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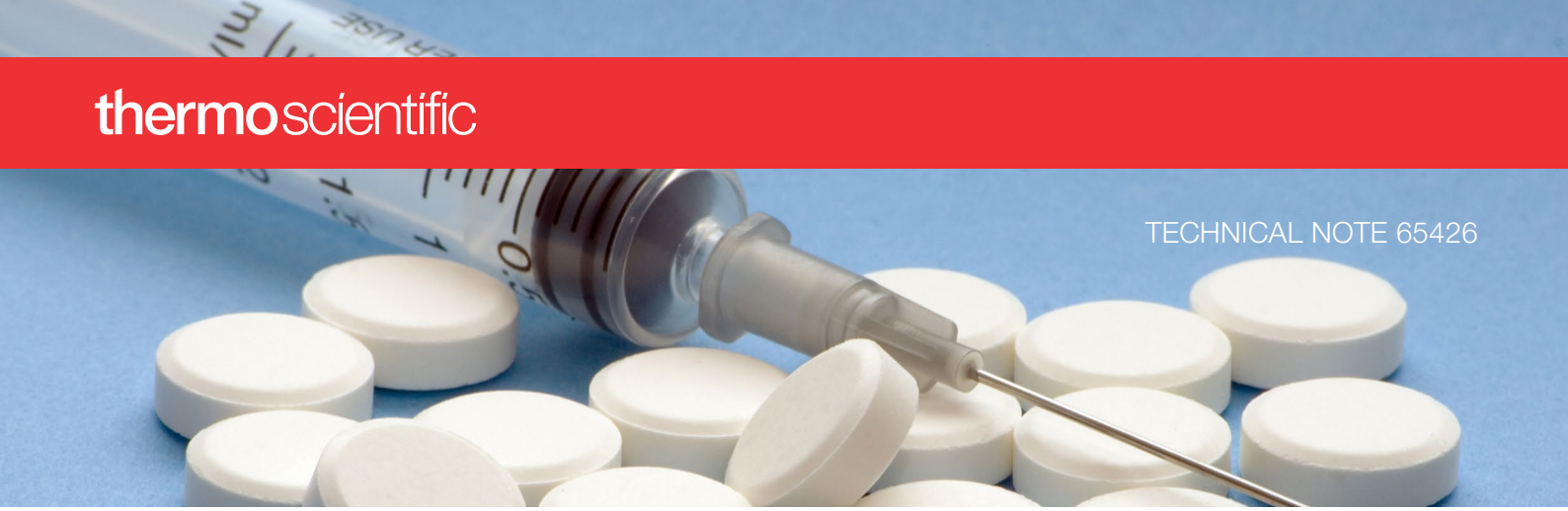

TECHNICAL NOTE 65426

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

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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.

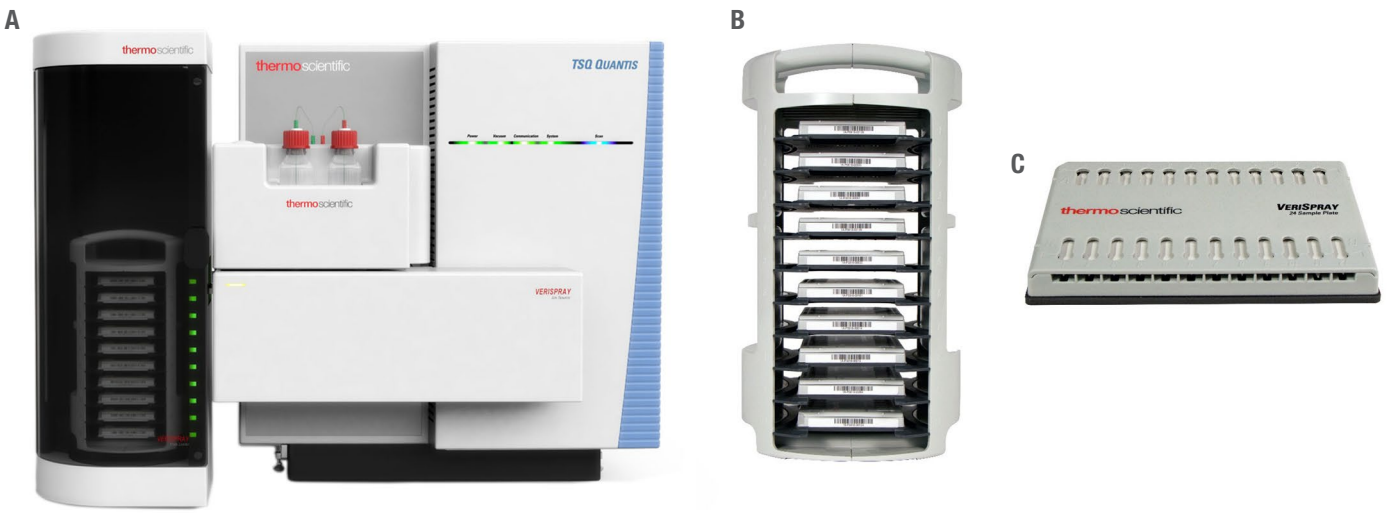

Figure 1. (A) VeriSpray ion source and plate loader mounted onto a TSQ Quantis MS, (B) Magazine, and (C) VeriSpray sample plate

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.

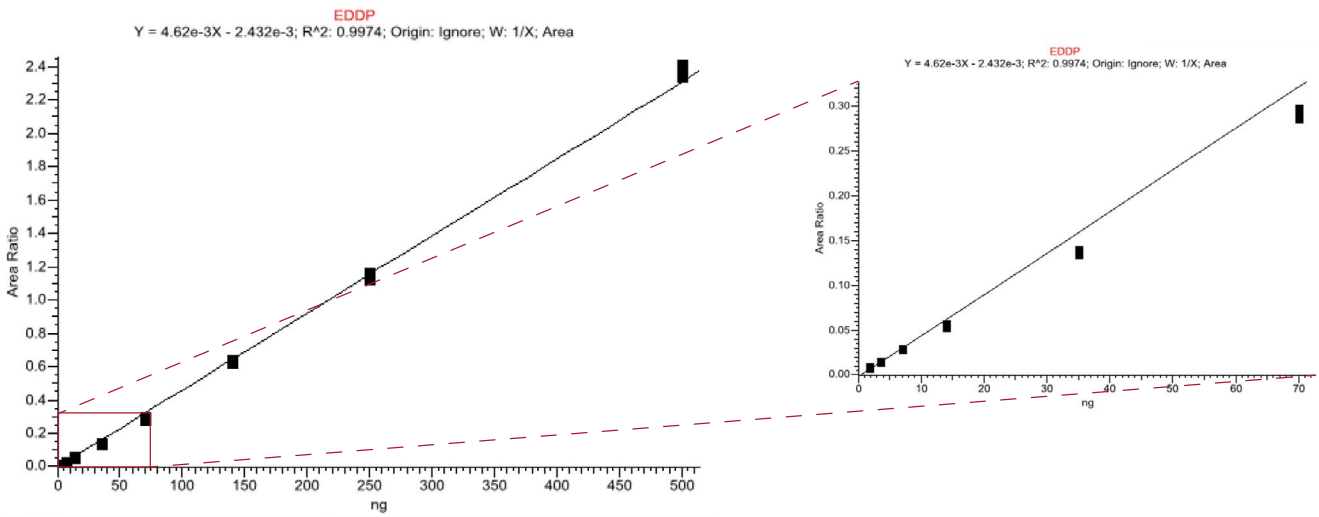

Figure 2. Overlays of four calibration curves of EDDP in blood showing excellent reproducibility. All individual calibration curves gave the same LLOQ of 3.5 ng/mL.

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

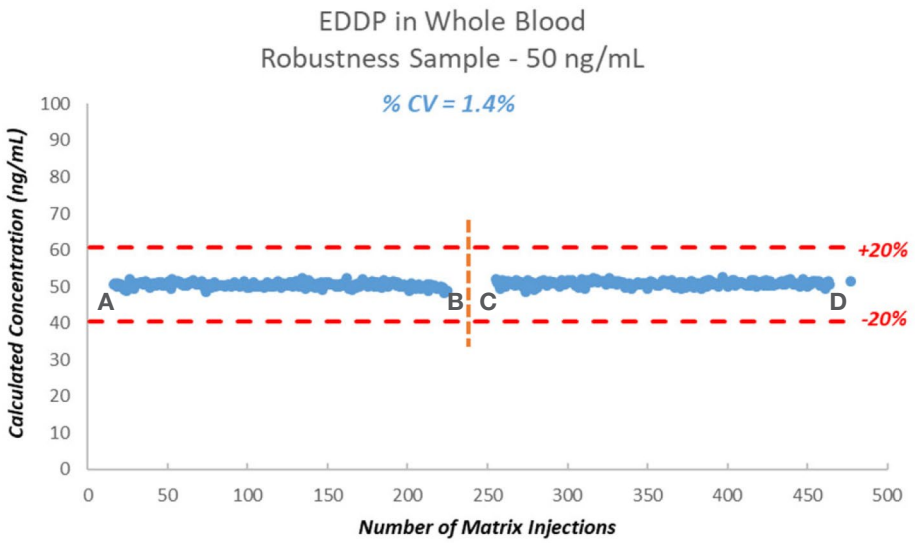

Figure 3. Precision for 50 ng/mL robustness sample of EDDP in whole blood over 480 injections. The orange mark represents the end of running one magazine of 10 sample plates. At this point the ion transfer tube was cleaned externally by wiping with a disposable wipe saturated with a mixture of water:methanol (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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