Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(1):277-282
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Extraction and determination of codeine phosphate in water samples by dispersive liquid-liquid microextraction coupled to UV-vis spectrophotometry

Malihe Samadi Kazemi*[1], Samira Boorumand[2] and Seyed Mahdi Moosavi Asad[3]

[1]*Department of Chemistry, Faculty of Sciences, Bojnourd Branch, Islamic Azad University, Bojnourd-Iran*
[2]*Department of Chemistry, Faculty of Sciences, Quchan Branch, Islamic Azad University, Quchan –Iran*
[3]*Faculty of Medicine, Mashhad University of Medical Science, Mashhad- Iran*

ABSTRACT

A sensitive method has been studied for the determination of codeine phosphate in water samples using dispersive liquid-liquid microextraction coupled with Uv-visible spectrophotometry. Parameters that affect on the extraction efficiency, such as kind and volume of the extraction and disperser solvent, extraction time, salt addition were investigated and optimized. Under the optimal conditions, the linearity of the method was obtained in the range 0.005-10 µg/mL with coefficient of (r^2) 0.9996. The limit of detection and relative standard deviation were 0.001 µg/mL (n=7) and 2.85 (c=2 µg/mL, n=5) respectively. Also, the proposed method was applied to the determination of codeine phosphate in water samples with satisfactory analytical results.

Key words: codeine phosphate, spectrophotometry, dispersive liquid-liquid microextraction, water samples.

INTRODUCTION

Codeine phosphate (7, 8-Didehydro-4, 5a-epoxy-3-methoxy-17- methylmorphinan-6a-ol) is predominant alkaloid in opium [1, 2]. It is considered as a pro-drug, metabolized to active compounds of morphine and codeine-6-glucoronide [3]. But, due to uncontrolled use, it is essential to develop an effective method for its determination in real samples. In many applications, techniques could be employed such as by GLC [4], TLC [5] and HPLC [6] but uv-vis spectrophotometry for its availability, simplicity, versatility, speed, accuracy, precision and cost-effectiveness is used in analytical chemistry especially for quantitative determination of different highly conjugated organic compounds and biological macro molecules. Also, due to the low concentrations of many analytes in the complex real samples a sample preparation step is necessary before measurements to improve the selectivity and sensivity [7].

Dispersive liquid-liquid microextraction (DLLME) is a new mode of LPME [8] that some of its remarkable advantage is simplicity of operation, rapidity, high recovery and high enrichment factor and low consumption of solvents and sample [9, 10].

Nowadays, the use of DLLME technique coupled to Uv-vis spectrophotometry has become very popular because of its usefulness, low cost and environmental friendliness.

In this study, we developed a dispersive liquid-liquid microextraction for the determination of codeine phosphate in water samples using uv-vis specrotometry.

MATERIALS AND METHODS

Apparatus and regents

Absorbance measurements were carried out a Uv-vis spectrophotometer model Jenway 6305 using 300µL quarts cells. A digital pH meter crison 20+ was used for all pH measurements. A centrifuge model Hettich universal was used to accelerate the phase separation.

Chloroform, 1, 2- dichloroethane, tetrachloride carbone, methanol (for spectroscopy), tetrahydrofouran, acetone (for spectroscopy) and acetonitrile (HPLC Grade) were used from Merck (Darmstadt, Germany). Also, codeine phosphate and sodium chloride were purchased from Merck (Darmstadt, Germany) company.

Codeine phosphate solution (1000 µg/mL) was prepared in methanol and the working solutions of codeine phosphate were prepared daily by proper dilution.

Dispersive liquid-liquid microextraction procedure

A 5mL of a standard solution or real sample was placed into an extraction vessel. Tetrahydrofuran (1mL) as a disperser containing 30µL tetrachloride carbone (as an extraction solvent) was rapidly injected into solution using a syringe. A cloudy solution was formed in the extraction vessel. In this step, the codeine phosphate in water sample was extracted into the fine droplets of tetrachloride carbone. The mixture was then centrifuged for 5min at 3000 rpm. After this process, the dispersed fine droplets of tetrachloride carbone were collected and were transported to a uv-vis spectrophotometer to measure its absorbance at λ_{max} (277 nm).

RESULTS AND DISCUSSION

Selection of extraction solvent and disperser solvent

Careful attention should be paid to selection of the extraction solvent. It should have higher density than water, good extraction properties for the compounds of interest and low solubility in water. Three solvents including tetrachloride carbone, chloroform and 1, 2- dichloroethane were considered for this purpose (Fig. 1).

Miscibility of a disperser with organic phase (extraction solvent) and aqueous phase (sample solution) is the most important factor for the selection of a disperser, in this study acetone, methanol, acetonitrile and tetrahydrofouran, which have this ability, are selected(Fig. 2).

According to the obtained results, tetrachloride carbone as the extraction solvent and tetrahydrofouran as the disperser solvent provided maximum absorbance for sample.

It should be noted that the density of tetrachloride carbone (1.587 g/cm^3 [11] more than chloroform and 1, 2-dichloroethane (1.253 g/cm^3 and 1.483 g/cm^3, respectively) [11], also the solubility of tetrachloride carbone is less than chloroform and 1, 2- dichloroethane; therefore, the selection of tetrachloride carbone as the extraction solvent is justified.

Table 1. Quantitative results from DLLME of codeine phosphate

Analyte	RSD%[a] (n=5)	EF[b]	LR[c] (µg.mL^{-1})	r^2 [d]	LOD[e](µg.mL^{-1})(n=7)
Codeine phosphate	2.85	45	0.005-10	0.9996	0.001

[a] Relative standard deviation; [b] Efficiency factor; [c] Linear range; [d] Correlation coefficient; [e] Limit of detection

Table .2. The application of presented method for determination of codeine phosphate in different water samples

Samples	spiked with 2.00 µg.mL^{-1}		spiked with 8.00 µg.mL^{-1}	
	concen found(µg.mL^{-1})		concen found(µg.mL^{-1})	
	mean(n=3)	Recovery(%)(n=3)	mean(n=3)	Recovery(%)(n=3)
Tap water	2.12±0.12	94.34±2.00	7.97±0.56	99.62±2.80
Beshghardash river	1.99±0.09	99.5±2.52	8.50±0.10	106.25±2.42

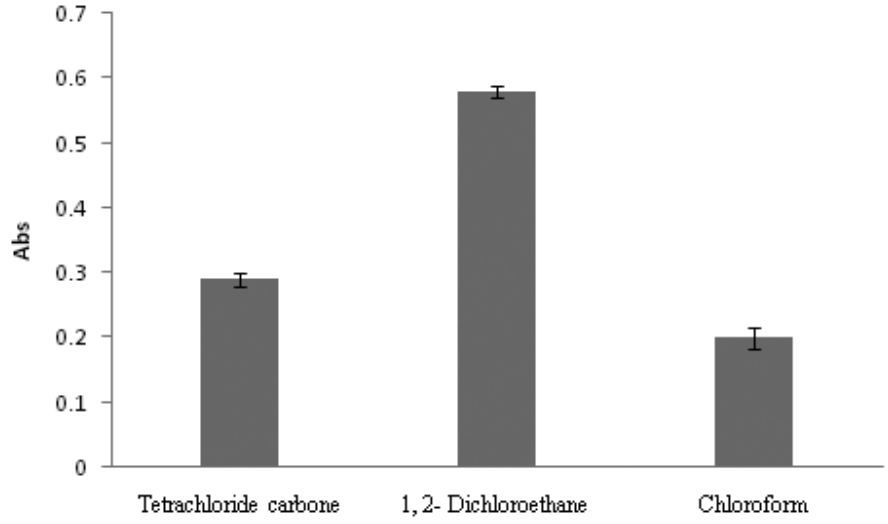

Figure 1. Effect of extraction solvent on absorbance of codeine phosphate in DLLME

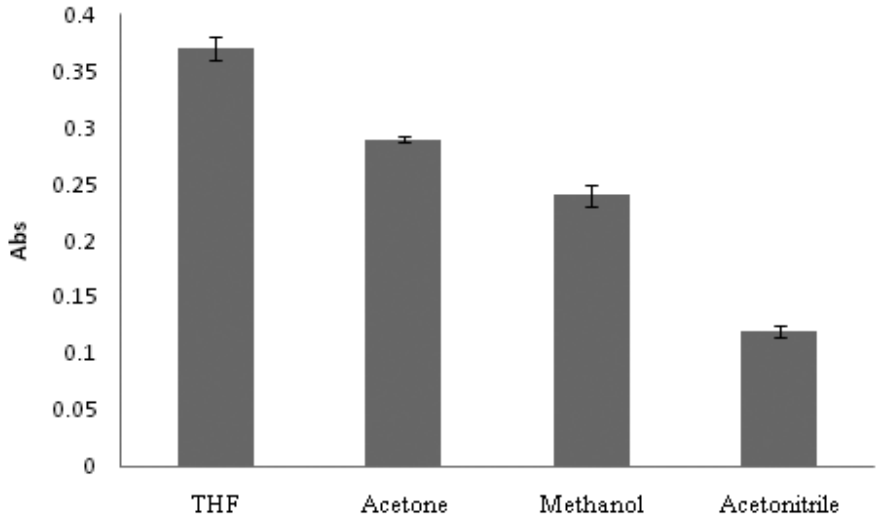

Figure 2. Effect of disperser solvent on absorbance of codeine phosphate in DLLME

Effect of volume of extractant

It is essential to select a volume of extraction solvent for the DLLME process. Different volumes of tetrachloride carbone (10, 30, 50, 70, 80 and 100 µL) were investigated. According to Fig. 3, the maximum of absorbance obtained at 30 µL, and with the increase of extractant volume, the absorbance due to the dilution effects of sample in sediment phase decreased.

Effect of disperser solvent volume

To study the effect of disperser volume on the absorbance of codeine phosphate, all experimental conditions were fixed except volume of tetrahydrofouran (0.5-1.5 mL). The results are shown in Fig. 4. According to the obtained results, the absorbance increased till 1mL and then decreased, this may be due to that the cloudy state is not formed well by increasing volume of tetrahydrofouran and thereby the extraction is disturbed. On the other hand, in the high volumes of disperser volume, stability of codeine phosphate in water increases, therefore, the absorbance decrease because of distribution coefficients decreasing. A 1mL volume was chosen as optimum volume for disperser.

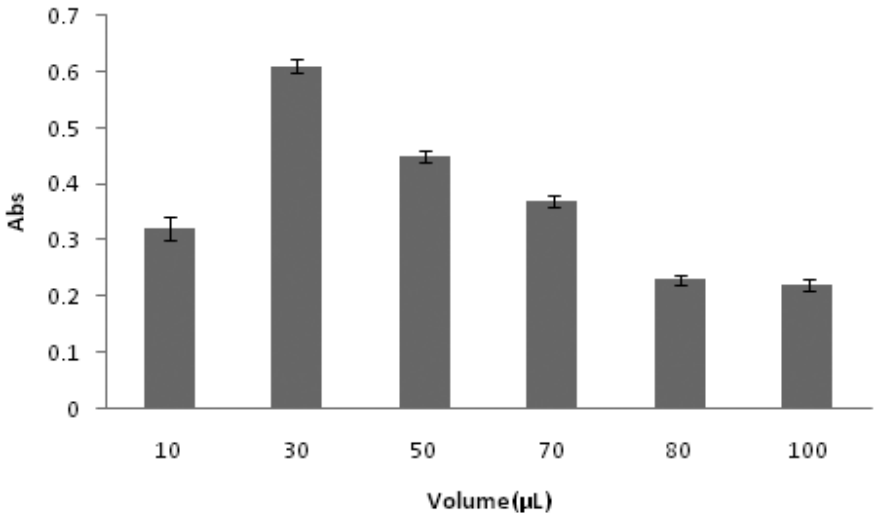

Figure 3. Effect of volume of extractant solvent on absorbance of codeine phosphate in DLLME

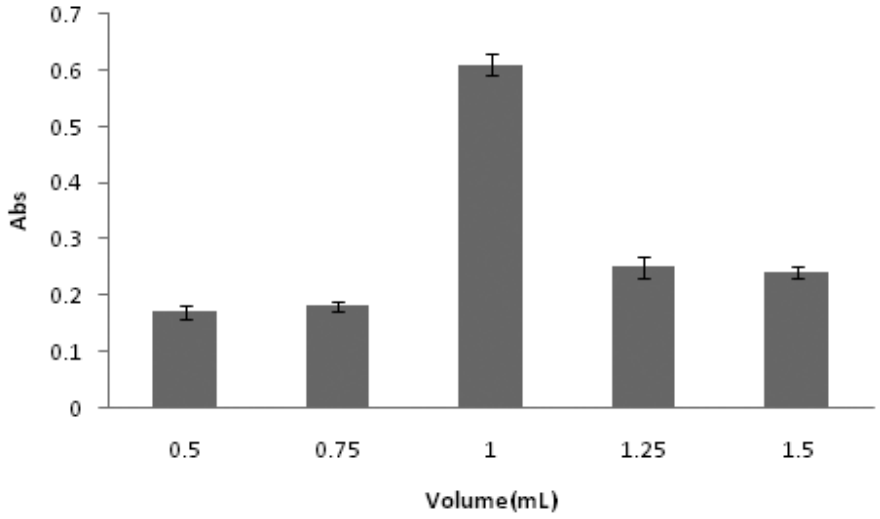

Figure 4. Effect of volume of disperser solvent on absorbance of codeine phosphate in DLLME

Effect of ionic strength

The addition of salt improves the extraction efficiency in many conventional extraction processes. Because the organic acceptor/ aqueous donor phase distribution coefficient can be enhanced by increasing the ionic strength of the aqueous sample [12-14]. This phenomenon helps to enhance the affinity of the acceptor phase for the analyte molecules. Sodium chloride is commonly added to analytical sample [14]. To investigate the effect ionic strength on DLLME performance, a number of experiments were performed by adding different amount of NaCl (0.4-4 %(w/v)) while other experimental conditions were kept constant. The results obtained showed that the salt had positive effect on the extraction efficiency of the codeine phosphate. The optimal concentration of NaCl was obtained at 2% (w/v).

Effect of extraction Time

In DLLME, extraction time is defined as interval time between injection the mixture disperser (tetrahydrofouran) and extraction (tetrachloride carbone) into the aqueous sample and starting to centrifuge. The formation of the

cloudy solution increase transition of the analytes (aqueous phase) to the extraction solvent. Subsequently, the equilibrium state is achieved quickly so, the extraction time is very fast. In this work the effect of extraction time was examined in the range 5- 20 min with constant experimental conditions. Fig. 5 shows the dependence of the absorbance of codeine phosphate on extraction time and according to this curve, the optimum of extraction time is 10 min.

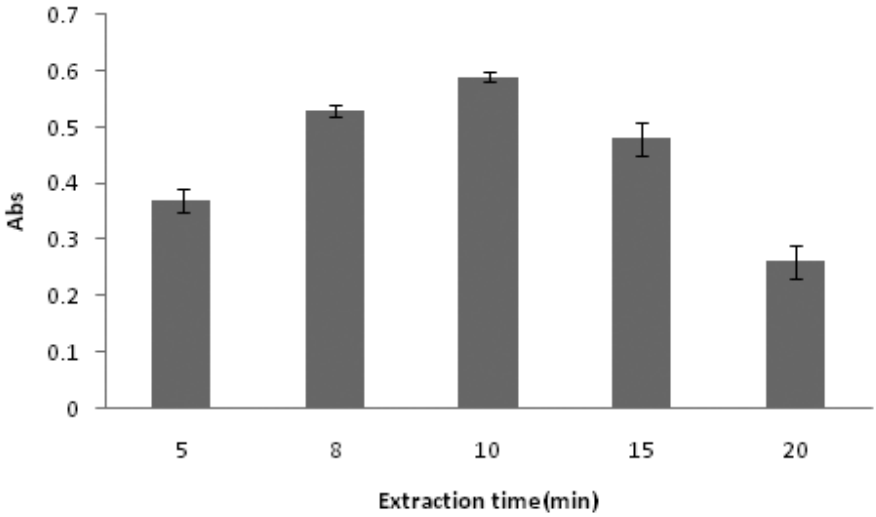

Figure 5. Effect of extraction time on absorbance of codeine phosphate in DLLME

Quantitative Analysis

The characteristic calibration data listed in Table. 1 was obtained under optimized conditions. The limit of detection (LOD) calculated based on $3S_b/m$ (where, S_b and m are the standard deviation of the blank and the slope of the calibration curve, respectively).

The preconcentration factor defined as the ratio of the concentrations of analyte in the settled phase and the aqueous sample solutions also, reproducibility based on relative standard deviation was studied by extracting a water sample spiked with 2 µg/mL of codeine phosphate

Real sample analysis

The proposed method was applied to the determination of codeine phosphate in water samples. Tap water was collected from Bojnourd, Iran. The other sample was collected from the Beshghardash River. The results show that the contents of codeine phosphate in the samples are all under the detection limit. Therefore, separate samples were spiked at two levels of the target compound. In order to test the present method, the relative recoveries of codeine phosphate were calculated and reported for the tap water and Beshghardash river water samples (Table. 2). These results demonstrate that the waters matrices have no effect on the DLLME procedure.

CONCLUSION

A dispersive liquid-liquid microextraction method coupled to Uv-vis spectrophotometry was used for determination codeine phosphate under the optimum condition (5mL water sample, 1mL tertrahydrofouran as disperser solvent containing 30µL tetrachloridcarbone as extraction solvent)

in water samples. The results show that this method is suitable for extraction and determination of codeine phosphate without sample matrices.

Acknowledgements

The authors would like to express their thanks to Bojnourd Branch, Islamic Azad University for their financial support.

REFERENCES

[1] T.V. Vree, R.T. Dongen, *Int J Clin Pract*., **2000**, 54 (6), 395.
[2] V. Srinivasan, D. Wielbo, I.R. Tebbett,, *Eur J Pain*., **1977**, 1(3), 185.
[3] S.C. Armstrong, K.L. Cozza, *Psychosomatics*., **2003**, 44(6), 515.
[4] K. Masumoto, Y. Tashiro, K. Matsumoto, A. Yoshida, M. Hirayama, S. Hayashi, *J Chromatogr*., **1986**, 381, 323.
[5] H.N. Al-Kaysi, M.S. Salem, *Anal Lett*., **1986**, 19, 915.
[6] M.L. Altun, T. Ceyhan, M. Kartal, T. Atay, N. Oezdemir, S. Cevheroglu, *J Pharm Biomed Anal*. **2001**, 25, 93.
[7] N. Shokoufi, F. Shemirani, F. Memarzadeh, *Anal. Chim. Acta*., **2007**, 601, 204.
[8] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, *J. Chromatoger. A*, **2006**, 1116, 1.
[9] A.V. Herrera-Herrera, M. Asensio- Ramos, J. Hernandez-Borges, M.A. Rodriguez-Delgado, *Trends Anal. Chem*., **2010**, 29, 728.
[10] C.B. Ojeda, F.S. Rojas, *Chromatographia*, **2009**, 69(11-12), 1149.
[11] Izutzu, K. *Electrochemistry in Nonaquoeous solutions*, Wiley-VCH, **2002**.
[12] S. Pedersen-Bjergaard, K.E. Rasmussen, *J. Chromatoger. A*, **2008**, 1184, 132.
[13] E. Psillakis, N. Kalograkis, *TrAC-Trends Anal. Chem*., **2003**, 22(10), 565.
[14] K.E. Rasmussen, S. Pedersen-Bjergaard, *Trends Anal. Chem*., **2004**, 23, 1.