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## MP50 MELTING RANGE OPERATION AND CALIBRATION SOP

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**1. PURPOSE:**

- 1.1. To provide the Quality Control (QC) 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 Stability samples utilizing the MP50 Melting Point Apparatus attached to P25 printer located in the Stroudsburg, PA and Bangor, PA QC Laboratories.
  - 2.1.1. Serial Number B412411844 is located in the Bangor, PA QC Laboratory.
  - 2.1.2. Serial Number B442145335 is located in the Stroudsburg, PA QC Laboratory.

**3. REFERENCES:**

- 3.1. BSI-SOP-0126 Laboratory Notebooks.:
- 3.2. Current USP
- 3.3. BSI-SOP-0131 Calibration.:
- 3.4. Mettler Toledo MP50 Melting Range Apparatus Manual
- 3.5. BSI-FRM-0863 MP50 Melting Range Calibration Procedure:

**4. EQUIPMENT:**

- 4.1. Mettler Toledo MP50 Melting Point Apparatus
- 4.2. Melting Point Capillary Tubes
- 4.3. Mortar and Pestle
- 4.4. Capillary Tube Packing Rod
- 4.5. Mettler Toledo P25 Printer

**5. PROCEDURE:**

- 5.1. Calibration:
  - 5.1.1. The MP50 must be calibrated on a bi-annual basis by a trained QC analyst to ensure the instrument is working properly.
    - 5.1.1.1. Utilize the MP50 Melting Range Calibration Procedure, BSI-FRM-0863.
  - 5.1.2. Allow the USP Traceable Reference standards to dry as per the preparation required on the respective certificate of analysis provided with the standard.
  - 5.1.3. Transfer a small amount of the USP Traceable Reference Standard to a clean, dry mortar and crush to a fine powder using a clean pestle.
    - 5.1.3.1. If the mortar and pestle has been recently washed, it may be dried in the oven to ensure complete dryness prior to analysis.
  - 5.1.4. Fill and pack a capillary tube to approximately 2.5-3.0 mm with the standard and insert into the MP50.
  - 5.1.5. Change the Manual settings as follows:
    - 5.1.5.1. Operation Mode: Melting Range
    - 5.1.5.2. Start Temperature: ~5°C below the expected start temperature for the Reference Standard
      - 5.1.5.2.1. It is very important that the range is below the expected start time of the melt so the onset melting temperature is clearly visualized.
    - 5.1.5.3. Waiting time: 10 s
    - 5.1.5.4. Heating Rate: 1.0°C/min
    - 5.1.5.5. End Temperature: ~3°C above the expected end temperature for the Reference Standard.

- 5.1.5.6. It is important that the end temperature is not too high to avoid degradation of the standard or decomposition.
- 5.1.5.7. T (iso): 0 s
- 5.1.5.8. End behavior: Start Temperature
- 5.1.5.9. Method Comment: USP Traceable Reference Standard's lot number and expiration date.
- 5.1.5.10. Print Report: Checked
- 5.1.5.11. Then press Start.
- 5.1.6. Test each USP Traceable reference standard (Vanillin, Phenacetin, Sulfanilamide, Succinic Acid, and Theophylline) to their respective specifications listed on each Certificate of Analysis. When using a secondary standard, utilize the USP primary standard acceptance criteria that the secondary standard is qualified with.
  - 5.1.6.1. 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.
- 5.1.7. If any standard fails to meet the specification, perform a two-point adjustment by following the steps below.
  - 5.1.7.1. Analyze the lowest (Vanillin) and highest (Theophylline) standard each in quadruplicate.
  - 5.1.7.2. Use the average results alongside the corresponding USP averages to perform the two-point adjustment.
  - 5.1.7.3. Use the mean value provided on the results print out for the low standard as the  $T_1$  Measured Value and the USP value as the  $T_1$  Nominal Value.
  - 5.1.7.4. Repeat this for the high standard and use those values for  $T_2$  Measured Value and  $T_2$  Nominal value, respectively.
  - 5.1.7.5. The instrument will use this data to determine an appropriate adjustment.
  - 5.1.7.6. Re-analyze all five USP traceable reference standards listed in Step 5.1.6.
- 5.2. Operation:
  - 5.2.1. Verify that the calibration is current.
  - 5.2.2. Log into the instrument by selecting the appropriate username.
  - 5.2.3. Reduce sample to a fine powder in a mortar and pestle prior to drying the sample, or analyzing the sample as-is.
    - 5.2.3.1. All Finished Good and Stability samples are 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 LOD procedure. Raw Material samples may be analyzed as-is, with the exception of Domestic TAC Tris Raw Material.
      - 5.2.3.1.1. Domestic TAC Tris Raw Material is to be dried as per the Loss on Drying method in the Tris Testing Methods prior to analyzing for melting range.
  - 5.2.4. Place the prepared sample into a capillary tube. A clean packing rod can be used to push residual sample down the capillary tube but should not enter approximately 2 cm from the closed end of the capillary tube. The sample should then be packed down to a height of 2.5-3.0mm 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.

- 5.2.5. Allow the instrument to reach the approximate start temperature for the current method. The instrument will beep once the initial temperature is reached.
- 5.2.6. Place the capillary tube containing the sample in the melting point apparatus.
- 5.2.7. Ensure that the packed sample is within the “min” and “max” lines on the instrument.
- 5.2.8. Do not force the capillary tube(s) into the apparatus; they should drop right in.
- 5.2.9. Select the correct method on the home screen, according to the product being analyzed.
  - 5.2.9.1. If the product does not have a method shortcut on the home screen, see page 23 of the Operating Instructions to create a manual method.
- 5.2.10. Press the start button.
- 5.2.11. When prompted, enter the lot number into the “Analysis Comments”.
- 5.2.12. Up to four samples of the same product may be analyzed at the same time.
- 5.2.13. Results will print upon completion.
  - 5.2.13.1. Printed results for all product other than Tris HCl may not be altered without proper documentation, i.e. Out of Specification Checklist.
- 5.2.14. For analyzing Tris HCl samples:
  - 5.2.14.1. Prepare the sample by following steps 5.2.1-5.2.11.
  - 5.2.14.2. Using the on-screen camera display follow these steps to determine a result: 5.2.14.2.1. Record the initial temperature as the temperature at which the sample begins to collapse in on itself.
    - 5.2.14.2.2. Record the final temperature as the temperature at which the sample is completely liquidated.
    - 5.2.14.2.3. Bubbles may form within the sample during melting. If the sample is completely liquidated and there are still bubbles present in the sample, the sample is still considered completely melted.
- 5.3. Record results in the appropriate laboratory documentation. Transcribe data to the appropriate Analytical Summary sheet.

## 6. ACCEPTANCE CRITERIA:

- 6.1. Each sample result should be 0.5°C – 1.5°C wide and meet the specification as required by the requested code.
  - 6.1.1. If a sample result is outside of this range, the result must be visually verified by the analyst, as well as the reviewer<sup>1</sup> of the analysis.
    - 6.1.1.1. **NOTE:** Laboratory management may verify visually determined or unusually wide melting ranges in real-time (during testing) to help minimize the time lapse between the visually determined result(s) and verification of the result(s). An initial and date of the manager or supervisor should be next to the visually called result for acknowledgement of the authenticity and accuracy of the data.
  - 6.1.2. Upon visual verification, if bubbles are observed that led to an incorrect automatic determination, the result should be visually (manually) determined using the available video data on the instrument display, refer to section 5.2.14.2.1. for more detail on visual determinations.

- 6.1.2.1. In the case that the analysis cannot be visually verified, an Out of Specification Checklist should be issued and a retest may be required at the discretion of the supervisor or QC Management.
- 6.1.3. If a sample result fails to meet the required specification, an Out of Specification Checklist should be initiated.
  - 6.1.3.1. Once the checklist is initiated, the result may be visually verified by the analyst performing the test.
  - 6.1.3.2. If the visually determined result is different than the printed result, it must be noted in the checklist. The visually determined result will stand as the official result.
  - 6.1.3.3. All visually determined results must be verified by the person performing the final review<sup>1</sup> of the documentation.
    - 6.1.3.3.1. <sup>1</sup>NOTE: Laboratory management may verify visually determined melting ranges in real-time (during testing) to help minimize the time lapse between the visually determined result(s) and verification of the result(s). An initial and date of the manager or supervisor should be next to the visually called result for acknowledgement of the authenticity and accuracy of the data.