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Journal of Pharmaceutical and Biomedical Analysis
24 (2001) 675–680

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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A simple HPLC method for quantitation of enalaprilat

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Received 29 May 2000; received in revised form 6 September 2000; accepted 14 September 2000

Abstract

A reversed-phase high performance liquid chromatography (HPLC) method with UV-detection has been developed for the determination of enalaprilat. The method produced linear response over the wide concentration range of 1–200 μg/ml, with an average accuracy of 97.35 ± 4.93%, as well as average intra- and iter-day variations of 3.72 and 5.18%, respectively. The limits of detection and quantitation of the method were 0.125 and 0.5 μg/ml, respectively. The method was selective with respect to resolution of the peaks of enalaprilat and enalapril maleate.

Keywords: Enalaprilat; High performance liquid chromatography; Reversed-phase HPLC; Drug analysis; Pharmaceutical analysis

1. Introduction

Enalaprilat, the pharmacologically active metabolite of enalapril, a widely used angiotensin converting enzyme inhibitor (ACEI), is available in intravenous injectable dosage forms, which are administered in the management of hypertension when rapid onset of action of drug is required and/or oral enalapril therapy is not practical [1–3]. Therefore, the availability of a suitable method for determination of enalaprilat in parenteral dosage forms may be of remarkable importance in conducting the in vitro quality control tests on preparations containing this drug. Because of some physicochemical difficulties inherent to this drug — mainly the lack of a well-discernible peak in the absorption spectrum of the drug — the development of chromatographic methods for quantitative analysis of enalaprilat suffers, to a considerable extent, from some limitations [4]. Consequently, only a limited success has been achieved regarding the quantitation of this drug and its prodrug, enalapril. In addition, because of the presence of two rotamers of the drug, most of the HPLC methods developed for analysis of this drug [4,5], including one presented in USP XXIV monograph of the drug [6], involve the use of high column temperatures and, as a result, special temperature-controlling devices are needed to overcome the peak-splitting problem. Nevertheless, a variety of methods mainly based on radioimmunoassay techniques [7–11], as well as measure-

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0731-7085/01/$ - see front matter
PII: S0731-7085(00)00446-5

ment of serum ACE activity as an indicator of drug concentration [12–18], have been developed to solve this problem. However, the application of these methods has been confined to the quantitation of the drug in biological fluids, mainly for pharmacokinetic studies [10,19–24] because of both the natures of the methods and also the cost effectiveness reasons. In the present study, a simple and available reversed-phase HPLC method, without any special requirements, has been developed and validated for assay of enalaprilat.

2. Materials and methods

2.1. Materials

Enalaprilat and enalapril maleate USP Reference standards (USPC Inc., Rockville, MD) were kindly donated by Dr Abidi Pharmaceutical Co. (Tehran, Iran). Acetonitrile (Merck Co., Art. No. 30) and phosphoric acid 89% (Merck Co., Art. No. 564) were prepared locally and were from HPLC and chemical laboratory grades, respectively.

2.2. Instrument and HPLC method

A series of parameters, consisting of composition and pH of mobile-phase, the column packing, flow rate and detection wavelength, were tested with respect to the location and shape of the enalaprilat peak in the corresponding chromatograms. As the final optimized method, a mobile-phase consisting of water–acetonitril–orthophosphoric acid (85% W/V) (90:10:1, v/v/v) with a pH* of 2.8 adjusted by the addition of NaOH concentrated solution, was delivered by a double-reciprocating pump (Waters, model 6000) with a flow rate of 0.7 ml/min. The separation was made using a pH-resistant Shodex C18 column (Shodex Rspak, D18-613, Showa Denko k.k., Japan). An ultraviolet detector (Waters, model 481) with a wavelength of 215 nm was used for detection and the outputs were processed and recorded by means of a compatible integrator (Waters, model 746) having a chart speed of 0.25 cm/min. The samples were injected to chromatograph using a Rheodyne injection devive (Rheodyne, model 7725I, CA) equipped with a 50 μl loop.

2.3. Sample preparation

A stock solution of 4 mg/ml of enalaprilat USP reference standard was prepared in distilled water and the concentrations of 1, 2, 4, 10, 25, 50, 100 and 200 μg/ml were prepared by diluting this solution with the proper amount of distilled water.

2.4. Standard curve

Samples prepared as described above were injected directly to chromatograph in three separate runs and in each case, the linear regression analysis was carried out on known concentrations of enalaprilat against the corresponding peak heights and the regression coefficient (r), slope and intercept of the resulted calibration curve were determined.

2.5. Analysis validation tests

2.5.1. Absolute recovery

Three samples from each of the concentrations used for construction of standard curve were prepared and the concentration of enalaprilat in each sample was determined using standard curve. Then, the percent ratios of measured concentration to known added concentration were calculated in each case.

2.5.2. Intra-day variations

In one day, all of the concentrations used for construction of standard curve were prepared as six replicates and analyzed by HPLC method. Then, the coefficients of variations (CV%) of responses were calculated in each case.

2.5.3. Inter-day variations

On 6 different days, samples from each of the concentrations used for construction of standard curve were prepared and analyzed by HPLC method. Then, the corresponding coefficients of variations were calculated.

2.5.4. Limits of detection and quantitation

For this purpose, 25 successive injections of distilled water (blank) were made and the average heights of noise peaks for each of the samples were determined. Then, the S.D. of the peak heights was calculated and multiplied by 3 and 10 to obtain limits of detection and quantitation, respectively. Finally, to validate the estimated values, six samples of each of the calculated concentrations were prepared and injected into the system.

2.5.5. Selectivity

To evaluate the selectivity of the method, an aqueous solution containing enalaprilat and enalapril maleate with concentrations of 20 μg/ml of the former and 10 μg/ml of the latter was injected to HPLC system.

2.5.6. Sample stability

To exploit the stability of analyte samples during analysis time and also upon storage for a limited time, samples with concentrations of 2, 25 and 200 μg/ml of enalaprilat were analyzed immediately after preparation as well as after 12, 24 and 72 h in room temperature. Then, the percent ratios of concentrations determined in each case to known added concentrations were calculated.

3. Results and discussion

3.1. Method development

To overcome the popularity problems of the HPLC methods presently used for analysis of enalaprilat (e.g. the need for temperature-controlling devices as well as thermo-resistant columns) and to develop an available and easy-to-use method for quantitative analysis of enalaprilat in dosage forms, a series of studies were conducted in our laboratory. We examined several HPLC method variables with respect to their corresponding effects on the results of the analysis. In our extensive preliminary experiments, using a μ-Bondapack C18 Column (3.9×150 mm, Waters, USA), a series of aqueous mobile-phases consisted of buffer solutions with different pH values in combination with different moderators including acetonitril, methanol and triethylamine with different volume fractions, were tested and only acetonitril in acidic pH condition showed an acceptable result. Furthermore, the results indicated that the peak shape and retention time of enalaprilat might be affected dramatically by both the composition and pH of the mobile-phase with only a limited range of both parameters giving the desirable results.

A set of column packings including C8, CN and C18 series with different particle sizes and lengths were tested and, consequently, the C18 packing (octadecyl-bonded silica) showed the best separation. However, because the pH of mobile phase was, to a considerable extent, acidic (pH = 2.8), we used a pH-resistant C18 packing (i.e. Shodex-D18 column) successfully for analysis. Among several flow-rates tested (0.5–2 ml/min) the rate of 0.7 ml/min was the best with respect to location and resolution of drug peak. Because of the widespread availability of UV detectors, our focus was primarily on this type of detector. A variety of wavelengths were examined for detection and, finally, the wavelength of 215 nm exhibited the best detection. A typical chromatogram produced by the developed HPLC method is shown in Fig. 1.

3.2. Method validation tests

3.2.1. Linearity

The peak heights showed linear correlation with enalaprilat concentration over the relatively wide range of 1–200 μg/ml with r^2, slope and intercept values of 0.999, 6.67 (S.D. = 0.60) and −1.00, respectively, with peak heights given in centimeters.

3.2.2. Accuracy

The mean absolute recovery values of the method throughout the linear range are shown in Table 1. From Table 1, it is obvious that the method is remarkably accurate and this ensures obtaining reliable results.

3.2.3. Precision

The intra- and inter-day variations of the method throughout the linear range of concentrations are shown in Table 2. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs.

3.2.4. Limit tests

The limits of detection (LOD) and quantitation (LOQ) of the method were 0.125 μg and 0.5 μg/ml, respectively and the CV% values of the peak heights of six successive injections in each case were 7.2 and 6.6%, respectively.

3.2.5. Selectivity

As demonstrated in Fig. 1, the developed HPLC method is selective in that it highly resolved the corresponding peaks of enalaprilat and enalapril maleate (the most chemically related compound to the drug) in the chromatogram.

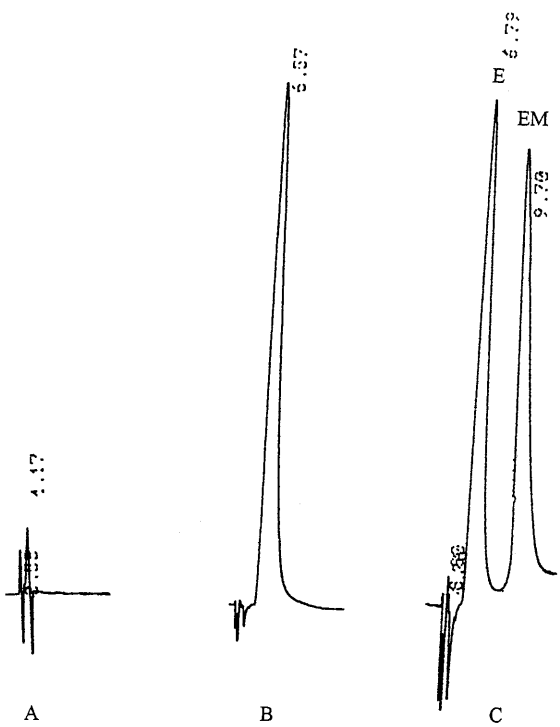

Fig. 1. Typical chromatograms of the HPLC method upon analysis of the distilled water (blank, A), enalaprilat aqueous solution (20 μg/ml, B) and aqueous solution containing enalaprilat (20 μg/ml) and enalapril maleate (10 μg/ml) (C). E, enalaprilat; EM, enalapril maleate.

Table 1
Absolute recovery of the HPLC method for determination of enalaprilat*

Added concentration (μg/ml)	Measured concentration (μg/ml)	Recovery (%)
1	0.93	93.00
2	1.94	97.05
4	3.66	91.41
10	9.42	94.20
25	26.36	105.44
50	51.04	102.08
100	94.81	94.81
200	201.60	100.80
Mean (S.D.)	97.35 (4.93)	

* $n = 3$.

3.2.6. Sample stability

The stability of samples with different concentrations of enalaprilat are shown in Table 3. These data showed that the drug has remarkable stability under test condition.

4. Conclusion

A simple and available HPLC method has been developed in this study for quantitation of

Table 2
Between- and within-run variations of HPLC method*

Concentration (μg/ml)	Within-run CV (%)	Between-run CV (%)
1	6.95	6.51
2	4.23	6.17
4	5.05	7.63
10	5.11	5.80
25	3.33	4.41
50	2.79	4.48
100	1.18	3.16
200	1.09	3.30
Mean	3.72	5.18

* $n = 6$.

Table 3
Sample stability of enalaprilat in the room temperature and analysis condition[a]

Concentration (μg/ml)	Percent recovered			
	Fresh sample	12 h sample	24 h sample	72 h sample
2	101.14 (2.01)[b]	98.47 (5.11)	96.29 (3.87)	96.67 (4.45)
25	98.57 (5.63)	97.35 (4.65)	95.28 (5.21)	96.84 (2.33)
200	103.35 (6.54)	98.71 (4.14)	95.54 (1.97)	98.76 (7.59)

[a] $n = 6$.
[b] Mean (S.D.).

enalaprilat in aqueous matrices. The most considerable advantage of this method in comparison to previously reported HPLC methods, is the lack of need for particular requirements (mainly column temperature programmer). The relatively short runtime is another advantage of this method. Because of the lack of an available and simple HPLC method with no special requirements, this method can be of remarkable importance for in-vitro quality control tests on finished products, as well as during the in-process control tests on parenteral preparations. However, the possible matrix effect(s) must be exploited before considering the method for any particular applications. The wide range of linearity with considerable accuracy, precision and selectivity allows the method to be used in order to assay enalaprilat during a variety of in vitro experiments including the development of new formulations as well as novel systems for the delivery of this drug. For example, the method has been used successfully by authors during experiments on an intravenous delivery system [25]. In addition, the remarkable selectivity of the method being itself validity indicating, allows the simultaneous determination of enalaprilat and enalapril maleate whenever needed. Studies are being performed on the method to obtain a suitable HPLC method for quantitative analysis of enalaprilat and its prodrug, enalapril, in biological fluids.

Acknowledgements

The authors acknowledge Dr Abidi Pharmaceutical Co. (Tehran, Iran) for providing us with USP reference standard of enalaprilat and also L. Hakemi for her remarkable technical assistance.

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