A method for determination of the Aerodynamic Droplet Size of Nebulised Colistimethate Sodium using a Next Generation Impactor with UHPLC-UV detection.

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Introduction

Nebulised colistimethate sodium is an antibiotic used to treat chronic pulmonary bacterial infections in patients with cystic fibrosis[1]. The product is supplied as a powder in a vial that is reconstituted in the clinical setting with an aqueous solution prior to nebulization. Colistimethate sodium is a prodrug of colistin, a poly cationic peptide (Figure 1). When reconstituted in aqueous solution, the prodrug undergoes hydrolysis and a range of partially sulfomethylated polymyxin chains are produced of varying molecular size. The Primary constituents of the drug are two Polymyxin chains called E1 and E2.

A method was required to assess and compare the aerodynamic droplet size delivered in order to support future in vitro studies of different nebulizer types. The selected method required reasonable sample throughput with a robust detection method as it is reported the aqueous solution has a relatively short shelf life. We discuss the key considerations made on route to establishing a final method for assessing the nebulized aerodynamic size distribution.

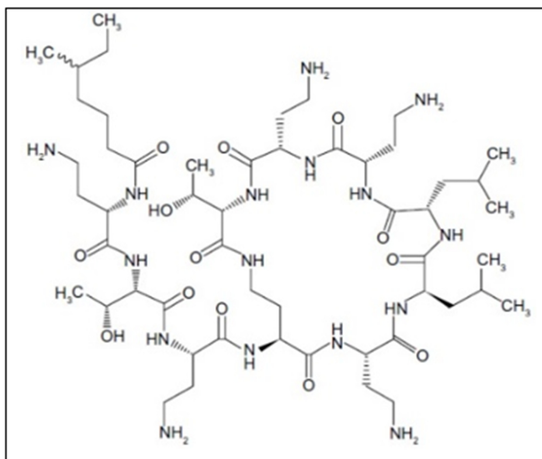

Figure 1 Colistin Chemical Structure (Koerner-Rettberg & Ballmann, 2014)

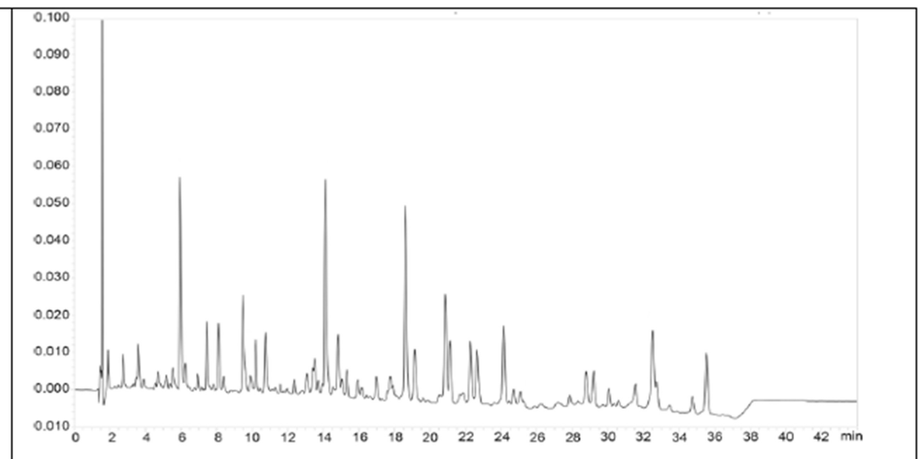

Figure 2 Colistimethate Sodium Reference Solution a, European Pharmacopeia, 10.7, 2022.

Objective

Develop a procedure that allows a simplified chromatographic assessment of the aerodynamic droplet size distribution of nebulised colistimethate sodium using UHPLC with UV detection. The method should be suitable for routine use in a quality control environment, replacing manual measurement procedures such as direct UV spectrophotometry or conductivity measurements currently employed in the laboratory.

Method Considerations

Based on in-house requirements and guidance in the USP chapter <1601>, cascade impaction by NGI was selected as the mechanism to measure the aerodynamic droplet size of the nebulised solution rather than the more rapid laser diffraction as USP <1601> recommends that laser diffraction methods should be validated against a cascade impaction method.[3]

UHPLC with UV detection was selected as chromatographic methods for assay/degradation products do exist in the European Pharmacopeia[4] and literature[5], which provided a good starting point for chromatographic analysis of the aerodynamic droplet size distribution. However, the hydrolysis of the molecule in to various polymyxin chains during reconstitution produces complex chromatography which is challenging to integrate and quantify (Figure 2).

However, it was felt if the integration/quantitation challenge can be resolved the advantages of UHPLC - rapid analysis, autosampler sequencing, and processing via a chromatographic data system (in this case Waters Empower 3) do offer advantages over other more manual/labour intensive methods such as UV spectrophotometry and conductivity detection used in other studies.

Experimental Parameters

Samples of commercially available colistimethate sodium for nebulisation (Colixin, Pharmis Biofarmaceutica, LDA) were reconstituted in 50:50 v/v water for injection/sodium chloride solution (0.9% w/v) prior to nebulisation.

Nebulisation parameters were established following guidance in <1601> of the US Pharmacopeia, using a flow rate of 15 L/min through the NGI. The NGI was chilled to +5°C for 90 minutes in an NGI chiller cabinet (Copley Scientific). A post NGI inline filter fitted with a Whatman GF/C filter (GE Healthcare) was used to collect any material not captured by the micro-orifice collector (MOC) of the NGI. During the development and validation, multiple AeroEclipse XL nebulisers powered by Ombra compressors were utilised (both Trudell Medical, international). Due to impactor overloading on some stages (see Figure 4 and 5), when the full sample volume was nebulised to extinction, a smaller representative sample had to be collected in the NGI. This was achieved by setting a 1 minute nebulisation duration per NGI collection.

UHPLC settings were derived from parameters suggested in literature for HPLC analysis of Colistimethate Sodium and adapted for UHPLC instruments. See Table 1 a) and b) for the UHPLC Parameters. A Waters Acquity system with TUV detector was used for the UHPLC analysis. The starting method used available commercial reference materials colistimethate sodium (United States Pharmacopeia, USP), and colistimethate E1 and E2 identification standards (European Pharmacopeia, EDQM).

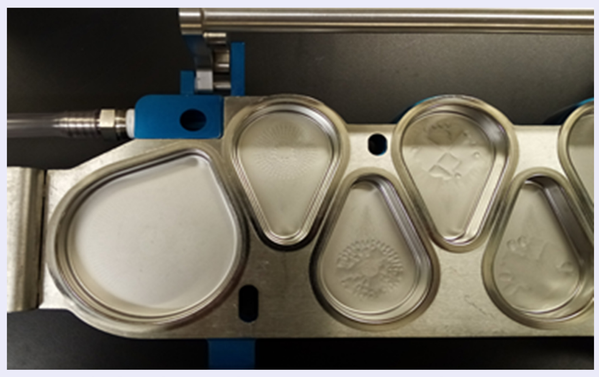

Figure 3. Overloaded Impactor post Nebulisation (4 minutes)

Figure 4. Typical Impactor loading (1 minute)

Table 1 a) System Settings

Column	Waters Acquity UPLC CSH C18, 1/7 µm, 150 x 2.1 mm
Guard Column	Waters VanGuard UPLC CSH C18, 1.7 µm 5x2.1 mm
Column Temperature	30 °C
Flow rate	0.3 ml/ minute
UV detector wavelength	210 nm
Run Time	44 min
Autosampler Temperature	5 °C

b) Mobile Phase Gradient

Time (mins)	Mobile Phase A	Mobile Phase B:	Gradient Slope
Initial	80%	20%	N/A
0 to 10	68%	32%	Linear
10 to 35	53%	47%	Linear
35 to 36	80%	20%	Linear
36 to 44	80%	20%	N/A

Mobile Phase A: 99.5:0.5% v/v 0.065 M Sodium dihydrogen phosphate (pH 6.5): Acetonitrile

Procedure and Results

Good chromatographic separations were obtained using the UHPLC parameters (Figure 5). However, the challenges presented by quantifying using the whole range of peaks in the chromatogram are magnified for an NGI method where deposition on some stages may lead to only the larger Polymyxin peaks being detected in NGI stages with lower drug deposition. As a result, a representative 6 peaks were selected that were deemed sufficiently large enough to be integrated in each chromatogram regardless of the loading on the specific NGI stages. (Table 2).

The areas of these peaks were summed to give a total peak area in order to generate a calibration curve. This procedure differs from the two peak approach reported by Metcalf et al. The six peak approach provided enhanced confidence that slight differences in integration of a peak - for example, due to baseline noise - did not significantly impact the sample quantitation. Once the peaks were selected the challenge of quantifying the colistimethate sodium level distributed on the NGI stages was addressed. Initially, quantitation against a commercial standard such as the USP reference material was considered; however, it was unclear whether the mix of peaks in the USP standard would reflect the same relative peak pattern present in a specific commercial batch of samples. Therefore, a decision was made to quantify the nebulised material against a standard curve generated using an un-nebulised sample from the same sample lot, using the assayed activity value established for the specific lot in International Units (IU). This procedure allowed the method to give a clear indication of the aerodynamic particle size distribution of the product and provide confidence that any difference in the distribution of polymyxin chains from different sources did not interfere with the quantitation of the nebulised material in the NGI. Control solutions of colistimethate E1 and E2 mixtures (Ph.Eur, EDQM) were injected as part of system suitability to monitor system performance and ensure consistent retention times.

The method was successfully validated. The calibration curve shows a linear response for colistimethate sodium concentrations between 100 IU/mL and 16,000 IU/mL (r2=1). Quantitation and recovery of 1,000,000 IU and 2,000,000 IU samples nebulised into the NGI was demonstrated to be accurate and precise (Table 3) with recovery from all stages of the NGI falling within the linear range. The method was demonstrated to be robust to small variations in mobile phase. Impactor wall losses were within acceptable limits (<5%) and overloading was controlled by use of the reduced nebulisation.

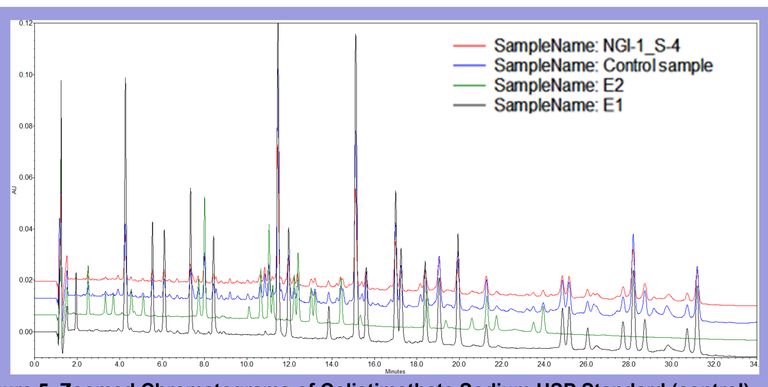

Figure 5. Zoomed Chromatograms of Colistimethate Sodium USP Standard (control) Polymyxin E1 and E2 Identification standards and Sample Recovered from NGI Stage 4

Table 2. Retention times of the 6 peaks selected for integration

Peak Name	Approximate Retention time
CMS E1-1	4.5
CMS E1-2	7.8
CMS E2-1	8.3
CMS E1-3	11.8
CMS E1-8	15.6
CMS E1-7	31.3

Table 3. Validation Precision and Intermediate Precision data

Analyst	Preparation	1 Million IU Strength			2 Million IU Strength		
		MMAD (µm)	GSD (µm)	% FPF < 5 µm	MMAD (µm)	GSD (µm)	% FPF < 5 µm
1	1	4.8	2.1	50.5	4.7	2.1	51.8
	2	4.3	2.1	56.6	4.3	2.0	57.1
	3	4.5	2.1	54.3	4.6	2.1	52.6
	4	4.6	2.2	53.3	4.2	2.1	57.5
	5	4.8	2.1	50.6	4.7	2.1	52.5
	6	4.3	2.1	56.7	4.4	2.1	54.7
	Mean (n=6)			53.7			54.3
	%RSD (n=6)			5			5
2	1	4.7	2.1	51.2	4.2	2.2	56.3
	2	5.0	2.6	48.4	4.5	2.1	53.4
	3	4.6	2.0	52.5	4.5	2.1	53.9
	4	4.5	2.1	53.5	4.6	2.1	52.6
	5	4.5	2.0	54.2	4.6	2.1	52.9
	6	4.7	2.1	50.8	4.8	2.1	50.9
	Mean (n=6)			51.8			53.3
	%RSD (n=6)			4			3
Mean (n=12) Analyst 1 and 2				52.7			53.8
%RSD (n=12) Analyst 1 and 2				5			4

Conclusion

A method to evaluate nebuliser performance by monitoring aerodynamic droplet size was established and validated for use in later studies where different nebuliser lots and models are to be compared for delivery of colistimethate sodium 1,000,000 IU and 2,000,000 IU products. The chromatographic method has since been validated for use in a method measuring the delivered dose of Colixin.

References

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2. Healen, A. M., Gray, W., Fuchs, E. J., Griffiss, J. M., Salata, R. A., & Blumer, J. (2012 Dec;). Stability of Colistimethate Sodium in Aqueous Solution. Antimicrobial Agents and Chemotherapy, 56(12): 6432–6433
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