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

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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.

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 amphetaminelike 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).

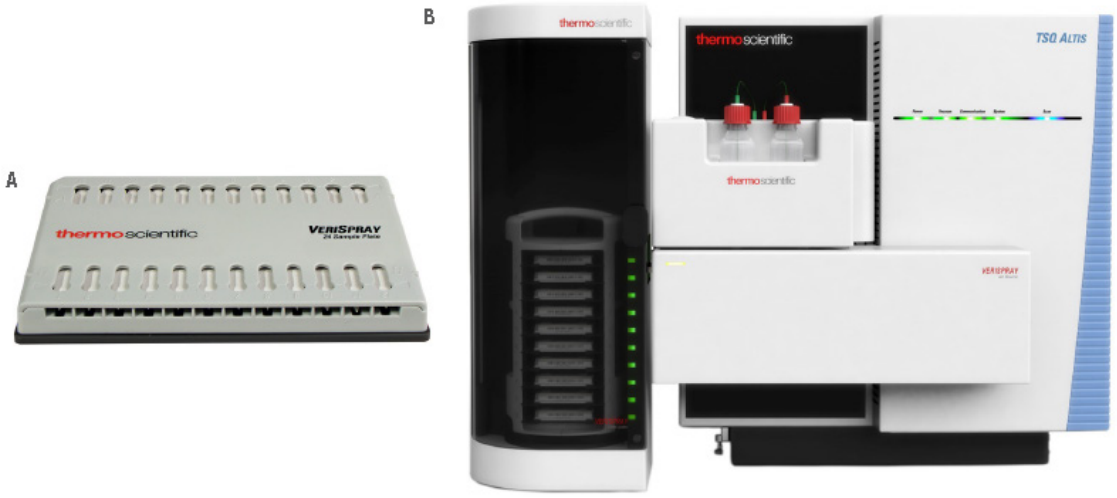

Figure 1. (A) VeriSpray sample plate and (B) VeriSpray PaperSpray system mounted to TSQ Altis triple quadrupole mass spectrometer

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.

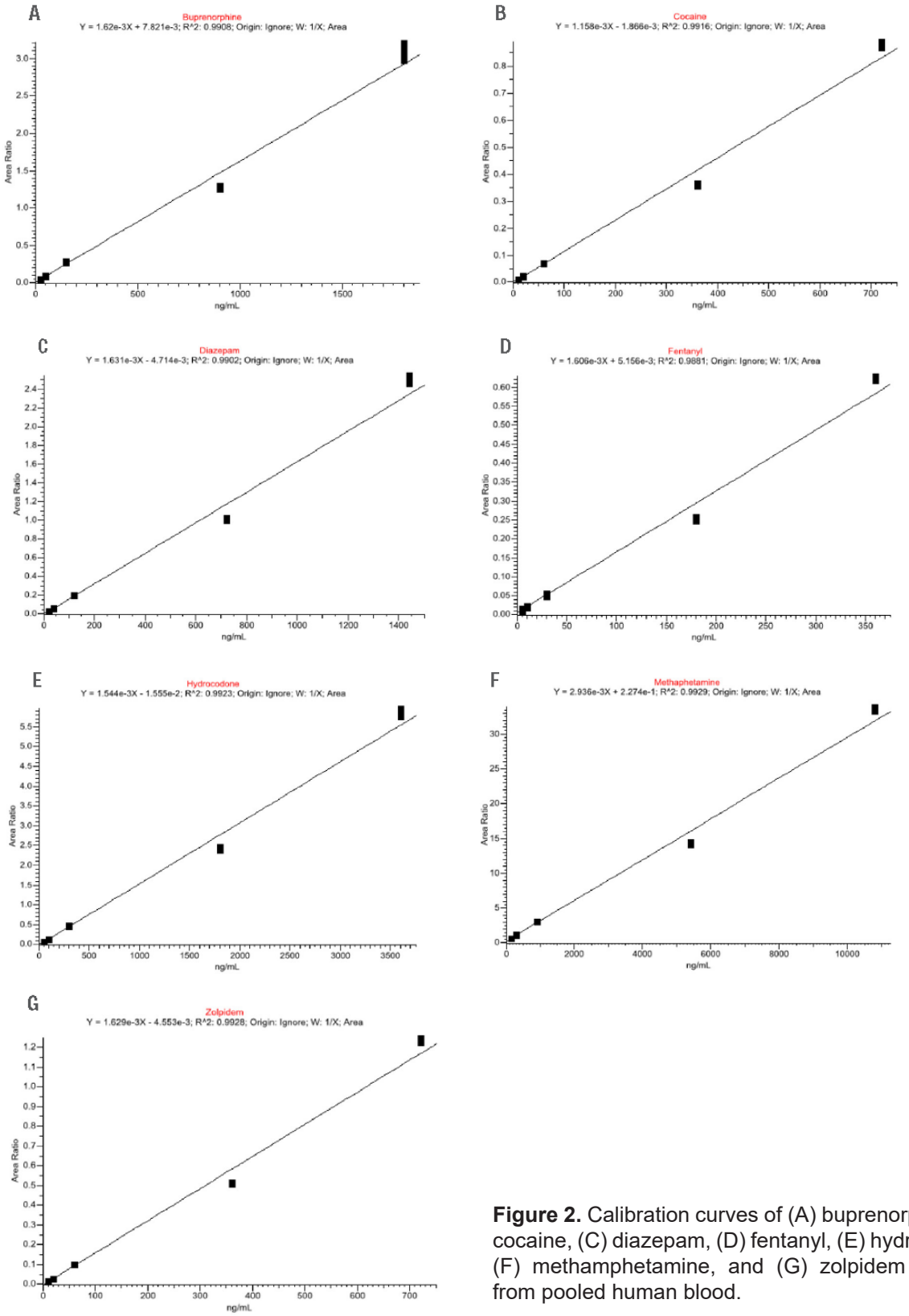

Figure 2. Calibration curves of (A) buprenorphine, (B) cocaine, (C) diazepam, (D) fentanyl, (E) hydrocodone, (F) methamphetamine, and (G) zolpidem obtained from pooled human blood.

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.

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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