ION LABS	Standard Operating Procedure **Determination of Lidocaine and Phenoxyethanol by HPLC using UV/VIS Spectroscopy**	**SOP Number** **D-783**	**Revision** **1**
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Written by/ Date	**Reviewed by/ Date**	**Approved by/ Date**
07/21/22	SAS 07/22/22	07/22/22
Title: Quality Systems Manager	**Title: Analytical Development Scientist**	**Title: QC Laboratory Director**

1.0 Purpose

The purpose of this procedure is to define the method for the quantification and identification of Lidocaine and Phenoxyethanol in raw materials and finished products by HPLC/UV.

2.0 Scope

The procedure applies to the identification and quantification of Lidocaine and Phenoxyethanol in raw materials and finished products by HPLC/UV, specifically in drug product **DCR00013**.

3.0 Responsibility

3.1 It is the responsibility of QC Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to ensure that this procedure is being followed.

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

4.0 Definitions

4.1 **HPLC/UV** – High Pressure Liquid Chromatography with Ultraviolet Detection

4.2 **QC** – Quality Control

4.3 **CofA** – Certificate of Analysis

4.4 **LDC** – Lidocaine

4.5 **POE** – 2-Phenoxyethanol

4.6 **DMA** – 2,6-Dimethylaniline HCl

4.7 **Euxyl® PE9010** – A preservative blend containing 90% Phenoxyethanol

4.8 **ACN** – Acetonitrile

4.9 **H_3PO_4** – 85% Phosphoric Acid

4.10 **H_2O** – Deionized water

5.0 References

5.1 MV-LAB-19-062 Validation of an Analytical Method for the Determination of Lidocaine by HPLC/UV

6.0 Supplies

6.1 Chemicals: All reagents are HPLC grade or better

6.1.1 LDC Reference Standard

6.1.2 POE Reference Standard

6.1.3 DMA

6.1.4 ACN

6.1.5 H_3PO_4

6.2 Glassware

6.2.1 Volumetric glassware as required for standard and sample preparations

6.3 Equipment

6.3.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.3.2 Analytical Balance

6.3.3 Centrifuge

6.3.4 Adjustable Pipette

7.0 Procedure

7.1 Mobile Phase Preparation

7.1.1 Mobile Phase A

7.1.1.1 Combine 1000 mL of H_2O with 1.0 mL of H_3PO_4

7.1.2 Mobile Phase B

7.1.2.1 ACN

7.1.3 Extraction Solvent

7.1.3.1 Combine 500 mL Water with 500 mL ACN

7.1.4 Diluent

7.1.4.1 Combine 900 mL of MP-A with 100 mL of CAN

7.1.5 Scale all mobile phases, extraction solvents and diluent as needed.

7.2 Standard Preparation

7.2.1 Standard solutions are stable for two days at room temperature.

7.2.2 Dilutions should be prepared using volumetric glassware.

7.2.3 Standard preparation may be scaled up as required. Scaling down is generally not recommended.

7.2.4 LDC Stock: Transfer 50 mg of LDC reference standard (or 61.6 mg of LDC•HCl monohydrate reference standard) into a 50-mL volumetric flask. Dissolve in and dilute to volume with Extraction Solvent.

7.2.5 POE Stock: Only prepare if quantifying POE. Use a pipet to transfer about 112.5 mg of POE reference standard directly into a 250-mL volumetric flask taking care to direct the standard to the bottom of the flask without touching the side. Dissolve in and dilute to volume with Extraction Solvent.

7.2.6 DMA Stock: Transfer 40 mg of DMA to a 25-mL volumetric flask. Dissolve in and dilute to volume with Extraction Solution. The DMA Stock does not expire

and may be kept in the refrigerator until needed. If additional peaks are observed in the chromatogram of the Working Standard, prepare a fresh DMA stock.

7.2.7 Working Standard: Transfer 4.0 mL of LDC Stock, 2.0 mL of POE Stock, and 1.0 mL of DMA Stock into a 100-mL volumetric flask. Dilute to volume with Diluent.

7.3 Sample Preparation

7.3.1 LDC is stable in sample preparations at room temperature for one day. Samples which require analysis of POE should be analyzed within 24 hours of preparation.

7.3.2 Finished Products: For topical semi-solid dosage forms: based on the label claim, transfer no less than 250 mg of the product into a suitably sized volumetric flask of no less than 25 mL to generate a concentration of LDC that is about 0.4 mg/mL and/or a concentration of POE that is about 0.09 mg/mL. Dilute the sample to 2/3 of the flask volume with Extraction Solvent and shake mechanically for 30 min or until the sample is **completely** dispersed. Dilute to volume with Extraction Solvent. Transfer a 5.0 mL aliquot of the completely dispersed sample into a 50-mL volumetric flask and dilute to volume with Diluent. Filter a portion through a 0.45 µm membrane discarding the first 3 mL.

Example: The product contains 4% LDC and 1% Euxyl® PE9010

Euxyl® PE9010 contains 90% POE

Prepare 50 mL of a 0.4 mg/mL LDC and 0.09 mg/mL POE solution

0.4 mg/mL LDC ÷ (4%/100%) × 50 mL = 500 mg

0.09 mg/mL POE ÷ (90%/100%) ÷ (1%/100%) × 50 mL = 500 mg

Dissolve 500 mg of sample in 50 mL of Diluent and filter

7.3.3 Raw Materials: For LDC determination, weigh no less than 40 mg of raw material sample into a suitably sized volumetric flask to generate a concentration of about 0.4 mg/mL Dissolve in and dilute to volume with diluent.

7.3.4 Perform further dilutions as required using diluent.

7.3.5 For finished products or raw materials being analyzed for the first time using this method, an in process validation is required to demonstrate spectral purity, baseline separation of peaks, and extraction efficiency as a part of system suitability before data can be reported using this method.

7.4 HPLC Parameters

7.4.1 Column: Kinetex XB-C18, 5µm, 4.6 mm x 150 mm

7.4.2 Column Temperature: 30 °C

7.4.3 Flow rate: 0.8 mL/min

7.4.4 Wavelength: 220 nm with 4 nm bandwidth

7.4.5 Injection Volume: 5 µL

7.4.6 Run Time: 15 minutes

7.4.7 Spectral Range (for Identification)- 210 nm to 360 nm

7.4.8 Gradient Profile:

Time	%MPA	%MPB
0	90	10
10	10	90
10.1	90	10
15	90	10

7.5 Recommended Sequence

7.5.1 Make at least 2 injections of the diluent.

7.5.2 Make five (5) injections of Standard Solution.

7.5.3 Make a single injection of each Sample Preparation.

7.5.4 Make a single injection of the Standard Solution after every ten (10) sample injections or at the end of a run.

7.6 System Suitability Requirements

7.6.1 The %RSD of the first five (5) standard injections is NMT 2.0%.

7.6.2 The %RSD of all standard injections is NMT 3.0%.

7.6.3 The average (n=5) tailing factor for LDC is NMT 1.5.

7.6.4 The average (n=5) resolution of DMA and LDC is NLT 1.8.

7.6.5 If present, any interference in the diluent should be subtracted out of the sample and standard peak areas.

7.7 Retention Times

7.7.1 DMA: RT = 4.23 min, RRT = 0.914

7.7.2 LDC: RT = 4.63 min, RRT = 1.000

7.7.3 POE: RT = 6.52 min, RRT = 1.408

7.8 Example calculations for determining finished product % label or raw material % purity

7.8.1 $$\% \text{ assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{Spl_{wt}} \times \frac{100}{LA}$$

R_u	Sample peak area
R_s	Mean standard peak area
Wt_{std}	Weight of reference standard in mg (correct for moisture if required)
V_{std}	Volume of the standard preparation accounting for dilutions in mL
P	Purity of the reference standard in percent
Spl_{wt}	Sample weight in mg
V_{spl}	Volume of the sample preparation accounting for dilutions in mL
LA	Label amount in percent (use 1 for raw materials)

7.9 Column Wash and Storage

7.9.1 Rinse the column with H_2O / ACN (80/20)

7.9.2 Store the column in H_2O / ACN (50/50)

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	06/26/19	New	N/A	S. Sassman
1	07/21/22	Scheduled review: updated logo and format.	CC-22-0295	K. Burris