J Therm Anal Calorim (2010) 102:317–321
DOI 10.1007/s10973-010-0807-8

Compatibility studies between nebicapone, a novel COMT inhibitor, and excipients using stepwise isothermal high sensitivity DSC method

J. P. Sousa e Silva · J. M. Sousa Lobo

Received: 27 January 2010 / Accepted: 13 April 2010 / Published online: 5 May 2010

Abstract Study of excipients incompatibility with drugs in an early phase of pharmaceutical development is still a persistent difficulty within the pharmaceutical industry. We examine here the compatibility between an experimental drug (nebicapone) and common excipients using differential scanning calorimetry (DSC), high sensitivity DSC (HSDSC) and a conventional heat stress test. The results obtained indicate that nebicapone may be compatible with lactose monohydrate and sodium croscarmellose but is incompatible with magnesium stearate. This study concludes that HSDSC, in stepwise isothermal mode, may be used as a potential tool for detecting excipient incompatibilities.

Keywords Nebicapone · HSDSC · Incompatibility · Drug–excipient interaction

Introduction

In simple terms the aim of pharmaceutical development is converting candidate drugs into dosage forms for registration and sale [1]. The selection of excipients, in early stages of pharmaceutical development, through drug–excipient compatibility testing is an important step in order to develop chemically stable dosage forms.

Nebicapone is a new catechol-*O*-methyltransferase inhibitor being developed for use as a levodopa-sparing agent in Parkinson's disease [2–4].

J. P. Sousa e Silva (✉) · J. M. Sousa Lobo
Faculty of Pharmacy, Pharmaceutical Technology Department, University of Porto, Rua Aníbal Cunha 164, 4050-047 Porto, Portugal
e-mail: paulo.silva@ff.up.pt

Differential scanning calorimetry (DSC) has been extensively used for evaluation of physico-chemical interactions between drug and excipients [5–12]. DSC is a thermal analysis method in which the heat flow is measured as a function of the temperature whilst the substance and the reference are submitted to a controlled variation of temperature.

The main advantage of this method for compatibility studies is its ability to quickly screen many excipients. This approach is based on the changes that occurred in the melting behaviour of substances under study, which may indicate chemical incompatibility [13]. However, due to the high temperature conditions required, the interpretation of the thermal events in order to detect incompatibilities at room temperature needs to be done cautiously to avoid erroneous conclusions.

High sensitivity DSC (HSDSC) operates on a similar basis to conventional DSC equipment, but can detect and measure subtle transitions that cannot be observed by standard DSC. The greater size of HSDSC pan sample and its higher sensitivity (±0.5 μW), approximately 10–100 fold more than conventional DSC, make it possible to detect baseline change below 1 μW [13, 14]. HSDSC is also known as Micro DSC. Some authors have indicated that stepwise isothermal HSDSC may be useful as a rapid and simple method to examine the compatibility of drugs with conventional excipients [13, 15]. But as far as we know there is very few studies published in the peer reviewed literature which provides evidence for the validity of method.

In this paper, HSDSC and DSC were used to study the possible incompatibility of nebicapone and some excipients that are often utilized in solid dosage forms. In order to compare and understand the thermoanalytical curves, the effect of 4 days at 373.15 K on samples identical to the

ones used in thermo analysis was evaluated by high performance liquid chromatography (HPLC).

This study intends to illustrate the potential of HSDSC run in stepwise isothermal mode as an alternative to studying excipients incompatibilities.

Experimental

Materials

Nebicapone was supplied by Bial, Portugal. Lactose monohydrate (DMV, Holland), sodium croscarmellose (Ac-Di-Sol®, FMC Europe N.V., Belgium) and magnesium stearate (Magnesia GmbH, Germany) comply with Ph. Eur.

Methods

The mixtures in a 1:1 (w/w) were prepared by mixing the drug and each excipient in a glass mortar.

DSC

Conventional DSC studies were conducted using a Setaram DSC 131 calibrated with indium. Samples of approximately 4 mg were placed in opened aluminium pans and heated at 10 K/min under nitrogen.

HSDSC

Samples of approximately 400 mg were loaded into the sample vessel of Setaram Micro DSC III. Stepwise isothermal studies were performed under nitrogen by increasing from 298.15 to 308.15 K at 0.6 K/min, followed by holding at 308.15 K for 1.30 h, then ramping from 308.15 to 313.15 K at 0.6 K/min, followed by holding at 313.15 K for 1.30 h, increasing from 313.15 to 333.15 K at 1 K/min, followed by holding for 1.30 h, and repeating the process at 353.15 K.

Heat stress test

Samples of drug, magnesium stearate, sodium croscarmellose and binary drug–excipient mixtures in equal parts were placed in a dryer at 373.15 K for 4 days.

Drug degradation was monitored using an HPLC method validated by the patent owner. The HPLC system consisted of a Lachrom L-7100 pump, an automatic injector model L-7250, a UV/VIS detector model L-7400 and an interface D-7000 all from Merck (Darmstadt, Germany). Peaks were integrated using the D-7000 HSM software. Chromatographic separation was performed, at room temperature, on a C8 Symmetry column (3.9 × 150 mm, 5 μm Waters, USA). The mobile phase used was water–acetonitrile (65:35) with 1% formic acid (pH 2.2 ± 0.1) at flow rate 1.2 mL/min. Samples (20 μL) of drug, magnesium stearate, sodium croscarmellose and binary drug–excipients mixtures were injected and detected at 280 nm.

Results and discussion

Thermoanalytical curves of nebicapone, lactose monohydrate, sodium croscarmellose and magnesium stearate and drug–excipient mixtures are shown in Fig. 1.

The drug curve showed a single sharp endothermic peak at 450.83 K, characteristic of its melting which is followed by thermal decomposition. The lactose DSC curve showed two endothermic peaks, the first (418.18 K) corresponding to dehydration of bond water and the second (485.36 K) to the melting point. The croscarmellose DSC trace exhibits a broad endotherm band in the 300–425 K range which may be attributed to the loss of adsorbed water. Commercially available magnesium stearate is a mixture of long-chained aliphatic acids with variable water content. This complicates the understanding of the magnesium stearate DSC curve. In the magnesium stearate DSC curve it is possible to identify a broad endotherm band (303–360 K) corresponding to dehydration, a small peak near 388 K which may be attributed to melting and an exothermic peak about 438 K that may relates to inorganic impurities, namely magnesium salts residues [16].

Interactions between compounds are derived or deduced from DSC curves by elimination of peaks or by the appearance of new ones. Modifications in peak shape, peak onset or peak maximum temperature may indicate an interaction, but it is necessary to bear in mind that some broadening of peaks is a result of mixing two different substances [17].

The thermoanalytical curve of nebicapone/croscarmellose shows a melting peak characteristic of the drug at 451.57 K and the endothermic effect due to loss of water in croscarmellose. This is generally considered as an absence of incompatibility in spite of some little differences in the peaks shape/height.

The curve of nebicapone/lactose monohydrate shows three endothermic peaks, one corresponding to the drug, at 450.79 K, and the others to lactose monohydrate de-hydratation and melting. This indicates that there is no incompatibility.

In the case of the binary mixture of drug and magnesium stearate the interpretation of the thermoanalytical curve should be done cautiously due to the overlap of the melting peak of nebicapone and the exothermic peak of excipient.

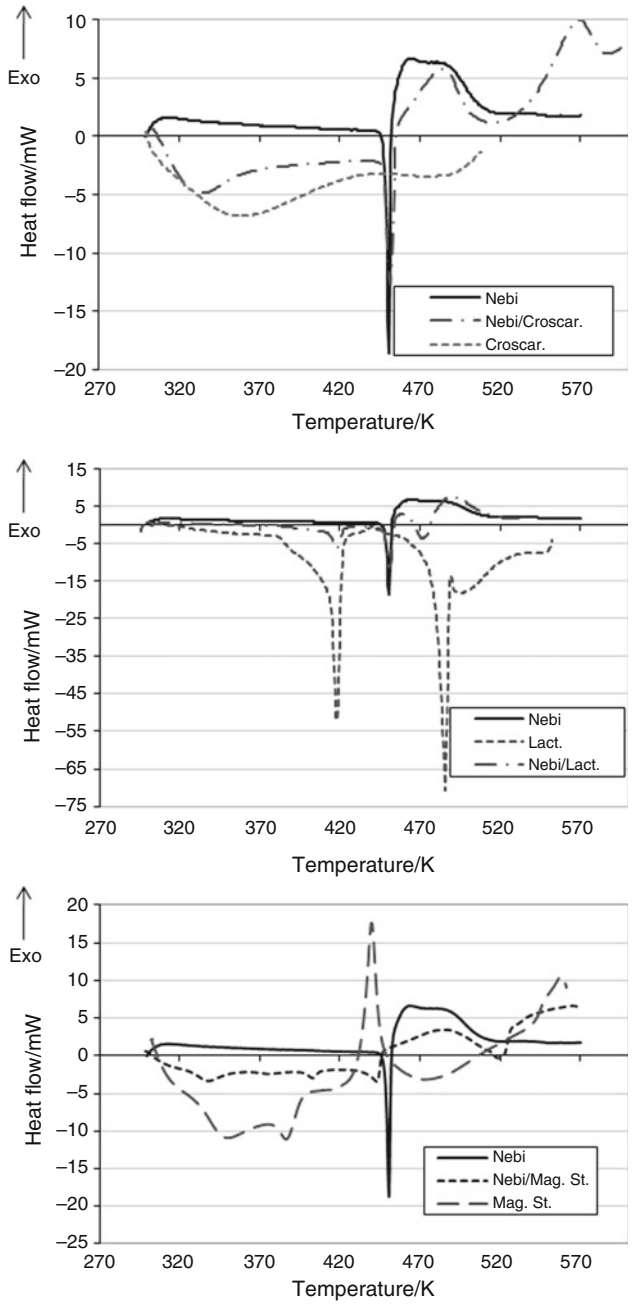

Fig. 1 DSC curves of pure drug, excipients and their 1:1 (w/w) mixtures. Nebicapone (Nebi), sodium croscarmellose (Croscar.), lactose monohydrate (Lact.) and magnesium stearate (Mag. St.)

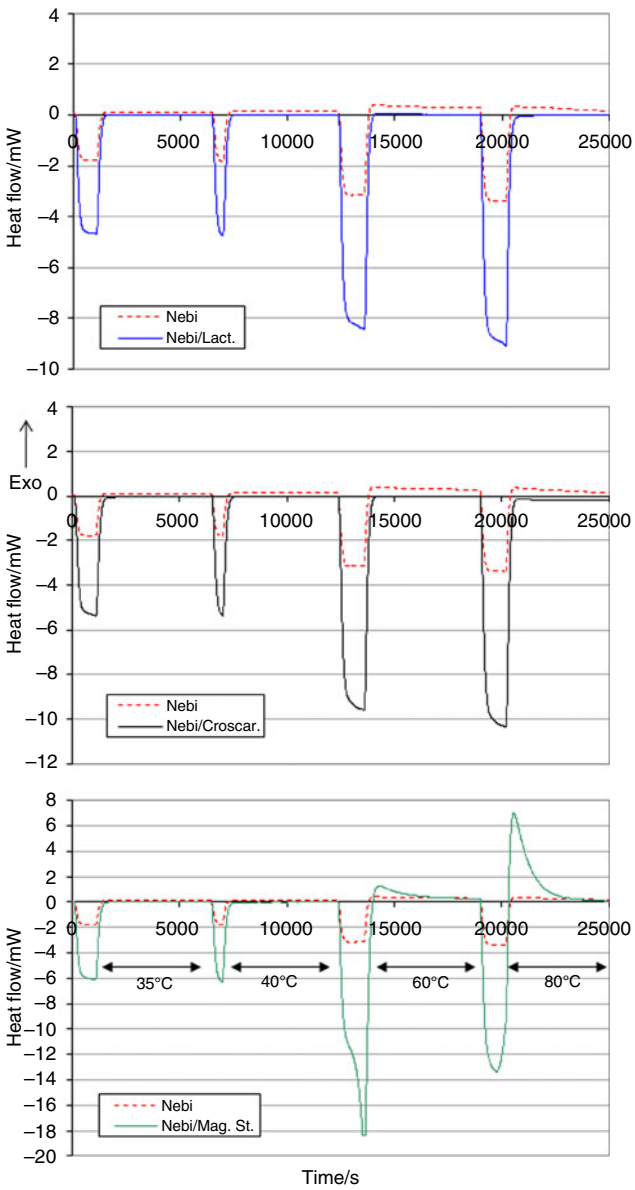

Fig. 2 Stepwise isothermal HSDSC runs using different increments (10, 5 and 20 K) and 1.30 h holding times for drug and binary drug/excipient mixtures 1:1 (w/w). Nebicapone (Nebi), sodium croscarmellose (Croscar.), lactose monohydrate (Lact.) and magnesium stearate (Mag. St.)

Nevertheless, the shift and substantial decrease in height of the endothermic peak of the drug (443.72 K) is apparent.

The effects of stepwise increasing temperature using HSDSC for nebicapone alone and 1:1 w/w lactose, magnesium stearate and croscarmelose are shown in Fig. 2. The isothermal steps end before the melting of any component.

The peaks in the heat flow curves (seen at the start of each isothermal period) are due to temperature re-equilibration. When the baseline heat flow is zero there is no difference in the energy flux behaviour between the sample and the reference [13]. The broad peaks during isothermal period can be attributed to chemical changes [15].

Nebicapone curve shows that there is no substantial difference in heat flux between the sample and the reference vessel although at 333.15 K and 353.15 K a small deviation of the baseline is perceptible.

With respect to magnesium stearate (the only excipient that exhibits a clear interaction) there is a report in literature (using a Setaram Micro DSC and similar stepwise

conditions, 5 K increments from 298.15 to 343.15 K with steps of 1 h) that refers that there is no deviation in heat flow from the baseline [13].

Examination of thermoanalytical curves of binary mixtures of drug/lactose and drug/croscarmelose indicate that no thermal events are taking place as can be seen by the null deviation from baseline.

However, it is evident that the sample containing magnesium stearate gives a clear exothermic effect, namely at 353.15 K, but already detectable at 333.15 K. The exothermic effect may be due to the oxidation of nebicapone as the main degradation product of the mixture nebicapone/magnesium stearate (impurity A), submitted to heat stress test, can be obtained by oxidation of nebicapone.

To verify if the interaction seen in the DSC curve corresponds to chemical incompatibility, HPLC analyses were performed on samples from the heat stress test.

The chromatograms obtained at the end of the study are presented in Fig. 3.

By the analysis of the chromatograms it is possible to verify that nebicapone alone did not suffer any detectable degradation, but when it is mixed with magnesium stearate decomposes substantially originating several degradants (total impurities, expressed in percentage of the total HPLC peaks area, are more than 80%). Neither the chromatogram of Nebicapone nor the chromatogram of magnesium stearate showed any significant degradation product.

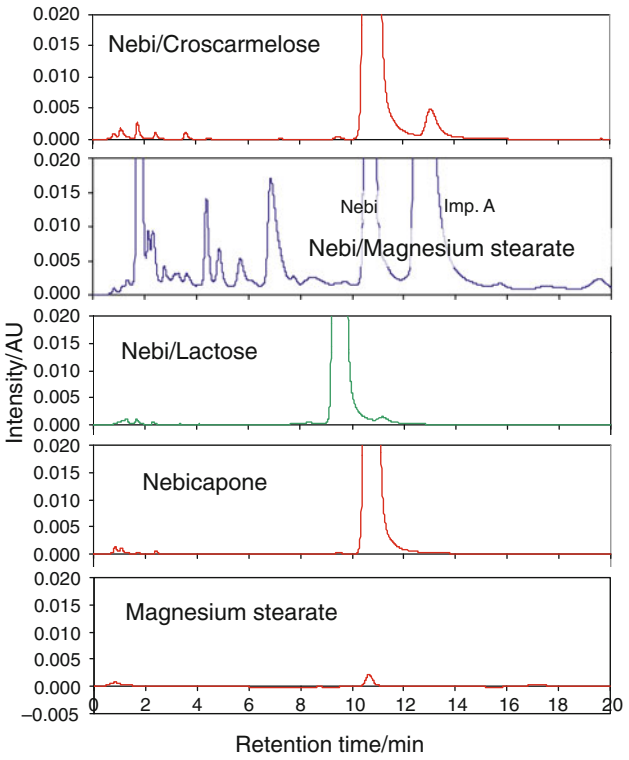

Fig. 3 HPLC chromatograms of nebicapone (Nebi), magnesium stearate and binary drug/excipient mixtures 1:1 (w/w): Nebi/sodium croscarmellose, Nebi/magnesium stearate and Nebi/lactose monohydrate obtained at the end of heat stress study. (*Imp*. impurity)

Besides the main degradation product was identified (impurity A is 1-(3,4-dihydroxy-5-nitrophenyl)-1,2-dioxo-2-phenyl-ethane) and it can be attributed to the oxidation of nebicapone. It also appeared in stability studies, at 298.15 K/60% RH, of formulations containing nebicapone, magnesium stearate and other excipients (data not shown).

In the case of the other excipients, lactose monohydrate and croscarmellose, the decomposition of nebicapone is insignificant. Total impurities, expressed in percentage of the total HPLC peaks area, are less than 1%.

Conclusions

Based on HSDSC results and supported by conventional heat stress study it was possible to show the chemical incompatibility between magnesium stearate and the drug. In the case of the other excipients there was no evidence of interaction in HSDSC studies, and the very low level of impurities detected by HPLC in stress test may support the absence of incompatibility at ambient temperature.

The results obtained in this study allow the selection of some excipients in order to be used in a solid dosage form of nebicapone. HSDSC results obtained are a contribution to confirm the utility of this method to study chemical incompatibilities. Besides it is easier and more reliable to verify the incompatibility in its thermoanalytical curves than in the curves of conventional DSC due to simpler interpretation of thermal events and lower temperature employed in stepwise isothermal mode.

Acknowledgements We would like to thank BIAL (Portela & Co S.A) in particular Cristina Santos and Escola Superior de Biotecnologia of UCP.

References

1. Gibson M. Pharmaceutical preformulation and formulation: a practical guide from candidate drug selection to commercial dosage form. Leicestershire, UK: Informa Health Care; 2001.
2. Silveira P, Vaz-da-Silva M, Almeida L, Maia J, Falcao A, Loureiro A, Torrão L, Machado R, Wright L, Soares-da-Silva P. Pharmacokinetic-pharmacodynamic interaction between BIA 3–202, a novel COMT inhibitor, and levodopa/benserazide. Eur J Clin Pharmacol. 2003;59(8-9):603–9.
3. Bonifacio MJ, Palma PN, Almeida L, Soares-da-Silva P. Catechol-*O*-methyltransferase and its inhibitors in Parkinson's disease. CNS Drug Rev. 2007;13(3):352–79.
4. Ferreira JJ, Almeida L, Cunha L, Ticmeanu M, Rosa MM, Januario C, Mitu CE, Coelho M, Correia-Guedes L, Morgadinho A, Nunes T, Wright L, Falcão A, Sampaio C, Soares-da-Silva P. Effects of nebicapone on levodopa pharmacokinetics, catechol-*O*-methyltransferase activity, and motor fluctuations in patients with Parkinson disease. Clin Neuropharmacol. 2008;31:2–18.

5. Marini A, Berbenni V, Pegoretti M, Bruni G, Cofrancesco P, Sinistri C, Villa M. Drug-excipient compatibility studies by physico-chemical techniques; the case of atenolol. J Therm Anal Calorim. 2003;73(2):547–61.
6. Ceschel GC, Badiello R, Ronchi C, Maffei P. Degradation of components in drug formulations: a comparison between HPLC and DSC methods. J Pharm Biomed Anal. 2003;32(4–5):1067–72.
7. Verma RK, Garg S. Selection of excipients for extended release formulations of glipizide through drug-excipient compatibility testing. J Pharm Biomed Anal. 2005;38(4):633–44.
8. Vueba ML, Batista de Carvalho LA, Veiga F, Sousa JJ, Pina ME. Influence of cellulose ether mixtures on ibuprofen release: MC25, HPC and HPMC K100M. Pharm Dev Technol. 2006;11(2):213–28.
9. Mora PC, Cirri M, Mura P. Differential scanning calorimetry as a screening technique in compatibility studies of DHEA extended release formulations. J Pharm Biomed Anal. 2006;42(1):3–10.
10. Stulzer HK, Rodrigues PO, Cardoso TM, Matos JS, Silva MA. Compatibility studies between captopril and pharmaceutical excipients used in tablets formulations. J Therm Anal Calorim. 2008;91(1):323–8.
11. Santos AF, Basílio ID, Souza FS, Medeiros AF, Pinto MF, Santana DP, Macêdo RO. Application of thermal analysis in study of binary mixtures with metformin. J Therm Anal Calorim. 2008;93(2):361–4.
12. Bernardi LS, Oliveira PR, Murakami FS, Silva MA, Borgmann SH, Cardoso SG. Characterization of venlafaxine hydrochloride and compatibility studies with pharmaceutical excipients. J Therm Anal Calorim. 2009;97(2):729–33.
13. Wissing S, Craig DQ, Barker SA, Moore WD. An investigation into the use of stepwise isothermal high sensitivity DSC as a means of detecting drug-excipient incompatibility. Int J Pharm. 2000;199(2):141–50.
14. Gaisford S, Buckton G. Potential applications of microcalorimetry for the study of physical processes in pharmaceuticals. Thermochim Acta. 2001;380(2):185–98.
15. McDaid FM, Barker SA, Fitzpatrick S, Petts CR, Craig DQ. Further investigations into the use of high sensitivity differential scanning calorimetry as a means of predicting drug-excipient interactions. Int J Pharm. 2003;252(1–2):235–40.
16. Bracconi P, Andres C, N'Diaye A, Pourcelot Y. Thermal analyses of commercial magnesium stearate pseudopolymorphs. Thermochim Acta. 2005;429(1):43–51.
17. Ford J, Timmins P. Pharmaceutical thermal analysis: techniques and applications. Chischester: Ellis Horwood Limited; 1989.