A STABILITY- INDICATING HPTLC METHOD FOR QUANTIFICATION OF ENALAPRIL MALEATE IN TABLETS

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ABSTRACT

A stability- indicating high performance thin layer chromatographic (HPTLC) method was developed and validated for the determination of enalapril maleate in tablets.

Chromatographic separation was achieved on precoated silica gel F 254 HPTLC plates using a mixture of 1-butanol, glacial acetic acid and water (12:3:5, v/v) as a mobile phase. Quantitative analysis was carried out at a wavelength of 207 nm. The method exhibited an adequate linearity ($r = 0.998$), selectivity, precision and accuracy.

Enalapril was subjected to oxidative, hydrolytic, thermal and photolytic stress conditions, and was found degradation significantly in basic stress condition only, during the time of the study. The degradation products were well resolved from main peak, proving the stability-indicating power of the method.

This method was successfully applied for quantitative analysis of enalapril maleate in tablets. Therefore, the method is suitable for quantitative determination and the stability studies of enalapril maleate in tablets.

Key words: enalapril, stability- indicating method, HPTLC, tablets.

INTRODUCTION

Stress testing is an important part of drug development process because it can help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. Chemical degradation may results in a loss of potency or an increase in drugs toxicity, so that clinical use of a medicine must be unacceptable if the degradation is relatively great¹. Stress testing helps to anticipate the behaviour of the drug substance when using it as a drug product. The nature of the stress testing depends on the individual drug substance and the type of drug product involved. Stress testing includes the effect of temperatures and other appropriate conditions such as humidity, light exposition, an others.¹⁻⁴

Enalapril is an angiotensin-converting (ACE) inhibitor. Enalapril is a prodrug and has little pharmacological activity until hydrolyzed in the liver to enalaprilat. Enalapril is the ethylester of enalaprilat. Figure 1 and Figure 2 shows the chemical structures of both compounds.

Figure 1. Chemical structure of enalapril.

Figure 2. Chemical structure of enalaprilat.

Enalapril is used alone or in combination with other classes of antihypertensive agents for the management of hypertension.

Enalapril maleate alone or in fixed combination with felodipine or hydrochlorothiazide is administered orally.

Enalapril maleate, unlike enalaprilat, is well absorbed following oral administration. Although enalaprilat is a more potent ACE inhibitor than enalapril, it is poorly absorbed from the GI tract because of his high polarity, with only about 3%-12% of an orally administered dose being absorbed. Approximately 55%-75% of an oral dose on enalapril maleate is rapidly absorbed from the GI tract in healthy individuals and hypertensive patients.

It is reported that enalapril drug substance or pharmaceutical preparation degrades to two major degradation products; enalaprilat and diketopiperazine derivative (DKP), under different storage conditions.⁵⁻⁷

The reported methods for quantitative determination of enalapril in pharmaceutical samples, including official method,² are by liquid chromatography with UV detection,⁸⁻⁹ by spectroscopy,¹⁰⁻¹¹ and by polarography.¹²

Although some high performance thin layer chromatography (HPTLC) methods were found in literature for quantitative determination of this drug in pharmaceutical samples,¹³⁻¹⁵ none of these HPTLC methods were stability-indicating.

High performance of HPTLC it is due to it fastness, solvent economy and high throughput of samples. Chromatography of samples simultaneously with standards it is another advantage, allowing to run up to 60 chromatographies simultaneously (both sides of the plate). Sensitivity of HPTLC it is normally in the range of nanograms in absorbance and picograms in fluorescence mode.

This paper deals with develop and validate a simple stability-indicating HPTLC method for enalapril maleate, and with the determination of its chemical stability under various ICH stress testing.

EXPERIMENTAL

Material

Instrumentation

HPTLC system: Spectrodensitometer Scanner 3 (Camag, Muttentz, Switzerland), equipped with software winCATS 1.4.2 (Camag). Band application device: ATS 3 automatic (Camag); twin trough chromatographic chamber (Camag) 10x10cm and 20x10 cm; and HPTLC glass backed plates 10x10 cm and 20x10 cm, pre-coated with silica gel F 254 (Merck, Darmstadt, Germany).

Reagents and chemicals

Standard of enalapril maleate was obtained from Sigma-Aldrich (St.Louis,

MO, USA). Standard of enalaprilat was obtained from USP (Rockville, MD, USA). 1-buthanol, glacial acetic acid and ethanol were from Merck (Darmstadt, Germany). Water was Milli-Q. All of the reagents were pro-analysis quality.

Methods

Standard Solution preparation

A stock solution of enalapril maleate was prepared in ethanol, to a concentration of 1 mg mL⁻¹. A stock solution of enalaprilat was prepared in ethanol at 1mg mL⁻¹. Diketopiperazine degradation product was produced from standard of enalapril maleate according to the USP method.²

Sample preparation

Pharmaceutical preparations were tablets, nominally containing 10 mg of enalapril maleate. 20 tablets were weighed and powered. A portion equivalent to 10 mg of enalapril maleate was accurately weighed and transferred to a 50 mL volumetric flask and diluted with water to volume (final concentration of enalapril 200 ng µL⁻¹). Then it was mixed with a vortex for 15 s, filtered and centrifuged for 4 min, and the supernatant was used for chromatography studies.

Chromatographic conditions

Chromatography was carried out on silica gel F254 HPTLC plates, previously washed with methanol and activated at 120°C during 20 minutes.

Sample application was done on 4mm bands using an automatic ATS 3 device (Camag). Sample and standard application volumens were 1 µL of each. Number of tracks depended of each assay. For the chromatographic development, 1-buthanol: glacial acetic acid: water (12:3:5, v/v) was used as mobile phase. The chamber was previously saturated. Migration distance was 8 cm. Densitometry readings were carried out using a scanner 3 Camag spectrodensitometer assisted by a computer equipped with software winCATS 1.4.2, and a deuterium lamp was used as the radiation source. Determinations were performed at a wavelength of 207 nm.

Validation

The parameters validated were linearity, detection limit, quantitation limit, repeatability, intermediate precision, accuracy and selectivity.

Validation was performed in compliance with the international standards.

2, 16-18

Stability-indicating capability of the HPLC assay/Stress testing

It was established by HPTLC analysis of all stressed samples and standard solutions of the main degradation products of enalapril (enalaprilat and diketopiperazine). The composition of the mobile phase was chose to optimize separation between enalapril and their degradation products.

Stress testing was carried out according to the ICH stability testing guidance.¹⁶

Hydrolysis:

It was established by forced decomposition of 5 mL of a solution of enalapril maleate 1 mg mL⁻¹, with 10 mL of 0.1 N hydrochloric acid and 10 mL of 0.1 N sodium hydroxide. Samples were heated on a hot plate at 60°C for 60 minutes, cooled to room temperature, diluted to 100 ng µL⁻¹ with ethanol, and analyzed.

Oxidation:

Oxidation of enalapril maleate was carried out in 3% H₂O₂ at a concentration of 2 mg mL⁻¹, at room temperature (25 ± 2°C) for 8 days and 80 °C for 6 hours, in the dark.

Temperature:

Solid enalapril maleate was exposed to dry heat at 50°C, 70°C, and 100°C, in an oven for 8 hours, taking samples each 2 hours. Samples were diluted with ethanol at 100 ng µL⁻¹, and analyzed.

Photostability:

A solution of enalapril maleate 2 mg mL⁻¹ in 1 mm layer in a petri-plate, was exposed to UV and VIS radiation for 3 hours. Samples were diluted with ethanol at 100 ng µL⁻¹, and analyzed.

Application of the method

The method was used for quantitative determination of enalapril maleate in tablets.

RESULTS AND DISCUSSION

Method validation

Linearity

The standard curve of enalapril was linear over the range of 200 ng µL⁻¹ to 1200ng µL⁻¹, which corresponded to 200 ng/band and 1200 ng/band, applying 1 µL of each solution to the chromatographic plates. Concentration solutions of 200-400-600-800-1000 and 1200 ng µL⁻¹ were analyzed. Each solution was spotted four times on one plate. Three replicates were used to establish the calibration plot and the correlation coefficient. The calibration plot relating the quantity of enalapril and peak area had a correlation coefficient of 0.998, indicating a linear relationship over the range investigated.

Detection and quantitation limits

The detection limit (LOD) was 23.78 ng/band and the quantitation limit (LOQ) was 72.01 ng/band, determined for three concentrations (25, 50 and 150 ng µL⁻¹), applying 1µL of each solution to the chromatographic plates, and using the equations: ¹⁶⁻¹⁷ LOD = 3.3s/b; LOQ = 10s / b, where s is the standard deviation of the response and “b” corresponds to the slope obtained from the linearity study of the method.

Precision

The intra-assay precision was determined by analysis of solutions of 200, 600 and 1000 ng µL⁻¹ on the same day, each of which was independently prepared and each of them being applied three times.^{2, 16} Intermediate precision (inter-assay precision) analyses at the same concentrations for three different days.

Precision analysis studies showed an intra-assay variation between 1.14% and 1.43% and an inter-assay variation between 1.27% and 3.67% (Table 1).

Table 1. Precision of the method.

Relative standard deviation (RSD)/ %		
Concentration (ng µL ⁻¹)	Intra-assay precision ^a	Intermediate precision ^b
200.00	1.43	3.67
600.00	1.58	3.16
1000.00	1.14	1.27

^a n= 27; analyzed on the same day (three solutions of each concentration, three spots each)

^b n= 81; analyzed on three different days (three solutions of each concentration, three spots each, prepared for 3 days)

Accuracy

The accuracy was determined by standard addition, applying the method to pharmaceuticals preparation (200ng µL⁻¹) to which known amounts of standard substance corresponding to 80%, 100% and 120% of the concentration expected in the samples were added. Three solutions of each concentration were prepared and three replicates were measured for each one. The accuracy was then calculated from the test results as the percentage of analyte recovered by the assay. The recovery obtained did not differ from the real value in more than 4.05% and is independent of the concentration with a minor coefficient of variation of 1.25% as shown in Table 2.

Selectivity

The selectivity of the method was evaluated through possible interference due to degradation products. For that, a mixture of enalapril, enalaprilat and enalapril diketopiperazine (DKP) was prepared at a concentration of 100 ng µL⁻¹, for each compound. Values for R_f were 0.52 for enalaprilat, 0.62 for enalapril, and 0.82 for DKP, as shown at Figure 3.

Table 2. Method accuracy.

Actual concentration (ng μL^{-1})	Found concentration (ng μL^{-1}) ^a	Accuracy/% ^b	RSD/% ^c
Intra-assay			
360.00	345.42 $\pm$ 3.80	95.95	1.10
400.00	395.60 $\pm$ 4.23	98.90	1.07
440.00	453.38 $\pm$ 4.67	103.04	1.03
Inter-assay			
360.00	348.12 $\pm$ 4.35	96.70	1.25
400.00	398.96 $\pm$ 4.47	99.74	1.12
440.00	452.32 $\pm$ 4.84	102.80	1.07

^a Each value is the mean $\pm$ standard deviation.^b (Founded concentration/ added concentration) $\times$ 100.^c Relative standard deviation (coefficient of variation). **Figure 3.** Selectivity of the method. Study with degradation products. Peak n°1: enalaprilat; peak n°2: enalapril; peak n°3: DKP. Axis X: retarding factor/Rf. Axis Y: absorbance unit/AU.

Also, the selectivity between enalapril and hydrochlorothiazide, the more common compound in the commercial mixtures of enalapril, was studied. The Rf was 0.52 for enalaprilat, 0.62 for enalapril, and 0.83 for hydrochlorothiazide (Figure 4).

Figure 4. Selectivity of the method. Study with hydrochlorothiazide. Peak n°1: enalaprilat; peak n°2: enalapril; peak n°3: hydrochlorothiazide. Axis X: distance ran by the compound/mm. Axis Y: absorbance unit/AU.**Stability-indicating capability of the HPTLC assay/Stress testing**

After treatment of enalapril with acid, none degradation product was found. However, after treatment with basic, peaks of enalaprilat and DKP were observed, and also one minor peak. All of these peaks were resolved from enalapril (Figure 5). When the drug was exposed to a forced degradation with UV or sunlight, and to forced degradation with peroxide or with temperature (50°C-70°C-100°C), no degradation product was found, during the time of the study. The degradation products for enalapril mentioned in the literature are enalaprilat and DKP.^{6, 19-21}

According to previous studies, enalapril degraded in various media, but we found degradation only under basic condition. This finding can be explained because the time of the study, and maybe with more time of drug exposition, degradation products could be found. In the hydrolysis study, samples were heated on a hot plate at 60°C for 60 minutes. In other study, total time for basic hydrolysis was 30 minutes, and total time for acid and neutral hydrolysis was 24 hours.²²

Figure 5. Study at forced degradation of enalapril with 0.1 N NaOH.

Peak n°1: enalaprilat; peak n°2: enalapril; peak n°3: enalapril; peak n°4: DKP. Axis X: retarding factor/Rf. Axis Y: absorbance unit/AU.

Application of the method

Table 3 shows the application of the method for quantitative determination of enalapril maleate in tablets by the proposed HPTLC method, using three different pharmaceutical products.

Table 3. Determination of enalapril in tablets.

Product	Content of enalapril (%) ^a
1	100.05 $\pm$ 3.25
2	97.00 $\pm$ 3.30
3	93.50 $\pm$ 3.38

^a Mean $\pm$ SD**CONCLUSIONS**

A stability-indicating HPTLC assay method for the determination of the chemical stability of enalapril maleate has been developed and validated, with appropriate precision, sensibility, accuracy and selectivity.

The method is stability-indicating since it allows the determination of enalapril maleate in the presence of its degradation products. Therefore, the proposed HPTLC method can be useful for stability studies of enalapril maleate in tablets. None stability-indicating HPTLC method for enalapril was found in literature. HPTLC method developed and validated in this work resulted fast, simple, efficient and easy to perform. HPTLC separation was obtained within few minutes and the method allows for a large number of samples to be measured simultaneously with a very good accuracy, sensitivity and precision. It is very important, specially in quality control. Therefore, this method is novelty and allows a fast and reliable analysis.

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