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# Fast Screening and Accurate Quantitation of Drugs of Abuse in Bioanalysis by CE-ESI-MS

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## Introduction

The quantitation of xenobiotics in bodily fluids is of great importance in many fields, such as clinical and forensic toxicology, therapeutic drug monitoring, metabolism studies, workplace drug testing, or doping analysis. CE represents an alternative technique to GC and LC for a large range of clinical and toxicological applications with numerous advantages, such as high separation efficiency, short analysis time, and low solvent and sample consumption. UV/Vis is the most widely used detection technique with CE configuration but suffers from a lack of sensitivity due to the narrow optical path length afforded by the internal diameter of the capillary. This lack of sensitivity, combined with the relatively low selectivity of UV/Vis detection, is considered a challenging issue for the determination of potentially low concentration xenobiotics in bodily fluids. This issue can be circumvented by using highly selective and sensitive MS detection.

In this study, CE was hyphenated for quantitative purposes to a highly sensitive Agilent 6490 Triple Quadrupole LC/MS system equipped with Jet Stream and ion funnel technologies. Electrospray ionization (ESI) is the most widespread ionization source for coupling CE with MS and was used here with the sheath-flow configuration. The sheath-flow interface is characterized by an additional make-up liquid flowing through a so-called triple-tube ESI sprayer that mixes with the CE effluent at the capillary tip, providing electrical contact at the outlet end, and the appropriate flow rate (µL range) and solvent conditions for ionization of the analytes. Recently, a new sprayer, which presents an adapted design compared to the standard triple-tube sprayer, has been designed and was used here for the hyphenation of CE with QqQ.

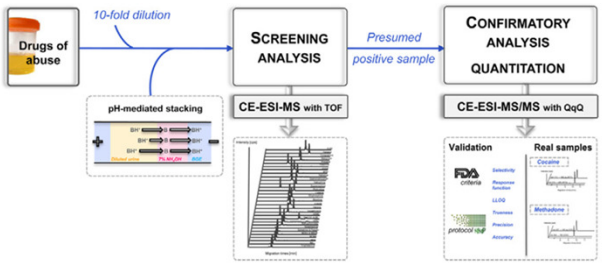


**Fig. 1:** Workflow of CE-MS sample analysis, from real or spiked sample to final validated results. Screening done by CE-TOF and Quantitation using CE-QqQ

## Experimental

### Sample Preparation

Blank pooled urine was obtained from a pool of six healthy Caucasian non-drug consumers and stored after collection at –20°C. Before analysis, the pooled urine was defrosted at ambient temperature, centrifuged at 10,000 rpm for 5 min and filtered through a 0.45-µm nylon filter (BGB Analytik AG, Böckten, Switzerland). Stock standard solutions of the solid analytes were prepared by dissolving each compound in MeOH to obtain a concentration of 1 mg $mL^{-1}$ and stored at 4°C until use. Blank pooled urine was spiked daily at desired concentrations. Toxicological samples were received from the Laboratory of Clinical Chemistry (Geneva Hospitals, Geneva, Switzerland) and stored at –20°C until use. Before analysis, samples were treated in the same manner in which the blank pooled urine was treated. For cocaine (COC) and methadone (MTD) quantitation, IS were spiked at 50 ng $mL^{-1}$ before dilution and injection. Two independent analyses were performed for each sample (n=2).

### BGE

The BGE consisted of 1 M formic acid at pH 1.8. The pH value was measured with a Seven Multi pH meter (Mettler-Toledo, Schwerzenbach, Switzerland). The BGE was prepared every four days.

### Capillary Electrophoresis

CE experiments were performed with a G7100 CE system from Agilent Technologies (Waldbronn, Germany). Separation was performed using a fused-silica capillary (BGB Analytik AG, Böckten, Switzerland) with a total length of 80 cm and an internal diameter of 50 µm.
For screening experiments, capillaries were coated with a commercial dynamic coating (CEofix™) compatible with MS. The capillary was conditioned daily with MeOH (5 min), water (5 min), 1 M NaOH (5 min), water (5 min), CEofix initiator (0.4 min with ESI source open), CEofix accelerator (0.4 min), and BGE (10 min) at 2 bar. Prior to each sample injection, the coated capillary was rinsed at 2 bar with BGE (3 min). The post-conditioning step was performed with water at 2 bar (2 min) and CEofix accelerator (1 min).
For quantitative experiments, an uncoated bare fused-silica capillary was used. Prior to each sample injection, the capillary was rinsed at 2 bar with BGE (3 min).
For screening and quantitation, a preplug of 7% $NH_4OH$ (*m/v*) was injected at 50 mbar for 10 s before hydrodynamic (HD) sample injection at 100 mbar for 150 s followed by a postplug injection of BGE at 50 mbar for 3 s. Experiments were carried out in positive polarity mode. A constant voltage of 30 kV with an initial ramping of 1667 V $s^{-1}$ (18 s) was applied, and the capillary temperature set at 25°C.

### Mass Spectrometry

For screening experiments, the CE instrument was coupled to a 6210 TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA) via a coaxial sheath flow ESI interface with a standard triple-tube sprayer from Agilent Technologies.
For quantitative experiments, the CE instrument was coupled to a 6490 Triple Quadrupole LC/MS system (Agilent Technologies) via the coaxial sheath flow interface, and an Agilent Jet Stream (AJS) source.

**For details see:** I. Kohler, et al., Highly sensitive capillary electrophoresis-mass spectrometry for rapid screening and accurate quantitation of drugs of abuse in urine, Anal. Chim. Acta 780 (2013) 101-109.

# Results and Discussion

### Fast Screening with CE-ESI-TOF/MS

Numerous drugs of abuse and respective phase I metabolites are weak basic compounds; thus, an acidic BGE was selected to ensure their maximal ionization for proper electrophoretic mobilities, according to their charge-to-size ratio. Volatile acids such as acetic and formic acids, with or without the presence of ammonium counterions, were considered at various concentrations and pH values. TFA was discarded due to potential ion suppression during the ESI process. The best results in terms of efficiency and selectivity for the separation of drugs of abuse were obtained with 1 M formic acid at pH 1.8.

Using fused-silica capillaries with low-pH BGE induces a very low electroosmotic flow (EOF), which results in long analysis times that can be deleterious in clinical or forensic toxicology, where a fast screening method is required. Moreover, the variability in migration times observed in the preliminary experiments with fused-silica capillaries using direct injection of diluted urine was critical for the discrimination and identification of compounds. The use of an anionic capillary coating was therefore selected to speed up the analysis due to the formation of a high and repeatable EOF, as well as to enhance the migration times' repeatability.

For injection, a stacking procedure was implemented to increase the quantity injected and to offset the loss of sensitivity caused by urine dilution. In contrast to other stacking procedures, which can be strongly dependent on the saline composition of the sample, a pH-mediated stacking procedure was applied for urine analysis. Samples were 10-fold diluted with BGE and water (1:1:8, *v/v/v*) prior to injection. This dilution allowed for (i) the normalization of urine pH, (ii) a full ionization of analytes before injection, and (iii) a consequent decrease of the sample conductivity.

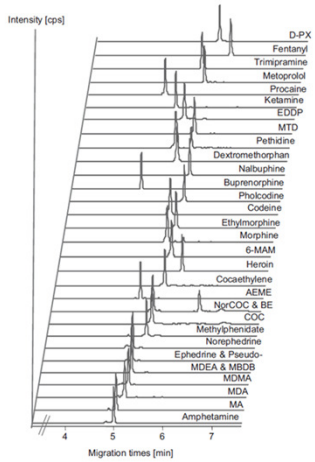


**Fig. 2:** Screening step: extracted ion electropherograms (±0.005 Da) of 33 compounds in urine at 100 ng mL$^{-1}$.

### Accurate Quantitation with CE-ESI-MS/MS

As a second step of the toxicological or forensic procedure, a highly sensitive and accurate confirmatory method is required to confirm or quantify compounds that have been positively identified by the screening method. In this context, CE was hyphenated to a highly sensitive QqQ mass spectrometer equipped with a novel source configuration, composed of a new triple-tube sprayer and a commercial Agilent Jet Stream source. The new triple-tube sprayer has a modified design consisting of a revised needle length and tip shape to place the needle position exactly in the center of the sprayer body.

The signal intensities, expressed by peak heights for MA and MTD, selected as model compounds for set-up comparison, were found to be significantly lower for the new ESI source and were explained by the relatively high value of the sheath gas flow rate which was selected due to instrumental constraints. Nevertheless, the use of isotopic IS correction was selected for quantitative purposes in CE-MS to lower the matrix effects (MEs) and to correct the lack of repeatability during injection. As a result, relative compound areas with deuterated IS correction did not show any significant difference between the two ESI sources for MA and MTD. Therefore, similar performance was observed for the new CE-MS configuration, i.e., new triple-tube sprayer in combination with new ESI source, compared to the conventional set-up.

With the mentioned separation conditions, the repeatability of the migration times was acceptable with RSDs between 3% and 10%, as for the intermediate precision with RSDs from 7% to 8%. The transitions were determined by compound infusion, and the most sensitive and specific transitions were selected for confirmation as well as quantitative purposes, i.e., *m/z* 304.1 → 182.0, *m/z* 307.1 → 185.0, *m/z* 310.2 → 265.1 and *m/z* 313.2 → 268.1 for COC, $d_3$-COC, MTD and $d_3$-MTD, respectively. With these conditions, LODs (expressed as the concentration where signal-to-noise ratio was superior to 3) were estimated at 2 ng mL$^{-1}$ for MTD and COC, which was considered an appropriate value for a simple injection of diluted samples without off-line sample preparation.

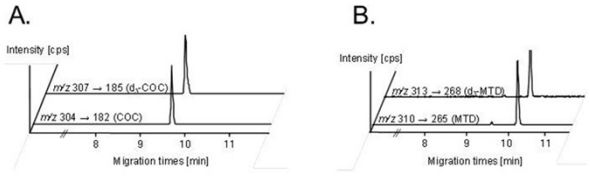


**Fig. 3:** Electropherograms obtained for toxicological samples in quantitative analysis. (A) Sample containing COC, (B) Sample containing MTD.

## Results and Discussion

| Validation criterion | COC | MTD | Validation criterion | COC | MTD |
|---|---|---|---|---|---|
| ***Trueness*** | | | ***Accuracy*** | | |
| Relative bias [%] | | | Lower/upper confidence limits of the total errors [%] | | |
| 10 ng/mL | 12.0 | 38.5 | 10 ng/mL | -1.1/25.1 | -25.5/102.5 |
| 25 ng/mL | 1.0 | 1.7 | 25 ng/mL | -10.5/12.4 | -14.7/18.0 |
| 500 ng/mL | -3.1 | -1.8 | 500 ng/mL | -12.5/6.3 | -10.1/6.5 |
| 1,000 ng/mL | 0.1 | 0.7 | 1,000 ng/mL | -7.4/7.6 | -6.2/7.6 |
| ***Precision*** | | | ***LLOQ [ng/mL]*** | 10 | 21 |
| Repeatability/intermediate precision [RSD, in %] | | | | | |
| 10 ng/mL | 5.7/5.7 | 21.0/27.8 | | | |
| 25 ng/mL | 5.0/5.0 | 7.1/7.1 | | | |
| 500 ng/mL | 4.1/4.1 | 2.8/3.6 | | | |
| 1,000 ng/mL | 3.0/3.3 | 3.0/3.0 | | | |

**Table 1:** Validation results for Cocaine and Methadone by CE-QqQ. LODs as low as 2 ng/mL were reached. LLOQ were determined as 10 and 21 ng/mL respectively.

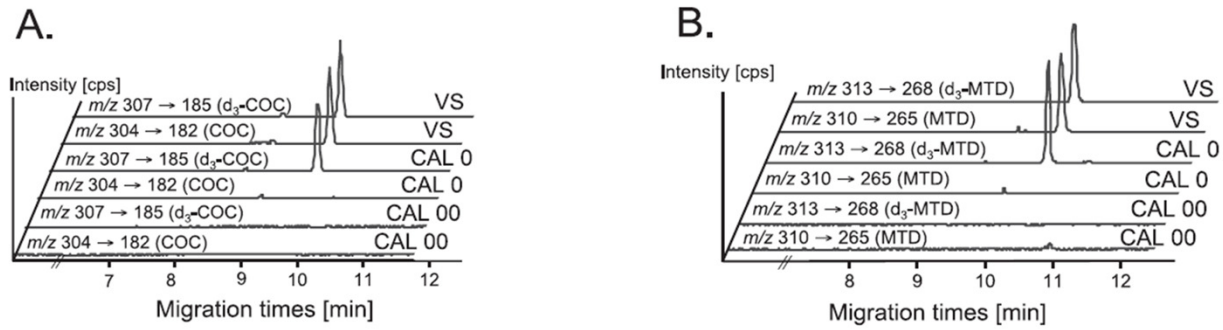


**Fig. 4:** Evaluation of the method selectivity. (A) Electropherograms obtained for COC by injecting blank pool urine (CAL 00), pooled urine spiked with $d_3$-COC at 50 ng $mL^{-1}$ (CAL 0) and pooled urine spiked with COC at 25 ng $mL^{-1}$ and $d_3$-COC at 50 ng $mL^{-1}$ (VS). (B) Electropherograms obtained for MTD by injecting blank pooled urine (CAL 00), pooled urine spiked with d3-MTD at 50 ng $mL^{-1}$ (CAL 0) and pooled urine spiked with MTD at 25 ng $mL^{-1}$ and $d_3$-MTD at 50 ng $mL^{-1}$ (VS).

## Conclusions

A fast and sensitive CE-ESI-MS two-step workflow was developed for the screening of drugs of abuse in urine samples prior to their quantitation. A CE-ESI-TOF/MS method was implemented for the screening step with a pH-mediated stacking procedure, which avoided a tedious off-line sample preparation with a simple urine dilution. The higher loading capacity (more than 20%) led to an increased sensitivity while maintaining strong efficiencies. The CE-ESI-TOF/MS method allowed for LODs as low as 2 ng $mL^{-1}$ for a varied set of common drugs of abuse and pharmaceutical compounds. The screening step was followed by compound quantitation by CE-ESI-MS/MS with a QqQ analyzer equipped with a new ESI source and a new triple-tube sprayer design, which did not show significant differences compared with the conventional ESI source and sprayer. The quantitative procedure was fully validated for COC and MTD according to reference guidelines based on selectivity, response function, trueness, precision, and accuracy. COC analysis was found to be accurate over the range of 10–1000 ng $mL^{-1}$, with accuracy included within the ±30% tolerance limits, and MTD analysis was accurate in the concentration range of 21–1000 ng $mL^{-1}$. The developed two-step strategy was eventually applied to the analysis of real cases and was found to be applicable for a fast and sensitive screening as well as for accurate quantitation in urine samples.