocin sample no. QOR04 (which had already been noticed to contain the lowest oxytocin amount of all investigated samples, see above) was found to contain only 0.004% benzyl alcohol, showing identical concentrations in all vials. This sample had been collected from a private wholesaler in Kigali. It is highlighted in Fig 3 by an arrow, and in S2 Table by bold print. Its batch number as well as primary and secondary packaging and package insert were identical to those from the 23 other samples which stated Jiangxi Xierkangtai as manufacturer (S2 Fig).

Within all other investigated samples by Jiangxi Xierkangtai only a single vial was found with a deviating concentration of benzyl alcohol. It belonged to a sample (no. QOR75) which

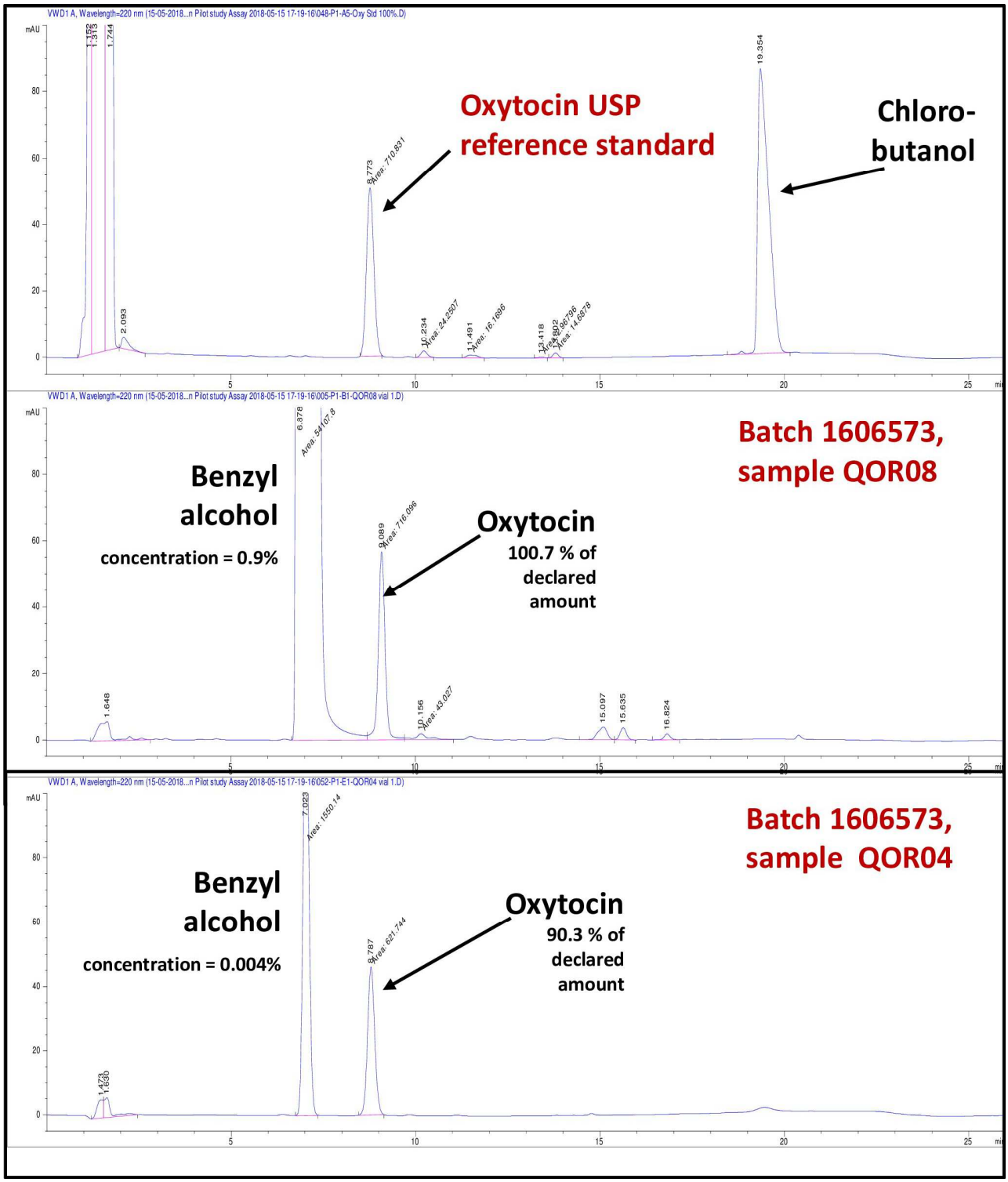

Fig 4. HPLC detection of the undeclared preservative benzyl alcohol, present in different concentrations in oxytocin samples of the same batch, stated to be manufactured by Jiangxi Xierkangtai Pharmaceuticals Co. Ltd (China).

https://doi.org/10.1371/journal.pone.0245054.g004

had been collected in a government district hospital (listed as facility no. 2 in S1 Table). That vial showed a concentration of 0.018% benzyl alcohol and an oxytocin content of 88.3% of the declared amount. The other two investigated vials of the same sample QOR075 carried the same batch number and had the same appearance, but showed a concentration of 0.9% benzyl alcohol and oxytocin contents of 99.7 and 100.2% of the declared amount, respectively.

Overview of collected misoprostol samples

Twenty-five samples of misoprostol tablets were collected. All of them had a stated content of 200 μg per tablet. These samples represented six brands and a total of ten batches (Table 3). As mentioned above for oxytocin, also three further wholesalers as well as the faith-based procurement agency BUFMAR were contacted, but had no additional brands or batches of misoprostol tablets in stock. The preparations listed in Table 3 therefore appear to represent most (or all) of the batches of misoprostol tablets which were in circulation in Rwanda at the time of sample collection.

The most frequently encountered preparation, representing ten of the 25 collected samples, was the originator product Cytotec®. For two of the three collected batches, the marketing authorization holder was Pfizer Holding (France), and for the third batch it was Continental

Table 3. List of misoprostol brands and batches collected in this study.

Brand name and stated manufacturer	WHO Pre-qualified/ SRA	Stated storage requirements	Batch N°	Manufacture/ Expiry Date	Number of samples
	SRA	No special storage requirements [c]	B15445 [a]	Dec 16 [d] / Nov 19	2
Cytotec® 200 μg			B17173 [a]	Jul 17 [d] / Jun 20	6
Piramal Healthcare UK Limited		Store at room temperature (15–25˚C)	B18097 [b]	Nov 17 [d] / Oct 20	2
United Kingdom					
Ace Miso®	WHO-PQ	Do not store above 30˚C, protect from light	ACE160963	Sep 16 / Aug 18	1
Acme Formulation Pvt. Ltd.					
India					
MIZO®	-	Store at a temperature not exceeding 30˚C at a dry place	E6SGFT010	Jun 16 / May 18	1
SYNOKEM Pharmaceuticals LTD India			E6SGLT004	Dec 16 / Nov 18	1
Misoprostol 200 mcg Tablets	WHO-PQ	Store at a temperature not exceeding 30˚C	45180301	Feb 18 / Feb 20	2
China Resources,					
ZIZHU Pharmaceuticals Co Ltd					
China					
C-stol®	-	Store below 30˚C. Protect from light and moisture	ERW-005	Mar 18 / Feb 21	3
CORONA Remedies Pvt Ltd					
India					
Cynomax®	-	Store at 20 to 25˚C in a dry area	M8TAB1801	May 18/ Apr 20	4
MAXTAR BIO-GENICS					
India			MTYX-1604	Aug 16 / Jul 18	3

WHO-PQ = WHO-prequalified medicine; SRA = manufactured in a country with stringent regulatory authority.

[a] Marketing authorization holder: Pfizer Holding, France.

[b] Marketing authorization holder: Continental Pharma Inc., Belgium.

[c] Package insert: "Tenir hors de la vue et de la portée des enfants. Pas de précaution particulière de conservation". I.e.: "Keep out of sight and reach of children. No special storage requirements."

[d] Manufacturing date not stated on packaging. Shelf-life listed according to information from the websites www.hpra.ie and www.medicines.org.uk/emc.

https://doi.org/10.1371/journal.pone.0245054.t003

Pharma Inc., Belgium (see Table 3). For all three batches, the stated manufacturer of the collected samples was Piramal Healthcare, based in the United Kingdom and therefore in a country with a stringent regulatory authority (SRA). Three further samples were WHO-prequalified medicines [38, 39] and had been produced by Acme Formulation Pvt Ltd, India, or by China Resources Zizhu Pharmaceutical Co, Ltd, China, respectively. The remaining 12 samples represented three generic preparations which were manufactured in India (Table 3), a country without an SRA, and were not WHO-prequalified.

Storage requirements were stated on the packaging and/or in the packaging insert of most misoprostol samples, but the indicated temperatures varied (Table 3): two samples were labeled for storage at 15–25˚C, seven samples at 20–25˚C, and eight samples at ≤ 30˚C. For the remaining eight samples, representing two of the three batches of the originator product Cytotec®, the package insert surprisingly stated: "No special storage requirements."

Misoprostol is very unstable and must be formulated as a 1% dispersion in hydroxypropyl methylcellulose (HPMC; hypromellose) to protect it from degradation [29]. For both the originator product and the WHO-prequalified product by Acme (India), exactly this formulation was stated in the package insert. Zizhu Pharmaceuticals, Co (China), listed hypromellose as an excipient for its product. The Indian company Synokem Pharmaceuticals Ltd (Mizo®) mentioned that misoprostol was contained as a "1% dispersion", but failed to mention what it was dispersed in. And notably, for the products by the Indian companies Corona Remedies Pvt., Ltd and Maxtar Bio-Genics, no information on excipients was given, and it remained unclear whether or not HPMC was contained therein.

The shelf life of four of the brands was given as two years, while for C-stol® (Corona Remedies, India) the stated shelf life was three years. For the originator medicine Cytotec® (Piramal Health Care, UK), only an expiry date but no manufacturing date was given on the packaging, but internet databases stated a shelf life of three years for this brand (see Table 3).

Misoprostol blister materials and storage conditions

While oxytocin degradation is primarily caused by elevated storage temperatures, misoprostol degradation is especially caused by exposure to moisture [27, 28]. Therefore, misoprostol tablets must be packaged in double-sided aluminium blisters, not in conventional plastic-aluminium blisters. Indeed, all collected samples were correctly packaged in double-sided aluminium blisters.

In the present study, storage temperatures were systematically recorded for oxytocin but not for misoprostol. However, the storage temperatures recorded at the 29 non-refrigerated oxytocin storage sites, as well as in four of the investigated retail pharmacies (S1 Table) may present a good approximation for the misoprostol storage temperatures in health facilities and drug outlets of Rwanda. As mentioned above, the recorded mean kinetic temperatures (MKTs) were moderate, and only in five out of 33 sites they slightly exceeded 25˚C (with recorded MKTs between 25.1 and 26.3˚C). From one of these sites (MKT 25.4˚C), a misoprostol sample with a stated storage requirement of 20–25˚C was collected (S1 Table), i.e. the MKT exceeded the demanded storage temperature by 0.4˚C. Therefore, in the present study no serious violations of the recommendations for packaging and for storage temperatures of misoprostol tablets were observed.

Chemical analysis of misoprostol samples

The presence of misoprostol was confirmed in all samples, but assay and dissolution testing revealed dramatic shortcomings in two of the six investigated brands. As shown in Fig 5, the assay results were within Ph. Int. specification (90–110% of the declared amount) for all samples of the originator product, of the two WHO-prequalified brands and of the product by

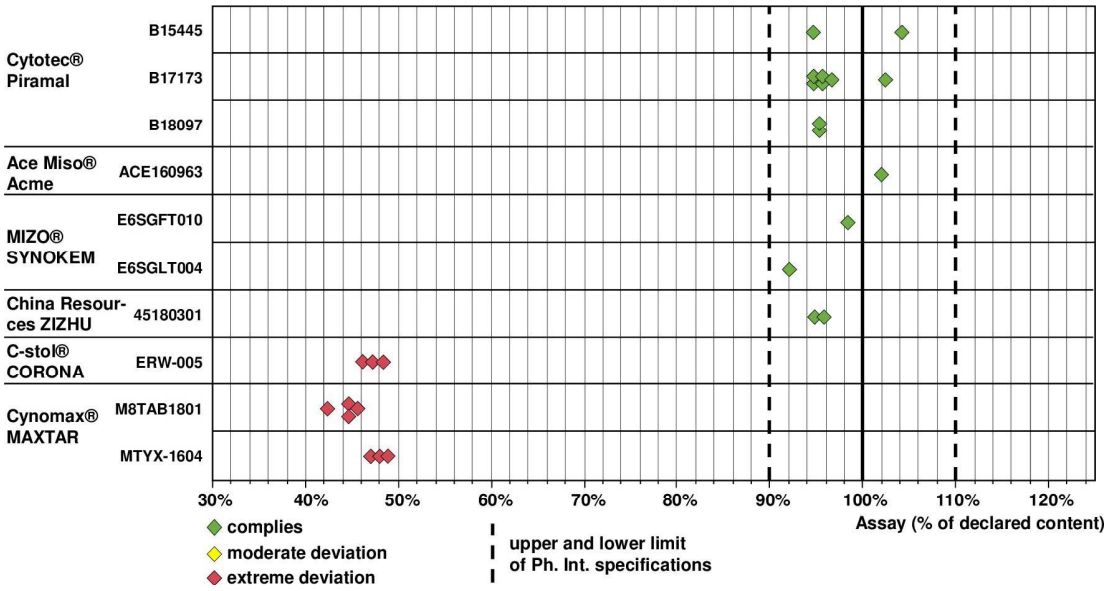

Fig 5. Content of misoprostol determined in each of the investigated samples.

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Synokem Pharmaceuticals, India. In sharp contrast, all three samples of C-stol® and all seven samples of Cynomax® showed less than 50% of the declared content, i.e. failed assay testing with extreme deviations. Therefore, an additional HPLC analysis was carried out for these samples, using the method of Ph. Int. for detection of related substances. This clearly showed large amounts of the typical degradation products of misoprostol (S3 Fig), suggesting that the low content of misoprostol in these two preparations was due to degradation of the API. The two investigated batches of Cynomax® had different ages at the time of analysis (7 and 22 months since the date of manufacture; S3 Table) and showed different API contents (mean 44.4% and 47.8% of the declared content). However, contrary to expectations the higher content was recorded for the older sample, indicating that the difference in content may not have been due to different age but due to differences in manufacture; different transport and storage conditions represent another possible reason.

All misoprostol samples were also tested for dissolution of the API. The International Pharmacopoeia demands that from misoprostol tablets at least 80% of the declared API amount must be released in 30 min under the defined conditions. As shown in Fig 6, all samples which had passed assay testing also passed dissolution testing. However, the samples of C-stol® and Cynomax®, which had been shown already in assay testing to contain less than 50% of the declared API amount, obviously failed dissolution testing with extreme deviations from USP specifications. From the C-stol® samples, approximately one quarter of the contained amount of the API did not dissolve, proving shortcomings in dissolution in addition to the extreme non-compliance in the assay. The results of the chemical analysis of each misoprostol sample, and the age of the samples at the time of analysis, are shown in S3 Table.

The two extremely non-compliant brands had been collected both from government and from faith-based health facilities, and from one retail pharmacy.

Product recall in Rwanda

The Rwanda Food and Drug Authority (RFDA) was alerted by the authors of this study about the two extremely substandard brands of misoprostol tablets by e-mail on December 2, 2018.

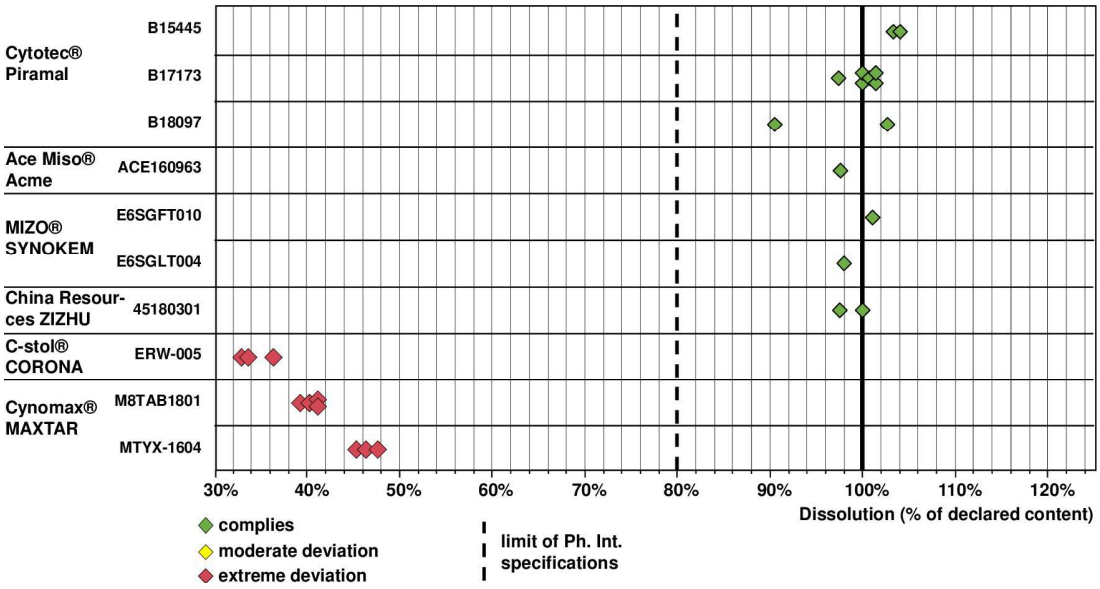

Fig 6. Dissolution of misoprostol determined in each of the investigated samples.

https://doi.org/10.1371/journal.pone.0245054.g006

RFDA invited the authors to a meeting at RFDA which took place on December 4, 2018. On December 7, 2018 RFDA issued an alert (Ref 079/Rwanda FDA/2018) requesting all suppliers and retailers to stop the distribution of these products and to put them in quarantine; all public and private hospitals, health centers, clinics and retail pharmacies were instructed to stop dispensing these products until investigations on the quality issues by Rwanda FDA were completed. Subsequently, RFDA issued a formal recall (Ref 0108/Rwanda FDA/2019 of 13 February 2019), stating that RFDA's investigations had revealed the indicted batches of misoprostol tablets to be substandard, and instructing all wholesalers, retailers, district pharmacies, public and private health facilities that the indicted batches should be returned to the supplier for suitable disposal.

Discussion

The present study showed excellent (100%) availability of oxytocin injections in the investigated government and faith-based health facilities. Also misoprostol tablets were available as oxytocics in all investigated hospitals, as foreseen by the Rwanda National List of Essential Medicines [9]. This proves remarkable success of the health authorities of Rwanda in assuring the availability of these life-saving commodities in hospitals and health centers.

Oxytocin storage inside or outside the refrigerator was found to exactly follow the manufacturers' instructions. For samples labeled for storage at 2–8˚C, the correct storage temperature was maintained well in most of the sites, with only few and probably inconsequential deviations. Again, this proves success of the health authorities of Rwanda, and stands in positive contrast to reports on oxytocin storage conditions in many other LMICs [19, 24, 25, 42–45]. A direct consequence of this success may be the fact that not a single sample of oxytocin was found which had an insufficient content of the API, again in contrast to reports from many other countries [22, 24, 25]. Of course, it cannot be excluded that knowledge of being monitored in this study may have influenced the behavior of health facility staff.

The number of different brands of oxytocin and misoprostol circulating in Rwanda at the time of sample collection was remarkably small. This may be related to the small market size of

Rwanda: many manufacturers and international distributors may not see sufficient economic incentive to engage in medicine sales in this country. Possibly, this can limit the ability of public and private stakeholders to select good-quality, affordable medicines in their procurement.

Out of ten brands collected in total (4 of oxytocin injections, 6 of misoprostol tablets), three were prequalified by WHO [38, 39], and three more were manufactured in countries with stringent regulatory authorities (SRAs) [37]. Together, these six brands represented 45% of the samples collected in this study, and notably all these samples passed the quality tests in this study with good results, indicating that prequalification by WHO, as well as production in a country with an SRA, is a reliable predictor of good quality of medicines.

In stark contrast, three out of the four brands which were neither WHO-prequalified nor produced in a country with an SRA showed serious quality deficiencies. Most alarmingly, every investigated sample of the misoprostol products Cynomax® (stated manufacturer Maxtar Bio-Genics, India) and C-stol® (stated manufacturer Corona Remedies, India) failed assay and dissolution testing with extreme deviations from the pharmacopeial specifications, likely to result in clinical inefficacy. Large amounts of misoprostol degradation products were detected in these two brands, indicating that the API had degraded to a large extent. Notably, the package inserts of both preparations did not mention whether or not HPMC (hypromellose) had been used in the formulation of these tablets, which is essential for misoprostol stability [29]. Also in Malawi, extremely substandard misoprostol brands, however from different stated manufacturers, have been found [24].

The quality deficiencies observed for the oxytocin injection stating Jiangxi Xierkangtai (China) as manufacturer were not as extreme as those observed for the above-mentioned misoprostol brands, but still alarming. All six samples of one of the two investigated batches of that oxytocin brand exceeded the content limit specified by USP, two of the samples even contained more that 120% of the stated amount. In pharmaceuticals with unstable APIs, a content slightly higher than 100% of the stated amount is often intentionally included to ensure that the content is still above the lower pharmacopeial limit towards the end of the shelf life. However, the pharmacopeias define an upper limit to avoid overdosing of the therapy, and this limit was clearly exceeded in the batch in question.

The oxytocin injections stating Jiangxi Xierkangtai as manufacturer were found to contain benzyl alcohol in a concentration of 0.9%. This compound in this concentration is perfectly acceptable as a preservative in parenteral preparations [39, 46]. However, in most countries regulations demand that the presence of such excipients must be declared in the package insert [47], but for the product in question no excipients were declared at all.

The most worrying observation regarding this product, however, was the detection of vials with a two-hundred-fold lower benzyl alcohol concentration, carrying the same batch number as the vials with 0.9% of that preservative. While this may not cause direct harm to a patient treated with that product, it indicates gross violations of good manufacturing practice, raising strong doubts also about other quality aspects of this product. This kind of problem has not been reported in previous studies of oxytocin quality. However, this problem may easily escape detection in a medicine quality study as it has been visible only in a small number of the investigated vials.

The oxytocin injections by Jiangxi Xierkangtai were labeled for non-refrigerated storage, as also is the case for oxytocin from many other manufacturers [19, 24, 42–44]. Obviously, the use of oxytocin products which do not have to be stored in a refrigerator appears to be an attractive option, especially in LMICs. However, recent studies have shown that oxytocin products labeled for non-refrigerated storage may not have any better stability than products labeled for refrigerated storage, and on the contrary may even be less stable [40, 44]. Therefore,

international stakeholders including WHO have issued the recommendation "Buy quality oxytocin, keep it cool" and recommended that all oxytocin products should be stored at 2–8˚C [16, 48], irrespective of the manufacturer's storage recommendation. Health authorities in Rwanda and elsewhere may consider this recommendation. A heat-stable formulation of the oxytocin analogue carbetocin has recently been added to the WHO Essential Medicines List, and in future it may become a further treatment option for post-partum hemorrhage for use in facilities where storage at 2–8˚C is problematic.

Several previous studies have reported problems of oxytocin quality in LMICs, but the present study shows for Rwanda a decidedly different situation than reported from other countries. The present data suggest that the Rwandan authorities have successfully assured the availability and (in most cases) the appropriate storage of oxytocin according to manufacturers' instructions. The detected problems of oxytocin and especially misoprostol quality must be addressed not so much by improved storage and transportation conditions of the medicines, but by improvements of the supplier qualification in medicine procurement. It may be considered whether for oxytocin and misoprostol, with their well-known problems of quality and stability, procurement should be restricted to WHO-prequalified medicines and to medicines manufactured in countries with stringent regulatory authorities. And as a simple "rule of thumb", the results of this study suggest that medicines for which the excipients are not stated in the package insert should be regarded as doubtful in quality.

So far, misoprostol quality has received much less attention in scientific studies than oxytocin quality [14]. However, the results of this study, of the study of Hall [28] and of a recent study from Malawi [24], show quality problems of different brands of misoprostol which are even much more serious than those reported for oxytocin, with API contents below (or far below) 50% of the stated amount, and this problem needs attention in future studies.

Rwanda has only recently established its national drug regulatory agency, i.e. the Rwanda Food and Drug Authority (RFDA), and this will certainly contribute to increased patient safety. The extremely quick and efficient action which RFDA took on the reported substandard misoprostol brands holds good promise for the future development of medicine quality in this country.

Supporting information

S1 Fig. Sample site & drug purchase record.
(PDF)

S2 Fig. Photos of two samples of oxytocin injections, carrying the same batch number but containing different concentrations of benzyl alcohol.
(PDF)

S3 Fig. HPLC analysis for related substances in two misoprostol samples.
(PDF)

S1 Table. List of included health facilities, number oxytocin vial and misoprostol tablets collected, and recorded oxytocin storage conditions.
(PDF)

S2 Table. Results of chemical analysis of all oxytocin samples.
(PDF)

S3 Table. Results of chemical analysis of all misoprostol samples.
(PDF)

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Author Contributions

Conceptualization: Thomas Bizimana, Nhomsai Hagen, Pierre Claver Kayumba, Lutz Heide.

Data curation: Thomas Bizimana, Nhomsai Hagen, Pierre Claver Kayumba, Lutz Heide.

Formal analysis: Thomas Bizimana, Nhomsai Hagen, Gesa Gnegel, Lutz Heide.

Funding acquisition: Pierre Claver Kayumba, Lutz Heide.

Investigation: Thomas Bizimana, Gesa Gnegel, Lutz Heide.

Methodology: Nhomsai Hagen, Gesa Gnegel, Pierre Claver Kayumba, Lutz Heide.

Project administration: Pierre Claver Kayumba, Lutz Heide.

Supervision: Nhomsai Hagen, Pierre Claver Kayumba, Lutz Heide.

Writing – original draft: Thomas Bizimana.

Writing – review & editing: Nhomsai Hagen, Pierre Claver Kayumba, Lutz Heide.

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SAMPLE SITE & DRUG PURCHASE RECORD

Name of survey site: _____ Date and time of visit: _____
Description of location: _____
Temperature data logger Location: _____ Serial number: _____
Photo taken (5): YES ☐ NO ☐

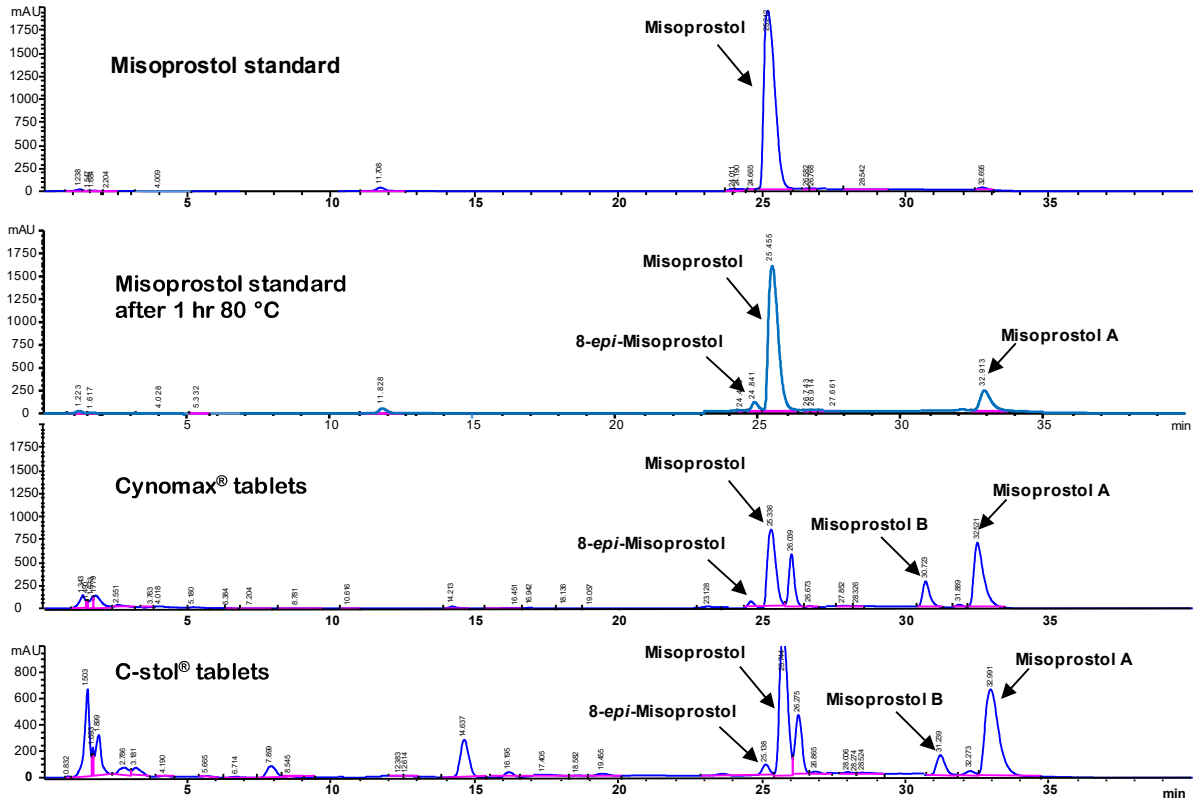
Drug samples collected	Desired quantity	Obtained quantity	QOR reference number	Reason for not obtaining the desired quantity	Brand name	Batch no	Expiry date	Manufacturer, country	Price in Rwf per tabs/vial (if applicable)	Storage conditions according to the manufacturer		Refrigerated (r) / not refrigerated (nr) in original package (op)/out of original package (nop) protected from light (pr) /not protected from light (npr)	1) Thermometer kept with oxytocics? 2) Temperature recorded daily? If yes, please take picture
										Temperature in °C	Relative humidity in %		
Misoprostol 0,2mg tab.	50											(r) ☐ (nr) ☐ (op) ☐ (nop) ☐ (pr) ☐ (npr) ☐	1) yes ☐ no ☐ 2) yes ☐ no ☐
Oxytocin 10 IU	10											(r) ☐ (nr) ☐ (op) ☐ (nop) ☐ (pr) ☐ (npr) ☐	1) yes ☐ no ☐ 2) yes ☐ no ☐

- **If samples are taken without original package, please take picture of original package (showing the name, batch number, expiry date, manufacturing date, name / address of manufacturer)!**
- Collected samples were replaced: YES ☐ NO ☐ Collected samples were paid for: YES ☐ (Attach receipt!) NO ☐
- Name of sampling person: _____ Signature: _____
- Name of person responsible for health facility: _____ Signature: _____

S1 Fig. Sample site & drug purchase record.

S2 Fig: Photos of two samples of oxytocin injections, carrying the same batch number but containing different concentrations of benzyl alcohol.

Top: Sample No. QOR04, containing 0.004% benzyl alcohol.
Bottom: Sample No. QOR05, containing 0.9% benzyl alcohol.



S3 Fig: HPLC analysis for related substances in two misoprostol samples.

Cynomax® tablets (batch M8TAB1801) and C-stol® tablets (batch ERW-005) were investigated. See Methods section for experimental details, and Results section for details of the investigated brands and batches.

S1 Table. List of included health facilities, number oxytocin vial and misoprostol tablets collected, and recorded oxytocin storage conditions

Facility No	Type of facility	District	Sampling site (maternity ward or storage room)	Number of collected misoprostol tablets	Misoprostol storage conditions stated on label	Number of collected oxytocin vials	Oxytocin storage conditions stated on label	Oxytocin storage site	Oxytocin storage shelf: mean kinetic temperature measured over 6 months (°C)	Refrigerator: mean kinetic temperature measured over 6 months (°C)
1	Government district hospital	Kigali city	maternity ward	50	20-25°C	10	2-8°C	refrigerator	-	**9.1**
			storage room	50; 50[e]	<30°C	10	room temperature [f]	shelf	25.3	-
2			storage room	50	<30°C	10	room temperature	shelf	24.2	-
3	Government referral hospital	Musanze	maternity ward	0	-	10	2-8°C	refrigerator	-	**8.5**
			storage room	60	<30°C	10	2-8°C	refrigerator	-	6.5
4	Faith-based district hospital	Bugesera	maternity ward	0	-	6	room temperature	shelf	26.3	-
			storage room	20	20-25°C	10	room temperature	shelf	23.4	-
5		Kamonyi	maternity ward	0	-	10	2-8°C	refrigerator	-	5.4
			storage room	51	<30°C	10	2-8°C	refrigerator	-	6.1
6		Karongi	maternity ward	0	-	10	2-8°C	cool box [a]	-	**15.8**[a]
			storage room	50	15°-25°C	10	2-8°C	refrigerator	-	not det.
7			maternity ward	10	15°-25°C	10	2-8°C	refrigerator	-	6.7
			storage room	50	<30°C	10	2-8°C	refrigerator	-	7.1
8		Muhanga	maternity ward	51	<30°C	10	room temperature	shelf	22.2	-
			storage room	50	20-25°C	10	room temperature	shelf	21.5	-
9	Government health center	Bugesera	maternity ward	0	-	5	room temperature	shelf	23.7	-
			storage room	13	20-25°C	10	room temperature	shelf	24.8	-
10			maternity ward	0	-	10	room temperature	shelf	26.0	-
			storage room	51	<30°C	10	room temperature	shelf	25.4	-
11		Kamonyi	maternity ward	0	-	8	room temperature	shelf	23.5	-
			storage room	0	-	10	room temperature	shelf	24.4	-
12			maternity ward	0	-	10	room temperature	shelf	not det.	-
			storage room	0	-	10	room temperature	shelf	25.0	-
13		Karongi	storage room	0	-	10	2-8°C	refrigerator	-	5.8
14			storage room	0	-	10	2-8°C	refrigerator	-	4.5
15		Kigali city	storage room	0	-	10	room temperature	shelf	23.7	-
16			maternity ward	0	-	10	room temperature	shelf	24.2	-
17		Muhanga	maternity ward	0	-	9	room temperature	shelf	22.3	-
			storage room	0	-	10	room temperature	shelf	21.4	-
18			storage room	0	-	10	room temperature	shelf	21.4	-
19		Musanze	storage room	0	-	10	2-8°C	refrigerator	-	7.3
20			storage room	0	-	10	2-8°C	refrigerator	-	5.7
21	Faith-based health center	Bugesera	maternity ward	0	-	10	room temperature	shelf	23.8	-
			storage room	0	-	10	room temperature	shelf	not det.	-
22			maternity ward	0	-	10	room temperature	shelf	25.1	-
			storage room	30	20-25°C	10	room temperature	shelf	**25.4** [g]	-
23		Kamonyi	storage room	0	-	10	room temperature	shelf	22.7	-
24			maternity ward	0	-	10	room temperature	shelf	22.2	-
			storage room	0	-	10	room temperature	shelf	22.1	-
25		Karongi	storage room	0	-	10	2-8°C	refrigerator	-	4.3
26			maternity ward	0	-	10	2-8°C	refrigerator	-	**-1.3**
27		Kigali city	storage room	0	-	6	2-8°C	refrigerator	-	6.5
28			storage room	0	-	10	2-8°C	refrigerator	-	**10.5**
29		Muhanga	maternity ward	0	-	9	room temperature	shelf	21.0	-
			storage room	0	-	10	room temperature	shelf	21.9	-
30			maternity ward	0	-	10	room temperature	shelf	22.7	-
			storage room	0	-	10	room temperature	shelf	22.4	-
31		Musanze	maternity ward	0	-	10	2-8°C	refrigerator	-	not det.
			storage room	0	-	10	2-8°C	refrigerator	-	5.1
32			storage room	0	-	10	2-8°C	refrigerator	-	4.7
33	Private clinic	Muhanga	storage room	0	-	10	room temperature	shelf	not det.	-
34	Retail pharmacy	Bugesera	storage room	25	no requirements	0	-	n.a.	24.1 [c]	-
35			storage room	29	no requirements	0	-	n.a.	24.0 [c]	-
36		Kigali city	storage room	50	no requirements	0	-	n.a.	24.2 [c]	-
37			storage room	28	no requirements	0	-	n.a.	23.9 [c]	-
38		Muhanga	storage room	50	no requirements	0	-	n.a.	-	-
39			storage room	48	20-25°C	0	-	n.a.	-	-
40		Musanze	storage room	50	no requirements	0	-	n.a.	-	-
41	Government central medical store	Kigali city	storage room	0	-	100;200[d,e]	2-8°C	refrigerator	-	not det.
42	Government district pharmacy	Burera	storage room	250[d]	<30°C	0	-	n.a.	-	-
43		Muhanga	storage room	200[d]	20-25°C	160[d]	room temperature	shelf	19.8	-
44	Private wholesaler	Kigali city	storage room	0	-	100[d]	room temperature	not det. [b]	-	-
45			storage room	0	-	100[d]	2-8°C	not det. [b]	-	-
46			storage room	252[d]	no requirements	100[d]	2-8°C	not det. [b]	-	-

Storage conditions which deviate from manufacturers' requirements are given in **bold, underlined** letters.
n.a. = not applicable
not det. = not determined (temperature logger not placed, lost after placement, or no data recorded).

[a] Oxytocin was stored in this maternity ward in a cool box, reportedly only ordered for immediate use.

[b] From private wholesalers, samples were purchased through a retail pharmacy using a mystery shopper approach. Therefore, storage conditions were not determined, and temperatures data loggers were not placed.

[c] In a few pharmacies, temperature loggers were placed although they did not stock oxytocin.

[d] Higher number of tablets/vials purchased as replacement samples and for additional stability testing.

[e] Two brands collected in one sampling site.

[f] Storage requirement stated on packaging: "Store in a cool dry place, away from light". Storage requirement stated on the package insert: "Store in a dark place at room temperature, protect from light."

[g] Exceeds storage temperature of misoprostol recommended by the manufacturer of the respective sample.

S2 Table. Results of chemical analysis of all oxytocin samples

Brand name and stated manu-facturer	Stated storage tempera-ture require-ment	Batch N°	Manu-facture/ Expiry Date	Faci-lity No.	Facility type	Site in facility	Mean assay (% of declared content)	RSD assay	Mean pH value	RSD pH value	Age of samples (months) [a]
Oxytocin injection; Jiangxi Xier-kangtai Pharma-ceutical Co. Ltd; China	Room tempe-rature	1606573	Jun 16/ Jun 19	2	gov. hospital	stor.	96.1% [b]	6.62% [c]	4.14	0.98%	30
				4	faith-b. hospital	mat.	102.7%	1.56%	4.03	0.48%	30
						stor.	105.5%	2.22%	4.00	0.68%	30
				8	faith-b. hospital	mat.	99.2%	0.68%	4.48	2.17	24
						stor.	99.9%	1.31%	4.40	0.96	24
				9	gov. HC	mat.	101.3%	0.55%	4.05	0.89%	30
						stor.	101.1%	1.66%	4.05	0.76%	30
				11	gov. HC	mat.	100.5%	1.73%	4.05	0.34%	30
						stor.	100.7%	1.73%	4.05	0.33%	30
				12	gov. HC	mat.	100.4%	2.29%	4.08	0.62%	30
						stor.	99.8%	1.29%	4.07	0.73%	30
				17	gov. HC	mat.	95.8%	1.71%	4.56	3.27	24
						stor.	97.9%	0.52%	4.50	1.33	24
				18	gov. HC	stor.	99.0%	0.88%	4.44	0.97	24
				23	faith-b. HC	stor.	99.1%	1.05%	4.12	0.18%	30
				24	faith-b. HC	mat.	100.4%	1.99%	4.06	0.48%	30
						stor.	95.6%	4.94%	4.19	4.38%	30
				29	faith-b. HC	mat.	98.2%	0.75%	4.46	0.73	24
						stor.	98.0%	0.79%	4.48	0.84	24
				30	faith-b. HC	mat.	99.5%	1.32%	4.38	1.18	24
						stor.	99.7%	1.18%	4.39	0.77	24
				33	private clinic	stor.	100.7%	0.96%	4.41	0.94	24
				43	distr. pharm.	stor.	101.5%	0.45%	4.06	0.32%	23
				44	**wholesaler**	**stor.**	**90.3% [b]**	**0.72%**	**4.08**	**0.84%**	**23**
		1604521	Apr 16/ Apr 19	1	gov. hospital	stor.	119.3%	2.09%	3.85	0.13%	32
				10	gov. HC	mat.	118.0%	1.98%	3.83	0.48%	32
						stor.	117.2%	0.52%	3.82	0.51%	32
				15	gov. HC	stor.	119.9%	3.25%	3.84	0.30%	32
				16	gov. HC	mat.	120.6%	2.24%	3.82	0.11%	32
				21	faith-b. HC	stor.	117.6%	1.02%	3.84	0.44%	32
						stor.	121.5%	1.61%	3.82	0.48%	32
				22	faith-b. HC	mat.	117.3%	1.38%	3.80	0.26%	32
						stor.	117.8%	2.31%	3.78	0.47%	32
Steroxine 10 IU/1 ml [d]; Labora-toires Sterop; Belgium	2-8°C	160042	Feb 16/ Jan 19	46	wholesaler	stor.	107.8%	0.62%	3.79	0.22%	27
		160269	Sep 16/ Aug 19	45	wholesaler	stor.	99.6%	1.11%	3.98	0.57%	20
Oxytocin 10 IU/ml; AS GRINDEKS, Latvia [e]	2-8°C	37611016	Oct 16/ Oct 20	1	gov. hospital	mat.	102.8%	1.63%	4.11	0.13%	26
				5	faith-b. hospital	mat.	102.1%	1.99%	4.09	0.15%	26
						stor.	101.9%	3.29%	4.10	0.22%	26
				20	gov. HC	stor.	99.9%	0.74%	4.11	0.10%	26
		37711116	Nov 16/ Nov 20	41	CMS	stor.	100.9%	0.82%	4.09	0.64%	18
Oxytocin 10; Rotex-medica GmbH Arznei-mittelwerk; Germany	2-8°C	70779A	Sep 17/ Sep 20	3	gov. hospital	mat.	104.1%	2.96%	4.06	0.39%	15
						stor.	102.0%	0.26%	4.02	0.20%	15
				6	faith-b. hospital	mat.	103.7%	2.09%	4.09	0.40%	15
						stor.	103.1%	1.53%	4.06	0.35%	15
				7	faith-b. hospital	mat.	103.9%	1.92%	4.02	0.10%	15
						stor.	103.8%	1.84%	4.03	0.10%	15
				13	gov. HC	stor.	103.8%	1.67%	4.02	0.19%	15
				14	gov. HC	stor.	104.2%	1.93%	4.05	0.30%	15
				19	gov. HC	stor.	102.6%	0.44%	4.00	0.10%	15
				25	faith-b. HC	stor.	103.6%	1.76%	4.02	0.24%	15
				26	faith-b. HC	mat.	103.9%	1.67%	4.01	0.19%	15
				27	faith-b. HC	stor.	105.9%	3.96%	3.97	0.19%	15
				28	faith-b. HC	stor.	105.2%	2.37%	3.99	0.21%	15
				31	faith-b. HC	mat.	103.4%	0.86%	4.00	0.14%	15
						stor.	105.4%	2.44%	3.99	0.19%	15
				32	faith-b. HC	stor.	104.6%	2.51%	4.01	0.20%	15
				41	CMS	stor.	102.4%	0.89%	3.99	0.47%	8

RSD = relative standard deviation; gov. = government; faith-b. = faith-based; HC = health center; CMS = government central medical store; stor. = storage room; mat. = maternity ward. The sample found to contain only 0.004 % benzyl alcohol is shown in **bold print**.

[a] Age of sample at time of analysis.
[b] Deviating concentration of the preservative benzyl alcohol observed, see main text.
[c] See text for explanation of the high standard deviation observed for this specific sample.
[d] Batch 160269 was labeled with the unbranded generic name "Oxytocin 10 IU/1 ml", all other information was identical as in batch 160042.
[e] Marketing authorization holder: Peckforton Pharmaceuticals Ltd., United Kingdom

S3 Table. Results of chemical analysis of all misoprostol samples

Brand name and stated manufacturer	Stated storage requirements	Batch N°	Manu-facture/ Expiry Date	Faci-lity no.	Facility type	Site in faci-lity	Mean assay (% of declared content)	RSD assay	Mean dissolution (% of declared content)	RSD disso-lution	Age of samples (months) at time of analysis
Cytotec® 200 µg, Piramal Healthcare UK Limited; United Kingdom [a]	No special storage requirements [b]	B15445 [a]	Dec 16[c] / Nov 19	38	retail pharm.	stor.	104.2%	0.13%	104.2%	0.61%	18
				40	retail pharm.	stor.	94.7%	0.27%	103.5%	2.79%	24
		B17173 [a]	Jul 17[c] / Jun 20	1	gov. hospital	stor.	95.9%	0.19%	100.1%	1.45%	17
				34	retail pharm.	stor.	94.8%	0.29%	101.2%	2.19%	17
				35	retail pharm.	stor.	94.6%	0.35%	100.1%	2.81%	17
				36	retail pharm.	stor.	94.8%	0.58%	100.8%	1.59%	17
				37	retail pharm.	stor.	95.6%	0.48%	101.5%	3.05%	17
				46	wholesaler	stor.	102.4%	1.13%	97.5%	2.82%	10
	Store at room temperature (15-25 °C)	B18097 [b]	Nov 17[c] / Oct 20	6	faith-b. hospital	stor.	95.1%	0.56%	90.6%	4.49%	13
				7	faith-b. hospital	mat.	95.5%	3.80%	102.8%	2.69%	13
Ace Miso®, Acme Formulation Pvt. Ltd.; India	Do not store above 30°C, protect from light	ACE160963	Sep 16/ Aug 18	42	gov. district pharm.	stor.	102.0%	0.44%	97.7%	2.59%	20
MIZO®, SYNOKEM Pharmaceuticals LTD; India	Store at a temperature not exceeding 30°C at a dry place	E6SGFT010	Jun 16/ May 18	8	faith-b. hospital	mat.	98.4%	0.02%	101.2%	3.51%	24
		E6SGLT004	Dec 16/ Nov 18	10	gov. HC	stor.	92.1%	1.62%	98.1%	3.18%	24
China resources, ZIZHU Pharmaceuticals Co Ltd; China	Store at a tempera-ture not exceeding 30 °C	45180301	Feb 18/ Feb 20	1	gov. hospital	stor.	95.8%	0.20%	97.7%	2.31%	10
				3	gov. hospital	stor.	94.8%	0.16%	100.1%	2.64%	10
C-stol®, CORONA Remedies Pvt Ltd; India	Store below 30°C. Protect from light and moisture	ERW-005	Mar 18/ Feb 21	2	gov. hospital	stor.	46.8%	1.33%	32.9%	15.61%	9
				5	faith-b. hospital	stor.	46.2%	0.14%	33.6%	6.82%	9
				7	faith-b. hospital	stor.	48.5%	2.96%	36.4%	4.53%	9
Cynomax®, MAXTAR BIO-GENICS; India	Store at 20° to 25°C in a dry area	M8TAB1801	May 18/ Apr 20	1	gov. hospital	mat.	42.5%	0.51%	39.8%	1.35%	7
				4	faith-b. hospital	stor.	44.6%	0.95%	39.3%	4.15%	7
				9	gov. HC	stor.	45.6%	1.07%	41.1%	3.53%	7
				22	faith-b. HC	stor.	44.9%	0.27%	41.2%	2.37%	7
		MTYX-1604	Aug 16/ Jul 18	8	faith-b. hospital	stor.	48.6%	0.06%	46.4%	3.36%	22
				39	retail pharm.	stor.	47.1%	0.04%	45.3%	1.57%	22
				43	gov. district pharm.	stor.	47.6%	0.24%	47.6%	4.49%	21

RSD = relative standard deviation
gov. = government
faith-b. = faith-based
HC = health center
pharm. = pharmacy
stor. = storage room
mat. = maternity ward.

[a] Marketing authorization holder: Pfizer Holding, France.

[b] Marketing authorization holder: Continental Pharma Inc., Belgium.

[c] Package insert: "Tenir hors de la vue et de la portée des enfants. Pas de précaution particulière de conservation". I.e.: "Keep out of sight and reach of children. No special storage requirements."

[d] Manufacturing date not stated on packaging. Shelf-life listed according to information from the websites www.hpra.ie and www.medicines.org.uk/emc.