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Research Article

DEVELOPMENT AND VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ATENOLOL ANDHYDROCHLORTHIAZIDE IN TABLET DOSAGE FORM

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ABSTRACT

The present work describes a simple, sensitive, accurate and rapid reverse phase high Performance liquid chromatographic method for simultaneous estimation and validation of Atenolol (ATN) and Hydrochlorothiazide (HCTZ) in tablet dosage form. The chromatographic separation was accomplished on Welchrom RP-C_{18} Column (250 mm X 4.6 mm; 5µm), Shimadzu LC-20AT Prominence Liquid Chromatograph and with a mixture of 10 mM Phosphate buffer (pH 3.0): acetonitrile (50:50, v/v). The flow rate was fixed at 1.0 mL/minute and the entire separation was carried out at an ambient temperature for the HPLC system, the analysis was performed using Shimadzu SPD-20A Prominence UV-Visible detector at 235 nm. The anti-hypertensive agents, ATN and HCTZ were separated within 4 minutes. ATN and HCTZ showed retention times of 2.257 min and 3.367 min respectively. The calibration plots were linear over the concentration range of 5-25 µg/mL for Atenolol (r^2 = 0.9999) and 2.5-12.5 µg/mL for Hydrochlorothiazide (r^2 = 0.9999). The method was very sensitive with regard to LOD and LOQ for Atenolol and Hydrochlorthiazide were found to be 0.0296 µg/mL and 0.6028 µg/mL; and 0.0899 µg/mL and 1.8269 µg/mL respectively. The method was statistically validated for its linearity, precision, accuracy, specificity, Robustness and ruggedness. The optimized method proved to be specific, robust and accurate for the quality control of ATN and HCTZ in bulk drug and pharmaceutical formulations.

Keywords: Atenolol, Hydrochlorthiazide, Isocratic RP-HPLC, UV detection, Validation.

INTRODUCTION

Atenolol (ATN)**(Figure 1)**and Hydrochlorthiazide (HZT)**(Figure 2)** aremainly aimed to treat prevention of high blood pressure, stroke and myocardial infarction. Atenolol (ATN) is a β1 receptor specific antagonist, while Hydrochlorothiazide (HCTZ)is a diuretic (water pill) used for treating high blood pressure (hypertension) and accumulation of fluid.Atenolol/HCTZ is an effective and well-tolerated combination antihypertensive therapy. It gives greater antihypertensive efficacy than eithercomponent used as monotherapy and may provide a useful treatment option in patients unable to attain blood pressure goal with monotherapy.

Thorough literature survey revealed that various analytical methods have been reported for determination of determination of either ATN or HCTZ separately or in combination with other drugs in pharmaceutical formulations or biological fluids which includes Spectrophotometry[1-3]HPLC[4-14], LS-MS/MS[15]. However, no method is reported for simultaneous

estimation of these two drugs by reverse phase HPLC. So the aim of the present study was development and validated RP-HPLC method for simultaneous determination of Atenolol andHydrochlorthiazide in combined dosage form. The method was validated in compliancewith ICH recommended guidelines [16].

EXPERIMENTAL

MATERIALS AND METHODS

Chemicals and Reagents

ATN and HCTZ of pharmaceutical grade were kindly supplied as gift samples for this research work by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals usedduring the research work are of analytical grade. Potassium dihydrogen orthophosphate was bought from Rankem Ltd., Mumbai, India. Acetonitrile (HPLC grade) and triethylamine (HPLC grade) purchased from Merck Pharmaceuticals Private Ltd., Mumbai, India. O-Phosphoric acid was also purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of consist of Ziblok H was purchased from local market manufactured by Mankind Pharma Ltd., New Delhi, India.

Instruments and Chromatographic conditions

Chromatographic separations were achieved by using Shimadzu LC-20AT Prominence Liquid Chromatograph comprising a LC-20AT VP pump, Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C_{18} column (4.6 mm i.d. X 250 mm, 5 micron particle size). 20 µL of sample was injected into the HPLC system. The HPLC system data acquisition was performed with "Spinchrom" software. Separations were executed on the reversed phase column comprising a mixture of 10 mM Phosphate buffer (pH adjusted to 3.0 using triethylamine) and Acetonitrile in ratio of 50:50 v/v as mobile phase. The mobile phase was set at a flow rate of 1 mL/minute and eluate was monitored at 235 nm. In addition, an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40) and UV-Visible Spectrophotometer (Systronics model 2203) were used in this present study.

Preparation of Reagents and Standards

Mobile phase

Accurately weighed 6.056 g of potassium dihydrogen orthophosphate in 445 mL of HPLC grade water to obtain 10 mM Phosphate buffer. To this buffer 55 mL of 0.1 M phosphoric acid was poured and mixed well. The pH of the solution was then adjusted to 3.0 with triethylamine. The above prepared buffer and acetonitrile were mixed in the proportion of 50:50 v/v. The mobile phase was then duly filtered through 0.45 µm nylon membrane vacuum filtration and properly degassed by sonication.

Preparation of Standard and working standard solutions

25 mg of ATN and12.5 mg of HCTZ were perfectly weighed separately and transferred into 100 ml of calibrated flasks. The drugs are then dissolved separately in sufficient mobile phase and the solutions were filtered through 0.45µ filter and then sonicated for about 10 minutes. This is the primary standard stock solution containing 500µg/mL of ATN and 250µg/mL of HCTZ.1 mL of the above said stock solution was taken in 10 mL calibrated flask and made up to 10 mL with mobile phase to get the 50 µg/mL of ATN and 25 µg/mL of HCTZ. To yield appropriate concentrations of working standards, aliquots from the stock solutions were appropriately diluted further with the same mobile phase to obtain the final working standard solution containing concentrations of 5-25 µg/mL of ATN and 2.5-12.5 µg/mL of AML.

Preparation of Sample solution

Twenty tablets ofZiblok H each containing 25 mg of ATN and 12.5 mg of HCTZ were correctly weighed and finely powdered. A quantity of powder equivalent to 25 mg of ATN and 12.5 mg of HCTZ was weighedand transferred to a 100 ml of calibrated flasks. To this 30 mL mobile phase was added and the drugs were extracted with mobile phase. The combined extracts were made up to 100 ml with mobile phase. The mixture was allowed to keep for 10 minutes with intermittent sonication to ensure total dissolution and filter through a 0.45 µm membrane filter.Eventually further dilutions were made to attain a concentrationof 5-25 µg/mL of ATN and 2.5-12.5 µg/mL of AML.This solution was utilized for the estimation of ATN and HCTZ in sample solution.

Selection of detection wavelength

Stocksolutions of ATN, HCTZ were prepared in mobile phase to get a concentration of 1 mg/ml. The stock solutions were further appropriately diluted with mobile phase to get a concentration of 25 µg/mL of ATN and 12.5 µg/mL of AML. Both the diluted solutions were scanned in the range of 200 - 400 nm in spectrum mode by using UV spectrophotometer.During study of the overlain

spectrum, it was found that these drugs showed optimum absorbance at 235 nm. Then the detector was chosen to 235 nm for monitoring the eluents. The overlain spectrum was recorded and is shown in **Figure 3.**

Validation of the proposed method

The developed method of analysis was validated as per the recommendations of ICH guidelines for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) and solution stability.

System suitability

The chromatographic systems utilized for analysis must pass the system suitability limits before sample analysis can commence. System suitability tests are an integral part of chromatographic method, which are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 50 and 12.5 μg/ml of ATN and HCTZ, respectively, to check the reproducibility of the system.**Table 1**shows optimum system suitability conditions.

Specificity

The specificity of the method was performed by separate injections of ATN and HCTZ standard and sample. The existence of large range of excipients and other additives generally present in the formulations in the determinations under optimum conditions was investigated. The general excipients such as lactose anhydrous, microcrystalline cellulose, purified talc, hydroxyl propyl methyl cellulose (HPMC) and magnesium stearate have been added to the placebo solution and injected and tested.

Linearity

Aliquots of primary working standard solution consisting ATN and HCTZ were diluted in such a way to get the eventual concentrations of ATN and HCTZ in the range of 5-25 μg/mL and2.5-12.5 μg/mL respectively. The solutions were injected using 20 μl and chromatograms were recorded. The calibration curves were constructed for the proposed assay methods by plotting average peak area versus concentrations and regression equations were computed for both the drugs.

Precision

Precision of the assay was done by repeatability (Intra-day) and Intermediate precision(Inter-day). The precision was evaluated by carrying out five independent assays of the sample. The intermediate precision was carried out by analyzing the assay six times on two different days. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0.

Accuracy (%Recovery studies)

The accuracy of the proposed method was determined by calculating the percentage recovery of ATN and HCTZ. For both the drugs, recovery studies were carried out by applying the method to drug sample to which known amount of ATN and HCTZ corresponding to 80, 100, and 120% of label claim had been added (standard addition method).

Robustness

To evaluate the robustness of HPLC method, a few parameters were deliberately changed. The parameters such as the slight variation in acetonitrile percentage composition in the mobile phase, flow rate, wavelength detection. One factor was changed at one time to estimate the effect.

Limit of detection and Limit of quantitation

Limit of Detection and Limit of Quantitation were calculated using following formula LOD = 3.3(SD)/S and LOQ = 10 (SD)/S, where SD = standard deviation of response (peak area) and S= slope of the calibration curve. The LOD and LOQ for the estimation of ATN were found to be 0.02969μg/mL and 0.08997μg/mL and for the estimation of HCTZ were0.6028μg/mL and 1.8269μg/mL respectively.

Stability of standard and sample solutions

In order to demonstrate the stability of both standard and sample solutions of ATN and HCTZ for during analysis, both solutions were analyzed over a period of 5 hours at room temperature. The results show that for solutions, the retention time and peak area of ATN and HCTZ remained almost unchanged (% RSD less than 2.0) and no significant degradation within the indicated period.

Application of method to marketed formulations

The proposed validated method was successfully applied to determination of the ATN and HCTZ in

the commercial tablet dosage form Ziblok H. 20 µL of above prepared sample solution was injected into HPLC and peak areas were measured under optimized chromatographic conditions. The assay was repeated for six times and the amount of the drug present per tablet was estimated from calibration equation.

RESULTS AND DISCUSSION

For getting suitable mobile phase for the analysis of the selected drugATN and HCTZ combination, various mixtures of acetonitrile and phosphate buffer were tested. Different column types and lengths were tried regarding other chromatographic parameters. C_{18} column with a 4.6 mm inner diameter, 250 mm length and 5 micron particle size was preferred. After some trials, it was found that the mobile phase consisting mixture of phosphate buffer (pH-3.0) and acetonitrile in a composition of 50:50, v/v, pH 3.0 at 1ml/min flow rate was optimized which gave two sharp well resolved peaks with minimum tailing factor for ATN and HCTZ at 235 nm in short runtime (6 min). The retention times for ATN and HCTZ were 2.257 minutes and 3.367 minutes respectively. UV overlain spectra of these drugs showed that these drugs absorbed appreciably at 235 nm, so that this wavelength was chosen as the detection wave length **(Figure 3).** The linearity of the method was determined at five concentration levels ranging from 5 to 25 µg/ml for ATN and 2.5 to 12.5µg/ml for HCTZ. The calibration curve data is shown in **Table 3.**The data of regression analysis of the calibration curve data is presented in **Table 4.** The regression equation obtained from linearity plot for ATN was Y = 36.79X+0.273 with r^2 value equal to 1and for HCTZ was Y = 42.37X+8.844 (R^2=0.9999) which shows that this method had good linearity. The representative chromatograms for calibration standards are shown from **Figure 6** to **Figure 10**. The calibration plot for ATN and HCTZ were shown in **Figure 12** and **Figure 13** respectively.The retention time of the ATN did not interfere with the retention time of HCTZ so the developed method was found to be specific since no significant peak was observed from the tablet excipients.**Figure 4** and **Figure 5** shows the chromatograms of blank and drug matrix (synthetic mixture) pertaining to combination drug of ATN and HCTZ. **Table 2**shows specificity data. The precision of the method was demonstrated by inter-day and intra-day variation studies and having RSD value less than 2.0 which indicate the method's precision. Results of the precision study are represented in the **Table 5.** Recovery studies **(Table 6)** of the method was found to begood within the overall mean % recovery of the tablet dosage form. Robustness was done by small deliberate changes in the chromatographic conditions like mobile phase flow rate, λ_{max}, mobile phase composition. The developed method was found to be robust as there were no marked changes in the chromatograms. The Robustness results are shown in **Table 7**which indicates that the small change in the conditions did not significantly affect the determination of ATN and HCTZ.The LOD and LOQ for ATN and HCTZ were found to be 0.0296 µg/mL and 0.0899 µg/mL; and 0.6028 µg/mL and 1.826 µg/mL, respectively. These results simply states that the method possesses relatively low values of LOD and LOQ. The proposed validated method was successfully applied to determine the assay of tablet and results are presented in **Table 8**. The representative sample chromatogram is shown in **Figure 11.** The assay results were found to be within the proposed limits. The results obtained for the combination of ATN and HCTZ were comparable with the corresponding labeled amounts. The mean assay values were found to be 100.73%±0.68 for ATN and 99.68%±0.51 for HCTZ. The results of the assay indicate that the method is sensitive for the assay of ATN and HCTZ without interference from the excipients used in these studies.

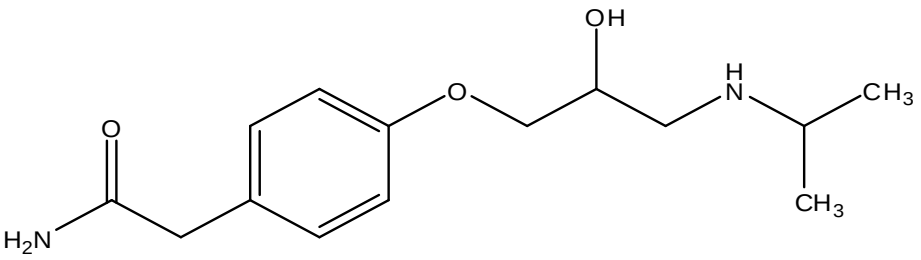

Fig. 1: Structure Atenolol investigated in the present study

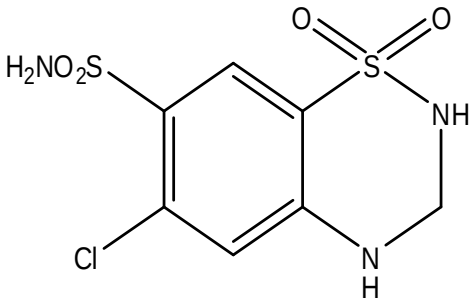

Fig. 2: Structure Hydrochlorothiazide investigated in the present study

Table 1: Optimized chromatographic conditions and system suitability parameters

Parameter	Chromatographic conditions	
Instrument	SHIMADZU LC-20AT Prominence liquid chromatograph	
Column	WELCHROM C_{18} Column (4.6 X 250mm, 5µm)	
Detector	SHIMADZU SPD-20A Prominence UV-Vis detector	
Diluents	10mM Phosphate Buffer(pH 3.0) : Acetonitrile (50:50, v/v)	
Mobile phase	10mM Phosphate Buffer(pH 3.0) : Acetonitrile (50:50, v/v)	
Flow rate	1mL/min.	
Detection wave length	UV at 235nm.	
Run time	5 minutes	
Column back pressure	155-158 kgf	
Temperature	Ambient temperature (25 ^{o}C)	
Injection Volume	20 µL	
	Atenolol	**Hydrochlorthiazide**
Retention time (t_R)	2.257 min.	3.367 min.
Theoretical plates[th.pl] (Efficiency)	4063	10683
Tailing factor (asymmetry)	1.060	1.063

Table 2: Specificity study

Name of the solution	Retention time, (t_R)min.
Mobile phase	No peaks
Placebo	No peaks
Solution containing a concentration of ATN, 25 µg/mL and HCTZ, 12.5 µg/mL.	Peaks at 2.257 min and 3.367 min for ATN and HCTZ respectively.

Table 3: Calibration data

Atenolol		Hydrochlorthiazide	
Concentration, µg/mL	Peak area, mV.s.	Concentration, µg/mL	Peak area, mV.s.
0	0	0	0
5	184.586	2.5	219.187
10	368.075	5	441.075
15	552.688	7.5	652.953
20	736.060	10.5	864.385
25	920.161	12.5	1053.663

Table 4: Linear Regression Data

Parameter	ATN	HCTZ
Detection wavelength(λ_{max})	UV at 235 nm	UV at 235 nm
Linearity range (µg/mL)	5-25 µg/mL	2.5-12.5 µg/mL
Regression equation (Y = aX + b)	Y = 36.79X + 0.273	Y = 42.37X + 8.844
Slope(a)	36.79	42.37
Intercept(b)	0.273	8.844
Standard error of slope (S_a)	0.67401	0.51133
Standard error of intercept (S_b)	10.2034	7.74058
Standard error of estimation (S_y)	0.331	10.6952
Regression coefficient (r^2)	0.9999	0.9999

#Average of 6 determinations; acceptance criteria < 2.0.

Table 5: Results of precision study

PRECISION STUDY	ATN	HCTZ
	%RSD	%RSD
INTRA-DAY	0.1141	0.0451
INTER-DAY	0.0894	0.0641

Acceptance criteria < 2.0.

Table 6: Recovery Data

Name of the drug	Mean % recovery* ± SD (n = 3 at each level)			% RSD
	80 %	100 %	120 %	
ATN	99.83 ± 0.023	99.96 ± 0.129	99.94 ± 0.015	0.132
HCTZ	99.76 ± 0.902	99.87 ± 1.777	99.15 ± 1.558	1.413

* Mean concentration at triplicate level.

Table 7: Robustness data

Parameter	Used	Retention time (t_R), min.		Plate count$		Peak asymmetry#		Remarks
		ATN	HCTZ	ATN	HCTZ	ATN	HCTZ	
Flow rate (±0.2 mL/min)	0.8 mL/min	2.251	3.361	4060	10680	1.209	1.269	*Robust
	1.2 mL/min	2.259	3.368	3069	10689	1.211	1.263	*Robust
Detection wavelength (±5 nm)	240 nm	2.251	3.361	4071	10690	1.234	1.222	Robust
	230 nm	2.257	3.369	4060	10676	1.216	1.210	Robust
Mobile phase composition (±2 % v/v)	52:48,% v/v	2.250	3.361	4057	10687	1.211	1.207	*Robust
	48:52, % v/v	2.258	3.369	4069	10699	1.256	1.227	*Robust

Acceptance criteria (Limits): #Peak Asymmetry < 1.5, $Plate count > 3000, *significant change in Retention time.

Table8: Assay results

Formulation	% Assay ± SD*	
	ATN	HCTZ
Ziblok H	100.73 % ± 0.68	99.68 % ± 0.51
Atenova-H	99.98 % ± 0.28	99.98 % ± 0.12

*Average of 6 determinations; SD is standard deviation.

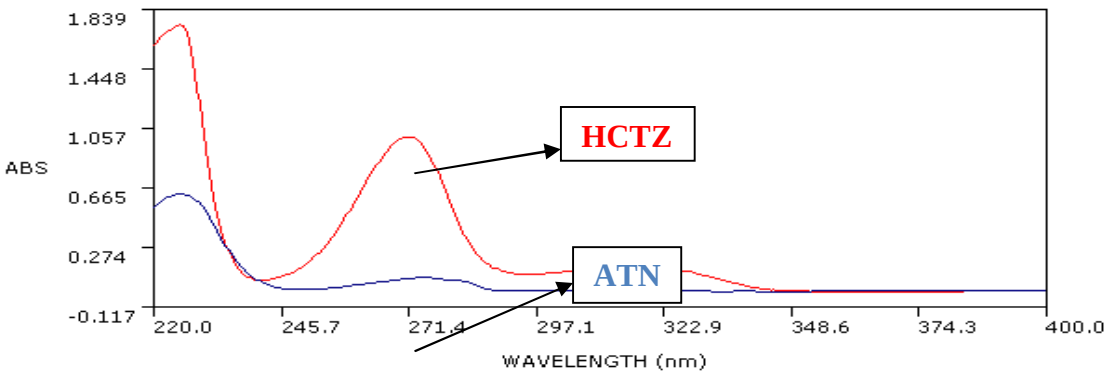

Fig. 3: Overlain Spectra of Atenolol and Hydrochlorthiazide

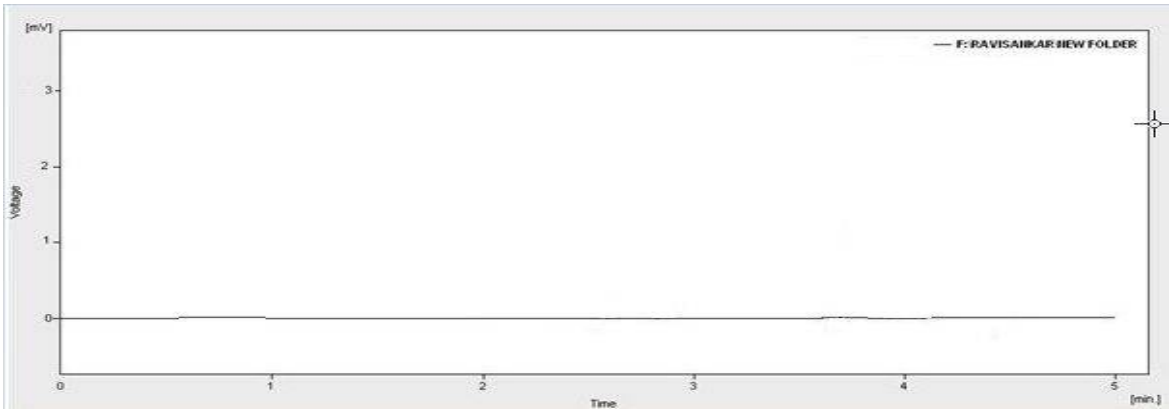

Fig.4: Chromatogram of blank solution (ATN and HCTZ).

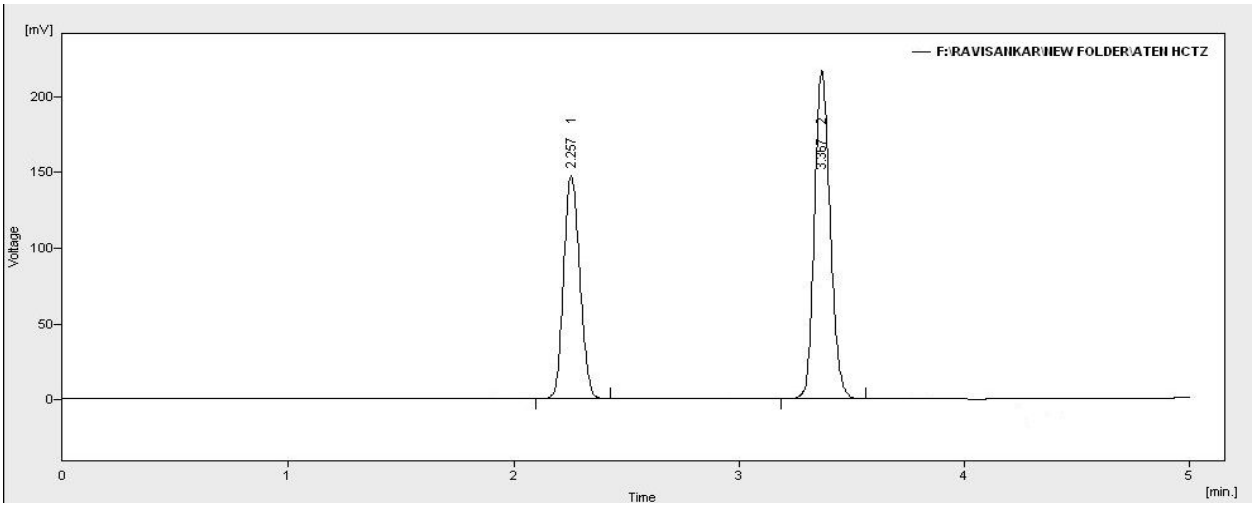

Fig. 5:Chromatogram of ATN and HCTZ synthetic drug

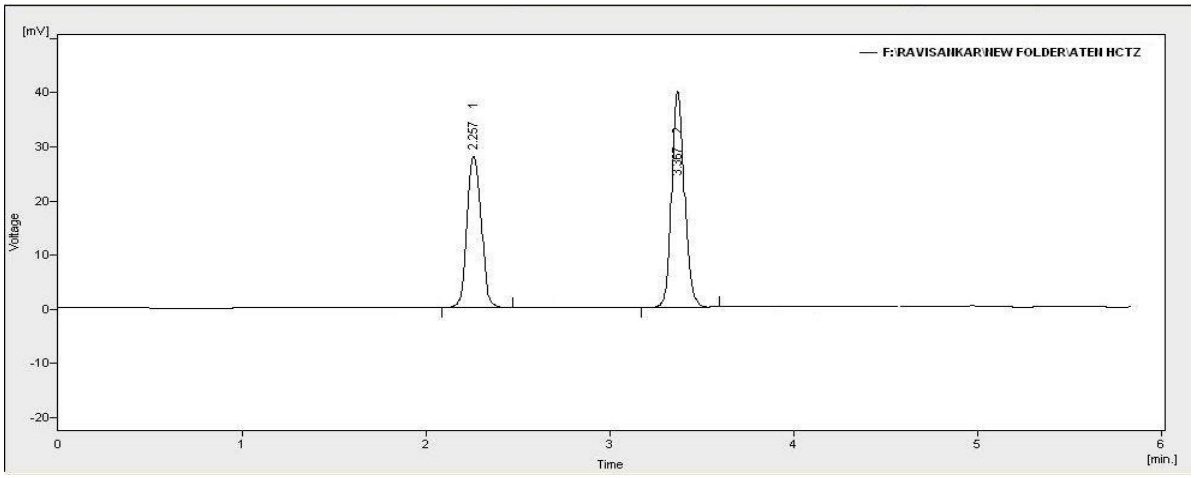

Fig. 6:Standard chromatogram of Atenolol (5 µg/mL) and Hydrocholorthaizide(2.5 µg/mL)

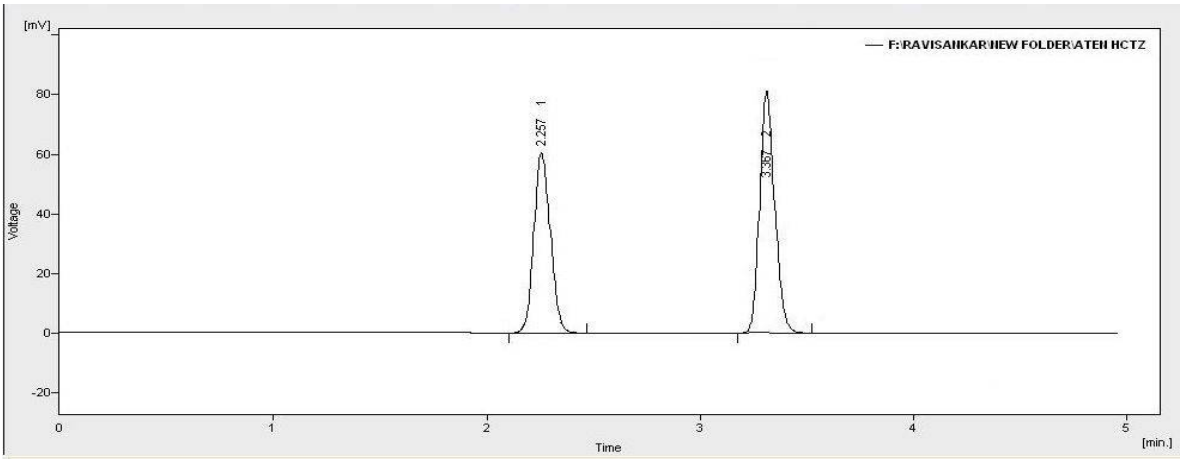

Fig. 7:Standard chromatogram of Atenolol (10 µg/mL) and Hydrochlorthiazide (5 µg/mL)

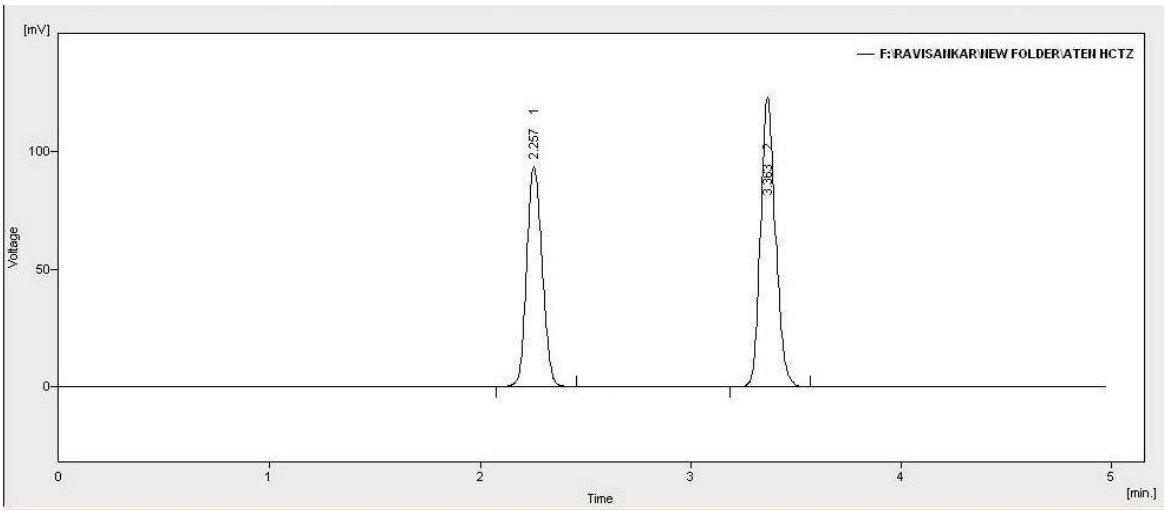

Fig.8:Standard chromatogram ofAtenolol (15 µg/mL) andHydrochlorthiazide(7.5µg/mL)

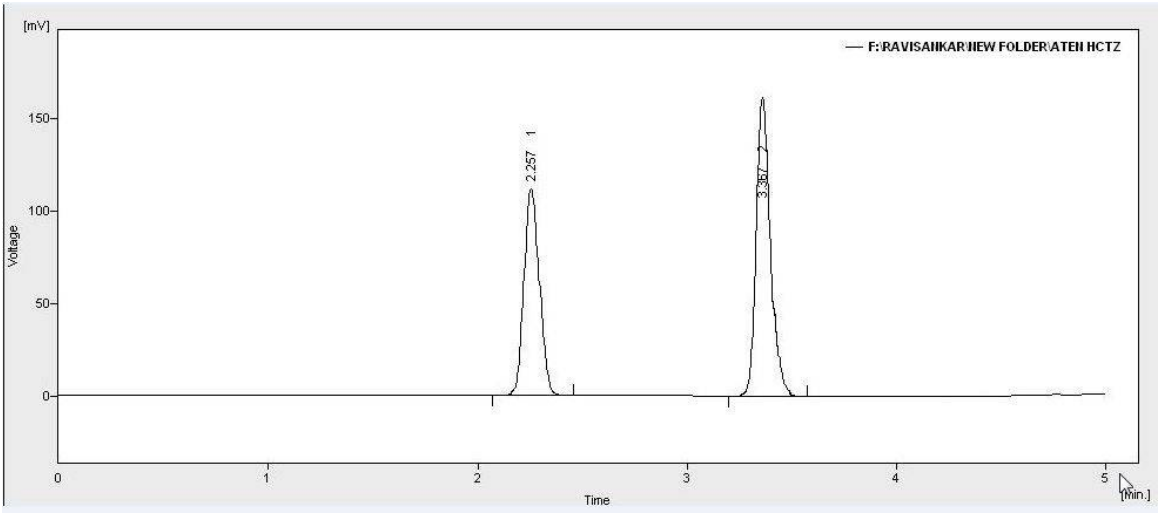

Fig.9: Standard chromatogram of Atenolol (20 µg/mL) andHydrochlorthiazide(10.5 µg/mL)

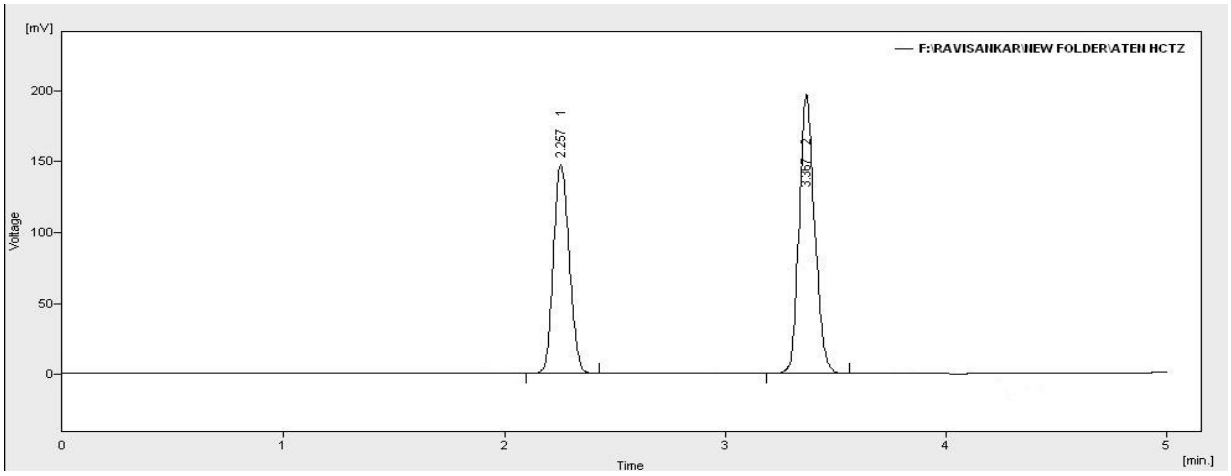

Fig. 10:Standard chromatogram of Atenolol (25 µg/mL) andHydrochlorthiazide(12.5 µg/mL)

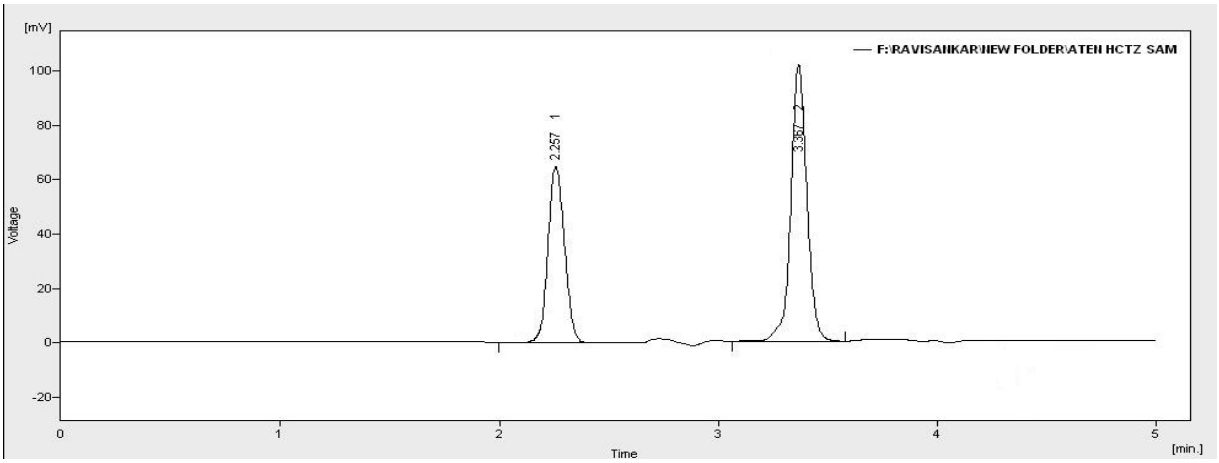

Fig. 11:Chromatogram of market formulation (ZiblokH tablets)

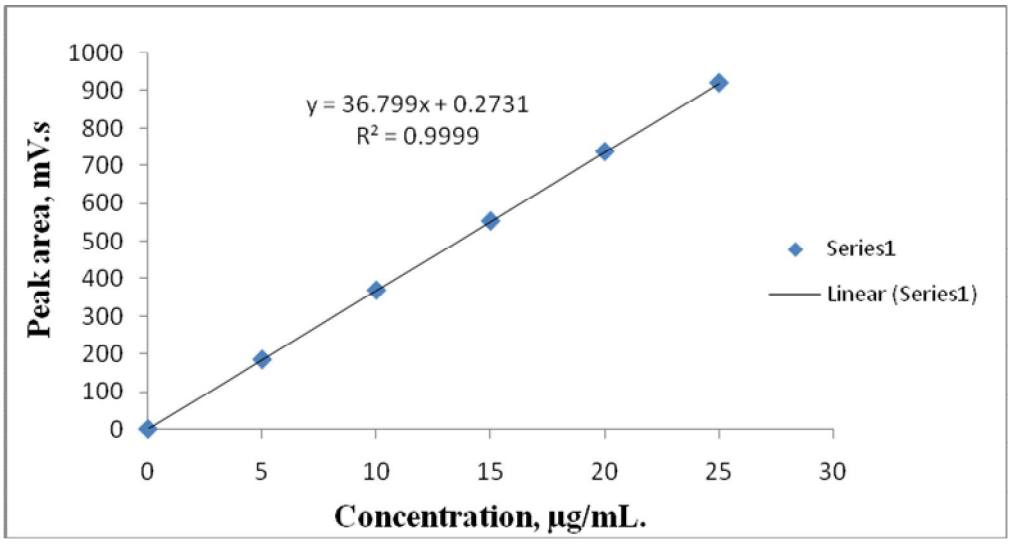

Fig.12:Calibration plot of Atenolol

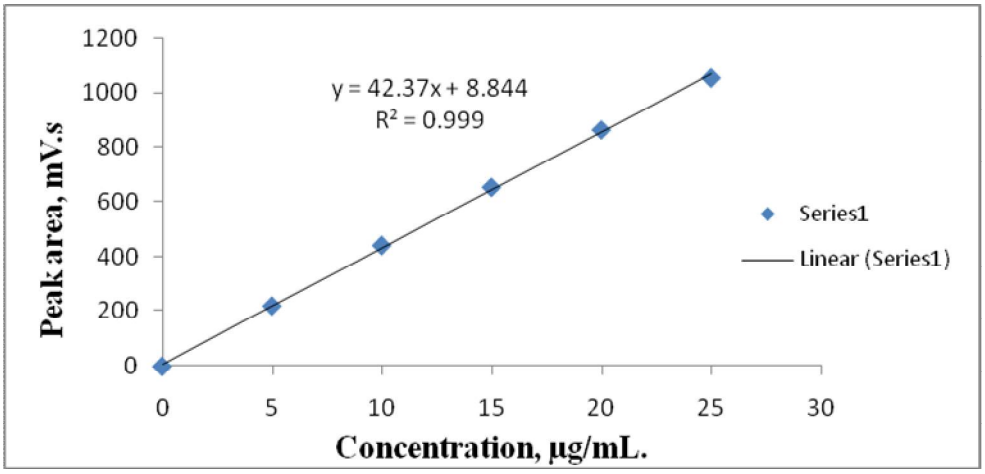

Fig.13: Calibration plot of Hydrochlorthiazide

CONCLUSION

The present proposed research study by the author describes the estimation of ATN and HCTZ available as combination tablet dosage forms and was carried out by utilizing RP-HPLC. The linearity of the proposed method was in the range of 5-25 µg/mL for ATN and 2.5-12.5µg/mL for HCTZ respectively. The method was very sensitive with regard to limit of detection The LOD and LOQ for ATN and HCTZ were found to be 0.0296 µg/mL and 0.0899 µg/mL; and 0.6028µg/mL and 1.826 µg/mL, respectively. The above said antihypertensive agents of total runtime of 5 minutes with an elution window of 1.5 minutes were achieved. The developed RP-HPLC method for the quantification of ATN and HCTZ was found to be simple, specific, highly sensitive, fast, economical, precise and extremely accurate with robustness. The developed method has several advantages like decorous linearity, less retention times and less solvent consumption which makes the method more economical. The developed RP-HPLC method for the estimation of ATN and HCTZ in combined dosage form is simple, quick, accurate, precise, linear and robust. Hence this present RP-HPLC method is widely suitable for the routine analysis of ATN and HCTZ in bulk powder and pharmaceutical tablets without any interference.

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REFERENCES

1. Thomas AB, Chavan UB, Nanda RK, Kothapalli LP, Deshpande AD, Jagdale SN and Dighe SB. Simultaneous spectrophotometric estimation of Hydrochlorothiazide, Atenolol and Losartan potassium in tablet dosage form. Hindustan Antibiot Bull. 2009;51(1-4):33-8.
2. Gupta KR, Tajne MR and Wadodkar SG. New spectrophotometric method for simultaneous determination of metoprololtartarate and hydrochlorthiazide in tablets. Indian J Pharm Sci. 2008;70(4):511-513.
3. Panzade PD and Mahadik KR. Simultaneous spectrophotometric estimation of lisinopril and Hydrochlorthiazide from combined dosage forms. Indian Drugs. 1999;36(3):21-23.
4. Belal F, Sharaf El-Din M ,Aly F, Hefnawy M and El-Awady M. Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations. J Chromat Separation Techniq. 2013;4(1):1-7.
5. Sachin Bhagwate and Gaikwad NJ. Stability Indicating HPLC Method for the Determination of Hydrochlorothiazide in Pharmaceutical Dosage form. Journal of Applied Pharmaceutical Science. 2013;3(2):88-92.
6. Shetkar PB and Shinde VM. Simultaneous determination of Enalapril maleate and Hydrochlorothiazide in tablets by RP-HPLC. Anal Lett. 1997;30:1143-52.
7. Rawool ND and Venkatchalam A. Analytical method for the simultaneous estimation of hydrochlorothiazide and metoprolol tartrate using RP HPLC. Indian JPharm Sci. 2011;73(2):219-23.
8. Baheti KG, Shah N and Shaikh S. Ion-pairing reverse-phase high performance liquid chromatography method for simultaneous estimation of atenolol and indapamide in bulk and combined dosage form. Indian J Pharm Sci. 2012;74(3):271-274.
9. Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT and Shah AK. A Stability-indicating High Performance Liquid Chromatographic Assay for the Simultaneous Determination of Atenolol and Lercanidipine Hydrochloride in Tablets. Indian J Pharm Sci. 2011;73(4):376-80.
10. Spanakis M and Niopas I. Determination of atenolol in human plasma by HPLC with fluorescence detection: validation and application in a pharmacokinetic study. J Chromatogr Sci. 2013;51(2):128-32.
11. Ravisankar P and DevalaRao G. Isocratic separation of four beta blockers with Amlodipine by C18 RP-HPLC. Application to Amlodipine determination in pharmaceutical dosage forms. Int Res J Pharm. 2013;4(6):88-96.
12. Ravisankar P, DevalaRao G and Krishna Chaitanya M. An improved RP-HPLC method for the simultaneous separation of four anti-hypertensive agents: application to assay of atenolol hydrochloride in bulk and tablet dosage form. Pharmanest. 2013;4(3):418-39.
13. Zecevic M, Zivanovic L, Agatonoric-Kustrin S and Minic D. HPLC determination of Amiloride, Methyldopa and Hydrochlorthiazide in pharmaceutical dosage form. J Pharm Biomed Anal. 2001;24:1019-25.
14. Ravisankar P, Swathi J, Santosh Kumar KVSand SrinivasaBabu P. Novel RP-HPLC method for simultaneous determination of OlmesartanMedoxomil, Amlodipine Besylate and Hydrochlorothiazide in tablet dosage form. IJBPR. 2014;5(12):927-936.
15. Kallem RR, Mullangi R, Hotha KK, Ravindranath L, Spoorthy Y and Seshagirirao J. Simultaneous estimation of amlodipine and atenolol in human plasma: a sensitive LC-MS/MS method validation and its application to a clinical PK study. Bioanalysis.2013;5(7):827-37.
16. ICH Q2 (R1), Validation of analytical procedures, Text and methodology International Conference on Harmonization, Geneva. 2005:1-17.