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## ANALYSIS

# VALIDATION OF UV DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF BENAZEPRIL HYDROCHLORIDE IN TABLETS AND EVALUATION OF ITS STABILITY

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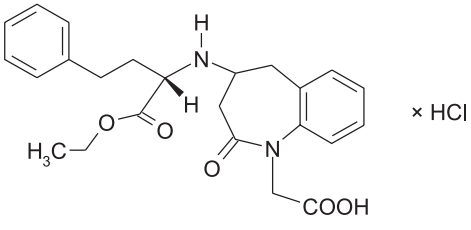
**Abstract:** The absorbance and first-, second- and third-order derivative UV spectroscopic methods were applied for the determination of benazepril hydrochloride (BEN) in model solutions and tablets, as well as the estimation of its stability in solid phase. Derivative UV spectroscopy and HPLC methods were tested for: precision, linearity, accuracy and repeatability. HPLC was used as a reference method. The study presents that derivative UV spectroscopy (the first and second derivative only) and HPLC can be successfully applied for the quantitative analysis of benazepril hydrochloride both pure and in pharmaceutical formulations. Although, the first and second derivative spectrophotometric methods are fast, precise and accurate, but they cannot be used for evaluation of purity and stability of BEN in pharmaceutical formulations (due to a lack of selectivity).

**Keywords:** benazepril hydrochloride (BEN), HPLC method, derivative UV spectroscopic methods, validation

Benazepril hydrochloride [BEN; (3S)-3-[(1S)-1-ethoxycarbonyl-3-phenylpropylamino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepin-1-yl]acetic acid hydrochloride:




is an antihypertensive drug, which belongs to the group of angiotensin convertase inhibitors. It acts on the renin-angiotensin-aldosterone system by enhibition of the conversion of the inactive angiotensin I to the highly potent vasoconstrictor – angiotensin II. It also reduces the degradation of bradykinin. BEN is applied in pharmacotherapy as a first choice drug for treatment of: arterial hypertension, ischemic heart disease, hypertrophy of the left heart ventricle and postinfarctial heart dysfunction (1-4).

Several analytical methods have been reported for the quantitative determination of BEN such as: VIS-spectrophotometry (5), high performance liquid chromatography (HPLC) with ultraviolet (UV) detection (6-9), gas chromatography/mass spectrometry (GC/MS) for BEN in human plasma (10) and HPTLC-densitometry for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide in their binary mixtures (6). Nevertheless, no official method for resolution and determination of impurities in BEN substance has been described in the European Pharmacopoeia or British Pharmacopoeia. Moreover, some of the methods mentioned above require expensive apparatus (eg. HPLC-GC) and a long sample assay (> 15 min). According to ICH requirements, methods for kinetic studies must be not only selective, precise and accurate but also fast and unexpensive.

Derivative UV spectrophotometry has been widely used as a tool for quantitative and control analysis in agricultural, pharmaceutical and biomedical fields (11-13). This technique offers various advantages over the conventional absorbance methods such as: the discrimination of the sharp spectral

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features over the large bands and the enhancement of the resolution of overlapping spectra. As a result, derivative spectrophotometry usually provides much better spectra than the traditional absorbance spectra.

The main aim of this investigation was to determine the suitability of UV-spectrophotometry based on measurement of absorbance and calculations of the first (D1), the second (D2) and the third derivative (D3) for determination of BEN in tablets and the evaluation of its stability. HPLC was used as a reference method. Each method under study (derivative UV spectrophotometry and HPLC) was validated according to the demands of the International Chemical Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use (14). The obtained results were subjected to statistical analysis.

## EXPERIMENTAL

### Material, reagents and apparatus

Benazepril hydrochloride was supplied by Novartis, each tablet contained 10 mg equivalent of benzazepril (Lotensin®10 mg). Other reagents used in the study were commercial preparations with a pro analysis grade of purity.

Spectrophotometric analyses were performed with Perkin-Elmer Lambda-6 UV WinLab Version 2.70.01 instrument. The UV spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 240 nm × $min^{-1}$, response time 0.5 s and fixed slit width 3 nm. Derivative spectra were automatically obtained by Perkin-Elmer Lambda-6 UV WinLab Version 2.70.01 ("peak-zero method"). The concentrations of BEN in its solutions in methanol were determined within wavelength range of 210-400 nm.

A Shimadzu liquid chromatograph consisting of: Rheodyne 7125, 100 mL fixed loop injector, UV-VIS SPO-6AV detector, LC-6A pump and C-RGA chromatopac integrator was used. High performance liquid chromatography was performed on a RP- LiChrospher 100 $C_8$ (size 10 mm, 250 mm × 4 mm I.D. Merck) column.

### Preparation of standard solutions and calibration curve

Standard solution (12 mg $mL^{-1}$) of BEN was prepared by dissolving appropriate amounts of BEN

Table 1. Statistical evaluation of methanolic BEN solutions calibration curves described by equations: $y = ax + b$ and $y = ax$.

| Measured value | λ [nm] | Calibration curve parameters $y = ax + b$ | Calibration curve parameters $y = ax$ |
|---|---|---|---|
| A | 205 | a ± Δa = 816 ± 33; $S_a$ = 14.17<br>b ± Δb = 0.045 ± 0.024; $S_b$ = 0.011<br>r = 0.9988<br>verification of coefficient<br>b significance hypothesis :<br>$t = b/S_b$ = 4.09 | a ± Δa = 869 ± 30; $S_a$ = 13.36<br>r = 0.9988<br>$b = 0, t > t_{\alpha,f}$<br>significant coefficient b value |
| D1 | 213 | a ± Δa = 12370 ± 283; $S_a$ = 122.5<br>b ± Δb = 0.1 ± 0.2; $S_b$ = 0.091<br>r = 0.9996<br>verification of coefficient<br>b significance hypothesis:<br>$t = b/S_b$ = 1.10 | a ± Δa = 12485 ± 261;<br>$S_a$ = 115.5<br>r = 0.9996<br>$b = 0, t < t_{\alpha,f}$<br>insignificant coefficient b value |
| D2 | 219 | a ± Δa = 164 ± 4; $S_a$ = 1.58<br>b ± Δb = 0.0023 ± 0.0027; $S_b$ = 0.0011<br>r = 0.9996<br>verification of coefficient<br>b significance hypothesis:<br>$t = b/S_b$ = 2.10 | a ± Δa = 167 ± 3; $S_a$ = 1.49<br>r = 0.9996<br>$b = 0, t < t_{\alpha,f}$<br>insignificant coefficient b value |
| D3 | 223 | a ± Δa = 29 ± 1; $S_a$ = 0.42<br>b ± Δb = 0.0023 ± 0.0007; $S_b$ = 0.0003<br>r = 0.9992<br>verification of coefficient<br>b significance hypothesis:<br>$t = b/S_b$ = 7.67 | a ± Δa = 32 ± 0.9; $S_a$ = 0.4<br>r = 0.9992<br>$b = 0, t > t_{\alpha,f}$<br>significant coefficient b value |
| **Conclusion**<br>Assessed $t = b/S_b$ (1.10 and 2.10) values for derivative spectroscopic methods D1, D2 are lower than critical value $t_{\alpha,f}$ = 2.228 for α = 0.05. It indicates that coefficient b = 0 and is statistically insignificant. However, $t = b/S_b$ (4.09 and 7.67) values for classic and derivative spectroscopy D3 are higher than critical value $t_{\alpha,f}$ = 2.228 for a = 0.05 and what indicates that b > 0 (statistically significant). | | | |

Table 2. Evaluation of accuracy for classic, derivative spectroscopy (D1, D2, D3) and reference method HPLC

| Model mixture | Recovery % | | | | |
|---|---|---|---|---|---|
| | A | D1 | D2 | D3 | Reference method |
| I | 102.3 ± 1.33<br>SD = 1.59<br>CV = 1.55% | 100.1 ± 1.57<br>SD = 1.87<br>CV = 1.87% | 99.40 ± 1.46<br>SD = 176<br>CV = 1.77% | 99.87 ± 2.08<br>SD = 2.49<br>CV = 2.51% | 99.28 ± 0.60<br>SD = 0.73<br>CV = 0.73% |
| II | 104.6 ± 1.65<br>SD = 1.98<br>CV = 1.89% | 99.38 ± 1.38<br>SD = 1.65<br>CV = 1.67% | 99.30 ± 1.26<br>SD = 1.50<br>CV = 1.51% | 98.09 ± 1.03<br>SD = 1.23<br>CV = 1.25% | 100.5 ± 0.87<br>SD = 1.05<br>CV = 1.04% |
| III | 102.0 ± 0.43<br>SD = 0.51<br>CV = 0.50% | 100.8 ± 1.16<br>SD = 1.38<br>CV = 1.37% | 99.62 ± 1.30<br>SD = 1.50<br>CV = 1.55% | 99.62 ± 1.30<br>SD = 1.50<br>CV = 1.55% | 100.4 ± 1,19<br>SD = 1.42<br>CV = 1.41% |

* each result is the average of three separate determinations.

Table 3. Evaluation of precision of HPLC and spectrophotometric methods (n = 10)

| Concentration added (μg mL$^{-1}$) | Measured value | Concentration found (mean ± S.D.[a]) (μg mL$^{-1}$) | | CV[b] (%) | |
|---|---|---|---|---|---|
| | | Inter-day | Intra-day | Inter-day | Intra-day |
| 1.20<br>6.00<br>12.00 | absorbance | 1.17 ± 0.049<br>6.09 ± 0.14<br>12.03 ± 0.55 | 6.07 ± 0.14 | 4.19<br>2.30<br>4.57 | 2.31 |
| | first-order | 1.19 ± 0.014<br>6.02 ± 0.035<br>12.09 ± 0.12 | 6.01 ± 0.11 | 1.18<br>0.58<br>0.99 | 1.80 |
| | second-order | 1.18 ± 0.009<br>6.05 ± 0.049<br>12.03 ± 0.15 | 6.03 ± 0.09 | 0.76<br>0.81<br>1.25 | 1.49 |
| | third-order | 1.12 ± 0.013<br>5.92 ± 0.32<br>11.93 ± 0.53 | 6.17 ± 0.28 | 1.16<br>5.41<br>4.44 | 4.53 |
| | HPLC | 1.19 ± 0.013<br>6.06 ± 0.07<br>12.02 ± 0.13 | 6.01 ± 0.06 | 1.09<br>1.15<br>1.08 | 0.09 |

[a] Standard deviation
[b] Coefficient of variation

in methanol. Stored at + 6°C in the dark, this standard solution was stable during the period of study. Ten concentrations of BEN varying from 1.2 mg mL$^{-1}$ to 12.0 mg mL$^{-1}$ were prepared by dilution of standard solution. The absorption spectra A, D1, D2 and D3 (absorbance, first-, second- and third order derivative spectra, respectively) of the solutions prepared at different concentrations were recorded against methanol. The calibration curve was checked in three consecutive days in solutions of the same concentration prepared from the standard solution.

**Procedure for tablets containing 10 mg BEN equivalent**

**Analysis of tablets**

Twenty tablets (LOTENSIN®) were accurately weighed and powdered in a mortar. An amount equivalent to one tablet (10 mg of BEN) was weighed and put with addition of 25.0 mL of methanol in 50 mL volumetric flask. The prepared mixture was sonicated for 15 min and filtered (solution I).

Table 4. Comparison of benazepril hydrochloride determination results in pharmaceutical preparation (LOTENSIN, 10 mg) obtained by means of UV spectroscopic methods D1, D2 with results obtained by means of HPLC method (reference method); F – Snedecor (F) test and t – Student *t*-test

| Method | Statistical evaluation of benazepril hydrochloride determination results in LOTENSIN tablets (mg/tab) | F ($F_\alpha = 3.79$) | t ($t_\alpha = 2.145$) |
|---|---|---|---|
| HPLC` (reference method) | $x \pm \Delta x = 10.11 \pm 0.09$<br>SD = 0.114<br>CV = 1.12% | — | — |
| D1 | $x \pm \Delta x = 10.17 \pm 0.14$<br>SD = 0.170<br>CV = 1.67% | 2.240<br>$F < F_\alpha$ | 0.927<br>$t < t_\alpha$ |
| D2 | $x \pm \Delta x = 10.16 \pm 0.20$<br>SD = 0.155<br>CV = 1.51% | 1.849<br>$F < F_\alpha$ | 0.822<br>$t < t_\alpha$ |

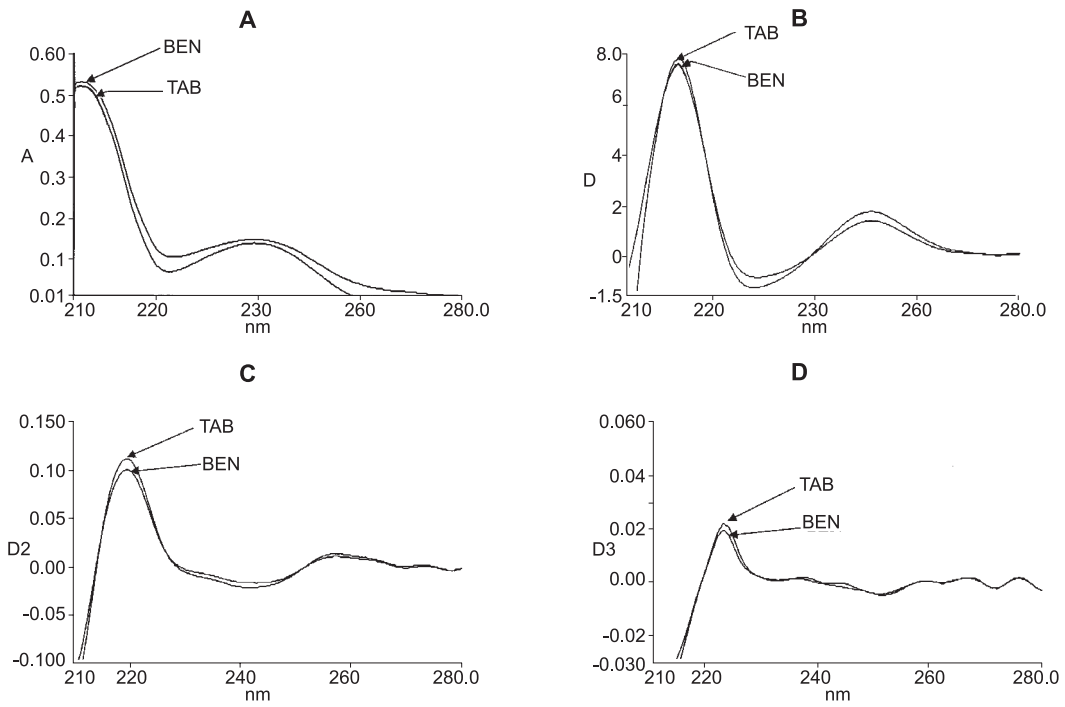


Figure 1. Zero- (A), first- (B), second- (C) and third- (D) order derivative spectrum of benazepril hydrochloride pure (BEN; 0.04 µg/mL) and extract of benazepril hydrochloride from tablets (TAB; 0.04 mg/mL) in methanol.

Spectrophotometric method: 0.5 mL of the solution I was diluted to 25.0 mL with methanol (solution II). The UV spectra A, D1, D2 and D3 (absorbance, first-, second- and third order derivative spectra, respectively) were recorded against methanol (n = 10).

HPLC method (reference method): a mixture of 1.0 mL of the solution I and 1.0 mL of I.S. – cetirizine dichloride (solution III) was analyzed by HPLC.

Parallelly, a reference solution of BEN in methanol at 0.4 mg $mL^{-1}$ concentration was prepared (solution B). A hundred mL of the samples were injected into the HPLC column and the emerging signals were recorded and analyzed: BEN and I.S. emerged with a retention time of approx. 10 min and 13 min,

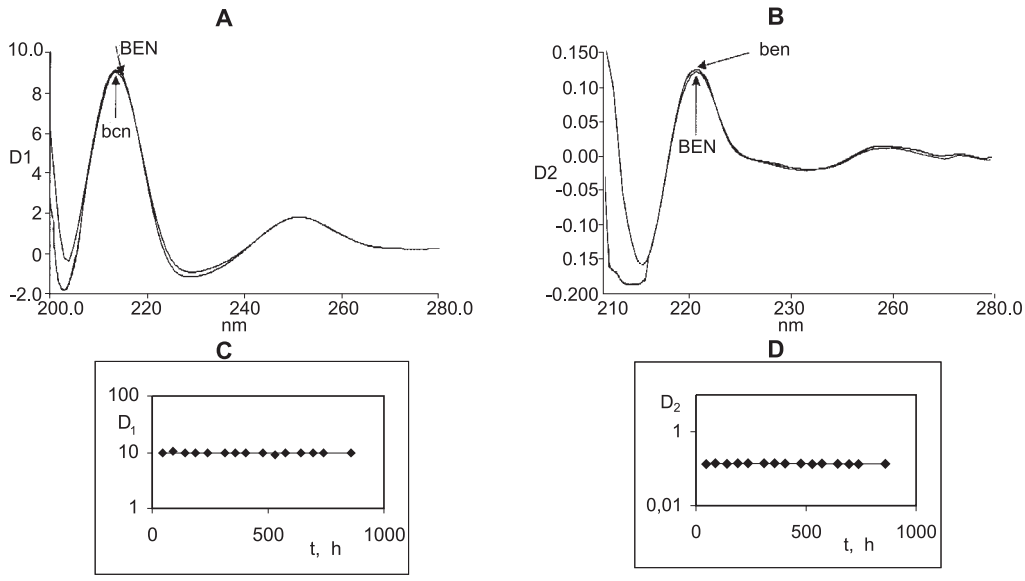


Figure 2. Spectra and relationships:
D1 = f(λ) the first derivative spectrum of methanol solution of benazepril hydrochloride at t = 0 (BEN) and after heating for 900 h at 363 K; RH = 76,4% (ben).
D2 = f(λ) the second derivative spectrum of benazepril hydrochloride at t = 0 (BEN) and after heating for 900 h at 363 K; RH = 76,4% (ben).
Semilogaritimc plot of the first derivative D1 = f(t) of BEN at T = 363 K, RH = 76,4%, t = 900 h).
Semilogaritmic plot of the second derivative D2 = f(t) of BEN at T = 363 K,
RH = 76,4%, t = 900 h).

respectively. The quantities of the drug were calculated using the regression equations of the calibration curves for the spectrophotometric and HPLC methods.

**Model mixtures for evaluation of accuracy of methods**

Model mixtures were prepared using LOTENSIN® tablets.

a) model mixture I: Ten LOTENSIN® tablets-ground in a mortar with addition of 50 mg of BEN; b) model mixture II: Ten LOTENSIN® tablets ground in a mortar with addition of 100 mg of BEN; c) model mixture III: Ten LOTENSIN® tablets ground in a mortar with addition of 150 mg of BEN.

Each mixture was ground with a hand pistle for 20 min. The recovery (in %; n = 10) of BEN for each mixture was calculated. The percentage recovery of BEN was calculated by comparison of the assayed and added concentrations (mg assayed/mg added × 100).

**Procedure for BEN kinetic study**

Exactly weighed amounts of BEN (0.0100 g) in opened, 5 mL glass vials were used for the determination of stability in the presence of relative humidity, RH = 76.4%. The samples were kept in desiccator in an automatically controlled heat chamber at 363 K. After fixed time intervals, samples of the investigated material were taken out from the heat chamber and quantitatively transferred into 25 mL measuring flasks and dissolved up to volume with methanol (solution L).

Spectrophotometric method: 0.5 mL of the solution L was diluted to 25.0 mL with methanol. The UV spectra A, D1, D2 and D3 (absorbance, first-, second- and third- order derivative spectra, respectively) were recorded against methanol.

HPLC method (reference method): One mL of the solution L was mixed with 1.0 mL of the internal standard solution. The obtained mixture was analyzed by HPLC method. The chromatograms were interpreted using the following dependence: $P_i/P_{I.S.} = f(t)$, where $P_i$ is the area of BEN signal, and $P_{I.S.}$-represent the area of I.S. (cetirizine dichloride).

## RESULTS AND DISCUSSION

**Spectrophotometric analysis**

The original UV, first, second, and third order derivative spectra of standard BEN solution and

BEN solutions prepared from tablets (8 mg $mL^{-1}$) are shown in Fig 1. The first, second and third order derivative spectra are characterized by a few peaks. Absorbance and derivative absorbance values of the spectra at 205 nm (A), 213 nm (D1), 219 nm (D2) and 223 nm (D3) were measured for the determination and evaluation of BEN stability.

The calibration curves were linear in the range from 1.2 mg $mL^{-1}$ to 12.0 mg $mL^{-1}$. The calibration curve is described by the equation: Y = aC + b, where "C" stands for BEN concentrations. The values of the intercept "b" were statistically insignificant for the first and second order derivative spectra. Table 1 shows the results of the statistical analysis of calibration curves for spectrophotometric method of BEN determination.

The recovery study conducted by the absorbance, first-, second- and third- order derivative UV spectrophotometric method was performed by adding the appropriate amounts of BEN: 50, 100 and 150 mg, respectively. The results of recovery analysis are presented in Table 2.

High recovery, about 100.0%, and low standard deviation was observed only for first and second derivative UV spectroscopic methods.

Eight samples at three different concentrations: low (1.20 μg $mL^{-1}$), medium (6.00 mg $mL^{-1}$) and high (12.00 μg $mL^{-1}$) were used to evaluate the precision.

To assess the repeatability (intra-day) ten samples were determined for BEN concentration of 6.00 μg $mL^{-1}$. Precision of this measurement was adequate only for the first and second derivative UV spectroscopic methods, CV(%) < 2.0% (Table 3).

The limits of detection (LOD) of BEN were estimated at 0.40 μg $mL^{-1}$ (A), 0.10 mg $mL^{-1}$ (D1), 0.6 μg $mL^{-1}$ (D2), 0.8 μg $mL^{-1}$ (D3) (signal-to-noise ratio of 3).

The limits of quantitation (LOQ) of BEN were estimated at 1.00 μg $mL^{-1}$ (A), 0.50 μg $mL^{-1}$ (D1), 1.20 μg $mL^{-1}$ (D2), 1.80 μg $mL^{-1}$ (D3) (signal-to-noise ratio of 10).

The process of validation results in conclusion that the most suitable methods for determination of benazepril in tablets are the UV spectrophotometry of the first and second derivative. The obtained results are presented in Table 4.

The second stage of the study was to apply the proposed spectrophotometric methods (first and second-order derivative) for evaluation of BEN stability in solid phase.

The UV spectra of methanol solutions of BEN subjected to the kinetic studies (the conditions are described in "Procedure for BEN kinetic study" above) do not differ from those of the methanol solutions of BEN not subjected to decomposition study. Changes in BEN concentration during time t were observed only in HPLC method. The results are presented in Fig. 2 and 3.

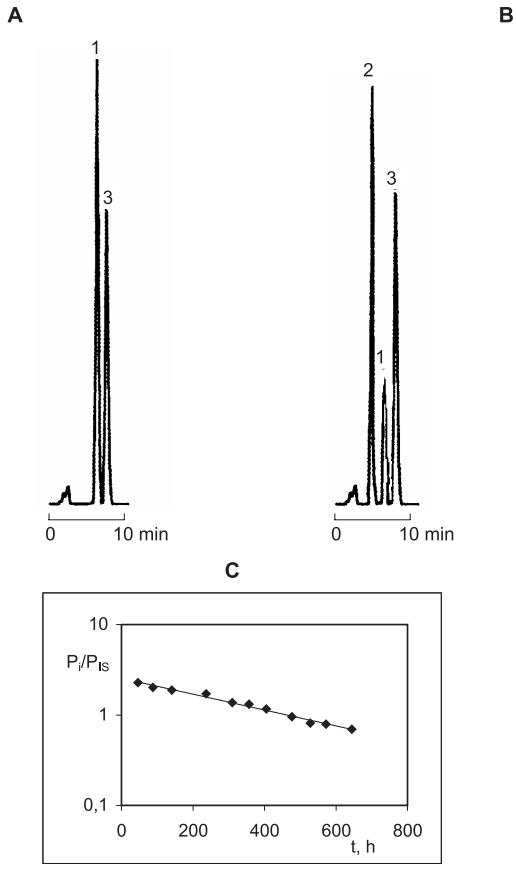


Figure 3. HPLC-chromatogram of methanol solution of BEN at t = 0 (A) and after heating for 630 h at 363 K, RH = 76,4% (B) and semilogaritimc plot of the $P_i/P_{IS}$ = f(t) of BEN at T = 363 K, RH = 76,4%, t = 700 h (C),

## CONCLUSION

As follows from the results presented above, the UV spectrophotometric methods based on measurements of the first and second derivative can be used for determinations of BEN in tablets. These methods satisfy the requirements such as: linearity (y = ax) – coefficient b is insignificant, precision and recovery.

The classic spectrophotometric method and method based on measurements of the third derivative do not satisfy the conditions of: linearity (corre-

lation is correct, but coefficient b is statistically significant), precision and recovery.

The results of BEN determination in tablets obtained by the UV spectrophotometric methods of the first and second derivative are in agreement with the BEN content declared by the manufacturer – NOVARTIS. The mean values of BEN in tablets determined by the UV spectrophotometric methods based on the first and second derivative measurements have been compared with those obtained by the HPLC – reference method. The *t*-Student test stated that they do not differ statistically. The precisions of these two methods compared by the F-Snedecor test were also not statistically different. Therefore, the UV spectrophotometric methods based on measurements of the first and second derivative are equivalent to the selective HPLC method.

Although the UV spectrophotometric methods based on measurements of the first- and second-order derivative were found to be suitable for determination BEN in tablets, they unfortunately were unsuitable for evaluation of the purity and stability of BEN. High recovery of BEN from the model mixtures prepared from LOTENSIN tablets and a lack of differences in the spectra of methanolic solutions of pure BEN and methanol extracts of tablets (Fig. 1) show that these methods are selective, both for BEN and substances forming pharmaceutical formulations. The UV spectrophotometry is unselective so the stability of BEN can be evaluated only by the HPLC method (there are no differences in spectra of BEN, even if, during kinetics study a loss of the substrate was 94%).

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