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Screening for new psychoactive substances in hair by ultrahigh performance liquid chromatography–electrospray ionization tandem mass spectrometry☆

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ABSTRACT

In the latest years, many new psychoactive substances (NPS) from several drug classes have appeared in the illicit drug market. Their rapid, sensitive and specific identification in biological fluids is hence of great concern for clinical and forensic toxicologists. Here is described a multi-analyte method for the determination of NPS, pertaining to different chemical classes (synthetic cannabinoids, synthetic cathinones, ketamine, piperazines and amphetamine-type substances—ATS) in human hair using ultrahigh performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) in electrospray ionization mode. We focused on a sample preparation able to extract the different classes of NPS. About 30 mg of hair was decontaminated and incubated overnight under sonication in different conditions depending on the type of analytes to be extracted: (a) with 300 μL of HCOOH 0.1% for cathinones, piperazines and ATS; (b) with 300 μL of MeOH for synthetic cannabinoids. Ten microliter of the extracts were then injected in UHPLC–ESI–MS/MS in MRM mode. The LODs varied from 2 pg/mg to 20 pg/mg. The method was linear in the range from the LOQ to 500 pg/mg and showed acceptable precision (%RSD < 15) and accuracy (%E < 15) for all the analytes. The method was finally applied on 50 samples from real forensic cases (driving license re-granting, postmortem toxicological analyses, workplace drug testing). In three samples we detected synthetic cannabinoids, in four samples cathinones or ephedrines, in two samples ketamine.

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1. Introduction

In the last decade many NPS with different chemical structures have appeared in the illicit drug market. These substances belong to different drug classes, including synthetic cannabinoids, synthetic cathinones, ketamines, phenethylamines, piperazines, substances not pertaining to any of these groups and plant-based materials. The easy distribution of NPS through the e-commerce and in the smart shops favored their rapid spreading worldwide. According to the 2013 World Drug Report, the number of NPS reported by member states to the United Nations Office on Drugs and Crime (UNODC) rose from 166 at the end of 2009 to 252 by mid-2012 [1], an increase of more than 50 per cent. For the first time, the number of NPS exceeded the total number of substances under international control.

Their identification in biological fluids/tissues is hence of great concern for forensic and clinical toxicologists, in order to evaluate the spread of NPS among population, and to diagnose intoxications and impairment due to the use of these substances. Analytical methods were developed for the identification of NPS in biological fluids, such as oral fluid [2–4], blood, plasma or serum [5–9], urine [10–12]. Head or body hair is a useful alternative biological matrix, allowing the determination of drugs that accumulate in keratinized tissues. Moreover hair samples permit a retrospective evaluation of the drug use history corresponding to several months before the actual sampling moment, depending essentially on hair length; this makes hair analysis a valuable tool to evaluate the spread of chronic use of drugs in a specific population. Other advantages linked to hair analysis are the easy, not invasive sample collection and the difficult sample adulteration. Furthermore, in hair (and in keratinized matrices in general) parent, un-metabolized drugs accumulate prevalently, in comparison with the corresponding

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metabolites [13]. Conversely, studies performed both *in vivo* and *in vitro* on some of the synthetic cannabinoids showed that they are extensively metabolized, and in many cases no parent compound is detectable in urine [14–18]. Thus, whereas the determination of synthetic cannabinoids in urine and blood will therefore be faced with the issue of identifying several metabolites often not well known, hair analysis can be focussed directly on the parent drug. Nevertheless, to date, only few studies deal with the determination of NPS in hair [19–24]. Two studies reported in the recent literature for the determination of synthetic cannabinoids used extraction methods based on incubation in concentrated sodium hydroxide solutions, providing the dissolution of the keratin matrix [19,20]. However, these procedures require a further extraction of the analytes from the aqueous solution, which increases the complexity of sample handling. Rust et al. described a two-step extraction of hair, the first one with absolute ethanol and the second with acidified ethanol for the screening of cathinones and piperazines [22]; the extracts were then evaporated to dryness and re-dissolved in mobile phase. They reported that the double extraction was necessary in order to extract all the compounds with adequate recovery. In another study, the analysis of cahinone, cathine and norephedrine was performed by incubation in an acidic aqueous solution and subsequent solid-phase extraction of the incubation mixture and derivatization prior to GC/MS analysis [25].

The present work was aimed at the development of a simple, high-throughput UHPLC–MS/MS screening method in MRM mode for the determination of NPS of different classes in hair matrix. The described method can be useful not only in the forensic investigation of NPS-related addiction histories, but also in epidemiological studies on the spread of NPS among specific safety-sensitive social groups, such as drivers and workers.

2. Materials and methods

2.1. Chemicals and reagents

1-[(5-Fluoropentyl)-1*H*-indol-3-yl]-(naphthalen-1-yl)methanone (AM22011), (2-Iodophenyl)(1-{[(2*S*)-1-methyl-2-piperidinyl]-methyl}-1*H*-indol-3-yl)methanone (AM2233), [1-(5-fluoropentyl)-1*H*-indol-3-yl](2-iodophenyl)-methanone (AM694), 1–naphthalenyl[4-(pentyloxy)-1-naphthalenyl]-methanone (CB13), (2-methyl-1-pentyl-1*H*-indol-3-yl)-1-naphthalenyl-methanone (JWH-007), (2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenyl-methanone (JWH-015), (1-butyl-2-methyl-1*H*-indol-3-yl)(1-naphthyl)methanone (JWH-016), (1-pentyl-1*H*-indol-3-yl)-1-naphthalenyl-methanone (JWH-018), (1-hexyl-1*H*-indol-3-yl)-1-naphthalenyl-methanone (JWH-019), 1-naphthalenyl(1-pentyl-1*H*-pyrrol-3-yl) methanone (JWH-030), (1-butyl-1*H*-indol-3-yl)-1-naphthalenyl-methanone (JWH-073), (4-methoxy-1-naphthalenyl)(1-pentyl-1*H*-indol-3-yl)methanone (JWH-081), (4-methoxy-1-naphthalenyl)(2-methyl-1-pentyl-1*H*-indol-3-yl)methanone (JWH-098), (4-methyl-1-naphthalenyl)(1-pentyl-1*H*-indol-3-yl)methanone (JWH-122), (1-hexyl-5-phenyl-1*H*-pyrrol-3-yl)(1-naphthalenyl)methanone (JWH-147), {1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl}(1-naphthalenyl)methanone (JWH-200), 2-(4-methoxyphenyl)-1-(1-phentyl-1*H*-indol-3-yl)-ethanone (JWH-201), 1-(1-pentyl-1*H*-indol-3-yl)-2-(2-methoxyphenyl)-ethanone (JWH-250), 2-(2-methylphenyl)-1-(1-pentyl-1*H*-indol-3yl)-ethanone (JWH-251), 2-(3-methoxyphenyl)-1-(1pentyl-1*H*-indol-3-yl)-ethanone (JWH-302), (5-(2-fluorophenyl)-1-pentylpyrrol-3-yl)-naphthalen-1-yl-methanone (JWH-307), (4-chloronaphthalen-1yl)(1-pentyl-1*H*-indole-3-yl)-methanone (JWH-398), (4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)-methanone (RCS4), 1-(1-(2-cyclohexylethyl)-1*H*-indol-3-yl)-2-(2-methoxyphenyl)-ethanone (RCS8), (4-methoxyphenyl)[(2-methyl)-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]-methanone (WIN48,098), 1-(3-chlorophenyl)piperazine (1mCPP), 1-(3,4-dimethylphenyl)-2-(methylamino)propan-1-one (3,4-dimethylmethcathinone or 3,4-DMMC), 1-(4-fluorophenyl)propan-2-amine (4-fluoroamphetamine or 4FA), 1-(4-methylphenyl)propan-2-amine (4-methyl amphetamine or 4MA), 2-(ethylamino)-1-(4-methylphenyl)propan-1-one (4methylethcathinone or 4MEC), benzylpiperazine (BZP), 2-(methylamino)-1-phenylbutan-1-one (buphedrone), 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one (butylone), (1S,2S)-2-amino-1-phenyl-propan-1-ol (cathine), (2S)-2-amino-1-phenyl-1-propanone (cathinone), (1R,2S)-2-(methylamino)-1-phenylpropan-1-ol (ephedrine), 2-(ethylamino)-1-phenylpropan-1-one (ethylcathinone), 1-(1,3-benzodioxol-5-yl)-2-(ethylamino)propan-1-one (ethylone), 1-(4-fluorophenyl)-2-(methylamino)propan-1-one (flephedrone), 2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one (ketamine), 1-(1,3-benzodioxol-5-yl)-*N*-methyl-2-butanamine (methylbenzodioxolylbutanamine or MBDB), 6,7-dihydro-5*H*-cyclopenta[f][1,3]benzodioxol-6-amine (5,6-methylenedioxy-2-aminoindane or MDAI), 1-(1,3-benzodioxol-5-yl)-2-pyrrolidin-1-yl-pentan-1-one (methylenedioxypyrovalerone or MDPV), (RS)-1-(4-methylphenyl)-2-methylaminopropan-1-one (mephedrone), 1-(4-methoxyphenyl)-2-(methylamino)propan-1-one (methedrone), 1-(1,3-benzodioxol-5-yl)-2-(methylamino)-propan-1-one (methylone), 1-[4-(methylthio)phenyl]propan-2-amine (4-methylthioamphetamine or MTA), 1-naphthalen-2-yl-2-pyrrolidin-1-yl-pentan-1-one (naphyrone), 2-(methylamino)-1-phenylpentan-1-one (pentedrone), 1-(1,3-benzodioxol-5-yl)-2-(methylamino)pentan-1-one (pentylone), (1S,2S)-2-(methylamino)-1-phenylpropan-1-ol (pseudoephedrine), 1-phenyl-2-propanamine D5 (amphetamine D5), ketamine D4, methylone D4, tetrahydrocannabinol-D3 (THC D3), JWH 210-D9 were supplied from LGC standards (Milan, Italy).

Water, acetonitrile, formic acid, acetone and methanol were purchased from 3V-Chemicals (Rome, Italy); ammonium formate was from Agilent (Agilent Technologies, Santa Clara, CA, USA). Tween 80, sodium hydroxide, hexane and ethyl acetate were from Sigma (Milan, Italy). All reagents and solvents were of LC/MS grade.

Standard compounds were stored according to supplier recommendations until their use.

2.2. Sample preparation

Two aliquots of 30 mg of hair were washed with 3 mL × 3 of a solution of TWEEN 80 × 0.1% for 3 min each, rinsed three times with 5 mL of distilled water and finally twice with 1 mL of acetone. After drying, each sample was cut with scissors into small pieces of 1 mm.

Two different extractions of analytes from keratin matrix were tested: incubation under sonication overnight at 45 °C with (A) 300 μL of methanol; (B) 300 μL of HCOOH 0.1%. The optimized procedure was as follows.

For the extraction of synthetic cannabinoids, one aliquot of hair samples was added with 10 μL of internal standard JWH 210-D9 (1 μg/mL), 300 μL of methanol and incubated under sonication overnight at 45 °C.

For the extraction of cathinones, ketamine, piperazines, stimulants and ATS, 30 mg of hair samples were added with 10 μL of a mixture of internal standards amphetamine D5, ketamine D4 and methylone D4 (1 μg/mL), 300 μL HCOOH 0.1% and incubated under sonication overnight at 45 °C.

2.3. Preparation of calibration curves

Individual methanolic stock solutions containing 1 mg/mL of each of the listed standards were used to prepare two working mixtures of standards at 1 μg/mL:

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