A stability study of amphotericin B, colistin and tobramycin in a hydrophilic suspension commonly used for selective decontamination of the digestive tract by HPLC and in vitro potency measurements

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ABSTRACT

Objectives A suspension for oral use which consists of three non-absorbable antibiotics (amphotericin B, colistin and tobramycin) is often used in clinical practice for the selective decontamination of the digestive tract (SDD) of patients in intensive care. Such a therapy is a preventive tool to minimise the risk of pneumonia and bacteraemia in intubated patients. The administration and the treatment results are controversially discussed. One limiting factor for a unique SDD treatment in the hospitals is a lack of adequate data regarding batch formula and stability for such a formulation. Since no detailed procedures, specifications or stability data are available for manufacturing this formulation there may be discrepancies regarding formulation and stability of suspensions prepared in different pharmacies. The aim of this research was to collect the physicochemical and microbiological stability data of a developed, stable standard formulation under defined storage conditions. The effectiveness of the SDD suspension should be preferably proven over a long period. This would help guarantee that all patients receive the same preparation, therefore, ensuring similar efficacy and improved safety.

Methods An adequate formulation composed of the registered, marketed medicinal product *Ampho-Moronal suspension* (Dermapharm AG, Germany) and a buffered, preserved aqueous solution of colistin and tobramycin both as sulfates has been developed. A stability study has been performed on two batches of the formulation. During the storage, samples were taken and compatibility was verified by physicochemical and microbiological testing in stability-indicating terms of colour, odour, flavour, pH, chemical and microbiological purity as well as in vitro potency. The test methods were built and tailored to be suitable, reliable and precise for the test needs.

Results The results show the physicochemical and microbiological stability of the described formulation for defined storage conditions.

Conclusions A standardised formulation with a proven stability for at least 6 months under fridge (5°C±3°C) conditions for the SDD of patients in intensive care was established.

substances as amphotericin B, colistin and tobramycin (see [table 1](#)) is often used for the selective decontamination of the digestive tract (SDD) of artificially (mechanically) ventilated intensive care patients.^{1–3}

There are currently no commercially available formulations on the market which are suitable for this purpose and the risk assessment of the preparation is the responsibility of the hospital pharmacists.

The lack of stability data, in particular, means that hospital pharmacists have no proper instruction for the suitable storage conditions of the preparation and they are forced to prepare before use. This is an undesirable situation since processing and storage time can influence the quality of the suspension.

A unique batch formula with known stability under defined storage conditions as well as a detailed manufacturing process is required to avoid differences in composition and stability between formulations prepared in different pharmacies. The assessment of the quality and the compatibility of the individual drug substances for pharmaceutical products prepared in non-industrial standards is mandatory.⁴ Furthermore, the knowledge acquired concerning stability and the establishment of a suitable shelf-life for the suspension allows hospital pharmacists to prepare larger quantities for storage, thus removing the necessity to prepare immediately before use. This will be a significant benefit to the pharmacists involved.

Therefore, an adequate formulation has been developed based on the registered, marketed medicinal product *Ampho-Moronal suspension* (Dermapharm AG, Germany).

The stability study was performed adapted from the stability guideline (CPMP/ICH/2736/99)⁵:

Stability: long-term and accelerated conditions for 6 months

Number batches: 2

Packaging material: 40 mL brown glass and 10 mL prefilled syringe

Temperature: 2°C–8°C (fridge conditions)

25°C (±2°C)/60% (±5%) relative humidity (RH)

Test frequency: 3 months

To perform stability studies, selective physicochemical and microbiological analysis of the relevant stability-indicating parameters in the

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INTRODUCTION

A suspension containing a combination of different, slightly absorbable or non-absorbable antimicrobial

Table 1 Overview of the used antibiotics

Name of the antibiotic	Antibiotic group	Produced by	Main field of application (antimicrobial potency against)
Colistin (=polymyxin E) (in form of colistin sulfate)	Polypeptides	Bacillus polymyxa var. colistinus	Gram-negative bacteria
Tobramycin (in form of tobramycin sulfate)	Aminoglycosides	Streptomyces tenebrarius	Gram-negative and gram-positive bacteria
Amphotericin B	Polymakrolides	Streptomyces nodosus	Yeasts and moulds

Table 2 Specification for the quality control of the investigated suspension

Parameter	Specification	Method
Properties		
Appearance and homogeneity	Homogeneous, viscous suspension, free from visible impurities and/or agglomerates	Visually
Colour	Orange	Visually
Odour	Like oranges and passion fruit	Organoleptic
pH value (25°C)	5.3–5.8	Ph.Eur. 2.2.3
Purity		
Amphotericin B	Sum of tetraens and heptaens ≤15.0% <i>Fingerprint comparison with the used active substance</i>	In-house method Ph.Eur. 2.2.29, HPLC
Colistin	Any impurity ≤4.0% Total ≤23.0% <i>Fingerprint comparison with the used active substance</i>	
Tobramycin	Deoxystreptamine-kanosaminide ≤0.5% Nebramine ≤0.5% Any unknown impurity ≤0.5% Total ≤2.5% <i>Fingerprint comparison with the used active substance</i>	In-house method Ph.Eur. 2.2.29, HPLC
In vitro potency		
Amphotericin B	Comparison to start value (test of equivalence)	In-house method
Colistin		Agar diffusion test
Tobramycin		Adapted from: USP<81> Ph.Eur. 2.7.2
Microbial purity		
	EP 5.1.4, aqueous preparations for oral use and oromucosal use	Ph.Eur. 2.6.12, Ph.Eur. 2.6.13

HPLC, high performance liquid chromatography

formulation were performed. On the basis of legal guidelines (Q6A and Q3B)^{6,7} and the knowledge derived from the Ph. Eur.⁸ and United States Pharmacopoeia (USP)⁹ monographs of the individual drug substances an appropriate specification has been set for the investigated suspension (see table 2).

Developed and validated methods were recently published to describe the purity of each suspension active pharmaceutical ingredient and with it to assess the suspension chemical stability.¹⁰

The stability of the described formulation regarding the microbial purity and the antimicrobial preservation efficacy has been evaluated using product-specific validated methods of the Ph.Eur.⁸

The determination of the in vitro potency is one of several methods which can be used to characterise the performance, and therefore, the efficacy of a finished (eg, topical) dosage form.^{11–13}

The determination of the in vitro potency is performed using the agar diffusion method, described in the pharmaceutical scientific literature as the most simple and old established in vitro

technique for semisolid and liquid preparations to test the liberation of the active ingredients (eg, antibiotics) out of the matrix.

The methods are in-house developed methods, only the principles of the agar diffusion test as well as the statistical testing design and equation approach are adapted from the Pharmacopoeias Ph.Eur. and USP as a convenient tool.^{8,9}

The testing approach is to determine the in vitro potency of the *complete formulation*.

This approach had been chosen as an adequate in vivo simulation of the potency as well as for the assessment of the preparation.

PREPARATION OF THE FORMULATION

The suspension is prescribed for the patient as a 10 mL dose four times daily. One dose contains 118.0 mg of colistin sulfate (corresponding to 100 mg of colistin base; Xellia Pharmaceuticals), 121.75 mg tobramycin sulfate (corresponding to 80 mg of tobramycin base; Chongqing Daxin) and 500 mg amphotericin B. The following excipients of the suspension are preservatives and are therefore also required to be declared:

9.5 mg methyl parahydroxybenzoate (EP), 3.0 mg propyl parahydroxybenzoate, 10.0 mg sodium benzoate and 7.5 mg sodium metabisulfite (Azelis Deutschland Pharma GmbH).

Aqua conservata is used as a standard formulation in the manufacture of the suspension.¹⁴ It contains 0.025% propyl parahydroxybenzoate and 0.075% methyl parahydroxybenzoate in water. To manufacture one dose, the declared quantities of colistin sulfate, tobramycin sulfate as well as 52.88 mg disodium phosphate dodecahydrate, 132.80 mg sodium dihydrogen phosphate (Merck dura GmbH, Germany) and 29.40 mg potassium chloride (VWR International GmbH or Kirsch Pharma GmbH) are dissolved in about 3 mL aqua conservans. The solution has to be checked for clarity. Next, 5.0 mL (corresponding to 5350 mg) of Ampho-Moronal suspension (Dermapharm AG, Germany) is added. The pH should be adjusted to between 5.3 and 5.8 with a solution of citric acid (20%) in aqua conservans. This pH range is in accordance with the buffer of Ampho-Moronal suspension and does not affect the efficacy, stability or solubility of colistin and tobramycin. The dose of suspension is finalised by dilution with 10.0 mL (10.5 g) with aqua conservans. The final suspension should be homogeneous and easily redispersible. All excipients should have Ph.Eur. quality.

ANALYTICAL METHODS

The prepared suspension was analytically investigated during the stability study. Details to the used physicochemical and especially chromatographic methods (reagents, sample preparation and measurements) can be found in a previous article to this topic.¹⁰

The used microbiological methods including the materials and reagents are described in the following paragraphs.

First, the *in vitro* potency test will be illustrated as the most important quality indicator for such a suspension.

The investigated formulation contains a *very high concentration* of all antibiotic ingredients with *overlapping scope of action*. The *in vitro* potency was tested with the *complete formulation, without further dilutions*.

Therefore, the criterion of the selection was the converse sensitivity/resistance of the microorganisms to the investigated antibiotics—sensitive to one of the substances and not sensitive to the others (see table 3).

The goal of the screening was to find preferably primary resistant species/strains.

It was necessary to perform a very expanded screening of scientific literature sources and of different species and strains for the detection of suitable microorganisms.^{15–21}

The established testing methods are specific according to the type of the examination (determination of the *in vitro* potency of the complete formulation) and the selected test microorganisms.

The in-house developed agar diffusion method has been applied as an adequate test tool. The statistical study design of the in-house agar diffusion method follows the USP, chapter <81>, item cylinder-plate assays.⁹

Accordingly, suitable media, incubation temperature and duration had been defined experimentally in-house, too. For details, see table 3.

The specificity and selectivity of the used test method have been confirmed in preliminary tests.

Any influence of both other antibiotics as well as the influence of the containing preservatives—methyl parahydroxybenzoate, propyl parahydroxybenzoate, sodium benzoate, sodium metabisulfite—to the specific test strain has been excluded experimentally.

Preparations containing various combinations of the relevant ingredients were tested for this purpose with the agar diffusion method using the selected test microorganisms.

The in-house method uses an unconventional testing approach, because the goal of the test is the comparison of the *in vitro* potency of the complete formulation (without any analytical sample preparation) at different time points.

This special parameter cannot be expressed in activity units as usual for results of antibiotic assays according to Pharmacopeias.

Therefore, the principle of the method here is the comparison of the diameters of inhibition zones, which are given by the quantities of the antibiotics in the formulation in relation to a constant value (=inhibition zones, induced by five defined concentrations of the appropriate reference standard) at each stability time point.

Different steps during the development were done for the above described approach.

First, the range of the five concentrations of the reference standard solutions giving inhibition zone diameters equal to those of the described fresh prepared original formulation was determined.

Second, adequate quantities of the inoculum for each microorganism to be incorporated into an appropriate volume of the used agar media were defined.

Table 3 Selected test microorganism for the *in vitro* potency test

	Test microorganism	Nutrient media	Incubation temperature and time	Reference standard substances
Colistin	<i>Achromobacter xylosoxidans</i> subsp. <i>Xylosoxidans</i> (ATCC 27061)	R2A broth and agar medium	35°C–37°C 24 hours	Colistine sulfate, lot G-1 (USP), 629 µg of colistin/mg dried substance
Tobramycin	<i>Providencia stuartii</i> (ATCC 33672)	Casein soya bean digest broth and agar medium	35°C–37°C 24 hours	Second International Standard for Tobramycin, 9800 IU per ampoule ('as is')
Amphotericin B	<i>Metschnikowia reukaufii</i> (DSMZ 70880)	Sabouraud 2% dextrose broth and agar medium	30°C–32°C 48 hours	Amphotericin B for microbiological assay CRS, batch 1 944 IU/mg (as is)
Supplied by	Leibnitz-Institut DSMZ Braunschweig, Germany, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH	MerckKGA/Millipore, Darmstadt, Germany, Oxoid Deutschland GmbH, Wesel, Germany		LGC, Central Warehouse Germany, Hannover, Germany European Directorate for the Quality of Medicines & Healthcare, Council of Europe, Strasbourg, France

CRS, chemical reference standard; DSMZ, deutsche sammlung von mikroorganismen und zellkulturen.

The quantity was chosen to allow sufficient growth within the incubation period and to give acceptable inhibition zones.

For testing, the inoculum was added to the agar medium that had been melted and cooled up to 45°C–50°C.

Afterwards the inoculated agar medium was poured immediately into standard Petri dishes to generate uniform layer.

Then, seven cavities of 6 mm diameter each were prepared in the agar layer of each plate using sterile punches of stainless steel.

The testing solutions were applied in these cavities in the order given in the USP, chapter <81>, see above.⁹

After the incubation time for each microorganism (see table 3) the diameters of the inhibition zones were measured with a precision of 0.1 mm using a calibrated digital calliper.

The single ‘relative value of potency’ of the formulation was calculated by interpolation from the standard curve using the log-transformed straight-line method with the least-squares fitting procedure according to USP.⁹

That means the inhibition zone diameters have been compared between the reference standard and the tested formulation.

Microbial determinations of antibiotics potency are subject to interassay as well as intra-assay variables; therefore, two or more independent assays are required for reliable estimation of the potency of a given sample.

So, eight separate determinations for each antibiotic have been accomplished at each testing time point during the stability time accordingly.

Statistical evaluation of the results of the in vitro potency has been performed using the test of equivalence (TOST: two one-sided tests).^{9 22}

The tests were conducted at a significance level of $\alpha=0.05$.

Using this statistical method it is important to define ‘sufficiently similar (equivalent)’ for the purpose of the study.

The acceptance limits were carefully set to accept the results in a 15% variation range δ from the start value.

This difference δ had been set on the basis of real specification limits of a typical antibiotic assay and the difference is also acceptable for reason of the unconventional approach of performing this test in opposite to the conventional antibiotic assay techniques.

All statistical analyses have been performed using Microsoft Excel 2010.

Beside the determination of the in vitro potency further microbiological tests were done to demonstrate the stability of the formulation.

The test for microbial purity has been carried out at the beginning and at the end of the stability study period.

The described non-sterile formulation has been classified as aqueous preparation for oral (and oromucosal) use according to the Ph.Eur. chapter 5.1.4.

The microbiological examination has been performed in accordance with Ph.Eur. chapter 2.6.12 (microbial enumeration tests) and 2.6.13 (test for specified microorganisms), respectively.⁸

The plate-count method (pour plate method) has been applied for the quantitative enumeration of the mesophilic bacteria and fungi that may grow under aerobic conditions.

Ten grams of the formulation has been used as a sample for the test. The sample has been suspended in buffered sodium chloride-peptone solution pH 7.0 containing neutralising agents for interfering preservatives. The recommended mediums, Casein soya bean digest agar and Sabouraud 4% dextrose agar have been used for the tests.

Casein soya bean digest broth has been mixed with a quantity of the prepared sample corresponding to 1 g sample for the determination of the absence of specified microorganisms and further processing as described in the Ph.Eur.⁸

MacConkey broth and agar as well as cetrimide agar and manitol salt agar has been applied as selective media for the test of the absence of specified microorganisms (here *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*).

The compositions of the used media follow the Ph.Eur.⁸ and they have been supplied by Merck/Millipore (Merck KGaA, Darmstadt, Germany).

The antimicrobial preservation efficacy test has been conducted with a sample of the test formulation with reduced content of preservatives simulating the potential decrease of these ingredients during the shelf-life.

The test of the preserved formulation in the multidose container has been performed according to Ph.Eur. 5.1.3.⁸

The compliance with the acceptance criteria for oral (and additionally oromucosal) preparations has been checked.

The test consists of challenging the preparation with a prescribed inoculum of test microorganisms (here *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404), storing the inoculated preparation at temperature range of 20°C–25°C (protected from light), withdrawing samples from the container at specified intervals of time and determine the microbiological quality in the samples.

The recommended media, Casein soya bean digest agar and Sabouraud 4% dextrose agar has been used for the tests by using the pour plate method (see test of microbial purity).

RESULTS

Physicochemical stability

Colour, odour and flavour as well as pH values (5.3–5.8) were unaltered during the storage time of 6 months under the defined storage conditions (2°C–8°C and 25°C, 60% RH).

The assessment of the chemical stability of the three individual active ingredients is preformed regarding the defined specification (see table 2).

Colistin

The stability of colistin in the investigated suspension is exemplarily shown in figure 1 and under different storage conditions. According to the Ph.Eur. monograph any impurity (excluding the sum of five colistin analogues) should not be <4% and the sum of unknown impurities should not be <23%.⁸ These two parameters can be used for the stability assessment of colistin in the investigated suspension.

Tobramycin

For the assessment of the purity profile of tobramycin during the stability study several impurities were considered and the specification limits were set according to the Ph.Eur. and USP monograph.^{8 9}

Potential impurities of tobramycin are known: kanosamine, nebramine (impurity B, EP), deoxystreptamine and deoxystreptamine-kanosaminide which may be formed by hydrolysis in acidic and basic solutions as well as through oxidation.^{8 9 23}

In table 4, the results for the purity profile of tobramycin for one batch under different storage conditions is exemplary shown.

These data are representative for the stability of colistin and tobramycin for all investigated batches and packing forms (glass bottle and prefilled syringe).

Figure 1 Stability of colistin at 25°C and 2°C–8°C (batch: 120214A, 40 mL glass bottle).

Table 4 Stability of tobramycin at 25°C and 2°C–8°C (batch: 120214A, 40 mL glass bottle)

Parameter	Specification (%)	0 month (%)	Storage condition	3 months (%)	6 months (%)
Deoxystreptamine-kanosaminide	≤0.5	<0.2	25°C, 60% RH 2°C–8°C	<0.2 <0.2	0.4 <0.2
Nebramine	≤0.5	<0.2	25°C, 60% RH 2°C–8°C	<0.2 <0.2	0.2 <0.2
Each unknown impurity	≤0.5*	0.5	25°C, 60% RH 2°C–8°C	0.7 0.7	0.9 0.8
Sum of all impurities	≤2.5	0.5	25°C, 60% RH 2°C–8°C	1.4 1.0	2.5 1.0

*One impurity can be limited between 0.5% and 1.0%.

Table 5 Purity of amphotericin B at 25°C and 2°C–8°C (batch: 120214A, 40 mL glass bottle)

Parameter	Specification (%)	Storage condition	Release	3 months	6 months
Amphotericin X (impurity B, Ph.Eur.)	≤4.0	25°C, 60% RH 2°C–8°C	0.48	0.31 0.40	0.47 0.56
Any other heptaen	≤2.0	25°C, 60% RH 2°C–8°C	1.31	1.44 1.38	1.47 1.30
Amphotericin A	≤5.0	25°C, 60% RH 2°C–8°C	2.41	2.24 2.03	2.11 2.05
Any other tetraen	≤1.0	25°C, 60% RH 2°C–8°C	0.15	0.17 0.13	0.13 0.13
Sum of tetraens and heptaens	≤15.0	25°C, 60% RH 2°C–8°C	10.5	10.7 10.4	8.9 8.6
Fingerprint comparison with the used active substance		25°C, 60% RH 2°C–8°C	Confirm	Confirm	Confirm

Amphotericin

The judgement of the impurity profile from the third active substance amphotericin B was also done in accordance to the limits of the Ph.Eur. monograph.⁸ Commercially available amphotericin B is manufactured by fermentation, which may produce biologically active analogue impurities as by-products like heptaenes and tetraenes.

The acceptable content of total impurities is limited by 15%. All impurity profiles measured (data shown in [table 5](#) and [figure 2](#)) demonstrate only small deviations in comparison to the used active substance and in any case the content of heptaenes and tetraenes achieve the acceptance limits of the Ph.Eur. monograph.⁸

In vitro potency and microbiological purity during the stability time

The in vitro potency of the described formulation is demonstrated as an example in [table 6](#) for one batch.

These data are representative regarding the stability for all investigated batches and packaging forms (glass bottle and pre-filled syringe).

The in vitro potency can be shown as equivalent after storage of 6 months at 2°C–8°C for the three active ingredients.

The formulation with reduced content of preservatives has been fulfilled the acceptance criteria for oral (and oromucosal) preparation as result of the test of antimicrobial efficacy of the preservation according to Ph.Eur. 5.1.3.

Figure 2 Chromatogram: purity profile amphotericin B (detection wavelength 408 nm); pink line: used active substance amphotericin B; black line: investigated suspension (lot 120214A) stored at 25°C, 60% RH for 6 months.

Table 6 Stability parameters at 25°C and 2°C–8°C (batch: 120214A, 40 mL glass bottle)

Parameter	Specification	0 month	Storage condition	3 months (%)	6 months (%)
In vitro potency of colistin*	Confirmation of the equivalence to the initial value (acceptable difference δ $\leq 15\%$)	Initial value has been determined =100%	25°C, 60% RH 2°C–8°C	<15 <15	>15 <15
In vitro potency of tobramycin*			25°C, 60% RH 2°C–8°C	<15 <15	<15 <15
In vitro potency of amphotericin B*			25°C, 60% RH 2°C–8°C	<15 <15	<15 <15
Microbiological purity	Ph.Eur. 5.1.4: acceptance criteria for aqueous preparation for oral (oromucosal) use TAMC: $\leq 10^2$ CFU/g TYMC: $\leq 10^1$ CFU/g <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> : absence in 1 g	TAMC: <10 CFU/g TYMC: <10 CFU/g <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> : not detected in 1 g	25°C, 60% RH 2°C–8°C	n.e. n.e.	TAMC: <10 CFU/g TYMC: <10 CFU/g <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> : not detected in 1 g

*The results of equivalence test show if the change of the in vitro potency exceed or do not exceed the defined limit. Therefore, the results are given only as $<\delta$ or $>\delta$. CFU, colony forming unit; n.e., not examined; TAMC, total aerobic microbial count; TYMC, total combined yeasts/mould count.

DISCUSSION

The unchanged pH values as well as no organoleptic changes support good stability and compatibility of the active substances in the investigated suspension under all conditions.

In contrast to this the purity profiles and the in vitro potency of the individual substances show differences.

It is obvious from figure 1 that colistin, a cyclic heptapeptide, in the investigated suspension is not stable at 25°C. Deamination, hydrolysis and racemisation are well known possible reactions for the degradation of polypeptides. Orwa *et al.*²⁴ identified the most degradation products of colistin solution at neutral or acid conditions as isomers through racemisation.

On the other side at 2°C–8°C temperature range the acceptance limits for the assessment of colistin stability are fulfilled during the whole testing period (6 months) and no change in the purity profile (fingerprint) is observed.

The in vitro potency has been changed during the stability time in different way for the examined antibiotic substance. There is a significant reduction at 6 months storage at 25°C, 60% RH for colistin. The equivalence test is not passed using the acceptable difference of $\delta \leq 15\%$ but at 2°C–8°C δ was calculated well below 15%, and therefore, the test criterion is fulfilled.

The in vitro potency results for colistin correlate well with the results of chemical stability.

The chemical stability of tobramycin is still ensured at 25°C after storage over 6 months. The values obtained (see table 4) for the known degradation products are still in accordance with specification limits for the desired Ph.Eur. quality of tobramycin.

After 6 months at 2°C–8°C temperature range storage condition there are only small variations in comparison to the impurity values at the beginning (0 month).

Brandl and Gu²³ reported rapid degradation of tobramycin under drastic pH values and at neutral pH extremely slow reactions in the absence of oxygen even at high temperatures.²⁵

The in vitro potency values have been reduced for tobramycin in comparison to the initial values. These tobramycin results support the previously reported incompatibilities of tobramycin with the anion excipient, especially carmellose sodium. A precipitation of tobramycin can be occurring in the presence of this substance at pH values <7.5 .

Because of the acceptable tobramycin purity levels the precipitant probably leads to decreased in vitro potency.^{8 14 26} But the defined acceptance equivalence criteria of $\delta \leq 15\%$ for the in

vitro potency test for tobramycin is still accomplished under storage conditions of 2°C–8°C and 25°C.

Although polyene macrolide antibiotics are known to be chemical and photosensitive amphotericin B is very stable in the investigated suspension.²⁵ No degradation and only small variation in the impurity profile has been observed. As described in the literature the oxidation process for the heptaene is weakened at neutral pH value.²⁷ This physicochemical stability of amphotericin B is in good correlation with the unchanged values observed in the in vitro potency test.

Additionally, it could be shown that the described formulation is stable regarding the microbiological purity for 6 months at 2°C–8°C and 25°C, storage in both types of primary containers.

CONCLUSION

This study established the physicochemical and microbiological stability of the described standardised formulation—suspension consisting the three antibiotics, amphotericin B, colistin and tobramycin, which is often used in clinical practice for SDD of patients in intensive care.

The developed standardised formulation contain Ampho-Moronal suspension (Dermapharm) and a buffered, preserved aqueous solution of colistin and tobramycin both as sulfates.

To prepare the suspension the quantities of colistin sulfate, tobramycin sulfate as well as the buffer substances sodium phosphate dodecahydrate, sodium dihydrogen phosphate and potassium chloride were dissolved in aqua conservans. Next, Ampho-Moronal suspension is added. The pH should be adjusted to between 5.3 and 5.8 with a solution of citric acid. The dose of suspension is finalised by dilution with aqua conservans.

The formulation can be manufactured in the clinical pharmacies in a simple way and stored at least for 6 months at the temperature range of 2°C–8°C.

What this paper adds?

What is already known on this subject?

- There are currently no commercially available formulations on the market which are suitable for the selective decontamination of the digestive tract (SDD) of patients in intensive care.
- The suspension is normally prepared repeatedly as required by the relevant hospital pharmacist.
- The lack of stability data, in particular, means that hospital pharmacists have no proper directions for the suitable storage of the preparation and are forced to prepare right before use. This is an undesirable situation since formulation and short-term storage differences may influence the safety and efficacy of the suspension.

What this study adds?

- The study established a standardised formulation for the clinical pharmacies and shows its physicochemical and microbiological stability under defined storage conditions.
- Therefore, the patients can receive the same preparation with ensured similar efficacy and improved safety.
- The comparability of study results with further clinical trials can be ensured due to the standardised formulation.

The preparation should be applied in further clinical trials for analysing the efficacy of SDD procedures due to the standardised formulation can ensure the comparability of the study results.

Contributors CP and SN planned and conducted the study and carried out the laboratory investigations. YR and RF manufactured the standardised formulation for the stability study. HG, RR and TJ supported the conduction of the study. The revision particularly the English language is conducted by Alaa Succer.

Competing interests None declared.

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