



Research Article



Determination of 2-Octanone in Biological Samples Using Liquid–Liquid Microextractions Followed by Gas Chromatography–Flame Ionization Detection

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ABSTRACT

Background: Analysis of chemicals in biological fluids is required in many areas of medical sciences. Rapid, highly efficient, and reliable dispersive and air assisted liquid–liquid microextraction methods followed by gas chromatography–flame ionization detection were developed for the extraction, preconcentration, and determination of 2-octanone in human plasma and urine samples.

Methods: Proteins of plasma samples are precipitated by adding methanol and urine sample is diluted with water prior to performing the microextraction procedure. Fine organic solvent droplets are formed by repeated suction and injection of the mixture of sample solution and extraction solvent into a test tube with a glass syringe. After extraction, phase separation is performed by centrifuging and the enriched analyte in the sedimented organic phase is determined by the separation system. The main factors influencing the extraction efficiency including extraction solvent type and volume, salt addition, pH, and extraction times are investigated.

Results: Under the optimized conditions, the proposed method showed good precision (relative standard deviation less than 7%). Limit of detection and lower limit of quantification for 2-octanone were obtained in the range of 0.1–0.5 $\mu\text{g mL}^{-1}$. The linear ranges were 0.5–500 and 0.5–200 $\mu\text{g mL}^{-1}$ in plasma and urine, respectively ($r^2 \geq 0.9995$). Enrichment factors were in the range of 13–37. Good recoveries (55–86%) were obtained for the spiked samples.

Conclusion: Preconcentration methods coupled with GC analysis were developed and could be used to monitor 2-octanone in biological samples.

Introduction

2-Octanone (molar mass of 128.2 $\text{g}\cdot\text{mol}^{-1}$) is a synthetic flavouring substance which is permitted to be used as a food additive. It could be used as a substrate in reduction to an optically pure alcohol which are used in enantioselective synthesis of pharmaceutical compounds.¹ It is one of the thermal decomposition products of hydroperoxy cyclic peroxides.² In addition it could be used for extraction of analytes from very dilute samples³ and also as a urinary biomarker of prostate cancer.⁴ Due to very low concentration of 2-octanone in biological samples, it should be pre-concentrated using appropriate procedures prior to analysis.

Preconcentration using solvent extraction methods are the most commonly used procedures for treatment of biological samples.⁵ In addition to preconcentration, the microextraction methods could also be employed for sample clean up purposes prior to analysis of organic⁶ or inorganic analytes⁷ in complex matrices like serum or urine.

Liquid-phase microextraction (LPME) is a solvent-minimized sample preparation approach in which only several microliters of an extracting solvent is required.⁸ A new liquid phase microextraction method, i.e. dispersive liquid-liquid microextraction (DLLME), was introduced by Assadi and co-workers.⁹ This high-performance microextraction

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method employs a ternary component solvent system, in which extraction and disperser solvents are rapidly injected into the aqueous sample to form a cloudy solution. The analytes enriched in the dispersed fine droplets of the extraction solvent is separated by centrifugation. DLLME is proved to be a simple, low-cost, and fast method using the small amount of the extraction solvent and the low sample volume together with a high enrichment factor (EF) for the analysis of different analytes. The use of relatively larger volumes of a disperser solvent is the most significant drawback of DLLME, because it reduces the polarity of aqueous phase which leads to increase the solubility of analytes into aqueous phase and decreases extraction efficiency. In order to resolve above mentioned problem, some disperser solvent-free techniques such as ultrasound-assisted emulsification microextraction¹⁰ and vortex-assisted liquid-liquid microextraction¹¹ were developed, in which the extraction solvent is dispersed into an aqueous sample through ultrasound irradiation or vortexing. However, without the use of an organic disperser solvent, the process of forming cloudy solution typically takes a significantly longer time than conventional DLLME method. In 2012, air-assisted liquid-liquid microextraction (AALLME)¹² was developed, which is a new version of the DLLME method. In AALLME, a few microliters of an extraction solvent is transferred into an aqueous phase containing the analytes. Fine organic solvent droplets are formed by repeated suction and injection of the mixture of the aqueous sample solution and the extraction solvent into a test tube with a glass syringe. By performing the predetermined cycles the turbidity of solution is increased and analytes are extracted into the organic phase. After centrifugation of cloudy solution, the extractant is settled down in the bottom of the centrifuge tube and used for further analysis. AALLME is a disperser solvent-free technique which is completely rapid. More recently, a new version of DLLME, so called syringe to syringe dispersive liquid phase microextraction was developed.¹³

In the present study, simple and rapid microextraction methods with improved sensitivity and reproducibility for the determination of 2-octanone in human plasma (using DLLME) and in urine (using AALLME) followed by gas chromatography flame ionization detection (GC-FID) were proposed. The effects of some experimental parameters, including the type and volume of the extraction solvent, salt addition, pH, and extraction times on the extraction efficiency are investigated. The performance of the optimized method was then evaluated and successfully applied to determine 2-octanone in biological samples spiked with standard solutions of the analyte.

Materials and Methods

Reagents and solutions

2-Octanone was purchased from Sigma-Aldrich (St. Louis, MO, USA). The tested extraction solvents were supplied from the following sources: carbon tetrachloride, 1,2-dichloroethane (1,2-DCE), 1,1,1-trichloroethane (1,1,1-TCE), and 1,1,2-trichloroethane (1,1,2-TCE) were from Merck (Darmstadt, Germany), 1-bromo-2-chloroethane was from Janssen Chimica (Beerse, Belgium), and chloroform (CHCl₃) was obtained from Scharlau (Barcelona, Spain). HPLC-grade methanol, sodium chloride, hydrochloric acid, and sodium hydroxide were purchased from Merck (Germany). De-ionized water (Shahid Ghazi Pharmaceutical Company, Tabriz, Iran) was used for preparation of aqueous solutions. A stock solution of 2-octanone (100 mg L⁻¹) was prepared in methanol and stored in a refrigerator at 4 °C. Working standard solutions were daily prepared by appropriate dilutions of the stock solution with de-ionized water. A standard solution of 2-octanone (100 mg L⁻¹) in chloroform was injected into GC-FID (three times in a day) and the obtained analytical signals (peak areas) were used for the calculation of EFs and extraction recoveries (ERs). A phosphate buffer (1.0 mol L⁻¹) was prepared by dissolving 39.0 g of sodium dihydrogen phosphate dehydrate (NaH₂PO₄·2H₂O) (Merck) in 250 mL deionized water, and its pH was adjusted at 2.0 by adding HCl 1.0 mol L⁻¹.

Instrumentation

GC analysis of 2-octanone was carried out using an Agilent 7890A gas chromatograph (Agilent Technologies, CA, USA) equipped with a split/splitless inlet system operated at 300 °C in a splitless mode (sampling time of 1 min) and an FID. Nitrogen (99.999%, Gulf Cryo, United Arab Emirates) was used as the carrier gas (at a constant flow of 1.2 mL min⁻¹) and make up gas (25 mL min⁻¹). Chromatographic separation was achieved on an HP-5 capillary column (30 m × 0.32 mm i.d. with a 0.25 μm stationary film thickness) (Hewlett-Packard, Santa Clara, USA). The oven temperature was programmed from 50 °C (held for 2 min) to 210 °C at a rate of 10 °C min⁻¹ and held at 210 °C for 3 min; then, the temperature was raised with a rate of 15 °C min⁻¹ to a final temperature of 290 °C that was held for 1 min. Chem Station software was used for data acquisition and processing. A 1-μL microsyringe (zero dead volume, Hamilton, Switzerland) was used for the injection of samples into GC. Injection volume was 1 μL and the FID temperature was maintained at 300 °C. Hydrogen gas was generated with a hydrogen generator (GLAIND-2200, Dani, Italy) for FID at a flow rate of 40 mL min⁻¹. Air flow rate for FID was 400 mL min⁻¹. A vortex from Labtron Company (Tehran, Iran) was used in sample preparation. A Metrohm

pH meter model 744 (Herisau, Switzerland) was used for pH measurements. Sigma centrifuge (Osterode, Germany) was used in protein precipitation step and Hettich centrifuge (Tuttlingen, Germany) was used for accelerating phase separation.

Samples

Plasma samples

Drug-free human plasma samples were obtained from the Iranian Blood Transfusion Research Center (Tabriz, Iran) and frozen in polypropylene microtubes (2-mL fractions) at -20 °C until analysis. To precipitate the proteins, 100 µL of plasma sample was mixed with 200 µL methanol. Then the obtained mixture was vortexed for 15 s and centrifuged for 7 min at 3000 rpm. Then 100 µL of the supernatant phase was removed and diluted with 4.9 mL sodium phosphate buffer (1 mol L⁻¹, pH 2.0) and used for further DLLME procedure. The remaining methanol from protein precipitation stage acts as a dispersive solvent and the method should be considered as a combination of DLLME and AALLME methods.

Urine samples

Drug-free urine samples were collected from healthy volunteers. Samples were collected in polypropylene tubes (2 mL fractions) and stored at -20 °C until analysis. The collected urine samples were centrifuged at 3000 rpm for 7 min. To reduce the matrix effect of urine sample the supernatant was diluted 5-fold with phosphate buffer (1.0 mol L⁻¹, pH 2.0) and then were subjected to the microextraction procedure.

AALLME procedure

5 mL of diluted plasma or urine sample (see Sections 2.2.1 and 2.2.2) was placed into a 10-mL glass test tube with conical bottom. Chloroform (75 µL) as an extraction solvent was added to the tube and then the mixture was repeatedly aspirated into a 5-mL glass syringe and then was expelled into the tube causing the solution to become turbid. The procedure was repeated for 4 times. After this process, the mixture was centrifuged at 3000 rpm for 7 min and fine droplets of the extractant were settled down in the bottom of the centrifuge tube (10 ± 1 µL). Finally, 1 µL of the sedimented phase was removed and injected into GC system for analysis.

Analytical parameters

Two main parameters, namely EF and ER, have been employed for evaluation of the proposed method. EF is defined as the ratio between the analyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_0) within the sample:

$$EF = \frac{C_{sed}}{C_0} \quad \text{Eq.(1)}$$

C_{sed} is calculated from a calibration curve obtained from direct injection of 2-octanone in the extraction solvent. ER is defined as the percentage of the total analyte amount (n_0) which is extracted into the sedimented phase (n_{sed}):

$$ER = \frac{n_{sed}}{n_0} \times 100 = \frac{C_{sed} \cdot V_{sed}}{C_0 \cdot V_{aq}} \times 100 = EF \times \frac{V_{sed}}{V_{aq}} \times 100 \quad \text{Eq.(2)}$$

Where V_{sed} and V_{aq} are volumes of the sedimented phase and aqueous solution, respectively.

Assay validation

For the validation of the recommended LLME methods in the determination of 2-octanone under the experimental conditions, the related analytical characteristics were calculated by employing the peak areas. The validation process of the present method was carried out following the Food and Drug Administration (FDA) guidelines. In order to do this, the calibration linearity, limit of detection (LOD), limit of quantification (LOQ), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), intra- and inter-day precisions, accuracy, relative recovery (RR%), stability (room temperature and freeze-thaw cycles) and robustness of the proposed methods were evaluated in plasma and urine samples. The mean of three calibration curves (produced on three different days) was used for linearity studies. All experiments were performed three times. LODs and LOQs were calculated on the basis of signal to noise ratio (S/N) of 3 and 10, respectively. S/N was estimated using measurements of the peak height relative to the baseline noise, and height values were consequently converted into concentrations through the height of the analyte peaks at the LLOQ. LLOQ and ULOQ terms are defined as the lowest and highest concentration levels of calibration curve that can be measured with an acceptable accuracy and precision. The intra- and inter-day precision were evaluated by assaying the quality control (QC) samples at three concentration levels and expressed as relative standard deviations (RSD). The accuracy of method was also determined by calculating relative errors (RE %) using the following equation:

$$RE(\%) = 100 \times \left(\frac{\text{Calculated conc.} - \text{nominal conc.}}{\text{nominal conc.}} \right) \quad \text{Eq.(3)}$$

The RR% were calculated as the ratio of the found concentration after extraction to the added concentration before extraction multiplied by 100. The stability of 2-octanone in samples was assessed by analyzing triplicate QC samples at different storage conditions: short term (12 h), room temperature and three freeze-thaw (-20 to 25°C) cycles. The concentrations following storage were compared with freshly prepared samples of the same concentrations. Furthermore, the robustness of the

method was evaluated by partial varying of some effective parameters in AALLME method such as extraction solvent volume, sample solution ionic strength and its pH at three levels.

Results and Discussion

In order to obtain the best extraction efficiency, some important experimental parameters that would influence the performance of LLME methods were investigated in details in the following sections. The parameters including extraction solvent type and volume, salt addition, pH, and extraction numbers were studied. To optimize the method, all extractions were initially carried out on human plasma spiked with 2-octanone then applied to spiked urine sample.

Selection of extraction solvent

The selection of extraction solvent is the most important experimental parameter of an LLME method. Generally, the extraction solvent has to possess insignificant solubility in water, high extraction capability of the interested analyte and good chromatographic behavior. Also, it should have different density from water to enable phase separation after extraction. Based on these facts, some organic solvents named chloroform, 1-bromo-2-chloroethane, carbon tetrachloride, 1,2-DCE, 1,1,1-TCE, and 1,1,2-TCE were examined. Different volumes of these solvents were tested to reach a volume of 10 ± 1 μL of the sedimented phase at the bottom of the test tube. The needed volumes for each solvent were chloroform 75 μL , 1-bromo-2-chloroethane 90 μL , carbon tetrachloride 35 μL , 1,2-DCE 70 μL , 1,1,1-TCE 35 μL , and 1,1,2-TCE 45 μL . The results indicate that chloroform is the most effective extraction solvent among the tested solvents and gave the highest extraction. Therefore, it was selected as the suitable extraction solvent for the subsequent experiments.

Extraction solvent volume

Extraction solvent volume is another important factor affecting volume of the sedimented organic phase, extraction efficiency and repeatability of the results obtained. To examine effect of the extraction solvent volume on the extraction performance, experiments involving different volumes of chloroform (70, 75, 80, 85, 90, and 100 μL) were done with the same extraction procedure while the other experimental conditions were kept constant. The results show that by increasing the extraction solvent volume from 75 to 100 μL , the peak areas decrease due to increase in volume of the sedimented phase from 10 to 22 μL which in turn leads to decrease in analyte concentration into the organic phase and EFs, too. It is noted that in the case of 70 μL extraction solvent volume or less, removal of the sedimented phase was difficult and

repeatability of the responses was also low. Therefore, further experiments were carried out with 75 μL of chloroform, which leads to obtain 10 ± 1 μL sedimented phase volume.

Effect of salt addition

Ionic strength affects the extraction efficiency by its influence on the solubility of analytes and viscosity of the aqueous phases. The effect of salt addition on the extraction efficiency of the LLME method was studied by adding sodium chloride in the range of 0–15 %, w/v, to the sample solution. Salt addition leads to an increase in volume of the sedimented phase by decreasing the solubility of extraction solvent into aqueous phase. Therefore, the experiments were performed using different volumes of the extraction solvent to achieve 10 μL of the sedimented phase volume (75, 71, 67, 62, 57, and 50 μL for 0, 2.5, 5.0, 7.5, 10, and 15 % NaCl, w/v, respectively). The obtained results reveal that by increasing NaCl concentration, analytical signals increase till 7.5 % and then remain almost constant. Therefore further experiments were performed in the presence of 7.5 %, w/v, NaCl.

Effect of pH

The effect of sample pH was investigated within a pH range of 1.0–6.0 with adjusting pH using solutions of 0.1 M HCl. At pH 1.0 the peak area decreased significantly compared to pH 2.0. The best extraction efficiency was obtained at pH 2.0; therefore, for subsequent experiments pH was adjusted to 2.0. To facilitate the pH adjustment, phosphate buffer (1.0 mol L⁻¹, pH 2.0) was used instead of HCl solution. The obtained results for both buffers were similar.

Effect of extraction cycles number

In an AALLME method, formation of fine droplets of the extraction solvent dispersed into aqueous phase is performed by repeatedly sucking extraction solvent and sample solution mixture into a glass syringe and then its injecting into a test tube. In AALLME the choice of syringe size depends on volume of extraction solvent and sample solution. The numbers of suction/injection cycles are considered as the extraction cycles. It was predictable that with increasing extraction cycles, extraction efficiency would be increased and then remained constant. Therefore to obtain the equilibrium status, the extraction cycles were studied in the range of 1–9 times. The results show that analytical signals increase with increasing the extraction cycles up to 4 and then decrease. That is because in high extraction cycles vaporization of the extraction solvent would be significant. Consequently, 4 times of extraction was selected for further studies. It is noted that this step is very rapid and takes less than 30 s.

Optimization of centrifugation time and speed

Centrifugation is substantial in order to obtain two separated phases after extraction. In order to achieve the best extraction efficiency, centrifugation time and speed were considered in the ranges of 3–9 min and 2000–6000 rpm, respectively. The obtained results showed that these parameters were less effective. Therefore, 3000 rpm and 7 min were selected as the optimal centrifuge rate and time, respectively, in the following studies.

Method validation**Linearity and calibration curves**

After optimization of all parameters, the calibration curves were constructed in 3 different days at seven/eight increasing levels and the average of three replicated curves was used for validation studies. The details of calibration curves and corresponding validation data (i.e., linear range, LOD, LOQ, LLOQ, and ULOQ) are presented in Table 1.

Assay precision and accuracy

Precision and accuracy of the methods were

assessed under the obtained conditions for both intra- and inter-days. These two parameters are expressed as the closeness of the individual measures of an analyte and deviation of mean test results from nominal concentrations, respectively. The repeatability and reproducibility of the proposed method, expressed as RSD %, were evaluated by performing the method on six repeated QC samples in a day (for intra-day assay) and four repeated QC samples in different days (for inter-days assay) at three (low, medium, and high) concentration levels. All RSD % values were less than 7.0 %. The accuracy of the method was determined by calculating the percentage deviation observed in the analysis of QC samples and expressed as the RE %. Inter- and intra-assay precisions along with accuracy for QC samples were listed in Table 2. These results demonstrate that the values are within the acceptable range recommended by FDA, and, hence, the developed method is sufficiently accurate and precise.

Table 1. Validation data of the proposed method for quantification of 2-octanone in human plasma and urine sample.

Sample	LR ^a	Slope (S.E.)	Intercept (S.E.)	r ^{2b}	Number of data points	LOD ^c	LOQ ^d	LLOQ ^e	EF ^f ± SD	ER% ^g ± SD
Plasma	0.5-500.0	56.7 (0.24)	2.4 (46.7)	0.9999	8	0.1	0.5	0.5	13 ± 1	86 ± 4
Urine	0.5-200.0	224.4 (0.81)	-98.8 (70.5)	0.9995	7	0.1	0.2	0.5	37 ± 1	55 ± 2

^a Linear range ($\mu\text{g mL}^{-1}$).

^b Coefficient of determination. ^c Limit of detection (S/N = 3) ($\mu\text{g mL}^{-1}$). ^d Limit of quantification (S/N = 10) ($\mu\text{g mL}^{-1}$). ^e Lower limit of quantification ($\mu\text{g mL}^{-1}$). ^f Enrichment factor ± standard deviation (n = 3). ^g Extraction recovery ± standard deviation (n = 3).

Table 2. Precision and accuracy of the method for determination of the 2-octanone in human plasma and urine sample.

Nominal concentration ($\mu\text{g mL}^{-1}$)	Intra-assay precision (RSD % ^a) (n = 6)	Accuracy (RE ^b %)	
		Inter-assay precision (RSD % (n = 4))	Accuracy (RE %)
Plasma			
0.5	5.6	-2.6	6.9
50.0	2.9	-5.4	3.3
500.0	1.2	0.3	1.2
Urine			
0.5	5.1	13.0	5.6
10.0	2.1	-0.7	2.9
200.0	0.9	0.1	1.0

^a Relative standard deviation. ^b Relative error.

Table 3. Relative recoveries of 2-octanone obtained by the developed method in human plasma and urine samples spiked at different concentrations.

Nominal concentration ($\mu\text{g mL}^{-1}$)	Found concentration ($\mu\text{g mL}^{-1}$) ± SD ^a	Relative recovery (RR%) ± SD
Plasma		
0.5	0.5 ± 0.1	93 ± 4
50.0	47.0 ± 0.5	94 ± 1
500.0	501.0 ± 5.0	100 ± 1
Urine		
0.5	0.6 ± < 0.01	113 ± 1
10.0	9.9 ± 0.2	99 ± 2
200.0	200.0 ± 1.0	100 ± < 1

^a Standard deviation (n=3).

Table 4. Stability data for 2-octanone in human plasma and urine samples.

Room temperature stability				Freeze–thaw stability		
Nominal concentration ($\mu\text{g mL}^{-1}$) (n = 3)	Found concentration ($\mu\text{g mL}^{-1}$) \pm SD	Accuracy (RE %)	Relative recovery (RR%) \pm SD	Found concentration ($\mu\text{g mL}^{-1}$) \pm SD	Accuracy (RE %)	Relative recovery (RR%) \pm SD
Plasma						
0.5	$0.5 \pm <0.1$	-3.2	97 ± 4	$0.5 \pm <0.1$	-1.4	99 ± 7
50.0	47.0 ± 1.0	-5.6	94 ± 2	48.0 ± 0.6	-3.9	96 ± 1
500.0	502.0 ± 8.0	0.4	100 ± 2	503.0 ± 2.0	0.7	$101 \pm <1$
Urine						
0.5	$0.6 \pm <0.1$	12.1	112 ± 1	$0.6 \pm <0.1$	12.4	112 ± 1
10.0	9.9 ± 0.3	-1.2	99 ± 3	10.0 ± 0.2	< -0.1	100 ± 2
200.0	199.0 ± 3.0	-0.5	99 ± 2	200.0 ± 1.0	-0.1	$100 \pm <1$

Table 5. Evaluation of method robustness for the extraction and analysis of 2-octanone in the spiked human plasma and urine samples with LLME-GC-FID method.

Level	Nominal concentration ($\mu\text{g mL}^{-1}$)	Found concentration ($\mu\text{g mL}^{-1}$) \pm SD (n = 3)	Accuracy (RE %)	Relative recovery (RR%) \pm SD
Plasma				
1	50.0	47.0 ± 1.0	-6.7	93 ± 2
2	50.0	47.0 ± 0.5	-6.1	94 ± 1
3	50.0	48.0 ± 1.0	-5.0	95 ± 2
Urine				
1	10.0	9.9 ± 0.2	-0.6	99 ± 2
2	10.0	9.9 ± 0.2	-0.9	99 ± 2
3	10.0	10.0 ± 0.1	0.1	100 ± 1

Level 1: pH = 1.9, extraction solvent volume: 60 μL , NaCl concentration: 7 %, w/v.
 Level 2: pH = 2.0, extraction solvent volume: 62 μL , NaCl concentration: 7.5 %, w/v.
 Level 3: pH = 2.1, extraction solvent volume: 64 μL , NaCl concentration: 8 %, w/v.

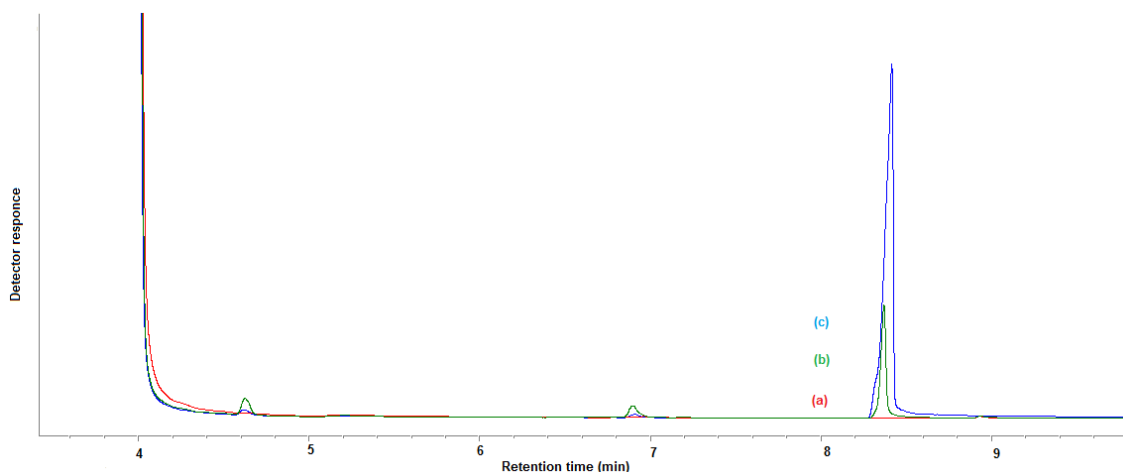


Figure 1. GC-FID chromatograms of: (a) plasma, (b) plasma spiked with $10 \mu\text{g mL}^{-1}$ of 2-octanone, and (c) standard solution (50 mg L^{-1}) prepared in chloroform. Chromatogram (c) was obtained by direct injection whereas in the cases of two other chromatograms the proposed LLME method was carried out on the samples and $1 \mu\text{L}$ of the sedimented phase was injected into the separation system.

Recovery

Recovery experiments are also performed in order to demonstrate method accuracy. For recovery study QC samples were spiked with three different levels (low, middle and high) of 2-octanone and subjected to the proposed method. Table 3 shows the RR%

data obtained during method validation. The calculated RRs were within the range of 93–113%; demonstrating the suitability of the sample preparation method for the analysis of 2-octanone in plasma and urine samples.

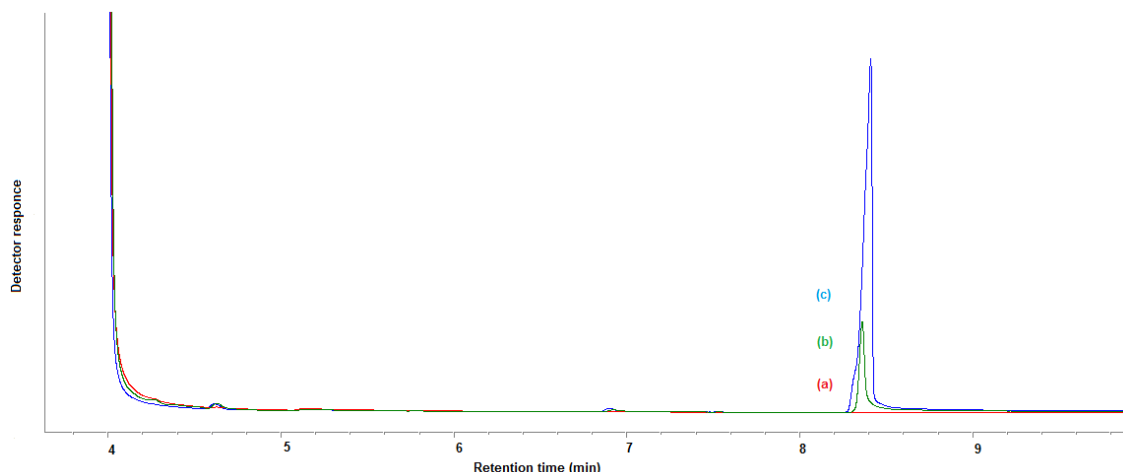


Figure 2. GC-FID chromatograms of: (a) urine, (b) urine spiked with $5 \mu\text{g mL}^{-1}$ of 2-octanone, and (c) standard solution (50 mg L^{-1}) prepared in chloroform. Chromatogram (c) was obtained by direct injection whereas in the cases of other chromatograms the proposed LLME method was carried out on the samples and $1 \mu\text{L}$ of the sedimented phase was injected into the separation system

Analyte stability

The stability of 2-octanone was assessed by analyzing triplicate QC samples, exposed to different storage conditions including room temperature ($25 \pm 2.0 \text{ }^\circ\text{C}$) for 12 h and three freeze-thaw cycles. No significant degradation of 2-octanone was observed under various storage conditions (Table 4).

Robustness

Robustness of the method was evaluated by different volumes of the extraction solvent (60, 62, and 64 μL), various pHs (1.9, 2.0, and 2.1) and different NaCl concentrations (7, 7.5, and 8%, w/v). The obtained results were comparable with each other and the differences among them were not significant (Table 5). Figures 1 and 2 show the chromatograms of blank and spiked biological samples injected to the system after microextraction as well as injection of standard solutions to the system.

Conflict of interests

The authors claim that there is no conflict of interest.

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