

응용 자료

## Analysis of Pharmaceutical Drugs Using the ACQUITY UPLC I-Class PLUS System and the Xevo TQ-S Mass Spectrometer

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This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

This technology brief assess the performance of the ACQUITY UPLC I-Class PLUS System coupled with the Xevo TQ-S Mass Spectrometer for the analysis of pharmaceutical drugs used in CYP inhibition assays compared with the ACQUITY UPLC I-Class System.

The ACQUITY UPLC I-Class PLUS System produced results comparable with the ACQUITY UPLC I-Class System for the analysis of pharmaceutical drugs. Retention time reproducibility was excellent with RSD below 0.5%. The ACQUITY UPLC I-Class PLUS System gives confident and reliable results to aid investigators in establishing DDI and CYP experiments in the process of evaluating new drugs.

### Benefits

The ACQUITY UPLC I-Class PLUS System with the Xevo TQ-S Mass Spectrometer provides reproducible and reliable results for the analysis of pharmaceutical drugs.

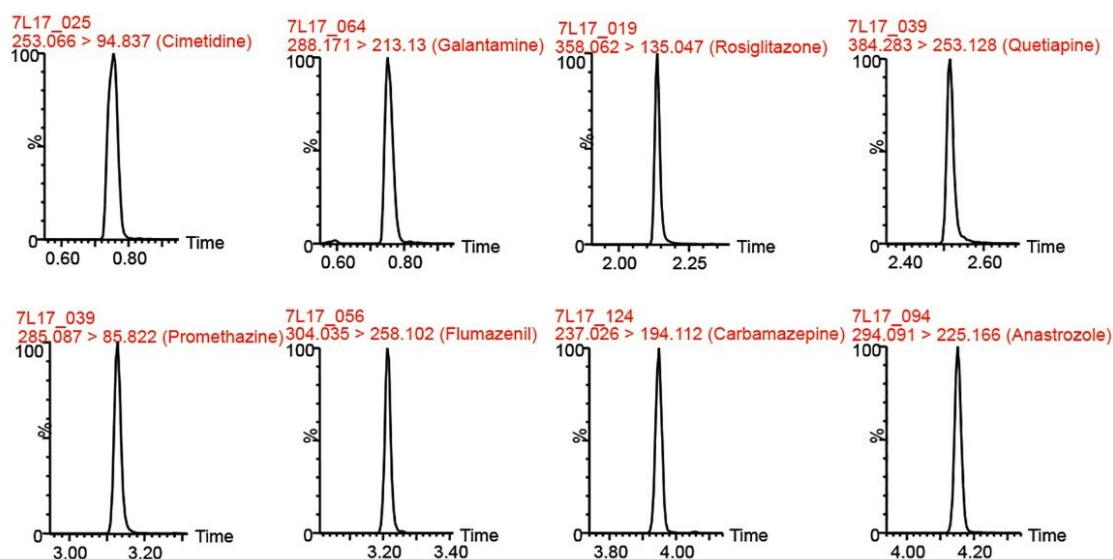
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## Introduction

Polypharmacy (use of multiple concurrent prescription drugs) in US adults is increasing with significant prevalence in elderly populations treating multiple disease states.<sup>1,2</sup> As the number of prescriptions increase for an individual, there is an increase for the risk of drug-drug interactions (DDI) primarily due to induction or inhibition of Cytochrome P450s (CYP) with these interactions leading to potentially dangerous side effects. CYP is a superfamily of enzymes that are, among others, responsible for the metabolic transformation of drug substances.<sup>3</sup> For this reason, it has become a requirement to include CYP inhibition assays to assess DDIs in new drug development.<sup>4,5</sup> Libraries of compounds with known metabolic fates and the investigational drug are incubated with liver microsomes or hepatocytes and after a time the sample is analyzed.<sup>6</sup> The data obtained gives a snapshot of the potential clinical consequences and gives investigators a framework for future studies.

In this work, we assess the performance of the ACQUITY UPLC I-Class PLUS System coupled with the Xevo TQ-S Mass Spectrometer for the analysis of pharmaceutical drugs used in CYP inhibition assays compared with the ACQUITY UPLC I-Class System.

## ACQUITY UPLC I-Class



## ACQUITY UPLC I-Class PLUS

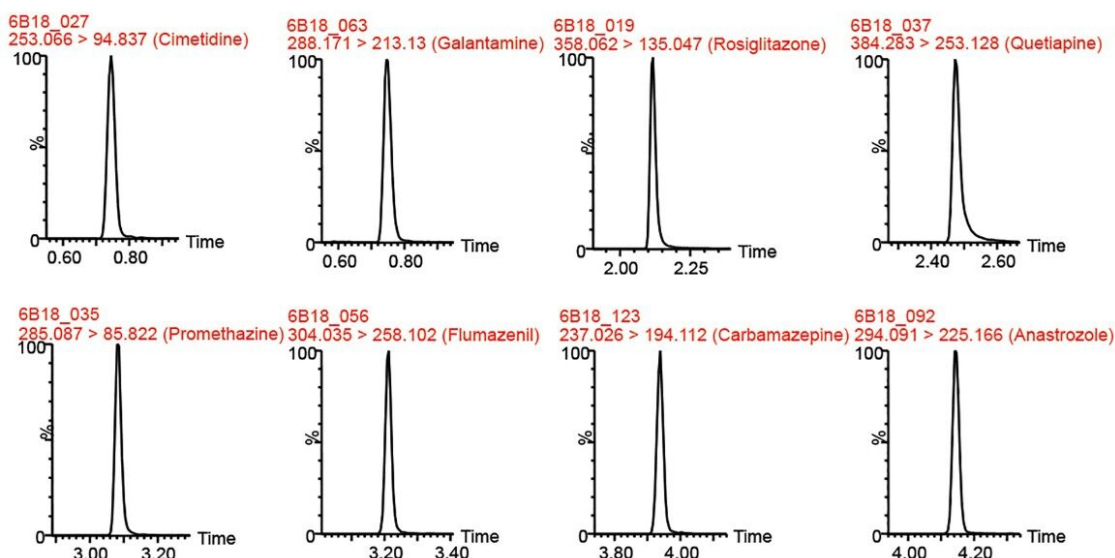


Figure 1. Chromatograms from representative compounds separated on the ACQUITY UPLC I-Class (top) and the ACQUITY UPLC I-Class PLUS (bottom) Systems.

## Results and Discussion

Compound libraries were purchased from Enzo Life Sciences (Farmingdale, NY, USA). The standards were

supplied in DMSO (96-well plate format) and were diluted to 1  $\mu$ M in 50:50 acetonitrile/water (v/v) for MRM optimization and 25 nM in 95:5 water/acetonitrile (v/v) for UPLC analysis. MRM optimization was performed using the MassLynx v4.1 application manager QuanOptimize. A UPLC separation was achieved on a CORTECS C<sub>18</sub>+, 1.6  $\mu$ m, 2.1 x 100 mm Column at a flow rate of 0.5 mL/min and gradient from 5% B to 95% B over 10 minutes, with a one-minute hold and re-equilibration for 10-column volumes at a total run time of 16 minutes. The mobile phase consisted of (A) water and (B) acetonitrile, each with 0.1% formic acid and injection volume was 5  $\mu$ L. The retention times were measured using the TargetLynx Application Manager, and were the average of five-replicate injections.

One hundred seven compounds commonly used in CYP inhibition assays were analyzed using the same column, mobile phase, and mass spectrometer by both the ACQUITY UPLC I-Class and the ACQUITY UPLC I-Class PLUS Systems. Figure 1 shows chromatograms for eight representative compounds on each LC system with tabulated results in Table 1. The retention times between the two systems were comparable for all the compounds tested. Retention time reproducibility is illustrated in Figure 2 for representative compounds, and was less than 0.6% and 0.5% RSD for the 107 compounds for the ACQUITY UPLC I-Class and ACQUITY UPLC I-Class PLUS Systems, respectively. These results demonstrate the reproducibility of the ACQUITY I-Class PLUS System and gives confidence in each analysis.

A. ACQUITY UPLC I-Class		
Compound	Retention time (min) n = 5	%RSD Retention time
Cimetidine	0.79	0.30%
Galantamine	0.80	0.01%
Rosiglitazone	2.18	0.01%
Quetiapine	2.55	0.10%
Promethazine	3.17	0.08%
Flumazenil	3.25	0.07%
Carbamazepine	3.99	0.001%
Anastrozole	4.19	0.004%

B. ACQUITY UPLC I-Class PLUS		
Compound	Retention time (min) n = 5	%RSD Retention time
Cimetidine	0.79	0.29%
Galantamine	0.79	0.26%
Rosiglitazone	2.16	0.003%
Quetiapine	2.52	0.10%
Promethazine	3.12	0.07%
Flumazenil	3.25	0.01%
Carbamazepine	3.98	0.004%
Anastrozole	4.19	0.003%

Table 1. Retention time and %RSD for the compounds separated on the ACQUITY UPLC I-Class (A) and the ACQUITY UPLC I-Class PLUS (B) Systems.

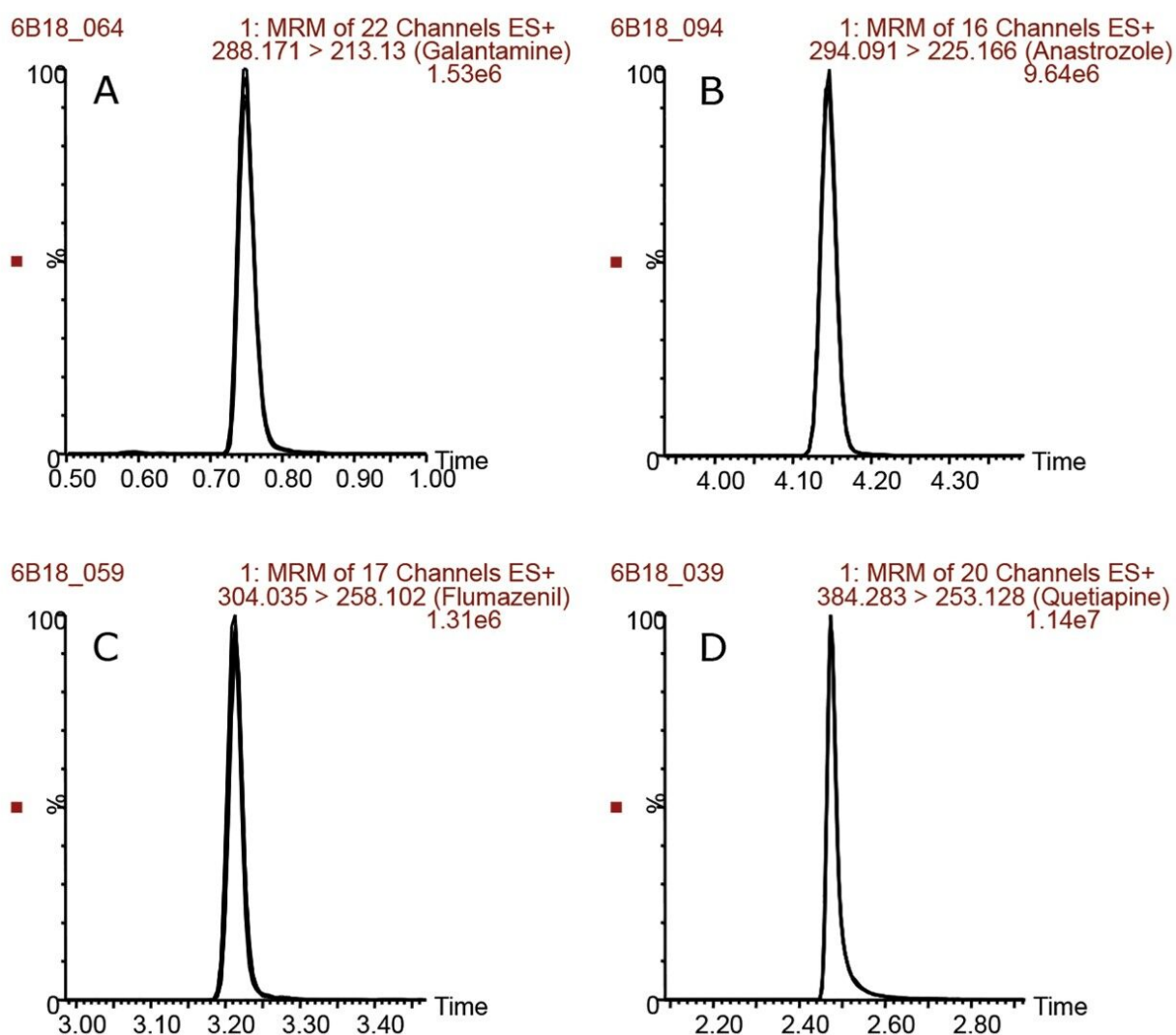


Figure 2. Chromatographic overlays of five replicate injections for galantamine (A), anastrozole (B), flumazenil (C), and quetiapine (D) on the ACQUITY UPLC I-Class PLUS System.

## Conclusion

The ACQUITY UPLC I-Class PLUS System produced results comparable with the ACQUITY UPLC I-Class System for the analysis of pharmaceutical drugs. Retention time reproducibility was excellent with RSD below 0.5%. The ACQUITY UPLC I-Class PLUS System gives confident and reliable results to aid investigators in establishing DDI and CYP experiments in the process of evaluating new drugs.

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## References

1. Kantor, E.D.; Rehm, C.D.; Haas, J.S.; Chan, A.T.; Giovannucci, E.L. Trends in prescription drug use among adults in the United States from 1999–2012. *JAMA*. 2015, 314(17), 1818–31.
2. Maher, R.L.; Hanlon, J.; Hajjar, E.R. Clinical consequences of polypharmacy in elderly. *Expert Opin Drug Saf*. 2014, 13(1), 57–65.
3. Danielson, P.B. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. *Curr Drug Metab*. 2002, 3(6), 561–97.
4. US Food and Drug Administration. Guidance for industry: *In Vitro* Metabolism and Transporter-Mediated Drug-Drug Interaction Studies. US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research, Rockville, MD, USA (2017).
5. US Food and Drug Administration. Guidance for Industry: Clinical Drug Interaction Studies–Study Design, Data Analysis, and Clinical Implications. US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research, Rockville, MD, USA (2017).
6. Riley, R.J.; Wilson, C.E. Cytochrome P450 time-dependent inhibition and induction: advances in assays, risk analysis and modelling. *Expert Opin Drug Metab Toxicol*. 2015, 11(4), 557–72.

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## Featured Products

ACQUITY UPLC I-Class PLUS System <<https://www.waters.com/134613317>>

Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <<https://www.waters.com/waters/134798856>>

MassLynx MS Software <<https://www.waters.com/513662>>

QuanOptimize <<https://www.waters.com/534330>>

TargetLynx <<https://www.waters.com/513791>>

### Available for purchase online

CORTECS UPLC C18+ Column, 90Å, 1.6 µm, 2.1 mm X 100 mm <

<https://www.waters.com/waters/partDetail.htm?partNumber=186007116>>

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