KINETICS OF OMEPRAZOLE DEGRADATION IN SOLID STATE

MARIA POPIELARZ-BRZEZIŃSKA

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

Abstract: The influence of temperature and relative air humidity on the stability of omeprazole (OME) in solid state was studied. Changes in the concentration were monitored by HPLC with UV detection. The method was validated; its selectivity, range of linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) were established. The linearity was satisfactory (r = 0.9998) in the concentration range from 0.5 to 14.0 mg/mL for a LiChrospher®100 RP-18 column (5 µm particle size, 100 mm × 4 mm, Merck, Darmstadt, Germany) and the mobile phase of methanol : 100 mM ammonium acetate (60:40 v/v). Degradation was conducted in solid phase at increased temperature (313 K to 393 K) and in the air humidity varying from 25% to 90% RH. The kinetic and thermodynamic parameters of OME decomposition in solid phase were determined.

Keywords: omeprazole, HPLC, stability, solid state

Omeprazole (OME) belongs to the medical drugs inhibiting the proton pump (H$^+$/K -ATP-ase inhibitors), applied in therapy of peptic stomach or duodenal ulcer, Zollinger-Ellison syndrome, stomach-esophagus reflux and eradication of *Helicobacter pylori*. This drug is a bioprecursor that is activated in strongly acidic environment. The active metabolite – sulfenamide – reacts with sulfhydryl H$^+$/K$^+$ groups of ATP-ase forming disulfide bridges and irreversibly blocking the enzyme. The drug is mainly metabolized in the liver. OME is fully metabolized by the P450 cytochrome system (CYP) (1–3).

In the analysis of inhibitor proton pump (IPP), the following methods: HPLC (4–7), UV-VIS (8), polarography (9) and TLC (10) were used. The study mainly focused in the determination of OME in body fluids. The literature does not refer to research into kinetics of OME for the substance in solid state. The study was undertaken to evaluate the stability of OME in the solid phase and establish the effect of temperature and relative air humidity on the process of OME degradation.

EXPERIMENTAL

Materials

Omeprazole (5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl}-1*H*-benzimida-zole), lot no. 111K1500; content of the active substance 99,8%; Sigma-Aldrich Chemie GmbH, Germany.

Flutamide, Sigma-Aldrich Chemie GmbH, Germany, was used as an internal standard (IS). Ammonium acetate was from POCh Gliwice and all other chemicals were obtained from Merck KGaA (Germany) and were of analytical or high-performance liquid chromatographic grade.

Equipment

Chromatographic separation and quantitative determination of omeprazole were performed by using a high-performance liquid chromatograph equipped with a UV detector. The analytical system consisted of a Rheodyne 7120 injector with a 20 µL loop. As the stationary phase a LiChrospher®100 RP-18 column, 5 µm particle size 100 mm × 4 mm (Merck, Darmstadt, Germany) was used. The UV detection was performed at 302 nm (Pye Unicam, England). A pump (Merck-Hitachi, Germany) and A/C transmitter with the software Chromed (Medson, Poland). The mobile phase consisted of methanol : 100 mM ammonium acetate (60:40, v/v). The flow rate was 1.0 mL/min.

Method validation

The method was validated according to the Guidelines of the International Conference on Harmonization (11).

* Corresponding author: e-mail: mpopiel@ump.edu.pl

Selectivity

Selectivity of the HPLC method was evaluated on the basis of measurements for the OME sample undegraded and two samples subjected to degradation at 373 K for 24 h and at 333 K and 76% relative air humidity for 24 h.

Linearity

The calibration curve of P_{OME}/P_{IS} = f(c) was drawn for OME concentrations in the range 0.5×10^{-2} -14.0×10^{-2} mg/mL

Precision

Precision of the method was determined for three concentrations of OME: 0.400, 0.200 and 0.100 mg/mL. The measurements were made on three subsequent days.

Limit of detection and limit of quantification

The LOD and LOQ values were calculated from the regression equation P_{OME}/P_{IS} = f(c), where LOD = 3.3 S_y/a and LOQ = $10S_y/a$. S_y and a values are the standard deviation and the slope, respectively, of the calibration curve.

Kinetic studies

Kinetic studies were performed at temperatures varied from 313 K to 393 K and at the relative air humidity varied from 25% to 90% in the thermal chambers. The exactly weighted samples (1 mg of
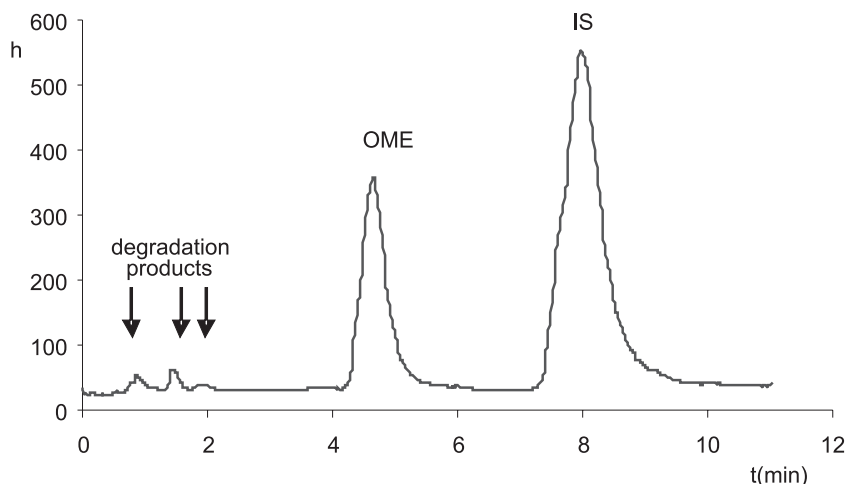

Figure 1. HPLC chromatograms for an authentic sample of omeprazole (OME), its three degradation products and the internal standard (flutamide, IS), maintained at 393 K in dry air for 24 h, where h is the height and t is the retention time of a peak
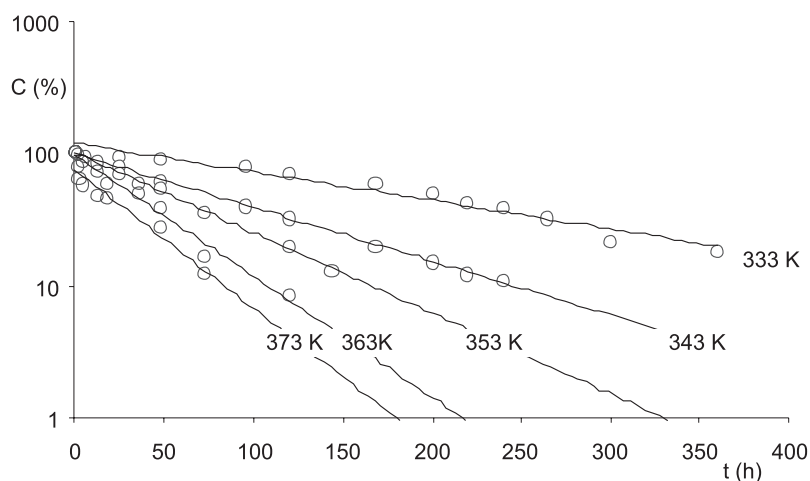

Figure 2. Semilogarithmic plots for degradation of OME in solid state in dry air at different temperatures
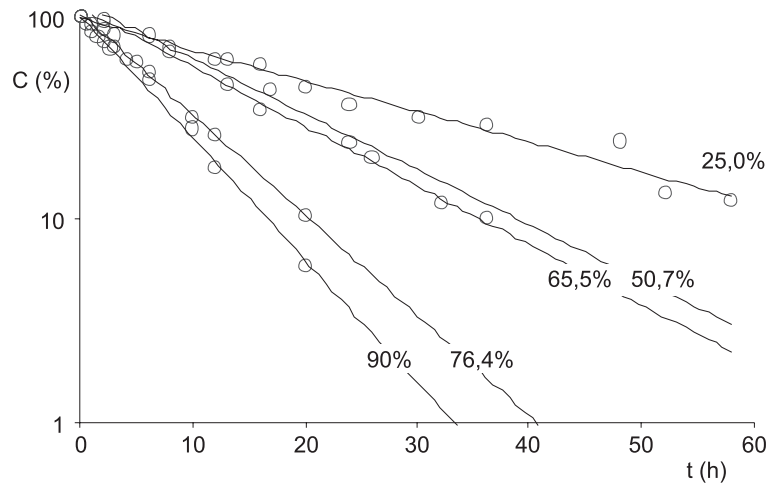
Figure 3. Semilogarithmic plots for degradation of OME in solid state at different humidity, at 353 K
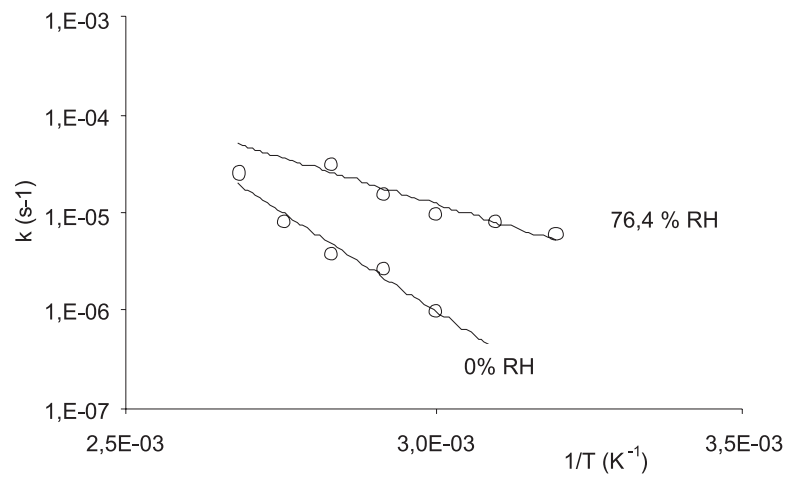
Figure 4. Semilogarithmic relationship for k = f(1/T) for the degradation of omeprazole (OME) solid sample in dry air and at 76.4% relative humidity, where k denotes the first order rate constant in s^{-1} and T the absolute temperature in kelvins (K)
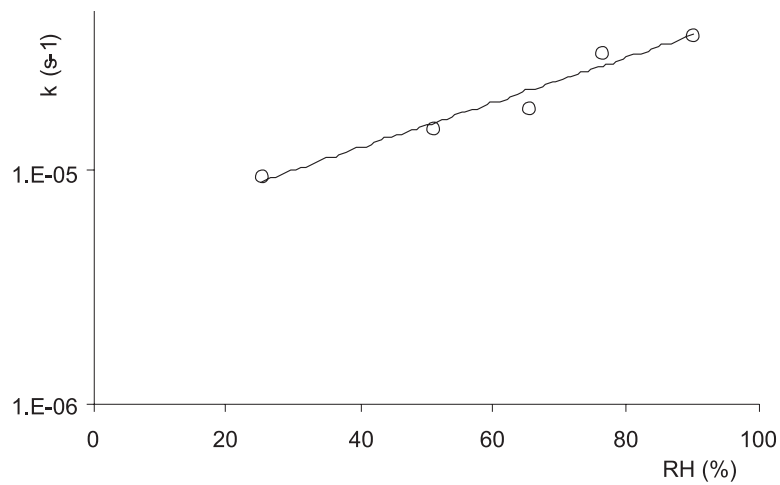
Figure 5. Semilogarithmic relationship ln k = f(RH%) for the degradation of OME in solid state, at 353 K

Table 1. Kinetic and thermodynamic parameters of the degradation of OME in the solid state.

Temp. [K]	$(k \pm \Delta k)$ [s^{-1}] $\times 10^5$	Statistic evaluation ln k = f(1/T)	Thermodynamic and kinetic parameters
RH = 0%			
333	0.10 ± 0.03	$a = -10251 \pm 1582$	$E_a = 85.2 \pm 13.2$ kJ/mol
343	0.26 ± 0.01	$s_a = 719.1$	$\Delta H = 82.8 \pm 13.2$ kJ/mol
353	0.39 ± 0.01	$b = 16.8 \pm 4.8$	$\Delta S = -104.8 \pm 39.5$ J/Kmol
363	0.79 ± 0.06	$s_b = 2.01$	$k_{293K} = 1.26 \times 10^{-8}$ s^{-1}
373	2.52 ± 0.69	$r = -0.9903$	$t_{0.1} = 97$ day
393	29.9 ± 5.10	$s_y = 0.266$	$t_{0.5} = 637$ day
RH = 76.4%			
313	0.59 ± 0.02	$a = -4407 \pm 1595$	$E_A = 36.64 \pm 13.3$ kJ/mol
323	0.82 ± 0.03	$s_a = 724.7$	$\Delta H = 34.20 \pm 13.3$ kJ/mol
333	0.98 ± 0.07	$b = 1.92 \pm 4.8$	$\Delta S = -228.8 \pm 42.9$ J/Kmol
343	1.61 ± 0.29	$s_b = 2.18$	$k_{293K} = 2.00 \times 10^{-6}$ s^{-1}
353	31.7 ± 1.00	$r = -0.9607$	$t_{0.1} = 15$ h
		$s_y = 0.207$	$t_{0.5} = 29$ h
RH [%]		**Statistic evaluation ln k = f(RH) 353 K**	
25.0	0.95 ± 0.13	$a = (2.20 \pm 0.55) \times 10^{-2}$	
50.9	1.51 ± 0.52	$s_a = 0.0025$	$k_{0\% RH} = 5.17 \times 10^{-6}$ s^{-1}
65.5	1.87 ± 0.34	$b = -12.17 \pm 0.39$	$t_{0.1} = 6$ h
76.4	3.17 ± 0.10	$s_b = 0.165$	$t_{0.5} = 37$ h
90.0	3.79 ± 0.34	$r = 0.9910$	

OME) were placed in vials of 5 mL capacity made of orange glass. The vials were placed in a sand bath or in desiccators with oversaturated solutions of inorganic salts (NaI, NaBr, NaCl, NaNO$_3$, ZnSO$_4$) (12) on the bottom. At appropriate time intervals the samples were taken out, cooled, weighted and dissolved in 10.0 mL of mobile phase. Then, 1.0 mL of the solution was taken and added to 1.0 mL of internal standard, mixed and subjected to HPLC analysis.

RESULTS AND DISCUSSION

Validation of the HPLC method

HPLC method is recommended by the European Pharmacopoeia VI and FP VIII for omeprazole determination. In this study the mobile phase was different than that recommended by the above pharmacopoeias.

This method is selective for OME, products of its decomposition and internal standard (Fig. 1). The chromatogram was recorded for the time 0–15 min. Five clearly separated and symmetric peaks were obtained. The OME peak corresponds to omeprazole of the retention time 4.5 min, IS peak corresponds to the internal standard – flutamide, of the retention time of 9.2 min, A, B, C peaks correspond to the products of OME decomposition of the retention times of 1.0 min, 1.5 min and 2.5 min.

In the concentration range studied (0.5×10^{-2} –14.0×10^{-2} mg/mL) the method is linear, r = 0.9998, the value of *b* (intercept) does not significantly differ from zero and is irrelevant, parameters of the calibration curve were calculated assuming the relation y = ax (n = 12, a = 2.95 ± 0.09). The calibration curve determined was described by equation y = (2.95+/–0.09)x.

Precision of the method was studied in three subsequent days. The recovery varied from 99.8 to 100.3%, whereas RSD changed from 0.52 to 1.26%. The OME concentration at which the peak of the substances analyzed was ten times higher than the level of noise was assumed as the limit of detection. In the conditions studied, the LOD parameter was 0.012 mg/mL, whereas the limit of quantification LOQ was 0.036 mg/mL.

Kinetic and thermodynamic parameters

Kinetic studies were carried out at elevated temperature (313–393 K), without the access of humidity and in the presence of humidity (25–90% RH). The OME samples (1 mg) changed the color from white of the samples not subjected to degradation to light-brown of the samples degraded at 393K for 24 h. After dissolution of the substance in a mixture of methanol : 100 mM ammonium acetate at the volume ratio of 60:40, the clear colorless or yellow solutions were obtained. Changes in OME concentration were recorded by HPLC with UV detection at 302 nm; the internal standard (flutamide) solution concentration was a 0.5 mg/mL. The chromatogram showed the OME signal of t_R = 4.5 min, the flutamide signal of t_R = 9.2 min and the signals of the decomposition products. The first product was observed for the samples stored at 393 K for 20 h, its retention time was t_R = 2.5 min. In humid atmosphere of 76% RH (353 K) this product was noted to appear already after 12 h of storage. The other two products were characterized by the retention times of t_R = 1.5 min and 1.0 min. The intensity of the peaks assigned to these products increased proportionally to the time of storage. Fig. 2 and 3 show a semilogarithmic relations describing the time changes in OME concentration in dry conditions (333–393 K) (Fig. 2) and at 25.0–90.0% RH at 353K (Fig. 3). The decomposition of OME in solid state can be described by the first order reaction (13). The rate constants of OME decomposition given in Table 1 prove high sensitivity of OME to the conditions applied. The relation between the rate constant of OME decomposition and temperature is given by the Arrhenius equation $\ln k = \ln A - E_A/RT$, where k = the rate constant of OME decomposition (s^{-1}), A is the frequency coefficient, E_a = the activation energy [J/mol], R = the gas constant (8.3144 J/K×mol), T = temperature [K]. The linear Arrhenius dependence $\ln k = f(1/T)$ (Fig. 4) was obtained for OME stored at 333–393 K in dry air and at 313–353 K at the relative air humidity of 76.4%. By approximation with the help of the least square method the frequency coefficient was calculated, which permitted estimation of the activation energy (E_a), enthalpy (DH) and entropy (DS) (Table 1) both under dry air conditions and at 76.4% RH. The presence of humidity had no effect on the mechanism of OME degradation in solid state. Analysis of the thermodynamic parameters of OME decomposition confirmed a significant effect of humidity on OME stability in solid state, so this substance must be stored in tightly closed boxes.

The effect of humidity on OME stability can be described by the equation: $\ln k = (2.20 \pm 0.55)´10^{-2}$ (RH%) – 12.17 ± 0.39. The slope a of the plot of ln k = f (RH) (Fig. 5) illustrates the effect of humidity on solid state OME stability, e^b (e = base of natural logarithms and b = coefficient) describes the OME stability at 0% RH (Table 1). The value of k = 3.89 ± 0.14) × 10^{-6} s^{-1} obtained experimentally at 353 K under dry conditions and k = 5.17 × 10^{-6} s^{-1} calculated from the relation ln k = a × RH + b at 353 K are not statistically significantly different.

CONCLUSION

According to the above discussed results of solid state OME stability investigation, this substance shows high sensitivity to temperature and relative air humidity.

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