Optimization of Atropine Extraction Process from *Atropa Belladonna* by Modified Bubble Column Extractor with Ultrasonic Bath

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ABSTRACT: *Modified Bubble Column Extraction with Ultrasonic Bath (BCE-UB) method was used to extract atropine from the stem and leaves of Atropa belladonna. Optimum condition were obtained with Kamada solvent which was chloroform-methanol-ammonia 15:15:1(v/v/v) as extraction solvent, the particle size of less than 350 µm, an extraction time of 23.95 min, a liquor to material ratio of 15.08 mL/g and an air flow of 6.31 mL/min.g. In this state, percent of extracted atropine was calculated which was equal to 6.81%. Percent of extracted atropine was 6.31% that showed a little difference compared to the predicted value. In order to study the effect of bubbles on the extraction rate, the same extraction with the previous method was performed in a stirred tank. Percent of atropine was 5.59%.*

KEYWORDS: *Atropine; Bubble column extraction; Uniform design; UV-Visible spectroscopy; Atropa belladonna.*

INTRODUCTION

Tropane alkaloids are a group of alkaloids and secondary metabolites which are widely used in medicine for their analgesic, anticholinergic, mydriatic, antispasmodic and antimuscarinic (parasympathetic inhibition) action. They are commonly found in plants of four families, named, Solanaceae, Erythtroxylaceae, Proteaceae and Convolvulaceae [1-4]. Solanaceae is a family of flowering plants that consist of some important agricultural crops, although many species are toxic plants. Many members of the Solanaceae family are used by human, and are important sources of food, spice and medicine [5]. *Atropa belladonna* belongs to the solanaceae family and the plant is a good source of Tropane alkaloids. Atropine is the main tropane alkaloids (usually the most plentiful) in Atropa belladonna, which results from racemization of (-)-hyoscyamine during the extraction process [6,7]. Atropine is well known as hallucinogenic and for its specific properties

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(mydriatic and muscarinic antagonist) and has been used for the cure of various illnesses such as Parkinson, gastrointestinal, parturition and cardiopathy diseases [8,9].

Many extraction methods including microwave-assisted extraction, pressurized solvent extraction, solid-phase microextraction, and supercritical fluid extraction were once reported to extract atropine from Solanaceae plants [10]. All of these methods have some limitations such as cost, availability, high extraction time and low yield.

The selection of extraction method depends on the active ingredients in the plants and the type of plant tissue. Usually, there are two kinds of limitations to improve the extraction rate. The extraction of active ingredients from plant cell to the surface is the first limitation and the extraction of the active ingredients from solid surface to solvent is the second one [11]. *Seidi & Yamini* [12] discussed in details the effect of ultrasonic waves on the plant cells. However, it can be briefly said that, the ultrasonic waves can increase the vibration of the cell, or even, break it down. This effect suggests that the ultrasonic waves can improve the extraction rate in an extraction process through overcoming the first limitation mentioned above.

A bubble column extractor is basically a cylinder-shaped vessel with a gas sparger at the lower part of the vessel. The gas is sparged in the form of bubbles into either a liquid phase or a liquid–solid suspension [13]. Currently, bubble columns have been extensively used for extraction of natural products [14,15]. In this device, bubbles were injected into liquid–solid system (made of the extraction solvent and plant material) to increase mass transfer rate [16-18]. Therefore, a modified bubble column extractor with ultrasonic bath may be a good choice, to overcome both aforementioned limitations.

It is noticeable to mention that, the contact area of particle-solvent is increased by reducing the particle size, and therefore enhances the extraction rate. However, when the particle size is lower than a limiting value, even if there is ultrasonic wave, a phenomenon called conglomeration causes to reverse the results [19]. Therefore, the particle size is an important parameter that must be optimized in the extraction process. One of the most important advantages of the bubble column extraction device is that the turbulence created by the bubbles increases the extraction rate and also the destruction of the local conglomerates. As a result, in a bubble column extraction device the particle size can be reduced to the smallest possible condition to increase the extraction rate.

In this respect, a bubble column extractor coupled with ultrasonic bath was designed and used to extract atropine from *Atropa belladonna* in this work. The effects of various extraction parameters such as extraction solvent, particle size, extraction time, liquor to material ratio, temperature and air flow were investigated on the extraction yields of atropine.

Different methods have been reported to measure the amount of atropine such as Gas Chromatography (GC), high performance liquid chromatography (HPLC) and Capillary Electrophoresis (CE) [20-24]. In this study Gas Chromatography (GC–FID) was used for qualitative analysis and UV-Visible spectroscopy was used for quantitative analysis of atropine that was more economical respect to the others. This method is based on measuring the amount of light that a sample (liquid and sometimes gas) absorbs in particular wavelength. Chloranilic acid reagent was used to create color with atropine [25-27]. Finally, the total amount of atropine was obtained per gram of powdered plant.

EXPERIMENTAL SECTION

Equipment

BCE device: bubble column extractor (made in the laboratory)

Experiments were performed in a bubble column extractor with internal diameter of 7.5cm and length of 75 cm. BCE device was made in the laboratory. The working volume of the column was 3 liters. Solvent and powdered plant materials (mesh number less than 45) were added to the bottom of extractor that was located inside the ultrasonic bath. The device consists of a cylindrical glass column with an air sparger at the bottom of the column. A condenser was located in the upper part of the column to prevent the loss of evaporated solvent. A fan (made in Mobinteb-Iran) was also supplied in order to transfer the air into the column. A valve and a flow meter were placed for controlling and measuring the flow rate of the air. Solvent and powdered plant entered the column and then air was sparged into the bed after adjusting the valve and the flow meter (Fig.1). Extraction times in all experiments were divided into 4 equal intervals so that the ultrasonic bath was turned on

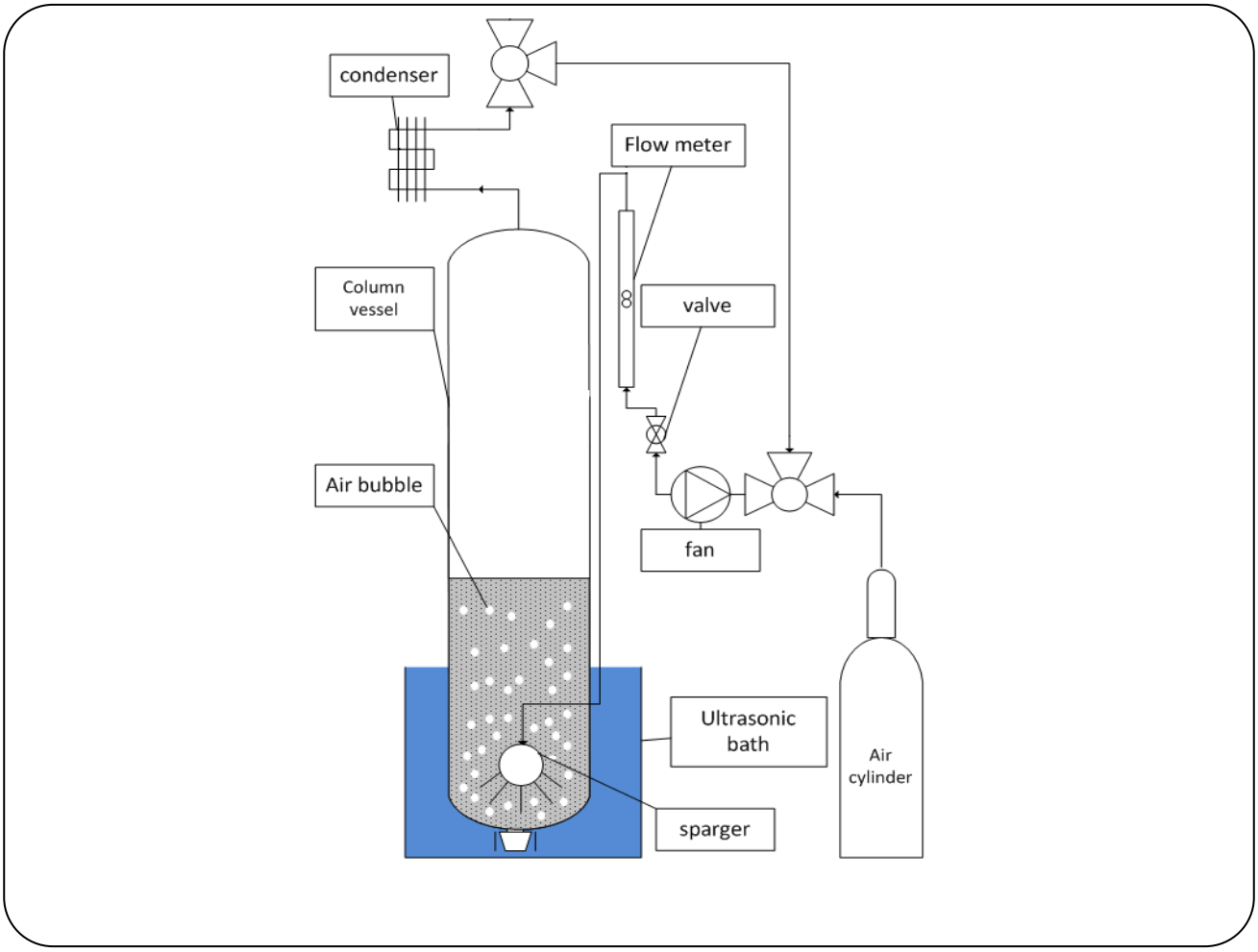


Fig. 1: Bubble column extractor coupled with ultrasonic bath; BCE- UB.

in the first and the third intervals and turned off in the second and the fourth intervals.

Ultrasound device

Ultrasound device was applied for the experiments, made in Germany, model of LC130H operated with frequency of 35 MHz. Because, in this frequency there is enough time for entering the bubbles into the cell wall structure and disturbing order of it.

Gas chromatography

as Chromatography (GC-FID) was used for qualitative analysis of extracted atropine. Capillary GC carried out using a Younglin Acm 6000 GC with HP-5(30m×0.25mm×0.25µm) column. Oven temperature was performed as follows: 50 ° C for 5 min and then heated to 240 ° C with a rate of 3° C/min; finally heated to 300 °C with a rate of 15° C/min and kept constant at 300° C for 3 min.; injector temperature 290° C; helium as the carrier gas with 0.8 milliliters per minute flow rate and a flame ionization detector. Quantitative data were obtained from FID area percentage data. The calculations were based on Area [mV×s][28].

Spectrophotometer UV–visible

Spectrophotometer UV–visible that was used for the experiments, was made by Human Corporation Company from X-ma 2000 type.

Reagents and Materials

Sulfuric acid, ammonia(25%), methanol and chloroform all in analytical grade were purchased from Merck Company (Germany). Atropine standard and chloranilic acid were purchased from Sigma-Aldrich company (USA).

Doubled distilled deionized water was used in all experiments. *Atropa belladonna* plants were cultivated and collected (2012 B.C) in Institute of Medicinal Plants-ACECR, Karaj, Iran. Plant materials were dried in shadow before extraction and analysis.

Extraction Procedure

Preparation of Plant for Extraction

The impurities and the pebbles were separated from the plant. It was placed in the shade for several days. Then, the leaves and the stems were separated from each other and after that were powdered separately. Leaves and stems of 5 samples were weighted to find the actual leave to stem weight ratio of plant materials. It was found that there was an about 1 to 1 ratio for the weight of leave to stem in the selected individual plants; and therefore, we prepared the samples with 1 to 1 weight ratio of leave to stem, in all experiments. The prepared samples were screened with the specified mesh sizes of 18, 30 and 45. Finally, the prepared samples with an equal leave to stem weight ratio and mesh sizes of 18, 30 and 45 were used separately to extract the *Atropa belladonna*.

Selection of Parameters

Many parameters can affect the extraction of the active ingredients from plants according to the type of plant and the extraction method. A number of them were selected as the most effective parameters according to the requirements and operational conditions and limitations of facilities**.** After the evaluation of these parameters, the ones that had the greatest effect on the rate of extraction were selected for optimization and other parameters were fixed at their best conditions. On the basis of primary tests and laboratory facilities, two experimental factors, namely, extraction solvent (Kamada solvent) and particle size of powdered plants (less than 354μm) were kept constant in all experiments. Extraction time (X_1), liquor to material ratio (X_2) and air flow (X_3) were used as factors (independent variables) and the extraction yield of atropine (Y) was used as response variable.

Extraction by Stirred Tank Extractor coupled with the Ultrasonic Bath (STE-UB)

Another Extraction was designed to study the effect of the bubbling. In this respect, the solvent and the powdered plant were added to the flask which is attached to a condenser and were stirred with the rate of 2400 rpm. This process was carried out with ultrasonic bath in optimal condition that was used in pervious method.

Extraction by Soxhlet extractor

For this part, a conventional Soxhlet extraction was used. An amount of 10g of powdered plant was prepared and placed inside the Soxhlet extractor, on the top of a round-bottom flask filled with 150 mL of solvent. The system was boiled using a bath boiler. This experiment was performed in three different times of 0.5h, 12h, and 6h. After filtration, the extract was maintained in 10mL flasks prior to UV-Visible spectroscopy analysis.

Experimental Design

The uniform design of experiments is a class of space filling design from number theory, developed by *Fang & Wang* [29]. This technique has been successful in many different fields such as chemical engineering and applied chemistry. In addition, the experimental design based on uniformity in comparison with other experimental methods like orthogonal design, factorial design, Box-Behnken design and response surface method [30-32] significantly reduces the number of experiments, and it is an appropriate method to study the unknown model of multi-level and multifactor [33]. In our work, a uniform design was used to design the extraction model for extraction of the atropine from *Atropa belladonna* plants based on regression analysis to predict the best extraction condition.

Different factors potentially can affect on the extraction process, and therefore the optimization of experimental condition is necessary in the development of BCE-UB method. In this respect, the effective factors such as solvent extraction, temperature and particle size as control parameters on the extraction method were identified and then, considered by tests. These factors were fixed at their best conditions. Three other factors namely, extraction time, liquor to material ratio and air flow as signal parameters were considered and their effect on the yield of atropine were investigated using a uniform design of $U_{30}(30\times15\times10)$[34,35].

Table 1: Table of standard solutions for atropine analysis by both techniques.

Standard atropine Conc (mg/L)	Chromatogram Area[mV×s]	Absorbance
500	977.82	0.767
400	740.15	0.601
300	564.27	0.424
200	361.03	0.273
100	196.32	0.12

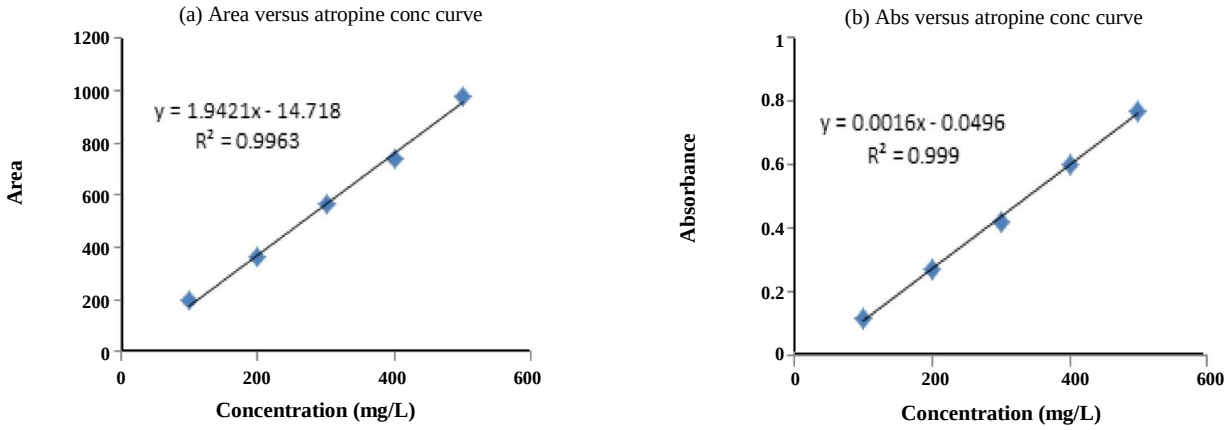


Fig. 2: Standard curves for atropine analysis techniques (a) GC-FID (b) UV-Visible.

Analysis

Qualitative analysis by GC–FID

Gas Chromatography (GC–FID) was used for qualitative analysis of extracted atropine. Comparison between GC-FID results for the standard solution of atropine and test solution confirmed the presence of atropine in the test solution of *Atropa belladonna L*.

Quantitative analysis using spectrophotometry

The complexometeric UV-visible spectroscopy was used for quantitative analysis of extracted atropine. This method is based on measuring of the amount of light that a sample of liquid and gas (some times) absorbs in a particular wave length. Chloranilic acid reagent was used to create color with atropine. Finally, the total concentration of atropine was obtained per gram of powdered plant. The accuracy of the spectrophotometric analysis was also confirmed by comparing the results of this method with the results of GC-FID. Based on these results, it can be concluded that the accuracy of UV–Visible spectroscopy is good compared to the GC-FID standard method.

For this purpose, 5 different concentrations of atropine standard solution were prepared (Table 1) and then standard curves were plotted by both techniques, GC-FID and UV–Visible spectroscopy (Fig. 2). It is important to emphasize that each experiment was repeated three times to ensure accuracy and precision. Three similar test solutions were prepared using same extraction method. Then, one of the test solutions was diluted twice and the other was diluted four times. These solutions were measured by both techniques. Results are listed in Table 2. According to the results, comparison of GC-FID with UV–Visible spectroscopy showed that the error is less than one percent.

Preparation of extracted solution after running the experiments

After the system was established in specified conditions, plant and extract were conducted to the bottom valve and filtered. Then, the extract was dried in a rotary evaporator.

The residue was dissolved in 25 mL of $CHCl_3$ and 25 mL of 1 N sulfuric acid and mixed thoroughly. Aqueous

Table 2: Table of unknown solutions for atropine analysis by both techniques.

	Chromatogram Area[mV×s]	Unknown atropine sample Conc (mg/L)	Absorbance	Unknown atropine sample Conc (mg/L)	%error
Sample 1	709.06	372.65	0.551	375.37	0.73
Sample 2	392.82	209.84	0.290	212.25	1.1
Sample 3	146.34	82.93	0.082	82.25	0.8

solution was separated from the $CHCl_3$ rich phase, and was basified (i.e. about pH=10) using 25% ammonium hydroxide.

Alkaloids were extracted once with 10 mL of $CHCl_3$ and twice with 5 mL of chloroform. After the addition of anhydrous Na_2SO_4, the organic solution was filtered to 100 mL flask. The solvent was removed by vacuum evaporation at 40°C and residue was dissolved in appropriate volume (10 mL) of methanol [36,37]. This solution is the unknown solution for the next experiments.

Spectrophotometry of Atropine –Chloranilic Acid Complex

The complexometeric UV-visible spectroscopy was used for quantitative analysis of extracted atropine. The extracted atropine was reacted with chloranilic acid to form a dye complex. The 0.005 M stock solution of chloranilic acid in methanol was used in all experiments. Also, 10 mL of 1000 µg/L standard atropine solution was prepared with methanol.

1.5 mL of stock solution of chloranilic acid in methanol was added to six 5 mL volumetric flasks and then 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mliliters of atropine standard solution were added to each of them, separately. All flasks were brought to volume. The first solution was a blank solution and the others were considered as the standard solution. The absorbance of the mentioned solutions was measured by UV-Visible spectrophotometer in λ_{max}=530 nm and the standard curve were plotted (Fig. 2).

RESULTS AND DISCUSSION

Atropine Identification by GC-FID

The GC-FID results confirmed the presence of atropine in the *Atropa belladonna L*. sample. Fig. 2 shows the chromatogram of atropine stock standard solution and unknown solution together obtained by GC-FID method (Fig. 3). 10 mL of 1000 µg/L standard atropine solution was prepared with methanol and was injected into the GC device that was removed from the column at Retention time= 7.87. Another sample was extracted from the plant and it was injected into the GC device. As a result, Obtained two peaks were similar that showed the presence of atropine in the *Atropa belladonna L*. sample.

Quantitative Analysis by Spectrophotometry of Atropine-Chloranilic Acid Complex

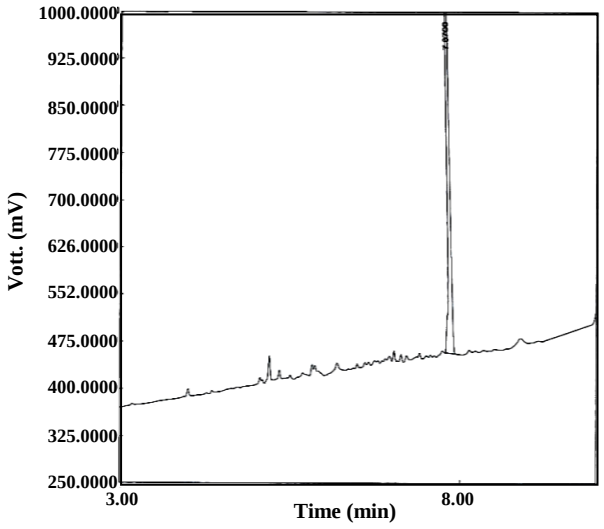
The complexometeric UV-visible spectroscopy was used for quantitative analysis of extracted atropine. 5 mL of the prepared unknown solution entered to 10 mL flasks and 3 mL of stock solution of chloranilic acid was added to it. The volume of the solution was reached to 5 mL by adding methanol, and the absorbance was measured using UV-visible spectrophotometer in λ_{max}=530nm. The concentration of the unknown solution was determined using the prepared standard curve. The obtained results are reported in Table 1.

Effect of Extraction Time, Liquor to Material Ratio and Air Flow

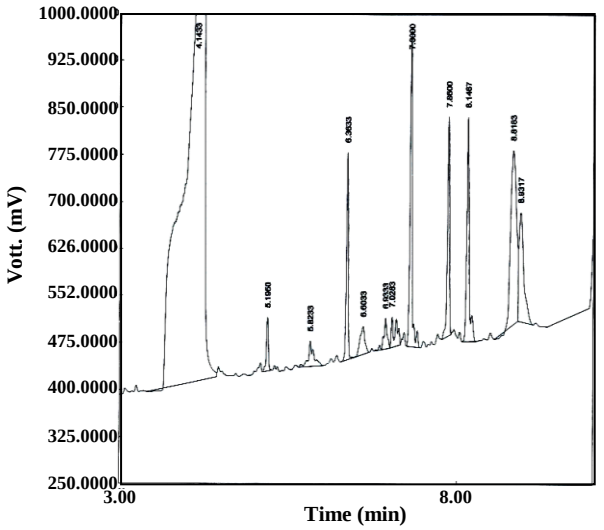
On the basis of primary tests and laboratory facilities, two experimental factors, namely, extraction solvent (Kamada solvent) and particle size of powdered plants (less than 354µm) were kept constant in all experiments. Extraction time (X_1), liquor to material ratio (X_2) and air flow (X_3) were used as factors (independent variables) and the extraction yield of atropine (Y) was used as response variable. The obtained results are reported in Table 3.

Different mathematical equations were used to reproduce the response against the independent factors. The best results were obtained when the following quadratic polynomial equation was used.

Reported data in Table 1, was screened carefully to find the best equation for representation of the response values as a function of the independent variables. Finally, it was found that the following quadratic polynomial type equation is the best:



Num	RT[min]	Area[mV×s]	Type	With[s]	Area%
1	7.8700	1877.5992	VP	18.0000	100



Num	RT[min]	Area[mV×s]	Type	With[s]	Area%
1	4.1433	15346.904	BB	61.7000	69.469
2	5.1950	167.8892	VV	8.7000	0.7600
3	5.8233	215.3894	VP	21.8000	0.9750
4	6.3633	602.3728	VP	12.2000	2.7267
5	6.6033	217.9455	VP	13.5000	0.9866
6	6.9333	167.4987	VP	14.1000	0.7582
7	7.0283	209.4387	VP	9.4000	0.9480
8	7.3000	1052.7566	VP	12.8000	4.7654
9	7.8600	709.0632	VP	8.2000	3.2096
10	8.1467	904.9514	VP	12.5000	4.0964
11	8.8183	1628.3219	VV	20.5000	7.3708
12	8.9317	869.1013	VP	13.4000	3.9341
Sum		22091.633			

Fig. 3: Chromatogram of standard solution of atropine versus solution extracted from the plants Atropa belladonna.

$$Y = \beta_1 X_1^2 + \beta_2 X_2^2 + \beta_3 X_3^2 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1 + \beta_8 X_2 + \beta_9 X_3 + \beta_1. \quad (1)$$

Where β_1 to β_{10} are coefficients. The parameters of the model were estimated by multiple regressions as follows (Table 4).

The goodness of this fitting was evaluated using the residual standard deviation and regression coefficient. Fig. 4 shows the observed values against predicted ones using Eq. (2).

$$Y = -0.000199X_1^2 - 0.00433X_2^2 - 0.00253X_3^2 + 0.000945X_1X_2 + 0.000824X_1X_3 - 0.00349X_2X_3 - 0.00992X_1 + 0.1300X_2 + 0.06484X_3 - 0.39756 \quad (2)$$

It can be seen from this figure, that the correlation is the best (R^2=0.9939). In addition, the statistical data of observed and predicted values revealed the residual standard deviation value is 0.0132. These results confirm that the numerical simulation is successful due to the goodness of the fitting between the observed and the predicted values. ANOVA results showed on the Table 5.

Moreover, Eq. (2) can be used to predict the response values in the experimental domain. The optimum extraction conditions were predicted by Eq. (2).

Based on the obtained results, it was found that the extraction rate increased with time of the process; however, after about 24 minutes the extraction rate was decreased. This observation might be referred to the evaporation of the solvent during the extraction process that decreased the available solvent for extraction; and subsequently the rate of extraction was decreased in long time periods.

Effect of liquor to material ratio was also studied and it was found that the extraction rate increased with increasing this factor to about 15g/mL and above this value, the extraction rate shows a steady and constant behavior. It seems that in this case the mass transfer to the solution was increased in the presence of the excess amount of solvent. However, the atropine content of the plant was limited and mass transfer reached its maximum condition which led to the constant behavior of the extraction rate. It should be noted that ultrasound waves was used in order to accelerate the penetration of atropine from plant cells to the surface of the powdered plants.

Table 3: Results of uniform experimental design for the extraction of atropine.

Exp. No.	Extraction time (min),X_1	Liquor to material ratio(mL/mg),X_2	Air flow ($mL.min^{-1}.g^{-1}$),X_3	Absorbance, A	yield of atropine (mg/g)% ,*Y*
1	4(2)	8(5)	16(8)	0.463	0.337
2	8(4)	12(9)	12(6)	0.501	0.543
3	12(6)	17(14)	8(4)	0.342	0.547
4	16(8)	6(3)	2(1)	0.380	0.212
5	20(10)	10(7)	18(9)	0.427	0.392
6	24(12)	15(12)	14(7)	0.378	0.527
7	28(14)	4(1)	10(5)	0.550	0.197
8	32(16)	8(5)	4(2)	0.419	0.308
9	36(18)	13(10)	20(10)	0.278	0.350
10	40(20)	17(14)	16(8)	0.284	0.466
11	44(22)	6(3)	12(6)	0.433	0.238
12	48(24)	11(8)	6(3)	0.370	0.379
13	52(26)	15(12)	2(1)	0.251	0.371
14	56(28)	4(1)	18(9)	0.029	0.026
15	60(30)	9(6)	14(7)	0.322	0.275
16	2(1)	13(10)	8(4)	0.503	0.591
17	6(3)	18(15)	4(2)	0.323	0.552
18	10(5)	7(4)	20(10)	0.321	0.213
19	14(7)	11(8)	16(8)	0.425	0.429
20	18(9)	16(13)	10(5)	0.396	0.586
21	22(11)	5(2)	6(3)	0.522	0.235
22	26(13)	9(6)	2(1)	0.406	0.337
23	30(15)	14(11)	18(9)	0.293	0.394
24	34(17)	18(15)	12(6)	0.325	0.555
25	38(19)	7(4)	8(4)	0.501	0.317
26	42(21)	12(9)	4(2)	0.404	0.447
27	46(23)	16(13)	20(10)	0.191	0.317
28	50(25)	5 (2)	14(7)	0.286	0.138
29	54(27)	10(7)	10(5)	0.370	0.345
30	58(29)	14(11)	6(3)	0.277	0.376

Table 4: Parameter estimates.

Term	Contrast	Lenth t-Ratio	Individual p-Value	Simultaneous p-Value
Intercept	-0.39756	0.04490	-8.85	<.0001
X2	0.12999	0.00968	13.49	<.00012
X1	-0.00992	0.00206	-4.82	<.0001
X3	0.06484	0.00617	10.51	0.0001
X2*X2	-0.00433	0.00039	-11.11	<.0001
X2*X1	0.00095	0.00017	5.45	0.0006
X1*X1	-0.00020	2.67E-05	-7.46	0.0759
X2*X3	-0.00349	0.00056	-6.24	0.8492
X1*X3	0.00082	0.00014	6.04	<.0001
X3*X3	-0.00253	0.00019	-13.80	<.0001

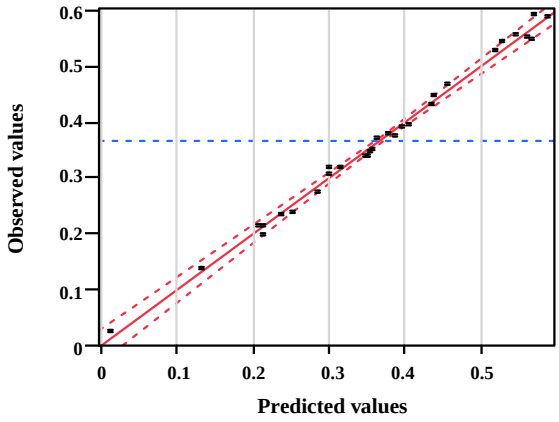


Fig. 4: Observed values vs. predicted values of atropine obtained from estimated model.

The optimum condition for the air flow was also found to be about 6.31 mL/min.g. On the one hand, it was obvious that increasing the air flow caused the increasing of the turbulence in the extraction system which resulted in the improvement of the extraction rate. On the other hand, the high values of the air flow resulted in the separation of the solid phase (the powdered plants) from the liquid phase (extraction solvent), and subsequently the extraction rate was reduced. Fig. 5 shows atropine concentration versus (X1) extraction time, (X2) liquor to material ratio and (X3) air flow and pairs of them versus (Y) yield.

Comparison of different extraction methods

There are several conventional methods to extract atropine from Solanaceae plants. In this research, another method was introduced to extract atropine from Solanaceae plants. To evaluate the efficiency, this method was compared with other conventional methods such as soxhlet and Stirred Tank Extractor coupled with Ultrasonic Bath (STE-UB). Percent of atropine was obtained 5.59% from with ultrasonic bath (STE-UB) method. This result indicates that the effect of bubbles on the extraction rate is good. The average yield of atropine from soxhlet methods for extraction times of 0.5 h, 6.0 h and 12.0 h were 0.339 mg/g, 0.674 mg/g and 0.670 mg/g, respectively. Assuming that the average percentage of atropine obtained by soxhlet method was completed at relatively long time (6.0 h and 12.0 h had approximately equal efficiency), the efficiency of this method was 94%. It is important to stress that each experiment was repeated three times to ensure accuracy and precision.

CONCLUSIONS

Based on the results obtained from the Uniform Design (UD) method used in this work, it was found that the optimum conditions for the process is the extraction time of 23.95 min, the liquor to material ratio of 15.08 mL/g and an air flow rate of 6.31 mL/min.g. After determining this condition, experiments were carried out in the nearest and the most possible condition. In this respect, an experiment was performed in extraction time of 24 min, a liquor to material ratio of 15 mL/g and an air flow of 6 mL/min.g. Under these optimum conditions, the average yield of atropine for 3 independent experiments was 0.631 mg/g, which showed

Table 5: Analysis of variance (ANOVA).

Source	Df	Sum of Squares	Mean Square	F Ratio	P robe > F
Model	9	0.573767	0.063752	364.60	<.0001
Error	20	0.003497	0.000175		
C. Total	29	0.577267			

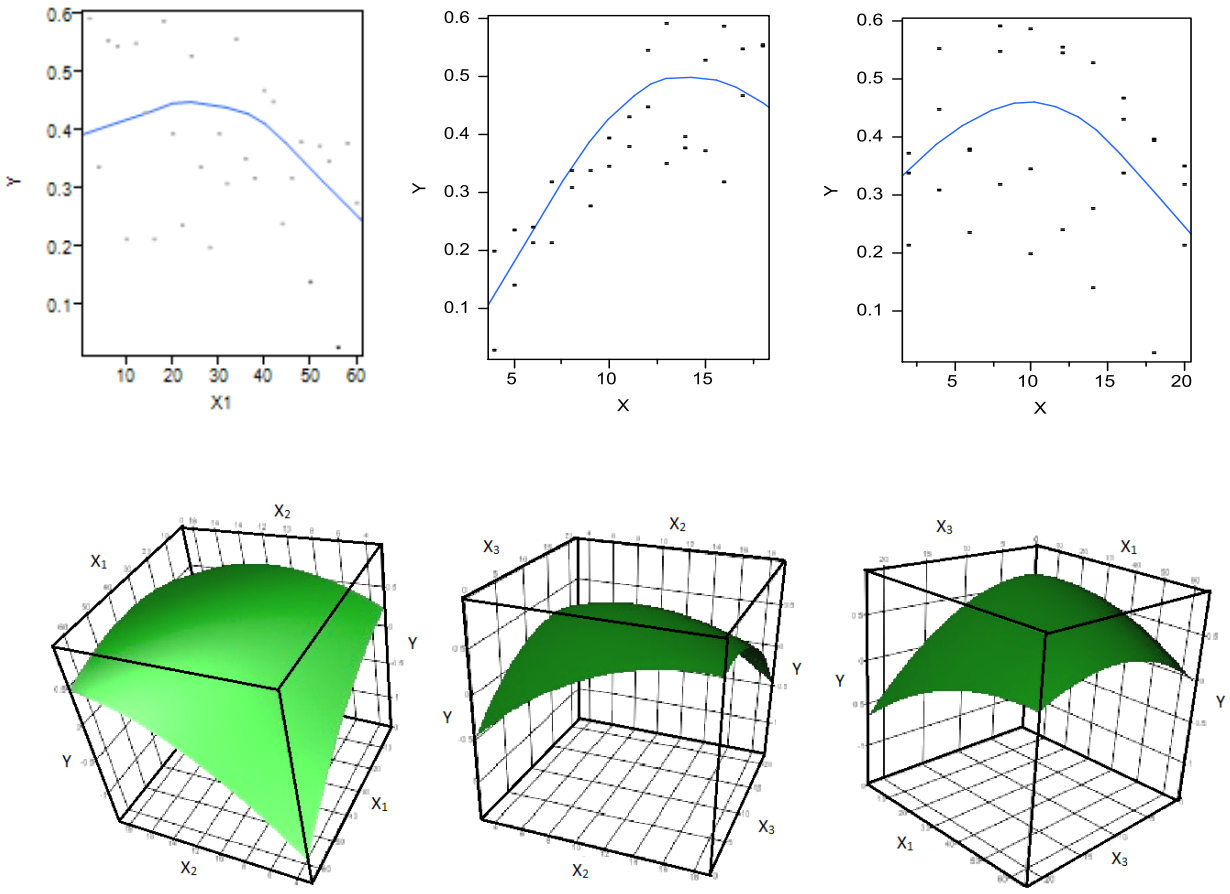


Fig. 5: Atropine concentration versus (X_1) extraction time (X_2) liquor to material ratio (X_3) air flow curves and pairs of them versus (Y) yield.

slight difference from the predicted value and therefore, the optimized results might be reliable.

In a similar condition, it was found that the percent of atropine which obtained by BCE-UB method was greater than STE-UB. These results showed that the bubbles might be increased the rate of extraction. Also, this method was fast and had a high extraction rate. Therefore, BCE is a fast and efficient method for the extraction of Atropine and other pharmaceutical ingredients in the pilot and industrial scales of herbal products.

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Nomenclature

BCE-UB	Bubble column extraction coupled with ultrasonic bath
CE	Capillary electrophoresis
GC–FID	Gas Chromatography–Flame Ionization Detector
HPLC	High performance liquid chromatography
M	Molarity
MHz	Megahertz
N	Normality
R.S.D.	Relative standard deviation
STE-UB	Stirred tank extractor coupled with ultrasonic bath
UD	Uniform design
X_1	Extraction time, min
X_2	Liquor to material ratio, mL/g
X_3	Air flow, L/min
Y	Extraction yield of atropine, mg/g
β_i	Coefficients of the equation, i=1, 2, 3, 4, 5, 6, 7, 8, 9, 10
λ_{max}	The wavelength at which the absorption is at a maximum

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