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Determination of 2-phenylethanol in Rose Water Using Dispersive Liquid-Liquid Microextraction with Gas Chromatography Flame Ionization Detection

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A simple and rapid microextraction procedure based on dispersive liquid-liquid microextraction (DLLME) coupled with gas chromatography-flame ionization detection (GC-FID) was developed for the extraction and analysis of 2-phenylethanol in rose water sample. In the proposed approach, carbon tetrachloride and ethanol were used as extraction and dispersive solvents, respectively. Some important parameters, such as extraction and disperser solvent and volume of them, extraction time, pH and salt effect were investigated. Under optimized conditions, a linear relationship was obtained in the range of 1.0-300.0 mg l^{-1}. The method detection limit was 0.1 mg l^{-1}. The relative standard deviations for the analysis of 2-phenylethanol were in the range of 1.5-2.4%. The relative recoveries of 2-phenylethanol at spiking levels of 10, 75 and 150 mg l^{-1} were 93.7, 96.9 and 97.2%, respectively. The enrichment factor of the proposed method was 123. The proposed method is a simple, fast, accurate, highly stable and selective and was applied for determination of 2-phenylethanol in rose water sample with satisfactory results.

Keywords: 2-Phenylethanol, Dispersive liquid-liquid microextraction, Gas chromatography-flame ionization detection, Rose water

INTRODUCTION

2-Phenylethanol, commonly known as phenethyl alcohol, is a compound with a great application mainly in food (soft drinks, candy and cookies) and cosmetic industries (its esters, especially phenylethyl acetate, are also valuable flavour and fragrance compounds) due to its characteristic fragrance and flavour [1-3]. 2-Phenylethanol can be occurred naturally in the essential oils of many flowers and plants [4,5]. In most cases, concentration of 2-phenylethanol is too low for direct extraction. One exception is rose oil [6]. Typical level of concentration of 2-phenylethanol in rose oil was found up to 60% [7]. It has been reported that other natural products containing 2-phenylethanol are foodstuffs whose production involves fermentation, such as tea leaves, cocoa, coffee, bread, wine, cider, beer, cheese and soy sauce [8,9].

Several methods have been reported for extraction of 2-phenylethanol from different samples, including supercritical fluid extraction (SFE) [10], solid phase extraction (SPE) [11], solid phase microextraction (SPME) [12-14], headspace-solid-phase microextraction (HS-SPME) [15, 16], liquid-liquid microextraction (LLME) [17] and stir bar sorptive extraction (SBSE) [18]. Liquid-liquid extraction (LLE) is a classical method for clean-up and pre-concentration of analytes. Conventional extraction methods based on LLE are time-consuming and need a large amount of organic solvents, which are dangerous for human health and the environment. In the last decades, microextraction methods such as liquid phase microextraction (LPME), single drop microextraction (SDME), ultrasound-assisted emulsification microextraction (USAEME) and dispersive liquid-liquid microextraction (DLLME) have attracted increasing attention as novel sample preparation techniques. These techniques are simple, low-cost, rapid, and require only very small sample and solvent consumption.

DLLME employs a mixture of a high-density solvent (extractant) and a water miscible, polar solvent (disperser).

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Acetone, methanol, ethanol and acetonitrile can be used as dispersers, whereas chlorinated solvents (*e.g.* chlorobenzene, carbon tetrachloride, tetrachloroethylene) are useful as extractants. When this solution is added to a water sample, a cloudy state, consisting of fine droplets of the extractant dispersed in the aqueous matrix, is formed. The large contact surface between the sample and the droplets of the extractant speeds up mass transference processes. After centrifuging the fine droplets of extraction solvent are sedimented in the bottom of the conical test tube.

DLLME was developed for the extraction of some organic compounds in aqueous matrices [19-23]. The main advantages of DLLME are: rapidity, high enrichment factor, high extraction recovery and simplicity of operation [24-26].

The aim of the present work is the development of a rapid, simple and sensitive DLLME method coupled with gas chromatography-flame ionization detection for the determination and analysis of 2-phenylethanol in rose water sample.

EXPERIMENTAL

Chemicals and Solvents

2-Phenylethanol with purity of >99%, benzaldehyde (Internal Standard, I.S.), hydrochloric acid, sodium hydroxide, sodium chloride, ethanol, methanol, acetone, dichloromethane, chloroform and carbon tetrachloride were obtained from Merck (Darmstadt, Germany). Acetonitrile was supplied from Acros (Belgium). Doubly distilled water was used in all experiments. Rose water was purchased from a local supermarket.

Instrumentation

Experiments were carried out using a gas chromatograph (GC-17 Shimadzu, Japan) equipped with a flame ionization detector (GC-FID) and a BP5 capillary column (25 m × 0.32 mm I.D., film thickness 0.25 µm). Helium (99.9999%) was used as the carrier gas. The inlet was operated in splitless mode. The oven temperature was programmed as follows: initial temperature 40 °C (held for 1 min) ramped at 20 °C min^{-1} to 250 °C (held for 10 min). The temperatures of injector and detector were set at 280 °C and 300 °C, respectively.

GC-MS analyses were performed using an Agilent gas chromatograph 7890A (Agilent, Little Falls, DE, USA) coupled with an electronically controlled split/splitless injection port and interfaced to a MSD-5975C mass selective detector. The gas chromatograph was equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness) purchased from J&W Scientific (Folsom, CA, USA). Helium (99.9999%) was used as carrier gas, with a flow rate of 1 ml min^{-1}. The oven temperature was programmed similarly as mentioned above. The injection was performed at 280 °C in split mode (ratio 20:1). The transfer line and ion source were set at 250 and 200 °C, respectively. The mass spectra worked in total-ion-scanning (TIC) mode and the electron impact energy was set at 70 eV. Mass range was from m/z 50-600 amu. The identification of analyte was done by matching its retention time against that of the standards and GC-MS.

Dispersive Liquid-liquid Microextraction Procedure

An aliquot (5 ml) of aqueous solution containing of 2-phenylethanol (20.0 mg l^{-1}) and I.S. (50.0 mg l^{-1}) and sodium chloride (4% (w/v) was poured in a 10-ml screw caped test tube with conical bottom. A 0.6 ml of ethanol, as disperser solvent, containing 25 µl of carbon tetrachloride (as extraction solvent) was rapidly injected into the sample solution with a 1.0-ml syringe (F-LC, SGE, Australia) and then the mixture was gently shaken for 1 min. A cloudy solution was formed in a test tube (the cloudy state was stable for a long time). The mixture was centrifuged for 10 min at 2000 rpm. The dispersed fine droplets of extraction solvent were sedimented in the bottom of conical test tube. Then, 1.0 µl of sedimented phase was removed using a 10-µl micro syringe (F-LC, SGE, Australia) and injected into the GC-FID or GC-MS.

RESULTS AND DISCUSSION

Effect of Type and Volume of Disperser Solvent

The selection of disperser solvent is a critical factor in DLLME. Ideally, the disperser solvent should be miscible both with extraction solvent and sample. Acetonitrile, ethanol, methanol and acetone were compared in the extraction of 2-phenylethanol. Figure 1 shows the results in the term of listing the percent recovery of 2-phenylethanol

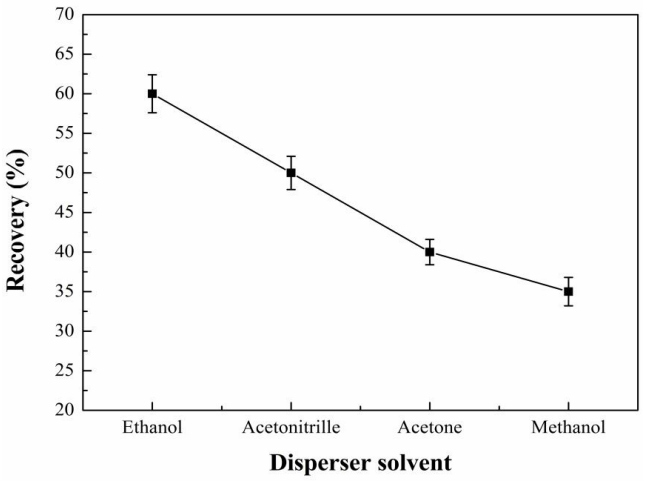

Fig. 1. Effect of the disperser solvent type on the recovery of 2-phenylethanol. Extraction conditions: volume of disperser solvent, 0.8 ml; volume of extraction solvent (CCl_4), 20 µl; concentration of NaCl, (0% w/v); pH, 7.0; extraction time, 1 min.

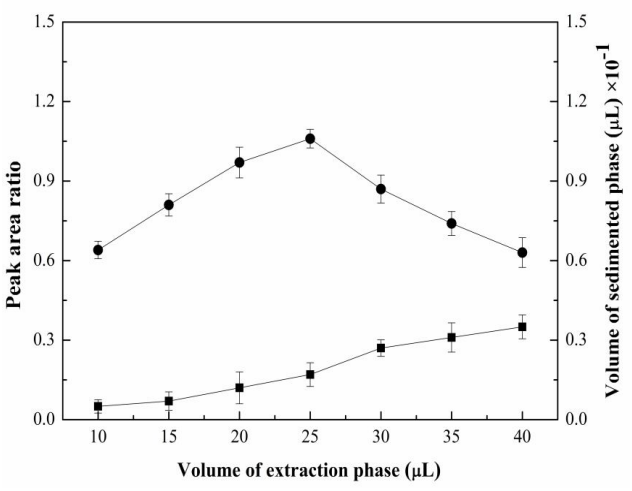

Fig. 2. Effect of the extraction solvent volume on the peak area ratio of 2-phenylethanol and relationship between initial extraction solvent volume and sedimented phase volume. Extraction conditions: volume of the disperser solvent (ethanol), 0.6 ml; concentration of NaCl, (0% w/v); pH, 7.0; extraction time, 1 min.

with different disperser solvents at fixed volume of carbon tetrachloride (20 µl, extraction solvent). As it can be seen, ethanol provides better extraction efficiency than other solvents.

The effect of disperser solvent volume on the peak area ratio is shown in Table 1. The results show that peak area ratio increased with increasing disperser solvent volume up to 0.6 ml. At lower volumes of ethanol, the cloudy suspension of CCl_4 droplets is not formed well, resulting in a decrease in the extraction efficiency [27]. At higher volumes of ethanol, the solubility of 2-phenylethanol in water increases and the extraction efficiency decreases [28]. However, the peak area ratio and the extraction efficiency decreased by further increase in the disperser solvent volume from 0.7 to 0.9 ml. A 0.6 ml of ethanol was used for the subsequent experiments.

Selection of Extraction Solvent

In DLLME, the selection of suitable organic solvents is based on the requirement of a higher density than that of water, the solvent's extraction capability for selected compounds, and good chromatographic behavior. The selection of an appropriate solvent is of high importance for the DLLME process [29,30]. Based on these considerations, dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$) and carbon tetrachloride (CCl_4) were compared for the extraction of 2-phenylethanol. The physical properties of extraction solvents are shown in Table 2 [31]. It was found that except for carbon tetrachloride-ethanol system, all other combinations of extraction and disperser solvents do not show stable cloudy solution. CH_2Cl_2 was completely dissolved in the aqueous solution and chloroform forms an unstable cloudy solution. Based on the above results, CCl_4 and ethanol were chosen as extraction and disperser solvents, respectively.

Effect of Extraction Solvent Volume

To optimize the effect of extraction solvent volume, a fixed volume of ethanol (0.6 ml) containing different volumes of CCl_4 in the range 10-40 µl were subjected to the same DLLME procedure. The results presented in Fig. 2 revealed that the analytical signal virtually increases with CCl_4 volume in the range of 10-25 µl. However, a further increase in CCl_4 volume from 30 µl to 40 µl results in a

Table 1. Effect of Volume of Disperser Solvent on the Peak Area Ratio of 2-Phenylethanol

Volume of disperser solvent (ml)	RSD (%)	Peak area ratio
0.4	2.3	0.56
0.5	1.9	0.79
0.6	1.2	0.91
0.7	2.0	0.74
0.8	1.4	0.55
0.9	1.3	0.47

Extraction conditions: water sample volume, 5.0 ml; disperser solvent, ethanol; extraction solvent (CCl_4) volume, 20 µl; concentration of NaCl, (0% w/v); pH, 7.0; extraction time, 1 min; volume of sedimented solvent, 12 µl.

Table 2. Physicochemical Properties of the Solvents Studied as Possible Extractants [31]

Solvents	Density 20 °C ($g\ ml^{-1}$)	Water solubility 20 °C ($g\ ml^{-1}$)
CH_2Cl_2	1.33	0.0130
$CHCl_3$	1.48	0.0080
CCl_4	1.59	0.0008

Table 3. Effect of Salt Addition on the Peak Area Ratio of 2-Phenylethanol

NaCl (w/v)%	Volume of sedimented phase (µl)	RSD (%)	Peak area ratio
0	17	2.3	1.06
2	18	1.8	1.13
4	18	1.7	1.25
6	19	1.1	1.21
8	20	2.1	1.19
10	21	2.0	1.16

Extraction conditions: water sample volume, 5.00 ml; disperser solvent, ethanol; volume of disperser solvent, 0.6 ml; extraction solvent, (CCl_4); volume of extraction solvent, 25 µl; pH, 7.0; extraction time, 1 min.

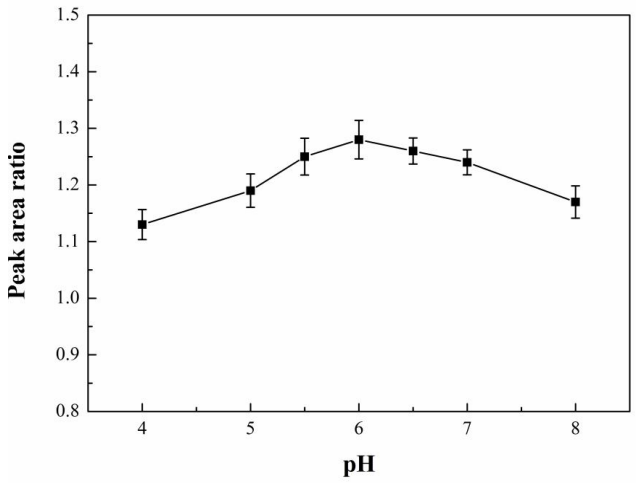

Fig. 3. Effect of pH on the peak area ratio of 2-phenylethanol. Extraction conditions: volume of the disperser solvent (ethanol), 0.6 ml; volume of the extraction solvent (CCl_4), 25 µl; concentration of NaCl, (4% w/v); extraction time, 1 min.

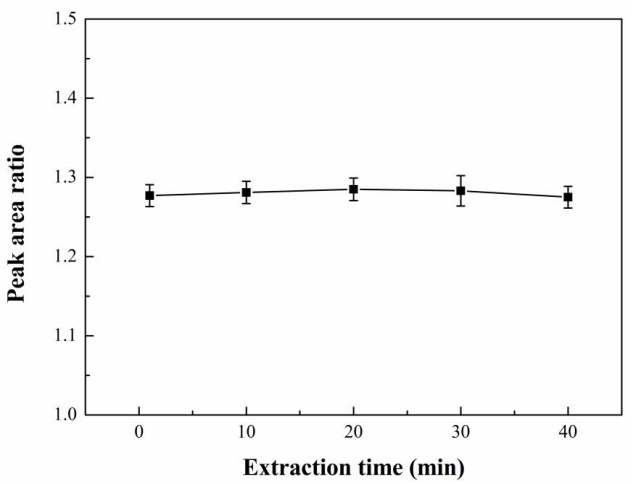

Fig. 4. Effect of the extraction time on the peak area ratio of 2-phenylethanol. Extraction conditions: volume of the disperser solvent (ethanol), 0.6 ml; volume of the extraction solvent (CCl_4), 25 µl; concentration of NaCl, (4% w/v); pH, 6.0.

decrease in the peak area ratio. This could be assigned to the formation of larger CCl_4 droplets and consequently increase in sedimented phase volume (Fig. 2). Hence, a 25 µl of extraction solvent volume was applied for the subsequent experiments.

Salt Addition

The effect of increasing the ionic strength of the aqueous sample was evaluated by adding NaCl (0-10%, w/v) into the water sample spiked with 2-phenylethanol at level of 20.0 mg l^{-1}. DLLME experimental conditions were the same as those described before. The results are summarized in Table 3. It is clear that by increasing the NaCl concentration from 0 to 4% solubility of analyte in aqueous solutions decreases due to the salting-out effect and peak area ratio increases. A further increase in NaCl from 4 to 10% results in an increase in the volume of the sedimented phase from 18.0 to 21.0 µl, due to the decrease in aqueous solubility of the extraction solvent in the presence of salt and the peak area ratio decreases [32].

Effect of pH

It is very important to optimize the pH of the aqueous solution because it determines the existing state of analytes, as well as the extraction efficiency of target compounds. The analytes needs to be in their neutral form for efficient partitioning from an aqueous phase in to a hydrophobic organic solvent [27]. In this study, the effect of varying pH values of the sample solution was examined in the range of 4.0-8.0 under the proposed method. The results exhibited in Fig. 3 indicate that the extraction efficiency is increased by increasing the pH up to 6.0 and then is decreased with further increase in pH. The reason behind is that changing the pH of the sample solution results in protonation or deprotonation of 2-phenylethanol, which can significantly affect its solubility in aqueous phase and decrease the amount of 2-phenylethanol in extractant phase. Thus, in the successive experiments the pH of the sample solution was adjusted to 6.0 with the use of hydrochloric acid and sodium hydroxide.

Effect of Extraction Time

The effect of extraction time (interval time between the injection of a mixture of disperser solvent and extraction solvent, before starting to centrifuge) on the performance of DLLME is considered as a key factor which must be studied

Table 4. Results from Determination of Precision and Recovery of 2-Phenylethanol by Standard Addition Method under Optimized Conditions (n = 3)

Added ($mg\ l^{-1}$)	Found ($mg\ l^{-1} \pm$ S.D.)	RSD (%)	Recovery (%)
00.0	5.5 ± 0.09	1.6	-
10.0	14.8 ± 0.22	1.5	93.7
75.0	78.2 ± 1.71	2.2	96.9
150.0	151.3 ± 3.63	2.4	97.2

Table 5. Comparison of Different Methods for the Determination of 2-Phenylethanol in Food Samples

Parameter	Ref. [15]	Ref. [16]	Proposed method
LR ($mg\ l^{-1}$)	0.03-7.5	1.0-200	1.0-300
LOD ($mg\ l^{-1}$)	0.02	0.1	0.1
RSD (%)	6	12	3>
Recovery (%)	98	104	93<
Time (min)	60	60	10
Matrix	Tomato	Wine	Rose water

and evaluated. Therefore, for evaluating this parameter, different extraction times (ranged from 1-40 min) with constant other experimental conditions were studied. According to the results (Fig. 4), this extraction method is time-independent, due to the infinitely large surface area between extraction solvent and aqueous phase. Therefore, this method is very fast which is the most important advantage of DLLME technique.

Method Validation

Under the above mentioned optimized experimental conditions, the proposed method was validated by linearity, precision, recovery, the limit of detection (LOD), limit of quantification (LOQ) and enrichment factor (EF). The calibration plot was found to be linear in the range of 1.0-300.0 mg l^{-1}, with a coefficient of determination (R^2) of 0.9997 (n = 9). For each concentration level, three replicate extractions were performed. LOD value was calculated as three times the standard deviation of ten replicate runs of aqueous sample spiked with low concentration of 2-phenylethanol (1.0 mg l^{-1}) [33]. The LOD value was 0.1 mg l^{-1}. LOQ value was calculated as ten times the standard deviation of ten replicate runs of aqueous sample spiked with low concentration of 2-phenylethanol. The LOQ value was 0.33 mg l^{-1}. The precision and the recoveries of 2-phenylethanol determined by standard addition method,

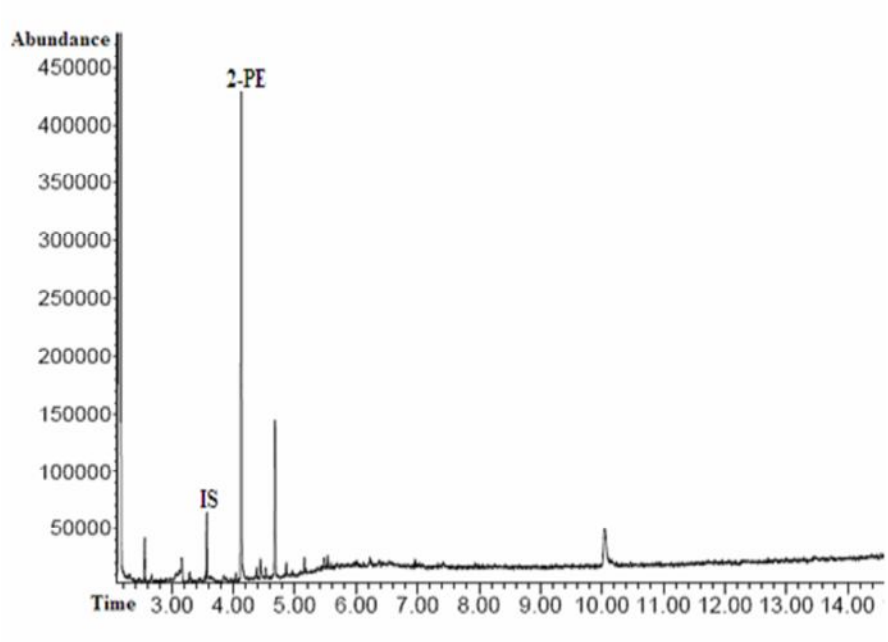

Fig. 5. Total ion chromatogram of unspiked rose water sample after DLLME obtained under optimized conditions.

were calculated by analyzing in replicate (n = 3) rose water sample spiked with three different concentration levels (10.0, 75.0 and 150.0 mg l^{-1}) of analyte. The relative standard deviations (RSDs) for the measured concentrations were in the range of 1.5-2.4% and the recoveries varied from 93.7% to 97.2%. The results are summarized in Table 4. Enrichment factor was defined as the ratio of the concentrations of 2-phenylethanol in sedimented phase and aqueous sample. The enrichment factor of the proposed method was 123.

Figure 5 shows GC-MS chromatogramic profile of a rose water sample extracted by the proposed DLLME method.

Table 5 indicates the linear range (LR), LOD, extraction time, RSD (%) and recovery (%) using headspace solid-phase microextraction-gas chromatography-mass spectrometric detection (HS-SPME-GC-MS) [15], headspace solid-phase microextraction-gas chromatography-flame ionization detection (HS-SPME-GC-FID) [16] and dispersive liquid-liquid microextraction-gas chromatography-flame ionization detection (DLLME-GC-FID, proposed method) methods for the determination of 2-phenylethanol in food samples. The proposed method provides similar quantification extraction efficiency, with advantages of being faster and lower limit of detection.

CONCLUSIONS

In the present study, a new mode of microextraction technique was described as a dispersive liquid-liquid microextraction (DLLME) which has been developed. DLLME provides high enrichment factor within a very short time. 2-Phenylethanol was employed as a model compound to assess the extraction procedure and was determined by GC-FID. The comparison of the new method with other methods demonstrated that DLLME is fast, simple, and inexpensive.

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