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|  | Standard Operating Procedure | | SOP Number D-1027 | Revision 0 |
| | Ninhydrin Testing for Boron Chelate 5% | | Effective Date | Page Page 1 of 3 |
| Written by/ Date <i>A. Dukles 01-21-25</i> | | Reviewed by/ Date <i>ATS 01/21/25</i> | | Approved by/ Date <i>PEC 01/21/25</i> |
| Title: Analytical Research Scientist | | Title: QC Laboratory Manager | | Title: QA/QC Director |

1.0 Purpose

- 1.1 The purpose of this SOP is to outline the systematic approach for the accurate identification and testing of boron chelates to ensure quality and consistency. This procedure applies to the identification of amino acids by means of a color change reaction.

2.0 Responsibility

- 2.1 It is the responsibility of QC and Analytical Chemists to follow this procedure.
- 2.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.
- 2.3 It is the responsibility of QC Laboratory to keep this procedure current with latest Ion Labs Practice.

3.0 Definitions

- 3.1 QC – Quality Control

4.0 Supplies

- 4.1 Ninhydrin, ACS reagent CAS:485-47-2
- 4.2 NaOH 1N, CAS:1310-73-2
- 4.3 HCl 3M, CAS:7647-01-0, 7732-18-5
- 4.4 Sodium phosphate dibasic dihydrate CAS:10028-24-7
- 4.5 Sodium phosphate monobasic dihydrate CAS: 1342-35-0
- 4.6 DI Water
- 4.7 Analytical Balance
- 4.8 Hot Plate

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|---|-------------------------|-----------------|-------------|
| Standard Operating Procedure Ninhydrin Testing for Boron Chelate 5% | SOP No D-1027 | Rev 0 | Page 2 of 3 |
|---|-------------------------|-----------------|-------------|

4.9 Pipettes

4.10 Thermometer

4.11 Timer

5.0 Procedure

5.1 Buffer and Test Solution Preparation

5.1.1 Prepare a 100ml of phosphate buffer.

5.1.1.1 Weigh ~0.69g of Sodium phosphate monobasic dihydrate and ~1.34 g of sodium phosphate dibasic dihydrate to 70mL of deionized water into a suitable container.

5.1.1.2 Stir the mixture using a magnetic stirrer until both salts are completely dissolved.

5.1.1.3 Measure the pH using a calibrated pH meter to a pH of 7.4. Add small amounts of 1M NaOH if the pH is too low. Add small amounts of 1M HCl if the pH is too high.

5.1.1.4 Dilute to volume. Prepare this solution fresh.

5.1.2 Prepare Ninhydrin TS

5.1.2.1 Weigh ~200mg of Ninhydrin in water to make 10mL.

5.1.2.2 Prepare this solution fresh.

5.2 Sample preparation

5.2.1 Weigh ~0.5g of boron chelate and transfer to a 100mL glass beaker

5.2.2 Add 20mL of phosphate buffer to the beaker

5.2.3 Stir the mixture using a magnetic stirrer for 15 minutes.

5.2.4 Add 20mL of 3M HCl to the boron chelate solution while stirring

5.2.5 Heat the mixture on a hot plate at 105°C for 3 hours. Place a watch glass over the beaker to ensure no evaporation.

5.2.6 Remove the beaker from the hot plate and allow to cool to room temperature.

5.2.7 Add 10mL of Ninhydrin TS to the cooled reaction mixture.

5.2.8 Mix thoroughly using a stir bar for 5 minutes.

5.2.9 Heat the mixture to ~70-75°C with continuous stirring

5.2.10 Using a pipette, add 5mL increments of 1M NaOH to the heated mixture while stirring until a distinct color change is observed.

6.0 Revision History

| Revision | Date | Description of Changes | CCR # | By |
|----------|----------|------------------------|-------|----------|
| 0 | 01/20/25 | New Procedure | N/A | A. Lukes |