

CHEMICAL STABILITY OF ENALAPRIL MALEATE DRUG SUBSTANCE AND TABLETS BY A STABILITY-INDICATING LIQUID CHROMATOGRAPHIC METHOD

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The chemical stability of enalapril drug substance and tablets was studied by a stability-indicating liquid chromatographic method. Stress testing was performed on drug substance under various conditions. Accelerated stability testing was carried out for different formulations of enalapril tablets. Chromatographic separation was achieved on a RP-18 column, using a mobile phase of methanol - phosphate buffer at 1.0 mL min⁻¹ and UV detection. Degradation of the drug substance was greater under hydrolytic conditions. After 180 days of accelerated stability testing most enalapril tablets showed more than 10% of degradation. Enalapril drug substance and tablets showed instability under stress and accelerated testing respectively, with possible implications on the therapeutic activity.

Keywords: enalapril; stability; liquid chromatography.

INTRODUCTION

The stability of a pharmaceutical preparation is in relation with its potency, therefore, with its therapeutic properties. Chemical degradation of the active constituent often results in a loss of potency, or in a few instances the degradation products may be toxic, so that clinical use of a pharmaceutical preparation can not be recommendable if the degradation is relatively high.¹

Different factors can affect the stability of drug substances and dosage form; these include intrinsic factors such as the molecular structure of the drug itself and environmental factors, such as temperature, light, humidity and oxygen.¹⁻³ For many pharmaceutical preparations, 90% of labelled potency is generally recognized as the minimum acceptable level.⁴

Enalapril (Figure 1) is an angiotensin-converting enzyme (ACE) inhibitor used in the treatment of hypertension. It is a prodrug which has an ester group in their molecular structure therefore it is susceptible to degradation, it is rapidly absorbed after oral administration as its maleate salt and has little pharmacologic activity until hydrolyzed in the liver to enalaprilat.⁵

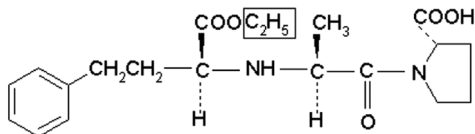


Figure 1. Molecular structure of enalapril. The structure enclosed within box is replaced with a hydrogen atom to form the active molecule in vivo (enalaprilat)

It is reported that enalapril degrades to two major degradation products; enalaprilat and diketopiperazine derivative (DKP).⁶⁻¹¹

Most of the reported methods for quantitative determination of enalapril in pharmaceutical samples,⁶⁻¹⁴ including official method,² are by liquid chromatography with UV detection using a high column temperature. Other techniques include liquid chromatography-mass spectrometry for analysis in biological samples.^{15,16}

The objectives of this study were to develop and validate a simple stability-indicating LC method for enalapril, to determine the chemical stability of enalapril maleate drug substance under various ICH (International Conference on Harmonization) stress testing, and to evaluate the stability of different formulations of enalapril tablets under accelerated storage conditions according to the ICH stability testing guidance.¹⁷

EXPERIMENTAL

Reagents and chemicals

Standard of enalapril maleate and salicylic acid (> 99.0% purity) were obtained from Sigma (St. Louis, MO, USA). Standard of enalaprilat was obtained from USP (Rockville, MD, USA). Methanol LC grade, KH₂PO₄, sodium hydroxide, hydrochloric acid and hydrogen peroxide p.a. grade were from Merck (Darmstadt, Germany). Enalapril maleate drug substance was obtained from Diprolab (Santiago, Chile). Seven commercial products of enalapril maleate tablets 10 mg were studied; three registered trade (RA, RB, RC) and three generic products (GA, GB, GC) were purchased from a Chilean pharmacy, and reference product (S) was purchased from the Argentine market. RA and GA were from the same manufacturer.

Instrumentation

For stability studies a climate chamber KBF-115 Binder (Tuttlingen, Germany) was used. Chromatography was performed by using a Perkin Elmer Series 200 liquid chromatograph (Norwalk, CT, USA) equipped with a manual injector, a 7125 Rheodyne injection valve (Cotati, CA, USA), and a 20-μL loop. An Applied Biosystems Model 785A programmable absorbance detector (Foster, CA, USA), and a Perkin Elmer Nelson Model 1022 data processor (Norwalk, CT, USA).

Chromatographic conditions

All analyses were performed at room temperature (23 ± 2 °C) under isocratic conditions. The separation was carried out using a

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Purospher® RP-18 column (150 mm x 4.6 mm, 5 µm; Merck, Darmstadt, Germany). The mobile phase consisted of methanol - phosphate buffer (pH 2.2; 0.01M) (55:45, v/v). The UV detection was made at 215 nm and the flow rate was 1.0 mL/min. Salicylic acid (60 µg/mL) was used as the internal standard.

Preparation of standard solutions

A stock solution of enalapril maleate and a stock solution of salicylic acid were independently prepared at about 2.0 and 3.0 mg/mL respectively in methanol. Standard solutions were prepared from the stock solution after adequate dilution with water. A stock solution of enalaprilat was prepared in methanol at 1 mg/mL. Diketopiperazine degradation product was produced from standard of enalapril maleate according to the USP method.²

Sample preparation

Ten tablets of enalapril maleate 10 mg were weighed and powdered. A portion equivalent to 10 mg of enalapril maleate was accurately weighed and transferred to a 50 mL volumetric flask. A portion of 1 mL of internal standard (3 mg/mL) was added (final concentration of 60 µg/mL), and diluted with water to volume (final concentration of enalapril; 200.0 µg/mL). Then they were vortexed for 15 s, filtered and centrifuged for 5 min, and the supernatant was chromatographed.

Stability-indicating capability of the LC assay

It was established by chromatographic analysis of all stressed samples (as explained in section *stress testing*) and standard solutions of the main degradation products of enalapril (enalaprilat and diketopiperazine). The composition, pH and the flow rate of the mobile phase were changed to optimize separation between enalapril, internal standard and the degradation products.

Method validation

The method was validated according to the ICH guidelines for validation of analytical procedures.¹⁸ The parameters validated were linearity, precision, accuracy, selectivity, detection and quantitation limits.

Stability studies

Stress and accelerated stability testing were carried out according to the ICH stability testing guidance.¹⁷

Stress testing

Enalapril maleate was stressed under various conditions; until to facilitate approximate 5-20% degradation.¹⁹ For each condition, a blank solution was prepared and was subjected to stress in the same manner as the drug, also it was prepared a control solution of enalapril maleate, which was stored without the stress condition. Prior to analysis, samples were diluted to 200 µg/mL.

Hydrolysis

It was established by forced decomposition of enalapril maleate 2 mg/mL in 0.1 N hydrochloric acid, water and 0.1 N sodium hydroxide. Samples of 5 mL for each time point were kept on a hot plate at 60 °C for basic hydrolysis and at 80 °C for acid and neutral hydrolysis, (total time for basic hydrolysis 30 min. Total time for acid and neutral hydrolysis 24 h), after which they have been cooled to room temperature, then they were transferred to a 50 mL volumetric flask, a 1 mL of internal standard (3.0 mg/mL) was added, then they

were diluted to volume and analyzed by the LC method. In order to determine the degradation kinetics, graphs of the log% residual versus time were obtained.

Oxidation

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

Temperature

Solid enalapril maleate was exposed to dry heat at 70 °C in an oven and under controlled temperature of 40 ± 2 °C and relative humidity of 75 ± 5% in a climate chamber, for 27 days.

Photostability

An aqueous solution of enalapril maleate 2 mg/mL and solid drug in 1 mm layer in a petri-plate, were exposed to UV and VIS radiation for 7 days. Dark controls were run simultaneously.

Accelerated stability testing

Enalapril maleate tablets 10 mg in their original packaging were stored under controlled temperature of 40 ± 2 °C and relative humidity of 75 ± 5% in a climate chamber. Samples were taken at 0, 30, 90 and 180 days and analyzed by LC to determine the enalapril amount. Each sample was analyzed in duplicate.

RESULTS AND DISCUSSION

Stability-indicating LC method development and optimization

The main target of the stability-indicating chromatographic method is to get the separation between enalapril and their degradation products. Reported LC methods for enalapril, used gradient and/or a high column temperature, which requires more sophisticated instrumentation and/or long stabilization times.^{2,6-11,13,14} These methods mainly used acetonitrile and phosphate buffers of various pH in the mobile phase, therefore, different proportions of acetonitrile and change in pH were tested (50:50, 40:60, 30:70 v/v; pH 2.2, pH 2.4, pH 2.6), but under these conditions peak shapes were not good. Then methanol was tested instead of acetonitrile, obtaining good peak shapes. Interference with methanol absorption at 215 nm was not observed. After that, different proportions of methanol using phosphate buffers of various pH and different flow rate, at room temperature of the column, were evaluated before the final chromatographic conditions were selected. The mobile phase that best resolved enalapril maleate, degradation products of enalapril and salicylic acid, with sharp peaks was methanol - phosphate buffer (pH 2.2; 0.01M) (55:45, v/v) at 1.0 mL/min. Figure 2 shows a chromatogram of stressed sample and internal standard, which prove the stability-indicating capability of the assay.

Method validation

Linearity

The calibration curve of enalapril maleate was linear over the concentration range of 50.0 to 300.0 µg/mL (6 different concentrations were used and each solution was injected 5 times). The equation of the standard curve based on the ratio of the peak heights of enalapril maleate / internal standard to the enalapril maleate concentration was $y = 0.0048 \pm 0.0001 x + 0.0088 \pm 0.0048$; $r^2 = 0.9989 \pm 0.0003$.

Precision

The intra-day precision was determined by analysis of 3 different

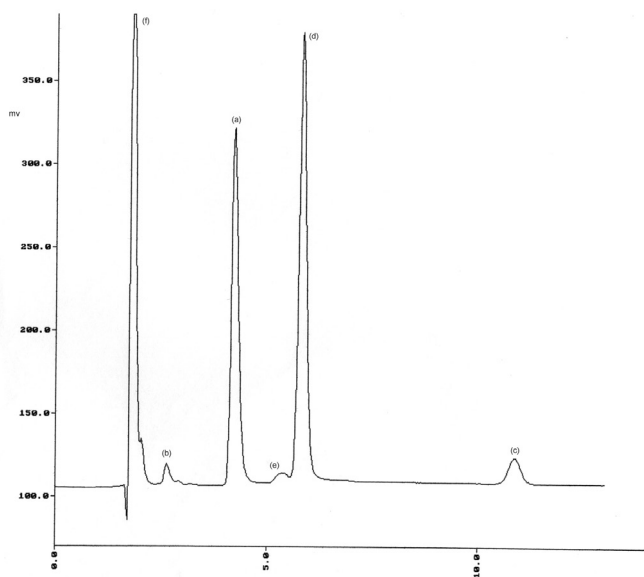


Figure 2. Representative chromatogram of stressed sample: enalapril (a), enalaprilat (b), diketopiperazine (c), salicylic acid (d), acidic degradation product (e) and maleic acid (f). 1 mV:0.001 AU

preparations in concentrations of 150.0, 200.0 and 250.0 $\mu\text{g/mL}$ on the same day. The inter-day precision was studied by comparing the assays on 3 different days. The results are shown in Table 1. The obtained values show a suitable precision for the analytical method.

Table 1. Precision determined during method validation

Concentration ($\mu\text{g/mL}$)	Relative standard deviation (%)	
	intra-day ^{a)}	inter-day ^{b)}
150.00	0.68	1.62
200.00	1.59	1.19
250.00	0.96	1.30

a) Analyzed on the same day (n=3). b) Analyzed on 3 different days (n=9).

Accuracy

To evaluate the accuracy of the method, recovery test were performed by adding known amounts of standard of enalapril maleate in the level 75, 100 and 125% of the enalapril maleate levels in the tablets (3 replicates of each level) to common tablet excipients (lactose, starch and magnesium stearate). The accuracy of the assay was determined by comparing the found amount with the added amount. The results obtained are shown in Table 2. The obtained values confirm the accuracy of the proposed method.

Table 2. Recovery percentage of enalapril determined during method validation

Sample level (%)	Added amount (mg)	Found amount (mg) ^{a)}	Recovery (%) ^{b)}
75	7.50	7.63 \pm 0.05	101.75
100	10.00	9.90 \pm 0.16	99.02
125	12.50	12.70 \pm 0.12	101.63

a) Mean \pm SD (n= 3). b) (Found amount/Added amount) x 100.

Selectivity

The method is selective towards enalapril, degradation products, internal standard, and maleic acid (from maleate salt) as shown in Figure 2. The selectivity was also evaluated by observing any interfer-

ence from excipients used in the tablets; therefore a sample of each commercial product was analyzed without degradation, showing no peaks that interfered with enalapril, degradation products or internal standard. These results proved the selectivity of the proposed method.

Detection and quantitation limits

The DL and QL were calculated by using the equations: $DL = 3.3 \times \sigma / S$; $QL = 10 \times \sigma / S$, where σ is the standard deviation of the response and S is the slope of the calibration curve. DL was 0.18 $\mu\text{g/mL}$ and QL was 0.56 $\mu\text{g/mL}$. These values are adequate for the enalapril determination in pharmaceutical samples.

Stability studies

Stress testing

Stress testing showed the formation of two principal degradation products identified as enalaprilat and diketopiperazine by comparing the chromatograms of stressed samples with chromatograms of standard solution of these compounds.

Hydrolysis

In all the studied stress conditions, the degradation of enalapril was higher under hydrolytic conditions, especially under alkaline stress; therefore degradation kinetics was obtained: After 30 min of hydrolysis in 0.1 N sodium hydroxide, the percentage of enalapril remaining was 7.5%. After 24 h of hydrolysis in water and 0.1 N hydrochloric acid, the percentage of enalapril remaining was 95.2 and 80.4% respectively. In the three studied conditions the degradation followed first-order kinetics. The degradation rate constant and the time at which concentration fell to 90% of the original concentration (t_{90}) are shown in Table 3.

Table 3. Degradation kinetics of enalapril under hydrolytic conditions

Stress condition	Temperature ($^{\circ}\text{C}$)	$K^{\text{a)}$	$t_{90}^{\text{b)}$
0.1 N NaOH	60	0.0914 1/min	1.15 min
Water	80	0.0018 1/h	57.20 h
0.1 N HCl	80	0.0090 1/h	11.73 h

a) Degradation rate constant. b) Time at which concentration fell to 90% of the original concentration

Under alkaline stress, enalapril degraded to enalaprilat, under neutral condition, enalapril degraded to enalaprilat and especially to diketopiperazine and under acidic stress enalapril degraded to enalaprilat, diketopiperazine and a degradation product with that appeared between enalapril and salicylic acid (the major degradation product was DKP).

Oxidation

Enalapril was found to be stable under oxidative stress.

Temperature

No decomposition was seen on exposure of solid drug powder to dry heat at 70 $^{\circ}\text{C}$ or 40 \pm 2 $^{\circ}\text{C}/75 \pm 5\%$ RH for 27 days.

Photostability

The exposure of an aqueous solution of enalapril to UV and VIS radiation for 7 days resulted in a slight degradation to enalaprilat. In solid state, no degradation was observed.

Accelerated stability testing

The results obtained in the accelerated testing showed that most enalapril maleate tablets 10 mg are highly susceptible to de-

Table 4. Accelerated stability testing of enalapril maleate tablets 10 mg

Product	0 days		30 days		90 days ^{a)}		180 days ^{a)}	
	(mg) ^{a)}	(%)	(mg) ^{a)}	(%)	(mg) ^{a)}	(%)	(mg) ^{a)}	(%)
S	9.97±0.05	99.7	9.82±0.08	98.2	9.80±0.01	98.0	9.79±0.08	97.9
RA	9.77±0.12	97.7	9.52±0.21	95.2	9.13±0.07	91.3	9.09±0.05	90.9
RB	9.23±0.13	92.3	8.69±0.13	86.9	7.99±0.08	79.9	7.77±0.19	77.7
RC	9.30±0.09	93.0	7.28±0.14	72.8	5.88±0.09	58.9	4.87±0.20	48.7
GA	9.22±0.15	92.2	9.12±0.09	91.2	9.07±0.02	90.7	9.03±0.19	90.3
GB	8.89±0.12	88.9	7.28±0.19	72.8	5.77±0.13	57.7	4.79±0.14	47.9
GC	9.13±0.08	91.3	8.09±0.16	80.9	5.01±0.14	50.1	3.19±0.08	31.9

S= reference product. R= registered trade products. G= generic products. Mean ± SD (n= 4).

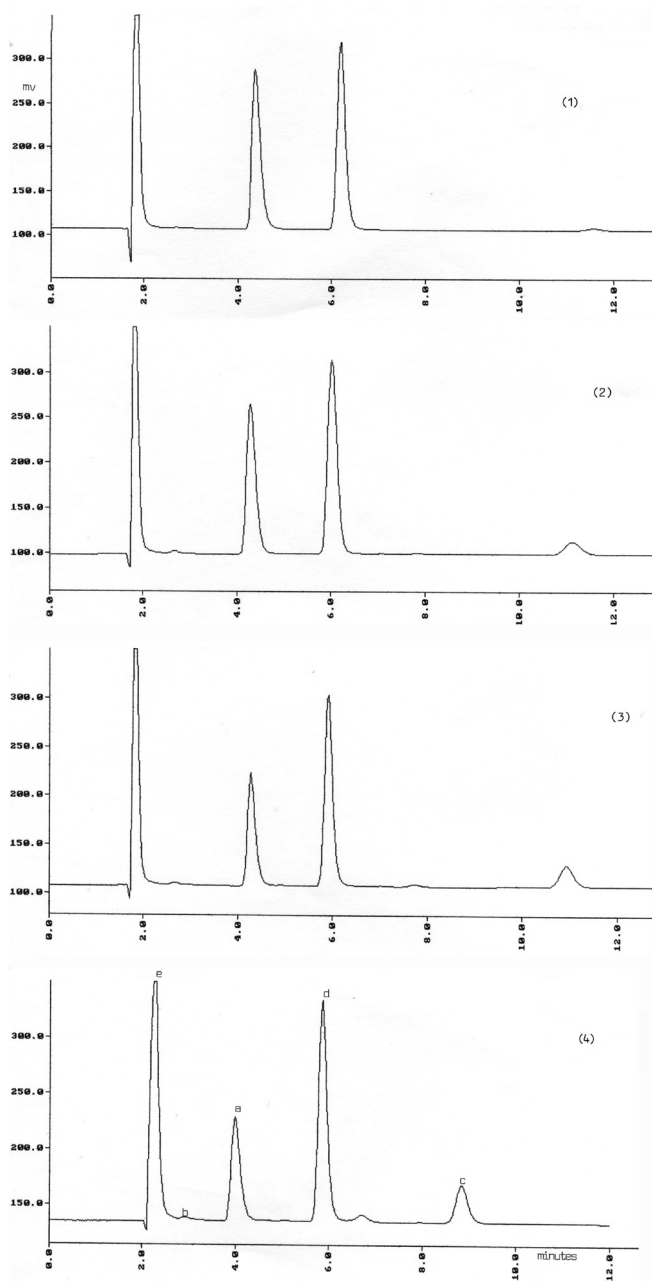


Figure 3. Representative chromatograms of enalapril maleate tablets 10 mg, product GB stored under 40 °C and 75% RH at 0 (1), 30 (2), 90 (3) and 180 (4) days: enalapril (a), enalaprilat, (b) diketopiperazine (c), salicylic acid (d) and maleic acid (e). 1 mV:0.001 AU

gradation. According to the United States Pharmacopeia,² enalapril maleate tablets must contain between 90-110% of the labelled amount, which corresponds to 9-11 mg. As show in Table 4, only reference product (S), registered trade product RA and generic product GA (both from the same manufacturer) are within these limits throughout the study. Even product GB is outside of specifications at the beginning of the study (day 0). These results are in accordance with previous work,¹⁰ but the study was in tablets from the Brazilian market.

Under accelerated conditions, enalapril tablets degraded gradually to enalaprilat and especially to diketopiperazine as a function of the time of exposure to higher temperature and humidity. Figure 3 shows chromatograms of product GB showing the gradual increase of enalapril degradation during the study.

CONCLUSIONS

A simple stability-indicating LC method to determine the chemical stability of enalapril maleate drug substance and tablets has been developed and validated.

The results obtained from the stress testing show that the degradation of enalapril maleate drug substance in solution is higher under hydrolytic conditions especially under alkaline stress, whereas in solid state (powder) is highly stable.

The results obtained in the accelerated testing show that most enalapril maleate tablets 10 mg are highly susceptible to degradation under moderate temperature and humidity (40 °C and 75% RH). Therefore, care should be taken in the manufacturing process and during storage of this product in order to avoid degradation, because amounts lower than 90%, the product does not accomplishes with the pharmacopeial requirements, and thus, it could result in diminution of the therapeutic activity and safety.

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