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DEVELOPMENT AND VALIDATION OF A UV SPECTROPHOTOMETRIC AND A RP-HPLC METHODS FOR MOEXIPRIL HYDROCHLORIDE IN PURE FORM AND PHARMACEUTICAL DOSAGE FORM

(Perkembangan dan Validasi Kaedah Spektrofotometri UL dan Kaedah KCPT Fasa Terbalik Bagi Analisis Moexipril Hidroklorida Dalam Keadaan Tulen dan Dalam Dos Farmaseutikal)

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Abstract

A simple and reliable UV spectrophotometric and high-performance liquid chromatography (HPLC) methods were developed and validated for Moexipril hydrochloride in pure form and pharmaceutical dosage form. The RP-HPLC method was developed on agilant eclipse C_{18}, (150 mm x 4.6 mm, 5 µm) with a mobile phase gradient system of 60% {methanol:acetonitrile (70:30% v/v)} : 40% 20mM ammonium acetate buffer pH 4.5 (v/v) and UV spectrophotometric method was developed in phosphate buffer pH 6.8. The effluent was monitored by SPD-M20A, prominence PDA detector at 210 nm. Calibration curve was linear over the concentration range of 10 –35 µg/ml and 1-9 µg/ml for RP-HPLC and UV with a regression coefficient of 0.999. For RP-HPLC method Inter–day and intra–day precision % RSD values were found to be 1.00078% and 1.49408% respectively. For UV method 0.73386% to 1.44111% for inter day 0.453864 to 1.15542 intra-day precision. Recovery of Moexipril hydrochloride was found to be in the range of 99.8538% to 101.5614% and 100.5297586% to 100.6431587% for UV and RP-HPLC respectively. The limits of detection (LOD) and quantification (LOQ) for HPLC were 0.98969 and 2.99907 µg/ml, respectively. The developed RP-HPLC and UV spectrophotometric method was successfully applied for the quantitative determination of Moexipril hydrochloride in pharmaceutical dosage.

Keywords: Moexipril hydrochloride, UV spectrophotometer, RP-HPLC, Pharmaceutical dosage form, Validation

Abstrak

Suatu kaedah mudah dan boleh dipercayai spektrofotometri UL dan kromatografi cecair berprestasi tinggi (HPLC) telah dibangunkan dan pengesahan untuk Moexipril hidroklorida dalam bentuk tulen dan dos farmaseutikal. Kaedah RP-HPLC dibangunkan pada agilant gerhana C 18, (150 mm x 4,6 mm, 5 µm) dengan sistem kecerunan fasa bergerak 60% {metanol: asetonitril (70:30% v / v)}: 40% 20mm penampan asetat ammonium pH 4.5 (v / v) dan kaedah spektrofotometri UV telah dibangunkan pada penimbal fosfat pH 6.8. Efluen telah dipantau oleh SPD-M20A, prominens pengesan PDA pada 210 nm. Lengkung penentukuran linear adalah pada julat kepekatan 10 - 35 µg/ml dan 1 - 9 µg/ml untuk RP-HPLC dan UV dengan nilai pekali regresi 0.999. Bagi RP-HPLC kaedah antara hari dan nilai %RSD di dapati antara 1.00078 % dan 1.49408% pada ketepatan masing-masing. Bagi kaedah UV nilai %RSD adalah antara 0.73386% kepada 1,44111% untuk hari manakala antara 0.453864 kepada 1.15542 ketepatan antara-hari. Perolehan semula Moexipril hidroklorida di dapati berada dalam julat 99.8538-101.5614% dan 100.529758 - 100.6431587% masing – masing bagi UV dan RP-HPLC. Had pengesanan (LOD) dan kuantifikasi (LOQ) untuk HPLC masing – masing adalah 0.98969 dan 2.99907 µg/ml. Kaedah yang dibangunkan RP-HPLC dan UV spektrofotometri telah berjaya digunakan untuk penentuan kuantitatif Moexipril hidroklorida dalam dos farmaseutikal.

Kata kunci: Moexipril hidroklorida, spektrofotometer UL, RP-HPLC, dos farmaseutikal , pengesahan

Introduction

Moexipril hydrochloride is a non-sulfhydryl containing precursor of the active angiotensin-converting enzyme (ACE) inhibitor moexiprilat and its structural formula as shown in Figure 1

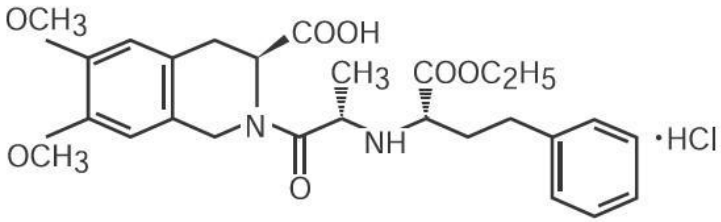

Figure 1. Structure of Moexipril Hydrochloride

Moexipril hydrochloride is a fine white to off-white powder. It is soluble (about 10% weight-to-volume) in distilled phosphate buffer (pH 3.8) at room temperature. This cannot be attributed to a chronic decrease in food consumption or increased physical activity, suggesting that the drug increases metabolic energy expenditure. Moexipril hydrochloride is a prodrug for moexiprilat, which inhibits ACE in humans and animals. The mechanism through which moexiprilat lowers blood pressure believed to be primarily inhibition of ACE activity [1]. There are various spectrophotometric methods developed for estimation of Moexipril hydrochloride [2,3]. Solvents used for the methods are comparatively of high cost. Hence there is a need for a simple, rapid, cost effective and reproducible method for assay of Moexipril hydrochloride in its dosage forms. Therefore, it was thought of interest to develop simple, accurate, fast and cost effective method for the analysis of Moexipril hydrochloride in its tablet formulation. This paper describes development and validation of simple, specific, sensitive, accurate and precise ultraviolet spectroscopic and RP-HPLC method for the estimation of Moexipril hydrochloride in bulk and its formulation.

Literature survey reveals that several analytical methods have been reported for the estimation of Moexipril hydrochloride by, UV [3] and RP-HPLC [4,5,6] method. From the analytical methods, it is possible to obtain the required information (about quality, purity, and concentration of the drug (analyte) in the dosage form) both qualitatively and quantitatively by the systematic approach. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw materials used and final products obtained meet the required specifications. The continuous and wider usage of same drugs report new toxicities and resistance. Under these conditions standard analytical procedures for some drugs may not available in pharmacopoeias. So, it becomes necessary to develop newer analytical methods [7] and Validate as per the guidelines of International Conference for Harmonization [8-13].

Materials and Methods

Chemicals and Reagents

An analytically pure sample of Moexipril hydrochloride was procured as gift sample from Glennmark Pharmaceutical. (Goa, India). HPLC grade Methanol, Acetonitrile and Water was procured from Sigma Aldrich and Merck Ltd., Mumbai, India. Tablet formulations UNIVSAC were procured from a local pharmacy with labeled amount of 7.5 mg per tablet. Phosphate buffer (pH 3.8) used for dilution was distilled in the laboratory.

Instrumentation and Analytical conditions

The HPLC system is of schimadzu, lc-20AD, a SPD-M20A prominence PDA detector, HPLC Pump LC-20AD pump and Injector Loop rheodyne, model No. 2767, Made in USA 20 µl volume loops. Data acquisition was performed by the LC solution software. Chromatographic analysis was performed on a agilent eclipse C-18 column with 150 mm x 4.6 mm i.d. and 5 µm particle size. The mobile phase consisted of 60% {methanol:acetonitrile (70:30% v/v)} : 20mM ammonium acetate buffer pH 4.5 (v/v) and was pumped gradientically at a flow rate of 1.0 ml/min and a column temperature of 25^{o}C. The injection volume was 5µl. The mobile phase was degassed and filtered through 0.22 µm membrane filter before pumping into HPLC system. The effluent was monitored by PDA

detector at 210 nm [10]. A double beam UV spectrophotometer (Shimadzu UV-1800) was used with 1 cm matched quartz cell with the λ at 222.0 nm (Fig 2). Phosphate buffer (pH 3.8) was selected as a solvent.

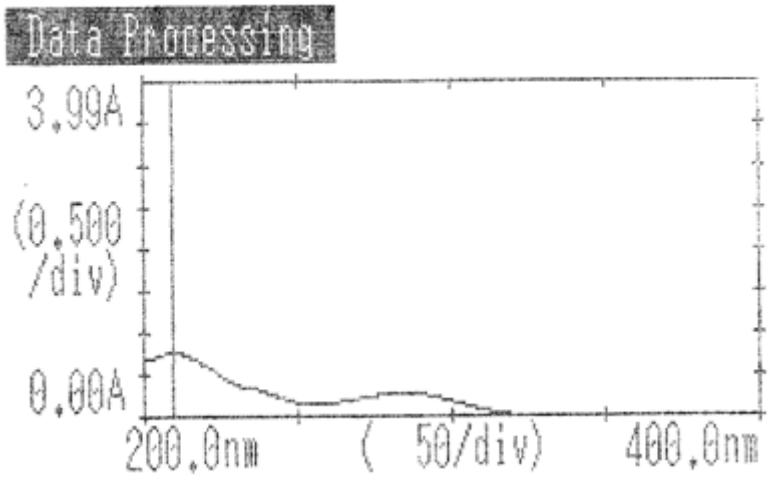

Figure 2. UV spectrum of Moexipril hydrochloride standard by zero order method

Preparation of Solutions For HPLC Method

Preparation of Mobile Phase (HPLC)

i. ***Mobile Phase A*** : (methanol:acetonitrile (70:30%) v/v): The mobile phase was prepared by mixing of methanol and acetonitrile in the ratio of 70: 30 % (v/v). The solution was then filtered through 0.45 µ membrane filter and degassed.
ii. ***Mobile Phase B*** : (20mM ammonium acetate buffer pH 4.5 v/v): Dissolve 136 gm of sodium acetate and 77 gm of ammonium acetate in water and dilute with water to 1000 ml. Add 250 ml of glacial acetic acid and mix.
iii. ***Optimized Mobile Phase***: The final mobile phase was prepared by mixing both mobile phase A and B in the ratio of 60:40 % (v/v).

Preparation of Standard Solutions

A stock solution of Moexipril hydrochloride was prepared by accurately weighing 7.5 mg of drug, transferring to 100 ml volumetric flask. Add about 30 ml of mobile phase and sonicate to dissolve it completely and make up volume up to mark with mobile phase (75µg/ml).

Preparation of Moexipril hydrochloride standard and sample Solutions

Preparation of Standard Solution

2 ml of the above stock solution was taken in 10 ml volumetric flask and thereafter made up to 10 ml with mobile phase to get a concentration of 30µg/ml. Mixed well and filter through Whatmann filter paper (No. 41).

Sample preparation

Weighed 5 Moexipril hydrochloride Tablets and calculate the average weight. Accurately weighed and transferred the sample equivalent to 7.5 mg of Moexipril hydrochloride into a 100 ml volumetric flask. Added about 30 ml of diluent and sonicated to dissolve it completely and make volume up to the mark with diluent. Mixed well and filtered through Whatmann filter paper (No.41). Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with the same solvent. Mixed well and filtered through whatmann filter paper (No.41).

Table 1. Assay Studies of Moexipril Hydrochloride by RP-HPLC method

Sample	Injections	Peak Area(mA)		Label Claim (mg)	Amount Found (mg)	% Assay
		Standard	Sample			
UNIVSAC®	1	2625947	2598798	7.5	7.42	98.96
UNIVSAC®	2	2625879	2618927	7.5	7.48	99.73
UNIVSAC®	3	2625897	2626786	7.5	7.50	100.03
	AVG	2625907.66	2614837	7.5	7.46	99.57
	STD DEV	35.23	14435.30	0	0.041	0.55
	%RSD	0.00134	0.552	0	0.553	0.55

Preparation of stock solutions for UV Spectroscopic Method

Standard Moexipril hydrochloride 100mg was weighed and transformed to a 100 ml volumetric flask and dissolved in 25 ml of phosphate buffer (pH 3.8). The flask was shaken and volume was made up to the mark with Phosphate buffer (pH 3.8) to give a solution containing 1000 µg/ml (Stock solution A). From this stock solution A, pipette out 5 ml and place into 50 ml volumetric flask. The volume was made up to the mark with phosphate buffer (pH 3.8) to give a solution containing 100 µg/ml (Stock solution B).

Selection of analytical concentration range

From the standard stock solution B of Moexipril hydrochloride, appropriate aliquots 0.1, 0.3, 0.5, 0.7, and 0.9 were pipetted out in 10 ml volumetric flasks and dilutions were made with phosphate buffer (pH 3.8) to obtain working standard solutions of concentrations from 1-9µg/ml. Absorbance for these solutions were measured at 210 nm. For standard solution analytical concentration range was found to be 1-9 µg/ml and overlain spectra was obtained.

Calibration curve for the Moexipril hydrochloride

Appropriate volumes of aliquots from standard Moexipril hydrochloride stock solution B were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with phosphate buffer (pH 3.8) to obtain concentrations of 1, 3, 5, 7 and 9 µg/ml. Absorbance value of each solution against phosphate buffer (pH 3.8) as a blank were measured at 210 nm. From that absorbance value, regression equation and correlation coefficient (r^2) are determined and presented (Fig.5).

Sample preparation for determination of Moexipril hydrochloride from Dosage form

Twenty tablets of formulation were weighed and finely powdered. The powder equivalent to 100 mg of Moexipril hydrochloride was accurately weighed. It was then transferred to volumetric flask of 100 ml capacity containing 25 ml of phosphate buffer (pH 3.8) and sonicated for 30 min. The flask was shaken and the solution was filtered through whatmann filter paper (No. 41) into 100 ml volumetric flask. Volume was made up to the mark with phosphate buffer (pH 3.8) to give a solution of 1000 µg/ml (Stock solution A). From this solution 5 ml was taken and placed in 50 ml volumetric flask. The volume was made up to the mark using phosphate buffer (pH 3.8) to give a solution of 100 µg/ml (Stock solution B). From the stock solution B, 5.0 ml was taken and diluted to 100 ml to give 5 µg/ml and it was further used for the estimation of Moexipril hydrochloride.

Results and Discussion

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 60% [methanol: acetonitrile (70:30% v/v)]: 40% 20mM ammonium acetate buffer pH 4.5 (v/v) accomplished at 210 nm by using an agilant eclipse C-18 column (150 mm x 4.6 mm, 5 µm). The retention time was 2.32 min at a flow-rate of 1.0 ml/min and the injection volume was 5 µl. (Figure 3) [15].

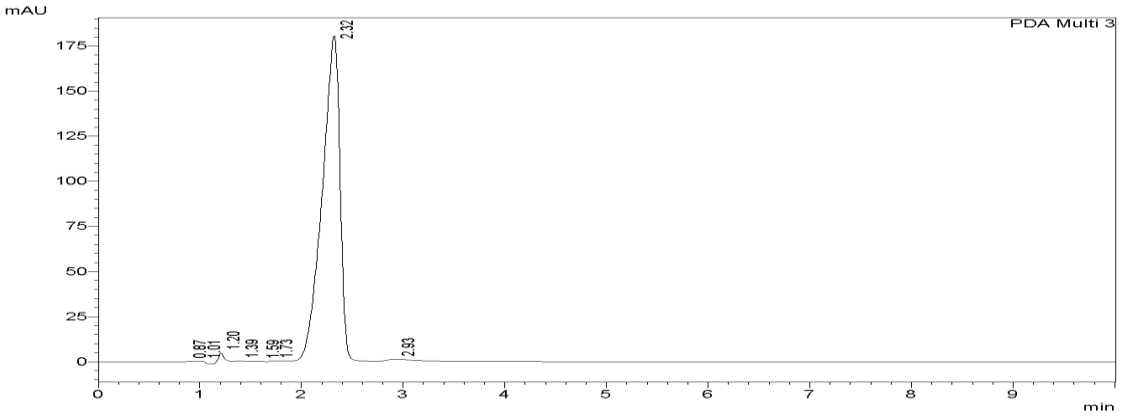

Figure 3. Chromatogram of Moexipril hydrochloride at 210 nm

Method Validation For HPLC Method

System suitability

A system suitability test of the chromatographic system was performed. Six replicate injections for a system suitability test were injected into the chromatographic system. Relative standard deviation and column efficiency for the five suitability injections were determined. For all sample analyses, the efficiency and %RSD were found ≥ 2000 Theortical plate and ≤ 2% respectively. USP tailing factor and capacity factor was found to be ≤ 1.5 (Table 2) [16].

Table 2. System suitability test for Moexipril hydrochloride

Name	Area	USP Plate Count	USP Tailing
Moexipril hydrochloride	2625865	5121.11	0.705

Linearity

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 10, 15, 20, 25, 30 and 35µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). Linearity result for Moexipril hydrochloride was summarized in Table 3.

Table 3. Linearity result for Moexipril hydrochloride

Series No	Concentration(µg/ml)	Peak Area(mA)
1	0	0
2	10	928722
3	15	1366565
4	20	1828807
5	25	2240392
6	30	2625947
7	35	3063605

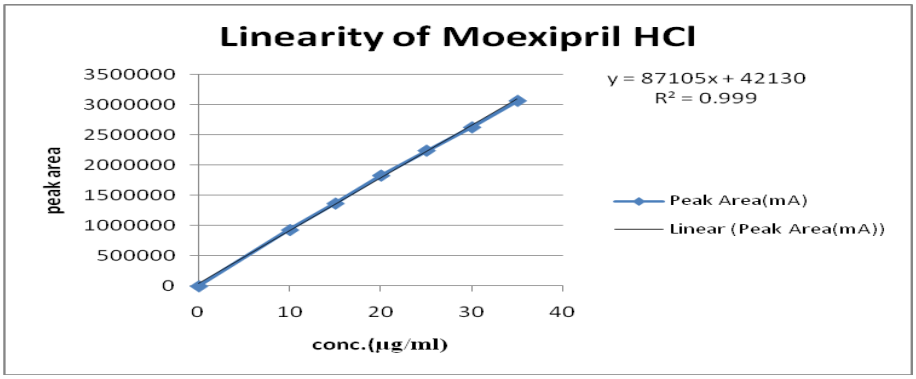

Figure 4. Calibration curve for Moexipril hydrochloride

Accuracy

Accuracy was performed in triplicate after spiking pure drug equivalent to 80, 100, and 120% of the standard concentration of Moexipril hydrochloride (30 µg/ml). The results obtained as summarized in Table 4 indicate that recovery was excellent, not less than 100% ± 2.

Table 4. Accuracy results for Moexipril hydrochloride

Series No	Percentage (%)	Peak Area	Amount Recovered	% Recovery
1	80	2201445	25.15	100.60
2	100	2612321	30.15	100.52
3	120	3083203	35.22	100.64

Sensitivity

Limit of detection (LOD) and quantification (LOQ) were estimated. LOD and LOQ values were found to be 0.9896 and 2.99 µg/ml respectively.

Precision

The precision of the method was demonstrated by interday and intra-day variation studies. In the inter-day studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. In the intra-day variation studies, six injections of standard solution were injected at different days. Percentage RSD was calculated presented in Table 5.

Reproducibility (Ruggedness)

In addition to intra and inter day precision reproducibility study was also carried out and it was checked by determining precision on the same instrument, but by a different analyst. Results of reproducibility are shown in Table 6.

Table 5. Precision (for *Inter day* and *Intra day*) results for Moexipril hydrochloride

Precision Inter Day			
Series No.	**Concentration (µg/ml)**	**Peak Area (mAU)**	**Retention Time (min)**
1	30	2625958	2.32
2	30	2572418	2.36
3	30	2599921	2.39
4	30	2634780	2.38
5	30	2636662	2.35
6	30	2592134	2.36
	AVG	2610312	2.36
	SD	26123.44	0.0244
	%RSD	1.000	1.03

Precision Intra Day			
Series No.	**Concentration (µg/ml)**	**Peak Area (mAU)**	**Retention Time (min)**
1	30	2661347	2.35
2	30	2582789	2.35
3	30	2642431	2.36
4	30	2562131	2.32
5	30	2582169	2.36
6	30	2592910	2.32
	AVG	2603963	2.34
	SD	38905.3	0.018619
	%RSD	1.494081	0.794551

Table 6. Ruggedness studies of Moexipril hydrochloride

Series No.	**Analyst 1 Area**	**Analyst 2 Area**
1	2673431	2656844
2	2692134	2676409
3	2625347	2702317
4	2701301	2673984
5	2682145	2686744
6	2673101	2645639
AVG	2674576.5	2673656.2
SD	26483.126	20337.31
% RSD	0.9901802	0.7606554

*Average of six determinations.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in wavelength and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results are shown in Table 7.

Table 7. Robustness studies of Moexipril hydrochloride

Condition	Modification	Mean Peak area ± SD	Mean Rt ± SD	Mean % RSD (for AREA)
Mobile Phase Flow Rate (ml/min)	0.9	3012284±43567.9	2.356±0.0057	1.446
	1.1	3012284±21774.7	2.373±0.0115	0.722
Wavelength (nm)	208	3012284.3±12490.35	2.363±0.0152	0.414
	212	3144309.7±10892.65	2.353±0.0351	0.346

*Average of three determinations

For UV Spectroscopic Method

Linearity

The linear regression equation and the statistical evaluation of the calibration plots for the analysis of authentic samples are listed. Under the described experimental conditions, linear correlations were obtained at the wavelength 210 nm over the concentration range of 1 - 9 µg/ml of Moexipril hydrochloride. The calculated correlation coefficient (r) of least square linear regression was found to be 0.999 for the zero order in Table 8.

Table 8. Absorbance values for calibration curve of Moexipril hydrochloride at 210 nm by zero order spectroscopy

Concentration (µg/ml)	Absorbance*	±S.D*
0	0	0
1	0.070667	0.000577
3	0.21	0.001
5	0.369333	0.001528
7	0.531333	0.001528
9	0.672667	0.002082
	Average of SD	±0.001343

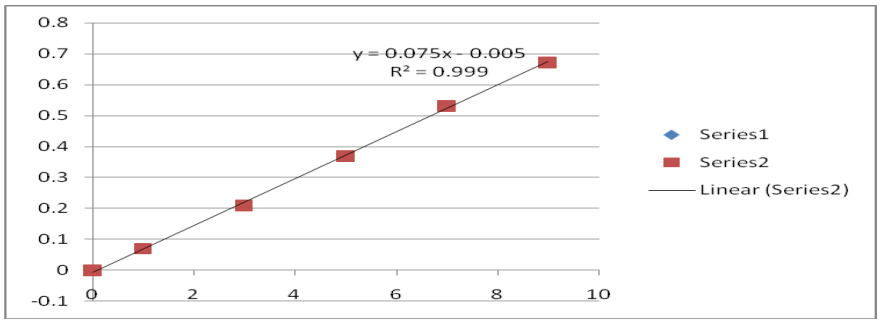

Figure 5. Calibration curve for Moexipril Hydrochloride at 210nm

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (50, 100, and 150%) of standard bulk sample of Moexipril hydrochloride within the linearity range were taken and were added to the pre-analyzed formulation of concentration 5 µg/ml and percentage recovery values were calculated. They were found to be present within the range. The accuracy results were obtained for zero order spectroscopy in Table 9.

Table 9. Recovery study data of Moexipril hydrochloride by Zero order spectroscopy

Level of recovery (%)	Amount of sample (µg/ml)	Amont of drug added (µg/ml)**	Amount of drug recovered (µg/ml)**	% Recovery ± S.D**
50	5	7.5	7.48	99.85±0.001169
100	5	10	10.02	100.26±0.00899
150	5	12.5	12.69	101.56±0.004491

** is average of three determinations

Precision

The precision of the proposed method was ascertained by determination of six replicates of same concentration of sample and standard for method precision and system precision. Both intraday precision and interday precision was carried out for zero order spectroscopy. The deviation between repeated readings was found to be present within the limit in Table 10.

Table 10. Precision study data of Moexipril hydrochloride by Zero order spectroscopy

Concentration (µg/ml)	Inter-day absorbance mean ± SD**	% RSD	Intra-day absorbance mean ± SD**	% RSD
1	0.0716±0.001	1.44	0.0706±0.0008	1.155
3	0.214±0.0017	0.8359	0.211±0.0012	0.5994
5	0.3738±0.0042	1.140	0.3758±0.0022	0.5929
7	0.5341±0.003	0.733	0.5336±0.0024	0.4538
9	0.6718±0.0056	0.844	0.6731±0.0041	0.6190

** is average of six determinations

Ruggedness

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. In the current study it was carried by two analysts for zero order spectroscopy. The results thus obtained by two analysts were not having considerable deviation in Table 11.

Table 11 Ruggedness study data of Moexipril hydrochloride by Zero order Spectroscopy

Label claim (mg)	Analyst 1		Analyst 2	
	Amount found (mg)	% Recovery ± SD**	Amount found (mg)	% Recovery ± SD**
7.5	7.39	98.57 ± 0.0025	7.40	98.75 ± 0.0024

** is average of six determinations

Limit of detection

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 1-9 µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value. The detection limit was found to be 0.05908µg/ml.

Limit of quantification

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve. The LOQ was found to be 0.17904 µg/ml.

Conclusion

The method was validated according to International Conference of Harmonization guidelines for validation of Moexipril hydrochloride by UV and RP-HPLC. The proposed method showed absorption maxima at 210 nm and obeyed Beer's law in the concentration range of 1-9 µg/ml and 10 to 35 µg/ml respectively. The limit of detection (LOD) was found to be 0.059084 µg/ml and limit of quantification (LOQ) to be 0.179042 µg/ml. The percentage recovery value indicates no interference from excipients used in formulation. The RP-HPLC method involved a mobile phase consisting of 60% {methanol:acetonitrile (70:30% v/v)} :40% 20mM ammonium acetate buffer pH 4.5 (v/v) accomplished at 210 nm. The retention time was 2.32 min at a flow-rate of 1.0 ml/min and the injection volume was 5 µl. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including linearity, accuracy, sensitivity, precision, ruggedness and robustness. The low value of percentage relative standard deviation shows that the developed method was precise. All statistical data prove validity of proposed method, which can be applied in industries for routine analysis of Moexipril hydrochloride drug from tablet.

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