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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

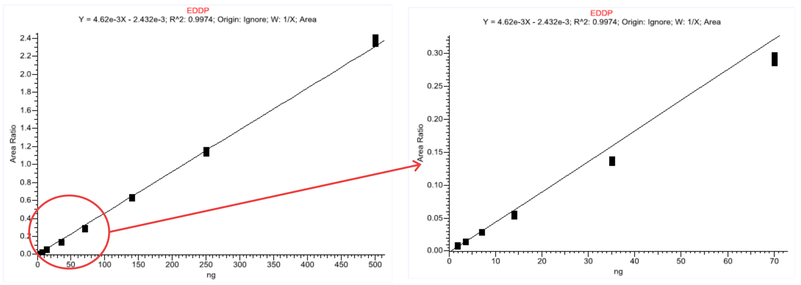
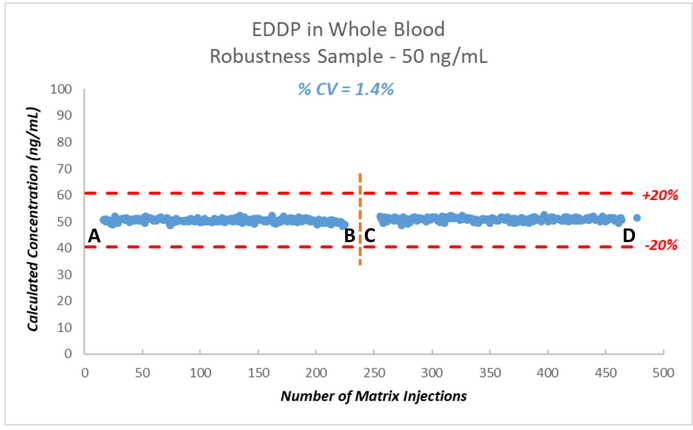


Table 5. Precision and accuracy of the EDDP calibrators in human whole blood

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

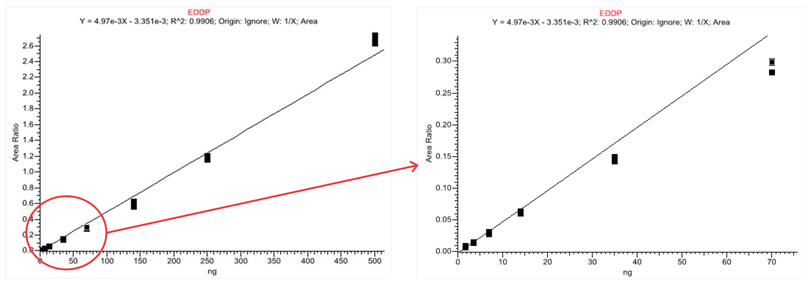


Figure 5. Precision of EDDP robustness sample in urine on TSQ Fortis MS over 480 samples. The orange line represents the end of first set of 10 sample plates, at which point the ion transfer tube was cleaned externally by wiping with a disposable wipe saturated with a mixture of water: methanol (1:1).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

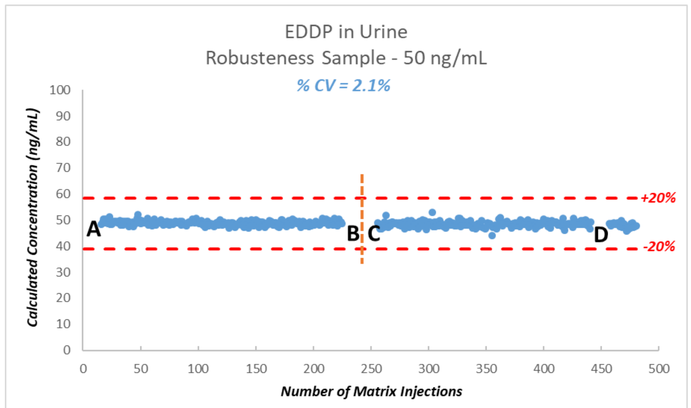


Table 6. Precision and accuracy of the EDDP calibrators in urine

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.

REFERENCES

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TRADEMARKS/LICENSING

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