Ind. J. Pharm. Edu. Res., 2024; 58(3):908-917.
https://www.ijper.org

Original Article

Development and Validation of a Novel Second Derivative UV Method for the Estimation of Antihypertensive Drugs in Tablet Dosage Form

Sonal Zambrekar, Leena Sawaikar*, Swati Keny, Pradnya Pal, Surashree Gaude

Department of Pharmaceutical Chemistry, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Ponda, Goa, INDIA.

ABSTRACT

Aim: The objective of the current work was to develop and statistically validate a second order derivative method for estimating atenolol in tablet dosage form using UV spectrophotometry. **Materials and Methods:** The method validation was done in accordance with ICH recommendations. Atenolol (BSC class III) and hydrochloride (BSC class II) were soluble in 0.1 N NaOH and gave stable absorbance with it, hence 0.1 N NaOH was chosen as a solvent. The wavelengths selected were 226 nm and 274 nm for atenolol and hydrochlorothiazide respectively. The proposed method was validated for parameters like linearity, precision, robustness, accuracy, limit of detection and limit of quantification. **Results:** The method was found to be linear with a correlation coefficient (R^2) of 0.999 and within a concentration range of 5-60 µg/mL for atenolol and 5-50 µg/mL for hydrochlorothiazide. The analysis of tablet formulation was carried out using second order derivative method and percentage mean assay was found to be 100.4% and 100.9% for atenolol and hydrochlorothiazide respectively. The method was statistically validated showed less % RSD indicating that method is precise, accurate and robust.

Keywords: Atenolol, Hydrochlorthiazide, UV spectroscopy, Second derivative, ICH guidelines.

Correspondence:
Dr. Leena Ajay Sawaikar
Department of Pharmaceutical Chemistry, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi-403401, Ponda, Goa, INDIA.
Email: mailto:sawaikarleena@gmail.com

Received: 16-04-2023;
Revised: 24-11-2023;
Accepted: 09-04-2024.

INTRODUCTION

Atenolol (ATEN), a beta-blocker is used to treat angina and high blood pressure. Hydrochlorothiazide (HCTZ) is a thiazide diuretic that increases the urine flow and prevents the retention of fluid in the body. It is used to treathigh blood pressure.[1]

Different researchers have employed different methods to estimate these two drugs in combination. The developed HPLC or HPTLC methods are time consuming and make use of costly solvents.[2-12] Further UV methods have also been developed for this in combination with other drugs.[13-16] Some UV methods have been developed for this combination however it makes use of methanol as a solvent.[17-20] Using 0.1 N NaOH as a solvent, the current study aimed to design and validate a second derivative UV method. The method is simple, fast, efficient, rapid and affordable for determining these two drugs in combined dosage form.

MATERIALS AND METHODS

Materials

Active Pharmaceutical Ingredients (API) used atenolol (Figure 1) and hydrochlorothiazides (Figure 2) were gifted by Zydus and Unichem Pvt. Ltd., respectively. The formulation ATEN H 25 was purchased from local pharmacy. It contained 50 mg of atenolol and 25 mg of hydrochlorothiazide 0.1 N NaOH was used as solvent for entire study.

Instrumentation and Equipment

UV spectrophotometer of model 1800 and Shimadzu make having UV probe as the software was used.

Methods

Choice of solvent

The drug solubility was assessed in a number of solvents like NaOH, ethanol, methanol etc. Depending upon the solubility and the solvent giving stable absorbance reading 0.1 N NaOH was selected for preparation of stock solutions.

Selection of the Wavelength

The selection of wavelengths for the estimation of atenolol and hydrochlorothiazide done by scanning standard solution

DOI: 10.5530/ijper.58.3.99

containing 10 µg/mL concentration in the UV region 200-400 nm by using 0.1 N NaOH as blank. The second order derivative spectra were obtained by making use of a derivative mode to examine the overlain spectra.

The amplitude of the second derivative spectrum of atenolol and hydrochlorothiazide was measured at 226.4 nm and 274 nm, respectively from the spectrum.

Preparation of Stock Solutions

A quantity equivalent to 25 mg of atenolol and hydrochlorothiazide was transferred in a 25 ml volumetric flask and dissolved in 0.1 N NaOH to attain a concentration of 1000 µg/mL respectively. Further dilutions were made using this standard stock solution.

Preparation of Calibration Curve

An aliquot of standard solutions of atenolol and hydrochlorothiazide was placed in a set of 10 mL volumetric flasks and the volume was adjusted with solvent, to get concentrations of 5-60 µg/mL and 5-50 µg/mL, respectively for both the drugs. The absorbance of the solution was recorded at a given wavelength and a graph of absorbance vs. concentration was plotted. The regression equation and correlation coefficient (r^2) were determined and Beer lambert's range was established.

Method Development and Validation[21]

Both the drugs were soluble in 0.1 N NaOH, so this was the solvent chosen for the method development. The method which was developed was then validated as per ICH guidelines

Linearity

The stock solutions were diluted to give a concentration of 5-60 µg/mL and 5-50 µg/mL for atenolol and hydrochlorthiazide to assess the linearity. Calculations of the detection and the quantification limits were based on this.

Precision

The precision of the method was determined by repeatability and intermediate precision.

Repeatability

Repeatability was carried out by analysing the formulation six times for the same concentration. Absorbance of these solutions

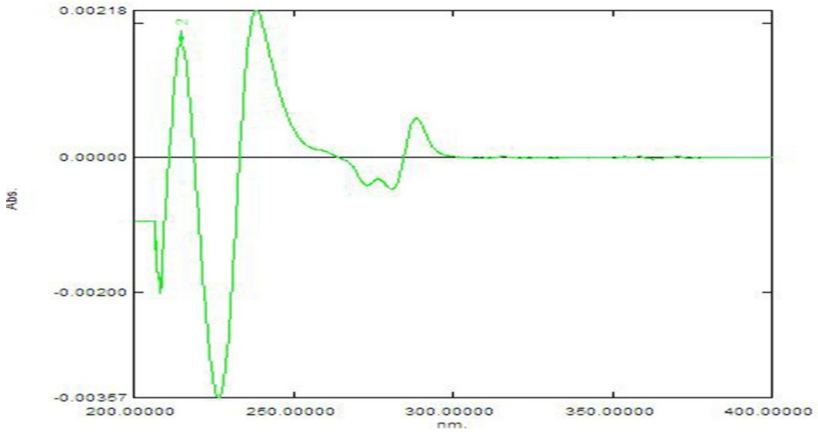

Figure 1: UV second derivative spectrum of atenolol.

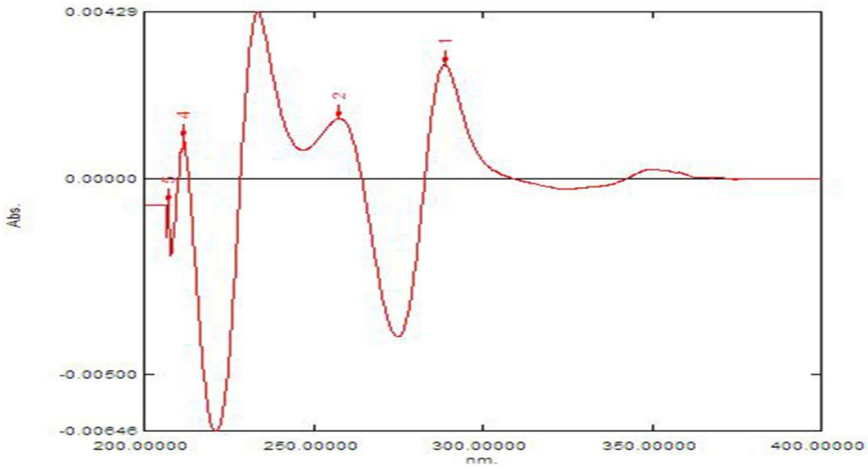

Figure 2: UV spectrum second derivative of hydrochlorthiazide.

was measured at predetermined wavelengths and calculation for the % relative standard deviation was done.

Intermediate Precision

The intermediate precision was checked by intra-day and inter-day analysis.

Intra-day

Intra-day precision was carried out by analysing the tablet formulation of the same concentration and repeating it for three times at different intervals i.e., morning, afternoon and evening on the same day. Absorbance of these solutions measured at predetermined wavelengths and % relative standard deviation was calculated.

Inter-day

Inter-day precision was carried out by the analysing the tablet formulation having same concentration and repeating it three times on three successive days. Absorbance of these solutions measured at predetermined wavelengths and % relative standard deviation was calculated.

Accuracy

Accuracy was checked by performing recovery studies by using pre-analysed sample solution and by evaluating % mean recovery of compounds. The known concentration of drug was added at different concentration level i.e., 80%, 100% and 120%.

In three different 10 mL volumetric flasks 2.5 mL pre-analysed solution was added and 2 mL, 2.5 mL, 3 mL of atenolol standard solution as well as 1 mL, 1.25 mL, 1.5 mL of hydrochlorothiazide standard solution was added and volume was adjusted up to the mark with 0.1 N NaOH. Control was used as blank. Absorbance of solution was recorded at selected wavelengths against blank and the percentage recoveries were calculated.

Robustness

Robustness of developed method was determined by performing the assay procedure. Three minor changes were considered in experimental conditions such as:

- Using a different UV spectrophotometric instrument,
- Performing analysis by different analyst,

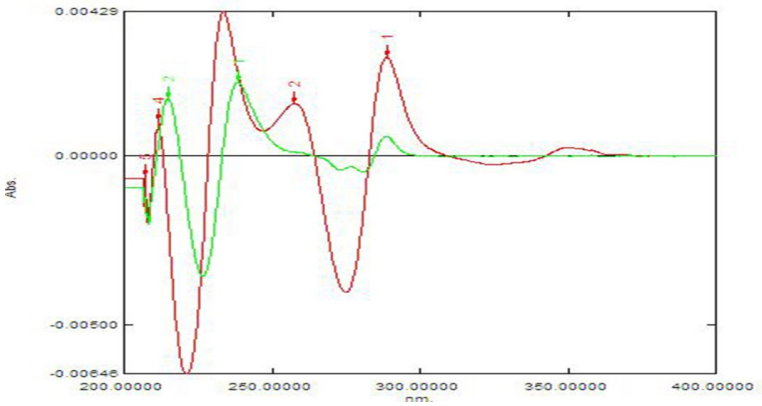

Figure 3: Overlain spectra of atenolol and hydrochlorthiazide.

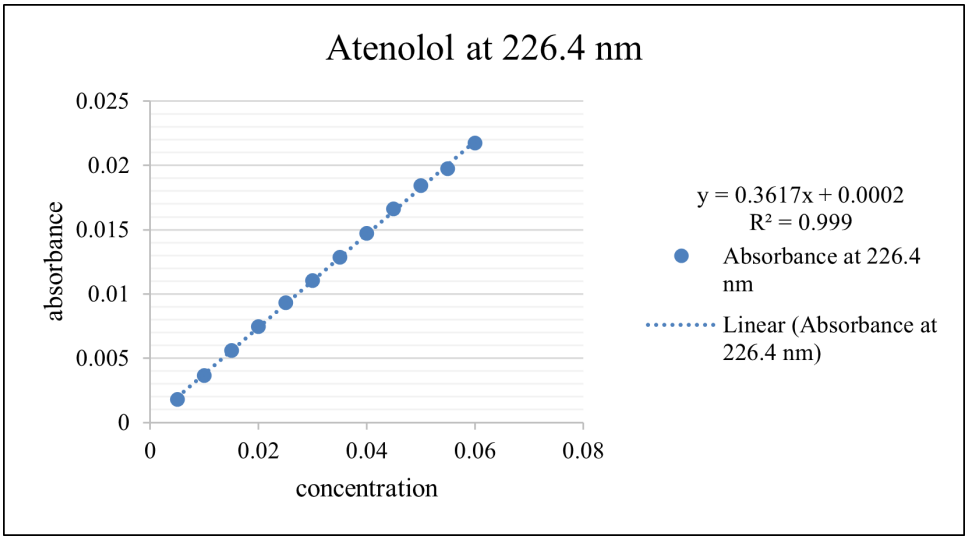

Figure 4: Calibration curve of atenolol.

- By making minor changes in wavelength being scanned (±2 nm).

Assay of Formulation

The tablet formulation was powdered and a quantity equivalent to 25 mg of atenolol was appropriately diluted to obtain the required concentration of 12.5 µg/mL of hydrochlorothiazide and 25 µg/mL of atenolol. The absorbance of these solutions was measured at all selected wavelengths. Drug content and percent purity was calculated.

A set of equations were framed for calculation of concentration of drugs by second order derivative method.[22]

After scanning the solutions in between 400 to 200 nm second order derivative spectra were obtained. The concentration of atenolol and hydrochlorothiazide present in tablet were calculated from the regression equation

$$Y = mx + c$$

Where,

Y=absorbance of drugs,

m=slope,

x=concentration of drugs,

c=intercept.

Calculation of concentration of atenolol at 226.4 nm

$$Y = 0.3617x + 0.0002$$

Where,

Y=absorbance of atenolol at 226.4 nm.

x=concentration of atenolol at 226.4 nm.

Calculation of concentration of hydrochlorothiazide at 274 nm

$$Y = 0.4351x - 0.0003$$

Where,

Y=absorbance of hydrochlorothiazide at 274 nm.

x=concentration of hydrochlorothiazide at 274 nm.

RESULTS

Method Development

Choice of solvent

Atenolol and hydrochlorothiazide were both dissolved separately in different solvents like NaOH, methanol, ethanol and distilled

Table 1: Linearity and range data of atenolol and hydorchlorthiazide.

Sl. No.	Parameters	Atenolol	Hydrochlorothiazide
		226.4 nm	274 nm
1	Linearity range (µg/mL)	5-60	5-50
2	Regression equation	Y= 0.3617x + 0.0002	Y= 0.4351x – 0.0003
3	Correlation coefficient	0.999	0.999
4	Slope	0.3617	0.4351
5	Intercept	0.0002	0.0003

Table 2: Repeatability data of atenolol and hydrochlorothiazide.

Repeatability (*n*=6)								
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
1	0.00510	0.00922	12.50	25.00	12.41	24.93	99.28	99.72
2	0.00512	0.00917	12.50	25.00	12.45	24.79	99.60	99.16
3	0.00522	0.00908	12.50	25.00	12.68	24.55	101.4	98.20
4	0.00518	0.00932	12.50	25.00	12.59	25.21	100.7	100.8
5	0.00511	0.00937	12.50	25.00	12.43	25.35	99.44	101.4
6	0.00505	0.00949	12.50	25.00	12.29	25.68	98.32	102.7
Mean							99.79	100.3
SD							1.095	1.628
% RSD							1.097	1.623

water. According to solubility characteristics, the common solvent for both drugs was found to be 0.1 N NaOH.

Choice of wavelength

Solutions of the working standard of both the drugs were scanned between 200-400 nm against the blank and the spectra of atenolol (Figure 1) and hydrochlorthiazide (Figure 2) were recorded. The choice of wavelength for analysis was made using concept of second order derivative method.

From overlain spectra, two wavelengths were selected: 226.4 nm absorbance due to atenolol only and 274 nm absorbance due to hydrochlorothiazide only. Hence 226.4 nm and 274 nm were selected as wavelengths for analysis which is depicted in Figure 3.

Method Validation

Linearity

Dilutions were made for a linearity range of 5-60 µg/mL for atenolol and 5-50 µg/mL for hydrochlorthiazide. The regression coefficient was found to be in the range (Table 1).

The calibration curve of atenolol and hydrochlorthiazide is displayed in (Figure 4 and Figure 5).

Precision

Analysis was performed by carrying out repeatability studies (Table 2), inter-day and intra-day precision studies (Table 3). According per the methodology, the solutions were made, and absorbance values were measured.

The precision of the method was confirmed from the % RSD value which was <2% for atenolol and hydrochlorthiazide.

Accuracy

The accuracy of the developed method was confirmed by a recovery study at three different concentrations (80%, 100%, and 120%). The % recovery at 80%, 100% and 120% for hydrochlorothiazide was found to be 99.85, 102.9, and 99.48% respectively and for atenolol was found to be 101.6, 99.53, and 98.92% respectively and is within the acceptable range of 95%-105%. The % RSD for atenolol and hydrochlorothiazide was found to be < 2%. Hence, the method developed was accurate (Table 4).

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

The slope and standard deviation of the calibration curve were used to determine LOD and LOQ. The developed method was found to be sensitive as seen in Table 5.

Table 3: Intra-day data for atenolol and hydrochlorothiazide.

Morning (*n*=3)								
Morning (n=3)	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	
1	0.00495	0.00902	12.50	25.00	12.06	24.38	96.48	97.52
2	0.00510	0.00909	12.50	25.00	12.41	24.57	99.28	98.28
3	0.00509	0.00915	12.50	25.00	12.38	24.74	99.04	98.96
Mean							98.26	98.25
SD							1.551	0.720
% RSD							1.579	0.733

Afternoon (*n*=3)								
Sl. No.	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**
1	0.00505	0.00928	12.50	25.00	12.29	25.10	98.32	100.4
2	0.00507	0.00919	12.50	25.00	12.34	24.85	98.72	99.40
3	0.00501	0.00935	12.50	25.00	12.20	25.29	97.60	101.1
Mean							98.21	100.3
SD							0.567	0.854
% RSD							0.577	0.851

Evening (*n*=3)								
Sl. No.	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**
1	0.00519	0.00940	12.50	25.00	12.61	25.43	100.8	101.7
2	0.00517	0.00946	12.50	25.00	12.57	25.60	100.5	102.4
3	0.00520	0.00941	12.50	25.00	12.64	25.46	101.1	101.8
Mean							100.8	101.9
SD							0.300	0.378
% RSD							0.297	0.371

Day 1 (*n*=3)								
Sl. No.	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**
1	0.00525	0.00921	12.50	25.00	12.75	24.91	102.0	99.64
2	0.00524	0.00928	12.50	25.00	12.73	25.10	101.8	100.4
3	0.00517	0.00944	12.50	25.00	12.57	25.54	100.5	102.1
Mean							101.4	100.7
SD							0.814	1.259
% RSD							0.802	1.250

Day 2 (*n*=3)								
Sl. No.	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**
1	0.00499	0.00931	12.50	25.00	12.15	25.18	97.20	100.7
2	0.00498	0.00934	12.50	25.00	12.13	25.26	97.04	101.0
3	0.00503	0.00937	12.50	25.00	12.25	25.35	98.00	101.4
Mean							97.41	101.0
SD							0.514	0.351
% RSD							0.527	0.347

Day 3 (*n*=3)								
Sl. No.	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**
1	0.00513	0.00916	12.50	25.00	12.47	24.77	99.76	99.08
2	0.00508	0.00927	12.50	25.00	12.36	25.07	98.88	100.2
3	0.00510	0.00923	12.50	25.00	12.41	24.96	99.28	99.84
Mean							99.30	99.70
SD							0.440	0.571
% RSD							0.443	0.573

Morning (*n*=3)

Table 4: Accuracy data for atenolol and hydrochlorothiazide.

Accuracy 80 % (*n*=3)								
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
1	0.00412	0.00750	10.00	20.00	10.15	20.18	101.5	100.9
2	0.00407	0.00752	10.00	20.00	10.04	20.23	100.4	101.1
3	0.00395	0.00765	10.00	20.00	9.767	20.59	97.67	102.9
Mean							99.85	101.6
SD							1.971	1.101
% RSD							1.974	1.083
Accuracy 100 % (*n*=3)								
Sl. No.	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**
1	0.00525	0.00928	12.50	25.00	12.75	25.10	102.0	100.4
2	0.00534	0.00908	12.50	25.00	12.96	24.55	103.6	98.20
3	0.00532	0.00925	12.50	25.00	12.91	25.02	103.2	100.0
Mean							102.9	99.53
SD							0.832	1.171
% RSD							0.808	1.177
Accuracy 120 % (*n*=3)								
Sl. No.	**Absorbance**		**Amount present (µg/mL)**		**Amount found (µg/mL)**		**% Purity**	
	274 nm	**226.4 nm**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**	**HCTZ**	**ATEN**
1	0.00610	0.01096	15.00	30.00	14.70	29.74	98.00	99.13
2	0.00627	0.01088	15.00	30.00	15.09	29.52	100.6	98.40
3	0.00622	0.01097	15.00	30.00	14.98	29.77	99.86	99.23
Mean							99.48	98.92
SD							1.339	0.453
% RSD							1.346	0.458

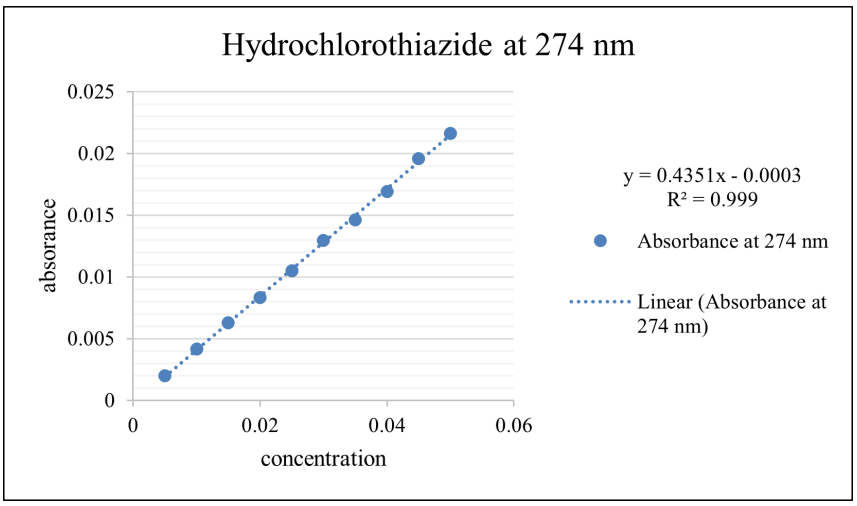

Figure 5: Calibration curve of hydrochlorthiazide.

Robustness

The typical variations studied under this parameter were UV instrument, analyst and wavelength. As seen above % RSD for atenolol and hydrochlorothiazide for change in UV instrument, analyst and wavelength found to be < 2%. This confirmed the robustness of the developed method (Table 6).

Assay

The above validated method can be employed to estimate atenolol and hydrochlorothiazide in combined dosage form. According to the methodology the solutions were made and absorbance values measured at predetermined wavelengths. The results of atenolol and hydrochlorothiazide were comparable with the corresponding labelled levels of marketed formulation and % assay was found to be 100.9% for hydrochlorothiazide and 100.4% for atenolol which was within the acceptable limit (95%-105%) (Table 7).

DISCUSSION

The above method was developed using 0.1 N NaOH as solvent. The two wavelengths were selected for estimation of drugs 274 nm for hydrochlorothiazide and 226.4 nm for atenolol. Method validation was performed according to ICH guidelines. The

Table 5: Results of LOD and LOQ.

Parameters	Hydrochlorothiazide	Atenolol
	274 nm	210 nm
LOD (µg/mL)	0.00011	0.00022
LOQ (µg/mL)	0.00035	0.00069

Table 6: Results of robustness: change in UV instrument results.

Change in UV instrument Change in UV instrument 1 (*n*=3)								
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
1	0.00493	0.00934	12.50	25.00	12.02	25.26	96.16	101.0
2	0.00498	0.00938	12.50	25.00	12.13	25.38	97.04	101.5
3	0.00497	0.00954	12.50	25.00	12.11	25.82	96.88	103.2
Mean							96.69	101.9
SD							0.468	1.153
% RSD							0.484	1.131
UV instrument 2 (*n*=3)								
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
1	0.00502	0.00936	12.50	25.00	12.22	25.32	97.76	101.2
2	0.00500	0.00933	12.50	25.00	12.18	25.24	97.44	100.9
3	0.00499	0.00942	12.50	25.00	12.15	25.49	97.20	101.9
Mean							97.46	101.3
SD							0.280	0.513
% RSD							0.288	0.506
Change in Analyst Analyst 1 (*n*=3)								
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
1	0.00493	0.00930	12.50	25.00	12.02	25.12	96.16	100.6
2	0.00495	0.00901	12.50	25.00	12.06	24.35	96.48	97.40

Change in UV instrument Change in UV instrument 1 (*n*=3)									
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
3	0.00494	0.00925	12.50	25.00	12.04	25.02	96.32	100.0
Mean							96.32	99.33
SD							0.160	1.700
% RSD							0.166	1.712
Analyst 2 (*n*=3)								
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
1	0.00502	0.00935	12.50	25.00	12.22	25.29	97.76	101.1
2	0.00499	0.00947	12.50	25.00	12.15	25.62	97.20	102.4
3	0.00501	0.00939	12.50	25.00	12.20	25.40	97.60	101.6
Mean							97.52	101.7
SD							0.288	0.655
% RSD							0.295	0.644
Change in wavelength Wavelength +2 nm (*n*=3)								
Sl. No.	Absorbance at 276 nm				Absorbance at 228.4 nm			
1	0.00507				0.00487			
2	0.00506				0.00492			
3	0.00510				0.00485			
Mean	0.00507				0.00488			
SD	0.00002				0.00003			
%RSD	0.410				0.738			
Wavelength -2 nm (*n*=3)								
1	0.00480				0.01165			
2	0.00478				0.01174			
3	0.00479				0.01161			
Mean	0.00479				0.01166			
SD	0.00001				0.00006			
%RSD	0.208				0.507			

Table 7: Results of the assay.

Assay (*n*=3)								
Sl. No.	Absorbance		Amount present (µg/mL)		Amount found (µg/mL)		% Purity	
	274 nm	226.4 nm	HCTZ	ATEN	HCTZ	ATEN	HCTZ	ATEN
1	0.00522	0.00921	12.50	25.00	12.68	24.91	101.4	99.64
2	0.00517	0.00929	12.50	25.00	12.57	25.13	100.5	100.5
3	0.00519	0.00936	12.50	25.00	12.61	25.32	100.8	101.2
Mean							100.9	100.4

linearity range of hydrochlorothiazide and atenolol were between 5-50 µg/mL and 5-60 µg/mL with correlation coefficient greater than 0.990. The % RSD calculated for precision, robustness and accuracy was found to be < 2%. The percent recoveries results confirm the accuracy of the method. LOD and LOQ study method was performed and observed to be sensitive. The proposed method has been proven to be sensitive, robust, precise and accurate.

The % assay of hydrochlorothiazide and atenolol in tablet formulation gives satisfactory results of 100.8% and 100.6% respectively, which were within the acceptance criteria.

CONCLUSION

The technique is easy, accurate, and precise, and therefore suitable for regular analysis of atenolol and hydrochlorothiazide in commercially available marketed tablets.

ACKNOWLEDGEMENT

All the authors are thankful to PES's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Ponda, Goa for providing the facilities to carry out the research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATEN: Atenolol; **HCTZ:** Hydrochlorthiazide; **UV:** Ultraviolet; **SD:** Standard deviation; **RSD:** Relative standard deviation; **LOD:** Limit of detection; **LOQ:** Limit of quantification.

SUMMARY

A novel method was developed and validated for the estimation of atenolol and hydrochlorthiazide in a marketed formulation using UV derivative spectroscopy. The method used 0.1N NaOH as a solvent. The second derivative spectra of each drug was taken and based on the overlain second derivative spectra of both the drugs 226 nm and 274 nm were the wavelengths selected. The method gave a good linear response with a correlation coefficient (R2) of 0.999 within a concentration range of 5-60 µg/mL for atenolol and 5-50 µg/mL for hydrochlorothiazide. The method was statistically validated and the marketed formulation ATEN H 25 was analysed. The percentage purity obtained was of 100.4% and 100.9% for atenolol and hydrochlorothiazide respectively.

REFERENCES

1. Korupolu P, Tata S, Baba KH. Development and validation of a stability indicating method for the simultaneous determination of atenolol and hydrochlorothiazide by HPLC. ijpda. 2013;1(1):49-60.
2. Hemdan A, Al-Tannak NF, Mohamed EH. Development of a multivariate model with desirability-based optimization for determination of atenolol and hydrochlorothiazide by eco-friendly HPLC method with fluorescence detection. J Sep Sci. 2022;45(4):824-31. doi: 10.1002/jssc.202100711, PMID 34910855.
3. Elshanawane AA, Mostafa SM, Elgawish MS. Development and validation of a reversed-phase high-performance liquid chromatographic method for the simultaneous determination of amiloride hydrochloride, atenolol, hydrochlorothiazide, and chlorthalidone in their combined mixtures. J AOAC Int. 2009;92(2):404-9. doi: 10.1093/jaoac/92.2.404, PMID 19485198.
4. Neelima MS, Gandhi BM, Raju VB, Sumanth KS, Srinivas K, Mounika P, *et al.* Development and validation of stability indicating reverse phase high-performance liquid chromatography method for simultaneous estimation of atenolol, hydrochlorothiazide and losartan in bulk and pharmaceutical dosage form. Asian J Pharm Clin Res. 2016;9(2):118-24.
5. Ravisankar P, Anusha RK, Pavan G. Development and validated RP-HPLC for simultaneous determination of atenolol and hydrochlorothiazide in tablet dosage form. IJPCBS. 2015;5(3):665-75.
6. Zaveri M, Khandhar A. Development and validation of a RP-HPLC for the simultaneous estimation of atenolol and hydrochlorothiazide in pharmaceutical dosage form. AJPRHC. 2010;2:248-52.
7. Chaudari V, Hussian S, Ubale M. A newer validated and stability indicating HPLC method for the estimation of atenolol and hydrochlorothiazide in bulk drug and dosage form. Int J Chem Stud. 2013;1(4):93-101.
8. Thomas AB, Chavan UB, Nanda RK, Kothapalli LP, Jagdale SN, Dighe SB, *et al.* Simultaneous RP-HPLC analysis of atenolol, hydrochlorthiazide and losartan potassium in a tablet formulation. Acta Chromatographica. 2010;22(2):219-26. doi: 10.1556/AChrom.22.2010.2.5.
9. Indrani B, Ghosh SK. Development and validation of stability indicating RP-HPLC method for the determination of atenolol and hydrochlorothiazide in combined solid dosage form. Int J Pharm Pharm Sci. 2015;7(2):489-92.
10. Eswarudu MM, Sakheena P, Lahari K, Babu PS, Eswaraiah MC. Validated RP-HPLC method for simultaneous estimation of atenolol, hydrochlorothiazide and losartan potassium in bulk and pharmaceutical dosage form. Asian J Pharm Res Dev. 2021;9(1):60-6. doi: 10.22270/ajprd.v9i1.913.
11. Chheta N, Gandhi SP, Rajput SJ. Development and validation of a stability indicating high performance liquid chromatographic method for atenolol and hydrochlorothiazide in bulk drug and tablet formulation. Int J Chem Technol. 2009;1(3):654-62.
12. Sharma R, Khanna S, Mishra GP. RP-HPLC method for simultaneous estimation of atenolol, hydrochlorothiazide and losartan in tablet dosage form. Chem Sci Trans. 2013;2(1):1-6.
13. Haque MA. Simultaneous estimation of atenolol and chlorthalidone as bulk and in tablet dosage form using UV-spectrophotometry. IOSJPBS. 2012;1(4):20-3. doi: 10.9790/3008-0142023.
14. Bari S, Sathe S, Jain P, Surana S. Spectrophotometric method for simultaneous estimation of atenolol in combination with losartan potassium and hydrochlorothiazide in bulk and tablet formulation. J Pharm Bioallied Sci. 2010;2(4):372-5. doi: 10.4103/0975-7406.72144, PMID 21180476. PMCID PMC2996073.
15. Thomas AB, Chavan UB, Nanda RK, Kothapaili LP, Deshpande AD. Simultaneous estimation of hydrochlorothiazide, atenolol and losartan potassium in tablet dosage form. 2009;8(3):139-41.
16. Archakam SC, Palur K, Arava PK. Multivariate analytical methods for simultaneous estimation of atenolol and hydrochlorothiazide in bulk and tablet dosage form. IJCAAP. 2021;6(4):205-8. doi: 10.18231/j.ijcaap.2021.037.
17. Antakli S, Nejem L, Joumaa MA. Simultaneous determination of atenolol and hydrocholrothiazide in tablets formulation by derivative spectrometry. Adv Chemother. 2020;17:73-9. doi: 10.24297/jac.v17i.8808.
18. Behera AK. Simultaneous spectrophotometric estimation of atenolol and hydrochlorothiazide in tablet dosage forms. Int J Chem Technol. 2010;2(4):1901-6.
19. Tarkase KN, Hapse SA, Shirsath AS, Kadaskar PT. Development and validation of spectrophotometric method for simultaneous estimation of atenolol and hydrochlorothiazide in pure and tablet dosage form. Int J Chem Technol. 2012;4(3):1146-50.
20. Antakali S, Nejem L, Joumaa MA. Simultaneous determination of atenolol and hydrochlorthiazide in tablet formulation by derivative spectroscopy. J Adv Chem. 2020;17(1):73-9. doi, doi: 10.24297/jac.v17i.8808.
21. Research C for de and. U.S. Food and Drug Administration. 2021;Q2(R1). Validation of analytical procedures: text and methodology guidance for industry [internet]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2r1-validation-analytical-procedures-text-and-methodology-guidance-industry.
22. Beckett AH, Stenlake JB. The practical pharmaceutical chemistry. Part II. New Delhi: CBS Publisher and distributors: 1997.

Cite this article: Zambrekar S, Sawaikar L, Keny S, Pal P, Gaude S. Development and Validation of a Novel Second Derivative UV Method for the Estimation of Antihypertensive Drugs in Tablet Dosage Form. Indian J of Pharmaceutical Education and Research. 2024;58(3):908-17.