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SUPRAS extraction approach for matrix-independent determination of amphetamine-type stimulants by LC-MS/MS

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ABSTRACT

Monitoring of amphetamine-type stimulant (ATS) confronts clinical labs with a high number of samples involving a variety of biological matrices. Liquid chromatography-tandem mass spectrometry (LC-MS/MS), routinely used for confirmation of ATS abuse, requires of laborious and matrix-dependent sample treatment methods, this increasing analysis time and cost. In this work, a universal and single-step sample treatment, based on supramolecular solvents (SUPRAS), was proposed for simplifying ATS confirmation in seven biological matrices. The SUPRAS was synthesized in situ in the sample (900 μL of basified oral fluid, urine, serum, sweat or breast milk or 50 mg of digested hair or fingernails) by the addition of hexanol (200 μL) and tetrahydrofuran (900 μL). The mixture was vortex-shaken and centrifuged and the SUPRAS extract was subsequently analyzed by positive ion mode electrospray LC-MS/MS. The method was fully validated for amphetamine (AMP), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), N-ethyl-3,4-methylenedioxyamphetamine (MDEA) and N-methyl-3,4-methylenedioxyamphetamine (MDMA). Maximum ion suppression or enhancement was 9% and 7%, respectively, and extraction recoveries (87–111%) and within- (0.1–6.7%) and between-day (0.3–9.7%) CVs were all within required values. The lower limits of quantification (LLOQ) for biological fluids (5 ng/mL), and hair and fingernails (100 ng/g) were all well below the cut-offs established by worldwide organizations. Confirmation of MDA was carried out in five urine samples that tested positive for ATS by immunoassay. The SUPRAS-LC-MS/MS methodology succeeded in developing a hitherto unexplored and universal tool for quantifying ATS in a comprehensive pool of biological matrices of interest in forensic and clinical samples.

1. Introduction

The need for new methodologies that allow the determination of amphetamine and their derivatives (MA, MDA, MDEA and MDMA) in biological matrices is supported by the alarming data provided by different worldwide reports [1]. The driving force causing expanding abuse of amphetamine-type stimulants (ATS) is their pharmacological activity on the central nervous system, linked with increasing of energy, endurance and sociability [2]. Around 2.1 million young adults used MDMA – "ecstasy"- worldwide during 2015 while for AMP and MA the numbers were around 1.3 million [3]. World Drug Report 2016 shows a rising trend in consumption being ATS the second most consumed illicit drugs [4].

ATS, similarly to other illicit drugs, are determined in a variety of biological matrices with very different purposes, including workplace testing, Driving Under the Influence of Drugs (DUID) programs, drug consumer follow-up, gestational or newborn exposure, post-mortem toxicology, drug facilitated sexual assaults, and so on [5]. Matrix selection depends on the purpose of the analysis as well as on the advantages and limitations that each matrix brings out [6,7]. Thus, it is important to consider the required detection time window, which may range from hours to a few days for biological fluids and from months to years for hair and fingernails [8]. This broader detection window has permitted the use of hair as an attractive matrix to assess gestational exposure [9]. On the other hand, sample collection convenience is essential for on-site drug testing. Sampling of oral fluid is preferred for workplace, antidoping testing and DUID programs because is non-invasive and less subject to adulteration than urine and, on the contrary than blood, it does not require specialized staff [10–12]. Correlation between drug concentration and pharmacodynamic effects is required for judicial settings and, in this sense, oral fluid and blood show better correlation with impairment performance that urine [13]. Breast milk is interesting for assessing newborn exposure [14] while sweat patches provide a qualitative record of drug consumption over the period of

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observation [15]. In short, clinical labs are routinely confronted with the analysis of a huge number of samples involving many different biological matrices.

ATS screening is mainly based on class specific immunoassays but the lack of specific drug identification and cross-reactivity with unrelated medications demands for drug confirmation and quantitation by a more selective analytical technique [16]. Traditionally, gas chromatography-mass spectrometry (GC-MS) has been used for ATS determination; however, the need for ATS derivatization has fostered the use of LC-MS/MS [7]. A critical point with this technique is its susceptibility to matrix effects, which often compromises sensitivity and selectivity and consequently the accuracy of its application [17,18]. As a result, sample treatment, which is matrix-dependent, often involves extensive, time-consuming, non-green, and unspecific procedures and consequently, mostly of the reported methods have been only validated for single biological matrices [19–26]. Therefore, the development of a unique, simple, and fast sample treatment, integrating both ATS extraction and cleaning-up of matrix interferences, and applicable to the major types of biological matrices of interest for the control of ATS abuse by LC-MS/MS, would be of interest for clinical and toxicological labs. In this work, we try to succeed this aim with the use of supramolecular solvents (SUPRAS).

SUPRAS are nanostructured liquids generated from colloidal solutions of amphiphiles by spontaneous processes of self-assembly and coacervation [27]. They are highly ordered systems showing well-differentiated regions. An outstanding feature is that their structure, composition and properties can be tailored at will by selecting the environmental conditions for amphiphile aggregation. In this way, water-induced SUPRAS with restricted-access properties (SUPRAS-RAM) have been synthesized from colloidal solutions of alkanols in tetrahydrofuran [28,29] giving solvents made up of inverted hexagonal aggregates, with the hydrophilic alcohol heads surrounding aqueous cavities and the lipophilic chains dissolved in tetrahydrofuran (Fig. 1). These different polarity regions imply that SUPRASs can interact in several ways with low-molecular weight solutes, whilst polysaccharides and proteins are excluded by size and precipitation, respectively [28]. Furthermore, the size of the aqueous cavity can be tuned controlling the initial conditions and, because of the non-covalent nature of their internal bindings, the tailor-made synthesis is completely reversible [28,29]. SUPRAS-RAM have been used in food and environmental analysis because of their high capacity to clean up complex matrices, rich in interferences, and to extract the target compounds with optimum recoveries [30–32]. These properties have been successfully proved for a wide range of chemicals, from very low (e.g. vitamin E) to high (e.g. hydrazine) polarity, demonstrating in this way that SUPRAS composition and nature can be tailored to match the target analytes [27]. In view of all these facts, it is the aim of this work to develop and validate a universal sample treatment platform, based on SUPRAS-RAM, which may allow the determination of ATS by LC-MS/MS in human biological matrices of toxicological and forensic interest.

2. Material and methods

2.1. Chemicals and reagents

ATS standards (AMP, MA, MDA, MDEA and MDMA), the internal standard (IS) methamphetamine-D14 (MA-D14), methanol, acetonitrile, ammonia (25%), sodium hydroxide and formic acid were supplied by Sigma-Aldrich. The reference materials (RM) Medidrug® DOA-I S low and Medidrug® WDT Confirm U − 25% were obtained from LGC Ltd. The first RM is a lyophilized serum that contains 28 drugs, including the target ATS at 25 µg/L, while the second RM is a lyophilized urine that contains 55 drugs including ATS (150 µg/L each) at concentrations that are − 25% of the recommended cut-off by the European Workplace Drug Testing Society (EWDTS). The RM DHF 2/12-A HA was purchased from ACQ Science. This RM is a powdered hair that contains 16 illegal drugs including ATS at the following concentrations: AMP (1170 ng/g), MA (797 ng/g), MDA (428 ng/g), MDEA (589 ng/g) and MDMA (1740 ng/g). Tetrahydrofuran was obtained from Panreac and 1-hexanol from Merck. All solvents were LC-MS grade. Type I water was

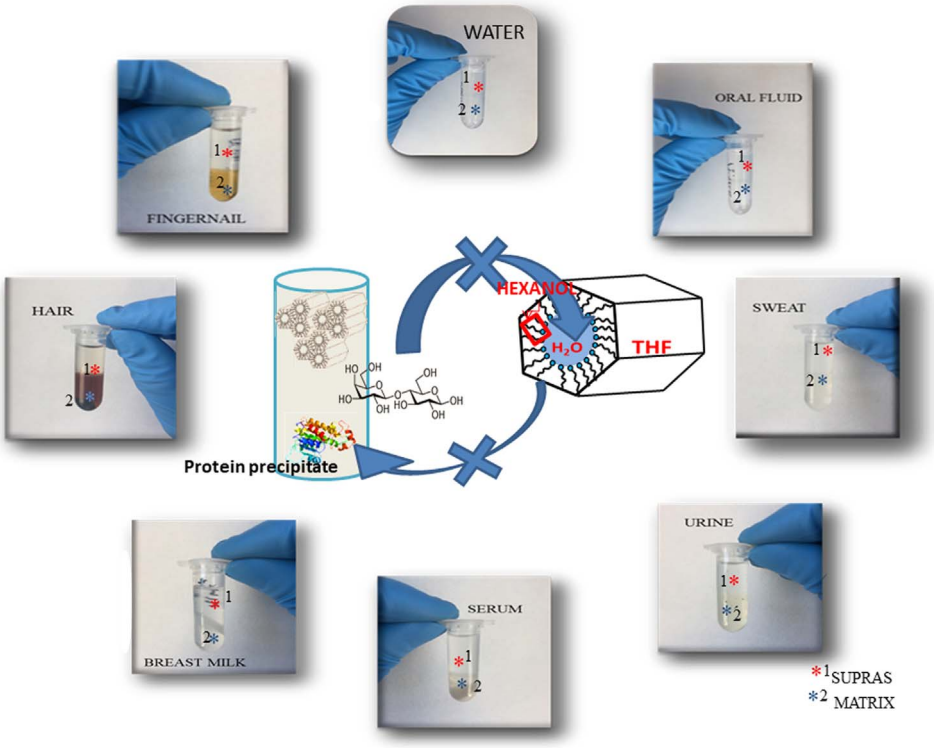

Fig. 1. Photographs corresponding to the extraction of ATS from a fortified aqueous solution and seven biological matrices with a hexanol-based SUPRAS-RAM and schematic showing the structure of the SUPRAS and the mechanisms for proteins and carbohydrates removal.