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Method Development Considerations for Paper Spray Mass Spectrometry - Direct Ionization Technique for Physiological Fluid Analysis

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ABSTRACT

Purpose: Method development workflow for paper spray technology, which allows for rapid detection of small molecules in physiological fluids, but deals with more complex samples due to lack of sample pre-treatment and no prior chromatography to the analysis.

Methods: Horse urine fortified with stimulant/amphetamine drugs. Prosolia Velox 360™ PaperSpray® ion source coupled to a triple quadrupole and Thermo Scientific™ Orbitrap™ mass spectrometers.

Results: The utility of paper spray technology coupled to a triple quadrupole mass spectrometer is here demonstrated for the purpose of anti-doping monitoring in horse racing.

INTRODUCTION

Sports doping in both humans and animals is a widespread problem. Techniques that are both quicker and simpler to use are of great interest as they require less training, making them attractive for compound screening and quantitation in many clinical research and forensic toxicology applications.

Paper spray is a direct ionization technique that simplifies the mass spectrometric analysis of compounds from physiological fluids. Qualitative and quantitative analyses of small molecules from complex matrices such as blood and urine are possible without time consuming sample pre-treatment and chromatography.

In this work, methamphetamine and other stimulant drugs were spiked in horse urine to demonstrate the potential of the technique to monitor sport doping in horse racing. Paper spray ionization presents challenges for mass spectrometric analysis due to the lack of specimen matrix clean-up and lack of chromatography. We outline a method development approach using paper spray Ionization mass spectrometry with this in mind.

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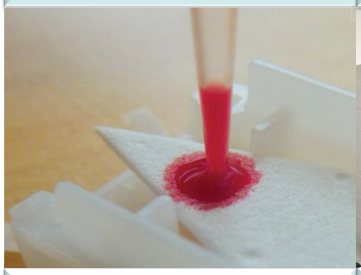
PAPER SPRAY – HOW DOES IT WORK

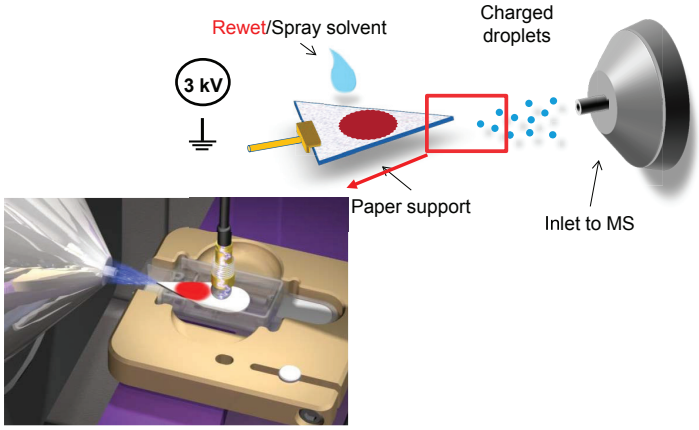
STEP 1
Blood sample is loaded onto a triangle-shaped paper and let air-dry



STEP 2
Cartridge is loaded onto the sample magazine

STEP 3
Automated addition of 2 solvents: solvent to rewet the sample and spray solvent to extract the sample and electrospray

STEP 4
A voltage is applied to the piece of paper and ionization occurs



MATERIALS AND METHODS

- A Prosolia Velox 360 PaperSpray system was used on a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer (MS).
- Paper spray technology relies on spotting samples on cellulose paper (Whatman® ET 31 grade) and drying them, with the paper retaining unwanted compounds from the sample matrix.
- Ions are generated directly from paper when an applied high voltage (3.5-5.0 kV) induces electrospray from the sharp tip of the rewetted paper. High percent organic is selected as the extraction/electrospray solvent. This is compatible with the analyte of interest and produces a stable electrospray signal, while not extracting background from the dried matrix, which is water soluble.

Mass Spectrometry Compound Optimization

- All MS compound tuning and optimization was done by paper spray ionization.
- Fragmentation spectra were generated using the product ion scan function on the TSQ Endura MS and using paper spray ionization, with a collision energy ramp (CER) of 10-40.
- The Collision Energies (CE) for the selected transitions were optimized using Selected Reaction Monitoring (SRM) with Q1, Q3 resolution of 0.4 FWHM.
- Eight microliters of horse urine were applied to the paper cartridge and allowed to dry at room temperature for at least 10 min. Velox 360 PaperSpray conditions: rewetting and extraction solvent 90/10 /0.01 acetonitrile/H_2O/acetic acid; sample rewetting 3 uL, extraction solvent 10 dispenses of 10 uL.
- The Velox 360 PaperSpray system was also coupled to a Thermo Scientific™ Q Exactive™ HF MS, using the high resolution and high mass accuracy (HRAM) MS spectra for clarification when calibration curves did not produce expected results.

Sample Preparation and Data Processing

- Seven analytes were spiked in horse urine with internal standards (IS) and spotted in one paper cartridge: amphetamine (d5 IS), methamphetamine (d5 IS), 6-MAM (d3 IS), MDEA (d5 IS), MDMA (d5 IS), benzoylecgonine (d8 IS) and clenbuterol (d9 IS), 0.5 to 1000 ng/mL concentrations, five replicates.
- Methamphetamine was selected as an example to explain matrix effects, spiked in solvent (50/50 MeOH/H_2O) and in horse urine at concentrations of 0.5 to 1000 ng/mL. Analyte area is normalized by deuterated internal standard (200 ng/mL) and the ratio plotted against concentration (five replicates at each concentration). Matrix blanks with and without internal standard were also analyzed.
- Analytes and IS were purchased from Cerilliant Corp. (Round Rock, Texas) and horse urine from Bioreclamation IVT (Hicksville, NY).
- Thermo Scientific™ Xcalibur™ platform tools were used for data processing and quantitative analysis.

RESULTS – Quantitative Analysis - Triple Quadrupole MS, seven drugs plus IS spotted per sample

Figure 1. A) Representative calibration curve (MDMA) for one of seven drugs spiked in horse urine. The ratio of analyte/IS is plotted against concentration, replicates of five. B) Typical paper spray chronograms for 5 ng/mL and 100 ng/mL spikes, uncorrected for IS. Data collected on a TSQ Endura mass spectrometer with Q1, Q3 resolution set at 0.4 FWHM.

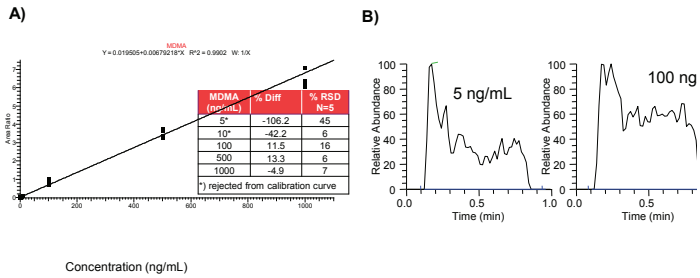


Figure 2. Calibration curves for six of the seven compounds spiked in horse urine. Selected SRM transitions provided least interference from matrix and best fits to a calibration curve. Data collected on a TSQ Endura mass spectrometer in replicates of five. Q1, Q3 resolution 0.4 FWHM.

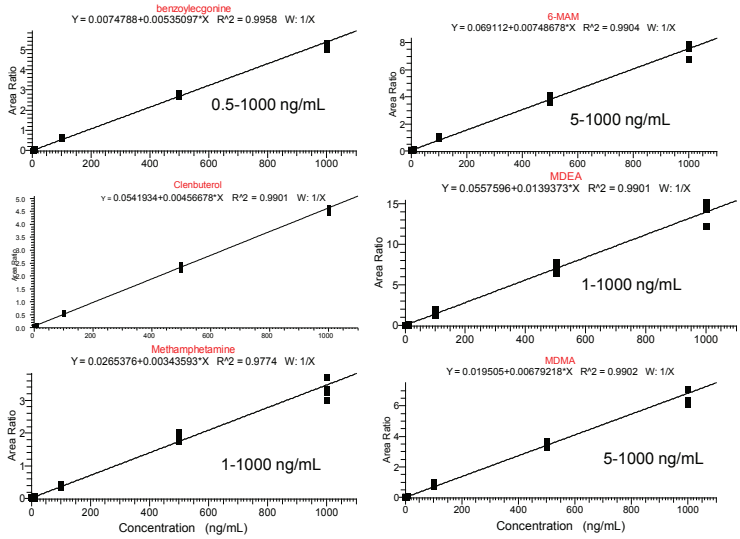
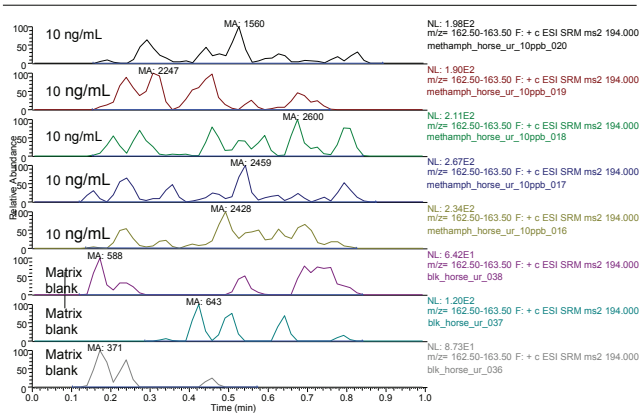


Table 1. Summary of SRM transitions plotted above, correlation coefficient R^2 for the linear regressions and limit of detection (LOD), limit of quantitation (LOQ) for six out of seven drugs spiked in horse urine.

7 Drugs Spiked in Horse Urine	m/z	SRM transition plotted	R^2	LOD (ng/mL)	LOQ* (ng/mL)
Amphetamine	136.112	119.1	0.9168	NA	NA
Benzoylecgonine	290.139	105, 150.2, 168	0.9958	0.5	10
Clenbuterol	277.087	132, 203	0.9982	0.5	10
6-MAM	328.154	211, 268.3	0.9904	5	10
MDEA	208.133	163	0.9901	1	10
MDMA	194.118	163	0.9902	5	10
Methamphetamine	150.128	119.1	0.9794	1	>10, but <100

* Limits of quantitation determined by %RSD values ≤ 20% and a signal to blank ratio of ≥4 (AUC), see Fig. 3. High variability found in amphetamine data, needs further investigation.

Figure 3. Areas under the curve for MDMA (SRM 194 to 163) at 10 ng/mL compared to horse urine blanks. AUC_{avg} analyte/AUC_{avg} Blank ≥ 4 (2259/534 = 4.2) was used to calculate LOQ. Data collected on a TSQ Endura mass spectrometer in replicates of five. Q1, Q3 resolution 0.4 FWHM.



RESULTS – Product Ion Scans: methamphetamine

We use methamphetamine as an example of how matrix effects can produce interference in ions, requiring proper attention when analyzing data by paper spray ionization.

In Fig.4, a comparison is made for methamphetamine spiked in solvent and in horse urine against matrix blanks. Usual methamphetamine fragment ions are observed (m/z 150 to 91.1, 119.1 and 122.1). Interferences brought on by the matrix can be observed for the SRM transition m/z 150 to 91.1 (minor) and m/z 150 to 122.1 as they show in the matrix blanks. A calibration curve using m/z 150 to 122 would produce a poor linear regression (Fig. 6D). The HRAM spectrum in Fig. 5 shows such interference peak in more detail. Please note that although the paper substrate is known to contribute to the MS baseline (1), it is ubiquitous in all the samples compared below.

Figure 4. Methamphetamine (m/z 150.1, 500 ng/mL) spiked in solvent (50/50 MeOH/H_2O) and horse urine compared to matrix blanks. Analyzed on a TSQ Endura MS by PaperSpray ionization. Q1, Q3 resolution 0.4 FWHM.

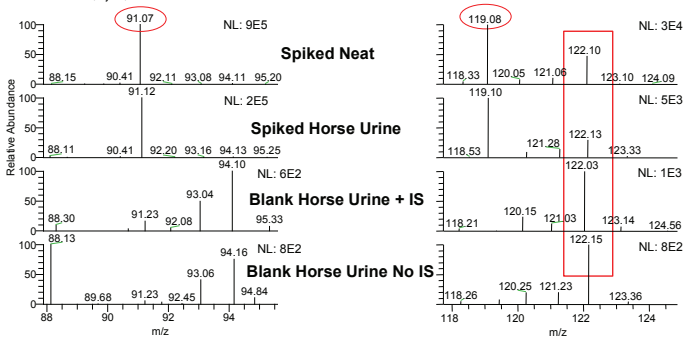
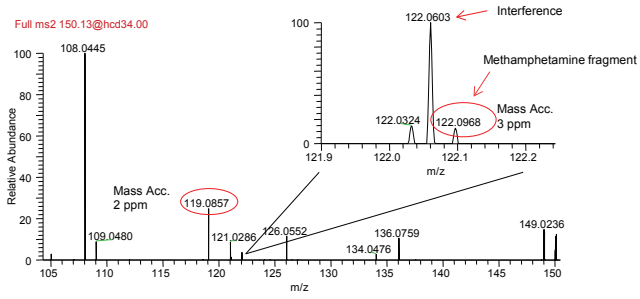


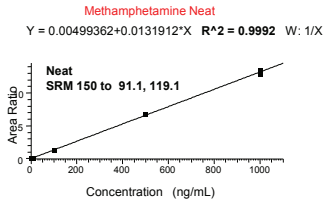
Figure 5. HRAM MS/MS spectra of methamphetamine spiked in urine (500 ng/mL) analyzed in a Q Exactive HF mass spectrometer. Resolving power for the MS/MS set at 17.5k (FWHM at m/z 200). The transition m/z 150 to 122 would not be useful in a triple quadrupole MS/MS experiment as the incorrect ion (m/z 122.0603) would be included (see also Fig. 4).



RESULTS – Quantitative Analysis - Triple Quadrupole MS, methamphetamine

Figure 6. Calibration curves for methamphetamine considering three different SRM transitions: m/z 150 to 91.1, 150 to 119.1 150 to 122.1 (refer to Fig. 4). A) Methamphetamine spiked in solvent, SRM m/z 150 to 91.1, 119.1 (no matrix effects). B) Methamphetamine spiked in horse urine, SRM m/z 150 to 119.1. C) Methamphetamine spiked in horse urine, but transition plotted is SRM m/z 150 to 91.1. D) Methamphetamine spiked in horse urine, but transition plotted is SRM m/z 150 to 122.1 .

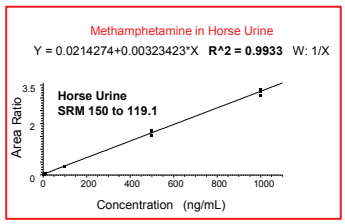
A)



Conc. (ng/mL)	% RSD n=5	% Difference
0.5	6	5.9
1	7	-8.9
5	<1	-8.7
10	2	-12.2
100	2	-6.2
500	<1	2.3
1000	2	-2.0

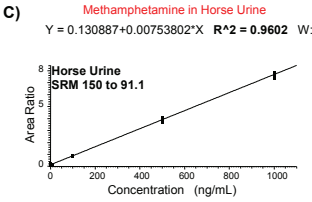
Better selectivity is obtained when the SRM transition does not contain contributions from the matrix, as shown by the higher correlation coefficient R^2 (0.9933 vs. 0.9602) and a slope intercept that is closer to the neat sample (0.02 to 0.005 vs. 0.13).

B)

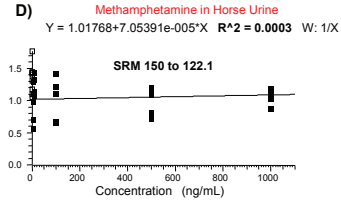


Conc. (ng/mL)	% RSD n=5	% Difference
1	13	38.0
5	8	-11.0
10	6	-20.8
100	2	-3.4
500	4	3.8
1000	2	1.8

C)



D)



CONCLUSIONS

- We have shown an easy-to-use technique (no sample preparation, no chromatography) for the quantitative analysis of drugs with potential use in animal sports doping monitoring.
- The use of paper spray technology coupled to a TSQ Endura triple quadrupole mass spectrometer allows answers in much shorter timeframes than is possible using liquid chromatography techniques. The driving force for use of a triple quadrupole mass spectrometer is maximum sensitivity and speed.
- Factors that affect the length in data acquisition with paper spray ionization, which, can range from 10 to 60 sec: screening or quantitative analysis, number of compounds to analyze, number of SRM transitions to monitor and number of scans per compound. One minute acquisitions were used in this study.
- Two other advantages of using paper spray ionization are small sample volumes (8-12 uL) and small amounts of solvent required.
- Understanding matrix contributions is the first step to enable successful use of paper spray ionization on a triple quadrupole mass spectrometer.
- Electrospray from paper generates MS spectra and SRM transitions that match those generated by infusion with nanoelectrospray source (1). However, the baseline noise level is higher for cellulose paper than for nanospray source which limits analytical sensitivity.
- LOQs between 10-100 ng/mL were achieved in this study for samples spiked in horse urine (Table 1). While 10-50 ng/mL might satisfy cutoff values for stimulant drugs in race animals, it is of interest to have a method that provides quantitation that is at least an order of magnitude lower. Further investigation of optimal extraction solvents and alternative substrates will help improve limits of quantitation and will be the subject of future studies.

REFERENCES

1. Manicke et al. Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots. IJMS 300, 2011 123-129.

ACKNOWLEDGEMENTS

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