European Medicines Agency
SCIENCE MEDICINES HEALTH

26 February 2015
EMA/CHMP/153652/2015

Assessment report

Review under Article 5(3) of Regulation EC (No) 726/2004

Polymyxin-based products

Procedure number: EMEA/H/A-5(3)/1384

Note

Assessment report as adopted by the CHMP with all information of a
commercially confidential nature deleted.

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1. Background information on the procedure

1.1. Referral of the matter to the CHMP

On 13 September 2013, the Agency's Executive Director requested the Committee for Medicinal Products for Human Use (CHMP) to provide an opinion under Article 5(3) of Regulation (EC) No 726/2004 on whether the current manufacturing, the quality control methods and the European Pharmacopoeia (*Ph. Eur.*) monographs for polymyxin-based products need to be revised. The CHMP was asked to give particular consideration to the need to establish limits/ranges for each of the subcomponents of colistimethate sodium, the suitability of the potency bioassay and the need to update and harmonise Module 3 (CMC).

The procedure started on 19 September 2013.

2. Scientific discussion

2.1. Introduction

The emergence of multi-drug resistant Gram-negative bacteria that cause nosocomial infections is a growing problem worldwide. Limited therapeutic options have led to an increased clinical use of colistin, a polymyxin antibiotic developed over 50 years ago and which has retained activity against a number of multi-drug resistant pathogens. This is possibly due to its limited clinical use, as the parenteral formulation quickly decreased in utilisation following authorisation in the 1960s, due to the existence of safer, less neurotoxic and nephrotoxic therapeutic options.

Polymyxins are currently listed among the critically important antimicrobials and in view of the importance of ensuring the availability of efficacious and safe antibiotics in order to efficiently respond to the threat posed by the spread of antimicrobial resistance, the European Medicines Agency initiated a review under Article 5(3) of Regulation (EC) No 726/2004 on 13 September 2013, requesting the Committee for Medicinal Products for Human Use (CHMP) to give its opinion on whether the current manufacturing process, quality control methods and *Ph. Eur.* monographs for polymyxin-based products need to be revised. The CHMP should give particular consideration to the need to establish limits/ranges for each of the subcomponents of colistimethate sodium (CMS), the suitability of the potency bioassay as currently described in the European Pharmacopoeia (*Ph. Eur.*) monographs and the need to update and harmonise the Quality dossier (Module 3).

Of note, recent clinical experience and the medical literature point to the urgent need to update the product information, in particular the indications, the dosage recommendations and the PK/PD information, as highlighted by recent reports of suboptimal efficacy and the emergence of colistin resistance, in particular when used as monotherapy. A parallel review under Article 31 of Directive 2001/83/EC was concluded in October, in the context of which the CHMP recommended a number of changes to the product information of polymyxin-based products.

The CHMP noted that only two manufacturers of the active substance colistimethate sodium are currently authorised in the EU: Xellia Pharmaceutical ApS (Xellia), which is the major supplier to EU marketing authorisation holders (MAHs) and Tarchomińskie Zakłady Farmaceutyczne "Polfa" S.A. (Polfa), which is understood to only supply one Polish MAH. In its assessment, the CHMP reviewed all available data, including data submitted during the procedure by the manufacturers and the MAHs in response to questions raised by CHMP. In the context of the procedure, the CHMP consulted the Quality Working Party (QWP). This assessment report considers the nomenclature, characterisations, manufacture, purity, control and stability of CMS active substance and finished product and presents a summary of the relevant data for the procedure.

The CHMP noted that *Ph. Eur.* monographs exist for both colistin and CMS (monographs 0320 and 0319 respectively). The monographs were first published in the British Pharmacopoeia in 1966 and were subsequently transferred to the *Ph. Eur.* but have remained largely unchanged since, with only minor amendments. Both monographs are therefore potentially extensively outdated and given the

resurgence in clinical use of polymyxin-based products, they may no longer be fit for purpose. A revision of the monographs should therefore be considered, if possible, to minimise variability of the active substance and thus ensure consistent quality of the finished product. The CHMP noted that a revision of the *Ph. Eur.* monograph for CMS is currently being undertaken by the European Directorate for the Quality of Medicines & HealthCare (EDQM).

2.2. Strength expression and potency

The CHMP reviewed the available data to determine the optimal way of expressing the strength and dose of polymyxin-containing finished products and noted the currently existing differences. The EU reference standard is declared in international units (IU)/mg, while the US reference standard is declared in µg activity/mg of colistin base activity (CBA). Accordingly, in clinical practice in the EU and in the European and British Pharmacopoeia, strength and vial contents are expressed as IU of CMS while in other parts of the world, such as North America and Australia, vial contents are expressed in mg of CBA, even though the finished product content is CMS.

The definition of an international unit of the drug is biological, i.e. 1 IU of colistin is defined as the amount of colistin that inhibits the growth of the *E. coli* 95 I.S.M. strain under standardised conditions. Based on historical information referenced in Martindale: The Extra Pharmacopoeia (29th edition), it is generally accepted that 1 mg of pure CBA has a potency of 30,000 IU, while 1 mg of CMS has a nominal potency of 12,500 IU. These conversions are valid whether the potency result is obtained from testing with US or EU reference material and the potency can therefore be converted from IU/mg to µg activity/mg and vice-versa. As a result, 1 million IU (MIU) of CMS is approximately equal to 80 mg CMS or 33.3 mg CBA. In the EU, this results in a conversion factor of 2.4 (80 mg CMS divided by 33.3 mg CBA) which means that 2.4 mg of CMS is required to obtain 1 mg of CBA. The CHMP noted that other sources (published *Ph. Eur.* monographs, Martindale, the US Pharmacopoeia (USP) and the 1st International Conference on Polymyxins held in Prato (Italy) in May 2013) identify other ratios, ranging from 2.36 to 2.67.

The CHMP was of the opinion that given the established use of international units in the EU, the strength and dosing recommendations in the EU SmPC and PL for CMS should continue to be expressed in international units, as any potential switch in strength expression risks causing confusion and potential administration errors even if accompanied by advice and educational material to stakeholders. However, the CHMP considered that the multiplicity of terms used is likely to lead to dosage and reporting errors and therefore decided to introduce a table indicating dose content conversions between CMS expressed in IU, CMS expressed in mg and CBA expressed in mg. Although only nominal and approximate, this table will raise awareness of the different ways of expressing dose and will be of relevance to prescribers who obtain additional information from medical literature or publications using different standards or ways of expressing dose or strength.

CMS conversion table

Potency		≈ mass of CMS (mg)*
IU	≈ mg CBA	
12,500	0.4	1
150,000	5	12
1,000,000	34	80
4,500,000	150	360
9,000,000	300	720

*   Nominal potency of the drug substance = 12,500 IU/mg

2.3. Synthesis, manufacturing and structure

Polymyxins are a group of naturally occurring multi-component cyclic polypeptide antibiotics produced by selected strains of the spore-forming soil bacterium *Paenibacillus polymyxa* (formerly known as *Bacillus polymyxa var. colistinus*). Five major, chemically distinct members of the group have been recognised and are designated as polymyxins A, B, C, D and E, of which B and E are available

commercially and approved in the EU. Polymyxin B is approved for topical use but only polymyxin E, usually referred to as colistin, is approved for oral, parenteral and inhalation use and is therefore the focus of this procedure. The fermentation process by which colistin is produced results in a complex mixture consisting of a family of similar compounds; two major polymyxins: E1 and E2, in addition to at least 30 minor polymyxins and related substances including polymyxins E1-I, E3 and E1-7MOA, which all have very similar physicochemical properties and only minor structural differences. Two forms of colistin are used clinically: colistin sulfate (colistin) for oral administration and its prodrug, colistimethate sodium (CMS), which may be considered inactive, for parenteral and inhalation use. Because colistin has a relatively high level of toxicity associated with parenteral administration, a process to manufacture the far-better tolerated product CMS for parenteral and inhalation use was developed.

Manufacturing the CMS active substance

The CHMP reviewed a description of the CMS manufacturing process, including information on the steps, specifications, purity tests and in-process controls. Colistin possesses five free primary amine groups, which can be substituted with methansulfonate. This is achieved through the reaction of colistin with formaldehyde and sodium hydrogen sulfite. The reaction is referred to as sulfomethylation and results in the formation of CMS. The derivatisation process as described in the literature is a two-stage synthesis process, resulting in the generation of methate and methansulfonate groups respectively. Although the USP monograph makes the assumption that all five amine groups are converted to form CMS, the available data indicates that the derivatisation process may not be complete and that the degree of sulfomethylation is variable and results in five progressively sulfomethylated derivatives. This is confirmed by electrophoresis data, which show a single spot for colistin, but five or more spots for CMS. The recovery and purification steps consist of extraction and precipitation of colistin, or of impurities, with the purpose of eliminating or strongly reducing impurities, so that the final CMS active substance complies with *Ph. Eur.* specifications for identity, potency, quality and purity.

The CHMP reviewed the critical process parameters and means to satisfactorily reduce variability. The MAH provided further information on the production steps involved to ensure consistent quality of the CMS active substance. As CMS is derived by sulfomethylation from a starting material consisting of a mixture of polymyxins, the control of the sulfomethylation level of the subcomponents is regarded as critical quality attribute. The degree of sulfomethylation is controlled by the amount of colistin to the reagent formaldehyde bisulfite ratio. The reagent-to-substrate ratio is the critical parameter in this process and the optimal ratio has been determined using design of experiments. Based on the assay of the colistin base starting material and the reagent-colistin ratio mentioned above, a certain amount of colistin base is added to the reaction mixture. In order to reduce variability due to a mismatch in reagent to substrate ratio, the reagent amount to be applied in a CMS production batch is automatically calculated from the assay amount of colistin in a dedicated spreadsheet. This assay is exclusively measured for CMS purposes. The colistin base dissolves as it is protonated by the acidified reagent solution, and the sulfomethylation starts. After some time, the required degree of sulfomethylation is reached. Since the CMS reaction mixture is self-buffered, a mismatch in reagent to substrate ratio will be indicated by the final pH measurement of the reaction mixture. The final reaction mixture is also subjected to a silicotungstic test to secure a minimum degree of sulfomethylation. The CMS product specification indirectly limits the allowable degree of sulfomethylation by the "total sulfite" measurement, in which the sulfomethyl groups are cleaved off the CMS, degraded to sulfite and measured by an iodometric titration.

Regarding the reaction parameters, the manufacturer clarified that the reaction temperature is not critical for the degree of sulfomethylation, but should still be within a defined range so as to ensure that reaction occurs and that starting material is not degraded. The pH of the reaction mixture has some impact on the reaction outcome, but this is controlled through the correct preparation of the formaldehyde bisulfite reagent solution. The CHMP considered the critical steps such as the degree of sulfomethylation to be adequately controlled by complying with the defined suitable colistin/reagent (formaldehyde bisulfite) ratio and pH. The information provided on the control of the degree of sulfomethylation was considered satisfactory and the CHMP was of the view that the manufacturers have sufficient experience of the process. The CHMP considered that a general production statement for inclusion in a *Ph. Eur*. monograph would not be appropriate. The suitability of a source of CMS can only be addressed on a case by case basis during assessment.

It was noted that using the molecular weight of colistin and assuming that all five amine groups are always included in the reaction, 1M of colistin base would convert to 1M of its methylsulfonic base

derivative. If so, 1162g of polymyxin E (average weight of polymyxin E1/E2), plus the weight of five sodium methylsulfonic components (116g) would yield 1742g of CMS derivative sodium salt. This is equivalent to 1g colistin base yielding 1.5g CMS, which can in turn be expressed as 2.4mg CMS from 1.5mg colistin base. This diverges from the previously established ratio based on potency of 2.4mg CMS being equivalent to 1mg colistin. Furthermore, in cases of incomplete derivatisation due to an incomplete sulfomethylation reaction (i.e. less than all five amine groups being derivatised), this divergence would increase. As a result, there is a discrepancy in equivalence between CMS and colistin depending on whether the calculation is based on chemistry or potency.

It is not possible to obtain a clear stoichiometric relationship between CMS and colistin, however from the data reviewed, including MAH submissions, it was clarified that the definition of the International Unit of activity is the same for both colistin and CMS, i.e. that one IU of potency of CMS is equivalent to one IU of potency of colistin. However, it was not considered to be of practical value to reflect this in the product information, as the potency per mg is not equivalent between CMS and colistin and in the case of CMS, because the activity is only available on total hydrolysis of CMS to colistin. Although this may be the case *in vitro*, the *in vivo* conversion is only approximately 30%, with the remaining 70% of CMS being excreted intact in the urine.

Structure of CMS

The CHMP noted that conflicting evidence exists regarding the structure of CMS. A number of published data sources e.g. the USP monograph and British Journal of Pharmacology (1964), 23, 552-574), state that the structure of CMS involves the mono-sulfomethylation of the amine groups, however data provided by the manufacturer refers to bis-sulfonylmethyl groups for the amine groups. The available data was reviewed and it was noted that the analytical methods specified in the pharmacopoeial monograph have been used since the 60s and do not reveal the multiplicity and actual structure of CMS.

Barnett et al.[1] further states that "Electrophoresis shows that the derivatives are composite, the components corresponding to mono- to pentasulfomethyl polymyxin". It is a fact that electrophoresis differentiates between sizes, binding and relative charges but cannot identify an exact molecular structure. The observation is, however, equally explainable by the presence of components corresponding to bis-sulfomethylated polymyxin. Barnett et al. based much of the theory behind the mono-sulfomethylation of the amine functionalities on a publication by Schiff in 1866[2]. However, the original Schiff publication basically deals with the reaction of alkyl and aryl aldehydes (formaldehyde was not included in this study) with aryl amines in the presence of sulfite, where the reaction patterns are different. A SciFinder search revealed that Barnett et al. was cited 11 times between 2012 and 2014 in large reviews by well-established groups doing research in the polymyxin area, which suggests that the Barnett et al. publication is the origin of the "established knowledge of CMS". In contrast, a 1969 publication by McMillan and Pattison[3] studied the transformation of CMS *in vitro* by exploring aqueous solutions of the material, and they suggested that neutral solutions of CMS comprise complex equilibria in which individual molecules are substituted by varying numbers of methanesulfonate or hydroxymethyl groups, possibly as many as 10, but three or four of these species more highly favoured than the rest. By using iodometric titration methods, they concluded on di-substitution of the amine groups, as supported by electrophoreogram data. This publication received only little attention with regard to structure elucidation of CMS and has only been cited 6 times according to SciFinder.

As most research groups within this field are interested in the dosing regimen of CMS and polymyxins and in the development of new polymyxin derivatives, the well-established structure proposed by Barnett et al. has never been questioned. A SciFinder search for the alkyl bis-sulfomethyl amine fragment $-CH_2N(CH_2SO_3)_2$ returns 158 hits, including several for recently developed, commercially available compounds. The manufacturer stated that this is supported by own data where mono-components from the sulfomethylated polymyxin E1 mixture were isolated by preparative HPLC. Structure elucidation of 8, 6 and 4 sulfomethylated polymyxin E1 components was performed by way of NMR, LC-MS and direct infusion MS. None of the amine groups were found to be mono-substituted

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[1] Barnett M, Bushby SRM, Wilkinson S, Brit. J. Pharmacol. (1964), 23, 552-574. Sodium Sulfomethyl derivatives of Polymyxins
[2] Schiff H, Justus Liebigs Annalen der Chemie, (1866), 140,1, 92-137, Eine neue Reihe Organischen Diamine,
[3] McMillan FH and Pattison IC, Journal of Pharmaceutical Sciences, (1969), 58, 6, 730-737. Sodium colistimethate I: Dissociations of aminomethanesulfonates in aqueous solution

and it was therefore concluded that the current established structure of CMS as suggested by Barnett et al. is very likely incorrect.

The CHMP agreed that the structure of CMS consists of five primary amine groups which are either non-substituted or bis-sulfomethylated. The current accepted structure of CMS, as mono-sulfomethylated derivatives, is therefore incorrect and the CHMP was of the opinion that this important information should be made widely known by updating the *Ph. Eur.* monograph accordingly. Consideration should also be given to informing the US Pharmacopoeia and international competent authorities.

The following recommendation is made to EDQM for revision of the CMS monograph:

The *Ph. Eur.* monograph for colistimethate sodium (CMS) should be revised to reflect the following current understanding of the substance's molecular structure:
"Colistimethate sodium is prepared from colistin by the action of formaldehyde and sodium hydrogen sulfite, to form a mixture of bis-sulfomethyl primary amine derivatives."

CMS manufacture - controls and characterisation, degree of sulfomethylation

The composition and purity of the colistin starting material used to manufacture CMS are considered critical quality attributes. The composition of colistin starting material should be equivalent or better than that described in the *Ph. Eur.* monograph for colistin sulfate. Data from manufactured batches of colistin starting material was provided, including data on composition, related substances and potency. The CHMP noted that each batch is controlled before its introduction into CMS production for compliance with relevant and appropriate quality requirements in accordance with the current colistin sulfate *Ph. Eur.* monograph. The content of related substances and content of polymyxins (composition) is verified by an HPLC method and the biological potency is evaluated for each batch. The data showed that the two principal components E1 and E2 consistently made up more than 80% of the composition in every batch and that the composition of these two components and the other main components did not vary by more than three times the standard deviation. Thus the manufacturing process for CMS was considered well controlled. However, the available data indicates that polymyxin E1 is present in significantly greater amounts than polymyxin E2 in the active substance produced by Xellia but these are not directly controlled in the monograph. Having compared the current monograph limits with the obtained results, the CHMP was of the view that the current monograph does not reflect process capability and that it would be appropriate to control the quantitative composition of each of these components. New limits for additional colistin subcomponents and tightened limits for some of the components based on historical data should be provided to the EDQM to support the already ongoing revision of the colistin monograph. Once the monograph has been revised, the quality specification for colistin starting material should be updated to reflect the revised *Ph. Eur.* monograph for colistin.

Each individual colistin component (e.g. polymyxin E1, E2, E3, E1-I, or E1-7 MOA) will generate a set of CMS sub-components identifiable by HPLC peaks. The HPLC method is based on the isolation of the two major sub-components of colistin (E1 and E2) at high purity (>90%) using preparative chromatography followed by conversion to E1-CMS and E2-CMS by sulfomethylation. Due to the low amount of the other sub-components, identification is more difficult and these are therefore categorised as related impurities. The SAX column method[4] presented in literature is not validated and is not considered suitable for the analysis of CMS, mainly because it is not capable of providing any qualitative or quantitative data and the sample handling does not provide stable samples.

The proposed HPLC method evaluates three important quality parameters of CMS; purity, composition and determination of related impurities. Purity and related impurities is mainly related to the starting material, colistin base, where variations in the starting material will have a direct effect on the purity of CMS. High purity CMS will have a high amount of polymyxin E1 and E2 sulfomethylated derivatives and low amounts of the other related polymyxin derivatives. Prior to synthesis of CMS, the purity and composition of the colistin base is tested using the *Ph. Eur.* method for colistin sulfate. The composition is related to the starting material (ratio of polymyxin E1/polymyxin E2 and related substances) and the CMS manufacturing process. The influence of the process parameters to the HPLC composition a design space for the product has been investigated and critical process parameters have been identified; their effect on the sulfomethylation is known. Evaluation of the composition is determined by analysing the content of specified peaks.

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[4] Li et al., Antimicrob Agents Chemother. 2003 April; 47(4): 1364-1370

It was determined that variations in the critical process parameters have a direct influence on the area percentage of the peaks, hence if they are not within the set limits, the product will contain an incorrect number of sulfomethyl groups which in turn may affect the *in vivo* pharmacokinetics and toxicity. The high number of peaks in the CMS chromatogram makes it close to impossible to integrate peaks below 0.05%. There are over 100 peaks with an area percentage above 0.05% and approximately 40 peaks above 0.5%. All peaks above 0.05% are included in the evaluation although an identity (impurity, CMS E1 or E2) is only assigned to peaks above 0.5%. Peaks above 0.5% with unknown origin are called related impurities. Hence, the entire baseline is integrated. Independent of the origin, the sum in area% of all peaks between 0.05% and 0.5% (approximately 15%) is subtracted from the total area% (100%). Additionally, all related impurities are subtracted from the purity (max 5%). This gives a purity of around 80%.

It is generally accepted that CMS does not possess antimicrobial activity itself but is instead hydrolysed to form the active antibiotic colistin. The rate of hydrolysis to colistin varies depending on how many of the five amine groups are sulfomethylated. This can potentially lead to variations/delays in achieving therapeutic drug concentrations because the time to achieving active colistin concentrations will differ between a colistin molecule bis-sulfomethylated once and a colistin molecule bis-sulfomethylated five times. Variations in the degree of sulfomethylation caused by different manufacturing processes could potentially give different rates of hydrolysis and lead to significant variation in the time to peak colistin concentrations and significant differences in colistin area under the concentration-time curve (AUC). This was confirmed by He et al., 2013[5] who demonstrated that rats receiving the same dose of CMS produced by four different manufacturers achieved difference in time to peak colistin concentrations ranging from 45 to 108 minutes with up to a 2-fold difference in colistin AUC. This is further complicated by the fact that CMS, unlike colistin, is primarily cleared renally. As a result, in patients with unimpaired renal function, the risk of drug clearance before achieving systemic colistin concentrations raises significant concerns and this needs to be taken into account in the dosing regimens. However variation in the rate of hydrolysis caused by variation in the degree of sulfomethylation could seriously affect these calculations and thus complicate dosing protocols for CMS.

There is therefore the possibility of a significant variation in the ratio of the different sulfomethylated polymyxins caused either by the different degrees of sulfomethylation or by different ratios of the initial polymyxin components. This can only be addressed by ensuring consistency in the ratio of the sulfomethylated polymyxins contained in the marketed CMS, by having a CMS specification where the ratio of polymyxins and degree of sulfomethylation is controlled. A method proposal has been submitted by the manufacturer.

In developing this method, the E1 and E2 components of colistin were isolated and derivatised into the respective CMS mono-components. A comparison of the HPLC profiles for the two components reveals that the sulfomethyl groups are distributed in the same way. Hence, the distribution of sulfomethyl groups is the same, independently of the polymyxin. It is therefore considered that the structural characterisation of one of the sulfomethylated polymyxins is sufficient to understand the overall sulfomethylation. The isolation and synthesis of specific CMS E1 peaks (components) followed by structural characterisation with NMR, MS, LC-MS and degradation studies have revealed a pattern in the CMS profile, with groups of peaks attributable to 10, 8, 6, 4 and 2 substituents for CMS E1 and to 8, 6 and 4 substituents for CMS E2. Because substituted amine groups are always bis-sulfomethylated, CMS contains up to ten substituents, in multiples of two. The variance within each group (8, 6 and 4 substitutions) shows that there are preferences in the position of substitution, otherwise all peaks within one group would be of the same size. Additionally, the structural elucidation work shows that there are free amine groups in CMS and that there are no mono-sulfomethylated amine groups. This makes it possible to estimate the degree of sulfomethylation of intact CMS.

In vitro degradation of CMS shows that the starting degree of sulfomethylation may play an important role in the release of colistin. Analysis using a modified HPLC method (LC-MS compatible) detected no formation of colistin for the upper sample (starting average degree of sulfomethylation is 10) but around 6% of free colistin for the lower sample (starting average degree of sulfomethylation around 6) after 20 hours of degradation. This suggests that no activity would be expected for the upper sample after 20 hours whereas the lower sample would show activity after 5 hours. Thus, the degree of

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[5] He H, Li JC, Nation RL, et al. Pharmacokinetics of four different brands of colistimethate and formed colistin in rats. J Antimicrob Chemother 2013;68:2311–7.

sulfomethylation for each individual API appears to play a significant role in the bioactivity (*in vivo* performance) of the product and this is characterised and controlled by the HPLC method.

Xellia submitted a composition analysis method using HPLC to evaluate interdependent key components for the, 8, 6, and 4 substituted components with limits including average, maximum, minimum and standard deviation for each of the six peaks based on data from 111 tested API batches. Only six peaks are proposed to be characterised – three for each polymyxin E1 and E2 derivative, with different degree of sulfomethylation (tetra-bis, tri-bis and di-bis). Due to the unstable nature of penta-bis-sulfomethylated CMS (10 sulfomethyl substituents) and its elution close to the void peak, evaluation of this peak is not recommended. Because the peaks are interdependent (for example, a reduced amount of the penta-sulfomethylated polymyxin E1 should result in an increase in the lower sulfomethylated peaks), the evaluation of the six selected peaks in the chromatogram will provide sufficient information on the CMS quality and its correlation to the critical process parameters and stability. In addition, any change in the sulfomethylation (whether through incorrect manufacturing or degradation of the sample) will be detectable using these peaks.

The CHMP recommended that calculation of purity, total related impurities and largest related impurity is kept in the updated monograph since this is to a large extent related to the colistin starting material. The CHMP agreed that the degree of sulfomethylation and the composition of the active compounds (polymyxin E components) are critical quality attributes that need to be adequately controlled in the *Ph. Eur.* monograph in order to ensure the safety and efficacy of polymyxin-based products. The current *Ph. Eur.* monograph only indirectly controls the degree of sulfomethylation through the Total Sulfite test – which determines the available sulfite, liberated from CMS by hydrolysis, by iodiometric titration and does not control the composition of polymyxin components.

The degree of sulfomethylation and the composition of the active compounds are, in turn, dependent on the composition of the colistin starting material and the method and control strategy of the synthesis of CMS from colistin. This can be addressed, in part, by ensuring that the starting material complies with *Ph. Eur.* requirements and by ensuring tighter controls of the colistin sulfate composition in its *Ph. Eur.* monograph. It was noted that composition data for colistin sulfate was only provided by the manufacturer Xellia and that it is not known whether CMS obtained from colistin starting material with a different polymyxin composition (but still compliant with the colistin sulfate *Ph. Eur.* monograph with respect to composition) would be equivalent in safety and efficacy to the CMS synthesised by Xellia, which has been qualified by use for many years. It was therefore acknowledged that problems may arise if other suppliers produce active substances using different fermentation processes, with resulting differences in composition and the proportion of polymyxin E1 and E2 components as only colistin starting material with a similar polymyxin composition to that sourced from Xellia is likely to comply with the chromatographic test determining the degree of sulfomethylation. A requirement with respect to colistin starting material would need to take this into account.

The following recommendation is made to EDQM for revision of the CMS monograph:

The *Ph. Eur.* monograph should include a statement that the composition and purity of colistin starting material, used in the synthesis of CMS, should be equivalent to that described in the *Ph. Eur.* monograph for colistin sulfate.

The composition of polymyxin E1 and E2 in colistin starting material, necessary to comply with the proposed chromatographic test for determining the degree of sulfomethylation, should also be stated.

And the following recommendation is made to EDQM for revision of the colistin sulfate monograph:

Polymyxin E1 and polymyxin E2 are the principle components of colistin sulfate, but are not directly controlled in the *Ph. Eur.* monograph.

Evidence from a European supplier indicates that polymyxin E1 is present is significantly greater amounts than polymyxin E2. It would therefore be appropriate that the quantitative composition of each of these components is controlled.

This recommendation is also necessary to ensure consistent quality of the colistimethate sodium (CMS) active substance, since colistin is used as a starting material for CMS manufacture.

It is acknowledged that this may be problematic if different suppliers exist, using different fermentation processes, giving rise to a diverse proportion of polymyxin E1 and E2 components.

The *Ph. Eur.* monograph should also provide some assurance of a consistent impurity profile. It is recommended that known single impurities are identified (at least by relative retention time).

The CHMP reviewed historical data on the composition of the CMS subcomponents in products for injection and noted that the method for determination of CMS composition was only developed and validated in 2011. A total of 19 batches of the finished product were analysed (93 individual analysis) with regard to composition and microbiological assay results. It was noted that according to the *Guideline on setting specification for related impurities in antibiotics* (EMA/CHMP/QWP/199250/2009 corr.), very complex impurity profiles such as for CMS, for which the identification of individual peaks is impossible, should at least be characterised by a descriptive specification based on a sufficient high number of manufactured batches. CMS is a complex substance of fermentation origin and the chromatogram of the active substance has been shown to contain over 100 or over 40 different peaks with an area percentage over 0.05% or 0.50%, respectively. As a result, the structural elucidation of the peaks detected in the obtained chromatogram has only partly been successful; nevertheless, the method was confirmed to be stability indicating and was validated. Due to the complex chromatogram, the setting of appropriate specifications for the chromatographic profile, i.e. the specification that would appropriately reflect and control the drug quality in terms of substance composition, purity and related impurities, was carefully evaluated.

From the chromatographs and information provided, the degree of sulfomethylation is characterised by either complete (all 5 primary amine groups) or partial (only 4, 3 or 2 primary amine groups) bis-sulfomethylation. No mono-sulfomethylated components have been identified, the amine groups are either bis-sulfomethylated or not sulfomethylated at all. The chromatographic system is such that the least sulfomethylated components have the longer retention times and each polymyxin subcomponent will produce one peak due to penta-sulfomethylated polymyxin, five peaks due to tetra-sulfomethylated polymyxin and ten peaks due to tri sulfomethylated polymyxin as well as other minor peaks due to di and mono sulfomethylated polymyxin. This explains the complex composition of CMS and the observed multi-peak chromatograms and could constitute a suitable rationale for peak selection and control using a chromatographic test for CMS.

In establishing new monograph tests, limits are usually set empirically from data provided by a preferably wide number of active substance manufacturers, whose active substance is used in products authorised and marketed in the EU. This is not the case for CMS, as discussed previously. From the data provided, Xellia's CMS consists predominantly of polymyxin E1 derivatives. The concerns associated with defining a proposal for chromatographic characterisation based on data from a single manufacturer, which may result in unnecessarily tight specification, were discussed with the QWP. The QWP was of the view that although revisions of monographs should ideally be based on data from several manufacturers, they also need to be based on data and in this particular, the inclusion in the monograph of a chromatographic method to characterise the CMS components, based on the method developed by Xellia, was therefore accepted.

As previously described, Xellia's chromatographic method only characterised six chromatographic peaks, three for each polymyxin E1 and E2 derivative. Each selected peak is a marker representative for tetra, tri or di-substituted derivatives. The limits proposed are based on historical batch data, with range set by mean ± 3S.D. The CHMP considered the revised proposal to be acceptable.

The following recommendation is made to EDQM for revision of the CMS monograph:

A chromatographic test should be included to characterise and control the degree of sulfomethylation.

Upper and lower limits should be set for representative peaks of the 4, 3 and 2 substituted bis-sulfomethylated derivatives of the principle polymyxin constituents, polymyxin E1 and polymyxin E2, based on available historical batch data.

Peaks should be chemically identified and characterised by chromatographic retention time.

The method of Xellia, the sole method proposed during the referral procedure, should be suitable.

Effect of subcomponents

The CHMP noted an investigation of the effect of CMS composition (sub-component ratios) on the safety and efficacy of colistin and CMS. The efficacy of the polymyxin E1 and E2 mono-components against a range of sensitive *A. baumannii* and multi-drug resistant *P. aeruginosa* and *K. pneumonia* strains was measured and found to be comparable to the colistin sulfate active substance. Likewise the efficacy of the colistin E1 and E2 mono-components was comparable to the equivalent subcomponents of the sulfomethylated CMS active substance. An *in vivo* study of the efficacy of CMS E1, CMS E2 and colistin against *P. aeruginosa* infections in neutropenic mice showed significant efficacy for all three entities when dosed at the equivalent to a normal therapeutic dose. As the E1 and E2 components represent on average > 75% of the content of the active components within colistin or CMS, it is not expected that there would be any variation in the antibacterial efficacy between different batches of CMS. Based on this limited evidence, the CHMP concluded that the efficacy of the E1 and E2 mono-components as determined by the minimum inhibitory concentration (MIC) values are comparable to that of the colistin sulfate and the sulfomethylated CMS.

Regarding safety, the limited post-marketing safety data did not reveal any effect of the variance in the subcomponent ratios on safety and efficacy. However, it was noted that the exact polymyxin component ratio and impurity profile of CMS produced from colistin is likely to vary depending on the strain of *P. polymyxa* used and this will result in potential variability in the toxicity of CMS depending on the source of colistin[6,7]. As a result, the safety of the Xellia CMS supported by years of product consistency cannot be extrapolated to CMS products produced by other manufacturers. Mohamed et al., 2012[8] have shown that polymyxin E1 and E2 have different protein binding in the blood which is caused by differences in the fatty acid side chain which in turn influences lipophilicity. Brink et al., 2014[9] also discuss the influence of polymyxin E composition and how it may affect heteroresistance (variable resistance profiles within a specific population of organisms) and cross-resistance. Hence, there is a great risk that variance in the polymyxin E composition will affect toxicity and bioactivity of CMS.

Free Colistin in CMS

The CHMP was of the opinion that a test for free colistin should be retained in the *Ph. Eur.* monograph and should be included in the CMS finished product specification. The current *Ph. Eur.* monograph includes a semi quantitative "wet chemistry" limit test for free colistin in CMS, based upon the degree of opalescence after addition of silicotungstic acid. This test is well established and is used to ensure completion of the synthesis i.e. absence of starting material in the final active substance. This test is also of value for finished product solution in-use stability assessment to show if degradation of CMS by hydrolysis has occurred. However, this test has not been changed since first publication of the monograph, and an alternative and more sensitive chromatographic method has been identified during this procedure. The CMS monograph test for free colistin should therefore be updated accordingly.

The following recommendation is made to EDQM for revision of the CMS monograph:

The degree of sulfomethylation and limit test for free colistin are considered critical quality attributes in CMS active substance and should be retained in the *Ph. Eur.* monograph.

These tests should be reviewed to take into account current analytical technology to replace the qualitative limit test for free colistin and the titrimetric quantitative test for total sulfite,

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[6] Barnett M, Bushby SRM, Wilkinson S, Brit. J. Pharmacol. (1964), 23, 552-574.Sodium Sulfomethyl derivatives of Polymyxins

[7] Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K International Journal of Antimicrobial Agents 25 (2005) 11–25 ;Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria

[8] Mohamed AF, Karaiskos I, Plachouras D, et al. Application of a Loading Dose of Colistin Methanesulfonate in Critically Ill Patients: Population Pharmacokinetics, Protein Binding, and Prediction of Bacterial Kill. *Antimicrobial Agents and Chemotherapy*. 2012;56(8):4241-4249. doi:10.1128/AAC.06426-11.

[9] Brink AJ, Richards GA, Colombo G, Bortolotti F, Colombo P, Jehl F. Multicomponent antibiotic substances produced by fermentation: implications for regulatory authorities, critically ill patients and generics. *Int J Antimicrob Agents*. 2014 Jan;43(1):1-6. doi:0.1016/j.ijantimicag.2013.06.013. Epub 2013 Aug

since both these tests are essentially unchanged since the *Ph. Eur.* monograph was first elaborated in the 1960s.

With respect to the test for free colistin, the published chromatographic analytical method by Wallace et. al., 2008, for free colistin in the presence of CMS, which employs pre-column derivatisation, could be suitable for adaption for use in the *Ph. Eur.* monograph.

Rate and extent of CMS hydrolysis to colistin

The CHMP noted that the conversion of CMS to colistin can occur both *in vitro* in aqueous solutions as well as *in vivo* in human plasma after administration. CMS exhibits low protein binding and is partially hydrolysed into colistin together with a complex, undefined mixture of sulfomethylated colistin derivatives. In aqueous solutions, the mixture is even more complex than *in vivo*. The available data indicates that *in vitro* conversion is temperature and concentration dependent, with higher conversion rates at lower, less clinically relevant, concentrations. The known conversion of the reconstituted finished product *in vitro* has potential implications for toxicity, as colistin is less safe and less well tolerated than CMS. While the hydrolysis of CMS may be complete in the *in vitro* bio-analytical assay for potency, it appears that conversion *in vivo* is only approximately 30%, with the majority (60-70%) being excreted intact in the urine. The current microbiological potency testing of CMS *in vitro* is therefore not considered to be a suitable control method.

Bergen et al.[10] demonstrated that CMS possesses no or little antimicrobial activity and that microbial assays are not useful in assaying biological samples. The authors also discuss the problem with the handling of CMS samples where it is difficult to determine whether the amount of colistin in a sample is caused by degradation or by the sample pre-treatment. The *Ph. Eur.* monograph determines potency by means of the microbiological assay of antibiotics, using the diffusion method, following incubation, for at least 18 hours, at pH 7.3 and a temperature between 35-39 °C. It is known that CMS is an inactive pro-drug, which converts to the active colistin by hydrolysis at uneven rates *in vitro*, particularly in aqueous environments at 37 °C. As no correlation between the microbiological assay and composition as analysed by HPLC has been established, the microbiological assay and determination of potency is dependent upon both the reference standard and the test sample undergoing hydrolysis at the same rate and extent during the incubation period. The use and the reliability of a microbiological assay to determine colistin levels was therefore questioned because CMS is the inactive prodrug of colistin and it is the colistin formed from CMS during incubation that actually provides the antimicrobial activity. Bioassay results may therefore be misleading, since some of the CMS present in the biological fluid will be converted to colistin during the 24 hour incubation period of the test and the microbial assay for CMS instead becomes a measurement of the formed colistin. The *in vitro* microbiological potency test was therefore not considered satisfactorily stability indicating.

The literature establishes that the formation of colistin in solution is dependent on factors such as concentration, time after dissolution, pH, media, and temperature. All these factors are fixed in the preparation of the finished product. Thus, if CMS is prepared in vial and diluted according to the SmPC there is negligible formation of colistin and the rate and amount of formed colistin should be constant. One factor that influences the rate and thereby also the amount of liberated colistin is the degree of sulfomethylation of the starting material, which is controlled by HPLC. If batch to batch consistency is achieved during product manufacturing, little to no difference would be seen in the rate and amount of formed colistin. Hence, determination of the rate and formation of colistin should be investigated and documented in the finished product development. The suggested HPLC method ensures a constant degree of sulfomethylation with a constant microbial activity.

The CHMP therefore considered that the rate and extent of release of colistin from CMS by hydrolysis are critical quality attributes and that a clear understanding of these is essential for defining the *in vitro* potency. However, the CHMP acknowledged that there are practical obstacles in developing an *in vitro* test under bio-relevant conditions, in particular if colistin content is determined chromatographically, given the complexity of both CMS and colistin chromatograms. Although the test would be complementary to the proposed additional chromatographic tests to determine the degree of sulfomethylation and free colistin, it is likely that it would only be developed by a pharmacopoeial body

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[10] Bergen PJ, Li J, Rayner CR, Nation RL. Colistin Methanesulfonate Is an Inactive Prodrug of Colistin against Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy. 2006;50(6):1953-1958. doi:10.1128/AAC.00035-06.

and not by industry. The CHMP therefore recommended to EDQM that an *in vitro* test to determine the rate and extent of hydrolysis of CMS to colistin, under bio-relevant conditions should be proposed for inclusion in the *Ph. Eur.* monograph.

Hydrolysis during finished product manufacture and the impact of lyophilisation

Powders for solution for injection or inhalation CMS finished product do not contain excipients. Some products are prepared by aseptic assembly of the sterile active substance. The finished product usually has a shelf-life of 3 years and is known to be stable as a dry powder. Therefore, the efficacy and safety of the finished product is principally determined by the quality of the active substance and the recommendations discussed above for the active substance have been made to minimise variability of the active substance and thus ensure consistent quality of the finished product.

However, for finished products manufactured using non-sterile CMS, with dissolution, filter sterilisation and subsequent lyophilisation, there is a risk of hydrolysis of CMS during manufacture, resulting in a decrease in the degree of sulfomethylation of the active substance and the possible release of the more toxic colistin. This should be addressed by appropriate risk assessment and risk mitigation steps. Such a change would not affect necessarily potency determination of the finished product, because the potency test is not considered a satisfactory stability indicating method, but the safety of the finished product may be adversely affected.

The CHMP therefore investigated the potential impact of lyophilisation on the chromatographic pattern of the finished product. Some products are manufactured by dosing sterile lyophilised CMS powder directly into vials that are subsequently sealed. Other products are manufactured by dosing CMS suspended in an aqueous solution into a vial with subsequent lyophilisation and sealing. This powder is then solubilised again at the time of administration. This may result in additional hydrolysis, which would be reflected in the HPLC profile. Having reviewed the available data, the CHMP considered it insufficient to determine whether the lyophilisation process affects the stability of the CMS product, as the analytical methods available were considered inadequate. The CHMP concluded that while many products are prepared by aseptic assembly, without additional lyophilisation and without any effect on the degree of sulfomethylation of the active substance, there is an increased risk of active substance degradation, with a change in the degree of sulfomethylation for products whose manufacture involves lyophilisation. Because the degree of sulfomethylation is a critical quality attribute, the CHMP recommended that the control strategy for CMS finished product should include a test to show that the degree of sulfomethylation is not affected by the manufacturing process.

The following recommendation is made to the marketing authorisation holders:

The *in vitro* microbiological potency test is not considered satisfactorily stability indicating.

CMS active substance in finished products, on release and throughout the shelf-life of the finished product, should comply with the CMS *Ph. Eur.* monograph, in particular with respect to the degree of sulfomethylation and the limit test for colistin in the CMS *Ph. Eur.* monograph, unless otherwise qualified and justified.

A test to monitor the rate and release of colistin, by hydrolysis, from CMS is also recommended.

For finished products manufactured by lyophillisation, given the known instability of CMS by hydrolysis to colistin, the manufacturing process should be supported by a risk assessment and risk mitigation steps to minimize hydrolysis.

2.4. Stability

The CHMP reviewed the stability of CMS active substance, in particular to assess whether any changes in the composition of CMS with a consequential impact on safety and efficacy could be identified. The CHMP noted that a method for analysing composition for stability control of the active substance was only recently implemented by the manufacturer and that no continuous control during shelf life is available. However, the analysis of samples from end-of-shelf life (5 years of stability) showed no deviation in composition, related impurities or sum of E1 and E2 compared to *Ph. Eur.* reference standards and recently manufactured batches. Moreover, the stability of the active substance may be

evaluated by assessment of stability results obtained for the finished dosage form and stability data from three CMS active substance batches stored under approved conditions were provided for assessment. The CHMP considered that based on the data, the finished product remains stable at room conditions and storage was not expected to have any impact on safety and efficacy of the finished product. The CHMP also noted results from long-term stability testing which were within the required limits of the specification developed on the basis of the *Ph. Eur.* CMS monograph and were considered to support the declared 18-month re-test period when stored at up to 25 °C and protected from light and moisture. The CHMP therefore considered CMS to be stable when stored as a freeze dried product and controlled to the current *Ph. Eur.* specification. The consistency of the sub-fractions between batches was considered reassuring. No historical trend data was provided, but this was accepted, given that such testing is additional to current *Ph. Eur.* requirements.

Regarding the stability of the reconstituted solution, the CHMP reviewed the results of an in-use stability test carried out with three batches of 1 MIU CMS powder for solution for injection or infusion. The vials were reconstituted with 5 ml of sterile water for injection or 0.9% saline solution and the obtained solution was stored at 5 °C for up to 24 hours. The E1-1 and E1-2 components decreased in all tested samples while the E1-6 and E1-7 components increased. It was concluded that early eluting peaks tend to decrease, while late eluting peaks tend to increase. However, all results were inside the proposed limits and it was therefore concluded that partial hydrolysis of CMS during 24 hours at 5 °C has no significant impact on safety and efficacy. Another test investigated the compatibility and stability of 1 MIU CMS powder for solution for injection, infusion and inhalation after dissolution in water for injection and in 0.9% saline solution. Extreme cases of the highest (500,000 IU/ml) and lowest (10,000 IU/ml) concentrations used in clinical practice were prepared and stored for 72 hours at 2 °C - 8 °C and at 22 °C - 27 °C. The stability criterion was active substance content reduced by 5% (a solution in which the content is reduced by less than 5% is considered stable) and the results showed that chemical stability was retained for 3 days when stored at 2 °C – 8 °C (refrigerator) and at 25 °C. However, for microbiological reasons and in accordance with the CPMP/QWP/159/96 guideline, the storage time should not be longer than 24 hours at 2 °C – 8 °C (refrigerator), unless the vial contents were dissolved in controlled and validated aseptic conditions.

While the data on the stability of CMS was considered reassuring, the CHMP noted that the amount of free colistin during in use reconstitution was not monitored. The CHMP therefore reviewed data on the conversion of CMS to free colistin in reconstituted suspensions and any evidence of safety concerns, including pain on injection. The conflicting data regarding the requirements for aqueous stability of CMS, depending on whether it is considered in the context of test conditions or in the context of therapeutic administration was considered to be due to a previously incomplete understanding of the nature of CMS solutions. This has been explored by Wallace et al., 2010[11,12] in a study to evaluate the extent of conversion of CMS to colistin in CMS pharmaceutical formulations under relevant conditions of storage and use, using an HPLC method and CMS sourced from the main EU manufacturer. Two commercial CMS formulations were investigated for stability with respect to colistin content. The CMS lyophilised powder for injection was stable (<0.1% of CMS present as colistin) for at least 20 weeks at 4 °C and 25 °C at 60% relative humidity. Once reconstituted, stability appeared to be dependent on concentration. When reconstituted with 2 ml of water to a CMS concentration of 200 mg/ml for injection, it remained stable (<0.1% colistin formed) for at least 7 days at both 4 °C and 25 °C. When further diluted to 4 mg/ml in a glucose (5%) or saline (0.9%) infusion solution as directed, CMS showed evidence of hydrolysis after 48 hours, in a temperature-dependent manner (<4% colistin formed at 25°C compared to 0.3% colistin formed at 4 °C). The solution for inhalation formulation (77.5 mg/ml), was stable at 4 °C and 25 °C for at least 12 months, as determined based on colistin content (<0.1%).

Wallace et al. characterised the self-assembly behaviour of both colistin and CMS in solution. In addition, the authors investigated whether micellisation could be a possible mechanism for the observed concentration-dependent stability of CMS. The degradation of CMS in solution over time was assessed by measuring the extent of colistin formation. CMS solutions were prepared at 0.052, 0.52, 5.2 and 52 mM in 0.9% (154 mM) saline solution. The authors considered that the marked change in the observed rates of CMS conversion to colistin between 0.52 and 5.2 mM is entirely consistent with the critical micelle concentrations (CMC) of 3.5 mM for CMS, as determined by light scattering. Based

[11] Wallace S, Li J, Rayner C, Coulthard K, Nation R. 2008. Stability of colistin methanesulfonate in pharmaceutical products and solutions for administration to patients. Antimicrob. Agents Chemother. 52:3047–3051
[12] Wallace, S. J., Li, J., Nation, R. L., Prankerd, R. J., Velkov, T., & Boyd, B. J. (2010). Self-assembly behaviour of colistin and its prodrug colistin methanesulfonate: implications for solution stability and solubilisation. Phys Chem B., 114(14): 4836–4840. doi:10.1021/jp100458x.

upon this observation, the micellisation of CMS would appear to afford some protection of the labile sulfomethyl groups from degradation. Assuming that micellisation is driven by attractions between the flexible fatty acid tails of CMS, the anionic, susceptible sulfomethyl groups would be orientated towards the exterior surface of the micelle. Therefore, the hydrolysis of susceptible sulfomethyl groups arranged within a micelle can be slower than that of the monomer dispersed in the bulk. The authors concluded that rapid conversion of CMS to colistin occurs below the CMC (60% over 48 h), while conversion above the CMC is less than 1%, in a 0.9% saline solution at 25°C. The authors calculated the CMC for CMS to be 3.5 milimolar (mM). From the mM definition ($10^{-3}/dm^3$ (L)) and the CMS relative molecular mass stated by the European active substance manufacturer, the CMC of 3.5 mM corresponds to 610.75 mg/100 ml, or 6.1 mg/ml, which in turn corresponds to approximately to 80,000 IU/ml, although it was noted that this figure can only be approximate, given the paucity of available data.

Dutkiewicz et al., 2002[13] demonstrated that the activity of water at micelle surfaces is different to that at the bulk and the high concentrations of the counter ions immobilised close to the micelle surface reduce the mobility of and deplete the water close to the micelle surface. All of this results in a considerably slower hydrolysis of the sulfomethyl groups of CMS existing as micelles, compared with CMS existing as monomers.

With regard to the effect of CMS micelle formation on the antimicrobial assay of CMS, Bergen et al., 2006 demonstrated that CMS is an inactive prodrug of colistin and that it is therefore reasonable to assume that rapid and complete hydrolysis of the colistin is required to achieve an accurate microbiological assay for colistin. The measured MICs for CMS are typically 1-2 mg/ml and are therefore below the CMC. It is therefore considered unlikely that CMS micelle formation will affect the routine assay of CMS, especially as CMS at a concentration of 1.3 mg/ml will hydrolyse completely within 9-10 hours, which is significantly less than the 18 hour time point for the reading of a microbial assay plate.

In conclusion, the CHMP agreed that the apparent discrepancies in the aqueous stability of CMS are related to the concentration and that at concentrations below the CMC, CMS can hydrolyse rapidly to its constituent polymyxin components while above the CMC, the surface effects of the micellisation appears to give CMS a degree of protection against hydrolysis of the sulfomethyl groups of the proportion of CMS existing as micelles. The concentrations used for the microbiological assay are below the CMC and therefore the CMS is considered to be approximately fully converted. The CHMP considered that the critical factors for the in-use stability of CMS and its conversion/hydrolysis to colistin to be temperature, concentration and time.

The CHMP concluded that CMS solutions are stable from the chemical and physical point of view during 24 hours at 2 to 8 °C but that rapid conversion of CMS to colistin occurs below the CMC of 6.1 mg/ml at 25 °C which corresponds approximately to 80,000 IU/ml, and is more or less the minimum dose recommended if the dilution is made with 2 ml of solvent. In addition, the minimum infusion concentration (according to CMC) should be 610.75 mg/100 ml or approximately 7,634,375 IU/100 ml at 25 °C, to avoid conversion to colistin. The loading dose of 9 MIU in 100 ml can be given by infusion, based on this study, but solutions of CMS with a concentration below 7 MIU in 100 ml are not stable and a rapid conversion of CMS to colistin may occur.

Based on the available data, the CHMP considered the chromatographic analytical method (derivatisation of colistin) to be stability indicating, in contrast to the in-use stability assessment by microbiological assay, which does not provide adequate evidence of stability. The reaction with silicotungstic acid is the limit test for free colistin, as stated in the *Ph. Eur.* CMS monograph and while no colistin was detected in the reconstituted solutions, which is considered reassuring, the CHMP noted that this may be due to inadequate sensitivity of the test.

The following recommendation is made to EDQM for revision of the CMS monograph:

The rate and extent of release of colistin, by hydrolysis, from CMS are considered critical quality attributes. A test to specify these attributes in the *Ph. Eur.* monograph should be developed.

It is known that these attributes are influenced by the degree of sulfomethylation and drug concentration.

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[13] Dutkiewicz E, Jakubowska A. Chemphyschem 2002;3:221.

Hydrolysis of colistimethate is significantly increased when reconstituted and diluted below its critical micelle concentration of about 80,000 IU per ml (6.16mgs per ml). It is therefore recommended that test conditions should be representative of CMS infusion administration e.g. a solution of CMS in 0.9% sodium chloride, at a concentration well below 80,000IU/ml.

The following recommendation is made to the marketing authorisation holders:

Investigation of in-use shelf life of reconstituted solutions of finished product should include assessment of changes in the degree of sulfomethylation, presence of free colistin. The rate and release of colistin should also be considered.

The size of the primary packaging should be sufficiently small so that on reconstitution, the concentration of CMS is well above its critical micelle concentration of about 80,000 IU per ml e.g. 10ml.

2.5. Changes to the product information

Based on this information, the CHMP considered that section 6.3 of the summary of product characteristics documents of all CMS products should be harmonised and revised. It was considered important to differentiate between the various formulations available when defining in-use stability recommendations for the SmPC. In addition, the chemical and physical stability of the solution should be considered separately from the conditions of administration and the potential microbiological risk of contamination. Regarding the physical and chemical stability, it was for example noted that due to different dosing and reconstitution volumes, the range of reconstituted solution concentrations for adult patients will differ greatly between solutions for infusion/injection (in the range 20,000 IU/ml to 200,000 IU/ml) and solutions for inhalation (in the range 333,333 IU/ml to 1,000,000 IU/ml). Consequently, the lowest solution concentration for inhaled products is significantly greater than that for infusion/injections products, meaning that a reconstituted inhaled solution is more stable than reconstituted solutions of infusion/injection products. Regarding conditions of administration, it was considered that in-use stability statements could only be defined for reconstituted CMS solutions with concentrations above the CMC and, as a precaution, at refrigerated conditions only, as the data suggests that some level of degradation may occur also above the CMC. An in-use stability statement for the solution for infusion was not considered possible; instead users should be instructed to use the solution immediately. Finally, regarding the potential microbiological risk of contamination, a statement "*From a microbiological point of view, unless the method of opening/ reconstitution/ dilution precludes the risk of microbial contamination, the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of user*" was recommended for products for solution for bolus injection and infusion. Because there is no requirement for inhalation products to be sterile, this statement is not applicable for products for inhalation by nebulisation, in accordance with CHMP/QWP/159/96 corr..

The CHMP also reviewed a number of SmPCs for CMS powder for solution for nebulisation, injection and infusion finished products, looking at the wording present in section 6.6. The reconstitution solvent was found to differ between the formulations. Given the available strengths of CMS finished products and the size of the vials, it is expected that on reconstitution, the CMS concentration will be above its CMC of about 80,000 IU/ml. The SmPCs describe reconstitution only with water for injections or 0.9% sodium chloride. For solutions for nebulisation, reconstitution can be done with water for injection to produce a hypotonic solution, with a 50:50 mixture of water for injection and 0.9% sodium chloride to product an isotonic solution or with 0.9% sodium chloride to product a hypertonic solution. For bolus injections, the injection volume should not exceed 10 ml; for infusions, the volume is usually 50 ml. For inhalation by nebulisation, the maximum volume is 4 ml. The CHMP also advised that the reconstitution should be gentle, to avoid frothing and that the size of the vial should be included in section 6.5.

3. Overall conclusion

The CHMP, having considered the matter and all available data, including responses by the marketing authorisation holders, is of the opinion that the *Ph. Eur.* monographs of colistin sulfate and colistimethate sodium should be updated and that the quality requirements for the CMS containing finished products should be changed.

With regards to the recommendations to update the *Ph. Eur.* monographs, the CHMP particularly emphasises that the colistin and colistimethate sodium subcomponents should be adequately controlled, to ensure consistency in the quality of the colistimethate sodium active substance ; the degree of sulfomethylation, the levels of free colistin and the rate and extent of colistin release by hydrolysis are considered critical quality attributes and should therefore be adequately controlled through appropriate tests with limits set based on the available historical data.

The CHMP also adopted some recommendations to the marketing authorisation holders of polymyxin-based medicinal products, including on the reconstitution of CMS and the associated instability of the solution. Sections 6.3, 6.5 and 6.6 of the summary of product characteristics should be revised for all formulations, as presented in the annex to this document (Appendix I – Polymyxin Article 5(3) - Recommendations to the MAHs).

Appendix I

Polymyxin Article 5(3) - Recommendations to the MAHs

Recommendations made to the marketing authorisation holders

Drug Product Dossier

The *in vitro* microbiological potency test is not considered satisfactorily stability indicating.

CMS active substance in finished products, on release and throughout the shelf-life of the finished product, should comply with the CMS *Ph. Eur.* monograph, in particular with respect to the degree of sulfomethylation and the limit test for colistin in the CMS *Ph. Eur.* monograph, unless otherwise qualified and justified.

A test to monitor the rate and release of colistin, by hydrolysis, from CMS is also recommended.

For finished products manufactured by lyophillisation, given the known instability of CMS by hydrolysis to colistin, the manufacturing process should be supported by a risk assessment and risk mitigation steps to minimize hydrolysis.

Investigation of in-use shelf life of reconstituted solutions of finished product should include assessment of changes in the degree of sulfomethylation, presence of free colistin. The rate and release of colistin should also be considered.

The size of the primary packaging should be sufficiently small so that on reconstitution, the concentration of CMS is well above its critical micelle concentration of about 80,000 IU per ml e.g. 10ml.

Changes to the Summary of Product Characteristics

For solutions for bolus injection, infusion and inhalation by nebuliser, the following changes are recommended to the summary of product characteristics document:

(a)   Section 6.3 to include

Hydrolysis of colistimethate is significantly increased when reconstituted and diluted below its critical micelle concentration of about 80,000 IU per ml.
Solutions below this concentration should be used immediately

For solutions for bolus injection or nebulisation, the chemical and physical in-use stability of reconstituted solution in the original vial, with a concentration ≥ 80,000 IU/mL, has been demonstrated for 24 hours at 2 to 8°C.

From a microbiological point of view, unless the method of opening/ reconstitution/ dilution precludes the risk of microbial contamination, the product should be used immediately.
If not used immediately, in-use storage times and conditions are the responsibility of user.

For solutions for infusion, which have been diluted beyond the original vial volume and / or with a concentration < 80,000 IU/mL, should be used immediately.

(b)   Section 6.5 to include the size of the vial.

(c)   Section 6.6 to include

For bolus injection:
Reconstitute the contents of the vial with not more than 10ml water for injection or 0.9% sodium chloride.

For infusion:
The contents of the reconstituted vial may be diluted, usually with 50ml 0.9% sodium chloride

For inhalation by nebuliser:

Reconstitute the contents of the vial with either water for injections to produce a hypotonic solution or a 50:50 mixture of water for injections and 0.9% sodium chloride to produce an isotonic solution or with 0.9% sodium chloride to produce a hypertonic solution.
The volume of reconstitution should be according to the instructions for use of nebuliser administration device, and is normally not more than 4ml.

(d)   Section 6.6 to also include

During reconstitution swirl gently to avoid frothing.

For single use only and any remaining solution should be discarded.