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Determination of antihypertensive drugs by using ratio difference spectrophotometric method

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RESEARCH ARTICLE

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ABSTRACT

An accurate, sensitive and time saving spectrophotometric method has been developed and validated for the determination of two antihypertensive drug mixtures. Mixture 1 contains spironolactone (SPIR), furosemide (FUR) and anthranilic acid (ANTH) (impurity of furosemide) and mixture 2 contains triamterene (TRI), hydrochlorothiazide (HCZ) and chlorothiazide (CZ) (impurity of hydrochlorothiazide). In mixture 1, the determination of drugs depends on dividing the spectrum of ternary mixture by the spectrum of 10 µg/mL of standard furosemide and then spironolactone and anthranilic acid were determined using the difference in amplitude between 242.3 and 254.6 nm, and between 250.8 and 242.4 nm in the ratio spectrum, respectively. On the other hand, furosemide could be determined by dividing the spectrum of ternary mixture by the spectrum of 10 µg/mL of standard spironolactone and then it was determined using the difference in amplitude between 244.8 and 229.7 nm in the ratio spectrum. In mixture 2, the determination of drugs depends on dividing the spectrum of ternary mixture by spectrum of 10 µg/mL of standard triamterene and then hydrochlorothiazide and chlorothiazide were determined using the difference in amplitude between 268.9 and 232.8 nm, and between 292.9 and 250.7 nm in the ratio spectrum, respectively. On the other hand, triamterene could be determined by dividing spectrum of ternary mixture by spectrum of 10 µg/mL of standard hydrochlorothiazide and then triamterene was determined using the difference in amplitude between 230.1 and 244 nm in the ratio spectrum. The developed analytical methods were validated regarding good accuracy and precision according to The International Conference on Harmonisation guidelines, and they were applied to pharmaceutical preparations in addition to laboratory prepared mixtures successfully. Statistically the results were compared with those obtained by reported method and no significant difference was found.

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1. Introduction

Spironolactone is 7a-acetylthio-3-oxo-17a-pregn-4-ene-21,17-carbolactone and its molecular formula is $C_{24}H_{32}O_4S$. It inhibits the action of aldosterone by competing for intracellular receptors of aldosterone in the distal tubule cells [1]. Furosemide is 4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid and its molecular formula is $C_{12}H_{11}ClN_2O_5S$. Furosemide is an anthranilic acid derivative, which is act as a strong diuretic because it decreases the active reabsorption of chloride. This diuretic is commonly used for the treatment of hypertension [1]. Anthranilic acid, 2-aminobenzoic acid, its molecular formula is $C_7H_7NO_2$. It is considered as a substrate of enzyme anthranilate hydroxylase in benzoate degradation [2]. The chemical structure of spironolactone, furosemide and anthranilic acid are shown in Figure 1.

The literature review shows that FUR was determined in its bulk powder by liquid chromatography and spectroscopy [3,4]. The binary mixture of SPIR and FUR was determined by some spectrophotometric methods variable angle scanning fluorescence spectrophotometry and derivative ratio method [5,6] and determined by reversed phase liquid chromatography [1,7].

Triamterene is 6-phenyl-2,4,7-triaminopteridine and its molecular formula is $C_{12}H_{11}N_7$. It has a relatively inefficient effect for treatment of antihypertensive when used alone; so it is used in combination with a potent diuretic (e.g. anthranilic acid or thiazide derivative) to give a synergistic action [8]. Hydrochlorothiazide is 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide is a thiazide class diuretic. Its molecular formula is $C_7H_8ClN_3O_4S_2$. It increases the elimination of sodium, chloride and water from body [9].

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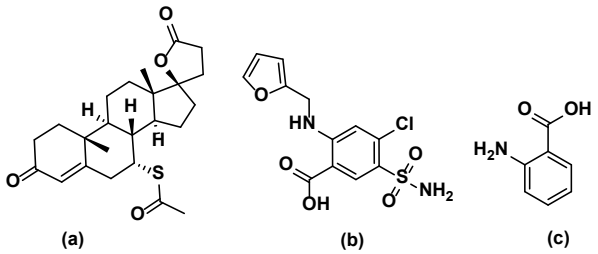

Figure 1. Chemical structure of spironolactone (**a**), furosemide (**b**) and anthranilic acid (**c**).

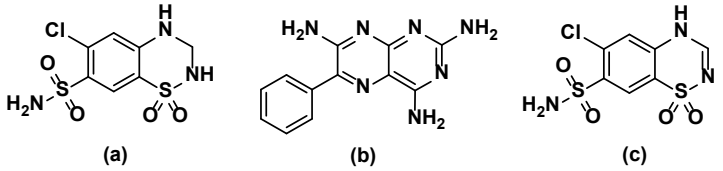

Figure 2. Chemical structure of hydrochlorothiazide (**a**), triamterene (**b**) and chlorothiazide (**c**).

Chlorothiazide, 6-chloro-2*H*-1,2,4-benzothia-diazine-7-sulphonamide-1,1-dioxide and its molecular formula is $C_7H_6ClN_3O_4S_2$ and it considered as specified impurities for HCZ which are synthetic impurity [9]. The chemical structure of triamterene, hydrochlorothiazide and chlorothiazide are shown in Figure 2.

The literature review shows that triamterene and hydrochlorothiazide were determined in their bulk powder by derivative ratio spectrophotometry [10] and liquid chromatography [8]. The aim of this work is to develop and validate a new analytical method for determination of the tertiary mixture triamterene and hydrochlorothiazide in presence of impurity of hydrochlorothiazide (chlorothiazide) as well as determination of spironolactone and furosemide in presence of impurity of furosemide (anthranilic acid) in bulk powder and pharmaceutical dosage form without interference or prior separation.

2. Experimental

2.1. Instrumentation

A double beam UV-visible spectrophotometer (Shimadzu, Japan) model UV-1601 PC with quartz cell of 1 cm and UV-PC personal software version 3.7 was used. The spectral band width is 2 nm and wavelength-scanning speed 2800 nm/min.

2.2. Materials

2.2.1. Pure samples

Standard of SPIR and FUR with purity of 99.8 and 99.7 %, respectively, according to manufacturer certificates of analysis were taken as grant from the Amoun Pharmaceutical Company (Cairo, Egypt), but the pure samples of TRI and HCZ with purity of 99.8 and 99.9%, were also taken as grant from Egyptian International Pharmaceutical Industries Co SAE (EIPICO) (Nasr city, Egypt). Anthranilic acid and chlorothiazide were purchased from (Sigma-Aldrich Chemie GmbH, Germany) with claimed purity of 98% according to the manufacturer certificates of analysis.

2.2.2. Pharmaceutical dosage form

Lasilactone® tablets (Batch No. 7EG007) were manufactured by Amoun Pharmaceutical Company (Cairo, Egypt). Each tablet is claimed to contain 100 mg of SPIR and 20 mg of FUR. Dyazide® tablets (Batch No. 622102E) were manufactured by Amoun Pharmaceutical Company (Cairo, Egypt). Each tablet is claimed to contain 37.5 mg of TRI and 25 mg of HCZ.

2.2.3. Solvents

Methanol, ethanol and acetonitrile of HPLC grade (Chromasolve®, Sigma-Aldrich Chemie GmbH, Germany) was used.

2.2.4. Solutions

Stock solutions of SPIR, FUR, ANTH, TRI, HCZ and CZ were prepared in methanol. They were prepared by dissolving 0.025 g of each drug in 25 mL of methanol in six separate calibrated flasks. Working solutions of the six drugs containing 100 µg/mL of each drug were prepared separately by diluting 2.5 mL of each stock solution to 25 mL by methanol.

2.3. Methods

2.3.1. Linearity

For determination of mixture 1:Accurate aliquots were transferred from working prepared solutions of SPIR, ANTH and FUR, and transferred to three separate series of 10 mL volumetric flasks and diluted with methanol to obtain concentrations of 10-50, 1-27 and 5-20 µg/mL, respectively. The calibration curves for SPIR, FUR and ANTH were constructed by plotting the ratio difference for each drug at its selected wavelengths against its corresponding concentrations and the regression equations were calculated. Zero order absorption spectra of the prepared samples were measured in the wavelength range of 200-400 nm as shown in Figure 3.

For determination of mixture 2: Accurate aliquots were taken from working solutions (100 µg/mL) of HCZ, CZ and TRI and transferred to three separate series of 10 mL volumetric flasks and diluted with methanol to obtain concentrations of 3-18, 3-15 and 3-30 µg/mL, respectively. The calibration curves for TRI, HCZ and CZ were constructed by plotting the ratio difference for each drug at its selected wavelengths against its corresponding concentrations and the regression equations were calculated.

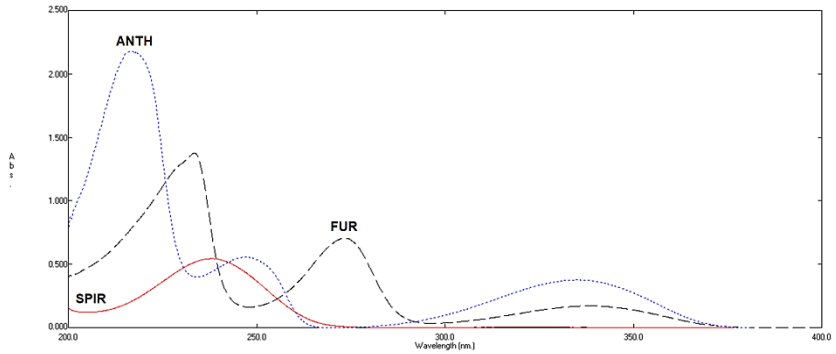

Figure 3. Zero order absorption spectra of 10 µg/mL of each of spironolactone (red), furosemide (black) and anthranilic (blue) using methanol as a blank.

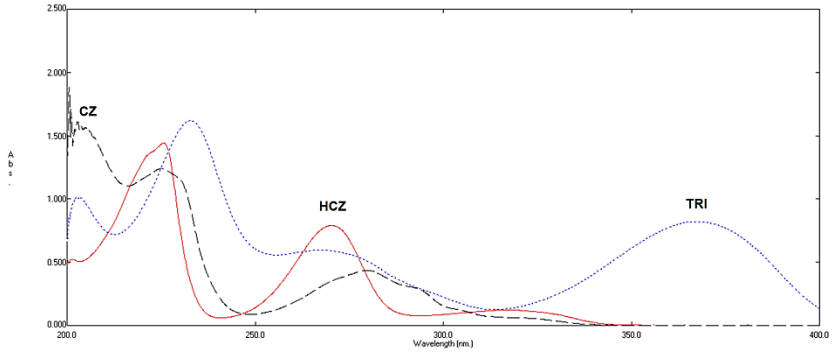

Figure 4. Zero order absorption spectra of 10 µg/mL of each of triamterene, hydrochlorothiazide and chlorothiazide using methanol as a blank.

Zero order absorption spectra of the prepared samples were measured in the wavelength range of 200-400 nm as shown in Figure 4.

2.3.2. Analysis of laboratory prepared mixtures

Mixtures containing different ratios of SPIR, FUR and ANTH and different ratios of TRI, HCZ and CZ were prepared in methanol. The procedure under linearity for each mixture then followed and the concentration of each component was calculated from its corresponding regression equation.

2.3.3. Application to pharmaceutical formulation

Ten tablets of Lasilactone® were weighed, powdered and mixed well; an accurate weight of the powdered tablets equivalent to 100 mg of SPIR and 20 mg of FUR was transferred into 100 mL volumetric flask. 75 mL of methanol was added and sonicated for 20 min, complete to the volume then filtered to obtain stock solution of 1 mg/mL of SPIR. The sample working solution of 100 µg/mL was prepared by diluting. The procedure under linearity for each drug was followed and concentrations of SPIR and FUR were calculated using the previously computed regression equation. Standard addition technique has been done by spiking the pharmaceutical formulation by known amount of standard drug powder. The recovery was calculated of the added standards after applying the proposed methods.

Ten tablets of Dyazide® were weighed, powdered and mixed well; an accurate weight of the powdered tablets equivalent to 37.5 mg of TRI and 25 mg of HCZ was transferred into 100 mL volumetric flask. 75 mL of methanol was added and sonicated for 20 min complete to the volume then filtered to obtain stock solution of 1 mg/mL of TRI. The sample working solution of 100 µg/mL was prepared by diluting. The procedure under linearity for each drug was followed and concentrations of TRI and HCZ were calculated using the previously computed regression equation. Standard addition technique has been done by spiking the pharmaceutical formulation by known amount of standard drug powder. The recovery was calculated of the added standards after applying the proposed methods.

3. Results and discussion

The aim of this work in this study is the development and validation of an accurate, selective and precise spectrophotometric method for resolving the overlapped spectra of SPIR, FUR and ANTH in mixture 1 and TRI, HCZ and CZ in mixture 2 with minimum sample preparation and data manipulation (Figures 3 and 4).

The principle of this analytical method is that the ratio difference at two wavelengths is directly proportional to the concentration of the drug of interest. Ratio difference technique has advantage to determine ternary and quaternary mixtures and also to eliminate derivative steps and so signal to noise ratio is enhanced.

3.1. Method development and optimization

There are many factors that affect selectivity and sensitivity of the developed methods such as: the solvent, the divisor concentration and the chosen wavelength. Different solvents were tried (Methanol, ethanol, acetonitrile, water, 0.1 N HCl and 0.1 N NaOH). Regarding method selectivity and sensitivity, methanol was the solvent of choice.

The divisor and its concentration greatly affect method selectivity, so divisors with different concentrations were tried. A divisor of 10 and 20 µg/mL for mixture 1 and 2, respectively, showed the best results.

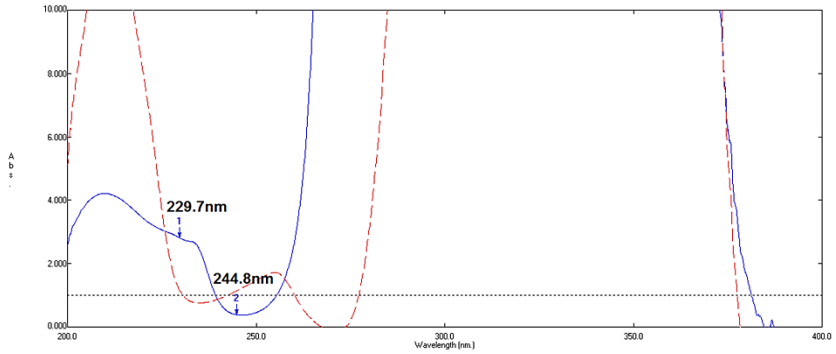

Figure 5. Division spectra of 10 µg/mL of each of spironolactone (black), furosemide (blue) and anthranilic (red) using standard spectrum of 10 µg/mL of spironolactone as a divisor and methanol as a blank.

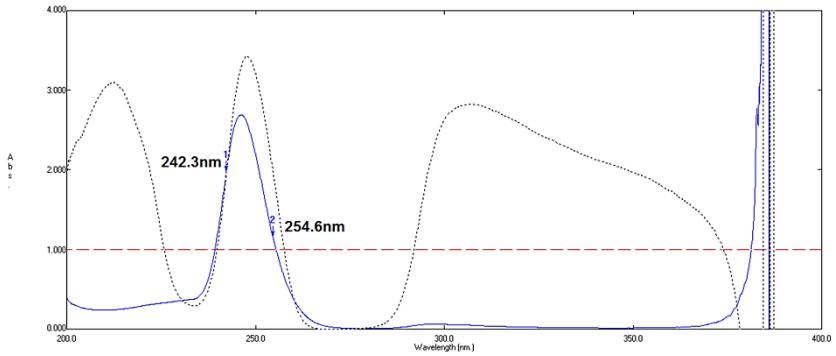

Figure 6. Division spectra of 10 µg/mL of each of spironolactone (blue), furosemide (red) and anthranilic (black) using standard spectrum of 10 µg/mL of furosemide as a divisor and methanol as a blank for the determination of spironolactone.

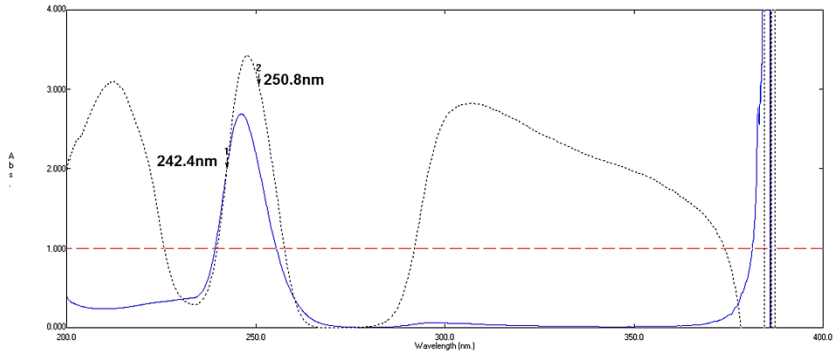

Figure 7. Division spectra of 10 µg/mL of each of spironolactone (blue), furosemide (red) and anthranilic (black) using standard spectrum of 10 µg/mL of furosemide as a divisor and methanol as a blank for the determination of anthranilic acid.

Calibration graphs were obtained by plotting the amplitude differences at the selected wavelengths versus the corresponding concentrations of the analyzed drugs from which regression equations were computed and found to be:

$Y = 0.2566 \times C - 0.1547$ $r^2 = 0.9999$ For FUR (1)

$Y = 0.0786 \times C + 0.0561$ $r^2 = 0.9999$ For SPIR (2)

$Y = 0.0982 \times C + 0.0160$ $r^2 = 0.9999$ For ANTH (3)

$Y = 0.0724 \times C + 0.0037$ $r^2 = 0.9999$ For HCZ (4)

$Y = 0.6305 \times C - 0.1264$ $r^2 = 0.9999$ For TRI (5)

$Y = 0.2393 \times C + 0.0509$ $r^2 = 0.9999$ For CZ (6)

where Y are the amplitude difference values at the selected wavelengths, C is the concentration in µg/mL and r^2 is the correlation coefficient.

The selection of wavelengths plays an important role during method development and optimization. Different wavelength pairs were tested where the wavelength pairs of 242.3 and 254.6, 244.8 and 229.7 and 242.4 and 250.8 nm were the pairs of choice for determination of SPIR, FUR and ANTH in mixture 1 and 230.1 and 244, 232.8 and 268.9, and 250.7 and 292.9 nm were the pairs of choice for determination of TRI, HCZ and CZ in mixture 2, respectively (Figures 5-10).

3.2. Method validation

Validation of the proposed spectrophotometric analytical method was done according to the International Conference of Harmonization (ICH) recommendations [11].

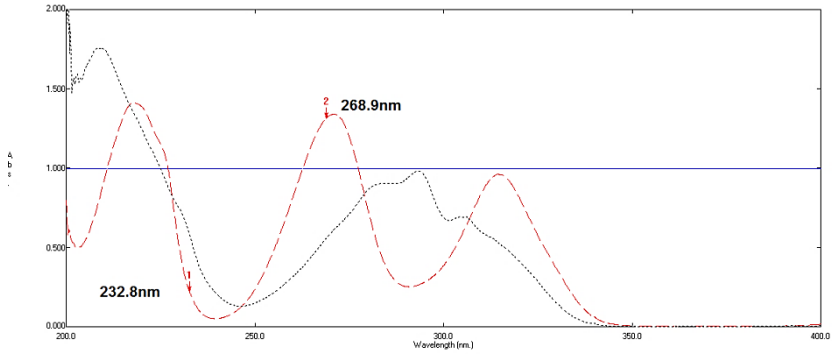

Figure 8. Division spectra of 10 µg/mL of each of hydrochlorothiazide (red), chlorothiazide (black) and triamterene (blue) using standard spectrum of 10 µg/mL of triamterene as a divisor and methanol as a blank for the determination of hydrochlorothiazide.

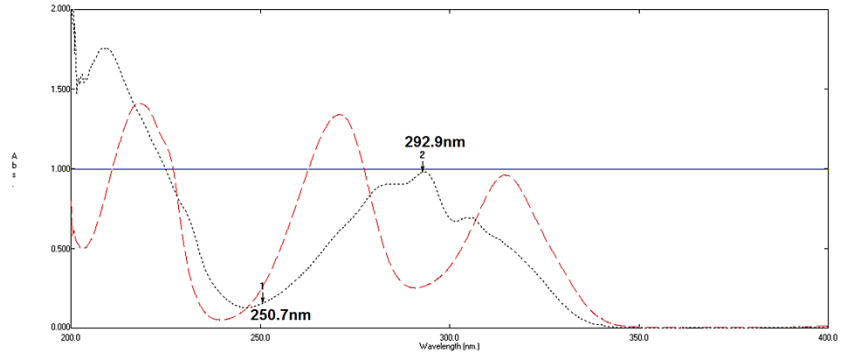

Figure 9. Division spectra of 10 µg/mL of each of hydrochlorothiazide (red), chlorothiazide (black) and triamterene (blue) using standard spectrum of 10 µg/mL of triamterene as a divisor and methanol as a blank for the determination of chlorothiazide.

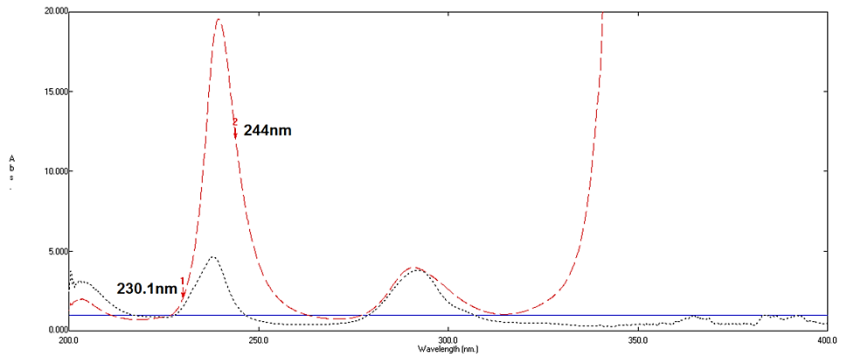

Figure 10. Division spectra of 10 µg/mL of each of hydrochlorothiazide (blue), chlorothiazide (black) and triamterene (red) using standard spectrum of 10 µg/mL of hydrochlorothiazide as a divisor and methanol as a blank.

3.2.1. Linearity and range

The linearity of the proposed spectrophotometric method was evaluated by analyzing different concentrations of FUR, SPIR, ANTH, TRI, HCZ and CZ. To developed method, Beer-Lambert's law was obeyed in the concentration range of 5-20, 10-50 and 1-27 µg/mL for FUR, SPIR and ANTH, respectively, in mixture 1 and 3-18, 3-30 and 3-15 µg/mL for HCZ, TRI and CZ, respectively, in mixture 2. The values of correlation coefficients were close to unity indicating good linearity. The regression parameters like the slope, intercept and the correlation coefficient were calculated and are presented in Table 1.

3.2.2. Accuracy

The accuracy of these analytical developed methods was computed as percentage recoveries of pure samples of the interested studied drugs. The concentrations of the drugs were computed from the corresponding regression equations, Table 1. Accuracy was further estimated by stratify the standard addition technique to Lasilactone® and Dyazide® tablets, where good recoveries were obtained revealing that there was no interference from excipients, Table 2.

3.2.3. Precision

Repeatability: Three concentrations of each drug of them were analyzed three times intra-daily using the proposed methods. Good results and acceptable standard deviations (SDs) were obtained, Table 1. The chosen concentrations were 5, 10, 13 µg/mL of FUR, 10, 13, 18 µg/mL of SPIR and 1, 5, 10 µg/mL of ANTH in mixture 1 and 3, 5, 9 µg/mL of

Table 1. Assay parameter and method validation for the determination of pure sample of SPIR, FUR, ANTH, TRI, HCZ and CZ by the proposed spectrophotometric methods.

Parameters	Mixture 1			Mixture 2		
	SPIR	FUR	ANTH	TRI	HCZ	CZ
Range (µg/mL)	10-50	5-20	1-27	3-30	3-18	3-15
Slope	0.079	0.257	0.098	0.630	0.072	0.239
Intercept	0.0561	-0.1547	0.0160	-0.1200	0.0037	0.0509
Correlation coefficient	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Accuracy Mean±SD	99.97±0.670	99.89±0.760	100.25±0.820	100.29±1.120	99.88±0.700	100.01±0.629
Precision repeatability [a]	1.01	1.13	1.01	1.44	0.916	0.732
Intermediate precision [b]	1.10	1.21	1.05	1.53	0.924	0.814

[a] The intraday (n = 3), average of three different concentrations repeated three times daily.
[b] The interday (n = 3), average of three different concentrations repeated three times in three successive days.

Table 2. Assay result for determination of SPIR, FUR, TRI and HCZ in pharmaceutical preparation using the proposed spectrophotometric methods *.

Pharmaceutical preparation										
Lasilactone®	FUR					SPIR				
	Taken (µg/mL)	Found (%)	Standard addition			Taken (µg/mL)	Found (%)	Standard addition		
	5	104.2	DF	5	98.3	35	99.9	DF	35	100.1
			PURE	5				PURE	5	
	7	98.9	DF	5	100.4	50	101.7	DF	35	99.4
			PURE	10				PURE	10	
	10	101.9	DF	5	99.0	35	100.5	DF	35	101.9
			PURE	10				PURE	15	
	Mean±SD	101.6±2.65	99.23±1.06			Mean±SD	100.7±0.91	100.46±1.28		
Dyazide®	TRI					HCZ				
	Taken (µg/mL)	Found (%)	Standard addition			Taken (µg/mL)	Found (%)	Standard addition		
	15	106.9	DF	5	98.6	10	98.9	DF	15	99.8
			PURE	8				PURE	3	
	30	107.0	DF	5	99.2	20	98.9	DF	15	101.6
			PURE	10				PURE	5	
	35	105.6	DF	5	101.5	15	100.6	DF	15	99.8
			PURE	15				PURE	7	
	Mean±SD	106.4±0.8	99.7±1.5			Mean±SD	99.4±0.9	100.4±1.03		

* DF: Dosage form concentration (µg/mL) and PURE: Pure drug concentration (µg/mL).

Table 3. Assay result for the determination of SPIR, FUR, ANTH, TRI, HCZ and CZ in synthetic mixtures using the proposed spectrophotometric method.

Drugs	Mixture 1					
	SPIR		FUR		ANTH	
Mix. no.	Taken (µg/mL)	Recovery (%)	Taken (µg/mL)	Recovery (%)	Taken (µg/mL)	Recovery (%)
1	25	101.01	5	98.88	2	100.3
2	30	99.69	5	98.01	1	101.8
3	35	99.8	7	99.2	4	102
4	50	101.5	7	99.4	2	99.2
5	37	102	10	101.5	1	99.7
6	40	99.4	10	101.1	4	99.2
Mean ±SD	100.66±1.25		100.02±1.40		100.30±1.25	
Drugs	**Mixture 2**					
	TRI		**HCZ**		**CZ**	
Mix. no.	**Taken (µg/mL)**	**Recovery (%)**	**Taken (µg/mL)**	**Recovery (%)**	**Taken (µg/mL)**	**Recovery (%)**
1	13	100.5	5	101.7	3	101.5
2	15	98.5	13	98	3	101.8
3	26	99.2	7	99.1	5	98.5
4	30	101.9	10	100.1	5	101.8
5	7	101.7	14	98.6	7	98.4
6	9	101.8	20	98.5	7	102
Mean ±SD	100.6±1.4		99.00±1.37		100.6±1.7	

TRI, 7, 9, 13 µg/mL of HCZ and 3, 5, 7 µg/mL of CZ in mixture 2.

Intermediate precision: The last steps were repeated inter-daily on three different days for the analysis of the chosen concentrations. Good results and acceptable SDs were obtained and presented in Table 1.

3.2.4. Determination of SPIR, FUR, TRI and HCZ in harmaceutical preparation using the proposed spectrophotometric method

This developed spectrophotometric method has the capability of determination of SPIR and FUR in their dosage form Lasilactone® tablets and determination of TRI and HCZ in their dosage form Dyazide® tablets. The validity of this spectrophotometric method was assessed by applying standard addition technique which also confirmed the accuracy of the proposed analytical spectrophotometric method (Table 2). Satisfactory results were obtained, Table 2.

3.2.5. Selectivity

The selectivity of this proposed spectrophotometric method was assessed by the analysis of different synthetic laboratory prepared mixtures containing different concentrations of SPIR, FUR, ANTH, TRI, HCZ and CZ in the range of their linearity, good results were obtained and presented in Table 3.

The results obtained by applying the proposed analytical spectrophotometric method was statistically compared with those obtained by the reported HPLC method, Table 4.

4. Conclusion

Spectrophotometric methods have been developed and validated for the determination of two antihypertensive mixtures. These analytical proposed methods are developed for determination of the spironolactone and furosemide in presence of the impurity of furosemide (anthranilic acid) and

Table 4. The statistical comparison of the results obtained by the analytical proposed methods and the established method.

Items	Lasilactone® tablet				Dyazide® tablet			
	Developed ratio difference method		Reported method [7]		Developed ratio difference method		Reported method [8]	
	SPIR	FUR	SPIR	FUR	TRI	HCZ	TRI	HCZ
Mean	99.97	99.89	101.40	99.11	100.29	99.88	99.73	100.52
SD	0.67	0.76	2.30	2.50	1.12	0.70	1.57	2.55
N	8	8	8	8	8	8	8	8
Variance	0.586	0.598	1.280	1.800	1.250	0.657	2.400	1.062
Student T test [a] (2.22)	1.60	0.60			0.29	0.18		
F value [b] (5.05)	0.100	0.080			0.191	0.266		

[a] Figures in parentheses represent the corresponding tabulated values of T at $p = 0.05$.
[b] Figures in parentheses represent the corresponding tabulated values of F at $p = 0.05$.

determination of triamterene and hydrochlorothiazide in presence of impurity of hydrochlorothiazide (chlorothiazide). The developed analytical methods were validated regarding good accuracy and precision according to The International Conference on Harmonisation guidelines. The developed methods have advantages of being simple, rapid, cost effective, less tedious and time saving as compared to chromatographic techniques. These methods can be easily and conveniently adopted for routine quality control analysis of the two mixtures.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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