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Article

# Determination of the melting temperature, heat of fusion, and purity analysis of different samples of zidovudine (AZT) using DSC

**Adriano Antunes Souza Araújo[1,*], Marília dos Santos Bezerra[1], Sílvia Storpirtis[2], Jivaldo do Rosário Matos[3]**

*[1]Departament of Physiology, Federal University of Sergipe, [2]Departament of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, [3]Departament of Fundamental Chemistry, Institute of Chemistry, University of São Paulo*

The determination of chemical purity, melting range, and variation of enthalpy in the process of characterizing medicines is one of the principal requirements evaluated in quality control of the pharmaceutical industry. In this study, the method of purity determination using DSC was outlined, as well as the application of this technique for the evaluation of commercial samples of zidovudine (AZT) (raw material) supplied by different laboratories. To this end, samples from six different laboratories (A, B, C, D, E, and F) and the standard reference (R) from the United States Pharmacopeia (USP) were analyzed. The DSC curves were obtained in the temperature range of 25 to 200 °C under the dynamic atmosphere of $N_2$ (50 mL $min^{-1}$), heating rate of β=2 °C $min^{-1}$, using an Al capsule containing approximately 2 mg of sample material. The results demonstrated that the standard reference presented a proportion of 99.83% whereas the AZT samples presented a variation ranging from 97.59 to 99.54%. In addition, the standard reference was found to present a temperature of onset of melting point of 122.80 °C. Regarding the samples of active agents provided by the different laboratories, a variation ranging from 118.70 to 122.87 °C was measured. In terms of $\Delta H_m$, the samples presented an average value of 31.12 kJ $mol^{-1.}$

**Uniterms**: Zidovudine/purity determination. Differential scanning calorimetry. Thermal analysis. Medicines/quality control. Medicines/qualitative analysis.

A determinação da pureza química, a faixa de fusão e a variação de entalpia envolvida no processo de caracterização de fármacos é um dos principais requisitos avaliados no controle de qualidade em indústrias farmacêuticas. Neste trabalho é feita uma breve abordagem sobre o método de determinação de pureza utilizando DSC, assim como a aplicação desta técnica para avaliação de amostras comerciais de zidovudina (AZT) (matéria-prima) fornecida por diferentes laboratórios. Para tal, foram analisadas amostras de seis diferentes laboratórios (A,B,C,D,E e F) e a substância química de referência (R) da United States Pharmacopeia (USP). As curvas DSC foram obtidas na faixa de temperatura entre 25 a 200 °C, sob atmosfera dinâmica de $N_2$ (50 mL $min^{-1}$), β=2 °C $min^{-1}$, utilizando cápsula de Al contendo aproximadamente 2 mg de amostra. De acordo com os resultados, pode-se observar que a substância química de referência apresentou teor igual a 99,83% e que as amostras de AZT apresentaram uma faixa de variação entre 97,59 e 99,54%. Pode-se verificar, ainda, que a substância química de referência apresentou uma temperatura onset de fusão igual a 122,80 °C. Para as amostras dos princípios ativos fornecidos pelos diferentes laboratórios, pode-se verificar uma faixa de variação entre 118,70 e 122,87 °C. No que se refere ao $\Delta H_m$, as amostras apresentaram valor médio de 31,12 kJ.$mol^{-1}$.

**Unitermos:** Zidovudina/determinação da pureza. Calorimetria exploratória diferencial. Análise térmica. Medicamentos/controle de qualidade. Medicamentos/análise qualitativa.

***Correspondence:** A. A. S. Araújo. Departamento de Fisiologia, Universidade Federal de Sergipe-UFS. Av. Marechal Rondom, s/n -49000-100 - São Cristóvão - Sergipe-Brazil. E-mail: adriasa2001@yahoo.com.br

## INTRODUCTION

Applications in the pharmaceutical field exploit thermal analysis as an important tool in the solution of problems involving the development, production, and quality control of medicines (Buckton *et al*., 1991). The principal applications in this area have included determining the degree of purity of drugs (Windmann, Scherrer, 1991), the conducting of the analyses of thermal stability and the kinetics of decomposition, the study of possible drug-drug and/or drug-excipient interactions in the pre-formulation phase (Iglesias *et al.,* 1998; Saesma *et al.,* 1991; Araújo *et al.,* 2003), and in the characterization of raw materials and finished products (Ford, Timmins, 1989; Canotilho *et al.*, 1992; Cammenga, Epple, 1995; Giron, 1998, 1999; Brown, Glass, 1999; Thompson, 2000).

Canotilho and collaborators (1992) reported that the resources and potential of thermal analysis in the pharmaceutical field are considerable not only for the study of polymorphism, but also for their versatility in other applications. These encompass characterization of polymorphs (Barbas *et al*., 2007), inclusion complexes and solid dispersants, studies of drug/drug compatibility and drug/excipients at the pre-formulation level (Kiss *et al.*, 2006), determination of chemical purity, study of reactions in solid state (thermal stability and kinetic parameters) (Araújo *et al*., 2005), analysis of solid dosage forms, quality control of drugs and medicines (as in the determination of the contents of ash and humidity, among others) (Araújo *et al.*, 2006), the evaluation of isomers and polymorphs, the analysis of raw materials (drugs and excipients) as well as finished dosage forms (emulsions, suppositories, microspheres, pills, liposomes, powders, creams, and gels) (Canotilho *et al*., 1992; Lvova *et al*., 1993).

The determination of the melting point using the differential scanning calorimetry (DSC) method has been satisfactorily used as a method of evaluating the degree of purity of a compound (Widmann, Scherrer, 1991). By means of DSC, the melting range can be determined for a substance, and based on the equation of Van't Hoff (Canotilho *et al*., 1992, Bezjak *et al*., 1992) (Equation 1) it is possible to determine the molar fraction of impurities $x_2$ (number of mols of impurities by the total mol number) contained in this material in relation to the melting range. However, this method can be applied only if $x_2$ lies between 0.95 and 0.999 (or 95 and 99.99 mol %).

$$T_m = T_o - x_2 \frac{R\,T_o^2}{\Delta H_m} \qquad \text{(Equation 1)}$$

where $T_m$ represents the melting temperature of impurities in the process of melting, since the greatest component of remaining crystalline solids is in equilibrium with the phase already melted; $T_o$ is the melting temperature of the main component expressed in Kelvin; R is the constant of gases (8.3143 J $K^{-1}$ $mol^{-1}$); $x_2$ is the molar fraction of impurities in liquid phase; $\Delta H_m$ is the heat of melting of the main component expressed in J $mol^{-1}$; $\Delta H_m$ ($R.T_o^2$) and is the denominated cryoscopic constant.

When the substance is heated, the collection of impurities is melted, forming a liquid phase. Above this temperature, the solid phase only consists of a pure substance. As the temperature decreases with the formation of the eutectic phase of impurities (Figure 1), the molar fraction of impurities in liquid phase $x_2$ is constantly diminished since the pure substance dissolves in eutectic solution within this temperature range (Giron, Goldbronn, 1995; Staub, Perron, 1974). This is expressed by Equation 2:

$$X_2 = x_2^* \cdot \frac{1}{F} \qquad \text{(Equation 2)}$$

in which $x_2^*$ is a fraction of the impurities in the liquid phase of the completely melted substance or in the original substance, and F is the melted fraction.

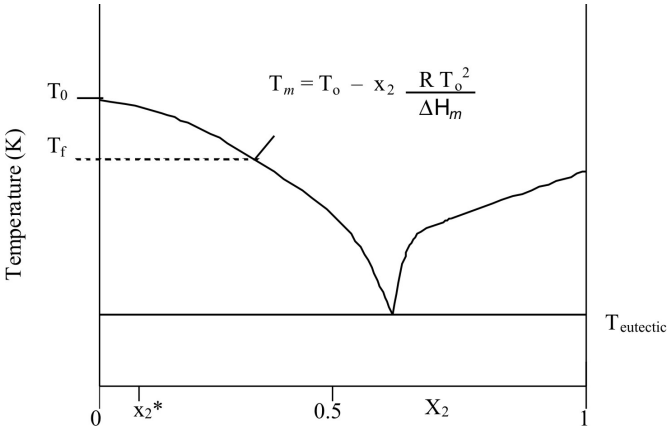


**FIGURE 1 -** Phase diagram of a eutectic mixture.

When Equation 2 is substituted in Equation 1 it gives Equation 3. This equation describes the linear correlation between $T_m$ and 1/F with an intercept of $T_o$ and slope of $x_2^*.R.T_o^2/\Delta H_m$ :

$$T_m = T_o - \frac{x_2^*\,R\,T_o^2}{\Delta H_f} \cdot \frac{1}{F} \qquad \text{(Equation 3)}$$

$x_2^*$– mol fraction of impurity in the original sample,
$T_o$ – melting temperature of the pure main component, in K,
$\Delta H_m$ – melting heat of the pure main component in J $mol^{-1}$,

F- fraction of sample melted at $T_m$,
R- gas constant, 8.314 J $mol^{-1}$ deg.

To obtain the graph of $T_m$ x 1/F from the DSC curve, some points, normally within the region between 10 and 50% of the peak, are evaluated. The fraction melted is the ratio of the partial area $\Delta H_i$ (measured from the beginning of the peak to temperature $T_m$) to the total area $\Delta H_{tot}$. The range between 10 and 50% of the peak is also used to exclude the highest concentration of impurities in liquid phase and to avoid errors in the region where the rate of melting is also greater. If the calculated values show correlation among each other then the curve produced by $T_m$ x 1/F will typically be represented by a straight line (Figure 2). The purity determination by DSC is explained by the theory of non-linearity, that is, the area where melting is normally initiated occurs due to a series of impurities melting before the main compound, thereby explaining a non-linear process. Thus, the accepted procedure for linearization of these data includes correction for this area (Blaine, Schoff, 1983). The melting temperature, melting heat, and purity were determined using Shimadzu TASYS software.

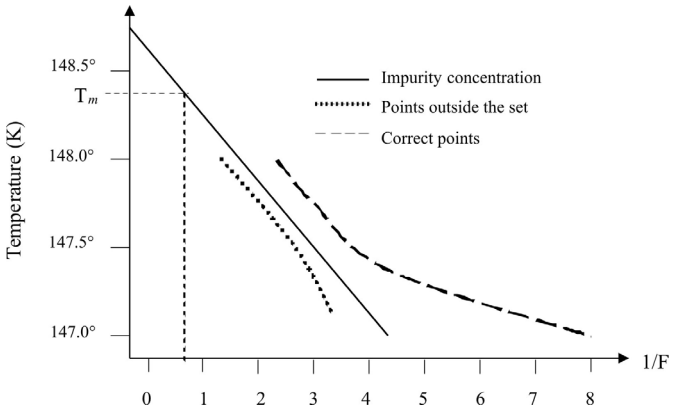


**FIGURE 2** - Temperature of melting vs. 1/F (melted fraction).

The determination of degree of purity utilizing DSC cannot be performed in the following situations: a) when the compounds decompose at the stage subsequent to melting; b) when the impurities form a eutectic with the principal component, that is, the impurities are soluble in the liquid phase of the principal component; c) for amorphous materials, since the melting peak is related to the variation of enthalpy in function of crystallinity; d) when the melting heat is independent of temperature; e) when other events take place close to melting point such as volatization or polymorphic transition (Blaine, Schoff, 1983).

As thermal analysis has stimulated great interest from investigators and scientists in the last few years regarding studies involving the quality control of drugs and medicines, the objective of this study was the use of DSC for determining the purity of zidovudine (AZT) of samples provided by different pharmaceutical laboratories. In this study, the following parameters were determined: purity, temperature of *onset* of melting ($T_{onset}$), variation of enthalpy occurring during melting, besides the effects of variation of mass and heating rate on the results.

## MATERIAL AND METHODS

### Material

The standard reference of AZT ($C_{10}H_{13}N_5O_4$; 3´-azido-2,3´-dideoxythymidine) was obtained from the United States Pharmacopeia (lot: F, N° cat. 72450). DSC analyses of six AZT samples supplied by Laboratório Farmacêutico do Estado de Pernambuco-LAFEPE, batch AZN001, Fundação para o Remédio Popular-FURP, batch 22459710, Indústria Química do Estado de Goiás-IQUEGO, batch 00100/001, Sanval Indústria Farmacêutica LTDA, batch AB934, Fundação Oswaldo Cruz-Farmanguinhos, batch AZL027, and Glaxo Wellcome Indústria Farmacêutica, lote 9054001, were carried out. These samples were randomly labeled A, B, C, D, E and F. The standard reference of AZT was called the R product.

### Methods

DSC curves were recorded in a DSC-50 cell (Shimadzu) using aluminum crucibles containing ~ 2 mg of samples, under dynamic nitrogen atmosphere (50 mL $min^{-1}$) and a heating rate of 2 °C $min^{-1}$ in the temperature range of 25 to 600 °C. The DSC cell was calibrated with indium (m.p. 156.6 °C; $\Delta H_m$ = 28.54 J $g^{-1}$). An empty pan sealed with a cover pan was used as a reference sample. The analyses were done in triplicate.

## RESULTS AND DISCUSSION

The endotherm of melting corresponds to the portion of the DSC curve that is far from the baseline, and, later, returns to it. The melting temperature, $T_{onset}$, is defined by the extrapolated beginning of the curve, being defined by the point of intersection of the tangent with the point of maximum slope, on the principal side of the peak with the base line extrapolated. Figure 3 shows the endotherm of melting of AZT (reference standard) obtained at the heating rate of 2 °C $min^{-1}$. The graph shows that the sample presents an onset temperature for melting of 122.80 °C with $\Delta H_m$ = 31.12 kJ $mol^{-1}$ and a purity of 99.83%. The 1/F

plot shows the melting temperature $T_m$ measured at several $\Delta H_i$ as a function of the reciprocal of the liquid fraction 1/F before and after the required linearization (Figure 4). This

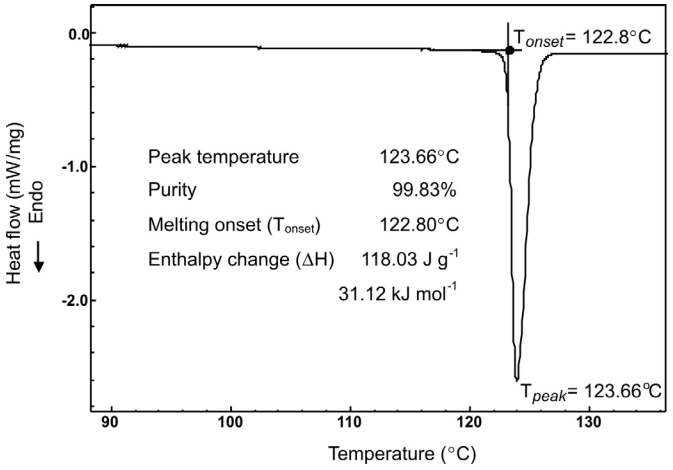


**FIGURE 3 -** DSC curves of AZT (standard reference) recorded in a dynamic nitrogen atmosphere (50 mL min$^{-1}$), and at a heating rate of 2 °C min$^{-1}$.

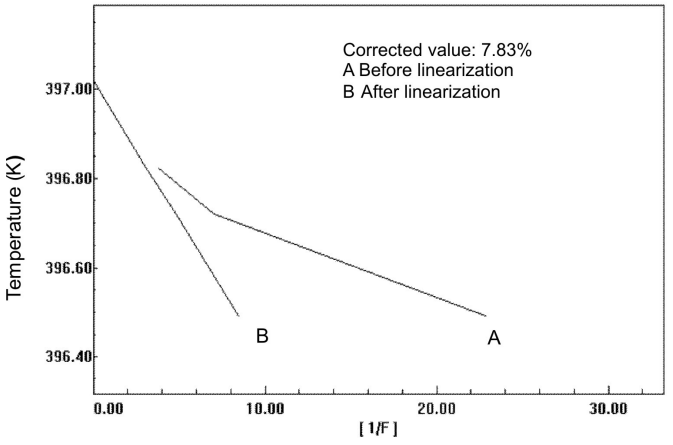


**FIGURE 4 -** The 1/F plot provides a visual assessment of the raw data and linearized function.

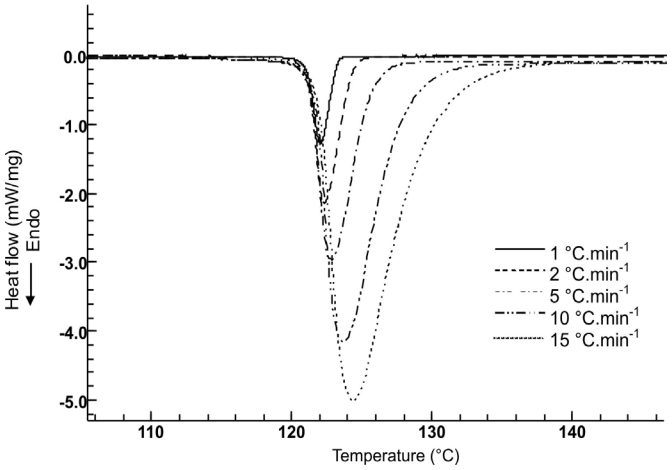


**FIGURE 5** - DSC curves of AZT (A sample) recorded in a dynamic nitrogen atmosphere (50 mL min$^{-1}$), and at a heating rate of 1, 2, 5, 10 and 15 °C min$^{-1}$.

The effect of heating rate on the purity determination, melting point, and enthalpy of melting was conducted using a sample mass of about 2 mg. To perform the analysis, sample B was randomly chosen for evaluation of the effects of heating rate and mass. Figure 5 shows the DSC curves of AZT (substance A) at heating rates of 1, 2, 5, 10, and 15 °C min$^{-1}$.This study demonstrated that the heating rate had a significant effect on the values obtained for purity, since the values of purity diminish as the heating rate increases (Table I). This observation can be explained by the fact that the Van´t Hoff equation is valid for conditions of equilibrium and therefore lower heating rates are recommended.

Figure 6 depicts the DSC curve of AZT (substance A) when the mass of the sample is varied by 0.58 to 7.44 mg, for a heating rate of 2 °C min$^{-1}$. In this case, the degree of purity, employing different values of sample mass, was above 99.53%. These results show clearly that the purity, melting heat, and temperature for the *onset* of melting undergo small variations when the sample mass is changed, as shown in Table II.

The representative DSC curves for the six AZT samples provided by laboratories A, B, C, D, E, and F (Figure 7). The determination of chemical purity of the samples

**TABLE I -** Peak temperature and enthalpy values of AZT (A sample) at different heating rates

| β (°C min$^{-1}$) | Purity (%) | $T_{onset}$ (°C) | $\Delta H_m$ (kJ mol$^{-1}$) |
|---|---|---|---|
| 1 | 99.61 | 120.36 | 29.18 |
| 2 | 99.53 | 120.34 | 29.35 |
| 5 | 99.49 | 120.46 | 28.38 |
| 10 | 99.23 | 120.77 | 29.07 |
| 15 | 98.76 | 120.87 | 29.91 |

**TABLE II -** Results of differential scanning calorimetry for AZT (A sample) at different weights.

| Weight (mg) | Purity (%) | $T_{onset}$ (°C) | $\Delta H_m$ (kJ.mol$^{-1}$) |
|---|---|---|---|
| 0.580 | 99.59 | 120.38 | 30.64 |
| 1.120 | 99.49 | 120.41 | 29.12 |
| 2.710 | 99.58 | 120.47 | 30.57 |
| 5.050 | 99.53 | 120.14 | 29.60 |
| 7.440 | 99.48 | 120.04 | 28.69 |

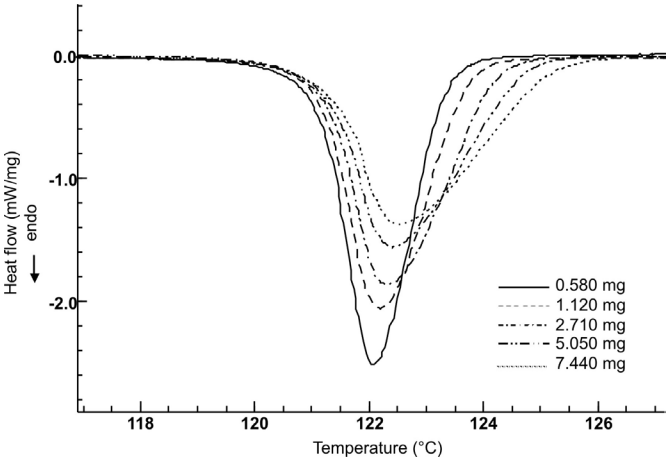


**FIGURE 6 -** DSC curves of AZT (A sample) using different weights recorded in a dynamic nitrogen atmosphere (50 mL min$^{-1}$), and at a heating rate of 2 °C min$^{-1}$.

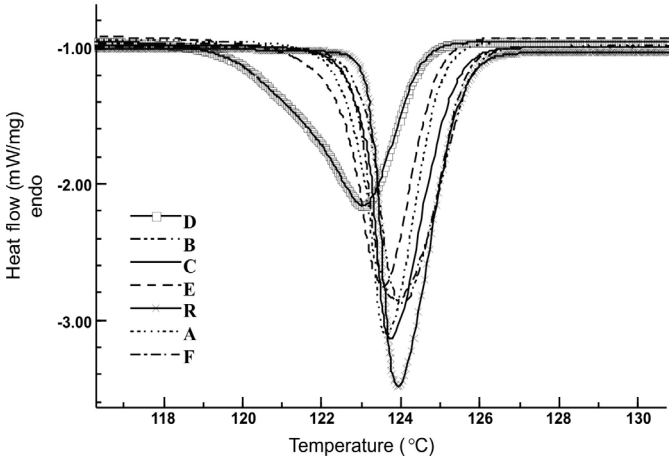


**FIGURE 7** - DSC curves of melting for different samples of AZT and standard reference using dynamic nitrogen atmosphere (50 mL min$^{-1}$), and a heating rate of 2 °C min$^{-1}$.

was done based on the correction factor (corrected value) shown for each in Table III. Consequently, the smaller the correction factor used, the greater the purity of the material. The degree of purity, the melting temperature, and the $\Delta H_m$ found in the analyses by DSC for the different samples of AZT are described in Table III.

The results were obtained using a low heating rate (2 °C min$^{-1}$) in order to better define the event. The data obtained evidences that the standard reference presented a degree of purity of 99.83%, and that the AZT samples presented a variation ranging from 97.59 to 99.54%. Given that the standard deviation is generally used to define intervals around the average, the results obtained for the tests in triplicate by DSC were shown to be sufficiently consistent and with a low dispersion of values. The results in Table I show that the standard reference presented a temperature for the *onset* of melting of 122.80 °C. Concerning the samples of active ingredients provided by the

**TABLE III** – Purity analysis, temperature and melting heat of different samples of AZT using DSC recorded in a dynamic nitrogen atmosphere (50 mL min$^{-1}$), and at a heating rate of 2 °C min$^{-1}$

| Products | Purity (%) | Correction factor (%) | $T_{onset}$ (°C) | $\Delta H_m$ (kJ mol$^{-1}$) |
|---|---|---|---|---|
| Standard reference | 99.83 ± 0.09 | 7.83 | 122.80 ± 0.76 | 31.12 ± 0.61 |
| A | 99.53 ± 0.14 | 10.92 | 120.81 ± 0.12 | 29.43 ± 0.96 |
| B | 99.54 ± 0.28 | 7.91 | 122.87 ± 0.83 | 29.54 ± 0.95 |
| C | 98.43 ± 0.10 | 12.76 | 121.82 ± 0.35 | 30.78 ± 1.13 |
| D | 97.59 ± 0.16 | 18.38 | 118.70 ± 0.43 | 29.97 ± 1.38 |
| E | 98.02 ± 0.11 | 15.88 | 121.19 ± 0.42 | 30.45 ± 1.08 |
| F | 99.01 ± 0.12 | 8.28 | 122.32 ± 0.86 | 32.33 ± 0.46 |

different laboratories, a variation of the range of melting of between 118.70 and 122.87°C is evident. In terms of $\Delta H_m$, the samples presented an average value of 31.12 kJ $mol^{-1}$.

The results obtained for product D show a greater proportion of impurity (2.41%) than did the remaining products, while a widening and shifting of the temperature *onset* of melting to a lower temperature was also observed, in this case to 118.70 °C.

## CONCLUSION

The determination of the purity of substances by DSC is considered a fast and reliable technique for eutectic systems, in which melting is not accompanied by thermal decomposition of the material. The main advantages of the technique over chromatography and spectroscopy are that analysis of all the impurities normally does not require standards except for the main compound, and permits simultaneous measurement of the melting range and variation of enthalpy. In this study, the comparison of six commercial samples of AZT medicine and the analysis of the chemical reference substance provided by the *United States Pharmacopeia* was conducted.

The results showed that the standard reference presented a proportion of 99.83% while the AZT samples presented a range of variation between 97.59 and 99.54%. Given that standard deviation is generally used to define intervals around the average, the obtained results for the tests in triplicate by DSC show sufficient agreement and a low dispersion of values. This can be verified even though the standard reference presented a temperature for the *onset* of melting of 122.80 °C. For the samples of active ingredients provided by the different laboratories, a variation ranging from 118.70 to 122.87 °C was ascertained. With regards to $\Delta H_m$, the samples presented an average value of 31.12 kJ.$mol^{-1}$. In conclusion, the number of studies published in the last few years, the speed in obtaining results, and the development of computer calculus programs have stimulated this technique and shown it to be promising.

This study demonstrated that the heating rate had a significant effect on the values obtained for purity, since the values of purity diminish as the heating rate increases. The results show clearly that the purity, melting heat, and temperature for the *onset* of melting undergo small variations when the sample mass is changed.

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