RESEARCH PAPER

Estimation of Ethylene Glycol and Diethylene Glycol in Multicomponent Cough Syrup Containing Salbutamol, Bromhexine and Guaifenesin by GC-FID

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ABSTRACT

Background: Multicomponent cough syrup is indicated for the clearance of thick, purulent sputum associated with acute, chronic, and complicated respiratory conditions, including bronchial asthma, bronchiectasis, and whooping cough. Each 5 mL of Multicomponent cough syrup contains 2 mg of salbutamol, 4 mg of bromhexine hydrochloride, and guaifenesin. Salbutamol acts as a selective β_2-adrenergic agonist, producing bronchodilation by relaxing the bronchial smooth muscles without significantly affecting cardiac β_1-receptors. Bromhexine hydrochloride acts as a mucolytic agent, liquefying and loosening mucus to facilitate its removal from the respiratory tract. Guaifenesin, an expectorant, enhances the volume and decreases the viscosity of bronchial secretions, thereby aiding in the expulsion of mucus and improving airway clearance.

Aim: The purpose of this study was to develop and validate a simple, rapid, and cost-effective gas chromatographic method with flame ionization detection (GC-FID) for the simultaneous quantification of ethylene glycol (EG) and diethylene glycol (DEG) in Multicomponent cough syrup. The analytical procedure involved sample dilution with water and analysis using an Agilent 8890 GC system equipped with a ZB-WAX capillary column (30 m × 0.530 mm, 1.0 µm film thickness), with nitrogen as the carrier gas at a flow rate of 1.5 mL/min. The oven temperature program was optimized as follows: initial temperature 100°C (0 min hold), ramped at 7.8°C/min to 180°C (14 min hold), followed by 30°C/min to 245°C (15 min hold). The injector and detector temperatures were both maintained at 250°C. Under these conditions, the retention times for ethylene glycol and diethylene glycol were found to be 12.0 minutes and 21.0 minutes, respectively.

Results and Discussion: The developed GC-FID method was validated according to ICH guidelines, and the parameters such as accuracy, precision, linearity, and robustness were found to comply with the established acceptance criteria. The statistical evaluation of results confirmed the reliability, reproducibility, and suitability of the proposed method for routine quality control analysis.

Conclusion: The validated GC-FID method provides a reliable, efficient, and economical approach for the simultaneous estimation of ethylene glycol and diethylene glycol in Multicomponent cough syrup. This procedure can be effectively employed for routine analytical testing and quality assurance in pharmaceutical laboratories.

Keywords: Ethylene glycol, Diethylene glycol, Multicomponent cough syrup, GC-FID, Method validation

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Introduction

Ethylene glycol (EG) [Fig. 1] is a colorless, odorless, sweet-tasting liquid with low vapor pressure, minimizing inhalation exposure under ambient conditions. It is miscible with water, alcohol, and acetone and is produced industrially from ethylene via ethylene oxide hydrolysis. Ethylene glycol has a molecular formula of $C_2H_6O_2$ and a boiling point of 197.3°C. It is widely utilized in antifreeze formulations, de-icing agents, and as a raw material in the manufacture of plastics, solvents, and paints. However, its sweet taste makes it dangerous, as accidental ingestion—especially by children or animals—can lead to severe

toxicity. Once ingested, EG is metabolized into glycolic acid and oxalic acid, both of which are toxic metabolites responsible for central nervous system depression, cardiotoxicity, and renal failure.

Diethylene glycol (DEG) [Fig. 2] is a clear, colorless, hygroscopic liquid with a slightly sweet taste and is miscible with water, alcohol, and acetone. It is synthesized by the reaction of ethylene oxide with ethylene glycol and may contain trace amounts of EG as an impurity. The compound has a molecular formula of $C_4H_{10}O_3$ and a boiling point of 244–245°C. DEG is frequently used as an industrial solvent and antifreeze component. Due to its similar viscosity, sweetness, and cost-effectiveness compared to glycerol and propylene glycol, DEG has been illegally substituted in pharmaceutical formulations, posing serious toxicological hazards.

Exposure to ethylene glycol or diethylene glycol and their toxic metabolites initially affects the central nervous system, followed by cardiac and renal complications. Several mass poisoning incidents have been documented globally due to contamination of medicinal syrups containing these compounds—such as those reported in Uzbekistan, Argentina, Bangladesh, India, and Nigeria—resulting in acute kidney failure and numerous fatalities among children. In response, regulatory authorities, including the USP–NF, have established a maximum permissible limit (NMT 0.10%) for EG and DEG in pharmaceutical preparations to ensure product safety and patient protection.

MATERIALS AND METHODS

Chemicals and reagents:

Ethylene glycol and Diethylene glycol standards and Multicomponent cough syrup sample were provided by Oman Pharmaceutical Products Co L.L.C. LCMS grade water was procured from Fischer scientific.

Solution preparations:

Diluent:
Water

Preparation of Sample solution:
Weigh 5 mL of sample into a 20 mL volumetric flask, add 5 mL of diluent, vortex to mix well for 1minute. Make up final volume with diluent, vortex to mix well for 1minute, filter through Whatman 0.45µm nylon w/GMF syringe filter and discard the first 3 mL of filtrate.

Preparation of Standard Stock Solution-1:
Weigh accurately about 100 mg of Ethylene glycol (EG) and Diethylene glycol (DEG) reference / working standard in a 20 mL volumetric flask. Dissolve, dilute to volume with diluent and mix well.

Preparation of Standard Stock Solution-2:
Transfer 2 mL of Standard Stock Solution-1 into a 20 mL volumetric flask. Dilute to volume with diluent and mix well.

Preparation of Standard Solution:
Transfer 1 mL of Standard Stock Solution-2 into a 20 mL volumetric flask. Dilute to volume with diluent and mix well.

Note:
The concentration of Standard Solution would be 0.100% with respective glycerol concentration in test sample (Multicomponent cough syrup contains 500mg/5 mL of glycerol. Considering the maximum daily dose of Bronkovent syrup as 20 mL for adults, the total daily intake of glycerol would be 2000 mg. Therefore, the standard concentration will be 25µg/mL i.e., 0.10% w.r.t test concentration) for Ethylene glycol (EG) and Diethylene glycol (DEG).

Chromatographic study:
Ethylene glycol (EG) and Diethylene glycol (DEG) content in all solutions were determined by GC-FID by using the chromatographic conditions as mentioned in Table No- 1.
The Chromatographic data were analyzed and Specificity, Linearity and range, method precision, and accuracy were determined.

Results and discussion:
The developed method for estimation of Ethylene glycol and Diethylene glycol in Multicomponent cough syrup by GC-FID were validated by using the following parameters:

System suitability:
For establishing the system suitability, the procedure described in the methodology was followed before starting the analysis. System suitability data has been presented in Table No- 2.

Specificity:
Specificity of the method was evaluated with respect to

interference from blank at the retention time of each standard, Placebo of Multicomponent cough syrup, Standard solution and Multicomponent cough syrup sample (un-spiked and spiked) preparation. Refer Fig. 5, 6, 7 and 8 for the chromatograms and specificity data has been presented in Table No- 3.

Linearity and range:

Standard solution containing Ethylene glycol and Diethylene glycol were prepared. Linearity study was demonstrated for Ethylene glycol and Diethylene glycol in the concentration range from six different concentrations LOQ (0.036%), 50% (0.050%), 100% (0.100%), 200% (0.200%), 300% (0.300%) and 500% (0.500%) the targeted concentrations with respective glycerol concentration in test sample (Multicomponent cough syrup contains 500mg/5 mL of glycerol. Considering the maximum daily dose of Bronkovent syrup as 20 mL for adults, the total daily intake of glycerol would be 2000 mg. Therefore, the standard concentration will be 25µg/ mL i.e., 0.10% w.r.t test concentration) for Ethylene glycol (EG) and Diethylene glycol (DEG).

The linearity was evaluated using the calibration curve plotted by average peak areas against concentrations to calculate coefficient of correlation and slope. In general, a value of correlation coefficient (r) > 0.990 is considered as the evidence of an acceptable fit for the data to the regression line.

The results obtained are presented in Table No-4 and 5 which demonstrates that the current method was linear for the three analytes in the range specified above with a correlation coefficient better than 0.990. The plots have been represented in Fig. 3 and 4.

Method Precision:

Precision was determined by preparing the standard, sample and spiked sample as per the methodology. The sample and spiked sample was prepared in six replicates and injected into the chromatography system. The content of each preparation was calculated and finally the %RSD of the six replicate preparations data has been presented in Table No- 6 and 7.

Accuracy:

The Accuracy of the methodology at LOQ, 100% and 500% of the specification limit, samples were analyzed as per methodology and % recovery at each spiked level was calculated. Prepare LOQ (0.036%), 100% (0.100%) and 500% (0.500%) concentration levels in triplicate Results are presented below in Table No-. 8 and 9.

CONCLUSION

This intended study concludes that the proposed method is economical, simple, sensitive and reliable. Also, it is found to be specific, linear, precise and accurate. Hence, it can be employed for the routine estimation of Estimation of Ethylene glycol and Diethylene glycol in Multicomponent cough syrup by GC-FID

ACKNOWLEDGEMENT:

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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Table No.1

Column	ZB-WAX, 30 m x 0.53 mm, 1.0µm (Part No: 7HK-G007-22)
Column Oven	Rate/min (°C) / Temp.°C / Hold - / 100 / 0 minutes 7.8 / 180 / 14 minutes 30 / 245 / 15 minutes
Control mode	constant flow
Flow rate	1.5 mL/minute
Carrier gas	Nitrogen
Injection volume	1.0 µL
Injection mode	Split
Split ratio	5:1
Liner	Ultra inert, Low PSI drop, wool (p/n 5190-2295)
Front Inlet temperature	250°C
FID temperature	250°C
FID Make-up gas flow(N_2)	30 mL/minute
FID H_2 flow	40 mL/minute
FID Air flow	350 mL/minute
Data rate	20 Hz
Save	On
Syringe wash solvent- A & B	Diluent

Table No. 2: System suitability – Standard solution

Injection #	**Ethylene glycol**	**Diethylene glycol**
	Area	**Area**
1	200663	196756
2	195981	192082
3	201298	197189
4	199799	205377
5	200188	198147
6	202741	201359
Mean	**200112**	**198485**

SD	**2271.15580**	**4506.46757**
%RSD	**1.1**	**2.3**

Table No. 3: Interference studies

Observation			
Name of Solution		**Interference**	**RT (Min)**
Blank		No interference	NA
Ethylene glycol (EG)		No interference	12.00
Propylene glycol (PG)		No interference	11.37
Diethylene glycol (DEG)		No interference	20.99
Standard solution	Ethylene glycol (EG)	No interference	12.01
	Diethylene glycol (DEG)	No interference	21.02
Placebo of Bronkovent syrup	Ethylene glycol (EG)	BQL	12.01
	Diethylene glycol (DEG)	No interference	ND
Unspike Bronkovent Sample Solution	Ethylene glycol (EG)	BQL	12.01
	Diethylene glycol (DEG)	No interference	ND
Spike Bronkovent Sample Solution	Ethylene glycol (EG)	No interference	12.01
	Diethylene glycol (DEG)	No interference	21.00

BQL- Below Quantification limit
ND- Not detected

Table No. 4: Linearity of Ethylene glycol

Name of linearity solution	**Concentration (%)**	**Mean area**
LOQ Level	0.036	67776
Level-2 (0.050%)	0.051	95521
Level-3 (0.100%)	0.102	185752
Level-4 (0.200%)	0.204	377974
Level-5 (0.300%)	0.306	567642
Level-6 (0.500%)	0.510	973669
Slope		**1906566.034**
Intercept		**-6117.556**
Correlation coefficient		**0.999**

Table No. 5: Linearity of Diethylene glycol

Name of linearity solution	**Concentration (%)**	**Mean area**

LOQ Level	0.036	60274
Level-2 (0.050%)	0.051	94754
Level-3 (0.100%)	0.102	189819
Level-4 (0.200%)	0.204	387280
Level-5 (0.300%)	0.306	594562
Level-6 (0.500%)	0.510	1016923
Slope		**2009749.395**
Intercept		**-14362.920**
Correlation coefficient		**0.999**

Table No. 6: Method Precision – Unspiked sample

Date of Analysis	17/10/2024	
Instrument ID	QC/INS/246	
Column No.	173	
Analyst	Satish. Samireddi	
Batch No.	4EC031A	
Sample ID#	**EG (in %)**	**DEG (in %)**
1	0.008 (BDL)	ND
2	0.007 (BDL)	ND
3	0.006 (BDL)	ND
4	0.009 (BDL)	ND
5	0.008 (BDL)	ND
6	0.007 (BDL)	ND
Mean	**0.007 (BDL)**	**ND**
SD	**-**	**-**
%RSD	**-**	**-**

ND- Not detected
BDL- below Detection limit

Table No. 7: Method Precision – Spiked sample

Date of Analysis	17/10/2024	
Instrument ID	QC/INS/246	
Column No.	173	
Analyst	Satish. Samireddi	
Batch No.	4EC031A	
Sample ID#	**EG (in %)**	**DEG (in %)**
1	0.093	0.102
2	0.093	0.107
3	0.093	0.108
4	0.087	0.106

5	0.089	0.104
6	0.091	0.110
Mean	**0.091**	**0.106**
SD	**0.00253**	**0.00286**
%RSD	**2.8**	**2.7**

Table No. 8: Accuracy of Ethylene glycol

Name of Solution	Preparation	Amount recovered (%)	Amount added (%)	% Recovery	% Mean and % RSD Recovery level	
Accuracy at LOQ level	1	0.037	0.036	102.8	**Avg**	**103.7**
	2	0.036	0.036	100.0	**SD**	**4.22256**
	3	0.039	0.036	108.3	**%RSD**	**4.1**
Accuracy at Specification level (0.100%)	1	0.093	0.102	91.2	**Avg**	**91.2**
	2	0.093	0.102	91.2	**SD**	**0.00000**
	3	0.093	0.102	91.2	**%RSD**	**0.0**
Accuracy at Higher level (0.500%)	1	0.443	0.511	86.7	**Avg**	**87.6**
	2	0.444	0.511	86.9	**SD**	**1.38924**
	3	0.456	0.511	89.2	**%RSD**	**1.6**

Table No. 9: Accuracy of Diethylene glycol

Name of Solution	Preparation	Amount recovered (%)	Amount added (%)	% Recovery	% Mean and % RSD Recovery level	
Accuracy at LOQ level	1	0.035	0.036	97.2	**Avg**	**101.9**
	2	0.037	0.036	102.8	**SD**	**4.27707**
	3	0.038	0.036	105.6	**%RSD**	**4.2**
Accuracy at Specification level (0.100%)	1	0.102	0.102	100.0	**Avg**	**103.6**
	2	0.107	0.102	104.9	**SD**	**3.15753**
	3	0.108	0.102	105.9	**%RSD**	**3.0**
Accuracy at Higher level (0.500%)	1	0.476	0.512	93.0	**Avg**	**93.8**
	2	0.481	0.512	93.9	**SD**	**0.75498**
	3	0.484	0.512	94.5	**%RSD**	**0.8**

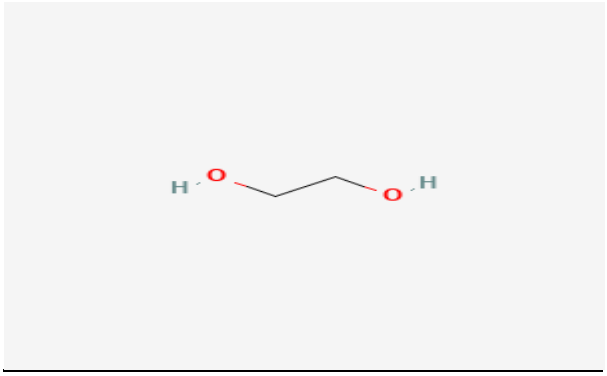

Figure 1 Chemical structure of Ethylene glycol

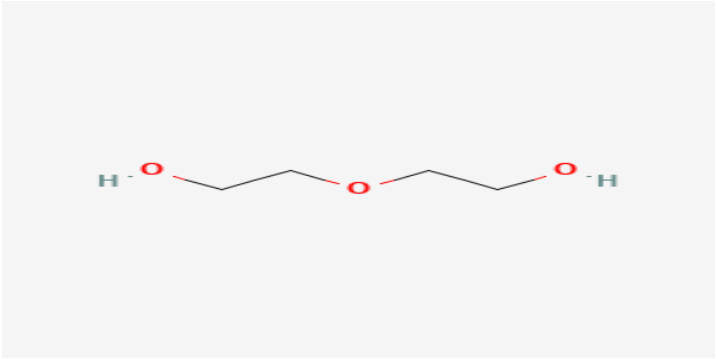

Figure 2 Chemical structure of Diethylene glycol

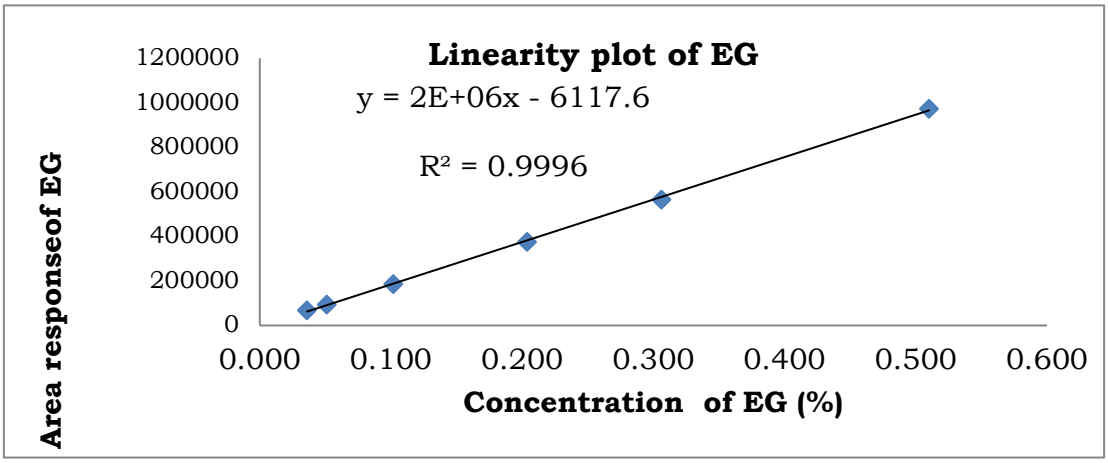

Figure 3: Linearity of Ethylene glycol

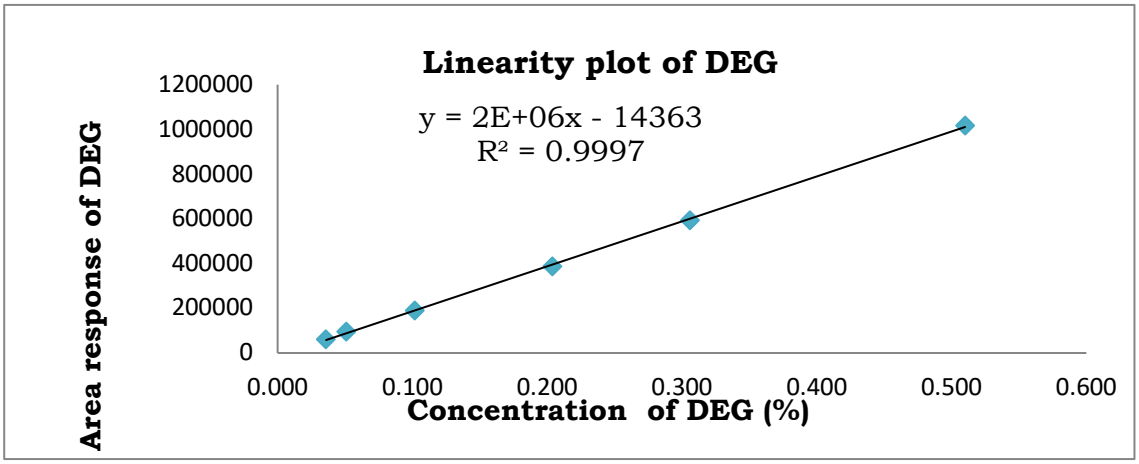

Figure 4: Linearity of Diethylene glycol

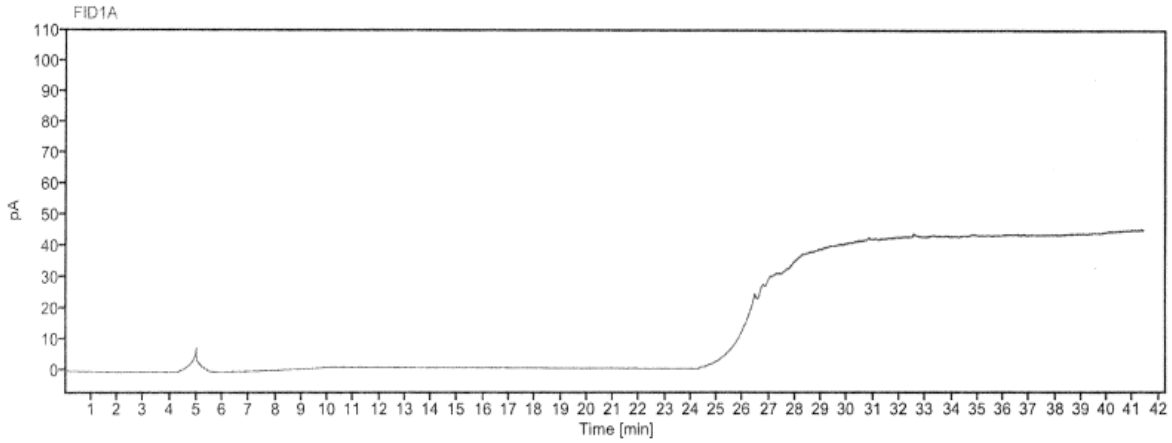

Figure 5: Blank Chromatogram

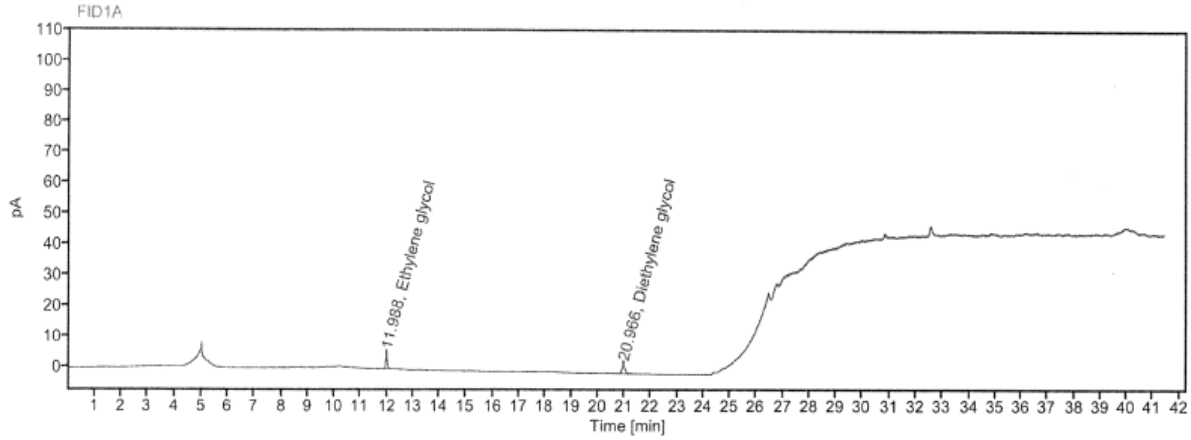

Figure 6: Standard Chromatogram

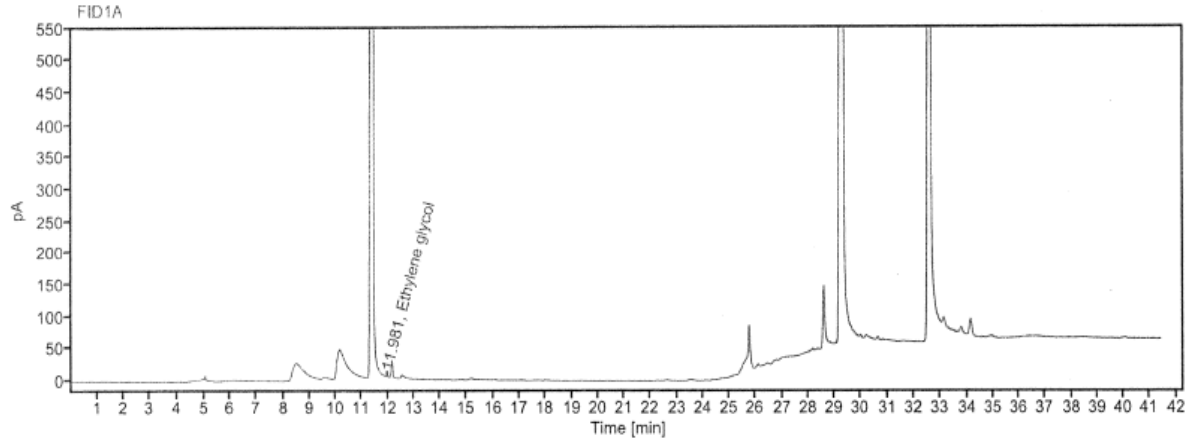

Figure 7: Sample Chromatogram

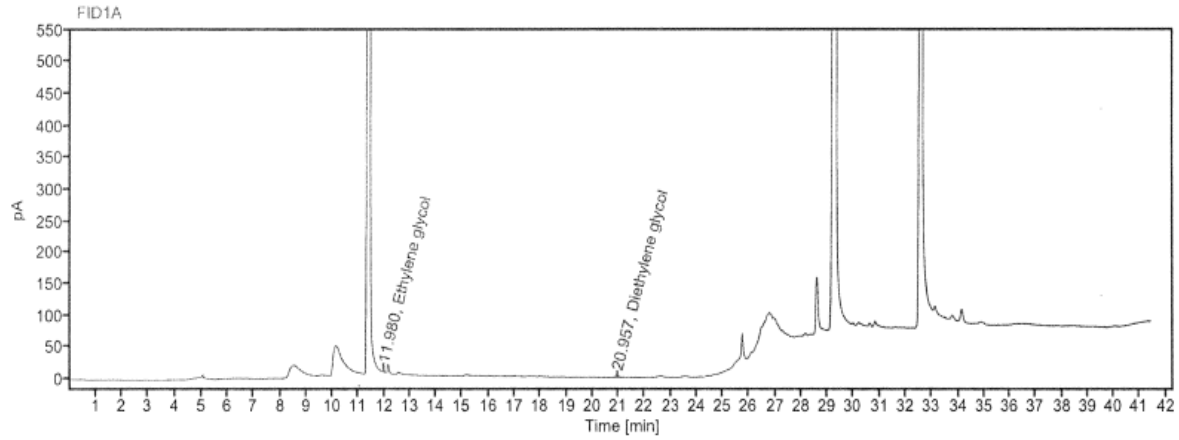

Figure 8: Spiked sample Chromatogram