Acta Poloniae Pharmaceutica – Drug Research, Vol. 61 No. 2 pp. 91–97, 2004
ISSN 0001-6837
Polish Pharmaceutical Society

BIOPHARMACY

THE INFLUENCE OF RELATIVE HUMIDITY AND TEMPERATURE ON STABILITY OF MOEXIPRIL HYDROCHLORIDE IN SOLID PHASE

BEATA STANISZ

Poznań, University of Medical Sciences, Department of Pharmaceutical Chemistry, 6 Grunwaldzka Str., 60–780 Poznań, Poland

Abstract: Kinetic and thermodynamic parameters of the decomposition of moexipril hydrochloride in solid phase in the absence and presence of humidity were calculated. The evaluation of stability of moexipril hydrochloride was followed by the HPLC method. The applied method was validated (evaluation of the following parameters: selectivity, linearity, precision, limit of detection (LOD), limit of quantification (LOQ) and repeatability). The effect of humidity on the stability of MHCl in solid phase at 363 K was described by the equation: $ln\ k_i = ax + b = (0.0676 \pm 0.016) \cdot RH\% - (15.53 \pm 0.78)$. Identification of degradation products of MHCl were carried out by the HPLC–MS method.

Keywords: moexipril hydrochloride (MHCl); solid phase; HPLC; HPLC–MS; kinetic and thermodynamic parameters

Moexipril hydrochloride (MHCl) {(3*S*)–2–[(2*S*)–*N*–[(1*S*)–1–carboxy–3–phenylpropyl]alanyl]–1,2,3,4–tetrahydro–6,7–dimethoxy–3–isoquinolinecarboxylic acid, 2–ethyl ester} belongs to a class of *N*–carboxyalkyl dipeptide angiotensin converting enzyme (ACE) inhibitor. Moexipril is a prodrug, which is hydrolyzed after absorption to the active diacid moexipril (moexiprilat). Both moexipril and moexiprilat inhibit ACE activity, although the potency of moexipril is much lower than that of moexiprilat. Moexipril and moexiprilat inhibit ACE activity and thereby prevent the formation of angiotensin II from angiotensin I (1–3).

The effect of organic solvents on the degradation kinetics, on the product formation and on the mechanism of MHCl transformation at 25°C has already been investigated and the results are presented in the paper of Gu L, Strickley R.G. et al. (4,5). The stability of MHCl was also studied in the presence of selected excipients (6).

The literature does not refer to research into the kinetics of MHCl in substance in solid phase. The aim of the study was to evaluate the influence of relative humidity and temperature on the stability of moexepril hydrochloride during storage of the substance.

EXPERIMENTAL

Materials and reagents

Moexipril hydrochloride was obtained from Schwarz–Pharma AG. Other chemical substances and reagents were products of Sigma Chemical Co.

HPLC method for the estimation stability of MHCl in solid phase

In this paper, a modified HPLC method was applied, used earlier for the study of stability of enalapril maleate and quinapril hydrochloride in solid phase (7,8).

A Merck analytical column (Hypersil MOS, 5 μm particle size, 250 mm × 4 mm i.d.) was used as the stationary phase. The mobile phase: acetonitrile – phosphate buffer {pH = 2.0, 0.001 mole/l} (50:50 v/v); flow rate of the mobile phase: 1.3 ml/min; internal standard: xylometazoline hydrochloride (methanolic solution 0.16 mg/ml); detector UV: 220 nm.

HPLC – MS method for the identification of decomposition product of MHCl

Column: Hypersil MOS, 5 μm particle size, 250 mm × 4 mm i.d. Merck; mobile phase: acetonitrile – water (90:10) with formic acid;, flow rate: 0.5 ml/min.

Validation method

For the validation test, the following substances were used: a sample of MHCl, a sample of MHCl heated to 373 K in dry air, and a sample of MHCl heated to 363 K in a humid atmosphere of 76.4% RH. During the validation study, the following parameters were evaluated:

selectivity: the applied method was selective for the MHCl (t_R = about 5 min) as well as for the internal standard (xylometazoline hydrochloride – t_R = about 7 min) in the presence of degradation products (t_R = about 2 and 3 min);

precision: it was were evaluated for 10 individual samples of 0.4 mg/ml MHCl, and the following results were obtained: mean value P_i/P_{is} = 2.3115, standard deviation = 0.0248, variation coefficient = 1.07%;

linearity: the calibration curve for MHCl was taken in methanol for solutions of concentrations varying from 0.0125 mg/ml to 0.4000 mg/ml. The internal standard (xylometazoline hydrochloride) was a methanolic solution at a concentration of 0.16 mg/ml. Next, 1.0 ml of the so obtained solution was mixed with 1.0 ml of the internal standard solution and subjected to analysis. Linearity was also examined for three succeeding days in solutions of the same concentration prepared from a stock solution. The equation for the calibration curve was: $y = (5.45 \pm 0.14) \times c$; for the equation $y = ac + b$, the value b was statistically insignificant). The calculated correlation coefficient was > 0.999, thus indicating good linearity;

limit of detection (LOD), limit of quantification (LOQ): in the described experimental conditions the limit of detection was approx. 6 µg/ml and the limit of quantification was 12 µg/ml;

repeatability of the method was tested by eight replicates and evaluated by the variation coefficient = 1.31%.

Conditions of the kinetic studies

For the experiments, 10 mg samples of MHCl were weighed into 5 ml open vials. To assess the effect of humidity on the stability of MHCl, the vials with MHCl were placed in desiccators containing aqueous saturated solutions of appropriate inorganic salts, which safeguarded the conditioned relative humidity: 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 up to the desired temperatures: 333 K, 343 K, 353 K, 358 K and 363 K.

To assess the stability of MHCl in dry air, the vials containing the studied substance were immersed in a sand bath, in a heat chamber adjusted to a temperature of 353 K, 358 K, 363 K, 373 K and 383 K.

During the experiment, the color of the substance changed from white through yellow to brown, all products of degradation were soluble in methanol. Each series comprised 7 – 12 samples.

After definite time intervals, determined by the 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 the total volume of 25.0 ml with methanol. To 1.0 ml of the so obtained solution 1.0 ml of the internal standard solution was added. The internal standard was added to the solutions at a constant concentration before investigation.

100 µl of the samples were fed to the HPLC column and the emerging signals were recorded.

The chromatograms were interpreted using the following dependence: $P_i/P_{i.s} = f(t)$; where P_i are the values of the MHCl, and $P_{i.s}$ represent the values of internal standard, t represent time.

RESULTS AND DISCUSSION

Rate constants of degradation reaction of MHCl

In presence of relative humidity (50.9% – 76.4%), the changes in concentration of MHCl occurred according to the first order reaction model; $c_0 \to 0$. The plots $\ln c_t = f(t)$ were linear (Figures 1 and 2) and the observed rate constants were calculated by the least squares method according to the equation: $\ln c_t = \ln c_0 - k \cdot t$,
where: c_t and c_0 represent the concentration of MHCl in time t and 0, respectively, k is the first–order rate constant (s^{-1}).

The following statistical parameters of the respective equations were computed: $a \pm \Delta a$,

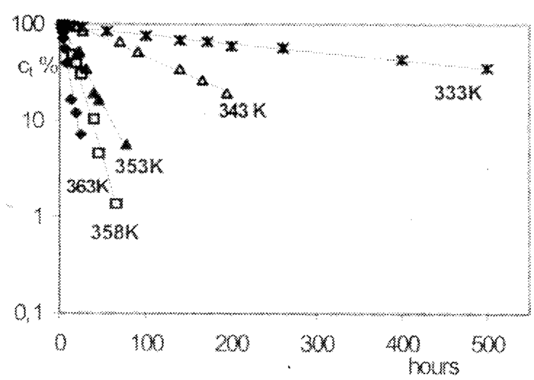


Figure 1. Semilogarithmic plots for the degradation of MHCl in solid phase at relative humidity RH ~76% at different temperatures.

Table 1. Kinetic and thermodynamic parameters of MHCl decomposition in solid phase at relative humidity RH = 76.4%

T(K)	10^5 k ± Δk, s^{-1}	–r	n	Statistical evalution ln k_i = f(1/T)	Thermodynamic parameters
			Relative humidity, RH = 76.4%		
333	0.106 ± 0.049	0.994	10	a ± Δa = –14067 ± 944	E_a= 116.96 ± 7.9 [kJ/mole];
343	0.333 ± 0.022	0.997	7	S_a = 296.9	$\Delta H^{\neq}$ = 114.51 ± 7.8
353	1.205 ± 0.14	0.995	7	b ± Δb = 28.5 ± 2.7	[kJ/mole];
358	1.994 ± 0.21	0.996	7	S_b = 0.849	$\Delta S^{\neq}$ = –8.29 ± 22.4
363	3.309 ± 0.31	0.994	9	r = –0.999	[J /(K × mole)]
			Dry air, RH = 0%		
353	0.106 ± 0.049	0.994	10	a ± Δa =–17473 ± 2729	E_a= 145.3 ± 22 [kJ/mole];
358	0.218 ± 0.022	0.997	7	S_a = 983	$\Delta H^{\neq}$ = 142.8 ± 25
363	0.293 ± 0.14	0.995	7	b ± Δb = 35.67 ± 7.46	[kJ/mole];
373	1.448 ± 0.21	0.996	7	S_b = 2.69	$\Delta S^{\neq}$ = 51.7 ± 183
383	4.983 ± 0.31	0.994	9	r = –0.995	[J/(K × mole)]

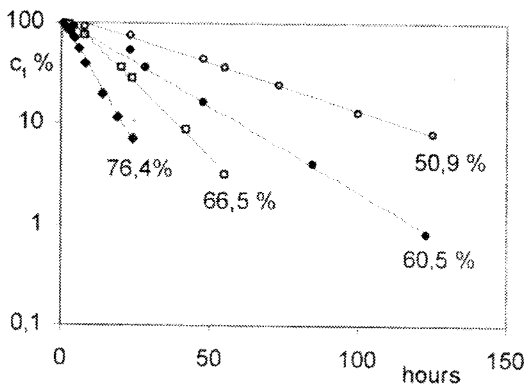


Figure 2. Semilogarithmic plots for the degradation of MHCl in solid phase at different humidity, temp. 363 K.

$b \pm \Delta b$, standard derivative (SD) and the coefficient of linear correlation. The values of Δa and Δb were computed for $f = n-2$ degrees of freedom, with $\alpha = 0.05$.

In dry air, the concentration changes of MHCl occurred according to the pseudo–first–order reaction model; $c_t \rightarrow c_e > 0$ (Figure 3a). The plots ln $(c_t - c_e) = f(t)$ were linear (Figure 3b) and the rate constants were calculated by the least squares method according to the equation:

$$ln\ (c_t - c_e) = ln\ (c_0 - c_e) - k \cdot t$$

where: c_t, c_e and c_0 represent the concentrations of MHCl in time t, de infinite and 0, respectively, k is the first–order rate constant (s^{-1}).

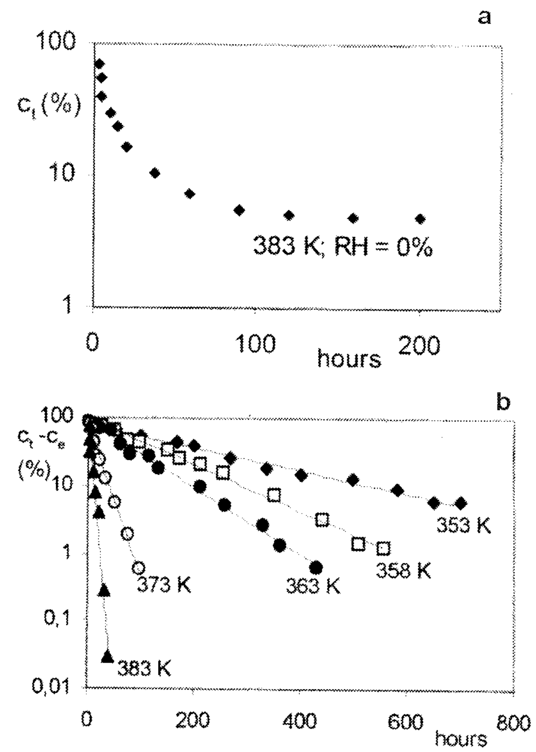


Figure 3. **a.** Changes of MHCl concentration during storage at RH = 0% at 383 K;
b. Semilogarithmic plots ln $(c_t - c_e)$ = f(t) for the degradation of MHCl in solid phase in dry air at different temperatures.

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 squares methods $a \pm \Delta a$, $b \pm \Delta b$, standard derivative (SD) and the coefficient of linear correlation. The values of Δa and Δb were computed for $f = n-2$ degrees of freedom, with $\alpha = 0.05$.

Thermodynamic parameters

The determined reaction rate constants were employed for the calculation of the Arrhenius relationship: $ln\ k_i = lnA - E_a/RT$, where k_i represent the respective reaction rate constants [s^{-1}], A = frequency coefficient, E_a = activation energy [J/mole],

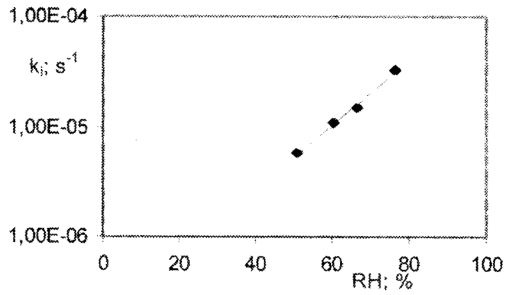


Figure 4. The effect of humidity on the stability of MHCl in solid phase.

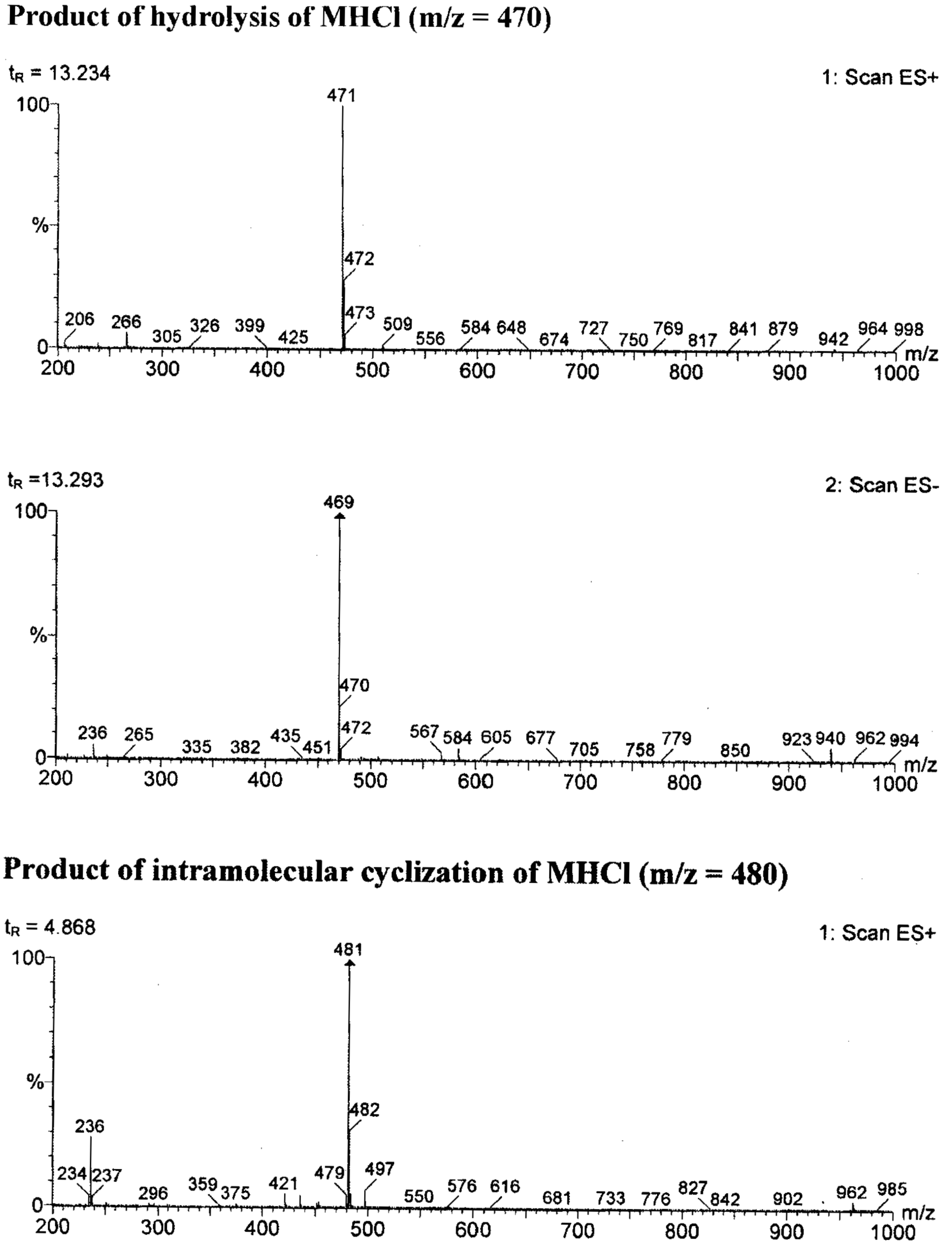
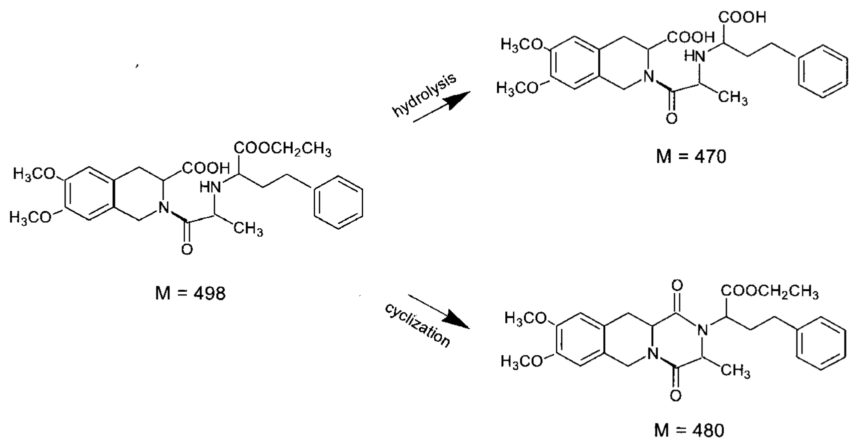


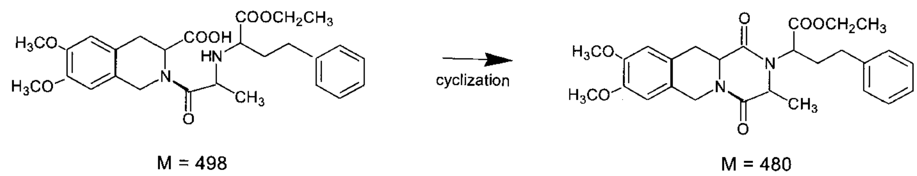
Figure 5. Mass spectra degradation products of MHCl.

Table 2. The effect of humidity on the stability of MHCl in solid phase at 363 K

RH%	10^5 k ± Δk [s^{-1}]	–r	n	Parameters of regression ln k_i = f(RH%)
50.9	0.578 ± 0.030	0.991	8	a ± Δa = 0.0676 ± 0.0163
60.5	1.085 ± 0.301	0.996	10	S_a = 0.00379
66.5	1.488 ± 0.303	0.991	7	b ± Δb = –15.53 ± 0.775
76.4	3.309 ± 0.314	0.994	9	S_b = 0.244
				r = 0.997



Scheme 1. Degradation of MHCl in solid state (experimental conditions: RH = 76.4%, temp. 363K).



Scheme 2. Degradation of MHCl in solid phase (experimental conditions: RH = 0%, temp. 373K).

R = universal gas constant (8.3144 J/(K × mole), T = temperature [K].

For the relationship $ln\ k_i = f(1/T)$ straight line plots were obtained for both the humid and dry conditions of sample exposure (Table 1). From the parameters of the plot $ln\ k_i = f(1/T)$ the following thermodynamic parameters of the decomposition reaction of MHCl 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.

Effect of humidity on the stability of MHCl

The effect of humidity on the stability of MHCl at 363 K, in the humidity range from 50.0% to 76.4%, is described by the equation: $ln\ k_i = ax + b$ = (0.0676 ± 0.0163) × RH% – (15.53 ± 0.775) (Figure 4). The slope (a = 0.0676 ± 0.0163) of the straight linear plot $ln\ k_i = f(RH\%)$ characterizes the effect of humidity on the stability of MHCl. This effect is similar to the effect of humidity on the stability of quinapril hydrochloride (a = 0.0589 ± 0.0086) (8). The value b obtained by extrapolation of this dependence for RH = 0% ($b = k_0$ = 1.802 $\cdot 10^{-7} s^{-1}$) is lower than the value k at 363 K in dry air conditions appointed empirically (k = 2.936 $\cdot 10^{-6} s^{-1}$). Such results point to the differences in the mechanism of degradation of MHCl occurring in the presence or absence of ambient humidity (Table 2).

Identification of the degradation products of MHCl (relative humidity 76% and temp. 363 K)

In the presence of relative humidity, the following signals were observed on HPLC–MS chromatogram: the MHCl signal at t_R = about 25 min, and two signals corresponding to the decomposition products of MHCl t_R = about 5 and 13 min.

Mass spectra of the degradation product of MHCl obtained by means of the technique of ESI allowed to define their molecular masses M = 470 (t_R = about 13 min; ES^+, m/z = 471; ES^-, m/z = 469) and M = 480 (t_R = about 5 min; ES^+, m/z = 481). The analysis of HPLC–MS spectra yielded the following conclusion: the product with M = 470 is moexipril diacid and the product with M = 480 is moexipril diketopiperazine (Scheme 1, Figure 5).

Identification of the degradation products of MHCl in dry air (RH = 0%, temp. 373 K)

In dry air, the following signals were observed on HPLC–MS chromatogram: the MHCl signal at t_R = about 25 min, and one signal corresponding to the decomposition product of MHCl t_R = about 5 min.

Mass spectra of the degradation product of MHCl obtained by means of the technique of ESI allowed to define their molecular masses M = 480 (t_R = about 5 min; ES^+, m/z = 481). The analysis of HPLC–MS spectra yielded the following conclusion: the product with M = 480 is moexipril diketopiperazine (Scheme 2, Figure 5).

CONCLUSIONS

The HPLC method was found to be selective, linear, accurate and precise for the evaluation of stability of MHCl in solid phase. In dry atmosphere (RH = 0%, in the temperature range from 343 K to 383 K), only moexipril diketopiperazine was the product of degradation of MHCl in solid phase.

In the presence of humidity (RH from 50.0% to 76.4%, in the temperature range from 333 to 363 K) moexipril diacid (moexiprilat) and moexipril diketopiperazine were the products of degradation of MHCl in solid phase. The conducted study on the stability of MHCl has shown that the investigated substance (in solid state) presents an appreciable stability at room temperature. The degradation constants of MHCl at 298 K when extrapolated from the Arrhenius equation, are equal to $k = 1.005 \cdot 10^{-10}\ s^{-1}$; $t_{10\%}$ = 12134 days; $t_{50\%}$ = 79783 days (RH = 0%) and $k = 7.013 \cdot 10^{-9}\ s^{-1}$; $t_{10\%}$ = 173 days; $t_{50\%}$ = 1143 days (RH = 76%).

Acknowlegements

I wish to thank the company SCHWARZ–PHARMA AG for supplying moexipril hydrochloride pure substance.

REFERENCES

1. Cawello W., Boekens H., Waitzinger J., Miller U.: Int. J. Clin. Pharmacol. Ther. 40, 9 (2002).
2. Zając M., Pawełczyk E.: Chemia Leków, Medical Academy, Publisher, Poznań 2000.
3. Brogden R.N., Wiseman L.R.: Drugs 55, 845 (1998).
4. Gu L., Strickley R.G.: Int. J. Pharm. 60, 99 (1990).
5. Strickley R.G., Visor G.C., Lin L.H., Gu L.: Pharm. Res. 6, 971 (1989).
6. Gu L., Strickley R.G., Ci L.H., Chowhan Z.T: Pharm. Res. 7, 379 (1990).
7. Stanisz B.: J. Pharm. Biomed. Anal. 31, 375 (2003).
8. Stanisz B.: Acta Polon. Pharm.–Drug Res. 60, 443 (2003).

Received: 13.11.2003