International Journal of Drug Development & Research
| October-December 2011 | Vol. 3 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands

FULL Length Research Paper
Covered in Index Copernicus with IC Value 4.68 for 2010

Application of TLC- Densitometry, First Derivative and Ratio Derivative Spectrophotometry for the Determination of Eprosartan Mesylate and Hydrochlorothiazide in Pharmaceutical Preparations

Emad M Hussien[a], Magda M Ibrahim[a], Yousry M Issa[b], Fatma M Abdel-Gawad[a*], Saadia Barakat[a]

[a]National Organization for Drug Control and Research, Giza, Egypt.
[b]Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt

Abstract

Three sensitive and accurate methods are described for the direct determination of eprosartan mesylate (EPM) and hydrochlorothiazide (HCT) in bulk powder and combined dosage form without prior separation. The first method is a first derivative spectrophotometry (^{1}D) using a zero-crossing technique of measurement at 240.7 nm for EPM and at 233.4 nm for HCT. The second method is the first derivative of ratio spectrophotometry (^{1}DD) where the amplitudes were measured at 237.0 nm for EPM and at 277.0 nm for HCT. The third method is based on TLC separation of the two drugs followed by densitometric measurements of their spots at 290 and 270 nm for EPM and HCT, respectively. The separation was carried out on silica gel 60 F_{254} using n-butyl acetate-ethanol-water-ammonia 33% (40:40:10:1, v/v/v/v) as mobile phase. The calibration curves were linear in the range 1.0-18.0, 2.0-18.0 µg mL^{-1} and 2.0-20.0 µg/spot for EPM and 1.0-9.0, 1.0-9.0 µg mL^{-1} and 2.0-9.0 µg/ spot for HCT using ^{1}D, ^{1}DD and TLC methods, respectively. The suggested methods were tested using laboratory-prepared mixtures and were successfully applied for the analysis of pharmaceutical preparations. The methods retained their accuracy and precision when the standard addition technique was applied. The results obtained by applying the proposed methods were statistically analyzed and compared with those obtained by the manufacturer's and USP methods for EPM and HCT, respectively.

*Corresponding author, Mailing address:
Fatma M. Abdel-Gawad
Email- fatmagawad@gmail.com

Key words:
First derivative spectrophotometry; Ratio derivative spectrophotometry; TLC- densitometry; Eprosartan mesylate; Hydrochlorothiazide

How to Cite this Paper:
Emad M Hussien, Magda M Ibrahim, Yousry M Issa, Fatma M Abdel-Gawad*, Saadia Barakat "Application of TLC- Densitometry, First Derivative and Ratio Derivative Spectrophotometry for the Determination of Eprosartan Mesylate and Hydrochlorothiazide in Pharmaceutical Preparations", Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 168-177

Article History:----------
Date of Submission: 23-07-2011
Date of Acceptance: 29-07-2011
Conflict of Interest: NIL
Source of Support: NONE

Introduction

Eprosartan mesylate (EPM), (E)-2-butyl-1-(p-carboxybenzyl)-α-2-thenylimidazole-5-acrylic acid methanesulphonate, is an angiotensin-II-receptor antagonist (ARA-II) with actions similar to those of losartan [1-3]. It is used in the management of hypertension. Few methods were reported for the determination of EPM. Analysis of eprosartan and other ARA-II alone or in combination with diuretics in pharmaceuticals was achieved using electrophoretic and chromatographic screening methods [4-6]. Eprosartan was also determined in human plasma by HPLC-UV [7, 8]. The chemometric tool combined with the HPLC-UV developed method allowed the separation and quantitation of eprosartan from human plasma with an adequate resolution and total analysis time of 1 h [9].

Hydrochlorothiazide (HCT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a diuretic of the class of benzothiadiazines. Many analytical methods were developed for the determination of HCT. These methods include spectrophotometry [10-13], chromatography [14-20] and capillary electrophoresis [21].

The eprosartan mesylate/hydrochlorothiazide mixture is not yet official in any pharmacopoeia. Recently, only one HPTLC method [22] has been reported for simultaneous analysis of eprosartan and hydrochlorothiazide in tablets. The purpose of the present study is to investigate the utility of first derivative, ratio derivative spectrophotometric and TLC- densitometric methods in the assay of eprosartan mesylate and hydrochlorothiazide in combination in the pharmaceutical preparations without the necessity of sample pretreatment. The methods had sufficiently good accuracy, precision and permitted a simple, time and money-saving assay of these compounds in mixtures.

Experimental

Apparatus and conditions

A LABOMED, UV Win 5.0, double beam UV-vis spectrophotometer (USA) with 1 cm quartz cuvettes and connected to an IBM-PC computer loaded with UV Win PC software and equipped with HP desk jet printer was used for all the absorbance measurements and treatment of data.

TLC (20x20 cm) aluminum plates precoated with silica gel 60 F_{254} were purchased from E. Merck (Darmstadt, Germany). The samples were applied to the plates using 20 µL Hamilton microsyringe.

A Shimadzu dual wavelength flying spot densitometer model CS-9301 was used. The experimental conditions of measurements were λ=290 and 270 nm for EPM and HCT, respectively, photomode: reflection, scan mode: zigzag and swing width: 16 nm.

The TLC plates were developed using n-butyl acetate-ethanol-water-ammonia 33% (40:40:10:1, v/v/v/v) as a mobile phase. For detection and quantitation, 10 µL of different concentrations of the standard solutions within the quantitative range were applied as separate spots 15 mm apart and 20 mm from the bottom of the TLC plate using 20 µL Hamilton microsyringe.

Chemicals and reagents

All chemicals and solvents were of analytical-reagent grade. Eprosartan mesylate (Solvay Pharmaceuticals, Zwitzerland) and hydrochlorothiazide (Arab Company for Gelatin and Pharmaceutical products, Egypt) were of pharmaceutical grade. Teveten tablets containing 600 mg of eprosartan free base per tablet and Teveten plus tablets containing 600 mg eprosartan free base plus 12.5 mg HCT per tablet (Solvay Pharma, S. A., Barceelona) were obtained from commercial sources.

Preparation of standard solutions

Stock standard solutions of each EPM and HCT were prepared separately by dissolving 100 mg each of EPM and HCT in 50 ml methanol (2.0 mg mL^{-1}). The standard solutions were prepared individually by

dilution of the stock solution (2.0 mg mL^{-1}) with methanol to reach working solution of 50.0 µg mL^{-1} for the two drugs (for spectrophotometric method). Solutions of concentrations 0.2-2.0 mg mL^{-1} of EPM and 0.2-0.9 mg mL^{-1} of HCT were prepared from stock standard solution of 2.0 mg mL^{-1} (for TLC method).

General procedures

Calibration graphs for first derivative spectrophotometric method

Aliquots of working standard solution (50.0 µg mL^{-1}) were transferred into a series of 10 ML volumetric flasks. For EPM 0.2-3.6 mL and HCT 0.2-1.8 mL were taken and the volume was completed to the mark with methanol. The first derivative spectra (^1D) of the two series solutions were scanned within the range 220-330 nm against methanol as a blank. The values of the ^1D amplitudes at 240.7 nm (zero-crossing of HCT) were measured for the determination of EPM, while the amplitudes at 233.4 nm (zero-crossing of EPM) were recorded for the determination of HCT.

Under the experimental conditions described, standard calibration graphs for EPM (1.0-18.0 µg mL^{-1}) and HCT (1.0-9.0 µg mL^{-1}) were constructed by plotting the ^1D values versus concentration and the regression equations were computed and recorded in Table 1.

Calibration graphs for the first derivative of ratio spectra (^1DD)

Aliquots from working solutions (50.0 µg mL^{-1}) equivalent to 20-180 µg of EPM and 10-90 µg of HCT were transferred into separate 10 ML volumetric flasks and the volume was completed to the mark with methanol. For EPM, each of the absorption spectrum was recorded and divided by the spectrum of HCT with a concentration 8.0 µg mL^{-1} as divisor. The first derivative of the ratio spectra were calculated with $\Delta\lambda$=21 nm and scaling factor (SF) =20. The peak amplitudes were measured at 237.0 nm and the values were plotted against the corresponding concentration of EPM and then the regression equation was obtained (Table 1).

For HCT, a similar procedure was followed with a concentration of 8.0 µg mL^{-1} EPM as a divisor. HCT was determined by measuring the amplitude value of the ratio spectra at 277.0 nm with $\Delta\lambda$ =21 and SF=100. The calibration graph was plotted representing the concentration versus the ^1DD amplitudes and the regression equation was computed and recorded in Table 1.

Calibration graphs for the TLC-densitometric determination

A series of standard solutions covering the range 0.2-2.0 mg mL^{-1} of EPM and 0.2-0.9 mg mL^{-1} of HCT were prepared from standard stock solutions (2.0 mg mL^{-1}).

A volume of 10 µL from each solution was applied consecutively to TLC plates until the concentration range 2.0-20.0 and 2.0-9.0 µg/spot for EPM and HCT was covered, respectively. Triplicate applications of the different concentrations were made for each solution. The peak areas were plotted against the corresponding concentration to obtain the calibration graph and the regression equation was thus obtained (Table 1).

Procedure for tablets

For all methods, twenty tablets were weighed, finely powdered and mixed thoroughly. An accurately weighed amount of powder equivalent to 100 mg each of EPM and HCT was individually transferred into a 50-mL volumetric flask, diluted with methanol, stirred for about 10 min and then completed to volume with methanol to obtain 2.0 mg mL^{-1} of drug. This solution was filtered to separate any insoluble matter, and the filtrate was collected in a clean flask. After filtration, working solutions were prepared by taking suitable aliquots of clear filtrate and diluted with methanol. The assay was completed as described under section 2.4. The contents of EPM

and HCT were calculated from the corresponding regression equations.

Due to the lower concentration of HCT in Teveten plus tablets (600 mg of eprosartan base + 12.5 mg of HCT), ^{1}D and ^{1}DD methods failed to determine HCT. On the contrary, TLC method was used successfully for determination of HCT in these tablets.

Results and Discussion

First derivative spectrophotometry (^{1}D)

EPM and HCT are new combination of antihypertensive drugs; they are used for the treatment of edema and hypertension. As shown in Fig.1, due to overlapping of the zero order spectra of EPM and HCT pure drugs, it was difficult to determine the two drugs simultaneously in a mixture. Thus, a conventional UV-spectrophotometer can not be used for their individual determination in binary mixture. On the contrary, the ^{1}D spectra of each pure drug showed zero-crossing points (Fig. 2), hence, the first derivative was used for the simultaneous determination of EPM and HCT in a mixture. In practice, the selected wavelength measurement is that which exhibits the best linear response, gives a zero or near zero intercept on the ordinate axis of the calibration graph and is less affected by the concentration of any other component. The shape of the first derivative spectra is adequate for determining EPM in the presence of HCT and vice versa (Fig. 2).

EPM was determined by measurement of its ^{1}D amplitude at the zero- crossing point of HCT (240.7 nm), while HCT was determined by measuring ^{1}D amplitude at the zero-crossing point of EPM (233.4 nm). Linear relationships between derivative amplitude and drug concentration were obtained over the concentration range of 1.0-18.0 µg mL^{-1} for EPM and 1.0-9.0 µg mL^{-1} for HCT. The regression equations were computed and listed in Table 1.

First-derivative of ratio spectra method (^{1}DD)

To optimize the simultaneous determination of EPM and HCT by using ^{1}DD method, it is necessary to test the influence of the variables: divisor standard concentration, Δλ and scaling factor (SF). All these variables were studied.

An accurate choice of divisor standard concentration is fundamental for several reasons [23], hence, we tested the method with various divisor concentrations. A standard spectrum of 8.0 µg mL^{-1} for both EPM and HCT was considered as suitable for the determination of the two drugs in a mixture. The absorption spectra of the mixtures prepared at different concentrations of EPM and HCT were recorded in the range 220-300 nm and stored in the IBM-PC. The stored spectra of the binary mixtures, EPM and HCT were divided by a standard spectrum of 8.0 µg mL^{-1} HCT. The ratio spectra were smoothed with Δλ=21 nm intervals and SF=20 and their first derivatives were traced with the same Δλ (Fig. 3). In the binary mixtures, the concentration of EPM was determined by measuring the amplitude at 237.0 nm corresponding to maximum wavelength. The regression equation was computed and listed in Table 1.

For the determination of HCT, the stored spectra of the mixtures are divided by 8.0 µg mL^{-1} of EPM, Fig. 4 shows the first derivative of the ratio spectra plotted with the intervals of Δλ= 21 nm and SF= 100. The contents of HCT in binary mixtures were determined by measuring the amplitudes at 277.0 nm corresponding to minimum wavelength. The regression equation was computed and grouped in Table 1.

The calibration graphs at 237.0 nm for EPM and 277.0 nm for HCT were obtained by plotting the values of the first derivative of the ratio spectra EPM/HCT and HCT/EPM, with variable concentrations of EPM and HCT, respectively. The proposed method is applicable over the concentration ranges 2.0-18.0 and 1.0-9.0 µg mL^{-1} for EPM and HCT, respectively (Table 1).

Statistical analysis of the data shows that the slope of the calibration graph for each drug was independent on the concentration of the other component of the mixture. This means that the ^{1}D and ^{1}DD amplitudes of the mixture were only a function of the concentration of the drug at the specified wavelength.

TLC-densitometric method

Planar chromatography with precise application of the samples and computer controlled evaluation and quantitation of the developed chromatograms, has been considered reliable for purity control and qualitative drug testing [24]. Experimental conditions, such as mobile phase composition, scan mode, speed and wavelength of detection were optimized to provide accurate and reproducible results for both EPM and HCT. The chosen scan mode was the zigzag mode and the wavelengths of scanning were 290 and 270 nm for EPM and HCT, respectively. The R_f values of the investigated drugs are 0.35 and 0.77 for EPM and HCT, respectively, obtained by the system containing n-butyl acetate-ethanol-water-ammonia 33% (40:40:10:1, v/v/v/v). The equilibration time required before development is important to achieve homogeneity of the atmosphere, thus minimizes the evaporation of the solvent from the TLC plate during the development, the saturation time of the tank has been optimized and found to be 30 min. Linear correlations were obtained between the area under the peak and the concentration of drug. The calibration curves were linear in the range of 2.0-20.0 and 2.0-9.0 µg/spot with LOQ of 0.49 and 0.42 µg mL^{-1} for EPM and HCT, respectively. The results are illustrated in Table 1.

Methods validation

Accuracy

The previously mentioned methods were repeated five times for different concentrations of pure samples. The concentrations were calculated each time from its corresponding regression equation. The mean recovery percentages are found to be 99.86, 100.92 and 100.17 % for EPM and 100.01, 100.21 and 99.89 % for HCT using ^{1}D, ^{1}DD and TLC methods, respectively (Table 1). The excellent recoveries obtained suggest the good accuracy of the methods.

Precision

To measure the degree of method repeatability (intraday precision), three different concentrations were freshly prepared and analyzed, each three times within the same day and in three successive days (interday precision). The results listed in Table 1, show that no significant difference for the assay, which tested within-day (repeatability) and between-day (reproducibility). The relative standard deviation (RSD) was less than 1% indicating high degree of precision of the proposed methods.

Selectivity

Method selectivity for each drug in presence of the other was achieved by preparing different mixtures of drugs within linearity range. The results obtained in Table 2 are good indication of the high selectivity of the three methods and their potential for the simultaneous determination of EPM and HCT from their mixtures.

Detection and quantitation limits

According to ICH recommendations [25] the approach based on the SD of the response and the slope of Beer's law was used for determining the detection and quantitation limits. The values of low limits of detection (LOD) and quantitation (LOQ) were assessed practically and listed in Table 1.

Standard addition method

Standard addition technique was applied to the analysis of the commercial tablets (Teveten and Teveten plus tablets) by adding known amounts of pure drug to a known concentration of preanalyzed tablets and the concentration of the pure drug was calculated from the corresponding regression equation of EPM (^{1}D, ^{1}DD and TLC methods) or HCT (TLC method). The recovery results in Table 3 illustrate the suitability of the methods for the

analysis of EPM or HCT in its dosage forms without interference from the common excipients.

Applications

The proposed methods were applied for the determination of EPM and HCT in bulk powder or commercial tablets of Teveten (for EPM) and Teveten plus tablets (for both EPM and HCT). Five replicate determinations were made.

Satisfactory results were obtained for EPM and HCT and were in good agreement with label claims. However, the ^{1}D and ^{1}DD methods failed to determine the concentration of HCT in Teveten plus tablets due to its low concentration relative to the concentration of eprosartan (600 mg of eprosartan base + 12.5 HCT per tablet). The ratio of EPM to HCT in Teveten plus tablets is 59:1. Only, the ^{1}D and ^{1}DD methods were used successfully for determination of HCT in presence of EPM in a laboratory prepared binary mixture when the ratio of EPM to HCT is 8:1 (Table 2). The results of EPM and HCT were compared with those obtained by the HPLC methods of Solvay Pharmaceutical Company for EPM and USP [26] for HCT, using t- and F- tests. The values at 95% confidence limit did not exceed the theoretical values of 2.306 and 6.39 for t- and F- tests, respectively, indicating no significant difference between the performance of these methods regarding accuracy and precision (Table 3).

Conclusion

TLC, first derivative (^{1}D) and ratio spectra derivative (^{1}DD) spectophotometry are suitable techniques for the analysis of commercial formulations containing combination of EPM and HCT. The most striking features of the proposed methods are their simplicity, sensitivity, selectivity and rapidity, which render them suitable for routine analysis in control laboratories. HCT can be determined in Teveten plus tablets using TLC method, while ^{1}D and ^{1}DD spectrophotometry failed to determine the lower concentration of HCT in these tablets. The proposed methods permit direct determination of the two drugs in bulk powder or in binary mixtures without previous separations. Moreover, they have many advantages over the other separation techniques such as HPLC method. With these methods, one can gain the advantages of speed and reduced cost without scarifying accuracy.

Table 1: Analytical data for the calibration graphs (n=7) for the determination of EPM and HCT using ^{1}D, ^{1}DD and TLC methods

Parameters	Eprosartan mesylate (EPM)			Hydrochlorothiazide (HCT)		
	^{1}D	^{1}DD	TLC	^{1}D	^{1}DD	TLC
Wavelenth,nm	240.7	237.0	290.0	233.4	277.0	270.0
Concentration range [a]	1.0-18.0	2.0-18.0	2.0-20.0	1.0-9.0	1.0-9.0	2.0-9.0
Regression equation (Y) [b]						
Slope (b)	0.213	6.450	0.344	0.676	4.749	1.013
Intercept (a)	0.064	0.060	3.670	0.050	0.084	4.599
SD of the slope [c]	0.004	0.005	0.007	0.002	0.009	0.001
SD of the intercept [c]	0.016	0.020	0.003	0.004	0.010	0.002
Correlation coefficient (r)	0.9999	0.9998	0.9996	0.9999	0.9995	0.9994
LOD (µg mL^{-1})	0.100	0.080	0.150	0.040	0.050	0.130
LOQ (µg mL^{-1})	0.330	0.260	0.490	0.140	0.170	0.420
Accuracy (n= 5) [d]	99.86±0.70	100.92±0.82	100.17±0.85	100.01±0.92	100.21±0.63	99.89± 0.46
Intraday precision (n=9) [d]	100.09±0.14	100.23±0.44	100.90±0.95	99.69±0.40	100.19±0.28	100.74±0.93
Interday precision (n=9) [d]	99.42±0.61	99.83±0.72	100.48±0.37	99.88±0.27	99.83±0.67	100.42±0.73

[a] Drug concentration ,µg mL^{-1} (^{1}D and ^{1}DD methods) and µg/spot (TLC method).
[b] Y = a + bC, where Y is the peak amplitude (^{1}D and ^{1}DD methods) or the peak area x 10^{-3} (TLC method) and C is the drug concentration.
[c] Standard deviation of the slope or the intercept (n=7).
[d] Mean ± RSD (%).

Table 2: Determination of eprosartan mesylate (EPM) and hydrochlorothiazide (HCT) in laboratory prepared mixtures using ^{1}D, ^{1}DD and TLC methods

Methods		Recovery% [a]					
		^{1}D		^{1}DD			TLC[b]
Added (µg mL^{-1})		240.7 nm	233.4 nm	237.0 nm	277.0 nm	290.0 nm	270.0 nm
EPM	HCT	EPM	HCT	EPM	HCT	EPM	HCT
8	1	99.35	99.53	99.90	99.49	100.20	99.68
8	2	100.12	100.07	100.88	100.43	100.05	100.32
8	4	100.24	100.53	100.33	100.70	100.16	100.15
8	6	101.40	100.55	100.35	99.61	100.54	99.55
8	8	101.13	99.83	99.94	100.48	100.36	100.08
Mean recovery (%)		100.45	100.10	100.28	100.14	100.26	99.96
RSD (%)		0.82	0.44	0.39	0.55	0.19	0.33
2	8	99.90	99.65	99.84	100.07	99.84	100.00
4	8	99.92	100.25	99.48	100.23	99.68	100.08
6	8	98.89	99.31	100.14	99.69	100.05	99.67
8	8	100.25	99.75	100.09	99.87	100.13	99.52
10	8	100.12	99.68	99.90	100.10	99.91	100.02
Mean recovery (%)		99.82	99.73	99.89	99.99	99.92	99.86
RSD (%)		0.54	0.34	0.26	0.21	0.18	0.25

[a] Each sample was analyzed three times.
[b] In µg/spot (TLC method).

Table 3: Results of the determination of EPM and HCT using ^{1}D, ^{1}DD and TLC methods and compared HPLC methods in bulk powder and in commercial tablets.

Sample	Test (n=5)	^{1}D	^{1}DD	TLC	HPLC
Eprosartan mesylate (EPM)					
Bulk powder	Mean±RSD (%)	100.14±0.58	100.62±0.80	100.40±0.85	99.97±0.41[a]
	t	0.54	1.62	1.02	(2.306)[b]
	F	2.00	3.81	4.30	(6.390)[b]
Teveten tablets	Mean± RSD (%)	100.43±0.69	100.80±0.73	99.54±0.94	100.13±0.47[a]
	t	0.80	1.73	1.26	
	F	2.16	2.41	4.00	
	Recovery± RSD (%)	99.90±0.84	100.18±0.96	99.92±0.95	
Teveten plus tablets	Mean± RSD (%)	99.57±0.43	99.28±0.35	99.87±0.96	99.73±0.55[a]
	t	0.67	1.54	0.28	
	F	1.64	2.47	3.05	
	Recovery± RSD (%)	99.75±0.44	99.93±0.31	99.76±0.35	
Hydrochlorothiazide (HCT)					
Bulk powder	Mean± RSD (%)	100.10±0.41	100.32±0.54	99.88±0.46	99.93±0.68[C]
	t	0.48	1.00	0.14	
	F	2.75	1.59	2.19	
Teveten plus tablets	Mean± RSD (%)	–	–	100.41±0.64	
	Recovery±RSD(%)	–	–	99.86±0.25	

[a]HPLC method of Solvay Pharmaceutical Company.
[b]Theoretical values of t- and F-tests at 95% confidence level .
[c]The United States Pharmacopoeia 2009 [26].

Fig. ıol.

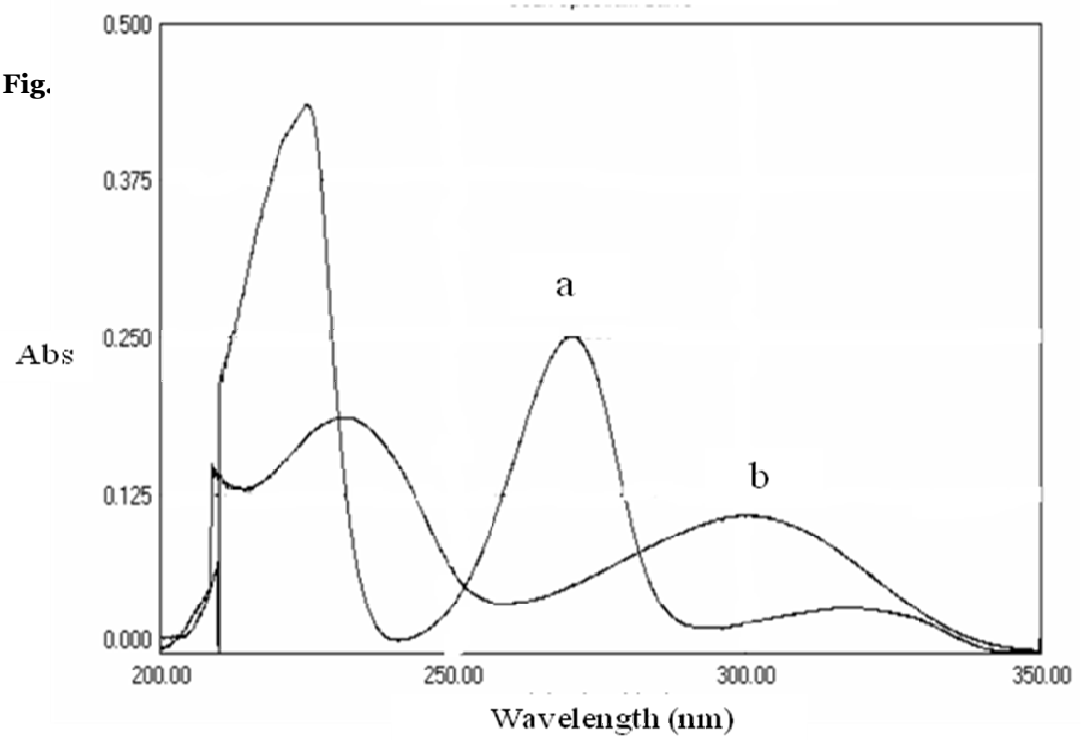

Fig.1: Zero order absorption spectra of: (a) 4 µg mL^{-1} HCT and (b) 4 µg mL^{-1} EPM in methanol.

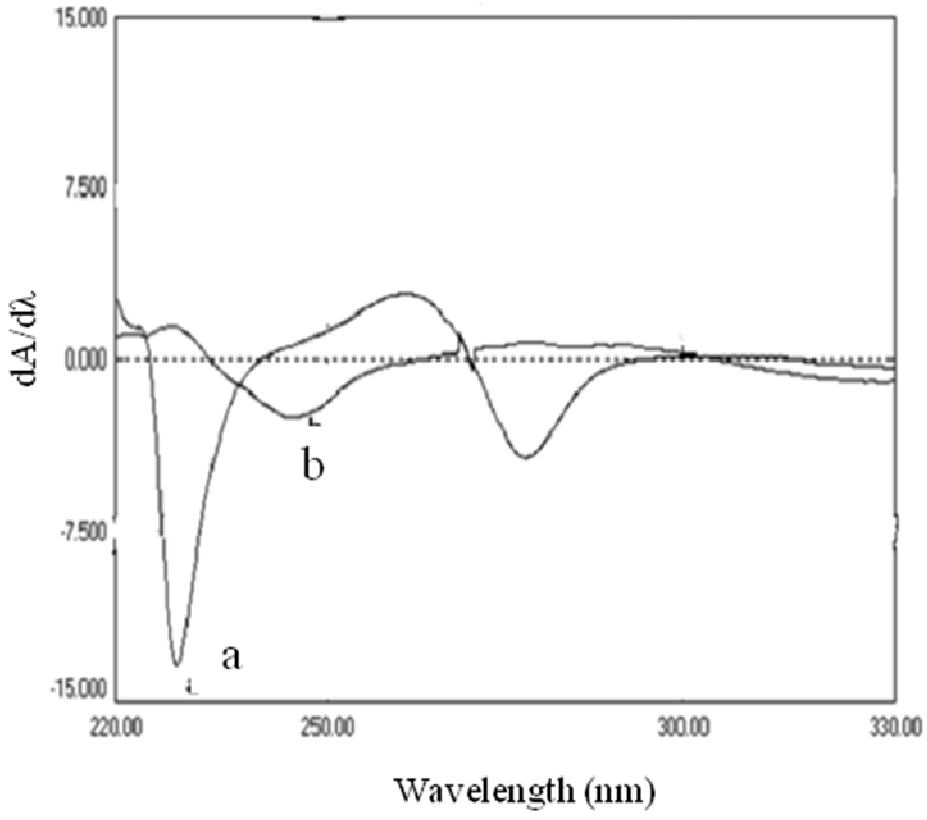

Fig.2: First derivative spectra (^{1}D) of: (a) 4 µg mL^{-1} HCT and (b) 4 µg mL^{-1} EPM in methanol.

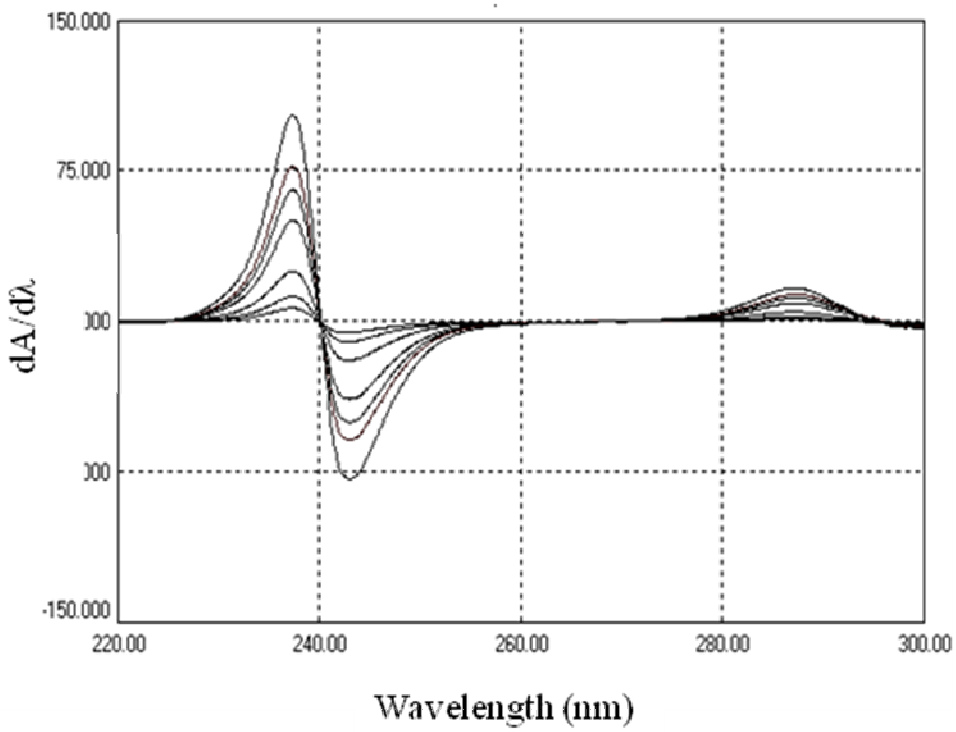

Fig.3: First derivative of ratio spectra (^{1}DD) for different concentrations of EPM (2.0- 18.0 µg mL^{-1}) using 8.0 µg mL^{-1} HCT as divisor in methanol at SF = 20 and Δλ = 21 nm.

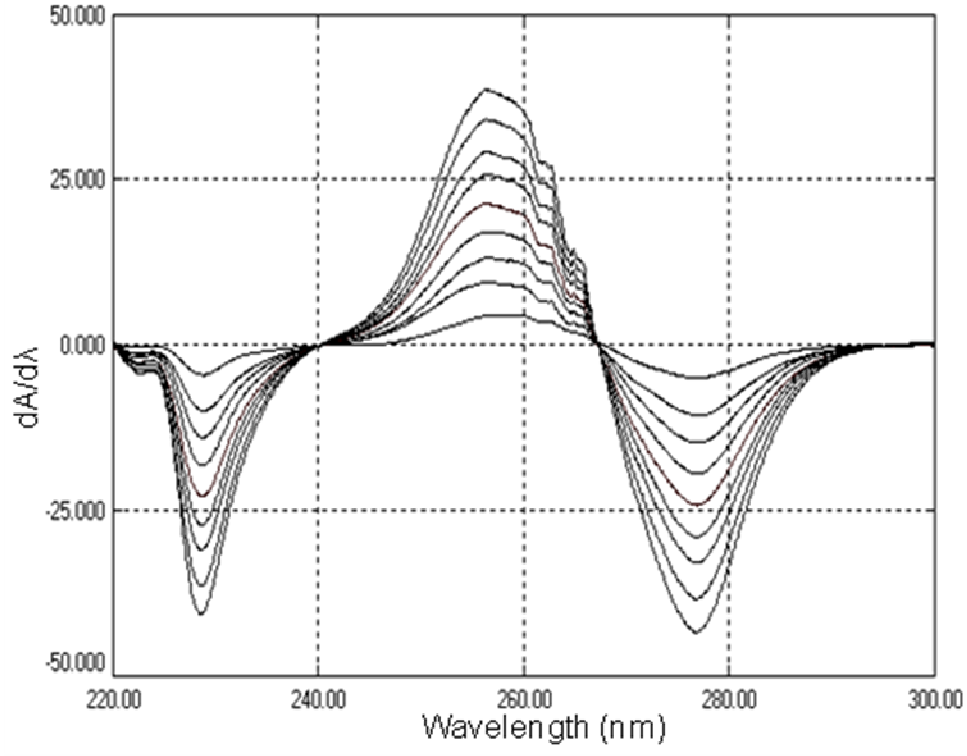

Fig.4: First derivative of ratio spectra (^{1}DD) of HCT (1.0-9.0 µg mL^{-1}) using 8.0 µg mL^{-1} EPM as divisor in methanol at SF=100 and Δλ =21 nm.

References

1) K. J. McClellan, J. A. Balfour, *Drugs*, 55, 713 (1998).
2) G. L. Plosker, R. H. Foster, *Drugs*, 60, 177 (2000).
3) G.W. Robbins, L. J. Scott, *Drugs*, 65, 2355 (2005).
4) S. Hillaert, T.R.M. De Beer, J.O. De Beer, W. Van den Bossche, *J. Chromatogr. A*, 984, 135 (2003).
5) S. Hillaert, W. Van den Bossche., *J. Chromatogr. A*, 979, 323 (2002).
6) S. Hillaert, W. Van den Bossche., *J. Pharm. Biomed. Anal.*, 31, 329 (2003).
7) D. E. Lundberg, C. R. Person, S. Knox, M. J. Cyronak., *J. Chromatogr. B*, 707, 328 (1998).
8) N. Ferreiros, G. Iriarte, R. M. Alonso, R. M. Jimenez, E. Ortiz, *J. Chromatogr. A*, 1119, 309 (2006).
9) N. Ferreiros, G. Iriarte, R. M. Alonso, R. M. Jimenez, *Talanta*, 69, 747 (2006).
10) C. Vetuschi, A. Giannandrea, *Anal. Lett.*, 36, 1051 (2003).
11) J. Joseph-Charles, S. Brault, C. Boyer, M.H. Langlois, L. Cabrero, J. P. Dubost, *Anal. Lett.*, 36, 2485 (2003).
12) N. Erk, *Pharmazie*, 58, 543 (2003).
13) N. Erk, *Pharmazie*, 58, 796 (2003).
14) S. A. Shah, I. S. Rathod, B. N. Suhagia, S.S. Savale, J. B. Patel, *J. AOAC Inter.*, 84, 1715 (2001).
15) E. Satana, S. Altinay, N. G. Goger, S. A. Ozkan, Z. Senturk, *J. Pharm. Biomed. Anal.*, 25, 1009 (2001).
16) S. A. Özkan, S. Akay, S. Cevheroglu, Z. Sentürk, *J. Liq. Chromatogr. Rel. Technol.*, 24, 869 (2001).
17) S. A. Özkan, *J. Liq. Chromatogr. Rel. Technol.*, 24, 2337 (2001).
18) N. Erk, *J. Pharm. Biomed. Anal.*, 24, 603 (2001).
19) G. Carlucci, G. Palumbo, P. Mazzeo, M. G. Quaglia, *J. Pharm. Biomed. Anal.*, 23, 185 (2000).
20) A. P. Argekar, J. G. Sawant, *Anal. Lett.*, 33, 869 (2000).
21) M. G. Quaglia, E. Donati, G. Carlucci, P. Mazzeo, S. Fanali, *J. Pharm. Biomed. Anal.*, 29, 981 (2002).
22) H.U. Patel, B.N. Suhagia, C.N. Patel, *Acta Chromatographica*, 21, 319 (2009).
23) A. El-Gindy, B. El-Zeany, T. Awad, M. M. Shaban, *J. Pharm. Biomed. Anal.*, 26, 203 (2001).
24) B. Benger, in: *Proceeding of the Six International Symposium on Instrumental planner Chromatography, Institute for Chromatography*, Bad Duerkheim 1991, p. 291.
25) The European Agency for the Evaluation of Medical Products. *ICH Topic Q2B Note for Guidance on Validation of Analytical Procedure: Methodology* GPMP/ICH/281/95, 1996.
26) The United States Pharmacopoeia, 32, NF 27, *United States Parmacopoeial Convention*, INC, USA 2009, p. 2567.