Pharmaceutical Sciences, 2023, 29(2), 246-251
doi:10.34172/PS.2022.14
https://ps.tbzmed.ac.ir/

Research Article

Determination of Benzo(α)pyrene in Infant Formula Using High Performance Liquid Chromatography and Dispersive Liquid–Liquid Microextraction

Akram Faridi[1,2], Mahboob Nemati[3,4,5*]

[1]*Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
[4]*Department of Pharmaceutical and Food Control, Tabriz University of Medical Sciences, Tabriz, Iran*
[5]*Halal Research Center of IRI, FDA, Tehran, Iran.*

Article Info

Article History:
Received: 14 December 2021
Accepted: 14 March 2022
ePublished: 31 March 2022

Keywords:
-Benzo(α)pyrene
-HPLC
-Infant formula milk
-Microextraction

Abstract

Background: Benzo(α)pyrene (BaP) is one of the most familiar polycyclic aromatic hydrocarbons (PAHs) to evaluate food safety and quality and can be present in infant formulas An analytical method for the extraction and quantification of BaP in infant formula milk has been established by dispersive liquid–liquid microextraction (DLLME) followed by high-performance liquid chromatography with fluorescence detector.

Methods: BaP was first extracted from matrices of infant formula milk via acetonitrile and then DLLME was used for further purification and preconcentration of target analyte.

Results: Under the optimum extraction conditions, 150 µL of dichloromethane as extraction solvent, 3 mL acetonitrile as disperser and cleaner solvent and 1 mL volume sample, the accuracy of the method was between 89-97%. The limits of detection and quantification were 0.12 and 0.35 ng/ml, respectively. There was a linear relation (R^2=0.998) between chromatographic peak area and concentrations in the range of 0.5 to 15 ng/mL.

Conclusion: The proposed method is applicable to the quantification of BaP in infant formula milk with acceptable accuracy and precision.

Introduction

Polycyclic aromatic hydrocarbons (PAHs), a kind of persistent organic pollutants that consist of two or more aromatic rings.[1] PAHs have been of excessive interest because of their mutagenicity, carcinogenicity, and the general ubiquity in the environment.[2] The carcinogenicity of PAHs is related to molecular complexity such as the number of benzenoid rings in their constructions and with the metabolic conversion to reactive diol-epoxide intermediates that can be attached via covalent binding to the target in ribonucleic acids, DNA, RNA and proteins.[3,4] Environmental PAHs can be presented into the food chain by both animals and plants. However, the occurrence of PAHs in food, mainly in animal-origin foodstuffs, can originate during thermal treatments that used in the manufacturing and preparation of foods.[5] Among these processing procedures, smoking, grilling, roasting, baking, drying and frying, are identified as the main source of potentially high level of food contamination [2,6,7]

Infant formulas, existing in liquid-concentrate, powder and ready-to-feed formulas, are artificial replacements mimicking human breast milk. They designed based on cow milk or soy milk for the consumption of infants. Contamination with PAHs are an important subject area in food safety and quality and can be present in infant formulas from raw materials to finished products [8-10]. The level of PAHs in powdered infant milk (infant formulae) can be correlated to both on drying conditions applied and the amount of environmental contamination in the region which milk was collected.[11]

Benzo(α)pyrene (BaP) is one of the most familiar PAHs, and it is extensively used as an indicator component in studies addressing the toxicity PAHs in natural communities due to its multiple-ring structure and potential harm to human health.[12,13] Based on epidemiological studies over the long term and carcinogenicity, mutagenicity, and mode of action, it was classified as a probable human carcinogen (group 2A). However, assessment by the International Agency for Research on Cancer (IARC) has been reclassified it as a human carcinogen (group 1).[14]

Following the scientific committee on food (SCF) opinion of the potential hazards to human health from

*Corresponding Author: Mahboob Nemati, E-mail: nematim@tbzmed.ac.ir

PAHs in food, the European Commission (EC) has introduced an amendment to EC regulation 466/2001 and set maximum levels for BaP in a number of foodstuff, such as infant formula and baby food with a limit of 1 µg/kg.[15]

To date, there are various separation techniques developed for the analysis of BaP in different solid samples using several detection chromatographic techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC).[16-19]

Owing to the existence of BaP in ultra-trace amounts ($ng.g^{-1}$ level) in food samples, which are characterized by complex matrices and high levels of unwanted material, determination of BaP directly from complex solid sample remains a serious challenge. Therefore, a primary extraction is required to adequately determine these compounds. Soxhlet and ultra-sonication extraction are conventional extraction techniques for solid samples with reasonably good recoveries. Nevertheless, these techniques have many disadvantages such as time consuming process and require a large volume of organic solvents. These unfavorable characteristic are a conflict with analytical principles and modern green chemistry extraction methods, such as pressurized liquid extraction (PLE),[20,21] supercritical fluid extraction[22] and microwave-assisted extraction (MAE),[23,24] have been developed for extracting target analytes from solid matrices. These techniques speed up extraction time and reduce the volume of solvents required. The analytical techniques must also be selective and sensitive for their purification, isolation and identification. Generally, after primary extraction, another cleanup and/or concentration step such as solid phase extraction (SPE)[25] and liquid-liquid extraction (LLE)[26] are required, particularly for complex sample matrices, in order to minimize or remove unwanted interferences co-extracted with the analyte and purify as well as concentrate the extract. However, these purification techniques are time-consuming, requires large amounts of organic solvents, and prone to contamination and the loss of sensitivity.[27] Dispersive liquid–liquid microextraction (DLLME) a new extraction technique based on the use of a ternary mixture of solvent systems, in which appropriate mixture of disperser and extraction solvents are speedily injected into the sample solution (aqueous phase) to form a cloudy solution.[28-30] The sediment phase after centrifugation is analyzed by chromatographic or spectroscopic analysis methods.

In the present study, we have focused on the development of a simple and sensitive analytical method based on the DLLME technique for removing unwanted interferences and extraction/preconcentration of BaP in infant formula milk. Therefore, special attention was given to the optimization DLLME parameters for enhanced sensitivity and extraction efficiency. BaP was first extracted from matrixes of infant formula milk via acetonitrile, then its eluents were utilized as a dispersant of the followed DLLME for further purification and concentration of target analyte. The proposed method was then validated and applied for the analyzing of the level BaP in infant formula milk.

Materials and Methods

Materials

Analytical grade acetonitrile and methanol were obtained from Duksan Pure Chemicals Co. (Gyeonggi-do, Korea). BaP was provided from Sigma Aldrich (St. Louis, MO, USA). Dichloromethane, n-hexane, acetone, chloroform were supplied from Merck (Darmstadt, Germany). Infant formula samples were purchased from pharmacies in Tabriz, Iran.

Preparation of solution

The standard stock solutions of BaP were prepared by dissolving an appropriate amount of analyte in acetonitrile (100 µg/mL). To obtain a working solution, they were diluted with mobile phase and stored at 4˚C in a refrigerator and used daily.

Instrument and chromatographic conditions

The analysis was performed on the KNAUER HPLC system equipped with FL–detector RF/10 AXL (Shimadzu, Japan), pump K-1000 (Knauer, Berlin, Germany) and Degasser Biotech model 2003 (USA). The chromatographic separation was performed at room temperature (25˚C), by injecting 20 µL on a RP-C_{18} (Hichrom) chromatography column (250 mm × 4.6 mm, 5 µm) with a mobile phase of acetonitrile:water (95:5, v/v) at a flow rate of 1.5 mL min^{-1}. The excitation/emission wavelengths were 290/430 nm for BaP. Centrifuging (Hettich Rotofix 32A) was applied at 6000 rpm to separate the mixture. Total run time for each sample injection was 10 min and the retention time for BaP was 6.5 min.

Sample preparation and DLLME Procedure

An amount of 1 g formula milk sample was weighed and dissolved in 10 mL deionized water (10% w/v) to obtain a homogenized milk sample. Then, 1 mL of the sample was transferred to the test tube, and were spiked with different concentrations of BaP in order to assess extraction variables under different conditions. Three mL acetonitrile was added to each test for protein precipitation and it was placed in ultrasonic bath for 1 min and centrifuged for 5 min at 6000 rpm. The supernatant phase containing analyte and acetonitrile were separated and transmitted to a test tube for the DLLME process. Dichloromethane as an extracting solvent (150 µL) was added to the mixture. It was gently shaken with vortex for 1 min and then injected into 5 mL of deionized water.

In the next step, a cloudy solution consequently from dispersion of fine droplets of dichloromethane via acetonitrile into aqueous solution was formed in the vial and target analytic (BaP) was extracted into the dichloromethane. To separate the extraction phase, the liquid mixture was centrifuged for 5 min at 6000 rpm. After this process, the dispersed fine droplets of dichloromethane were sedimented at the bottom of the falcon. The sedimented phase separated using a Hamilton syringe, and evaporated by a nitrogen stream. Finally, the

residue was re-dissolved in 100 µl of mobile phase and 20 µl of the sample by a loop was injected into the HPLC system.

Real samples

Fifteen various infant formula samples from the different brands were provided from pharmacies in Tabriz, Iran, and the developed method was used for BaP quantification.

Results and Discussion

Analysis of BaP in infant formula milk samples is a challenging issue because of its affinity to the fat and proteins and their extremely low concentrations. Therefore, it is crucial to perform an optimized extraction method prior to instrumental analysis. In a DLLME method, the type and volume of extraction and disperser solvent have a considerable effect on extraction recovery.

Optimization of the DLLME method

Selection of extraction solvent

Three organic solvents having these qualifications were considered: chloroform, n-hexane and dichloromethane. The method of extracting was performed with each of the mentioned solvents. The sediment phase volume after DLLME was high for dichloromethane compared with n-hexane and chloroform. Moreover, it has a lower boiling point (39 °C) in compared with n-hexane and chloroform and the time of extraction was minimum. Thus, dichloromethane was selected as the best extraction solvent for BaP.

The evaluation of extraction solvent volume

To evaluate the effect of extraction solvent volume on the recovery of extraction, various volumes of dichloromethane between 150-300 µL were investigated. The sample volume less than 150 µL did not give an appropriate sediment phase and the maximum response was obtained when the volume of dichloromethane was 150 µL. Therefore, this volume was selected as a suitable volume for extraction (Figure 1).

Selection of dispersive solvent

In this work, acetonitrile, methanol and acetone were evaluated as dispersive solvents. For this purpose, the extraction process was carried out with the mentioned solvents. Obtained solution after protein precipitation by acetonitrile was cleaner than methanol and acetone and the cloudy solution was formed better than other solvents. Consequently, acetonitrile was selected as the best solvent for the protein precipitation and as the dispersive solvent.

The evaluation of dispersive solvent volume

The effect of volume of the disperser solvent i.e. acetonitrile was studied in the range of 2-3 mL along with the 150 µL dichloromethane as the extraction solvent. The obtained results showed the extraction efficiency enhanced when the volume of acetonitrile increased up to 3 mL, and then decreased according to an increased volume of acetonitrile for the target analytes. The reason might be that a cloudy state could not form well in a low volume of acetonitrile, which consequently resulted in low extraction efficiency. Thus, an acetonitrile volume of 3 mL was selected as the best disperser solvent volume (Figure 2).

Evaluation of method validation

The linearity of the calibration curve was constructed using the assays of the spiked sample of BaP solutions to infant formula in eight concentrations (0.5, 1.0, 2.5, 3.5, 5.0, 7.5, 10.0 and 15.0 ng/mL). The calibration curve was linear for these concentrations (R^2=0.998). To assess the precision and accuracy of the suggested technique, intraday and interday repeatability values were studied by evaluating tree replicate samples spiked with BaP at four levels (0.5, 3.5, 7.5 and 15 ng/ml) on the same day and three different days, respectively, and each concentration was done in triplicate. The precision of the method was estimated by the relative standard deviation (RSD %). In order to evaluate the accuracy, infant formula milk samples were spiked at four levels (0.5, 3.5, 7.5 and 15 ng mL^{-1}) of BaP. Accuracy values were calculated using the following

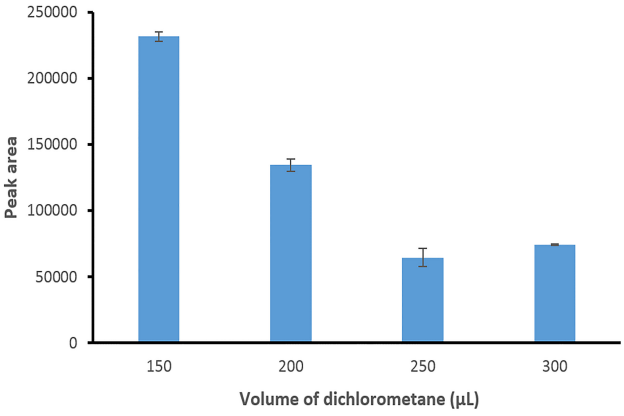

Figure 1. Effect of the dichloromethane volume (as extraction solvent) on the extraction of BaP by DLLME (concentration of BaP, 10 ng/mL; dispersant solvent, acetonitrile; volume, 3 mL)

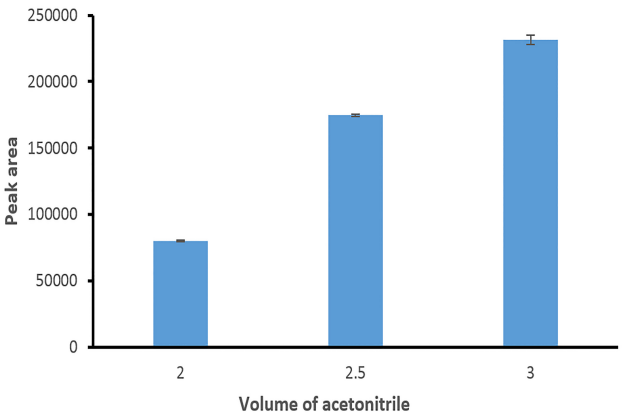

Figure 2. Effect of the acetonitrile volume (as dispersive solvent) on the extraction of BaP by DLLME (concentration of BaP, 10 ng/mL; extraction solvent, dichloromethane; volume, 150 µL.

Table 1. Validation data of the developed analytical method for quantification of BaP in infant formula.

Linear range (ng mL^{-1})	R^2	RSD%		LOQ (ng mL^{-1})	LOD (ng mL^{-1})	Accuracy (%)
		Intra- day(n=3)	inter-day(n=3)			
0.5-15	0.998	0.44-2.90	0.23-7.76	0.115	0.345	89-97

Table 2. Comparison of the proposed extraction methods for quantification of BaP.

Method	Matrix	Time[a]	amount of sample (g)	Reference
LLE–HPLC–FLD	Infant formula	More than 240 min	10	[10]
LLE–HPLC–FLD-DAD	Infant formula	More than 30 min	10	[9]
DLLME–HPLC	Milk sample	46 min	2	[31]
SPE–HPLC	Human breast milk	-	-	[12]
DLLME–HPLC–FLD	Infant formula	25 min	0.1	This work

[a]The required time for extraction
LLE: Liquid-liquid extraction
FLD: Fluorescence detector
DAD: Diode array detector
SPE: Solid Phase extraction

formula: Accuracy= 100× (found value/nominal value).

Limit of detection (LOD) and limit of quantification (LOQ) were evaluated by 3 and 10 folds of the standard deviation of intercept of calibration curve to the slope, respectively.[32] The results were listed in Table 1. RSD% values were between 0.23–7.76% for intraday (n = 3) and 0.44–2.9 % for interday (n = 3) which indicate the precision of the developed method. A linear calibration curve was plotted by analyzing the infant formula samples spiked with 0.5 to 15 ng/ mL BaP. BaP with a correlation coefficient equal to 0.998. LOD and LOQ for BaP were 0.115 and 0.350 ng/mL, respectively. The values of accuracy percent for infant formula samples using the suggested method were 89% to 97%. These data confirm the acceptable accuracy and precision and it indicates the validity of the developed method.

Comparison of the method with other procedures

The proposed DLLME-HPLC-FLD method for the determination of BaP in infant formula was compared with the other analyzed methods in the literature and the results were presented in Table 2. It was found that the extraction time and required sample for the suggested method was much lower than that of the other techniques. It offered benefits such as low cost, simple, rapid, efficient, and reproducible that can be applied for clean-up and pre-concentration of BaP from infant formula.

Application of the DLLME methodology to real sample

Fifteen samples of infant formula milk were collected from different brands of pharmacy in Tabriz city (Iran) and were analyzed with the developed DLLME method. The results showed all samples were free of BaP contamination (< LOD).

Conclusion

A DLLME-HPLC-FLD procedure was developed for quantification and rapid extraction of BaP at very low levels in infant formula samples. A clean separation based on the obtained chromatogram is achieved without matrix interference. The proposed technique has advantages such as low consumption of organic solvent, simplicity, economical and higher sensitivity and shorter extraction time. The results indicate that the method is appropriate for the quantification of BaP in infant formula samples.

Acknowledgments

This study was funded by the Vice Chancellor for Research of Tabriz University of Medical Sciences (58254), Tabriz, Iran, for financial support, and it is a part of A. Faridi's M.S. thesis registered at Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Author Contributions

AF: acquisition of data, drafting the manuscript, MN: design of the work, analysis of data, revising the manuscript. All authors have read and agreed to the published version of the manuscript and they are accountable for all aspects of the work.

Conflict of Interest

The authors report no conflicts of interest.

Reference

1. Huang Y, Wei J, Song J, Chen M, Luo Y. Determination of low levels of polycyclic aromatic hydrocarbons in soil by high performance liquid chromatography with tandem fluorescence and diode-array detectors. Chemosphere. 2013;92(8):1010-6. doi:10.1016/j.chemosphere.2013.03.035
2. Samanta SK, Singh OV, Jain RK. Polycyclic aromatic hydrocarbons: Environmental pollution and bioremediation. Trends Biotechnol. 2002;20(6):243-8. doi:10.1016/S0167-7799(02)01943-1

3. Kim K-H, Jahan SA, Kabir E, Brown RJ. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int. 2013;60:71-80. doi:10.1016/j.envint.2013.07.019
4. Moorthy B, Chu C, Carlin DJ. Polycyclic aromatic hydrocarbons: From metabolism to lung cancer. Toxicol Sci. 2015;145(1):5-15. doi:10.1093/toxsci/kfv040
5. Wenzl T, Simon R, Anklam E, Kleiner J. Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the european union. Trends Analyt Chem. 2006;25(7):716-25. doi:10.1016/j.trac.2006.05.010
6. Abdel-Shafy HI, Mansour MS. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egypt J Pet. 2016;25(1):107-23. doi:10.1016/j.ejpe.2015.03.011
7. Lee S, Ho K, Chan L, Zielinska B, Chow JC. Polycyclic aromatic hydrocarbons (PAHs) and carbonyl compounds in urban atmosphere of hong kong. Atmos Environ. 2001;35(34):5949-60. doi:10.1016/S1352-2310(01)00374-0
8. Iwegbue CM, Edeme JN, Tesi GO, Bassey FI, Martincigh BS, Nwajei GE. Polycyclic aromatic hydrocarbon concentrations in commercially available infant formulae in nigeria: Estimation of dietary intakes and risk assessment. Food Chem Toxicol. 2014;72:221-7. doi:10.1016/j.fct.2014.06.026
9. Ciecierska M, Obiedziński M. Polycyclic aromatic hydrocarbons in infant formulae, follow-on formulae and baby foods available in the polish market. Food Control. 2010;21(8):1166-72. doi:10.1016/j.foodcont.2010.01.013
10. Cho H-K, Shin H-S. Evaluation of polycyclic aromatic hydrocarbon contents and risk assessment for infant formula in korea. Food Sci Biotechnol. 2012;21(5):1329-34. doi:10.1007/s10068-012-0175-1
11. Kishikawa N, Wada M, Kuroda N, Akiyama S, Nakashima K. Determination of polycyclic aromatic hydrocarbons in milk samples by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2003;789(2):257-64. doi:10.1016/s1570-0232(03)00066-7
12. Gazioglu I, Tekkeli SEK. Development and validation of a hplc method for the determination of benzo (a) pyrene in human breast milk. Food Sci Biotechnol. 2017;26(2):319-22. doi:10.1007/s10068-017-0043-0
13. Ebrahimi V, Eyvazi S, Montazersaheb S, Yazdani P, Hejazi MA, Tarhriz V, et al. Polycyclic aromatic hydrocarbons degradation by aquatic bacteria isolated from khazar sea, the worlds largest lake. Pharm Sci 2021;27(1):121-30. doi:10.34172/PS.2020.28
14. Humans IWGotEoCRt. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum. 2010;92:1-853.
15. EU Commission, Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs, Official Journal European Communities L77. 2001. https://op.europa.eu/en/publication-detail/-/publication/52b2484d-39e0-4aa9-ba19-4b13a887bb1c
16. Conchione C, Purcaro G, Conte LS, Moret S. Solid-phase microextraction with gas chromatography and mass spectrometry determination of benzo (a) pyrene in microcrystalline waxes used as food additives. J Sep Sci. 2015;38(10):1749-54. doi:10.1002/jssc.201401246
17. Zhang X, Cai X, Li R. [Rapid determination of benzo [a] pyrene in foods by ultra performance liquid chromatography-triple quadrupole mass spectrometry with atmospheric pressure chemical ionization]. Se Pu. 2017;35(6):608-12. Chinese. doi:10.3724/SP.J.1123.2017.02021
18. Ledesma E, Rendueles M, Díaz M. Spanish smoked meat products: Benzo (a) pyrene (bap) contamination and moisture. J Food Compos Anal. 2015;37:87-94. doi:10.1016/j.jfca.2014.09.004
19. Olatunji OS, Fatoki OS, Opeolu BO, Ximba BJ. Determination of polycyclic aromatic hydrocarbons [PAHs] in processed meat products using gas chromatography–flame ionization detector. Food Chem. 2014;156:296-300. doi:10.1016/j.foodchem.2014.01.120
20. Choi M, Kim Y-J, Lee I-S, Choi H-G. Development of a one-step integrated pressurized liquid extraction and cleanup method for determining polycyclic aromatic hydrocarbons in marine sediments. J Chromatogr A. 2014;1340:8-14. doi:10.1016/j.chroma.2014.03.015
21. Mercier F, Gilles E, Saramito G, Glorennec P, Le Bot B. A multi-residue method for the simultaneous analysis in indoor dust of several classes of semi-volatile organic compounds by pressurized liquid extraction and gas chromatography/tandem mass spectrometry. J Chromatogr A. 2014;1336:101-11. doi:10.1016/j.chroma.2014.02.004
22. Han Y, Ren L, Xu K, Yang F, Li Y, Cheng T, et al. Supercritical fluid extraction with carbon nanotubes as a solid collection trap for the analysis of polycyclic aromatic hydrocarbons and their derivatives. J Chromatogr A. 2015;1395:1-6. doi:10.1016/j.chroma.2015.03.038
23. Mahmoudpour M, Mohtadinia J, Mousavi M-M, Ansarin M, Nemati M. Application of the microwave-assisted extraction and dispersive liquid–liquid microextraction for the analysis of PAHs in smoked rice. Food Anal Methods. 2017;10(1):277-86. doi:10.1007/s12161-016-0579-2
24. Mahmoudpour M, Pilevar Z, Javanmardi F, Taram F, Mousavi M-M. PAHs in toasted bread: determination using microwave-assisted extraction and dispersive liquid–liquid microextraction followed by high-performance liquid chromatography. Anal Methods. 2018;10(20):2398-404. doi:10.1039/C8AY00475G
25. Ma J, Xiao R, Li J, Yu J, Zhang Y, Chen L. Determination

of 16 polycyclic aromatic hydrocarbons in environmental water samples by solid-phase extraction using multi-walled carbon nanotubes as adsorbent coupled with gas chromatography–mass spectrometry. J Chromatogr A. 2010;1217(34):5462-9. doi:10.1016/j.chroma.2010.06.060

26. Pena MT, Casais MC, Mejuto MC, Cela R. Development of an ionic liquid based dispersive liquid–liquid microextraction method for the analysis of polycyclic aromatic hydrocarbons in water samples. J Chromatogr A. 2009;1216(36):6356-64. doi:10.1016/j.chroma.2009.07.032
27. Song X, Li J, Liao C, Chen L. Ultrasound-assisted dispersive liquid–liquid microextraction combined with low solvent consumption for determination of polycyclic aromatic hydrocarbons in seawater by GC–MS. Chromatographia. 2011;74(1-2):89-98. doi:10.1007/s10337-011-2048-9
28. Rezaee M, Assadi Y, Hosseini M-RM, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid–liquid microextraction. J Chromatogr A. 2006;1116(1-2):1-9. doi:10.1016/j.chroma.2006.03.007
29. Soltanmohammadi F, Mogaddam MRA, Khoubnasabjafari M, Jouyban A. Development of salt induced liquid–liquid extraction combined with amine based deep eutectic solvent-dispersive liquid–liquid microextraction; an efficient analytical method for determination of three anti-seizures in urine samples. Pharm Sci. 2020;26(3):323-31. doi: 10.34172/PS.2020.14
30. Mohebbi A, Farajzadeh MA, Jouyban A, Nemati M, Afshar Mogaddam MR. Development of sodium sulfate induced water based dispersive liquid-liquid microextraction for the extraction of four tricyclic antidepressants in urine samples prior to their determination by gas chromatography–mass spectrometry. Pharm Sci. 2021;27(1):76-85. doi:10.34172/PS.2020.24
31. Mahmoudpour M, Mohtadinia J, Ansarin M, Nemati M. Dispersive liquid–liquid microextraction for HPLC-UV determination of PAHs in milk. J AOAC Int. 2016;99(2):527-33. doi:10.5740/jaoacint.15-0169
32. Ershadi S, Shayanfar A. Are LOD and LOQ reliable parameters for sensitivity evaluation of spectroscopic methods? J AOAC Int. 2018;101(4):1212-3. doi:10.5740/jaoacint.17-0363