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Short communication

Simultaneous determination of moexipril hydrochloride and hydrochlorothiazide in tablets by derivative spectrophotometric and high-performance liquid chromatographic methods

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Abstract

Two new simple and selective assay methods have been presented for the binary mixtures of moexipril hydrochloride (MOEX) and hydrochlorothiazide (HCTZ) in pharmaceutical formulations. The first method depends on second-derivative ultraviolet spectrophotometry with zero-crossing measurements at 215 and 234 nm for MOEX and HCTZ, respectively. The assay was linear over the concentration ranges 1.0–11.0 μg ml^{-1} for MOEX and 0.5–9.0 μg ml^{-1} for HCTZ. The determination limits for MOEX and HCTZ were found to be 1.0 and 0.5 μg ml^{-1}, respectively; while the detection limits were 0.2 μg ml^{-1} for MOEX and 0.1 μg ml^{-1} for HCTZ. The second method was based on isocratic reversed-phase liquid chromatography by using a mobile phase acetonitrile–20 mM phosphate buffer (pH 4.0) (50:50, v/v). Lisinopril was used as an internal standard (IS) and the substances were detected at 212 nm. The linearity range for both drugs was 0.5–12.0 μg ml^{-1}. The determination and detection limits were found to be 0.100 and 0.010 μg ml^{-1} for MOEX and 0.025 and 0.005 μg ml^{-1} for HCTZ, respectively. The proposed methods were successfully applied to the determination of these drugs in synthetic mixtures and commercially available tablets with a high percentage recovery, good accuracy and precision.

Keywords: Moexipril hydrochloride; Hydrochlorothiazide; UV-derivative spectrophotometry; High-performance liquid chromatography

1. Introduction

Moexipril hydrochloride (MOEX), (3S)-2-[(2S)-2-{[(1S)-1-(ethoxycarbonyl)-3-phenyl-propyl]amino}-1-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (Fig. 1a), is a new potent orally active non-sulfhydryl angiotensin-converting enzyme

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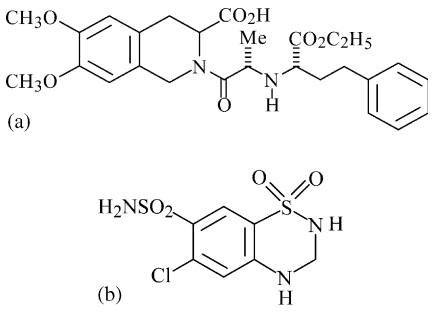

Fig. 1. Chemical structure of MOEX (a) and HCTZ (b).

(ACE) inhibitor for the treatment of hypertension and congestive heart failure [1]. MOEX is administered alone or together with antihypertensive, diuretic hydrochlorothiazide (HCTZ). Combined therapy with MOEX and HCTZ had a significantly greater blood pressure reduction than with the same dosage of the drug alone [1].

HCTZ, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide (Fig. 1b), is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical preparations which decreases active sodium reabsorption and reduces pheripheral vascular resistance.

Only a gas chromatographic-mass spectrometric method [2] has been reported for MOEX and its active metabolite moexiprilate in human plasma. On the other hand, several analytical procedures have been described for the individual determination of HCTZ, by using electrochemical [3,4] spectrophotometric [5–7], flow injection [8] and HPLC methods [9,10] and jointly with other pharmaceutical substances including spectrophotometric [11,12], polarographic [13], HPTLC-dansitometric [14] and HPLC [14,15] procedures.

So far, no method has been described for the determination of MOEX in pharmaceutical formulations. Therefore, it is required simple, precise, accurate and reliable methods that can be applied in quality control laboratories for its determination alone or in the presence of HCTZ. For this porpuse, UV-derivative spectrophotometric and liquid chromatographic assay methods have been developed in this study. These methods were especially chosen because of their usage in the determination of drugs in pharmaceutical preparations in pharmacopoeias.

2. Experimental

2.1. Chemicals

MOEX (99.85%, Batch No: 2010620151) was kindly supplied by Adeka Pharm. Ind. (Istanbul, Turkey). HCTZ (99.99%, Batch No: NX019-Re-1) and internal standard (IS), lisinopril (99.44%, Batch No: 3000699001) were obtained from Bristol-Myers Squibb Co. (Istanbul, Turkey) and Sanovel Pharm. Ind. (Istanbul, Turkey), respectively. Commercially available Uniretic® tablets are products of Schwarzpharma (Germany); each tablet was labelled to contain 7.5 mg of MOEX and 12.5 mg of HCTZ.

Analytical grade potassium dihydrogen phosphate, phosphoric acid, methanol and HPLC grade acetonitrile were purchased from Merck (Darmstadt, Germany). Bidistilled water was used.

2.2. Apparatus

A Shimadzu UV-160 A UV-visible spectrophotometer was used under the following operating conditions; derivative mode ^2D ($d^2A/d\lambda^2$), scan speed 1500 nm min^{-1}, scan range 200–350 nm, slit width 2 nm and derivation interval ($\Delta\lambda$) 2.8 nm. Derivative spectra were automatically obtained using 1 cm quartz cell by Shimadzu UV-160 A system software.

The HPLC system (Therma Separation Products, Texas, USA) consisted of P 4000 solvent-delivery system equipped with a Reodyne injection valve with a 20 μl loop and UV 3000 detector (Therma Separation Products) set at 212 nm. Integrations and system parameters were controlled by the SN-4000 automation system software (Therma Separation Products). Chromatographic separation was achieved isocratically on a Luna C_{18} column (250 × 4.6 mm I.D., 5 μm, Phenomenex) fitted with guard column (4 × 3 mm I.D., Phenomenex) packed with the same material and maintained at ambient temperature. The mobile phase was 20 mM phosphate buffer

(pH 4.0)–acetonitrile (50:50, v/v) with a flow rate of 1.0 ml min^{-1}.

2.3. Solutions

About 25.0 mg of MOEX and HCTZ were accurately weighed and dissolved in 50 ml of methanol. Two series standard solutions were obtained by diluting of the stock solutions.

IS stock solution was prepared by dissolving 50.0 mg of lisinopril in 50 ml of methanol. Appropriate dilution was made to obtain the working solution (100 μg ml^{-1}).

The stock solutions were stored at 4 °C and were stable for a month.

Phosphate buffer was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in 500 ml of water. The pH was adjusted to 4.0 with 50% phosphoric acid and the volume was made up to 1000 ml with water.

2.4. Procedures

2.4.1. Derivative spectrophotometry

2.4.1.1. Calibration. Appropriate volumes of the stock solutions were transferred into two sets of 10 ml calibrated flasks and the volumes were adjusted with methanol. The first series contained varying concentrations of MOEX (1.0, 2.0, 4.0, 8.0 and 11.0 μg ml^{-1}) and a constant concentration of HCTZ (5 μg ml^{-1}). The second series contained varying concentrations of HCTZ (0.5, 1.0, 2.0, 4.0, 8.0 and 11.0 μg ml^{-1}) and a constant concentration of MOEX (5 μg ml^{-1}). The second derivative spectra (^2D) of these standard solutions were scanned against a methanol blank between 200 and 320 nm. The values of the derivative amplitudes at 215 nm ($^2D_{215}$, zero-crossing point of HCTZ) and 234 nm ($^2D_{234}$, zero-crossing point of MOEX) were measured for the determination of MOEX and HCTZ, respectively. The concentrations of each compound versus their derivative amplitudes were plotted in order to obtain the calibration graphs.

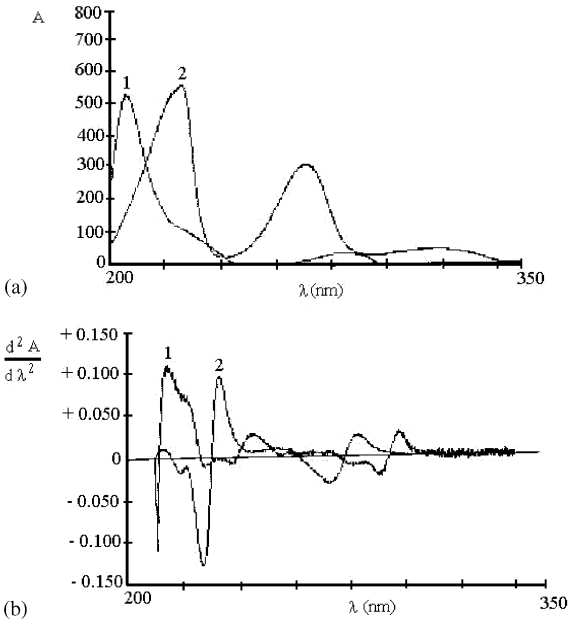

Fig. 2. (a) Zero-order absoption and (b) second-derivative spectra of MOEX (1) and HCTZ (2) (5 μg ml^{-1} of each two drugs in methanol).

2.4.2. HPLC method

2.4.2.1. Calibration. Standard solutions of MOEX and HCTZ were prepared by diluting of the stock solutions with mobile phase. The final concentrations of each drug were the same for 0.5–12.0 μg ml^{-1}. These solutions also contained IS at 7.5 μg ml^{-1}. HPLC analysis was carried out with 20 μl aliquots of the standard solutions under the conditions described above. The chromatograms were evaluated on the basis of MOEX or HCTZ/IS ratios of the peak areas.

2.5. Assay of pharmaceutical preparations

Ten tablets were separately weighed and finely powdered. About 60 ml of methanol was added to accurately weighed amount of the powder equivalent to the median mass of one tablet in a 100 ml calibrated flask. The mixture was shaken mechanically and sonicated in ultrasonic bath totally 30 min and diluted to volume with methanol and then filtered using 0.45 μm nylon syringe filter (Titan, Eatontown, NJ).

For the derivative spectrophotometric measurements appropriate dilutions were made with methanol so that the final concentrations of MOEX and HCTZ were 3.0 and 5.0 μg ml^{-1}, respectively.

For the HPLC measurements the filtrate was diluted with the mobile phase to give a final concentration of 6.0 μg ml^{-1} of MOEX and 10.0 μg ml^{-1} of HCTZ. This solution also contained 7.5 μg ml^{-1} of IS.

The sample solutions were assayed using the procedures described above and the quantity of MOEX and HCTZ were calculated from the regression equations constructed for both the methods.

3. Results and discussion

Direct UV-absorption measurements were found to be inapplicable to the analysis of MOEX and HCTZ in binary mixtures because of the spectral interference (Fig. 2a). Derivative spectrophotometry is a favourable technique to solve this problem. Therefore, first, second, third and fourth derivative spectra of methanolic solutions of the two drugs were recorded. The second derivative spectra presented spectral features which can be used for the simultaneous determination of MOEX and HCTZ (Fig. 2b). The zero-crossing method was used in this study with satisfactory results. The experiments showed that the second derivative amplitudes at 215 and 234 nm are proportional to the MOEX and HCTZ concentrations, respectively.

Calibration curves were constructed by plotting ^2D ($d^2A/d\lambda^2$) values against corresponding concentrations in the range of 1.0–11.0 μg ml^{-1} for MOEX and 0.5–9.0 μg ml^{-1} for HCTZ. The regression equations were calculated as $^2D_{215} = 0.0248\ C + 0.0198$ ($r = 0.9999$) for MOEX and $^2D_{234} = 0.1063\ C - 0.0014$ ($r = 0.9999$) for HCTZ.

As a second simultaneous analysis method for these drugs and to check the UV-derivative spectrophotometric results, an HPLC method was also developed. The optimum chromatographic conditions were examined to get a good separation of the drugs. Successful attempts were performed isocratically on a reversed-phase column. The best chromatographic separation was obtained with acetonitrile–20 mM phosphate buffer (pH 4.0) (50:50, v/v) mobile phase system. UV-detector was set at 212 nm where the two drugs have the same molar absorbtivity. Retention times were 4.47 and 3.36 min for MOEX and HCTZ, respectively, and 2.1 min for IS (Fig. 3).

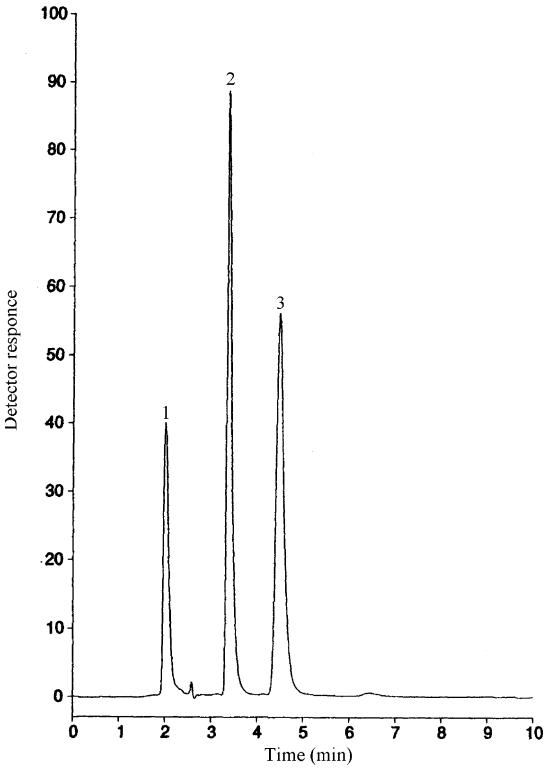

Fig. 3. Chromatogram obtained with the mixture of IS (1), HCTZ (2) and MOEX (3) (7.5 μg ml^{-1} of IS, 8 μg ml^{-1} of HCTZ and MOEX).

The equations of the calibration curves were obtained from linear regression analysis of the peak area ratios of MOEX or HCTZ to IS versus the concentration. The linearity was observed in the concentration range of 0.5–12.0 μg ml^{-1} for each of the two drugs. Regression equations of the calibration curves for MOEX and HCTZ were calculated as $A_{MOEX}/A_{IS} = 0.2651C - 0.0067$ ($r = 0.9999$) and $A_{HCTZ}/A_{IS} = 0.2506\ C - 0.0094$ ($r = 0.9999$), respectively.

Table 1
Recovery data obtained different mixtures by using derivative spectrophotometric method

Mixture number	MOEX[a]			HCTZ[b]		
	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)
1	2.00	1.96	98.00	9.00	9.09	101.00
2	3.00	2.98	99.33	5.00	5.05	101.00
3	4.50	4.50	100.00	7.50	7.50	100.00
4	9.00	9.07	100.78	3.00	2.98	99.33
5	8.00	8.01	100.13	4.00	4.02	100.50
6	4.00	4.04	101.00	8.00	8.06	100.75
7	10.00	9.93	99.30	1.00	1.02	102.00
8	11.00	10.83	98.46	6.00	6.04	100.67
9	6.00	6.03	100.50	7.00	7.03	100.43
10	7.50	7.58	101.07	1.50	1.52	101.33

[a] $x = 99.86$; R.S.D. = 1.06.
[b] $x = 100.70$; R.S.D. = 0.72.

Table 2
Recovery data obtained different mixtures by using HPLC method

Mixture number	MOEX[a]			HCTZ[b]		
	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)
1	1.00	0.95	95.00	12.00	11.71	97.58
2	2.00	1.99	99.50	12.00	12.05	100.42
3	3.00	2.96	98.67	5.00	4.83	96.60
4	4.00	4.07	101.75	8.00	8.03	100.38
5	5.00	5.05	101.00	7.00	7.08	101.14
6	6.00	6.03	100.50	10.00	10.09	100.90
7	7.00	7.03	100.43	11.00	11.01	100.09
8	8.00	7.99	99.88	4.00	3.98	99.50
9	9.00	9.01	100.11	3.00	2.89	96.33
10	12.00	11.89	99.08	1.00	1.04	104.00

[a] $x = 99.59$; R.S.D. = 1.86.
[b] $x = 99.69$; R.S.D. = 2.05.

Under the experimental conditions used, the determination limits for MOEX and HCTZ were found to be 1.0 and 0.5 μg ml^{-1}, respectively; while the detection limits were 0.2 μg ml^{-1} for MOEX and 0.1 μg ml^{-1} for HCTZ for derivative spectrophotometric method. The determination and detection limits were found to be 0.100 and 0.010 μg ml^{-1} for MOEX and 0.025 and 0.005 μg ml^{-1} for HCTZ, respectively, for HPLC method.

In order to assess the validity and applicability of the proposed methods, recovery studies were performed by analysing synthetic mixtures of each drug in different ratios. The mean percentage recoveries ($\pm$R.S.D.) were found to be 99.86$\pm$1.06 for MOEX and 100.70$\pm$0.72 for HCTZ in derivative spectrophotometric (Table 1), 99.59$\pm$1.86 for MOEX and 99.69$\pm$2.05 for HCTZ in HPLC (Table 2) methods.

The precision and accuracy of the methods were assessed by carrying out six replicate determinations of three different concentrations (high, medium and low) of MOEX and HCTZ both on within-day and day-to-day (Table 3). RSD values were less than 3.06 and 3.57% for derivative

Table 3
Within-day and day-to-day precision and accuracy the derivative spectrophotometric and HPLC methods ($n = 6$)

Drugs	Derivative spectrophotometric method				HPLC method			
	Added (μg ml^{-1})	Found (μg ml^{-1})	R.S.D. (%)	RME (%)	Added (μg ml^{-1})	Found (μg ml^{-1})	R.S.D. (%)	RME (%)
MOEX	Within-day				Within-day			
	3.00	2.99	1.10	−0.33	2.00	2.01	2.20	0.50
	7.50	7.58	0.49	1.07	6.00	6.02	1.64	0.33
	11.00	10.92	1.03	−0.73	12.00	11.99	1.96	−0.08
	Day-to-day				Day-to-day			
	3.00	2.98	1.78	−0.67	2.00	1.99	3.50	−0.50
	7.50	7.59	1.15	1.20	6.00	6.05	1.93	0.83
	11.00	10.93	1.04	−0.73	12.00	11.97	2.38	−0.25
HCTZ	Within-day				Within-day			
	1.50	1.52	3.06	1.33	3.00	2.92	3.00	−2.61
	5.00	5.03	0.97	0.60	8.00	8.04	3.57	0.50
	9.00	9.09	0.85	1.00	12.00	12.03	0.47	0.25
	Day-to-day				Day-to-day			
	1.50	1.53	2.71	2.00	3.00	2.93	2.72	−2.33
	5.00	5.03	1.23	0.60	8.00	8.01	3.33	0.13
	9.00	9.09	1.12	1.00	12.00	12.00	1.35	−0.00

Table 4
Assay results of tablets containing 7.5 mg MOEX and 12.5 mg HCTZ[a]

Drugs	Statistical value	Derivative spectrophotometric method		HPLC
MOEX	Mean	7.43		7.44
	S.D.	0.10		0.06
	R.S.D.	1.35		0.81
	t		0.20	
	F		2.78	
HCTZ	Mean	12.62		12.70
	S.D.	0.07		0.07
	R.S.D.	0.55		0.55
	t		2.08	
	F		1.00	

[a] $n = 6$; $P = 0.05$; $t = 2.23$; $F = 5.05$.

spectrophotometric and HPLC methods, respectively, indicating good precision and there was no significant difference between the assays which were tested using the both methods on the same day or different days. The relative mean errors were less than 1.67 and −2.61% for derivative-spectrophotometric and liquid chromatographic methods, respectively, indicating good accuracy.

The proposed methods were applied to the determination of MOEX and HCTZ in tablets and the results were statistically compared with each other using Student's *t*- and *F*-ratio tests (Table 4). At 95% confidence level there was no significant difference between two methods with the respect of mean values and standard deviations (S.D.).

4. Conclusion

This is for the first time that UV-derivative spectrophotometric and liquid chromatographic methods are being reported for the determination of MOEX alone or together with HCTZ in the

pharmaceutical formulations. HPLC (0.1 μg ml^{-1}) is ten times more sensitive than derivative spectrophotometric method (1.0 μg ml^{-1}) for MOEX whereas first method (0.025 μg ml^{-1}) is 20 times more sensitive than the latter (0.5 μg ml^{-1}) for HCTZ. There is no significant difference in terms of precision between the both methods. Only a few minutes (< 5.0 min) are required for the analysis in the developed methods. Being simple, specific, of good accuracy and high precision, the proposed methods are suitable for the routine analysis and quality control of these drugs in tablets. Additionally, the high separation efficiency of the HPLC method may be an advantage for the analysis of MOEX and HCTZ in biological samples. This matter is subjected to investigation.

Acknowledgements

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