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Application News

Gas Chromatograph GC-2010 Pro

Analysis of Ethylene Glycol and Diethylene Glycol in Glycerin, Propylene Glycol and Sorbitol via GC-FID in Accordance with USP Monographs

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User Benefits

- GC-2010 Pro offers advanced capabilities for the detection of EG and DEG contamination in the raw materials (glycerin, propylene glycol and sorbitol) used for manufacturing medicinal syrup.
- The configuration proposed in the application news exceeds the requirement specified in the USP monographs.
- GC-2010 Pro coupled with the AOC-20i+s Plus autosampler can obtain highly reproducible results using the proposed workflow.

■ Introduction

Glycerin, propylene glycol (PG) and sorbitol are commonly used as excipients in medicinal syrup during formulation. Their global supply chains are vital to both the pharmaceutical and consumer healthcare industries, as these 3 raw materials are widely used by these industries. Ensuring the safety and quality of these raw materials in the supply chains is a significant challenge to the authorities, as evidenced by some incidents, such as reports of deaths in Indonesia, Gambia [1] and Uzbekistan [2] due to the contamination of ethylene glycol (EG) and diethylene glycol (DEG) in medicinal syrup.

Several companies in Indonesia have been implicated for their involvement in the distribution of contaminated raw materials to pharmaceutical companies [3]. To prevent the recurrence of contaminated medicinal syrup from reaching the general public, more stringent multi-level Quality Control (QC) and checks must be in place during the manufacturing of the medicinal syrup. **Figure 1** displays the suggested checks that could be implemented. The scope of the QC check must start from the manufacturing of raw materials till the end of the finished products. Following the release of two applications news for the detection of EG and DEG in finished medicinal syrup [4,5], this application news is published to show determination of ethylene glycol (EG) and diethylene glycol (DEG) in pharmaceutical raw materials: glycerin, propylene glycol (PG) and sorbitol. This was performed in accordance with the United States Pharmacopoeia (USP) monographs, using a gas chromatograph with flame ionization detector.

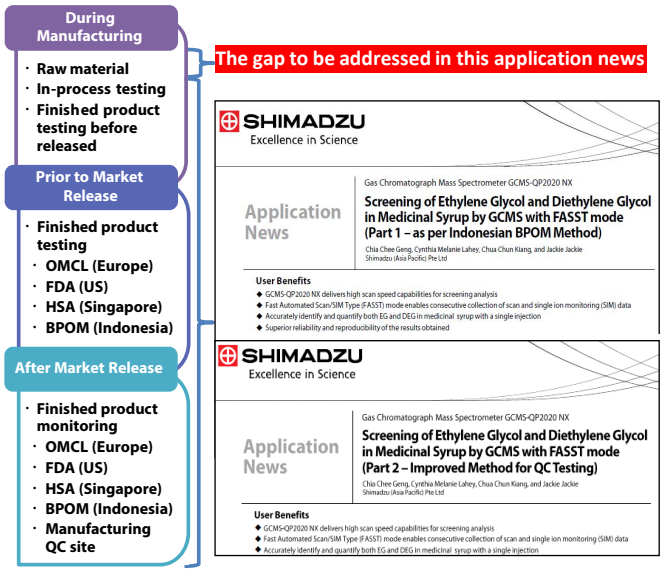

Figure 1. The scope covered by the application news released earlier and the gap to be addressed in this application news

■ Measurement Conditions

This study employed Shimadzu's GC-2010 Pro coupled with the AOC-20i+s Plus autosampler with flame ionization detector (FID), while PEAK Scientific's Precision SL 100 Hydrogen Generator was utilized to generate hydrogen for FID (**Figure 2**).

The analytical conditions utilized for the analysis, in accordance with the method outlined by the USP [6,7,8], are provided in **Table 1**. 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 contaminating the GC system. Additionally, in the sorbitol analysis, the final column oven temperature program was adjusted to 270 °C (instead of 300 °C) due to the column-recommended specification.

■ USP Requirements

USP has established specific compendia tests for detecting contaminants such as EG and DEG in glycerin, PG and sorbitol. The analytical methods specified in the USP monographs are based on GC-FID. The criteria to be met, as specified in the monographs are stated in **Table 2.**

Figure 2. Shimadzu's GC-2010 Pro with AOC™-20i+s Plus and PEAK Scientific's Precision SL 100 Hydrogen Generator

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

System Configuration			
Raw Material	**Glycerin**	**Propylene Glycol (PG)**	**Sorbitol**
GC System	GC-2010 Pro		
Auto Injector	AOC™-20i+s Plus		
Syringe	5 µl syringe [P/N: 221-75173]		
Column	SH-624 (USP G43 phase) 30 m x 0.53 mm I.D. x 3.0 µm df [P/N: 221-75865-30]	SH-624 (USP G43 phase) 30 m x 0.53 mm I.D. x 3.0 µm df [P/N: 221-75865-30]	SH-1701 (USP G46 phase) 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 liner with glass wool) (Split ratio = 10)		
Injector Temperature	220 °C	220 °C	240 °C
Injection Volume	0.5 µL	0.5 µL	1.0 µL
Carrier Gas	Helium		
Flow Control Mode	Linear velocity		
Column Flow Rate	4.5 mL/min	4.5 mL/min	3.0 mL/min
GC Oven Parameters			
Column Oven Temperature Program	Initial temp. 100 °C (hold for 4 min) - Increase to 120 °C with a rate of 50 °C/min (hold for 10 min) - Increase to 220 °C with a rate of 50 °C/min (hold for 6 min)	Initial temp. 100 °C (hold for 4 min) - Increase to 120 °C with a rate of 50 °C/min (hold for 10 min) - Increase to 220 °C with a rate of 50 °C/min (hold for 6 min)	Initial temp. 70 °C (hold for 2 min) - Increase to 270 °C with a rate of 50 °C/min (hold for 5 min)
FID Parameter			
Detector Temperature	250 °C	250 °C	300 °C

■ Preparation of Standard

Standard solution preparation

EG, DG, glycerin and PG of USP grade were prepared in accordance with USP monographs.

Glycerin Standard

Glycerin standard solution was prepared in methanol with a final concentration of 2.0 mg/mL USP glycerin, 0.10 mg/mL 2,2,2-trichloroethanol (Internal Standard, IS), 0.05 mg/mL USP EG and 0.05 mg/mL USP DEG.

PG Standard

PG standard solution was prepared in methanol with a final concentration of 2.0 mg/mL USP PG, 0.10 mg/mL 2,2,2-trichloroethanol (Internal Standard, IS), 0.05 mg/mL USP EG and 0.05 mg/mL USP DEG.

Sorbitol Standard

Diluent was first prepared by mixing acetone and water (96:4). Sorbitol standard solution was prepared in diluent with a final concentration of 0.08 mg/mL USP EG and 0.08 mg/mL USP DEG.

■ Preparation of Sample

Sample Solution Preparation

Raw material samples for glycerin, PG, and sorbitol were prepared as per USP monographs.

Glycerin Sample

Glycerin sample was prepared in methanol with a final concentration of 50 mg/mL glycerin raw material and 0.10 mg/mL 2,2,2-trichloroethanol (IS).

PG Sample

PG raw material sample was prepared in methanol with a final concentration of 50 mg/mL glycerin raw material and 0.10 mg/mL of 2,2,2-trichloroethanol (IS).

Sorbitol Sample

Diluent was first prepared by mixing acetone and water (96:4). Sorbitol raw material sample was prepared by adding 2.0 g of sorbitol raw material to a 25-mL volumetric flask. Then, 1.0 mL of diluent was added, and the mixture was vortexed for 3 minutes. The remaining diluent was added in three equal portions, with 3-minute vortexing upon each addition, until the mark of the volumetric flask was reached. A portion of the supernatant layer was passed through a 0.45-µm nylon filter. The initial 2 mL filtrate was discarded, and the remaining filtrate was collected for sample analysis. (Note: acetone was used for the precipitation of sorbitol).

■ Preparation of Spiked Sample

Spiked Sample Solution Preparation

To simulate a sample that exceeds the USP acceptance criteria, USP EG and USP DEG were intentionally spiked into the raw material samples.

Glycerin Spiked Sample

Glycerin spiked sample was prepared in methanol with a final concentration of 50 mg/mL glycerin raw material, 0.10 mg/mL 2,2,2-trichloroethanol (IS), 0.055 mg/mL USP EG and 0.055 mg/mL USP DEG.

PG Spiked Sample

PG spiked sample was prepared in methanol with the final concentration of 50 mg/mL PG raw material, 0.10 mg/mL 2,2,2-trichloroethanol (IS), 0.055 mg/mL USP EG and 0.055 mg/mL USP DEG.

Sorbitol Spiked Sample

Diluent was first prepared by mixing acetone and water (96:4). Sorbitol spiked sample solution was prepared by adding 2.0 g of sorbitol raw material to a 25-mL volumetric flask. USP EG and USP DEG were then spiked into the sample, each at a final concentration of 0.088 mg/mL. Then, 1.0 mL of diluent was added and the mixture was vortexed for 3 minutes. The remaining diluent was added in three equal portions, with 3-minute vortexing upon each addition, until the mark of the volumetric flask was reached. A portion of the supernatant layer was passed through a 0.45 µm nylon filter. The initial 2 mL filtrate was discarded, and the remaining filtrate was collected for sample analysis.

■ Results and Discussion

System Suitability

The System Suitability Test (SST) requirements are summarized in **Table 2**. The SST is based on the specific peak resolution between the analytes mentioned in **Table 2**.

Glycerin and PG methods use a wide bore column of 0.53 mm ID (**Table 1**), resulting in low column head pressure. Using the analytical conditions in **Table 1**, methanol (diluent for glycerin and PG) produces a high expansion volume at low pressure. This could create backflash which potentially contaminates the instrument. Hence, the injection volume was decreased to 0.5 µl for glycerin and PG analyses. USP states that when the injection volume is reduced, special attention needs to be given to these [9]:

- Repeatability of the peak response
- The impurities (EG and DEG) should be reliably detected at the concentration limit

Thus, in addition to the criteria in **Table 2**, the following would be determined for glycerin and PG methods:

- %RSD of EG and DEG peak response ratios
- Signal-to-noise ratio of EG and DEG peak response at 0.1%

Glycerin Standard

Figure 3a depicts the chromatogram for glycerin standard. The peaks observed are well-separated among the analytes of interest. RRT of EG, IS, DEG and glycerin were reported as 0.3, 0.6, 0.8 and 1.0, respectively (**Table 3**). The observed RRT was consistent with the reference RRT in the USP monograph. The average resolution between DEG and glycerin peaks was reported as 15.9 (**Table 4**), thus fulfilling the USP requirement that the resolution between those 2 peaks must be greater than 1.5.

Table 2. USP requirements for the analysis of glycerin, propylene glycol and sorbitol

USP Requirements	Glycerin	Propylene Glycol (PG)	Sorbitol
Resolution	≥ 1.5 between DEG and glycerin	≥ 5 between EG and PG	≥ 30 between EG and DEG
Acceptance Criteria	• Peak response ratio of EG or DEG in the sample is not more than the peak response ratio in the standard* (which corresponds to ≤ 0.10% of EG or DEG in the sample) • RT^+ of glycerin peak in sample solution should correspond to the RT^+ of glycerin peak in standard	• Peak response ratio of EG or DEG in the sample is not more than the peak response ratio in the standard* (which corresponds to ≤ 0.10% of EG or DEG in the sample) • RT^+ of PG peak in sample solution should correspond to RT^+ PG peak in standard	• Peak area of EG or DEG in sample is not more than the peak area of EG or DEG in standard (which corresponds to ≤ 0.10% of EG or DEG in the sample)

*Refer to Calculation section for the determination of peak response ratio. $^+$Denotes retention time.

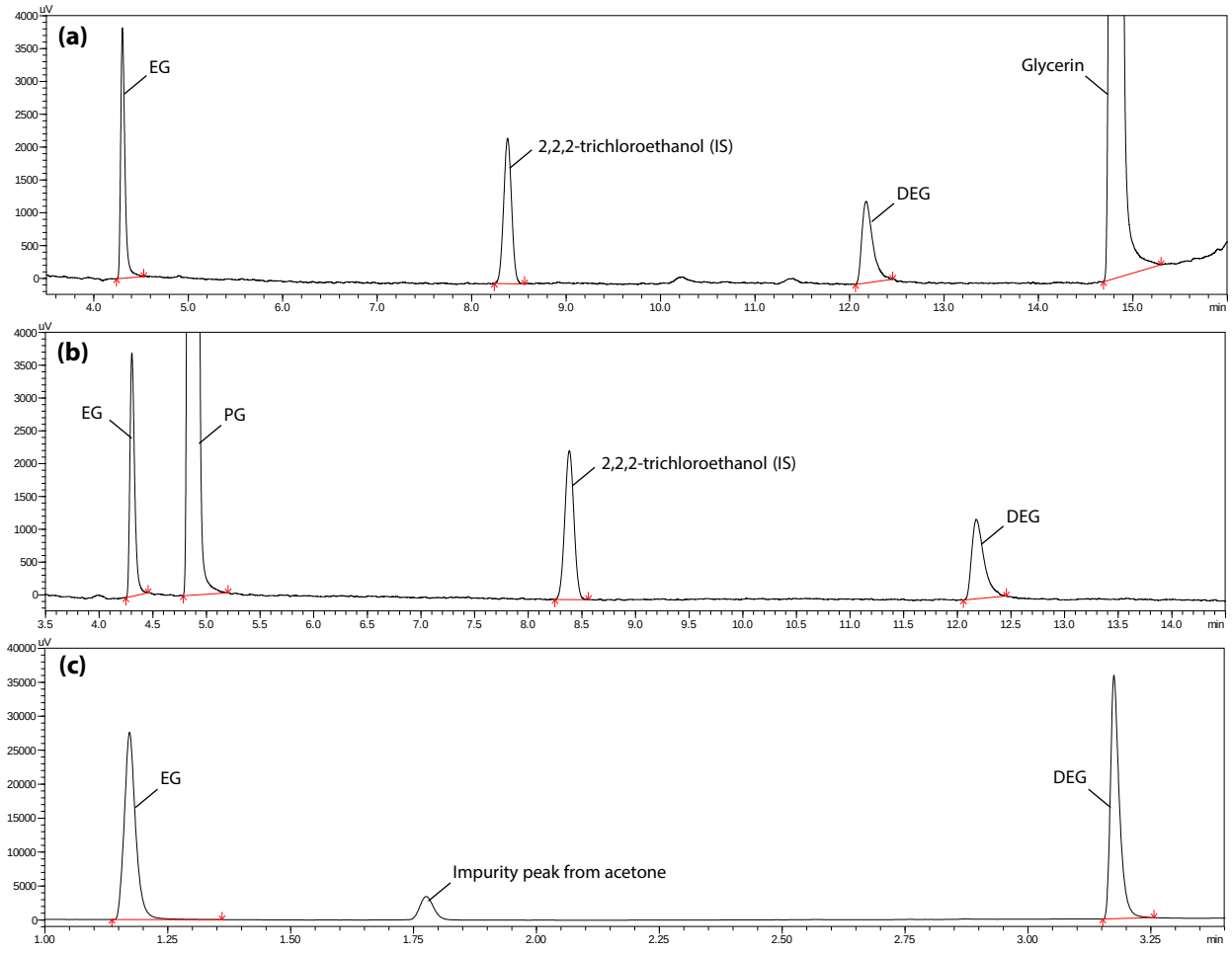

Figure 3. Standard chromatograms of ethylene glycol (EG) and diethylene glycol (DEG) in **(a)** glycerin standard, **(b)** PG standard and **(c)** sorbitol standard

Table 3. Relative retention times (RRT) and retention times (RT) of the glycerin, PG and sorbitol standards

Standard Solution	Glycerin Standard (RRT*)				PG Standard (RRT*)				Sorbitol Standard (RT+)/min	
Injection	EG	IS#	DEG	Glycerin	EG	PG	IS#	DEG	EG	DEG
1	0.3	0.6	0.8	1.0	0.9	1.0	1.7	2.5	1.2	3.2
2	0.3	0.6	0.8	1.0	0.9	1.0	1.7	2.5	1.2	3.2
3	0.3	0.6	0.8	1.0	0.9	1.0	1.7	2.5	1.2	3.2
4	0.3	0.6	0.8	1.0	0.9	1.0	1.7	2.5	1.2	3.2
5	0.3	0.6	0.8	1.0	0.9	1.0	1.7	2.5	1.2	3.2
USP reference¤	0.3	0.6	0.8	1.0	0.8	1.0	1.7	2.4	DEG elutes after EG	

*Denotes Relative Retention Time. +Denotes Retention Time. #Denotes Internal Standard. ¤ Relative retention time provided in USP monograph for reference purpose.

Table 4. System Suitability Requirement for Resolution of the glycerin, PG and sorbitol standards

Standard Solution	Glycerin Standard	PG Standard	Sorbitol Standard
Injection	Resolution (between DEG & Glycerin)	Resolution (between EG & PG)	Resolution (between EG & DEG)
1	15.8	7	54
2	16.0	7	53
3	15.9	7	54
4	15.9	7	54
5	16.1	7	54
Average	15.9	7	54
USP Requirement	≥ 1.5	≥ 5	≥ 30

Table 5. Repeatability (*n*=5) of the glycerin, PG and sorbitol standards

Standard Solution	Glycerin Standard				PG Standard				Sorbitol Standard	
Injection	Peak Response Ratio (EG/IS#)	S/N+ (EG)	Peak Response Ratio (DEG/IS#)	S/N+ (DEG)	Peak Response Ratio (EG/IS#)	S/N+ (EG)	Peak Response Ratio (DEG/IS#)	S/N+ (DEG)	Peak Area (EG)	Peak Area (DEG)
1	0.913	147.55	0.819	27.62	0.856	112.89	0.718	28.57	44,525	45,205
2	0.866	87.18	0.768	25.62	0.846	93.07	0.716	37.26	43,719	44,689
3	0.914	125.46	0.770	25.88	0.858	91.55	0.741	43.22	44,537	45,205
4	0.877	95.85	0.788	23.57	0.848	86.28	0.734	26.34	44,391	45,309
5	0.897	129.92	0.794	28.15	0.861	87.68	0.718	27.60	44,630	45,360
Average	0.893	117.19	0.788	26.17	0.854	94.29	0.725	32.60	44,361	45,154
Std. Dev.*	0.021		0.021		0.006		0.011		368.468	268.174
%RSD	2.39%		2.65%		0.77%		1.57%		0.83%	0.59%

*Denotes Standard Deviation. #Denotes Internal Standard. +Denotes Signal-to-Noise Ratio

The repeatability of the experiment was assessed by analyzing five replicate injections (*n*=5) of the glycerin standard. The average peak response ratio of EG to IS (EG/IS) was observed to be 0.893 and 0.788 (**Table 5**) for peak response ratio of DEG to IS (DEG/IS). Highly precise repeatability was observed from the repeated injections. For EG/IS and DEG/IS ratios, %RSD of 2.39% and 2.65% were obtained, respectively (**Table 5**). The average signal-to-noise (S/N) ratios for the EG and DEG peaks were 117.19 and 26.17, respectively. Given the low %RSD and the high S/N ratios, the method reliably detects EG and DEG impurities at the limit of 0.10%.

PG Standard

Figure 3b depicts the chromatogram for the PG standard. The peaks were well-resolved among the analytes of interest, with the RRT of EG, PG, IS and DEG reported as 0.9, 1.0, 1.7 and 2.5, respectively (**Table 3**).

It is worth noting that, as a reference, the RT for PG in the USP monograph was stated to be 4 min. The RT for PG was observed to be 4.87 min in our experiment. Thus, the observed RRTs for EG and DEG obtained in our experiment were off by 0.1 from the value provided in the USP monograph (**Table 3**). Such minor deviation is within expectation due to the observed column-to-column variations. The average resolution between EG and PG peaks was found to be 7 (**Table 4**), thus satisfying the USP requirement (resolution between those 2 peaks should be not less than 5). An average peak response ratio to the internal standard, for EG and DEG obtained was 0.854 (EG/IS) and 0.725 (DEG/IS) respectively (**Table 5**). For the repeated injections (*n*=5), %RSD of 0.77% and 1.57% were observed for EG/IS and DEG/IS, respectively (**Table 5**). The average S/N ratios for the EG and DEG peaks were 94.29 and 32.60, respectively. These results demonstrate high precision and reliability in detecting EG and DEG impurities.

Sorbitol Standard

Figure 3c depicts the chromatogram for the sorbitol standard. The peaks of interest were well-resolved as shown. An unknown peak observed at around RT 1.78 min was attributed to the impurities intrinsically present in the acetone used. The RT of EG and DEG was observed at 1.2 and 3.2 min respectively (**Table 3**). The average (*n*=5) resolution of the peak between EG and DEG obtained was observed to be 54 (**Table 4**). This result obtained exceeds the USP requirement by almost twice (**Table 2**).

It was observed that the average (*n*=5) peak areas obtained were 44361 and 45154 for EG and DEG respectively (**Table 5**). %RSD for the repeated injections (*n*=5) obtained was observed to be 0.83% and 0.59% for EG and DEG peak areas respectively (**Table 5**), demonstrating that a high degree of precision was successfully achieved.

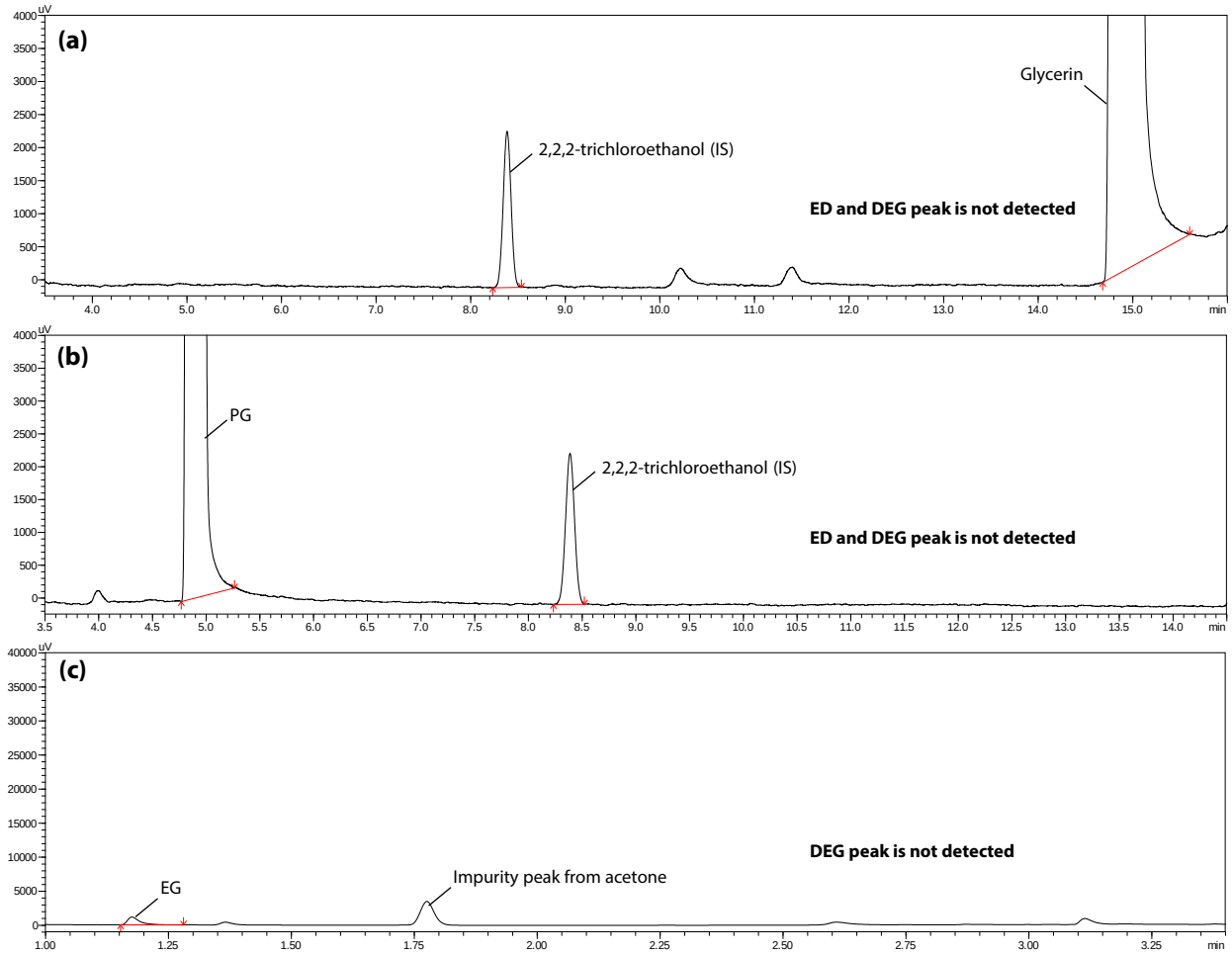

Figure 4. Chromatograms of **(a)** glycerin sample, **(b)** PG sample and **(c)** sorbitol sample

Analysis of Sample

Glycerin Sample and PG Sample

Figure 4a-b shows the chromatograms of raw material samples, i.e. glycerin and PG. **Table 6** summarizes the average RT of peaks of interest during the analysis, for its standard and sample. Comparison between the standard and sample during the analysis for glycerin raw material, a slight shift in RT of around 0.228 min was observed (**Table 6**). This was mainly due to the difference in the amount of glycerin that was originally present in both the standard and sample. As depicted in **Figure 5a**, glycerin's peak area was much larger in the sample as compared to the standard, this resulted in the shift in the apex of the peak, which is detected as RT. However, the shift is very slight and does not pose any difficulty during peak identification. This effect is also observed in PG (**Figure 5b**) but to a lesser degree. Thus, for PG, the shift in peak is much less. Hence glycerin and PG peaks were successfully identified in the sample, and their observed RTs were similar to their respective standards. In summary, the USP requirement for the acceptance criteria that requires the RT of glycerin and PG in the sample to be in strong agreement with the standard is successfully achieved in this experiment.

EG and DEG peaks were not detected in these analyses. Thus, these 2 raw materials samples cleared the requirement set by USP.

Table 6. Retention time of glycerin and PG in standard and sample

	Glycerin	PG
Standard Average RT (*n*=5) /min	14.824	4.872
Sample Average RT (*n*=3) /min	15.052	4.931

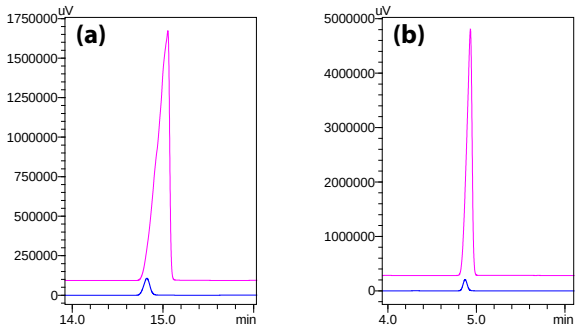

Figure 5. Overlay of standard (blue) and sample (pink) for **(a)** glycerin and **(b)** PG peak

Sorbitol Sample

Figure 4c depicts the chromatogram for the analysis of the sorbitol raw material. DEG peak was not detected, but EG peak was detected in the sample. The average peak area for EG in the sorbitol sample was less than 2200 (**Table 7**). This result indicates that the detected peak area of EG in the sorbitol sample was lower than the average EG peak area observed in the sorbitol standard analyzed earlier (44361). As the peak areas of EG and DEG in the sorbitol sample were not more than the peak areas in the standard, it can be concluded that this sorbitol sample complies with the requirements set by the USP.

Table 7. Summarized results of EG detected in sorbitol sample

Test Sample	Sorbitol Sample
Injection	Peak Area (EG)
1	2,293
2	2,063
3	2,101
Average	2,153
Sorbitol Standard Sample's Average Peak Area	44,361*

*This result obtained from **Table 5**

Analysis of Spiked Samples

To validate the method, we simulated samples that exceed the acceptance criteria set by USP monographs. All raw material samples were spiked with 0.11% of USP EG and USP DEG each, a slightly higher amount than the specified limit (0.10%). These spiked samples were then analyzed using the same USP method described in **Table 1**.

Figure 6 a-c presents the chromatograms of the spiked samples for glycerin, PG and sorbitol, respectively. **Table 8** summarizes EG and DEG's peak response ratios or peak areas across all spiked samples. The results clearly indicate that the peak response ratios of EG/IS and DEG/IS in the glycerin and PG spiked samples surpass the average peak response ratios

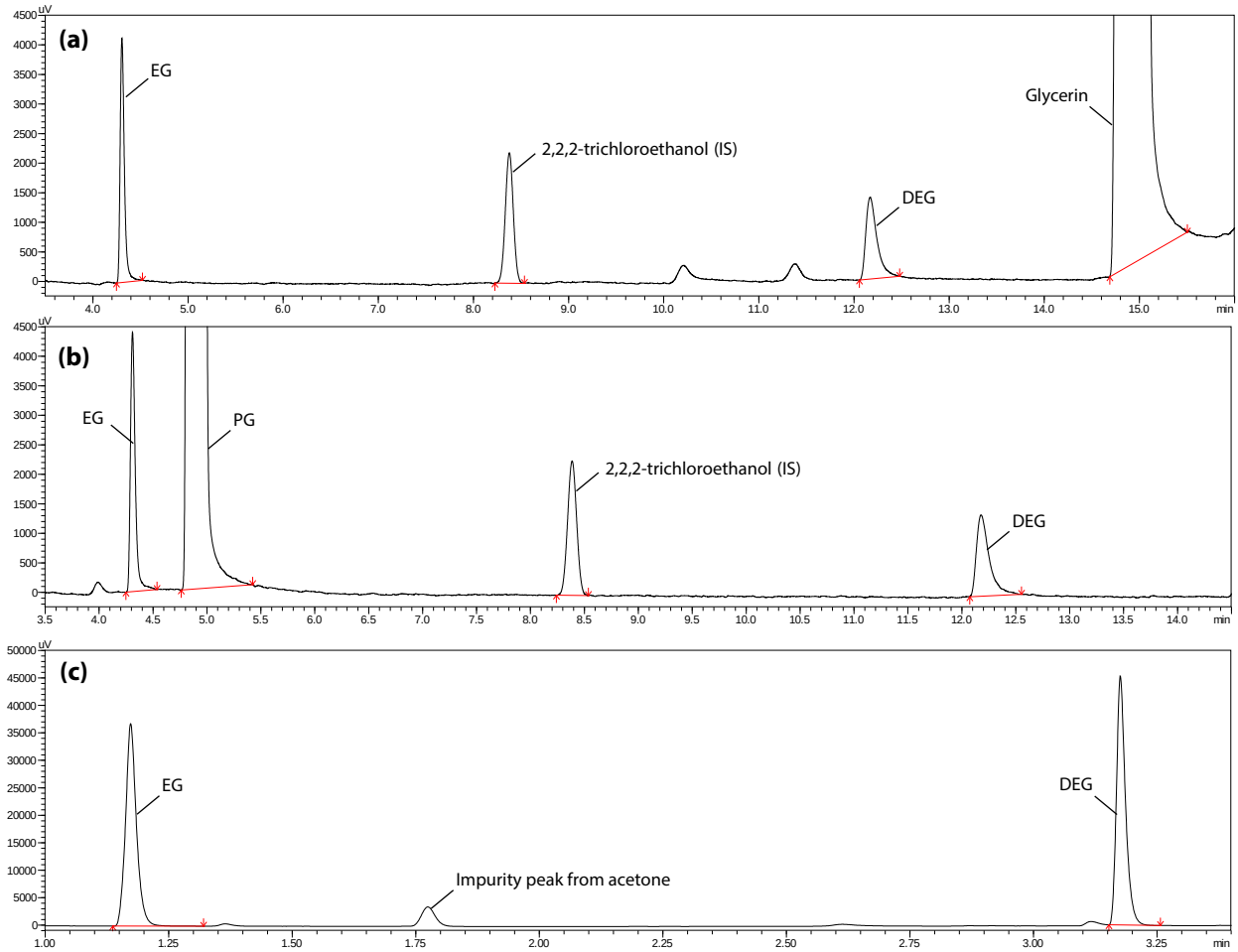

Figure 6. Chromatograms of **(a)** glycerin spiked sample, **(b)** PG spiked sample and **(c)** sorbitol spiked sample

Table 8. Summarized result of EG and DEG detected in glycerin, PG and sorbitol spiked samples

Spiked Sample	Glycerin Spiked Sample		PG Spiked Sample		Sorbitol Spiked Sample	
Injection	Peak Response Ratio (EG/IS#)	Peak Response Ratio (DEG/IS#)	Peak Response Ratio (EG/IS#)	Peak Response Ratio (DEG/IS#)	Peak Area (EG)	Peak Area (DEG)
1	0.984	0.869	0.989	0.819	56,281	59,366
2	0.974	0.879	1.026	0.848	55,736	58,958
3	0.992	0.898	1.000	0.815	56,180	59,422
Average	0.983	0.882	1.005	0.827	56,066	59,249
Standard Average	0.893*	0.788*	0.854*	0.725*	44,361*	45,154*

*These results obtained from **Table 5**. #Denotes Internal Standard.

observed in the standards. Similarly, for the sorbitol spiked sample, the average peak areas of EG and DEG exceeded that of the standard.

Calculation

The RRT of interest analytes for both the standard and sample in the glycerin and PG are calculated using the formula below:

$$RRT = \frac{\textit{Retention Time of the Peak of Interest}}{\textit{Retention Time of the Reference Peak}}$$

Note: Reference peak refers to the examined substance (glycerin or PG).

The peak response ratio of EG and DEG to the 2,2,2-trichloroethanol (IS) for both the standard and sample in the glycerin and PG are calculated using the formulae below:

$$\textit{Peak Response Ratio for EG} = \frac{\textit{Peak Area EG}}{\textit{Peak Area of 2,2,2-trichloroethanol}}$$

$$\textit{Peak Response Ratio for DEG} = \frac{\textit{Peak Area DEG}}{\textit{Peak Area of 2,2,2-trichloroethanol}}$$

■ Conclusion

The application news successfully demonstrates the capability of Shimadzu's GC-2010 Pro coupled with the AOC-20i+s Plus autosampler to perform the analysis of EG and DEG in raw materials (glycerin, PG and sorbitol) to be used for the manufacturing of medicinal syrup. The system exhibited remarkable performance, as evidenced by the superior resolution observed among the analytes peak of interests, meeting the SST requirement specified in respective raw materials monographs. Great precision of the analytical results was also obtained, as seen from the low %RSD. Our proposed setup ensures the safety of raw materials used in the pharmaceutical and healthcare industries.

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04-AD-0293-EN First Edition: Oct 2023

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