Drug Screening by Paper Spray Mass

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Overview

- **Paper Spray Mass Spectrometry** is a method for direct analysis of biofluid samples, including d**ried blood spots.** No sample preparation is needed
- Goal: develop a **drug screening** method for post-mortem forensic toxicology
  - Screen for 154 commonly encountered drugs and drug metabolites, including amphetamines, analgesics, anesthetics, anticonvulsants, antidepressants, antipsychotics, barbiturates, benzodiazepines, and opiates.
- A representative subset of the more challenging targets was selected for initial assessment and method development

Introduction

- An MS front-end is available that attaches to MS in place of the HPLC system and commercial electrospray/APCI source
- ~10 µL of sample is applied to a single-use cartridge containing the paper substrate
- An extraction/spray solvent is applied to the cartridge
  - Solvent wicks through the paper and performs an extraction from the dried biofluid sample
  - Electrospray ionization is induced directly from the paper tip
- Advantages: no chromatography, no sample preparation, small sample volume, no carry-over, no solvent waste, low solvent consumption
- Disadvantages: lower selectivity (no chromatography), higher detection limits

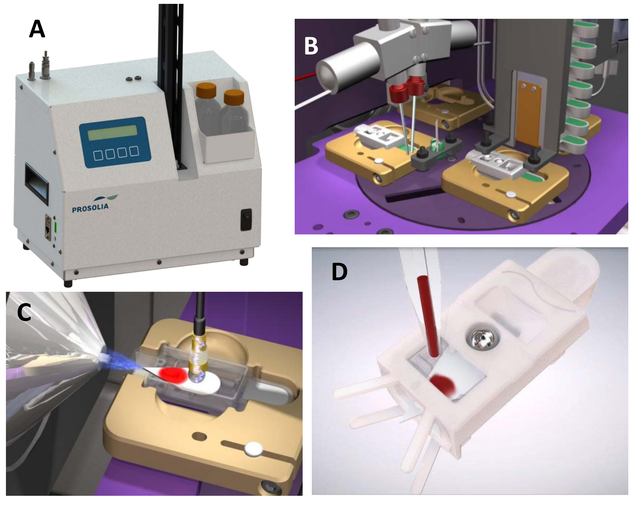


Figure 1.
A.Paper spray autosampler (Velox 360, Prosolia Inc.)

B.Inside autosampler - moving cartridges between stations

C.Paper spray cartridge in the analysis position

D.Paper spray cartridge with blood sample applied

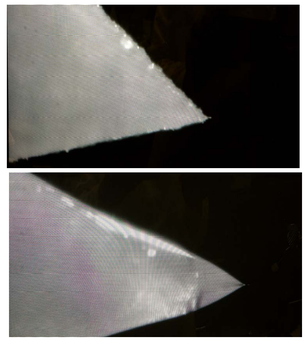


Figure 2.
Top: Wetted paper tip, no HV applied. Viewed with camera mounted on MS

Bottom: Paper after spray voltage is applied. Taylor cone is visible

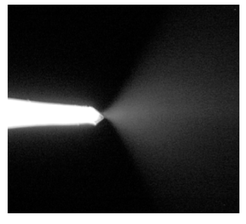
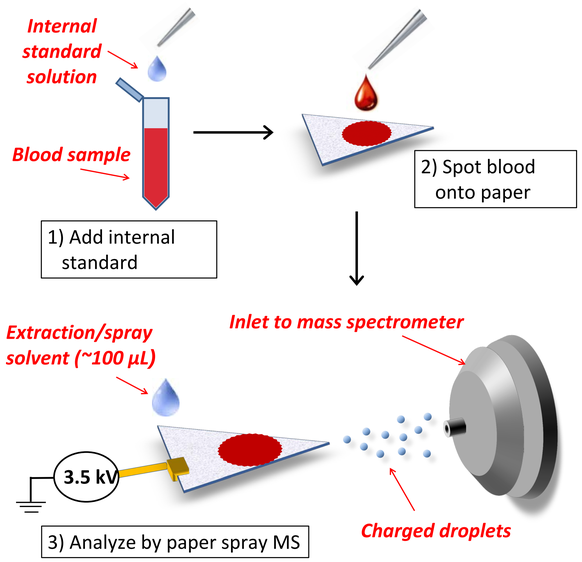


Figure 3. Picture of cone-jet generated from paper

Methods



Procedure

Sample loading
10-20 µL of blood sample was pipetted onto the sample and allowed to dry. The blood sample can be mixed with an internal standard solution prior to spotting if desired.

Extraction and ionization
~100µL solvent is applied to the back of the paper. The solvent acts as both the extraction solvent and the electrospray solvent. Typical solvents include methanol or acetonitrile mixed with water (<10%) and acetic acid (<0.1%). Extraction step takes about 60 seconds.

Detection
The cartridge is positioned about 5mm in front of the inlet to the MS. Spray voltage of 3-5 kV is applied to the paper. Analyte signal is normally seen immediately. Signal duration is about 60 seconds.

Results

1. Drug Screening in Urine – Exact Mass on the Exactive

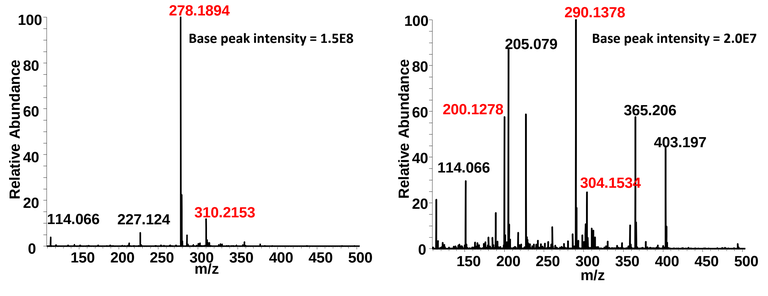


Figure 4. Two spectra obtained from paper spray MS of urine samples obtained from a methadone clinic. Analysis was performed on a Thermo Exactive MS

Compound	Theoretical m/z $(M+H)^+$
Ecgonine methyl ester	200.1281
Benzoylecgonine	290.1387
Cocaine	304.1543
EDDP	278.1903
Methadone	310.2165

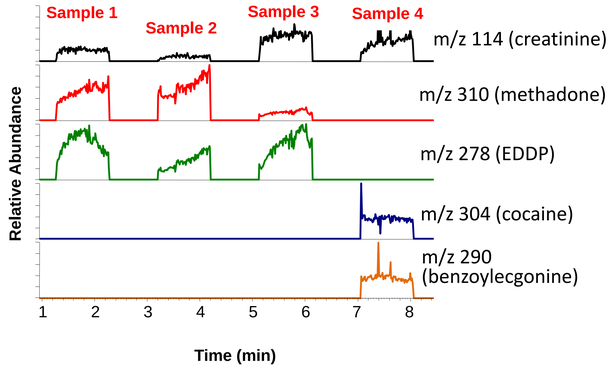


Figure 5. Extracted ion chronograms (5ppm window) for several compounds detected from the urine. Four samples are shown on each chronogram

lass Spectrometry: a Direct Analysis Approach

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Drug Screening in Blood – MS/MS on a Triple Quad

Table 1. Results for a representative subset of challenging drugs analyzed from human whole blood

- ❑Challenging drugs were selected for initial assessment
  - ➢ Poor ionizers or low cutoff levels
- ❑Screening cutoffs were set by consultation with AIT Laboratories
- ❑Potential interferences with the same nominal mass were obtained from drugbank.ca and hmdb.ca (drug origin only)

Drug	Target Cutoff (ng/mL)	SRM Transition	Signal to Blank at Cutoff	Potential Interferences (HMDB.ca)	Excluded Interferences
Alprazolam	5	309→281	8	Pinazepam Fluoxetine glucuronide 8-Hydroxycarteolol	Oxybuprocaine[1] Phenylbutazone[2] Indecainide[3]
		309→205	8		
7-Aminoclonazepam	10	286→121	11	Morphine Same as morphine	Same as morphine
		286→94	6		
Amphetamine	50	136→91	64	Adenine (136→119)	Homocysteine[1]
		136→119	31		
Buprenorphine	1	468→414	2	4-Hydroxytamoxifen sulfate	Tobramycin[3] Tiropramide[3]
		468→396	2		
Clonazepam	10	316→214	3	Codeine N-oxide Rotigotine Saxagliptin	Bromazepam[1] Efavirenz[1] Alizapride[1] Mitiglinide[3] Chlorprothixene[3]
		316→241	5		
Cocaethylene	50	318→196	68	beta-oxycodol N2-Monodes-methylnizatidine Arbutamine	Nilutamide[2] Nateglinide[2] Tetrabenazine[3] Butenafine[1]
		318→82	51		
Fentanyl	1	337→105	2	Captopril-cysteine disulfide	Acebutolol[1] Berberine[1] Acetyl-α-methylfentanyl[3] N-Desmethyl rosuvastatin[1]
		337→188	6		
Gabapentin	500	172→137	44	N.F.	Metronidazole[1] Rasagiline[1]
		172→119	48		
Ketamine	100	238→125	48	2-Amino-5-benzoylbenzimidazole	N.F.
		238→89	37		
Meprobamate	2000	219→180	67	N-despropyl ropinirole	Mephenytoin[3] N-Acetylserotonin[1]
		219→158	34		
Morphine	20	286→152	5	Norcodeine Hydromorphone Norhydrocodone Letrozole Isothipendyl N-Monodesmethyl-rizatriptan Mepyramine	7-Aminoclonazepam[1] Cladribine[1] fludarabine[1] Faropenem[1] Probenecid[2]
		286→165	6		
Normeperdine	25	234→56	8	N,N-Didesmethyltramadol	Dopamine 3/4-O-sulfate[3] Lomustine[2] p-Chlorobenzene sulfonyl urea[2]
		234→42	5		
Topiramate	2000	362→265	67	Methylhydroxygliclazide 7-Hydroxygliclazide Alogliptin Methylergonovine nor-Levomethadyl acetate	Disopyramide[1] Hexetidine[1] Cefacetrile[2] Noracymethadol[3]
		362→207	66		
Zolpidem	10	308→235	45	2-oxobrimonidine Alcaftadine Ibopamine Hydroxyterbinafine	Nitazoxanide[2] Glutathione[1] Tolnaftate[1]
		308→92	13		

Excluded interferences

[1]MS/MS spectra from the literature or database was compared to the target MS/MS spectra. There was no interference at the two target SRM channels. Main spectral source was Thermo's mzcloud
[2]compound is a poor ionizer and is unlikely to give appreciable MS signals
[3]compound is not available in the US and is also not an abused drug

Potential interferences

❑Not enough information to exclude. Compounds will be tested for interferences if commercially available
❑The underlined compounds are known to interfere with the target compounds at one or both SRM channels

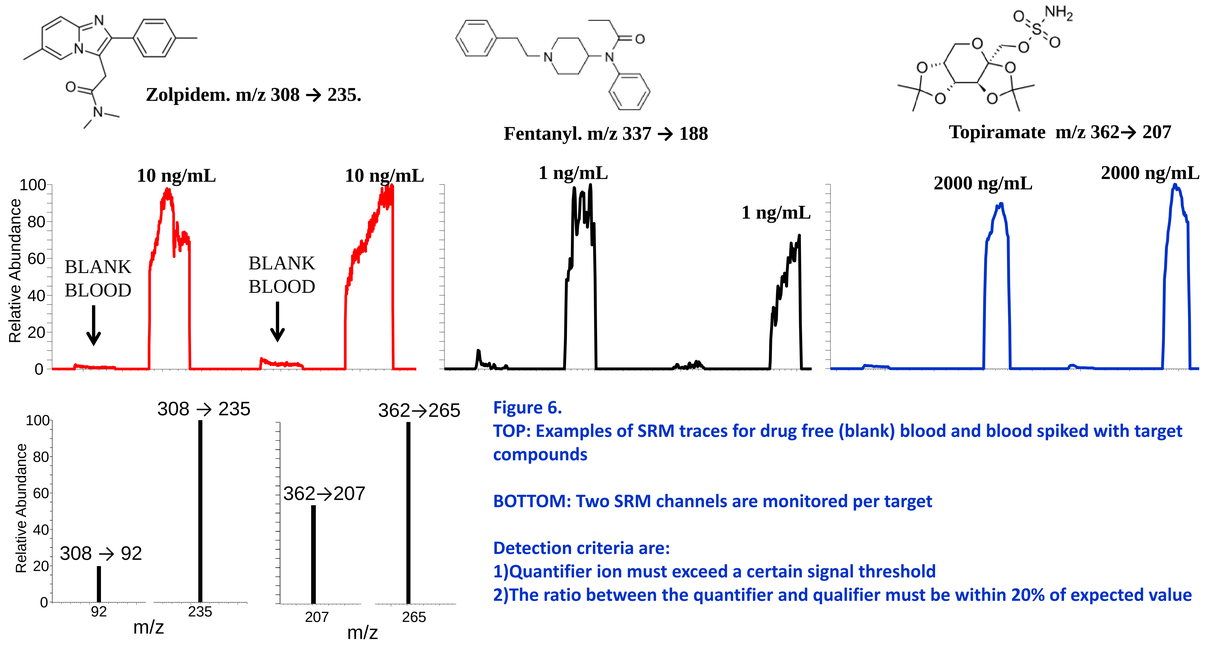

Figure 6.
TOP: Examples of SRM traces for drug free (blank) blood and blood spiked with target compounds

BOTTOM: Two SRM channels are monitored per target

Detection criteria are:
1)Quantifier ion must exceed a certain signal threshold
2)The ratio between the quantifier and qualifier must be within 20% of expected value

Conclusions

- ❑ From a subset of the most challenging drugs, paper spray MS/MS showed adequate sensitivity for 11 of the 14 targets for direct analysis of dried blood spots
- ❑ Only modest improvement is required to have adequate sensitivity for the other 3 targets.
- ❑ Within-class interferences, especially for opiates, is likely to occur
- ❑ No other source of false positives have been identified for this subset
- ❑ Next steps:
  - ➢ Refine method to improve sensitivity (sample volume, extraction solvent)
  - ➢ Test remaining compounds to ensure adequate detection limits
  - ➢ Use database searching to identify possible interfering compounds for the remaining target compounds. Experimentally test when needed.
  - ➢ Develop negative ion mode method (naproxen, salicylic acid, valproic acid, furosemide, hydrochlorothiazide, thiopental)

Acknowledgements

This project was supported by Award No. 2014-R2-CX-K007 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice
Thanks to Kevin Shanks MS, D-ABFT-FT for help in setting cutoff levels and target compounds