Turk J. Pharm. Sci. 5 (2) 53-66, 2008

Original Article

QUANTITATIVE DETERMINATION OF HYDROCHLOROTHIAZIDE AND SPIRONOLACTONE IN TABLETS BY SPECTROPHOTOMETRIC AND HPLC METHODS

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Abstract

In this study, simple, sensitive and reliable spectroscopic methods (absorbance ratio and Vierordt) were compared with HPLC method developed for quantitative determination of spironolactone (SPL) and hydrochlorothiazide (HCT) in commercial tablets.

260 nm was chosen as the isosbestic point in the absorbance ratio method. For Vierordt method; A_1^1 values were calculated at 242 and 269 nm for both substances and used for quantitative analysis of HCT and SPL in its binary mixture. Linearity ranges for HCT and SPL was 2-15 μg/mL and 2-12 μg/mL respectively for both methods. The relative standard deviations for absorbance ratio and Vierordt-methods were found to be 1.58 % and 1.32 % for HCT, 1.26 % and 1.52 % for SPL respectively. In HPLC method, HCT, SPL and Mefrusid (MFS) as an internal standard were determined by isocratic system using water-methanol-phosphate buffer (pH 3.0 ± 0.1) (71:25:4 v/v/v) as mobile phase and with Luna.C_{18} column. Linear concentration range was 5-25 μg/mL, 2-15 μg/mL for HCT and SPL respectively, and the relative standard deviations were found to be 1.39 % for HCT and 1.44 % for SPL respectively. Therefore, it is concluded that two spectroscopic methods and HPLC method can be used in routine simultaneous quantitative analyses of HCT-SPL in commercial tablets.

Keywords: *Hydrochlorothiazide, Spironolactone, Absorbance ratio, Vierordt, HPLC, Quantitative determination.*

Hidroklortiyazid ve Spironolakton İçeren Tabletlerde Spektrofotometrik ve Yüksek Basınçlı Sıvı Kromatografisi Yöntemleri İle Kantitatif Tayinler

Bu çalışmada hidroklortiyazid (HCT) - spironolakton (SPL) in tabletlerde kantitatif tayin için basit, duyarlı ve güvenilir spektrofotometrik yöntemler tarafımızdan geliştirilen yüksek basınçlı sıvı kromotografisi (YBSK) yöntemi ile karşılaştırılmıştır. Absorbans oranları yönteminde isobestik nokta olarak 260 nm seçilmiştir. Vierordt yönteminde ise her iki etken madde nin de 242 ve 269 nm'lerdeki A_1^1 değerleri hesaplanmıştır. Her iki yöntem de HCT ve SPL için doğrusal konsatrasyon aralığı sırası ile 2-15 μg/mL ve 2-12 μg//mL olarak bulunmuştur. Bağıl standart sapma değerleri absorbans oranları ve Vierordt yönteminde sırası ile HCT için % 1.58 ve % 1.32, SPL için % 1.26 ve % 1.52 olarak bulunmuştur.

YBSK yönteminde HCT, SPL ve Mefrusid (MFS, internal standart), Luna 18 kolonu üzerinde isokratik olarak su-metanol-fosfat tamponu (pH: 3 ± 0,1) (71:25:4 v/v/v) hareketli faz sisteminde tayin edilmiştir. Doğrusal konsantrasyon aralığı HCT ve SPL için sırası ile 5-25 μg/mL ve 2-15 μg/mL olarak saptanmıştır. Bağıl standart sapma değerleri HCT ve SPL için sırası ile % 1.39 ve % 1.44 olarak hesaplanmıştır.

Sonuç olarak önerilen iki farklı spektroskopik ve YBSK yöntemi HCT-SPL içeren tabletlerin rutin kantitatif analizlerinde kullanılabilir olduğu gösterilmiştir.

Anahtar kelimeler: *Hidroklorotiyazid, Spironolakton, Absorbans oranları, Vierordt, YBSK, Kantitatif tayin*

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INTRODUCTION

Spironolactone (SPL), (7α, 17α)-7-(acethylthio)-17-hydroxy-3-oxopregn-4-ene-21-carboxylic acid; a γ-lactone steroid with a structure resembling to that of the natural adrenocortical hormon, aldesterone, acts on the distal portion of the renal tubule as a competitive antagonist of aldesterone. It acts as potassium sparing diuretic increasing sodium and water excretion and reducing potassium excretion (1,2).

Hydrochlorothiazide (HCT), 6-chloro-3,4-dehydro-2H-1,2,4-benzothiadiazine-7-sulfonamid-1,1-dioxide, is moderately potent diuretic and exert their diuretic effect by reducing the reabsorbtion of electrolytes from renal tubules thereby increasing the excretion of sodium and chloride ions, consequently of water. Commercial preparations of SPL-HCT combinations have usually been used in the treatment of refractory oedema associated with heart failure and essential hypertension (1,2).

HCT has been used in combination with several drugs. Quantitative analysis of the binary mixtures containing HCT-Benazepril HCl is achieved by spectrophtometry (3-7), chemometry (8), TLC (9) and HPLC (3,5,9,10). In addition, quantitative analysis of binary mixtures of HCT- Amiloride has also been determined by spectrophotometry (11-14) and HPLC (15,16). HCT-Enalapril maleat mixtures are analyzed by derivative spectrophotometry (17,18) and HPLC (17-19).

The amount of HCT and Lisinopril in mixt solutions has been determined by spectrophotometry (20). HCT-Cilazepril combination is performed by spectrophotometry (21), voltametry (22), HPLC (23). The content of HCT – Fosinopril is determinated by spectrophotometry (24), derivative spectrophotometry and HPLC (25). HCT-Lasortan potassium combining tablets are analyzed by HPLC (26,27). The determination of HCT-Spironolactone in samples has been carried out by spectrophotometry (28-31), colorimetry, chemometric technique (32) and HPLC (33-35).

EXPERIMENTAL

Reagents and Chemicals

HCT and SPL were kindly donated by ARİS (Ali Raif İlaç Sanayi, Istanbul-Turkey). MFS used as the internal standard was obtained from Bayer Health Care (Istanbul-Turkey). All solvents and chemicals were of analytical grade in spectrophotometric methods and HPLC grade in HPLC method, all of them were purchased from Merck Company (Germany).

Instruments

HPLC Chromatograph (Thermo-Electron Corporation) equipped with a Finnigan Serveyor PDA detector, and UV-Vis Spectrophometer (A Beckman DU 650 series), double beam with a fixed slit width (2 nm) and 1 cm quartz cell employed over the range 200-400 nm, were used in the quantitative analysis of the samples.

Pharmaceutical Samples

Tablet formulation Aldactazide® (ARİS Istanbul Turkey) containing 25 mg HCT-25 mg SPL (Batch No 3D-318) and 50 mg HCT-50 mg SPL (Batch No 5B-226) were purchased from local pharmacies in Ankara-Turkey.

METHODS

Spectroscopic methods

Vierordt Method

The following stock solutions of HCT and SPL were prepared for the determination of A_1^1 (1 %, 1 cm) values.

Solution-HCT_1: 0.1 % w/v solution of HCT was prepared in methanol.

Solution-HCT_2: 10 ml of solution-HCT_1 was diluted to 100 ml with 0.1 N HCl.

Solution-SPL_1: 0.1 % w/v solution of SPL was prepared in methanol.

Solution-SPL_2: 10 ml of solution-SPL_1 was diluted to 100 ml with 0.1 N HCl.

20-30-40-60-80-100 µg/mL of HCT in 0.1 N HCl were prepared from the solution-HCT_1 by appropriate dilutions. The absorbances of solutions were measured at 242 nm. A series of 4-6-8-10-12 µg/mL of HCT in 0.1 N HCl were prepared from solution-HCT_2, and absorbances of these solutions were measured at 269 nm.

20-40-60-80-100 µg/mL solution of SPL in 0.1 N HCl were prepared by appropriate dilutions of from solution-SPL_1. The absorbances of prepared solutions were measured at 269 nm. The solution of SPL at concentration range of 5-10-15-20-25 µg/mL in 0.1 N HCl were also prepared from solution-SPL_2 by appropriate dilutions, and absorbance values of each solution were measured at 242 nm.

Absorbance Ratio Method

For obtaining standard calibration mixture of solution-HCT_2 and solution-SPL_2 at five different concentrations, different volumes of solution-HCT_2 and solution-SPL_2 were transferred into 100 ml volumetric flasks, and diluted to the volume with 0.1 N HCl (Table 1). The absorbances of these solutions were measured at 269, 242 and 260 nm (isosbestic point).

Table 1. Selected concentrations of HCT and SPL to prepare calibration graphs in both HPLC and Spectroscopic methods

Spectroscopic Methods			HPLC Method		
Synth. Stand. Mixture	**HCT (µg/mL)**	**SPL (µg/mL)**	**Synth. Stand. Mixture**	**HCT (µg/mL)**	**SPL (µg/mL)**
1	2	8	1	2.5	10
2	4	6	2	5	15
3	5	5	3	10	5
4	6	4	4	15	10
5	8	2	5	20	5
			6	25	2

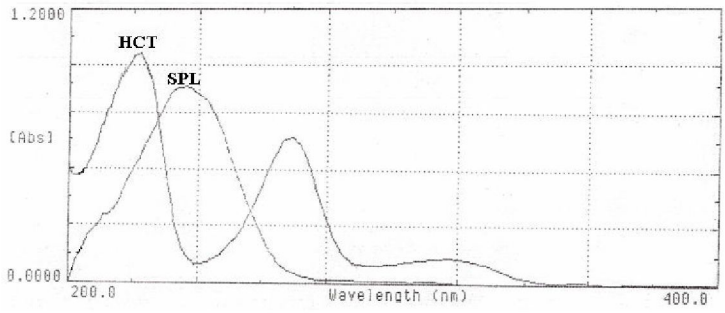

Figure 1. Zero-order spectra of HCT (10 μg/mL) and SPL (20 μg/mL) in 0.1 N HCl

HPLC method

Chromatographic condition

Chromatographic separation was carried out on Luna.C_{18} (250 x 2.6 mm, 5 μ) reversed phase column. HCT, SPL and MFS were separated by isocratic system using a mobile phase composed of water-methanol-phosphate buffer (pH 3.0 ± 0.1) (71:25:4 v/v/v). The mobile phase was prepared daily and filtered through an Altech 45 mm, 0.45 μm membrane and degassed for 15 minutes. Sample volumes of 10 μL were injected and the column eluent was monitored at 240 nm for twelve minutes.

Calibration for HPLC method

Stock solutions of 0.01% w/v HCT, SPL and 0.005 % (w/v) internal standard (MSF) were prepared in the mobile phase system. For obtaining standard calibration mixtures of stock solution HCT and SPL at six different concentrations, appropriate volumes of stock solutions of HCT and SPL were transferred into 10 mL volumetric flasks. Then 1 mL of internal standard solution was added to each flask and diluted with mobile phase.

Standard solutions of HCT and SPL were prepared at the concentration range of 2.5-25 μg/mL and 2-15 μg/mL, respectively (Table 1). Internal standard concentration was fixed at 5 μg/mL for each standard mixture and HPLC injections of 10 μL were made in triplicate for calculation of regression equations. The peak area ratios of active substances to internal standard were plotted against corresponding concentration of HCT and SPL separately.

Sample preparation

Twenty tablets were weighed and powdered. A portion of the powder equivalent to about 50 mg HCT was weighed accurately and transferred to a 50 mL volumetric flask and stirred with 40 mL methanol on a magnetic stirrer for 15 minutes. The solution was filtered and diluted up to 50 mL with methanol (Sample solution A). 2 mL of this solution was pipeted into 100 mL volumetric flask and completed with 0.1 N HCl to the point. Absorbance of the solution was measured at 269, 242 and 260 nm for absorbance ratio, and at 269 and 242 nm for Vierordt method.

For HPLC, 1 mL sample solution A and 1 mL internal standard solution (0.025 % w/v MFS in methanol) were pipeted into a 50 mL volumetric flask and completed with mobile phase to the point. 10 μL of sample solution was injected into the HPLC column.

RESULT AND DISCUSSION

The aim of this study was to develop new, simple, accurate, reproducible and sensitive spectrophotometric and HPLC methods for the simultaneous determination of HCT and SPL in commercial tablets.

In absorbance ratio method, 260 nm was chosen as the isosbestic point. Absorbance ratios of A_{269}/A_{260} for HCT were calculated by measuring the absorbances of different concentration of HCT and SPL at the wavelengths of 269, 242 and 260 nm. Then, using these absorbance ratios, the linear calibration curves were calculated and utilized for quantitative determination of HCT and SPL. To calculate regression equations, concentration ratios of C_{HCT} / $C_{HCT} + C_{SPL}$ for HCT, and the ratios of C_{SPL} / $C_{HCT} + C_{SPL}$ were used as the X-axis values. For the Y-axis values the ratios of A_{269} / A_{260} and A_{242} / A_{260} were used for HCT and SPL, respectively. Values for regression equations were given Table 2.

Table 2: The linear regression values of binary synthetic mixture (HCT and SPL) by spectroscopic and HPLC methods.

	Absorbance Ratio Method		Vierordt Method		HPLC Method	
Parameters	HCT	SPL	HCT	SPL	HCT	SPL
Linearitiy range (µg/mL)	2 – 15	2 – 12	2 – 15	2 – 12	5 – 25	2 –15
Regression equation*						
Slope (a)	1.2469	1.8186	-	-	0.04802	0.4412
St. error of slope	$2.73x10^{-3}$	$1.45x10^{-3}$	-	-	$1.07x10^{-4}$	$4.9x10^{-4}$
Intercept (b)	0.6013	0.0129	-	-	-0.0514	-0.510
St. error of intercept	$7.15x10^{-4}$	$1.12x10^{-4}$	-	-	$1.16x10^{-4}$	$6.68x10^{-4}$
Correlation coefficient (r^2)	0.9987	0.9982	-	-	0.9992	0.9986
Limit of quantitation (LOQ) (µg/mL)	1.5	0.8	1.5	0.8	0.5	0.2
Limit of dedection (LOD) (µg/mL)	0.5	0.3	0.5	0.3	0.2	0.1

* y=ax+b where;
(x) concentration ratio of HCT/HCT+SPL and SPL/HCT+SPL in absorbance ratio method
(y) absorbance ratio of A_{269}/A_{260} for HCT, A_{242}/A_{260} for SPL in absorbance ratio method
(x) concentration of HCT and SPL and (y) peak area ratios HCT/MFS and SPL/MFS in HPLC method

For the quantification of HCT and SPL concentrations in aliquots according to the absorbance ratio method, the following equation was used.

$$c = \frac{Q - b}{a} \times \frac{A_{iso}}{a_{iso}} \times 10^3$$

c = µg/mL

Where, Q is the ratio of A_{269} / A_{260} and A_{242} / A_{260} for HCT and SPL, respectively. (a) is the previously determined slope value for both of the active substances. (b) is the previously determined intercept value for both of the active substances. (A_{iso}) is the absorbance determined at 260 nm (isosbectic point) and (a_{iso}) is absorbtivity value determined at the isosbestic point. (a_{iso} was determined as 31.3 for this study)

The Vierordt method is another spectrophotometric method used for the determination of HCT and SPL in commercial tablets. The A^1_1 (1 %, 1 cm) values of HCT and SPL in 0.1 N HCl were determined at 269 and 242 nm, respectively. Determined α_1, α_2, β_1, β_2 values for HCT and SPL are listed in Table 3.

Table 3: A^1_1 values and other spectroscopic parameters for HCT and SPL in Vierordt method

α_1	α_2	β_1	β_2	$a = \alpha_2 / \alpha_1$	$b = \beta_2 / \beta_1$	m
618	53.5	75.8	452	0.0865	5.96	A_2 / A_1

α_1 = HCT 269 nm A^1_1 value in 0.1 N HCl, α_2 = HCT 242 nm A^1_1 value in 0.1 N HCl
β_1 = SPL 269 nm A^1_1 value in 0.1 N HCl, β_2 = SPL 242 nm A^1_1 value in 0.1 N HCl
A_1 = Total absorbance of binary mixture at 269 nm in 0.1 N HCl
A_2 = Total absorbance of binary mixture at 242 nm in 0.1 N HCl

Vierordt equations for HCT (A) and SPL (B) are shown below:

$$c_{HCT} = \frac{A_1}{\alpha_1\ 10^{-3}} \frac{b - m}{b - a} \qquad \textbf{(A)}$$

$$c_{SPL} = \frac{A_2}{\beta_2\ 10^{-3}} \frac{b(m - a)}{m(b - a)} \qquad \textbf{(B)}$$

c = mg/100 mL

. In HPLC method, MFS was chosen as the internal standard and calculated regression equations for each substance are given in Table 2. A mixture of water-methanol-phosphate buffer (pH 3.0) (71:25:4 v/v/v) was found to be an appropriate mobile phase for adequate separation of active substances and the internal standard. The separation profile in HPLC is shown in Figure 2.

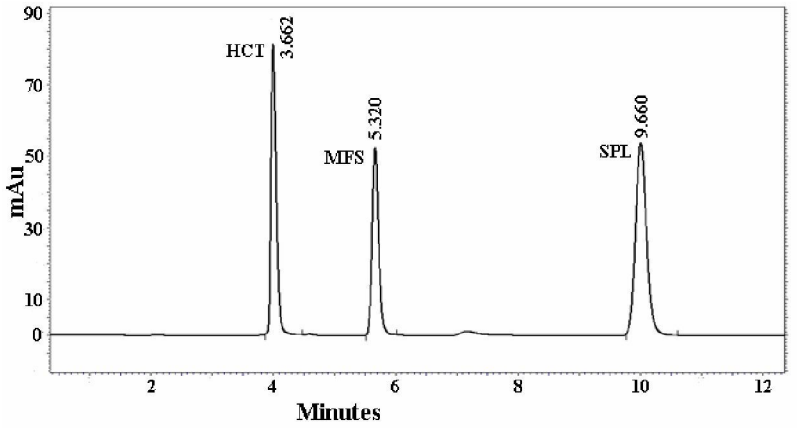

Figure 2. Chromatogram for a commercial tablet (20 μg/mL HCT, 20 μg/mL SPL and 5 μg/mL MFS)

Linearity

Absorbance Ratio Method

To examine the linearity parameter for validation of this method, two stock solutions of HCT_2 and SPL_2 were prepared in 0.1 N HCl at a concentration of 100 μg/mL separately. For obtaining standard calibration mixtures of solution-HCT_2 and solution-SPL_2, five different concentrations of each substance were prepared (Table 1). Absorbances of these solutions were measured at 269, 232 and 260 nm.

The statistical parameters and regression equations calculated from the calibration curves along with the standard error of the slope and intercept for HCT and SPL separately (Table 2).

HPLC Method

Standard solutions of HCT and SPL were prepared within the concentration range of 2.5-25 μg/mL and 2-15 μg/mL, respectively by using the related stock slutions. Internal standard (MFS) concentration was fixed at 5 μg/mL for each mixture. All appropriate dilutions were done with the mobile phase. 10 μL of each synthetic mixture was injected in triplicates in all applications were done. The peak area ratios of active substances to the internal standard were plotted against corresponding concentrations of HCT and SPL. The statistical parameters and regression equations for HCT and SPL were given in Table 2.

Recovery

Recovery experiments were conducted to determine the accuracy of the proposed methods. The studies were performed at a concentration of 10 μg/mL HCT + 10 μg/mL SPL and different concentrations of synthetic binary mixtures in 0.1 N HCL for Absorbance ratio and Vierordt methods (Table 4 and 5).

For HPLC method, the synthetic mixture at a concentration of 12 μg HCT + 8 μg SPL/mL prepared in the mobile phase was utilized. The mean recovery and RSD values were found to be 99.5 %, 2.26; 99.3 %, 1.63 and 99.6 %, 1.39 for absorbance ratio, Vierordt and HPLC methods for HCT, respectively. These values were found to be 98.9 %, 1.9; 98.3 %, 1.45 and 99.2 %, 1.44 for the mentioned three methods respectively for SPL (Table 4). The other recovery study was conducted on the synthetic mixtures at different concentration levels and the percentage recovery values and RSD % of HCT and SPL were calculated (Table 5).

Table 4: The result of recovery values of binary synthetic mixtures by proposed methods.

	Absorbance Ratio Method (μg/mL)		Vierordt Method (μg/mL)		HPLC Method (μg/mL)	
	HCT	**SPL**	**HCT**	**SPL**	**HCT**	**SPL**
	9.73	9.58	9.91	9.96	11.9	7.80
	9.80	9.78	9.79	9.81	11.84	7.97
	9.92	9.90	9.81	9.85	12.07	7.91
	10.24	10.06	10.17	9.89	12.23	7.93
	10.28	10.15	9.95	10.01	12.05	8.12
	9.95	9.85	9.87	9.96	11.78	8.05
	9.75	9.92	9.90	9.64	11.81	7.82
Mean	9.95	9.89	9.93	9.83	11.95	7.94
Recovery %	99.5	98.9	99.3	98.3	99.6	99.2
SD	0.225	0.188	0.162	0.144	0.164	0.116
RSD %	2.26	1.90	1.63	1.45	1.39	1.44

Table 5: The results of percentage recovery values different concentrations of binary synthetic mixtures by proposed method

	Added (μg/mL)		Found (μg/mL)		Recovery %	
Absorbance	HCT	SPL	HCT	SPL	HCT	SPL
Ratio	3	7	3.04	6.94	101.4	99.1
Method	5	5	4.93	5.03	98.4	100.6
	7	3	6.96	2.91	99.4	97
Mean	-	-	-	-	99.7	98.9
SD	-	-	-	-	1.58	1.24
RSD %	-	-	-	-	1.58	1.26
Vierordt	3	7	3.02	6.91	100.7	98.7
Method	5	5	4.9	4.96	98	99.2
	7	3	6.97	3.06	99.6	102
Mean	-	-	-	-	99.3	99.6
SD	-	-	-	-	1.31	1.52
RSD %	-	-	-	-	1.32	1.52
	5	5	4.97	4.93	99.1	98.7
HPLC	15	7.5	14.7	7.32	97.8	97.6
Method	25	15	25.4	15.2	101.6	101.3
	15	5	14.86	4.9	99.1	98.7
	15	10	15.04	9.85	100.3	98.5
	15	20	15.22	20.06	101.5	101.9
	5	10	5.06	9.79	101.2	97.9
	20	10	19.7	9.96	98.5	99.6
	25	10	24.65	10.12	98.6	101.2
Mean	-	-	-	-	99.7	99.5
SD	-	-	-	-	1.43	1.59
RSD %	-	-	-	-	1.43	1.60

Precision and Accuracy

The precision and accuracy of the assay was ascertained based on the analysis of binary synthetic mixtures. Sample concentrations of HCT and SPL were 3 – 7 μg/mL for the two spectroscopic methods in these methods.

In HPLC method, sample concentrations for HCT and SPL were 5 – 25 μg/mL and 5 – 20 μg/mL, respectively. Five replicate samples at each concentration were analyzed on three consecutive days and five replicate samples were analyzed on a third day after which inter- and intra-day means. Standard deviation and relative standard deviation were calculated by statistical methods. Results obtained in synthetic binary mixture were given in Table 6 and 7.

Table 6: Inter-day precision and accuracy for the determination of synthetic binary mixtures

	Added (μg/mL)		Found (μg/mL)		Precision				Accuracy	
					HCT		SPL		Bias %*	
	HCT	SPL	HCT	SPL	SD	RSD %	SD	RSD %	HCT	SPL
Abs.	3	7	2.96	6.94	0.072	2.43	0.131	1.88	-1.33	-0.86
Ratio	5	5	4.92	5.03	0.097	1.97	0.105	2.09	-1.60	0.6
Method	7	3	6.96	2.91	0.122	1.75	0.069	2.37	-0.71	-1.66
Vierordt	3	7	3.02	6.91	0.077	2.54	0.128	1.83	0.66	-1.28
Method	5	5	4.90	4.96	0.104	2.12	0.110	2.21	-2	-0.8
	7	3	6.97	3.06	0.115	1.65	0.066	2.15	-0.43	2
HPLC	5	5	4.97	4.93	0.087	1.75	0.077	1.56	-0.6	-1.4
Method	15	7.5	14.70	7.32	0.254	1.73	1.400	1.91	1	2.4
	25	12	24.65	11.74	0.313	1.27	2.100	1.79	-1.4	-2.16

*Bias: (Found – Added / Added) x 100, n=5

Table 7: Intra-day precision and accuracy for the determination of synthetic binary mixtures

	Added (μg/mL)		Found (μg/mL)		Precision				Accuracy	
					HCT		SPL		Bias %*	
	HCT	SPL	HCT	SPL	SD	RSD %	SD	RSD %	HCT	SPL
Abs.	3	7	2.94	7.12	0.084	2.86	0.151	2.12	-2	-1.71
Ratio	5	5	5.09	4.92	0.160	3.15	0.143	2.90	-1.80	-1.60
Method	7	3	7.17	3.05	0.220	3.07	0.084	2.75	2.43	1.66
Vierordt	3	7	2.96	6.93	0.059	1.99	0.187	2.70	-1.20	-1
Method	5	5	4.94	4.90	0.136	2.75	0.156	3.18	-1.20	-2
	7	3	7.05	7.10	0.223	3.15	0.219	3.08	0.714	1.43
HPLC	5	5	5.03	4.96	0.078	1.55	0.107	2.15	0.60	-0.80
Method	15	7.5	14.15	7.61	0.278	1.94	0.082	1.15	-0.86	1.47
	25	12	25.6	14.87	0.254	0.90	0.278	1.87	2.40	-0.87

*Bias: (Found – Added / Added) x 100
n: 5

Results of commercial preparations by application of three methods proposed in this study are shown in Table 8.

Table 8: Results obtained in commercial samples by using spectroscopic and HPLC methods

	Absorbance Ratio Method Found (mg)	Vierordt Method Found (mg)	HPLC Method Found (mg)
Sample A			
HCT mean[a] ± SD [b]	24.6 ± 0.46	24.5 ± 0.52	24.7 ± 0.29
SPL mean ± SD	25.2 ± 0.39	24.8 ± 0.63	25.1 ± 0.36
Sample B			
HCT mean[a] ± SD [b]	49.3 ± 0.92	49.8 ± 0.99	50.2 ± 0.85
SPL mean ± SD	49.9 ± 1.21	50.1 ± 1.15	49.9 ± 0.79

Sample A: Label claim 25 mg HCT + 25 mg SPL/tablet
Sample B: Label claim 50 mg HCT + 50 mg SPL/tablet
[a]: Result calculated are average of ten experiments for each technique
[b]: Standard deviation

Table 9: Statistical comparison of results in proposed methods.

	Sample 25 mg				Sample 50 mg			
	HCT		SPL		HCT		SPL	
	t Test	F Test	t Test	F Test	t Test	F Test	t Test	F Test
Vierordt-Abs. Ratio	0.437	2.65	0.582	3.05	0.805	1.95	0.875	4.15
Vierordt-HPLC	0.772	4.54	0.505	4.85	0.902	3.87	0.615	3.80
Abs. Ratio -HPLC	0.312	3.76	0.618	5.18	0.775	5.60	0.912	4.25

n: 10-2 = 8, p=0.05, $t_{\text{teoritical value}}$ 1.86, $F_{\text{teoritical value}}$ 6.39

The results obtained for Aldactazide® tablet (25 and 50 mg) were compared with Student's *t* test and Fisher test statistically. These results showed that the differences between the results of the methods were statistically insignificant (Table 9).

CONCLUSION

The content of HCT and SPL were simultaneously determined using spectroscopic and HPLC methods. Synthetic binary mixtures as well as commercial tablets were conveniently assayed.

Erk, N., (Ref. 31) applied the Vierordt method to the binary mixture of Spirinolactone and Hydrochlorothiazide in her study and the applied method is completely different from our suggested Vierordt method.

In our applied Vierordt method; (a) and (b) values calculated from A_1^1 ratios of active substances at maximum absorption wavelength (269 nm and 242 nm) and total absorption ratios at 269 and 242 nm were used as (m) value in Table 3. Concentration of active substances were calculated from Vierordt's formula using (a), (b) and (m) value (equation A and B). In Erk's study (31), calculated A_1^1 values for HCT and SPL at 272 and 240 nm (Ref. 31, Table 4) did not show correlation with spectrum given in Ref. 31, Fig. 1.6. Also, it isn't possible to reach the results given in Table 5, 6 and 7 (Ref. 31) by using Erk's calculated A_1^1 values.

Accurate and precise results were obtained by the proposed spectroscopic methods for the determination of HCT and SPL in pharmaceutical formulations. These methods are simple, rapid and inexpensive.

Results obtained by using spectroscopic methods did not show statistically significant difference when compared with our suggested HPLC method. In addition, in our HPLC method, we applied internal standard (Mefrusid) and analyses were carried out within twelve minutes. The resolution between HCT and SPL is R_T = 8.2 and the relative standard deviation for replicate injections are 1.15 %. The relative retention times are 1.0 min. for HCT and 2.63 min. for SPL.

The proposed spectroscopic and HPLC methods were successfully applied to the simultaneous quantitative determination of HCT and SPL in commercial preparations, tablet, marketed in Turkey.

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Received: 13.07.2007
Accepted: 02.04.2008