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Research Article

DERIVATIVE RATIO, ISOSBESTIC POINT, FACTORIZED ABSORPTIVITY AND BIVARIATE SPECTROPHOTOMETRIC DETERMINATION OF ATENOLOL AND CHLORTHALIDONE

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ABSTRACT

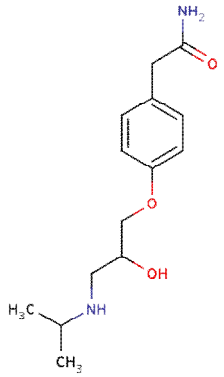
Four methods are developed for simultaneous determination of Atenolol and Chlorthalidone without previous separation. The first method depends on first derivative of the ratios spectra by measurements of the amplitudes at 235 nm for Atenolol and (236,249 nm) forChlorthalidone.The second method depends on measuring the absorbance at the isosbestic point at 283.5 nmfor the total concentration of both drugs while the concentration of Chlorthalidone is determined by direct spectrophotometric method at λ_{max} 250 nm in the presence of Atenolol, the concentration of Atenolol is calculated by subtraction. The third method is factorized absorptivity method at which both drugs are determined at more than one isospestic point (265, 284.5 nm).The fourth method involved application of the bivariate calibration algorithm for spectrophotometric simultaneous determination of the mixture. The suggested procedures are validated using laboratory prepared mixtures and are successfully applied for the analysis of pharmaceutical preparations. The methods retained their accuracy and precision when the standard addition technique is applied. The results obtained are statistically analyzed and compared with those obtained by the reference method.

Keywords: Atenolol and Chlorthalidone, First derivative of the ratio spectra.

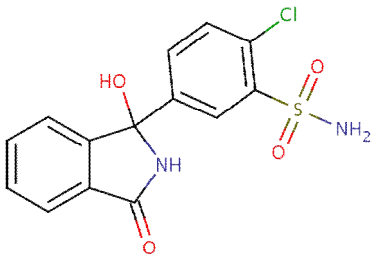
1.INTRODUCTION

Atenolol and Chlorthalidoneare formulated together to be highly effective in the treatment ofhypertension. Atenolol (ATN), chemically known as 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy)benzeneacetamide[1] (Fig.1A), is a β1-selective (cardio selective) adrenoreceptor antagonist drug used foranti-angina treatment to relieve symptoms, improve tolerance and as an anti-arrhythmic to helpregulate heartbeat and infections. It is also used in management of alcohol withdrawal, in anxietystates, migraine prophylaxis, hyperthyroidism and tremors[2]. The drug is official in IndianPharmacopoeia[3] which describes a UV-spectrophotometric method and in British Pharmacopoeia[4] which recommends high performance liquid chromatographic (HPLC) methodfor its determination. Several methods have been reported for the determination of ATN inpharmaceutical dosage forms and include diffuse reflectance spectroscopy[5], HPLC[6-16], highperformance thin layer chromatographic (HPTLC)[17],ultra performance liquid chromatography(UPLC)[18], gas chromatography (GC)[19],charge transfer complex formation[20], flourimetry[21], differential scanning calorimetry (DSC) and thermogravimetry (TG)[22], electrophoresis[23-24],voltammetry[25], ion-selective electrode (ISE) based potentiometry[26],atomic absorptionspectrometry (AAS)[27], kinetic spectrophotometry, titrimetry[28-32] and chemometry with Amiloride, Timolol and Hydrochlorothiazide[33].

Chlorthalidone(CLT) chemically, 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide[34](Fig.1B), is an antihypertensive diuretic used for treating edema secondary to congestive heart insufficiency. The drug is slowly absorbed in the gastrointestinal tract and excreted virtually unaltered[35]. The drug is official in United State Pharmacopoeia(IP)[36] and in IndianPharmacopoeia (USP)[37].CLT has been determined in urine[38,39] and plasma by reversed phaseliquid chromatography[40] and in various pharmaceutical formulations by micellar LC[41]. CLT also has been quantitated in mixtures with other drugs by derivative spectrophotometry[42] and with Atenolol by TLC densitometric and chemometric methods[43,44], HPLC in human plasma[45], in breast milk[46] and by uv-spectrophotometry alone or with Atenolol[47,48].



(A)



(B)

Fig. 1: Chemical structures of (A) Atenolol and (B) Chlorthalidone

The aim of this work is to develop new spectrophotometric methods for resolving this mixture with spectral interfering problems, without preliminary separation. Thesuggested methodsare very simple as Atenolol is determined by direct spectrophotometry and Chlorthalidoneis determined by simple mathematical calculation, also the methods used did not require any sophisticated instrumentation such as HPLC which requires expensive equipment and materials

2.Experimental

2.1.Apparatus

Spectrophotometer: SHIMADZU UV-1800 PC, dual beam UV–visible spectrophotometer with two matched 1 cm quartz cells, connected to an IBM compatible personal computer (PC) and an HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version (3.7) was used to process the absorption and the derivative spectra. The spectral band width was 0.2 nm with wavelength scanning speed of 2800 nm min−1,Japan.

2.2.MATERIALS

2.2.1.Pure samples

Atenolol and Chlorthalidonewere kindly supplied by E.I.P.I.CO, 10th of Ramadan CityandSigma,Egypt.

2.2.2.Market samples

Tenedonetablets (Sigma), Egypt. It is labeled to contain (50 and 25 mg) Atenolol and Chlorthalidone, respectively per tablet.

2.3.Chemicals and reagents

All chemicals are of analytical grade and the solvents are of spectroscopic grade. Methanol, (E-Merck, Germany).

2.4.Standard solutions

Stock solutions

Atenolol and Chlorthalidonestock solutions (100µg ml^{-1}) are prepared by weighing accurately 0.01gm of each powder into two separate 100 ml volumetric flasks. Methanol (50 ml) is added, shaken for a few minutes and completed to volume with the same solvent.

3.Determination of linearity range

3.1. First derivative of the ratio spectrophotometric method (DD1)

For determination of Atenolol, the spectra of Atenolol of different concentrations range(5-30µg ml−1) are divided on the spectra of different concentrations of Chlorthalidone range(5-30 µg ml−1) then the first derivative of the ratio spectra are obtained taking Δλ=10 nm. The calibration curves of Atenolol first derivative ratio are obtained at the peak at 235 nm. After comparing the results, the spectrum of Chlorthalidone 15 µg ml−1 is chosen as adivisor

as giving the least intercept and best correlation coefficient. For determination of Chlorthalidone , the spectra of Chlorthalidone of different concentrations range (5-25μg ml−1) are divided on the spectra of different concentrations of Atenolol range(5-30 μg ml−1) then the first derivative of the ratio spectra are obtained taking Δλ=10 nm. The calibration curves of first derivative ratio of Chlorthalidoneare obtained at the peaks at 236,249nm. After comparing the results, the spectrum of Atenolol 25μg ml−1 chosen as adivisor as giving the least intercept and best correlation coefficient.

3.2.Isosbestic spectrophotometric method

The zero order absorption spectra of 50 μg/mlof Atenolol and Chlorthalidone were recorded and the spectrum of 25 μg/mlof ATE/CLT in a mixture was recorded.Aliquots from stock solutions ATE and CLT equivalent to final concentration 10-100 μg/mlof ATE and 10-90 μg/mlof CLTare transferred into two separate sets of 10 ml volumetric flasks and completed to the mark with methanol. The zero order absorption spectra are recorded for both drugs using methanol as a blank; then the absorbance measured at 283.5nm (isosbestic point) for ATE and CLT and 250 nm for CLT. Two calibration curves are constructed for each wavelength relating the absorbance to the corresponding drug concentrations and the regression equations are computed.

3.3. Factorized absorptivity method

The zero order absorption spectra of 50 μgml-1 of ATE and 25 μg/ml CLT were recorded. Aliquots from stock solution of ATE equivalent to final concentration 50-90μgml-1 are transferred into10 ml volumetric flasks and completed to the mark with methanol. The zero order absorption spectra are recorded using methanol as a blank; then the absorbance measured at 284.5, 265 nm (two isosbestic points). Two calibration curves are constructed for each wavelength relating the absorbance to the corresponding drug concentrations and the regression equations are computed.

Aliquots from stock solution CLT equivalent to final concentration 10-90μg/ml are transferred into10 ml volumetric flasks and completed to the mark with methanol. The zero order absorption spectra are recorded using methanol as a blank; then the absorbance measured at 284.5, 265 nm (two isosbestic points) and at 248nm λmax of CLT. Three calibration curves are constructed for each wavelength relating the absorbance to the corresponding drug concentrations and the regression equations are computed.

3.4.Bivariate method

Aliquots of standard ATE and CLT equivalentto final concentration 10-35μg/ml and5-30μgml-1, respectively are transferred separately into two sets of 10 ml volumetric flasks then diluted to volume with methanol. Calibration curves at differentwavelengths 220,225, 230 and 235nm are constructedand the regression equation at each wavelength is calculated. From both sets ofregression equations, the sensitivity matrices K was calculated, the optimum pair of wavelengths chosen is(225 and 235 nm) to carry out the determination and theregression equations used in the bivariate algorithm are deduced.

4.Laboratory-prepared mixtures

Accurate aliquots equivalent to final concentration (10-100 μg/ml) of ATEare transferred from its stock solution into a series of 10 ml volumetric flasks and portions equivalent to final concentration (10-90 μg/ml) are transferred from CLTstock solution then added to the same flasks and volumes are completed to mark with methanol and mixed well to make different ratios.

5.Procedures

5.1.First derivative of the ratio spectrophotometricmethod (DD1)

According to the theory of the ratio spectra derivative method. The stored UV absorptionspectra of standard solutions of Atenolol weredivided by a standard spectrum of Chlorthalidone(15 μg/ml) wavelength-by-wavelength. The first derivativecalculated for the obtained spectra with Δλ = 10 nm.The amplitudes at 235 nm are measured and foundto be linear to the concentrations of Atenolol.

ForChlorthalidone, the stored UV absorption spectra of standardsolutions of Chlorthalidoneare divided by a standard spectrum of Atenolol (25μg/ml) wavelength-by-wavelength. The first derivative calculated for theobtained spectra with Δλ = 10 nm. The amplitudes at (236,249 nm)are measured and found to be linear to theconcentration of Chlorthalidone.

5.2.Isosbestic spectrophotometric method

Absorbance of the spectra of laboratory prepared mixtures containing different ratios of ATE and CLTare measured at 250nm corresponding to the contents of CLT only and at283.5 nm, corresponding to the total content

of ATE and CLT in the mixture. The concentration of CLT alone and the total concentration of the two drugs are calculated from their corresponding regression equations; then by subtraction of CLT concentration from the total mixture concentration, the actual concentration of ATE in the mixture obtained.

5.3.Factorized absorptivity method

Absorbance of the spectra of laboratory prepared mixtures containing different ratios of ATE and CLTare measured at 248nm corresponding to the contents of CLTonly and at284.5 and at 265 nm, corresponding to concentration of ATE and CLT in the mixture. The concentration of CLTalone and the total concentration of the two drugs are calculated from their corresponding regression equations; then by subtraction of CLTconcentration from the total mixture concentration then multiplying the result by 2, the actual concentration of ATE in the mixture obtained.

5.4.Bivariate method

Different volumes (0.5-2.5 ml) of ATE (100µg/ml) aretransferred and mixed with (0.5-2.5 ml) ofCLT in a set of 10mlvolumetric flaks. The volume is completed to mark with methanol, and the absorbance ofeach mixture is recorded at 225 and 235 nm. The concentrations of the two drugs arecalculated using Kaiser method[49].

6.Assay of pharmaceutical formulations

Tenedone tablets: Ten Tenedone tablets accurately weighed. one tablet contain 50mg Atenolol (content I) and 25 mg Chlorthalidone (content II). The tablets powdered and the weight of one tablet transferred into a 100 ml beaker, sonicated in 20 ml methanol for 10 min and filtered into a 100 ml volumetric flask. The residue washed three times using 20 ml methanol each time and the volume completed to the mark with methanol forming tablet stock solution that contain Atenolol(50mg/100ml) and Chlorthalidone (25 mg/100ml) then make dilution to this tablet stock solution, take 20 ml and complete to 100 ml with methanol to form solution of concentration (10mg/100ml), content I(Atenolol) prepared. Take 40 ml from this stock solution and complete to 100 ml with methanol to form solution of concentration (10 mg/100ml), content II(Chlorthalidone).

For first derivative of ratio method: aliquots (0.5,1,1.5 ml) equivalent to final concentration(5,10,15 µg/ml) are separately transferred from authentic drug(100 µg/ml) to 10 ml volumetric flasks and add (1ml), equivalent to final concentration 10 µg/ml, from content (I) and diluted with methanol then aliquots (0.5,1,1.5 ml) are separately transferred from authentic drug to 10 ml volumetric flasks and add (1ml), equivalent to final concentration 10 µg/ml , from content (II) and diluted with methanol.

For isosbestic point and factorized absorptivity methods: aliquots (3,4,5 ml) equivalent to final concentration (30,40,50 µg/ml) are separately transferred from authentic drug (100 µg/ml) to 10 ml volumetric flask and add 2ml equivalent to final concentration 20 µg/ml , from content(I) and diluted with methanol then aliquots (3,4,5 ml) are separately transferred from authentic drug to 10 ml volumetric flask and add 2ml of working tablet solution,equivalent to final concentration 20 µg/ml , from content(II) and diluted with methanol.

For bivariate method: aliquots (0.5,1,1.5 ml) from both authentic drugs (100 µg/ml) equivalent to final concentration (5,10,15µg/ml) are separately transferred to 10 ml volumetric flasks and3ml from tablet stock solution equivalent to final concentration 30µg/ml were added to each flask and diluted with methanol .

The general procedures under linearity are followed.The validity of the methods assessed by applying the standard addition technique.

7. RESULTS AND DISCUSSION

Analytical methods for the determination of binary mixture without previous separation are of interest.As shown in Fig. 2, the zero-order spectra of standard drugs are found to be overlapped making their simultaneous determination difficult.

7.1.DD1 method

The main parameters that affect the shape of the derivative ratio spectra are the concentration of the standardsolution used as a divisor and the wavelength intervals over which the derivative is obtained (Δ λ). These parameters need to be optimized to give a well resolved large peak with good selectivity and higher sensitivity. The obtained ratio spectra (Fig. 3, 4)were differentiated with respect to wavelength to afford the first derivative ratio spectra.Good measurements could be obtained at the 235nm for ATE and at 236, 249 nm for CLT (Fig. 5, 6). Effect of the wavelength intervals revealed that Δ λ=10 nm was the most suitable interval for measurement of both drugs with scaling factor of 1. Increasing that interval led to a less sensitive peak.

7.2.Isosbestic spectrophotometric method

Chlorthalidone can be determined by direct measurement of absorbance at 250 nm since ATE show neglected absorbance while the

absorption spectra of ATE and CLT showed severe overlap, which makes the determination of Atenolol concentration in the mixture more difficult figure(2). By applying the proposed method to the spectral data of the mixture, both Atenolol and Chlorthalidone concentrations could be determined without any interference figure (7).At the isosbestic point the mixture of drugs acts as a single component and gives the same absorbance value as pure drug. Thus, by measuring the absorbance value at the chosen isosbestic point 283.5 nm (Aiso) (Fig. 7), the total concentration of both ATE and CLT could be calculated, while the concentration of CLTin the mixture could be calculated, without any interference at 250 nm. Thus, the concentration of ATEcould be calculated by subtraction.Linearcorrelation obtained between the absorbance values and the corresponding concentrations of both drugs at their corresponding wavelengths. The regression equations are:

A (iso) =0.004C – 0.035 r = 0.9999 at 283.5 nm

A =0.0096C – 0.0297 r = 0.9999 at 250 nm

Where *A* is the absorbance, *C* is the concentration of the drug in μg ml−1 and *r* is the correlation coefficient.

The proposed method is applied for the determination of both drugs in tablets, applying standard addition technique.

7.3. Factorized absorptivity method

This method is used to improve the isosbestic point in this mixture so if we draw the spectra of certain concentration of ATE and CLT concentration half that of ATE we will have new isosbestic point like shown in figure(8).

7.4. Bivariate method

Bivariate calibration spectrophotometric method is a direct method which is proposedfor the resolution of mixtures. The principle of bivariate calibration is themeasurement of two components (A and B) at two selected wavelengths($\lambda1$, $\lambda2$) toobtain two equations[49]:

$A_{AB1}=m_{A1} \cdot C_A+m_{B1} \cdot C_B+e_{AB1}$

$A_{AB2}=m_{A2} \cdot C_A+m_{B2} \cdot C_B+e_{AB2}$

The resolution of each equation set allows the evaluation of CA and CB values:

$C_B=m_{A2}(A_{AB1}-e_{AB1})+m_{A1}(e_{AB2}-A_{AB2}) / m_{A2}m_{B1}-m_{A1}m_{B2}$

$C_A=(A_{AB1}-e_{AB1}-m_{B1}C_B) / m_{A1}$

Where C_A and $C_B$are the concentration of Atenolol and Chlorthalidone ,respectively m_{A1},m_{A2} are the slope values of Atenololat $\lambda1$, $\lambda2$; m_{B1}, m_{B2} are the slope values of Chlorthalidoneat $\lambda1$, $\lambda2$; A_{AB1},$A_{AB2}$are the absorbance of the binary mixture at $\lambda1$, $\lambda2$; e_{AB1}, $e_{AB2}$are the sumof the intercepts of the two drugs at $\lambda1$, $\lambda2$, respectively.This simple mathematic algorithm allows the resolution of the two components by measuringthe absorbance of their mixture at the two selected wavelengths and using the parameters ofthe linear regression functions evaluated individually for each component at the samewavelengths.In order to apply the bivariate method in the resolution ofAtenolol and Chlorthalidone, the absorbance of the two components at nine different selected wavelengths is recorded in the region of overlapping; 220,225,230 and 235 nm. Fig.9. The calibration curve equations and their respective linear regressioncoefficients are obtained directly with the aim of ensuring that there isa linearrelationship between the absorbance and the corresponding concentration. All of thecalibration curves at the selected wavelengths showed a satisfactory linear regressioncoefficient ($r > 0.9985$).

The method of Kaiser[48] is usedfor the selection of the optimum wavelengthsset, which assured the best sensitivity and selectivityof the determination. A series of sensitivitymatrices *K* are created for each binarymixture and for every pair of pre-selected wavelengths:

$$K=[m_{A1} \quad m_{B1}] [m_{A2} \quad m_{B2}]$$

Where*mA1, mA2* are the slopes, which are consideredas the sensitivity parameters of the componentA at two selected wavelengths (1, 2) andm_{B1}, $m_{B2}$are the parameters for the componentB. The resolution of these matrices is calculated:

$$K=(m_{A1}*m_{B2})-(m_{A2}*m_{B1})$$

The values of K obtained and the values of the absolute selectivity of **Kaiser's Determinant** ($K\times10^5$) are obtained and used as the optimization criterion; the wavelengths set selected isthat with the highest absolute matrix determinantvalue.For the bivariate determination of Atenolol and Chlorthalidone, 225 and235 nm are found to give the maximum value of K and thus can be used for the analysis, Table2.

8.Quantification, accuracy and precision

The characteristic parameters andthe linear regression equations together with correlation coefficients, slope,intercept,R.S.D. of slope and intercept, repeatability (within day) and reproducibility (between-day) obtained for each drug are collected in (Table 1). There are no significant difference for the assay, which is tested within-day (repeatability) and between-days (Reproducibility). In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by

analyzing laboratory prepared mixtures of Atenolol and Chlorthalidone with different composition ratio (Table 3). Results obtained are compared with the reference methods[50, 51] by student's t-test and variance ratio F-test (Table 4). The calculated values did not exceed the theoretical ones.

9.CONCLUSION

For routine analytical purposes, it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with good accuracy and precision, either in laboratory prepared mixtures or in commercial pharmaceutical dosage form. Spectrophotometric methods can generate large amounts of data within a short period of analysis. The proposed methods provide a clear example of the high resolving power and low cost while HPLC method is more specific, it needs expensive equipment and materials. The results demonstrate the usefulness of the methods, which are simple, safe, sensitive, precise, accurate, inexpensive and non-polluting so the proposed methods could be used in routine and quality control analysis of Atenolol and Chlorthalidonein pharmaceutical preparations containing them.

8. Satisfactory results were obtained (Tables 1-4):

Table 1: Spectral data for determination of Atenolol and Chlorthalidone by DD1, Isosbestic point, Factorized absorptivity and Bivariate methods

Drug	Atenolol						Chlorthalidone					
Methods / Validation Parameters	Bivariate		DD1	Isosbestic point	Factorized absorptivity		Bivariate		DD1		Isosbestic point	Factorized absorptivity
Linearity range (µg/ml)	20-35	10-35	5-30	10-100	50-90	50-90	5-25	5-30	5-25	10-25	20-90	10-80
Wavelength (nm)	225	235	235	283.5	265	284.5	225	235	236	249	250	248
Slope	0.0372	0.017	0.0022	0.0049	0.0035	0.0034	0.0719	0.05	0.0938	0.1596	0.0095	0.0118
Intercept	-0.1442	-0.125	-0.0016	-0.035	-0.0361	-0.054	-0.1319	-0.1312	-0.1016	-0.0393	-0.0269	0.0107
Correlation coefficient	0.9998	1	0.9998	0.9999	0.9996	0.9997	0.9999	0.9999	0.9998	1	0.9999	0.9999
LOD (µg/ml)	0.46	0.19	1.14	0.59	0.5	0.55	0.6	1.5	1.2	0.4	0.02	0.95
LOQ (µg/ml)	1.5	0.67	3.8	1.98	1.7	1.8	2.1	5.1	4	1.4	0.07	3.2
S.E.	0.15	0.07	0.38	0.198	0.169	0.182	0.2	0.51	0.399	0.14	0.278	0.32
Repeatability (R.S.D. %)		1.93	1.38	1.4	1.7	0.38		0.96	1.03	0.65	1.59	1.9
Reproducibility (R.S.D. %)		1.3	0.67	1.47	1.19	0.5		0.68	1.67	1.9	1.55	0.27

Table 2: Application of the method of Kaiser for the selection of the wavelengths set for the ATE–CTD the absolute values of determinants of sensitivity matrices ($K \times 10^5$)

λ1, λ2	220	225	230	235
220	0	1.07	-	-
225	-	0	-	-
230	1.56	8	0	-
235	19.69	64.14	54.1	0

Table3: Inter-day and intra-day accuracy and precision determination of Atenolol and Chlorthalidone by the proposed methods

	Drug	Atenolol					Chlorthalidone				
	Method	DD1	Isosbestic Point	Factorized absorptivity		Bivariate	DD1		Isosbestic Point	Factorized absorptivity	Bivariate
	Wavelength(nm)	235nm	283.5 nm	284.5 nm	265 nm	225, 235 nm	236 nm	249 nm	250 nm	248 nm	225, 235 nm
	Weight taken(µg)	20	60	40	40	20	10	10	30	20	10
	Validation Parameters										
	%Recovery / Experiment										
Inter-day	1	101.1	97.96	99.05	99.76	99.8	102	102.4	100.5	100.97	102
	2	99	99.67	101.5	97.7	97.5	102.7	102.2	101.8	101.3	103.7
	3	102.3	99.67	101.5	97.7	99.8	100.2	100.92	101.98	100.55	102
	Mean	100.8	99.1	97.6	98.4	99.03	101.6	101.84	101.4	99.9	102.57
	S.D.	1.4	0.99	0.42	1.2	1.33	1.3	0.8	0.8	0.38	0.98
	R.S.D.	1.38	0.99	0.43	1.2	1.34	1.3	0.8	0.8	0.37	0.96
	%Recovery / Experiment										
Intra- day	1	102.3		99	97.7		102.3		101.8		
	2	101.1	97.62	99	97.7	99.8	102.3	100.2	101.5	100.55	
	3	101.7	97.97	101.5	96.7	99.8	100.2	100.2	101.28	100.97	102
	4		97.97	101.5	96.7	97.5		101.5		101.4	102
	5			101.5	97.7					100.97	103.2
										100.97	
	Mean	101.4	97.85	100.5	97.3	99.03	101.6	100.6	101.5	100.97	102.40
	S.D.	0.68	0.2	1.2	0.49	1.33	1.2	0.75	0.26	0.27	0.69
	R.S.D.	0.67	0.21	1.2	0.50	1.34	1.2	0.75	0.26	0.27	0.68

Table 4: Statistical data for determination of Atenolol and Chlorthalidone in authentic (single), laboratory prepared mixture and pharmaceutical dosage form using DD1, Isosbestic point, Factorized absorptivity, Bivariate and Reference methods

Methods Parameters (Mean ±S.D., N, V, t-test, F-test)	DD1 235nm (ATN) 236,249 nm (CLT)	Isosbestic point (283.5,250) nm	Factorized absorptivity method (284.5,265,248) nm	Bivariate method (225,235) nm	Reference method [50,51]
Authentic Atenolol (single)	100±0.85 N=5 V=0.9 t=0.76(2.305)* F=1.76(6.69)*	101±0.6 N=9 V=0.4 t=1.42(2.201)* F=1.28(8.85)*	100±0.41 N=5 V=0.21 t=0.1(2.305)* F=2.4(8.12)*	100±0.3 N=4 V=0.09 t=0.36(2.447)* F=5.67(8.28)*	100±0.71**[50]** N=4 V=0.51
			100±0.38 N=5 V=0.18 t=0.11(2.305)* F=2.8(9.12)*	100 ±0.11 N=3 V=0.01 t=0.33(2.571)* F=51(9.66)**	
Authentic Chlorthalidone (single)	100±0.89 N=5 V=1 t=0.85(1.86)* F=2.5(6.69)*	100.05±0.74 N=7 V=0.54 t=1.2(2.228)* F=1.35(4.63)*	100±0.84 N=7 V=0.82 t=0.95(2.228)* F=2.05(4.53)*	99.94±.37 N=4 V=0.14 t=1.08(2.305)* F=2.86(6.69)*	99.55±0.63**[51]** N=5 V=0.4
	100±0.25 N=3 V=0.09 t=1.13(2.447)* F=4.4(6.94)*			100.2±1.12 N=6 V=1.25 t=1.15(2.202)* F=3.125(5.19)*	
Laboratory prepared mixture for Atenolol	100±0.98	99±1.41	99±1.56 102±1.98	99.10±1.29	
Laboratory prepared mixture for Chlorthalidone	100±1.34 101±0.75	102±1.19	100±1.73	101.1±0.82	
Standard addition technique for Atenolol	100±1.7	100±0.94	100±0.77 100±0.52	100.5±0.75	
Standard addition technique for Chlorthalidone	100±1.06	101±0.38	100±0.8	98.17±0.63	

*Tabulated values of t and F at p = 0.05

**There is significance difference between the proposed and reference methods indicate that the proposed methods are more precise, since they have the smallest variance values.

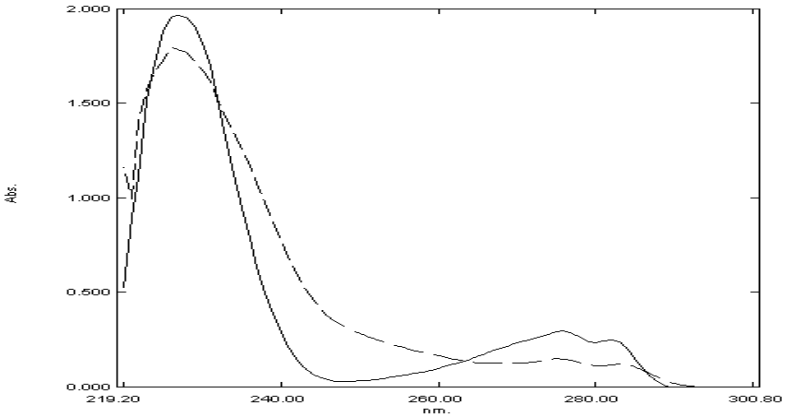


Fig. 2: Absorption spectra of Atenolol (___) 50 µg/ml and Chlorthalidone (– –)25µg/ml

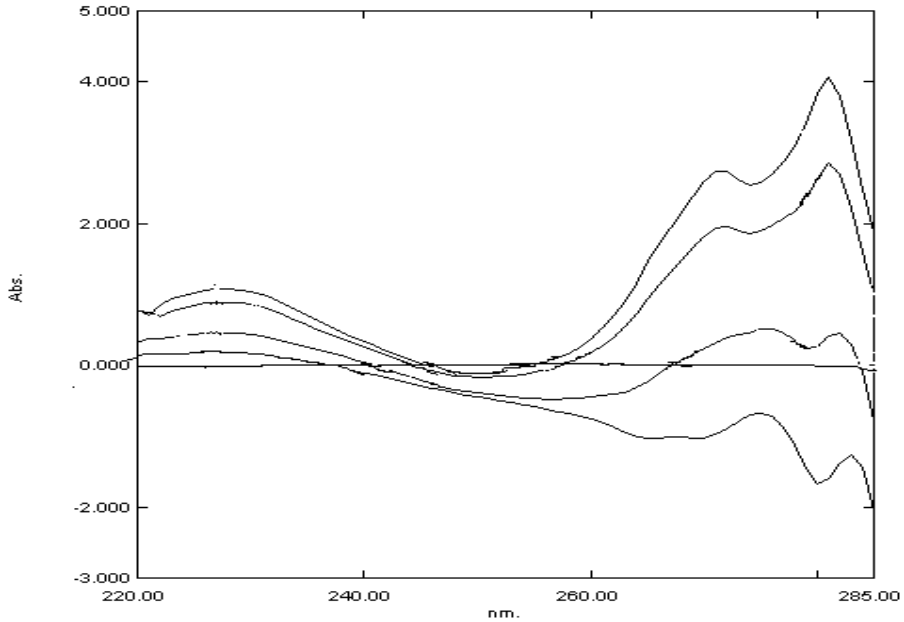


Fig. 3: Ratio spectra of Atenolol (5-30µg/ml), using the spectrum of Chlorthalidone 15 µg/ml as divisor

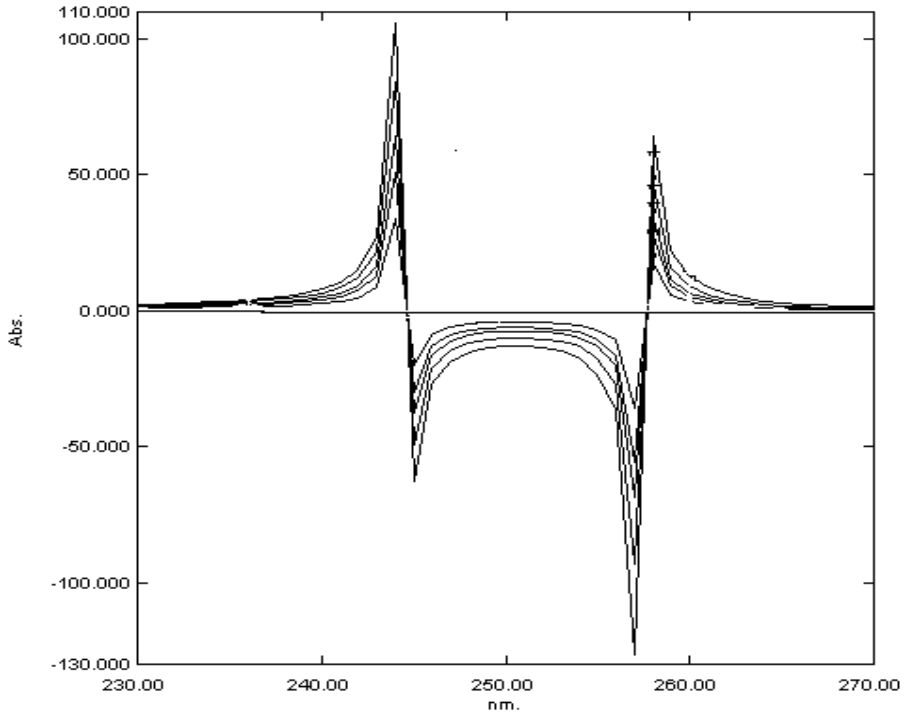


Fig. 4: Ratio spectra of Chlorthalidone (5-25µg/ml), using the spectrum of Atenolol 25 µg/ml as divisor

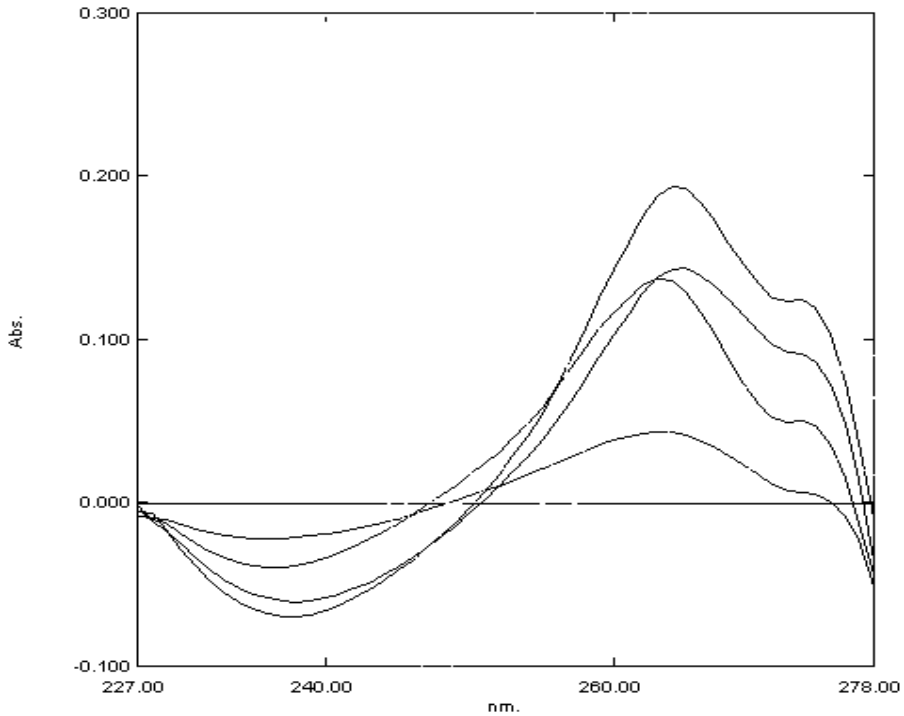


Fig. 5: First derivative of the ratio spectra of Atenolol (5-30 µg/ml). Divisor is 15µg/ml Chlorthalidone

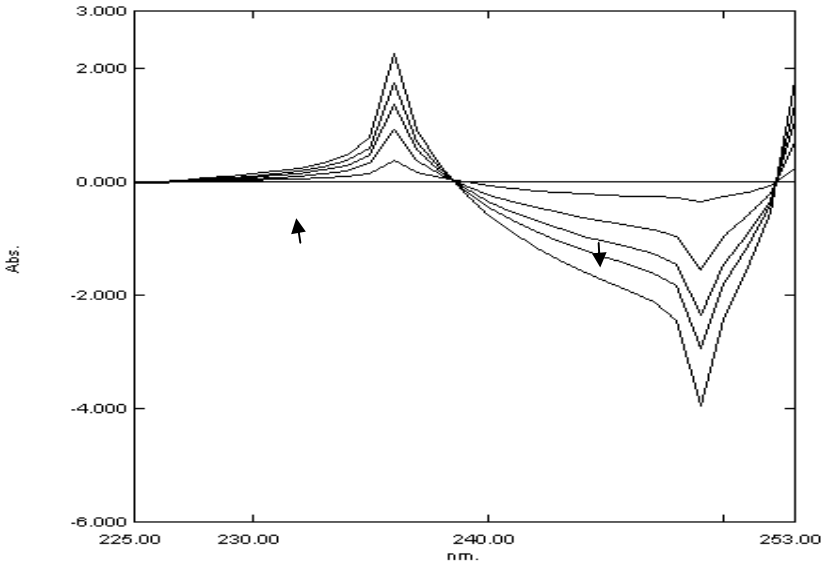


Fig.6: First derivative of the ratio spectra of Chlorthalidone (5-25µg/ml) Divisor is 25µg/ml Atenolol

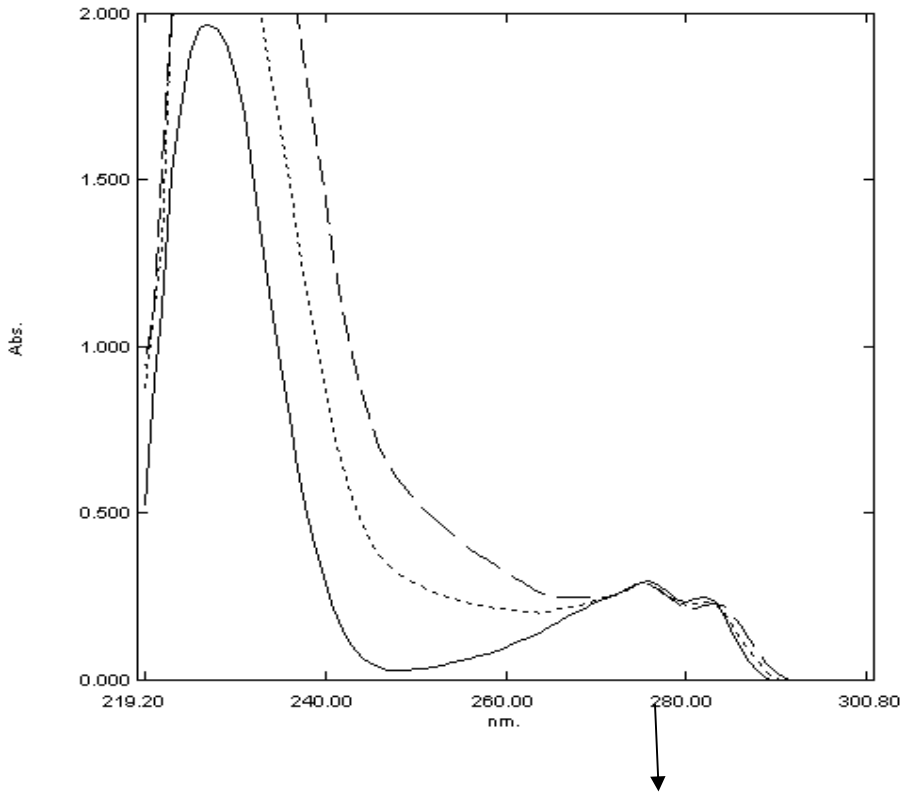


Fig. 7: Zero order absorption spectra of 50 µg ml−1 of Atenolol (___), 50 µg ml−1 of Chlorthalidone (– —)and (1:1) mixture containing 25 µg ml−1 of each (- - - -) using methanol as a blank

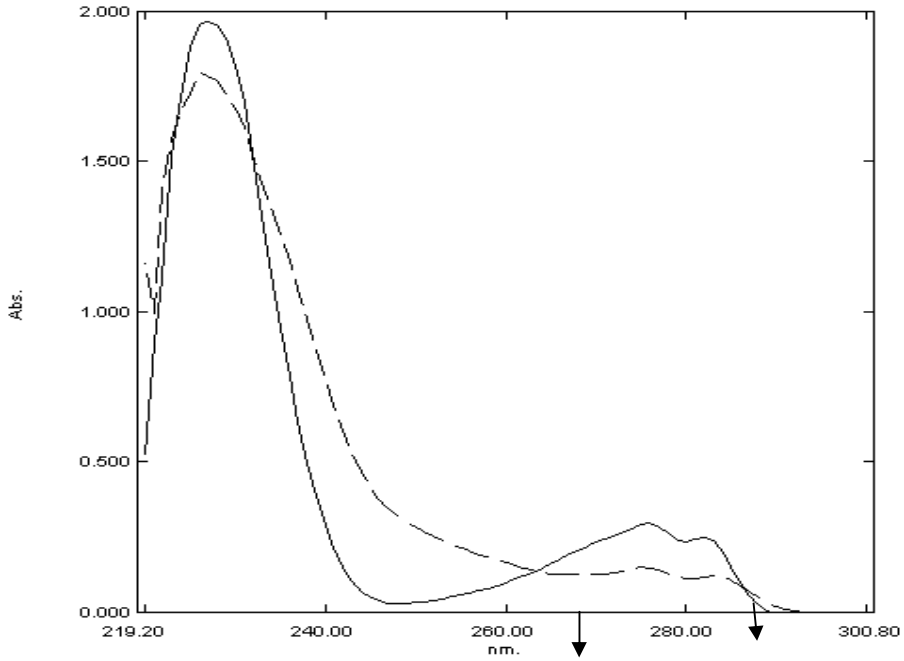


Fig. 8: Absorption spectra of Atenolol (___), 50µg/ml and Chlorthalidone (– —)25 µg/ml

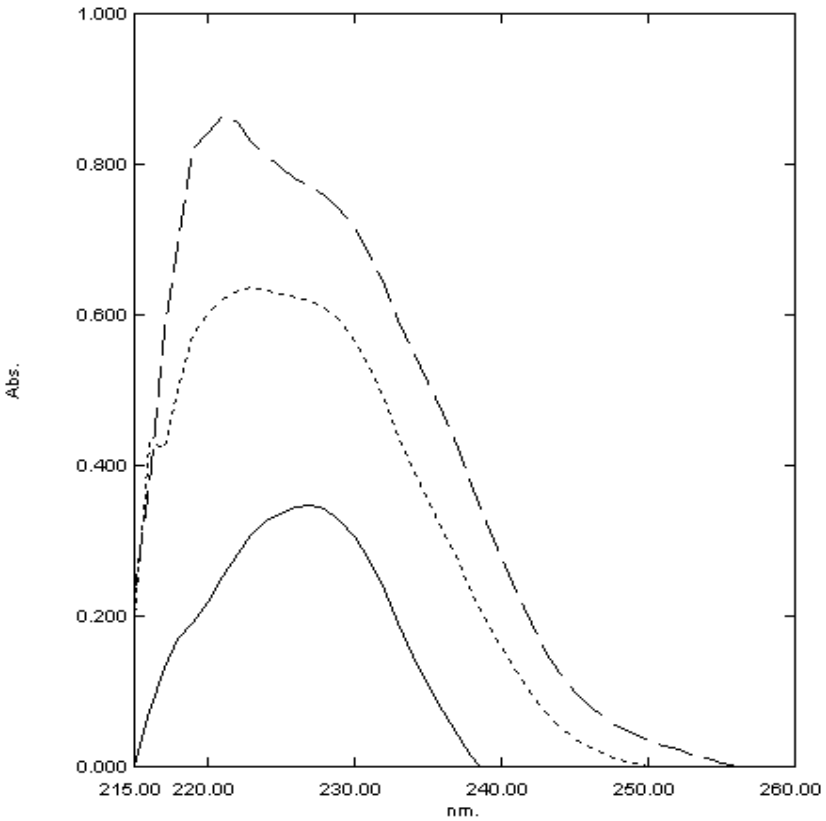


Fig. 9: Zero order absorption spectra of 12.5 μg ml−1 of Atenolol (___), 12.5 μg ml−1 of Chlorthalidone (– –)and (1:1) mixture containing 6.25 μg ml−1 of each (- - - -) using methanol as a blank

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