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

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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).

• The separation of the analytes was performed using an Acquity BEH C18 column (50 mm, 1.7 µm) with a 0.20 µm frit filter before the column.

• 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).

• A novel sensitive, simple, and high throughput UPLC

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).

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.

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

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

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.

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