Simultaneous Quantification of Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin in Human Plasma Using SPE and UPLC-MS/MS Analysis

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Abstract

This application details the simultaneous extraction and quantification of Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin from plasma using a simple, mixed-mode SPE sample preparation in the μElution format, and subsequent UPLC-MS/MS analysis.

The analytical sensitivity and excellent performance of this method can be attributed to use of a highly specific extraction using mixed-mode SPE, high resolution chromatographic separation with an ACQUITY HSS C18 Column on an ACQUITY UPLC I-Class System, and high MS sensitivity of the Xevo TQ-S micro Mass Spectrometer. With its simplicity and excellent performance, this fit-for-purpose method shows promise to support drug research and development as well as clinical research.

Benefits

- Simultaneous and rapid extraction of Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin
- Fast, simple, and selective sample preparation using mixed-mode SPE in the μElution format
- Rapid analysis with five minute run time using UPLC Technology
- Linear, accurate, and precise results for all analytes
- High analytical sensitivity detection using the Xevo TQ-S micro Mass Spectrometer
Introduction

Saxagliptin and the fixed dose combination of Daxagliptin/Dapagliflozin are orally active, selective, long-acting, and reversible dipeptidyl-peptidase 4 (DPP4) inhibitors, used for the treatment of type 2 diabetes mellitus. DPP4 inhibitors enhance levels of active glucagon-like peptide 1 (GLP-1) and other incretins, and facilitate glucose-dependent insulin secretion. In addition, GLP-1 inhibits glucagon release, slows gastric emptying, reduces appetite, and regulates the growth and differentiation of the insulin producing β cells in pancreatic islets. In this application note, we describe a simple method for the simultaneous quantification of saxagliptin and its major active metabolites, 5-Hydroxy Saxagliptin and Dapagliflozin in human plasma. This method uses a fast, selective sample preparation in the 96-well format and high-throughput UltraPerformance Liquid Chromatography tandem mass spectrometry (UPLC-MS/MS) analysis to achieve lower limits of quantification in the sub ng/mL range.
Experimental

Sample preparation

Commercially available human plasma was spiked with saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin at various concentrations (0.146–300 ng/mL). Calibration curve standards were prepared in duplicate to check the reproducibility, while six replicates were prepared for the QC and blank (non-spiked) plasma samples. No internal standard was used. A 300 µL aliquot of each of the prepared plasma samples were pretreated with 2% formic acid in water and mixed. The pretreated plasma sample was extracted using an Oasis MCX 96-Well µElution Plate according to the protocol in Figure 1. Following extraction, samples were injected for LC-MS/MS analysis.

UPLC conditions

LC system: ACQUITY UPLC I-Class
Column: ACQUITY UPLC HSS C<sub>18</sub>, 2.1 x 100 mm, 1.8 µm (P/N 1860004864)
Column temp.: 40 °C
Sample temp.: 10 °C
Injection volume: 10 µL
Mobile phase A: 2 mM ammonium acetate in water
Mobile phase B: Acetonitrile
Flow rate: 0.4 mL/min
LC gradient: Start at 5% B and hold for 1 min, linear ramp to 95% B for 2.5 min, hold for 3.5 min, and return to initial condition by 4 min
Run time: 5 min
MS conditions for positive mode

Mass spectrometer: Xevo TQ-S micro

Mode: ESI+/ESI

Capillary voltage: 3.0 KV (+), 2.8 kV (-)

Desolvation temp.: 550 °C

Cone gas flow: 150 L/h

Desolvation gas flow: 900 L/h

Collision cell pressure: 3.8 X e-3 mbar

Data management

Chromatography software: MassLynx

Quantification software: TargetLynx
Results and Discussion

LC-MS/MS quantification of the extracted samples was performed on an ACQUITY UPLC I-Class FTN System equipped with a binary solvent manager, column manager and sample manager couple to a Xevo TQ-S micro tandem quadrupole mass spectrometer. Reversed-phase chromatographic separation of saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin was performed with an ACQUITY HSS C18 Column (1.7 µm, 2.1 x 100 mm) maintained at 40 °C, at a flow rate of 0.4 mL/min using a linear gradient with ammonium acetate buffer and acetonitrile as the organic modifier. The column effluent was monitored by positive and negative ion electrospray MS/MS using multiple reaction monitoring (MRM). The MRM transitions used for quantitation of Saxagliptin, and 5-Hydroxy Saxagliptin were in positive mode 316.22>180.19, 332.30>196.20, and Dapagliflozin in negative mode with transition 467.22>329.15 respectively.

Simultaneous extraction of Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin from plasma was achieved using a single SPE extraction method with Oasis MCX, a mixed-mode sorbent in a 96-well µElution plate format. Use µElution plate format facilitated fast sample processing, while use of the mixed-mode SPE enhanced selectivity of the extraction. Full details of the SPE extraction procedure are highlighted in Figure 1. Using this simple extraction method, analyte recoveries of 100%, 71%, and 59% were achieved for Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin, respectively.

Assessment of the calibration and quality control (QC) results indicate that this method developed herein is linear, accurate, and precise. For all three analytes, with three full inter and intra-day precision and accuracy batches, plasma matrix responses were linear over the entire calibration range with $R^2$ values >0.99 using 1/x2 weighted regression. Figures 2–4 show the calibration curves, while Tables 1–3 summarize the data from these curves.

At the same time, QC statistics easily met regulatory guidelines, with average precision values ≤10% and QC accuracy ranges of 95–105%, for all analytes at all QC levels (Table 4). In addition, excellent S/N ratios were achieved for Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin at their respective LLOQ levels. This data can be seen in Figures 5–7.
Conclusion

This application details the simultaneous extraction and quantification of Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin from plasma using a simple, mixed-mode SPE sample preparation in the µElution format, and subsequent UPLC-MS/MS analysis. The method described herein achieves LLOQs of 0.150, 0.200, and 0.600 ng/mL, respectively. The analytical sensitivity and excellent performance of this method can be attributed to use of a highly specific extraction using mixed-mode SPE, high resolution chromatographic separation with an ACQUITY HSS C18 Column on an ACQUITY UPLC I-Class System, and high MS sensitivity of the Xevo TQ-S micro Mass Spectrometer. With its simplicity and excellent performance, this fit-for-purpose method shows promise to support drug research and development as well as clinical research.
References


3. FDA Guidance for Industry for Bioanalytical Method Validation, CDER.
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