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Quantitative determination of acetylsalicylic acid in commercial drugs using DSC

Comparison with titration and UV spectrophotometric methods

Luigi Campanella · Valentina Micieli · Mauro Tomassetti · Stefano Vecchio

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Abstract In this article, the quantitative determination of acetylsalicylic acid (ASA) contained in two of the most commercially available pharmaceutical formulations was performed using differential scanning calorimetry (DSC) after a thermoanalytical characterization and a preliminary test on compatibility of ASA with three of the most commonly considered excipients [cellulose (CE), starch (ST) and sodium saccharin (SS)]. Finally, the analytical results obtained were compared with those derived by titrimetry and two UV spectrophotometric methods: i.e. a 'direct method' and a method based on first-order derivative UV spectra.

Keywords Acetylsalicylic acid · Determination · Thermal analysis · DSC · Titrimetry · UV spectrophotometry

Introduction

In this research, a new analytical method was used to determine the acetylsalicylic acid (ASA) content of a number of commercially available pharmaceutical specialties. In spite of the more commonly used thermogravimetry [1], this method is based on differential scanning calorimetry (DSC), the use of which was recently proposed [2] by the present authors.

For this purpose, a brief preliminary study was made using binary mixtures to detect any possible interactions between the active component and the excipients. It was found that any interactions between ASA and the excipients tested were not particularly evident (at least for low excipient/ASA ratios and essentially taking as reference the endothermic melting peak). It was also found that the melting enthalpy value determined for pure ASA was in good agreement with the corresponding values obtained for the ASA present in the drugs tested.

The next step was therefore the analytical determination of the ASA content of two commercially available drugs. The proposed method is based on the measurement of the melting enthalpy of increasing quantities of ASA, using the values therefore obtained to construct a calibration straight line. In practice, the melting enthalpy values (ΔH_{fus}) were obtained by plotting the area of the DSC melting peak as a function of a weighed quantity of ASA. The results obtained were validated also by comparing them with the values obtained titrimetrically (Italian Pharmacopoeia approved method) [3] and by applying a spectrophotometric method described in the literature and in the United States and European Pharmacopoeias [4, 5]. As far as the calorimetric determination was concerned, several methodological variants of previously described methods such as DSC peak deconvolution were also taken into consideration, as well as the first derivative method in the case of the spectrophotometric approach. Several years ago, one of the present authors proposed [6] this calorimetric method, which was recently reappraised and successfully used by the authors of this article who made further studies and experiments involving thermal analysis as was deemed necessary to evaluate the suitability of the method. In

L. Campanella · M. Tomassetti (✉)
Department of Chemistry, Sapienza University of Rome, P.le A. Moro, 5, 00185 Rome, Italy
e-mail: mauro.tomassetti@uniroma1.it

V. Micieli · S. Vecchio
Department of Chemical Engineering Materials and Environment, Sapienza University of Rome, Via del Castro Laurenziano 7, 00161 Rome, Italy

particular, in the present case, a prior calorimetric analysis of binary mixtures (ASA/excipients) was performed together with a detailed study of the temperatures and melting enthalpies of the ASA contained in these mixtures.

On the other hand, ASA, together with sodium salicylate, was the first non-steroidal anti-inflammatory drug (NSAID) to be used in clinical practice in the early twentieth century [7]. ASA, in particular, is considered as the prototype of NSAIDs; despite the introduction of new congeners, it is still the most commonly used analgesic–antipyretic–antiinflammatory drug and is used as a paragon against which to measure the activity of other peripheral analgesics. Even though there is no lack of analytical methods for its determination (for instance, the chromatographic ones [4, 8–10]), the possible development of very fast and low cost methods can always be considered an attractive alternative, above all, for rapid routine testing.

Experimental

Apparatus

The TG/DSC and DTG measurements were performed using a Stanton-Redcroft STA 625 simultaneous thermoanalyzer interfaced with an IBM compatible computer running Stanton-Redcroft Data Acquisition System, Trace 2 software (version 4).

UV absorption spectra were recorded on a Perkin-Elmer Lambda 16 spectrophotometer interfaced with a PC running UV-Winlab software to record the absorption spectra, derived spectra and for mathematical processing. Suprasil quartz cuvettes with a 1.0-cm optical path were used.

Materials

Sodium hydroxide, phenolphthalein, methyl orange, phenol red, potassium hydrogen phthalate, sodium carbonate, hydrochloric acid, all of analytical reagent grade were purchased from Fluka (Buchs SG, Switzerland) and used 'as is' without any further treatment or purification. ASA (analytical grade) was also purchased from Fluka, while all the excipients [starch (ST), cellulose (CE) and sodium saccharin (SS)] were obtained from the Pharmacy Department of 'Sapienza, University of Rome' (Italy).

The 'nominal' content of ASA concerning the two pharmaceutical specialties tested in the present research, conventionally identified as F1 and F2, which are the most commonly used from a commercial point of view, are 93.6 and 84.3% w/w, respectively. Each package of the two different drugs was purchased at a local pharmacy, and the following excipients were declared to be present: ST and CE in the drug F1, while ST, CE, SS, raspberry flavour (RF) in the drug F2. The drug specialties were analysed 'as is', without any pre-treatment except the careful grinding and homogenization of at least 20 tablets of each test drug.

Methods

TG/DSC measurements and experimental determination of T_{fus} and ΔH_{fus}

The TG and DSC measurements were made using a simultaneous thermoanalyzer interfaced with an IBM compatible computer running ad hoc software (see 'Experimental' section). About 7 mg of sample were used for each TG/DSC experiment, operating in a stream of air (about 50 cm^3 min^{-1}) at a heating rate of 10 K min^{-1}. TG/DSC experiments carried out in a stream of argon under the same operating conditions showed that the DSC curves referring to the melting peak were practically identical to those obtained in a stream of air (Fig. 1). This implies that the enthalpy values referring to the melting processes investigated were not appreciably affected by the surrounding atmosphere. The second endothermic peak, which may be seen in the DSC curves in Fig. 1, is partly superimposed to the melting one and is ascribable to the thermal degradation of ASA, although in the past several different mechanisms were proposed to explain this behaviour [11–13]. Temperature calibration was achieved by exploiting the melting temperatures of known standard substances, such as: indium, lead, tin, zinc and benzoic acid [14]. For the purpose of TG calibration (ordinate), using the same software running on the computer interfaced with the instrument, a calibration curve was constructed (counts vs. mg) when exactly known masses were introduced into the TG/DSC equipment. Heat flow calibration was performed by recording two DSC experiments under the same experimental conditions: the first with empty crucibles,

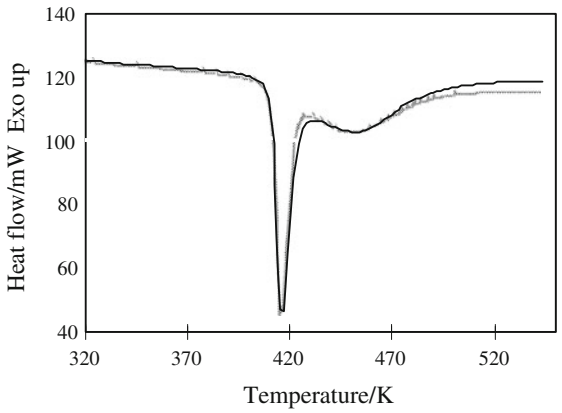

Fig. 1 DSC curves of pure ASA at heating rate of 10 K min^{-1} under a stream of: air (*thin line*), argon (*dotted line*)

while the second with a sapphire crystal placed inside the sample crucible, the heat flow of which was very accurately known over a wide temperature range. The difference between the heat flow values at each temperature was used to construct a calibration curve (counts vs. mW). At the conclusion of each test performed on the various samples, the software was used, taking into account the baseline of the DSC curve peak, to obtain the values referring to the melting temperature and the melting enthalpy. The same software running on the computer was used to determine the area of the DSC peaks.

Titrimetric method

In order to validate the calorimetric method proposed in this study for the quantitative determination of ASA, the titrimetric method described in the Italian Pharmacopoeia [3] was used as it is the most conventional analytical method for this determination.

For the titrimetric determination of the drugs, to an aliquot of powder obtained by grinding the tablets (as reported in 'Experimental' section) accurately weighed out and containing about 500 mg of ASA, 10 mL of ethyl alcohol and 30 mL of a 0.5-mol L^{-1} sodium hydroxide solution (previously standardized with potassium hydrogen phthalate) were added. The resulting solution was brought to the boil for 10 min. The alkali excess was then back-titrated with a 0.5-mol L^{-1} HCl solution (previously standardized with sodium carbonate) using phenol red as indicator.

A typical back-titration curve for the titrimetric analysis of a solution of pure ASA is shown in Fig. 2a. The blank for these analyses was prepared by adding 10 mL of ethyl alcohol to 30 mL of a 0.5-mol L^{-1} NaOH solution and bringing to the boil for 10 min. The solution was then back-titrated using a 0.5-mol L^{-1} HCl solution and phenol red as indicator. The same procedure was followed to back-titrate the drug specialties tested.

Spectrophotometric method

The calorimetric method was further validated using the spectrophotometric method described in the United States Pharmacopoeia [4] and in the European Pharmacopoeia [5] as an alternative to titrimetric quantitative determination. Several different solutions with increasing concentration of the active component were therefore prepared to be subjected to spectrophotometric analysis. For this purpose, about 10 mg of ASA was weighed out and dissolved in 50 mL of distilled water. The solution was then placed in a sonicator for 15 min and made up to 100 mL with distilled water. The absorbance of the solution thus prepared was measured at the relative absorption peak, namely at $\lambda = 274$ nm. The same procedure was followed for the drug specialties which, after being ground to powder and having weighed out suitable quantities, were used to prepare solutions having the same nominal concentration of a solution containing 10 mg of pure ASA. These solutions were then analysed using the UV spectrophotometer, recording the absorbance at $\lambda = 274$ nm, measured against a blank consisting of distilled water subjected to sonication for 15 min.

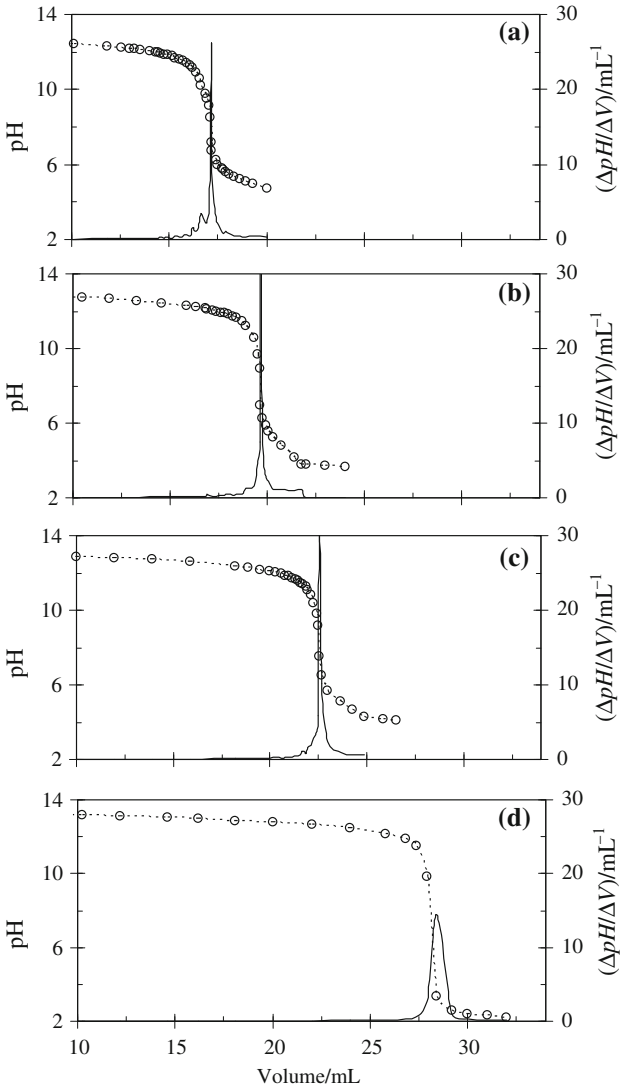

Fig. 2 **a** Back titrimetric curves and first-order derivative curves of aqueous solutions containing pure ASA, **b** drug F1, **c** drug F2 and **d** blank

Results

Preliminary compatibility test using thermal analysis

First, the TG/DTG and DSC curves of pure ASA and of the three principal excipients contained in the two drugs were

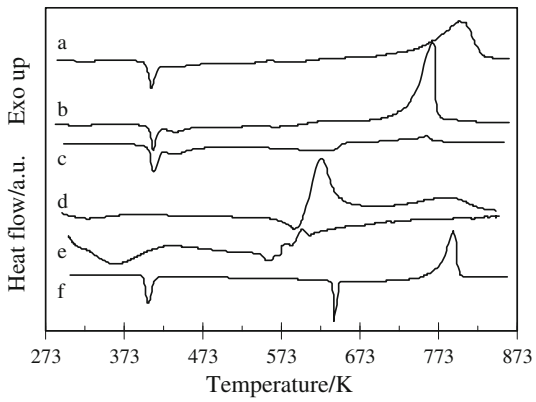

Fig. 3 DSC curves at heating rate of 10 K min^{-1} under *a* stream of air of drug F2, *b* drug F1, *c* pure ASA, *d* pure cellulose, *e* pure starch and *f* pure sodium saccharin

recorded. ST and CE were contained both in the drug marked F1 and in that marked F2; SS was instead present only in the specialty denoted as F2. The raspberry aroma was assumed to have practically no effect concerning the interaction with ASA detectable by DSC experiments as only negligible amounts of this excipient are contained in the drug F2. The DSC curves of drugs F1 and F2, obtained at a heating rate of 10 K min^{-1}, are set out in Fig. 3, where they are compared with that of pure ASA and with those of the pure excipients (ST, CE and SS) present in the same drugs.

A glance at the DSC curves set out in Fig. 3 seems to show that, as far as ST and CE are concerned, no significant interactions occur, at least in the temperature range up to about 550 K, that is 100 K higher than melting temperature. For both drugs, which contains one or two of these two excipients, the ASA melting peak occurs at 408.7 K, i.e. the same value was found for pure ASA (see Table 1) and that is in excellent agreement with the value of 408.1 K (for pure ASA) reported in literature [15]. Actually, any transition corresponding to the decomposition of the two excipients is in practice evidenced up to about 550 K.

On the other hand, the presence of a melting peak at 396.5 K in the case of SS, which is fairly close to the melting peak of the active component itself (408.7 K), seems to evidence that a partial superimposition of the SS peak over the ASA melting peak in the DSC curve of drug F2 is possible. However, a careful examination of the DSC curves of drug F2 in Fig. 3 revealed no significant interactions. To this end, it was also observed that a very similar situation occurred when the UV absorption peak of an ASA aqueous solution at $\lambda = 274$ nm was used for the purpose of quantitative analysis. Indeed, by observing the UV spectra of both pure ASA and SS shown in Fig. 4a, it is evident that solutions of ASA and SS have relative absorption peaks that are very close together, around 270 nm. Therefore, the presence of SS might have produced a positive interference also in this case if the relative ASA absorption peak had been used to determine the concentration of the ASA solutions in the two drugs examined.

Table 1 Melting temperature and enthalpy of fusion of pure ASA, in comparison with those referred to the solid binary mixtures

Samples	Content of ASA/% w/w	T_{fus}/K (RSD $\leq$ 0.5)	ΔH_{corr}/J g^{-1} [a] (RSD $\leq$ 1.5%)
ASA	100	408.7	184.1
ASA/CE	50	410.4	187.0
ASA/CE	93	409.8	180.3
ASA/ST	50	411.2	187.0
ASA/ST	93	410.7	185.1
ASA/SS	50	405.8	180.5
ASA/SS	93	406.2	183.3

CE cellulose, *ST* starch, *SS* sodium saccharin

[a] $\Delta H_{corr} = (\Delta H_{fus}/\text{percentage of ASA in the sample}) \cdot 100$

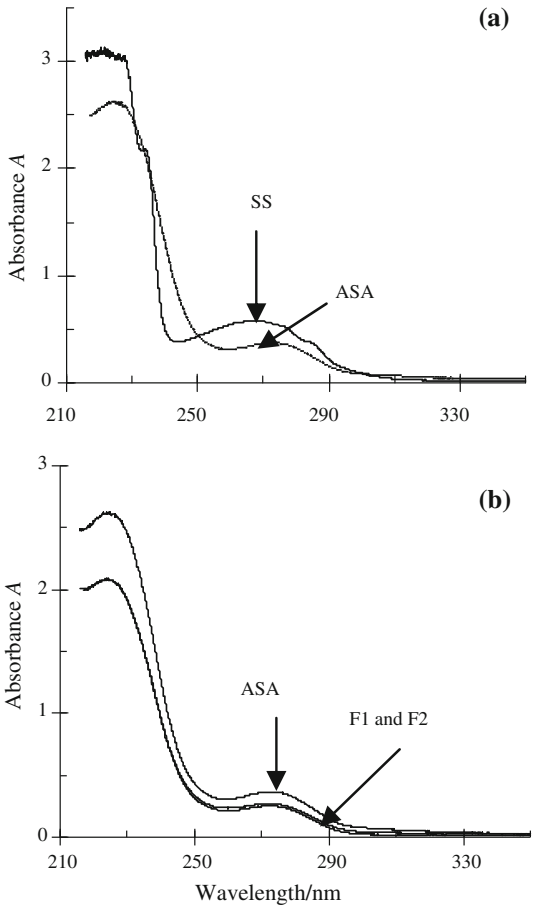

Fig. 4 **a** Absorbance spectra of aqueous solution of ASA (74.0 g L^{-1}) and sodium saccharin (79.0 g L^{-1}) and **b** ASA (74.0 g L^{-1}), F1 and F2 (both at concentration of 72.0 g L^{-1})

Nevertheless, by observing the UV absorption curves of solution containing the drugs F1 and F2, of which only the latter contains SS, as shown in Fig. 4b, it is clear that these two curves may be considered practically superimposable even though only one of the two drugs contains SS. Also the comparison between these two curves and the UV spectrum of pure ASA revealed no differences, which led us to believe that the ASA melting peak in drug F2 is unaffected by the presence of sodium SS as the latter is evidently present only in very low concentrations, less than 0.25%. This may be inferred from even a summary calculation based on the absorbance of UV spectra at $\lambda = 274$ nm for the solutions of the two drugs and of the pure active component, as shown in Fig. 4b. Indeed, the slight difference that occurs in absorbance in Fig. 4b is due solely to the small difference in active component concentration between the measured pure ASA solution and the solutions obtained by dissolving the ASA contained in the two drugs.

In order to make a more thorough analysis of the possibility to apply the calorimetric method to drugs containing these excipients and the active component considered, also some binary mixtures of ASA/ST, ASA/CE and ASA/SS were prepared in different ratios (weight by weight).

The thermal behaviour of these binary mixtures was then investigated using the DSC technique under a heating rate of 10 K min^{-1}. The different DSC curves of the ASA/ST, ASA/CE and ASA/SS mixtures and of the two pure components up to at least 550 K are summarized in Fig. 5a–c. A comparison of these curves shows that they display the same thermal behaviour.

However, differences in the DSC curves of the binary mixtures examined in comparison with that of pure ASA, which could presumably be observed at higher temperatures (see Fig. 3), involve mainly exothermic effects ascribable to the decomposition of ASA and excipients, and certainly are not referred to the ASA melting process, which takes place at much lower temperatures (around 408.7 K). In the temperature range up to 550 K, the DSC curves of all the ASA/excipient binary mixtures considered are practically very similar to those of pure ASA (Fig. 5a–c).

As stated in a previous article [2], in order to evaluate any interaction between the active component (ASA) and the excipients present in the test drugs from a calorimetric standpoint but on a quantitative basis, the melting temperatures and enthalpies of samples of pure ASA and that contained in the binary mixtures were experimentally derived from the DSC curves shown in Fig. 5 and summarized in Table 1.

No significant differences were found in the temperatures and melting enthalpies of the ASA/CE, ASA/ST and ASA/SS mixtures containing more than 93% w/w of ASA

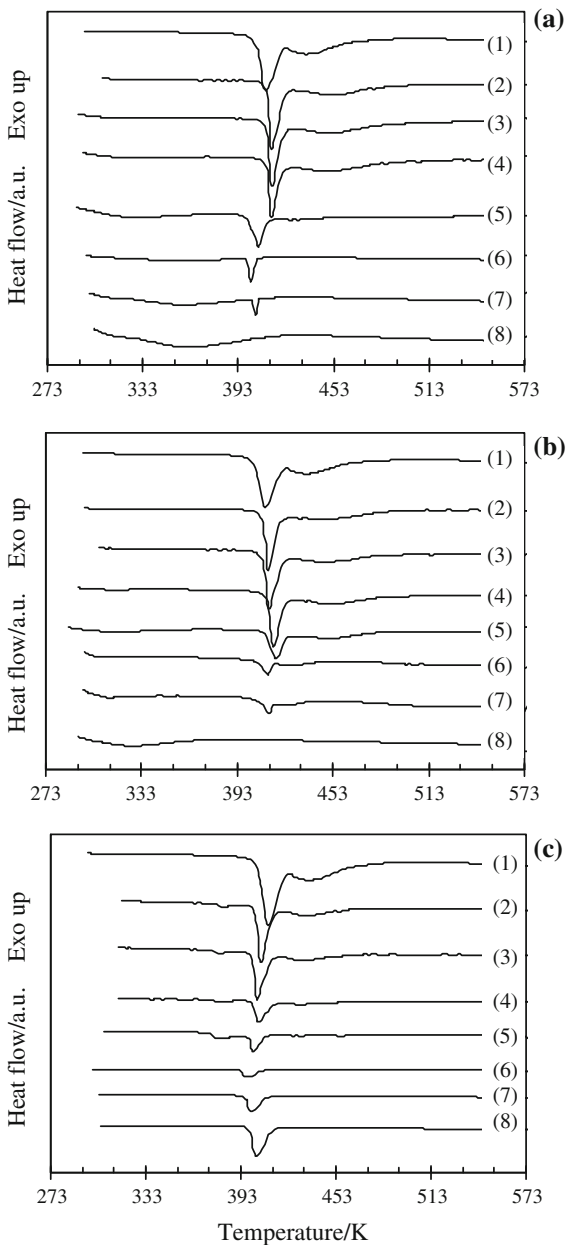

Fig. 5 **a** DSC curves at heating rate of 10 K min^{-1} under a stream of air of binary mixtures ASA/starch, **b** ASA/cellulose and **c** ASA/sodium saccharin. The ASA nominal content in the sample considered (expressed in % w/w) is: (1) 100%, (2) 93%, (3) 90%, (4) 85%, (5) 50%, (6) 30%, (7) 10%, (8) 0%

compared with those of pure ASA. Only in the case of ASA/SS mixtures, a slight drop in melting temperature was observed. However, it must be borne in mind that the percentage of SS in these mixtures is still at least 10 times greater than that actually present in drug F2, as previously estimated by UV measurement. However, also in the case of binary mixtures with high ASA content (whose composition is 50% w/w excipient/active component), the small differences observed in the ΔH_{fus} and T_{fus} values,

compared with those of pure ASA, seem to be more ascribable to a random nature rather than to 'systematic causes'. These results lead to the conclusion that CE, ST and SS (at least when the latter is present in low percentages in drug F2) have no substantial effect on the ASA melting peak. Consequently, the calorimetric method may be correctly applied (at least in the case of the two drugs tested herein).

We also took an interest in solving the problem of the possible interference of salicylic acid (SA), which should be present in commercial drugs because of the ASA hydrolysis. To this end, a further series of DSC experiments were carried out on binary mixtures ASA/SA (prepared by increasing the mass percentage of ASA in the mixtures by gently mixing powders of pure components in an agate mortar). As expected from literature results [16], a eutectic between ASA and SA was observed, whose melting enthalpy decreases by increasing the ASA content, becoming less than 1% of the melting enthalpy of pure ASA for mass percentage of ASA higher than 99.5% w/w. As commercial drugs are usually considered expired when degradation products of their active components were found to be at concentration higher than 0.1–0.2% w/w, it can be concluded that SA cannot be considered an interference when ASA is quantitatively determined in its commercial pharmaceutical formulations using the calorimetric method described in the present study, as even an ASA concentration $\leq$0.5% w/w is too low to produce relevant error in the measurement of the melting enthalpy (its enthalpy contribution practically lie within the standard deviation of the calorimetric method).

Acetylsalicylic acid determination by the calorimetric method

After studying the thermal behaviour of ASA and the excipients contained in the two drugs and having investigated the possibility of applying the calorimetric method to formulations containing ASA, the practical application of the DSC method to the two drugs F1 and F2 was investigated. In the first instance, a calibration curve was constructed using the melting enthalpy (ΔH_{fus}) as parameter, the value of which (expressed in J) was determined directly from the measurement of the area of the DSC melting peak of ASA, from which the ΔH_{fus} (in J) was obtained for samples consisting of increasing quantities of pure weighted ASA. For this purpose samples ranging between 2 and 20 mg of ASA were carefully weighed out. Each sample was then subjected to DSC analysis and the respective melting peaks recorded.

In the DSC curve of pure ASA reported in Fig. 6a the typical melting peaks for increasing quantities of ASA are shown, whereas Fig. 6b and c shows those related to the

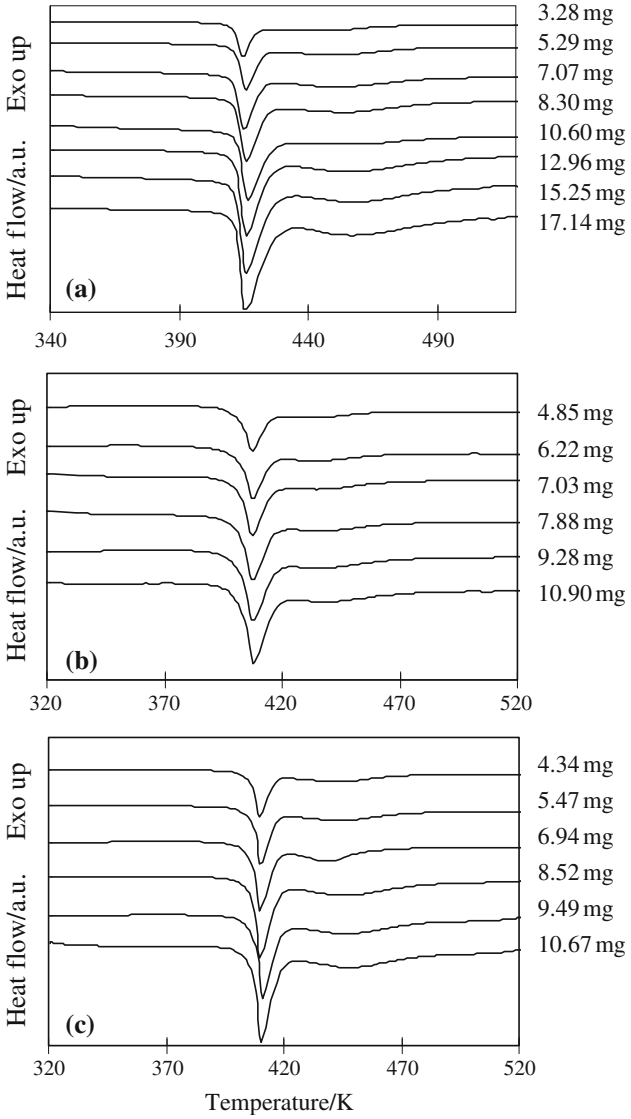

Fig. 6 **a** DSC curves representing the melting peak of increasing samples size of pure ASA, **b** drug F1 and **c** drug F2

two drugs tested containing ASA, recorded over the temperature range around the ASA melting point. In all cases examined, six determinations of the enthalpy of fusion were obtained by measuring the areas of the melting peaks of different weighed quantity of each drug, and the resulting values were used to obtain 6% of ASA for each drug studied using both the equations associated with the calibration straight lines and the equation referring to the calibration straight line obtained by measuring the melting ΔH of the deconvoluted peaks (Table 2). Finally, in each case the percentage of ASA in each considered drug was calculated as a mean of the six values determined. Figure 7a shows the calibration curve (ΔH_{fus} as a function of increasing quantities of pure ASA) and the respective confidence interval. The ΔH_{fus} values were obtained by integrating the area of each melting peak; the software

Table 2 Regression and statistical parameters related to the calibration curves obtained by the calorimetric method without (*on the left*) and with (*on the right*) deconvolution of melting DSC peak

Regression equation	ΔH_{fus}/J = (0.1709 ± 0.003)M/mg − (0.044 ± 0.037)	ΔH_{fus}/J = (0.1335 ± 0.002)M/mg − (0.002 ± 0.007)
Linearity range/mg	(3.28–17.1)	(3.28–17.1)
R^2	0.99768	0.99801
Confidence level (α)	0.95	0.95
Student t value	1.94	1.94
Confidence interval of slope/J mg^{-1}	(0.1651–0.1767)	(0.1296–0.1374)
Confidence interval of intercept/J	(−0.116–0.028)	(−0.016–0.012)
LOD[a]/mg	0.65	0.16

[a] Limit of detection

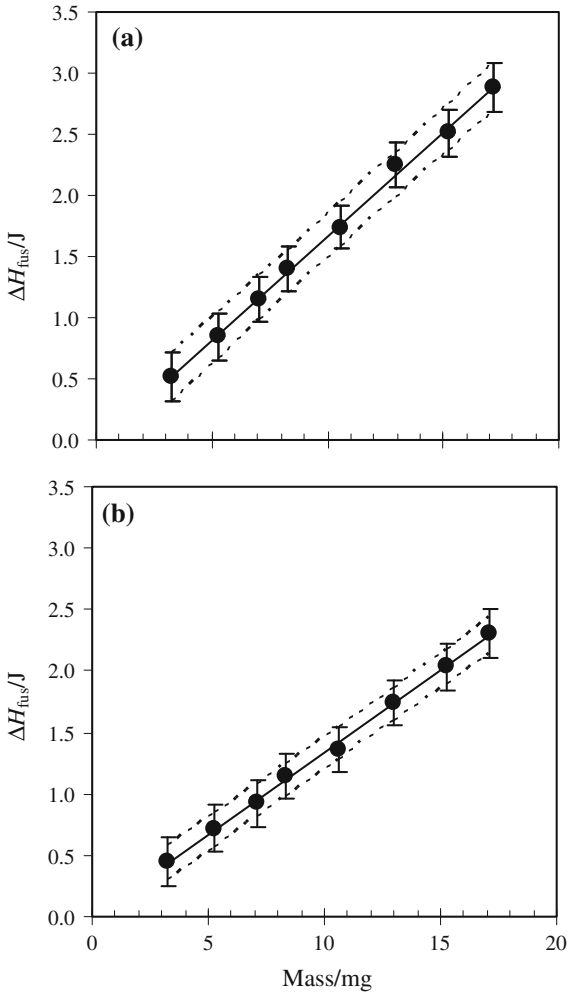

Fig. 7 **a** Calorimetric calibration curves obtained using the melting enthalpy (ΔH_{fus}) as a function of the mass M of pure ASA samples calculated using non-deconvoluted DSC peaks and **b** calculated using deconvoluted DSC peaks

applied enable to express these area values in J units using proper conversion factors. These values were then plotted versus the sample mass. Table 2 shows the equations of the regression straight lines obtained, the squares of the linear correlation coefficients, the confidence levels, Student's t values and the limits of detection (LOD), defined as $3\cdot(\sigma_{int}/S)$ [16], where σ_{int} is the standard deviation of the intercept and S is the slope of the regression line considered. Figure 7b thus shows the calibration curve obtained by plotting the ΔH_{fus} obtained by integrating the area of the deconvoluted melting peak for samples consisting of increasing quantities of pure ASA. Since, in Fig. 8a, it was observed that the melting and first decomposition peaks of ASA are partly superimposable, albeit only marginally, it was attempted to perform a deconvolution of the above-mentioned peaks, as shown in Fig. 8b. This was done after having constructed the baseline using the typically sigmoid-shaped trend of the respective TG curve and having ensured that the values of the heat flow corresponding to the initial and final temperatures of the non-deconvoluted DSC peak corresponded to those of the TG baseline, while the values referring to the intermediate temperatures were proportional to the recorded weight loss. This deconvolution was then carried out on all the curves obtained for the various ASA weighed samples. In the case of values

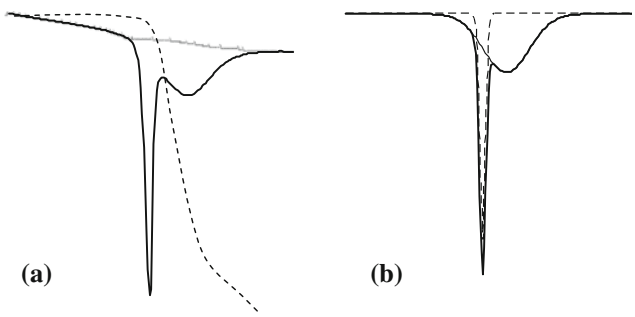

Fig. 8 **a** Experimental DSC curves representing the melting peak of pure ASA (*solid line*), before and after **b** deconvolution. The DSC baseline used for deconvolution (*dot lines* in plot **a**) was obtained using the shape of the corresponding experimental TG curve (*dashed lines* in plot **a**), while the melting DSC curve after deconvolution (*dashed lines*) is shown in plot **b**

Table 3 Results of the calorimetric determination of ASA in the dosage forms examined and comparison with the nominal content of ASA using the non-deconvoluted and deconvoluted DSC peaks

Dosage forms	Content of ASA/% w/w				
	(*a*)	(*b*) ± SD ($n \geq 6$)	(*c*) ± SD ($n \geq 6$)	$\Delta = [(b - a)/a] \cdot 100/\%$	$\Delta = [(c - a)/a] \cdot 100/\%$
F1	93.6	88.1 ± 1.6	90.4 ± 2.0	−5.9	−3.4
F2	84.3	82.3 ± 1.7	84.1 ± 1.7	−2.4	−0.2

(*a*) The percentage of nominal ASA content,
(*b*) The percentage of ASA content obtained using the calorimetric method,
(*c*) The percentage of ASA content obtained using the calorimetric method after melting peak deconvolution

obtained using the calibration curve, in which the melting enthalpy was computed after deconvolution of DSC peak area (Fig. 7b), it may be observed an increase in the goodness of linear fit (a better linear correlation), albeit slightly (see Table 2), which gives a summary of the new analytical data obtained. Repeated DSC scans were made of increasing quantities of each of the two commercially available drugs F1 and F2, after they were carefully ground up and homogenized. The experimental values of the ASA percentages obtained using alternatively the calorimetric calibration curves reported in Fig. 7a or b were compared in Table 3 with the nominal values provided by the manufacturers. It should be noted that, using the calibration straight line that utilizes the ΔH_{fus} obtained by means of the deconvoluted peaks, the difference between the experimental value and the nominal one is always lower than 3.5% for either drug. Indeed, for the drug F2, this difference is as low as 0.2%, while, without peak deconvolution, the difference is as high as about 6% in the case of drug F1.

Acetylsalicylic acid determination by the titrimetric method

The analytical data obtained, determining the ASA percentage in the same drugs by the calorimetric method, were compared with those obtained using the titrimetric method recommended by the Italian Pharmacopoeia [3]. This consists of a long-standing classic method, whose application, however, takes much longer than the calorimetric method. The operating conditions have been reported in detail in 'Experimental' section, describing the experimental methods used. Figures 2b–d, respectively, show the typical back-titration curves referring to ASA (for drugs F1 and F2) and the relative blank curve. On the basis of the volume of the HCl aqueous solution (about 20 mL)

Table 4 Results of the titrimetric determination of ASA in the dosage forms examined and comparison with the nominal content of ASA

Dosage forms	Content of ASA/% w/w		
	(*a*)	(*b*) ± SD ($n \geq 3$)	$\Delta = [(b - a)/a] \cdot 100/\%$
F1	93.6	92.1 ± 1.8	−1.6
F2	84.3	87.4 ± 1.5	3.7

(*a*) The percentage of nominal ASA content
(*b*) The percentage of ASA content obtained using the titrimetric method

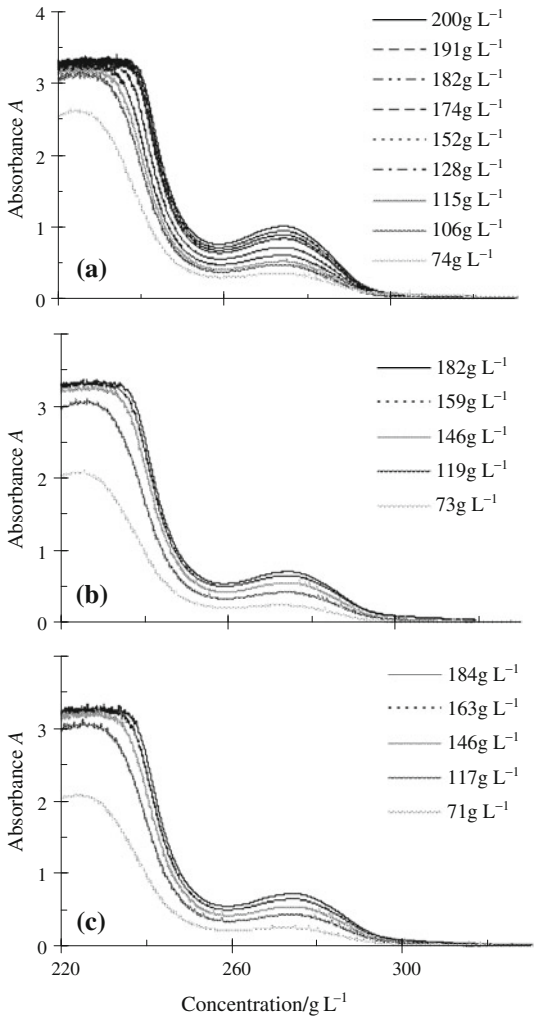

Fig. 9 **a** Absorbance UV spectra related to increasing concentration of aqueous solution, for pure ASA, **b** drug F1 and c drug F2

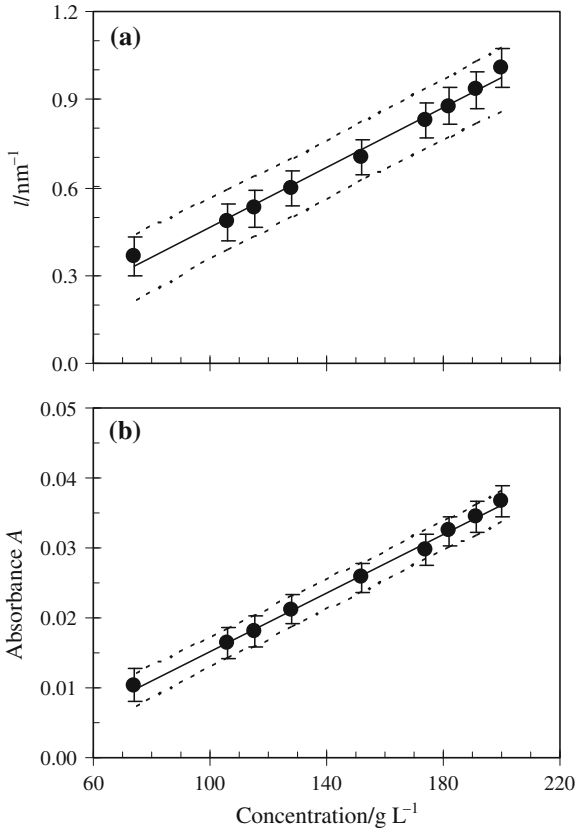

Fig. 10 **a** UV spectrophotometric calibration curves of pure ASA solutions at 274 nm as a function of increasing ASA concentration (g L^{-1}) direct method and **b** first-order derivative method

required in each case to back-titrate the excess NaOH until the indicator changed colours, the percent ASA contained in drugs F1 and F2 was calculated. The results of these determinations are set out in Table 4, together with the nominal percentages, to make a comparison of the results reported in Table 3. Using this method, the difference between the experimentally determined ASA content and the nominal value does not exceed about 4% for either drug.

Acetylsalicylic acid determination by the spectrophotometric methods

In order to further compare the analytical data referring to the percentage of ASA contained in the two drugs tested obtained using the calorimetric method, a well-known spectrophotometric method, suggested by the European and American Pharmacopoeias [4, 5], was applied.

First of all, absorbance UV spectra concerning solutions of increasing concentrations of pure ASA obtained as described in 'Experimental' section were recorded and reported in Fig. 9a. Using the absorbance values, read off at $\lambda = 274$ nm, the calibration straight line shown in Fig. 10a was constructed, the regression equation of which, together with the respective statistical parameters, is set out in Table 5 (column on the left). Also in this case, the uncertainties associated to the regression parameters were expressed in terms of standard deviation.

Consequently, five aqueous solutions of increasing concentrations of each drug were prepared following the procedure described in 'Experimental' section. After the solutions of drugs F1 and F2 had been prepared, their UV absorption spectra were recorded and set out in Fig. 9b and c. After reading off the absorbance of each solution at $\lambda = 274$ nm and using the equation of the calibration straight line (Fig. 10a) set out in Table 5 (column on the left), the concentration of the ASA contained in each of the two pharmaceutical specialties was calculated as a mean of five values obtained for each solution (see Table 6). Observing the results obtained, as reported in the column on the left of Table 6, it may be seen that the percentage values found are always less than the declared nominal values and that, in one of the two cases, the difference between nominal value and observed value is about 12%.

As in several cases documented in the literature [9, 10], the results are improved by performing derivative analysis, it was attempted to apply the first-order derivative method hoping this approach will improve the results obtained.

Table 5 Regression and statistical parameters related to the calibration curve obtained by the direct (*on the left*) and first-order derivative (*on the right*) spectrophotometric method

Regression equation	$A = (0.005\mathit{1} \pm 0.0002)C$/g L^{-1} $- (0.048 \pm 0.028)$	l/nm$^{-1} = (0{,}000\mathit{208} \pm 0{,}000\mathit{004})C$/g L^{-1} $- (0.00\mathit{554} \pm 0.00\mathit{060})$
Linearity range/g L^{-1}	(74–200)	(74–200)
R^2	0.9907*3*	0.9975*7*
Confidence level (α)	0.95	0.95
Student t value	1.90	1.90
Confidence interval of slope	(0.0047–0.0055)/g^{-1} L	(0.000*200*–0.000*216*)/nm L g^{-1}
Confidence interval of intercept	(−0.10*1*–0.005)	[(−0.00*668*)–(−0.00*440*)]/nm^{-1}
LOD[a]/g L^{-1}	16.5	8.*65*

[a] Limit of detection

Table 6 Results of both direct and first-order derivative spectrophotometric determinations of ASA in the dosage forms examined and comparison with the nominal content of ASA

Dosage forms	Content of ASA/% w/w				
	(*a*)	(*b*) ± SD ($n \geq 5$)	(*c*) ± SD ($n \geq 5$)	$\Delta = [(b - a)/a] \cdot 100/\%$	$\Delta = [(c - a)/a] \cdot 100/\%$
F1	93.6	82.5 ± 2.3	82.8 ± 7.9	−11.9	−11.5
F2	84.3	83.8 ± 2.3	83.3 ± 8.6	−0.6	−1.2

(*a*) The percentage of nominal ASA content

(*b*) The percentage of ASA content obtained using the spectrophotometric method

(*c*) The percentage of ASA content obtained using the first-order derivative spectrophotometric method

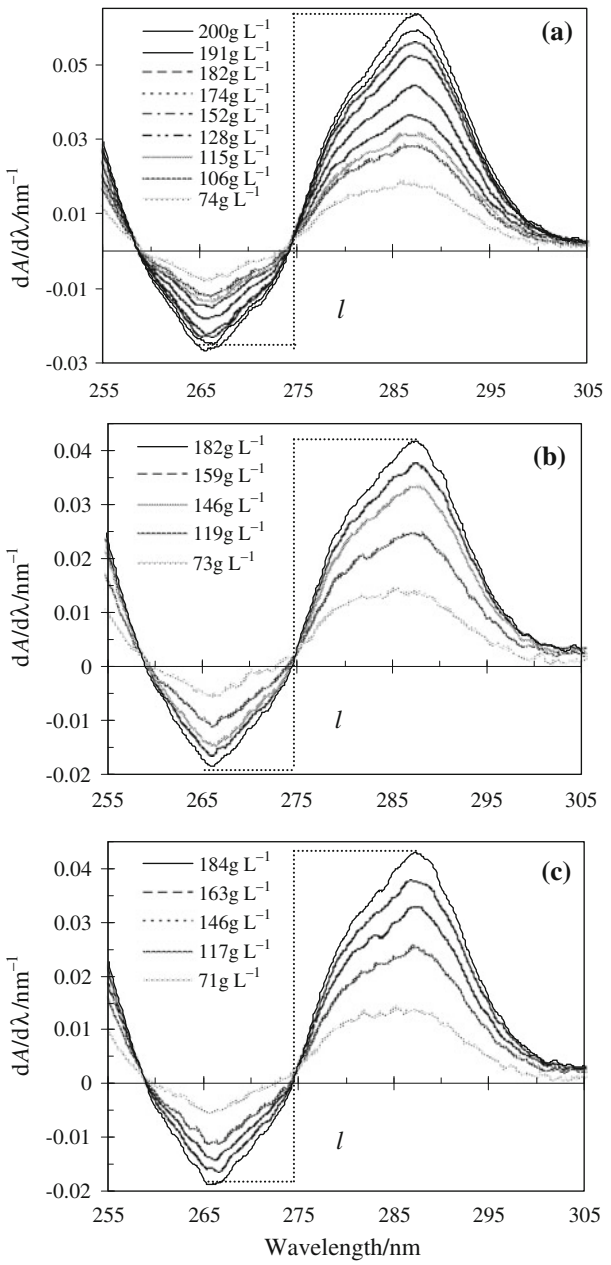

Fig. 11 **a** First-order derivative UV spectra at different concentrations for aqueous solutions of pure ASA, **b** drug F1 and c drug F2. The value of the difference between the maximum (at 288 nm) and the minimum (at 266 nm) is denoted as '*l*'

Figure 11 shows the first-order derivative curves for solutions of increasing concentration of pure ASA and for the solutions of the two drugs containing ASA. Figure 10b thus shows the calibration curve obtained using the first-derivative method, by plotting the difference '*l*' between the values related to the maximum (at $\lambda = 288$ nm) and the one at the minimum (at $\lambda = 266$ nm) of the first-order derivative curve as a function of increasing concentration of the pure ASA solutions.

The regression equation and the principal regression parameters referring to the method cited are given in Table 5 (column the right), while the analytical results as a mean of five determinations in solutions with increasing concentration of each drug obtained using this method together with a comparison with the nominal ASA content are summarized in Table 6. As can be seen, using the first-order derivative method, the agreement between the nominal and the value determined for drug F1 is only slightly improved, while the agreement is actually worsened in the case of drug F2.

Discussion and conclusions

As it is already reported in the literature [2], the results show that the application of the calorimetric analysis method to determine the active component contained in a drug specialties demands that a preliminary investigation be carried out regarding the occurrence of possible interactions between the active component and any excipients [16–20], but how the study may be performed also by means of thermal analysis. From the analysis of the DSC curves and of the data referring to the melting enthalpies obtained, it can actually be evaluated whether the calorimetric method may or not be applied to a given commercially available drug [2]. In the case of the drugs tested in the present research, the calorimetric method and the titrimetric method proposed by the Italian Pharmacopoeia, the results are substantially comparable as regards precision of the measurements while the spectrophotometric method produces higher standard deviations. In addition, as far as the agreement with the nominal values declared by the manufacturers is concerned, the calorimetric method and the titrimetric method are

practically equivalent, even though the calorimetric method has slightly better results ($\Delta\% \leq 3.5$), in comparison with those of the titrimetric method ($\Delta\% \leq 4$). In the spectrophotometric method, on the other hand, agreement with the nominal value is less satisfactory ($\Delta\%$ for the first of the two drugs tested was actually in the order of 11–12%).

On the other hand, the titrimetric method, based on the back-titration of an excess of NaOH with hydrochloric acid, was found to be more dependent on the care and experience of the operator; as well as being more laborious to perform, it also demands an accurate standardization of the reagents. Furthermore, the spectrophotometric method is considered to be relatively cheap and quite rapid as it is not based on separation techniques. However, it is quite prone to interference (as measurement is performed in the UV range) due to both the presence of small impurities or turbidity as well as to possible hydrolysis or decomposition, as indeed in the case of ASA [3, 21], and to the presence of given excipients, such as SS, if contained in non-negligible concentrations. It has already been seen that SS has an UV absorption peak at wavelengths very close to 274 nm, i.e. where also the ASA UV absorption peak, which is used for the quantitative spectrophotometric measurements, occurs. However, as discussed in 'Results' section, SS may not represent an interference when, as in the case studied herein, it is contained in a very low percentage.

In the present research, however, the limits of the spectrophotometric method appear to be linked to the low solubility of the ASA and the ease with which it is hydrolyzed, rather than to the presence of possible interfering components. For this reason, therefore, the application of methodological variants, such as the first-order derivative method, gave limited advantages as the use of the latter method is known to be advantageous above all in the case in which evident turbidity or positive interferences must be abated [22], but this does not seem to be a problem encountered in the present research. Finally, the calorimetric method may be said to be very 'robust' as small variations in the operating parameters, such as in heating rate and even the limits of integration of the deconvoluted melting peak area, have no appreciable effect on the result of the measurement. In addition, another advantage of the calorimetric method is that it is not influenced by any hydrolytic process since the calorimetric measurements are carried out directly on a solid powder.

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