Sheathless Capillary Electrophoresis - Mass Spectrometry:
The High Sensitivity Porous Sprayer (HSPS) for the Forensic Analysis of Drugs and Metabolites

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Introduction

In Forensic Drug Analysis there is always a need for increased sensitivity to provide better detection in challenging casework. Samples may only be obtained hours or days after the crime has been committed and the drugs may have been metabolized or the metabolites excreted. In this work we apply a prototype sheathless interface for Capillary Electrophoresis-ESI-Mass Spectrometry (CE-MS) to the objective of routine screening for drugs and metabolites at sub-therapeutic levels in biofluids. Levels of detection (LOD) for a custom designed Test Mixture were determined for 15 drugs and illustrated for one well known drug, diphenhydramine (DPH). Extracts of spiked human urine were analyzed and the LOD was determined for each of the 15 drugs. The ruggedness of the HSPS interface was tested in automated batch analysis of the spiked urine extracts and further in Case Studies on the antiemtic drugs, metoclopramide, and in an exploration of the urine metabolic profile after ingestion and metabolism of the antimalarial drug, chloroquine.

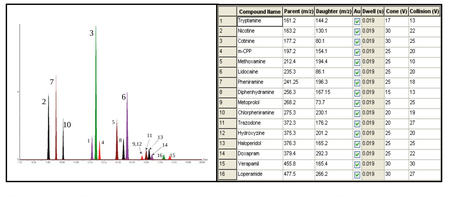
Material and Methods

Chemicals:
All chemicals were Reagent Grade and were purchased from VWR International.

Drug and Metabolite Standards:
Standards were purchased from Cerilliant Corporation, Round Rock, TX, USA. Stock solutions prepared at a concentration of 1 mg/mL and were further diluted to 5, 1 or 0.1 ng/µL in 5 mM Ammonium Formate (pH 2.85).

Operating Conditions:

Capillary	90 cm bare-fused silica,150µm o.d., 30 µm i.d., at 200-300 V/cm, 2.4 to 2.8 µamps
Temperatures	Capillary and sample storage = 25 ºC
Background Electrolyte (BGE)	50 mM Ammonium Formate, pH 2.85
Sample Introduction	Hydrodynamic 16s at 34.5 mBar or Electrokinetic 10kV for 16s
CE Instrument	Beckman Coulter PA 800 Enhanced
MS Instrument	Waters Xevo TQ with MassLynx 4.1
Sheathless Interface	High Sensitivity Porous Sprayer (HSPS) Prototype (Beckman Coulter, Brea, CA).
Conductive Liquid	0.7% Formic Acid
ESI Voltage	1.2 to 1.8 kV
HSPS Conditioning	The capillaries were initially conditioned with MeOH, water, 1N NaOH, water and BGE.



	Compound Name	Parent (m/z)	Daughter (m/z)	Acq Dwell (s)	Cone (V)	Collision (V)
1	Pyridamine	181.2	144.2	0.019	17	13
2	Nicotine	163.2	130.1	0.019	30	22
3	Cotinine	177.2	80.1	0.019	30	25
4	m-CPP	197.2	154.1	0.019	25	20
5	Methocramine	212.4	194.4	0.019	25	10
6	Lidocaine	235.3	86.1	0.019	25	20
7	Pheniramine	241.25	196.3	0.019	25	18
8	Diphenhydramine	256.3	167.15	0.019	15	13
9	Metoprolol	268.2	73.7	0.019	25	25
10	Chlorpheniramine	275.3	230.1	0.019	20	19
11	Trazodone	372.3	176.2	0.019	20	27
12	Hydroxyzine	375.3	201.2	0.019	25	20
13	Haloperidol	376.3	165.2	0.019	25	25
14	Doxapram	379.4	292.3	0.019	25	22
15	Verapamil	455.8	165.4	0.019	30	30
16	Loperamide	477.5	266.2	0.019	30	27

Figure 1: Drug/Metabolites Test Mixture and MRM Conditions

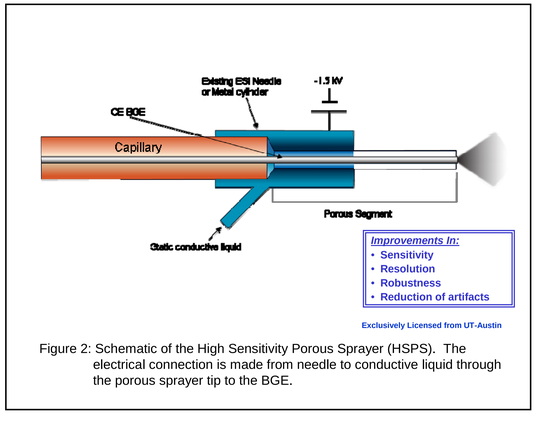


Figure 2: Schematic of the High Sensitivity Porous Sprayer (HSPS). The electrical connection is made from needle to conductive liquid through the porous sprayer tip to the BGE.

Results

1. A Drug and Metabolite Test Mixture utilizing Multiple Reaction Monitoring (MRM) of 15 drugs and an internal standard on a Tandem Quadrapole MS was used in the evaluation (Figure 1).

2. A sheathless prototype HSPS (Figure 2) was used to interface CE and ESI-MS.

3. Linearity and LOD determinations were done using serial dilutions of aqueous solutions of the Test Mixture. The LOD values for all components were found to be less than 1 ng/mL. See Figure 3 for an example component from the Test Mixture, diphenhydramine (DPH) .

4. Urine samples were spiked with the Test Mixture and internal standard, doxapram, and were processed with a standard liquid-liquid extraction protocol (1).

5. As an illustration of the required detection sensitivity in Forensic Drug Analysis, the common drug, diphenhydramine, was selected from the urine analysis results. Levels after therapeutic dosing of the drug typically result in levels of 20 ng/mL in blood (2) and are usually an order of magnitude higher in urine (Figure 4).

6. Detection at lower levels (sub-therapeutic) is important in cases where a significant period of time has elapsed after intake of the drug and collection of the samples, e.g. sexual assaults, impaired driving.

7. Diphenhydramine (DPH) was easily detected at an LOD and LOQ of 0.25 ng/mL of urine. This is a factor of 80 times lower than the required detection level of 20 ng/mL after therapeutic administration (Figure 5).

8. The HSPS interface was further tested for ruggedness on positive urine samples provided by volunteers in two case studies.

9. In Case Study 1 (Figure 6), urine was provided by a volunteer after a minor surgical procedure. The antiemetic drug, metoclopramide, was easily detected and confirmed (3 ions provided by 2 MRMs) after therapeutic administration. The results were obtained in automated runs of urine extracts without any matrix related problems.

10. Case Study 2 illustrates the resolution of chloroquine and metabolites provided by CE and maintenance of this resolution by the HSPS interface (Figure 7). Chloroquine and four of its metabolites were detected in all samples over a four day collection period. This confirmed that the parent drug was excreted unchanged and in high amounts, thus providing protection against malaria in a travel dosing scenario.

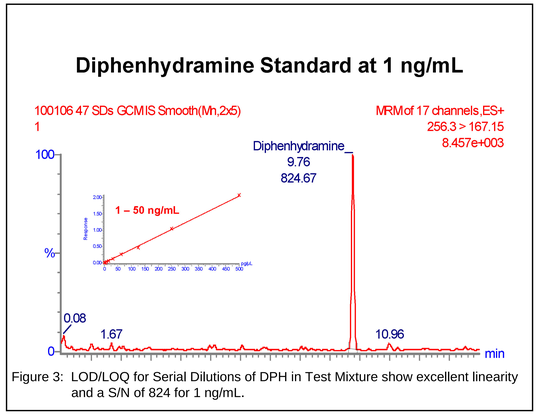


Figure 3: LOD/LOQ for Serial Dilutions of DPH in Test Mixture show excellent linearity and a S/N of 824 for 1 ng/mL.

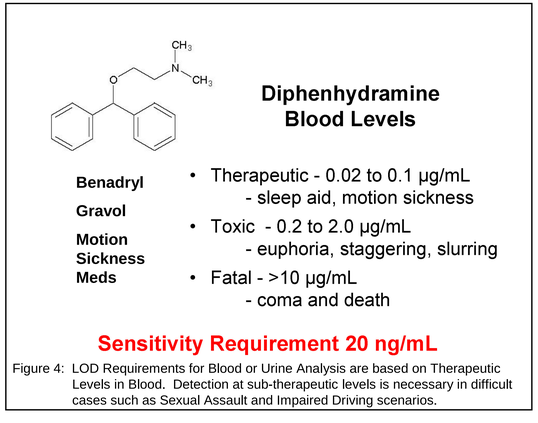


Figure 4: LOD Requirements for Blood or Urine Analysis are based on Therapeutic Levels in Blood. Detection at sub-therapeutic levels is necessary in difficult cases such as Sexual Assault and Impaired Driving scenarios.

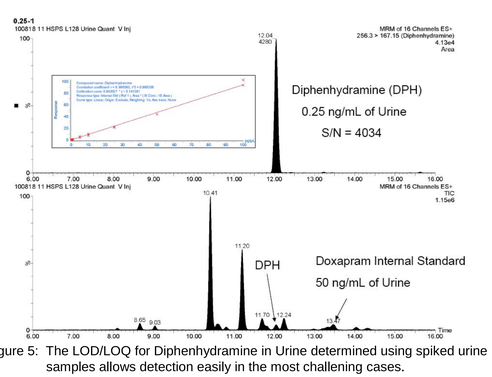


Figure 5: The LOD/LOQ for Diphenhydramine in Urine determined using spiked urine samples allows detection easily in the most challening cases.

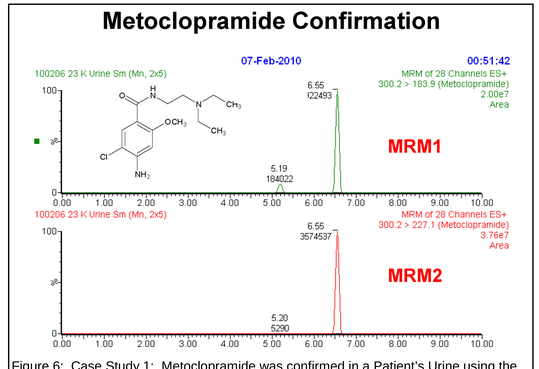


Figure 6: Case Study 1: Metoclopramide was confirmed in a Patient's Urine using the widely accepted criteria of the presence of three ions including the M+1 and the two most prominent daughter ions.

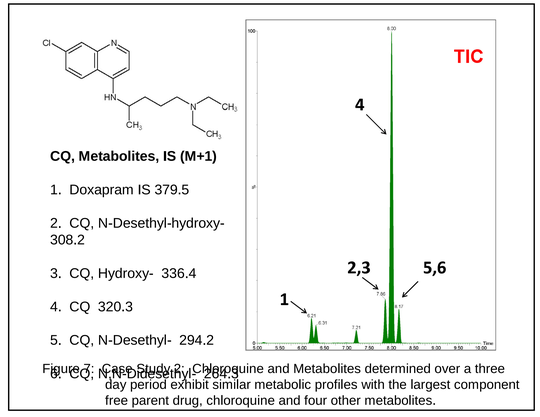


Figure 7: Case Study 2: Chloroquine and Metabolites determined over a three day period exhibit similar metabolic profiles with the largest component free parent drug, chloroquine and four other metabolites.

Conclusions

A High Sensitivity Porous Sprayer (HSPS) has been integrated into a sheathless interface for CE-ESI-MS. The HSPS provides superior transfer of analytes to the mass spectrometer and maintains the intrinsic attributes of capillary electrophoresis such as resolution. Routine detection of common drugs in biological fluids for forensic laboratories is facilitated by this advancement and can be applied to the most challening of forensic drug cases. The process is robust and automated and should be of great value to analytical laboratories that work with charged analytes.

References

1. Hudson, J.C. et al., Can. Soc. Forens. Sci., 31(1) 1 - 29 (1998).
2. Baselt, R.C. Disposition of Toxic Drugs and Chemicals in Man, pp. 489-492, 8th Edition, Biomedical Publications, Foster City, California, 94404 (2008).

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