Sensitive Method for the Quantitative Determination of Ergotamine in Tablet Dosage Form by High-Performance Liquid Chromatography Using Bromocriptine as Internal Standard

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Authors' contributions

This work was carried out in collaboration with all authors. Author S.A. designed the study, wrote the protocol, and wrote the first draft of the manuscript, read and approved the final manuscript. Author S.O. performed the statistical analysis, managed the analyses of the study, managed the literature searches.

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ABSTRACT

Aim: To develop and validate a selective, sensitive and simple RP-HPLC method for the determination of ergotamine tartrate (ET) in pharmaceutical dosage forms.
Study Design: All variables were studied to optimize the chromatographic conditions.
Place and Duration of Study: Department of Chemistry, Faculty of Science, Aleppo University, Aleppo, Syria during seven months.
Methodology: The chromatographic separation of ET and bromocriptine mesylate (BCM, was used as internal standard) was achieved on a reversed phase BDS Hypersil C_8 column (250×4.6 mm i.d., 5 μm particle size) with a mobile phase consisted of MeOH-HCOOH 0.1 M (70:30, *v/v*), pumped at a flow rate 1.0 mL min^{-1} and detected at 320 nm.
Results: The retention times were 8.30 and 10.93 min for ET and BCM, respectively. The validation of the proposed method was carried out for specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. Linearity range was 3.0-1400.0 μg mL^{-1} with limits of detection and quantification values of 0.18 and 0.58 µg mL^{-1}, respectively. The precision of the method was demonstrated using intra- and inter-day assay RSD values which were less than 2.35% in all instances, while the relative percentage error was less than 1.99% (*n*=6). No interference from any components of pharmaceutical dosage forms or degradation products was observed.
Conclusion: The developed method was found to be selective, accurate, precise, robust and could be applied to the quantitative analysis of ET in raw material and tablets.

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Keywords: Ergotamine tartrate; liquid chromatography; pharmaceutical dosage forms.

1. INTRODUCTION

Ergotamine tartrate, bis [(6a*R*,9*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxo-octahydro-8*H*-oxazolo [3,2-*a*] pyrrolo [2,1-*c*] pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide] tartrate. The drug indicated as a therapy for vascular headache, migraine and migraine variants. Ergotamine has a direct stimulating effect on the smooth muscle of peripheral and cranial blood vessels [1]. Literature survey reveals that ergotamine tartrate is official in U.S.P. [2] and B.P. [3]. Few methods for the determination of ergotamine tartrate have been reported. RP-HPLC coupled with UV [4-10] or fluorescence detection [11-14] and HPTLC [15-17] methods have been proposed for the determination of ergotamine tartrate in biological fluids and pharmaceutical products. For the analysis of ergotamine tartrate in human plasma a gas chromatography–mass spectrometry [18] and triple-sector quadrupole mass spectrometry [19] have also been reported. Capillary electrophoresis [20,21], chemiluminescence [22] and fluorimetric [23-26] methods for the determination of ergotamine tartrate in different pharmaceutical preparations, either alone or with other active ingredients, have been described. Various amperometric [27] and spectrophotometric [28,29] methods have been reported for the determination of ergotamine tartrate from its individual formulations.

The objective of this work was to develop and validate a sensitive and reliable analytical method using reversed phase-high performance liquid chromatography (RP-HPLC) with a simple composition and low cost of mobile phase, which was used for the first time in this work, for determination of ergotamine tartrate in raw material and tablets. Moreover, the lower solvent consumption leads to an environmentally friendly chromatographic procedure. The method serves as an alternative to the methods described in pharmacopoeias.

2. EXPERIMENTALS

2.1 Equipment

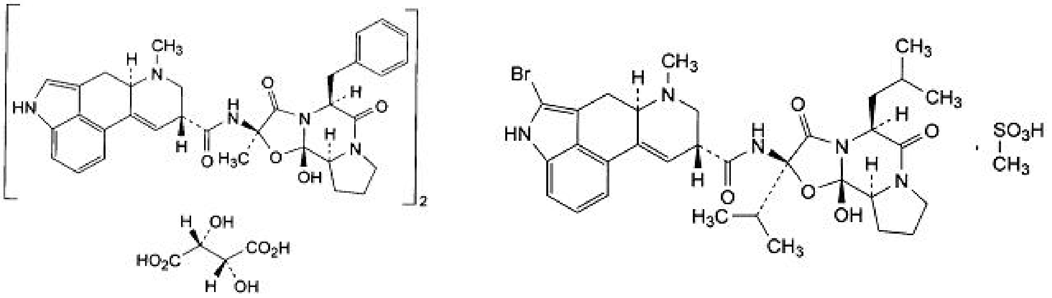
A high performance liquid chromatographic system consisted of Hitachi (Japan) Model L-2000 equipped with a binary pump (model L-2130, flow rate range of 0.000-9.999 mL min^{-1}), degasser and a column oven (model L-2350, temperature range of 1-85 ^{o}C). All samples were injected (10 μL) using a Hitachi L-2200 autosampler (injection volume range of 0.1-100 μL). Elutions of all analytes were monitored at 218 nm by using a Hitachi L-2455 absorbance detector (190-900 nm) containing a quartz flow cell (10 mm path and 13 μL volume). Each chromatogram was analyzed and integrated automatically using automation system software.

2.2. Materials and chemicals

Ergotamine tartrate (ET) was supplied by IVAX, Czech ($(C_{33}H_{35}N_5O_5)_2.C_4H_4O_6$ = 1311.39 g/mol), and its purity was found to be 99.80% according to the pharmacopoeial method [3]. The internal standard (bromocriptine mesylate, BCM, $C_{32}H_{40}BrN_5O_5.CH_3SO_3H$ = 750.60 g/mol) was obtained from Divi's Laboratories Limited, India, and its purity was found to be 99.87% according to the pharmacopoeial method [3]. The structure formulas of ET and BCM are shown in Fig. 1.

All solvents used were of HPLC grade, Merck, Germany. All other chemicals employed were of analytical grade, Merck, Germany. Tablets containing ET: Balergot-C (Balsam Pharma

Co., Syria), Asia Migraine (Asia Pharmaceutical Industries, Syria) and Medergot (Unipharma, Syria) each tablet was labeled to contain 1 mg of ergotamine tartrate and 100 mg of caffeine.



Ergotamine tartrate **Bromocriptine mesilate**

Fig. 1. Structural formula of ET and BCM (I.S.)

2.3. Chromatographic conditions and measurement procedure

Chromatographic separation was achieved on a reversed phase BDS Hypersil C_8 column (250×4.6 mm, 5 μm particle size, Thermo, Germany). The mobile phase was a mixture of 0.1 M HCOOH: methanol (30:70 *v/v*) and was filtered through a 0.45 μm nylon-membrane filter and degassed by ultrasonic agitation prior to use. The mobile phase was prepared weekly and was delivered at a flow rate of 1.0 mL min^{-1}. The injection volume was 10 μL. Peak areas were measured and HPLC analysis was conducted at ambient temperature (25 ^{o}C).

2.4. Standard solutions and calibration graphs

Standard solution of ET was prepared by direct weighing of standard substance with subsequent dissolution in methanol. The concentration of the stock standard solution was 2.0 mg mL^{-1}. Stock standard solution of BCM 1.0 mg mL^{-1} was prepared by dissolving appropriate amount of the compound in methanol. These solutions were stored in the dark at 2-8° C and were found to be stable for one month at least. A series working standard solutions of ET (3.0-1400.0 μg mL^{-1}) were prepared by diluting the stock standard solution with the mobile phase. In each sample 200.0 μg mL^{-1} of BCM was added. Standard solutions were found to be stable during the analysis time.

To construct the calibration curve of ET five replicates (10 μL) of each working standard solution were injected immediately after preparation into the column and the peak area of the chromatograms were measured. Then, the mean peak area ratio of ET to that of the internal standard was plotted against the corresponding concentration of ET (3.0-1400.0 μg mL^{-1}) to obtain the calibration graph (Table 1).

2.5. Assay procedure for dosage forms

Twenty tablets containing ET were weighed and finely pulverised. Five accurately weighed quantities of this powder, equivalent to 5.0 mg of ET, were placed in 25 mL separated volumetric flasks with 20 mL of methanol. The mixtures were sonicated for 10 min. Then, the sample solutions were filtered and the filtrates were diluted with methanol to 25 mL in volumetric flasks containing 200.0 μg mL^{-1} of the internal standard BCM. Finally, 10 μL of each sample was injected into the column. Peak area ratios of ET to that of BCM were then measured for the determinations. ET concentrations in the samples were then calculated using peaks data and standard curves.

2.6. Optimization procedure

On the basis of the optimization procedure the following factors were selected and tested in the experimental design: (A) volume percent of methanol (60-80%), (B) concentration of HCOOH (0.05-1.0 M). Factor levels are given in parenthesis. Experimental design indicates that the best conditions for separation of ET from internal standard (I.S.) are at mobile phase composition: HCOOH solution (0.1 M): methanol (30:70, *v/v*).

2.7. Validation

The standard curve was a plot of the peak area ratios of ET–BCM versus the corresponding concentrations of ET in the standard curve samples. The linearity of the standard curve was evaluated using least-squares linear regression analysis. To determine recovery of ET at concentrations of 3, 50, 200, 500, 700, 1000 and 1400 μg mL^{-1} and of BCM at the concentration used in the assay (200 μg mL^{-1}) from bulk or formulations, an identical set of standards prepared in the mobile phase was analyzed. Absolute recoveries at each concentration were measured by comparing the response of pre-treated standards with the response of standards which had not been subjected to sample pre-treatment. Intra- and inter-day coefficients of in variation of the assay were determined by the analysis of five samples at each concentration on the same day and of five samples at each concentration on 6 different days, respectively. The limit of quantification for this assay is defined as the lowest concentration of ET that can be detected.

3. RESULTS AND DISCUSSION

3.1. Chromatography

The goal of this study was to develop HPLC assay for the analysis of ET drug in pharmaceutical dosage form. Initial studies to develop HPLC assay involved the use of C_{18} and C_8 columns with various mobile phases containing acetonitrile- or methanol-aqueous formic acid solutions. The chromatographic separations were performed on a BDS Hypersil C_8 column, since it produced sharp and symmetrical peaks. The final selective HPLC mobile phase consisting of MeOH-HCOOH. The effect of composition of the mobile phase on the retention time of ET and the internal standard, BCM, was investigated. Results of the effect of methanol in the mobile phase are presented in Fig. 2. An increase in the percentage of methanol decreases the retention of compounds; ET and BCM. Increasing methanol percentage to more than 80% ET peak is eluted with the solvent front, while at methanol percentage lower than 65% the elution of BCM peak is seriously delayed. The optimum methanol percentage was found to be 70%. The effect of pH in the chromatographic elution of both compounds was also investigated by changes the concentration values of the aqueous component of the mobile phase from 0.05 to 1.0 M. For all experimental concentration values, the drugs are eluted in order of ET and BCM. A concentration value of 0.1 M HCOOH was chosen for the optimum separation of the compounds, as at this concentration the analyte peaks were well defined and resolved. The optimum wavelength for detection was at 320 nm, at which the best detector responses for all substances were obtained. The specificity of the HPLC method is illustrated in Fig. 3 where complete separation of the compounds was observed. ET was eluted at 8.30 min, while the internal standard BCM was eluted at 10.93 min.

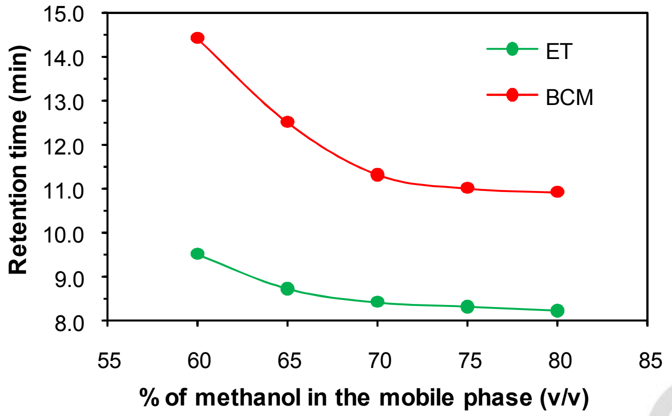


Fig. 2. Plots of the retention time vs. methanol percentage in the mobile phase of ET and BCM.

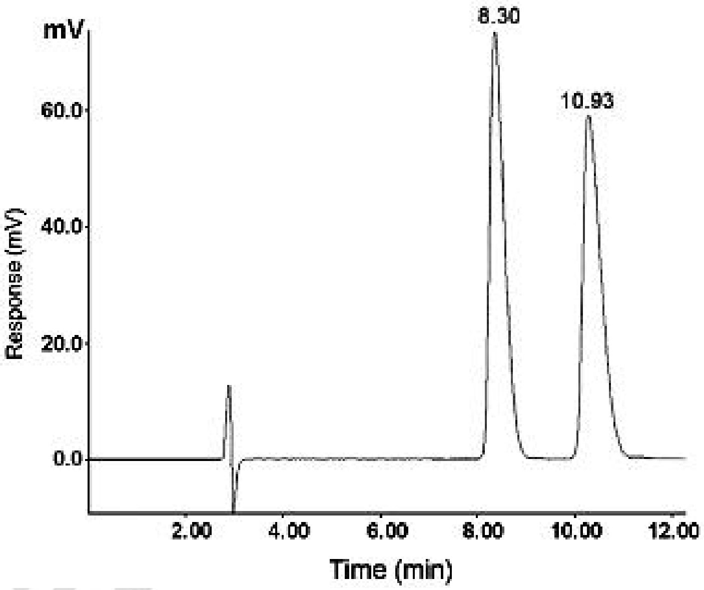


Fig. 3. A typical chromatogram of a mixture of ET (200.0 μg mL^{-1}) and the internal standard BCM (200.0 μg mL^{-1}) at retention times 8.30 and 10.93 min, respectively. Chromatographic conditions: RP-HPLC on a BDS Hypersil C_8 column; mobile phase: HCOOH 0.1 M:methanol (30:70, *v/v*); flow rate 1.0 mL min^{-1} and a UV detector at 320 nm.

3.2. Linearity and limits of quantification and detection

Standard curve of ET was linear over the concentration range 3.0-1400.0 μg mL^{-1}. Straight line for ET was obtained, when the area of the peaks were plotted versus concentration (Table 1). Also, Linear relationship was obtained between the peak area ratio of ET to that of BCM (I.S.) and the corresponding concentration of ET (3.0-1400.0 μg mL^{-1}), as shown by the equation presented in Table 1 [30]. The minimum levels at which the investigated compound can be reliably detected (limit of detection, LOD) and quantified (limit of quantitation, LOQ) were determined experimentally. LOD was expressed as the concentration of drug that generated a response to three times of the signal to-noise (S/N) ratio, and LOQ was 10 times of the S/N ratio. The LOD of RSP attained as defined by IUPAC [31], $\mathrm{LOD}_{(k=3)} = k \times S_a/b$ (where b is the slope of the calibration curve and S_a is the standard deviation of the intercept), was found to be 0.18 μg mL^{-1}. The LOQ was also attained according to the IUPAC definition, $\mathrm{LOQ}_{(k=10)} = k \times S_a/b$, and was found to be 0.58 μg mL^{-1}.

Table 1. Calibration data for the estimation of ET by HPLC.

Parameters	Ergotamine tartrate
Optimum concentration range, μg mL^{-1}	3.0-1400.0
Regression equation*	$A_{\mathrm{ET}} = 0.7189C_{\mathrm{ET}} + 2.4081$
Correlation coefficient (R^2)	0.9999
Standard deviation of slope	0.0053
Standard deviation of intercept	0.0094
Regression equation**	$R_{\mathrm{ET/BCM}} = 0.005C_{\mathrm{ET}} – 0.0094$
Correlation coefficient (R^2)	0.9998
Standard deviation of slope	2.6×10^{-4}
Standard deviation of intercept	3.1×10^{-4}
limit of detection (LOD), μg mL^{-1}	0.18
limit of quantification (LOQ), μg mL^{-1}	0.58

* Regression equation for the peak area of ET vs. concentration of ET in μg mL^{-1}.

** Regression equation for the ratio of peak area of ET to that of I.S. (BCM) vs. concentration of ET in μg mL^{-1}.

3.3. System suitability

The system suitability was determined by making five replicate injections and analyzing each solute for their peak area, resolution and peak tailing factor. The system suitability requirements for 200.0 µg mL^{-1} of ET in the presence of 200.0 µg mL^{-1} of internal standard was a %RSD for peak area less than 0.65, a peak tailing factor less than 1.3 and an Rs greater than 4.0 between adjacent peaks. This method met these requirements. The results are shown in Table 2.

Table 2. System suitability parameters.

Parameters	Ergotamine tartrate	Bromocriptine mesylate
Theoretical plates (N)	6292	5459
Resolution factor (Rs)	-	4.73
Tailing factor (T)	1.07	1.25
Capacity factor (k)	4.53	6.42
% RSD for five injections	0.58	0.62

3.4. Accuracy and Precision

The precision and accuracy of the method were evaluated by intra- (analysis of standard solutions of ET in replicates of five in the same day) and inter-day (analysis of standard solutions of ET in replicates of five on 6 different days from day 1 to 30 after preparation) assay variance (Table 3). The standard deviation, relative standard deviation, recovery and relative percentage error of different amounts tested were determined, as recorded in Table 3. The accuracy of the method is indicated by the excellent recovery (99.76-101.99%) and the precision is supported by the low standard deviation. Table 3 shows that the percent error of the method was always less than 1.99%; therefore, it was concluded that the procedure gives acceptable accuracy and precision for the analyte.

The reproducibility of the method was calculated in terms of percent relative standard deviation % R.S.D. from the response level of 10 different solutions at concentration level of 50.0 μg ml^{-1} and the value was found to be 1.07%.

Table 3. Accuracy and precision of within and between run analysis for the determination of ET by HPLC.

Nominal concentration (μg mL^{-1})	Assayed concentration (μg mL^{-1})			
	Mean±SD	**RSD (%)**	**Recovery (%)**	**E_r (%)**
Intra-day (n=6)				
3.00	3.05±0.06	1.97	101.67	1.67
50.00	50.23±0.53	1.06	100.46	0.46
200.00	201.77±1.69	0.84	100.89	0.89
500.00	503.18±3.57	0.71	100.64	0.64
700.00	703.72±4.43	0.63	100.53	0.53
1000.00	1019.90±6.01	0.59	101.99	1.99
1400.00	1410.83±7.75	0.55	100.77	0.77
Inter-day (n=6)				
3.00	3.04±0.07	2.35	101.33	1.33
50.00	49.88±0.63	1.28	99.76	-0.24
200.00	200.73±1.85	0.92	100.37	0.37
500.00	499.86±4.35	0.87	99.97	-0.03
700.00	701.23±4.21	0.60	100.18	0.18
1000.00	1007.56±5.44	0.54	100.76	0.76
1400.00	1403.55±6.60	0.47	100.25	0.25

3.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of the concentration of HCOOH in the mobile phase, flow rate, percentage of methanol in the mobile phase and column temperature. The standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 4).

Table 4. Results of robustness study.

Factor	**Level**	**Ergotamine tartrate**	
		Mean % assay (*n* = 5)	**% RSD of results**
HCOOH in mobile phase (M)	0.05	99.9	0.88
	0.15	100.6	0.79
Flow rate (mL/min)	0.9	99.8	0.74
	1.1	100.5	0.51
Column oven temperature (^{o}C)	20	100.4	1.07
	30	99.8	1.64
% of methanol	65	100.3	0.64
	75	100.9	0.69

3.6. Stability studies

Stability studies were carried out at laboratory temperature for a month to find potential stability problems of the drug in the formulations. Samples were analyzed at intervals of 0, 1, 5, 15 and 30 days. The results obtained are given in Table 5. The percent RSD values between subsequent readings gave an indication of the stability of the drug in the formulations.

Table 5. Stability study for ET drug in different formulations.

formulations	Time (days)	Amount found[a] (mg)	Recovery %	±RSD %
Balergot-C (1 mg ET/tab)	0	1.02	102.00	0.38
	1	1.03	103.00	0.52
	5	1.02	102.00	0.31
	15	1.01	101.00	0.62
	30	1.01	101.00	0.57
Asia Migraine (1 mg ET/tab)	0	1.03	103.00	0.97
	1	1.02	102.00	0.84
	5	1.02	102.00	0.71
	15	0.99	99.00	0.66
	30	0.98	98.00	0.76
Medergot (1 mg ET/tab)	0	1.01	101.00	0.78
	1	1.00	100.00	0.48
	5	0.99	99.00	0.69
	15	0.99	99.00	0.71
	30	0.97	97.00	0.46

[a] Five independent analyses.

3.7. Application of the Assay

The applicability of the proposed method was assayed by analyzing commercial tablets. The assays were carried out as described under the experimental procedure for dosage forms. In all the preparations, the amount of ET was obtained by direct measurement using the standard calibration curve. For the sake of comparison, the ET content of the pharmaceutical preparations was also determined by the reference method [7]. In all of the pharmaceutical preparations, the results were found to be very good (Table 3), thus confirming the validity of the proposed method. Three pharmaceutical formulations of ET (1 mg/tab) were analyzed. Figure 4 illustrates a typical chromatogram of ET (200 µg mL^{-1}) in the methanol prepared from Balergot-C tablets in the presence of BCM (200 µg mL^{-1}). Table 3 shows the results of the determination of three local pharmaceutical preparations using the proposed method and reference method. Good agreement with results obtained by the reference method was observed.

A statistical comparison between results obtained from both the proposed and reference methods was carried out. The calculated *t*- and *F*-values did not exceed the theoretical values at the 95% confidence level, indicating the absence of any difference between the two methods.

The procedure described here provides a rapid method for determination of ET in bulk and dosage forms because of its simplicity, accuracy and reproducibility. It also provides practical and significant economic advantages over other instrumental methods. The method is, thus, suitable for routine analysis of ET tablets without interference from the other active ingredients caffeine, excipients and additives such as starch, glucose, lactose and magnesium stearate.

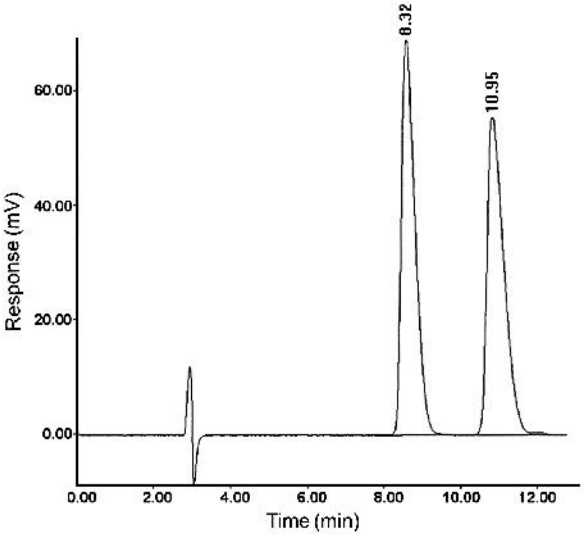


Fig. 4. A typical chromatogram of a mixture of ET (200 μg mL^{-1}) and BCM (200 μg mL^{-1}) in the methanol prepared from BALERGOT-C tablets. Chromatographic conditions: RP-HPLC on a BDS Hypersil C$_8$ column; mobile phase: HCOOH 0.1 M:methanol (30:70, *v/v*); flow rate 1.0 mL min^{-1} and a UV detector at 320 nm.

Table 6. Determination of ET in tablets by the proposed and official methods.

Sample	**Recovery (%)^a ± SD**	
	Ergotamine tartrate	
	Proposed method	**Official method [7]**
Pure	100.08±0.26	99.80±0.23
t-value	1.97	
F-value	1.40	
Balergot-C		
Mean±S.D.^{a}	102.32±0.27	99.94±0.13
t-value ^{b}	2.56	2.96
F-value ^{b}	4.31	
Asia Migraine		
Mean±S.D.^{a}	101.62±0.33	100.04±0.29
t-value ^{b}	2.04	2.83
F-value ^{b}	1.29	
Medergot		
Mean±S.D.^{a}	100.95±0.23	100.18±0.19
t-value ^{b}	1.86	2.04
F-value ^{b}	1.46	

^{a}Five independent analyses.
^{b}Theoretical values for *t* and *F*-values at five degree of freedom and 95% confidence limit are *t* = 2.776 and *F* = 6.26.

4. CONCLUSION

The proposed high-performance liquid chromatographic method has been evaluated over the linearity, precision, accuracy and selectivity and proved to be convenient and effective for the quality control of ergotamine tartrate in pharmaceutical formulations. The measured signal was shown to be precise, accurate and linear over the concentration range tested (3.0-1400.0 $\mu g\ mL^{-1}$) with a limit of detection of 0.18 $\mu g\ mL^{-1}$ and a correlation coefficient better than 0.9998. The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulations excipients and other active ingredients caffeine in the estimation. Moreover, the lower solvent consumption along with the short analytical run time of 11.0 min leads to an environmentally friendly chromatographic procedure.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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