Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 627–631

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

The use of differential scanning calorimetry for the purity verification of pharmaceutical reference standards

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ARTICLE INFO

Article history:
Received 26 September 2008
Received in revised form 3 December 2008
Accepted 5 December 2008
Available online 30 December 2008

Keywords:
Pharmaceuticals
Reference standards
Certification
Differential Scanning Calorimetry (DSC)
Purity

ABSTRACT

Reference standards are routinely used in pharmaceutical industry to determine strength, content, and the quality of drug products, active pharmaceutical ingredients (API), preservatives, antioxidants and excipients. Traditionally, chromatographic techniques such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) in conjunction with other analytical techniques have been used to determine the purity and strength of a specific lot of a compound for the purpose of qualifying the lot to use as a reference standard. The assigned purity of the reference standard for a wide variety of compounds can be verified using an absolute method such as Differential Scanning Calorimetry (DSC). In this paper, purity of 16 reference standards was determined by DSC and the results were then compared to the purity values that were obtained using HPLC and other analytical techniques. The results indicate that the purity obtained from DSC analysis is comparable to the chromatographic purity for organic compounds that are at least 98% pure. Use of DSC for purity determination is not appropriate if a compound lacks sharp melting point, decomposes in the defined temperature range or exhibits other thermal event(s) which interfere with the melting point of the compound. The use of DSC as an alternative and or complementary method to verify the purity of a compound as part of the pharmaceutical reference standard certification process is discussed.

1. Introduction

Reference standards are used for qualitative, semi-quantitative and quantitative analysis. Reference standards are critically important in pharmaceuticals, environmental and petrochemical fields. In the pharmaceutical industry, reference standards are routinely used to determine the strength and quality of drug products, active pharmaceutical ingredients (APIs), preservatives, antioxidants and excipients. Reference standards are also used in routine instrument calibration and qualification. It is a mandatory requirement by the United States (U.S.) Food and Drug Administration (FDA), compendia and other national health regulatory agencies throughout the world that reference standards used in the pharmaceutical industry must be thoroughly characterized and evaluated using appropriate analytical methods. Depending on the chemical nature and the intended use of a reference standard, various analytical tests are performed to characterize and certify or qualify a reference standard. Understandably, reference standards that are used for quantitative purpose undergo extensive testing and evaluation, whereas limited testing may be sufficient for reference standards that would be used for semi-quantitative and or qualitative purpose only. Quantitative reference standards can be certified either as a primary reference standard or as a secondary reference standard. The purity of a primary reference standard is determined through extensive chemical and or physical testing and full characterization of the assigned lot, whereas the purity of a secondary reference standard is determined by the chromatographic or spectroscopic analysis against a primary reference standard (e.g., a compendial reference standard or an in-house primary reference standard). Recently, the European Pharmacopoeia (Ph.Eur.) added a new general chapter (5.12. Reference Standards; Section 4) that provides guidance for the establishment of reference standards [1]. As indicated in the aforementioned Ph.Eur. general chapter, the purity of primary reference standards assigned using chromatographic or spectroscopic methods must be independently verified by methods such as Differential Scanning Calorimetry (DSC) or phase solubility, where appropriate.

Differential Scanning Calorimetry has been used for various applications in the pharmaceutical industry. These applications include, studying polymorphism of APIs, evaluating the stability/storage conditions of drug products/APIs/raw materials [2], the quantitation of pharmaceutical crystal forms [3] and for the purity determination of crystalline organic compounds [4–8]. One of the main advantages of purity analysis by DSC is that it does not require a corresponding reference standard. Other advantages of DSC analysis are minimal sample requirement and shorter total analysis

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doi:10.1016/j.jpba.2008.12.030

time as compared to a typical chromatographic analysis. For purity factor determination of the pharmaceutical reference standards, typical tests performed are chromatographic analysis, Residue on Ignition (ROI), Loss on Drying (LOD) and/or Karl Fischer. Total time spent for these analyses (specifically for chromatographic analysis) can vary from a day to several days depending on the analyte and the method. In addition, several grams of the test sample is needed for LOD, ROI and Karl Fischer tests. This presents a significant challenge as some of the pharmaceutical reference standards are only available in very limited quantities (few hundred milligrams to several grams). In contrast, DSC purity determinations are less time consuming (typically less than an hour) and only require very limited sample (~1–5 mg). It should be noted further that HPLC, which plays a pivotal role in the analysis of pharmaceutics, has certain limitations. First and foremost, there is no single detector that can detect all types of analytes. In the majority of HPLC analyses a UV detector is used. Impurities which do not have a chromophore or do not absorb at the detecting wavelength will not be detected in such analyses. In addition, all impurities may not have the same response factor. If appropriate correction factors are not applied, results calculated will not be accurate. In case of DSC, all impurities irrespective of their UV absorptivity contribute equally towards analyte purity provided they are soluble in melt.

The principle behind the DSC technique is that impurities which are soluble in melt but not in solid (i.e., do not form solid solutions) cause a depression in the melting point of the analyte. The modified Van't Hoff equation, which relates the fraction of the impurities (in mole%) to the melting point depression, is used to estimate the sample purity (%mole/mole). A detailed discussion regarding theoretical aspect of this phenomenon has been reported elsewhere [8]. It should be noted that when DSC purity (%mole/mole) is compared to the purity obtained using other approaches (mass balance or assay against an official certified reference standard, both of which give material purity in %w/w), it is assumed that molecular weight of the impurities are very similar to the main analyte. This is a valid assumption for small molecules, as most of the impurities are either related compounds of the main analyte, starting material or degradation products. In addition, it is assumed that the amount of inorganic impurities is negligible unless DSC purity values are appropriately corrected for the inorganic impurities. Inorganic impurities, which are ionic in nature, are insoluble in organic compound's melt (non-ionic) thus do not contribute to melting point depression. Drozdzewska et al. analyzed several polycyclic aromatic hydrocarbons (PAH), using DSC and demonstrated that DSC purity values were comparable (within 1.5%) to the reported purity values of the PAH reference standards, especially when DSC results were corrected for inorganic impurities [9].

In this paper, the purity obtained by DSC for 16 reference standards is compared to the purity obtained using HPLC and other analytical techniques These reference standards are used in the identification and assay of drug products, active pharmaceutical ingredients (API), API intermediate and pharmaceutical excipients. The purity values of these reference standards were obtained from the results of chromatographic analysis and other analytical tests (e.g., Residue on Ignition, Karl Fischer or Loss on Drying, Residual Solvents, Thermogravimetric analysis, etc.), as applicable. The results obtained from our studies clearly indicate that Differential Scanning Calorimetry can be successfully used as a complementary and or an alternative technique to verify the purity of a compound/reference standard during the certification or re-certification of a pharmaceutical reference standard, provided that the material is at least 98%, and does not decompose or has any interfering thermal event with its melting endotherm.

2. Experimental

2.1. Materials

All materials used were either manufactured within Schering-Plough or obtained from commercial vendors such as Sigma–Aldrich (St. Louis, MO) or Fisher Scientific (USA). The reagents used to prepare mobile phases for the HPLC analyses were all HPLC grade, and were obtained from Fisher Scientific (USA).

2.2. Differential Scanning Calorimetry

The DSC analysis of the samples was conducted using Q1000 (TA Instruments, New Castle, DE, USA) equipped with 50-position autosampler and digital mass flow controller. The Q1000 has Tzero™, an advanced DSC technology that automatically corrects for thermal lag associated with heat flow [4], a problem that previously affected the accuracy of DSC measurements. The instrument was calibrated using indium and sapphire NIST traceable certified standards as recommended by the vendor. Samples (1–5 mg) were hermetically sealed in aluminum crucibles to prevent any mass loss due to moisture. All sample measurements (in triplicate) were performed at a heating rate of 1.0 °C/min using nitrogen as a purge gas (flow rate = 50 mL/min). The DSC purity results are reported as the average of the triplicate determinations.

2.3. Thermo gravimetric analysis

The Thermo gravimetric analysis (TGA) of the samples was conducted using Q500 (TA Instruments, New Castle, DE, USA) where applicable. The instrument was calibrated using the nickel, alumel and certified micro weights (all traceable to NIST) as recommended by the vendor. The sample (5–10 mg) measurement was performed at a heating rate of 10.0 °C/min (start temperature = room temperature) using nitrogen as a purge gas (flow rate = 60 mL/min).

2.4. HPLC analyses and determination of sample purity (potency)

The High Performance Liquid Chromatography (HPLC) analysis of the samples was conducted using a Waters Alliance 2695 HPLC system (Water Corp., Milford, MA) equipped with dual wavelength UV detector. Material purity was calculated either following a mass balance approach [i.e., chromatographic purity × (100 − inorganic impurities − residual solvents or moisture)/100] or against an external reference standard (e.g., a compendial standard). The purity values were obtained for these reference standards using chromatographic techniques in conjunction with other analytical tests (Residue on Ignition, Karl Fischer or Loss on Drying, Thermogravimetric analysis, etc.), as applicable. Either Schering-Plough or compendial test methods were employed for purity determination as part of the reference standard certification process.

3. Results and discussion

3.1. Reproducibility/precision for DSC purity analysis

The DSC purity analysis was performed for 16 pharmaceutical reference standards. Representative melting endotherms (DSC scan) for two reference standards are shown in Figs. 1 and 2. Among the 16 reference standards analyzed, 12 were APIs, 2 were API intermediates and remaining 2 were commonly used excipients. These reference standards were either certified as a primary (i.e., using mass balance approach) or secondary (purity assigned against an official primary reference standard) reference standard. All the samples were analyzed by DSC in triplicate ($n = 3$). The average result, standard deviation (SD) and uncertainty in DSC purity results

Table 1
Precision and accuracy of purity obtained via DSC.

Compound name	Type	T_m (°C)[a]	DSC purity (%)[c]	Standard deviation (%)[b]
Caffeine	API	235.9	99.72 ± 0.50	0.44
Azatadine Maleate	API	146.2	97.95. ± 0.40	0.35
Salicylic Acid	API	158.6	98.99 ± 0.35	0.31
Aspirin	API	129.0	99.26 ± 0.34	0.30
Perphenazine Intermediate (PTPH)	Intermediate	44.0	98.90 ± 0.32	0.29
Labetalol Hydrochloride	API	178.6	99.24 ± 0.23	0.20
Perphenazine	API	95.8	98.93 ± 0.18	0.16
Tolnaftate	API	110.4	99.75 ± 0.07	0.07
N-Methyl desloratadine	Intermediate	118.9	99.78 ± 0.05	0.05
Betamethasone Dipropionate	API	175.9	99.15 ± 0.04	0.03
Bisacodyl	API	132.4	99.16 ± 0.03	0.03
Propylparaben	Excipient	96.5	99.96 ± 0.03	0.03
Loratadine	API	133.8	99.68 ± 0.02	0.02
Methylparaben	Excipient	125.8	99.99 ± 0.02	0.02
Acetaminophen	API	168.9	99.95 ± 0.01	0.01
Ethinyl Estradiol	API	183.4	99.89 ± 0.00	0.00

[a] Melting onset temperature; average of three measurements.
[b] Standard deviation from three measurements.
[c] Average of three measurements; all values are reported at 95% confidence level.

at 95% confidence level [9,10] are reported in Table 1. The uncertainty in DSC purity results at 95% confidence was calculated using Eq. (1):

$$\text{uncertainty} = \frac{\pm Z \times \text{SD}}{\sqrt{N}} \quad (1)$$

where $Z = 1.96$; SD, standard deviation; N, number of replicate; see reference [10] for additional information about above equation.

The uncertainty in DSC purity measurements for nine reference standards (Tolnaftate, N-Methyl desloratadine, Betamethasone Dipropionate, Bisacodyl, Propylparaben, Loratadine, Methylparaben, Acetaminophen and Ethinyl estradiol) was within 0.1%. This supports high reproducibility/precision for DSC analysis, especially when the analyte has high purity and does not have thermal events that interfere with the melting endotherm. For all other seven reference standards, the DSC purities were within 0.5% of the average value. The melting onset (T_m) and the DSC purities are reported in Table 1.

In the case of Azatadine Maleate, the uncertainty in purity value at 95% confidence level was 0.4%. The representative DSC scan of Azatadine Maleate is shown in Fig. 3. As shown in the DSC scan, Azatadine Maleate decomposes right after melting. This observation was further confirmed by TGA analysis of the Azatadine Maleate. As shown in the inset of Fig. 3, Azatadine Maleate starts decomposing around 150 °C, right after its melting onset temperature (146.2 °C). Higher uncertainty in Azatadine Maleate DSC purities can be associated to the sample decomposition. A similar decomposition event was observed for Labetalol Hydrochloride as shown in Fig. 4. In the decomposition region of the Labetalol Hydrochloride DSC endotherm, sharp spikes were also apparent in the DSC analysis. These spikes are attributed to the volatilization of entrapped gases or moisture from the sample pan after melting. Caffeine and salicylic acid samples started decomposing during the melting, which resulted in less reproducible results (see Table 1).

3.2. Effect of heating rate on DSC purities

In general a fast heating rate (as high as 10 °C/min) is recommended for samples that decompose when heated [11]. On the other hand, a slow heating rate is preferred for pure samples that melt without decomposition [11]. To evaluate the effect of a slow heating rate, three reference standards (Methylparaben, N-Methyl desloratadine and Perphenazine) where analyzed at 0.2 °C/min. As shown in Table 2, there was no difference in DSC purity results for Methylparaben and N-Methyl desloratadine at a slower heating

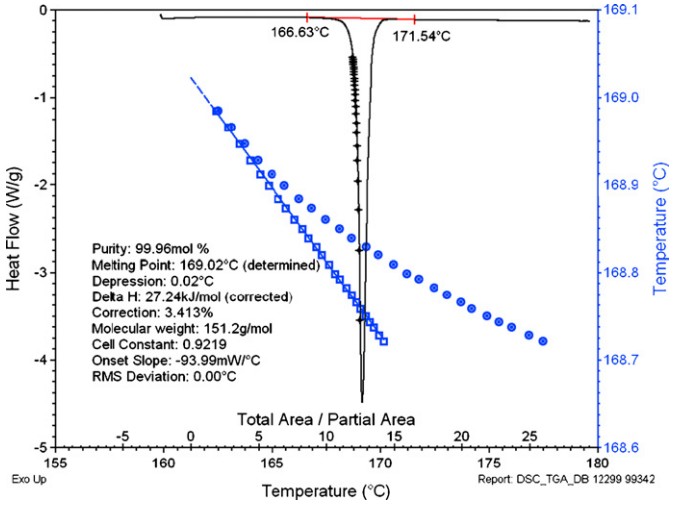

Fig. 1. DSC scan and purity analysis for the Acetaminophen Reference Standard (heating rate = 1.0 °C/min, sample weight = 2.11 mg).

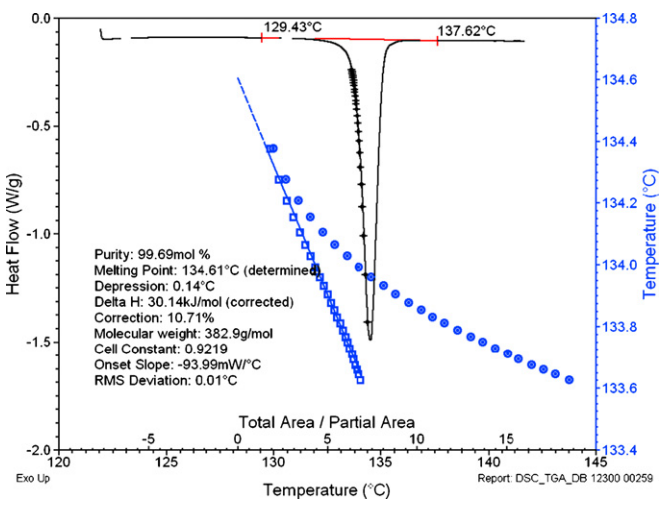

Fig. 2. DSC scan and purity analysis for Loratadine Reference Standard (heating rate = 1.0 °C/min, sample weight = 1.35 mg).

Table 2
Effect of slow heating rate on DSC purities.

Compound name	0.2 °C/min		1.0 °C/min		
	Purity[b] (%)	Standard deviation[a]	Purity[b] (%)	Standard deviation[a]	DSC purity difference (%)
Methylparaben	99.96	0.01	99.99	0.02	0.0
N-Methyl desloratadine	99.80	0.05	99.78	0.05	0.0
Perphenazine	99.30	0.02	98.93	0.16	0.4

[a] Standard deviation from three DSC purity values.
[b] Average of three measurements.

rate. The DSC purity obtained for perphenazine, however, was 0.4% higher at slower heating rate. It is recommended that the user perform sample analysis at different heating rates in order to get high confidence in the DSC purity values. It should be noted that if part of the sample is amorphous, the exothermic transition associated with re-crystallization of amorphous portion may impact the melting endotherm. In such cases sample may be heated beyond the exothermic transition associated with re-crystallization, followed by a slow cooling to obtain a crystalline material for further DSC purity analysis.

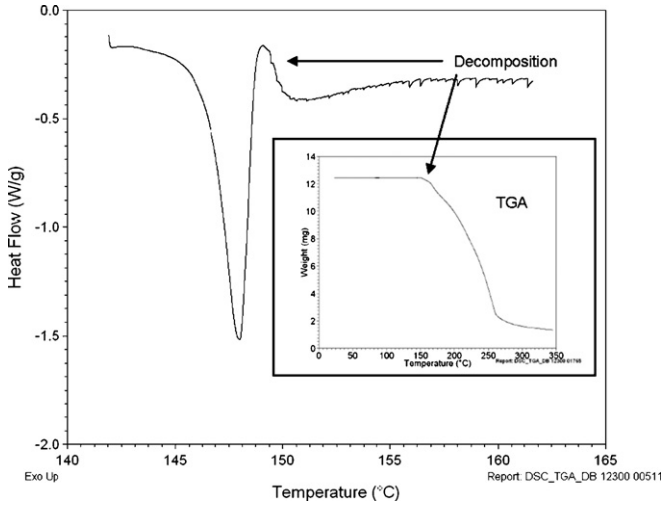

Fig. 3. DSC and TGA (inset) scans of Azatadine Maleate Reference Standard (heating rate = 1.0 °C/min, sample weight = 1.24 mg) displaying decomposition beyond 150 °C).

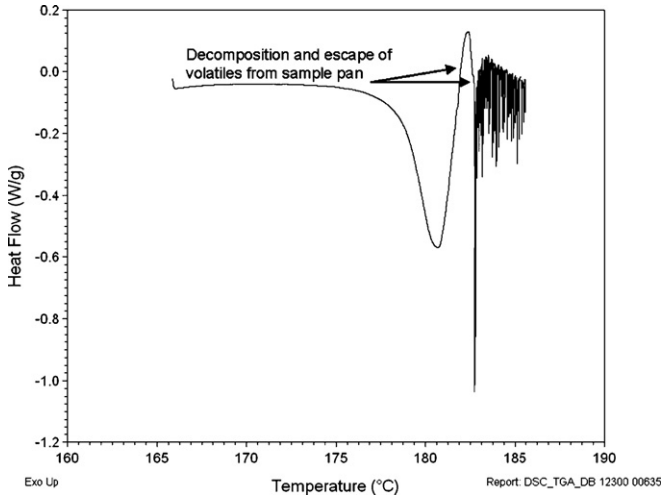

Fig. 4. DSC scan of Labetalol Hydrochloride Reference Standard (heating rate = 1.0 °C/min, sample weight = 2.18 mg).

3.3. Comparison of DSC and certified purities

The DSC purity values were compared to the certified purity values obtained using chromatographic techniques in conjunction with other analytical tests (Residue on Ignition, Karl Fischer or Loss on Drying, Thermogravimetric analysis, etc.), as applicable. Purity difference between the DSC purity and certified reference purity was calculated using Eq. (2):

$$\text{purity difference} = \frac{|\text{certified purity} - \text{DSC purity}|}{\text{certified purity}} \times 100 \qquad (2)$$

Table 3 and Chart 1 show purity difference between DSC purity and certified reference standard purity values. The purity difference was less than 1.0% for 11 reference standards (Betamethasone Dipropionate, Propylparaben, Bisacodyl, Salicylic Acid, Caffeine, Ethinyl Estradiol, Tolnaftate, Loratadine, Acetaminophen, Labetalol Hydrochloride and Perphenazine) out of sixteen reference standards evaluated. In addition, the purity difference for other 4 reference standards [Methylparaben, Azatadine Maleate, Aspirin and N-Methyl desloratadine] was between 1.0% and 2.0%. It should be noted that Methylparaben, Azatadine Maleate and Aspirin were certified as secondary reference standards (i.e., purity was assigned by performing assay against an official reference standard) which tend to have higher variability as compared to those certified as primary reference standards (mass balance approach). Thus, the purity difference higher than 1.0% is not surprising for these reference standards. The anticipated purity difference for all primary reference standards, except N-Methyl desloratadine, was very small (0.3% or less). The N-Methyl desloratadine, which is an API related compound, showed a purity difference of 1.8%. This is partially because the reported purity of the N-Methyl desloratadine sample was only 98.0%. This is consistent with the expectation that DSC accuracy decreases as sample purity decreases. Only the Perphenazine Intermediate (a qualitative reference standard) showed significant purity difference (4.4%). This was because of the very low certified purity of Perphenazine Intermediate (94.8%).

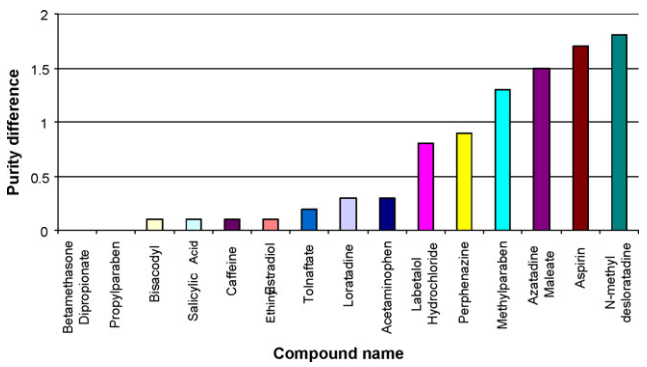

Chart 1. Purity differences between the DSC and the certified purities for 15 reference standards. Purity difference for Perphenazine Intermediate, a qualitative standard, is not included in this chart.

Table 3
Comparison of DSC purity with the purity results obtained via chromatographic and other analytical techniques.

Compound name	Standard type	Certified purity (%)	DSC purity (%)	Purity difference (%)[a]
Betamethasone Dipropionate	Primary	99.2	99.2	0.0
Propylparaben	Primary	100.0	100.0	0.0
Bisacodyl	Secondary	99.3	99.2	0.1
Salicylic Acid	Secondary	99.1	99.0	0.1
Caffeine	Secondary	99.6	99.7	0.1
Ethinyl Estradiol	Primary	100.0	99.9	0.1
Tolnaftate	Secondary	100.0	99.8	0.2
Loratadine	Primary	100.0	99.7	0.3
Acetaminophen	Secondary	99.7	100.0	0.3
Labetalol Hydrochloride	Secondary	100.0	99.2	0.8
Perphenazine	Secondary	99.8	98.9	0.9
Methylparaben	Secondary	98.7	100.0	1.3
Azatadine Maleate	Secondary	99.5	98.0	1.5
Aspirin	Secondary	100.0	98.3	1.7
N-Methyl desloratadine	Primary	98.0	99.8	1.8
Perphenazine Intermediate	Primary[b]	94.8	98.9	4.4

[a] Refer to Section 3.3 for equation used for these calculations.
[b] For qualitative use only.

4. Conclusion

Differential Scanning Calorimetric analyses of 16 pharmaceutical reference standards were conducted for purity determination and to assess if DSC can be used as an alternative technique (compared to the traditional techniques) for the purpose of purity determination of the reference standards. The statistical analysis of DSC results showed consistently good reproducibility. Comparisons were made between the purity obtained via DSC with the purities obtained via HPLC and other analytical techniques. To the best of our knowledge, this is the first report in which such comparisons for pharmaceutical reference standards have been made.

For reference standards with purity greater than or equal to 98.0%, the relative percent difference between the reported purity and the DSC purity was less than 2.0%. Typically, for the high purity reference standards, the DSC purity is within 1% of the purity obtained via chromatography and other analytical techniques. The DSC purity of a qualitative reference standard (which is used for identification only and has a purity of 94.8%) varied greater than 2.0% compared to the 94.8% purity obtained by other methods. This difference is consistent with the theory and principle of purity estimation by DSC (i.e., the DSC purity is consistent with the purity obtained by other methods if the material is at least 98% pure). As less than 2.0% difference between the DSC and HPLC purities was obtained for 15 quantitative reference standards, it can be concluded that DSC technique can serve as an important alternative method to verify the purity of pharmaceutical reference standards and can be routinely used as part of the reference standard certification process. Once the initial certification work is conducted using traditional testing, recertification work can be conducted using DSC for purity determination which would save tremendous amount of laboratory analysts' time because multiple types of tests are typically needed in traditional testing including HPLC, GC etc., to determine the purity. However, users should be aware of the fact that DSC results are only reliable for compounds which are highly pure (98% or above), have sharp melting points, and do not have thermal events that interfere with their melting endotherms. Furthermore, DSC analysis should only be performed to verify the purity of the reference standards whose purity has already been assigned using other analytical techniques as recommended in European Pharmacopoeia's general chapter on the reference standards.

Acknowledgment

The authors would like to thank all the analytical scientists in Global Quality Services—Analytical Sciences group (Schering-Plough) for their support during this study.

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