A simple and sensitive validated method for quantitation of toxic impurities-Ethylene glycol and Diethylene glycol in Pharmaceutical Ingredient-Glycerin 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 to 160% for Ethylene Glycol and LOQ to 160% for Diethylene Glycol demonstrating excellent linearity with correlation coefficients (r^2) of 1.000. Sensitivity analyses revealed low limits of detection (LOD) and limits of quantification (LOQ) for both components with GC-FID achieving LODs of 13 ppm for EG and 5 ppm for DEG. Precision and accuracy assessments showed that the method provided consistent results, with relative standard deviations (% RSD) below 5%. Accuracy was evaluated through recovery rates for EG and DEG, which ranged from 80% 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, Glycerin, Quality control.

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 ppm) (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 over LOQ to 160%, demonstrating strong linear relationships with correlation coefficients (r^2) of 1.000 for both EG and DEG, which verifies the methods' linearity. Accuracy was evaluated through recovery rates for EG and DEG, which ranged from 80% 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 5%, 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 harmful 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 Glycerin. 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 harmful 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:

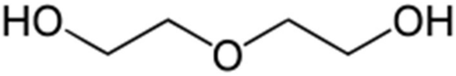
1.3.1.2 Molecular Formula and Molecular Weight

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

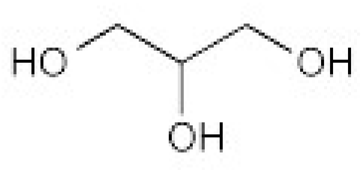
Molecular Formula: $C_4H_{10}O_3$
Molecular Weight: 106.12 g/mol

1.4 Chemical Information of Pharmaceutical ingredient/excipient

1.4.1. **Name:** Glycerin

Chemical Names: Glycerol, 1,2,3-Proapnaetriol

Chemical Structure:



1.4.2 Molecular Formula and Molecular Weight

Molecular Formula: $C_3H_8O_3$
Molecular Weight: 92.09 g/mol

2.0 Methodology

2.1 Chemicals and Reagents

Component Name	Source	Batch /Lot No.	Potency/Purity (%)
Diethylene Glycol RS	Sigma-Aldrich	LRAC0277 & LRAB0287	99.8
Ethylene Glycol RS	Sigma-Aldrich	LRAB3269	99.9
2,2,2-Trichloroethanol (Internal standard)	Sigma-Aldrich	STBH5424	99.8
Glycerin RS	Sigma-Aldrich	LRAB7812	99.8
Glycerin-Excipient	LDC	21148-365	99.8

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 0.32 mm, 1.8 µm film thickness, Equivalent to USP G43 stationary phase). The carrier gas was helium, with a flow rate of 2.72 ml/min (constant flow mode). The injector temperature was set to 220°C, and the detector (FID) temperature was set to 230°C. The oven temperature program was as follows: an initial temperature: 100°C and hold for 4.0 minutes and 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 held for 6 minutes at 220°C. The injection volume was 4.0 µL, and the split ratio was 1:1.

2.4 Preparations

2.4.1 Diluent Preparation

Methanol

2.4.2 Internal Standard Solution

Weighed accurately about 250 mg of 2,2,2-Trichloroethanol into 100 mL volumetric Flask. Diluted to volume with diluent and mixed well.
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:

Preparation of Stock Solution-1: (Glycerin)
Weighed accurately about 250 mg of Glycerin RS 25 mL volumetric flask. Added about 10 mL of diluent and sonicated for about 1 minute. Diluted to volume with diluent and mixed well.

Preparation of Stock Solution-2: (Ethylene glycol and Diethylene glycol)
Weigh accurately each about 50 mg of Ethylene Glycol RS and Diethylene Glycol RS into 50 mL volumetric flask. Add about 10 mL of diluent and sonicate for about 1 minute. Dilute to volume with diluent and mix well.

Preparation of Working Standard Solution:
Pipette out each 10.0 mL of Stock Solution-1, 2.5 mL of Stock Solution-2 and 2.0 mL of Internal Standard Solution into a 50 mL volumetric flask. Diluted to volume with diluent. (About 2mg/mL of Glycerin, 0.05 mg/ml of Ethylene glycol and Diethylene glycol and 0.1mg/mL of 2,2,2-Trichloroethanol).

2.4.4 Sample Preparation

Accurately weighed and transferred about 1500 mg of sample into 25 mL volumetric flask. Added about 10 mL of methanol and sonicate to dissolve. Pipette out 1.0 mL of internal standard solution into same volumetric flask. Dilute to volume with diluent and mixed well. (About 60mg/mL of Glycerin and 0.1mg/mL of 2,2,2, -Trichloroethanol).

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 1.3 and 1.1 respectively, which demonstrates the method is precise and consistent.[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 concentrations ranging from LOQ to 160% for Ethylene Glycol and LOQ to 160% 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 to be 1.000. 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 1 and Table 5, Figure 2].

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. Calculated 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-3,4,5].

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 5.0%. The difference between method precision and intermediate precision results was found within the acceptance criteria of not be more than 5.0% [Table 7,8,9]. 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 10,11]. 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 12].

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 2.72 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 13,14,15,16]. Hence the method is robust.

4.0 Analysis of Pharmaceutical Samples

The validated GC method was applied to the analysis of various pharmaceutical products such as Glycerin as an Excipient. Each sample was analyzed to ensure reliability. The concentrations of EG and DEG in the samples were quantified based on the standard concentration 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 concentration (ppm).

5.0 Results

5.1 System Precision

Table 1: System Precision

	SampleName	RT	Name	Response
1	Standard	3.386	Ethylene Glycol	0.846947
2	Standard	3.380	Ethylene Glycol	0.838545
3	Standard	3.344	Ethylene Glycol	0.861589
4	Standard	3.373	Ethylene Glycol	0.846221
5	Standard	3.376	Ethylene Glycol	0.851746
6	Standard	3.342	Ethylene Glycol	0.868022
Mean				0.852178
% RSD				1.3

	SampleName	RT	Name	Response
1	Standard	9.294	Diethylene Glycol	0.955451
2	Standard	9.289	Diethylene Glycol	0.934402
3	Standard	9.271	Diethylene Glycol	0.959137
4	Standard	9.283	Diethylene Glycol	0.953007
5	Standard	9.287	Diethylene Glycol	0.952606
6	Standard	9.271	Diethylene Glycol	0.964298
Mean				0.953150
% RSD				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	13	0.0013	6	38	0.0038	17
Diethylene Glycol	5	0.0005	4	16	0.0016	13

5.3 Precision at LOQ Level

Table 3: Precision at LOQ Level

Component Summary For Response

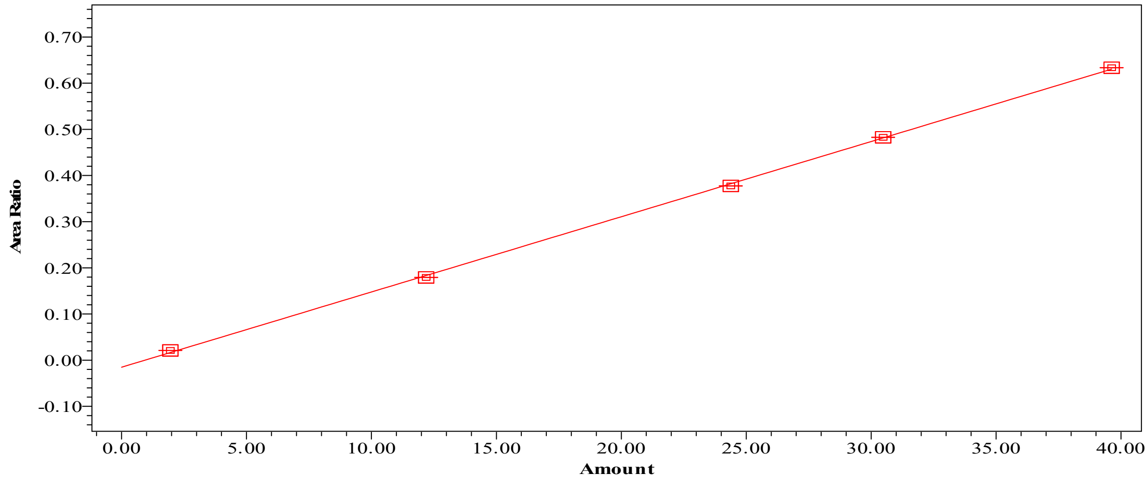
	SampleName	**Ethylene Glycol**	**Diethylene Glycol**
1	LOQ Solution	0.032650	0.014053
2	LOQ Solution	0.033812	0.014324
3	LOQ Solution	0.031541	0.014308
4	LOQ Solution	0.032384	0.014280
5	LOQ Solution	0.031880	0.014950
6	LOQ Solution	0.031820	0.013477
Mean		0.032348	0.014232
% RSD		2.5	3.3

5.4 Linearity and Range

Table 4: Linearity data for Ethylene Glycol

Linearity Level (%)	**Amount (%)**	**Response**
LOQ (8%)	0.0033	0.021028
50	0.0203	0.179300
100	0.0406	0.377546
125	0.0508	0.482733
160	0.0660	0.633463

Figure 1: Linearity Plot for Ethylene Glycol

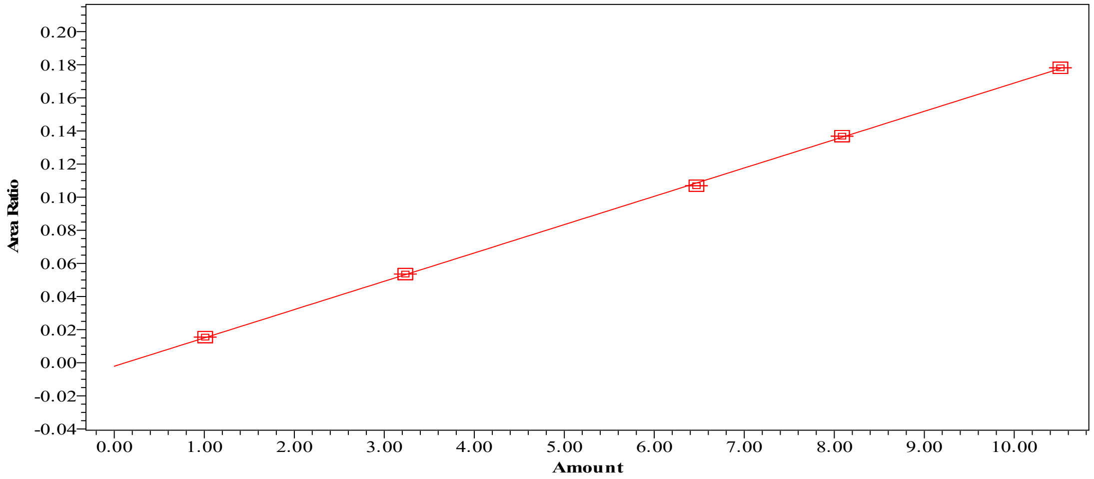


Name: Ethylene Glycol; R^2 1.000; Equation Y = 1.63e-002 X - 1.53e-002; A-Intercept -0.015; B-Slope 0.016

Table 5: Linearity data for Diethylene Glycol

Linearity Level (%)	Amount (%)	Response
LOQ (15%)	0.0017	0.015456
50	0.0054	0.053553
100	0.0108	0.106894
125	0.0135	0.136875
160	0.0175	0.178157

Figure 1: Linearity Plot for Diethylene Glycol



Name: Diethylene Glycol; R^2 1.000; Equation Y = 1.71e-002 X - 2.13e-003; A-Intercept -0.002; B-Slope 0.017

Name	Correlation Coefficient (r^2)
Ethylene Glycol	1.000
Diethylene Glycol	1.000

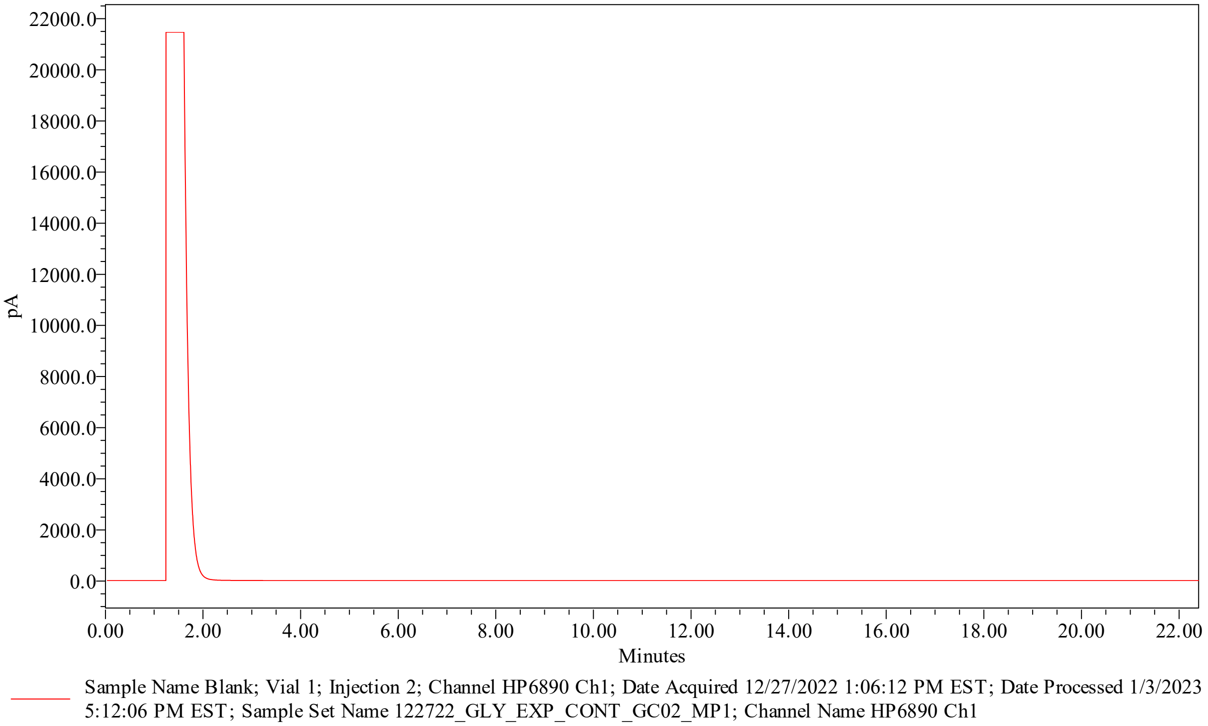
5.5 Method Precision

Table 2:Method Precision

Component Summary For PercentImpurity_RM

	SampleName	Ethylene Glycol	Diethylene Glycol
1	Method Precision-1	0.0433	0.0083
2	Method Precision-2	0.0430	0.0078
3	Method Precision-3	0.0433	0.0080
4	Method Precision-4	0.0443	0.0087
5	Method Precision-5	0.0444	0.0085
6	Method Precision-6	0.0437	0.0084
Mean		0.0437	0.0083
% RSD		1.3	4.1

Figure 3: Typical Chromatogram of Blank (Diluent):



Sample Name Blank; Vial 1; Injection 2; Channel HP6890 Ch1; Date Acquired 12/27/2022 1:06:12 PM EST; Date Processed 1/3/2023 5:12:06 PM EST; Sample Set Name 122722_GLY_EXP_CONT_GC02_MP1; Channel Name HP6890 Ch1

Figure 4: Typical Chromatogram of Standard:

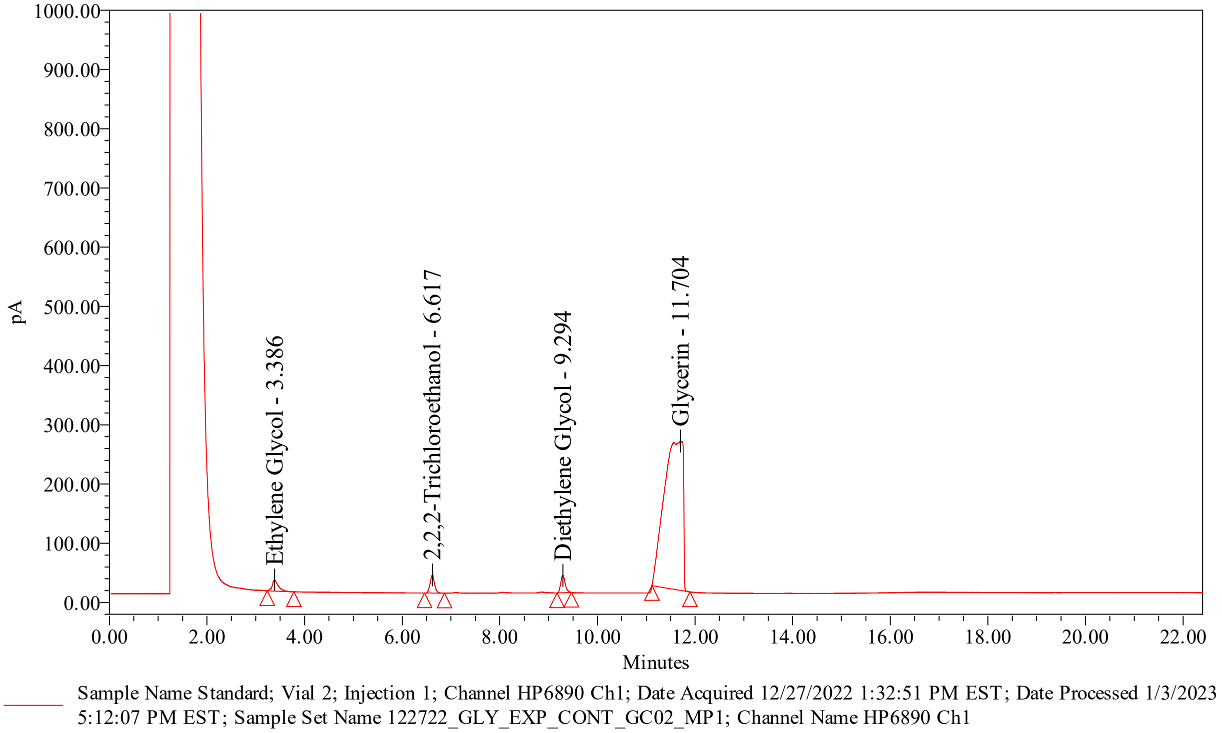
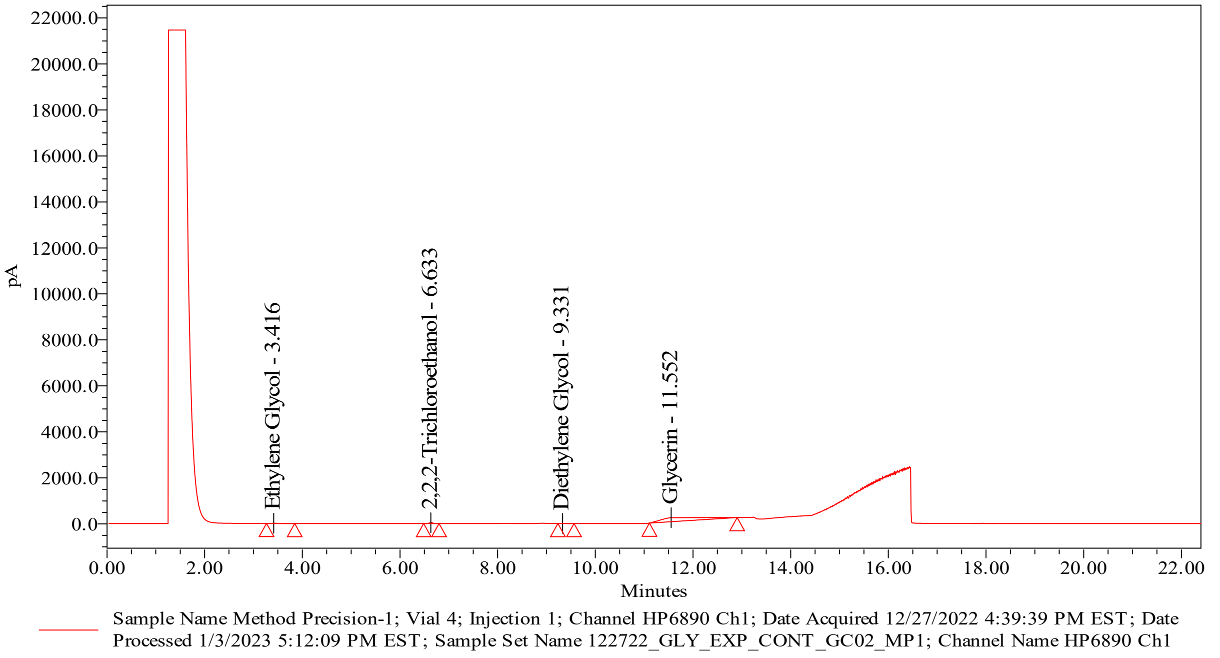


Figure 5: Typical Chromatogram of Sample (Method Precision)



5.6 Intermediate Precision (Ruggedness)

Table 3: Method Precision (Analyst-1)

Component Summary For PercentImpurity_RM

	SampleName	**Ethylene Glycol**	**Diethylene Glycol**
1	Method Precision-1	0.0433	0.0083
2	Method Precision-2	0.0430	0.0078
3	Method Precision-3	0.0433	0.0080
4	Method Precision-4	0.0443	0.0087
5	Method Precision-5	0.0444	0.0085
6	Method Precision-6	0.0437	0.0084
Mean		0.0437	0.0083
% RSD		1.3	4.1

Table 8: Intermediate Precision (Analyst 2)

Component Summary For PercentImpurity_RM

	SampleName	**Ethylene Glycol**	**Diethylene Glycol**
1	Intermediate Precision-1	0.0434	0.0092
2	Intermediate Precision-2	0.0450	0.0089
3	Intermediate Precision-3	0.0447	0.0085
4	Intermediate Precision-4	0.0445	0.0083
5	Intermediate Precision-5	0.0453	0.0087
6	Intermediate Precision-6	0.0448	0.0083
Mean		0.0446	0.0086
% RSD		1.5	3.9

Table 9: Intermediate Precision and Reproducibility

Name	**% Impurity**		**% Difference**	**Overall % RSD**
	Analyst-1	**Analyst-2**		
Ethylene Glycol	0.0437	0.0446	0.0009	1.4
Diethylene Glycol	0.0083	0.0086	0.0003	4.0

5.7 Method Accuracy

Table 10: Recovery Study of Ethylene Glycol

	Sample Name	Amount Added (%)	Amount Found (%)	%Recovery
1	LOQ Rec-1	0.0038	0.0030	80
2	LOQ Rec-2	0.0038	0.0030	80
3	LOQ Rec-3	0.0038	0.0031	82
Mean				81
4	100% Rec-1	0.0415	0.0375	90
5	100% Rec-2	0.0415	0.0372	90
6	100% Rec-3	0.0415	0.0376	91
7	100% Rec-4	0.0415	0.0383	92
8	100% Rec-5	0.0415	0.0383	92
9	100% Rec-6	0.0415	0.0378	91
Mean				91
10	160% Rec-1	0.0706	0.0641	91
11	160% Rec-2	0.0706	0.0649	92
12	160% Rec-3	0.0706	0.0649	92
Mean				92
Overall Mean				89
Overall %RSD				4.7

Table 11: Recovery Study of Diethylene Glycol

	Sample Name	Amount Added (%)	Amount Found (%)	%Recovery
1	LOQ Rec-1	0.0016	0.0013	81
2	LOQ Rec-2	0.0016	0.0013	81
3	LOQ Rec-3	0.0016	0.0013	81
Mean				81
4	100% Rec-1	0.0119	0.0110	93
5	100% Rec-2	0.0119	0.0112	94
6	100% Rec-3	0.0119	0.0111	93
7	100% Rec-4	0.0119	0.0108	91
8	100% Rec-5	0.0119	0.0112	94
9	100% Rec-6	0.0119	0.0110	92
Mean				93
10	160% Rec-1	0.0179	0.0176	99
11	160% Rec-2	0.0179	0.0175	98
12	160% Rec-3	0.0179	0.0178	100
Mean				99
Overall Mean				92
Overall %RSD				6.6

5.8 Specificity

Table 12: Specificity Study

Name of the impurity/solvent	**Retention Time (RT) in Minutes-Approx.**
Diluent (Methanol)	1.9
Ethylene Glycol	3.3
2,2,2-Trichloroethanol	6.6
Diethylene Glycol	9.3
Glycerin	11.5

5.9 Robustness

Table 4: Robustness Study-Column Oven Temperature at 95°C (-5°C)

Component Summary For Response

	SampleName	**Ethylene Glycol**	**Diethylene Glycol**
1	Standard	0.834085	0.912848
2	Standard	0.833687	0.913024
3	Standard	0.839284	0.915028
4	Standard	0.841738	0.914035
5	Standard	0.836292	0.917191
6	Standard	0.835995	0.924107
Mean		0.836847	0.916039
% RSD		0.4	0.5

Table 5: Robustness Study-Column Oven Temperature at 105°C (+5°C)

Component Summary For Response

	SampleName	**Ethylene Glycol**	**Diethylene Glycol**
1	Standard	0.797712	0.932794
2	Standard	0.800866	0.922258
3	Standard	0.786288	0.909911
4	Standard	0.787644	0.925660
5	Standard	0.797811	0.915718
6	Standard	0.798215	0.918545
Mean		0.794756	0.920814
% RSD		0.8	0.9

Table 6: Robustness Study-Flow at 2.22 (-0.2 mL/min)

Component Summary For Response

	SampleName	Ethylene Glycol	Diethylene Glycol
1	Standard	0.786615	0.927042
2	Standard	0.793795	0.916985
3	Standard	0.795210	0.911381
4	Standard	0.804249	0.919124
5	Standard	0.806118	0.928168
6	Standard	0.786067	0.902157
Mean		0.795342	0.917476
% RSD		1.1	1.1

Table 7: Robustness Study-Flow at 3.22 (-0.2 mL/min)

Component Summary For Response

	SampleName	Ethylene Glycol	Diethylene Glycol
1	Standard	0.817978	0.930810
2	Standard	0.826786	0.924971
3	Standard	0.813123	0.940915
4	Standard	0.816243	0.935049
5	Standard	0.820453	0.920153
6	Standard	0.823393	0.930418
Mean		0.819663	0.930386
% RSD		0.6	0.8

6.0 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-Glycerin. 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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