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Simultaneous Determination of Moexipril Hydrochloride and Hydrochlorothiazide by RP-HPLC and Ratio Spectra Derivative Spectrophotometric Methods in Pure, Pharmaceutical Dosage Forms and Biological Fluids

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ABSTRACT

A simple and reliable UV spectrophotometric and high-performance liquid chromatographic (HPLC) methods were developed and validated for simultaneous determination of moexipril hydrochloride (MOE) and hydrochlorothiazide (HCT) in pure form and pharmaceutical dosage forms and biological fluids (serum and urine). The first method was a RP-HPLC method which was performed using ACE Generix 5 C18 column (5 µm practical size, 25 cm x 4.6 mm i.d.). The flow rate, the injection volume and the detection wavelength were 1.0 mL min^{-1}, 20 µL and 200 nm, respectively. The mobile phase consisted of 0.05 mol L^{-1} pentane sulfonic acid sodium salt at pH 3 and acetonitrile (50:50, v/v). The retention times for MOE and HCT drugs were found to be 4.21 ± 0.15 min and 2.98 ± 0.07 min, respectively. The second method was ratio spectra derivative (1DD) spectrophotometric method. Calibration curve was linear over the concentration range of 0.1-150 µg mL^{-1} and 0.1-300 µg mL^{-1} using RP-HPLC method and 2.0-11.0 µg mL^{-1} and 1.0-12.0 µg mL^{-1} using UV method for MOE and HCT drugs, respectively. The analytical validation and recovery study were performed to confirm the accuracy of the proposed methods. The methods were validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The developed RP-HPLC and UV spectrophotometric methods were successfully applied for the simultaneous determination of moexipril hydrochloride and hydrochlorothiazide drugs in pharmaceutical dosage forms and biological fluids (serum and urine).

Keywords: Moexipril hydrochloride; Hydrochlorothiazide; Ratio spectra derivative (1DD) spectrophotometry; RP-HPLC; Pharmaceutical dosage form; Validation

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INTRODUCTION

Moexipril hydrochloride (MOE) has the IUPAC name of (3S)-2-[(2S)-2-{[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino}-1-oxopropyl]-1,2,3,4 tetrahydro -6,7dimethoxy-3-isoquinoline carboxylic acid hydrochloride. Its structure is given in (Figure 1). Its chemical formula is $C_{27}H_{34}N_2O_7$. HCl and the molecular weight is 535.03 g mol^{-1} for MOE salt and its melting point is 141-161^oC [1].

Moexipril hydrochloride is a long-acting non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor, developed for the treatment of hypertension and congestive heart failure. It works by relaxing blood vessels, causing them to widen. Lowering high blood pressure helps prevent strokes, heart attacks and kidney problems [2]. Moexipril is a

pro-drug of moexiprilat, which inhibits ACE in humans and animals. In biological systems it is rapidly de-esterified by esterases, resulting in its active metabolite moexiprilat [3].

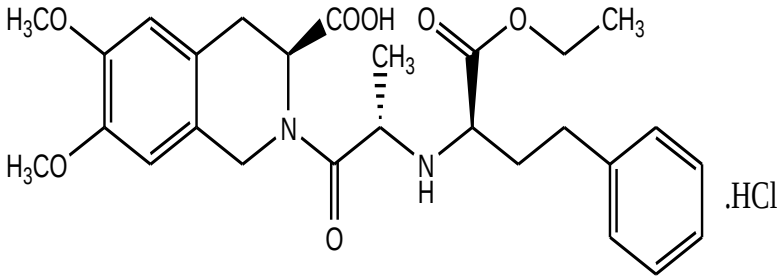

Figure 1. Chemical structure of moexipril hydrochloride

Hydrochlorothiazide (HCT) has the IUPAC name of 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. Its structure is given in (Figure 2). Its chemical formula is $C_7H_8ClN_3O_4S_2$ and the molecular weight is 297.74 g mol^{-1} and its melting point is 273-275^oC [4].

Hydrochlorothiazide is a first-line diuretic drug of the thiazide class used as antihypertensive drug by inhibits active chloride reabsorption and reduces peripheral vascular resistance. HCT is a calcium-sparing diuretic, meaning it can help the body get rid of excess water while still keeping calcium [5].

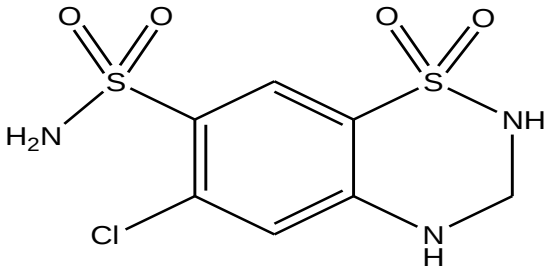

Figure 2. Chemical structure of hydrochlorothiazide

Literature survey for moexipril hydrochloride showed that there were few methods for the determination of moexipril hydrochloride either individually or in combination with hydrochlorothiazide and also in presence of its degradation products or in human plasma or urine. These methods included high performance liquid chromatography (HPLC) [2,6-10], thin layer liquid chromatography (TLC) [11], spectrophotometry [3,9,12-15], derivative spectrophotometric methods [6,8], colorimetric method [13], electrometric methods [16,17] and LC-MS method [18].

In the present study, RP-HPLC and ratio derivative spectrophotometric methods were developed and validated for the simultaneous estimation of moexipril hydrochloride and hydrochlorothiazide in pure form, in their pharmaceutical formulations and biological fluids (serum and urine). The proposed HPLC and first derivative spectrophotometric methods were compared to an official method [19]. The proposed methods can be successfully applied for routine analysis measurements of both drugs with satisfactory results which are important for the quality control of pharmaceutical products.

EXPERIMENTAL

Instruments

RP-HPLC measurements were performed on an Agilent HPLC instrument (Agilent, USA) 1200 series Rapid Resolution (RR), Binary pump SL (Model G1312B Agilent 1200 series; Agilent Technologies) connected with auto sampler SL (Model G1329B Agilent 1200 series; Agilent Technologies). Thermostat column compartment SL (Model 1316B, Agilent 1200 series; Agilent Technologies) equipped with a diode array detector DAD SL (Model G1315C, Agilent 1200 series; Agilent Technologies).

For spectrophotometric method, a Shimadzu UV-Visible recording spectrophotometer (Model UV-2450) with 1 cm quartz cuvettes and connected to an IBM-PC computer loaded with UV Win PC software was used for all the absorbance measurements and treatment of data.

Chemicals and Reagents

All chemicals and reagents used were of high purity available and were of analytical and HPLC grade. The reagents were of analytical grades. Acetonitrile and methanol HPLC grades were purchased from Poch, Poland. Orthophosphoric acid 85% (Analytical grade, ADWIC, Egypt) and pentane sulfonic acid sodium salt (obtained from Sigma-Aldrich) were used.

Materials and Samples

Moexipril hydrochloride (MOE) reference standard material was kindly provided by Mina Pharm Company for Pharmaceutical Industry, Egypt. Its potency was found to be 99.9%.

Hydrochlorothiazide (HCT) reference standard material was kindly provided by Boehringer Ingelheim Pharma Company, Egypt. Its potency was found to be 99.9%.

Primox plus tablets were labeled to contain 15 mg of moexipril hydrochloride and 25 mg of hydrochlorothiazide per tablet and produced by Mina Pharm Company for Pharmaceutical Industry, Egypt. They were purchased from the local market.

Chromatographic Conditions

The chromatographic separation was performed on 5 µm ACE Generix 5 C_{18} column (length 250 mm, i.d. 4.6 mm). The mobile phase consisted of 0.05 $molL^{-1}$ pentane sulfonic acid sodium salt (pH 3.0) and acetonitrile in the ratio of (50:50 v/v); pH value was adjusted using 0.2 $molL^{-1}$ orthophosphoric acid. It pumped at room temperature at a flow rate of 1 mL min^{-1}. The column was conditioned for at least 30 min and the injection volume was 20 µL. Ultraviolet (UV) detection was performed at 200 nm.

Preparation of Solutions for HPLC Method

Preparation of mobile phase: 4.35 g of pentane sulfonic acid sodium salt was weighed, dissolved and diluted to 500 mL with deionized water and pH was adjusted to 3.0 by using 0.2 mol L^{-1} orthophosphoric acid. Then 500 mL of the buffer was mixed with 500 mL of acetonitrile. The prepared mobile phase was filtered through a 0.45 µm filter under vacuum, degassed and sonicated for 5 min. The mobile phase was used as diluent.

MOE standard stock solution: 25 mg of moexipril hydrochloride (MOE) working standard was accurately weighed and transferred to 100 mL clean dry volumetric flask, dissolved and completed to the mark with mobile phase to get a stock standard solution of 0.25 mg mL^{-1}.

HCT standard stock solution: 50 mg Of HCT working standard was accurately weighed and transferred to 100 mL clean dry volumetric flask, dissolved and completed to the mark with mobile phase to get a stock standard solution of 0.5 mg mL^{-1}.

Standard working solution: 30 mL Of stock standard solution of MOE (0.25 mg mL^{-1}) and 25 mL of stock standard solution of HCT (0.5 mg mL^{-1}), respectively, were transferred to 100 mL cleaned dried volumetric flask and dissolved with mobile phase to get final standard solution with concentrations 75 µg mL^{-1} of MOE and 125 µg mL^{-1} of HCT.

Construction of calibration curve for MOE and HCT drugs: A two series of standard solutions were prepared by diluting MOE stock standard solution (0.25 mg mL^{-1}) and HCT stock standard solution (0.5 mg mL^{-1}) with the mobile phase to reach a concentration range of 0.1-150 and 0.1-300 µg mL^{-1} for MOE and HCT drugs, respectively. Triplicate of each drug solution were injected. Calibration curves were constructed and the regression equations were computed by plotting the peak area against the corresponding concentration in µg mL^{-1}.

Assay of pharmaceutical preparation: Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 15 mg of MOE and 25 mg of HCT was transferred into a 100 mL clean dry volumetric flask. Add about 70 mL of mobile phase and sonicate to dissolve it completely and then make volume up to the mark with the same solvent. Aliquots of 5 mL of this solution after filtration and transferred to 10 mL volumetric flask and completed to the volume with the same solvent (mobile phase) to obtain solutions with concentrations 75 µg mL^{-1} of MOE and 125 µg mL^{-1} of HCT. Such drug solution was stable for a period of 3 days when kept in refrigerator at about 4 °C.

Preparation of drugs in serum and urine samples: For serum preparation, a two series of standard solutions were prepared by diluting MOE stock standard solution (250 µg mL^{-1}) and HCT stock standard solution (500 µg mL^{-1}) with the mobile phase to reach a concentration range of 2.5-150 and 3-250 µg mL^{-1} for MOE and HCT drugs, respectively. Serum standards were prepared by spiking 500 µL of each working standard of each drug to 500 µL of human control serum followed by 3 mL of acetonitrile, after shaking for 5 min each standard was Centrifuged for 2 min at 1500 rpm then transferred to a 10 mL volumetric flask and the solution diluted with mobile phase to the appropriate volume to reach a concentration range of 0.25-15 and 0.3-25 µg mL^{-1} for MOE and HCT drugs, respectively.

For urine preparation, a two series of standard solutions were prepared by diluting MOE stock standard solution (250 µg mL^{-1}) and HCT stock standard solution (500 µg mL^{-1}) with the mobile phase to reach a concentration range of 75-225 and 3-250 µg mL^{-1} for MOE and HCT drugs, respectively. Urine standards were prepared by spiking 1 mL of each working standard of each drug to 1 mL of drug-free urine, followed by 3 mL of acetonitrile, and the solutions were allowed to stand for 2 min. Five mL of mobile phase were added to each solutions to reach the final concentration range of 7.5-22.5 and 0.3-25 µg mL^{-1} for MOE and HCT drugs, respectively.

Preparation of standards solutions for spectrophotometric method: Standard stock solutions of MOE or HCT (100 µg mL^{-1}) were prepared by dissolving 10 mg of each drug in methanol in 100 mL volumetric flask and diluted to the mark with the solvent. The calibration curves of the respective drugs were prepared by appropriate dilution using methanol to reach concentration ranges of 2.0-11.0 and 1.0-12.0 µg mL^{-1} for MOE and HCT drugs, respectively. All solutions were stable for a period of 3 days when kept in a refrigerator at about 4 °C.

Preparation of tablet sample solutions: Ten tablets were weighed and finely powdered. An equivalent weight of one tablet was transferred to a 100 mL volumetric flask. 70 mL of methanol was added and sonicated for 15 min, and then the solution was made up to the mark with methanol. The solution was diluted with methanol to obtain the concentration ranges of both drugs. All solutions were stable for a period of 3 days when kept in a refrigerator at about 4 °C.

Preparation of drugs in serum and urine samples: 1 mL Of free drug urine or plasma was transferred into two series of separatory funnels and then to each was added 1 mL of 2.0-11.0 and 1.0-12.0 µg mL^{-1} for MOE and HCT drugs, respectively, followed by the addition of 3 mL acetonitrile. After shaking each funnel for 5 min, the aqueous layer was transferred to a centrifuge tube. Centrifuged for 2 min at 1500 rpm, then transferred to a 10 mL volumetric flask and the solution diluted with methanol to the appropriate volume. The procedure described above was applied.

RESULTS AND DISCUSSION

HPLC Method

Method development and optimization: The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of 0.05 mol L^{-1} pentane sulfonic acid sodium salt at pH 3 adjusted by o-phosphoric acid and acetonitrile (50 : 50, v/v) accomplished at 200 nm by using an ACE Generix 5 C18 column (5 µm practical size, 25 cm x 4.6 mm i.d.).The retention times for MOE and HCT drugs were found to be 4.21 ± 0.15 min and 2.98 ± 0.07 min, respectively, at a flow-rate of 1.0 ml min^{-1} and the injection volume was 20 µl. Figures 3 and 4 show the chromatograms of the used drugs and their dosage forms. The difference in the retention time between the two drugs enable successful determination of both drugs simultaneously without the need for their prior separation, from each other.

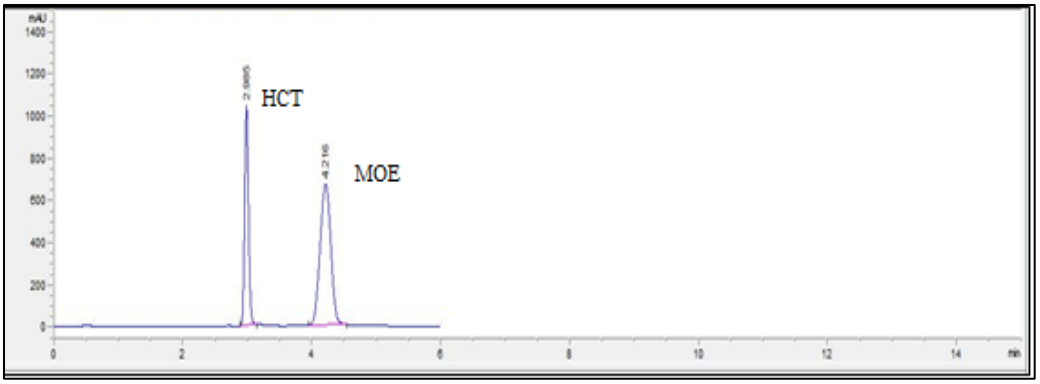

Figure 3. Typical chromatogram of moexipril HCl (75 µg mL-1) and hydrochlorothiazide (125 µg mL-1) mixed standard

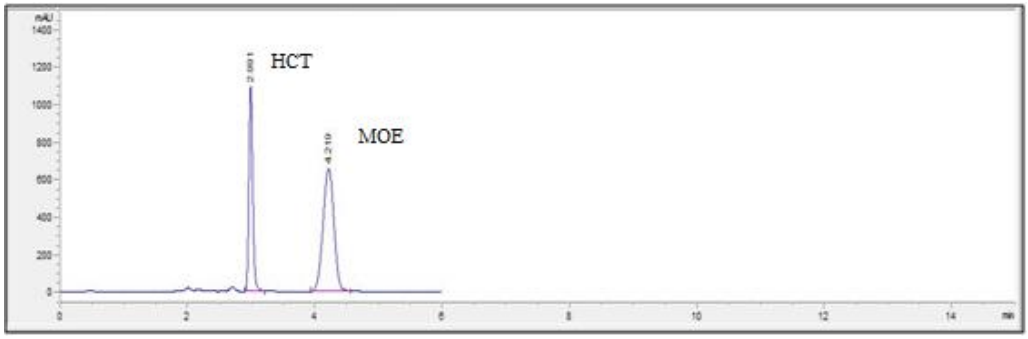

Figure 4. HPLC chromatogram of (75 µg mL-1) of MOE and (125 µg mL-1) of HCT in primox plus tablets (15 mg MOE/25 mg HCT per tablet)

System suitability tests: System suitability testing parameters were calculated according to USP [20] to ensure that the chromatographic systems were working correctly during the analysis after optimizing the chromatographic conditions using different mobile phases, pH, and flow rates. Selectivity factor (α), resolution (R), column efficiency, tailing factor (T) and relative standard deviation peak area of ten replicate injections were parameters to be checked during the analysis as represented in Table 1.

Table 1. Summary of validation parameters for system suitability test for simultaneous determination of mixture of MOE and HCT drugs by the proposed HPLC method

Parameter	Moexipril HCl	Hydrochlorothiazide	Reference values
Retention time (min)	4.216	2.985	–
Selectivity (α)	3.502	–	α > 1
Resolution (R)	5.853	–	R >2
Tailing factor (T)	0.980	1.141	T ≤ 2
RSD% of peak area	0.474	0.527	<1, n=10
Theoretical plates (N)	3524	11988	>2000

Method Validation

Linearity and range: Calibration curves were constructed representing the relationship between integrated peak areas and the corresponding concentrations in the range of 0.1-150 and 0.1-300 µg mL^{-1} for MOE and

HCT, respectively, as shown in Figures 5 and 6. The characteristic parameters for the regression equation were computed as illustrated in Table 2.

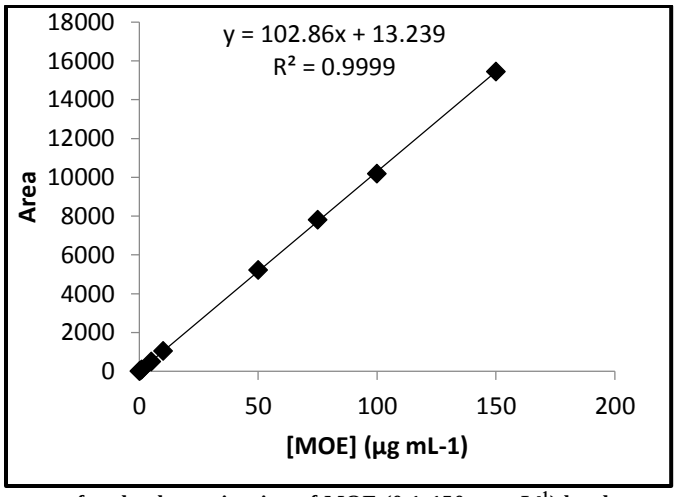

Figure 5. Calibration curve for the determination of MOE (0.1-150 µg mL^{-1}) by the proposed HPLC method

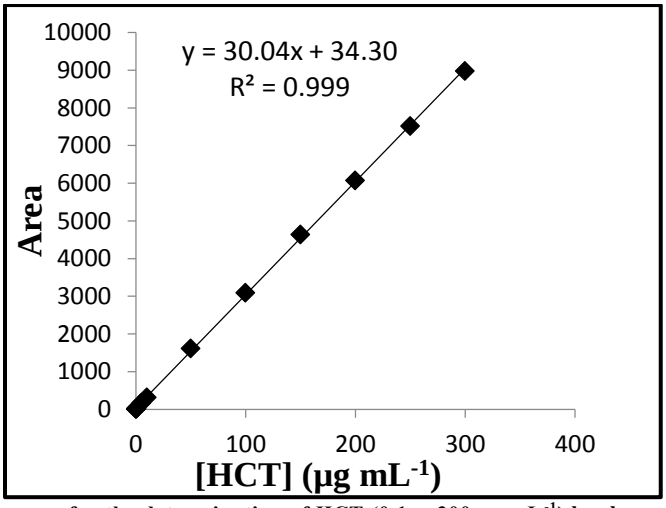

Figure 6. Calibration curve for the determination of HCT (0.1 – 300 µg mL^{-1}) by the proposed HPLC method

Table 2. Linearity parameters for the determination of MOE and HCT by proposed HPLC method

Parameters	Moexipril HCl	Hydrochlorothiazide
Linearity range, µg mL^{-1}	0.1-150	0.1-300
LOD, µg mL^{-1}	0.018	0.007
LOQ, µg mL^{-1}	0.0594	0.0248
Regression equation	y=102.86x + 13.239	y=30.048x + 34.305
Slope	102.86	30.048
SE of slope	3.47279E-05	0.000165598
Intercept	13.239	34.305

SE of intercept	0.24066411	0.689044015
Regression coefficient (R^2)	0.9999	0.9998
SE of estimation	0.550072355	1.813374016

Precision: The repeatability of the proposed method was evaluated by assaying three samples solutions of MOE or HCT within the same day and under the same experimental conditions (intra-day). The precision was evaluated by assaying solutions on three consecutive days (Inter-day). Peak areas were determined and the precision was expressed as %RSD < 2. From the data obtained in Table 3, the developed RP-HPLC method was found to be precise.

Table 3. Intraday and interday precisions of the proposed HPLC method for the determination of MOE and HCT drugs in their mixture

Drugs	**Concentration ($\mu g\ mL^{-1}$)**	**Intra-day precision**		**Mean recovery% ± SD**	**Mean RSD%**
		Amount Found[a] ($\mu g\ mL^{-1}$)	**Recovery[a] %**		
MOE	5	5.024	100.48	100.54 ± 0.57	0.57
	75	75.01	100.01		
	100	101.14	101.14		
HCT	4	3.996	99.89	99.37 ± 0.49	0.49
	80	79.14	98.92		
	200	198.57	99.29		
		Inter-day precision			
MOE	5	5.024	100.48	100.28 ± 0.18	0.18
	75	75.17	100.23		
	100	100.13	100.13		

HCT	4	3.983	99.58	99.58 ± 0.48	0.48
	80	80.04	100.05		
	200	198.21	99.10		

[a]mean of three different samples for each concentration

Limits of detections and quantifications: The detection and quantification limits were calculated based on the standard deviation of the response and the slope of the calibration curves, as follows:

$LOD=3.3 \times \sigma/S$, (1)

$LOQ=10 \times \sigma/S$, (2)

Where σ is the standard deviation of the response and S is the slope of the regression line of each calibration curve. The low values of LOD indicated the sensitivity of the method for both drugs as shown in Table 2.

Accuracy: The accuracy of the method was determined by calculating recoveries of MOE and HCT drugs using standard addition method. Known amounts of MOE and HCT drugs were added to a pre-quantified sample solution (primox plus tablets), and the amount of MOE and HCT drugs were estimated by measuring the peak areas and draw relation between concentration and area once when standard alone and the other when adding sample (Table 4 and Figure 7).

Table 4. Standard addition data of MOE and HCT drugs for the proposed method

Drugs	Label claim/tablet (µg mL^{-1})	Amount of standard added (µg mL^{-1})	Amount found (µg mL^{-1})[a]	Recovery%[(a)]	Mean Recovery% ± SD	RSD (%)
MOE In Primox plus Tablets	10	2.5	2.481	99.24	99.85 ± 0.44	0.44
		5	4.992	99.84		
		7.5	7.489	99.85		
		10	10.02	100.24		
		15	14.92	99.49		
		20	20.08	100.43		
HCT	25	6.25	6.16	98.59	99.40 ± 0.63	0.64

In Primox plus Tablets		12.5	12.43	99.41		
		18.75	18.51	98.75		
		25	24.92	99.69		
		37.5	37.59	100.25		
		50	49.86	99.73		

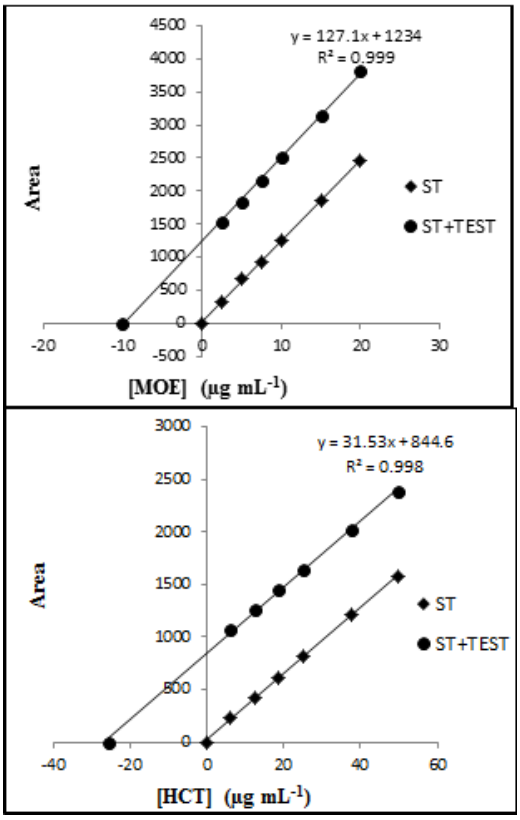

Figure 7. Standard addition plots of MOE and HCT drugs

Comparison of proposed method with reference method: The results obtained by applying the proposed chromatographic method were statistically compared to those of the official HPLC (USP) method [19] used for MOE and HCT analysis in a binary mixture drug (primox plus tablets). It is concluded that with 95% confidence, there is no significant difference between them since the calculated t- and F- values were less than the theoretical values; as presented in Table 5.

Table 5. Statistical analysis of data obtained for the determination of MOE and HCT drugs in their dosage form

Pharmaceuticals	Parameters	MOE		HCT	
		Proposed method	Reported method	Proposed method	Reported method
Primox plus Tablets	Mean	100.26	99.70	100.33	99.89
	SD	0.529	0.857	0.692	0.792
	Variance	0.280	0.734	0.479	0.627
	SE	0.570	0.922	0.679	0.777
	t-test*	1.350	–	1.036	–
	F-test*	2.621	–	1.309	–

*Tabulated t- and F- values at 95% confidence level (n=6) were 2.228 and 5.05, respectively

Robustness: The robustness of a method is the ability to remain unaffected by small changes in parameters. To determine the robustness of the developed method, experimental conditions were purposely altered. The flow rate and pH values were changed by ± 0.2 units and the mobile phase (buffer: acetonitrile v/v) was changed by ± 1. The results were provided in (Table 6). Form these results; it was observed that there was no significant changes in the chromatogram obtained which demonstrate that the developed HPLC method was robust.

Table 6. System suitability parameters and robustness in normal and changed condition for the determination of MOE and HCT drugs by the proposed HPLC method

System suitability parameters / Robustness	Drugs	Resolution	Retention time (min)	Theoretical plates	Tailing factor	Selectivity	Recovery%
Optimum condition	MOE	5.853	4.216	3524	0.980	3.502	99.85
	HCT	-	2.985	11988	1.141	-	99.40
Flow rate (mL min^{-1})	MOE	6.673	5.419	4344	0.998	3.402	98.95
		5.293	3.450	3038	0.987	3.532	100.20

0.8 1.2	**Mean ± SD**	5.94 ± 0.69	4.36 ± 0.99	-	0.99 ± 0.01	3.48 ± 0.07	99.58 ± 0.88
	HCT	- -	3.764 2.484	14512 9787	1.175 1.169	- -	98.87 99.98
	Mean ± SD	-	3.08 ± 0.65	-	1.16 ± 0.02	-	99.43 ± 0.78
Buffer pH 2.8 3.2	**MOE**	5.683 6.023	4.106 4.328	3034 3450	0.959 0.991	3.482 3.492	99.55 99.96
	Mean ± SD	5.853 ± 0.17	4.22 ± 0.11	-	0.98 ± 0.02	3.49 ± 0.01	99.76 ± 0.29
	HCT	- -	3.019 3.014	12255 11912	1.122 1.149	- -	100.18 100.02
	Mean ± SD	-	3.01 ± 0.02	-	1.14 ± 0.01	-	100.1 ± 0.11
Buffer: Acetonitrile 51:49 49:51	**MOE**	6.343 5.973	4.596 4.212	3087 3692	0.963 0.983	3.742 3.502	98.92 99.58
	Mean ± SD	6.06 ± 0.26	4.34 ± 0.22	-	0.98 ± 0.01	3.58 ± 0.14	99.25 ± 0.47
	HCT	- -	3.043 2.985	11834 11984	1.151 1.172	- -	99.14 99.85
	Mean ± SD	-	3.00 ± 0.03	-	1.15 ± 0.02	-	99.50 ± 0.50

First Derivative Ratio Spectra Method (^{1}DD)

Selection of Δλ and SF: In optimization stage of the experimental conditions for derivative ratio spectrophotometry, the effect of Δλ and scaling factor (SF) were examined to find the best derivative spectra. The most suitable values of Δλ were found to be 4.0 and 8.0 for MOE and HCT drugs, respectively, for derivative ratio spectrophotometric method. These values of Δλ permitted a maximum sensitivity with highly smoothing spectra, which were needed in

such measurements. It was found that SF values vary according to the selected drug and they were 20 and 10 for MOE and HCT drugs, respectively, for derivative ratio spectrophotometric method.

Effect of divisor concentration: The absorption spectra of standard solutions of MOE drug with different concentrations (2.0 - 11.0 µg mL^{-1}) were recorded. The spectra obtained were divided several times by the spectrum of working standard solution of HCT each time with different concentrations 2.0, 4.0, 6.0 and 8.0 µg mL^{-1} in order to obtain the most suitable concentration used as a divisor in the determination of MOE ratio spectra. The first derivative then was applied under certain selected instrumental parameters as: Δλ=4, SF=20 and wavelength range of 200 ─ 320 nm. Similarly, the above studies were carried out on different concentrations of HCT drug (1.0 - 12.0 µg mL^{-1}). The spectra obtained were divided several times by the spectrum of working standard solutions of MOE each time with different concentrations 3.0, 6.0 and 10.0 µg mL^{-1} in order to obtain the most suitable concentration used as a divisor in the determination of HCT ratio spectra. The first derivative then was applied under certain selected instrumentation parameters as: Δλ=8, SF=10 and wavelength range of 200 ─ 360 nm. Finally, the first derivative of ratio spectra (^{1}DD) method was applied by choosing the concentration of 4.0 µg mL^{-1} for HCT (Figures 8 and 9) and 6.0 µg mL^{-1} for MOE (Figures 10 and 11) as a convenient divisor concentration for the determination of both MOE and HCT drugs, respectively, where they had a good signal to noise ratio with good slope and intercept values and more repeatable results.

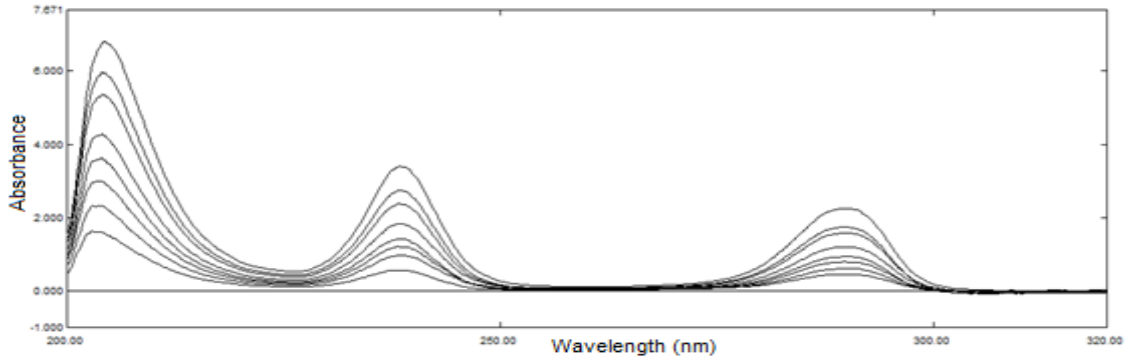

Figure 8. Ratio spectra for MOE drug (2 ─ 11 µg mL^{-1}) divided by zero-order absorption spectrum of HCT drug (4 µg mL^{-1}) as a best divisor

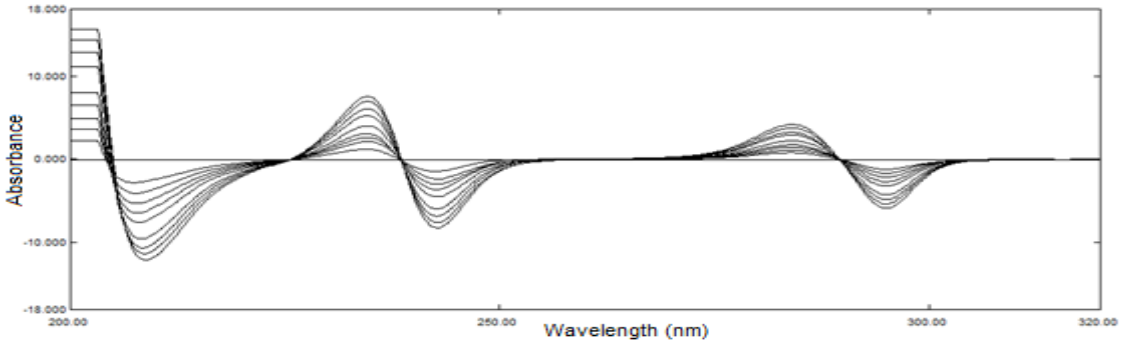

Figure 9. First derivative of ratio spectra ^{1}DD for different concentrations of MOE drug (2 ─ 11 µg mL^{-1}) using HCT drug (4 µg mL^{-1}) as a divisor

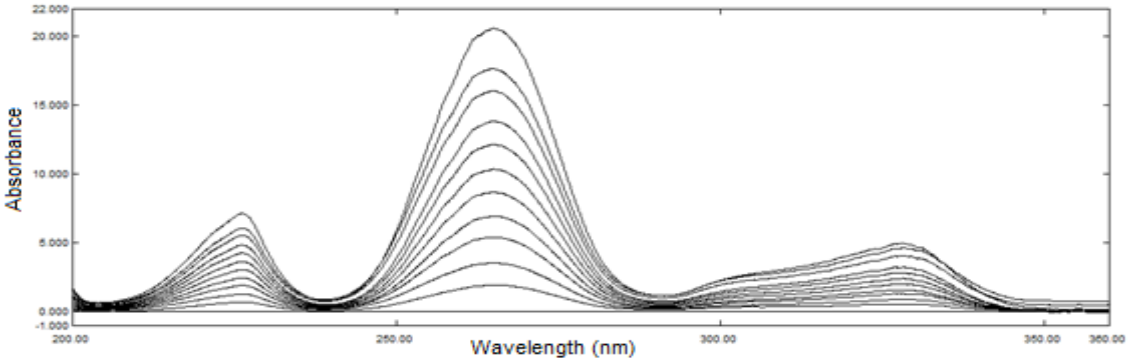

Figure 10. Ratio spectra for HCT drug (1 ─ 12 µg mL^{-1}) divided by zero-order absorption spectrum of MOE drug (6 µg mL^{-1}) as a best divisor

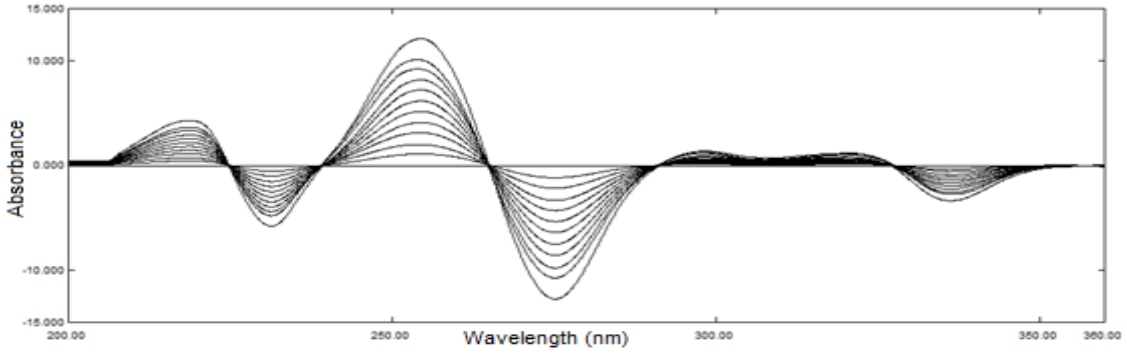

Figure 11. First derivative of ratio spectra ^{1}DD for different concentrations of HCT drug (1 ─ 12 µg mL^{-1}) using MOE drug (6 µg mL^{-1}) as a divisor

Method Validation

Linearity and selection of wavelengths: The linearity was evaluated for each drug by analyzing a series of different concentrations of each MOE and HCT drugs. Calibration curves were constructed by plotting the peak amplitudes at the selected wavelengths versus concentration ranges of the studied drugs. Linearity of the calibration curves was obeyed in the concentration range of 2.00-11.0 µg mL^{-1} for MOE at 242.6, 284.0 and 295 nm as shown in (Figure 9), and 1.00-12.0 µg mL^{-1} for HCT at 218.8, 231.3, 254.4, 275.2 and 336.1 nm as shown in (Figure 11). The following (Figures 12 and 13) were showing the calibration curves of MOE and HCT drugs, respectively, at each wavelength.

The analysis of these graphs using least squares method was made for the slope, intercept and correlation coefficient. Finally, the validation of ^{1}DD method for the determination of MOE and HCT drugs in a binary mixture was applied by choosing the working wavelengths at 295 nm for MOE and at 275.2 nm for HCT, where they had the most suitable values of the regression equations parameters. The analytical data of these wavelengths were grouped in Table 7. LOD and LOQ were determined according to ICH recommendation which were also grouped in Table 7.

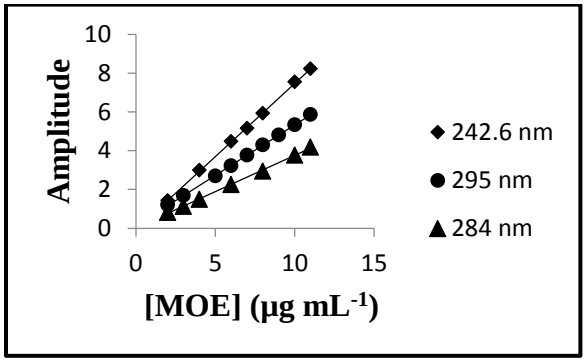

Figure 12. Calibration curves for the determination of MOE using HCT 4 µg mL^{-1} as a divisor in ^{1}DD method at 242.6, 295 and 284 nm

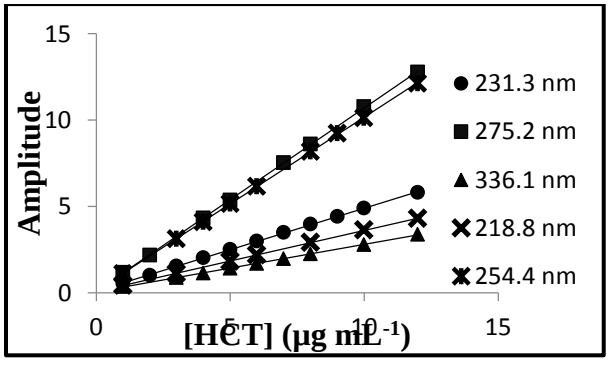

Figure 13. Calibration curves for the determination of HCT using MOE 6 µg mL^{-1} as a divisor in ^{1}DD method at 231.3, 275.2, 336.1, 218.8 and 254.4 nm

Precision: To measure the degree of method repeatability (intraday precision), three different concentrations were analyzed in triplicate within the same day and in three successive days (interday precision) for MOE and HCT drugs separately. The percentage relative standard deviation RSD were less than 2.0% which indicated high degree of precision of the proposed method as shown in Table 8.

Accuracy: The accuracy of the method was determined by calculating recoveries of MOE and HCT drugs by standard addition method. Known amounts of MOE and HCT drugs were added to a pre-quantified sample solution (primox plus tablets), and the amount of MOE and HCT drugs were estimated by measuring the peak areas and draw relation between concentration and area once when standard alone and the other when adding sample (Table 9 and Figure 14).

Table 7. Linearity parameters for the determination of MOE and HCT drugs using ^{1}DD method

Parameters	Moexipril HCl	Hydrochlorothiazide
Wavelength at peak amplitude (nm)	295	275.2
Linearity range, µg mL^{-1}	2.00-11.0	1.00-12.0
LOD, µg mL^{-1}	0.252	0.120
LOQ, µg mL^{-1}	0.832	0.396
Regression equation	Y=0.5203X+0.1246	Y=1.062X + 0.0843
Slope	0.5203	1.062
SE of slope	0.008767951	0.003577136
Intercept	0.1246	0.0843
SE of intercept	0.034634699	0.027224747
Regression coefficient (R^2)	0.9999	0.9998
SE of estimation	0.039657391	0.038534311

Table 8. Intraday and interday precisions for the determination of MOE and HCT drugs using ^{1}DD method

Drugs	Concentration (µg mL^{-1})	Intraday precision		Mean recovery% ±	Mean RSD%
		Amount Found [a] (µg	Recovery [a] %		

		mL^{-1})		**SD**	
MOE (295 nm)	3.00 5.00 8.00	2.983 4.946 7.960	99.44 98.91 99.50	99.28 ± 0.32	0.33
HCT (275.2 nm)	3.00 6.00 8.00	2.964 5.975 7.945	98.80 99.59 99.31	99.23 ± 0.40	0.40
		Interday precision			
MOE (295 nm)	3.00 5.00 8.00	3.010 4.965 8.025	100.33 99.30 100.31	99.98 ± 0.59	0.59
HCT (275.2 nm)	3.00 6.00 8.00	2.989 5.971 7.973	99.63 99.51 99.66	99.60 ± 0.08	0.08

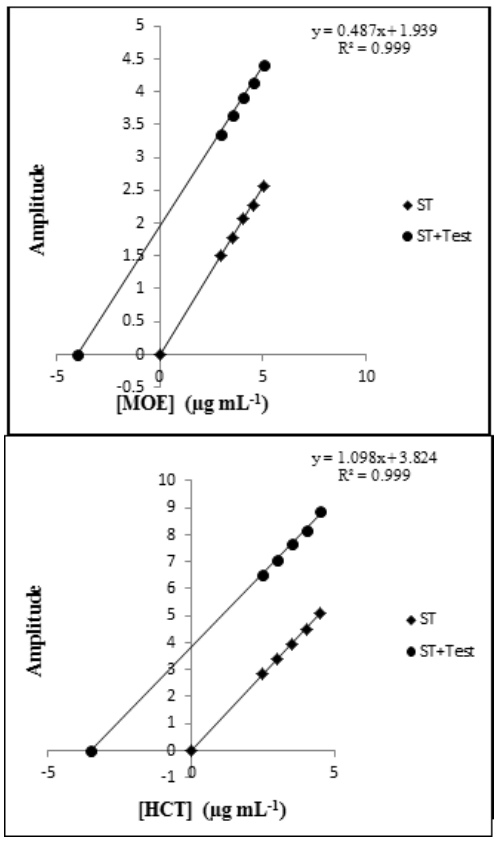

Figure 14. Standard addition plots of MOE and HCT drugs

Table 9. The standard addition method for the determination of MOE and HCT drugs using 1DD method

Drugs	Label claim/tablet (µg mL^{-1})	Amount of standard added (µg mL^{-1})	Amount found (µg mL^{-1})[a]	Recovery %[a]	Mean Recovery% ± SD	RSD (%)
MOE In Primox plus Tablets	4.0	3.0	2.98	99.48	100.36 ± 0.80	0.80
		3.5	3.798	99.95		
		4.0	4.05	101.27		
		4.5	4.498	99.96		
		5.0	5.06	101.14		
HCT In Primox plus	3.5	2.5	2.51	100.57	100.63 ± 0.76	0.76
		3.0	3.01	100.27		

Tablets		3.5	3.52	100.66		
		4.0	3.99	99.80		
		4.5	4.58	101.86		

Comparison of ^{1}DD method with HPLC method: By applying the proposed 1DD method in primox plus tablets for the analysis of MOE and HCT drugs simultaneously and the results obtained were statistically compared with those obtained by the HPLC method. The results obtained from both methods were shown in (Table 10).

Student's t-test and F-test values at 95% confidence level did not exceed their theoretical values of 2.228 and 5.05, respectively, indicating no significant difference between the performance of the methods regarding to accuracy and precision. The results were illustrated in Table 10.

Table 10. Statistical comparison between the results obtained by the proposed ^{1}DD method and the HPLC method for the analysis of MOE and HCT in primox plus tablets

Pharmaceuticals	**Parameters**	**MOE**		**HCT**	
		Proposed method	**Reported method**	**Proposed method**	**Reported method**
Primox plus Tablets	**Mean**	99.56	100.26	100.05	100.33
	SD	0.759	0.529	0.849	0.692
	Variance	0.577	0.280	0.722	0.479
	SE	0.839	0.585	0.651	0.531
	t-test	1.834	-	0.629	-
	F-test	2.059	-	1.506	-

Application to Serum and Urine

The proposed HPLC and ^{1}DD methods were applied to determine both MOE and HCT drugs in biological fluids such as human serum and urine. The results obtained are summarized in (Table 11). The results summarized showed that the proposed methods were accurate for the determination of MOE and HCT in urine and serum samples without interferences from the coformulated adjuvants as indicated by the percentage recovery values.

Table 11. Determination of both MOE and HCT drugs in human serum and human urine using HPLC and ^{1}DD methods

Sample		HPLC method			^{1}DD method		
		Mean recovery[a] (%)	Variance	RSD[a] %	Mean recovery[a] (%)	Variance	RSD[a] %
Human serum	MOE	97.8	0.063	0.257	96.2	0.058	0.221
	HCT	100	0.093	0.306	97.4	0.031	0.128
Human urine	MOE	99.5	0.106	0.327	98.5	0.072	0.284
	HCT	98.1	0.036	0.193	101.3	0.114	0.426

[a]Mean of three different samples for each concentration.

CONCLUSION

The developed HPLC and 1DD methods for the determination of MOE and HCT drugs in pharmaceutical formulation were linear, reproducible, accurate and specific. The methods were validated to the requirements of ICH guidelines and the results were satisfactory. The developed analytical methods can be easily used for the routine analysis particularly when large numbers of samples were encountered. The developed methods were found to be specific as there was no interference of the excipients. The developed methods offered several advantages such as rapid, cost effective, simple sample preparation steps and sensitive.

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