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CESI-MS Analysis of Biofluids of Basic Drugs and Metabolites

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Introduction

Capillary Electrophoresis (CE) has been used for over 15 years to screen biofluids, which include whole blood, serum, plasma and urine.[1-4] Confirmation and quantification of the presence of a drug or metabolite detected by CE has traditionally been done independently after the screening process by gas, liquid chromatography or sheathflow CE[5] coupled to Mass Spectrometry (MS). In this paper, the successful use of an integrated CE and ESI process known as CESI, is described in the context of the analysis of biofluids for drugs and their metabolites. The sensitivity is greatly improved by the use of this very low flow process. This hyphenated technique can provide screening, confirmation and quantification of drugs and metabolites in a single process with detection at 0.25 ng/mL or lower in biofluid.

Experimental

Chemicals and standards

Drug and metabolite standards were obtained from Cerilliant Corporation, Round Rock, TX, USA; Sigma-Aldrich, St. Louis, MO, USA; and USP, 12601 Twinbrook Parkway, Rockville, MD, USA. All standards were dissolved in 5 mM ammonium formate at pH 2.85 buffer at the concentration indicated in Table 1. Quantitative analysis used a spiking mixture at 3 times the concentration shown in Table 1 in each milliliter of biofluid. Doxapram was used as Internal Standard (IS), and it was added separately at 50 nanograms per mL of biofluid.

Reagents

Distilled and deionized (DDI) water was obtained from a Barnstead Nanopure Infinity ultrapure water system. Rinse solutions of methanol, 1N sodium hydroxide and or 0.1N hydrochloric acid were all reagent grade and were obtained from VWR Scientific, Bridgeport, NJ, USA. The Background Electrolyte (BGE) and Conductive Liquid (CL) solutions were prepared as follows: BGE (50 mM ammonium formate, Alfa Aesar P/N A10699 at pH 2.85) was prepared by diluting 962.5 microliters of formic acid (98%, EMD P/N FX0440-5) with DDI water to a final volume of 500 mL, and then adjusting its pH to 2.85 with a 50 mM ammonium formate solution (0.813 g ammonium formate dissolved in 250 mL of DDI water). CL (0.7% formic acid) was prepared by diluting 714 microliters of formic acid (98% purity) with DDI water to a final volume of 100 mL. Alternatively, the BGE can be used as the conductive liquid. Both BGE and CL solutions were stored at 2°C to 8°C when not in use. Both solutions were brought to ambient temperature and sonnicated before use. The BGE was filtered through a 0.2 µm membrane filter just prior to use.

#	Compound	Conc.	Source
		ng/µL	Cer-Cerilliant S – Sigma-Alrich USP – United States Pharmacopeia
1	Nicotine	3	N-008 Cer
2	Pheniramine	1	P-045 Cer
3	Chlorpheniramine	1	C-036 Cer
4	Tryptamine	1	246557 S
5	Cotinine	3	C-016 Cer
6	m-CPP	1	C-089 Cer
7	Methoxamine	2	M 6524 S
8	Diphenhydramine	1	D-015 Cer
9	Lidocaine	1	L-018 Cer
10	Hydroxyzine	1	H8885 S
11	Metoprolol	1	M-123 Cer
12	Trazodone	1	T-030 Cer
13	Haloperidol	1	H-030 Cer
14	Doxapram IS	1	USP 1225000
15	Verapamil	1	V-002 Cer
16	Loperamide	1	L 4762 S

Table 1: Drug and metabolite performance standard with source information.

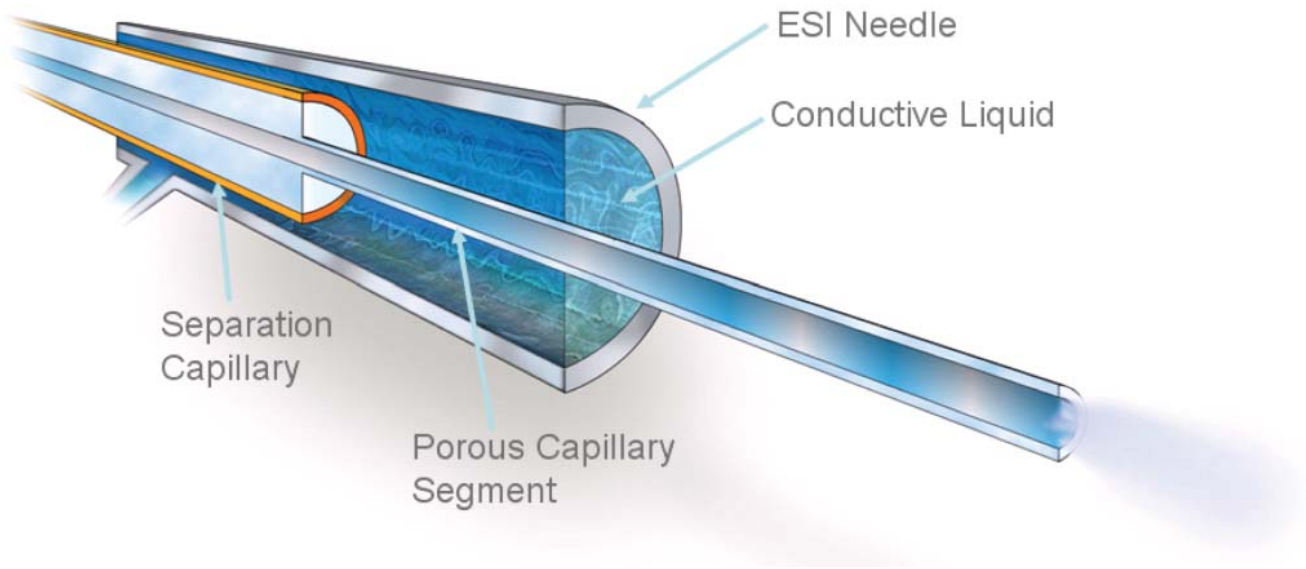


Figure 1. Schematic of the OptiMS capillary.

Instrument configurations

CE instrument

All experiments were performed using a prototype CESI 8000 High Performance Separation – ESI Module* (Beckman Coulter, Inc., Brea, CA, USA). Data were acquired and the instrument was controlled by CESI 8000 Software. Prototype OptiMS Silica Surface Capillary Cartridges (150 µm OD, 30 µm ID, 90 cm long) with a porous tip were obtained from Beckman Coulter. All separations were performed at a capillary temperature of 20°C. The system sample storage temperature was set to a temperature of 10°C. Only applied voltage and CE current data were acquired on the CE.

MS instrument

MS data were acquired on a Waters Xevo tandem mass spectrometer (Waters Corporation, Milford, MA, USA). The instrument was operated in ESI positive ion mode with a capillary voltage range from 1200 to 1400V. MS/MS was performed using Multiple Reaction Monitoring (MRM) (Table 2) with argon as the collision gas at 0.15 mL/min.

OptiMS capillary

The OptiMS capillary (Figure 1) used in this work features a porous tip, which was created from a 150 µm OD, 30 µm ID capillary with the polyimide removed from the tip area. This combined ESI source, interface and separation capillary, are enclosed in a cartridge with a stainless steel needle in a retractable tip assembly (Figure 2). Electrical contact was made between the background electrolyte in the capillary through the porous section of the capillary tip and finally through a conductive liquid which fills the space between the needle and porous tip. The conductive liquid is supplied using pressure rinsing from the outlet side of the CESI instrument.

#	Compound	Precursor ion	Product ion	Cone voltage	Collision energy
		(m/z)	(m/z)	(V)	(eV)
1	Nicotine	163.2	130.1	30	22
2	Pheniramine	241.2	196.3	25	18
3	Chlorpheniramine	275.3	230.1	20	19
4	Tryptamine	161.2	144.2	17	13
5	Cotinine	177.2	80.1	30	25
6	m-CPP	197.2	154.1	25	20
7	Methoxamine	212.4	194.4	25	10
8	Diphenhydramine	256.3	167.2	15	13
9	Lidocaine	235.3	86.1	25	20
10	Hydroxyzine	375.3	201.2	25	20
11	Metoprolol	268.2	73.7	25	25
12	Trazodone	372.3	176.2	20	27
13	Haloperidol	376.3	165.2	25	25
14	Doxapram	379.4	292.3	25	22
15	Verapamil	455.8	165.4	30	30
16	Loperamide	477.5	266.2	30	27

Table 2: MRM transitions and conditions for the performance standard.

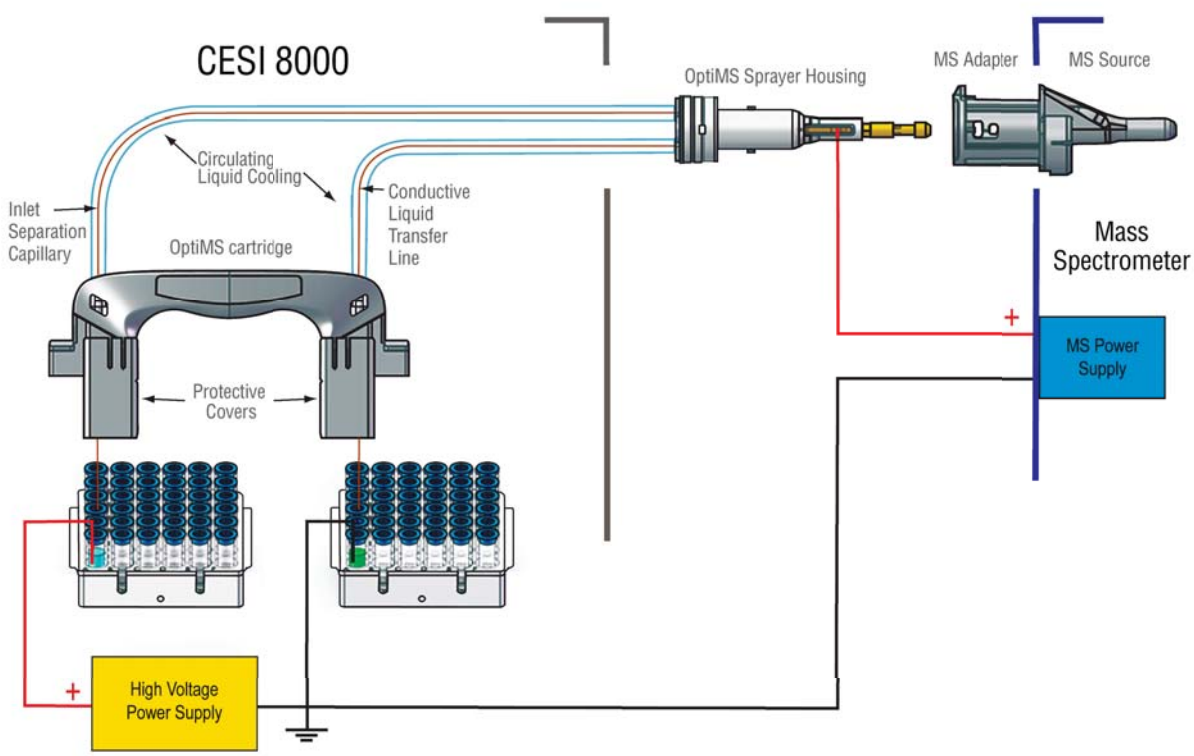


Figure 2: Schematic of the OptiMS Cartridge and retractable tip assembly. The data was generated on a prototype similar to the one shown above.

Preparation of the OptiMS silica surface capillary

CE conditioning method

New OptiMS capillaries were conditioned by rinsing, in sequence, with methanol to remove organic material from capillary manufacturing, DDI water to remove the methanol, 1N NaOH to fill the capillary and condition its surface during a 60 min. wait period. DDI water was used to remove NaOH and, finally, a long rinse with background electrolyte (BGE) was used to condition the capillary surface (Figure 3). This conditioning method was performed with the porous sprayer retractable tip, which was immersed in DDI water. **Note:** This conditioning method is used once on a new sprayer. However, this same method can be repeated as necessary as a cleaning procedure or to improve separation performance.

CE separation method

The sample components were separated by CE using the following method (Figure 4). First, the capillary was rinsed twice with BGE rinses, a three-minute rinse followed by a five-minute rinse, both at 50 psi. This double rinse ensured that there was no carryover of sample from one run to the next. The conductive liquid (CL) supply to the needle surrounding the porous tip was replenished prior to each run (line 3 of Figure 4) from the outlet side of the CE instrument by a reverse pressure rinse. The sample was electrokinetically injected at the inlet side using a 10kV voltage injection of 16 seconds. The sample injection was followed by a short 0.5 psi pressure injection of BGE for 10 seconds to ensure good quantitative reproducibility of injections. During all steps in the separation method, the conductive liquid capillary installed in the outlet side of the instrument was positioned in BO:A1. The vials were incremented every 12 runs to prevent carryover and buffer depletion effects.

Shutdown methods

Following the completion of a sequence of runs, the capillary was preserved for future use by different methods, depending on the situation.

Short-term storage (<24 hrs.)

The housing containing the sprayer tip was immediately removed from the system and submerged in DDI water overnight to be used the next day.

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	10.00 min	BI:F1	BO:A1	forward	Methanol 10 min.
2		Rinse - Pressure	50.0 psi	5.00 min	BI:F2	BO:A1	forward	Water rinse 5 min.
3		Rinse - Pressure	50.0 psi	2.00 min	BI:F3	BO:A1	forward	NaOH rinse 60 min.
4		Wait		60.00 min	BI:F3	BO:A1		
5		Rinse - Pressure	50.0 psi	10.00 min	BI:F2	BO:A1	forward	Water rinse 10 min.
6		Rinse - Pressure	50.0 psi	5.00 min	BI:F4	BO:A1	forward	Buffer Rinse 5 min.
7	0.00	Separate - Pressur	50.0 psi	60.00 min	BI:F4	BO:A1	forward	Pressure separation - rinse with housing in water.
8	60.00	Stop data						
9	60.00	End						
10								

Figure 3: Time program for new capillary conditioning methods.

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	3.00 min	BI:A1	BO:A1	forward, In vial inc 12	BGE rinse
2		Rinse - Pressure	50.0 psi	5.00 min	BI:B1	BO:A1	forward, In vial inc 12	2nd BGE rinse to prevent carryover
3		Rinse - Pressure	75.0 psi	0.75 min	BI:B1	BO:A1	reverse, In vial inc 12	Conductive liquid replenishment from outlet vial
4		Relay On					1: 0.05	Start MS acquisition
5		Inject - Voltage	10.0 KV	16.0 sec	SI:A1	BO:A1	Override, normal polarity	Voltage Injection
6		Inject - Pressure	0.5 psi	10.0 sec	BI:C1	BO:A1	No override, forward, In / Out vial inc 12	Buffer plug added
7	0.00	Separate - Voltage	30.0 KV	21.00 min	BI:D1	BO:A1	1.00 Min ramp, normal polarity, In vial inc 12	Separation step normal polarity
8	0.25	Relay On					1: 0.05	Start next MS file (moves to Data Acquisition line).
9	1.00	Relay On					1: 0.05	Start MS data acquisition.
10	21.00	Stop data						
11	21.00	End						
12								

Figure 4: Time program for separation method.

Long-term storage (>24 hrs.)
The separation capillary was rinsed sequentially with DDI water and methanol, followed by the application of voltage across the capillary filled with methanol to remove any ionic material that might be present in the porous sprayer tip (Figure 5). Then, the separation capillary was dried by flushing with air. The CL capillary was rinsed with water, methanol and air. The retractable housing end was sealed with Parafilm and the whole assembly was stored at 2°C to 8°C.

Unattended shutdown
Use the time program below (Figure 6) to decrease the rinse flow, thus preventing the capillary from drying out while maintaining the current MS operating conditions.

Buffer tray configuration
Vials containing 1.5 mL of reagents were placed in the buffer trays prior to the use of the conditioning, separation or shutdown methods (Figure 7). All vials were capped with blue caps. An empty capped vial was used to rinse capillaries with air.

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:F2	BO:A1	forward	Rinse with water forward; BO:A1 contains CL.
2		Rinse - Pressure	50.0 psi	5.00 min	BI:F1	BO:A1	forward	Rinse with methanol forward; BO: contains CL.
3	0.00	Separate - Voltage	20.0 KV	10.00 min	BI:F1	BO:A1	1.00 Min ramp, normal polarity	Voltage across separation capillaryl; normal polarity.
4	10.00	Rinse - Pressure	50.0 psi	5.00 min	BI:F5	BO:A1	forward	Pressure rinse with air.
5	15.00	Rinse - Pressure	50.0 psi	5.00 min	BI:F5	BO:F2	reverse	Pressure rinse CL line, reverse with water.
6	20.00	Rinse - Pressure	50.0 psi	5.00 min	BI:F5	BO:F1	reverse	Pressure rinse CL line, reverse with methanol.
7	25.00	Rinse - Pressure	50.0 psi	5.00 min	BI:F5	BO:F5	reverse	Pressure rinse CL line, reverse with air.
8	30.00	End						
9								

Figure 5: Time program for storage method.

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:A1	BO:A1	forward	BGE Rinse.
2		Rinse - Pressure	50.0 psi	2.00 min	BI:A1	BO:A1	reverse	Conductive Liquid Fill.
3	0.00	Separate - Pressur	5.0 psi	720.00 min	BI:C1	BO:A1	forward	Low Pressure Rinse for 6 hours.
4	720.00	Stop data						
5	720.00	End						
6								

Figure 6: Time program for unattended shutdown method.

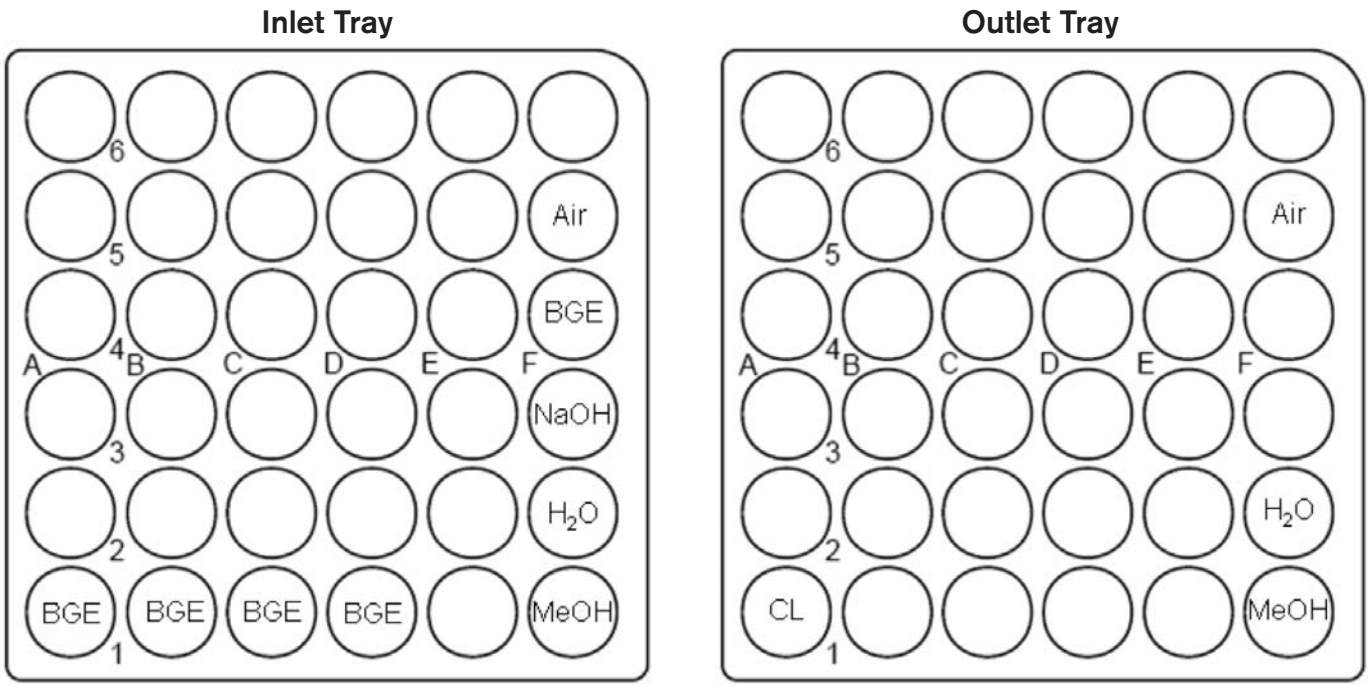


Figure 7: Buffer tray vial configuration for all methods.

Evaluating system performance by running the performance standard test mix

An assessment of the performance of the CESI-MS system must be made before running any samples. In this work, the separation was optimized for all 16 drugs and metabolites covering the useful mobility range of compounds with molecular weights under 525 Da (Figures 8 and 9).

Running the CESI-MS System

In order to ensure that high quality data are acquired, all the components of the CESI-MS system must be working properly. Each component should be checked separately and then optimized as an integrated system. Follow these six steps to ensure that high performance is achieved.

Step 1. Qualify and calibrate the instruments
Both the CESI 8000 High Performance Separation – ESI Module and the MS instrument must be maintained and calibrated prior to integrating them via CESI. This process requires testing each instrument independently to ensure that they are operating within manufacturers' specifications.

Step 2. Install and prepare the interface
Install the previously-conditioned OptiMS Capillary. Use an infusion standard to optimize the location of the sprayer tip relative to the entrance of the MS source. The optimal X-Y-Z sprayer position will generate the highest signal response from the mass spectrometer.

Step 3. Test the CESI-MS system for performance
Access the sprayer performance by monitoring the CE electrical current generated during the separation, the minimum ESI voltage required to establish a stable spray, the results from the infusion standard X-Y-Z positioning and the MS data from a performance standard. The performance standard described in this article is recommended as the separation has been optimized for drug and metabolite screening. This mixture should be run daily and the results stored to form the basis for long-term comparison of the performance of individual interfaces and the overall system. See "System Performance" and Figures 8 and 9.

Step 4. Prepare samples from the biofluid
Best results are obtained from an extract of the biofluid using a preconcentration protocol. Methods such as liquid-liquid extraction (LLE), and solid-phase extraction (SPE) provide suitable extracts for CESI-MS. Established LLE protocol[3, 8] using urine is outlined in Table 3. This procedure can also be used for whole blood, plasma and serum.

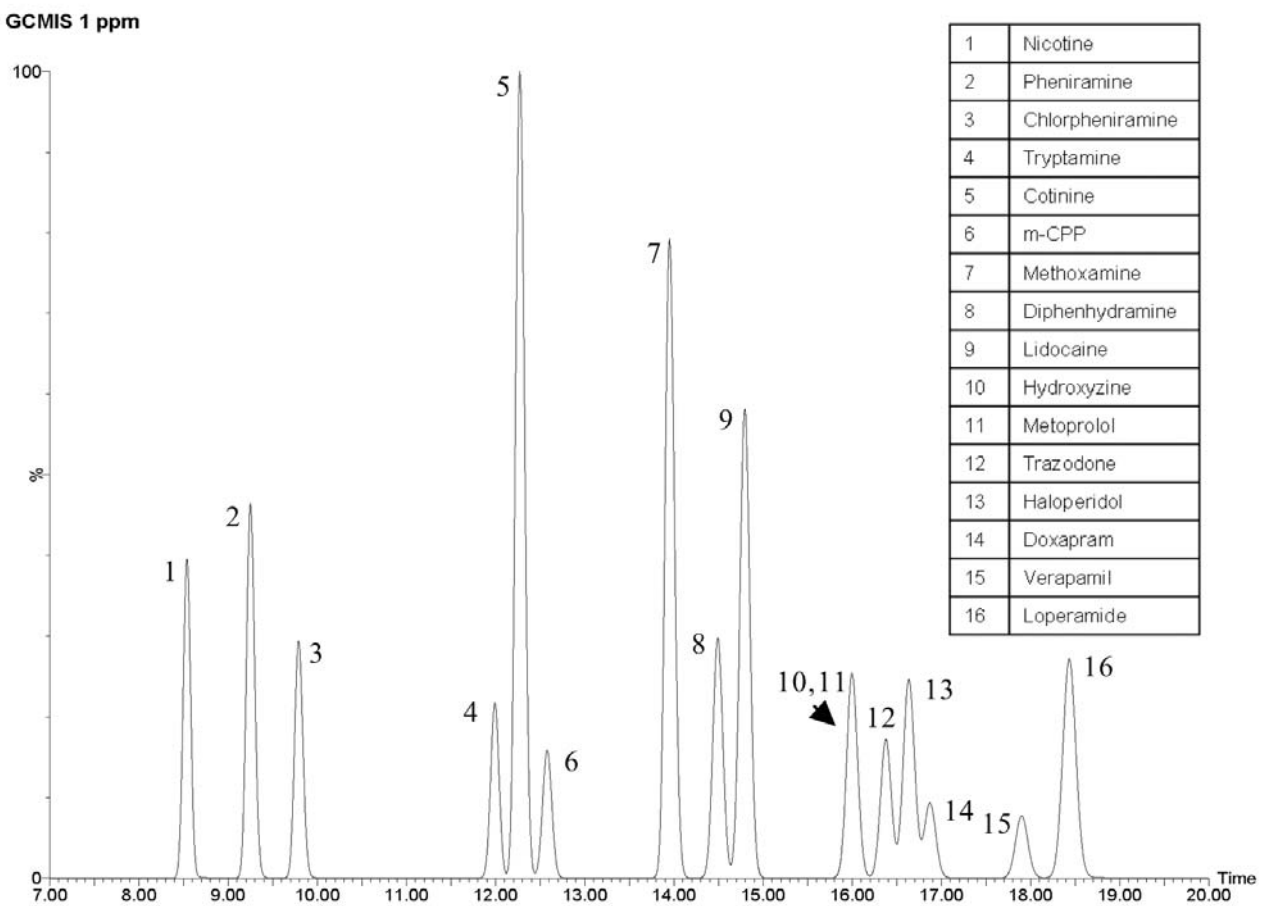


Figure 8: Total Ion Chromatogram (TIC) of the performance standard test mix.

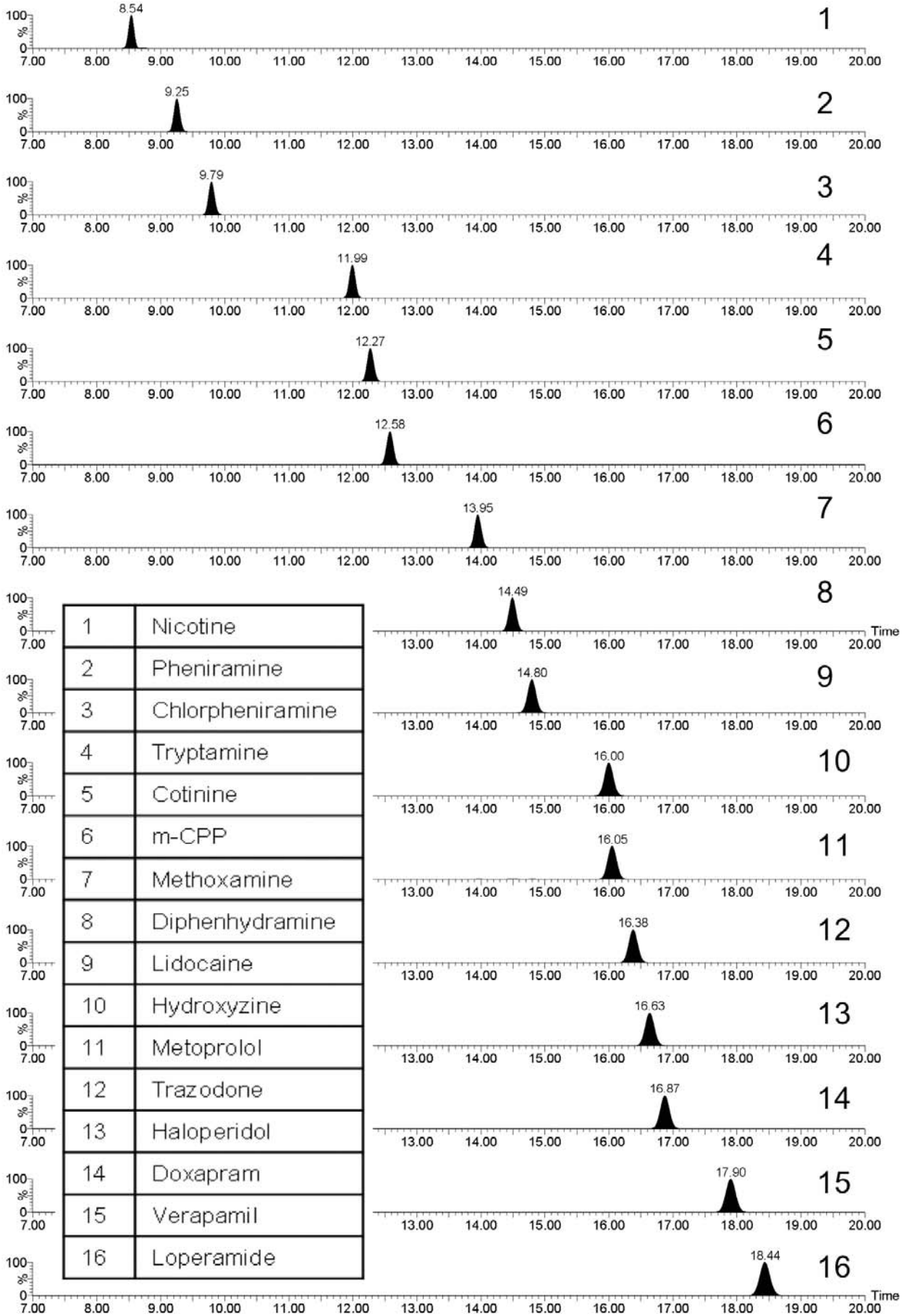


Figure 9: MRM chromatograms for the performance standard of 16 compounds.

Urine** Concentration	Volume of Standard Sol'n	Conc. of Standard Sol'n	Volume of IS Sol'n	Conc. of Internal Standard Sol'n
100	20 µL	5 ng/µL	10 µL	5 ng/µL
50	10 µL	5 ng/µL	10 µL	5 ng/µL
25	5 µL	5 ng/µL	10 µL	5 ng/µL
10	10 µL	1 ng/µL	10 µL	5 ng/µL
5	5 µL	1 ng/µL	10 µL	5 ng/µL
1	10 µL	0.1 ng/µL	10 µL	5 ng/µL
0.5	5 µL	0.1 ng/µL	10 µL	5 ng/µL
0.25	2.5 µL	0.1 ng/µL	10 µL	5 ng/µL
Blank + IS	N/A	N/A	10 µL	5 ng/µL
Blank	N/A	N/A	N/A	N/A

*See Standards and Table 1. **In duplicate.

Table 3: Design of Experiment (DOE) for spiked urine samples.

Protocol

Quantitative Analysis of Biofluids – An Example Using Spiked Urine Samples.*

Drug-free urine samples (1 mL each) were spiked with the performance standard to yield concentrations ranging from 0.25 to 250 ng/mL of urine. Doxapram Internal Standard (IS, 50 ng/mL) was added to each tube, except the Blank (Table 3).

Extraction Procedure for Biofluids

The extraction of the drugs and metabolites from urine was done using a LLE method.[3] The procedure involved the following steps:

1. Add an internal standard, (Doxapram, 50 ng) to 1 mL urine aliquots in 15 mL screw-capped tubes and vortex for 5 seconds.
2. Spike standards into 1 mL of drug-free urine samples for use as calibrators and vortex.
3. Add 200 µL of concentrated ammonium hydroxide (28-30%, EMD P/N AX1303-6) to each tube followed by vortexing.
4. Add 5 mL of 1-Chlorobutane to each tube. Cap the tubes and shake on a flat-bed, rotary shaker or similar device for 10 minutes.
5. Centrifuge the samples at 1500 rpm (Beckman Coulter Allegra X-22R equipped with SX4250 rotor) for 10 minutes under refrigeration (0°C – 4°C).
6. Decant the organic layer into 12x75 mm culture tubes and evaporate under nitrogen (or other suitable evaporation system) just to dryness. Do not over evaporate because it will result in the loss of volatile drugs (e.g. nicotine).
7. Dissolve the residue in 100 µL of 5 mM ammonium formate, pH 2.85 by vortexing followed by centrifuging the 12x75 tubes at 1200 rpm for 2 minutes.
8. Transfer the sample solution to 200 µL PCR vials; cap and centrifuge at 14,000 rpm for 20 minutes to precipitate any particulate material.
9. Position the samples in the CESI 8000 inlet sample tray and inject using an applied voltage of 10 kV for 16s followed by a short BGE pressure injection for 10s at 0.5psi.

Step 5. Prepare data acquisition control sequences

The CESI 8000 provides the sample automation needed to collect a series of runs. The sequence (Figure 10) uses a method for voltage injection from the performance standard and reconstituted urine samples. A corresponding sequence or sample table from the MS software must be synchronized with the sequence of the CESI 8000 (a portion of the table is shown in Figure 11). The MS sample table controls the operation of the applied ESI voltage – turning it on while rinsing the interface, off while injecting using the CE voltage, and back on when collecting acquisition data.

Step 6. Data processing

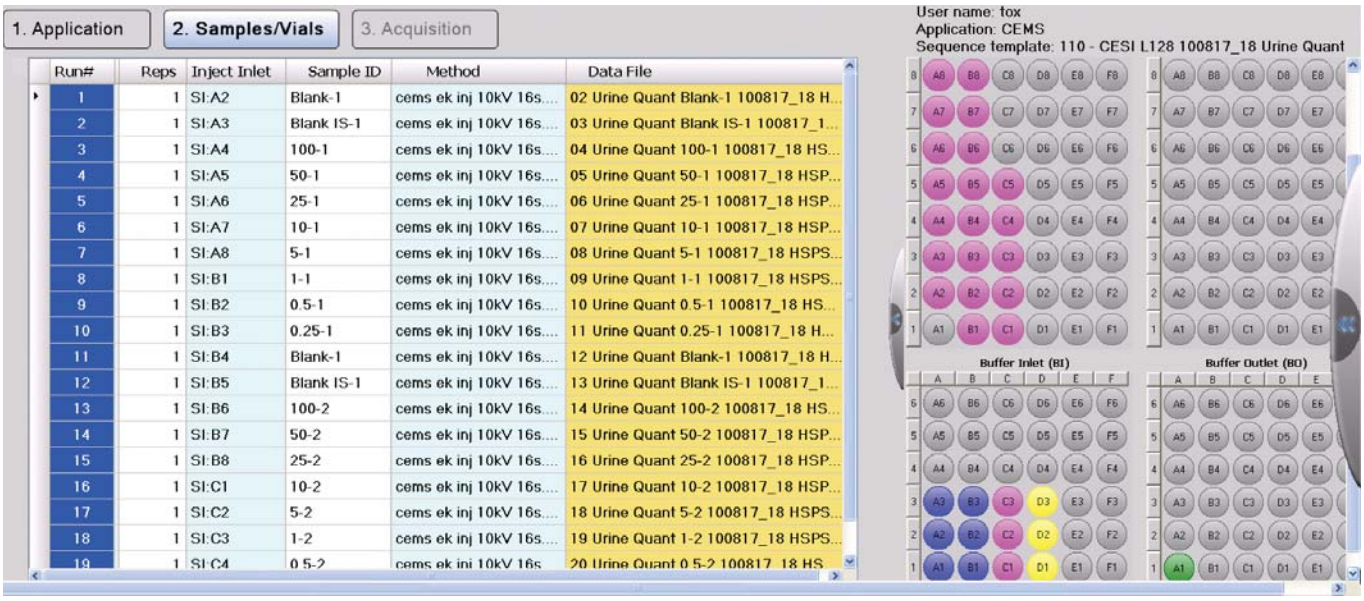
Data collected by the mass spectrometer software can be manually processed or, preferably, automatically via modifications to the sample table after the full data acquisition is complete. Lines in the sample table which are only used to control the ESI voltage are removed and an example is shown in Figure 12.

Step 7. Interpretation of results

In this work, the results for the quantitative urine extracts provide assessment parameters such as linearity and limit of detection (LOD).

Results

1. Sample acquisition was controlled by the CESI 8000 sequence (Figure 10).



Run#	Reps	Inject Inlet	Sample ID	Method	Data File
1	1	SI:A2	Blank-1	cems ek inj 10kV 16s....	02 Urine Quant Blank-1 100817_18 H...
2	1	SI:A3	Blank IS-1	cems ek inj 10kV 16s....	03 Urine Quant Blank IS-1 100817_1...
3	1	SI:A4	100-1	cems ek inj 10kV 16s....	04 Urine Quant 100-1 100817_18 HS...
4	1	SI:A5	50-1	cems ek inj 10kV 16s....	05 Urine Quant 50-1 100817_18 HSP...
5	1	SI:A6	25-1	cems ek inj 10kV 16s....	06 Urine Quant 25-1 100817_18 HSP...
6	1	SI:A7	10-1	cems ek inj 10kV 16s....	07 Urine Quant 10-1 100817_18 HSP...
7	1	SI:A8	5-1	cems ek inj 10kV 16s....	08 Urine Quant 5-1 100817_18 HSPS...
8	1	SI:B1	1-1	cems ek inj 10kV 16s....	09 Urine Quant 1-1 100817_18 HSPS...
9	1	SI:B2	0.5-1	cems ek inj 10kV 16s....	10 Urine Quant 0.5-1 100817_18 HS...
10	1	SI:B3	0.25-1	cems ek inj 10kV 16s....	11 Urine Quant 0.25-1 100817_18 H...
11	1	SI:B4	Blank-1	cems ek inj 10kV 16s....	12 Urine Quant Blank-1 100817_18 H...
12	1	SI:B5	Blank IS-1	cems ek inj 10kV 16s....	13 Urine Quant Blank IS-1 100817_1...
13	1	SI:B6	100-2	cems ek inj 10kV 16s....	14 Urine Quant 100-2 100817_18 HS...
14	1	SI:B7	50-2	cems ek inj 10kV 16s....	15 Urine Quant 50-2 100817_18 HSP...
15	1	SI:B8	25-2	cems ek inj 10kV 16s....	16 Urine Quant 25-2 100817_18 HSP...
16	1	SI:C1	10-2	cems ek inj 10kV 16s....	17 Urine Quant 10-2 100817_18 HSP...
17	1	SI:C2	5-2	cems ek inj 10kV 16s....	18 Urine Quant 5-2 100817_18 HSPS...
18	1	SI:C3	1-2	cems ek inj 10kV 16s....	19 Urine Quant 1-2 100817_18 HSPS...
19	1	SI:C4	0.5-2	cems ek inj 10kV 16s	20 Urine Quant 0.5-2 100817_18 HS...

Figure 10: CESI 8000 sequence for urine calibration.

2. MS data were acquired using the MassLynx sample table, a part of which is shown in Figure 13.

	File Name	File Text	MS Tune File	MS File
1	discard 1		cems_on	cap_on
2	discard 2		cems_off	cap_off
3	100818 01 HSPS L128 Urine Quant V Inj	GCM IS 1 ppm	cems_hsps	GCM plus Doxapram
4	discard 3		cems_on	cap_on
5	discard 4		cems_off	cap_off
6	100818 02 HSPS L128 Urine Quant V Inj	Blank-1	cems_hsps	GCM plus Doxapram
7	discard 5		cems_on	cap_on
8	discard 6		cems_off	cap_off
9	100818 03 HSPS L128 Urine Quant V Inj	Blank IS-1	cems_hsps	GCM plus Doxapram
10	discard 7		cems_on	cap_on
11	discard 8		cems_off	cap_off
12	100818 04 HSPS L128 Urine Quant V Inj	100-1	cems_hsps	GCM plus Doxapram
13	discard 9		cems_on	cap_on
14	discard 10		cems_off	cap_off
15	100818 05 HSPS L128 Urine Quant V Inj	50-1	cems_hsps	GCM plus Doxapram

Figure 11: MassLynx sample table for control of the ESI voltage and data acquisition.

Sample data files are acquired in sets of three as illustrated in Figure 11. The first file is used to keep the ESI voltage on during rinsing the capillary. The second file is used to turn the ESI voltage off during injection and the following BGE injection. The third file in a set of three is the collection of the actual sample data.

After data acquisition was complete, the data were processed using the sample table is shown in Figure 12. Lines in the original sample table which control the ESI voltage were removed before processing.

	File Name	File Text	MS Tune File	MS File	Conc A
1	100818 02 HSPS L128 Urine Quant V Inj	Blank-1	cems_hsps	GCM plus Doxapram	0
2	100818 03 HSPS L128 Urine Quant V Inj	Blank IS-1	cems_hsps	GCM plus Doxapram	0
3	100818 04 HSPS L128 Urine Quant V Inj	100-1	cems_hsps	GCM plus Doxapram	100
4	100818 05 HSPS L128 Urine Quant V Inj	50-1	cems_hsps	GCM plus Doxapram	50
5	100818 06 HSPS L128 Urine Quant V Inj	25-1	cems_hsps	GCM plus Doxapram	25
6	100818 07 HSPS L128 Urine Quant V Inj...	10-1	cems_hsps	GCM plus Doxapram	10
7	100818 08 HSPS L128 Urine Quant V Inj...	5-1	cems_hsps	GCM plus Doxapram	5
8	100818 09 HSPS L128 Urine Quant V Inj	1-1	cems_hsps	GCM plus Doxapram	1
9	100818 10 HSPS L128 Urine Quant V Inj...	0.5-1	cems_hsps	GCM plus Doxapram	0.5
10	100818 11 HSPS L128 Urine Quant V Inj...	0.25-1	cems_hsps	GCM plus Doxapram	0.25
11	100818 12 HSPS L128 Urine Quant V Inj...	Blank-2	cems_hsps	GCM plus Doxapram	0
12	100818 13 HSPS L128 Urine Quant V Inj	Blank IS-2	cems_hsps	GCM plus Doxapram	0
13	100818 14 HSPS L128 Urine Quant V Inj	100-2	cems_hsps	GCM plus Doxapram	100
14	100818 15 HSPS L128 Urine Quant V Inj	50-2	cems_hsps	GCM plus Doxapram	50
15	100818 16 HSPS L128 Urine Quant V Inj	25-2	cems_hsps	GCM plus Doxapram	25
16	100818 17 HSPS L128 Urine Quant V Inj	10-2	cems_hsps	GCM plus Doxapram	10
17	100818 18 HSPS L128 Urine Quant V Inj	5-2	cems_hsps	GCM plus Doxapram	5
18	100818 19 HSPS L128 Urine Quant V Inj	1-2	cems_hsps	GCM plus Doxapram	1
19	100818 20 HSPS L128 Urine Quant V Inj	0.5-2	cems_hsps	GCM plus Doxapram	0.5
20	100818 21 HSPS L128 Urine Quant V Inj	0.25-2	cems_hsps	GCM plus Doxapram	0.25

Figure 12: MassLynx sample table for data processing.

3. Linearity was assessed for the 15 drugs and metabolites using Peak Area Ratios (PAR) to the internal standard, doxapram.

4. As examples, the Total Ion Chromatogram of the urine extract and the MRMs for four representative compounds, pheniramine, m-CPP, diphenhydramine and loperamide are shown below:

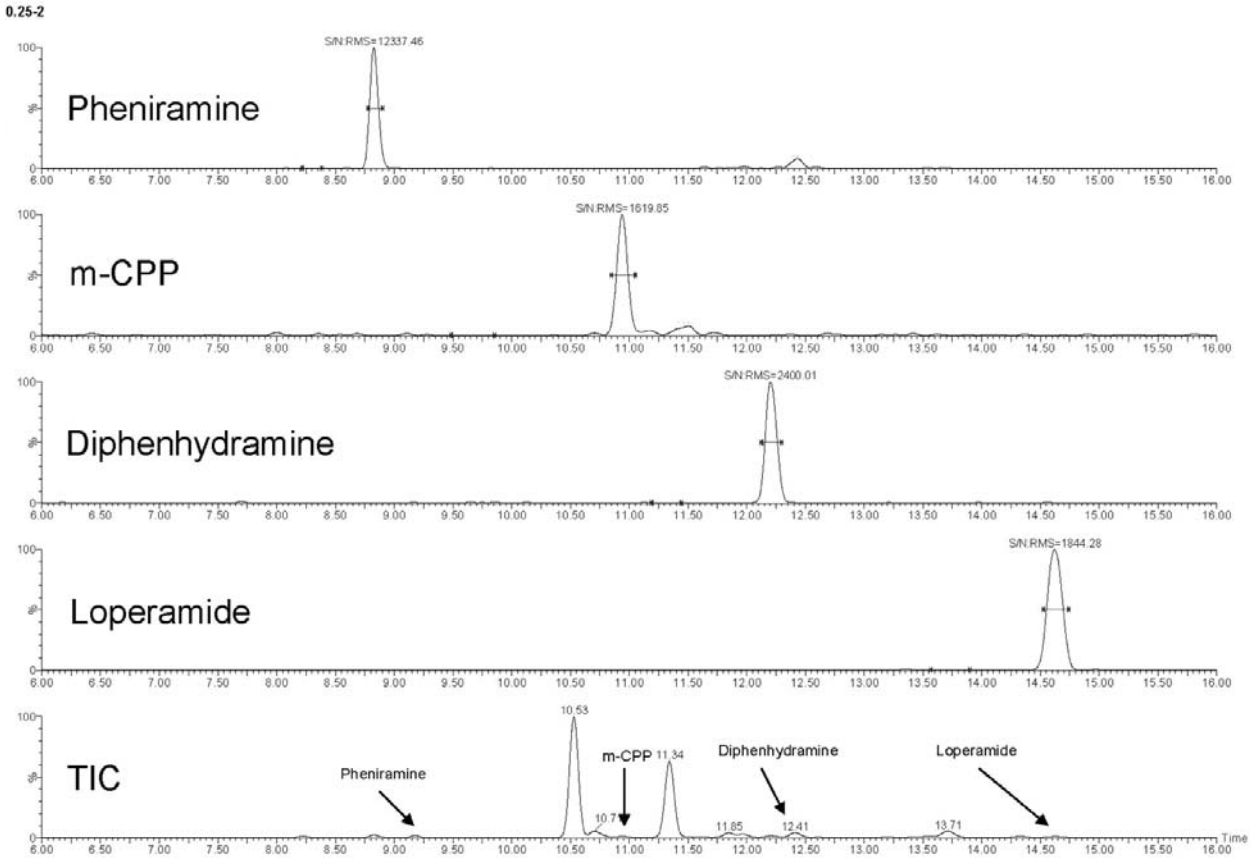


Figure 13: TIC and MRMS for four representative drugs and metabolites.

Calibration results are summarized in Table 4. Methoxamine and metoprolol were excluded from the table as they are poorly ionized in positive ESI MS. Tryptamine was also excluded as it is an endogenous compound always present in urine. It is important not to over evaporate the residues because nicotine and cotinine may be lost with prolonged drying. The observed limit of detection was 0.25 ng/mL of urine (the low calibrator) for the drugs and metabolites quantitated. This limit of detection is well below sub-therapeutic levels and will provide the detection limits needed for the most challenging of forensic case work.

Compound	RMT*	Calibration Curve	R	R^2
Nicotine	0.604	0.198 x – 0.035	0.966	0.934
Pheniramine	0.641	1.080 x + 0.156	0.996	0.993
Chlorpheniramine	0.670	0.661 x + 0.097	0.997	0.995
Cotinine	0.795	0.219 x + 0.828	0.998	0.996
m-CPP	0.808	0.493 x + 0.088	0.996	0.993
Diphenhydramine	0.897	0.940 x + 0.128	0.995	0.991
Lidocaine	0.910	2.128 x + 0.431	0.997	0.993
Hydroxyzine	0.965	0.235 x + 0.017	0.997	0.995
Trazodone	0.982	0.379 x + 0.048	0.997	0.994
Haloperidol	0.991	0.581 x + 0.104	0.997	0.994
Verapamil	1.042	0.673 x + 0.056	0.997	0.994
Loperamide	1.062	0.591 x + 0.044	0.997	0.993

*RMT – Relative Migration Time

Table 4: Linear calibration data for drugs and metabolites.

Summary and Conclusions

This article contains the information necessary to analyze basic drugs and metabolites in forensic casework by CESI-MS. The use of a performance standard is detailed along with sources of the individual components. CE and MS methods are included and explained in detail. Steps to successfully integrate CE with MS are outlined. The results obtained spiked urine analysis of a mixture of drugs and metabolites with an internal standard demonstrates the linearity and limits of detection possible with the use of this system. The response was linear from 0.25 to 50 ng per mL of urine. The LOD and LOQ were both 0.25 ng/mL for 12 of the 15 drugs quantitated. The procedures, methods and protocol outlined in the article are suitable for quantitation of forensically significant drugs.

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B2011-12537
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