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First derivative ratio spectrophotometric, HPTLC-densitometric, and HPLC determination of nicergoline in presence of its hydrolysis—induced degradation product

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Abstract

Three methods are presented for the determination of Nicergoline in presence of its hydrolysis-induced degradation product. The first method was based on measurement of the first derivative of ratio spectra amplitude of Nicergoline at 291 nm. The second method was based on separation of Nicergoline from its degradation product followed by densitometric measurement of the spots at 287 nm. The separation was carried out on HPTLC silica gel F_{254} plates, using methanol–ethyl acetate–glacial acetic acid (5:7:3, v/v/v) as mobile phase. The third method was based on high performance liquid chromatographic (HPLC) separation and determination of Nicergoline from its degradation product on a reversed phase, nucloesil C_{18} column using a mobile phase of methanol–water–glacial acetic acid (80:20:0.1, v/v/v) with UV detection at 280 nm. Chlorpromazine hydrochloride was used as internal standard. Laboratory prepared mixtures containing different percentages of the degradation product were analysed by the proposed methods and satisfactory results were obtained. These methods have been successfully applied to the analysis of Nicergoline in Sermion tablets. The validities of these methods were ascertained by applying standard addition technique, the mean percentage recovery $\pm$R.S.D.% was found to be 99.47 ± 0.752, 100.01 ± 0.940, 99.75 ± 0.740 for the first derivative of ratio spectra method, the HPTLC method and the HPLC method, respectively. The proposed methods were statistically compared with the manufacturer's HPLC method of analysis of Nicergoline and no significant difference was found with respect to both precision and accuracy. They have the advantage of being stability indicating. Therefore, they can be used for routine analysis of the drug in quality control laboratories.

Keywords: Nicergoline; Derivative ratio spectrophotometry; HPTLC; HPLC method; Vasodilator; Basic or acidic degradation

1. Introduction

Nicergoline is a semi-synthetic ergot derivative that is used as cerebral and peripheral vasodilator

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[1]. It is a yellowish–white crystalline powder with melting point 136–138 °C. It is practically insoluble in water, soluble in ethanol and chloroform, slightly soluble in ether [2].

Nicergoline is extensively metabolized in the body mainly by hydrolysis and N^1 demethylation to give 10α–methoxy-1,6-dimethylergolin-8-yl-methanol and 10α-methoxy-6-methylergolin-8-yl-methanol, which are excreted in the urine as free and conjugated metabolites [2].

It was reported that, Nicergoline in solid state was stable to heat, moisture, and light except, for a faint coloration. In aqueous solution it was sensitive to alkali, forming degradation products, but it was comparatively stable at pH 4–8 [3].

Different colorimetric [4,5] methods were reported for the analysis of Nicergoline in pure form and pharmaceutical formulations using different reagents including tropeolin and *p*-dimethylaminobenzaldehyde. Also a spectrophotometric method for the identification of Nicergoline was reported [6].

Nicergoline was determined by differential pulse polarography [7,8] in its pharmaceutical dosage form. Also it was determined titrimetrically [9], by capillary zone electrophoresis [10] and by mass spectrometry [11].

Several radioimmunoassays were developed for the determination of Nicergoline and its metabolites in plasma and biological fluids [12–15].

Different chromatographic techniques were applied for the detection and determination of Nicergoline. These techniques include TLC [16–23] and HPLC [24–33] methods.

The International Conference of Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires that, stress testing be carried out to elucidate the inherent stability characteristics of the active substance [34]. It suggests that the degradation products that are formed under a variety of conditions should be identified and degradation pathways established. Susceptibility to hydrolysis is one of the required tests.

Being an ester, Nicergoline is expected to degradate in bad storage conditions through the breakage of the ester linkage. Since no method has been reported in the literature for the determination of Nicergoline, in presence of its hydrolysis-induced degradation products in the pharmaceutical dosage form (in vitro). Therefore, it was thought necessary to develop stability indicating methods for the analysis of Nicergoline in raw materials and in pharmaceutical dosage form.

In the present work, study of the acidic and alkaline hydrolysis of Nicergoline has been made. The hydrolysis products have been isolated and their structures have been elucidated by IR and NMR spectrometry techniques. This was followed by the development of three stability indicating methods for determining Nicergoline, in the presence of its hydrolysis-induced degradation product using first-derivative ratio spectrophotometry, HPTLC-densitometry and HPLC. The three proposed methods were used for the determination of Nicergoline in pharmaceutical dosage form (tablets) without any interference from the excipients normally used in tablet formulations.

2. Experimental

2.1. Samples

2.1.1. Pure nicergoline

Provided by Chemical Industries Development (CID), Giza–Egypt. Under license of Farmitalia Carlo Erba, Erbamont Group, Milano, Italy. The purity of the drug was certified and analyzed to be 99.88% according to the manufacturer's method [33].

2.1.2. Market samples

Sermion tablets produced by Chemical Industries Development (CID), Giza, Egypt. Under license of Farmitalia Carlo Erba, Erbamont Group, Milano, Italy. Batch No 598101, 600101, 706301, each tablet claimed to contain 10 mg Nicergoline.

2.2. Standard preparations

2.2.1. Nicergoline standard solutions

(i) For first derivative ratio method (^{1}DD): standard solutions of Nicergoline in the con-

centration range 10–70 μg ml^{-1} were prepared in ethanol

(ii) For HPTLC-densitometric method: standard solutions of Nicergoline in the concentration range 5–45 μg ml^{-1} were prepared in ethanol

(iii) For HPLC method: standard solutions of Nicergoline in the concentration range 5–80 μg ml^{-1} were prepared in mobile phase and mixed with 300 μg ml^{-1} of chlorpromazine.

2.2.2. Preparation of degradation product

Accurately weighed 500 mg of Nicergoline (I) were dissolved and were dissolved in 20 ml ethanol then 100 ml 1 N NaOH was added. The solution was refluxed on a heating mantle at 90 °C for 5 h then cooled. A white precipitate was formed in the solution, which was filtered. Part of this precipitate was dissolved in ethanol, spotted on a HPTLC plate and developed using methanol–ethyl acetate–glacial acetic acid (5:7:3, v/v/v). One spot other than that of Nicergoline was observed ($R_f = 0.286$). This precipitate was the degradation product of Nicergoline (II), it was washed with water, dissolved in ethanol and recrystalized.

A small portion of the refluxed solution, remaining was diluted with ethanol spotted on a HPTLC plate and developed using the previously mentioned solvent system. Another spot other than that of Nicergoline and Degradate (II) was observed. To separate this degradation product, 1 N HCl was added to the refluxed solution remaining till about pH 5 where, a white precipitate was formed which was filtered, washed with water and dried. This precipitate was found to be sparingly soluble in NaOH, insoluble in ethanol, chloroform and ether. This precipitate was the other degradation product of Nicergoline (III).

The above procedure can be done using 1 N HCl instead of 1 N NaOH in hydrolysis.

The stock solution of (II) was prepared by dissolving 25 mg of (II) in 25 ml ethanol (for the ^{1}DD method and HPTLC method) or methanol (for the HPLC methods).

Standard solutions of (II) were prepared from the above stock solution in the concentration range 5–40 μg ml^{-1} in ethanol for the ^{1}DD method.

2.2.3. Laboratory prepared mixtures of (I) and (II)

Several mixtures of (I) and (II) were prepared so that the degradation product (II) in these mixtures would be in the concentration range of 10–90% of Nicergoline (I) in ethanol for ^{1}DD method and HPTLC method and in mobile phase together with 300 μg ml^{-1} of chlorpromazine hydrochloride in HPLC method.

2.2.4. Sermion tablets standard solutions

Twenty weighed tablets were ground together of each of the selected batches of Sermion. A portion of the powder equivalent to 100 mg of (I) was weighed accurately, dissolved in and diluted to 100 ml with ethanol (for the ^{1}DD method, HPTLC method) or methanol (for the HPLC method). The sample solution was then filtered. From this stock solution different standard solutions were prepared in the concentration ranges as described under preparation of nicergoline standard solutions.

2.3. C- Reagents

1. Deionized water for HPLC was prepared by double glass distillation and filtration through a 0.45-um membrane filter.
2. The methanol used was HPLC grade (E. Merk, W. Germany).
3. Ethanol, ethyl acetate, methanol and acetic acid were of analytical grade.

2.4. Apparatus

1. A double-beam Shimadzu (Japan) 1601PC UV–visible spectrophotometer connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}.The absorbance spectra of test and ref-

erence solutions were recorded in 1 cm quartz cells over the range 200–400 nm.

2. A Shimadzu dual wavelength flying spot densitometer Model CS-9301 was used. The experimental conditions of the measurements were: wavelength, 287nm; photomode, reflection; scan mode, zigzag; swing width, 16. The HPTLC plates used were 10×10 cm, glass plates pre-coated with silica gel F_{254}, purchased from Macherey Nagel (Germany).The samples were applied to the plates using 25 μl Hamilton microsyringe.
3. The HPLC (shimadzu Japan) instrument was equipped with an isocratic Pump model LC-10 AS, Rheodyne 7161 injector with a 20 μl loop and a UV–variable wavelength detector model SPD-10A, separation and quantitation were made on a 250×4.6 mm nucleosil C_{18} RP column (10 μm particle size). The detector was set at $\lambda = 280$ nm. Data acquisition was performed on a model C-R7A integrator (Shimadzu, Japan)
4. The IR spectra were recorded as potassium bromide disk on Shimadzu IR 435 spectrophotometer and expressed in cm^{-1}.
5. The proton NMR spectra were carried out on Varian Gemini 200 MHz spectrometer and chemical shifts were expressed in δ (ppm).

3. Procedures

3.1. The first derivative ratio method

The absorption spectra of standard solutions of Nicergoline (I) and the degradation product (II), were recorded against a reagent blank of ethanol between 200 and 400 nm and were stored in the computer. The stored spectra of Nicergoline (I) were divided by the stored spectrum of the degradation product (II) of concentration 15 μg ml^{-1} and the first derivative of the ratio spectra were obtained with $\delta\lambda = 4$ nm and scaling factor of ten. The content of Nicergoline (I) was calculated from the measurement at 291 nm. The same procedure was used to determine the content of (I) in the laboratory prepared mixtures and in the different batches of sermion tablets.

3.2. The HPTLC-densitometric method

Ten microlitres of each standard solution of (I) were applied to the HPTLC plates as compact spots 10 mm apart and 10 mm from the bottom of the plate using 25 μl Hamilton microsyringe. The HPTLC plates were developed in methanol–ethyl acetate–glacial acetic acid (5:7:3, v/v/v) as mobile phase. The plates were developed by ascending chromatography over a distance of 7–8 cm. The chromatographic tank was saturated with mobile phase in the usual mode. After development the plates were dried in air, scanned for Nicergoline at 287 nm as described under the instrumental parameters and the peak areas were measured. The same procedure was used to determine the content of (I) in the laboratory prepared mixtures and in the different batches of Sermion tablets.

3.3. The HPLC method

The mobile phase of HPLC was prepared by mixing methanol–water–glacial acetic acid in the ratio (80:20:0.1, v/v/v). After that it was filtered using a 0.45 μm membrane filter (Millipore, Milford, MA, USA) and degassed by ultrasonic vibrations prior to use.

Twenty microlitres injections, were made for each concentration of the standard solutions of (I) at a flow rate of 1.3 ml min^{-1} and the peak areas ratios were calculated. The samples were also filtered using 0.45 μm disposable filters and all determinations were performed at ambient temperature. The same procedure was used to determine the content of (I) in the laboratory prepared mixtures and in the different batches of Sermion tablets.

3.4. The reference method [33]

Nicergoline was determined by HPLC method, using nucleosil C_8 column and a mobile phase of acetonitrile: solution pH 6 (1.74 gm of potassium phosphate dibasic anhydrous, dissolve and dilute to a volume of 900 ml with deionized water, add 1 ml triethylamine and adjust to a pH of 6 with 85% phosphoric acid) in a ratio of (58:42 v/v).

The flow rate was 1.6 ml min^{-1} and the detection was at 280 nm

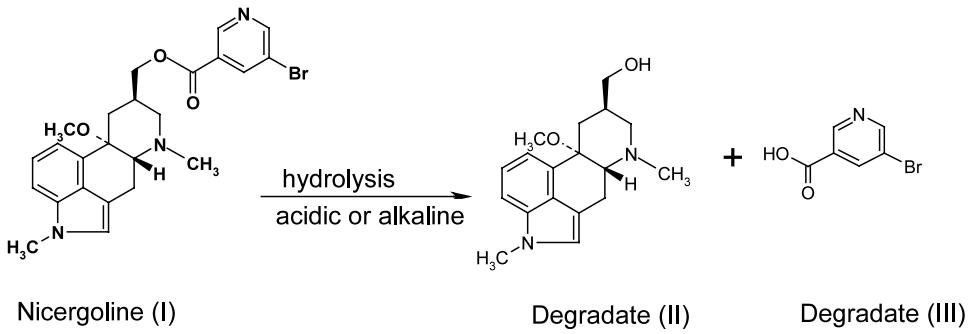
4. Results and discussion

4.1. Identification of the degradation products

Nicergoline (I) readily hydrolyses in acidic or alkaline medium to give 10α-methoxy-1,6-dimethylergolin-8-ylmethanol (degradation product II) and bromonicotinic acid (degradation product III) as shown in Scheme 1. Different concentrations of NaOH and HCl were tried and it was found that 0.1 N solutions of both were capable to produce hydrolysis but 1 N solutions were used for the preparation of the degradation products to ensure complete hydrolysis in a short time.

The structures of the degradation products produced (II, III) were confirmed by IR and NMR spectrometry. Also the melting points of the two degradates were tested and found to be 221–223 °C for Degradate (II) and 190–192 °C for Degradate (III).

Degradate (III) is sparingly soluble in alkaline solutions as NaOH showing poor absorbance at 218 nm and also its insolubility in alcohol, ether and chloroform accounted for its exclusion from the preparations of the stability indicating assays



Scheme 1. Hydrolysis of Nicergoline.

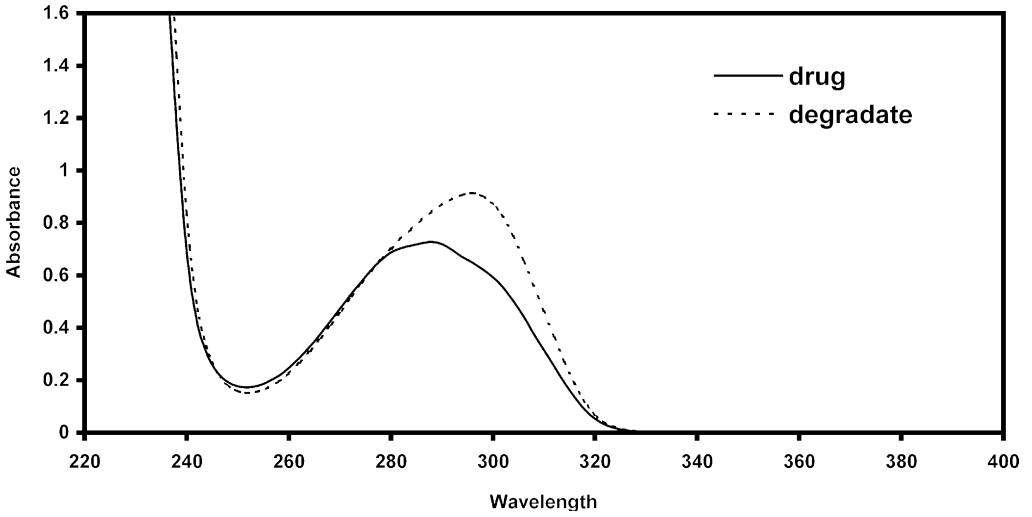


Fig. 1. Absorption spectra of Nicergoline and its hydrolytic degradate (40 μg ml^{-1}each).

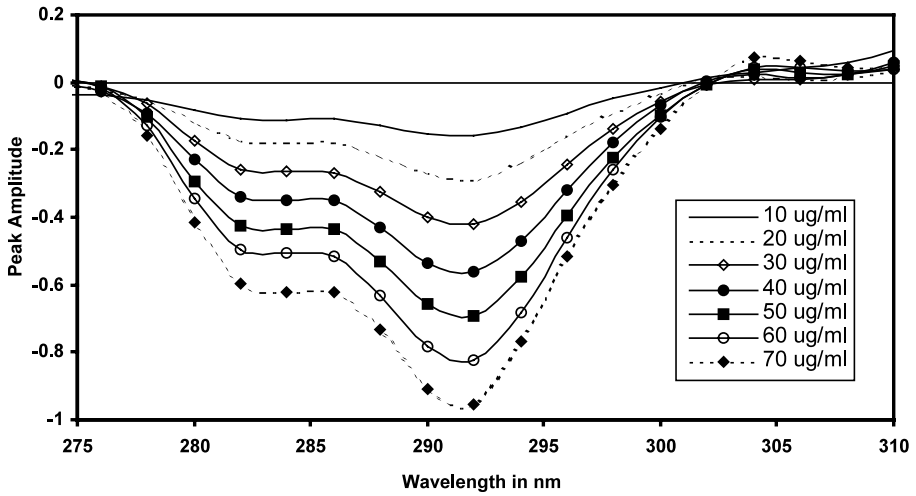


Fig. 2. First-derivative of the ratio spectra of Nicergoline (10–70 μg ml^{-1}) Using 15 μg ml^{-1} of its hydrolytic degradate as a divisor.

4.2. Assay parameters

4.2.1. The first derivative ratio method

Zero-order absorption spectra of Nicergoline (I) and its Degradate (II) show severe overlapping (Fig. 1) so that no direct measurement of any of the two compounds in presence of the other is possible. Thus, Nicergoline was quantified in the presence of Degradate (II) by first derivative of ratio-spectra. This method resulted in suitable regression equation for the drug.

An accurate choice of standard divisor and working wavelength are of capital importance for several reasons [35–38] hence, the method was tested with various divisor concentrations. In measurements the spectrum of 15 μg ml^{-1} of Nicergoline Degradate (II) was used as a standard divisor for the determination of Nicergoline. This assured the best compromise in terms of sensitivity, repeatability and signal to noise ratio.

A linear relationship was found between the peak amplitudes and the concentration of Nicergoline at 291 nm in the range of 10–70 μg ml^{-1} (Fig. 2) from which the linear regression equation was calculated to be:

$$^{1}DD = 0.013321C + 0.02443 \quad (r = 0.9999)$$

where, ^{1}DD is the peak amplitude of the first derivative of ratio spectra at 291 nm, C is the corresponding concentration in μg ml^{-1} and r is the correlation coefficient.

By this equation it was possible to determine Nicergoline with mean recovery percentage of 99.97 ± 0.596.

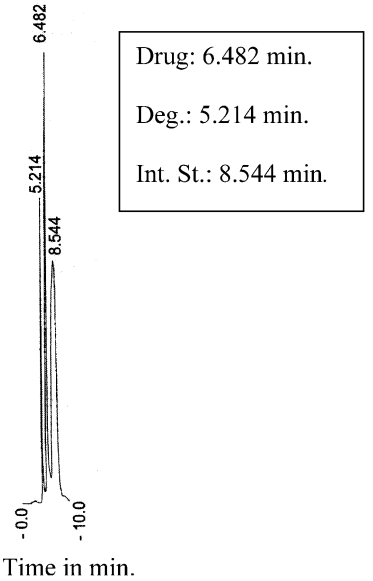


Fig. 3. Chromatogram of 20 μl injection of Nicergoline, its hydrolytic degradate (I) and chlorpromazine hydrochloride as internal standard.

Table 1
Determination of laboratory prepared mixtures of Nicergoline by the proposed methods (^{1}DD, HPTLC and HPLC)

% Degradation	^{1}DD			Densitometry			HPLC		
	Concentration (μg ml^{-1})			Concentration (μg ml^{-1})			Concentration (μg ml^{-1})		
	Intact drug	Degradate	Intact drug recovery %[a]	Intact drug	Degradate	Intact drug recovery %[a]	Intact drug	Degradate	Intact drug recovery %[a]
10	45	5	100.51	36	4	99.94	72	8	99.84
20	40	10	99.38	32	8	100.63	64	16	100.81
30	35	15	99.63	28	12	101.18	56	24	101.43
40	30	20	100.50	24	16	100.54	48	32	100.62
50	25	25	101.36	20	20	99.90	40	40	99.68
60	20	30	100.45	16	24	99.81	32	48	98.21
70	15	35	120.93[b]	12	28	98.75	24	56	99.76
80	10	40	143.90[b]	8	32	101.25	16	64	98.32
90	10	90	138.60[b]	5	45	99.60	8	72	99.63
70	12	28	125.84[b]						
70	21	49	140.22[b]						
Mean ± R.S.D.			100.30 0.710			100.18 0.799			99.81 1.069

[a] Average of five different determinations.
[b] These values were rejected.

Table 2
Quantitative determination of Nicergoline in pharmaceutical formulation by the proposed methods

Sermion tablets	Mean ± R.S.D.%[a]		
	1DD	Densitometry	HPLC
B.N. 598101	99.89 ± 0.670	100.22 ± 0.854	99.97 ± 0.910
B.N. 600101	99.74 ± 0.458	99.55 ± 0.797	99.73 ± 0.891
B.N. 706301	100.16 ± 0.691	100.42 ± 0.780	99.95 ± 0.638

B.N., Batch number.
[a] Average of five different determinations.

4.3. The HPTLC-densitometric method

Instrumental planar chromatography, with precise sample application and computer-controlled evaluation and quantification of the developed chromatograms, has been considered as reliable tool for purity control and quantitative drug testing [39]. Experimental conditions, such as mobile phase, scan mode and wavelength of detection, were optimized to provide accurate, precise and reproducible results for determination of (I) in the presence of (II). The chosen scan mode was the zigzag mode and the wavelength of scanning was chosen to be 287 nm. The greatest differences between the R_f values of the two compounds (0.464 and 0.286 for (I) and (II), respectively), were obtained by the system containing ethyl acetate: methanol: acetic acid mixture in the ratio of 7: 5: 3 v/v/v.

By the use of HPTLC plates better resolution was obtained with a migration distance of about 50% when compared with the regular TLC plates, this is due to the smaller grain size, thinner coating than conventional layers and thus less sample was used to obtain the same results. Also these plates show improved optical properties, which allows better accuracy during densitometric evaluation [40]. For all these advantages in spite of high cost, the quantitative evaluation of the drug with its degradate was performed on HPTLC silica gel plates (10 × 10 cm).

A linear correlation was obtained between the peak area and concentrations of Nicergoline in the range of 5–45 μg ml^{-1} from which the linear regression equation was calculated to be:

$$A = 0.3316C + 0.1263 \quad (r = 0.9999)$$

where, A is the peak area of Nicergoline, C is the corresponding concentration in μg ml^{-1} and r is the correlation coefficient.

By this equation, it was possible to determine Nicergoline with mean recovery percentage of 99.94 ± 0.895.

4.4. The HPLC method

Nicergoline was determined by different HPLC methods either alone or with its metabolites in the body as mentioned before in the literature review. The aim of this work was to develop a simple and stability indicating isocratic HPLC assay for the analysis of Nicergoline in the presence of its hydrolyzed degradation product either in bulk powder or the pharmaceutical preparation.

Several trials have been carried out to obtain a good separation between Nicergoline and Nicergoline Degradate (II). These trials involved the use of different mobile phases with different flow rates

Table 3
Results of application of standard addition technique to the determination of nicergoline by the proposed methods

Method	Amount taken (μg ml^{-1})[a]	Authentic added (μg ml^{-1})	Recovery mean ± R.S.D.%[b]
1DD	20	10–50	99.47 ± 0.752
Densitometry	10	10–30	100.01 ± 0.940
HPLC	20	5–60	99.75 ± 0.740

[a] Sermion tablets batch number 598101.
[b] Average of five different determinations.

Table 4
Statistical comparison between the results of the proposed methods and the manufacturer's HPLC method

	[1]DD	HPTLC	HPLC	Manufacturer's method (HPLC)
Pure sample				
Mean ± S.D.	99.97 ± 0.595	99.94 ± 0.895	99.71 ± 0.793	99.88 ± 0.607
n	7	9	5	7
Variance	0.355	0.801	0.628	0.368
Student's *t*-test	0.280 (1.782)	0.152 (1.761)	0.579 (1.812)	
F	1.041 (4.280)	2.174 (4.150)	1.707 (4.530)	99.98 ± 0.446
n	7	9	5	7
Variance	0.448	0.732	0.827	0.199
Student's *t*-test	0.296 (1.782)	0.671 (1.761)	0.030 (1.812)	
F	2.257 (4.280)	3.684 (4.150)	4.163 (4.530)	
Batch No. 598101				
Mean ± S.D.	99.89 ± 0.670	100.22 ± 0.856	99.97 ± 0.910	99.98 ± 0.446
n	7	9	5	7
Variance	0.448	0.732	0.827	0.199
Student's *t*-test	0.296 (1.782)	0.671 (1.761)	0.030 (1.812)	
F	2.257 (4.280)	3.684 (4.150)	4.163 (4.530)	
Batch No. 600101				
Mean ± S.D.	99.74 ± 0.457	99.55 ± 0.793	99.73 ± 0.889	99.52 ± 0.545
n	7	9	5	7
Variance	0.209	0.629	0.790	0.297
Student's *t*-test	0.818 (1.782)	0.085 (1.761)	0.638 (1.812)	
F	1.422 (4.280)	2.117 (4.150)	2.661 (4.530)	
Batch No. 706301				
Mean ± S.D.	100.16 ± 0.692	100.42 ± 0.783	99.95 ± 0.638	100.12 ± 0.579
n	7	9	5	7
Variance	0.479	0.613	0.407	0.336
Student's *t*-test	0.117 (1.782)	0.847 (1.761)	0.720 (1.812)	
F	1.428 (4.280)	1.829 (4.150)	1.214 (4.530)	

N.B: Figures between parenthesis are the corresponding tabulated values ($P = 0.05$).

and ratios. The best peak shape and separation was obtained upon using methanol: water: acetic acid in the ratio of 80:20:0.1 (v/v/v) and a flow rate of 1.3 ml min^{-1}. The retention time for Nicergoline was found to be 6.5 ± 0.2 min and Nicergoline Degradate (II) at 5.2 ± 0.2 min, thus indicating complete separation between both compounds as shown in Fig. 3.

The calibration curve representing the relation between the concentration of Nicergoline versus the peak area ratio was constructed. Results show linear relationship in the range of 5–80 μg ml^{-1} from which the linear regression equation was calculated

$$A = 0.01021C + 0.02232 \quad (r = 0.9999)$$

where, A is the peak area ratio of Nicergoline, C is the corresponding concentration in μg ml^{-1} and r is the correlation coefficient.

By this equation, it was possible to determine Nicergoline with mean recovery percentage of 99.71 ± 0.795.

4.5. Accuracy and precision of the proposed methods

To assess accuracy and precision of the proposed methods, a series of laboratory prepared mixtures were assayed by the proposed procedures. The results contributed to the good performance of the first derivative ratio method up to 60% presence of Nicergoline Degradate (II) with a mean recovery percentage ± relative standard deviation of 100.30 ± 0.710. Above this concentration of Degradate (II) inaccurate results for the determination of Nicergoline were obtained.(Table 1)

For the HPTLC method and the HPLC

method the results of the analysis were not affected by the presence of up to 90% degradate(II),(as shown in Table 1). The mean percentage recovery of Nicergoline (I) was found to be 100.18 ± 0.799 for the HPTLC method and 99.81 ± 1.069 for the HPLC method.

The detection limits of (I) in the three proposed methods were found to be 1.49, 1.07and 0.96 μg ml^{-1} for ^{1}DD, HPTLC and HPLC methods, respectively. The relative sensitivity, based on detection limit, was calculated. The HPLC method, was found to be more sensitive than the ^{1}DD and HPTLC methods; and the HPTLC method was found to be more sensitive than the ^{1}DD method.

4.6. Tablet analysis

The proposed procedures were successfully applied for the analysis of Nicergoline in its pharmaceutical dosage form. No interferences due to excipients was detected in the spectra or chromatograms produced. The results of analysis in Table 2 indicate that the proposed assays can be used for the quantitation of Nicergoline in its tablets. Furthermore, the validities of the proposed procedures were assessed by applying standard addition technique (Table 3).

Results obtained by applying the proposed procedures, were statistically compared with those obtained by adopting the manufacturer's method [33].Table 4 shows that the calculated t and F values are less than the theoretical ones, confirming accuracy and precision at 95% confidence level.

Different parameters for the validation of the assays were calculated following a scheme of assay validation of H.G. Brittain [41] and the results are presented in Table 5.

Table 5
Results of assay validation obtained by applying the proposed methods

Parameter	Method		
	Derivative ratio	Densitometry	HPLC
Accuracy			
Mean ± R.S.D.%	99.97 ± 0.596	99.94 ± 0.895	99.71 ± 0.795
Precision			
Interday[a]	0.842	1.456	1.095
Intraday[a]	1.053	1.917	1.308
Specificity[b]			
Student's t-test	1.350 (1.796)	0.804 (1.746)	0.392 (1.782)
F	1.432 (4.390)	1.252 (3.440)	1.810 (6.040)
L.O.D.[c]	1.49	1.07	0.96
L.O.Q.[d]	4.50	3.25	2.90
Linearity			
Slope	0.0133	0.3316	0.0102
S.E. of Slope	0.633×10^{-4}	2.04×10^{-3}	0.345×10^{-4}
Intercept	0.0244	0.1263	0.0223
S.E. of Intercept	0.283×10^{-2}	5.753×10^{-2}	0.169×10^{-2}
Correlation coefficient	0.9999	0.9999	0.9999
Range	10–70 (μg ml^{-1})	5–45 (μg ml^{-1})	5–80 (μg ml^{-1})

N.B: Figures between parenthesis are the corresponding tabulated values ($P = 0.05$).

[a] $n = 12$.

[b] In the presence of Nicergoline Degradate (II).

[c] Limit of detection, calculated from the results of analysis of pure powder using the following equation: 3.3 (S.D./S) where, S.D. is the residual standard deviation of the regression line, S is the slope.

[d] Limit of quantitation, calculated from the results of analysis of pure powder using the following equation: 10 (S.D./S), where, S.D. is the residual standard deviation of the regression line, S is the slope.

5. Conclusion

The proposed HPTLC and HPLC methods provide simple, accurate and reproducible quantitative analysis for the determination of Nicergoline in pharmaceutical tablets and in the presence of its hydrolysis-induced degradation product. Also the ^{1}DD method can be used successfully in the presence of the degradation product up to 70% of the initial concentration. The HPLC method was found to be more sensitive than the ^{1}DD and HPTLC methods. While the ^{1}DD method has the advantages of lower cost, rapid and environment protecting. The HPTLC method, is simple and uses a minimal volume of solvents, compared with the HPLC method. The three proposed methods are suitable for quality control laboratories, where economy and time are essential.

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