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Systematic assessment of different solvents for the extraction of drugs of abuse and pharmaceuticals from an authentic hair pool

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ABSTRACT

Hair analysis has been established as a prevalent tool for retrospective drug monitoring. In this study, different extraction solvents for the determination of drugs of abuse and pharmaceuticals in hair were evaluated for their efficiency. A pool of authentic hair from drug users was used for extraction experiments. Hair was pulverized and extracted in triplicate with seven different solvents in a one- or two-step extraction. Three one- (methanol, acetonitrile, and acetonitrile/water) and four two-step extractions (methanol two-fold, methanol and methanol/acetonitrile/formate buffer, methanol and methanol/formate buffer, and methanol and methanol/hydrochloric acid) were tested under accurately equal experimental conditions. The extracts were directly analyzed by liquid chromatography–tandem mass spectrometry for opiates/opioids, stimulants, ketamine, selected benzodiazepines, antidepressants, antipsychotics, and antihistamines using deuterated internal standards. For most analytes, a two-step extraction with methanol did not significantly improve the yield compared to a one-step extraction with methanol. Extraction with acetonitrile alone was least efficient for most analytes. Extraction yields of acetonitrile/water, methanol and methanol/acetonitrile/formate buffer, and methanol and methanol/formate buffer were significantly higher compared to methanol. Highest efficiencies were obtained by a two-step extraction with methanol and methanol/hydrochloric acid, particularly for morphine, 6-monoacetylmorphine, codeine, 6-acetylcodeine, MDMA, zopiclone, zolpidem, amitriptyline, nortriptyline, citalopram, and doxylamine. For some analytes (e.g., tramadol, fluoxetine, sertraline), all extraction solvents, except for acetonitrile, were comparably efficient. There was no significant correlation between extraction efficiency with an acidic solvent and the pka or log P of the analyte. However, there was a significant trend for the extraction efficiency with acetonitrile to the log P of the analyte. The study demonstrates that the choice of extraction solvent has a strong impact on hair analysis outcomes. Therefore, validation protocols should include the evaluation of extraction efficiency of drugs by using authentic rather than spiked hair. Different extraction procedures may contribute to the scatter of quantitative results in inter-laboratory comparisons. Harmonization of extraction protocols is recommended, when interpretation is based on same cut-off levels.

1. Introduction

Analysis of hair for drugs of abuse and pharmaceuticals is a well-established technique in clinical and forensic toxicology. Accuracy and precision of the analytical method are tested by validation, however, usually using spiked hair samples instead of authentic hair. To assess quality of the method, guidelines recommend the use of authentic hair samples for evaluation of extraction efficiency. Moreover, laboratories should participate in proficiency tests in which each laboratory analyzes the same sample by their own hair testing protocol [1,2]. These inter-laboratory comparisons usually show a considerable variance of quantitative results [3–7].

The commonly established workflow for hair sample preparation consists of segmentation, decontamination, cutting/pulverization, extraction, optional clean-up, and analysis [8,9]. Analytical parameters potentially affecting extraction are particle size, extraction time, energy (temperature/shaking/ultrasonication), type of solvent, solvent volume, and frequency of extraction steps. Reducing particle size by pulverization of hair has been shown to significantly increase the extraction yield of ethyl glucuronide [10–13] and different drugs of abuse [14]. In a recent publication,

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Mueller et al. revealed extraction solvent and temperature as most significant factors for the determination of ethyl glucuronide in hair [15].

Commonly used solvents for drug extraction from hair are methanol, basic methanol, acidic methanol, acidic or basic aqueous or buffer solutions, aqueous natrium hydroxide, and solvent mixtures [8,9]. Extraction by enzymatic digestion or with urea or thioglycolate are rarely used [8,9]. Considering the current trend towards multi-analyte procedures, the choice of the extraction solvent is a critical step.

The aim of this study was to evaluate different solvents, such as methanol, acetonitrile, acetonitrile/water, acidic aqueous solvent mixtures, and acidic methanol, for their efficiency to extract various drugs of abuse and pharmaceuticals from an authentic hair pool.

2. Materials and methods

2.1. Reference substances, chemicals, solvents

All analytical standards used for calibration were purchased from Sigma–Aldrich (Buchs, Switzerland) or Lipomed AG (Arlesheim, Switzerland). Ammonium formate (analytical grade), formic acid (99% for analysis), and methanol were purchased from Sigma–Aldrich (Buchs, Switzerland). Acetonitrile was obtained from Chemie Brunschwig AG (Basel, Switzerland). Water used for the preparation of the mobile phase was processed by a PURELAB Option-Q system by ELGA LabWater (Labtec Services AG, Villmergen, Switzerland).

2.2. Authentic hair pool

Excess hair samples from the routine from known drug users were collected and pooled. Drug concentrations of hair samples were considered to result in final concentrations in the low to medium range of the calibration curve (Table 1). The pooled hair samples were washed consecutively by water, acetone, and hexane by shaking for two minutes in each solvent [16]. Hair was cut into snippets and homogenized by our in-house multi-step sieving procedure which was optimized following the first report by Moench et al. [12]. For the approval of the pool, analysis was performed on three days in duplicates by our routine lab procedure.

2.3. Evaluation of extraction solvents

Extraction solvents were chosen from the literature or method development at our lab and tested in a one- (E1) or two-step (E2) extraction in triplicate, respectively. The following solvents were tested as one-step procedures (E1_1, E1_2): methanol (control 1) [17–20], acetonitrile (E1_1) [21,27], and a mixture of acetonitrile/water (1:1, *v/v*, E1_2). In the two-step procedures (E2_1; E2_2; E2_3), the first extraction was performed with methanol, and the following solvents were tested in the second step: methanol (control 2), methanol/acetonitrile/5 mM formate buffer pH 3.5 (1/1/2, *v/v*, E2_1) [22], methanol/1 mM ammonium formate buffer pH 3.5 (1/1, *v/v*, E2_2) [23], and methanol containing 1.4% hydrochloric acid (*v/v*, E2_3) [16]. Moreover, hydrolysis of 6-monoacetylmorphine to morphine was investigated during acidic extraction conditions with E2_3.

Table 1
Drug concentrations in the authentic hair pool, concentrations of internal standards, and concentration ranges of the calibration curve of the routine lab procedure.

Analyte	Concentration (pg/mg) ± relative standard deviation (%) determined by routine procedure (n = 6)	Internal standard	Concentration of internal standard (pg/mg)	Concentration range of calibration curve (pg/mg)
Morphine	2330 ± 8.4	Morphine-D3	1000	50–50000
6-Monoacetylmorphine	3870 ± 20	6-Monoacetylmorphine-D3	1000	10–10000
Codeine	400 ± 8.4	Codeine-D3	1000	10–10000
6-Acetylcodeine	620 ± 14	6-Monoacetylmorphine-D3	1000	50–50000
Hydromorphone	45 ± 28	Oxycodone-D3	1000	10–10000
Hydrocodone	13 ± 28	Oxycodone-D3	1000	10–10000
Tramadol	130 ± 37	13C-Tramadol-D3	1000	10–10000
Methadone	3300 ± 7.9	Methadone-D9	1000	100–100000
EDDP	200 ± 14	EDDP-D3	1000	50–50000
Cocaine	12700 ± 9.6	Cocaine-D3	1000	100–100000
Benzoylecgonine	8620 ± 14	Benzoylecgonine-D3	1000	10–10000
Norcocaine	100 ± 23	Cocaine-D3	1000	10–10000
Cocaethylene	450 ± 12	Cocaethylene-D3	1000	10–10000
MDMA	6880 ± 9.9	MDMA-D3	1000	10–10000
MDA	410 ± 15	MDA-D5	1000	10–10000
Amphetamine	5930 ± 15	Amphetamine-D6	1000	50–50000
Ketamine	93 ± 7.9	Ketamine-D4	1000	10–10000
Methylphenidate	678 ± 16	Methylphenidate-D9	1000	10–10000
Zopiclone	15 ± 26	Zopiclone-D4	1000	10–10000
Zolpidem	1033 ± 8.5	Zolpidem-D6	200	10–10000
Diazepam	135 ± 17	Diazepam-D5	1000	10–10000
Nordazepam	49 ± 22	Diazepam-D5	1000	10–10000
7-Aminoclonazepam	84 ± 12	7-Aminoclonazepam-D4	200	10–10000
Lorazepam	11 ± 24	Lorazepam-D4	200	10–10000
Amitriptyline	82 ± 4.9	Trimipramine-D3	1000	1–1000
Nortriptyline	86 ± 21	Clomipramine-D3	1000	10–10000
Bupropion	458 ± 5.6	Ketamine-D4	1000	1–1000
Citalopram	240 ± 11	Citalopram–D6	1000	10–10000
Fluoxetine	196 ± 23	Fluoxetine–D6	1000	50–50000
Mirtazapine	58 ± 14	Quetiapine-D8	1000	10–10000
Sertraline	158 ± 20	Venlafaxine-D6	1000	1–1000
Trazodone	16 ± 21	Trazodone-D6	1000	10–10000
Quetiapine	166 ± 28	Quetiapine-D8	1000	50–50000
Diphenhydramine	4800 ± 8.5	Diphenhydramine-D3	1000	1–1000
Doxylamine	1055 ± 12	Quetiapine-D8	1000	10–10000