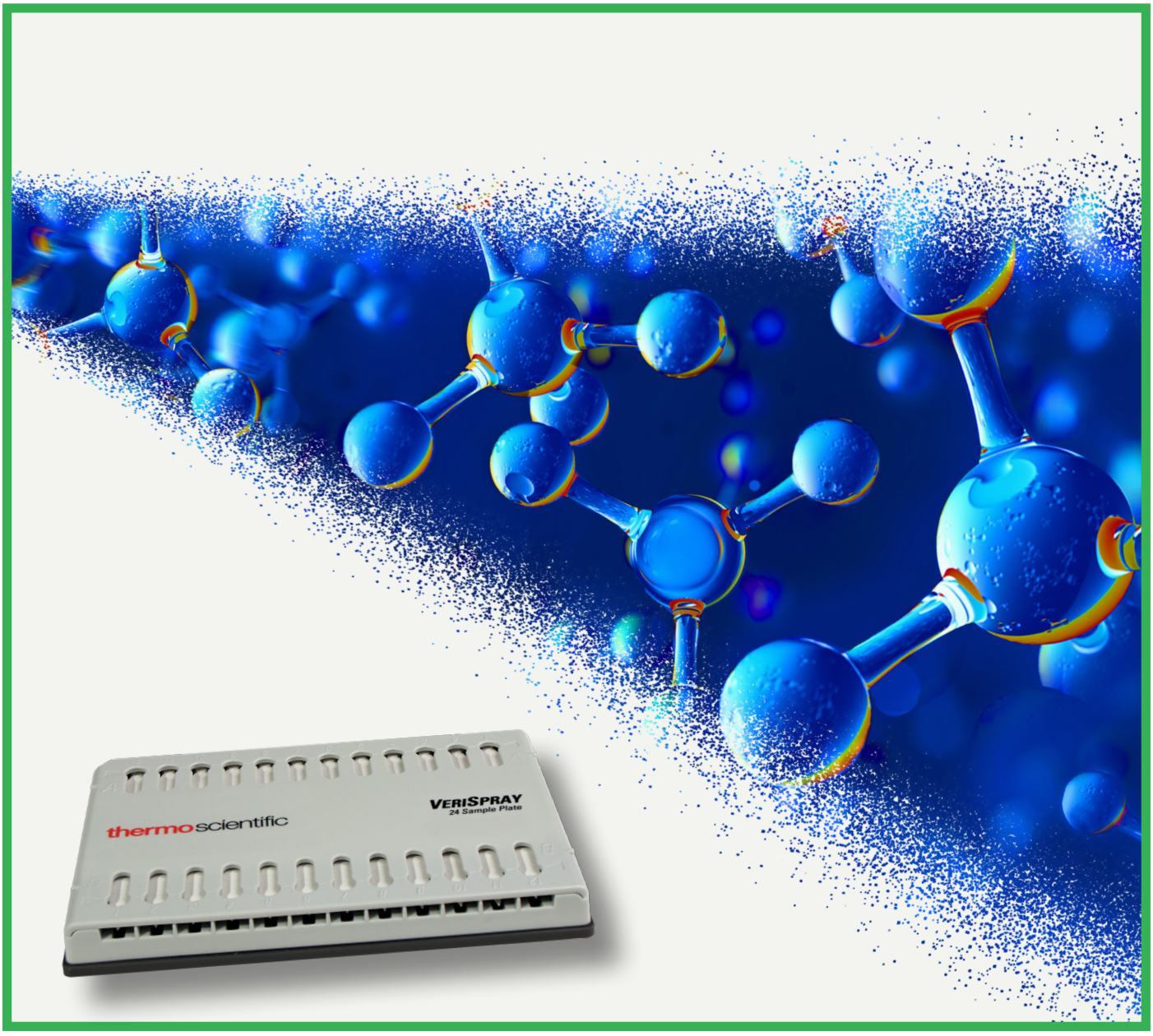
Paper spray ionization: The faster path to mass spec analysis

Save time and reduce costs through direct sample analysis of biological fluids



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Introduction

Common concerns with using conventional chromatography-based MS include time-consuming protocols, experiment backlogs, high costs per sample, equipment downtime for maintenance and the need for experienced technicians.

Using paper spray ionization such as the Thermo Scientific™ VeriSpray™ PaperSpray ion source, researchers in clinical and forensic toxicology labs with high throughput requirements can now perform direct Mass Spectrometry (MS)-based analyses with minimal solvent consumption and sample preparation, thereby saving costs per test and reducing the time involved.

The VeriSpray ion source solution involves fast, easy and direct introduction of samples to the mass spectrometer via electrospray ionization. By simply adding samples and solvent to paper cartridges and loading them into the system, the VeriSpray ion source technology minimizes time-consuming sample preparation steps and offers a simplified workflow, unlike chromatography processes.

In recent years, paper spray-MS has garnered interest in the fields of forensic toxicology and clinical research due to its robust quantitative analysis, high-throughput capabilities, short turnaround times and time-saving workflows. In this eBook, we provide case studies and proof-of-concept experiments that corroborate the varied benefits of employing paper spray ionization methods.

Minimal sample preparation

Paper spray ionization in mass spectrometry employs a “dilute and shoot” method, requiring minimal sample preparation steps, such as derivatization and reducing/removing simpler sample purification processes. In a method optimized to analyze immunosuppressant drugs, 10 µl of human blood was directly spotted on sample plates, minimizing sample preparation that chromatography protocols would otherwise require. Cyclosporin A, Everolimus and Tacrolimus were analyzed using a 2-minute method directly from the dried blood spots.

In another method developed for quantitative analysis of antifungal drugs, 4 µl of serum samples spiked with internal standards were spotted on the VeriSpray

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cartridge and allowed to dry at ambient temperature for 30 minutes. The drugs – voriconazole, itraconazole, and posaconazole – were analyzed in a 2-minute protocol that included the extraction step, as well as the MS analysis.

Quick turnaround time for screening and quantitation

Researchers investigated the potential of paper spray ionization along with a triple quadrupole mass spectrometer as a screening tool for commonly encountered drugs of abuse in a study at Indiana University–Purdue University Indianapolis. The sampling plate, with 24 sample tips, each holding a separate dried blood spot, was analyzed in minutes, offering quick turnaround times to screen drugs, an important consideration for forensic and clinical labs.

Another proof-of-concept research method, testing the rapid screening ability of the PaperSpray technology on 19 drugs of abuse demonstrated how the system analyzed 480 injections with no loss in sensitivity or reproducibility.

High-throughput quantitative analysis:

The short analysis times offered by paper spray ionization serves as a high-throughput quantitative assay, rapidly processing samples and saving time for clinical researchers. In a technical method for quantitative analysis of EDDP (2-ethylidene- 1,5-dimethyl-3,3-diphenylpyrrolidine), an opioid metabolite, human whole blood samples were analyzed. Without the need for sample pre-treatment, separation, sample clean-up or dilution, the system maintained sensitivity, robustness and quantitative accuracy over all of the 480 samples.

Another method tested the high-throughput capabilities of the PaperSpray-MS system in EDDP quantitation, this time using urine samples. Upon analyzing 480 urine samples, researchers reported that the system runs for extended periods of time without the need for maintenance and yielded no significant loss in signal.

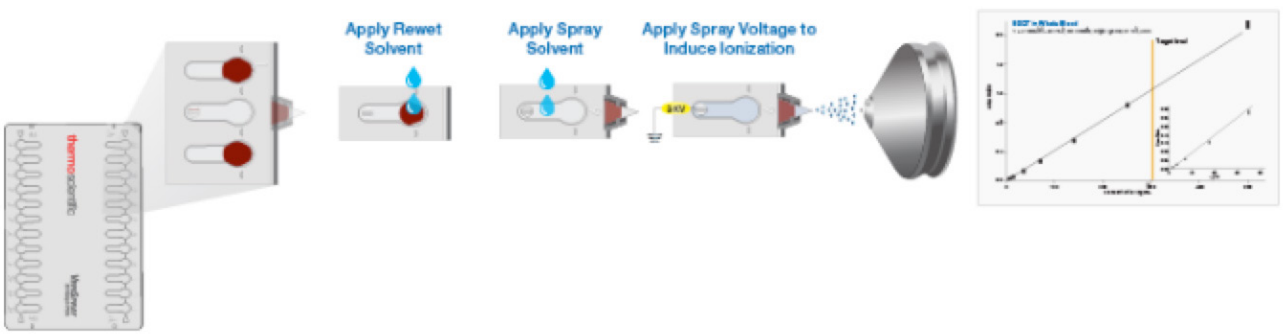
Improved selectivity and limits of detection

One way to circumvent the potential low signal-to-noise ratio in paper spray applications is to couple it with high-field asymmetric ion mobility spectrometry (FAIMS). A differential ion mobility technique, FAIMS enables greater selectivity of ions entering the mass spectrometer. Coupling PaperSpray with FAIMS in a proof-of-concept method analyzing the antidepressant drug, amitriptyline, reduced background interferences and increased signal-to-noise ratio, resulting in improved limits of detection.

Another study quantifying immunosuppressants in human whole blood compared calibration curves obtained using the PaperSpray technology with and without FAIMS. Without coupling to FAIMS, the lowest calibration level for two of the immunosuppressants, tacrolimus and everolimus, was excluded due to being close to the background signal. With the FAIMS interface coupled, however, the background signal was significantly reduced. The data showed accuracy and linearity across the measured range.

A recent webinar on paper spray-MS explores the background of paper spray ionization in greater detail and digs deeper into potential applications, along with workflows and additional examples.

With simple workflows, faster analyses and reduced cost/sample, the PaperSpray ion source technology is an excellent option for every clinical research and forensic toxicology laboratory looking for faster, accurate, robust, and sensitive analyses.



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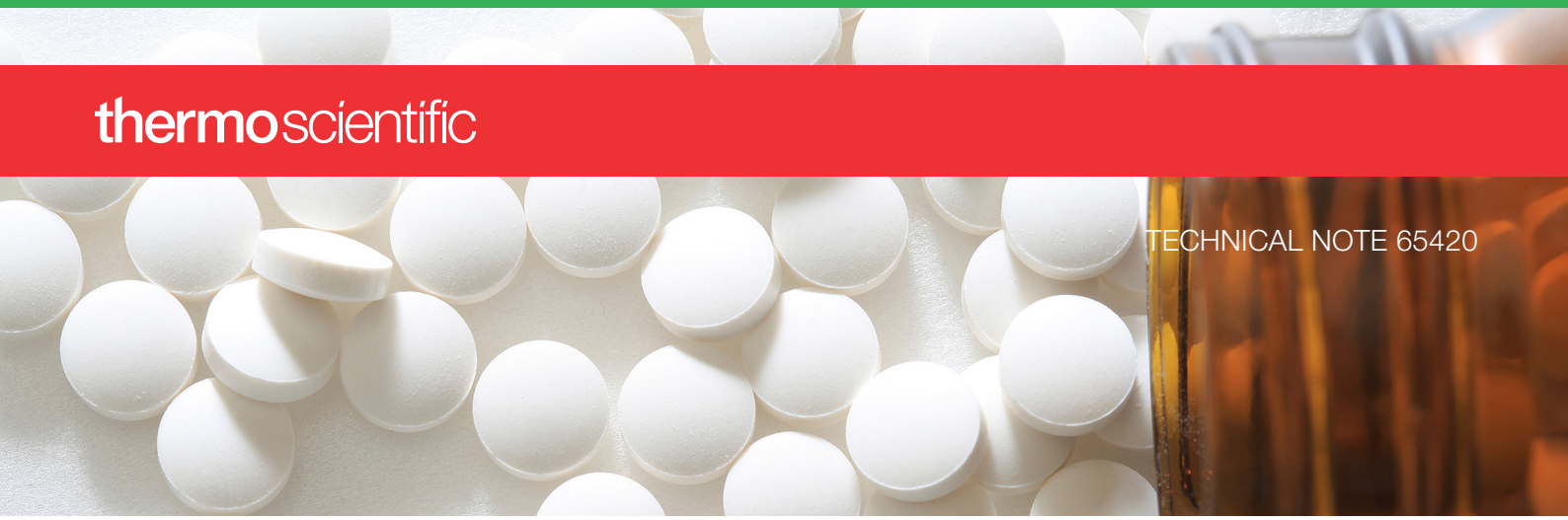

TECHNICAL NOTE 65420

Detection of controlled substances in blood samples using the VeriSpray ion source with TSQ Altis MS for clinical research and forensic toxicology

Authors

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Keywords

Illicit drugs, opiates, opioids, amphetamines, PS-MS/MS, TSQ Altis MS, VeriSpray PaperSpray ion source, TraceFinder, forensic toxicology, benzodiazepines

Application benefits

- Quick turn-around time
- Reduced cost per sample, increased ease-of-use and robustness
- Six drugs of abuse analytes in single quantitative method

Goal

To develop a robust, sensitive, reliable, and reproducible PaperSpray-mass spectrometry workflow for detection of illicit drugs in blood for clinical research and forensic toxicology using the Thermo Scientific™ TSQ Altis™ mass spectrometer connected with the Thermo Scientific™ VeriSpray™ PaperSpray system

Introduction

The abuse of controlled substances is a serious ongoing problem worldwide, causing significant societal disruption and economic damage. One part of the overall strategy to mitigate the effects from the abuse of drugs requires high-performance methodologies for the screening and quantitation of these substances in biological matrices. Modern forensic toxicological and clinical research laboratories need simpler methods that provide higher throughput and faster analysis for the screening and quantitation of drugs of abuse.

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PaperSpray technology combined with triple quadrupole mass spectrometry is an ideal choice for rapid drug screening and quantitation in clinical research and forensic toxicology applications for two main reasons. First, studies have demonstrated that low ng/mL or lower detection limits are obtainable directly from blood, which is sufficient for the detection of relevant drugs at target concentrations. Second, reduce the burden on the laboratory by simplifying method development, reducing the amount of bench work and thus decreasing time to result. Reports in the literature for screening by PaperSpray MS include the detection of amphetamine-like designer drugs in oral fluid,[1] agrichemicals in fruit,[2] targeted triple quadrupole based screening,[3] and use of HR-MS/MS for urine[4] and blood screening[5].

Triple quadrupole mass spectrometers are unit-resolution instruments that achieve high selectivity by monitoring characteristic collision induced dissociation (CID) fragment ions. When operated in selected reaction monitoring (SRM) mode, the instruments give high sensitivity and robust quantitation.

In this study, we investigated the VeriSpray PaperSpray ion source coupled to a triple quadrupole mass spectrometer as a drug screening tool for applications in clinical research and forensic toxicology. Experiments were carried out using the VeriSpray PaperSpray ion source on a TSQ Altis triple-stage quadrupole mass spectrometer. The VeriSpray system enables robust, rapid, and automated PaperSpray analysis. Sample storage, extraction, and ionization all take place on VeriSpray sampling plates. Biofluids are spotted and dried directly on the sampling plate. The plates contain 24 individual PaperSpray tips, each of which analyzes a separate sample (Figure 1A). Analysis of the plate is carried out automatically via the VeriSpray ion source (Figure 1B) in a matter of minutes. To demonstrate proof-of-concept, controlled substances commonly encountered in clinical research and forensic toxicology were tested (cocaine, diazepam, fentanyl, hydrocodone, methamphetamine, and zolpidem).

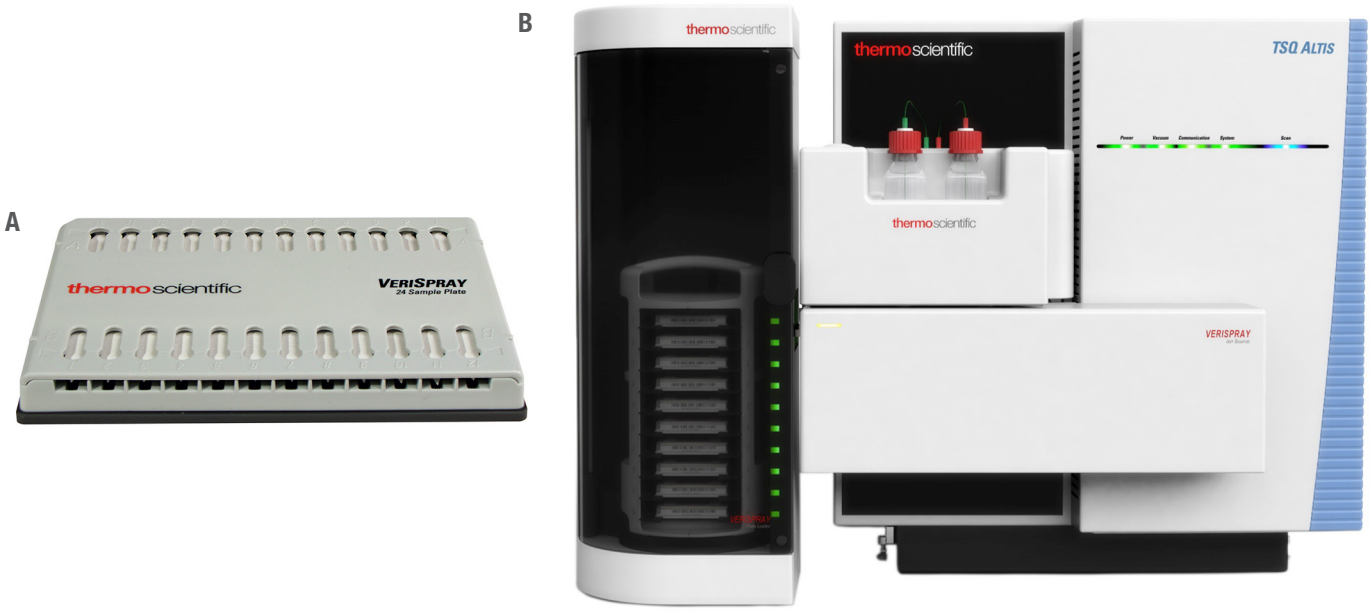

Figure 1. (A) VeriSpray sample plate and (B) VeriSpray PaperSpray system mounted to TSQ Altis triple quadrupole mass spectrometer

Experimental

Sample preparation

The method was adapted from a previous PaperSpray method for drug detection[5]. Calibration standards were prepared in pooled human blood. Working solutions at 20x concentration were prepared in methanol by serial dilution and spiked into blood the day of analysis. Blood samples (100 µL) were mixed with 300 µL of aqueous internal standard solution containing isotopically labeled analogs of each of the analytes. A 6 µL aliquot of each blood sample was then spotted onto a VeriSpray cartridge and allowed to dry at room temperature for 1 hour or in an incubator for 20 minutes at 40 ºC.

PaperSpray and MS conditions

The PaperSpray solvents (both sample rewet and spray solvents) were acetonitrile/acetone/water 0.01% acetic acid (85:10:5), applied according to the settings in Table 1. The TSQ Altis triple quadrupole mass spectrometer was used for detection. The experimental conditions were optimized with a time dependent spray voltage of 3.8 kV, a cycle time of 0.8 s, and resolution of 0.7 Da FWHM for both Q1 and Q3. The source parameters and SRM table along with the critical MS features for all target analytes are listed in Tables 2 and 3, respectively. The optimum RF lens settings and collision energies for the product ions were determined by infusion of the individual standards into the mass spectrometer.

Table 1. VeriSpray solvent application parameters. Each rewetting and solvent dispense is 10 µL.

Rewetting dispense delay	
Dispense	Delay (s)
1	1
Solvent dispense delay	
Dispense	Delay (s)
1	1
2	1
3	1
4	1
5	3
6	3
7	5
8	5
9	5
10	5
11	7
12	7
13	7
14	7
15	7

Table 2. (A) Source parameters for analysis of Illicit drugs on the TSQ Altis triple quadrupole mass spectrometer

Ion Source Parameter	Value
Spray Voltage	Time Dependent
Positive Ion	3800 V
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	300 °C
CID Gas	2 mTorr

Table 2. (B) Time dependent spray voltage

Time (min)	Voltage (V)
0	0
0.1	3800
1.1	0

Table 3. Optimized mass spectrometer transitions for the Illicit drugs in blood with acquisition time of 1.2 min and positive polarity for each sample

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Methamphetamine	150.1	91.1	21	91
	150.1	119.1	12	91
Methamphetamine-D5	155.1	92.1	21	91
	155.1	121.1	12	91
Diazepam	285.1	193.1	33	223
	285.1	222.0	28	223
Diazepam-D5	290.1	198.1	33	223
	290.1	227.0	28	223
Hydrocodone	300.1	171.1	40	207
	300.1	199.1	31	207
Hydrocodone-D6	306.1	174.1	40	207
	306.1	202.1	31	207
Cocaine	304.1	150.1	26	172
	304.1	182.2	21	172
Cocaine-D3	307.1	153.1	26	172
	307.1	185.2	21	172
Zolpidem	308.2	235.2	36	228
	308.2	263.2	27	228
Zolpidem-D6	314.2	235.2	36	228
	314.2	263.2	27	228
Fentanyl	337.4	105.1	38	200
	337.4	188.1	24	200
Fentanyl-D5	342.4	105.1	38	200
	342.4	188.1	24	200
Buprenorphine	468.4	396.2	40	299
	468.4	414.3	35	299
Buprenorphine-D4	472.4	400.2	40	299
	472.4	415.3	35	299

Data acquisition and analysis

Data acquisition and processing were conducted using Thermo Scientific™ TraceFinder™ software version 4.1. Limits of detection were calculated by the formula $3*s_b/m$, where s_b is the standard error of the intercept and m is the slope of the calibration line.

Results and discussion

The seven controlled substances were successfully quantitated simultaneously as shown in Figure 2. The correlation coefficient (R^2) for each calibration curve was greater than 0.98, indicating good linearity. The detection limits (Table 4) are below the concentrations normally encountered in forensic toxicology with the exception of buprenorphine. Total analysis time for the dried blood spots was approximately two minutes. This included the extraction step as well as the mass spectrometric detection, both of which take place automatically using the VeriSpray sample plate.

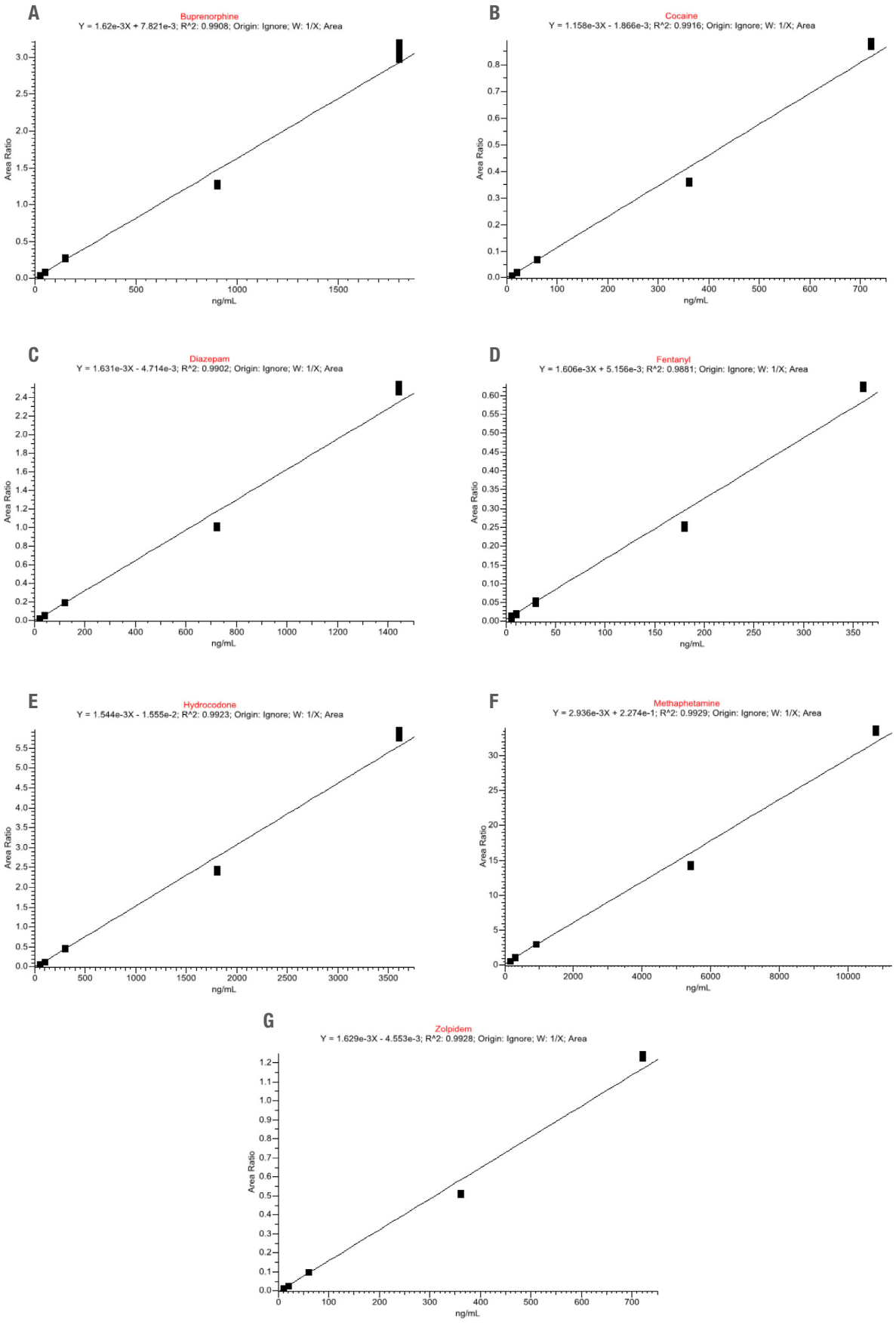

Figure 2. Calibration curves of (A) buprenorphine, (B) cocaine, (C) diazepam, (D) fentanyl, (E) hydrocodone, (F) methamphetamine, and (G) zolpidem obtained from pooled human blood

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Table 4. Limits of detection (LOD) and calibration curve correlation coefficient (R^2) from human blood obtained using the VeriSpray system

Compound	LOD (ng/mL)	R^2
Buprenorphine	13	0.9909
Cocaine	5	0.9916
Diazepam	11	0.9902
Fentanyl	3	0.9881
Hydrocodone	23	0.9923
Methamphetamine	68	0.9928
Zolpidem	5	0.9928

Conclusions

PaperSpray MS on the VeriSpray sampling plates and ion source was capable of accurate quantitation of controlled substances in human blood for clinical research and forensic toxicology. Analysis was fast and simple, requiring no sample pretreatment or separations.

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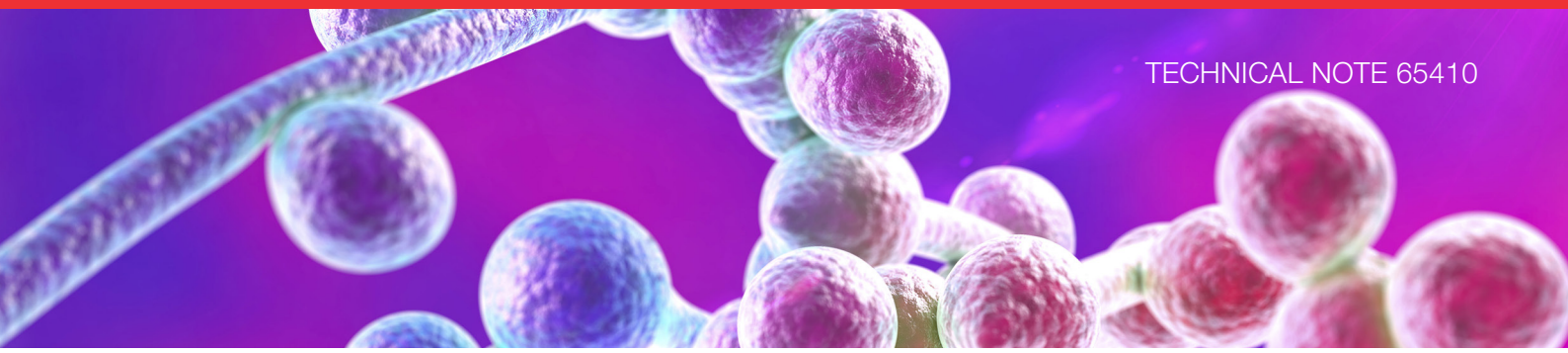

TECHNICAL NOTE 65410

Quantitative analysis of antifungal drugs using PaperSpray tandem mass spectrometry for clinical research

Authors

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[2]Thermo Fisher Scientific, San Jose, CA

Keywords

Antifungal drugs, PS-MS/MS, voriconazole, itraconazole, posaconazole, TSQ Altis, VeriSpray, TraceFinder, clinical research

Application benefits

- Simple, no sample preparation and fast analysis
- Three antifungal drugs in a single quantitative method

Goal

To develop a reliable and reproducible PaperSpray-mass spectrometry workflow for quantitative analysis of antifungal drugs in serum for clinical research using a Thermo Scientific™ TSQ Altis™ mass spectrometer with the Thermo Scientific™ VeriSpray™ PaperSpray ion source

Introduction

Voriconazole, itraconazole, and posaconazole (Figure 1) are drugs used to treat serious, invasive fungal infections. Invasive fungal infections are highly prevalent in individuals with seriously compromised immune defenses, including those on immunosuppressive drugs following organ or bone marrow transplant, or those undergoing chemotherapy for cancer treatment. Using antifungal drugs in pediatric populations is especially challenging because of the highly variable pharmacokinetics among individuals and across different ages. Adjustment of dose based on rapid and accurate drug monitoring results helps to achieve control of infection in clinical research.

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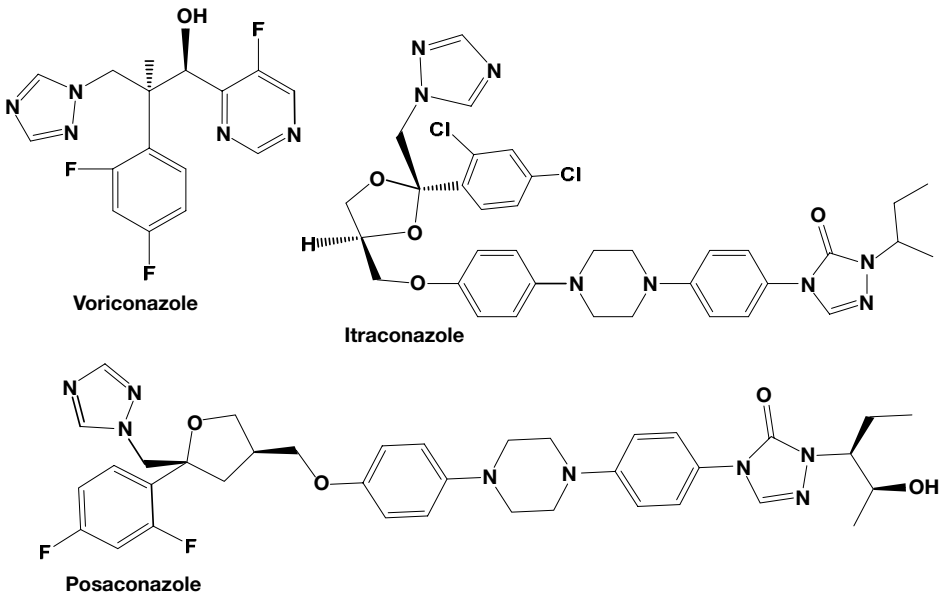

Figure 1. Antifungal drug structures

PaperSpray mass spectrometry, first described in 2010,[1] is a method for performing rapid, direct analysis of biological fluids spotted on paper or another porous substrate. In the PaperSpray technique, extraction and ionization of one or more analytes occur from a porous substrate containing the sample. Several reports in literature have demonstrated quantitative analysis of small molecules, including therapeutic and illicit drugs, directly from biological fluids.[2] PaperSpray MS has garnered significant interest because of its lack of sample preparation and shorter turn-around time while still maintaining good quantitative performance and sensitivity.

In this study, we describe the quantitation of three antifungal drugs (posaconazole, itraconazole, and voriconazole) using the VeriSpray PaperSpray ion source on a TSQ Altis triple-stage quadrupole mass spectrometer. The VeriSpray system enables robust, rapid, and automated PaperSpray analysis. Sample storage, extraction, and ionization all take place on VeriSpray sampling plates containing 24 individual paper spray tips, each of which analyzes a separate sample (Figure 2A). Analysis of the plate is carried out automatically via the VeriSpray ion source (Figure 2B) directly from the dried biofluid spots within minutes.

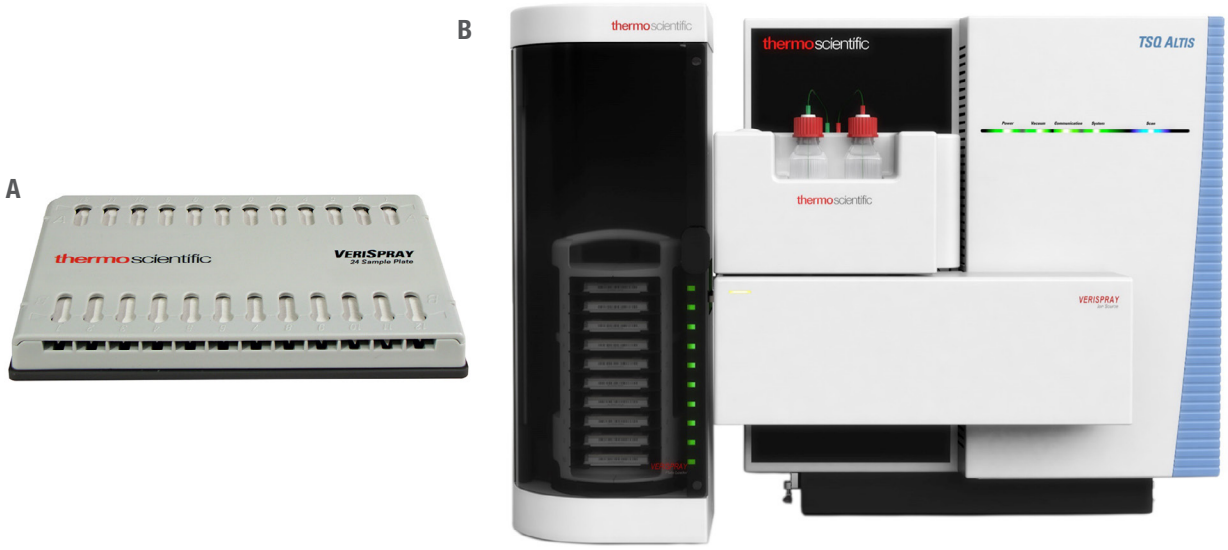

Figure 2. (A) VeriSpray sample plate and (B) VeriSpray PaperSpray system mounted to TSQ Altis triple quadrupole mass spectrometer

Experimental

Sample preparation

Calibration curve standards in the range of 0.1 to 10 µg/mL were prepared in pooled human serum. Working solutions at 20× concentration were prepared in methanol by serial dilution and spiked into serum on the day of analysis. Serum samples were mixed with an internal standard solution containing isotopically labeled voriconazole and itraconazole. A suitable isotopically labeled analog of posaconazole was not available; isotopically labeled itraconazole was used as its internal standard instead. Serum samples (4 µL) were then spotted on the Thermo Scientific™ VeriSpray™ cartridge and allowed to dry at ambient temperature for 30 minutes.

PaperSpray and MS conditions

For PaperSpray ionization, the VeriSpray system was used. The PaperSpray solvents (both sample rewet and spray solvents) were acetonitrile/acetone/water 85/10/5 with 0.01% acetic acid, applied according to the settings in Table 1. The TSQ Altis triple-stage quadrupole mass spectrometer was used for the analyses. The experimental conditions were optimized with a spray voltage of 4.2 kV, a cycle time of 0.8 s, and both Q1 and Q3 resolution of 0.7 Da FWHM. The source parameters and SRM table along with the critical MS features for all target analytes are listed in Tables 2 and 3, respectively. The optimum RF lens settings and collision energies for the product ions were determined by infusion of the individual standards into the mass spectrometer.

Data acquisition and analysis

Data acquisition and processing were conducted using Thermo Scientific™ TraceFinder™ software version 4.1. Limits of detection were calculated by the formula $3*s_b/m$, where s_b is the standard error of the intercept and m is the slope of the calibration line.

Table 1. VeriSpray solvent application parameters. Each rewetting and solvent dispense is 10 µL.

Rewetting dispense delay	
Dispense	Delay (s)
1	1
Solvent dispense delay	
Dispense	Delay (s)
1	1
2	1
3	1
4	1
5	3
6	3
7	5
8	5
9	5
10	5
11	7
12	7
13	7
14	7
15	7

Table 2. (A) MS conditions

Ion Source Parameter	Value
Spray Voltage	Time Dependent
Positive Ion	4200 V
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	350 °C
CID Gas	2 mTorr

Table 2. (B) Time dependent spray voltage

Time (min)	Voltage (V)
0	0
0.1	4200
1.1	0

Table 3. Optimized SRM transitions for the antifungal drugs in serum with acquisition time of 1.2 min and positive polarity for each sample

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Voriconazole	350.1	224.1	19	59
Voriconazole	350.1	263.1	25	59
Voriconazole	350.1	281.2	16	59
Posaconazole	701.3	344.1	43	117
Posaconazole	701.3	370.2	43	117
Posaconazole	701.3	614.3	35	117
Itraconazole	705.2	335.1	42	115
Itraconazole	705.2	348.1	40	115
Itraconazole	705.2	392.2	36	115
Voriconazole D3	353.0	224.2	20	54
Voriconazole D3	353.0	266.2	25	54
Voriconazole D3	353.0	284.1	18	54
Itraconazole D4	709.2	339.3	43	119
Itraconazole D4	709.2	352.0	40	119
Itraconazole D4	709.2	396.4	36	119

Results and discussion

The three antifungal drugs were successfully quantitated simultaneously (Figure 3). The correlation coefficient (R^2) for each calibration curve was greater than 0.99, indicating good linearity. The detection limits (Table 4) are well below the concentrations normally found in research of fungal infections, and the assay measurement range is comparable to that offered by reference lab HPLC-MS/MS analysis. Total analysis time for the dried serum spots was about 2 minutes. This included the extraction step as well as the mass spectrometry analysis, which both take place automatically using the VeriSpray sample plate.

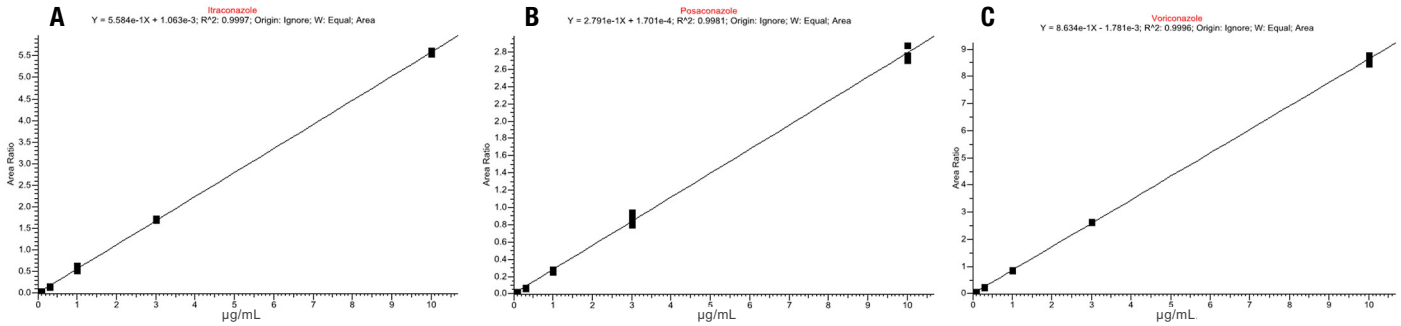

Figure 3. Calibration curves of (A) itraconazole, (B) posaconazole, and (C) voriconazole obtained from pooled human serum

Table 4. PaperSpray quantitative results in serum

Target	R^2	Limits of Detection in Serum (ng/mL)	Target Range
Itraconazole	0.9990	26	>500 ng/mL (local infection) >1000 ng/mL (systemic infection)
Posaconazole	0.9973	43	~1000 ng/mL (trough)
Voriconazole	0.9996	16	~1000 ng/mL (steady-state)

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Conclusions

PaperSpray mass spectrometry with the VeriSpray ion source was capable of accurate quantitation of antifungal drugs in human serum. In addition to providing fast analysis, this easy-to-implement workflow requires no additional sample pretreatment or separations. The linear dynamic range of the assay encompassed the range normally measured for clinical research.

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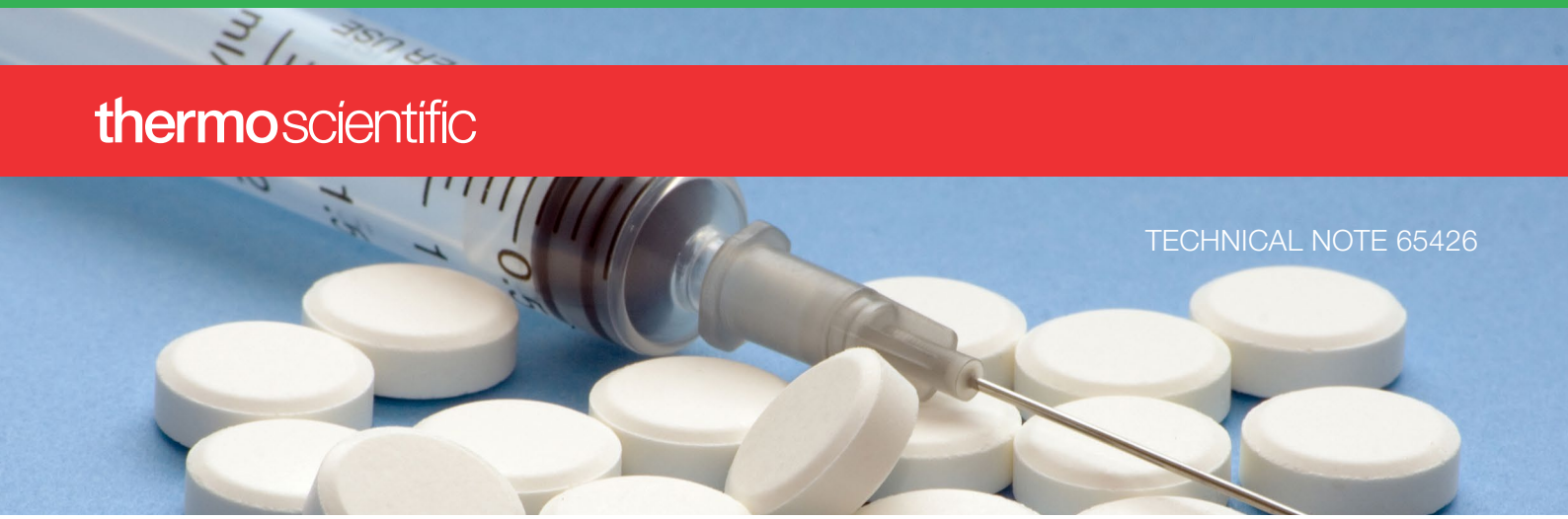

TECHNICAL NOTE 65426

Robustness characterization of a quantitative whole blood assay using PaperSpray technology for clinical research

Authors

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Thermo Fisher Scientific, San Jose, CA

Keywords

PaperSpray, EDDP, TSQ Quantis MS, VeriSpray system, TraceFinder, clinical research

Application benefits

- Ability to analyze 480 blood samples while maintaining sensitivity, accuracy and precision
- Robust high-throughput analysis of whole blood without the need for sample preparation

Goal

Demonstrate robustness of a quantitative whole blood assay for clinical research, without the need for sample clean-up or dilution. Sensitivity is maintained and quantitation remains unaffected over a total of 480 analyses of whole blood samples, or the equivalent of two fully loaded Thermo Scientific™ VeriSpray™ magazines, using the Thermo Scientific™ TSQ Quantis™ mass spectrometer connected to the Thermo Scientific™ VeriSpray™ PaperSpray ion source.

Introduction

PaperSpray mass spectrometry, first described in 2010[1], is an analytical technique where the sample is spotted onto a strip of paper and is extracted and ionized directly at the ion source of the mass spectrometer. This is done by first taking the dried sample and rewetting it with an organic-aqueous mixture and then applying a voltage with the aid of a spray solvent to facilitate ionization and introduction into the mass spectrometer. A chronogram of the ion current is collected for a short duration, usually one minute or less, and the area under the detected ion current is integrated and used to quantify the detected analyte.

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PaperSpray technology provides significant benefits for high-throughput applications because analysis times are short and little to no sample preparation is required. The new VeriSpray PaperSpray ion source has a capacity of up to 240 samples, which are analyzed in an automated fashion, meaning a walk-away run time of up to 8 hours. The VeriSpray plate loader and magazine hold up to 10 VeriSpray sample plates, each equipped with 24 paper strips, for flexible capacity. Figure 1 shows the VeriSpray system installed on a triple quadrupole mass spectrometer, removable magazine that holds 10 sample plates and VeriSpray sample plate.

In this technical note, we describe a method for quantitative and robust analysis of EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), an opioid metabolite, in human whole blood for clinical research using the VeriSpray Paper Spray ion source coupled with a TSQ Quantis mass spectrometer.

Experimental

Sample preparation

EDDP was spiked into human whole blood at various concentrations to yield a nine-point calibration curve ranging from 1.75 to 500 ng/mL and three quality control samples at concentrations of 50, 100, and 200 ng/mL. Two sets of 240 samples (a total of 480 samples), consisting of calibrators, qualifying samples, and robustness samples, were spotted onto 20 VeriSpray sample plates at a sample volume of 8 µL each. The samples were oven-dried for 30 minutes at a temperature of 45 °C. Calibration curves and QC samples (single analysis, no replicates) were placed at the beginning and end of each set of 240 samples, yielding a total of four calibration curves with QC samples over the 16-hour run. A robustness sample at the same concentration as QC1 was spotted on all other paper strips.

PaperSpray and MS conditions

Sample storage, extraction, and ionization all take place on VeriSpray sample plates that contain 24 individual paper strips. Each strip is used for an individual sample. For this analysis, 480 samples of human whole blood spiked with EDDP and its internal standard EDDP-d3 were spotted onto a total of 20 VeriSpray plates. Data from a one-minute method was collected for each sample. The first set of 10 plates (240 samples) was analyzed in an unattended fashion. After the first set was finished, the magazine was loaded with the second set of 10 plates, which again was run in an automated and unattended procedure. In between the two sets of 10 plates each, the outer surface area of the TSQ Quantis ion transfer tube was cleaned by wiping it using a disposable wipe soaked with a 1:1 mixture of water and methanol. Neither the TSQ Quantis ion transfer tube nor the VeriSpray PaperSpray ion source needed to be removed from the mass spectrometer for this procedure.

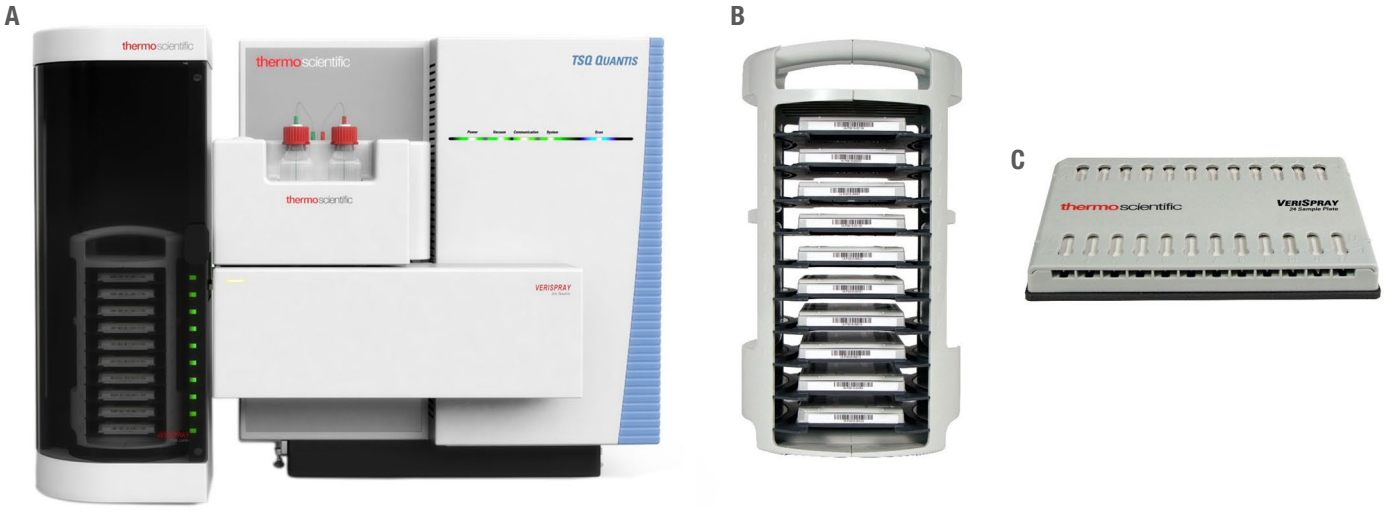

Figure 1. (A) VeriSpray ion source and plate loader mounted onto a TSQ Quantis MS, (B) Magazine, and (C) VeriSpray sample plate

The paper spray solvents (both sample rewet and spray solvents) were methanol/water 95/5 with 0.01% acetic acid, applied according to the settings in Table 1. The experimental conditions of the mass spectrometer were optimized with a spray voltage of 3.8 kV, a cycle time of 0.8 s, and Q1 and Q3 resolution at 0.7 and 1.2 Da FWHM, respectively. The source parameters and SRM table with the critical MS features for all target analytes are listed in Tables 2 and 3, respectively. The optimum RF lens settings and collision energies for the product ions were determined by infusion of the individual standards into the mass spectrometer. No sweep gas or sweep cone was used. The paper tip to MS inlet distance was set to 6.5 mm to maintain system robustness without compromising the system sensitivity.

Table 1. VeriSpray solvent application parameters. Each rewetting and solvent dispense is 10 µL.

Rewetting dispense delay	
Dispense	Delay (s)
1	1
1	1
Solvent dispense delay	
Dispense	Delay (s)
1	1
2	1
3	1
4	1
5	5
6	5
7	5
8	5
9	5
10	5
11	0

Table 2. (A) MS conditions

Ion Source Parameter	Value
Spray Voltage	Time Dependent
Positive Ion	3800 V
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	350 °C
Q1 Resolution	0.7
Q3 Resolution	1.2
CID Gas	2 mTorr

Table 2. (B) Time dependent spray voltage

Time (min)	Voltage (V)
0	0
0.1	3800
1.1	0

Data acquisition and analysis

Data acquisition and processing were conducted using Thermo Scientific™ TraceFinder™ software version 4.1. The area ratio of EDDP to EDDP-d3 was plotted against concentration, and all four calibration curves containing nine calibration points were analyzed both independently and combined. Robustness sample precision was plotted for 50 ng/mL of EDDP in human whole blood over all 20 sample plates (480 analyses) or two full magazines.

Table 3. Optimized SRM transitions for EDDP and EDDP-d3

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
EDDP	278.288	234.208	31.50	206
	278.288	158.208	44.93	206
	278.288	186.208	35.75	206
EDDP-d3	281.288	234.208	32.04	202
	281.288	157.179	52.35	202
	281.288	189.208	36.51	202

Results and discussion

The lower limit of quantification (LLOQ) for EDDP was 3.5 ng/mL as defined by the lowest calibration standard analyzed that yielded ±20% accuracy and ≤15% CV. All four calibration curves combined yielded an LOQ of 3.5 ng/mL, with 2.6% precision ≥4.1% accuracy. The overlaid calibration curves are shown in Figure 2. The precision for all four curves combined was under 4% for each individual concentration level. Precision and accuracy values of the calibrators and QC samples are listed in Table 4 and Table 5. Consistent performance was demonstrated by monitoring the calculated concentration of the analyte. The calculated precision for the EDDP robustness sample (420 samples of 50 ng/mL) is 1.4%, showing that the VeriSpray ion source and the VeriSpray sample plates deliver highly reproducible analytical performance (Figure 3). Wiping the ion transfer tube with a disposable wipe soaked with water and methanol was sufficient to remove all visible traces of residue and produced reproducible data between the first 240 samples (first full magazine) and second 240 injections (second full magazine), demonstrating the robustness of the method.

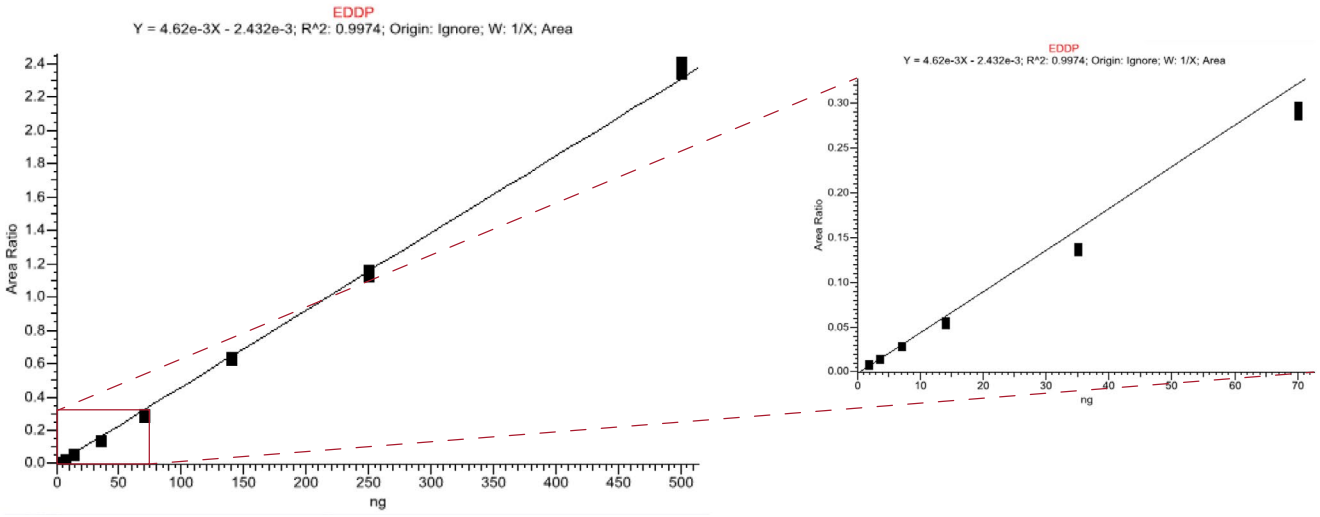

Figure 2. Overlays of four calibration curves of EDDP in blood showing excellent reproducibility. All individual calibration curves gave the same LLOQ of 3.5 ng/mL.

Table 4. Precision and accuracy of calibrators (all four curves combined) for EDDP within the 480 analyses

Theoretical concentration (ng/mL)	Calculated concentration (ng/mL)	Accuracy	%RSD
3.5	3.64	4.1	2.6
7	6.79	-3.0	2.2
14	12.3	-12.1	3.8
35	30.3	-13.6	1.7
70	63.3	-9.6	1.9
140	138	-1.8	1.2
250	249	-0.3	1.5
500	516	3.2	1.4

Table 5. Precision and accuracy of QC samples (four replicates per concentration) for EDDP within the 480 analyses

QC Level	Theoretical concentration (ng/mL)	Calculated concentration (ng/mL)	Accuracy	%RSD
QC 1	50	50.6	1.2	1.6
QC 2	100	106	5.8	1.4
QC 3	200	208	3.9	1.9

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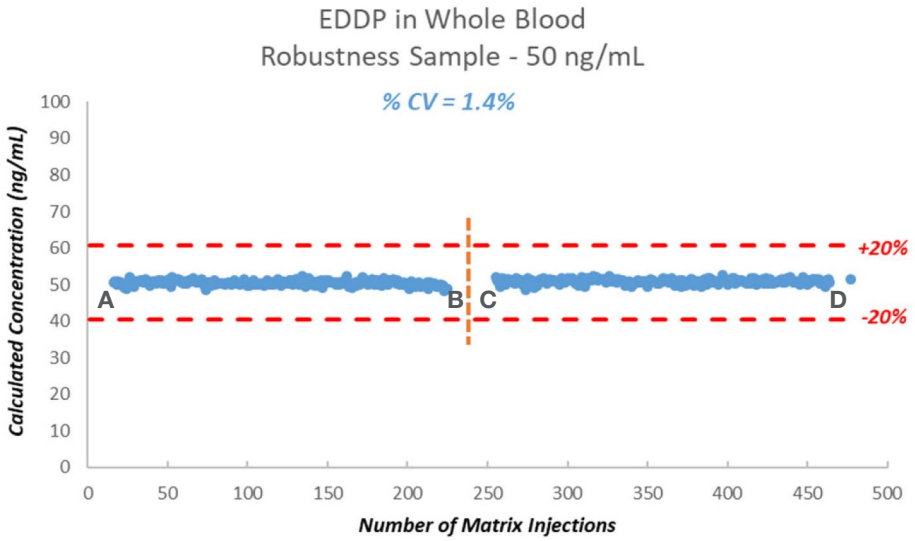

Figure 3. Precision for 50 ng/mL robustness sample of EDDP in whole blood over 480 injections. The orange mark represents the end of running one magazine of 10 sample plates. At this point the ion transfer tube was cleaned externally by wiping with a disposable wipe saturated with a mixture of water:methanol (1:1). Then, another VeriSpray magazine was run, containing 10 more sample plates. The points A, B, C, and D indicate the places in the sequence where calibration curves were run.

Conclusions

The new VeriSpray sample plates and VeriSpray ion source deliver accurate and robust quantitative results for measurement of EDDP in human whole blood for clinical research. Analysis was fast and simple, requiring no sample pretreatment or separation, and extended over 480 analyses without the need for extensive maintenance.

Reference

1. Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z., Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry. *Angewandte Chemie International Edition* **2010,** *49* (5), 877–880.

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TECHNICAL NOTE 73307

Combined PaperSpray and FAIMS technology for rapid quantification of immunosuppressants in whole blood for clinical research

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Keywords: VeriSpray, PaperSpray, FAIMS Pro, improved S/N, TSQ Altis, immunosuppressant

Goal

The goal of this technical note is to show the synergy between PaperSpray and FAIMS technology in quantifying the concentration of immunosuppressants in whole blood for clinical research.

Introduction

The rapid quantification of immunosuppressant drugs from blood is a key interest in the clinical research community. Common methods use immunoassays or LC/MS. Immunoassays are expensive, may suffer from antibody cross-reactivity, and have limited dynamic range. While LC/MS run times can be very short, protein crash and other sample preparation and clean-up steps lengthen the overall duration of the analytical method and solvent waste is generated.

PaperSpray-MS is a technique for rapidly quantifying analytes in dried matrix spots such as urine or whole blood. Little or no sample preparation is required, and sample analysis times are 2 minutes or less. The new Thermo Scientific™ VeriSpray™ PaperSpray ion source system utilizes PaperSpray technology to make clinical research workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. The VeriSpray system consists of the VeriSpray ion source and the Thermo Scientific™ VeriSpray™ plate loader (Figure 1A). The VeriSpray plate loader holds up to 10 VeriSpray sample plates (Figure 1B). Each VeriSpray sample plate contains

24 single-use paper strips (12 on each side, A and B, Figure 1C). The plate loader allows the full 10-plate magazine to be run without user intervention.

(A)

(B)

(C)

Figure 1. (A) VeriSpray ion source and plate loader, FAIMS Pro interface mounted onto TSQ Altis MS, (B) plate loader magazine, and (C) VeriSpray sample plate

Since PaperSpray is a direct analysis technique with no chromatographic separation and minimal sample cleanup, MS signals can have high background, which may limit the LOQ due to the signal-to-noise (S/N). Field asymmetric ion mobility spectrometry (FAIMS) is a technique that enhances selectivity of an analytical method by adding an additional dimension of separation based on ion mobility. It operates by applying an asymmetric waveform between a set of electrodes. Alternating between high and low field strengths impacts mobility of ions through a carrier gas. By applying an optimized compensation voltage (CV), target ions pass through the electrodes, while ions not of analytical interest are neutralized on the electrode walls.

By combining PaperSpray and FAIMS technology, the background noise can be reduced and the signal-to-noise ratio enhanced, thereby achieving lower limits of detection. Herein we analyze cyclosporin A, tacrolimus, and everolimus in whole blood using the new VeriSpray PaperSpray ion source, both with and without the Thermo Scientific™ FAIMS Pro™ interface.

Experimental

Three immunosuppressants—cyclosporin A, tacrolimus, and everolimus—were spiked into whole human donor blood at calibration levels ranging from 10 to 1600 ng/mL for cyclosporin A, 0.5 to 80 ng/mL for tacrolimus, and 2.5 to 80 ng/mL for everolimus. Their corresponding internal standards—cyclosporin A-D_4, tacrolimus-$^{13}C,D_2$, and everolimus-D_4—were also spiked into the blood samples at 640 ng/mL, 32 ng/mL, and 32 ng/mL, respectively. Ten microliters of each blood sample were spotted onto VeriSpray sample plates and oven-dried for 30 min at 45 °C. Five replicates of each calibration level were analyzed, both with and without the FAIMS Pro interface installed.

Dried plates were placed in the VeriSpray ion source plate loader. Before analysis, the source applies a rewet solvent directly onto the dried sample spot to extract analytes. Next, a spray solvent is dispensed onto the paper, and a high voltage is applied to the paper to facilitate spray and ion formation. A mixture of 60% methanol, 40% chloroform, and 0.1% sodium acetate was used as both the rewet and spray solvent. The wetting protocol and delays between solvent dispenses are shown in Table 1.

Data were acquired on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer coupled to the VeriSpray ion source with and without the FAIMS Pro interface using Thermo Scientific™ Xcalibur™ software. Two optimized transitions were monitored per analyte (Table 2)

Table 1. VeriSpray solvent application parameters. Each rewet and spray solvent dispense is 10 µL.

Rewet solvent dispense		Spray solvent dispense	
Dispense	Delay (s)	Dispense	Delay (s)
1	5	1	1
		2	1
		3	1
		4	1
		5	5
		6	5
		7	5
		8	5
		9	5
		10	5
		11	5
		12	10
		13	10
		14	10
		15	10

Table 2. Optimized SRM transitions and CV parameters for cyclosporin A, tacrolimus, everolimus, and their corresponding internal standards: cyclosporin A-D_4, tacrolimus-$^{13}C,D_2$, and everolimus-D_4

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	CV (V)
Tacrolimus	826.471	616.387	34.91	106	-22
Tacrolimus	826.471	443.304	46.58	106	-22
Tacrolimus-$^{13}C,D_2$	829.487	619.417	35.45	113	-22
Everolimus	980.57	389.292	55	163	-26
Everolimus	980.57	409.292	53.99	163	-26
Everolimus-D_4	984.599	393.321	54.45	161	-26
Cyclosporin A	1224.831	1112.917	55	223	-15
Cyclosporin A	1224.831	1207.042	55	223	-15
Cyclosporin A-D_4	1228.859	1112.774	55	153	-16

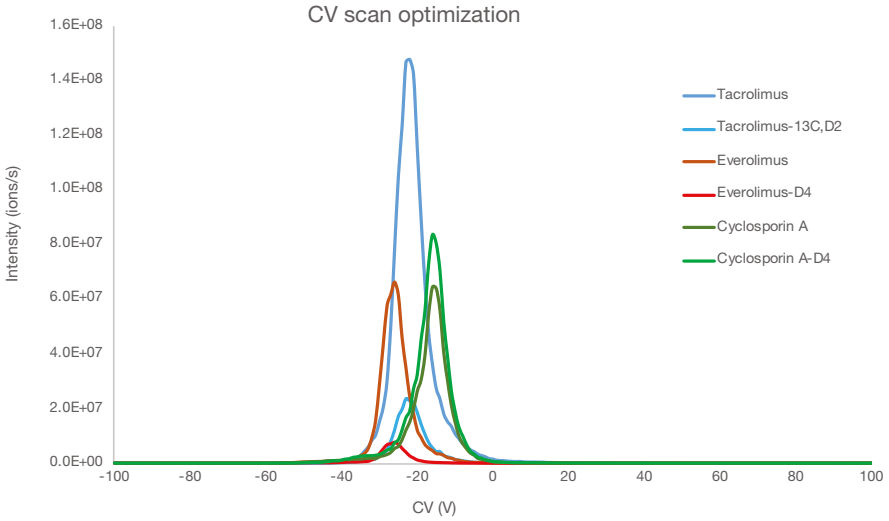

Figure 2. Scan of CVs from -100 to 100 V to determine optimized CV values for immunosuppressants and their internal standards

at a collision gas pressure of 1.5 mTorr. The ion transfer tube temperature was set to 350 °C. The distance of the paper tip with respect to the ion transfer tube, or FAIMS Pro entrance plate, was as follows: 5 mm without the FAIMS Pro interface, 2.5 mm with the FAIMS Pro interface. Data was acquired for 1 min per sample. The spray voltage, which was set at 3400 V for both experiments with and without the FAIMS Pro interface, was turned on at 0.1 min and turned off at 0.9 min to produce a chronogram. Chronograms were integrated using Thermo Scientific™ TraceFinder™ software to determine the area-under-the-curve (AUC). A summary of the TSQ Altis system settings are in Table 3.

The CV (compensation voltage) for each compound was optimized by infusion with the HESI source (Table 2 and Figure 2). The FAIMS Pro interface was operated in normal resolution mode at 100 °C and no additional user gas flow was set.

Table 3. (A) TSQ Altis MS parameters for the analysis of immunosuppressants and (B) time-dependent spray voltage settings

(A)

TSQ Altis MS Parameter	Value
Spray voltage	Time-dependent
Positive ion	3400 V
Sweep gas	0 Arb
Ion transfer tube temperature	350 °C
Q1 resolution	0.7
Q3 resolution	1.2
CID gas	1.5 mTorr

(B)

Time (min)	Voltage (V)
0	0
0.1	3400
0.9	0

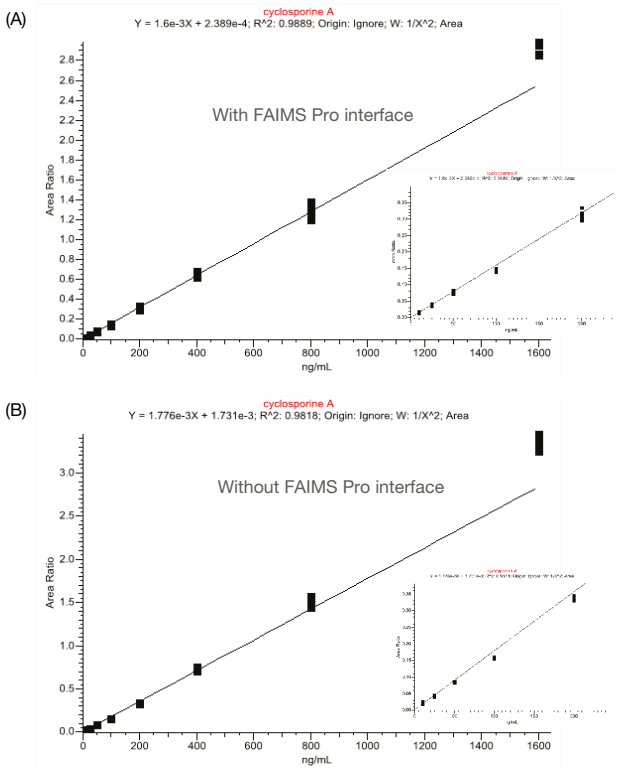

Figure 3. (A) Cyclosporin A calibration curve with FAIMS Pro interface and (B) without FAIMS Pro interface. Inset: calibration levels from 10 to 200 ng/mL

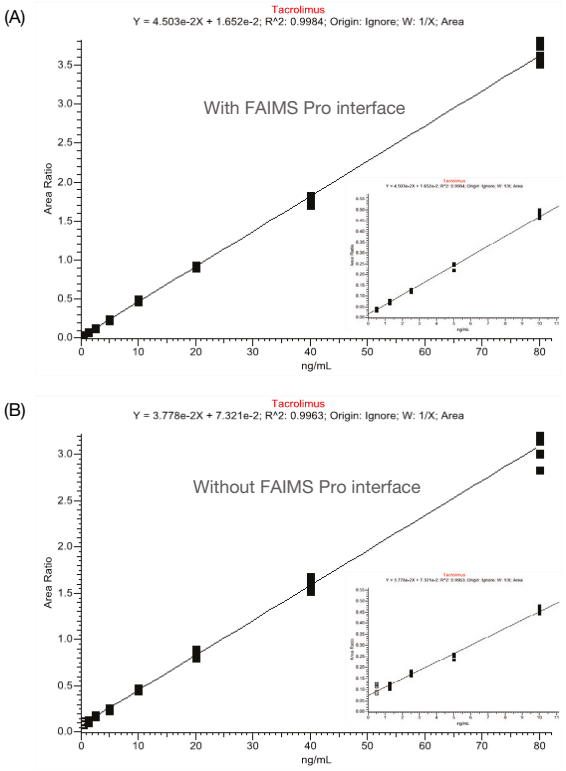

Figure 4. (A) Tacrolimus calibration curve with FAIMS Pro interface and (B) without FAIMS Pro interface. Open circles are excluded calibration levels that had accuracy ≥20%. Inset: calibration levels from 0.5 to 10 ng/mL

Results and discussion

The VeriSpray ion source was used to extract analytes from whole human blood and introduce ions into the mass spectrometer with very little sample preparation. Calibration curves were constructed for cyclosporin A, tacrolimus, and everolimus in human whole blood, acquired with the VeriSpray ion source both without and with the FAIMS Pro interface (Figures 3–5). With the FAIMS Pro interface installed, good precision, accuracy, and linearity were achieved for each immunosuppressant across the measured range. Without the FAIMS Pro interface, the lowest calibration level for tacrolimus and everolimus was excluded based on poor accuracy; this is due to this calibration level being close to the background signal.

With the FAIMS Pro interface, the LOQ (limit of quantitation) was equal to or lower than the LOQ without the FAIMS Pro interface. LOQs were determined based on the following criteria: S/N at the LOQ must be ≥4, precision and accuracy at the LOQ must be <15% and ≤20%, respectively, and the ion ratio of the target ion AUC to confirming ion AUC must be consistent. The LOQs for tacrolimus and everolimus were lowered to 0.5 ng/mL from 10 ng/mL and 5 ng/mL from 20 ng/mL, respectively, when the FAIMS Pro interface was used. The LOQ for cyclosporin

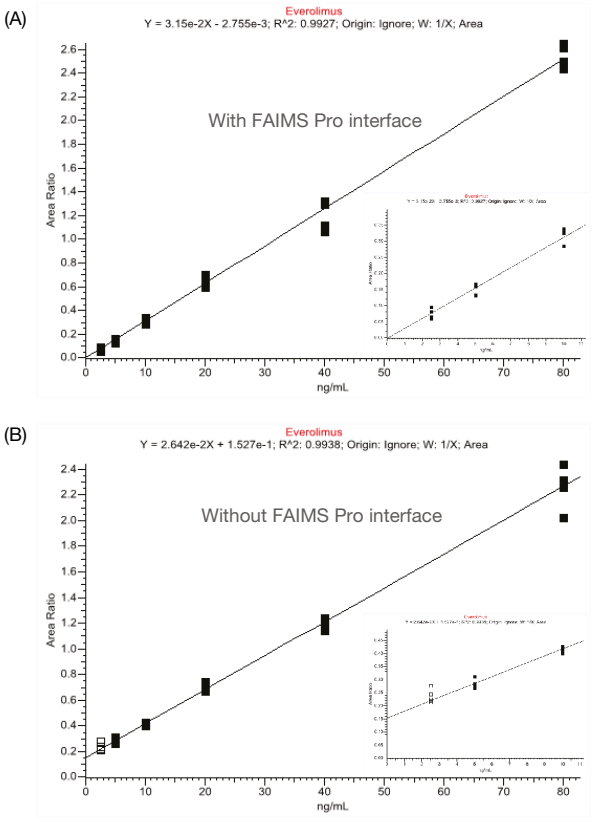

Figure 5. (A) Everolimus calibration curve with FAIMS Pro interface and (B) without FAIMS Pro interface. Open circles are excluded calibration levels that had accuracy ≥20%. Inset: calibration levels from 2.5 to 10 ng/mL

Table 4. Clinically relevant target level for tacrolimus, everolimus, and cyclosporin A and the LOQ (ng/mL) obtained using the VeriSpray ion source with and without FAIMS Pro interface

Compound	Target range (ng/mL)	LOQ with FAIMS Pro interface (ng/mL)	LOQ (ng/mL)
Tacrolimus	5-20	0.5	10
Everolimus	3-8	5	20
Cyclosporin A	100-400	25	25

Table 5. Comparison of average blank AUC, average cal level AUC, and S/N for immunosuppressants (A) with FAIMS Pro interface and (B) without the FAIMS Pro interface. Cal level is 800 ng/mL for cyclosporin A and 40 ng/mL for tacrolimus and everolimus.

(A)

With FAIMS Pro interface	Blank AUC	Cal AUC	S/N
Cyclosporin A	8.1	332528	41053
Tacrolimus	585	136378	233
Everolimus	27	27549	1024

(B)

Without FAIMS Pro interface	Blank AUC	Cal AUC	S/N
Cyclosporin A	1494	932426	624
Tacrolimus	11434	217741	19
Everolimus	8579	55477	6.5

A remained at 25 ng/mL because the LOQ was limited by the ion ratio not the S/N (Table 4). With the FAIMS Pro interface, the ranges of quantitation cover the clinically relevant ranges of immunosuppressants in human whole blood.

The FAIMS Pro interface filters interfering compounds based on differences in ion mobility. This feature is particularly useful for PaperSpray samples, which have no chromatographic separation. With the FAIMS Pro interface, the LOQs improved due to significant reduction in background signal of the matrix blank. Table 5 shows the average blank AUC, an average high cal level (800 ng/mL for cyclosporin A, and 40 ng/mL for tacrolimus and everolimus) AUC, and the S/N for samples with and without the FAIMS Pro interface. The high cal level AUCs show some reduction in signal, which is expected. However, the many-fold decrease in the blank signal leads to an improvement in the signal-to-noise. For cyclosporin A and everolimus, the FAIMS Pro interface almost entirely eliminates the transmission of interfering ions in the matrix blank. In Figures 6-8, example chronograms of the matrix blank and the LOQ calibrator for each immunosuppressant with and without the FAIMS Pro interface are shown. All chronograms have the typical square shape that results

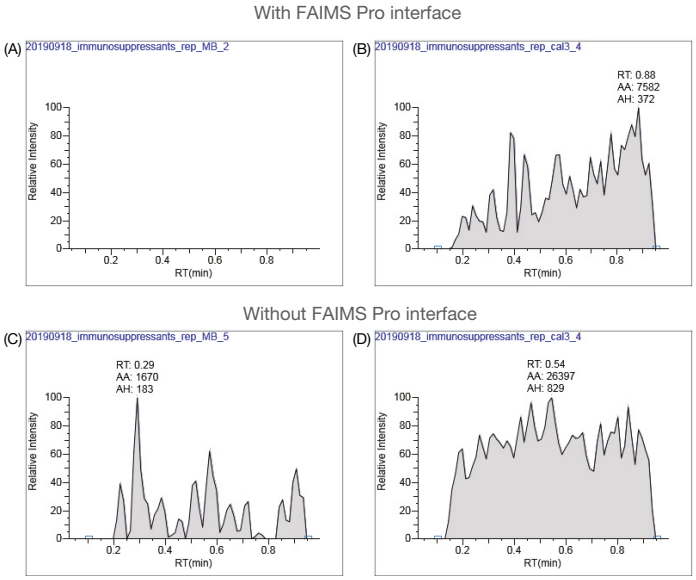

Figure 6. Cyclosporin A chronograms: A) matrix blank with FAIMS Pro interface, B) 25 ng/mL calibrator with FAIMS Pro interface, C) matrix blank, D) 25 ng/mL calibrator

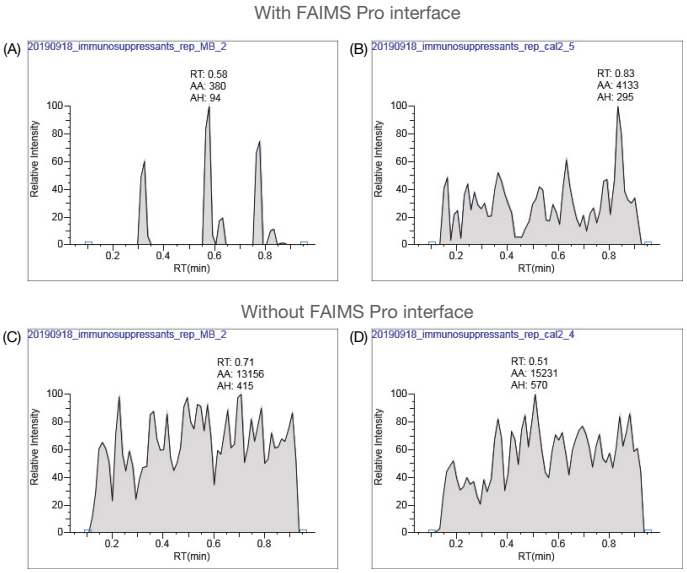

Figure 7. Tacrolimus chronograms: A) matrix blank with FAIMS Pro interface, B) 0.5 ng/mL calibrator with FAIMS Pro interface, C) matrix blank, D) 0.5 ng/mL calibrator

from ion generation only when voltage is applied except those for matrix blanks with the FAIMS Pro interface. The chronograms of matrix blanks with the FAIMS Pro interface have low intensity signal spikes or no signal at all because of the reduction in the transmission of interfering ions.

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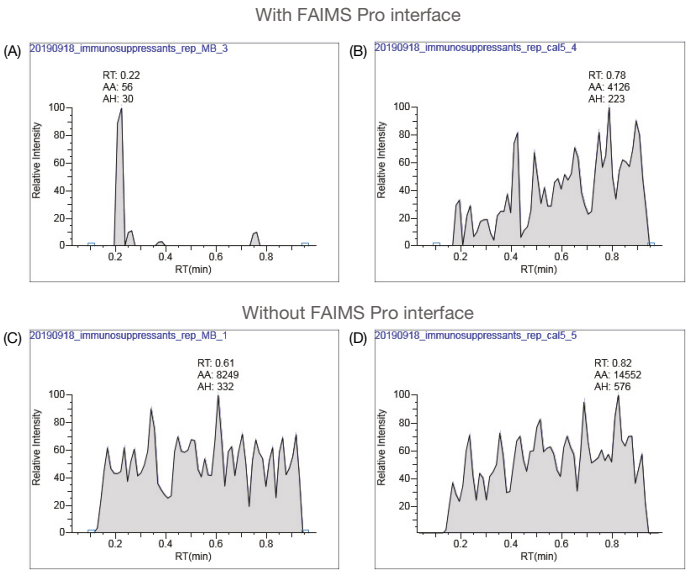

Figure 8. Everolimus chronograms: A) matrix blank with FAIMS Pro interface, B) 5 ng/mL calibrator with FAIMS Pro interface, C) matrix blank, D) 5 ng/mL calibrator

Conclusion

The VeriSpray ion source extracts immunosuppressants from whole human blood and introduces ions into the mass spectrometer with little or no sample preparation. When using the FAIMS Pro interface, the background signal is significantly reduced and the LOQ is lowered. The combination of FAIMS Pro and PaperSpray technologies yields an easy-to-use, sensitive, fast analytical method for clinical research.

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TECHNICAL NOTE 73300

Improving limit of quantification in clinical research by combining PaperSpray with FAIMS technology

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Thermo Fisher Scientific, San Jose, CA

Keywords: VeriSpray, PaperSpray, FAIMS, improved S/N, TSQ Altis, drug monitoring research

Application benefits

- Fast analysis with no sample preparation
- Reduced background interference
- Improved LOD and LOQ in matrix samples with no sample preparation

Goal

In PaperSpray analysis, signal-to-noise is often the limiting factor in achieving lower LODs and LOQs. The goal of this technical note is to show the benefit of coupling PaperSpray with FAIMS technology to reduce background interferences and increase signal-to-noise, thereby lowering the limit of detection.

Introduction

PaperSpray mass spectrometry (PS-MS) is a rapid technique for analysis of compounds directly from unprocessed dried sample spots. Because minimal to no sample preparation is required, the technique is particularly beneficial for biological sample matrices, which normally require time-consuming and labor-intensive sample cleanup when analyzed by LC-MS. However, because of the lack of sample cleanup, PaperSpray can produce a high background ion signal. High background noise can limit the signal-to-noise ratio (S/N) and compromise the LOQ and LOD of the method. Field asymmetric ion mobility spectrometry (FAIMS) is a technique that enhances

selectivity of an analytical method by adding an additional dimension of separation based on differential mobility. It operates by applying an asymmetric waveform between a set of electrodes. Alternating between high and low field strengths impacts the mobility of ions through a carrier gas. By applying an additional, optimized offset voltage (compensation voltage, or CV), ions of particular mobility pass through the electrodes, while background ions get neutralized on the electrode walls.

By combining PaperSpray and FAIMS technology, background noise can be reduced, and signal-to-noise ratio enhanced. Here, we demonstrate this principle by analyzing the antidepressant drug amitriptyline using the new Thermo Scientific™ VeriSpray™ PaperSpray ion source, both with, and without the Thermo Scientific™ FAIMS Pro™ interface.

Experimental

Amitriptyline was spiked into donor human blood at concentrations ranging from 0.1 to 500 ng/mL to construct calibration curves. Amitriptyline-d_3 was used as internal standard and spiked into the blood at 50 ng/mL. QC samples were prepared at three additional concentrations (20, 100, and 400 ng/mL). Ten microliters of each sample were spotted onto VeriSpray sample plates and oven-dried for 30 min at 45 °C. Each concentration was spotted onto three different paper strips. A mixture of 95% methanol, 5% water, and 0.01% acetic acid was used as both rewet and spray solvent. The wetting steps are shown in Table 1.

Table 1. VeriSpray solvent application parameters. Each rewetting and solvent dispense is 10 µL.

Rewetting dispense delay		Solvent dispense delay	
Dispense	Delay (s)	Dispense	Delay (s)
1	1	1	1
		2	1
		3	1
		4	1
		5	2
		6	5
		7	5
		8	5
		9	5
		10	0

Data was acquired on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer coupled to the VeriSpray ion source and the FAIMS Pro interface. Three transitions were monitored per compound (Table 2), at a collision gas pressure of 2 mTorr. The ion transfer tube temperature was set to 350 °C. Data were acquired for 2 min per sample. The spray voltage was turned on at 0.1 min and turned off at 1.9 min to create a chronogram, which can easily be integrated (Figure 1). The spray voltage was set to 4 kV for runs without the FAIMS Pro interface, and 3.3 kV for runs with the FAIMS Pro interface. The distance of the paper tip with respect to the ion transfer tube, or FAIMS Pro entrance plate, was as follows: 4.5 mm without the FAIMS Pro interface, 2.4 mm with the FAIMS Pro interface (Figure 2). When the FAIMS Pro interface was in use, the CV was optimized by infusion with the HESI source. The optimum value for both amitriptyline and amitriptyline-d_3 was -29.5 V. The CV optimization scan for amitriptyline is shown in Figure 3. The FAIMS Pro interface was operated in normal resolution mode, and no additional user gas flow was set.

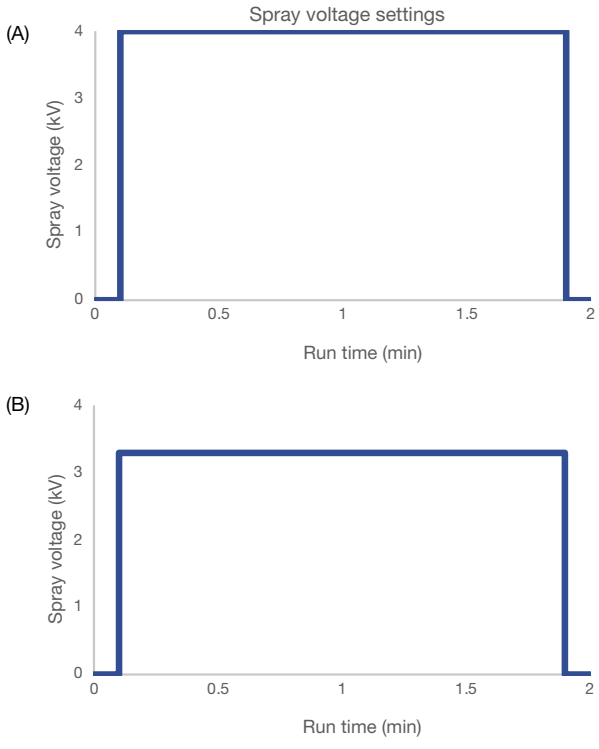


Figure 1. Time-dependent spray voltage settings for the analyses (A) without the FAIMS Pro interface, (B) with the FAIMS Pro interface

Table 2. Optimized mass spectrometer transitions for amitriptyline and the deuterated internal standard amitriptyline-d_3

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Amitriptyline	278.2	117.1	24	65
	278.2	191.1	26	65
	278.2	233.1	18	65
Amitriptyline-d_3	281.2	117.1	24	62
	281.2	191.1	26	62
	281.2	233.1	18	62

(A)

(B)

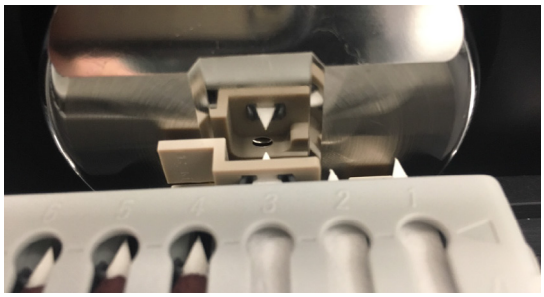


Figure 2. (A) VeriSpray PaperSpray system and FAIMS Pro interface mounted to TSQ Altis triple quadrupole mass spectrometer, (B) VeriSpray tip positioned in front of FAIMS Pro entrance plate

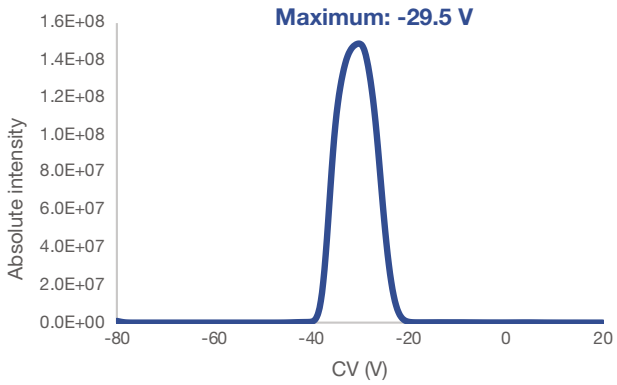
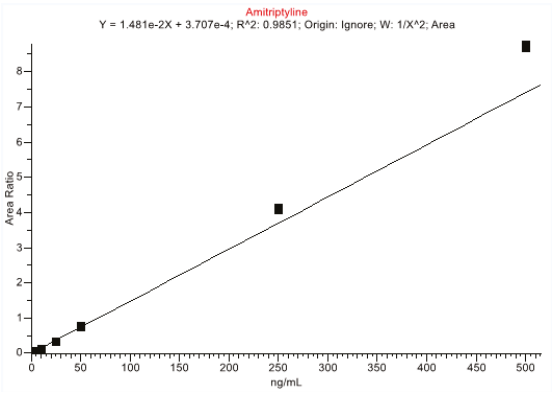


Figure 3. CV Scan for amitriptyline (*m/z* 278.2, acquired in SIM mode), resulting in an optimized CV value of -29.5 V

Results and discussion

Calibration curves were generated for amitriptyline in human whole blood, acquired both without and with FAIMS technology (Figure 4). Both curves were linear over the measured concentration range. LOQs were based on the following criteria: precision and accuracy at the LOQ must be ≤15% and within ±20%, respectively. Further, the S/N at the LOQ must be ≥4. Without the FAIMS Pro interface, an LOQ of 2.5 ng/mL was obtained. With the FAIMS Pro interface, the LOQ was 1.0 ng/mL (Table 3).

(A) Amitriptyline without FAIMS



(B) Amitriptyline with FAIMS

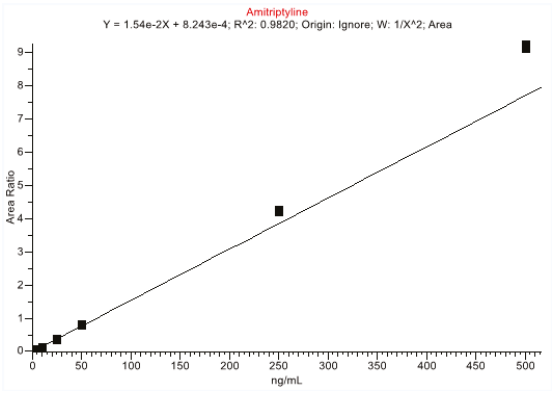


Figure 4. Calibration curves for amitriptyline, obtained from human whole blood (A) without the FAIMS Pro interface, and (B) with the FAIMS Pro interface

Table 3. LOQ obtained for amitriptyline, without and with the FAIMS Pro interface

	Without FAIMS	With FAIMS
LOQ (ng/mL)	2.5	1.0

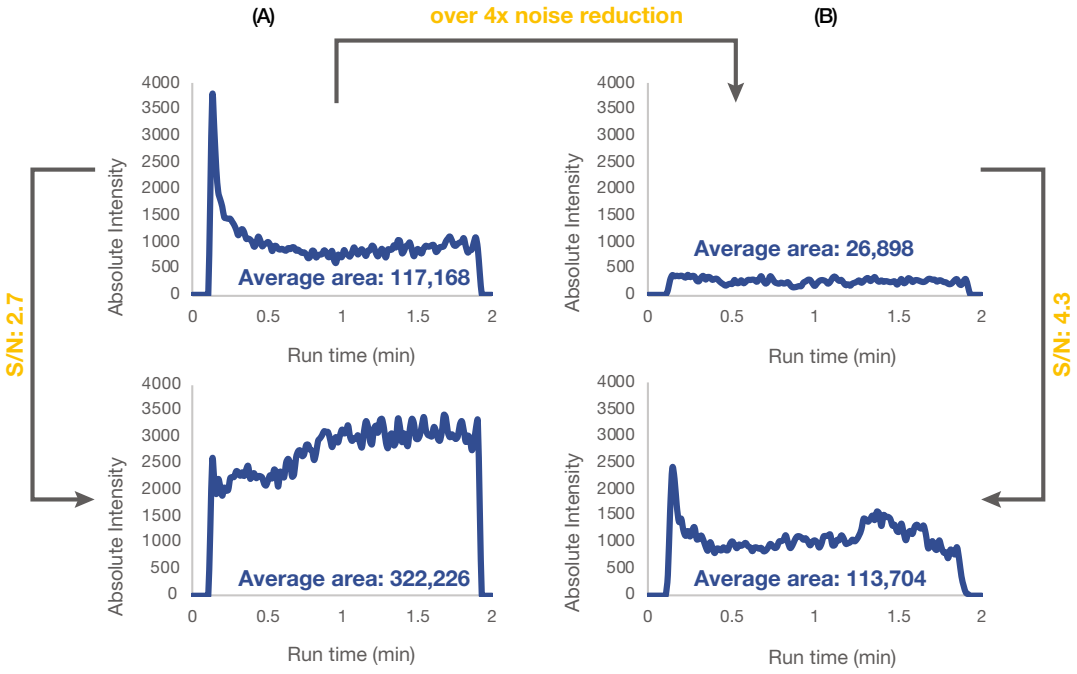


Figure 5. Chronograms of a matrix blank (top) and 1 ng/mL amitriptyline in whole blood (bottom), acquired (A) without the FAIMS Pro interface, and (B) with the FAIMS Pro interface. Because of significant reduction in background interference, the data obtained with the FAIMS Pro interface meets the LOQ requirement of S/N ≥ 4 at a concentration of 1 ng/mL.

Figure 5 shows a comparison of the chronograms of both a 1 ng/mL sample and a matrix blank sample obtained both without and with the FAIMS Pro interface. The background signal in the matrix blank (absolute area under the trace) obtained with the FAIMS Pro interface was over four times lower compared to the background obtained without the FAIMS Pro interface. Because ions of different structures have different ion mobilities through the FAIMS Pro device, it acts as a filter, which limits, or even eliminates, transmission of interfering ions. Although the signal for amitriptyline decreased slightly with the FAIMS Pro interface attached, the background decreased significantly more, thereby raising the S/N from 2.7 to 4.3 at a concentration of 1 ng/mL. This S/N improvement demonstrates the effectiveness of the FAIMS Pro interface in reducing background interferences, which cannot be separated by mass spectrometry alone.

Table 4 shows the precision of each calibrator sample, without and with the FAIMS Pro interface. For both experiments, %RSDs obtained are well below 10%. Table 5 shows the intra-day precision and accuracy of the QC samples. The precision was under 2% for three replicates, and accuracy was within ±20%. For the run without the FAIMS Pro interface, additional inter-day precision and accuracy were obtained using the mid-level QC sample (Table 6). On all three days, the precision was under 1% RSD and the accuracy was within ±12%.

Table 4. Precision of calibrator samples for amitriptyline, without and with the FAIMS Pro interface

Concentration (ng/mL)	%RSD	
	Without FAIMS	With FAIMS
1.0	N/A	7.1
2.5	3.6	9.9
5.0	0.5	0.7
10.0	1.4	0.4
25.0	0.7	0.4
50.0	1.2	0.9
250.0	0.8	1.0
500.0	0.4	0.6

thermo scientific

Table 5. Intra-day precision and accuracy of QC samples (three replicates per concentration) for amitriptyline, without and with the FAIMS Pro interface

Concentration (ng/mL)	%RSD		% Difference	
	Without FAIMS	With FAIMS	Without FAIMS	With FAIMS
20	0.5	1.7	- 15.7	- 10.2
			- 15.0	- 12.5
			- 15.5	- 13.0
100	0.2	0.2	- 10.0	- 9.3
			- 10.3	- 9.7
			- 10.2	- 9.5
400	0.2	1.0	1.7	1.1
			1.8	2.6
			2.2	3.0

Table 6. Inter-day precision and accuracy of the mid-level QC sample (100 ng/mL) on three consecutive days (three replicates per day) for amitriptyline, without the FAIMS Pro interface

Day	%RSD	% Difference
1	0.2	- 10.0
		- 10.3
		- 10.2
2	0.9	- 11.4
		- 10.0
		- 11.3
3	0.7	- 11.3
		- 10.2
		- 10.2

Conclusion

Combining FAIMS and PaperSpray technology leads to improved S/N and lowers the LOQ for amitriptyline, measured directly from human whole blood. Using the FAIMS Pro interface for this type of clinical research analytical method makes it possible to take advantage of short turnaround times and ease-of-use offered by the VeriSpray ion source, without limiting analytical method performance arising from interferences.

Acknowledgements

We would like to thank Rae Ana Snyder, Satendra Prasad, Michael Belford, and Kristine Van Natta for helpful discussions.

Find out more at **thermofisher.com/FAIMSPro**
thermofisher.com/VeriSpray
thermofisher.com/Altis

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Analysis of Immunosuppressant Drugs directly from Whole Blood using PaperSpray Technology

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ABSTRACT

Purpose: Minimizing sample preparation for the analysis of immunosuppressant drugs using PaperSpray technology for clinical research.

Methods: Cyclosporin A, Everolimus and Tacrolimus were analyzed using a 2-minute method directly from dried blood spots on Thermo Scientific™ VeriSpray™ PaperSpray ion source sample plates.

Results: Very good linearity and precision were achieved for all compounds. Cyclosporin A and Tacrolimus meet the clinical research range in whole blood. Everolimus exhibits high background signal which currently limits the LOQ. Strategies to further reduce interfering signals are currently in development.

INTRODUCTION

Immunosuppressant drugs are of significant interest in the clinical community. Various analysis methods have been published, the majority of them requiring a protein crash followed by LC/MS analysis [Ref 1]. LC/MS run times for this assay can be relatively short, e.g., 4.5 minutes and under [Ref 2]. However, extensive sample preparation and clean-up are necessary for LC injections, increasing the duration of the workflow.

PaperSpray technology is a rapid analysis technology specifically suitable for clinical research samples. Quick sample turnaround times of 2 minutes or less make it very competitive compared to traditional LC/MS-based techniques. Minimal sample preparation is required for analysis of dried urine or blood spots from a piece of triangular shaped paper. A rewet solvent is applied directly onto the dried sample spot to extract analytes. Next, a spray solvent is dispensed onto the paper, and a high voltage is applied to the paper to facilitate spray and ion formation.

The new VeriSpray PaperSpray ion source system uses PaperSpray technology to make clinical research workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. The VeriSpray system consists of the VeriSpray ion source and the VeriSpray plate loader (Figure 1, left). The VeriSpray plate loader holds up to 10 VeriSpray sample plates (Figure 1, right). Each VeriSpray sample plate contains 24 paper strips (12 on each side, A and B). Through Thermo Scientific™ Xcalibur™ software sequence setup, the full magazine can be run in an automated way.

Here we demonstrate a PaperSpray method for the analysis of immunosuppressant drugs from a single whole blood spot for research purposes.

Figure 1. VeriSpray ion source with VeriSpray plate loader (left) and VeriSpray sample plate (right).

MATERIALS AND METHODS

Sample Preparation

Human blood samples were spiked with the respective drugs and incubated at 4 C over night. On the next day, isotopically labelled internal standards were added and a volume of 10 uL was spotted on VeriSpray sample plates.

Method and Data Analysis

The spray solvent and inlet capillary temperature were optimized (see results section). Data were acquired for 2 minutes per sample, and 4 replicates of each calibrator and QC level were measured. Compounds were detected on a Thermo Scientific™ TSQ Quantis™ Triple Quadrupole mass spectrometer. Four transitions were monitored per compound, with a cycle time of 1.5 seconds and a collision gas pressure of 2.5 mTorr. The spray voltage was 3.6 kV, applied from 0.05 to 1.95 min and the distance paper tip to inlet was approximately 5.5 mm. Thermo Scientific™ TraceFinder™ software, version 4.1 was used for data analysis.

METHOD OPTIMIZATION

Method of spiking the internal standard

Four different internal standard solutions were made which resulted in the same final internal standard concentration in blood, but used different spiking volumes. The following ratios (blood / IS solution) were spiked: IS solution 1: 5:1, IS solution 2: 1:1, IS solution 3: 1:2, IS solution 4: 1:3. %RSD values were obtained for the absolute areas and the response ratio (analyte / internal standard), and are summarized in Table 1. Because IS solution 3 gave the best %RSD values for both, absolute areas and area ratios, it was chosen for further method optimization.

Table 1. %RSD of the absolute area and response ratio (analyte/internal standard) obtained across 3 replicates per condition.

	Cyclosporin A		Everolimus		Tacrolimus	
c (analyte) in blood	400 ng/mL		20 ng/mL		20 ng/mL	
c (IS) in blood	300 ng/mL		15 ng/mL		15 ng/mL	
	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio
IS solution 1	29.6	10.9	13.6	1.8	20.5	5.5
IS solution 2	31.5	2.5	15.6	5.4	33.3	7.6
IS solution 3	34.8	3.1	9.6	4.2	24.5	2.2
IS solution 4	54.3	12.4	31.8	9.1	35.4	3.3

Solvent system optimization

Table 2. %RSD of the response ratio (analyte/internal standard) and %RSD of the absolute areas obtained across 3 replicates per condition. Each solvent contains 0.1% sodium acetate.

	Cyclosporin A		Everolimus		Tacrolimus	
c (analyte) in blood	400 ng/mL		20 ng/mL		20 ng/mL	
c (IS) in blood	300 ng/mL		15 ng/mL		15 ng/mL	
	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio
100% methanol	34.9	1.2	21.3	6.3	32.4	7.6
80% meth., 20% chlorof.	33.8	6.3	24.5	8.1	29.5	9.9
60% meth., 40% chlorof.	29.4	3.4	26.4	1.9	26.5	3.4

Best results were obtained for a solvent composition of 60% methanol, 40% chloroform, and 0.1% sodium acetate (see Table 2).

Inlet capillary temperature

For optimization of the inlet capillary temperature (Table 3) IS solution 3 was used and the spray solvent was 60% methanol, 40% chloroform and 0.1% sodium acetate.

Table 3. %RSD of the response ratio (analyte/internal standard) and %RSD of the absolute areas obtained across 3 replicates per condition.

	Cyclosporin A		Everolimus		Tacrolimus	
c (analyte) in blood	250 ng/mL		12.5 ng/mL		12.5 ng/mL	
c (IS) in blood	300 ng/mL		15 ng/mL		15 ng/mL	
	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio	%RSD absolute	%RSD ratio
325 C	50.0	1.5	39.4	11.2	39.3	1.5
350 C	36.2	5.9	19.3	4.7	28.0	4.2
375 C	14.2	2.7	28.7	9.4	11.9	4.5
400 C	35.3	6.1	22.1	4.6	32.4	9.5

Because Everolimus is the most challenging compound to detect using this method, the condition that produced the best result for Everolimus (400 C) was chosen for the final method.

RESULTS

Cyclosporin A and Tacrolimus

The final experiment was carried out using the following optimized conditions:

- Ratio (Blood / IS solution): 1:2
- Solvent: 60% methanol, 40% chloroform, 0.1% sodium acetate in rewet and spray solvent
- Inlet capillary temperature: 400 C

The resulting calibration curves for Cyclosporin A and Tacrolimus are shown in Figures 2 and 3.

Figure 2. Calibration curve for Cyclosporin A

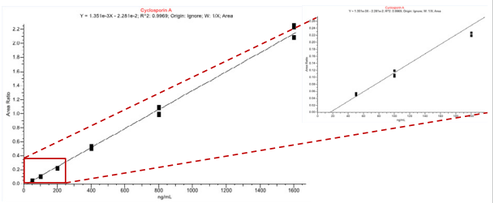
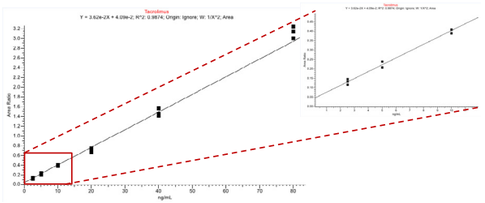


Figure 3. Calibration curve for Tacrolimus



For both, Cyclosporin A and Tacrolimus, precision and accuracy values meet the LOQ requirement (lower than 15% and 20% respectively) at all calibrator and QC levels, including the lowest calibrator level (see Tables 4 and 5). Resulting LOQs are:

Cyclosporin A: 50 ng/mL

Tacrolimus: 2.5 ng/mL

Table 4. Precision and accuracy of calibrator samples.

Level	Cyclosporin A			Tacrolimus		
	c (ng/mL)	%RSD	% Difference	c (ng/mL)	%RSD	% Difference
1	50	4.1	< 13.6	2.5	9.8	< 17.8
2	100	6.8	< 7.8	5	8.0	< 9.5
3	200	2.1	< 10.8	10	2.9	< 4.0
4	400	3.4	< 4.0	20	4.7	< 12.4
5	800	5.6	< 7.5	40	5.4	< 5.6
6	1600	4.3	< 5.8	80	3.6	< 10.8

Table 5. Precision and accuracy of QC samples.

Level	Cyclosporin A			Tacrolimus		
	c (ng/mL)	%RSD	% Difference	c (ng/mL)	%RSD	% Difference
1	160	1.9	- 4.9	8	3.1	- 9.8
			- 7.5			- 1.7
			- 5.6			- 6.8
			- 8.4			- 4.9
2	250	3.7	- 10.1	12.5	1.7	- 4.7
			- 2.7			- 1.2
			- 8.1			- 5.0
			- 5.8			- 2.3
3	500	0.8	- 11.7	25	3.1	1.2
			- 11.5			- 1.4
			- 10.9			- 6.3
			- 10.3			- 1.3

Everolimus

The resulting calibration curve and precision values for Everolimus are shown below. Good linearity and precision was achieved (see Figure 4 and Table 6). However, because of significant contribution of background signal, the accuracy was not sufficient to meet clinical research criteria.

Figure 4. Calibration curve for Everolimus.

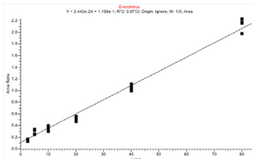


Table 6. Precision of calibrator and QC samples.

Level	Everolimus	
	c (ng/mL)	%RSD
Cal 1	2.5	11.6
Cal 2	5	14.9
Cal 3	10	13.2
Cal 4	20	6.8
Cal 5	40	4.6
Cal 6	80	5.2
QC 1	8	7.6
QC 2	12.5	5.3
QC 3	25	5.9

In order to meet clinical research criteria for Everolimus, as well as Sirolimus, the background signal coming from paper needs to be further reduced. Strategies to achieve this are currently in development.

CONCLUSIONS

- PaperSpray minimizes the need for sample preparation. Mixing internal standard with the sample, spotting samples on the sample plates and drying them are the only required steps.
- Quantification of Cyclosporin A and Tacrolimus is easily achievable in the required measurement range, with the necessary precision and accuracy.
- Further cleanup of the paper and sample plates is under investigation to reduce background signals for Everolimus.

REFERENCES

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2. A. Buchwald et al., *BMC Clin. Pharmacol.* **2012**, 12: 2.

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TRADEMARKS/LICENSING

PO65512-EN0519S

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Robust Quantitative Analysis of EDDP by PaperSpray Mass Spectrometry

Neloni R Wijeratne, Stephanie Samra, Cornelia Boeser, Katherine Walker **Thermo Fisher Scientific, San Jose, CA**

ABSTRACT

Purpose: Development of robust workflow for quantitation of EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine) in human whole blood and urine using Thermo Scientific™ TSQ mass spectrometers coupled to the new Thermo Scientific™ VeriSpray™ PaperSpray™ Ion Source and Plate Loader.

Methods: EDDP calibrators, controls, and robustness samples were prepared in human whole blood or urine and spotted onto 10 sample plates. A set of calibrators and controls were run at the beginning and end of the sequence, to monitor systems quantitative capability during this robustness study.

Results: The lower limits of quantification (LLOQ) for EDDP in both blood and urine were 3.5 ng/mL and robustness samples showed excellent accuracy and precision over the full run of 10 plates in both blood and urine.

Figure 1. (A) VeriSpray ion source and plate loader mounted to TSQ Quantis mass spectrometer (B) VeriSpray magazine and (c) VeriSpray sample plate

INTRODUCTION

Since its introduction nine years ago in Graham Cook's lab,[1] PaperSpray ionization has been applied to many areas: from clinical research and research & development labs to food safety and environmental markets.[2-4] As the name implies, a strip of paper is used to introduce the sample into the mass spectrometer. Because little to no sample preparation is required and analysis times are short, PaperSpray technology provides significant benefits for high-throughput applications.

Using the new Thermo Scientific VeriSpray system, up to 240 samples can be analyzed in an unattended fashion. The VeriSpray Plate Loader and magazine holds up to 10 VeriSpray sample plates. Each VeriSpray sample plate is equipped with 24 paper strips. Figure 1 shows a picture of the VeriSpray system, magazine and VeriSpray sample plate.

In this study, we developed a two minute analytical method that detects EDDP, a primary opioid metabolite of methadone, using PaperSpray mass spectrometry. This workflow eliminates the need for sample preparation and enables quick turnaround times needed in clinical research.

MATERIALS AND METHODS

Sample Preparation

- 20 µL of spiking solution (final concentration range for calibrators: 1.75 – 500 ng/mL, final concentrations for control samples: 50, 100 and 200 ng/mL) and 5 µL of internal standard (final concentration of 200 ng/mL EDDP-d3) were added to 1 mL of human whole blood or urine
- The robustness sample was prepared by spiking 40 µL working solution (final concentration of 50 ng/mL) and 10 µL of internal standard (final concentration of 200 ng/mL EDDP-d3) were added to 2mL of human whole blood or urine
- Samples were put on a blood shaker for 20-30 minutes (whole blood) or vortexed for 1 min (urine)
- Two sets of 240 samples of EDDP in blood or urine (2 magazines, 480 samples total) consisting of calibrators, controls and robustness sample were spotted on to the sample plates (spotting volume is 8 µL for whole blood and 5 µL for whole blood)
- Sample plates were oven-dried at a temperature of 45 ° C for 5 mins and 30 mins for urine and blood respectively.

PaperSpray Conditions

- For EDDP in human whole blood

  Rewetting (20 µL) and Spraying (110 µL) solvents were both 95/5 Acetonitrile/Water 0.01% acetic acid

- For EDDP in urine

  Rewetting (20 µL) and Spraying (110 µL) solvents were both 90/10 Acetonitrile/Water 0.1% acetic acid

Mass spectrometry

The EDDP study in human whole blood was carried out on a TSQ Quantis mass spectrometer connected with the VeriSpray system. Table 1 and 2 shows the MS source parameters and critical MS features for EDDP respectively. No sweep gas or sweep cone was used. The paper tip to MS inlet distance was set to 6.5 mm to maintain system robustness without compromising the system sensitivity.

Table 1. (a) TSQ Quantis MS source conditions for EDDP analysis (b) time dependent spray voltage settings

(a)

Ion Source Parameter	Value
Spray Voltage	Time Dependent
Positive Ion	3800 V
Ion Transfer Tube Temperature	350 ° C
Q1 Resolution	0.7
Q3 Resolution	1.2
CID Gas	2 mTorr

(b)

Time (min)	Voltage (V)
0	0
0.1	3800 V
0.95	0

Table 2. Optimized MS transitions for EDDP on TSQ Quantis MS with acquisition time of 1.0 min

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
EDDP	278.29	234.21	31.5	206
	278.29	158.21	44.9	206
	278.29	186.21	35.7	206
EDDP-d3	281.29	234.21	32.0	202
	281.29	157.18	52.3	202
	281.29	189.21	36.5	202

The EDDP study in urine was carried out on a Thermo Scientific™ TSQ Fortis™ MS coupled to the Thermo Scientific VeriSpray system. Both the rewet and spray solvents were 90/10 Acetonitrile/water 0.1% acetic acid and the same volumes were applied as in blood analysis.

Table 3. (a) TSQ Fortis MS source conditions for EDDP analysis (b) time dependent spray voltage settings

(a)

Ion Source Parameter	Value
Spray Voltage	Time Dependent
Positive Ion	4000 V
Ion Transfer Tube Temperature	325 ° C
Q1 Resolution	0.7
Q3 Resolution	1.2
CID Gas	2 mTorr

(b)

Time (min)	Voltage (V)
0	0
0.1	4000 V
0.95	0

Table 4. Optimized MS transitions for EDDP on TSQ Fortis MS with acquisition time of 1.0 min

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Tube Lens (V)	Source Fragmentation (V)
EDDP	278.29	234.21	31.5	85	14.7
	278.29	158.21	44.9	85	14.7
	278.29	186.21	35.7	85	14.7
EDDP-d3	281.29	234.21	32.0	85	14.7
	281.29	157.18	52.3	85	14.7
	281.29	189.21	36.5	85	14.7

Samples were analyzed by, first, extraction of the sample spot by application of rewet solution. Then spray solvent is added to carry the sample to the paper tip by capillary action. Finally, spray voltage is applied under ambient conditions to induce ionization that introduces sample into the MS. Once the first set of 240 samples was analyzed in an unattended fashion, the magazine is loaded with the second set of 10 sample plates, which again is ran in an unattended manner. In between the 2 sets the external surface area of the ion transfer tube was wiped using disposable wipe soaked with a water: methanol (1:1) mixture. Thermo Scientific™ TraceFinder™ 4.1 software was used for data processing.

RESULTS

EDDP in Human Whole Blood

We were able to achieve linear range of 3.5 – 500 ng/mL for EDDP in whole blood. The lower limit of quantification (LLOQ) on the TSQ Quantis for EDDP was 3.5 ng/mL, as defined as the lowest calibration standard analyzed that yielded < 20% accuracy and < 15% CV for 4 replicate samples. The overlaid 4 calibration curves are shown in Figure 2. All four calibration curves yielded the same LLOQ for EDDP. The precision and accuracy of the robustness sample, 50 ng/mL, met the method requirements and has a RSD of 1.4% for the calculated concentration throughout 480 samples. Wiping the ion transfer tube with a disposable wipe soaked with water and methanol was sufficient to remove all visible traces of blood and produced reproducible data between the first 240 injections (first full magazine) and latter 240 injections (second full magazine).

Figure 2. Overlaid 4 calibration curves of EDDP in human whole blood showing excellent reproducibility. All calibration curves gave the same LLOQ of 3.5 ng/mL for EDDP in whole human blood

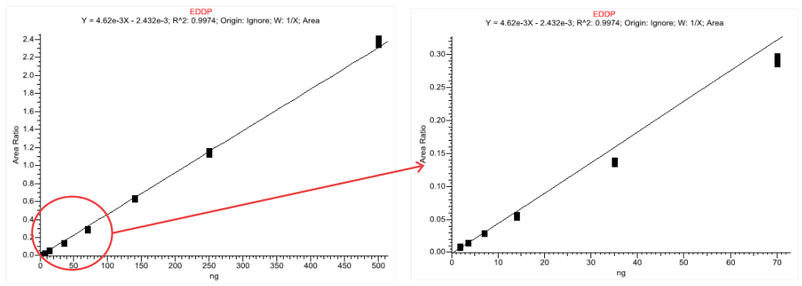
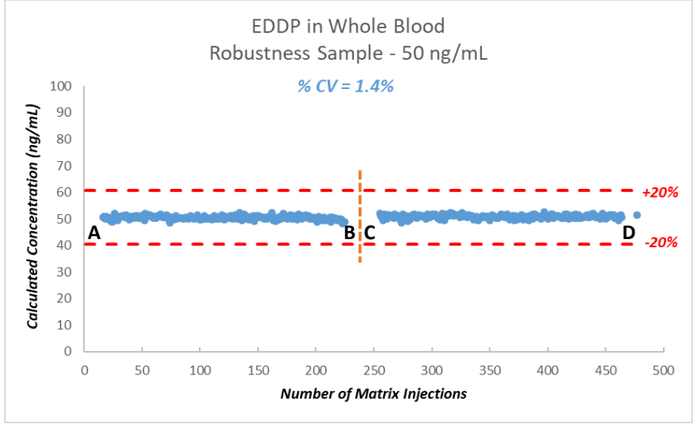


Table 5. Precision and accuracy of the EDDP calibrators in human whole blood

Theoretical Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy	%RSD
3.5	3.64	4.1	2.6
7	6.79	-3.0	2.2
14	12.3	-12.1	3.8
35	30.2	-13.6	1.7
70	63.3	-9.6	1.9
140	138	-1.8	1.2
250	249	-0.3	1.5
500	516	3.2	1.4

Figure 3. Precision of EDDP robustness sample in whole blood on TSQ Quantis MS over 480 samples. The orange line represents the end of first set of 10 sample plates, at which point the ion transfer tube was cleaned externally by wiping with a disposable wipe saturated with a mixture of water: methanol (1:1). The points A, B, C and D represent where in the sequence calibration curves were run.



EDDP in Urine

The lower limit of quantification (LLOQ) for EDDP in urine was 3.5 ng/mL 4 replicate samples. Figure 4 shows the overlaid 4 calibration curves. The precision and accuracy of the robustness sample, 50 ng/mL, met the method requirements and had an RSD of 2.1% for the calculated concentration throughout 480 samples. The precision and accuracy for all the calibrators are listed in Table 6.

Figure 4. Overlaid 4 calibration curves of EDDP in urine showing excellent reproducibility. All calibration curves gave the same LLOQ of 3.5 ng/mL for EDDP in urine

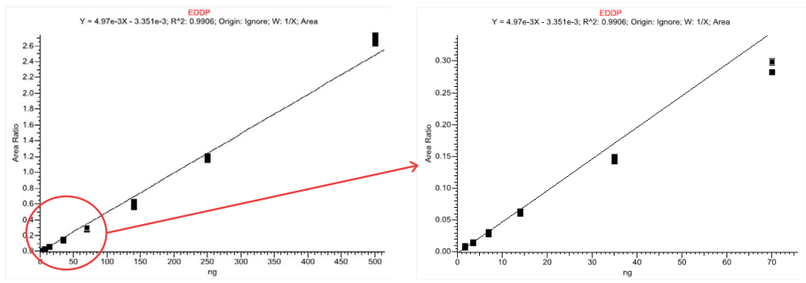


Figure 5. Precision of EDDP robustness sample in urine on TSQ Fortis MS over 480 samples. The orange line represents the end of first set of 10 sample plates, at which point the ion transfer tube was cleaned externally by wiping with a disposable wipe saturated with a mixture of water: methanol (1:1). The points A, B, C and D represent where in the sequence calibration curves were run.

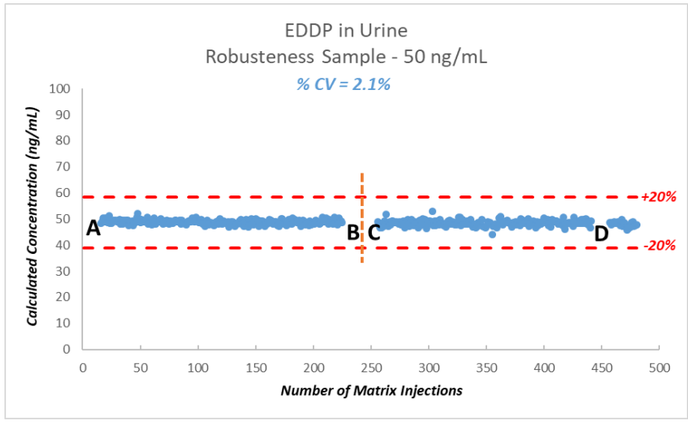


Table 6. Precision and accuracy of the EDDP calibrators in urine

Theoretical Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy	%RSD
3.5	3.67	4.9	1.7
7	6.80	-2.9	5.7
14	13.2	-5.5	2.7
35	30.2	-13.7	2.1
70	60.2	-14.0	2.8
140	123	-12.3	5.0
250	238	-4.6	1.6
500	543	8.7	1.6

CONCLUSIONS

- PaperSpray mass spectrometry is an alternative or complimentary method for many clinical research applications.
- Here we have demonstrated that this method is robust and able to run for an extended period of time without the need for maintenance and with no significant loss in signal, both critical requirements for any routine analytical method.
- While the method proved to be reproducible over 480 samples without any significant loss in signal, we believe the full potential of this technique has not been realized and more work will be done to improve the overall performance of the assay.

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2. N. E. Manicke et al., *J. Am. Soc. Mass Spectrom.*, **2011**, *22*, 1501-1507.
3. H. Evard et al., *J. Food Compos. Anal.*, **2015**, *41*, 221-225.
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TRADEMARKS/LICENSING

Drugs of Abuse Screening and Quantification Using Paper Spray Technology

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ABSTRACT

Purpose: Paper spray is a rapid analysis technique which is particularly beneficial for the analysis of compounds in biological matrices, such as blood and urine. The technique offers a strong advantage over traditional methods which rely on chromatographic separation with high solvent usage and time required for sample preparation and analysis.

Method: A new high-throughput and automated paper spray system was used to generate data for 19 drugs of abuse for screening and quantification and 1 drug metabolite used for demonstration of system robustness.

Results: Here, we demonstrate that paper spray technology can be used as a rapid way to screen and quantitate drugs of abuse in blood and urine in a robust and reproducible manner.

Figure 1. VeriSpray ion source with VeriSpray plate loader (left) on a Thermo Scientific™ TSQ Quantis™ and VeriSpray sample plate (right).

MATERIALS AND METHODS

Sample Preparation for Robustness

EDDP was spiked into whole human blood at various concentrations to yield a nine point curve ranging from 1.75 to 500 ng/mL and three quality controls at concentrations of 50, 100, and 200 ng/mL. A total of 480 samples, consisting of either calibrators or quality controls, were spotted onto the paper cartridges at 8 µL each and were oven dried for 30 minutes at a temperature of 50 ºC. Sample plates were then loaded onto the Thermo Scientific™ VeriSpray plate loader, which holds up. to 10 paper spray plates (24 paper strips per plate), and connected to a VeriSpray Ion source and a Thermo Scientific™ TSQ Quantis™ mass spectrometer where it was then analyzed and assessed for robustness.

Method for Robustness

The paper spray solvents (for both sample rewet and spray solvents) were methanol/water 95/5 with 0.01 % acetic acid. Calibration curves and QC samples (single analysis, no replicates) were placed at the beginning and end of each set of 240 samples, yielding a total of four calibration curves with QC samples over the 8-hour run. A robustness sample at the same concentration as QC1 was spotted on all other paper strips. A total of 10 plates (240 samples) were run in an unattended and automated fashion, followed by wiping the outer surface of the transfer tube and then loading and starting the next 10 plates. Three transitions were monitored per compound, with a cycle time of 0.8 seconds and a collision gas pressure of 2 mTorr. The spray voltage was 3.8 kV, applied from 0.1 to 1.1 min, the inlet capillary temperature of 350 ºC. , and the distance paper tip to inlet was 5 mm. Thermo Scientific™ TraceFinder™ software, version 4.1 was used for data analysis.

Sample Preparation for Drug Screening and Quantitation

For the screening method, a total of 19 compounds from the following compound classes: opiates, amphetamines, cocaine and PCP, were spiked into human donor urine. Four concentration levels were prepared: at target, 2 times higher than cutoff, 5 times lower than cutoff, and blank. Isotopically labelled internal standards were added. For the quantitation method, a total of 29 compounds were spiked into human donor urine. A total of 11 concentration levels were prepared at levels ranging from 0.5 to 400 ng/mL. Isotopically labelled internal standards were added. Eight microliters of the spiked urine sample was spotted onto VeriSpray sample plates for analysis.

Method for Drug Screening and Quantitation

The spray solvent was 90% acetonitrile, 10% water, and 0.01 % acetic acid. Data was acquired for 1 minute per sample, and each concentration level was measured 5 times for screening and 3 times for quantification. Compounds were detected on a Thermo Scientific™ TSQ Quantis™ mass spectrometer for screening and Thermo Scientific™ TSQ Altis™ for quantitation. Three transitions were monitored per compound, with a cycle time of 1.5 seconds (screening assay) or 1 second (quantification) and a collision gas pressure of 2 mTorr. The spray voltage was 3.8 kV, applied from 0.1 to 0.9 min, the inlet capillary temperature was 325 ºC for TSQ Quantis and 400 ºC for TSQ Altis.

ROBUSTNESS RESULTS

Table 1. Precision and accuracy (all four curves combined) for EDDP within the 480 analysis.

Theoretical Concentration (ng/mL)	Calculated Concentration (ng/mL)	% Accuracy	% RSD
3.5	3.64	4.1	2.6
7	6.79	-3.0	2.2
14	12.3	-12.0	3.8
35	30.3	-13.6	1.7
70	63.3	-9.6	1.9
140	138	-1.8	1.2
250	249	-0.3	1.5
500	516	3.2	1.5

Table 2. Precision and accuracy of QC samples (four replicates per concentration) for EDDP within the 480 analyses.

QC Level	Theoretical Concentration (ng/mL)	Calculated Concentration (ng/mL)	% Accuracy	% RSD
QC 1	50	50.6	1.2	1.6
QC 2	100	106	5.8	1.4
QC 3	200	208	3.9	1.9

Figure 2. Overlays of four calibration curves of EDDP in blood showing excellent reproducibility. All individual calibration curves gave the same LLOQ of 3.5 ng/mL.

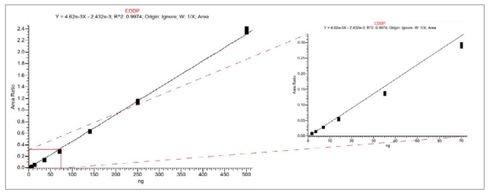
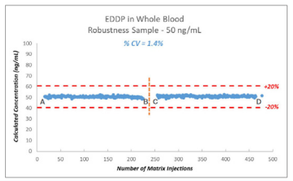


Figure 3. Precision for 50 ng/mL robustness sample of EDDP in whole blood over 480 injections. The orange mark represents the end of running one magazine of 10 sample plates. At this point the ion transfer tube was cleaned externally by wiping with a disposable wipe saturated with a mixture of water:methanol (1:1). Then, another VeriSpray magazine was run, containing 10 more sample plates. The points A, B, C, and D indicate the places in the sequence where calibration curves were run. The ion transfer tube was never removed and the run continued shortly after wiping the outer surface.

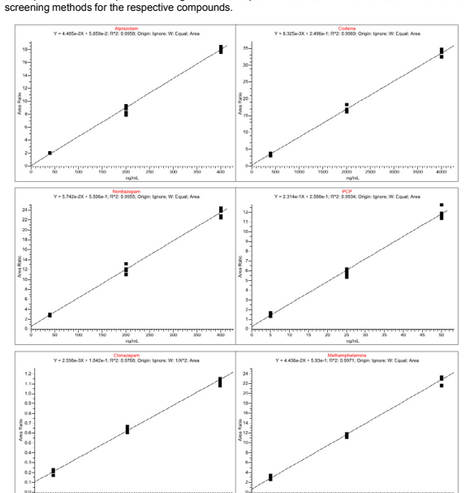


SCREENING RESULTS

Table 3. AUC Counts and S/N Ratios at the Respective Cutoff Levels. AUC for cutoff was observed with > 5 S/N ratio for each of the screened drug compound.

Compound	Low 5x < Cutoff	Cutoff	High 2x > Cutoff	AUC at Cutoff	AUC Matrix Blank	S/N Ratio
Oxycodone	20	100	200	185,630	21,235	8.7
Clonazepam	40	200	400	487,321	86,841	5.6
Methadone	30	150	300	80,066,278	156,898	510.3
Alprazolam	40	200	400	5,593,279	25,924	215.8
Cocaine	20	100	200	21,482,707	52,199	411.6
Oxymorphone	20	100	200	245,704	27,850	8.8
Codeine	400	2,000	4,000	7,714,065	27,537	280.1
Hydrocodone	20	100	200	3,233,438	28,707	108.9
Benzoylecgonine	20	100	200	3,737,256	32,319	115.6
Oxazepam	40	200	400	691,260	18,350	37.7
Morphine	400	2,000	4,000	2,497,123	18,172	137.4
Diazepam	40	200	400	5,729,539	54,682	104.8
Nordiazepam	40	200	400	1,919,336	29,434	65.2
PCP	5	25	50	4,375,303	169,618	25.8
MDMA	50	250	500	14,237,904	36,872	386.1
MDA	50	250	500	1,014,502	166,613	6.1
Methamphetamine	50	250	500	27,860,611	660,915	42.2
Amphetamine	50	250	500	7,105,503	1,454,938	4.9
Hydromorphone	20	100	200	1,388,447	41,053	33.8

Figure 3. Calibration curves for selected compounds for drug screening. Calibration curves are semi-quantitative, three-point screening curves. Low point is less than 5 times the cutoff levels for drug screening methods for the respective compounds.

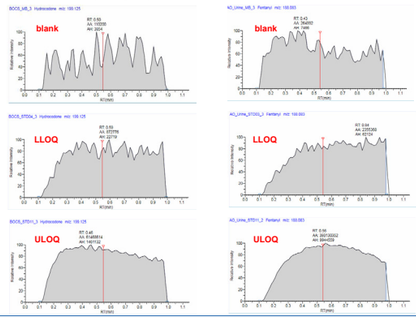


QUANTIATION RESULTS

Table 4. Limit of Quantitation (ng/mL) for each quantitated drug with respective cutoff limits.

Analyte	Cutoff in Urine (ng/mL)	VeriSpray LOQ in Urine (ng/mL)
Benzodiazepines		
Alprazolam	10	2.5
Diazepam	10	5
Clonazepam	10	10
Midazolam	20	2.5
Nordiazepam	20	2.5
Temazepam	10	5
Zolpidem	10	0.5
Zolpidem Phenyl-4-carbo acid	10	2.5
Opiates		
Codeine	20	2.5
Hydrocodone	20	5
Hydromorphone	20	20
Morphine	20	20
Norhydrocodone HCl	20	10
Noroxycodone	20	50
Oxymorphone	20	10
Cocaine		
Cocaine	20	5
Benzoylecgonine	20	20
Sedatives		
Diphenhydramine HCl	10	1
N-ethylpentylone HCl	10	2
Ketamine HCl	10	2.5
(+)-Norketamine HCl	10	5
PCP (Phencyclidine)	10	5
Amphetamines		
(+)-Amphetamine	50	50
(+)-MDMA	50	10
MDEA	50	2.5
Opioids		
EDDP	25	0.5
Methadone	20	2.5
Tramadol	20	5
Fentanyl	1	2.5

Figure 4. Chronograms for Hydrocodone and Fentanyl at Blank, LLOQ (5 and 2.5 ng/mL respectively) and ULOQ (400 ng/mL).



QUANTITATION RESULTS

Figure 5. Calibration Curve for Hydrocodone.

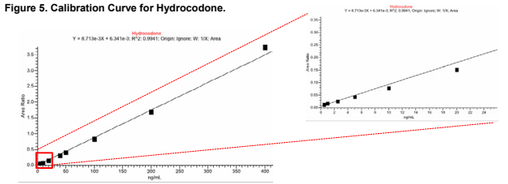


Figure 6. Calibration Curve for Fentanyl.

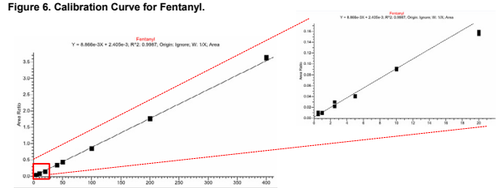


Table 5. Precision and Accuracy of Calibrators for Hydrocodone and Fentanyl.

Concentration (ng/mL)	%RSD		% Difference	
	Hydrocodone	Fentanyl	Hydrocodone	Fentanyl
2.5	0.026	16.72	< 4.34	< 8.80
5	0.90	1.27	< 6.33	< 6.92
10	0.51	1.30	< 2.62	< 4.96
20	1.08	1.59	< 8.32	< 12.26
40	1.12	0.48	< 10.44	< 1.71
50	0.45	0.43	< 6.45	< 1.93
100	1.09	0.18	< 5.67	< 3.73
200	0.11	0.47	< 4.43	< 1.22
400	0.33	0.29	< 5.57	< 2.34

CONCLUSIONS

- Paper spray technology is well suitable for fast drugs of abuse screening, because of short analysis times and the ability to analyze many compounds within a minute window.
- Robustness testing demonstrated that the system can analyze 480 injections with no loss in sensitivity or reproducibility. Robustness testing was operated in an unattended fashion for 240 injections (over 8 hours of runtime) prior to minimal cleaning and loading of an additional 240 samples (10 plates of 24 samples each)
- The system is compatible with all OptaMax NG ion sources including the TSQ Quantis and TSQ Altis as demonstrated here with Robustness, Screening, and Quantitative assays.
- Paper spray technology is robust, reproducible, and sensitive for drug screening and quantitation.

TRADEMARKS/LICENSING

Research Method.

ThermoFisher SCIENTIFIC

Featured Products

VeriSpray PaperSpray Ion Source

The Thermo Scientific™ VeriSpray™ PaperSpray Ion Source is a fully automated, high-throughput, direct ionization technique used with the latest Thermo Scientific™ TSQ triple quadrupole mass spectrometers. It is designed to enable direct sample analysis capability, eliminating the need for chromatographic based separation, simplifying your workflow, getting results faster, and reducing cost per test. The VeriSpray system is fully integrated into instrument control software and application-specific software, and is capable of running proprietary VeriSpray PaperSpray sample plates, for single or high-throughput applications.

"The VeriSpray PaperSpray ion source allows us to perform simple paper spray mass spectrometry (PS-MS) methods in our research programs. It is an elegant approach that provides a direct sampling strategy, a simple cleanup step and a disposable interface, all in one sampling platform. In particular, PS-MS eliminates the potential for carry-over and contamination — crucial for our development of rapid drugs-of-abuse screening assays used in harm-reduction drug testing. With its universal well-plate geometry and intelligent autoloader, VeriSpray has surpassed any limitations with regard to throughput, making it suitable for high-throughput, sensitive, quantitative, direct drug analyses."

Dr. Chris Gill, Professor of Chemistry at Vancouver Island University and co-director of Applied Environmental Research Laboratories

Thermo Scientific TSQ Altis Triple Quadrupole Mass Spectrometer

"Great! Would like to recommend for targeted analysis."

Rating: 5 ★★★★★

Application Area: Targeted Proteomics

"The instrument is great as compared to the previous version of triple quadrupole from Thermo. It has improved signal to noise ratio. The software is user friendly."

Sandip Chavan, Moffitt Cancer Centre, Florida, U.S.

FAQ

What is the maximum sample volume that you can load on the paper?
Depending on the viscosity of the matrix, it can be 8-10 µL of whole blood and 5-12 µL of urine. These amounts will prevent the paper from becoming too saturated.

What is the flow rate?
There is no conventional flow rate because it is not liquid chromatography, but the rewet and spray solvents that are used are dispensed at 10 µL/min.

Can you do quantitation without internal standards?
We highly suggest that you use internal standards. Like all quantitation, it is better if you have an internal standard so it can account for variability in the paper, ion suppression and ionization efficiency.

Have you tried any large molecules, such as peptides?
Yes, 1-2 peptides have successfully been monitored using paper spray-mass spectrometry

How many drugs can you analyze at once?
Up to 53 drugs and internal standards have been analyzed with 2-5 transitions each, resulting in a maximum of ~250 transitions at one time.

How long can you keep the paper? Is it degradable?
The paper is very stable and can be stored at room temperature. It is very portable and easily shipped to clinics and laboratories.

What instruments are compatible?
The VeriSpray PaperSpray ion source is compatible with Thermo Scientific Triple Quadrupole Mass Spectrometer instrumentation. For further clarification, contact your local representative.

How easy is it to shift between LC and the VeriSpray PaperSpray Ion Source?
It consists of a very simple transition, which can be completed in as little as five minutes.

With all analytical assays, robustness is important. How many samples can you run without cleaning or performing maintenance on the mass spectrometer?
Up to 480 samples have been consistently analyzed without the need for maintenance. The source can be cleaned with 1:1 methanol:water. It is also a good practice to clean the ion transfer tube, and tips like these are included in your instrument maintenance SOPs.

How many analytes can be used with one internal standard?
This is left up to the discretion of the user and should be set up like any other quantitation experiment.

Additional Resources

Focused analyses

- LCMS Targeted Quantitation Clinical Research Workflow Solutions
- LCMS Targeted Quantitation Forensic Toxicology Workflow Solutions

Technology details

- VeriSpray PaperSpray Ion Source Product Brochure
- VeriSpray PaperSpray Ion Source Product Specification
- Thermo Scientific TSQ Altis Triple Quadrupole Mass Spectrometer
- Thermo Scientific TSQ Quantis Triple Quadrupole Mass Spectrometer

Video

- See the VeriSpray PaperSpray Ion Source in Action

Learn more and request more information about VeriSpray PaperSpray Ion Source
https://www.thermofisher.com/verispray