ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 107–116

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Kinetics of the acidic and enzymatic hydrolysis of benazepril HCl studied by LC

M. Gana, I. Panderi, M. Parissi-Poulou, A. Tsantili-Kakoulidou *

Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis Zografou, GR-157 71 Athens, Greece

Received 13 March 2001; received in revised form 16 May 2001; accepted 29 May 2001

Abstract

A reversed-phase high-performance liquid chromatographic (HPLC) method was developed and validated for the kinetic investigation of the chemical and enzymatic hydrolysis of benazepril hydrochloride. Kinetic studies on the acidic hydrolysis of benazepril hydrochloride were carried out in 0.1 M hydrochloric acid solution at 50, 53, 58 and 63 °C. Benazepril hydrochloride appeared stable in a pH 7.4 phosphate buffered solution at 37 °C and showed susceptibility to undergoing in vitro enzymatic hydrolysis with porcine liver esterase (PLE) in a pH 7.4 buffered solution at 37 °C. Benazeprilat appeared to be the major degradation product in both (chemical and enzymatic) studies of hydrolysis. Statistical evaluation of the proposed HPLC methods revealed their good linearity and reproducibility. Relative standard deviation (R.S.D.) was less than 4.76, while detection limits for benazepril hydrochloride and benazeprilat were 13.0×10^{-7} and 9.0×10^{-7} M, respectively. Treatment of the kinetic data of the acidic hydrolysis was carried out by non-linear regression analysis and k values were determined. The kinetic parameters of the enzymatic hydrolysis were determined by non-linear regression analysis of the data using the equation of Michaelis–Menten.

Keywords: Benazepril; Benazeprilat; Acidic-hydrolysis; Enzyme kinetics; Porcine liver esterase; Reversed-phase high performance liquid chromatography; Kinetic measurements

1. Introduction

Benazepril hydrochloride (3-[1-(ethoxycarbonyl)-3-phenyl-(1*S*)-propylamino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid monohydrochloride) [1], is a new angiotensin-converting enzyme inhibitor, which is shown to be effective in the treatment of hypertension and congestive heart failure [2–4]. The compound, a dicarboxylic acid monoethylester, is a prodrug [5]. Its main metabolic route involves hydrolytic cleavage of the ester linkage leading to its active carboxylic acid metabolite, benazeprilat (3-[1-(carbonyl)-3-phenyl-(1*S*)-propylamino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid) [6,7]. Structures of the compounds are presented in Scheme 1.

* Corresponding author. Fax: +30-1-72-74747.
E-mail address: tsantili@pharm.uoa.gr (A. Tsantili-Kakoulidou).

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00508-8

The ester linkage of benazepril could be cleaved chemically or enzymatically by hydrolases or proteins displaying an esterase-like activity. The in vitro studies of the rate of hydrolysis of medicinal esters under a variety of conditions (non-enzymatic or enzymatic), is a prerequisite for the development and rationalization of therapy. Several authors have studied the hydrolysis of a variety of medicinal esters, by chromatographic [8–12] spectrophotometric methods using chromogenic substrates [13] and spectrofluorimetric [14] methods. An automated analytical system has also been reported allowing the enzymatic studies of medicinal esters using microdialysis sampling technique coupled with reversed phase high-performance liquid chromatography (HPLC) [15].

To follow the pharmacokinetics of benazepril, different analytical methods have been developed. Gas chromatography–mass spectrometry (GS–MS) was used for the determination of benazepril and its major metabolite benazeprilat in human plasma and urine [16,17]. An enzyme inhibition HPLC method and a radioenzymatic method were used for the specific determination of benazeprilat in biological fluids [18]. A HPLC method, using a chiral AGP column, has also been reported for the stereoselective analysis of benazepril and its steroisomers [19]. A number of HPLC assays have also been described in the literature for the determination of benazepril hydrochloride in pharmaceuticals [20–24]. To our knowledge, no article related to degradation kinetics of this drug has ever been mentioned in literature. The objective of this work was to investigate the stability of benazepril hydrochloride at the pH of the stomach since this drug is administered orally. As a prodrug it should be a good substrate for the porcine liver esterase (PLE) but it should be resisted to acidic hydrolysis. Thus, it would be of particular interest to study kinetics of the acidic and enzymatically catalyzed hydrolysis of this compound. A HPLC method was developed, allowing the simultaneous determination of benazepril and its major degradation product benazeprilat. The method was further applied to kinetic studies of this compound in non-enzymatic and enzymatic conditions.

2. Experimental

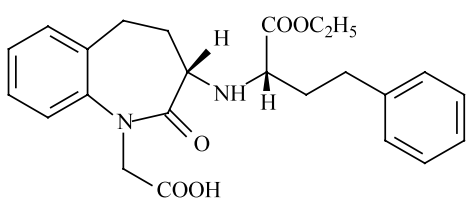
2.1. Equipment

A HPLC system consisted of a GBC Model LC1126 pump and a Rheodyne Model 7725i injector with a 5-μl loop, which were coupled to a GBC Model LC1210 ultraviolet–visible (UV–vis) detector operated at 250 nm. Data acquisition was performed using WinChrom chromatography software package, ChemWin, version 1.2.

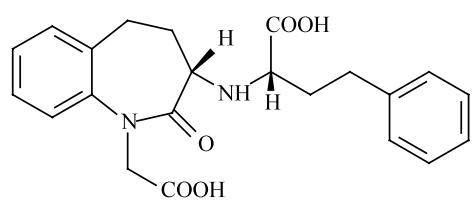
A thermostated Heto water bath with a precision of ($\pm$0.2 °C) was used for the kinetic studies, while all pH measurements were performed on a pH meter (Metrohm, model 654 Herisau).

2.2. Materials and reagents

Solvents were of HPLC grade and were purchased from Lab-Scan Science Ltd., Ireland. Sodium hydrogen phosphate, 2-H_2O, sodium dihydrogen phosphate, hydrochloric acid, perchloric acid and sodium hydroxide (all analytical-reagent grade) were purchased from E. Merck, Darmstadt, Germany. Purified porcine



I



II

Scheme 1. Structure of benazepril hydrochloride I, and benazeprilat, II.

liver carboxylesterase suspension, PLE, labeled to contain 15 mg of protein per ml, 250 U ml^{-1}, in a 3.2 M $(NH_4)_2SO_4$ solution of pH 8.0, was purchased from Sigma Aldrich Chemie, Steinheim, Germany (Cat. No E2884) and stored at 4 °C. Water was deionized and further purified by means of a Milli-Q Plus Water Purification System, Millipore Ltd. Benazepril hydrochloride and benazeprilat of pharmaceutical purity grade were kindly provided by Novartis Pharma AG, Basel, Switzerland. All substances were used without any further purification.

2.3. Stock and working standard solutions

Stock standard solutions of benazepril hydrochloride, Bz, 2.17×10^{-4} M, benazeprilat, Bzlat, 2.52×10^{-4} M, were prepared by dissolving appropriate amounts of the compounds in methanol. These solutions were stored in the dark under refrigeration at 4 °C and were found to be stable for several weeks.

Two series of mixed working standard solutions of Bz and Bzlat in a ratio (1:1) were prepared by the appropriate dilution of the above mentioned stock standard solutions in the appropriate mobile phase.

2.3.1. Buffer solutions

Phosphate buffer (pH 7.40, 25 mM NaH_2PO_4 and 25 mM Na_2HPO_4, $I = 0.05$) was used in all enzymatic studies.

2.4. Chromatographic conditions and measurement procedure

2.4.1. HPLC method for the acidic hydrolysis

Chromatographic separation was performed on a reversed phase BDS C-18 column (250 × 3.0 mm i.d., 5 μm particle size) Shandon Scientific Ltd., (Chesire, UK). The mobile phase consisted of 0.020 M disodium hydrogen phosphate, adjusted to pH 3.0 with phosphoric acid, and methanol (40:60, v/v), was filtered through a 0.20 μm nylon membrane filter (GelmanSciences Ltd.) and degassed under vacuum prior to use. A flow rate of 0.35 ml min^{-1} with a column inlet pressure of 2000 psi was used in order to separate benazepril and its degradation products. Peak heights were measured and HPLC analysis was conducted at ambient temperature.

2.4.2. Calibration procedure

Calibration curves of Bz and Bzlat were conducted using the series of working standard solutions described previously. The concentration range tested for the study of the acidic hydrolysis, ranged from 1.08×10^{-5} to 8.68×10^{-5} M for Bz and from 1.26×10^{-5} to 10.08×10^{-5} M, for Bzlat. The linearity of the HPLC assay for the enzymatically catalyzed hydrolysis was determined over the range 0.22×10^{-5} to 8.68×10^{-5} M for Bz and from 0.25×10^{-5} to 10.08×10^{-5} M, for Bzlat. All solutions were analyzed immediately after their preparation. Triplicate 5-μl injections were made of each solution and peak area of each compound were plotted against the corresponding to obtain the calibration graph.

The over-all precision and accuracy of the HPLC assay was evaluated by analyzing four series of mixed standard solutions of Bz and Bzlat, at concentrations of 0.22×10^{-5}, 1.08×10^{-5}, 4.34×10^{-5} and 8.68×10^{-5} M for Bz and 0.25×10^{-5}, 1.26×10^{-5}, 5.04×10^{-5} and 10.08×10^{-5} M for Bzlat. The relative standard deviation (% R.S.D.) was determined in order to assess the precision of the assay, while the accuracy was expressed by the relative percentage error (E_r%).

2.5. Kinetic investigation of the acidic hydrolysis

A 2.0 ml aliquot of stock methanolic solution of Bz was transferred to a 25-ml volumetric flask and diluted to volume with 0.1 M hydrochloric acid. This solution was transferred to a two-necked round-bottomed flask. One neck of the flask was fitted with a reflux condenser and samples were collected from the other neck. The entire flask assembly was submerged in a thermostated water-bath and the temperature was allowed to equilibrate prior to the addition of Bz solution. This procedure was performed at 50, 53, 58 and 63 °C.

During the kinetic study at predetermined time intervals, 200 μl aliquots were removed from the

flask and 200 μl of water were added followed by vigorous mixing. A volume of 5 μl was quantitatively injected into the analytical column.

Treatment of kinetic data was carried out by the software package Minsq program (version 4.03, Micro-Math Scientific Software, Salt Lake City, UT) using the least squares and simplex subroutines.

2.6. Kinetic investigation of the enzymatic hydrolysis

An appropriate aliquot of the PLE suspension was transferred to a 10-ml volumetric flask and diluted to volume with the phosphate buffer. This solution was transferred to a two-necked round-bottom flask; one neck of the flask was fitted with a reflux condenser and samples were collected from the other neck. The enzyme solution was then incubated for 60 min, at 37 ± 0.2 °C in a thermostated water-bath. The reaction was initiated by adding the appropriate aliquot of the Bz stock standard solution into the flask. At appropriate time intervals, determined by the rate of the reaction, 120 μl aliquots were removed from the flask and the reaction was stopped by the addition of a 10 μl aliquot of $HClO_4$ (70% w/v) [25]. Samples were then vortexed thoroughly for 30 s and a 10 μl aliquot of 18 N NaOH was added in order to reduce the acidity of the solution prior to the chromatographic assay.

For the Michaelis–Menten kinetic evaluation, four series of PLE stock solutions (2.25×10^{-2}, 3.00×10^{-2}, 3.75×10^{-2} and 4.50×10^{-2} mg ml^{-1}) were prepared. The final concentration of Bz in the reaction solutions ranged from 5.42×10^{-6} to 8.68×10^{-5} M.

The initial linear appearance of Bzlat was plotted as a function of time. The slope of each plot was regarded as the initial rate of the reaction, v_o, expressed in mmol min^{-1}. The kinetic parameters V_{max} and k_m were determined by iterative non-linear optimization using the equation of rectangular hyperbola corresponding to Michaelis–Menten kinetics [26,27].

The non-enzymatic hydrolysis rate of Bz was also studied in a phosphate buffered solution of pH 7.4 at 37 ± 0.2 °C. The stability of Bz was adequate for measurement of enzymatic hydrolysis, without chemical hydrolysis interfering.

3. Results and discussion

3.1. Chromatographic characteristics

The chromatographic separations were performed on a BDS C-18 column with a 3.0-mm internal diameter. In recent years, there has been an increasing interest in columns with reducing internal diameter and packed with smaller particles [28,29]. The reduction in column diameter from a standard 4.6- to 3.0-mm i.d. leads to a concomitant reduction of solvent usage, as they operate at lower flow rates (0.30–0.50 ml min^{-1}). Another very practical aspect of the choice of a 3.0-mm i.d. column is the drastic reduction in peak volumes, which are related to the low flow rate [30]. Consequently, for an equal injected mass the solute concentration is greater which results in an increase in the sensitivity compared with columns with diameters of 4.6-mm i.d. This advantage is extremely important, as during the chemical and enzymatically catalyzed hydrolysis several degradation products may be present in trace concentrations.

The effect of composition and pH of the mobile phase on the retention time of Bz and Bzlat, was investigated. Results of the effect of methanol concentration in the mobile phase are presented in Fig. 1. Increasing methanol concentration to more than 65% Bzlat peak is eluted with the solvent front. At methanol concentration lower than 55%, the elution of Bzlat peak is seriously delayed, but the retention time of Bz peak is increased dramatically. The optimum methanol concentration was found to be 60%. The effect of pH in the chromatographic elution of both compounds is related to the degree of ionization. The pK_a values of both compounds were calculated using ACD/pK_a predictor software. A decrease of pH values from 4.0 to 2.5 causes a concomitant decrease of the retention times of Bz due to an increase in the protonation of the nitrogen atom in the benzazepine ring ($pK_a = 5.02$). The same decrease in pH lead to a delay in the elution of Bzlat due to

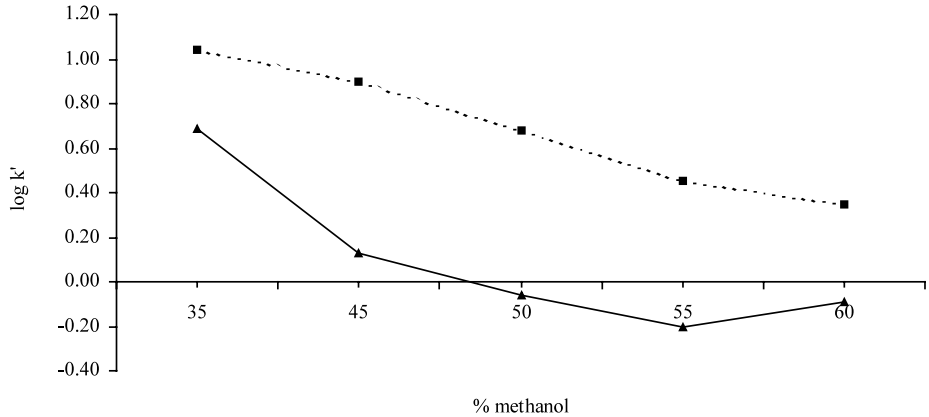


Fig. 1. Effect of methanol concentration on the capacity factor, k, of benazepril hydrochloride (broken line, ■) and benazeprilat (solid line, ►). Column, C-18 BDS column (250 × 3.00 mm i.d.); eluent, 0.020 M sodium hydrogen phosphate $2H_2O$ (pH 3.0) and methanol; flow rate, 0.35 ml min^{-1}; detection wavelength 250 nm.

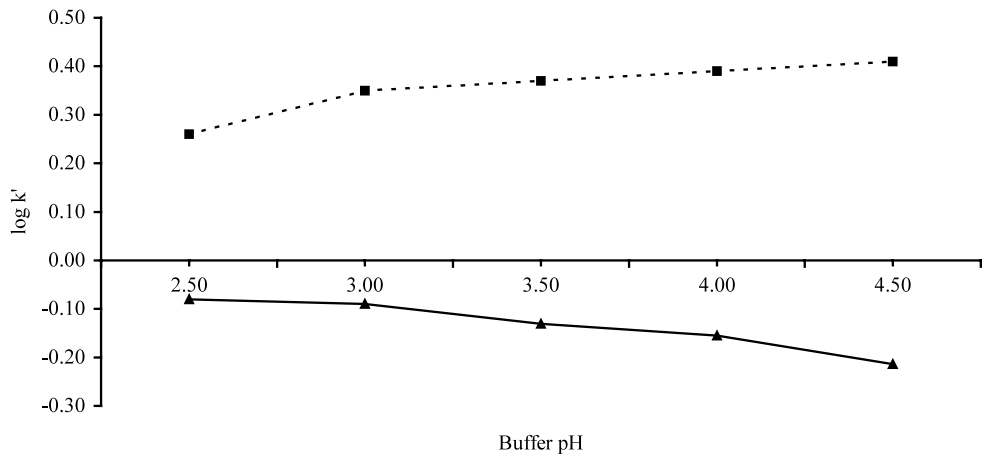


Fig. 2. Effect of mobile phase pH on the capacity factor, k, of benazepril hydrochloride (broken line, ■) and benazeprilat (solid line, ►). Column, C-18 BDS column (250 × 3.00 mm i.d.); eluent, 0.020 M sodium hydrogen phosphate $2H_2O$–methanol (40:60, v/v); flow rate, 0.35 ml min^{-1}; detection wavelength 250 nm.

the reduction in the ionization of the second carboxyl group ($pK_a = 2.2$; Fig. 2). A pH value of 3.0 was found to be the optimum, as at this pH the analyte peaks were well defined and resolved. Bzlat peak is adequately delayed from the solvent front, while this pH is well compatible with the column lifetime. The optimum wavelength for detection was at 250 nm, at which the best detector responses for all the substances were obtained.

The specificity of the HPLC method is illustrated in Fig. 3 where complete separation of the compounds was observed during the kinetic study of the acidic hydrolysis of Bz. Both compounds, Bz and Bzlat, are baseline separated from each other with retention times of 5.08 and 9.35 min, respectively. Three other degradation products are also eluted at 6.23, 7.23 and 14.95 min, respectively. A typical chromatogram obtained during the enzymatic hydrolysis is presented in Fig. 4, Bz and Bzlat eluted at 5.05 and 9.17 min, respectively.

3.2. Linearity and reproducibility

Under the experimental conditions described in Section 2, linear relationships were observed between the peak area of each compound and the corresponding concentration as described in Table 1. The correlation coefficient (r) and the standard error of the estimate (S_r) of the calibration lines are also given, along with S.D. of the slopes and the intercepts.

In order to further evaluate the linearity of the proposed method, five calibration equations were constructed over a period of 4 weeks. The average regression equation for Bz:

$$S_{Bz} = 44.25(\pm 1.53)C_{Bz} + 2.71(\pm 0.95)$$

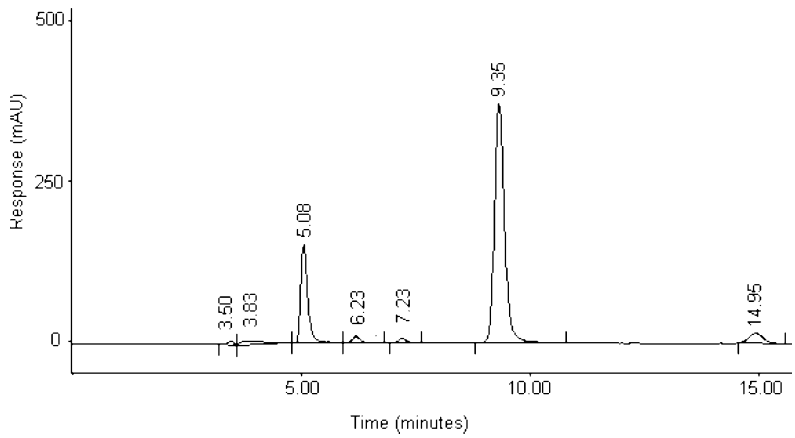


Fig. 3. Representative chromatogram of a mixture of benazeprilat and benazepril hydrochloride at retention times 5.08 and 9.35 min, respectively, and three other degradation products eluted at retention times 6.23, 7.23 and 14.95 min. The chromatogram was obtained during the acidic hydrolysis and the chromatographic conditions were, reversed-phase HPLC on a C-18 BDS column (250 × 3.00 mm i.d.); mobile phase, 0.020 M sodium hydrogen phosphate $2H_2O$ (pH 3.0) and methanol (40:60, v/v); flow rate 0.35 ml min^{-1}; detection wavelength 250 nm.

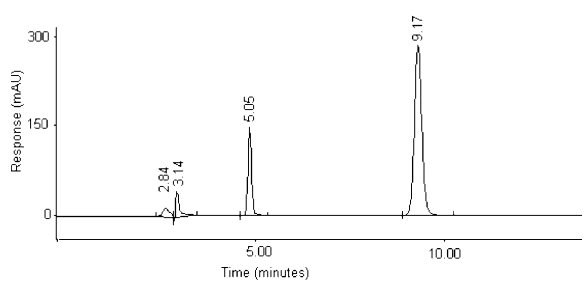


Fig. 4. Representative chromatogram of a mixture of benazeprilat and benazepril hydrochloride at retention times 5.05 and 9.17 min, respectively. The chromatogram was obtained during the enzymatic hydrolysis and the chromatographic conditions were, reversed-phase HPLC on a C-18 BDS column (250 × 3.00 mm i.d.); mobile phase, 0.020 M disodium hydrogen phosphate (pH 3.0) and methanol (40:60, v/v); flow rate 0.35 ml min^{-1}; detection wavelength 250 nm.

Table 1
Analytical parameters of the calibration curves of benazepril hydrochloride and benazeprilat by HPLC

Linearity range ($\times 10^{-5}$ M)[a]		Regression equations	r[b]	S.D.[c]		S_r[d]
Bz	Bzlat			Slope	Intercept	
(*I*)[e]						
1.08–8.68	1.26–10.08	$A_{Bz} = 42.66C_{Bz} - 5.83$	0.9998	0.36	1.85	2.32
1.08–8.68	1.26–10.08	$A_{Bzlat} = 44.15C_{Bzlat} - 0.64$	0.99991	0.22	1.33	1.67
(*II*)[f]						
0.22–8.68	0.25–10.08	$A_{Bz} = 44.96C_{Bz} + 2.77$	0.9991	0.28	1.06	2.41
0.22–8.68	0.25–10.08	$A_{Bzlat} = 45.06C_{Bzlat} + 1.93$	0.99992	0.23	0.99	2.27

[a] Bz, benazepril hydrochloride; Bzlat, benazeprilat.
[b] Correlation coefficient.
[c] S.D. of slope and intercept.
[d] S.E. of the estimate.
[e] Peak area of each compound, A, vs. the appropriate concentration ($\times 10^{-5}$ M), C; six standards.
[f] Peak area of each compound, A, vs. the appropriate concentration ($\times 10^{-5}$ M), C; ten standards.

Table 2
Accuracy and precision of within and between run analysis for the determination of benazepril hydrochloride and benazeprilat by HPLC

Nominal concentration ($\times 10^{-5}$ M)		Assayed concentration ($\times 10^{-5}$ M)					
Benazepril	Benazeprilat	Benazepril			Benazeprilat		
		Mean ± S.D.	R.S.D.%[a]	E_r%[b]	Mean ± S.D.	R.S.D.%[a]	E_r%[b]
Intra-day ($n = 5$)							
0.22	0.25	0.21 ± 0.01	4.76	−4.5	0.24 ± 0.01	4.17	−4.0
1.08	1.26	1.10 ± 0.02	1.82	1.8	1.27 ± 0.01	0.79	0.8
4.34	5.04	4.31 ± 0.01	0.23	−0.7	5.03 ± 0.02	0.40	−0.2
8.68	10.08	8.64 ± 0.01	0.12	−0.5	10.14 ± 0.01	0.10	0.6
Inter-day ($n = 5$)							
1.08	1.26	1.11 ± 0.03	1.80	2.8	1.28 ± 0.02	1.56	1.6
4.34	5.04	4.30 ± 0.04	0.93	−0.9	5.01 ± 0.02	0.40	−0.6
8.68	10.08	8.71 ± 0.02	0.23	0.3	9.91 ± 0.03	0.30	−1.7

[a] Percentage R.S.D.
[b] Relative percentage error = [(assayed concentration − nominal concentration)/nominal concentration] × 100.

and for Bzlat:

$$S_{Bzlat} = 46.40(\pm 0.73)C_{Bzlat} + 1.63(\pm 1.07)$$

where S is the peak area of each compound $\times 10^4$ and C is the appropriate concentration expressed in M $\times 10^5$.

The slopes of the calibration equations for Bz and Bzlat had R.S.D. values of 3.4 and 1.6, respectively, while the correlation coefficient invariably exceeded 0.9992.

Intra-day data for the precision and accuracy of the method given in Table 2, indicate R.S.D.% = 0.12–4.76 and $E_r\% = -4.5 - 1.8$ for Bz and R.S.D.% = 0.10–4.17 and $E_r\% = -4.0 - 0.8$ for Bzlat. Moreover, the inter-day R.S.D.% values (Table 2) for the determination of Bz and Bzlat were ranged from 1.80 to 0.23 and −1.7 to 2.8, respectively.

The limits of detection a, $LOD_{(k=3)} = k \times S_a/b$ where b is the slope of the calibration graph and

S_a is the S.D. of the blank signal, as defined by International Union of Pure and Applied Chemistry (IUPAC) [31], were found to be 13.0×10^{-7} and 9.0×10^{-7} M, for Bz and Bzlat, respectively.

The statistical evaluation of the proposed HPLC method revealed its good linearity and reproducibility and proved suitable for the study of the acidic and enzymatic hydrolysis of Bz.

3.3. Kinetic investigation of the acidic hydrolysis

Accelerated kinetic measurements, of the acidic hydrolysis of Bz, were performed at 50, 53, 58 and 63 °C. The Bz concentration decreased exponentially with time, following a first order reaction. Fig. 5 shows plots of the decay of Bz versus time under different temperatures.

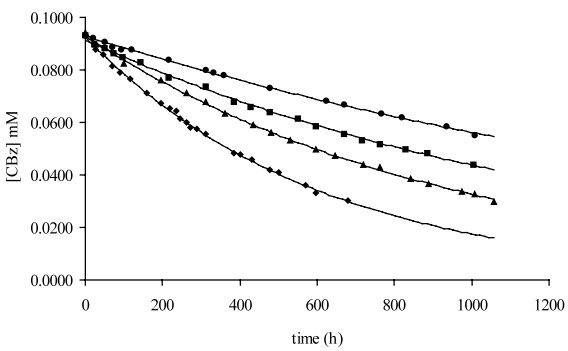


Fig. 5. Typical apparent first order plots of the accelerated hydrolysis of benazepril hydrochloride at 50 (●), 53 (■), 58 (▲) and 63 (◆) °C; all four in HCl 0.1 M.

Table 3
Results of kinetic investigation of the acidic-hydrolysis of benazepril in HCl 0.1 N

θ (°C)	$(k \pm s) \times 10^4$ (h^{-1})	Correlation coefficient r (n)[a]	$t_{1/2}$ (h)
50	4.94 ± 0.03	0.9991 (17)	1403
53	6.68 ± 0.16	0.996 (19)	1037
58	10.40 ± 0.09	0.9993 (20)	666
63	16.55 ± 0.15	0.9992 (22)	419

[a] Correlation coefficient, r, is a statistical parameter which is the correlation coefficient of determination calculated by MINSQ, and n is the number of experimental points included in the non-linear fit (concentration of benazepril hydrochloride vs. time) made by MINSQ.

Values of reaction rate constants, k, were estimated after treatment of the results by a non-linear parametric method (MINSQ). The mathematical model used was $C = pe^{-kt}$, applied to first and pseudo-first order reaction kinetics; where C represents the concentration of Bz at time t and p is a constant factor depending on experimental conditions. Results of the rate constants are presented in Table 3. Correlation coefficient indicate that a good fit between calculated plots and experimental data was achieved. Arrhenius plot was drawn, according to equation:

$$k = Ae^{-E_a/RT}$$

where k is the reaction rate constant expressed in h^{-1}, A the constant, E_a the activation energy expressed in kcal mol^{-1} and T is the absolute temperature. The activation energy E_a was found to be 19.90 kcal mol^{-1} with a correlation coefficient of 0.99994, k and $t_{1/2}$ values at 37 °C were found to be 1.36×10^{-4} h^{-1} and 5095 h, respectively. These results prove that Bz is, as expected, highly stable in acidic media.

3.4. Kinetic investigation of the enzymatic hydrolysis

In order to investigate the enzymatic hydrolysis of Bz the uncatalyzed rate was evaluated in phosphate buffer pH 7.4 at 37 °C. It was found that Bz remained stable for at least 30 min.

The kinetic parameters V_{max} and K_m of Bz in the presence of porcine liver carboxylesterase were calculated from the Michaelis–Menten rectangular hyperbola using non-linear least-squares procedure. Results are presented in Table 4 and indicate that were obeyed over the concentration range of $2.25–3.75 \times 10^{-2}$ mg ml^{-1}. At these enzyme concentrations, K_m remains constant while V_{max} is linearly related to enzyme concentration, E_o, with a correlation coefficient of 0.9991. At $E_o = 4.50$ mg ml^{-1} deviation from linearity of V_{max} against E_o along with an increase of K_m value is observed, indicating the limits for Michaelis–Menten kinetics. Fig. 6 shows an example of Michaelis–Menten kinetics as obtained under three different concentrations of PLE.

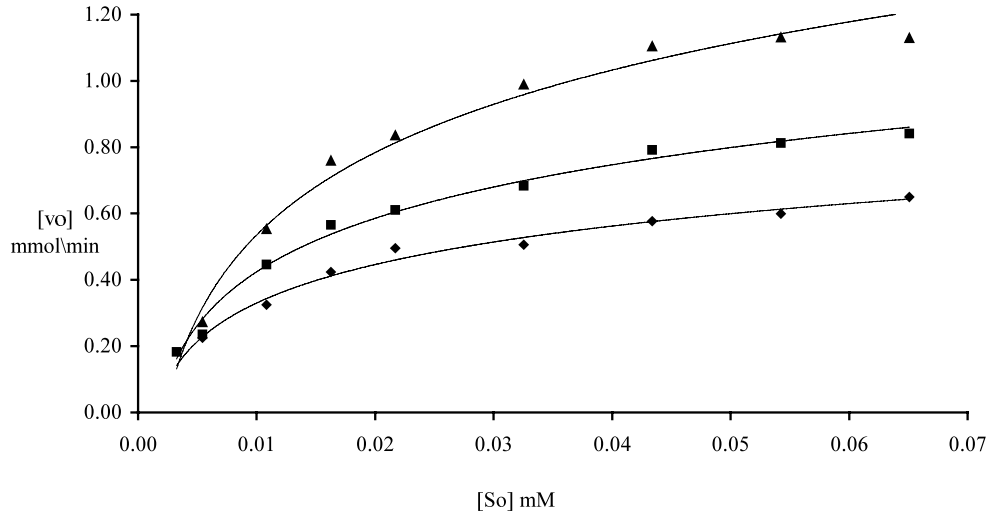


Fig. 6. Michaelis–Menten kinetics as obtained under the following concentrations of porcine liver carboxylesterase 2.25×10^{-2} (◆), 3.00×10^{-2} (■), and 3.75×10^{-2} (▲); S_o is the concentration of benazepril hydrochloride in mM and v_o the initial velocity in mmol min^{-1}.

Table 4
Kinetic parameters of benazepril in the presence of four different concentrations of porcine liver carboxylesterase, as calculated from the Michaelis–Menten equation

E_o (mg ml^{-1})[a]	V_{max} (mmole min^{-1})	K_m (mM)	r (n)[b]
2.25×10^{-2}	0.739 ± 0.034	0.0127 ± 0.0018	$r = 0.992$ (8)
3.00×10^{-2}	1.098 ± 0.031	0.0154 ± 0.0013	$r = 0.996$ (9)
3.75×10^{-2}	1.513 ± 0.077	0.0169 ± 0.0031	$r = 0.992$ (8)
4.50×10^{-2}	2.712 ± 0.041	0.0324 ± 0.0091	$r = 0.995$ (8)

[a] Concentration of porcine liver carboxylesterase.
[b] Correlation coefficient; n is the number of experimental points.

In conclusion, the proposed HPLC method was evaluated over the linearity, reliability and specificity and proved to be convenient and effective for the degradation kinetic studies of benazepril hydrochloride. The advantage of lower solvent consumption, due to the low flow rates that have been used leads to an environmentally friendly chromatographic procedure. Moreover, it offers a short analytical run time of 15.0 min and achieved a good resolution between Bz, Bzlat and degradation products. The method permits the estimation of the reaction rate constants, k, of the acidic hydrolysis of Bz in the presence of degradation products, as well as the determination of Michaelis–Menten parameters and initial rate constant in the presence of PLE. The method could be applied to study the hydrolytic activity of PLE in other substrates.

References

[1] J.W.H. Watthey, J.L. Stanton, M. Desai, J.E. Babiarz, B.M. Finn, J. Med. Chem. 28 (1985) 1511–1516.
[2] J.A. Balfour, K.L. Goa, Drugs 42 (1991) 511–539.
[3] S. Boutelant, A. Francillon, J.P. Siche, L. Cocco-Guyomarchh, J.M. Mallion, Therapie 50 (1995) 313–318.
[4] C. Le Feuvre, A. Francillon, J.F. Renucci, L. Cocco-Guyomarch, M. Muller, P. Peuplier, L. Poggi, Therapie 51 (1996) 27–34.
[5] F. Waldmeier, K. Schmid, Arzneim. Forsch./Drug Res. 39 (1989) 62–67.
[6] G. Kaiser, R. Ackermann, H.P. Gschwind, I.M. James, D. Sprengers, Biopharm. Drug Disposition 11 (1990) 753–764.
[7] J.R. Wade, D.M. Hughes, A.W. Kelman, A. Howie, P.A. Meredith, J. Pharm. Sci. 82 (1993) 471–474.
[8] A. Durrer, B. Walther, A. Racciati, B. Testa, J. Chromatogr. 495 (1989) 256–262.
[9] A.H. Kahns, P.B. Jensen, N. Mork, H. Bundgaard, Acta Pharmacol. Nord. 1 (1989) 327–336.
[10] R. Gokhale, A.M. Grider, R. Gupte, J.D. Wood, J. Pharm. Sci. 79 (1990) 63–65.

[11] G.N. Wernly-Chung, J.M. Mayer, A. Tsantili-Kakoulidou, B. Testa, Int. J. Pharm. 63 (1990) 129–134.
[12] L.G. Rajewski, A.A. Stinnett, V.J. Stella, E.M. Topp, Int. J. Pharm. 82 (1992) 205–213.
[13] O.S. Wolfbis, A. Gurakar, Clin. Chim. Acta 164 (1987) 329–337.
[14] A. Salvi, J.M. Mayer, P.A. Carrupt, B. Testa, J. Pharm. Biomed. Anal. 15 (1996) 149–155.
[15] J. Zhou, E.C. Shearer, J. Hong, C.M. Riley, R.L. Schowen, J. Pharm. Sci. 14 (1996) 1691–1698.
[16] G. Kaiser, R. Ackerman, W. Dieterle, J.P. Dubois, J. Chromatogr. 419 (1987) 123–131.
[17] A. Sioufi, F. Pommier, G. Kaiser, J.P. Dubois, J. Chromatogr. 434 (1988) 239–246.
[18] P. Graf, F. Frueh, K. Schmid, J. Chromatogr. 425 (1988) 353–361.
[19] R. Girilli, F. La Torre, J. Chromatogr. 818 (1998) 53–60.
[20] F. Barbato, P. Morrica, F. Quaglia, Farmaco 49 (1994) 457–460.
[21] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, J. Pharm. Biomed. Anal. 16 (1997) 431–438.
[22] A. Gumieniczek, L. Przyborowski, J. Liquid Chromatogr. 20 (1997) 2135–2142.
[23] I. Panderi, M. Parissi-Poulou, J. Pharm. Biomed. Anal. 21 (1999) 1017–1024.
[24] T. Radhakrishna, D. Sreenivas Rao, K. Vyas, G. Om Reddy, J. Pharm. Biomed. Anal. 22 (2000) 641–650.
[25] E. Jensen, H. Bundgaard, Acta Pharm. Nord. 3 (4) (1991) 243–247.
[26] J.E. House, Principles of Chemical Kinetics, Brown Publishers, USA, 1997, pp. 178–185 Chapter 6.
[27] C.W. Wharton, R. Eisenthal, Molecular Enzymology, Blackie, Glasgow and London, 1981, pp. 275–309 Chapter 10.
[28] F.J. Yang, Chromatogr. Sci. 14 (1989) 17–41.
[29] D.N. Mallet, B.J. Law, J. Pharm. Biomed. Anal. 9 (1) (1991) 53–57.
[30] U.D. Neue, HPLC Columns. Theory, Technology and Practice, Wiley, New York, 1997, pp. 49–53.
[31] J.N. Miller, Analyst 116 (1991) 1–14.