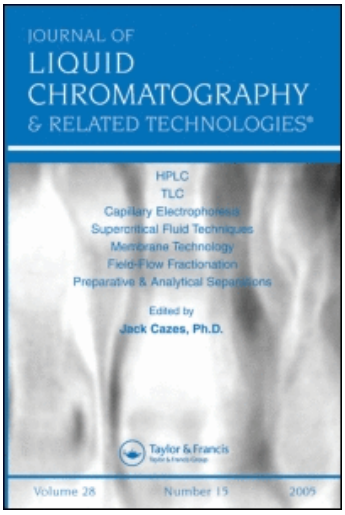

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Thin Layer Chromatographic Scanner Method for the Determination of Some Natural Products in Raw Materials and Pharmaceutical Preparations

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THIN LAYER CHROMATOGRAPHIC SCANNER METHOD FOR THE DETERMINATION OF SOME NATURAL PRODUCTS IN RAW MATERIALS AND PHARMACEUTICAL PREPARATIONS

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ABSTRACT

The suggested method depends upon the determination of lobeline, khellin and emetine hydrochloride. The method is simple, accurate, precise, and depends on the quantitative TLC spectrodensitometric evaluation of lobeline I, khellin II, and emetine hydrochloride III. Excellent separation with discrete spots was quickly obtained. The proposed method determines 8.50-22.00 $mg.mL^{-1}$; 0.02-0.14 $mg.mL^{-1}$, and 0.8-3.6 $mg.mL^{-1}$ with mean percentage accuracies of 99.34% ± 0.49%, 99.99% ± 0.62%, and 99.69% ± 0.50% for I, II, and III respectively.

The proposed method was successfully applied to the analysis of I, II, and III in raw materials, and to pharmaceutical preparations. The results obtained were compared statistically with those obtained by applying the official methods, furthermore, the validity of the results was assessed by applying the standard addition technique.

INTRODUCTION

Recently back to nature theory has been extensively thought about and applied; as a result the use of phytopharmaceuticals is widely spread all over the world. So special attention must be paid to the methods of analysis of these preparations. The action of lobelia inflata L. (Lobeliaceae) is due chiefly to lobeline. It is frequently used in the treatment of bronchial asthma and chronic bronchitis.[1]

Khellin is the major constituent of Ammi visnaga L.fruits (Umbelliferea). It is used as smooth muscle relaxant, vasodilatator, and antispasmodic specially for renal colic to remove urinary calculi.[2] Emetine hydrochloride is an alkaloid obtained from the roots of Cephaelis Ipecacuanha, (Rubiaceae). It is used as an expectorant, emetic, and in the treatment of amebic diseases.[3]

Several methods have been reported for the determination of lobeline in pharmaceutical preparations colorimetry,[4-6] U.V,[7] HPLC,[8] and polarography.[9] Many methods have been proposed for the determination of khellin gravimetry,[2,10] colorimetry,[11-13] U.V.,[14,15] fluorimetry,[16,17] HPLC,[18,19] and NMR.[20,21] Different methods have been proposed for the assay of emetine hydrochloride colorimetry,[22,23] U.V.,[24] fluorimetry,[25] gas chromatography,[26] and HPLC.[27]

No literature revealed the application of spectrodensitometry for analysis. So the work described in this paper was undertaken to apply a simple, rapid, and precise densitometric method for the determination of I, II, and III in raw materials and in their pharmaceutical preparations.

EXPERIMENTAL

Materials

For lobeline

Lobeliae extract in a concentration of 0.750-1.000% was kindly supplied by Nile Co.Egypt.

Lobestra syrup: Nile Co., Egypt, Batch No. D, Hoo6. Each 15mL syrup claimed to contain: KI 0.25 gm, tincture lobelia 0.80mL, tincture belladonna 0.20 mL, tincture stramonium 0.60mL, tincture scilla 0.50 mL, syrup tolu, 3mL.

Broncho syrup: Mepaco Co. Egypt, Batch No. 04113. Each 100 mL syrup claimed to contain: lobelia tincture 10mL, squill tincture 3mL, liquorice extract 20 mL, stramonium tincture 3 mL.

For khellin

Khellin bulk powder was kindly supplied by Memphis Co. Egypt.

Glucolynamine ampoule, Memphis Co.Egypt, Batch No. 295190. Each 10mL claimed to contain khellin 0.030 gm., dihydroxypropyl theophylline 0.150 gm., theophylline 0.075 gm., dextrose 2.00gm.

Khellalgine ampoule, Misr Co.Egypt, Batch No. 55025. Each 5mL claimed to contain: khellin 0.050 gm, atropine sulphate 0.300 mg, analgin 1.00 gm, hyoscine hydrobromide 0.03 mg, phenobarbital 12mg, aqueous base to 5mL.

Psorvityl tablet, Memphis Co.Egypt, Batch No. 491007. Each tablet claimed to contain: khellin 25mg.

Uricol effervescent granules, Pharco Co Egypt., Batch No. 1610. Each 100 gm granule claimed to contain: hexamine 10 gm, piperazine citrate 3.8 gm, khellin 36.6 mg.

Khellalgine suppository, Misr Co. Egypt, Batch No. 627082. Each suppository claimed to contain: khellin 0.050 gm, atropine sulphate 0.500 mg, analgin 0.250 mg, papaverine hydrochloride 0.050 gm, phenobarbital sodium 0.030 mg, fatty base to 1.800 gm.

For emetine hydrochloride

Emetine hydrochloride bulk powder was kindly supplied by Kahira Co., Egypt.

Solvin Syrup: Arab Co. Egypt, Batch No. 345123. Each 100 mL syrup claimed to contain: bromhexine hydrochloride 80mg, extract senega 2 mL, tincture ipeca 5mL, syrup tolu. 2 mL, anise oil 0.035 mL.

Bronchistal Syrup: Kahira Co., Batch No. 550974. Each 15 mL syrup claimed to contain: ammonium chloride 0.1 gm, chlorpheniramine maleate 0.003 gm, tincture ipeca 0.6 mL, sodium comphosulphonate 0.2 gm.

Preparation of Standard Solutions

* Lobeline 43.7 mg. mL^{-1} in chloroform: Transfer a measured volume of 50 mL lobelia extract to a 500 mL separating funnel containing 100 mL ethanol 60%, acidify with dilute sulphuric acid and wash two times with 50 mL chloroform. Reject the chloroformic layer, alkalinize the aqueous layer with ammonia solution 25%, and extract with successive quantities of chloroform 4x50 mL until complete extraction of the alkaloid is effected (Mayer's test). Wash the combined chloroform extract with 25 mL of water and evaporate on a water bath to about 5 mL and transfer quantitatively into a 10-mL volumetric flask and complete to volume with chloroform.

* Khellin 0.2 mg. mL^{-1} in methyl alcohol.

* Emetine hydrochloride 2 mg. mL^{-1} in methyl alcohol.

Reagents

All chemicals and reagents used through out this work were of analytical grade and the solvents are of spectroscopic grade.

1. Sulphuric acid, 10%, aqueous solution, (Prolabo, Paris, France).
2. Ammonia solution 25% aqueous solution, (Prolabo Paris, France).
3. Chloroform, (Merck, Darmstadt Germany).
4. Methyl alcohol (Merck, Darmstadt, Germany).
5. Ethyl alcohol 95%, 60%, (Prolabo, Paris, France).
6. Ethyl acetate (Merck, Darmstadt, Germany).

Apparatus

Shimadzu-dual wavelength flying spot scanning densitometer, CS-9000. List of parameters photomode: reflection, scan mode zigzag and beam size 12. Wave length 285 nm, 250 nm and 230 nm. Recorded parameters: Abscissa scale XL.

UV Lamp-short wavelength.

TLC plates - 20x20 cm with 0.25 mm thickness silica gel G60 F_{254} (Merck).

Procedure

Construction of calibration curves for I, II and III

Transfer accurately aliquot portions (44-175 mg), (0.1-0.7 mg), (2-8mg) of I, II, and III from its standard solutions into a separate series of 5-mL volumetric flasks and complete to volume with chloroform for I and methanol for II and III. Apply 25μL of each solution to a separate precoated thin layer chromatographic plate (20x20cm) using a micro pipette. Place the plate in a chromatographic tank previously saturated for one hour with developing mobile phase, methanol: ammonia 25% (100:1.5 v/v) for I and III and ethyl acetate for II. Develop the plates by ascending chromatography through a distance of 16 cm, dry at room temperature and scan at 285, 250, 230 nm for I, II, and III respectively. Plot the calibration curve representing the relationship between the recorded area under the peak and the corresponding concentration.

Assay of pharmaceutical preparations

Lobeline: for syrups

Shake the bottle vigorously then transfer, accurately, 40 mL of syrup to separating funnel and complete as under lobeline in preparation of standard solution starting with the words "acidify with dilute sulphuric acid..." Calculate the concentration from the regression equation. Results obtained are shown in Table 2.

Khellin

Ampoules

Make the appropriate dilution of ampoules in methanol to a concentration 0.2 $mg.mL^{-1}$ complete as under construction of calibration curves starting with the words"Transfer accurately aliquot portions (0.1-0.7 mg).." Calculate the concentration from the regression equation. Results obtained are shown in Table 3.

Suppositories

Weigh accurately ten suppositories in a small beaker, melt on a water bath with stirring and cool. Extract an amount of the melted and cooled suppositories equivalent to 50mg of khellin with 3x15 mL hot methanol. Cool in a refrigerator each time, filter into 50-mL volumetric flask, and complete to volume with methanol. Complete as under ampoules staring with the words "Make the appropriate dilution in methanol..." Calculate the concentration from the regression equation. Results obtained are shown in Table 3.

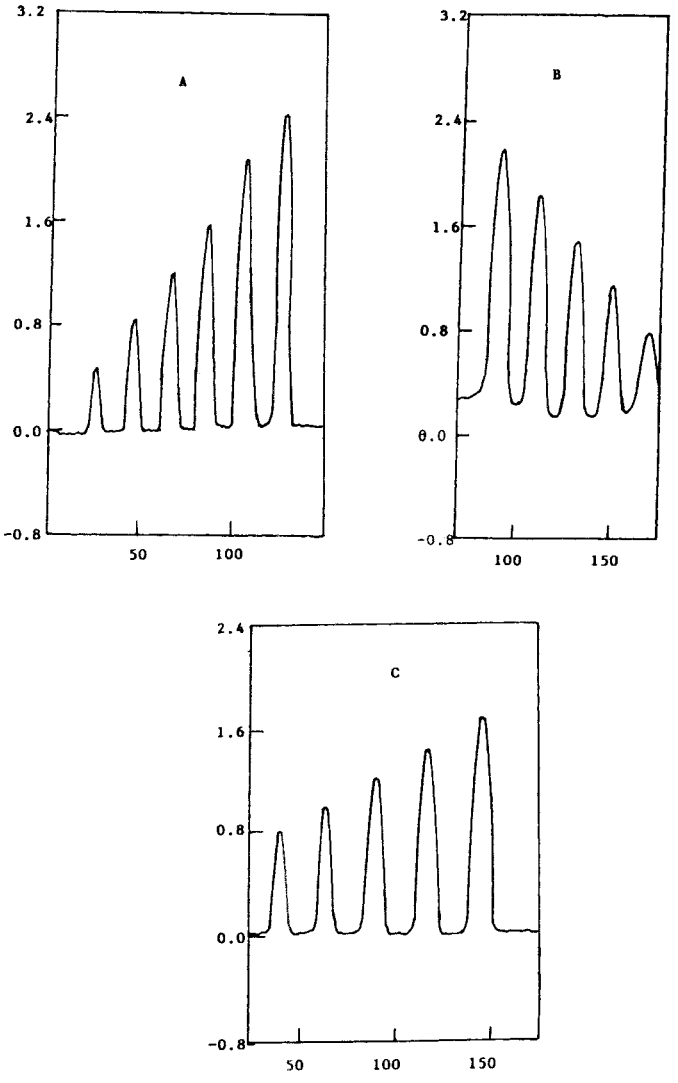

Figure 1. Scanning profile of TLC chromatogram A-Lobeline, B-Khellin, C-Emetine.

Effervescent granules

Weigh accurately 5 gm of finely powdered effervescent granules, extract with 3x15 mL methanol, filter into a 50-mL volumetric flask, and complete to volume

with methanol. Complete as under ampoules starting with the words "Make the appropriate dilution in methanol..." Calculate the concentration from the regression equation. Results obtained are shown in Table 3.

Tablets

Thoroughly powder and mix the contents of twenty tablets of khellin. Weigh accurately an amount of the powder equivalent to 50 mg of khellin, dissolve in 50-mL methanol, complete to volume with methanol, filter, and complete as under ampoules starting with the words "Make the appropriate dilution in methanol...." Calculate the concentration from the regression equation. Results obtained are shown in Table 3.

Emetine hydrochloride: for syrups

Transfer accurately measured 100 mL. syrup, dilute with about 50 mL water, then alkalinize with dilute ammonia and shake with successive portions of ether 3x40 mL. Wash the combined ether extracts with 10 mL water and reject the water. Shake the ether layer with successive portions of 0.1 N sulphuric acid 3x20 mL, mix the acid liquids, and wash with three successive portions each of about 10 mL chloroform then reject the chloroform. Alkalinize the acid liquid with dilute solution of ammonia and shake with 4x20 mL. chloroform.

Filter the chloroform and evaporate under vacuum to dryness. Dissolve the residue in chloroform to obtain a solution containing 2 $mg.mL^{-1}$ and complete as under construction of calibration curves for I, II, and III starting with the words "Transfer accurately aliquot portion...."

RESULTS AND DISCUSSION

The present work is concerned with the development of a simple method for the determination of lobeline, khellin, and emetine hydrochloride in raw materials and in pharmaceutical formulations. The proposed method is based on separation of the studied materials on TLC using methanol-ammonia 25% (100: 1.5 v/v) for I and III and ethyl acetate for II as solvent system. The chromatograms can be scanned densitometry at 285 nm, 250 nm, and at 230 nm for I, II, and III, respectively, see Fig 1.

By applying this technique a linear correlation was obtained between the area under the peak and the corresponding concentration, from which the linear regression equation was found to be:

Table 1

Application of the Spectrodensitometric Method for the Determination of Lobeline I, Khellin II and Emetine Hydrochloride III in Raw Materials

Sample No.	Lobeline I Found %*	Khellin II Found %*	Emetime Hydrochloride III Found %*
1	99.51	100.34	100.22
2	98.62	100.50	99.79
3	99.01	99.96	99.51
4	100.20	100.21	100.02
5	99.35	98.94	98.92
Mean	99.34	99.99	99.69
S.D.	± 0.49	±0.62	±0.50

*Average of five different experiments.

Table 2

Comparison Between the Proposed Method and the Official Method B.P 1988[29] for the Detemination of Lobeline in its Pharmaceutical Preparations

Preparations	Densitometric Method Found % ± S.D	Standard Addition Recovery % ± S.D*	Official Method Found %± S.D.
Lobestra Syrup B.N. D, Hoo6	100.10± 0.89	100.01 ± 0.23	99.77± 0.56
Broncho Syrup B.N. 04113	99.70 ± 0.58	99.89 ± 0.45	99.15 ± 0.62

*Average of five different experiments.

$A = 0.81\ C + 0.86$ $r = 0.9997$ I
$A = 0.02\ C + 0.40$ $r = 0.9995$ II
$A = 0.4\ C + 0.24$ $r = 0.9992$ III

where "A" is the area under the peak and "C" is the corresponding concentration in mg. mL^{-1} and "r" is the correlation coefficent.

Table 3

Comparison Between the Proposed Method and the Official Method E.p 1984[2] for the Determination of Khellin in its Pharmaceutical Preparations

Preparations	Densitometric Method Found % ± S.D	Standard Addition Recovery % ± S.D*	Official Method Found %± S.D.
-Glucolynamine ampules β.N. 295190	99.29 ± 0.21	99.98 ± 0.61	100.58 ± 0.52
-Khellalgine ampoules β.N. 552025	99.23± 0.42	100.20 ± 0.36	100.12 ± 0.19
-Psorvityl Tablets β.N. 491007	100.15 ± 0.60	99.79 ± 0.37	99.51 ± 0.32
-Uricol efferevescent granules β.N. 1610	100.36 ± 0.42	98.91 ± 0.54	99.17 ± 0.22
-Khellalgine Suppositories β.N. 627082	99.59 ± 0.74	100.05 ± 0.25	99.62 ± 0.45

*Averages of five different experiments.

The proposed densitometric method was applied to the determination of I,II and III with the mean percentage recovery 99.43 ± 0.49, 99.99% ± 0.62 and 99.69% ± 0.50 of pure I,II, and III respectively, Table 1.

The proposed method was successfully applied to the determination of I,II, and III in their pharmaceutical formulations and satisfactory results were obtained. The validity of the suggested method was further assessed by applying the standard addition technique. Results obtained are presented in Tables 2, 3, 4.

Table 4

Comparison Between the Proposed Method and the Official Method β.P. 1988[29] for the Determination of Emetine Hydrochloride in its Pharmaceutical Preparations

Preparations	Densitometric Method Found % ± S.D	Standard Addition Recovery % ± S.D*	Official Method Found %± S.D.
-Solvin Syrup B.N. 345123	98.67 ± 0.31	100.04 ± 0.61	99.25 ± 0.67
-Bronchistal Syrup B.N. 550974	99.05 ± 0.24	98.59 ± 0.46	98.26 ± 0.91

*Average of five different experiments.

Table 5

Statistical Comparison Between the Determination of I, II and III by the Proposed Method and the Official Methods

	Lobeline I		Khellin II		Emetine Hydrochloride III	
	Proposed Method	Official Method	Proposed Method	Official Method	Proposed Method	Official Method
Mean %	99.34	99.56	99.99	100.25	99.69	99.63
S.D.	0.49	0.29	0.62	0.67	0.50	0.42
Variance	0.24	0.08	0.38	0.45	0.25	0.18
F. ratio	3.00		1.18		1.39	
t. Test	0.87		0.63		0.21	

N = 5, P = 0.05, F. tabulated = 6.4, t. Tabulated = 2.306.

The results obtained were statistically compared with those of the official methods.[2,29] Table 5 shows that the calculated t and F values are less than the theoretical values indicating that there is no significant difference between the two methods with respect to both precision and accuracy.

The TLC scanning method has the advantage of measuring the absorbance of I,II, and III directly on the plate, hence avoiding loss of materials and giving savings in time and effort. It determines I, II, and III also in their pharmaceutical preparations without interferences of either the excepients or other constituent formulated in their preparation and this indicated by the percentage recovery of added standard I,II, and III to the pharmaceutical preparations.

One of the important advantages of this method is that it requires only a simple and relatively inexpensive apparatus depending on the laboratory facilities available.

Dealing with natural products, pharmaceutical preparations, and their raw materials in the present work, the authors believe that the results obtained with scanning densitometry are sufficentely good to merit replacemant of the tedious, non specific, and less sensitive pharmacopoeial methods.

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