APPLICATION BRIEF

Pharmaceutical

Analysis of an Antibiotic Ophthalmic Solution by UHPLC/SQ MS

An expired ophthalmic solution of trimethoprim and polymyxin B sulphates was analyzed for degradants using UHPLC/SQ MS. The ophthalmic solution is used to treat bacterial eye infections and is active against a variety of gram negative and gram positive bacteria. An assay to detect degradants formed during storage beyond the expiration date of this diluted antibacterial ophthalmic solution is described.

Experimental Conditions

Target Analytes: Polymyxin B1, B2

Sample Preparation Conditions

An expired ophthalmic solution containing a mixture of polymyxin B sulphates and trimethoprim was diluted 1:10 in HPLC grade water and injected on column.

Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15
Column:	Grace® VisionHT™-HL column (2.1 mm x 50 mm, 1.9 µm)
Flow Rate:	0.8 mL/min with a post column split of 0.4 mL/min into MS
Injection Volume:	1 µL/min in partial fill mode
Mobile Phase:	A: water with 0.1% formic acid B: acetonitrile with 0.1% formic acid

Gradient:

Time (min)	%A	%B
0	90	10
1	80	20
5	50	50

Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Positive mode
Scan Range:	100-1500 m/z
Scan Rate:	2000 u/sec
Capillary Exit Voltage:	100 V and 150 V

Results

Polymyxin B is a cyclic peptide antibiotic which is usually present as a mixture of B1 and B2 sulphates. The structure of polymyxin B1 sulphate is shown in Figure 1.

The mass spectrum of polymyxin B1 at a low capillary exit voltage showed the intact molecule with multiple charges on it (Figure 2). Multiple charge information is very useful for this type of biomolecule to further confirm the molecular weight information.

The mass spectrum of polymyxin B1 analyzed at a higher capillary exit voltage resulted in fragmentation of the cyclic antibiotic to yield characteristic fragments of the molecule (Figure 3).

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The extracted ion chromatogram of polymyxin B1 molecular ion showed the antibiotic to elute at different times during the chromatographic run. However, the spectra of polymyxin B1 was identical at these different retention times suggesting they are most likely stereoisomers formed by isomerization of L with D amino acids. A similar scenario was observed with polymyxin B2. To determine if these stereoisomers are formed at higher temperatures, the sample was heated to 40 °C overnight and then analyzed by UHPLC/SQ MS. The results showed increased formation of some of the stereoisomers of polymyxin B1 and B2 (data not shown).

Conclusions

UHPLC/SQ MS analysis of an expired ophthalmic solution containing diluted concentrations of cyclic antibiotics of polymyxin B showed several stereoisomers of B1 and B2. These isomers increased upon heating of the sample, suggesting racemic mixtures can be formed in the ophthalmic solution during storage under non-refrigerated conditions.

The Flexar SQ 300 MS ionization capabilities allowed us to obtain molecular ion information even on a labile structure like that of polymyxin B. Multi-charged species were also obtained confirming the molecular ion.

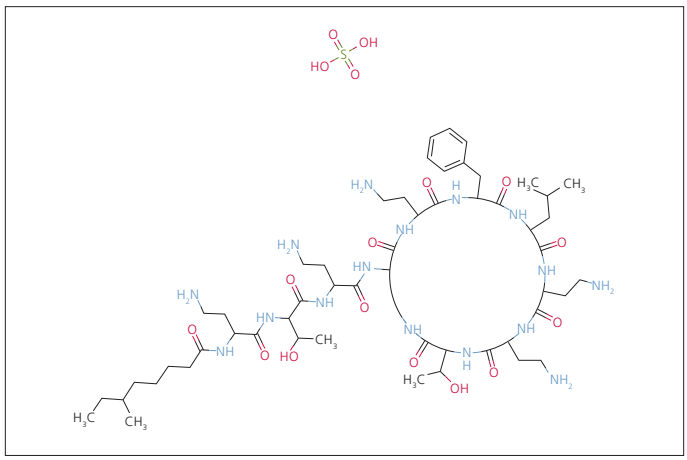

Figure 1. Structure of polymyxin B1 sulphate. Polymyxin B1 has a terminal 6-methyloctanoyl group and polymyxin B2 sulphate has a terminal 6-methylheptanoyl group.

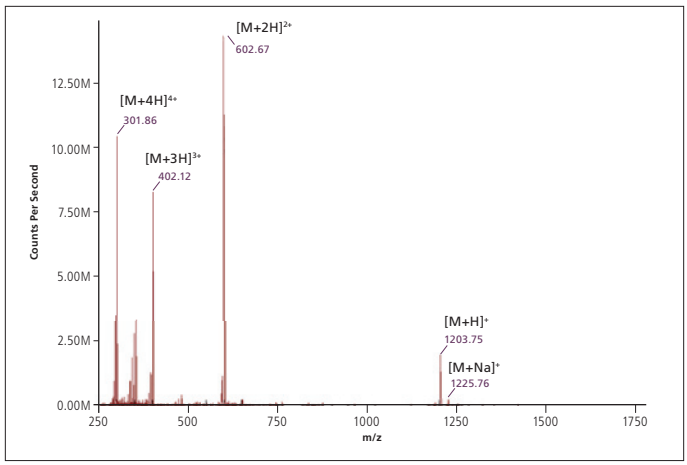

Figure 2. Mass spectrum of polymyxin B1 showing intact molecule with multiple charges analyzed at a low capillary exit voltage of 100 V. The observed $[M+H]^+$ of the intact molecule matches the calculated monoisotopic $[M+H]^+$ of 1203.75.

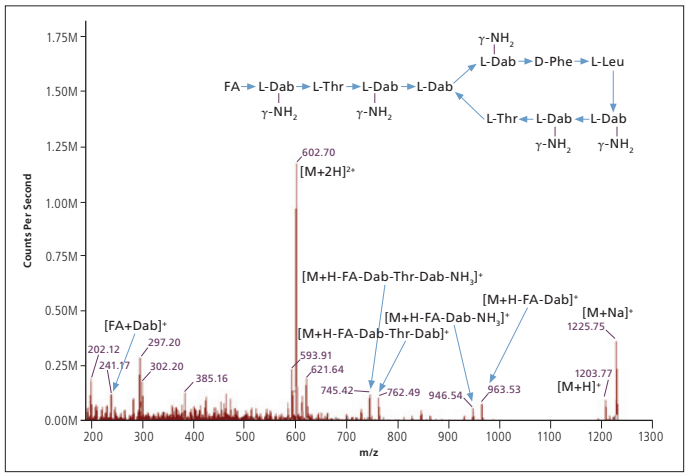

Figure 3. Collision Induced Dissociation (CID) spectrum of polymyxin B1 obtained at a higher capillary exit voltage of 150 V. Annotations: FA is the fatty acid 6-methyloctanoyl group; Dab is α, γ-diaminobutryic acid; amino acids are represented as three letter notations.

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