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High-Performance Thin-Layer Chromatography for the Determination of Certain Antihypertensive Mixtures

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Abstract

A new simple, precise, accurate and selective high-performance thin-layer chromatographic (HPTLC) method has been developed for the simultaneous determination of five mixtures: atenolol and chlorthalidone (Mix. I), enalapril maleate and hydrochlorothiazide (Mix. II), amiloride hydrochloride and hydrochlorothiazide (Mix. III), atenolol, chlorthalidone and amiloride hydrochloride (Mix. IV) and atenolol, hydrochlorothiazide and amiloride hydrochloride (Mix. V) in bulk powders and in pharmaceutical dosage forms. The methods consist of dissolving the drugs in methanol and spotting these solutions on a thin layer Merck HPTLC plates (0.25 mm thickness) pre-coated with 60 GF_{254} silica gel on aluminum sheet as the stationary phase, using dioxane:acetonitrile:1-propanol:hexane (30:18:23:1;v/v/v/v), ethylacetate:chlo-roform:methanol:acetic acid (11:8:7.5:1.5; v/v/v/v), ethylacetate:chloroform:1-propanol:25% ammonia solution (12:9:1:0.2; v/v/v/v), dioxan:acetonitrile:1-propanol:tetrahydrofuran (20:13 :4:15; v/v/v/v) and dioxane:ethylacetate: acetonitrile:1-propanol (10:7:5.5:3 v/v/v/v) as the mobile phases for mixtures I, II, III, IV and V, respectively. Detection was carried out densitometrically using UV detector at 283, 266, 257, 226 and 362 for atenolol, chlorthalidone, enalapril maleate, hydrochlorothiazide and amiloride hydrochloride, respectively. Calibration curves were linear in the range 1-100 μg ml^{-1} with correlation coefficients not less than 0.9996. The percentage recoveries ranged from 98.3±1.42 to 100.8±0.79.

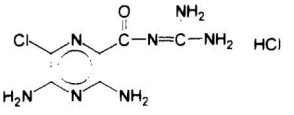
Keywords

atenolol; chlorthalidone; enalapril maleate; hydrochlorothiazide; amiloride hydrochloride; HPTLC.

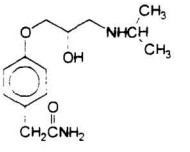
1. Introduction

Amiloride hydrochloride is a potassium-conserving drug that possess weak diuretic and hypertensive activity [1]. Recent methods have been reported for determination of

amiloride hydrochloride include; spectrophotometric [2-4] and chromatographic [5-8] techniques.

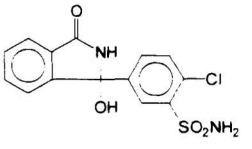


Amiloride hydrochloride



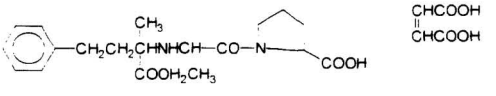
Atenolol

Atenolol is a beta-adrenergic receptor blocking agent, indicated in the management of hypertension [9]. Voltametric [10], spectrophotometric [11-13] and chromatographic [14-16] techniques have been used for the assay of atenolol.



Chlorthalidone

Chlorthalidone is a thiazide derivative. It has a very long duration of action, therefore it is often used to treat hypertension [17]. Fluorometric [18], and chromatographic [19-24] techniques have been employed for determination of chlorthalidone.

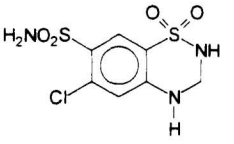


Enalapril maleate

Enalapril maleate is indicated for the treatment of hypertension. It is effective alone or in combination with other antihypertensive agents [25]. Several methods have been reported for determination of enalapril maleate. Recent methods include, spectrophotometric [26,27] and chromatographic [28-30] techniques.

Hydrochlorothiazide is one of well-known thiazide diuretics, it counteracts the sodium and water retention observed with other agents used in treatment of hypertension

[1]. Several methods have been reported for determination of hydrochlorothiazide include, spectrophotometric [31-33] and chromatographic [34-36] techniques.



Hydrochlorothiazide

Only two techniques were reported for the simultaneous determination of the above mixtures, the first one is a HPLC technique used for separation of atenolol from chlorthalidone [37, 38], enalapril maleate from hydrochlorothiazide [37, 39] and amiloride hydrochloride from hydrochlorothiazide [37]. The second one is a derivative spectrophotometric technique used for analysis for all the studied mixtures [40].

In the present paper, HPTLC has been recommended for the assay of mixtures of the above-mentioned drugs in laboratory prepared mixtures and in pharmaceutical dosage forms.

2. Experimental

2.1. Apparatus

I. Dual wavelength Shimadzu flying-spot CS-9000 densitometer with video display and high-speed, high-quality, parallel-head printer/ plotter.

II. Hamilton Microliter syringe: 25 μl, CH-7402 Bonad 42- Switzerland.

III. Pre-coated TLC plates, silica gel 60 GF_{254}, (20 x 20 cm), 0.25 mm thickness (Alugram).

IV. A Shimadzu UV1601, UV-Visible Spectrophotometer (Tokyo, japan)

2.2. Materials

Pharmaceutically pure chlorthalidone obtained from (Sigma Co., Egypt); atenolol was kindly supplied by (Memphis Co., Egypt); Enalapril maleate from (Egyptian Int. Pharm. Indu. Co., Egypt) and hydrochlorothiazide and amiloride hydrochloride obtained from (Kahira Pharm. and Chem. Ind. Co., Egypt).

2.3. Pharmaceutical preparations

The commercial dosage forms subjected to analysis were Tenedone 100® tablets, Batch No. 0038 (Sigma Co., Egypt) and Blokium-Diu® tablets, Batch No. 001875 (M.U.P. Co., Egypt). Both of them labeled to contain 100 mg atenolol and 25 mg chlorthalidone per tablet. Co-Rnitec® tablets, Batch No. 23540 (Merck) and Ezapril –Co® tablets Batch No. 0010349 (Multipharma Co., Egypt).Both of them labeled to contain 20 mg enalapril maleate and 12.5 mg hydrochlorothiazide per tablet. Moduretic® tablets, Batch No. 0011188 (Kahira Pharm. and Chem. Ind. Co., Egypt), Yostiretic® tablets, Batch No. 1850 (Amoun Co., Egypt). Both of them labeled to contain 50 mg hydrochlorothiazide and 5 mg amiloride hydrochloride. Teklo® tablets, Batch No. 00123049 (Acapi Co., Egypt), labeled to contain 100 mg atenolol, 25 mg chlorthalidone and 5 mg amiloride hydrochloride. Atenoretic® capsules, Batch No. 0412096 (SIGMA pharm. Ind. Co., Egypt), labeled to contain 50 mg atenolol, 25 mg hydrochlorothiazide and 2.5 mg amiloride hydrochloride.

2.4. Reagents

All solvents and mobile phases were of analytical reagent grade. Dioxane, acetonitrile, propanol, hexane and ethylacetate (Prolabo), ammonia solution 25% (Adwic), chloroform, methanol, acetic acid and tetrahydrofuran (Merck).

2.5. Mobile phases

I. Dioxane: acetonitrile: 1-propanol: hexane (30:18:23:1; v/v/v/v).

II. Ethylacetate: chloroform: methanol: acetic acid (11:8:7.5:1.5; v/v/v/v).

III. Ethylacetate: chloroform: 1-propanol: 25% ammonia solution (12:9:1:0.2; v/v/v/v).

IV. Dioxan: acetonitrile: 1-propanol: tetrahydrofuran (20:13:4:15; v/v/v/v).

V. Dioxane: ethylacetate: acetonitrile: 1-propanol (10:7:5.5:3 v/v/v/v)

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

Table 1

R_f values of the studied mixtures

Mixture I		Mixture II		Mixture III	
Compound	R_f	Compound	R_f	Compound	R_f
Atenolol	0.79	Enalapril maleate	0.25	Amiloride HCl	0.20
Chlorthalidone	0.31	Hydrochlorothiazide	0.58	Hydrochlorothiazide	0.60

Mixture IV		Mixture V	
Compound	R_f	Compound	R_f
Atenolol	0.86	Atenolol	0.86
Chlothalidone	0.30	Hydrochlorothiazide	0.59
Amiloride HCl	0.19	Amiloride HCl	0.11

2.6. Standards solutions

50 mg% methanolic stock solutions of chlorthalidone, atenolol, enalapril maleate, hydrochlorothiazide and amiloride hydrochloride were prepared.

3. Procedures

3.1. Construction of calibration curves

Aliquot portions from each stock solution were transferred into 10 ml calibrated flasks. The flasks were made up to volume with methanol to form working solutions, which contain 0.5 mg ml^{-1} of the cited drugs. 1-5, 5-25, 20-100, 4-20 and 10-50 µg for amiloride hydrochloride, chlorthalidone, atenolol, enalapril maleate and hydrochlorothiazide, respectively, are accurately transferred to thin layer chromatographic plates (20 x 20 cm) using 25 µl Hamilton syringe. Spots are spaced 2 cm apart from each other and 2 cm from the bottom edge of the plate. The plates are developed in chromatographic tank previously

saturated at least two hours with the developing mobile phases (I, II, III, IV and V), by ascending chromatography through a distance of 6 cm at room temperature. The spots were air dried and measured at suitable wavelengths 283, 266, 257, 226 and 362 for atenolol, chlorthalidone, enalapril maleate, hydrochlorothiazide and amiloride hydrochloride, respectively. The drugs under reflection photo mode and zigzag scan mode conditions are scanned.

The areas under the peaks are recorded. The calibration curves are constructed by plotting area under the peak versus the corresponding concentrations.

3.2. Assay of laboratory prepared mixtures

Stock methanolic laboratory prepared mixtures of 100 mg% atenolol and 25 mg% chlorthalidone (Mix. I), 20 mg% enalapril maleate and 12.5 mg% hydrochlorothiazide (Mix. II), 5 mg% amiloride hydrochloride and 50 mg% hydrochlorothiazide (Mix. III), 100 mg% atenolol, 25 mg% chlorthalidone and 5 mg% amiloride hydrochloride (Mix. IV) and 100 mg% atenolol, 50 mg% hydrochlorothiazide and 5 mg% amiloride hydrochloride (Mix. V) were prepared.

Different aliquots of each stock solution were transferred into 10 ml calibrated flasks. The flasks were made up to volume with methanol to form the working solutions of the laboratory prepared mixtures which contain 1.0 and 0.25 mg of atenolol and chlorthalidone, respectively (Mix. I), 0.20 and 0.12 mg of enalapril maleate and hydrochlorothiazide, respectively (Mix. II), 0.05 and 0.50 mg of amiloride hydrochloride and hydrochlorothiazide, respectively (Mix. III), 1.0, 0.25 and 0.05 mg atenolol, chlorthalidone and amiloride hydrochloride, respectively (Mix. IV) and 1.0, 0.50 and 0.05 mg atenolol, hydrochlorothiazide and amiloride hydrochloride, respectively. Apply 2-10 µl of the prepared mixtures to a silica gel plates and proceed as detailed under 3.1. construction of calibration curves, starting from " spots are spaced .....".

Record the area under the peaks and calculate the concentration of drugs from the regression equation or referring to calibration curves.

3.3. Application to pharmaceutical preparations

3.3.1. Assay of tablets and capsules

The contents of twenty tablets and capsules of the drugs were thoroughly ground. A quantity equivalent to the labeled active constituents was accurately weighed into a 100 ml

volumetric flasks, completed to volume with the appropriate solvent and the procedure was completed as under 3.1. and 3.2.

4. Results and discussion

In the present paper, spectrodensitmetry was examined for the assay of mixtures of the above-mentioned drugs.

The proposed method was used for separating atenolol from chlorthalidone (Mix. I), enalapril maleate from hydrochlorothiazide (Mix. II), amiloride hydrochloride from hydrochlorothiazide (Mix. III), atenolol from chlorthalidone and from amiloride hydrochloride (Mix. IV) and atenolol from hydrochlorothiazide and from amiloride hydrochloride (Mix. V). The method depends on the difference of the R_f values of the analyzed drugs (Table 1). Various common excipients and coloring matter did not interfere in the separation process.

4.1. Linear regression equations

A calibration graph (peak area ratio versus concentration) for each component was constructed. It was linear at low concentrations and curved at high concentrations. It was valid from 1-5, 5-25, 20-100, 4-20 and 10-50 µg for amiloride hydrochloride, chlorthalidone, atenolol, enalapril maleate and hydrochlorothiazide, respectively, from which the linear regression equations were computed and found to be:

For mixture I:

$Y = 0.548 + 8.52\ X$ ($r = 0.9999$) for atenolol

$Y = 0.321 + 12.09\ X$ ($r = 0.9998$) for chlorthalidone

For mixture II:

$Y = 0.142 + 3.99\ X$ ($r = 0.9996$) for enalapril maleate

$Y = 0.853 + 9.59\ X$ ($r = 0.9998$) for hydrochlorothiazide

For mixture III:

$Y = 0.609 + 4.30\ X$ ($r = 0.9999$) for amiloride hydrochloride

$Y = 0.902 + 10.11\ X$ ($r = 0.9998$) for hydrochlorothiazide

For mixture IV:

$Y = 0.598 + 7.98\ X$ ($r = 0.9998$) for atenolol

$Y = 0.289 + 11.51\ X$ ($r = 0.9999$) for chlorthalidone

$Y = 0.598 + 5.12\ X$ ($r = 0.9997$) for amiloride hydrochloride

For mixture V:

$Y = 0.575 + 6.99\ X$ ($r = 0.9996$) for atenolol

$Y = 0.799 + 10.21\ X$ ($r = 0.9997$) for hydrochlorothiazide

$Y = 0.600 + 3.98\ X$ ($r = 0.9999$) for amiloride hydrochloride

Where X is the concentration of related drug (μg/spot), r is the correlation coefficient and Y is the area under the peak.

4.2. Statistical analysis

Statistical analysis of the results obtained by the proposed methods are compared with results of the reported and official methods [37 and 40] (Tables 2-6) at a 95% confidence level, the calculated t and F values do not exceed the tabulated ones, revealing equal precision and accuracy.

Table 2

HPTLC analysis of atenolol and chlorthalidone (Mix. I), in a laboratory prepared mixture and in pharmaceutical dosage forms.

		Atenolol		Chlorthalidone	
		HPTLC	Compared[37]	HPTLC	Compared[37]
Laboratory prepared	$\bar{X}$	100.8	100.9	100.2	100.6
Mixture	S.D.	0.79	0.85	0.88	0.92
	V	0.62	0.72	0.77	0.85
	t	0.07		0.45	
	F	1.16		1.10	
Blokium Diu® tablets	$\bar{X}$	99.0	98.6	99.3	99.6
	S.D.	1.50	1.61	1.00	1.10
	V	2.25	2.59	1.00	1.21
	t	0.37		0.34	
	F	1.15		1.21	
Tenedone® tablets	$\bar{X}$	99.0	98.9	98.3	98.2
	S.D	1.25	1.50	1.42	1.30
	V	1.56	2.25	2.01	1.69
	t	0.10		0.03	

	F	1.44	1 78

Three and six determinations were used for the reported and the HPTLC methods. respectively. The tabulated values of t and F at 95% confidence limit are t=2.23 and F=5.79.

Table 3

HPTLC analysis of enalapril maleate and hydrochlorothiazide (Mix. II), in a laboratory prepared mixture and in pharmaceutical dosage forms.

		Enalapril maleate		Hydrochlorothiazide	
		HPTLC	Compared[40]	HPTLC	Compared[40]
Laboratory prepared	X̄	100.00	99.95	99.99	100.3
Mixture	S.D	1.98	1.95	1.49	1.34
	V	3.92	3.80	2.22	1.80
	t	0.03		0.29	
	F	1.03		1.23	
Co-Renitec® tablets	X̄	99.89	100.1	100.02	100.3
	S.D.	1.10	1.14	0.89	0.92
	V	1.21	1.30	0.79	0.85
	t	0.25		0.41	
	F	1.07		1.07	
Ezapril-Co® tablets	X̄	100.01	99.98	99.54	99.40
	S.D	1.90	1.85	1.11	1.08
	V	3.61	3.42	1.23	1.17
	t	0.08		0.07	
	F	1.06		1.05	

Three and six determinations were used for the reported and the HPTLC methods. respectively. The tabulated values of t and F at 95% confidence limit are t=2.23 and F=5.79.

Table 4

HPTLC analysis of amiloride hydrochloride and hydrochlorothiazide (Mix. III), in a laboratory prepared mixture and in pharmaceutical dosage forms.

	Amiloride hydrochloride		Hydrochlorothiazide	
	HPTLC	Compared[40]	HPTLC	Compared[40]

Laboratory prepared	X̄	99.89	100.00	99.82	99.32
Mixture	S.D	0.99	1.04	1.81	1.74
	V	0.98	2.10	3.28	3.02
	t	0.07		0.37	
	F	2.14		1.08	
Moduretic® tablets	X̄	100.0	199.6	99.00	99.2
	S.D	1.00	1.15	0.99	0.95
	V	1.00	1.32	0.98	0.90
	t	0.51		0.27	
	F	1.32		1.09	
Yostiretic® tablets	X̄	100.55	100.0	100.35	100.60
	S.D	1.00	0.98	1.10	1.04
	V	1.00	0.96	1.21	1.08
	t	0.07		0.31	
	F	1.04		1.08	

Three and six determinations were used for the reported and the HPTLC methods, respectively. The tabulated values of t and F at 95% confidence limit are t=2.23 and F=5.79

Table 5

HPTLC analysis of atenolol, chlorthalidone and amiloride hydrochloride (Mix. IV), in a laboratory prepared mixture and in Teklo® tablets.

		Atenolol	Chlorthalidone	Amiloride hydrochloride	
Laboratory prepared	X̄	99.97	100.80	100.20	
mixture	S.D	1.10	1.02	1.50	
	V	1.21	1.04	1.10	
	Teklo® tablets		X̄ 98.59	99.05	99.25
	S.D	1.00	1.02	1.11	
	V	1.00	1.04	1.23	

Table 6

HPTLC analysis of atenolol, hyddrochlorothiazide and amiloride hydrochloride (Mix. V), in a laboratory prepared mixture and in Atenoretic® capsules.

		Atenolol	Hydrochlorothiazide	Amiloride hydrochloride
Laboratory prepared	X̄	99.89	100.35	100.60

mixture	S.D.	1.10	1.10	1.04
	V	1.21	1.21	1.08
Atenoretic® capsules	X	99.85	98.99	99.31
	S.D.	1.49	0.92	1.08
	V	2.22	0.85	1.17

4.3. Validity

The validity of the proposed method was also checked by applying the standard addition technique. (Tables 7-11).

Table 7

Application of the standard addition technique to the analysis of Blokium Diu® and Tenedone® tablets.

Tablets	Atenolol			Chlorthalidone		
	Claimed amount taken	Added µg/spot	Recovery %	Claimed amount taken	Added µg/spot	Recovery %
Blokium Diu®	2.0	1.0	100.1	0.5	2.0	100.3
tablets	µg/spot	1.5	99.85	µg/spot	2.5	98.92
		2.0	98.96		3.0	99.62
		2.5	100.0		3.5	101.2
		3.0	99.31		4.0	98.56
Tenedone®	4.0	3.0	98.36	1.0	3.0	99.96
tablets	µg/spot	4.0	99.66	µg/spot	4.0	99.20
		5.0	100.8		5.0	100.6
		6.0	98.79		6.0	99.00
		7.0	100.0		7.0	98.99

Table 8

Application of the standard addition technique to the analysis of Co-Renitec® and Ezapril-Co® tablets.

Tablets	Enalapril maleate	Hydrochlorothiazide

	Claimed amount taken	Added µg/spot	Recovery %	Claimed amount taken	Added µg/spot	Recovery %
Co-Renitec®	4.0	0.5	99.60	2.5	0.5	100.90
tablets	µg/spot	1.5	100.2	µg/spot	1.0	99.99
		2.5	100.0		1.5	99.80
		3.5	99.28		2.0	99.61
		4.5	99.53		2.5	98.50
Ezapril-Co®	4.0	0.5	97.99	2.5	1.5	100.3
tablets	µg/spot	1.0	99.66	µg/spot	2.0	99.58
	1.5	99.90		2.5	99.62	
		2.0	99.08		3.0	100.3
		2.5	99.44		3.5	99.95

Table 9

Application of the standard addition technique to the analysis of Moduretic® and Yostiretic® tablets.

Tablets	Amiloride hydrochloride			Hydrochlorothiazide		
	Claimed amount taken	Added µg/spot	Recovery %	Claimed amount taken	Added µg/spot	Recovery %
Moduretic®	0.5	2.0	99.14	5.0	1.0	99.00
tablets	µg/spot	3.0	99.83	µg/spot	1.5	99.53
		4.0	99.00		2.0	100.9
		5.0	99.35		2.5	100.3
		6.0	98.39		3.0	99.99
Yostiretic®	0.5	1.0	99.00	5.0	1.5	98.98
tablets	µg/spot	2.0	100.2	µg/spot	3.0	100.0
		3.0	101.0		4.5	100 4
		4.0	99.27		6.0	99 15
		5.0	98.82		7 5	99.99

Table 10

Application of the standard addition technique to the analysis of Teklo® tablets.

Tablets	Atenolol			Chlorthalidone			Amiloride hydrochloride		
Claimed amount taken	Added µg/spot	Recovery %	Claimed amount taken	Added µg/spot	Recovery %	Claimed amount taken	Added µg/spot	Recovery %
Teklo® tablets 5.0 µg/spot	2.0	100.0	2.5 µg/spot	0.5	99.87	0.5 µg/spot	0.1	99.89
	4.0	99.82		1.0	99.85		0.2	100.6
	6.0	99.23		1.5	99.92		0.3	99.36
	8.0	99.33		2.0	99.02		0.4	100.2
	10.0	100.1		2.5	100.0		0.5	98.59

Table 11

Application of the standard addition technique to the analysis of Atenoretic ® capsules.

Capsules	Atenolol			Hydrochlorothiazide			Amiloride hydrochloride		
Claimed amount taken	Added µg/spot	Recovery %	Claimed amount taken	Added µg/spot	Recovery %	Claimed amount taken	Added µg/spot	Recovery %
Atenoretic® capsules 5.0 µg/spot	2.0	99.98	2.5 µg/spot	0.5	98.97	0.5 µg/spot	0.1	100.0
	4.0	100.02		1.0	99 12		0.2	99.52
	6.0	99.95		1.5	99.33		0.3	99.55
	8.0	99.33		2.0	98.97		0.4	99.29
	10.0	99.50		2.5	100.3		0.5	99.00

5. Conclusion

From the above study, it is concluded that the proposed method is highly sensitive. Because a variable wavelength UV detector can be used, each substance can be determined of very low concentration of each substance. Furthermore, the method is rapid and less expensive than HPLC method.

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