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Simultaneous Determination Of Atenolol And Hydrocholrothiazide In Tablets Formulation By Derivative Spectrometry

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ABSTRACT

The derivative spectrophotometric method was developed and applied for the simultaneous determination of Atenolol (ATE) and Hydrochlorothiazide (HCT) in Tablets formulations. The first derivative spectrophotometric (^{1}DS) method was applied for the determination of (ATE) and (HCT), respectively. (ATE) was determined at 271.9 nm ($^1D_{271.9}$) and (HCT) was determined at 279.3 nm ($^1D_{279.3}$). Linearity showed a good correlation coefficients R^2 = 0.9994 and R^2 = 0.9989 for (ATE) and (HCT), respectively. Linearity ranges were (10 – 280) µg/mL for (ATE) and (2 – 20) µg/mL for (HCT). The limit of detection (LOD) and limit of quantification (LOQ) were to be 2.77 µg/mL and 8.38 µg/mL for (ATE), 0.52 µg/mL and 1.59 µg/mL for (HCT), respectively. The proposed first derivative method was successfully applied to determine (ATE) and (HCT) in one Syrian trademark drug such as: (NORMOTIC 100 mg (ATE) and 25 mg (HCT)/tab.). All studied samples showed that the drug level was conformed to British Pharmacopeia.

KEYWORDS: Atenolol, Hydrochlorothiazide, Derivative Spectrophotometry**.**

INTRODUCTION

Atenolol (ATE) chemically, 4-(2-hydroxy-3-isopropyl aminopropoxy)-phenyl acetamide is a β-adrenoreceptor blocking agent, primarily used in hypertension, angina pectoris and myocardial infraction. It mainly acts by inhibition of rennin release and angiotensin-2 & aldosterone production. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilizing properties.

Hydrochlorothiazide (HCT), 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, which is widely used in antihypertensive pharmaceutical preparations, reduces active sodium reabsorption and peripheral vascular resistance.

The review of the literature revealed that no method is yet reported for the simultaneous estimation of both drugs in combined dosage forms. Present work describes two simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of (ATE) and (HCT) in tablets formulation[1].

Thus, various methods have been proposed to determine the amount of (ATE) and (HCT) in some pharmaceutical formulations, such as reverse phase high performance liquid chromatographic method (RP-HPLC)[2-3-4], high performance liquid chromatographic method (HPLC)[5], spectrophotometric method (UV)[1-6-7], Liquid chromatographic method (LC)[8], Ultra performance liquid chromatographic method (ULPC)[9] are successfully applied to determine the two compounds.

The aim of this work is to develop a simple and accurate spectrophotometric method for simultaneous determination of (ATE) and (HCT) in one pharmaceutical formulations without prior treatment by derivative spectrophotometry (^{1}DS).

MATERIALS AND METHODS

Apparatus

All spectral measurements were carried out using a T80+, UV/Vis spectrophotometer PG instrument Ltd (UK), connected to computer, quartz cells 1 cm. Ultrasonic bath Daihan (China), and stirrer Velp Scientifica (Europe).

Chemical regents:

Methanol from LOBAL Chemie (INDIA), Hydrochloric acid from SURCHEM PRODUCTS LTD (ENGLAND), Hydrochlorothiazide purity 99.38 % was obtained from China and Atenolol purity 100.42 % was obtained from India, Double distilled water.

Stock standard preparation

Stock solution 1.5 × 10^{-2} M of Atenolol (M_W = 266.341 g/mol) was prepared by dissolving 400 mg of Atenolol standard material in volumetric flask 100 mL of Methanol. The working standard solutions were prepared by appropriate dilutions of stock solution 1.5 × 10^{-2} M with (0.1 N) HCl to give concentrations between (10 - 280) □g/mL of (ATE).

Stock solution 6.76 × 10^{-4} M of Hydrochlorothiazide (M_W = 297.73 g/mol) was prepared by dissolving 20 mg of Hydrochlorothiazide equivalent to 20.125 mg (after taking the purity in consideration) in volumetric flask 100 mL of Methanol. The working standard solutions were prepared by appropriate dilutions of stock solution 6.7 × 10^{-4} M with (0.1 N) HCl to give concentrations between (2 - 20) □g/mL of (HCT).

Calibration Curve

To construct the calibration curve, ten standard solutions for each concentration were prepared and the absorbance was measured of each solution five times.

Sample preparation

One Syrian product was studied:

Ten NORMOTIC tablets were weighed and finely powdered and an accurate weight equivalent to one tablet 100 mg (ATE) and 25 mg (HCT) was accurately weighed and dissolved in 25 mL Methanol. The sample solution was filtered through a filter papers (Whatman 3, England). Then 0.1 mL was taken to 10 mL volumetric flask and adjusted to volume with (0.1 N) HCl. It was theoretically equivalent to 40 □g/mL of (ATE) and 10 □g/mL of (HCT).

RESULTS AND DISCUSSION

Absorption spectra of the standard raw material 120 □g/mL (ATE) and 10 □g/mL (HCT) solutions were recorded within a wavelength range of (265 - 300) nm against the blank: (Methanol + (0.1N) HCl). It was noticed that (ATE) and (HCT) cannot be determined by direct measurement, because of the overlapped spectra.

On the other hand, derivative spectrophotometry showed more resolution, where the determination of (ATE) and (HCT) mixture was possible without pretreatment.

The first derivative spectrum at zero-crossing point was used to determine (ATE) in the presence of (HCT) at 271.9 nm

"Fig. 1, a", and determine (HCT) in the presence of (ATE) at 279.3 nm "Fig. 1, b".

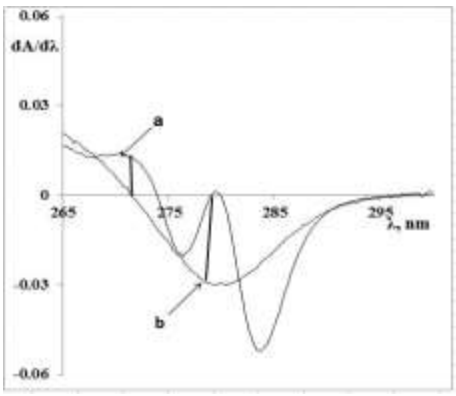

Fig. 1: First derivative spectra of: a- (ATE), b- (HCT).

METHOD VALIDATION

The validity of the proposed method was assessed by accuracy (reported as recovery percentage), precision (reported as RSD %), linearity (evaluated by regression equation), limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The concentration linearity of (ATE) was in the range (10 - 280) □g/mL at 271.9 nm by $^1D_{271.9}$," Figs. 2,3" and the concentration linearity of (HCT) was in the range (2 - 20) µg/mL at 279.3 nm by $^1D_{279.3}$, "Figs. 4, 5".

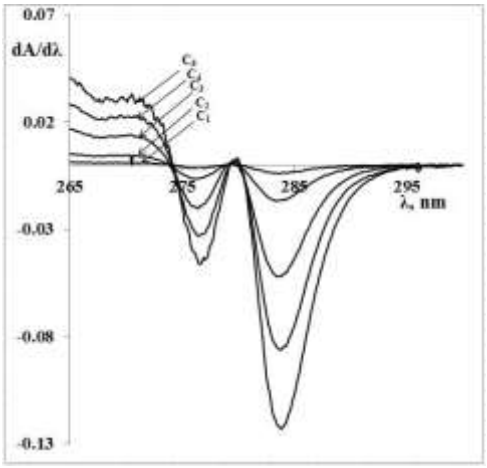

Fig. 2: First derivative spectra of (ATE): C_1: 10 µg/mL, C_2: 40 µg/mL, C_3: 120 µg/mL, C_4: 200 µg/mL, C_5: 280 µg/mL.

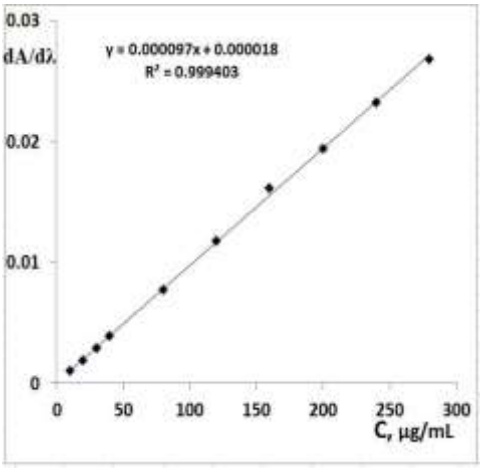

Fig. 3: Calibration curve for (ATE): C_1: 10 µg/mL, C_2: 20 µg/mL, C_3: 30 µg/mL, C_4: 40 µg/mL, C_5: 80 µg/mL, C_6: 120 µg/mL, C_7: 160 µg/mL, C_8: 200 µg/mL, C_9: 240 µg/mL, C_{10}: 280 µg/mL. n = 5 for each concentration.

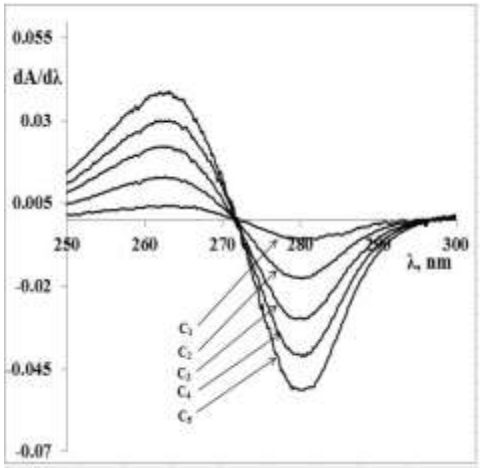

Fig. 4: First derivative spectra of (HCT): C_1: 2 µg/mL, C_2: 6 µg/mL, C_3: 10 µg/mL, C_4: 14 µg/mL,

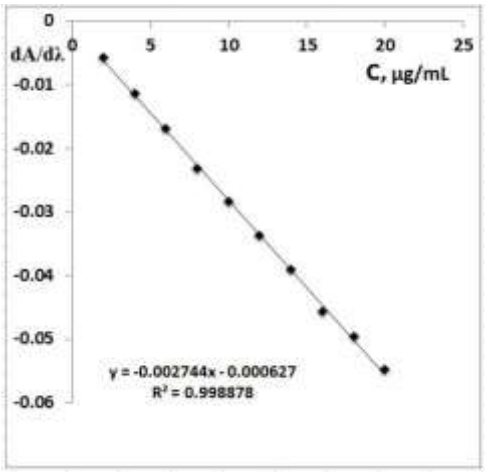

Fig. 5: Calibration curve for (HCT). C_1: 2 µg/mL, C_2: 4 µg/mL, C_3: 6 µg/mL, C_4: 8 µg/mL,

C_5: 18 μg/mL.

C_5: 10 μg/mL, C_6: 12 μg/mL,

C_7: 14 μg/mL, C_8: 16 μg/mL,

C_9: 18 μg/mL, C_{10}: 20 μg/mL.

n = 5 for each concentration.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated in "table 1" using the following equations:

$$\text{LOQ} = \frac{10 \times SD}{m}$$

$$\text{LOD} = \frac{3.3 \times SD}{m}$$

Where SD is the standard deviation of y-intercepts (a) of regression lines and (b) is the slope of the equitation of calibration curve, y = a + b x.

Table 1: Statistical data for calibration graphs

Method	Analyte	Selected wavelength (nm)	Linearity rang □g/mL	Correlation coef. (R2)	LOD □g/mL	LOQ □g/mL
DS	ATE	$^1D_{271.9}$	10 – 280	0.9994	2.77	8.38
DS	HCT	$^1D_{279.3}$	2 – 20	0.9989	0.52	1.59

Accuracy

To determine the precision and accuracy of the proposed method, five replicate determinations were carried out on three different concentrations of standards (ATE) and (HCT). The validation results are shown in "table 2".

Table 2: Method validation for the simultaneous determination of Atenolol and Hydrochlorothiazide by the ^{1}DS

DS	Pharmaceutically Raw material	Theoretical concentration (µg/mL)	observed concentration $\bar{x}$ (µg/mL)	SD µg/mL	Precision RSD (%)	Accuracy (%)
$^1D_{271.9}$	Atenolol	80	82.28	2.06	2.50	102.85
		160	165.17	2.00	1.21	103.23
		240	244.35	1.52	0.62	101.81
$^1D_{279.3}$	Hydrochlorothiazide	4	3.99	0.03	0.75	99.75
		8	8.33	0.10	1.20	104.12
		12	12.27	0.15	1.22	102.25

Accuracy (%) = (observed concentration/theoretical concentration) ×100.

$\bar{x}$ Five separate determinations were performed and calculated the mean.

Precision

In order to demonstrate the precision of the proposed method, intra-day and inter-day variability were performed at three different concentrations (40, 120, and 200 □g/mL) for Atenolol and (6, 10, and 14 □g/mL)

for Hydrochlorothiazide at the same day and at three different days. Method precision was tested in terms of RSD % for both intra-day and inter-day precisions.

The precision was ascertained by carrying out five replicates of standard Atenolol and Hydrochlorothiazide under study and the mean was calculated. The RSD % results were not more than 4.51 %, 3.58 % for (ATE) and (HCT) respectively during the determination in one day and the RSD % results were not more than 4.54 %, 3.45 % for (ATE) and (HCT) respectively during the determination in three days, where the method is considered very precise.

Recovery

The recovery was studied by three addition standards of each (ATE) and (HCT) in the **NORMOTIC** product. "Table 3" presents the recovery result for the NORMOTIC Syrian trademark drug.

Table 3: Recovery for NORMOTIC Syrian trademark drug.

Product	pharmaceutical compounds	Sample µg/mL	Added µg/mL	Total Found $\bar{x}$ µg/mL	Recovery %	SD µg/mL	RSD %	Recovery Average %
NORMOTIC 100 mg (ATE) and 25 mg (HCT)/tab.	ATE	30	24	53.63	98.46	3.59	3.65	**98.94**
			30	59.68	98.93	3.49	3.53	
			36	65.79	99.42	3.50	3.52	
	HCT	7.5	6	13.59	101.5	0.79	0.78	**100.42**
			7.5	14.99	99.87	0.53	0.53	
			9	16.49	99.89	1.12	1.12	

$\bar{x}$ Five separate determinations were performed and calculated the mean.

Application

The developed method was applied for quantitative determination for (ATE) and (HCT) in NORMOTIC Syrian trademark as tablets formulation. The sample was prepared as described in the section of sample preparation. Quantitative analysis was done by using calibration curves.

The obtained results are summarized in "table 4" for five different NORMOTIC batches. The concentrations of detected (ATE) and (HCT) in the NORMOTIC product was within the allowed limits under the British Pharmacopeia[10]. The relative standard deviations RSD % (n = 5) of the quantitative results were in the range of 2.50 – 4.74 % and 0.95 – 2.18 % for (ATE) and (HCT), respectively.

Table 4: Results of (ATE) and (HCT) in (NORMOTIC 100 mg (ATE) and 25 mg (HCT)/tab), for five different batches.

No. of batches	ATE 100 mg/tab.				HCT 25 mg/tab.			
	Result dose $\bar{x}$ mg/tab.	SD mg/tab.	RSD %	Per %	Result dose $\bar{x}$ mg/tab.	SD mg/tab.	RSD %	Per %
1	102.62	2.57	2.50	102.62	25.12	0.39	1.55	100.48
2	103.14	3.82	3.70	103.14	25.25	0.38	1.50	101.00
3	98.33	3.77	3.83	98.33	25.12	0.24	0.96	100.48
4	101.46	3.46	3.41	101.46	25.22	0.55	2.18	100.88

5	99.01	4.69	4.74	99.01	25.42	0.36	1.42	101.68
Range mg/tab	**98.33 – 103.14**				**25.12 – 25.42**			
Range Per %	**98.33 – 103.14**				**100.48 – 101.68**			
Range RSD %	**2.50 – 4.74**				**0.96 – 2.18**			

$\bar{x}$ Five separate determinations were performed and calculated the mean.

According to British Pharmacopeia, the tablets must contain not less than 92.5 percent and not more than 107.5 percent of labeled amount for (ATE) and the tablets must contain not less than 92 percent and not more than 107 percent of labeled amount for (HCT). So the obtained results are conformed to British Pharmacopeia [10].

CONCLUSION

Atenolol (ATE) and Hydrochlorothiazide (HCT) combination were estimated in one local pharmaceutical product by the first derivative spectrum method, using the zero-crossing point.

The active substances (ATE) and (HCT) in NORMOTIC pharmaceutical product were within the permissible limits set by the British Pharmacopeia legislation. The proposed method for estimating the binary mixture (ATE) and (HCT) is accurate, sensitive, simple, straight forward in quantitative analysis without prior chemical treatment. In general the dosage ratio of (ATE) to (HCT) is bigger in all pharmaceutical tablets formulations.

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