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Determination of opiates, amphetamines and cocaine in whole blood, plasma and urine by UHPLC-MS/MS using a QuEChERS sample preparation

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Sylvain DULAURENT[1], Mikaël LEVI[2], Jean-michel GAULIER[1], Pierre MARQUET[1,3] and Stéphane MOREAU[2]

[1] CHU Limoges, Department of Pharmacology and Toxicology, Unit of clinical and forensic toxicology, Limoges, France ;

[2] Shimadzu France SAS, Le Luzard 2, Boulevard Salvador Allende, 77448 Marne la Vallée Cedex 2

[3] Univ Limoges, Limoges, France

CHU Centre hospitalier universitaire Limoges

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Introduction

The determination of drugs of abuse (opiates, amphetamines, cocaine) in biological fluids is still an important issue in toxicology, in cases of driving under the influence of drugs (DUID) as well as in forensic toxicology. At the end of the 20th century, the analytical methods able to determine these three groups of narcotics were mainly based on a liquid-liquid-extraction with derivatization followed by GC-MS. Then LC-MS/MS was proposed, coupled with off-line sample preparation. Recently, on-line Solid-Phase-Extraction coupled with UHPLC-MS/MS was described, but in our hands it gave rise to significant carry-over after highly concentrated samples. We propose here another approach based on the QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) sample preparation principle, followed by UHPLC-MS/MS.

Methods and Materials

This method involves 40 compounds of interest (13 opiates, 22 amphetamines, as well as cocaine and 4 of its metabolites) and 18 isotopically labeled internal standards (designed with *) (Table1).

Table 1: list of analyzed compounds with their associate internal standard (*)

Cocaine and metabolites	Amphetamines or related compounds	Opiates
• Anhydroecgonine methylester • Benzoylecgonine* • Cocaethylene* • Cocaine* • Ecgonine methylester*	• 2-CB • 2-CI • 4-MTA • Ritalinic acid • Amphetamine* • BDB • Ephedrine* • MBDB • m-CPP • MDA* • MDEA* • MDMA* • MDPV • Mephedrone • Metamphetamine* • Methcathinone • Methiopropamine • Methylphenidate • Norephedrine • Norfenfluramine • Norpseudoephedrine • Pseudoephedrine	• 6-monoacetylmorphine* • Dextromethorphan • Dihydrocodeine* • Ethylmorphine • Hydrocodone • Hydromorphone • Methylmorphine* • Morphine* • Naloxone* • Naltrexone* • Noroxycodone* • Oxycodone* • Pholcodine

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To 100 µL of sample (urine, whole blood or plasma) were added isotopically labeled internal standards (in order to improve method precision and accuracy) at 20 µg/L in acetonitrile (20 µL), and 200 µL of acetonitrile. After a 15 s shaking, the mixture was placed at -20°C for 10 min. Then approximately 50 mg of QuEChERS salts ($MgSO_4$/NaCl/Sodium citrate dehydrate/Sodium citrate sesquihydrate) were added and the mixture was shaken again for 15 s and centrifuged for 10 min at 12300 g. The upper layer was diluted (1/3; v/v) with a 5 mM ammonium formate buffer (pH 3). Finally, 5 µL were injected in the UHPLC-MS/MS system. The whole acquisition method lasted 5.5 min.

UHPLC conditions (Nexera MP system, figure 1)

Column	: Restek Pinnacle DB PFPP 50x2.1 mm 1.9 µm
Mobile phase A	: 5mM Formate ammonium with 0.1% formic acid in water
B	: 90% CH_3OH/ 10% CH_3CN (v/v) with 0.1 % formic acid
Flow rate	: 0.474 mL/min
Time program	: B conc. 15% (0-0.16 min) - 20% (1.77 min) - 90% (2.20 min) – 100% (4.00 min) – 15% (4.10-5.30 min)
Column temperature	: 50 °C

MS conditions (LCMS-8040, figure 1)

Ionization	: ESI, Positive MRM mode
Ion source temperatures	: Desolvation line: 300°C Heater Block: 500°C
Gases	: Nebulization: 2.5 L/min Drying: 10 L/min
MRM Transitions	: 2 Transitions per compounds were dynamically scanned for 1 min except pholcodine (2 min)
Pause time	: 3 msec
Loop time	: 0.694 sec (minimum 17 points per peak for each MRM transition)

Figure 1: Shimadzu UHPLC-MS/MS Nexera-8040 system

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Results

Chromatographic conditions

The analytical conditions allowed the chromatographic separation of two couples of isomers: norephedrine and norpseudoephedrine; ephedrine and pseudoephedrine (figure 2). A typical chromatogram of the 58 compounds is presented in figure 3.

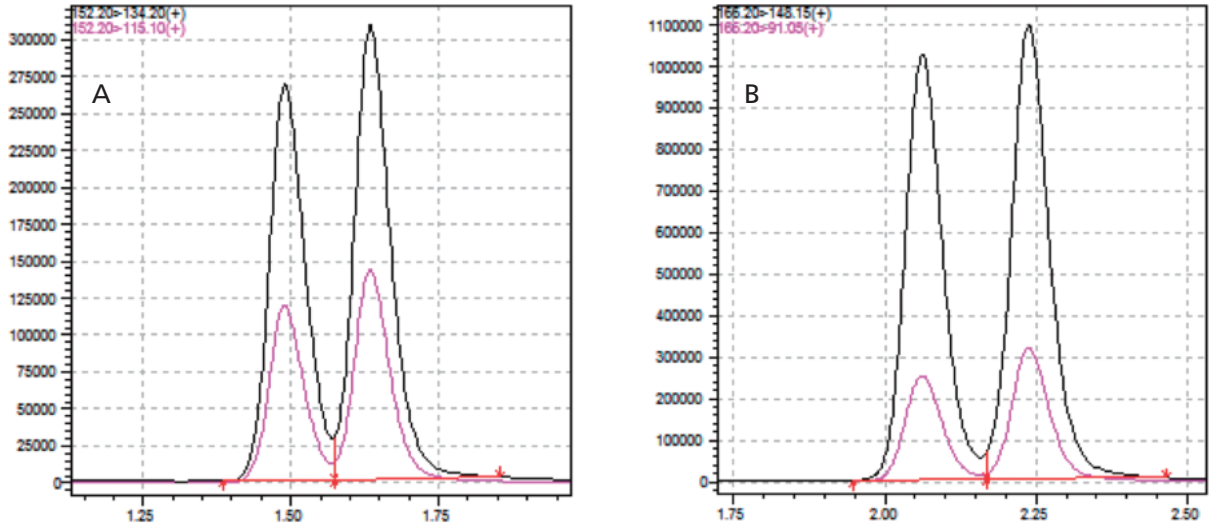

Figure 2: Chromatograms obtained after an injection of a 5 µL whole blood extract spiked at 200 µg/L.
Order of retention - A: norephedrine and norpseudoephedrine / B: ephedrine and pseudoephedrine

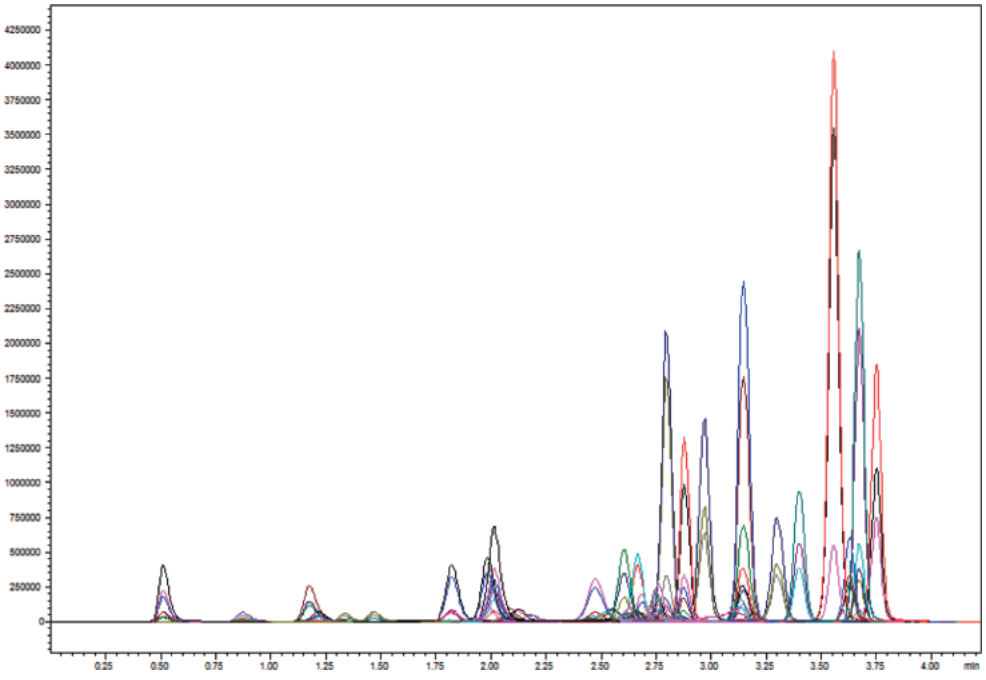

Figure 3: Chromatogram obtained after an injection of a 5 µL whole blood extract spiked at 200 µg/L

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Extraction conditions

As described by Anastassiades et al. J. AOAC Int 86 (2003) 412-31, the combination of acetonitrile and QuEChERS salts allowed the extraction/partitioning of compounds of interest from matrix. This extraction/partitioning process is not only obtained with whole blood and plasma-serum where deproteinization occurred and allowed phase separation, but also with urine as presented in figure 4.

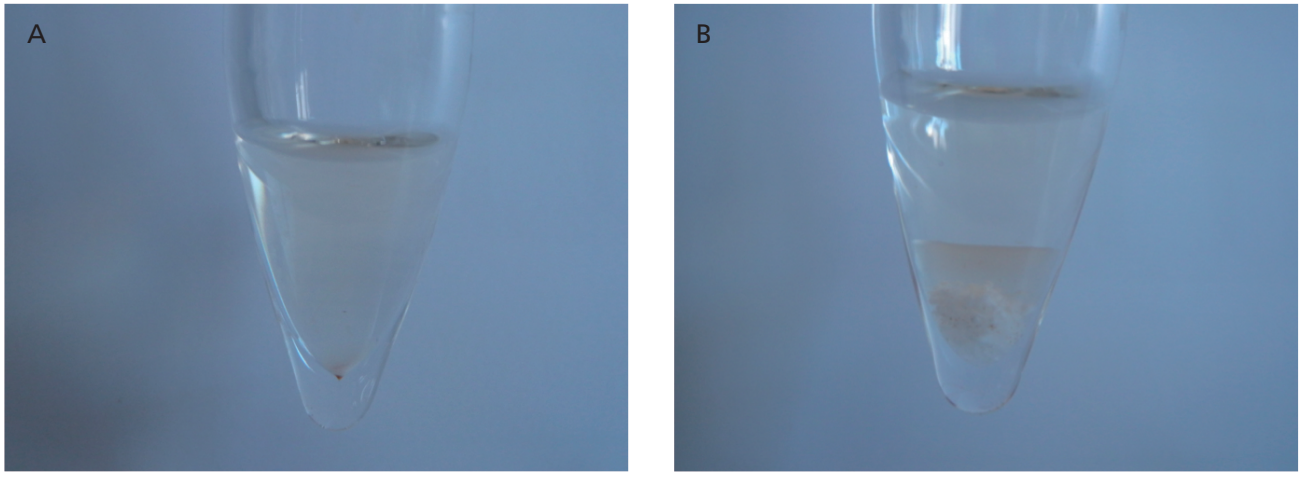

Figure 4: influence of QuEChERS salts on urine extraction/partitioning: A: acetonitrile with urine sample lead to one phase / B: acetonitrile, QuEChERS salts and urine lead to 2 phases.

Validation data

Among the 40 analyzed compounds, 38 filled the validation conditions in term of intra- and inter-assay precision and accuracy were less than 20% at the lower limit of quantification and less than 15% at the other concentrations.
Despite the quick and simple sample preparation, no significant matrix effect was observed and the lower limit of quantification was 5 µg/L for all compounds, while the upper limit of quantification was set at 500 µg/L. The concentrations obtained with a reference (GC-MS) method in positive patient samples were compared with those obtained with this new UHPLC-MS/MS method and showed satisfactory results.
Contrary to what was already observed with on-line Solid-Phase-Extraction, no carry-over effect was noted using the present method, even when blank samples were injected after patient urine samples with analytes concentrations over 2000 µg/L.

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Conclusions

- Separation of two couples of isomers with a run duration less than 6 minutes and using a 5 cm column.
- Quick sample preparation based on QuEChERS salts extraction/partitioning, almost as short as on-line Solid Phase Extraction.
- Lower limit of quantification compatible with determination of DUID.
- No carry over effect noticed.

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