Research Article

Development and Validation of High Performance LCMS Methods for Estimation of Enalapril and Enalaprilat in Presence of R-S Lercanidipine

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Accepted on: 13-06-2016; **Finalized on:** 31-07-2016.

ABSTRACT

The aim of present study is development and validation of analytical methods for estimation of Enalapril and Enalaprilat in presence of R-S Lercanidipine in API, method in LCMS. Enalapril is a prescription medication that acts as an angiotensin-converting-enzyme (ACE) inhibitor used in the treatment of hypertension, diabetic nephropathy, and some types of chronic heart failure presently commercially available and marketed as Vasotec in US, Enaladex and Renitec in some other countries, and Enacard for veterinary use. Drug showed linearity in the concentration range of 1.578 ng/ml to 421.497 ng/ml for Enalapril and 1.181 ng/ml to 236.641 ng/ml for Enalaprilat with correlation coefficient consistently greater than 0.99 for Enalapril and Enalaprilat. Different parameters such as linearity, range, precision, accuracy, ruggedness and robustness, limit of detection (LOD) and limit of quantification (LOQ) were used for validation of the method. The results were found to be acceptable as per the guidelines of International Conference on Harmonization (ICH). The method is found to be novel, rapid, linear, precise, accurate, robust and rugged and can be successfully applied for the routine analysis of Enalapril and Enalaprilat in presence of R-S Lercanidipine. The method is also found to be useful and economical.

Keywords: Enalapril, Enalaprilat, R-S Lercanidipine, LCMS, Validation, ICH.

INTRODUCTION

This research paper relates to development and validation of LCMS methods for estimation of Enalapril and Enalaprilat in presence of R-S Lercanidipine in API. The IUPAC name is (2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino} propanoyl]pyrrolidine-2-carboxylic acid. It is a prescription medication that acts as an angiotensin-converting-enzyme (ACE) inhibitor used in the treatment of hypertension, diabetic nephropathy, and some types of chronic heart failure presently commercially available and marketed as Vasotec in US, Enaladex and Renitec in some other countries, and Enacard for veterinary use. Enalapril is generic and available globally under many brands. Its molecular formula is C20H28N2O5 with average molecular weight 376.447 g/mol. It is almost white to off white crystalline powder, Solubility (g/ml): alcohol 0.08, methanol 0.20 with melting point 143-144.5 deg C and pKa value 2.97. [2-3] Chemical structure of Enalapril is given below:

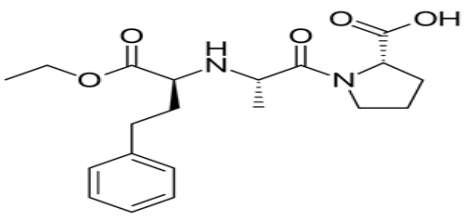

Figure 1

Enalapril is used alone or in combination with other medications to treat high blood pressure. It is also used in combination with other medications to treat heart failure. Enalapril is in a class of medications called angiotensin-converting enzyme (ACE) inhibitors. It works by decreasing certain chemicals that tighten the blood vessels, so blood flows more smoothly and the heart can pump blood more efficiently.[2]

ACE converts the peptide hormone angiotensin I to angiotensin II. One of the actions of angiotensin II is the vasoconstriction of blood vessels, resulting in an increase in blood pressure. ACE inhibitors such as enalapril prevent this effect. Enalapril has been shown to lower the death rate in systolic heart failure. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.[2]

Literature survey reveals (Ryan DF, 2005, Daniel DP, 2008, Madhududan Bachute T, 2013 and Ramineni SK 2013) determination of Imiquimod by HPLC and UV Spectroscopy methods.[1-5]

However, as to our best knowledge, none of the methods LCMS has been validated in all the parameters for determination of this drug in presence of the other drug with a simultaneous method of determining drug and metabolite.

Hence, the aim of present investigations is to develop and validate simple, rapid, accurate, economical and convenient bio analytical methods for determination of Enalapril and Enalaprilat in presence of R-S Lercanidipine.

Validation can be defined as establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

Data obtained from method validation can be used to evaluate the quality, consistency and reliability of analytical method. It is an indispensable part of any good analytical practice.[5]

The Necessity for Analytical Methods to be Validated

The main objective of method validation is to demonstrate the reliability of a particular method for the determination of an analyte concentration in a specific biological matrix, such as blood, serum, plasma, urine, or saliva.

Moreover, if an anticoagulant is used, validation should be performed using the same anticoagulant as for the study samples.

Generally a full validation should be performed for each species and matrix concerned.

Full validation of bioanalytical methods is important:

1) During development and implementation of a novel bioanalytical method.
2) For analysis of a new drug entity.
3) For revisions to an existing method that add metabolite quantification.

Advantages of Bioanalytical Method Validation

It results in level of confidence to the developer as well as to the user.

The validation process might be a costly and time consuming exercise but results are found to be inexpensive and lead to better time management in the end. It also eliminates annoying repetitions.

It helps to absorb the shock of minor changes in analytical setup due to unavoidable reasons such as supplier or grade of reagent, and pays more than spent on the bioanalytical method validation.[6]

The objective of bioanalytical procedure proposed should be clearly understood since this will govern the validation parameters which need to be evaluated.

Typical validation parameters to be considered are Linearity, Concentration Range, Accuracy, Precision (Repeatability, Intermediate Precision), Ruggedness, Matrix Effect, Selectivity, Robustness, Limit of Detection and Limit of Quantification.[4-7]

MATERIALS AND METHODS

Materials

Enalapril Maleate and Enalapril D5 Maleate (IS), was supplied by Clearsynth, Mumbai. Enalaprilat dehydrate, Enalaprilat D5 (IS), was supplied by Clearsynth, Mumbai. Methanol was obtained from Finar chemicals Ltd., Ahmadabad, India. The LCMS instrument used here was AB Sciex, API 4000.

Selection of Solvent

Solubility of drug was checked in different solvents and LCMS spectra recorded.

Preparation of Stock and Working Standard Solutions for Pure Drug

Enalapril

Transfer accurately weighed (about 5 mg) Enalapril into a 5 ml volumetric flask and dissolve in Milli-Q water.

Make up the volume using Milli-Q water and vortex. Concentration of the resultant solution will be about 1000 µg/ml.

Enalaprilat

Transfer accurately weighed (about 5 mg) Enalaprilat into a 5 ml volumetric flask and dissolve in Milli-Q water.

Make up the volume using Milli-Q water and vortex. Concentration of the resultant solution will be about 1000 µg/ml.

This solution was further diluted with methanol to get working standard solution of 10µg/ml of drug.

Preparation of sample solutions: (Methanol: Water:: 50:50 v/v)

500 ml of Methanol, 500 ml of Milli-Q water into a 1000 ml of reagent bottle, mix and sonicate.

Chromatographic Conditions

Mobile phase: (Methanol: 80:20 v/v)

800 ml of Methanol and 200 ml of buffer into a 1000 ml of reagent bottle, mix well and sonicate.

Injection Volume: 5 µl

Column: HYPERSIL GOLD C8, 150cm×4.6mm, 3.0µm

Flow rate: 1.0 ml/min, with splitter.

Preparation of diluent: Buffer1 (0.1 % Formic Acid solution)

1.0mL of Formic acid in to 1000mL volumetric flask and makeup the volume with of Milli-Q water.

Buffer2 (2% Acetic Acid solution)

2.0mL of Formic acid in to 100mL volumetric flask and makeup the volume with of Milli-Q water.

Preparation of Standard Stock Solution

Enalapril D5

Transfer accurately weighed (about 5 mg) Enalapril D5 into a 5 ml volumetric flask and dissolve in Milli-Q water.

Make up the volume using Milli-Q water and vortex. Concentration of the resultant solution will be about 1000 µg/ml.

Enalaprilat

Transfer accurately weighed (about 5 mg) Enalaprilat into a 5 ml volumetric flask and dissolve in Milli-Q water.

Make up the volume using Milli-Q water and vortex. Concentration of the resultant solution will be about 1000 µg/ml.

10mg accurately weighed drug was put into 10ml volumetric flask containing 5 ml of diluent and sonicated for 10 mins.

Then the volume was adjusted with more diluent up to the mark.

Preparation of Sample Solutions

Sample solutions of different conc. from 81.0325 to 0.1580µg/ml were prepared from above stock solution and diluted with mobile phase.

Parameters of Validation

Linearity and Range

The linearity of an analytical method is its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range.

The range of an analytical method is the interval between upper and lower analyte concentration in sample including these concentrations for which it has been established that the analytical method has a suitable level of linearity, accuracy and precision. To establish the linearity and range of proposed methods, various aliquots of standard solution of drug were prepared from stock solution and analyzed.

Precision

Precision studies were carried out to establish the repeatability and reproducibility of proposed methods by using six replicates of same concentration of sample solution.

Repeatability

It was determined by preparing six replicates of same concentration of sample and their area measured.

Reproducibility

Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision. The results were indicated as %RSD.

Accuracy

Accuracy of a method is the degree to which observed results correspond to true value of analyte in the sample. The determination was done at three different levels. Three samples of each level were prepared and total 9 determinations done as per ICH conditions. The samples were analyzed and their area measured and results indicated as % RSD.

Robustness/Ruggedness

Robustness/Ruggedness of an bioanalytical procedure are measure of its ability to remain unaffected by small changes in method parameters and provide an indication of its reliability.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts at the same operational and environmental conditions. The respective absorbance/ area noted and results indicated as % RSD.

Robustness

To check the robustness of proposed methods: analysis was carried out at two different temperatures, room temperature and at 18°C and respective absorbance measured. The results were indicated as % RSD.

Similarly analysis was carried out at three different wavelengths and flow rates and respective area measured. The results were indicated as % RSD.

LOQ AND LOD

Limit of detection (LOD) is the minimum quantity of analyte in sample that can be detected. Limit of quantification (LOQ) is the minimum quantity of analyte in sample that can be quantitatively determined by suitable precision and accuracy. LOQ and LOD were determined using equations: LOQ-10σ/S and LOD-3.3σ/S where σ is the standard deviation of response and S slope of related calibration curve.

RESULTS AND DISCUSSION

Linearity and Range

Linearity was established by preparing an eight-point standard calibration curve in K2EDTA human plasma covering the concentration range 1.578 ng/ml to 421.497 ng/ml for Enalapril and 1.181 ng/ml to 236.641 ng/ml for Enalaprilat using (Enalapril D5 and Enalaprilat D5 respectively) as internal standards.

Calibration standards were prepared and six batches of precision and accuracy were analysed (Table 1 and 2). Calibration curves were calculated by least-squares linear regression analysis of the response ratios (analyte/IS) in calibration standards with $1/x^2$ weighting. A representative calibration curve for Enalapril and Enalaprilat in K2EDTA human plasma are shown in Figure 2 and 3.

Recovery

Recovery of Enalapril and Enalaprilat from K2EDTA human plasma was determined by comparing peak areas of extracted QCL, QCM and QCH samples with peak areas

determined from freshly prepared un extracted (aqueous) samples prepared at similar concentrations in mobile phase. Mean overall % recovery was 93.42% and overall %CV was 5.19% for Enalapril and % recovery was 78.58% and %CV was 1.63% for Enalaprilat and % recovery was 96.17% and %CV was 4.19% for IS (Enalapril D5) and % recovery was 73.72% and %CV was 3.57% for IS (Enalaprilat D5).

Table 1: Summary of the Experimental Parameters of Enalapril in K2EDTA Human Plasma

Experimental Parameters	Results
Analyte	Enalapril
Biological Matrix	K2 EDTA Human Plasma
Specificity and Selectivity % CV	2.90%
Analytical range	1.578 to 421.497 ng/ml
Sensitivity	
Precision, Accuracy	3.08%, 106.91%
Recovery	
Enalapril	
% CV, % Recovery	5.91%, 93.42%
Enalapril D5	
% CV, % Recovery	4.19%, 96.17%

Table 2: Summary of the Experimental Parameters of Enalaprilat in K2EDTA Human Plasma

Experimental Parameters	Results
Analyte	Enalaprilat
Biological Matrix	K2 EDTA Human Plasma
Specificity and Selectivity % CV	3.27%
Analytical range	1.181 to 236.641ng/ml
Sensitivity	
Precision, Accuracy	5.32%, 90.69%
Recovery	
Enalaprilat	
% CV, % Recovery	1.63%, 78.58%
Enalaprilat D5	
% CV, % Recovery	3.57%, 73.72%

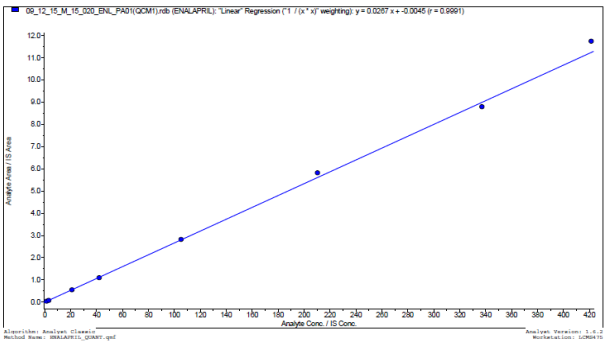

Figure 2: Representative Calibration Curve for Enalapril in Human Plasma (K_2EDTA)

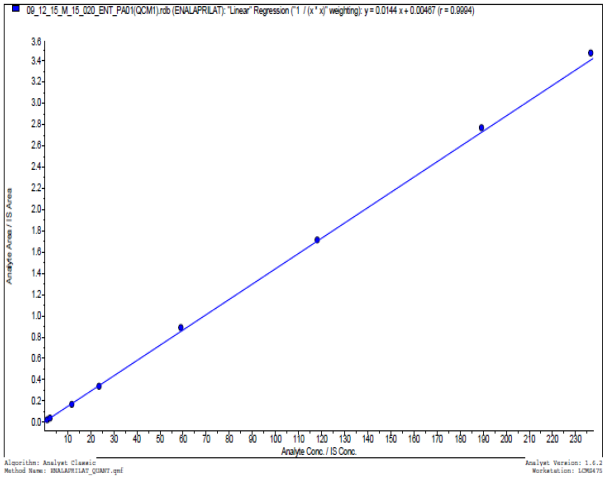

Figure 3: Representative Calibration Curve for Enalaprilat in Human Plasma (K_2EDTA)

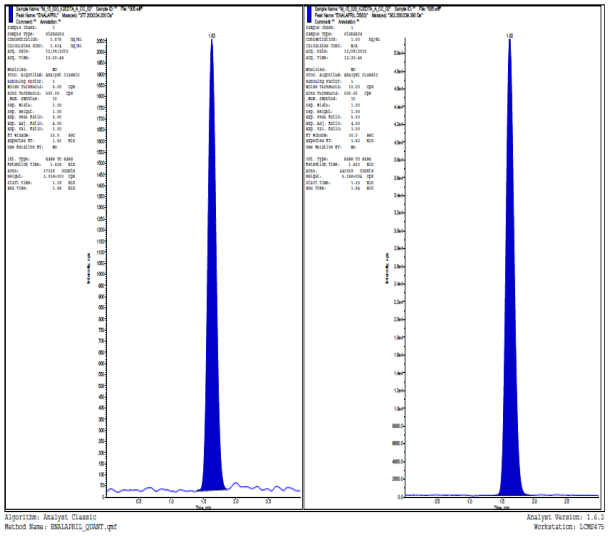

Figure 4: Chromatogram of the LLOQ Calibration Curve Standard for Enalapril with Internal Standard (Enalapril D5)

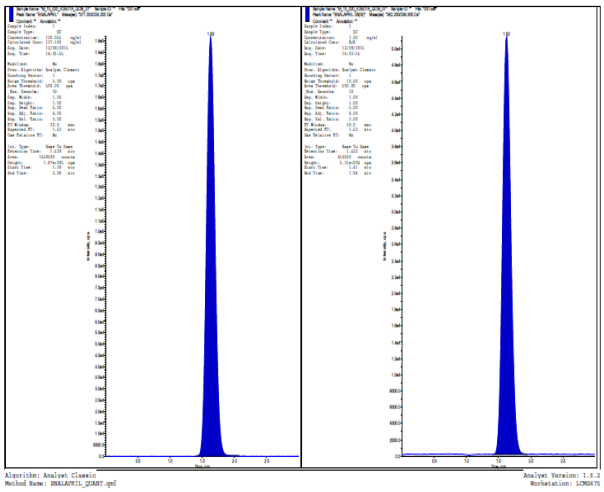

Figure 5: Chromatogram of the LLOQ Calibration Curve Standard for Enalaprilat with Internal Standard (Enalaprilat D5)

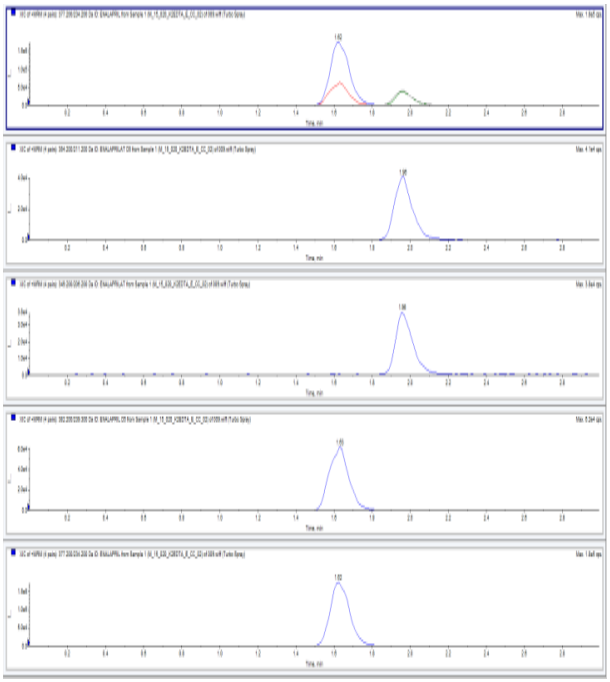

Figure 6: Chromatographic separation of Enalapril, Enalaprilat and Enalapril D5 & Enalaprilat D5 (Internal Standards)

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Source of Support: Nil, **Conflict of Interest:** None.