RESEARCH ARTICLE

High-performance liquid chromatography as an assay method for the investigation of conditions of enalapril maleate extraction by organic solvents

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Abstract

Introduction: Modern methods of isolating drugs from biological material are based on the individual physic-chemical properties of the compounds, so the choice of optimal conditions for isolation from biological objects, cleaning extracts of impurities are a pressing issue for improving existing and developing new methods of analytical and bioanalytical analysis. The objective of this research was to select the optimal conditions for the extraction of enalapril maleate (EM) by organic solvents from water solutions in dependence on pH solutions. **Materials and Methods:** The chromatographic analysis of enalapril performed on liquid chromatograph ACQUITY Arc System. **Results:** The extraction of enalapril by organic solvents from water solutions in dependence on pH solutions has been conducted. The quantitative determination of enalapril by high-performance liquid chromatography methods has been conducted. **Conclusion**: As a result of studies, we have found that the optimal extragent is chloroform, which is extracted at pH 5–90.74% and methylene chloride is extracted at pH 4–81.39%. We have found that EM least extracted with hexane, so these conditions may be cleaned extract from coextractives impurities.

Key words: Enalapril maleate, extraction, high-performance liquid chromatography, organic solvents

INTRODUCTION

Enalapril maleate (EM) tablets formulate in lactose matrix containing magnesium stearate lubricant have revealed an enhance in the disintegration times at very high humidities. The drug degrades slightly to form diketopiperazine by dehydration and the diacid enalaprilat by hydrolysis and these products enhance with temperature.[1] EM, as the maleate salt, is well absorbed following oral administration and comprehensively hydrolyzed to enalaprilat, its bioactive form. EM is an angiotensin-converting enzyme inhibitor and it is used in the treatment of cardiovascular diseases, which are known to be the principal cause of mortality in present stages. Cardiovascular diseases for which EM is prescribed include hypertension, left ventricular systolic dysfunction, and myocardial infarct, and it is also known to significantly retard renal function loss associated with diabetic nephropathy, which is caused by an amalgamation of diabetes mellitus and hypertension.[2] Any analytical definition includes the following steps: Sample preparation, proper chemical analysis, and statistical processing of the results of the analysis. Sample preparation is a complex of rational actions over the object of analysis to transform the test into a form acceptable for further analysis. Sample preparation is an important step in pharmaceutical analysis and is needed to improve the metrological characteristics of the analysis: Improving accuracy, reliability, correctness and reproducibility of the determination, expanding the range of values studied, accelerating the test, and reducing the error

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of the results of the analysis.[3] The nature of the sample preparation is determined by the nature of the sample and the analytical method used for further analysis. Depending on the nature, aggregate state, sample concentration, and method of analysis, various sampling procedures are used: Moisture removal, grinding, decomposition, dissolution, melting, elution, removal of the matrix, dilution, concentration, etc. For the purification of substances from impurities, as well as for the separation of mixtures of substances, extraction is used. This method is based on the different solubility of the substances in the suitable solvent, or in two non-mixing solvents.[4] Modern methods of isolating drugs from biological material based on the individual physicochemical properties of the compounds, so the choice of optimal conditions for isolation from biological objects, cleaning extracts of impurities are a pressing issue for improving existing and developing new methods of analytical and bioanalytical analysis.[5,6]

Our aim was to select the optimal conditions for the extraction of EM by organic solvents from water solutions in dependence on pH solutions.

MATERIALS AND METHODS

Chemical and Reagents

The chemicals used in all experiments were obtained from Sigma (Bangalore, India) and Merck (Mumbai, India), MEM (Hi-Media), EM, and dimethyl sulfoxide. All of other chemicals and reagents were obtained from Sigma-Aldrich**.**

The main quantitative measure of extraction is the degree of extraction (R) - the ratio of the extracted material to the total (initial) of the substance in the aqueous solution. Amount of API was determined experimentally using high-performance liquid chromatography (HPLC) method. We have chosen organic solvents due their use in the pharmaceutical analysis of drugs for the isolation and purification of extracts from biological material such as hexane, chloroform, methylenchloride.

To investigate the degree of extraction of EM from aqueous solutions of organic solvents used standard solutions, which was prepared with concentrations. The standard solution concentration of EM is 10 μg/mL in 0.01 mol/l sodium hydrochloride. Assay performed using previously developed conditions.[5-20] Research of enalapril extraction conditions with aqueous solutions of organic solvents was performed by the following procedure: The number of separating funnels made in 8.00 mL of buffer solutions and in 200 mL of standard solution of enalapril, to the resulting mixture was added 10.00 mL appropriate organic solvent. Mixtures shaken in separating funnel for 5 min and left for 10 min to separate the layers.

Organic layers were collected in a beaker and evaporated in a water bath to dryness, which was dissolved in 5 mL of ethanol, quantitatively transferred to a volumetric flask and 10.0 mL of solvent was adjusted to the mark. Amount of API was determined experimentally using HPLC method. The experiment was performed 3 times for each pH studied for organic solvents. Need pH created using universal buffer Britona-Robinson (pH = 1.8) and 0.2 M sodium hydroxide from 2.0 to 12.0. The pH installed in the application of pH meter - pH 150 MI (2011, Russia).

The chromatographic analysis of EM performed on liquid chromatograph ACQUITY Arc System.

Chromatography was performed on liquid chromatography with spectrophotometric detector under the following conditions:

- Grace platinump C8 EPS column (4.6 mm i.d. × 250 mm, 5 μm);
- Mobile phase: Acetonitrile: Buffer solution pH 2.2 (25/75, v/v);
- The rate of mobile phase: 2.0 mL/min;
- Column temperature: 30°C;
- Detection wavelength: 215 nm.

RESULTS

The extractions of EM by organic solvents from water solutions in dependence on pH solutions were conducted. The different solvent extracted samples from the EM were analyzed using HPLC. For elaboration on the method, the chromatograms of the standard solution of enalapril [Figure 1], as well as the dependence of the intensity peaks on the retention time were obtained and analyzed.

DISCUSSION

HPLC is a versatile, robust, and widely used technique for the isolation of chemical products.[21] Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study.[22] To understand the purpose of HPLC analytical method, it is necessary to consider the applications of HPLC in pharmaceutical analysis. There is wide variety of application throughout the processing of new drugs, from the initial drug discovery to manufacture of formulated products which will administer to the patients.[23-25]

In developing technique for enalapril, to find the appropriate HPLC conditions, various columns and isocratic and gradient mobile phase systems were tried, and successful attempts were performed using Grace Platinump C8 EPS column (4.6 mm i.d. × 250 mm, 5 μm). The mobile phase consists of acetonitrile and phosphate buffer solution (pH 2.2) in the proportion (25/75, v/v) at a flow rate of 2.0 mL/min with ultraviolet detection at 215 nm.

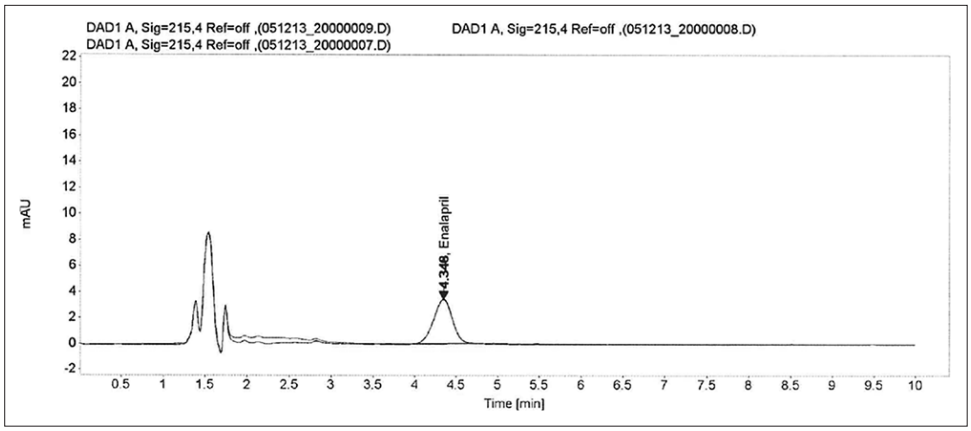

Figure 1: Chromatogram of the standard solution of enalapril by high-performance liquid chromatography in terms of the quantification of enalapril maleate

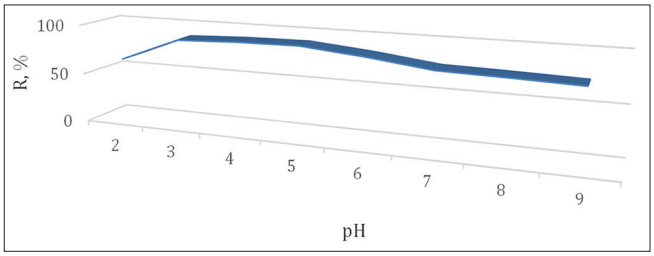

Figure 2: The dependence of the degree of extraction of enalapril on pH solutions and nature of organic solvents (chloroform)

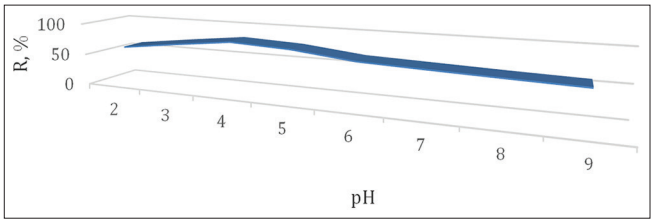

Figure 3: The dependence of the degree of extraction of enalapril on pH solutions and nature of organic solvents (methylenchloride)

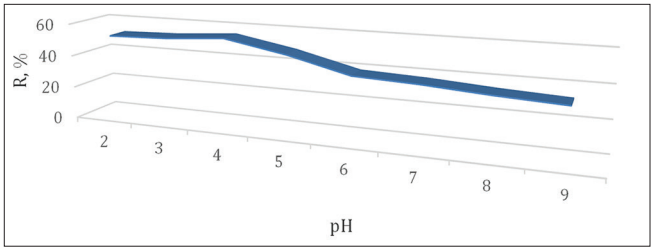

Figure 4: The dependence of the degree of extraction of enalapril on pH solutions and nature of organic solvents (hexane)

The results of the current study of enalapril degree of extraction of various organic solvents, depending on the pH clearly indicate that the extraction of the drugs takes place already almost in acidic solutions.

Results of the present study are revealed in Figures 2-4 and indicate that EM is extracted with used organic solvents. The area of maximum extraction for most solvents is observed at pH 4–5. The obtained data testify that the optimal solvents for the extraction of EM in the process of separating it from objects of biological origin are chloroform and methylene chloride. The amount of substance released by chloroform is 90.74% (at pH 5) and methylene chloride 81.39% (at pH 4).

Considering that hexane extracts very small amount of EM from aqueous solutions, it can be used to purify aqueous extract containing the preparation.

CONCLUSION

The extraction of EM by organic solvents from water solutions in dependence on pH solutions has been conducted. As a result of studies, we have found that the optimal extragent is chloroform, which is extracted at pH 5–90.74% and methylene chloride is extracted at pH 4–81.39%. We have found that EM least extracted with hexane, so these conditions may be cleaned extracts from coextractives impurities.

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