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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR MOEXIPRIL HYDROCHLORIDE IN BULK DRUGS AND PHARMACEUTICAL DOSAGE FORMS

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Abstract

The objective of the current study was to develop a validated, sensitive, specific and stability-indicating reverse phase HPLC method for the quantitative determination of Moexipril Hydrochloride and its potential impurities. The determination was done for active pharmaceutical ingredient and its pharmaceutical dosage form in the presence of degradation products, and its process-related impurities. The drug was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation per International Conference on Harmonization (ICH) prescribed stress conditions to show the stability-indicating power of the method. Significant degradation was observed during acid, base, oxidative, Thermal and photo stress studies. The chromatographic conditions were optimized using an impurity-spiked solution and the samples generated from forced degradation studies. In the developed HPLC method, the resolution between Moexipril Hydrochloride and its process-related impurities was found to be greater than 2.0. Regression analysis shows an r value (correlation coefficient) of greater than 0.999 for Moexipril Hydrochloride and it's all the seven impurities. The chromatographic separation was achieved on a phenyl stationary phase. The method employed a linear gradient elution and the detection wavelength was set at 210nm. Mobile phase consists of Solution A as buffer (10 mM Potassium dihydrogen phosphate, pH adjusted to 2.8 using phosphoric acid) and Solution B consists of acetonitrile and water in the ratio of 95:5 v/v, delivered at a flow rate of 1.2 mL min^{-1}. The HPLC gradient program was set as: time (min) / % Solution B: 0/10, 20/80, 20.1/10 and 25/10. The stress samples were assayed against a qualified reference standard and the mass balance was found to be close to 99.5%. The developed Reverse phase HPLC method was validated with respect to linearity, accuracy, precision and robustness.

Key words: Moexipril Hydrochloride; HPLC; Forced degradation; Validation; Stability indicating; LCMS.

1. Introduction

Moexipril hydrochloride is a potent orally active non-sulfhydryl angiotensin converting enzyme inhibitor (ACE) which is used for the treatment of hypertension and congestive heart failure, chemically as (3S)-2-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid and its structural formula is $C_{27}H_{34}N_2O_7.HCl$.

Moexipril is available as a prodrug moexipril hydrochloride, and is metabolized in the liver to form the pharmacologically active compound moexiprilat, caused by hydrolysis of an ethyl ester group [1] of Moexipril. Moexipril is incompletely absorbed after oral administration, and its bioavailability is low, accounting for 22% of unchanged drug [2]. The long pharmacokinetic half-life and persistent ACE inhibition of Moexipril, allows once-daily administration [3].

Few methods were available in literature for the analysis of Moexipril Hydrochloride. Much of the work in the literature has focused on the assessment of Moexipril and its metabolites and in other cases quantification of Moexipril in the formulations and in combination products, using various techniques like liquid chromatography, differential pulsed voltammetry, spectrophotometry and hyphenated techniques like liquid chromatography coupled with mass sectrometry [4-12].

No LC methods were reported in major pharmacopeia like USP, EP, JP and BP.

Extensive literature survey reveals there is no rapid stability-indicating LC method for determination of related substances and for quantitative estimation of Moexipril Hydrochloride in bulk drugs and pharmaceutical dosage forms. The purpose of the present research work was to develop a suitable, single and rapid stability-indicating HPLC method for the determination of Moexipril Hydrochloride and its related substances.

Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible method for the determination of Moexipril Hydrochloride and all the seven impurities in bulk drug samples and in pharmaceutical dosage forms along with method validation as per ICH norms. The stability tests were also performed on both drug substances and drug product as per ICH norms [13-18].

2. Experimental

2.1. Chemicals

Samples of Moexipril Hydrochloride and its related impurities were obtained as gratis sample from Sebondscience Labs (Hyderabad, India) (Fig.1). Commercially available 7.5 mg of Moexipril Hydrochloride tablets (Univasc®) were purchased. HPLC grade Acetonitrile, analytical reagent grade Potassium dihydrogen phosphate, and phosphoric acid were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples and impurities used in this study were of greater than 99.0% purity.

Fig.1: Chemical structures and labels of Moexipril Hydrochloride and its impurities.
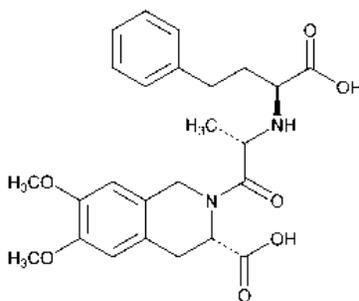
Moexipril Hydrochloride:
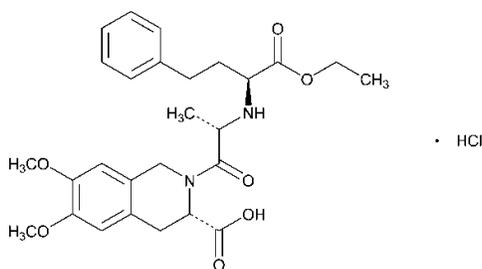
2-[2-(1-Ethoxycarbonyl-3-phenyl-propylamino)-propionyl]-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid, hydrochloride

Molecular Formula: $C_{27}H_{34}N_2O_7 \cdot HCl$
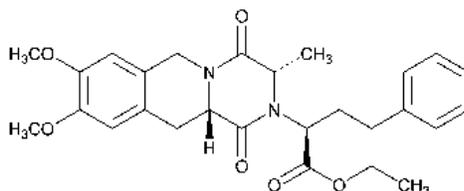
Molecular Weight: 535.03

Imp-1:

2-[2-(1-Carboxy-3-phenyl-propylamino)-propionyl]-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid

Molecular Formula: $C_{25}H_{30}N_2O_7$

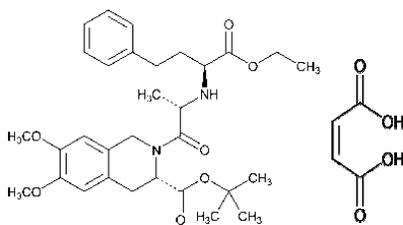Molecular Weight: 470.51

Imp-2:

2-(8,9-Dimethoxy-3-methyl-1,4-dioxo-1,3,4,6,11,11a-hexahydro-pyrazino[1,2-b]isoquinolin-2-yl)

-4-phenyl-butyric acid ethyl ester

Molecular Formula: $C_{27}H_{32}N_2O_6$

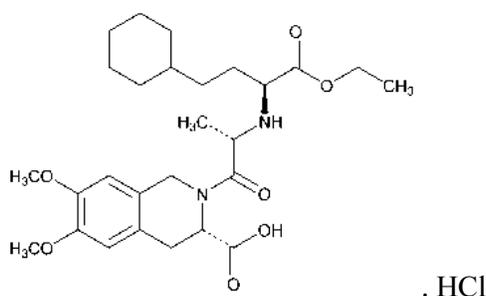Molecular Weight: 480.55

Imp-3:

2-[2-(1-Ethoxycarbonyl-3-phenyl-propylamino)-propionyl]-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid tert-butyl ester, maleate

Molecular Formula: $C_{31}H_{42}N_2O_7 . C_4H_4O_4$

Molecular Weight: 670.75

Imp-4:
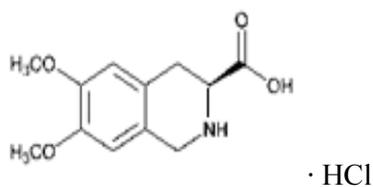

. HCl

2-[2-(3-Cyclohexyl-1-ethoxycarbonyl-propylamino)-propionyl]-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquin,

hydrochlorideoline-3-carboxylic acid

Molecular Formula: $C_{27}H_{40}N_2O_7 \cdot HCl$

Molecular Weight: 541.08

Imp-5:

· HCl

6,7-Dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid, hydrochloride

Molecular Formula: $C_{12}H_{15}NO_4 \cdot HCl$

Molecular Weight: 273.71

Imp-6:

2-(1-Carboxy-ethylamino)-4-phenyl-butyric acid ethyl ester

Molecular Formula: $C_{15}H_{21}NO_4$

Molecular Weight: 279.33

Imp-7:

.HCl

6, 7-Dimethoxy-2-[2-(1-methoxycarbonyl-3-phenyl-propylamino)-propionyl]-1,2,3,4-tetrahydro-isoquinoline-3-

carboxylic acid, hydrochloride

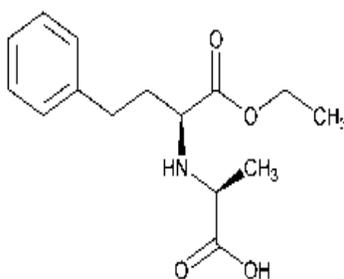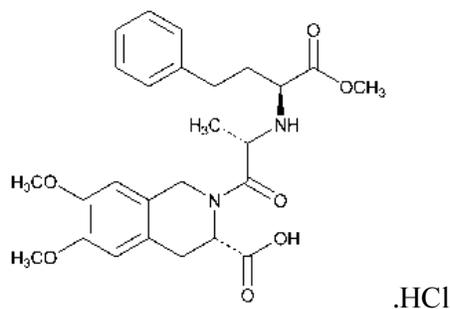Molecular Formula: $C_{26}H_{32}N_2O_7 \cdot HCl$

Molecular Weight: 521.00

2.2. Equipment

The HPLC system, used for method development, forced degradation studies and method validation was Waters 2695 binary pump plus auto sampler and a 2996 photo diode array detector with Empower software (Waters Corporation, MA, USA). The output signal was monitored and processed using Empower software on Pentium computer (Digital equipment Co). Waters Quattro Micro Triple quad LCMS was used with MassLynx software to monitor the output signal. Water bath equipped with temperature controller was used to carry out degradation studies for all solution. Photo stability studies were carried out in a photo stability chamber (Mack Pharmatech, Hyderabad, India). Thermal stability studies were performed in a dry air oven (Mack Pharmatech, Hyderabad, India).

2.3. Chromatographic Conditions

The chromatographic column used was Agilent Zorbax SB phenyl column (150 x 4.6) mm with 3.5 µm particles. Buffer consists of 10 mM Potassium dihydrogen phosphate, pH adjusted to 2.8 using phosphoric acid. Mobile phase consists of Solution A as buffer and Solution B consists of acetonitrile and water in the ratio of 95:5 v/v. The flow rate of the mobile phase was 1.2 mL min^{-1}. The HPLC gradient program was set as: time (min) / % solution B: 0/10, 20/80, 20.1/10 and 25/10. The column temperature was maintained at 35°C and the detection was monitored at a wavelength of 210 nm. The injection volume was 10 µL. Water was used as diluent. The concentration is 300 µg mL^{-1} for related substances method and 100 µg mL^{-1} for Assay method.

2.4. Preparation of Solutions

2.4.1. Preparation of Standard Solutions

A stock solution of Moexipril Hydrochloride (3.0 mg mL^{-1}) was prepared by dissolving appropriate amount in water. Working solutions were prepared from above stock solution for related substances determination and assay determination, respectively. A stock solution of impurities (mixture of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7) at a concentration of 300 µg mL^{-1} was prepared in a mixture of Water and Acetonitrile (90:10 v/v).

2.4.2. Preparation of Sample Solutions

Univasc® tablets contain 7.5 mg of Moexipril Hydrochloride. The inactive ingredients present in Univasc® were lactose, magnesium oxide, crospovidone, magnesium stearate and gelatin. The film coating contains hydroxypropyl cellulose, hypromellose, polyethylene glycol 6000, magnesium stearate, titanium dioxide, and ferric oxide. Twenty Univasc tablets (7.5 mg) were weighed and the average weight was calculated. The tablets were powdered in a

mortar and a sample of the powder equivalent to 30 mg of the active pharmaceutical ingredient (Moexipril Hydrochloride) was transferred to 100 mL volumetric flask. Approximately 75 mL water was added and the flask was placed on rotatory shaker for 10 min and sonicated for 10 min to dissolve the material completely. The solution was then diluted to 100 mL and centrifuged at 3,000 rpm for 10 min. The supernatant was collected and filtered through a 0.22 μm pore size Nylon 66-membrane filter. The filtrate was used as sample solution.

2.5. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the Moexipril Hydrochloride in the presence of its impurities namely imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 and degradation products was determined by developed HPLC method. Forced degradation studies were also performed on Moexipril Hydrochloride to provide an indication of the stability indicating property and specificity of the proposed method [13-18]. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (105°C), acid hydrolysis (0.1N HCl), base hydrolysis (0.1 NaOH) and oxidation (3% H_2O_2). For heat study period was 2days and for light studies, study period was to illuminate the sample for 1.2 million Lux hours, where as for acid, base and peroxide hydrolysis the test period was 48 h. Peak purity of stressed samples of Moexipril Hydrochloride was checked by using 2996 Photo diode array detector of Waters Corporation, MA, USA. Stressed samples were also analyzed by LCMS to verify the homogeneity of the Moexipril peak.

2.6. Analytical Method Validation

The developed chromatographic method was validated for linearity, precision, accuracy, sensitivity, robustness and system suitability.

2.6.1. Precision

The precision of the related substance method was checked by injecting six individual preparations of (300 μg mL^{-1}) Moexipril Hydrochloride spiked with 0.10% each of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7. The %RSD area of each imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 was calculated. Precision study was also determined by performing the same procedures on a different day (interday precision).

The intermediate precision (ruggedness) of the method was also evaluated using different analyst, different column and different instrument in the same laboratory.

Assay method precision was evaluated by carrying out six independent assays of test sample of Moexipril Hydrochloride against qualified reference standard. The %RSD of six assay values obtained was calculated. The intermediate precision of the assay method was evaluated by different analyst and by using different instrument from the same laboratory.

2.6.2. Sensitivity

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7, calculated the %RSD for the areas of each impurity.

2.6.3. Linearity and Range

Linearity test solutions for assay method were prepared from stock solution at five concentration levels from 50 to 200% of assay analyte concentration (50, 75, 100, 150 and 200 µg mL-1).

A linearity test solution for related substance method was prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration levels. From LOQ to 200% of the permitted maximum level of the impurity (i.e. LOQ, 0.0375, 0.075, 0.1125, 0.15, 0.225 and 0.3%) was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses.

Linearity was checked for three consecutive days in the same concentration range for both assay and related substance method and calculated the %RSD Value of the slope and Y-intercept of the calibration curve. Upper and lower levels of range were also established.

2.6.4. Accuracy

The accuracy of the assay method was evaluated in triplicate at five concentration levels, i.e., 50, 75, 100, 150 and 200µg mL^{-1} in bulk drugs and pharmaceutical dosage forms. At each concentration, three sets were prepared and injected in triplicate. The percentage of recovery was calculated at each level.

The accuracy of the related substance method was evaluated in triplicate at 0.075, 0.1125, 0.15, 0.225 and 0.3% of the analyte concentration (300 µg mL^{-1}). The percentage of recoveries for imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 were calculated.

2.6.5. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (R_s) between Moexipril Hydrochloride, imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 were evaluated. The flow rate of the mobile phase was 1.2 mL min^{-1}. To study the effect of flow rate on the developed method, 0.1 units of flow was changed (i.e. 1.1 and 1.3 mL min^{-1}). The effect of column temperature on the developed method was studied at 30°C and 40°C instead of 35°C. The effect of pH on resolution of impurities was studied by varying ± 0.1 pH units (i.e. buffer pH altered from 2.8 to 2.7 and 2.9). In all the above varied conditions, the components of the mobile phase were held constant.

2.6.6. Solution Stability and Mobile Phase Stability

The solution stability of Moexipril Hydrochloride in the assay method was carried out by leaving the test solutions of samples in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed at 6 h intervals up to the study period against freshly prepared standard solution. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 6 h intervals up to 48 h. Mobile phase prepared was kept constant during the study period. The %RSD of assay of Moexipril Hydrochloride was calculated for the study period during mobile phase and solution stability experiments.

The solution stability of Moexipril Hydrochloride and its related impurities were carried out by leaving both spiked sample and un-spiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Content of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 was determined at every 6 h interval, up to the study period. Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions, for every 6 h interval. Content of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

3. Results and Discussion

3.1. Method Development and Optimization

The HPLC method carried out in this study aimed at developing chromatographic system capable of eluting and resolving Moexipril Hydrochloride from its process related impurities and degradation products that comply with the general requirements for system suitability. Initial trials were done on X Bridge C18 column (100 mm × 3 mm i.d., particle size 3.5 μm) using mobile phase consisting of Solution A as Buffer (10 mM di-potassium hydrogen phosphate, pH adjusted to 7.5 with phosphoric acid) and solution B as Acetonitrile with the gradient as (time (min) / % solution B): 0/0, 2/5, 15/60, 20/60, 20.1/0, 25/0 at flow rate 1.0 mL min^{-1}. All the peaks got separated but because of the varied polarities of impurities, longer retention times were unavoidable. The impurity-5 being more polar and acidic in nature is the early eluter and Impurity-3 because of its non-polarity is the late eluter of the related compounds. Studied the separation and peak shape by varying pH from 7.5 to 2.5 with phosphate buffer, and observed that, as the pH is decreasing towards 2.5, the peak shapes of Moexipril, impurity-4 and impurity-7 became broad and impurity-1 is co-eluting with Moexipril. Different buffers such as Formic acid, Trifluoroacetic acid were also tried with different gradient methods to check the peak shape and separation in acidic pH. But poor peak shapes were still unavoidable and at 210nm, with the Formic acid and Trifluoroacetic acid buffer solutions, a negative base line drifting is also observed. Changed the column to Agilent Zorbax SB CN and studied the separation and observed that impurity-5 is early eluting. Changed the column to Agilent Zorbax SB phenyl and observed that the impurity-5 is retained satisfactorily. Obtained better separations and peak shapes with mobile phase consisting of Solution A as 10 mM Potassium phosphate, pH adjusted to 2.8 with phosphoric acid and Solution B as Acetonitrile. To improve the baseline introduced 5% water to solution B and as a part of further optimizing the method increased flow rate to 1.2mL per minute and the column temperature to 35°C and obtained good resolution between all the impurities and Moexipril Hydrochloride . The % of acetonitrile played a key role in the retention times and resolution between impurities.

After many logical trials, chromatographic condition was established such that which could be suitable for separation of drug and seven known impurities along with the degradation products.

In the optimized conditions the chromatographic column used was Agilent Zorbax SB phenyl column (150 x 4.6) mm with 3.5 μm particles, maintained at 35°C temperature. Mobile phase consists of Solution A as buffer (10 mM

Potassium dihydrogen phosphate, pH adjusted to 2.8 using phosphoric acid) and Solution B consists of acetonitrile and water in the ratio of 95:5 v/v, delivered at a flow rate of 1.2 mL min^{-1}. The HPLC gradient program was set as: time (min) / % solution B: 0/10, 20/80, 20.1/10 and 25/10. The detection was monitored at a wavelength of 210 nm. The Retention times observed for Moexipril, imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 are about 10.8, 8.1, 14.1, 14.8, 12.4, 2.9, 6.9 and 10.0 minutes respectively. Under the finalized conditions Moexipril Hydrochloride and its known impurities were well separated with a resolution of greater than 2. The system suitability results are given in Table 1.

Table-1: System Suitability report.

Compound	USP Resolution(R_s)	USP Tailing factor (T)	No of theoretical plates USP tangent method (N)
Imp-5	-	1.0	9072
Imp-6	29.7	0.8	36546
Imp-1	5.7	1.0	16059
Imp-7	7.3	1.0	22918
Moexipril	2.8	1.3	25640
Imp-4	6.3	1.0	41244
Imp-2	7.5	0.8	95054
Imp-3	3.7	0.9	108087

3.2. Results of Forced Degradation Studies

3.2.1. Degradation in Acidic solution

The drug was exposed to 0.1 N HCl at 50°C for 2 h 30 minutes. Moexipril Hydrochloride has shown significant sensitivity towards the treatment of 0.1 N HCl. The drug gradually undergone degradation with time in 0.1 N HCl and prominent degradation was observed (~27%).

3.2.2. Degradation in Basic solution

The drug was exposed to 0.1 N NaOH. Moexipril Hydrochloride has shown very significant sensitivity towards the treatment of 0.1 N NaOH. The drug has undergone degradation within no time in 0.1 N NaOH and prominent degradation was observed (~4%).

3.2.3. Oxidative Conditions

The drug was exposed to 3% hydrogen peroxide at 50°C for 30 minutes. Moexipril Hydrochloride has shown significant sensitivity towards the treatment of 3% hydrogen peroxide and the drug gradually undergone prominent degradation (~27 %).

3.2.4. Photo stress stability

The drug in solution state was exposed to 66000 Lux hours of UV light. Moexipril Hydrochloride has shown significant sensitivity towards the illumination of UV light and the drug gradually undergone prominent degradation (~20 %).

Interestingly the drug in solid state was stable under photo stress conditions.

3.2.5. Thermal stress stability

The drug was exposed to 105°C for 4 hours. Moexipril Hydrochloride has shown significant sensitivity towards the thermal condition. The drug gradually undergone degradation with time and prominent degradation was observed (~5%).

DP-1 was common degradation product found in Oxidative and photo degradation. Labeling made by logical matching of all chromatograms for retention time and wavelength for those peaks. (See Fig. 2).

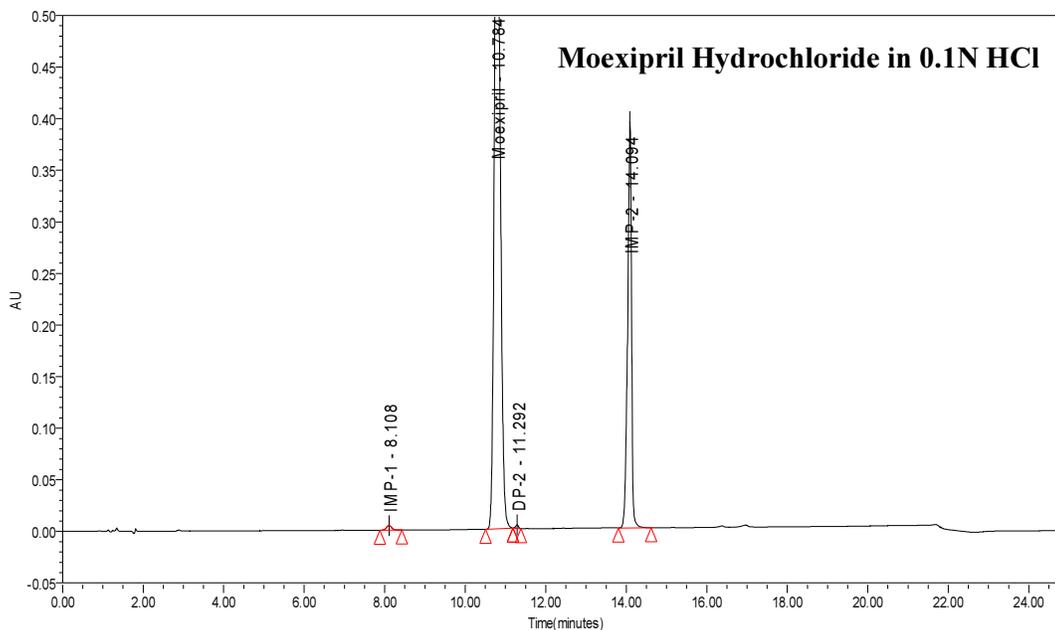**Fig-2: Typical chromatogram of stressed Moexipril Hydrochloride samples.**
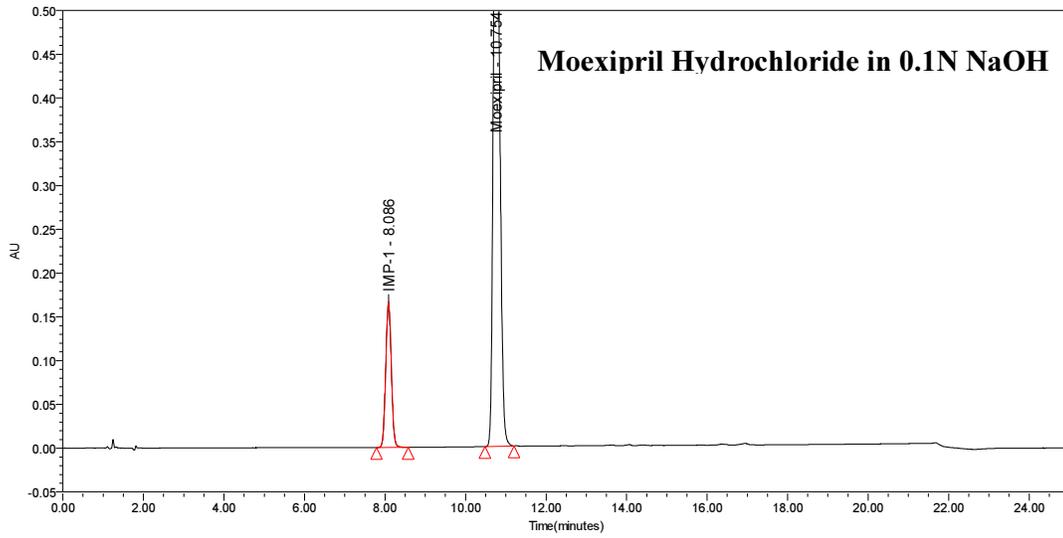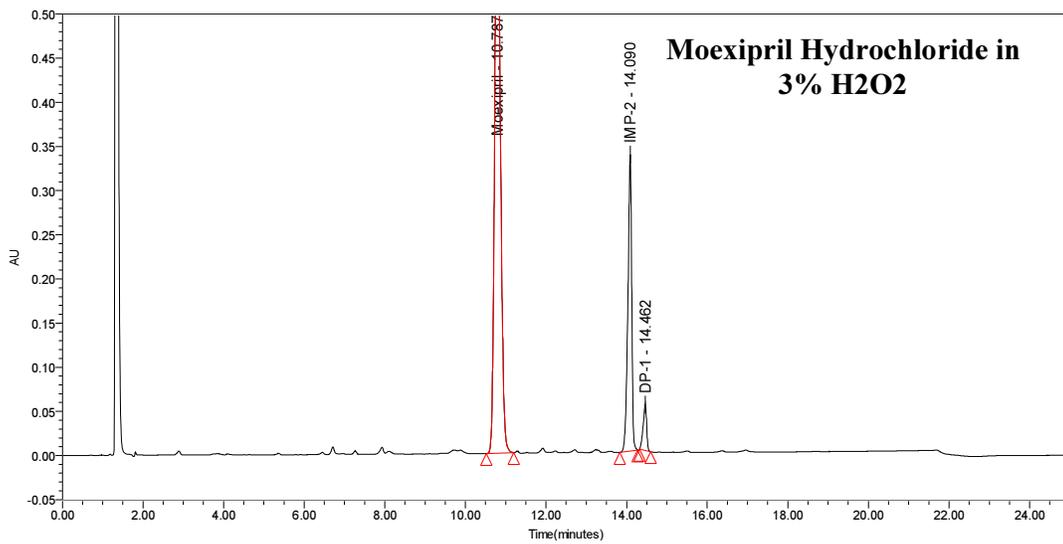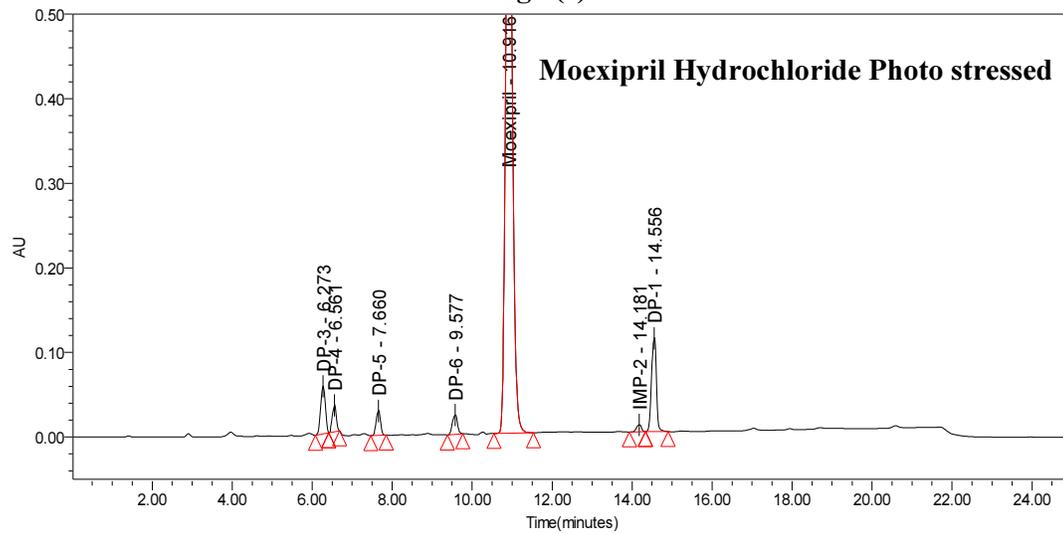

Fig 2(a)

Fig 2(b)
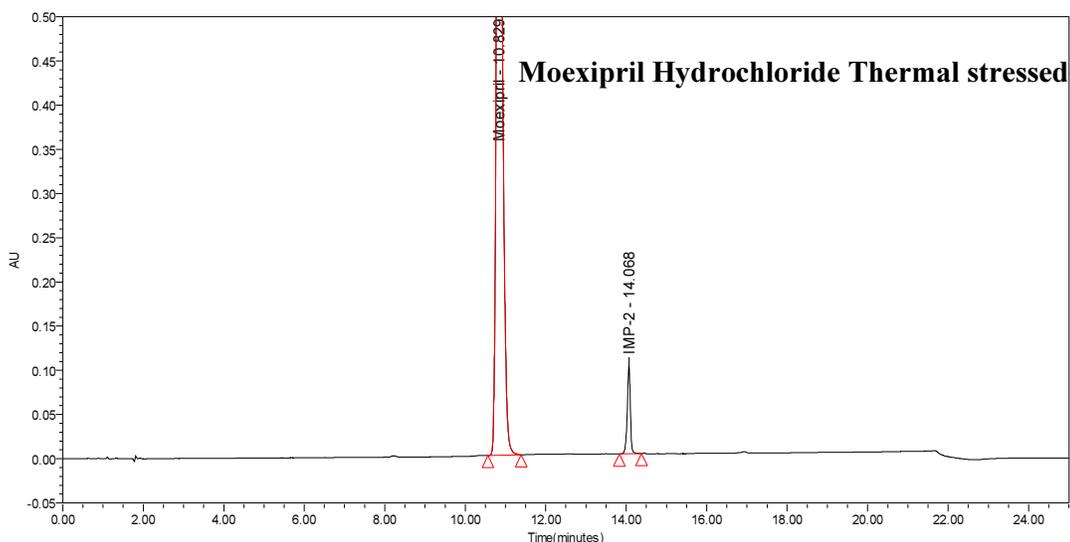

Fig 2(c)

Fig 2(d)

Fig 2(e)

From the degradation studies, Peak purity test results derived from PDA detector, LCMS analysis of stressed samples confirmed that the Moexipril Hydrochloride peak was homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.5%. No degradants were observed after 20 min in the extended runtime of 20 min of all the Moexipril Hydrochloride samples. The developed HPLC method was found to be specific in the presence of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6, imp-7 and its degradation products confirm the stability indicating power of the developed method.

3.3. Method validation

3.3.1. Precision

The %RSD of assay of Moexipril Hydrochloride during assay method precision study and intermediate precision study was 0.4 and the %RSD of area of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6, imp-7 in related substance method precision study were within 2.0 , confirming the good precision of the developed analytical method.

3.3.2. Sensitivity

The limit of detection of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 were 0.004, 0.002, 0.003, 0.005, 0.002, 0.006 and 0.004% (of analyte concentration, i.e., 300 μg mL^{-1}) respectively for 10 μL injection volume. Under the same conditions, the LOQ were 0.012, 0.006, 0.009, 0.014, 0.007, 0.018 and 0.013% (of analyte concentration, i.e. 300 μg mL^{-1}) respectively.

The precision at LOQ concentration for imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 were below 2%.

3.3.3. Linearity and Range

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 50-200 μg mL^{-1} and the correlation coefficient obtained was greater than 0.999. The result shows an excellent correlation existed between the peak area and concentration of the analyte.

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.3% for imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7. The correlation coefficient obtained was greater than 0.999 for all the impurities. The result shows an excellent correlation existed between the peak area and concentration of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7.

At all concentration levels, standard deviation of peak area was significantly low and RSD was below 1.0%. Analysis of residuals indicated that residuals were scattered within ± 2 % with respect to 100% concentration response. Linearity was checked for related substances over the same concentration ranges on three consecutive days and the %RSD of the slopes and Y-intercept of the calibration plots were with in 2.3 and 5.0 respectively. The range of the method was found from LOQ to 0.3% of the analyte concentration (300 μg mL^{-1}).
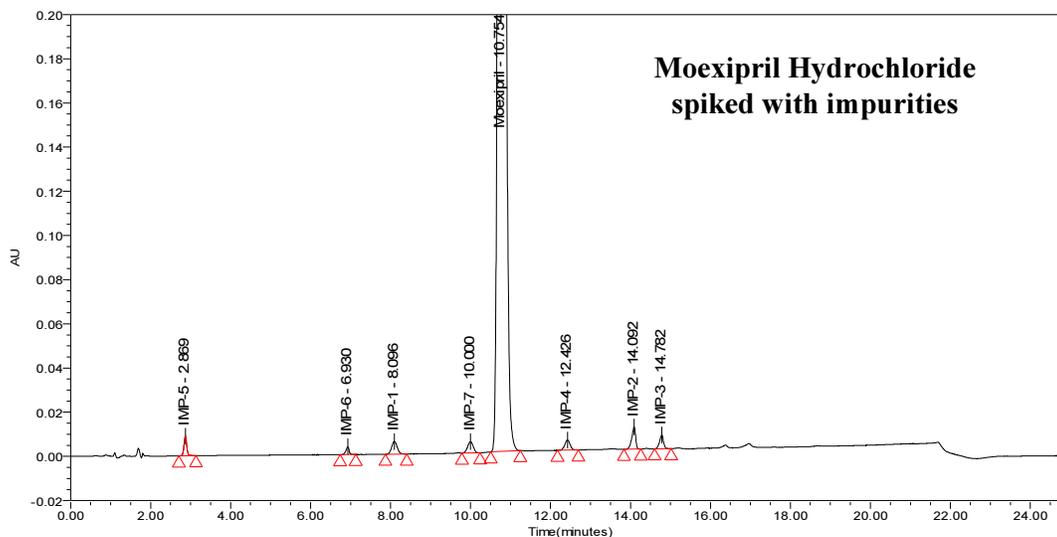
3.3.4. Accuracy

The percentage recovery of Moexipril Hydrochloride in bulk drug samples ranged from 98.9-100.3% and in pharmaceutical dosage forms ranged from 99.4-101.3% (Table 2). The percentage recovery of imp imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 in bulk drug samples ranged from 99.4 to 100.8% (Table 3). HPLC chromatograms of spiked sample with all seven impurities in Moexipril Hydrochloride bulk drug sample are shown in Fig 3(a).

Table-2: Results of Accuracy study for Bulk drugs and Pharmaceutical dosage forms.

Added (μg) (*n*= 3)	%Recovery for Bulk drugs	%RSD for Bulk drugs	%Recovery for Pharmaceutical dosage forms	%RSD for Pharmaceutical dosage forms
50	99.4	0.1	99.4	0.2
75	100.3	0.2	101.3	0.3
100	99.5	0.1	100.6	0.1
150	98.9	0.3	99.7	0.2
200	99.2	0.2	100.4	0.3

n =3, Number of determinations

Fig 3: Typical chromatogram of Moexipril Hydrochloride spiked with impurities at 0.1% level.

Table-3: Results of Accuracy study for impurities.

No. of Accuracy level (*n*= 3)	%imp-1	%imp-2	% imp-3	%imp-4	%imp-5	%imp-6	%imp-7
Accuracy at 50%	99.5	99.7	100.1	99.5	100.3	100.2	100.2
Accuracy at 75%	99.7	100.3	99.7	100.1	100.5	100.8	100.6
Accuracy at 100% level	100.2	100.5	99.5	99.8	99.8	100.4	99.8
Accuracy at 150% level	99.6	99.6	100.3	100.5	99.7	99.5	99.4
Accuracy at 200% level	100.3	100.2	99.8	99.4	100.5	100.1	100.7

n =3, Number of determinations

3.3.5. Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate, pH and column temperature) revealed that the resolution between closely eluting impurities, namely imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 was always greater than 2.0, illustrating the robustness of the method.

3.3.6. Solution Stability and Mobile phase Stability

The %RSD of assay of Moexipril Hydrochloride during solution stability and mobile phase stability experiments was within 1.0. No significant changes were observed in the content of imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and imp-7 during solution stability and mobile phase stability experiments. The solution stability and mobile phase

stability experiments data confirms that sample solutions and mobile phase used during assay and related substance determination were stable up to the study period of 48 h.

3.3.7. Assay analysis

Analysis was performed for different batches of Moexipril Hydrochloride in both bulk drug samples (n=3) ranged from 99.88-99.96 and dosage forms (n=3) ranged from 100.8-102.5%.

4. Conclusion

The HPLC method developed for Assay and related substance determination of Moexipril Hydrochloride in both bulk drugs and pharmaceutical dosage forms is precise, accurate, sensitive and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of Moexipril Hydrochloride samples.

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