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PLACE OF DSC PURITY ANALYSIS IN PHARMACEUTICAL DEVELOPMENT

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Abstract

DSC purity analysis is based on thermodynamic phase diagrams for substances (purity ≥98%) which undergo a melting point. Impurities which have eutectic behaviour with the analyte are determined together.

DSC purity analysis obtained from a single melting event of a 1–2 mg sample is, therefore, extremely attractive for the global assessment of eutectic impurities. The main advantages in early development lie in the very small amount of material necessary and the very fast analysis time.

However, the DSC purity analysis cannot replace chromatographic methods which deliver specific individual levels of impurities. Furthermore, a complete validation of a DSC purity method is difficult and time consuming. Despite these limitations, DSC is the best support for the development of chromatographic methods, for purity profile and stability assessment during pharmaceutical development.

Parameters of purity determination and validation aspects are discussed. Examples of use in pharmaceutical development are given.

Keywords: DSC, eutectic point, pharmaceutical development, purity analysis

Introduction

The basis of any calorimetric purity method is the relationship between the melting depression of a substance and the level of impurities according to van't Hoff's law. The purity is readily calculated from the DSC curve of a single melting event of some mg of the substance without the need for reference standard of the drug substance and its impurities.

The DSC impurity analysis is described in USP XXII. Some difficulties may be encountered, but with modern equipment including robotic systems and data aquisition, the DSC purity analysis is a state of the art technique for pharmaceutical development.

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Instrument

Differential scanning calorimeters PERKIN-ELMER DSC-2 or DSC-7 Robotic system, with removable 48 position sample caroussel and pneumatically controlled robotic sampling arm and with PE 7700 computer, have been used.

Performance of the instrument is checked with standards for the temperature (4-nitrotoluene, naphthalene, benzil, acetanilide, diphenylacetic acid, indium, anisic acid, 2-chloro-anthraquinone, tin, anthraquinone, lead and zinc) and with the calorimetric standards (naphthalene, indium, 2-chloroanthraquinone) for the calorimetric signal.

Sample pans and covers in aluminium are heated at 400°C in order to eliminate impurities e.g. lubricants resulting from their manufacture. Sample pans are crimped (volatile impurities can escape) or tightly sealed (volatile sample pan = vsp).

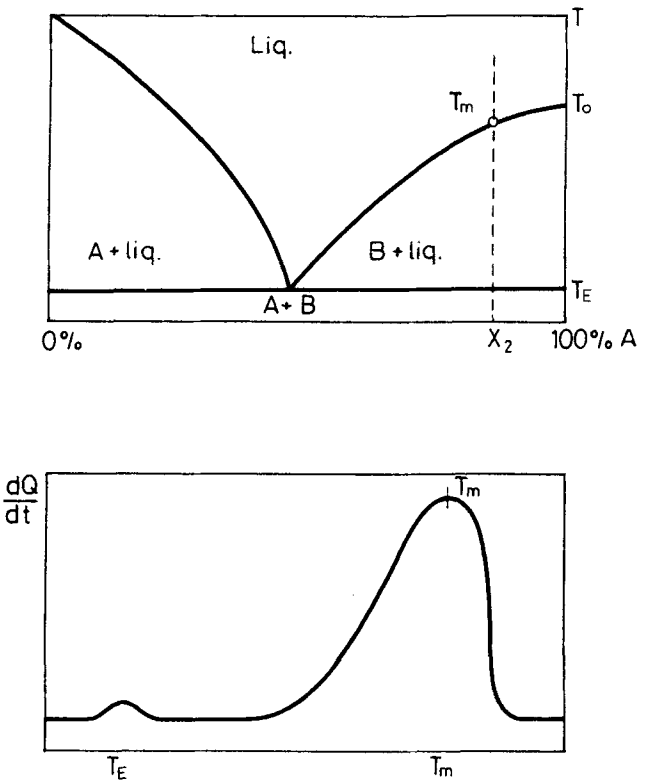

Fig. 1 Binary phase diagram with eutectic behaviour and corresponding DSC curve

Purity analysis is performed under pure nitrogen atmosphere (99.99%, flow 15–20 ml/min). A DSC scan of indium (PERKIN-ELMER, purity >99.999%) is performed under the same conditions.

Purity determination and validation

Principle of purity determination

The determination of purity by means of DSC is based on the assumption that impurities depress the melting point of a pure material according to the eutectic phase diagram behaviour [1–18].

Figure 1 shows the phase diagram for the two component mixture with so called eutectic point. At the eutectic point E, (for example 40% A, 60% B), the crystals A and B melt together at the temperature T_E, below the melting temperature of the pure compounds. If a mixture of A and B (containing e.g. 90% A) is heated, the melting of eutectic mixture (which is 40% in A) is observed initially, until all of B is melted. During the melting of the eutectic (40% A, 60% B) a part of A is melted with B, with the corresponding amount 2/3×10% of A, i.e. 6.66% of A.

Then as the temperature increases, pure A melts between T_E and T_m. T_m is the end of the melting. For the corresponding DSC curve, an endotherm at the eutectic temperature is observed, then the melting of crystals A occurs. The effect of impurity on the DSC curve is a melting depression and a broadening of the melting curve.

The amount of impurities is calculated from the melting point depression $\Delta T = T_o - T_m$. The van't Hoff's law for diluted solutions is

$$x = \frac{(-\Delta T \times \Delta H_f)}{RT_o^2} \tag{1}$$

where x is the mole fraction of impurities, ΔT the melting point depression, ΔH_f the melting point of pure material, T_m the melting of the analyte, T_o the melting point of the pure compound and R the gas constant.

The DSC procedure does not directly measure ΔT, but can be used to calculate it from the melting curve. At the eutectic point, all of B is in the liquid phase. During the melting of A after the eutectic point the concentration of B varies in the liquid phase. This causes the broadening of the DSC curve (Fig. 2).

With no solid solution formation, the concentration of impurity in the liquid phase at any temperature during the melting is inversely proportional to the fraction melted at that temperature, and the melting-point depression is directly proportional to the mole fraction of impurity. A plot of the observed analyte

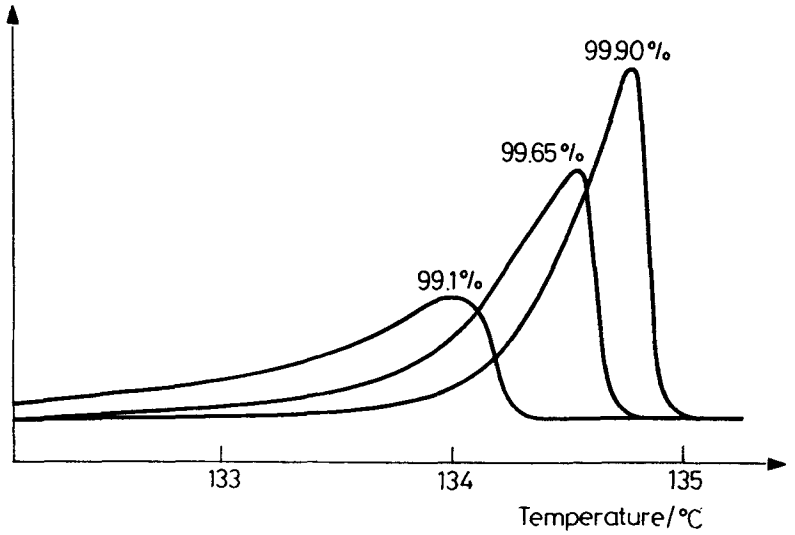

Fig. 2 Influence of purity on the shape of the DSC melting curve

temperature, T_i *vs.* the reciprocal of the fraction melted $1/F_i$ at temperature T_i should yield a straight line with the slope equal to the melting-point depression $(T_o - T_m)$. The theoretical melting point of the pure compound is obtained by extrapolation to $1/F_i = 0$:

$$T_i = T_o - \frac{RT_o^2 \times (1/F_i)}{\Delta H_f} x \tag{2}$$

The relation (2) may be expressed:

$$T_i = T_o - \Delta T(1/F_i) \tag{3}$$

Substituting the experimentally obtained values for ΔT, ΔH_f, and T_o in Eq. (1) yields the mole fraction of the total eutectic impurities, which, when multiplied by 100, gives the mole percentage of total eutectic impurities.

The temperature of each point T_i is the sample temperature, not the programmed temperature. Due to the thermal lag, a correction depending on the instrument has to be done for each point. For the power compensated DSC, the calculation is as follows:

$$T_i = T_{read} + 156.6 - T_{indium} + 273.3 - dQ/dt \times R_o \tag{4}$$

T_{read} is the temperature on the abscissa for each point i, R_o is the thermal constant expressed in °C/mw if the signal is in mw. T_{indium} is the observed melting

point of indium. R_o is determined from the slope of the melting curve of pure indium.

The melting curve is divided into small portions and each area S_i calculated. The melted fraction F_i,

$$F_i = \frac{S_i}{S_{total}} \tag{5}$$

is calculated for each point and the curve T_i is plotted as a function of $1/F_i$, where T_i is the temperature at fraction F_i (Fig. 3).

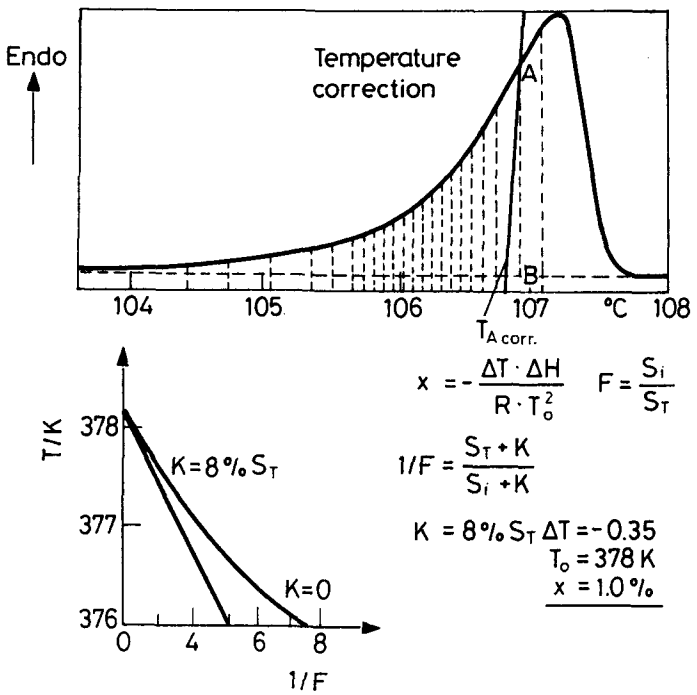

Fig. 3 Procedure of DSC purity analysis

The slope ΔT and the ordinate T_o can be calculated.

Partly because of the lack of the eutectic-point detection, the curve is not a straight line, and a correction factor K must be added to each fraction of the curve. Formation of solid solution or artefacts during melting may be also responsible.

$$F_i = \frac{S_i + K}{S_{total} + K} \tag{6}$$

Sondack (12) proposed a quick method with only 3 points of the curve.

$$X = \frac{M}{RT_o^2 m} \times \frac{T_3 - T_1}{\dfrac{1}{\Delta H_1 + K} - \dfrac{1}{\Delta H_3 + K}}$$

$$K = \frac{\Delta H_3 \dfrac{T_3 - T_2}{T_2 - T_1} - \Delta H_1 \dfrac{\Delta H_3 - \Delta H_2}{\Delta H_2 - \Delta H_1}}{\dfrac{\Delta H_3 - \Delta H_2}{\Delta H_2 - \Delta H_1} - \dfrac{T_3 - T_2}{T_2 - T_1}}$$

M molecular mass of the main component

m sample mass in mg

$\Delta H_{1,2,3}$ partial heats of fusion at T_1, T_2, T_3 (or S_1, S_2, S_3)

K correction for linearization.

Software from manufacturers mostly use iterative linearization which gives the best value of K with 10–30 points (Figs 3, 4).

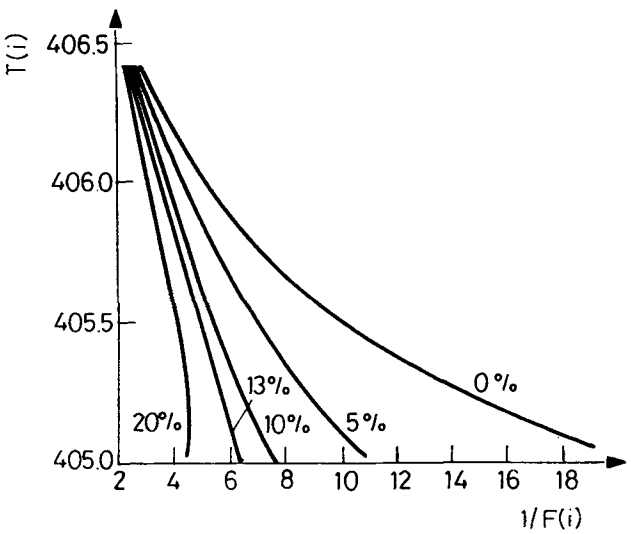

Fig. 4 Influence of the correction factor for the linearizations of the plot $T_i = f(1/F_i)$

Characteristics of this determination are as follows:

– Impurities are measured which have an eutectic behaviour (i.e. solubles in the liquid phase and insolubles in the solid phase)

– The sum of impurities must be ≤2%

– The result is expressed in mole % without knowledge of impurities

– Pure material is not needed

– Small amounts (1–2 mg or less) of material are used

– If decomposition occurs during melting it can give erroneous results
– The purity results are obtained after less than 1 h.

Other methods have been described, but are not generally used, including: addition of pure compound to sample, use of the maximum of the derivative of the melting point, use of RC calculation, analysis of the peak form or calibration curves [10, 16–18].

Product parameters

Thermal and thermodynamic equilibrium should be attained as far as possible, therefore the sample size should be 1–3 mg, and the lower the heating rate, the better the equilibration.

Heating rate between 1 and 5 deg·min^{-1} is recommended.

For very pure material a low heating rate is better. It should be increased for impure samples [4] in order to measure accurately the start of the peak.

The particle size affects the thermal conductivity. Since milling may decrease the crystallinity of the substance, some errors may be introduced, if the analyte is milled in a mortar.

Impurities in liquid state have to be miscible with the liquid melt of the drug substance and give ideal solutions. If enantiomers form conglomerates, the antipode will be measured. Since 95% racemate formation generally occurs, the purity method described here does not determine antipodes. Diastereoisomers have often eutectic behaviour.

The type of impurities (multiple phase diagrams), the vapor pressure during melting, possible parallel transitions during melting (sublimation, polymorphism, desolvation), reaction between impurities in the liquid state, degradation of impurities or drug substance during melting, the particle size of the sample and the homogeneity of the sample may contribute to possible difficulties originating from the sample and its impurities.

The type of pan is very important. For substances which sublimate a sealed pan is better. It is preferable to use pans with holes or crimped pans since solvent including water may be eliminated before the melting point.

In the case of salts, one should take into consideration the excess of acid or base which may act as eutectic impurity (Fig. 5).

A. A. van Dooren and B. W. Müller [1] and J. L. Ford and P. Timmins [15] discussed in detail the validity of van't Hoff's law. The stepwise method proposed [7–11], where the temperature is first increased and then kept constant in order to obtain the best equilibrium, is not used in the instrument software.

For samples which undergo some decomposition during melting, high heating rates have been suggested [9]. In order to prevent oxidation, purity analysis should always be carried out under nitrogen atmosphere.

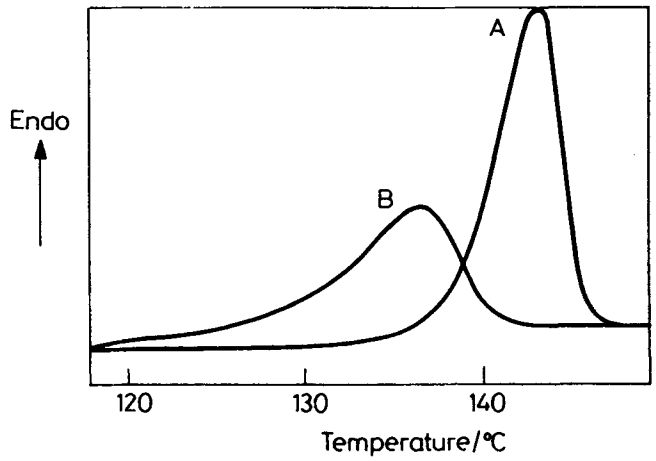

Fig. 5 Case of salts: an excess of the base or of the acid part may be determined as impurity. The corresponding phase diagram has been controlled: eutectic behaviour A: batch A of hydrogen maleate of the drug substance. B: batch A spiked with the corresponding base of the drug substance

If decomposition, sublimation or a change in the specific heat occur at the end of melting, the calculation of the S_{total} may be difficult. Considering that

$$\Delta Hf = \frac{S_{total} \times M}{\text{sample mass}} \times \text{unit} \tag{7}$$

Eq. (2) may be expressed:

$$T_i = T_o - \frac{RT_o^2 \times \text{sample mass} \times 1 / S_i}{M \times \text{unit}} \times x \tag{8}$$

and the plot $T_i = f(1/S_i)$ allows the calculation of T_o, the slope, then x.

In any case, if the total area is inaccurate this will have no impact on the accuracy of the calculation of ΔT and T_o according to Eq. (3).

The error will have impact only on the value of ΔH_f. An error of 20%, for instance, would have an impact of 20% on the molar impurity.

Examples of the influence of polymorphic behaviour on the purity analysis

Case of propyphenazone

The purity analysis method was optimized using batches from manufacturer 1 at a heating rate of 2.5 deg·min^{-1}, where only one melting peak was observed. We received samples from manufacturer 2, which had too high a

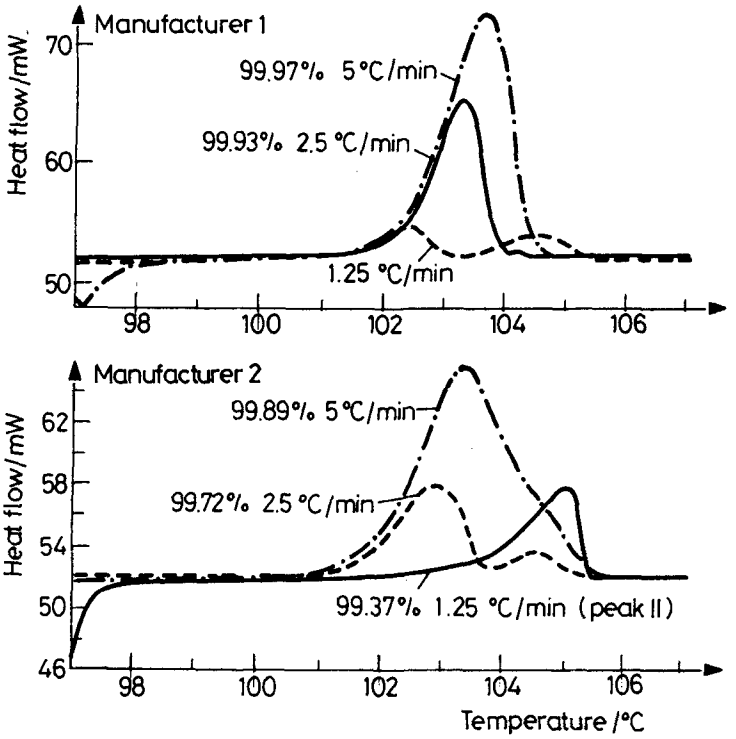

Fig. 6 Polymorphism of propyphenazone. Influence of the heating rate and the kinetic behaviour of the polymorphic transition on the purity determination

melting point according to the pharmacopoeia method. The DSC scans showed a second peak after the first melting peak.

The polymorphic behaviour of propyphenazone [19–20] was studied. The form melting at higher temperatures (Modification II) is due to a reversible enantiotropic transition which occurs in the melt.

Modification II was manufactured by heat treatment and an X-ray diffraction method for quantitative analysis of form II in commercial batches was developed [21].

Figure 6 shows the influence of the heating rate on the DSC scans of samples of manufacturer 1 or manufacturer 2 with a 30 µl sample pan with holes.

For both manufacturers the slow heating rate of 1.2 deg·min^{-1} is not suitable for purity measurements. The transition in the second modification takes place in the melt. At a higher heating rate no transition takes place in the sample from manufacturer 1.

For this sample purity results are not affected by the heating rate: Sample from manufacturer 1: 10 deg·min^{-1}: 99.99%, 5 deg·min^{-1}: 99.97%, 2.5 deg·min^{-1}: 99.93%.

For the sample from manufacturer 2, purity results are a lower at slower heating rate.

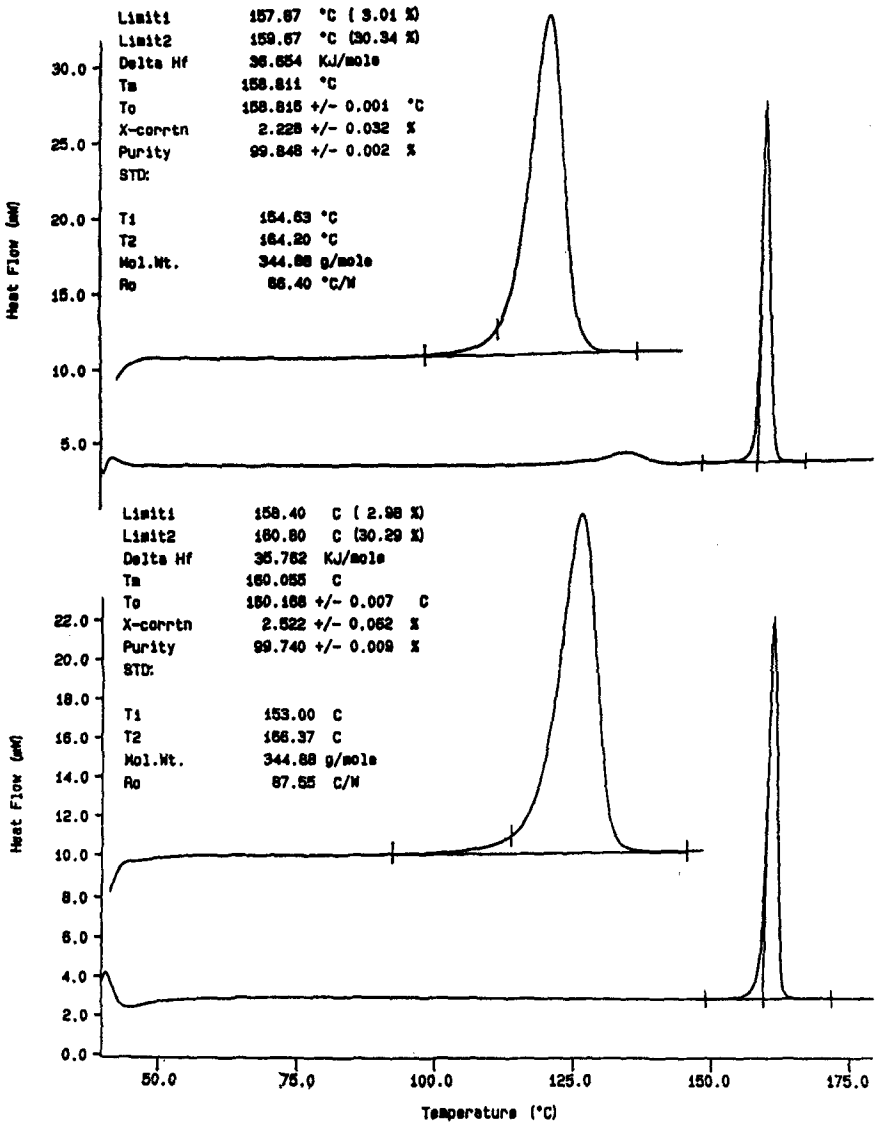

Fig. 7 Polymorphic transition before the melting point: no influence on the purity calculation in the case of oxybuprocaine. Upper curve: modification A, lower curve: modification B

Sample from manufacturer 2: 10 deg·min^{-1}: 99.91%, 5 deg·min^{-1}: 99.89%, 2.5 deg·min^{-1}: 99.72%.

Case of oxybuprocaine

Figure 7 demonstrates that purity analysis in not affected by the endothermic transition A→B which takes place before the melting point for the modification A.

Instrument parameters

The accuracy of the DSC scan is dependent on the instrument and should be checked. The DSC peak slope is different in the power compensated DSC (PERKIN-ELMER) and in the heat-flux DSC (temperature is the primary measurement, therefore, the calorimetric calibration of the instrument should be given by the manufacturer).

Since the sample mass has to be small, the instrument has to be sufficiently sensitive.

The calibration of the thermal constant of the instrument and, therefore, the correction of each temperature T_i has to be calculated, e.g., with pure indium in the same type of pan.

For the PERKIN-ELMER DSC-7 for example, the thermal constant varies from 50 to 90°C/W depending on the aluminium pan and the sample holder. The impact on this determination is demonstrated in the example in Table 1.

The temperature does not greatly affect the accuracy of the level of impurities. A 3°C error would give an error of 2% in the value of x.

Data acquisition and processing

The number of points used in the calculations, the peak sensitivity, the base line drift and the base line noise have direct impact on the precision of the results.

The Sondack method [12] with only 3 points of the melting curve is not recommended.

Important parameters are the linearization procedure of the plot $T_i = f(1/F_i)$ and the part of the melting curve which is used. The lower part of the melting curve corresponding to the high $1/F_i$ values has a high effect on the correction factor K (Fig. 4), but since it is the beginning of the melting, the integration is less accurate.

The upper part of the melting curve corresponding to low $1/F_i$ values does not play a big role in the correction of K but is measured with higher precision.

Table 1 Influence of the value of the thermal constant R_o on the impurity result for a new drug substance with a melting point of 184°C. Heating rate 5 deg·min^{-1}

	R_o / °C·Watt^{-1}	% Impurity
Batch 1	60.0	0.26
	83.7	0.24
	100.0	0.22
Batch 2	60.0	0.65
	83.7	0.63
	100.0	0.61

Table 2 Influence of the area of calculation for Darodipine and Sorbitol

	Area of calculation					
Darodipine batch 81905 1.2 deg·min^{-1}						
	3–30 %	4–40 %	5–40 %	6–50 %	3–55 %	3–60 %
Impurity %	0.24	0.25	0.26	0.26	0.24	0.25
K/%	3.5	3.7	4	4	3.4	3.5
ratio % upper limit/ maximum height	43	59	59	73	78	84
Sorbitol batch 88834 5 deg·min^{-1}						
	3–30 %	5–40 %	5–45 %	3–50 %	5–50 %	3–60 %
Impurity %	1.20	1.85	1.95	1.38	2.01	1.46
K/%	5.6	9.1	9.5	9.7	6.3	6.5
ratio % upper limit/ maximum height	50	63	67	75	75	86

Table 3 Comparison of calculations methods for a batch of Darodipine

Sandoz Giron	99.74 %
DSC-2 Data station	99.74 %
DSC-7 MLR method	99.76 %
DSC-7 STD method	99.78 %
Sondack method	99.70 %

The upper part is limited by the peak maximum indicating that the whole sample is melted. We suggest that the part of the melting peak with a height >80% of peak maximum is not used.

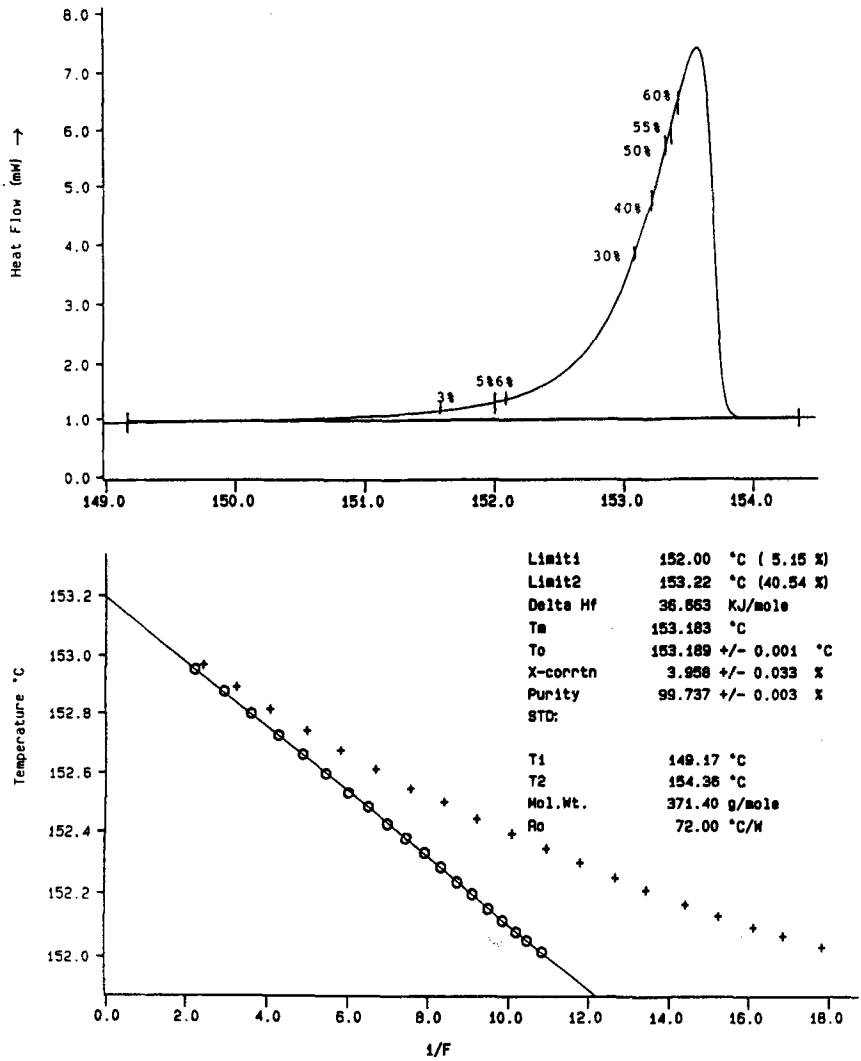

Fig. 8 Darodipine. DSC curve and parts of area used for calculations of Table 2. Example of the plot $T_i = f(1/F_i)$ and calculation for the area 5–40%

Since calculation is very quick, we suggest that different parts of the curve, e.g. 3–30%, 3–40% and 3–50% are used systematically and that the results are compared. Change of purity results with the area of calculation is an indication of parallel phenomena such as a decomposition, transition, sublimation etc.

Table 2 gives purity results using different areas of the curve for Darodipine which exhibits an ideal melting and for Sorbitol which decomposes during melt-

ing. Figure 8 shows the corresponding curves of the PERKIN-ELMER software for Darodipine.

Table 3 gives a comparison between our own interative procedure (1972) using a Sandoz interface and a Hewlett Packard 9830 calculator (Sandoz Giron), 3 different software from PERKIN-ELMER and the Sondack method for another batch of this drug substance.

A system suitability check of each instrument with substance of given purity would be useful. Table 4 gives the purity results of a batch of Darodipine which was found to be stable throughout the time. Use of such a compound is suggested for checking the instrumental parameters.

Table 4 Checking of performance of DSC purity calculation system using a batch of Darodipine with the same method (system suitability)

Date	Instrument	Impurity %
1985	DSC-2 and software	0.32 %
1990	DSC-7 and software	0.28 %
1991	DSC-7 and software	0.31 %
1992	DSC-7 and software	0.26 %

NBS standards of phenacetine doped with *p*-aminobenzoic acid are available.

Robotic systems

Robotic systems are very attractive for efficient use of DSC purity analysis [22]. The reproducibility of the results may be affected by the position of the pan in the sample holder, or by the integrating systems.

Table 5 gives examples which show the reproducibility which can be obtained by the DSC-7 robot of PERKIN-ELMER.

Development of purity method and validation

Step 1

Study of the melting process first provides information on its reversibility: comparison of the DSC purity of a second scan after cooling. For a sample of Darodipine the first calculation gave a purity result of 99.82%. After the sample was cooled to room temperature, a second scan was performed immediately. A purity of 99.97% was obtained, but the enthalpy was only 20% of the theoretical value (36 kJ/mole). Therefore, pure drug substances crystallize first! We repeated the experience and performed the second DSC run waiting 2 h at room

temperature after cooling. The purity value obtained was 99.84% with an enthalpy of 30 kJ/mole.

Table 5 Reproducibility of results obtained by the DSC-7 robot of PERKIN-ELMER

Physostigmine base: heating rate, 5 deg·min^{-1}; ΔH = 33 kJ·mol^{-1}

Weight	Onset °C	Purity
2.197	104.3	99.69
2.196	104.1	99.64
2.109	104.1	99.64
2.109	104.2	99.65
2.188	104.2	99.60
2.222	104.1	99.66
2.186	104.1	99.60
1.110	104.1	99.70
3.153	104.0	99.70
4.241	104.2	99.69

Mean = 104.15°C; SD = 0.08; RSD = 0.08%; n = 10

Butalbital: heating rate 2.5 deg·min^{-1}; ΔH = 25 kJ·mol^{-1}

Weight	Onset °C	Purity
1.583	139.0	99.84
1.561	138.9	99.85
1.578	138.9	99.88
1.540	138.9	99.90
1.527	138.8	99.90
1.537	139.2	99.86
1.519	139.0	99.88
1.070	139.0	99.88
2.098	139.0	99.84
3.069	139.1	99.88
4.047	139.4	99.96
5.082	139.3	99.96

Mean = 139.04°C; SD = 0.18; RSD = 0.13%; n = 12

Therefore, we do not recommend that purity analysis is calculated on a second scan only.

Since most organic substances do not behave ideally and remain in a glassy state after cooling, thermogravimetric analysis or chromatographic analysis allows the checking of decomposition during melting.

Table 6 Comparison of results at different heating rates demonstrating degradation during melting

Heating rate/deg·min^{-1}	20	10	5
Purity %	98.7	98.0	96.9

The comparison of DSC analysis at different heating rates may also indicate degradation (Table 6).

Step 2

Development of a purity analysis begins with the knowledge of the influence of the experimental conditions

- sample pan
- sample mass
- heating rate
- temperature and holding time of the start of the DSC scan
- Part of the curve for the plot $T_i = f(1/F_i)$.

Table 7 Influence of the parameters on the purity results for a batch of Darodipine with ideal melting behaviour

Influence of the heating rate Weight 1 mg		Influence of the sample mass (1.25 deg·min^{-1})	
deg·min^{-1}	Purity/%	Mass/mg	Purity/%
0.31	99.6 (5)	1.060	99.5 (8)
0.62	99.6 (2)	2.017	99.6 (0)
1.25	99.5 (8)	3.003	99.6 (5)
2.50	99.5 (7)	5.015	99.6 (8)
5.00	99.5 (8)	6.942	99.7 (2)
10.00	99.6 (4)		

Table 7 shows such a program for Darodipine with ideal melting. The same results were obtained with a crimped pan or a sealed pan.

Table 8 deals with drug substances with partial decomposition during melting. For both substances higher purity values are obtained with increasing sample mass. Reproducibility of the method in terms of purity may be very high, see Tables 5 and Table 9.

Table 8 Influence of the parameters on the purity results of drug substances with partial decomposition during melting

Influence of the heating rate		Influence of the sample mass (5 deg·min^{-1})	
deg·min^{-1}	Purity/%	Mass/mg	Purity/%
Substance 1 area 3–30 %			
1.25	99.90	1	99.77
2.50	99.89	2	99.81
5.00	99.89	3	99.84
10.00	99.52	4	99.97
20.00	99.56	5	99.94
Substance 2 area 3–40 %			
1.25	98.90	1	99.71
2.50	98.70	2	99.72
5.00	99.68	3	99.76
10.00	99.69	4	99.74
20.00	99.68	5	99.84

Table 9 Results of the standard deviation corresponding to Table 7

Standard deviation with 1.25 deg·min^{-1}	
For purity	0.03 %
For impurities	3 %
Individual values	99.59, 99.62, 99.62, 99.65, 99.59, 99.57, 99.64, 99.62

Step 3

For validation of a DSC purity method, the biggest problem is checking the thermodynamical relationship between impurities and substance.

Since most substances do not melt again after a first DSC run, the method of producing standard mixtures may be problematic. Mixtures formed in situ in the sample pan are not homogeneous and the first melting curve is often erroneous, especially for solid-solution. Evaporation of the solvent of mixtures of fixed compositions is a time consuming procedure, but it is attractive because it reproduces the manufacturing procedure. Table 10 shows the results of such a procedure. However, as demonstrated in Fig. 9, one may encounter problems of polymorphism.

Table 10 Results of DSC purity analysis of mixtures obtained by mixing in solid state (method 1) or in solution (method 2) and comparison to phase solubility analysis (PSA) Fluproquazone + 1% by-product

	Sample	Mixture
PSA	0.0 %	0.73 % (method 1)
DSC	0.07 %	0.8 % (method 1)
DSC	0.07 %	0.8 % (method 2)

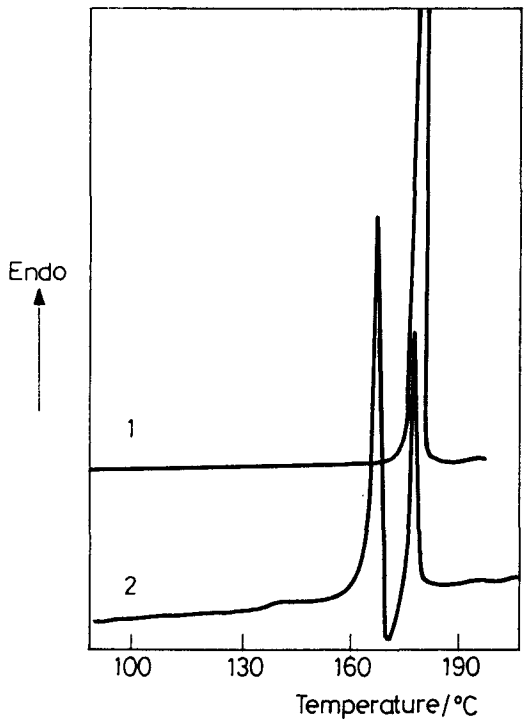

Fig. 9 Mixture of drug substance and 2% by-product recrystallised in order to reproduce manufacturing process: difficulty for purity analysis because of a new polymorphic form

Mixing different levels of impurities with drug substance before the introduction of an aliquot into the sample pan is the easiest way.

The calculations have to be expressed in mole %. As demonstrated in Tables 11–14 not all the impurities have eutectic behaviour.

For salts an additional step is to manufacture samples of impurities with the same salt forming agent.

Table 11 Results after spiking of by-products of Darodipine

Sample	Amount spiked/ mole %	Purity theoretical/ %	Purity found/ %
Sample	–	–	99.70
Sample + 1 % BP 1	0.7	99.0	99.65
Sample + 1 % BP 2	2.3	97.4	98.30

Table 12 Accuracy of the method for an indole derivative in the hydrochloride salt form. Melting point: 283°C (heating rate 10 deg·min^{-1})

	Amount spiked/ mole %	Purity theoretical	Purity found
Drug substance	–	–	99.6 %
1 % BP 1	2.3 %	97.3 %	98.4 %
1 % BP 2	2.0 %	97.6 %	99.1 %
1 % Base	1.1 %	98.5 %	98.5 %

Table 13 Accuracy of the method for a benzopyran derivative. Melting point: 156°C. (Heating rate 5 deg·min^{-1})

	Purity/ mole % theoretical	Purity Range 3–30 %	Purity Range 5–40 %
Batch 1	–	99.7 %	99.5 %
Standard deviation (n=7)		0.05 %	
Batch 2	–	99.9 %	99.9 %
Batch 2 + 1 % BP 1	98.9 %	99.25/99.36 %	99.1/99.3 %
Batch 2 + 1 % BP 2	98.5 %	98.8/98.2 %	98.7/97.9 %
Batch 2 + 1 % BP 3	96.9 %	98.9 %	98.3 %

Another way to check validity of DSC purity determination is to compare the results with other techniques, as shown in Tables 15–17 for different drug substances. This can only be done at the end of the development of the chromatographic methods. The results are not expressed in the same units: mole% in DSC and mass % in chromatography.

In conclusion, the development of a purity method with the Steps 1 and 2 is easy to carry out, especially with robotic systems and data processing. Step 3 is very often difficult and is highly time consuming. Steps 1 and 2 should be done with the first batch used in toxicological experiments.

Table 14 Accuracy of the DSC purity analysis for a hydrogene tartrate salt of a phenyl carbonate derivative (calculation in 3–30% melting area)

Sample	Amount spiked/ mole %	Purity theoretical/ %	Purity found/ ($\bar{x}_2$)
Drug substance	–	–	99.70
+ 0.2 % base	0.3	99.4	99.66
+ 0.5 % base	0.8	98.9	99.74
+ 1 % base	1.6	98.1	99.75
+ 0.2 % BP 1	0.5	99.2	99.45
+ 0.5 % BP 1	1.2	98.5	99.08
+ 1 % b BP 1	2.4	97.3	99.00
+ 0.2 % BP 2	0.2	99.5	99.58
+ 0.5 % BP 2	0.5	99.2	99.35
+ 1 % BP 2	1.0	98.7	99.20
+ 0.2 % BP 3	0.2	99.5	99.75
+ 0.5 % BP 3	0.5	99.2	99.62
+ 1 % BP 3	1.0	98.7	99.43

Table 15 Comparison of methods for the amount of impurities of several batches of hydrogene tartrate salts of phenyl carbonate derivative

Batch	DSC (mole)	HPLC (mass)	TLC (mass)
92909	0.26 %	0.10 %	–
91908	0.50 %	0.93 %	1.0 %
91907	0.36 %	0.20 %	0.2 %
91906	0.25 %	0.20 %	0.2 %
91905	0.20 %	0.26 %	0.2 %
90904	0.50 %	0.66 %	0.6 %
88903	0.0 %	0.0 %	0.0 %
Recontrol	0.20 %	0.0 %	0.0 %
88902	0.20 %	0.0 %	0.0 %
Recontrol	0.10 %	0.0 %	0.0 %

For research sample which are available in very small amounts, such a study is often not possible and generally a medium heating rate (e.g. 5 deg·min^{-1} or 10 deg·min^{-1}) can be used, taking into account that the result is not validated.

Table 16 Comparison of methods for the amount of impurities of two batches of a naphthyridine derivate

Batch	DSC (mole)	HPLC (mass)
1	0.24 %	0.65 %
2	0.63 %	0.65 %

Table 17 Comparison of methods after optimization of chromatographic methods for Fluoroquazon

Batch	DSC	HPLC	TLC	PSA
80906	0.1 %	0.10 %	0.18 %	0.00 %
80907	0.1 %	0.15 %	0.20 %	0.10 %
80908	0.1 %	0.20 %	0.20 %	0.30 %
80909	0.1 %	0.20 %	0.20 %	0.25 %
80910	0.1 %	<0.1 %	0.10 %	0.10 %
81901	0.12 %	0.20 %	0.20 %	0.20 %
81902	0.1 %	0.20 %	0.20 %	0.20 %
81903	0.1 %	0.20 %	0.20 %	0.20 %
81904	0.1 %	<0.1 %	<0.1 %	0.14 %
81905	0.1 %	<0.1 %	<0.1 %	0.20 %

Table 18 Advantage of DSC purity analysis for a lactone instable in solutions. Results given as impurities

Batches	TLC	NMR	DSC	HPLC
76901	Sum: 0.7 %	<0.15 % Acid	Sum: 0.1 %	0.1 % Acid
76902	1.5 % Acid	1.3 % Acid	Sum: 0.6 %	0.9 % Acid
76903	2.0 % Acid	1.6-1.9 % Acid	Sum: 0.9 %	1.1 % Acid

Examples of use in pharmaceutical development

Amount of the analyte

Purity analysis may be successfully performed with very small amounts of substance. For Darodipine it is possible with the slow heating rate of 1.2 deg·min^{-1} to determine precise values with 0.2 mg of substance (Fig. 10). With a heating rate of 20 deg·min^{-1}, measurement is feasible with 0.03–0.05 mg!

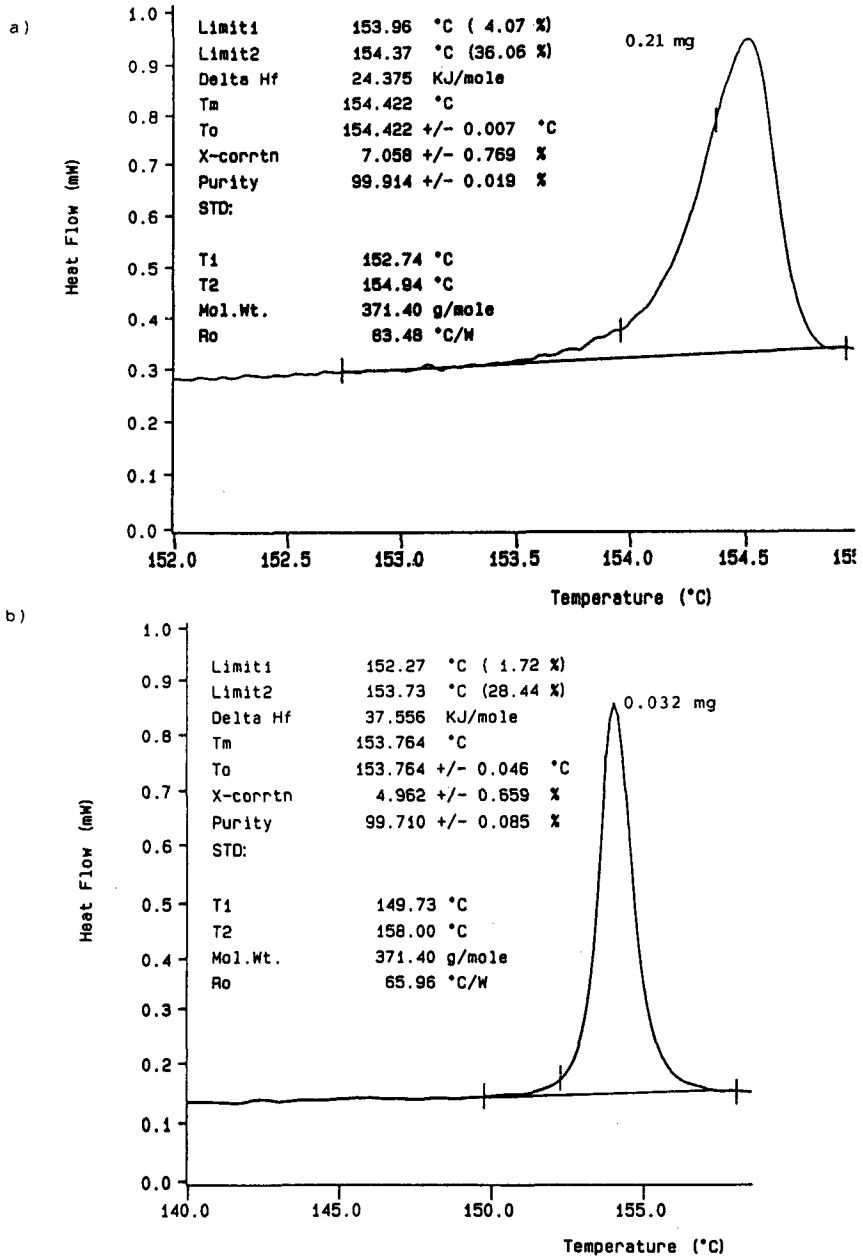

Fig. 10 DSC purity experiments: a) 0.21 mg Darodipine batch 85911 and 1.2 deg·min^{-1} heating rate. 0.58 mg: 99.82%, 1.017 mg: 99.85%; b) 0.03 mg Darodipine batch 87912 and 20 deg·min^{-1} heating rate

This is an advantage for the analysis of radio labelled material used in pharmacokinetics studies. Purity may also be determined for laboratory samples for comparison of for samples of by-products which are available in very small amounts.

Homogeneity or batches can be checked easily (Fig. 11).

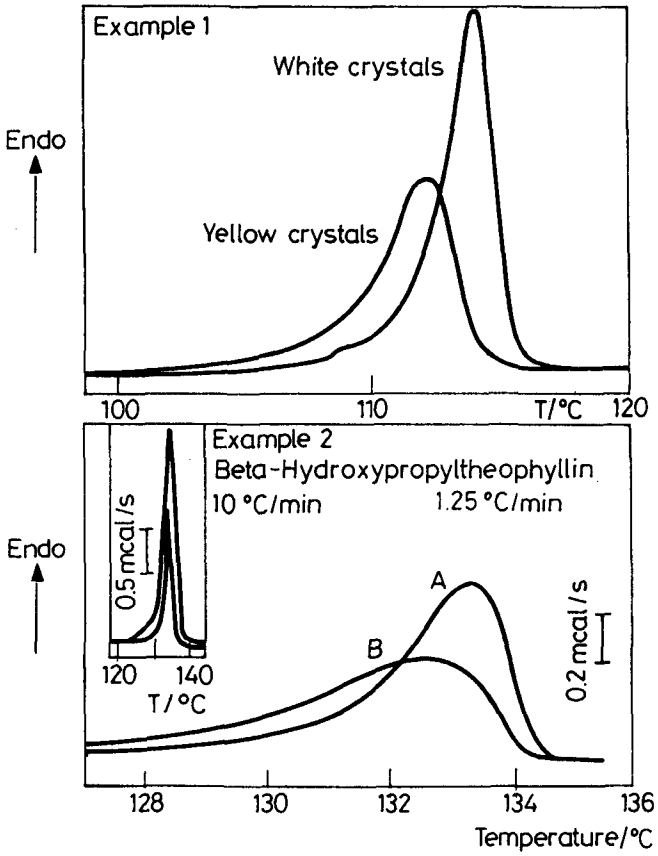

Fig. 11 Inhomogeneity of samples demonstrated by DSC

Support for chromatographic techniques

The example of β-hydroxypropyltheophylline given in Fig. 12 is our first experience with DSC purity analysis. We had to check a TLC procedure. The DSC purity analysis was the best argument to prove the inadequacy of the purity procedure.

Artefacts in chromatography can be quickly detected in the development phase as demonstrated in Table 18 for a lactone instable in solutions. TLC and NMR procedures induced artefacts. By using DSC and HPLC, purity was cor-

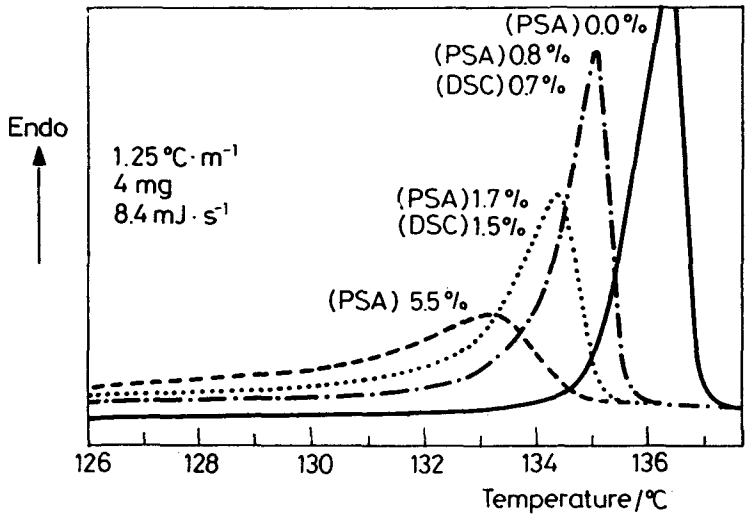

Fig. 12 Comparison of samples of β-hydroxypropyltheophylline of different manufacturers: The TLC procedure did not reveal any impurity. (PSA = phase solubility results)

rectly assessed. For a batch of hydrochlorothiazid an artefact in the HPLC analysis could be demonstrated by using the DSC purity result.

For very early development it is not important to deliver validated data. The information about global purity is the most important advantage of DSC whether the substance is 98% or 99.9% (Table 19).

Table 19 Combination of DSC with other techniques to solve a problem of unknown impurity

Batch	DSC	TLC			HPLC	PSA
		a	b	c		
1	0.10 %	0.15 %	<0.15 %	<0.15 %	n.p.	n.p.
2	0.20 %	0.20 %	<0.15 %	<0.15 %	n.p.	n.p.
3	0.12 %	0.25 %	<0.15 %	0.15 %	n.p.	n.p.
4	1.50 %	0.10 %	>1 %	2 %	1.40 %	1.70 %

n.p. = not performed

Figure 13 deals with two batches of a drug substance. The DSC curves demonstrated a difference of quality. Since some degradation occurs during melting the purity results obtained at 10 deg·min^{-1} were questionable (99.5% *vs.* 98.5%). No by-products were detected by HPLC and TLC. Since we were convinced that new by-products were present in batch 2, we changed the method (*pH* of the mobile phase and gradient): new impurities were separated. After isolation it appeared that one impurity had a HPLC UV factor of 5!

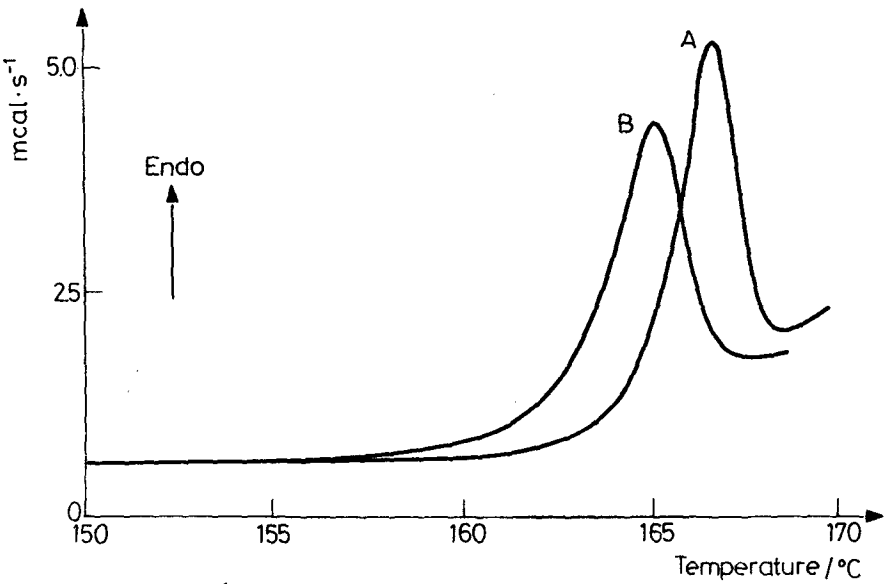

Fig. 13 Support of DSC for chromatographic method. Indole derivative with decomposition on melting. Batch A: 99.5%, batch B: 98.5%

In development every batch is analyzed by DSC. If the purity result is lower than the result given by chromatography, the DSC information (if no artefacts possible) is correct; if the purity result of chromatography methods is lower, some impurities do not behave as eutectic impurities.

Monitoring of purification

If the DSC purity is low, purification via crystallization is possible. The combination of DSC purity analysis with purification via equilibration of an excess of drug substance with solvent (principle of phase solubility analysis) permits the finding of the appropriate solvent of purification. The substance which is not dissolved is purer, and the solution is enriched in impurities. The enriched solution is used for chromatographic study. Such an example is given in Fig. 14.

The sample delivered in Fig. 15 was analyzed by HPLC but the impurity was not separated as demonstrated by DSC. Our combined technique allowed the purification of the batch and the enrichment allowed the isolation of the impurity and a quick optimization of the HPLC purity method. 4% of a by-product was determined in the sample.

A quick check of purification control is also easy (Fig. 16).

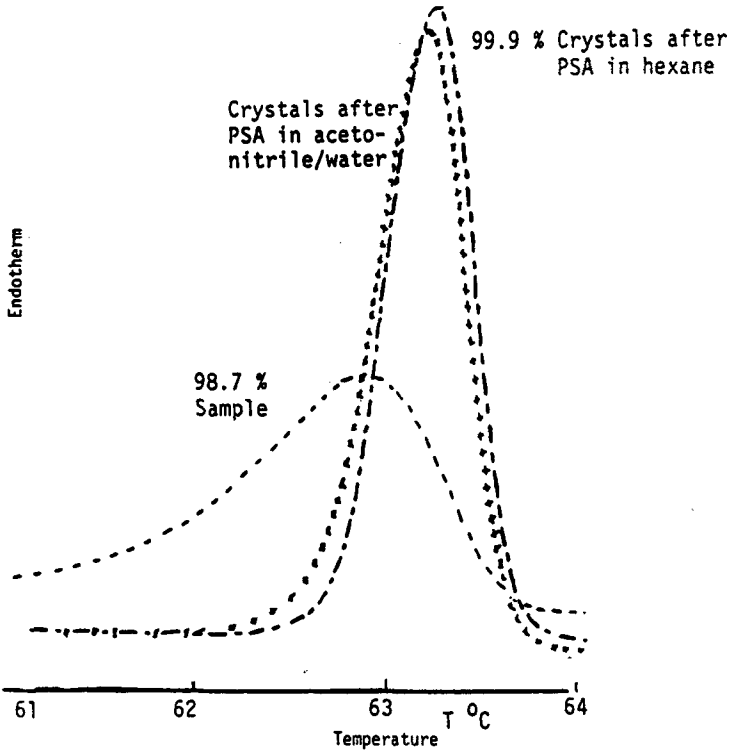

Fig. 14 Monitoring of purification. Combination of DSC and equilibration (PSA principle) to detect impurities and to purify samples

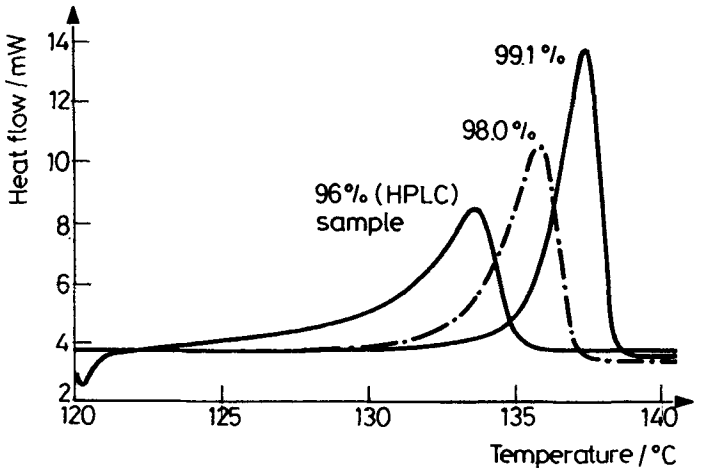

Fig. 15 Optimization of HPLC and purification procedure by means of DSC purity analysis

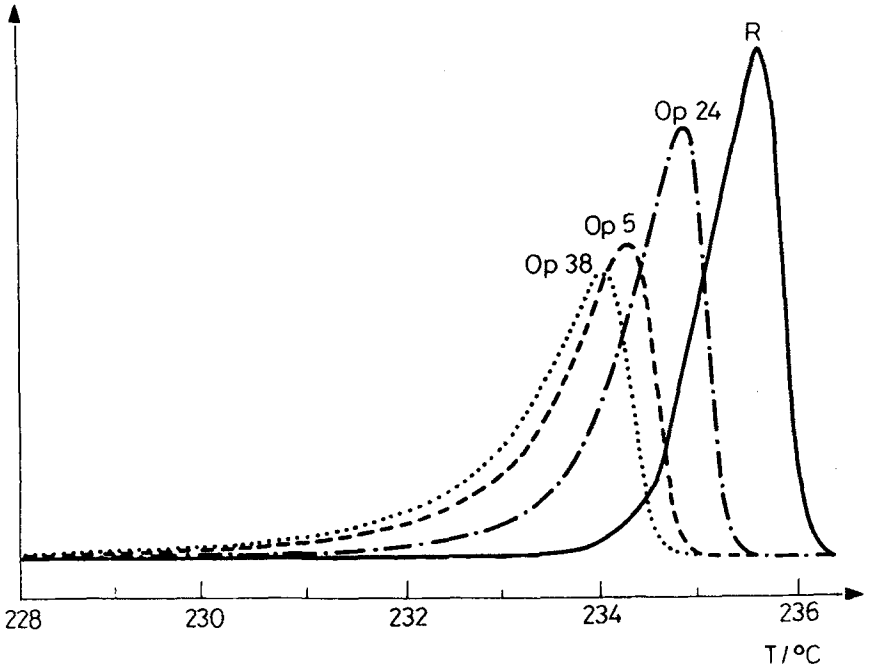

Fig. 16 Purification process control

Stability behaviour

In the early phase of development it is mandatory that the bulk drug substance is stored properly, otherwise all activities are questionable.

Figure 17 deals with a very unstable steroid for which not absolute method for assay, such as titration, was possible.

The assay was performed against a reference material. Due to the lack of detection of degradation products, the sensitivity of the substance to heat was not known and also the reference decomposed. The DSC analysis of batch 73901 which was manufactured 6 months earlier revealed poor quality.

In parallel the stability samples were checked by DSC (Fig. 18), which revealed the sensitivity of the drug to heat and to light.

A new reference was purified and the drug substance stored in a deep-freezer.

By using a new spray reagent the TLC purity method was subsequently optimized. Table 20 shows a comparison of all methods.

DSC purity analysis is used in our preformulation program. Figure 19 deals with a recent example. The drug substance is a fumarate and the degradation products were not separated from the fumaric peak with the HPLC procedure.

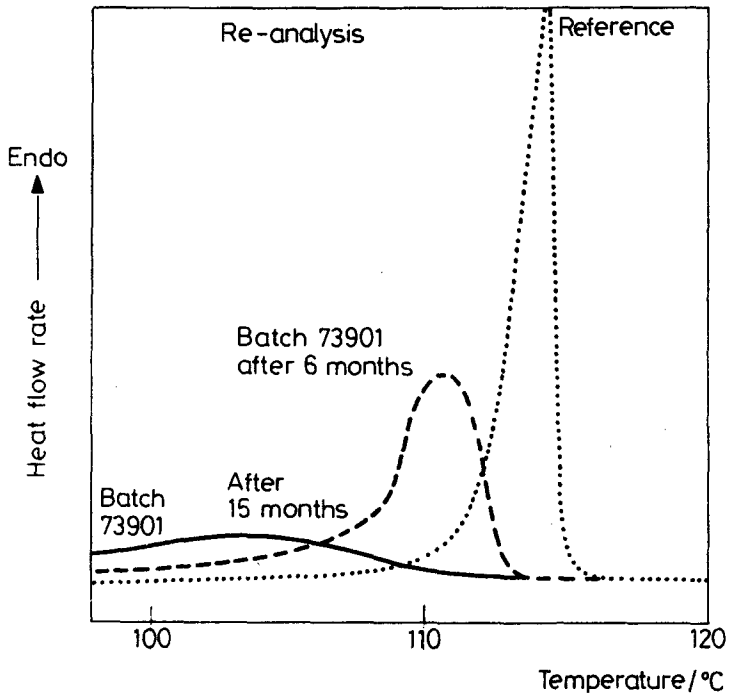

Fig. 17 Detection of degradation products of a steroid

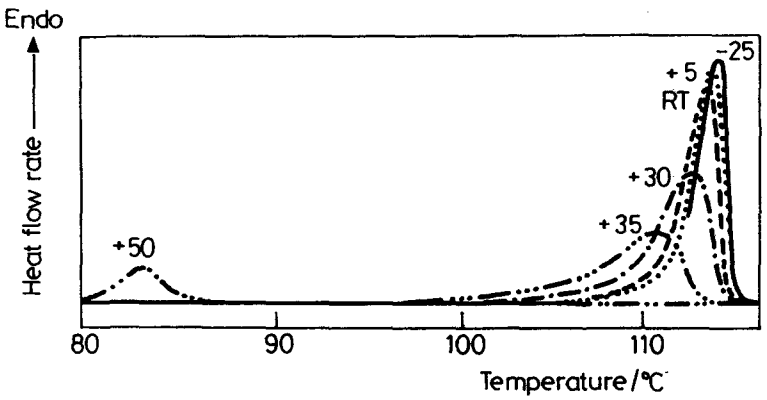

Fig. 18 Long-term stability screening of the steroid. DSC curves after 3 months at different temperatures

The stability behaviour of drug substance may be influenced by unknown factors, for example catalyst, particle size. Figure 20 shows how to use DSC purity analysis for batchwise prediction of stability.

Table 20 Comparison of DSC purity results with other analytical methods for an instable steroid. Assay are performed with the pure new reference stored in deep-freezer

Sample	Assay (UV)	Specific Assay (TLC)	DSC Purity	PSA	TLC Purity
1	95.3 %	92.0 %	<95.0 %		No impurity
2	87.5 %	87.5 %	Not calculable		"
3	100.0 %		99.3 %	98 %	"
4	99.7 %	98.0 %	99.1 %	<98 %	"
5	99.7 %		99.7 %		"
					New TLC
6	99.7 %	99.3 %	99.4 %		99.0 %
7	99.3 %	99.1 %	99.1 %		98.7 %
8	99.8 %	100.8 %	99.7 %		99.6 %

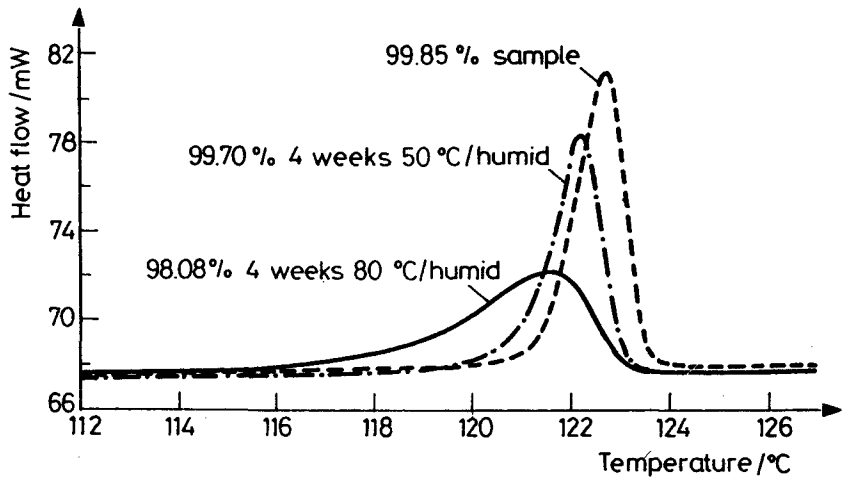

Fig. 19 Optimization of the HPLC for degradation products

Figure 21 illustrates the use of DSC purity analysis in the first stability screening of drug substances. For the first case high sensitivity to light was observed. The drug substance was not sensitive to heat, oxygen or moisture but was sensitive to light. In the second case degradation occurs only in the presence of humidity.

DSC purity analysis is used not only to reveal instability. It also supports the results of chromatographic analysis of long term stability results, as demonstrated in Table 21.

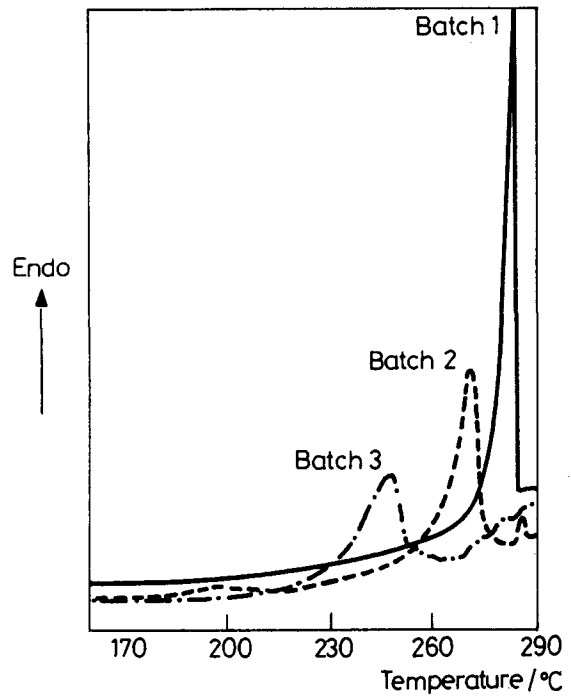

Fig. 20 DSC curves of stability samples demonstrating the different stability behaviour of batches of a drug substance stored in tropical climate (30°C/75% r.h.)

Control of starting material and intermediate

A change of quality in starting materials or intermediate may have disastrous consequences for the quality of the drug substance.

Figure 22 shows a comparison of the quality of three manufacturers for a key intermediate of synthesis.

Table 21 Use of DSC for the analysis of long-term stability samples

Substance	DSC purity	Storage	
	initial value	30°C/75 % r. h. time	DSC purity
Isradipine	99.8 %	4 years	99.80 %
Proquazone	99.8 %	4 years	99.80 %
Clemastine hfu	99.8 %*	7 years	99.80 %
Darodipine	99.8 %	5 years	99.75 %

*Sample stored in deep freezer

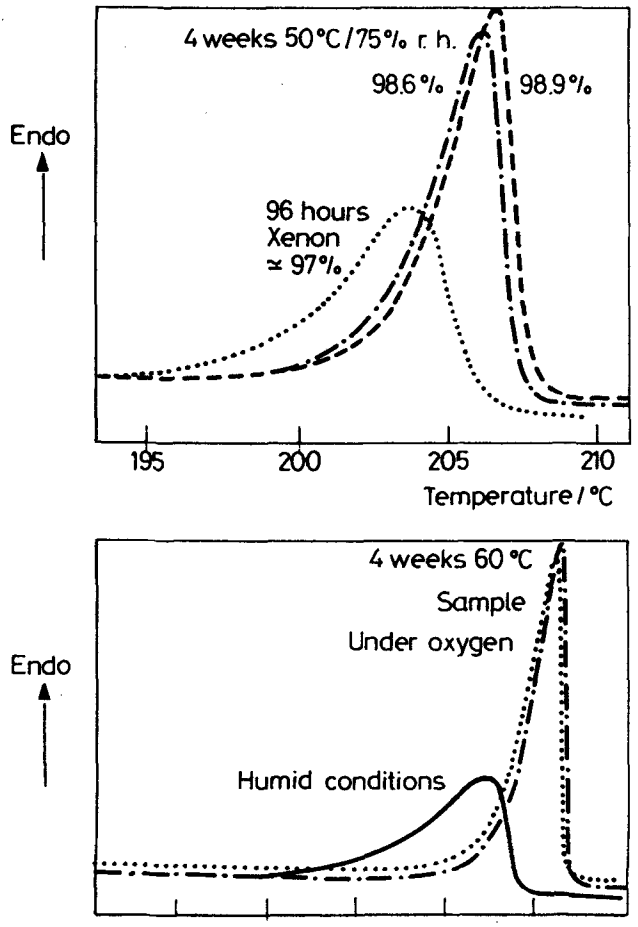

Fig. 21 Choice of packing conditions of drug substances after stress experiments under nitrogen, oxygen, humidity and light exposure

Change of synthesis. Purity profile

Very often a change of synthesis implies a change of chromatographic methods. The example given in Fig. 23 demonstrates the use of DSC to support purity evaluation. Batch 10 contained a new impurity not determined by TLC nor HPLC. After optimization of chromatography urea derivatives were found. These could be removed by a change in the process.

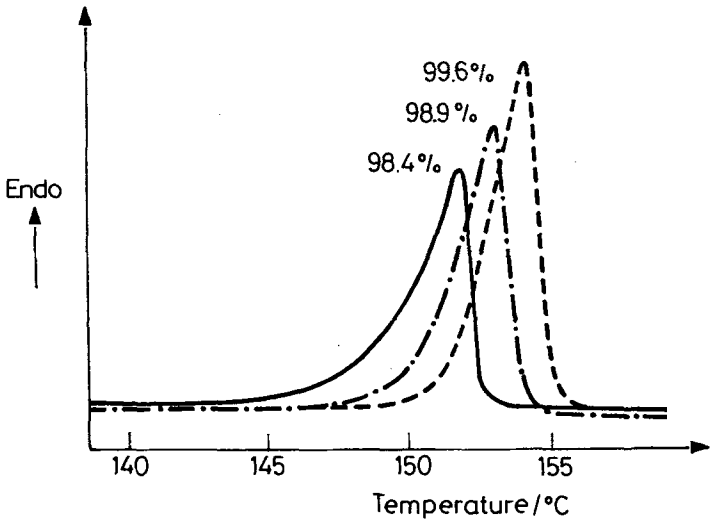

Fig. 22 DSC for quality comparison of ethoxycarbonyl-3-phenylpropy-L-alanine from 3 different manufacturers

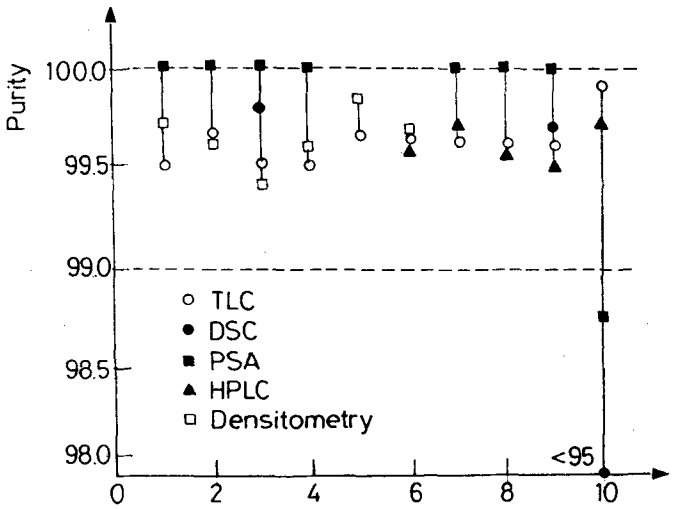

Fig. 23 Comparison of DSC with other techniques to detect relevant changes in synthesis. Change of synthesis for batch 10. DSC is in mol%

Purity of reference standards and pharmacopoeia substances

The purity level of reference standards is easily determined. Since DSC analysis is particularly valuable for samples of high purity, it should be applied

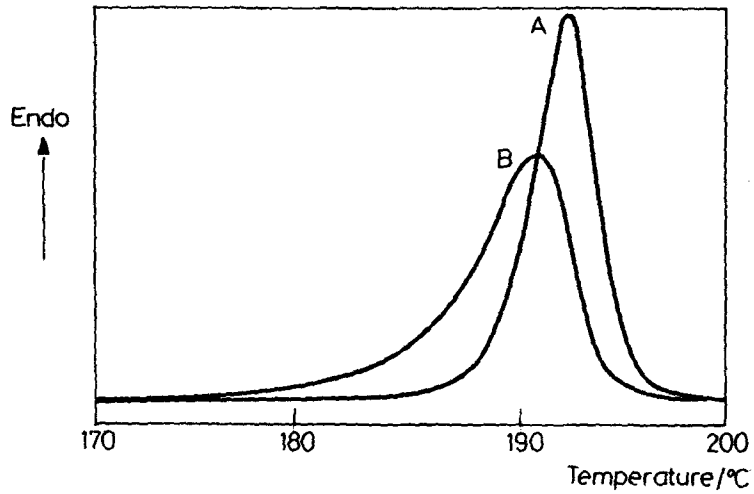

Fig. 24 Use of DSC purity analysis to detect cross contamination of a batch at the client site. Sample size: 1.5 mg; Heating rate: 20 deg·min^{-1}; A: analysis sample; B: contaminated sample

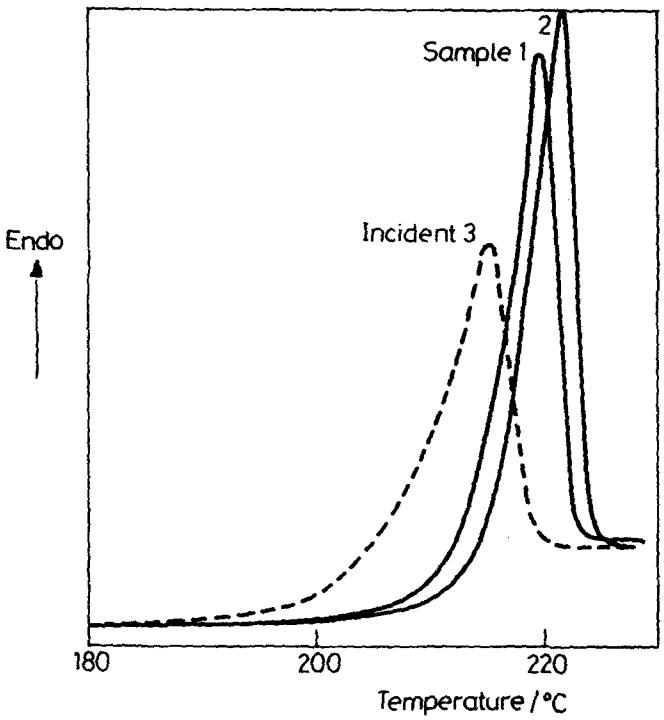

Fig. 25 Use of DSC purity scan to detect a purification incident. Scan 20 deg·min^{-1}. On the laboratory scale sample 1 was purified in sample 2. On the pilot-scale a contamination occurred leading to impure sample 3

systematically. We confirmed the purity of our analytical standards, for example benzoic acid for titration.

We suggested replacing the determination of the melting point by DSC analysis [24]. We analyzed 65 different drug substances on the market and were able to calculate the purity for half of them.

For dinatrium benzoate a batch did not pass the titration test. The DSC analysis could demonstrate a mistake in the titration experiment.

Analysis: DSC: 99.5%, Titration: 100.0%

Re-analysis: DSC: 99.5%, Titration: 97.4%, New titration: 100.1%

Contamination

DSC purity analysis can detect very quickly problems of contamination by comparing retained samples and samples delivered to other sources as demonstrated in Fig. 24.

Figure 25 deals with a contaminant introduced in a purification.

Conclusion

The progress in instrumentation, in data acquisition, and robotics makes the DSC purity analysis a 'state of the art method' for pharmaceutical analysis.

However, it is limited to eutectic impurities and a proper validation is difficult. The advantages of the method lie in the fact that it complements the chromatographic techniques, it has a short analysis time and the amount of analyte is small.

DSC provides the best support for development of chromatographic methods, for purity profile and stability knowledge during development of new entities up to registration.

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Zusammenfassung — Die DSC-Reinheitsanalyse basiert auf thermodynamischen Phasendiagrammen für Substanzen (Reinheit ≥98%) mit einem Schmelzpunkt. Verunreinigungen mit eutektischem Verhalten werden gemeinsam bestimmt.

Die DSC-Reinheitsanalyse aus einem einzelnen Schmelzvorgang einer Probe von 1–2 mg ist deshalb extrem attraktiv für eine globale Einschätzung eutektischer Verunreinigungen. Die Hauptvorteile liegen in dem sehr geringen Probemengenbedarf und des sehr schnellen Analysevorganges.

Die DSC-Reinheitsanalyse kann jedoch chromatografische Methoden nicht ersetzen, die spezifische Einzelniveaus von Verunreinigungen liefern. Weiterhin ist ein vollständiger Beweis der DSC-Reinheitsmethoden schwierig und zeitaufwendig. Trotz dieser Grenzen ist DSC die beste Unterstützung zur Entwicklung chromatografischer Methoden, für Reinheitsprofile und Stabilitätseinschätzungen in der Pharmaforschung.

Die Parameter für die Reinheitsbestimmung und für Beweisaspekte werden diskutiert. Beispiele aus der Pharmaforschung werden gegeben.