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REVIEW ARTICLE

Application of Microextraction Methods to Extract and Determine the Occupational Analytes from Urine Samples: A Brief Review

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ABSTRACT

Inexpensive and simple microextraction methods with high efficiency are highly recognized approaches for sample preparation in the analysis of pollutant compounds. Therefore, the present study was aimed to review the studies conducted by Iranian researchers on the use of microextraction methods to determine the occupational analytes from the urine sample. In the current review study, we used keywords, including microextraction, determine, extract, analytes, and urine samples among published articles by Iranian researchers from 2000 to 2019 in databases of Google Scholar, ISC, SID, Magiran, Web of Science, ScienceDirect, PubMed, and Scopus. Then, the extracted articles during the past 20 years were categorized and analyzed according to the title, author name, publication year, study method, study type, and evaluation results. The results of reviewing the selected articles were discussed in terms of several topics. They included optimization of affecting factors method efficiency and extraction efficiency, optimization of parameters affecting extraction performance, application of the optimized method for real samples, and comparison of the proposed method with other procedures. The developed methods in the selected articles were found to be fast, simple, with minimum solvent consumption, short extraction time, and environmentally friendly that can be used as alternatives to conventional methods.

KEYWORDS: *Microextraction Methods, Extract and Determine, Occupational Analytes, Urine Samples, Brief Review*

INTRODUCTION

Human biomonitoring has been recognized as an efficient and cost-effective means to measure human exposure to environmental and occupational compounds. Human biomonitoring considers all sources and routes of intake, making it an ideal approach for conducting the health risk assessment [1-2]. Biological samples contain very complex compounds that interfere with the analysis and measurement processes. The small amounts of occupational and environmental contaminants cannot

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be measured even by the advanced analytical instruments, and they are often incompatible with analyzing processes. Therefore, it is necessary to develop susceptible and specific methods to measure such contaminants in biological samples with strong repeatability and high accuracy [3].

As demonstrated in Figure 1, the analysis process consists of several main steps, including sampling, sample preparation, separation, quantification, statistical evaluation, and decision-making based on the results [2]. Sample preparation is one of the essential steps in the analysis process that mainly involves the extraction steps, leading to the separation of desired species from the sample, pre-concentration for injection into the device, and an increase in the sensitivity and conversion of the analytes to a suitable form for quantitative and qualitative identification.

The choice of the preparation method depends on the working conditions, type of sample, and extraction phase [4-5]. Sample preparation for separating organic compounds from aqueous solutions is a time-consuming and controversial step in sample analysis methods [6]. Common methods for extracting compounds from aqueous media (i.e., liquid-liquid and solid-phase extraction) have such disadvantages as high consumption of toxic organic solvents, significant chemical additives, using complex equipment, requiring high amounts of secondary residues and high costs, having pre-filtration problems, and being time-consuming. The above-mentioned problems led to developing the micro-extraction methods to simplify sample preparation techniques [5-7-8].

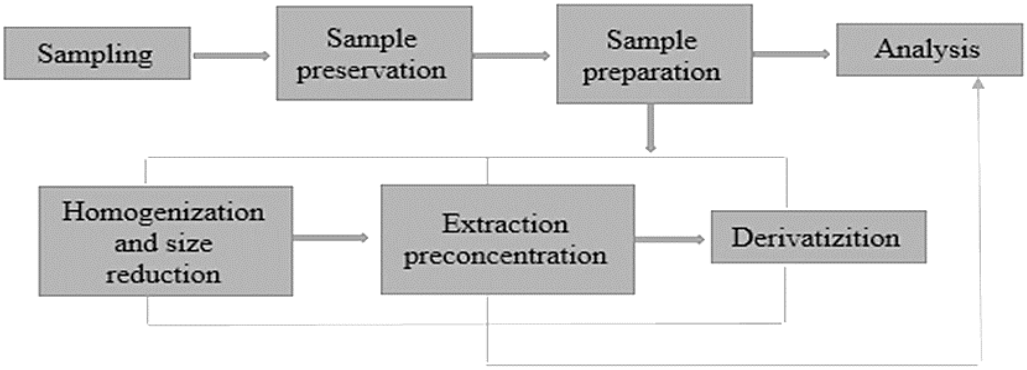

Fig 1. The Main Stages of the Analysis Process

The modern sample preparation techniques, in the liquid or solid phases, are often simpler, faster, and more selective with less consumption of reagent at the same time [5-9]. For example, in the liquid phase microextraction (LPME) method, an analyte can be extracted from a sample containing an aqueous phase by a small amount of water-insoluble solvent (acceptor phase) [10-12]. Ideally, sample preparation techniques should be fast, inexpensive, easy, and compatible with analysis devices [13]. Nowadays, special attention has been paid to some micro-extraction methods such as solid and LPME so as to develop them [14]. The application of micro-extraction methods for evaluating occupational exposure to chemicals makes it possible to determine the lowest amount of contaminants in the work environments [15]. The aim of this study was to briefly review studies conducted by Iranian researchers to investigate the application of microextraction methods for extracting and determining analytes from urine samples.

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Experimental:

This review study has investigated the application of microextraction methods for extraction and determination of occupational analytes from urine samples. To collect the required data, six available electronic databases were used, including Google Scholar, ISC, SID, Magiran, Web of Science, Science Direct, PubMed, and Scopus. The search was performed using such keywords as microextraction methods, extraction, determining, occupational analytes, and urine samples. At each stage, the searched articles in each database were fed into the endnote software.

In the first stage, a total of 162 documents related to the topic were entered into the software. In the second stage, a framework was selected for the study based on the review of published studies from the years 2000 to 2019. Therefore, the relevant documents before this period were deleted and 98 documents remained. After eliminating the duplicated records, 91 documents remained for the systematic review. In the next step, the titles of these articles were carefully reviewed, in which 33 irrelevant articles were deleted. After reviewing their abstracts, another 58 documents were excluded due to their irrelevant methodology. In addition, the full text of the five articles could not be accessed. Thus, they were also excluded from the review process. In the screening step, after studying the full texts, seven further articles were found not to be closely related to the subject in terms of purpose and method. A diagram of the study selection process has been presented in Figure 2.

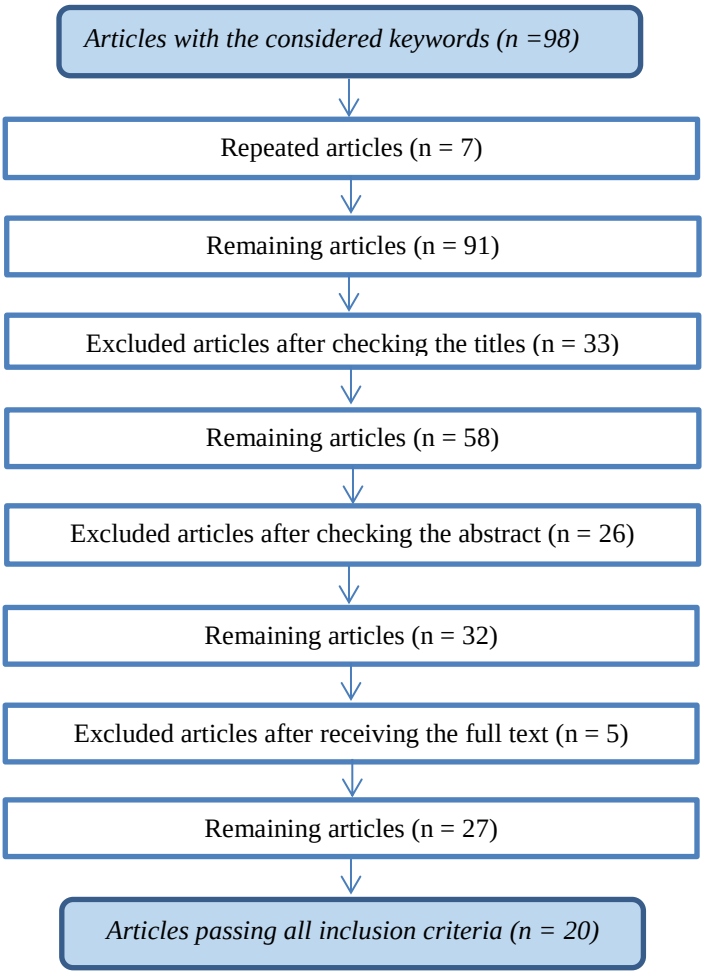

Fig 2. Flowchart of the Literature Review.

Table 1. Studies published in Persian journals

Year	Summary of the study method	Summary of results	Reference
2019	DLLME technique coupled with high-performance liquid chromatography equipped with uv detector was developed for trace extraction and determination of diazinon pesticide in human urine samples	A DLLME procedure was successfully developed for the extraction of diazinon from human urine samples.	[16]
2010	Extraction of ethylbenzene in minimal amounts was performed using the SP from the upper space in water and urine samples and analysis by GC.	Ethylbenzene solution was performed under optimized conditions with a very high detection limit.	[18]
2019	At first, a DLLME method for the extraction of chlorpyrifos in the urine sample was designed. Then, the Taguchi model was used to screen and investigate the role of effective factors on the extraction of chlorpyrifos from the urine. A central composite design was used to examine the interaction of these factors	This method can be used as a simple, fast, inexpensive, sensitive, and precise method for chlorpyrifos analyzing urine specimens in clinical and forensic toxicology laboratories.	[19]
2011	A mixture of extracting and dispersing solvents was used to perform the extraction process. The studied variables were evaluated and optimized to achieve cadmium uptake and recycling efficiency from biological samples.	The optimized method was successfully used to extract cadmium metal from urine samples. The pH of the sample and the volume of the extracted solvent were essential variables in the extraction of cadmium from the urine sample.	[20]

In the present study, a researcher reviewed the literature to identify inclusion and exclusion criteria based on the title and abstract. After removing the irrelevant articles, the full text of the remaining articles was investigated. Next, the desired results were extracted considering a number of focused parameters and then handed over to another researcher for review and revision if needed. Finally, 20 articles were selected to be examined for extracting the results.

RESULTS AND DISCUSSION

Among the studies selected for the review, four were published in Persian journals and 16 articles were published in English language journals. All of the studies were performed under laboratory conditions and their purpose was to use microextraction methods to extract and determine analytes from urine samples. Tables 1 and 2 illustrate the studies with the mentioned criteria

Table 2. Studies published in English journals.

Year	Summary of the study method	Summary of results	Reference
2017	A selective, fast, and easy-to-use procedure was developed to determine MA in urine samples for the first time. The new procedure is based on MIMEPS, the combination of a molecularly imprinted polymer (MIP) and microextraction by packed sorbent (MEPS).	The MIMEPS–HPLC–UV procedure is recommended as an alternative for the biomonitoring of workers exposed to ethylbenzene and/or styrene.	[21]
2020	In this study, report for the first time developing an analytical method based on three metal-organic frameworks (MOF), including UiO-66, UiO-66-NH2, and UiO-66-NH2 @ Fe3O4-SiO2 in microextraction by packed sorbent (MEPS) extracting trans, trans-muconic acid (tt-MA) in urine.	The results indicated that this technique was a sensitive, fast and reusable method and can be applied to extract and determine trace amounts of urinary tt-MA in the urine matrix.	[22]
2017	This study, for the first time, a novel and easy-to-use analytical method based on molecularly imprinted polymer in microextraction by packed sorbent, followed by high-performance liquid chromatography with ultra-violet detection (MIMEPS-HPLC-UV) was developed to determine tt-MA in urine samples.	The developed method is suggested as an alternative to existing conventional SPE methods for biomonitoring of benzene-exposed subjects.	[23]
2019	In this study, two hybrid metal-organic frameworks, including MOF-5@ Fe3O4-NH2 and MOF-5@ SBA-15, for the first time, were synthesized and combined with microextraction by packed sorbent (MEPS) to extract mandelic acid (MA) from urine samples.	The results implied that the proposed technique is a fast and sensitive procedure for extracting and determining MA from urine samples.	[24]
2019	For the analysis of hippuric methyl acids in human urine samples; in this study, a new method based on metal-organic framework of MIL-53-NH2 (Al) in microextraction by packed sorbent (MEPS) was developed	The results indicated that this method was selective, sensitive, rapid, and efficient for the extraction of urinary MHAs	[25]
2016	For the first time, hollow-fiber liquid-phase microextraction combined with high-performance liquid chromatography–ultraviolet was used to extract trans,trans-muconic acid in urine samples of workers who had been exposed to benzene.	The method was successfully applied to the analysis of t,t-MA in real urine samples.	[26]
2018	The authors described a new application of amino-functionalized KIT-6 for dispersive ultrasonication-assisted micro solid-phase extraction of hippuric acid (HA) and methyl hippuric acid (MHA) from human urine and water samples.	The method was successfully implemented for the sensitive determination of HA and MHA in (spiked) human urine samples.	[27]

2015	Factors affecting solid phase extraction (SPE) of trans, trans-muconic acid (t,t-MA), as a benzene biomarker, including sample pH, sample concentration, sample volume, sample flow rate, washing solvent, elution solvent, and type of sorbent, were evaluated. Extracted samples were determined by HPLC-UV (high-performance liquid chromatography-ultraviolet).	This study shows an efficient sample preparation procedure for muconic acid as a benzene biomarker, as a solid phase extraction method using bonded silica has more advantages than LLE.	[28]
2019	Dispersive liquid-liquid micro-extraction (DLLME) technique coupled with high-performance liquid chromatography equipped with ultraviolet detector (HPLC-UV) developed for trace extraction and determination of malathion pesticide in human urine samples	DLLME procedure was successfully developed for the extraction of malathion from human urine samples.	[29]
2019	DLLME, coupled with high-performance liquid chromatography equipped with the ultraviolet detector, extracted chlorpyrifos pesticide in human urine samples. Different affecting parameters on the efficiency of the method were optimized using one factor at a time method	Compared to other extraction techniques, the optimized DLLME resulted in some advantages such as shorter extraction time, high extraction efficiency, and good enrichment factor for the extraction of chlorpyrifos from human urine samples.	[30]
2006	This study describes headspace solid-phase microextraction (HS-SPME) optimization followed by GC-FID for benzene in spiked urine.	The headspace solid-phase microextraction, GC-FID technique provides a relatively simple, convenient, practical procedure that was successfully applied to determine benzene in spiked urine.	[31]
2019	2,5-Hexanedione (2,5-HD), the primary metabolite of n-hexane, was extracted from urine samples using cold fiber headspace solid-phase microextraction based on thermoelectric cooling and analyzed with gas chromatography equipped with a flame ionization detector (GC-FID).	The method was shown to be rapid, sensitive, and easier than conventional methods for quantitative analysis of 2, 5-HD in urine samples.	[32]
2017	Quantitatively applied a cold fiber solid-phase microextraction device based on a cooling capsule as a cooling unit and CO2 as a coolant to analyze BTEX in aqueous samples.	The method was successfully applied to the determination of BTEX in urine samples with good recovery.	[33]
2011	A new solid-phase microextraction fiber based on alumina/titania sol–gel-coated on copper wire for headspace sampling of chlorinated organic solvents (chloroform, carbon tetrachloride, trichloroethene, and tetrachloroethene) from urine samples is introduced.	The proposed fiber has high capacity and demonstrates a fast sampling of chlorinated organic solvents from urine samples with high sensitivity.	[34]

Generally, sampling and preparation of the analyses were the most time-consuming step in the process. The quality of these steps dramatically affected the success of analyzing a complex matrix. Different studies have been conducted to find more efficient extractions to replace conventional methods [16]. These methods have been reviewed in the following:

Optimization of factors affecting the efficiency and recovery of extraction methods:

In any extraction method, the factors that affect efficiency and recovery must be optimized. The response surface methodology (RSM) is commonly applied to determine the optimal conditions and factors. More than two levels were considered for each factor. Therefore, the central composite design (CCD) is considered in RSM design for this purpose [17]. A complete or fractional two-level factorial experimental design (Taguchi or Platelet-Borman designs) was used to investigate the effects of independent factors and their interactions [18]. These models can be applied to eliminate ineffective variables and to reduce the number of experiments required to optimize the method. Table 3 shows the essential optimized parameters in the selected studies.

The essential optimized factors in the selected studies include:

A) Extraction solvent and its volume:

Choosing a proper extraction solvent is very important in microextraction methods. Several important factors should be taken into consideration in the selection of organic solvents. Distribution constant and good selectivity was the most critical parameters in selecting the extraction solvent. The solvent should be insoluble or slightly soluble in water, with a high affinity for extracting and dissolving the desired compound, and compatible with the analysis device [19]. In addition, the extraction solvent in the absorption wavelength region should lack any absorption. This solvent should have low volatility and a melting point close to room temperature [20]. Increasing the volume of the solvent enhances the volume of the final organic phase and decreases the desired analyte concentration as well as the extraction efficiency; hence, the solvent volume must be optimized.

B) Disperser solvent and its volume:

The main criteria for selecting the dispersing solvent is high miscibility in the extracted and sample solutions. It can quickly create droplets of solvent in the sample. In addition, the type of disperser solvent affects the viscosity of the binary solvent and the rate of dispersion into the sample solution. The dispersing solvent should reduce the surface tension of the extracting solvent and disperse it in droplets in the aqueous phase to provide more surface area for the contact of the extracting solvent and the sample solution. This increases the transfer of target compounds from the aqueous phase to the organic phase [20]. The volume of disperser solvent directly affects the formation of the cloudy solution, the degree of dispersion of the extracting solvent in the aqueous phase, and the extraction efficiency. A low volume of the solvent cannot correctly disperse the extracting solvent into the aqueous phase; therefore, the cloudy solution could not be entirely formed.

C) Washing solvent and its volume:

In the solid-phase extraction process, a washing solution was used to purify the adsorbent from the interfering compounds to detect the analyte precisely. The washing solution can separate the interfering compounds without separating the analyte from the adsorbent bed. At this stage, the rest of the unwanted and minor interventions in the adsorbent bed can be washed away so that better detection of the target analyte would be achieved. The concentration and pH of the washing solvent were essential factors in reducing leakage of target analytes [21-22].

D) Ionic strength of the sample solution:

Adding salt due to its high solubility in water helps remove the analyte from the aqueous phase of the sample [30]. The addition of salt affects the extraction efficiency by changing the boundary phase properties and reducing the solubility of hydrophilic compounds in the aqueous phase [23]. In extraction methods, adding salt to the aqueous solutions causes two interactions. The presence of salt increases the ionic strength, decreases the analyte solubility in the aqueous phase, and transfers them into the extracting solvent, which increases the extraction efficiency. The addition of salt to the sample can reduce the solubility

Table 3. The essential optimized parameters in the studies.

Selective articles	Optimized parameters						
Sabet [17]	Extraction solvent (CCL4)	Extraction solvent volume	Dispersive solvent (methanol)	Dispersive solvent volume	Centrifuge or stirring speed	Centrifuge or stirring time	PH
Heidari [39]	Extraction time	Centrifuge or stirring speed	Ionic strength (add salt)	Sample volume	Extraction temperature	pH	-
Mohammadzaheri [46]	Extraction solvent (Toluene)	Extraction solvent volume	Dispersive solvent (Methanol)	Dispersive solvent volume	Ionic strength (add salt)	-	-
kamgoo[47]	Extraction solvent (n-decan)	Extraction solvent volume	Dispersive solvent (ethanol)	Dispersive solvent volume	Extraction time	Centrifuge or stirring speed and time	Ionic strength (add salt) and PH
Soleimani1 [48]	Sample volume and Sorbent amount	PH	Extraction cycles	Washing solvent	Washing solvent volume	Elution solvent	Elution solvent volume and ratio
Rahimpoor [49]	Sample volume	Extraction cycles	Washing solvent (Water- ethanol)	Washing solvent volume and ratio	Elution solvent (acetonitrile-acetic acid)	Elution solvent volume and ratio	Sorbent amount
Soleimani1 [50]	Sample volume and Sorbent amount	pH	Extraction cycles	Washing solvent (water)	Washing solvent volume	Elution solvent (ethanol-acetic acid)	Elution solvent volume and ratio
Rahimpoor [51]	Extraction cycles	PH	Washing solvent (water)	Washing solvent volume and ratio	Elution solvent (methanol-nitric acid)	Elution solvent volume and ratio	Sorbent amount
Pirmohammadi [52]	Extraction cycles	Washing solvent (Water- methanol)	Elution solvent (acetic acid-methanol)	Elution solvent volume and ratio	Sorbent amount	-	-
Ghamari [53]	Sample volume	Extraction temperature	pH	-	-	-	-
Behbahani [54]	Centrifuge or stirring time	PH	Extraction cycles	Elution solvent (methanol-NH4OH)	Elution solvent ratio	Sorbent amount	-

Shahtaheri [21]	Sample volume	PH	Washing solvent (acetic acid)	Washing solvent ratio	Elution solvent (acetic acid)	Elution solvent volume and ratio	Sorbent amount
Ramin [29]	Extraction solvent (CS2)	Extraction solvent volume	Dispersive solvent (acetonitrile)	Dispersive solvent volume	Centrifuge or stirring speed and time	Sample volume	PH
Ramin [28]	Extraction solvent (CCl4)	Extraction solvent volume	Dispersive solvent (methanol)	Dispersive solvent volume	Centrifuge or stirring speed and time	PH	-
Shahtaheri [31]	Extraction time	Desorption time &temperature	Extraction temperature	Centrifuge or stirring speed	Ionic strength (add salt)	Sample volume	PH
Pourbakhshi [55]	Extraction time	Desorption time &temperature	Ionic strength (add salt)	Sample volume	Extraction temperature	-	-
Tajik [56]	Extraction time	Ionic strength (add salt)	Sample volume	Extraction temperature	-	-	-
Farhadi [57]	Extraction time	Desorption time &temperature	Sample volume	Extraction temperature	-	-	-
Heidari [58]	Extraction time	Desorption time &temperature	Centrifuge or stirring speed	Ionic strength (add salt)	Sample volume	Extraction temperature	-
Sargazi [59]	-	-	-	-	-	-	-

of the extraction solvent in the aqueous phase and increase the volume of the accumulated organic phase, leading to a reduction in the analyzing signal due to high dilution [24-25]. It has been found that the presence of salt in water samples can be ineffective in the extraction process in some developed methods [26].

E) PH of the sample solution:

This variable was important because it induces a change in the ionic or molecular nature of the target analyte, affecting the extraction rate. Theoretically, adjusting the pH level of the sample medium can reduce the solubility of analytes in water and increase their extraction efficiency [27-28]. To investigate the impact of this factor on the extraction rate, the pH level of the sample solution was fixed by hydrochloric acid or sodium hydroxide in the desired range. Then, the extraction was performed and compared [29].

F) Sample volume:

The sensitivity of extraction methods was proportional to the amount of analyte in the sample, so it was expected that the amount of analyte extraction will increase, along with the increase in the sample volume. Theoretically, if the sample volume was significantly higher than the adsorbent capacity in some micro-extraction methods, there would be no increase in extraction rate with increasing sample volume [27].

G) Extraction temperature:

The extraction temperature affects the mass transfer rate and the analytes distribution coefficient [25]. The optimum temperature can accelerate mass transfer and increase the contact between the surface of the extractant and the solution [30]. In the micro-extraction method, from the headspace by the fiber coating, increasing the temperature of the sample solution can increase the analyte vapor pressure. Accordingly, the analyte concentration increases in the space above the sample. Therefore, the separation of the analyte between the sample and its upper space and reaching the equilibrium point can be accelerated. The analyte distribution constant between the upper sample space and the fiber coating was also temperature-dependent. By increasing temperature, particularly at high temperatures, the cohesion of the analyte with the fiber coating decreases [27-31].

H) Recovery temperature:

The potential fiber decomposition (in thermal recovery) affects the assessment of recovery temperature. Fiber with high-temperature stability makes it possible to assess the recovery of fiber and improves thermal recovery. In the solid-phase microextraction method, increasing the temperature can reduce the recovery time and the amount of analyte remaining on the fiber (after completing the heat recovery stage). In this way, a complete recovery of thermally stable compounds is achieved. Further, an optimal recovery temperature was determined by comparing the peaks obtained from chromatography [27].

J) Stirring speed and sample mixing:

In many extraction processes, stressing the sample matrix by shaking, stirring, ultrasound, or microwave radiation was necessary to improve mass transfer and reduce extraction time. In the hollow-fiber liquid-phase microextraction (HF-LPME) method, sample stirring facilitates the analyte diffusion from the donor phase to the receiver phase by the organic solvent. Extraction efficiency was calculated at different speed rates (in rpm) of sample mixing generated by a magnetic stirrer on a hot plate stirring device [25-32-33].

K) Extraction time:

Time was an essential parameter for extraction because the mass transfer was a time-dependent process. When the system is in the equilibrium state, the maximum extraction efficiency was obtained. As long as the extraction conditions were reproducible, it was unnecessary to have a perfect equilibrium to achieve accurate and correct analysis [25]. Extraction time was an essential parameter in the HF-LPME method. It affects the target analyte differentiation coefficient between the donor and organic phases and between the organic and receiver phases [33].

L) The amount of adsorbent:

The amount of adsorbent was another effective parameter in the efficiency of micro-extraction methods with the solid adsorbent (i.e., the solid-phase diffusion microextraction method). The effect of the

amount of adsorbent on the extraction efficiencydepends on the volume of the sample solution ratio to the solid phase of the adsorbent. At low adsorbent values, the phase ratio was low, providing a low extraction efficiency. As adsorbent increases, both the phase ratio and the extraction efficiency increase as well [34]. Therefore, the amount of adsorbent was one of the parameters that needs optimization.

3.2) Validity of the optimized method:

To evaluate the validity of the optimized method, the analytical features including accuracy, precision, linearity range (LR), the limit of detection (LOD), the limit of quantification (LOQ), selectivity, correlation coefficient (R2), relative standard deviation (RSD%), enrichment factor (EF), and extraction recovery (ER) were calculated using optimized variables. The essential analytical features calculated in the selected studies have been presented in Table 4.

Table 4. The essential analytical features calculated in the studies include

Selective articles	Validated parameters	Instruments	Analyte
Sabet [17]	linearity range (LR), Limit of quantification (LOQ), Limit of detection (LOD), Enrichment factor (EF), Extraction recovery (ER), Relative standard deviation (RSD%), Correlation coefficient (R2)	DLLE technique coupled with high performance liquid chromatography equipped with ultra violet detector	pesticide diazinon in urine samples
Heidari [39]	RSD%, R2	headspace solid phase microextraction followed by gas chromatography equipped with a flame ionization detector	toluene at trace level in spiked urine
Mohammadzaheri [46]	LR, LOQ, LOD, ER, RSD%, R2	Dispersive Liquid-Liquid Microextraction Method and HPLC-PDA.	chlorpyrifos in the urine sample
kamgoo[47]	LOQ, LOD, ER, EF, RSD%, R2	DLLME SFOD method	cadmium in biological samples
Soleimani1[48]	LR, LOQ, LOD, ER, RSD%, R2	The chromatographic system was a Knauer HPLC system (Berlin, Germany) equipped with a K-2600 ultraviolet detector,	mandelic acid
Rahimpoor [49]	LR, LOQ, LOD, ER, RSD%, R2 Accuracy,	UiO-MEPS procedure combined with high-performance liquid chromatography (HPLC).	Trans, trans-muconic acid (tt-MA) in urine.
Soleimani1[50]	LR, LOQ, LOD, ER, RSD%, R2 Accuracy,	liquid chromatography with ultraviolet detection	trans, trans-muconic acid (tt-MA) in urine

Rahimpoor [51]	LR, LOQ, LOD, ER, RSD%, R2	MOF-MEPS procedure combined with high performance liquid chromatography	mandelic acid
Pirmohammadi [52]	LR, LOQ, LOD, ER, EF, RSD%, R2 Accuracy,	HPLC–UV analysis	urinary methyl hippuric acids
Ghamari [53]	LOQ, LOD, ER, EF, RSD%, R2, LR	hollow-fiber liquid-phase microextraction combined with high-performance liquid chromatography–ultraviolet	trans, trans-Muconic Acid as a Biomarker of Benzene
Behbahani [54]	LR, LOQ, LOD, ER, RSD%, R2	HPLC with UV detection.	hippuric acid and methyl hippuric acid, two biomarkers for toluene and xylene exposure
Shahtaheri [21]	LR, ER RSD%, R2	High Performance Liquid Chromatographic (HPLC)	Muconic Acid as a Biomarker of Occupational Exposure to Benzene
Ramin [29]	LR, LOQ, LOD, ER, EF, RSD%, R2	Dispersive liquid-liquid micro-extraction (DLLME) technique coupled with high-performance liquid chromatography equipped with ultraviolet detector (HPLC-UV)	Malathion Pesticide in Urine Samples
Ramin [28]	LR, LOQ, LOD, ER, EF, RSD%, R2	DLLME, coupled with high performance liquid chromatography equipped with ultra violet detector,	chlorpyrifos in human urine samples
Shahtaheri [31]	LR, LOQ, RSD%, R2	(HS-SPME) followed by GC-FID	benzene in spiked urine
Pourbakhshi [55]	LR, LOQ, LOD, ER, RSD%, R2	cold fiber head space solid-phase microextraction (CF-HS-SPME) based on thermoelectric cooling and analyzed with gas chromatography equipped with a flame ionization detector (GC-FID)	2,5 HEXANDION IN URINE

Tajik [56]	LR, LOD, RSD%, R2	cooling/heating-assisted headspace solid-phase microextraction	BTEX in urine samples
Farhadi [57]	LR, LOD, ER, RSD, R2	HS-SPME was performed with a 6890N gas chromatograph	chlorinated organic solvents from urine
Heidari [58]	LR, LOD, R2	The GC apparatus	Toluene
Sargazi [59]	LR, LOQ, LOD, EF, ER, RSD%, R2	liquid–liquid microextraction and GC-FID	di(2-ethylhexyl) phthalate and its metabolite in human urine samples

The essential analytical features calculated in the studies include:

A) Accuracy:

The accuracy of the developed method was defined as the degree to which the results obtained from the analytical method were close to the actual value. This figure of merit was calculated using the extraction recovery as follows [35-36]:

A (%) = ER (%)-100 Equation (1)

B) Precision:

The degree of repeatability or similarity of the experimental results was determined by an individual at different times and days of the week. The method precision was calculated through the repeatability criterion in one day (intra-day) and in three consecutive days (inter-day) [37-38]. The RSD% was used to evaluate the repeatability and precision of a proposed method [39].

C) The LOD and the LOQ:

The lowest concentration of an analyte that can be detected in a matrix was known as LOD. The lowest concentration that can be measured by acceptable precision was defined as LOQ. Signal to noise ratio was used to determine LOD and LOQ. The ratios of 3: 1 and 10: 1 were used to determine LOD and LOQ, respectively [40-42].

D) Linear dynamic range (LDR)

LDR expresses the upper and lower limits of the analyzing method, demonstrating a significant and linear relationship between the analyte concentration and the peak area in a chromatogram using the technique. In other words, it indicates the applicable range of the analyzed methods. The linearity of the analysis method was evaluated by plotting the concentration-surface area under the curve at different concentrations of the analyte [36].

E) ER:

ER for the analyte refers to the difference between the amount of analyte added and recovered from the standard sample by the method. The ER was calculated as follows:

$$ER\ (\%) = \frac{Peak\ area\ (sample)}{Peak\ area\ (standard)} \times 100$$

Equation (2)

F) Selectivity:

Analytical selectivity was related to the extent to which the method can be used to determine a specific analyte in mixtures or matrices without interfering with other spices with similar behavior [43]. Selectivity was usually examined by studying the capacity of the method to measure the analyte in samples in which different potential interferences have been intentionally introduced, including those factors that are likely to be present in the samples [44].

G) EF

The ER was calculated by comparing the peak area of the standard solution of analytes with the peak area in the sample solution after performing the proposed extraction method [39].

3.3) Method efficiency in real samples:

The efficiency of the proposed method for measuring the analyte or analytes in real samples under optimal conditions should be evaluated in a proposed method.

The results demonstrated the capability of the proposed extraction method to determine the desired compound or compounds.

CONCLUSION

As described in this review study, there were many significant studies conducted by Iranian researchers on the use of microextraction methods to extract and determine the occupational analytes from urine samples. Based on the results of the selected articles in this study, to use microextraction methods for analyzing urine samples, first, the factors affecting the efficiency of the extraction method should be optimized. These include elution solvent, dispersive solvent, and ionic strength of the sample, PH of the sample solution, sample volume, extraction temperature, recovery temperature, stirring speed, extraction time, and other influential factors. In the next step, to evaluate the practical applicability of the optimized method, the analytical figures of merit (i.e., LOD/LOQ, linearity, accuracy, inter-and intra-day precisions, ER, and the like) must be investigated for analytes under optimal conditions. Finally, the efficiency of the developed method for extracting analytes from real samples will be examined. The developed methods in the selected articles were fast, simple, with minimum solvent consumption, short extraction time, and environmentally friendly that can be used as alternatives to conventional methods.

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CONFLICT OF INTEREST

There is no conflict of interest.

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