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Research article

A novel application of hydrotropic solubilization for quantitative estimation and validation of Atenolol and Hydrochlorothiazide in tablet dosage form

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ABSTRACT

Simple, sensitive, economical analytical methods are described for the determination of Atenolol and hydrochlorothiazide in bulk and tablet formulations. In the present investigation, 4M Ammonium acetate solution (Hydrotropic agent) was employed to solubilizing, Atenolol and hydrochlorothiazide (a poorly water soluble drug), from fine powder of its tablets to carryout spectrophotometric analysis. The proposed methods namely simultaneous equation method (Method 1) and absorbance ratio method (Method-2). λmax for Atenolol and hydrochlorothiazide is 224.5 nm and 272.5 nm respectively. Both Atenolol and hydrochlorothiazide obey Beer's law in the concentration range of 10-50 µg/ml (r2=0.9992) and 5-25 µg/ml (r2=0.9994) in 4 M Ammonium acetate (Hydrotropic agent) respectively.

Keywords: Hydrotropic solubilization, Spectrophotometry methods, ammonium acetate, ICH guideline.

INTRODUCTION

Hydro trophy is a solubilization process whereby addition of large amount of second solute results in an increase in aqueous solubility of another solute. Spectrophotometric estimation of poorly water soluble drugs generally requires the use of organic solvents, acid or base. Various organic solvents like methanol, 95%ethanol, cycloheaxne and 1, 4-dioxane etc are generally used for solubilization of poorly water soluble drugs. High cost and error in analysis due to volatility are the drawbacks of organic solvents. These drawbacks can be avoided by the use of hydrotropic solubilizing agents such as sodium salicylate, sodium benzoate, sodium lauryl sulphate, sodium glycinate, sodium gentisate, nicotinamide, urea sodium acetate, sodium citrate, Ammonium acetate, etc. Atenolol1 (Figure 1) is an antihypertensive, antianginal and antiarrhythmic drug. Chemically, it is 4-(2-hydroxy-3- isopropyl aminopropoxy)-phenyl acetamide. The Indian Pharmacopoeia describes non-aqueous titration method for the assay of atenolol. UV Spectroscopy (2, 3), reversed phase HPLC (4-6) and HPTLC, New chiral normal phase HPLC are few of the methods reported in literature for the analysis of atenolol with other drugs [3, 4]. Hydrochlorothiazide (Fig:2) 6-chloro-3, 4-dihydro-2H-1, 2,4 benzothiadiazine- 7-Sulphonamide 1, 1-dioxide, is a diuretic, which inhibits active chloride re-absorption at the early distal tubule via the Na-cl co-transporter, resulting in an increase in the excretion of sodium chloride and water. UV Spectroscopy absorbance Ratio Spectroscopy,

Derivative Spectrophotometry HPLC and HPTLC methods are reported for simultaneous estimation of Hydrochlothiazide in combined dosage form. No method has been reported for the estimation of Atenolol (ATN) and Hydrochlorothiazide (HCT) in combined dosage form by using hydrotropic solubilization technique. Present work emphasizes on the quantitative estimation of Atenolol and Hydrochlorothiazide in their combined dosage form hydrotropic solubilization

MATERIAL AND METHOD

Apparatus

The proposed work was carried out on a Systonic UV-visible spectrophotometer (model UV-2203 series), having double beam detector configuration with 1 cm matched quartz smple cells, Cyclo Mixer, centrifuge apparatus were used [1].

Reagents and standards

Reference standard of atenolol and hydrochlorothiazide was generous gift from Aristo pharmaceutical pvt. Ltd., Mandideep, Bhopal (India). Ammonium acetate was obtained from Oxford Laboratory, Mumbai. Commercial tablets marketed combination of atenolol (50mg) and hydrochlorothiazide (25mg) is ATEN-H 25 (Zydus cadila) were procured from the local drug market [2].

Method 1- Simultaneous Estimation method

Preliminary solubility studies

Solubility of both drugs was determined at 32±1 °C. An excess amount of drug was added to screw capped 30 ml glass vials containing different aqueous systems viz. 4M Ammonium acetate and distilled water, buffer (pH 8.2) Enhancement of solubility of drug was more than 6 fold. This enhancement of solubility was due to the hydrotropic solubilization phenomenon [5, 6].

Preparation of standard stock, calibration curve and binary mixture solution

Preparation of Standard Stock Solution

An accurately weighed powder sample equivalent to 100 mg of Atenolol and Hydrochlorothiazide individualy was transferred to 100 ml of volumetric flask containing 20 mL 4M Ammonium Acetate solution. The flask was sonicated for about 20 min to solubilize the drug and the volume was made up to mark with distilled water and get concentration 1000 µg mL-1. The solution was filtered through Whatmann filter paper No 41 [7].

Preparation of Working Standard Solution

1 ml of stock solution of Atenolol and Hydrochlorothiazide was separately in 10 mL-1 volumetric flask and volume makeup with distilled water to get the concentration of 100 µg mL-1. Taking 1 mL from working standard solution 100 µg mL-1 and further diluted with distilled water to get concentration range 10, 20, 30,40,50 µg mL-1 for Atenolol and 5, 10, 15, 20, 25 µg mL-1 for Hydrochlorothiazide was analyzed on UV spectrophotometer [8].

Preparation of the Calibration Curves of the Drug

Each of the standard drug solutions were injected 3 times and the mean absorbance of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve. A typical chromatogram (figure 1, 2) and the calibration curve (figure 3, 4) were obtained. The result of Optical Parameter of Atenolol and hydrochlorothiazide is shown in Table No. 1 [9].

Figure 1: Selection of lmax of atenolol

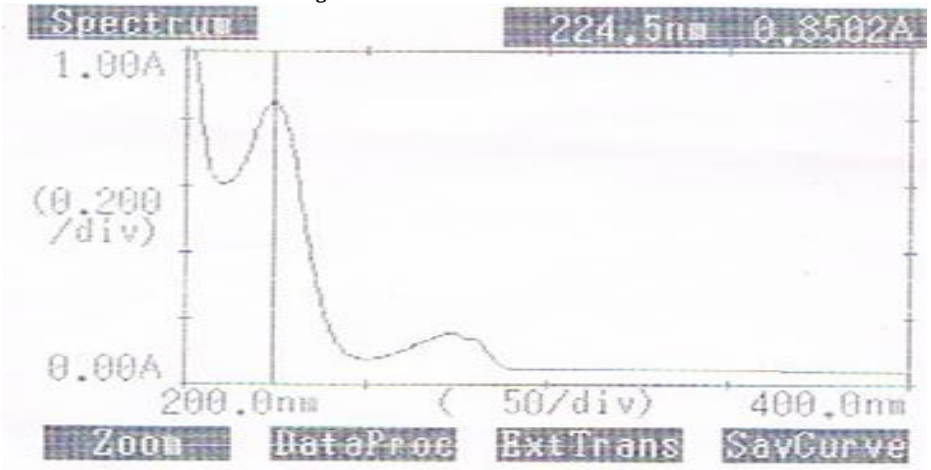


Figure 2: Selection of lmax of hydrochlorothiazide

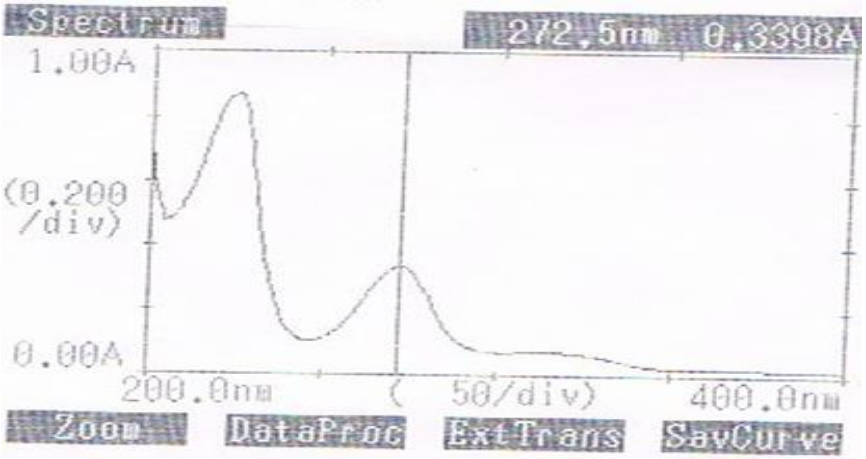


Table 1: Result of optical parameter of atenolol and hydrochlorothiazide

Parameters	**Atenolol**	**Hydrochlorothiazide**
λ_{max}	224.5 nm	272.5 nm
Beer's law limit (µg/mL)	10-50 µg/ml	5-25 µg/ml
Regression equation	Y = 0.0219x + 0.0011	Y = 0.0315x + 0.0016
Correlation Coefficient (r^2)	0.9992	0.9994
Molar Absorptivity(L $mol^{-1}cm^{-1}$)	6.23 X 10^4	0.0152 X 10^4
Sandell's Sensitivity µg/mL 0.00 absorbance unit	0.234 X 10^4	3.02 X 10^4

Figure 3: Standard Calibration Curve of Pure Atenolol

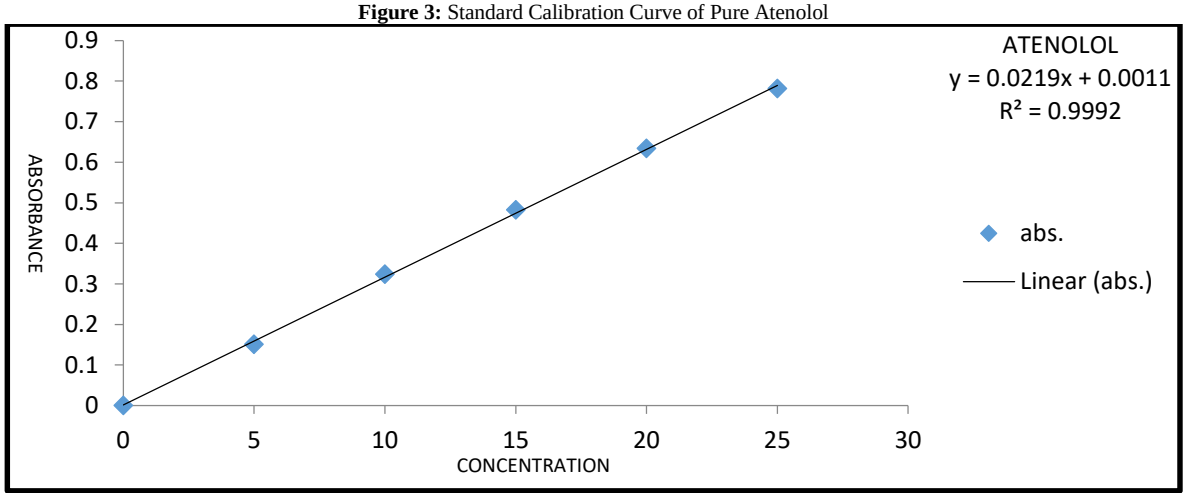
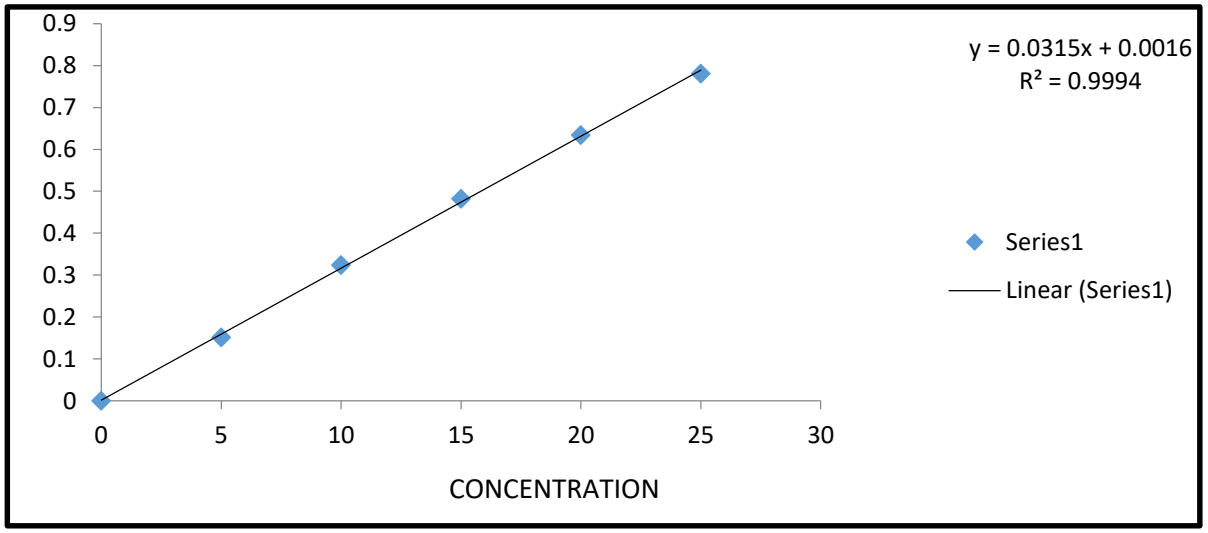


Figure 4: Standard Calibration Curve of Pure



Preparation of Analysis of Tablet Formulation

Marketed tablet ATEN-H 25 (Zydus cadila) was selected for formulation analysis. Twenty tablets were accurately weighed and crushed to obtain fine powder and accurately weight 100 mg of Atenolol and hydrochlorothiazide was transferred to a 100 ml volumetric flask containing 20 mL-1 of 4M Ammonium Acetate solution was added and shaken for about 10 min. to solublized the drug present in the tablet powder volume was made up to the mark with distilled water, and filtered and 10 ml filtered was taken in 10 ml volumetric flask and volume was made up to mark with distilled water. Further 5 ml of this solution was taken and diluted up to 10 ml with distilled water. Absorbance was recorded at 224.5 nm and 272.5 nm was measured and against blank (Table2) [10].

Table 2: Result of tablet analysis

Brand Name	**Atenolol**		**Hydrochlorothiazide**	
	Label Claim (mg)	% Purity	Label Claim (mg)	% Purity*
ATEN-H 25	50	99.00	25	98.98

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to absorbance of analyte in the sample. The calibration curve was plotted after analysis of five different concentrations (from 10 to 50 µg/ ml and 5 to 25 µg/ml) and absorbance's for each concentration was recorded thrice and mean absorbance was calculated for Atenolol and

hydrochlorothiazide respectively. The regression equation, correlation coefficient of the standard curve of the drug is shown in Figure No. 4 and 5 for Atenolol and hydrochlorothiazide respectively [11].

Accuracy

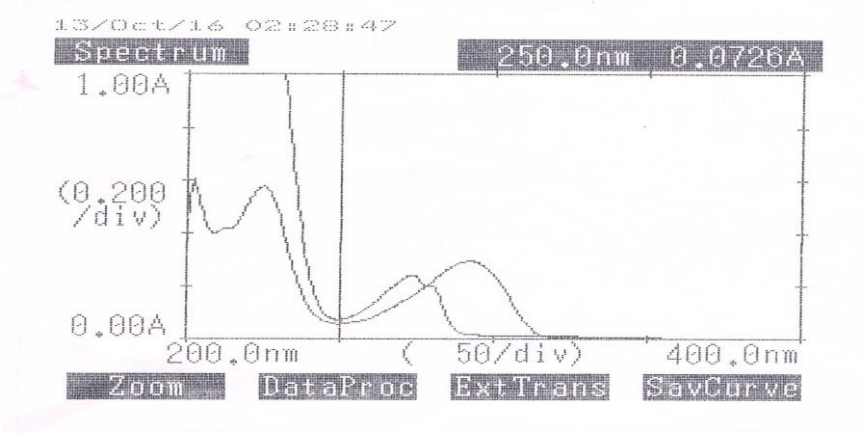
Recovery studies were performed to validate the accuracy of developed method. To pre-analyzed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed and result was shown in and statistical validation of recovery studies shown in (Table no.3) [12].

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis (Table no.4). Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out [13].

Figure 5: Isoabsorptive point



Results of Accuracy

Table 3: Recovery studies for accuracy of formulation

Level of Recovery (%)	80		100		120	
	ATN	HCZ	ATN	HCZ	ATN	HCZ
Amount Present (mg)	25 25 25	10 10 10	25 25 25	10 10 10	25 25 25	10 10 10
Amount of Std. Added (mg)	20 20 20	8 8 8	25 25 25	10 10 10	30 30 30	12 12 12
Amount Recovered (mg)	19.9 20.0 19.8	8.0 7.8 7.9	25.2 25.0 24.9	10.0 10.2 9.8	30.0 29.9 30.0	12.1 12.0 11.9
% Recovery	99.5 100.0 99.0	100.0 97.5 98.7	100.8 100.0 99.6	100.0 102 98.0	100.0 99.6 100.0	100.8 100.0 99.1

Result of Precision

Repeatability

Table 4: Results of analysis data of tablet formulation

Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.	% RSD
ATN	50	49.45	98.9	0.342	0.265
HCZ	25	24.64	98.5	0.777	0.194

Intermediate Precision

Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods. The statistical analysis method was carried out and the data is presented in (Table no.5) [14].

Analyst to Analyst

Table 5: Result of analyst to analyst precision

Analyst	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.	% RSD	Analyst	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.
	ATN	HCZ	ATN	HCZ	ATN	HCZ	ATN	HCZ	ATN	HCZ
1	50	25	50.20	25.00	100.40	100.00	0.141	0.070	0.028	0.017
2	50	25	49.90	24.80	99.80	99.2	0.070	0.106	0.014	0.026

Method 2 - Employing Absorbance Ratio Method
(Q-Analysis)

Q -a b sorbance method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λmax of one of the two components. The standard stock solution and calibration curve were prepared at described in method 1. From the overlain spectra of Hydrochlorothiazide (20 µg/ ml) and Atenolol (10 µg/ ml), two wavelengths at 250.0 nm (isoabsorptive point) and at 274.5 nm (λ max of Hydrochlorothiazide) were selected for the formation of Qabsorbance shown in Figure No. 4. The absorbances of 250.0 nm (isoabsorptive point) and λ max of Hydrochlorothiazide at 274.5 nm and the absorptivity coefficients of each drug at both wavelengths were determined. Result of analysis of tablets formulation is shown in Table No. 6 [15].

Table 6: Assay of atenolol and hydrochlorothiazide in tablet formulation (method 2)

Brand Name	Atenolol		Hydrochlorothiazide	
	Label Claim (mg)	% Purity	Label Claim (mg)	% Purity*
ATEN-H 25	50	99.80	25	98.60

Validation of Method 2 - Absorbance Ratio Method (Q-Analysis)

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to absorbance of analyte in the sample. The calibration curve was ploted after analysis for five different concentrations (from 10 to 50 µg/ ml and 5 to 25 µg/ ml) and absorbances for each concentration was recorded three times, and mean absorbance was calculated for Atenolol and hydrochlorothiazide respectively. The regression equation, correlation coefficient of the standard curve of the drug is shown in Figure No.3 and 4 for Atenolol and hydrochlorothiazide respectively [16].

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre-analysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed and result is shown in Table No. 7 [17].

Precision Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to Statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out and result is shown in Table No. 8 [18].

Results of Validation Parameters:-
Results of Accuracy

Table 7: Recovery studies for accuracy of ATN and HCZ by method 2 absorbance ratio method (Q-Analysis)

Level of Recovery (%)	80		100		120	
	ATN	HCZ	ATN	HCZ	ATN	HCZ
Amount Present (mg)	25	10	25	10	25	10
	25	10	25	10	25	10
	25	10	25	10	25	10
Amount of Std. Added (mg)	20	8	25	10	30	12
	20	8	25	10	30	12
	20	8	25	10	30	12
Amount Recovered (mg)	19.7	8.02	25.3	10.2	30.2	12.4
	20.2	7.90	25.1	10.0	29.8	12.2
	19.8	7.80	24.5	9.78	30.0	11.8
% Recovery	98.5	100.2	101.2	102	100.6	103
	101	98.7	100.4	100	99.3	101.6
	99.0	97.5	98.0	97.8	100.0	98.3

Result of Precision:
Repeatability

Table 8: Results of analysis data of tablet formulation (method 2)

Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.	% RSD
ATN	50	49.42	98.84	0.401	0.402
HCZ	25	24.56	98.24	0.777	0.194

Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilutions was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods for which the result is shown in Table No.9 [19].

Analyst to Analyst

Table 9: Result of Analyst to Analyst Precision (method 2)

Analyst	Label claim mg/tab		Amount found* mg/tab		Label claim (%)		S.D.		% RSD	
						HCZ	ATN	HCZ	ATN	HCZ
1	50	25	50.12	25.10	100.20	100.40	0.068	0.108	0.112	0.167
2	50	25	49.89	24.90	99.78	99.60	0.070	0.106	0.124	0.180

DISCUSSION

This "A Novel Application of Hydrotropic Solubilization for Quantitative Estimation and Validation of Atenolol and Hydrochlorothiazide in Tablet Dosage Form". Is start from solubility of drug's here Table no. 3, 4. Shows the solubility of Atenolol and Hydrochlorothiazide in different solvents, methanol and ethanol was selected for the solubility of drug because it is cheap and it is easily available. Fig. 8, 9, shows the scanning result of Pure API's of Atenolol and Hydrochlorothiazide when it was scan in UV range i.e. 200 – 400 nm and the λ_{max} was found to be 224.5 nm and 272.5 nm respectively. Shows the standard curve of Atenolol and Hydrochlorothiazide Pure Regression equation was found from that standard curve, R^2 value is 0.9956 for Atenolol and 0.9967 for Hydrochlorothiazide so it is Linear. Shows the optical characteristics of Pure Atenolol and Hydrochlorothiazide UV -Spectrophotometry are Beer's law limit (10-50µg/mL) and (5-25µg/mL) respectively, Regression equation Y = 0.0216x + 0.0014 for Atenolol and , Y = 0.0309 x + 0.0043 for Hydrochlorothiazide, Correlation Coefficient (r^2) 0.9956 for Atenolol and 0.9967 for Hydrochlorothiazide, Molar Absorptivity (L mol^{-1} cm^{-1}) 6.23 X 10^4 for Atenolol and 0.0152 X 10^4 for Hydrochlorothiazide, Sandal's Sensitivity(µg/mL 0.001 absorbance unit) 0.234 X 10^4 for Atenolol and3.02 X 10^4 for Hydrochlorothiazide were calculated. Shows the result of Analysis of two different Marketed Tablet formulations, the RSD of the Average of 3 determinations was found to be below 1. So that method can be used for the simultaneous estimation of Atenolol and Hydrochlorothiazide from its Tablet dosage form. Shows the Recovery results on addition of Standard drug. The Mean percentage Recovery was found and RSD was also found to be below 1. Shows the Repeatability results of Marketed Tablet formulations. The Formulation was analyzed and S.D and RSD was found to be within the Range. Shows the Intraday and Interday Precision results for combined dosage form by UV Spectrophotometry. The Mean value for the Intraday and Interday precision was found. S.D and RSD are less than 1. The absorbance ratio method of Atenolol and Hydrochlorothiazide from its tablet dosage form. Shows the Recovery results on addition of Standard drug. shows, The Mean percentage Recovery was found and RSD was also found to be below 1. Shows the Repeatability results of Marketed Tablet formulations. The Formulation was analyzed and S.D and RSD was found to be within the Range. Shows the Intraday and Interday Precision results for combined dosage form by UV Spectrophotometry. The Mean value for the Intraday and Interday precision was found. S.D and RSD are less than 1. So the method was found to be more accurate without any interference.

CONCLUSION

The proposed method is new, simple, cost effective, accurate, safe, and precise, Ecofriendly can be successfully employed in the routine analysis of Atenolol and hydrochlorothiazide in bulk and tablet dosage form. There was no interference with 4M Ammonium Acetate solution (as hydrotropic agent) with other excipients. Decisive advantage is that the organic solvent was precluded but not at the expense of accuracy. There is a good scope for other poorly water soluble drugs which may be tried to get soluble in hydrotropic solubilizing agent to carry out their spectrophotometric analysis excluding the use of costlier and unsafe organic solvents. Hydrotropy may find wide use in development of aqueous formulations of poorly water soluble drugs in future. It can therefore be concluded that use of their methods can save much time and money and they can be with accuracy.

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