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Quantitation of valproic acid in pharmaceutical preparations using dispersive liquid-liquid microextraction followed by gas chromatography-flame ionization detection without prior derivatization

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Dispersive liquid-liquid microextraction (DLLME), coupled with gas chromatography-flame ionization detection (GC-FID), has been successfully used for the extraction and determination of valproic acid (VPA) in pharmaceutical preparations. In the developed method, an appropriate mixture of extracting and disperser solvents was rapidly injected into an aqueous sample. Having formed a cloudy solution, the mixture was centrifuged and then the extracting solvent was sedimented at the bottom of a conical test tube. The extract was then injected into a GC system directly, without any further pretreatment. Initially, microextraction efficiency factors were optimized and the optimum experimental conditions found were as follows: tetrachloroethylene (9.0 μL) as extracting solvent; acetone (1.0 mL) as disperser solvent; 5 mL acidic aqueous sample (pH 1) without salt addition. Under the selected conditions, the calibration curve showed linearity in the range of 0.1–5.0 mg/L with regression coefficient corresponding to 0.9998. The limit of detection was found to be 0.05 mg/L. Finally, the method was applied for the determination of VPA in two different pharmaceutical preparations. A reasonable intra-assay (3.9–10.8%, $n = 3$) and inter-assay (5.6–11.4%, $n = 3$) precision illustrated the good performance of the analytical procedure. The protocol proved to be rapid and cost-effective for screening purposes.

Keywords: dispersive liquid-liquid microextraction; gas chromatography-flame ionization detection; valproic acid; pharmaceutical preparation

Introduction

Valproic acid (2-propylvaleric acid, VPA) is an anticonvulsant and mood-stabilizing agent widely used in the treatment of epilepsy, bipolar disorder. and prophylaxis of migraine headaches.[1–5] Because of these properties, in the recent years, VPA production and use have increased significantly. Therefore, the drug monitoring in various matrixes is of great importance.

The analytical methods used in the determination of VPA are mainly high performance liquid chromatography (HPLC)[6–12] and capillary electrophoresis (CE).[13–19] Additionally, due to the volatility of VPA, gas chromatography (GC) is a common tool for the analysis, offering unrivalled high resolution.[20–23] Other methods have been reported including chemical ionization mass spectrometry,[24] isotope dilution mass spectrometry,[25] colorimetric determination[26] and also potentiometry ion selective sensors.[27,28] The drug in complex matrixes was determined by these techniques usually after laborious manipulation of the sample including extraction, evaporation, and derivatization before the instrumental analysis.

Recent research activities are oriented towards the development of efficient, economical, and miniaturized sample preparation methods. The invention of solid phase microextraction (SPME) by Pawliszyn *et al*.[29] basically initiated the interest for microextraction techniques in analytical chemistry. SPME satisfies most of the requirements of a good sample preparation technique, including simplicity of use, automation, and low consumption of materials.[30] Therefore, it has been used for many applications consisting of environmental, biological, and pharmaceutical monitoring.[31]

An alternative solvent-minimized sample preparation approach to complement SPME appeared in the middle-to-late 1990s;[32–34]

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liquid-phase microextraction (LPME) utilizes only a small amount of solvent (low microlitre range) for concentrating analytes from aqueous samples. It is simply a miniaturized format of liquid-liquid extraction (LLE). It overcomes many of the disadvantages of LLE as well as some of those of SPME (e.g. non-dependence on a commercial supplier and sample carryover). LPME is simple to implement and use, it's generally fast, and is characterized by its affordability and reliance on widely available apparatuses or materials.[35,36] The applications of LPME in environmental and biological analysis have been described in several papers.[37–40]

Recently, Assadi *et al*. developed a simple and novel LPME method, namely dispersive liquid-liquid microextraction (DLLME)[41]. It is based on ternary component solvents system and consists of two steps. (1) The injection of an appropriate mixture of extracting and disperser solvent into aqueous samples containing the analytes. In this step, the extracting solvent is dispersed into the aqueous sample as very fine droplets and the analytes are enriched into it. Owing to the infinitely large surface area between the extracting solvent and the aqueous sample, the equilibrium state is achieved quickly and the extraction is independent of time. This is the most important advantage of this method. (2) The centrifugation of the cloudy solution. After centrifugation, the determination of the analytes in the sedimented phase can be performed by a proper instrumental analysis. Rapidity, operation simplicity, and low cost are some of the advantages of this method.

The aim of the present study is to assess the DLLME method suitability for the determination of VPA in pharmaceutical preparations. The factors affecting the microextraction efficiency were studied in detail and the optimal conditions were established. The method was then validated for quantitative purposes, and applied to real sample analysis in combination with gas chromatography-flame ionization detection (GC-FID).

Experimental

Reagents

Sodium valproate (SV) was kindly supplied by Roozdaroo Pharmaceutical Company (Tehran, Iran). A stock solution of SV (500 mg/L) was prepared by dissolving proper amounts of pure substance in methanol, stored at 4 °C. The working solutions were prepared by diluting the stock solution with water. Double-distilled water, used for preparing the working solutions, was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA). All of organic solvents as well as hydrochloride acid (HCl), sodium hydroxide (NaOH), sodium chloride and hexanoic acid (as internal standard, I.S.) were of analytical grade and purchased from Merck (Darmstradt, Germany).

Instrumentation

The analysis was performed on a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) HP 7890 series GC, equipped with FID and split/splitless injector. The chromatographic data were recorded using HP Chemstation software, which was controlled by Microsoft Windows NT. The analytes were separated on a 30 m × 0.32 mm i.d. × 0.25 µm film thickness BP-10 gas chromatographic column (14% Cyanopropyl phenyl dimethylpoly siloxane) from J&W Scientific (Folsom, CA, USA) with the following oven temperature program: the initial temperature was 50 °C, held 1 min, and increased to 140 °C at a rate of 10 °C/min, then raised to 250 °C at a rate of 40 °C/min and finally held at the latter temperature for 15 min. The injector and detector temperatures were set at 250 °C and 280 °C respectively. The injection port was operated at a split ratio of 1 : 2. The gas flow rates were as follows: carrier (Helium, 99.999%) 2.0 mL/min, make-up (Nitrogen, 99.999%) 30 mL/min, hydrogen and air (for FID) 40 and 300 mL/min, respectively.

Extraction procedure

A 5 mL acidic aqueous sample, to assure that all dissolved SV would be in the form of VPA, was transferred into a 10-mL screw-capped glass test tube with conic bottom, and spiked at particular levels of the analyte. To adjust the pH at the desired value, a micropipette was used for addition of an appropriate amount of HCl. Acetone (1.0 mL) as the disperser solvent, containing 9.0 µL tetrachloroethylene (extracting solvent) was injected rapidly into the sample solution using a 2.0 mL syringe (gastight, Hamilton, Reno, USA). In this step, a cloudy solution (water/acetone/tetrachloroethylene) was formed in the test tube and the analyte in the water sample was extracted into fine tetrachloroethylene droplets. The mixture was then centrifuged for 5 min at 4000 rpm. The volume of the sedimented phase, measured by a 10 µL microsyringe, was about 5.0 µL. Of which 1.0 µL was withdrawn by a 5.0 µL microsyringe (SGE, Victoria, Australia) and immediately injected into the GC system.

Results and Discussion

There are various parameters affecting the DLLME performance and efficiency, including aqueous sample pH, the nature and volume of the extracting and the disperser solvents, as well as the ionic strength. These parameters were investigated and the optimal conditions were then established. A one-at-a-time strategy was employed to optimize the influential factors in this method. To screen the mentioned parameters, PF was calculated using the following expression:

$$PF = \frac{C_{sed}}{C_0} \quad (1)$$

where, C_{sed} and C_0 are the concentration of analyte in sedimented phase and initial concentration of analyte in aqueous sample solution respectively. These parameters were known except for C_{sed}. The C_{sed} calculation was conducted by the direct injection of VPA standard solutions in tetrachloroethylene with the concentrations in the range of 10–500 mg/L.

A fixed concentration, 0.50 mg/L of the analyte, was used in the optimization process. In order to improve the precision and accuracy of the method in all of experiments hexanoic acid with the concentration of 5.0 mg/L was used as I.S. and added into the sample solution before the extraction process. All quantifications, made in this study, were based on the relative peak area of the analyte to I.S. from the average of three replicate measurements.

Selection of the extracting solvent

The selection of an appropriate extracting solvent is very important to achieve good recovery and high preconcentration factor for the target compound.[39,41,42] The extracting solvent has to meet four requirements; it should demonstrate (1) higher density than water; (2) good chromatographic behaviour; (3) extraction capability of the interested compounds; and (4) low solubility in water.[43–45] Chlorobenzene, carbon tetrachloride, and tetrachloroethylene

Table 1. Efficiency of different extracting solvents evaluated for extraction of VPA by DLLME.[a]

	Chlorobenzene, mean ± S.D.[b] ($n = 3$)	Tetrachloroethylene, mean ± S.D. ($n = 3$)	Carbon tetrachloride, mean ± S.D. ($n = 3$)
PF	26 ± 3	38 ± 5	29 ± 4

[a] Extraction conditions: aqueous sample volume, 5 mL (pH 3); disperser solvent (acetone) volume, 1.0 mL;
[b] Standard deviation.

Table 2. Efficiency of different disperser solvents evaluated for extraction of VPA by DLLME.[a]

	Acetone, mean ± S.D.[b] ($n = 3$)	Methanol, mean ± S.D. ($n = 3$)	Acetonitrile, mean ± S.D. ($n = 3$)
PF	69 ± 6	55 ± 6	60 ± 7

[a] Extraction conditions: aqueous sample volume, 5 mL (pH 1); disperser solvent volume: 1.0 mL; extracting solvent, tetrachloroethylene.
[b] Standard deviation.

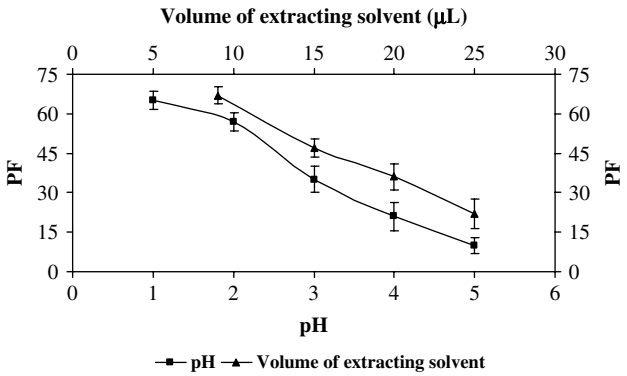

Figure 1. The effects of pH of the sample solution and extracting solvent volume on the PF obtained from DLLME. Extraction conditions: aqueous sample volume, 5 mL; extracting solvent, tetrachloroethylene; disperser solvent (acetone) volume, 1.0 mL.

were initially examined in order to find the most suitable extracting solvent for DLLME. For this purpose, a series of acidic sample solutions (pH = 3 was preliminary chosen, unless the following optimization process produced otherwise) were studied by using 1.0 mL acetone containing different volumes of the extracting solvent to achieve 5.0 µL volume of the sedimented phase. Therefore, 9.0, 15.0, and 13.0 µL of tetrachloroethylene, chlorobenzene, and carbon tetrachloride were used, respectively. The results in Table 1 revealed that tetrachloroethylene presented the highest PF in comparison with the other two solvents. Therefore, tetrachloroethylene was selected as the optimal extracting solvent.

Effect of the sample pH

VPA ($pK_a = 4.8 \pm 0.2$ [46]) exists in neutral (un-ionized) form at low pH, thus it has more tendencies to get dissolved in water and its extraction efficiency would be reduced. Different amounts of HCl and NaOH were added to the sample solution to investigate the effect of pH on the PF. Since pH 1 provided the highest extraction efficiency (Figure 1), it was selected for the subsequent experiments.

Selection of the disperser solvent

The miscibility of the disperser solvent in the organic phase (extracting solvent) and the aqueous phase (sample solution) is the main point for the selection of the disperser solvent.[47–49] Acetonitrile, acetone, and methanol, illustrating the above ability, were initially chosen for this purpose. In order to achieve the sedimented phase with a volume around 5.0 µL, a series of aqueous sample solutions were injected by adding 1.0 mL of acetonitrile, acetone, and methanol, as disperser solvent, containing 10.0, 9.0, and 11.0 µL tetrachloroethylene, respectively. Based on the results demonstrated in Table 2, acetone was selected as the optimal disperser solvent for the further studies.

Effect of the extracting and disperser solvent volumes

In order to evaluate the effect of the extracting solvent volume on the extraction efficiency, additional experiments were performed using 1.0 mL acetone containing different tetrachloroethylene volumes (9.0, 15.0, 20.0 and 25.0 µL). It is clear that by increasing the volume of extracting solvent, the volume of the sedimented phase increases (5.0 to 20.0 µL) and, as a result, the PF decreases (Figure 1). Taking all the above-mentioned points into account, the volume of 9.0 µL of tetrachloroethylene was selected in the following studies.

The variation in the acetone volume (disperser solvent) causes changes in the volume of the sedimented phase. To avoid this problem and in order to achieve a constant volume of the sedimented phase, the volumes of acetone and tetrachloroethylene were changed simultaneously. The experimental conditions were fixed and included the use of different acetone volumes (0.5, 1.0, 1.5 and 2.0 mL), each of which contained 7.5, 9.0, 11.5, and 13.5 µL tetrachloroethylene, respectively. At this step, the volume of sedimented phase was relatively constant (5.0 ± 0.3 µL). It seems that at low volumes of acetone, the cloudy state is not fully formed, and thus the PF is low; while at higher volumes of acetone, the solubility of the analytes in the aqueous samples increases. The PF decreases because of a decrease in the distribution coefficient of the analytes. Taking

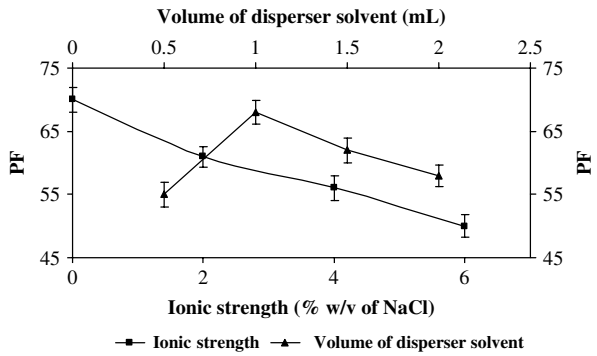

Figure 2. The effects of ionic strength and disperser solvent volume and on the PF obtained from DLLME. Extraction conditions: aqueous sample volume, 5 mL (pH 1) extracting solvent, tetrachloroethylene; disperser solvent, acetone.

Table 3. Quantitative data obtained after the DLLME-GC-FID determination of VPA in pharmaceutical preparations

Parameter	Analytical feature
Limit of detection (LOD, mg/L)	0.05
Linear range (LR, mg/L)	0.1–5.0
Regression coefficient (r^2)	0.9998
Regression equation	$A_r{}^a = 136\ C^b - 3.22$
Preconcentration factor (PF, at concentration level of 0.5 mg L^{-1}, n = 3)	67

[a] Relative peak area.
[b] Concentration (mg/L).

all the mentioned points into consideration, a constant volume of acetone (1.0 mL) has been selected as the optimal value (Figure 2).

Effect of the ionic strength

The addition of salt to an analytical sample can potentially cause the formation of hydration spheres, which reduce the amount of water available to dissolve the analyte in water. This effect resulted in an increase in analyte recovery in microextraction procedures.[50–52] The effect of increasing the ionic strength of the water samples evaluated by adding NaCl (0–6%, w/v) in the water sample, already spiked into with the analyte at the level of 0.5 mg/L. DLLME experimental conditions were the same as those described before. The plot of PF versus ionic strength has been shown in Figure 2. It is clear that by increasing the concentration of NaCl, because of the decrease in solubility of extracting solvent in the presence of the salt, the volume of sedimented phase increases leading to decrease in the PF. Thus, no addition of salt was employed for the further experiments.

Evaluation of the method performance

To assess the practical applicability of the method, the optimized conditions were adopted in the evaluation of linear range (LR), correlation coefficient (r^2), limit of detection (LOD) and PF. For each level, three similar extractions were performed. The results are summarized in Table 3. As shown, the LR varied in the range of 0.1–5.0 mg/L with r^2 corresponding to 0.9998. The LOD, defined as the analytical signal which is larger than the blank by multiple three of the variation in the blank, was found to be 0.05 mg/L. Furthermore, the mean value of PF was found to be 67 ($n = 3$) at the concentration level of 0.5 mg/L.

Table 4. The results obtained from the analysis of real samples

Sample	Concentration (mg/L)	VPA (mg/L) Added	VPA (mg/L) Found ($n = 3$)
Syrup	0.2	0	0.22 ± 0.02
Tablet	0.5	0	0.47 ± 0.05

Analysis of pharmaceutical preparations

The proposed method was applied for the analysis of VPA in the tablet and syrup samples. Both samples were dissolved in water and then diluted for preparing the solutions containing 0.5 and 0.2 mg/L of VPA, respectively. Having applied the standard addition method, the mean concentrations ($n = 3$) of VPA were found to be 0.47 and 0.22 mg/L in the tablet and syrup sample solutions, respectively. The analytical results are summarized in Table 4. The DLLME-GC-FID chromatograms of VPA at different variety of concentration levels in the aqueous and real samples are depicted in Figures 3 and 4. It is noteworthy that the percent relative inter-day and intra-day standard deviation (RSD %) based on three replicate determinations varied in the ranges of 3.9–10.8 and 5.6–11.4%, respectively.

Conclusion

This research article outlines the successful application of the DLLME technique, combined with GC-FID for qualitative and quantitative analysis of VPA in some pharmaceutical preparations. Compared to conventional sample preparation methods,

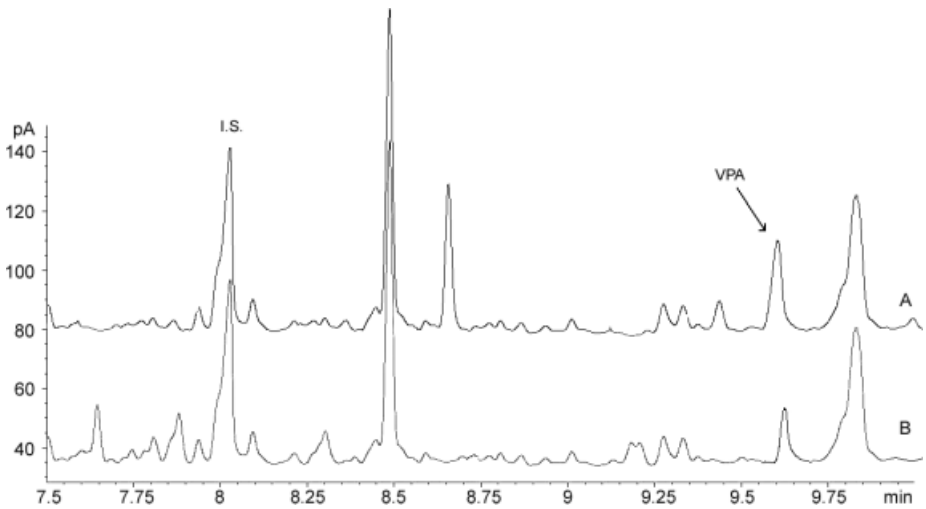

Figure 3. The DLLME-GC-FID chromatograms of VPA at the concentration level of 0.5 mg/L in the tablet sample (A) and that of VPA at the concentration level of 0.2 mg/L in the syrup sample (B). Extraction conditions: aqueous sample volume, 5 mL (pH 1); extracting solvent, tetrachloroethylene; disperser solvent (acetone) volume, 1.0 mL.

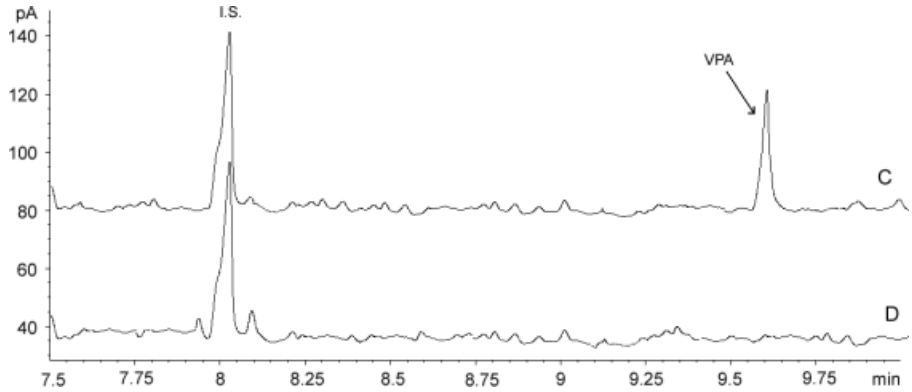

Figure 4. The DLLME-GC-FID chromatogram of VPA at the spiked level of 0.5 mg/L in the aqueous sample (C) and the blank one (D). Extraction conditions: aqueous sample volume, 5 mL (pH 1); extracting solvent, tetrachloroethylene; disperser solvent (acetone) volume, 1.0 mL.

the current protocol has numerous advantages, such as simplicity, low cost, ease of operation, no possibility of sample carryover, and a very short analysis time. Moreover, there was no need for evaporation of solvent and derivatization of the analyte prior to injection into the GC. Putting all the benefits together, it possesses great potential to be employed routinely in the quality control process.

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