1.0 Purpose

The purpose of this procedure is to define the process for optimization of existing HPLC methods for specific products.

2.0 Scope

This procedure applies to the optimization of existing HPLC methods for specific products that are not amenable to analysis using the method as written..

3.0 Responsibility

3.1 It is the responsibility of QC Chemists and/or Analytical Development to understand and work within the guidelines of this procedure.

3.2 It is the responsibility of QC Laboratory Management and/or Analytical Development to ensure compliance with this procedure and to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **QC** – Quality Control

4.2 **HPLC** – High Performance Liquid Chromatography

4.3 **UV** – Ultraviolet Detection

5.0 References

5.1 D-126, SOP, Non-Conforming Results in the QC Laboratory

5.2 C-201, SOP, Deviation and Investigation Procedure

6.0 Overview

6.1 Because of the large number of products manufactured at Ion Labs, analyte specific HPLC methods cannot be validated for every product. In some cases, the sample preparation instructions in an analyte specific HPLC method may not perform as expected for a given product. When such a case is identified, a product specific method optimization may overcome the method deficiency. Product specific method optimization may be triggered by certain events including, but not limited to:

6.1.1 An out of specification test result which was determined to be "Minor" as defined in D-126 Non-Conforming Results in the QC Laboratory was obtained for either finished product release or stability testing.

6.1.2 A new product, which has not previously undergone testing, is expected to present incompatibility with a HPLC method due to a unique characteristic of the product such as sample matrix, label claim, serving size, or dosage form.

6.2 After successful completion of a product specific method optimization, the Test Details section of the product profile will be updated to reflect the method optimization. At a minimum, the Test Details should include:

6.2.1 Instructions for preparing the sample in accordance with the optimal conditions.

6.2.2 Instructions for preparing the working standard at the concentration used during optimization.

6.2.3 A list of any deviations to the existing method that are required to obtain reliable results for the specific product/analyte (e.g. alternate wavelength, injection volume, or diluent). Any deviation outlined in the Test Details is henceforth no longer considered a deviation and does not require documentation by SOP C-201 Deviation and Investigation Procedure.

6.2.4 If applicable, the maximum time for which the sample preparation is stable.

6.3 The results generated during product specific method optimization shall not be used for finished product release. To ensure this, the following guidelines will be adhered to:

6.3.1 Sequences generated during product specific method optimization shall not be stored in the same project folder as those generated for finished product release.

6.3.2 Sequence names and sample names for sequences generated during product specific method optimization shall not include a batch number. The product name, formula number, or test identifier (e.g. LOWCONC, MIDCONC, HIGHCONC) should be used instead.

6.4 If it is determined that two formulas are sufficiently similar, a single method optimization can be performed for the set or a reduced version of the criteria listed herein may be appropriate. Scientific justification is required.

7.0 Procedure

7.1 Sample preparation and/or method parameters which may need optimization are listed below. Only Accuracy and Specificity are required. It is up to the discretion of the analyst to decide which other parameters may benefit from optimization.

7.2 Accuracy

7.2.1 Spike a placebo with authentic reference standard at a level corresponding to that expected in the finished product formulation.

7.2.2 Alternatively: prepare an unspiked sample and a spiked sample, and determine the recovery of spiked analyte. Both the spiked and unspiked samples should be within the linear range of the method.

7.2.3 Acceptance Criteria

7.2.3.1 In general, the spike recovery should be within the range 95% - 105%. More or less stringent acceptance criteria may be used depending on product requirements. For example, if the product contains a very small amount of the target analyte with a large (40%) overage added, an acceptance criteria of 90% - 110% may be more appropriate. Scientific justification is required.

7.3 Specificity

7.3.1 Using the optimal conditions determined during optimization, the assay should meet the following criteria for Specificity.

7.3.2 Acceptance Criteria

7.3.2.1 The retention time of the target analyte in the sample chromatogram differs from that of the reference standard by no more than 0.3 min. If the retention time is greater than 15 min, then the difference may be no more than 3%.

7.3.2.2 For HPLC/UV: The spectral match of the target analyte in the sample chromatogram with that of the reference standard is no less than 900.

7.4 Extraction Conditions

7.4.1 For samples that require extraction: perform the analytical test method using different extraction conditions.

7.4.2 Sample preparation may consist of any combination of shaking, stirring, heating, and/or sonication. Extraction conditions that may be varied include sonication time, shaking time, intensity of shaking, stir time, intensity of stirring, extraction temperature, and/or protection from light.

7.4.3 The optimal extraction condition is usually the one which results in the highest assay value. However, all data collected should be evaluated as a whole to determine if this is true. For example, a very vigorous extraction condition may result in extraction of a previously unobserved interfering compound resulting in inflated assay value. This would be evident in an unsatisfactory spectral match to the reference standard.

7.5 Sample Concentration

7.5.1 Perform the analytical test method at different sample concentrations within the validated linear range of the method.

7.5.2 The optimal sample concentration is usually the one which results in the assay value with highest percent of label claim. As discussed above, this may not always be true. For example, a high sample concentration may result in poor

chromatography with co-elution causing overestimation of the assay value. This would be evident from the chromatography and from reduced spectral match.

7.6 Solution Stability

7.6.1 For analytes that have been observed to be or are expected to be unstable in solution, perform repeat injection of the sample over the course of at least three hours.

7.6.2 The composition of the extraction solvent/diluent may require adjustment if solution stability in the method specified diluent is not sufficient.

7.6.3 Acceptance Criteria

7.6.3.1 The sample preparation is stable as long as the change in measured concentration does not change relative to the initial concentration by more than 5.0%. More or less stringent criteria may be used with scientific justification as discussed above for Accuracy.

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	12/20/22	New procedure	N/A	S. Sassman
1	06/06/23	A report is no longer required, using a purposely adulterated sample is no longer required, only specificity and accuracy optimizations will be required. Update logo and format.	CC-23-0273	S. Sassman