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Nota de aplicación

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-peptidas 4 (DPP4) inhibitors, used for the treatment of type 2 diabetes mellitus.[1] 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 differention of the insulin producing β cells in pancreatic islets.[2] 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.

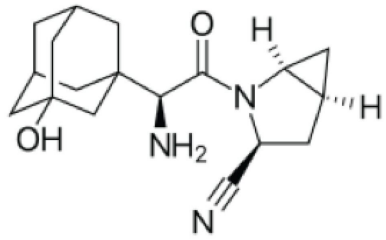

Figure 1. Saxagliptin.

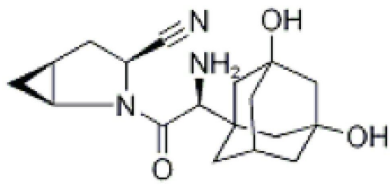

Figure 2. 5-Hydroxy Saxagliptin.

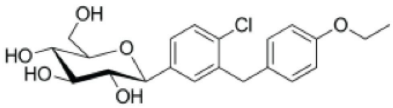

Figure 3. Dapagliflozin.

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_{18}, 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 C_{18} 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.

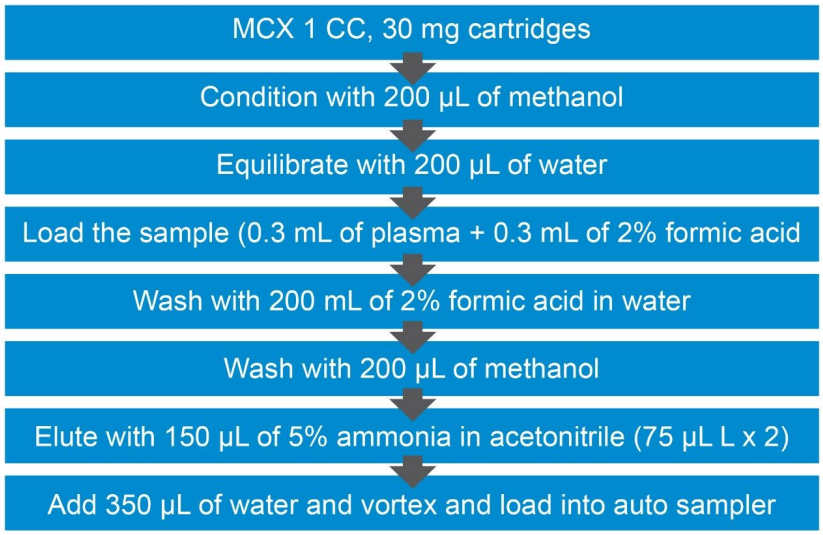

Figure 1. Oasis MCX SPE extraction protocol for Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin extracted from human plasma.

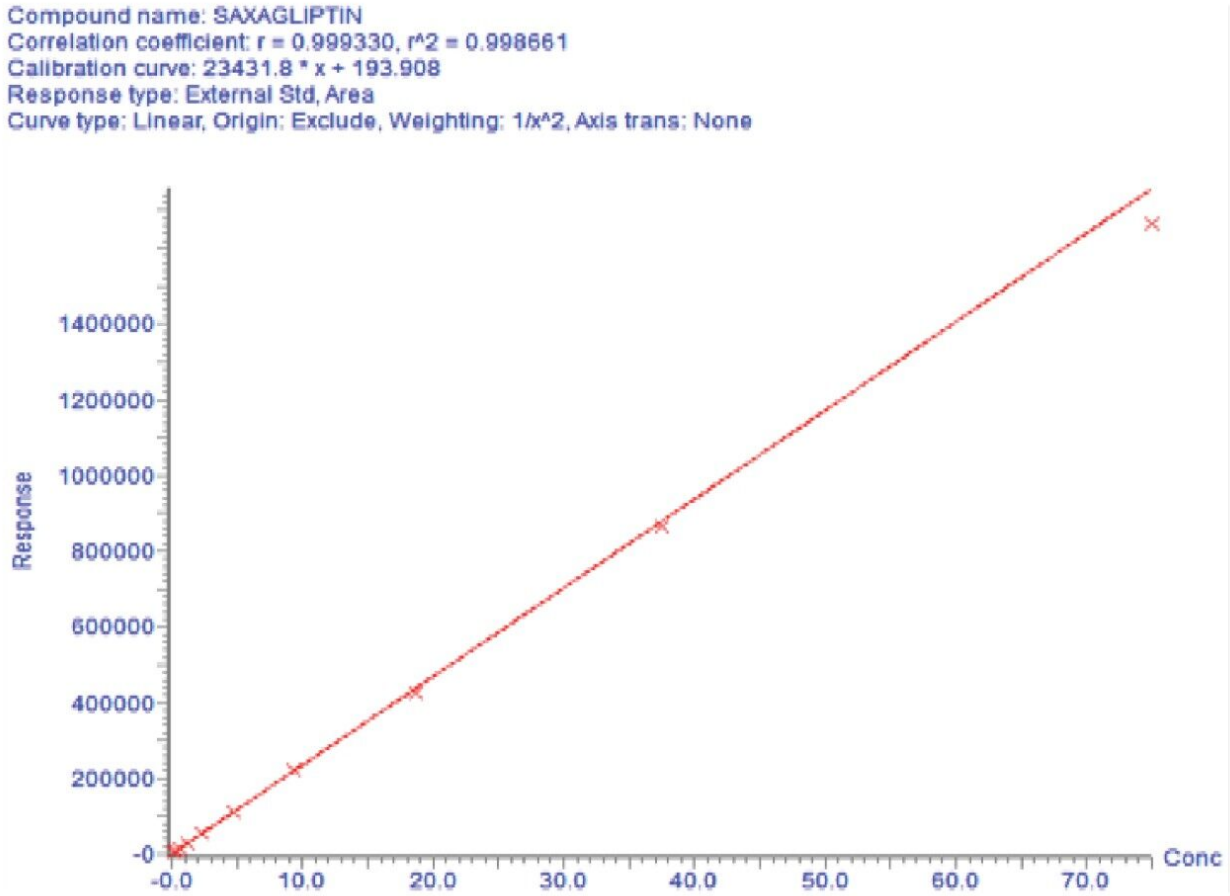

Figure 2. Example calibration curve (0.148–75 ng/mL) for Saxagliptin extracted from human plasma.

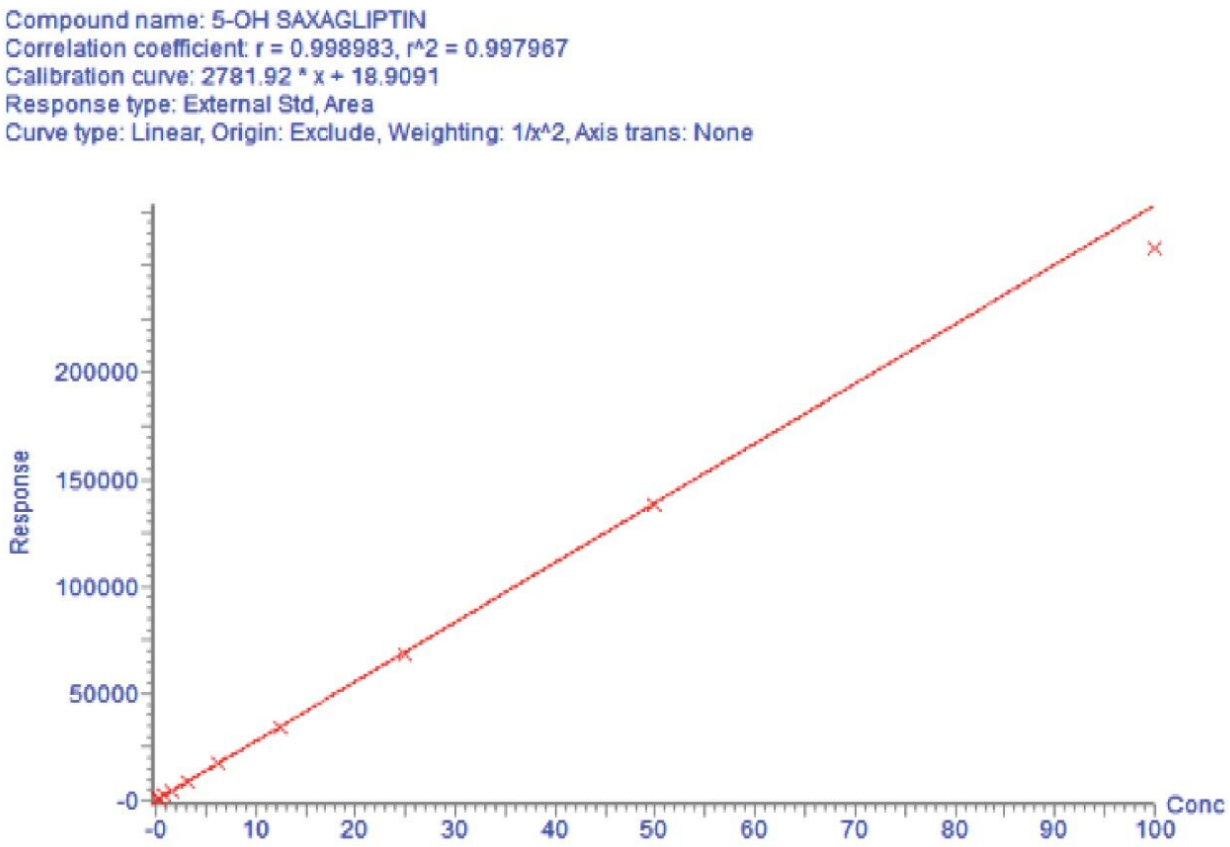

Figure 3. Example calibration curve (0.195–100 ng/mL) for 5-Hydroxy Saxagliptin extracted from human plasma.

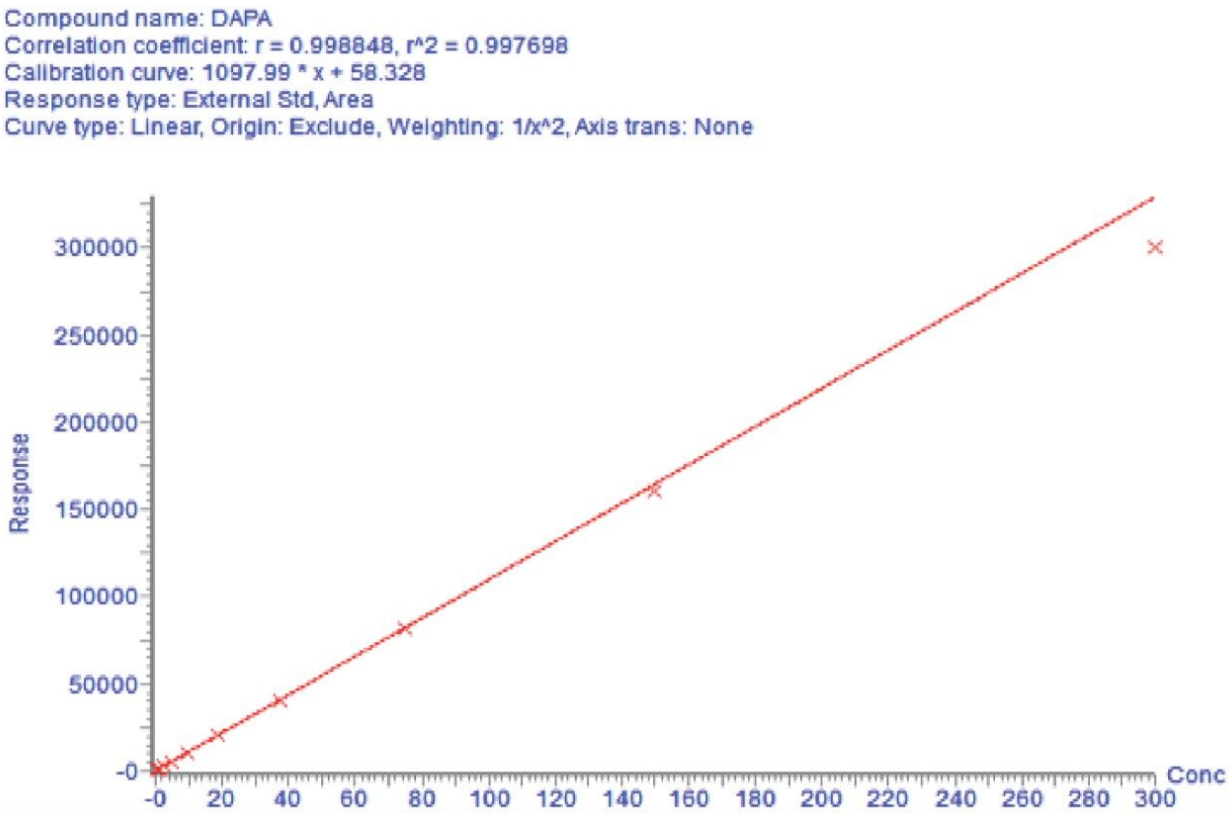

Figure 4. Example calibration curve (0.586–300 ng/mL) for Dapagliflozin extracted from human plasma.

ID	RT	Area	Inj. vol	Std. conc.	Conc.	Type	%Rec
Blank	2.31	68	10.00			Blank	
CC-01	2.30	3524	10.00	0.146	0.142	Standard	97.0
CC-02	2.30	7349	10.00	0.293	0.305	Standard	104.2
CC-03	2.30	14035	10.00	0.586	1.000	Standard	100.8
CC-04	2.30	28939	10.00	1.172	1.227	Standard	104.7
CC-05	2.30	55504	10.00	2.344	2.360	Standard	100.7
CC-06	2.30	112151	10.00	4.688	4.778	Standard	101.9
CC-07	2.30	221226	10.00	9.375	9.433	Standard	100.6
CC-08	2.30	425762	10.00	18.750	18.162	Standard	96.9
CC-09	2.30	864778	10.00	37.500	36.898	Standard	98.4
CC-10	2.30	1665019	10.00	75.000	71.050	Standard	94.7
Blank	2.30	521	10.00		0.014	Blank	
LLOQ-1	2.30	3485	10.00	0.146	0.140	QC	95.9
LLOQ-2	2.30	3348	10.00	0.146	0.135	QC	91.9
LLOQ-3	2.30	3458	10.00	0.146	0.139	QC	95.1
LLOQ-4	2.30	3471	10.00	0.146	0.140	QC	95.5
LLOQ-5	2.30	3445	10.00	0.146	0.139	QC	94.7
LLOQ-6	2.30	3429	10.00	0.146	0.138	QC	94.3
LQC-1	2.30	13803	10.00	0.586	0.581	QC	99.1
LQC-2	2.30	14166	10.00	0.586	0.596	QC	101.8
LQC-3	2.30	14431	10.00	0.586	0.608	QC	103.7
LQC-4	2.30	14136	10.00	0.586	0.595	QC	101.6
LQC-5	2.30	14424	10.00	0.586	0.607	QC	103.6
LQC-6	2.30	14146	10.00	0.586	0.595	QC	101.6
MQC-1	2.30	111356	10.00	4.688	4.744	QC	101.2
MQC-2	2.30	111702	10.00	4.688	4.759	QC	101.5
MQC-3	2.30	111924	10.00	4.688	4.768	QC	101.7
MQC-4	2.30	111676	10.00	4.688	4.758	QC	101.5
MQC-5	2.30	111580	10.00	4.688	4.754	QC	101.4
MQC-6	2.30	111098	10.00	4.688	4.733	QC	101.0
HQC-1	2.30	872024	10.00	37.500	37.207	QC	99.2
HQC-2	2.30	875118	10.00	37.500	37.339	QC	99.6
HQC-3	2.30	879400	10.00	37.500	37.522	QC	100.1
HQC-4	2.30	871324	10.00	37.500	37.177	QC	99.1
HQC-5	2.30	872099	10.00	37.500	37.210	QC	99.2
HQC-6	2.30	878483	10.00	37.500	37.483	QC	100.0

Table 1. Saxagliptin precision and accuracy for calibration and QC samples extracted from plasma; linear dynamic range achieved was 0.148–75 ng/mL.

ID	RT	Area	Inj. vol	Std. conc.	Conc.	Type	%Rec
Blank	2.68	44	10.00			Blank	
CC-01	2.66	677	10.00	0.586	0.564	Standard	96.2
CC-02	2.66	1402	10.00	1.172	1.224	Standard	104.4
CC-03	2.66	2739	10.00	2.344	2.442	Standard	104.2
CC-04	2.66	5331	10.00	4.688	4.802	Standard	102.4
CC-05	2.66	10800	10.00	9.375	9.783	Standard	104.3
CC-06	2.66	20859	10.00	18.750	18.944	Standard	101.0
CC-07	2.66	40820	10.00	37.500	37.123	Standard	99.0
CC-08	2.66	82064	10.00	75.000	74.687	Standard	99.6
CC-09	2.66	160839	10.00	150.000	146.432	Standard	97.6
CC-10	2.66	300477	10.00	300.000	273.607	Standard	91.2
Blank	2.68	25	10.00			Blank	
LLOQ-1	2.66	662	10.00	0.586	0.550	QC	93.8
LLOQ-2	2.66	694	10.00	0.586	0.579	QC	98.8
LLOQ-3	2.66	648	10.00	0.586	0.537	QC	91.7
LLOQ-4	2.66	697	10.00	0.586	0.581	QC	99.2
LLOQ-5	2.66	662	10.00	0.586	0.550	QC	93.9
LLOQ-6	2.66	699	10.00	0.586	0.583	QC	99.5
LQC-1	2.66	2783	10.00	2.344	2.482	QC	105.9
LQC-2	2.66	2726	10.00	2.344	2.429	QC	103.6
LQC-3	2.66	2819	10.00	2.344	2.514	QC	107.3
LQC-4	2.66	2774	10.00	2.344	2.473	QC	105.5
LQC-5	2.66	2620	10.00	2.344	2.333	QC	99.5
LQC-6	2.66	2813	10.00	2.344	2.509	QC	107.0
MQC-1	2.66	21027	10.00	18.750	19.097	QC	101.9
MQC-2	2.66	21405	10.00	18.750	19.442	QC	103.7
MQC-3	2.66	21438	10.00	18.750	19.471	QC	103.8
MQC-4	2.66	20973	10.00	18.750	19.048	QC	101.6
MQC-5	2.66	21030	10.00	18.750	19.100	QC	101.9
MQC-6	2.67	21380	10.00	18.750	19.419	QC	103.6
HQC-1	2.66	162747	10.00	150.000	148.169	QC	98.8
HQC-2	2.66	163596	10.00	150.000	148.942	QC	99.3
HQC-3	2.66	162882	10.00	150.000	148.292	QC	98.9
HQC-4	2.66	163882	10.00	150.000	149.203	QC	99.5
HQC-5	2.66	162706	10.00	150.000	148.132	QC	98.8
HQC-6	2.66	164596	10.00	150.000	149.853	QC	99.9

Table 2. 5-Hydroxy Saxagliptin precision and accuracy for calibration and QC samples extracted from plasma; linear dynamic range achieved was 0.195–100 ng/mL.

ID	RT	Area	Inj. vol	Std. conc.	Conc.	Type	%Rec
Blank	2.68	44	10.00			Blank	
CC-01	2.66	677	10.00	0.586	0.564	Standard	96.2
CC-02	2.66	1402	10.00	1.172	1.224	Standard	104.4
CC-03	2.66	2739	10.00	2.344	2.442	Standard	104.2
CC-04	2.66	5331	10.00	4.688	4.802	Standard	102.4
CC-05	2.66	10800	10.00	9.375	9.783	Standard	104.3
CC-06	2.66	20859	10.00	18.750	18.944	Standard	101.0
CC-07	2.66	40820	10.00	37.500	37.123	Standard	99.0
CC-08	2.66	82064	10.00	75.000	74.687	Standard	99.6
CC-09	2.66	160839	10.00	150.000	146.432	Standard	97.6
CC-10	2.66	300477	10.00	300.000	273.607	Standard	91.2
Blank	2.68	25	10.00			Blank	
LLOQ-1	2.66	662	10.00	0.586	0.550	QC	93.8
LLOQ-2	2.66	694	10.00	0.586	0.579	QC	98.8
LLOQ-3	2.66	648	10.00	0.586	0.537	QC	91.7
LLOQ-4	2.66	697	10.00	0.586	0.581	QC	99.2
LLOQ-5	2.66	662	10.00	0.586	0.550	QC	93.9
LLOQ-6	2.66	699	10.00	0.586	0.583	QC	99.5
LQC-1	2.66	2783	10.00	2.344	2.482	QC	105.9
LQC-2	2.66	2726	10.00	2.344	2.429	QC	103.6
LQC-3	2.66	2819	10.00	2.344	2.514	QC	107.3
LQC-4	2.66	2774	10.00	2.344	2.473	QC	105.5
LQC-5	2.66	2620	10.00	2.344	2.333	QC	99.5
LQC-6	2.66	2813	10.00	2.344	2.509	QC	107.0
MQC-1	2.66	21027	10.00	18.750	19.097	QC	101.9
MQC-2	2.66	21405	10.00	18.750	19.442	QC	103.7
MQC-3	2.66	21438	10.00	18.750	19.471	QC	103.8
MQC-4	2.66	20973	10.00	18.750	19.048	QC	101.6
MQC-5	2.66	21030	10.00	18.750	19.100	QC	101.9
MQC-6	2.67	21380	10.00	18.750	19.419	QC	103.6
HQC-1	2.66	162747	10.00	150.000	148.169	QC	98.8
HQC-2	2.66	163596	10.00	150.000	148.942	QC	99.3
HQC-3	2.66	162882	10.00	150.000	148.292	QC	98.9
HQC-4	2.66	163882	10.00	150.000	149.203	QC	99.5
HQC-5	2.66	162706	10.00	150.000	148.132	QC	98.8
HQC-6	2.66	164596	10.00	150.000	149.853	QC	99.9

Table 3. Dapagliflozin precision and accuracy for calibration and QC samples extracted from plasma; linear dynamic range achieved was 0.586–300 ng/mL.

At the same time, QC statistics easily met regulatory guidelines,[3] 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.

Accuracy and precision									
	Saxagliptin			5-hydroxy Saxagliptin			Dapagliflozin		
QC level	Mean calculated concentration (N=6) ng/mL	% mean (N=6) accuracy	% RSD	Mean calculated concentration (N=6) ng/mL	% mean (N=6) accuracy	% RSD	Mean calculated concentration (N=6) ng/mL	% mean (N=6) accuracy	%RSD
LLOQ	0.138	94.6	1.35	0.185	94.7	7.80	0.563	96.2	3.50
LQC	0.597	101.9	1.65	0.814	104.8	1.85	2.456	104.7	2.45
MQC	4.752	101.4	0.26	6.377	102.8	0.72	19.262	102.7	1.03
HQC	37.323	99.5	0.40	50.012	99.2	0.47	148.431	99.2	0.24

Table 4. Summary of quality control results for Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin extracted from human plasma.

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 C_{18} 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

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720006186, January 2018