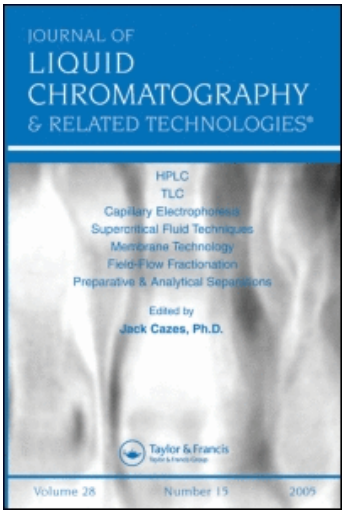

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Liquid Chromatographic Studies of the Stability of Benazepril in Pure Form and in Tablets

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Liquid Chromatographic Studies of the Stability of Benazepril in Pure Form and in Tablets

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ABSTRACT

Selective, linear (coefficient of linear correlation = 0.999) and accurate HPLC method for evaluation of stability of benazepril (BEN) in solid phase has been applied. Liquid chromatography was performed with a column Hypersil MOS (5 μm particle size, 250 mm × 4 mm, Merck), the mobile phase used was mixture of acetonitrile–phosphate buffer (50 : 50 v/v), flow rate of 1.1 mL/min, and internal standard: cetirizine dichloride (methanolic solution 0.3 mg/mL). The effluent was monitored on a UV detector at 215 nm. The influence of humidity and temperature on the stability of BEN in solid phase has been studied. The thermodynamic parameters, at 293 K of degradation of BEN hydrochloride in solid phase, E_a (kJ/mol) = 121.16 ± 15.7, $\Delta H^{\neq}$(kJ/mol) = 118.67 ± 15.7,

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$\Delta S^{\neq}$ [J/(K × mol)] = −28.99 ± 44.5 for RH = 76.4% and E_a (kJ/mol) = 85.2 ± 15.9, $\Delta H^{\neq}$ (kJ/mol) = 82.7 ± 15.9, $\Delta S^{\neq}$ [J/(K × mol)] = −139.2 ± 41.3 for RH = 0% have been calculated.

Key Words: Benazepril hydrochloride; Cetirizine dichloride; HPLC and HPLC-MS method; Stability in solid phase.

INTRODUCTION

Benazepril (BEN) hydrochloride is a new angiotensin-converting enzyme (ACE) inhibitor and it is used in the treatment of hypertension. The BEN hydrochloride is a prodrug.[1] Its main metabolic route involves hydrolytic cleavage of the ester linkage to its active carboxylic acid metabolite, benazeprilat (BAT).[2,3]

The study of the solid state reactivity of four model ACE inhibitors: spirapril hydrochloride,[4] quinapril hydrochloride,[4,5] enalapril maleate,[6] and moexipril hydrochloride[7,8] have been conducted previously. Solid phase reactions of the drug substance belonging to a class of dipeptide ACE inhibitors are of interest, however, the literature does not mention kinetic studies of BEN in solid phase. Detailed evaluation of BEN stability in solid phase is pivotal, therefore, it is used in treatment, predominantly in tablets. The following analytical chromatographic techniques were used in the previous studies for the determination of BEN capillary gas chromatography,[9] thin layer chromatography,[10,11] and liquid chromatography.[12–16] However, no simple HPLC methods were reported for the stability studies of BEN in solid phase and an official method for the evaluation of purity of BEN has not yet been described in any pharmacopoeia.

The aim of this paper was to describe a new selective HPLC method for evaluation of the stability of BEN in pure form and in tablets, to estimate the effect of temperature and relative humidity on the degradation of BEN, to determine the kinetic equations describing the concentration changes of BEN as a function of time, and the thermodynamic parameters of the reaction. Moreover, identification of the product of BEN degradation in solid phase was intended.

EXPERIMENTAL

Materials and Reagents

The BEN hydrochloride and BAT were obtained from Novartis International Pharmaceutical Ltd and Department of Pharmaceutical

Chemistry, Poznan University of Medical Sciences, respectively; each tablet was claimed to contain 10 mg of BEN and typical excipients (lactose, starch, talc, methylhydroxypropylocellulose, hydroxypropylocellulose, yellow iron oxidate, and silica gel). Cetirizine dichloride (I.S.) was provided by BIOFARM. HPLC-grade methanol and acetonitrile were purchased from Merck Co., Germany. Other chemical substance and reagents were the products of Sigma Chemical Co.

Instrumental and Chromatographic Conditions

The BEN and BAT were analyzed using a HPLC (Shimadzu Scientific instruments), consisting of a Rheodyne (7125, 100 μL) fixed loop injector, detector (UV-VIS SPO-6AV), pump (LC-6A), and integrator (C-RGA chromatopac). An analytical column (Hypersil MOS, 5 μm particle size, 250 mm × 4 mm Merck) was used as the stationary phase. The mobile phase was acetonitrile–phosphate buffer (50 : 50 v/v), mobile phase rate of flow 1.1 mL/min, and UV detector set at 215 nm.

Phosphate buffer solution was prepared by dissolving 0.0680 g potassium dihydrogen phosphate in 450 mL of water. The pH was adjusted to 2.0 with phosphoric acid and the volume was made up to 500'mL with water.

The study was performed using a liquid chromatograph, which was not equipped with a thermo stating column and autosampler, therefore, the technique employing an internal standard had to be used. Cetirizine dichloride was applied as an internal standard, neutralizing the error inherent in sample injection and eliminating random errors.

The product of BEN degradation in solid phase—BAT was analyzed using a HPLC-MS (Waters micromass ZQ) with photodiode array detector (Waters 996). LC separations were made on a Hypersil MOS column, 5 μm particle size, 250 mm × 4 mm at 35°C. The flow rate of the mobile phase, which consisted of methanol–water–formaldehyde (49 : 50 : 0.5 v/v/v), was 0.5 mL/min. The mobile phase was filtered through a 0.22 μm filter and degassed by ultrasound. The injection volume was 100 μL. Recorded mass range m/z was from 100 to 1000, ionization ES^+ and ES^-.

HPLC-MS analysis was performed in the Laboratory of Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland.

Stock and Working Standard Solutions

Stock standard solutions of BEN hydrochloride, 9.43×10^{-4} mol/L and BAT, 1.01×10^{-3} mol/L, 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 4 weeks. Mixed working standard solutions of BEN and BAT in a ratio (1 : 1) were prepared by the appropriate dilution of the above mentioned stock standard solutions in the methanol.

Calibration Procedure

Calibration curves of BEN and BAT were conducted using the series of working standard solutions described previously. The concentration range was from 9.47×10^{-5} mol/L to 9.43×10^{-4} mol/L for BEN and from 1.01×10^{-4} mol/L to 1.01×10^{-3} mol/L for BAT. For the analysis, 1.0 mL of solutions were mixed with 1.0 mL of the cetirizine dichloride (I.S.). All solutions were analyzed immediately after their preparation. Triplicate 100 μL injections were made for each concentration and chromatographed under the conditions described above. The calibration curve was obtained by plotting the peak area ratios of BEN and BAT to cetirizine dichloride (I.S.) corresponding to the BEN and BAT concentration.

Precision and Accuracy

Day-to-day precision and accuracy were evaluated by using eight samples of three different concentrations at low (for BEN 9.47×10^{-5} mol/L and for BAT 1.01×10^{-4} mol/L), medium (for BEN 5.66×10^{-4} mol/L and for BAT 6.06×10^{-4} mol/L), and high (for BEN 9.43×10^{-4} mol/L and for BAT 1.01×10^{-3} mol/L) concentrations, which were prepared and analyzed on the same day. Sample-to-sample variability was assessed using eight samples of three different concentrations at low (for BEN 9.47×10^{-5} mol/L and for BAT 1.01×10^{-4} mol/L), medium (for BEN 5.66×10^{-4} mol/L and for BAT 6.06×10^{-4} mol/L), and high (for BEN 9.43×10^{-4} mol/L and for BAT 1.01×10^{-3} mol/L) concentrations analyzed on three different days, over a period of a week.

Analysis of Pure BEN

The study of stability of BEN in solid phase was performed by means of the stress degradation test (in the temperature range from 343 to 393 K, at a relative humidity 215.0–76.4%), which determines kinetic and thermodynamic relationships. Samples of BEN (10.00 mg) were accurately weighed

into 5 mL vials. To assess the stability of BEN in relative humidity RH = 0%, the vials containing the studied substance were immersed in a sand bath that was placed in a heat chamber adjusted to temperatures of 378, 383, 388, and 393 K.

To assess the effect of humidity on the stability of BEN, the vials with BEN were placed in exsicators containing saturated aqueous solutions of appropriate inorganic salts, which safeguarded the desired relative humidity of the ambient air, sodium iodide (RH = 25.0%), sodium bromide (RH = 50.9%), potassium iodide (RH = 60.5%), sodium nitrate (RH = 66.5%), and sodium chloride (RH = 76.4%); and inserted in a heat chamber set to 363 K.

Samples destined for investigation of the effect of temperature at a relative humidity of 76.4% were placed in desiccators containing aqueous saturated solutions of sodium chloride and inserted in heat chambers set to the desired temperatures of 343, 348, 353, 358, and 363 K. Each series was comprised of 9–15 samples.

After definite time intervals, determined by rate of degradation, the respective vials were taken out of the chamber, cooled to room temperature, and the contents dissolved in methanol. The so obtained solution was quantitatively transferred into a measuring flask and made up to a total volume of 25.0 mL with methanol. To 1.0 mL of the solution, 0.5 mL of solution of internal standard was added. The chromatograms were interpreted using the following dependence: $P_{BEN}/P_{IS} = f(t)$ and $P_{BAT}/P_{IS} = f(t)$; where P_{BEN} and P_{BAT} are areas of the BEN and BAT signal and P_{IS} represents the values of I.S. (cetirizine dichloride).

Analysis of BEN in Pharmaceutical Dosage Forms

Kinetic studies of conditions were in compliance with recommendations of the International Chemical Harmonization.[17]

Tablets with BEN were placed (in blister and nonblister) in a desiccators containing a saturated aqueous solution of sodium chloride (RH = 76.4%) and inserted in heat chambers set to the desired temperature, 318 K.

At respective time intervals, five tablets with BEN (in blister and nonblister) were withdrawn from the desiccators and after cooling to room temperature, they were transferred into measuring flasks (50 mL) and 3.0 mL of water was added to the flasks; their contents were shaken until the tablet disintegrated. Then, 22.0 mL of methanol was added and the contents were extracted by means of shaking for 15 min. The extracts were then filtered, and such obtained solutions were analyzed by HPLC. For the analysis, 1.0 mL of this solution was mixed with 1.0 mL of the internal standard (cetirizine dichloride).

The evolving signals were recorded over a time span from 1 to 10 min. The following formula was adopted to calculate the drug content/tablet in g

$$\frac{(P_i \times C \times V)}{P_{ST}} \qquad P_i = \frac{P_i}{P_{IS}} \quad \text{and} \quad P_{ST} = \frac{P_{ST}}{P_{IS}}$$

where P_i, P_{ST}, and P_{IS} are the areas peaks of BEN, standard solution of BEN, and internal standard, respectively; C is the concentration of BEN of the standard solution (0.04%) and V is the dilution factor for the sample.

RESULTS AND DISCUSSION

HPLC Method Characteristics

Concentration changes of BEN under the experimental conditions were assessed by means of HPLC. The method was validated in compliance with ICH guidelines Q2B.[18,19] The applied method was selective for the BEN (t_R about 6 min), as well as for the internal standard (cetirizine dichloride—t_R about 8 min), in the presence of the degradation product (t_R about 3 min). The typical excipients included in the drug formulation do not interfere with selectivity of the method. The analysis of the chromatogram of BEN and its degradation product and I.S., revealed the following efficiencies of the column: for BEN $N = 4986$, product degradation $N = 5672$, and I.S. $N = 3191$ (where N represents theoretical plate number). The separation factors between BEN and degradation product = 5.4 and BEN and cetirizine dichloride (I.S.) = 2.6. The selectivity of the HPLC method is illustrated in Fig. 1.

The equation for calibration curves was $Y = aC + b$. All calibration plots showed excellent linearity with correlation coefficients of better than 0.999; b was statistically nonsignificant. Table 1 shows calibration characteristics for the peak area ratio of varying amounts of BEN and BAT to a constant amount of cetirizine dichloride (I.S.) (0.15 mg/mL) (Table 1). Limit of detection (LOD) and limit of quantitation (LOQ) were experimentally determined and they are also presented in Table 1.

Intra-day data for the precision and accuracy of the HPLC method given in Table 2, indicate recovery % = 99.12 − 100.11 and RME% = −0.88 − 0.11 for BEN and recovery% = 99.01–100.99 and RME% = −0.91 − 0.59 for BAT. Moreover, the inter-day recovery % = 99.64 − 100.63 and RME% = −0.36 − 0.63 for BEN and recovery% = 99.34 − 100.99 and RME% = −0.66 − 0.99 for BAT (Table 2). The stability of the standard

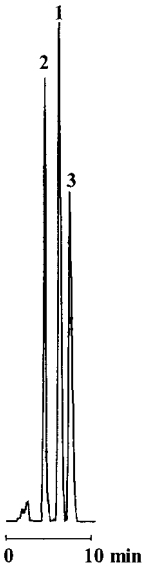


Figure 1. HPLC chromatogram for the analysis of degradation solution of BEN (363 K, 76.4%RH). Peak 1: BEN, peak 2: BAT-decomposition product, and peak 3: internal standard. Chromatographic conditions are described in the text.

solution was determined over 48 hr. The standard solution was stored at 25 °C under laboratory light conditions. The standard solution was analyzed at 0, 24, and 48 hr against freshly made standard solution. Less than 1.5% difference was observed, which demonstrates that the standard solution was stable for up to 48 hr, when stored at ambient temperature under laboratory light conditions.

Kinetic Investigation of the BEN in the Presence of Increased Humidity

The concentration of BEN changes in the presence of increased humidity according to a first-order reaction model. In the course of $t \rightarrow \infty$, the values of $c_t \rightarrow 0$. The plots $\ln c_t = f(t)$ were linear. Figure 2(a and b) shows the concentrations changes of BEN at time t. The observed rate constants were calculated by the least-square method according to the equation

$$\ln c_t = \ln c_0 - k \times t$$

Table 1. Statistical analysis of calibration curves.

Parameters for BEN	
Linearity range (mol/L)	9.47×10^{-5}–9.43×10^{-4}
Regression equation (Y)[a]	
Slope $a \pm \Delta a$	1676 ± 59
Standard deviation of the slope (SD_a)	25
Intercept $b \pm \Delta b$	0.025 ± 0.035
Standard deviation of the intercept (SD_b)	0.015
Correlation coefficient (r)	0.999
n	10
Relative standard deviation (%)[b]	0.761
LOD[c]	3.15×10^{-5}
LOQ[c]	9.47×10^{-5}
Parameters for BAT	
Linearity range (mol/L)	1.01×10^{-4}–1.01×10^{-3}
Regression equation (Y)[a]	
Slope $a \pm \Delta a$	1566 ± 56
Standard deviation of the slope (SD_a)	24
Intercept $b \pm \Delta b$	0.025 ± 0.035
Standard deviation of the intercept (SD_b)	0.014
Correlation coefficient (r)	0.999
n	10
Relative standard deviation (%)[b]	0.723
LOD[c]	3.33×10^{-5}
LOQ[c]	1.01×10^{-4}

[a]$Y = aC + b$, where C is concentration of BEN and BAT in mol/L and Y is the BEN and BAT peak area to cetirizine dichloride (I.S.) peak area ratio.
[b]Three replicate samples.
[c]The values were experimentally calculated.

where c_t and c_0 represent concentration of BEN at time t and 0, respectively, k is the first-order rate constant.

Kinetic Investigation of the BEN in Relative Humidity RH = 0%

The chromatographic peaks of analyzed samples of BEN incubated in relative humidity RH = 0%, over a period of time from $t \rightarrow t_\infty$, showed a decline from $c_t \rightarrow c_e$. After some time, a steady state of the reaction was reached and the plots of $\ln(c_t - c_e) = f(t)$ presented straight lines, Fig. 2(c).

Table 2. Accuracy in the assay determination of LIS.

Day of analysis	Nominal concentration $\times 10^{-4}$(mol/L)	Assayed concentration $\times 10^{-4}$(mol/L)	Recovery (%)	SD	RME (%)
		BEN			
Intra-day					
0 day	0.947	0.948 ± 0.029	100.11	0.19	0.11
	5.66	5.61 ± 0.11	99.12	0.69	−0.88
	9.43	9.42 ± 0.98	99.89	0.73	−0.11
Inter-day					
1 day	0.947	0.945 ± 0.021	99.79	0.11	−0.21
	5.66	5.68 ± 0.19	100.35	0.71	0.35
	9.43	9.44 ± 0.91	100.11	0.91	0.11
	0.947	0.949 ± 0.019	100.21	0.29	0.21
2 day	5.66	5.64 ± 0.16	99.64	0.85	−0.36
	9.43	9.49 ± 1.08	100.63	0.98	0.63
		BAT			
Intra-day					
0 day	1.01	1.00 ± 0.10	99.01	0.14	−0.91
	6.06	6.12 ± 0.55	100.99	0.56	0.99
	10.1	10.16 ± 0.95	100.59	0.84	0.59
Inter-day					
1 day	1.01	1.02 ± 0.12	100.99	0.13	0.99
	6.06	6.02 ± 0.45	99.34	0.64	−0.66
	10.1	10.08 ± 0.88	99.80	0.92	−0.20
	1.01	1.01 ± 0.09	100.00	0.19	0.00
2 day	6.06	6.07 ± 0.56	100.17	0.35	0.17
	10.1	10.06 ± 0.94	99.60	0.88	−0.40

Note: SD, standard deviation; RME, relative mean error.

The rate constant of the pseudo-first-order reaction was calculated from the equation

$$\ln(c_t - c_e) = \ln(c_0 - c_e) - k_{obs}\, t$$

For the interpretation of the straight curves plotted from $\ln(c_t - c_e) = f(t)$, the following statistical parameters of the respective equations were computed by means of the minimal square methods a ± Δa, b ± Δb, standard deviation, and the coefficient of linear correlation. The values of Δa and Δb were computed for $f = n - 2$ degrees of freedom, with $\alpha = 0.05$. Rate constants are presented in Table 3.

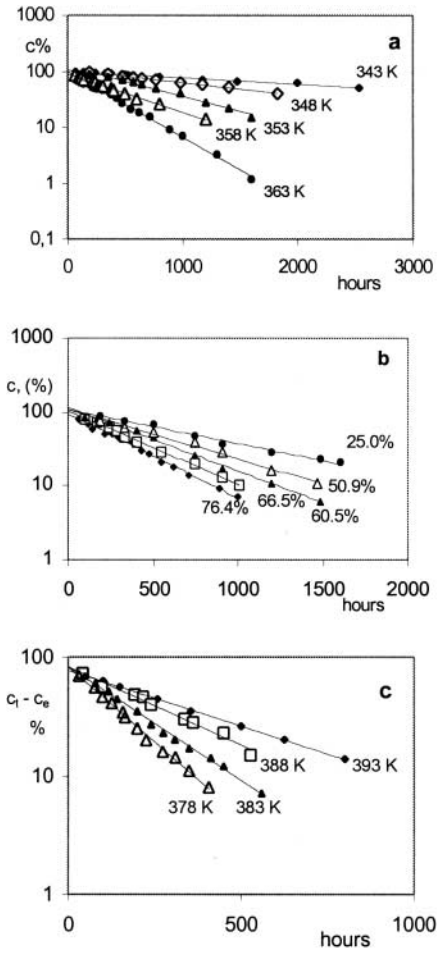


Figure 2. Semi-logarithmic plots $c_t = f(t)$ for the degradation of BEN in solid phase (a) in relative humidity RH ~76% at different temperatures; (b) at different humidity, temperature 363 K; and (c) in relative humidity RH = 0% at different temperatures.

Thermodynamic Parameters

The determined reaction rate constants were employed for the calculation of the Arrhenius relationship: $\ln k_i = \ln A - E_a/RT$, where k_i represents the respective reaction rate constants (sec^{-1}), A, frequency coefficient, E_a,

Table 3. Kinetic and thermodynamic parameters of BEN decomposition in solid phase in relative humidity RH = 76.4%.

T (K)	$k \pm \Delta k$ (sec^{-1}) $\times 10^7$	$-r$	n	Statistical evaluation $\ln k_i = f(1/T)$	Thermodynamic parameters
Relative humidity, RH = 76.4%					
343	0.710 ± 0.057	0.991	15	$a \pm \Delta a = -14572 \pm 1888$	$E_a = 121 \pm 16$ (kJ/mol)
348	1.285 ± 0.14	0.992	10	$SD_a = 594$	
353	2.034 ± 0.22	0.992	10	$b \pm \Delta b = 25.9 \pm 5.4$	$\Delta H^{\neq} = 118 \pm 16$ (kJ/mol)
358	4.254 ± 0.20	0.997	15	$SD_b = 1.7$	
363	7.273 ± 0.22	0.998	18	$r = -0.998$	$\Delta S^{\neq} = -29 \pm 44$ [J/(K × mol)]
298	2.912×10^{-10} [a]				
Relative humidity, RH = 0%					
378	5.704 ± 0.74	0.994	8	$a \pm \Delta a = -10246 \pm 1918$	$E_a = 85 \pm 16$ (kJ/mol)
383	7.652 ± 0.82	0.993	9	$SD_a = 603$	
388	11.80 ± 0.54	0.998	12	$b \pm \Delta b = 12.7 \pm 4.9$	$\Delta H^{\neq} = 83 \pm 16$ (kJ/mol)
393	15.60 ± 1.18	0.994	12	$SD_b = 1.6$	
298	3.843×10^{-10} [a]			$r = -0.997$	$\Delta S^{\neq} = -139 \pm 41$ [J/(K × mol]

[a]The values were calculated with Arrhenius equation.

Note: SD_a, standard deviation of slope regression; SD_b, standard deviation of value b; r, coefficient of linear correlation.

activation energy (J/mol), R, universal gas constant [8.3144 J/(K mol)], T, temperature (K).

For the relationship $\ln k_i = f(1/T)$, straight line plots were obtained for both humid and dry conditions of sample exposure. From the parameters of the plot $\ln k_i = f(1/T)$, the following thermodynamic parameters of the reaction of decomposition of BEN in solid phase pertaining to either conditions of sample incubation, i.e., in dry air and in an atmosphere of RH = 76.4% were calculated: the activation energy (E_a), enthalpy ($\Delta H^{\neq}$), and entropy ($\Delta S^{\neq}$) for the temperature 293 K. The thermodynamic parameters are shown in Table 3.

Influence of Humidity on the Stability of BEN

The effect of humidity on the stability of BEN at 363 K, in the humidity range from 25.0% to 76.4%, is described by the equation

$$\ln k_i = ax + b = (0.0176 \pm 0.0024)\text{RH\%} - (15.49 \pm 0.141)$$

The influence of humidity on the stability of BEN was presented in Table 4. The slope ($a = 0.0176 \pm 0.00242$) of the straight linear plot $\ln k_i = f(\text{RH\%})$ characterizes the effect of humidity on the stability of BEN. This effect was similar to the effect of humidity on the stability of enalapril maleate ($a = 0.0196 \pm 0.0033$).[6]

Table 4. The effect of humidity on the stability of BEN in solid state at 363 K.

RH%	$k \pm \Delta k$ $(\text{sec}^{-1}) \times 10^7$	$-r$	n	Regression parameters $\ln k_i = f(\text{RH\%})$
25.0	2.96 ± 0.32	0.9983	18	$a \pm \Delta a = 0.0176 \pm 0.00024$
50.9	4.39 ± 0.53	0.9966	10	$\text{SD}_a = 0.000076$
60.5	5.52 ± 0.40	0.9961	10	$b \pm \Delta b = -15.5 \pm 0.14$
66.5	6.06 ± 0.41	0.9944	7	$\text{SD}_b = 0.0044$
76.4	7.27 ± 0.22	0.9940	8	$r = 0.997$

Note: SD_a, standard deviation of slope regression; SD_b, standard deviation of value b; r, coefficient of linear correlation.

Kinetic Interpretation of the Decomposition of BEN in Solid Phase

The rate constants of BEN degradation and formation of the product did not show any statistically significant differences (Table 5). The limit of product concentration at time t, $c_{e,P} = 9.48 \times 10^{-4}$ mol/L and the BEN concentration at time $t = 0$, $c_{0,BEN} = 9.43 \times 10^{-4}$ mol/L was comparable. This indicates that degradation of BEN leads to obtain a single product without the presence of any other parallel process (BEN $\rightarrow$ product) in the absence and the presence of humidity. The result is given in Table 5.

Product Identification of the Degradation of BEN in Solid Phase

In the presence of relative humidity and dry air, the following signals were observed on HPLC–ESI(+)–MS–TIC and HPLC–ESI(+)–MS–TIC chromatograms: $t_R =$ about 14 and 19 min (Figs. 3 and 4).

The analysis of HPLC–ESI(+)–MS spectra yielded the following conclusion: the compound with $m/z = 425$; $t_R = 19$ min was BEN ($M = 424$) and the compound with $m/z = 397$; $t_R = 14$ min was BAT ($M = 396$). The analysis of HPLC–ESI(−)–MS spectra yielded the following conclusion: the compound with $m/z = 423$; $t_R = 19$ min was BEN ($M = 424$) and the compound with $m/z = 395$; $t_R = 14$ min was BAT ($M = 396$); see also Figs. 3 and 4. Degradation of BEN in solid phase was according to Sch. 1.

In conclusion, the HPLC method was successfully applied for evaluation of stability of BEN in solid phase. The proposed method gives good resolution between BEN and its degradation product and I.S. with short analysis time

Table 5. The first-order rate constants for the degradation of benazepril hydrochloride and the rate constants of formation its product.

Conditions research	Symbol	$k \pm \Delta k$ (sec^{-1})	$k_1 \pm \Delta k_1$ (sec^{-1})
383 K RH = 0%	BEN [M = 424] BAT [M = 396]	$(7.652 \pm 0.82) \times 10^{-7}$	$(7.618 \pm 0.50) \times 10^{-7}$
363 K RH = 76%	BEN [M = 424] BAT [M = 396]	$(7.273 \pm 0.22) \times 10^{-7}$	$(7.276 \pm 0.42) \times 10^{-7}$)

Note: BEN, benazepril; BAT, benazeprilat; k, rate constant of degradation of BEN; k_1, rate constant of formation of BAT.

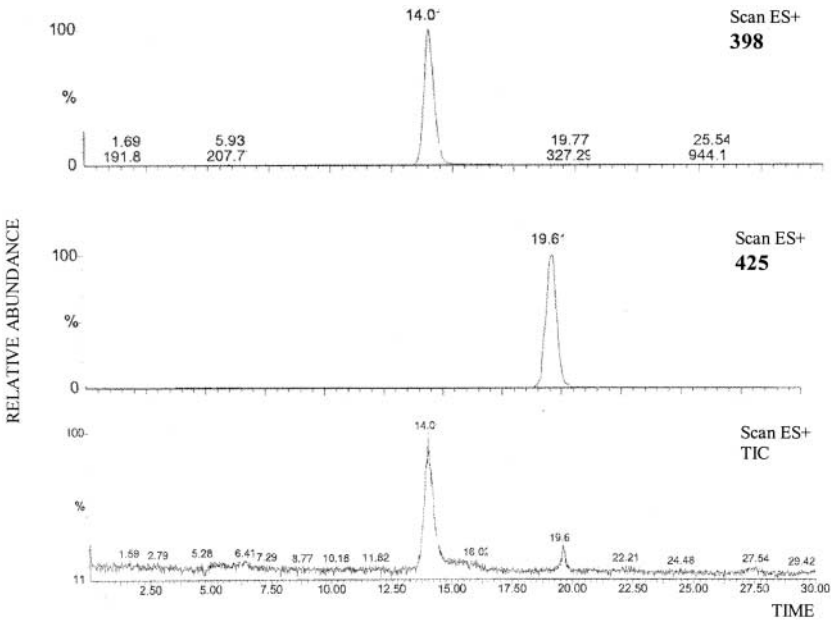


Figure 3. HPLC–ESI(+)–MS–TIC, and HPLC–ESI(+)–MS–SCAN chromatogram for the analysis of degradation solution of BEN (363 K, 76.4%RH). Chromatographic conditions are described in the text.

(< 10 min). The statistical evaluation of the HPLC method revealed its good linearity and accuracy and precision, and proved suitable for the study of the degradation of BEN in solid phase (see Fig. 1). My own observations indicated that the investigated substance, BEN, presented appreciable stability at room temperature (298 K; Table 3). In relative humidity, RH = 0%, at temperatures ranging from 373 to 393 K, and in relative humidity, RH from 25.0% to 76.4%, at temperatures ranging from 343 K to 363 K, the degradation of BEN to BAT proceeds according to a first-order reaction.

The previous studies of drugs, which belong to a class of dipeptide ACE inhibitors (quinapril hydrochloride, moexipril hydrochloride, enalapril maleate, and spirapril hydrochloride) in solid phase, have proven that these compounds undergo intramolecular cyclization and hydrolysis very easily. Kinetic studies of BEN in solid state, as presented, indicates that under the study conditions, BEN undergoes hydrolysis; however, the product of intramolecular cyclization has not been observed (Figures 1, 3, 4). It is assumed that the chemical structure of the BEN molecule does not allow the intramolecular cyclization.

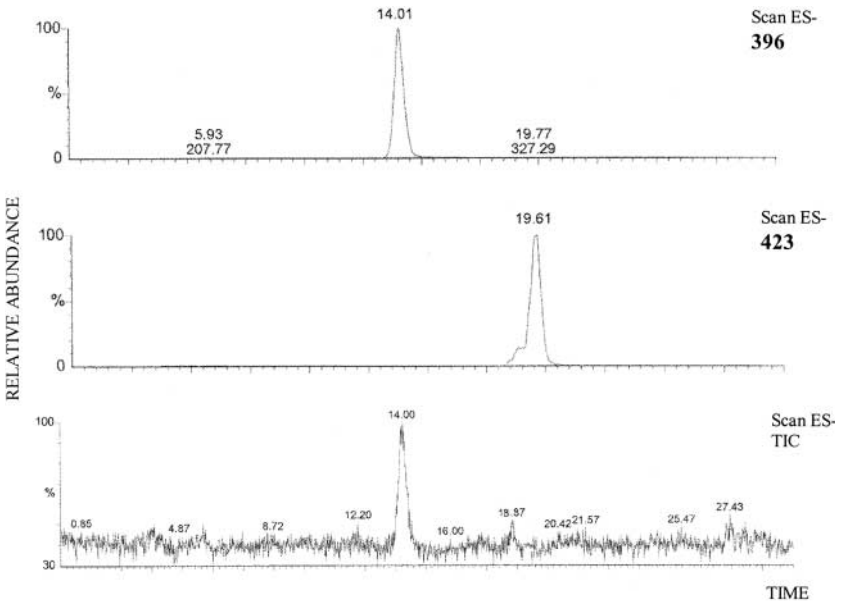
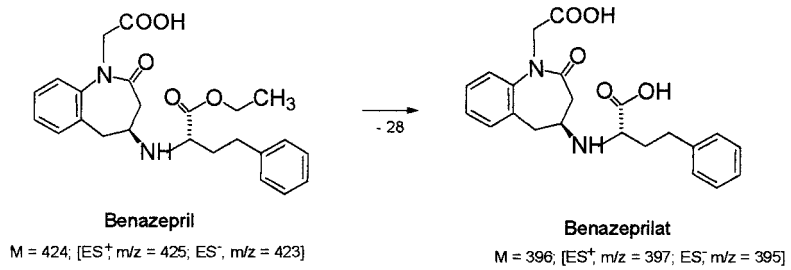


Figure 4. HPLC–ESI(−)–MS–TIC and HPLC–ESI(−)–MS–SCAN chromatogram for the analysis of degradation solution of BEN (363 K, 76.4%RH). Chromatographic conditions are described in the text.



Scheme 1. Degradation of BEN in solid phase.

Over the study period (196 days, T = 318 K, RH = 76.4%), degradation of BEN in tablets has not been observed; initial content of BEN in tablets (10.17 ± 0.11 mg), content of BEN in tablets after 196 days (10.15 ± 0.09 mg). Under the study conditions, the appearance of tablets with BEN has not been changed.

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