Clinical Diagnostics Compendium

Volume 2

SCIEX

The Power of Precision

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SCIEX Clinical Diagnostics Compendium Volume 2

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Introduction

SCIEX Clinical Diagnostics Compendium Volume 2

Introducing new technology into the clinical laboratory is a difficult process as clinicians are pressured to deliver reliable and timely patient test results under challenging regulatory and financial circumstances. As such, they are constantly seeking innovative technologies as a solution to achieve better outcomes and lower costs, while simplifying usability.

Mass spectrometry is the perfect solution and the gold standard for accuracy, but those available to clinical labs have been highly complex with significant barriers to adoption. As an analytical technique, liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers high specificity and is being used in an increasing number of clinical laboratories—especially in toxicology and endocrinology. The uniqueness of mass spectrometry lays in its high selectivity, which enables direct identification of molecules based on the mass-to-charge ratios as well as fragmentation patterns.

At SCIEX, our affordable benchtop platforms offer high sensitivity, low detection limits and high specificity, leading to better data than alternative testing methods to support clinicians with confident results.

This Clinical Diagnostics Compendium aims to illustrate the advantages of SCIEX *in vitro* diagnostic medical devices.

Here we feature application notes for the SCIEX Citrine® Systems coupled with the Jasper® HPLC System as well as the Triple Quad™ 4500MD Systems. Whether you are new to SCIEX or a current customer, a novice or an expert in IVD LC-MS/MS, you will find this compendium beneficial. For additional information on all our other medical devices, software as well as details on our service and support, visit our **website**.

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Why Choose SCIEX for Your Clinical Diagnostic Applications?

At SCIEX, we understand that delivering fast and accurate results is a top priority for clinical testing laboratories. Regardless of the medical setting, having absolute confidence that your testing methods meet diagnostic needs is paramount. As such, our team is committed to developing IVD LC-MS/MS instrumentation and reagents; backed by SCIEX support to meet that demand.

There are various analytical techniques in the clinical laboratory, but mass spectrometry offers distinct advantages in delivering more accurate and reliable results for challenging assays. While mass spectrometry is generally accepted as a more sensitive and accurate method, widespread adoption is still limited in clinical diagnostic laboratories due to the inherent complexities of this technology.

As opposed to high-volume, automated assays in the clinical laboratory, mass spectrometry remains a more challenging technology. Historically, it has focused on a small number of tests and has made a considerable impact in a variety of different applications where it is now the analytical method of choice. But as with all advanced technology, implementing mass spectrometry can be a complicated process presenting various obstacles that have made many labs shy away from adoption.

However, there's no denying that mass spectrometry adoption is accelerating across a broader range of clinical applications. The technology offers increased specificity beyond conventional analytical techniques for challenging analytes delivering reliable results with reduced interferences. Another inherent advantage of mass spectrometry is that the technology allows you to capture a multitude of information within a single analysis. The large dynamic range also allows compounds at low and high concentrations to be detected without additional sample preparation. Together, this means that a large number of analytes can be detected in a single injection, providing a broad panel of results, reducing the pre-analytic steps required and enabling faster time to results.

These are just a few examples of how you can get faster results with increased efficiency, with a robust technology that supports enhanced uptime and is rugged enough for a high volume of clinical tests. With the ability to improve test accuracy while also reducing turnaround time and capturing lost revenue in send-out testing, it is easy to see why clinical laboratories want to adopt mass spectrometry.

But, making the transition hasn't seemed quite so easy, until now.

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Lowering The Barrier to Adoption

At SCIEX, we have invested decades in understanding and solving the complex analytical challenges. Together with our customers, we have transformed diagnostic workflows by implementing solutions anchored on our mass spectrometry technology.

SCIEX is providing clinical solutions encompassing hardware, software, services, training, and most importantly: after-sales support. Large or small, hospital, reference or pathology laboratory, SCIEX Diagnostics will provide you with a solution tailored to the needs of your clinical team.

Suitable for a wide range of clinical applications, the proven solutions and robust systems from SCIEX have gained the attention and trust of clinicians across the globe. Giving clinicians the confidence that the right diagnostic and treatment decisions can be made, quickly.

The Future of Clinical Diagnostics is in Your Hands

Due to its sensitivity and selectivity, LC-MS/MS is considered the most advanced method for quantification in complex biological matrices including plasma, serum, urine, and many others.

When it comes to routine clinical analysis, most laboratories favor analyzers that work straight out of the box with so-called “locked” pre-programmed methods. However, many analytical techniques are challenged with meeting the necessary performance in detecting trace levels of analytes in complex matrices with high accuracy and precision. When detecting and quantifying analytes at ultra-low concentrations or when challenged by interferences, nothing else comes close to mass spectrometry.

We believe LC-MS/MS should be accessible to clinical laboratories for relevant qualitative and quantitative tests. It is superior compared to alternative analytical techniques, but the advanced capabilities of the technique need to be easy to adopt and simple to use. SCIEX instruments and software have been built specifically to make this a reality: designed to offer users a simple, streamlined experience from sample to results. The SCIEX clinical diagnostic portfolio includes medical device mass spectrometers and fully integrated LC-MS/MS systems which are powered by intuitive software.

Whether you are looking for routine or more advanced analysis, we offer the best of both worlds. With our medical device LC-MS/MS portfolio, you can be confident that you’re equipped with tools that are simple for routine analysis and sophisticated enough to build and develop custom laboratory methods. We take pride that our instruments and systems are developed with the flexibility to handle a variety of clinical applications. SCIEX mass spectrometers and fully integrated LC-MS/MS systems has your lab covered, facilitating both clinical diagnostic testing as well as academic or clinical research.

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Why SCIEX Medical Devices? Here are 4 Good Reasons

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Citrine® MS/MS *in vitro* Medical Device Analytical Performance

The Power of Precision

Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for Free Triiodothyronine and Free Thyroxine

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze Free Triiodothyronine (FT3) and Free Thyroxine (FT4) in serum matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Serum samples and calibrators were processed using the following conditions:

Sample Prep Conditions
Centrifugal Filtration (MWCO 10kDa)

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C18
Mobile Phase A: Water/Acetic Acid
Mobile Phase B: Methanol/Acetic Acid
Flow Rate: 0.6ml/min
Injection Volume: 50ul
Gradient: Linear gradient over 10 minutes
Retention Time: 5.4 (FT3) and 5.7 (FT4) minutes

Mass Spectrometry Conditions
Method Duration: 10 minutes
Polarity: Positive ESI
Transitions: 652-606 (FT3), 778 – 732 (FT4)
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (units)	%CV (at LLOQ)	S/N
Free T3	0.5-100 pg/ml	12.3	7.9*
Free T4	0.5-100 pg/ml	11.2	8.1*

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) for FT3 and FT4.

*Calculated using a Peak-to-Peak algorithm

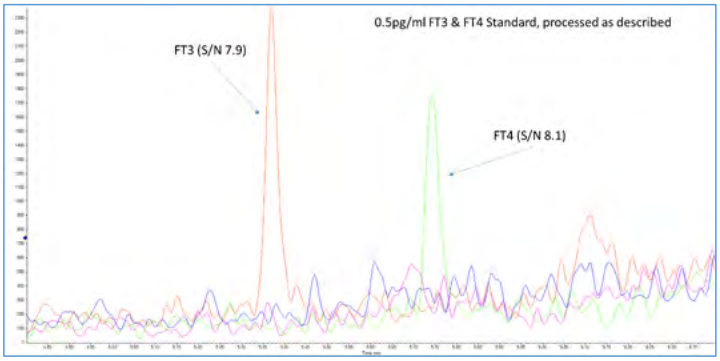

Figure 1. Chromatogram of calibration standard for FT3 and FT4 at 0.5 pg/mL (0.77 and 0.64 pmol/L respectively) using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

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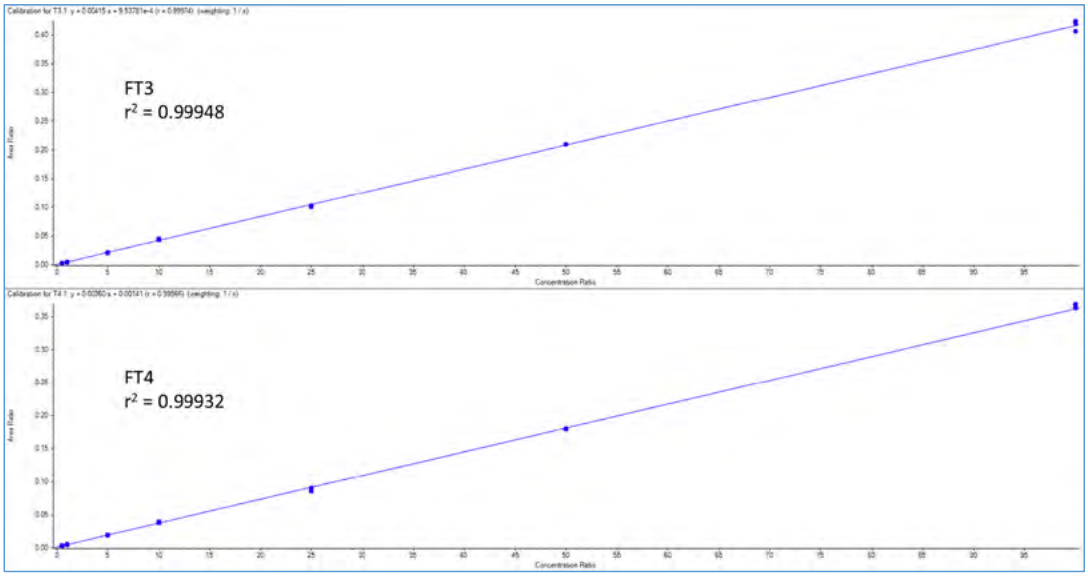

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for FT3 and FT4 in serum (r^2>0.999 for both compounds) evaluated over the concentration range in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: FT3 and FT4 showed r^2 values of >0.999 for the range measured in serum.

Reproducibility: At the LLOQ (0.5pg/ml), the precision (%CV) was <13% for both FT3 and FT4, determined by n=3 replicates. CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for quantitation of FT3 and FT4 in serum matrix.

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Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for 1,25-Dihydroxyvitamin D3 and D2

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze 1,25-dihydroxyvitamin D3 and 1,25-dihydroxyvitmain D2 in serum matrix.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum spiked with 1,25-dihydroxyvitamin D was processed using the following conditions:

Sample Prep Conditions
Immunopurification (Immunodiagnosticsystems) of 500 µL serum, followed by derivatization using the SCIEX Amplifex™ Diene Reagent derivatization chemistry.

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C18
Mobile Phase A: Water/Formic Acid
Mobile Phase B: Methanol/Formic Acid
Flow Rate: 0.6ml/min
Injection Volume: 50ul
Gradient: Linear 5-98% B over 10 minutes
Retention Time: 3.5-4 minutes

Mass Spectrometry Conditions
Method Duration: 10 minutes
Polarity: Positive Electrospray
Transitions: Compound Dependent
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound (units)	Range	%CV (at LLOQ)	S/N
1,25-Dihydroxyvitamin D3 (pg/mL)	5-200	1.2%	20
1,25-Dihydroxyvitamin D2 (pg/mL)	5-200	4.8%	20

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) for each compound evaluated.

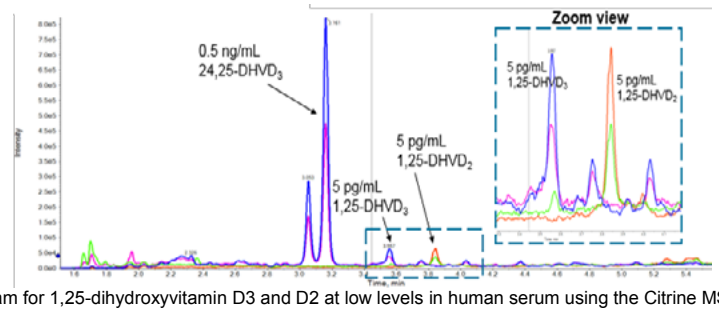

Figure 1. Chromatogram for 1,25-dihydroxyvitamin D3 and D2 at low levels in human serum using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

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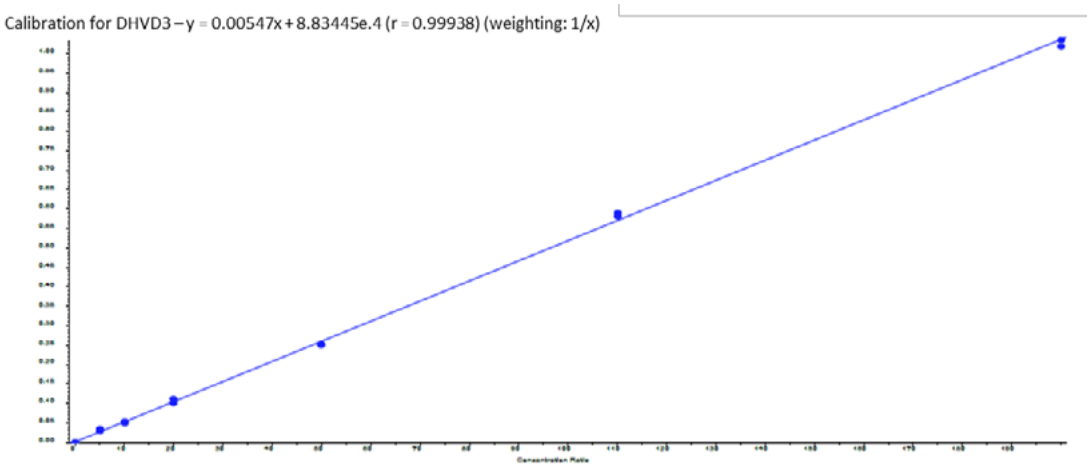

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for 1,25-dihydroxyvitamin D3 in serum (r^2=0.9987) evaluated over the concentration range in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: 1,25-dihydroxyvitamin D2 and D3 showed r^2 values of >0.998 for the range measured in serum.

Reproducibility: At each LLOQ, the precision (%CV) was <5% for 1,25-dihydroxyvitamin D3 and D2, determined by n=3 replicates (Table 1). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for 1,25-dihydroxyvitamin D3 and D2 quantitation in serum matrix.

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Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for Steroids, Water-Soluble Vitamins and Fat-Soluble Vitamins.

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze steroids, water-soluble vitamins and fat-soluble vitamins in serum matrix.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum spiked with each compound evaluated was processed using the following conditions:

Sample Prep Conditions
Steroids (ST): Liquid/Liquid (L/L) extraction with methyl tert-butyl ether.

Water-Soluble Vitamins (WS): L/L extraction with cold acetonitrile, dry under nitrogen, reconstitute in water.

Fat-Soluble Vitamins (FS): L/L extraction with 50/50 hexane/ethyl acetate, dry under nitrogen, reconstitute in methanol.

Liquid Chromatography Conditions
Column (Phenomenex): Kinetex C18 *(ST)*, C18 *(WS)*, Phenyl Hexyl *(FS)*

Mobile Phase A: Water/NH_4F *(ST),*
Water/NH4FA, FA *(WS),*
Water/FA *(FS)*

Mobile Phase B: Methanol/NH_4F *(ST),*
Methanol *(WS),* Methanol + 0.1% FA *(FS)*

Flow Rate (mL/min): 0.6 *(ST)*, 0.7 *(WS)*, 0.4 *(FS)*
Injection Volume (µL): 20 *(ST)*, 10 *(WS)*, 5 *(FS)*
Gradient: 5-98% B *(ST)*, 5-99% B *(WS)*, 20-99% B *(FS)*
Retention Time: Compound Dependent, from 5-9 min
Mass Spectrometry Conditions
Method Duration (min): 12 *(ST)*, 5 *(WS)*, 5 *(FS)*
Polarity: ESI with Pos/Neg Switching
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatograms of the compounds evaluated utilizing the described method are shown in Figures 1-3. Calibration curves over the defined concentration ranges for each compound are shown in Figure 4.

Compound	Range (ng/mL)	%CV (at LLOQ)	S/N
Thiamine	0.25-100	0.4%	207
Riboflavin	0.41-100	1.6%	87
Nicotinamide	0.07-102	2.7%	69
Nicotinic acid	0.1-100	11.3%	28
Pantothenic acid	0.5-1000	0.4%	118
Biotin	0.05-100	6.2%	9
Folic acid	10-1000	5.0%	11
Cyanocobalamin	0.2-100	1.7%	16
Retinol	10-2000	2.0%	15
β-Carotene	12-1200	2.7%	34
Cholecalciferol	1-100	2.3%	55
Ergocalciferol	4-100	3.1%	260
α-Tocopherol	200-20000	1.0%	1040
Phylloquinone	0.064-20	1.3%	41
Aldosterone	1-10,000	8.7%	44*
Estradiol	1-10,000	9.2%	31*
Estriol	1-10,000	18.4%	143
Estrone	1-10,000	4.6%	394
Androstenedione	1-10,000	8.7%	14*
Corticosterone	1-10,000	10.8%	6
Cortisol	1-10,000	13.5%	11
Cortisone	1-10,000	5.6%	30*
11-Deoxycortisol	1-10,000	4.0%	30*
21-Dexoycortisol	1-10,000	3.2%	4
DHEA	1-10,000	19.4%	15
17-Hydroxyprogesterone	1-10,000	12.0%	45*
21-Hydroxyprogesterone	1-10,000	5.4%	12*
Prednisone	1-10,000	9.0%	26
Testosterone	1-10,000	3.7%	22

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) for each compound evaluated.

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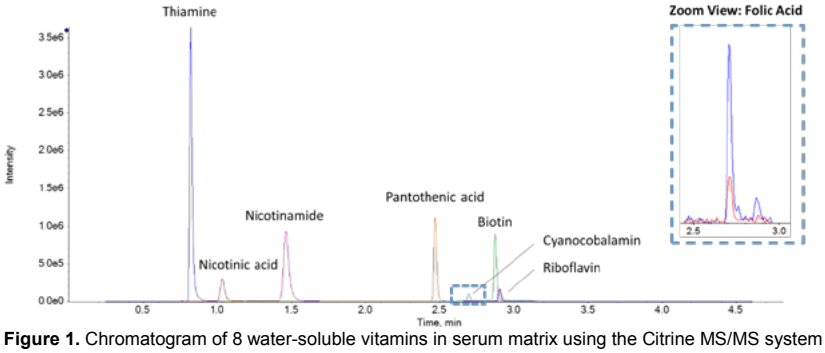

Figure 1. Chromatogram of 8 water-soluble vitamins in serum matrix using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

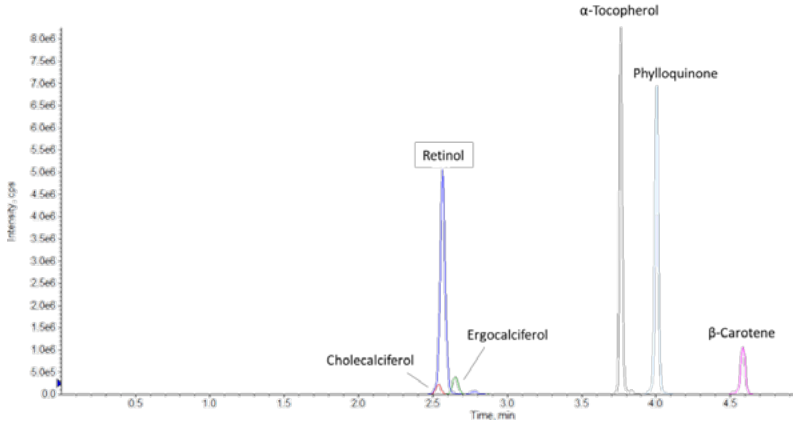

Figure 2. Chromatogram of 6 fat-soluble vitamins in serum matrix using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

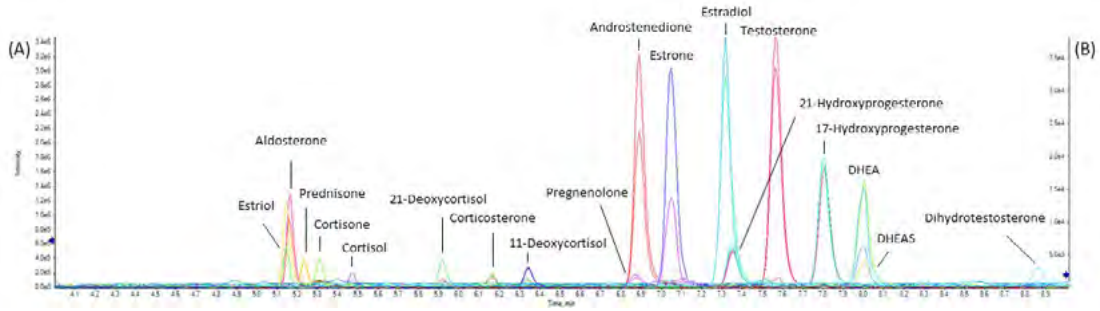

Figure 3. Chromatogram of 18 steroid compounds extracted from serum employing rapid polarity switching (10 ms) between positive and negative ESI modes. Here, (A) compounds analyzed in positive mode and (B) in negative mode are displayed from a single injection, where two MRM transitions were monitored per compound with 10+ points across the peaks for all compounds.

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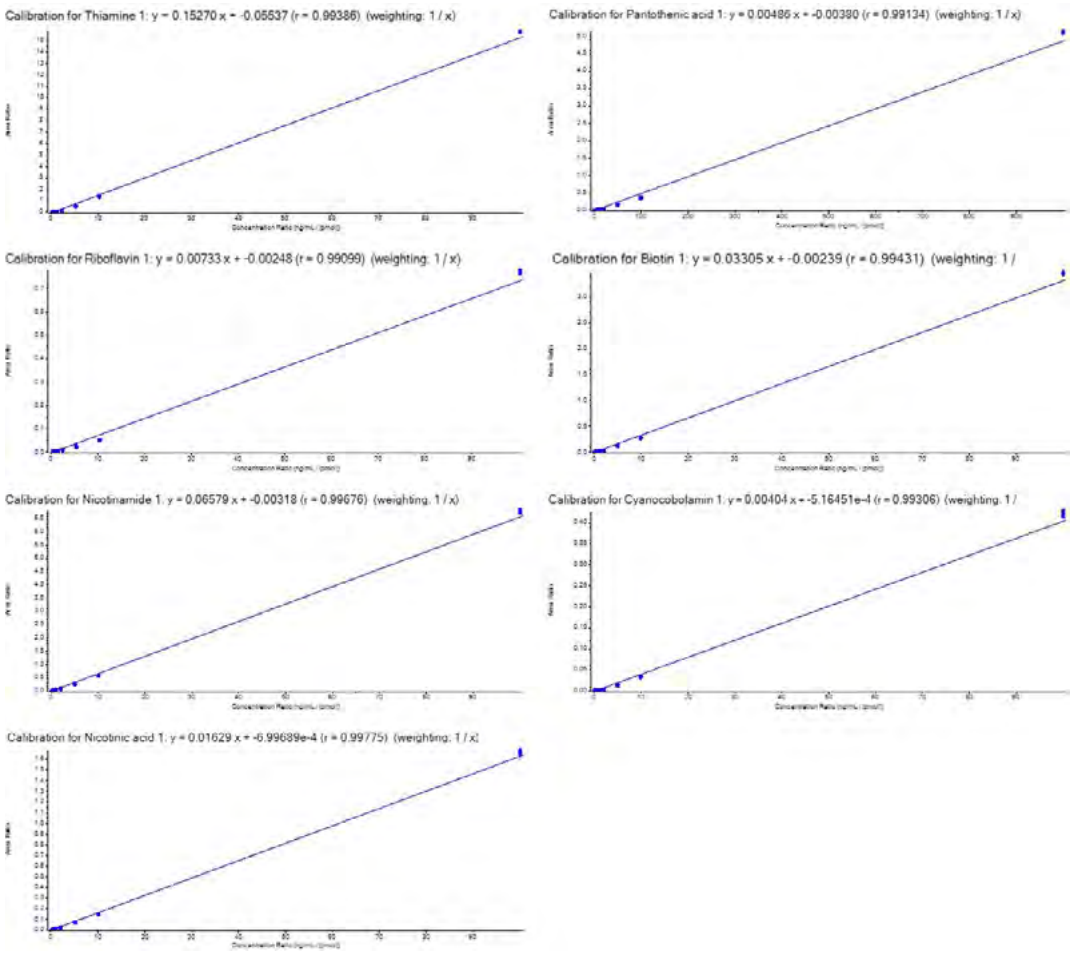

Figure 4. Calibration curves using ordinary least-squares regression and 1/x weighting for water-soluble vitamins in serum evaluated over the concentration ranges in Table 1.

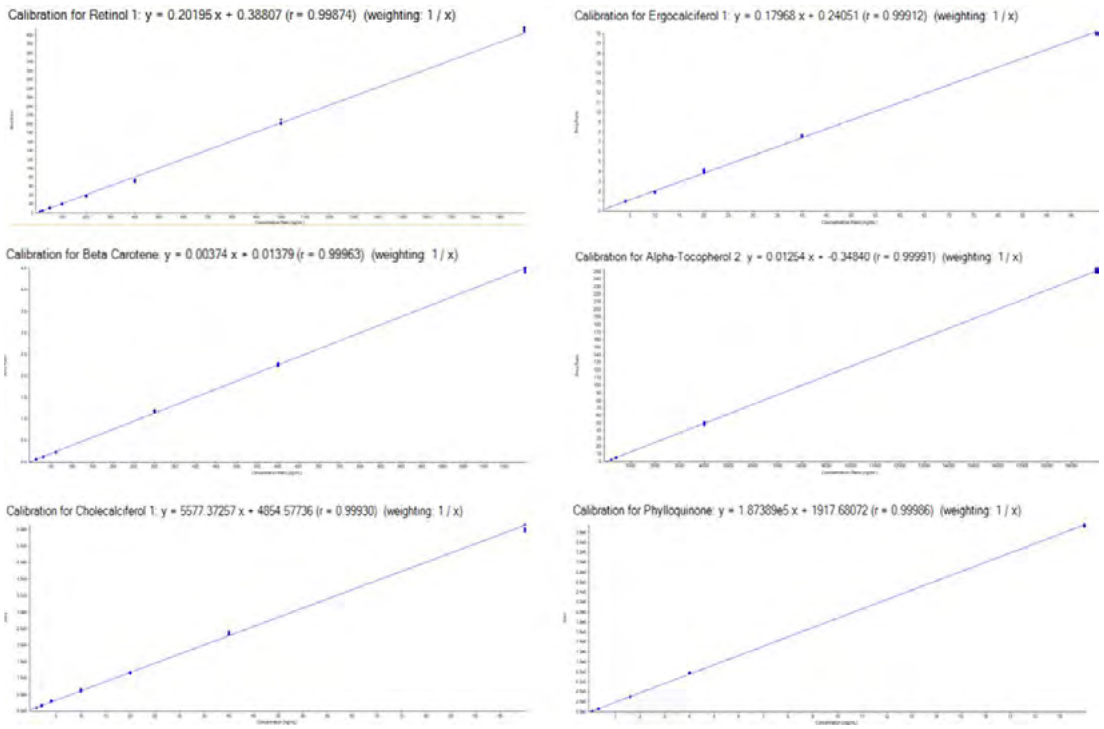

Figure 5. Calibration curves using ordinary least-squares regression and 1/x weighting for fat-soluble vitamins in serum evaluated over the concentration ranges in Table 1.

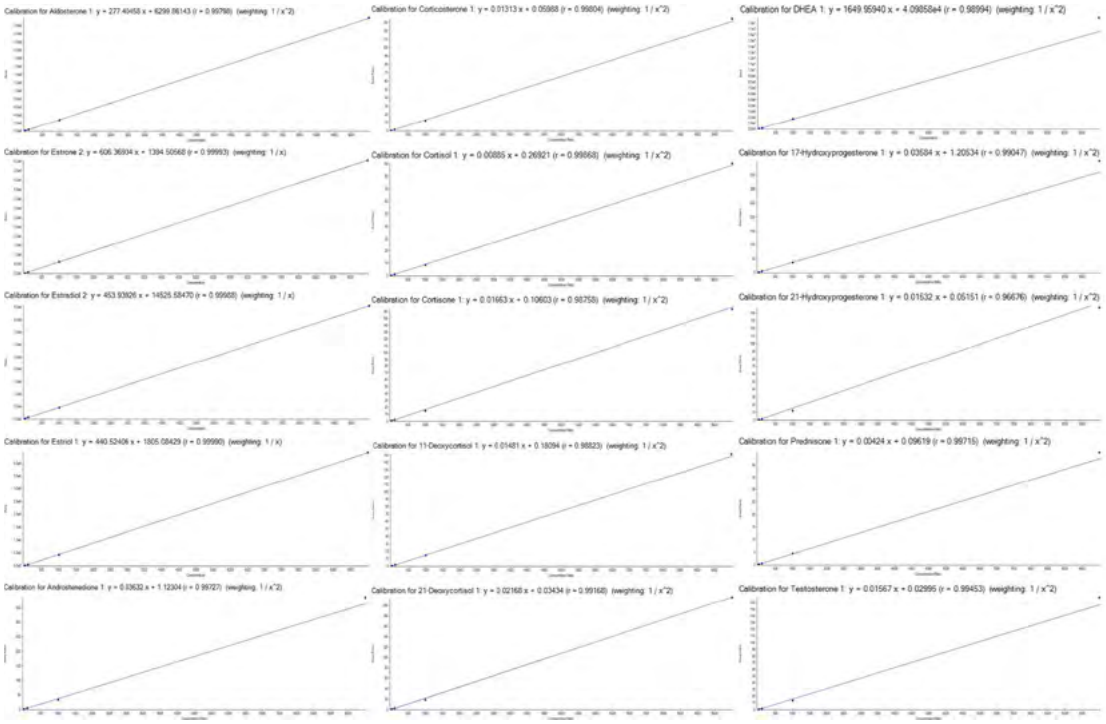

Figure 6. Calibration curves for aldosterone, estradiol, estriol, estrone, androstenedione, corticosterone, cortisol, cortisone, 11-deoxycortisol, 21-deoxycortisol, DHEA, 17-hydroxyprogesterone, 21-hydroxyprogesterone, prednisone, testosterone in serum matrix, demonstrating a dynamic range of at least five orders of magnitude.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds evaluated are shown in figures 4-6 over the concentration ranges in Table 1. Here, all water-soluble and fat-soluble vitamins demonstrated r-values >0.99 (1/x weighting) and the steroids exhibited a linear dynamic range of at least five orders of magnitude for the ranges measured in serum.

Reproducibility: At each LLOQ, the precision (%CV) was <20% for the compounds evaluated, as determined by n=3 replicates (Table 1). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for steroids, water-soluble vitamins and fat-soluble vitamins in serum matrix.

Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for 90+ Drug Compounds in Human Urine

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze more than 90 drug compounds (Table 1) in human urine matrix monitoring over 200 MRM transitions (including internal standards).

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available urine spiked with the drug compounds evaluated (Cerilliant) was processed using the following conditions and an optimized scheduled MRM™ algorithm:

Sample Prep Conditions
Hydrolysis of 500 µL urine sample was performed using IMCS Rapid Hydrolysis Buffer and IMCSzyme (30-60 min at 55 °C). Following hydrolysis, 200 µL methanol and water was added before centrifugation (21,000 *xg* for 10 min.)

Liquid Chromatography Conditions
Column: Phenomenex Kinetex Phenyl-Hexyl and SecurityGuard ULTRA Phenyl cartridge.
Mobile Phase A: Water/Ammonium Formate
Mobile Phase B: Methanol/Formic Acid
Flow Rate: 1 mL/min
Injection Volume: 5 uL
Gradient: Linear 5-98% B over 6.5 minutes
Retention Time: 0.9-4.1 minutes

Mass Spectrometry Conditions
Method Duration: 5 minutes
Polarity: Positive/Negative Electrospray
Scheduled MRM detection window: 20 seconds
Transitions: Compound Dependent
Source Conditions: Flow rate-optimized

Results

Chromatogram of the 93 drug compounds evaluated utilizing the described method conditions, is shown in Figure 1. Results of precision studies, including the observed %CV at the concentration indicated for each of six representative compounds are shown in Table 1. Calibration curves of these representative compounds over the defined concentrations are illustrated in Figure 2. The list of all compounds evaluated, including the internal standards, the concentration range over which calibration curves were constructed, and the linear regression coefficients (r) are shown in Table 2.

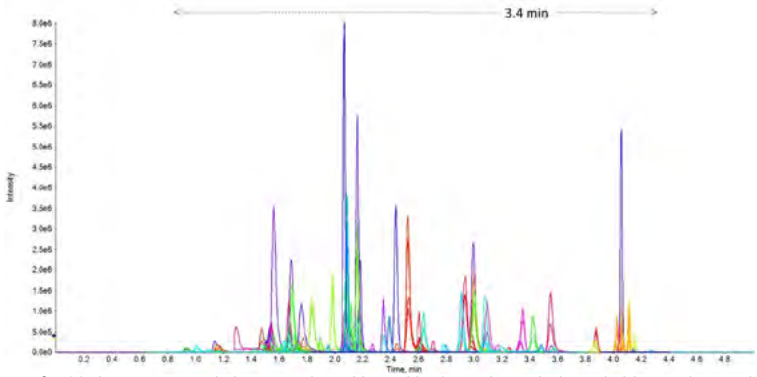

Figure 1. Chromatogram for 93 drug compounds human urine with positive/negative polarity switching and an optimized scheduled MRM algorithm, using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

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Compound	Concentration (ng/mL)	%CV
6-MAM	10	6.4
Amphetamine	100	2.3
Buprenorphine	20	7.6
Morphine	50	1.8
Nordiazepam	50	3.9
THC-COOH	100	9.6

Table 1. Percent coefficient of variation (%CV) at the lower-limit of quantitation (LLOQ) for six representative drug compounds as determined by n=3 replicates.

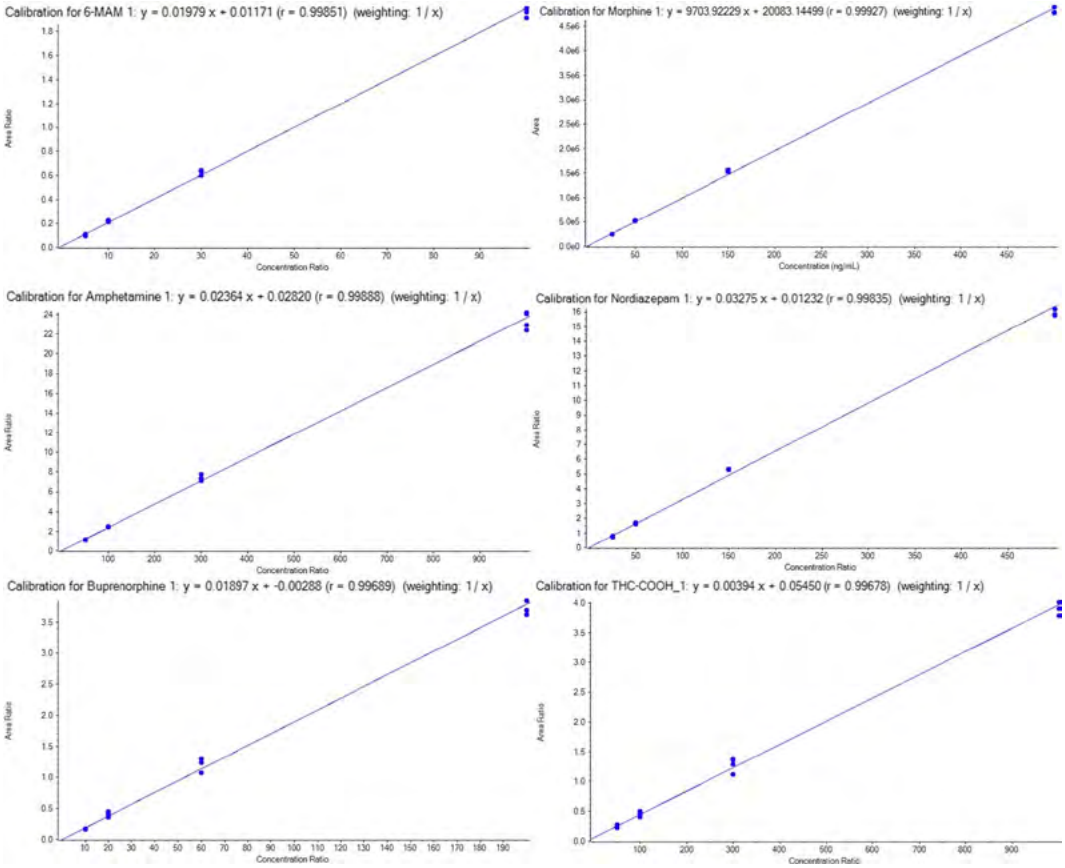

Figure 2. Calibration curves of six representative compounds (6-MAM, amphetamine, buprenorphine, morphine, nordiazepam, and THC-COOH) using ordinary least-squares regression and 1/x weighting in urine (r > 0.996) over the concentration range in Table 1.

List of more than 90 drug compounds and internal standards evaluated in urine matrix.

Compounds	Internal Standards	Range (ng/mL)	Regression Coefficient (r)
6-MAM	6-MAM-d3	5-100	0.99851
7-Aminoclonazepam		25-500	0.99931
7-Hydroxymitragynine		5-100	0.99505
Acetyl Fentanyl		1-20	0.99862
Alpha-Hydroxyalprazolam		25-500	0.99762
Alpha-Hydroxymidazolam		25-500	0.99773
Alpha-Hydroxytriazolam		25-500	0.99642
Alpha-PPP		5-100	0.99692
Alpha-PVP		5-100	0.99649
Alprazolam		25-500	0.99621
AM-22014-OH pentyl		5-100	0.99350
Amitriptyline		25-500	0.99831
Amobarbital/pentobarbital		50-1000	0.99890
Amphetamine	Amphetamine-d5	50-1000	0.99888
Benzoylecgonine	Benzoylecgonine-d3	25-500	0.99839
Buphedrone		5-100	0.99798
Buprenorphine	Buprenorphine-d4	10-200	0.99689
Butabarbital		50-1000	0.99861
Butalbital	Butalbital-d5	50-1000	0.99758
Carisoprodol	Carisoprodol-d7	50-1000	0.99036
Clomipramine		25-500	0.99698
Codeine	Codeine-d6	25-500	0.99688
Cotinine		25-500	0.99848
Cyclobenzaprine		25-500	0.99780
Desalkylflurazepam		25-500	0.99809
Desipramine		25-500	0.99849
Desmethyldoxepin		25-500	0.99763
Dextromethorphan		25-500	0.99703
Diazepam		25-500	0.99788
Dihydrocodeine		25-500	0.99965
Doxepin		25-500	0.99853
EDDP		50-1000	0.99462
Fentanyl	Fentanyl-d5	1-20	0.99912
Gabapentin		50-1000	0.99722
Hydrocodone	Hydrocodone-d6	25-500	0.99923

Hydromorphone	Hydromorphone-d6	25-500	0.99676
Imipramine		25-500	0.99800
JWH-0184-OH pentyl	JWH184-OH pentyl-d5	5-100	0.99929
JWH-018 pentanoic acid		5-100	0.99965
JWH-0196-OH hexyl	JWH196-OH hexyl-d5	5-100	0.99943
JWH-0733-OH butyl		5-100	0.99973
JWH-073 butanoic acid		5-100	0.99873
JWH-0815-OH pentyl		5-100	0.99356
JWH-1225-OH pentyl		5-100	0.99950
JWH-2105-OH pentyl		5-100	0.99897
JWH-2504-OH pentyl		5-100	0.99965
Lorazepam		25-500	0.99700
MDA		50-1000	0.99719
MDEA		50-1000	0.99809
MDMA		50-1000	0.99860
MDPV	MDPV-d8	5-100	0.99924
Meperidine	Meperidine-d4	25-500	0.99633
Mephedrone	Mephedrone-d3	5-100	0.99631
Meprobamate	Meprobamate-d7	50-1000	0.99346
Methadone	Methadone-d3	50-1000	0.99792
Methamphetamine	Methamphetamine-d5	50-1000	0.99862
Methedrone		5-100	0.99966
Methylone	Methylone-d3	5-100	0.99971
Methylphenidate		25-500	0.99912
Midazolam		25-500	0.99789
Mitragynine	Mitragynine-d3	5-100	0.99948
Morphine	Morphine-d6	25-500	0.99927
Naloxone		25-500	0.99920
Naltrexone		25-500	0.99920
N-desmethyltapentadol		25-500	0.99863
Norbuprenorphine		10-200	0.99972
Norcodeine		25-500	0.99726
Nordiazepam	Nordiazepam-d5	25-500	0.99835
Norfentanyl		1-20	0.99837
Norhydrocodone		25-500	0.99749
Normeperidine		25-500	0.99786
Noroxycodone		25-500	0.99801
Norpropoxyphene		50-1000	0.99642

Nortriptyline	Nortriptyline-d3	25-500	0.99820
O-Desmethyltramadol		25-500	0.99870
Oxazepam		25-500	0.99816
Oxycodone	Oxycodone-d6	25-500	0.99756
Oxymorphone	Oxymorphone-d3	25-500	0.99927
PCP		12.5-250	0.99769
Phenobarbital		50-1000	0.99774
Pregabalin		50-1000	0.99688
Propoxyphene		50-1000	0.99720
Protriptyline		25-500	0.99801
Ritalinic Acid		25-500	0.99959
Secobarbital	Secobarbital-d5	50-1000	0.99765
Sufentanil		1-20	0.99917
Tapentadol		25-500	0.99976
Temazepam		25-500	0.99732
THC-COOH	THC-COOH-d3	10-200	0.99692
Tramadol		25-500	0.99923
Zolpidem		25-500	0.99768

Table 2. List of more than 90 drug compounds and internal standards, the concentration ranges (ng/mL) over which calibration curves were constructed, and the linear regression coefficients (r) for each analyte.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All drug compounds evaluated showed r values of >0.99 for the range measured in urine.

Reproducibility: The precision (%CV) was <10% for 6-MAM, amphetamine, buprenorphine, morphine, nordiazepam, and THC-COOH as determined by n=3 replicates (Table 2). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for the quantitation of more than 90 drug compounds and internal standards (over 200 MRMs) in human urine matrix.

Citrine® QTRAP® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for 11-nor-9-Carboxy-THC (THC-COOH) in Hair

The SCIEX Citrine QTRAP MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze TCH-COOH in hair matrix.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available hair spiked with THC-COOH was processed using the following conditions:

Sample Prep Conditions
Alkaline digestion followed by liquid-liquid extraction

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C18
Mobile Phase A: Water/Acetic Acid
Mobile Phase B: Methanol/Acetic Acid
Flow Rate: 0.5 mL/min
Injection Volume: 20 µL
Gradient: Linear gradient over 10 minutes
Retention Time: 5.9 minutes

Mass Spectrometry Conditions
Method Duration: 8 minutes
Polarity: Negative ESI, QTRAP
Transitions: MRM³ 343-299-245
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatogram of THC-COOH evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (units)	%CV (at LLOQ)	S/N
THC-COOH	0.1-1 pg/mL	8.45%	26

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) for THC-COOH.

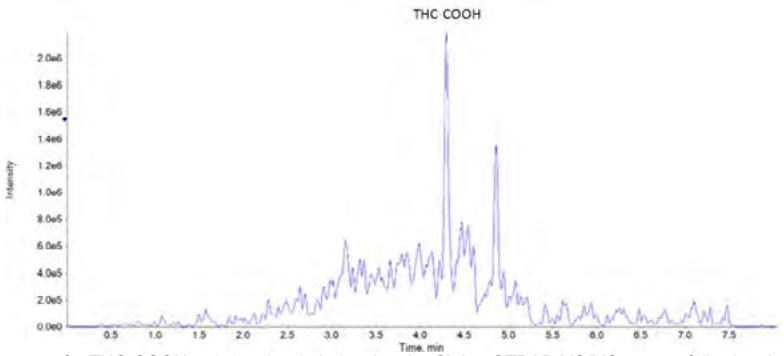

Figure 1. Chromatogram for THC-COOH at 0.1 pg/mg in hair using the Citrine QTRAP MS/MS system following the sample preparation and LC-MS/MS conditions.

Contents

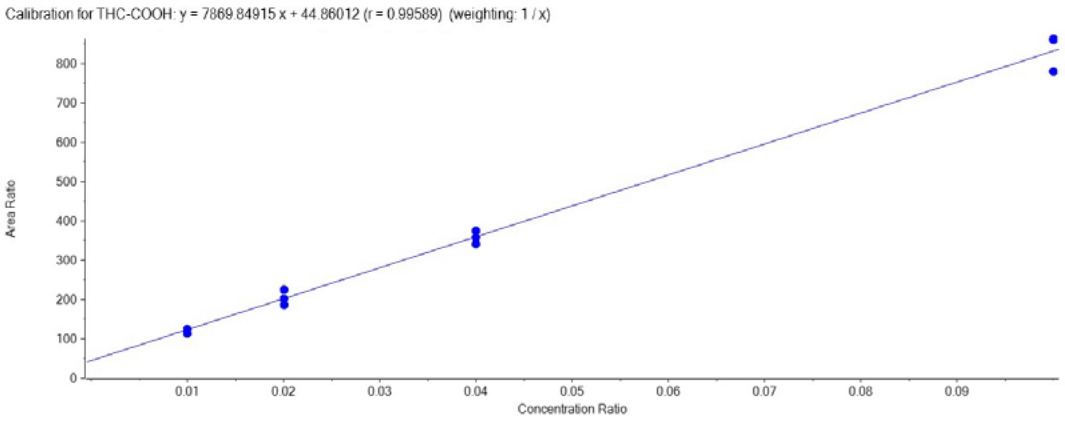

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for THC-COOH in hair (r > 0.996) evaluated over the concentration range in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: THC-COOH showed r values of >0.996 for the range measured in hair, as given in Table 1.

Reproducibility: At the LLOQ, the precision (%CV) was 8.4% for THC-COOH, as determined by n=3 replicates (Table 1). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine QTRAP MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for THC-COOH quantitation in hair matrix.

Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for Aldosterone

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze aldosterone in serum matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum spiked with aldosterone was processed using the following conditions:

Sample Prep Conditions
Liquid-Liquid Extraction with MTBE

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C8
Mobile Phase A: Water/Ammonium Fluoride
Mobile Phase B: Methanol/Ammonium Fluoride
Flow Rate: 0.6ml/min
Injection Volume: 20ul
Gradient: Linear 5-98% B over 12 minutes
Retention Time: 5.9 minutes

Mass Spectrometry Conditions
Method Duration: 12 minutes
Polarity: Positive Negative ESI
Transitions: 359-189
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (units)	%CV (at LLOQ)	S/N
Aldosterone	1-10000 pg/ml	8.7%	44*

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) for aldosterone.

*S/N calculated by subtracting recorded S/N measured in a blank extract from the recorded compound S/N.

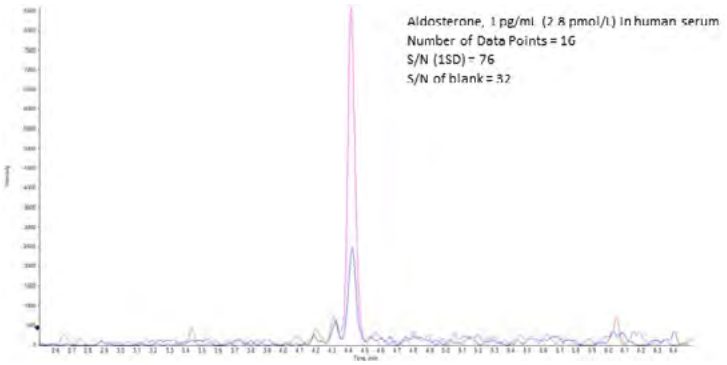

Figure 1. Chromatogram for aldosterone at 1 pg/mL (2.8 pmol/L) in human serum using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

Contents

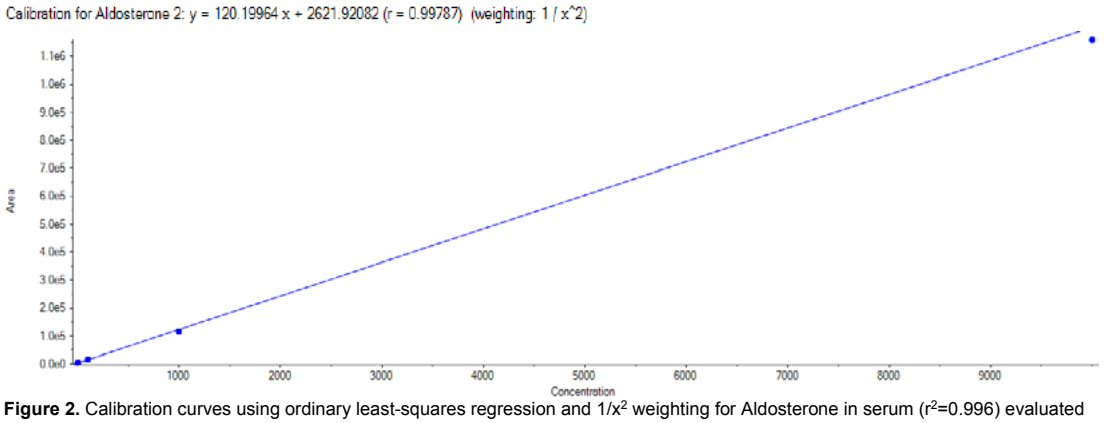

Figure 2. Calibration curves using ordinary least-squares regression and $1/x^2$ weighting for Aldosterone in serum (r^2=0.996) evaluated over the concentration range in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: Aldosterone showed r^2 values of >0.995 for the range measured in serum.

Reproducibility: At the LLOQ, the precision (%CV) was 8.8% for aldosterone, determined by n=3 replicates at the LLOQ of 1 pg/mL (Table 1). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for quantitation of aldosterone in serum matrix.

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Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for Total Testosterone

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze total testosterone in serum matrix.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum spiked with testosterone was processed using the following conditions:

Sample Prep Conditions
Liquid-Liquid Extraction with MTBE

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C8
Mobile Phase A: Water/Ammonium Fluoride
Mobile Phase B: Methanol/Ammonium Fluoride
Flow Rate: 0.6ml/min
Injection Volume: 20ul
Gradient: Linear 5-98% B over 12 minutes
Retention Time: 5.9 minutes

Mass Spectrometry Conditions
Method Duration: 12 minutes
Polarity: Positive ESI
Transitions: 289-97
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (units)	%CV (at LLOQ)	S/N
Testosterone	0.5-10000 pg/mL	8.1%	13

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) for testosterone.

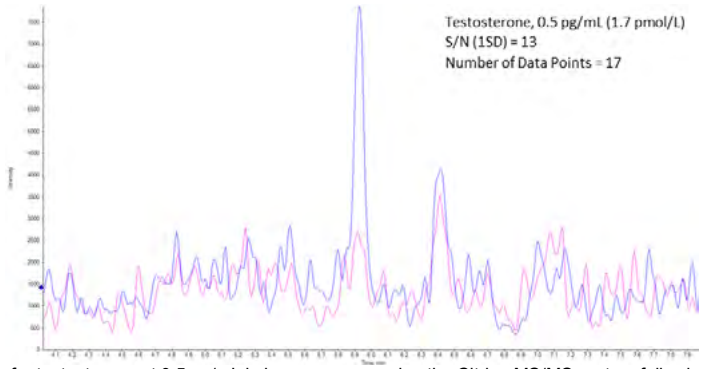

Figure 1. Chromatogram for testosterone at 0.5 pg/mL in human serum using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

Contents

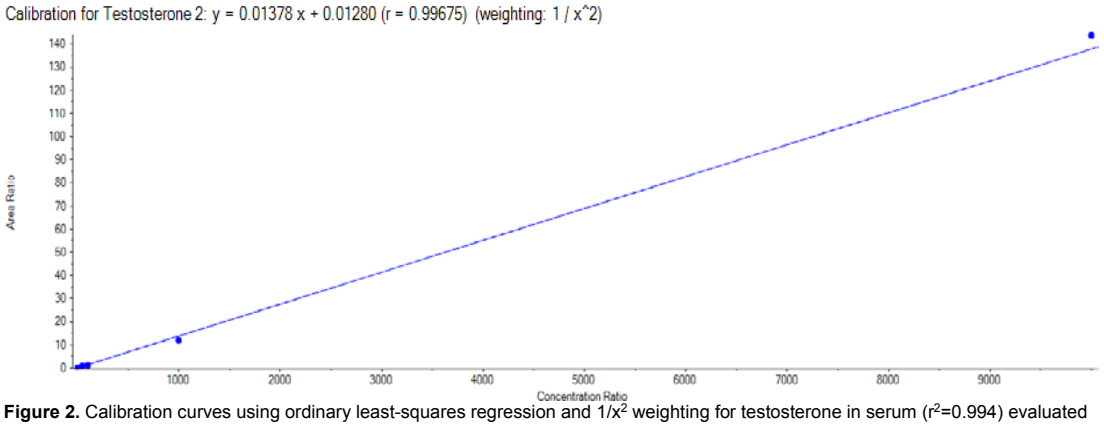

Figure 2. Calibration curves using ordinary least-squares regression and $1/x^2$ weighting for testosterone in serum (r^2=0.994) evaluated over the concentration range in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: Testosterone showed r^2 values of >0.996 for the range measured in serum, as given in Table 1.

Reproducibility: At the LLOQ, the precision (%CV) was 8.12% for Testosterone, determined by n=3 replicates (Table 1). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for total testosterone quantitation in serum matrix.

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Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for Estrone, Estradiol, and Estriol

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze estrone, estradiol, and estriol in serum matrix

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum spiked with estrone, estradiol, and estriol was processed using the following conditions:

Sample Prep Conditions
Liquid-Liquid Extraction (LLE) with MTBE

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C8
Mobile Phase A: Water/Ammonium Fluoride
Mobile Phase B: Methanol/Ammonium Fluoride
Flow Rate: 0.6ml/min
Injection Volume: 20ul
Gradient: Linear 5-98% B over 12 minutes
Retention Time: 5.9 minutes

Mass Spectrometry Conditions
Method Duration: 12 minutes
Polarity: Negative ESI
Transitions: Compound dependent
Source Conditions: Flow rate-optimized

Results

Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2. Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1.

Compound	Range (units)	%CV (at LLOQ)	S/N
Estrone	1-10,000 pg/mL	4.6	394
Estradiol	1-10,000 pg/mL	9.2	30.5*
Estriol	1-10,000 pg/mL	18.4	143

Table 1. Measured concentration range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) for each compound evaluated.

*S/N calculated by subtracting recorded S/N measured in a blank extract from the recorded compound S/N.

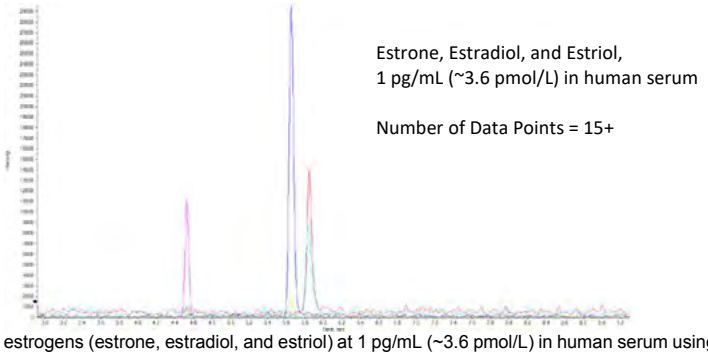

Figure 1. Chromatogram for estrogens (estrone, estradiol, and estriol) at 1 pg/mL (~3.6 pmol/L) in human serum using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

Contents

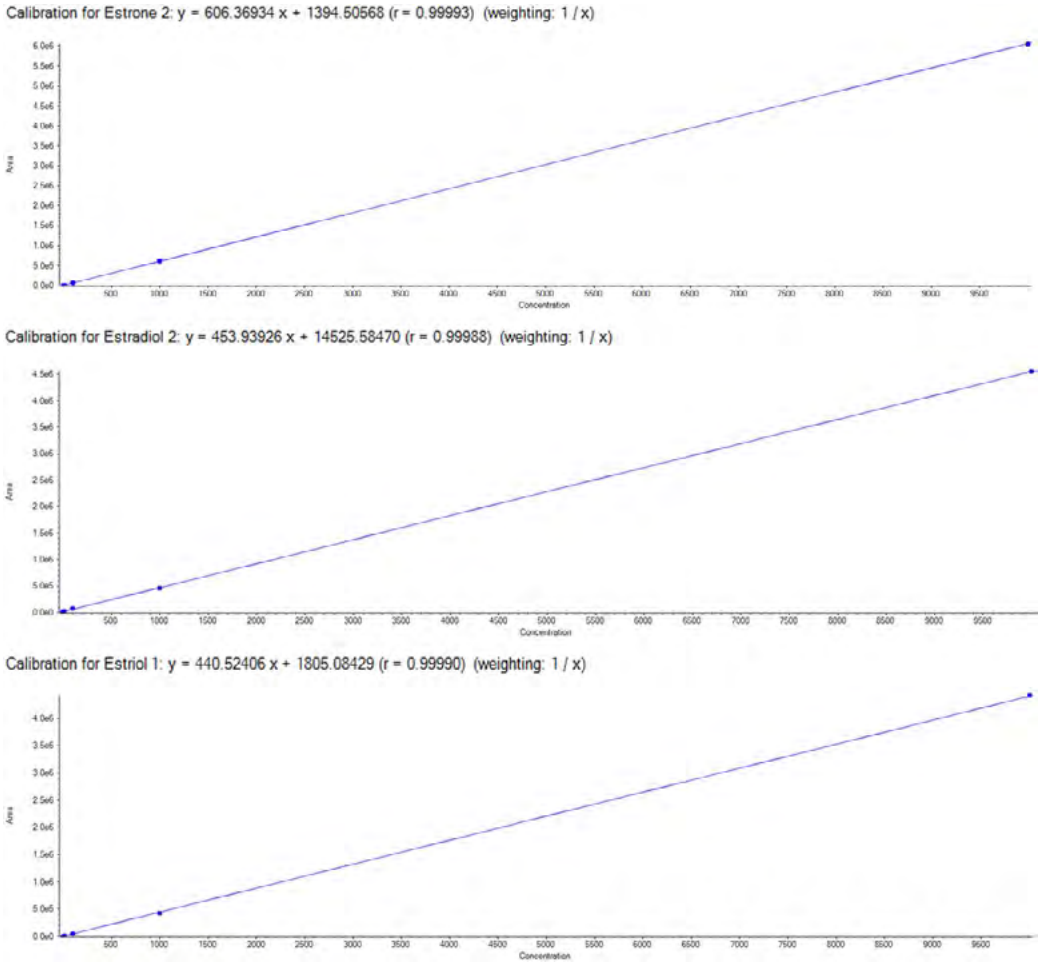

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for estrone, estradiol and estriol in serum (r > 0.999) evaluated over the concentration range in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All estrogens showed r values of ≥0.999 for the range measured in serum (Table 1).

Reproducibility: At 1 pg/mL, the precision (%CV) was 4.6% for estrone, 9.2% for estradiol, and 18.4% for estriol, as determined by n=3 replicates (Table 1). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for quantitation of estrogens in serum matrix.

Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for Cortisol, 11-Deoxycortisol, 21-Deoxycortisol, 17-Hydroxyprogesterone, and Androstenedione.

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

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This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze cortisol, 11-deoxycortisol, 21-deoxycortisol, 17-hydroxyprogesterone, and androstenedione in serum matrix.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum spiked with the compounds evaluated was processed using the following conditions:

Sample Prep Conditions
Liquid-Liquid Extraction of 200 µL sample with MTBE

Liquid Chromatography Conditions
Column: Waters Acquity HSS T3
Mobile Phase A: Water + Formic Acid + Ammonium Acetate
Mobile Phase B: Methanol + Formic Acid + Ammonium Acetate
Flow Rate: 0.6 mL/min
Injection Volume: 20 µL
Stepped Gradient: 45-98% B over 4.5 minutes
Retention Time: 1.8 – 3.2 minutes

Mass Spectrometry Conditions
Method Duration: 4.5 minutes
Polarity: Positive Electrospray
Transitions: Compound Dependent
Source Conditions: Flow rate-optimized

Results

Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2. Analytical performance statistics including the concentration range evaluated and results from precision experiments (n=3 replicates), are shown in Table 1.

Compounds	Range (ng/mL)	%CV (at lowest cal)
Cortisol	0.1-500	10%
11-Deoxycortisol	0.1-100	11%
21-Deoxycortisol	0.1-100	10%
17-Hydroxyprogesterone	0.1-500	5%
Androstenedione	0.1-500	1%

Table 1. Measured range, percent coefficient of variation (%CV) at lowest calibrator (cal), for each compound evaluated.

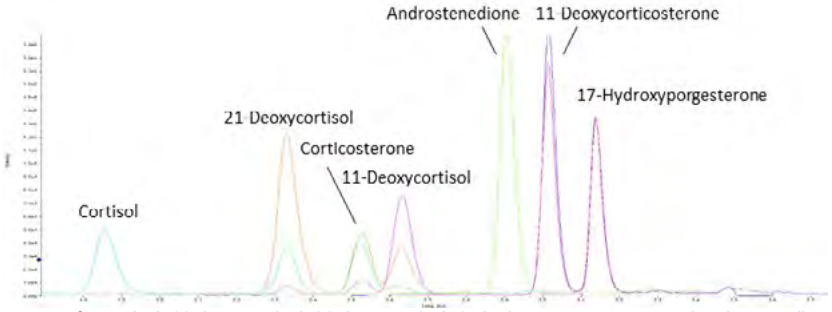

Figure 1. Chromatogram for cortisol, 11-deoxycortisol, 21-deoxycortisol, 17-hydroxyprogesterone, and androstenedione at 0.1 ng/mL in human serum using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions. Chromatographic resolution of 17-hydroxyprogesterone from its isobaric interference, 11-deoxycorticosterone, was achieved in a 4.5 minute run time. Additionally, 21-deoxycortisol, 11-deoxycortisol and the interference corticosterone, were fully separated.

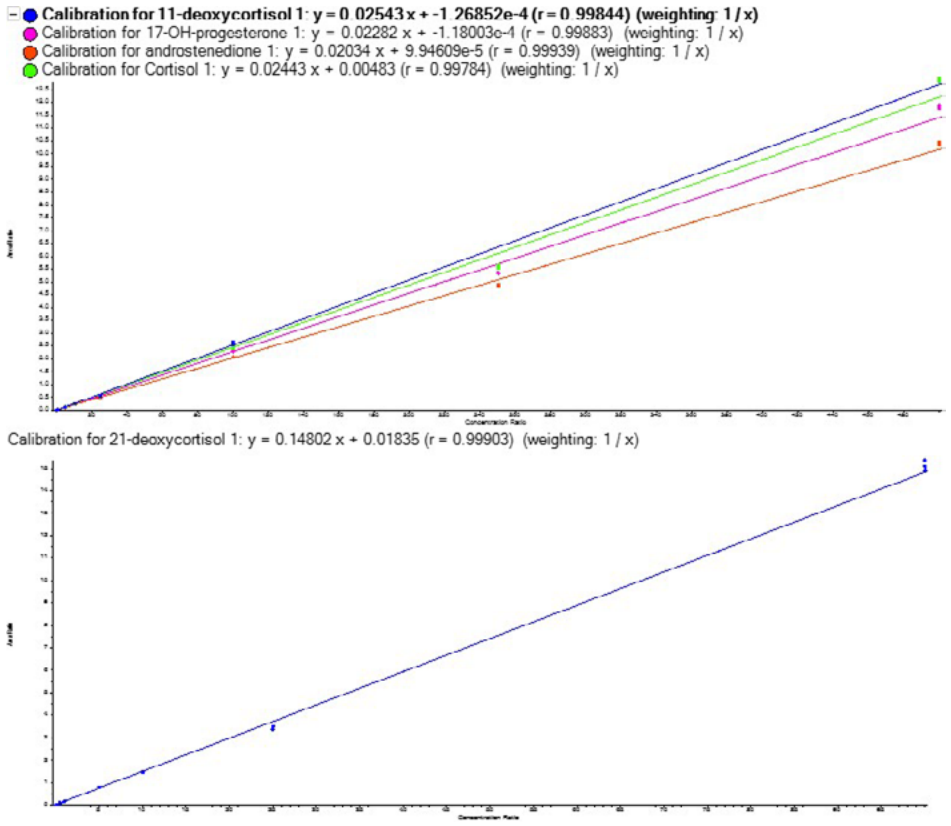

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for (a) cortisol, 11-deoxycortisol, 17-hydroxyprogesterone, androstenedione, and (b) 21-deoxycortisol in serum (r > 0.998) evaluated over the concentrations in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: Linearity for all calibration curves showed r values of >0.998 for the range measured in serum.

Reproducibility: For the lowest calibrator evaluated, the precision (%CV) was <11% for the compounds evaluated, as determined by n=3 replicates (Table 1). CV data is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for cortisol, 11-deoxycortisol, 21-deoxycortisol, 17-hydroxyprogesterone, and androstenedione in serum matrix.

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Citrine® MS/MS Analytical Performance Data Sheet

Illustration of Analytical Performance for Simultaneous Analysis of Aldosterone, Estradiol, Estriol, Estrone, Androstenedione, Corticosterone, Cortisol, Cortisone, 11-Deoxycortisol, 21-Deoxycortisol, DHEA, 17-Hydroxyprogesterone, 21-Hydroxyprogesterone, Prednisone, Testosterone

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze aldosterone, estradiol, estriol, estrone, androstenedione, corticosterone, cortisol, cortisone, 11-deoxycortisol, 21-deoxycortisol, DHEA, 17-hydroxyprogesterone, 21-hydroxyprogesterone, prednisone, and testosterone in serum matrix.

Materials and Methods

The Citrine MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum spiked with the compounds listed was processed using the following conditions:

Sample Prep Conditions
Liquid-Liquid Extraction of 500 µL sample with MTBE

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C18
Mobile Phase A: Water/NH_4F
Mobile Phase B: Methanol/NH_4F
Flow Rate: 0.6 mL/min
Injection Volume: 20 µL
Gradient: Linear, 5-98% B over 12 minutes
Retention Time: Compound Dependent, from 5-9 minutes

Mass Spectrometry Conditions
Method Duration: 12 minutes
Polarity: ESI with Pos/Neg Switching
Transitions: Compound Dependent
Source Conditions: Flow rate-optimized

Results

Analytical performance including the concentration range, precision (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatogram of the compounds evaluated is shown in Figure 1.

Compounds	Range (pg/mL)	%CV (at LLOQ)	S/N (at LLOQ)
Negative Polarity			
Aldosterone	1-10,000	8.7%	44*
Estradiol	1-10,000	9.2%	31*
Estriol	1-10,000	18.4%	143
Estrone	1-10,000	4.6%	394
Positive Polarity			
Androstenedione	1-10,000	8.7%	14*
Corticosterone	1-10,000	10.8%	6
Cortisol	1-10,000	13.5%	11*
Cortisone	1-10,000	5.6%	30*
11-Deoxycortisol	1-10,000	4.0%	30*
21-Dexoycortisol	1-10,000	3.2%	4
DHEA	1-10,000	19.4%	15
17-Hydroxyprogesterone	1-10,000	12.0%	45*
21-Hydroxyprogesterone	1-10,000	5.4%	12*
Prednisone	1-10,000	9.0%	26
Testosterone	1-10,000	3.7%	22

Table 1. Measured concentration range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and signal-to-noise (S/N) at LLOQ for each compound evaluated. *S/N calculated by subtracting recorded S/N measured in a blank extract from the recorded compound S/N.

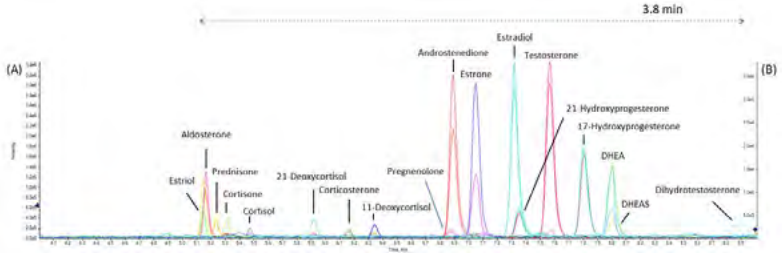

Figure 1. Chromatogram of 18 steroid compounds extracted from serum employing rapid polarity switching (10 ms) between positive and negative ESI modes. Here, (A) compounds analyzed in positive mode and (B) in negative mode are displayed from a single injection, where two MRM transitions were monitored per compound with 10+ points across the peaks for all compounds.

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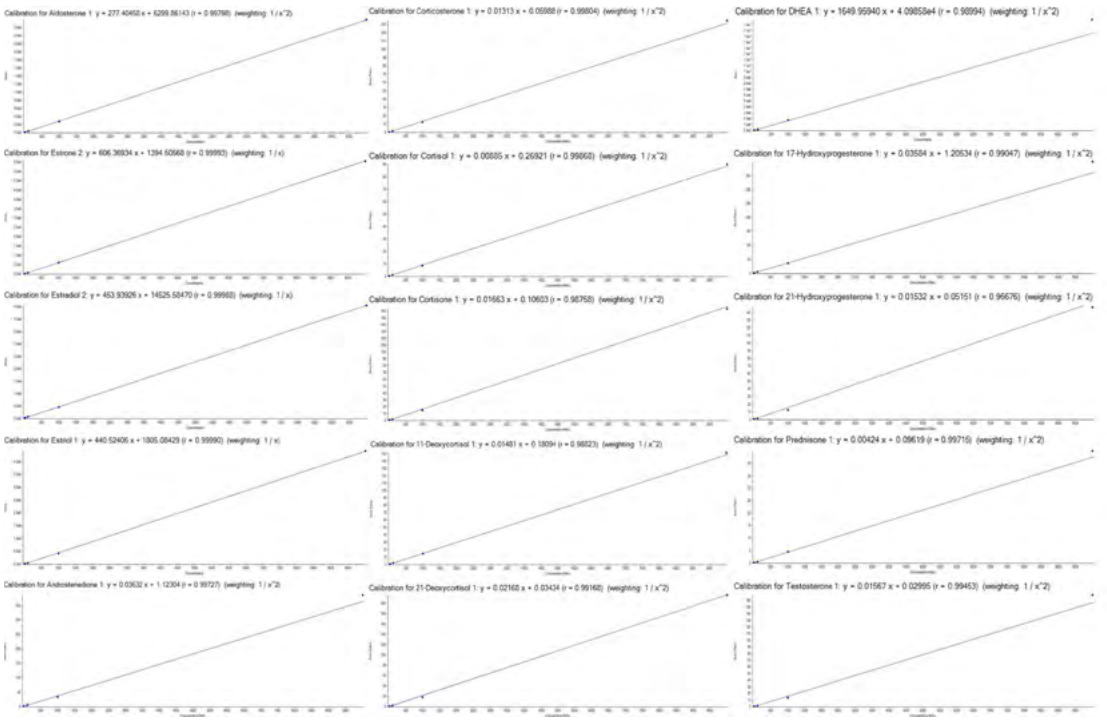

Figure 2. Calibration curves for aldosterone, estradiol, estriol, estrone, androstenedione, corticosterone, cortisol, cortisone, 11-deoxycortisol, 21-deoxycortisol, DHEA, 17-hydroxyprogesterone, 21-hydroxyprogesterone, prednisone, testosterone in serum matrix, demonstrating a dynamic range of at least five orders of magnitude.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds exhibited a linear dynamic range of at least five orders of magnitude for the ranges measured in serum.

Reproducibility: At each LLOQ, the precision (%CV) was <19.4% for all compounds evaluated, as determined by n=3 replicates (Table 1). CV data for all compounds evaluated is based on calculated concentration with internal standard.

The Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for Aldosterone, Estradiol, Estriol, Estrone, Androstenedione, Corticosterone, Cortisol, Cortisone, 11-Deoxycortisol, 21-Deoxycortisol, DHEA, 17-Hydroxyprogesterone, 21-Hydroxyprogesterone, Prednisone, and Testosterone in serum matrix.

Contents

Citrine® MS/MS Analytical Performance Data Sheet

Illustration of analytical performance for long term robustness of serum extracts

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Citrine LC-MSMS System to analyze extracted serum samples over a period of extended continuous operation.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The Citrine LC-MS/MS System was controlled, and data processed using Analyst™ MD Software 1.6.3. Serum samples spiked at a mid-range concentration with Testosterone and Aldosterone, and were processed and analysed as described below.

Sample Prep Conditions
Protein precipitation with methanol

Liquid Chromatography Conditions
Column: Phenomenex Kinetex C8
Mobile Phase A: WaterAmmonium Fluoride
Mobile Phase B: Methanol/Ammonium Fluoride
Flow Rate: 0.6ml/min
Injection Volume: 20ul
Gradient: Linear gradient over 2.5 minutes

Mass Spectrometry Conditions
Method Duration: 10 minutes
Polarity: Switching between Positive and Negative ESI
Transitions: Compound optimized
Source Conditions: Flow rate-optimized

Results

Robustness data, assessed using unadjusted peak area data without internal standard correction (n=3500), acquired over a 6-day period of continuous 24/7 instrument operation, without operator intervention is shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Metric plots of individual peak area data are shown in Figure 2.

Compound	Peak Area Mean	Peak Area Standard Deviation	Peak Area CV
Testosterone	458700	22080	4.8
Aldosterone	64560	4415	6.8

Table 1. Uncorrected peak area statistical data for each compound analyzed.

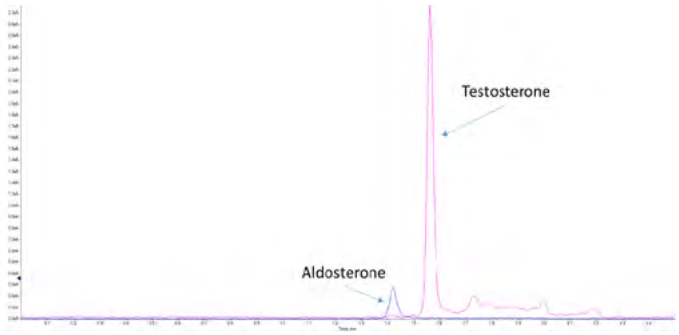

Figure 1. Chromatogram for all evaluated compounds using the Citrine System following the sample preparation and LC-MS/MS conditions.

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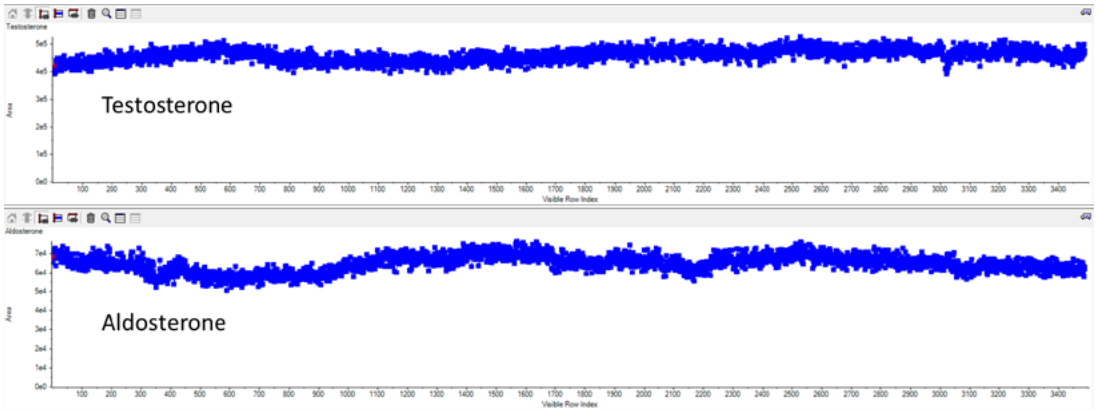

Figure 2. Metric plots of individual, uncorrected peak area values (n=3500) used to generate statistical data in Table 1.

Conclusions

Based on the above performance testing, all CVs based on uncorrected (no internal standard) peak area data for all compounds were <7%. Individual values were recorded as 4.8% for Testosterone and 6.8% for Aldosterone.

The Citrine System exhibited capability to deliver reproducible analytical performance in extracted serum over a 6-day continuous (24hr/day) period of operation for a series of 3500 injections without operator intervention.

4500MD LC-MS/MS Medical Device System Analytical Performance

SCIEX
The Power of Precision

Contents

Triple Quad™ 4500MD System Analytical Performance Data Sheet

Illustration of Analytical Performance for Metanephrine, Normetanephrine and 3-Methoxytyramine

The SCIEX Triple Quad 4500MD system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD System to analyze Metanephrine, Normetanephrine and 3-Methoxytyramine (3-MT) in plasma matrix

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The Triple Quad 4500MD System was controlled and data processed using Analyst® MD Software 1.6.3. Plasma samples calibrators and QCs were processed using a commercially available kit (Chromsystems, Munich, Germany):

Sample Prep Conditions
Solid Phase Extraction as per kit

Liquid Chromatography Conditions
Column: Provided in Kit
Mobile Phase A: Provided in Kit
Mobile Phase B: Provided in Kit
Flow Rate: 1.0 mL/min
Injection Volume: 25 µL
Gradient: Linear, 0-100% B over 5 min
Retention Time: Compound dependent: 3.2-3.6 min

Mass Spectrometry Conditions
Polarity Positive: ESI
Transitions: Compound Dependent
Source Conditions: Flow Rate-Optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=3 replicates), and signal-to-noise (S/N) are shown in Table 1. Chromatograms of the compounds evaluated utilizing the described method are shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (units)	%CV (Cal 1)	S/N* (Cal 1)
Metanephrine	24.9-2738 ng/L	5.2	38.8
Normetanephrine	27.1-4159 ng/L	7.1	10.4
3-Methoxytyramine	14.4-2646 ng/L	5.6	13.4

Table 1. Measured range, percent coefficient of variation (%CV) and signal-to-noise (S/N) Metanephrine, Normetanephrine and 3-Methoxytyramine.

* Calculated by a peak-to-peak algorithm

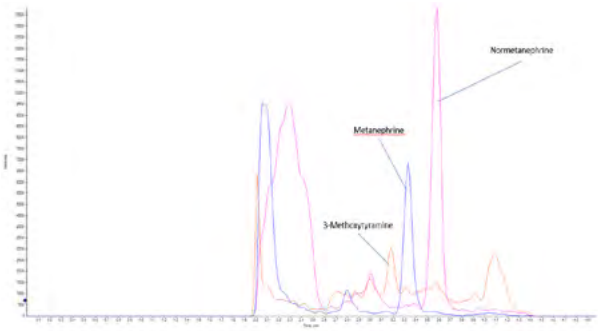

Figure 1. Chromatogram of extracted commercial QC 1 for all evaluated compounds using the Triple Quad 4500MD System following the sample preparation and LC-MS/MS conditions.

IVD-MKT-02-9750-A

Contents

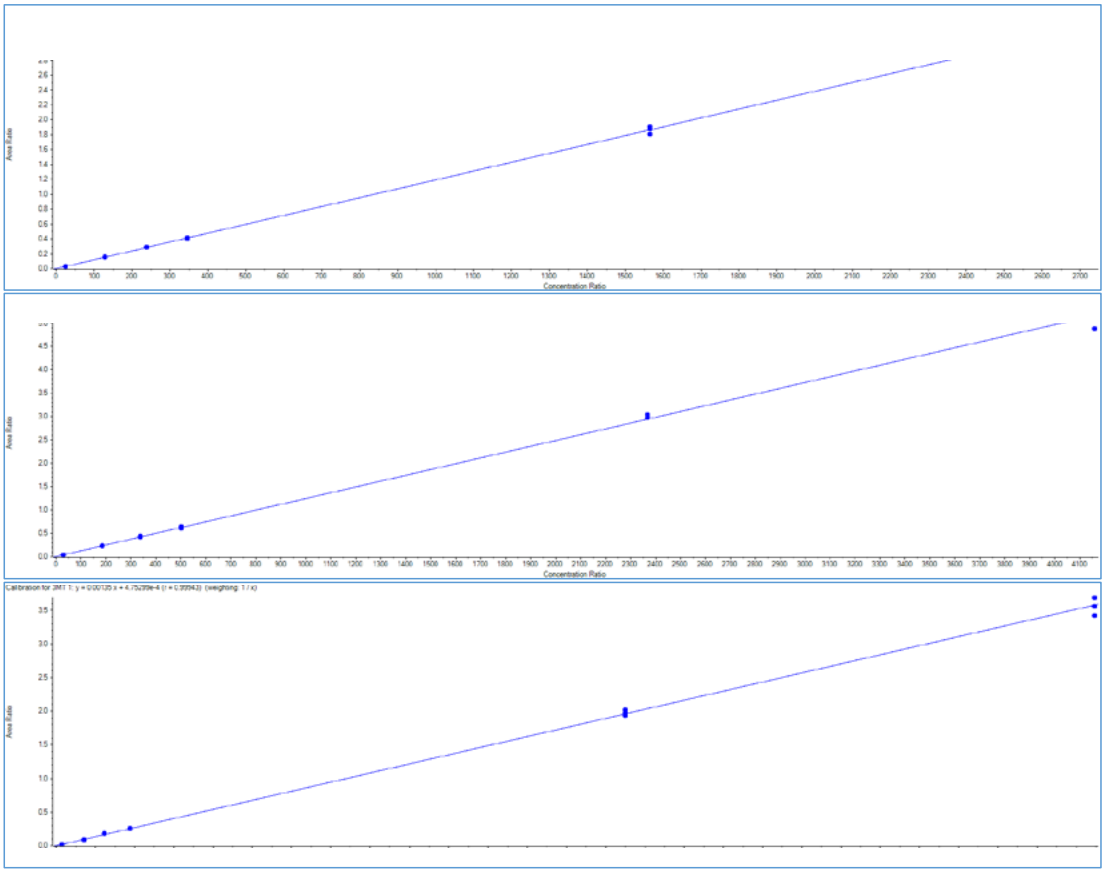

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for all the compounds evaluated over the defined concentration ranges in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds exhibited a linear dynamic range of greater than 3 orders of magnitude. Linearity (r2) is shown to be ≥ 0.999 for all analytes.

Reproducibility: At the lowest calibrator measured, the precision (%CV) was <6% for all compounds evaluated as determined by n=3 replicates. CV data is based on calculated concentration with internal standard.

Sensitivity: The lowest concentration calibrator extracted and analysed showed signal:noise values (based on a peak-to-peak algorithm) of >10:1 for all analytes

The SCIEX Triple Quad 4500MD LC-MSMS System exhibited capability to deliver sensitive and reproducible analytical performance for Metanephrine, Normetanephrine and 3-Methoxytyramine in plasma matrix.

IVD-MKT-02-9750-A

SCIEX Triple Quad™ 4500MD System Analytical Performance Data Sheet

Illustration of Analytical Performance for Testosterone, Androstenedione, Cortisone, Cortisol, 11-Deoxycortisol, Corticosterone, 17-Hydroxyprogesterone, DHEA, and Progesterone

The SCIEX Triple Quad 4500MD LC-MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD LC-MS/MS system to analyze the compounds Testosterone, Androstenedione, Cortisone, Cortisol, 11-Deoxycortisol, Corticosterone, 17-Hydroxyprogesterone, DHEA, and Progesterone in serum matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The Triple Quad 4500MD LC-MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum calibrators and quality controls (UTAK) containing the compounds of interest were processed using the following conditions:

Sample Preparation Conditions
Protein precipitation of 100 µL sample with ZnSO4 in methanol, centrifugation and direct injection.

Liquid Chromatography Conditions
Column: Phenomenex® Kinetix ® C8
Mobile Phase A: Water
Mobile Phase B: 95% Methanol
Flow Rate: 0.6 mL/min
Injection Volume: 50 µL
Gradient: Linear, 10-100% B over 6.5 min
Retention Time: Compound dependent: 3.0-4.5 min

Mass Spectrometry Conditions
Method Duration: 6.5 min
Polarity Positive: APCI
Transitions: Compound Dependent
Source Conditions: Flow Rate-Optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=6 replicates), and functional sensitivity are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (units)	%CV at LLOQ	Functional Sensitivity*
Testosterone	10-1000 ng/dL	13%	4.12
Androstenedione	5-500 ng/dL	14.8%	1.20
Cortisone	0.06-6 µg/dL	8.2%	0.0033
Cortisol	0.025-25 µg/dL	17.5%	0.0064
11-Deoxycortisol	4-400 ng/dL	13.5%	0.77
Corticosterone	10-1000 ng/mL	6.7%	16.85
17OH-Progesterone	10-1000 ng/dL	13.9%	2.18
DHEA	1-10 ng/mL	9.6%	0.41
Progesterone	0.25-25 ng/mL	10.3%	0.20

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and functional sensitivity for each compound evaluated.

**Functional sensitivity is defined here as the lowest concentration achieving a CV of 20%.*

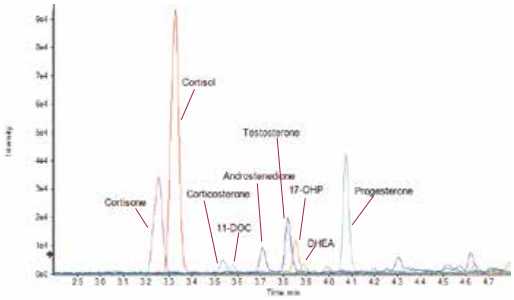

Figure 1. Chromatogram for all evaluated compounds using the SCIEX Triple Quad 4500MD LC-MS/MS system following the sample preparation and LC-MS/MS conditions.

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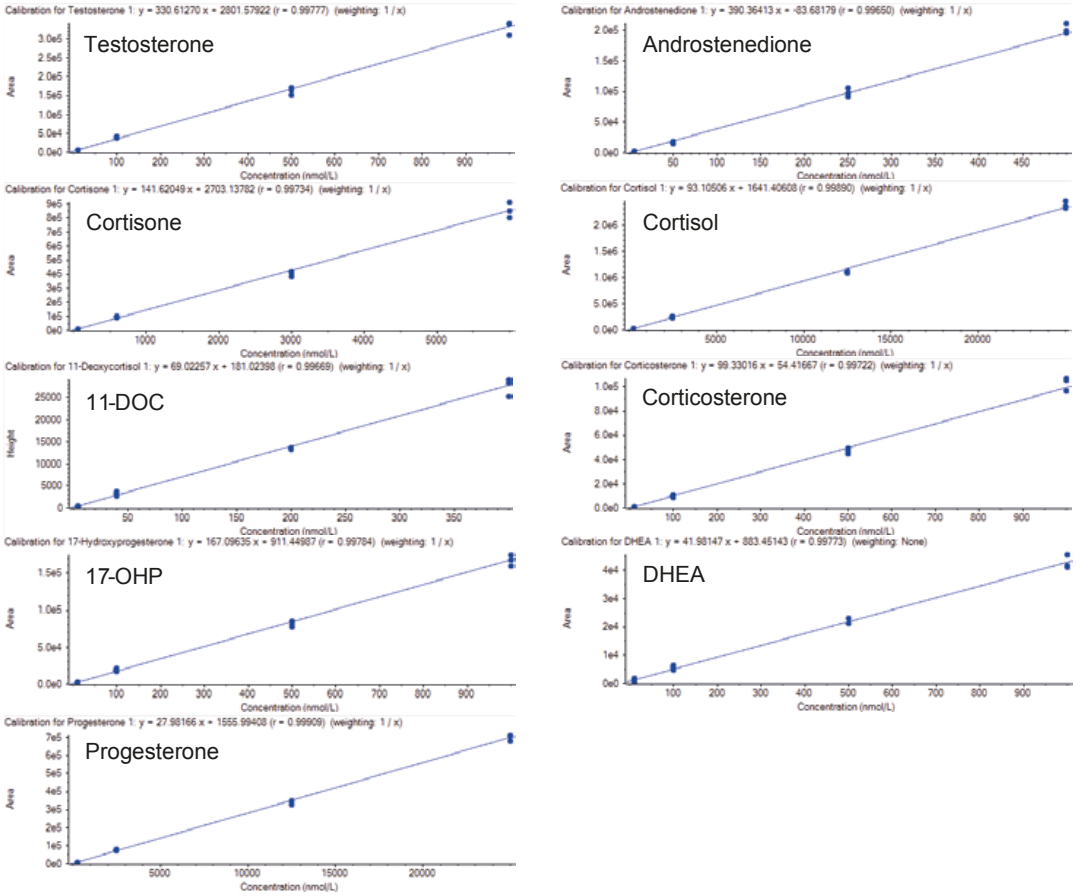

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for all the compounds evaluated over the defined concentration ranges in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds exhibited a linear dynamic range of at least 3 orders of magnitude.

Reproducibility: At each LLOQ, the precision (%CV) was <18% for all compounds evaluated as determined by n=6 replicates. CV data is based on calculated concentration without internal standard.

Sensitivity: Functional sensitivity, as defined by a CV of <20%, was <5 ng/dL for all compounds with the exception of Cortisol (6.4 ng/dL) and Corticosterone (16.85 ng/dL).

The SCIEX Triple Quad 4500MD LC-MS/MS system exhibited capability to deliver sensitive and reproducible analytical performance for Testosterone, Androstenedione, Cortisone, Cortisol, 11-Deoxycortisol, Corticosterone, 17-Hydroxyprogesterone, DHEA, and Progesterone in serum matrix.

IVD-MKT-02-8586-B

SCIEX Triple Quad 4500MD™ System Analytical Performance Data Sheet

Illustration of Analytical Performance for Methylmalonic Acid in Serum

The SCIEX Triple Quad 4500MD system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD system to analyze the compounds methylmalonic acid in serum matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials & Methods

The SCIEX Triple Quad 4500MD system was controlled and data processed using Analyst® MD Software 1.6.3. A commercially available kit for the analysis of methylmalonic acid in serum (Instruchemie B.V.) was processed using the following conditions:

Sample Prep Conditions
Protein precipitation as per kit instructions

Liquid Chromatography Conditions
Column: Phenomenex Luna Omega
Mobile Phase A: Provided in Kit
Mobile Phase B: Provided in Kit
Flow Rate: 0.65 – 0.8 mL/min
Injection Volume: 20 µL
Gradient: Linear, 5-100% B over 4 min
Retention Time: 1.15 min

Mass Spectrometry Conditions
Method Duration: 4 minutes
Polarity: Positive ESI
Source Conditions: Flow Rate-Optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=6 replicates), and functional sensitivity are shown in Table 1. Chromatogram of methylmalonic acid utilizing the described method is shown in Figure 1. Calibration curve over the defined concentration range for methylmalonic acid is illustrated in Figure 2.

Compound	Range (nmol/L)	%CV at LLOQ	Functional Sensitivity*
Methylmalonic Acid	216-1430	6.1%	0.23

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and functional sensitivity for methylmalonic acid.

**Functional sensitivity is defined here as the lowest concentration achieving a CV of 20%.*

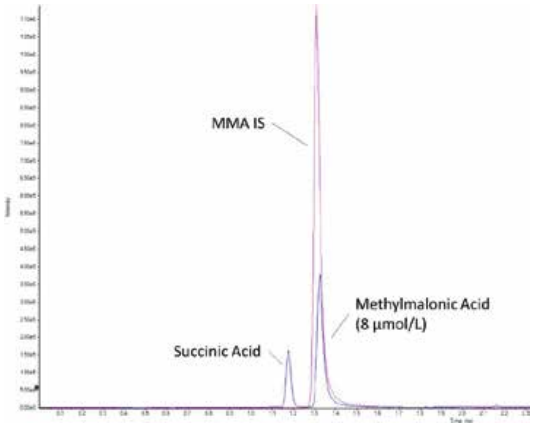

Figure 1. Chromatogram for methylmalonic acid and its deuterated internal standard using the SCIEX Triple Quad 4500MD system following the sample preparation and LC-MS/MS conditions.

IVD-MKT-02-8586-B

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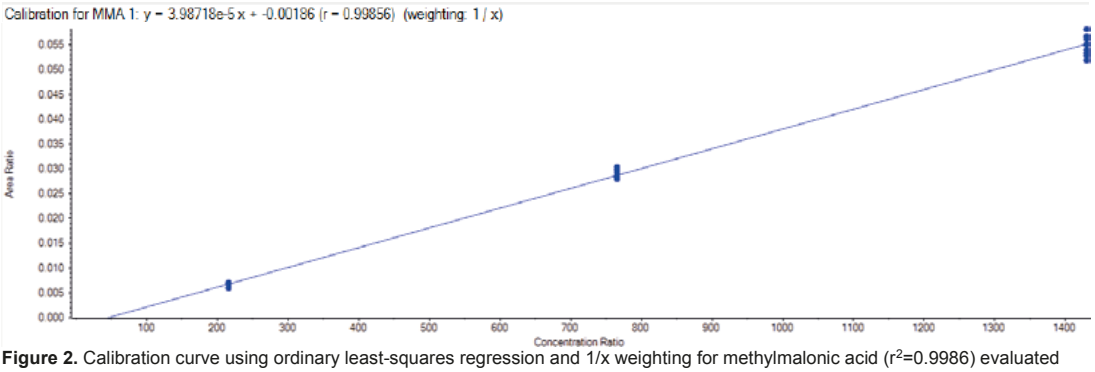

Figure 2. Calibration curve using ordinary least-squares regression and 1/x weighting for methylmalonic acid (r^2=0.9986) evaluated over the defined concentration range in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: MMA exhibited a linear dynamic range of approximately 3 orders of magnitude.

Reproducibility: The precision (%CV) was 6.1% for MMA as determined by n=6 replicates at the LLOQ. CV data is based on calculated concentration with internal standard.

Sensitivity: Functional sensitivity, as defined by the lowest measureable concentration with a CV of <20%, was <0.3 nmol/L for MMA.

The SCIEX Triple Quad 4500MD system exhibited capability to deliver sensitive and reproducible analytical performance of MMA in serum matrix.

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SCIEX Triple Quad™ 4500MD System Analytical Performance Data Sheet

Illustration of analytical performance for Caspofungin, Itraconazole, Hydroxyitraconazole, Voriconazole, and Fluconazole

The SCIEX Triple Quad 4500MD LC-MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD LC-MS/MS system to analyze the compounds Caspofungin, Itraconazole, Hydroxyitraconazole, Voriconazole, and Fluconazole in serum matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The SCIEX Triple Quad 4500MD LC-MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available serum calibrators and quality controls (UTAK) containing all compounds of interest were processed using the following approach:

Sample Prep Conditions
Protein Crash with Methanolic Zinc Sulphate solution using 100ul plasma, centrifugation and direct injection

Liquid Chromatography Conditions
Column: Phenomenex® Kinetex® Biphenyl
Mobile Phase A: Water/0.1% Formic Acid
Mobile Phase B: Methanol/0.1% Formic Acid
Flow Rate: 0.6ml/min
Injection Volume: 25ul
Gradient: Linear 5-98%B over 5 minutes
Retention Time: Compound dependent 1.5-3 minutes

Mass Spectrometry Conditions
Method Duration: 5 minutes
Polarity: Positive electrospray
Transitions: Compound dependent
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=6 replicates), and functional sensitivity are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (µg/mL)	%CV at LLOQ	Functional Sensitivity*
Caspofungin	0.09-1.8	10.6%	0.078
Fluconazole	0.2-10	16.5%	0.142
Hydroxyitraconazole	0.05-1	12.0%	0.067
Itraconazole	0.05-1	18.3%	0.174
Voriconazole	0.005-2.5	7.6%	0.033

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and functional sensitivity for each compound evaluated.

**Functional sensitivity is defined here as the lowest concentration achieving a CV of 20%.*

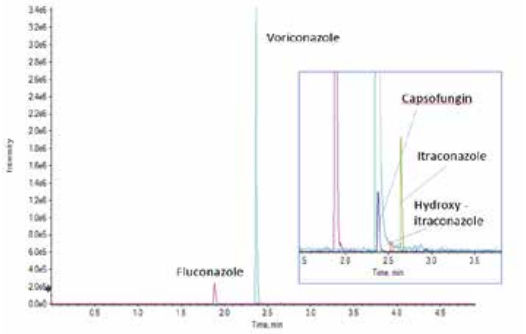

Figure 1. Chromatogram for all evaluated compounds using the SCIEX Triple Quad 4500MD system following the sample preparation and LC-MS/MS conditions.

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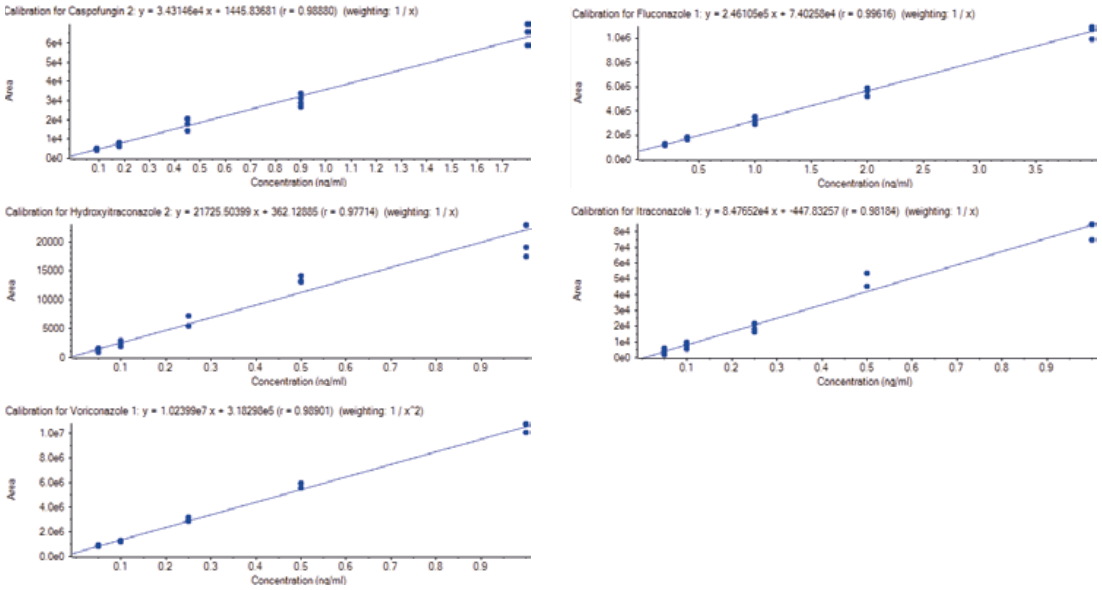

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for all the compounds evaluated over the defined concentration ranges in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds exhibited a linear dynamic range of at least 3 orders of magnitude.

Reproducibility: At each LLOQ, the precision (%CV) was <19% for all compounds evaluated as determined by n=6 replicates. CV data for all compounds evaluated is based on calculated concentration without internal standard.

Sensitivity: Functional sensitivity, as defined by a CV of <20%, was <0.2 µg/mL for all evaluated compounds.

The SCIEX Triple Quad 4500MD LC-MS/MS system exhibited capability to deliver sensitive and reproducible analytical performance for Caspofungin, Itraconazole, Hydroxyitraconazole, Voriconazole, and Fluconazole in serum matrix.

IVD-MKT-02-8586-B

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SCIEX Triple Quad™ 4500MD System Analytical Performance Data Sheet

Illustration of Analytical Performance for Methamphetamine, Morphine, Benzoylecgonine, Methadone, Phencyclidine, Amphetamine, and Oxazepam in Oral Fluid

The SCIEX Triple Quad 4500MD LC-MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD LC-MS/MS system to analyze the compounds Methamphetamine, Morphine, Benzoylecgonine, Methadone, Phencyclidine, Amphetamine, and Oxazepam in oral fluid matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The SCIEX Triple Quad 4500MD LC-MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available urine calibrators and quality controls (Bio-Rad) containing all compounds of interest were processed using the following conditions:

Sample Preparation Conditions
Dilution with Water/Acetonitrile solution using 200µL sample, followed by direct injection

Liquid Chromatography Conditions
Column: Phenomenex® Kinetex® Biphenyl
Mobile Phase A: Water/0.1% Formic Acid
Mobile Phase B: Acetonitrile/0.1% Formic Acid
Flow Rate: 0.7mL/min
Injection Volume: 5µL
Gradient: Gradient 10-85%B over 7 min
Retention Time: Compound dependent: 2.9-4.8 min

Mass Spectrometry Conditions
Method Duration: 7 min
Polarity: Positive electrospray
Transitions: Compound dependent
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=6 replicates), and functional sensitivity are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (ng/mL)	%CV at LLOQ	Functional Sensitivity*
Methamphetamine	0.75-750	9.5%	0.704
Morphine	1.5-1500	14.0%	0.757
Benzoylecgonine	0.225-225	16.4%	0.425
PCP	0.19-19	19.7%	0.224
Methadone	0.225-225	7.0%	0.083
Amphetamine	2.25-225	11.2%	0.520
Oxazepam	0.225-225	17.8%	0.262

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and functional sensitivity for each compound evaluated.

**Functional sensitivity is defined here as the lowest concentration achieving a CV of 20%.*

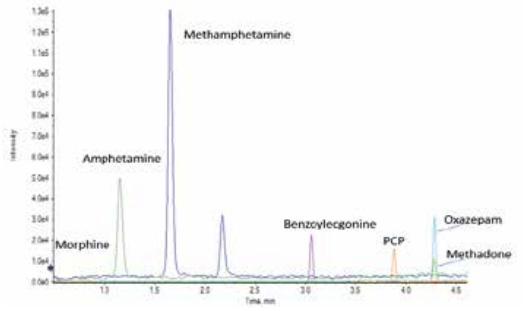

Figure 1. Chromatogram for all evaluated compounds using the SCIEX Triple Quad 4500MD system following the sample preparation and LC-MS/MS conditions.

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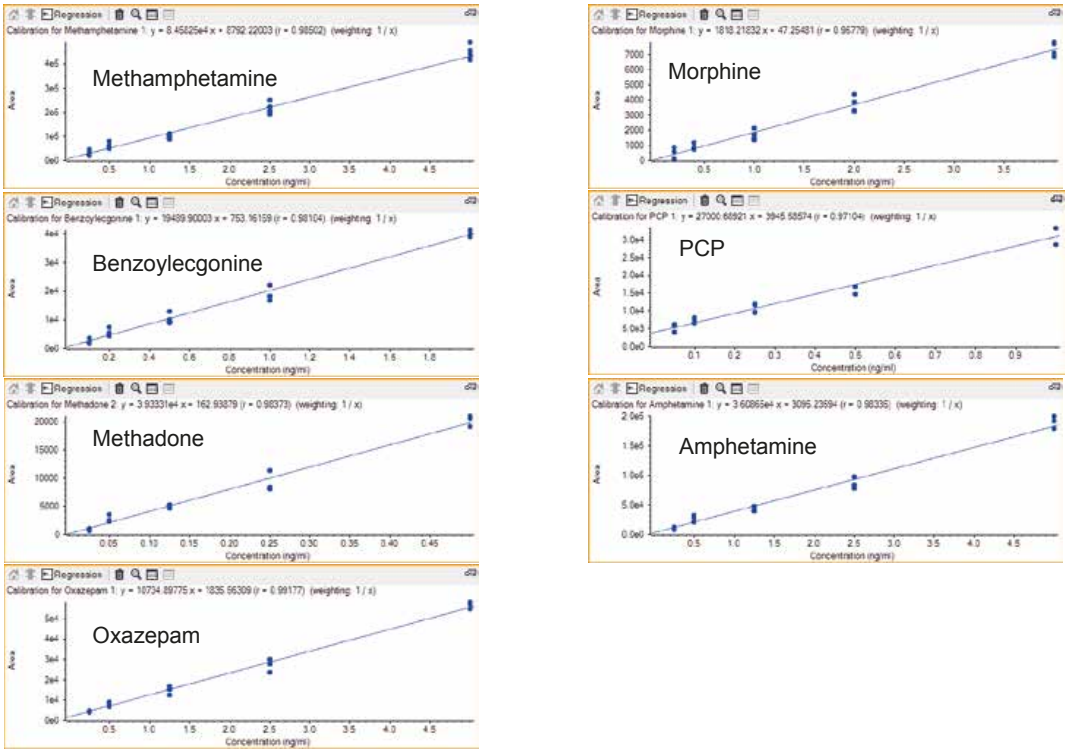

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for all the compounds evaluated over the defined concentration ranges in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds exhibited a linear dynamic range of at least 3 orders of magnitude.

Reproducibility: At each LLOQ, the precision (%CV) was <18% for all compounds evaluated as determined by n=6 replicates. CV data for all compounds evaluated is based on calculated concentration without internal standard.

Sensitivity: Functional sensitivity, as defined by a CV of <20%, was <0.8 ng/mL for all evaluated compounds.

The SCIEX Triple Quads 4500MD system exhibited capability to deliver sensitive and reproducible analytical performance for Methamphetamine, Morphine, Benzoylecgonine, Methadone, Phencyclidine, Amphetamine, and Oxazepam in oral fluid matrix.

IVD-MKT-02-8586-B

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SCIEX Triple Quad™ 4500MD System Analytical Performance Data Sheet

Illustration of Analytical Performance for Methamphetamine, Morphine, Benzoylecgonine, Methadone, Phencyclidine, Propoxyphene, and Methaqualone in Urine

The SCIEX Triple Quad 4500MD LC-MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD LC-MS/MS system to analyze the compounds Methamphetamine, Morphine, Benzoylecgonine, Methadone, Phencyclidine, Propoxyphene, and Methaqualone in urine matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The SCIEX Triple Quad 4500MD LC-MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available urine calibrators and quality controls (Bio-Rad) containing all compounds of interest were processed using the following approach:

Sample Preparation Conditions
Dilution with water/Acetonitrile solution using 200µL sample, followed by direct injection

Liquid Chromatography Conditions
Column: Phenomenex® Kinetex® Biphenyl
Mobile Phase A: Water/0.1% Formic Acid
Mobile Phase B: Acetonitrile/0.1% Formic Acid
Flow Rate: 0.7mL/min
Injection Volume: 5µL
Gradient: Gradient 10-85%B over 7 min
Retention Time: Compound dependent 2.9-4.8 min

Mass Spectrometry Conditions
Method Duration: 7 min
Polarity: Positive Electrospray
Transitions: Compound Dependent
Source Conditions: Flow Rate-Optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=6 replicates), and functional sensitivity are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (ng/mL)	%CV at LLOQ	Functional Sensitivity*
Methamphetamine	0.75-750	15.3%	1.99
Morphine	1.5-1500	14.2%	6.27
Benzoylecgonine	0.225-225	16.3%	85.14
PCP	0.19-19	12.2%	3.43
Methadone	0.225-225	15.4%	0.58
Propoxyphene	2.25-225	13.9%	7.81
Methaqualone	0.225-225	12.3%	0.11

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and functional sensitivity for each compound evaluated.

**Functional sensitivity is defined here as the lowest concentration achieving a CV of 20%.*

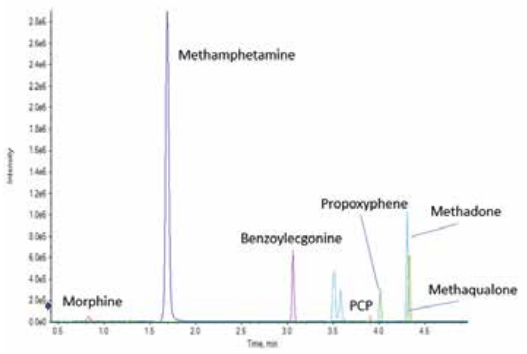

Figure 1. Chromatogram for all evaluated compounds using the SCIEX Triple Quad 4500MD LC-MS/MS system following the sample preparation and LC-MS/MS conditions.

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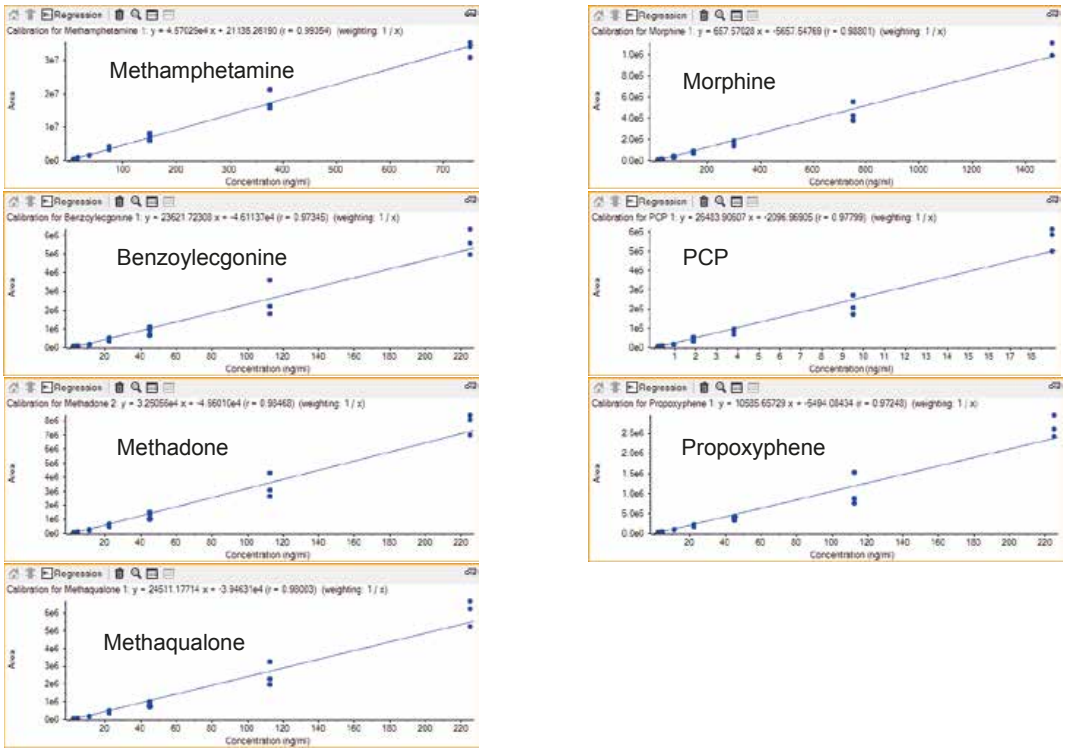

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for all the compounds evaluated over the defined concentration ranges in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds exhibited a linear dynamic range of at least 3 orders of magnitude.

Reproducibility: At each LLOQ, the precision (%CV) was <17% for all compounds evaluated as determined by n=6 replicates. CV data for all compounds evaluated is based on calculated concentration without internal standard.

Sensitivity: Functional sensitivity, as defined by a CV of <20%, was <8 ng/mL for all compounds with the exception of Benzoylecgonine (85.1ng/mL)

The SCIEX Triple Quad 4500MD LC-MS/MS system exhibited capability to deliver sensitive and reproducible analytical performance for Methamphetamine, Morphine, Benzoylecgonine, Methadone, Phencyclidine, Propoxyphene, and Methaqualone in urine matrix.

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SCIEX Triple Quad™ 4500MD System Analytical Performance Data Sheet

Illustration of Analytical Performance for Vitamin B1 & Vitamin B6 in Whole Blood

The SCIEX Triple Quad 4500MD LC-MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD LC-MS/MS system to analyze the compounds Vitamin B1 & Vitamin B6 in whole blood matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials & Methods

The SCIEX Triple Quad 4500MD LC-MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. A commercially available kit for the analysis of Vitamin B1 and B6 in whole blood (Instruchemie B.V.) was processed using the following conditions:

Sample Prep Conditions
As per kit instructions, protein precipitation based

Liquid Chromatography Conditions
Column: Phenomenex Luna C18
Mobile Phase A: Provided in Kit
Mobile Phase B: Provided in Kit
Flow Rate: 0.65 – 0.8 mL/min
Injection Volume: 20 µL
Gradient: Linear 10-97% B over 3 minutes
Retention Time: 0.95 min (B1), 1.05 min (B6)

Mass Spectrometry Conditions
Method Duration: 3 minutes
Polarity: Positive ESI
Transitions: Compound Dependent
Source Conditions: Flow Rate-Optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=6 replicates), and functional sensitivity are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (nmol/L)	%CV at LLOQ	Functional Sensitivity*
Vitamin B1 (TDP)	39-890	1.3	4.45
Vitamin B6 (PLP)	21-550	5.9	6.31

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and functional sensitivity for each compound evaluated.

**Functional sensitivity is defined here as the lowest concentration achieving a CV of 20%.*

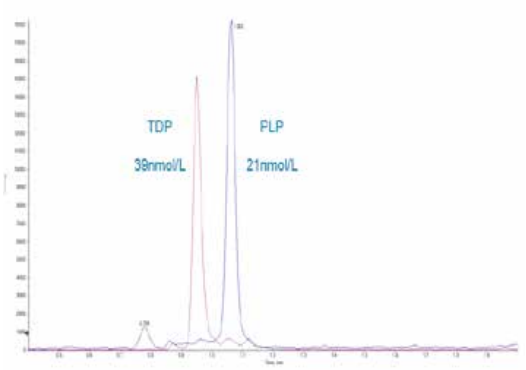

Figure 1. Chromatogram for all evaluated compounds using the SCIEX Triple Quad 4500MD system following the sample preparation and LC-MS/MS conditions.

IVD-MKT-02-8586-B

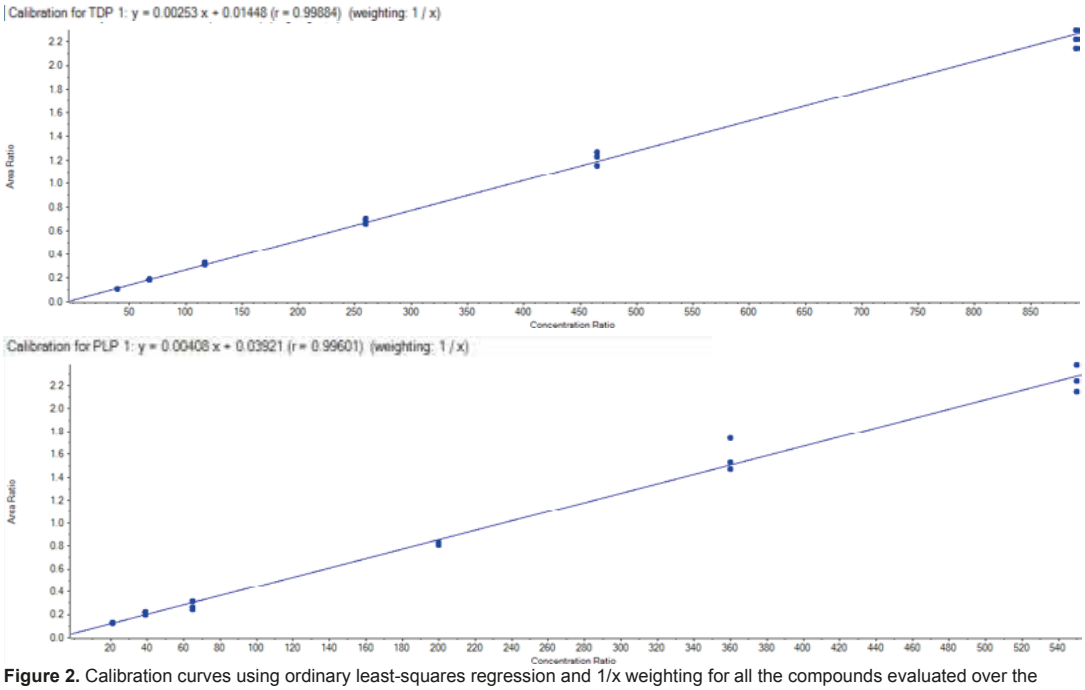

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for all the compounds evaluated over the defined concentration ranges in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: Compounds exhibited a linear dynamic range of approximately 3 orders of magnitude.

Reproducibility: At each LLOQ, the precision (%CV) was <6% for compounds evaluated as determined by n=3 replicates. CV data for all compounds evaluated is based on calculated concentration with internal standard.

Sensitivity: Functional sensitivity, as defined by a CV of <20%, was <7 nmol/L for evaluated compounds.

The SCIEX Triple Quad 4500MD system exhibited capability to deliver sensitive and reproducible analytical performance Vitamin B1 and B6 in whole blood matrix.

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Contents

SCIEX Triple Quad™ 4500MD System Analytical Performance Data Sheet

Illustration of analytical performance for Cyclosporin A, Tacrolimus, Sirolimus, and Everolimus

The SCIEX Triple Quad 4500MD LC-MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified, and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Triple Quad 4500MD LC-MS/MS system to analyze the compounds Cyclosporin A, Tacrolimus, Sirolimus, and Everolimus in whole blood matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and Methods

The Triple Quad 4500MD LC-MS/MS system was controlled and data processed using Analyst® MD Software 1.6.3. Commercially available whole blood calibrators and quality controls (Chromsystems) containing the compounds of interest were processed with the conditions:

Sample Prep Conditions
Precipitation with kit extraction solutions using 50µL whole blood, centrifugation and direct injection

Liquid Chromatography Conditions
Column: Commercial kit
Mobile Phase A: Commercial kit
Mobile Phase B: Commercial kit
Flow Rate: 1-2.2mL/min
Injection Volume: 25µL
Gradient: Linear 9-100%B over 1.65 min
Retention Time: Compound dependent 1-1.2 min

Mass Spectrometry Conditions
Method Duration: 1.65 minutes
Polarity: Positive electrospray
Transitions: Compound dependent
Source Conditions: Flow rate-optimized

Results

Analytical performance statistics including the concentration range evaluated, precision experiments (n=4 replicates, analyzed in triplicate), and functional sensitivity are shown in Table 1. Chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Compound	Range (µg/L)	%CV at LLOQ	Functional Sensitivity*
Cyclosporin A	4-2000	4.2%	1.1
Tacrolimus	0.4-100	5.1%	0.43
Sirolimus	0.4-100	6.6%	0.47
Everolimus	0.4-100	5.8%	0.14

Table 1. Measured range, percent coefficient of variation (%CV) at lower-limit of quantitation (LLOQ), and functional sensitivity for each compound evaluated.

**Functional sensitivity is defined here as the lowest concentration achieving a CV of 20%.*

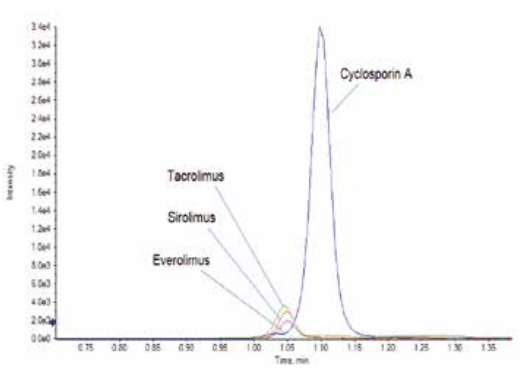

Figure 1. Chromatogram for all evaluated compounds using the SCIEX Triple Quad 4500MD system following the sample preparation and LC-MS/MS conditions.

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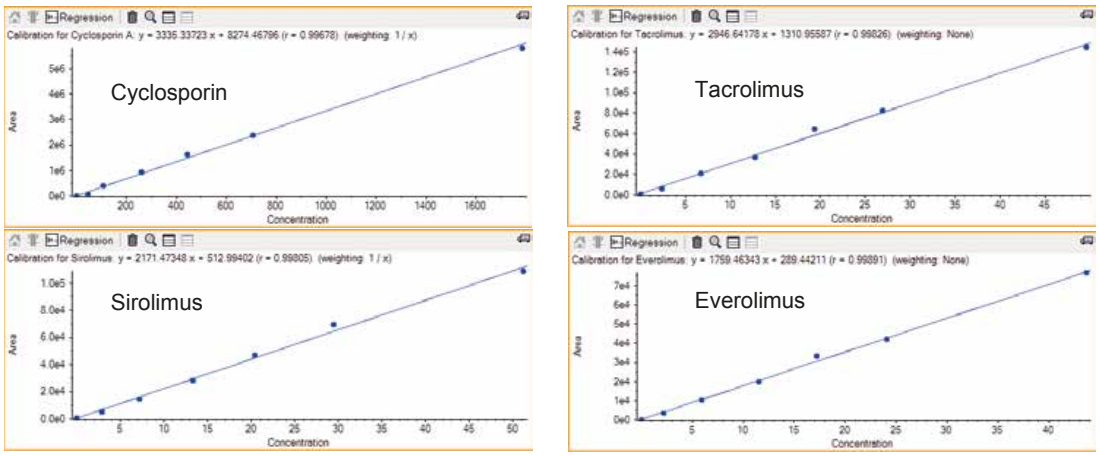

Figure 2. Calibration curves using ordinary least-squares regression and 1/x weighting for all the compounds evaluated over the defined concentration ranges in Table 1.

Conclusions

Based on the above performance testing, the following results were obtained:

Assay Linearity: All compounds exhibited a linear dynamic range of at least 3 orders of magnitude.

Reproducibility: At each LLOQ, the precision (%CV) was <7% for all compounds evaluated as determined by n=4 replicates, analyzed in triplicate. CV data for all compounds evaluated is based on calculated concentration with internal standard.

Sensitivity: Functional sensitivity, as defined by a CV of <20%, was <1.2 µg/L for all evaluated compounds.

The SCIEX Triple Quad 4500MD system exhibited capability to deliver sensitive and reproducible analytical performance for Cyclosporin A, Tacrolimus, Sirolimus, and Everolimus in whole blood matrix.

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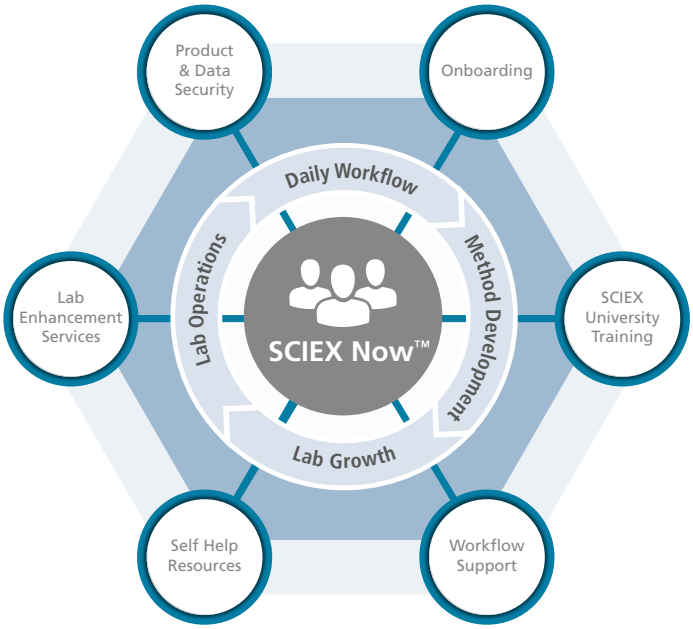

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