Development of LC-MS/MS Method in Plasma for Colistin and Colistimethate

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Overview

- Colistin is an antibiotic from the polymyxin class, effective against most Gram-negative bacilli.
- Colistin consists of a mixture of the cyclic polypeptides Colistin A and B.
- Colistin is administered as the produrug Colistimethate Sodium (CMS) due to high toxicity of free colistin.
- CMS is hydrolyzed in vivo to a variety of polymethanesulfonated compounds, and eventually, free colistin
- A novel LC-MS/MS method was developed for measuring free colistin and total colistin (which includes CMS and all partially hydrolyzed derivatives) in rat plasma.

Development Challenges

Total Method

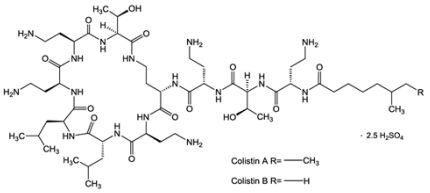
- CMS and its methanesulfonate derivatives must be fully hydrolyzed to free colistin.

Free Method

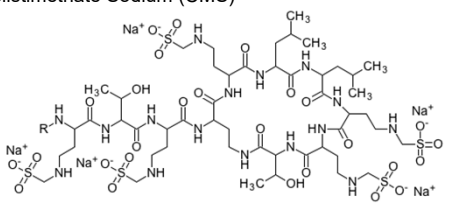
- Hydrolysis of CMS to colistin at any step during processing creates an artificially high free colistin measurement.
  - CMS is very unstable in plasma. Samples must be chilled to prevent unwanted hydrolysis to free colistin.
  - CMS in plasma extracts may hydrolyze to free colistin while waiting for injection. Free colistin extraction recovery should be maximized while minimizing recovery of CMS.
  - Low pH during sample processing could cause unwanted hydrolysis.

Structures

Colistin



Colistimethate Sodium (CMS)



Instrumentation

Waters Acquity UPLC		**Thermo Scientific TSQ Vantage MS**	
Run Time:	3.8 min	Ion Source:	HESI
Column Temp.:	30ºC	Spray Voltage:	1000
Autosampler Temp.:	7.5ºC	Ion Transfer Tube Temp:	250 ºC
Injection Volume:	20 µL	Vaporizer Temp:	400 ºC
Flow Rate:	0.4 mL/min	Sheath Gas	40
Mobile Phase:	A: Water-Formic Acid, 100-0.5 B: Acetonitrile Gradient 5% to 30% B over 2.5 min	Aux Gas	12
Analytical Column:	Waters Acquity UPLC® BEH C18, 1.7 µm, 2.1 x 50 mm	Resolution	Unit/Unit

SRM Table

Compound	Polarity, charge state	Precursor (m/z)	Product (m/z)	Collision Energy (V)	S Lens (V)
Colistin A	Positive (2+)	578.40	100.95	27	136
Colistin B	Positive (2+)	585.43	100.95	27	141
Polymyxin B	Positive (2+)	602.43	100.95	27	140

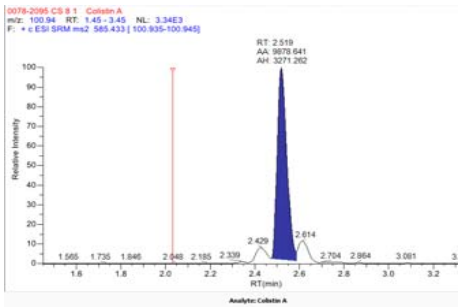
Extraction Method

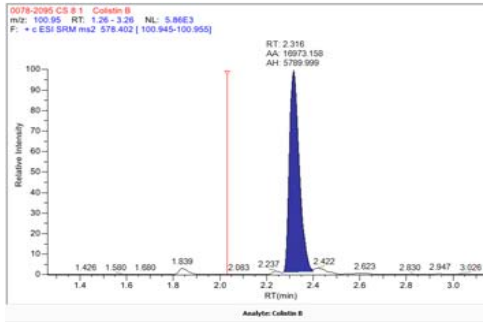
- 50-50,000 ng/mL colistin in K2EDTA rat plasma (18.9-18,900 ng/mL Colistin A; 31.1-31,100 ng/mL Colistin B)
- 50 µL plasma aliquot; the same sample is analyzed two times: once for total and once for free colistin
- Polymyxin B is the internal standard.

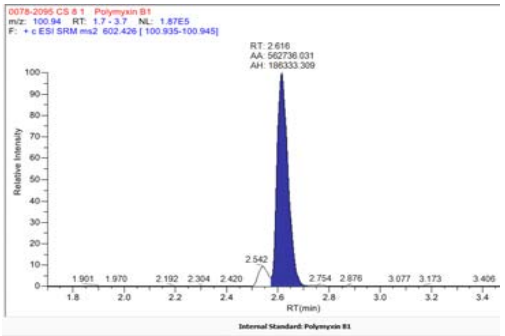
- Total Method: dilute H_2SO_4 is added to the plasma aliquot for 15 minutes, then quenched with NaOH prior to SPE.
  - 100% hydrolysis of CMS to free colistin is achieved.
- Free Method: No hydrolysis step; hydrolysis of CMS must be prevented.
- Free and total methods use the same solid phase extraction (SPE) procedure.

- Waters Oasis weak cation exchange (WCX) µElution SPE
  - Samples are diluted and loaded/washed on the sorbent with ammonium formate buffer (50 mM pH 9).
    - CMS is polyanionic, and is unretained and washed through the sorbent.
    - Free colistin is polycationic, and is retained on the sorbent.
  - Colistin and Polymyxin B are eluted with 50 µL water-ACN-TFA, 25-75-1. Strongly acidic TFA is needed for recovery of >80%.
    - No risk of unwanted hydrolysis since CMS has been washed away.
  - Extracts of CMS spiked plasma samples show little increase in colistin response over several days, indicating that CMS was effectively removed by using pH 9 buffer during extraction.

LLOQ Calibration Standards







Conclusions

A method for quantitating both free and total colistin A and B in rat plasma has been developed. A unique approach using specific pH control during SPE extraction controls the hydrolysis of colistimethate to colistin. Hydrolysis is quantitative using H_2SO_4 when measuring total colistin, while hydrolysis is minimized when measuring free colistin. Recovery of both analytes and the internal standard using the weak cation exchange SPE is high and reproducible.