Simultaneous Determination Of Atenolol And Hydrochlorothiazide In Tablets Formulation By Derivative Spectrometry

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ABSTRACT

The derivative spectrophotometric method was developed and applied for the simultaneous determination of Atenolol (ATE) and Hydrochlorothiazide (HCT) in Tablets formulations. The first derivative spectrophotometric (1DS) method was applied for the determination of (ATE) and (HCT), respectively. (ATE) was determined at 271.9 nm (1D 271.9) and (HCT) was determined at 279.3 nm (1D 279.3). Linearity showed a good correlation coefficients R² = 0.9994 and R² = 0.9989 for (ATE) and (HCT), respectively. Linearity ranges were (10–280) μg/mL for (ATE) and (2–20) μg/mL for (HCT). The limit of detection (LOD) and limit of quantification (LOQ) were to be 2.77 μg/mL and 8.38 μg/mL for (ATE), 0.52 μg/mL and 1.59 μg/mL for (HCT), respectively. The proposed first derivative method was successfully applied to determine (ATE) and (HCT) in one Syrian trademark drug such as: (NORMOTIC 100 mg (ATE) and 25 mg (HCT)/tab.). All studied samples showed that the drug level was conformed to British Pharmacopeia.

KEYWORDS: Atenolol, Hydrochlorothiazide, Derivative Spectrophotometry.

INTRODUCTION

Atenolol (ATE) chemically, 4-(2-hydroxy-3-isopropyl aminoproxy)-phenyl acetamide is a β-adrenoreceptor blocking agent, primarily used in hypertension, angina pectoris and myocardial infarction. It mainly acts by inhibition of rennin release and angiotensin-2 & aldosterone production. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilizing properties.

Hydrochlorothiazide (HCT), 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, which is widely used in antihypertensive pharmaceutical preparations, reduces active sodium reabsorption and peripheral vascular resistance.

The review of the literature revealed that no method is yet reported for the simultaneous estimation of both drugs in combined dosage forms. Present work describes two simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of (ATE) and (HCT) in tablets formulation³.

Thus, various methods have been proposed to determine the amount of (ATE) and (HCT) in some pharmaceutical formulations, such as reverse phase high performance liquid chromatographic method (RP-HPLC)²-³-⁴, high performance liquid chromatographic method (HPLC)⁵, spectrophotometric method (UV)¹-⁶-⁷, Liquid chromatographic method (LC)⁸, Ultra performance liquid chromatographic method (ULPC)⁹ are successfully applied to determine the two compounds.

The aim of this work is to develop a simple and accurate spectrophotometric method for simultaneous determination of (ATE) and (HCT) in one pharmaceutical formulations without prior treatment by derivative spectrophotometry (1DS).

MATERIALS AND METHODS

Apparatus

All spectral measurements were carried out using a T80+, UV/Vis spectrophotometer PG instrument Ltd (UK), connected to computer, quartz cells 1 cm. Ultrasonic bath Daihan (China), and stirrer Velp Scientifica (Europe).
Chemical regents:
Methanol from LOBAL Chemie (INDIA), Hydrochloric acid from SURCHEM PRODUCTS LTD (ENGLAND), Hydrochlorothiazide purity 99.38 % was obtained from China and Atenolol purity 100.42 % was obtained from India, Double distilled water.

Stock standard preparation
Stock solution $1.5 \times 10^{-2}$ M of Atenolol ($M_W = 266.341$ g/mol) was prepared by dissolving 400 mg of Atenolol standard material in volumetric flask 100 mL of Methanol. The working standard solutions were prepared by appropriate dilutions of stock solution $1.5 \times 10^{-2}$ M with (0.1 N) HCl to give concentrations between (10 - 280) Dg/mL of (ATE).

Stock solution $6.76 \times 10^{-4}$ M of Hydrochlorothiazide ($M_W = 297.73$ g/mol) was prepared by dissolving 20 mg of Hydrochlorothiazide equivalent to 20.125 mg (after taking the purity in consideration) in volumetric flask 100 mL of Methanol. The working standard solutions were prepared by appropriate dilutions of stock solution $6.7 \times 10^{-4}$ M with (0.1 N) HCl to give concentrations between (2 - 20) Dg/mL of (HCT).

Calibration Curve
To construct the calibration curve, ten standard solutions for each concentration were prepared and the absorbance was measured of each solution five times.

Sample preparation
One Syrian product was studied:
Ten NORMOTIC tablets were weighed and finely powdered and an accurate weight equivalent to one tablet 100 mg (ATE) and 25 mg (HCT) was accurately weighed and dissolved in 25 mL Methanol. The sample solution was filtered through a filter papers (Whatman 3, England). Then 0.1 mL was taken to 10 mL volumetric flask and adjusted to volume with (0.1 N) HCl. It was theoretically equivalent to 40 Dg/mL of (ATE) and 10 Dg/mL of (HCT).

RESULTS AND DISCUSSION
Absorption spectra of the standard raw material 120 Dg/mL (ATE) and 10 Dg/mL (HCT) solutions were recorded within a wavelength range of (265 - 300) nm against the blank: (Methanol + (0.1N) HCl). It was noticed that (ATE) and (HCT) cannot be determined by direct measurement, because of the overlapped spectra.

On the other hand, derivative spectrophotometry showed more resolution, where the determination of (ATE) and (HCT) mixture was possible without pretreatment.

The first derivative spectrum at zero-crossing point was used to determine (ATE) in the presence of (HCT) at 271.9 nm

"Fig. 1, a", and determine (HCT) in the presence of (ATE) at 279.3 nm "Fig. 1, b".

![Fig. 1: First derivative spectra of: a- (ATE), b- (HCT).](image-url)
METHOD VALIDATION

The validity of the proposed method was assessed by accuracy (reported as recovery percentage), precision (reported as RSD %), linearity (evaluated by regression equation), limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The concentration linearity of (ATE) was in the range (10 - 280) μg/mL at 271.9 nm by 1D271.9, “Figs. 2,3” and the concentration linearity of (HCT) was in the range (2 - 20) μg/mL at 279.3 nm by 1D279.3, “Figs. 4, 5”.

Fig. 2: First derivative spectra of (ATE):

C₁: 10 μg/mL, C₂: 40 μg/mL,
C₃: 120 μg/mL, C₄: 200 μg/mL,
C₅: 280 μg/mL.

Fig. 3: Calibration curve for (ATE):

C₁: 10 μg/mL, C₂: 20 μg/mL,
C₃: 30 μg/mL, C₄: 40 μg/mL,
C₅: 80 μg/mL, C₆: 120 μg/mL,
C₇: 160 μg/mL, C₈: 200 μg/mL,
C₉: 240 μg/mL, C₁₀: 280 μg/mL.

n = 5 for each concentration.

Fig. 4: First derivative spectra of (HCT):

C₁: 2 μg/mL, C₂: 6 μg/mL,
C₃: 10 μg/mL, C₄: 14 μg/mL,

Fig. 5: Calibration curve for (HCT):

C₁: 2 μg/mL, C₂: 4 μg/mL,
C₃: 6 μg/mL, C₄: 8 μg/mL,
$C_5: 18 \mu g/mL,$ $C_6: 10 \mu g/mL,$ $C_6: 12 \mu g/mL,$
$C_7: 14 \mu g/mL,$ $C_8: 16 \mu g/mL,$
$C_8: 18 \mu g/mL,$ $C_9: 20 \mu g/mL.$

$n = 5$ for each concentration.

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

LOD and LOQ were calculated in “table 1” using the following equations:

$LOQ = 10 \times SD_{m}$

$LOD = 3.3 \times SD_{m}$

Where $SD$ is the standard deviation of $y$-intercepts (a) of regression lines and (b) is the slope of the equitation of calibration curve, $y = a + b \times$.

Table 1: Statistical data for calibration graphs

| Method | Analyte | Selected wavelength (nm) | Linearity range $\mu g/mL$ | Correlation coef. (R$^2$) | LOD $\mu g/mL$ | LOQ $\mu g/mL$ |
|--------|---------|--------------------------|-----------------------------|---------------------------|----------------|----------------|
| DS     | ATE     | $\lambda_1 279.9$       | 10 – 280                    | 0.9994                    | 2.77           | 8.38           |
| DS     | HCT     | $\lambda_2 279.3$       | 2 – 20                      | 0.9989                    | 0.52           | 1.59           |

Accuracy

To determine the precision and accuracy of the proposed method, five replicate determinations were carried out on three different concentrations of standards (ATE) and (HCT). The validation results are shown in “table 2”.

Table 2: Method validation for the simultaneous determination of Atenolol and Hydrochlorothiazide by the $\lambda_1$DS

| DS     | Pharmaceutically Raw material | Theoretical concentration ($\mu g/mL$) | observed concentration $\bar{X} (\mu g/mL)$ | SD $\mu g/mL$ | Precision RSD (%) | Accuracy (%) |
|--------|-------------------------------|--------------------------------------|---------------------------------------------|---------------|-------------------|--------------|
| $\lambda_1 271.9$ | Atenolol                 | 80                                   | 82.28                                       | 2.06          | 2.50              | 102.85       |
|        |                               | 160                                  | 165.17                                      | 2.00          | 1.21              | 103.23       |
|        |                               | 240                                  | 244.35                                      | 1.52          | 0.62              | 101.81       |
| $\lambda_1 279.3$ | Hydrochlorothiazide          | 4                                    | 3.99                                        | 0.03          | 0.75              | 99.75        |
|        |                               | 8                                    | 8.33                                        | 0.10          | 1.20              | 104.12       |
|        |                               | 12                                   | 12.27                                       | 0.15          | 1.22              | 102.25       |

Accuracy (%) = (observed concentration/theoretical concentration) ×100.

$\bar{X}$ Five separate determinations were performed and calculated the mean.

Precision

In order to demonstrate the precision of the proposed method, intra-day and inter-day variability were performed at three different concentrations (40, 120, and 200 $\mu g/mL$) for Atenolol and (6, 10, and 14 $\mu g/mL$).
for Hydrochlorothiazide at the same day and at three different days. Method precision was tested in terms of RSD % for both intra-day and inter-day precisions.

The precision was ascertained by carrying out five replicates of standard Atenolol and Hydrochlorothiazide under study and the mean was calculated. The RSD % results were not more than 4.51 %, 3.58 % for (ATE) and (HCT) respectively during the determination in one day and the RSD % results were not more than 4.54 %, 3.45 % for (ATE) and (HCT) respectively during the determination in three days, where the method is considered very precise.

**Recovery**

The recovery was studied by three addition standards of each (ATE) and (HCT) in the NORMOTIC product. “Table 3” presents the recovery result for the NORMOTIC Syrian trademark drug.

**Table 3: Recovery for NORMOTIC Syrian trademark drug.**

| Product | pharmaceutical compounds | | Total Found | Recovery % | SD µg/mL | RSD % | Recovery Average % |
|---------|---------------------------|---|----------------|-------------|----------|--------|---------------------|
| NORMOTIC 100 mg (ATE) and 25 mg (HCT)/tab. | ATE | 30 | 24 | 53.63 | 98.46 | 3.59 | 3.65 | 98.94 |
| | | | 30 | 59.68 | 98.93 | 3.49 | 3.53 | |
| | | | 36 | 65.79 | 99.42 | 3.50 | 3.52 | |
| | HCT | 7.5 | 6 | 13.59 | 101.5 | 0.79 | 0.78 | 100.42 |
| | | | 7.5 | 14.99 | 99.87 | 0.53 | 0.53 | |
| | | | 9 | 16.49 | 99.89 | 1.12 | 1.12 | |

Five separate determinations were performed and calculated the mean.

**Application**

The developed method was applied for quantitative determination for (ATE) and (HCT) in NORMOTIC Syrian trademark as tablets formulation. The sample was prepared as described in the section of sample preparation. Quantitative analysis was done by using calibration curves.

The obtained results are summarized in “table 4” for five different NORMOTIC batches. The concentrations of detected (ATE) and (HCT) in the NORMOTIC product was within the allowed limits under the British Pharmacopeia. The relative standard deviations RSD % (n = 5) of the quantitative results were in the range of 2.50 – 4.74 % and 0.95 – 2.18 % for (ATE) and (HCT), respectively.

**Table 4: Results of (ATE) and (HCT) in (NORMOTIC 100 mg (ATE) and 25 mg (HCT)/tab), for five different batches.**

| No. of batches | ATE 100 mg/tab. | HCT 25 mg/tab. |
|----------------|-----------------|----------------|
| Result dose × mg/tab. | SD mg/tab. | RSD % | Per % | Result dose × mg/tab. | SD mg/tab. | RSD % | Per % |
| 1 | 102.62 | 2.57 | 2.50 | 102.62 | 25.12 | 0.39 | 1.55 | 100.48 |
| 2 | 103.14 | 3.82 | 3.70 | 103.14 | 25.25 | 0.38 | 1.50 | 101.00 |
| 3 | 98.33 | 3.77 | 3.83 | 98.33 | 25.12 | 0.24 | 0.96 | 100.48 |
| 4 | 101.46 | 3.46 | 3.41 | 101.46 | 25.22 | 0.55 | 2.18 | 100.88 |
Range mg/tab 98.33 – 103.14 25.12 – 25.42
Range Per % 98.33 – 103.14 100.48 – 101.68
Range RSD % 2.50 – 4.74 0.96 – 2.18

According to British Pharmacopeia, the tablets must contain not less than 92.5 percent and not more than 107.5 percent of labeled amount for (ATE) and the tablets must contain not less than 92 percent and not more than 107 percent of labeled amount for (HCT). So the obtained results are conformed to British Pharmacopeia.

CONCLUSION
Atenolol (ATE) and Hydrochlorothiazide (HCT) combination were estimated in one local pharmaceutical product by the first derivative spectrum method, using the zero-crossing point.

The active substances (ATE) and (HCT) in NORMOTIC pharmaceutical product were within the permissible limits set by the British Pharmacopeia legislation. The proposed method for estimating the binary mixture (ATE) and (HCT) is accurate, sensitive, simple, straightforward in quantitative analysis without prior chemical treatment. In general the dosage ratio of (ATE) to (HCT) is bigger in all pharmaceutical tablets formulations.

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REFERENCES
1. Tarkase K N, Hapse S A, Shirsath A S, Kadaskar P T. Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Atenolol and Hydrochlorothiazide in Pure and Tablet Dosage Form. International Journal of Chem Tech Research. 2012; 4(3): 1146-1150.
2. Zaveria M, Khandharb A. Development and validation of a RP-HPLC for the simultaneous estimation of atenolol and hydrochlorothiazide in pharmaceutical dosage forms. jprhc. 2010; 2(3): 248-252.
3. Zaveri M, Amit K. Development and Validation of a RP-HPLC for the Simultaneous estimation of Atenolol and Hydrochlorothiazide in Pharmaceutical Dosage Forms. International Journal of Advances in Pharmaceutical Sciences. 2010; 1 (2010): 167-171.
4. Sa’sa’ S I, Jalal I M, Khalil H S. Determination of Atenolol Combinations with Hydrochlorothiazide and Chlorthalidone in Tablet Formulations by Reverse-Phase HPLC. Journal of Liquid Chromatography.2006; 11(8):1673-169.
5. Neelima M S, Gandhi B M, Raju V B, Sumanth K S, Mounika K S, Lakshmi P J N. 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): 118-124.
6. Bari S, Sathe Sh, Jain P, Surana S. Spectrophotometric method for simultaneous estimation of atenolol in combination with losartan potassium and hydrochlorothiazide in bulk and tablet formulation. Journal of Pharmacy and Bioallied Sciences October-December. 2010; 2(4): 372-275.
7. Behera A K. Simultaneous Spectrophotometric Estimation of Atenolol and Hydrochlorothiazide in Tablet Dosage Forms. International Journal of Chem Tech Research. 2010; 2(4): 1901-1906.
8. Yadav S S, Rao J R. Micellar liquid chromatographic analysis for simultaneous determination of atenolol and hydrochlorothiazide in tablet dosage form. Int J Pharm Pharm. 2013; 5(4), 63-67.
9. Durga Rao D N V, Satyanarayana S S S, Ramakoti Reddy Y K, Makkanti. Simultaneous Determination of Losartan Potassium, Atenolol and Hydrochlorothiazide in Pharmaceutical Preparations by Stability-Indicating UPLC. Chromatographia. 2009; 3(4): 647-651.

10. British Pharmacopeia 2010.