SIMULTANEOUS DETERMINATION OF AMILORIDE HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICALS BY DERIVATIVE SPECTROPHOTOMETRY

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Abstract

Method is established for simultaneous determination of Amiloride hydrochloride (Amilo) and hydrochlorothiazide in tablets. The method is based on employing normal and derivative spectrophotometry using the zero order via described by measuring the absorbance at λ 361 nm and the first order derivative spectra at peak λ 340 and valley λ 382 nm for (Amilo), while the zero-crossing method is applied for hydrochlorothiazide. The first order derivative absorption spectra at valleys λ 285 and λ 239 nm were used for hydrochlorothiazide. No interferences found between both determined and those of matrices. A good accuracy and precision of simultaneous determination of (Amilo), and hydrochlorothiazide were confirmed by statistical analysis. The percentage recovery of the individual drags under the established conditions is ranged from 97.79% to 101.5 %. Linearity is maintained within a wide concentration range from 2.00 mg/L to 40.00 mg/L for Amiloride hydrochloride and hydrochlorothiazide. The detection limit was 0.5 and 1.0 mg/L for Amiloride hydrochloride and hydrochlorothiazide respectively.

Keywords: Amiloride Hydrochloride, Hydrochlorothiazide drug analisis, Derivative Spectrophotometry.

Introduction

Amiloride Hydrochloride, an antikaliuretic-diuretic agent, is a pyrazine-carbonyl-guanidine[1] that is unrelated chemically to other known antikaliuretic or diuretic agents[2,3] .It is known chemically as 3, 5 - diamino - 6 - chloro- N - (diaminomethylene) pyrazine carboxamide monohydrochloride, dihydrate and its structural formula as in Figure 1a. [4].

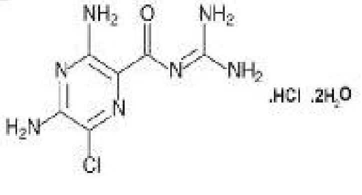


Fig. (1a): Structure Formula of Amiloride Hydrochloride.

Hydrochlorothiazide is diuretic agent. It is known chemically as 6-chloro-3,4-dihydro-2*H*-1,2,4- benzothiadiazine-7-sulphonamide 1,1 -dioxide and its structural formula as in Fig.(1b). [4]

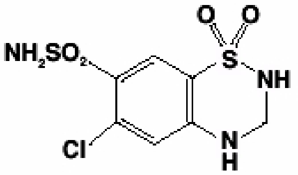


Fig. (1b): Structure Formula of Hydrochlorothiazide.

Amiloride Hydrochloride was determined in tablets or biological fluids using fluorescence spectrometry [5, 6]. Sequential injection analysis technique (SIA)[7], simple and fast derivative spectrophotometry [8, 9,], chromatographic methods HPLC and GC/MS [10-13], voltammetry[14], potentiometry.[15] and UV spectrophotometry coupled with new chemometric regression techniques [16, 17].

Several analytical methods, were developed for the determination Hydrochlorothiazide, either alone or is combining with other drugs, including LC–MS[11,19,20]. UV derivative spectrophotometric method21], UV-spectrophotometry coupled with new chemometric regression techniques [16,17], capillary electrophoresis [22, 23], and HPLC [24–26].

Technological and scientific progress has led to the development of numerous synthetic drugs. It is therefore imperative to dispose of analytical methods to determine these drugs both in the quality control manufacturing phase of the pharmaceutical formulations and their determination in the human body. Derivative UV spectroscopy has been widely used as a tool for quantitative analysis, characterization, and quality control in agricultural, pharmaceutical, and biomedical fields [27].

In this paper a new spectrophotometric method for simultaneous determination of Amiloride Hydrochloride and hydrochlorothiazide is presented. Due to interferences in zero-order spectra and significant differences in concentration of both constituents in the. preparation, derivative spectrophotometry was used for quantitative analysis by using first order at λ 340 and λ 382 nm for Amiloride Hydrochloride. An attempt was made to find suitable derivatives and wavelength for quantitative analysis for hydrochlorothiazide at which both drugs show no interference by using first derivative spectra with zero-crossing method at λ 285 and λ 239 nm. As no similar analyses were found in available literature it seems justifiable to develop a simple, quick and easily available spectrophotometric method for drug quality control purposes. This method differ from other it don't need any chemical treatment and both drugs can be determined directly in a single sample without using any separation process.

Experimental

Instruments and Equipments:

Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZU (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version 1.10), using 1.00 cm quartz cells was used for measuring the absorption signals, Ultra sonic devise (ultrasonicator) for dissolving samples, (SONOREX), (W. Germany), Ultra pure water manufacturing devise, (TORAYPURE), model LV-08 (Japan).

Chemicals:

Pure Amiloride hydrochloride (C6H8CIN7O.HCl.2H2O F.W.302.12) and Hydrochlorothiazide (HCT) $C_7H_8ClN_3O_4S_2$; F.W. 297.74) were obtained as a gift from the State Company of Drug Industries and Medical Appliances (Samara- IRAQ-SDI).

All commercial drugs such as Saluretic tablets (Amiloride hydrochloride 5mg + Hydrochlorothiazide 50mg) made in Cairo-Egypt, Co-Amilozid (5/50 mg tablets) from TEVA UK Limited and the same content in Maduratic Indian tablets. Esidrex (25) from NOVARTIS Switzerland contains 25 mg Hydrochlorothiazide only were purchased from the local drug store.

Preparation of Stock and working Standard Solutions

1. Stock solutions of (500 or 250 mg/L) were prepared by dissolving an accurately weighed amount (50 or 25 mg) of the pure studied drugs in about 80 mL of the deionized water in 100 mL volumetric flask .using ultra sonic devise (ultrasonicator) for dissolving samples, The solutions are then made up to the mark with deionized water.
2. Two series of pure single standards drugs (0.2-100.0 mg/L) were prepared by diluting stock solutions with deionized water.
3. Solutions for binary mixtures of standard drugs Amiloride hydrochloride and hydrochlorothiazide solutions were prepared by two series;

First series of mixture solutions was prepared by using a fixed concentration of (10 mg/L) for Amiloride hydrochloride with different concentrations (2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, and 40 mg/L) of Hydrochlorothiazide, while the second series of mixture contains a fixed concentration (20 mg/L) of hydrochlorothiazide with different concentration of (2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, and 40 mg/L) of Amiloride hydrochloride.

General Procedure for Sample Preparation:-

Ten tablets were weighted and crushed for each drug, mixed in a mortar. Accurately weight of one tablet from each drug which dissolved by 50 mL deionized water and using ultrasonicator for~10min. The obtained suspensions were filtered and the clear solutions were taken and filed up to 100 mL the resulting solution~contain 50 mg/L Amloride hydrochloride and 500 mg/L Hydrochlorothiazide and 250 mg/L respectively, then by dilution with deionized water desired concentrations were obtained.

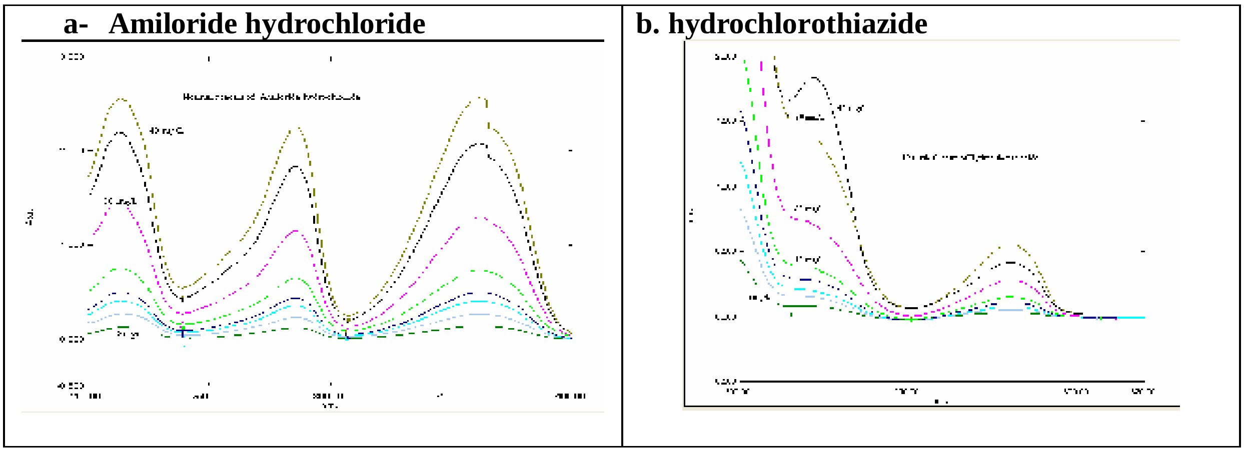
UV-Visible Measurement:

The absorption spectra of the Amiloride hydrochloride, and Hydrochlorothiazide were measured from 600 to 200 nm against distilled water as blank. The wavelength at absorption maximum (λ_{max}) was identified. The first derivative spectra ^{1}D, have been taken from normal spectrum for each drug by the computer via a SHIMADZU UV probe data system program and the parameters S and $\Delta\lambda$, by which the absorbance and smoothing the spectra were proportional directly. The suitable wavelengths peaks (P) and valley (V) at (λ_{max}) were identified for standard drugs at zero crossing for each to other. The calibration curves of first derivative were constructed and used to determine the concentrations of each drug simultaneously.

Results and Discussion

The zero-order (normal spectrum) absorption spectra recorded for standard solutions. There are three absorption maxima for Amiloride hydrochloride, wavelengths at 213, 285.5 and 361 nm as shown in Fig.(2a), and for hydrochlorothiazide there is one clear absorbance maxima at 280 nm as shown in Fig. (2b).

Normal spectrum can be used to determine of pure Amiloride hydrochloride at wavelengths 213, 285.5 and 361 nm. The calibration curves for standard solutions (2-40 mg/L) Amiloride hydrochloride was constructed, which regression line gave linear equation with slope and the correlation coefficient and molar absorptivity (ε). The data are summarized in Table(1).


a- Amiloride hydrochloride

b. hydrochlorothiazide

Fig.(2): Normal spectra of a- Amiloride hydrochloride and b- hydrochlorothiazide.

Table(1)
Statistical Evaluation for the Calibration Graphs of amiloride hydrochloride for different wavelength.

Range of conc. mg/L	*Wavelength nm*	*equation*	*r*	*Molar absorptivity(ε)*
2 - 40	361.0	Y=0.06962x -0.01889	0.9995	21.05474×10^3
2 - 40	285.5	Y=0.05616x-0.01273	0.9999	16.96706×10^3
2 - 40	213.5	Y=0.06492x-0.01004	0.9994	19.61363×10^3

Normal spectrum can be also used to assay of Amiloride hydrochloride in the presence Hydrochlorothiazide in the mixture contains of a greater than 90 % Hydrochlorothiazide at λ 361 nm, the percentage relative error and RSD not exceed than 1.65 and 0.782 respectively. The relative errors for the mixtures listed in Table (3).

Normal spectrum cannot be used to determine of Hydrochlorothiazide when they present in mixture, due to interference between the spectra, as shown in Fig.(3).

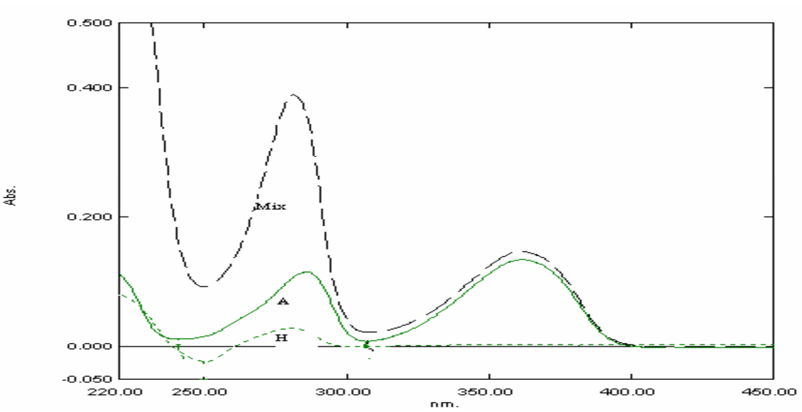
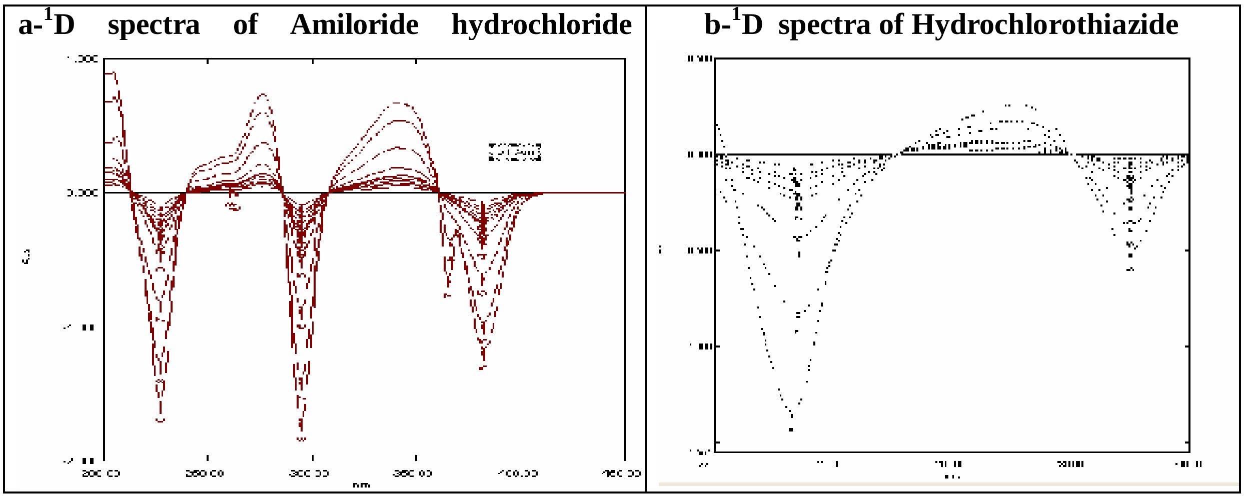


Fig.(3): Normal spectra for 2mg/L Amiloride , 2 mg/L Hydrochlorothiazide and the mixture of 2 A+ 2 H mg/L.

Therefore, UV derivative can be used in this case, as shown in Fig.(4), first derivative may be used to determine Amiloride hydrochloride in the presence Hydrochlorothiazide at peak λ 340 nm and valley λ 382 nm. The calibration curve was constructed of ^{1}D for standard solutions (2-40 mg/L) Amiloride hydrochloride gave linear equation with slope and the correlation coefficient listed in Table(2). Also the relative error RSD not exceeds than ± 1.650 and 0.522 respectively. The relative errors for the mixtures in the mixture contains a greater than 90 % Hydrochlorothiazide listed in Table (3).


a-^{1}D spectra of Amiloride hydrochloride

b-^{1}D spectra of Hydrochlorothiazide

Fig. (4): First derivative spectra:- a- Amiloride hydrochloride, b- hydrochlorothiazide.

Table(2)

Statistical Evaluation for the Calibration Graphs of amiloride hydrochloride for first derivative wavelength.

Range of conc. mg/L	*Wavelength nm ^{1}D*	*equation*	*r*
2 - 40	P=340	Y=0.01716x -0.0013	0.9997
2 - 40	V=382	Y=0.02997x-0.00331	0.9998

Table (3)

The relative error for determination of Amiloride hydrochloride solutions using normal spectra at 361 nm and first derivative with scale factor=10 and Δλ= 5 at peak 340 nm and valley 382 nm.

Am+H mg/L	*Normal at 361*		*^{1}D at P=340 nm*		*^{1}D at V= 382nm*	
	mg/L Am. found	*Relative error%*	*mg/L Am. found**	*Relative error%*	*mg/L Am.found**	*Relative error%*
2.00+0.00	1.987	-0.65	2.031	+1.55	1.992	-0.40
5.00+0.00	5.054	+1.08	5.023	+0.46	5.029	+0.58
10.00+0.00	9.887	-1.13	10.12	+1.2	10.118	+1.18
20.00+0.00	20.211	+1.05	19.89	-0.55	19.911	-0.44
2.00+20.00*	2.033	+1.65	2.027	+1.35	1.992	-0.40
3.00+20.00*	3.027	+0.90	3.032	+1.07	3.026	+0.86
4.00+20.00*	4.036	+0.90	4.045	+1.12	4.061	+1.52
5.00+20.00	5.127	+2.54	5.023	+0.46	5.026	+0.52
6.00+2000	6.104	+1.73	6.021	+0.35	6.096	+1.60
10.00+20.00	9.972	-0.03	10.127	+1.27	10.234	+2.34
20.00+20.00	20.124	+0.62	19.924	-0.38	20.439	+2.19
30.00+20.00	30.297	+0.99	29.720	-0.93	30.3	+1.00
40.00+20.00	39.764	-0.59	39.047	-2.38	39.60	-1.00
10.00+2.00	10.037	+0.37	10.126	+1.26	10.132	+1.32
10.00+3.00	10.119	+1.19	9.996	-0.04	10.134	1.34
10.00+400	10.138	+1.38	9.992	-0.08	9.834	-1.66
10.00+10.00	9.986	-0.14	9.97	-0.30	9.834	-1.66
10.00+20.00	9.998	-0.02	10.00	0.00	10.034	+0.34
10.00+30.00	10.115	+1.15	10.137	1.37	10.101	+1.01
10.00+40.00	10.076	+0.76	10.137	1.37	10.063	+0.63

**** These Solutions Contain ~ the same ratio of drugs and RSD% not exceed 0.782 and 0.522 for normal and ^{1}D respectively.***

First derivative ^{1}D spectrum used to determine Hydrochlorothiazide in the presence of Amiloride hydrochloride in the mixtures as shown in Fig.(5) by using zero crossing method[1] at valleys λ 239 and λ 285 nm. The calibration curve was constructed for standard Hydrochlorothiazide solutions (2-40 mg/L Hydrochlorothiazide) gave a linear equation with slope and the correlation coefficient was listed in Table (4).

And the relative errors and standard deviation for each concentration represents an average of at least three measurements is between 0.587-1.272. The results are listed in Table (5) that the zero crossing method at valleys 239 and 285.nm used for determination Hydrochlorothiazide in presence of Amiloride hydrochloride with good results.

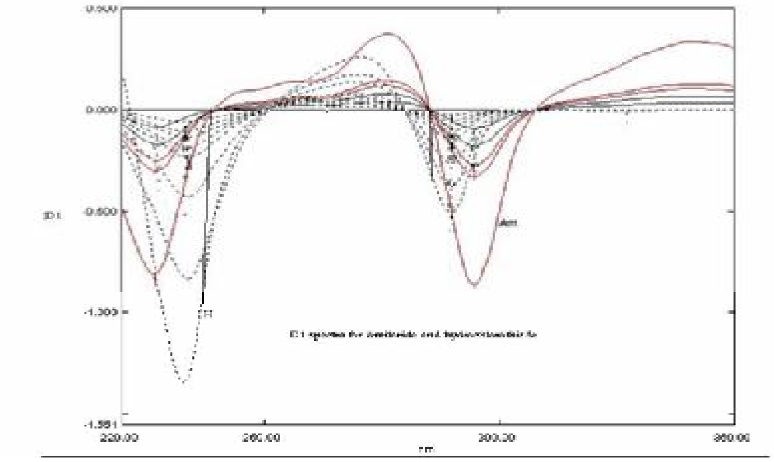


Fig. (5): ^{1}D amiloride hydrochloride and Hydrochlorothiazide zero crossing for determining Hydrochlorothiozide at valley 285 nm and 239 nm.

Table (4)
First derivative of Hydrochlorothiazide and the linear equations with correlation coefficient of calibration curves for first derivative zero crossing wavelengths for Hydrochlothiozide.

Range of conc. mg/L	*Wavelength nm*	*equation*	*r*
6 - 40	285.0	Y=-0.00659x -0.00058	0.9996
2 - 40	239.0	Y=-0.01443x -0.01120	0.9995

Specificity

To find an effect of matrix constituents on the results of determination, comparative analysis was carried out for standard solution containing active components at concentrations (2 Am+20H) mg/L comparable to those of the analyzed drug contain the same concentration ,They show the same normal spectra Fig.(5).

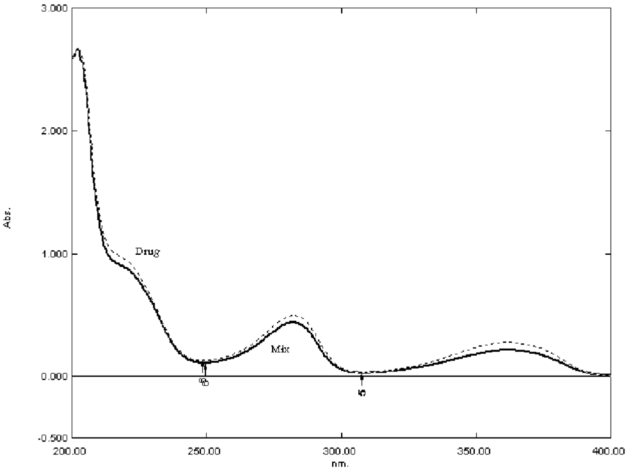


Fig.(6) :Normal spectra for analyzed preparation drug(……) and standard solution mixture(___).

Detection limit

The detection limit was established from analysis of solutions of decreasing concentrations of analyzed substances. It was found that the detection limits were 0.5 and 1.0 mg/L for Amiloride hydrochloride and hydrochlorothiazide respectively.

Analysis of Pharmaceutical Sample:

Accuracy of the proposed method was assisted by determining Amiloride's and Hydrochlorothiazide solutions using the proposed methods with the suitable conditions (scale factor=10 and $\Delta\lambda = 5$) and the data obtained for pharmaceutical samples were listed in Table (6) and (7) respectively for Maduratic and Saluretic tablets.

Table (5)

The relative error for determination hydrochlorothiazide solutions using first derivative with scale factor=10 and $\Delta\lambda = 5$ at valley 239 nm and valley 285 nm.

H+Am mg/L	P=239 nm		V= 285nm	
	mg/L H. found*	Relative error%	mg/L H. .found*	Relative error%
2.00+0.00	1.989	-0.55	2.011	+0.55
4.00+0.00	4.037	+0.925	3.963	-0.925
6.00+0.00	5.969	-0.517	5.979	-0.350
8.00+0.00	8.086	+1.075	8.111	+1.261
10.00+0.00	10.026	+0.26	10.141	+1.41
20.00+0.00	20.111	+0.555	20.215	+1.075
30.00+0.00	30.312	+1.04	29.822	-0.593
40.00+0.00	39.749	-0.627	40.307	+0.767
20.00+2.00	20.122	+0.61	20.312	+1.560
20.00+4.00	20.211	+1.055	20.201	+1.005
20.00+6.00	20..232	+1.160	20..298	+1.490
10.00+10.00	9.974	-0.260	10.178	+1.780
20.00+10.00	19.956	-0.220	19.898	-0.510
30.00+10.00	29.779	-0.737	30.367	+1.223
40.00+10.00	39.421	-1.44	40.231	+0.578

**The standard deviation for at least three measurements is between (0.587-1.272).*

Table (6)

Sample analyses of Maduratic, Co-Amilozid and Saluretic tablets pharmaceutical Amiloride using normal and derivative spectrophotomtry.

Pharmaceutical sample	Method	Conc.m mg/L	Conc.mg/L Average. Found*	Confidence limits for the mean mg/Lt 95%	Repeatability RSD%	RC%
Maduratic tablets	Normal at λ 361 nm	4.000	3.888	3.888±0.1556	1.614	97.200
	^{1}D spectra at v λ 382 nm	4.000	3.911	3.911±0.0948	0.977	97.775
	^{1}D spectra at p λ 340 nm	4.000	3.897	3.897±0.0809	0.837	97.425
Co-Amilozid	Normal at λ 361 nm	4.000	4.114	4.114±0.0578	0.567	102.850
	^{1}D spectra at v λ 382 nm	4.000	3.992	3.992±0.0578	0.685	99.800
	^{1}D spectra at p λ 340 nm	4.000	3.999	3.999±0.0351	0.354	99.975
Saluretic tablets	Normal at λ 361 nm	4.000	3.891	3.891±0.0767	0.795	97.275
	^{1}D spectra at v λ 382 nm	4.000	3.883	3.883±0.0695	0.721	97.075
	^{1}D spectra at p λ 340 nm	4.000	3.914	3.914±0.0605	0.623	97.850

Average of three determinations

Table (7)

Sample analyses of Maduratic, Co-Amilozid, Saluretic and Esidre tablets pharmaceutical Hydrochlorothiazide using derivative spectrophotomtry.

Pharmaceutical sample	*Method*	*Conc.m mg/L*	*Conc.mg/L Average. Found**	*Confidence limits for the mean mg/L t 95%*	*Repeata bilityRS D%**	*RC%*
Maduratic tablets	**^{1}D spectra at v λ 239 nm**	20.00	19.266	19.266±0.1123	0.235	96.330
	^{1}D spectra at p λ 285 nm	20.00	19.305	19.305±0.275	0.574	96.525
Co-Amilozid	**^{1}D spectra at v λ 239 nm**	20.00	20.272	20.272±0.3244	0.645	101.360
	^{1}D spectra at p λ 285 nm	20.00	20.354	20.354±0.3294	0.652	101.770
Saluretic tablets	**^{1}D spectra at v λ 239 nm**	20.00	19.925	19.925±0.2616	0.529	99.625
	^{1}D spectra at p λ 285 nm	20.00	19.765	19.765±0.4002	0.816	98.825
Esidrex	**^{1}D spectra at v λ 239 nm**	20.00	20.51	20.511±0.3915	0.769	102.555
	^{1}D spectra at p λ 285 nm	20.00	20.412	20.412±0.11842	0.234	102.060

****Average of three measurement.***

Conclusions

A fast and accurate method for determining Amiloride hydrochloride and hydrochlorothiazide was developed by using derivative spectrophotometry. The advantage of this method is that both constituents can be determined directly in a single sample without the need to be separated. It was also found that auxiliary drug components had no effect on the results of determination obtained under the established conditions.

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الخلاصة

استخدمت الطريقة المتعاقبة لتقديركل من الأميلورايد هيدروكلورايد والهيدركلوروثايزايد في المستحضرات الصيدلانية المنفردة و المزدوجة بالطرق الطيفية العادية أو المشتقة. قدرالأميلورايد هيدروكلورايد بالطريقة العادية عن طريق قياس الامتصاص عند طول موجي 361 نم.أما المشتقة الأولى فعند الطول الموجي 340 و 382 نم. أما الهيدركلوروثايزايد فتم تقديره باستعمال المشتقة الأولى عند التقاطع الصفري عند الطول الموجي285 و 239 نم على التوالي بدون تداخل بين جميع مكونات الأدوية. النتائج الأحصائية تساند دقة وصحة الطرق المستعملة .حيث بلغت النسبة المسترجاع بالمئة اثبتت بين 97.79% - 101.5% و ان الخطية 2 – 40 ملغم/لتر لكل من الأميلورايد هيدروكلورايد والهيدركلوروثايزايد .وكان حد الكشف 0.5 و 1.0 ملغم/لتر لكل من الأميلورايد هيدروكلورايد والهيدركلوروثايزايد على التوالي.