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Chiral Quantitative Analysis of Loperamide in a State of Acid Degradation by HPLC Method

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Research Article

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ABSTRACT

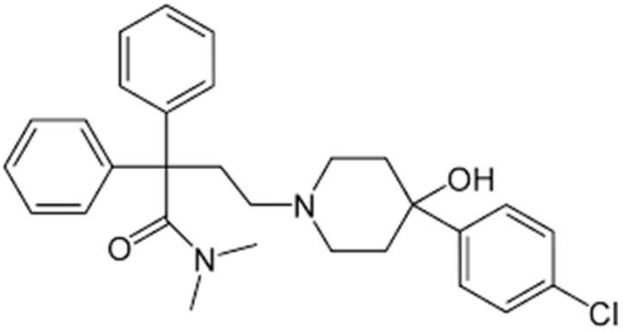
This work describes the stability of Loperamide® hydrochloride in different pH solutions with different buffer species. High-Performance Liquid Chromatography (HPLC) is an efficient method of Loperamide® hydrochloride, but the direct analysis procedure is not suitable for the determination of Loperamide® hydrochloride in the presence of its acid degradation product or its impurity. The aim is to develop and validate a new feasible, sensitive and specific analytical procedure using HPLC on chiral columns polysaccharides, namely, CSPsCHIRALCEL OJ-3R, CHIRALCELOD-RH, and CHIRALPACK AD-3R using various mobile phases. According to the results of the chiral separation of Loperamide, for the CHIRALCEL® OJ-3R column, Loperamide is effective compared to the columns CHIRALCEL® OD-RH, CHIRALPACK® AD-3R.

Keywords: Loperamide®; Chiral Columns; HPLC; Degradation; Stability

INTRODUCTION

Nowadays, living beings suffer from various diseases due to their lifestyle habits. Today, medicine is the first aid preferred for every disease. The usage of medicine has been practiced from prehistoric times to the present. It is an ancient thought of development in medicines [1]. A drug is considered to be practically stable when, within a determined period, its essential properties do not change or change at most intolerable proportions; in addition, it is understood that the medicinal product must be stored under appropriate and prescribed conditions of temperature, humidity and light exposure and that a suitable container has been used. Loperamide® is an antidiarrheal drug available over-the-counter since the Food and Drug Administration (FDA) reported it safe. In contrast to other μ-receptor agonists, Loperamide® specifically affects the myenteric gastrointestinal network [2]. This drug affects the peripheral μ receptors, inhibiting its passage through the blood-brain barrier, which prevents evident cerebral symptoms; theoretically, it does not have the potential to be abused [3].
Loperamide $C_{29}H_{33}ClN_2O_2$(4-[4-chlorophenyl].-4-hydroxy-N-dimethyl-alpha,alphadiphenyl-1-piperidine-butanamide hydrochlori de) is a phenylpiperidine derivative that was synthesized in 1969 and approved by the US Food and Drug Administration in 1976 [4-6]. Its chemical structure is related to diphenoxylate, haloperidol, and meperidine (Figure 1), but it has minimal analgesic activity and does not produce euphoria at standard doses [7-10]. Loperamide®is administered orally and is moderately absorbed (about 40%) from the gastrointestinal tract to undergo first-pass metabolism in the liver and excretion in the faces *via* the bile as inactive conjugates (acombination of sulfoandglucurono). The analysis of Loperamide® in bulk drugs and pharmaceutical products is well described in the literature and is mentioned in either the European or US Pharmacopoeias [11]. High-Performance Liquid Chromatography (HPLC) is an efficient method for the analysis of Loperamide® hydrochloride.

Figure 1. 1Loperamide structure.



Goran S. Nikolic et al. used a C-18 column with a mobile phase of 0.1% sodium octane sulfonate, 0.5% triethylamine, and 0.1% ammonium hydroxide in water: acetonitrile (45:55v/v) to study the quantitative analysis of Loperamide® in the presence of its acid degradation products. Phosphoric acid was used to adjust the pH of the mobile phase to 3.2. The method demonstrated high sensitivity and linearity over a concentration range of 10 to 100 g cm^{-3} [12,13]. This work aims to develop a new RP-HPLC method for determining Loperamide hydrochloride in the presence of its acid degradation products using chiral columns as stationary phase based on polysaccharides, as well as a study of the drug's degradation and kinetics under two conditions (temperature and acidity) in order to carry out a kinetic control sheet of this drug.

MATERIALS AND METHODS

Chemicals and reagents

For the experimentation step, n-heptane, 2-propanol, n-hexane, methanol and ethanol HPLC grad were supplied by Chemminova (Harboore, Denmark) and RiedeldeHaën (Sleeze, Germany).

HPLC system: Shimadzu's LC-2030 series was used in this study, and it was outfitted with an automatic injector of a 1-100 μL sample loop, a double pump system with a vacuum degassing unit, and a Shimadzu Ultraviolet (UV) detector [14-17]. The LC Lab solution software collected, stored, and analyzed chromatographic data (Shimadzu, Tokyo, Japan). The injection volume was 10 μL, and the UV wavelength was adjusted to match the λ_{max} of each compound. Chromatographic separations were performed at 25˚C in isocratic mode with different flow rates.

Chiral Stationary Phases (CSPs): For further investigation, three different polysaccharides, CSPsCHIRALCEL OJ-3R,

CHIRALCELOD-RH, and CHIRALPACK AD-3R, were purchased from Chiral Technologies Europe (Illkirch, France).

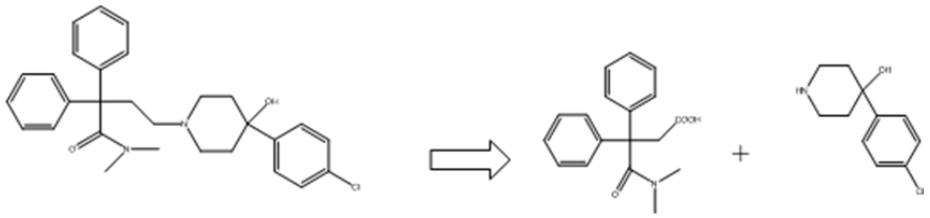
Sample preparation

The Loperamide® sample was prepared by dissolution of 2 mg Loperamidetabletsin 40 ml of distilled water.
The pH and temperature degradation of Loperamide were adjusted after selecting the best stationary phase, the CHIRALCEL® OJ-3R column, and the acetonitrile 100% mobile phase.

RESULTS AND DISCUSSION

Loperamide analysis in bulk drugs and pharmaceuticals is well described in the literature and is cited in European and American pharmacopeias [18]. The stability of Loperamide hydrochloride in various pH solutions with various buffer species has been described in the literature. Although High-Performance Liquid Chromatography (HPLC) is an effective method for analyzing Loperamide hydrochloride, it is ineffective for determining Loperamide hydrochloride in the presence of its acid degradation product or impurity [19-21]. This research aims to create and validate a new, feasible, and sensitive analytical procedure for the Chiral analysis of this drug and its degradation products. The HPLC is appropriate for analyzing Loperamide hydrochloride in the presence of its degradation products in drug quality control or regulatory laboratories (Figure 2).

Figure 2. Diagram of the Loperamide hydrochloride degradation process in acid solutions.



Loperamide HPLC analysis was performed using four stationary phases (Figure 3). Three exhibit poor separation quality (variable resolution between 0.038 and 2.01). Otherwise, CHIRACEL® OD-RH separation. When 100% acetonitrile was used as the mobile phase, a good selectivity factor α=1.82 and a high-resolution Rs=4.27were obtained. Table 1 summarizes all other chromatographic results.

Figure 3. Loperamide HPLC analysis chromatogram on the CHIRACEL® OD-RH column with mobile phase (100% acetonitrile), T=25°C, flow rate.

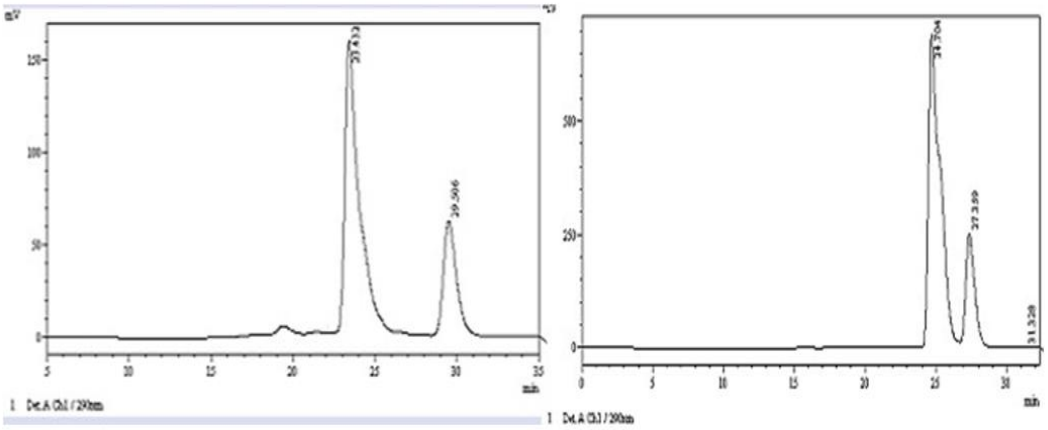


Table 1. Result of the separation of Loperamide on the chiral OD-RH® column.

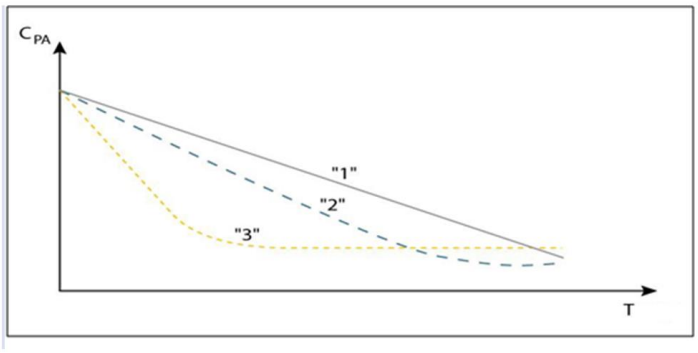
CSP	Mobile phase	T_1	T_2	K_1	K_2	Rs	A	%
Chiralcel® OD-RH	Acetonitrile 100%	23.432	29.506	0.33	0.36	4.272	1.82	21.772
		26.528	30.816	0.926	1.092	2.375	1.195	64.764
	2-Propanol 100%	-	-	-	-	-	-	-
	Ethanol/ACN (20/80)%	24 .45	26.459	0.952	1.54	0.038	1.622	16.91
	Methanol /ACN20/80%	3.96	5.96	1.506	1.506	0.05	1.155	1.081
		25.9	27.9	2.319	3.195	0.19	1.378	51.425
	Hexane/2-propanol20/80%	23.28	25.283	0.688	0.92	0.972	1.348	29.382
		24.49	26.497	1.076	1.681	1.957	1.563	61.382
	Hexane/2-propanol50/50%	-	-		-	-	-	-
	Hexane/2-propanol 60/40%	23.638	27.138	0.088	0.138	0.246	1.571	10.227
		23.036	27.536	0.265	0.447	0.11	1.687	18.68
		24.794	28.294	0.265	0.47	0.11	1.775	18.68
		25.854	29.354	0.426	0.686	0.306	1.611	52.789

Determination of the reaction's order

We Plotted the Curve (PA) to study the change in concentration of Loperamide (PA) as a function of time and at constant temperature (50°C) (Figure 4). There are several plot possibilities. Equation 1 expresses the speed V as follows:

$$V=-dC/dt= K.Cn \quad (1)$$

Figure 4. PA concentration plots as a function of time.



Zero-order reaction: (Figure 4, plot 1)

If n=0: -dC/dt=K (constant), the reaction rate is independent of the concentration of the substance studied: V=constant= K slope of the curve. By integrating this equation, we obtain the equation of the graph C=f (t), which is a straight line:

$$C = C_0 - K\,t \quad (2)$$

Where, C_0 and C are PA concentrations at a time (t0) and (t), and K is the speed constant (slope of the line).

Reaction of order 1: (Figure 4, 2nd plot)

The degradation rate is high at first and then decreases as the concentration decreases, as follows:

$$n=1 \Rightarrow -dC/dt=K.C. \quad (3)$$

By integrating this equation, we obtain the equation of the asymptote:

$$C=C_0.\ e^{-Kt} \quad (4)$$

By expressing this equation, we obtain a line of slope:

$$K.\ Log\ C=-(Kt/2.303)+log\ C_0 \quad (5)$$

Reaction of order 2: (Figure 4, 3rd plot)

In this type of reaction, two molecules are involved in the degradation process (example: Interaction between AP and excipient) -dC /dt=K.C2. The degradation rate is a function of the square of the AP concentration. By integrating the preceding equation, we obtain the equation of a hyperbola: C=f (t). We make sure that the reaction is of order 2 by checking whether the graph 1/C as a function of time is indeed a line: 1/C=f (t), of slope K.

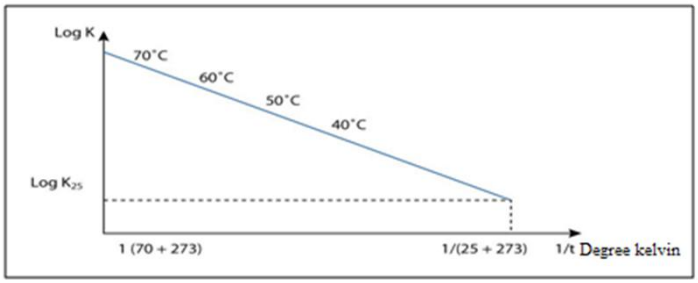
Determination of the constant degradation rate at 25°C

We exposed the drug to high temperatures (40°, 50°, 60°, 70°...) and determined the degradation rate constant at each temperature. Based on the Arrhenius law, we drew the curve (Figure 5):

$$log\ K= f\ (1/T),\ K=A\ e\text{-}Ea/RT \Rightarrow log\ K=log\ A\text{-}Ea/2.303\ RT \quad (6)$$

Where, A is the constant, Ea is theactivation energy, T is theabsolute temperature in Kelvin, and R is the perfect gas constant.

Figure 5. Constant correlation of degradation rate as a function of applied temperatures.



Estimation of the validity period

From the estimated degradation rate constant K 25, and depending on the order of the degradation reaction, the shelf-life D of the drug is calculated as follows:

If the reaction is of order 0: D=X C0/100.K25 If the reaction is of order 1: D=(2,303/K25). Log (100/100-X). If the reaction is of order 2: D=[X/(100-X)]. 1/(Co.K25) X: being the drop in the titer of the active principle.

Real-time studies must confirm this provisional validity date.

Study of the stability of Loperamide under harsh degradation conditions

Study of the effect of temperature on Loperamide degradation: Table 2 summarizes the results of the Loperamide degradation kinetics as a function of temperature (50°C).

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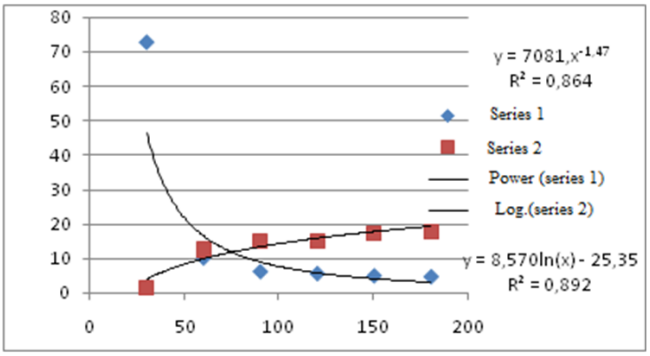
Table 2. The results of the degradation kinetics of Loperamide by the effect of temperature (50°C).

Time (min)	30	60	90	120	150	180
% Lopéramide	72.82	10.12	6.15	5.56	4.93	4.65
% Product 1	1.51	12.59	15.05	15	17.29	17.7
% Product 2	1.57	6.51	8.06	20.29	22.55	22.47

According to the Loperamide degradation kinetics (Table 2), there is a very rapid decrease during the first hour of dropping the aqueous solution of this drug to 50°C, from 72.82% to 10.12% Loperamide. Moreover, a slow decreasing variation in the second hour and a very slow, almost constant variation in the third hour can be noted. In addition, according to the graph's tendency curve, (PA)=f (t) (series 1, R^2=0.864) (Figure 6), which gives a hyperbolic equation confirming that this degradation in the mixture governs the kinetics of a first-order reaction.

Figure 6. The effect of temperature (50°C) on the kinetics of Loperamide degradation.

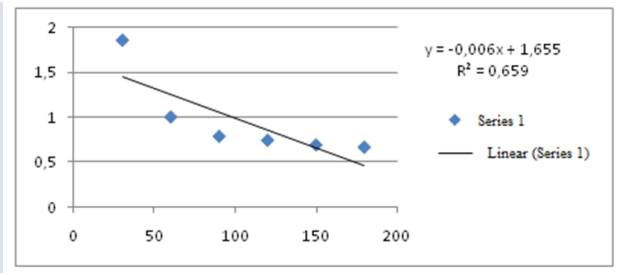


To confirm that this degradation is of kinetic order 1 (-dC/dt=KC), we draw the graph log C as a function of time, and we will check that we obtain a straight line: log C=f (t), of slope K with K: Rate constant of this degradation reaction (Table 3 and Figure 7).

Table 3. The results of the degradation kinetics of Loperamide by the effect of temperature.

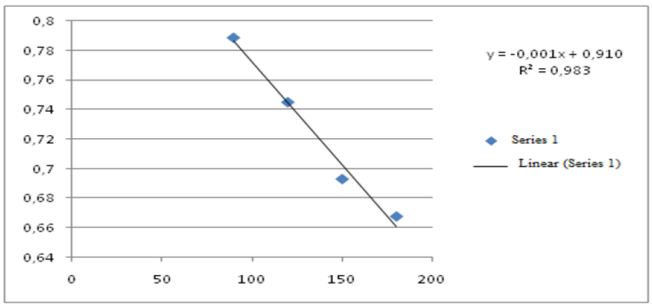
Time (min)	30	60	90	120	150	180
% Lopéramide	72.82	10.12	6.15	5.56	4.93	4.65
Log (% Lopéramide)	1.8622	1.0051	0.7888	0.745	0.6928	0.6674

Figure 7. Determination of the kinetic order of the reaction and the rate constant by the graph: log C=f (t) (R^2=0.659).



Because of this divergence in the tendency line for the six points of this graph, we aimed to validate the graph's approach to a line for the last four points (Figure 8).

Figure 8. Determination of the kinetic order of the reaction and the rate constant by the graph: log C=f (t) (R^2=0.983).



We notice that the tendency curve is closer to R^2=0.983. This result confirms that the degradation kinetics of Loperamide are first order, and according to the equation that determines this kinetics:

$$\text{Log C} = -(Kt/2.303) + \log C0$$

We get a straight line with slope K. Therefore, the rate constant of this reaction is of the order:

$$K = 1.6.\ 10^{-3}\ s^{-1}$$

Study of the effect of acidity on the degradation of Loperamide

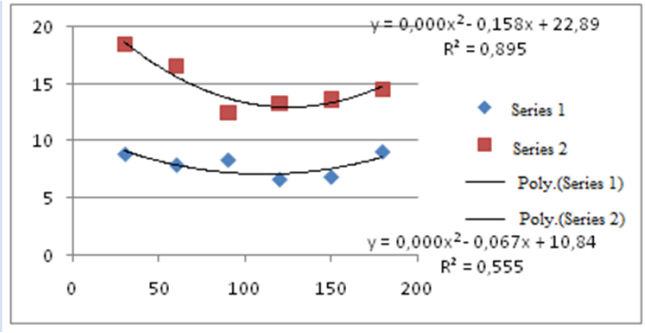
Table 4 summarizes the results of the Loperamide degradation kinetics as a function of acidity (PH=5.2).

Table 4. Results of the degradation kinetics of Loperamide by the effect of acidity (PH = 5.2).

Time (min)	30	60	90	120	150	180
% Loperamide	8.85	7.89	8.33	6.59	6.82	9.07
% Product 1	18.5	16.58	12.54	13.34	13.67	14.59
% Product 2	3.24	1.39	1.19	2.14	1.7	2.01

According to the results of the Loperamide degradation kinetics under the effect of acidity (Table 4), we observed a slow decrease in the composition of Loperamide in the first ninety minutes. Then, over the next ninety minutes, there is a slight increase (Figure 9). The tendency curve (PA)=f (t) (series 1, R^2=0.895) yields a polynomial equation that confirms that this degradation in mixture governs the kinetics of an order 2 reaction.

Figure 9. Degradation kinetics of Loperamide by the effect of acidity (PH=5.2). Series 1:% Loperamide=f (t), Series 2:% product 1=f (t).

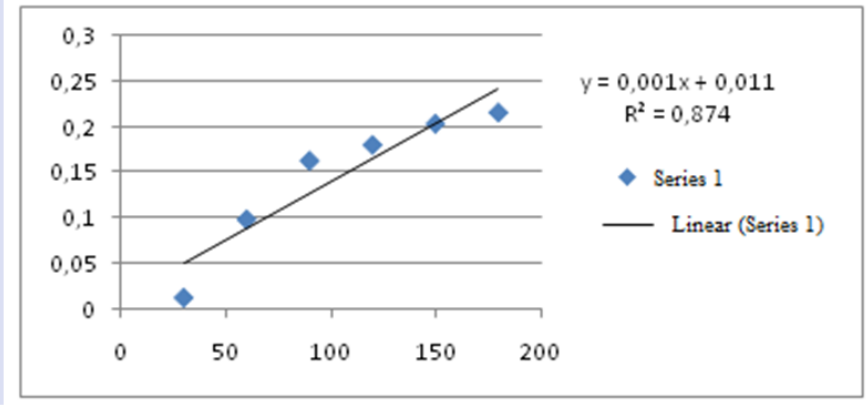


To confirm that this degradation is of kinetic order 2, we drew Graph 1/C as a function of time and checked for a straight line: 1/C=f (t), slope K with K: Rate constant of this degradation reaction (Table 5, Figure 10).

Table 5. The results of the degradation kinetics of Loperamide by the effect of acidity (pH=5.2).

Time (min)	30	60	90	120	150	180
% Lopéramide	8.85	7.89	8.33	6.59	6.82	9.07
1/(% Lopéramide)	0.0137	0.0988	0.1626	0.1798	0.2028	0.215

Figure 10. Determination of the reaction's kinetic order.



The determination of the kinetic order of the reactionand the rate constant by the graph is 1/C=f (t). The trend curve (1/C).= f (t) (series 1, R^2=0.874) gives an equation of a straight line (y=0.001x+0.011) (Figure 10). Hence the degradation kinetics of Loperamide in an acidic medium is of order 2. Moreover, the rate constant of this reaction is equal to the slope of this line (Figure 10). Therefore, the rate constant is of the order k=2.5. 10^{-3} L. $mol^{-1}.s^{-1}$

CONCLUSION

The chiral separation of Loperamide® using CHIRALCEL® OJ-3R, CHIRALCEL® OD-RH, and CHIRALPACK® AD-3R columns revealed that each chiral column has a unique ability for chiral separation of this drug. The rotatory power calculated shows that the active ingredient of this drug is achiral, and its isomers, due to the conformation, are not isolable at room temperature and also due to the rapid change of axial and equatorial position. The chair form of the piperidine partis the most stable structure of Loperamide®, and the phenyls are found in the equatorial position, making them farther away with minimal interaction. We studied its degradation and developed an analysis method indicative of its stability to control this active molecule's stability data. The degradation kinetics were investigated using acid and thermolytic degradation stress conditions. This work successfully investigated the potential degradation products that could form due to the drug's poor storage conditions. The degradation process also allowed us to develop a selective and sensitive HPLC analysis method that allows us to control the quality of Loperamide® and has been pre-validated by determining the kinetic orders of probable degradation.

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