Development and Validation of Different Spectrophotometric and High-Performance Thin-Layer Chromatographic Methods for the Determination of Fosinopril Sodium, Hydrochlorothiazide, and Chlorothiazide as Hydrochlorothiazide Impurity

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Key Words:

Fosinopril sodium
Hydrochlorothiazide
Chlorothiazide
Ratio difference
Mean-centered ratio spectra
Thin-layer chromatography–densitometry

Summary

Three simple, sensitive, and validated methods were developed for the quantitative determination of fosinopril sodium (FOS) and hydrochlorothiazide (HCZ) in the presence of an HCZ impurity, chlorothiazide (CZ). The first method (I) was the ratio difference spectrophotometric method (RD), in which a standard spectrum of 8 µg mL^{-1} HCZ was used as a divisor, and the difference in amplitude values at 204.6 and 231.2 nm and 290 and 302.6 nm was used for the determination of FOS and CZ, respectively. Meanwhile, for the determination of HCZ, a standard spectrum of 6 µg mL^{-1} CZ was the chosen divisor, and the amplitude difference at 275 nm and 293.6 nm was selected for the calculation of its concentrations. The second method (II) was mean centering of ratio spectra spectrophotometric method (MCR), which depended on the implementation of the mean-centered ratio spectra in two successive steps and the measurement of the amplitudes of the mean-centered second ratio spectra at 243.4 nm for CZ and peak-to-peak amplitudes at 215.6 and 215.8 nm for FOS and at 223.8 and 224 nm for HCZ. On the other hand, the third method (III) was thin-layer chromatography (TLC)–densitometry at which the chromatographic separation of this ternary mixture was performed using pre-activated silica gel 60 F_{254} TLC plates and a developing system mixture consisting of ethyl acetate–chloroform–methanol–formic acid (60:40:5:0.5, by volume) with ultraviolet (UV) scanning at 215 nm. The developed methods were validated according to the International Conference of Harmonization (ICH) guidelines and were successfully used for the determination of FOS and HCZ in their pharmaceutical formulations. Also, a statistical comparison between the developed methods and the reported HPLC method was attained. Using Student's *t*-test and *F*-test, the results confirmed that there was not any significant difference between them regarding accuracy and precision.

1 Introduction

Fosinopril sodium (FOS) is chemically identified as sodium (2S,4S)-4-cyclohexyl-1-(2-{[2-methyl-1-(propionyloxy) propoxy](4-phenylbutyl)phosphoryl}acetyl)pyrrolidine-2-carboxylate [1] **(Figure 1).** It is the only angiotensin-converting enzyme (ACE) inhibitor which contains phosphate group, which makes it a safer choice than other ACE inhibitors for the treatment of hypertension and some types of chronic heart failure, especially for patients with impaired kidney function as it is eliminated from the body by both renal and hepatic pathways [2].

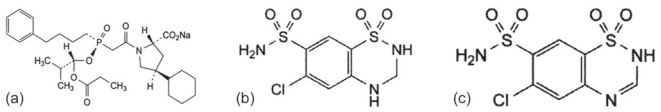

Figure 1

The chemical structures of fosinopril sodium (a), hydrochlorothiazide (b), and chlorothiazide (c).

Hydrochlorothiazide (HCZ) is chemically identified as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [1] (Figure 1). It is a thiazide diuretic drug, which acts by inhibiting the reabsorption of water in the nephron. Chlorothiazide (CZ) is chemically identified as 6-chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [1] (Figure 1). It is reported to be the common process impurity of HCZ [1]. Also, CZ was found to be pharmacologically less active than HCZ and was reported to be incompletely and variably absorbed comparing to the parent drug, HCZ.

The combination of ACE inhibitor (FOS) and thiazide diuretic (HCZ) has been noticed to have advantages over monotherapy for the treatment of hypertension [3]. It was reported that ACE inhibitors inhibit the counterregulatory rise in the angiotensin II level produced as a result of stimulation of the renin–angiotensin system caused by thiazide diuretics [4]. Additionally, in combined medication, the patient needs lower dose of each drug than monotherapy, leading to reduce the risk of dose-related

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side effects [3]. On the other hand, the presence of impurities such as CZ in the pharmaceutical preparations may influence the efficacy and safety of the product.

After reviewing the literature extensively, some methods have been found for the determination of FOS and HCZ in their binary mixture. They were determined by different spectrophotometric methods including multiwavelength UV spectrophotometry [5], fourth derivative ultraviolet (UV) spectrophotometry [6], derivative differential spectrophotometry [7], ratio spectra derivative spectrophotometry [7], absorbance ratio spectrophotometry [7], and high-performance liquid chromatography (HPLC) [6, 8, 9] methods. On the other hand, they were determined along with other drugs by capillary electrophoresis [10], HPLC [11–13], and ultra-performance liquid chromatography (UPLC) [13].

From the previous literature review, it was clear that there is no reported method for the determination of FOS, HCZ, and CZ in their ternary mixture. Thus, in this work, three rapid, sensitive, efficient, and validated spectrophotometric and thin-layer chromatography (TLC)–densitometric methods were developed for the first time, for the determination of FOS, HCZ, and CZ. The developed methods have advantages of being time- and cost-effective as well as highly sensitive and specific.

2 Experimental

2.1 Instruments

For spectrophotometric methods, a double beam UV–visible spectrophotometer (Shimadzu, Kyoto, Japan), model UV-1601 PC, was used. The light path length was 1 cm, and a quartz cell was used. This spectrophotometer was connected to an IBM-compatible computer. It was turned on by using UVPC personal spectroscopy software, version 3.7, and MATLAB®, version 6.5 [14], was used for the proposed mean centering of ratio spectra spectrophotometric (MCR) method.

For TLC–densitometric method, a CAMAG (Muttenz, Switzerland) TLC Scanner 3 S/N 130319 run by winCATS software (CAMAG) was used. Absorbance mode was used as the scanning mode, and the scanning speed was adjusted at 20 mm s^{-1}. A sample applicator Linomat IV with a 100-µL syringe (CAMAG) was used. In addition, deuterium lamp was used as the radiation source. The band width was 6 mm, while the slit dimensions were 3 mm × 0.45 mm. The output appeared as a chromatogram with integrated peak area. High-performance thin-layer chromatographic (HPTLC) aluminum plates precoated with 0.25 mm silica gel 60 F_{254} (Merck, Darmstadt, Germany) with diameters of 10 cm × 20 cm were used. A Sonix IV SS-Series ultrasonicator (Newtown, CT, USA) was used for dissolving and mixing the prepared solutions. A digital balance with 4 digits was used for preparing the accurate weights (Sartorius AG, Göttingen, Germany).

2.2 Samples

2.2.1 Pure Samples

Fosinopril sodium (FOS) and hydrochlorothiazide (HCZ) were kindly obtained from SmithKline Beecham Egypt L.L.C. (Giza, Egypt). Their purity was found to be 100.14% and 100.01%, respectively, according to supplier certificates of analysis. Chlorothiazide with a claimed purity of 99.56% was purchased from Sigma-Aldrich (Steinheim, Germany) and brought to us by the Egyptian International Center (EIC, Cairo, Egypt) for import and export.

2.2.2 Marketed Samples

Monozide™ (10/12.5) tablets (batch No. 108803) manufactured by SmithKline Beecham Egypt L.L.C. labeled to contain 10 mg of FOS and 12.5 mg of HCZ per tablet were used.

Monozide™ (20/12.5) tablets (batch No. 157072) manufactured by SmithKline Beecham Egypt L.L.C labeled to contain 20 mg of FOS and 12.5 mg of HCZ per tablet were used.

2.3 Chemicals and Solvents

Methanol (HPLC-grade) was purchased from Sigma-Aldrich and brought to us by the Egyptian International Center for import and export. Ethyl acetate, chloroform, and formic acid (analytical grade with an acceptable purity) were purchased from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt).

2.4 Solutions

Stock standard solutions of FOS, HCZ and CZ were prepared in methanol in the concentration of 1 mg mL^{-1}.

Working standard solutions:

A) For spectrophotometric methods: working standard solutions of FOS, HCZ (0.1 mg mL^{-1}), and CZ (0.05 mg mL^{-1}) were prepared by proper dilutions of their particular stock standard solutions using methanol as a solvent.

B) For TLC–densitometric method: working standard solutions of HCZ (0.5 mg mL^{-1}) and CZ (0.2 mg mL^{-1}) were prepared by proper dilutions of their particular stock standard solutions using methanol as a solvent.

2.5 Laboratory-Prepared Mixtures for Spectrophotometric Methods

The working standard solutions of FOS, HCZ, and CZ were used for the preparation of different mixtures containing different ratios of them (taking into consideration the marketed pharmaceutical formulation ratio) using methanol as a solvent.

3 Procedure

3.1 Spectral Characteristics of Fosinopril Sodium, Hydrochlorothiazide, and Chlorothiazide

The absorption spectra of 10 µg mL^{-1} each of FOS, HCZ, and CZ were recorded from 200 to 400 nm using methanol as a solvent **(Figure 2).**

3.2 Construction of Calibration Curves

3.2.1 For Spectrophotometric Methods

Into 3 different sets of 10-mL volumetric flasks, different accurate aliquots containing 40–350, 20–150, and 20–150 µg of FOS, HCZ, and CZ, respectively, were individually transferred

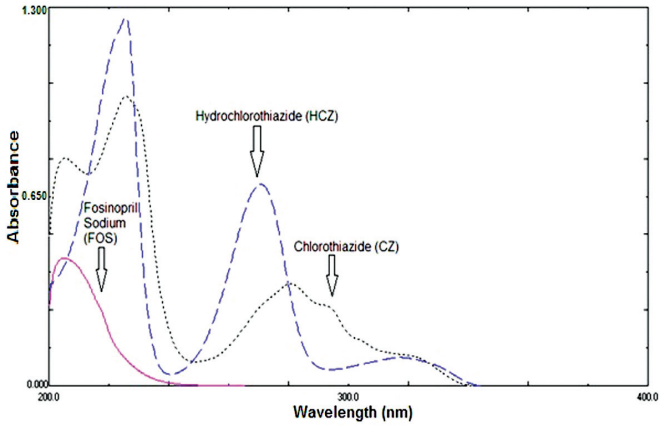


Figure 2

Zero-order absorption spectra of 10 µg mL^{-1} each of fosinopril sodium (——), hydrochlorothiazide (----), and chlorothiazide (·····) by using methanol as a solvent.

from their working solutions; then, by using methanol, the volume was adjusted. For each set, the absorbance spectra were recorded in the range of 200–400 nm.

3.2.1.1 For Ratio Difference Spectrophotometric Method (RD) (Method I)

The obtained spectra each of FOS and CZ were divided by a standard spectrum of 8 µg mL^{-1} HCZ as a divisor, and then, the amplitudes values at 204.6 and 231.2 nm and 290 and 302.6 nm in the produced division spectra of FOS and CZ, respectively, were measured. On the other hand, the absorption spectra each of HCZ were divided by a standard spectrum of 6 µg mL^{-1} CZ as a divisor, and then, the amplitudes values at (275 and 293.6) nm in the produced division spectra of HCZ were measured. Linear relationships were constructed between the amplitudes difference at the selected wavelengths and the corresponding concentrations of each component.

3.2.1.2 For Mean Centering of Ratio Spectra Spectrophotometric Method (MCR) (Method II)

For the determination of FOS, its stored spectra in the range of 200–300 nm were divided by a spectrum of 8 µg mL^{-1} CZ to give the first ratio spectra, which was then mean-centered. Then, the resultant vectors were divided by the mean-centered ratio of ($\alpha_{8\mu g/mL\ HCZ}/\alpha_{8\mu g/mL\ CZ}$), and then, the mean centering of the second ratio spectra was achieved. In the same way, for the determination of HCZ, its scanned spectra in the range of 200–300 nm were divided by 8 µg mL^{-1} CZ to obtain the first ratio spectra, which was then mean-centered. Then, the obtained vectors were divided by the mean-centered (MC) ratio of ($\alpha_{15\mu g/mL\ FOS}/\alpha_{8\mu g/mL\ CZ}$), and then, the mean centering of the second ratio spectra was achieved. In the same way, for the determination of CZ, 8 µg mL^{-1} HCZ and 15 µg mL^{-1} FOS were the first and second divisors, respectively. Then, the recorded values of the mean-centered second ratio spectra at 243.4 nm for CZ, 215.6 and 215.8 nm (peak to peak) for FOS, and 223.8 and 224 nm (peak to peak) for HCZ were plotted against the corresponding concentrations of each component. Finally, the calibration curves were established, and the regression equations were calculated.

3.2.2 For Thin-Layer Chromatographic Densitometric Method (TLC) (Method III)

Different aliquots of FOS, HCZ, and CZ equivalent to 0.1–1 mg mL^{-1}, 0.02–0.3 mg mL^{-1}, and 0.02–0.2 mg mL^{-1}, respectively, were accurately transferred from their corresponding stock standard solutions into 3 sets of 10-mL volumetric flasks. Then, the volume was completed to the mark with methanol. Application of 10 µL from each sample was made in triplicates on the TLC plates. The chromatographic elution was carried out in a chromatographic tank left for 30 min for saturation with a developing system consisting of ethyl acetate–chloroform–methanol–formic acid (60:40:5:0.5, by volume). The developed plates were air-dried and then scanned at 215 nm. Then, the integrated peak areas were obtained for each component, and calibration curves were constructed by plotting the mean integrated peak areas against the corresponding concentrations for each component, and finally, their regression equations were established.

3.3 Analysis of Laboratory-Prepared Mixtures

For the developed spectrophotometric methods, the above-mentioned procedures given under Construction of Calibration Curves were followed but using the recorded spectra of the laboratory-prepared mixtures, and the previously obtained regression equations were used for calculating the concentrations of FOS, HCZ, and CZ.

3.4 Application to Pharmaceutical Formulation

For preparing stock solutions (1 mg mL^{-1}) for each pharmaceutical formulation, 10 tablets each of Monozide™ 10/12.5 and Monozide™ 20/12.5 were separately weighed, grinded, and then mixed well. An amount weighed from tablet formulations of Monozide™ 10/12.5 or Monozide™ 20/12.5, equivalent to 10 mg of FOS (containing also 12.5 mg of HCZ) or 20 mg of FOS (containing also 12.5 mg of HCZ), respectively, were transferred separately into two 25-mL volumetric flasks. An amount of 15 mg methanol was added, and then, samples were sonicated for 15 min, then cooled well, and filtered. After that, the volume was accurately adjusted with methanol. Sample working solutions (0.1 mg mL^{-1}) were then prepared using methanol from which several dilutions within a linearity range of each method were prepared, and the developed methods were applied to calculate the concentrations of the studied drugs. Standard addition method was then performed to assess the accuracy of the methods.

4 Results and Discussion

The presence of a drug's impurities can happen under several conditions like the manufacturing process, packaging of the formulations, or during their storage. Thus, the principles for their acceptance within certain limits depend on accurate pharmaceutical studies or recognized safety data [15].

The presence of the drug's impurities may affect the safety and efficacy of the pharmaceutical formulation. Thus, the pharmacopeias and the ICH established restrictive requirements for the accepted levels of such impurities in pharmaceutical products.

CZ was reported to be a HCZ process impurity in the British Pharmacopoeia [1], which was found to have less pharmacological activity than the parent drug, HCZ. One of the main problems during analysis of HCZ in the presence of its impurity (CZ) is the structural similarity between them, which lead to similar chromatographic behavior and UV absorption between HCZ and CZ, hindering their simultaneous analysis.

Also, fosinopril sodium is the only angiotensin-converting enzyme (ACE) inhibitor that has a phosphate group in its structure. This group allows its elimination from the body by both renal and hepatic pathways. Thus, this makes it a safer choice than other ACE inhibitors for the treatment of hypertension and some types of chronic heart failure, especially for patients with impaired kidney function [2].

From the literature review, there is no reported spectrophotometric or TLC–densitometric method for the determination of FOS, HCZ, and CZ in their ternary mixture. Thus, the goal of this work was to develop and validate new, sensitive, precise, specific, and selective methods for the appropriate determination of the cited drugs in the presence of HCZ impurity (CZ) in their laboratory-prepared mixtures and for the determination of the main drugs in their marketed pharmaceutical formulation with short analysis time and cost.

UV–Vis spectrophotometry is a rapid, inexpensive, and familiar technique used for the quality control of pharmaceutical preparations. Spectrophotometric methods are widely applicable analytical methods due to their simple procedures, as they do not need any previous separation steps and their widely spread instrument does not need sophisticated apparatus or high-cost solvents. Therefore, spectrophotometric methods provide an alternative tool for resolving mixtures with overlapping spectra in quality-control laboratories.

Two different spectrophotometric methods, namely, ratio difference (RD) and mean centering of ratio spectra (MCR), were chosen for the determination of FOS, HCZ and CZ in their ternary mixture without preliminary separation in bulk and in their combined pharmaceutical formulations.

On the other hand, TLC is a commonly used analytical method, which provides rapid analysis and efficient separation method for the determination of various mixtures from different classes of compounds. It has many advantages over other chromatographic methods, such as short analysis time, since numerous samples can be developed on the same run, and its low-cost chemicals [16]. All these make TLC a suitable choice for different applications in the analytical, biomedical, and pharmaceutical fields.

4.1 Method Development and Optimization

4.1.1 Ratio Difference Spectrophotometric Method (RD)

As shown in **Figure 2,** there was a severing spectral overlap between HCZ and CZ in the region of 200–350 nm. On the other hand, FOS showed absorbance only in the region of 200–250 nm, at which HCZ and CZ were severely overlapped with it. Depending on their UV spectral characteristics, the ratio difference method was developed, at which HCZ and CZ were measured at the region where no interference from FOS was found, while FOS was measured in the region which showed UV contributions.

This method (RD) resolved the spectral overlap between the studied components without needing any derivatization steps. It depended on dividing the scanned spectrum of the ternary mixture (for example, $x + y + z$) by the standard spectrum of one of the constituent components (for example, x) to obtain a new ratio spectrum. For determination of component y (for example), 2 wavelengths in the obtained ratio spectrum were chosen, at which the ratio difference was zero for each of x and z, while the difference was significant for y. Calibration curve for y was constructed by plotting the ratio difference between the selected wavelengths for pure y against its corresponding concentrations. Components x and z were then determined in the same way. In this method, the divisor and its concentration played a significant role in method specificity. Different concentrations including 6, 8, 10, and 15 µg mL^{-1} each of CZ, HCZ, and FOS were tried as divisors. Standard spectra of 8 µg mL^{-1} HCZ and 6 µg mL^{-1} CZ were the chosen divisors. For the determination of FOS and CZ in the ternary mixture, a standard spectrum of 8 µg mL^{-1} HCZ was used as a divisor, and the ratio difference between 204.6 and 231.2 nm was used for measuring FOS (zero difference for each of CZ and HCZ) **(Figure 3a).** Meanwhile, the difference between 290 and 302.6 nm was used for measuring CZ (the difference was zero for each of FOS and HCZ) (Figure 3a). In the same way, HCZ was determined in the ternary mixture after dividing by the standard spectrum of 6 µg mL^{-1} CZ and then measuring the ratio difference between 275 and 293.6 nm (zero difference for each of CZ and FOS) **(Figure 3b).** Calibration

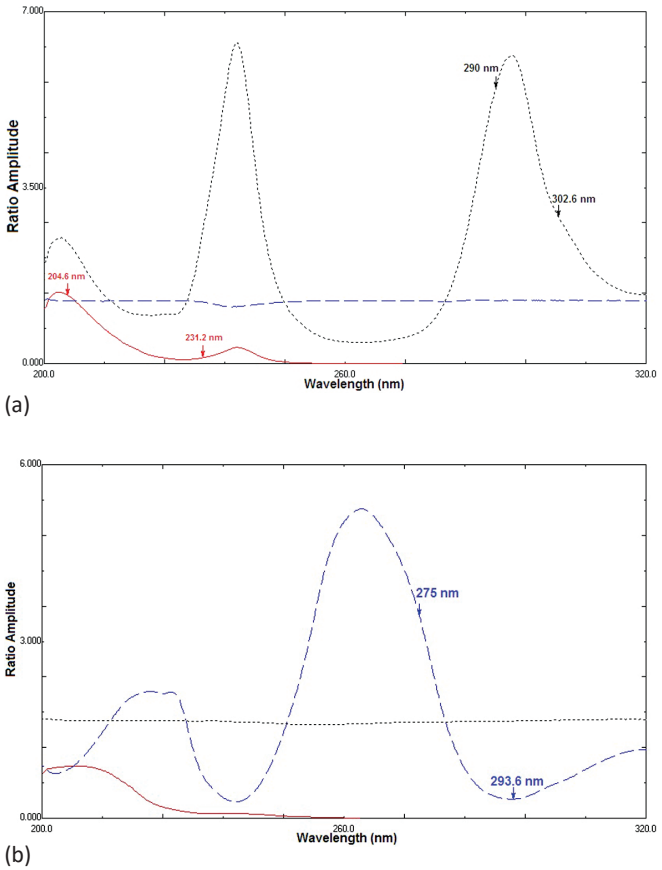


Figure 3

Ratio spectra of 10 µg mL^{-1} each of fosinopril sodium (——), hydrochlorothiazide (----), and chlorothiazide (·····) by using (a) 8 µg mL^{-1} of hydrochlorothiazide as a divisor and (b) 6 µg mL^{-1} of chlorothiazide as a divisor.

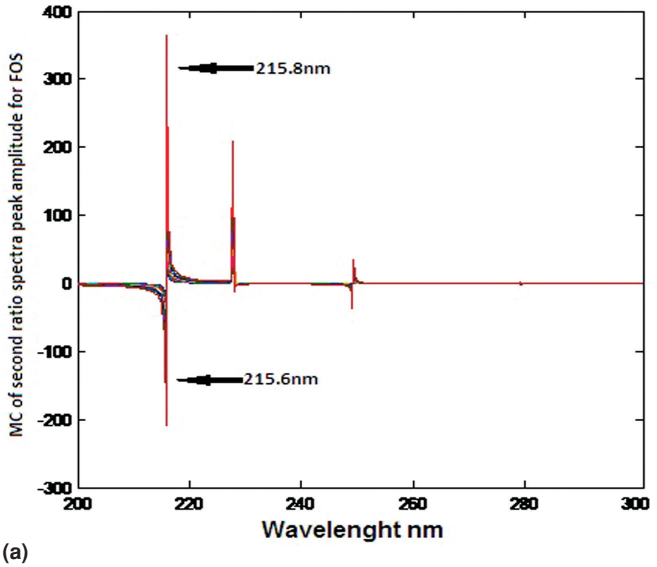
curves for each of the studied components were constructed, relating the ratio difference at the selected wavelengths and their corresponding concentrations.

4.1.2 Mean Centering of Ratio Spectra Spectrophotometric Method (MCR)

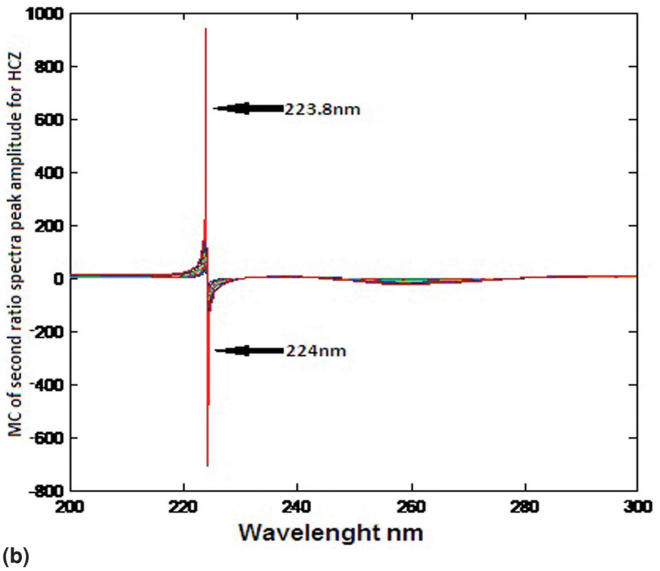
Mean centering of ratio spectra in two successive steps was used for resolving the overlapped spectra of different mixtures [17–22]. In this work, it was used for resolving the overlap between HCZ, CZ, and FOS without needing any derivatization steps, leading to enhancement in the signal-to-noise ratio [23]. The full mathematical elucidation of this method was firstly illustrated by *Afkhami* and *Bahram* [24, 25]. In this method, for resolving a ternary mixture of ($x + y + z$) and for measuring x (as an example), the spectrum of the ternary mixture was divided firstly by the standard spectrum of y (first divisor), and the obtained ratio spectrum was mean-centered. The obtained first mean-centered ratio spectrum was then divided by mean-centered ratio (z/y) to obtain the second ratio spectrum, which was then mean-centered. In the obtained second mean centering of ratio spectrum, a wavelength maximum or minimum was chosen to determine the component x (no interference from both y and z). The remaining components y and z could be determined in the same way. In the studied ternary mixture, the standard spectra of 8 μg mL^{-1} CZ and 8 μg mL^{-1} HCZ were used as the first and second divisors, respectively, for the determination of FOS. Meanwhile, the standard spectra of 8 μg mL^{-1} CZ and 15 μg mL^{-1} FOS were used as the first and second divisors, respectively, for the determination of HCZ. In addition, for measuring CZ, the standard spectra of 8 μg mL^{-1} HCZ and 15 μg mL^{-1} FOS were used as the first and second divisors, respectively. The selected amplitudes were 215.6 and 215.8 nm (peak to peak) for the determination of FOS, 223.8 and 224 nm (peak to peak) for the determination of HCZ, and 243.4 nm for measuring CZ **(Figure 4).**

4.1.3 Thin-Layer Chromatographic–Densitometric Method (TLC–Densitometry)

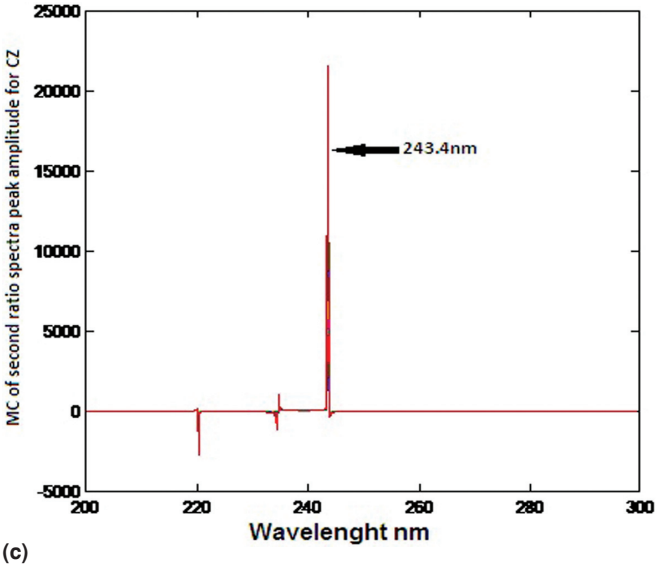
TLC method is a very widespread chromatographic method used in separating numerous mixtures [26–31]. In order to separate the 3 studied components, various trials were examined to choose the most suitable developing system. Firstly, different developing systems consisting of either ethyl acetate–chloroform (in several ratios including 80:20, 70:30, and 60:40) or methanol–chloroform (in several ratios including 75:25, 70:30, and 60:40) were tested. It was observed that ethyl acetate–chloroform (60:40, *V/V*) resulted in good resolution among HCZ and CZ, as well as unresolved peaks between FOS and HCZ. Secondly, different small ratios of methanol were added as trials to improve the resolution between FOS and HCZ, where an amount of 5 mL was found to be sufficient to improve the resolution between FOS and HCZ without affecting the separation between HCZ and CZ. Thirdly, to improve the symmetry of the FOS peak, different ratios of glacial acetic acid, formic acid, ammonia, or tri-ethylamine solutions were individually added to the last developing system. It was observed that addition of 0.5 mL formic acid solution was enough to remove the tail and improve the FOS peak shape. Finally, suitable separation among the 3 studied components could be achieved from using a developing system mixture of ethyl acetate–chloroform–methanol–formic acid (60:40:5:0.5, by volume), where



(a)



(b)



(c)

Figure 4

The mean centering of second ratio absorption spectra of (a) fosinopril sodium (FOS) in the range of 4–35 μg mL^{-1}, (b) hydrochlorothiazide (HCZ) in the range of 2–15 μg mL^{-1}, and (c) chlorothiazide (CZ) in the range of 2–15 μg mL^{-1}.

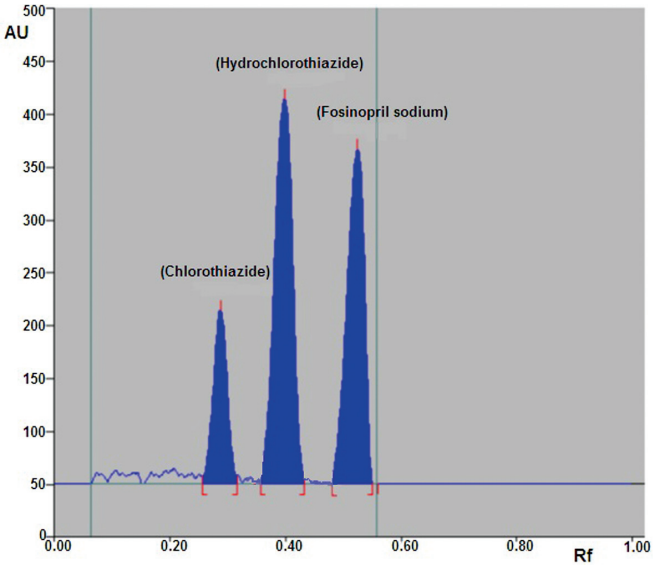


Figure 5

TLC–densitogram of chlorothiazide, hydrochlorothiazide, and fosinopril sodium using ethyl acetate–chloroform–methanol–formic acid (60:40:5:0.5, by volume) as the developing system.

the obtained R_F values were 0.29, 0.39, and 0.53 for CZ, HCZ, and FOS, respectively **(Figure 5).** Scanning at several wavelengths was also tested (208 nm, 210 nm, 215 nm). UV scanning at 215 nm gave acceptable sensitivity with lower detector noise for all the studied components. Also, slit dimensions and intervals between bands were optimized, where slit dimensions of 3 mm × 0.45 mm were selected, and the bands were separated from each other by 5 mm and by 10 mm apart from the bottom edge of the plate.

4.2 Method Validation

According to the International Conference on Harmonization (ICH) guidelines [32], method validation was carried out.

4.2.1 Linearity and Range

The linearity of the developed method was proved by analyzing numerous concentrations of FOS, HCZ, and CZ in triplicate. It was achieved in the ranges of 4–35, 2–15, and 2–15 µg mL^{-1} for FOS, HCZ, and CZ, respectively (for RD and MCR spectrophotometric methods), and in the ranges of 1–10, 0.2–3, and 0.2–2 µg per band for FOS, HCZ, and CZ, respectively (for TLC–densitometric method). The obtained regression parameters, such as slope, intercept, and correlation coefficients, are presented in **Table 1.** The values of correlation coefficients confirmed that the developed methods were linear within the studied ranges.

4.2.2 Limits of Detection and Limits of Quantification (LOD and LOQ)

For the determination of the limits of detection and quantification, FOS, HCZ, and CZ concentrations located in the lower part of the calibration curves were used, and the following equations were applied: LOD = 3.3 × SD / B and LOQ = 10 × SD / B, where SD is the standard deviation of the response and B is the obtained slope of the corresponding calibration curve. The values of LOD and LOQ are shown in Table 1, ensuring that the methods have high sensitivity.

Table 1

Regression and analytical parameters obtained from the developed methods used for the determination of fosinopril sodium, hydrochlorothiazide, and chlorothiazide.

Parameter	RD			MCR			TLC–densitometry		
	FOS	HCZ	CZ	FOS	HCZ	CZ	FOS	HCZ	CZ
Calibration range	4–35	2–15	2–15	4–35	2–15	2–15	1–10	0.2–3	0.2–2
	µg mL^{-1}			µg mL^{-1}			µg per band		
Slope	0.1104	0.3168	0.3209	16.317	107.8	1408.4	1.3785	4.6662	5.5267
Intercept	0.1421	0.0928	−0.5386	−2.8617	43.488	527.88	1.1536	2.1228	1.0468
Correlation coefficient	0.9998	0.9999	0.9998	0.9998	0.9998	0.9998	0.9999	0.9997	0.9997
Accuracy	99.79	99.90	99.86	99.90	100.02	100.07	100.21	100.09	99.86
Repeatability *(%RSD)*[a]	0.63	0.88	0.86	0.73	0.95	1.15	1.22	1.08	0.94
Intermediate precision *(%RSD)*[b]	1.41	1.03	1.22	1.17	1.01	1.24	1.53	1.45	1.35
LOD[c]	1.27	0.73	0.69	1.11	0.84	0.72	0.28	0.09	0.05
LOQ[d]	3.83	2.46	2.30	3.59	2.79	2.41	0.86	0.35	0.16

[a]The intra-day precision (n = 9), average of three different concentrations repeated three times within day
[b]The inter-day precision (n = 9), average of three different concentrations repeated three times on three successive days
[c]LOD = (SD of the response / slope) × 3.3
[d]LOQ = (SD of the response / slope) × 10

4.2.3 Accuracy

The accuracy of the developed methods was examined by their application for the determination of different concentrations of the pure samples from the studied components within their ranges of linearity. By using their related regression equations, the concentrations were calculated, and then the recovery percentages were computed. The results of testing accuracy of the methods are given in Table 1, where they were near to 100%, confirming the high accuracy of the developed methods. Also, the technique of the standard addition was applied to prove method accuracy; the results illustrated in **Table 2** confirmed that the excipients did not interfere.

4.2.4 Precision

It was studied on 2 levels, by testing both repeatability and intermediate precision. Repeatability was examined through analysis of different three concentrations of pure samples from the selected components in triplicate on the same day. The chosen

Table 2

Determination of fosinopril sodium and hydrochlorothiazide in the tablets was achieved by the developed methods and also with the application of a standard addition technique.

Pharmaceutical formulation	Method		Component	Taken [µg mL^{-1}]	Recovery% ± SD[a)]	Standard addition technique			
						Added [µg mL^{-1} or µg per band]	Found [µg mL^{-1} or µg per band][b)]	Recovery %	Mean ± SD
Monozide™ (10/12.5) tablets claimed to contain 10 mg of FOS and 12.5 mg of HCZ (batch No. 108803)	Spectrophotometric methods	RD	FOS	8.00	98.11 ± 1.95	6.00 8.00 10.00	5.87 7.92 9.99	97.86 99.06 99.99	98.96 ± 1.05
			HCZ	10.00	97.31 ± 1.46	2.00 3.00 4.00	1.99 2.98 3.97	99.99 99.28 99.32	99.53 ± 0.40
		MCR	FOS	8.00	99.67 ± 1.90	6.00 8.00 10.00	5.96 7.98 10.02	99.35 99.73 100.15	99.74 ± 0.40
			HCZ	10.00	99.60 ± 1.93	2.00 3.00 4.00	2.01 2.95 3.97	100.67 98.47 99.19	99.44 ± 1.12
	TLC–densitometry		FOS	2.00	97.97 ± 1.84	–	–	–	–
			HCZ	2.50	97.68 ± 1.22	–	–	–	–
Monozide ™ (20/12.5) tablets claimed to contain 20 mg of FOS and 12.5 mg of HCZ (batch No. 157072)	Spectrophotometric methods	RD	FOS	8.00	99.19 ± 1.60	6.00 8.00 10.00	5.97 7.98 9.88	99.55 99.79 98.81	99.38 ± 0.51
			HCZ	5.00	100.84 ± 1.33	3.00 4.00 5.00	2.98 3.96 5.003	99.45 99.08 100.05	99.52 ± 0.50
		MCR	FOS	8.00	98.17 ± 1.85	6.00 8.00 10.00	5.96 8.03 9.86	99.35 100.40 98.56	99.52 ± 0.50
			HCZ	5.00	99.98 ± 1.88	3.00 4.00 5.00	2.98 4.01 4.96	99.41 100.27 99.17	99.49 ± 0.37
	TLC–densitometry		FOS	1.60	100.75 ± 1.78	1.30 1.60 1.80	1.29 1.58 1.803	99.29 98.63 100.17	99.36 ± 0.77
			HCZ	1.00	99.20 ± 1.75	0.80 1.00 1.20	0.79 1.01 1.19	98.71 100.61 99.18	99.50 ± 0.99

[a)]Average of 6 determinations
[b)]Average of 3 determinations

Table 3

Results obtained from determination of FOS, HCZ, and CZ in laboratory synthetic–mixtures using the developed spectrophotometric methods.

No. of mixtures	CZ% (to pure HCZ)	Claimed [taken µg mL^{-1}]			RD			MCR		
		FOS	HCZ	CZ	FOS	HCZ	CZ	FOS	HCZ	CZ
1[a)]	20%	8.00	10.00	2.00	100.99	100.64	97.16	101.60	100.60	98.92
2[b)]	20%	16.00	10.00	2.00	99.69	101.30	98.41	101.10	102.28	99.28
3	14%	6.00	15.00	2.10	97.06	100.97	100.99	98.68	101.52	101.73
4	50%	8.00	8.00	4.00	101.55	102.48	98.60	100.72	97.77	100.42
5	40%	8.00	6.00	2.40	97.02	101.97	97.59	98.67	98.50	98.50
6	25%	6.00	12.00	3.00	98.72	101.67	99.16	99.89	101.46	100.46
Mean ± *%RSD*					99.17 ± 1.94	101.50 ± 0.66	98.65 ± 1.37	100.11 ± 1.24	100.35 ± 1.81	99.88 ± 1.20

[a)]Ratio of FOS and HCZ in Monozide™ 10/12.5 tablets
[b)]Ratio of FOS and HCZ in Monozide™ 20/12.5 tablets

concentrations for RD and MCR spectrophotometric methods were 10, 20, and 30 µg mL^{-1} (for FOS) and 6, 10, and 15 µg mL^{-1} (for HCZ), and 8, 10, and 15 µg mL^{-1} (for CZ). On the other hand, the chosen concentrations for TLC–densitometric method were 1, 4, and 8 µg per band (for FOS) and 0.5, 1.5, and 3 µg per band (for HCZ), and 0.2, 1, and 2 µg per band (for CZ). For the determination of the intermediate precision, the analysis was reiterated on 3 sequential days using the same illustrated concentrations. The calculated relative standard deviation values *(%RSD)* were within the agreeable values (Table 1).

4.2.5 Specificity and Selectivity

The specificity of the spectrophotometric methods was assessed by their application to several laboratory-prepared mixtures containing different concentrations of FOS, HCZ, and CZ. Satisfactory results were observed, shown in **Table 3.** On the other hand, the specificity of the TLC–densitometric method was ensured by its application to different mixtures containing the cited components in order to assess good chromatographic separation with good peak shapes, as shown in Figure 5. Moreover, the determined results obtained from the application of these methods to pharmaceutical formulations containing FOS and HCZ (Table 2) affirmed the method selectivity, and the additives did not make any interference**.**

4.2.6 Robustness

This parameter was made mainly for the TLC–densitometric method and used to prove that the method remained unaffected by little deliberate changes in the method parameters. The studied parameters were: methanol volume (±0.5 mL), formic acid volume (±0.05 mL), saturation time (±5 min), and also scanning wavelength (±2 nm). Then the effect of these variations on the R_F values were measured and expressed as *%RSD.* The values given in **Table 4** affirmed that the developed method is robust.

4.2.7 System Suitability Testing Parameters

These parameters were examined mainly for the TLC–densitometric method, which was used to evaluate the performance of the system either before or during the analysis of the studied components. It was performed by measuring some parameters such as resolution, selectivity, and symmetry factors. Reasonable results were obtained as given in **Table 5** [33].

Table 4

Experimental results of robustness that were estimated for the developed TLC–densitometric method during the determination of the studied components.

Parameters	FOS	HCZ	CZ
	%RSD[a)]		
5 mL methanol ± 0.5 mL	0.55	0.24	0.39
0.5 mL formic acid ± 0.05 mL	0.94	0.87	0.69
Saturation time ± 5 min	0.35	0.44	0.28
Scanning wavelength ± 2 nm	0.17	0.25	0.40

[a)]Relative standard deviation *(%RSD)* of the change in R_F

Table 5

System suitability testing parameters were obtained for the developed TLC–densitometric method.

Parameters	TLC–densitometric method			Reference values [33]
	CZ	HCZ	FOS	
Symmetry factor	1	1.04	1	~1
Resolution (R_s)	2.05	2.04		>1.5
Selectivity (α)	1.46	1.34		>1
Retention factor (R_F)	0.29	0.39	0.53	–

Table 6

Statistical comparison was made between the obtained results by the developed methods and that of the reported method for the determination of fosinopril sodium and hydrochlorothiazide in pure powder form.

Component	Spectrophotometric methods				TLC–densitometry		Reported method[a] [8]	
	RD		MCR					
	FOS	HCZ	FOS	HCZ	FOS	HCZ	FOS	HCZ
Mean	99.79	99.90	99.90	100.02	100.21	100.09	99.81	99.83
SD	1.44	0.96	1.65	1.58	1.23	1.29	0.84	1.09
Variance	2.07	0.92	2.72	2.50	1.51	1.66	0.71	1.19
N	7	7	7	7	7	7	7	7
t-Test (2.447)[b]	0.9797	0.9013	0.8996	0.7972	0.4938	0.6947	–	–
F-Test (4.284)[b]	2.9155	1.2935	3.8310	2.1008	2.1268	1.3950	–	–

[a]Reversed-phase (RP)-HPLC method to estimate FOS and HCZ using a reversed-phase C18 column as the stationary phase and a mixture of methanol–water (40:60, *V*/*V*), adjusted to pH 4 with 10% *o*-phosphoric acid, as the mobile phase. Also, the flow rate was adjusted to 1 mL min^{-1}, and the detection wavelength was 245 nm [8]

[b]The numbers between the parentheses represent the corresponding tabulated values of *t* and *F* at probability of 0.05

4.3 Application to Pharmaceutical Formulations

After development and optimization of the proposed methods, they were applied for the determination of FOS and HCZ in Monozide™ 10/12.5 and Monozide™ 20/12.5 tablet formulations following the instructions illustrated for each method. The results were declared as percentage recoveries, and they were within the acceptable limits (90%–110%) (Table 2). In addition, the results of the standard addition technique (Table 2) affirmed the accuracy of the developed methods and also proved that the tablets excipients made no interference during the measurement of the studied components.

4.4 Statistical Analysis

The results acquired from the analysis of pure samples of the studied components by using the developed methods were compared statistically with those obtained from using the reported HPLC method for FOS and HCZ [8]. According to the Student's *t*-test and *F*-test assessment, there was no significant difference between them at 95% confidence limits **(Table 6).**

5 Conclusion

Two selective spectrophotometric methods and the TLC–densitometric method have been used for the first time for the analysis of FOS, HCZ, and the HCZ impurity (CZ) in their ternary mixture. The developed RD and MCR spectrophotometric methods have the advantage of high-resolution power without needing any derivatization steps, which enhance the signal-to-noise ratio. On the other hand, the TLC–densitometric method has the advantage of high sensitivity with low analysis time since various samples can be developed at the same time. All the developed methods have high selectivity and are time- and cost-effective methods. Additionally, the validation of the methods was performed according the ICH guidelines, and the illustrated values ensured their validity. These suggested methods can be used effectively in quality control laboratories for the quantitative estimation of the cited components.

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