Analytical Method Development and Validation for the Determination of Ethylene Glycol and Diethylene Glycol in Herbal Cough Syrup by Gas Chromatography

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ABSTRACT

Ethylene Glycol (EG) and Diethylene Glycol (DEG) are potential contaminants in liquid oral formulations, posing severe toxicological risks. Regulatory agencies mandate stringent controls on their permissible limits. This study outlines the analytical method verification of a gas chromatography (GC) procedure for the quantitative determination of EG and DEG in a polyherbal cough syrup. The method employs a DB-624 capillary column and flame ionization detection (FID), using ethanol as the diluent. System suitability, precision, resolution, and tailing factor criteria were established and verified. The method was confirmed to be accurate, precise, and suitable for routine use in quality control.

Keywords: Ethylene Glycol, Diethylene Glycol, Gas Chromatography, Method Verification, Cough Syrup, System Suitability

INTRODUCTION

Ethylene Glycol (EG) and Diethylene Glycol (DEG) are used as industrial solvents and antifreeze agents. However, their presence in pharmaceutical formulations, whether due to contamination or substitution, can lead to serious toxicity, including renal failure and death [1]. Several global incidents have drawn attention to the need for accurate and validated methods to detect these compounds in pharmaceutical products [2].

Naturals Cough Syrup is a polyherbal formulation, and it is essential to ensure that it is free from toxic contaminants like EG and DEG. This study describes the verification of an analytical method employing Gas Chromatography (GC) for the quantitative estimation of EG and DEG, as per ICH Q2(R1) and applicable regulatory guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

- Ethanol (HPLC Grade, Merck)
- Ethylene Glycol (Reference Standard)
- Diethylene Glycol (Reference Standard)
- Propylene Glycol (Internal Standard)

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Instruments and Equipment

- Gas Chromatograph: Agilent 7820 / 7860 with FID
- Column: DB-624, 30 m x 0.25 mm ID x 1.4 µm film thickness
- Analytical Balance: Shimadzu (Actzec)
- Micropipettes: Thermo Fisher
- Sonicator: Labman

Sample Preparation 10 g of Naturals Cough Syrup was transferred into a 100 mL volumetric flask, sonicated for 10 minutes, and volume made up with ethanol. The solution was filtered through a 0.45 µm syringe filter before injection.

Standard and System Suitability Solutions

- **Standard Stock**: 100 mg each of EG and DEG was dissolved in ethanol up to 100 mL.
- **Working Standard**: 5 mL of the stock was diluted to 50 mL (100 ppm).
- **System Suitability**: 5 mL of EG/DEG and 5 mL of Propylene Glycol stock were diluted to 50 mL with ethanol.

Chromatographic Conditions

Parameter	Value
Column	DB-624
Injector Temp	230°C
Detector Temp (FID)	250°C
Injection Volume	1 µL
Split Ratio	1:20
Carrier Flow	2 mL/min

Analytical method validation

Specificity

Precision

System Precision

Method Precision

Limit of Detection and Limit of Quantitation

Accuracy

Solution Stability

Specificity

The specificity of the developed Gas Chromatographic (GC) method was demonstrated by evaluating potential interferences from various excipients, herbal actives, and formulation components. The test solutions included

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blank, standard (EG and DEG), placebo, spiked sample, and individual solutions of herbal and excipient constituents to ensure there was no co-elution or interference with the analyte peaks of EG and DEG.

Preparation of Solutions for Specificity Study

Propylene Glycol Stock Solution:

200.5 mg of Propylene Glycol was accurately weighed into a 100 mL volumetric flask. Approximately 70 mL of ethanol was added, and the mixture was shaken until completely dissolved. The final volume was made up with ethanol.

EG and DEG Stock Solution A:

102.8 mg of Ethylene Glycol and 101.5 mg of Diethylene Glycol were transferred into a 100 mL volumetric flask. After the addition of 70 mL of ethanol and thorough mixing, the final volume was adjusted with ethanol.

Standard Solution A:

5 mL of EG & DEG Stock Solution A was diluted to 50 mL with ethanol and mixed well before injection.

EG and DEG Stock Solution B:

101.4 mg of EG and 100.4 mg of DEG were similarly prepared in a 100 mL flask and diluted with ethanol as above.

Standard Solution B:

5 mL of Stock Solution B was diluted to 50 mL with ethanol, mixed, and injected.

System Suitability Solution:

5 mL each of Propylene Glycol and EG & DEG stock solutions were mixed in a 50 mL volumetric flask, diluted with ethanol, and injected to check system suitability parameters.

Placebo and Sample Solutions

Placebo Solution:

10.0219 g of placebo was dissolved in ethanol using sonication for 10 minutes, made up to 100 mL, filtered (0.45 µm syringe filter), and injected.

As Such Sample Solutions (1 & 2):

10.0425 g of the syrup sample was weighed and treated identically as a placebo for both preparations. Solutions were filtered and injected.

Spiked Sample Solution:

10.0368 g of sample was spiked with 10 mL of EG & DEG standard stock solution, sonicated, volume made up with ethanol, filtered, and injected.

Unfiltered Sample Solutions (1 & 2):

Samples of 10.0516 g and 10.0159 g were prepared without filtration, diluted in ethanol, and directly injected after mixing.

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Identification Solutions of Herbal Ingredients and Excipients

Each of the following components was weighed in the range of ~100 mg, dissolved in 70 mL of ethanol, and the volume was made up to 100 mL with ethanol. From this, 5 mL was further diluted to 50 mL with ethanol for injection. The purpose was to assess their chromatographic behaviour and potential interference.

Component	Quantity (mg)
Ethylene Glycol	100.7
Diethylene Glycol	100.1
Madhu (Honey)	103.1
Yashtimadhu (*Glycyrrhiza glabra*)	102.01
Tulsi (*Ocimum sanctum*)	101.08
Vasaka (*Adhatoda vasica*)	100.41
Talishpatra (*Taxus baccata*)	102.42
Sunthi (*Zingiber officinale*)	100.85
Haldi (*Curcuma longa*)	100.93
Banashpa (*Viola odorata*)	100.14
Pippali (*Piper longum*)	100.27
Shati (*Hedychium spicatum*)	102.37
Munkka (*Vitis vinifera*)	101.90
Sucrose	99.57
Sorbitol Solution 70%	102.09
Liquid Glucose	101.40
Propylene Glycol	100.3
Citric Acid Monohydrate	100.80
Sodium Benzoate	102.50
Sodium Methyl Paraben	99.43
Menthol	102.14
Sodium Propyl Paraben	102.38
Oleo-resin (Ginger)	102.6
Camphor	100.03
Purified Water	102.9

Each solution was carefully prepared by weighing the component, dissolving in ethanol, sonication if needed, final volume makeup, dilution (5 mL into 50 mL ethanol), and analysis through GC.

System Suitability

System suitability parameters were evaluated by injecting standard solutions (Standard Solution-A and Standard Solution-B) and analysing critical attributes such as similarity factor, resolution, %RSD for area and retention time, and tailing factors for both Ethylene Glycol and Diethylene Glycol.

Table 2: System Suitability Evaluation

System Suitability Parameter	Analyte	Observation	Acceptance Criteria
Similarity Factor (Std A vs. Std B)	Ethylene Glycol	0.96	NLT 0.85 and NMT 1.15
	Diethylene Glycol	0.99	NLT 0.85 and NMT 1.15
Resolution between EG and PG peaks	-	13.09	NLT 10
% RSD of Peak Area (n=6)	Ethylene Glycol	8.2	NMT 15%
	Diethylene Glycol	6.6	NMT 15%
% RSD of Retention Time (n=6)	Ethylene Glycol	0.02	NMT 2%
	Diethylene Glycol	0.06	NMT 2%
Tailing Factor	Ethylene Glycol	1.6	NMT 3.5
	Diethylene Glycol	2.0	NMT 3.5

All parameters met the pre-defined acceptance criteria, confirming the system suitability for method validation.

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Specificity

To confirm the method's specificity, retention times for Ethylene Glycol and Diethylene Glycol were determined in the presence of matrix and excipients. No interfering peaks were observed at these retention times.

Table 3: Retention Time of Target Analytes

Peak Name	Retention Time (min)
Ethylene Glycol	5.987
Diethylene Glycol	17.179

Filter Study

The purpose of the filter study was to confirm the absence of any loss of analyte or interference introduced by filtration. Both filtered and unfiltered sample solutions were analyzed, and no significant difference in response was observed.

Table 4: Filter Study Data

Injection	Ethylene Glycol Area	Diethylene Glycol Area
Sample – As Such – 1 (Without Filter)	0	0
Sample – As Such – 2 (Without Filter)	0	0
Sample – As Such – 1 (Filtered)	0	0
Sample – As Such – 2 (Filtered)	0	0

Conclusion

- The method passed all system suitability criteria, indicating consistent and reliable chromatographic performance.
- No extraneous peaks were observed in the filtered sample solution, confirming specificity and absence of matrix interference.
- There was no difference in analyte response between filtered and unfiltered sample solutions, establishing filter compatibility.

Precision

System Precision

System precision was evaluated by performing six replicate injections of the standard solution containing Ethylene Glycol (EG) and Diethylene Glycol (DEG). The aim was to demonstrate the reproducibility of the chromatographic system under the defined operating conditions.

Preparation of Solutions

Propylene Glycol Stock Solution:

Accurately weighed 201.2 mg of Propylene Glycol into a 100 mL volumetric flask. Added 70 mL ethanol, mixed thoroughly, and diluted to volume with ethanol. The solution was shaken well before use.

EG & DEG Stock Solution-A:

Transferred 100.4 mg of Ethylene Glycol and 102.1 mg of Diethylene Glycol into a 100 mL volumetric flask. Dissolved in 70 mL of ethanol and diluted to volume with ethanol. The solution was shaken well to ensure homogeneity.

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Standard Solution-A:

5 mL of EG & DEG Stock Solution-A was diluted to 50 mL with ethanol, shaken well, and injected.

EG & DEG Stock Solution-B:

Prepared similarly using 101.1 mg of EG and 101.9 mg of DEG in ethanol to a final volume of 100 mL.

Standard Solution B:

5 mL of EG & DEG Stock Solution-B was diluted to 50 mL with ethanol and injected.

System Suitability Solution (EG, DEG & Propylene Glycol):

Combined 5 mL each of Propylene Glycol and EG & DEG stock solutions in a 50 mL volumetric flask. Diluted to volume with ethanol and injected to verify suitability.

System Suitability Results

Table 5: System Suitability Parameters

Parameter	**Analyte**	**Observation**	**Acceptance Criteria**
Similarity Factor (Standard A vs. B)	Ethylene Glycol	1.01	NLT 0.85 and NMT 1.15
	Diethylene Glycol	1.02	NLT 0.85 and NMT 1.15
Resolution between EG and Propylene Glycol peaks	-	12.96	NLT 10
% RSD of Area (n=6)	Ethylene Glycol	2.2	NMT 15%
	Diethylene Glycol	4.4	NMT 15%
% RSD of Retention Time (n=6)	Ethylene Glycol	0.02	NMT 2%
	Diethylene Glycol	0.05	NMT 2%
Tailing Factor	Ethylene Glycol	1.7	NMT 3.5
	Diethylene Glycol	2.3	NMT 3.5

System Precision Data

Table 6: Precision – Retention Time and Peak Area (n = 6)

Injection	**Ethylene Glycol**		**Diethylene Glycol**	
	Retention Time (min)	Area	Retention Time (min)	Area
Standard Solution_01	5.988	190,875	17.174	190,241
Standard Solution_02	5.990	191,365	17.192	195,484
Standard Solution_03	5.989	191,505	17.192	191,174
Standard Solution_04	5.988	191,949	17.173	192,114
Standard Solution_05	5.988	193,698	17.174	189,143
Standard Solution_06	5.989	196,172	17.189	194,394
Average	**5.989**	**192,594**	**17.182**	**192,092**
% RSD	**0.01**	**1.0**	**0.06**	**1.3**

Conclusion

- All **system suitability parameters** complied with pre-defined acceptance criteria, indicating a stable and reliable chromatographic system.
- The **% Relative Standard Deviation (RSD)** for peak areas of Ethylene Glycol and Diethylene Glycol in six replicate injections was found to be **1.0%** and **1.3%**, respectively, well within the acceptable limit of **15%**.

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- The **% RSD for retention times** was **0.01%** for EG and **0.06%** for DEG, which is significantly below the **2%** acceptance threshold.
- These results confirm the **system precision** of the method for simultaneous estimation of EG and DEG in the matrix.

Method Precision

Method precision, or repeatability, was evaluated by preparing and analyzing six individual spiked sample solutions of **Wet Cough Syrup** from a single batch. Each sample was spiked with known concentrations of Ethylene Glycol (EG) and Diethylene Glycol (DEG), and the % Relative Standard Deviation (%RSD) for both analytes was calculated to assess the consistency of the method under normal operating conditions.

Preparation of Solutions

Propylene Glycol Stock Solution:

Accurately weighed 201.2 mg of Propylene Glycol into a 100 mL volumetric flask. Added ~70 mL ethanol, mixed well, and made up to volume with ethanol. The solution was shaken thoroughly.

EG & DEG Stock Solution-A:

Transferred 100.4 mg of Ethylene Glycol and 102.1 mg of Diethylene Glycol into a 100 mL volumetric flask, dissolved in ethanol (70 mL), and made up to volume with ethanol. The solution was shaken well.

Standard Solution-A:

5 mL of EG & DEG Stock Solution-A was diluted to 50 mL with ethanol, mixed, and injected.

EG & DEG Stock Solution-B:

Weighed 101.1 mg of EG and 101.9 mg of DEG into a 100 mL flask, prepared as above.

Standard Solution B:

5 mL of Stock Solution-B was diluted to 50 mL with ethanol and injected.

System Suitability Solution (EG, DEG & Propylene Glycol):

5 mL each of EG & DEG and Propylene Glycol stock solutions were diluted to 50 mL with ethanol, mixed well, and injected.

Preparation of Spiked Sample Solutions

Six individual spiked samples were prepared by weighing approximately 10 g of cough syrup and adding 10 mL of EG & DEG Standard Stock Solution-A, followed by dilution to 100 mL with ethanol.

Table 7: Spiked Sample Preparations

Sample Name	Weight (g)	Stock Solution Added (mL)	Final Volume (mL)	Remarks
Spiked Sample Solution_1	10.0390	10	100	Prepared as per Section 6.7
Spiked Sample Solution_2	10.1637	10	100	
Spiked Sample Solution_3	10.0226	10	100	
Spiked Sample Solution_4	10.0335	10	100	
Spiked Sample Solution_5	10.0069	10	100	
Spiked Sample Solution_6	10.1333	10	100	

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Results

System Suitability Evaluation

Table 8: System Suitability Parameters

Parameter	Analyte	Observation	Acceptance Criteria
Similarity Factor (Standard A vs. B)	Ethylene Glycol	1.01	NLT 0.85 and NMT 1.15
	Diethylene Glycol	1.02	NLT 0.85 and NMT 1.15
Resolution between EG and Propylene Glycol peaks	-	12.96	NLT 10
% RSD of Area (n=6)	Ethylene Glycol	1.3	NMT 15%
	Diethylene Glycol	5.4	NMT 15%
% RSD of Retention Time (n=6)	Ethylene Glycol	0.01	NMT 2%
	Diethylene Glycol	0.06	NMT 2%
Tailing Factor	Ethylene Glycol	1.7	NMT 3.5
	Diethylene Glycol	2.3	NMT 3.5

Method Precision – Spiked Sample Results

Table 9: Recovery and %RSD of Spiked Samples (n = 6)

Sample	Ethylene Glycol (% Content)	Recovery (%)	Diethylene Glycol (% Content)	Recovery (%)
Spiked Sample 1	0.103	103.0	0.113	112.5
Spiked Sample 2	0.108	107.8	0.117	117.1
Spiked Sample 3	0.110	109.6	0.118	118.2
Spiked Sample 4	0.109	109.1	0.119	118.6
Spiked Sample 5	0.110	109.8	0.119	119.1
Spiked Sample 6	0.109	109.1	0.119	119.3
Average	**0.108**	**108.1**	**0.117**	**117.5**
% RSD	**2.4**	**2.4**	**2.2**	**2.2**

Conclusion

- All **system suitability parameters** were within acceptable limits, confirming chromatographic system performance.
- The %RSD of **Ethylene Glycol** and **Diethylene Glycol** content across six replicate spiked samples was **2.4%** and **2.2%**, respectively—both significantly below the acceptable limit of **15%**.
- Recovery values for both analytes ranged between **103.0% to 119.3%**, indicating the method is accurate for the quantification of EG and DEG at the specified concentration levels.
- These results confirm that the method is precise and reproducible for the analysis of Ethylene Glycol and Diethylene Glycol in **Wet Cough Syrup**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for **Ethylene Glycol (EG)** and **Diethylene Glycol (DEG)** were determined to establish the sensitivity of the analytical method. These parameters were calculated based on the residual standard deviation of the response and the slope of the calibration curve generated from standard solutions in the concentration range of 5% to 30% of the standard concentration.

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Predicted LOD and LOQ Values Table 10: Calculated LOD and LOQ

Analyte	LOD (% w/w)	LOQ (% w/w)
Ethylene Glycol	0.001	0.003
Diethylene Glycol	0.001	0.003

Preparation of Solutions

Propylene Glycol Stock Solution:

201.2 mg of Propylene Glycol was dissolved in ethanol and made up to 100 mL.

EG & DEG Stock Solution-A:

100.4 mg of EG and 102.1 mg of DEG were dissolved in ethanol and made up to 100 mL.

Standard Solution-A & B:

5 mL of the stock solution was diluted to 50 mL with ethanol.

System Suitability Solution:

5 mL each of Propylene Glycol and EG/DEG stock solutions were diluted to 50 mL.

LOD Solution:

10.0245 g of placebo was spiked with 1.0 mL of Standard Stock Solution-A, sonicated, diluted to 100 mL with ethanol, filtered (0.45 µm), and injected.

LOQ Solution:

10.0359 g of placebo was spiked with 3.0 mL of Standard Stock Solution-A, processed as above, and injected.

Results

System Suitability

Table 11: System Suitability Criteria

Parameter	Analyte	Observation	Acceptance Criteria
Similarity Factor (Standard A vs. B)	EG / DEG	1.01 / 1.02	NLT 0.85 – NMT 1.15
Resolution (EG vs. Propylene Glycol)	-	12.96	NLT 10
% RSD of Peak Area (n=6)	EG / DEG	2.2 / 4.4	NMT 15%
% RSD of Retention Time (n=6)	EG / DEG	0.02 / 0.05	NMT 2%
Tailing Factor	EG / DEG	1.7 / 2.3	NMT 3.5

Limit of Detection (LOD) Table 12: LOD S/N Ratios

Injection	EG S/N Ratio	DEG S/N Ratio
LOD_1	20.07	7.05
LOD_2	16.55	6.68
LOD_3	15.55	7.40

Observation: Signal-to-noise ratios for both analytes exceed 3, confirming detectability at the LOD level.

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Limit of Quantitation (LOQ)

Table 13: LOQ Precision and S/N Ratios (n = 6)

Injection	EG RT (min)	EG Area	EG S/N	DEG RT (min)	DEG Area	DEG S/N
LOQ_1	6.004	51,538	70.76	17.193	65,948	25.28
LOQ_2	6.004	50,179	72.07	17.194	66,718	25.98
LOQ_3	6.003	52,145	70.74	17.189	66,128	25.20
LOQ_4	6.002	50,413	72.44	17.191	64,528	25.39
LOQ_5	6.002	50,445	69.07	17.199	67,413	24.82
LOQ_6	6.001	52,243	73.01	17.187	64,636	25.26
Average	**6.003**	**51,161**	—	**17.181**	**65,895**	—
%RSD	**0.02**	**1.8**	—	**0.02**	**1.7**	—

Conclusion

- The **system suitability parameters** met all acceptance criteria, validating the chromatographic system's performance.
- The **S/N ratio for LOD** was consistently **> 3**, and for **LOQ**, it was **> 10**, satisfying regulatory thresholds.
- The **%RSD of area** for EG and DEG in six injections at the LOQ level was **1.8%** and **1.7%**, respectively, well below the 15% threshold.
- The **%RSD of retention time** was **0.02%** for both EG and DEG, confirming consistent chromatographic behaviour.
- These results affirm the method's **sensitivity and precision** at low analyte concentrations.

Accuracy / Recovery

The accuracy of the developed gas chromatographic method was evaluated for the estimation of **Ethylene Glycol (EG)** and **Diethylene Glycol (DEG)** in *Natural Cough Syrup*. Accuracy studies were conducted by recovery experiments at four levels: **LOQ, 50%, 100%, and 150%** of the specification concentration. Recovery was calculated by comparing the measured concentration of spiked samples with the theoretical concentration.

Preparation of Solutions

Propylene Glycol Stock Solution:

201.2 mg of Propylene Glycol was transferred into a 100 mL volumetric flask, dissolved in 70 mL of ethanol, and the volume was made up with ethanol. The solution was mixed thoroughly.

EG & DEG Stock Solution-A:

100.4 mg of EG and 102.1 mg of DEG were weighed and similarly diluted to 100 mL with ethanol.

Standard Solutions A and B:

5 mL of EG & DEG stock solution was diluted to 50 mL with ethanol and mixed well before injection.

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System Suitability Solution:

A mixture of 5 mL of Propylene Glycol stock solution and 5 mL of EG & DEG stock solution was diluted to 50 mL with ethanol and injected.

Spiking Stock Solution:

107.4 mg of EG and 108.3 mg of DEG were dissolved and diluted to 100 mL with ethanol to prepare the standard stock solution for spiking.

Sample Solutions:

The placebo matrix was spiked with the standard stock solution at defined volumes to achieve

the desired % levels. Sample weights and spiking details are summarised in the respective tables below.

Results

System Suitability

Table 14: System Suitability Parameters

Parameter	Analyte	Observation	Acceptance Criteria
Similarity Factor (Standard A vs. B)	EG / DEG	1.01 / 1.02	0.85 – 1.15
Resolution between the EG and Propylene Glycol peaks	-	12.96	NLT 10
% RSD of peak areas (n = 6)	EG / DEG	2.3 / 6.7	NMT 15%
% RSD of retention time (n = 6)	EG / DEG	0.03 / 0.06	NMT 2%
Tailing Factor	EG / DEG	1.7 / 2.3	NMT 3.5

Accuracy / Recovery of Ethylene Glycol Table 15: Accuracy of Ethylene Glycol

Level	Sample No.	Area	Recovery (%)	Mean Recovery (%)	RSD (%)
As Such	1	ND	NA	NA	NA
	2	ND	NA		
	3	ND	NA		
LOQ	1	73517	118.9	115.1	3.0
	2	69462	112.4		
	3	70405	113.9		
50%	1	108457	105.3	105.7	0.4
	2	109052	108.9		
	3	109254	106.1		
100%	1	211092	102.9	103.3	0.5
	2	212776	103.3		
	3	213907	103.8		
150%	1	322894	104.5	102.5	1.7
	2	314748	101.8		
	3	312999	101.3		

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Accuracy / Recovery of Diethylene Glycol Table 16: Accuracy of Diethylene Glycol

Level	Sample No.	Area	Recovery (%)	Mean Recovery (%)	RSD (%)
As Such	1	ND	NA	NA	NA
	2	ND	NA		
	3	ND	NA		
LOQ	1	72948	119.3	118.0	1.3
	2	71138	116.4		
	3	72305	118.3		
50%	1	113281	111.2	111.3	1.1
	2	114683	112.6		
	3	112098	110.0		
100%	1	219952	107.9	109.3	1.1
	2	224189	110.0		
	3	224130	110.0		
150%	1	341233	111.6	109.7	1.5
	2	333045	109.0		
	3	331845	108.6		

Conclusion

- System suitability parameters were found to be within acceptance limits.
- EG and DEG were not detected in the unspiked (as such) samples, confirming specificity.
- At the LOQ level, recovery for both EG and DEG was within **70–130%**, as expected.
- At 50%, 100%, and 150% spiking levels, the recovery ranged from **80–120%**, under ICH Q2(R1) guidelines.
- %RSD for each level was less than 15%, confirming **repeatability and reliability** of the recovery procedure.

Solution Stability

The **solution stability** of the *as-such* sample and spiked sample solutions was evaluated by comparing the chromatographic responses of freshly prepared solutions with those stored in the autosampler tray over a period of 24 hours. Both **Ethylene Glycol (EG)** and **Diethylene Glycol (DEG)** were monitored for any significant change in content or chromatographic behaviour.

Preparation of Solutions

- **Propylene Glycol Stock Solution**

201.7 mg of Propylene Glycol was accurately weighed into a 100 mL volumetric flask. Approximately 70 mL of ethanol was added, the solution was mixed thoroughly, and the final volume was made up with ethanol.

- **EG & DEG Stock Solution-A**

100.9 mg of Ethylene Glycol and 100.7 mg of Diethylene Glycol were dissolved and diluted to 100 mL with ethanol.

- **Standard Solutions A & B**

5 mL of EG & DEG stock solution was diluted to 50 mL with ethanol, mixed well, and injected.

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- **System Suitability Solution**

A mixture of 5 mL each of Propylene Glycol stock solution and EG & DEG stock solution was diluted to 50 mL with ethanol, mixed thoroughly, and injected.

- **As Such Sample Solution**

10.0138 g of sample was weighed into a 100 mL volumetric flask. Ethanol was added (approximately 70 mL), and the solution was sonicated for 10 minutes. The final volume was made up with ethanol, filtered using a 0.45 µm syringe filter, and injected.

- **Spiked Sample Solution**

10.0194 g of sample was weighed and spiked with 10 mL of standard stock solution in a 100 mL volumetric flask. Ethanol was added, and the solution was sonicated for 10 minutes. The final volume was adjusted with ethanol, filtered, and injected.

Results

System Suitability

Table 17: System Suitability Parameters

Parameter	Analyte	Observation	Acceptance Criteria
Similarity Factor (Standard A vs. B)	EG / DEG	0.99 / 0.98	0.85 – 1.15
Resolution between EG and Propylene Glycol	-	12.82	NLT 10
% RSD of Peak Area (n = 6)	EG / DEG	4.3 / 6.2	NMT 15%
% RSD of Retention Time (n = 6)	EG / DEG	0.03 / 0.03	NMT 2%
Tailing Factor	EG / DEG	1.7 / 1.9	NMT 3.5

Stability Study Results

Table 18: Stability of Ethylene Glycol

Time Point	Area	% Difference	Content (%)	% Difference
Initial	210958	NA	0.110	NA
2 hours	214301	-1.57	0.111	-0.90
4 hours	210759	0.09	0.110	0.00
8 hours	211691	-0.35	0.110	0.00
12 hours	216628	-2.65	0.113	-2.69
18 hours	226174	-6.96	0.118	-7.02
24 hours	227025	-7.34	0.118	-7.02

Table 19: Stability of Diethylene Glycol

Time Point	Area	% Difference	Content (%)	% Difference
Initial	218163	NA	0.112	NA
2 hours	224838	-3.01	0.115	-2.64
4 hours	212976	2.41	0.109	2.71
8 hours	217302	0.40	0.111	0.90
12 hours	214104	1.88	0.110	1.80
18 hours	224205	-2.73	0.115	-2.64
24 hours	226155	-3.60	0.116	-3.51

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Conclusion

- All **system suitability parameters** met the acceptance criteria, indicating a robust and reproducible method.
- The **solution stability** of both Ethylene Glycol and Diethylene Glycol was confirmed for a minimum of 24 hours.
- The observed **differences in content and area responses** were within ±15%, demonstrating solution stability as per analytical validation norms.
- No extraneous or degradant peaks were observed in any chromatogram.
- The % relative difference between initial and periodic interval results remained well below 60%, confirming chemical stability of the analytes in the matrix.

FINAL CONCLUSION

Based on the results obtained from the method verification and validation study, the analytical method for the estimation of **Ethylene Glycol** and **Diethylene Glycol** in *Naturals Cough Syrup* is verified and found to be suitable for its intended purpose. All evaluated parameters, including specificity, precision, accuracy, linearity (if applicable), LOD, LOQ, robustness, and solution stability, comply with standard regulatory expectations (ICH Q2(R1)).

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