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Research Article

Simultaneous estimation of atenolol and chlorthalidone in combine tablet dosage form by absorption ratio method using UV-Vis spectrophotometry

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ARM

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 absorption ratio method as a quantification parameter. The absorption ratio method (ARM) involves measurement of absorbance of sample solution is measured at 240.0 nm (Isobestic Point) and 251.0 nm (λ_{max} of CTN) and based on E 1% 1cm values at these wavelengths two set of equations were framed. 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 ARM 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.

1. Introduction

The 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 absorbance ratio method (ARM) absorbances are measured at two wavelengths, one is being wavelength (λ_1) of equal absorptivity of two components i.e. an isoabsorptive point and other being λ max of one of the component[16]. From the literature review[7-13] it has been found that only three analytical methods for the above combination have been reported. Therefore the attempt is made to develop simple, accurate, precise rapid and economical UV-VIS spectrophotometric method 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.

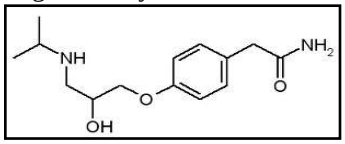

Figure 1 Chemical Structure of Atenolol

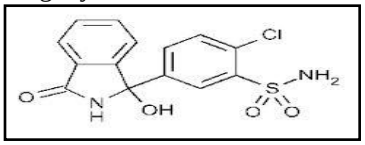

Figure 2 Chemical Structure of Chlorthalidone

2. Experimental

2.1 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.

2.2 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.

2.3 Selection of Common Solvent

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

2.4 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.

2.5 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.**

2.6 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 227.0 nm (λ_{max} of ATN) then the calibration curve (absorbance *vs* concentration) was plotted shown in **Figure 4** absorbances are shown in **Table 1**.

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 251.0 nm (λ_{max} of CTN) then the calibration curve (absorbance *vs* concentration) was plotted shown in **Figure 5** absorbances shown in **Table 2.**

2.7 Determinations of E (1% 1cm) Values at Selected Wavelengths

Aliquot portions of 0.4 mL of standard stock solutions of ATN (A) were transferred to five separate 10.0 mL volumetric flask and then the volume was made up to the mark with methanol, to get 5 dilutions having concentrations of (40.0 µg/mL of ATN) respectively. similarly aliquot portions of 0.1 mL of standard stock solutions of CTN (B) were transferred to five separate 10.0 mL volumetric flask and then volume was made up to the mark with methanol, to get 5 dilutions having concentration of (10.0 µg/mL of CTN) respectively. Then the absorbances of these solutions were measured at the wavelengths of 227.0 nm, 251.0 nm and 240.0 nm.

E (1%1cm) values of drugs were calculated using following formula:

E (1%1cm) = Absorbance / Concentration (g / 100 mL)

The results of E (1%1cm) values of both the drugs shown at selected wavelengths shown in **Table 3.**

Absorption Ratio Method (ARM) In this method absorbance of sample solution is measured at 240.0 nm (Isobestic Point) and 251.0 nm (λ_{max} of CTN) and based on E (1% 1cm) values at selected wavelengths a set of two equations are framed which are given below.

$$C_{ATN} = \frac{Qm - Qy}{Qx - Qy} \times \frac{A}{ax} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

$$C_{CTN} = \frac{Qm - Qx}{Qy - Qx} \times \frac{A}{ay} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where,

$$Qm = \frac{\text{Absorbance of sample at 251.0 nm}}{\text{Absorbance of sample at 240.0 nm}}$$

$$Qx = \frac{\text{Absorptivity of ATN at 251.0 nm}}{\text{Absorptivity of ATN at 240.0 nm}}$$

$$Qy = \frac{\text{Absorptivity of CTN at 251.0 nm}}{\text{Absorptivity of CTN at 240.0 nm}}$$

A is the absorbance of mixture at 240.0 nm and ax, ax2 and ay, ay2 are E (1%, 1 cm) of ATN and CTN at 240.0 nm and 251.0 nm and Qm= A2/A, Qy = ay2/ay and Qx = ax2/ax.

2.8 Analysis of the Standard 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 251.0 nm, 240.0 nm. Then these measured absorbances put in equation (1) & (2) for **(ARM).**

2.9 Analysis of the Marketed Formulation by Proposed Method

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 251.0 nm, 240.0. Then these measured absorbances put in equation (1) & (2) for **(ARM).**

2.10 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 6.**

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. The absorbances of the resultant solution were measured at 251.0 nm and 240.0 nm. Then these measured absorbances put in equation (1) & (2) for **(ARM)** The results are shown in **Table 7.**

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. The absorbances of the resultant solutions were measured at 251.0 nm and 240.0 nm. Then these measured absorbances put in equation (1) & (2) for **(ARM).** The results are shown in **Table 7.**

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 these solutions at 251.0 nm and 240.0 nm. Then these measured absorbances put in equation (1) & (2) for **(ARM)**. The results are shown in **Table 8.**

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 9.**

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 50^0after addition of 2.0 mL of 0.1 N NaOH.
- At 50^0after addition of 2.0 mL of 0.1 N HCL.
- At 50^0after 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. The absorbances of the resultant solutions were measured at 251.0 nm and 240.0 nm. Then these measured absorbances put in equation (1) & (2) for **(ARM).** The results are shown in **Table 10.**

3. Results and Discussion

Study of Overlain Spectra and Determination of λ $_{max}$ of ATN & CTN

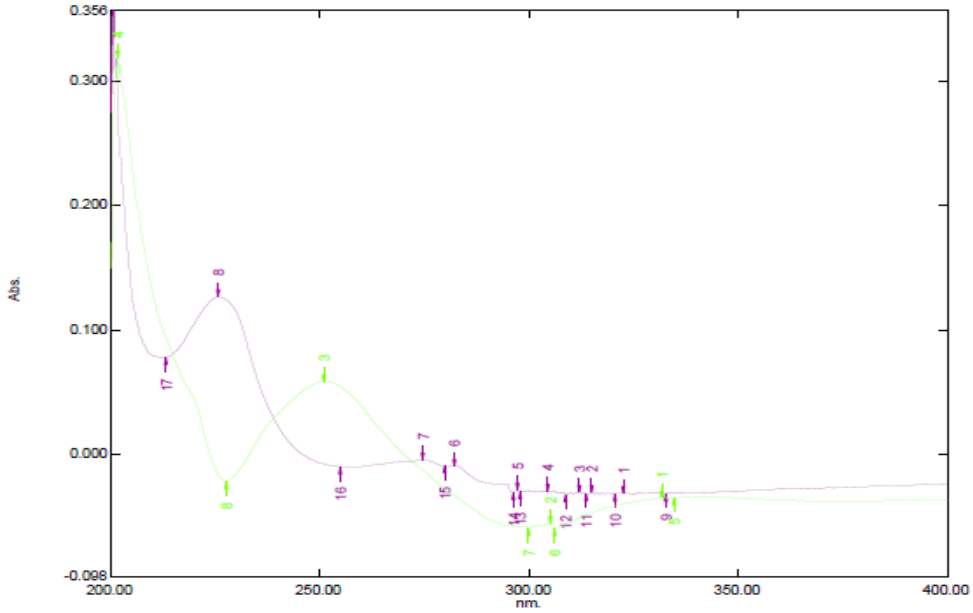

Figure 3 Overlain Spectra of ATN and CTN

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**

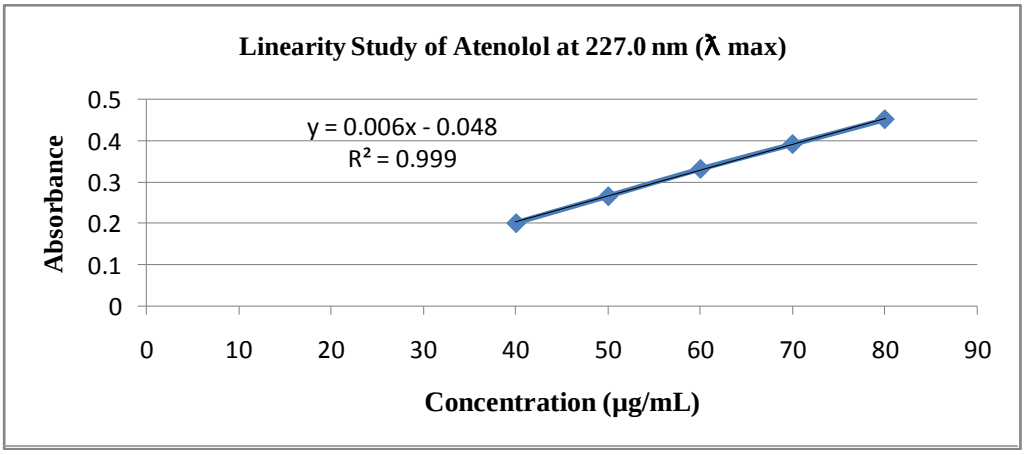

Figure 4 Calibration Curve of ATN at 227.0 nm (λ $_{max}$)

Table 1 Linearity Study of ATN at 227.0 nm (λ_{max})

Sr. No	Concentration (µg/mL)	Absorbance at 227.0 nm
1	40	0.201
2	50	0.266
3	60	0.332
4	70	0.392
5	80	0.452

Linear regression data from the calibration curve indicates good linear relationship between concentration and absorbance at the concentration range of **(40-80 µg/mL for ATN)** respectively. The linear equation for the calibration curve was **y = 0.0006x-0.048**. The r^2 value was **0.999** which nearly equals to unity.

- **CTN**

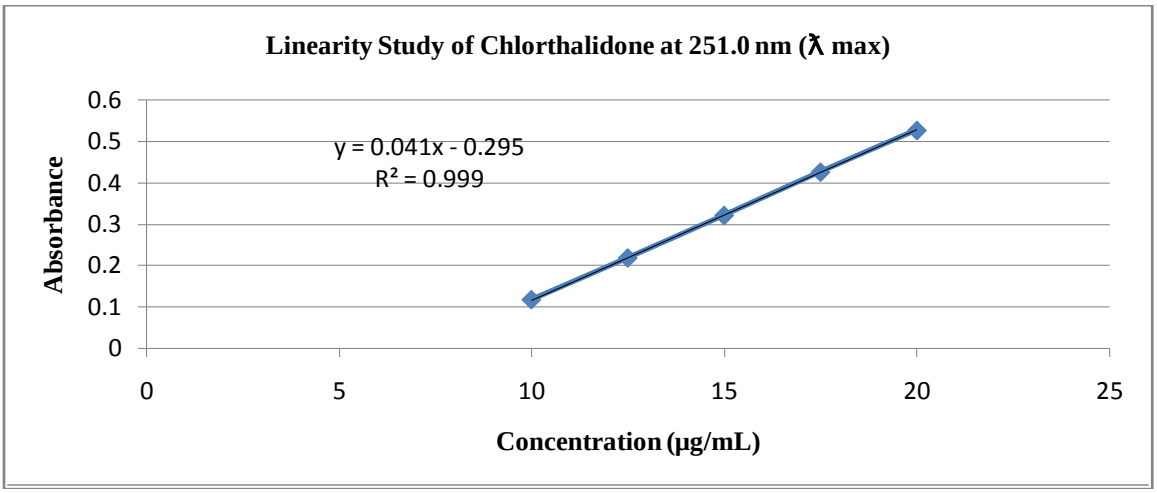

Figure 5 Calibration Curve of CTN at 251.0 nm (λ_{max})

Table 2 Linearity Study of CTN at 251.0 nm (λ_{max})

Sr. No	Concentration (µg/mL)	Absorbance at 251.0 nm
1	10	0.117
2	12.5	0.218
3	15	0.321
4	17.5	0.426
5	20	0.527

Linear regression data from the calibration curve indicates good linear relationship between concentration and absorbance at the concentration range of **(10-20 µg/mL for CTN)** respectively. The linear equation for the calibration curve was **y = 0.041x-0.295**. The r^2 value was **0.999** which nearly equals to unity.

Determinations of E (1% 1cm) Values at Selected Wavelengths

The absorbances of the solutions having concentration of 40.0 µg/mL of ATN & 10.0 µg/mL of CTN were recorded at wavelength 227.0 nm, 240.0 nm and 251.0 nm and E (1%, 1cm) values of drugs were calculated and results were shown in **Table 3.** The **SD** value in all the determinations was **NMT 2** indicating fineness of the results obtained.

Table 3 E (1% 1cm) Values of ATN & CTN at 227.0, 251.0, 240.0 nm.

Sr. No	E (1% 1cm) at 227.0 nm		E (1% 1cm) at 251.0 nm		E (1% 1cm) at 240.0 nm	
	ATN	CTN	ATN	CTN	ATN	CTN
1	595	3250	760	2590	552.5	3090
2	596.1	3255	762	2592	552.7	3093
3	595.3	3253	763	2594	553	3092
4	595.7	3251	762	2591	552.1	3090
5	595.4	3253	760	2590	552.5	3091
Mean	595.5	3252.4	761.4	2591.4	552.56	3091.2
SD	0.41	1.94	1.34	1.67	0.32	1.30

Analysis of Standard Laboratory Mixtures

Six laboratory mixtures were analyzed having concentration of (40.0 µg/mL of ATN & 10 µg/mL of CTN) respectively. And their absorbances were measured at 227.0, 251.0 nm for (SEM).

Table 4 Results of Analysis of Standard Laboratory Mixtures by (ARM)

Analysis of Standard Laboratory Mixtures By (ARM)						
Sr. No	Amount Taken [µg/mL]		Amount Found [µg/mL]		% Amount Found	
	ATN	CTN	ATN	CTN	ATN	CTN
1	40	10	39.7	9.7	99.25	97
2	40	10	40.2	10.2	100.5	102
3	40	10	39.5	9.8	98.75	98
4	40	10	40.2	10.2	100.5	102
5	40	10	39.8	10.3	99.5	103
6	40	10	40.2	9.7	100.5	97
		Mean	39.93	9.98	99.83	99.83
		SD	0.307	0.278	0.769	2.78
		%RSD	0.770	2.79	0.770	2.79

Analysis of Marketed Formulation Six sample solutions of marketed formulation were analyzed having concentration of (40.0 µg/mL of ATN & 10.0 µg/mL of CTN) respectively. And their absorbances were measured at 227.0, 251.0 nm for (SEM).

Table 5 Results of Marketed Formulation Analysis by (ARM)

Analysis of Marketed Formulation By (ARM)						
Sr. No	Amount Taken [µg/mL]		Amount Found [µg/mL]		% Amount Found	
	ATN	CTN	ATN	CTN	ATN	CTN
1	40	10	39.7	10.2	99.25	102
2	40	10	39.8	10.2	99.5	102
3	40	10	39.7	9.8	99.25	98
4	40	10	40.5	9.8	101.25	98
5	40	10	39.8	9.8	99.5	98
6	40	10	39.7	9.85	99.25	98.5
		Mean	39.86	9.91	99.66	99.41
		SD	0.31	0.22	0.78	2.01
		%RSD	0.787	2.24	0.787	2.02

The proposed methods when applied to marketed formulation the **% drug estimated** for **ARM** was **99.66 & 99.41 (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[2]

1. Accuracy this is performed on the basis of recovery studies by standard addition method. Standard solutions of pure drugs (ATN & CTN) were added in different levels i.e. 80%, 100 %, 120%.

Table 6 Results of Recovery Studies by (ARM)

Recovery Studies by (ARM)								
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) by ARM** 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 7 Results of Precision Studies Intra & Inter Day by (ARM)

Precision Studies Intra & Inter Day By (ARM)					
		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	40.2	0.52	39.6	0.5
	40	41		39.8	
	40	39.8		40	
CTN	10	10.2	1.73	9.7	1.83
	10	9.8		10.1	
	10	10.1		10.2	

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.**

3. Repeatability

Table 8 Results of Repeatability Studies by (ARM)

Repeatability Studies By (ARM)				
Drug	**Amount Taken [µg/mL]**	**Mean Amount Found [µg/mL] (n=5)**	**%Amount Found (n=5)**	**%RSD**
ATN	40	39.8	99.5	0.284
CTN	10	9.91	99.1	0.285

4. Ruggedness

Table 9 Results of Ruggedness Studies by (ARM)

Ruggedness (ARM)							
Sr. No	**Amount Taken [µg/mL]**		**Analyst**	**Amount Found [µg/mL]**		**%Amount Found**	
	ATN	**CTN**		**ATN**	**CTN**	**ATN**	**CTN**
1	40	10	Analyst I	39.85	9.93	99.625	99.3
2	40	10	Analyst II	40.1	9.87	100.25	98.7
			Mean	39.97	9.9	99.93	99
			SD	0.176	0.04	0.441	0.424
			%RSD	0.442	0.43	0.442	0.428

5. Specificity

Table 10 Results of Specificity Studies by (ARM)

Specificity (ARM)								
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	37.5	9.4	93.75	94
2	40	10	0.1 N NaOH	50^0c	38.3	9.5	95.75	95
3	40	10	3% H_2O_2	50^0c	35	9.6	87.5	96
4	40	10	Normal	R.T	39.2	9.8	98	98
				Mean	37.5	9.575	93.75	95.75
				SD	1.80	0.17	4.51	1.70
				%RSD	4.81	1.78	4.81	1.78

Specificity studies are performed by exposing the tablet powder samples to different stress conditions (Acid, Base, Oxidative, Room temperature). The % Amount found for **SEM** was **93.75 & 95.75 (ATN & CTN).** Such deviations from the results given Table 5 indicate method is capable of estimating intact drug contents free of interference from degradation product.

4. 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 methods 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 methods were validated as per the ICH guidelines.

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