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: alterations 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 become 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-
enced that in the course of its decomposition two
degradation products are formed (diketopiperazine
derivative and imidaprilat) (15). It was also estab-
lished that the degradation rate of pure IMD acceler-
ates 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 estab-
lished, which enabled the evaluation of the degrada-
tion rate constant of IMD in tablets and the assess-
ment of thermodynamic parameters of its decompo-
sition. 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 (blis-
ter) under the conditions of elevated relative humid-
ity (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).

EXPERIMENTAL

Material and reagents

Pure imidapril hydrochloride in the form of
substance was kindly provided by Jeleniogorskie
Zakłady Farmaceutyczne (Poland). Sodium chlo-
ride, 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; calci-
um hydrogen phosphate, anhydrous; maize starch,
pregelatinized; lactose monohydrate; croscarmel-
lose sodium; glycerol distearte.

Chromatographic conditions

In this study, a Shimadzu liquid chromato-
graph 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 col-
umn nor with autosampler, therefore, in order to
neutralize the error inherent during sample injection
and eliminate random errors, the technique employ-
ing 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 KH₂PO₄ 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 com-
pleted 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 solu-
tions 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
Kinetics studies

The following series of IMD tablets (10 mg) were prepared: whole tablets in PVC/PVDC/Al blisters, whole tablets 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 benzoica, 0.20 mg/mL (solution P) and injected onto the HPLC column.

In order to calculate the content of IMD in each sample [mg] the following formulae were adopted:

\[ X_{\text{tabl/mg}} = \frac{(P_i \times c \times V)}{P_{\text{IS}} \times M}; \]

or

\[ X_{\text{tabl/mg}} = \frac{(P_i \times c \times V \times M)}{(P_{\text{IS}} \times m)}; \]

where: \( P \) = peak area of IMD in studied sample, \( P_i \) = relative peak area of IMD in studied sample, \( P_{\text{MID}} \) = area of pure IMD peak, \( P_{\text{STI}} \) = relative area of IMD standard solution peak, \( P_{\text{IS}} \) = 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 \( P_{\text{MID}}/P_{\text{IS}} \) 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: \( P_{\text{MID}}/P_{\text{IS}} = (34.02 \pm 1.1)x \).

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 \( P/P_{\text{IS}} = 1.431 \); 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.
this case, the relationship $c_t = f(t)$ was found to be non-linear, however, it was observed that as time approaches infinity ($t \to \infty$), the detected concentration decreases to the constant value $c_\infty$ ($c_t \to \infty$) (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: $\ln (c_t \cdot c_\infty) = \ln (c_0 \cdot c_\infty) - k_t$, where $c_\infty$, $c_\infty$, and $c_0$ represent the concentration of IMD in tablets in time $t$, $t_\infty$, 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 1. Qualitative and quantitative composition of model mixtures for accuracy assessment.

<table>
<thead>
<tr>
<th>Model mixture</th>
<th>Portion of tablet mass (g)</th>
<th>Portion of pure IMD [B]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.0000</td>
<td>0.0250</td>
</tr>
<tr>
<td>II</td>
<td>1.0000</td>
<td>0.0500</td>
</tr>
<tr>
<td>III</td>
<td>1.0000</td>
<td>0.0750</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>T [K]</th>
<th>$10^4 k \pm k$, s$^{-1}$</th>
<th>$r$</th>
<th>$n$</th>
<th>Statistical evaluation</th>
<th>Thermodynamic</th>
<th>Kinetic*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$E_a$ [kJ/mol]</td>
<td>$t_{0.1}$</td>
</tr>
<tr>
<td>IMD in the form of whole tablets stored without blisters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>1.541 ± 0.172</td>
<td>0.993</td>
<td>8</td>
<td>$a \pm \Delta a = -4110 \pm 1191$</td>
<td>341.8 ± 99.0</td>
<td>21 days</td>
</tr>
<tr>
<td>318</td>
<td>1.738 ± 0.129</td>
<td>0.997</td>
<td>7</td>
<td>$b \pm \Delta b = -2.61 \pm 16.6$</td>
<td>316.9 ± 123.8</td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>2.300 ± 0.204</td>
<td>0.995</td>
<td>7</td>
<td>$S_a = 3.74$</td>
<td>266.7 ± 211.9</td>
<td></td>
</tr>
<tr>
<td>333</td>
<td>3.101 ± 0.395</td>
<td>0.992</td>
<td>7</td>
<td>$S_b = 3.84$</td>
<td>266.7 ± 211.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$r = -0.996$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMD in the form of halved tablets stored without blister</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>2.177 ± 0.164</td>
<td>0.997</td>
<td>8</td>
<td>$a \pm \Delta a = -3457 \pm 774$</td>
<td>287.5 ± 64.4</td>
<td>12 days</td>
</tr>
<tr>
<td>318</td>
<td>2.459 ± 0.220</td>
<td>0.996</td>
<td>8</td>
<td>$b \pm \Delta b = -4.36 \pm 2.40$</td>
<td>262.7 ± 89.2</td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>2.750 ± 0.235</td>
<td>0.995</td>
<td>9</td>
<td>$S_a = 243$</td>
<td>262.7 ± 89.2</td>
<td></td>
</tr>
<tr>
<td>333</td>
<td>3.991 ± 0.397</td>
<td>0.996</td>
<td>7</td>
<td>$S_b = 0.756$</td>
<td>262.7 ± 89.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$r = -0.995$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMD in the form of whole tablets stored in blisters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>0.691 ± 0.071</td>
<td>0.995</td>
<td>8</td>
<td>$a \pm \Delta a = -11174 \pm 282$</td>
<td>929.1 ± 23.4</td>
<td>513 days</td>
</tr>
<tr>
<td>318</td>
<td>1.967 ± 0.184</td>
<td>0.997</td>
<td>7</td>
<td>$b \pm \Delta b = 18.3 \pm 0.88$</td>
<td>904.3 ± 48.3</td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>5.770 ± 0.306</td>
<td>0.999</td>
<td>7</td>
<td>$S_a = 88.7$</td>
<td>904.3 ± 48.3</td>
<td></td>
</tr>
<tr>
<td>333</td>
<td>4.30 ± 3.95</td>
<td>0.994</td>
<td>8</td>
<td>$S_b = 0.27$</td>
<td>904.3 ± 48.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$r = -0.999$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Kinetic parameters at the temperature 293 K. $S_a = \text{standard deviation of slope} ; S_b = \text{standard deviation of value b} ; r = \text{coefficient of linear correlation} ; t_{0.1} = \text{shelf life}.
Kinetics of degradation of imidapril hydrochloride in finished...

...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: 
\[ t \to t_0, c_t \to c_0, \] 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: \( \pm \Delta a, \pm \Delta b, \) standard deviation of slope \( S_a, \) standard deviation of intercept \( S_b, \) and the coefficient of linear correlation \( r. \)

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 \( T = 293 \text{ K, RH} = 76.4\%. \)

The mean IMD content in tablet is 0.01040 g. The IMD content determined after 360-day period of storage under 293 K/60%RH 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.

### 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 \( (CV = 0.64\%). \)

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

<table>
<thead>
<tr>
<th>Time [days]</th>
<th>Contents of IMD in tablets [g]</th>
<th>Statistical assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.01040 ± 0.00012</td>
<td>The mean IMD content in tablet is 0.01040 g. The IMD content determined after 360-day period of storage under 293 K/60%RH 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.</td>
</tr>
<tr>
<td>30</td>
<td>0.01041 ± 0.00016</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.01049 ± 0.00018</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>0.01068 ± 0.00014</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.01046 ± 0.00019</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>0.01040 ± 0.00015</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>0.01044 ± 0.00017</td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>0.01039 ± 0.00016</td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>0.01046 ± 0.00014</td>
<td></td>
</tr>
<tr>
<td>360</td>
<td>0.01042 ± 0.00019</td>
<td></td>
</tr>
</tbody>
</table>

The mean IMD content in tablet is 0.01040 g. The IMD content determined after 360-day period of storage under 293 K/60%RH 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.

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The adoption of the above kinetic equations were computed using least square method, and the following statistical parameters for each equation were assessed: \( \pm \Delta a, \pm \Delta b, \) standard deviation of slope \( S_a, \) standard deviation of intercept \( S_b, \) and the coefficient of linear correlation \( r. \)

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 \( T = 293 \text{ K, RH} = 76.4\%. \)

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

**Acknowledgments**

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