Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 2 pp. 193–201, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

RAPID AND SIMPLE STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CILAZAPRIL IN PURE SUBSTANCE AND PHARMACEUTICAL FORMULATION IN COMPARISON WITH CLASSIC AND DERIVATIVE SPECTROPHOTOMETRIC METHODS

SYLWIA K. PASZUN, BEATA. STANISZ* and WOJCIECH PAWŁOWSKI

Department of Pharmaceutical Chemistry, K. Marcinkowski University of Medical Sciences, 6 Grunwaldzka St., 60-780 Poznań, Poland

Abstract: The present study describes development and subsequent validation of high performance liquid chromatographic method (HPLC) in comparison with spectrophotometric methods (classic, first, second and third order derivative) for determination of pure cilazapril in substance and pharmaceutical preparation. The main aim of this study was to find the method suitable not only for determination of cilazapril, but additionally useful in degradation kinetic study. Only the HPLC method is stability indicating. The HPLC method utilizes LiChroCART® 250-4 HPLC-Cartridge, LiChrospher® 100 RP-18 (5 μm) column, at ambient temperature, eluted at the flow rate 1.0 mL/min. The mobile phase consists of acetonitrile, methanol and phosphate buffer (pH 2.0) (60:10:30, v/v/v). Wavelength of detection is set at 212 nm. Benzocaine is used as an internal standard. The second and third order derivative spectrophotometric methods can be applied for the cilazapril analysis in substance and tablet, but not for stability evaluation (the lack of selectivity towards degradation product).

Keywords: cilazapril, HPLC, spectrophotometry, drug stability

The angiotensin-converting enzyme inhibitors (I-ACE) are established in therapies for the treatment of hypertension and heart failure. They prevent conversion of angiotensin I into angiotensin II leading to decreased circulating levels of angiotensin II and aldosterone. As an indirect result of angiotensin-converting enzyme inhibition, blood pressure is lowered. I-ACEs are used in monotherapy as well as combined with thiazide diuretics or β-antagonists (1). Cilazapril is one of the I-ACEs. Daily dose of cilazapril varies between 0.5–5 mg, with the maximum daily dose of 5 mg (2). Cilazapril is a pro-drug. *In vivo* it is hydrolyzed to biologically active form – cilazaprilat, which acts as an angiotensin-converting enzyme inhibitor (3).

Cilazapril (Fig. 1) chemically is [1*S*-[1α,9α(*R**)]]-9-[[1-ethoxycarbonyl-3-phenyl-propyl]amino]octa-hydro-10-*oxo*-6*H*-pyridazino[1,2*a*][1,2]diazepine-1-carboxylic acid monohydrate. It exists in pharmaceutical formulations in the form of tablets. The expiry date of cilazapril preparations is shorter than 5 years, what makes the drug interesting from degradation kinetics point of view. The purpose of stability testing is to provide evidence on how quality of a drug substance varies with the time under the influence of variety of environmental factors such as temperature, humidity and light. The choice of the analytical method in stability study is essential. The chosen method must selec-

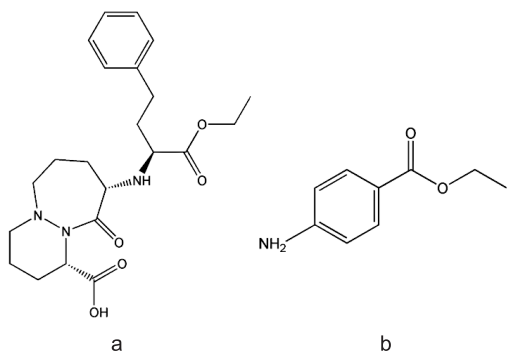

Figure 1. Structural formulas of **a** – cilazapril and **b** – benzocaine (internal standard, IS)

* Corresponding author: e-mail: bstanisz@ump.edu.pl; phone:+48618546645; fax +48618546652.

tively determine the drug coexisting with degradation products (4).

Literature survey for cilazapril analysis revealed methods based on different techniques. Various methods for determining levels of cilazapril in biological samples have been developed including: enzyme immunoassay in serum or plasma (5), liquid chromatography/positive ion tandem mass spectrometry method in human plasma (6), HPLC with amperometric (7) and photometric (8) detection in urine, capillary zone electrophoresis in urine (9) and gradient RP-high performance liquid chromatography in the presence of hydrochlorothiazide in urine (10). Polish Pharmacopoeia VIII presents HPLC method for purity assessment of cilazapril (11). Methods applied for the analysis of cilazapril in pharmaceutical formulations include: HPLC with amperometric (7) and photometric (8) detection, capillary zone electrophoresis (9), amperometric biosensor enantioselective analysis of S-cilazapril (12) and voltammetric determination (13). Spectrophotometry (14–16) and capillary electrophoresis (17) were used for determination of cilazapril in the presence of hydrochlorothiazide. Methods for determination of cilazapril in a mixture with other I-ACEs were also developed and are listed below: HPTLC coupled with densitometry (18), square wave voltammetric method (19), HPLC with spectrophotometric detection (20) and capillary electrophoresis (21, 22). As it results from the literature, till now no data about stability indicating method for cilazapril has been developed.

The present study is the first time report on stability indicating assay of pure cilazapril in the presence of its degradation product by HPLC method with spectrophotometric detection. The method's conditions were optimized in terms of mobile phase to achieve proper separation of analyzed sample. This is challenging, because during stability study the active substance is exposed to high temperature and high humidity. The proposed method is able to separate the drug from its degradation product generated during the stress test. It is not only selective, precise and accurate, but also fast (large number of samples), cheap and characterized by low toxicity. Furthermore, HPLC apparatuses are widely used in chemical and pharmaceutical laboratories. The developed method was validated according to ICH guidelines (23).

The paper also presents the spectroscopic methods (classic and derivative) for determination of pure cilazapril in substance and pharmaceutical preparation. Cilazapril exhibits a weak benzene chromophore, which cause low absorptivity values. As a consequence, poor sensitivity can be achieved by conventional spectroscopic methods. Therefore, derivative mode was applied to improve the selectivity of UV spectrophotometric determinations (24). Recording the first and higher order derivative spectra was beneficial in our study, while it resulted in improved resolution of overlapping peaks and greater precision in determination of λ_{max} (25). Though, spectrophotometric methods cannot be applied in kinetics study (lack of selectivity towards the degradation product): it is expected that this methods could serve the stability study on condition of prior separation of the substrate and the product of decomposition.

EXPERIMENTAL

Apparatus

A chromatographic system consisted of a Shimadzu LC-6A Liquid Chromatograph pump with a 7725 Rheodyne value injector 20 μL fixed loop equipped with a Shimadzu SPD-6AV UV-VIS Spectrophotometric Detector. The detector was set at 212 nm and peak areas were integrated by Shimadzu C-R6A Chromatopac integrator. Columns (LiChroCART® 250-4 HPLC-Cartridge, LiChrospher® 100 RP-18 (5 μm) and RP-8 (10 μm), Merck, Darmstadt, Germany) worked at ambient temperature.

Spectrophotometric analysis was carried out on Perkin–Elmer UV-Vis Lambda 20 double beam spectrophotometer connected to the computer loaded with Perkin-Elmer Lambda-6 UV WinLab Version 2.70.01 software. Analyses were performed using 1 cm quartz cells at a scan speed of 240 nm/min, response time 0.5 s, a slit width 1.0 nm.

Chemicals

Cilazapril was kindly supplied by Biofarm (Poznań, Poland, batch number 1621816). Methanol and acetonitrile were of HPLC grade (Merck, Darmstadt, Germany). All other chemicals were of analytical reagent grade. Potassium phosphate monobasic was purchased from POCh, Gliwice, Poland. Water used was freshly bidistilled. A commercial pharmaceutical preparation (Inhibace® 5 mg, Roche) is characterized by declared content: cilazapril 5 mg per tablet.

Procedures

Mobile phase preparation

Phosphate buffer at pH 2.0: the solution of 0.0681 g of KH_2PO_4 (M = 136.09 g/mol) was prepared in 500 mL volumetric flask by dissolving in

400 mL water, adjusting it to desired pH value by 80% orthophosphoric acid and final filling up the volume with water. The obtained phosphate buffer (pH 2.0) was mixed with the acetonitrile and methanol in the desired volume ratios (Tab. 1). Prepared mobile phases were filtered and degassed before use.

Sample preparation

Prepared methanolic solutions for spectrophotometric method were recorded as the UV spectra A, D1, D2 and D3 (classic, first, second and third order derivative spectra) against the methanol as the blank. Solutions for HPLC study were mixed with IS (internal standard) in the ratio: 1 mL of analyzed solution and 0.5 mL 20.0 μg/mL solution of IS. A volume of 20 μL of prepared sample was injected into the column.

Precision study

The methods for intra-day precision, both for spectrophotometric and HPLC methods, were evaluated on three concentration levels: low, medium and high. Cilazapril concentrations for spectrophotometric methods were 10.0 μg/mL, 20.0 μg/mL, 40.0 μg/mL and for the HPLC method 100.0 μg/mL, 200.0 μg/mL and 400.0 μg/mL. Concentrations mentioned above were obtained by dilution of cilazapril standard solution with methanol. Inter-day precision was evaluated in five consecutive days on 400 mg/mL methanolic cilazapril solution for HPLC method and 40 mg/mL for spectrophotometric methods. RSD (relative standard deviation) [%] was checked.

Linearity study

Standard solution 800.0 μg/mL of cilazapril was prepared daily by dissolving appropriate amount of substance in methanol. Solutions for calibration curves, for spectrophotometric and HPLC methods, were obtained by serial dilution of standard solution of cilazapril with the same diluent to yield the desired assay concentrations.

For spectrophotometric methods, concentrations were prepared from 4.0 μg/mL to 48.0 μg/mL. The calibration curves were the plots relating A, D1, D2 and D3 as a function of cilazapril concentration measured at analytical wavelength selected for each method.

For the HPLC method, concentrations at higher level (10.0 to 480.0 μg/mL) were selected. Calibration curve was the relationship between the

Table 1. Method optimization for HPLC analysis of cilazapril.

Column	Mobile phase (v/v/v)			Flow rate [mL/min]	Detection λ [nm]	Observations	Result
	CH_3CN	CH_3OH	phosphate buffer (pH 2.0)				
RP – 8	50	-	50	1.0	218	Wide, low peaks analysis time ~14 min.	Rejected
	60	-	40	1.0	212	Wide peaks analysis time ~11 min.	Rejected
	60	-	40	1.2	212	Wide peaks analysis time ~10 min. high pressure	Rejected
	50	20	30	1.0	212	Wide peaks analysis time ~14 min.	Rejected
	50	20	30	1.2	212	Wide peaks analysis time ~11 min. high pressure	Rejected
	60	10	30	1.0	212	Wide peaks analysis time ~13 min.	Rejected
RP – 18	50	20	30	1.0	212	Narrow, high peaks analysis time ~13 min.	Rejected
	60	10	30	1.2	212	Narrow, high peaks analysis time ~13 min. high pressure	Rejected
	60	10	30	1.0	212	Narrow, high peaks analysis time ~ 9 min.	Accepted

peak area ratios of cilazapril against IS to the corresponding cilazapril concentrations.

Detection and quantitation limits (LOD, LOQ, respectively) were established on basis of the standard deviation of the response and the slope LOD = 3.3 s_a/a; LOQ = 10 s_a/a.

Selectivity study

Selectivity was established on the basis of comparison of pure samples with those obtained from pharmaceutical formulation (Inhibace® 5 mg). For kinetic study, selectivity was checked towards degradation product of cilazapril.

Accuracy study

The methods' accuracy was verified by analyzing samples fortified with 50, 100 and 150% of claimed drug content and the evaluation of their recovery. The recovery study was performed by using model mixtures prepared from Inhibace® 5 mg tablets: mixture I – 20 tablets (Inhibace® 5 mg) grounded in mortar with a portion of 50.0 mg of cilazapril, mixture II – 20 tablets (Inhibace® 5 mg) grounded in mortar with a portion of 100.0 mg of cilazapril, mixture III – 20 tablets (Inhibace® 5 mg) grounded in mortar with a portion of 150.0 mg of cilazapril. From each model mixture, nine samples weighing about 100.0 mg were prepared by dissolving in 25 mL of methanol. Samples were mechanically shaken for 30 min and filtered through dense, narrow pores, slow-filtering filter paper circles No. 390 for quantitative analysis (Filtrak GmbH, Barenstein, Germany). The clear supernatant was analyzed. Accuracy was evaluated as a recovery [%] of cilazapril from each sample. The results were presented as the mean value of recovery ± SD from three model mixtures (I–III).

Tablet content analysis

Twenty tablets (Inhibace® 5 mg) were accurately weighed and powdered in mortar. A simple equivalent to one tablet (with 5 mg of cilazapril content) was weighed and transferred into 50 mL volumetric flask. Then, with addition of 25 mL of methanol obtained mixture was mechanically shaken for 30 min and was filtered through dense, narrow pores, slow-filtering filter paper circles No. 390 for quantitative analysis. The clear filtrate was analyzed. The obtained in the course of analysis cilazapril content was verified with the claimed drug content.

Optimization of chromatographic conditions

The chromatographic conditions were optimized by different means. Different columns, mobile phase composition, detection wavelengths and flow rate were checked (Tab. 1).

Pilot degradation kinetic study

To obtain cilazapril with its product of decomposition, samples of cilazapril were subjected to stress test. The test was performed on accurately weighed 0.0100 g cilazapril samples under conditions of increased temperature T = 363 K and relative humidity RH = 76.4 %. Environment of relative humidity at 76.4% was provided in desiccator by saturated solution of inorganic salt (NaCl, M = 58.45 g/mol). Cilazapril samples were kept in conditions described above for specified period of time and then subjected to HPLC and spectrophotometric analyses.

RESULTS AND DISCUSSION

Chromatographic method analysis

Optimization of chromatographic system

Optimization of composition of chromatographic system was based on an adequate selection of chromatographic column and a composition of mobile phase that will guarantee satisfactory chromatographic separation of cilazapril and its product of decomposition, both in pure substance and in pharmaceutical preparations.

Cilazapril is a drug with a structural relationship to enalapril (26). Though, the HPLC conditions were developed on the basis of the existing method for determination of the enalapril maleate in stability study, in which a RP-8 column was used with acetonitrile-KH_2PO_4 solution (pH 2.2, 0.001 M) (50:50, v/v) (27). This method was a further optimization of method developed for determination of enalapril maleate, felodipine and their degradation products (28). Under conditions of mobile phase mentioned above, cilazapril and enalapril maleate should behave in a similar way. They both possess net positive charge at pH = 2.0, while their pKa values of the carboxyl groups are 2.97 (28) and 2.35 (29) for enalapril maleate and cilazapril, respectively. Under these conditions, cilazapril and its degradation product were eluted in less than 15 min, but the peak's shapes were not satisfactory in terms of their sharpness and symmetry, although, this separation formed good base for further optimization. An idea of methanol addition to acetonitrile and phosphate buffer (pH = 2.0) mobile phase and usage of RP-18 column was taken from another HPLC method developed for determination of cilazapril and cilazaprilat in urine (8). Addition of methanol to mobile phase did not affect the reten-

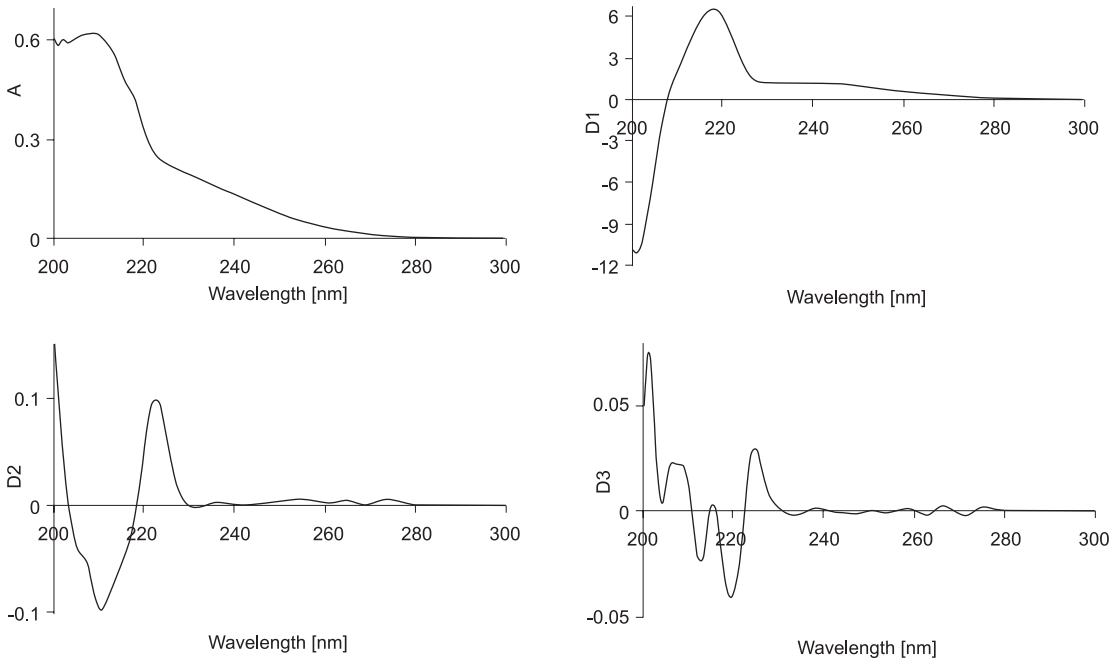

Figure 2. The absorbance (A), first (D1), second (D2) and third (D3) order derivative spectra of 20 µg/mL cilazapril methanolic solution

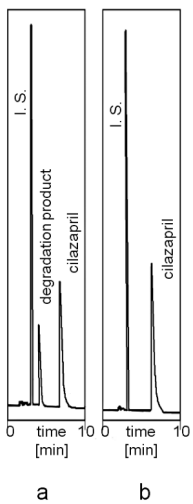

Figure 3. Selectivity of HPLC method: **a** – chromatogram of cilazapril decomposed during the stress test RH = 76.4%, T = 363 K, t = 30 h with IS; **b** – chromatogram of pure sample of cilazapril with IS

tion times, but changed the shape of chromatogram. Peaks were sharper and symmetric. Addition of methanol caused the rise in peak height and shorten the width of the peaks, for both, cilazapril and its product of decomposition. The maximum absorbance of cilazapril is located at 212 nm in methanol (Fig. 2) and at this analytical wavelength good selectivity was achieved and thus it was used throughout this work. The internal standard proper for these chromatographic conditions was benzocaine. The pH = 2.0 of the phosphate buffer was crucial for good separation. Lower pH values of prepared buffer lowered retention times of the product of cilazapril degradation leading to lack of its separation from IS.

The optimal chromatographic separation was performed using LiChroCART® 250-4 HPLC-Cartridge, LiChrospher® 100 RP-18 (5 µm) column (Merck, Darmstadt), at ambient temperature, eluted at the flow rate 1.0 mL/min. The mobile phase consisted of acetonitrile-methanol-phosphate buffer (pH 2.0) (60:10:30; v/v/v). The wavelength was set at 212 nm and these conditions gave reasonably good response (Tab. 1).

Validation

The HPLC method was selective towards cilazapril, its degradation product and IS. The retention times were respectively: ~8.9, ~3.1 and ~2.8 min (Fig. 3). The method was also selective towards the ingredients of Inhibace® tablet – no changes in chromatogram shape were observed in comparison to chromatogram of pure cilazapril sample.

The HPLC method is characterized by good linearity, precision and accuracy. The validation parameters are presented in Table 2.

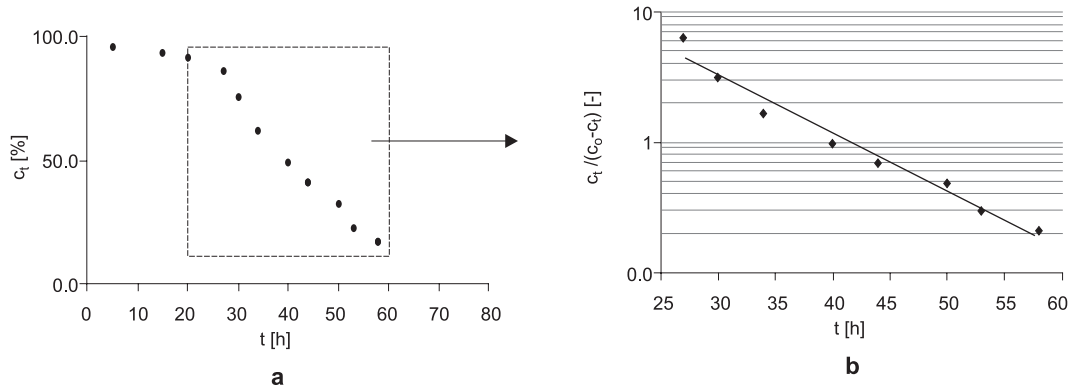

Figure 4. Relationships describing changes of cilazapril concentration during the stress test (T = 363 K, RH = 76.4%, t = 0–70 h): **a** – sigmoidal curve c = f(t) , **b** – linear relationship for degradation of cilazapril plotted according to Prout-Tompkins equation ln $c_t/(c_0-c_t)$ = f(t)

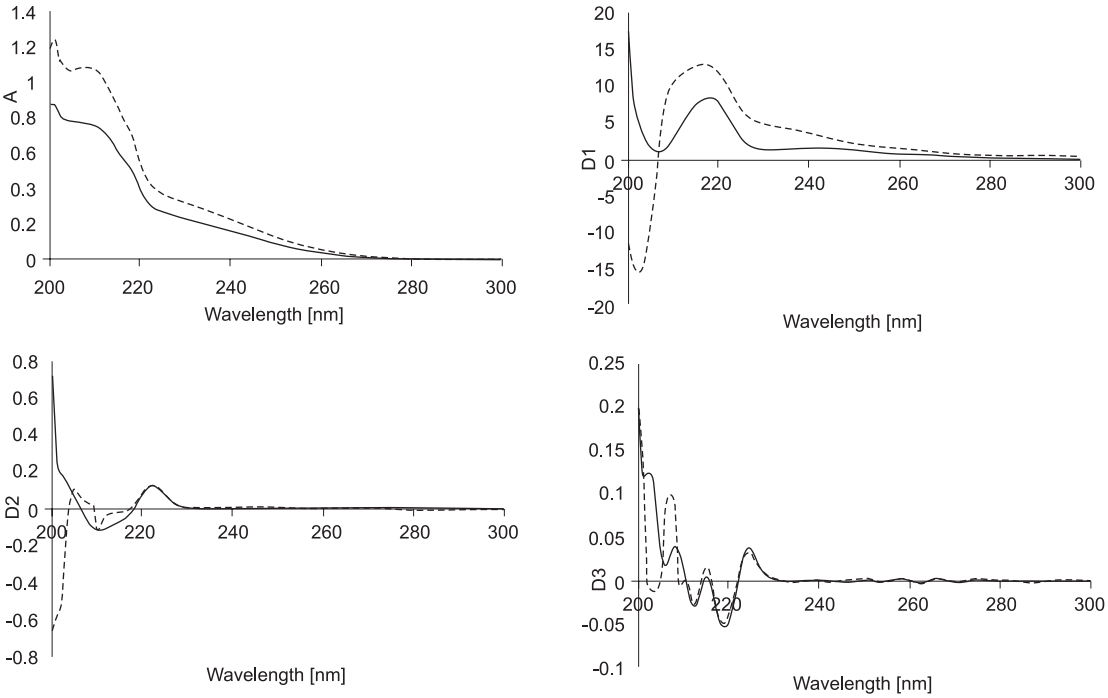

Figure 5. Selectivity of spectroscopic methods towards tablet content. Spectra of 20 mg/mL cilazapril methanolic solution (——) and 20 mg/mL cilazapril methanolic solution obtained from Inhibace® 5 mg tablets (- - - - -)

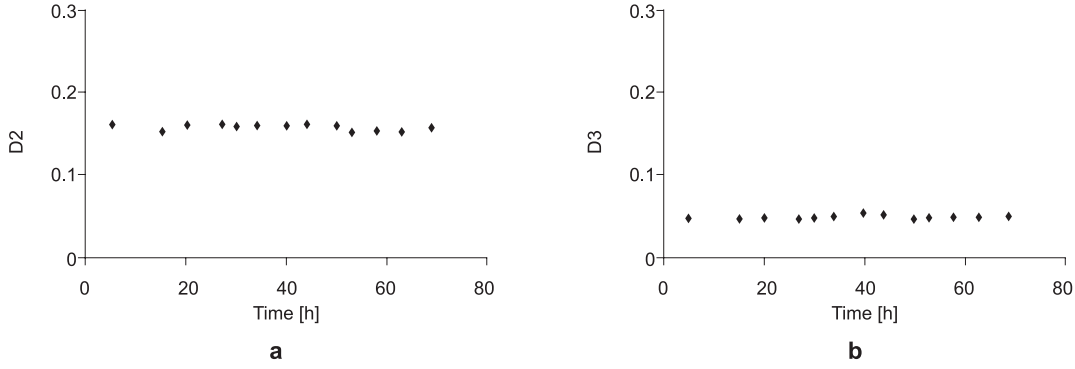

Figure 6. Selectivity of D2 and D3 spectrophotometric method towards degradation product. Samples of cilazapril during stress test (T = 363 K, RH = 76.4%) assessed by **a** – D2, **b** – D3 spectrophotometric method

Table 2. Validation parameters of HPLC and A, D1, D2, D3 spectrophotometric methods.

Parameters	**HPLC**	**A**	**D1**	**D2**	**D3**
Range [µg/mL]	10.0 – 480.0	4.0 – 48.0	4.0 – 48.0	4.0 – 48.0	4.0 – 48.0
LOD [µg/mL]	0.0278	0.0566	0.0458	0.0540	0.0730
LOQ [µg/mL]	0.0843	0.1716	0.1389	0.1637	0.2213
Regression equation			***y = ax+b***		
a ± Δa	0.00526 ± 0.0010	0.02958 ± 0.00115	0.34371 ± 0.01151	0.00527 ± 0.00020	0.00144 ± 0.00008
s_a	0.00005	0.00051	0.00509	0.00009	0.00003
b ± Δb	-0.00257 ± 0.02600 [1]	0.05486 ± 0.03185 [2]	0.12216 ± 3.1925 [1]	-0.00188 ± 0.00564 [1]	0.00088 ± 0.00215 [1]
s_b	0.01181	0.01408	0.14113	0.00249	0.00095
Regression equation			***y = ax***		
a ± Δa	0.00526 ± 0.00010	-	0.34758±0.01092	0.00521 ± 0.00019	0.00147 ± 0.00007
s_a	0.00004	-	0.00483	0.00009	0.00003
r	0.999	0.999	0.999	0.999	0.998
Precision RSD [%] Intra-day [3] Inter-day	 0.94, 0.95, 1.05 0.76	 > 2 > 2	 > 2 > 2	 1.91, 1.35, 0.88 0.52	 1.58, 1.13, 1.02 1.36
Accuracy Recovery[%]	98.74 ± 0.81	-	-	100.03 ± 0.15	99.13 ± 0.70
Inhibace 5 mg analysis [g]	0.0049 ± 0.0001	-	-	0.0050 ± 0.0001	0.0048 ± 0.0001

[1] intercept b statistically insignificant (Student *t*-test, $\alpha = 0.05$). [2] intercept b statistically significant (Student *t*-test, $\alpha = 0.05$). [3] concentrations of cilazapril 100.0, 200.0, 400.0 µg/mL for HPLC method and 10, 20, 40 µg/mL for spectrophotometric methods.

Pilot kinetic study

Changes in concentration of cilazapril occurred during the drug incubation in the atmosphere of increased relative humidity (RH = 76.4%) and temperature (T = 363 K). Degradation curve, which describes changes in cilazapril concentrations against the time, has characteristic sigmoidal shape (Fig. 4) what indicates the process of autocatalytic reaction. An intermediate product functions as a catalyst in the autocatalytic reaction. For the interpretation of this process the Prout-Tompkins equation is used (3):

$$\ln c_t/(c_0-c_t) = \text{const} - kt$$

where c_0 = cilazapril concentration at zero time, c_t = cilazapril concentration at time t, k = degradation rate constant and const is a constant related to the introduction period.

Classic and spectra derivative spectrophotometic method analysis

Choice of analytical wavelengths

Measurements of absorbance and derivative spectra were done against methanol. The derivative spectra were obtained by means of peak-zero method. Selections of analytical wavelengths were performed by examining the UV spectra of 20.0 μg/mL cilazapril solution. Values of λ_{max} for A, D1, D2 and D3 spectra measured in the range 200–300 nm were 212, 218, 222 and 224 nm, respectively (Fig. 2). The λ_{max} values selected for each method were further used during the validation study and sample assay.

Validation

Linearity of each spectrophotometric method was established in the range 4.0–48.0 mg/mL. In the linearity evaluation, Lambert-Beer law was used, according to which the absorbance is linear with the concentration. Therefore, plots of A, D1, D2 and D3 as a function of cilazapril concentration *f(c)* are linear. With the exception of classic spectrophotometric method, all of them can be described by the equation $y = ax$, while intercept b is statistically insignificant (Student t-test). All evaluated validation parameters of A, D1, D2 and D3 methods are presented in Table 2.

Validation of spectrophotometric method gave satisfactory results only for D2 and D3 methods. The A and D1 methods were rejected as unsuitable for cilazapril determination study due to low precision, low accuracy (both A and D1) and, mentioned above, lack of $y=ax$ relationship of calibration curve for classic spectrophotometric method. Therefore, only D2 and D3 methods were chosen for further analysis.

Spectrophotometric methods selectivity was established towards the ingredients of tablet and degradation product (the latter described in the next section). Figure 5 shows that only D2 and D3 method is selective towards tablet ingredients – the same signal at λ_{max} is selectivity indicating.

Pilot degradation kinetic study

D2 and D3 methods selectivity towards degradation product was established on the basis of comparison of pure cilazapril samples with those decomposed during stress test. Results were presented as a signal obtained by D2, D3, respectively, in time t (Fig. 6). No changes in D2 or D3 signal was observed. Although, the spectrophotometric methods (D2, D3) can be successfully used for determination of cilazapril in substance and tablets, they cannot be used for stability evaluation of cilazapril. Analysis of disintegrated samples of cilazapril during stress test showed no changes in D2, D3 values, while the HPLC assay of the same samples confirmed the degradation of cilazapril (Fig. 4).

CONCLUSIONS

In conclusion, the stability indicating HPLC method with UV detection has been developed and validated for analysis of cilazapril. Based on peak purity results, obtained from the analysis of degraded samples using described method, it can be concluded that the absence of coeluting peak along with the main peak of cilazapril indicated that the developed method is specific for estimation of cilazapril in the presence of degradation products. Further, the proposed method has good selectivity, precision and accuracy. Even though no attempt was made to indentify the degraded products, the proposed method can be used as a stability indicating method for assay of cilazapril. The D2 and D3 spectrophotometric methods can be applied for stability study of cilazapril only after previous separation of the substrate and the product(s) of decomposition.

Pilot kinetic study indicates autocatalytic way of cilazapril decomposition. The cilazapril kinetic study will be continued in terms of influence of humidity and temperature on cilazapril stability, mechanism of degradation and analysis of degradation products.

REFERENCES

1. Golan D.E., Tashijan A.H., Armstrong E.J., Armstrong A.W.: Principles of pharmacology: the pathophysiologic basis of drug therapy.

Lippincott Williams & Wilkins, Philadelphia 2007.
2. Szucs T.: Drugs 41, 18 (1991).
3. Zając M., Pawełczyk E., Jelińska A.: Chemistry of drugs (Polish). AM Poznań, 2006.
4. Pawełczyk E., Hermann T.: Principles of drug stability (Polish). PZWL, Warszawa 1982.
5. Tanaka H., Yoneyama Y., Sugawara M., Umeda I., Ohta Y.: J. Pharm. Sci. 76, 224 (1987).
6. Heon-Woo L., Wan-Su P., Sung-Hee C., Min-Hwan K., Ji-Hyung S. et al.: Talanta 71, 62 (2007).
7. Prieto J.A., Jimenez R.M., Alonso R.M.: J. Chromatogr. B 714, 285 (1998).
8. Prieto J.A., Jimenez R.M., Alonso R.M., Oritz E.: J. Chromatogr. B 754, 23 (2001).
9. Prieto J.A., Akesolo U., Jimenez R.M., Alonso R.M.: J. Chromatogr. A 916, 279 (2001).
10. Vujic Z., Crevar M., Obradovi V., Kuntic V., Uskokovic-Markovic S.: Chromatographia 70, 1221 (2009).
11. Polish Pharmacopoeia VIII, PZWL, Warszawa 2008.
12. Aboul-Enein H.Y., Stefan R-I., Radu. G-L.: Pharm. Dev. Technol. 2, 251 (1999).
13. Tamer U., Ozcicek P.N., Atay O., Tildiz A.: J. Pharm. Biomed. Anal. 29, 43 (2002).
14. Erk N., Onur. F.: Anal. Lett. 29, 1963 (1996).
15. Erk N.: J. Pharm. Biomed. Anal. 20, 155 (1999).
16. Dinc E., Baleanu D.: J. Pharm. Biomed. Anal. 30, 715 (2002).
17. Hillaert S., De Grauwe K., Van den Bossche W.: J. Chromatogr. A 924, 439 (2001).
18. Kowalczuk D., Pietraś R., Hopkała H.: Chromatographia 60, 245 (2004).
19. Prieto J.A., Jimenez R.M., Alonso R.M.: Farmaco, 58, 343 (2003).
20. Gumieniczek A., Przyborowski L.: J. Liq. Chromatogr. Relat. Technol. 20, 2135 (1997).
21. Hillaert S., Van den Bossche W.: J. Chromatogr. A 895, 33 (2000).
22. Hillaert S., Vander Heyden Y., Van den Bossche W.: J. Chromatogr. A 978, 231 (2002).
23. International Conference on Harmonization (ICH). Validation of Analytical Procedures: Methodology, Geneva 1996.
24. Bonazzi D., Gotti R., Andrisano V., Cavrini V.: J. Pharm. Biomed. Anal. 16, 431 (1997).
25. Gordon M.H., Macrae R.: Instrumental Analysis in the Biological Sciences, Blackie Academic & Professional, Glasgow 1987.
26. Saito H, Minami M.: Antihypertensive drugs today. VSP Press, Utrecht, Tokyo 1992.
27. Stanisz, B.: J. Pharm. Biomed. Anal. 31, 375 (2003).
28. Qin Q-Z, DeMarco J., Ip D.P.: J. Chromatogr. A 707, 245 (1995).
29. Venn R. F.: Principles and practice of bioanalysis, Taylor & Francis, London, New York 2000.

Received: 12. 01. 2011