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Simple novel spectrophotometric methods for the determination of two antihypertensive mixtures containing hydrochlorothiazide in pharmaceutical dosage forms

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ABSTRACT

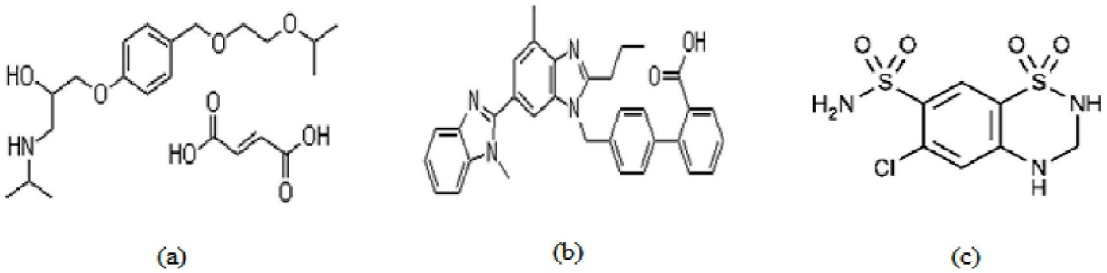
Three simple, rapid, accurate and precise spectrophotometric methods have been developed for the simultaneous determination of telmisartan/ hydrochlorothiazide and bisoprolol fumarate/ hydrochlorothiazide binary mixtures. The first is an isosbestic method for determination of telmisartan/ hydrochlorothiazide mixture in a concentration range of 2-40 $\mu g.ml^{-1}$ for both drugs. The absorbance of the zero order spectra at 266 nm (isosbestic point) was corresponding to the total concentration, while telmisartan was selectively determined from the amplitudes of the first derivative spectra (D^1) at 323 nm. The second is a ratio subtraction method for bisoprolol fumarate determination in presence of hydrochlorothiazide by measuring the absorbance at 224.4 nm where hydrochlorothiazide (10 $\mu g.ml^{-1}$) used as a divisor. The linearity was achieved over concentration range of 2-30 $\mu g.ml^{-1}$. The third is a dual wavelength method for hydrochlorothiazide determination in presence of bisoprolol fumarate without any interference in a concentration range of 1-20 $\mu g.ml^{-1}$. This was performed by measuring the absorbances difference (ΔA) at 266 and 277.8 nm, where the absorbances values of bisoprolol fumarate were the same. The proposed methods have been validated as per ICH guideline and were found to be valid and suitable for the assay of the cited drugs in raw materials and combined dosage forms.

KEYWORDS

Telmisartan;
Bisoprolol fumarate;
Hydrochlorothiazide;
Isosbestic point;
Ratio subtraction;
Dual wavelength.

INTRODUCTION

Bisoprolol fumarate (BF), 1-[4-(2-Isopropoxyethoxymethyl) phenoxy]-3-isopropylaminopropan-2-ol fumarate (2:1) salt, is a selective β1 adrenergic receptor blocker[1]. It is given in the management of hypertension and angina pectoris[2] (Figure 1a). Telmisartan (TLEM) is 4'[(1, 4'-Dimethyl-2'-propyl [2, 6'-bi-1H-benzimidazol]-1'-yl) methyl] [1, 1'-biphenyl]-2-carboxylic acid, acts as an angiotensin II receptor blockers, used mainly for treatment of hypertension[2] (Figure 1b), while, hydrochlorothiazide (HCTZ) is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide 1, 1-dioxide, a drug belonging to thiazide diuretics class of antihypertensive drugs. It is effective in the treatment of hypertension[1]



(a) (b) (c)

Figure 1: Chemical structure of: (a) bisoprolol fumarate, (b) telmisartan and (c) hydrochlorothiazide.

(Figure 1c).

Several methods have been reported in literature for the determination of bisoprolol fumarate including electrochemical methods alone[3] or in combination with other β blockers[4,5], high-performance liquid chromatographic methods in pharmaceutical formulations alone[6] or with other cardiovascular drugs as celiprolol, oxprenolol[7] and HCTZ[8,9], in waste water[10], in river water[11]and in human plasma and urine alone[12-15] or in combination with HCTZ [16,17] and other cardiovascular drugs[18,19]. Alone or in combination with HCTZ, telmisartan has been determined by derivative spectrophotometric[20], spectrofluorimetric[20], electrochemical[21,22], immunoassay[23], titrimetric[24], capillary zone electrophoretic[25-27], high performance thin layer chromatographic[20] and high performance liquid chromatographic[28-33] methods.

The main goal of this work is to establish simple, rapid, accurate, precise, and low cost spectrophotometric methods for the simultaneous determination of telmisartan/ hydrochlorothiazide and bisoprolol fumarate/ hydrochlorothiazide binary mixtures which can be adopted for the routine quality control analysis of the investigated drugs without prior separation in raw material and pharmaceutical preparations.

EXPERIMENTAL

Instrumentation

Double-beam UV-Visible spectrophotometer (Shimadzu, Japan) model UV-1601 PC with quartz cell of 1 cm path length, connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used. The spectral bandwidth was 0.2 nm and the wavelength scanning speed was 1000 nm.min^{-1}. The measurements were done at 25.0 ˚C, using $\Delta\lambda$= 4 nm and scaling factor of 10 for computing first derivative (D^1).

Materials and reagents

TLEM was kindly supplied by National Organization for Drug Control and Research-Egypt and certified to contain 99.90% while, BF and HCTZ were kindly provided by Hikma Pharmaceuticals-Egypt and certified to contain 99.70% and 99.75%, respectively.

Micardis plus® tablets (Boehringer Ingelheim Pharmaceutical Company) containing 40 mg TELM and 12.5 mg HCTZ per tablet. Concor plus® tablets (Serono Pharmaceutical Company) containing 5 mg BF and 12.5 mg HCTZ per tablet.

All chemicals and reagents used through this work are of spectroscopic analytical grade. Methanol (Sigma-Aldrich, Germany) and Sodium hydroxide (BDR) (Prolabo), aqueous 0.1M were used.

Standard solutions and laboratory prepared mixtures

Stock standard solutions

Stock standard solutions of TELM, BF and HCTZ, each having concentration of 1.0 mg.ml^{-1}, were prepared by dissolving 100.0 mg of each drug in sufficient amount of 0.1 M NaOH and methanol for TELM/ HCTZ and BF/HCTZ mixtures, respectively. The volumes were completed to 100-ml volumetric flask with the same solvent.

Working standard solutions

5 ml of each TELM, BF and HCTZ standard stock solution (1.0 mg ml^{-1}) was transferred separately into 100 ml volumetric flask and the volume was completed to the mark with 0.1 M NaOH and methanol for TELM/ HCTZ and BF/HCTZ mixtures, respectively to obtain 50.0 µg ml^{-1} working solution each.

Laboratory prepared mixtures

Mixtures containing different concentrations of each

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drug working solution (50.0 μg ml^{-1}) were prepared using 0.1 M NaOH and methanol for TELM/HCTZ and BF/HCTZ mixtures, respectively as diluents.

PROCEDURES

Construction of calibration curves

Isosbestic method

Accurately measured volumes (0.4 – 8.0 ml) of each of TELM and HCTZ working solution (50.0 μg.ml^{-1}) were transferred separately into a series of 10-ml volumetric flasks, diluted to the volume with 0.1M NaOH. The zero order absorption spectra of each solution were recorded against 0.1M NaOH and stored; then the absorbance at the isosbestic point (A_{iso}) was measured at 266 nm for both drugs. Two calibration curves were constructed relating the absorbance at the selected wavelength to the corresponding drug concentration. While for TELM, the amplitude of first derivative spectrum (D^1) at 323 nm were recorded using $\Delta\lambda$ = 4 nm and scaling factor of 10 and plotted against their corresponding concentrations. The regression equations were then computed.

Ratio subtraction method

Accurately measured volumes (0.4 – 6.0 ml) of BF working solution (50.0 μg.ml^{-1}) were transferred into a series of 10-ml volumetric flasks, diluted to the volume with methanol. The calibration curve, representing the relationship between the absorbance of zero order spectra at λ_{max} = 224.4 nm for BF and the corresponding concentration, was plotted and the linear regression equation was computed.

Dual wavelength method

Accurately measured volumes (0.2 – 4.0 ml) of HCTZ working standard solution (50.0 μg.ml^{-1}) were transferred into a series of 10-ml volumetric flasks, diluted to the volume with methanol. The zero order absorption spectra were then recorded using methanol as a blank. The calibration curve, representing the relationship between the difference in the absorbance of zero order spectra of HCTZ at λ= 266 nm and λ= 277.8 nm and the corresponding concentration, was plotted and the linear regression equation was computed.

Application to pharmaceutical formulations

An accurately weighed portion equivalent to one tablet of the mixed contents of twenty Micardis plus® and Concor plus® tablets was separately transferred into a 50-ml volumetric flask using about 25 ml 0.1 M NaOH (in Micardis plus®) and methanol (in Concor plus®), shaking for 15 minutes and completed to volume with the same solvent. The contents of the flask were mixed well and filtered. Aliquots of the filtrate were diluted to obtain final concentration within the linear ranges mentioned in TABLE 1 and then assayed using the proposed methods.

RESULTS AND DISCUSSION

Spectrophotometric characteristics

The zero order spectra of the selected drug mixtures show high degree of spectral overlapping which interfered with their simultaneous determination (Figure 2 and Figure 3). This spectral overlapping is sufficient to demonstrate the resolving power of the proposed spectrophotometric methods which can resolve bands overlapping, without prior separation.

Isosbestic method

An isosbestic point is the wavelength at which the absorbances of two or more species are the same. At the isosbestic point, the mixture of drugs acts as a single component and gives the same absorbance value as pure drug. The zero order spectra of TELM and HCTZ

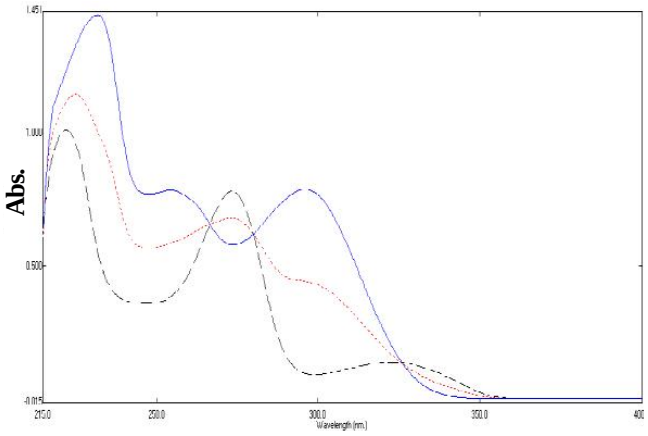

Figure 2 : Zero order spectra for telmisartan (—), hydrochlorothiazide (——) (16.00 μg.ml^{-1} each) and their mixture (….) (8.00 μg.ml^{-1} of each of telmisartan and hydrochlorothiazide) using 0.1M NaOH as a blank.

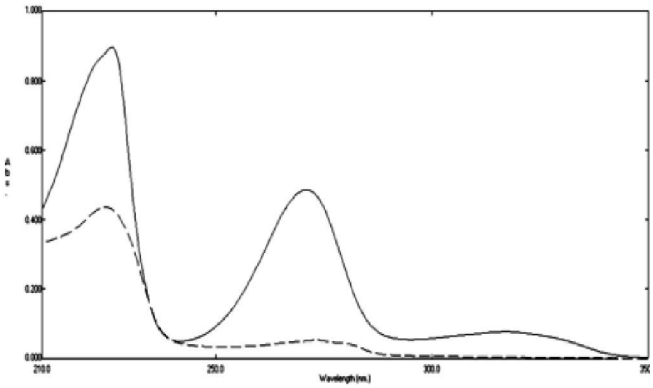

Figure 3 : Zero order spectra for bisoprolol (——), hydrochlorothiazide (—) (10.00 µg.ml^{-1} each) using methanol as a blank

showed three isosbestic points (Figure 2). Thus, by measuring the absorbance value of the mixture at the chosen isosbestic point 266 nm (A_{iso}), the total concentration of both TELM and HCTZ could be calculated. While the first derivative (D^1) spectra (Figure 4) of TELM and HCTZ showed better resolution that allowed the determination of the concentration of TELM in the mixture, without any interference from HCTZ. $\Delta\lambda$ was optimized to give a well resolved peak and thus high selectivity was obtained. $\Delta\lambda$ = 4 nm was selected without smoothing and scaling factor was 10.

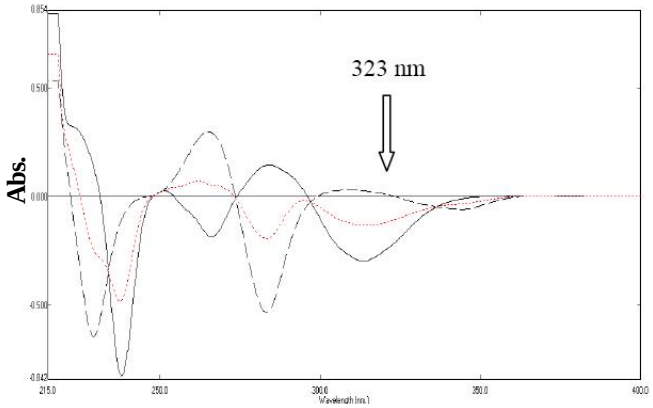

Figure 4: First order derivative spectra for telmisartan (—), hydrochlorothiazide (——) (16.00 µg.ml^{-1}, each) and their mixture (….) (8.00 µg.ml^{-1} of each of telmisartan and hydrochlorothiazide).

TELM was determined by measuring the values of the D^1 amplitudes at 323 nm that corresponds to zero crossing of HCTZ. And then the concentration of HCTZ could be calculated by subtraction, thus both drugs were measured simultaneously in their mixtures.

Ratio subtraction method

The spectrum of HCTZ is extended more than that of BF which permits the use of ratio subtraction technique for the determination of BF without any interference from HCTZ.

The determination of BF could be achieved by scanning the zero order absorption spectra of the mixtures (Figure 3), then dividing them by a carefully chosen concentration (10.0 µg.ml^{-1}) of HCTZ (I^2 = divisor) to produce a new ratio spectra that represents II/I2 + constant, as shown in (Figure 5); then, subtraction of the absorbance values of these constants (I/I2) in the plateau as shown in (Figure 6) followed by multiplication of the obtained spectra by the divisor (I2) as shown in (Figure 7); finally, the original spectra of BF (II), which were used for direct determination of BF (II) at 224.4 nm, could be obtained. This can be summarized as follows:

(II + I) / I2 = II/I2 + II/I2 = II/I2 + constant

II/I2 + constant - constant = II/I2

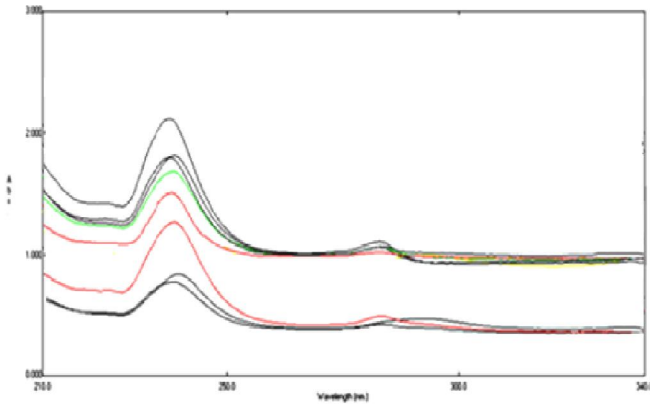

Figure 5 : Zero order absorption spectra for different mixtures of bisoprolol and hydrochlorothiazide using hydrochlorothiazide (10 µg.ml^{-1}) as a divisor.

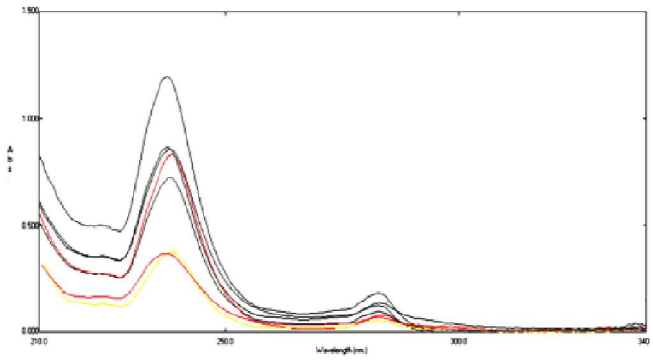

Figure 6 : Zero order absorption spectra for different mixtures of bisoprolol and hydrochlorothiazide using hydrochlorothiazide (10 µg.ml^{-1}) as a divisor after subtraction of constant.

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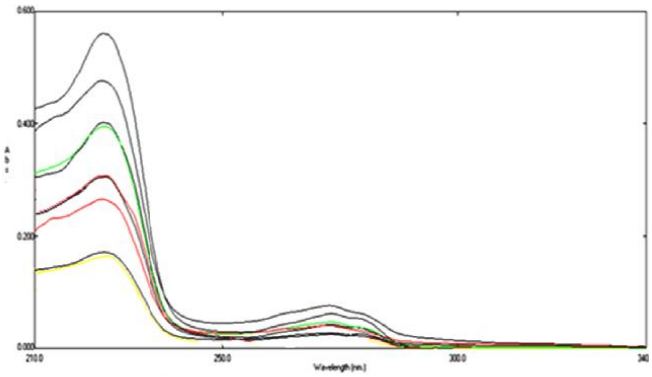

Figure 7 : Zero order absorption spectra for different mixtures of bisoprolol and hydrochlorothiazide using hydrochlorothiazide (10 µg.ml^{-1}) as a divisor after subtraction of constant and multiplying with the divisor.

II/I2 * I2 = II

The constant can be determined directly from the curve (II + I)/I2 (Figure 5) by the straight line which is parallel to the wavelength axis in the region where (I) is extended. Careful choice of the divisor (I2) is of great importance. So different concentrations of HCTZ were tried, the best one was 10.00 µg.ml^{-1} as it gave the best compromise in terms of sensitivity, repeatability and signal to noise ratio.

Dual wavelength method

The absorbances values of BF were the same at 266 and 277.8 nm, so the absorbance difference at these two wavelengths was corresponding to HCTZ concentration without any interference of BF (Figure 3).

METHOD VALIDATION

Linearity

Under the described experimental conditions, standard calibration curves were constructed for TELM, BF and HCTZ in the two mixtures versus concentration. The statistical parameters and regression equations calculated from the calibration graphs are shown in TABLE 1. The results obtained showed that linearity of the calibration graphs and the compliance with Beer's law were validated, as illustrated by the high values of correlation coefficients of regression equations and the small values of intercepts.

Limits of detection and quantification

In accordance with the formulae given by Miller[34], the limit of detection, LOD = 3.3 S/b and the limit of quantification, LOQ = 10 S/b, where S is the standard deviation of intercept and b is the slope of the calibration graph. The detection and quantification limits were calculated and presented in TABLE 1.

Accuracy

The methods were repeated for different concentrations of pure samples at the low, medium and high concentration levels, each repeated three times. The concentrations were calculated each from its corresponding regression equation. The mean recovery percentages and the standard deviations were then calculated and the results show good accuracy as were shown

TABLE 1 : Regression equations and statistical parameters for the determination of the two mixtures in pure powder form using the proposed spectrophotometric methods

Parameters	Isosbestic point			Ratio subtraction	Dual wavelength
	TELM		HCTZ	BF	HCTZ
	λ_{266}	λ_{323}	λ_{266}		
Linearity range (µg/ml)	2 - 40	2 - 40	2 - 40	2 - 30	1 - 20
LOD (µg/ml)	0.42	0.25	0.32	0.15	0.13
LOQ (µg/ml)	1.26	0.77	0.97	0.46	0.39
Intercept (a)	0.0081	0.0028	0.0073	-0.0004	-0.0039
Slope (b)	0.0411	0.0135	0.0417	0.0425	0.0125
Correlation coefficient (r)	0.9998	0.9999	0.9999	0.9999	0.9999
S_a	0.00517	0.001035	0.00405	0.00196	0.00049
S_b	0.000271	0.0000542	0.000199	0.000119	0.0000515

Regression equation: A = a + bc, where A is the absorbance, a is the intercept, b is the slope and c is the concentration; S_a: standard deviation of intercept; S_b: standard deviation of slope.

TABLE 2 : Accuracy and precision for the determination of TELM/HCTZ and BF/HCTZ mixtures using the proposed spectrophotometric methods

Parameters	Isosbestic point			Ratio subtraction	Dual wavelength
	TELM		HCTZ	BF	HCTZ
	λ_{266}	λ_{323}	λ_{266}		
Accuracy [a]	99.76 ± 0.83	99.99 ± 0.44	99.74 ± 0.70	100.03 ± 0.82	99.80 ± 0.52
Specificity [b]	100.23 ± 1.21		99.31 ± 0.75	99.81 ± 0.79	99.62 ± 0.89
Precision:					
Repeatability [c]	100.43 ± 0.78	99.72 ± 1.03	99.18 ± 0.82	99.23 ± 1.37	100.70 ± 0.69
Intermediate precision [c]	99.76 ± 1.12	100.54 ± 0.62	99.16 ± 0.81	99.90 ± 1.85	99.45 ± 0.97

[a] Mean ± SD; [b] Mean ± RSD%.; [c] Mean ± RSD%

in TABLE 2.

Precision

To measure the degree of methods repeatability, three concentrations of each drug in each mixture were analyzed using the previously mentioned methods three times within the same day and in three successive days. The results showed good precision expressed as percentage relative standard deviation (TABLE 2)

Selectivity and specificity

This was assessed through analysis of different synthetic mixtures containing TELM with HCTZ or BF with HCTZ with different ratios by the proposed methods. The concentration of each drug was calculated from the corresponding regression equation and then the mean

TABLE 3 : Simultaneous determination of telmisartan (TELM) and hydrochlorothiazide (HCTZ) in pharmaceutical formulation by isosbestic point method

Product	Isosbestic point %Recovery*		Standard addition technique			
			TELM		HCTZ	
	TELM	HCTZ	Added (µg/ml)	% Recovery*	Added (µg/ml)	% Recovery*
Micardis 40 plus®	99.75 ± 0.90	99.6± 0.68	5	99.4	1	99.00
			6	100.67	2	98.50
			8	99.75	2.5	100.40
			9	100.60	3	98.27
			10	100.50	4	99.25
Mean ± SD				100.18 ± 0.56		99.08 ± 0.83
RSD%				0.56		0.84

* Average of three determinations.

TABLE 4 : Determination of bisoprolol fumarate (BF) and hydrochlorothiazide (HCTZ) in pharmaceutical formulation by the proposed methods.

Product	BF by ratio subtraction			HCTZ by dual wavelength		
	% Recovery*	Standard addition		% Recovery*	Standard addition	
		Added (µg/ml)	% Recovery*		Added (µg/ml)	% Recovery*
Concor 5 plus®	98.20 ± 0.28	4	101.20	100.18 ± 0.75	5	99.71
		5	99.40		6	98.52
		6	101.07		7	98.43
		10	100.30		8	99.90
Mean ± SD			100.49 ± 0.83			99.25 ± 0.72
% RSD			0.83			0.73

* Average of three determinations.

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recovery percentages and the relative standard deviations were calculated. The results showed good specificity as shown in TABLE 2.

Standard addition technique

Standard addition technique was applied to the commercial tablets, by adding TELM and HCT (in case of Micardis plus® tablets) or by adding BF and HCT (in case of Concor plus® tablets) to a known concentration of the studied drugs. The mean recovery percentages and the relative standard deviations results, presented in TABLE 3 and TABLE 4, showed the high reproducibility and accuracy of the proposed methods.

Comparison of the proposed methods with the reported method

The results obtained by applying the proposed methods were compared statistically with those obtained from a reported method [35] for TELM/HCTZ mixture and USP pharmacopeial method[9,36] for BF/HCTZ mixture. It is clear that the calculated *t* and *F* values are less than the tabulated ones indicating that there is no significant difference between the methods (TABLE 5).

TABLE 5 : Statistical comparison of the results obtained by applying the proposed methods and the reference methods for the analysis of the pure drugs.

Value	TELM		HCTZ		BF		HCTZ	
	Isosbestic point	Reference method*	Isosbestic point	Reference method*	Ratio subtraction	USP method	Dual wavelength	USP method
Mean	99.76	99.27	99.74	99.48	100.03	100.68	99.80	99.57
SD	0.68	0.51	0.33	0.38	0.82	0.83	0.52	1.00
N	8	6	7	6	7	6	7	6
Variance	0.54	0.26	0.49	0.14	0.67	0.69	0.27	1.00
t –test	1.38 (2.18)	-	0.85 (2.26)	-	1.37 (2.23)	-	0.52 (2.36)	-
F value	2.68 (4.87)	-	3.42 (4.95)	-	1.02 (5.05)	-	3.62 (4.38)	-

Values in parenthesis are the theoretical values of t and F at P = 0.05; * Inertsil ODS-C18 (250mmX4.6mm) using (40: 60, v/v) phosphate buffer of pH 3.0 and acetonitrile in an isocratic program with flow rate 1.0 ml/min and UV detection was performed at 271 nm. The retention times observed were 5.79 min and 2.85 min for telmisartan and hydrochlorothiazide respectively.

ASSAY OF PHARMACEUTICAL PREPARATIONS

The results obtained were satisfactory and in good agreement with the label claim (TABLE 3, TABLE 4). The results of analysis of the pharmaceutical formulations suggested that there is no interference from any excipients.

CONCLUSION

The proposed methods provide simple, rapid, accurate and reproducible quantitative analysis for the determination of HCTZ with TELM or BF as binary mixtures in dosage forms, without any interference from excipients or prior separation. They are also direct since they estimate each drug independent of the other. In addition, they offer the advantage of time and cost saving as well as simplicity of reagents and apparatus. The methods are validated and found suitable for quality control laboratories, where economy and time are essential.

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