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Article

Determination of Eprosartan Mesylate and Hydrochlorothiazide in Tablets by Derivative Spectrophotometric and High-Performance Liquid Chromatographic Methods

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Two new simple and selective assay methods have been presented for the analysis of eprosartan mesylate (EPR) and hydrochlorothiazide (HCT) in pharmaceutical formulations. The first method is based on first-derivative ultraviolet spectrophotometry with zero-crossing measurements at 246 and 279 nm for EPR and HCT, respectively. The assay was linear over the concentration ranges 3.0–14.0 μg/mL for EPR and 1.0–12.0 μg/mL for HCT. The quantification limits for EPR and HCT were found to be 1.148 and 0.581 μg/mL, respectively, while the detection limits were 0.344 μg/mL for EPR and 0.175 μg/mL for HCT. The second method involved isocratic reversed-phase liquid chromatography using a mobile phase composed of acetonitrile–10 mM phosphoric acid (pH 2.5) (40:60, *v*/*v*). Olmesartan was used as internal standard and the substances were detected at 272 nm. The linearity ranges were found to be 0.5–30 and 0.3–15.0 μg/mL for EPR and HCT, respectively. The limits of detection were found to be 0.121 μg/mL for EPR and 0.045 μg/mL for HCT. The limits of quantification were found to be 0.405 and 0.148 μg/mL for EPR and HCT, respectively. The proposed methods were successfully applied to the determination of commercially available tablets with a high percentage of recovery and good accuracy and precision.

Introduction

Eprosartan mesylate (EPR) (Figure1A), monomethanesulfonate of (E)-2-butyl-1-(p-carboxybenzyl)-α-2-thienylmethylimidazole-5-acrylic acid, is a highly selective, nonpeptide angiotensin-II antagonist. The compound has been shown to inhibit angiotensin-II, including vasoconstriction in preclinical species, and to cause reductions in systolic and diastolic blood pressure at peak effect after dosing in clinical patients. It belongs to the ARA-II family. These are safe and effective agents for the treatment of hypertension and heart failure, either alone or in combination with diuretics (1, 2). The combination of EPR and hydrochlorothiazide (HCT) can be effectively and safely used in patients (3). Two methods have been reported for the determination of EPR in pharmaceutical preparations: ultraviolet (UV)-spectrophotometry (4) and high-performance liquid chromatography (HPLC) (5).

HCT (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide) (Figure 1B) is a potent orally diuretic and antihypertensive agent related to chlorothiazide. Several analytical methods have been published on teh determination of HCT in tablets using flow injection (6), spectrophotometric (7–9), densitometric (10), HPLC (7–9, 10–13), electrophoretic (14, 15) and polarographic (16) methods.

In our literature survey, only one high-performance thin layer chromatography (HPTLC) method was observed for simultaneous analysis of these drugs in pharmaceutical preparations (17). HPTLC has the advantage of operation simplicity and low cost. However, HPLC is a more commonly used method in quality control laboratories because of its high sensitivity and precision. The derivative UV-spectrophotometric method is also very simple and does not require any reagent, pH-adjustment or extraction procedure. For this purpose, a zero-crossing first-derivative spectrophotometric method and an HPLC method were developed for analysis of EPR and HCT in mixtures without prior separation. Additionally, the proposed methods are shown to be useful in determination of both drugs in combination tablet formulations.

Experimental

Apparatus

Spectrophotometric measurements were performed by using a Shimadzu UV-160 A spectrophotometer with 1-cm glass cells.

The HPLC analyses were performed on a Thermo Separation Products Liquid Chromatograph that consisted of a P4000 solvent delivery system equipped with a Rheodyne injection valve with a 20-μL loop, a UV3000 detector set at 272 nm and an SN4000 automation system software. Chromatographic separation was achieved isocratically at a temperature of 30°C on an ACE 5 CN column (4.6 mm I.D. × 200 mm, 5 μm; Aberdeen, Scotland). The mobile phase was composed of acetonitrile–10 mM phosphoric acid (pH 2.5; 40:60, *v*/*v*) with a flow rate of 1.0 mL/min.

Reagents and solutions

EPR was supplied by Solvay Pharmaceuticals (Netherlands). HCT was supplied Abdi Ibrahim Pharmaceuticals (Istanbul, Turkey). Their pharmaceutical preparation Teveten Plus tablet, containing 600 mg of EPR and 12.5 mg of HCT per tablet, were obtained from a local drugstore. All chemicals and reagents were of analytical-reagent grade.

For both methods, portions (100 mg each) of standard EPR (calculated as a base) and HCT were weighed and transferred to 100-mL volumetric flasks and dissolved in methanol, and further dilutions were made with methanol at concentrations of 100 μg/mL each to obtain standard solutions of EPR and HCT. Before measuruements, final dilutions were made with the acetonitril–water to 50:50 and 40:60 (for derivative spectrometry and HPLC methods, respectively).

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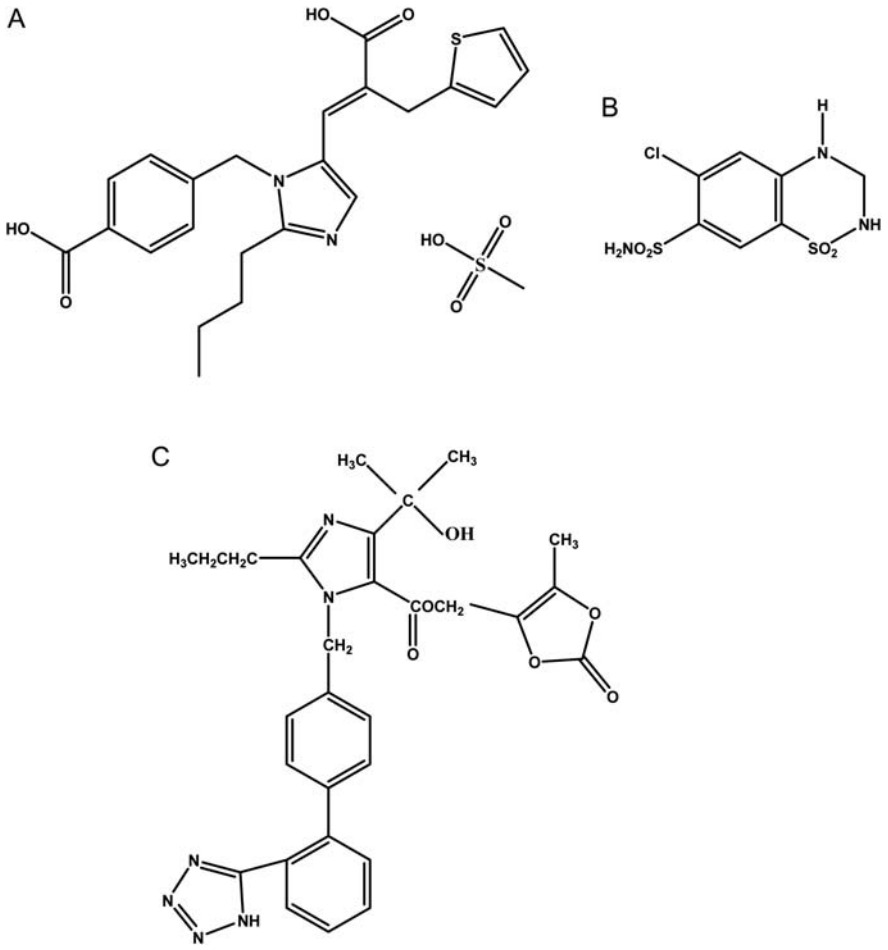

Figure 1. Chemical structures: eprosartan mesylate (A); hydrochlorothiazide (B); olmesartan medoxomil (IS) (C).

Internal standard (IS) stock solution was prepared by dissolving 50.0 mg of olmesartan in 50 mL of methanol. The stock solutions were stored at 4°C and were stable for a month.

General procedure

Derivative spectrophotometric method

Different aliquots of drug solution (0.05–0.7 mL) were transferred to 5-mL volumetric flasks to provide a final concentration range of 1.0–10.0 μg/mL for HCT and 3.0–14.0 μg/mL for EPR, and the volume was diluted to final volume with acetonitril–water (50:50).

The first derivative spectra of these standard solutions were scanned against an acetonitrile–water (50:50) blank between 200 and 400 nm. The values of the derivative amplitudes at 246 nm and 279 nm were measured for the determination of EPR and HCT, respectively. The concentrations of each compound versus their derivative amplitudes were plotted to establish calibration graphs.

HPLC method

Standard solutions of EPR and HCT were prepared by dilution of the stock solutions with acetonitrile–water (40:60, *v*/*v*). The final concentrations of each drug were between 0.5–30 and 0.3–15.0 μg/mL for EPR and HCT, respectively. These standard solutions also contained an IS at 50 μg/mL. The chromatograms were evaluated on the basis of EPR–IS or HCTZ–IS ratios of the peak areas.

Assay procedure for tablets

For the spectrophotometric method, 20 tablets were weighed and finely powdered. Powder equivalent to 60 mg EPR and 1.25 mg HCT was accurately weighed and transferred to a 100-mL volumetric flask. Methanol (75 mL) was transferred to the volumetric flask and and then extraction was performed mechanically for 20 min and sonication for 20 more min. The dilution was made with methanol to reach a solution containing 600 μg/mL EPR and 12.5 μg/mL HCT (Solution 1), which was used for the determination of HCT. One milliliter of this solution was transferred to a 100-mL volumetric flask. The volume was diluted with acetonitril–water, 50:50 and 40:60 (for derivative spectrometry and HPLC methods, respectively) to a final volume to have a solution containing 6 μg/mL EPR and 0.125 μg/mL HCT (Solution 2), which was used for the determination of EPR only. The difference between the amount of EPR and HCT in the tablet formulation was very high;

therefore, measurements were performed at different dilution levels. First, HCT was measured at a low dilution level, then EPR was measured at a higher dilution level.

Results and Discussion

Method development

A zero-crossing first-derivative spectrophotometric method and an HPLC method were developed for the analysis of EPR and HCT in combination tablet preparations.

Direct UV-absorption measurements were found to be inapplicable to the analysis of EPR and HCT in simultaneous analysis because of the spectral interference (Figure 2). On the other hand, derivative spectrophotometry based on a mathematical transformation of the zero-order curve into the derivative spectra can overcome this problem (18–23). The EPR and HCT were prepared in different solvents (acetonitrile, methanol and water) and mixtures of the solvents. The best results were found with acetonitrile–water (50:50). Using memory channels, the first to fourth order derivative spectra were overlapped. The first order derivative spectrum was selected based on available zero crossing points. As shown in Figure 3C, the wavelength 243 nm was selected for the determination of EPR (where the derivative response for HCT was zero) and 279 nm was selected for the determination of HCT (where the derivative response for EPR was zero).

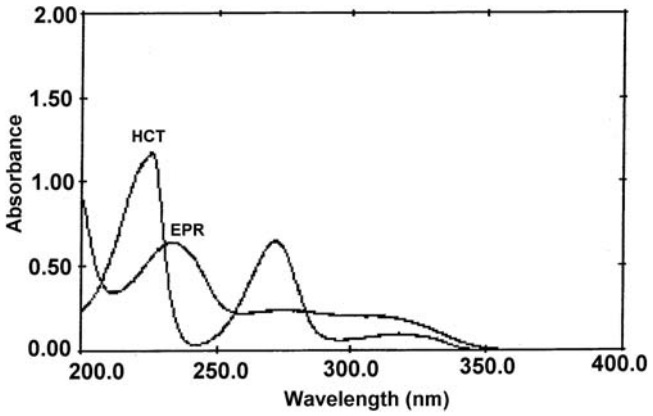

Figure 2. Overlaid zero-order spectra of eprosartan (10 μg/mL) and hydrochlorothiazide (10 μg/mL) in acetonitrile–water (50:50).

As a new alternate method for analysis of these drugs and to verify the results of the developped UV-derivative spectrophotometric method, an HPLC method was also developed. For a good chromatographic separation, some parameters were tested. A C18 and a reversed-phase CN column were used for the column selection. Trials showed that the reversed-phase CN column gave symmetrical and sharp peaks. Acidic and aqueous mobile phase were tested, and acidic mobile phase provided good results. Therefore, *o*-phosphoric acid solution (10 mM) was preferred as the acidic solution. Acetonitrile was chosen as the organic modifier. A mobile phase composition of acetonitrile–phosphoric acid (pH: 2.5), 40:60 (*v*/*v*) at a flow rate of 1.0 mL/min was used to achieve a good resolution. Olmesartan, telmisartan, valsartan, irbesartan were tested as IS, and olmesartan was chosen as IS. For quantitative analytical purposes, the wavelength was set at 272 nm (Figure 2). Retention times of the drugs obtained under these

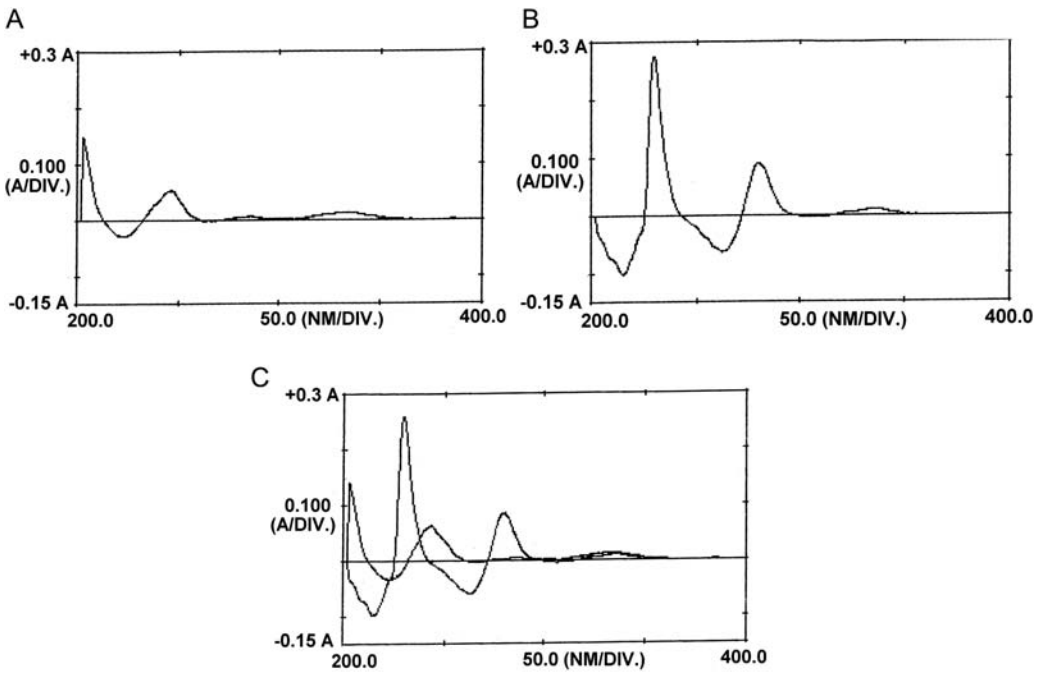

Figure 3. First-derivative spectrum of EPR in acetonitrile–water (50:50) (A); first-derivative spectrum of HCT in acetonitrile–water (50:50) (B); overlaid first-derivative spectra of EPR (10 μg/mL) and HCT (10 μg/mL) in acetonitrile–water (50:50) (C).

conditions are 4.2, 5.9 and 7.5 for HCT, EPR, and IS, respectively (Figure 4).

Method validation

Linearity range

For derivative spectrophotometry, calibration curves were constructed by plotting two-dimensional (2D) values against corresponding concentrations in the range of 1.0–10.0 and 3.0–14.0 μg/mL for HCT and EPR, respectively. The regression equations were calculated as $^2D_{243} = / \ 0.0086 \ C + 0.0025$ ($r^2 = 0.9995$) for HCT and $^2D_{279} = 0.0054C - 0.0025$ ($r^2 = 0.9997$) for EPR.

For HPLC analysis, the equations of the calibration curves were obtained from linear regression analysis of the peak area ratios of EPR to IS or HCT to IS versus the concentration of related substance. The linearity ranges were found to be 0.5–30 and 0.3–15.0 μg for EPR and HCT, respectively. Regression equations of the calibration curves for EPR and HCT were calculated as $A_{EPR}/A_{IS} = / \ 0.1214C + / \ 0.0125$ ($r^2 = 0.9992$) and $A_{HCT}/ A_{IS} = / \ 0.0593C + / \ 0.0195$ ($r^2 = 0.9999$), respectively.

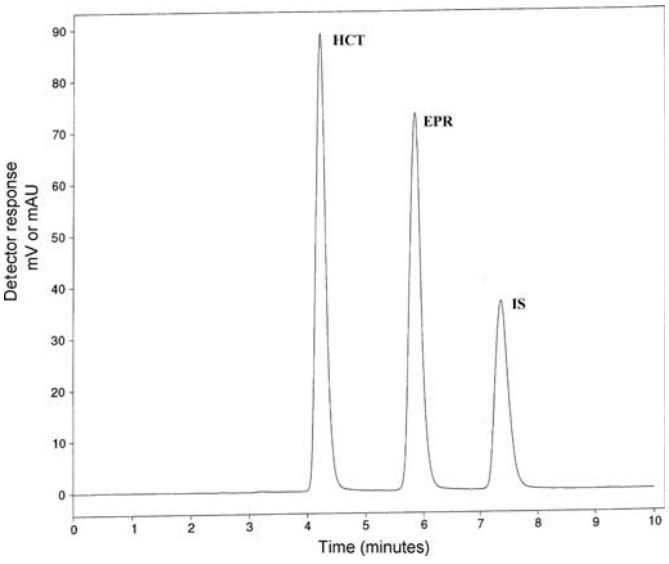

Figure 4. Chromatogram obtained with the mixture of HCT, EPR and IS (15, 30 and 50 μg/mL, respectively).

LOD and LOQ

The limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula LOD or LOQ = κSDa/b, where κ = 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. For derivative spectrophotometric method, the LOD were found to be 0.345 μg/mL for EPR and 0.174 μg/mL for HCT, and the LOQ were found to be 1.148 μg/mL for EPR and 0.581 μg/mL for HCT. For the HPLC method, the LOD for EPR and HCT were found to be 0.121 and 0.045 μg/mL, respectively, and the LOQ were 0.405 μg/mL for EPR and 0.148 μg/mL for HCT.

The results of some analytical parameters of the proposed methods are given in Table I.

Precision

The inter-day and intra-day precision were examined by analysis of drugs for the same day and seven consecutive days (each n = 5). The relative standard deviation (RSD) values found for teh developed methods were 0.64–1.18% for intra-day precision and 0.95–1.29% for inter-day precision, indicating good precision.

Recovery

To check the accuracy of the proposed methods, the standard addition technique was applied. A different amount of pure sample solution was added to four different concentrations of the standard drug solution and assayed. The percent recovery of the added standard to the assay samples was calculated from:

$$Recovery\% = [(C_t - C_u)/C_a] \times 100$$

Where C_t is the total concentration of the analyte found; C_u is the concentration of the analyte present in the formulation; and C_a is the concentration of the pure analyte added to the formulation. The results of analysis of the commercial dosage forms and the recovery study are shown in Table II. The average percent recoveries for all methods were quantitative (99.80–101.20%), indicating good accuracy of the methods.

Table I
Results of Analytical Parameters for Proposed Methods

Parameter	Derivative spectrophotometric method		HPLC method	
	EPR	HCT	EPR	HCT
Linearity range* (μg/mL)	3.0–14.0	1.0–12.0	0.50–30.0	0.3–15.0
Regression equation†				
Slope ± SD	0.0054 ± 0.0001	0.0086 ± 0.00007	0.0593 ± 0.0004	0.1214 ± 0.0004
Intercept ± SD	−0.0025 ± 0.00062	0.0025 ± 0.0005	0.0195 ± 0.0024	0.0125 ± 0.0018
Correlation coefficient, r^2	0.9997	0.9995	0.9999	0.9992
LOD (μg/mL)	0.344	0.174	0.121	0.045
LOQ (μg/mL)	1.148	0.581	0.405	0.148

*n = 5 correspond to replicate analysis for each level.
†$A = a + bC$ (where C is the concentration of drug in μg/mL for both methods, A is the absorbance at λ_{max} for the derivative spectrophotometric method and peak area ratios of EPR or HCT to IS versus for HPLC method).

Table II
Results of Recovery Studies by Standard Addition Method

	Amount removed (μg/mL)*	Amount added (μg/mL)	Total amount found† (μg/mL) (Mean ± S.Dc)	Recovery (%)	RSD (%)
Derivative spectrophotometric method					
For EPR		3.0	5.003 ± 0.037	100.10	0.73
	2	5.0	7.034 ± 0.079	100.68	1.12
		7.0	9.002 ± 0.068	100.03	0.75
		12.0	14.125 ± 0.128	101.04	0.91
For HCT		1.0	3.004 ± 0.032	100.40	1.07
	2	3.0	5.009 ± 0.045	100.30	0.90
		7.0	8.996 ± 0.0723	99.94	0.80
		10.0	12.104 ± 0.089	101.04	0.74
HPLC method					
For EPR		0.50	10.506 ± 0.087	101.20	0.83
	10.0	2.0	11.996 ± 0.112	99.80	0.93
		12.0	22.115 ± 0.187	100.96	0.85
		20.0	30.214 ± 0.401	101.07	1.33
For HCT		0.3	5.304 ± 0.065	101.33	1.23
	5.0	1.5	6.507 ± 0.087	100.47	1.34
		6.0	11.012 ± 0.093	100.20	0.85
		15.0	20.123 ± 0.115	100.82	0.57

*Teveten Plus tablet, containing 600 mg of EPR and 12.5 mg of HCT per tablet.
†Five independent analyses.

Table III
Results from Robustness Experiments for HPLC Method

Condition	Value	Recovery (%)		RSD (%)	
		EPR	HCT	EPR	HCT
Flow rate (mL/min)	0.95	100.73	100.83	1.16	1.07
	1.05	100.68	100.33	1.29	1.20
Mobile phase composition	55	100.45	100.27	1.10	1.15
Percent acetonitrile	45	100.72	100.70	0.87	1.24
Column temperature (°C)	29	100.20	100.50	0.74	0.93
	31	100.45	100.67	0.82	0.75

Stability

The stability of drug standard solutions was tested at several storage conditions (room temperature in the dark for 24 h; autosampler conditions for 24 h and 4°C for 1 month). Stability studies indicated that the samples were stable when kept at room temperature for 24 h, in autosampler conditions for 24 h and refrigerated at 4°C for 1 month. Under all conditions tested, EPR and HCT were found to be stable.

Robustness

The robustness of the HPLC method was evaluated by changing the flow-rate, column oven temperature and acetonitrile and water phase contents of the mobile phase,. The mobile phase proportions were changed from 55:45 (acetonitrile–acidic solution) to 45:55; column temperature was changed from 29 to 31°C; and the flow rate was changed from 0.95 to 1.05 mL/min. These changes had no significant effect on peak area. Low RSD values were indicative of the robustness of the method (Table III).

System suitability parameters of study are given in Table IV.

Table IV
System Suitability Parameters of the Study

Compounds	Capacity factor	Resolution*	HETP*	Tailing factor*	Asymmetry factor
EPR	11.03	4.07	0.0234	1.57	1.50
HCT	7.52	5.07	0.0358	1.53	1.47
OLM	7.68	4.07	0.0212	1.42	1.38

*USP calculations.

Table V
Statistical Evaluations of the Results Obtained by the Proposed Methods for the Assay of Drugs in Pharmaceutical Preparations ($n = 5$)

Statistical value	EPR		HCT	
	Derivative spectrophotometic method	HPLC method	Derivative spectrophotomic method	HPLC method
Mean*	603.36	605.70	12.09	12.07
Recovery (%)	100.56	100.95	100.72	100.62
RSD (%)	0.98	0.64	0.39	0.47
t-Test of significance†	0.02		0.34	
F-test of significance†	2.36		1.43	

*Five independent analyses.
†$p = 0.05$, $t = 2.23$, $F = 5.05$.
‡Teveten Plus tablet, containing 600 mg of EPR and 12.5 mg of HCT per tablet.

Applications of the methods

The applicability of the proposed methods was tested by the determination of drugs in their pharmaceutical preparations. The results of the HPLC method were statistically compared with those obtained by the derivative UV spectrophotometric method. The Student's *t*-test and variance ratio *F*-test revealed no significant difference in terms of averages and SDs (Table V).

Conclusion

A first-derivative spectrophotometric method and an LC method were developed for the determination of EPR and HCT in the presence of each other. The proposed first-derivative spectrophotometric method is simple, practical, inexpensive and fast. The HPLC method has rapid and simple mobile phase and sample preparation steps, improved sensitivity and a short chromatographic run time. Both methods were validated and the acquired validation parameters indicated that the proposed method is selective, precise, accurate and robust, and hence, suitable for routine analysis and quality control of these drugs in tablets.

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