Ultrasound-Assisted Emulsified Microextraction Based on Deep Eutectic Solvent for Trace Residue Analysis of Metribuzin in Urine Samples

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Deep eutectic solvent was used as extraction solvent to develop and optimize a new sample preparation method for the determination of metribuzin in urine samples. In order to determine the optimal values of the effective factors in the deep eutectic solvent-based ultrasound assisted emulsification microextraction method, six effective parameters were selected. The design of experiments was performed using the one-variable-at-a-time method. Totally, 96 experimental runs were performed, and the samples were analyzed using high-performance liquid chromatography with a UV detector. Under the optimum conditions, the calibration curve for metribuzin was linear in the concentration range of 5 to 500 µg L⁻¹ for urine samples. The accuracy and reproducibility of the introduced method were determined using the relative recovery (RR %) and relative standard deviation (RSD %) tests on the fortified urine samples. RR % and RSD % were found to be 96.3–101.7 % and 3.2–7.6 %, respectively. The limit of quantification and the limit of detection were obtained 5 and 0.8 µg L⁻¹, respectively.

Keywords: deep eutectic solvents, sample preparation, ultrasound assisted emulsified microextraction, metribuzin, urine, high performance liquid chromatography

Due to the world population increase and limitations in the production of food products, pesticides are a group of chemical substances extensively used to eliminate, keeping away, or removing any kind of insect, rodent, fungi, or other types of annoying organisms. Although pesticides have a great impact on improving agricultural productivity and increase of food production, the processes of production, formulation, storage, transformation, and marketing of crops and also the wide application of these substances cause occupational exposures, environmental contamination, and the presence of their residuals in food [1-3]. Small amount of pesticides can also cause the occurrence of symptoms, diseases, and irreparable poisonings in humans. Thus, trace monitoring of these compounds is of great importance from occupational and environmental perspectives [4].

Metribuzin (4-amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one) is one of the triazine herbicides used for protecting agricultural products against the irregular growth of weeds [5, 6]. This herbicide has a long persistency in soil and also it has been found in surface and groundwater, as it benefits from high solubility in the aqueous medium making it to be exist in water resources [7, 8]. Human and animal studies have shown evidence of a relationship between exposure to this pesticide and genotoxic effects, cellular changes, and the occurrence of some disorders in the immune system, liver, kidney, and thyroid [9, 10].

Considering the extensive use of pesticides, development of fast and reliable technique for trace residue analysis of these compounds in various media such as biological, food, fruit, and vegetable samples is of great importance [11-13]. Among the instrumental methods, high-performance liquid chromatography (HPLC) instrument is an appropriate option for analyzing trace amount of pesticides in different samples, such as water, urine, and fruits [14].

Sample preparation is considered an important step in the process of analysis. The main goals of sample preparation are elimination of interfering agents, preconcentration of the desired analyte, and transforming the analyte (if required) to a more appropriate extraction solvent. In addition, using
smaller sample volume, improvement of selectivity in the extraction process, automation, reducing the consumption of organic solvents and laboratory equipment have been proposed recently [15].

In the past, two methods of liquid-liquid extraction [16] and solid-phase extraction [17, 18], were often used for extraction and pre-concentration of analytes from the sample matrices. Recently, novel procedures, in which, low amount of organic solvents are used, have been developed. For example, in the liquid phase microextraction (LPME) method, the extraction of analyte from a sample containing the liquid phase is performed using small amount of water-insoluble solvent [19]. The LPME technique is divided into three main categories including single-drop microextraction (SDME), hollow fiber liquid-phase microextraction (HF-LPME), and dispersive liquid-liquid microextraction (DLLME) [20].

In 2006, in response to the need for a fast, cost-effective, and environmentally friendly sample preparation technique, DLLME method was introduced by Rezaei et al. The DLLME method is applicable for a variety of samples such as soil, urine, and food. In this procedure the extraction process is based on the tendency of the analyte to the aquatic sample phase and the organic phase of the extractor solvent [21, 22]. Considering the advantages of the relatively novel DLLME method over the previous methods, its attraction has been retained for the analytical toxicologists, and, in comparison with the other sample preparation techniques, it has a significant share of studies. However, DLLME suffers from some limitations. The first and most important drawback is the application of an extra organic solvent as a disperser solvent, which causes entrance of the added solvent into the environment. Also, the required time of preparation is extended due to the numerous steps during the sample preparation process. Although by manual injection of the extractor solvent into the sample, the tiny droplets and cloudy phase are rapidly created, the contact surface between the aqueous phase of the sample solution and the organic phase of the extractor solvent is not in its maximum range [23].

In addition to the benefits that the DLLME method poses, the ultrasound-assisted emulsified microextraction (UA-EME) technique has other advantages such as the ease of forming small emulsions, creating the maximum contact surface between the aqueous phase of the sample and the organic phase of the extractor solvent. Moreover, ultrasound application eliminate disperser solvent consuming and avoids increasing the sample and wastewater volumes [24].

The only limitation of this technique is using organic solvents as the extractor solvent. The organic halogenated solvents, especially chlorinated solvents (e.g., carbon tetrachloride, chloroform, trichloroethylene, chlorodibromomethane, etc.), which are usually used as the extractor solvent, have some disadvantages such as toxicity, incompatibility with the environment, and high price [25]. In order to eliminate this limitation, which can be considered as the last remaining limitation for solvent-based microextraction methods, development of a novel extraction method using environment friendly extraction solvent is essential.

The deep eutectic solvents (DESs) were first developed in 2003 by Abbott et al. for using in oil, gas, and biomass industries. DES is produced by combining two natural substances, between which the hydrogen bond forms during synthesize process. The substance with free electron pairs is known as hydrogen bonding donor (HBD) and the substance with empty orbit is known as hydrogen bond acceptor (HBA). One or both raw materials may be solid, however the resulting product of such combination has a liquid form and much lower melting point comparing to the initial substances [26]. In comparison to the other solvents, the DESs have advantages such as more compatibility to the environment as well as biodegradability, nontoxicity, and also it is more inexpensive. Moreover, the physicochemical properties of the DESs, such as density, viscosity, conductivity, surface tension, and etc., can be engineered and changed as needed [27, 28]. Thus, with the assessment of the feasibility of applying DESs instead of organic solvents in sample preparation methods, it would be possible to reach a novel method.

The aim of this study was the development of DES-UAEME method as a rapid, easy, inexpensive, effective, safe, and environment friendly sample preparation procedure for determination of trace metribuzin in urine samples, in which, the substitution of chlorinated organic solvents (COSs) with DESs is considered to eliminate the drawbacks of previous sample preparation methods.

**Experimental part**

**Chemicals.** Metribuzin standard was obtained from Sigma-Aldrich (Germany). The analytical grade of organic solvents, including chlorobenzene, carbon tetrachloride, chloroform, methanol, and acetonitrile, were purchased from Merck (Germany). Also, chemical substances of choline chloride, phenol, sodium chloride, tetrahydrofuran (THF) with the purity of higher than 99 %, and buffer solutions at different pH were bought from Merck (Germany). Double distilled water was provided by Purite purification system.

**Instruments.** In order to analyze the samples, a high-performance chromatography system, equipped with a K-1001 pump and a K-2006 ultraviolet detector (Knauer, Germany), was used. The chromatography conditions were a C18 column (L= 150 mm, ID=4.6 mm), a mobile phase of methanol (70 %), and water (30 %), pump flow-rate of 1.0 mL min⁻¹, column temperature of 25°C, and ultraviolet wavelength 290 nm, respectively. Fourier transform-infrared spectrometer (Thermo AVATAR, USA) was used.
to identify functional groups of DESs. The other equipment and tools used in this study include a double ionized water distillation machine (PURITE, USA), micro sampler (Socorex, Germany), digital scale (Sartorius, Germany), ultrasonic bath (Sono, Switzerland), magnetic stirrer (Chiltern, USA), digital thermometer (TP3001, China), pH meter (Metrohm, Switzerland), centrifuge (Hettich zentrifugen Rotofix 32, China). All glassware used in this study were washed with acetone and deionized water and dried in an oven at 50°C.

**Preparation of stock and standard solutions**

The stock solution with the concentration of 1000 mg L\(^{-1}\) was prepared in acetonitrile and kept in the refrigerator at 4°C. The standard solutions with different concentrations were prepared daily by diluting the stock solution with deionized water.

**Synthesis of DES**

In order to synthesis DES, choline chloride ((2-Hydroxyethyl) trimethylammonium chloride) as the HBA, and phenol as the HBD were combined in the specified molar ratios at 50°C for 5 min, to obtain three hydrophobic DES with different structures.

Considering the addition of phenol molar ratio in preparation of DES, the viscosity of the DES solution can be decreased. In this way, the solution with the ratio of 1:1 has the highest viscosity and the solution with the ratio of 1:4 has the lowest viscosity, so that the solution with the ratio of 1:1 has a very high viscosity, Therefore, the ratios of 1:2 (DES1), 1:3 (DES2), and 1:4 (DES3) were selected for the investigation.

**Ultrasound-assisted emulsification microextraction**

a) 400 µL of the extraction solvent (DES1) was injected through a syringe into a 15 mL sample tube containing 10 mL of sample. b) Then, 400 µL of THF as an emulsifier agent was injected into the solution and at this stage a cloudy solution, which prove the formation of insoluble self-aggregation in nano and molecular dimensions was formed. The cloudy solution was subjected to ultrasonication to guarantee well distribution of extraction solvent droplets in aqueous phase. c) In the next step, the solution was centrifuged and two separated phases were obtained. The phase containing the analyte was separated using a syringe and poured into a new tube and was dried under a nitrogen stream. d) Finally, the residual substance was dissolved in acetonitrile and was injected to HPLC-UV for quantification. Fig. 1 shows the steps of the UA-EME method.

**Optimization procedure**

In order to determine the optimal values and levels of the effective factors in the extraction of metribuzin via UA-EME method, six effective parameters including the type of extraction solvent, extraction solvent volume, the amount of added salt, sample pH, time of exposure with ultrasonic (sonication time), and centrifugation time were selected. The design of experiments was performed using the one-variable-at-a-time method, and each parameter was tested in different levels and three replicates to select the optimal values. In each step of optimization, all the effective variables are kept constant except the desired parameter which is changed in different levels of the experimental range to determine its optimal value. The experiment steps were done with three replicates and the average values were reported as results. Totally, 96 steps were performed for optimization of the effective factors in the extraction of metribuzin from standard samples. Table 1 shows the variables with corresponding levels in the optimization steps. In all steps of the experiment, standard samples with a concentration of 1000 ng L\(^{-1}\) and a volume of 10 mL were used.

![Fig. 1 The steps of the UA-EME method.](image)
Results and discussion

**FT-IR**

In order to make the DES, a hydrogen bond should be formed between choline chloride and phenol. To investigate the formation of this bond, the analysis of the Fourier Transform Infrared Spectra (FT-IR) related to choline chloride and pure phenol as well as their resulting solvent was performed. The obtained results are presented in Fig. 2. The vibrations related to the O-H and C-N bonds in the structure of pure choline chloride were in the range of 2356 and 1092 cm$^{-1}$, respectively (Fig. 2a). Absorption in the important bonds that present in the analysis of pure phenol FT-IR spectra were O-H (3260 cm$^{-1}$), C=C (1472 and 1598 cm$^{-1}$), and C-O (1224 cm$^{-1}$), respectively (Fig. 2b). The characteristic peaks of both substances were present in the DES spectrum (Fig. 2c). All peaks were approximately in the same range as the peaks of the pure material, except for the peak associated with the O-H bond, which was in the pure phenol spectra in the range of 3260 cm$^{-1}$ and transferred to the range of 3236 cm$^{-1}$. This could indicate the sharing of oxygen atom electrons to form the hydrogen bond between phenol and choline chloride during the formation of DES.

**Selection of the extraction solvent (DESs versus COSs)**

In the UA-EME process, the extraction solvent must have certain properties such as insolubility in water, and the ability to extract the desired compound as well as the appropriate chromatographic behavior. In order to develop the application of DES in the mentioned method, three solvents, DES1, DES2, and DES3 which their compositions were described in the previous section were tested in addition to three mentioned organic solvents including chlorobenzene (C$_6$H$_5$Cl), carbon tetrachloride (CCl$_4$), and chloroform (CHCL$_3$). Therefore, 6 parallel levels were examined.

![Fig. 2 FT-IR spectra of choline chloride (a), phenol (b) and DES1 (c).](image-url)
Based on the obtained results, during the application of chlorobenzene and chloroform as the extraction solvent, after the formation of the cloudy phase and in the step of separating the extraction solvent through the centrifuge, the whole volume of the added extraction solvent has not separated which could be due to their small amount of solubility in water. This issue reduced the extraction ability of these solvents in comparison to the other solvents. Overall, among the six studied extraction solvents, DES1 and carbon tetrachloride showed the highest relative recovery (RR), however, due to the advantages of DES compared to the organic solvents, DES1 was selected as the optimum extraction solvent for the next steps (Fig. 3a).

**Selecting the volume of extraction solvent**

In this step, to investigate the effect of extraction solvent volume on the analyte extraction recovery, the experiments were performed using various volumes in 6 parallel levels ranged from 100 to 600 µL of DES1. The lower range of extractant solvents volume was considered to be 100 µL, equivalent to HPLC injection volume. On the other side, due to the dilution effect, applying a high volume of extraction solvent cause a decrease in the enrichment factor. To prevent this, the upper level of the extracting solvent was considered to be 600 µL. The maximum recovery was obtained when 400 µL of DES1 was employed as extracting solvent. In the higher solvent values, the recovery rate remained constant, therefore, a volume of 400 µL was selected as optimum value (Fig. 3b).

**The effect of salt addition**

Due to the fact that ionic compounds have the highest solubility in water, the addition of salt to the solvent facilitates the analyte removal from the aqueous sample. This variable is called ionic power. In order to study the effect of adding salt, six different values of sodium chloride in the range of 0-25 W/V% were applied.

**Fig. 3** Steps to optimize the factors affecting extraction: a - selection of the extraction solvent type; b - selecting the volume of extraction solvent; c - the effect of salt addition; d - effect of sample pH; e - effect of sonication time; f - effect of centrifugation time.
The obtained results showed that, by the increase of salt concentration, the extraction rate diminishes, and the highest analyte extraction rate was obtained when no salt was added to the sample. It is because of the increase in sample viscosity that caused a reduction in the analyte penetration coefficient. Therefore, 0 % was selected as the optimum value and the next steps of the optimization were carried out without the salt addition (Fig. 3c).

**The effect of sample pH**

Through the adjustment of pH, the analyte molecules could be directed towards the ionization or molecular forms. In order to easier extraction of the analyte from aqueous samples (water-based samples), it must be directed towards molecularization. With the addition of an appropriate amount of hydrochloric acid or sodium hydroxide to the sample, metribuzin extraction rate was studied. For this purpose, the pH of the sample was examined at 6 levels in the range of 2-12. The highest extraction rate was achieved in around pH = 6. Metribuzin has a pKa equal to 1 (dissociation constant (Ka) = 0.1) [29]. To any extent that the dissociation constant is less than 1, the desired analyte has a greater molecular form in the water. Thus, the pH value of 6 was chosen for the next steps (Fig. 3d).

**Effect of sonication time**

In the sonication step, the test tube containing the sample and extraction solvent was placed in the ultrasonic bath to achieve maximum contact between the extraction solvent and sample solution. The effect of sonication time on the extraction efficiency was investigated in the 6 levels ranged 2-12 minutes. After 4 minutes, the extraction efficiency was constant. Therefore, 4 minutes was selected as the optimal sonication time for the next steps of the experiment (Fig. 3e).

**Effect of centrifugation time**

The centrifugation time in the UA-EME method is an important step to separate the extraction solvent from the sample. During this step, the cloudy phase of the sample disappears and two separate and clear phases are created. The effect of centrifuge time and speed on the extraction efficiency was investigated at 6 levels of the time ranges from 2-12 minutes using speed of 2500-4000 rpm. After 8 mins, the extraction efficiency was constant and the maximum extraction efficiency was obtained at the speed of 4000 rpm. Thus, the time of 8 minutes and the speed of 4000 rpm were chosen as the optimum values for the next steps (Fig. 3f).

**Analytical performance**

In order to draw the calibration curve, blank urine samples of unexposed individuals were diluted at a ratio of 1:2 in deionized water and then spiked with certain concentrations. Then, the prepared samples were extracted via the developed preparation method. The sketched calibration curve was linear in the concentration range of 5 to 500 µg L⁻¹ (n=5) and desirable linearity (R²=0.999) was achieved. The limit of quantification (LOQ), based on the lowest detected analyte concentration with 85-115 % accuracy and precision ≤10 % in five consecutive injections, and also the limit of detection (LOD, signal/noise = 3) were obtained 5 and 0.8 µg L⁻¹, respectively. The analytical figures achieved for the developed technique confirmed the proper efficiency, linearity, and reproducibility of this process for the quantification of metribuzin in urine samples.

**Application assay**

In order to assess the applicability and efficiency of the developed DES-USAEME method for the real urine samples, the spiked urine samples of unexposed persons with standard metribuzin concentrations of 5, 50, and 150 µg L⁻¹ were prepared. According to the developed method and setting the effective parameters based on the optimized condition, the samples were analyzed day to day (6 consecutive days) and in one day (n=6) and the relative recovery (RR%) values were calculated at the mentioned standard concentrations. The RR% values in the intra-day (repeatability) and inter-day (reproducibility) situations were in the range of 97.5 to 101.7 and 96.3 to 99.5 percent, respectively.

Also, in order to assess the reproducibility and repeatability, the RSD % values were calculated using the concentrations ranges of 5, 50, and 150 µg L⁻¹. The obtained results for RSD % were in the range of 3.2 to 7.6 percent which confirms the acceptable precision of the developed method. The results of relative recovery (RR %) and relative standard deviation (RSD %) have reported in Table 2. Overall, the results achieved in the recovery and precision confirm that the proposed method has concentrated and purified the desired analyte from the complex matrix of the real urine sample with high accuracy and precision and confirms the validity of the method.

**Comparison to other sample preparation methods**

Comparison of the obtained results in the present study to the techniques reported in other studies for analysis of metribuzin and the other triazine toxins in different samples has been briefly reported in Table 3. In the majority of methods, the organic solvents have been used as the extraction or elution solvent which is toxic for the analyte and also could enter the environment as waste and may cause ecosystems damages [5, 9, 17]. While, in the developed method a green solvent with a volume much less than other methods (the used volume of DES solvent was 2.5-80 times less than the other methods) was used. In addition, the present method requires less equipment, chemicals, skills, and costs than the other presented studies. Therefore, the current developed method can be widely used in the laboratories of developing countries.
Table 2. The results of analysis, accuracy and precision of metribuzin determination in urine.

| Real sample | Spiked levels (μg L⁻¹) | RR ± SD (%), (n=6) | Spiked metribuzin (μg L⁻¹) | measured mean ± SD (μg L⁻¹) | precision (% RSD) | accuracy (% RE) |
|-------------|------------------------|---------------------|---------------------------|-----------------------------|------------------|----------------|
| Urine       | 0                      | N.D.                | 0                         | 0                           | -                | -              |
|             | 5                      | 97.5 ± 7.6          | 96.3 ± 6.2                | 5                           | 4.875 ± 0.38     | 7.79           | 2.5            |
|             | 50                     | 99.1 ± 6.5          | 99.5 ± 5.1                | 50                          | 49.55 ± 3.25     | 6.55           | 0.9            |
|             | 150                    | 101.7 ± 5.1         | 99.8 ± 3.2                | 150                         | 152.55 ± 7.65    | 5.01           | 1.7            |

* N.D. - Not detected

Table 3. Comparison of the LOD, linear range and RSD of different methods for determination of metribuzin.

| Sample | Method           | LOD (μg L⁻¹) | Linear range (μg L⁻¹) | RSD (%) | Ref.      |
|--------|------------------|--------------|-----------------------|---------|-----------|
| Soil   | HS-SPME¹, GC-MS  | 0.1–2 (ng g⁻¹) | 5-200                 | 11-22   | (1)       |
| Soil   | MAE², HPLC-UV    | 300          | 1000-19000            | 6.32    | (30)      |
| Urine  | MI-SPE³, HPLC-UV | 5.7          | 20-120                | 3.58    | (9)       |
| Olive oil | MSPD⁴, GC-MS | 10–60 (µg kg⁻¹) | NR                    | 3.3-9.9 | (17)      |
| Urine  | DES-UAE-ME, HPLC-UV | 0.8       | 5-500                 | 1.5-4   | This study |

1. Headspace solid-phase microextraction; 2. Microwave-assisted extraction; 3. Molecularly imprinted solid-phase extraction; 4. Matrix solid-phase dispersion extraction.

Comparison of RSD %, MDL, and LOQ values of the proposed method to the similar methods [1, 17], revealed that, the current method using a relatively inexpensive equipment (HPLC) has considerable high sensitivity, accuracy, and reproducibility. Moreover, the present developed technique benefits from important advantages such as environmental compatibility, simplicity, and cost-effectiveness over the previous works. Finally, the current work is an appropriate method for extraction and pre-concentration of metribuzin from biological samples of exposed individuals.

Conclusions

In the present study, the DES-UA-EME technique was successfully developed for the extraction of metribuzin from urine samples and subsequent quantification by high-performance liquid chromatography. Different variables affecting the extraction process of this compound were investigated and optimized. Employing a solvent with lower toxicity and volume is an important advantage for a sample preparation method as this developed method possessed and enjoyed such advantages. The obtained results illustrated that, in addition to having better performance and lower volume requirement, the deep eutectic solvent could replace toxic organic solvents in sample preparation methods. Using ultrasonic, the formation of tiny extraction solvent droplets in the sample was remarkably increased; Therefore the contact area (surface) between the analyte and solvent was increased which resulted in a high extraction efficiency and a decrease in extraction time. In comparison to the other sample preparation methods, the proposed technique has the advantages of shorter extraction time, simplicity, and applicability in laboratories with less equipment.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Orazbayeva, D.; Koziel, J.A.; Trujillo-Rodriguez, M. J.; et al. Polymeric ionic liquid sorbent coatings in headspace solid-phase microextraction: A green sample preparation technique for the determination of pesticides in soil. *Microchem. J.* 2020, 157, 104996. https://doi.org/10.1016/j.microc.2020.104996.

2. Rani, L.; Thapa, K.; Kanojia, N.; Sharma, N.; et al. An extensive review on the consequences of chemical pesticides on human health and environment. *J. Clean. Prod.* 2020, 124657. https://doi.org/10.1016/j.jclepro.2020.124657

3. Pourbabaki, R.; Khadem, M.; Samiei, S.; et al. The protective effect of rosemary in mitigating
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oxidative stress induced by Chlorpyrifos in rat kidney. *J. Health. Safety. work.* 2020, W. 10 (2), 148-159. http://jhsw.tums.ac.ir/article-1-6298-en.html.

4. Su, D.; Li, H.; Yan, X.; et al. Biosensors based on fluorescence carbon nanomaterials for detection of pesticides. TrAC. Trends. *Anal. Chem.* 2020, 116126. https://doi.org/10.1016/j.trac.2020.116126.

5. Shah, J.; Jan, M. R.; Ara, B.; et al. Extractive spectrophotometric method for determination of metribuzin heribicide and application of factorial design in optimization of various factors. *J. Hazard. Mater.* 2009, 164(2-3), 918-22. https://doi.org/10.1016/j.jhazmat.2008.10.100.

6. Wahla, A. Q.; Anwar, S.; Mueller, J. A.; et al. Immobilization of metribuzin degrading bacterial consortium MB3R on biochar enhances bioremediation of potato vegetated soil and restores bacterial community structure. *J. Hazard. Mater.* 2020, 390, 121493. https://doi.org/10.1016/j.jhazmat.2019.121493.

7. Gaona, L.; Bedmar, F.; Gianelli, V.; et al. Estimating the risk of groundwater contamination and environmental impact of pesticides in an agricultural basin in Argentina. *Int. J. Environ. Sci. Technol.* 2019, 16, 6657-6670. https://doi.org/10.1007/s13762-019-02267-w.

8. Viti, M. L.; Mendes, K. F.; dos Reis, F. C.; et al. Characterization and metabolism of bound residues of three herbicides in soils amended with sugarcane waste. *Sugar. Tech.* 2020, 1-15. https://doi.org/10.1007/s12355-020-00884-1.

9. Heravizadeh, O. R.; Khadem, M.; Nabizadeh, R.; et al. Synthesis of molecular imprinted polymer nanoparticles followed by application of response surface methodology for optimization of metribuzin extraction from urine samples. *Chem. Pap.* 2018, 72(12), 3057-3068. https://doi.org/10.1007/s11696-018-0546-z.

10. Kostopoulou, S.; Ntatsi, G.; Arapis, G.; et al. Assessment of the effects of metribuzin, glyphosate, and their mixtures on the metabolism of the model plant Lemna minor L. applying metabolomics. *Chemosphere.* 2020, 239, 124582. https://doi.org/10.1016/j.chemosphere.2019.124582.

11. Khoshkhalagh, A. H.; Beygizadeh, M.; Golbabaei, F.; et al. Isotherm, kinetic, and thermodynamic studies for dynamic adsorption of toluene in gas phase onto porous Fe-MLL-101/OAC composite. *Environ. Sci. Pollut. Res.* 2020, 27, 44022-44035. https://doi.org/10.1007/s11356-020-1297-y.

12. Khoshkhalagh, A. H.; Golbabaei, F.; Beygizadeh, M.; et al. Toluene adsorption on porous Cu-BDC@ OAC composite at various operating conditions: optimization by response surface methodology. *RSC. Adv.* 2020, 10, 35582-35596. https://doi.org/10.1039/DORA06578A.

13. Zuluaga, M.; Yathe, G. L.; Rosero-Moreano, M.; et al. Multi-residue analysis of pesticides in blood plasma using hollow fiber solvent bar microextraction and gas chromatography with a flame ionization detector. *Environ. Toxicol. Pharmacol.* 2020, 103556. https://doi.org/10.1016/j.etap.2020.103556.

14. Lawrence, J. F. High-Performance Liquid Chromatography of Pesticides: Analytical Methods for Pesticides and Plant Growth Regulators. *Academic press Inc.* 2016, Vol. 12. [Book].

15. Nasiri, M.; Ahmadzadeh, H.; Amiri, A. Sample preparation and extraction methods for pesticides in aquatic environments: A review. TrAC. Trends. *Anal. Chem.* 2020, 123, 115772. https://doi.org/10.1016/j.trac.2019.115772.

16. Mahara, B. M.; Borossay, J.; Torkos, K. Liquid–liquid extraction for sample preparation prior to gas chromatography and gas chromatography–mass spectrometry determination of herbicide and pesticide compounds. *Microchem. J.* 1998, 58, 31-38. https://doi.org/10.1006/mchj.1997.151.

17. Ferrer, C.; Gómez, M. J.; García-Reyes, J. F.; et al. Determination of pesticide residues in olives and olive oil by matrix solid-phase dispension followed by gas chromatography/mass spectrometry. *Chromatogr. A.* 2005, 1069(2),183-194. https://doi.org/10.1016/j.chroma.2005.02.015.

18. Poole, C. F. New trends in solid-phase extraction. *TrAC. Trends. Anal. Chem.* 2003, 22, 362-373. https://doi.org/10.1016/S0165-9936(03)00605-8.

19. He, Y. Recent advances in application of liquid-based micro-extraction: A review. *Chem. Pap.* 2014, 68(8), 995-1007. doi:10.2478/s11696-014-0562-6.

20. Lambropoulou, D. A.; Albanis, T. A. Liquid-phase micro-extraction techniques in pesticide residue analysis. *J. Biochem. Biophys. Methods.* 2007, 70(2), 195-228. https://doi.org/10.1016/j.jbbm.2006.10.004.

21. Pourhossein, M.; Shahtaheri, S. J.; maleckkhani, H.; et al. Optimization of dispersive liquid liquid microextraction method for determination of trace salivary melatonin using high performance liquid chromatography. *Iran. Occup. Health.* 2017, 14(4), 94-85. http://ioh.tums.ac.ir/article-1-1825-en.html.

22. Pourhossein, M.; Shahtaheri, S. J.; Mazloumi, A.; et al. Dispersive Liquid–Liquid Microextraction for the Determination of Salivary Melatonin as a Biomarker of Circadian Rhythm. *J. Anal. Chem.* 2018, 73(10), 966-72. https://doi.org/10.1134/S106193481810009X.

23. Zgola-Grześkowiak, A.; Grześkowiak, T. Dispersive liquid-liquid microextraction. *TrAC. Trends. Anal. Chem.* 2011, 30(9), 1382-1399. https://doi.org/10.1016/j.trac.2011.04.014.

24. Ozcan, S.; Tor, A.; Aydin, M. E. Application of ultrasound-assisted emulsification-micro-extraction for the analysis of organochlorine pesticides in waters. *Water. res.* 2009, 43(17), 4269-4277. https://doi.org/10.1016/j.watres.2009.06.024.

25. Jordan, A.; Stoy, P.; Sneddon, H. F. Chlorinated Solvents: Their Advantages, Disadvantages, and Alternatives in Organic and Medicinal Chemistry. *Chem. Rev.* 2021, 121 (3), 1582-1622. DOI: 10.1021/acs.chemrev.0c00709.
26. Smith, E. L.; Abbott, A. P.; Ryder, K.S. Deep eutectic solvents (DESs) and their applications. Chem. Rev. 2014, 114(21), 11060-11082. https://doi.org/10.1021/cr300162p.

27. Soltanmohammadi, F.; Jouyban, A.; Shayanfar, A. New aspects of deep eutectic solvents: extraction, pharmaceutical applications, as catalyst and gas capture. Chem. Pap. 2021, 75, 439–453. https://doi.org/10.1007/s11696-020-01316-w.

28. Vanda, H.; Dai, Y.; Wilson, E. G. et al. Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents. C R Chim. 2018, 21(6), 628-638. https://doi.org/10.1016/j.crci.2018.04.002.

29. Li, G.; Meng, X.; Wang, J.; et al. A low-cost and high-efficiency carbazole-based porous organic polymer as a novel sorbent for solid-phase extraction of triazine herbicides in vegetables. Food. chem. 2020, 309, 125618. https://doi.org/10.1016/j.foodchem.2019.125618.

30. Shah, J.; Jan, M. R.; Ara, B. Quantification of triazine herbicides in soil by microwave-assisted extraction and high-performance liquid chromatography. Environ. Monit. Assess. 2011, 178(1), 111-119. https://doi.org/10.1007/s10661-010-1676-0.