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Thermal characterization of lovastatin in pharmaceutical formulations

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Abstract Thermogravimetry (TG) and differential scanning calorimetry (DSC) are useful techniques that have been successfully applied in the pharmaceutical industry to reveal important information regarding the physicochemical properties of drug and excipient molecules such as polymorphism, stability, purity, and formulation compatibility among others. In this study, lovastatin was studied by TG, DSC, and other techniques such as Fourier transform infrared spectroscopy, optical microscopy, X-ray diffraction, chromatography, and mass spectrometry. Lovastatin showed melting point at 445 K and thermal stability up to 535 K. It presented morphological polymorphism, which in the drug has the same unit cell, but with different crystal habits. Preservative excipient butylhydroxyanisole (BHA) causes amorphization of lovastatin crystallites and, therefore is incompatible with lovastatin. Degradation by hydrolysis was observed under neutral, acid, and basic conditions. The active degradation product, lovastatin hydroxyacid, was obtained after neutral and basic hydrolysis.

Keywords Lovastatin · Thermal analysis · Dosage form · Pharmaceutical formulations

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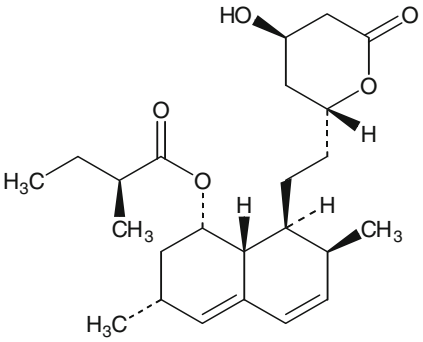
Introduction

Several reports in the literature demonstrate the importance of thermal analysis by thermogravimetry (TG) and differential scanning calorimetry (DSC) in the characterization, polymorphism identification, purity evaluation of drugs, compatibility studies for the pharmaceutical formulation, stability, and drugs thermal decomposition [1–12].

Hypercholesterolemia is characterized by elevated plasma levels of low-density lipoprotein-cholesterol (LDL-c). This results in premature atherosclerosis and in increased risk of cardiovascular disease, which is the major cause of morbidity and mortality around the globe [13–15]. The chance of developing a coronary event without effective management in men and women was at least 50 and 30% higher, respectively [16]. Therefore, diagnosis and treatment of hypercholesterolemia with statins is important for cardiovascular risk reduction [17]. In fact, the antilipemic statin drugs are widely consumed in Brazil and most of them are chemically derived from lovastatin (I) with minor chemical modifications.

Lovastatin, $C_{24}H_{36}O_5$, is a white crystalline powder with a melting point at 447.5 K, under N_2 atmosphere. It presents maximum UV absorptions at 231, 238, and 247 nm in acetonitrile. It is quite soluble in chloroform (350 mg/mL), soluble in acetone (47 mg/mL), sparingly soluble in acetonitrile (28 mg/mL), methanol (28 mg/mL), ethanol (16 mg/mL), and n-propanol (11 mg/mL) [18]. Lovastatin

is practically insoluble in water (0.4 mg/mL) and has a partition coefficient, Log P (octanol/water), of 4.26 [18, 19].



(I)

Drugs as lovastatin, with low solubility and high permeability belongs to the Class II in the biopharmaceutics classification system (BCS), in which, dissolution process is the rate-limiting step for the absorption, and IVIVC (in vivo–in vitro correlation) can be expected [19–21]. Hence, it is important to evaluate drug features, such as the presence of polymorphism, stability, and compatibility of the pharmaceutical formulation, since any changes can directly influence its bioavailability.

Therefore, the aim of this study was to evaluate the characterization of lovastatin using a variety of techniques including TG and DSC, thermal optical analysis (TOA), optical microscopy, Fourier transform infrared spectroscopy (FTIR), chromatography, mass spectrometry, and X-ray diffraction (XRD). Formulation compatibility studies, identification of polymorphism, and degradation products were carried out to help understanding the solid-state characterization, consequently, evaluate the quality control for this important active pharmaceutical ingredient.

Experimental

Lovastatin characterization was performed using TG, DSC, and IR. TG curves (thermobalance TGA50H, Shimadzu) used conditions were heating rate 10 K/min, from room temperature up to 1023 K, nitrogen flow rate 50 mL/min, with a mass of 5.0 mg in an alumina crucible. DSC curves (DSC50 calorimeter, Shimadzu) were obtained under nitrogen flow rate 50 mL/min, heating rate 10 K/min from room temperature up to 673 K. The aluminum crucible was partially closed with about 0.5–1.0 mg of sample. The assessment of purity by DSC was made by Van't Hoff equation using the Shimadzu Purity Determination Program Software, version 2.20.

The FTIR experiments were carried out using a Spectrum One Perkin Elmer spectrometer. The spectrum was obtained using the KBr disks, with resolution of 0.5 cm^{-1}, and 10 scans.

Compatibility studies were performed by DSC technique, considering three local market formulations containing 20 mg of lovastatin: two generics (A and B) and one reference dosage form (C). The excipients of each formulation were (i) A—citric acid, ethanol, corn starch, butylhydroxyanisole (BHA), indigo blue dye, magnesium stearate, povidone 30 (Kollidor K30®), microcrystalline cellulose, and ascorbic acid; (ii) B—BHA, lactose, starch, microcrystalline cellulose, magnesium stearate, and indigo; and (iii) C—lactose hydrate, pregelatinized starch, micronized cellulose, magnesium stearate, and butylhydroxyanisole (BHA).

All ingredients listed for the tablets development were individually evaluated by DSC. In addition, 1:1 ratio binary mixtures of drug to each excipient, the commercial pharmaceutical formulations and placebo (of Formulation B) were tested to evaluate lovastatin thermal behavior and the formulations compatibility. For the search for drug–excipient interactions in the binary mixtures 5 mg of the drug was used with the same amount of excipient, to maximize the probability of observing an interaction. Then, multicomponent mixtures, as it occurs in dosage forms were evaluated [22–24].

The search for polymorphs occurrence began with the drug recrystallization under different conditions using the following solvents: dichloromethane, methanol, ethanol, water, acetone, and hexane; room (303 K) and cooled (263 K) temperatures; saturated and diluted solutions were used. Analysis was performed by DSC, thermo-optical analysis (TOA) (FP90 and FP82OA Mettler Toledo), optical microscopy coupled with camera (Siedentopf), and X-ray powder diffraction (XRD). For the XRD experiments, a Geigerflex Rigaku diffractometer with cobalt tube (CoK_{α}), operating voltage at 32.5 kV and 25.0 mA current was used. A D5000 Siemens diffractometer (CuK_{α} tube, 40 kV and 30 mA) equipped with graphite monochromator for sample under spinning, 60 rpm, with steps of 0.01°/2θ with constant time of 20 s per increment was used to obtain crystal refinement by the Rietveld method [25–27].

Chromatographic studies using HPLC/UV-DAD (HP1200, Agilent) and HPLC/MS–MS (Quattro LC, Micromass) in the electrospray ionization (ESI) positive mode were performed. The search for identifying degradation products after drug stress conditions could possibly be correlated with the degradation products from the incompatibilities found by thermoanalytical studies of DSC. The stress conditions (intrinsic stability) of lovastatin was systematically investigated after 4 h of exposure under distinct conditions: (i) dry heat at 378 K, (ii) reflux over steam bath in water, (iii) in NaOH 1 M, (iv) in HCl 1 M, (v) in aqueous solution of H_2O_2 3%, and (vi) UV light (254 nm).

HPLC/UV-DAD method used was developed and optimized according to lovastatin monograph described in the United States Pharmacopeia [25]. Chromatographic conditions used were: column RP8 (OS, 250 × 4 mm, 5 µm, Merck), mobile phase: acetonitrile/phosphoric acid 0.1% V/V (65:35); 1.5 mL/min, injection volume of 10 µL; UV-DAD detection: λ 238 nm, 303 K, samples concentration 40 µg/mL in acetonitrile [28–30].

Results and discussion

After DSC and TG drug characterization, lovastatin (Fig. 1, bottom; Fig. 2, curve 1, respectively) showed melting point at 445 K with characteristic endotherm peak and fusion heat (ΔH) of 87.6 J/g, and the thermal decomposition begins after fusion and showed thermal stability up to 503 K. The infrared spectrum showed no wavenumber displacement compared to the reference spectrum, registered in British Pharmacopoeia, and displayed major bands at 1603, 1590, 1564, 1248, 1188, and 1109 cm^{-1} [19, 31].

Generally, in compatibility studies, thermal analysis techniques allow the prior choice of more stable pharmaceutical formulations at very short time. This can be observed through by the evaluation of the interactions that may occur, first in their binary mixtures and afterward in their multicomponent mixtures. The quality of the provided information along with the speed of analysis is welcome and desirable for the pharmaceutical industry, but it does not substitute conventional stability studies implied by regulatory agencies [32]. The DSC curves profile from compatibility studies may show changes in the fusion range, shape or area of the peaks, as well as, appearance or disappearance of thermal events after mixing two components. They may indicate interactions or chemical reactions that must be confirmed by other analytical techniques.

In the DSC curves for the binary mixture of lovastatin with each described excipient (Fig. 1), changes at the

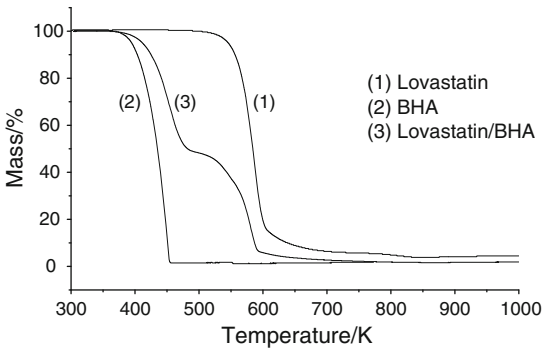


Fig. 2 TG curves for (1) lovastatin, (2) BHA, and (3) their binary mixture (1:1)

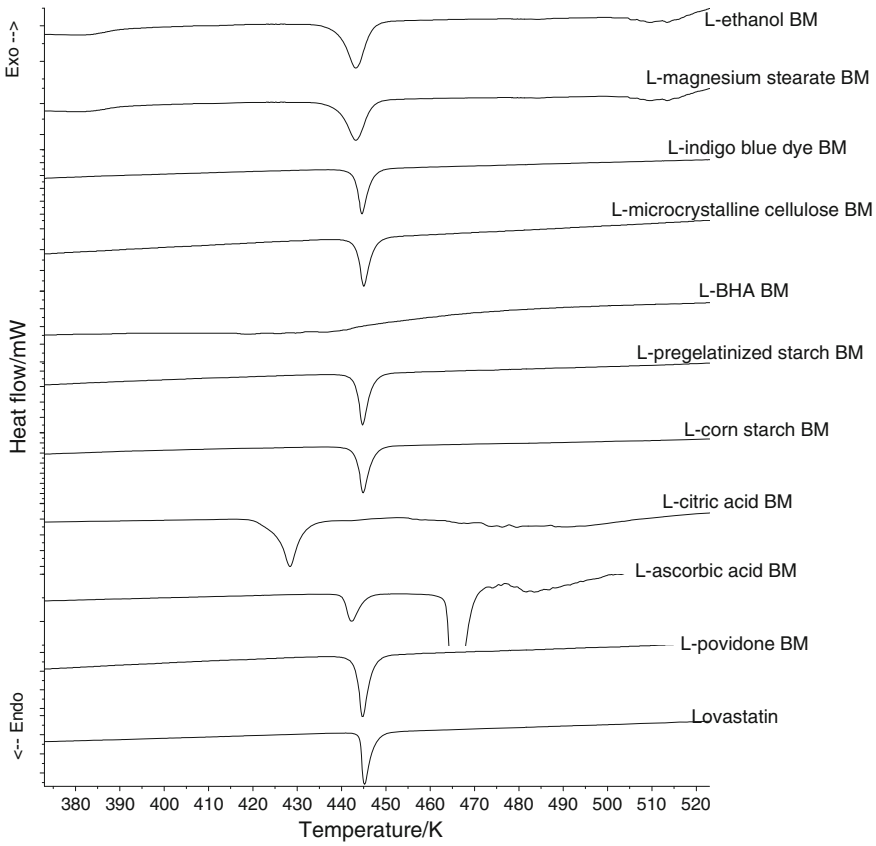


Fig. 1 DSC curves for lovastatin and its binary mixtures (BM, 1:1)

melting point of the drug were observed for the correspondent lovastatin/BHA and lovastatin/citric acid mixtures. In the mixture lovastatin:BHA (1:1), the drug melting point disappeared, indicating a possible drug incompatibility. For the lovastatin/citric acid evaluation using TOA, it was observed that this excipient melts around 428 K, before that of the lovastatin. Because lovastatin dissolves in it, this can explain the disappearance of the drug melting peak for such binary mixture by DSC. This observation does not characterize itself as an incompatibility neither as a chemical reaction. For the other excipients tested, the drug full merger remained the same with a small change in ΔH (J/g). However, the most significant thermal event occurred in the same temperature range, with little change according to the curves presented in Fig. 1, these small changes may indicate the occurrence of weak interactions in lovastatin tablets. When pharmaceutical formulations or multicomponent mixtures were analyzed by DSC, the event of lovastatin fusion disappears as happened to the mix lovastatin/BHA.

In order to better assess the drug interaction with BHA, a new evaluation by TG (Fig. 2) were conducted. However, the events of mass loss in the TG of the mix lovastatin/BHA did not show any incompatibility, as a result of the sum of the individual events, suggesting that there is no chemical reaction.

Analyzing the lovastatin/BHA mixture by XRD (Fig. 3), it was observed that there was a drug amorphization after physical contact for a short time period of 10 days in 1:1 ratio. It suggested that the BHA does not have a chemical reaction with the drug, but, there is a chemical interaction between them. Similarly, the amorphization process of antilipemic simvastatin is already known after contact with PVP (poly(N-vinyl pyrrolidone)) [33]. However, as the concentration of BHA (0.2%) in the dosage form is very small, it may explain the fact that the pharmaceutical product does not present amorphization in larger scales.

The search for identification of lovastatin polymorphism began with DSC analysis at different temperature rates. Heating rates of 2 and 20 K/min under nitrogen atmosphere, from room temperature up to 453 K, revealed no crystalline transition events and there were no double melting peaks. In principle, this discharges the presence of polymorphs in lovastatin. Recrystallization was performed under different conditions, such as different solvents, temperatures, and solution saturations. By optical microscopy, differences in crystal morphologies were observed. The crystals obtained from water and from acetone presented a tabular form, while the crystals from methanol and from ethanol presented an acicular (needle) form (Fig. 5, in box).

With the aim of identifying the polymorphic forms, DSC curves were obtained (Fig. 4) to acicular and tabular forms, no crystalline transition events and there were no double melting peaks, indicating no polymorphism. It can be observed that the crystals have the same melting point and temperatures with purity exceeding 99% for acicular and tabular crystals, according to Van't Hoff equation [34–36].

By powder XRD analysis it was possible to observe that there were differences between the crystallographic material from the acicular and tabular crystals type (Fig. 5). There are differences regarding the intensity in specific plans for reflection, showing the same crystal unit cell with some preferred orientation in the development of macroscopic crystallites in different ways.

The experimental diffraction patterns were fitted by Rietveld algorithm, and the structure due to both diffraction experiments is the tabular crystal form for lovastatin (Fig. 6, Table 1). Lovastatin did not show the classic polymorphism, nevertheless, it presented a distinctive morphology. It presented the same unit cell dimensions under different crystal habits, indicating that different solute–solvent interactions occur during the preferential crystallization process. This polymorphism does not alter

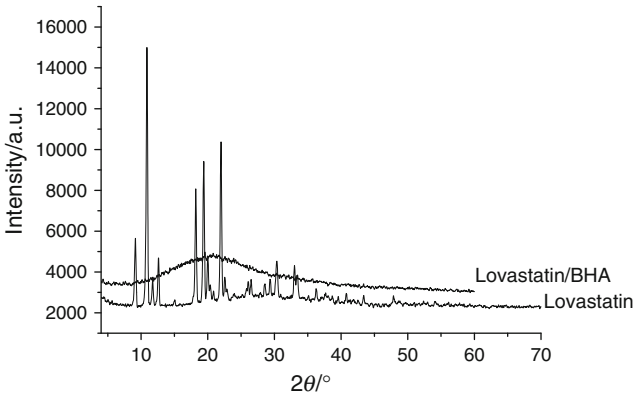


Fig. 3 XRD crystalline profile obtained for lovastatin and binary mixture lovastatin/BHA

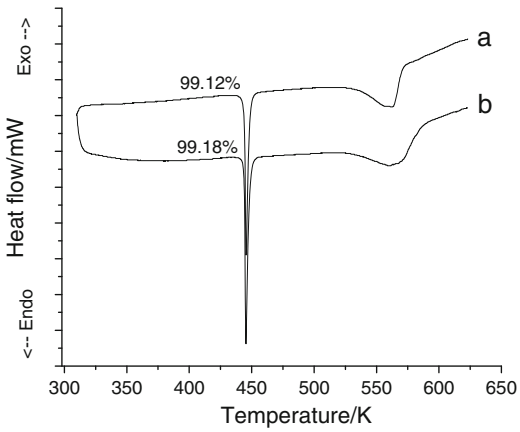


Fig. 4 DSC results obtained for lovastatin crystals in (*a*) acicular and (*b*) tabular form

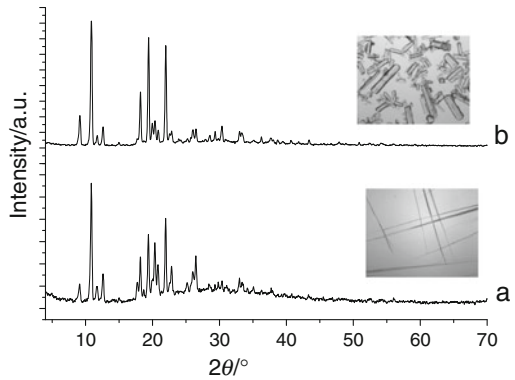


Fig. 5 XRD results obtained for lovastatin crystals in (*a*) acicular and (*b*) tabular form, and images obtained by optical microscopy (100×)

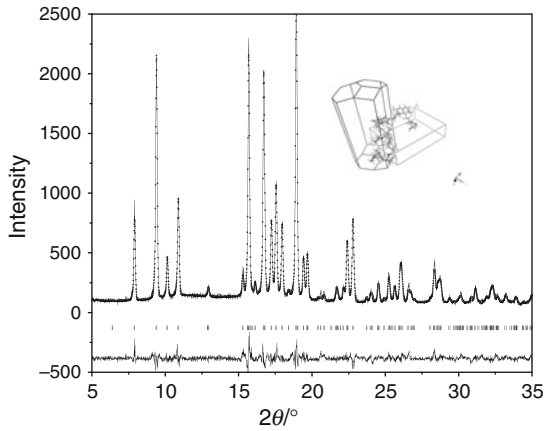


Fig. 6 Powder X-ray diffraction of lovastatin, acicular form, fitted by the Rietveld method. *Insert*: packing diagram of lovastatin and morphological habit

Table 1 Fitted X-ray parameters for lovastatin by Rietveld method

Parameter	Results
Lattice	Orthorhombic
Space group	$P_{21\ 21\ 21}$
a	22.59364 ± 0.04907
b	17.65434 ± 0.03816
c	6.08425 ± 0.01322
α	90.00000°
β	90.00000°
γ	90.00000°
Final R_{wp}	9.46%
Final R_p	7.19%

its physicochemical properties. Both forms have the same stability, solubility, and reactivity.

A HPLC/UV-DAD method was validated for lovastatin in the presence of degradation product: capacity factor (k') of 11.96, peak symmetry (As) of 1.04, theoretical plates/column (N) of 5089, repeatability and intermediate precision (RSD less than 2%), accuracy intra-day and inter-days with percentage recovery of 100.04 and 100.31% were satisfactorily obtained. Linear correlation coefficient (r) was greater than 0.99 in the range of 1–60 μg/mL. Detection limit of 0.05 μg/mL and quantification limit of 0.15 μg/mL. Selectivity studies, performed after drug stress conditions, and robustness were appropriate.

The chromatograms of lovastatin obtained before and after exposure to each stress conditions can be seen in Fig. 7. Authors have reported that lovastatin undergoes hydrolytic degradation in acid and basic conditions, producing more than one product in each condition and these products present an UV spectra quite similar to that of the drug, suggesting that the structure of the drug chromophore still remains after its degradation [37]. Generally, as the retention time of an analyte peak is located as close as possible to the retention time of a reference peak, as well as, it shows spectra with similarity index (SI) greater than 0.99, the peaks refer to a similar compound [38].

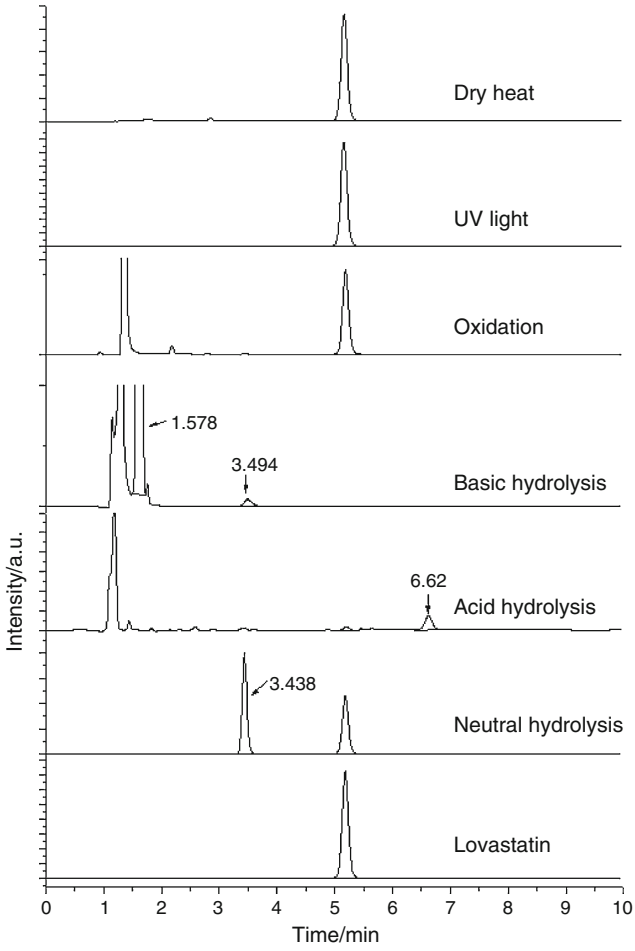
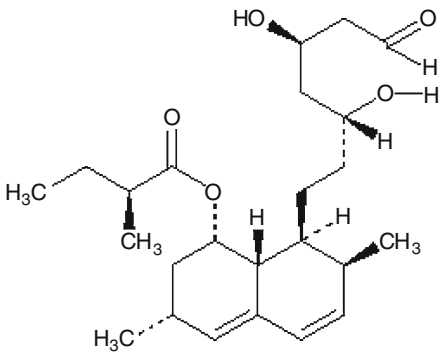


Fig. 7 Lovastatin chromatogram before (*bottom*) and after stress conditions: neutral hydrolysis; acid hydrolysis; basic hydrolysis; oxidation; exposure to UV light; and exposure to temperature (dry heat)

As shown in Fig. 7, lovastatin presented degradation when subjected to neutral, acid, and basic hydrolysis, as reported in [38]. The retention time (t_R) peak at 1.351 min, shown after oxidation, refers to the peak of hydrogen peroxide. Spectra of possible degradation products peaks were compared to lovastatin spectrum using the SI. In Fig. 8a, the sample under neutral hydrolysis shows a degradation product in t_R 3.438 min, which has an identical spectrum to that of lovastatin (t_R 5.182 min). Its similarity index (SI) 0.9997, indicating that both chromophores are the same. The samples under acid hydrolysis degraded, and t_R at 6.62 min does not refer to chromophore structure similar to the lovastatin, as it presents SI 0.0250 to lovastatin (Fig. 8b). There were no observed degradation products peaks with spectra similar to the drug, as reported in [38]. For the sample under basic hydrolysis, which was also completely degraded, the degradation products peaks related to t_R at 1.578 and 3.494 min showed SI 0.9501 and 0.9995, respectively, to lovastatin (Fig. 8c, d).

After intrinsic stability tests and observations on hydrolytic degradation of lovastatin, the drug was subjected to physical contact (4 h, 333 K) with BHA and citric acid excipients, which were given incompatible by DSC, to assess and identify a possible degradation product by HPLC. However, no degradation product peak was formed and there was no reduction of lovastatin peak area, showing that no chemical reaction between lovastatin and BHA or citric acid occurred in these conditions.

Analysis by HPLC/MS–MS was performed using the same analytical conditions than those for the validated method, except that phosphoric acid was replaced by formic acid because equipment adjustments. The degradation product peak under neutral hydrolysis at t_R 3.438 min and under basic hydrolysis at t_R 3.494 min, refers to lovastatin hydroxyacid (II) $M/Z\,(M^* + 1)$ 423.6 g/mol. Lovastatin hydroxyacid has a higher polarity than lovastatin, what explain its lower retention time in chromatographic analysis using a reversed phase column. The ultraviolet spectra similarity of the hydroxyacid with lovastatin is due to the presence of the same chromophore structure (diene) in both molecules.



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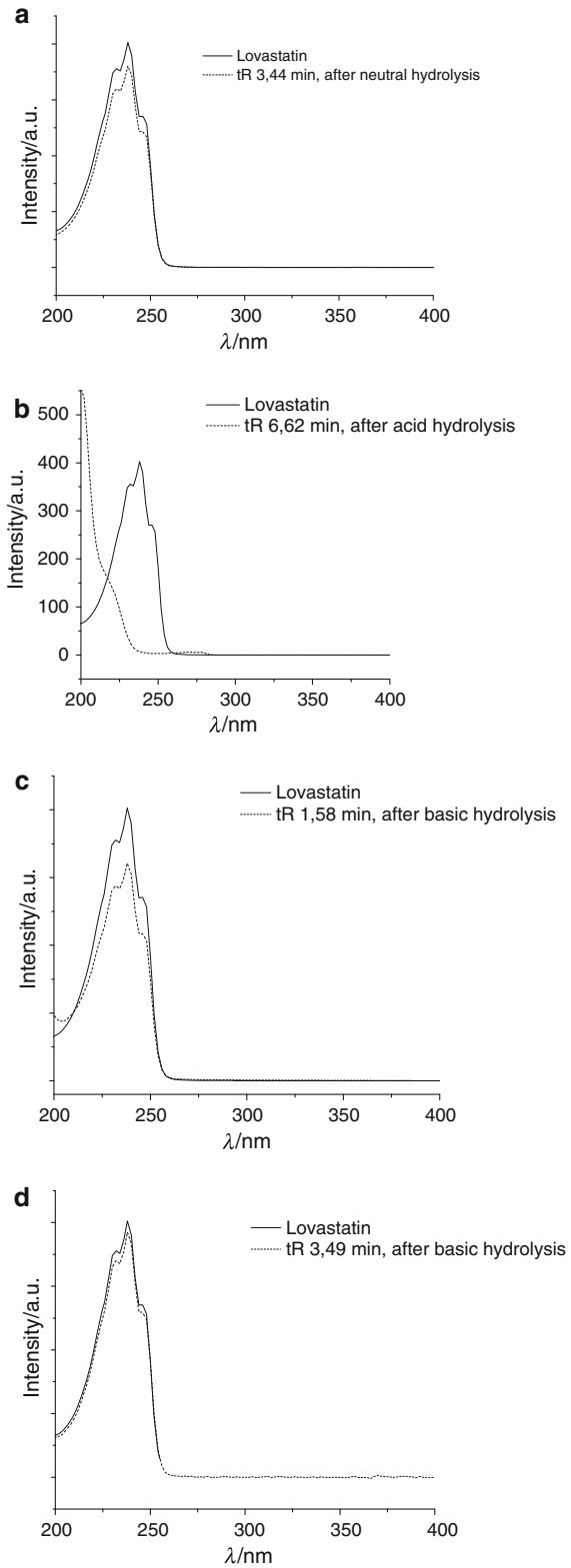

Fig. 8 Overlay of UV spectra of lovastatin (t_R 5.18, k' 11.96) before (—) and after stress conditions (– – –) obtained for degraded solutions under (**a**) neutral hydrolysis (t_R 3.4, k' 7.5), (**b**) acid hydrolysis (t_R 6.62, k' 15.55), (**c**) basic hydrolysis (t_R 1.6, k' 3.0), (**d**) basic hydrolysis (t_R 3.5, k' 7.75)

Conclusions

Lovastatin pro-drug showed thermal stability up to 503 K, melting point at 445 K, and total degradation at 535 K. The drug was compatible in the presence of all evaluated excipients, except for BHA, which triggers a process of amorphization. Lovastatin does not show classic polymorphism, however distinctive morphology, with the same unit cell and different crystal habits may occur. This morphological polymorphism does not alter its physicochemical properties and, thus, both forms, crystalline tabular or needle, have the same stability, solubility, and reactivity. Lovastatin degrades under neutral, acid, and basic hydrolysis. The active degradation product under neutral and basic hydrolysis is the lovastatin hydroxyacid.

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References

1. Craig DQM, Reading M. Thermal analysis of pharmaceuticals. New York: CRC Press; 2007.
2. Wendlandt WW. Thermal analysis. 3rd ed. New York: Wiley; 1986.
3. Gabbott P. Principles and applications of thermal analysis. Oxford: Blackwell Publishing; 2008.
4. Caira MR, Foppoli A, Sangalli ME, Zema L, Giordano F. Thermal and structural properties of ambroxol polymorphs. J Therm Anal Calorim. 2004;77:653–62.
5. Freitas MN, Alves R, Matos JR, Marchetti JM. Thermal analysis applied in the osmotic tablets pre-formulation studies. J Therm Anal Calorim. 2007;87:905–11.
6. Hassan MA, Salem MS, Sueliman MS, Najib NM. Characterization of famotidine polymorphic forms. Int J Pharm. 1997;149:227–32.
7. Laszcz M, Kosmacinska B, Korczak K, Smigielska B, Glice M, Maruszak W, Groman A, Beczkowicz H, Zelazko L. Study on compatibility of imatinib mesylate with pharmaceutical excipients. J Therm Anal Calorim. 2007;88:305–10.
8. Leitão MLP, Canotilho J, Cruz MSC, Pereira JC, Souza AT, Redinha JS. Study of polymorphism from DSC melting curves: polymorphs of terfenadine. J Therm Anal Calorim. 2002;68:397–412.
9. Macedo RO, Nascimento TG, Veras JWE. Compatibility and stability studies of propranolol hydrochloride binary mixtures and tablets for TG and DSC-Photovisual. J Therm Anal Calorim. 2002;67:483–9.
10. Oliveira GGG, Ferraz HG, Matos JSR. Thermoanalytical study of glibenclamide and excipients. J Therm Anal Calorim. 2005;79:267–70.
11. Porter WW III, Elie SC, Matzger AJ. Polymorphism in carbamazepina cocrystals. Cryst Growth Des. 2008;8:14–6.
12. Stulzer HK, Rodrigues PO, Cardoso TM, Matos JSR, Silva MAS. Compatibility studies between captopril and pharmaceutical excipients used in tablets formulations. J Therm Anal Calorim. 2008;91:323–8.
13. Hebert PR, Gaziano JM, Chan KS, Hennekens CH. Cholesterol lowering with statins drugs, risk of stroke and total mortality. An overview of randomized trial. JAMA. 1997;278:313–21.
14. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Semes R. Cholesterol treatment trialists (CTT) collaborators: efficacy and safety of cholesterol-lowering treatment: propective meta-analysis of data from 90,056 participants in 14 randomized trials of statins. Lancet. 2005;366:1267–78.
15. Ward S, Lloyd JM, Pandor A, Holmes M, Ara R, Ryan A, Yeo W, Payne N. A systematic review and economic evaluation of statins for the prevention of coronary events. Health Technol Assess. 2007;11:1–160.
16. Scientific Steering Committee on behalf of the Simon Broome Register Group. Mortality in treated heterozygous familial hypercholesterolaemia: implications for clinical management. Atherosclerosis. 1999;142:105–12.
17. Rodenburg J, Vissers MN, Wiegman A, Trotsenburg ASP, Graaf A, Groot E, Wijburg FA, Kastelein JJP, Hutten BA. Statin treatment in children with familial hypercholesterolemia: the younger, the better. Circulation. 2007;116:664–8.
18. The Merck Index, Version 12:3 [Cd-Rom], Chapman & Hall, New York; 1999.
19. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons: in pharmaceuticals, body fluids and postmortem material, vol. 2. 3rd ed. London: Pharmaceutical Press; 2004.
20. Amidon GL, Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennernas H, Hussain AS, Junginger HE, Stavchansky SA, Midha KK, Shah VP. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. Mol Pharm. 2004;1:85–96.
21. FDA—Food and Drug Administration. Guidance for industry. Dissolution testing of immediate release solid oral dosage forms. FDA, CDER, 1997.
22. Balestrieri F, Magri AD, Magri AL, Marini D, Sacchini A. Application of differencial scanning calorimetry to the study of drug-excipient compatibility. Thermochim Acta. 1996;285:337–45.
23. Bazzo GC, Segatto Silva GC. Estudo termoanalítico de comprimidos revestidos contendo captopril através de termogravimetria (TG) e calorimetria exploratória diferencial (DSC). Braz J Pharm Sci. 2005;41:315–22.
24. Cides LCS, Araújo AAS, Santos-Filho M, Matos JR. Thermal behaviour, compatibility study and decomposition kinetics of glimepiride under isothermal and non-isothermal conditions. J Therm Anal Calorim. 2006;84:441–5.
25. Carini JP, Pavei C, Silva APC, Machado G, Mexias AS, Pereira VP, Fialho SL, Mayorga P. Solid state evaluation of some thalidomide raw materials. Int J Pharm. 2009;372:17–23.
26. Sperandeo NR, Karlsson A, Cuffini S, Pagola S, Stephens PW. The crystal structure and physicochemical characteristics of 2-hydroxy-N-[3(5)-pyrazolyl]-1,4-naphthoquinone-4-imine, a new antitrypanosomal compound. AAPS Pharm. Sci Technol. 2005;6:E655–63.
27. Tedesco E, Giron D, Pfeffer S. Crystal structure elucidation and morphology study of pharmaceuticals in development. Cryst Eng Commun. 2002;4:393–400.
28. The United States pharmacopeia. 31st ed. Rockville: United States Pharmacopeial Convention; 2008.
29. ICH Q2 (R1)—International conference on harmonization of technical requirements for the registration of drugs for human use. Validation of analytical procedures: text and methodology Q2(R1), Nov, 2005. http://www.ich.org/LOB/media/MEDIA417.pdf.
30. Snyder LR, Kirkland JJ, Glajch JL. Pratical HPLC method development. 2nd ed. New York: Wiley; 1997.
31. British pharmacopoeia 2007, Version 11.0 [Cd-Rom], London: The Stationery Office; 2007.
32. ICH Q1A (R2)—International conference on harmonization of technical requirements for the registration of drugs for human

use. Stability testing of new drug substances and products Q1A(R2), Feb, 2003. http://www.ich.org/LOB/media/MEDIA419.pdf.
33. Sundaram V, Kharkar MR, Yarraguntla SR, Gudipati S, Mandava VNBR. Amorphous simvastatin. US patent application publication, US 2006/0223882 A1, Oct. 5; 2006.
34. Macedo RO, Nascimento TG, Aragão CFS, Barreto Gomes AP. Application of thermal analysis in the characterization of antihypertensive drugs. J Therm Anal Calorim. 2000;59:657–61.
35. Ferguson HF, Frurip DJ, Pastor AJ, Peerey LM, Whiting LF. A review of analytical applications of calorimetry. Therm Acta. 2000;363:1–21.
36. Rodrigues PO, Cardoso TFM, Silva MAS, Matos JR. Aplicação de técnicas termoanalíticas na caracterização, determinação da pureza e cinética de degradação da zidovudina (AZT). Acta Farm Bonaer. 2005;24:383–7.
37. Lueje AA, Pastine J, Squella JA, Nunez-Vergara LJ. Assessment of the hydrolytic degradation of lovastatin by HPLC. J Chil Chem Soc. 2005;50:639–46.
38. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons: in pharmaceuticals, body fluids and postmortem material, vol. 1. 3rd ed. London: Pharmaceutical Press; 2004.