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Determination of Atenolol and Hydrochlorothiazide in Pharmaceutical Preparation Using RP-HPLC

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Abstract: A (Reversed Phase High Performance Liquid Chromatography) (RP-HPLC) method was Development for the determination of Atenolol (ATE) and Hydrochlorothiazide (HCT) in tablets formulations. Isocratic Elution Chromatography analysis was achieved with (methanol: phosphoric acid) (pH = 4) in ratio of 70:30 (v/v) as mobile phase, the analytical is done by C_{18} column and UV detector at 220 nm, pump flow rate was 1.2 mL/min and sample injection volume 20 µL. Retention time was less than 3 min. The method was validated with respect to (linearity, accuracy, specificity, precise, LOD and LOQ). Linearity showed a good correlation coefficients R^2 = 0.9993 and R^2 = 0.9997 for (ATE) and (HCT), respectively ranges were (20 – 100) µg/mL for (ATE) and (3 – 15) µg/mL for (HCT). (The limit of detection) (LOD) and (limit of quantification) (LOQ) were to be 2.60 µg/mL and 7.87 µg/mL for (ATE), 0.25 µg/mL and 0.77 µg/mL for (HCT), respectively. The proposed method was successfully applied to analysis individual or mixture of (ATE) and (HCT) in Syrian trademark drugs. All studied samples showed that the drug levels were conformed to British Pharmacopeia. Finally, the method is simple, selective and suitable for routine quality control analysis.

Keywords: (HPLC), (Atenolol), (Hydrochlorothiazide).

تقدير اتينولول وهيدروكلوروثيازيد في المستحضر الصيدلاني باستعمال الطور العكسي لكروماتوغرافيا السائل عالي الأداء

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المستخلص: تم تطوير طريقة كروماتوغرافية سائلة عالية الأداء ذات الاطوار المعكوسة لتقدير الأتينولول وهيدروكلوروثيازيد في المستحضرات الصيدلانية. تم اجراء التحليل بالازاحة الايزوكراتية باستخدام طور متحرك (ميثانول: حمض الفوسفور) ودرجة الحموضة pH = 4 وبنسبة مزج (v/v) (30: 70)، تم التحليل باستخدام عامود C_{18} ومكشاف أشعة فوق بنفسجية عند الطول الموجي 220 nm سرعة الجريان 1.2 mL/min وحجم العينة المحقونة 20 µL. لم يتجاوز زمن الاحتجاز ثلاث دقائق، درسنا خطية المركبات المدروسة. تم التأكد من صلاحية الطريقة من خلال (الخطية، الصحة، النوعية، الدقة، حد الكشف وحد التحديد الكمي) فكانت معاملات الارتباط جيدة R^2 = 0.9993 و R^2 = 0.9997 كلا من ايتانولول وهيدروكلوروثيازيد على التسلسل والمجال الخطي (20 - 100) µg/mL للأتينولول و(3 - 15) µg/mL لهيدروكلوروثيازيد. بلغ حد الكشف وحد التحديد الكمي للأتانولول 2.60 µg/mL و 7.87 µg/mL

اما لهيدروكلوروثيازيد فكان 0.25 µg/mL و 0.77 µg/mLعلى التسلسل. تم تطبيق الطريقة المقترحة بنجاح على بعض المستحضرات الصيدلانية السورية بشكل افرادي أو مزيج من المركبين. كافة النتائج توافقت مع المجالات المسموح بها ضمن دستور الأدوية البريطاني. أخيراً الطريقة بسيطة وانتقائية ومناسبة للتحاليل ضبط الجودة الروتينية.

الكلمات المفتاحية: (أتينولول)، (هيدروكلوروثيازيد)، (كروماتوغرافيا).

INTRODUCTION.

Atenolol (ATE) chemically, 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy]-benzeneacetamide Fig. 1-a, is a β-adrenoreceptor blocking agent, primarily used in hypertension, angina pectoris and myocardial infraction. It mainly acts by inhibition of (Rennin) release and (Angiotensin-2) and (Aldosterone) production.

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

The review of the literature revealed that method is not reported for the simultaneous estimation of both drugs in combined dosage forms.

Thus, various methods have been reported in the literature for the analysis of (ATE) and (HCT) in some pharmaceutical formulations, such as (Reverse Phase High Performance Liquid Chromatographic) method (RP-HPLC)[2-3-4-5], Spectrophotometric method (UV)[1-6], Liquid Chromatographic method (LC)[7], (Ultra Performance Liquid Chromatographic) method (ULPC)[8], (Capillary Electrophoresis) method[9-10], Voltammetric method[11-12-13] are successfully applied to determine the two compounds (ATE) and (HCT).

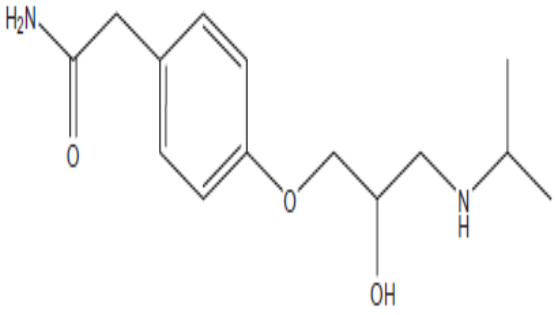


Fig. 1 –a: ATENOLOL: ($C_{14}H_{22}N_2O_3$), M.wt = 266.341 g/mol

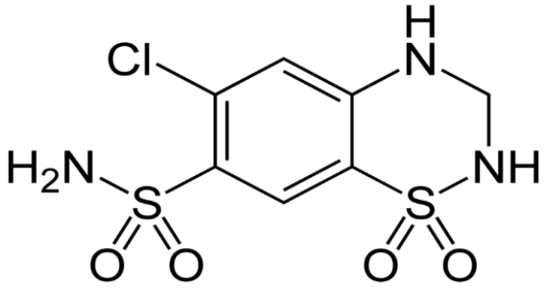


Fig. 1 –b: HYDROCHLOROTHIAZIDE: ($C_7H_8CLN_3O_4S_2$), M.wt = 297.73 g/mol

MATERIALS AND METHODS.

Apparatus:

HPLC analysis was performed on an YL 9100 HPLC system (Korea) device accessories were reported in table 1. Chromatographic analysis were obtained by using SGE Analytical Science clc-C_{18}

column (SGE Analytical Science, Australia). Ultrasonic bath (Daihan, USA), analytical balance TE64 Sartorius (Germany) sensitivity 0.01 mg. Germany digital pipettes (Isolab).

Table (1) Device Accessories.

Instrument	Model
Binary pump	YL9111
Vacuum degasser series	YL9101
Column compartment	YL9130
UV/Vis. Detector	YL9120

Chemical regents:

Solvents and materials that used in this work, water (Merck, Germany), methanol (Chm-lab, Belgium), all were HPLC grade. Phosphoric acid (Isolab, Germany) GR analytical grade, Hydrochlorothiazide purity 99.38 % was obtained from China and Atenolol purity 100.42 % was obtained from India.

Stock standard preparation:

Stock solution 3.75×10^{-3} M of Atenolol ($M._{wt}$ = 266.3 g/mol) has been prepared by dissolving 100 mg of (ATE) standard material in volumetric flask 100 mL of Methanol. The working (Standard Solutions) have been prepared by appropriate dilutions of stock solution 3.75×10^{-3} M with mobile phase to give concentrations between (20 - 100) μg/mL of (ATE).

Stock solution 3.36×10^{-4} M of Hydrochlorothiazide ($M._{wt}$ = 297.7 g/mol) has been prepared by dissolving 10 mg of (HCT) equivalent to 10.06 mg (after taking the purity in consideration) in volumetric flask 100 mL of Methanol. The working (Standard Solutions) have been prepared by appropriate dilutions of stock solution 3.36×10^{-4} M with mobile phase to give concentrations between (3 - 15) μg/mL of (HCT).

Chromatographic system:

Stationary phase is SGE Analytical Science clc-C_{18} column (250 × 4.6 mm i.d., 5 µm). Mobile phase (methanol: water) in ratio of (70:30) V/V, pH = 4, prepared by addition phosphoric acid.

Calibration Curve:

To construct the calibration curve, for each concentration five standard solutions were prepared and measured the area peaks five times for each solution

Samples preparation:

One Syrian product was studied:

Ten NORMOTIC tablets were have been weighed and finely powdered than an accurate weight equivalent to one tablet 100 mg (ATE) and 25 mg (HCT) was dissolved in 25 mL Methanol.

The sample solution filtered through a filter paper (Whatman 3, England).

Then 0.1 mL of the last solution has been taken to 10 mL volumetric flask and adjusted to volume with (mobile phase). It was theoretically equivalent to 40 μg/mL of (ATE) and 10 μg/mL of (HCT).

RESULTS AND DISCUSSION.

Chromatogram of the standard solutions of mixture (ATE) and (HCT), under optimized method conditions showed that well separated (ATE) from (HCT) with a good resolution, fig. 2. The time of analysis was achieved in less than 3 min.

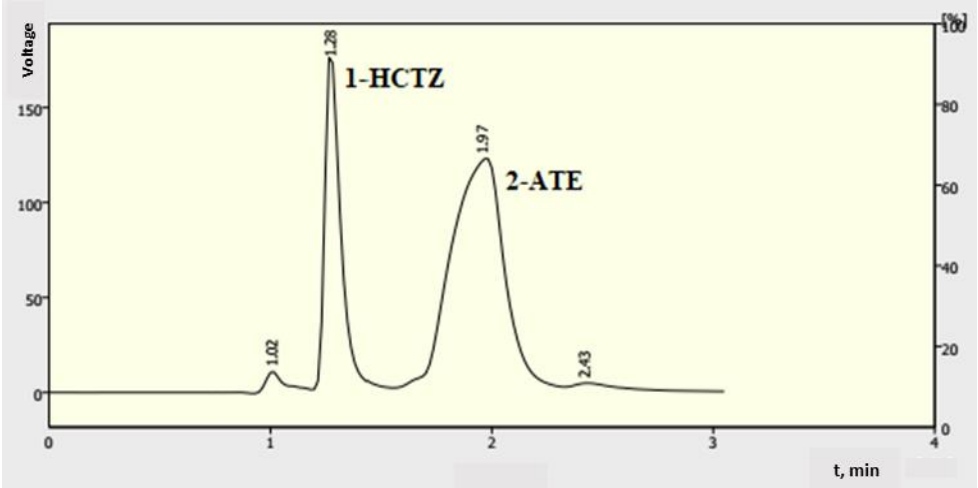
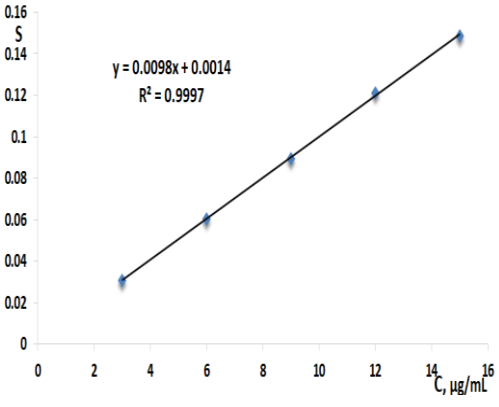


Fig.(2) Chromatogram of standard solutions showing separated peaks:
1- (HCT) = 9 µg/mL, 2- (ATE) = 60 µg/mL.

METHODS VALIDATION:



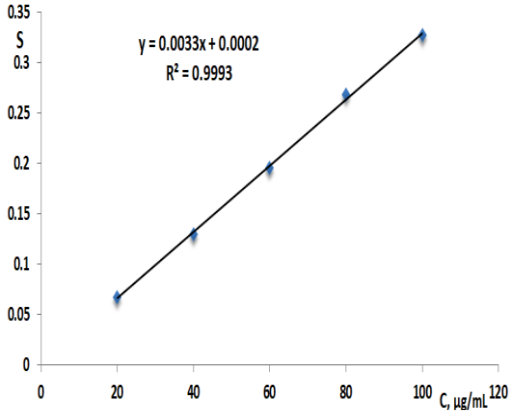


Fig. 3: Linear relationship for (HCT):

C1: 3 μg/mL, C2: 6 μg/mL,
C3: 9 μg/mL, C4: 12 μg/mL,
C5: 15 μg/ml.
n = 5 for each concentration.

Fig. 4: Linear relationship for (ATE):

C1: 20 μg/mL, C2: 40 μg/mL,
C3: 60 μg/mL, C4: 80 μg/mL,
C5: 100 μg/mL.
n = 5 for each concentration.

Linearity:

The concentration linearity S = f(C) of (HCT) where S: a peak surface and C: standard concentration. The concentration linearity was in the range (3 - 15) μg/mL, fig. 3, and the concentration linearity of (ATE) was in the range (20 - 100) µg/mL, fig. 4.

Limit of detection (LOD) and Limit of quantification (LOQ):

The (LOD) and (LOQ) were obtained from the calibration curves. The (LOD) and (LOQ) were calculated based on the standard deviation (SD) of the Y-intercepts of regression lines for five calibrations curves, it was to be 0.002651 for atenolol and 0.000751 for hydrochlorothiazide and (the slope m) of the average of five calibrations curves, it was to be 0.003367 for atenolol and 0.009767 for hydrochlorothiazide, using the formulae 3.3 x SD / m and 10 x SD /m respectively. The (LOD) and (LOQ) concentrations were reported in table 2.

Table (2) Validation parameters for (ATE) and (HCT).

Component	Linearity (µg/mL)	Regression equation*	Retention time (min)	LOQ* (µg/mL)	LOD* (µg/mL)
(ATE)	20 - 100	Y = 0.0033 x + 0.0002	1.97	7.87	2.60
(HCT)	3 - 15	Y = 0.0098 x + 0.0014	1.28	0.77	0.25

*n = 5

Accuracy:

To determine the precision and accuracy of the proposed method, five replicate determinations has been carried out on three different concentrations of standards (ATE) and (HCT). The validation results are shown in table 3.

Table (3) Method validation for the simultaneous determination of Atenolol and Hydrochlorothiazide.

Pharmaceutically Raw material	Theoretical concentration (µg/mL)	observed concentration (µg/mL)	SD µg/mL	Precision RSD (%)	Accuracy (%)
Atenolol	40	39.60	0.40	1.01	99.00
	60	61.20	1.64	2.68	102.00

Pharmaceutically Raw material	Theoretical concentration (µg/mL)	observed concentration $\bar{x}$ (µg/mL)	SD µg/mL	Precision RSD (%)	Accuracy (%)
	80	81.05	0.48	0.59	101.31
Hydrochlorothiazide	6	6.03	0.10	1.66	100.50
	9	9.06	0.10	1.10	100.67
	12	12.12	0.09	0.74	101.00

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

$\bar{x}$ mean of five replicated determinations,

Precision:

In order to demonstrate the precision of the proposed method, (Intra-day) and (Inter-day) variability studies performed at three different concentrations (40, 60, 80 μg/mL) for (ATE) and (6, 9, 12 μg/mL) for (HCT) at the same day in two hours' time interval and at three days. Method precision was tested in terms of RSD % for both intra-day and inter-day precisions.

The precision has been ascertained by carrying out five replicates of standard (ATE) and (HCT) under study and the mean was calculated. The RSD % results were not more than 2.43 %, 1.78 % for (ATE) and (HCT) respectively during the determination in one day and the RSD % results were not more than 2.43 %, 2.34 % for (ATE) and (HCT) respectively during the determination in three days, where the method is considered very precise.

Recovery:

The recovery studied by three addition standards (80%, 100%, and 120%) for each (ATE) and (HCT) in the NORMOTIC product. Table 4 presents the recovery results for the NORMOTIC Syrian trademark drug.

Table (4) Recovery for NORMOTIC Syrian trademark drug.

Product	Pharmaceutical compounds	Sample µg/mL	Added µg/mL	Total found $\bar{x}$ µg/mL	Recovery %	SD µg/mL	RSD %	Recovery Average %
NORMOTIC 100 mg (ATE) and 25 mg (HCT)/tab.	ATE	25.42	20.8	46.19	99.86	1.49	1.49	**99.00**
			26.0	51.08	98.69	1.16	1.18	
			31.2	56.14	98.46	1.77	1.80	
	HCT	6.81	5.2	12.11	101.92	2.70	2.65	**101.25**
			6.5	13.42	101.69	1.99	1.96	
			7.8	14.62	100.13	0.73	0.73	

$\bar{x}$ Mean for five replicates.

RSD % is reported to recovery values.

Specificity:

(ATE) and (HCT) have been determined in NORMOTIC sample without any interference. So, (ATE) and (HCT) peaks were completely determine with a good resolution and specificity, proved by recovery results, arrived to approximately 100%, as seen in fig. 5.

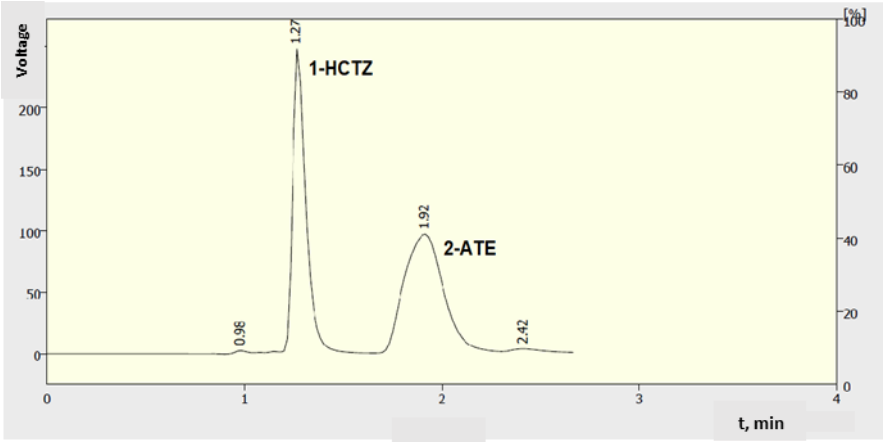


Fig. (5) Chromatogram of "NORMOTIC" tablets showing separated peaks:

1- (HCT) = 10 µg /mL, 2- (ATE) = 40 µg /mL.

Application:

The method has been applied for quantitative determination for (ATE) and (HCT) in NORMOTIC Syrian pharmaceutical product NORMOTIC for five different batches for each one. The samples were prepared as mention before in the section of sample preparation. Quantitative analysis has been done by using direct calibration curves. The obtained results are summarized in table 5 In general, the concentrations of detected (ATE) and (HCT) in the NORMOTIC product was within the allowed limits under the British Pharmacopeia. According to British Pharmacopeia, the tablets must contain not less than 92.5 % and not more than 107.5 % of labeled amount for (ATE); and the tablets must contain not less than 92 % and not more than 107 % of labeled amount for (HCT). So, the obtained results are conformed to British Pharmacopeia. The relative standard deviations RSD % (n = 5) of the quantitative results were in the range of 1.86 – 3.59 % and 0.95 – 2.36 % for (ATE) and (HCT), respectively.

Table (5) 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 $\bar{x}$ mg/tab.	SD mg/tab.	RSD %	Per %	Result dose $\bar{x}$ mg/tab.	SD mg/tab.	RSD %	Per %
1	98.35	1.83	1.86	98.35	26.21	0.25	0.95	104.84
2	97.28	3.49	3.59	97.28	25.71	0.53	2.06	102.84
3	96.84	2.60	2.68	96.84	25.41	0.54	2.13	101.64
4	97.38	2.78	2.85	97.38	25.79	0.30	1.16	103.16
5	97.50	2.77	2.84	97.50	25.47	0.60	2.36	101.88
Range mg/tab	96.84 – 98.35				25.41 – 26.21			
Range RSD %	1.86 – 3.59				0.95 – 2.36			
Range Per %	96.84 – 98.35				101.64 – 104.84			

$\bar{x}$ Mean for five replicates.

CONCLUSION.

The proposed HPLC method are direct, specific, precise and accurate for simultaneous determination of Atenolol and Hydrochlorothiazide in NORMOTIC tablets formulation.

The active substances (ATE) and (HCT) in NORMOTIC pharmaceutical product were within the permissible limits set by the British Pharmacopeia legislation.

The described method is suitable for routine analysis and quality control of pharmaceutical preparation either (ATE) alone or in combination with (HCT).

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