J Therm Anal Calorim (2012) 108:207–212
DOI 10.1007/s10973-011-1770-8

Application of thermal analysis to the study of anti-tuberculosis drug compatibility. Part 1

Edilene P. Lavor · Fátima Duarte Freire · Cícero Flávio S. Aragão · Fernanda N. Raffin · Túlio Flávio A. de Lima e Moura

Received: 4 November 2010 / Accepted: 24 June 2011 / Published online: 7 September 2011

Abstract Worldwide Brazil is among one of the 22 countries with high rates of tuberculosis placing this disease as a priority for the Government Health Policies in this country. Studies with the main tuberculostatic drugs rifampicin, isoniazid, pyrazinamide, and ethambutol, aiming the development of fixed-dose combination formulations (FDCs) have been performed. The aim of this study was to evaluate the thermal behavior of these drugs by DSC, TG/DTG, and DTA in order to predict possible physical and chemical interactions between tuberculostatics. DSC and DTA curves suggested incompatibility and/or interactions among drug preparations resulting from new thermal events, as well as the disappearance and shift of the melting point of the drugs. TG/DTG curves of drug mixtures presented different profiles from those observed for the individually tested drugs, supporting the evidence of drug incompatibility and indicating that mixtures are less stable when compared to the drugs alone.

Keywords Tuberculostatic drugs · Interaction · Fixed dose combination · Thermal analysis

E. P. Lavor
Laboratório de Desenvolvimento de Medicamentos, Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal Do Rio Grande Do Norte—UFRN, R. Brigadeiro Cordeiro de Farias, s/n Petrópolis, CEP 59010-180 Natal, RN, Brazil

F. D. Freire
Laboratório de Desenvolvimento de Medicamentos, Programa de Pós-Graduação em Desenvolvimento e Inovação Tecnológica em Medicamentos, Universidade Federal do Rio Grande Do Norte—UFRN, R. Brigadeiro Cordeiro de Farias, s/n, Petrópolis, Natal, RN CEP 59010-180, Brazil

C. F. S. Aragão · F. N. Raffin · T. F. A. de Lima e Moura (✉)
Laboratório de Desenvolvimento de Medicamentos, Departamento de Farmácia, Universidade Federal Do Rio Grande Do Norte—UFRN, R. Brigadeiro Cordeiro de Farias, s/n, Petrópolis, Natal, RN CEP 59010-180, Brazil
e-mail: moura@ufrnet.br

Introduction

According to data from the Brazilian Ministry of Health and the World Health Organization (WHO) [1, 2], Brazil occupies the 18th position among the countries with high rates of tuberculosis which supports a great current interest in the study of the tuberculostatic drugs.

Rifampicin is a first line drug recommended by WHO in the treatment of tuberculosis, however, the reduced bioavailability from fixed-dose combination (FDCs) products of anti-tuberculosis drugs due to the stability issues [3] is a problem in the treatments using this drug. The thermal properties of a drug can be studied using a combination of thermal analysis which detects polymorphic tendencies, early prediction drug–drug interaction and drug-excipient incompatibility in early stages of formulation development, since it has direct influence on the stability of materials. The deficiency in delivery of the drug has serious implications that can result in the therapeutic failure and hence in the development of drug resistance [4–6].

Recent studies have focused on the development of rapid methods for checking the compatibility of active pharmaceutical ingredients (API) and inert ingredients-excipients during the early stages of the development process. These rapid methods of analysis are based on thermal analysis. Thermal analysis has been applied to any technique that involves the measurement of specific properties of the materials as a function of controlled temperature. It is used in

the pharmaceutical industry as a method fast and reliable for quality control and development of new pharmaceutical formulations in the pré-formulation studies [7–13].

The evaluation of drug stability and their possible physical and chemical interaction involves the incompatibility study, as can affect the chemical nature, the stability and bioavailability of drugs and, consequently, therapeutic efficacy and their safety. Differential scanning calorimetry (DSC) has been proposed as a rapid method to examine the possibility of physicochemical interactions, between drug(s) and drugs and excipient(s), predicting eutectic behavior of drugs and excipients for preparation of a phase diagram. Differential thermal analysis (DTA) predicting polymorphic forms and study thermal behavior kinetic analysis through thermogravimetry/derivative thermogravimetry curves (TG/DTG) since it is often necessary to predict degradation rates at marketing temperatures on accelerated processes studied at elevated temperatures [14–21].

Agrawal et al. [22] characterized solid-state properties of standard form I, form II, amorphous, and commercial samples of rifampicin using differential scanning calorimetry (DSC) and thermogravimetric analysis (TG). However, studies on the thermal behavior of isoniazid and ethambutol by DSC are scarcely found. Alves [23] studied thermal properties of standard form I and II of rifampicin and isoniazid using DSC, TG/DTG to verify interaction between drugs with results indicating interaction with both polymorphs. In this study, the TG/DTG curves showed that the polymorph I is more thermally stable than polymorph II. The thermal behavior of isoniazid and rifampicin and binary mixtures of polymorphs I and II of rifampicin with isoniazid when also studied using DSC showed that both polymorphs of rifampicin had probable interaction with isoniazid [15].

In addition, a DSC procedure has been developed for quantitation of R,S diastereoisomer of ethambutol dihydrochloride in bulk drug samples and marketed antituberculosis formulations, involving the determination of the enthalpy associated with polymorphic transitions of ethambutol [24]. On the other hand, the thermal behavior of pyrazinamide has not been reported by DSC.

Studies of thermal decomposition were executed by TG/DTG and DTA for isoniazid, pyrazinamide, and ethambutol. Three stages of thermal decomposition for all studied compounds were distinguished. On the DTA curves only endothermic peaks appeared due to the melting, however, in some cases small weight loss occurred due to evaporation of molten compounds. In the second stage of decomposition one or more intermediate products are formed. Propanol and ethylenediamine were formed from the isoniazid destruction, while pyrazine, and cyanic acid are formed from the pyrazinamide due to the reorganization of its structure [25].

The aim of this study was to characterize and evaluate the thermal behavior of first-line drugs for tuberculosis treatment and their binary mixtures by DSC, DTA, and TG/DTG.

Experimental

Rifampicin (RIF—Novartis), isoniazid (INH—Second Pharma), pyrazinamide (PZA—Davilson), and ethambutol (ETA—Hildose) samples used in this study were purchased by the Nucleus of Research on Food and Pharmaceuticals NUPLAM, Natal, Brazil).

Samples and binary mixtures

RIF, INH, PZA, and ETA samples and their binary mixtures at 1:1(w/w) rate were investigated by DSC, DTA, and TG/DTG. The powders were sieved and homogenized for 10 min using the method of geometric dilution. DSC curves were recorded in a Shimadzu DSC-60 cell using aluminum crucibles containing 2 mg of samples under dynamic N_2 atmosphere (flow rate: 50 mL min^{-1}) and 10 °C min^{-1} heating rate at a 25–400 °C temperature range. The DSC cell was calibrated with indium (m.p. 156.6 °C) and zinc (m.p. 419.6 °C). TG/DTG and DTA curves were recorded using a Shimadzu DTG-60H cell. About 4 mg of samples were placed in alumina sample holders and analyzed under dynamic synthetic air (flow rate: 50 mL min^{-1}) and 10 °C min^{-1} heating rate at a 25–900 °C temperature range. TG/DTG and DTA cell was calibrated with indium and zinc.

Results

Analysis of the drugs

The DSC, DTA, and TG/DTG curves obtained for the drugs alone are presented in Fig. 1. The DSC and DTA curves for RIF (Fig. 1a) showed a melting endotherm (181–196 °C) followed by an exothermic event characteristic of the solid–liquid–solid transition from the polymorphic form II crystal to form I (196–208 °C) according to the literature [15, 22, 23]. The decomposition process was indicated by two exothermic peaks at 242–269 and 346–408 °C. The TG/DTG curve for RIF showed that the substance was thermally stable up to 190 °C and decomposition occurred in four steps with mass loss of 5.5, 11.5, 24.3, and 54.6% at 190–222, 222–272, 272–396, and 396–609 °C, respectively. The main thermal events of the drugs are shown in Table 1.

The DSC and DTA curves for INH (Fig. 1b) showed a melting endothermic event from 170 to 176 °C followed by

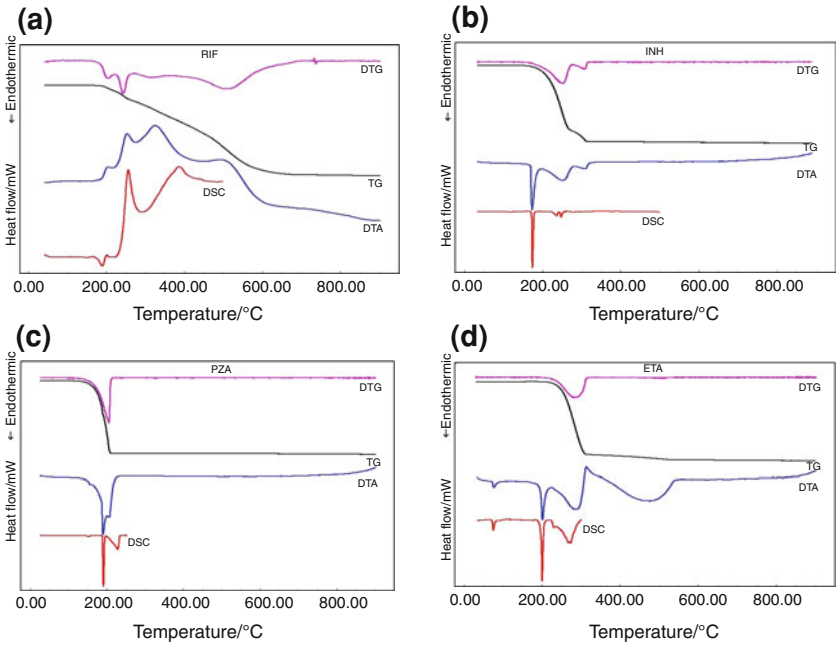

Fig. 1 DSC, TG/DTG, and DTA curves of the tuberculostatic drugs: **a** RIF. **b** INH. **c** PZA. **d** ETA

Table 1 The main thermal events found in the DSC curves of tuberculostatic drugs

Samples	DSC: transition temperature (onset–endset, °C) (enthalpy, ΔH = J/g)			
	Endotherm 1	Exotherm 1	Exotherm 2	Exotherm 3
RIF	181–196 ($\Delta H = -11$)	196–208 ($\Delta H = 3$)	242–269 ($\Delta H = 112$)	346–408 ($\Delta H = 103$)

Samples	DSC: transition temperature (onset–endset, °C) (enthalpy, ΔH = J/g)		
	Endotherm 1	Endotherm 2	Endotherm 3
INH	170–176 ($\Delta H = -256$)	227–236 ($\Delta H = -31$)	244–251 ($\Delta H = -26$)
PZA	147–152 ($\Delta H = -5$)	188–194 ($\Delta H = -177$)	219–233 ($\Delta H = -195$)
ETA	72–79 ($\Delta H = -20$)	196–204 ($\Delta H = -110$)	–

two decomposition endothermic peaks between 227–236 and 244–251 °C. In TG/DTG curves the thermal decomposition started along the fusion and occurred in the following two stages at 170–275 °C with mass loss of 83.8% and at 275–322 °C with mass loss of 14.4%.

The DSC and DTA curves for PZA (Fig. 1c) showed the polymorphic transition endotherm between 147–152 °C characteristic of the α polymorphic form [19, 26], the melting endotherm between 188–194 °C and the decomposition endothermic event (219–239 °C). It could be observed in the TG/DTG curves that the decomposition occurred in one step between 150–212 °C with mass loss of 96.2%.

The DSC and DTA curves for ETA (Fig. 1d) showed an endothermic polymorphic transition from 72–79 °C, characteristic of polymorphic transitions of the SS-isomer [24]. The melting endothermic peak occurred between 196–204 °C followed by the endothermic decomposition (225–278 °C). In the TG/DTG curves the decomposition occurred in two stages, with mass loss of 88.9 and 6.9% and between 232–314 and 314–546 °C, respectively.

Analysis of the binary mixtures

The DSC, DTA, and TG/DTG curves obtained for the binary mixtures of the tuberculostatic drugs are presented in Figs. 2. The main thermal events of the binary mixtures are shown in Table 2. In the DSC and DTA curves for the RIF + INH preparation (Fig. 2a) there was a shift of the melting point of INH between 166–173 °C beyond the disappearance of the RIF melting endothermic event. The absence of the melting endothermic event of the RIF can be attributed to the dissolution of the polymorph II in the melted INH and/or interaction with INH during its merge, since its melting endothermic event was shifted to lower temperatures [23]. The thermal behavior of the mixture suggests an interaction between the substances and indicated the decreased thermal stability of the mixture

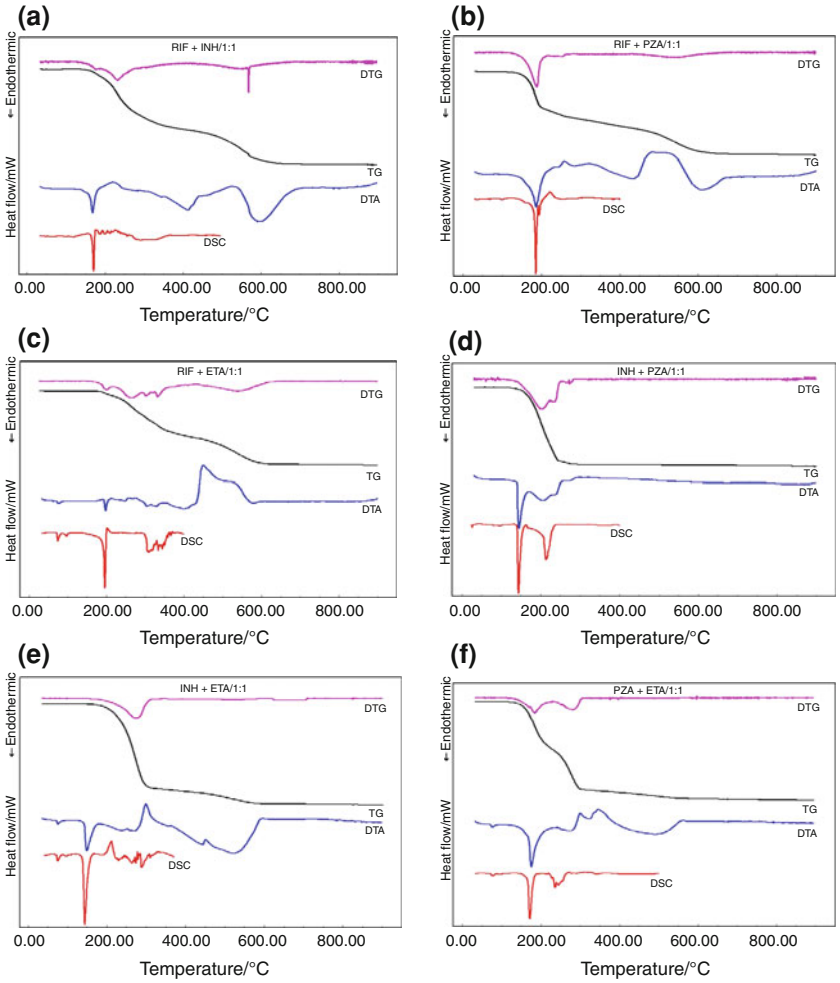

Fig. 2 DSC, TG/DTG, and DTA curves of the binary preparations with antituberculosis drugs: **a** RIF + INH, **b** RIF + PZA, **c** RIF + ETA, **d** INH + ETA and INH + ETA, **f** PZA + ETA

Table 2 The main thermal events found in the DSC curves of the binary mixtures of first-line drugs for tuberculosis treatment

Samples	Thermal events [DSC: transition temperature (onset–endset, °C) (enthalpy, ΔH = J/g)]		
	Event 1	Event 2	Event 3
RIF + INH	166–173 ($\Delta H = -92$)	–	–
RIF + PZA	183–189 ($\Delta H = -83$)	191–200 ($\Delta H = -12$)	203–232 ($\Delta H = 34$)
RIF + ETA	72–79 ($\Delta H = -8$)	193–198 ($\Delta H = -51$)	199–209 ($\Delta H = 7$)
INH + PZA	141–151 ($\Delta H = -221$)	208–228 ($\Delta H = -235$)	–
INH + ETA	72–78 ($\Delta H = -9$)	140–151 ($\Delta H = -155$)	200–217 ($\Delta H = 39$)
PZA + ETA	73–83 ($\Delta H = -8$)	168–177 ($\Delta H = -149$)	

compared to the drug alone [15]. The TG/DTG curves showed different thermogravimetric profile and the thermal decomposition occurred 24 and 44 °C below the temperatures observed for INH and RIF, respectively, reinforcing the likely interaction between the drugs. The decomposition occurred in three steps between 146–185, 185–279, and 279–660 °C with corresponding mass loss of 6.2, 39.3, and 53.6%. At first, this event at lower temperature can be attributed to the displacement of the melting point of INH, indicating a decrease in thermal stability of the mixture compared to the drug alone [15, 23]. Moreover, the disappearance of the RIF melting and decomposition events leads to the suspicion of a eutectic mixture formation.

The DSC and DTA curves for the preparation containing RIF and PZA (Fig. 2b) showed a melting endothermic peak between 183–189 °C, probably due to the merge of the two

substances, followed by the endothermic and exothermic decomposition events between 191–200 and 203–232 °C of PZA and RIF, respectively. The solid–solid transition between 147–150 °C of the α polymorphic form is not present. The TG/DTG curves showed that the thermal decomposition occurred at 19 °C below the temperature observed for PZA and at 59 °C bellow the one for RIF presenting different thermogravimetric profile from that observed for each substance. The decomposition occurred in three steps: between 131–215, 215–263, and 263–641 °C with mass loss of 45.6, 7.9, and 44.7%, respectively. In this analyzes the curves suggest probable interaction between PZA and RIF, however, Bhutani et al. [27] reported that RIF-PZA mixture shows no interaction because the mixture showed to be physically and chemically stable.

DSC and DTA curves for the RIF-ETA mixture (Fig. 2c) showed the endothermic polymorphic transition event of ETA (72–79 °C) followed by a melting endotherm from 193 to 198 °C, which can be attributed to the melting of the substance, since in this range of temperature both substances merge and the exothermic event of decomposition of RIF (199–209 °C) as well as the endothermic event of decomposition of the mixture occur. The curves TG/DTG showed that the decomposition started at 170 °C, approximately 62 °C below the temperature observed for the ETA and 20 °C below the one for RIF alone, and occurred in five steps between 170–213 °C (5.8%), 213–295 °C (27.2%), 295–324 °C (10.3%), 324–435 °C (20.6%), and 435–626 °C (35.5%) (TG/DTG curves). The curves suggest a probable interaction; however, the hypothesis of eutectics formation should not be discarded. On the other hand, studies of interaction studies under accelerated conditions of temperature and humidity, where the RIF is in combination with ETA presents 10% degradation for both drugs and the identification of 3-formylrifamycin as the major degradation product [27]. The TG/DTG curves of the mixture of RIF-ETA show decomposition temperature compared to the drugs alone, which corroborate with these results, denoting that the association of these drugs decrease stability of both.

The DSC and DTA curves for INH and PZA (Fig. 2d) showed a melting endothermic peak from 141 to 151 °C not characteristic to any of the substances and the second event of decomposition of the mixture (208–228 °C). The melting endothermic peak at lower temperatures, around 29 and 47 °C concerning INH and PZA, for onset temperatures, suggests the occurrence of interaction between the drugs or eutectic formation. The TG/DTG curves showed three steps of the thermal decomposition between 140–222 °C (70.8%), 222–252 °C (23.1%), and 252–284 °C (3.9%) which started at about 30 °C below the temperature observed for INH and 79 °C below the one for PZA reinforcing the assumption of interaction between the drugs.

The DSC and DTA curves for the INH and ETA preparation (Fig. 2e) showed endothermic polymorphic transition peak of ETA between 72–78 °C, and the endothermic peak between 140–151 °C, which are not characteristic of any of the analyzed substances, followed by exothermic and endothermic decomposition events between the range of 200–217 and 285–296 °C ($\Delta H = -19$ J g^{-1}).

The TG/DTG curves for the INH and ETA preparation showed mass loss between 179–322 (83.3%) and 322–590 °C (14.9%). The shift of the endothermic peak in the DSC curve, at about 30 °C concerning the INH alone and 56 °C for ETA, the lack of fusion events of INH and PZA and the appearance of exothermic peak (not characteristic to any of the studied substances), suggest a probable interaction between them which is reinforced by the different thermogravimetric profile observed. Hence, the polymorphic α form of PZA decomposition probably begins before the PZA melting, which shows the solid–solid phase transitions upon heating [19]. Studies of interaction performed by Bhutan et al. [27] and Singh et al. [6] reported that the mixture between INH and ETA becomes liquid under accelerated conditions of temperature and relative humidity, demonstrating physical instability due to the dissolution of the drugs upon gaining moisture. A loss indicated by the decrease in the total content was observed for INH and ETA in the amounts of 15 and 11%, respectively. Chemical interaction was also observed between these two drugs.

The DSC and DTA curves for the PZA and ETA preparation (Fig. 2f) showed the endothermic polymorphic transition peak of ETA between 73 and 83 °C and an endothermic peak in the range of 168–177 °C. This peak which is not characteristic to any of the studied substances appears at 20 and 28 °C bellow to melting endothermic peak of the PZA and ETA, respectively. The endothermic peak at 150 °C, characteristic of the α polymorphic form of the PZA, could not be observed which may suggest interaction between the drugs. The TG/DTG curves showed thermal decomposition at about 30 ℃ below that observed for PZA and 112 °C for ETA and occurred in three steps between 120–215, 215–307, and 307–560 °C with mass loss of 44.4, 45.6, and 9.2%, respectively, showing thermal instability. Bhutani et al. [27], reported that interaction occurred between PZA and ETA, resulting in the loss of ETA to an extent of 12%. These results indicate that the mixtures are less stable then each drug alone, which can lead to problems such as reduced bioavailability, impairment of therapy, and thus development of drug resistance.

Conclusions

The DSC, DTA, and TG/DTG curves for binary mixtures of tuberculostatics drugs suggest incompatibilities and/or interactions due to the emergence of new thermal events, as well as the disappearance and shift of the melting point of the drugs. These results indicate that the mixtures are less stable then each drug alone, which can lead problems such as reduced bioavailability, impairment of therapy, and thus development of drug resistance. Studies to evaluate the influence of these findings over the biopharmaceutical problems are necessary to guarantee the efficacy and safety of the antituberculosis medicine.

Acknowledgements The authors wish to thank NUPLAM/UFRN for the supply the samples and CAPES for a master fellowship.

References

1. World Health Organization. WHO report 2007. Global tuberculosis control: surveillance, planning, financing, Geneva. World Health Organization; 2007. http://www.who.int/tb/publications/global_report/2008/pdf/fullreport.pdf. Accessed 11 Jan 2010.
2. World Health Organization. Ministério da Saúde. Secretaria de Vigilância em Saúde. Programa Nacional de Controle da Tuberculose—PNCT, 2008. http://www.cve.saude.sp.gov.br. Accessed 2 Feb 2010.
3. Singh S, Bhutani H, Mariappan TT. Quality problems of anti-tuberculosis fixed-dose combinations (FDCs): a way forward. Ind J Tuberc. 2006;5:201–5.
4. Bottom R. The role of modulated temperature differential scanning calorimetry in the characterisation of a drug molecule exhibiting polymorphic and glass forming tendencies. Int J Pharm. 1999;192:47–53.
5. McDaid FM, Barker SA, Fitzpatrick S, Petts CR, Craig DQM. Further investigations into the use of high sensitivity differential scanning calorimetry as a means of predicting drug-excipient interactions. Int J Pharm. 2003;252:235–40.
6. Singh S, Bhutani H, Mariappan TT, Kaur H, Bajaj M, Pakhale SP. Behavior of uptake of moisture by drugs and excipients under accelerated conditions of temperature and humidity in the absence and the presence of light. Part 1: pure anti-tuberculosis drugs and their combinations. Int J Pharm. 2002;245:37–44.
7. Cheng SZD, Li CY, Calhoun BH, Zhu L, Zhou WW. Thermal analysis: the next two decades. Thermochim Acta. 2000;355:59–68.
8. Procópio JVV, Souza VG de, Costa RA da, Correia LP, Souza FS de, Macêdo RO. Application of thermal analysis and pyrolysis coupled to GC/MS in the qualification of simvastatin pharmaceutical raw material. J Therm Anal Calorim 2010. doi:10.1007/s10973-010-1274-y.
9. Vemuri NM, Chrzan Z, Cavatur R. Use of isothermal microcalorimetry in pharmaceutical preformulation studies. Part I. Monitoring crystalline phase transitions. J Therm Anal Calorim. 2004;78:47–54.
10. Cavatur R, Vemuri NM, Chrzan Z. Use of isothermal microcalorimetry in pharmaceutical preformulation studies. Part III. Evaluation of excipient compatibility of a new chemical entity. J Therm Anal Calorim. 2004;78:63–72.
11. Pinto MF, de Moura EA, De Souza FS, Macêdo RO. Thermal compatibility studies of nitroimidazoles and excipients. J Therm Anal Calorim. 2010;102:323–9.
12. Nunes RS, Semaan FS, Riga AT, Cavalheiro ETG. Thermal behavior of verapamil hydrochloride and its association with excipients. J Therm Anal Calorim. 2009;97:349–53.
13. 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.
14. Freire FD, Aragão CFS, de Le Moura TA, Raffin FN. Compatibility study between chlorpropamide and excipients in their physical mixtures. J Therm Anal Calorim. 2009;97:355–7.
15. Freire FD, Aragão CFS, de Le Moura TA, Raffin FN. Thermal studies of isoniazid and mixtures with rifampicin. J Therm Anal Calorim. 2009;97:333–6.
16. Avula SG, Alexander K, Riga AT. Predicting eutectic behavior of drugs and excipients by unique calculations. J Therm Anal Calorim. 2010;99:655–8.
17. Aigner Z, Heinrich R, Sipos E, Farkas G, Ciurba A, Berkesi O, Szabó-Révész P. Compatibility studies of aceclofenac with retard tablet excipients by means of thermal and FT–IR spectroscopic methods. J Therm Anal Calorim. 2010. doi:10.1007/s10973-010-1105-1.
18. Tita B, Fulias A, Szabadai Z, Rusu G, Bandur G, Tita D. Compatibility study between ibuprofen and excipients in their physical mixtures. J Therm Anal Calorim. 2010. doi:10.1007/s10973-010-1188-8.
19. Tita D, Fulias A, Tita B. Thermal stability of ketoprofen-active substance and tablets. J Therm Anal Calorim. 2010. doi:10.1007/s10973-010-1187-9.
20. Castro RAE, Maria TMR, Évora AOL, Feiteira JC, Silva MR, Beja AM, Canotilho J, Eusébio MES. A new insight into pyrazinamide polymorphic forms and their thermodynamic relationships. Cryst Growth Des. 2010;10:274–82.
21. Bertol CD, Cruz AP, Stulzer HK, Murakami FS, Silva MAS. Thermal decomposition kinetics and compatibility studies of primaquine under isothermal and non-isothermal conditions. J Therm Anal Calorim. 2010;102:187–92.
22. Agrawal S, Ashokraj Y, Bharatam PV, Pillai O, Panchagnula R. Solid-state characterization of rifampicin samples and its biopharmaceutic relevance. Eur J Pharm Sci. 2004;22:127–44.
23. Alves R. Estudo termoanalítico e de compatibilidade fármaco-excipiente de rifampicina e alguns medicamentos utilizados na terapêutica da tuberculose. Dissertation, Postgraduate in in drugs and medicines, USP, 2007.
24. Prasad B, Bhutani H, Kumar V, Singh S. A new validated differential scanning calorimetric procedure for monitoring the less active r, s isomer of ethambutol dihydrochloride in bulk drug samples and anti-tuberculosis formulations. Pharm Forum. 2007;33:326–33.
25. Wesolowski M, Konarski T. Characteristics of the thermal decomposition of antituberculosis drugs. J Therm Anal Calorim. 1995;45:1199–204.
26. Takaki Y, Sasada Y, Watanabe T. The crystal structure of 2-pyrazinamide. Acta Crystallogr. 1960;13:693–702.
27. Bhutani H, Singh S, Jindal KC. Drug-drug interaction studies on first-line anti-tuberculosis drugs. Pharm Dev and Techn. 2005;10:517–24.