RESEARCH ARTICLE

Dispersive Liquid-liquid Microextraction (DLLME)-Spectrophotometric Determination of Procaine Hydrochloride in Pharmaceutical Preparations

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ABSTRACT

An efficient, simple, fast, economical, and selective dispersive liquid-liquid microextraction (DLLME) coupling with the spectrophotometric determination of Procaine hydrochloride (Pro.) in pharmaceutical preparation was introduced. The developed method is based on Schiff's base condensation reaction in an alkaline medium between sodium 1,2-naphthaquinone-4-sulfonate and the amino group of pro. to give an orange-red color (λ=485 nm). The method permits the determination of Pro. over a concentration range of 0.0005–0.2 µg.mL^{-1}. Moreover, the limit of detection and the limit of quantification were 0.0003 µg.mL^{-1} and 0.01µg.mL^{-1} for Pro. Std, respectively. Good recoveries of Pro. std., and drugs ranging from 95.4 to 102.4% were obtained. The proposed method was successfully applied for the determination of Pro. in pharmaceutical preparations.

Keywords: Dispersive liquid-liquid microextraction, Microextraction, Procaine, Pharmaceutical Preparation, Spectrophotometry

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INTRODUCTION

Local anesthetic procaine (Pro.) was first synthesized in 1905, chemically known as 2-diethyl aminoethyl-4- aminobenzoate hydrochloride (Figure 1) and its formula $C_{13}H_{20}N_2O_2$ with a molecular weight of 272.8. Pro. usually applied as an antibacterial drug alone or with penicillin. Moreover, it acts as a nerve block as it halting the generation and conduction of nerve impulses signal pain. In addition, it is sometimes used in obstetrics, relief pain in the lower back and tooth extraction.[1,2]

Various analytical methods such as chromatography,[3] high-performance liquid-chromatography,[4-7] chemiluminescence,[8] polarography,[9] electrophoreses,[10,11] atomic absorption,[12] flow injection analysis,[13,14] fluorimetry[15,16] sequential injection analysis,[17,18] colorimetry,[19] and spectrophotometric[20-23] have been reported for the determination of Pro. in pharmaceutical preparations.

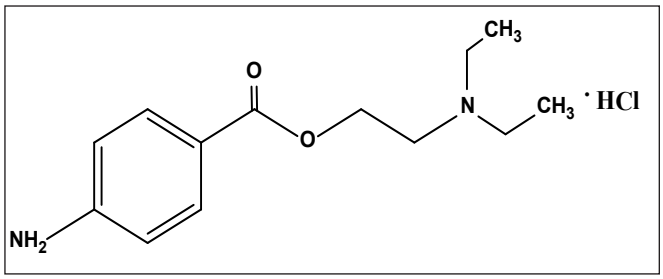

Figure 1: The structural formula of Pro.

However, some of the reported methods have high sensitivity and low detection limit, but they require expensive apparatus and reagents and are sometimes time-consuming. Therefore, the development of a simple and rapid analysis method is still required.

One of the microextraction techniques, named DLLME, was first reported by Rezaee *et al.*[24] This technique has immediately attracted special attention and becomes widely used in the field of sample preparation. The main advantages of this technique are high enrichment factor, rapidity, low cost and environmental benignity. Moreover, short reaction time and equilibrium state is achieved quickly.[25] DLLME has been reported for the quantitative determination of some pesticides,[26] drugs and ions.[27-33] Therefore, the present study aims to develop a spectrophotometric method equipped with dispersive liquid-liquid microextraction for the quantitation of Pro. in pure and pharmaceutical preparations. The reaction of the suggested method is based on Schiff's base condensation reaction in an alkaline medium between sodium 1,2-naphthaquinone-4-sulfonate (NQS) and the amino group of Pro orange-red (λ=485 nm).

The optimum conditions of the suggested method for the analysis of pharmaceutical preparations and real sample were studied. As per our best knowledge, It is the first study about the combination of DLLME with UV-Vis. for the quantitative determination of Pro.

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Experimental

Apparatus

All absorption measurements were performed using a Shimadzu 1800 UV-Vis spectrophotometer (Japan) with 1 centimeter quartz microcells. A HERMLE centrifuge (Germany) was used for the phase separation process.

MATERIALS AND SOLUTIONS

All chemicals and reagents used were of analytical reagent grade. Methanol, acetonitrile, ethanol, acetone Carbon tetrachloride CCl_4, chloroform $CHCl_3$, methylene chloride CH_2Cl_2 and ethylene chloride $C_2H_4Cl_2$, were supplied from BDH (England). Pure Procain HCl Std. was provided by Samarra Drug Industry (Iraq).

Procaine Stock Solution (1000 µg mL^{-1})

A 0.1 gm amount of pure Procaine. HCl was dissolved in amount of distilled water then the solution was made up to 100 mL in a volumetric flask with same solvent.

NQS Solution (0.01 M)

Prepared freshly by dissolving 0.065 g in distilled water and diluted to 25 mL in a calibrated flask. A solution of 0.005 M was prepared by suitable dilution.

Sodium Bicarbonate (0.1 M)

Prepared by dissolving 0.84 g in distilled water and diluted to 100 mL in a calibrated flask.

General Procedure

In 15 mL centrifuge tube, 0.5 mL of 0.2 M $NaHCO_3$ was added to 0.75 mL of NQS (5×10^{-3} M) and then mixed with aliquot amount (8–800 µL) of the working solution of Pro. (10 ppm). The solution was heated at 60°C in water bath for 8 minutes to form orange-red product. The colored solution was cooled, diluted to 10 mL with distilled water and left for 5 minutes. A mixture of 750 µL methanol and 250 µL chloroform was injected rapidly to induce cloudy solution formation. The mixture was then centrifuged at 4000 rpm for 5 minutes. After that, the fine droplets of chloroform were joined together and sedimented at the bottom of the conical test tube. After removing the whole aqueous phase, the organic layer was transferred using a microsyringe, placed into the quartz microcell, and its absorbance was measured at 485 nm against the blank. The latter was run under the same procedure without adding Pro. The steps of the suggested DLLME procedure are summarized in Figure 2.

Procedure for the Pharmaceutical Formulations

Procaine benzylpenicillin injections from different sources were analyzed. An accurately weighed portion from mixed three vials powder for each sample (equivalent to about 0.01 gm of procaine HCl) was dissolved in distilled water and completed to the mark. Additional appropriate solutions of pharmaceutical preparations were made up by simple dilution with distilled water. A 0.4 mL of the prepared solution was introduced to the DLLME procedure and used for the analytical applications.

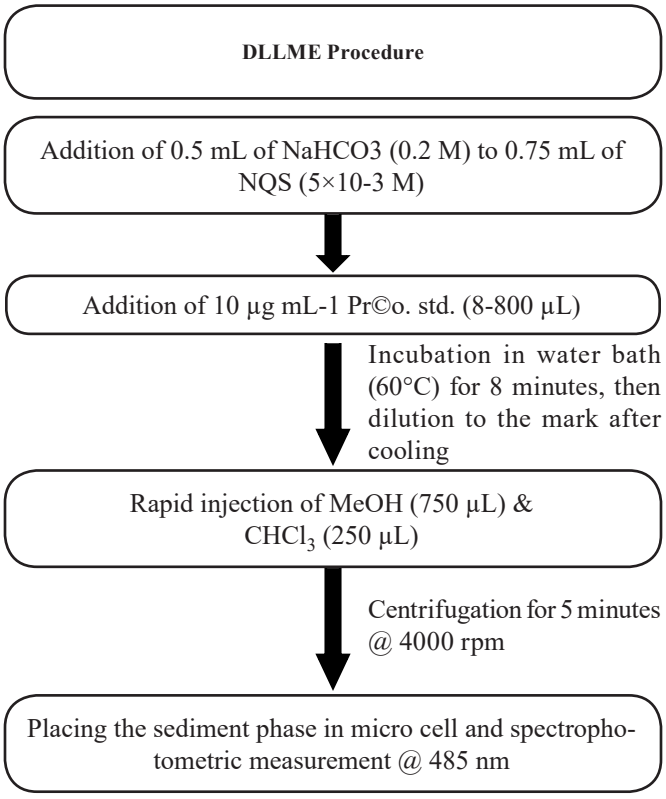


Figure 2: Steps of Dllme Procedure for the Analysis of Pro.

RESULT AND DISCUSSION

Absorption Spectra

Figure 3 shows the absorption spectra of the sample solution containing different concentrations of Pro. that tested according to the suggested DLLME procedure. The product results from the reaction between NQS reagent and Pro. to give an orange-red colored Schiff's base with maximum absorption at 485 nm. The proposed mechanism (shown in Scheme 1) involves the reaction of NH^2 group of Pro. (I) with NQS (II) in the presence of sodium bicarbonate in an aqueous medium to form an orange-red colored product (III) that found agreement with the mechanism found in the litterateur.[34] Hence this wavelength was used for all subsequent measurements.

Optimization of the Reaction Conditions

The effect of various parameters on the absorption intensity of the formed product was studied and optimized.

The effect of different bases such KOH, NaOH, $NaHCO_3$, and NH_4OH (0.1 M) on the maximum absorbance of the product has been studied. It was found that sodium bicarbonate is the most suitable alkaline medium for maximum absorbance and was used in all the subsequent experiments.

The effect of various concentrations 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 M of $NaHCO_3$ on the absorbance were tested. The investigations showed that 0.2 M gave a maximum absorbance; therefore, it was chosen for further experiments.

Consequently, the effect of different volumes of 0.2M of $NaHCO_3$ on the maximum absorbance was investigated by changeable the volume between (0.25–2.0 mL) and fixing the

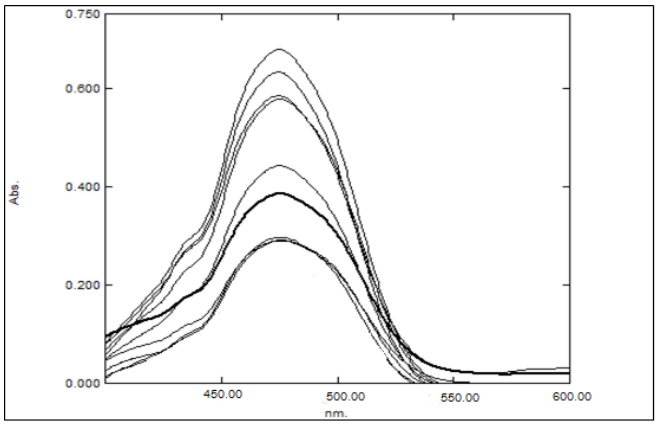
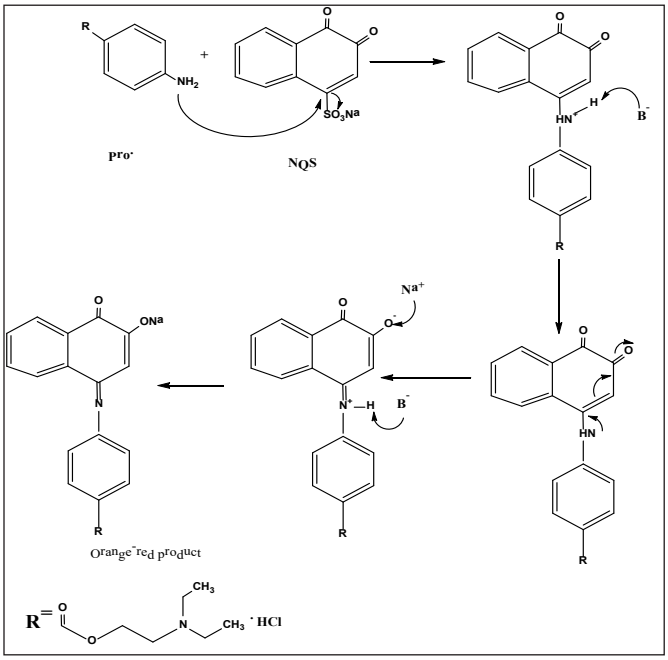


Figure 3: UV-Visible absorbance spectra of sedimented phase in the presence of different concentration of Pro.



Scheme 1: A schematic reaction mechanism for the reaction between Pro. and NQS

other parameters (Figure 4a). It was found that the highest absorbance was obtained using 0.5 mL gave of $NaHCO_3$ and was selected for the subsequent experiments (Figure 4b).

Various concentration of NQS solution (1×10^{-3}–1×10^{-2} M) was tested to a fixed amount of procaine HCl. The results show that 5×10^{-3} M solution is enough to develop the color to its full intensity, which is considered optimum.

To study the effect of NQS amount on color development, the amount ranging from 0.25 mL to 2.50 mL was submitted to the proposed procedure. It was found that the absorbance of the product increased with the rise of the amount of 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS). When the amount of NQS was more than 0.75 mL, the absorbance reached the maximum intensity and remained stable. Therefore, 0.75 mL of NQS was selected as the optimum amount for later experiments (Figure 4c).

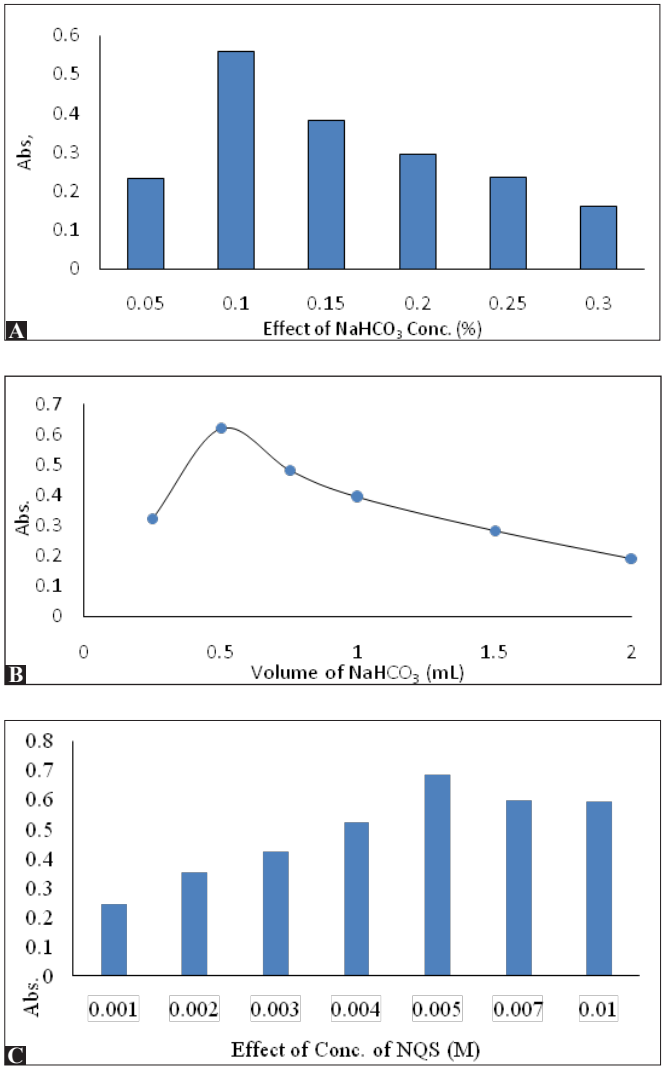


Figure 4: Optimization of experimental conditions of a) conc. of $NaHCO^3$ b) volume of $NaHCO^3$ and c) Conc. of NQS

Effect of temperature and heating time were studied and optimized at temperature ranged 30–70°C. Maximum absorbance was found after 5 minutes at 60°C and was stable for a further 30 minutes. Above 60°C, the absorbance decreases, indicating dissociation. Hence, 8 minutes at 60°C is recommended for the proposed method.

Optimization of DLLME Method

Type of Extraction and Disperser Solvents

In DLLME technique, the essential points for selecting the extraction solvent were low solubility in water, capable of extracting

For the response above, several chlorinated solvents such as carbon tetrachloride CCl_4, chloroform $CHCl_3$, methylene chloride CH_2Cl_2, and ethylene chloride $C_2H^4Cl_2$, mixed with low cost and toxic as a disperser solvent (i.e., methanol, acetonitrile, ethanol, and acetone) were examined. Figure 5 shows how the mixture of methanol 750 µL and chloroform (250 µL) gave higher absorbance. Thus, these solvents were chosen as optimum.

The Volume of Extraction and Dispersive Solvents

To test the effect of extraction and disperser solvents volume, a series of solutions containing different volumes of chloroform (200-400 µL) plus methanol (750 µL) were studied. The results show that when the volume of extraction solvent is increased, the volume of sediment phase absorbance increases. Therefore, 250 µL was selected in the next experiment. The effect of the volume of disperser solvent was also examined using various volumes of acetonitrile (600–1600 µL), containing (250 µL) of the extraction solvent. According to the results obtained, the absorbance increases on the increase of volume of disperser solvent from 600 to 800 µL. However, the absorbance was dropped probably thereafter due to solubility increase of the analyte in the water sample.[25] Thus, 800 µL of acetonitrile was selected.

Effect of Ionic Strength and Extraction Time

The effect of ionic strength was evaluated by adding different amounts of NaCl (0–10%, w/v) into the sample solution. The results reveal that the absorbance decreases with the increase in NaCl amount from 0 to 10% (w/v). This was due to the decrease in the solubility of the extraction solvents in the aqueous solution. Accordingly, the procedure was performed without adding the salt.

The effect of the extraction time" interval time between the injection of the mixture of solvents (extraction and dispersive solvent) in an aqueous sample and stating to centrifuged was studied. The mixture was centrifuged for 1–20 minutes. The results showed that the variations of the absorbance versus the extraction time were not remarkable. This was because the surface area between the extraction solvent and the aqueous layer was infinitely large. Thereby, the method was rapid; this was the most important advantage of DLLME technique.

Effect of Centrifugation Time and Speed

Centrifugation was required for the separation of the organic solvent from the aqueous phase. To attain the best extraction efficiency, the centrifugation time and speed were optimized in the range of 1–10 minutes and 1000–6000 rpm, respectively. The results indicated that the separation was complete within 5 min using a rotation speed of 4000 rpm.

Method Validation

Table 1 summarizes all analytical characteristics of the proposed method. Under the optimum conditions, the linear range was 0.0005–0.2 µg mL^{-1}. Limit of detection (LoD) and quantification (LoQ) was calculated using the following equation: $\frac{3Sb}{m}$ and $\frac{10Sb}{m}$ (where S^b and m are the standard deviations of the blank and slope of a calibration graph, respectively).

The precision of the method was attributed to its repeatability and reproducibility. Intra-day repeatability and inter-day reproducibility were determined using four different concentrations of Pro. Std., and drug. Five replicate measurements for each level were conducted. Acceptable precision was reflected from the RSD% values for the intra-day (0.14–1.41%) and inter-day (0.16–1.66%) (Table 2).

The accuracy of the method was performed at four concentration levels for Pro. Std., and drug. Five replicates were investigated at 0.005, 0.01, 0.1, and 0.16 µg mL^{-1} for each of the samples. The results showed that high recovery values obtained ranged from 95.4 to 102.4% (Table 2).

APPLICATION

The performance of the suggested DLLME method was evaluated by determining Pro. level in four types of different injection samples containing procaine. The results indicate high recovery values compression with the British pharmacopeia method using the usual calibration method (Table 3). This is probably due to the interaction of the benzylpenicillin that is present with procaine injections. Whereas good recoveries were obtained after applying a standard additions method (Table 3). The proposed method was compared as well successfully with the British pharmacopeia's standard method.

The calculated values for the F-test and t-test analysis, at 95% confidence level was found F (2.231) and t (0.253) did not exceed the critical values of F = 19.00 and t = 2.776. This indicates no significant differences between the proposed DLLME method and the official method concerning

Table 1 Analytical data of DLLME method

	Analytical feature	
Parameters	*Before DLLME*	*After DLLME*
Regression equation	0.0404x-0.1436	4.7789x+0.0289
Correlation coefficient, r	0.9989	0.9993
Linearity percentage, r2%	99.78	99.86
Linear range (µg mL^{-1})	5–40	0.0005–0.2
ε^a (L mol-1 $cm^{-1)}$	9.6×103	1.13×106
LOD^b (µg mL^{-1})	4.38	0.0003
LOQ^c (µg mL^{-1})	14.45	0.001
S^d (µg cm^{-2})	0.025	209x10-6

[a]Molar absorptivity, [b]Limit of detection, [c]Limit of quantification, and [d]Sandell's sensitivity

Table 2: Accuracy and precision of the proposed DLLME method

Conc. (µg mL^{-1})	*Intra-day Repeatability (RSD%, n=5)*		*Inter-day Reproducibility (RSD%, n=15)*		*Rec. (n=5)*	
	Std.	Drug	Std.	Drug	Std.	Drug
0.0005	1.11	1.35	0.98	1.24	95.4	98.3
0.01	0.32	0.46	0.65	0.58	96.5	99.7
0.1	0.11	0.31	0.38	0.45	100.1	101.3
0.18	0.08	0.15	0.21	0.17	100.3	102.4

Table 3: Application of the proposed DLLME method for the determination of procaine in different samples

	Procaine HCl (μg.ml^{-1})		*Rec. %*
Sample	*Present*	*Found*	*(RSD%)*
Acacaine injections (600 mg of Procaine Penicillin)-Acay production	0.10	0.098	98.0 (1.23)
Procaine injections (300 mg of Procaine Penciliine, 100 mg of benzyl pencilline)-Pakistan production.	0.10	0.101	101.0 (0.89)
Procaine Injections 100 mg of Procaine pencilline)-Sammara-Iraq.	0.10	0.096	96.0 (0.35)
Procaine benzyl penicillin injection (300 mg) Procaine penicillin)-Indian.	0.10	0.102	102.0 (1.09)

Table 4: Recoveries values of calibration method, standard addition method and British pharmacopeia method.

	Standard addition method			*British pharmacopeia method*
	Concentration of procaine HCl (μg mL^{-1})			
Sample	*Present*	*Found*	*Rec.% (RSD%)*	*Rec.% (RSD%)*
Acacaine injections (600 mg of Procaine Penicillin)-Acay production	10	10.12	101.0 (0.48)	98.99 (0.32)
Procaine injections (300 mg of Procaine Penciliine, 100 mg of benzyl pencilline)-Pakistan production.	10	9.88	98.8 (0.62)	100.2 (0.54)
Procaine Injections 100 mg of Procaine pencilline)-Sammara-Iraq.	10	9.91	99.1 (1.06)	99.56 (0.68)
Procaine benzyl penicillin injection (300 mg Procaine penicillin)-Indian.	10	10.09	10.9 (0.85)	101.1 (0.73)

precision and accuracy in the determination of Procaine in pharmaceutical preparations.

CONCLUSIONS

The current paper introduces the DLLME method for the extraction and preconcentration of Pro. in pharmaceutical preparations before the determination of Pro. by spectrophotometry. The developed DLLME method provides an efficient separation with low detection limits and quantification of 0.0003 and 0.01 μg mL^{-1}, respectively. The combination of DLLME with UV-Vis spectrophotometry gave a fast and low-cost procedure for the determination of Pro. without requiring sophisticated instruments, such as electrophoresis and HPLC.

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