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SIMULTANEOUS ESTIMATION OF ATENOLOL AND HYDROCHLOROTHIAZIDE IN COMBINED DOSAGE FORM BY UV-SPECTROPHOTOMETRIC METHODS

MANZOOR AHMED*, NADEEM JAMADAR and A. SATISHKUMAR SHETTY[a]

Department of Pharmaceutical Analysis, National College of Pharmacy, SHIMOGA – 577201 (K.S.) INDIA
[a]Department of Pharmaceutical Analysis, National College of Pharmacy, Balaraj Urs Road, SHIMOGA – 577201 (K.S.) INDIA

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ABSTRACT

Two simple, precise and accurate derivative spectrophotometric methods have been developed and validated for the simultaneous estimation of Atenolol (ATN) and Hydrochlorothiazide (HCTZ) in bulk and pharmaceutical formulation. Method A is Area Under Curve method, which involved the measurement of area between 221 to 231 nm and 265 to 275 nm for the estimation of Atenolol and Hydrochlorothiazide respectively. Method B applied Second order derivative spectrophotometry, which involved measuring the absorbance values at 226 nm and 270 nm of second derivative spectrum, without mutual interference with a linearity range of 2-10 µg/mL and 1-5 µg/mL for the estimation of Atenolol and Hydrochlorothiazide, respectively for both the methods. Results of analysis were statistically reported and were found to be satisfactory.

Key words: Atenolol, Hydrochlorothiazide, Area Under Curve, Second order derivative.

INTRODUCTION

Atenolol, chemically (RS)-4-2 (2-hydroxy-3-isopropylamino propoxy) phenylacetamide is a selective beta-1 adrenergic receptor antagonist[1]. It is used in the treatment of cardiovascular diseases such as angina, hypertension, cardiac arrhythmias and myocardial infarctions. Atenolol competitively blocks beta-adrenergic receptors in the heart and juxtoglomerular apparatus. They lead to decreased heart rate decreasing the work load by the heart. They do not produce coronary vasodilation but lead to a shift and redistribution of coronary circulation to the ischemic areas. It decreases the release of renin from the kidney, thus lowering the blood pressure[2]. It is official in IP[3].

Hydrochlorothiazide, chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfanamide 1,1-dioxide belongs to the thiazide class of diuretics. It is used for the treatment of edema associated with heart (congestive heart failure), liver (hepatic cirrhosis), renal (nephrotic syndrome, chronic renal failure and glomerulonephritis) diseases. Hydrochlorothiazide acts on the kidneys to reduce sodium (Na) reabsorption in the distal convulated tubule[4]. It is official in IP. The combination of Atenolol and Hydrochlorothiazide is useful in the treatment of hypertension and congestive heart failure (CHF).

Available online at www.sadgurupublications.com
E-mail: ms_manzoor@yahoo.com, nadeemahmed.156@gmail.com; Mo.: +919448204746; Fax: 08182 2737796

On literature survey, it was found that HPLC[5], and spectrophotometric[6] methods have been reported by using methanol as a solvent for the simultaneous estimation of Atenolol and Hydrochlorothiazide in combined dosage forms. In view of the need for a suitable method for routine analysis in combined formulations, attempt was made to develop simple, precise, accurate spectrophotometric methods for simultaneous estimation of titled drugs and extend it for their determination in formulation using methanol as solvent.

EXPERIMENTAL

Instrument and materials

A Shimadzu UV-1700 UV/-Vis Spectrophotometer was used with 1 cm match quartz cell. Tablets of 50 mg of Atenolol and 25 mg of Hydrochlorothiazide were procured from local pharmacy. Standard Atenolol and Hydrochlorothiazide were obtained as gift samples from Microlabs Pvt. Ltd., Bangalore and Watson Pharmaceuticals Pvt. Ltd., Mumbai. AR grade methanol was used as solvent throughout the experiment.

Preparation of Standard solution

Both the pure drugs of about 100 mg were weighed accurately and dissolved in 100 mL of methanol to give the standard stock solution of 1000 µg/mL (Stock A). Aliquots of standard stock solution were pipetted out and suitably diluted with methanol to get a final concentration of the standard solution.

Method A: Area Under Curve method [7]

This method is applicable when there is no sharp peak or broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between AUC and concentration. For the selection of analytical wavelength suitable dilutions of Atenolol (2-10 µg/mL) and Hydrochlorothiazide (1-5 µg/mL) of the standard stock solutions (100 µg/mL) of both were prepared separately and scanned in the range of 200-400 nm.

For Area under Curve method, the sampling wavelength ranges selected for estimation of Atenolol and Hydrochlorothiazide are 221-231 nm (λ_1-λ_2) and 265-275 nm (λ_3-λ_4) respectively. Sample solutions were prepared (2-10 µg/mL) and their area under the curve was measured at the selected wavelength ranges.

Method B: Second Order Derivative Method [8,9]

The standard solutions of both the drugs were scanned in the spectrum mode from 400-200 nm. These spectrums were converted to second order derivative spectra by using derivative mode in UV probe software 2.0. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected should be such that at each wavelength the absorbance difference between the components should be as large as possible. From the examination of the second derivative spectra of Atenolol and Hydrochlorothiazide, 226 nm (λ_1) and 270 nm (λ_2) were selected as working wavelengths for the second order derivative spectroscopy.

Analysis of Tablet Dosage form

Twenty tablets of Atenolol and Hydrochlorothiazide (Aten–H 25) in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet

powder equivalent to 50 mg of Atenolol was weighed which also contains 25 mg of Hydrochlorothiazide and transferred to 100 mL volumetric flask and dissolved in 50 mL methanol and the content was kept in ultrasonicator for 25 min. Finally the volume was made up to the mark with methanol. The solution was filtered through Whatmann filter paper No. 41 which gave a concentration of 1000 µg/mL and this solution was used as stock 'A' solution. From the above stock 'A' solution, 5 mL of the aliquot was pipetted out and was transferred to a 50 mL volumetric flask. The volume was made upto 50 mL with methanol to obtain a solution with final concentration of Atenolol and Hydrochlorothiazide, 2 to 10 µg/mL from the above prepared concentrations select any one concentration or final concentration i.e., 5 µg/mL and 10 µg/mL for Hydrochlorothiazide and Atenolol, respectively. Six different mixtures containing 5 µg/mL of Hydrochlorothiazide and 10 µg/mL of Atenolol were prepared as above and analyzed at the selected analytical wavelengths, 270.0 nm and 226.0 nm and their results were statically validated.

Validation of the methods

All the methods were validated according to ICH guidelines by carrying out analysis of six replicate samples of tablet. Recovery studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to previously analyzed tablet powder sample. From the amount of drug found, percentage recovery was calculated.

RESULTS AND DISCUSSION

The estimation of Atenolol and Hydrochlorothiazide in tablet formulation was found to be accurate and reproducible with a linearity of 2-10 µg/mL and 1-5 µg/mL, respectively for both the methods and the correlation coefficient 0.999 and 0.999 for the methods A and B, respectively. The optical characteristics such as linearity range, molar absorptivity, percentage relative standard deviation of recovery studies and precision in each method were calculated and the results were reported in Table 1. Also the regression characteristics like slope (m), intercept (c) and correlation coefficient (r) were calculated and are presented in Table 1. The accuracy of the methods at different levels i.e. 80%, 100% and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value was less than 2, an indicative of the accuracy of the methods.

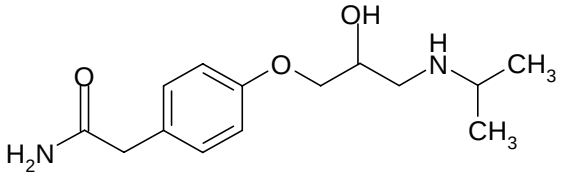

Fig. 1: Chemical Structure of Atenolol

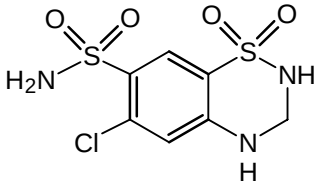

Fig. 2: Chemical Structure of Hydrochlorothiazide

The absorption spectra of Atenolol, Hydrochlorothiazide and formulation by Area Under Curve method are reported (Fig. 3, 4 and 5) and calibration curve was plotted (Fig. 6, 7 and 8).

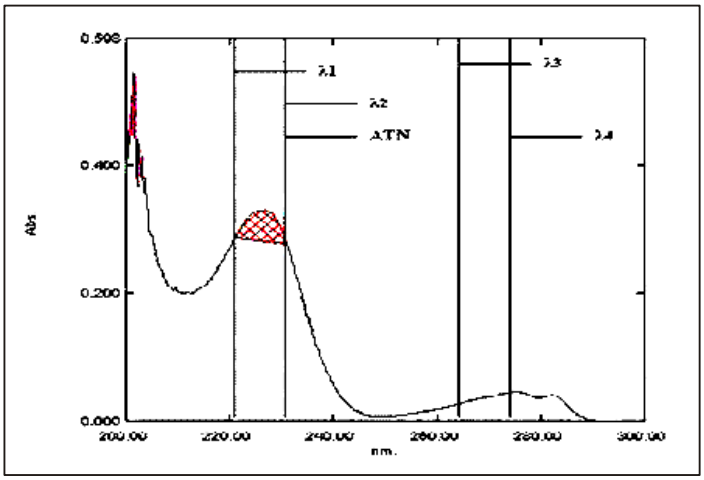

Fig. 3: Spectra showing Area Under Curve for Atenolol in the wavelength range, 221(λ_1) to 231 (λ_2) nm and 265 (λ_3) to 275 (λ_4)

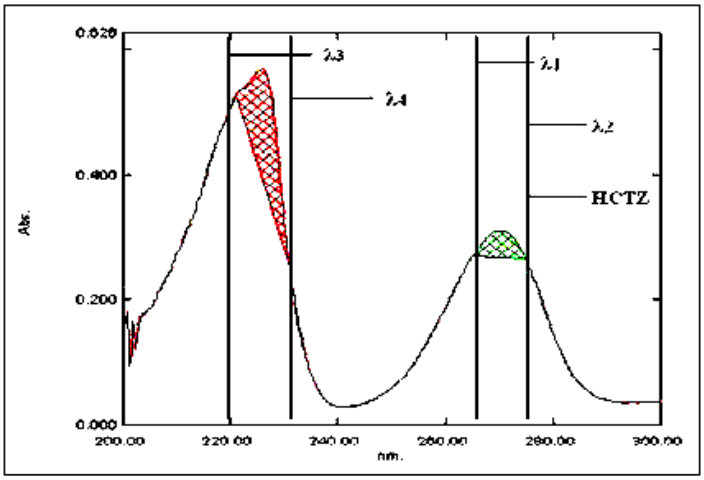

Fig. 4: Spectra showing Area Under Curve for Hydrochlorothiazide in the wavelength range of 265 (λ_1) to 275 (λ_2) nm and 221 (λ_3) to 231 (λ_4)

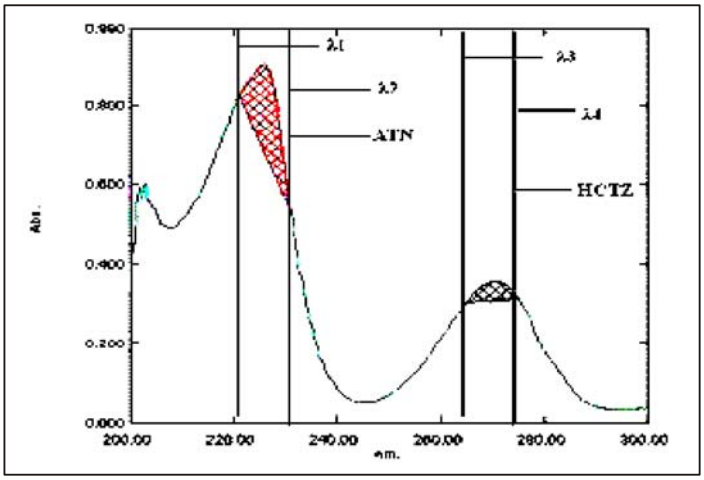

Fig. 5: Spectra showing Area Under Curve for mixture of Atenolol and Hydrochlorothiazide in the wavelength range, 221 (λ_1) to 231 (λ_2) nm and 265 (λ_3) to 275 (λ_4) nm in the Tablet dosage form

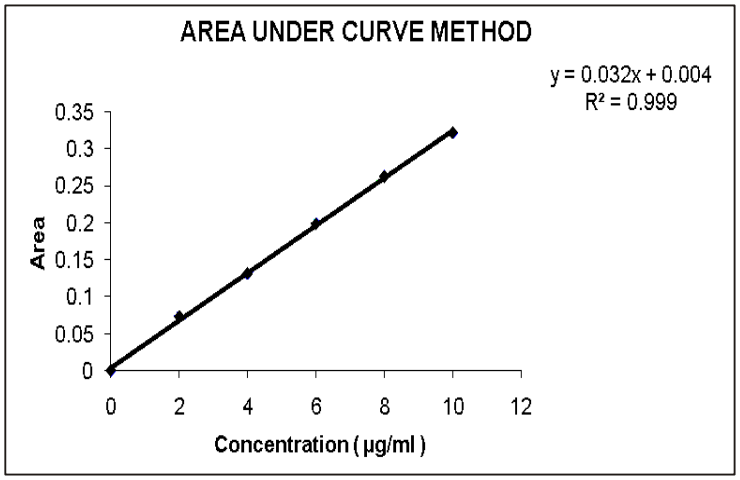

Fig.6: Calibration curve of for Atenolol at 221 to 231 nm.

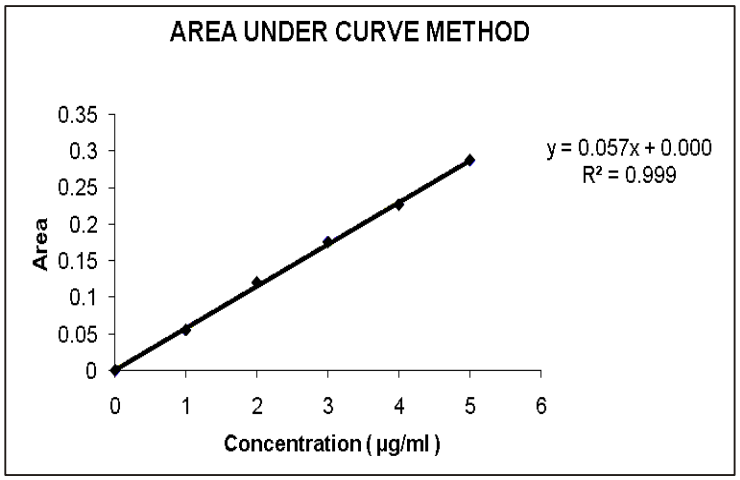

Fig.7: Calibration curve for Hydrochlorothiazide at 265 to 275 nm.

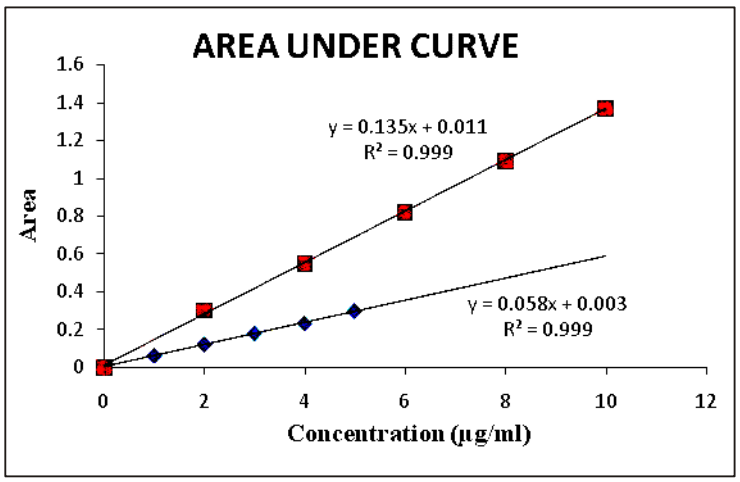

Fig. 8: Calibration curve for Formulation

The spectra of Atenolol, Hydrochlorothiazide and formulation are reported by Second order derivative method (Fig. 9, 10 and 11) and calibration curve was plotted (Fig. 12, 13 and 14).

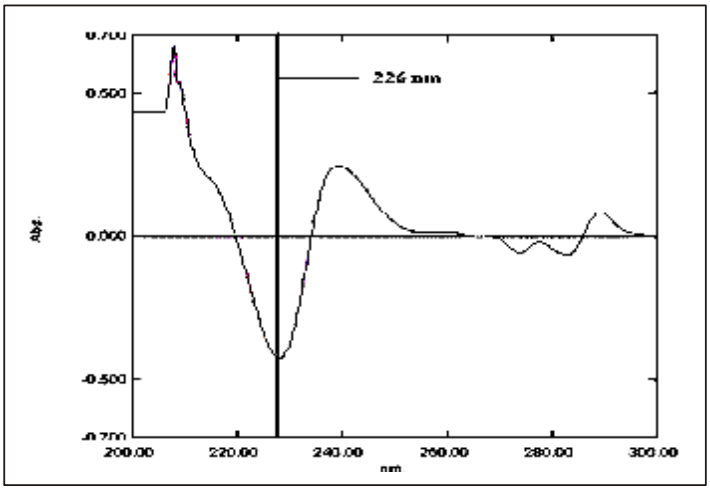

Fig. 9: Second order derivative spectra of Atenolol at 226 nm.

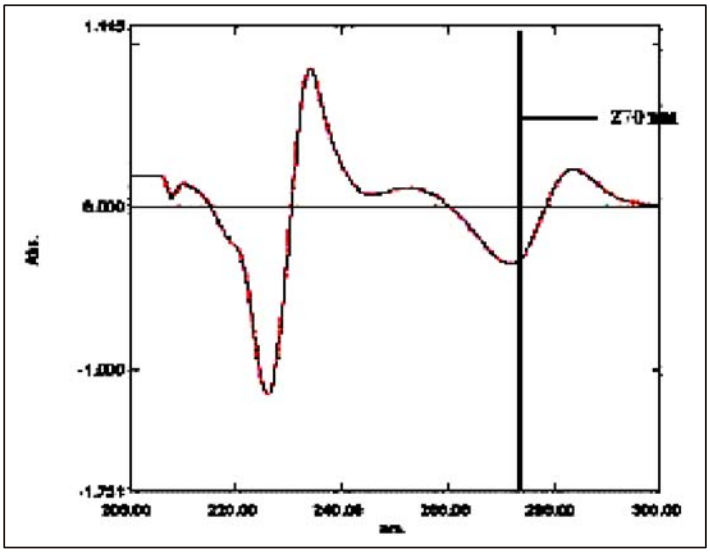

Fig. 10: Second order derivative spectra of Hydrochlorothiazide at 270 nm

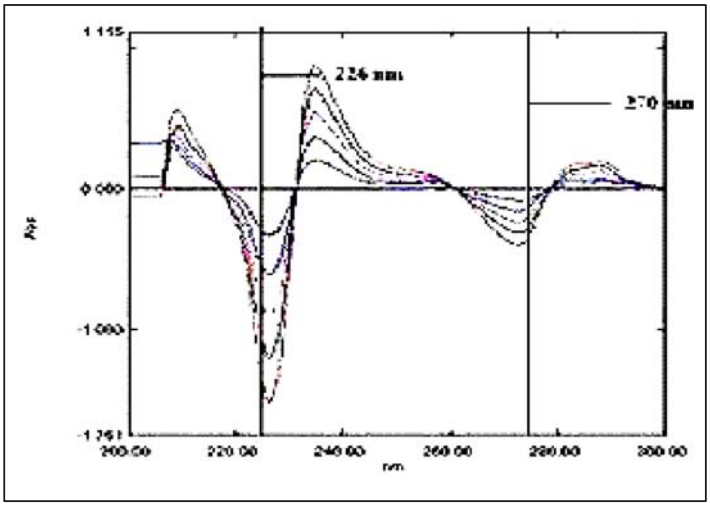

Fig. 11: Second order derivative spectra of Mixture at 226 and 270 nm

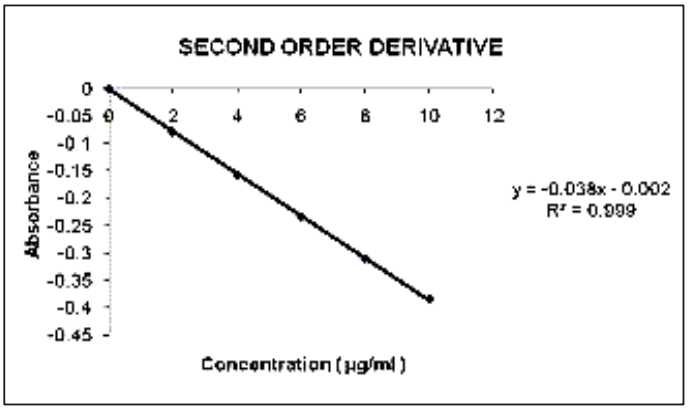

Fig. 12: Calibration curve for Atenolol at 226 nm.

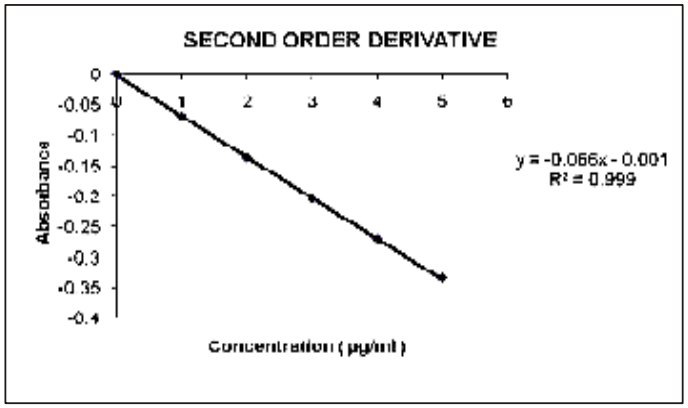

Fig. 13: Calibration curve for Hydrochlorothiazide at 270 nm.

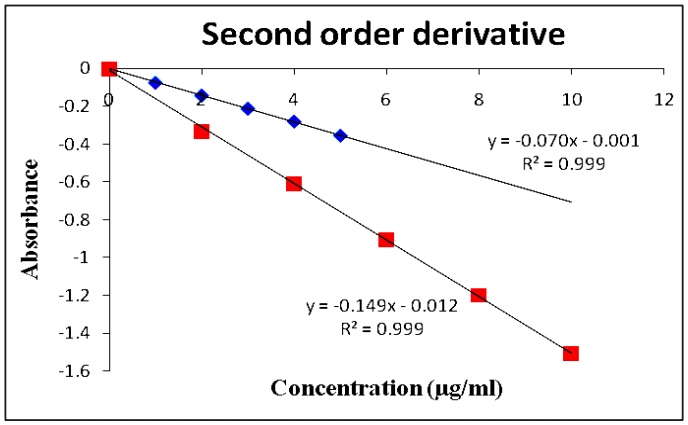

Fig. 14: Calibration curve for Formulation.

Table 1: Optical characteristics and other parameters for Method A

Parameters	Atenolol	Hydrochlorothiazide
λ_{max} / wavelength range (nm)	221-231	265-275
Linearity range (µg/mL)	2-10	1-5
Coefficient of Correlation	0.999	0.999

Cont…

Parameters	Atenolol	Hydrochlorothiazide
Slope* (m)	0.135	0.058
Intercept* (c)	0.011	0.003
Accuracy (% RSD)		
80%	0.4619	0.8284
100%	0.3772	0.2507
120%	0.7538	0.1809
Precision (%RSD)		
Intra-day	0.3760	0.4567
Inter-day	0.9179	0.4378
LOD	0.077	0.051
LOQ	0.235	0.154

*y = mx + c; when x is the concentration in µg/mL and y is absorbance unit.

Table 1: Optical characteristics and other parameters for Method B

Parameters	Atenolol	Hydrochlorothiazide
Wavelength range (nm)	226	270
Linearity range (µg/mL)	2-10	1-5
Coefficient of Correlation	0.999	0.999
Slope* (m)	-0.149	-0.070
Intercept* (c)	-0.012	-0.001
Accuracy (%RSD)		
80%	0.2730	0.2867
100%	0.2982	0.2982
120%	0.2827	0.2945
Precision (%RSD)		
Intra-day	0.4696	0.2864
Inter-day	0.6927	0.4630
LOD	0.086	2.231
LOQ	0.261	6.761

Table 2: Results of formulation

Method	Brand name	Label claim of ATN (mg)	Label claim of HCTZ (mg)	Amount found for ATN (mg)	Amount found for HCTZ (mg)	% Recovery ± SD** for ATN	% Recovery ± SD** for HCTZ
A	Aten-H25	50	25	49.79	24.93	99.13 ± 0.9933	99.72 ± 0.3123
B		50	25	49.91	24.93	99.82 ± 0.1740	99.72 ± 0.3123

**Average of six determinations

CONCLUSION

The proposed two spectrophotometric methods were found to be simple, accurate and precise and inexpensive and can be used for routine analysis of Atenolol and Hydrochlorothiazide in bulk and its formulation.

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