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ORIGINAL ARTICLE

OPTIMIZED OF DISPERSIVE LIQUID-LIQUID MICRO EXTRACTION AND UV-VIS SPECTROPHOTOMETER FOR DETERMINATION OF CODEINE IN WATERS

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ABSTRACT: In this work study, a new simple and reliable method for rapid extraction and determination of codeine in water was developed by dispersive liquid–liquid micro extraction pre-concentration and UV-Visible spectrophotometer. In this work chloroform and methanol selected for extractor solvent and disperser solvent respectively. The effective parameters of DLLME such as volume of extractor solvent, pH, and concentration of salt optimized with central composite design (CCD). The optimal conditions were: volume of extractor solvent 140; concentration of the salt,2.88 and pH, 9.00. The correlation coefficient (R2) was higher than0.99. The calibration graph was linear in the range of 200-800µg/L with the detection limit of 18µg/L for codeine. The relative standard deviation (RSD, *n*=5) for the extraction and determination of 500µg/L of codeine in the aqueous samples was 1.9%. The method was successfully applied to determination of codeine in the real water samples and relative recoveries (97.2-97.9 %) were achieved.

KEYWORD: dispersive liquid – liquid micro extraction, experimental design, codeine

INTRODUCTION

Codeine is a narcotic, painkiller, anti-cough, antihypertensive, anti-diarrheal, hypnagogic and generally relieving. It is usually administrated in order to kill pain and ease off cough. It is the second more contained of opium, after morphine, which consist of %3 opium. In regular medication, this combination would be in form of codeine-phosphate and administrated as part of aspirin or paracetamol (Chen *et al.,* 2008; Mitra, 2003).

There is a lot of ways to measure the amount of codeine such as fluid-chromatography with high performance by UV detector, fluid-chromatography with high performance by diode detector, fluid-chromatography with high performance by mass-spectrometric detector, fluid-chromatography by mass-spectrometric detector, gas-chromatography by mass-spectrometric detector, capillary electrophoresis, absorbency visible-ultraviolet spectrophotometer, voltammeter and microchip electrophoresis by electrochemist detector (Shamsipur and Fattahi, 2011; Hu *et al.,* 2011; Bjork *et al.,* 2010; Concheiro *et al.,* 2008; Barroso *et al.,* 2010; Hindson *et al.,* 2007; Vlasova *et al.,* 2008; Pournaghi-Azar and Saadatirad, 2008; Pournaghi-Azar and Saadatirad, 2010).

In this study, we used broadcast liquid-liquid micro extraction, absorbency visible-ultraviolet photometer spectrum for measuring codeine in watery samples. The first usage of the two mentioned methods was done by Shokufi and his colleagues for determining palladium and cobalt exist in some watery samples (Shokoufi *et al.,* 2007). In this study, codeine spectrum was used for extracting and optimization in wavelength of 270nanometer. In first stage, for extracting and thickening we used broadcast liquid-liquid micro extraction, because this method is simple and fast, also it can be used for extracting and pre-thickening a wide range of organic compounds. Other benefits include low cost, efficiency and its compatibility with the environment and finally, it will give chance to examine all the influential factors of extraction by central composite design to determine the line of optimized extraction.

The main aim of this study is to develop the method of extracting and determining codeine in watery samples. The results obtained show that liquid-liquid micro extraction method is more efficient for analyzing codeine exist in watery samples. In addition to that, effective parameters on extraction efficiency have been examined by experimental design method. Also, effective parameters on extraction efficiency were checked and hence the optimal conditions were set.

MATERIALS AND METHODS

2.1. Liquid – liquid micro extraction method

5 milliliter of codeine dilute with 500 microgram per liter concentration has been placed in a

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conical-bottom test tube and then a compound consist of 0.5 milliliter methanol as a dispersive solvent, 140 micro liter chloroform which was injected by a 1milliliter-syringe to the solution. The output solution is produced as a extracting solvent. In this stage, the solvent had been speeded within the whole solution and appeared as a very small drop on the watery solution and so upper solution was created. Therefore, the codeine exist in the solution is extracted by chloroform drops and in the second stage, for isolation between organic phase and watery phase, this solution was centrifuged for about 3 minutes with a velocity of 4000 r/m. Eventually, organic phase will get precipitate and apart by a 100-microliter syringe to analyze it in absorbency visible-ultraviolet photometer spectrum in the next stage.

2.2. Choosing a dispersive solvent

Solubility of solvent in organic phase and watery phase is the main factor of choosing a dispersive solvent (Shokoufi *et al.*, 2007). In this study, acetone, methanol and acetonitrile which are soluble either in both organic phase and watery phase were taken as a dispersive solvents. For choosing dispersive solvent, a series of codeine samples were prepared and then 0.5 milliliter dispersive solvent containing of 140 micro liter extracting solvent, chloroform, were added to the watery solution, 5 milliliter volume. But chloroform-acetonitrile compound did not result a constant cloudy phase however chloroform-methanol and chloroform-acetone compounds have made an adequate cloudy phase. Methanol was used as a dispersive solvent as its compound with chloroform had shown more absorption in compare to combination of acetone with chloroform.

2.3. Choosing extractor solvent

Choosing extractor solvent by broadcast liquid-liquid micro extraction is very important. Extractor solvent should have conditions such as high density in compare to water, low solidity in water and has a capability of extracting a specific material. In this study, chloroform has a density of 1.48 gram/milliliter, tetra chloromethane has a density of 1.59 gram/milliliter and tetra ethylene chloride has a density of 1.62 gram/milliliter are used to be extractor solvents. Different amount of volumes of extractors were taken in order to get an equal precipitated phase's volume for each one of them, 50micro liter, as following, 140 micro liter of chloroform, 120 micro liter of tetra chloromethane, 85 micro of tetra ethylene chloride. Chloroform has been chosen as an extractor solvent as its compound had had the most absorption.

2.4. Optimization

A mathematical model can be predicted by respond-level method in which linear and quadratic effects were approximated. Central Composite Design is a common method which has been applied in this stage, in order to improve efficiency and gain more reliable respond. For setting optimal conditions, there is a need of effective factors as well as a detailed-quantitative model to predict the way of respond to the factors. A central composite design is used which is orthogonal and rotatable and also it has resulted to a suitable respond level of quadratic multi-exponential model (Morgan and Deming, 1987; Brereton, 2007).

RESULTS AND DISCUSSION

Since the number of factors is three, extractor solvent volume, PH and salt concentration are to affect extraction efficiency therefore f=3. The formulae below calculate the number of factorial points and axis points which are, in order, 8 and 6 as shown below:

$$\alpha = \sqrt[4]{N_f} = \sqrt[4]{8} = \pm 1.682$$

$N_f = 2^f = 2^3 = 8$ factorial points

$N_a = 2f = 2*3 = 6$ axis points

For obtaining central composite design, orthogonal and rotatable, first of all axis point, a, is calculated by the following formulae and hence the number of central repetition, N_C.

$$1.682 = \sqrt{\frac{\sqrt{(8+6+N_C)8} - 8}{2}}$$

According to the above equation, N_C is 9 so there is 9 times repetition in the centre and the number of required experiment for examining all of three factors are 23 as calculated below:

$$N = N_F + N_C + N_A = 8+9+6 = 23$$

In another hand, in order to control odd parameter, the experiments were divided into two group of factors level and design matrix which are shown in tables number 1 and 2.

Design-expert was used to analyze data and draw graphs and the results are shown in table number 3. The value of the "F" of the model equal 1220.34 which means that the model is less likely to bear errors, possibility of only %0.01. Segments which have a value of "F" with a prob<0.0500 are defined as very important and those which have a prob>0.10 are ignorable. Therefore, parameters such as volume of extractor solvent, salt concentration, PH and the

reactivity between the extractor solvent and salt are substantial.

When the number of points taken for designing the model is more than enough and some of them have been duplicated, then an approximation of net error and LOF (lock of fit error) can be achieved. LOF error is not a good sign of the model being sufficient and should not be used to predict response, However it may be used for those segments which F value is high and prob<0.10. "LOF" value has no relationship with net error. For instance, if "LOF" equal 2.38, there will be a probability of %15.67 for occurring error. So, for detecting a relationship between these factors and the consequences following by them, a model including sufficient initial data, in which "F" and "R" are both high and low standard error, should be taken. According to the results, statistical model including three main effects, three effect of two-factor interaction and three phrase square or curvature which calculated as following.

$$Y = b_0 + b_1V + b_2P + b_3C + b_4VP + b_5VC + b_6PC + b_7V^2 + b_8P^2 + b_9C^2$$

b_0= +0.0074; b_1=+0.48; b_2=+0.055; b_3=+0.020; b_4= -0.016; b_5=-0.015; b_6=+0.0032; b_7=+0.0001; b_8=+0.11; b_9=-0.015

The above equation, positive and negative coefficient for main effects have affected the final response very clearly in which the higher the sheer coefficient value, the higher effect it will have on the response. In another hand, negative coefficients have an inverse effect. In order to interpret reactants graphically, 3-dimential graphs have been used. This method is useful to illustrate the relationship between the response and experimental level of each factor. These graphs have been drawn as response versus two other experimental factors, which have been held in central point. Figure 1, response increases when PH increases for all values of extractor solvent volume. Also the response increases when extractor solvent volume increases for all values of PH. positive interaction co-efficiency of these two parameters, proves this fact, b_5. Figure 2, the amount of response decreases when salt concentration increases for all values of extractor solvent volume. Because, as the salt increases, the extractor solvent reactivity in watery phase decreases and in the result, the volume of deposited phase increases and being diminished cause the decrease in thickness and response rate. Finally, by utilizing Design-Expert software version 7.1.3, the optimal point has been determined including 140microliter of extractor solvent (chloroform), PH 9, %2.88 W-V of salt concentration.

3.1. Actual Sample Analysis

For analyzing actual samples, the method came up with three solution-sample of codeine tablet and then a determined concentration (40 microgram per liter) of standard codeine has been put on. Comparative retrieval is calculated. Extraction comparative retrieval has been done by broadcast liquid-liquid micro extraction method and achieved within the interval %97.9-97.2 and the result arranged in table 4.

CONCLUSION

The mentioned method, broadcast liquid-liquid micro extraction, include visible-ultraviolet photometer spectrum can be used for codeine extraction, pre-thickening and codeine isolation prevailing in watery samples using simple matrix (water). Parameters which affect the method efficiency and model equation have been examined by response level. Optimal conditions and the model equation is derived by central component design. According to the procedure and the result obtained, this method has many advantages such as simplicity, velocity, low-cost, sustainability and suitability for a wide range of organic compounds. Finally, this method was used for analyzing actual codeine samples and the results suggest that the method is sufficient for extracting and analyzing the samples.

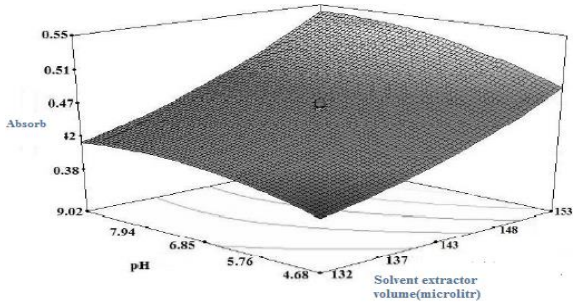

Figure 1: non Important interactive effects between extractor solvent volume with pH and how to change that the response (absorbance)

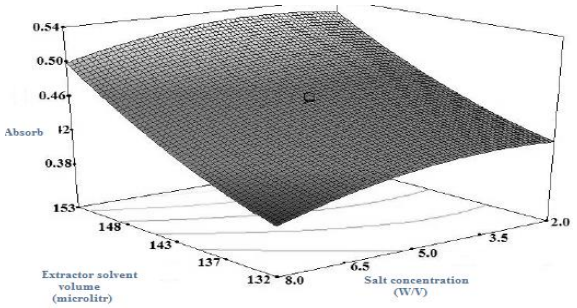

Figure 2: Important interactive effects between extractor solvent volume with salt concentration and how to change that the response (absorbance).

Table 1: Parameters, symptoms and factors level for Central composite design

Parameter	Symptoms	Factors level				
		+ α	+1	0	-1	-α
Solvent extractor volume	E	160	154	143	132	125
PH	P	10.5	9.0	6.7	4.7	3.2
Salt concentration(W/V)	C	10.0	8.0	5.0	2.0	0.0

Table 2: Matrix of Central composite design

Tests No.	E	P	C	Absorption
1	132	0.9	2	43.0
2	143	9.6	5	47.0
3	132	7.4	2	40.0
4	132	7.4	8	36.0
5	153	7.4	8	46.0
6	143	7.4	5	46.0
7	143	9.6	5	46.0
8	153	7.4	2	49.0
9	132	0.9	8	38.0
10	153	0.9	8	52.0
11	143	9.6	5	46.0
12	153	0.9	2	54.0
13	143	2.3	5	37.0
14	143	5.10	5	45.0
15	143	9.6	5	46.0
16	125	9.6	5	39.0
17	143	9.6	0	44.0
18	143	9.6	5	46.0
19	143	9.6	5	46.0
20	143	9.6	5	46.0
21	5	9.6	5	46.0
22	10	9.6	10	39.0
23	5	9.6	5	58.0

Table 3: Analysis of variance for level response quadratic model

Source / Model	Sum of squares	Degree of freedom	Average of Square	F value	P value	Importance
	0.060	9	0.0067	192.09	<0.0001	Significant
E	0.04	1	0.042	1192.43	<0.0001	
P	0.0056	1	0.0056	159.60	<0.0001	
C	0.0036	1	0.0036	102.63	<0.0001	
EP	0.0004	1	0.0004	12.50	<0.0041	
EC	0.0001	1	0.0001	2.27	0.1576	
PC	0.0000	1	0.0000	0.11	0.7461	
E^2	0.0020	1	0.0020	57.82	<0.0001	
P^2	0.0034	1	0.0034	98.24	<0.0001	
C^2	0.0036	1	0.0036	101.49	<0.0001	
Residual	0.0004	12	<0.0001		0.1567	
LOF	0.0003	5	0.0001	2.28	<0.0001	Not significant
Net error	0.0002	7	<0.0001			

Table 4: Relative efficiency for codeine extraction from real samples

Samples	Real concentration (µg/L)	Added concentration (µg/L)	Found concentration (µg/L)	relative efficiency
1	25.8±1.253	40	96.7±0.286	2.97
2	3.00±0.47	40	12.8±1.334	8.97
3	19.3±1.347	40	14.1±1.380	9.97

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