Determination of Melting Points According to Pharmacopeia

Application Note #4

Introduction

The development and manufacturing of pure chemicals requires that close attention be paid to purity, quality, stability and safety to ensure that the final product performs as intended.

One of the analytical techniques applied to the characterization of pure chemicals and pharmaceutical drugs (from raw material, to scale-up, to finished form) is the melting point (MP) determination. Carefully choosing the MP determination procedure is important for generating certifiable results for chemical quality control (QC) and quality assurance (QA).

In addition to following well-defined guidelines for Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP), pharmaceutical QC/QA labs must also follow multiple strict chemical analysis protocols set forth by local, national and even international Pharmacopeias.

Analytical QC/QA laboratories must calibrate their MP instrumentation on a regular basis against certified reference standards (CRSs), to determine whether their instruments are in accordance with the specific requirements defined by their local, national and international standards laboratories.

This application note describes some of the most widely accepted Pharmacopeia protocols for MP determinations, and also includes a comprehensive listing of CRSs commonly used for the calibration/validation of MP instrumentation.

Important! Pharmacopeia procedures and CRSs are routinely updated, supplemented, reformulated and revised. Use the information in this application note for reference only, and always consult the latest Pharmacopeia publications and supplements for up-to-date information on MP determination protocols and certification procedures.

U.S. Pharmacopeia

The United States Pharmacopeia (USP, http://www.usp.org/) is a non-governmental, non-profit organization comprising of volunteer scientists. It publishes the *U.S. Pharmacopeia and National Formulary* (USP-NF) which contains the official, legally recognized standards for pharmaceutical manufacturing.

MP Protocol

The MP Determination procedure is described in section <741>, p. 2033-2034 of the USP25-NF20 US Pharmacopeia. Five procedures for the determination of melting range or temperature are provided, varying in accordance with the nature of the substance.

USP-compatible capillaries are specified for MP determinations: 10 cm length, 0.8 - 1.2 mm internal diameter and 0.2 - 0.3 mm wall thickness. Capillary tubes must be charged with sufficient amount of the dry powder to form a column in the bottom of the tube 2.5 - 3.5 mm high when packed down as tightly as possible by tapping on a solid surface. The most common MP procedure (Class Ia, Apparatus I) requires inserting the capillary with the sample into the heating block 5°C below its expected MP and ramping at 1 +/- 0.5 °C/minute until the melt is complete. The MP range is recorded at the end of the melt.

The USP methodology calls for measuring the onset and the clear point of the melt. For “visual-only determinations”, the start of the melt (i.e. onset point) is defined as the temperature at which the column of the substance under test is observed to collapse definitely against the side of the capillary tube (i.e. collapse point). According to USP, the clear point of a compound (i.e. the temperature at which the sample becomes completely liquid) is recognized as the “single” MP of a substance.

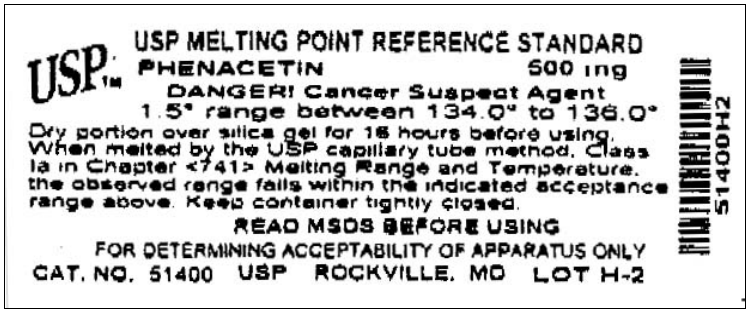
Automated instruments relying on bulk optical properties (i.e. absorption or reflection) are included in the general methodology. However, OptiMelt has automation capabilities that go beyond the automation options considered within section <741>, Apparatus II, of the USP-NF monograph. With an OptiMelt system, a user can carefully adjust the detection thresholds of the Digital Image Processor to precisely match all visual observations. The built-in digital camera effectively replaces the operator’s eyes and performs an unattended “visual” determination of the melt.

USP Standards

The US Pharmacopeial Convention is the recommended source of CRSs for US Pharmacopeia protocols. A catalog is available as a download from their website (www.usp.org).

USP Pharmacopeial Convention CRSs.

Each MP CRS is provided with a detailed label, listing: (1) chemical name, (2) MP range, (3) recommended drying procedure, (4) catalog/batch number, and (5) USP MP procedure required for the determination of acceptability of MP instrumentation. The acceptance MP ranges are based on the Class Ia MP Procedure in Chapter <741> of the USP monograph.


USP MELTING POINT REFERENCE STANDARD
USP™ PHENACETIN 500 mg
DANGER! Cancer Suspect Agent
1.5° range between 134.0° to 136.0°
Dry portion over silica gel for 16 hours before using.
When melted by the USP capillary tube method, Class Ia in Chapter <741> Melting Range and Temperature, the observed range falls within the indicated acceptance range above. Keep container tightly closed.
READ MSDS BEFORE USING
FOR DETERMINING ACCEPTABILITY OF APPARATUS ONLY
CAT. NO. 51400 USP ROCKVILLE, MD LOT H-2
51400H2

Label for USP CRS: Phenacetin

An alternative source of USP standards is LGC PromoChem located in the UK (www.lgcpromochem.com).

USP MP Standards			
Chemical Name	Nom. MP (°C)	USP Part No.	LGC Part No.
Vanillin	83	1711009	USP1711009
Acetanilide	116	1004001	USP1004001
Phenacetin	136	1514008	USP1514008
Sulfanilamide	166	1633007	USP1633007
Sulfapyridine	193	1635002	USP1635002
Caffeine	237	1086006	USP1086006

Tip:

- USP CRSs have wide acceptance MP ranges and generally do not deliver the accuracy levels required to take full advantage of the temperature measurement accuracy and resolution of OptiMelt. A common calibration strategy is to use USP standards for calibration checks and determination of acceptability against USP protocols, but to use WHO standards instead (see next section) to adjust the temperature scale if recalibration is deemed necessary.
- Any OptiMelt MP apparatus carefully calibrated against WHO standards should test "acceptable for use" against all current USP standards. Consult the "OptiMelt Compliance" section later in this document for additional details.

Calibration Procedure

The accuracy and acceptability of a MP apparatus should be checked at regular intervals by the use of three or more of the six USP MP Reference Standards, preferably those that melt nearest the melting temperatures of the compounds being tested. Any apparatus or method, with either oil or metal oven, capable of achieving the required accuracy is compatible with this certification procedure.

USP standards rely on the USP capillary method, Class Ia, in chapter <741> of the USP Monograph. Powder and dry the reference standard sample according to the procedure listed on its label. Insert the capillary with the CRS sample 5 °C below its expected MP and ramp at 1°C/minute until the melt is completed. Determine the MP range of the MP standard using a visual determination. The start of the melt is defined as the temperature at which the column of the substance under test is observed to collapse definitely against the side of the capillary tube (i.e. collapse point). The clear point (i.e. the

temperature at which the sample becomes completely liquid) is also recorded as the “single” MP of that substance.

A MP apparatus is considered calibrated and acceptable for use if the measured MP range falls within the acceptance limits of the melting range assigned to the CRS by USP. If deviations *beyond the accuracy of the instrument* are detected, the instrument’s temperature scale must be recalibrated.

Tip:

- OptiMelt includes automation capabilities that make it feasible to determine the melting range of a substance without requiring direct visual observation.
- Whenever possible, use the MeltView software package to visualize your melts and to store a record of the melt process. Being able to replay a melt is not only important to improve the accuracy of your results, but also to provide definitive documentation in accordance to GLP requirements.

International Pharmacopeia

The World Health Organization (WHO) developed and maintains the International Pharmacopeia, and it coordinates the efforts of the WHO Collaborating Centre for Chemical Reference Substances.

MP Protocol

The International Pharmacopeia, 3rd Edition, Volume 1: General Methods of Analysis, describes methods and procedures for the quality control of pharmaceutical substances. The Physical Methods section includes protocols for the determination of melting temperatures and melting ranges. Electrically heated metal ovens are compatible with the capillary method of the International Pharmacopeia monograph.

Before use, the sample substances must be finely powdered and carefully dried, for instance in a vacuum desiccator over silica gel for 24 hours.

The capillary with sample must be brought into contact with the heating medium at a temperature 5°C below the expected lower limit of the melting range. The temperature is ramped about 1°C per minute.

The melting range starts when the substances begin to collapse (onset point or collapse point) and ends at the temperature where the samples are completely molten (clear point).

The clear point is recognized as the “single” MP of the substance provided the instructions listed in the International Pharmacopoeia, and particularly the heating rate, is closely followed.

WHO Standards

WHO CRSs are required to calibrate instruments and methods for determination of MPs against the method of the International Pharmacopoeia.

The most direct source of WHO CRSs is the *WHO Collaborating Centre for Chemical Reference Substances* (www.apl.apoteket.se/who), which is also recommended by the European Pharmacopeia.

MP standards are provided with certificates of calibration including: (1) analytical purity data, (2) assigned MP (i.e. clear point at 1°C/min) and (3) Directions for Use.

WHO MP standards can also be obtained from LGC Promochem (www.lgcpromochem.com). LGC Promochem is currently the leading supplier of reference standards in Europe.

WHO Collaborating Centre for Chemical Reference Substances CRSs

The MPs of the WHO CRSs have been laid down on the basis of the results obtained in a collaborative study according to the capillary method of the International Pharmacopeia, 2nd ed.

WHO Certified Standards (LGC PromoChem)			
Compound	**MP (°C) (#)**	**Part No.**	**Nom. MP Range (°C) (&)**
Azobenzene	69	WHO9930217	67.8 - 68.8
Vanillin (*)	83	WHO9930438	81.7 - 83.0
Benzil	96	WHO9930222	94.8 - 96.0
Acetanilide	116	WHO9930201	114.4 - 115.7
Phenacetin (*)	136	WHO9930380	134.7 - 135.9
Benzanilide	165	WHO9930221	163.5 - 164.7
Sulfanilamide	166	WHO9930422	164.7 - 165.9
Sulfapyridine	193	WHO9930423	190.8 - 192.1
Dicyanodiamide	210	WHO9930286	191.7 - 192.7
Saccharin	229	WHO9930411	227.2 - 229.3
Caffeine (*)	237	WHO9930235	235.8 - 237.0
Phenolphthalein	263	WHO9930382	261.5 - 263.0

(#) Clear point

(*) Standard Substance included in SRS CRS Kit O100MPS

(&) H. Bervenmark et. al., Bull. Wld. Hlth. Org. 28(1963)175-188.

O100MPS CRS Kit

Stanford Research Systems supplies a CRS Kit (SRS Part # O100MPS) consisting of three chemicals with specified MP temperatures assigned in accordance with International Pharmacopeia (WHO) procedures.

O100MPS CRSs are provided to calibrate your OptiMelt's temperature scale for determination of MPs against the method of the International Pharmacopoeia and provides traceability to International Pharmacopoeia reference standards.

Tip:

- Use the O100MPS kit to check and readjust the temperature scale calibration of your OptiMelt every 6 months.
- Any OptiMelt MP apparatus carefully calibrated against WHO standards should test "acceptable for use" against all current USP standards and procedures. Consult the "OptiMelt Compliance" section later in this document for additional details.

Calibration Procedure

The MP ranges of three or more CRSs must be determined, at a heating rate of 1°C/min. The measured clear points are compared against their assigned values (i.e. certified clear points) and if deviations (beyond the accuracy of the instrument) are detected, the temperature scale of the instrument must be recalibrated.

Reliance on very accurate clear point temperatures, and a standard heating rate of 1°C/min, assures compatibility with routine MP determinations, and full enjoyment of the accuracy specifications of OptiMelt.

Tip:

- USP CRSs have wide certified melting ranges and often do not provide the accuracy levels required to take full advantage of the temperature measurement accuracy and resolution of OptiMelt. A common calibration strategy is to reserve USP standards for calibration checks and determination of acceptability against USP guidelines, but to use WHO standards instead to adjust the temperature scale if recalibration is deemed necessary.

- Any OptiMelt MP apparatus carefully calibrated against WHO standards should test “acceptable for use” against all current USP standards and procedures. Consult the “OptiMelt Compliance” section later in this document for additional details.

British Pharmacopeia

The British Pharmacopeia is an authoritative collection of standards for UK medicines and is an essential reference point for everyone involved in UK research, development or manufacturing.

Important!

- Following the unification of Europe (i.e. European Community), the British Pharmacopeia is being superseded by the more widely recognized European Pharmacopeia.

MP Protocol

The British Pharmacopeia (BP) MP determination procedure was written in 1988 and relies on the meniscus point (Method II in Appendix V, A92) to record the MP of a substance; the first observation of a definite meniscus is what is interpreted as the “single” MP of the sample.

Tips

- The main disadvantage of this procedure is the subjectivity of meniscus point determinations. Even experienced chemists, looking simultaneously at the same melt, will often disagree on the exact temperature value of meniscus formation.

- There is an alternative procedure (Method I) that records the clear point instead, but it is not regarded as a widely recognized protocol in the UK.

British Standards

Two sources of **CRSs** are recommended by the British Pharmacopeia:

LGC Promochem CRSs

(1) National Physical Laboratory, Teddington TW11 0LW, England (www.npl.co.uk).
(2) LGC Promochem (www.lgcpromochem.com). LGC Promochem is currently the leading supplier of reference standards in Europe.

Calibration Procedure

Calibration is very straight forward (particularly when using LGC CRSs) since the procedure is clearly listed in the Certificate of Measurement of each compound. The user must determine the MP of three or more standards, at the specified heating rate, and compare the MP results (automatic or manual) to those recorded in the calibration certificates. If deviations beyond the accuracy specification of the MP Apparatus are detected, the instrument must be recalibrated.

Tip

- It is important to keep in mind that the records supplied by the LGC are generated in a stirred-liquid-bath heating stand. There is a general acceptance that there will always be a small difference in the results of a clear point determination performed with a metal oven, compared to the more traditional (i.e. oil based) MP determination method mentioned in the British Pharmacopeia standard. The difference is usually very small and generally within the accuracy of the standard at low heating rates. However, this

small difference must be included as one of the sources of error in the measurement.

European Pharmacopeia

The European Community has a unified European Pharmacopeia (EP), which was founded by eight states (Belgium, France, Germany, Italy, Luxembourg, Netherlands, Switzerland and the UK) in 1964. This organization is recognized as one of the main authorities on quality and safety of medicines in Europe, and it has taken precedence over many national (and local) conventions such as the British, French and Italian pharmacopeias.

Mandatory in 30 member states including the European Union countries; 16 countries and the World Health Organization are observers at the European Pharmacopeia Commission. Certain observer states officially implement (in whole or in part) the standards of the European Pharmacopeia.

MP Protocol

The MP procedure is described in the Physical and Physicochemical Methods section of the Ph. Eur. Monograph. (4th edition), under the title: Melting Point-capillary method 2.2.14. Consult the latest Ph. Eur. and its supplements for up-to-date guidelines.

European Standards

According to the Ph. Eur. Monograph (4^{th} edition), you must procure MP standards directly from the World Health Organization Collaborating Centre for Chemical Reference Standards. WHO Standards can also be obtained from LGC Promochem (www.lgcpromochem.com).

Calibration Procedure

Calibration is very straight forward (particularly when using LGC CRSs) since the procedure is clearly listed in the Certificate of Measurement of each compound. The user must determine the MP of three or more standards, at the specified heating rate, and compare the MP results (automatic or manual) to those recorded in the calibration certificates. If deviations beyond the accuracy specifications of the instrument are detected, the instrument must be recalibrated.

OptiMelt Compliance

OptiMelt was carefully designed to assure compliance with modern National and International Pharmacopeia requirements.

Stanford Research Systems supplies a CRS Kit (SRS Part # O100MPS) consisting of three chemicals with specified MP temperatures assigned in accordance with International Pharmacopeia (WHO) procedures and with traceability to WHO MP standards.

Tip:
O100MPS CRSs are provided to calibrate your OptiMelt's temperature scale for determination of MPs against the method of the International Pharmacopoeia and provides traceability to International Pharmacopoeia reference standards.

Any OptiMelt MP apparatus carefully calibrated against WHO traceable standards should test "acceptable for use" against all current USP standards and procedures.

The following table is an example of a typical OptiMelt carefully calibrated against WHO MP Standards and subsequently checked for acceptability against USP CRSs. In all cases, the measured MP ranges fell within the acceptance limits assigned to the MP standards by the USP certifications laboratory.

Chemical Name	Assigned MP (°C)	Measured MP (°C)
WHO MP Standards (Calibrated)		
Vanillin	83.2 (#)	83.2
Phenacetin	136.0	136.2
Caffeine	237.2	237.1
USP MP Standards (Checked)		
Vanillin	81.0 – 83.0 (1.5) (*)	82.1 - 82.9 (0.8)
Acetanilide	113.5 – 115.5 (1.5)	114.1 – 115.3 (1.2)
Phenacetin	134.0 – 136.0 (1.5)	134.4 – 135.4 (1.0)
Sulfanilamide	164.2 – 165.8 (1.0)	164.7 – 165.5 (0.8)
Sulfapyridine	190.0 – 192.0 (1.5)	190.8 – 192.1 (1.3)
Caffeine	235.5 – 237.0 (1.0)	235.9 – 236.8 (0.9)

(#) Clear points. Standard capillaries (O100MPC) were used.
(*) Acceptable limits for the MP range, followed by (maximum acceptable MP range). USP capillaries were used.

Quality Control Glossary

Pharmacopeia (also spelled: Pharmacopoeia)

The Pharmacopeia (also called Medical Handbook) is a publication containing official regulations and protocols. It provides detailed information on the nature and storage of drugs and their ingredients, and how they are to be tested, analyzed and dispensed by the pharmacist.

An International Pharmacopeia has been compiled by the World Health Organization. National Pharmacopeias have also been retained as supplements to enable quick and flexible introduction of new pharmaceutical standards on a national level.

The use of the title, or subtitle, of a Pharmacopeia Monograph in connection with a drug implies that the product conforms to the specifications of that monograph.

Good Laboratory Practice

Good Laboratory Practice (GLP) deals with the organization, process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported. GLP data are intended to promote the quality and validity of test data.

The GLP regulations originated in the pharmaceutical industry in the USA. The rest of the world rapidly embraced the concept of quality assurance so that the Organization for Economic Cooperation and Development (OECD) published an international version of the GLP testing methods in 1981.

All GLP regulations include detailed descriptions of the structure and maintenance of their test equipment. Instruments used to produce, measure and verify data must be installed, tested and calibrated according to strict guidelines, and written documentation must be prepared and stored for all inspections and audits performed.

One of the most important principles of the GLP is the requirement for reproducible documentation of the results of any analysis. The so-called “5-W-rule”: Who did What, When, With What and Why?

GLP imposes great demands on the documentation of MP determinations. For that reason, OptiMelt offers the possibility of storing multiple analysis methods (up to 24) and MP reports (up to 8). Since GLP demands verifiable documentation of the results of analysis, OptiMelt also has the ability to transfer its stored MP reports to a printer or a host PC.

The MeltView software package included with OptiMelt can handle real-time image transfer, allowing you to display and store high-resolution digital images of

the samples (including relevant information such as temperature, time and date) on your computer screen during analysis. All sample images transferred to the PC are bundled together as a single package and automatically stored in the computer's hard disk when the test is completed. This option provides the most powerful and definitive documentation infrastructure available from any commercial MP apparatus. Stored image packages may be recalled at any time, and melts can be played back frame-by-frame or as movie, by simple moving a cursor back and forth with your mouse. Being able to replay a test after the fact is an invaluable tool for GLP documentation, fine tuning of results, and for laboratory demonstrations in educational settings.

For detailed information on GLP guidelines consult:

(1) Ludwig Huber, "A primer -Good laboratory practice and current good manufacturing practice", Publication # 5968-6193E, Agilent Technologies, Germany, March 2000; and

(2) Ludwig Huber, "Validation and Qualification in Analytical Laboratories", Interpharm Press, Buffalo Grove, IL, USA, 1998.

Good Manufacturing Practice

Good Manufacturing Practice (GMP) regulations are applicable in production and in analysis associated with production. GMP is that part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use.

Published GLP and GMP regulations have a significant impact on the daily operation of an analytical lab.

For detailed information on GMP guidelines consult:
Michael Anisfeld, "International Drug GMPs", Interpharm Press, Buffalo Grove, IL, USA, August 1993.

21 CFR Part 11

21 CFR Part 11 is the United States Food and Drug Administration's (FDA) requirement for electronic record keeping (i.e. part of the GMP guidelines set forth by the FDA). These are the rules under which an electronic document can be considered equivalent to a paper document. In broad terms, this includes software validation, password protection of data and macros, time-stamped audit trail capability, and various password and security measures.

The FDA regulation in 21 CFR Part 11, effective since August 20, 1997, specifies how companies in FDA-governed industries must handle electronic records and

electronic signatures. The regulation does not mandate the use of electronic records or signatures. Rather, it simply outlines the requirements that must be met by medical device, drug, and biologic manufacturers that do choose to use them. The regulation applies to all aspects of the research, clinical study, maintenance, manufacturing, and distribution of medical products.

Collaborative efforts between FDA and the regulated industries (initiated in 1992) were the origin of 21 CFR Part 11. The regulation is grounded in the agency's belief that the new data technologies have become so pervasive that the use of electronic records and signatures will inevitably become universal. It is designed essentially to minimize the possibility of data misappropriation. Part 11 focuses on ensuring the authenticity of data, the integrity of data and systems, the confidentiality of data (particularly with respect to clinical trials and blood banks), and the no repudiation of electronic signatures.

No software or hardware vendor can claim that his or her products are certified Part 11 compliant. A vendor, instead, can say that he has all of the Technical Controls for 21 CFR Part 11 compliance built in to his product. Remember, it is the responsibility of the user to implement (correctly and consistently) the Procedural and Administrative Controls and use products with the correct Technical Controls for overall Part 11 compliance.

Related Web Links

Name	URL
United States Pharmacopeia	www.usp.org
British Pharmacopeia	www.Pharmacopeia.org.uk
LGC Promochem (*)	www.lgcpromochem.com
European Pharmacopeia	www.pheur.org
Japanese Pharmacopeia	http://jpdb.nihs.go.jp/jp14e/
International Pharmacopeia	www.who.int
FDA Website	www.fda.gov
21 CFR Part 11	www.21cfrpart11.com
Laboratory compliance and regulations.	www.labcompliance.com
R.T. Corp	www.RT-Corp.com

(*) LGC Promochem has the widest selection of standards, and offers worldwide distribution through a network of accredited representatives (including R.T. Corp in the US). Twelve very pure (>99.9% mol%) organic compounds, with very sharp MPs covering the range 52 °C to 285 °C, are available to satisfy the requirements of the US, WHO, British and many other European pharmacopeias.