BIOSPECTRA
100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

MP50 MELTING RANGE OPERATION, VERIFICATION, AND CALIBRATION SOP

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide the Laboratory Analysts with a procedure to determine the melting range of Raw Materials (RM), Finished Goods (FG), and stability samples.

2. SCOPE:

2.1. Applies to the measurement of the melting range for all RM, FG, and Supplier Approval, Development, and Stability samples utilizing the MP50 Melting Point Apparatus attached to P25 printer located in the Stroudsburg, PA and Bangor, PA Laboratories.
  2.1.1. Serial Number B412411844 is located in the Bangor, PA Laboratory.
  2.1.2. Serial Number B442145335 is located in the Stroudsburg, PA Laboratory.

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Testing, or designee, is responsible for the content of this procedure.
3.2. The Laboratory Manager, or other qualified designated individual, is responsible for the implementation and training of this procedure.
3.3. All Laboratory Analysts and designated, trained personnel are responsible for performing and complying with the requirements of this procedure.
3.4. It is the responsibility of the Laboratory Systems unit to review and approve data generated during the execution of this procedure.

4. REFERENCES:

4.1. BSI-FRM-0863, MP50 Melting Range Verification and Calibration Procedure
4.2. BSI-SOP-0126, Laboratory Notebooks
4.3. BSI-SOP-0131, Calibration
4.4. Current USP <741> Melting Range or Temperature, General Chapter
4.5. Mettler Toledo MP50 Melting Range Apparatus Manual

5. EQUIPMENT AND REAGENTS:

5.1. Capillary Tube Packing Rod
5.2. Mettler Toledo MP50 Melting Point Apparatus
5.3. Melting Point Capillary Tubes
5.4. Mortar and Pestle
5.5. Mettler Toledo P25 Printer
5.6. Vanillin USP Reference Standard, or USP-Traceable equivalent
5.7. Phenacetin USP Reference Standard, or USP-Traceable equivalent
5.8. Sulfanilamide USP Reference Standard, or USP-Traceable equivalent
5.9. Succinic USP Acid Reference Standard, or USP-Traceable equivalent
5.10. Theophylline USP Reference Standard, or USP-Traceable equivalent

6. PROCEDURE:

6.1. Operation:

6.1.1. Ensure that the temperature verification is current.
6.1.2. Log into the instrument by selecting the appropriate username.
6.1.3. Using a mortar and pestle, reduce sample to a fine powder prior to drying the sample, or analyze the sample "as-is".
6.1.3.1. All Finished Good and Stability samples will to be dried prior to analysis. Dry the sample over a suitable desiccant for a minimum of 16 hours, or dry at a temperature and time according to the product's Loss on Drying (LOD) procedure.
6.1.3.2. Raw Material samples may be analyzed "as-is", with the exception of Domestic TAC Tris Raw Material.
6.1.3.2.1. Prior to analyzing Domestic TAC Tris Raw Material for melting range, supplied by Advancion (Angus), dry the sample as according to the LOD method defined the Tris Testing Methods.
6.1.4. Place the prepared sample into a capillary tube. If needed, use a clean packing rod to push residual sample down the capillary tube being careful to keep the rod greater than approximately 2 cm from the closed end of the capillary tube. The sample should then be packed down to a height of 2.5 - 3.0 mm by gentle tapping on a solid surface. The height measured should be representative of the sample and not the capillary tube height since the height of the closed end may vary.
6.1.5. Allow the instrument to reach the approximate start temperature for the current method. The instrument will beep once the initial temperature is reached.
6.1.6. Place the capillary tube containing the sample in the melting point apparatus.
6.1.7. NOTE: Do not force the capillary tube(s) into the apparatus; they should drop right in.
6.1.8. Ensure that the packed sample is within the "min" and "max" lines on the instrument.
6.1.9. Select the correct method on the home screen, according to the product being analyzed.
6.1.9.1. If the product does not have a method shortcut on the home screen, refer to page 23 of the Operating Instructions to create a manual method.
6.1.10. Press the Start button.
6.1.11. When prompted, enter the lot number into the "Analysis Comments."
6.1.12. Up to four samples of the same product may be analyzed at the same time.
6.1.13. Results will print upon completion.
6.1.13.1. See Section 7, "Acceptance Criteria and Result Reporting," for reporting results.
6.1.14. Record results in the appropriate laboratory documentation. Transcribe data to the appropriate Analytical Summary sheet.
6.1.15. If a capillary tube breaks and cannot be extracted in the typical manner, the following actions will be performed:
6.1.15.1. The event must be documented in the appropriate instrument logbook.
6.1.15.2. Laboratory management will be notified immediately and must observe this section of the procedure.
6.1.15.3. Remove the insulation glass and capillary guide according to Section 6.2.2.1. of the Mettler Toledo MP50 Melting Range Apparatus Manual.
6.1.15.4. Gently manipulate the instrument to extract the broken capillary tube.
6.1.15.5. Reassemble the insulation glass and capillary guide.
6.1.15.6. Following these actions, an abbreviated verification must be performed, utilizing the lowest (Vanillin) and highest (Theophylline) standards in quadruplicate, according to Step 6.6.1 of BSI-FRM-0863, "MP50 Melting Range Operation, Verification and Calibration Procedure."

6.1.15.6.1. If all measurements meet the accepted melting range specification, to the standard, the instrument may be returned to use.

6.1.15.6.2. If any measurement fails to meet the accepted melting ranges, the results of this verification will be used to perform the Calibration, according to Steps 6.6.2 – 6.6.6 of BSI-FRM-0863 "MP50 Melting Range Verification and Calibration Procedure".

6.2. **Instrument verification**:

6.2.1. The MP50 will be verified on a monthly basis, by a trained Laboratory Analyst, to ensure the instrument is working properly.

6.2.1.1. Utilize form BSI-FRM-0863, "MP50 Melting Range Operation, Verification and Calibration Procedure", or a notebook if the procedure cannot be printed.

6.2.2. Allow the USP Traceable Reference Standards to dry as per the preparation required on the respective Certificate of Analysis (COA) provided with the standard.

6.2.3. Transfer a small amount of the Reference Standard to a clean, dry mortar, and crush to a fine powder using a clean pestle.

6.2.3.1. If the mortar and pestle has been recently washed, it must be dried in the oven to ensure complete dryness prior to analysis.

6.2.4. Fill and pack a capillary tube to approximately 2.5-3.0 mm with the standard and insert into the MP50.

6.2.5. Change the Manual settings as follows:

6.2.5.1. Operation Mode: Melting Range

6.2.5.2. Start Temperature: ~5°C below the expected start temperature for the Reference Standard

6.2.5.2.1. It is very important that the range is below the expected start time of the melt so the onset melting temperature is clearly visualized.

6.2.5.3. Waiting time: 10 s

6.2.5.4. Heating Rate: 1.0°C/min

6.2.5.5. End Temperature: ~3°C above the expected end temperature for the Reference Standard.

6.2.5.6. It is important that the end temperature is not too high to avoid degradation of the standard or decomposition.

6.2.5.7. T (iso): 0 s

6.2.5.8. End behavior: Start Temperature

6.2.5.9. Method Comment: USP Traceable Reference Standard's lot number and expiration date.

6.2.5.10. Print Report: Checked

6.2.5.11. Then press Start.

6.2.6. Test each USP Traceable reference standard (Vanillin, Phenacetin, Sulfanilamide, Succinic Acid, and Theophylline) to their respective specifications listed on each Certificate of Analysis.

6.2.6.1. Ensure each USP Traceable standard to be used is current and within the valid use date by referencing the USP Reference Standards Catalog and ensuring it is traceable to the most current lot or, in the event a new lot is issued, that the old lot is still within the USP designated expiration date.

6.2.7. If any standard fails to meet the specification, a calibration must be performed according to Section 6.3.

6.3. **Calibration:**

6.3.1. A two-point calibration will be performed:

6.3.1.1. At a frequency of no less than (NLT) a bi-annual basis (every 6 months), or sooner as needed.

6.3.1.2. If any standard in the Verification fails to meet the specification.

6.3.2. The lowest (Vanillin) and highest (Theophylline) standard will each be analyzed in quadruplicate.

6.3.3. The two-point calibration is achieved using the mean measured results of the Reference Standards (RS) and the mean of the corresponding RS ranges, as calculated from each Certificate of Analysis (COA).

6.3.4. In the instrument software, the analyst will enter results as follows:

6.3.4.1. T Nominal 1 = Mean Vanillin RS value, calculated from COA range

6.3.4.2. T Measured 1 = Mean of the measured results of all four Vanillin RS replicates

6.3.4.3. T Nominal 2 = Mean Theophylline RS value, calculated from COA range

6.3.4.4. T Measured 2 = Mean of the measured results of all four Theophylline RS replicates

6.3.4.5. NOTE: The mean of the four, RS replicates is taken from the instrument results printout(s).

6.3.5. The instrument uses this data to determine an appropriate adjustment.

6.3.6. Following this calibration, the system must be verified by re-analyzing all five USP traceable reference standards listed in Step 6.2.6. according to section 6.2.

6.3.6.1. If all standard measurements meet the specifications, the instrument may be used, if needed.

6.3.6.2. If any standards fail to meet the specification, the instrument must be tagged as Out of Calibration (or alike) and Laboratory management must be notified for further instruction.

7. ACCEPTANCE CRITERIA AND RESULT REPORTING:

7.1. Method Acceptance Criteria:

7.1.1. Sample results must be:

7.1.1.1. 0.5°C – 1.5°C wide

7.2. Sample Acceptance Criteria:

7.2.1. Sample results must:

7.2.1.1. meet the specification for the respective product code

7.3. Results for Finished Goods, Raw Material, and Stability, meeting all above criteria, will be reported from the instrument printout, unless otherwise specified in the steps below.

7.4. VISUAL VERIFICATION AND VISUAL DETERMINATION:

7.4.1. *Visual Verification* refers to the review of the available video data on the instrument display in order to confirm the validity of the instrument printout result.

7.4.1.1. If during a Visual Verification, it is observed that the instrument incorrectly identified the start or end of the melt, for example, if a bubble is observed in the capillary, a Visual Determination must be performed.

7.4.2. *Visual Determination* refers to manual determination of the melting point by the analyst using the available video data on the instrument display, rather than the instrument result displayed on the printout.

7.4.3. Using the on-screen camera display, follow these steps to determine a result:

7.4.3.1. Record the initial temperature as the temperature at which the sample begins to collapse in on itself.

7.4.3.2. Record the final temperature as the temperature at which the sample is completely liquified.

7.5. If either a Visual Verification or Visual Determination is performed by an analyst, a Visual Verification must also be performed by a Reviewer or Laboratory management.
  7.5.1. NOTE: A Reviewer or Laboratory management may visually verify melting ranges in real-time (during testing) to help minimize the time lapse between the result(s) and review of the result(s). In this event, the Reviewer or Laboratory Management representative must initial and date next to the result, as acknowledgement of the authenticity and accuracy of the data.
7.6. In the case that the analysis cannot be visually verified by a Reviewer or Laboratory management, an OOS Checklist must be initiated and a retest may be required at the discretion of Laboratory management.
  7.6.1. For samples that are no longer available for analysis, a discrepancy must be issued.
7.7. If results reported on the instrument printout, meet the Method Acceptance Criteria, but fail to meet the Sample Acceptance Criteria, a Visual Verification must be performed by an Analyst and either a Reviewer or Laboratory management, to determine if the sample results from the instrument printout are valid. No OOS checklist is required.
  7.7.1. If the instrument result is confirmed to be Out-of-Specification (OOS) through Visual Verification, the following actions will be taken:
    7.7.1.1. Laboratory management must be immediately notified.
    7.7.1.2. an OOS checklist will be initiated.
  7.7.2. If the Visual Verification does not confirm the OOS result:
    7.7.2.1. the instrument printout results are determined to be invalid
    7.7.2.2. the Analyst will proceed to Visual Determination of the melting point. Refer to Section 7.4., above.
7.8. If results reported on the instrument printout meet the Sample Acceptance Criteria, but fail to meet the Method Acceptance Criteria, a Visual Verification is required to determine if the sample results from the instrument printout are valid.
  7.8.1. If the instrument result for Method Acceptance Criteria is confirmed to be valid, through Visual Verification, the instrument reported melting point will be reported.
  7.8.2. If the instrument result for the Method Acceptance Criteria is not determined to be invalid through Visual Verification, a Visual Determination must be performed by an Analyst and either a Reviewer or Laboratory management. If the Visual Determination meets Sample Acceptance Criteria, the visually-determined results will be reported.
7.9. Product specific Instructions:
  7.9.1. For **Bis Tris** (BTRI) Melting Point Determinations:
    7.9.1.1. Per USP <741> Melting Range or Temperature, the "beginning of the melting can be accurately established, and it is to be reported as the *melting point*", when "the melting process is accompanied by simultaneous decomposition, which is visually evidenced as a side event like darkening of the material, charring, bubbling, or other incident".
    7.9.1.2. When this occurs, a Visual Verification by a Reviewer or Laboratory management is required.
    7.9.1.3. No OOS checklist is required.
  7.9.2. For **Urea** Melting Point Determinations, per JP:
    7.9.2.1. Per JP, melting point will be recorded as the final temperature at which the sample is completely liquified (end of melt).
  7.9.3. Materials that often require Visual Determination are **Tris Hydrochloride** (THCL) and **Uridine** (URID).
    7.9.3.1. If the instrument correctly measures these materials (both Method and Sample Acceptance Criteria are met), the instrument printout results will be reported.
    7.9.3.2. If the instrument did not correctly measure these materials, as determined by Visual Verification, a Visual Determination will be performed.