| ION LABS QUALITY FIRST | Standard Operating Procedure<br>**Determination of Ethylene Glycol and Diethylene Glycol by GC-FID** | **SOP Number**<br>**D-1002** | **Revision**<br>**0** |
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| | | **Effective Date**<br>08/14/21 | **Page**<br>**1 of 5** |

| **Written by/ Date** | **Reviewed by/ Date** | **Approved by/ Date** |
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| SAS 06/21/21 | [illegible] 07/23/21 | [illegible] 07/26/21 |
| **Title: Analytical Development Scientist** | **Title: Analytical Development Manager** | **Title: Quality Control Laboratory Director** |

## 1.0 Purpose

1.1 The purpose of this SOP is to define the method for the determination of ethylene glycol and diethylene glycol in raw materials and finished products by GC-FID.

## 2.0 Scope

2.1 This procedure applies to the determination of ethylene glycol and diethylene glycol in raw materials and finished products in the QC Laboratory at Ion Labs.

2.2 This procedure does not apply to the limit of ethylene glycol and diethylene glycol in glycerin, propylene glycol, and sorbitol raw materials.

## 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/Analytical Development to keep SOP aligned with current practices.

## 4.0 Definitions

| | | |
|---|---|---|
| 4.1 | **QC** | Quality control |
| 4.2 | **GC-FID** | Gas chromatography with flame ionization detection |
| 4.3 | **CoA** | Certificate of analysis |
| 4.4 | **EG** | Ethylene glycol |
| 4.5 | **DEG** | Diethylene glycol |
| 4.6 | **TCE** | 2,2,2-trichloroethanol |
| 4.7 | **PTFE** | Polytetrafluoroethylene |

## 5.0 References

5.1 PRTCL-21-0021: Validation of an Analytical Method for the Determination of Ethylene Glycol and Diethylene Glycol by GC-FID

5.2 PRTCL-20-0015: Verification of an Analytical Method for the Limit of Diethylene Glycol and Ethylene Glycol in Glycerin and Propylene Glycol Raw Materials

## 6.0 Supplies

6.1 Chemicals (all chemicals ACS reagent grade or better)

6.1.1 Ethylene Glycol Reference Standard

6.1.2 Diethylene Glycol Reference Standard

6.1.3 TCE

6.1.4 Methanol

6.2 Compressed Gases (use ultra-high purity gases)

6.2.1 Hydrogen

6.2.2 Helium

6.2.3 Air

6.2.4 Nitrogen

6.3 Supplies and Glassware

6.3.1 Volumetric glassware as required for standard and sample preparation

6.3.2 2-mL GC vials with PTFE lined closures

6.3.3 2-mL Eppendorf centrifuge tubes

6.3.4 Pipet Tips

6.4 Equipment

6.4.1 Agilent 7890 GC with FID detector

6.4.2 Analytical Balance

6.4.3 Adjustable Pipet

6.4.4 Centrifuge

## 7.0 GC Conditions

| | | |
|---|---|---|
| 7.1 | Column: | Rxi-624SilMS, 30 m x 0.32 mm x 1.8 µm or equivalent |
| 7.2 | Inlet Liner: | Restek, 4.0 mm ID x 6.3 mm OD x 78.5 mm length straight liner with glass wool or equivalent |
| 7.3 | Inj Temp: | 220 °C |
| 7.4 | Det Temp: | 250 °C |
| 7.5 | Equil Time: | 0.5 min |

7.6 Flow Rate: 2.5 mL/min

7.7 Run Time: 22.4 min

7.8 Split ratio: 10:1

7.9 Septum purge: 3 mL/min

7.10 Air flow: 350 mL/min

7.11 Fuel flow: 30 mL/min

7.12 Makeup flow: 30 mL/min (column + makeup = constant)

7.13 Injection Volume 1 µL

7.14 Injection Type Standard

7.15 Plunger Speed Fast

7.16 Wash Solvent Methanol

7.17 Temperature Ramp:

| Ramp Rate (°C/min) | Temp (°C) | Hold Time (min) |
|---|---|---|
| N/A | 100 | 4 |
| 50 | 120 | 10 |
| 50 | 220 | 6 |

## 8.0 Diluent

8.1 Transfer 65 µL of TCE to a 1-L media bottle.

8.2 Add 1000 mL of methanol

8.3 Mix well.

## 9.0 Stock Standard Preparation

9.1 Transfer 200 ± 2 mg of EG reference standard to a small beaker or vial.

9.2 Transfer 200 ± 2 mg of DEG reference standard to a small beaker or vial.

9.3 Use small portions of *Diluent* to quantitatively transfer the EG and DEG into a 50-mL volumetric flask.

9.4 Dilute to volume with *Diluent* and mix well.

## 10.0 Working Standard Preparation

10.1 Transfer 0.625 mL of *Stock Standard* to a 50-mL volumetric flask.

10.2 Dilute to volume with *Diluent* and mix well.

## 11.0 Sample Preparation

11.1 The validated linear range of the method is 2 – 200 mcg/mL. The sample preparation must be within the linear range.

11.2 To manage large volumes, the sample can be initially dissolved in a smaller volume that is within the solubility range and a portion further diluted to bring the analyte concentration into the linear range of measurement.

11.3 For finished products, pool at least 10 dosage units and homogenize. Based on the dosage weight (for powders), fill weight (for capsules) or tablet weight and the limit level, weigh no less than 20 mg of the pooled dosages into a suitably sized volumetric flask to generate a concentration within the validated linear range, add *Diluent* to about 2/3 of the flask volume and shake for at least 20 minutes. Dilute to volume with *Diluent*, and sonicate for 5 minutes.

11.4 For raw materials, based on the limit level, weigh no less than 20 mg of sample into a suitably sized volumetric flask to generate a concentration within the linear range, add *Diluent* to about 2/3 of the flask volume and shake for at least 20 minutes. Dilute to volume with *Diluent*, and sonicate for 5 minutes.

11.5 If particulates remain in sample preparations, filter through a 0.45 µm membrane, discarding a portion before collecting the sample for analysis. Alternatively, centrifuge to remove particulates.

## 12.0 Recommended Sequence

12.1 Make two injections of Diluent

12.2 Make five injections of Working Standard

12.3 Make a single injection of each Sample Preparation

## 13.0 System Suitability Requirements

13.1 No significant (>0.5%) interfering peaks are present in the blank injection.

13.2 The %RSD of the peak area ratio in five consecutive injections of the Working Standard is NMT 10.0%.

## 14.0 Retention Times

| | | |
|---|---|---|
| 14.1 | EG | 3.4 min |
| 14.2 | TCE | 6.8 min |

14.3 DEG 9.4 min

## 15.0 Example Calculations

$$\text{Assay (\%)} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{Spl_{wt}} \times 100$$

$R_u$ Sample peak area ratio

$R_s$ Mean Working Standard A peak area ratio (5 injections)

$Wt_{std}$ Weight of reference standard used to prepare Working Standard (mg)

$P$ Purity of reference standard from the CoA (% w/w)

$V_{std}$ Volume of Working Standard (mL)

$V_{spl}$ Volume of Sample Solution (mL)

$Spl_{wt}$ Sample weight (mg)

## 16.0 Revision History

| Revision | Date | Description of Changes | CCR # | By |
|---|---|---|---|---|
| 0 | 05/29/20 | New | N/A | S. Sassman |