

RESEARCH ARTICLE

SPECTROSCOPICAL METHOD FOR ESTIMATION OF ATENOLOL AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

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Received: 12 Aug, 2021/Revision: 13 Sep, 2021 /Accepted: 11 Nov, 2021

ABSTRACT: Introduction: A sensitive and validated spectroscopic estimation of Atenolol and Hydrochlorothiazide in pharmaceutical dosage form, without prior separation, by three different techniques (Simultaneous Equation, Dual Wavelength Method, and Derivative Spectroscopic Method) has been developed. Method: The works were carried out on Shimadzu electron UV1800 double beam UV-Visible spectrophotometer. The absorption spectra of reference and test solutions were carried out in 1 cm matched quartz cell over the range of 200 - 400 nm. The linearity ranges for Atenolol and Hydrochlorothiazide were 2-10 µg/ml and 1-5 µg/ml. Conclusion: The results of the analysis have been validated statistically and by recovery studies. The proposed procedures are rapid, simple, require no preliminary separation steps, and can be used for routine analysis of both drugs in quality control laboratories.

KEYWORD: Atenolol, Hydrochlorothiazide, UV spectroscopy, and Validation.

INTRODUCTION:

Chemically, Atenolol (Figure 1) is (RS)-4-2-(2-hydroxy-3-isopropyl amino propoxy) phenylacetamide. It is a selective beta-1 adrenergic receptor antagonist. 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 juxtaglomerular apparatus. They lead to decreased heart rate decreasing the workload of the heart. They do not produce coronary vasodilatation but lead to a shift and redistribution of the coronary circulation to the ischemic areas.

It decreases the release of renin from the kidney, thus lowering blood pressure. ^[1] Chemically, Hydrochlorothiazide (Figure 1) is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfanamide 1,1-dioxide. It is a diuretic used for the treatment of edema associated with heart (congestive heart failure), liver (hepatic cirrhosis), renal (nephritic syndrome, chronic renal failure, and glomerulonephritis) diseases. Hydrochlorothiazide acts on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. ^[2]

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Atenolol and Hydrochlorothiazide is a combination of two blood-pressure lowering medicines. These combinational tablets improve the patient's condition by reducing heart rate and making the heart more efficient at pumping blood and lowering blood pressure by removing extra water and certain electrolytes from the body.^[3]

Literature survey reveals that one Spectrophotometric method^[4-14] has been reported for the estimation of Atenolol and Hydrochlorothiazide in pharmaceutical formulations. This paper aimed to explore the possibility of using techniques of simultaneous equation method, dual-wavelength method, and derivative spectroscopic method for quantifying Atenolol and Hydrochlorothiazide simultaneously in their mixture forms. The proposed methods are simple, convenient, precise, accurate, and economical than the reported method and validated as per ICH guidelines.^[15,16]

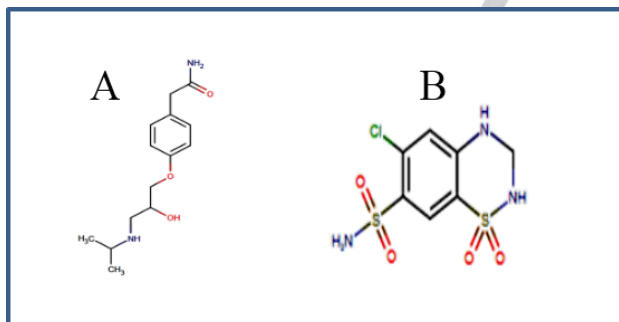


Figure 1. Chemical structures of analytes Chemical structure of A. Atenolol and B. Hydrochlorothiazide

EXPERIMENTAL METHOD:

Instrumentation: To develop a UV spectroscopic method for simultaneous estimation of Atenolol and Hydrochlorothiazide, Shimadzu electron UV1800 double beam UV-Visible spectrophotometer was used. The instrument is equipped with a silicon photodiode detector.

Chemicals and solvents: The working standards of Atenolol and Hydrochlorothiazide were provided as gift samples from Spectrum Pharma Research

Solutions, Hyderabad, India. Combined Atenolol and Hydrochlorothiazide tablets were purchased from the Local Pharmacy Market.

Preparation of standard stock and working standard solutions of Atenolol: 100 mg of Atenolol was weighed into 100 ml volumetric flask and dissolved in NaOH and then dilute up to the mark with NaOH to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100 µg/ml and was kept as the stock solution. The prepared stock solution was diluted with NaOH solution to get working standard solutions of concentrations 2-10 µg/ml.

Preparation of standard stock and working standard solutions of Hydrochlorothiazide: 100 mg of Hydrochlorothiazide was weighed and transferred into 100 ml volumetric flask and dissolved in NaOH and then make up to the mark with NaOH to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100 µg/ml and was kept as the stock solution. The prepared stock solution was diluted with NaOH solution to get working standard solutions of concentrations 1-5 µg/ml.

Simultaneous equation method (Method-I): For a multi-component system consisting of two components X and Y, each of which absorbs at the $\lambda_{\max}$ of the other, λ_1 is being the wavelength of maximum absorbance of X ($\lambda_{\max}$) and λ_2 being the wavelength of maximum absorbance of Y ($\lambda_{\max}$). In such cases, it can be possible to determine both the components by the simultaneous equation method. Marketed formulations of Atenolol and Hydrochlorothiazide were procured. The absorbance of the final sample solution was measured against NaOH as blank at 224 nm and 219 nm for quantization of Atenolol and Hydrochlorothiazide, respectively. The amount of Atenolol and Hydrochlorothiazide present in the sample solutions was determined by solving the following simultaneous equations.

$$C_x = (A_1 a_{Y2} - A_2 a_{Y1}) / (a_{X1} a_{Y2} - a_{X2} a_{Y1})$$

$$C_y = (a_{X1} A_2 - a_{X2} A_1) / (a_{X1} a_{Y2} - a_{X2} a_{Y1})$$

Where, A_1 , A_2 are abs. of components, a_{X1} , a_{X2} are absorptivity of the first drug at λ_1 and λ_2 respectively; a_{Y1} , a_{Y2} are absorptivity of the second drug at λ_1 and λ_2 respectively.

Dual-Wavelength Method (Method-II): The utility of dual-wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the component of interest and an unwanted interfering component by the principle of difference in the absorbance between two points on the mixture spectrum. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The prerequisite for the dual-wavelength method is the selection of two such wavelengths where the interfering component shows the same absorbance whereas the component of interest shows a significant difference in absorbance with concentration. Based on this criterion, two wavelengths 233.8 nm and 266.8 nm were selected as λ_1 and λ_2 for the estimation of Atenolol as Hydrochlorothiazide shows the same absorbance at these wavelengths. Similarly, wavelengths 271.30 nm and 242.20 nm were selected as λ_3 and λ_4 for the estimation of Hydrochlorothiazide as Atenolol shows the same absorbance at these wavelengths. For calibration curve, the standard stock solutions of these drugs were diluted in the concentration range of 2-10 $\mu\text{g/ml}$ (2, 4, 6, 8 and 10 $\mu\text{g/ml}$), 1-5 /ml (1, 2, 3, 4 and 5 $\mu\text{g/ml}$) for Atenolol and Hydrochlorothiazide respectively. Absorbances were recorded at selected wavelengths.

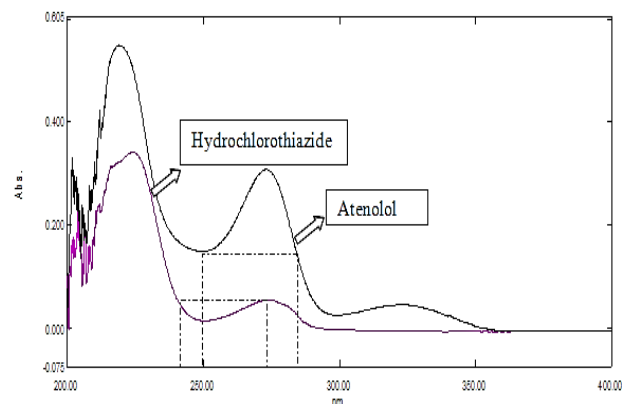


Figure 2. Selection of wavelengths for dual-wavelength method

Derivative Spectroscopic Method (Method-III): Derivative spectrophotometry involves the conversion of a normal spectrum (fundamental, zeroth-order, or D spectrum) to its first, second or higher derivative spectrum by differentiating absorbance of a sample concerning for to wavelength λ for higher accuracy.

$$[A] = f(\lambda): \text{zero order}$$

$$[dA/d\lambda] = f(\lambda): \text{first order}$$

$$[d^2A/d\lambda^2] = f(\lambda): \text{second order}$$

The strong positive & negative bands with maximum and minimum at the same wavelength of an absorption band as an inflection point in absorbance band governs the odd (first & third) derivative spectrum whereas the strong positive & negative band with minimum or maximum at the same wavelength as λ_{max} of absorbance band governs the even (second & fourth) derivative spectrum.

Number of bands = Derivative order + 1

The amplitude (D) is directly proportional to the concentration of analyte provided Beer's law is obeyed by the D^o spectrum.

In first-order derivative spectroscopy, zero-crossing point for both drugs is found and the wavelengths are selected in a manner such that at the zero-crossing of one drug, the other drug should show substantial absorbance.

The obtained zero-order absorption spectra of Atenolol and Hydrochlorothiazide were converted to first-order derivative spectra (Figure 3) by using transformation mode. After observing the first-order derivative spectra, zero-crossing points of drugs were selected for the analysis of other drugs. The first wavelength selected was 221 nm for Atenolol (zero crossing) and the second wavelength selected was 249 nm for Hydrochlorothiazide (zero-crossing).

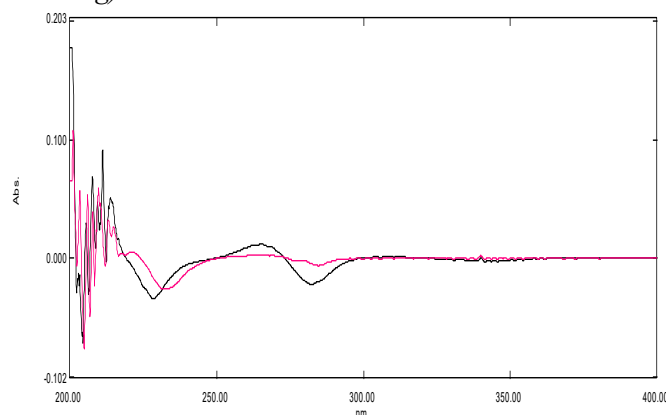


Figure 3. First derivative overlay spectrum of Atenolol and Hydrochlorothiazide

A Calibration curve for Atenolol at 249 nm (zero Crossing for Hydrochlorothiazide) and Hydrochlorothiazide at 221 nm (zero Crossing for Atenolol) was developed to calculate the concentration of a sample

VALIDATION:

Linearity: A stock solution was prepared by dissolving 100 mg of the drugs in 100 ml of solvent. Then from the stock solution, dilutions of various concentrations from 2 to 10 $\mu\text{g/ml}$ were prepared for Atenolol and 1 to 5 $\mu\text{g/ml}$ were prepared for Hydrochlorothiazide. Each dilution was analyzed in series to construct the calibration curves. The absorbance of each dilution was noted and plotted against the concentration of each dilution.

Accuracy: Accuracy was determined by calculating the % recovery of Atenolol and

Hydrochlorothiazide by the standard addition method. The pre-analyzed sample solutions (4 $\mu\text{g/ml}$ of Atenolol and 2 $\mu\text{g/ml}$ of Hydrochlorothiazide) were spiked with standard drug solutions at three different levels- 50, 100, and 150 %. The resulting mixtures were reanalyzed using the proposed method. The experiment was conducted in triplicates accuracy was reported as % recovery.

Precision: The precision of the proposed method was calculated by conducting intermediate precision.

1. Intra-Day Precision: The intra-day precision was determined by estimating the corresponding absorbance of the drug solution (in triplicates) three times on the same day.

2. Inter-Day Precision: The inter-day precision was established by analyzing the drug solution (in triplicates) on three different days.

3. Analyst-Analyst: Analyst to analyst precision was established by analyzing the drug solution by the different analysts.

The standard deviation, % relative standard deviation, and estimated concentrations based on the standard curve were reported for each set of data.

Robustness: The robustness of the developed method was determined by analyzing the drug solution in triplicates by varying the wavelength (± 2). Robustness is reported in %RSD.

LOD and LOQ: the detection limit and the quantization limit of the drug are calculated by using the calibration curve standards. The detection limit and the quantization limit were calculated from the equation $3.3\sigma/S$ and $10\sigma/S$ respectively, where σ is the standard deviation of y-intercept and S is the slope of the calibration curve.

Specificity: The specificity of the developed method was seen by analyzing solutions containing excipients and pure drugs and demonstrating that

the result is unaffected by the presence of the excipients present in it.

Assay: 20 tablets were taken and weighed accurately. Then the tablets are crushed to powder. The weight of tablet contents equivalent to 100 mg of Atenolol and Hydrochlorothiazide was calculated and were taken into a 100 ml volumetric flask. 50 ml of NaOH was added to it and sonicated for 10 minutes and diluted up to the mark to prepare 1 mg/ml solutions. From these solutions, 10 µg/ml dilutions of the drugs were prepared and their absorbances were taken. From the absorbance of the drug solutions, the amounts of each drug in the sample solutions were computed. The results were compared with the label claim of Atenolol and Hydrochlorothiazide in tablet dosage forms. From the results, the average %Assay was calculated.

RESULTS:

Linearity: The calibration curves (Figure 4) drawn by using the proposed method were found to be linear in the range (2-10 µg/ml Atenolol and 1-5 µg/ml Hydrochlorothiazide). Table 1 shows the calibration data with regression coefficient and %RSD was found to be less than 2.

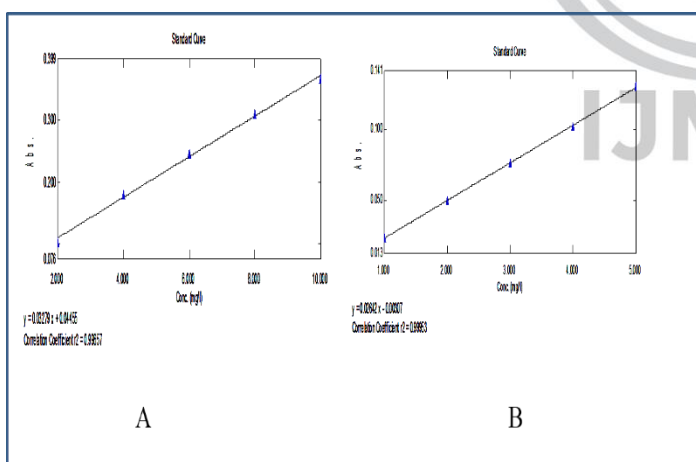


Figure 4. Calibration curves of analytes Calibration curves of A. Atenolol and B. Hydrochlorothiazide

Accuracy (recovery): Accuracy of the methods was determined by using the standard addition method and %recovery was found in the range 98-

101% and %RSD was within the range for both drugs. Table 1 shows the results of accuracy studies.

Limit of Detection and Limit of Quantification: LOD and LOQ were calculated from the calibration curves of the drugs and the results are shown in Table 1.

% Assay: %Assay of the formulation was calculated by three different techniques and %recovery was found in the range 98-101% and %RSD was within the range for both drugs. Table 1 shown the results of %assay studies.

Precision: Intra-day precision was assessed by analyzing the drug at 3 different times on the same day. The method passed the test as the %RSD was found to be less than 2. Inter-day precision was assessed by analyzing the drug for three different days. The method passed the test as the %RSD was found to be less than 2. Analyst to analyst Precision was conducted by two different analysts at the same experimental conditions. Results are shown in Table 1.

Robustness: Robustness studies were performed by varying the detection wavelength (± 2). The method was found to be robust. Results are shown in Table 2.

Result of specificity: Specificity studies were performed by spiking the formulation and standard drug using two-tailed unpaired t-tests.

Table 1. Summarized results of linearity, accuracy, LOD, LOQ, %assay and Precision

PARAMETERS	Simultaneous	Dual Wavelength				First Order Derivative	
	Equation						
	Atenolol	Hydro-chlorothiazide	Atenolol	Hydro-chlorothiazide	Atenolol	Hydro-chlorothiazide	
Detection Wavelength	224 nm	219 nm	233.8 nm 266.8 nm	271.30 nm 242.20 nm	249 nm	221 nm	
Linearity and range	2-10 µg/ml	1-5 µg/ml	2-10 µg/ml	1-5 µg/ml	2-10 µg/ml	1-5 µg/ml	
Correlation coefficient	0.076	0.076	0.012	0.028	0.076	0.076	
Slope	0.003	0.003	0.004	0.001	0.003	0.003	
Intercept	0.996	0.999	0.998	0.999	0.999	0.999	
Accuracy	100.21	100.77	99.19	100.04	98.67	99.67	
Limit of detection	0.13	0.11	0.13	0.11	0.13	0.11	
Limit of quantitation	0.39	0.35	0.39	0.35	0.39	0.35	
% Assay	100.9±0.49	97.3±0.20	98.0±0.45	100.4±0.48	99.1±0.42	94.9±0.71	
Intra-day (n=3)	97.63±0.80	97.10±1.02	96.00±0.88	97.13±0.83	97.13±0.83	96.43±5.36	
Inter-day (n=3)	87.66±10.41	92.63±7.42	95.36±5.15	101.40±1.78	81.53±17.38	87.36±14.31	
Analyst-Analyst	101.40±0.12	99.33±0.66	100.16±0.76	100.86±2.70	101.0 ±0.81	99.23 ±0.88	

Table 2. Results of robustness studies

λ_{max} (±2 nm)	Concentration (µg/ml)	Observed absorbance			Mean	SD	%RSD
Atenolol							
224+2	10	0.91	0.9	0.88	0.896	0.0152	1.7
224-2		0.9	0.91	0.9	0.903	0.0070	0.78
Hydrochlorothiazide							
219+2	5	0.324	0.333	0.331	0.329	0.0047	0.47
219-2		0.330	0.329	0.337	0.332	0.0043	0.43

DISCUSSION:

The proposed methods were validated and found to be simple, sensitive, accurate and precise as per ICH guidelines^[15,16]. The methods are don not required any separation step is required. The % RSD for the validation parameters was found to be less than 2%. Hence proposed method may be used for routine analysis of these drugs in pharmaceutical dosage forms. Accuracy of proposed method was confirmed by performing accuracy studies that showed the results within the range. Precision of proposed UV method was confirmed by performing intra-day and inter-day precision. Results were well within acceptance criteria that indicate excellent scope of the method for the determination of simultaneous estimation of Atenolol and Hydrochlorothiazide in pharmaceutical dosage forms and bulk. Even though, so many methods are there for the estimation of the drugs. There must be scope of future development of analysis in the means of economy and time saving.

CONCLUSION:

The proposed methods may find practical applications as a quality-control tool in the simultaneous analysis of the two drugs in combined dosage forms in quality-control laboratories.

ACKNOWLEDGEMENTS:

The authors are thankful to Spectrum Pharma Research Solutions., Hyderabad for providing gift samples, and the authors are also thankful to V. V. Institute of Pharmaceutical Science, Gudlavalluru for providing the necessary facilities to carry out the research work.

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Cite of article: Sai Datri A, Lakshmana Rao A, Chandini U, Anil Kumar V, Prudhvi Raj V, Veera Naga Anjali V. Spectroscopical method for estimation of atenolol and hydrochlorothiazide in pharmaceutical dosage form. *Int J Med Lab Res*. 2021;6(3):44–51. <http://doi.org/10.35503/IJMLR.2021.6306>

CONFLICT OF INTEREST: Authors declared no conflict of interest

SOURCE OF FINANCIAL SUPPORT: Nil

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