Artigo

PREDICTION OF ANTI-DIABETIC ALOGLIPTIN STABILITY BY ISOTHERMAL STUDIES

Charise Dallazem Bertol[a,b,c,*], Gabriel Felimberti[b], Karini da Rosa[b], Eliamar Ceresoli Rizzon[d], Rafael Nicolay Pereira[e], Luiz Carlos Kreutz[f] and Pedro Eduardo Froelich[c]

[a]Curso de Farmácia, Universidade de Passo Fundo, 99010-052 Passo Fundo – RS, Brasil

[b]Programa de Pós-Graduação em Envelhecimento Humano, Universidade de Passo Fundo, 99010-052 Passo Fundo – RS, Brasil

[c]Laboratório de Química Farmacêutica, Universidade Federal do Rio Grande do Sul, 90610-000 Porto Alegre – RS, Brasil

[d]Instituto de Ciências Exatas e Geociências, Universidade de Passo Fundo, 99010-052 Passo Fundo – RS, Brasil

[e]Laboratório de Controle de Qualidade, Universidade Federal de Santa Catarina, 88040-370 Florianópolis – SC, Brasil

[f]Programa de Pós-Graduação em Bioexperimentação, Universidade de Passo Fundo, 99010-052 Passo Fundo – RS, Brasil

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Alogliptin (ALG) benzoate is an oral hypoglycemic drug that works as a DPP-4 inhibitor to prevent incretin degradation. Forced stability studies use high temperatures to decompose drugs and estimate their behavior at low temperatures. We aimed to evaluate the thermal stability of ALG using two techniques: isothermal thermogravimetry (TGA) and degradation in an oven followed by liquid chromatography (LC-PDA) analysis. ALG was subjected to 150, 155, 160, 165, and 170 °C up to 10% of mass loss in isothermal TGA. In the oven, the drug was submitted to 130, 140, 150, 155, 160, and 170 °C. Kinetic parameters were calculated with the Arrhenius model. ALG followed zero-order kinetics, in which the degradation rate did not depend on reagent concentration. Activation energy ranged from 31.0 to 35.9 kcal mol^{-1}. The degraded drug was less toxic in a cytotoxicity assay in CRIB cells than the undegraded drug. The TGA method is faster and more practical than the oven followed by LC-PDA, and the data present a correlation. Here we described the kinetic parameters of ALG degradation, improving the knowledge about the drug and assisting in developing new formulations from the drug.

Keywords: alogliptin; thermal analysis; LC-PDA; TGA; cytotoxicity.

INTRODUCTION

Diabetes is a chronic disease with serious complications, such as cardiovascular disease, blindness, kidney failure, lower limb amputation, oral complications, and a higher risk of developing infections. Type 2 diabetes (T2D) accounts for 90% of cases.[1] Among T2D treatments are dipeptidyl peptidase-4 (DPP-4) inhibitors. The DPP-4 enzyme inactivates the incretin glucagon-like peptide-1 and glucose-dependent insulinotropic hormone. After food ingestion, enteroendocrine cells secrete incretins, stimulating insulin secretion in a glucose-dependent manner.[2-4] DPP-4 inhibitors prevent incretin degradation, enhancing the effect of these hormones. Alogliptin (ALG) benzoate is a potent DPP-4 inhibitor with high selectivity and affinity (Figure 1).[5]

Temperature, humidity, light, oxygen, and some excipients are drug degradation factors.[6,7] Drug degradation reduces potency and safety due to the potential formation of toxic products.[7,8] Determining degradation kinetics is essential before predicting the storage time of a formulation.[9]

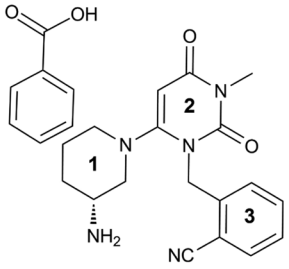

Figure 1. *Chemical structure of alogliptin benzoate, showing piperidine in ring 1, pyrimidine in ring 2, and benzonitrile in ring 3*

Forced stability studies use high temperatures to decompose drugs and, from there, estimate their behavior at low temperatures.[10] At high temperatures (in shorter periods), degradation rates are more easily verified.[11] The modeling of results is based on the Arrhenius equation (Equation 1), which describes the effect of temperature on decomposition.[6,10-13]

$$LnK = Ln\ A + (-Ea/R) \times (1/T) \quad (1)$$

where Ea is the activation energy, A is the Arrhenius frequency factor, R is the gas constant (8.314 J mol^{-1}), and T is the temperature (Kelvin).

Other investigations report using the Arrhenius equation to determine the kinetic parameters of stability studies. The kinetic stability of an MLNA oncology solid-state drug was evaluated using the moisture-modified Arrhenius equation and JMP statistical software. The authors stored the drug at 60 °C/5% RH, 80 °C/9% RH, and 60 °C/75% RH (RH: relative humidity), for eight weeks. The drug was evaluated by DSC, TGA, dynamic vapor sorption (DVS), and XRPD UHPLC with UV detection was used to monitor the degradation. Zero-order degradation curves were obtained for mild conditions (37 °C/75% RH and 60 °C/5% RH). As for more stressed conditions, the first part of the degradation curve was linear and may come close to zero-order. The latter part of the reaction showed slower and more complex kinetics, probably due to isoconversion.[14] Monoclonal antibody stability was also evaluated in accelerated stability studies (up to six months) combined with a first-order degradation kinetics model. Long-term stability (up to three years) was also investigated. The data originated from different temperatures,

*e-mail: charise@upf.br

usually from intended (5 °C), accelerated (25 °C), and stress (40 °C) conditions. The Arrhenius equation was used to evaluate kinetic parameters. The accelerated studies showed robustness, speed, and accuracy of long-term kinetic stability predictions, justifying the shelf-life extrapolation to some biologic agents such as monoclonal antibodies.[15]

Thermogravimetry (TGA) is a faster technique to determine decomposition. TGA evaluates mass losses according to the temperature or time, and the first TGA derivative (DTGA) represents the mass change rate (dm dt^{-1}).[16] Thermal analysis does not replace a classic stability program consisting of long observation times required by regulatory agencies for drug registrations. However, it helps understand the degradation process, detect problems, and obtain effective, stable, and safe formulations.[10,17]

We aimed to determine the degradation kinetics parameters of ALG using two methods and compare them. TGA and an oven followed by liquid chromatography (LC) coupled to a photodiode array detector (PDA) were used. Cytotoxicity was also assessed. Determining cell viability is essential to verify the safety of impurities or degradation products.[18]

EXPERIMENTAL

Materials

ALG was purchased from China (Lanospharma Laboratories Co., Ltd, produced by Rosewa Holding Group Co., Limited) and identified and quantified by IR spectroscopy and differential scanning calorimetry (98.85 ± 0.199% RSD),[19] respectively. Acetonitrile HPLC grade was obtained from Vetec (Rio de Janeiro, RJ, Brazil). All other chemicals were of analytical grade.

Isothermal degradation kinetics by thermogravimetry (TGA)

TGA experiments were performed in a Shimadzu TGA-50 thermobalance using platinum crucibles containing approximately 4 mg of the sample under a dynamic nitrogen atmosphere (100 mL min^{-1}). The equipment was previously calibrated with calcium oxalate. ALG was formerly heated to 110 °C and then cooled to remove moisture. The isothermal study was performed by submitting the sample to isothermal temperatures of 150, 155, 160, 165, and 170 °C. The sample remained at these temperatures until reaching 10% of mass loss. The heating rate was 10 °C min^{-1}.

Thermal degradation in an oven followed by LC-PDA

The drug (approximately 200 mg of the sample for each temperature) was submitted to 130, 140, 150, 155, 160, and 170 °C in an oven (Biopar). Aliquots were removed and analyzed with LC-PDA at predetermined times. The determinations were performed in triplicate.

Liquid chromatography analysis used a Flexar Perkin Elmer LC containing a binary pump, self-sampler, and PDA detector at 275 nm. The mobile phase was a mixture of 10 mmol L^{-1} of ammonium acetate buffer, pH of 3.5, and acetonitrile (10:90); a flow of 0.8 mL min^{-1}; and Nano Separation Technologies (NST) C8 column (250 × 4.6 mm, 5 µm). The stability-indicating method was previously validated.[20] The data was integrated with Chromera Workstation software.

Data treatment

The remaining mass (%) or content (%) *versus* time (h) of the degraded drug was plotted to determine the reaction order. The zero-, first-, and second-order graphs were obtained by plotting the mass or content *versus* time, the logarithm of the mass or content *versus* time, and 1/mass or content *versus* time, respectively. The R-value closest to one defined the reaction order representing the best fitting of the data. After determining the reaction order, degradation rate constants (K) were calculated for each temperature. The Arrhenius graphical method (ln K *versus* 1/temperature (K) plot) was used to calculate the rate constant at 25 °C (K_{25}), *Ea*, and *A*.

Cytotoxicity of undegraded and degraded drugs

The cytotoxicity of undegraded and degraded drugs (170 °C, two hours) was evaluated with the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)[21] and CRIB (cells resistant to infection with BVDV), a lineage derived from MDBK (Madin-Darby bovine kidney, ATCC CCL22). The drugs were prepared at a concentration of 10 mg mL^{-1} in Roswell Park Memorial Institute (RPMI) medium and filtered through a 0.22 µm membrane before the test. The cells were cultured in RPMI medium and supplemented with 5% fetal bovine serum (FBS) and 1.25 mg mL^{-1} of gentamicin in sterile flasks (25 cm^2) at 37 °C and 5% CO_2 (CO_2 incubator, Minitub, Porto Alegre, RS, Brazil). The cells were expanded every three days by dispersing the cell monolayer with 1 mL of trypsin (2.5 mg mL^{-1}) in a laminar flow hood (TROX, Model FLV) and seeding new flasks.

The MTT assay was conducted in 96-well plates. Monolayers of CRIB were dispersed and adjusted to 4.5×10^5 cells mL^{-1}. Then, 100 µL aliquots were distributed into each well and incubated for 24 hours. The cell medium was then removed, and the undegraded and degraded drugs were added to the wells at 5.0, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 mg mL^{-1} in RPMI medium containing antibiotics, in quintuplicates. The RPMI medium without drug was used as the control. The plates were incubated for 96 hours under 5% CO_2. The supernatant from each well was discarded, and 50 µL of MTT (1 mg mL^{-1} in RPMI) was added to each well and incubated at 37 °C for four hours. MTT was removed, 100 µL of dimethyl sulfoxide was added per well, and the plates were shaken for five to 10 minutes and incubated for 15 minutes. Absorbances were read (optical density - OD) at 550 nm in an ELISA reader (Anthos 2010). The percentage of viable cells was calculated for evaluating cell viability (Equation 2):

$$\textit{Cell viability}\ (\%) = OD_{\textit{treated cells}}/OD_{\textit{control cells}} \times 100 \qquad (2)$$

Control cells were 100% viable. The OD values were converted into percentages and plotted against the different drug concentrations to determine the cytotoxic concentration for 50% of cell culture (CC 50%).

RESULTS AND DISCUSSION

The temperatures used in the techniques relate to ALG melting and decomposition processes. ALG melts to 179.4-187.2 °C (Tpeak = 183.33 °C), followed by a decomposition process.[19,20] Therefore, degradation kinetics studies should be performed before degradation. The last temperature used (170 °C) is close to these processes (melting and degradation).

Isothermal degradation kinetics by thermogravimetry (TGA)

Figure 2 shows the time required to lose 10% of mass at each temperature. Under isothermal conditions, the higher the temperature, the earlier the mass loss.

The degradation rate followed zero-order kinetics for the first three studied temperatures (150, 155, and 160 °C), and the other two

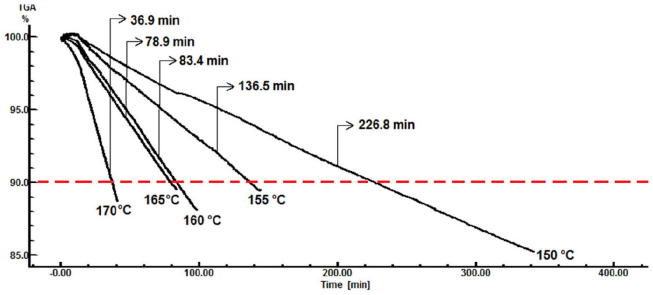

Figure 2. *ALG isothermal thermogravimetric curves at 150, 155, 160, 165, and 170 °C, under nitrogen atmosphere*

temperatures followed first- and second-order kinetics (Table 1). The correlation coefficient (R) values were similar, probably due to the low mass loss percentage (close to 10%). Furthermore, drugs may sometimes follow zero-order kinetics and later adopt first-order kinetics. Based on the isoconversion concept, pharmaceuticals are usually a mixture of forms with small amounts of rapidly reacting forms, including an amorphous phase and/or crystalline defects, and large amounts of slowly reacting crystalline phase. The initial linear part of degradation kinetics can be simplified and approximated as zero-order.[14]

Table 1. Correlation coefficient (R) values from plotting the graphs of zero- (mass (%) *vs.* time (h)), first- (logarithm of the mass (%) *vs.* time (h)), and second- (1/mass (%) *vs.* time (h)) order at different temperatures

Temperature (°C)	Zero-order R	First-order R	Second-order R
150	–0.9998	–0.9997	–0.9995
155	–0.9997	–0.9994	–0.9989
160	–0.9999	–0.9998	–0.9992
165	–0.99985	–0.99999	–0.99996
170	–0.9992	–0.9994	–0.9994

Kinetic parameters were calculated according to the zero-order reaction. The degradation rate (K_0) at each temperature was calculated (Equation 3).[9]

$$dC/dt = -K_0 \quad (3)$$

where dC/dt is the rate of change between drug concentration (dC) over time (dt).

Ea was calculated (Equation 4) from the Arrhenius model (Equation 1):

$$Inclination = -Ea/R \quad (4)$$

where *R* is the constant of perfect gases.

A was obtained with Equation 5:

$$Interception = ln\ A \quad (5)$$

The calculated K_0 (Equation 3) for each studied temperature was used to obtain the Arrhenius plot, which was achieved with Ln K_0 (min^{-1}) *versus* 1/temperature (Kelvin). Then, the line equation was obtained: y = −15,620.856x + 33.83 (R^2 = 0.953), which calculated *Ea* (Equation 4), degradation rate at 25 °C (K_{25}) (replacing "x" with 1/298), and *A* (Equation 5). The resulting values were *Ea* = 31,038.64 cal mol^{-1}, $K_{25} = 8.45 \times 10^{-9}$ min^{-1}, and $A = 4.94 \times 10^{14}$ min^{-1}.

Other studies obtained *Ea* after plotting the graphic containing the Ln time *versus* 1/T (K). The time presented in the graph is required to lose 5, 8, or 10% of the mass and is related to the expiration date.[22-24] The correlation coefficient close to 1 in the Ln time × 1/T (K) graph follows a zero-order reaction.[22,25] In our study, this Ln time *versus* 1/T plot was obtained using the time required to lose 10% of the mass. The line equation was y = 1,6175x – 32,844 (R^2 = 0.9816), and *Ea* was 32,139.7 cal mol^{-1}, similar to the previous value.

Thermal degradation in an oven followed by LC-PDA

The thermal degradation in an oven followed by LC-PDA was performed to complement and understand the results obtained with TGA and verify a potential correlation. TGA evaluated the mass loss, and LC analyzed the content loss, which confirmed the zero-order reaction.

The degradation rate was greater than 50% in an oven at all temperatures (Figure 3). The zero-, first-, and second-order graphs were plotted, and *R* values were determined (Table 2). The *R* values closest to 1 defined the reaction as zero-order, as in the isothermal TGA method.

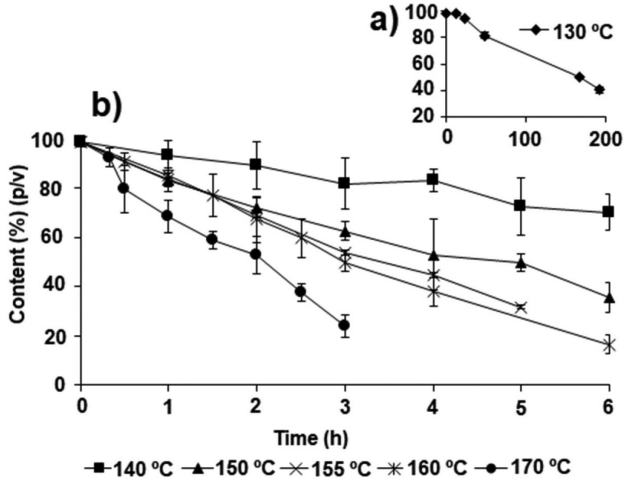

Figure 3. *a) Remaining ALG content (%) submitted to 130 °C in an oven followed by LC-PDA. b) Remaining ALG content (%) submitted to 140, 150, 155, 160, and 170 °C in an oven followed by LC-PDA*

Table 2. Correlation coefficient (R) values from plotting the graphs of zero- (content (%) *vs.* time (h)), first- (log of the content (%) *vs.* time (h)), and second- (1/content (%) *vs.* time (h)) order at different temperatures

Temperature (°C)	Zero-order R	First-order R	Second-order R
130	–0.9963	–0.9947	–0.9847
140	–0.9816	–0.9788	–0.9738
150	–0.9895	–0.9888	–0.9597
155	–0.9968	–0.9931	–0.9612
160	–0.9946	–0.9841	–0.9120
170	–0.9914	–0.9750	–0.9128

The Arrhenius plot was obtained from the K_0 values for each temperature (Equation 5): Ln K (h^{-1}) *versus* 1/T (Kelvin). Then, the line equation was obtained (y = −18083x + 44.567, R^2 = 0.835), which calculated K at 25 °C (K_{25}), obtaining 1.004×10^{-7} h^{-1}. *Ea* and *A* were also calculated (Equations 4 and 5), resulting in 35,930.18 cal mol^{-1} and 2.26×10^{19} h^{-1}, respectively.

The chromatograms showed degradation product formation in all temperatures (retention time (RT) of around 7.5 minutes) (called peak 2) after ALG elution (RT = 7.0 minutes) (Figure 4). Another

peak appeared before drug elution (RT about 3.0 minutes) (called peak 1). However, the peak purity analysis by the PDA showed that peak 1 was impure. The purity of peak 2 was compatible with a single substance, but the formation rate of this degradation product was not linear, meaning that, as the drug content decreased, the degradation product did not form in the same proportion, making it hard to monitor the degradation product alone to calculate the kinetic parameters. Lu *et al*. (2016)[26] showed, after thermal hydrolysis, a loss of the amino group and nitrogen displacement in the ALG piperidine ring (Figure 1) occurs, forming a product with polarity similar to ALG and RT similar to the drug. This study did not identify the degradation product (peak 2), but a product with polarity similar to ALG might have formed, that is, similar to the degradation product 2 - [[6-(3-piperidinylamino)-3,4-dihydro-3-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]methyl]-benzonitrile formed after thermal hydrolysis, as described by Lu *et al*. (2016).[26]

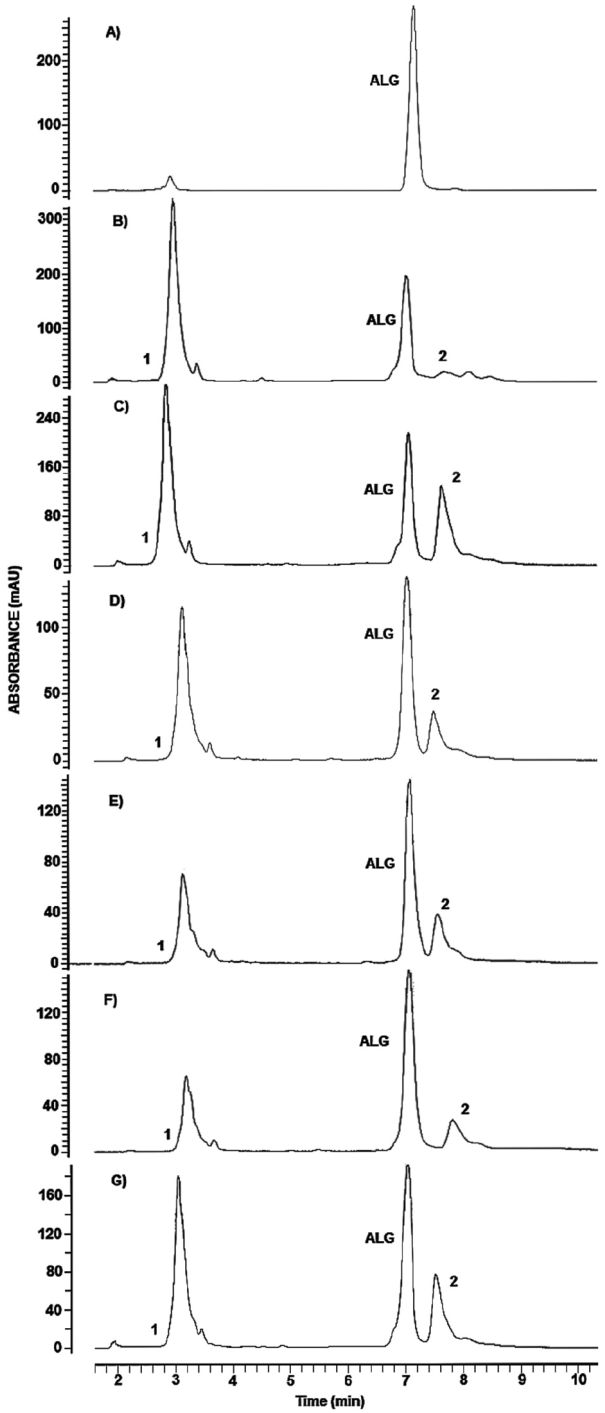

Figure 4. *Chromatograms of A) ALG without degradation. ALG exposed to: B) 130 °C for 168 hours (remaining content of about 50%), C) 140 °C for five hours (remaining content of about 70%), D) 150 °C for five hours (remaining content of about 50%), E) 155 °C for four hours (remaining content of about 45%), F) 160 °C for 2.5 hours (remaining content of about 60%), and G) 170 °C for two hours (remaining content of about 50%). Peaks 1 and 2 are the degradation peaks 1 and 2*

The Ln of the rate constants obtained at 150, 155, 160, and 165 °C by degradation in an oven followed by LC-PDA (Ln K of the content loss) and TGA (Ln K of the mass loss) were compared, resulting in a positive correlation (Figure 5). The coefficient of determination (R^2) value shows a correlation of 92% (0.9225 × 100) between the response of variables x and y.

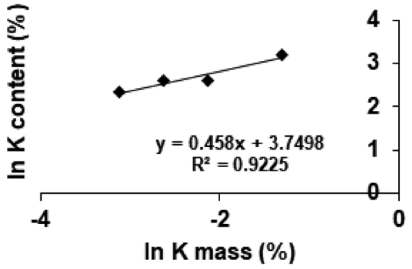

Figure 5. *Comparison of Ln of the rate constants obtained at 150, 155, 160, and 165 °C with TGA and in an oven followed by LC-PDA*

Our study used different methodologies to determine the kinetic parameters of ALG. *Ea* values were similar in all methods, but *A* and K_{25} were different. *Ea* is the activation energy for the degradation reaction. *A* (Arrhenius frequency factor) is the frequency of collisions between the molecules of reactants. K_{25} is the degradation rate at 25 °C and can be used to calculate shelf-life. Studies are often performed at high temperatures, and the data are extrapolated to 25 °C. The differences in data (*A* and K_{25}) can relate to the degradation rate obtained by the techniques. The degradation in TGA was lower, affecting the data.

The TGA method is common to evaluate the kinetics of solids due to the ease and speed of data collection. The isothermal method takes longer than the non-isothermal, but it provides reliable results. Several studies using the isothermal method calculated the kinetic parameters with a mass loss of up to 10%. However, an optimal mass loss should be higher than 10%. Using the Arrhenius method to estimate storage time presents a limitation, in which degradation rates lower than 10% impair the distinction between kinetic reaction orders.[9]

TGA allows determining kinetic parameters such as *Ea*, but the expiration date is not always informed.[22,23,27-29] Using high temperatures to estimate drug degradation usually provides unrealistic expiration date values because it is a heterogeneous system and should not be extrapolated to room temperature.

It is essential to determine and understand the degradation process of a drug to prevent unexpected reactions that will affect the produced formulations. This data reveals what is the drug degradation process and whether the degradation rate will depend on the drug alone or on some degradation product potentially formed in the process.[10] For instance, if the drug follows first-order kinetics, the degradation rate depends on the concentration of one of the reactants (*i.e.*, the higher the concentration, the higher the degradation rate). ALG follows zero-order kinetics, in which the degradation rate does not depend on reagent concentration and is constant.

Studies using kinetics to evaluate drug degradation have also been encouraged to use biological products to predict decomposition

behavior.[15] Studies under accelerated or forced conditions may provide valuable support data for establishing the expiration date, promoting product stability information for future product development, assisting in analytical method validation for the stability program, generating data that may help elucidate drug degradation profiles, and helping reveal degradation patterns. Therefore, these changes should be monitored under the proposed storage conditions.[30] The European Medicine Agency determines that the definition of shelf-life and storage conditions of investigational medicinal products should be based on the stability profile, and extrapolations (at higher temperatures) may be used.[31]

In this study, only temperature was evaluated as a degradation factor, but other factors can lead to drug degradation, such as light, oxidation, and humidity. ALG contains the pyrimidine ring (Figure 1, ring 2), and nitrogen is linked to two carbonyls, which may be susceptible to hydrolysis in the presence of moisture. The double bonds (alkenes) in the molecule may be prone to oxidation and photodecomposition reactions. As a solution, ALG undergoes hydrolysis and oxidation, as observed in previous specificity studies, and the drug is more sensitive to acid, basic, and neutral hydrolysis.[20] Lu *et al.* (2016)[26] showed the introduction of hydroxyl in pyrimidine ring (Figure 1, ring 2) after an oxidation reaction, piperidine ring (Figure 1, ring 1) is lost after acid hydrolysis, pyrimidine and piperidine rings (Figure 1, rings 1 and 2) are lost after basic hydrolysis, nitrile group of the benzyl ring is lost and there is the insertion of amide (Figure 1, ring 3) after basic hydrolysis and oxidation, and amine group is loss of piperidine (Figure 1, ring 1) after thermal degradation. Zhou *et al.* (2014)[32] describe the same impurity as Lu *et al.* (2016)[26] after acid hydrolysis. Zhou *et al.* (2014)[32] and Zhang *et al.* (2015)[33] showed the loss of the nitrile group and insertion of amide after hydrolysis, while Lu *et al.* (2016)[26] demonstrate the formation of this impurity after basic hydrolysis and oxidation. These reactions occur in a liquid medium and cannot be directly compared to reactions in a solid medium, but it is worth knowing the possible degradation routes.

Cytotoxicity of undegraded and degraded drugs

The degraded drug was less toxic than the undegraded one (Figure 6). Degradation occurred at 170 °C for two hours. Then, there was a 50% drug loss and about 15% degradation product formation. CC50% was 1,354.6 μg mL^{-1} and 2,147.46 μg mL^{-1} for undegraded ALG and degraded ALG, respectively. Determining cytotoxicity is vital to assess the biological safety of impurities, including degradation products.[18]

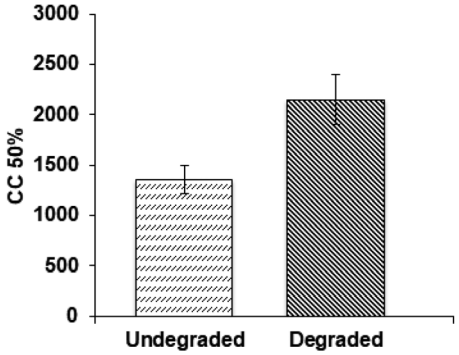

Figure 6. *Cytotoxic concentration for 50% of cell culture (CC 50%) of undegraded and degraded (170 °C for two hours) ALG*

The cytotoxicity of the degradation product of other drugs has also been studied. Degraded meropenem (in the solid state, in neutral and alkaline solutions) showed higher toxicity *in vitro* than undegraded meropenem in human mononuclear cells.[34] As for oxaliplatin, the degradation product showed toxicity similar to the drug in a human adenocarcinoma cell line.[35]

CONCLUSIONS

The results obtained with thermoanalytical methods are correlate to chromatographic techniques, considering the degradation rate from the mass loss (TGA) is directly related to the chemical degradation rate obtained in an oven followed by LC-PDA. However, TGA is faster and more practical than the chromatographic method for routine analyses and understanding the kinetic parameters of the drug. ALG degradation follows zero-order kinetics because the degradation rate does not depend on reagent concentration, remaining constant. Finally, the degraded drug showed lower toxicity in the cytotoxicity assay in CRIB cells.

REFERENCES

1. International Diabetes Federation (IDF); *About Diabetes*, 2023. [Link] accessed in April 2023
2. Drucker, D. J.; Nauck, M. A.; *Lancet* **2006**, *368*, 1696. [Crossref]
3. Gerich, J.; *Diabetes Res. Clin. Pract.* **2010**, *90*, 131. [Crossref]
4. Hanna, A.; Woo, V.; Yale, J.; Cooke, E. M.; *Can. J. Diabetes* **2012**, *36*, 9. [Crossref]
5. Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; Skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L.; *J. Med. Chem.* **2007**, *50*, 2297. [Crossref]
6. Kommanaboyina, B.; Rhodes, C. T.; *Drug Dev. Ind. Pharm.* **1999**, *25*, 857. [Crossref]
7. Bajaj, S.; Sakhuja, N.; Singla, D.; Bajaj Principal, S.; *J. Appl. Pharm. Sci.* **2012**, *02*, 129. [Crossref]
8. Huynh-Ba, K.; *Handbook of Stability Testing in Pharmaceutical Development*, 2009th ed.; Springer: New York, 2009.
9. Florence, A. T.; Attwood, D.; *Princípios Físico-Químicos em Farmácia*, 2ª ed.; Pharmabooks: São Paulo, 2011.
10. Clancy, D.; Hodnett, N.; Orr, R.; Owen, M.; Peterson, J.; *AAPS PharmSciTech* **2017**, *18*, 1158. [Crossref]
11. Blessy, M.; Patel, R. D.; Prajapati, P. N.; Agrawal, Y. K.; *J. Pharm. Anal.* **2014**, *4*, 159. [Crossref]
12. Ball, D. W.; *Físico-Química*, 1ª ed.; Cengage Learning: São Paulo, 2006.
13. Alsante, K. M.; Ando, A.; Brown, R.; Ensing, J.; Hatajik, T. D.; Kong, W.; Tsuda, Y.; *Adv. Drug Delivery Rev.* **2007**, *59*, 29. [Crossref]
14. Fu, M.; Perlman, M.; Lu, Q.; Varga, C.; *J. Pharm. Biomed. Anal.* **2015**, *107*, 370. [Crossref]
15. Kuzman, D.; Bunc, M.; Ravnik, M.; Reiter, F.; Žagar, L.; Bončina, M.; *Sci. Rep.* **2021**, *11*, 1. [Crossref]
16. Giron, D.; *Pharm. Sci. Technol. Today* **1998**, *1*, 191. [Crossref]
17. Rodante, F.; Vecchio, S.; Tomassetti, M.; *J. Pharm. Biomed. Anal.* **2002**, *29*, 1031. [Crossref]
18. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH); *Q3a (R2): Impurities in New Drug Substances*; 2006. [Link] accessed in May 2023
19. Bertol, C. D.; Pereira, R. N.; Mendes, C.; Paulino, A. S.; Silva, M. A. S.; Froehlich, P. E.; *J. Therm. Anal. Calorim.* **2017**, *130*, 1175. [Crossref]
20. Bertol, C. D.; Friedrich, M. T.; Carlos, G.; Froehlich, P. E.; *J. AOAC Int.* **2017**, *100*, 400. [Crossref]
21. Takeuchi, H.; Baba, M.; Shigeta, S.; *J. Virol. Methods* **1991**, *33*, 61. [Crossref]
22. Murakami, F. S.; Bernardi, L. S.; Pereira, R. N.; Valente, B. R.; Vasconcelos, E. C.; Carvalho Filho, M. A. S.; Silva, M. A. S.; *Pharm. Chem. J.* **2009**, *43*, 716. [Crossref]

23. Bertol, C. D.; Cruz, A. P.; Stulzer, H. K.; Murakami, F. S.; Silva, M. A. S.; *J. Therm. Anal. Calorim.* **2010**, *102*, 187. [Crossref]
24. Felix, F. S.; da Silva, L. C. C.; Angnes, L.; Matos, J. R.; *J. Therm. Anal. Calorim.* **2009**, *95*, 877. [Crossref]
25. Gomes, A. P. B.; Souza, F. S.; Macêdo, R. O.; *J. Therm. Anal. Calorim.* **2003**, *72*, 545. [Crossref]
26. Lu, Y.; Yang, D.; Li, Z.; Hang, T.; Song, M.; *J. Pharm. Biomed. Anal.* **2016**, *128*, 253. [Crossref]
27. Burnham, L.; Dollimore, D.; Alexander, K. S.; *Thermochim. Acta* **2002**, *392–393*, 127. [Crossref]
28. Tita, B.; Fulias, A.; Marian, E.; Tita, D.; *Rev. Chim. (Bucharest, Rom.)* **2009**, *60*, 524. [Link] accessed in April 2023
29. Medeiros, A. C. D.; de Cervantes, N. A. B.; Gomes, A. P. B.; Macedo, R. O.; *J. Therm. Anal. Calorim.* **2001**, *64*, 745. [Crossref]
30. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH); *ICH Topic Q 5 C: Stability Testing of Biotechnological/Biological Products*, 1996. [Link] accessed in April 2023
31. European Medicines Agency (EMEA); *Guideline on the Requirements for the Chemical and Pharmaceutical Quality Documentation Concerning Investigational Medicinal Products in Clinical Trials*, 2017. [Link] accessed in April 2023
32. Zhou, Y.; Zhou, W.; Sun, L.; Zou, Q.; Wei, P.; Ouyang, P.; *J. Sep. Sci.* **2014**, *37*, 1248. [Crossref]
33. Zhang, K.; Ma, P.; Jing, W.; Zhang, X.; *Asian J. Pharm. Sci.* **2015**, *10*, 152. [Crossref]
34. Mendez, A.; Chagastelles, P.; Palma, E.; Nardi, N.; Schapoval, E.; *Int. J. Pharm.* **2008**, *350*, 95. [Crossref]
35. Jerremalm, E.; Hedeland, M.; Wallin, I.; Bondesson, U.; Ehrsson, H.; *Pharm. Res.* **2004**, *21*, 891. [Crossref]