T1330-03-019

LC/MS/MS Method for the quantification of Metronidazole in skin dialysate

Benjamin Kuzma[1], Sharareh Senemar[1] , Richard DePinto[2], Grazia Stagni[1]

1 Division of Pharmaceutical Sciences, Long Island University, Brooklyn, NY,

2 Waters Corp, Milford, MA

CONTACT INFORMATION: HS 623 75 DeKalb Ave, Brooklyn NY 11201 Tel. 203-843-5490 email: Benjamin.kuzma@my.liu.edu

aaps PharmSci 360 ADVANCING PHARMACEUTICAL SCIENCES, CAREERS, AND COMMUNITY

BACKGROUND

Metronidazole (MTZ)

- Molecular formula: $C_6H_9N_3O_3$
- Water solubility > 1 mg/mL;
- LogP -0.02; pKa 2.38

Analytical Sensitivity

- Intradermal microdialysis facilitates the monitoring of drug concentration in the dermis, which is the site of action for many topically applied products
- The success of this technique is dependent upon the availability of a highly sensitive and selective analytical method.

PURPOSE

- To develop and validate a highly sensitive, selective, and reproducible Liquid Chromatography – Mass Spectrometry method for the drug Metronidazole (MTZ) and the internal standard (IS) Deuterated Metronidazole (D_3-MTZ).

METHODS

Preparation of Standard Solutions

- A "calibration stock" solution was prepared from the MTZ and D_3–MTZ stock solution (1 mg/ml) to obtain a concentration of 1,000 ng/mL.
- Dilution were made from the "calibration stock" using lactated ringer solution to obtain concentrations of: 200 ng/mL, 100 ng/mL, 50 ng/mL, 20 ng/mL, 4 ng/mL, 2 ng/mL, 0.8 ng/mL, 0.4 ng/mL.
- The calibrators were prepared in 1.5mL Eppendorf centrifuge tubes and stored at 25°C.

Chromatographic Conditions

- Waters Acquity BEH C18 column (2.1 x 50mm, 1.7um). The column temperature was 40°C.
- Gradient mobile phase 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile(Solvent B). (See Table 1)
- Flow rate of 0.6ml/min The total run time was 3 minutes and the injection volume was 1ul.
- Dialysate samples are in lactated ringer (LR) and do not require a clean-up step. However, to prevent salt accumulation in the MS, the LR salts were diverted to waste.

MS/MS Detection

- Analysis was performed on a Waters Xevo TQS tandem mass spectrometry system using positive ion electrospray with MRM detection
- The transitions monitored for quantification were the protonated molecular ions 172 [M+H+] → 128 for Metronidazole and 175 [M+H+] → 131 for D_3 Metronidazole, respectively.

RESULTS

MTZ and D_3-MTZ Calibration Curve

- The assay was linear from 0.4 ng/ml to 200 ng/ml (R^2> 0.99). The curve was weighted using the 1/Y scheme.
- The LOD was found to be 0.05 ng/mL while the LLOQ was 0.4 ng/mL. The ULOQ was 200 ng/mL.
- No interference was observed for *in-vivo* dialysate samples spiked with various concentrations of MTZ. The mean analyte response of the blank matrix was 0.6% of the LLOQ response.
- Neither MTZ or D_3-MTZ were detected in their respective transitions, indicating no cross analyte interference.
- The deviation of the QCs across multiple runs spanning 3 days were less than 6%.

Stability

- The stock solution was confirmed to be stable for 1 month at 25 ℃
- The bench top stability confirmed the samples to be stable for 3 days.
- Demonstrated to be stable on ice for 3 days and followed by 7 days at 25 ℃

Time	Flow rate (ml/min)	%A	%B
Initial	0.6	100	0
1.00	0.6	100	0
1.50	0.6	10	90
1.80	0.6	100	0

Table 1: Gradient program to detect MTZ and D_3-MTZ

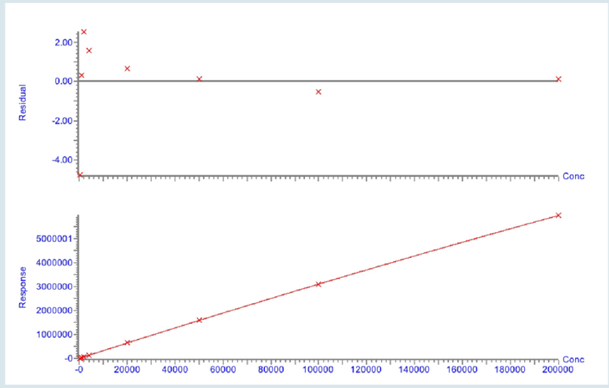

Figure 1: The top figure is an example plot of the residuals while the bottom figure is an example of a calibration curve used in analysis of samples

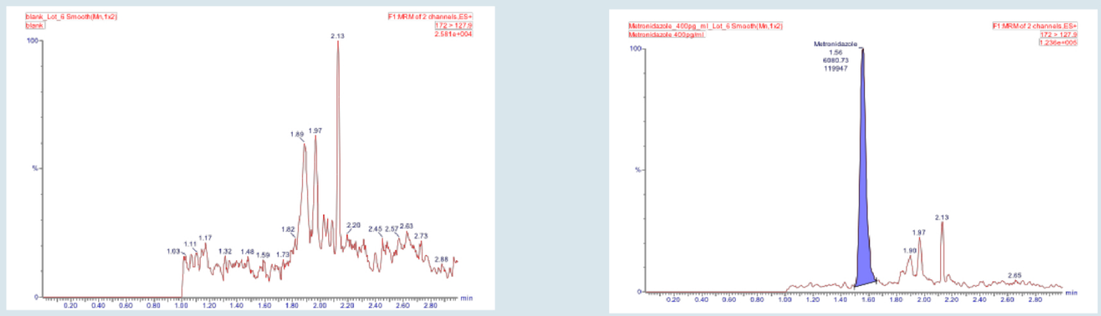

Figure 2: Selectivity of the MTZ and D_3-MTZ analysis The left figure is an blank LR sample indicating no interference at the measured transition. The right figure indicates the quantification of the LLOQ (0.4 ng/ml) for Metronidazole

CONCLUSIONS

- A UPLC-MS/MS for the quantification of Metronidazole (MTZ) and D_3-Metronidazole (D_3-MTZ) was developed and validated.
- The quantitation of both MTZ and D_3-MTZ in Yucatan mini-pig skin dialysate samples is free from interference, giving confidence and reliability to concentrations detected.
- The ability to quantify D_3- MTZ allows it to be used as a dMD performance marker and correct MTZ concentrations.
- The method was **successful** applied to pharmacokinetic studies of metronidazole in dermis dialysate using microdialysis.

REFERENCES

1. Shippenberg, T.S. and A.C. Thompson, *Overview of microdialysis.* Curr Protoc Neurosci, 2001. **Chapter 7**: p. Unit7 1.
2. Swartz, M. and I.S. Krull, *Handbook of analytical validation*. 2012, Boca Raton, FL: CRC Press. xiv, 206 p.

FUNDING / GRANTS

Funding for this project was made possible, in part, by the Food and Drug Administration through grant 1U01FD005862. The views expressed in this abstract/poster do not reflect the official policies of the U.S. Food and Drug Administration or the U.S. Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

LIU Pharmacy