Determination of Moxifloxacin in Human Plasma and Feces by LC-MS/MS

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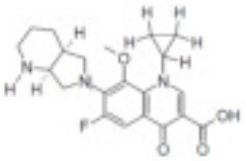
INTRODUCTION

Da Volterra devised a novel colon-targeted adsorbent, DAV132, to be associated with antibiotics to prevent their impact on the microbiota, resulting in emergence and dissemination of resistant bacteria, antibiotic-associated diarrhea, and Clostridioides difficile infection. Da Volterra previously showed DAV132 ability to deliver a powerful adsorbent in the late ileum of human volunteers. Moxifloxacin is an antibiotic used to treat a number of bacterial infections. To support a clinical study of DAV132, a plasma method is developed and validated. As the amount of antibiotics in feces (unbound to feces particles) is a critical parameter in this study, a method in feces to determine the free fraction was developed and validated according to FDA and EMA guidelines.



Moxifloxacin, $C_{21}H_{24}FN_3O_4$, 401.43 g/mol

IS: H = D

DIFFICULTIES WITH FECES ANALYSIS

- Inhomogeneity
- Additional sample preparation (a new sample is created)
- Back calculation to concentration per kilogram
- Feces has to be handled with care because of intestinal bacteria

VALIDATION RESULTS

Bioanalytical method validations where performed according to FDA and EMA guidelines.

Assay range: 10.0 – 5000 ng/mL for both plasma and feces extract (40.0 to 20,000 ng/gram feces).

plasma

Level	LLOQ 10.0 ng/mL	LQC 30.0 ng/mL	MQC 500 ng/mL	HQC 4000 ng/mL
Precision (%CV)	5.3%	3.8%	1.7%	1.2%
Accuracy (%RE)	-2.1%	+9.7%	-8.0%	+3.6%

Feces

Level	LLOQ 10.0 ng/mL	LQC 30.0 ng/mL	MQC 500 ng/mL	HQC 4000 ng/mL
Precision (%CV)	3.1%	1.9%	1.8%	1.6%
Accuracy (%RE)	+2.3%	+0.1%	-4.4%	+1.2%

All other validation items were also within criteria

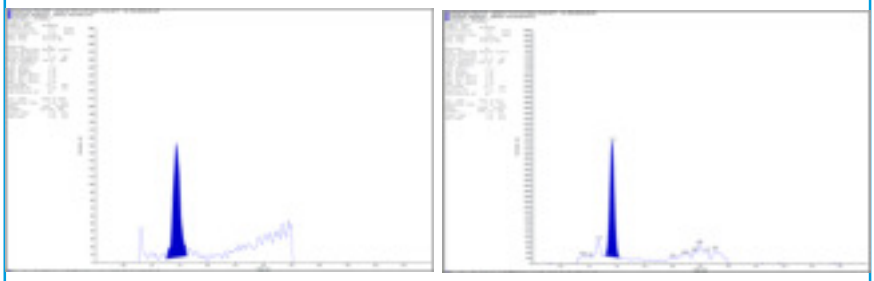
MATERIALS AND METHODS

Plasma Sample preparation: protein precipitation was applied using 50.0 µL sample and 400 µL ACN:FA (100:1 v/v). After centrifuging the extracts were evaporated to dryness and reconstituted in 200 µL Milli-Q:ACN:FA (85 : 15 : 1 (v/v/v)).

Feces sample preparation: liquid liquid extraction was applied using 100 µL feces filtrate and 3 mL TBME. The organic layer is evaporated to dryness and reconstituted.

Column: Phenomenex Gemini 5 µM C18 110 Å , 50 x 2 mm @ 600 µl/min. the gradient is 85% Mobile phaseThe start of A, after half a minute it decreases to 25% Mobile phase A followed by a flush step. ESI in positive mode was applied monitoring: 402.3 / 384.4 (Moxifloxacin), 407.4 / 389.3 (Moxifloxacin-d5) using a Sciex API4000 mass spectrometer.

CHROMATOGRAMS AT THE LLOQ LEVEL



Plasma Feces filtrate

CONCLUSION

The methods for the determination of moxifloxacin in human plasma and free moxifloxacin in human feces where successfully validated. The methods were applied for a clinical study (data not shown).
In this clinical study the free moxifloxacin fecal concentration as well as other clinical endpoints will be used to evaluate the clinical efficacy of DAV132 to protect the intestinal microbiota from the antibiotic-induced dysbiosis and prevent clinical manifestation of this dysbiosis in patients.

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