

응용 자료

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

### 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.

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## 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>

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).

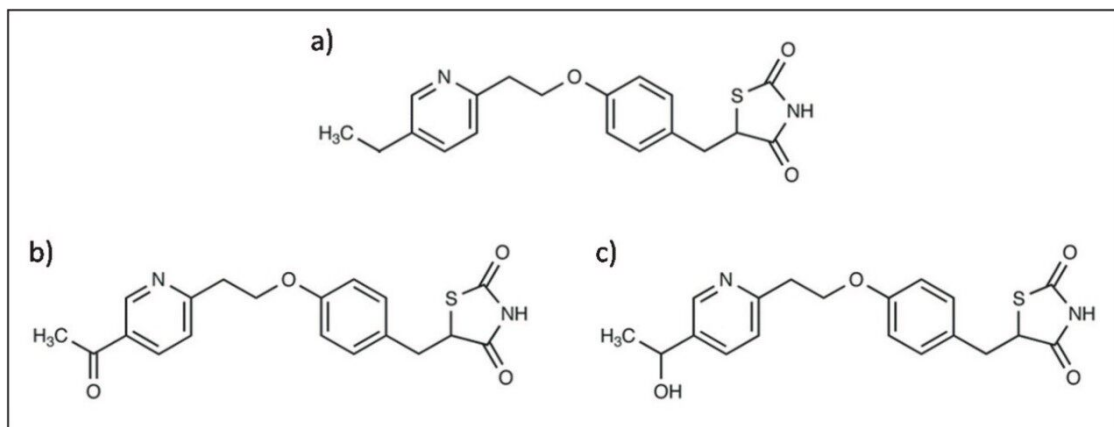


Figure 1. Structure of (a) pioglitazone, (b) keto pioglitazone, and (c) hydroxy pioglitazone.

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.

## Experimental

### Sample Description

Samples were prepared using an Oasis 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 was previously conditioned and equilibrated with methanol (200  $\mu$ L) and water (200  $\mu$ L). The sample was washed with a 5% methanol/water solution, and then eluted with a 50  $\mu$ L and subsequently 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 UPLC BEH C<sub>18</sub> 2.1 x 50 mm, 1.7- $\mu$ m Column. The column was operated under gradient conditions over 2 min at a flow rate of 600  $\mu$ L/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<sub>4</sub>-pioglitazone: 361 > 138

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

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

## Data integration and calculation software

UNIFI Scientific Information System

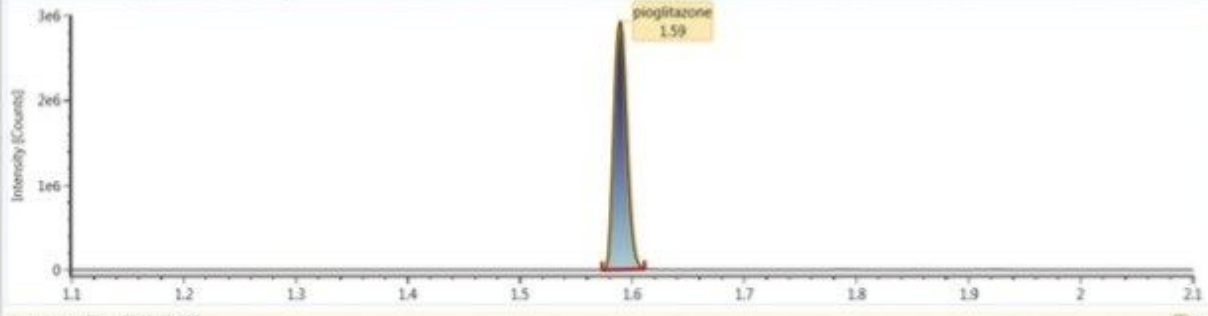
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## Results and Discussion

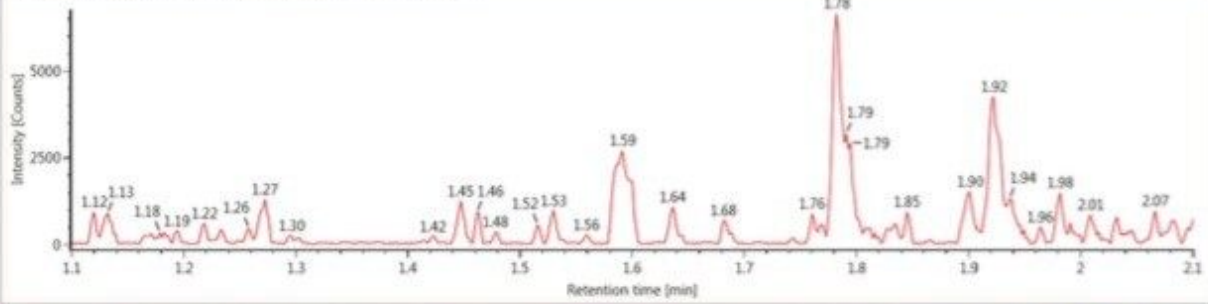
Pioglitazone, keto pioglitazone, and hydroxy pioglitazone eluted with retention times of 1.59, 1.35, and 1.34 minutes, respectively, as shown in Figure 2. This data reveals very symmetrical peaks produced by the chromatography system with a width at the base of approximately 3 s for all three compounds. 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.

Chromatograms

Item name: 29Jun2012\_J5\_022  
Channel name: Integrated : Smoothed : 1: QUAD MRM 357>134 30eV ESI+

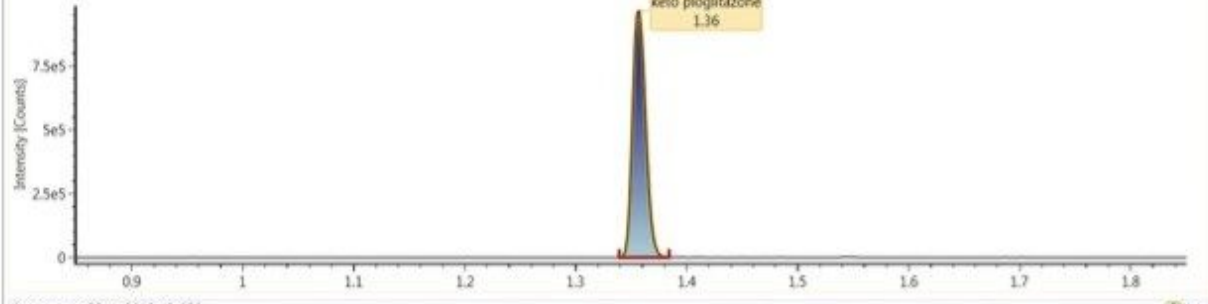


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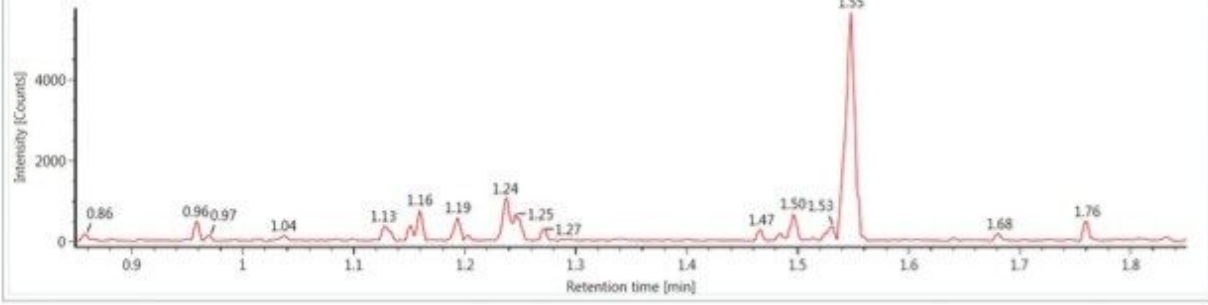


Chromatograms

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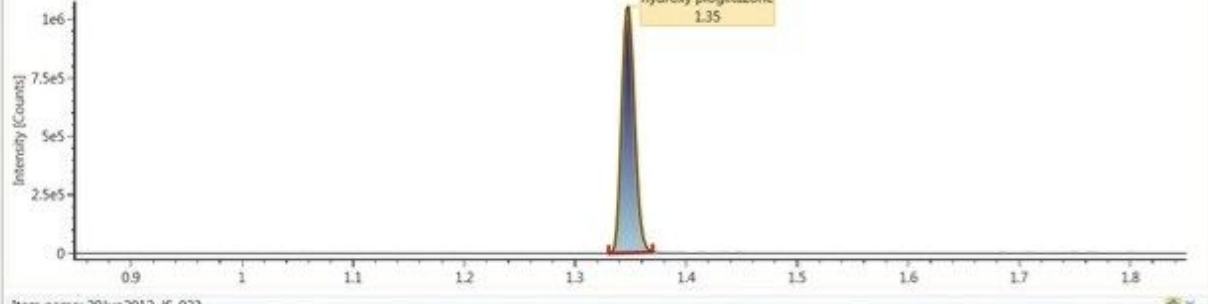


Item name: 29Jun2012\_J5\_023  
Channel name: Integrated : Smoothed : 1: QUAD MRM 371>148 30eV ESI+



Chromatograms

Item name: 29Jun2012\_J5\_022  
Channel name: Integrated : Smoothed : 1: QUAD MRM 373>150 30eV ESI+



Item name: 29Jun2012\_J5\_023  
Channel name: Integrated : Smoothed : 1: QUAD MRM 373>150 30eV ESI+



- 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.

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## References

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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ACQUITY UPLC H-Class PLUS System <<https://www.waters.com/10138533>>

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