

Development an Efficient and Sensitive Magnetic Dispersive Solid Phase Microextraction Technique for Preconcentration of Amphetamine and Methamphetamine Determined by High-Performance Liquid Chromatography and Liquid Chromatography-Tandem Mass Spectrometry in Dietary Supplements

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Abstract

Background: The sport and weight management supplements are prime target for adulteration. Amphetamines are widely known to cause appetite suppression and encourage weight loss. Amphetamines also abused by athletes to improve their performance. Objective: A rapid and simple magnetic dispersive solid phase extraction method was proposed in order to isolate amphetamine (AM) and methamphetamine (MET) from complex dietary supplements. Method: A high-performance liquid chromatography-ultraviolet (HPLC-UV) method with precision and accuracy better than 10% was proposed to detect AM and MET in the range of 300-1500 ng/ml and 500-2000 ng/ml, respectively. Results: Confirmation study of AM and MET was accomplished by ion-spray LC-MS/MS using multiple reaction monitoring (MRM) mode. The LC-MS/MS method was validated in the range of 5-100 ng/ml and 1-200 ng/ml for AM and MET, respectively. Conclusion: Sixteen dietary supplements comprising both weight management and sport supplements were tested with the developed methods. AM and MET were detected in four samples.

Keywords: Magnetic dispersive solid phase extraction, Amphetamines, Dietary supplement, Adulteration, Mass spectroscopy

Introduction

In recent years, dietary supplements have become more popular all over the world because in public opinion they are safer and healthier than synthetic drugs. As athletes and consumers are interested to over-the-counter products to help them lose weight, build muscle, and having endless amounts of energy, companies try to meet the required demands (1). Adulteration of dietary supplements with synthetic drugs for enhancing efficiency of these products include a wide range of adulterants such as appetite suppressors, energy consuming/enhancing drugs, stimulants, antidepressants, anxiolytics, diuretics, and laxatives (2). Consumers might be subject to the risks of drug–drug interactions with other medications, potential overdosage from simultaneously using similar prescribed drugs, or experiencing un-attributable side-effects that pose a threat to their health. Adulterated products not only endanger subjects' long term health, however also motivate illegal acts. Therefore, it is of prime to propose a rapid screening and quantification method in order to discover the synthetic banned materials in dietary supplements.

Amphetamine (AM) and methamphetamine (MET) are central nervous system (CNS) stimulant and speed up the performance of brain. AM is a man-made compound and its natural origin has rarely reported (3). Medications containing amphetamines are prescribed for narcolepsy, obesity, and attention deficit/hyperactivity disorders (4). These drugs are included in banned list by anti-doping agencies but they have been misused by some athletes because of their psychological and performance-enhancing effects. Weight loss is one of the most researched and marketed topics all over the world. One of the products that are well known to suppress appetite and cause weight loss is amphetamines due to increased cardiovascular events, the drug was withdrawn from the market in 1997 (5). Manufacturers of dietary supplements marketed to promote weight loss are adulterated with amphetamines in their dietary supplements. While these medications suppress appetite, most people are not

aware of the detrimental side effects of amphetamines, including hypertension, tachycardia, arrhythmias, and in certain instances acute myocardial infarction (6). Like most stimulants, amphetamines may prompt extensive feelings of euphoria and can be addictive and uses for purposes other than medication (7).

Many of dietary supplements have complex mixtures, such as biological proteins, carbohydrates and analysis generally requires clean-up prior to quantification of chemical species. Applying appropriate preconcentration methods in order to improve the signal to noise ratio as well as providing a clean extract is an applicable solution when using less sensitive detectors such as UV.

Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are the most popular sample preparation processes. Compared to LLE, SPE provides more benefits, including shorter processing time and lower consumption of hazardous solvents. Moreover, it resolves the difficulties of phase separation in LLE, resulting in inaccurate extract volume and unclean sample (8-12). The sorbent is the heart of SPE. In conventional SPE, the liquid phase might percolate through the solid particles; hence, they are usually recommended for single use only (13). Recently magnetic dispersive solid phase extraction (MDSPE) become one of the most interesting microextraction methods (14). Among the variety of sorbents, graphene and its derivatives show a set of remarkable characteristic such as ultra high surface area (2630 m²/g), ease of formation of non-covalent π - π interactions, stability and high capability for manipulation by different functional groups (15, 16).

This work applies a fast and simple MDSPE for complex dietary supplements in order to investigate presence of adulterants such as AM and MET. In the present work, a rapid and simple HPLC-UV method was validated for routine qualitative and quantitative analysis of AM and MET. A sensitive LC/MS-MS method was developed to further confirmation the

presence of the suspect adulterants. A range of popular diet pills and sports supplements (16 brands) were provided from local market and checked by developed DMSPE-LC/MS-MS method.

Experimental

Reagents and Materials

The pure substance of AM and MET were supplied by Sigma. Graphite powder, KH_2PO_4 , $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, KOH, NaOH, NH_3 (25%), H_2SO_4 , H_2O_2 , KMnO_4 and HCl were purchased from Merck Chemicals (Darmstadt, Germany). Acetonitrile, acetone and methanol (all in HPLC grade) were purchased from Duksan Co. Ltd. (Ansan, South Korea). Double distilled water was prepared from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran).

Preparation of standard solutions

AM and MET were prepared in concentrations of 1000 $\mu\text{g}/\text{mL}$ in methanol. Standard solutions of analytes were made by further dilution of the stock solution with appropriate volumes of methanol. Standard solutions were prepared freshly and stock solution was stored at 4 °C.

Apparatus and chromatographic condition

HPLC-UV analysis was handled by an Agilent (Germany) apparatus equipped with a UV detection system and a injector consisting of a 20 μL loop. Separation was performed on an analytical C_{18} column (10 μm particle diameter, 4.6 mm i.d. \times 15 cm) (Agilent, Germany) at room temperature. The mobile phase was acetonitrile/phosphate buffer solution (10mM) at a ratio of 15/85 (V/V) and final pH of 3.5 were used in the isocratic mode at a flow rate of 1 ml/min.

LC/MS-MS apparatus, MS parameters, and chromatographic conditions

Separation was carried out using an Agilent HPLC system. The separation was performed on an Atlantis HILIC Silica column (150 mm × 2.1 μm i.d., 3 μm particle size, Milford, MA).

HPLC separation was with an isocratic of 25 mM aqueous ammonium formate and acetonitrile (18:82, v/v) adjusted to pH 3 with formic acid. The optimized flow rate was 0.25 ml/min and the column temperature was maintained at room temperature.

Detection of ions was performed using a triple quadrupole tandem mass spectrometer in positive mode. The mass spectrometer was operated in positive ESI mode with MS/MS ion monitoring acquisition using two transitions per compound. Optimization of the ion source and MS/MS parameters for data acquisition was performed by direct infusion of a 1 μg/mL of AM and MET into the ESI source of the mass spectrometer. The protonated molecules were fragmented by collision-activated dissociation with nitrogen as the collision gas. ESI of AM and MET gave the protonated molecular ion of m/z 135.8 and m/z 149.9 which corresponds to the molecular ion $[M+H]^+$ of AM and MET, respectively. The MRM transitions of m/z 135.8 > 90.85 and m/z 149.9 > 90.85 were monitored for AM and MET at a dwell time of 200 ms per transition. The ESI instrumental settings were as follows: probe temperature, 500 °C; ion gas 1, 50 psi; ion gas 2, 50 psi; ion spray potential, 5000 V; collision gas, 8 psi; curtain gas, 28 V; and collision cell exit potential, 15 eV. The relative intensities were calculated as the ratio of intensity of the quantification transition to that of the qualifier transition (the most abundant MRM transition).

Synthesis of magnetic graphene oxide (GO@Fe₃O₄)

GO was synthesized by improved Hummer's method which describes a green synthesis. This modification help reducing toxic gases such as NO₂ and N₂O₄ (17, 18). Commercial graphite powder (0.5 g) and concentrated H₂SO₄ (12 mL) were place into a conical flask while stirring in an ice bath. Then, KMnO₄ (1.5 g) was added gradually at three portions into solution and

the resultant mixture was stirred in an oil bath while the temperature adjusted at 35 °C. The rate of the KMnO_4 addition was carefully controlled to prevent the sudden rise of mixture temperature as well as unwanted reactions. After stirring for 30 min, the color of the solution changed to light brown and distilled water (15 mL) was added slowly to the resulting mixture and the solution was stirred for another 30 min at 90 °C. Then, H_2O_2 (1 mL of a 30% solution) was added to the mixture as a reaction terminator, changing the color of mixture from dark brown to yellowish brown. The obtained precipitation was discarded by centrifuge and washed repeatedly with HCl 3% (3×15 mL) in order to eliminate the impurities and metal ions. Then, the residual was thoroughly washed with distilled water until the pH of the solution turned to neutral. The gel-like residual was dried in an oven at 70 °C and the dark brown GO was obtained.

$\text{GO}@Fe_3O_4$ composite was synthesized by chemical co-precipitation method (19). First, GO suspension (3 mg/mL) was prepared by adding GO in water and well-dispersed in an ultrasonic bath for 30 min. Then, 10 mL aqueous solution containing of 100 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 45 mg of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was gradually added to the mixture under the nitrogen atmosphere. The mixture was stirred vigorously for 30 min. Ammonia solution (25%) was added dropwise into the mixture until pH reached to about 11. The temperature of the mixture was elevated to 80 °C and after about 2 h shaking, the temperature was decreased and the solution was cooled back at room temperature. Precipitated black residue separated by magnetic field and then washed with distilled water for several times and the resulted black solid was dried in an oven at 70 °C. The characterization of $\text{GO}@Fe_3O_4$ was reported in our previous work (14).

Dispersive micro solid phase extraction (DMSPE) procedure

Accurate amount of each sample was prepared under following method; 10 mg of GO@Fe₃O₄ accurately weighted and dispersed into 5 mL of sample (pH 10) and placed in an ultrasonic bath for 3 min to enhance the drug-adsorbent interaction chance. Analytes-loaded magnetic nanoparticles (MNPs) were separated by applying an external magnetic field rapidly and the supernatant was discarded. Subsequently, 300 μL of acetone as a desorption solvent added to isolated particles and were sonicated for 3 min. Then the adsorbents were separated as described in extraction performance and the supernatant was loaded into separation apparatus for quantification.

Sample preparation

The samples were provided from local drugstore (Etminan, Tabriz). These samples were mainly in three forms including capsules, tablets and powder. For the sample preparation of capsules, the husks were removed and the powder was homogenized. All samples were smashed into small pieces and accurate amount of each sample (0.1 g) transferred to a 5 ml capped vial for described DMSPE process.

Results

The well distribution of the target analytes between the MNPs and sample solution is the major question for determining adequate levels of AM and MET in samples. Matrix and co-existing materials could deteriorate the sensitivity of the instrumental analysis by increasing the signal background and matrix effect; therefore, an optimized pre-concentration process is necessary to overcome this analytical inconvenient. In this sense, several important variables were considered in the MDSPE study, including the kind and amount of adsorbent, the pH of the sample solution, adsorption time and rate, type and volume of the eluting solvent, desorption time, pH and sample volume. The optimization of above mentioned variables was done using one-parameter-at-a-time method. The variable optimization was

performed by extracting 5 mL sample containing 0.1 $\mu\text{g}/\text{ml}$ of AM and MET as described in experimental section with the corresponding condition of each assay.

Selection of optimal sorbent type and amount

In order to obtain maximum extraction capacity two types of adsorbents were assessed for extraction of AM and MET. MNPs and Fe_3O_4 were compared as adsorbents. The extraction efficiency of the MNPs was significantly higher than that of Fe_3O_4 . It might be attributed to presence of hydrophilic functional groups (such as carboxyl and hydroxyl) on the surface of MNPs which helps to the well dispersion of magnetic adsorbent in sample medium that provide a wide surface to load the analytes on the adsorbent. Therefore, MNPs used for the next experiments. In order to observe the effect of adsorbent amount on the extraction efficiency, different amounts of MNPs were added to the sample solution and the recoveries were calculated (Figure 1a). The observation showed that by increasing the MNPs amounts from 10 mg up to 40 mg, the extraction recovery remained almost constant. In another way, accessible sites in 10 mg of adsorbents were enough for the maximum extraction efficiency. Consequently, 10 mg of the MNPs was selected for all next experiments. In comparison with the conventional SPE, MDSPE offers a great surface to volume ratio, therefore, extraction sites can be achieved with rather fewer amounts of adsorbent.

The effect of extraction time

In MDSPE, the extraction time is defined as the time interval between the contact of analytes and adsorbent and the beginning of separation process using a magnetic field. The extraction time was set in the range of 3 to 10 min and the observations in Figure 1b showed that 3 min is enough for complete maximum extraction of analytes. In 3 min the extraction process reaches to the equilibrium and further increasing the time does not have any positive effect on the extraction efficiency. According to the findings, this extraction approach is almost time-

independent due to a wide surface area assessable for extraction. Therefore, this method is carried out very fast, which is one of the main features of MDSPE. The adsorption capacity (Q_e) of C/MNPs was calculated according to following equation:

$$Q_e = \left(\frac{(C_0 - C_e)V}{M} \right) \times 100$$

Where C_0 and C_e are the initial and equilibrium concentrations of the analyte in the sample solution ($\mu\text{g/mL}$), respectively, V is the volume of sample solution (mL), and m is the mass of MNPs (g). The adsorption capacity was calculated 0.35 mg/g.

The effect of desorption parameters

In MDSPE, solvents that have the ability to desorb the analyte from the adsorbent can be used as desorption solvent. Therefore, acetonitrile, acetone and methanol were examined as desorption solvents and the effect of these solvents on the performance of MDSPE were observed. For this purpose, different experiments were set-up using 800 μL of each desorption solvent and the recoveries were calculated. Findings showed that result with acetone (93.66 \pm 3.51%) is higher in comparison to others. Thus, we chose acetone among these solvents for the next operations. After finding acetone as an elution solvent, it is important to evaluate its volume on the extraction efficiency. For selecting the optimized volumes of desorption solvent, various experiments were done by using different volumes of acetone (300-900 μL). Figure 1c shows the plot of the signal of target analytes versus the volume of acetone. According to experiments, 300 μL of acetone showed a maximum extraction recovery. The extraction efficiency decreased by continuous increasing of acetone volume that might be attributed to the dilution of the sample. Therefore, 300 μL of acetone was opt as most suitable desorption volume. To complete the desorption of AM and MET from the adsorbent, it is of prime to set a suitable desorption time. The ultrasonication time was varied

within a range of 3–10 min and 3 min was enough to ensure consistent and complete elution of the analytes among operations.

Effect of pH

The pH of sample solution determines the state of analytes. The effect of pH was evaluated thoroughly in the range of 8-12. As shown in Figure 1d, extraction efficiency is increased with increasing the pH from 8 to 10 and then decrease by further elevating the pH. Observation showed (Data not shown) that at pH 10, (zeta potential=-41mV) has its maximum amount prevented the agglomeration between adsorbent sheets. This phenomenon is accordance with the presence of negative charges on the adsorbent due to the ionic form functional groups such as carboxylic acids in higher pHs. Usually, it is accepted that the zeta value more than ± 30 mV falls into a highly stable system category (20). The more negative charge on the adsorbent derived more repulsion forces help to overcome aggregation interactions. Moreover, pK_a value of AM and MET is around 10 due to the presence of primary amine and are mostly in their ionized form caused to electrostatic interactions between analyst and adsorbent enhancing the extraction efficiency.

Sample volume

The sample volume was evaluated in the range of 2 to 10 mL. As shown in Figure 1e, the recovery increases up to 5 mL and then decreased in larger volumes. It might be related to weak dispersibility of adsorbent in larger size of sample solution. Therefore, a sample volume of 5 mL was selected for subsequent experiments.

*****Figure 1 a,b,c,d,e*****

Method validation

While the HPLC-UV method is simple and robust, it is sometimes difficult to analyze the target analytes if the amount of they are traceable or the matrices have complex mixtures that interfere with the retention time. In this case, the sensitive LC-MS/MS method can be a good candidate as a confirmative method, although it is almost expensive set-up compared with HPLC. Basically, LC-MS/MS was used to identify and confirm unmatched peaks in the HPLC chromatogram. In particular, LC-MS/MS provided structural information of the compound represented by the unknown peak (21). When the HPLC-UV separation mode was used for the analysis of dietary supplements, it was observed that some of the extracts had a peak with a retention time matching that of AM and MET. These peaks should be more investigated using a confirmatory method. The identity these peaks were resolved with the use of the LC-MS/MS method described in experimental section. The chromatograms of LC/MS-MS method. Figure 2 shows chromatograms of AM and MET obtained from LC/MS-MS.

The need for such a method was essential since there are several compounds shared the same retention time pattern in HPLC-UV analysis and were not confirmed by the UV used in original method.

*****Figure 2*****

Analysis of AM and MET in sample matrix is carried out using calibration curve were prepared using DMSPE. The matrix effect in the quantitative analysis was large enough that it could not be ignored. Consequently, we prepared the calibration standards by adding AM and MET working standard solution into blank matrices. Table I shows details of mathematical equation at linear range. Limit of detection (LOD) and limit of quantification (LOQ), which was determined considering the corresponding concentration to produce a signal 3 and 10 times the standard deviation of the blank, respectively. The LODs ranged

from 70 to 15 ng/ml, while the LODs ranged from 0.1 to 0.5 ng/ml for HPLC/UV and LC-MS/MS, respectively.

*****Table I*****

Repeatability and accuracies were calculated in interday and intraday on three different levels of spiked samples. As was shown in Table II, the proposed method is precise and accurate as the average RSD% was below 10%. No well-defined peak was observed with the blank matrix when using this method. DMSPE purify the complex matrix well and bypass the impurities such as non-polar components in the solution. Based on a 95% confidence level, the intercepts of the calibration curves were not significantly different from zero. Therefore, no systematic error is found for this value.

*****Table II*****

Real samples

The developed method was applied to sixteen different dietary supplements provided from local markets, and they were capsule, tablet, or powder forms. Once the extraction conditions were optimized by employing the LC/MS-MS, the method was used to asses a simultaneous analysis of AM and MET in commercial samples of natural weight loss medicines and dietary supplements. The details of the samples are summarized in Table III. Due to the large variation between the matrices analyzed, the standard addition samples were constructed to quantity and the recoveries were calculated. Extraction recovery was also investigated and the extraction recoveries in different formulations were from 88% to 110% for powder forms and from 81% to 116% for tablet and capsule forms using LC-MS/MS.

*****Table III*****

Discussion

Table IV lists a review of the presented methods for the determination of AM and MET with different separation method. For the sake of comparison, the present approach was included as the last entry in Table II. Our method was sensitive enough to detect the analytes using a LC/MS-MS system. Present approach offers outstanding advantages; it allows avoiding using large amounts of hazardous solvents and a time-consuming extraction operation. Moreover, the DMSPE design made further efforts for complex matrix. All these results indicate that the proposed method based on DMSPE/ LC/MS-MS is a sensitive fast, reproducible and simple method that can successfully be used for the pre-concentration and determination of AM and MET as adulterants in dietary supplements. In conventional SPE, solid particles are immobilized generally on a supporting phase such as cartridge, fiber, and disk. This method resolves the difficulties of conventional cartridges preparation (loading, washing, and elution), resulting in inaccurate extract volume and unclean sample and all these steps were done less than 15 min and with consuming only 300 acetone. The equilibrium between adsorbent and analytes reaches very fast due to the high surface of nanoadsorbent.

*****Table IV*****

Conclusion

Unfortunately, the undesirable effects of some banned additives in a few of dietary supplement come at a high price to consumer health and safety. This is why researchers have focused to find and control substances and adulterants that can be lurking in some sports and weight management products. In this study, the presence of AM and MET in solid dietary supplements were successfully accomplished with the assistance of MDSPE/LC-MS-MS.

This newly-developed method is sensitive, simple, rapid and efficient as it did not need to time-consuming cartridges preparation in conventional SPE.

Ethical compliance

Present work has not involved in human participants and/or animals.

Conflict of interest

Declared none.

Figure caption

Figure 1. Optimization parameters for magnetic dispersive solid phase extraction method

Figure 2. Chromatograms of amphetamine and methamphetamine

References

1. Phua D, Zosel A, Heard K. Dietary supplements and herbal medicine toxicities—when to anticipate them and how to manage them. *International journal of emergency medicine*. 2009;2(2):69-76.
2. Rocha T, Amaral JS, Oliveira MBP. Adulteration of dietary supplements by the illegal addition of synthetic drugs: a review. *Comprehensive Reviews in Food Science and Food Safety*. 2016;15(1):43-62.
3. Pawar RS, Grundel E, Fardin-Kia AR, Rader JI. Determination of selected biogenic amines in *Acacia rigidula* plant materials and dietary supplements using LC–MS/MS methods. *Journal of pharmaceutical and biomedical analysis*. 2014;88:457-466.
4. Heal DJ, Smith SL, Gosden J, Nutt DJ. Amphetamine, past and present—a pharmacological and clinical perspective. *Journal of Psychopharmacology*. 2013;27(6):479-496.
5. Connolly HM, Crary JL, McGoon MD, Hensrud DD, Edwards BS, Edwards WD, Schaff HV. Valvular heart disease associated with fenfluramine–phentermine. *New England Journal of Medicine*. 1997;337(9):581-588.

6. Karch SB, Stephens BG, Ho C-H. Methamphetamine-related deaths in San Francisco: demographic, pathologic, and toxicologic profiles. *Journal of Forensic Science*. 1999;44(2):359-368.
7. Cruickshank CC, Dyer KR. A review of the clinical pharmacology of methamphetamine. *Addiction*. 2009;104(7):1085-1099.
8. Zygler A, Wasik A, Namieśnik J. Retention behaviour of some high-intensity sweeteners on different SPE sorbents. *Talanta*. 2010;82(5):1742-1748.
9. Hamidi S, Soltani S, Jouyban A. A dispersive liquid–liquid microextraction and chiral separation of carvedilol in human plasma using capillary electrophoresis. 2015.
10. Hamidi S, Jouyban A. Capillary electrophoresis with UV detection, on-line stacking and off-line dispersive liquid–liquid microextraction for determination of verapamil enantiomers in plasma. *Analytical Methods*. 2015;7(14):5820-5829.
11. Hamidi S, Jouyban A. Pre-concentration approaches combined with capillary electrophoresis in bioanalysis of chiral cardiovascular drugs. *Pharm Sci*. 2015;21:229-243.
12. Hamidi S, Alipour-Ghorbani N. Liquid Phase Microextraction of Biomarkers: A Review on Current Methods. *Journal of Liquid Chromatography & Related Technologies*. 2017(just-accepted).
13. Ashri NY, Abdel-Rehim M. Sample treatment based on extraction techniques in biological matrices. *Bioanalysis*. 2011;3(17):2003-2018.
14. Jouyban A, Hamidi S. Dispersive micro solid phase extraction using carbon-based adsorbents for sensitive determination of verapamil in plasma samples coupled with CE. *Journal of Separation Science*. 2017.
15. Liu Q, Shi J, Jiang G. Application of graphene in analytical sample preparation. *TrAC Trends in Analytical Chemistry*. 2012;37:1-11.
16. Allen MJ, Tung VC, Kaner RB. Honeycomb carbon: a review of graphene. *Chemical reviews*. 2009;110(1):132-145.
17. Hummers Jr WS, Offeman RE. Preparation of graphitic oxide. *Journal of the American Chemical Society*. 1958;80(6):1339-1339.
18. Chen J, Yao B, Li C, Shi G. An improved Hummers method for eco-friendly synthesis of graphene oxide. *Carbon*. 2013;64:225-229.
19. Zeng S, Gan N, Weideman-Mera R, Cao Y, Li T, Sang W. Enrichment of polychlorinated biphenyl 28 from aqueous solutions using Fe₃O₄ grafted graphene oxide. *Chemical engineering journal*. 2013;218:108-115.
20. Bhattacharjee S. DLS and zeta potential—What they are and what they are not? *Journal of Controlled Release*. 2016;235:337-351.
21. Ai K, Liu Y, Lu L. Hydrogen-bonding recognition-induced color change of gold nanoparticles for visual detection of melamine in raw milk and infant formula. *Journal of the American Chemical Society*. 2009;131(27):9496-9497.
22. Beck O, Leine K, Palmkog G, Franck J. Amphetamines detected in exhaled breath from drug addicts: a new possible method for drugs-of-abuse testing. *Journal of analytical toxicology*. 2010;34(5):233-237.
23. Beck O, Stephanson N, Sandqvist S, Franck J. Detection of drugs of abuse in exhaled breath from users following recovery from intoxication. *Journal of analytical toxicology*. 2012:bks079.
24. Beck O, Stephanson N, Sandqvist S, Franck J. Detection of drugs of abuse in exhaled breath using a device for rapid collection: comparison with plasma, urine and self-reporting in 47 drug users. *Journal of breath research*. 2013;7(2):026006.
25. Miranda-G E, Sordo M, Salazar AM, Contreras C, Bautista L, Garcia AER, Ostrosky-Wegman P. Determination of amphetamine, methamphetamine, and hydroxyamphetamine derivatives in urine by gas chromatography-mass spectrometry and its relation to CYP2D6 phenotype of drug users. *Journal of analytical toxicology*. 2007;31(1):31-36.
26. Raikos N, Christopoulou K, Theodoridis G, Tsoukali H, Psaroulis D. Determination of amphetamines in human urine by headspace solid-phase microextraction and gas chromatography. *Journal of Chromatography B*. 2003;789(1):59-63.

27. Laznickova J, Dedicová M, Vorel F. [Determination of amphetamine and methamphetamine in human hair using gas chromatography/mass spectrometry]. *Soudni lekarstvi/casopis Sekce soudního lekarstvi Cs lekarske spolecnosti J Ev Purkyne*. 2000;45(2):26-29.
28. Miyaguchi H, Inoue H. Determination of amphetamine-type stimulants, cocaine and ketamine in human hair by liquid chromatography/linear ion trap–Orbitrap hybrid mass spectrometry. *Analyst*. 2011;136(17):3503-3511.
29. Kwak J-H, Choe S, Pyo JS. Determination of Methamphetamine and Amphetamine in Hair by Mixed-Mode Anion Exchange Solid Phase Extraction and Liquid Chromatography–Tandem Mass Spectrometry. *Analytical Letters*. 2015;48(16):2533-2543.
30. Stout PR, Horn CK, Klette KL. Rapid simultaneous determination of amphetamine, methamphetamine, 3, 4-methylenedioxyamphetamine, 3, 4-methylenedioxymethamphetamine, and 3, 4-methylenedioxyethylamphetamine in urine by solid-phase extraction and GC-MS: a method optimized for high-volume laboratories. *Journal of analytical toxicology*. 2002;26(5):253-261.
31. Meng L, Zhang W, Meng P, Zhu B, Zheng K. Comparison of hollow fiber liquid-phase microextraction and ultrasound-assisted low-density solvent dispersive liquid–liquid microextraction for the determination of drugs of abuse in biological samples by gas chromatography–mass spectrometry. *Journal of Chromatography B*. 2015;989:46-53.
32. Kumazawa T, Hasegawa C, Hara K, Uchigasaki S, Lee XP, Seno H, Suzuki O, Sato K. Molecularly imprinted solid-phase extraction for the selective determination of methamphetamine, amphetamine, and methylenedioxyphenylalkylamine designer drugs in human whole blood by gas chromatography-mass spectrometry. *Journal of separation science*. 2012;35(5-6):726-733.
33. Wu TY, Fuh MR. Determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, and 3,4-methylenedioxymethamphetamine in urine by online solid-phase extraction and ion-pairing liquid chromatography with detection by electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2005;19(6):775-780.
34. Villamor J, Bermejo A, Fernandez P, Tabernero M. GC-MS Determination of Amphetamines in Human Urine. *Analytical letters*. 2005;38(5):781-790.
35. Westphal F, Franzelius C, Schäfer J, Schütz HW, Rochholz G. Development of a validated method for the simultaneous determination of amphetamine, methamphetamine and methylenedioxyamphetamines (MDA, MDMA, MDEA) in serum by GC-MS after derivatisation with perfluorooctanoyl chloride. *Accreditation and quality assurance*. 2007;12(7):335-342.
36. Fernández MdMR, Wille SM, Samyn N, Wood M, López-Rivadulla M, De Boeck G. High-Throughput Analysis of Amphetamines in Blood and Urine with Online Solid-Phase Extraction-Liquid Chromatography—Tandem Mass Spectrometry. *Journal of analytical toxicology*. 2009;33(9):578-587.
37. Meng P, Fang N, Wang M, Liu H, Chen DD. Analysis of amphetamine, methamphetamine and methylenedioxy-methamphetamine by micellar capillary electrophoresis using cation-selective exhaustive injection. *Electrophoresis*. 2006;27(16):3210-3217.
38. Hyotylainen T, Siren H, Riekkola ML. Determination of morphine analogues, caffeine and amphetamine in biological fluids by capillary electrophoresis with the marker technique. *Journal of chromatography A*. 1996;735(1-2):439-447.
39. Boatto G, Faedda MV, Pau A, Asproni B, Menconi S, Cerri R. Determination of amphetamines in human whole blood by capillary electrophoresis with photodiode array detection. *Journal of Pharmaceutical and Biomedical Analysis*. 2002;29(6):1073-1080.
40. Wernly P, Thormann W. Analysis of illicit drugs in human urine by micellar electrokinetic capillary chromatography with on-column fast scanning polychrome absorption detection. *Analytical chemistry*. 1991;63(24):2878-2882.
41. Tagliaro F, Manetto G, Bellini S, Scarcella D, Smith FP, Marigo M. Simultaneous chiral separation of 3, 4-methylenedioxymethamphetamine (MDMA), 3, 4-methylenedioxyamphetamine (MDA), 3, 4-methylenedioxyethylamphetamine (MDE),

- ephedrine, amphetamine and methamphetamine by capillary electrophoresis in uncoated and coated capillaries with native β -cyclodextrin as the chiral selector: Preliminary application to the analysis of urine and hair. *Electrophoresis*. 1998;19(1):42-50.
42. Wei F, Fan Y, Zhang M, Feng YQ. Poly (methacrylic acid-ethylene glycol dimethacrylate) monolith in-tube solid-phase microextraction applied to simultaneous analysis of some amphetamine derivatives in urine by capillary zone electrophoresis. *Electrophoresis*. 2005;26(16):3141-3150.
43. Aturki Z, D'Orazio G, Fanali S, Rocco A, Bortolotti F, Gottardo R, Tagliaro F. Capillary electrochromatographic separation of illicit drugs employing a cyano stationary phase. *Journal of Chromatography A*. 2009;1216(17):3652-3659.
44. Zhang L, Wang R, Yu Y, Zhang Y. Capillary electrophoresis with laser-induced fluorescence and pre-column derivatization for the analysis of illicit drugs. *Journal of Chromatography B*. 2007;857(1):130-135.
45. Gottardo R, Bortolotti F, De Paoli G, Pascali JP, Mikšik I, Tagliaro F. Hair analysis for illicit drugs by using capillary zone electrophoresis-electrospray ionization-ion trap mass spectrometry. *Journal of Chromatography A*. 2007;1159(1):185-189.
46. dos Santos VB, Daniel D, Singh M, do Lago CL. Amphetamine and derivatives in natural weight loss pills and dietary supplements by capillary electrophoresis-tandem mass spectrometry. *Journal of Chromatography B*. 2016;1038:19-25.