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SPECTRODENSITOMETRIC DETERMINATION OF CERTAIN DIUTRETIC MIXTURES

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ABSTRACT

A simple and direct spectrodensitometric method has been developed for the qualitative and quantitative determination of three pairs of diuretic mixtures: i- spironolactone with hydrochlorothiazide, ii- captopril with hydrochlorothiazide and iii- spironolactone with furosemide p-Chloroacetanilide was used as internal standard in quantitative determination. The method was applied for the determination of these drugs in different pharmaceuticel dosage forms; Aldactazide, Capozide tablets and Fructone capsules. The results indicated high accuracy and reproducability.

INTRODUCTION

Hydrochlorothiazide, spironolactone, furosemide and captopril have been recommended as diuretic as well as hypotensive[1]. Several different techniques and reagents are described for their determination, UV and derivative spectrophotometry were successfully used for the determination of these compounds as two-component mixtures or single ingredient[2-6]. A quantitative NMR method was described for the analysis of furosemide[7]; while, hydrochlorothiazide and related compounds were determined using the polarographic technique[8]. Infrared spectroscopy also was used for the determination of furosemide[10]. Gas chromatography was used for the determination of captopril[11]. In addition, HPLC technique was used for the determination of these compounds[12].

In th meantime, direct quantitation of hydrochlorothiazide on the plates densitometry[13] and fluorometry[14-16] in pharmaceutical analysis was also reported.

In the present paper, spectrodensitometry has been recommended for the assay of two-component mixtures of the above mentioned drugs.

EXPERIMENTAL

Material:

Hydrochlorothiazide and Spironolactone were kindly provided by Kahira Co., Egypt. Captopril from Squibb Co., Egypt. Furosemide from Memphis Co., Egypt. p-Chloroacetanilde from Aldrich Chemical Company. Aldactazide tablets from Kahira Co., labelled to contain 25 mg Spironolactone and 25 mg Hydrochlorothiazide per tablet, Average weight of one tablet is 373 mg. Capozide tablets from Squibb Co., labelled to contain 50 mg Captopril and 25 mg Hydrochlorothiazide per tablet. Average weight of one tablet is 300 mg. Fructone capsules from Memphis Co., labelled to contain 20 mg. Furosemide and 50 mg Spironolactone per capsule. Average weight of one capsule is 142 mg.

Standard solutions:

Spironolactone: 25 mg. 100 ml^{-1} of chloroform.

Hydrochlorothiazide: 25 mg, 100 ml^{-1} of acetone.

Captopril: 25 mg. 100 ml^{-1} of ethanol.

Furosemide: 20 mg. 100 ml^{-1} of acetone.

p-Chloroacetanilide: 40 mg 100 ml^{-1} of ethanol.

Apparatus:

Shimadzu CS-9000, dual wavelength flying-spot scanner.
Hamilton Microliter syringe, CH-7402 Bonad 42-Switzerland.

Chromatographic condition:

HPTLC silica gel F-254, precoated plates 20 X 20 cm from Merck, Germany.

Mobile phase:

Mixture I: Toulene-ethyl acetate-methanol (7: 2.5: 0.5).
Mixture II: Chlorform-ethyl acetate-dioxan-methanol (8:8:1:1) .
Mixture III: Toulene-ethyl acetate-methanol (8:4:1).

The developed distance was about 6 cm, and the R_f values are listed in Table 1.

Spectrophotometric conditions:

The spots were measured at λ_{max} 238, 269,225,276 and 254 nm for spironolactone, hydrochlorothiazide, captopril, furosemide and p-chloroacetanilide, respectively in reflectance mode.

Procedure:

1- For pure components:

Different aliquots from each stock solution were transferred into 10 ml calibrated flask 1 ml of the internal standard was added to each flask. The flasks were made up to volume with the appropriate solvent to form the working solution which contains 0.025 mg ml^{-1} of spironolactone, hydrochlorothiozide and captopril, and 0.02 mg ml^{-1} of furosemide.

Exactly 2 µl aliquot of each component was spotted by micropipette, on HPTLC-F254 plates scored into 1 cm lanes. The plates were developed with a suitable developing system for about 6 cm from the base line. The spots were air dried and measured at the suitable wavelength. Calibration curve was made by plotting the area ratio versus concentration (µg per spot).

2- For Aldactazide and capozide tablets:

Twenty tablets were weighed and finely powdered. A weight of the powder equivalent to one tablet (373 mg for Aldactazide and 300 mg for capozide) was dissolved in 80 ml of chloroform: acetone (1:1), and acetone: ethanol (1:1) for Aldactozide and capozide, respectively. After 5 min., shaking, solutions were filtered in 100 ml volumetric flasks and completed to volume with the appropriate solvent. The procedure was completed as described above.

3- For Capsules:

The contents of 20 capsules were weighed, and the average weight of one capsule was determined. Aquantity equivalent to one capsule (142 mg) was dissolved in 80 ml of chlorform : acetone (1:1) and filtered if necessary and completed to 100 ml. The procedure was completed as mentioned before.

RESULTS AND DISCUSSION

The proposed method has been used for separating spironolactone, from hydrochlorothiazide; captopril from hydrochlorothiazide and spironolactone from furosemide. It was found that various common excipients and coloring matter e.g. in captopril tablets did not interferre in

the separation process as shown in Fig. 1,2,3. The R_f values for the compounds are shown in Table 1.

p-Chloroacetanilide was chosen as optimum internal standard with suitable R_f value.

A calibration graph (peak area ratio versus concentration) for each component was constructed. It was rectilinear at low concentration and curved at high concentration. It was valid from 0.05 to 0.25 $\mu g\ \mu l^{-1}$ for hydrochlorothiazide, from 0.05 to 0.5 $\mu g\ \mu l^{-1}$ for spironolactone and captopril, from 0.04 to 0.24 $\mu g\ \mu l^{-1}$ for furosemide.

The regression equation for each component was listed as follows:

For mixture I:

$Y = -0.125 + 4.88\ x$ r = 0.996 for Spironolactone.

$Y = 0.588 + 17.39\ x$ r = 0.993 for Hydrochlorothiazide.

For mixture II:

$Y = 0.0021 + 0.993\ x$ r = 0.992 for Captopril.

$Y = -0.103 + 4.38\ x$ r = 0.998 for Hydrochlorothiazide.

For mixture III:

$Y = 0.01 + 1.56\ x$ r = 0.994 for Spironolactone.

$Y = 0.83 + 18.99\ x$ r = 0.993 for Furosemide.

Where: x is the concentration in micrograms.

r is the correlation coefficient.

Y is the peak area ratio.

The suggested method has been assessed by determination of authentic mixtures in ratios as those claimed in the pharmaceutical preparation, then applied to the determination of Aldactozide and

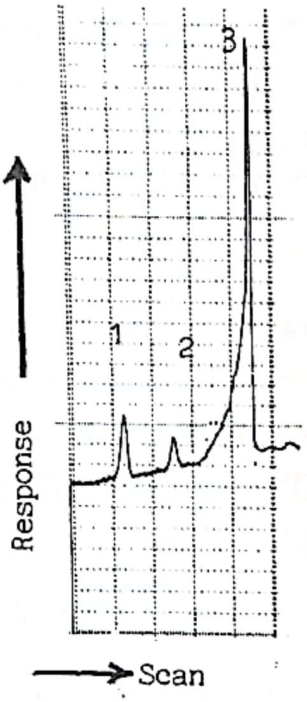

Fig. 1- Spectrodensitometer Chromatogram of 1: hydrochlorothiazide; 2: p-Chloroacetanilide; 3: spironolactone measured at 238 nm.

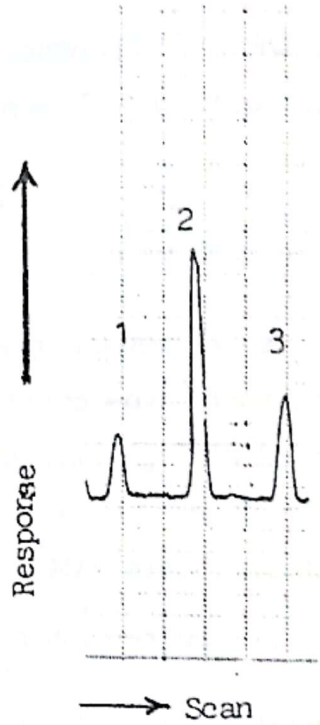

Fig. 2- Spectrodensitometer Chromatogram of 1: hydrochlorothiazide; 2: captopril; 3: p-Chloroacetanilide; measured at 225 nm.

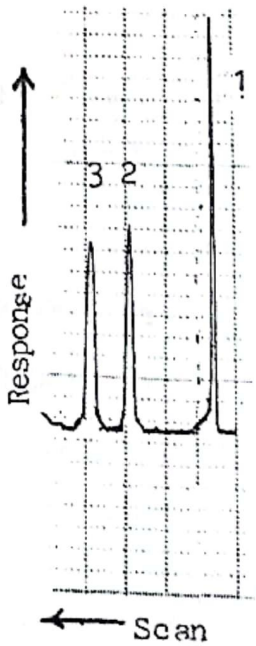

Fig. 3- Spectrodensitometer Chromatogram of 1: furosemide; 2: p-Chloroacetanilide; 3: spironolactone measured at 276 nm.

Capozide tablets and fructone capsules, the results are shown in Tables 2,3 and 4.

From the above study, it can be concluded that the proposed method is highly sensitive as the UV detector used at variable wavelengths. Therefore each substance can be determined at its λ_{max} and this allows determination of very low concentrations for each substance; 0.05 µg from hydrochlorothiazide, captopril and spironolactone and 0.04 µg from furosemide.

Statistical analysis of the results shows that the proposed method is accurate (as indicated from the relative error and percentage recovery) and precise (as shown from the relative standard deviation) (Tables 2-4). Furthermore, the method is rapid and less expensive than HPLC.

Table (1): R_f values of the three studied diuretic mixtures.

Mixture I		Mixture II		Mixture III	
Compound	**R_f***	**Compound**	**R_f***	**Compound**	**R_f***
Spironolactone	0.57	Captopril	0.66	Spironolactone	0.81
Hydrochlorothiazide	0.18	Hydrochlorothiazide	0.33	Furosemide	0.24
p-Chloroacetanilide	0.40	p-Chloroacetanilide	0.83	p-Chloroacetanilide	0.63

* Measured using a fluorescence quenching technique except in case of Captopril which was measured by using iodine.

Table (2): Analysis of Aldactazide tablets using the proposed HPTLC method.

Spironolactone		Hydrochlorothiazide	
Conc. claimed µg µl^{-1}	% Recovery*	Conc. claimed µg µl^{-1}	% Recovery*
0.05	100.9	0.05	99.2
0.01	99.3	0.01	98.7
0.15	100.7	0.15	99.1
0.20	101.3	0.20	99.8
0.25	100.7	0.25	99.6
Mean	100.6		99.3
SD	0.76		0.43
RSD	0.75		0.44
E rel.	0.6		0.6

* Mean of four experiments.

Table (3):Analysis of Fructone capsule using the proposed HPTLC method.

Spironolactone		Hydrochlorothiazide	
Conc. claimed µg µl-1	% Recovery	Conc. claimed µg ml-1	% Recovery
0.08	99.1	0.1	102.4
0.12	98.5	0.2	103.1
0.16	101.3	0.3	101.3
0.20	99.4	0.4	99.5
0.24	99.3	0.5	100.6
Mean	99.52		101.4
SD	1.055		1.43
RSD	1.06		1.41
E rel.	0.48		1.40

Table (4): Determination of Capozide tablets using the proposed HPTLC method.

Hydrochlorothiazide		Captopril	
Conc. claimed $\mu g\ \mu l^{-1}$	% Recovery*	Conc. claimed $\mu g\ \mu l^{-1}$	% Recovery*
0.05	100.9	0.1	102.7
0.10	100.8	0.2	101.6
0.15	99.5	0.3	102.3
0.20	99.2	0.4	100.4
0.25	100.7	0.5	101.1
Mean	100.2		101.6
SD	0.80		0.93
RSD	0.80		0.90
E rel.	0.2		1.6

* Average of 4 experiments.

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تقدير مكونات بعض المخاليط المدرة للبول بقياس الكثافة الضوئية على كروماتوجرافيا الطبقة الرقيقة ذو التقنيه العالية

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تم استحداث طريقة جديدة للتقدير الكمى والنوعى لثلاثة من المخاليط المدره للبول ، المخلوط الأول يحتوى على سبايرونولاكتون مع هيدروكلوروثيازير ، والثانى يحتوي على الكابتوبريل مع الهيدروكلوروثيازيد أما الثالث فيتحتوى على السبايرونولاكتون مع الفيوروزيمايد .

حيث تم قياس الكثافة الضوئية على رقائق كروماتوجرافيا ذات التقنية العالية لكل مكون دون تداخل من المواد الملونة أو أى مكونات أخرى الموجوة فى المستحضرات الصيدلية التى تحتوى على هذه المخاليط مثل اقراص الكابوزايد والالداكتازيد وكبسولات الفركتون . وقد تم استخدام الباراكلورواسيتانيليد كمادة قياسية داخلية في هذه المخاليط . وقد وجد أن هذه الطريقة المقترحة سريعة ومباشرة وذات حساسية عالية .