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A Reproducible Method for the Quantification of Pioglitazone and Metabolites in Human Plasma Using the ACQUITY UPLC H-Class System and Xevo TQD MS with UNIFI

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APPLICATION BENEFITS

A high sensitivity method was developed for the analysis of pioglitazone and its two active metabolites, keto pioglitazone and hydroxy pioglitazone, in human plasma. The extremely low carryover exhibited by the ACQUITY UPLC® H-Class System allows the full sensitivity of the Xevo® TQD Mass Spectrometer to be utilized.

WATERS SOLUTIONS

Xevo TQD Mass Spectrometer

ACQUITY UPLC H-Class System

UNIFI® Scientific Information System

Oasis® HLB Extraction Plate

KEY WORDS

Pioglitazone, thiazolidinedione, diabetes, insulin, lipid, glucose, keto pioglitazone, hydroxy pioglitazone, metabolites, plasma

INTRODUCTION

Pioglitazone is part of the thiazolidinedione class of drugs used in the treatment of diabetes through hypoglycemic action. It selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) to modulate the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism.[1]

Following oral administration ranging from 15 to 45 mg, the dosed compound undergoes hepatic metabolism with CYP2C8, and to a lesser degree CYP3A4, to give rise to the following two active metabolites: keto pioglitazone and hydroxy pioglitazone. Both metabolites are present at higher systemic concentrations than the parent compound at steady state, reached seven days after dosing. At steady state, in patients with type 2 diabetes, pioglitazone comprises approximately 30% to 50% of the peak total pioglitazone serum concentrations (pioglitazone plus active metabolites) and 20% to 25% of the total area under the curve (AUC).

In this application note, we report the development of a highly sensitive solid phase extraction, and LC/MS/MS assay using the Xevo TQD for the analysis of pioglitazone and the two active metabolites in human plasma with an assay sensitivity of 10 pg/mL.

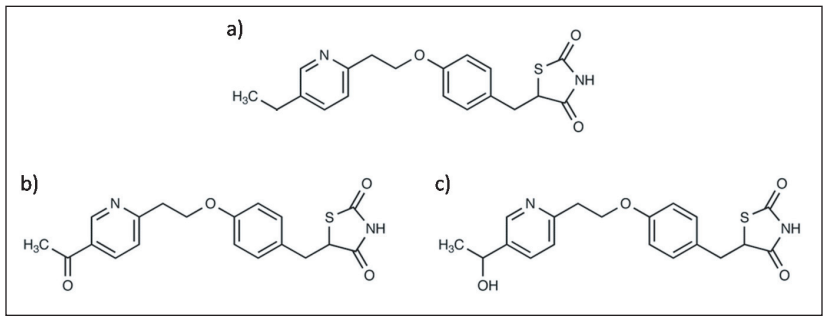

Figure 1. Structure of (a) pioglitazone, (b) keto pioglitazone, and (c) hydroxy pioglitazone.

EXPERIMENTAL

Sample Description

Samples were prepared using an Oasis HLB µElution solid phase extraction plate. The plasma samples, measuring 300 µL, were mixed with 20 µL of internal standard solution (deuturated analogues of all three compounds) and 300 µL of 2% phosphoric acid. The samples were applied to the solid phase extraction plate, which was previously conditioned and equilibrated with methanol (200 µL) and water (200 µL). The sample was washed with a 5% methanol/water solution, and then eluted with a 50 µL and subsequently 25 µL aliquot of methanol. Samples were further diluted with 75 µL of water prior to injection.

Method Conditions

The analysis was performed on an ACQUITY UPLC H-Class System. A 10-µL aliquot of the sample was injected onto an ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7-µm Column. The column was operated under gradient conditions over 2 min at a flow rate of 600 µL/min. The mobile phases used were 0.1% ammonium hydroxide and methanol. The column effluent was monitored using a Xevo TQD Mass Spectrometer operated in multiple reaction monitoring (MRM) positive ion electrospray mode.

The transitions monitored included the following:
Pioglitazone: 357 > 134
Keto pioglitazone: 371 > 148
Hydroxy pioglitazone: 373 > 150
d_4-pioglitazone: 361 > 138
d_4-keto pioglitazone: 375 > 152
d_5-hydroxy pioglitazone: 378 > 154

Data integration and calculation software

UNIFI Scientific Information System

RESULTS AND DISCUSSION

Pioglitazone, keto pioglitazone, and hydroxy pioglitazone eluted with retention times of 1.59, 1.35, and 1.34 minutes, respectively, as shown in Figure 2. This data reveals very symmetrical peaks produced by the chromatography system with a width at the base of approximately 3 s for all three compounds. The narrow peak width and the symmetrical nature allow for efficient processing and peak integration. The data displayed in Figure 2 illustrates the injection of an extracted plasma blank injection, immediately following analysis of the 1000 pg/mL standard. This data demonstrates that there is no discernible carryover in the blank chromatogram (the baseline has been magnified) for any of the compounds. The extremely low carryover exhibited by the ACQUITY UPLC H-Class System allows the full sensitivity of the Xevo TQD Mass Spectrometer to be exploited.

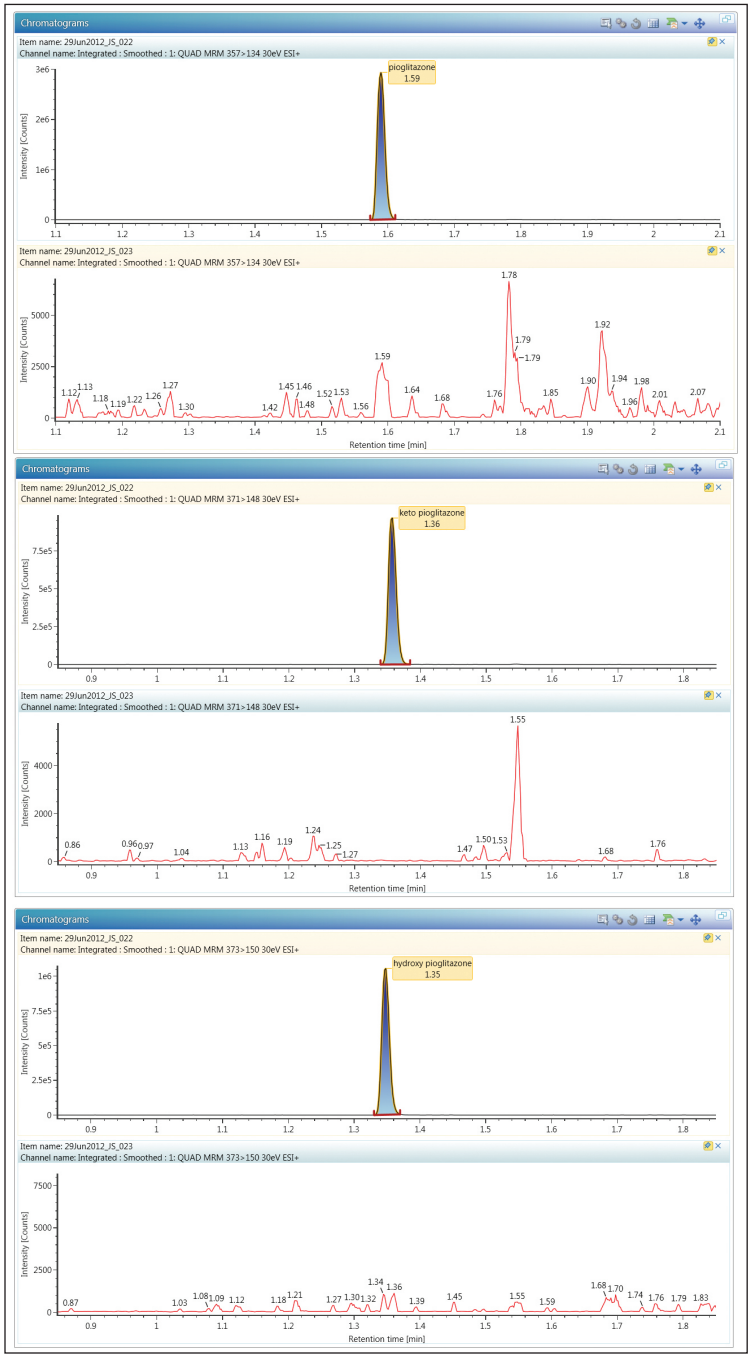

Figure 2. LC/MS/MS chromatogram of extracted 1000 pg/mL standard and blank for pioglitazone (top), keto pioglitazone (middle), and hydroxy pioglitazone (bottom).

The lower limit of quantification (LLOQ) for the assay was 10 pg/mL for all three analytes. Calculated signal-to-noise values for the LLOQ were 44:1 for pioglitazone, 21:1 for keto pioglitazone, and 58:1 for hydroxy pioglitazone, as shown in Figure 3. A typical calibration curve obtained for the assay of pioglitazone is shown in Figure 4. The correlation coefficient ranged between 0.997 and 0.999 for the three compounds using a 1/x weighting linear regression. The single day accuracy and precision data are displayed in Tables 1 through 3 for quality control (QC) samples prepared at four levels spanning the calibration range, and extracted in replicates of five. The validation data shows that the coefficient of variation for the parent and two metabolites ranged from 8.4% to 13.4% for the 10 pg/mL LLOQ with a bias between -5.7% and 1.8%. For the high QC (800 pg/mL), the coefficient of variation ranged from 1.1% to 4.4% with a bias between -0.1% to 0.5%.

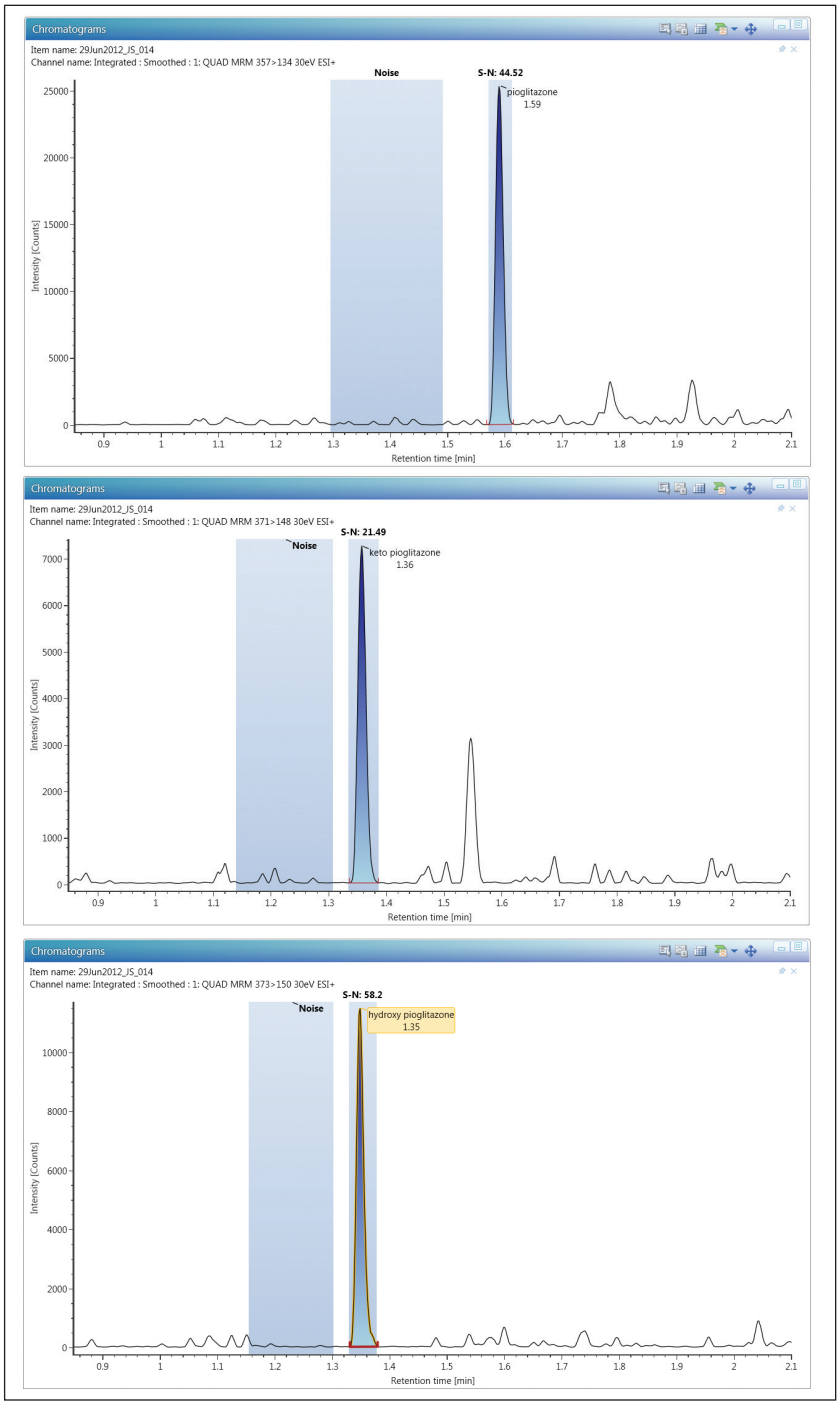

Figure 3. LLOQ (10 pg/mL) signal-to-noise values for pioglitazone (top), keto pioglitazone (middle), and hydroxy pioglitazone (bottom).

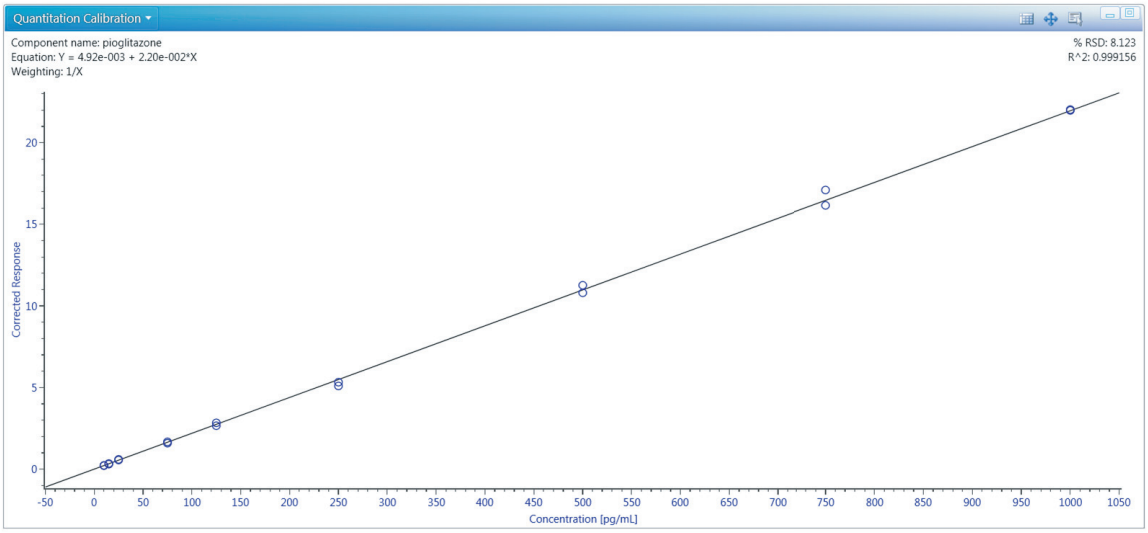

Figure 4. Representative calibration line for the LC/MS/MS quantification of pioglitazone.

	QC LLOQ 10 pg/mL	QC Low 30 pg/mL	QC Mid 300 pg/mL	QC High 800 pg/mL
	8.98	30.6	311	775
	9.52	31.3	288	771
	9.49	33.4	310	836
	11.2	30.2	286	843
	9.96	27.7	312	780
Mean	9.82	30.6	302	801
St Dev	0.821	2.03	13.3	35.5
%CV	8.4	6.6	4.4	4.4
%Bias	1.8	-2.1	-0.5	-0.1

Table 1. Intra-day QC accuracy/precision statistics for pioglitazone.

	QC LLOQ 10 pg/mL	QC Low 30 pg/mL	QC Mid 300 pg/mL	QC High 800 pg/mL
	12.4	30.0	327	788
	8.86	30.6	315	789
	9.46	32.0	315	819
	10.3	31.1	322	798
	9.85	33.0	325	800
Mean	10.2	31.3	321	799
St Dev	1.37	1.21	5.56	12.6
%CV	13.4	3.9	1.7	1.6
%Bias	-1.7	-4.4	-7.0	0.1

Table 2. Intra-day QC accuracy/precision statistics for keto pioglitazone.

	QC LLOQ 10 pg/mL	QC Low 30 pg/mL	QC Mid 300 pg/mL	QC High 800 pg/mL
	11.4	32.1	298	789
	11.5	32.0	287	793
	8.65	29.6	300	802
	11.8	29.5	298	809
	9.58	31.0	323	788
Mean	10.6	30.8	301	796
St Dev	1.37	1.27	13.1	8.78
%CV	13.0	4.1	4.4	1.1
%Bias	-5.7	-2.8	-0.4	0.5

Table 3. Intra-day QC accuracy/precision statistics for hydroxy pioglitazone.

CONCLUSIONS

- A high sensitivity method has been developed for the analysis of pioglitazone and its two active metabolites in human plasma using the Xevo TQD.
- The assay showed excellent intra-day accuracy and precision for QCs prepared at four concentration levels.
- The lower limit of quantification was determined to be 10 pg/mL with a %CV and bias, both well below the required +/- 20% required for assay validation.
- The carryover was determined to be significantly less than 20% of the LLOQ in an extracted blank, following the injection of a high concentration standard.

Reference

1. Baughman TM, Graham RA, Wells-Knecht K, Silver IS, Tyler LO, Wells-Knecht M, Zhao Z. Metabolic activation of pioglitazone identified from rat and human liver microsomes and freshly isolated hepatocytes. *Drug Metabolism and Disposition.* 2005; 33: 733-738.

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