

Nota applicativa

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Antiepileptic Drugs

Waters Corporation

For *in vitro* diagnostic use. Not available in all countries.

Introduction

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System enables the quantification of organic compounds in human biological liquid matrices.

This document describes a test of the analytical performance of the ACQUITY UPLC I-Class/Xevo TQD IVD system for the analysis of 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, perampanel, phenobarbital, phenytoin, pregabalin, primidone, retigabine, tiagabine, topiramate, valproic acid, and zonisamide in plasma.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx Software v4.2 and the data processed using the TargetLynx XS Application Manager. Calibrators and quality controls were prepared by spiking commercially available reference material in plasma and the samples were processed using the following

conditions:

Sample Preparation Conditions

A 50- μ L sample was processed with methanol and centrifuged, then subsequently diluted with water prior to analysis.

LC Conditions

Column:	CORTECS C ₈ , 2.7 μ m, 2.1 mm \times 50 mm
Mobile phase A:	2 mM ammonium acetate in water
Mobile phase B:	2 mM ammonium acetate in methanol
Flow rate:	0.5 mL/min
Gradient:	95% A initial, hold for 0.20 minutes; gradient 6 until 75% A at 1.50 min, hold until 2.50 min; gradient 6 until 30% A at 4 min; gradient 6 until 5% A at 4.01 min, hold until 4.50 min; gradient 6 to 95% A at 4.51 min, then hold until 5.0 min

MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.75 FWHM)
Acquisition mode:	MRM
Polarity:	ESI(+/-)



Figure 1. The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System.

Results and Discussion

Chromatographic selectivity of a range of antiepileptic drugs using the ACQUITY UPLC I-Class/Xevo TQD IVD System is illustrated in Figure 2. Performance characteristics of the antiepileptic drugs are shown in Table 1.

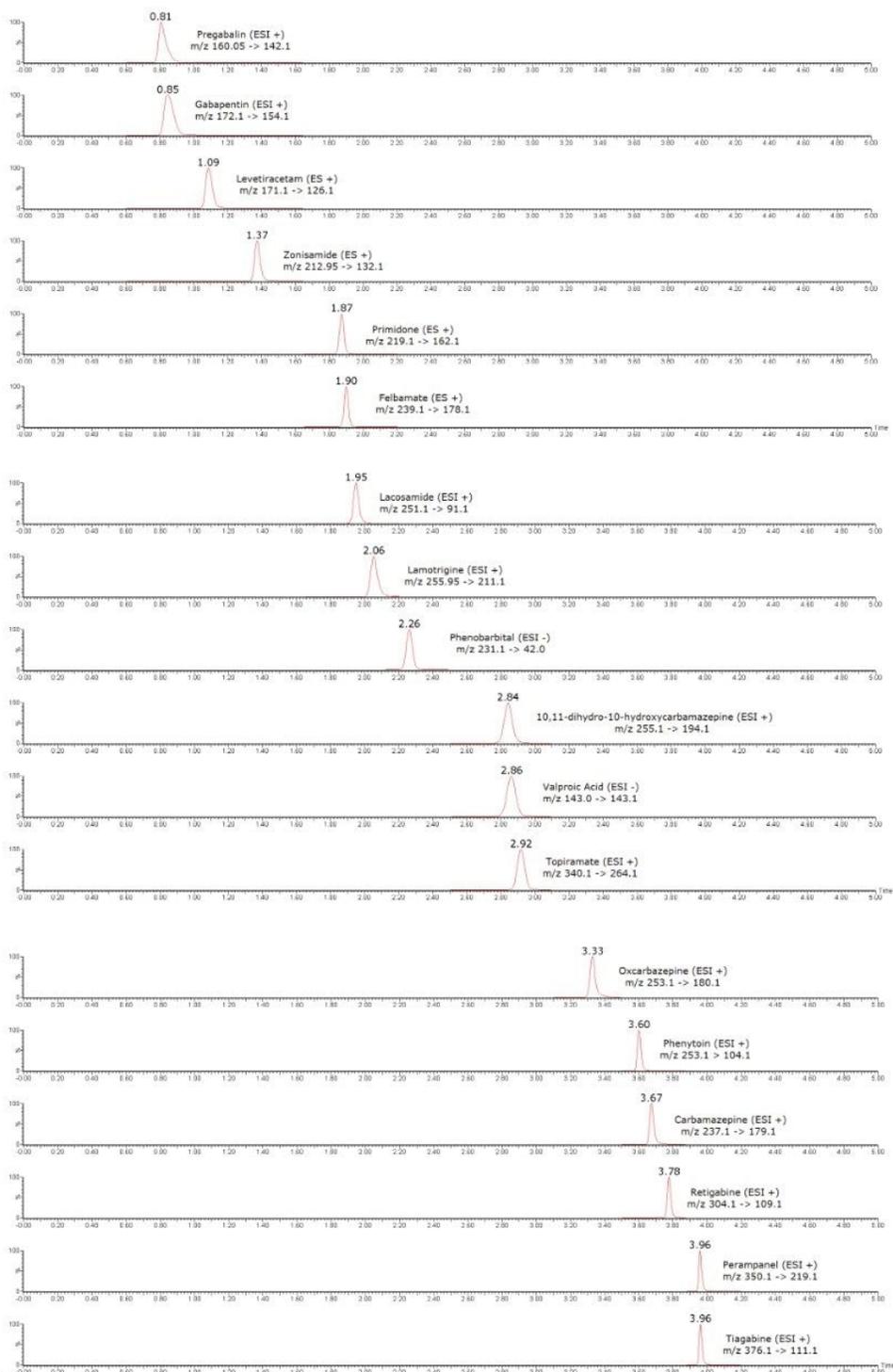


Figure 2. Chromatographic selectivity of a range of antiepileptic drugs using the ACQUITY UPLC I-Class/Xevo

Compound	Calibration range* (µg/mL)	LLOQ (µg/mL)	Linear range (µg/mL)	Total precision	Repeatability	EQA mean bias
10,11-dihydro-10-hydroxycarbamazepine	1-100	0.5	0.752-130	≤6.7%	≤4.7%	0.7%
Carbamazepine	1-100	0.5	0.752-130	≤5.8%	≤5.0%	-2.0%
Felbamate	1-100	0.75	0.752-130	≤6.8%	≤6.2%	10.6%
Gabapentin	1-100	0.5	0.752-130	≤6.6%	≤4.9%	-2.2%
Lacosamide	1-100	1	0.752-130	≤5.9%	≤4.8%	7.0
Lamotrigine	1-100	0.9	0.752-130	≤6.5%	≤5.3%	-0.3
Levetiracetam	1-100	0.5	0.752-130	≤5.8%	≤4.4%	0.9
Oxcarbazepine	0.1-10	0.075	0.0752-13	≤9.3%	≤6.3%	N/A
Perampanel	0.1-10	0.075	0.0752-13	≤5.8%	≤4.4%	-0.8
Phenobarbital	1-100	1	0.752-130	≤8.6%	≤8.4%	-6.1
Phenytoin	1-100	0.5	0.752-130	≤9.5%	≤9.1%	5.4
Pregabalin	0.1-10	0.075	0.0752-13	≤6.7%	≤6.1%	-6.3
Primidone	1-100	0.5	0.752-130	≤6.6%	≤5.2%	0.6
Retigabine	0.1-10	0.075	0.0752-13	≤6.5%	≤5.2%	N/A
Tiagabine	0.01-1	0.0075	0.00752-1.3	≤8.4%	≤7.3%	-4.4
Topiramate	1-100	0.75	0.752-130	≤5.7%	≤4.6%	0.7
Valproic Acid	2-200	1.5	1.5-260	≤6.9%	≤4.5%	1.4
Zonisamide	1-100	0.5	0.752-130	≤6.7%	≤4.8%	-2.6

Table 1. Performance characteristics of the analytes evaluated. *Calibration Range was defined by linear fit where $r^2 > 0.995$ for phenobarbital, topiramate, and zonisamide; for all other analytes a quadratic fit was used. LLOQ was defined by $S/N (PtP) > 10$ with $\%RSD \leq 20\%$ and $\leq 15\%$. $\%RSD$ at LLOQ determined through analytical sensitivity experiments performed over five occasions ($n=50$). Total precision and repeatability of QCs performed over five occasions in plasma ($n=25$). EQA mean bias determined by comparison of obtained values to the assigned value ($n=10$ for 10,11-dihydro-10-hydroxycarbamazepine, felbamate, gabapentin, lacosamide, levetiracetam, perampanel, pregabalin, tiagabine, topiramate, and zonisamide; $n=30$ for carbamazepine, lamotrigine, phenobarbital, phenytoin, primidone, and valproic acid; and oxcarbazepine and retigabine were not included in the scheme).

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver an

analytically sensitive and precise method for the analysis of antiepileptic drugs in plasma.

Disclaimer

The analytical performance data presented here is for illustrative purposes only. Waters does not recommend or suggest analysis of the analytes described herein. These data are intended solely to demonstrate the performance capabilities of the system for analytes representative of those commonly analyzed using liquid chromatography and tandem mass spectrometry. Performance in an individual laboratory may differ due to a number of factors, including laboratory methods, materials used, intra-operator technique, and system conditions. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the analytes in this analysis.

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[MassLynx MS Software](https://www.waters.com/513662)

[TargetLynx](https://www.waters.com/513791)

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