Highly sensitive CE-MS for rapid screening and accurate quantitation of drugs of abuse in urine

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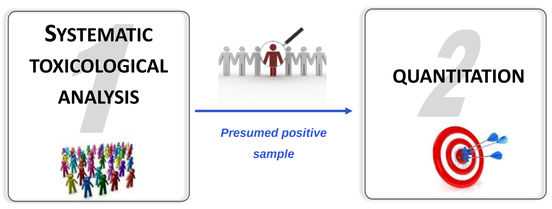
INTRODUCTION

In clinical and forensic toxicology, the determination of xenobiotics in biosamples relies on the use of a two-step methodology, consisting first in a systematic toxicological analysis (STA) to identify the compound(s), followed by a confirmatory quantitative step in case of positive result.



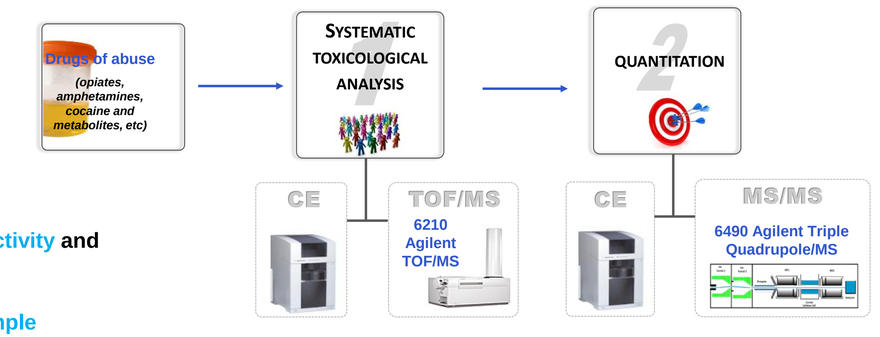
STA usually consists in a immunoassay determination for a rapid sample screening, followed by a chromatographic confirmation (GC-MS, LC-MS).

Quantitation is performed by chromatography-tandem mass spectrometry.

In this study, an alternative strategy to chromatographic methods is presented for the determination of drugs of abuse in urine samples, based on a two-step capillary-electrophoresis-mass spectrometry (CE-MS) workflow.

CE-MS

- High efficiency, selectivity and sensitivity
- Low solvent and sample consumption (nL range injected)

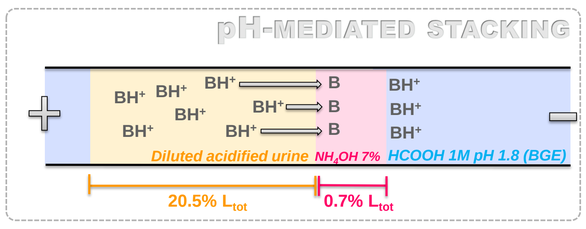


SYSTEMATIC TOXICOLOGICAL ANALYSIS

1 Injection

A simple dilution of the sample was considered to increase the analysis throughput by avoiding any offline sample preparation. Urine was 10-fold diluted with background electrolyte (BGE) and water (1:1:8, *v/v/v*) to (i) decrease the sample conductivity, (ii) ensure a full ionization of the analytes before injection, and (ii) normalize the urine pH.

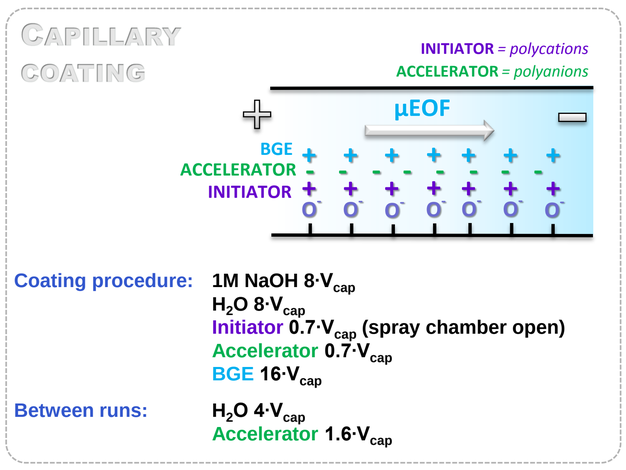
A pH-mediated stacking was implemented with the injection of a small preplug of 7% NH_4OH to increase the loading volume (20.5% of the capillary length, 322 nL) and overcome the loss of sensitivity brought by urine dilution.



2 Capillary coating

Using fused-silica capillaries with low-pH BGE induces a very low electroosmotic flow (EOF), resulting in long analysis time.

To increase the analysis throughput, capillaries were coated with a commercial and MS-compatible anionic bilayer coating (***CEofix™ MS compatible coating kit, Analis, Belgium),*** inducing a high and repeatable EOF.

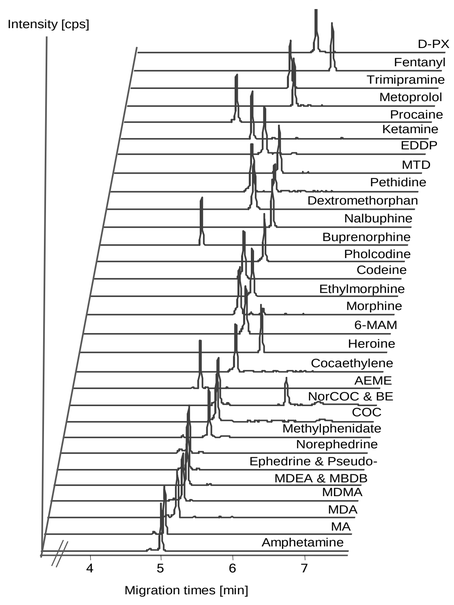


Coating procedure:
1M NaOH 8·V_{cap}
H_2O 8·V_{cap}
Initiator 0.7·V_{cap} (spray chamber open)
Accelerator 0.7·V_{cap}
BGE 16·V_{cap}

Between runs:
H_2O 4·V_{cap}
Accelerator 1.6·V_{cap}

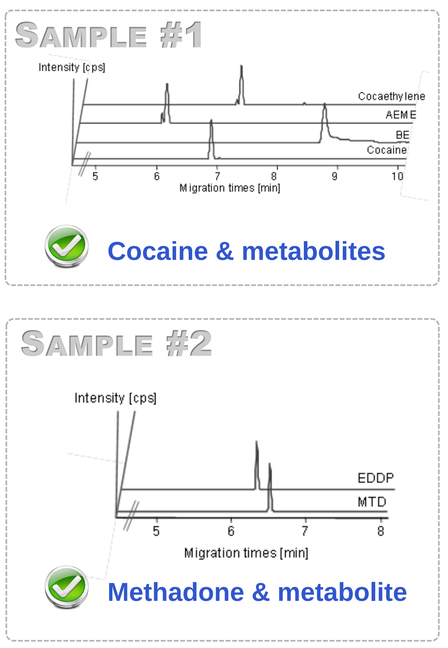
CE-ESI-TOF/MS

Extracted ion electropherograms (±0.005Da) of 33 compounds spiked in urine at 100 ng/mL.



- < 10 min/sample
- LODs as low as 2 ng/mL
- RSDs for migr. times < 1%

3 Real samples

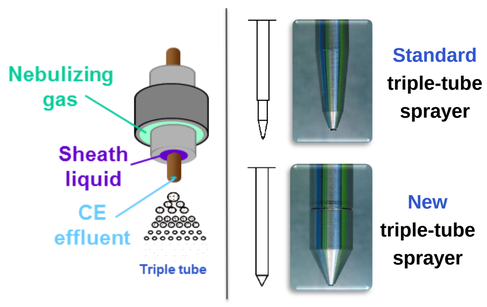


QUANTITATION

For the quantitative step, CE was hyphenated to a highly sensitive Triple Quadrupole MS equipped with a novel source configuration, composed of a new triple-tube sprayer (sheath-flow interface) and a new ESI source. The same BGE and injection conditions as for the screening step were used. The quantitative performance of the CE-ESI-MS/MS method was evaluated with cocaine (COC) and methadone (MTD) selected as model compounds, and their respective deuterated analogues (d_3-COC and d_3-MTD) used as internal standards. The developed CE-ESI-MS/MS method was fully validated according to SFSTP protocols and Guidance of Food an Drug Administration for bioanalytical method validation with evaluation of selectivity, response function, lower limit of quantitation (LLOQ), trueness, precision, and accuracy.

1 Source configuration

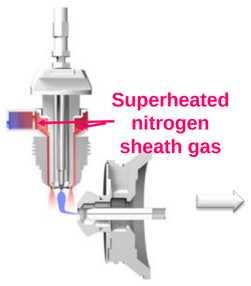
SPRAYER



Standard triple-tube sprayer

New triple-tube sprayer

ESI SOURCE

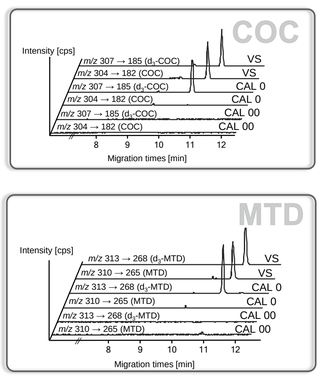


Agilent Jet Stream (AJS) source

Similar performance was observed for the new configuration (new triple-tube sprayer and AJS source) compared to the conventional set-up.

2 Validation

SELECTIVITY



CAL 00

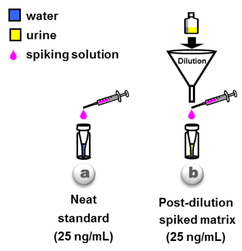
CAL 0 — d_3-COC/ d_3-MTD

VS — d_3-COC/ d_3-MTD COC/MTD (25 ng/mL)

→ Selectivity

MATRIX EFFECT

In order to determine the influence of potential co-migrating interferents on the ionization, matrix effect (ME) was quantified according to the methodology proposed by Matuszewski *et al.*



b/a Matrix effect (ME)

	ME (*n* = 3)
COC	92 ± 9%
MTD	73 ± 5%

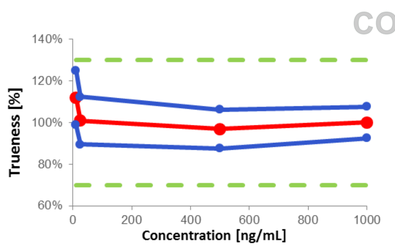
→ Calibration within the matrix

Matuszewski et al., Anal. Chem. 75 (2003) 3019

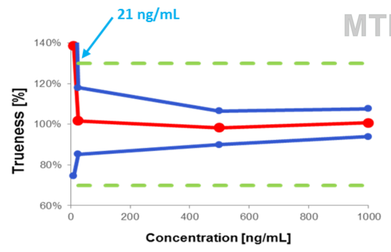
Three independant series (*j* = 3)

Calibration standards (CS) (*k* = 3, *n* = 2) Within the matrix

Validation standards (VS) (*k* = 4, *n* = 4) Within the matrix



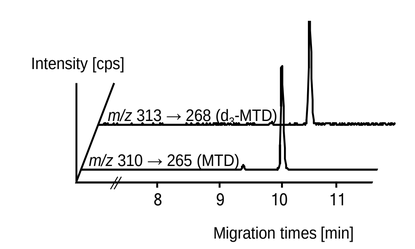
LLOQ: 10 ng/mL
Validated range: 10 – 1'000 ng/mL
acceptance limits ±30%



LLOQ: 21 ng/mL
Validated range: 21 – 1'000 ng/mL
acceptance limits ±30%

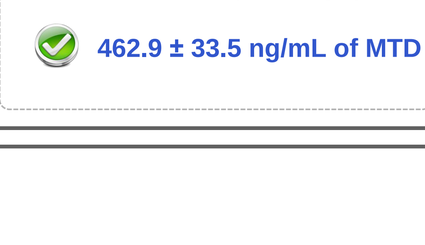
3 Real samples

SAMPLE #1



41.0 ± 6.4 ng/mL of COC

SAMPLE #2



462.9 ± 33.5 ng/mL of MTD

CONCLUSIONS

A fast and sensitive two-step workflow based on CE-MS was developed for the determination of drugs of abuse in urine samples.

For the STA, a CE-ESI-TOF/MS method was implemented with a pH-mediated stacking procedure, avoiding any offline sample preparation and increasing the analysis throughput. Coated capillaries were used to increase this throughput and enhance the migration times repeatability (RSDs < 1%). Less than 10 min were required for each sample, and limits of detection as low as 2 ng/mL were obtained.

Compounds quantitation was performed by CE-ESI-MS/MS with a Triple Quadrupole MS. 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 accurate over the range 10 – 1'000 ng/mL, with accuracy included within the ±30% tolerance limits, while MTD was accurate in the concentration range of 21 – 1'000 ng/mL.

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I. Kohler et al., Anal Chim Acta 780 (2013) 101–109