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KINETICS OF DEGRADATION OF IMIDAPRIL HYDROCHLORIDE IN FINISHED DOSAGE FORMULATIONS

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Abstract: This study investigates the impact of relative air humidity and temperature on the stability of imidapril hydrochloride (IMD) tablets. For this purpose the forced degradation test was used and the following environmental conditions were employed: RH = 76.4% and the temperature range of 313 – 333 K. For the determination of IMD content in the analyzed samples a reversed-phase high performance liquid chromatography (RP-HPLC) technique was used. Three series of tablets were prepared: whole-blistered tablets, whole-bare tablets and halved-bare tablets, in order to analyze the influence of different in-home storage habits on IMD tablets' quality. In the course of the study, the degradation of IMD was observed in each series of tablets. The kinetic mechanisms and the thermodynamic parameters of these reactions were established. It was evidenced that halved IMD tablets stored without immediate packaging retain their quality only for 12 days while tablets stored according to label recommendations are stable for 513 days.

Keywords: imidapril hydrochloride (IMD), degradation study, tablets, HPLC method

Chemical stability, defined as an ability to maintain the identity, strength and purity under variety of environmental conditions throughout shelf life, is the most important aspect of quality assurance in pharmaceutical industry (1-3). The extent to which a drug product remains within its specification criteria depends on its reactivity, which is demonstrated by its liability to degradation by various chemical reactions, such as: hydrolysis, solvolysis, dehydration, isomerization, racemization, elimination, oxidation, reduction, etc. (4). Drug's chemical degradation is an unfavorable effect leading to deterioration of its quality, mainly by loss of active ingredient, formation of degradation impurities or loss of excipients activity. Further clinical consequences of drug's instability involve: alternations of its bioavailability, potency or toxicity (5). Therefore, the comprehensive stability testing, including the evaluation of drug-container compatibility, and the determination of optimal storage conditions, have became a legal requirement for approval of any formulated drug intended for human use (1-3).

Unfortunately, low patient compliance with label-storage recommendations is prevalent, which was confirmed in various research articles (6-9). A common, in-home practice involves use of weekly- or monthly-medication organizers, and storage of whole or halved tablets in damaged immediate packaging or even without immediate packaging under the high-moisture conditions. This supposedly increases their rate of degradation, however, there are no studies evaluating the extent to which such procedures impair their quality. For this reasons the authors have decided to investigate the influence of improper storage on the rate of degradation of one antihypertensive pharmaceutical, imidapril hydrochloride.

The selected drug belongs to angiotensin converting enzyme inhibitors (ACE-I) which are one of the major pharmaceutical classes used in renovascular and essential hypertension and congestive heart failure (10, 11). It is administered orally in the form of off-white, oblong, biconvex tablets, which can be divided into equal parts (12). The degradation studies, performed for pure IMD in solid state, evi-

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denced that in the course of its decomposition two degradation products are formed (diketopiperazine derivative and imidaprilat) (15). It was also established that the degradation rate of pure IMD accelerates under the conditions of increased temperature and relative humidity (13, 14). The above findings indicate that the stability of IMD tablets could also be adversely impacted by improper storage, which explains the necessity of conduction of the present study. Thus, our main purpose was to determine the effect of temperature and elevated humidity on the degradation rate of formulated IMD in the presence of typical excipients. For this reason, the kinetic equations describing the IMD concentration changes in tablets as a function of time were established, which enabled the evaluation of the degradation rate constant of IMD in tablets and the assessment of thermodynamic parameters of its decomposition. The present study was performed using forced degradation test. The adopted analytical approach involved the storage of whole and halved tablets with and without immediate packaging (blister) under the conditions of elevated relative humidity (76.4% RH) and within the temperature range of 313–333 K. The concentration changes of IMD were assayed by reversed-phase high performance liquid chromatography (RP-HPLC) which was selected due to its established applicability to solid state IMD studies (13, 14).

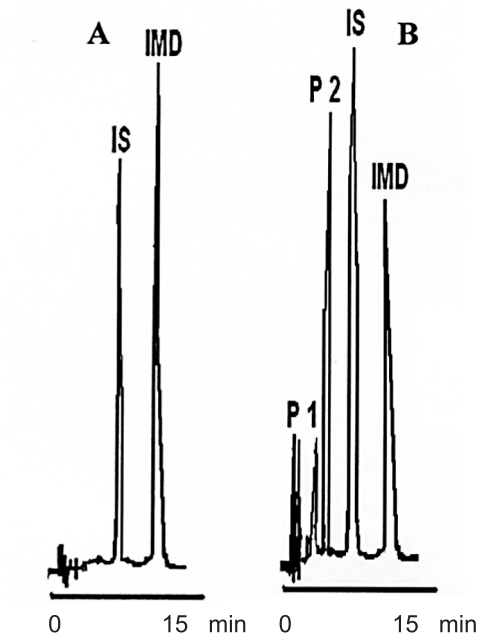

Figure 1. HPLC chromatogram of an extract of (**A**) undegraded IMD tablets (**B**) IMD tablets stored without final packs at 323 K in a moist atmosphere of 76.4% RH for 30 days. **IMD**: t_R ~ 13 min; **P1** and **P2**: products of degradation of IMD – t_R ~ 3 min and 5 min; **IS**: internal standard t_R ~ 8 min

EXPERIMENTAL

Material and reagents

Pure imidapril hydrochloride in the form of substance was kindly provided by Jeleniogorskie Zakłady Farmaceutyczne (Poland). Sodium chloride, potassium dihydrogen phosphate, benzocaine were purchased from Sigma-Aldrich Co. (Germany). Methanol (HPLC grade) was purchased from Merck (Germany).

The studied finished dosage form – imidapril hydrochloride tablets, had the following qualitative composition: imidapril hydrochloride 10 mg; calcium hydrogen phosphate, anhydrous; maize starch, pregelatinized; lactose monohydrate; croscarmellose sodium; glycerol distearate.

Chromatographic conditions

In this study, a Shimadzu liquid chromatograph equipped with UV-VIS SPD-6AV detector, LC-6A pump and CR-6A chromatopac integrator was used. A Merck analytical column (LiChrospher RP-18, 5 μm particle size, 250 mm × 4 mm i.d.) was applied as a stationary phase. The employed mobile phase consisted of: methanol - phosphate buffer (30 : 70, v/v), and its flow rate was 1.2 mL/min. The apparatus was not equipped with thermostating column nor with autosampler, therefore, in order to neutralize the error inherent during sample injection and eliminate random errors, the technique employing an internal standard (a methanolic solution of benzocaine 0.2 mg/mL) had to be used. The UV detector was set at 216 nm (Fig. 1).

The aqueous phosphate buffer preparation

An exact amount of 0.0680 g of KH_2PO_4 was weighted and dissolved in 450 mL of water. The pH of the obtained solution was adjusted to 2.0 with 80% *orto*-phosphoric acid and the volume was completed with water to 500 mL.

Stock solution and calibration graph

Stock solution was prepared by dissolving 80.0 mg of IMD in 100.0 mL of methanol. Standard solutions were obtained by diluting the stock solution with methanol to the following concentrations: 0.04, 0.08, 0.16, 0.24, 0.32, 0.40 and 0.48 mg/mL. Portions of 1.0 mL of each standard solution were mixed with 0.5 mL of internal standard and injected

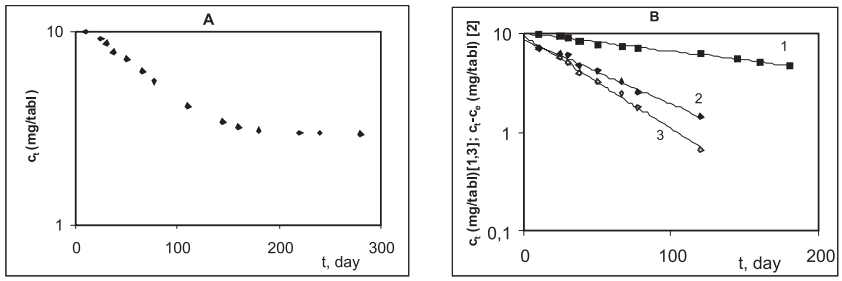

Figure 2. Semilogarithmic plot $c_t = f(t)$ for the decomposition of IMD tablets stored without blister (**A**), and linear plots $\ln c_t = f(t)$ for the decomposition of whole IMD tablets stored in blister (**B**, line 1), $\ln (c_t - c_\infty) = f(t)$ for the decomposition of whole IMD tablets stored without blister (**B**, line 2) and $\ln c_t = f(t)$ for the decomposition of halved IMD tablets stored without blister (**B**, line 3) under the conditions of T = 318 K, RH = 76.4%

in triplicate onto the chromatographic column. The injection volume was 20 µL. The obtained chromatograms were analyzed, and the measured peak areas for each sample were plotted against corresponding concentrations of IMD to construct the calibration graph: $P_{IMD}/P_{IS} = f(c)$, where P_{IMD} is IMD peak area and P_{IS} is internal standard peak area.

Kinetic studies

The following series of IMD tablets (10 mg) were prepared: whole tablets in PVC/PVDC/Al blisters, whole tables without immediate packaging and halved tablets without immediate packaging. The obtained samples were placed into desiccator containing saturated aqueous solution of sodium chloride (RH = 76.4%), and heated to the following temperatures: 313, 318, 323 and 333 K over different time intervals. After heating, one tablet or two halves were withdrawn from the desiccator and cooled to room temperature. Halved tablets were weighted. The samples were subsequently transferred into 50 mL-volumetric flask, dissolved in 3.0 mL of water and diluted with 22.0 mL of methanol. The obtained samples were shaken for 15 min and filtered. The aliquots of 1.0 mL of the filtered solutions (solution P) were mixed with 0.5 mL of methanolic solution of benzocaine, 0.20 mg/mL (solution P_i) and injected onto the HPLC column. The injection volume was 20 µL. The evolving signals were recorded over a time range of 2-15 min. In parallel, a reference methanolic solution of IMD at concentration of 0.4 mg/mL was prepared (solution P_{ST}). One milliliter of the solution P_{ST} was mixed with 0.5 mL of internal standard (solution P_{STi}). The aliquots of 20 µL of the solution P_{STi} were injected onto the HPLC column.

In order to calculate the content of IMD in each sample [mg] the following formulae were adopted: $X_{(tabl/mg)} = (P_i \times c \times V)/ P_{STi}$; or $X_{(tabl/mg)} = (P_i \times c \times V \times M)/(P_{STi} \times m)$; for $P_i = P/P_{IS}$ and $P_{STi} = P_{IMD}/P_{IS}$, where: P = peak area of IMD in studied sample, P_i = relative peak area of IMD in studied sample, P_{IMD} = area of pure IMD peak, P_{STi} = relative area of IMD standard solution peak, P_{IS} = area of IS peak, c = the concentration of IMD in the standard solution (0.040%) and V = the dilution factor for the sample, M = average tablet mass, and m = weighted amount.

RESULTS AND DISCUSSION

Validation of RP-HPLC method

RP-HPLC method is an established analytical approach to determination of IMD in solid state (14). The employed analytical system enabled a complete separation of IMD in the presence of its decomposition products and IS, confirming method's selectivity (Fig. 1). A straight-line relationship (r = 0.999) between the measured signal P_{IMD}/P_{IS} and IMD concentration in model mixtures in a range of 0.040 to 0.480 mg/mL was observed, and the regression equation was found to be the following: $P_{IMD}/P_{IS} = (34.02 \pm 1.1)x$.

Intercept *b* was statistically insignificant, S_y was 0.02 and S_a was 0.49.

The precision of the method was evaluated by the analysis of eight individual samples. The following parameters were calculated: mean value $P/P_{IS} = 1.431$; SD = 0.0091; CV = 0.64%.

Degradation rate constants of IMD in tablets

In the temperature range of 313–343 K, under the conditions of increased humidity (76.4% RH), two different kinetic mechanisms of IMD tablets' degradation were observed. For the series of whole tablets stored without immediate packaging the reversible first-order reaction was determined. In

Table 1. Qualitative and quantitative composition of model mixtures for accuracy assessment.

	Model mixture I	Model mixture II	Model mixture III
Portion of tablet mass (g) [A]	1.0000	1.0000	1.0000
Portion of pure IMD [B]	0.0250	0.0500	0.0750
Total A + B	1.0250	1.0500	1.0750

Table 2. Kinetic and thermodynamic parameters of the decomposition of IMD tablets stored with and without immediate packaging.

T [K]	10^7 k ± k, s^{-1}	r	n	Statistical evaluation $\ln k_i = f(1/T)$	Parameters	
					Thermodynamic	Kinetic*
IMD in the form of whole tablets stored without blisters						
313 318 323 333	1.541 ± 0.172 1.738 ± 0.129 2.300 ± 0.204 3.101 ± 0.395	0.993 0.997 0.995 0.992	8 7 7 7	a ± Δa = -4110 ± 1191 S_a = 374 b ± Δb = -2.61 ± 16.6 S_b = 3.84 r = -0.996	E_a [kJ/mol] = 341.8 ± 99.0 $\Delta H^{\neq}$ [kJ/ mol] = 316.9 ± 123.8 $\Delta S^{\neq}$ [J/(K· mol)] = 266.7 ± 211.9	k = (5.889 ± 0.45)$10^{-8}s^{-1}$ $t_{0.1}$ = 21 days
IMD in the form of halved tablets stored without blister						
313 318 323 333	2.177 ± 0.164 2.459 ± 0.220 2.750 ± 0.235 3.991 ± 0.397	0.997 0.996 0.995 0.996	8 8 9 7	a ± Δa = -3457 ± 774 S_a = 243 b ± Δb = -4.36 ± 2.40 S_b = 0.756 r = -0.995	E_a [kJ/mol] = 287.5 ± 64.4 $\Delta H^{\neq}$ [kJ/mol]= 262.7 ± 89.2 $\Delta S^{\neq}$ [J/(K·mol)] = -281.2 ± 224.9	k = (9.542 ± 0.63)$10^{-8}s^{-1}$ $t_{0.1}$ = 12 days
IMD in the form of whole tablets stored in blisters						
313 318 323 333	0.691 ± 0.071 1.967 ± 0.184 5.770 ± 0.306 41.30 ± 3.95	0.995 0.997 0.999 0.994	8 7 7 8	a ± Δa = -11174 ± 282 S_a = 88.7 b ± Δb = 18.3 ± 0.88 S_b = 0.27 r = -0.999	E_a [kJ/mol] = 929.1 ± 23.4 $\Delta H^{\neq}$ [kJ/mol]= 904.3 ± 48.3 $\Delta S^{\neq}$ [J/(K· mol)] = -92.9 ± 237.7	k = (2.378 ± 0.19)$10^{-9}s^{-1}$ $t_{0.1}$ = 513 days

* Kinetic parameters at the temperature 293°K. S_a = standard deviation of slope; S_b = standard deviation of value b; r = coefficient of linear correlation; $t_{0.1}$ = shelf life.

this case, the relationship $c_t = f(t)$ was found to be non-linear, however, it was observed that as time approaches infinity ($t \rightarrow \infty$), the detected concentration decreases to the constant value c_∞ ($c_t \rightarrow \infty$) (Fig. 2A), and therefore, the subtraction technique could be employed to obtain the linear plot $\ln (c_t - c_\infty) = f(t)$ (Fig. 2B line 2). The reaction was described by the following kinetic equation: $\ln (c_t - c_\infty = \ln (c_0 - c_\infty) - k\,t$, where: c_t, c_∞ and c_0 represent the concentration of IMD in tablets in time t, t_∞ and t_0, respectively, and k is first-order reaction rate constant.

On the contrary, for the series of tablets stored in blisters and for the series of halved tablets stored without blister, first-order reaction model was evi-

Table 3. Contents of IMD in tablets stored under 293K/60%RH.

Time [days]	Contents of IMD in tablets [g]	Statistical assessment
0	0.01040 ± 0.00012	The mean IMD content in tablet is 0.01040 g. The IMD content determined after 360-day period of storage under 293 K/60RH in immediate packaging was 0.01042 g, which is not statistically different. This indicates that within this period of time, under applied conditions, IMD degradation does not occur.
30	0.01041 ± 0.00016	
60	0.01049 ± 0.00018	
90	0.01068 ± 0.00014	
120	0,01046 ± 0.00019	
150	0,01040 ± 0.00015	
180	0,01044 ± 0.00017	
220	0,01039 ± 0.00016	
280	0,01046± 0.00014	
360	0,01042 ± 0.00019	

denced, and it was described by the following equation: $\ln c_t = \ln c_0 - k\,t$, where: c_t and c_0 represent the concentration of IMD in tablets in time t and t_0, respectively, and k is first-order reaction rate constant. In this case the following relationship was established: $t \rightarrow 0$, $c_t \rightarrow 0$, and therefore the plots $\ln c_t = f(t)$ were linear (Fig. 2B, line 1 and 3).

Basing on the above kinetic equations, the appropriate regression equations were computed using least square method, and the following statistical parameters for each equation were assessed: $a \pm \Delta a$, $b \pm \Delta b$, standard deviation of slope S_a, standard deviation of intercept S_b, and the coefficient of linear correlation r. The values Δa and Δb were calculated for f = n -2 degrees of freedom, with a = 0.05. The kinetic parameters of the above reactions, i.e.: reaction rate constants k, half-life $t_{0.5}$ and shelf-life $t_{0.1}$, were established for the following environmental conditions T = 293 K, RH = 76.4%. Tablet's shelf life was defined as the time period necessary for a drug to decompose to 90% of its initial concentration at a specific temperature. The obtained results are demonstrated in Table 1.

The calculation of thermodynamic parameters for IMD tablets decay

The Arrhenius equation was used to describe the relationship between the reaction rate constant and temperature: $\ln k_i = \ln A - E_a/RT$, where: k_i = reaction rate constant in temperature i (s^{-1}), A = frequency coefficient, E_a = energy of activation [J mol^{-1}], R = universal gas constant (8.3144 J K^{-1} mol^{-1}), T = temperature (K).

The straight line plot $\ln k_i = f(1/T)$ was obtained for each reaction. Basing on the transition state theory, also the following thermodynamic parameters of the observed reactions were calculated: energy of activation (E_a), enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) for temperature 293 K. The obtained results are presented in Table 1.

DISCUSSION AND CONCLUSION

The present degradation study of formulated IMD was performed by means of validated RP-HPLC method, which was evidenced to be selective (Fig. 1), linear (r = 0.999) and precise (CV = 0.64%). The adopted analytical protocol involved the evaluation of IMD degradation rate changes, depending on different storage methods that are representative for popular, in-home storage habits. For this reason, three series of tablets were tested: whole tablets in blister, whole tablets without blister and halved tablets without blister. They were stored in high-moisture (RH = 76.4%) and high-temperature (313–343 K) environment. Under the applied analytical conditions the physical appearance of the investigated tablets did not change and also no interactions between pharmaceutical formulation and packaging components occurred. The regular loss of IMD content with time was evidenced in each series of tablets and the kinetic mechanism of these reactions was established. It was found that degradation of blister-packaged tablets and halved-bare tablets followed the first-order kinetics while the whole-bare tablets decomposed according to reversible-first order kinetics. The kinetic parameters of the above reactions, such as: degradation rate constant k, half-life $t_{0.5}$ and shelf-life $t_{0.1}$ were calculated for the following conditions T = 293°K, RH = 76.4%. The analysis of the obtained data show that the degradation rate constant of IMD in halved tablets

stored without immediate packaging (e.g., in monthly-medication organizers or glasses) increases substantially, $k = (9.542 \pm 0.63)\ 10^{-8}\ s^{-1}$, when compared to degradation rate constant of blistered tablets $k = (2.378 \pm 0.19)\ 10^{-9}\ s^{-1}$. The estimated shelf-life of the halved tablets was 12 days while the shelf-life of tablets stored according to label recommendations was $t_{0.1} = 513$ days. The same parameters calculated for the whole tablets stored without blister were the following: $k = (5.889 \pm 0.45)\ 10^{-8} s^{-1}$ and $t_{0.1} = 21$ days. To compare, the kinetic parameters (k, s^{-1}) of degradation of pure IMD in the form of powder at temperature 293°K and 76.4% RH were the following: $(1.36 \pm 0.16)\ 10^{-9}\ s^{-1}$ (15).

It was finally evidenced that pure IMD in the form of powder was more stable than IMD in bare tablets or halved tablets, however, it was less stable than IMD in blistered tablets. It can be therefore concluded that the process of formulation stabilizes the investigated compound, supposedly by the presence of excipients. Also the change of kinetic mechanism of degradation was observed. Pure IMD decomposes according to autocatalytic first-order reaction model (14) while its tablets' degradation follows first-order kinetics. Similar degradation kinetics was evidenced for binary mixture of IMD and magnesium stearate (1 : 1, w/w) (13). The above findings emphasize the importance of proper drug storage. It was shown that only commercial immediate packaging ensures the satisfactory protection from moisture, which seems to be the main reason for IMD degradation. The halved IMD tablets stored without immediate packaging are considered to be expired after 12 days since the loss of their active ingredient reaches 10%, which is unfortunately impossible to detect visually because of absence of any physical changes in tablets' appearance.

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