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SIMULTANEOUS ESTIMATION OF ATENOLOL AND CHLORTHALIDONE IN COMBINE TABLET DOSAGE FORM BY DUAL WAVELENGTH METHOD USING UV-VIS SPECTROPHOTOMETRY

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ABSTRACT

A simple, precise, reproducible, accurate, economical & rapid UV-VIS Spectrophotometric method have been developed and validated for the simultaneous estimation of ATN and CTN in tablet dosage form. This paper describes the dual wavelength method as a quantification parameter. In this method the two wavelengths are selected for each drug in way that the differences in absorbances are zero for another drug. ATN has equal absorbance at 232.0 & 218.0 nm which are used for the quantification of CTN while CTN has equal absorbance at 260.0 nm & 242. 0 nm which are used for the quantification of ATN. The developed method obeys the beers law in the concentration range of 40-80µg/mL for ATN and 10-50 µg/mL for CTN. The recovery studies shows %RSD for ATN 0.21 and for CTN 1.34 by this method. The results of analysis have been validated statistically for accuracy, precision, repeatability, specificity and ruggedness. The method was successfully applied to the determination of these drugs in pharmaceutical dosage form.

KEYWORDS: ATN, CTN, UV-VIS, Spectrophotometry, Dual Wavelength, Assay Method.

INTRODUCTION

UV- VIS spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances, which potentially interfere in the assay. If the formula of the samples is known, the identity and concentration of the interfering substance are known and the extent of interference in the assay may be determined. The U.V. Spectrophotometric techniques for multicomponent samples is the property that at all wavelengths the absorbance of a solution is the sum of absorbance of the individual components or The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell. In dual wavelength method is based on the photometric modes of functioning. This method involves the estimation of two components, each time considering the second one to be the interfering one. A prior criterion for this method is the existence of two such wavelengths where interfering component shows same absorption, spectra should be overlapping partially.[16] From the literature review[7,13] it has been found that only three analytical methods for the above combination have been reported and no paper of dual wavelength method for above combination is reported. Therefore the attempt is made to develop simple, accurate, precise rapid and economical UV-VIS spectrophotometric method based on dual wavelength parameters for determination of Atenolol (ATN) and Chlorthalidone (CTN) in combine dosage form. Atenolol [Figure 1] Chemically is RS)-2-{4-[2-Hydroxy-3-(propan-2 ylamino)propoxy]phenyl}acetamide. It is white to almost white powder used as anti- hypertensive having solubility in methanol and water, sparingly soluble in ethanol. While chlorthalidone [Figure 2] chemically is (RS)-2-chloro-5-(3-Hydroxy-1-oxoisoindolin-3-yl) benzenesulphonamide.[5,6,14,15] It is white to yellowish white crystalline and practically odorless. Used as anti-hypertensive having solubility in methanol and insoluble in water, slightly soluble in ethanol.

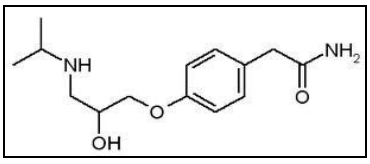

Fig. 1 Chemical Structure of Atenolol

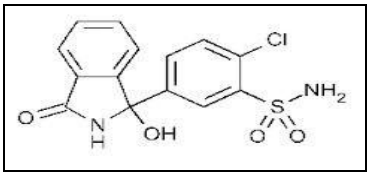

Fig.2 Chemical Structure of Chlorthalidone

MATERIALS AND METHODS

Reagents & Chemicals

Standard samples of ATN & CTN were received as gift samples from the leben laboratories akola (Maharashtra) and IPCA Laboratories mumbai (Maharashtra). The marketed formulation Tenoric (IPCA Laboratories) was purchased from the local market containing ATN 50 mg and CTN 12.5 mg and all the chemicals used were are of analytical grade.

Instruments

A Shimadzu UV- VIS double beam spectrophotometer with model UV 1700 and software UV probe 2.33was used for spectral and absorbance measurement. Analytical balance of citizen model CY 104 (microanalytical balance) was used for weighing purpose also the ultrasonicator servewell instruments model RC-SYSTEM MU-1700 used for sonication purpose.

Selection of Common Solvent

Methanol was selected as common solvent after assessing the solubilities of both the drugs in different solvents.

Preparation of Standard Stock Solutions

Standard Stock Solution (A)

Accurately weighed quantity of ATN (10.0 mg) was transferred to 10.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (1000 µg/mL of ATN). The resultant solution was then sonicated for 10.0 min in ultrasonicator.

Standard Stock Solution (B) Accurately weighed quantity of CTN (10.0 mg) was transferred to 10.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (1000 µg/mL of CTN). The resultant solution was then sonicated for 10.0 min in ultrasonicator.

Study of Overlain Spectra and Determination of λ_{max} of ATN & CTN

Aliquot portions of 0.1 mL of standard stock solutions of ATN (A) and CTN (B) were transferred to the two separate 10.0 mL volumetric flask and then the volume was made up to the mark with methanol to get final concentration of (10.0 µg/mL of ATN & CTN) respectively. Both the solutions were scanned separately in the range of 400-200 nm against methanol as blank. The resulting overlain spectrum is shown in **Figure 3.**

Selection of Wavelengths for Dual Wavelength Method

For elimination of interferences, dual analytical wavelengths were selected in a way to make the absorbance difference zero for one drug in order to analyse the other drug. The difference in absorbance at 232.0 nm (G1) and 218.0 nm (G2) was zero for atenolol, so G1 - G2 were used to analyze chlorthalidone whereas the difference in absorbance at 260.0 nm (G3) and 245.0 nm (G4) was zero for chlorthalidone and hence, G3 - G4 were used to analyze atenolol.

Linearity Study

ATN

aliquot portions of 0.4 to 0.8 mL of standard stock solution of ATN (A) were transferred to five 10.0 mL volumetric flasks and then the volume was made up to the mark with methanol to get final concentrations in the range of (40 to 80 µg/mL of ATN) respectively. The absorbances of these solutions were measured at 260.0 & 245.0nm (G3-G4) then the calibration curve (absorbance difference *vs* concentration) was plotted shown in **Figure 4.**

CTN

aliquot portion of 0.1, 0.12, 0.15, 0.17,0.2 mL of standard stock solution of CTN (B) were transferred to five 10.0 mL volumetric flasks and then the volume was made up to the mark with methanol to get final concentrations of (10.0, 12.5, 15.0, 17.5, 20.0 µg/mL of CTN) respectively. The absorbances of these solutions were measured at 232.0 & 218.0 nm (G1-G2) then the calibration curve (absorbance difference *vs* concentration) was plotted shown in **Figure 5.**

Analysis of Physical Laboratory Mixtures

Standard laboratory mixtures were prepared by accurately weighed 50.0 mg of ATN and 12.5 mg of CTN (as per labeled requirement of marketed formulation) was transferred to 50.0 mL volumetric flask and dissolved in sufficient quantity of methanol. Then the volume was made up to the mark with methanol. The resultant solution was then sonicated in ultrasonicator for 10.0 min. Aliquot portion of 0.4 mL was then transferred to six separate 10.0 mL volumetric flask and then volume was made up to the mark with methanol to get final concentrations of (40.0 µg/mL of ATN and 10.0 µg/mL of CTN) respectively. Then the absorbances of resultant solutions were measured at 232.0 nm, 218.0 nm, 260.0 nm, 245.0 nm. The results are shown in **Table 1.**

Analysis of Marketed Formulation

Ten tablets were weighed accurately and ground to fine powder. An accurately weighed quantity of Tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) were transferred to 50.0 mL of volumetric flask and dissolved in sufficient amount of methanol. Then the volume was made up to the mark with methanol. The resultant solution was then filtered through whatman filter paper (no. 41). The filtered solution was then sonicated in ultrasonicator for 10.0 min. aliquot portion of 0.4 mL was then transferred to the six separate 10.0 mL volumetric flask and then the volume was mad up to the mark with methanol to get final concentrations of (40.0 µg/mL of ATN and 10.0 µg/mL of CTN) respectively. Then the absorbances of resultant solutions were measured at 232.0 nm, 218.0 nm, 260.0 nm, 245.0 nm. The results are shown in **Table 2.**

METHOD VALIDATION

1. Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. To the preanalysed sample solution (40.0 µg/mL of ATN & 10.0 µg/mL of CTN) a known amount of standard solutions of pure drugs (ATN & CTN) were added in different levels i.e. 80%, 10.00 %, 120%. The results of recovery studies are shown in **Table 3.**

2. Precision

2.1 Intra- Day Precision

It was determined by analyzing the 3 different solutions having concentration **(**40.0 µg/mL of ATN & 10.0 µg/mL of CTN) at 3 different times over a period of day. Then the absorbances of resultant solutions were measured at 232.0 nm, 218.0 nm, 260.0 nm, 245.0 nm. The results are shown in **Table 4.**

2.2 Inter- Day Precision

It was determined by analyzing the 3 different solutions having concentration **(**40.0 µg/mL of ATN & 10.0 µg/mL of CTN) at 3 days over a period of week. Then the absorbances of resultant solutions were measured at 232.0 nm, 218.0 nm, 260.0 nm, 245.0 nm. The results are shown in **Table 4.**

3. Repeatability

Repeatability was determined by analyzing solution having concentration (40.0 µg/mL of ATN & 10.0 µg/mL of CTN) respectively for 5 times, by measuring the absorbances of resultant solutions were measured at 232.0 nm, 218.0 nm, 260.0 nm, 245.0 nm. The results are shown in **Table 5.**

4. Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions, the results are shown in **Table 6.**

5. Specificity

Accurately weighed quantity of tablet powder equivalent to (50 mg of ATN & 12.5 mg of CTN) was transferred to four separate 50.0 Ml of volumetric flask and was stored for 24 h under the following different conditions.

- At 50^{0}after addition of 2.0 mL of 0.1 N NaOH.
- At 50^{0}after addition of 2.0 mL of 0.1 N HCL.
- At 50^{0}after addition of 2.0 mL of 3% H_2O_2.
- At room temperature (normal).

The samples were diluted with methanol and then volume was made up to the mark and filtered through whatman filters (No. 41). Aliquot of the filtrate was diluted with methanol so as to get concentration equivalent to 40.0 µg/Ml of ATN and 10.0 µg/Ml of CTN. Then the absorbances of resultant solutions were measured at 232.0 nm, 218.0 nm, 260.0 nm, 245.0 nm. The results are shown in **Table 7.**

RESULTS AND DISCUSSION

Study of Overlain Spectra and Determination of λ_{max} of ATN & CTN

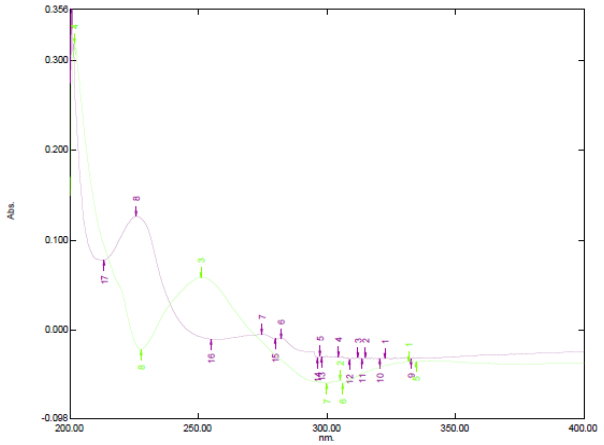

Fig. 3 Overlain Spectra of ATN and CTN

The overlain spectrum was recorded by taking methanol as a blank. The overlain spectrum showed that the ATN & CTN shows their maximum absorbance at **227.0 & 251.0 nm.** While the **240.0 nm** was the point of equal absorbance i.e. **Isobestic point.**

Linearity Study

- **ATN**

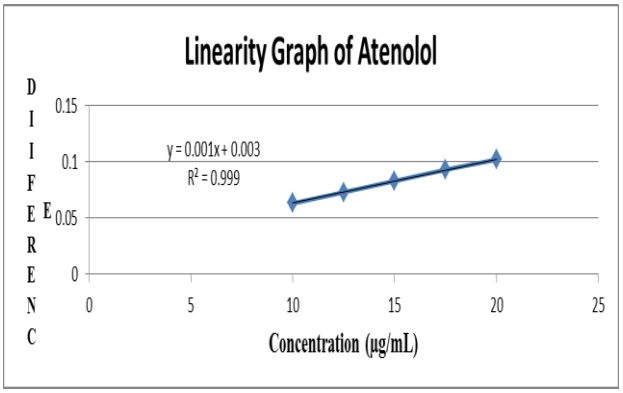

Fig. 4 Calibration Curve for ATN

- **CTN**

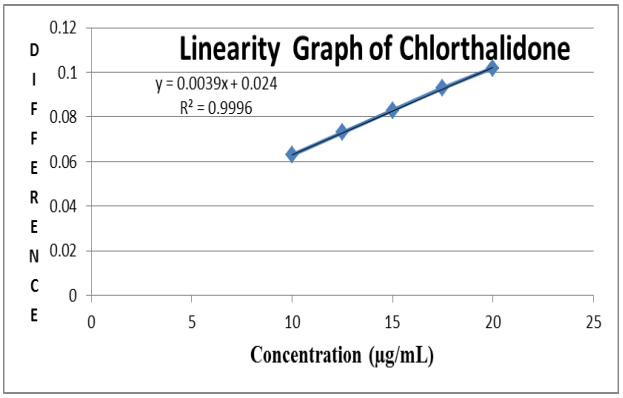

Fig.5 Calibration Curve for CTN

Linear regression data from both the calibration curve indicates good linear relationship between concentration

and absorbance difference at the concentration range of **(10-20 µg/mL for CTN) & (40-80 µg/mL for ATN)** respectively. The linear equation for the calibration curve for ATN was y = 0.001x + 0.003 and for CTN y = 0.003x + 0.024 with R^2 0.999 for both drugs which nearly equals to unity.

Analysis of Physical Laboratory Mixtures

Table 1 Results of Physical Laboratory Mixtures Analysis

Analysis of Standard Laboratory Mixtures						
Sr.No	**Amount Taken [µg/mL]**		**Amount Found [µg/mL]**		**% Amount Found**	
	ATN	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**
1	40	10	39.9	9.9	99.75	99
2	40	10	40.2	10	100.5	100
3	40	10	39.9	9.9	98.75	99
4	40	10	40.5	10.2	101.25	102
5	40	10	39.8	10	99.5	100
6	40	10	40	9.9	100	99
		Mean	39.93	9.98	100.12	99.83
		SD	0.307	0.278	0.647	1.16
		%RSD	0.770	2.79	0.646	1.17

Analysis of Marketed Formulation

Table 2 Results of Marketed Formulation Analysis

Analysis of Marketed Formulation						
Sr.No	**Amount Taken [µg/mL]**		**Amount Found [µg/mL]**		**% Amount Found**	
	ATN	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**
1	40	10	39.7	10.1	99.25	101
2	40	10	39.8	10.2	99.5	102
3	40	10	39.9	9.9	99.75	99
4	40	10	40	9.8	100	98
5	40	10	39.9	9.9	99.75	99
6	40	10	40	9.9	100	99
		Mean	39.88	9.96	99.70	99.66
		SD	0.11	0.15	0.293	1.50
		%RSD	0.293	1.51	0.302	1.51

The proposed methods when applied to marketed formulation the **% drug estimated** was **99.70 & 99.66 (ATN & CTN)** while **% RSD** was NMT more than **2** for both the methods. Indicating specific nature of developed methods free from the interference from the excipients in these tablets. It also showed that the developed method was successfully applied to analysis of marketed formulation.

METHOD VALIDATION

1. Accuracy

Table 3 Results of Recovery Studies

Recovery Studies								
Sr. No	**Pre-analysed Sample Solution [µg/mL]**		**Excess Pure Drug Added [µg/mL]**		**Recovery [µg/mL]**		**% Recovery**	
	ATN	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**	**ATN**	**CTN**
1	40	10	32	8	32	8	100	100
2	40	10	40	10	39.9	9.9	99.75	99
3	40	10	48	12	47.8	12.2	99.58	101.6
						Mean (n=3)	99.77	100.22
						SD (n=3)	0.209	1.34
						%RSD (n=3)	0.21	1.34

The mean % recovery found was **99.77 & 100.22 (ATN & CTN)** while **% RSD** was NMT more than **2** for both the methods indicating that the developed methods were found to be sufficiently **accurate.**

2. Precision

Table 4 Results of Precision Studies

Precision Studies Intra & Inter Day					
		Intra-Day [n=3]		Inter-Day [n=3]	
Drugs	Amount Taken [µg/mL]	Amount Found [µg/mL]	%RSD	Amount Found [µg/mL]	% RSD
ATN	40	40	0.62	40	0.56
	40	40		39.8	
	40	39.9		40	
CTN	10	10.2	1.83	9.9	1.75
	10	10		10.1	
	10	10.1		10.1	

The precision is always expressed as the % RSD around mean value. Here by both the methods **%RSD for Intra-Day and Inter-Day precision for the estimation (ATN & CTN) is NMT 2%.** Hence the developed methods were found to be sufficiently **precise.**

2. Repeatability

Table 5 Results of Repeatability Studies

Repeatability Studies				
Drug	Amount Taken [µg/mL]	Mean Amount Found [µg/mL] (n=5)	%Amount Found (n=5)	%RSD
ATN	40	39.9	99.75	0.188
CTN	10	9.91	99.1	0.151

3. Ruggedness

Table 6 Results of Ruggedness Studies

Ruggedness								
Sr. No	Amount Taken [µg/mL]		Analyst	Amount Found [µg/mL]		%Amount Found		
	ATN	CTN		ATN	CTN	ATN		CTN
1	40	10	Analyst I	39.9	9.9	99.75		99
2	40	10	Analyst II	40	9.85	100		98.5
			Mean		39.95	9.87	99.87	98.75
			SD		0.07	0.03	0.176	0.35
			%RSD		0.176	0.35	0.177	0.358

5. Specificity

Table 7 Results of Specificity Studies

Specificity								
Sr.No	Amount Taken [µg/mL]		Condition		Amount Found [µg/mL]		% Amount Found	
	ATN	CTN	Medium	Temperature	ATN	CTN	ATN	CTN
1	40	10	0.1 N HCL	50^0c	37.59	9.5	93.97	94
2	40	10	0.1 N NaOH	50^0c	38.5	9.7	96.25	95
3	40	10	3% H_2O_2	50^0c	36	9.6	90	96
4	40	10	Normal	R.T	39.2	9.8	98	98
				Mean	37.82	9.65	95.55	96.5
				SD	1.38	0.12	3.45	1.29
				%RSD	3.65	1.33	3.65	1.33

Specificity studies are performed by exposing the tablet powder samples to different stress conditions (Acid, Base, Oxidative, Room temperature). The % Amount found was **95.55 & 96.5 (ATN & CTN).** Such deviations from the results given Table 2 indicate method is capable of estimating intact drug contents free of interference from degradation product.

CONCLUSION

The developed UV-VIS spectrophotometric method was found to be simple, accurate, sensitive, precise, specific, economical and rapid. The method is very simple and involving no complicated sample preparations. The developed UV-VIS spectrophotometric method provides suitable quantification of ATN and CTN without any positive and negative interference from the excipients indicating its highly specific nature. The developed UV-VIS spectrophotometric methods are found to be linear over wider concentration range. Therefore the developed UV-VIS spectrophotometric method can be applied for routine quantitative and qualitative analysis of ATN & CTN in bulk and pharmaceutical dosage form like tablets. The developed UV-VIS spectrophotometric method was validated as per the ICH guidelines.

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