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Application News

Brevis™ GC-2050 Gas Chromatograph

Analysis of Ethylene Glycol and Diethylene Glycol in Glycerin, Propylene Glycol and Sorbitol Solutions (Indian Pharmacopoeia Monographs)

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User Benefits

- Brevis GC offers advanced capabilities for the detection of EG and DEG contamination in the raw materials (glycerin, propylene glycol, and sorbitol solutions) used for manufacturing medicinal syrup.
- Brevis GC coupled with the AOC-30i autosampler can obtain highly reproducible results using the proposed workflow.
- The configuration proposed in the application news exceeds the requirement specified in the IP monographs.

■ Introduction

Ethylene glycol (EG) and diethylene glycol (DEG) are classified as toxic alcohols. These chemicals are colorless, odorless, and water-soluble. There have been multiple incidents involving the deaths of children due to medicinal cough syrups contaminated with these impurities. These impurities often originated from the raw materials (such as glycerin, propylene glycol, and sorbitol solutions) or excipients used in their formulation (1-2). Substandard and falsified raw materials frequently enter the excipient supply chain, causing significant challenges for drug manufacturers and regulatory bodies. The complexity of the global supply chain, which often involves importing raw materials from various countries, worsened this issue. Therefore, it is crucial to frequently test these raw materials upon receipt to prevent the reoccurrence of such incidents and protect public health.

This application news demonstrates the use of GC-FID for analyzing EG and DEG contaminants in glycerin, propylene glycol (PG), and sorbitol solutions (70%) (both crystallizing and non-crystallizing) in accordance with the Indian Pharmacopoeia (IP) monographs. Shimadzu's Brevis GC, featuring a compact design with a width that is approximately 35% smaller than existing GC models, is used in this experiment (Fig. 1). This design allows the Brevis GC to occupy less space in the laboratory and to be eco-friendly with reduced power consumption, while maintaining analytical performance and enhancing productivity.

Fig. 1 Shimadzu's Brevis™ GC with AOC™-30i

■ Analytical Conditions

Table 1 shows the analytical conditions utilized for the analysis, in accordance with IP monographs. A notable modification was implemented to the injection volume used for analyzing glycerin and PG. The injection volume was reduced from 1.0 µL to 0.5 µL to avoid the risk of backflash that could lead to contaminate the GC system. Additionally, in the sorbitol solutions analysis, the final column oven temperature was adjusted to 270 °C (instead of 300 °C) due to the column-recommended specification.

Table 1 System configuration and analytical conditions for the analysis of EG and DEG in glycerin, propylene glycol, and sorbitol solution

System Configuration			
Raw Material	**Glycerin**	**Propylene Glycol (PG)**	**Sorbitol Solution (Crystallising & Non-Crystallising)**
GC System	Brevis™ GC		
Auto Injector	AOC™-30i		
Syringe	5 µL syringe [P/N: 221-75173]		
Column	SH-1701 30 m x 0.32 mm I.D. x 1.0 µm df [P/N: 221-75782-30]	SH-624 30 m x 0.53 mm I.D. x 3.0 µm df [P/N: 221-75865-30]	SH-1701 15 m x 0.32 mm I.D. x 0.25 µm df [P/N: 221-75780-15]
Injector Parameters			
Injection Mode	Split mode (using deactivated split glass insert with glass wool)		
Split Ratio	20	10	10
Injection Temperature	220 °C	220 °C	240 °C
Injection Volume	0.5 µL	0.5 µL	1.0 µL
Carrier Gas	Nitrogen		
Flow Control Mode	Linear velocity		
Flow Rate at Initial Oven Temp.	2.05 mL/min	4.5 mL/min	3.0 mL/min
Linear Velocity	38 cm/sec	38.1 cm/sec	56.2 cm/sec
GC Oven Parameters			
Column Oven Temperature Program	• Initial temp. 100 °C • Increase to 220 °C with a rate of 7.5 °C/min (hold 10 min)	• Initial temp. 100 °C (hold 4 min) • Increase to 120 °C with a rate of 50 °C/min (hold 10 min) • Increase to 220 °C with a rate of 50 °C/min (hold 6 min)	• Initial temp. 70 °C (hold 2 min) • Increase to 270 °C with a rate of 50 °C/min (hold 5 min)
FID Parameter			
Detector (FID) Temp.	250 °C	250 °C	300 °C
Makeup Gas	N_2 24 mL/min		
Detector Gas	H_2 32 mL/min, Air 200 mL/min		

■ Preparation of Reference Solutions

All the reference solutions required for each raw material were prepared in accordance with the respective IP monographs.

Glycerin Reference Solution (a) (50 ppm)

Reference solution (a) was prepared in methanol with a final concentration of 0.005 % w/v for each of glycerin, EG, and DEG.

Glycerin Reference Solution (b) (500 ppm)

Reference solution (b) was prepared in methanol with a final concentration of 0.05 % w/v for each of glycerin, EG, and DEG.

PG Reference Solution

Reference solution was prepared in methanol with a final concentration of 0.2 % w/v PG, 0.01 % w/v 2,2,2-trichloroethanol (Internal Standard, IS), 0.005 % w/v EG and 0.005 % w/v DEG.

Sorbitol Reference Solution (for Crystallising and Non-crystallising 70 % Sorbitol Solution)

Solvent mixture was first prepared by mixing acetone and water (96:4). The reference solution was prepared in solvent mixture with a final concentration of 0.008 % w/v EG and 0.008% w/v DEG.

[Note: Both crystallising & non-crystallising sorbitol solutions (70 %) use the same reference solution]

■ Preparation of Test Solutions

All the raw materials for glycerin, PG, sorbitol solution (70 %) (crystallising), and sorbitol solution (70 %) (non-crystallising) were prepared in accordance with its respective IP monographs.

Glycerin Test Solution

Test solution was prepared in methanol with a final concentration of 5.0 % w/v glycerin raw sample.

PG Test Solution

Test solution was prepared in methanol with a final concentration of 5.0 % w/v of PG raw sample and 0.01 % w/v of 2,2,2-trichloroethanol (Internal Standard, IS).

Sorbitol Test Solutions (for Crystallising and Non-crystallising 70 % Sorbitol Solution)

Each test solvent was prepared by adding 2.0 g of sorbitol solution in 25 mL volumetric flask, followed by 1.0 mL solvent mixture (acetone and water, 96:4) with 3 minutes vortexed. Adding the remaining portion of solvent mixture (split into 3 portions) to fill up the flask, with about 3 minutes vortexed after each addition. Finally, filtered the supernatant layer with a 0.45 µm nylon filter.

[Note: Test solutions for both crystallising & non-crystallising sorbitol solution (70 %) were prepared using the same method]

■ IP Requirements

Compliance of IP monograph for these raw materials (glycerin, propylene glycol, and sorbitol solution) includes a limit test of ethylene glycol (EG) and diethylene glycol (DEG) as part of the identity test. The IP monographs specify system suitability requirements and acceptance criteria for EG and DEG for each raw material (Table 2). Glycerin requires the use of two reference solutions to fulfill the system suitability test. The acceptance criteria for EG or DEG in each raw material are set not to exceed 0.10 %, determined by comparing their peaks with those of the reference solution.

Table 2 IP requirements for the analysis of EG and DEG in glycerin, propylene glycol, and sorbitol solution

IP Requirements	Glycerin	Propylene Glycol (PG)	Sorbitol Solution (Crystallising & Non-Crystallising)
Resolution	≥ 40 (between EG and DEG)*1 ≥ 10 (between DEG and glycerin)*1	≥ 5 (between EG and PG)	≥ 30 (between EG and DEG)
Repeatability (%RSD)	≤ 10 %*2	-	-
Acceptance Criteria	Peak area of EG or DEG in the test solution is not more than the EG or DEG peak area in the reference solution (a) (corresponds to ≤ 0.10 % of EG or DEG in the sample)	Peak response ratio of EG or DEG in the test solution is not more than the EG or DEG peak response ratio in the reference solution (corresponds to ≤ 0.10 % of EG or DEG in the sample)	Peak area of EG or DEG in the test solution is not more than the EG or DEG peak area in the reference solution (corresponds to ≤ 0.10 % of EG or DEG in the sample)

*1 Denotes reference solution (b)
*2 Denotes reference solution (a)

■ Results and Discussion

System Suitability

The System Suitability Test (SST) requirements for IP are listed in Table 2 for each raw material. Achieving good peak separation and repeatable chromatograms are crucial to meet IP requirements and accurately determine the peak areas of target analytes. Fig. 2 illustrates well-separated peaks with good peak shape for all three raw material reference solutions. The overlay chromatograms of six repeatability injections of the reference solution displayed highly repeatable results, demonstrating the stability of Brevis GC oven in achieving consistent results.

Glycerin Reference Solution

Reference Solution (a), containing 50 ppm of each analyte, was used to assess repeatability and served as the reference solution for peak area comparisons. The retention times (RT) for EG, DEG, and glycerin were observed as 3.0, 6.6, and 7.8 min, respectively (Table 3). These RTs align with the IP monograph elution order, where EG peak elutes first, followed by DEG and then glycerin. The repeatability of peak area (n=6) for EG, DEG, and glycerin were obtained with %RSD of 0.44%, 1.36%, and 1.00%, respectively (Table 4).

Reference Solution (b) containing 500 ppm of each analyte was used to determine the peak resolution between specific analytes as specified in the IP monograph. The average resolution between the EG and DEG peaks was 63, and between DEG and glycerin peaks was 18 (Table 5), thus meeting the IP requirement of not less than 40 and 10, respectively.

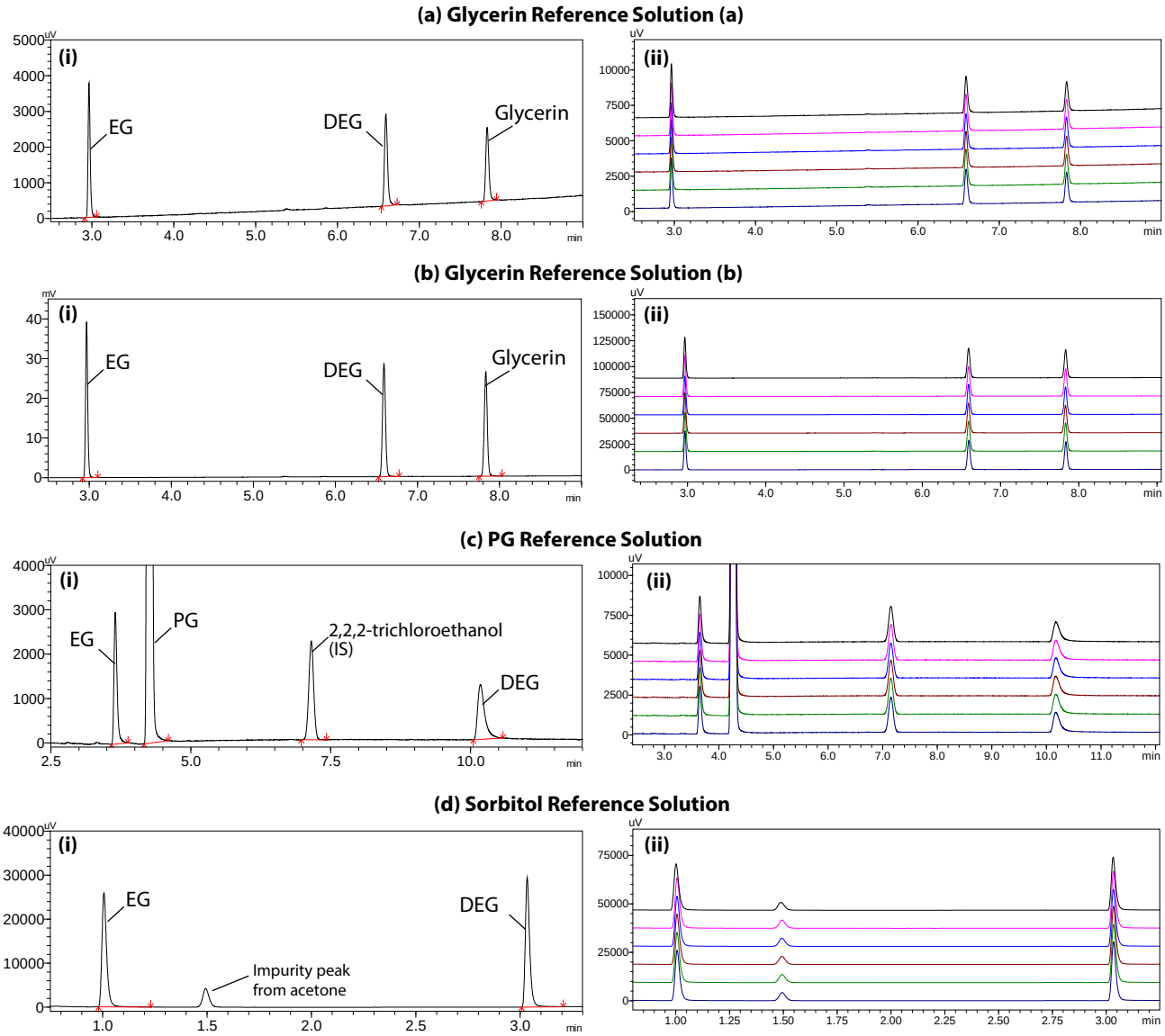

Fig. 2 **(i)** Chromatograms of ethylene glycol (EG) and diethylene glycol (DEG) in **(a)** glycerin reference solution (a), **(b)** glycerin reference solution (b), **(c)** PG reference solution, and **(d)** sorbitol reference solution; **(ii)** Overlay chromatograms of the respective reference solution in each raw material (*n*=6)

Table 3 Relative retention times (RRT) and retention times (RT) of reference solution of glycerin, PG, and sorbitol solution

Reference Solution	**Glycerin Reference Solution (a) (RT*)/min**			**PG (RRT⁺)**				**Sorbitol Solution (RT*)/min**	
Injection	EG	DEG	Glycerin	EG	PG	IS#	DEG	EG	DEG
1	3.0	6.6	7.8	0.9	1.0	1.7	2.4	1.0	3.0
2	3.0	6.6	7.8	0.9	1.0	1.7	2.4	1.0	3.0
3	3.0	6.6	7.8	0.9	1.0	1.7	2.4	1.0	3.0
4	3.0	6.6	7.8	0.9	1.0	1.7	2.4	1.0	3.0
5	3.0	6.6	7.8	0.9	1.0	1.7	2.4	1.0	3.0
6	3.0	6.6	7.8	0.9	1.0	1.7	2.4	1.0	3.0
IP reference elution order	EG, DEG, then glycerin			0.8	1.0	1.7	2.4	EG then DEG	

*Denotes Retention Time. ⁺Denotes Relative Retention Time. #Denotes Internal Standard.

PG Reference Solution

The relative retention time (RRT) of EG, PG, IS, and DEG were observed at 0.9, 1.0, 1.7, and 2.4, respectively (Table 3). The RRT values for all analytes were matched with IP monograph guidelines, except for EG, which deviated by 0.1. The slight deviation is within expected limits due to column-to-column variations. The repeatability of the peak response ratio for EG/IS and DEG/IS had %RSD of 0.90% and 1.44%, respectively (Table 4). The average resolution between EG and PG peaks was found as 6.5 (Table 5), exceeding the IP requirement of not less than 5.0.

Sorbitol Reference Solution (for 70% Sorbitol Solution)

The IP monographs for both crystallising sorbitol solution (70 %) and non-crystallising sorbitol solution (70 %) use the same analytical conditions (Table 1) and Reference Solution, sharing the same SST and acceptance criteria (Table 2). Therefore, one reference solution is prepared for both crystallising and non-crystallising sorbitol solutions. The RTs for EG and DEG peaks were eluted at 1.0 and 3.0 min, respectively (Table 3), align with the IP monograph elution order where DEG elutes after EG peak.

In term of repeatability, %RSD of EG and DEG peak areas were 0.59% and 0.91%, respectively (Table 4). Meanwhile, the average resolution between the EG and DEG peaks was observed as 51.8 (Table 5), meeting the IP requirement of not less than 30.0.

Based on the generated results for all raw materials, the workflow with Brevis GC demonstrated high degree of precision, excellent repeatability, and peak resolution, meeting IP requirements for EG and DEG impurities in these raw materials.

Table 4 Repeatability (*n*=6) of reference solution for glycerin, PG, and sorbitol solution

Reference Solution	**Glycerin (Reference Solution (a))**			**PG**		**Sorbitol Solution**	
Injection	Peak Area (EG)	Peak Area (DEG)	Peak Area (Glycerin)	Peak Response Ratio (EG/IS#)	Peak Response Ratio (DEG/IS#)	Peak Area (EG)	Peak Area (DEG)
1	6,774	6,800	5,507	0.837	0.814	40,718	43,437
2	6,721	6,656	5,647	0.845	0.805	40,779	43,727
3	6,706	6,702	5,575	0.849	0.808	41,111	44,312
4	6,718	6,685	5,657	0.830	0.808	40,927	44,261
5	6,744	6,697	5,602	0.847	0.815	41,206	44,426
6	6,691	6,520	5,564	0.848	0.783	41,326	44,344
Average	6,725	6,677	5,592	0.842	0.805	41,011	44,085
Std. Dev.*	29.395	90.743	55.836	0.008	0.012	242.524	403.300
%RSD	0.44%	1.36%	1.00%	0.90%	1.44%	0.59%	0.91%

*Denotes Standard Deviation. #Denotes Internal Standard.

Table 5 Resolution (*n*=6) of specific peaks for glycerin, PG, and sorbitol solution

Reference Solution	**Glycerin (Reference Solution (b))**		**PG**	**Sorbitol Solution**
Injection	Resolution (EG & DEG)	Resolution (DEG & Glycerin)	Resolution (EG & PG)	Resolution (EG & DEG)
1	63	19	6.6	52.2
2	63	18	6.6	51.5
3	63	19	6.6	51.6
4	64	18	6.5	51.6
5	61	18	6.5	51.9
6	62	18	6.5	51.9
Average	63	18	6.5	51.8
IP Requirement	≥ 40	≥ 10	≥ 5.0	≥ 30.0

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

The injection volumes of glycerin and PG analyses were reduced to 0.5 µL in order to prolong the column lifespan and prevent potential contamination due to backflash. Referring to the General Chapter of IP, adjustment of injection volume of the test is allowed if the detection and repeatability are satisfactory. Excellent repeatability result with low %RSD was discussed and presented (Table 4). In term of detection, LOQ and LOD were determined experimentally by analyzing 6 repeated injections of known concentration reference solutions.

The LOQ was identified with a minimum signal-to-noise ratio (S/N) of 10. Table 6 shows the LOQ values for EG and DEG peaks. For the EG peak, the LOQ was determined to be 7.5 ppm, while for PG and sorbitol solutions, it was 10 ppm. For the DEG peak, the LOQ was not more than 15 ppm. These concentrations correspond to ≤0.02% and ≤0.03% of EG and DEG in the raw sample, indicating that the workflow can accurately quantify concentrations below the 0.10% threshold specified by the IP acceptance criteria (Table 2).

The determination of LOD was set with a minimum S/N of 3. The LOD for both EG and DEG was determined to be not more than 10 ppm, with a minimum average S/N of 8 across all reference solutions (Table 7). These results correspond to ≤0.02% of EG and DEG in the raw sample, demonstrating the workflow's maintained its high sensitivity in detecting EG and DEG.

Table 6 LOQ (*n*=6) of the glycerin, PG, and sorbitol solution using reference solution

Reference Solution	**LOQ**					
	Ethylene Glycol (EG)			**Diethylene Glycol (DEG)**		
	Conc. (ppm)	%RSD (n=6)	Average S/N	Conc. (ppm)	%RSD (n=6)	Average S/N
Glycerin	7.5	2.75	14	7.5	5.34	21
PG	10	3.30	16	15	2.81	14
Sorbitol Solution	10	4.11	27	10	3.59	106

Table 7 LOD (*n*=6) of the glycerin, PG, and sorbitol solution using reference solution

Reference Solution	**LOD**			
	Ethylene Glycol (EG)		**Diethylene Glycol (DEG)**	
	Conc. (ppm)	Average S/N	Conc. (ppm)	Average S/N
Glycerin	5	10	5	11
PG	5	8	10	8
Sorbitol Solution	7.5	14	5	29

Analysis of Raw Sample

The workflow was applied to analyze 4 raw samples: raw glycerin, raw PG, raw crystallising sorbitol solution (70 %), and raw non-crystallising sorbitol solution (70 %) in accordance with IP monographs test solution preparation described earlier in this application news.

Fig. 3 shows the chromatograms of all the tested raw materials samples. No EG and DEG peaks were detected in both in either glycerin or PG raw samples. For the sorbitol solutions, EG peaks were detected in both the crystallising and non-crystallising raw samples (Fig. 3 and Table 8). However, the detected EG peak areas were not more than the peak area in the Sorbitol Reference Solution. Hence, the results of all raw samples were determined to be PASS.

Since the acceptance criteria is well established by IP monographs, Shimadzu's multi-data report (MDR) allows users to prepare an automatic test report to generate a simple "Pass"/"Fail" judgment, which is available in LabSolutions DB/CS.

■ Conclusion

In this study, the analysis of EG and DEG in three types of raw materials was successfully performed using the compact Brevis GC coupled with AOC-30i. Despite its small size, the Brevis GC demonstrated excellent capability in analyzing EG and DEG impurities in the raw materials according to the IP monographs, making it an ideal and more eco-friendly solution for this application.

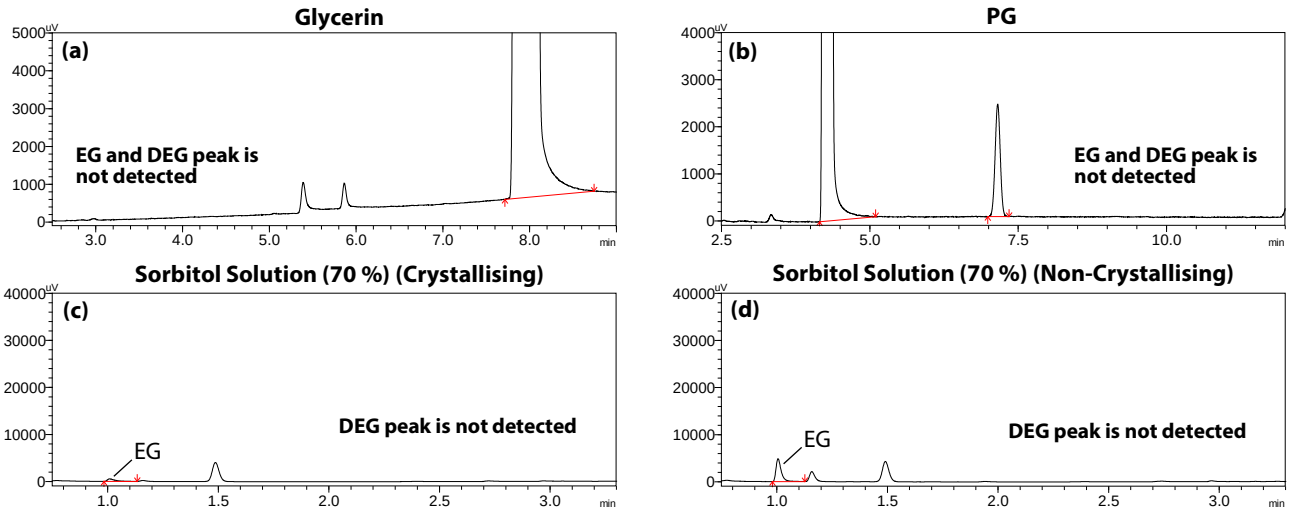

Fig. 3 Chromatograms of raw sample of **(a)** glycerin, **(b)** PG, **(c)** sorbitol solution (70 %) (crystallising), and **(d)** sorbitol solution (70 %) (non-crystallising)

Table 8 Summarized results of EG and DEG detected in each type of raw sample

Sample	Glycerin			PG			Sorbitol Solution (Crystallising)			Sorbitol Solution (Non-Crystallising)		
	Peak Area			Peak Area Ratio			Peak Area					
	EG	DEG	Result	EG/IS	DEG/IS	Result	EG	DEG	Result	EG	DEG	Result
Reference Solution*	6,725#	6,677#	-	0.842	0.805	-	41,011	44,085	-	41,011	44,085	-
Raw Sample	ND+	ND+	**Pass**	ND+	ND+	**Pass**	1,419	ND+	**Pass**	8,122	ND+	**Pass**

*Denotes average value from 6 injections. #Denotes result from reference solution (a). +Denotes not detected.

■ References

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2) Exclusive: WHO investigating links between cough syrup deaths, considers advice for parents. Reuters, https://www.reuters.com/business/healthcare-pharmaceuticals/who-investigating-links-between-cough-syrup-deaths - considers-advice-parents/, [Accessed 24 June 2024]

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