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A simple and sensitive validated method for quantitation of toxic impurities-Ethylene glycol and Diethylene glycol in Pharmaceutical Ingredient-Sorbitol NF by Gas chromatography

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Abstract

Ethylene glycol (EG) and Diethylene glycol (DEG) are hazardous compounds that can pose significant health risks if present in pharmaceutical products more than permissible limits. This study aims to develop and validate sensitive and accurate gas chromatography (GC) method for the quantification of Ethylene glycol (EG) and Diethylene glycol (DEG) in pharmaceutical ingredients. Calibration curves for EG and DEG were established over a concentration range of LOQ (29 µg/mL) to 800 µg/mL for Ethylene Glycol and LOQ (12 µg/mL) to 220 µg/mL for Diethylene Glycol demonstrating excellent linearity with correlation coefficients (r^2) exceeding 0.995. Sensitivity analyses revealed low limits of detection (LOD) and limits of quantification (LOQ) for both components with GC-FID achieving LODs of 9 µg/mL for EG and 6 µg/mL for DEG. Precision and accuracy assessments showed that the method provided consistent results, with relative standard deviations (% RSD) below 5% and recovery rates ranging from 97% to 100%. Application of the method to various pharmaceutical ingredients such as Sorbitol NF confirmed that all tested samples contained EG and DEG levels below regulatory limits set by the FDA and EMA. The results demonstrated that the developed GC method is precise, accurate, rugged, robust, reliable, and suitable for routine quality control to ensure the safety of pharmaceutical products. These findings underscore the importance of implementing stringent quality control measures to prevent toxic contamination and safeguard public health.

Keywords: Ethylene glycol, Diethylene glycol, Gas chromatography, Pharmaceutical Ingredients, Method Validation, ICH, FDA, Sorbitol NF.

1.0 Introduction

Ethylene glycol (EG) and diethylene glycol (DEG) are two toxic compounds that have garnered significant attention due to their potential for contamination in pharmaceutical products. These compounds are primarily used in industrial applications, including antifreeze, coolants, and solvents. Their presence in pharmaceutical products, however, poses severe health risks, which include renal failure, metabolic acidosis, and neurological damage (Barceloux et al., 1999; Schep et al., 2009). Historical instances of DEG contamination in pharmaceutical products have resulted in numerous fatalities, emphasizing the critical need for reliable detection

and quantification methods to prevent such tragedies.

1.1 Background and Toxicology

The history of pharmaceutical contamination with EG and DEG is marked by several tragic incidents that have highlighted the dire need for stringent quality control measures. One of the most notorious cases occurred in the 1930s in the United States, where the use of DEG as a solvent in an elixir led to the deaths of over 100 people, primarily children. This incident was a pivotal moment in the history of drug regulation, leading to the establishment of the Federal Food, Drug, and Cosmetic Act of 1938, which mandated pre-market safety testing of drugs (Wax, 1995).

More recently, similar incidents have been reported in various parts of the world. In 1990, over 300 children in Haiti died after consuming paracetamol syrup contaminated with DEG (O'Brien et al., 2009). Similar cases were reported in Nigeria in 2008 and in Panama in 2006, where contaminated cough syrups caused numerous fatalities (Schep et al., 2009). These incidents underscore the critical need for continuous monitoring and stringent quality control measures in the pharmaceutical industry to prevent such tragedies.

EG and DEG are both highly toxic when ingested. EG is metabolized in the body to glycolic acid and oxalic acid, which can cause metabolic acidosis, renal failure, and central nervous system depression (Jacobsen & McMartin, 1986). DEG, on the other hand, is metabolized to diglycolic acid, which is particularly nephrotoxic and can lead to severe kidney damage (Schep et al., 2009). The acute toxicity of these compounds necessitates their strict regulation and control in pharmaceutical products.EG and DEG are structurally similar to glycerin and propylene glycol, both of which are commonly used in the pharmaceutical industry as excipients. This structural similarity has led to inadvertent contamination during the manufacturing process. EG and DEG are metabolized in the body to toxic metabolites, including glycolic acid, glyoxylic acid, and oxalic acid, which can cause metabolic acidosis and renal failure (Jacobsen & McMartin, 1986).

Ingestion of EG leads to symptoms that progress from inebriation to metabolic acidosis and renal failure. DEG has a similar toxicity profile but is even more nephrotoxic than EG. Cases of DEG poisoning have been reported globally, often associated with contaminated pharmaceuticals (O'Brien et al., 2009; McGeehin et al., 1998).

1.2 Regulatory Standards

To mitigate the risks associated with EG and DEG contamination, regulatory bodies such as the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have established guidelines and permissible limits for these contaminants in pharmaceutical products. According to the International Council for Harmonization (ICH) guideline Q3C, the permissible limit for DEG in pharmaceutical products is set at 0.2% (2000 µg/mL) (FDA, 2020; EMA, 2018). These guidelines necessitate the development and implementation of precise analytical methods to ensure that pharmaceutical products comply with safety standards.

In this study, the development and validation of analytical method for the detection and quantification of Ethylene glycol (EG) and Diethylene glycol (DEG) in pharmaceutical ingredients were conducted in accordance with the International Council for Harmonization (ICH) guidelines and the United States Pharmacopeia (USP) standards. Emphasizing these guidelines ensures that the methods are robust, reliable, and compliant with international regulatory requirements.

The ICH guidelines provide a comprehensive framework for the validation of analytical methods. Specifically, ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology was rigorously followed. The methods were tested for specificity to ensure their ability to unequivocally assess EG and DEG in the presence of other components, such as excipients and potential reagents. Calibration curves were established over a wide

concentration range (12 to 800 µg/mL), demonstrating strong linear relationships with correlation coefficients (r^2) exceeding 0.995 for both EG and DEG, which verifies the methods' linearity. Accuracy was evaluated through recovery rates for EG and DEG, which ranged from 97% to 100%, indicating high accuracy. Precision was assessed by evaluating intra-day and inter-day precision, with the percent relative standard deviation (% RSD) consistently below 3%, confirming the reproducibility of the method. The limits of detection (LOD) and quantitation (LOQ) for developed Gas chromatography (GC) method was determined. Also, demonstrated the methods' sensitivity in detecting trace amounts of EG and DEG. Additionally, the robustness of the method was assessed by changing small and deliberate variations in method parameters and observed the effect on suitability and results.

The United States Pharmacopeia (USP) provides specific methods and acceptance criteria for the analysis of contaminants in pharmaceutical products. Relevant USP chapters and sections referenced in this study include USP <467> Organic Volatile Impurities / Residual Solvents, which specifies limits for residual solvents, including methods for detecting and quantifying organic volatile impurities and other toxic impurities such as EG and DEG. The methods developed in this study adhere to the guidelines outlined in this chapter, ensuring compliance with USP standards. Acceptance criteria were also met, as the concentration of EG and DEG in pharmaceutical samples was compared against the permissible limits specified by the USP, with all samples found to be within these limits. Additionally, USP <621> Chromatography provides guidelines for chromatographic methods, including system suitability, calibration, and validation requirements. The method developed in this study complies with these guidelines, ensuring accurate and reliable chromatographic analysis. Adherence to ICH guidelines and USP standards ensures that the analytical method developed in this study is validated according to international regulatory expectations. This compliance is crucial for several reasons. Regulatory approval for pharmaceutical products requires manufacturers to demonstrate that their products meet stringent safety and quality standards, and validated methods according to ICH and USP guidelines are essential for this approval. Consistent application of validated methods ensures the reliability and accuracy of results, contributing to the overall quality assurance process in pharmaceutical manufacturing. By adhering to these guidelines, the methods ensure that pharmaceutical products are free from harmful levels of contaminants, thereby protecting consumer health.

The rigorous development and validation of the GC method for EG and DEG analysis, following ICH guidelines and USP standards, underscores the robustness and reliability of this method. The study highlights the importance of compliance with international regulatory frameworks to ensure the safety and quality of pharmaceutical products. Implementing this validated method in routine quality control will help prevent toxic contaminations and safeguard public health.

The primary objective of this research is to develop and validate sensitive and accurate analytical method for the detection and quantification of Ethylene glycol (EG) and Diethylene glycol (DEG) in pharmaceutical ingredients using Gas chromatography (GC). This method aim to ensure compliance with the guidelines and permissible limits set by regulatory bodies such as the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for EG and DEG in pharmaceutical products.

A key focus of the study is to assess the specificity, precision, sensitivity and accuracy of the GC method in detecting and quantifying low levels of EG and DEG in pharmaceutical ingredient such as Sorbitol NF. This involves constructing calibration curves for EG and DEG, establishing their linearity over a wide concentration range, and determining the limits of detection (LOD) and quantification (LOQ) for both compounds. By doing so, we aim to ensure that the method is robust and reliable for routine analysis in quality control laboratories.

Another significant objective of this research is to highlight the importance of stringent quality control measures

in the pharmaceutical industry. By emphasizing continuous monitoring and stringent quality assurance practices, we aim to mitigate the risks associated with EG and DEG contamination, thereby enhancing the safety and efficacy of pharmaceutical products. Ultimately, this research aims to contribute to public health safety by providing reliable analytical techniques that can be used in quality control laboratories to monitor and prevent the presence of toxic contaminants like EG and DEG in pharmaceutical products.

1.3 Chemical Information of impurities (Ethylene Glycol and Diethylene Glycol)

1.3.1 Name: Ethylene Glycol (EG)

1.3.1.1 Chemical Name and Structure

Chemical Names: Ethane-1,2-diol; 1,2-ethanediol
Chemical Structure:

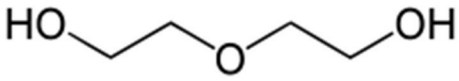
1.3.1.2 Molecular Formula and Molecular Weight

Molecular Formula: $C_2H_6O_2$
Molecular Weight: 62.07 g/mol

1.3.2 Name: Diethylene Glycol (DEG)

1.3.2.1 Chemical Name and Structure

Chemical Names: 2,2′-Oxydiethanol; Ethylene diglycol; Diglycol.
Chemical Structure:



1.3.2.2 Molecular Formula and Molecular Weight

Molecular Formula: $C_4H_{10}O_3$
Molecular Weight: 106.12 g/mol

1.4 Chemical Information of Pharmaceutical ingredient/excipient

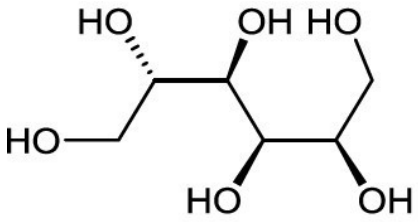
1.4.1 Name

Sorbitol Solution Non-Crystallizing, NF

1.4.2 Chemical Name and Structure

1.4.3 Chemical Names: (2R,3R,4R,5S)-hexane-1,2,3,4,5,6-hexol

Chemical Structure: Structure of Sorbitol



1.4.4 Molecular Formula and Molecular Weight

Molecular Formula : $C_6H_{14}O_6$
Molecular Weight : 182.17 g/mol.

2.0 Methodology

2.1 Chemicals and Reagents

Component Name	Source	Batch /Lot No.	Potency/Purity
Diethylene Glycol RS	Sigma-Aldrich	LRAC0277	99.8%
Ethylene Glycol RS	Sigma-Aldrich	LRAC2089	99.9%
2,2,2-Trichloroethanol (Internal standard)	Sigma-Aldrich	STBJ9934	99.9%
Sorbitol NF	Ingredion	7659090204	N/A

2.2 Instrumentation

The quantitative analysis of EG and DEG was performed using Gas chromatography (GC). The GC system used was an Agilent 6890N (Agilent Technologies) equipped with a flame ionization detector (FID).

2.3 Chromatographic Conditions (GC Parameters)

The GC analysis was performed using an Agilent DB-624 capillary column (30 m x 0.53 mm, 3 µm film thickness, Equivalent to USP G-43 stationary phase). The carrier gas was helium, with a flow rate of 4.0 ml/min. The injector temperature was set to 220°C, and the detector temperature was set to 230°C. The oven temperature program was as follows: an initial temperature of 100°C, hold for 4 minutes. Increased to 120° C with a rate of 50°C/min and hold for 10 minutes at 120°C. Followed by an increase to 220°C at a rate of 50°C/min, and hold for 6 minutes at 220°C. The injection volume was 4.0 µL, and the split ratio was 1:2.

2.4 Preparations

2.4.1 Diluent-1 Preparation

Methanol

2.4.2 Diluent-2 (Internal Standard)

Weighed accurately about 78 mg of 2,2,2-Trichloroethanol into 100 mL volumetric flask containing about 40 mL of Diluent-1. Diluted to volume with Diluent-1 and mix well. Pipetted out 1.0 mL of above solution into 100 mL volumetric flask. Diluted to volume with Diluent-1 and mixed well.

2.4.3 Standard Preparation

Preparation of Stock Solution:

Weighed accurately about 50 mg of Diethylene Glycol RS and 120 mg of Ethylene Glycol RS into a 100-mL volumetric flask containing about 40 mL of Diluent-1. Diluted to volume with Diluent-1 and mixed well.

Preparation of Intermediate Standard Solution:

Pipetted out 2.0 mL of Stock Solution into a 100-mL volumetric flask. Diluted to volume with Diluent-1 and mixed well.

Preparation of Standard Solution:

Pipetted out 7.0 mL of Intermediate Standard Solution into a 25-mL volumetric flask. Diluted to volume with Diluent-2 and mixed well (Concentration of about 28 ppm of Diethylene Glycol and 67 ppm of Ethylene Glycol with respect to concentration of sample preparation).

2.4.4 Sample Preparation

Weighed accurately and transferred about 2.5g of Sorbitol NF sample into 50 mL volumetric flask. Pipetted out 25.0 mL of Diluent-2 into the same volumetric flask and vortex for 1 minute. (Do not make up to the volume). Filtered the supernatant layer using 0.45 µm Nylon filter by discarding first two (2) mL of the filtrate. Transferred supernatant layer into liquid injection GC vial for injection.

3.0 Method Validation

3.1. System Precision

A standard solution was prepared as per the method and injected. Percent relative standard deviation for peak areas of Diethylene Glycol and Ethylene Glycol from six (6)-replicate injections of the standard solution was calculated and reported.
The % RSD of six (6) replicate injections of standard peak response of Ethylene glycol and Diethylene glycol observed to be 1.9 and 2.9 respectively, which demonstrates the method is precise and consistent. USP Tailing for Ethylene Glycol and Diethylene Glycol standard solution is found to be less than 1.5, except one reading [Table-1].

3.2 Sensitivity and Detection Limits

Serially diluted Ethylene Glycol and Diethylene Glycol to lower levels and determined the Limit of detection (LOD) and Limit of Quantitation (LOQ) values by signal to noise ratio method. The signal to noise (S/N) ratio

for LOD should be NLT 3 and for LOQ should be NLT 10.
The obtained LOD and LOQ values demonstrated that the method is highly sensitive for the determination of Ethylene Glycol and Diethylene Glycol [Table-2].

3.3 Precision at LOQ Level

Six (6) replicates of LOQ solution preparation were injected into GC system. The %RSD for areas of Ethylene Glycol and Diethylene Glycol from six (6)-replicate injections of the LOQ solution were calculated. The %RSD for peak responses of Ethylene Glycol and Diethylene Glycol from six (6)-replicate injections of LOQ preparation should be NMT 10.0%.
The %RSD for peak response of Ethylene Glycol and Diethylene Glycol from six (6) replicate injections of LOQ preparation met the acceptance criteria of not more than 10.0% and hence the method is precise at LOQ level [Table-3].

3.4 Linearity and Range

Calibration curves for EG and DEG were constructed by plotting the peak response against the concentration of the analyte solutions. Solutions of Diethylene Glycol and Ethylene Glycol at varying concentrations ranging from LOQ to 1200% for Ethylene Glycol and LOQ to 800% for Diethylene Glycol were injected into Gas chromatograph system. The linearity graph was plotted as amount versus peak response. The correlation coefficients (r^2) for both compounds were found more than 0.995. The linear regression data shows that the method is linear over the entire concentration range of Ethylene Glycol and Diethylene Glycol and it is adequate for its intended concentration range. The high correlation coefficients indicate excellent linearity, suggesting that the methods are reliable for quantifying these compounds over a wide concentration range. [Table 4, Figure 2 and Table 5, Figure 3].

3.5. Method Precision

Precision of the method was determined by injecting, six (6)-individual sample solutions of Sorbitol solution by spiking Diethylene Glycol at about specification level. The samples were prepared as per the method.Calculate the content of Diethylene Glycol and Ethylene Glycol in method precision sample. The relative standard deviation (RSD) for the results from six (6) sample solutions met the acceptance criteria of NMT 5.0% and hence, the method is precise [Table 6]. Typical chromatograms [Figure-4,5 and 6].

3.6 Intermediate Precision (Ruggedness)

Intermediate Precision of the method was determined by injecting, six (6)-individual sample solutions Sorbitol solution by spiking Diethylene Glycol at about specification level by a second analyst on a different day. The samples were prepared as per the method.
Calculated the content of Diethylene Glycol and Ethylene Glycol in Intermediate Precision sample. The percent relative standard deviation (%RSD) for the results from six (6) sample solutions found within the acceptance criteria of not more than 10.0%. The difference between method precision and intermediate precision results was found within the acceptance criteria of not be more than 10.0% [Table 7,8]. Hence, method is precise and rugged.

3.7 Method Accuracy

The recovery was performed by spiking varying amounts of Ethylene Glycol and Diethylene Glycol. The samples were prepared as per the method and injected. % Recovery found within acceptance criteria of between 75% and 125%. The overall %RSD for all determinations was found within 10.0% [Table 9,10]. Hence the method is accurate.

3.8 Specificity

Blank and standard solutions of Ethylene Glycol, and Diethylene Glycol prepared and injected into the chromatographic system for identification and to check the interference of diluent with the Diethylene Glycol and Ethylene Glycol peaks. No interference observed from diluent. All solvents were well separated from each other [Table 11].

3.9 Robustness

Variation in important chromatographic parameters such as column oven temperature ± 5°C (Procedural temperature 100°C), carrier gas flow ±0.5 ml/min (Procedural flow 4 mL/min and inject six (6)-replicates of standard preparation for each parameter and compared the system suitability. The percent RSD for solvent peak response from six (6)-replicate injections of standard solution was found less than 10.0% and met the system suitability. No significant change observed in system suitability with deliberate changes over column temperature, Carrier gas flow [Table 12,13,14,15 and 16]. Hence the method is robust.

3.10 Filter Study

The sample was filtered by discarding 0mL, 2 mL, 4 mL, 6 mL and 8 mL of the filtrate by using 0.45µm Nylon filter and calculated the content. The difference in the content of Ethylene glycol and Diethylene glycol results from the filtered sample solutions are less than 10.0% for fractions beyond the volume to be discarded [Table 17]. Based on filter study data, it is concluded that samples should be filtered through 0.45µm Nylon filter after discarding the first 2mL of filtrate.

4.0 Analysis of Pharmaceutical Samples

The validated GC method was applied to the analysis of various pharmaceutical products used Sorbitol NF as Excipient in product formulation. The concentrations of EG and DEG in the samples were quantified based on internal standard method, and the results were compared with the permissible limits set by regulatory bodies.

4.1 Data Analysis

All data were processed and analyzed using Waters Empower-3 software. The results were presented as mean standard deviation (SD), percent standard deviation (% RSD) and recoveries.

5.0 Results and Summary

5.1 System Precision

Table 1:System Precision

Component Summary For Response

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.980116	0.782789
2	Standard	2	1.918155	0.756823
3	Standard	3	1.921219	0.796848
4	Standard	4	1.945698	0.800705
5	Standard	5	1.872856	0.758961
6	Standard	6	1.953535	0.746482
Mean			1.931930	0.773768
% RSD			1.9	2.9

Component Summary For USP Tailing

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.2	1.2
2	Standard	2	1.2	1.2
3	Standard	3	1.2	1.2
4	Standard	4	1.2	1.2
5	Standard	5	1.2	1.2
6	Standard	6	1.6	1.1

5.2 Sensitivity and Detection Limits

Table 2: LOD and LOQ values

Name of the Component	LOD			LOQ		
	Amount (ppm)	Amount (%)	S/N	Amount (ppm)	Amount (%)	S/N
Ethylene Glycol	9	0.0015	5	29	0.0029	14

Diethylene Glycol	6	0.0006	7	12	0.0012	15

5.3 Precision at LOQ Level

Table 3: Precision at LOQ Level

Component Summary For Response

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	LOQ Precision	1	0.592180	0.262365
2	LOQ Precision	2	0.570308	0.263902
3	LOQ Precision	3	0.566909	0.233381
4	LOQ Precision	4	0.596543	0.261320
5	LOQ Precision	5	0.570231	0.247216
6	LOQ Precision	6	0.578450	0.241613
Mean			0.579103	0.251633
% RSD			2.2	5.1

5.4 Linearity and Range

Table 4: Linearity data for Ethylene Glycol

S.No.	Sample Name	Response	Amount (ppm)
1	LOQ - Linearity	0.562475	29.1812
2	55% - Linearity	0.845452	38.9083
3	85% - Linearity	1.544856	58.3624
4	100% - Linearity	1.765341	68.0894
5	200% - Linearity	3.223287	155.6330
6	1200% - Linearity	18.993983	778.1651

Figure 2: Linearity Plot for Ethylene Glycol

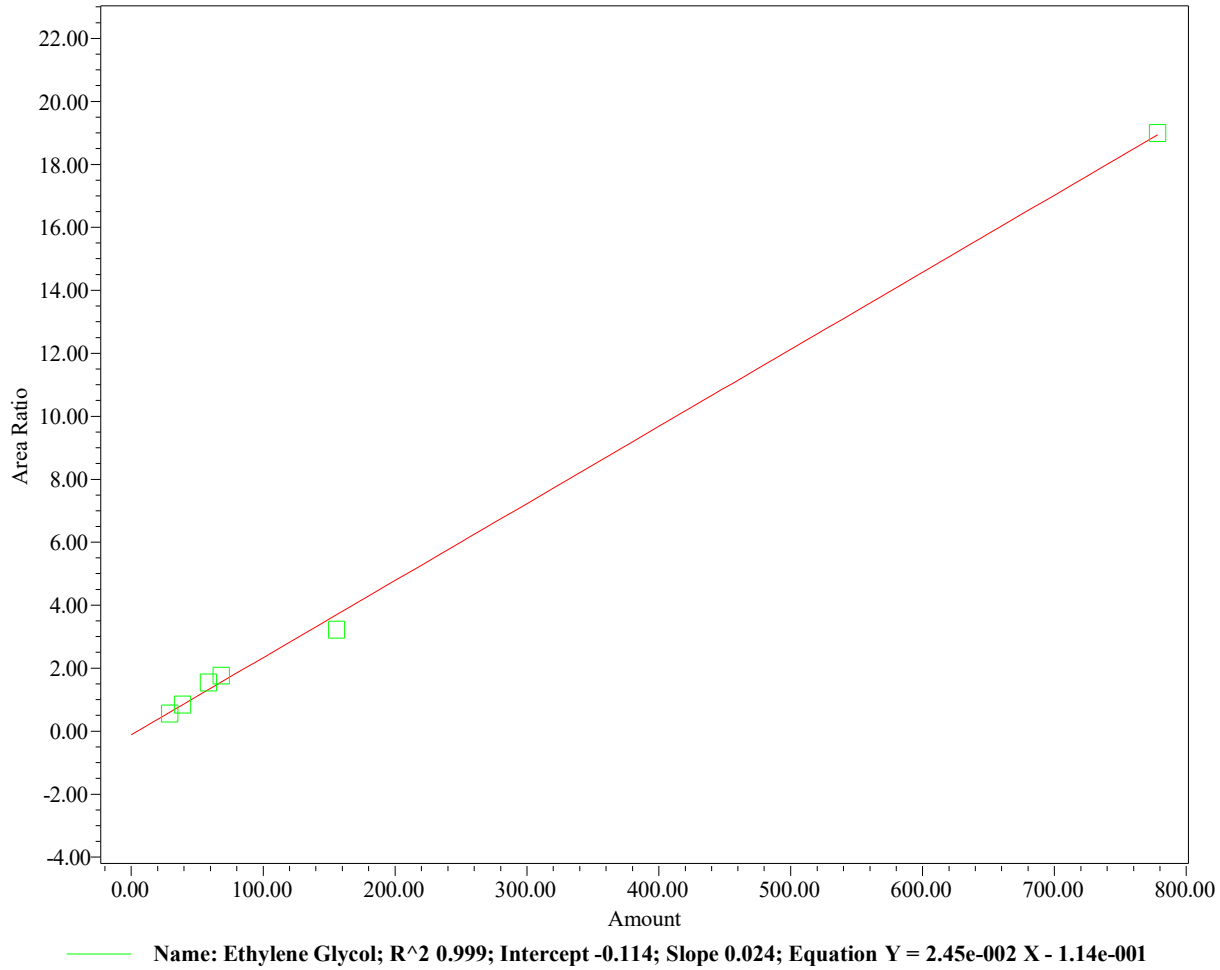
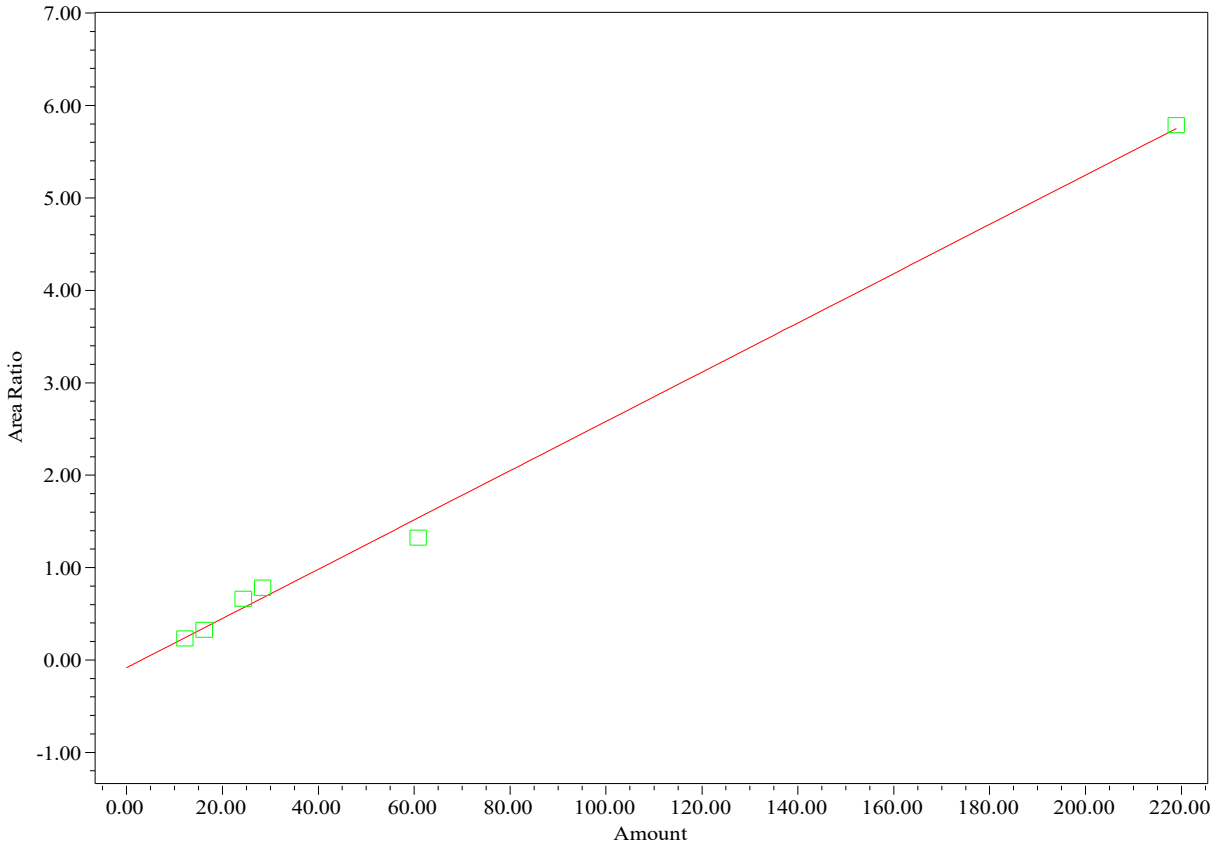


Table 5: Linearity data for Diethylene Glycol

S.No.	Sample Name	Response	Amount (ppm)
1	LOQ - Linearity	0.232656	12.1628
2	55% - Linearity	0.327352	16.2171
3	85% - Linearity	0.662225	24.3257
4	100% - Linearity	0.781162	28.3799
5	200% - Linearity	1.324238	60.8141
6	800% - Linearity	5.787757	218.9309

Figure 3: Linearity Plot for Diethylene Glycol



Component Name	**Correlation Coefficient Square** (r^2)
Ethylene Glycol	0.999
Diethylene Glycol	0.997

5.5 Method Precision

Table 2:Method Precision

Component Summary For Residual_solvent_PPM

	SampleName	Ethylene Glycol	Diethylene Glycol
1	Method Precision-1	85	34
2	Method Precision-2	86	36
3	Method Precision-3	87	38
4	Method Precision-4	85	35
5	Method Precision-5	84	35
6	Method Precision-6	88	35
Mean		86	35
% RSD		1.7	3.5

Figure 1:Typical Chromatogram of Blank (Diluent):

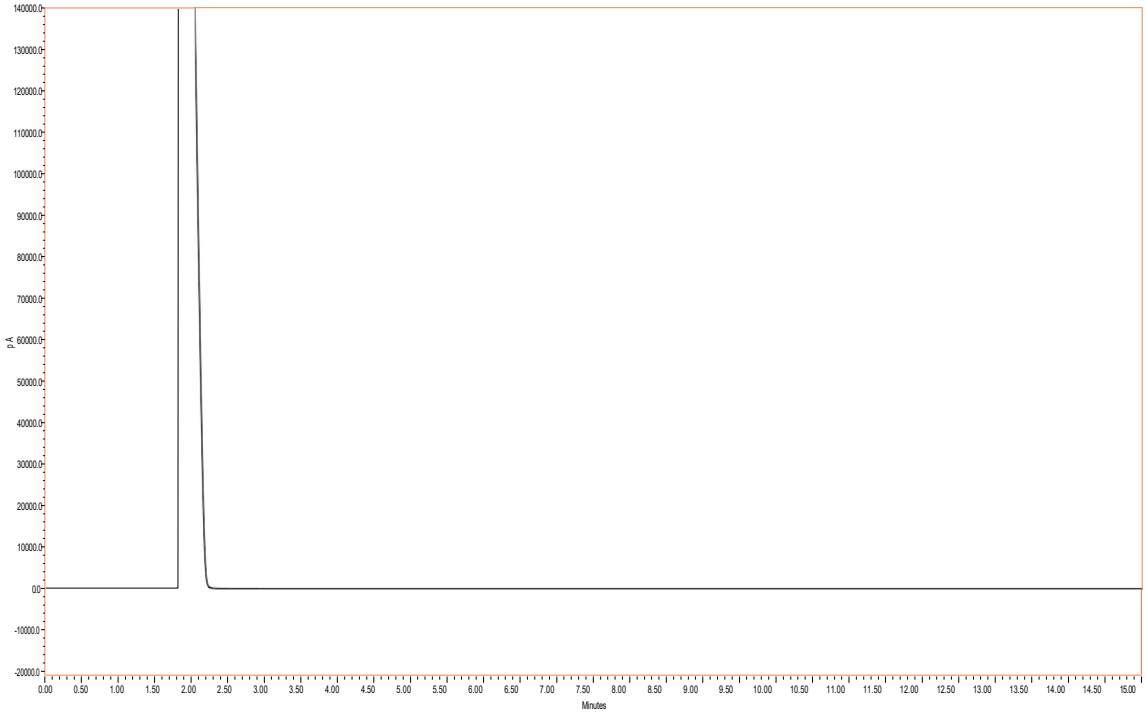
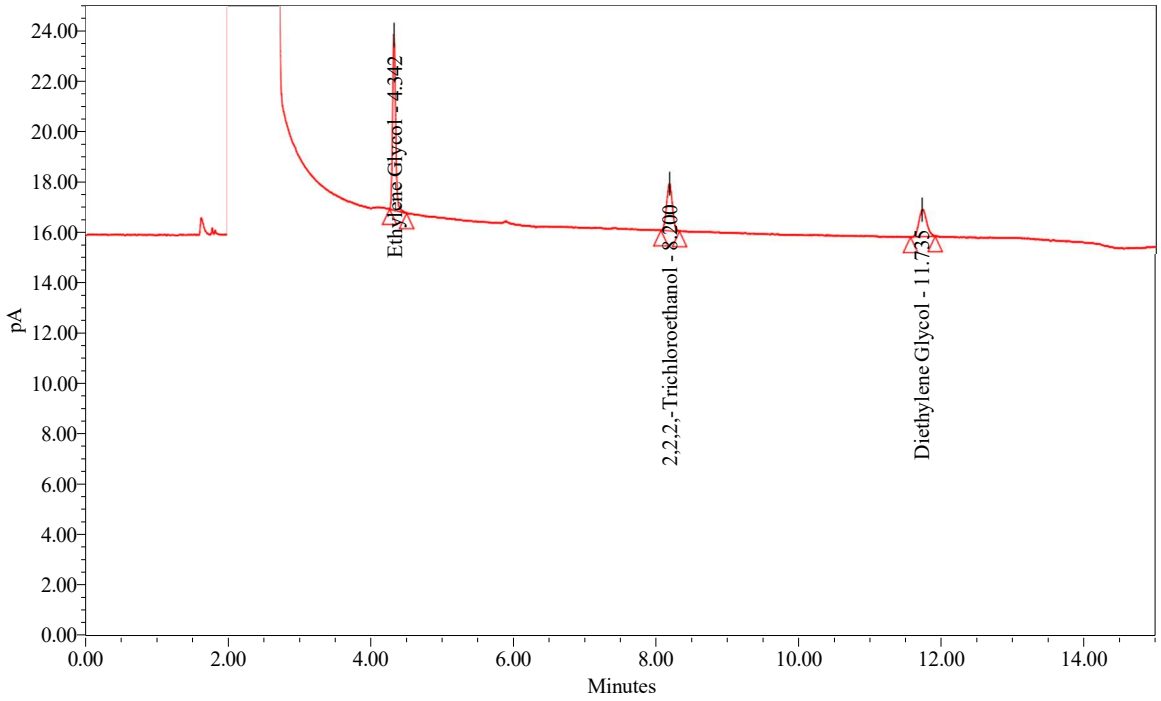
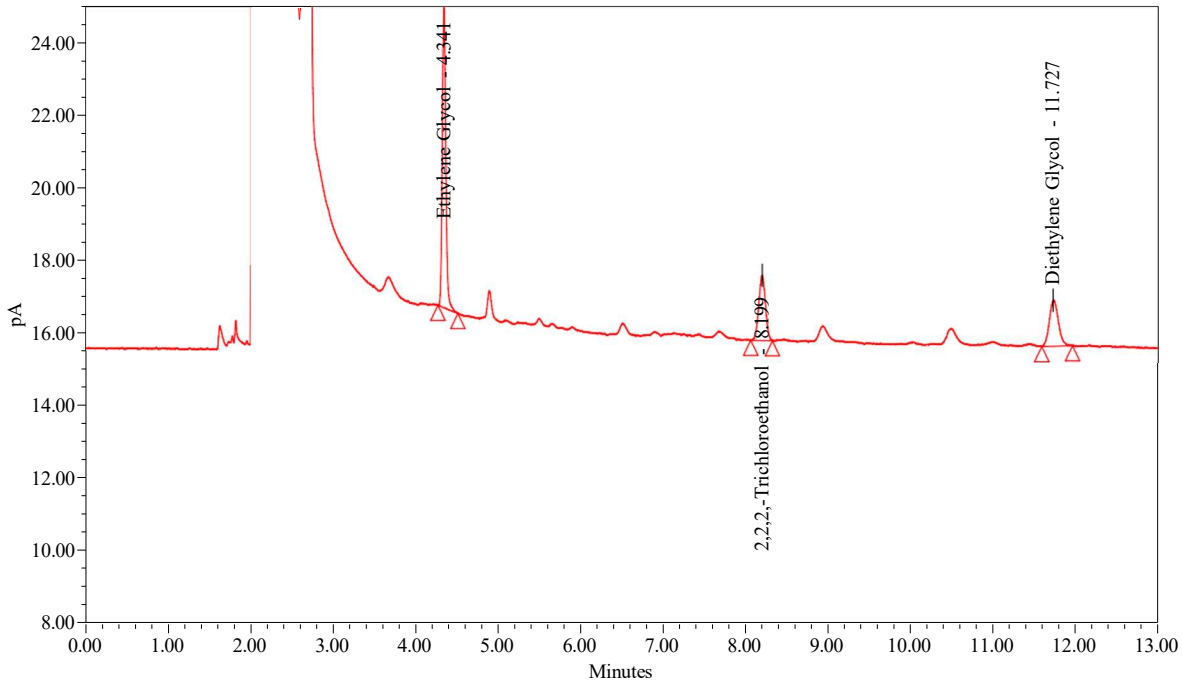


Figure 2: Typical Chromatogram of Standard:



Sample Name Standard; Vial 3; Injection 1; Channel HP6890 Ch1; Date Acquired 6/9/2021 12:10:38 PM EDT; Date Processed 6/11/2021 11:11:25 AM EDT; Sample Set Name 060921_SORBITO_EG_DEG_GC01_REC

Figure 6: Typical Chromatogram of Sample (Method Precision-Spiked)



Sample Name Method Precision-1; Vial 10; Injection 1; Channel HP6890 Ch1; Date Acquired 6/9/2021 9:58:56 PM EDT; Date Processed 6/11/2021 11:11:28 AM EDT; Sample Set Name 060921_SORBITO_EG_DEG_GC01_REC

5.6 Intermediate Precision (Ruggedness)

Table 3: Method Precision (Analyst-1 on Day-1)

Component Summary For Residual_solvent_PPM

	SampleName	Ethylene Glycol	Diethylene Glycol
1	Method Precision-1	85	34
2	Method Precision-2	86	36
3	Method Precision-3	87	38
4	Method Precision-4	85	35
5	Method Precision-5	84	35
6	Method Precision-6	88	35
Mean		86	35
% RSD		1.7	3.5

Table 4: Intermediate Precision (Analyst-2 on Day-2)

Component Summary For Residual_solvent_PPM

	SampleName	Ethylene glycol	Diethylene glycol
1	Intermediate Precision -1	86	33
2	Intermediate Precision -2	86	34
3	Intermediate Precision -3	84	33
4	Intermediate Precision -4	89	34
5	Intermediate Precision -5	92	36
6	Intermediate Precision -6	89	36
Mean		88	34
% RSD		3.0	3.4

Name	Content (ppm)		%Difference in content	
	Ethylene Glycol	Diethylene Glycol	Ethylene Glycol	Diethylene Glycol
Analyst-1	86	35	2.3	2.9
Analyst-2	88	34		

5.7 Method Accuracy

Table 5: Recovery Study of Ethylene Glycol

Amount_ Added (ppm): 29.1812

	Sample Name	Area	Amount_ Added (ppm)	Corr_Amt_ Found (ppm)	% Recovery
1	LOQ Rec-1	15.139444	29.1812	25.5716	88
2	LOQ Rec-2	16.192942	29.1812	24.0722	82
3	LOQ Rec-3	13.335609	29.1812	24.0393	82
Mean					84
% RSD					3.6

Amount_ Added (ppm): 68.0894

	Sample Name	Area	Amount_ Added (ppm)	Corr_Amt_ Found (ppm)	% Recovery
1	Method Precision-1	24.863287	68.0894	69.8400	103
2	Method Precision-2	26.140794	68.0894	71.4613	105
3	Method Precision-3	26.220630	68.0894	72.4600	106
4	Method Precision-4	31.140272	68.0894	69.8335	103
5	Method Precision-5	25.642933	68.0894	69.3136	102
6	Method Precision-6	27.186715	68.0894	72.8315	107
Mean					104
% RSD					2.1

Amount_ Added (ppm): 778.1651

	Sample Name	Area	Amount_ Added (ppm)	Corr_Amt_ Found (ppm)	% Recovery
1	Rec EG 800 ppm & DEG 200 ppm -1	237.104716	778.1651	639.9642	82
2	Rec EG 800 ppm & DEG 200 ppm -2	213.092105	778.1651	651.6706	84
3	Rec EG 800 ppm & DEG 200 ppm -3	242.560322	778.1651	636.8417	82
Mean					83
% RSD					1.2

Table 6: Recovery Study of Diethylene Glycol

Amount_ Added (ppm): 12.1628

	Sample Name	Area	Amount_ Added (ppm)	Corr_Amt_ Found (ppm)	% Recovery
1	LOQ Rec-1	4.501646	12.1628	12.4890	103
2	LOQ Rec-2	4.970612	12.1628	12.4139	102
3	LOQ Rec-3	3.832554	12.1628	11.6127	95
Mean					100
% RSD					4.0

Amount_ Added (ppm): 28.3799

	Sample Name	Area	Amount_ Added (ppm)	Corr_Amt_ Found (ppm)	% Recovery
1	Method Precision-1	9.688422	28.3799	34.3182	121
2	Method Precision-2	10.340832	28.3799	35.5065	125
3	Method Precision-3	10.876820	28.3799	37.6643	133
4	Method Precision-4	12.305994	28.3799	34.8009	123
5	Method Precision-5	10.178035	28.3799	34.7389	122
6	Method Precision-6	10.302057	28.3799	34.5528	122
Mean					124
% RSD					3.5

Amount_ Added (ppm): 218.9309

	Sample Name	Area	Amount_ Added (ppm)	Corr_Amt_ Found (ppm)	% Recovery
1	Rec EG 800 ppm & DEG 200 ppm -1	82.444583	218.9309	236.9253	108
2	Rec EG 800 ppm & DEG 200 ppm -2	70.929913	218.9309	230.8595	105
3	Rec EG 800 ppm & DEG 200 ppm -3	81.103016	218.9309	226.7413	104
Mean					106
% RSD					2.2

Parameter	Ethylene Glycol	Diethylene Glycol
Overall % Recovery	94	114
Overall % RSD	11.8	10.5

5.8 Specificity

No interference observed from diluent. All components were well separated from each other.

Table 11

Name of the Component	Retention time (RT)
Diluent (Methanol)	About 2 minutes
2,2,2-Trichloroethane (Internal standard)	About 8 minutes
Ethylene Glycol	About 4 minutes
Diethylene Glycol	About 11 minutes

5.9 Robustness

Table 12: Robustness Study-Normal Condition

Component Summary For Response

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.980116	0.782789
2	Standard	2	1.918155	0.756823
3	Standard	3	1.921219	0.796848
4	Standard	4	1.945698	0.800705
5	Standard	5	1.872856	0.758961
6	Standard	6	1.953535	0.746482
Mean			1.931930	0.773768
% RSD			1.9	2.9

Component Summary For USP Tailing

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.2	1.2
2	Standard	2	1.2	1.2
3	Standard	3	1.2	1.2
4	Standard	4	1.2	1.2
5	Standard	5	1.2	1.2
6	Standard	6	1.6	1.1

Table 13: Robustness Study-Column Oven Temperature Minus (95°C)

Component Summary For Response

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.902174	0.783642
2	Standard	2	1.877308	0.767861
3	Standard	3	1.860179	0.730552
4	Standard	4	1.807873	0.684101
5	Standard	5	1.850560	0.719100
6	Standard	6	1.829186	0.682985
Mean			1.854547	0.728040
% RSD			1.8	5.7

Component Summary For USP Tailing

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.3	1.2
2	Standard	2	1.2	1.2
3	Standard	3	1.3	1.2
4	Standard	4	1.3	1.2
5	Standard	5	1.4	1.2
6	Standard	6	1.3	1.1

Table 147: Robustness Study-Column Oven Temperature Plus (105°C)

Component Summary For Response

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.821469	0.752346
2	Standard	2	1.746745	0.701884
3	Standard	3	1.860178	0.795177
4	Standard	4	1.818227	0.728576
5	Standard	5	1.709831	0.698760
6	Standard	6	1.783301	0.764071
Mean			1.789958	0.740136
% RSD			3.1	5.1

Component Summary For USP Tailing

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.2	1.2
2	Standard	2	1.3	1.1
3	Standard	3	1.3	1.1
4	Standard	4	1.2	1.2
5	Standard	5	1.3	1.2
6	Standard	6	1.2	1.2

Table 15: Robustness Study-Carrier gas flow Minus (3.5mL/min)

Component Summary For Response

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.760667	0.670346
2	Standard	2	1.770861	0.702436
3	Standard	3	1.698694	0.660267
4	Standard	4	1.750110	0.749128
5	Standard	5	1.729831	0.693187
6	Standard	6	1.738024	0.720917
Mean			1.741365	0.699380
% RSD			1.5	4.7

Component Summary For USP Tailing

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.2	1.1
2	Standard	2	1.2	1.1
3	Standard	3	1.2	1.1
4	Standard	4	1.2	1.1
5	Standard	5	1.2	1.1
6	Standard	6	1.2	1.2

Table 8: Robustness Study-Carrier gas flow Plus (4.5mL/min)

Component Summary For Response

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.716653	0.689379
2	Standard	2	1.751596	0.741199
3	Standard	3	1.693763	0.723951
4	Standard	4	1.783645	0.751434
5	Standard	5	1.769832	0.754596
6	Standard	6	1.796475	0.739615
Mean			1.751994	0.733363
% RSD			2.3	3.3

Component Summary For USP Tailing

	SampleName	Inj. No.	Ethylene Glycol	Diethylene Glycol
1	Standard	1	1.2	1.2
2	Standard	2	1.2	1.1
3	Standard	3	1.2	1.1
4	Standard	4	1.2	1.2
5	Standard	5	1.2	1.1
6	Standard	6	1.2	1.1

5.10 Filter Study

Table 17: Filter Study with 0.45µm Nylon filter

S.No.	Sample Name	% Difference	
		EG	DEG
1	Centrifuge	Not applicable	Not applicable
2	0.45µm Nylon-0mL discard	3.4	2.9
3	0.45µm Nylon-2mL discard	1.1	0.0
4	0.45µm Nylon-4mL discard	1.1	2.9
5	0.45µm Nylon-6mL discard	1.1	8.8
6	0.45µm Nylon-8mL discard	2.3	8.8

6.0 Summary of Results

<table>
<tr><th>Validation Parameter and Acceptance Criteria</th><th>Summary of Result</th></tr>
<tr><td>System Precision The percent relative standard deviation (%RSD) for the peak area from six (6) replicate injections of Ethylene Glycol and Diethylene Glycol standard solution should be NMT 5.0. USP Tailing for</td><td><table><tr><th>Name</th><th>%RSD</th><th>USP tailing</th></tr><tr><td>Ethylene Glycol</td><td>1.9</td><td>1.2</td></tr><tr><td>Diethylene Glycol</td><td>2.9</td><td>1.2</td></tr></table></td></tr>
</table>

<table>
<tr><th>Validation Parameter and Acceptance Criteria</th><th>Summary of Result</th></tr>
<tr><td>Ethylene Glycol and Diethylene Glycol standard solution should be NMT 2.0</td><td></td></tr>
<tr><td>Linearity and Range
The correlation coefficient square (r^2) should be not less than (NLT) 0.99.</td><td>
<table>
<tr><th>Name</th><th colspan="2">Concentration (ppm)</th><th>r^2</th></tr>
<tr><td>Ethylene Glycol</td><td></td><td></td><td></td></tr>
<tr><td>Diethylene Glycol</td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>Limit of Quantitation (LOQ) and Limit of</td><td>
<table>
<tr><th rowspan="2"></th><th colspan="4">Concentration</th><th colspan="2">S/N</th></tr>
<tr><th colspan="2">LOD</th><th colspan="2">LOQ</th><th rowspan="2">LOD</th><th rowspan="2">LOQ</th></tr>
<tr><th>(ppm)</th><th>(%)</th><th>(ppm)</th><th>(%)</th></tr>
<tr><td>EG</td><td>9</td><td>0.0015</td><td>29</td><td>0.0029</td><td>5</td><td>14</td></tr>
<tr><td>DEG</td><td>6</td><td>0.0006</td><td>12</td><td>0.0012</td><td>7</td><td>15</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Validation Parameter and Acceptance Criteria</th><th>Summary of Result</th></tr>
<tr><td>Detection (LOD)

S/N for LOD should be not less than 3 and for LOQ should be not less than 10.</td><td></td></tr>
<tr><td>Precision at LOQ level The % RSD for response from replicate injections for LOQ should be NMT 10.0.</td><td><table><tr><th>Name</th><th>% RSD</th></tr><tr><td>Ethylene Glycol</td><td>2.2</td></tr><tr><td>Diethylene Glycol</td><td>5.1</td></tr></table></td></tr>
</table>

<table>
<tr><th>Validation Parameter and Acceptance Criteria</th><th>Summary of Result</th></tr>
<tr><td>Method Precision
a. Calculate the content of Diethylene Glycol and Ethylene Glycol in method precision sample.
b. The percent relative standard deviation (% RSD) for the results from six (6) sample solutions should be NMT 5.0.</td><td><table><tr><th>Name</th><th>Content (ppm)</th><th>% RSD</th></tr><tr><td>Ethylene Glycol</td><td>86</td><td>1.7</td></tr><tr><td>Diethylene Glycol</td><td>35</td><td>3.5</td></tr></table></td></tr>
<tr><td>Intermediate Precision (Ruggedness)
a. Calculate the content of Diethylene Glycol and Ethylene Glycol in precision sample.
b. The percent relative standard deviation (% RSD) for the results from six (6) sample solutions should be NMT 5.0%.
c. The difference between method precision and intermediate precision results should be no more than 10.0%.</td><td><table><tr><th>Name</th><th>Content (ppm)</th><th>%RSD</th><th>%Difference</th></tr><tr><td>Ethylene Glycol</td><td>88</td><td>3.0</td><td>2.3</td></tr><tr><td>Diethylene Glycol</td><td>34</td><td>3.4</td><td>2.9</td></tr></table></td></tr>
<tr><td>Method Accuracy
a. %Recovery should be between 6 75% and 125%.
b. The overall %RSD for all determinations should be NMT 15.0%.</td><td><table><tr><td rowspan="2">Name</td><td colspan="3">%Recovered</td></tr><tr><td>LOQ</td><td>100%</td><td>1200%</td></tr><tr><td>Ethylene Glycol</td><td>84</td><td>104</td><td>83</td></tr><tr><td>Name</td><td>LOQ</td><td>100%</td><td>800%</td></tr><tr><td>Diethylene Glycol</td><td>100</td><td>124</td><td>106</td></tr></table>Overall % RSD: 11.8 for Ethylene Glycol
Overall % RSD: 10.5 for Diethylene Glycol</td></tr>
<tr><td>Specificity
a. No interference should be observed from diluent.
b. All solvents should be well separated.</td><td>a. No interference was observed from diluent.
b. All solvents were well separated from each other.</td></tr>
</table>

<table>
<tr><th>Validation Parameter and Acceptance Criteria</th><th>Summary of Result</th></tr>
<tr><td>Robustness All the system suitability requirements must be met. Include the cautionary statement based on the results.</td><td>All the system suitability requirements met for variations in oven temperature ± 5°C and Flow ± 0.5 mL/min.</td></tr>
<tr><td>Filter Study The content of Ethylene glycol and Diethylene glycol results from the filtered sample solution differ by NMT 10.0%</td><td>
<table>
<tr><th rowspan="2">Name</th><th colspan="2">% Difference in Content</th></tr>
<tr><th>Ethylene Glycol</th><th>Diethylene Glycol</th></tr>
<tr><td>Centrifuge</td><td>Not applicable</td><td>Not applicable</td></tr>
<tr><td>0mL discard</td><td>3.4</td><td>2.9</td></tr>
<tr><td>2mL discard</td><td>1.1</td><td>0.0</td></tr>
<tr><td>4mL discard</td><td>1.1</td><td>2.9</td></tr>
<tr><td>6mL discard</td><td>1.1</td><td>8.8</td></tr>
<tr><td>8mL discard</td><td>2.3</td><td>8.8</td></tr>
</table>
Based on above results, it is concluded that sample solution should be filtered through 0.45µm Nylon filter by discarding first 2 mL of filtrate.
</td></tr>
</table>

Validation Parameter and Acceptance Criteria	Summary of Result
for fractions beyond the volume to be discarded.	

7.0 Conclusion

This sensitive and accurate method was developed and validated using Gas Chromatograph (GC) for the detection and quantification of Ethylene Glycol and Diethylene Glycol content in pharmaceutical ingredient-Sorbitol NF. This method demonstrated excellent sensitivity, linearity and high precision and accuracy, making this method suitable for routine quality control analysis. The application of this method to real pharmaceutical samples confirmed their compliance with safety standards, highlighting their effectiveness in ensuring the safety and quality of the pharmaceutical products and safeguard public health.

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