IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 5 Ver. V (Sep. – Oct. 2017), PP 46-52
www.iosrjournals.org

Simultaneous Determination of Three Different Alkaloids: Scopolamine, Hyoscyamine and Lobeline in Pharmaceutical Dosage Forms by UFLC Method

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Abstract*: A rapid and accurate UFLC method was developed for determination of scopolamine, hyoscyamine and lobeline in herbal cough mixture found in herbal product (BN) purchased from local market. Reversed phase chromatographic analyses was carried out under gradient conditions using column ACE, C18 (150 x 4.6 mm); packed with 5 µm diameter particles. The mobile phase was Acetonitrile (A) and (buffer was composed 0.1% phosphoric acid and 0.1% sodium lauryl sulfate in distilled water) (B). The gradient mobile phase was as follows: 35% (A): 65% (B) for 10 min; then 80% (A): 20% (B) for 12 min; then 35% (A): 65% (B) for 5 min. The flow rate was 2.0 ml/min. The injection volume was 20 µl. The detection wavelength (λ_{max}) was 205 nm using a photodiode array detector. Linearity of the method was established over the concentration ranges of 80 – 500 µg.ml^{-1} for scopolamine hydrobromide trihydrate with a retention time about 5.77 minutes, 18 – 112.5 µg.ml^{-1} for hyoscyamine sulfate with a retention time about 7.52 minutes and 20 – 125 µg.ml^{-1} for lobeline sulfate with a retention time about 17.74 minutes. Correlation coefficients was greater than 0.99. The recovery level of scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate were 100.02%, 100.02% and 99.96%; respectively. The method described is quite suitable for routine analysis of herbal extracts and in pharmaceutical preparations.*

Keywords: *UFLC, Alkaloids, Lobelia, Datura, Tinctures, Method validation.*

Date of Submission: 07-09-2017 Date of acceptance: 07-10-2017

I. Introduction

Tropane alkaloids are natural compounds having in common, the 8-aza-bicyclo [3.2.1] octane structure. They mainly occur in the Solanaceae, Erythroxylaceae and Convolvulaceae families (1-2). In the last few decades, more than 250 natural tropane alkaloids have been isolated from the different plant taxa and their biological properties have been the subject of many studies (3-4). Because of numerous pharmacological activities, tropane alkaloids are considered as an important class of natural products and some of which such as (-)-hyoscyamine (Fig. 1A), the more stable enantiomer of atropine and scopolamine (Fig. 1B) are widely used in therapeutics. Tropane alkaloids are competitive antagonists of the muscarinic acetylcholine receptor and classified as anticholinergic agents (5). Because of the high cost of the industrial synthesis, tropane alkaloids are extracted from the plants of Solanaceae family and the investigation for new sources is still on going. So far, a number of analytical methods including gas chromatography (GC) (6-7), gas chromatography-mass spectrometry (GC/MS) (8-9), LC/MS (10), high performance liquid chromatography (HPLC) (11), thin layer chromatography (TLC), (12) and capillary electrophoresis (CE) (13) have been used for determination of tropane alkaloids. More recently, analysis of tropane alkaloids and related compounds has been reviewed (14). Lobeline (Fig. 1C), which chiefly the alkaloid present in lobelia, has peripheral and central effects similar to those of nicotine. Lobelia has been used mainly in preparations aimed at relieving respiratory-tract disorders. Lobeline has been given by mouth as the hydrochloride or sulfate as a smoking deterrent. Lobelia has been used similarly given either by mouth or incorporated into herbal cigarettes.

Therefore, the aim of the present study was to determine main alkaloids, scopolamine, hyoscyamine and lobeline present in different herbal extracts and pharmaceutical formulations by reversed phase HPLC.

II. Materials and Methods

I. Chemicals and reagents

Reference standards of scopolamine hydrobromide trihydrate from Acros organics (USA), hyoscyamine sulfate from USP (Germany) and lobeline sulfate from ChromaDex (USA). HPLC grade Acetonitrile from Romil (England). Water for chromatography was purchased from Merck (Germany). Ortho-Phosphoric acid 85% was HPLC grade from Fluka chemicals (Germany). Sodium lauryl sulfate from El Nasr Pharmaceutical Chemicals Co. (Egypt).

II. Equipment and chromatographic conditions

The UFLC system consisting of Shimadzu LC-20XR, Prominence (Kyoto, Japan) equipped with model LC-20AD pump, PDA detector (SPD-M20A, Japan) was used for the analysis. Peak areas were integrated using a Shimadzu LC solution (Version 5.41.240) software program. A NSXX sonics ultrasonic bath (NS-A-12-7H, Germany) was used for degassing the mobile phase.

Experimental conditions were optimized on ACE C18 (150 x 4.6 mm, 5 µm) and the flow rate of the mobile phase was 2.0 ml/min. The mobile phase was consisting Acetonitrile (A) and Buffer (B). The buffer was prepared by adding 1 ml phosphoric acid and 1 gm sodium lauryl sulfate in 1000 ml distilled water. The composition gradient was: 35% (A): 65% (B) for 10 min; then 80% (A): 20% (B) for 12 min; then 35% (A): 65% (B) for 5 min. Analysis was performed with injection volume of 20 µl using PDA detection at 205 nm. Mobile phase was filtered using 0.45 µm nylon membrane filter (UK). The optimized chromatographic condition is shown in Table 1.

III. Preparation of standard stock and standard solution

1. Standard stock solutions:

50 mg of scopolamine hydrobromide trihydrate were dissolved in 5 ml distilled water in 10 ml volumetric flask, then complete to volume with distilled water and sonicate for 10 minutes (standard solution A).

45 mg of hyoscyamine sulfate were dissolved in 10 ml distilled water in 20 ml volumetric flask, then complete to volume with distilled water and sonicate for 10 minutes (standard solution B).

25 mg of lobeline sulfate were dissolved in 10 ml distilled water in 20 ml volumetric flask, then complete to volume with distilled water and sonicate for 10 minutes (standard solution C).

2. Mixed Standard solution:

Reference standard solutions were prepared and further diluted with distilled water to contain a mixture of scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate in the linearity range from 80 – 500 $\mu g.ml^{-1}$, 18 – 112.5 $\mu g.ml^{-1}$ and 20 – 125 $\mu g.ml^{-1}$ respectively. The solutions were filtered through a 0.45 µm nylon filter prior analysis.

IV. Preparation of sample solution

The content of three bottles of herbal cough mixture (BN) (Each 5 ml of herbal product (Y) contains 0.5 ml lobelia tincture and 0.15 ml datura tincture), were mixed well; then 20 gm of syrup was accurately transferred into 50 ml volumetric flask and sonicated for 10 min, Diluted to volume with distilled water.

V. Preparation of tinctures

Accurately weighing 10 gm of datura tincture and 10 gm of lobelia tincture were accurately transferred into 50 ml volumetric flask and sonicated for 10 min, Diluted to volume with distilled water.

VI. Linearity

Linear calibration plots of the proposed method were obtained over concentration ranges of 80 - 500 $\mu g.ml^{-1}$ (80, 280, 400, 440 and 500 $\mu g.ml^{-1}$) for scopolamine hydrobromide trihydrate, 18 – 112.5 $\mu g.ml^{-1}$ hyoscyamine sulfate (18, 63, 90, 99 and 112.5 $\mu g.ml^{-1}$) and 20 – 125 $\mu g.ml^{-1}$ (20, 70, 100, 110 and 125 $\mu g.ml^{-1}$) for lobeline sulfate. Triplicate injections were made for each standard solution.

VII. Accuracy

Accuracy was evaluated by standard addition method of scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate. This method known amounts of scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate were added to the previously analysed sample solution and then experimental and true values were compared. Three levels were made corresponding to 70%, 100% and 110% of the nominal analytical concentration.

VIII. Precision

Repeatability was studied by determination of intra-day and inter-day precision. Intra-day precision was determined by injecting five replicates of three different concentrations on the same day and inter-day precision was determined by injecting the same solutions for three consecutive days. Relative standard deviation (RSD%) of the peak area was then calculated to represent precision.

IX. Robustness

Premeditate variations were performed in the experimental conditions of the proposed method to assess the method robustness. For this intention, minor changes were made in mobile phase composition, flow rate and pH of buffer solution. The effect of these changes on chromatographic parameters such as retention time, tailing factor and number of theoretical plates was then measured.

X. Limit of detection (LOD) and limit of quantitation (LOQ)

Limits of detection (LOD) were calculated according to the expression 3.3σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. Limits of quantification (LOQ) were established by using the expression 10σ/S. LOD and LOQ were experimentally verified by injections of pure standard at the LOD and LOQ concentrations.

III. Results and Discussion

The variations in the composition of the mobile phase and dissimilar stationary phases had substantial influences on peak shape, tailing factor, retention factor, theoretical plates and resolution. The aim of this work is to develop a method that can be applied successfully for separation and quantification of the studied herbal cough mixture. A simple, selective, sensitive and accurate UFLC method was adopted for the simultaneous determination of scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate, either separate or in pharmaceutical preparations. There was a clear resolution between scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate with retention time of 5.77, 7.52 and 17.74 minutes; respectively. (Figs. 2 and 3) showed that there were no interferences at the retention times for scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate due to the placebo. Method validation was performed according to ICH guidelines (15).

Linearity of the proposed method was evaluated and it was evident in the concentration range of 80.0-500 $\mu g.ml^{-1}$ for scopolamine hydrobromide trihydrate, 18-112.5 $\mu g.ml^{-1}$ for hyoscyamine sulfate and 20-100 $\mu g.ml^{-1}$ for lobeline sulfate. Good linearity was evident by the high value of the correlation coefficient are shown in (Figs. 4, 5 and 6). The correlation between the analyte concentration and peak area is described by linear regression equations with high value of correlation coefficient (r) all results were listed in Table 2. The regression equations were calculated and found to be:

$Y1 = 13.6, C1 – 71.4 \times 10^3$, r1 = 0.99965

$Y2 = 17.4, C2 – 12.7 \times 10^3$, r2 = 0.99873

$Y3 = 32.4, C3 – 1.9 \times 10^3$, r3 = 0. 99938

Where Y1, Y2 and Y3 are the peak area/10^3, C1, C2 and C3 are scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate concentrations in $\mu g\ ml^{-1}$ respectively and r1, r2 and r3 are the correlation coefficients.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by making serial dilutions. LOD was found to be 6.74 $\mu g.ml^{-1}$, 4.52 $\mu g.ml^{-1}$ and 3.15 $\mu g.ml^{-1}$ for scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate, respectively (signal to noise ratio of 3:1). LOQ was found to be 20.42 $\mu g.ml^{-1}$, 13.71 $\mu g.ml^{-1}$ and 9.53 $\mu g.ml^{-1}$ for scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate; respectively (signal to noise ratio of 10:1).

Accuracy and recovery of the method was assured by applying the standard addition technique on different pharmaceutical dosage forms where good recoveries were obtained as shown in Table 8 revealing no interference from excipients and good accuracy of the proposed method.

Three levels of solutions (70, 100 and 110%) of the nominal analytical concentrations were prepared and analysed by the developed method.

Intra-day precision was determined by injecting five standard solutions of three different concentrations on the same day and inter-day precision was determined by injecting the same solutions for three consecutive days. Relative standard deviation (RSD%) of the peak area was calculated to represent precision. Results of intra-day and inter-day precision are presented in Table 3.

Robustness of the method was performed by slightly varying chromatographic conditions. The results showed that slight variations in chromatographic conditions had a negligible effect on the chromatographic parameters (Tables 4, 5 and 6).

System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as whole. System suitability is used to ensure system performance before or during the analysis of the drugs. System suitability was checked by calculating the asymmetry factor (A_S), tailing factor (T), theoretical plates (N) and resolution (R), where the system was found to be suitable as shown in Table 7.

IV. Conclusion

The validated UFLC method developed for the quantitative determination of scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate in the tinctures and liquid cough mixture found in the Egyptian market. The developed method was validated by testing its linearity, accuracy, precision, specificity, limits of detection and quantitation. This method enables simultaneous determination of scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate because of good separation and resolution of the chromatographic peaks. As a result, the proposed UFLC method could be adopted for the quantitative routine analysis.

Acknowledgement

The authors are grateful to Arab Company Pharmaceutical Company (Mepaco-Medifood), El Sharkia, Egypt for her ultimate support for research and development team.

Also, we would like to express our gratitude and appreciation for Dr. Adel El Khabiry; Chairman and Managing director, (Mepaco-Medifood) for his continuous and sincere encouragement, everlasting patience and overwhelming care for research team throughout our work.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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III. Tables and Figures

Tables

Table (1): Optimized chromatographic conditions

Parameters	Conditions
Stationary Phase	ACE, C18, 150 x 4.6 mm, 5 µm
Mobile Phase	Acetonitrile and Buffer (Gradient) The composition gradient was: 35% (A): 65% (B) for 10 min; then 80% (A): 20% (B) for 12 min; then 35% (A): 65% (B) for 5 min.
Flow Rate (ml min^{-1})	2.0
Run Time (min)	25.0
Column Temperature (^{0}C)	Ambient (25^{0}C)
Injection Volume (µl)	20
Detection Wavelength (nm)	205nm
Retention Time of Scopolamine (min)	5.77
Retention Time of Hyoscyamine (min)	7.52
Retention Time of Lobeline (min)	17.74

Table (2): Characteristics of the proposed methods used in assay of scopolamine, hyoscyamine and lobeline.

Parameters	Scopolamine	Hyoscyamine	Lobeline
Linearity range/µg ml^{-1}	80.0-500	18-112.5	20-125
Slope	13.6 x 10^3	17.4 x 10^3	32.4 x 10^3
Intercept (a)	-71.4 x 10^3	-12.7 x 10^3	-1.9 x 10^3
Correlation coefficient	0.99965	0.99873	0.99938
Detection limit/µg mL^{-1}	6.74	4.52	3.15
Quantification limit/µg mL^{-1}	20.42	13.71	9.53
Capacity factor	0.00	0.32	2.06
Tailing factor	1.10	1.12	1.25
Theoretical plate no.	4818	4315	4983

Regression equation: Y= a + bC, where Y is the area under peak, a is the intercept, b is the slope and C is the concentration.

Table (3): Intra-day and inter-day precision of the proposed UFLC method.

Drugs	Actual concentration (µg mL^{-1})	Intra-day precision measured concentrations (µg.ml^{-1}) ± SD; RSD (%)	Inter-day precision measured concentrations (µg.ml^{-1}) ± SD; RSD (%)
Scopolamine	280	280.54 ± 0.67; 0.24	279.72 ± 0.44; 0.16
	400	400.25 ± 0.11; 0.03	400.88 ± 0.41; 0.10
	440	440.34 ± 0.74; 0.17	440.49 ± 0.68; 0.15
Hyoscyamine	63	63.04 ± 0.18; 0.28	63.40 ± 0.55; 0.86
	90	89.95 ± 0.83; 0.92	90.35 ± 0.26; 0.29
	99	98.79 ± 0.96; 0.97	98.93 ± 0.60; 0.60
Lobeline	70	69.79 ± 0.73; 1.05	70.37 ± 0.44; 0.63
	100	100.10 ± 0.34; 0.34	100.02 ± 0.58;0.58
	110	110.21 ± 0.35; 0.31	110.36 ± 0.60; 0.54

Table (4): Robustness study of scopolamine.

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Column temperature (20^0C)	100.12	5.73	4820	1.12
Column temperature (25^0C)	99.89	5.78	4815	1.10
Column temperature (30^0C)	100.18	5.71	4831	1.18
Flow rate (1.8 mL min^{-1})	99.93	6.12	4833	1.11
Flow rate (2.0 mL min^{-1})	99.81	5.79	4823	1.13
Flow rate (2.2 mL min^{-1})	100.25	5.63	2824	1.10
Buffer (pH 2.6)	99.76	5.81	4536	1.15
Buffer (pH 2.8)	99.90	5.79	4841	1.14
Buffer (pH 3.0)	99.13	5.78	4817	1.11

Table (5): Robustness study of hyoscyamine.

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Column temperature (20^0C)	100.28	7.55	4318	1.17
Column temperature (25^0C)	100.02	7.54	4322	1.12
Column temperature (30^0C)	100.25	7.50	4317	1.10
Flow rate (1.8 mL min^{-1})	99.98	7.72	4318	1.12
Flow rate (2.0 mL min^{-1})	99.93	7.58	4320	1.16
Flow rate (2.2 mL min^{-1})	100.13	7.17	2321	1.13
Buffer (pH 2.6)	100.43	7.61	4317	1.13
Buffer (pH 2.8)	99.99	7.56	4316	1.14
Buffer (pH 3.0)	99.78	7.64	4311	1.12

Table (6): Robustness study of lobeline.

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Column temperature (20^0C)	99.19	17.75	4988	1.28
Column temperature (25^0C)	100.14	17.79	4997	1.22
Column temperature (30^0C)	100.02	17.78	4964	1.25
Flow rate (1.8 mL min^{-1})	99.63	17.97	4972	1.23
Flow rate (2.0 mL min^{-1})	99.72	17.76	4983	1.26
Flow rate (2.2 mL min^{-1})	99.86	17.51	4991	1.31
Buffer (pH 2.6)	100.34	17.61	4986	1.27
Buffer (pH 2.8)	99.97	17.70	4987	1.24
Buffer (pH 3.0)	99.68	17.67	4985	1.22

Table (7): Summary of system suitability tests.

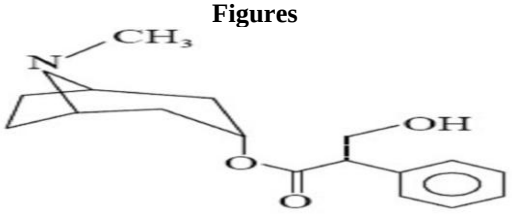
Parameters	Scopolamine	Hyoscyamine	Lobeline
T	1.23	1.16	1.31
R[b]	—	5.72	33.43
N	4822	4331	4981
AS	1.12	1.19	1.28
RSD[a] (peak areas)	0.83	0.72	0.53
RSD[a] (retention time)	0.16	0.21	0.15

T, Tailing factor; N, no. of theoretical plates; R, resolution factor; As, asymmetry factor.
[a] RSD for five determinations.
[b] The resolution factor (R) calculated to the nearest peak in order.

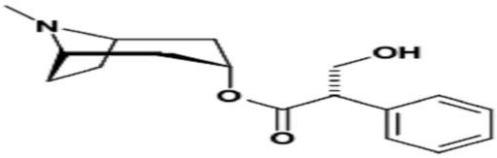
Table (8): Accuracy of the proposed UFLC method.

Level (%)	Scopolamine			Hyoscyamine			Lobeline		
	Amount of drug spiked (mg)	Found (mg)	Recove (%) ry (n=3)	Amount of drug spiked (mg)	Found (mg)	Recove (%) ry (n=3)	Amount of drug spiked (mg)	Found (mg)	Recovery (%) (n=3)
70	280.19	280.24	100.02	63.24	63.29	100.08	70.91	70.85	99.92
100	400.84	400.83	100.00	90.92	90.97	100.05	100.44	100.38	99.94
110	440.72	440.96	100.05	99.81	99.73	99.92	110.28	110.31	100.03
	Average Recovery		100.02	Average Recovery		100.02	Average Recovery		99.96
	SD		0.03	SD		0.09	SD		0.06
	% RSD		0.03	% RSD		0.09	% RSD		0.06

Figures



(A)



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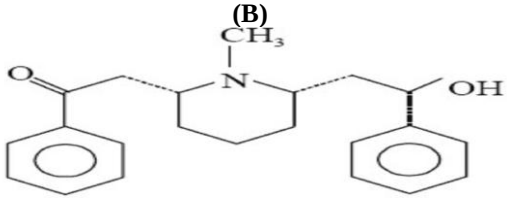

(C)

Fig. (1): Chemical structures of hyoscyamine (A), scopolamine (B) and lobeline (C).

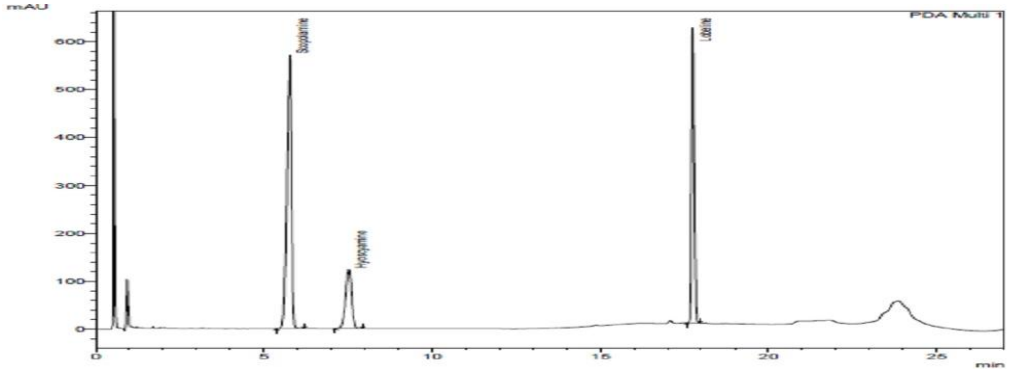

Fig. (2): A typical chromatogram for scopolamine hydrobromide trihydrate, hyoscyamine sulfate and lobeline sulfate standard drug.

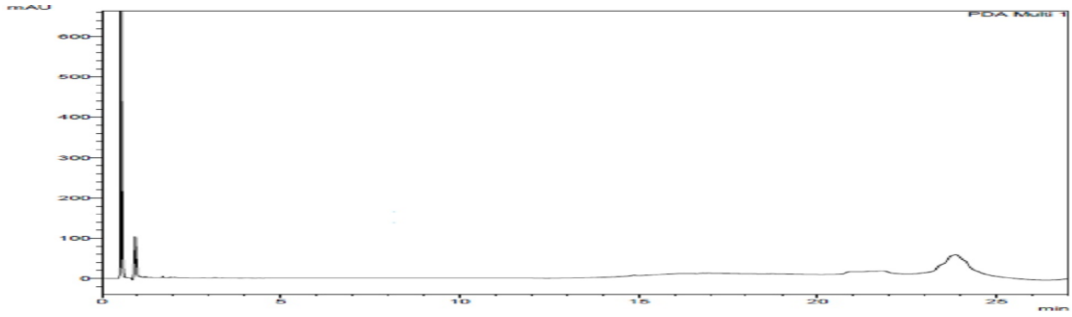

Fig. (3): UFLC chromatogram of placebo

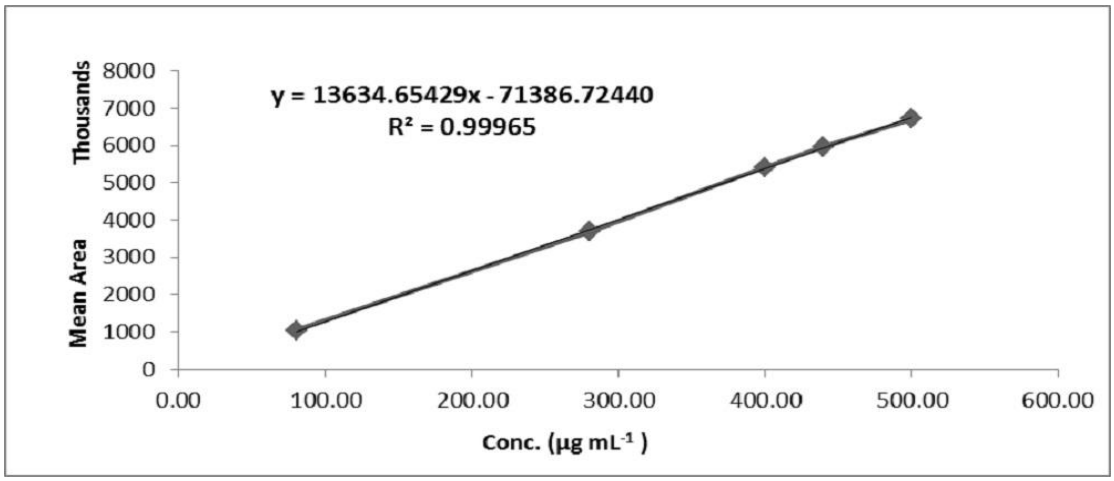

Fig. (4): Calibration curve of scopolamine hydrobromide trihydrate.

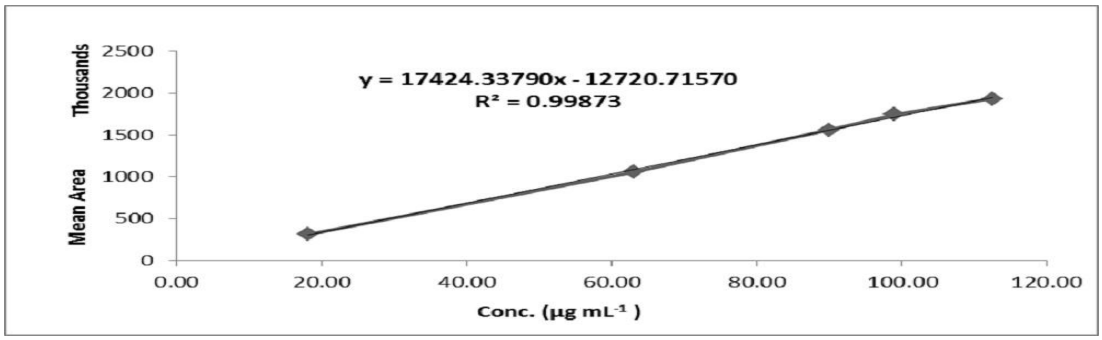

Fig. (5): Calibration curve of hyoscyamine sulfate.

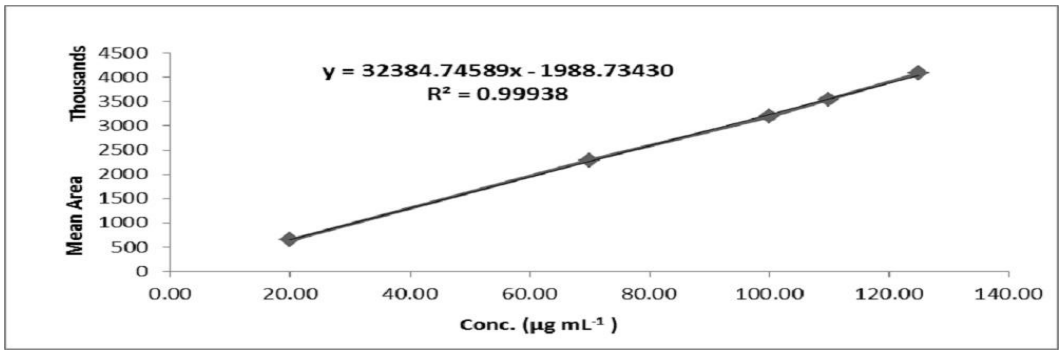

Fig. (6): Calibration curve of lobeline sulfate.

Mohamed A. Mahmoud . "Simultaneous Determination of Three Different Alkaloids: Scopolamine, Hyoscyamine and Lobeline in Pharmaceutical Dosage Forms by UFLC Method." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) , vol. 12, no. 5, 2017, pp. 46–52.