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RESEARCH PAPER

QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood

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Abstract Modern LC-MS/MS instruments have sensitivity and scanning velocity high enough to analyze many different compounds in single runs. Consequently, the sample preparation procedure has become the bottleneck for developing efficient, rapid, and cheap multi-compound methods. Here, we examined one-step sample preparation based on quick, easy, cheap, effective, rugged, and safe (QuEChERS) salts to set up and validate a LC-MS/MS method for the simultaneous determination of 35 drugs of abuse and their metabolites in whole blood. Despite large differences in physicochemical properties, this simplified QuEChERS extraction method yielded satisfactory recoveries (until 96 %) for the 35 molecules. The amounts of QuEChERS salts had no influence on extraction yield. Chromatographic separation was obtained in less than 6 min. LLOD and LLOQ were 3 and 5 ng/mL, respectively. The procedure was successfully validated and then applied to 253 cases of driving under the influence of drugs (DUID), collected over a 6-month period.

Keywords QuEChERS · Drugs of abuse · Mass spectrometry

Introduction

Opiates, amphetamines and analogs, and cocaine are the most widely used drugs of abuse (DOA) second to cannabis. In most countries, the determination of these DOAs (and their metabolites) is required in several contexts: forensics, clinics, as well as driving under the influence of drugs. For toxicology laboratories receiving many such samples to analyze, the easy and rapid detection and determination of these compounds in biological fluids is crucial.

LC-MS/MS methods are widely used for the simultaneous measurement of multiple DOAs in human matrices, due to their sensitivity and specificity [1–7]. Additionally, they overcome the need for time-consuming derivatization of the older GC-MS methods, while sample preparation is generally performed using solid-phase extraction (SPE) that remains time-consuming and expensive (cartridges and solvents). Quicker and simpler sample preparation may help save time and money.

QuEChERS, an acronym for "quick, easy, cheap, effective, rugged, and safe," is a sample preparation procedure that is widely used for the determination of pesticides. This acronym was coined for the first time in 2003, in the conclusion of an article written by Anastassiades et al. dedicated to the determination of pesticide residues in fruits and vegetables [8]. Roughly speaking, QuEChERS consists of adding salts to the analyzed matrix, previously mixed in a polar solvent (acetonitrile, ACN). Originally, "QuEChERS extraction" required two steps: (i) an extraction-partitioning step where the matrix was mixed with ACN before adding anhydrous $MgSO_4$ and NaCl (in order to dry the organic phase and allowing the separation of the two phases) and (ii) a dispersive SPE cleanup where the remaining impurities (contained in fruits and vegetables) are removed by an "adsorbent" such as primary-secondary amines (PSA). Numerous papers reporting the use of this kind of preparation procedure have been published in the field of environmental toxicology [9].

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However, only a few studies have been published so far using this concept for the determination of drugs or toxic compounds in human biofluids [10–13].

The aims of the present study were (i) to develop and validate an LC-MS/MS method based on a QuEChERS extraction, for the quantification of 35 DOAs and metabolites with very different physicochemical characteristics in whole blood, and (ii) to test the robustness of this method in routine laboratory conditions.

Material and methods

Chemicals and reagents

The 35 compounds analyzed are presented in Table 1.

Norfenfluramine was supplied by Sigma-Aldrich (Saint-Quentin Fallavier, France). MDPV was obtained by a police seizure.

1,3-Benzodioxolylbutanamine (BDB), dextromethorphan, norpseudoephedrine, pholcodine, dihydrocodeine D_3, and MDMA D_5 were supplied by Lipomed (Arlesheim, Switzerland).

The following compounds were purchased from Cerilliant (Round Rock, TX, USA): 2-CB, 6-monoacetylmorphine (6-MAM), ritalinic acid, amphetamine, anhydroecgonine methylester, cocaethylene, cocaine, dihydrocodeine, ecgonine methylester, ephedrine, ethylmorphine, hydrocodone, hydromorphone, MDEA, MDMA, methamphetamine, methiopropramine, methylmorphine, methylphenidate, morphine, naloxone, naltrexone, noroxycodone, oxycodone, mephedrone, 6-monoacetylmorphine D_3, amphetamine D_5, benzoylecgonine D_3, ecgonine methylester D_3, ephedrine D_3, MDA D_5, MDEA D_5, methamphetamine D_5, naloxone D_5, naltrexone D_3, noroxycodone D_3, and oxycodone D_3.

Benzoylecgonine, MDA, norephedrine, pseudoephedrine, methcathinone, cocaethylene D_3, cocaine D_3, methylmorphine D_3, and morphine D_3 were supplied by LGC Standards, Molsheim, France. Methanol was purchased from Carlo Erba Reactifs (Val de Reuil, France).

Formic acid, ammonium formate, and acetonitrile were purchased from Biosolve (Dieuze, France).

Pure water was obtained using a Millipore Integral purification system (Saint Quentin en Yvelines, France). Drug-free blood was obtained by *the Etablissement Français du Sang* (EFS, Limoges, France).

Commercial internal quality controls in blood were supplied by ACQ Science (Rottenburg-Hailfingen, Germany). "High" and "low" concentration levels were used.

Ready-to-use QuEChERS salts (4 g $MgSO_4$/1 g NaCl/1 g sodium citrate dihydrate/0.5 sodium citrate sesquihydrate) were supplied by UCT (Bristol, USA).

Samples preparation

Samples were stored at −20 °C until extraction. Twenty microliters of isotopically labelled internal standards (IS) prepared at a concentration of 200 ng/mL in ACN was added to 100 μL of whole blood sampled with an anticoagulant (EDTA). Secondly, 200 μL of ACN stored at −20 °C was added to obtain a final volume of 320 μL.

The mixture was then vortexed for 30 s. After 10 min, 40 mg of QuEChERS salts was added (see paragraph below). The mixture was then shaken again and centrifuged for 10 min at 18,200 g. Fifty microliters of the upper layer (out of an approximate total volume of 200 μL) was directly transferred in an injection vial before being diluted (1/3; *v*/*v*) in a 5 mM ammonium formate/0.1 % formic acid buffer. Finally, 5 μL was injected in the LC-MS-MS system.

Six calibration standards prepared in whole blood (5, 10, 50, 100, 200, and 500 ng/mL) were obtained by adding an appropriate volume of working standard solutions (from 10 to 50 μL in ACN).

In the laboratory, it was observed that the mean quantity of salt added by an experienced operator using a spatula was 40 mg, with values ranging from 10 to 70 mg. The impact of this salt amount was assessed by comparing the extraction recovery obtained for each compound spiked at 200 ng/mL in whole blood, when adding 10, 40, and 70 mg of salts (three replicates). One-way analysis of variance (ANOVA) was performed by considering 40 mg as reference.

LC-MS/MS conditions

The chromatographic system consisted in two Shimadzu LC-30 AD pumps (Nexera *X*2), a CTO 20AC oven, and a SIL-30 AC-MP autosampler (Shimadzu, Marne-la-Vallée, France). Chromatographic separation was performed using a Pinnacle DB PFPP, 1.9 μm (50 × 2.1 mm I.D.) column (Restek, Lisses, France), using a gradient of (A) 5 mM ammonium formate/0.1 % formic acid buffer and (B) ACN as mobile phase (constant flow rate of about 0.47 mL/min), as follows: 0.00–0.16 min, 15 % (B); 0.16–1.77 min, 15 to 20 % (B); 1.77–2.20 min, 20 to 90 % (B); 2.20–4.00 min, 90 to 100 % (B); 4.00–4.10, 100 to 15 % (B); and 4.10–5.30 min, column equilibration with 15 % (B). The oven temperature was set at 50 °C.

A Shimadzu 8050 triple quadrupole mass spectrometer (Marne-la-Vallée, France) was used in the positive electrospray ionization mode. The main common parameter settings were as follows: interface voltage, value registered in the tuning file (obtained after the mass calibration); nebulizing gas flow, 3 L/min; heating gas flow, 10 L/min; interface temperature, 300 °C; desolvatation line (DL) temperature, 250 °C; heat block temperature, 400 °C; and drying gas flow, 10 L/min. Multiple-reaction monitoring (MRM) transitions and specific parameters are presented in Table 1.

Table 1 MRM transitions, optimized parameters, and retention time for the 35 compounds of interest and their 18 internal standards

Compounds	Precursor ion		Product ion						Retention time (min)
			Quantitation			Confirmation			
	m/z	Q1 pre-bias (V)	*m/z*	Collision energy (V)	Q3 pre-bias (V)	*m/z*	Collision energy (V)	Q3 pre-bias (V)	
Acetyl-6-morphine	328.20	−24	165.20	−40	−29	211.10	−25	−22	2.58
Acetyl-6-morphine D_3	331.20	−24	165.15	−43	−17	211.10	−27	−22	2.58
Amphetamine	136.20	−14	91.10	−19	−17	119.05	−15	−12	2.48
Amphetamine D_5	141.20	−15	93.10	−18	−17	124.15	−14	−13	2.48
Anhydroecgonine methylester	182.20	−19	118.05	−24	−20	91.10	−25	−29	1.19
BDB	195.20	−13	178.10	−12	−17	136.15	−17	−23	3.07
Benzoylecgonine	290.20	−20	168.25	−18	−18	77.10	−54	−30	2.55
Benzoylecgonine D_3	293.20	−21	171.10	−19	−30	85.20	−32	−30	2.55
Cocaethylene	318.00	−22	196.30	−22	−21	82.25	−32	−19	3.84
Cocaethylene D_3	321.10	−12	199.25	−21	−22	85.20	−32	−16	3.84
Cocaine	304.00	−15	182.15	−20	−19	82.15	−31	−30	3.62
Cocaine D_3	307.30	−22	185.15	−19	−20	85.25	−31	−15	3.62
Dextromethorphan	272.30	−13	215.15	−25	−23	213.05	−29	−22	4.08
Dihydrocodeine	302.30	−14	199.10	−33	−20	128.10	−55	−13	2.07
Dihydrocodeine D_3	305.30	−15	199.15	−35	−21	128.30	−55	−25	2.07
Ecgonine methylester	200.20	−14	182.20	−16	−20	82.15	−24	−29	0.60
Ecgonine methylester D_3	203.20	−14	185.25	−18	−13	85.20	−26	−30	0.60
Ephedrine	166.20	−17	148.15	−18	−30	91.05	−35	−30	1.83
Ephedrine D_3	169.20	−17	151.25	−14	−16	91.20	−33	−17	1.83
Ethylmorphine	314.30	−15	153.15	−47	−28	165.20	−44	−30	2.82
Hydrocodone	300.20	−21	199.05	−32	−21	153.05	−50	−29	2.71
Hydromorphone	286.20	−20	185.05	−31	−20	157.05	−44	−28	1.18
MDA	180.20	−19	105.15	−22	−11	77.15	−39	−13	2.73
MDA D_5	185.20	−13	168.15	−13	−18	110.15	−22	−11	2.73
MDEA	208.20	−14	163.20	−15	−29	132.95	−21	−24	3.10
MDEA D_5	213.00	−23	163.15	−14	−30	105.20	−18	−18	3.10
MDMA	194.20	−14	163.10	−14	−29	133.15	−19	−24	2.85
MDMA D_5	199.20	−21	165.15	−15	−18	107.15	−25	−11	2.85
MDPV	276.20	−20	126.20	−25	−24	175.10	−22	−28	3.73
Mephedrone	178.00	−12	160.15	−15	−17	145.15	−18	−27	2.94
Methamphetamine	150.20	−30	91.10	−23	−16	119.10	−17	−20	2.79
Methamphetamine D_5	155.20	−30	92.20	−20	−28	121.10	−15	−25	2.79
Methcathinone	164.20	−11	131.25	−15	−26	105.20	−25	−18	2.04
Methiopropamine	155.90	−28	97.05	−23	−18	58.10	−13	−21	2.06
Methylmorphine	300.20	−14	165.05	−42	−17	215.10	−27	−23	2.03
Methylmorphine D_3	303.20	−14	215.25	−26	−20	181.20	−37	−17	2.03
Methylphenidate	234.20	−11	84.10	−23	−30	56.15	−50	−22	3.50
Morphine	286.20	−29	152.10	−55	−27	165.20	−45	−18	0.88
Morphine D_3	289.20	−14	165.15	−42	−27	152.05	−55	−28	0.88
Naloxone	328.20	−23	310.10	−20	−22	212.10	−42	−22	2.10
Naloxone D_5	333.20	−12	315.20	−20	−22	258.10	−29	−27	2.10
Naltrexone	342.20	−25	324.10	−22	−23	55.05	−39	−20	2.65
Naltrexone D_3	345.30	−16	327.15	−22	−23	270.15	−28	−29	2.65
Norephedrine	152.20	−29	134.20	−15	−27	115.10	−25	−21	1.32
Norfenfluramine	204.20	−22	159.15	−20	−30	108.95	−38	−19	3.80

Table 1 (continued)

Compounds	Precursor ion		Product ion						Retention time (min)
			Quantitation			Confirmation			
	m/z	Q1 pre-bias (V)	*m/z*	Collision energy (V)	Q3 pre-bias (V)	*m/z*	Collision energy (V)	Q3 pre-bias (V)	
Noroxycodone	302.20	−22	284.05	−16	−20	187.05	−24	−13	2.47
Noroxycodone D_3	305.20	−22	287.15	−17	−20	190.10	−25	−20	2.47
Norpseudoephedrine	Same as norephedrine								1.46
Oxycodone	316.20	−23	298.05	−20	−21	256.10	−27	−28	2.62
Oxycodone D_3	319.20	−23	301.10	−19	−21	259.10	−26	−27	2.62
Pholcodine	399.10	−30	114.05	−36	−21	70.20	−53	−12	2.49
Pseudoephedrine	Same as ephedrine								1.99
Ritalinic acid	220.20	−23	84.20	−22	−30	56.10	−48	−22	2.58

Validation procedure for whole blood

The laboratory is working towards accreditation by the International Standards Organization (ISO) 15189 standard (accreditation number: 8-2607). According to this accreditation, the validation protocol and the set of acceptance criteria were as follows:

- Precision and accuracy: The intra-assay precision (coefficient of variation, CV%) and accuracy (bias) had to be assessed at 5, 50, and 500 ng/mL for all compounds, after extraction and analysis of six different spiked whole-blood samples (compound-free human whole blood) for each level. A similar experiment had to be performed to assess the inter-assay precision and accuracy, except that a set of calibrating samples had to be analyzed each day for 6 days, in place of six different spiked whole-blood samples. Acceptance criteria were intra-assay and inter-assay precision (CV%) and an accuracy less than 20 %.
- Linearity: Calibration graphs of the compounds of interest-to-internal standard peak area ratios of the quantification transition versus expected concentration had to be constructed using quadratic regression with $1/X$ weighting. A value greater than 0.99 was expected for the coefficient of determination (r^2).
- Lower limits of quantification and lower limits of detection: The lower limit of quantification (LLOQ) was defined as the lowest concentration of compound that could be measured with both an intra-assay and inter-assay precision (CV%) and an accuracy less than 20 %. The lower limit of detection was calculated based on a signal-to-noise ratio >3.

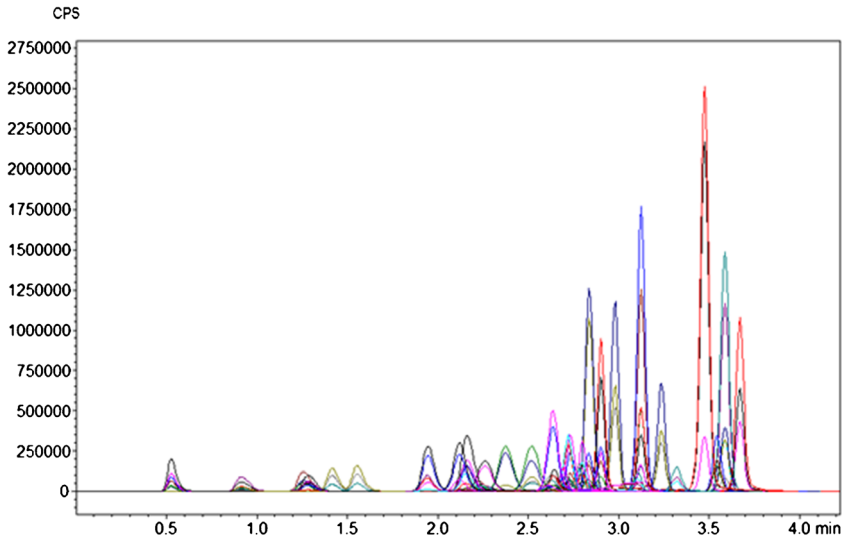

Fig. 1 Chromatogram of whole blood spiked at 100 ng/mL for each of the 35 compounds of interest and the 18 internal standards

Table 2 Main parameters of the validation protocol

Compound	Associated IS	Recovery (%) (%RSD)		Intra-assay precision (%) (%RSD)			Inter-assay precision (%) (%RSD)		
		5 ng/mL ($n=6$)	500 ng/mL ($n=6$)	5 ng/mL ($n=6$)	50 ng/mL ($n=6$)	500 ng/mL ($n=6$)	5 ng/mL ($n=6$)	50 ng/mL ($n=6$)	500 ng/mL ($n=6$)
Acetyl-6-morphine	Acetyl-6-morphine D_3	89.4 (10.7)	83.0 (3.5)	18.1 (12.2)	7.84 (15.93)	−7.7 (5.6)	0.1 (16.1)	−9.2 (15.2)	7.6 (15.1)
Amphetamine	Amphetamine D_5	66.1 (5.5)	72.2 (1.7)	4.3 (13.0)	3.30 (13.35)	−0.4 (5.2)	1.8 (11.8)	−2.7 (13.0)	−0.8 (10,5)
Anhydroecgonine methylester	Benzoylecgonine D_3	69.4 (6.3)	70.9 (4.4)	−2.2 (8.1)	8.76 (7.62)	−1.5 (5.8)	−2.4 (14.3)	−7.7 (12.8)	−1.4 (11.3)
BDB	MDMA D_5	85.1 (8.9)	73.1 (7.1)	12.1 (6.8)	−4.00 (16.95)	−8.6 (12.4)	2.7 (12.1)	−0.6 (16.7)	−1.1 (13.7)
Benzoylecgonine	Benzoylecgonine D_3	64.9 (7.3)	67.9 (2.4)	4.1 (9.9)	2.08 (12.00)	−2.4 (4.7)	−0.8 (9.0)	−5.6 (7.7)	0.9 (11.1)
Cocaethylene	Cocaethylene D_3	77.6 (3.2)	89.4 (2.3)	4.5 (3.1)	−1.88 (3.97)	0.4 (6.7)	−0.66 (10.6)	−4.8 (8.3)	0.0 (11.6)
Cocaine	Cocaine D_3	75.5 (4.1)	88.5 (1.3)	7.1 (4.2)	0.96 (3.92)	3.1 (10.4)	−0.2 (15.9)	−5.6 (9.8)	0.4 (10.4)
Dextromethorphan	Morphine D_3	83.3 (9.0)	88.0 (7.6)	−4.7 (9.2)	−8.94 (11.77)	−7.6 (8.5)	3.0 (19.3)	−1.7 (14.3)	0.5 (18.0)
Dihydrocodeine	Dihydrocodeine D_3	60.0 (7.8)	68.3 (5.6)	4.9 (18.8)	8.76 (7.87)	1.2 (8.2)	−0.6 (15.4)	0.2 (11.9)	−0.3 (9.9)
Ecgonine methylester	Ecgonine methylester D_3	53.1 (4.2)	55.7 (1.9)	−1.9 (13.2)	1.68 (14.38)	−2.4 (5.8)	−1.1 (8.0)	−4.7 (12.7)	−0.4 (6.9)
Ephedrine	Ephedrine D_3	63.9 (3.9)	70.2 (4.6)	1.4 (5.7)	4.34 (7.68)	0.0 (5.1)	−2.8 (9.1)	−5.3 (6.2)	−2.1 (9.5)
Ethylmorphine	Methylmorphine D_3	82.3 (15.9)	79.8 (9.3)	−17.8 (18.4)	9.10 (11.60)	−6.9 (13.7)	−2.4 (16.5)	−5.7 (5.2)	−6.0 (14.7)
Hydrocodone	Noroxycodone D_3	77.4 (14.9)	79.8 (10.8)	3.1 (18.9)	−17.14 (7.77)	−3.6 (11.7)	−0.4 (15.1)	2.9 (11.8)	−7.4 (7.2)
Hydromorphone	Morphine D_3	106 (5.6)	80.1 (6.3)	7.7 (10.7)	−11.90 (15.78)	−6.2 (4.6)	−0.4 (16.4)	−3.4 (11.0)	−3.4 (11.4)
MDA	MDA D_5	79.4 (14.1)	70.4 (5.68)	1.4 (13.5)	10.92 (13.00)	−0.1 (6.7)	−4.5 (8.7)	−9.0 (10.3)	−1.4 (12.9)
MDEA	MDEA D_5	71.3 (6.1)	80.1 (2.3)	−4.8 (4.7)	−0.90 (2.42)	−7.5 (4.4)	−1.1 (9.9)	−4.1 (11.1)	−1.5 (10.3)
MDMA	MDMA D_5	78.4 (14.4)	79.4 (5.3)	15.3 (4.6)	10.94 (2.95)	12.5 (12.1)	6.9 (11.5)	−2.2 (15.1)	−0.3 (18.1)
MDPV	Metamphetamine D_5	72.9 (11.7)	85.8 (6.0)	6.4 (4.4)	−3.92 (8.71)	−2.4 (8.4)	5.7 (10.3)	−5.7 (8.9)	1.7 (9.8)
Mephedrone	Metamphetamine D_5	72.5 (14.9)	80.7 (10.6)	9.7 (5.3)	19.40 (9.43)	−12.4 (9.3)	12.2 (6.4)	−5.7 (14.5)	−1.0 (17.4)
Methamphetamine	Metamphetamine D_5	61.9 (14.4)	74.8 (1.4)	10.4 (9.6)	−15.78 (11.42)	−4.5 (17.5)	0.3 (13.2)	−6.0 (11.7)	−3.0 (16.8)
Methcathinone	Metamphetamine D_5	63.9 (10.1)	75.3 (8.1)	8.9 (10.2)	−4.48 (10.74)	−6.5 (10.1)	4.7 (8.3)	−9.8 (8.9)	3.2 (9.5)
Methiopropamine	Amphetamine D_5	74.8 (6.8)	72.4 (2.1)	13.9 (8.8)	9.86 (7.26)	3.2 (7.0)	−2.8 (11.0)	−1.6 (13.1)	3.6 (13.0)
Methylmorphine	Methylmorphine D_3	59.3 (7.8)	76.8 (2.7)	−4.9 (17.3)	11.06 (9.82)	−7.7 (10.4)	5.1 (16.6)	0.9 (9.9)	−5.1 (14.2)
Methylphenidate	Metamphetamine D_5	67.9 (10.7)	82.1 (7.4)	3.3 (4.6)	−12.32 (8.72)	−1.8 (9.1)	9.7 (6.5)	−8.6 (14.5)	1.7 (11.8)
Morphine	Morphine D_3	81.2 (5.4)	62.6 (1.9)	9.8 (10.2)	−4.00 (12.54)	−4.8 (3.0)	−5.7 (13.1)	−4.8 (6.4)	0.5 (8.3)
Naloxone	Naloxone D_5	96.0 (3.8)	89.5 (2.2)	12.8 (9.3)	10.98 (15.27)	−1.6 (6.4)	5.8 (6.5)	−9.7 (10.9)	−1.7 (11.2)
Naltrexone	Naltrexone D_3	92.0 (6.9)	85.5 (5.6)	19.3 (8.3)	−10.36 (15.58)	−3.0 (11.1)	−1.0 (15.1)	−4.0 (9.4)	−2.5 (11.2)
Norephedrine	Ephedrine D_3	54.4 (10.4)	52.3 (1.9)	−3.9 (11,9)	−0.42 (7.97)	−5.8 (5.9)	0.5 (15.3)	−5.7 (7.9)	−1.9 (11.3)
Norfenfluramine	Metamphetamine D_5	64.4 (16.8)	79.1 (5.1)	19.6 (5.1)	−4.30 (8.77)	3.7 (12.6)	17.0 (5.7)	4.1 (14.6)	5.2 (16.6)
Noroxycodone	Noroxycodone D_3	53.1 (14.7)	67.3 (5.4)	18.3 (7.2)	−15.32 (11.12)	−8.3 (9.7)	6.6 (10.7)	−4.4 (11.2)	−6.3 (9.3)
Norpseudoephedrine	Ephedrine D_3	60.0 (4.2)	64.4 (5.0)	2.7 (5.6)	−1.34 (10.87)	−3.7 (8.4)	1.6 (14.6)	−2.8 (10.8)	−3.8 (7.0)
Oxycodone	Oxycodone D_3	62.5 (10.1)	74.2 (3.2)	10.9 (8.1)	8.82 (6.01)	−4.0 (6.0)	−3.4 (9.3)	−2.6 (11.9)	−0.7 (9.7)
Pholcodine	Morphine D_3	83.6 (6.9)	56.3 (10.2)	0.7 (6.4)	7.52 (18.05)	−5.5 (12.0)	5.1 (16.0)	1.3 (18.9)	−2.2 (17.9)
Pseudoephedrine	Ephedrine D_3	66.5 (6.2)	69.7 (1.4)	−2.8 (3.9)	−10.94 (7.89)	−2.8 (6.8)	1.8 (10.7)	−1.3 (11.9)	−1.6 (6.4)
Ritalinic acid	Metamphetamine D_5	34.5 (19.3)	40.3 (13.9)	18.2 (7.3)	−10.20 (13.50)	−11.2 (6.5)	3.1 (19.3)	−0.2 (15.4).	−8.4 (19.4)

IS internal standard, *RSD* relative standard deviation

- Recovery: Extraction recovery had to be determined at the LLOQ and at the upper LOQ (ULOQ), 5 and 500 ng/mL. The analyte/internal standard peak area ratios obtained after extraction ($n=6$) had to be compared to those of whole blood (DOA-free) spiked after extraction. CV% in the extraction recovery had to be less than 20 %.
- Stability: The potential degradation of DOAs was explored by analyzing, twice a week during 1 month, the commercial quality controls stored at −20 °C.

Matrix effects and endogenous interferences for whole blood

The matrix effect had to be evaluated for the 35 molecules using 6 different whole-blood and 6 purified water samples. In each case, the QuEChERS extraction had to be performed before spiking the extract with all compounds at 200 ng/mL. Potential ion suppression or enhancement was explored by comparing the signal of each molecule of interest observed in the matrix and in purified water.

Six other whole-blood samples were analyzed to explore the presence of endogenous interferences.

Application to real patient samples

Finally, the whole analytical procedure was tested in 253 cases of driving under the influence of drugs collected over a 6-month period, where the 38 compounds were screened in whole blood.

Results

Following the developed extraction procedure, the preparation of a classical batch that includes 6 calibration standards, 2 internal quality controls, and 10 patient samples required less than 45 min for an experienced operator. When testing salt quantities from 10 to 70 mg for a single sample, no significant modifications in the extraction recovery (maximal difference, 11 %) were observed for the 35 compounds.

The chromatographic separation of the 35 compounds was obtained in 6 min, with retention time from 0.6 min for ecgonine methylester to 4.1 min for dextromethorphan. Figure 1 represents a chromatogram obtained for a whole-blood sample spiked at 100 ng/mL.

As presented in Table 2, acceptance criteria were obtained for both the intra-assay and the inter-assay precision and accuracy. The CV% values in the extraction recovery were also less than 20 % for the 35 compounds. Using quadratic regression with a $1/X$ weighting, the coefficients of determination of the calibration curves between 5 and 500 ng/mL were higher than 0.99 for all compounds. According to these results, the LLOQ was considered to be 5 ng/mL for all the compounds. A common LLOD of 3 ng/mL was obtained.

Concerning the matrix effects, we reported that the signals of each molecule of interest observed in the matrix and those obtained in purified water were not significantly different: Differences ranged from −3.5 to +19.9 % in the signal intensity. We did not observe any impact of potential endogenous interferences.

When stored at −20 °C, the quality controls showed no degradation for the DOAs they contain.

Among the 253 whole-blood samples extracted from the routine activity of the lab, 74 were positive. Opiates were detected in 48 samples, cocaine and its metabolites in 14 samples, and amphetamines or analogs in 19 samples. An example is presented in Fig. 2. In this case, cocaethylene, ecgonine methyl ester, benzoylecgonine, morphine, MDA, and MDMA were detected at concentrations of 6, 40, 380, 16, 31, and 458 ng/mL, respectively.

Discussion

Multiple sample preparation procedures have already been published for the determination of cocaine and metabolites, amphetamines and analogs, and opiates in biological samples: liquid-liquid extraction [1, 2], off-line solid-phase extraction

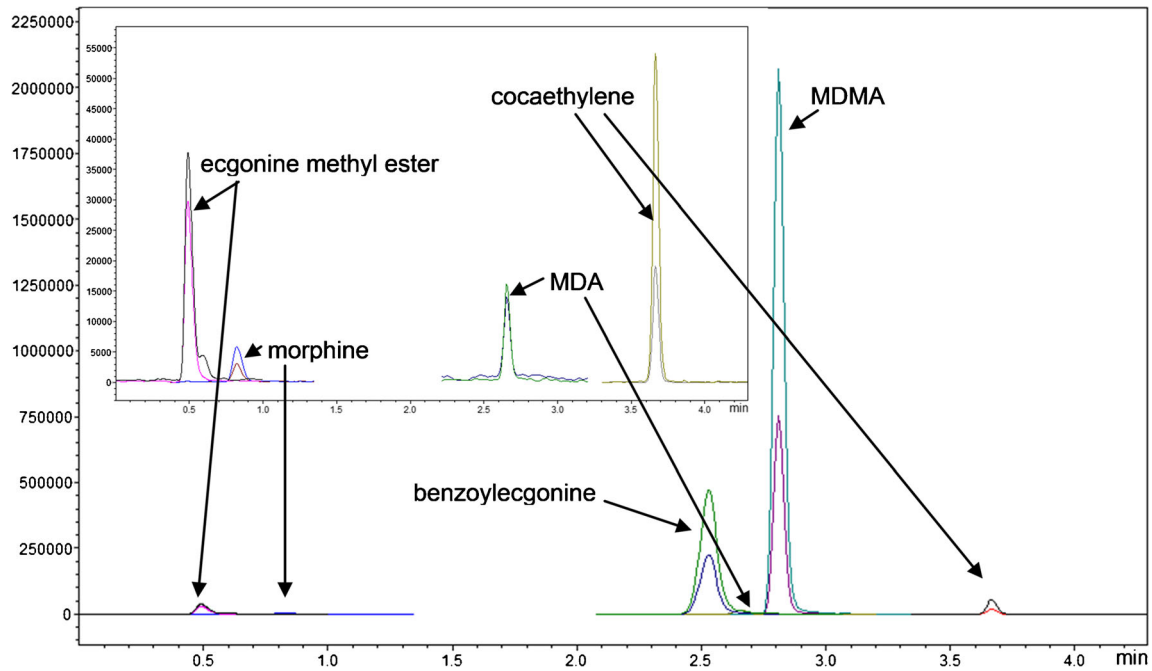

Fig. 2 Chromatogram of a whole-blood sample collected in a case of driving under the influence of drugs. Compounds and concentrations were morphine, 16 ng/mL; benzoylecgonine, 380 ng/mL; ecgonine methyl ester, 40 ng/mL; cocaethylene, 6 ng/mL; MDA, 31 ng/mL; and MDMA, 458 ng/mL

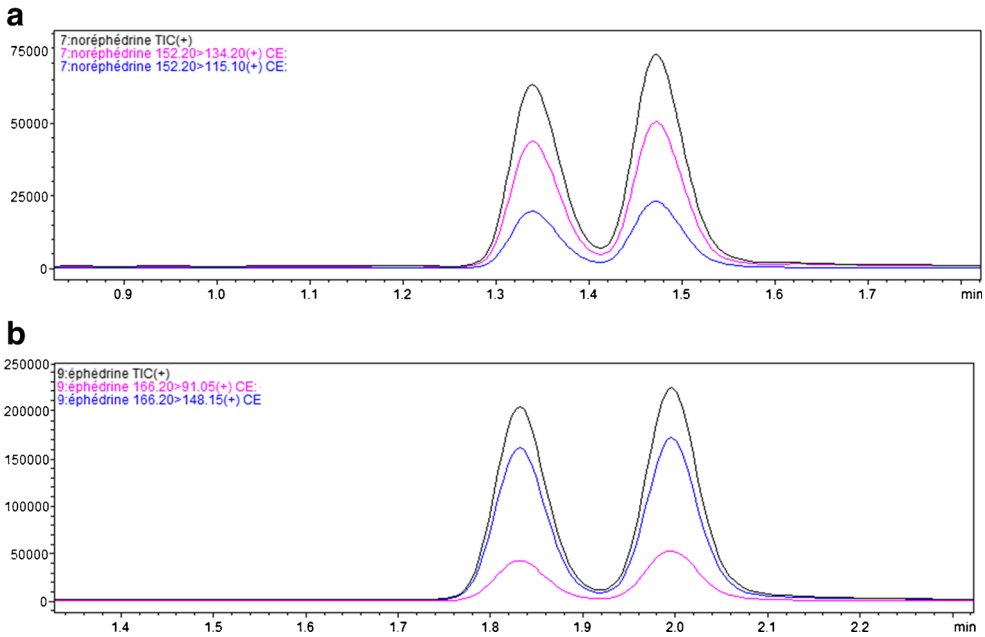

Fig. 3 Chromatogram of two couples of isomers in whole blood spiked at 100 ng/mL. **A** norephedrine (*left*) and norpseudoephedrine (*right*). **B** Ephedrine (*left*) and pseudoephedrine (*right*)

[2, 3], and on-line solid-phase extraction or 2D chromatography [4, 5]. Over the years, all three approaches have been applied in our lab. On the one hand, liquid-liquid and solid-phase extractions provide good sensitivity and clean extracts but were time-consuming as they require multiple steps (agitation, loading, elution, centrifugation, and evaporation). On the other hand, 2D chromatography can offer shorter manual sample preparation and can be automated, but in the used conditions, it was not easy to set up an on-line solid-phase extraction for the simultaneous determination of the most polar (ecgonine methylester) and of the most non-polar (cocaine) compounds.

In 2006, Plössl et al. [10] proposed a method derived from the QuEChERS approach developed for the pesticide residues analysis [8], and applied it to 40 drugs. More recently, Usui et al. have reported QuEChERS applications to determine drugs in whole blood [11]. Briefly, their method needed both an extraction and a dispersive-solid-phase extraction step, which is more time-consuming than the present procedure. Matsuda et al. have proposed a double extraction of 100 μL of whole blood with modified QuEChERS salts followed by evaporation of the organic phase to dryness, for the determination of a limited number of analytes (amphetamine and methamphetamine, and 11 drugs) [12]. The authors reported good results, but their procedure included a time-consuming concentration step. Anzilloti et al. have used liquid-liquid extraction followed by evaporation to dryness for the determination of 21 DOAs and benzodiazepines in blood, using a tenfold higher whole-blood volume than that proposed in the present study [6]. They only used QuEChERS for the cleanup step with primary-secondary amine dispersive sorbent and achieved an LLOQ of 2 ng/mL.

In the present study, the pentafluorophenyl columns provided good separation of all the DOAs (Figs. 1 and 3), probably due to the π-π interactions with the combination of methanol and the pentafluorophenyl phase. In the aqueous mobile phase, adding acetonitrile improved peak shape, while adding formic acid shortened the retention time of all compounds. In preliminary steps, several chromatographic columns were with C18. Even with particles less than 2 μm in diameter, a complete separation of the 35 compounds was not achieved, in particular for ephedrine, pseudoephedrine, and their metabolites (norephedrine and norpseudoephedrine).

According to recommendations of the French Society of Analytical Toxicology (SFTA) [13], a LLOQ of 5 ng/mL was determined for the 35 drugs or metabolites. Zhang et al. proposed a LLOQ value of 0.025 ng/mL, together with an upper limit of quantification (ULOQ) of 0.5 ng/mL, for the determination of 12 illicit drugs [14]. From these authors, such an ULOQ renders necessary the dilution of most of positive forensic cases (2- to 1000-fold). In the present study, we have proposed a ULOQ of 500 ng/mL. Among the 253 whole-blood samples analyzed, none needed to be diluted.

Conclusion

We developed and validated a one-step extraction procedure that eliminates any concentration step, followed by LC-MS/MS, for the determination of 35 drugs of abuse or their metabolites. This method has been successfully applied to more than 250 suspected cases of driving under the influence of drugs. Such a procedure may be suitable for many other

compounds and confirms that QuEChERS are actually quick, easy, cheap, effective, rugged, and safe.

Compliance with ethical standards All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

For this type of study, formal consent is not required.

Conflict of interest The authors declare that they have no competing interests.

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