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QUANTITATIVE ANALYSIS OF LOPERAMIDE HYDROCHLORIDE IN THE PRESENCE ITS ACID DEGRADATION PRODUCTS

The aim of this work was to develop a new RP-HPLC method for the determination of loperamide hydrochloride in the presence of its acid degradation products. Separation of loperamide from degradation products was performed using ZORBAX Eclipse XDB C-18, column with a mobile phase consisting of 0.1% sodium-octansulphonate, 0.05% triethylamine, 0.1% ammonium hydroxide in water:acetonitrile (45:55 v/v). The mobile phase was adjusted to pH 3.2 with phosphoric acid. The method showed high sensitivity with good linearity over the concentration range of 10 to 100 µg cm⁻³. The method was successfully applied to the analysis of a pharmaceutical formulation (Loperamide, Zdravlje-Actavis, Serbia) containing loperamide hydrochloride with excellent recovery. The loperamide hydrochloride degradation during acid hydrolysis and kinetics investigation was carried out in hydrochloric acid solutions of 0.1, 1.0 and 1.5 mol dm⁻³, at different temperatures (25 and 40 °C), by monitoring the parent compound itself. The first order reaction of loperamide degradation in acid solution was determined. The activation energy was estimated from the Arrhenius plot and it was found to be 38.81 kJ mol⁻¹ at 40 °C. The developed procedure was successfully applied for the rapid determination of loperamide hydrochloride in pharmaceutical formulation (Loperamide, Zdravlje-Actavis, Serbia) and in the presence of its acid degradation products.

Loperamide hydrochloride (4-(*p*-chlorophenyl)-4-hydroxy-*N,N*-dimethyl-diphenyl-1-piperidine butyramide hydrochloride), is an opiate agonist widely used as an effective drug for the control and symptomatic relief of acute non-specific diarrhea [1]. More recently, it has also been reported that loperamide could have some interest as an antihyperalgesic agent reducing pain without any central nervous system side effects [2]. Loperamide is orally administered and is moderately absorbed (about 40%) from the gastrointestinal tract to undergo first-pass metabolism in the liver and excretion in the feces *via* the bile as inactive conjugates (sulfo- and glucurono-combination).

The analysis of loperamide in bulk drugs and pharmaceutical products is well described in the literature [3–12] and it is mentioned either in the European [13] and the United States Pharmacopoeias [14]. In literature, stability of loperamide hydrochloride has been described in different pH solutions with various buffer species [15]. The high performance liquid chromatography (HPLC) was an official method in any pharmacopoeia for the analysis of loperamide hydrochloride, but this procedure is not suitable for the determination of loperamide hydrochloride in the presence of its acid degradation product or impurity.

The present work aimed to develop and validate a new RP-HPLC feasible, sensitive and specific analytical procedure, suitable for application in a drug quality control or regulatory laboratory analysis of loperamide

hydrochloride, in the presence of its degradation products (Fig. 1).

The analysis of degradation products obtained during the acid hydrolysis in hydrochloric acid solutions with various concentrations, at different temperatures, was presented in this paper. The developed analytical method was validated as per International Conference on Harmonization guidelines [16] and Serbian requirements [17]. Statistical tests were performed on validation data [18]. The validation consists of testing the method selectivity towards components and the assessment of method precision and accuracy [17,20] at different concentration levels over the range investigated as well as the confirmation of the limit of quantization (*LOQ*) and the method linearity [17–19]. Adaptation of the proposed procedure for the analysis of the available dosage form, including expired ones, is also an important task in order to solve problems encountered in quality control. Moreover, kinetic studies and accelerated stability experiments to predict expiry dates of pharmaceutical products necessitate such methods.

EXPERIMENTAL

Samples

Standards substances of loperamide hydrochloride, 4-(4-chlorophenyl)piperidin-4-ol and 4-(4-hydroxy-4-phenylpiperidin-1-yl)-*N,N*-dimethyl-2,2-diphenylbutanamide were kindly supplied by Zdravlje-Actavis, Leskovac. Loperamide tablets were kindly supplied by Zdravlje-Actavis, Leskovac. Each tablet is claimed to contain 2 mg of loperamide hydrochloride.

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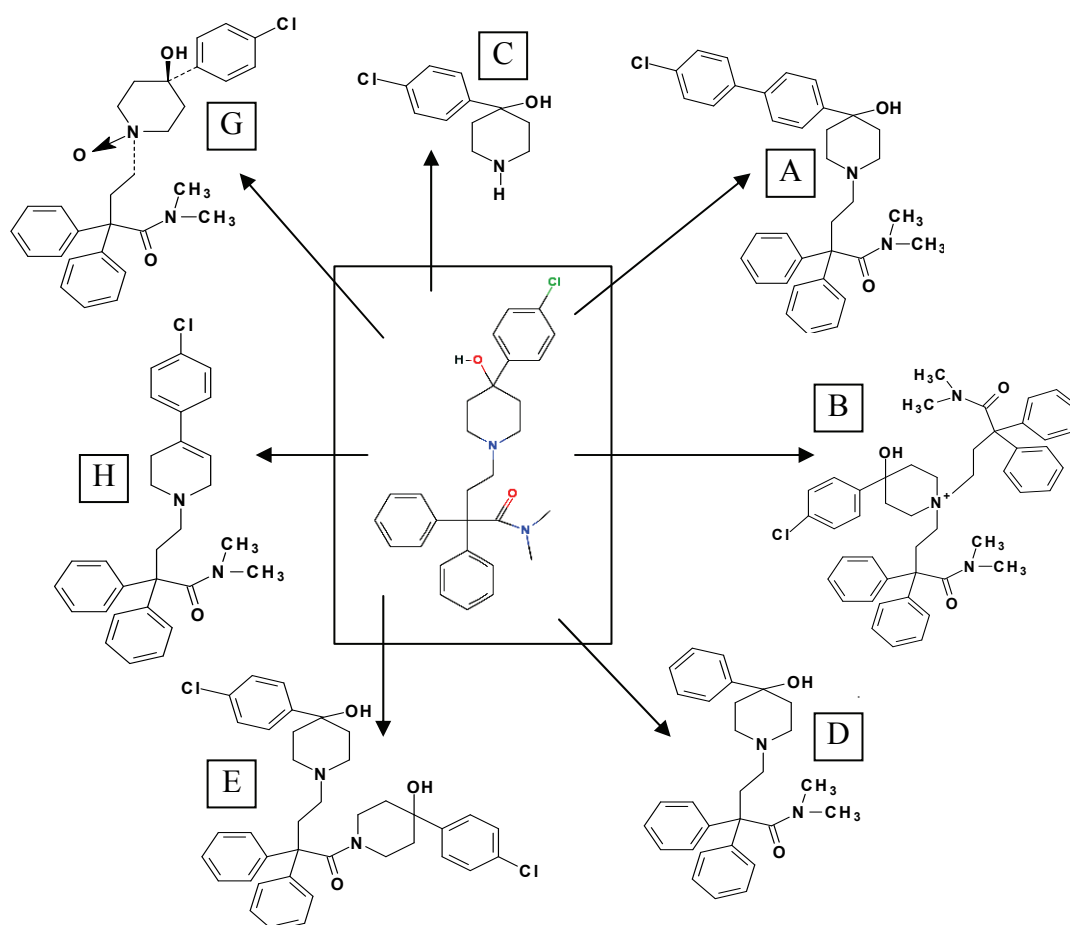


Fig. 1. The potential impurities and degradation products of loperamide hydrochloride.

Reagents

All chemicals used were of analytical grade and deionized water was HPLC grade. Hydrochloric acid, sodium octansulphonate, triethylamine, ammonium hydroxide and acetonitrile for HPLC were obtained from Merck.

Apparatus

The method development was performed with an Agilent 1100-Series HPLC system consisting of an Agilent 1100-Series with DAD detector and Agilent 1100-Series autosampler using a 20 μ L sample loop (Faculty of Technology, Leskovac). The system was controlled and data analyses were performed with the Agilent HPLC Data Analysis software. The assays (repeatability) were performed with another LC system consisting of an Agilent 1100-Series binary pump and Agilent 1100-Series DAD detector (Zdravlje-Actavis, Leskovac). The detector was set at 226 nm and the peak areas were integrated automatically by the computer using the Agilent HPLC Data Analysis software program. The separation was carried out at ambient temperature using a ZORBAX Eclipse XDB-C18 column, (4.6 mm $\times$ 250 mm, 5 μ m). All

the calculations concerning the quantitative analysis were performed with the external standardization by measuring the peak areas.

Chromatographic conditions

RP-HPLC analysis was performed by isocratic elution with a flow rate of 1.5 cm³ min⁻¹. A mobile phase consisting of 0.1% sodium-octansulphonate, 0.05% triethylamine, 0.1% ammonium hydroxide (buffer) in water:acetonitrile (45:55 v/v). All solvents were filtered through a 0.45 μ m millipore filter. Volumes of 20 μ L of the solutions and samples prepared were injected into the column. Quantification was effected by measuring at 226 nm as established from the 3-D chromatogram. Throughout the study, the suitability of the chromatographic system was monitored the efficiency column and peak asymmetry.

Procedures

For preparing different concentrations, aliquots of the stock solution were transferred into a series of 10 cm³ standard volumetric flasks and the volumes were made with the respective media. Ten different concen-

trations were prepared in the range of 10–100 $\mu\text{g cm}^{-3}$ of loperamide hydrochloride in mobile phase for a standard curve. The final concentrations of loperamide hydrochloride in the samples were calculated by comparing the sample and standard peak obtained with the average of three injections of standard solutions.

For studying the kinetic order of the reaction: in a 100 cm^3 volumetric flask, dissolve 4 mg of loperamide hydrochloride in 1 mol dm^{-3} HCl and complete to the mark with the same solvent. Transfer this solution into another clean dry conical flask and reflux in a thermostatically controlled water bath at 40 °C for 30 min. Take 1 cm^3 samples at 5 min intervals and neutralize with 1 cm^3 of 1 mol dm^{-3} NaOH. Inject the solutions in the liquid chromatograph using the chromatographic conditions described above. The concentration of loperamide hydrochloride is calculated from the regression equation. Plot the log % of loperamide hydrochloride remaining against time.

For studying the effect of HCl concentration on the reaction rate: in a 100 cm^3 volumetric flask, dissolve 4 mg of loperamide hydrochloride in 0.1, 1.0 and 1.5 mol dm^{-3} HCl and complete to the mark with the same solvent. Transfer this solution into another clean dry conical flask and reflux in a thermostatically controlled water bath at 40 °C for 30 min. Take 1 cm^3 samples at 5 min intervals and then complete as described in section for kinetic order of the reaction. Plot the log % of loperamide hydrochloride remaining against time for different concentration of HCl and calculate the rate constant.

For studying the effect of temperature on the reaction rate: dissolve two portions of 4 mg of loperamide hydrochloride in 100 cm^3 volumetric flasks and complete the volume with 0.1, 1.0 and 1.5 mol dm^{-3} HCl, respectively. Transfer these solutions into other clean dry conical flasks and then reflux in a thermostatically controlled water bath at 25 and 40 °C for 30 min. Take 1 cm^3 samples at 5 min intervals and then complete as described in section for kinetic order of the reaction. Plot the log % of loperamide hydrochloride remaining against time for different temperatures. Also, plot the Arrhenius plot for the effect of temperature on the rate of hydrolysis.

Procedure for tablets

A total of 10 tablets of studied pharmaceutical preparation (Loperamide, Zdravlje-Actavis, Leskovac, Serbia) containing loperamide hydrochloride were weighed and finely powdered using a pestle and mortar. An accurately weighed quantity of the resulting powder, equivalent to 2 mg (weight of one tablet) of loperamide hydrochloride was dissolved in 10 cm^3 of ethanol. Then it was filtered directly into a 10 cm^3 standard volumetric flask. For HPLC determination, aliquots (1 cm^3) of loperamide hydrochloride were taken and suitably diluted with the mobile phase in order to get a 20 $\mu\text{g cm}^{-3}$ con-

centration and the samples were injected into the chromatograph.

RESULTS AND DISCUSSION

High performance liquid chromatographic analysis

A simple isocratic high-performance liquid chromatographic method is described for the determination of loperamide hydrochloride in the presence of its degradation products without prior separation. Foremost, to optimize the HPLC assay parameters, the type of column and its dimension, mobile phase condition, and choice of wavelength of detection were investigated. Different types of stationary phase XDB-C8 and a ZORBAX Eclipse XDB-C18 column with different dimensions and particles size were used. It was found that the ZORBAX Eclipse XDB-C18 column (4.6 mm×250 mm), with a particle size of 5 μm gave the most suitable resolution. Satisfactory separation of used standards was obtained with a mobile phase consisting of 0.1% sodium octansulphonate, 0.05% triethylamine and 0.1% ammonium hydroxide in water:acetonitrile (45:55 v/v).

Corresponding HPLC chromatogram of standard loperamide hydrochloride and the HPLC chromatograms of its products after acid hydrolysis (0.1 and 1 mol dm^{-3} HCl) at 40 °C, recorded at 226 nm, are shown in Fig. 2.

The obtained compounds during acid hydrolysis were identified by use adequate standards. At retention time of 1.672 min was identified loperamide hydrochloride. At 1.265 min was noticeable its acid degradation products 4-(4-chlorophenyl) piperidin-4-ol (Fig. 2c), which was obtained during acid hydrolysis with 1 mol dm^{-3} HCl. But at 1.457 min was detected 4-(4-hydroxy-4-phenylpiperidin-1-yl)-*N,N*-dimethyl-2,2-diphenylbutanamide (Fig. 2d), product of acid hydrolysis with 0.1 mol dm^{-3} HCl. Based on identified degradation products (impurity C and D, Fig. 1), the proposed scheme for preparing the degradation products of loperamide hydrochloride by acid hydrolysis is shown in Fig. 3.

The chromatographic parameters, such as efficiency column and peak asymmetry were reconsidered for the loperamide hydrochloride standard (Fig. 2b). According to the obtained value of Number of Theoretical Plates ($N = 6705$), the conclusion is that the efficiency column is satisfactory ($HETP = 0.0372$). The asymmetry peak value of 0.717 indicates that the peak is not ideally symmetric, that is, it is not Gause's peak. Having in mind that $W_{ab} < W_{bc}$, this means that there is a certain interaction between the stationary phase and the investigated component.

The excellent linearity was obtained between the peak areas and the concentrations. The linear regression equation (Eq. (1)) obtained with a regression coefficient, r , of 0.9972 was:

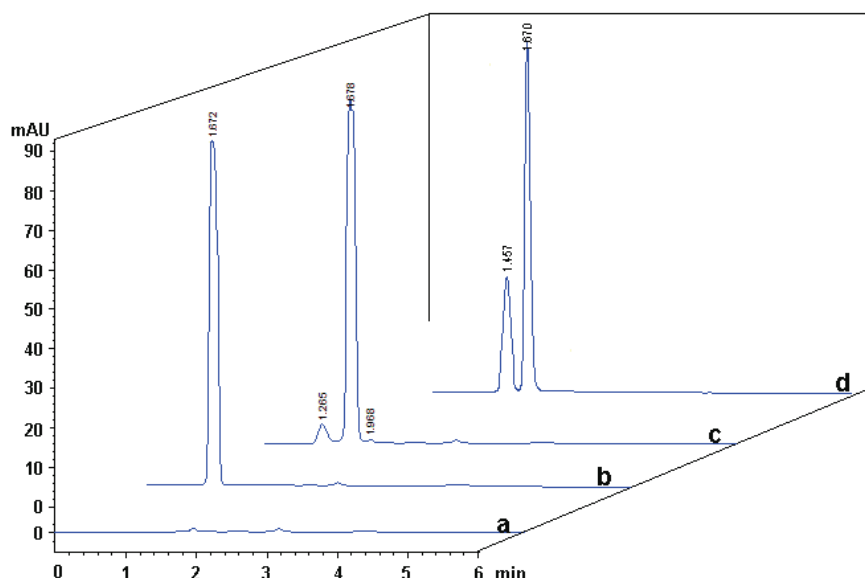


Fig. 2. The HPLC chromatograms at 226 nm of: blank solution (a), loperamide hydrochloride standard (b), and products during acid hydrolysis in 0.1 and 1 mol dm⁻³ of HCl at 40 °C. It is noticeable loperamide hydrochloride at $t_r = 1.672$ min and degradation products 4-(4-chlorophenyl) piperidin-4-ol (1 mol dm⁻³ HCl) at $t_r = 1.265$ min (c) and 4-(4-hydroxy-4-phenylpiperidin-1-yl)-N,N-dimethyl-2,2-diphenylbutanamide (0.1 mol dm⁻³ HCl) at $t_r = 1.457$ min (d).

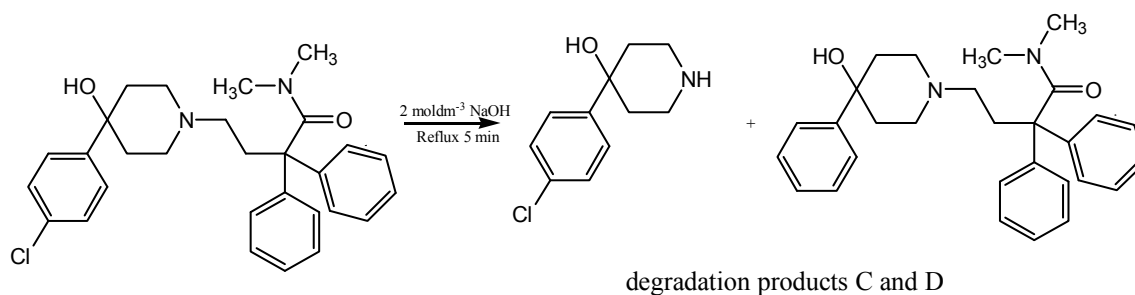


Fig. 3. Proposed scheme of loperamide hydrochloride degradation process in acid solutions.

$$A_{226} = 29377.27c \text{ (mg cm}^{-3}\text{)} + 137.44 \quad (1)$$

Beer's law was obeyed in the range of 10–100 $\mu\text{g cm}^{-3}$. The chromatogram of loperamide hydrochloride was not changed in the presence of common excipients used in the pharmaceutical preparations. The chromatogram of the pure drug sample was matched with the formulation samples in mobile phase. The calculated t -values of 1.368 were found to be less than that of the tabulated t -values (2.225). Therefore, the proposed analytical method is specific and selective for the drug. The linearity range for loperamide hydrochloride estimation was found to be 10–100 $\mu\text{g cm}^{-3}$ ($r = 0.9972$). Goodness

of the fit of the regression equations was supported by high regression coefficient values. The accuracy ranged from 20 to 60 $\mu\text{g cm}^{-3}$ (Table 1).

The excellent mean % recovery values, close to 100%, and their low standard deviation values ($SD < 1.0$) represent high accuracy of the analytical methods. The validity and reliability of the proposed methods was assessed by the recovery studies.

The validity and reliability of the proposed methods was further assessed by recovery studies via standard addition method. The mean % recoveries (RSD) for concentration of 20 $\mu\text{g cm}^{-3}$ are shown in Table 2.

Table 1. Accuracy and the precision data method for the developed method ($n = 10$)

Level	Estimated concentration ^a , $\mu\text{g cm}^{-3}$			Mean recovery $\pm SD$, %	Accuracy ^b , %
	Range	Mean $\pm SD$	RSD , %		
LC (20 $\mu\text{g cm}^{-3}$)	18.9–20.8	19.85 $\pm$ 0.137	0.683	99.25 $\pm$ 0.137	–0.75
IC (40 $\mu\text{g cm}^{-3}$)	38.1–42.3	40.21 $\pm$ 0.337	0.672	100.42 $\pm$ 0.337	0.42
HC (60 $\mu\text{g cm}^{-3}$)	58.9–59.4	59.15 $\pm$ 0.179	0.303	98.58 $\pm$ 0.179	–1.42

^aEstimated concentration of loperamide hydrochloride was calculated by linear regression equation; ^baccuracy is given in % relative error (=100(predicted concentration – nominal concentration)/nominal concentration)

Table 2. Standard addition of loperamide hydrochloride for accuracy ($n = 10$) for $20 \mu\text{g cm}^{-3}$ of the drug in formulation

Pure drug added, $\mu\text{g cm}^{-3}$	Total drug found $\pm$ SD, $\mu\text{g cm}^{-3}$	Recovery $\pm$ RSD, %
0	19.85 ± 0.137	99.25 ± 0.683
10	29.92 ± 0.32	99.73 ± 0.58
20	40.35 ± 0.45	100.87 ± 0.64
30	49.87 ± 0.42	99.74 ± 0.53

These results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical methods.

Precision was determined by studying the repeatability and the intermediate precision. Repeatability (*RSD*) ranged from 20 to $60 \mu\text{g cm}^{-3}$ (Table 3).

The repeatability results indicated the precision under the same operating conditions over a short interval of time and the inter-assay precision. The intermediate precision expresses within-laboratory variations in different days and in different instruments. In the intermediate precision study, *RSD* values were not more than 2.0% in all the cases. *RSD* values found for the proposed analytical method were well within the acceptable range indicating that the method have excellent repeatability and the intermediate precision.

LOD and *LOQ* were found to be 0.72 and $2.38 \mu\text{g cm}^{-3}$ for loperamide hydrochloride.

The assay value of loperamide hydrochloride found in preparation was 98.20% with standard deviation not more than 0.0091%. Assay values of formulations were same as mentioned in the label claim indicating that the interference of excipient matrix is insignificant in estimation of loperamide hydrochloride by proposed analytical methods. The estimated drug content with low values of standard deviation established the precision of the proposed method. The calculated Student's *t*-values (1.98) did not exceed the tabulated values (theoretical values at 95% confidence limits is $t = 2.225$).

Kinetics of the degradation

The loperamide hydrochloride degradation during acid hydrolysis and kinetics investigation was carried out in HCl solutions of 0.1, 1.0 and 1.5 mol dm^{-3} , at different temperatures (25 and 40°C), by monitoring the parent compound itself.

For loperamide hydrochloride hydrolysis in 1 mol dm^{-3} of HCl at 40°C the linear relationship was obtained by plotting the log concentrations of the remaining against time (Fig. 4).

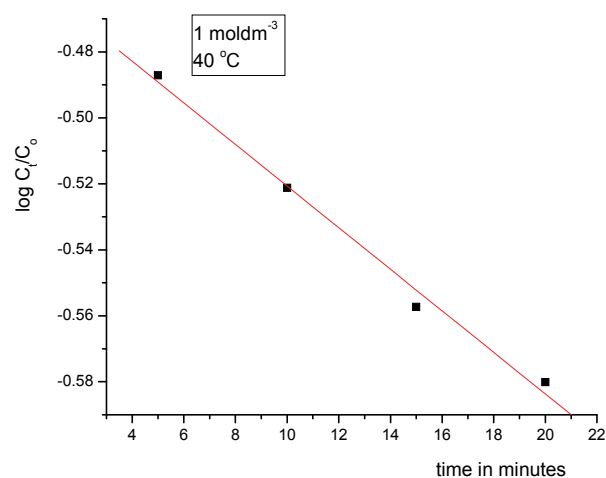


Fig. 4. First order plot of the hydrolysis of loperamide hydrochloride with 1 mol dm^{-3} HCl at 40°C ($y = -0.0063x - 0.4576$, $r = -0.9959$).

Since the hydrolysis was performed in a large excess of HCl (1 mol dm^{-3}), it follows a pseudo-first order reaction rate [21] which is the term used when two reactants are involved in the reaction but one of them is in such a large excess (*i.e.* HCl) that any change in its concentration is negligible compared with the change in concentration of the other reactant (*i.e.* drug).

Different parameters that affect the rate of the reaction were studied. The effect of temperature was studied by conducting the reaction at different temperatures using different concentrations of the acid solution (Figs. 5–8).

Table 3. System precision study ($n = 10$)

Concentration, $\mu\text{g cm}^{-3}$	Estimated concentration intra-day repeatability %RSD, $n = 10$			Intra-instrument repeatability % RSD, $n = 10^*$
	Day 1	Day 2	Day 3	
20	19.85 (0.683)	19.94 (0.672)	20.48 (0.512)	18.96 (0.889)
40	39.85 (0.05)	40.32 (0.212)	39.88 (0.08)	40.35 (1.408)
60	60.55 (0.183)	60.51 (0.842)	60.15 (0.083)	60.34 (1.547)

*Calculation: $RSD(\%) = 100 \frac{S}{\bar{x}}$, $S = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$

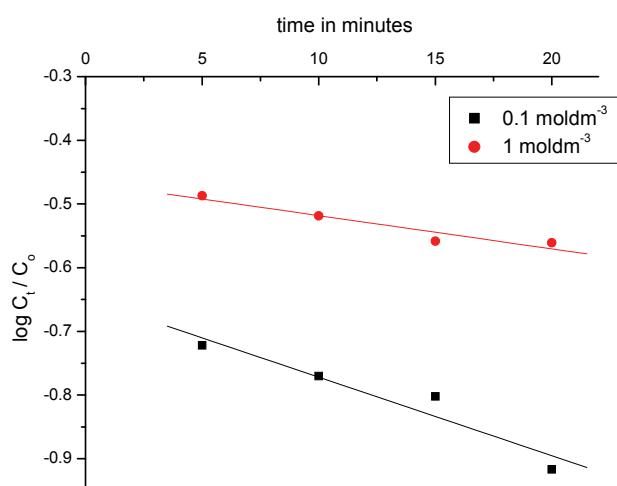


Fig. 5. First order plot of the hydrolysis of loperamide hydrochloride with 0.1 and 1 mol dm⁻³ HCl at 25 °C

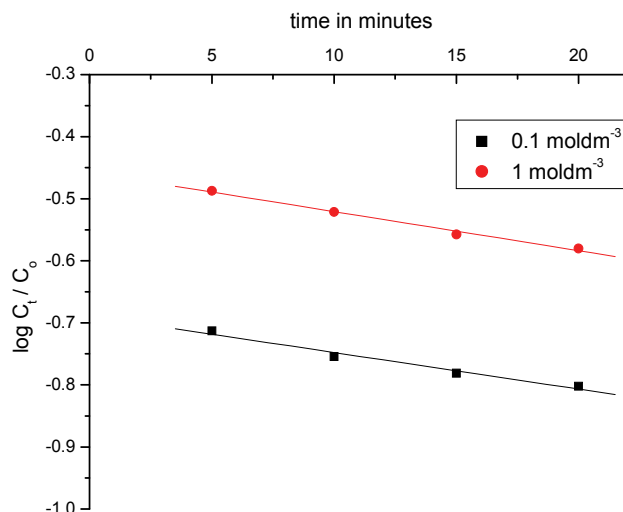


Fig. 6. First order plot of the hydrolysis of loperamide hydrochloride with 0.1 and 1 mol dm⁻³ HCl at 40 °C.

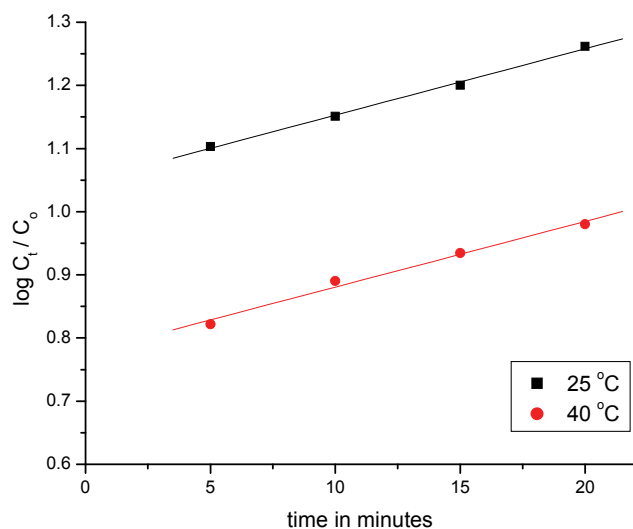


Fig. 7. First order plot of the loperamide hydrochloride, represented by 4-(4-chlorophenyl)piperidin-4-ol, with 1 mol dm⁻³ HCl at different temperatures.

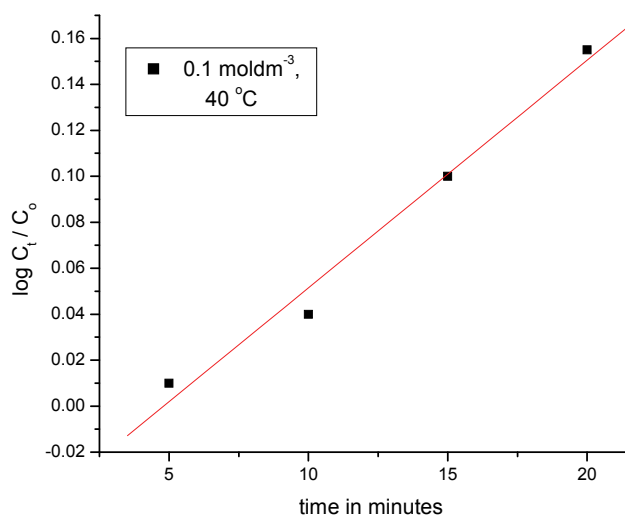


Fig. 8. First order plot of the loperamide hydrochloride, represented by 4-(4-hydroxy-4-phenylpiperidin-1-yl)-N,N-dimethyl-2,2-diphenylbutanamide, with 0.1 mol dm⁻³ HCl at 40 °C.

At each temperature, the rate constant was calculated and then log of rate constant was plotted against the reciprocal of the temperature (Arrhenius plot, Fig. 9) to demonstrate the effect of temperature on the rate constant. The activation energy was determined by calculating the rate constant [22] from the following equation:

$$\log \frac{k_2}{k_1} = \frac{E_a}{2.303R} \frac{T_2 - T_1}{T_2 T_1} \quad (2)$$

where E_a is the activation energy, T_1 and T_2 are the two temperatures degrees in Kelvin, R is the gas constant, and k_1 and k_2 are the rate constants at the two temperatures used.

The calculated E_a was found to be 38.81 kJmol⁻¹, which was a comparatively low value for esters, suggesting the instability in acid medium. Different concentration of HCl solutions were used to study the hydrolysis increased with an increasing HCl concentration, although the effect was minor compared to the effect of temperature (Figs. 5–8) and (Tables 4 and 5). In conclusion, the acid hydrolysis of loperamide hydrochloride and its acid degradation products 4-(4-chlorophenyl) piperidin-4-ol and 4-(4-hydroxy-4-phenylpiperidin-1-yl)-N,N-dimethyl-2,2-diphenylbutanamide was found to follow a pseudo first order reaction rate. Also, the reaction rate increases in the temperature and the strength of the acid solution.

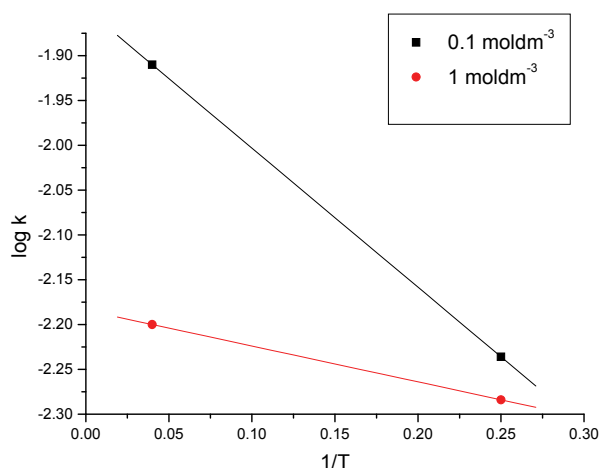


Fig. 9. Arrhenius plot for the hydrolysis of loperamide hydrochloride with 0.1 and 1 mol dm⁻³ HCl.

CONCLUSION

A new RP-HPLC method was developed and validated for the determination of loperamide hydrochloride in the solid pharmaceutical products. The analytical method is a simple, sensitive and selective method suitable for application in a drug manufacturing quality control or regulatory analysis laboratory of loperamide hydrochloride either in the pure powdered form or available pharmaceutical dosage forms. Moreover, a stability-indicating HPLC assay method was developed for the identification and separation of loperamide, its potential impurity or degradation products. At retention time of 1.672 min loperamide hydrochloride was identified. At 1.265 min was noticeable its acid degradation products 4-(4-chlorophenyl)-piperidin-4-ol, which was obtained during acid hydrolysis with 1 mol dm⁻³ HCl. But at 1.457 min was detected 4-(4-hydroxy-4-phenylpiperidin-1-yl)-*N,N*-dimethyl-2,2-diphenylbutanamide, product of acid hydrolysis with 0.1 mol dm⁻³ HCl.

The acid hydrolysis of loperamide hydrochloride was found to follow a pseudo first order reaction rate. Also, the reaction rate increases with the increase of temperature and concentration of HCl. Loperamide was found to be highly susceptible to 1 mol dm⁻³ HCl. However, general degradation from different cases (oxidation and UV-B photolysis) is possible but needs to be studied further by the developed HPLC method.

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Table 4. Kinetic and statistical data of loperamide hydrochloride with different concentrations of HCl at different temperatures

Temperature, °C	<i>a</i> or <i>k</i> (the rate constants), s ⁻¹	<i>b</i>	<i>r</i>
0.1 mol dm ⁻³			
25	0.0123	-0.6487	-0.9606
40	0.0058	-0.689	-0.9875
1 mol dm ⁻³			
25	0.0052	-0.4659	-0.9578
40	0.0063	-0.4577	-0.9956

Table 5. Kinetic and statistical data of 4-(4-chlorophenyl)piperidin-4-ol with 1 mol dm⁻³ HCl at different temperatures

Temperature, °C	<i>a</i> or <i>k</i> (the rate constants), s ⁻¹	<i>b</i>	<i>r</i>
1 mol dm ⁻³			
25	0.0105	1.0476	0.9980
40	0.0104	0.7765	0.9940

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IZVOD

KVANTITATIVNA ANALIZA LOPERAMID HIDROHLORIDA U PRISUSTVU NJEGOVIH KISELIH DEGRADACIONIH PRODUKATA

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²Farmaceutsko-hemijska industrija Zdravlje-Actavis, Vljakova 199, Leskovac

(Naučni rad)

Cilj rada bio je razvoj nove RP-HPLC metode za određivanje loperamid hidrohlorida u prisustvu njegovih kiselih degradacionih produkata. Odvajanje loperamid hidrohlorida od degradacionih produkata postignuto je upotrebom ZORBAX Eclipse XDB C-18 kolone sa mobilnom fazom koja sadrži 0,1% natrijum-oktansulfonat, 0,05% trietilamin, 0,1% amonijum hidroksid u vodi:acetonitril (45:55 v/v). pH mobilne faze podešen je na 3,2 pomoću fosforne kiseline. Metoda je pokazala visoku osetljivost sa dobrom linearnošću u opsegu koncentracije od 10 do 100 µg cm⁻³. Metoda je takođe uspešno primenjivana za analizu farmaceutskog preparata (Loperamid, Zdravlje-Actavis, Srbija) koji sadrži loperamid hidrohlorid. Degradacija loperamid hidrohlorida u toku kisele hidrolize i kinetička ispitivanja izvedena su rastvorima hlorovodonične kiseline koncentracije 0,1; 1,0 i 1,5 mol dm⁻³, na različitim temperaturama (25 i 40 °C). Degradacija loperamid hidrohlorida u kiselim rastvorima pokazala je reakciju prvog reda. Energija aktivacije izračunata po Arenijusovoj jednačini iznosila je 38,81 kJ mol⁻¹ na 40 °C. Razvijana metoda uspešno je primenjivana za brzo određivanje loperamid hidrohlorida u farmaceutskoj formulaciji (Loperamid, Zdravlje-Actavis, Srbija) i u prisustvu njegovih kiselih degradacionih produkata.

Ključne reči: Loperamid-hidrohlorid

• Degradacioni produkti • HPLC • Kinetika

Key words: Loperamid hydrochloride • Degradation products • HPLC • Kinetics