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A Simple And Sensitive Validated Method For Quantitation Of Toxic Impurities-Ethylene Glycol And Diethylene Glycol In Pharmaceutical Ingredient-Propylene Glycol 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 5 ppm to 755 ppm for Ethylene Glycol and 5 ppm to 212 ppm 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 3 ppm for both EG and DEG. Precision and accuracy assessments showed that the method provided consistent results, with relative standard deviations (% RSD) below 5%. Application of the method to various pharmaceutical ingredients such as Propylene glycol 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, rugged, robust 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, Propylene glycol NF, 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. 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 and sensitivity of the GC method in detecting and quantifying low levels of EG and DEG in pharmaceutical ingredient such as Propylene glycol 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 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:

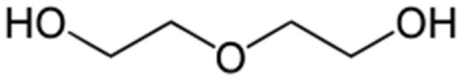
1.3.1.2 Molecular Formula and Molecular Weight

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

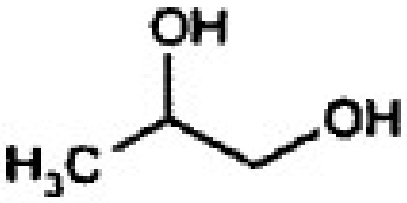
Molecular Formula: $C_4H_{10}O_3$
Molecular Weight: 106.12 g/mol

1.4 Chemical Information of Pharmaceutical ingredient/excipient

1.4.1. Name: Propylene glycol

Chemical Names: Propane-1,2-diol, 1,2-Propanediol.

Chemical Structure:



1.4.2 Molecular Formula and Molecular Weight

Molecular Formula: $C_3H_8O_2$
Molecular Weight: 76.095 g/mol

2.0 Methodology

2.1 Chemicals and Reagents

Name	Source	Batch/Lot #	Potency/Purity (%)
Diethylene Glycol RS	Sigma-Aldrich	LRAC0277	99.8
Ethylene Glycol RS	Sigma-Aldrich	LRAC2089	99.9
Propylene Glycol	Sigma-Aldrich	LRAC6494	99.8
Propylene Glycol	DOW Chemicals	D207JCNPG1	99.9

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).

2.3.1 Chromatographic Conditions (GC Parameters)

The GC analysis was performed using an Agilent DB-5 Capillary column (60 m x 0.53 mm, 5 µm film thickness, Equivalent to USP G27 stationary phase). The carrier gas was helium, with a flow rate of 2.5 ml/min (constant flow mode). The injector temperature was set to 230°C, and the detector (FID) temperature was set to 260°C. The oven temperature program was as follows: an initial temperature: 180°C and hold for 10.0 minutes and increased to 250° C with a rate of 20°C/min and hold for 5 minutes at 250°C. The injection volume was 0.2 µL, and the split ratio was 1:2.

2.4 Preparations

2.4.1 Diluent Preparation

Propylene glycol

2.4.2 Standard Preparation

Preparation of Stock Solution:

Weighed accurately about 25 mg each of Diethylene Glycol and Ethylene Glycol RS into 25 mL volumetric flask. Added about 15 mL of diluent, mixed well. Diluted to volume with diluent and mixed well.

Preparation of Standard Solution:
Pipette out 1.0 mL of stock solution into 100 mL volumetric flask. Diluted to volume with diluent and mixed well. (about 10 ppm of Diethylene Glycol and Ethylene Glycol with respective sample concentration).

2.4.3 Sample Preparation

Fill the GC vial with the sample.

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 1.6 and 3.4 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 5 ppm to 755 ppm for Ethylene Glycol and 5 ppm to 212 ppm 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 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 7,8,9]. Hence, method is precise and rugged.

3.7 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 10].

3.8 Robustness

Variation in important chromatographic parameters such as column oven temperature ± 5°C (Procedural temperature 180°C), carrier gas flow ±0.5 ml/min (Procedural flow 2.5 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 15.0% and met the system suitability. No significant change observed in system suitability with deliberate changes over column

temperature, Carrier gas flow [Table 11,12,13,14]. 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 Propylene glycol 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	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Diethylene Glycol	9.039	4.628918
2	Standard	2	Diethylene Glycol	9.034	4.343752
3	Standard	3	Diethylene Glycol	9.057	4.596358
4	Standard	4	Diethylene Glycol	9.060	4.709678
5	Standard	5	Diethylene Glycol	9.065	4.351546
6	Standard	6	Diethylene Glycol	9.057	4.620050
Mean					4.541717
% RSD					3.4

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Ethylene Glycol	5.545	6.218622
2	Standard	2	Ethylene Glycol	5.557	6.319657
3	Standard	3	Ethylene Glycol	5.564	6.501374
4	Standard	4	Ethylene Glycol	5.566	6.332588
5	Standard	5	Ethylene Glycol	5.571	6.329396
6	Standard	6	Ethylene Glycol	5.567	6.235999
Mean					6.322939
% RSD					1.6

5.2 Sensitivity and Detection Limits

Table 2: LOD and LOQ values

Name	LOD		LOQ	
	Amount (ppm)	S/N	Amount	S/N
Ethylene Glycol	3 ppm	9	5 ppm	21
Diethylene Glycol	3 ppm	6	5 ppm	12

5.3 Precision at LOQ Level

Table 3: Precision at LOQ Level

Component Summary For Area

	SampleName	Injection No.	Ethylene Glycol	Diethylene Glycol
1	LOQ Precision	1	3.720797	2.138374
2	LOQ Precision	2	3.536767	2.070341
3	LOQ Precision	3	3.606358	1.923750
4	LOQ Precision	4	3.617095	1.801545
5	LOQ Precision	5	3.262773	2.314013
6	LOQ Precision	6	3.626465	2.177776
Mean			3.561709	2.070967
% RSD			4.4	8.9

5.4 Linearity and Range

Table 4: Linearity data for Ethylene Glycol

	SampleName	Name	Amount (ppm)	Area
1	Linearity LOQ	Ethylene Glycol	5.3946	3.732709
2	Linearity EG&DEG~80%	Ethylene Glycol	8.6314	4.790030
3	Linearity EG&DEG~100%	Ethylene Glycol	10.7892	5.132247
4	Linearity EG&DEG~300%	Ethylene Glycol	32.3676	13.051628
5	Linearity EG&DEG~1000%	Ethylene Glycol	107.8920	40.374526
6	Linearity EG-7000% ; DEG~2000%	Ethylene Glycol	755.2440	277.862498

Figure 1: Linearity Plot for Ethylene Glycol

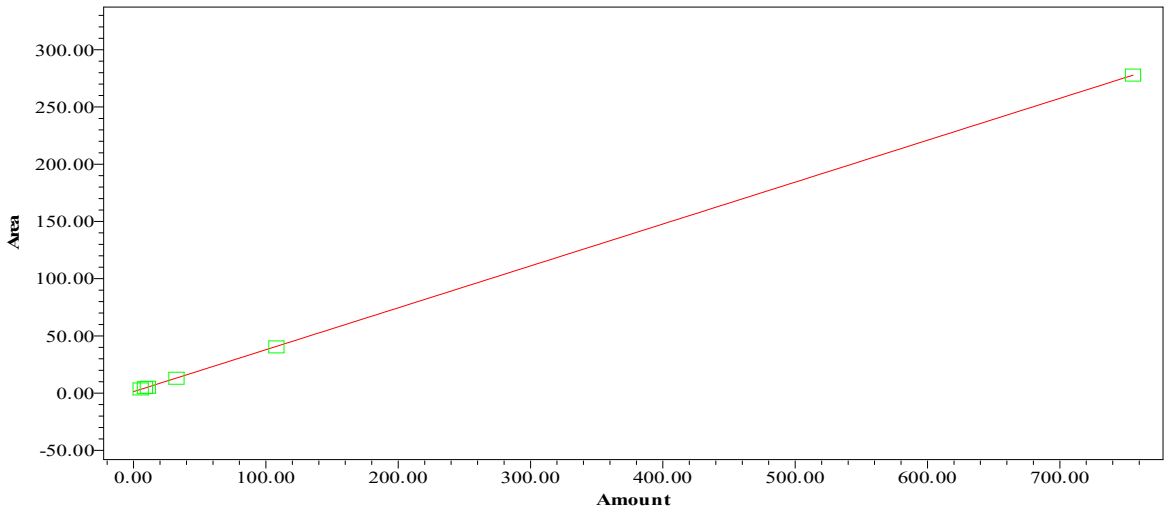
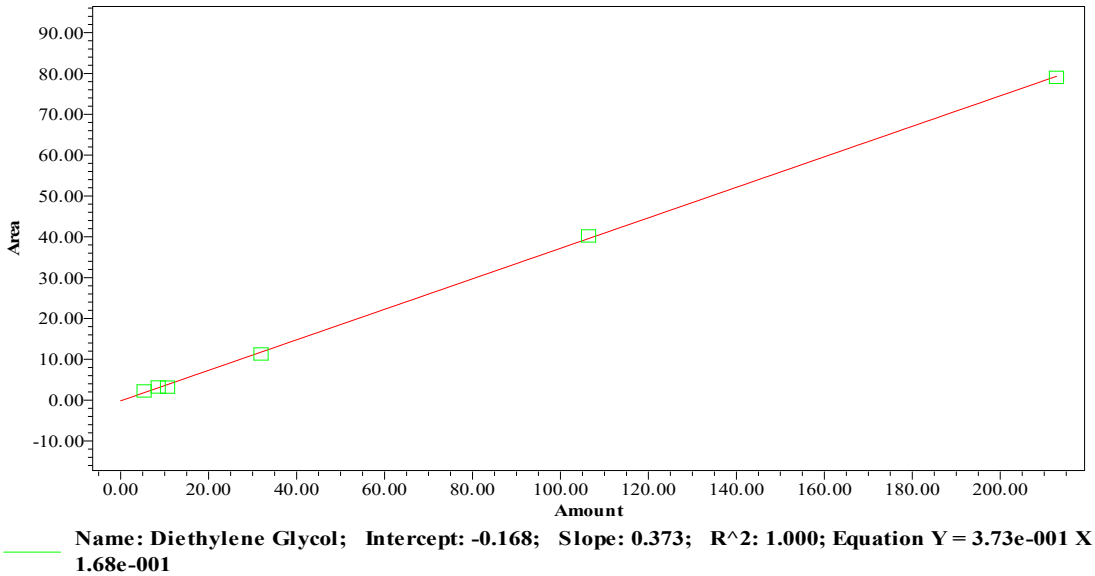


Table 5: Linearity data for Diethylene Glycol

	SampleName	Name	Amount (ppm)	Area
1	Linearity LOQ	Diethylene Glycol	5.3193	2.240341
2	Linearity EG&DEG~80%	Diethylene Glycol	8.5109	3.230889
3	Linearity EG&DEG~100%	Diethylene Glycol	10.6387	3.200125
4	Linearity EG&DEG~300%	Diethylene Glycol	31.9160	11.311540
5	Linearity EG&DEG~1000%	Diethylene Glycol	106.3868	40.225600
6	Linearity EG-7000% ; DEG~2000%	Diethylene Glycol	212.7736	79.049853

Figure 1: Linearity Plot for Diethylene Glycol



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

5.5 Method Precision

Table 2: Method Precision

Sample Name	Content (ppm)	
	Ethylene Glycol	Diethylene Glycol
Method Precision Sample-1	2	Not detected
Method Precision Sample-2	2	Not detected
Method Precision Sample-3	2	Not detected
Method Precision Sample-4	2	Not detected
Method Precision Sample-5	2	Not detected
Method Precision Sample-6	2	Not detected
Mean	2	Not applicable
%RSD	4.5	Not applicable

Representative chromatograms for blank, standard and sample were depicted below:

Figure 3: Typical Chromatogram of Blank

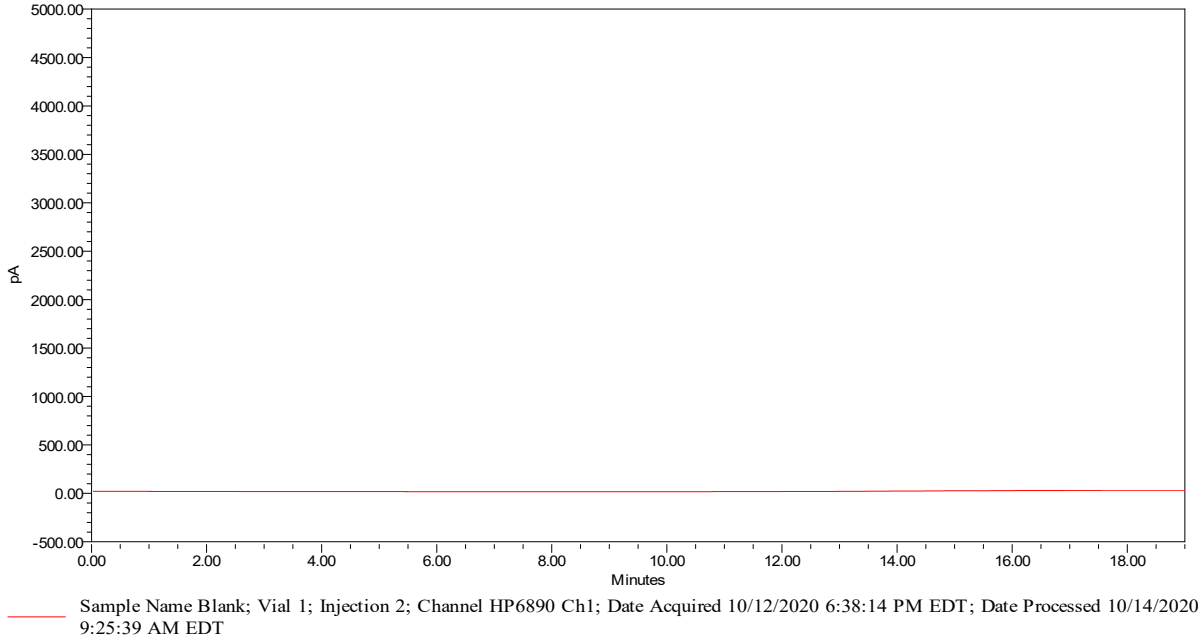
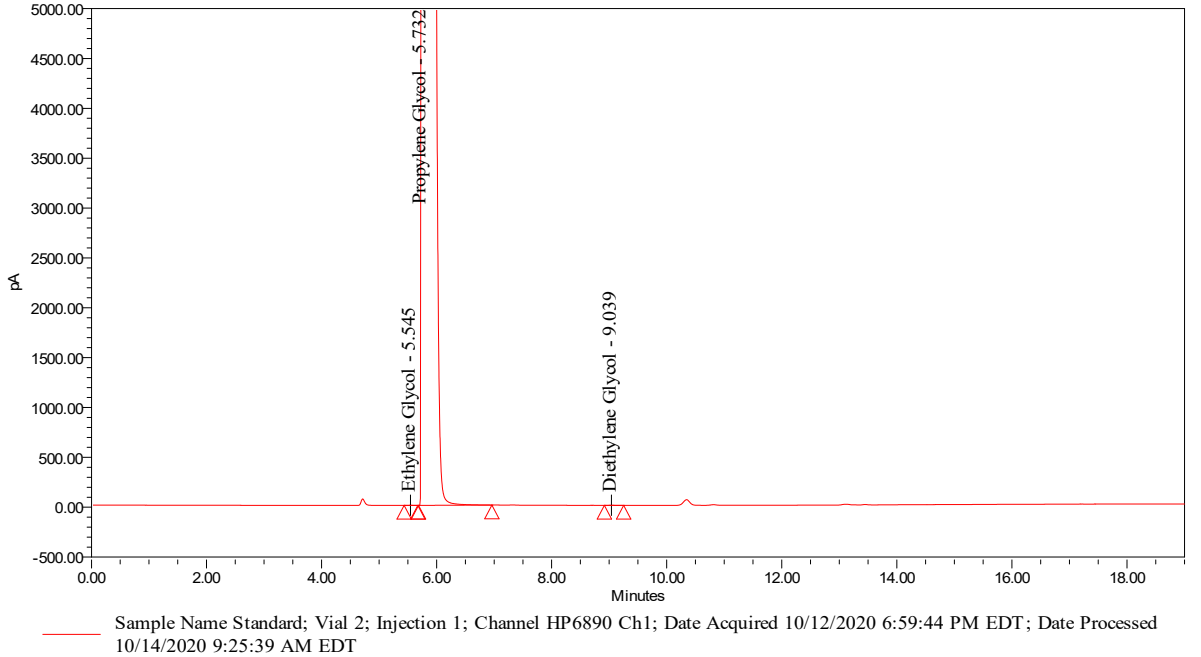


Figure 4: Typical Chromatogram of Standard

A: Full Scale Chromatogram of Standard (RT: 0.00 to 19.00 Minutes)



B: Zoom Scale Chromatogram of Standard (RT: 4.00 to 10.00 Minutes)

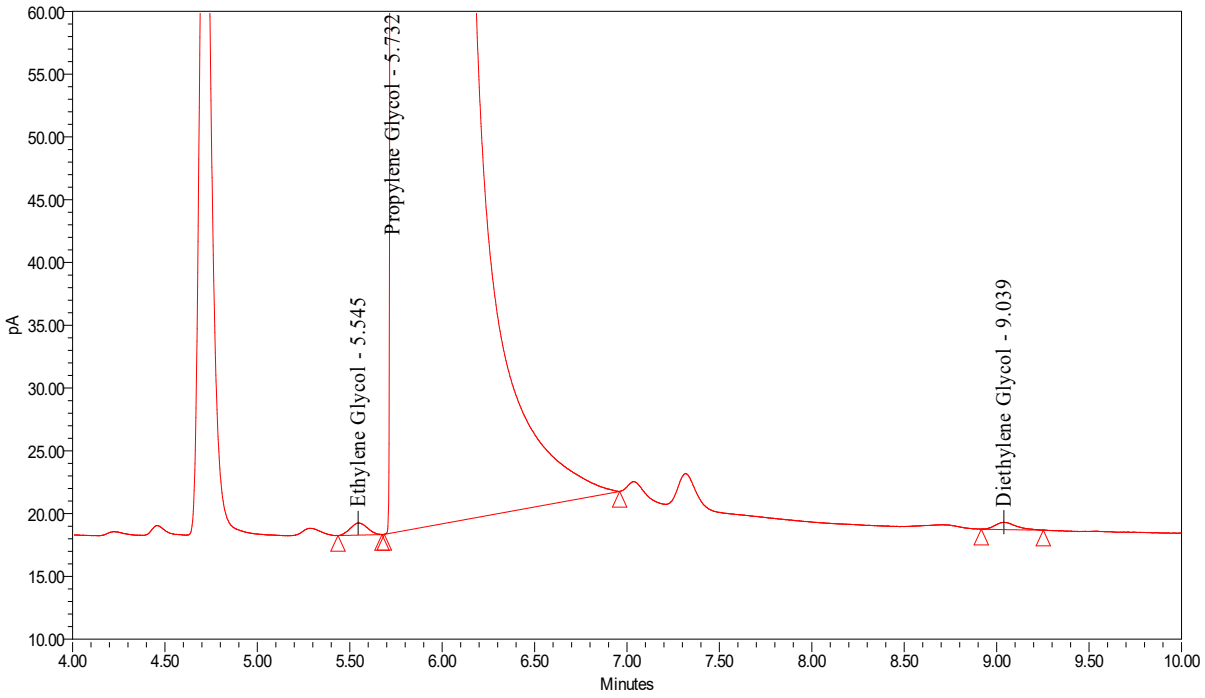
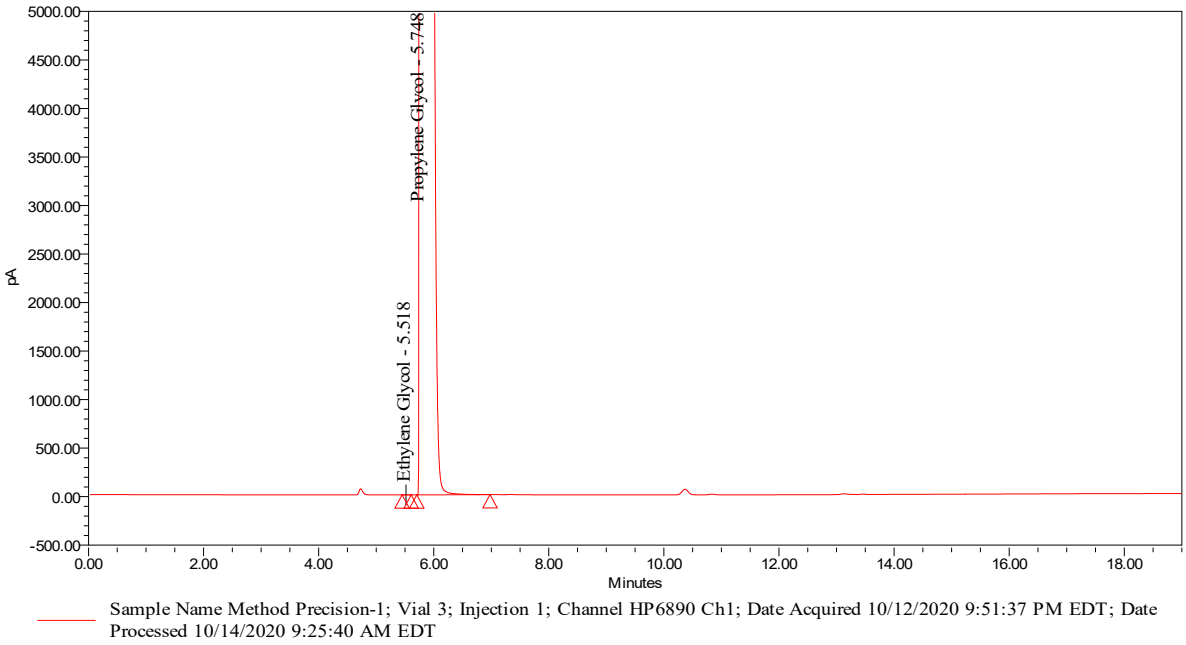
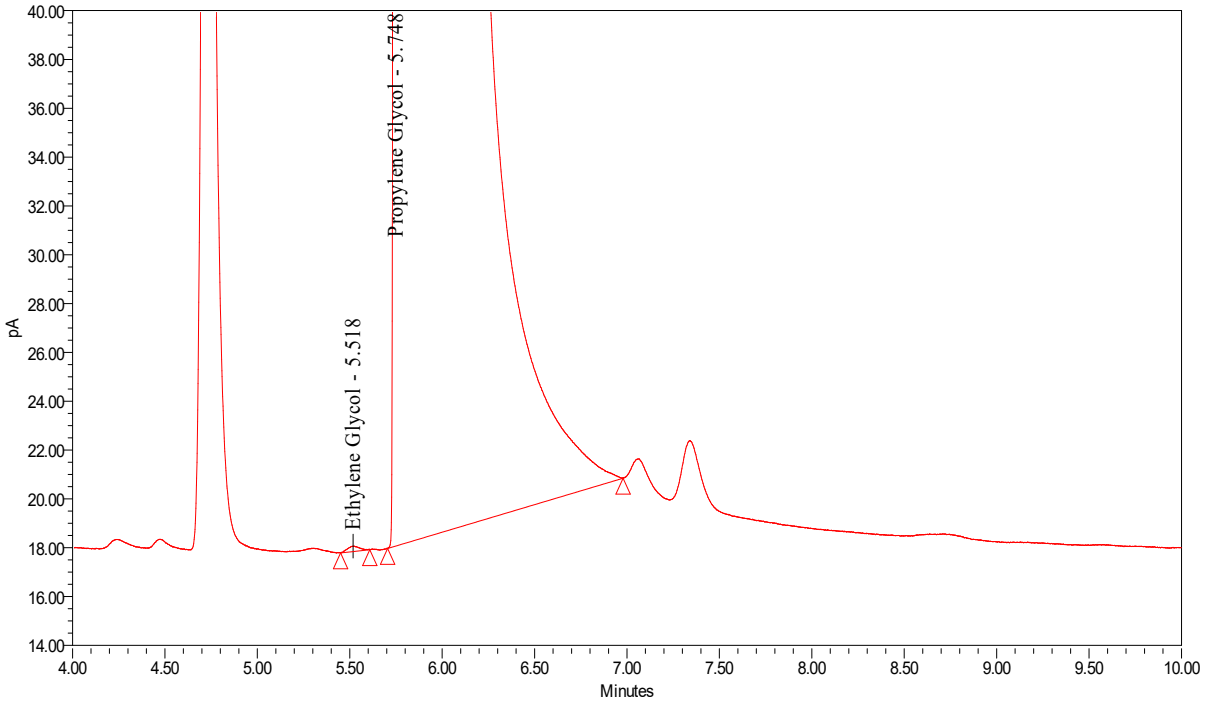


Figure 5: Typical Chromatogram of Method Precision Sample

A: Full Scale Chromatogram of Method Precision Sample (RT: 0.00 to 19.00 Minutes)



B: Zoom Scale Chromatogram of Method Precision Sample (RT: 4.00 to 10.00 Minutes)



5.6 Intermediate Precision (Ruggedness)

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

Sample Name	Content (ppm)	
	Ethylene Glycol	Diethylene Glycol
Method Precision Sample-1	2	Not detected
Method Precision Sample-2	2	Not detected
Method Precision Sample-3	2	Not detected
Method Precision Sample-4	2	Not detected
Method Precision Sample-5	2	Not detected
Method Precision Sample-6	2	Not detected
Mean	2	Not applicable
%RSD	4.5	Not applicable

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

Sample name	Content (ppm)	
	Ethylene Glycol	Diethylene Glycol
Intermediate Precision-1	4	Not detected
Intermediate Precision-2	3	Not detected

Intermediate Precision-3	4	Not detected
Intermediate Precision-4	3	Not detected
Intermediate Precision-5	3	Not detected
Intermediate Precision-6	3	Not detected
Mean	3	Not applicable
%RSD	10.0	Not applicable

Table 9: Intermediate Precision and Reproducibility

Name	**% Impurity (solvent)**		**Difference**	**Overall % RSD**
	Analyst-1	**Analyst-2**		
Ethylene Glycol	2 ppm	3 ppm	1ppm	7.3
Diethylene Glycol	ND	ND	N/A	N/A

ND: Not detected. N/A: Not applicable.

5.7 Specificity

Table 10: Specificity Study

Name of the impurity/solvent peak	**Retention Time (RT) in Minutes-Approx.**
Ethylene glycol	5.5
Propylene glycol	5.7
Diethylene glycol	9.0

5.8 Robustness

Table 51: Robustness Study-Column Oven Temperature Minus (175°C)

	SampleName	**Injection No.**	**Name**	**Retention Time (min)**	**Area**
1	Standard	1	Ethylene Glycol	5.837	5.833927
2	Standard	2	Ethylene Glycol	5.837	5.101075
3	Standard	3	Ethylene Glycol	5.834	5.171330
4	Standard	4	Ethylene Glycol	5.837	5.163789
5	Standard	5	Ethylene Glycol	5.837	5.644756
6	Standard	6	Ethylene Glycol	5.842	5.109179
Mean					5.337343
% RSD					6.0

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Diethylene Glycol	9.895	2.168392
2	Standard	2	Diethylene Glycol	9.879	1.891080
3	Standard	3	Diethylene Glycol	9.886	2.381916
4	Standard	4	Diethylene Glycol	9.889	2.024229
5	Standard	5	Diethylene Glycol	9.889	2.058108
6	Standard	6	Diethylene Glycol	9.891	2.396365
Mean					2.153349
% RSD					9.4

Table 12: Robustness Study-Column Oven Temperature Plus (185°C)

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Ethylene Glycol	5.550	5.139938
2	Standard	2	Ethylene Glycol	5.553	5.085283
3	Standard	3	Ethylene Glycol	5.550	5.315973
4	Standard	4	Ethylene Glycol	5.551	5.244442
5	Standard	5	Ethylene Glycol	5.551	5.101932
6	Standard	6	Ethylene Glycol	5.551	5.046290
Mean					5.155643
% RSD					2.0

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Diethylene Glycol	8.764	2.655386
2	Standard	2	Diethylene Glycol	8.768	2.901955
3	Standard	3	Diethylene Glycol	8.757	2.144888
4	Standard	4	Diethylene Glycol	8.769	2.127839
5	Standard	5	Diethylene Glycol	8.771	2.171194
6	Standard	6	Diethylene Glycol	8.772	2.484481
Mean					2.414290
% RSD					13.3

Table 63: Robustness Study-Carrier gas Flow Plus 0.5 mL/min (3.0 mL/min)

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Ethylene Glycol	4.902	5.087462
2	Standard	2	Ethylene Glycol	4.904	5.122935
3	Standard	3	Ethylene Glycol	4.907	5.148287
4	Standard	4	Ethylene Glycol	4.906	5.046233
5	Standard	5	Ethylene Glycol	4.903	5.117307
6	Standard	6	Ethylene Glycol	4.906	5.163563
Mean					5.114298
% RSD					0.8

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Diethylene Glycol	8.017	2.524621
2	Standard	2	Diethylene Glycol	8.013	2.488938
3	Standard	3	Diethylene Glycol	8.015	3.201966
4	Standard	4	Diethylene Glycol	8.010	2.600871
5	Standard	5	Diethylene Glycol	8.010	2.794430
6	Standard	6	Diethylene Glycol	8.008	2.712138
Mean					2.720494
% RSD					9.6

Table 74: Robustness Study-Carrier gas Flow Minus 0.5mL/min (2.0 mL/min)

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Ethylene Glycol	5.892	5.402948
2	Standard	2	Ethylene Glycol	5.892	5.435060
3	Standard	3	Ethylene Glycol	5.886	5.232649
4	Standard	4	Ethylene Glycol	5.896	5.103720
5	Standard	5	Ethylene Glycol	5.892	5.400816
6	Standard	6	Ethylene Glycol	5.893	5.263165
Mean					5.306393
% RSD					2.4

	SampleName	Injection No.	Name	Retention Time (min)	Area
1	Standard	1	Diethylene Glycol	9.625	1.724973
2	Standard	2	Diethylene Glycol	9.622	1.848651
3	Standard	3	Diethylene Glycol	9.624	1.694757
4	Standard	4	Diethylene Glycol	9.623	1.938954
5	Standard	5	Diethylene Glycol	9.632	1.666568
6	Standard	6	Diethylene Glycol	9.630	2.297232
Mean					1.861856
% RSD					12.7

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-Propylene glycol. This method demonstrated excellent sensitivity, linearity and high precision and 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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