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Validated stability-indicating TLC method for the determination of noscapine

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A sensitive, selective, precise and stability-indicating thin-layer chromatographic (TLC) method was developed and validated for the analysis of noscapine, both as a bulk drug and in its formulation. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of chloroform-methanol (10 : 0.5 v/v). Densitometric analysis of noscapine and its degradation products was carried out in the absorbance mode at 254 nm. This system was found to give compact symmetrical spots for noscapine (R_f value 0.85 ± 0.04). Noscapine was subjected to acid and alkali hydrolysis, oxidation and photo degradation.

The drug undergoes photo degradation and also degrades under acidic and basic conditions. The prepared degradation products were identified and verified through infrared (IR) and mass spectral analyses. The degraded products were also well resolved from the pure drug with significantly different R_f values and they were quantitatively determined. The method was validated for linearity, precision, robustness, limit of detection (LOD), limit of quantitation (LOQ), specificity and accuracy. Linearity was found to be in the 1.0–10.0 μg, 0.4–3.2 μg, 1.0–9.0 μg and 0.5–5.0 μg/band ranges for noscapine, cotarnine, meconine and opionic acid, respectively. The polynomial regression analysis for the calibration plots showed a good polynomial relationship with r^2 of 0.9998, 9989, 9996 and 0.9997 for noscapine and its three degradation products, cotarnine, meconine and opionic acid, respectively. Statistical analysis proves that the method is repeatable and specific for the estimation of noscapine. As this approach could effectively separate the drug from its degradation products it can be employed as a stability-indicating method in Quality Control laboratories.

Keywords: densitometry; noscapine; stability-indicating method; thin layer chromatography (TLC)

Introduction

An ideal stability-indicating method quantifies a drug and resolves its degradation products. Thin-layer chromotography (TLC) has become a routine analytical technique due to the advantage that several samples can be run simultaneously using a small amount of mobile phase, in contrast with high-performance liquid chromotography (HPLC), thus lowering the time and cost per analysis.

Noscapine ((3S)-6,7-Dimethoxy-3-[(5R)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo[4,5-g]isoquinolin-5-yl]isobenzofuran-1(3H)-one), as shown in Figure 1, is an opioid agonist alkaloid present in plants of the papaveraceae family. It was also known as narcotine although it has no significant pain-killing properties.[1] It was grouped with papaverine as a benzylisoquinoline. Noscapine is primarily used for its antitussive effects as it has a sigma receptor agonist activity. Anticancer activity was recently reported.[2] The metabolism of noscapine was studied in rabbits, rats and humans.[3] Three metabolites were isolated and identified: meconine, cotarnine and hydrocotarnine. Meconine and cotarnine are the major metabolites, while hydrocotarnine was excreted to less than 1% of the total metabolite concentration. Meconine and cotarnine are produced from noscapine by treating the drug with dilute nitric acid,[4] as shown in Figure 2. On the other hand, upon treatment of noscapine with dilute sulphuric acid, two degradation products were produced, cotarnine and opionic acid (Figure 3).[5] Several methods were reported for the analysis of noscapine either as a constituent in cough mixtures or along with other related opiate alkaloids by capillary electrophoresis,[6–11] voltammetry[12–13] and flow injection chemiluminesence.[14] High-performance liquid chromatography (HPLC) was also introduced to separate it from related alkaloids[15–20] and to determine it in cough mixture.[21]

In this work, a sensitive and precise stability-indicating TLC densitometric method is proposed for the determination of noscapine in the presence of its degradation products – cotarnine, meconine and opionic acid – after their preparation, isolation, purification and identification. The method was validated according to ICH guidelines.[22]

Noscapine has been determined by TLC, both with its related alkaloids and in an antitussive preparation.[23–27] No article has been reported for the stability-indicating chromatographic determination of noscapine either in bulk powder or in pharmaceutical dosage forms.

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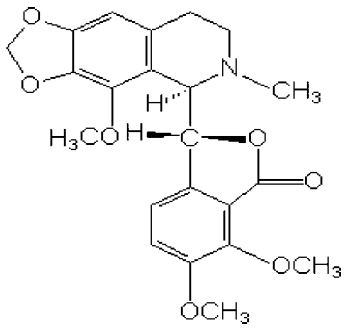

Figure 1. Chemical structure of noscapine.

Experimental

Instruments

- ☐ Camag TLC scanner 3 S/N 130 319 with winCATS software.
- ☐ Camag Linomat 5 autosampler (Switzerland).
- ☐ Camag microsyringe (100 µl).
- ☐ Precoated silica gel aluminium plates 60 F254, ALLUGRAM® SIL G/UV 254 (Machenary-Nagel, Germany), 20 × 20 cm with 0.2 mm thickness.
- ☐ Laboratory-prepared TLC glass plates, prepared by accurately weighing 25 gm of silica gel F_{254} in a glass stoppered conical flask; 50 mL of distilled water was added to the powder, shaken rapidly and poured onto the centre of a clean glass plate. The plates were left overnight and activated in the oven at 120 °C for about one hour.

Chemicals and solvents

Methanol (E. Merck, Darmstadt, Germany), chloroform (Adwic), isopropyl alcohol (E. Merck, Darmstadt, Germany), benzene (Adwic), sulphuric acid, dilute aqueous solutions (Prolabo) and nitric acid, dilute aqueous solution (Prolabo). All chemicals used were of analytical grade and solvents were of spectroscopic grade.

Samples

Pure samples

Noscapine was kindly supplied by the Egyptian International Pharmaceutical Industries Company (EIPI Co.). Its purity is 99.32% according to the manufacturer.

Degradation products of noscapine

Cotarnine, meconine and opionic acid were prepared, isolated, purified and identified.

Pharmaceutical dosage form

The Tusscapine® syrup was manufactured by Delta Pharm S.A.E. Batch numbers 05038 and 05036. It was labelled as containing 15 mg of noscapine per 5 mL of solution (0.3%).

Solutions

Standard solution

Noscapine (1 mg.mL^{-1}) was prepared by accurately weighing 10 mg of noscapine into 10 mL calibrated volumetric flask and the volume was completed with methanol.

Solutions of the degradation products

- ☐ Cotarnine (1 mg.mL^{-1}) was prepared by accurately weighing 10 mg of cotarnine in methanol in a 10-mL calibrated measuring flask.
- ☐ Meconine (1 mg.mL^{-1}) was prepared by accurately weighing 10 mg of meconine in methanol in a 10-mL calibrated measuring flask.
- ☐ Opionic acid (1 mg.mL^{-1}) was prepared by accurately weighing 10 mg of opionic acid in methanol in a 10-mL calibrated measuring flask.

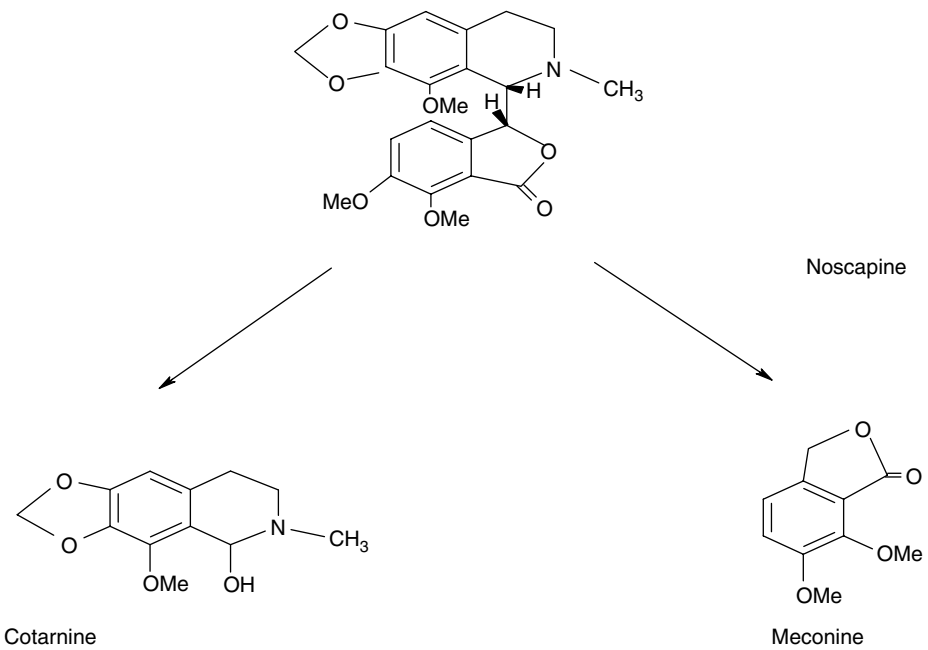

Figure 2. Schematic diagram showing noscapine degradates on treatment with dilute nitric acid.

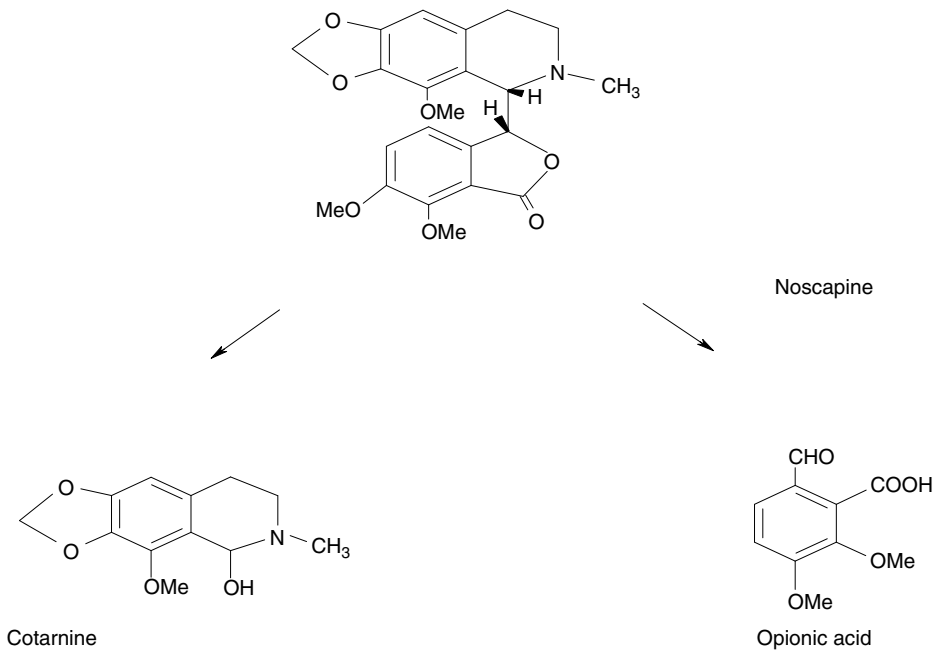

Figure 3. Schematic diagram showing noscapine degradates on treatment with dilute sulphuric acid.

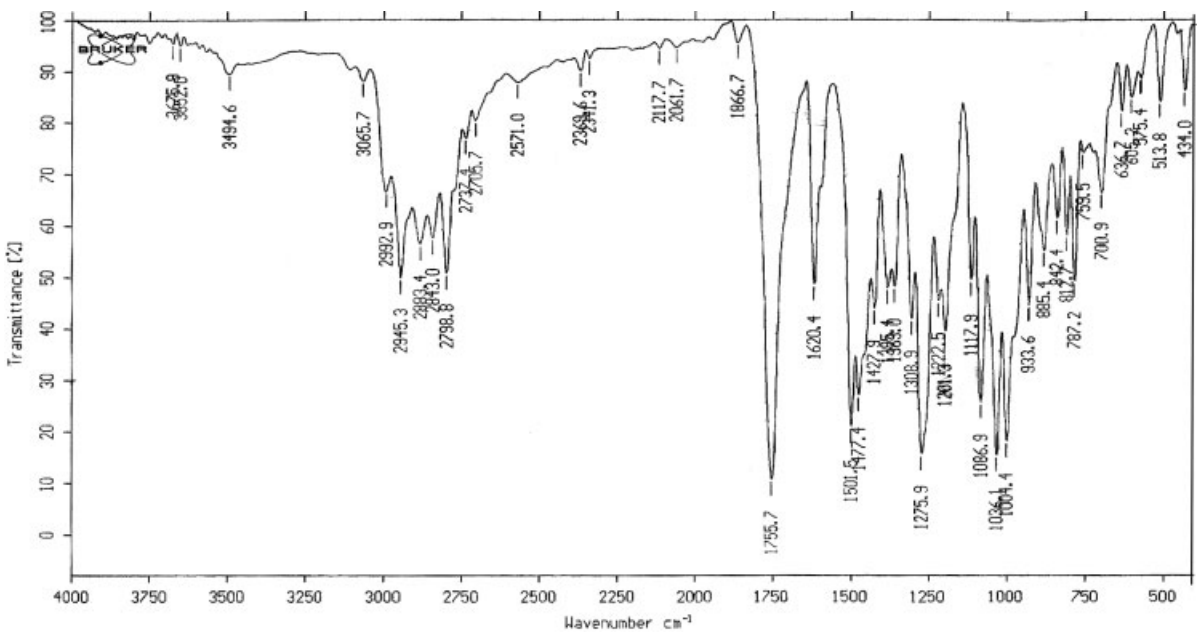

Figure 4. Infrared spectrum of noscapine.

Pharmaceutical sample solutions

Tusscapine® syrup was labelled as containing 15 mg of noscapine per 5 mL. Aliquots of 20 mL were transferred separately into 100 mL measuring flasks. The volumes were completed with methanol.

Preparation of noscapine degradation products

Preparation of cotarnine and meconine

Accurately weighed noscapine (0.5 g) was refluxed with 20 mL of dilute nitric acid for 3 hours. Complete degradation was confirmed by TLC through the disappearance of drug spot using chloroform/methanol 10 : 0.5 v/v.

Preparation of cotarnine and opionic acid

Accurately weighed noscapine (0.5) was refluxed with 20 mL of dilute sulphuric acid for 2 hours. Complete degradation was confirmed by TLC through the disappearance of drug spot using chloroform/methanol 10 : 0.5 v/v.

Separation of the prepared degradation products

The prepared degradate solutions were neutralized with ammonia. The products were then extracted with chloroform several times, as confirmed by TLC. The chloroform extracts were concentrated and applied as bands to the laboratory prepared silica glass plates.

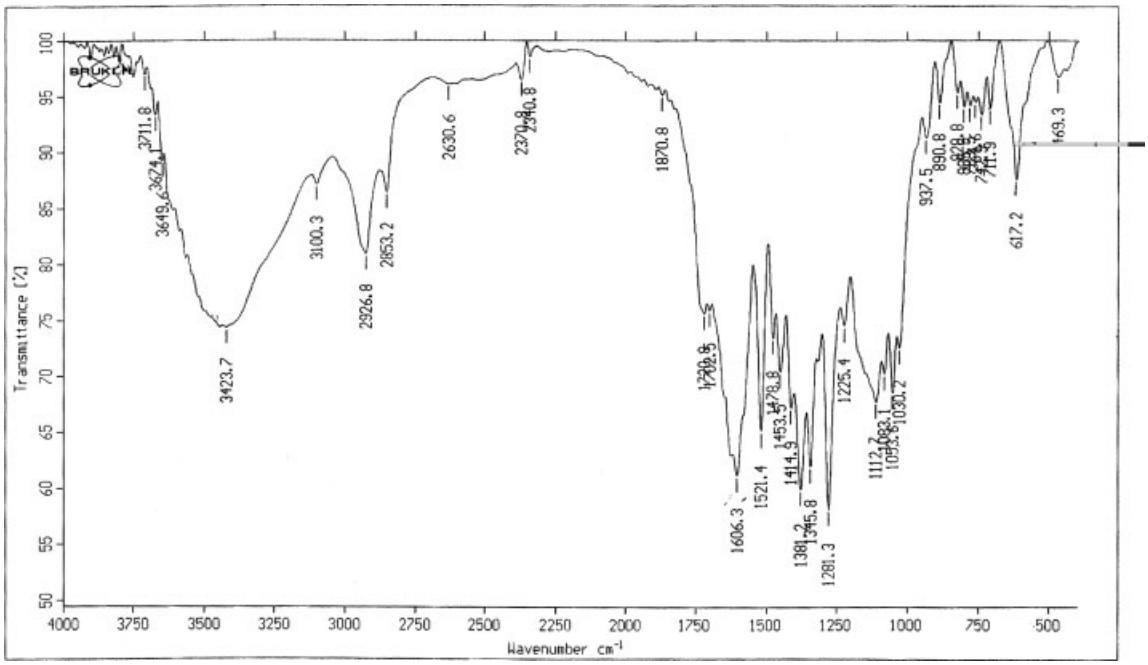

Figure 5. Infrared spectrum of cotarnine.

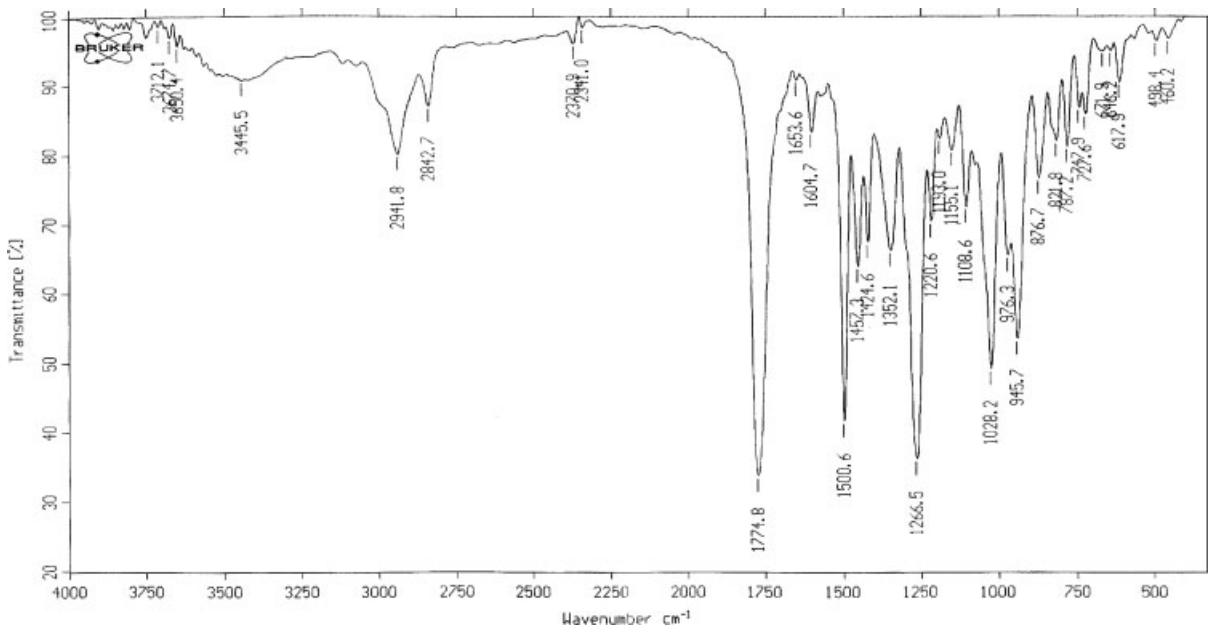

Figure 6. Infrared spectrum of meconine.

The development mobile phase is chloroform: methanol 10 : 0.5 v/v and dried at room temperature. The bands corresponding to each component were scratched, dissolved in methanol. The solutions were stirred, filtered and the solvent was allowed to evaporate. The separated degradation products were subjected to IR and mass spectral analyses for subsequent identification.

TLC densitometry

Chromatographic conditions

The analysis was performed on a precoated silica 20× 10 cm TLC aluminium sheet. The plates were prewashed with methanol and dried at 60 °C for 5 min prior to sample application. Samples were applied in the form of bands. Band length was 4 mm, dosage speed was 150 nl/s and the bands were applied 20 mm apart from each other and 15 mm from the bottom edge.

Linear ascending development was performed in a chromatographic tank previously saturated with chloroform-methanol (10 : 0.5 v/v) for one hour at room temperature. The migration distance was 90 mm from the lower edge. The developed plates were air dried and scanned at 254 nm under the following instrumental conditions:

- source of radiation: deuterium lamp

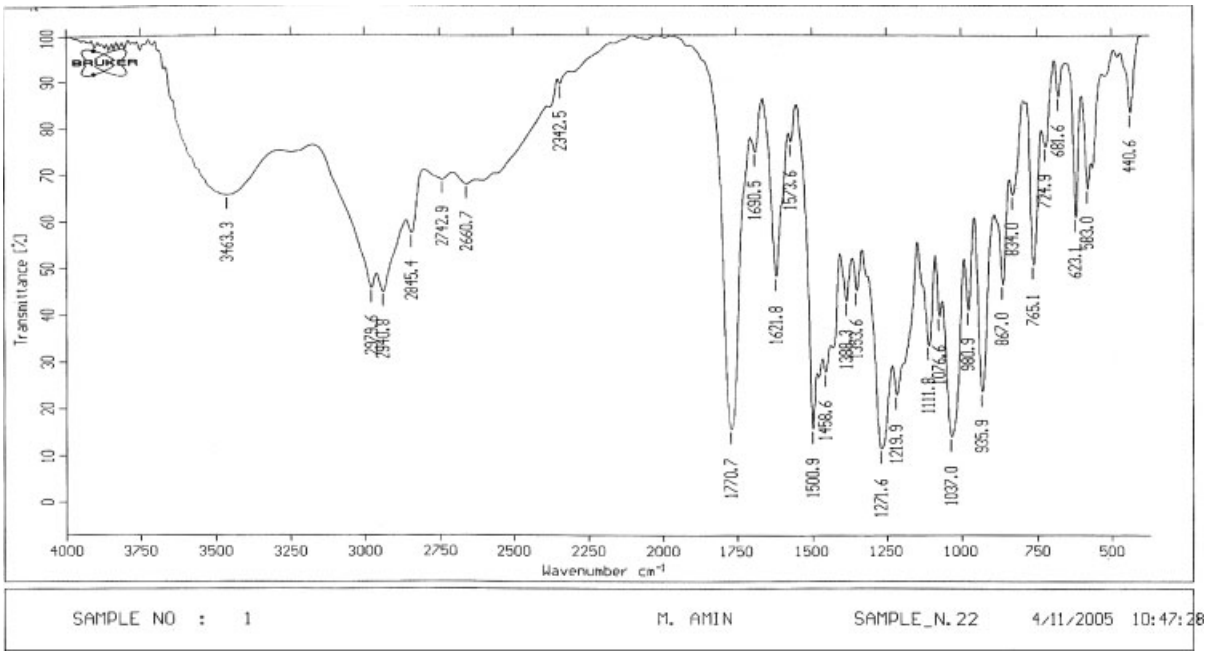

Figure 7. Infrared spectrum of opionic acid.

- ☐ scan mode: absorbance mode
- ☐ slit dimension: 3 mm × 0.45 mm
- ☐ scanning speed: 20 mm/s
- ☐ output: chromatogram and integrated peak area.

Method validation

Linearity

Different aliquots (1–10 µl) of standard solution of noscapine (1mg.mL^{-1}) were applied on the TLC plates. The calibration curve was constructed relating the integrated area under the peak in triplicates to the corresponding concentrations of noscapine as µg/band .

Accuracy

The procedure mentioned above, under 'linearity', was repeated for five different concentrations of standard solution in triplicate. The concentrations were calculated from the calculated regression equation or by comparison with standard applied on the same plate, mean recoveries and RSD% were calculated.

Precision

Intraday repeatability was obtained from the RSD% value by repeating the assay four times in the same day. Intermediate precision (interday precision) was assessed by the assay of samples on different days. The intraday and interday variation for the determination of noscapine was carried out at two concentration levels: 3 µg and 5 µg per band.

Specificity

Specificity was ascertained by analysing samples containing noscapine, cotarnine, meconine and opionic acid. The spot for noscapine in the prepared samples was confirmed by comparing R_f values and spectra of the spot with that of a standard noscapine solution.

Standard addition technique

The specified chromatographic conditions were adopted to the analysis of noscapine in Tusscapine® syrup, the standard addition technique was applied and the concentrations were calculated from the corresponding regression equation or by comparison with standard on the same plate. The percentage recoveries were then calculated.

Robustness

Several factors must be studied to test the robustness of the method as the mobile phase composition, the amount of the mobile phase, the time of the activation of the plates after prewashing with methanol before chromatography and the time for spotting and from chromatography to scanning. Each factor was varied slightly and the effects of the results were examined. The robustness of the method was studied at concentrations of 3 µg and 5 µg per band.

Limit of detection and limit of quantitation

The detection limit (LOD) of an analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value, while the quantitation limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted four times and the chromatographic conditions were adopted.

Detection of related impurities

In order to determine the related impurities a large volume of noscapine standard solution was applied (20 µl) on the TLC plate and the chromatogram was run as described under the chromatographic conditions.

Results and Discussion

Cotarnine and meconine are the main metabolites of noscapine and are produced from the action of dilute nitric acid on the drug, while noscapine is easily cleaved by dilute sulphuric acid or hydrochloric acid with the formation of two degradation products, cotarnine and opionic acid. While treatment of the drug with sodium hydroxide have the same effect of light and hydrogen peroxide on noscapine with the formation of the three degradation products . Cotarnine is considered as both a metabolite and degradation product of noscapine.

Preparative TLC was used for the separation of the prepared degradation products. After complete separation and purification of the three compounds, they were subjected to IR (Figures 4–7) and mass spectral analyses. Interpretable results were obtained. The assignments of cotarnine, meconine and opionic acid were based on comparison of the IR spectral data for the separated compounds with those for the intact drug (Table 1). Mass spectra of cotarnine, meconine and opionic acid are represented in Figures 8–11. The spectrum of cotarnine is characterized by its molecular ion peaks at m/z 235.5 (63.64%) and that of meconine at m/z 193 (70.22%). Opionic acid spectra are characterized by the molecular ion peaks at m/z at 207 (35%) and 399 (62.3%), which we suggest relate to the opionic acid and its anhydride, respectively (Figures 10–11). During the separation of cotarnine and meconine mixtures on the silica plates, it was observed that when the samples were left for a time before development, another spot appears, having the same R_f value as opionic acid. Apparently, meconine in a solid thin film can be easily oxidized by atmospheric oxygen to produce opionic acid (Figure 12). This suggestion was confirmed by subjecting the newly formed compound to IR and Mass spectral analyses which verify that the obtained compound is opionic acid.

The TLC procedure was optimized to develop a stability-indicating method. Different solvent systems were tried to achieve the best separation of the four components – ethyl acetate: methanol: ammonia (17 : 2:1 v/v); methanol: ammonia (10 : 0.15 v/v); chloroform: methanol (9 : 1 v/v)). Upon using the first two

Table 2. Parameters of system suitability of the developed TLC method

	Symmetry factor	Resolution[a] (Rs)	Capacity factor (K)	Selectivity[a] (α)
Noscapine	0.96		0.176	
Cotarnine		9.440	26.00	147.72
meconine		2.857	1.174	6.670
Opionic acid		1.789	0.515	2.926

[a] The parameters were calculated using noscapine as reference.

Table 1. Assignment of the IR characteristic bands of noscapine, cotarnine, meconine and opionic acid

Wavenumber (cm^{-1})				Assignment
Noscapine	Cotarnine	Meconine	Opionic acid	
2900	2900	2900	2900	Aliphatic and aromatic C–H absorptions Carboxylic O–H stretching
–	–	–	3600-2400	Alcoholic O–H stretching
–	3600-3100	–	–	C═O stretching
1755	–	1775	1770	

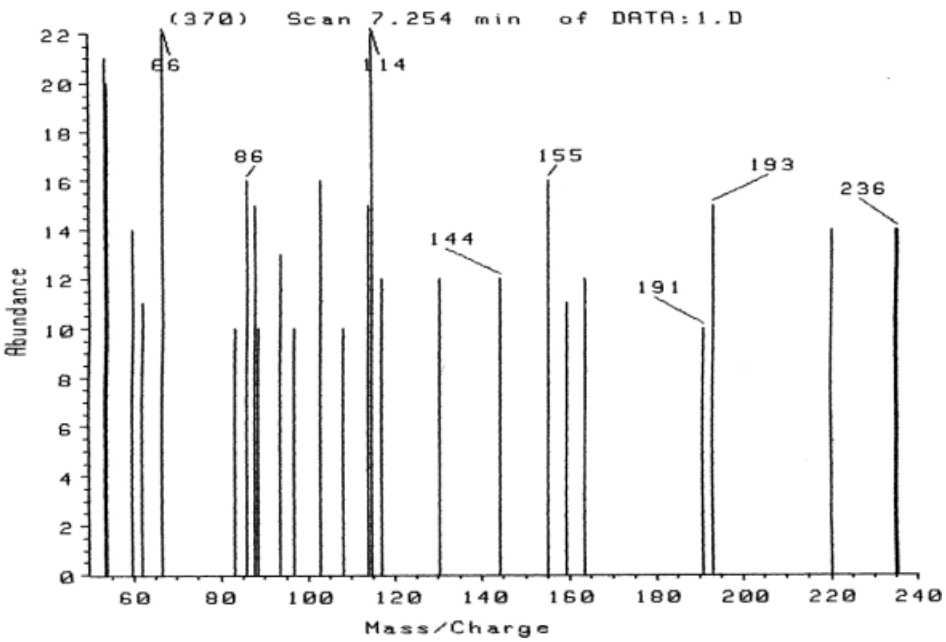

Figure 8. Mass spectrum of cotarnine.

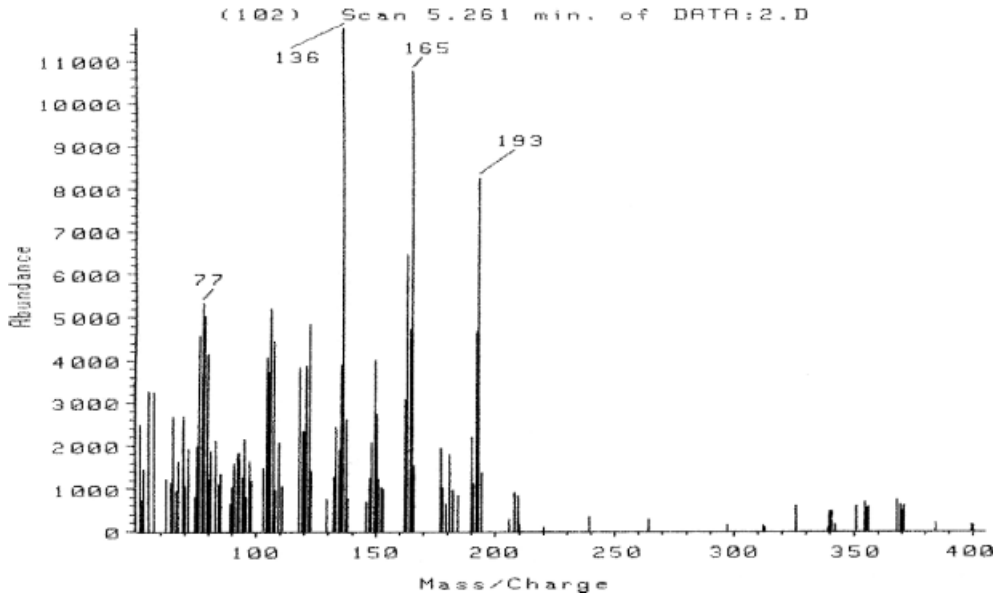

Figure 9. Mass spectrum of meconine.

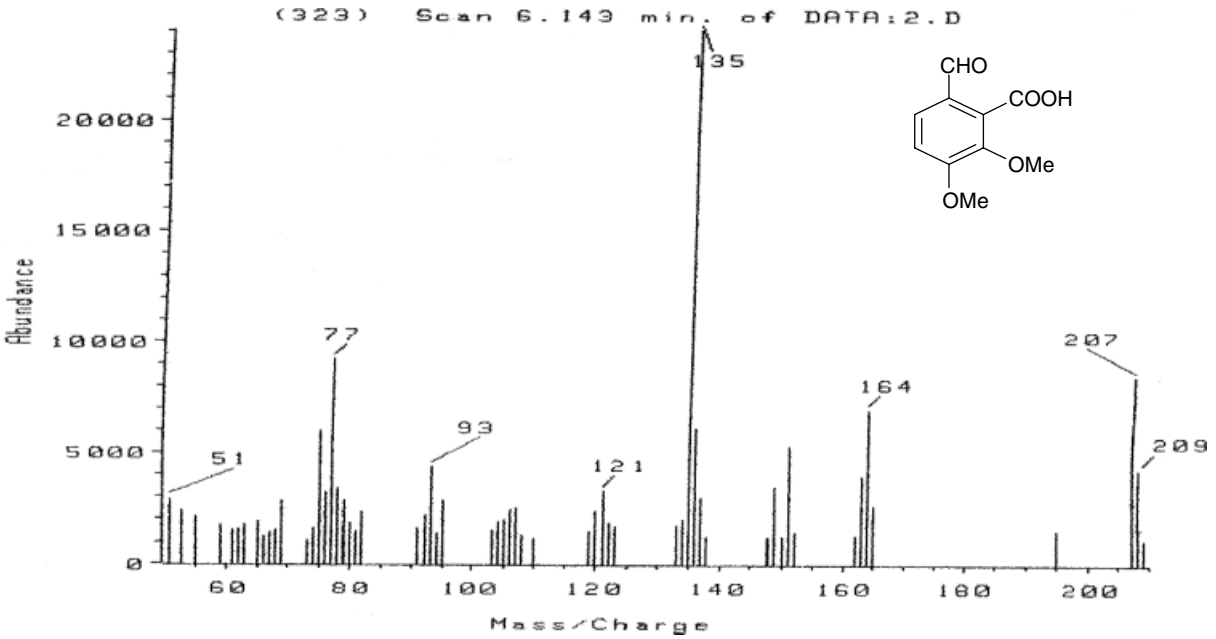

Figure 10. Mass spectrum of opionic acid.

systems, the R_f values were almost the same and the compounds could not be separated from each other. On the other hand, when the third one was used the separation was good but with tailing of the cotarnine spot. A modification was made to prevent tailing of the separated spots. Satisfactory separation was achieved using chloroform: methanol (10 : 0.5v/v). This separation allows for the determination of noscapine at 254 nm without any interference from its degradites, with a sharp and symmetrical peak (Figure 13). Figures 14–15 show the chromatograms of the nitric and sulphuric acid degradates, respectively. The resolved peak of noscapine from its degradation products is also presented (Figure 16).

The system suitability parameters, including resolution (R_s), peak symmetry, capacity factor (k) and selectivity (α), were calculated. The resolution was always above two, the selectivity more than one and an accepted value for symmetry factor was obtained (Table 2).

In order to find if any decomposition occurs during spot development, two-dimensional chromatography using the same solvent system was used. No decomposition was observed in either of the two dimensions, indicating the stability of the resolved drug spot during development.

Quantitative determination of the three degradation products was achieved by applying several volumes of the degradate solutions. The integrated peak areas were subjected to polynomial regression analysis. The three degradates show good a polynomial relationship in the range of 0.4–3.2 µg, 1.0–9.0 µg and 0.5–5.0 µg bands for cotarnine, meconine and opionic acid respectively.

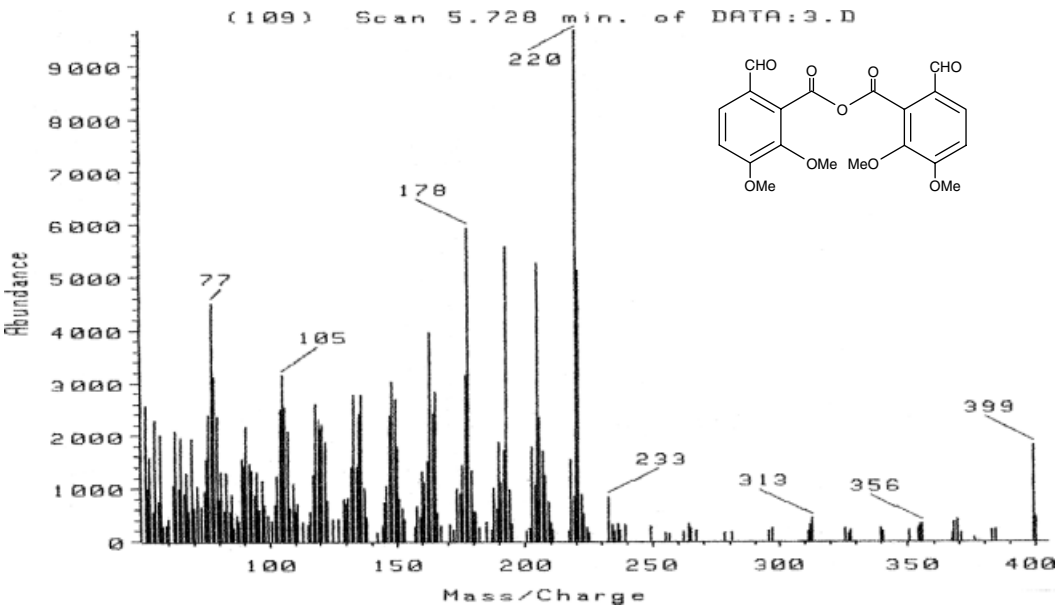

Figure 11. Mass spectrum of opionic acid anhydride.

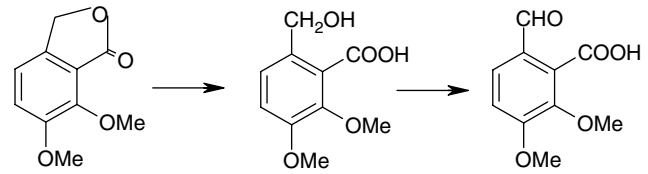

Figure 12. The suggested oxidation of meconine to opionic acid by atmospheric oxygen in the solid state.

Table 3. Summary of the validation parameters of the proposed TLC method

Parameter	Data
Linearity range (µg per band)	1.0–10.0
Coefficient 1[a] ± SE	$-0.063 \times 10^3 \pm 0.035$
Coefficient 2[b] ± SE	$-0.213 \times 10^3 \pm 0.015$
Intercept [c] ± SE	$0.098 \times 103 \pm 0.020$
Correlation Coefficient ± SE	0.9998 ± 0.030
Limit of detection (µg per band)	0.180
Limit of quantitation (µg per band)	0.900
Mean ±RSD %	100.30 ± 0.889
Precision (RSD%)	
Intra-day (n = 4)	0.890
Intermediate precision. Inter-day (n = 4)	1.030
Robustness	robust
Specificity	specific

Following a polynomial regression $A = a x^2 + b x + c$
Where, A is the integrated peak area, x is the concentration of noscapine (µg/band), a and b are coefficients 1 and 2, respectively, c is the intercept.

Validation parameters

The linearity was evaluated by determining the standard solution at ten different levels 1–10 µg per band, integrated peak areas and concentrations was subjected to polynomial regression (Figure 17). The regression equation and correlation coefficient were calculated (Table 3). While the accuracy expressed as percentage recoveries are shown in Table 4, good accuracy of the developed method was indicated by the results obtained.

The proposed method shows good intraday and intermediate precision expressed by the RSD% values in Table 3. Several parameters were used to test the robustness of the proposed method, the standard deviation of peak areas for each parameter was calculated, the low RSD% values shown in Table 5 indicate the robustness of the method. The LOD and the LOQ were found to be 0.18 µg and 0.90 µg per band, respectively. The LOD of the three degradates was calculated and found to be 0.086 µg, 0.348 µg and 0.095 µg for cotarnine, meconine and opionic acid respectively.

The peak purity of noscapine was determined by comparing the spectra at peak start (s), peak apex (m), and peak end (e) positions of the spot, r (s, m) = 0.9988 and r (m, e) = 0.9992. Good correlation was obtained between standard and sample spectra of noscapine (r = 0.9998).

The proposed method was used for estimation of the noscapine concentration in pharmaceutical dosage form and the accuracy was further assessed by applying the standard addition technique, the mean recoveries and RSD% are presented in Table 6.

There was no interference from the excipients commonly present in the syrup as methyl and propyl parabens; they were well separated from noscapine spot, R_f values 0.45. The results were compared to those obtained by applying the HPLC method (Table 7).[28] As shown in Figure 18, two additional spots were observed, I and II at (R_f = 0.05 and 0.39). The R_f value of the first spot exactly matches with that of cotarnine, while the second spot has a slightly different R_f from that of meconine. However, this spot has an absorption spectrum similar to that of meconine. Therefore it might be possible that during processing or storage the drug may have undergone little hydrolysis.

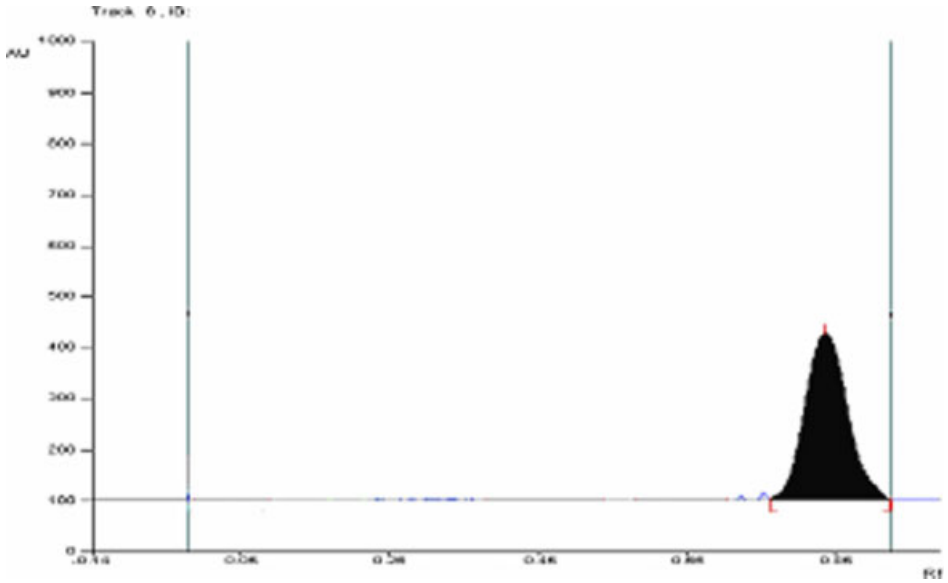

Figure 13. TLC chromatogram of standard noscapine (10 µg/band) (Rf = 0.85 ± 0.04), mobile phase chloroform: methanol (10: 0.5 v/v).

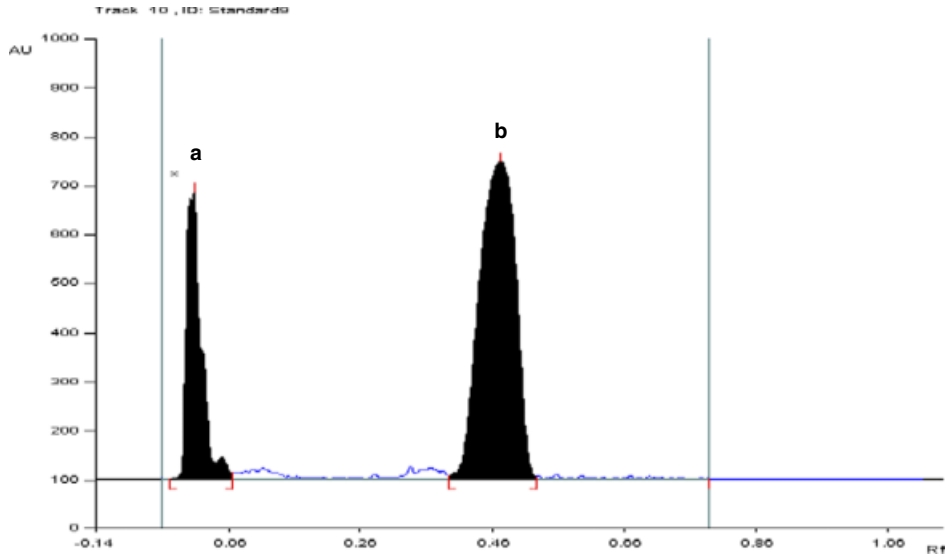

Figure 14. TLC chromatogram of nitric acid induced degradation products: (a) cotarnine (5 µg/band) and (b) meconine (8 µg/band).

Table 4. Accuracy represented as percent recoveries of noscapine by the proposed TLC method

Taken (µg/band)	Found (µg/band)	% Recovery (µg/band)
1.5	1.49	99.33
3.5	3.53	100.86
5.5	5.60	101.82
7.5	7.46	99.47
9.5	9.43	99.26
Mean ± RSD %		100.15 ± 1.139

Table 5. Parameters used to test the robustness of the proposed TLC method

Parameter	SD	RSD%
Mobile phase composition	1.981	1.350
Amount of mobile phase	1.250	0.982
Plate pretreatment	0.982	0.743
Time from spotting to chromatography	0.651	0.530
Time from chromatography to scanning	0.470	0.373

Conclusion

A simple, accurate and precise stability-indicating TLC method was used for the determination of noscapine in the presence of its degradation products. It was applied to the powder, to a pharmaceutical formulation and to mixtures of noscapine and its degradation products. Neither the degradation products nor the excipients interfered with the determination, indicating that the proposed approach could be successfully applied as a stability-indicating method, as well as for the determination of the

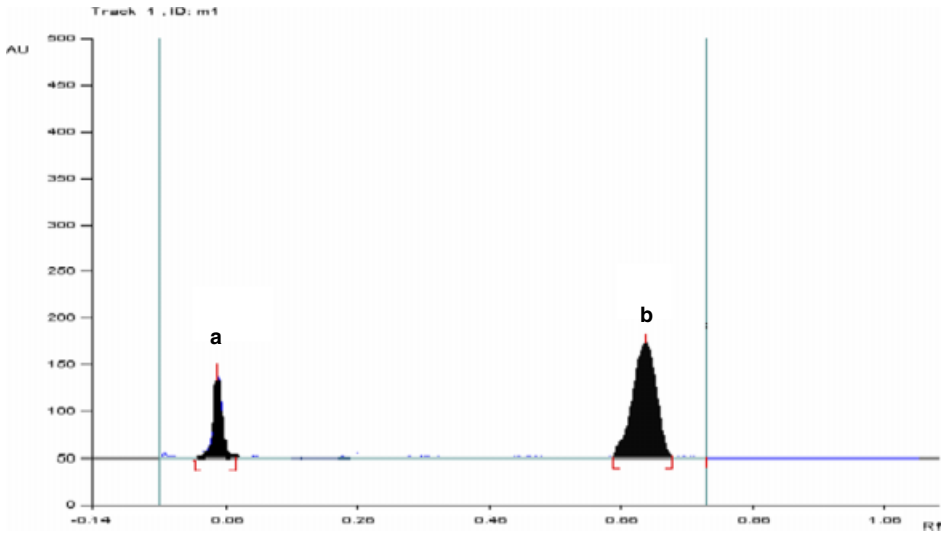

Figure 15. TLC chromatogram of sulphuric acid induced degradation products: (a) cotarnine (3 μg/band) and (b) opionic acid (5 μg/band).

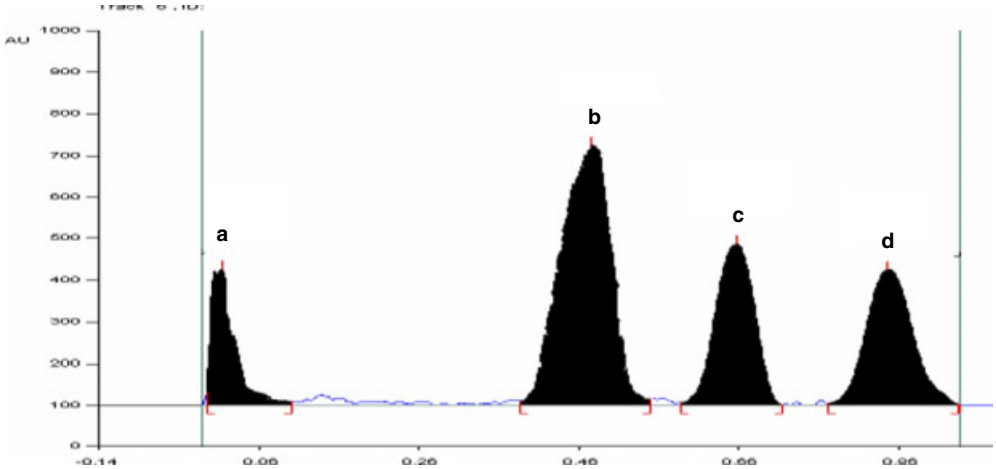

Figure 16. TLC chromatogram of a resolved mixture (a) cotarnine (3.2 μg/band): (b) Meconine (8 μg/band), (c) opionic acid (5 μg/band) and (d) noscapine (10 μg/band).

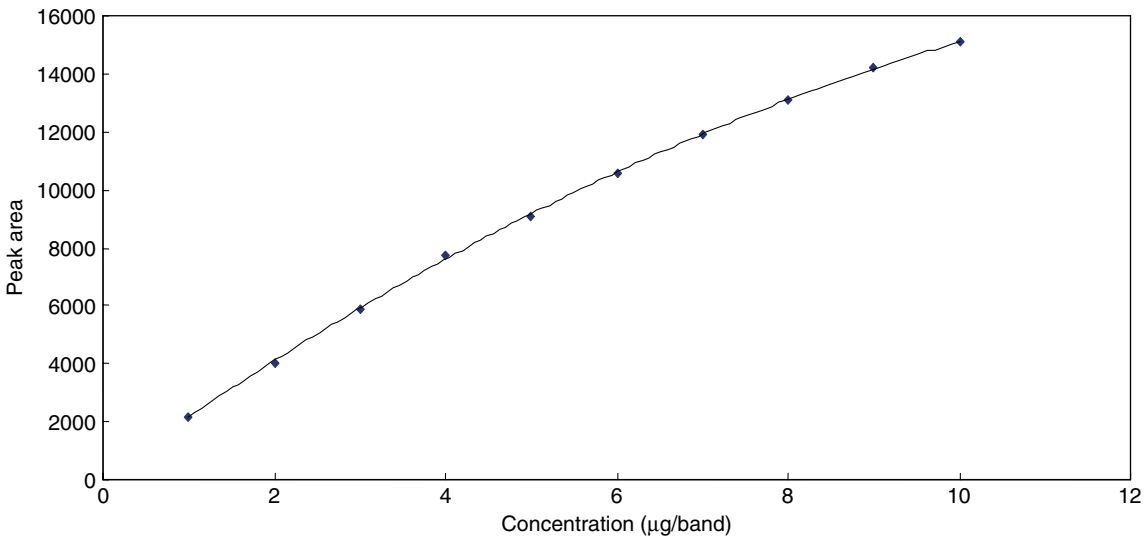

Figure 17. Second order calibration curve of noscapine in the concentration range of 1–10 μg/band.

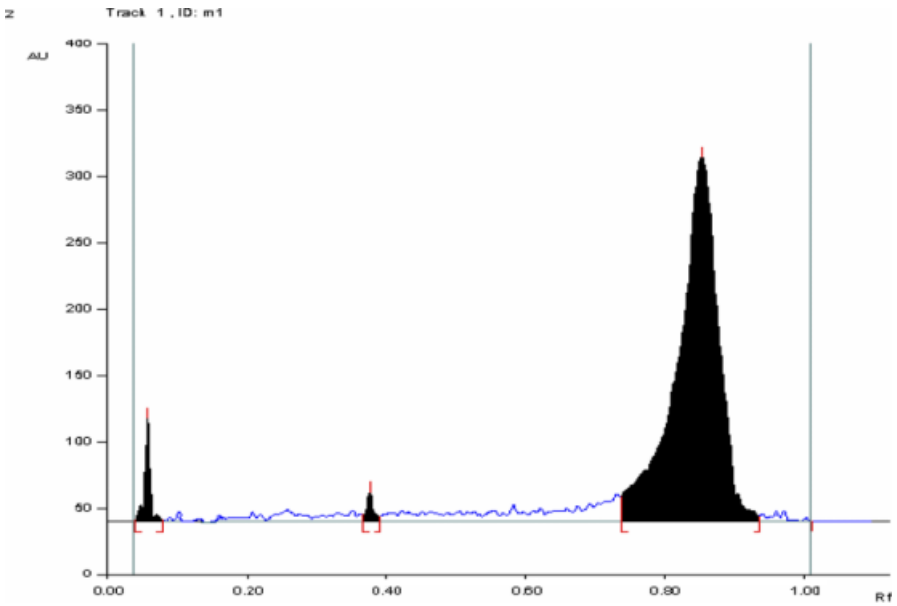

Figure 18. TLC chromatogram of noscapine (20 µ g/band) and its related impurities I and II.

Table 6. Determination of noscapine in Tusscapine® syrup by the proposed TLC method and application of standard addition technique

Tusscapine® syrup (15 mg noscapine per 5 ml)	% Recovery± RSD%	Standard addition technique		
		Amount added (mg)	Amount found (mg)	% Recovery
		72	73.03	101.43
		60	59.75	99.58
Batch no. 05038	100.13 ± 0.682	48	48.91	101.89
			Mean ± RSD%	100.97 ± 1.211
		72	72.74	101.03
		60	59.54	99.23
Batch no. 05036	98.83 ± 0.749	48	47.98	99.96
			Mean ± RSD%	100.07 ± 0.902

Table 7. Results of analysis of Tusscapine® syrup[a] by the proposed TLC method and statistical comparison with HPLC method[28]

	Sample no.	Noscapine	
		HPLC	TLC
Batch no. 05038	1	98.78	99.98
	2	97.90	100.78
	3	98.79	100.34
	4	100.01	99.03
	5	98.99	100.54
	Mean	98.89	100.13
	±SD	0.753	0.683
	RMSEP	0.075	0.046
	Degree of freedom	8	
	t-test (2.306)[b]	0.026	
	F-test (6.380)[b]	1.214	
Batch no. 05036	1	98.98	99.76
	2	99.05	98.78
	3	100.65	100.73
	4	98.09	100.32
	5	99.23	99.54
	Mean	99.20	99.83
	±SD	0.923	0.749
	RMSEP	0.083	0.041
	Degree of freedom	8	
	t-test (2.306)*	0.274	
	F-test (6.380)*	1.519	

HPLC separation using C8 column reversed-phase column using mobile phase of 0.025 M phosphate buffer (pH 4.5) : acetonitrile 60 : 40 (v/v) with flow-rate of 1.0 ml.min^{-1} and the detection wavelength was set at 211 nm.

[a] Tusscapine syrup claimed to contain 15 mg of noscapine per 5 ml syrup.

[b] The values in the parenthesis are the corresponding theoretical values at p = 0.05.

degradation products. The suggested method can be successfully applied for quality control and routine analysis.

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