[APPLICATION DATA BRIEF NOTEBOOK]

Clinical LC-MS/MS Systems: Analytical Capabilities

Application Data Briefs

Waters

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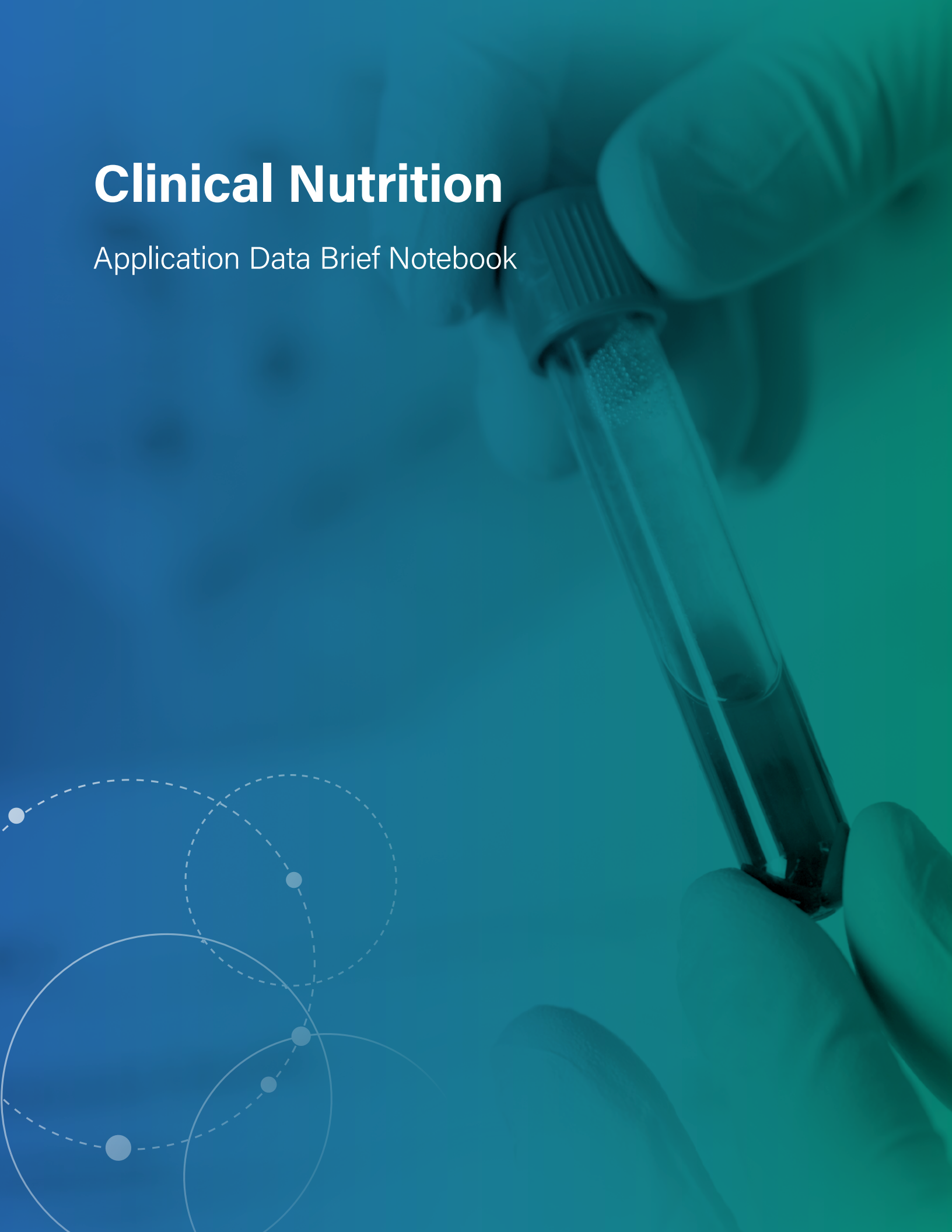
Clinical Nutrition

Application Data Brief Notebook



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Application Note

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System: Analytical Performance for Phylloquinone

Stephen Balloch, Lisa J. Calton, Gareth Hammond

Waters Corporation

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

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

Abstract

Vitamin K1 (phylloquinone) analysis using electrospray ionization mass spectrometry is challenging due to the hydrophobic nature of the molecule and lack of ionization sites. An additional issue is the low concentration of vitamin K1, which may be as low or even lower than 0.1 ng/mL in serum.

A new clinical research method for the analysis of vitamin K1 in serum has been developed using UPLC-MS/MS with electrospray ionization. 200 µL sample was processed with ethanol and centrifuged. The supernatants were loaded onto Oasis PRiME HLB µElution plates, washed, eluted, and a solvent exchange carried out prior to analysis.

Benefits

- A lower limit of the measuring interval (LLMI) of 0.05 ng/mL in serum
- Short run time (3.7 minutes injection-to-injection)

Introduction

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro 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 TQ-S micro IVD System for the analysis of vitamin K1 in serum.

Results and Discussion

A chromatogram illustrating the chromatography of the vitamin K1 analysis is shown in Figure 1. Performance characteristics of vitamin K1 on the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System are shown in Table 1.

Compound	Range (ng/mL)	LLOQ (ng/mL)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Vitamin K1	0.1–20	0.05	7.6%	≤5.4%	≤4.4%	7.5%

Table 1. Performance characteristics of vitamin K1. Range defined by linear fit where r^2 >0.995. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. %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 serum (n=25). EQA mean bias determined by comparison of obtained values to All Laboratory Trimmed Mean.

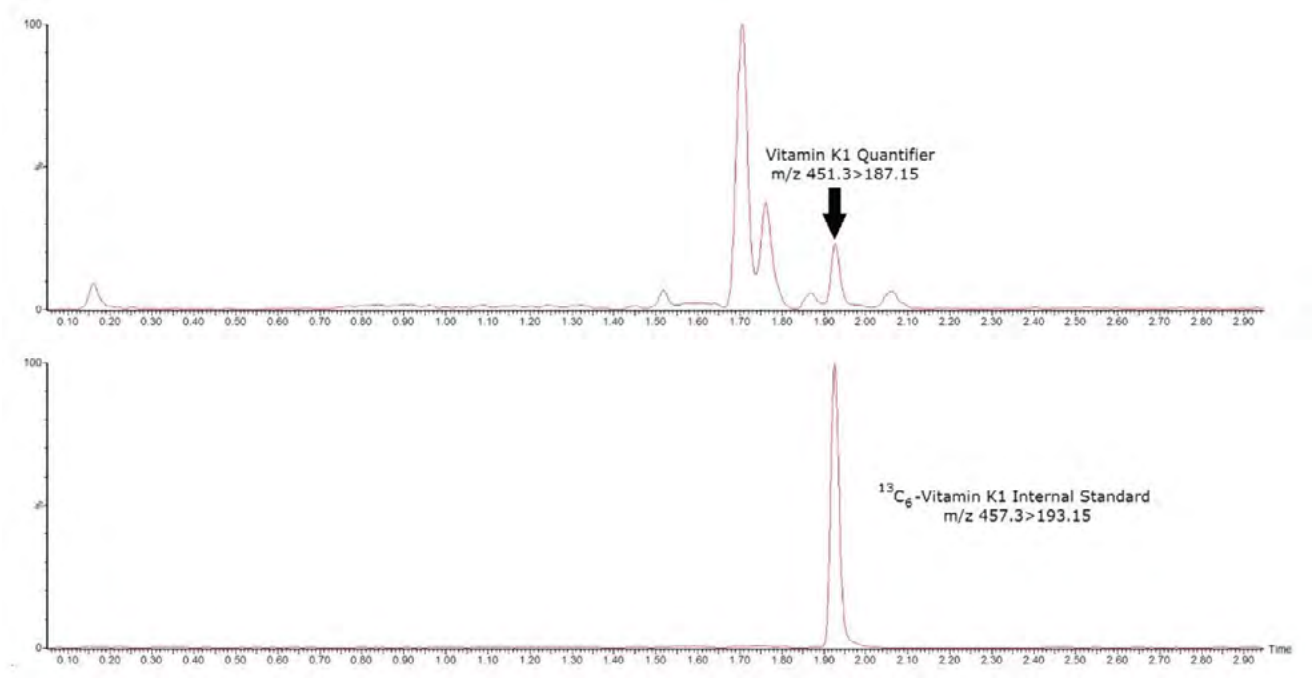


Figure 1. Chromatogram showing the analysis of a sample containing 0.14 ng/mL of vitamin K1 using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System has demonstrated the capability to deliver analytically sensitive, accurate, and precise performance for vitamin K1 in serum.

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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQ-S micro IVD System

MassLynx (IVD) Mass Spectrometry Software with TargetLynx (IVD) <
https://www.waters.com/waters/nav.htm?cid=134834177>

720007170, February 2021

Waters™

Application Note

ACQUITY™ UPLC™ I-Class/Xevo™ TQ-S micro IVD System: Analytical Performance for an Organic Acid

Waters Corporation

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

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

Abstract

This document describes a test of the analytical performance of the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System for the analysis of methylmalonic acid (MMA) in serum.

Introduction

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

Figure 1. The Waters ACQUITY UPLC I-Class System and Xevo TQ-S micro Mass Spectrometer.

Experimental

The ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System was controlled by MassLynx™ IVD (v4.2) and the data processed using the TargetLynx™ Application Manager. Calibrators were prepared by spiking commercially available material in 1% (w/v) bovine serum albumin (BSA) in phosphate buffered saline (PBS), and quality controls (QCs) in both plasma and serum. Samples were processed using the following conditions:

Sample Preparation Conditions

A 100-µL sample and internal standard were added to an Ostro™ protein precipitation and phospholipid removal plate. Samples were precipitated with 1% formic acid in acetonitrile, mixed in-well and then eluted into a collection plate, evaporated, and reconstituted prior to analysis.

LC Conditions

Column:	ACQUITY UPLC CSH™ C_{18}, 1.7 µm, 2.1 mm × 100 mm, with in-line filter
Mobile phase A:	Water with 0.2% formic acid
Mobile phase B:	Acetonitrile with 0.2% formic acid
Flow rate:	0.45 mL/min
Gradient:	1% B over 0.3 minutes, 1–20% B over one minute, 20% over 0.2 minutes, 95% B over 0.5 minutes

MS Conditions

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

Results and Discussion

Performance characteristics of MMA using the Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD system is shown in Table 1.

Analytical sensitivity of the system for analyzing extracted MMA serum samples is illustrated in Figure 1.

Compound	Range (nmol/L)	LLOQ (nmol/L)	%RSD at LLOQ	Total precision	Repeatability	Mean bias
MMA	21–1270	21	11.1%	≤6.3%	≤5.5%	-0.8%

Table 1. Performance characteristics of MMA. Range defined by linear fit where r^2>0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. % RSD at LLOQ determined through analytical sensitivity experiments performed over three occasions (n=30). Total Precision and repeatability of plasma and serum QCs. performed over five occasions (n=25). Mean bias determined through Bland-Altman comparison of calculated concentrations to an independent LC-MS/MS method for MMA. (Note: To convert SI units to conventional mass units, divide by 8.47 for MMA (nmol/L to ng/mL).)

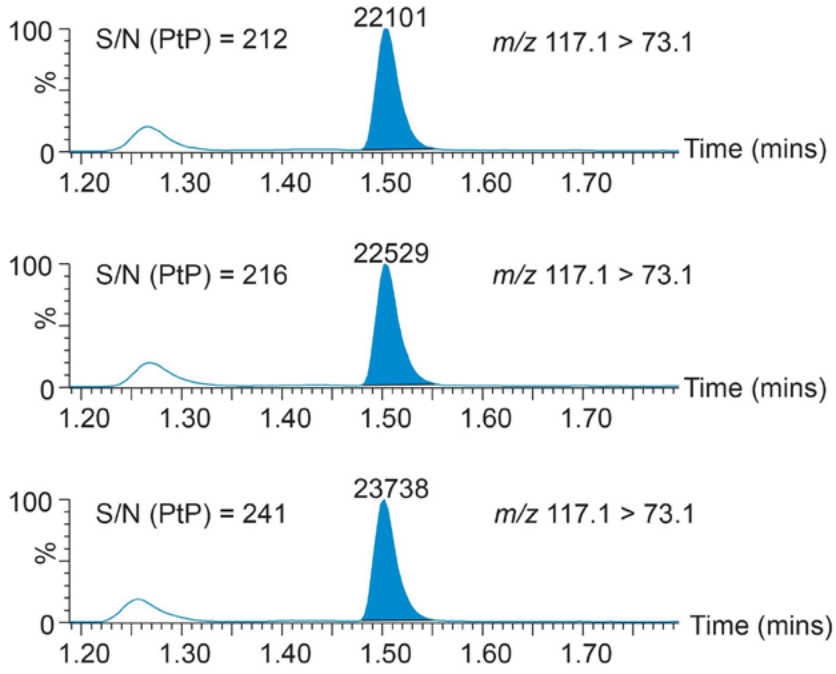


Figure 2. Chromatogram to show S/N (PtP) of extracted MMA control samples at 264 nmol/L using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System has demonstrated the capability to deliver analytically sensitive and selective performance with excellent precision and accuracy for the analysis of methylmalonic acid.

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.

Featured Products

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System <https://www.waters.com/waters/nav.htm?cid=134873687>

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

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

720006805, March 2020

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Fat Soluble Vitamins

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 vitamin A and vitamin E in serum.

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

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD Software (v4.2) and the data was processed using the TargetLynx Application Manager. In-house calibrators and quality controls were prepared by spiking commercially available reference material in serum. The samples were processed using the following conditions:

Sample Preparation Conditions

A 100-µL sample was processed with ethanol and water and centrifuged. The supernatants were diluted then loaded onto Oasis PRiME HLB µElution plates, washed, and eluted prior to analysis.

LC Conditions

Column:	ACQUITY UPLC HSS PFP, 1.8 µm, 2.1 mm × 50 mm
Mobile phase A:	2 mM ammonium acetate + 0.1% formic acid in water
Mobile phase B:	2 mM ammonium acetate + 0.1% formic acid in methanol
Flow rate:	0.4 mL/min
Gradient:	65–98% B over 2 minutes, 98% B for 0.55 minutes, 65% A for 0.95 minutes

MS Conditions

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

Results and Discussion

Chromatograms illustrating the chromatographic selectivity of vitamins A and E are shown in Figures 1 and 2. Performance characteristics of vitamins A and E on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1.

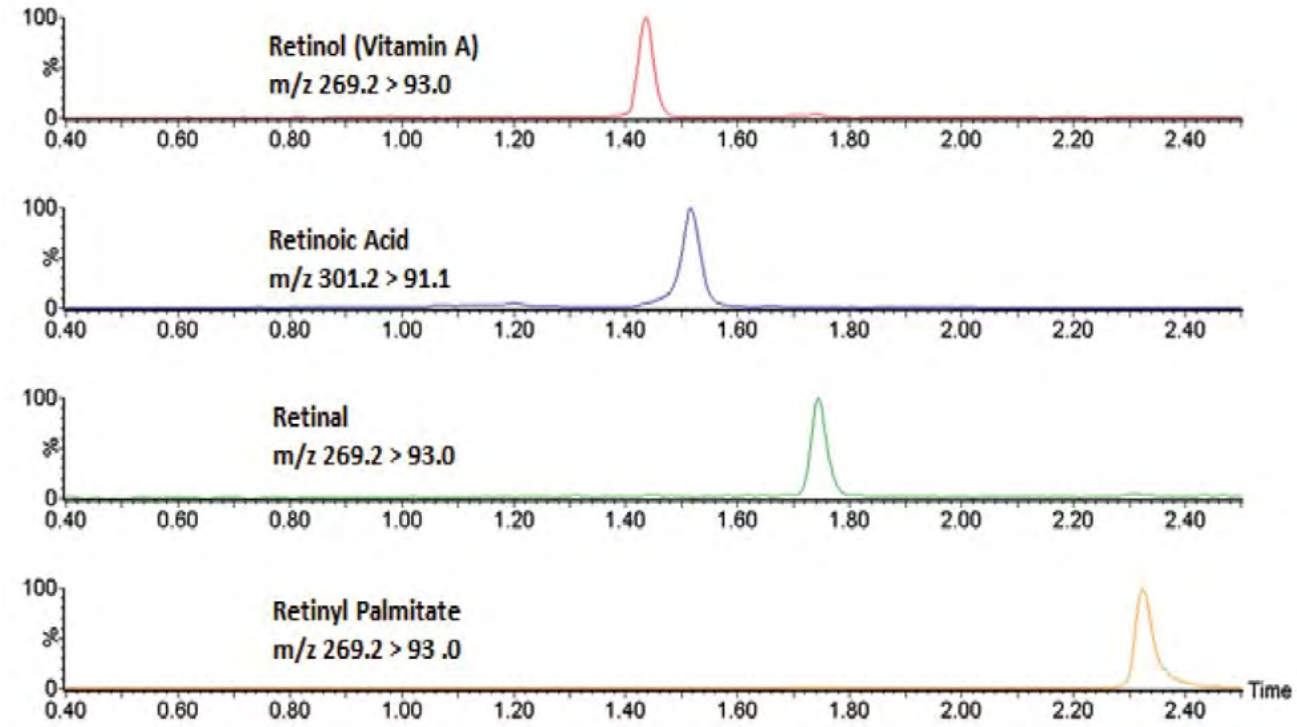


Figure 1. Chromatographic separation of vitamin A from metabolites and structurally similar compounds using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

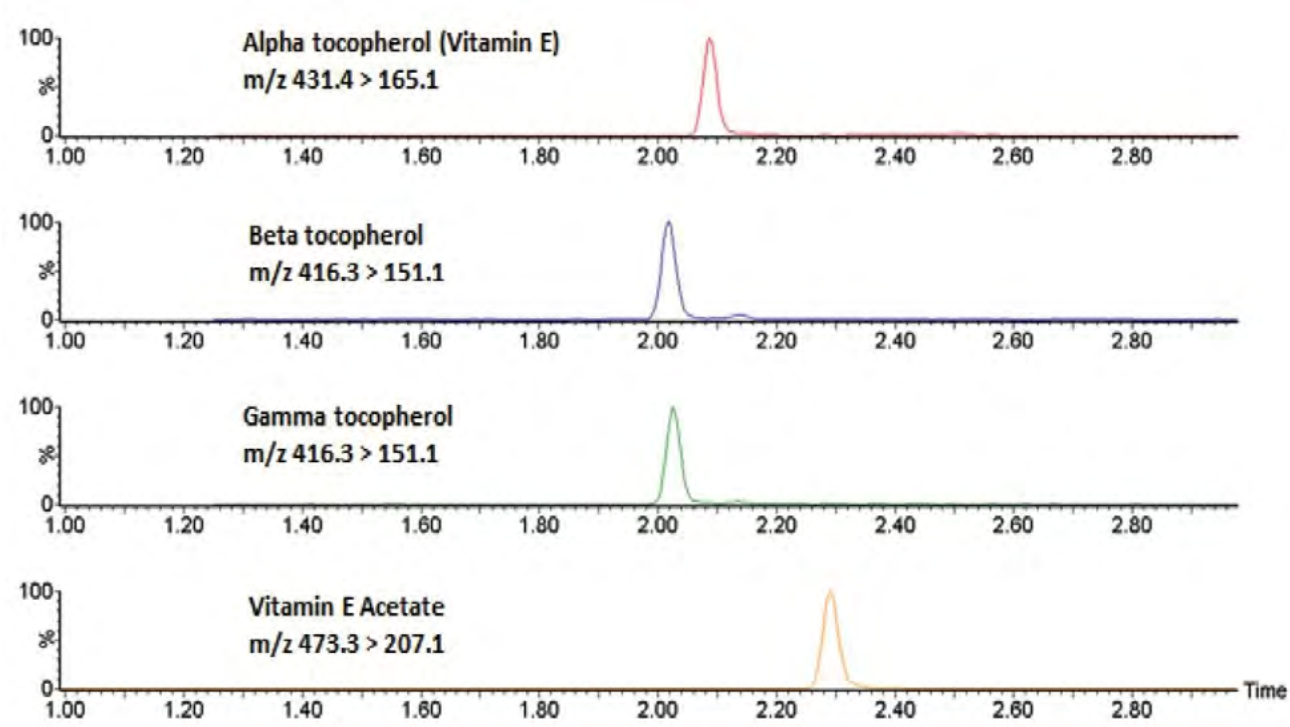


Figure 2. Chromatographic separation of vitamin E from structurally similar compounds using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Compound	Range (ng/mL)	LLOQ (ng/mL)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Vitamin A	100–2000	50	9.6%	≤5.4%	≤4.8%	-7.0%
Vitamin E	2100–21100	1100	14.7%	≤6.9%	≤3.9%	-10.9%

Table 1. Performance characteristics of vitamins A and E. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%; %RSD at LLOQ determined through analytical sensitivity experiments performed over three occasions (n=30). Total precision and repeatability of QCs performed over five occasions in serum (n=25). EQA mean bias (Bland-Altman agreement) determined by comparison of obtained values to all laboratory trimmed mean.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver analytically sensitive, accurate, and precise performance for vitamins A and E in serum.

Disclaimer

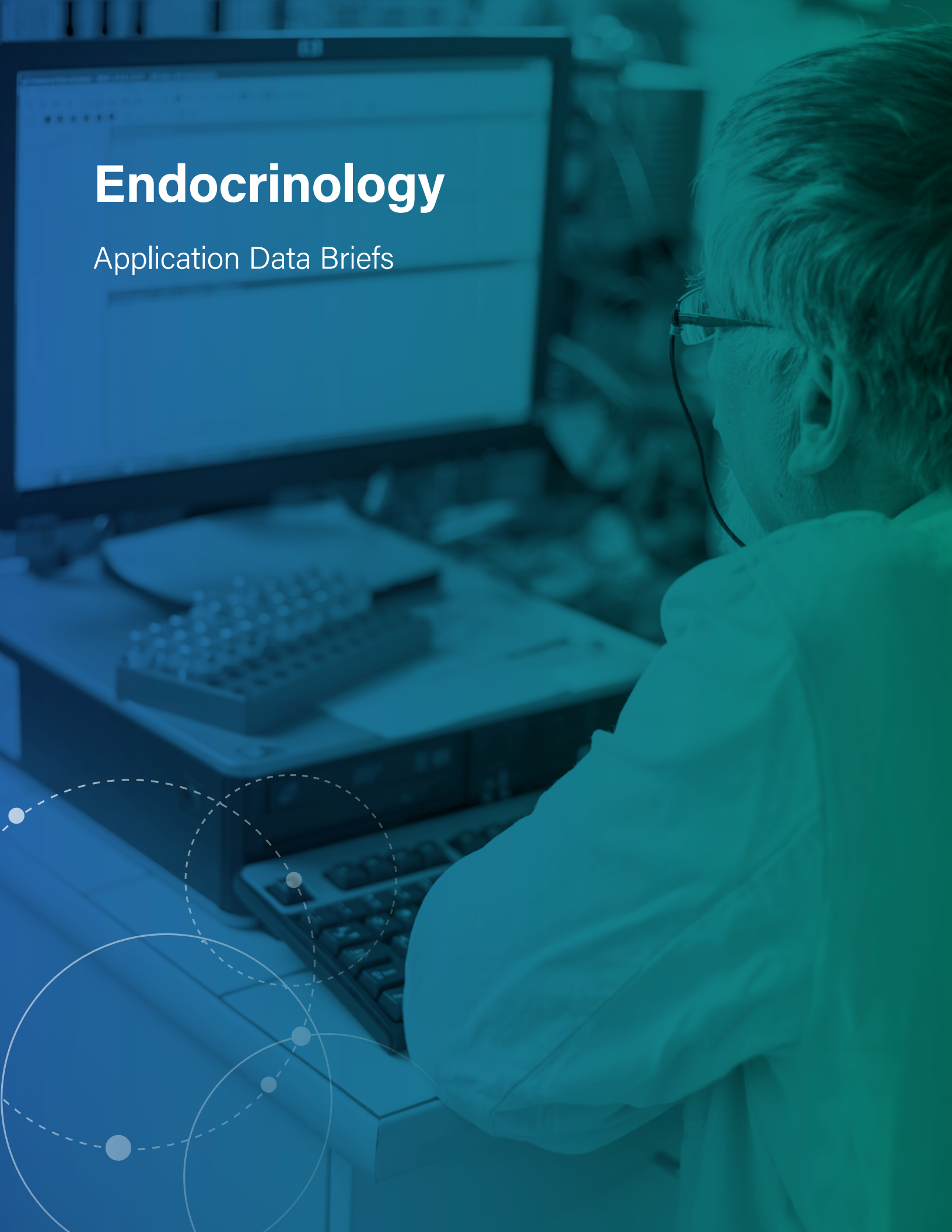
The analytical performance data presented here is for illustrative purposes only. Waters does not recommend or suggest analysis of the analytes described herein. These data are intended solely to demonstrate the performance capabilities of the system for analytes representative of those commonly analyzed 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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

MassLynx (IVD) Mass Spectrometry Software with TargetLynx (IVD) <
https://www.waters.com/waters/nav.htm?cid=134834177>

720006728, December 2019



Endocrinology

Application Data Briefs

Waters™

Application Note

Metrological Traceability of the Waters MassTrak Endocrine Steroid Calibrator and Quality Control Sets

Dominic Foley, Padhraic Rossiter, Norma Breen, Lisa J. Calton

Waters Corporation

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

Abstract

LC-MS/MS methods in clinical laboratories are often based on validated laboratory developed tests (LDTs), which comply with local regulatory guidelines and international standards. In some geographies, laboratories are required to use metrologically traceable calibration materials to aid in compliance with ISO 15189:2012 *Medical laboratories–Requirements for quality and competence*. Therefore, metrological traceability has been incorporated in to the design, development, and manufacture of the Waters MassTrak Endocrine Steroid Calibrator and Quality Control Sets (IVD), aiding laboratories in their compliance to ISO 15189, and providing confidence in the accuracy and harmonization of results when using validated LC-MS methods.

In this application brief, we provide an overview of the Waters MassTrak Endocrine Steroid Calibrator and Quality Control Sets and their accuracy and comparisons across lot-to-lot testing.

Benefits

- Metrologically traceable calibrators and QCs that aid laboratories in their compliance to ISO 15189
- Confidence in the accuracy steroid hormones and provides a path to laboratory method harmonization
- Lyophilized calibrators and QCs that reduce sample preparation time

Introduction

LC-MS/MS methods in many clinical laboratories are based on Laboratory Developed Tests (LDTs), which can involve significant manual preparation in the pre-analytical workflow. This can include the preparation and characterization of in-house calibrator and QC samples by the laboratory technician, which may result in errors and inaccuracies of the materials, leading to reductions in lab efficiency. In addition, to help adhere to local regulatory guidelines and international standards such as ISO 15189, there is a need for metrologically traceable materials to improve the accuracy of results. The use of metrologically traceable materials for calibration of LC-MS/MS methods will also provide a pathway towards laboratory harmonization, particularly if these materials are manufactured to high standards with minimal variability across different manufacturing lots.

The Waters MassTrak Endocrine Steroid Calibrator and Quality Control Sets (IVD) (Figure 1) contains a range of steroid hormones in lyophilized serum that have been sourced to obtain the highest level of metrological traceability available. Cortisol, testosterone, 17-hydroxyprogesterone, and progesterone are value assigned by reference measurement procedures. Dehydroepiandrostenedione sulfate (DHEA-S), 21-deoxycortisol, corticosterone, 11-deoxycortisol, androstenedione, 11-deoxycorticosterone, dehydroepiandrosterone (DHEA), and dihydrotestosterone (DHT) are gravimetrically prepared from certified reference material in stripped serum. All steroid hormone concentrations were confirmed with independent QCs and proficiency testing (PT) or External Quality Assessment (EQA) schemes where available.

Figure 1. The Waters MassTrak Endocrine Steroid Calibrator and Quality Control Sets.

Results and Discussion

Improvements in Laboratory Efficiency

The MassTrak Endocrine Steroid Calibrator and Quality Control Sets have been designed and manufactured to assist laboratories in their compliance with ISO15189. A key benefit is the improvements in laboratory efficiency gained from using ready to use (following reconstitution) metrologically traceable calibrator and QC materials. Table 1 highlights the significant time and resource savings made when using commercial calibrators and QCs, by eliminating the multiple steps a laboratory performs when preparing in-house calibrators.

Preparation of in-house calibrators	MassTrak Endocrine Steroid Calibrator Set
Source certified reference material	✓
Source matrix	✓
Gravimetric preparation of calibrators	✓
Stability evaluation	✓
Calibrator accuracy evaluation	✓
Lot-to-lot evaluation	✓
Measurement of uncertainty	✓

Table 1. Improvements in Laboratory Efficiency.

Metrological Traceability

The metrological traceability associated with the MassTrak Endocrine Steroid Calibrator and Quality Control Sets uses two processes. Primary in-house standards for androstenedione, 11-deoxycortisol, 21-deoxycortisol, corticosterone, 11-deoxycorticosterone, DHEA-S, DHEA, and DHT are traceable to CRM (NIST traceable) material with values based on gravimetric assignment (Figure 2). Secondary in-house standards for cortisol, testosterone, 17-OHP, and progesterone are traceable to the University of Ghent and Rfb reference measurement procedures with values based on a secondary reference measurement procedure assignment (Figure 3). The primary and secondary standards are used to generate and assign concentrations to the MassTrak Endocrine Steroid Calibrator and Quality Control Sets.

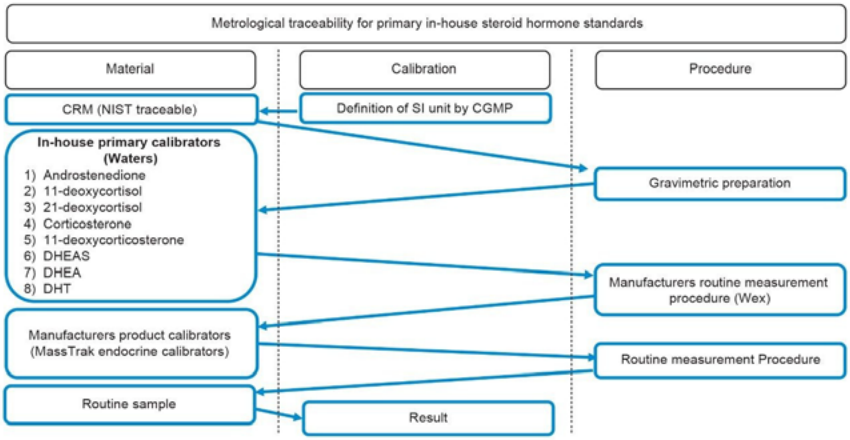


Figure 2. Metrological traceability for androstenedione, 11-deoxycortisol, 21-deoxycortisol, corticosterone, 11-deoxycorticosterone, DHEAS, DHEA, and DHT in the MassTrak Endocrine Steroid Calibrator and Quality Control Sets.

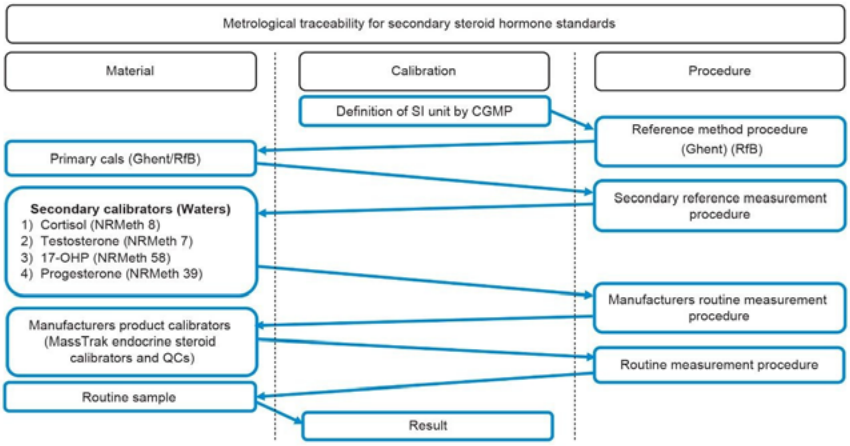


Figure 3. Metrological traceability for cortisol, testosterone, 17-OHP, and progesterone in the MassTrak Endocrine Steroid Calibrator and Quality Control Sets.

Accuracy and Lot-to-Lot Comparisons

The accuracy of the calibrator set was determined through evaluation of in-house QC and External Quality Assessment (EQA) samples. Three lots of manufacturing material for the calibrators were evaluated for accuracy and mean deviations from the assigned values at low and high concentrations following LC-MS/MS analysis using the routine measurement procedure. Results are shown in Figure 4. This data not only demonstrates the accuracy of the calibrators but also the lot-to-lot reproducibility of the manufacturing process, which is important if laboratories seek to maintain their harmonization standards overtime using different manufacturing lots.

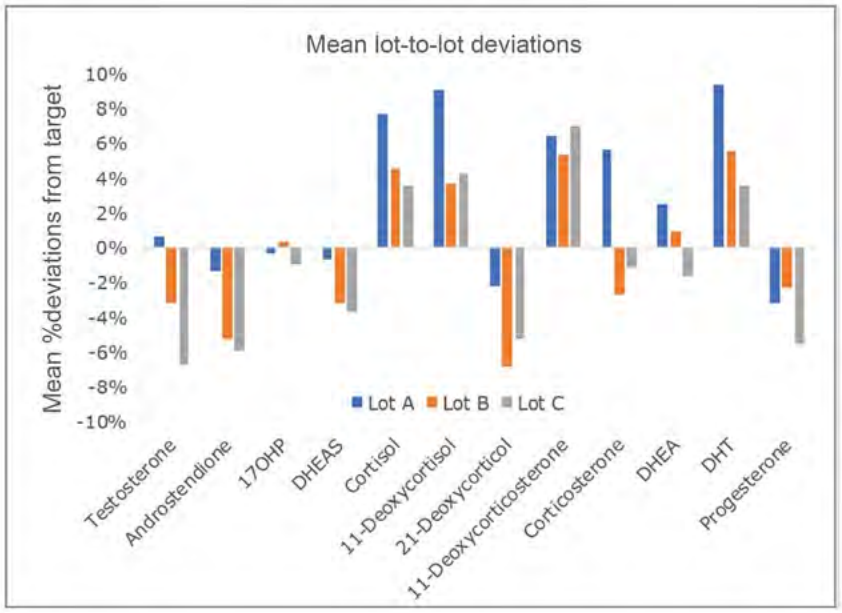


Figure 4. Lot-to-lot comparison of mean deviations from assigned values for manufacturing lots A–C for the steroid hormones demonstrating the accuracy of the MassTrak Endocrine Steroid Calibrator Set.

Conclusion

Metrological traceability of the MassTrak Endocrine Steroid Calibrator and Quality Control Sets has been established aiding laboratories in their compliance to ISO 15189. The assay's accuracy and precision have been confirmed through the use of independent QCs and proficiency testing (PT) schemes.

Disclaimer

MassTrak Endocrine Steroid Calibrator and Quality Control Sets are not available for sale in all countries. For information on availability, please contact your local sales representative.

Featured Products

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

MassTrak Endocrine Steroid Calibrator and Quality Control Sets </nextgen/dk/en/products/standards-and-reagents/masstrak-endocrine-steroid-calibrator-and-quality-control-sets.html>

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

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

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

720007404, Revised April 2022

Waters™

Application Note

Illustration of the Analytical Performance of the Waters MassTrak Endocrine Steroid Calibrators and QC Set Using the ACQUITY UPLC I-Class and Xevo TQ-S micro Mass Spectrometer

Dominic Foley, Lisa J. Calton

Waters Corporation

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

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

Abstract

This document describes a test of the analytical performance of the MassTrak Endocrine Steroid Calibrator and QC Set (IVD) using the Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Introduction

The Waters MassTrak Endocrine Steroid Calibrator and QC Set (IVD) contains metrological traceable steroid hormones in lyophilized human serum, which can be used for quantification of DHEA-S, cortisol, 21-deoxycortisol, corticosterone, 11-deoxycortisol, androstenedione, 11-deoxycorticosterone, testosterone, DHEA, 17-OHP, DHT, and progesterone. The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System enables the quantification of compounds in biological matrices.

Experimental

The ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System was controlled by MassLynx IVD Software (v4.2) and the data processed using the TargetLynx XS Application Manager. Calibrators and Quality Controls were prepared by reconstituting the MassTrak Endocrine Steroid Calibrator and QC Set (IVD) following the Instructions for Use (IFU) and the samples were processed using the following conditions:

Sample Preparation Conditions

125 µL sample was processed with internal standard, methanol, diluted with water and centrifuged. An aliquot of each sample was transferred to the collection plate and the remaining sample was loaded onto Oasis MAX µElution Plates, washed and eluted into the collection plate prior to analysis.

Liquid Chromatography Conditions

Column:	CORTECS C_8, 2.1 mm x 100 mm, 2.6 µm with VanGuard Pre-Column
Mobile phase A:	0.1 mM Ammonium fluoride in water
Mobile phase B:	0.1 mM Ammonium fluoride in methanol
Flow rate:	0.3 mL/min

Gradient:	40% B over 1.25 minutes, 40–52.5% B over 1.75 minutes, 52.5% B for 1 minutes, 52.5%–65% B over 1 minutes, 65% B for 1.25 minutes, 95% B for 0.75 minutes

Mass Spectrometry Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.5 FWHM)
Acquisition mode:	MRM
Polarity:	ESI (+/-)

Results and Discussion

Performance characteristics of the steroid hormones on the Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System are shown in Table 1. Analytical sensitivity of the system for analysing extracted steroid hormone samples is illustrated in Figure 1.

Compound	Range (nmol/L)	Mean r^2	Mean S/N (PtP) at Cal 1	Total precision	Repeatability	Mean bias
DHEA-S	61–29392	0.994	186	≤4.0%	≤4.0%	7.8%
Cortisol	3–1388	0.997	277	≤2.8%	≤2.8%	2.5%
21-Deoxycortisol	0.14–141	0.996	26	≤7.7%	≤5.8%	–
Corticosterone	0.14–141	0.995	52	≤7.7%	≤7.7%	–
11-Deoxycortisol	0.14–141	0.999	86	≤3.6%	≤3.4%	–
Androstenedione	0.17–169	0.999	79	≤3.9%	≤3.9%	3.4%
11-Deoxycorticosterone	0.03–59	0.996	20	≤3.6%	≤3.6%	–
Testosterone	0.05–74	0.995	59	≤7.3%	≤5.2%	2.1%
DHEA	0.90–224	0.994	27	≤7.7%	≤6.1%	–
17-OHP	0.15–293	0.995	55	≤3.7%	≤3.7%	-6.9%
DHT	0.10–8.2	0.998	16	≤5.2%	≤4.9%	6.6%
Progesterone	0.08–164	0.998	42	≤3.5%	≤3.2%	-1.2%

Table 1. Performance characteristics of the steroid hormones. The calibration ranges are based on assigned values for the steroid hormones. The mean linear fit (r^2) and S/N (PtP) were calculated over five runs. Total precision and repeatability of QCs performed over five runs (n=25). Mean bias was determined through Altman-Bland comparison of calculated concentrations to EQA mass spectrometry mean values for cortisol, DHEA-S 17-OHP, testosterone, and androstenedione; all method mean (ALTM) value for progesterone; and to RCPA QAP MS mean value for DHT.

Note: To convert SI units to conventional mass units divide by 2.76 for cortisol (nmol/L to ng/mL), 2.71 for DHEA-S (nmol/L to ng/mL), 2.89 for 21-deoxycortisol, corticosterone, and 11-deoxycortisol (nmol/L to ng/mL), 3.49 for androstenedione (nmol/L to ng/mL), 3.03 for 11-deoxycorticosterone and 17-OHP (nmol/L to ng/mL), 3.47 for testosterone (nmol/L to ng/mL), 3.47 for DHEA (nmol/L to ng/mL, 3.45 for DHT (nmol/L to ng/mL), and 3.18 for progesterone (nmol/L to ng/mL).

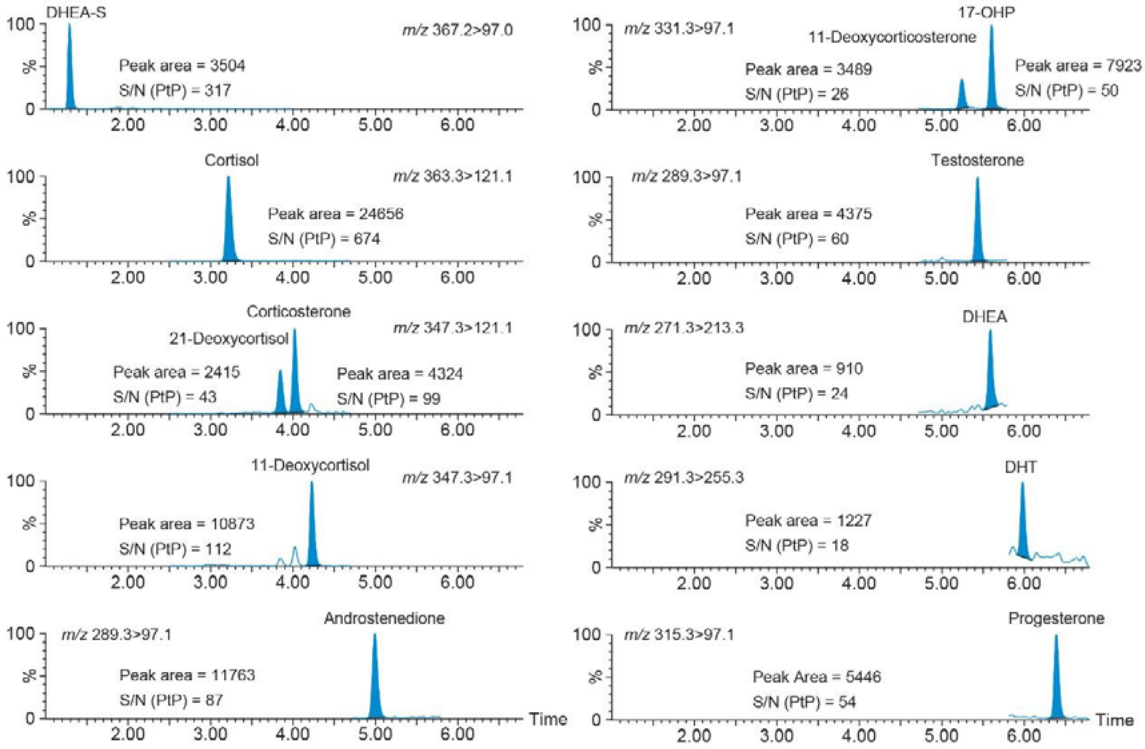


Figure 1. Performance characteristics of the extracted C1 calibrator from the MassTrak Endocrine Steroid Calibrator set analyzed using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Conclusion

The Waters MassTrak Endocrine Steroid Calibrators and QC Set (IVD) in combination with the Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System has demonstrated the capability to deliver analytically sensitive and selective performance with excellent precision and accuracy for DHEA-S, cortisol, 21-deoxycortisol, corticosterone, 11-deoxycortisol, androstenedione, 11-deoxycorticosterone, testosterone, DHEA, 17-OHP, DHT, and progesterone in serum.

Disclaimer

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Featured Products

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System <https://www.waters.com/waters/nav.htm?cid=134873687>

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

MassTrak Endocrine Steroid Calibrator and Quality Control Sets </nextgen/is/en/products/standards-and-reagents/masstrak-endocrine-steroid-calibrator-and-quality-control-sets.html>

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

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

720007372, September 2021

Waters™

Application Note

Confidence in Your Calibrators: MassTrak Endocrine Steroid Calibrators and Quality Control Sets for the LC-MS/MS Analysis of Steroid Hormones

Dominic Foley, Lisa J. Calton

Waters Corporation

Abstract

The routine analysis of steroid hormones is critical in understanding the function of metabolic pathways that impact sexual characteristics, inflammation and blood pressure. Liquid Chromatography-Mass Spectrometry (LC-MS) is fast becoming a sought-after technique in steroid analysis due to the advantages it provides over traditional ligand-binding techniques. These benefits include improvements in analytical sensitivity and selectivity, and the capability of multi-analyte quantitative detection in a single run. However, many LC-MS methods lack harmonization or standardization. The Waters MassTrak Endocrine Steroid Calibrator and Quality Control Sets (IVD) contain metrologically traceable calibrators, aiding laboratories in their compliance to ISO 15189, and provide confidence in the accuracy of results when using validated LC-MS methods.

The MassTrak Endocrine Steroid Calibrator and Quality Control Sets performance was demonstrated using the ACQUITY UPLC I-Class PLUS and Xevo TQ-S micro Triple Quadrupole Mass Spectrometer and an in-house developed LC-MS/MS methodology.

Benefits

- Metrologically traceable calibrators and QCs that aid laboratories in their compliance to ISO 15189
- Confidence in the accuracy steroid hormones and provides a path to laboratory method harmonization
- Lyophilized calibrators and QCs that reduce sample preparation time

Introduction

The analysis of steroid hormones is critical in our understanding of the dysfunction of steroid biosynthetic pathways that impact sexual characteristics, inflammation, and blood pressure.

Analysis of steroid hormones have traditionally been performed using ligand-binding techniques. These techniques can be analytically sensitive and highly automatable, providing high throughput of samples. However, the technique is held back by the inability to detect panels of analytes and is impacted by problems with selectivity of the reagents being used, therefore affecting the reliability of the result, particularly at lower steroid hormone concentrations. More recently, LC-MS/MS has become a sought-after technique in steroid analysis, as it has been established that it can overcome the limitations observed in ligand-binding methods, while providing similar levels of analytical sensitivity.

LC-MS/MS methods in clinical laboratories are often based on laboratory developed tests (LDTs), validated to local regulatory guidelines. These guidelines are constantly evolving and there is increasing demand for all aspects of clinical methods to comply with these changing regulations. This includes the calibrator and QC materials used to generate and independently check the accuracy of the calibration within the method. There is a growing need for metrological traceable calibration materials to replace the in-house prepared materials to aid in compliance with ISO 15189.

The Waters MassTrak Endocrine Steroid Calibration and Quality Control Sets (IVD) (Figure 1) contains a range of steroid hormones in lyophilized serum that have been sourced to obtain the highest level of metrological traceability available. In order to demonstrate the quality of materials found in this product, we have shown the proof of concept performance of the materials using solid phase extraction (SPE) and separation and detection of the samples using the ACQUITY UPLC I-Class PLUS with Xevo TQ-S micro Triple Quadrupole Mass Spectrometry.

Figure 1. The Waters MassTrak Endocrine Steroid Calibration and Quality Control Sets.

Experimental

The MassTrak Endocrine Steroid Calibrator and Quality Control Sets contain the following steroid hormones in lyophilized serum: dehydroepiandrosterone sulfate (DHEA-S), cortisol, 21-deoxycortisol, corticosterone, 11-deoxycortisol, androstenedione, 11-deoxycorticosterone, testosterone, dehydroepiandrosterone (DHEA), 17-hydroxyprogesterone (17-OHP), dihydrotestosterone (DHT), and progesterone. Assigned concentrations for the calibration range and QCs are found in Table 1.

Steroid hormone	Calibrator range (nmol/L)	QCs (nmol/L)
DHEA-S	61–29392	527, 4770, 19061
Cortisol	3–1388	30, 279, 931
21-Deoxycortisol	0.14–141	0.64, 6.2, 94
Corticosterone	0.14–141	0.65, 6.4, 93
11-Deoxycortisol	0.15–144	0.64, 6.3, 94
Androstenedione	0.17–169	0.74, 7.4, 114
11-Deoxycorticosterone	0.03–59	0.11, 1.1, 40
Testosterone	0.05–74	0.18, 1.7, 52
DHEA	0.90–224	2.9, 28, 110
17-OHP	0.15–293	0.66, 6.2, 207
DHT	0.10–8.2	0.35, 3.4, 5.8
Progesterone	0.08–164	0.40, 3.6, 114

Table 1. Concentration ranges of the MassTrak Endocrine Steroid Calibrator and Quality Control Sets.

The calibrators and QCs are reconstituted following the instructions for use (IFU), prior to sample preparation and analysis.

Sample Description

Sample preparation was performed using protein precipitation, followed by Solid Phase Extraction (SPE).

Protein Precipitation

To 125 µL of serum sample, 25 µL of internal standard (SIL) in 50/50 (v/v) methanol/water was added and mixed for 1 minute. 250 µL of methanol was added, followed by mixing for 5 minutes. The sample was diluted with 550 µL water prior to mixing for 1 minute and centrifugation for 10 minutes at 5000 g.

Solid Phase Extraction

An Oasis MAX µElution Plate was conditioned and equilibrated with 150 µL methanol and water, respectively. 625 µL of supernatant was loaded on to the SPE plate and 10 µL of supernatant (for DHEA-S) was directly transferred to the 1 mL 96-well collection plate. Washes were performed using 150 µL 1% formic acid in 10% acetonitrile, then 150 µL 1% ammonia in 10% acetonitrile. Samples were eluted with 35 µL 60% acetonitrile into the 1 mL 96-well collection plate, already containing 10 µL of protein precipitation supernatant. 35 µL of 50 mM ammonium bicarbonate pH 7.4 (0.03% acetic acid) was added and the mixed for 1 minute and sealed prior to injection onto the LC-MS/MS system.

LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS FTN
Sample needle:	30 µL
Column:	CORTECS C_8, 90 Å, 2.1 mm x 100 mm, 2.7 µm
Precolumn:	CORTECS C_8 VanGuard Cartridge, 2.1 mm x 5 mm, 2.7 µm
Column temp.:	50 °C
Sample temp.:	8 °C
Injection volume:	25 µL
Flow rate:	0.3 mL/min
Mobile phase A:	0.1 mM Ammonium fluoride in water
Mobile phase B:	0.1 mM Ammonium fluoride in methanol
Run time:	7.8 minutes

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.300	60	40	Initial
1.25	0.300	60	40	6
3.00	0.300	47.5	52.5	6
4.00	0.300	47.5	52.5	6
5.00	0.300	35	65	6
6.25	0.300	10	90	11
7.00	0.600	60	40	11
7.60	0.300	60	40	11

MS Conditions

MS system:	Xevo TQ-S micro Triple Quadrupole Mass Spectrometry
Ionization mode:	Positive/Negative ESI
Capillary voltage:	2.5 kV

MRM Parameters

Analyte	MRM		Cone (V)	Collison (V)	Scan window (min)
DHEA-S	367.2>97.0	Quantifier	40	30	1.00–2.00
	367.2>80.0	Qualifier	40	80	
	373.2>98.0	SIL	40	30	
Cortisol	363.3>121.1	Quantifier	40	24	2.50–4.70
	363.3>91.1	Qualifier	40	24	
	366.3>124.1	SIL	40	24	
21-Deoxycortisol	347.3>121.1	Quantifier	40	24	2.50–4.70
	347.3>311.2	Qualifier	40	14	
	351.3>121.1	SIL	40	24	
Corticosterone	347.3>121.1	Quantifier	40	24	2.50–4.70
	347.3>97.1	Qualifier	40	24	
	351.3>121.1	SIL	40	24	
11-Deoxycortisol	347.3>97.1	Quantifier	40	24	2.50–4.70
	347.3>109.1	Qualifier	40	24	
	350.3>100.1	SIL	40	24	
Androstenedione	287.3>97.1	Quantifier	70	24	4.71–5.80
	287.3>109.1	Qualifier	70	24	
	290.3>100.1	SIL	70	24	
11-Deoxycorticosterone	331.3>97.1	Quantifier	70	22	4.71–5.80
	331.3>109.1	Qualifier	70	22	
	334.3>100.1	SIL	70	22	
Testosterone	289.3>97.1	Quantifier	70	22	4.71–5.80
	289.3>109.1	Qualifier	70	22	
	292.3>100.1	SIL	70	22	
DHEA	271.3>213.3	Quantifier	40	14	4.71–5.80
	271.3>197.3	Qualifier	40	16	
	274.3>216.3	SIL	40	14	
17-OHP	331.3>97.1	Quantifier	70	22	4.71–5.80
	331.3>109.1	Qualifier	70	22	
	334.3>100.1	SIL	70	22	
DHT	291.3>255.3	Quantifier	40	14	5.80–6.80
	291.3>159.2	Qualifier	40	18	
	294.3>258.3	SIL	40	14	
Progesterone	315.3>97.1	Quantifier	70	24	5.80–6.80
	315.3>109.1	Qualifier	70	24	
	318.3>100.1	SIL	70	24	

Method Events

Time	Event	Action
0.01	Flow state	Waste
1.01	Flow state	LC
6.75	Flow state	Waste

Data Management

MS software: MassLynx v4.2 with TargetLynx XS

Results and Discussion

Chromatographic separation of the twelve steroid hormones was achieved using the CORTECS C_8, 2.7 µm, 2.1 mm x 100 mm Column, with baseline resolution of steroid hormone isomers which cannot be differentiated with MRM alone (Figure 2). This includes separation of 21-deoxycortisol, corticosterone and 11-deoxycortisol, in addition to the isomeric pair of 11-deoxycorticosterone and 17-OHP.

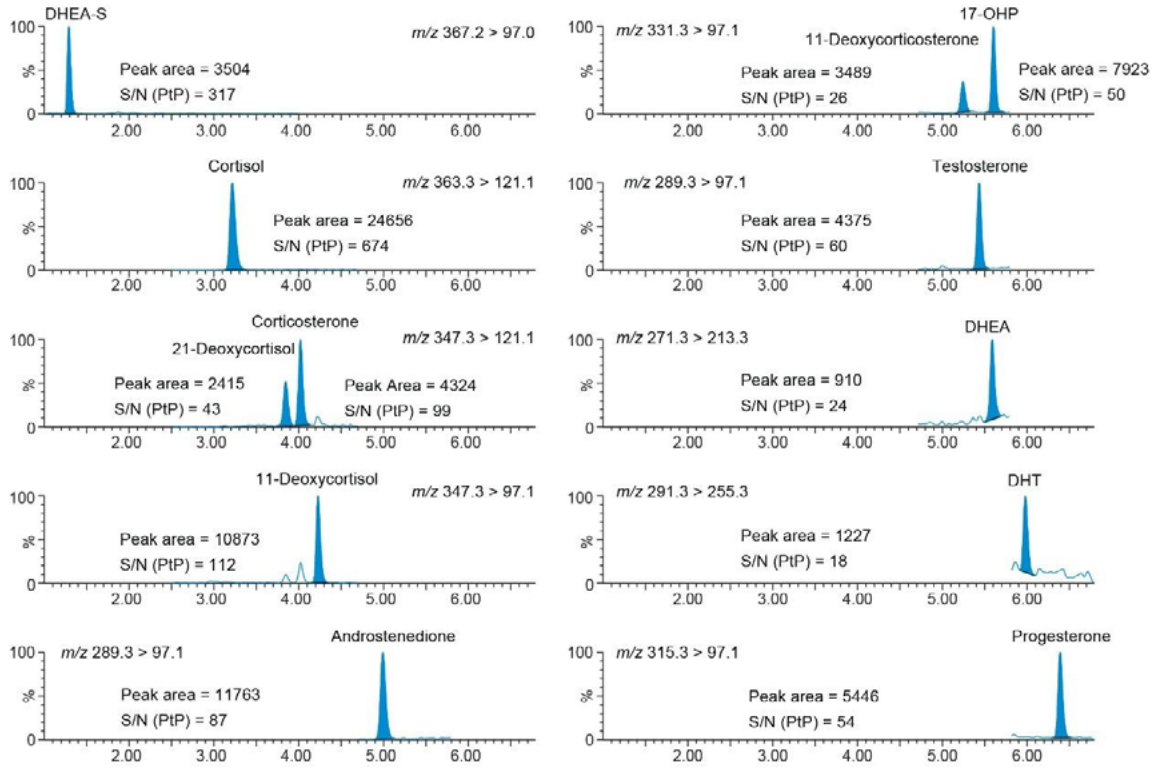

Figure 2. Performance characteristics of the extracted C1 calibrator from the MassTrak Endrocine Steroid Calibrator Set analyzed using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Linearity of the calibration ranges was demonstrated with mean r^2 values for the calibration lines >0.994 across the 12 steroid hormones. Analytical sensitivity of the method was determined through the signal:noise (S/N) evaluation of the low calibration (C1) standard For the steroid hormones. The S/N (PtP) was >10 at each of calibrator 1 concentrations across five analytical runs. This is summarized in Table 2 and an example of the S/N at the low calibrator can also be seen in Figure 2.

Compound	Calibrator range (nmol/L)	Mean r^2	Mean S/N (PtP) at Cal 1
DHEA-S	61–29392	0.994	186
Cortisol	3–1388	0.997	277
21-Deoxycortisol	0.14–141	0.996	26
Corticosterone	0.14–141	0.995	52
11-Deoxycortisol	0.15–144	0.999	86
Androstenedione	0.17–169	0.999	79
11-Deoxycorticosterone	0.03–59	0.996	20
Testosterone	0.05–74	0.995	59
DHEA	0.90–224	0.994	27
17-OHP	0.15–293	0.995	55
DHT	0.10–8.2	0.998	16
Progesterone	0.08–164	0.998	42

Table 2. Summary of calibration linearity and analytical sensitivity performance of the steroid hormones in the MassTrak Endocrine Steroid Calibrator Set.

Total precision and repeatability were determined by extracting and quantifying five replicates of three level QC material per day over five separate days (n=25). Low, mid and high concentrations were 527, 4770, and 19061 nmol/L for DHEA-S; 30, 279, and 931 nmol/L for cortisol; 0.64, 6.2, and 94 nmol/L for 21-deoxycortisol; 0.65, 6.4, and 93 nmol/L for corticosterone; 0.64, 6.3, and 95 nmol/L for and 11-deoxycortisol; 0.74, 7.4, and 114 nmol/L for androstenedione; 0.11, 1.1, 40 nmol/L for 11-deoxycorticosterone; 0.66, 6.2, and 207 nmol/L for 17-OHP; 2.9, 28, and 110 nmol/L for DHEA; 0.35, 3.4, and 5.8 nmol/L for DHT, and 0.40, 3.6, and 114 nmol/L for progesterone. Total precision and repeatability were determined to be ≤7.7% CV across all steroid hormones at the three QC concentrations (Figure 3).

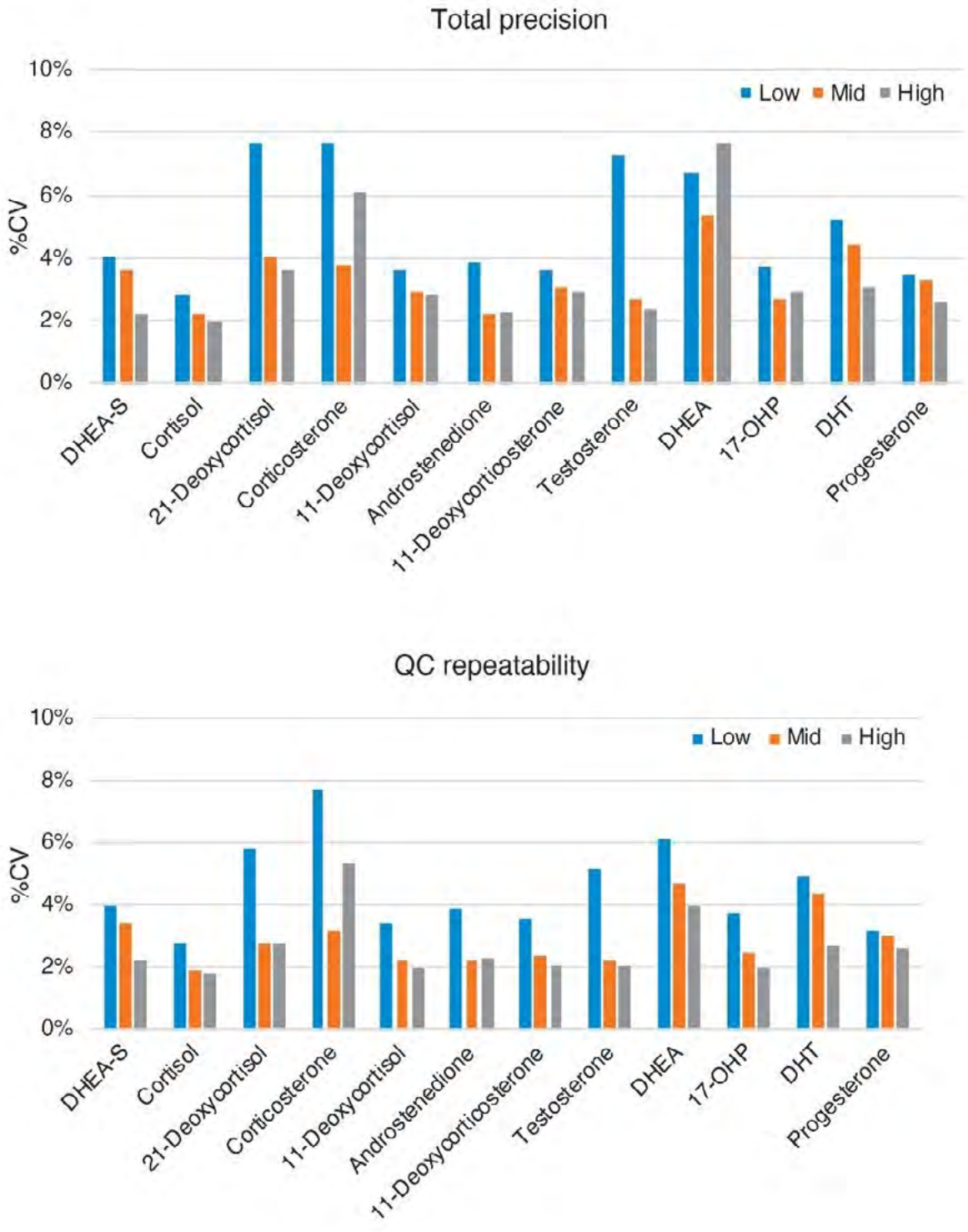

Figure 3. Total precision and repeatability for the analysis of the twelve steroid hormones in the MassTrak Endocrine Steroid Quality Control Set.

In addition, the accuracy of the QCs was evaluated in comparison to the calibrators over the five analytical runs. The mean accuracy for the QCs across the 12 steroid hormones at the three concentrations ranged from 91.0%–112.4% (Table 3).

	QC Accuracy		
Analyte	Q1	Q2	Q3
DHEAS	91%	102%	112%
Cortisol	102%	106%	98%
21-Deoxycortisol	99%	100%	109%
Corticosterone	102%	96%	108%
11-Deoxycortisol	103%	100%	103%
Androstenedione	100%	97%	103%
11-Deoxycorticosterone	104%	98%	103%
Testosterone	106%	107%	99%
DHEA	102%	100%	100%
17-OHP	105%	101%	99%
DHT	109%	108%	111%
Progesterone	102%	105%	97%

Table 3. Accuracy of the MassTrak Endocrine Steroid Quality Control Set analyzed in replicates of five at three concentrations over five analytical runs.

Accuracy was assessed for DHEA-S, cortisol, androstenedione, testosterone, 17-OHP, and progesterone through the analysis of EQA samples from UK NEQAS. The data obtained was compared to the mass spectrometry method mean for the samples (ALTM for progesterone, as LC-MS values were unavailable) and Altman-Bland agreement was performed on the data set. Altman-Bland agreement for DHEA-S, cortisol, androstenedione, testosterone, 17-OHP, and progesterone provided a mean method bias within ±7.8%, demonstrating excellent agreement with the EQA method values for the steroid hormones (Figures 4a-f).

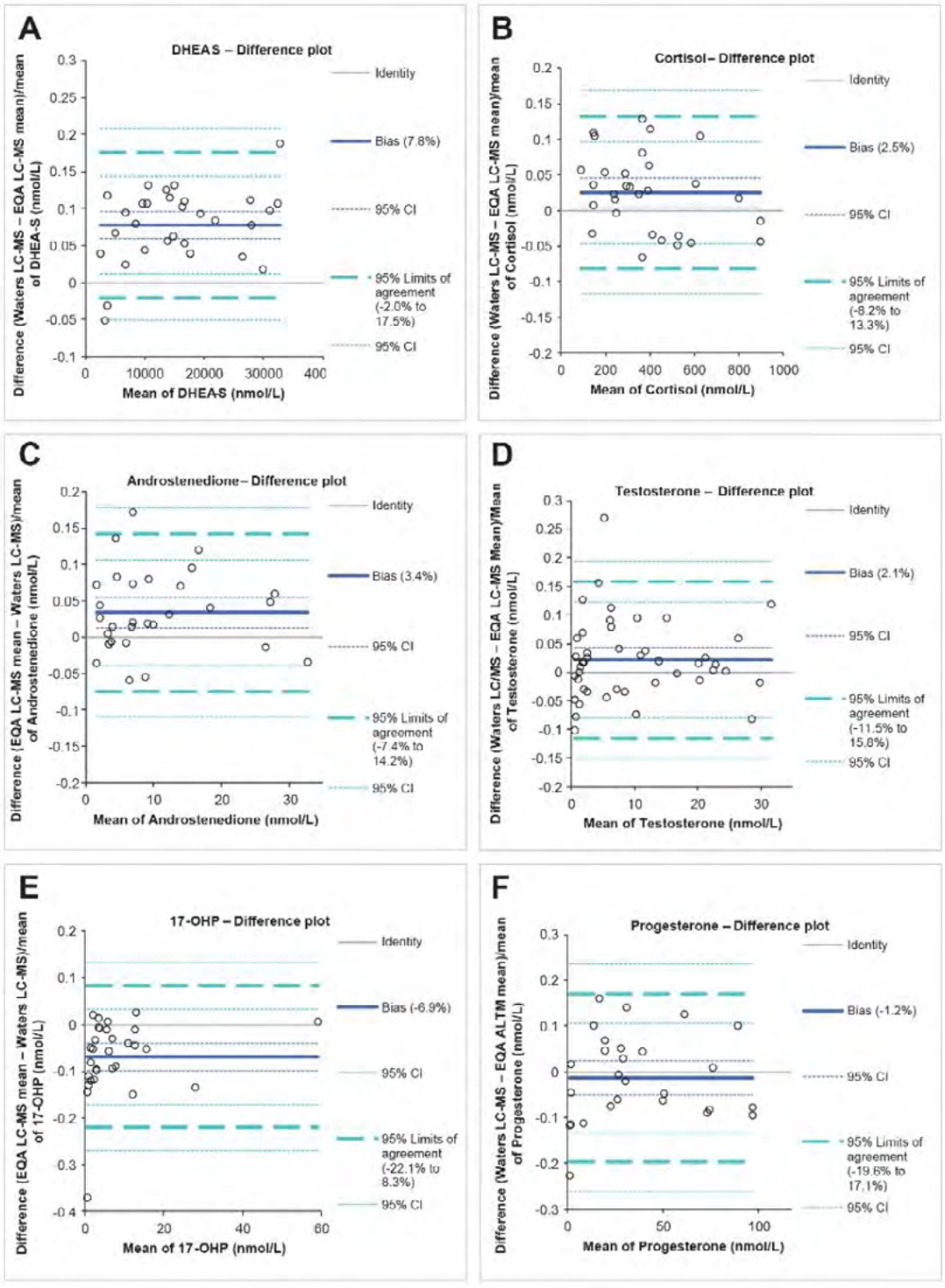

Figure 4. Altman-Bland agreement comparing the Waters LC-MS/MS method to the EQA scheme MS method mean for: (a) DHEA-S, (b) cortisol, (c) androstenedione, (d) testosterone, (e) 17-OHP, and (f) progesterone (ALTM mean).

Accuracy for DHT was assessed through the analysis of Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP) samples (n=4). Altman-Bland agreement demonstrated a mean bias of 6.6% (range -1% to 13%) compared to the all laboratory mean (n≥8).

Conclusion

Through this proof of performance evaluation, it has been demonstrated the MassTrak Endocrine Steroid Calibrator and Quality Control Sets (IVD) can provide precise and accurate quantification of the 12 steroid hormones in serum.

The ACQUITY UPLC I-Class PLUS with Xevo TQ-S micro Triple Quadrupole Mass Spectrometer is able to provide sufficient analytical sensitivity to analyze low levels of the steroid hormones in the set by using only 125 µL sample volume. Excellent levels of precision across the calibration range have been demonstrated with total precision and repeatability of ≤7.7% CV. In addition, the accuracy of the QC set was established with accuracies ranging from 91.0%–112.4%. An indication of metrological traceability through agreement to EQA samples was also shown, with the method providing excellent agreement to samples from the EQA (DHEA-S, cortisol, androstenedione, testosterone, 17-OHP, and progesterone) and RCPA (DHT) with mean method bias within ±7.8% compared to method mean values from the schemes.

Disclaimer

This method is an example of an application using the instrumentation, software and consumables described in this document. This method has not been cleared by any regulatory entity for diagnostic purposes. The end user is responsible for completion of the method development and validation. MassTrak Endocrine Steroid Calibrator and Quality Control Sets are not available for sale in all countries. For information on availability, please contact your local sales representative.

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/134798856>

MassTrak Endocrine Steroid Calibrator and Quality Control Sets </nextgen/in/en/products/standards-and-reagents/masstrak-endocrine-steroid-calibrator-and-quality-control-sets.html>

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

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

720007401, October 2021

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQ-XS IVD System: Analytical Performance for a Mineralocorticoid

Waters Corporation

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

Introduction

The Waters ACQUITY UPLC I-Class/Xevo TQ-XS 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 TQ-XS IVD System for the analysis of aldosterone in plasma.

Figure 1. The Waters ACQUITY UPLC I-Class System and Xevo TQ-XS Mass Spectrometer.

Experimental

The ACQUITY UPLC I-Class/Xevo TQ-XS IVD System was controlled by MassLynx IVD (v4.2) and the data processed using the TargetLynx Application Manager. Calibrators and quality controls were prepared by spiking commercially available reference material in stripped serum, and the samples were processed using the following conditions:

Sample Preparation Conditions

200 µL sample was precipitated with $ZnSO_4$/methanol, diluted, and centrifuged. Samples were loaded onto Oasis MAX µElution Plates, washed, and eluted prior to analysis.

LC Conditions

Column:	CORTECS C_{18}, 2.7 µm, 2.1 × 100 mm with VanGuard Pre-column
Mobile phase A:	Water with 0.05 mM ammonium fluoride
Mobile phase B:	Methanol
Flow rate:	0.5 mL/min
Gradient:	35% B over 1 min, 35–60% B over 0.7 min, 60% for 0.5 min, 95% B for 0.8 min

MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.5FWHM)
Acquisition mode:	MRM
Polarity:	ESI-

Results and Discussion

Performance characteristics of aldosterone using the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System is shown in Table 1. Analytical sensitivity of the system for analyzing extracted aldosterone plasma samples is illustrated in Figure 2.

Compound	Range (pmol/L)	LLOQ (pmol/L)	%RSD at LLOQ	Total precision	Repeatability	Mean Bias
Aldosterone	8–4161	8	6.1%	≤6.3%	≤6.0%	-3.2%

Table 1. Performance characteristics of aldosterone. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. % RSD at LLOQ determined through analytical sensitivity experiments performed over five occasions (n=40). Total precision and repeatability of QCs performed over five occasions (n = 25). Mean bias determined through Altman-Bland comparison of calculated concentrations to EQA mass spectrometry mean values for aldosterone.

Note: To convert SI units to conventional mass units, divide by 2.774 for aldosterone (pmol/L to pg/mL).

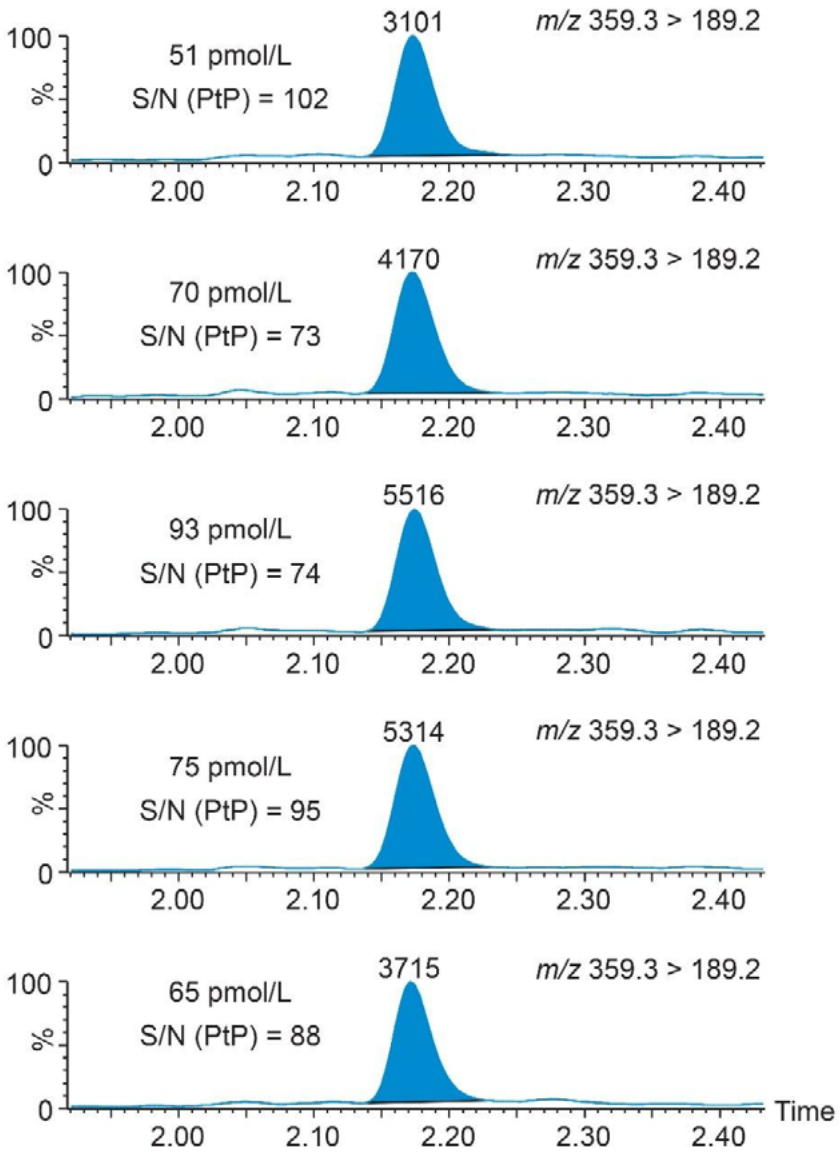

Figure 2. Chromatograms to show S/N (PtP) of extracted plasma aldosterone samples using the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQ-XS IVD System has demonstrated the capability to deliver analytically sensitive and selective performance with excellent precision and accuracy for the analysis of aldosterone 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.

Featured Products

ACQUITY UPLC I-Class/Xevo TQ-XS IVD System <
https://www.waters.com/waters/nav.htm?&cid=135034342>

MassLynx (IVD) Mass Spectrometry Software with TargetLynx (IVD) <
https://www.waters.com/waters/nav.htm?cid=134834177>

720006678, September 2019

Waters™

Application Note

ACQUITY UPLC I-Class with Xevo TQ-XS IVD System: Analytical Performance for Estrogens

Waters Corporation

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

Introduction

The Waters ACQUITY UPLC I-Class with Xevo TQ-XS 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 with Xevo TQ-XS IVD System for the analysis of 17β-estradiol (E2) and estrone (E1) in serum.

Figure 1. The Waters ACQUITY UPLC I-Class System and Xevo TQ-XS Mass Spectrometer.

Experimental

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

Sample Preparation Conditions

A 250 µL sample was processed with hexane, ethyl acetate, and centrifuged. Samples were transferred, evaporated to dryness, and reconstituted in methanol and water prior to analysis.

LC Conditions

Column:	CORTECS Phenyl, 2.7 µm, 2.1 × 50 mm
Mobile phase A:	0.05 mM ammonium fluoride in water
Mobile phase B:	Methanol
Flow rate:	0.3 mL/min
Gradient:	10% B for 0.5 min, 40%–70% B over 3.0 min, 98% B for 0.5 min, 10% B for 0.5 min

MS Conditions

Resolution:	MS1 (0.7 FWHM), MS2 (0.7 FWHM)
Acquisition mode:	MRM
Polarity:	ESI-

Results and Discussion

Chromatographic separation of E2 and E1 on the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System is illustrated in Figure 2, with low level samples of E2 shown in Figure 3. Performance characteristics of E2 and E1 are shown in Table 1.

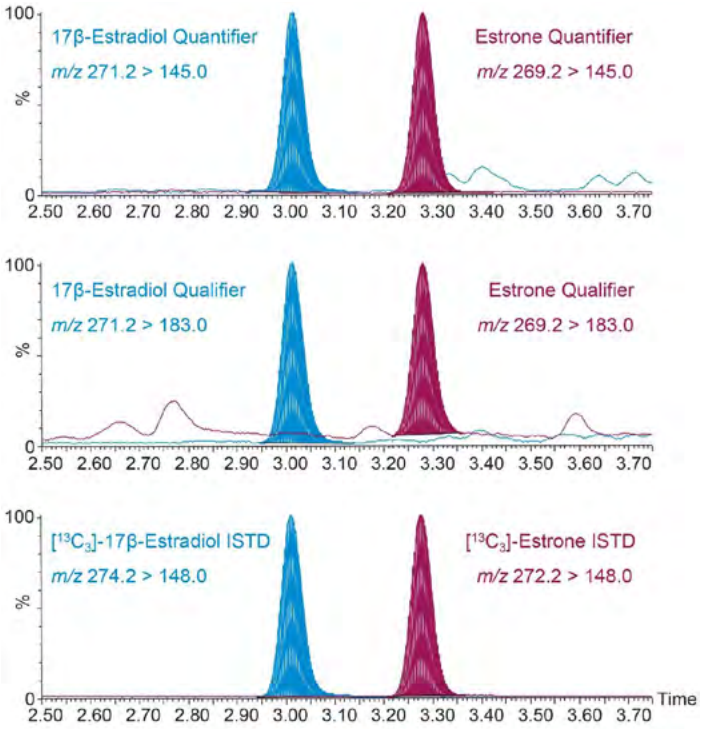

Figure 2. Chromatographic separation of E2 and E1 in a sample using the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System.

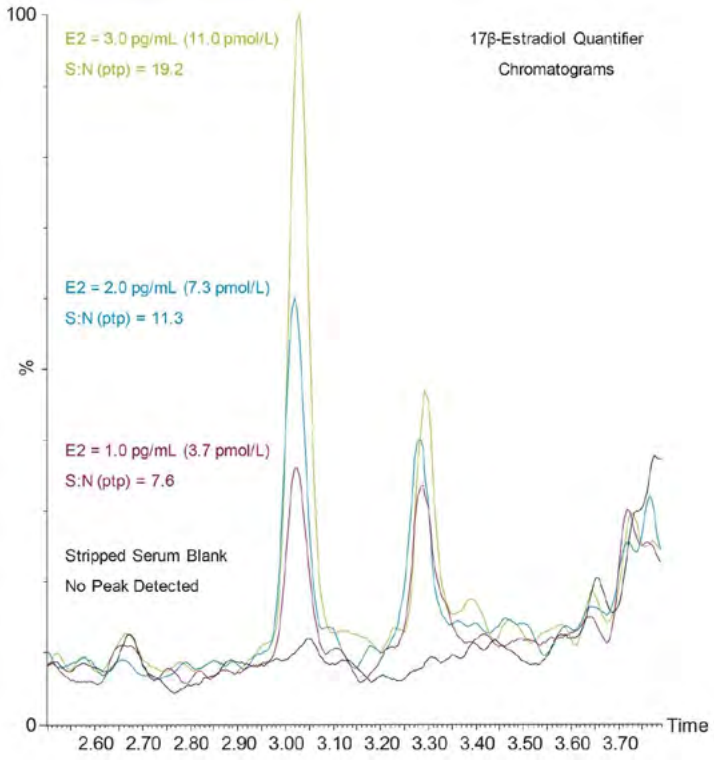

Figure 3. Stripped serum spiked with low levels of E2 using the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System.

Compound	Range (pmol/L)	LLOQ (pmol/L)	Total precision	Repeatability	CDC HoSt mean bias	EQA LC-MS mean bias
17β-Estradiol (E2)	11.1–3700	11.1	≤4.5%	≤4.5%	7.0%	1.7%
Estone (E1)	7.4–3700	7.4	≤3.5%	≤4.8%	N/A	N/A

Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where r^2 > 0.99. LLOQ defined by allowable precision of 20% and signal-to-noise ratio of >10:1, from samples performed over five days with one run per day (n = 25). Total precision and repeatability of samples performed over five occasions with one run per day (n = 25). Central for Drugs Control Hormone Standardization Program (CDC HoSt) mean bias determined from assigned values. EQA LC-MS mean bias determined from LC-MS method means of UK NEQAS Estradiol Programme.

Note: To convert SI units to conventional mass units divide by 3.671 for E2 (pmol/L to pg/mL) and 3.699 for E1 (pmol/L to pg/mL).

Conclusion

The Waters ACQUITY UPLC I-Class with Xevo TQ-XS IVD System has demonstrated the capability to deliver analytical sensitivity, precision, and accuracy for the analysis of 17β-estradiol and estrone in serum.

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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQ-XS IVD System

MassLynx (IVD) Mass Spectrometry Software with TargetLynx (IVD) <
https://www.waters.com/waters/nav.htm?cid=134834177>

720006679, November 2019

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Steroid Prohormones

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 25-hydroxyvitamin D2 (25OHD2) and 25-hydroxyvitamin D3 (25OHD3) in serum.

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by spiking commercially available reference material in stripped serum and the samples were processed using the following conditions:

Sample Preparation Conditions

150 µL sample was processed with zinc sulphate, methanol, and centrifuged. Samples were loaded onto Oasis HLB µElution plates, washed, and eluted prior to analysis.

LC Conditions

Column:	ACQUITY UPLC BEH Phenyl (IVD) 1.7 µm, 2.1 mm × 50 mm
Pre-column:	ACQUITY UPLC Column In-Line Filter
Mobile phase A:	2 mM ammonium acetate + 0.1% formic acid in water
Mobile phase B:	2 mM ammonium acetate + 0.1% formic acid in methanol
Flow rate:	0.45 mL/min
Gradient:	65–80% B over 2.5 minutes, 80% B over 0.2 minutes, 98% B for 0.8 minutes

MS Conditions

Resolution:	MS1 (0.7 FWHM), MS2 (0.85 FWHM)
Acquisition mode:	MRM
Polarity:	ESI+

Results and Discussion

Performance characteristics of 25OHD2 and 25OHD3 are shown in Table 1. Chromatographic separation of 25OHD2 and 25OHD3 on the ACQUITY UPLC I-Class/Xevo TQD IVD System is illustrated in Figure 1.

Compound	Range (nmol/L)	LLOQ (nmol)	Total precision	Repeatability	DEQAS mean bias
25OHD2	10–375	3.0	≤7.3%	≤6.5%	N/A
25OHD3	10–375	5.6	≤6.6%	≤6.0%	2.1

Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where r^2>0.99. LLOQ defined by allowable precision and bias of 15% and 10% respectively, from samples performed over three days with two runs per day (n=90). Total Precision and Repeatability of samples performed over 20 occasions with two runs per day (n=80). Vitamin D External Quality Assessment Scheme (DEQAS) Mean Bias determined from NIST assigned DEQAS values.

Note: To convert SI units to conventional mass units divide by 2.423 for 25OHD2 (nmol/L to ng/mL) and 2.496 for 25OHD3 (nmol/L to ng/mL).

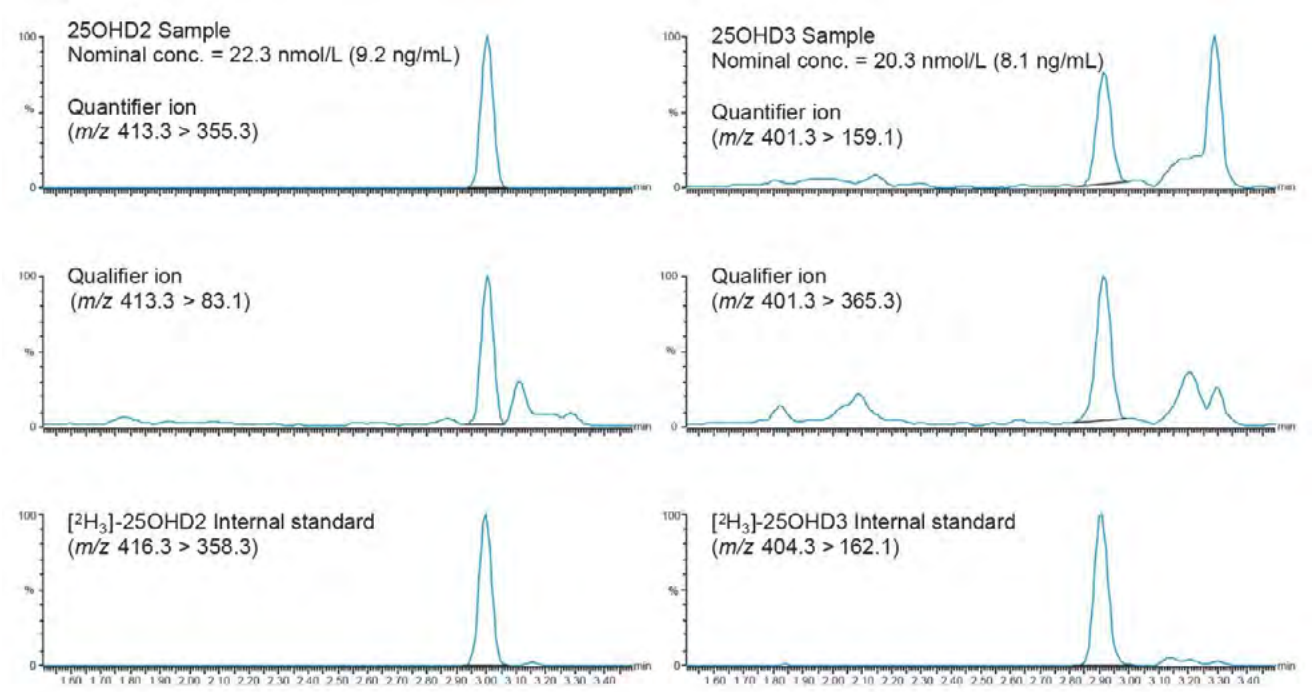

Figure 1. Chromatographic separation of 25OHD2 and 25OHD3 in a low level sample using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to quantify 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in serum with precision and accuracy.

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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

MassLynx (IVD) Mass Spectrometry Software with TargetLynx (IVD) <
https://www.waters.com/waters/nav.htm?cid=134834177>

720006356, August 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Progestogens and Androgens

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 testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP), and dehydroepiandrosterone sulfate (DHEAS) in serum.

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by spiking commercially available reference material in stripped serum and the samples were processed using the following conditions:

Sample Preparation Conditions

100 µL sample was precipitated with methanol, diluted with water, and centrifuged. Samples were loaded onto Oasis PRiME HLB µElution plates, washed, and eluted prior to analysis.

LC Conditions

Column:	ACQUITY UPLC HSS T3 (IVD) 1.8 µm, 2.1 mm × 50 mm
Pre-column:	VanGuard HSS T3 1.8 µm, 2.1 mm × 5 mm
Mobile phase A:	2 mM ammonium acetate + 0.1% formic acid in water
Mobile phase B:	2 mM ammonium acetate + 0.1% formic acid in methanol
Flow rate:	0.6 mL/min
Gradient:	45% B over one minute, 45–65% B over 2.5 minutes, 98% B for 0.5 minutes

MS Conditions

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

Results and Discussion

Performance characteristics of the steroid hormones on the Waters ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. Analytical sensitivity of the system for analyzing the steroid hormones in plasma is illustrated in Figure 1.

Compound	Range (nmol/L)	LLOQ (nmol)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Testosterone	0.17–69	0.17	12.0%	≤4.7%	≤3.6%	-0.5%
Androstenedione	0.17–69	0.17	9.1%	≤6.3%	≤5.2%	0.4%
17-OHP	0.76–303	0.76	9.2%	≤8.2%	≤8.2%	-5.0%
DHEAS	140–54000	140	7.0%	≤3.9%	≤2.7%	5.8%

Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD <20%. % RSD at LLOQ determined through analytical sensitivity experiments performed over three occasions (n=30). Total Precision and Repeatability of QCs performed over five occasions in stripped serum (n=30). EQA Mean Bias determined through Altman-Bland comparison of calculated concentrations to EQA mass spectrometry mean values.

Note: To convert SI units to conventional mass units divide by 3.470 for testosterone (nmol/L to ng/mL), 3.494 for androstenedione (nmol/L to ng/mL), 3.028 for 17-OHP (nmol/L to ng/mL) and 2.716 for DHEAS (nmol/L to ng/mL).

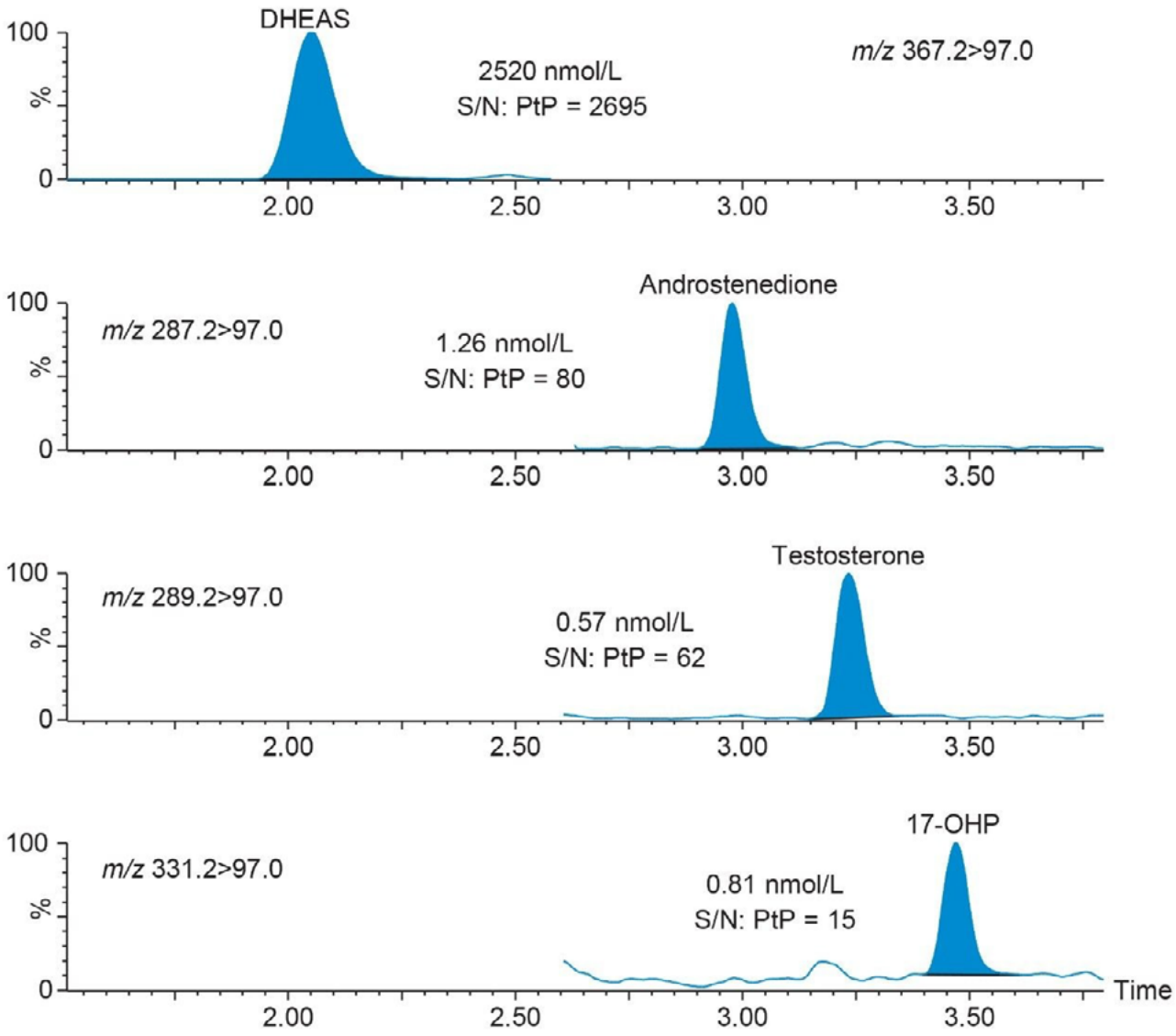

Figure 1. Low steroid hormone concentrations in serum containing DHEAS, androstenedione, testosterone, and 17-OHP.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver analytically sensitive, selective performance with excellent precision and accuracy for testosterone, androstenedione, 17-OHP, and DHEAS in serum.

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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

MassLynx (IVD) Mass Spectrometry Software with TargetLynx (IVD) <
https://www.waters.com/waters/nav.htm?cid=134834177>

720006355, August 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System: Analytical Performance for Progestogens and Androgens

Waters Corporation

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

Introduction

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro 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 I-Class/Xevo TQ-S micro IVD System for the analysis of dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), testosterone, androstenedione, 17-hydroxyprogesterone, and progesterone in serum.

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by spiking commercially available reference material in stripped serum and the samples were processed using the following conditions:

Sample Description

Sample preparation conditions 100 µL sample was processed with methanol, diluted with water, and centrifuged. Samples were loaded on Oasis MAX µElution plates, washed, and eluted prior to analysis.

LC Conditions

Column:	CORTECS UPLC C_{18} 1.6 µm, 2.1 mm × 50 mm
Mobile phase A:	0.05 mM Ammonium fluoride in water
Mobile phase B:	Methanol
Flow Rate:	0.25 mL/min
Gradient:	40% B over 0.5 minutes, 40–70% B over 3.5 minutes, 95% B for 0.5 minutes

MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.5 FWHM)
Acquisition mode:	MRM
Polarity:	ESI+

Results and Discussion

Performance characteristics of the steroid hormones on the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System are shown in Table 1. Analytical sensitivity of the system for analysing extracted steroid hormone samples is illustrated in Figure 1.

Compound	Range (nmol/L)	LLOQ (nmol/L)	%RSD at LLOQ	Total precision	Repeatability	Mean bias
DHT	0.086–34	0.086	13%	≤6.5%	≤6.4%	4.9%
DHEA	1.0–69	0.35	11%	≤4.7%	≤4.4%	-
Testosterone	0.017–69	0.017	11%	≤5.3%	≤2.9%	-1.4%
Androstenedione	0.087–349	0.035	18%	≤5.4%	≤3.7%	0.2%
17-OHP	0.076–303	0.030	17%	≤4.4%	≤4.0%	-5.6%
Progesterone	0.064–64	0.016	15%	≤4.5%	≤4.1%	-

Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. %RSD at LLOQ determined through analytical sensitivity experiments performed over five occasions (n=40). Total precision and repeatability of QCs performed over five occasions (n=25). Mean bias determined through Bland-Altman comparison of calculated concentrations to RCPA QAP target values for DHT and EQA mass spectrometry mean values for testosterone, androstenedione, and 17-OHP.

Note: To convert SI units to conventional mass units divide by 3.45 for DHT (nmol/L to ng/mL), 3.47 for DHEA (nmol/L to ng/mL), 3.47 for testosterone (nmol/L to ng/mL), 3.49 for androstenedione (nmol/L to ng/mL), 3.03 for 17-OHP (nmol/L to ng/mL), and 3.18 for progesterone (nmol/L to ng/mL).

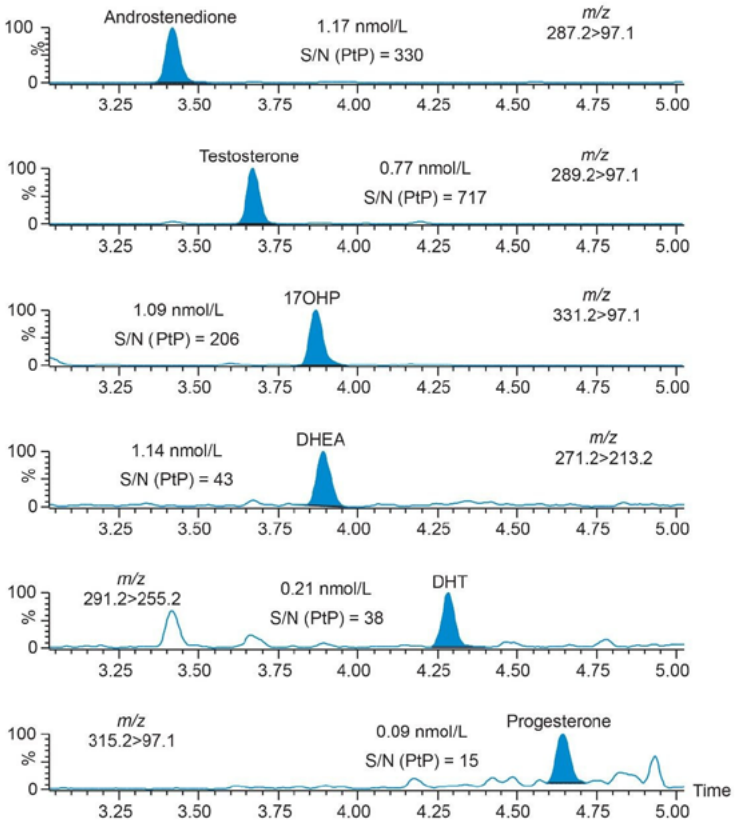

Figure 1. Performance characteristics of low concentration serum steroid hormone samples using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Conclusion

The ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System has demonstrated the capability to deliver analytically sensitive and selective performance with excellent precision and accuracy for DHT, DHEA, testosterone, androstenedione, 17-hydroxyprogesterone, and progesterone in serum.

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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQ-S micro IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories < https://www.waters.com/waters/en_US/Mass-spectrometry-software-for-easy-LC-MS-analysis-in-clinical-laboratories/nav.htm?locale=en_US&cid=134834177>

720006324, August 2021

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System: Analytical Performance for Androgens, Progestogens and Glucocorticoids

Waters Corporation

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

Introduction

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro 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 TQ-S micro IVD System for the analysis of testosterone, androstenedione, 17-hydroxyprogesterone, dehydroepiandrosterone sulfate, cortisol, 11-deoxycortisol, and 21-deoxycortisol in serum.

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by spiking commercially available reference material in stripped serum and the samples were processed using the following conditions:

Sample Description

100 µL sample was precipitated with methanol, diluted with water, and centrifuged. Samples were loaded on Oasis PRiME HLB µElution plates, washed, and eluted prior to analysis.

LC Conditions

Column:	ACQUITY UPLC HSS T3 (IVD) 1.8 µm, 2.1 × 50 mm
Pre-column:	VanGuard HSS T3 1.8 µm, 2.1 × 5 mm
Mobile phase A:	2 mM Ammonium acetate +0.1% formic acid in water
Mobile phase B:	2 mM Ammonium acetate +0.1% formic acid in methanol
Flow rate:	0.6 mL/min
Gradient:	45% B over one minute, 45–65% B over 2.5 minutes, 98% B for 0.5 minutes

MS Conditions

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

Results and Discussion

Analytical selectivity of the chromatographic separation is illustrated in Figure 1. Performance characteristics of the steroid hormones on the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System are shown in Table 1.

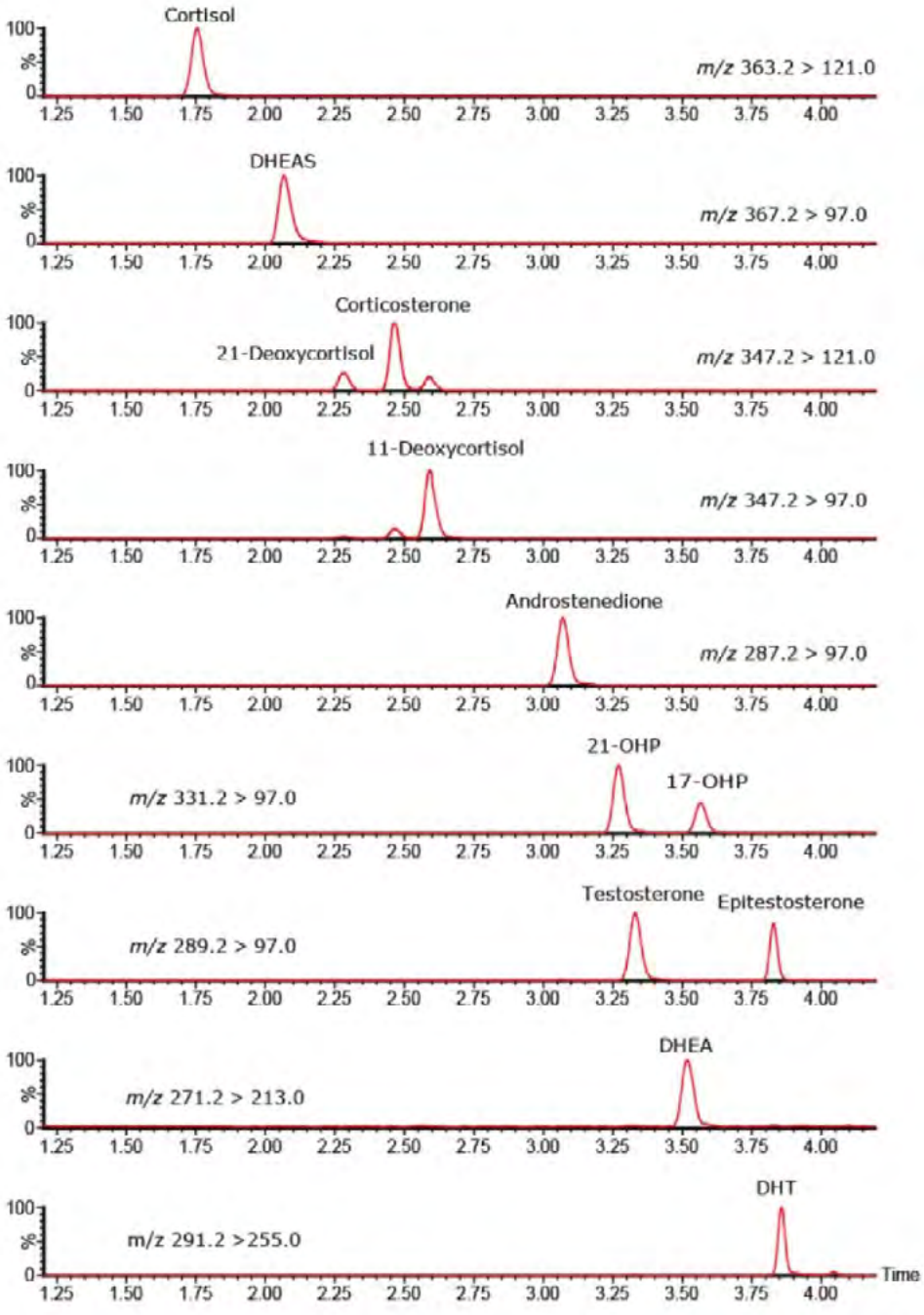

Figure 1. Chromatographic selectivity of a range of steroids using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Compound	Range (nmol/L)	LLOQ (nmol)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Testosterone	0.1–69	0.10	4.9%	≤6.3%	≤3.2%	-0.1%
Androstenedione	0.09–349	0.09	9.1%	≤6.0%	≤4.0%	-5.1%
17-OHP	0.19–757	0.19	8.2%	≤5.3%	≤3.4%	5.2%
DHEAS	65–43000	65	4.0%	≤7.6%	≤3.9%	-5.8%
Cortisol	0.69–1380	0.69	13.2%	≤7.3%	≤7.3%	1.0%
11-Deoxycortisol	0.72–144	0.72	14.1%	≤5.7%	≤3.5%	-
21-Deoxycortisol	0.72–144	0.72	9.9%	≤6.9%	≤5.2%	-

Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where r2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD <20%. %RSD at LLOQ determined through analytical sensitivity experiments performed over three occasions (n=30). Total precision and repeatability of QCs performed over five occasions in stripped serum (n=25). EQA mean bias determined through Altman-Bland comparison of calculated concentrations to EQA mass spectrometry mean values.

Note: To convert SI units to conventional mass units divide by 3.470 for testosterone (nmol/L to ng/mL), 3.494 for androstenedione (nmol/L to ng/mL), 3.028 for 17-OHP (nmol/L to ng/mL), 2.716 for DHEAS (nmol/L to ng/mL), 2.761 for cortisol (nmol/L to ng/mL), and 2.889 for 11-deoxycortisol and 21-deoxycortisol (nmol/L to ng/mL).

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System has demonstrated the capability to deliver analytically sensitive, selective performance with excellent precision, and accuracy for testosterone, androstenedione, 17-hydroxyprogesterone, dehydroepiandrosterone sulfate, cortisol, 11-deoxycortisol, and 21-deoxycortisol in serum.

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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQ-S micro IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories < https://www.waters.com/waters/en_US/Mass-spectrometry-software-for-easy-LC-MS-analysis-in-clinical-laboratories/nav.htm?locale=en_US&cid=134834177>

720006266, August 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System: Analytical Performance for a Mineralocorticoid

Waters Corporation

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

Introduction

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro 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 TQ-S micro IVD System for the analysis of aldosterone in plasma.

ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by spiking commercially available reference material in stripped serum and the samples were processed using the following conditions:

Sample Preparation Conditions

200 µL sample was processed with $ZnSO_4$/methanol, diluted, and centrifuged. Samples were loaded onto Oasis MAX µElution plates, washed, and eluted prior to analysis.

LC Conditions

Column:	CORTECS UPLC C_{18} 1.6 µm, 2.1 mm × 100 mm
Mobile phase A:	Water
Mobile phase B:	Methanol
Flow rate:	0.4 mL/min
Gradient:	40% B over one minute, 40–60% B over one minute, 60% for 0.3 minutes, 95% B for 0.5 minutes

MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.5 FWHM)
Acquisition mode:	MRM
Polarity:	ESI (-)

Results and Discussion

Performance characteristics of aldosterone using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System is shown in Table 1. Analytical sensitivity of the system for analyzing extracted aldosterone plasma samples is illustrated in Figure 1.

Compound	Range (pmol/L)	LLOQ (pmol/L)	S/N at LLOQ	Total precision	Repeatability
Aldosterone	42–4161	42	37	≤7.2%	≤7.0%

Table 1. Performance characteristics of aldosterone. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. S/N at LLOQ determined using the mean S/N (PtP) at the low calibrator over five occasions. Total precision and repeatability of QCs performed over five occasions in plasma (n=25).
Note: To convert SI units to conventional mass units divide by 2.774 for aldosterone (pmol/L to pg/mL).

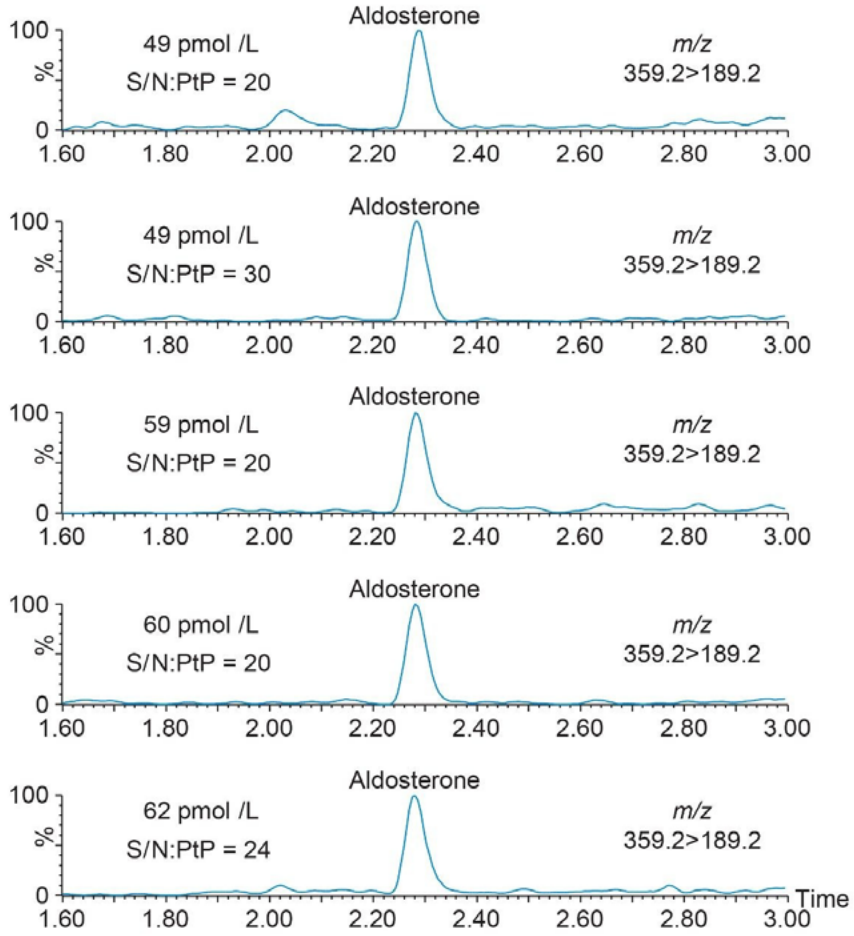

Figure 1. Performance characteristics of extracted plasma aldosterone samples using the ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro IVD System has demonstrated the capability to deliver analytical sensitivity and precision for the analysis of aldosterone 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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQ-S micro IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories <
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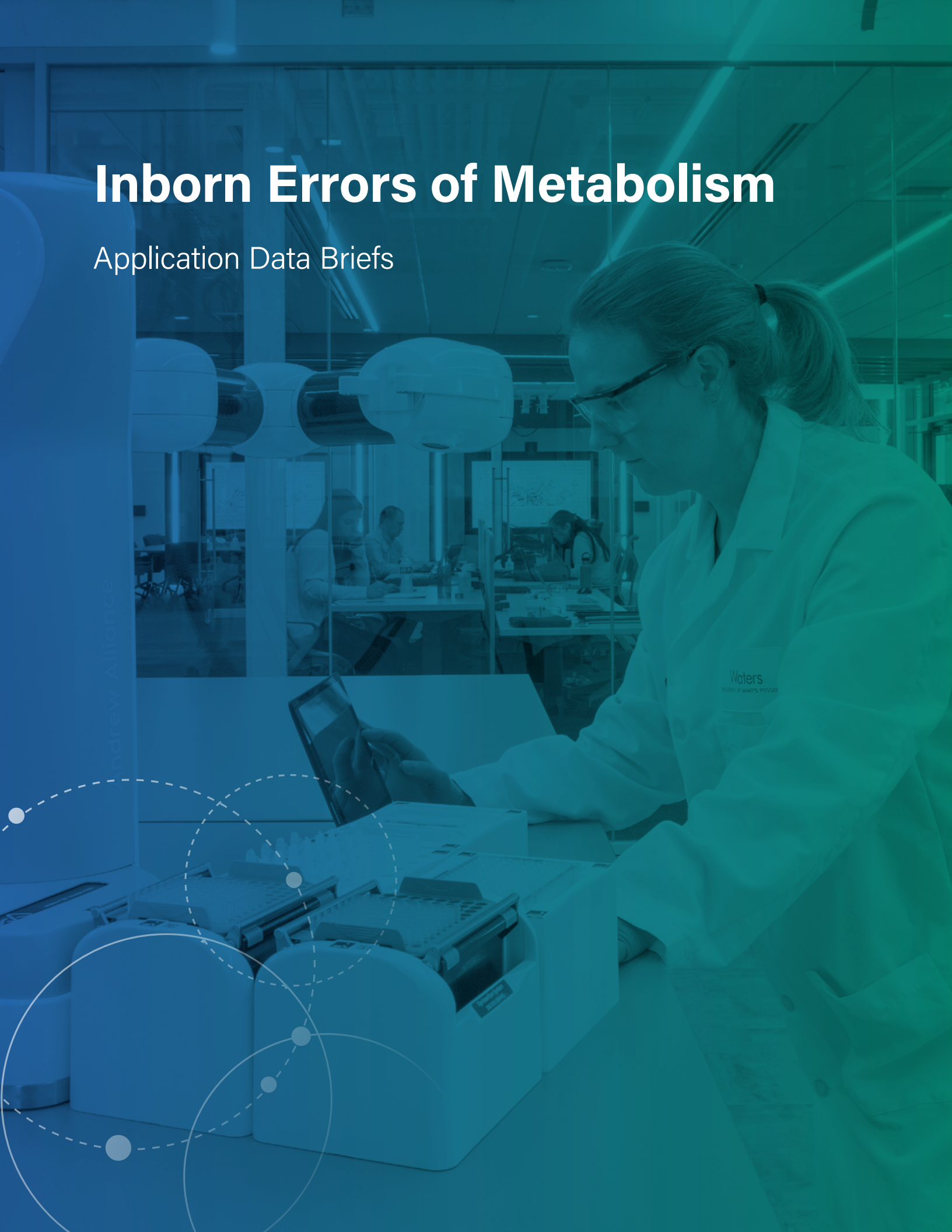
720006312, August 2018

Inborn Errors of Metabolism

Application Data Briefs



Waters™

Application Note

RenataDX Screening System: Analytical Performance for Butyl Esters of Amino Acids, Free Carnitine, and Acylcarnitines in Dried Blood Spots

Waters Corporation

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

Introduction

The Waters RenataDX Screening System enables flowinjection analysis and quantification of organic compounds in biological matrices.

This document describes a test of the analytical performance of the RenataDX Screening System for the analysis of butyl esters of amino acids, free carnitine, and acylcarnitines in dried blood spots.

RenataDX Screening System.

Experimental

Dried blood spot (DBS) control samples were extracted. Derivatized extracts from DBS control samples were analyzed with the RenataDX Screening System, under the control of MassLynx IVD Software (v4.2), with data processed using IonLynx Application Manager.

Sample Description

A single 3-mm diameter DBS punch was incubated in a methanol-based internal standard solution. After the incubation period, the samples were transferred to a new 96-well microtitre plate for derivatization with n-butanol HCl. Sample residue was reconstituted in mobile phase.

Flow-injection analysis conditions

System tubing:	~1 meter PEEK (0.005" ID) with post injection valve inline filter (2 µm pore size)

Mobile phase A:	80% Acetonitrile$_{(aq)}$ with 0.05% (v/v) formic acid
3777C wash 1:	20% Methanol$_{(aq)}$
3777C wash 2:	80% Acetonitrile$_{(aq)}$ with 0.05% (v/v) formic acid
Flow rate:	Variable flow rate from 150 µL/min to 15 µL/min, with 500 µL/min flush

MS Conditions

Resolution:	MS1 (0.70 FWHM), MS2 (0.70 FWHM)
Acquisition mode:	MRM
Polarity:	ESI+

Results and Discussion

The imprecision of extraction and analysis of amino acids and acylcarnitines is illustrated in Tables 1 and 2. The Peak-to-Peak (PtP) Signal-to-Noise ratio (S/N) is shown, as an indication of the analytical sensitivity of the system.

Compound	Endogenous			QC1			QC2		
	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)
Glycine	187	4.1	57.5	435	10.4	5070	824	5.8	864
Alanine	195	6.2	411	695	9.3	10700	856	6.5	1800
Proline	N/D	N/A	N/A	269	9.7	1880	614	6.4	756
Valine	70.8	6.5	113	279	9.5	672	523	6.5	1232
Leucine	67.4	6.6	479	299	10.0	7260	534	6.8	2000
Phenylalanine	30.0	7.2	384	152	9.1	6400	508	8.0	1860
Citrulline	10.8	6.3	609	57.5	8.5	30.2	272	6.0	3300
Tyrosine	145	2.6	116	291	5.6	1140	626	4.7	3290
Methionine	10.9	7.2	68.6	58.3	12.0	165	187	7.8	1170
Arginine	4.30	13.0	34.8	24.7	4.1	557	44.1	1.2	852

Table 1. Performance characteristics of the amino acid analytes. Between-batch imprecision experiments were performed over five occasions (n=25); µM in whole blood, accounting for the dilution of the DBS material into the extraction solution; endogenous=DBS from a single donor; QC1 and 2 of commercial origin; N/D=not detected; N/A=not applicable.

Compound	Endogenous			QC1			QC2		
	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)
Free carnitine (C0)	11.8	6.4	281	39.5	10.1	862	117	8.5	1150
Acetylcarntine (C2)	9.5	5.7	197	24.1	9.3	2410	70.1	5.5	1870
Propionylcarnitine (C3)	0.77	7.2	78.7	4.43	9.9	796	14.3	7.2	674
Hydroxyvalerylcarnitine (C4OH)	0.04	16.4	2.49	N/S	N/A	N/A	N/S	N/A	N/A
Butyrylcarnitine (C4)	0.07	13.9	20.3	0.77	7.3	116	4.01	5.3	207
Isovalerylcarnitine (C5)	0.06	9.3	8.61	0.40	8.9	55.3	2.01	7.1	637
Glutarylcarnitine (C5DC)	N/D	N/A	N/A	0.43	10.5	17.4	2.30	6.5	143
Hydroxyisovalerylcarnitine (C5OH)	0.32	7.7	38.7	N/S	N/A	N/A	N/S	N/A	N/A
Hexanoylcarnitine (C6)	0.09	9.8	7.67	0.44	7.5	52.7	2.09	6.4	91.6
Octanoylcarnitine (C8)	N/D	N/A	N/A	0.39	12.8	80.4	2.28	8.5	428
Decanoylcarnitine (C10)	N/D	N/A	N/A	0.26	14.8	106	1.46	12.1	217
Dodecanoylcarnitine (C12)	N/D	N/A	N/A	0.39	13.7	52.2	2.29	13.5	668
Tetradecanoylcarnitine (C14)	N/D	N/A	N/A	0.41	11.7	164	2.23	11.4	342
Palmitoylcarnitine (C16)	0.70	9.3	160	4.17	11.3	394	14.1	8.8	2510
Octadecanoylcarnitine (C18)	0.55	8.6	79.4	2.46	9.0	617	10.4	8.1	516

Table 2. Performance characteristics of the free carnitine and acylcarnitine analytes. Between-batch imprecision experiments were performed over five occasions (n=25); µM in whole blood, accounting for the dilution of the DBS material into the extraction solution; endogenous=DBS from a single donor; QC1 and 2 of commercial origin; N/S=not supplemented; N/A=not applicable; N/D=not detected, i.e. imprecision ≥20%CV ± S/N (PtP)≤3.

Conclusion

The Waters RenataDX Screening System has demonstrated the capability to measure a subset of butyl esters of amino acids, free carnitine, and acylcarnitines. The endogenous concentration of arginine was near the limit of detection of the RenataDX Screening System.

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 flow-injection analysis 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.

Featured Products

RenataDX Screening System <https://www.waters.com/waters/nav.htm?cid=134986073>

720006348, July 2021

Waters™

Application Note

RenataDx Screening System: Analytical Performance for Amino Acids, Free Carnitines and Acylcarnitines in Dried Blood Spots

Waters Corporation

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

Introduction

The Waters RenataDX Screening System enables flowinjection analysis and quantification of organic compounds in biological matrices.

This document describes a test of the analytical performance of the RenataDX Screening System for the analysis of amino acids, free carnitines, and acylcarnitines in dried blood spots.

RenataDX Screening System.

Experimental

Extracted dried blood spot (DBS) control samples were analyzed with the RenataDX Screening System, under the control of MassLynx IVD Software (v4.2), with data processed using IonLynx Application Manager.

Sample Description

A single 3-mm diameter DBS punch was incubated in a methanol-based internal standard solution. After the incubation period, the samples were transferred from the extraction plate to a clean 96-well microtitre plate.

Flow-Injection Analysis Conditions

System tubing:	~1 meter PEEK (0.005" ID) with post injection valve inline filter (2 µm pore size)
Mobile phase A:	80% Acetonitrile$_{(aq)}$ with 0.05% (v/v) formic acid
3777C wash 1:	20% Methanol$_{(aq)}$
3777C wash 2:	80% Acetonitrile$_{(aq)}$ with 0.05% (v/v) formic acid
Flow rate:	Variable flow rate from 150 µL/min to 15 µL/min, with 500 µL/min flush

MS Conditions

Resolution:	MS1 (0.70 FWHM), MS2 (0.70 FWHM)
Acquisition mode:	MRM
Polarity:	ESI+

Results and Discussion

The imprecision of extraction and analysis of amino acids and acylcarnitines is illustrated in Tables 1 and 2. The Peak-to-Peak (PtP) Signal-to-Noise ratio (S/N) is shown, as an indication of the analytical sensitivity of the system.

Compound	Endogenous			QC1			QC2		
	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)
Glycine	218	13.7	18.5	605	11.3	5.19	1030	10.0	56.7
Alanine	229	10.2	101	851	9.72	652	928	7.08	156
Proline	81.1	11.7	71.1	314	9.07	178	669	7.24	200
Valine	54.2	9.92	157	239	9.36	593	405	7.60	468
Leucine	88.2	9.52	179	384	10.1	618	564	6.98	1232
Phenylalanine	33.4	9.95	106	179	9.47	798	506	6.54	1789
Citrulline	N/D	N/A	N/A	58.0	17.0	11.3	264	10.9	44.6
Tyrosine	34.5	9.40	19.9	191	9.00	269	513	7.70	671
Methionine	10.5	10.2	28.0	77.9	8.53	94.4	244	7.57	151
Arginine	N/D	N/A	N/A	34.9	10.4	21.6	123	7.24	103

Table 1. Performance characteristics of the amino acid analytes. Between-batch imprecision experiments were performed on five occasions (n=25); µM in whole blood, accounting for the dilution of the DBS material into the extraction solution; endogenous=DBS control from a single donor; QC1 and 2 of commercial origin; N/D=not detected i.e. imprecision ≥20%CV ± S/N (PtP)≤3; N/A=not applicable.

Compound	Endogenous			QC1			QC2		
	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)
Free carnitine (C0)	10.9	10.9	79.8	38.9	10.7	199	99.9	7.91	756
Acetylcarntine (C2)	6.93	9.63	98.1	17.7	9.42	343	52.4	6.94	890
Propionylcarnitine (C3)	0.66	9.50	70.2	3.99	10.5	296	11.5	6.77	2376
Malonyl / Hydroxyvalerylcarnitine (C3DC/C4OH)	0.04	16.6	15.7	N/S	N/A	N/A	N/S	N/A	N/A
Butyrylcarnitine (C4)	0.07	11.8	14.3	0.72	8.91	106	3.32	6.22	867
Isovalerylcarnitine (C5)	0.04	11.6	8.44	0.40	11.5	63.9	1.81	8.31	106
Glutarylcarnitine (C5DC)	N/D	N/A	N/A	0.48	13.4	12.3	2.12	14.6	34.8
Methylmalonyl / Hydroxyisovalerylcarnitine (C4DC/C5OH)	0.37	9.72	106	0.15	10.1	21.7	0.22	8.51	49.4
Hexanoylcarnitine (C6)	N/D	N/A	N/A	0.41	11.7	176	2.07	7.52	407
Octanoylcarnitine (C8)	N/D	N/A	N/A	0.44	12.8	86.7	2.23	9.48	268
Decanoylcarnitine (C10)	N/D	N/A	N/A	0.40	15.7	386	2.00	11.9	654
Dodecanoylcarnitine (C12)	N/D	N/A	N/A	0.40	15.5	249	2.04	11.0	504
Tetradecanoylcarnitine (C14)	0.03	18.6	9.40	0.45	13.1	413	2.13	9.63	1025
Palmitoylcarnitine (C16)	0.68	13.3	387	4.08	11.3	248	12.3	8.30	816
Octadecenoylcarnitine (C18)	0.49	12.1	78.3	2.21	9.24	131	8.15	6.98	427

Table 2. Performance characteristics of the free carnitine and acylcarnitine analytes. Between-batch imprecision experiments were performed over five occasions (n=25); µM in whole blood, accounting for the dilution of the DBS material into the extraction solution; endogenous=DBS from a single donor; QC1 and 2 of commercial origin; N/S=not supplemented; N/A=not applicable; N/D=not detected, i.e. imprecision ≥20%CV ± S/N (PtP)≤3.

Conclusion

The Waters RenataDX Screening System has demonstrated the capability to measure a subset of amino acids, free carnitines, and acylcarnitines. The endogenous concentration of some analytes was at the limit of detection of the RenataDX System.

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 flow-injection analysis 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.

Featured Products

RenataDX Screening System <https://www.waters.com/waters/nav.htm?cid=134986073>

720006349, July 2021

Therapeutic Drug Monitoring

Application Data Briefs

Waters™

Application Note

Metrological Traceability of the Waters™ MassTrak™ Immunosuppressant Calibrator and Quality Control Sets

Gareth Hammond, Niamh Stafford, Norma Breen, Stephen Balloch, Lisa J. Calton

Waters Corporation

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

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

Abstract

Within clinical laboratories, there is a requirement to follow local regulatory guidelines and international standards when developing and validating laboratory developed tests (LDTs). Furthermore, in some geographies, the implementation of ISO 15189:2012 *Medical laboratories -- Requirements for quality and competence* necessitates that laboratory use metrologically traceable calibration materials. Therefore, metrological traceability and accuracy of results are integral components of the design, development and manufacture of the Waters MassTrak Immunosuppressant Calibrator and Quality Control Sets (IVD), aiding laboratories in the harmonization of their results when using validated LC-MS/MS methods.

In this application brief, we provide an overview of the Waters MassTrak Immunosuppressant Calibrator and Quality Control Sets and their accuracy and comparisons across lot-to-lot testing.

Benefits

- Lyophilized calibrators and quality controls (QC), with established shelf-life and stability data, that reduce sample preparation time
- Confidence in the accuracy of immunosuppressants, measurement of uncertainty and lot-to-lot and provides a path to laboratory method harmonization

Introduction

Clinical laboratories adhere to local regulatory guidelines and international standards such as ISO 15189 that requires the laboratory to demonstrate quality and competence. The manufacture of calibration and QC materials to high standards and quality processes which are metrologically traceable provides a pathway towards laboratory harmonization with minimal variability across different manufacturing lots. LC-MS/MS methods in many clinical laboratories are based on LDTs, which can involve significant manual preparation in the pre-analytical workflow. This can include the preparation and characterization of in-house calibrator and QC materials by the laboratory technician, which may result in errors and inaccuracies of the materials, leading to reductions in lab efficiency.

The Waters MassTrak Immunosuppressant Calibrator and Quality Control Sets (IVD) (Figure 1) contain cyclosporine, everolimus, sirolimus, and tacrolimus in lyophilized human whole blood that have been sourced to obtain the highest level of metrological traceability available. There are no SI traceable reference materials or reference measurement procedures for these immunosuppressants. All immunosuppressant concentrations were confirmed with independent International Proficiency Testing (IPT) scheme samples and secondary reference materials in whole blood (sirolimus and tacrolimus only).

Figure 1. The Waters MassTrak Immunosuppressant Calibrator and Quality Control Sets.

Results and Discussion

Improvements in Laboratory Efficiency

The MassTrak Immunosuppressant Calibrator and Quality Control Sets have been designed and manufactured to increase efficiencies in the pre-analytical workflow by providing ready-to-use (following reconstitution) metrologically traceable calibrator and QC materials. The key benefits, highlighted in Table 1, include the significant time and resource savings made by eliminating the multiple steps a laboratory performs when preparing in-house calibrators. The lyophilized reagents, prepared using well-documented manufacturing and quality processes, demonstrate good lot-to-lot variability and longer shelf-life (up to three years) and in-use stability (up to 31 days) than traditional in-house reagents.

Preparation of in-house calibrators	MassTrak immunosuppressant calibrator set
Source solid materials	✓
Source matrix	✓
Gravimetric preparation of calibrators	✓
Stability evaluation	✓
Calibrator accuracy evaluation	✓
Lot-to-Lot evaluation	✓
Measurement of uncertainty	✓

Table 1. Improvements in Laboratory Efficiency.

Metrological Traceability and Accuracy

Pursuant to ISO 15189, there are no higher-order reference procedures or reference materials for the immunosuppressants. Therefore, to aid laboratory harmonization, the MassTrak Immunosuppressant Calibrator and Quality Control Sets are confirmed using secondary reference materials (results are shown in Table 2).

ERM summary: % bias from target		
Analyte/ Lot	3519	2517
Sirolimus (ERM-DA111)	-9.3	-4.1
Tacrolimus (ERM-DA110)	-1.3	-2.6

Table 2. Accuracy of ERM reference materials.

Lot-to-Lot Comparisons

The accuracy of the calibrator set was determined through evaluation of in-house QC and IPT samples (spanning the calibration range). Three lots of manufacturing material for the calibrators were evaluated for accuracy by measurement of IPTs using a routine LC-MS/MS measurement procedure. The mean %deviation from the IPT target values as measured by each lot of calibrator material was determined to demonstrate accuracy (to the harmonized IPT scheme). Results are shown in Figure 2 which not only demonstrates the accuracy of the calibrators but also the lot-to-lot reproducibility of the manufacturing process, which is important if laboratories seek to maintain their harmonization standards overtime using different manufacturing lots.

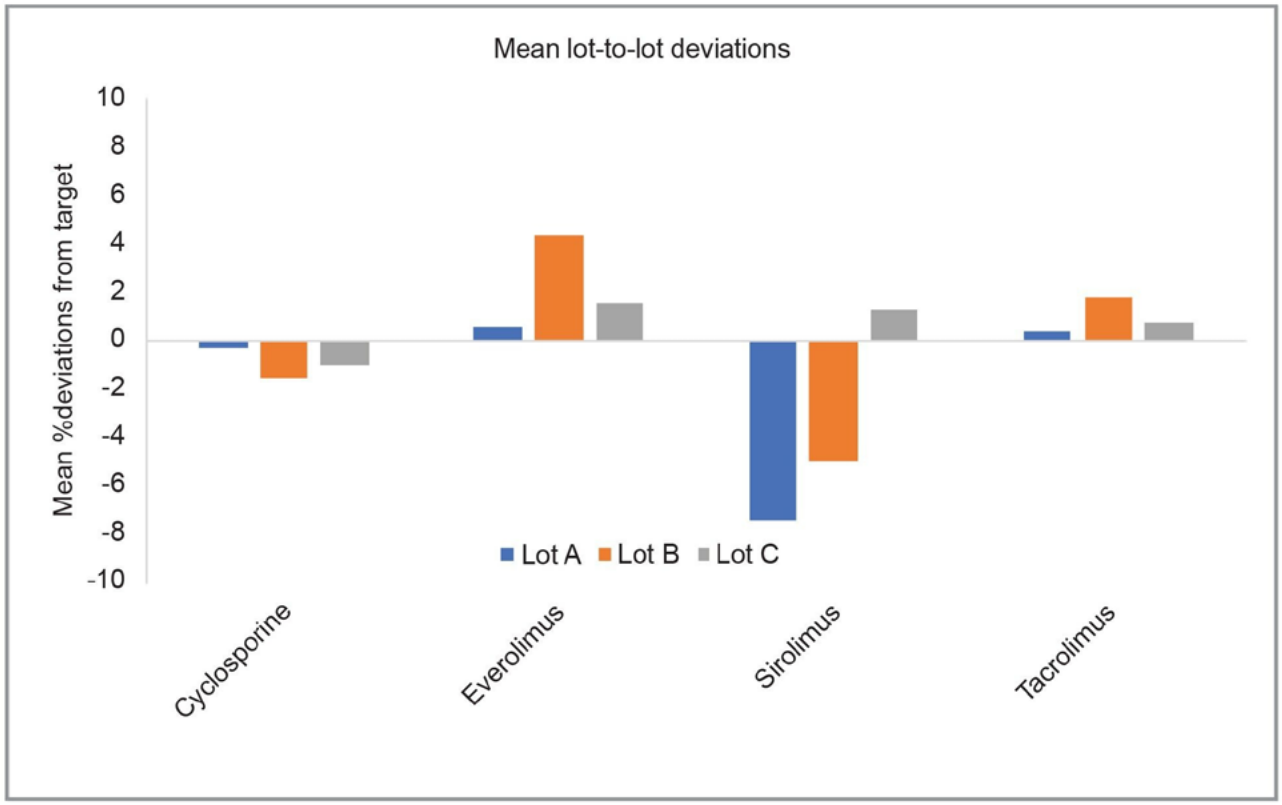


Figure 2. Lot-to-lot comparison of mean % deviations from IPT target values for manufacturing lots A-C for the immunosuppressants demonstrating the accuracy of the MassTrak Immunosuppressant Calibrator Set.

Conclusion

Metrological traceability of the MassTrak Immunosuppressant Calibrator and Quality Control Sets has been established aiding laboratories in their compliance to ISO 15189. The accuracy and lot-to-lot reproducibility have been confirmed through the use of independent international proficiency testing schemes. In addition, the laboratory workflow has been improved by removing time-consuming, low value tasks, which frees qualified staff for more important tasks.

Disclaimer

MassTrak Immunosuppressant Calibrator and Quality Control Sets are not available for sale in all countries. For information on availability, please contact your local sales representative.

Featured Products

MassTrak Immunosuppressant Calibrator, Quality Control, and Internal Standard Sets < /nextgen/lt/en/products/standards-and-reagents/masstrak-immunosuppressant-calibrator-quality-control-and-inter.html>

720007583, April 2022

Waters™

Application Note

Confidence in Your Calibrators: MassTrak™ Immunosuppressant Calibrator and Quality Control Sets for the LC-MS/MS Analysis of Cyclosporine, Everolimus, Sirolimus, and Tacrolimus

Stephen Balloch, Lisa J. Calton, Gareth Hammond

Waters Corporation

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

Abstract

The immunosuppressive drugs cyclosporine, everolimus, sirolimus and tacrolimus have historically been measured using immunoassay. However variable accuracy at low concentrations coupled with specificity issues due to cross-reactivity of antibodies with other components, such as metabolites, can cast doubt on results. This phenomenon is well documented in the literature. As such, many clinical laboratories increasingly analyse these drugs using liquid chromatography with tandem mass spectrometry (LC-MS/MS), for which they require reliable, reproducible calibrators, and quality control sets (QCs) for confidence in their results.

Waters™ MassTrak Immunosuppressant Calibrator and QC Sets (IVD) provide confidence in the accuracy and aid harmonization of results when using validated LC-MS/MS methods.

The MassTrak Immunosuppressant Calibrators and Quality Control Sets performance was demonstrated using the ACQUITY™ UPLC™ I-Class FL and Xevo™ TQ-S micro Triple Quadrupole Mass Spectrometer and an in-house developed LC-MS/MS methodology.

Benefits

- Guiding principles described in ISO17511 adhered to for value assignment
- Confidence in the accuracy of immunosuppressants and provides a path to laboratory method harmonization
- Lyophilized calibrators and QCs that reduce sample preparation time

Introduction

Routine analysis of immunosuppressant drugs in whole blood by immunoassay is highly automatable and affords analytical sensitivity, however issues relating to selectivity and multiplexing remain. As such, reliability of results, particularly at low concentrations can be a concern. Latterly, LC-MS/MS has become increasingly more-widely adopted for immunosuppressant analysis in order to overcome these known limitations.

LC-MS/MS methods in clinical laboratories are often based on laboratory developed tests (LDTs), validated to local regulatory guidelines. These guidelines are constantly evolving and there is increasing demand for all aspects of clinical methods to comply with these changing regulations. This includes the calibrator and QC materials used to generate and independently check the accuracy of the calibration within the method.

The Waters MassTrak Immunosuppressant Calibration and Quality Control Sets (IVD) (Figure 1) contains cyclosporine, everolimus, sirolimus, and tacrolimus in lyophilized whole blood that have been sourced to obtain the highest level of metrological traceability available. In order to demonstrate the quality of materials found in this product, we have shown the proof-of-concept performance of the materials using protein precipitation and separation and detection of the samples using the ACQUITY UPLC I-Class FL with Xevo TQ-S micro Triple Quadrupole Mass Spectrometer.

Figure 1. The Waters MassTrak Immunosuppressant Calibration and Quality Control sets.

Experimental

The Waters MassTrak Immunosuppressant Calibration and Quality Control Sets contain the following immunosuppressant drugs in lyophilized whole blood: cyclosporine, everolimus, sirolimus, and tacrolimus. Assigned concentrations for the calibration range and QCs are found in Table 1.

Immunosuppressant drug	Calibrator range (ng/mL)	Nominal QC concentrations (ng/mL)
Cyclosporine	25-1500	150, 400, 900
Everolimus	1-30	2, 8, 22
Sirolimus	1-30	2, 8, 22
Tacrolimus	1-30	2, 8, 22

Table 1. Concentration ranges of the MassTrak Immunosuppressant Calibrator and Quality Control Sets. The calibrators and QCs are reconstituted following the instructions for use (IFU), prior to sample preparation and analysis.

Sample Description:

Sample preparation was performed by using protein precipitation

Protein Precipitation:

To 50 µL of whole blood sample, 200 µL of 0.1 M aqueous zinc sulfate was added and mixed for five seconds. 500 µL of internal standard (ISTD) was added, followed by mixing for twenty seconds. Samples were then centrifuged for two minutes at 4696 g.

LC Conditions:

System:	ACQUITY UPLC I-Class with FL
Needle:	20 µL
Loop:	50 µL
Column:	ACQUITY UPLC HSS C_{18} SB Column; 1.8 µm, 2.1 x 30 mm (p/n: 186004117)

Column temp.:	55 °C
Sample temp.:	8 °C
Injection volume:	20 µL
Injection mode:	PLNO, with Load Ahead enabled
Mobile phase A:	Water + 2 mM ammonium acetate + 0.1% formic acid
Mobile phase B:	Methanol + 2 mM ammonium acetate + 0.1% formic acid
Weak wash:	Water:methanol 95:5 (v:v), 600 µL
Strong wash:	Water:methanol:acetonitrile:IPA 1:1:1:1 (v:v:v:v), 200 µL
Seal wash:	Water:methanol 80:20 (v:v)

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.45	50	50	Initial
0.2	0.45	50	50	1
0.6	0.45	0	100	6
1.2	0.80	50	50	11

MS Conditions

MS system:	Xevo TQ-S micro Triple Quadrupole Mass Spectrometer
Ionization mode:	ESI+
Capillary voltage:	0.8 kV

MRM Parameters

Analyte	MRM	ID	Cone (V)	Collision (V)
Cyclosporine	1219.9>1202.8	Quantifier	35	18
	1219.9>1184.8	Qualifier	35	34
	1231.9>1214.8	ISTD	35	18
Everolimus	975.6>908.6	Quantifier	35	16
	975.6>926.6	Qualifier	35	10
	981.6>914.6	ISTD	35	16
Sirolimus	931.6>864.6	Quantifier	35	16
	931.6>882.6	Qualifier	35	10
	809.6>756.6	ISTD	35	20
Tacrolimus	821.6>768.6	Quantifier	35	20
	821.6>786.6	Qualifier	35	16
	809.6>756.6	ISTD	35	20

Method Events

Time (min)	Event	Action
0	Flow state	Waste
0.6	Flow state	LC
1.4	Flow state	Waste

Results and Discussion

The four immunosuppressive drugs were chromatographed using the ACQUITY UPLC 2.1 mm x 30 mm HSS C_{18} SB Column.

Figure 2 shows an example chromatogram of calibrator 1 (25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus, and tacrolimus).

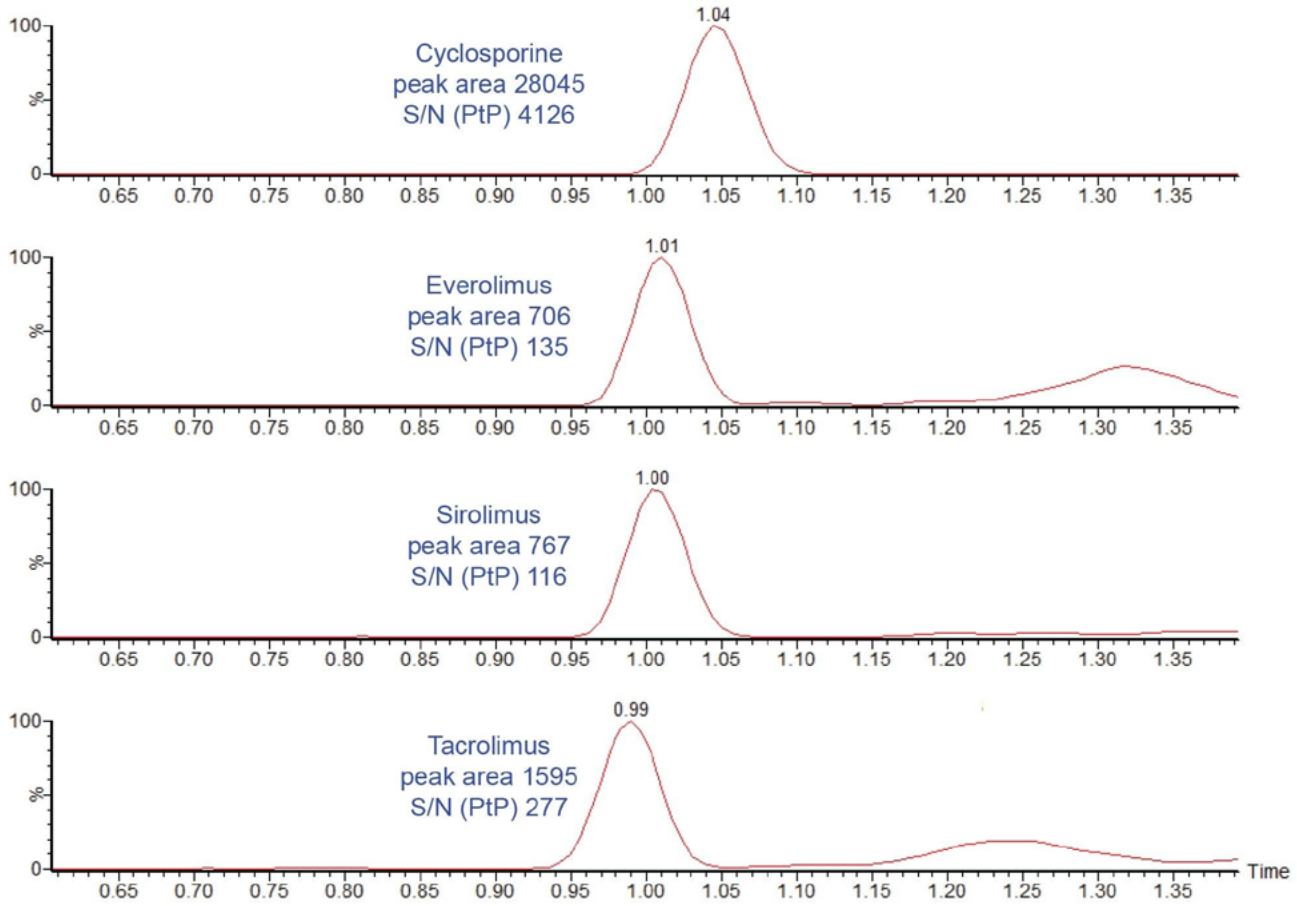


Figure 2. Performance characteristics of the extracted C1 calibrator from the MassTrak Immunosuppressant Calibrator Set analyzed using the ACQUITY UPLC I-Class/Xevo TQ-S micro System. Linearity of the calibration ranges was demonstrated with mean r^2 values for the calibration lines ≥0.9988 across the four immunosuppressant drugs. Analytical sensitivity of the method was determined through the signal:noise (S/N) evaluation of the low calibration (C1) standard for the immunosuppressant drugs. The S/N (PtP) was ≥10 at each of calibrator 1 concentrations across several analytical runs. This is summarized in Table 2 and an example of the S/N at the low calibrator can also be seen in Figure 2.

Analyte	Calibrator range (ng/mL)	Mean r^2	Mean S/N PtP at Cal 1
Cyclosporine	25.2–1474.7	0.9996	4525
Everolimus	1.1–31.8	0.9990	77
Sirolimus	1.0–26.5	0.9988	95
Tacrolimus	1.1–31.5	0.9994	149

Table 2. Summary of calibration linearity and analytical sensitivity performance of the immunosuppressant drugs in the MassTrak Immunosuppressant Calibrator Set.

Total precision and repeatability were determined by extracting and quantifying five replicates of three level QC materials per day over five separate days (n=25). Low, mid, and high concentrations were 154.6, 391.6, and 888.2 ng/mL for cyclosporine; 2.2, 8.4, and 22.6 ng/mL for everolimus; 1.9, 7.3, and 19.4 ng/mL for sirolimus and 2.2, 8.4, and 22.8 ng/mL for tacrolimus. Total precision and repeatability were determined to be ≤7.1% CV across all immunosuppressant drugs at the three QC concentrations (Figure 3).

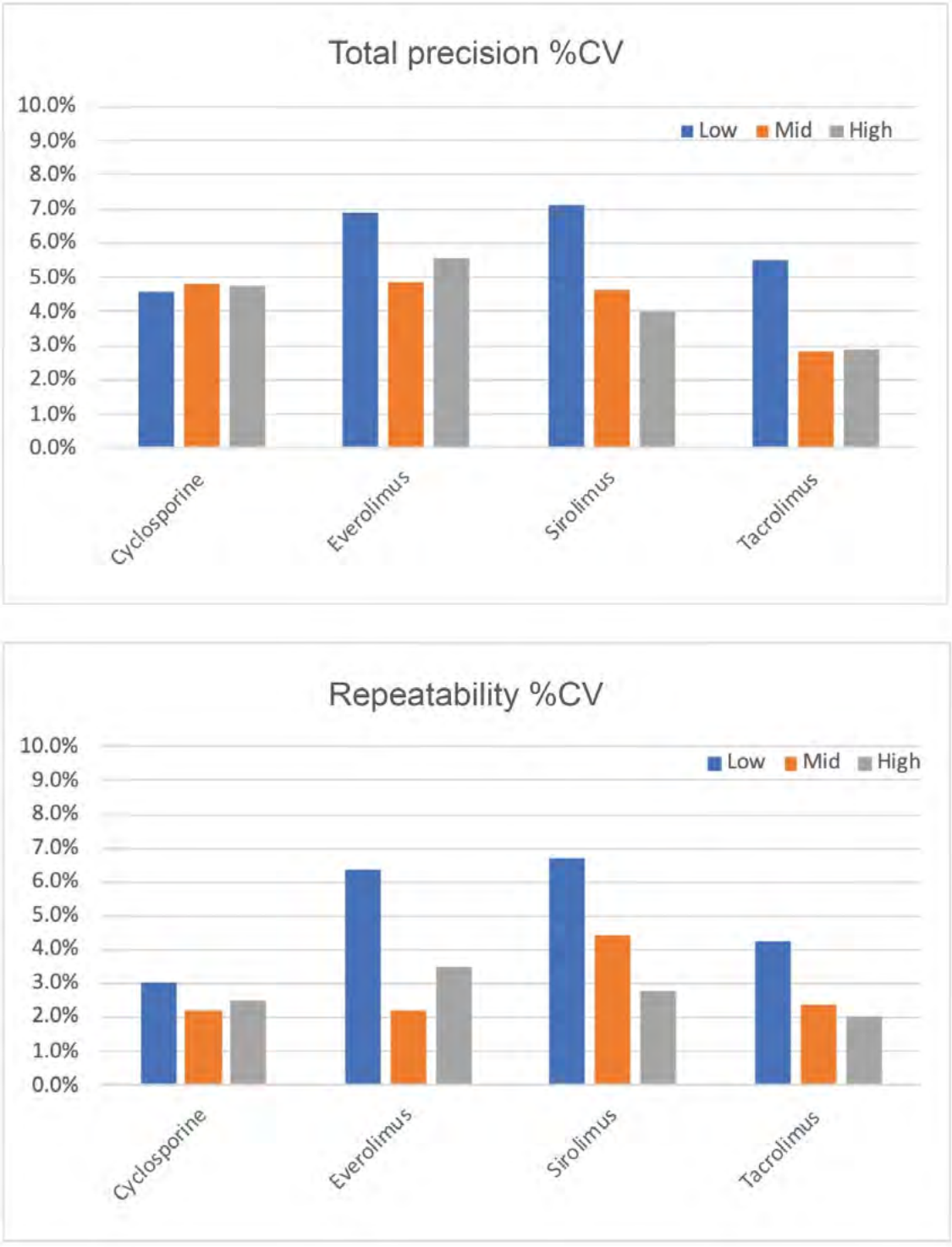


Figure 3. Total precision and repeatability for the analysis of the four immunosuppressant drugs in the MassTrak Immunosuppressant Quality Control Set.

In addition, the accuracy of the QCs was evaluated in comparison to the calibrators over the five analytical; runs. The mean accuracy for the QCs across the four immunosuppressant drugs ranged from 94.5–103.6% (Table 3).

	QC Accuracy		
Analyte	**Q1**	**Q2**	**Q3**
Cyclosporine	99.6%	101.0%	102.7%
Everolimus	100.0%	103.6%	103.1%
Sirolimus	100.0%	94.5%	95.4%
Tacrolimus	100.0%	101.2%	103.1%

Table 3. Accuracy of the Waters MassTrak Immunosuppressant Quality Control set analyzed in replicates of five at three concentrations over five analytical runs.

Accuracy was assessed for the four immunosuppressant drugs through the analysis of EQA samples from UK NEQAS. The data obtained was compared to the mass spectrometry method mean for the samples and Altman-Bland agreement was performed on the data sets. Altman-Bland agreement for cyclosporine, everolimus, sirolimus and tacrolimus provided a mean method bias within ±7.4%, demonstrating excellent agreement with the EQA method values for the immunosuppressant drugs (Figures 4a-d).

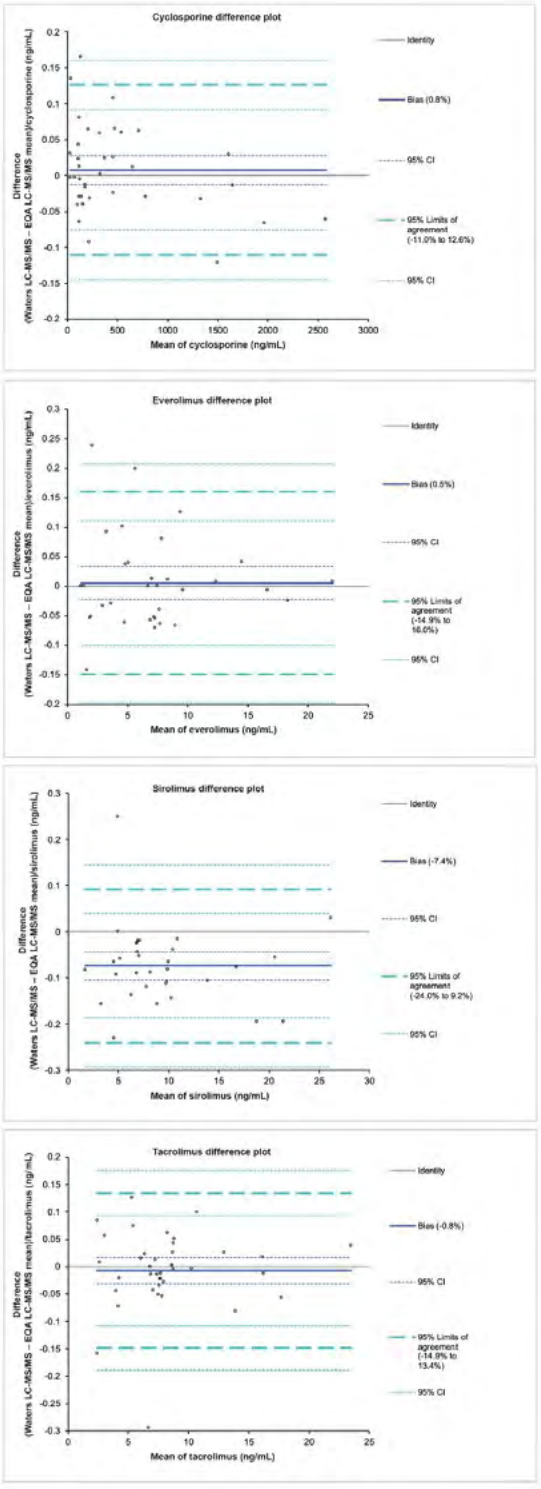


Figure 4. Altman-Bland agreement comparing the Waters LC-MS/MS method to the EQA scheme MS method mean for (a) cyclosporine (b) everolimus (c) sirolimus (d) tacrolimus.

Conclusion

Through this proof of performance evaluation, it has been demonstrated the MassTrak Immunosuppressant Calibrator and Quality Control Sets (IVD) can provide precise and accurate quantification of the four immunosuppressive drugs in whole blood.

The ACQUITY UPLC I-Class FL with Xevo TQ-S micro Triple Quadrupole Mass Spectrometer is able to provide sufficient analytical sensitivity to analyse lowest required concentrations using only 50 µL sample volume. Excellent levels of precision across the calibration range have been demonstrated with total precision and reproducibility ≤7.1% CV. In addition, the accuracy of the QC set was established with accuracies ranging from 94.5–103.6%. An indication of metrological traceability through agreement to EQA samples was also shown, with the method providing excellent agreement to EQA samples, with mean method bias ±7.4% compared to method mean values from the schemes.

Disclaimer

This method is an example of an application using the instrumentation, software and consumables described in this document. This method has not been cleared by any regulatory entity for diagnostic purposes. The end user is responsible for completion of the method development and validation. MassTrak Immunosuppressant Calibrator and Quality Control Sets are not available for sale in all countries. For information on availability, please contact your local sales representative.

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/134798856>

MassTrak Immunosuppressant Calibrator, Quality Control, and Internal Standard Sets </nextgen/si/en/products/standards-and-reagents/masstrak-immunosuppressant-calibrator-quality-control-and-inter.html>

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

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

720007582, April 2022

Waters™

Application Note

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

Stephen Balloch, Lisa J. Calton, Gareth Hammond

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 ampicillin, azithromycin, cefazolin, cefepime, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, clindamycin, daptomycin, flucloxacillin, linezolid, meropenem, piperacillin, sulbactam, and tazobactam 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 µL sample was processed with methanol and centrifuged, then subsequently diluted with acidified water prior to analysis.

LC Conditions

Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 mm x 100 mm
Mobile phase A:	0.1% ammonia in water
Mobile phase B:	Methanol
Flow rate:	0.5 mL/min
Gradient:	90% A initial, gradient 6 until 0% A at 3.00 minutes, hold until 4.00 minutes, 90% A gradient 6 at 4.10 minutes, then hold until 5.00 minutes

Gradient

Time (minutes)	Flow (mL/min)	% Mobile phase A	% Mobile phase B	Curve
Initial	0.5	90	10	Initial
3.00	0.5	0	100	6
4.00	0.5	0	100	6
4.01	0.5	90	10	6
5.00	0.5	90	10	6

MS Conditions

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

Results and Discussion

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

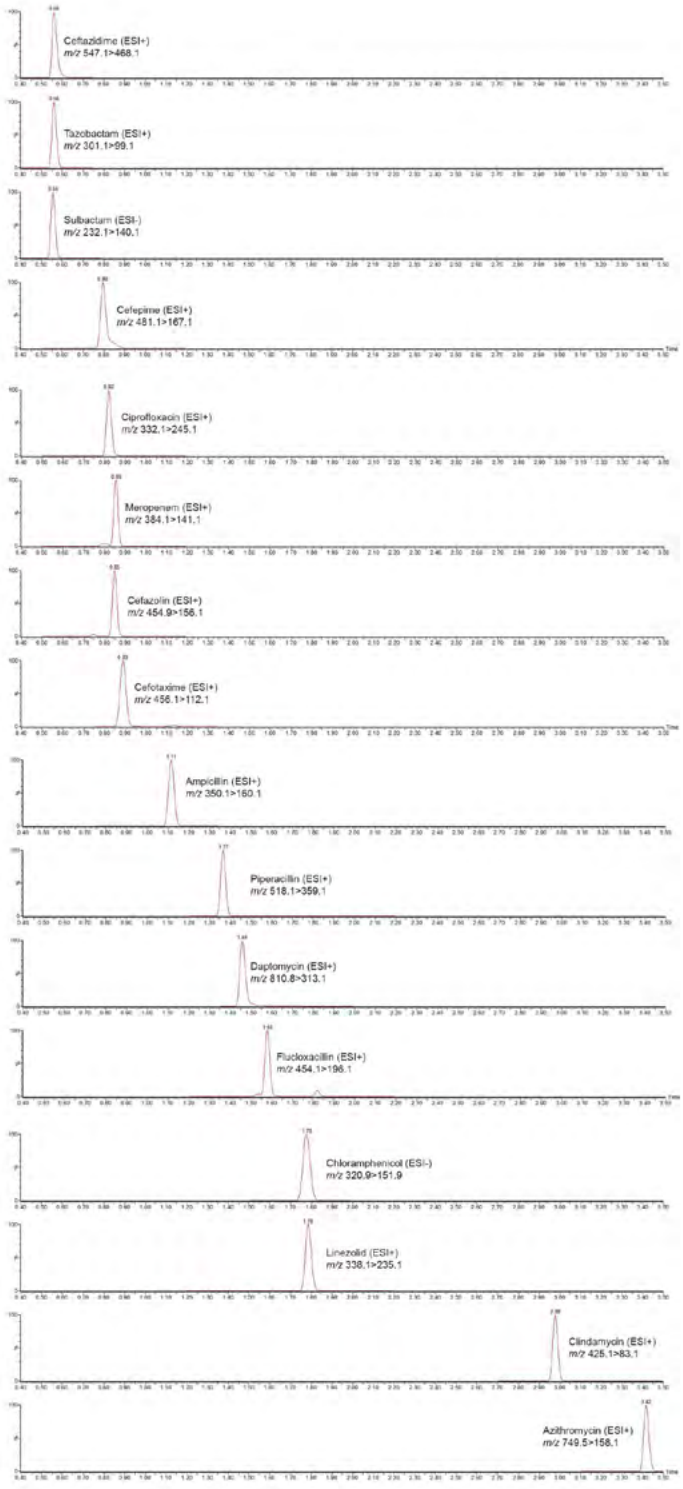


Figure 1. Chromatographic selectivity of a range of antibiotics using the ACQUITY UPLC I-Class/ Xevo TQD IVD System.

Compound	Calibration range* (µg/mL)	LLOQ (µg/mL)	Linear range µg/mL	Total precision	Repeatability
Ampicillin	0.5–50	0.375	0.5–50	≤7.8%	≤7.6%
Azithromycin	0.005–0.5	0.00375	0.00374–0.650	≤8.3%	≤2.6%
Cefazolin	1–100	1	0.748–130	≤12.2%	≤11.6%
Cefepime	1–100	0.9	0.748–130	≤11.5%	≤11.1%
Cefotaxime	0.5–50	0.375	0.374–65	≤9.2%	≤4.1%
Ceftazidime	1–100	0.75	0.975–101	≤8.2%	≤4.7%
Chloramphenicol	0.5–50	0.375	0.374–65	≤10.9%	≤4.2%
Ciprofloxacin	0.1–10	0.075	0.0748–13	≤12.5%	≤6.1%
Clindamycin	0.1–10	0.075	0.0975–10.1	≤5.8%	≤2.8%
Daptomycin	2–200	1.5	1.76–231	≤7.6%	≤4.3%
Flucloxacillin	1–100	1	0.748–130	≤9.6%	≤5.5%
Linezolid	0.5–50	0.375	0.374–65	≤6.5%	≤4.1%
Meropenem	1–100	0.9	0.975–130	≤12.4%	≤10.6%
Piperacillin	2–200	0.5	1.5–260	≤9.3%	≤3.6%
Sulbactam	1–100	0.75	0.748–130	≤8.6%	≤8.6%
Tazobactam	0.5–50	0.375	0.488–50.7	≤11.2%	≤8.1%

Table 1. Performance characteristics of the analytes evaluated.

**Calibration Range was defined by linear fit where r^2 >0.995 for cefepime, daptomycin, piperacillin and sulbactam; for all other analytes a quadratic fit was used. LLOQ defined by S/N (PtP) >10 with %RSD ≤20% and ≤15%. %RSD at LLOQ determined through analytical sensitivity experiments performed over five occasions (n=50). Total precision and repeatability of QCs performed over 5 occasions in plasma (n=25). Data was collected in two runs.*

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to analyze a panel of antibiotics 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.

Featured Products

- ACQUITY UPLC I-Class/Xevo TQD IVD System <https://www.waters.com/waters/nav.htm?cid=134831492>
- MassLynx MS Software <https://www.waters.com/513662>
- TargetLynx <https://www.waters.com/513791>

720007394, October 2021

Waters™

Application Note

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-µL sample was processed with methanol and centrifuged, then subsequently diluted with water prior to analysis.

LC Conditions

Column:	CORTECS C_8, 2.7 µm, 2.1 mm × 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.

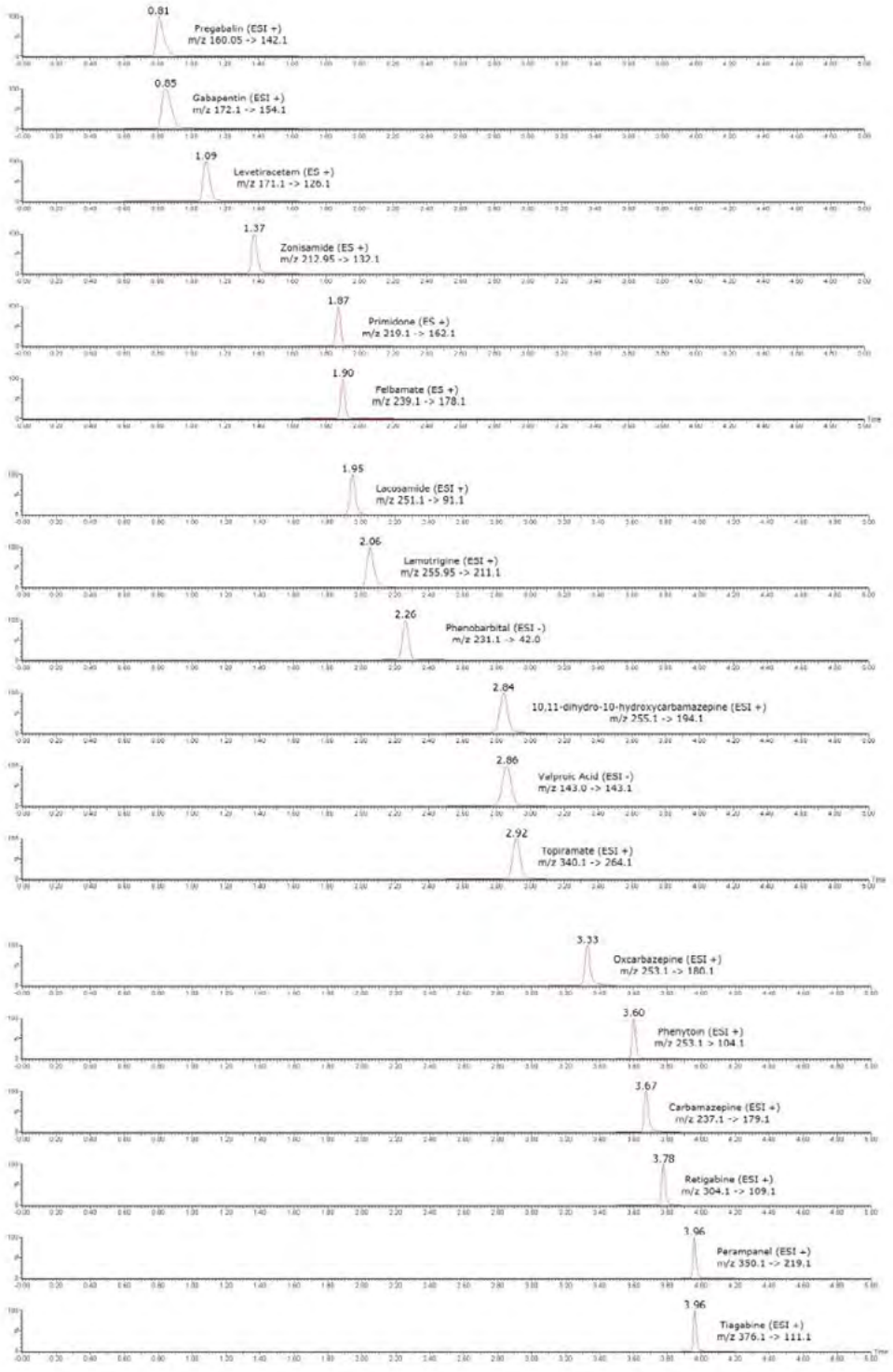


Figure 2. Chromatographic selectivity of a range of antiepileptic drugs using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

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 ≤20% and ≤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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

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

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

720006834, April 2020

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for an Inosine-5'-Monophosphate Dehydrogenase Inhibitor

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 mycophenolic acid in plasma.

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Commercial calibrators and Quality Controls were used as well as in-house calibrators prepared by spiking commercially available reference material in plasma. The samples were processed using the following conditions:

Sample Preparation Conditions

50 µL sample was processed with zinc sulphate/methanol and centrifuged. The supernatants were transferred to a collection plate for analysis.

LC Conditions

Column:	ACQUITY UPLC HSS C_{18} SB 1.8 µm, 2.1 mm × 30 mm
Mobile phase A:	2 mM Ammonium acetate+0.1% formic acid in water
Mobile phase B:	2 mM Ammonium acetate+0.1% formic acid in methanol
Flow rate:	0.7 mL/min
Gradient:	30–40% B over 0.75 minutes, 40–75% B over 0.85 minutes, 98% B for 0.4 minutes

MS Conditions

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

Results and Discussion

Performance characteristics of mycophenolic acid on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. A chromatogram illustrating the selectivity of the mycophenolic acid analysis is shown in Figure 1.

Compound	Range (µg/mL)	LLOQ (µg/mL)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Mycophenolic acid	0.1–20	0.075	5.6%	≤5.3%	≤3.6%	≤5.1%

Table 1. Performance characteristics of mycophenolic acid. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. %RSD at LLOQ determined through analytical sensitivity experiments performed over three occasions (n=30). Total precision and repeatability of QCs performed over five occasions in plasma (n=25). EQA mean bias determined by comparison of obtained values to HPLC-MS method mean.

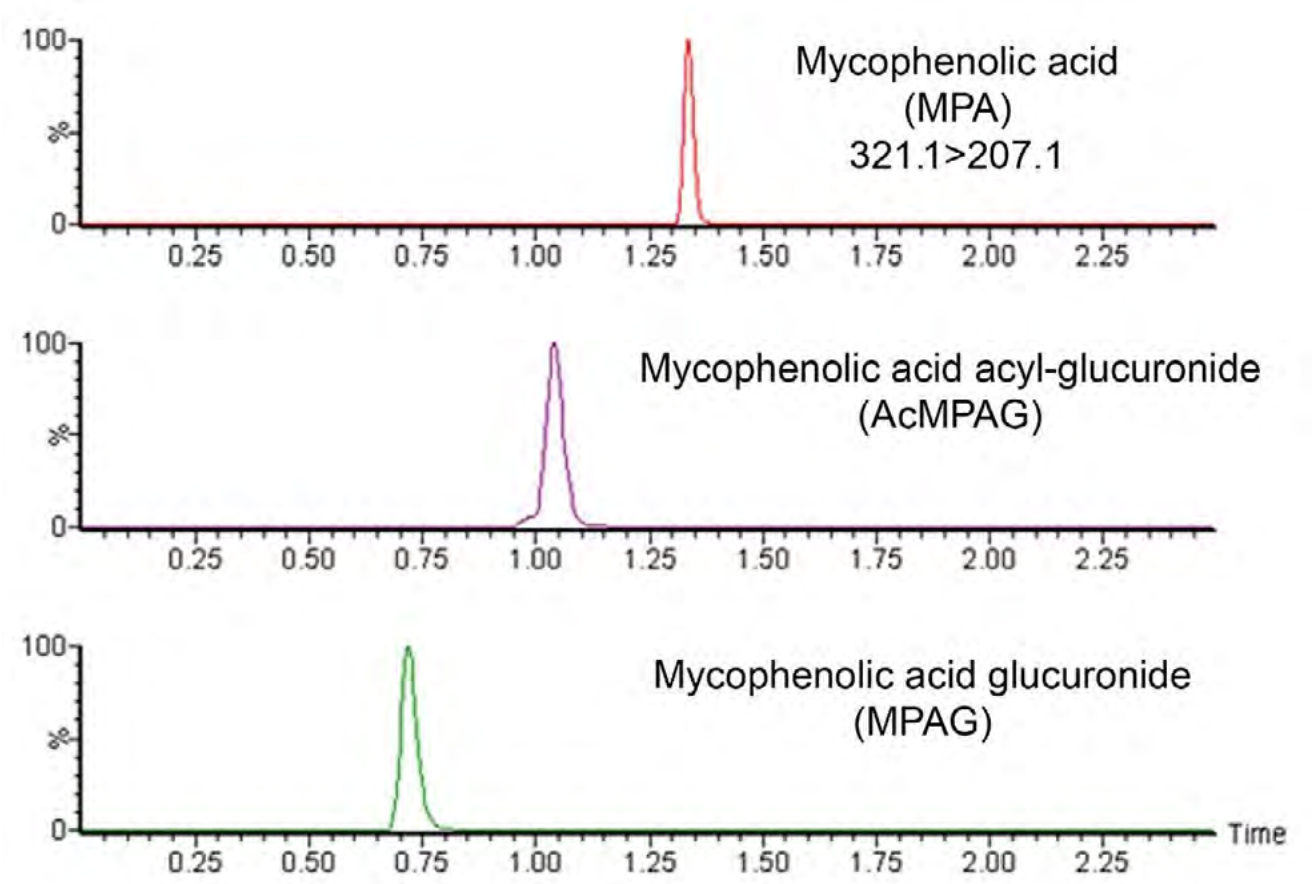


Figure 1. Chromatographic separation of mycophenolic acid from its metabolites using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver analytically sensitive, accurate, and precise performance for mycophenolic acid 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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories <
https://www.waters.com/waters/nav.htm?cid=134834177>

720006340, August 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for an Alkylating Agent

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 busulfan in plasma.

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD system was controlled by MassLynx IVD (v4.1) and the data processed using the TargetLynx 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

50 µL sample was processed with methanol and centrifuged, then subsequently diluted with water prior to analysis.

LC Conditions

Column:	ACQUITY UPLC HSS T3 (IVD) 1.8 µm, 2.1 mm x 50 mm
Mobile phase A:	2 mM Ammonium acetate+0.1% formic acid in water
Mobile phase B:	2 mM Ammonium acetate+0.1% formic acid in methanol
Flow rate:	0.6 mL/min
Gradient:	10% B for 0.5 minutes, 10–98% B over 1.0 minute, 98% B for 0.5 minutes, 10% B for 0.5 minutes

MS Conditions

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

Results and Discussion

Performance characteristics of busulfan on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. A chromatogram illustrating the analytical sensitivity of the busulfan analysis is shown in Figure 1.

Compound	Range (µg/mL)	LLOQ (µg/mL)	%RSD at LLOQ	Total precision	Repeatability
Busulfan	0.025–5	0.02	16.0	≤7.3%	≤5.1%

Table 1. Performance characteristics of busulfan. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. %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).

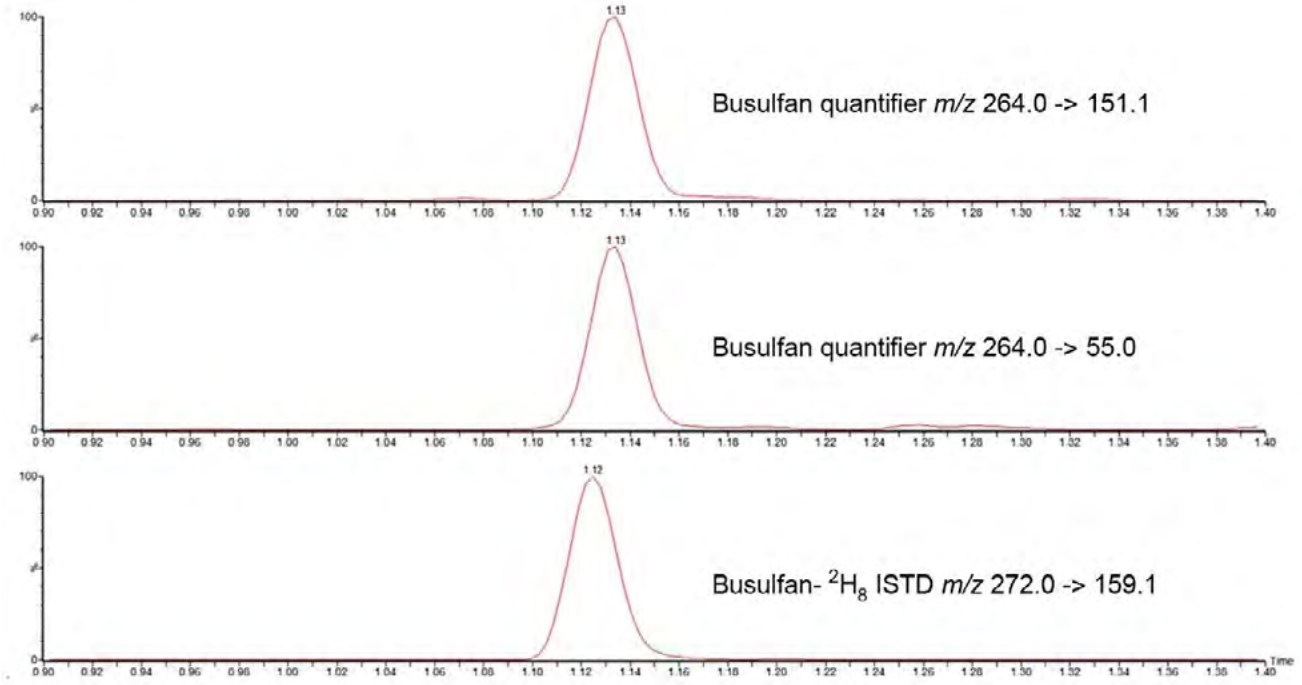


Figure 1. Chromatogram showing the analysis of busulfan using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver analytical sensitivity and precision for the analysis of busulfan 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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories <
https://www.waters.com/waters/nav.htm?&cid=134834177>

720006310, August 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for an Antifolate Agent

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 methotrexate in plasma.

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by spiking commercially available reference material in plasma. The samples were processed using the following conditions:

Sample Preparation Conditions

50 µL sample was processed with methanol and centrifuged, then subsequently diluted with water prior to analysis.

LC Conditions

Column:	ACQUITY UPLC HSS C_{18} SB 1.8 µm, 2.1 mm × 30 mm
Mobile phase A:	2 mM Ammonium acetate + 0.1% formic acid in water
Mobile phase B:	2 mM Ammonium acetate + 0.1% formic acid in methanol
Flow rate:	0.4 mL/min
Gradient:	77% A isocratic for 5 min

MS Conditions

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

Results and Discussion

Performance characteristics of methotrexate on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. Analytical sensitivity of the chromatographic separation is illustrated in Figure 1.

Compound	Range (µmol/L)	LLOQ (µmol/L)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Methotrexate	0.025–10	0.0025	15.7	≤5.5%	≤4.0%	-5.7%

Table 1. Performance characteristics of methotrexate. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. %RSD at LLOQ determined through analytical sensitivity experiments performed over three occasions (n=30). 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 gravimetric reference value.

Note: To convert SI units to conventional mass units divide by 2.2005 (µmol/L to µg/mL).

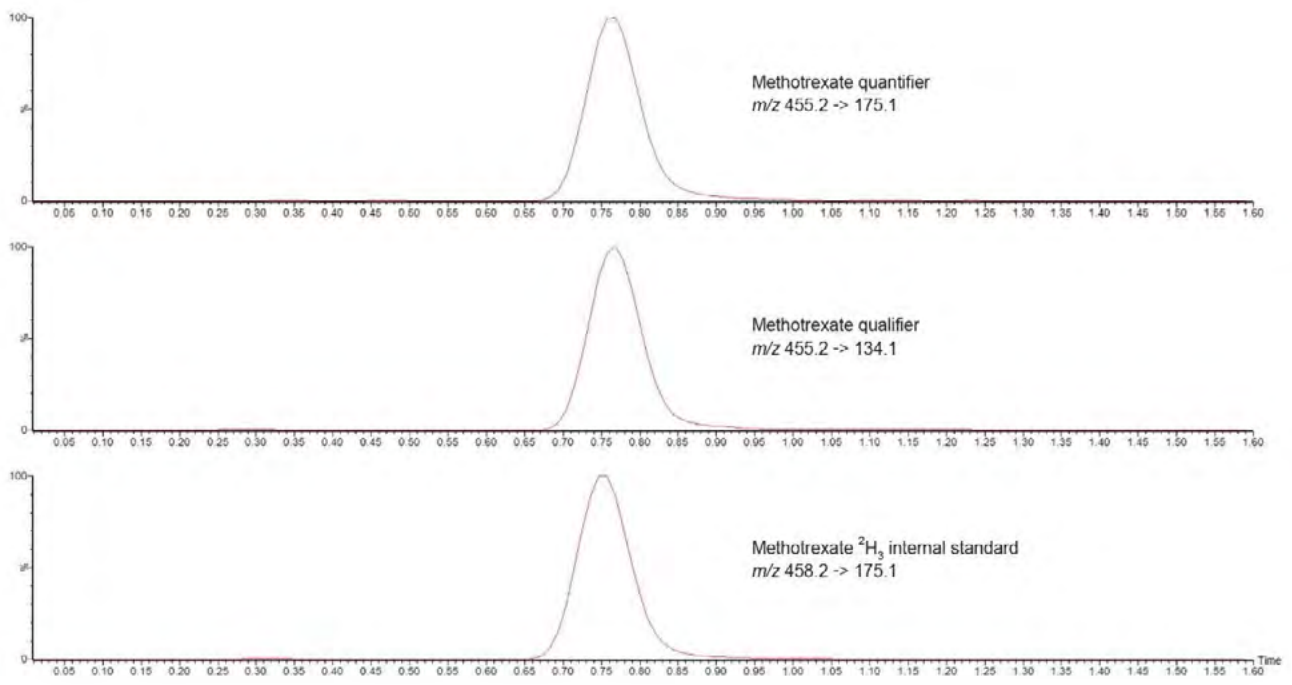


Figure 1. Chromatogram showing the analysis of methotrexate using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver an analytically sensitive and precise method for methotrexate 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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories <
https://www.waters.com/waters/nav.htm?cid=134834177>

720006318, August 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Azole Antifungals

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 fluconazole, hydroxyitraconazole, itraconazole, posaconazole, voriconazole, and voriconazole-N-oxide in serum.

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by spiking commercially available reference material in serum and the samples were processed using the following conditions:

Sample Description

50 µL sample was processed with methanol and centrifuged, then subsequently diluted with water prior to analysis.

LC Conditions

Column:	ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 mm × 30 mm
Mobile phase A:	2 mM Ammonium acetate + 0.1% formic acid in water
Mobile phase B:	2 mM Ammonium acetate + 0.1% formic acid in methanol
Flow rate:	0.8 mL/min
Gradient:	75% A initial, gradient 7 until 97% B at 2.1 minutes, then hold 75% Auntil 2.5 minutes

MS Conditions

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

Results and Discussion

Performance characteristics of the azole antifungals on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. Analytical selectivity of the chromatographic separation is illustrated in Figure 1.

Compound	Range (µg/mL)	LLOQ (µg/mL)	%RSD at LLOQ	Total precision	Repeatability
Fluconazole	0.5–100	0.3750	13.1	≤2.7%	≤2.6%
Hydroxyitraconazole	0.05–10	0.0500	18.8	≤11.5%	≤10.0%
Itraconazole	0.05–10	0.0375	17.2	≤8.9%	≤8.6%
Posaconazole	0.05–10	0.0500	15.2	≤7.7%	≤5.2%
Voriconazole	0.05–10	0.0250	15.8	≤2.6%	≤1.5%
Voriconazole N-Oxide	0.05–10	0.0375	16.1	≤5.4%	≤3.5%

Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. % RSD at LLOQ determined through analytical sensitivity experiments performed over three occasions (n=30). Total precision and repeatability of QCs performed over five occasions in stripped serum (n=25).

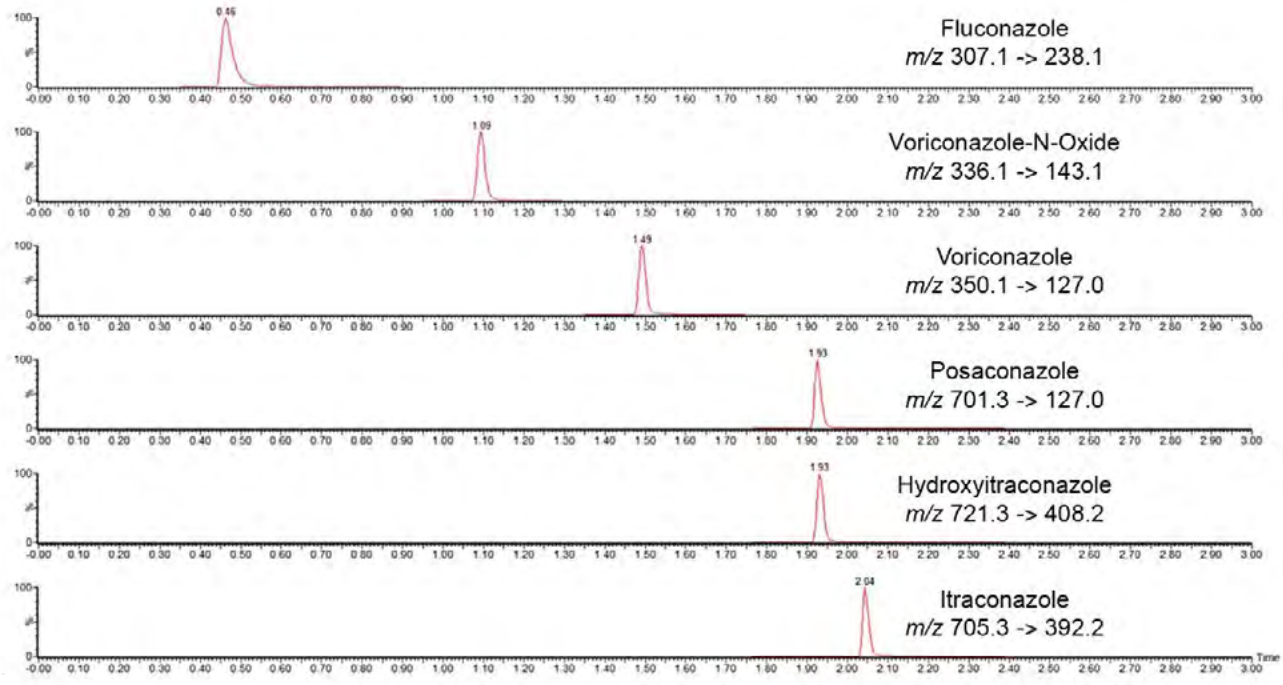


Figure 1. Chromatographic separation of a range of azole antifungals using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver an analytically sensitive and precise method for fluconazole, hydroxyitraconazole, itraconazole, posaconazole, voriconazole, and voriconazole-N-oxide in serum.

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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories < https://www.waters.com/waters/en_US/Mass-spectrometry-software-for-easy-LC-MS-analysis-in-clinical-laboratories/nav.htm?locale=en_US&cid=134834177>

720006319, June 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for a Thymidylate Synthase Inhibitor

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 5-fluorouracil in plasma.

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD (v4.1) and the data processed using the TargetLynx 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

50 µL sample was processed in a liquid-liquid extraction with acidified ethyl acetate followed by a solvent exchange to 0.1% formic acid in water prior to analysis.

LC Conditions

Column:	ACQUITY UPLC HSS PFP 1.8 µm, 2.1 mm × 100 mm
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Flow rate:	0.4 mL/min
Gradient:	2% B for 0.5 minutes, 2–60% B over 1.5 minutes, 98% B for 0.5 minutes, 98% A for 0.5 minutes

MS Conditions

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

Results and Discussion

Performance characteristics of busulfan on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. A chromatogram illustrating the analytical sensitivity of the busulfan analysis is shown in Figure 1.

Compound	Range (ng/mL)	LLOQ (ng/mL)	%RSD at LLOQ	Total precision	Repeatability
5-fluorouracil	20–2000	7.5	20.0	≤9.0%	≤7.2%

Table 1. Performance characteristics of 5-fluorouracil. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. %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).

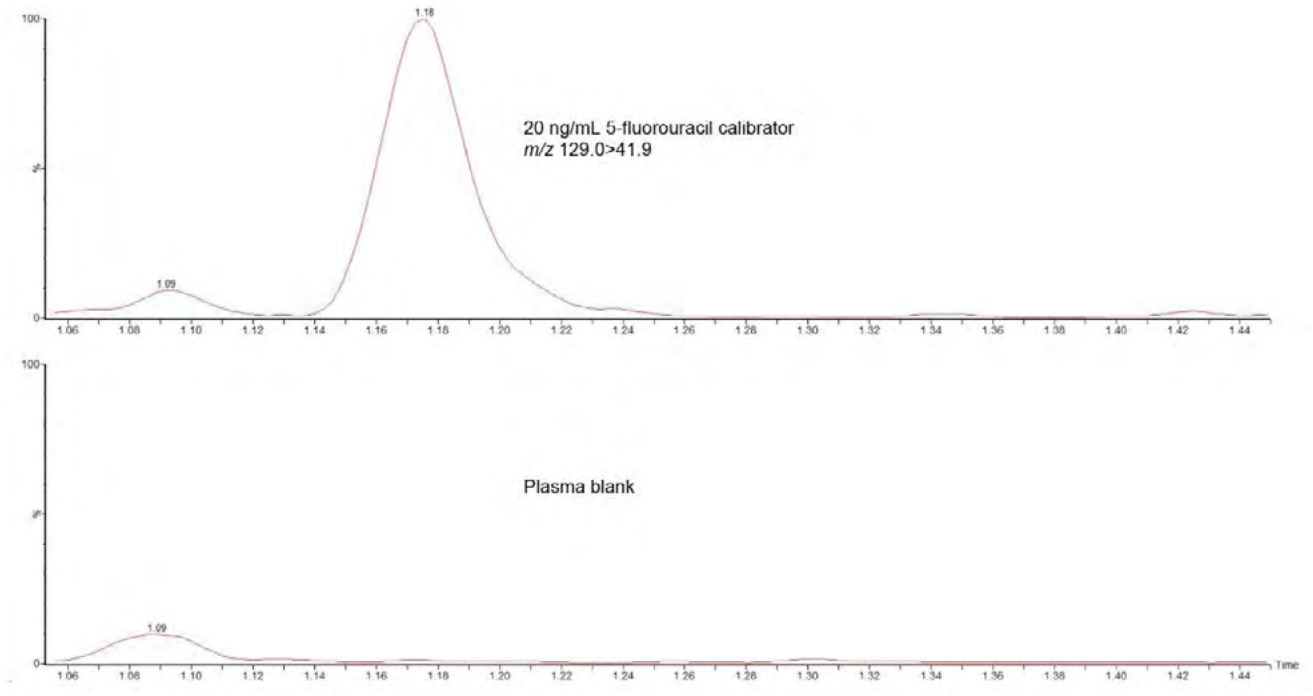


Figure 1. Chromatogram showing the analysis of 5-fluorouracil using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver analytical sensitivity and precision for the analysis of 5-fluouroracil 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.

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQD IVD System

Mass Spectrometry Software to Facilitate LC-MS Analysis in Clinical Laboratories <
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720006305, August 2018

Waters™

Application Note

ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Immunosuppressive Agents

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 cyclosporine, everolimus, sirolimus, and tacrolimus in whole blood.

Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD (v4.1) and the data processed using the TargetLynx Application Manager. Whole blood Calibrators and Quality Controls were processed using the following conditions:

ACQUITY UPLC I-Class/Xevo TQD IVD System.

Sample Preparation Conditions

50 µL sample was processed with aqueous zinc sulphate, acetonitrile, and centrifuged. Each analyte was analyzed individually.

LC Conditions

Column:	ACQUITY UPLC HSS C_{18} SB 1.8 µm, 2.1 mm x 30 mm
Mobile phase A:	2 mM Ammonium acetate+0.1% formic acid in water
Mobile phase B:	2 mM Ammonium acetate+0.1% formic acid in methanol
Flow rate:	0.4 mL/min
Gradient:	50% B for 0.2 minutes, 50–100% B over 0.4 minutes, hold 100% B for 0.6 minutes, equilibrate with 50% B for 0.6 minutes at 0.6 mL/min

MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (1.2 FWHM)
Acquisition mode:	MRM
Polarity:	ESI (+)

Results and Discussion

Performance characteristics of cyclosporine, everolimus, sirolimus, and tacrolimus on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. Analytical sensitivity of the system is illustrated in Figure 1.

Compound	Range (ng/mL)	LLOQ (ng/mL)	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Cyclosporine	25–1500	5	7.0	≤5.7%	≤1.8%	-0.2%
Everolimus	1–30	0.5	6.5	≤7.7%	≤4.7%	-11.9%
Sirolimus	1–30	1	12.7	≤9.1%	≤6.1%	-6.1%
Tacrolimus	1–30	0.5	17.5	≤6.3%	≤2.6%	+1.6%

Table 1. Performance characteristics of cyclosporine, everolimus, sirolimus, and tacrolimus. Range defined by linear fit where r^2 >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤20%. % 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 whole blood (n=25). EQA mean bias determined by comparison of obtained values to the LC-MS all laboratories trimmed mean (LC-MS ALTM) value (n=33). Note: The EQA mean bias for everolimus (-11.9%) is based on the returned EQA results for just two laboratories.

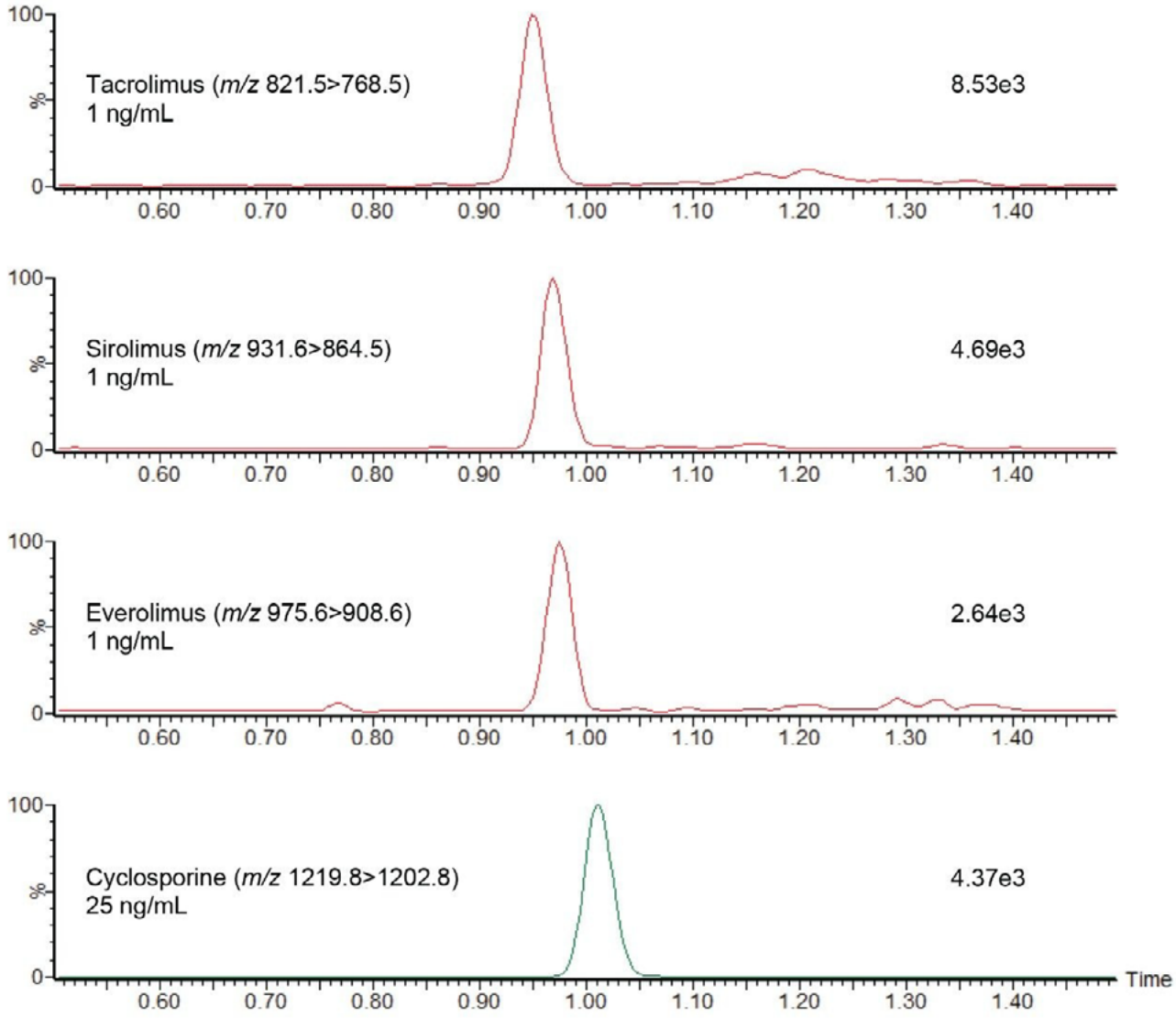

Figure 1. Chromatogram showing the analysis of 25 ng/mL cyclosporine and 1 ng/mL everolimus, sirolimus, and tacrolimus using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver analytical sensitivity, accuracy, and precision for the analysis of cyclosporine, everolimus, sirolimus, and tacrolimus in whole blood.

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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