Original Article

Analysis of Drugs of Abuse in Hair Sample by LC/MS-MS

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Abstract

Due to its prolonged detection window, hair has become an important material for drug analysis. It is suitable for the detection of frequent drug abuse (DOA) dependence and other toxicological considerations. The advancement in chromatographic techniques especially liquid chromatography-tandem mass spectrometry (LC/MS-MS) makes detecting even traces of a drug in hair possible. Therefore, LC/MS-MS is progressively replacing gas chromatographic techniques as a tool for screening and confirmation making it the method of choice for hair analysis. In the present study, a method to determine common drugs of abuse in hair samples is described.

Human hair was tested for a range of different classes of drugs of a forensic and toxicological nature including selected amphetamines, cannabinoids, and benzodiazepines. For extraction purposes, the hair samples were decontaminated using dichloromethane, grounded, and treated with 1 M sodium hydroxide, followed by n-hexane/ethyl acetate extraction using liquid-liquid extraction (LLE). Following extraction from hair samples, drug screening employed liquid chromatography coupled with tandem mass spectrometric (LC/MS-MS). The screening method (for >20 drugs) was calibrated with a tailored drug mixture and was validated for 11 selected drugs for this study. A shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 µm) column was used for analysis. The total instrument run time was 30 minutes with no noted matrix interferences. Hair samples were screened using this new method and samples were confirmed positive for several drugs, mainly drugs of abuse.

The LC/MS-MS method described in the present study has proved to be simple and robust for the determination of drugs in hair. It is currently used for real samples in the Forensic Laboratory of Jordan.

Keywords: Drugs of abuse, Hair sample, High-performance liquid chromatography, Mass spectrometry

Introduction

The analysis of biological matrices to detect various types of drugs is in great demand within forensic investigations as it provides essential proof of past drug exposure[1]. Monitoring of these substances can be performed on different biological specimens including blood, urine, and hair. Blood and urine have been used as the main body fluids for drug screening in most clinical and forensic laboratories and remain the matrices of choice to detect recent exposure[2-4].

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Hair analysis has been acknowledged as the best method that enables the evaluation of long-term exposure to illegal drugs[5-12]. Gas chromatography-mass spectrometry (GC/MS), and liquid chromatography-tandem mass spectrometry (LC/MS-MS) have dominated over other analytical techniques as screening and confirmation methods in hair analysis[13 &14]. For samples to be adequate for GC/MS, sample preparation requires a derivatization step before instrumental analysis [2&3]. LC/MS-MS has been largely used in forensic laboratories to determine a wide range of compounds in biological specimens[15]. Owing to the decreasing costs, growing effectiveness, and absence of derivatization step, LC/MS-MS instrumentation is progressively replacing GC/MS in forensic routine analysis[16]. The introduction of Liquid Chromatography and the advancement in triple quadrupole design allow fast data throughput, excellent sensitivity, and selectivity, making it the best technique of choice in forensic investigations. In this paper, we describe a new LC/MS-MS screening method for the simultaneous determination of 11 illicit, psychotropic, and hypnotic drugs in human hair including; amphetamine, ethylamphetamine, methamphetamine, diazepam, alprazolam, bromazepam, THC, amitriptyline, gabapentin, olanzapine.

The combination of an easy-to-apply sample extraction procedure with the LC/MS-MS technique proved to be simple, fast, accurate, and highly sensitive, allowing for the simultaneous detection of several drugs of abuse.

Materials and Method

Materials

1. Sample

Blank hair samples were used for the development and validation of the procedure and were obtained from healthy volunteers. Authentic hair samples were obtained from forensic and toxicology cases.

2. Reagents and Solvents:

Methanol and water (HPLC Grade) were purchased from Carlo Erba (Milan, Italy). Ammonium format (analytical grade, 99% min assay) from ALFA ASER, Ward Hill, Massachusetts, U.S.). Dichloromethane, Ethyl acetate, Isopropanol, and N-hexane; all were analytical grade. Analytical Reference Standards were brought from LIPOMED, (U.S.). All drugs together with internal standards were provided by the forensic science laboratory of Jordan.

3. Calibrant, Standards, and Stock Solutions.

Stock solutions of individual analytes were prepared by dissolving or diluting them in methanol obtaining a concentration of 1 mg/mL. Individual stock solutions were diluted to prepare a standard solution by mixing individual drugs in methanol arriving at a concentration of 5 µg/mL for selectivity experiments. A mixed working solution of non-deuterated compounds at 5 µg/mL in methanol was used for the preparation of calibrators and 'in-house QCs' for all the compounds. Calibrant samples were prepared using blank hair. Spiked hair sample solution was prepared from the dilution of stock solutions. The test solution prepared consisted of analytes of 5 components which is used to check the sensitivity and accuracy of the method. A mixed Internal Standards working solution of 0.4 µg/mL for deuterated standards was also prepared in methanol. Working solutions were stored at 4 °C for 6 months. Their stability was checked weekly as they were routinely used in the analysis of other biological matrices where external quality controls are analyzed in the same batch of samples

4. Analytical Equipment and Supplies

The LC system is Shimadzu UHPLC-LC20, the Analytical Column is Shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 um), and the detector is LCMS 8030 Tandem mass from Shimadzu Scientific Instruments Inc.

Method

Sample Preparation

To the hair sample 4 mL of dichloromethane was added, mix vortex-mixed for 3 minutes. The solvent is discarded and the process is repeated. The hair sample was dried at room temperature under gentle nitrogen flow. Hair was cut into small pieces, and 100 mg of the sample was put in a 10-mL screw-capped glass tube. 1 mL of 1M sodium hydroxide (NaOH) solution was added to the sample and was incubated

at 95 °C for 20 min. 3ml of n-hexane/ethyl acetate 90:10 (v/v) was used for the extraction process, the sample was then centrifuged at 5000rpm for 5min. The organic phase was transferred into a glass tube, and the extraction was repeated. The dried organic phase under nitrogen flow at 40°C was reconstituted with 100µL of methanol and transferred into a vial. Analysis of the sample was carried out on LC/MS-MS using the instrument operating conditions indicated in Table 1.

Table 1: Analytical Conditions

LC Conditions:			
Analytical Column	**Shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 um)**		
Mobile phase A	**10 mmol/L Ammonium formate-water**		
Mobile phase B	**Methanol**		
Gradient Program	**Time (Min)**	**Mobile phase A (%)**	**Mobile Phase B (%)**
	0	95	5
	15	5	95
	20	5	95
	20.1	95	5
	30	95	5
Flow rate	0.3 mL/Min		
Injection volume	5 µL		
Column oven temperature	40°C		
MS Conditions:			
Nebulizing Gas Flowrate	1.5 L/min		
Drying gas Flowrate	10 L/min		
DL Temperature	250°C		
Block heater Temperature	400°C		
Ionization mode	ESI		

Notes:

Extraction

For the results to be reproducible, it is advantageous to quantitatively extract all of the drugs from the hair matrix. To ensure that all of the analytes present in the sample are extracted from the sample and measured, it is preferred that the hair be completely dissolved. Therefore, the hair sample was incubated with a strong alkali solution at optimal temperature for an adequate time. All analytes were stable during incubation. The aqueous solution was then extracted with a mixture of n-hexane/ethyl acetate. The same sample was re-extracted a second time to ensure that a high recovery of all detected drugs in the sample was achieved. Cloudy or turbid extracts should be centrifuged before analysis. In analytes at a concentration above the calibration range, the extracts should be diluted to avoid errors in the quantitative analysis.To remove external contamination from the exterior of the hair, hair samples were washed before analysis.

LC System

To achieve reliable data and high performance in the Liquid Chromatography (LC) system, only high-purity organic solvents, and reagents of LC/MS grade are recommended. 10% of isopropanol in 50:50 v/v solution of water–Methanol was used to wash the LC system to avoid carry-over phenomena that could affect the high sensitivity of LC/MS.

Instrument's Operating Conditions.

Liquid Chromatography:

LC separations were performed on Shimadzu LC20 supported with an LCMS 8030 Tandem mass

spectrometer. Five microliters were injected into the LC system for a chromatographic run of 30 min using a Shim-pack FC-ODS (2.0 mm I.D. x 150 mm L, 3 um) (Shimadzu, Japan) and a gradient mixture of 10 mmol/L Ammonium formate-water/Methanol.

***MS-MS System*:**

Mass spectrometry: MS data were collected in positive electrospray ionization (ESI) mode on the Triple Quadrupole Mass by Shimadzu Scientific Instruments Inc. After complete validation, this method has been successfully applied to many forensic cases, one of which is reported.

Method Validation:

Standard calibration curves were obtained by spiking aliquots of negative control hair samples with a standard mixture at the concentration levels (ng/mg hair) of 0.01, 0.05, 0.10, 1, and 5, which were extracted as described above. Within-run precision was determined using these assays.

Method validation was conducted by applying calibration curves based on peak-height ratios of standard concentration vs analytes and internal standards. Limits of quantification (LOQ) were estimated by analyzing drug-free hair samples with various concentrations of analytes. Repeated injections (n = 5) were performed on a single day to establish the intra-day precision (% CV), The extraction efficiency of the compounds was determined by spiking blank hair samples with 10ng/mg drugs.Recoveries were determined by measuring peak heights obtained for the analytes added after the incubation step with those of the analytes added before sample preparation. The detection limits ((LOD) were evaluated by decreasing the concentration of the drugs spiked in drug-free hair until a response equivalent to three times the background noise was observed. Quantitation was based on the ratio of the peak areas of the analyte to the internal standards. The internal standard was in both cases added after incubation. High concentrations of selected drugs were incubated in drug-free hair samples to investigate the specificity of the method.

Results

To generate a standardized protocol for detecting drugs of abuse in hair samples of long-term users, a hair sample was processed for preparation by extraction followed by LC/MS-MS analysis. The present study focuses on the development of a method for extraction and analysis.

Extraction analysis

The results of the extraction recovery study are presented in Table 3. The recoveries ranged from 65% to 92% which are considered sufficient for screening purposes[17]. Extraction is not an issue as long as the sensitivity of the instrument is enough and reproducibility is lower than 15%. These results are acceptable considering this is a quantitative method dealing with 11 compounds in hair samples including drugs of abuse. However, the results obtained with the analysis of samples demonstrated that the extraction procedure is adequate for the study of forensic and toxicological samples. In the experiment performed to assess matrix effects (with 4 hair samples from different origins), no significant matrix effect was observed for any compound.

Calibration analysis

Calibration curves were made for each compound. The calibration ranges were selected according to the cut-offs recommended by the Society of Hair Testing (SoHT)[18] and focused on possible concentrations found in single-dose cases. The intra-assay imprecision for the 'in-house QC was satisfactory, with RSDs lower than 8% for all the compounds (Relative standard deviations (RSD) and LOQs ranged between 1.5% and 7.6% and 0.015-0.025 respectively as presented in Table 3.

LC/MS-MS analysis.

Figure 1 below shows the chromatographic profile of the analytes of DOA detected in a hair sample from a subject who reported past drug abuse consumption. For purposes of quantitating the drugs detected in hair extracts, liquid chromatography/ tandem mass spectrometry using selected ion monitoring was carried out. The ions that were selected for monitoring were chosen according to their relatively high abundance as can be seen in Table 2, which consists of monitored ions for each drug from an extract of the hair sample from a drug user. The analysis has indicated the presence of specific peaks at retention times as shown in Table 2.

In this case study, the real hair sample was analyzed to evaluate the robustness of the developed DOA screening method.

The selectivity of LC/MS-MS analysis implemented in this study has enabled the detection of 11 drugs in a hair sample collected from a single subject. No interferences were observed after the study of the blank hair samples, ensuring the selectivity of the method (Fig. 1).

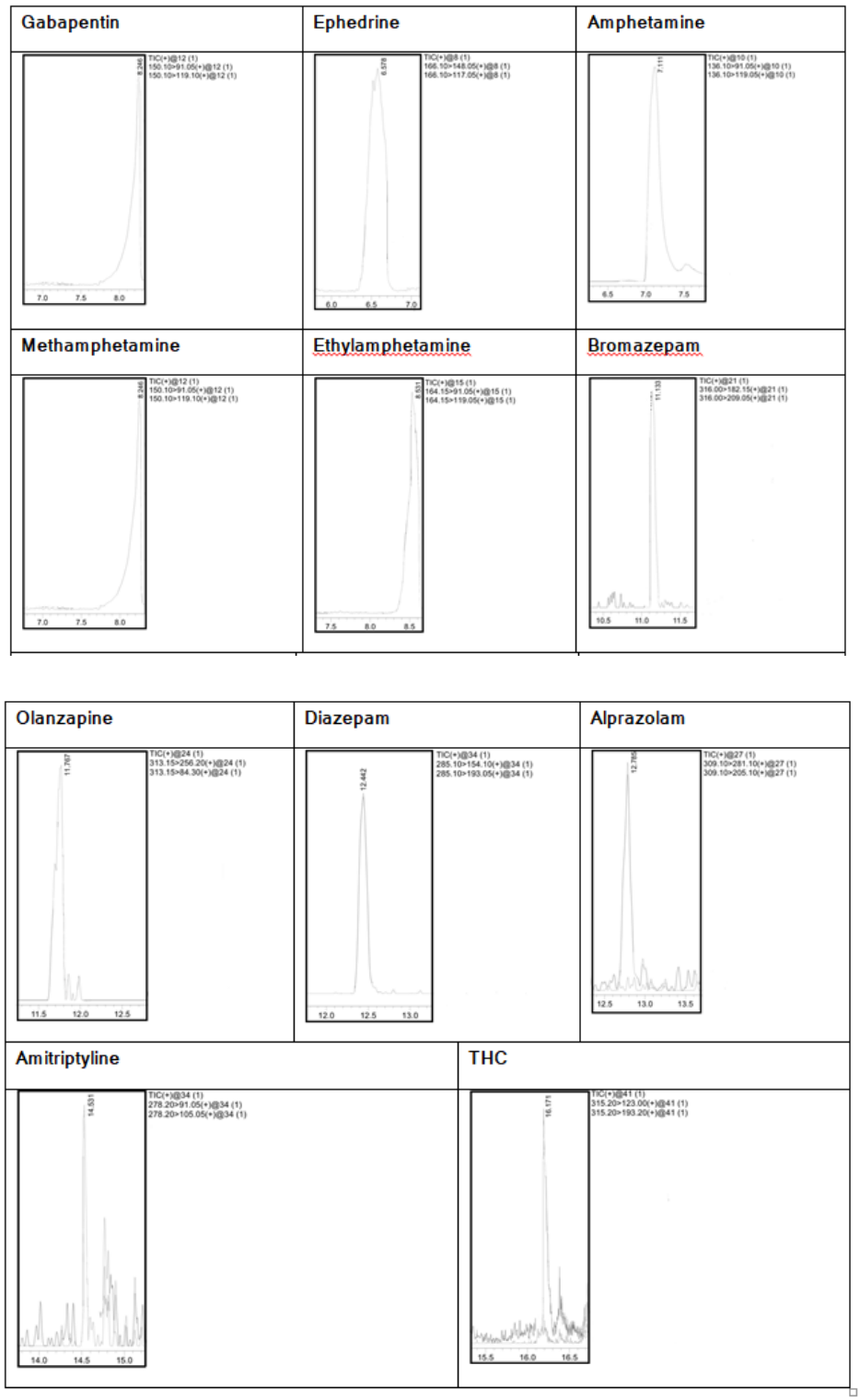

Figure 1: Chromatographic profile of the drugs of abuse by LC/MS-MS analysis.

Table 2: Retention time and MS characteristic of subject hair sample's analytes

Analyte	RT (Min)	Precursor Ion (m/z)	Product Ion Quantifier (m/z)	Product Ion Qualifier (m/z)
Illicit drugs				
Amphetamine	7.1110	136.10	91.05	119.05
Ethylamphetamine	8.5310	164.50	91.05	119.05
Ephedrine	6.5780	166.10	148.05	117.05
Methamphetamine	8.2460	150.10	91.050	119.05
THC	16.171	315.20	123.00	193.20
Psychotropic drugs				
Amitriptyline	14.531	278.20	91.050	105.05
Gabapentin	5.4860	172.15	154.10	55.00
Olanzapine	11.767	313.15	256.20	84.30
Hypnotic Drugs				
Alprazolam	12.785	309.10	281.10	205.10
Bromazepam	11.133	316.00	182.15	209.05
Diazepam	12.442	285.10	154.10	193.05

Table 3. Summary of validation results

No.	Analyte	LOQ (ng/mg)	Intraday RSD (%)	Recovery (%)
1	Amphetamine	0.020	3.3	91
2	Ethylamphetamine	0.015	2.5	92
3	Ephedrine	0.025	2.9	87
4	Methamphetamine	0.020	2.3	92
5	THC	0.020	5.6	76
6	Amitriptyline	0.015	4.3	85
7	Gabapentin	0.020	1.5	79
8	Olanzapine	0.010	2.8	81
9	Alprazolam	0.020	7.6	65
10	Bromazepam	0.020	6.9	67
11	Diazepam	0.025	7.5	69

Discussion

Hair is generally accepted as the sample of choice for drug abuse testing because hair has a longer window (months) in comparison to blood (hours to days) and urine (days to weeks), and can therefore be used as an integral material.

The aim of screening methods is firstly to decline negative samples in a large number of samples. This is achieved with low-cost immunological tests such as RIA[19]and ELIZA[20]. Another aim of screening tests is to use two separate and distinctive methods to confirm a positive result. So the main goal is that results will have added quality by using chromatography.

Quantitative results from genuine samples analyzed with the LC/MS-MS confirmation method are shown in Figure 1. Another quality of chromatography is that one can establish a screening method to measure exactly what the confirmation method does. The positive case under investigation has revealed a formerly unknown drug user by analyzing his hair sample. Hair analysis in such cases can reveal both known drug use as well as a prolonged period of use and in some overdose cases.

The LC/MS-MS method was developed for the rapid screening of abused drugs in human hair in patients who were investigated for drug abuse. This

method used sample preparation to extract DOAs from the hair sample. The use of the wash step was to eliminate false-positive results. The results show that this new competitive LC/MS-MS method to detect drugs of abuse in human hair has been successfully established. It was fully validated and provided a viable approach for the analysis of drugs in a hair sample to monitor long-term exposure with a high level of selectivity and robustness. The analytical method described in this study is suitable for a wide spectrum of chemically different drugs including; illicit, hypnotics, and psychotropic drugs. LC/MS-MS screening method enabled the simultaneous determination of 11 different compounds present in a single sample of hair. It has been already established for the analysis of authentic hair samples in the Forensic laboratory in Jordan.

Conclusions

The presented extraction method, followed by LC/MS-MS analysis appears adequate for drug monitoring use because it is highly sensitive and is very easy to apply. The developed method can identify and confirm 11 substances, including THC, within a single analytical run. This method can monitor patients for drug abuse with the minimum sample collected. The routine analysis using the developed method has proved that this method is an effective tool for determining abused drugs in human hair from patients who were monitored for drug abuse. The results provide medical professionals with thorough information about drug abuse or setbacks in drug abuse and can be used for patient-careful treatment to improve patient health.

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Declaration: The authors declare that there is no conflict of interest and that the data presented in the manuscript is the result of work conducted in the Department of Chemical and Biological Analysis of the Forensic Laboratory of Jordan.

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References

1. Kintz P, Salomone A, Vincenti M (2015) Hair analysis in clinical and forensic toxicology. Academic, Cambridge. https://www.elsevier.com/books/hair-analysis-in-clinical-andforensic-toxicology/kintz/978-0-12-801700-5.
2. Alabdalla MA. (2021) Solid-Phase Extraction and GC/MS Confirmation of Heroin Abuse in Urine. YJES. 2021;18(1):33-36. doi:10.53370/001c.28863
3. Alabdalla M. (2012) Confirmation of cannabis abuse by GC-MSIndian Journal of Forensic Medicine and Toxicology. 2012; 6:36- 38. https://www.researchgate.net/ publication/282709044.
4. Alabdalla MA. (2005) HPLC-DAD for analysis of different classes of drugs in plasma. Journal of Clinical Forensic Medicine. 2005;12(6):310-315. DOI: 10.1016/j.jcfm.2005.05.002.
5. Vincenti M, Salomone A, Gerace E, Pirro V (2013) Role of LC/MS-MS in hair testing for the determination of common drugs of abuse and other psychoactive drugs. Bioanalysis 5:1919–1938. DOI: 10.4155/bio.13.132
6. Vincenti M, Salomone A, Gerace E, Pirro V (2012) Application of mass spectrometry to hair analysis for forensic toxicological investigations. Mass Spectrum Rev 32:312– 332. DOI: 10.1002/mas.21364.
7. Barbosa J, Faria J, Carvalho F, Pedro M, Queirós O, Moreira R, Dinis-Oliveira RJ (2013) Hair as an alternative matrix in bioanalysis. Bioanalysis 5:895–914. doi: 10.4155/bio.13.50.
8. Pragst F, Balikova M (2006) State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 370:17–49. DOI: 10.1016/j.cca.2006.02.019.
9. Di Corcia D, D'Urso F, Gerace E, Salomone A, Vincenti M (2012) Simultaneous determination in the hair of multiclass drugs of abuse (including THC) by ultra-high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B 899:154–159. DOI: 10.1016/j.jchromb.2012.05.003.
10. Pragst, M. Rothe, K. Spiegel, and F. Sporkert. (199) Illegal and therapeutic drug concentration in hair segments-a timetable of drug exposure. Forensic 5ci. Rev. 10:81- 111 (1998). https://pubmed.ncbi.nlm.nih.gov/ 26255716/.
11. Villain, M. Cirimele, V., and Kintz. P. Hair analysis in toxicology. Clin. Chem. Lab. Med. 42:1265-1272 (2004). DOI: 10.1515/CCLM.2004.247.
12. Ropero-Miller. J. A decade revisited-the forensic and clinical applications of hair testing. Forensic Sci. Rev. 19" 50-67 (7 A.D.). https://pubmed.ncbi.nlm.nih.gov/26247283/.

13. Cheze, M. Duffort, G. Deveaux, M. and Pepin, P. (2005) Hair analysis by liquid chromatography-tandem mass spectrometry in toxicological investigation of drug-facilitated crimes: report of 128 cases over the period June 2003-May 2004 in metropolitan Paris. Forensic Sci. Int. 153: 3-I 0 (2005). DOI: 10.1016/j.forsciint.2005.04.021.

14. Villain, M. Concheiro, M. Cirimele, V and Kintz. P. Screening method for benzodiazepines and hypnotics in the hair at pg/mg level by liquid chromatography-mass spectrometry-mass spectrometry. J. Chromatogr. B Analyst. Technol. Biomed. Life Sci: 825: 72- 78 (2O05). DOI: 10.1016/j.jchromb.2004.12.036.

15. Maurer. H.H. (2005) Multi-analyte procedures for screening for and quantification of drugs in blood, plasma, or serum by liquid chromatography-single stage or tandem mass spectrometry (LC-MS or LC-MS-MS) relevant to clinical and forensic toxicology. Clin. Biochem. 38:310-318 (2005). DOI: 10.1016/j.clinbiochem.2005.01.014

16. Saito K, Saito R, Kikuchi Y, Iwasaki Y, Ito R, Nakazawa H (2011) Analysis of drugs of abuse in biological specimens. J Health Sci 57:472–487. doi.org/10.1248/jhs.57.472.

17. Kumar D., Gautam N., Alnouti Y.(2022) Analyte recovery in LC/MS-MS bioanalysis: an old issue revisited. 1198, 15(3) 2022, https://doi.org/10.1016/j.aca.2022.339512

18. Cooper G., Moeller M., Kronstrand R., (2008) Forensic Sci. Int. 176 (2008) 9–12. [19] https://doi.org/10.1016/j.forsciint.2007.07.019

19. Baumgartner W.A., Hill V.A., (1993) Sample preparation techniques, Forensic Sci. Int. 63 (1993) 121–135.doi: 10.1016/0379-0738(93)90266-d.

20. Negrusz A., Moore C., Deitermann D., Lewis D., Kaleciak K., Kronstrand R., Feeley B., Niedbala R.S., (1999) Highly sensitive micro-plate enzyme immunoassay screening and NCI-GC–MS confirmation of flunitrazepam and its major metabolite 7-aminoflunitrazepam in hair, J. Anal. Toxicol. 23-1999 429–435. doi: 10.1093/jat/23.6.429.