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Mohammed E. Draz *
Analytical Chemistry Department, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt.
Email: mohamad_deraz9999@yahoo.com
Telephone: 00201143935352

QR Code for Mobile users

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Simultaneous Determination of Some Antihypertension Drugs in Their Binary Mixtures by Simple Spectrophotometric Methods

Eglal A. Abdelaleem[a], Ibrahim A. Naguib[a], Hala E. Zaazaa[b] and Mohammed E. Draz[c]*
[a] Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University,62111 Beni-Suef, Egypt.
[b] Analytical Chemistry Department, Faculty of Pharmacy, Cairo University ,Kasr El-Aini St., 11562 Cairo, Egypt.
[c] Analytical Chemistry Department, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt.

Abstract
Two simple, accurate and cost effective dual wavelength (Method I) and mean centering of ratio spectra MCR (Method II) spectrophotometric methods are developed and validated for determination of two drug combinations. The first comprises Hydrochlorothiazide (HCZ) and Benazepril Hydrochloride (BZ) and the second comprises HCZ and Candesartan Cilexetil (CAN) in their bulk powder and pharmaceutical tablets. In method (I), two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. For BZ/HCZ, mixture (I), BZ had equal absorbance values at 224.2 and 252.9 nm, therefore these two wavelengths were used to determine HCZ. Similarly, 243.8 and 293.8 nm were selected to determine BZ, where HCZ had equal absorbance values. For CAN/HCZ, mixture (II), HCZ is determined at 242.2 and 264.4 nm while CAN is determined at 257.8 and 280 nm. In method (II), absorption spectra of each drug were recorded, divided by suitable divisor and the obtained ratio spectra were mean centered. The concentrations of the proposed drugs were then determined from the calibration curves obtained by measuring amplitudes at 274 and 240.2 nm for HCZ and BZ respectively for mixture (I) and 274 and 240.4 nm for HCZ and CAN respectively for mixture (II). The developed methods were validated according to ICH guidelines demonstrating good accuracy and precision. The results were statistically compared with those obtained by reported methods indicting no significant difference and ability of methods to be used for routine analysis of proposed drugs.

Keywords: Hydrochlorothiazide, Benazepril Hydochloride, Candesartn Cilexetil, Dual wavelength, Mean centering of ratio spectra, spectrophotometry.

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1. INTRODUCTION

Hydrochlorothiazide (HCZ), 6-Chloro-3,4-dihydro-2H-l,2,4-benzothiadiazine-7-sulfonamide l,l-dioxide **(Fig. 1a)**,[1] is an antihypertensive diuretic agent used for management of hypertension. Benazepril hydrochloride (BZ) **(Fig. 1b)**, (3S)-1-(Carboxymethyl)-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]-amino]-2,3,4,5-tetrahydro-1H-[1]benzazepin-2-one, hydrochloride, is an angiotensin-converting enzyme (ACE) inhibitor used in the treatment of hypertension and heart failure[2]. Candesartan cilexetil (CAN), (±)-1-(cyclohexyloxycarbonyloxy) ethyl-2-ethoxy-1-[[2´-(1Htetrazol-5-yl) biphenyl-4-yl]methyl]benzimidazole-7-carboxylate **(Fig. 1c)**, is antihypertensive drug. It is used, either alone or in combination with other drugs, to treat high blood pressure [3]. Formulation of HCZ with either BZ or CAN increases the antihypertensive effect.

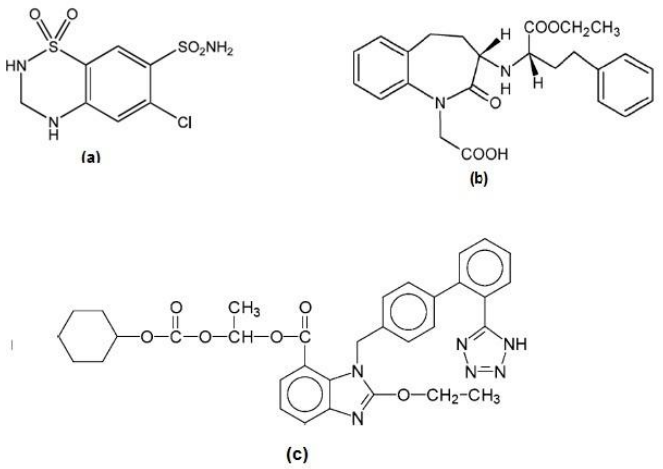

Figure 1: Chemical structures of (a) Hydrochlorothiazide, (b) Benazepril Hydrochloride, (c) Candesartan cilexetil.

The literature survey revealed different methods for simultaneous determination of HCZ and BZ as binary mixture such as spectrophotometric [4-6], reversed phase high performance liquid chromatography (RP-HPLC) [7-10], thin layer chromatographic (TLC)-densitometric [7,8] and chemometric [11,12] methods. HCZ and CAN were determined by spectrophotometric [13, 14], RP-HPLC [1518], TLC [16,19] and capillary electrophoresis [20] methods.

This work concerns with development and validation of two spectrophotometric method, dual wavelength and mean centering of ratio spectra spectrophotometric methods for determination of the suggested drugs. the suggested methods have the advantages of saving time and cost when compared to published chromatographic and spectrophotometric method and also provide a simple, rapid and sensitive way for simultaneous analysis of HCZ and BZ or CAN in their combined dosage form without derivatization steps.

2. Experimental

2.1. Instruments

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. All data analysis was performed using PLS-Toolbox 2.0 running under MATLAB®, version 6.5 [21].

2.2. Materials

2.2.1. Pure standards

Standard HCZ**,** BZ and CAN with claimed purity of 99.6 %, 99.7 % and 99.8 % respectively according to manufacturer certificate were kindly supplied by Sigma Pharmaceuticals Industries (El Monofeya, Egypt).

2.2.2. Pharmaceutical dosage forms

Cibadrex ® tablets batch No. (Y0006) were manufactured by Novartis Pharma S.A.E (Cairo, Egypt). Each tablet is claimed to contain 20 mg of BZ and 25 mg of HCZ.

Atacand Plus® tablets batch No. (130030D2) were manufactured by AstraZeneca, Egypt under license of AstraZeneca, Sweden. Each tablet is claimed to contain 16 mg of CAN and 12.5 mg of HCZ.

2.2.3. Solvents

Methanol HPLC grade (CHROMASOLVE®, Sigma - Aldrich Chemie GmbH, Germany).

2.2.4. Standard solutions

a. Standard stock solution of HCZ, BZ and CAN were prepared in methanol in the concentration of 1 mg/ml.

b. Standard working solutions of HCZ, BZ and CAN were prepared in methanol in the concentration of 0.1 mg/ml.

2.3. Procedures

2.3.1. Spectral characteristics and wavelength selection

The absorption spectra of 9 µg/ml for each of HCZ and BZ for mixture (I) and 7 µg/ ml for each of HCZ and CAN for mixture (II) were recorded over the range 200-350 nm using methanol as blank. The spectra were observed for selection of the suitable wavelengths for dual wavelength spectrophotometric method (Fig.2).

2.3.2. Construction of calibration curves

2.3.2.1. Dual wavelength method

Different aliquots equivalent to 10-280, 30-510 and 30-450 µg of HCZ, BZ and CAN were separately

transferred from their respective standard working solutions (0.1 mg/ml) into three separate series of 10-ml volumetric flasks and the volume completed using methanol to obtain final concentrations ranges of 1-28, 3-51 and 3-45 µg/ml for HCZ, BZ and CAN respectively. The prepared solutions were scanned in the range of 200 – 350 nm. For mixture (I), absorbance values at 224.2 and 252.9 nm (for HCZ) and at 243.8 and 293.8 nm (for BZ) were measured. HCZ was determined by plotting the difference in absorbance values at 224.2 and 252.9 nm (difference is zero for BZ) against its corresponding concentrations. Similarly for determination of BZ, the difference in absorbance values at 243.8 and 293.8 nm (difference is zero for HCZ) was plotted against the corresponding concentrations. For mixture (II), absorbance values at 242.2 and 264.4 nm (for HCZ) and at 257.8 and 280 nm (for CAN) were measured to determine HCZ and CAN as performed with mixture (I).

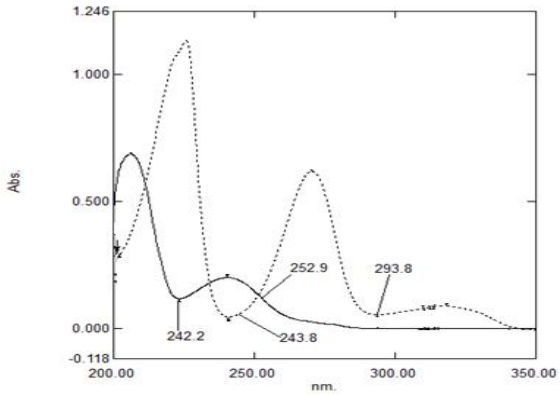

Fig 2.A. Zero order spectra of 9 µg/ml for each of HCZ (...) and BZ (___).

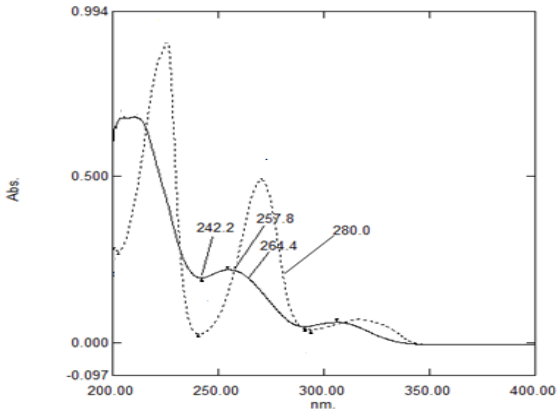

Fig 2.b. Zero order spectra of 7 µg/ml for each of HCZ (...) and CAN (___).

Figure (2). Spectra of HCZ, BZ and CAN using methanol as solvent.

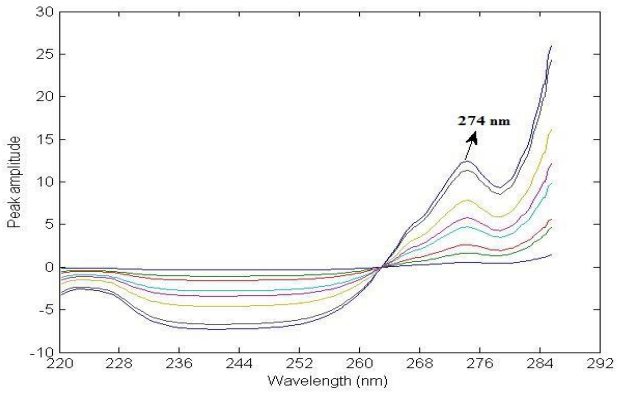

Figure 3: Mean centered ratio spectra of HCZ (1-24 µg/ml) using 42 µg/ml of BZ as a divisor and methanol as solvent.

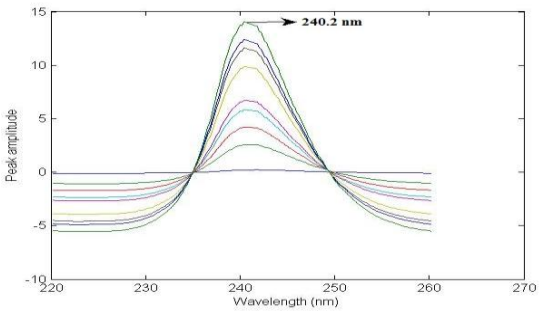

Figure 4: Mean centered ratio spectra of BZ (1-51 µg/ml) using 11 µg/ml of HCZ as a divisor and methanol as solvent.

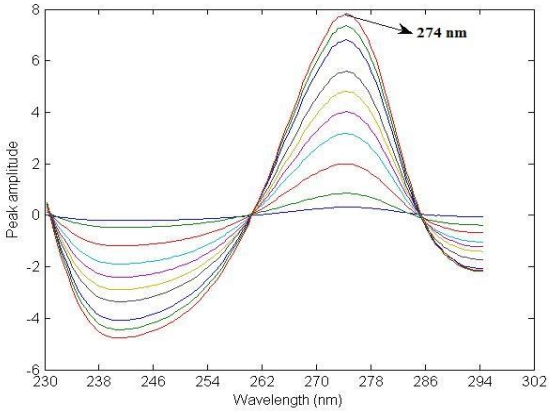

Figure 5: Mean centered ratio spectra of HCZ (1-28 µg/ml) using 6 µg/ml of CAN as a divisor and methanol as solvent.

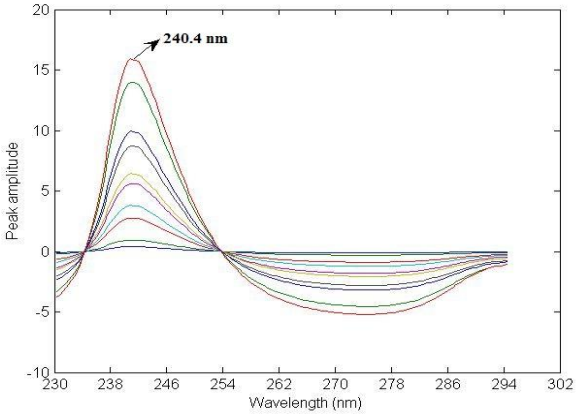

Figure 6: Mean centered ratio spectra of CAN (1-52 µg/ml) using 14 µg/ml of HCZ as a divisor and methanol as solvent.

2.3.2.2. Mean centering of ratio spectra (MCR) method **:** For mixture (I), aliquots of HCZ equivalent to 10-240 µg were accurately transferred from its standard working solution (0.1 mg/ml) into a set of 10 ml measuring flasks and the volume was adjusted using methanol to obtain final concentration range of 1-24 µg/ml. The absorption spectra of the prepared solutions were recorded in the range of 220-285.2 nm and divided by the standard spectrum of 42 µg/ml of BZ to obtain the ratio spectra which were then mean centered.

By the same way the spectra of different concentrations of BZ in the range of 1-51 µg/ml were recorded in the range of 220-260 nm, divided by the standard spectrum of 11µg/ml of HCZ and then the obtained ratio spectra were mean centered. For mixture (II), different concentrations of HCZ in the range of 1-28 µg/ml were recorded in the range of 230-294 nm then the stored spectra were divided by the standard 6 µg/ml spectrum of CAN and then mean centered. CAN can be determined in the range of 1-52 µg/ml by the same way using 14 µg/ml of HCZ as divisor. Calibration curves of the proposed drugs were constructed by plotting amplitude values of their respective mean centered ratio spectra at 274, 240.2 and 240.4 nm for HCZ, BZ and CAN respectively against their corresponding concentrations.

2.3.3. Analysis of laboratory prepared mixtures

Different laboratory preparations of the two combinations containing different ratios of (HCZ and BZ) and (HCZ and CAN) were mixed and the procedures under construction of calibration curves for each method were followed. Concentrations of HCZ, BZ and CAN in the prepared samples were calculated from the computed regression equations.

2.3.4. Analysis of pharmaceutical dosage forms

Twenty tablets of each Cibadrex® and Atacand Plus® tablets were powdered and mixed well. Accurately weighed amount of the powdered tablets equivalent to 100 mg of HCZ, BZ and CAN were separately transferred into 100 ml volumetric flasks. 50 ml methanol was added and ultrasonicated for 30 min, cooled and flasks were completed to volume to obtain 1 mg/ml stock solution and then the solution was filtered. Appropriate dilutions of the prepared solution were made to prepare its working solution (0.1 mg/ml) and the procedures under construction of calibration curves were followed.

2.3.4.1. Recovery studies

Recovery studies were carried were carried out by applying the standard addition technique. Known amount of pure studied drugs were separately added to a definite amount of the powdered tablets, the prepared samples were then analyzed as under construction of calibration curves and the percentage recoveries were then calculated.

3. Result and discussion

The two proposed combinations under investigation act as antihypertensive drugs and are used to treat severe diseases such as hypertension and heart failure. The main problem of univariate spectrophotometric multicomponent analysis is the simultaneous determination of two or more compounds in the same mixtures without preliminary separation. Several univariate spectrophotometric methods have been used for resolving mixtures of compounds with overlapping spectra. In this work; two simple spectrophotometric methods, namely dual wavelength and mean centering of ratio spectra methods have been described for analysis of HCZ with either BZ or CAN in their respective pharmaceutical dosage form which have the advantage of no need to any derivatization or sophisticated manipulation steps like other spectrophotometric methods also less cost than published chromatographic methods.

3.1. Dual wavelength method

The developed dual wavelength method provides simple method for selective determination of HCZ, BZ and CAN in their binary mixtures using their zero order absorption spectra. The principle of this method is that the absorbance difference at two wavelengths on the spectra is directly proportional to the concentration of component of interest, with no interference from other components[22]. The pre-requisite for dual wavelength method is the selection of two wavelengths where the interfering component shows the same absorbance value and the component of interest shows significant difference in absorbance with concentration.

Selection of suitable wavelengths plays an important role with respect to selectivity and sensitivity; hence different wavelengths were tried but the best results regarding selectivity and sensitivity were obtained by using the absorbance difference values at 224.2 and 252.9 nm for determination of HCZ where BZ has zero absorbance difference and using absorbance difference values at 243.8 and 293.8 nm for determination of BZ where HCZ has zero absorbance values, for mixture (I). Similarly absorbance difference values at 242.2 and 264.4 nm were recorded for determination of HCZ and at 257.8 and 280 nm for determination of CAN, for mixture (II).

Linear correlations were obtained between absorbance difference values at selected wavelengths for each drug and their corresponding concentrations in range of 1-22 and 3-51 µg/ml for HCZ and BZ respectively for mixture (I) and 1-28 and 3-45 µg/ml for HCZ and CAN for mixture(II). The regression equations for the proposed method were calculated and found to be:

Parameters	Dual wavelength method				Mean centering of ratio spectra (MCR) spectrophotometric method			
	Mixture (I)		Mixture (II)		Mixture (I)		Mixture (II)	
	HCZ	BZ	HCZ	CAN	HCZ	BZ	HCZ	CAN
Calibration range (µg/ml)	1-22	3-51	1-28	3-45	1-24	1-51	1-28	1-52
Slope	0.0990	0.0190	0.0490	0.0180	0.5160	0.2740	0.2800	0.3070
Intercept	0.0320	0.0170	0.0120	0.0050	0.0840	0.0490	0.0460	0.0620
Correlation coefficient	0.9997	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Accuracy (%) ± SD	100.96 ± 1.51	101.51 ± 0.94	101.26 ± 0.75	100.41 ± 1.35	100.33 ± 1.17	100.73 ± 0.85	100.57 ± 0.92	100.35 ± 1.29
Precision								
Repeatability (RSD %)[a]*	1.33	1.48	0.69	0.93	1.32	1.12	1.09	1.43
Intermediate precision (RSD %)[b]*	0.82	1.73	1.28	1.39	1.64	1.11	1.20	1.76
Specificity (%) ± SD	101.39 ± 0.45	100.34 ± 1.89	100.27 ± 1.45	99.52 ± 1.14	99.60 ± 0.40	102.04 ± 0.39	99.35 ± 1.19	99.03 ± 1.61
LOD**	0.31	0.86	0.29	0.26	0.31	0.26	0.33	0.28
LOQ**	0.93	2.61	0.87	2.79	0.94	0.81	0.94	0.89

Table 1. Regression and validation parameters of the proposed methods for determination of HCZ, BZ and CAN.

* a.The intraday precision (n=3), average of three different concentrations repeated three times within day.

b. The interday precision (n=3), average of three different concentrations repeated three times in three successive days.

** Limit of detection and quantitation are determined via calculations LOD = (SD of the response/slope) × 3.3; LOQ = (SD of the response/slope) × 10.

Dual wavelength method				
	Cibadrex® tablets B.N (Y006)		Atacand Plus® tablets B.N (130030D2)	
	HCZ	BZ	HCZ	CAN
Taken (µg/ml)	12.5	10	12.5	16
Found[a] % ± S.D	100.77 ±1.55	100.52 ±1.00	105.70 ±1.27	103.94 ±0.76
Added (µg/ml)	3	5	4	5
	5	15	5	8
	8	20	8	10
% recovery[b]	103.10	102.47	102.25	101.60
	100.89	100.33	101.80	100.00
	101.40	100.00	100.38	99.70
Mean ± S.D	101.80 ± 1.16	100.93 ± 1.34	101.48 ±0.98	100.43 ± 1.02
Mean centering ratio (MCR) spectrophotometric method				
	Cibadrex® tablets B.N (Y0006)		Atacand Plus® tablets B.N (130030D2)	
	HCZ	BZ	HCZ	CAN
Taken (µg/ml)	12.5	10	12.5	16
Found[a] % ± S.D	99.51 ±1.51	100.07 ±1.17	105.33 ±1.15	103.65 ±0.92
Added (µg/ml)	3	5	4	5
	5	15	5	8
	8	20	8	10
% recovery[b]	101.84	99.38	102.05	102.56
	98.80	102.20	102.00	98.66
	102.30	101.69	99.48	101.36
Mean ± S.D	100.98 ±1.90	100.09 ±1.50	101.18 ±1.47	100.86 ±1.99

Table. 2. Quantitative determination of HCZ and BZ in Cibadrex® tablets and HCZ and CAN in Atacand Plus® tablets by the proposed Dual wavelength and MCR spectrophotometric methods and application of standard addition technique.

[a]: average of six experiments.

[b]: average of three experiments.

Parameters		Dual wavelength method.		MCR spectrophotometric method.		Reported HPLC method [7].	
		HCZ	BZ	HCZ	BZ	HCZ	BZ
Cibadrex® tablets B.N (Y0006)	Mean %	100.77	100.52	99.51	100.07	100.83	99.59
	SD	1.55	1.00	1.51	1.17	1.63	0.59
	n	6	6	6	6	6	6
	Student *t*-test	0.059 (2.228)*	1.960 (2.228)*	1.448 (2.228)*	0.901 (2.228)*	-----	-----
	F- value	1.007 (5.050)*	2.950 (5.050)*	1.174 (5.050)*	3.934 (5.050)*	-----	-----
		Dual wavelength method		MCR spectrophotometric method.		Reported HPLC method [17].	
		HCZ	CAN	HCZ	CAN	HCZ	CAN
Atacand Plus® tablets B.N (130030D2)	Mean %	105.70	103.94	105.33	103.65	105.06	103.65
	SD	1.27	0.76	1.15	0.92	1.27	1.36
	n	6	6	6	6	6	
	Student *t*-test	0.876 (2.228)*	0.457 (2.228)*	0.386 (2.228)*	0.011 (2.228)*	-----	-----
	F- value	1.014 (5.050)*	3.207 (5.050)*	1.220 (5.050)*	2.163 (5.050)*	-----	-----

Table 3: Statistical analysis of the proposed Dual wavelength and MCR spectrophotometric methods and reported methods for determination of HCZ, BZ and CAN in their pharmaceutical formulations.
*Figures in parentheses represent the corresponding tabulated of *t* and *F* at P = 0.05.

Cibadrex® tablets B.N (Y0006)				
Method	n	HCZ Mean ± RSD %	n	BZ Mean ± RSD %
Dual wavelength	6	100.77 ± 1.54	6	100.52 ± 0.99
MCR	6	99.51 ± 1.52	6	100.07 ± 1.17
Reported HPLC [7]	6	100.83 ± 1.62	6	99.59 ± 0.59
F- value	1.3516 (3.6823)*		1.445 (3.6823)*	
P- value	0.2886		0.2667	
Atacand Plus® tablets B.N(130030D2)				
Method	n	HCZ Mean ± RSD %	n	CAN Mean ± RSD %
Dual wavelength	6	105.70 ± 1.20	6	103.94 ±0.73
MCR	6	105.33 ± 1.09	6	103.65 ± 0.88
Reported HPLC [17]	6	105.06 ± 1.21	6	103.65 ± 1.31
F- value	0.4115 (3.6823)*		0.1506 (3.6823)*	
P- value	0.6700		0.8615	

Table 4. Statistical analysis of the results obtained by applying the two proposed dual wavelength and MCR methods and the reported one on Cibadrex® and Atacand Plus® tablets using one way ANOVA.
*Figures in parentheses represent the corresponding tabulated of F at P < 0.05.

For the first mixture:
Y = 0.0990X+0.0320 r = 0.9997 for HCZ.
Y= 0.0190X + 0.0170 r = 0.9998 for BZ.
For the second mixture:
Y = 0.0490X+0.0120 r = 0.9999 for HCZ.
Y = 0.0180X+0.0050 r = 0.9999 for CAN.
Where Y is an absorbance difference value at selected wavelengths, X is a corresponding concentration and r is a correlation coefficient.

3.2. Mean centering of ratio spectra (MCR) spectrophotometric method.

The developed MCR method is based on the mean centering of ratio spectra; the mathematical explanation of the developed method was illustrated by *Afkhami and Bahram* [23]. This method was applied for resolving binary and ternary mixtures in the complex samples with unknown matrices [23]. In order to optimize the developed MCR method, different parameters were tested. Since the wavelength range taken has great effect on the obtained mean centering ratio spectra, different wavelength ranges were tested and the best results

were obtained when using the wavelength range from (220-285.2 nm) and (220-260 nm) for HCZ and BZ respectively to determine mixture (I) and using (230-294 nm) for both HCZ and CAN to determine mixture (II). Also the effect of divisor concentration on the selectivity of the method was checked by testing several concentrations of each HCZ, BZ and CAN. For mixture (I), the best results regarding sensitivity and selectivity were obtained by using 11 and 42 µg/ml each of HCZ and BZ respectively as divisors. For mixture (II), reproducible and good results were obtained upon using 14 and 6 µg/ml for determination of HCZ and CAN respectively as divisors.

Beer's Lambert law was obeyed in the range of 1-24 µg/ml at 274 nm and 1-51 µg/ml at 240.2nm for HCZ and BZ respectively to determine mixture (I) and in the range of 1-28 µg/ml at 274 nm and 1-52 µg/ml at 240.4 nm for HCZ and CAN respectively to determine mixture (II), Fig (3-6). The regression equations for the proposed method were calculated and found to be:

For the first mixture:

$Y = 0.5160X+0.0840$ $r = 0.9999$ for HCZ at 274 nm.

$Y= 0.2740X + 0.0490$ $r = 0.9999$ for BZ at 240.2 nm.

For the second mixture:

$Y = 0.2800X+0.0460$ $r = 0.9999$ for HCZ at 274 nm.

$Y = 0.3070X+0.0620$ $r = 0.9999$ for CAN at 240.4 nm.

Where Y is peak amplitudes value at selected wavelengths, X is a corresponding concentration and r is a correlation coefficient.

The selectivity of the proposed methods was evaluated by analysis of different laboratory prepared mixtures containing different ratios of the suggested drugs, where satisfactory results were obtained, (Table 1).

The developed spectrophotometric methods were also applied for determination of HCZ and BZ in Cibadrex® tablets and HCZ and CAN in Atacand Plus® tablets without interferences from tablets excipients and satisfactory results were obtained. The validity of the methods was further assessed by applying standard addition technique which also confirmed the accuracy of the proposed methods (Table 2). The results obtained by applying the proposed methods were statistically compared with those obtained by applying the reported HPLC method [7] for determination of Cibadrex® tablets and with obtained by applying reported HPLC method [17] for determination of Atacand Plus® tablets and no significance differences were obtained between them (Table 3). Furthermore, statistical analysis of the results obtained by the proposed methods and reported methods were carried out using one way ANOVA at ($P < 0.05$). Calculated F-value was found to be less than tabulated F-value (Table 4). The test ascertains that the proposed methods are as precise and accurate as the reported HPLC methods [7,17] and are comparable to one another.

3.3. Method validation

Validation of the methods was carried out according to ICH recommendation [24].

3.3.1. Linearity and range

The calibration range for HCZ, BZ and CAN was established through considerations of the practical range necessary according to adherence to Beer-lambert's law to give accurate, precise and linear results. Linearity ranges of HCZ, BZ and CAN are shown in (Table 1).

3.3.2. Accuracy

Accuracy of the proposed methods was calculated as the percentage recoveries of blind pure samples of the studied drugs. The concentrations were calculated from the corresponding regression equations and the results are shown in (Table1). Accuracy was further assessed by applying the standard addition technique to Cibadrex® and Atacand Plus® tablets, where good recoveries were obtained revealing no interference from excipients and good accuracy (Table 2).

3.3.3. Precision

3.3.3.1. Repeatability. Three concentrations (7, 12, 19 µg/ml of HCZ) and (20, 30, 40 µg/ml of BZ) for mixture (I) and (7, 12, 19 µg/ml) of both HCZ and CAN to determine mixture (II) were analyzed three times intra-daily using the proposed methods. Good results and acceptable relative standard deviations (RSDs) were obtained, (Table 1).

3.3.3.2. Intermediate precision. The previous procedures were repeated inter-daily on three different days for the analysis of the chosen concentrations. Good results and acceptable RSDs were obtained, (Table 1).

3.3.4. Selectivity

Selectivity of the proposed methods was assessed by the analysis of different synthetic laboratory prepared mixtures containing different ratios of (HCZ and BZ) and (HCZ and CAN) within their linearity ranges. Satisfactory results are shown in (Table 1).

3.3.5. LOD and LOQ

ICH recommendations [24] were followed to calculate the LOD and LOQ values of HCZ, BZ and CAN. Low LOD and LOQ values indicate the high sensitivity of the proposed methods (Table 1).

4. Conclusion

The developed methods have advantages over the published methods in being more simple, rapid, cost effective and data processing steps are not time consuming. Spectrophotometric methods can be regarded as a useful alternative to chromatographic techniques in the routine quality control analysis of pharmaceutical formulations allowing rapid

determination at relatively low cost. The developed dual wavelength and mean centering of ratio spectra (MCR) spectrophotometric methods were successfully applied for simultaneous determination of (HCZ and BZ) and (HCZ and CAN) as binary mixtures in their combined marketed dosage forms. On the developed dual wavelength method, once the equations were constructed, analysis requires only measuring the absorbance value at the selected wavelengths followed by few simple calculations. On the other hand the MCR method does not need any derivatization steps or complex algorithms, so the developed methods can be easily and conveniently adopted for routine quality control analysis of HCZ, BZ and CAN.

5. References

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