ISSN : 0974-7419 Volume 14 Issue 10

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 14(10) 2014 [369-377]

Stability study of the angiotensin converting enzyme inhibitor moexipril and its simultaneous determination with hydrochlorothiazide and moexiprilat by reversed phase chromatography in pharmaceutical formulation and plasma

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ABSTRACT

A sensitive, reproducible, and rapid stability indicating RP-HPLC method was developed and subsequently validated for simultaneous determination of moexipril (MOX), its active metabolite moexiprilat (MOXT), and hydrochlorothiazide (HCTZ) in bulk powder, pharmaceutical formulation, and human plasma, using benazepril (BENZ) as an internal standard (IS). The method uses Inertsil C18 column (250 x 4.6mm, 5μ) and acetonitrile-potassium hydrogen phosphate buffer pH = 6.2 (40/60 v/v) as a mobile phase. The flow rate was 1.0 mL/min and the detection wavelength was 282 nm. A detailed validation of the method was performed following the ICH guidelines and the standard curves were found to be linear in the range of 5-100, 5-100, and 1–100 μg/mL for MOX, MOXT, and HCTZ, respectively. Statistical comparison was done between the proposed method and the reported one where no significant difference was found between the two methods.

KEYWORDS

Moexipril;
Moexiprilat;
Hydrochlorothiazide;
Stability indicating;
RP-HPLC.

INTRODUCTION

Moexipril (3S)-2-[(2S)-2-[[(1S)-1-(Ethoxycarbonyl)-3–phenylpropyl]amino]-1–oxopropyl]-1,2,3,4–tetrahydro–6,7–dimethoxy–3–isoquinolinecarboxylic acid[1] is an antihypertensive drug, which belongs to the group of angiotensin convertase inhibitors. Moexipril hydrochloride is a long-acting nonsulfhydryl angiotensin-converting enzyme (ACE) inhibitor, developed for the treatment of hypertension and congestive heart failure. 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[2,3].

Diuretics, in particular Hydrochlorothiazide (HCTZ), are often used in association with other drugs in the management of hypertension in patients with ischemic heart disease. Thiazides affect the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride in approximately

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equivalent amounts. Indirectly, the diuretic action of HCTZ reduces plasma volume, with consequent increase in urinary potassium loss, plasma renin activity, aldosterone secretion and decrease in serum potassium[4].

It is reported that MOX was identified by HPLC[5-7], spectrophotometry[8,9], gas chromatography mass spectrometry[10], and liquid chromatography tandem mass spectrometry[11].

Also HCTZ was determined simultaneously with other combinations by HPLC[12-16], or spectrophotometry[17-19].

Primox® plus, a drug in the Egyptian market, is a combination of MOX and HCTZ and is used for the treatment of hypertension. Literature survey revealed that there are no available reported methods for simultaneous determination of Moexipril, its active metabolite Moexiprilat, and Hydrochlorothiazide. So, there was a need for a rapid, accurate, and reproducible method for simultaneous determination of the mixture.

In this study, Moexipril was subjected to acidic, basic, and oxidative degradation, where all the degradation products were separated completely from the studied analytes. Also, a complete structure elucidation of the degradation products was done. The manuscript also describes simultaneous quantitation of MOX, MOXT, and HCTZ in bulk powder, pharmaceutical formulation, and application of the method to human plasma.

EXPERIMENTAL

Materials and reagents

- MOX, HCTZ, and BENZ (IS) were kindly supplied from National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). The purity of the standards was certified to be higher than 99%. Structures of the compounds are shown in Figure 1.
- Primox® plus commercial tablets, labeled to contain 15 mg MOX and 25 mg HCTZ batch number GN 520034, GN 520047, GN 520080
- Methanol: HPLC-grade (Sigma-Aldrich).
- Acetonitrile: HPLC-grade (Sigma-Aldrich).
- Double distilled water was used.
- Other reagents were of analytical-grade.

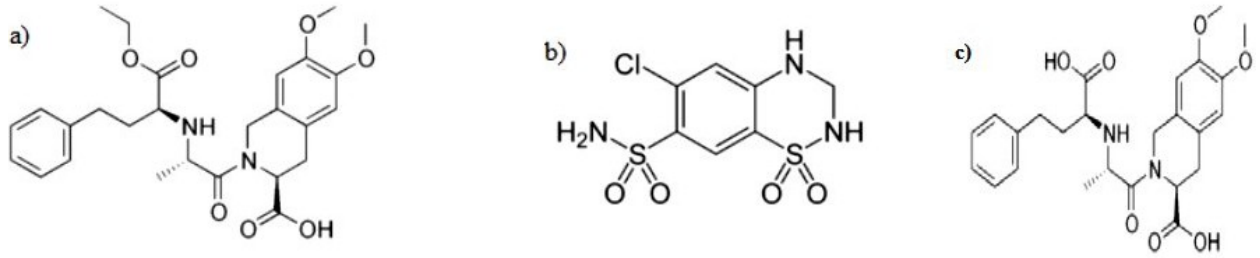

Figure 1 : Chemical structure for a)Moexipril, b)Hydrochlorothiazide, c)Moexiprilat

Instrument

RP-HPLC

Analysis was performed on a chromatographic system Jasco LC-Net II/ADC (Japan) equipped with UV detector (UV-2070 plus), isocratic pump (PU-2080 plus) and 4-line degasser (DG-2080-54). A chromatographic separation was achieved by Inertsil C-18, 250 x 4.6mm, 5µ analytical column. Data acquisition was made with ChromNAV software.

Mass Spectrometer for structure elucidation of the degradation product

This is done by MS/MS detection in positive ion mode using a MDS Sciex (Foster City, CA, USA) API-3200 MS/MS triple quadrupole mass spectrometer, equipped with a Turbo ion spray interface at 350°C. The common parameters, nebulizer gas (GS1), heater gas (GS2) and collision activated dissociation gas (CAD), were set at 30, 40, and 5 psi, respectively. The compound parameters, declustering potential (DP), collision energy (CE) and collision exit potential (CXP) were set at 56, 31, and 12 V respectively. The analytical data were processed using Analyst software (version 1.4.2).

PROCEDURE

Stability study of MOX

Basic degradation

100 mg of Mox was weighed, transferred to a conical flask and 10 mL 0.1 N NaOH was added. Then the flask was refluxed for 0.5 hour. After reflux, the flask was neutralized by 0.1 N HCl, and completed to 100 mL by distilled water. The complete degradation was confirmed using HPLC and mass spectrometry.

Acidic degradation

100 mg of mox was weighed, transferred to a conical flask and 10 mL 0.1 N HCl was added. Then the flask was refluxed for 3 hours. After reflux, the flask was neutralized by 0.1 N NaOH, and completed to 100 mL by distilled water. The complete degradation was confirmed using HPLC and mass spectrometry.

Oxidative degradation

100 mg of mox was weighed, transferred to a conical flask and 20 mL 30% H_2O_2 was added. Then the flask was refluxed for 0.5 hour. After reflux, the flask was completed to 100 mL by distilled water. The complete degradation was confirmed using HPLC.

Chromatographic conditions

Separation of the three analytes and the IS was done using Inertsil C18 column (250 x 4.6mm, 5µ column) as a stationary phase. The mobile phase used was acetonitrile-potassium hydrogen phosphate buffer pH = 6.2 adjusted by 0.1 N NaOH (40/60 v/v). The flow rate was 1 mL/min and the detection wavelength was 282 nm.

Preparation of standard stock and working solutions

Primary stock solutions of MOX, HCTZ, and BENZ internal standard (IS) (all at 1.0 mg/mL) were separately prepared by dissolving 100 mg of each standard powder in the least amount of methanol and completed to the volume by distilled water, where MOXT was prepared by degradation [as explained earlier under basic degradation]. Primary stock solutions were diluted with the mobile phase to prepare standard working solutions of MOX, MOXT, HCTZ, and BENZ (100 µg/mL). All solutions were stored at 4°C, and equilibrated to room temperature before use.

Linearity and calibration standards of the pure bulk powder

Accurately measured aliquots of MOX, MOXT, and HCTZ were transferred from their working standard solution (0.1 mg/mL) into three series of 10-ml volumetric flasks and complete to volume with the mobile phase (acetonitrile-potassium hydrogen phosphate buffer (40/60 v/v) pH = 6.2 adjusted by 0.1 N NaOH). The calibration samples consist of six concentrations of MOX (5 – 100 µg/mL), MOXT (5-100 µg/mL), and HCTZ (1–100 µg/mL). The samples were injected separately along with BENZ (IS) into the Inertsil C18 column under a flow rate of 1 mL/min. The relative peak area of each analyte was recorded against its concentration, the linearity curves were constructed and the regression equations computed.

Validation

Accuracy

The accuracy of the results was checked by applying the proposed methods for determination of three replicates of different concentrations of the analytes. The concentrations were obtained from the correspond-

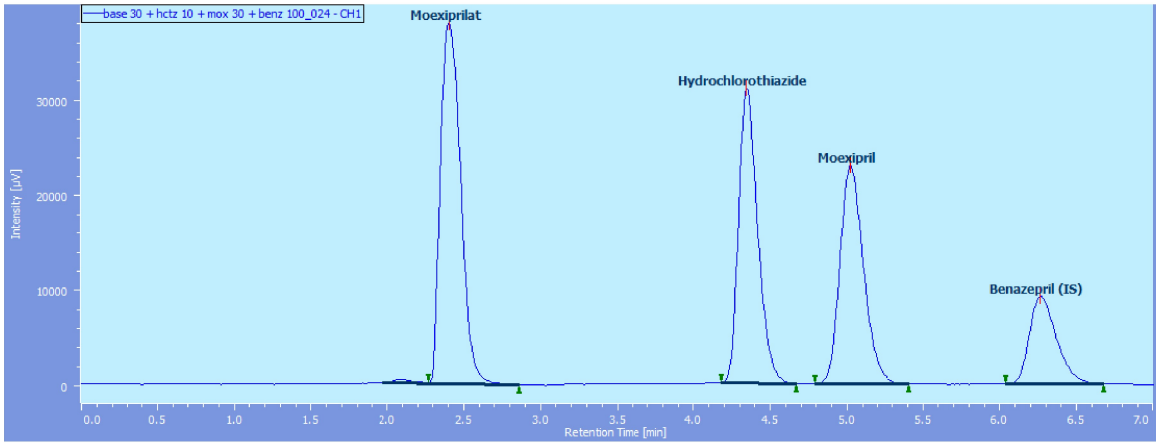

Figure 2 : HPLC chromatogram showing the complete resolution of the analytes

ing regression equations, from which the percentage recoveries suggested good accuracy of the proposed method.

Precision

Repeatability

Three concentrations of the analytes were analyzed three times intra-daily using the proposed method under the same experimental conditions. The relative standard deviations were calculated.

Reproducibility (Intermediate precision)

The previous procedures were repeated inter-daily on three different days for the analysis of the three chosen concentrations. The relative standard deviations were calculated.

Laboratory prepared mixtures (Selectivity)

Solutions containing different ratios of the analytes were prepared by transferring accurately measured aliquots from their standard working solutions into a series of 10-ml volumetric flasks and the volume was completed to the mark with mobile phase. The final concentration ranges were 5 - 20 µg/mL for MOX, 10 - 25 µg/mL for MOXT, and 5-20 µg/mL for HCTZ. The chromatograms of these different laboratory prepared mixtures were recorded and the procedure under linearity was then followed. Concentrations of the analytes in the prepared samples were calculated from the corresponding computed regression equations.

Application to pharmaceutical formulation

To determine the content of MOX and HCTZ in commercial tablets (Primox® plus) (each tablet labeled to contain 15 mg mox and 25 mg HCTZ), 20 tablets were weighed and finely powdered. A portion of powder equivalent to one tablet was weighed accurately and transferred to a 100-ml beaker. 50 ml of methanol was added, stirred using a magnetic stirrer for 15 min and filtered through 0.5µm Whatman filter paper into a 100-ml volumetric flask. The residue was washed three times each with 10 ml of methanol and the solution was completed to the mark with the same solvent. From the above prepared solution, further dilutions were prepared in the obtained linearity ranges using the mobile phase. The general procedure described under linearity was followed to determine the concentration of both drugs in the prepared dosage form solution. The analysis was done in triplicates. Concentrations of MOX and HCTZ in the prepared samples were calculated from the corresponding computed regression equations.

Application to spiked human plasma sample

Aliquot volumes from the standard working solutions of MOX, MOXT, and HCTZ were added on 500 µL human plasma, then 30 µL IS was added. Then, 3 mL of acetonitrile was added, the samples were mixed on a vortex for 1 min, followed by centrifugation for 10 min at 10,000 rpm. The organic phase solution was transferred to a clean tube and evaporated to dryness under a gentle stream of nitrogen gas at 40 æ%C. The residue was reconstituted with 100 µL mobile phase, and 20 µL was injected. Then, the same procedure was repeated but spiking was done after the extraction step to calculate the recovery %.

RESULTS AND DISCUSSION

This manuscript describes the use of a RP-HPLC method to quantify MOX, its active metabolite MOXT, and HCTZ in the pharmaceutical dosage form. Also the paper describes the application of the proposed method to determine the three analytes in spiked human plasma without interference form endogenous plasma constituents. All the determinations were done in a short run time and with high sensitivity.

Method development

Different organic modifiers proportions and different buffers with different pH's were tried. Our goal is to obtain optimum resolution, symmetric peak shape, reasonable run time, and best sensitivity. Methanol and acetonitrile were tried, methanol caused

TABLE 1: System suitability parameters of the proposed RP-HPLC method

Parameter	MOX	MOXT	HCTZ
Retention time	5	2.4	4.3
Resolution*	4.167	8.231	2.701
Tailing factor	1.235	1.368	1.33
Column Capacity	3.55	1.18	2.91
Column efficiency (No. of theoritical plates)	5238	1484	6017
HETP	0.005	0.017	0.004

*** Resolution is calculated relative to the next peak**

longer run time where the run time extended to more than 20 min. Phosphate buffer with different pH's (3, 5, 7) were tried. The optimum resolution and peak shape obtained with acetonitrile-potassium hydrogen phosphate buffer pH = 6.2 adjusted by 0.1 N NaOH (40/60 v/v) as a mobile phase. The flow rate for better resolution and rapid separation was adjusted to 1mL/min. Also, two types of stationary phases C8 and C18 were investigated for the optimum resolution of the analytes' peaks, however the more hydrophobic Inertsil C18 was found to be more superior in separating analytes' peaks in a reasonable run time. Full separation of the three peaks of MOX, MOXT, and HCTZ was obtained, where the retention time of the analytes were 5, 2.4, and 4.3 min., respectively, where the retention time for BENZ (IS) was 6.2 min. as shown in (Figuer 2). System suitability parameters are shown in TABLE 1.

Stability of MOEXIPRIL

Basic degradation

MOX with a molecular weight (499.4) is an ester pro-drug which is hydrolyzed in vivo by esterase enzyme to the pharmacologically active biacid MOXT with a molecular weight (471.0). So to obtain this active form, Hydrolysis of MOX was tried by exposing to 0.1 N NaOH and refluxing for 0.5 hour. In basic hydrolysis, the ester bond was broken, which was confirmed by MS. The complete degradation was confirmed by HPLC (with the disappearance of MOX peak at retention time 5. and appearance of the MOXT at retention time 2.4. (Figure 3), and mass spectrometry (with the appearance of m/z for MOXT at 471, and disappearance of m/z for MOX at 499.4) as shown in (Figure 4).

Acidic degradation

Hydrolysis of MOX was tried by exposing to 0.1 N HCl and refluxing for 3 hours. In acidic hydrolysis, the ester and amide bonds were broken, which was confirmed by MS. The complete degradation was confirmed by HPLC (with the disappearance of MOX peak at retention time 5. and appearance of two peaks at 2.1 and 3.8 for degradation product 1 and 2 (Figure 5), also mass spectrometry was done (with the appearance of m/z for degradation products 1 and 2 at 252.2 and 238.2, respectively, and disappearance of m/z for MOX at 499.4) as shown in (Figure 6).

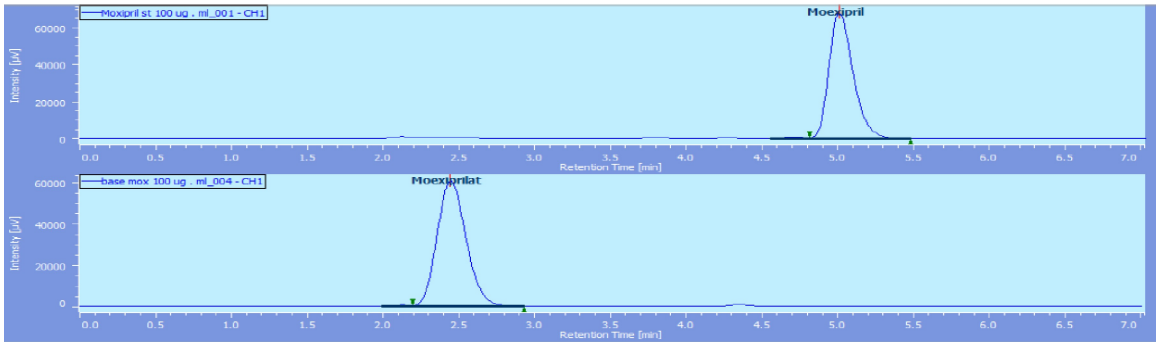

Figure 3 : HPLC chromatogram of MOX and MOXT, confirming the complete degradation of MOX by basic hydrolysis

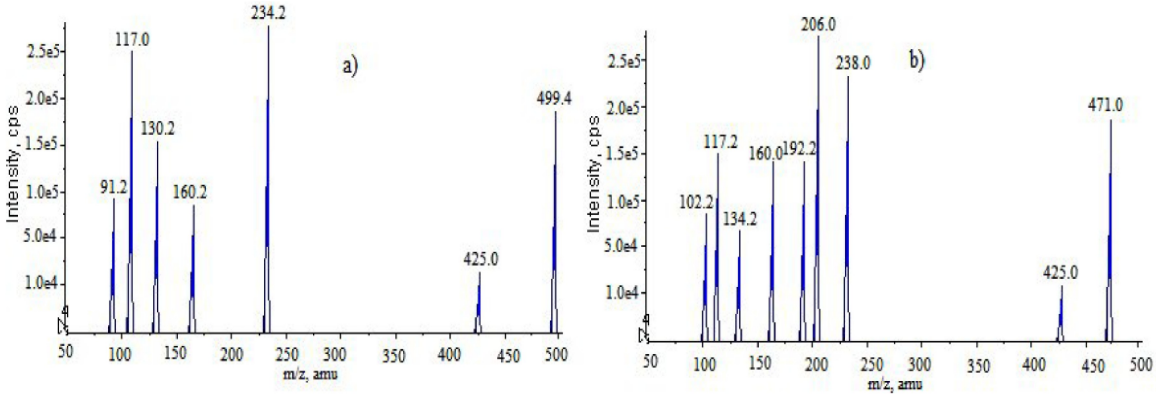

Figure 4 : MS spectra for a) Moexipril, and b) Moexiprilat

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Oxidative degradation

Degradation of MOX was tried by exposing to 30 % H_2O_2 and refluxing for 0.5 hour. The complete degradation was confirmed by HPLC (with the disappearance of MOX peak at retention time 5. and appearance of oxidative degradation products starting from retention time 1.9 (Figure 7).

Degradation Scheme for basic and acidic degradation pathway was suggested as shown in (Figure 8).

Validation

Validation of the proposed methods was assessed according to ICH guidelines[20]. Validation was done relative to linearity and range, accuracy, precision, and

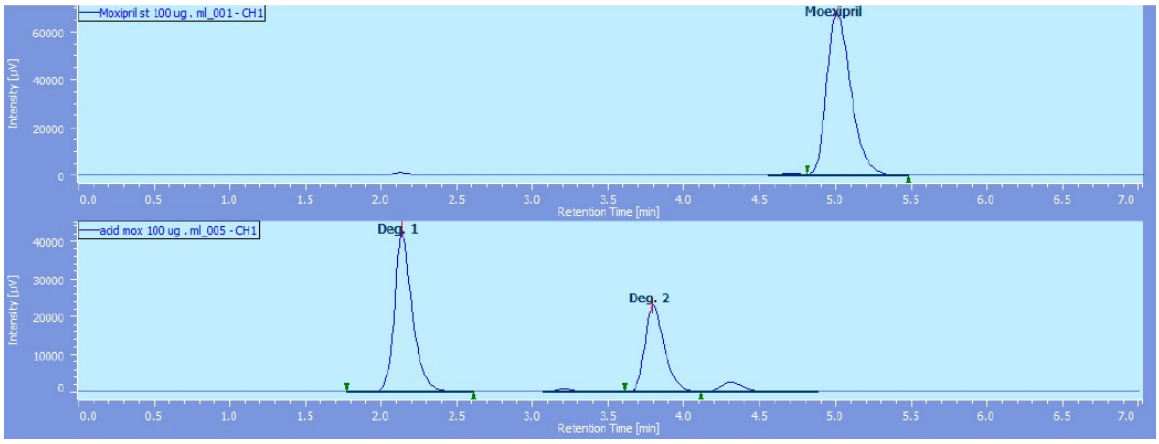

Figure 5 : HPLC chromatogram of MOX and MOX degradation products, confirming the complete degradation of MOX by acidic hydrolysis

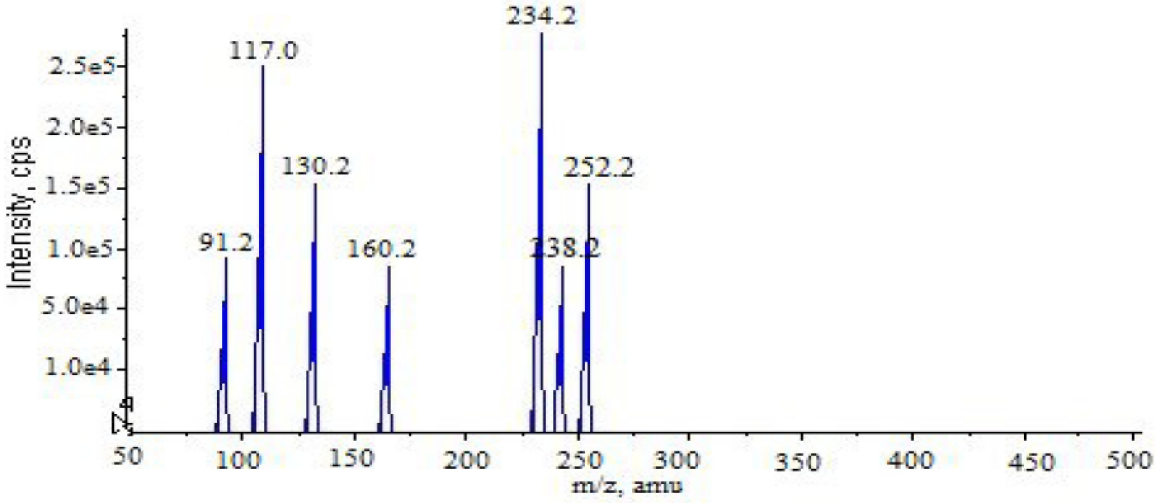

Figure 6 : MS spectrum for the acid degradation products of MOX

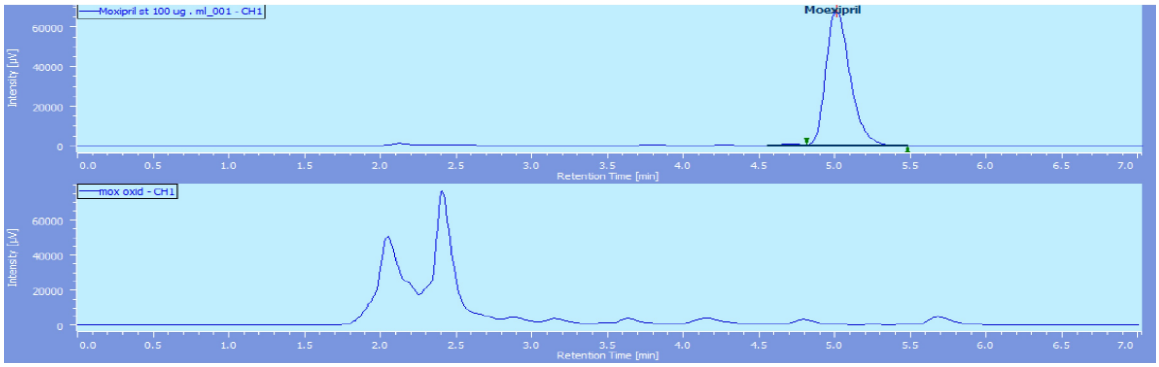

Figure 7 : HPLC chromatogram of MOX and oxidative degradation products showing complete degradation

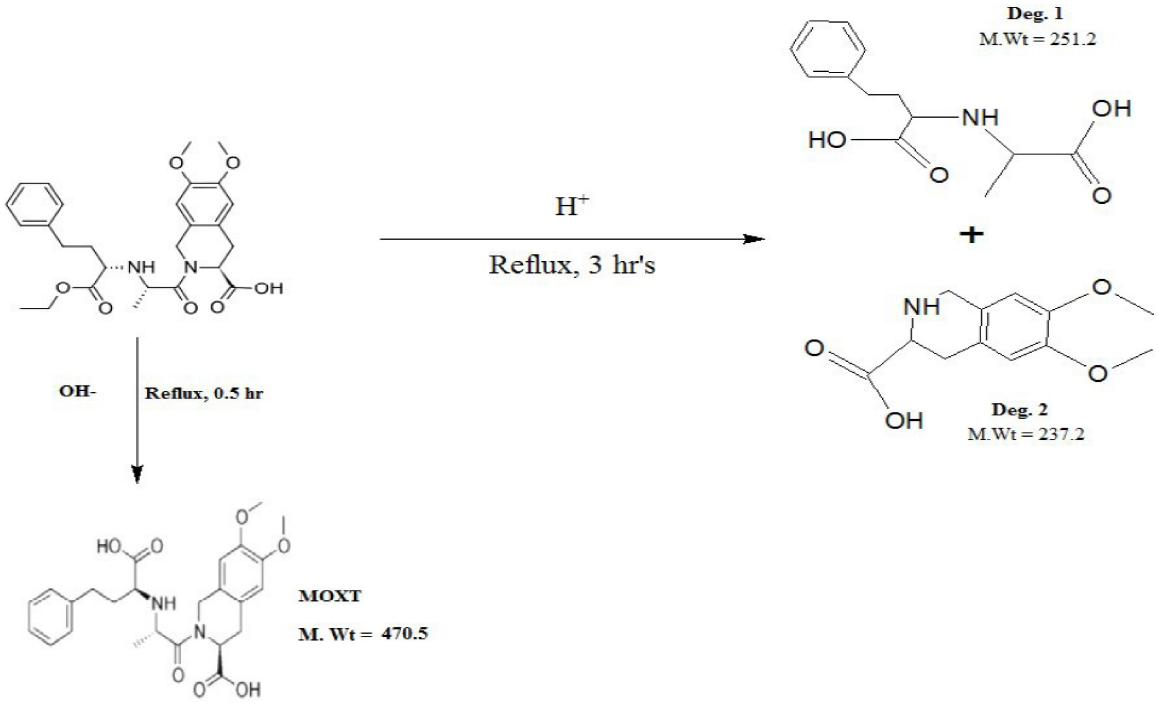

Figure 8 : Suggested degradation pathway for acidic and basic degradation of MOX

TABLE 2 : Validation parameters of the proposed chromatographic method

Parameter	MOX	MOXT	HCTZ
Range µg/mL	5-100	5-100	1-100
Regression Equation	y = 0.0354x + 0.0142	y = 0.0324x + 0.0171	y = 0.0724x + 0.0475
Correlation coefficient (r)	0.9998	0.9997	0.9999
Accuracy[a]	99.87 ± 1.024	100.27 ± 0.784	100.37 ± 1.241
Repeatability[b]	99.21 ± 1.541	100.74 ± 1.314	99.71 ± 1.284
RSD%	1.553	1.304	1.287
Intermediate precision[c]	99.74 ± 1.274	101.21 ± 1.342	101.41 ± 1.475
RSD%	1.277	1.325	1.454

a: Three concentrations of each analyte (10, 15, and 25 µg/mL), repeated three times for each concentration; b: Intra-day (n=3), average of three concentrations of the analytes (10, 15 & 25 µg/mL) repeated 3 times within the same day; c: Inter-day (n=3), average of three concentrations of the analytes (10, 15 & 25 µg/mL)) repeated 3 times in three consecutive days

TABLE 3 : Determination of the three analytes in their laboratory prepared mixtures by the proposed method

Conc µg/mL			MOX	MOXT	HCTZ
MOX	MOXT	HCTZ	(Recovery % ± SD)	(Recovery % ± SD)	(Recovery % ± SD)
20	10	5	100.75± 0.124	98.72 ± 0.514	100.47 ± 0.426
15	15	10	101.41 ± 0.251	99.52 ± 0.241	98.93 ± 1.463
10	20	15	99.35 ± 0.451	100.14 ± 0.841	99.25 ± 0.814
5	25	20	98.72 ± 0.364	99.47 ± 0.365	100.41 ± 0.647

All Calculations were done in triplicates

selectivity. All the validation parameters are shown in TABLES 2 and 3.

Application of the method in assay of tablets

The proposed method was applied for the determination of MOX and HCTZ in their combined pharmaceutical formulation (Primox® tablets). The validity of the methods was assessed by applying the standard addition technique (TABLE 4). It shows that the de-

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TABLE 4 : Determination of moexipril and hydrochlorothiazide in primox tablets by the proposed method and application of the standard addition technique

Product	Claimed	Standard addition			Recovery (Mean ± SD)	
Primox	µg/mL	Added µg/mL	Found µg/mL	Recovery %	Proposed method	standard addition
Moexipril	15	5	4.95	99.00	99.85 ± 0.412	99.84 ± 1.134
		10	9.94	99.40		
		15	15.17	101.13		
Hydrochlorothiazide	25	5	4.97	99.40	100.14 ± 1.364	100.59 ± 1.152
		10	10.17	101.70		
		15	15.1	100.67		

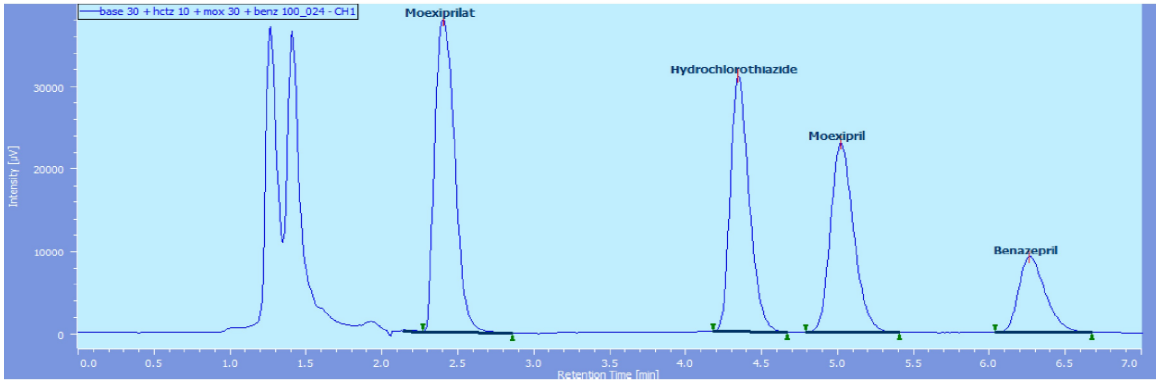

Figure 9 : HPLC chromatogram of spiked human plasma sample showing no interference

TABLE 5 : Determination of the analytes in spiked human plasma by the proposed RP-HPLC method

Spiked Concentration µg/mL			Recovery % ± SD*		
MOX	MOXT	HCTZ	MOX	MOXT	HCTZ
5	5	10	80.14 ± 4.712	84.21 ± 3.634	79.34 ± 3.532
15	15	20	80.47 ± 2.734	84.91 ± 2.647	78.47 ± 5.753
25	25	30	81.54 ± 5.796	83.71 ± 2.243	79.64 ± 5.724

***The mean percentage recovery of 3-separate determinations**

TABLE 6 : Statistical comparison for the results obtained by the proposed methods and the reference methods [16] for the determination of Moexipril and Hydrochlorothiazide in pure powder form

Parameter	Moexipril		Hydrochlorothiazide	
	Reference Method*	RP-HPLC	Reference Method*	RP-HPLC
Mean	99.54	99.87	99.25	100.37
S.D	0.985	1.024	0.895	1.241
N	6	6	6	6
Variance	0.970	1.049	0.801	1.540
Student t (2.23)		0.568		1.793
F test (5.05)		1.081		1.922

*** utilizes A column having 150 mm x 4.6 mm i.d. in isocratic mode with mobile phase containing acetonitrile:phosphate buffer (45:55; adjusted to pH 3) was used. The flow rate was 0.6 mL/min and effluent was monitored at 215 nm**

veloped methods are accurate and specific for determination of the cited drugs in presence of dosage form excipients.

Application to spiked human plasma sample

The chromatographic method was applied for the determination of MOX, MOXT, and HCTZ in biologi-

cal fluids. Drug free human plasma was obtained from healthy volunteer, and then Spiked with MOX, MOXT, and HCTZ. The method showed no interference from endogenous plasma constituents as shown in Figure 9. The recovery data are presented in (TABLE 5).

Statistical analysis

Results obtained by the proposed method for the determination of pure samples of MOX, MOXT and HCTZ are statistically compared to those obtained by the reported methods. The calculated t and F values were found to be less than their corresponding theoretical ones confirming good accuracy and excellent precision (TABLE 6).

Figures in parenthesis are the corresponding tabulated values at p = 0.05.

STABILITY

MOX, MOXT, and HCTZ working solutions showed no changes up to 2 weeks when stored at room temperature.

CONCLUSION

In summary, we developed and validated a highly sensitive, specific, reproducible and high-throughput stability indicating chromatographic methods for simultaneous quantification of MOX, MOXT, and HCTZ in bulk powder and pharmaceutical formulation. Also the chromatographic method was applied on spiked human plasma. According to the validation parameters, we concluded that the developed method could be useful for quality control laboratories and bioequivalent studies with desired precision and accuracy.

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