Development of a high-throughput UPLC–MS/MS method for the simultaneous determination of fexofenadine and olmesartan in human serum

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OVERVIEW

- The pharmacokinetics of the pharmacologic probe drugs olmesartan and fexofenadine are commonly used to phenotype transporter pathways in humans.
- These studies require accurate and precise measurement, so we developed the first LC-MS/MS method that quantifies fexofenadine and olmesartan simultaneously.
- The method utilizes small sample volume (50 µl), fast sample preparation, and was comprehensively validated using the FDA Guidance for Bioanalytical Method Validation.

INTRODUCTION

- Fexofenadine and olmesartan are prescription drugs with a unique pharmacokinetic profile. They are eliminated *in vivo* entirely by drug transporters, and this has created a niche for their use as pharmacologic probes to phenotype various transporter pathways.
- While there are LC-MS/MS methods to quantify these analytes individually, to date there are no published methods that quantify both analytes.
- A novel UPLC-MS/MS assay was developed to simultaneously quantify fexofenadine and olmesartan in human serum. To demonstrate the application of the method, the concentrations of fexofenadine and olmesartan were determined in human serum samples obtained from a patient who was administered both drugs orally.

METHODS

- Serum samples (50 µL) undergo protein precipitation with methanol-internal standard solution.
- The separation of the analytes was performed using an Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 µm) with a 0.20 µm frit filter before the column.
- The mobile phase consisted of water with 0.1% formic acid (solvent A) and acetonitrile (solvent B).
- A gradient elution at a flow rate of 0.5 mL/min was run on an Acquity UPLC I-class (Waters) with a total run time of 4 minutes.
- The analytes were detected in positive ion mode with selected reaction monitoring (SRM) using a heated electrospray ionization (HESI) source for ionization on a triple quadrupole mass spectrometer (TSQ Quantum Ultra, Thermo Scientific).

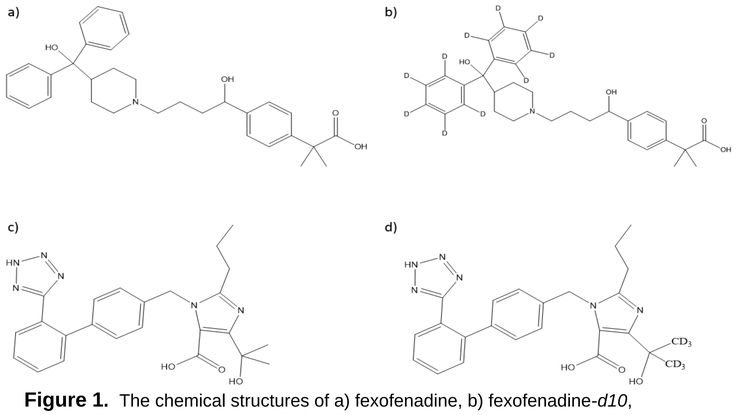

Figure 1. The chemical structures of a) fexofenadine, b) fexofenadine-*d10*, c) olmesartan, and d) olmesartan-*d6*.

Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Collision Energy (V)	Tube Lens (V)
Fexofenadine	502.000	466.580	27	71
Olmesartan	447.300	207.000	23	72
Fexofenadine-*d10*	512.410	476.220	27	112
Olmesartan-*d6*	453.040	207.090	30	62

Table 1. SRM parameters for the analytes and internal standards.

RESULTS

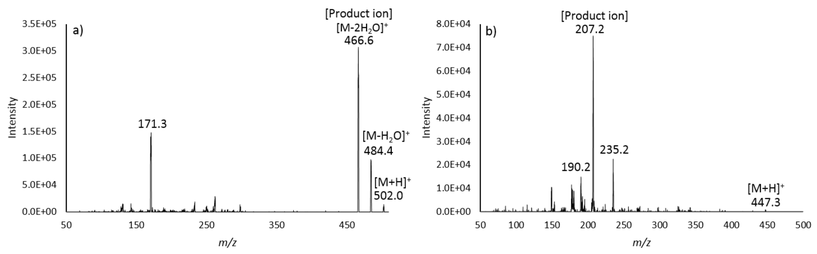

Figure 2. The UPLC-MS/MS spectra for a) fexofenadine and b) olmesartan. The most intense fragments were used for quantitation for fexofenadine (*m/z* 466.6) and olmesartan (*m/z* 207.2).

Parameter	Fexofenadine	Olmesartan
LOD (ng/mL)	0.10	0.30
LLOQ (ng/mL)	1.0	1.0
Linear range (ng/mL)	1.0-500	1.0-500
Slope (n = 3)	0.0006 ± 0.0001	0.0027 ± 0.0001
Intercept (n = 3)	0.0003 ± 0.0001	0.0009 ±0.0001
Correlation coefficient (R^2, n = 3)	0.9979 ± 0.0015	0.9963 ±0.0023

Table 2. Results of the assay validation including LOD, LLOQ, and linear range**.** The slope, intercept, and correlation coefficient are presented as mean ±standard deviation.

Analyte	Level	Nominal Concentration (ng/mL)	Intra-day[a]		Inter-day[b]	
			% Deviation	% CV	% Deviation	% CV
Fexofenadine	LLOQ	1.0	-7.4	12.2	-4.7	9.9
	LQC	3.0	-0.6	9.8	-1.9	8.0
	MQC	100	-9.1	3.0	-3.4	7.6
	HQC	400	-5.5	5.0	-5.5	6.5
Olmesartan	LLOQ	1.0	-3.7	3.2	-3.5	3.7
	LQC	3.0	-6.4	4.5	-5.2	5.3
	MQC	100	2.6	4.4	6.2	5.2
	HQC	400	-4.1	2.9	2.0	5.0

[a] Three replicates for LLOQ; 12 replicates for QCs.
[b] Nine replicates for LLOQ; 24 replicates for QCs.

Table 3. Intra- and inter-day accuracy (%deviation) and precision (%CV) for LLOQ and QCs.

Analyte	QC Level	Nominal Concentration (ng/mL)	Recovery (%, mean)	Matrix Effect (%, mean)
Fexofenadine	LQC	3.0	95.99	111
	MQC	100	87.91	104
	HQC	400	93.76	98.6
Olmesartan	LQC	3.0	105.1	96.6
	MQC	100	97.0	99.6
	HQC	400	101.9	93.5

Table 4. Recovery and matrix effect of fexofenadine and olmesartan from human serum (n = 3).

RESULTS

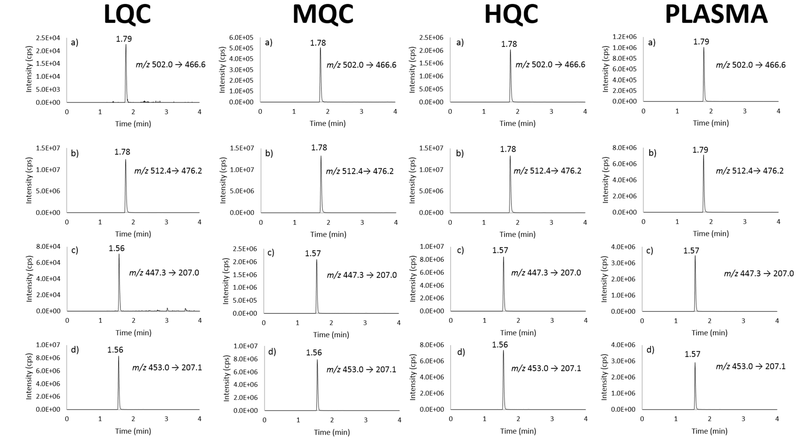

Figure 3. Extracted ion chromatograms (EICs) for a) fexofenadine, b) fexofenadine-d10, c) olmesartan, and d) olmesartan-d6 for the low, middle, and high quality control samples as well as in a human plasma from a patient who ingested oral fexofenadine and olmesartan.

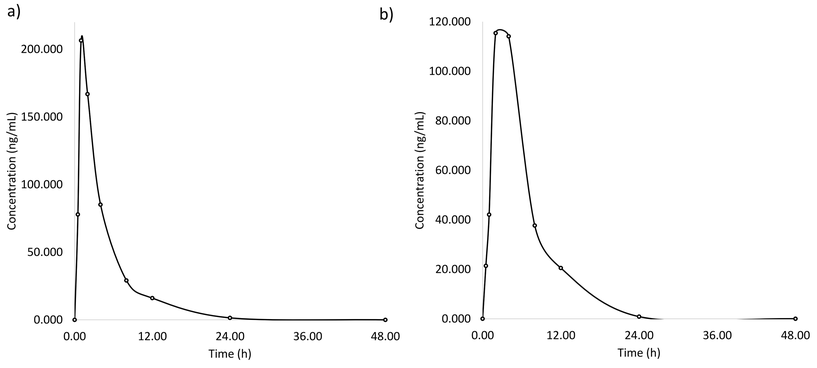

Figure 4. Plasma concentration vs. time curves for a patient after a single oral dose of a) fexofenadine (60 mg) and b) olmesartan (10 mg).

CONCLUSIONS

- A novel sensitive, simple, and high throughput UPLC-MS/MS assay for the simultaneous quantification of fexofenadine and olmesartan in human serum was developed and comprehensively validated according to FDA guidelines.
- The assay has several advantages over existing assays including small sample volume requirements, minimal sample preparation, high-throughput capacity, accuracy, and precision.
- The assay is also advantageous given that fexofenadine and olmesartan are probe substrates to assess transporter function, and studies often assess multiple transporter pathways simultaneously.
- The method is currently being applied to measure fexofenadine and olmesartan in serum for a clinical study.

ACKNOWLEDGEMENT

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