

## A Reproducible Method for the Quantification of Pioglitazone and Two Active Metabolites – Keto Pioglitazone and Hydroxy Pioglitazone – in Human Plasma Using Xevo TQD MS and the ACQUITY UPLC H-Class System

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## Abstract

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 the pioglitazone and the two active metabolites in human plasma with an assay sensitivity of 10 pg/mL.

## Benefits

Waters Oasis micro-elution plates, ACQUITY UPLC H-Class System, and an advanced tandem quadrupole mass spectrometer (Xevo TQD) were used for the development of a sensitive method for quantification of pioglitazone in human plasma. This application note addresses some critical challenges in the world of bioanalysis – developing a robust and reproducible method to quantify small molecules with the desired sensitivity.

## 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- $\gamma$ ) to modulate the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism.<sup>1</sup>

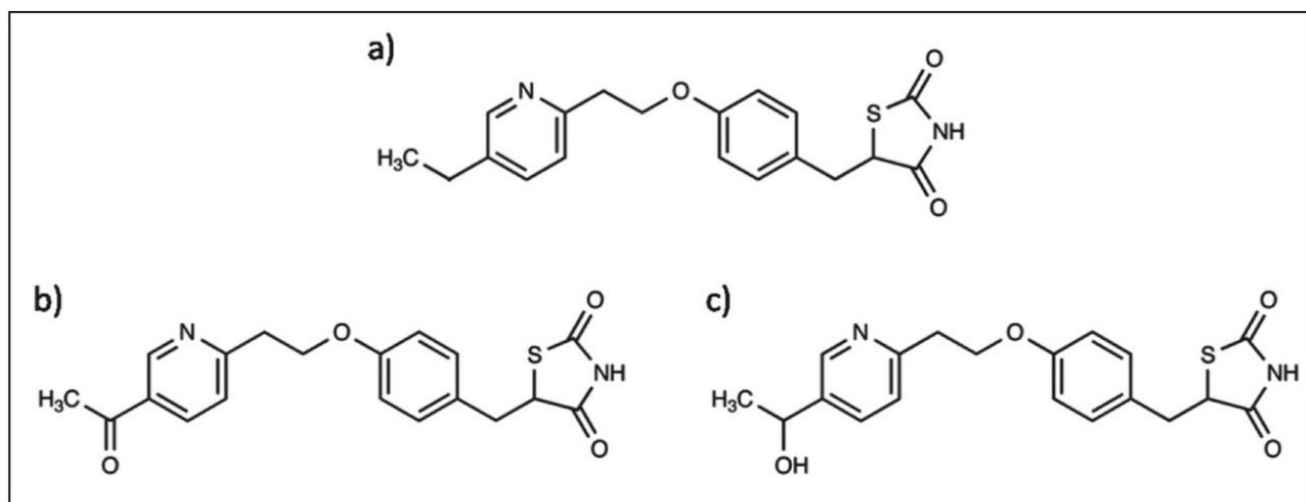


Figure 1. Structure of pioglitazone (a), keto pioglitazone (b), and hydroxy pioglitazone (c).

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 two active metabolites; keto pioglitazone and hydroxy pioglitazone. Both metabolites are present at higher systemic concentrations than the parent compound at steady state, which is 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 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 the pioglitazone and the two active metabolites in human plasma with an assay sensitivity of 10 pg/mL.

## Experimental

### Sample Description

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

### Method Conditions

The analysis was performed on an ACQUITY UPLC H-Class System. A 10- $\mu$ L aliquot of the sample was injected onto an ACQUITY BEH C<sub>18</sub> 2.1 x 50 mm, 1.7  $\mu$ m column. The column was operated under gradient conditions over 2 minutes at a flow rate of 600  $\mu$ L/min. 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 were:

Pioglitazone: 357 > 134

Keto pioglitazone: 371 > 148

Hydroxy pioglitazone: 373 > 150

d<sub>4</sub>-pioglitazone: 361 > 138

d<sub>4</sub>-keto pioglitazone: 375 > 152

d<sub>5</sub>-hydroxy pioglitazone: 378 > 154

## 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 shows the peaks produced by the chromatography system are very symmetrical, and have a width at the base of approximately 3 seconds 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. We can see from this data that there is no discernable carryover in the blank chromatogram (in which 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.

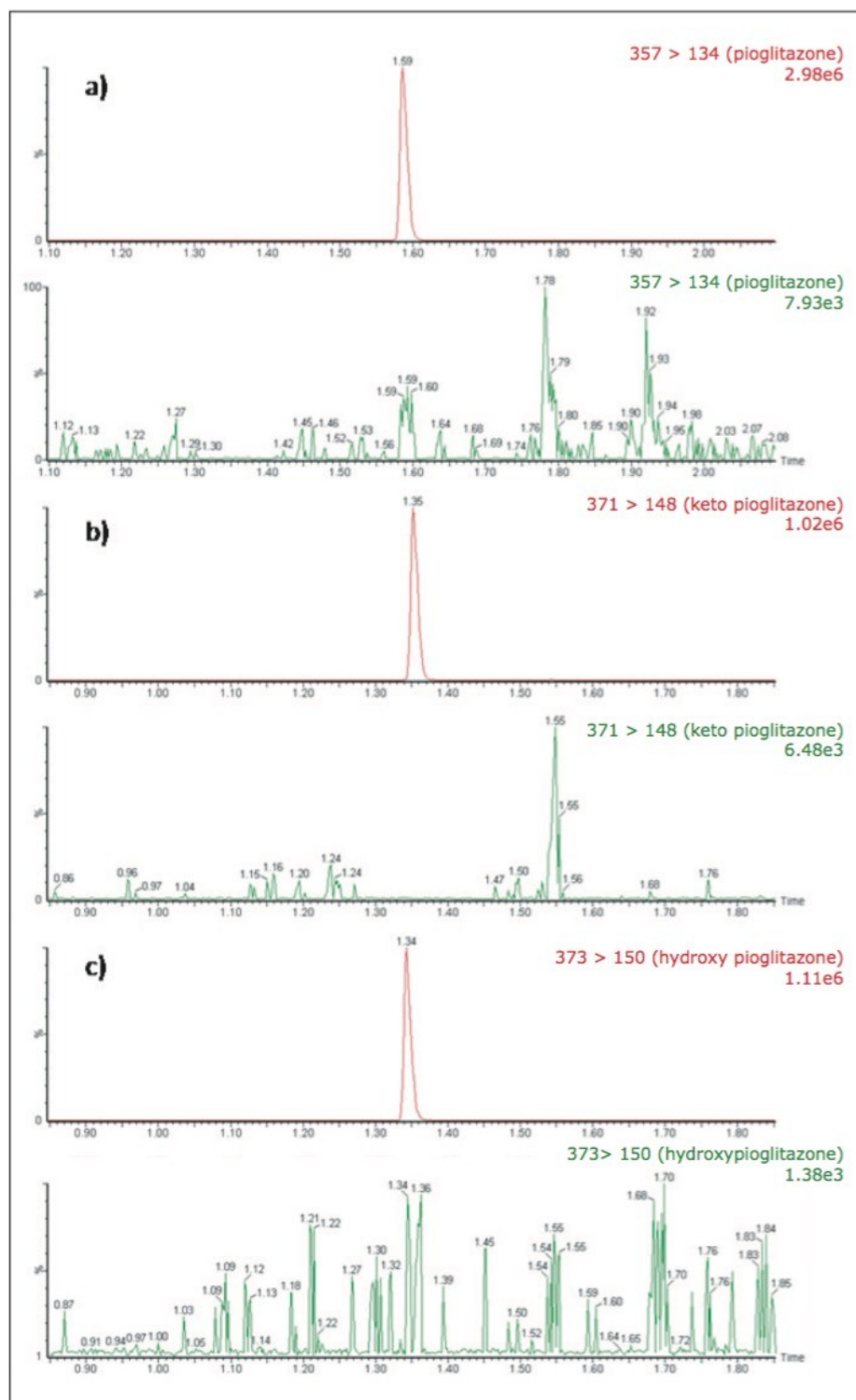


Figure 2. LC-

MS/MS chromatogram of extracted 1000 pg/mL standard and blank for pioglitazone (a), keto

pioglitazone (b) and hydroxy pioglitazone (c).

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 50:1 pioglitazone/ hydroxy pioglitazone, and 22:1 keto 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 is displayed in Tables 1 through 3 for quality control (QC) samples prepared at four levels, spanning the calibration range, and extracted in replicates of 5. 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%.

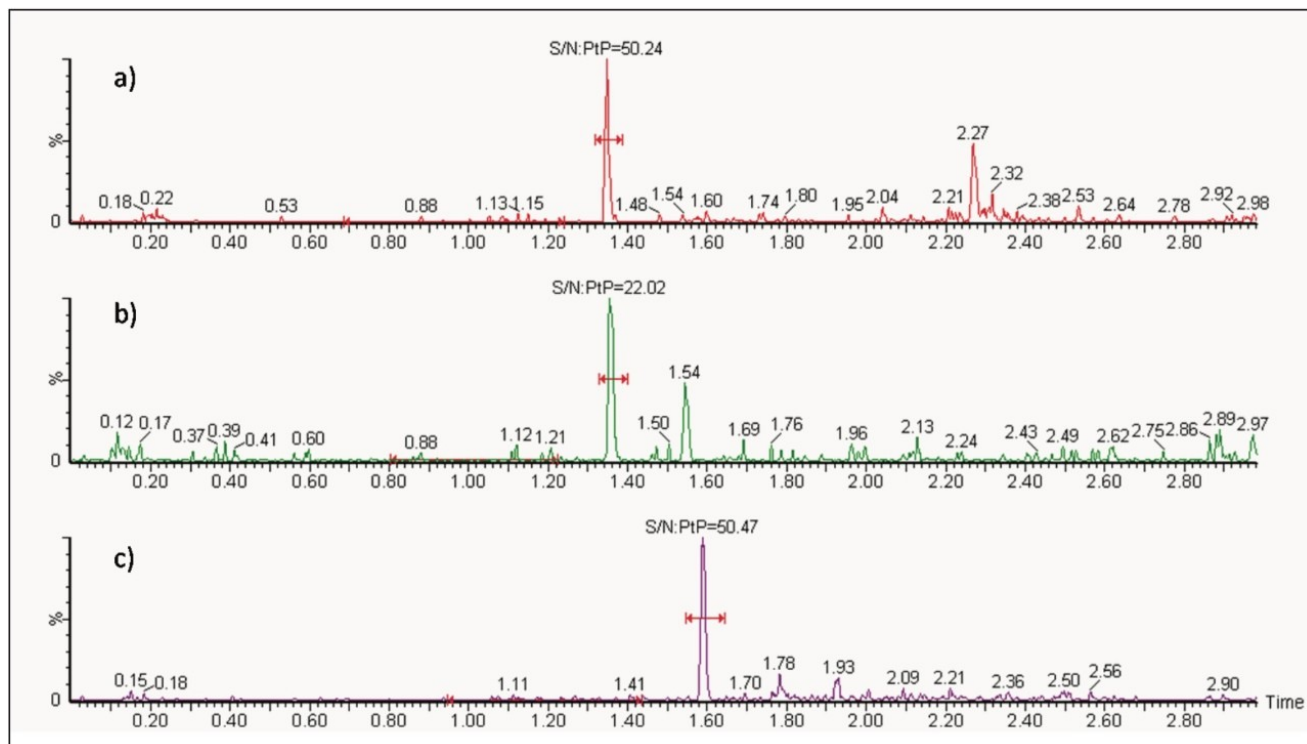


Figure 3. LLOQ (10 pg/mL) signal-to-noise values for pioglitazone (a), keto pioglitazone (b), and hydroxy pioglitazone (c).

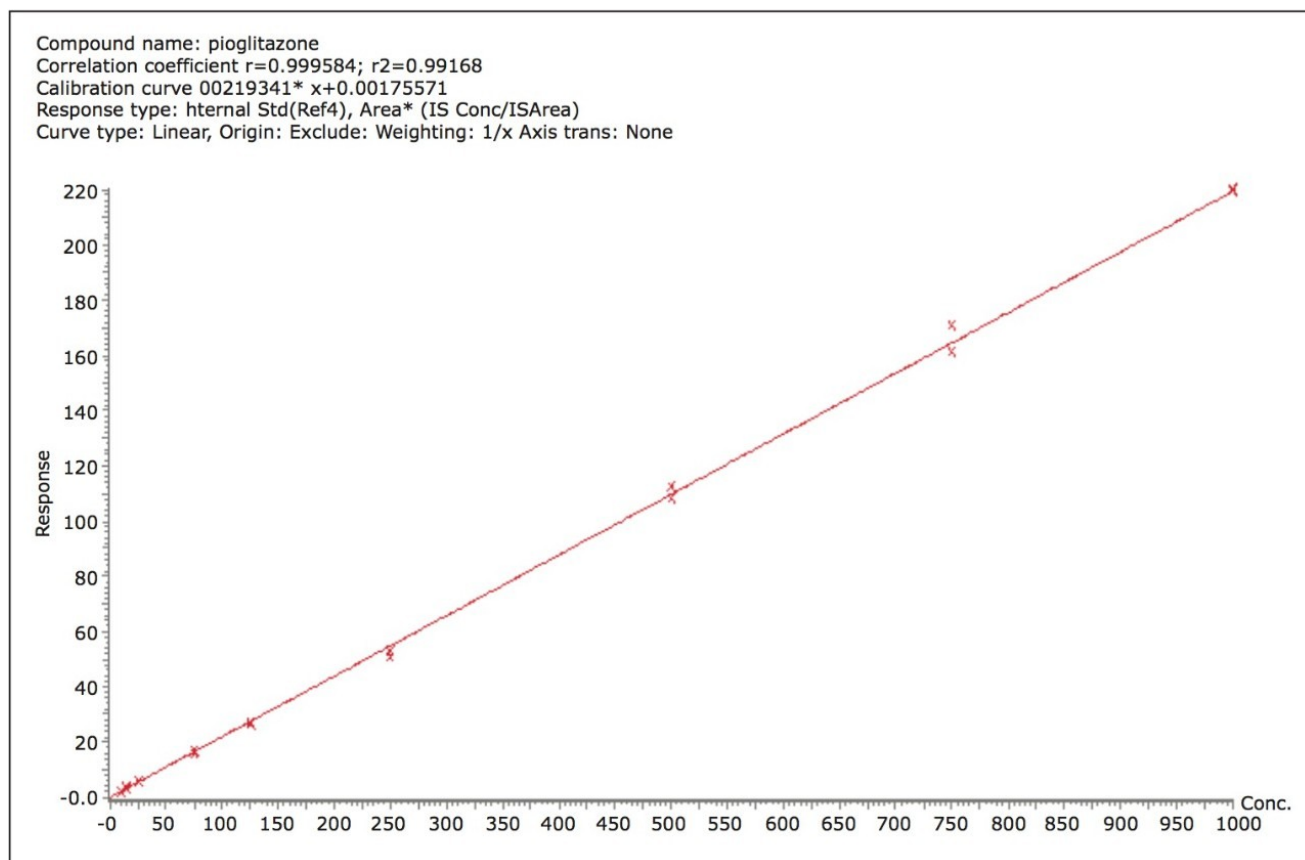


Figure 4. Representative calibration line for the LC-MS/MS quantification of pioglitazone.

	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: 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: 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: Hydroxy pioglitazone.

## Conclusion

- 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.

## References

1. Baughman TM, Graham RA, Wells-Knecht K, Silver IS, Tyler LO, Wells-Knecht M, and Zhao Z. *Drug Metabolism and Disposition*, 2005; 33, 733-738.

## Featured Products

- [ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>](https://www.waters.com/10138533)
- [Xevo TQD Triple Quadrupole Mass Spectrometry <https://www.waters.com/134608730>](https://www.waters.com/134608730)

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