APPLICATION NOTE

Gas Chromatography

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GC-FID: Limit of Diethylene Glycol and Ethylene Glycol according to USP Propylene Glycol Monograph

Introduction

Propylene glycol, also known as methyl ethyl glycol, 1,2-propanediol, or propane-1,2-diol, is a synthetic, clear, colorless liquid widely used as an additive or as an antifreeze in the chemical, food, cosmetics, and pharmaceutical industries.[1, 2] Propylene glycol is an important excipient for increasing the solubility and stability of prescribed and over-the-counter medicines. Moreover, due to its ability to absorb water, propylene glycol facilitates maintaining moisture in certain medicines and topical formulations like creams.[1]

Ethylene glycol and diethylene glycol are considered toxic to human health and both are commonly present as impurities in propylene glycol.[3] In response to these toxic considerations and adhering to the recommendations from US Food and Drug Administration (USFDA), United States Pharmacopoeia (USP) revised its monograph[4] for propylene glycol in 2010. This monograph addresses the toxicity concerns by specifying the limit for ethylene glycol and diethylene glycol while using propylene glycol as an inactive ingredient to meet quality standards in pharmaceutical products.[4, 5] This updated monograph identifies propylene glycol and defines a limit test for ethylene glycol and diethylene glycol through Gas Chromatography/Flame Ionization Detection (GC-FID) using an internal standard peak response quantitation method to then measure ethylene glycol and diethylene glycol content in propylene glycol samples.

This application note shows the performance of the PerkinElmer GC 2400™ System with FID for the analysis of propylene glycol quality according to the updated USP monograph, while demonstrating superior performance and a 40% improvement to the required resolution.

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Instrumentation

The PerkinElmer GC 2400 System, with a capillary split/splitless (CAP) injector and PerkinElmer Elite 624 analytical column provided a streamlined solution for the evaluation of propylene glycol quality. The GC 2400 System was configured with a PerkinElmer AS 2400™ Liquid Sampler and a FID, enabling a reliable platform for quantifying ethylene glycol and diethylene glycol as impurities for USP grade propylene glycol analysis.

PerkinElmer Simplicity Vision runs on the detachable touchscreen for real time monitoring and live status checks, when connected to the laboratory network, optimizing time, and increasing the productivity of the lab.

The PerkinElmer GC 2400 System

Experimental

A PerkinElmer Elite 624 column 30 m X 0.53 mm X 3.0 µm was installed in the injector and conditioned according to practices in the PerkinElmer Capillary Column Installation Quick Care guide. The GC conditions required for the analysis are listed in Table 1 and are as per USP monograph for propylene glycol. Methanol (Purge and Trap grade) was purchased from Millipore Sigma and used as a solvent for standard and sample preparation. USP-grade propylene glycol, ethylene glycol, diethylene glycol, 2,2,2 trichloroethanol (internal standard), and commercial USP-grade propylene glycol (used as sample) were purchased from Millipore Sigma.

Standard Preparation

A standard was prepared as per the following:

2.0 mg/mL of USP propylene glycol, 0.050 mg/mL of USP ethylene glycol, 0.050 mg/mL of USP diethylene glycol, and 0.10 mg/mL of 2,2,2-trichloroethanol (as internal standard) in methanol.

Sample Preparation

A sample was prepared with 50 mg/mL commercial USP-grade propylene glycol and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol.

Spiked Sample: The commercial USP-grade propylene glycol sample was intentionally spiked with ethylene glycol and diethylene glycol standards to mimic a real life non-USP grade sample under test. A spiked sample was prepared in methanol with 50 mg/mL commercial USP-grade propylene glycol, 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard), and approximately 0.050 mg/mL each of USP ethylene glycol and USP diethylene glycol.

Table 1: Chromatography conditions.

GC Parameters			
Instrument	PerkinElmer GC 2400 System		
Column	PerkinElmer Elite-624 30 m X 0.53 mm X 3.0 µm (N9316207)		
GC Oven Parameters	**Initial**	**Ramp**	**Final**
	100° C (4 min)	50° C/min	120° C (10 min)
	120° C	50° C/min	220° C (6 min)
AS 2400 Liquid Sampler Parameters			
Syringe Size	5 µL (N6402556)		
Injection Volume	1.0 µL		
Injection Speed	Normal		
Number of Plunges	6		
Sample Wash	2		
Sample Wash Volume	50%		
Pre-wash	0		
Post-Wash	0		
Viscosity Delay	2 seconds		
Injector Parameters			
Type	Capillary Split/Splitless, Septum Flow: 3 mL/min		
Temperature	220° C		
Carrier/mode	Helium/Constant Flow mode		
Flow Rate (mL/min)	4.5 mL/min		
Split Ratio	10:1		
Liner	Deactivated glass liner 4mm I.D. with deactivated wool (N9306233)		
FID Detector Parameters			
Type	FID		
Temperature	250° C		
Hydrogen	30 mL/min		
Air	400 mL/min		
Data rate	10 pt/sec		

Consumables

Product Description	Part Number
Elite 624 30 M X 0.53 mm X 3.0 µm	N9316207
4 mm ID Capillary Split /Splitless Deactivated Glass Liners with Deactivated Wool (green), Pkg. 5	N9306233
Advanced Green Inlet Septum, Pkg. 10	N9306218
5 µL Autosampler Syringe, Pkg. 1	N6402556
Graphite Vespel Capillary Column Ferrules 0.8 mm ID, Pkg. 10	09920107
Ceramic Column Cutter, Pkg. 10	N9301376
O-ring for Glass PSS Liner, Pkg. 10	09200714
2 mL Clear Glass 9 mm Screw Top Vial with Write-On Patch, Liquid Autosampler Vials, Pkg. 100	N9307801
9mm blue screw caps with PTFE/SIL Liner (Liquid Autosampler Caps), Pkg. 100	N9306202
Triple Filter (Hydrogen & Nitrogen), Pkg. 1	N9306110
Moisture/Hydrocarbon Trap (Air), Pkg. 1	N9306117
Triple Filter (Helium), Pkg. 1	N9306106
CAP Injector Gold Seal, Pkg. 1	N6400900

Data Acquisition

Instrument control and data analysis was performed using PerkinElmer SimplicityChrom™ CDS Software (version 2.0) which allows streamlined instrument setup, data acquisition, and processing. SimplicityChrom CDS Software supports compliance with Title 21 of the Code of Federal Regulations (CFR), Part 11.

Results and Discussion

Retention Time and Peak Identification

Figure 1 shows a standard chromatogram obtained under the parameters set in Table 1. Retention time (RT) identification is critical when using FID as it is a universal detector for hydrocarbon analysis where response is proportional to the number of carbon atoms. The PerkinElmer 2400 GC's advanced Pneumatic Pressure Controller (PPC) provides highly repeatable separations, allowing reliable identification of compounds by RT. Table 2 shows the RT and relative retention time (RRT), and it aligns to that reported in the USP monograph. RT % relative standard deviations (%RSD) of 0.02% to 0.04% were obtained for the components as shown in Table 2. USP monograph also states the RRT for each component against that of propylene glycol to provide peak identification for the users. USP literature values for RRT are specified in Table 2 for each component. Good accuracy in terms of RRT to USP literature value were obtained further validating the precise and reproducible temperature and flow control of the PerkinElmer 2400 GC System.

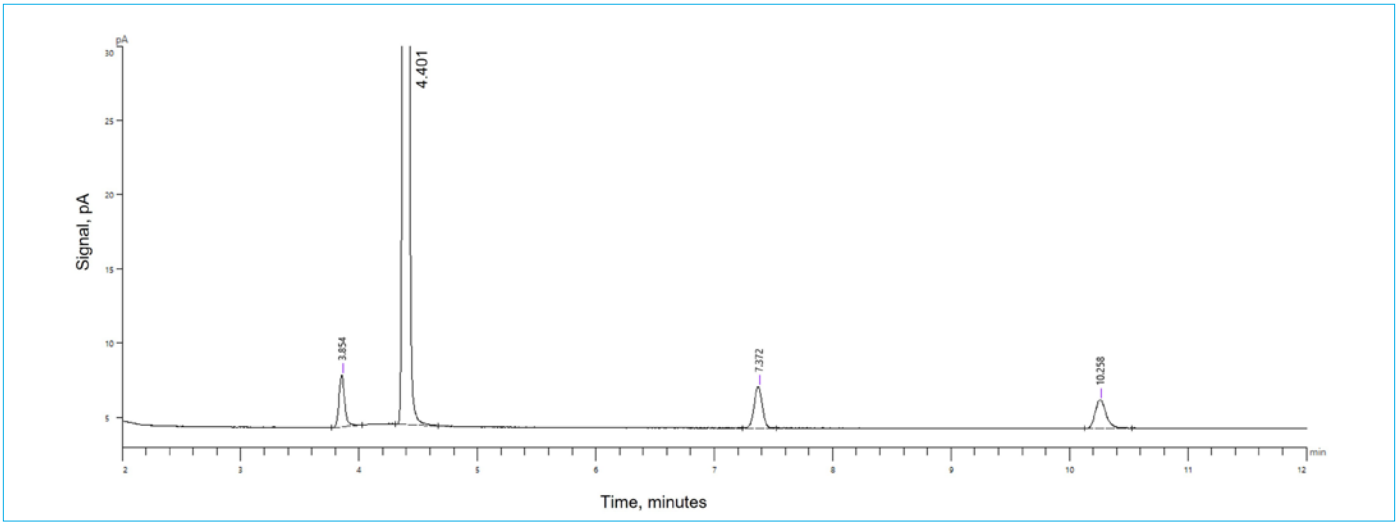

Figure 1: Standard Chromatogram containing 0.050 mg/mL of USP ethylene glycol (RT 3.854 min), 2.0 mg/mL of USP propylene glycol (RT 4.401 min), 0.10 mg/mL of 2,2,2-trichloroethanol as the internal standard (RT 7.372 min) and 0.050 mg/mL of USP diethylene glycol (RT 10.258 min), in methanol.

Table 2: Retention time (RT) and Relative Retention Time (RRT) for Standard Solution.

	Ethylene Glycol	Propylene Glycol*	2,2,2-trichloroethanol (Internal Standard)	Diethylene Glycol
Trial 1 RT (min)	3.855	4.402	7.371	10.261
Trial 2 RT (min)	3.855	4.400	7.370	10.260
Trial 3 RT (min)	3.851	4.399	7.367	10.258
Trial 4 RT (min)	3.854	4.401	7.371	10.261
Trial 5 RT (min)	3.853	4.399	7.367	10.256
Avg. RT (min)	3.854	4.400	7.369	10.259
RSD	0.043%	0.030%	0.028%	0.021%
Trial 1 RRT	0.9	1.0	1.7	2.3
Trial 2 RRT	0.9	1.0	1.7	2.3
Trial 3 RRT	0.9	1.0	1.7	2.3
Trial 4 RRT	0.9	1.0	1.7	2.3
Trial 5 RRT	0.9	1.0	1.7	2.3
Avg. RRT	0.9	1.0	1.7	2.3
USP Literature RRT[4]	0.8	1.0	1.7	2.4

** Propylene glycol is used as a reference peak for RRT calculations.*

System Suitability Requirement for Resolution

USP propylene glycol monograph states a system suitability (SST) requirement for resolution of not less than (NLT) 5 between ethylene glycol and propylene glycol. Using the Elite 624 column with GC 2400 System, SST requirement was exceeded with a reported resolution value of 7. Using SimplicityChrom CDS Software, the SST parameters are easy to select, and such calculations can be tailored to individual or group peaks alike giving more customization flexibility to the user. Table 3 shows resolution values were exceeded, with an average improvement to the resolution by 40%.

Table 3: Resolution between propylene glycol and ethylene glycol.

	USP Resolution between Ethylene Glycol and Propylene Glycol
Trial 1 Resolution	7.543
Trial 2 Resolution	7.370
Trial 3 Resolution	7.375
Trial 4 Resolution	7.345
Trial 5 Resolution	7.382
Avg. Resolution	7.403
RSD	1.074%
USP Literature Resolution[4]	NLT 5

Peak Area Ratio

Repeatability for standard injections is presented in Table 4 along with the peak area ratio for ethylene glycol with respect to 2,2,2-trichloroethanol (Internal Standard) and similarly, peak area ratio for diethylene glycol with respect to 2,2,2-trichloroethanol (Internal Standard). These peak area ratios relative to the internal standard in the standard solution are used as a limit value to quantitate these impurities in sample solution as explained later in sample analysis.

Table 4: Peak area repeatability of standard injections along with peak area ratio for ethylene glycol and diethylene glycol.

	Ethylene Glycol Peak Area	Propylene Glycol Peak Area	2,2,2-trichloroethanol (Internal Standard) Peak Area	Diethylene Glycol Peak Area	Peak Area Ratio of Ethylene Glycol w.r.t (Internal Standard)	Peak Area Ratio of Diethylene Glycol w.r.t (Internal Standard)
Trial 1	12.136	665.988	13.522	12.670	0.898	0.937
Trial 2	12.211	666.075	13.511	12.643	0.904	0.936
Trial 3	12.269	659.284	13.448	12.644	0.912	0.940
Trial 4	12.553	670.391	13.534	12.470	0.928	0.921
Trial 5	12.410	663.500	13.423	12.404	0.925	0.924
Avg	12.316	665.047	13.488	12.566	0.913	0.932
RSD	1.350%	0.611%	0.363%	0.960%	1.422%	0.913%

Sample Analysis

Commercial USP-grade propylene glycol from Millipore Sigma was used as a test sample. The sample was prepared as vide supra then analyzed as per the test conditions specified in Table 1.

USP specifies that if a peak is present in the sample at the retention time of ethylene glycol or diethylene glycol, then it needs to be quantified based on peak area ratio of these impurities with respect to the internal standard peak area, as in the following equation.

$$Peak\ Response\ Ratio\ for\ Ethylene\ Glycol = \frac{Peak\ Area\ Ethylene\ Glycol\ in\ Sample}{Peak\ Area\ of\ Internal\ Standard\ in\ Sample}$$

$$Peak\ Response\ Ratio\ for\ Diethylene\ Glycol = \frac{Peak\ Area\ Diethylene\ Glycol\ in\ Sample}{Peak\ Area\ of\ Internal\ Standard\ in\ Sample}$$

Limit for Ethylene Glycol

USP states the acceptance criteria for a ethylene glycol peak if present in a sample be calculated as the peak response ratio of ethylene glycol relative to the internal standard (2,2,2-trichloroethanol) in a sample solution be not more than (NMT) the peak response ratio of ethylene glycol relative to the internal standard (2,2,2-trichloroethanol) in the standard solution.

Limit for Diethylene Glycol

USP states the acceptance criteria for a diethylene glycol peak if present in a sample be calculated as the peak response ratio of diethylene glycol relative to the internal standard (2,2,2-trichloroethanol) in sample solution be not more than (NMT) the peak response ratio of diethylene glycol relative to the internal standard (2,2,2-trichloroethanol) in the standard solution.

Sample Analysis

The analyzed propylene glycol commercial USP-grade sample did not present any peak at the RT for ethylene glycol and diethylene glycol. Hence, it meets the USP criteria for the limit of ethylene glycol and diethylene glycol and can be considered as USP grade propylene glycol as marketed by the supplier. An overlay chromatogram of propylene glycol commercial USP-grade sample and standard injection is presented in Figure 2. As seen, there are no peaks detected at the RT of either of the impurities in question. The identity of propylene glycol is confirmed based on the RT of the propylene glycol peak in the sample solution corresponds to that of the standard solution.

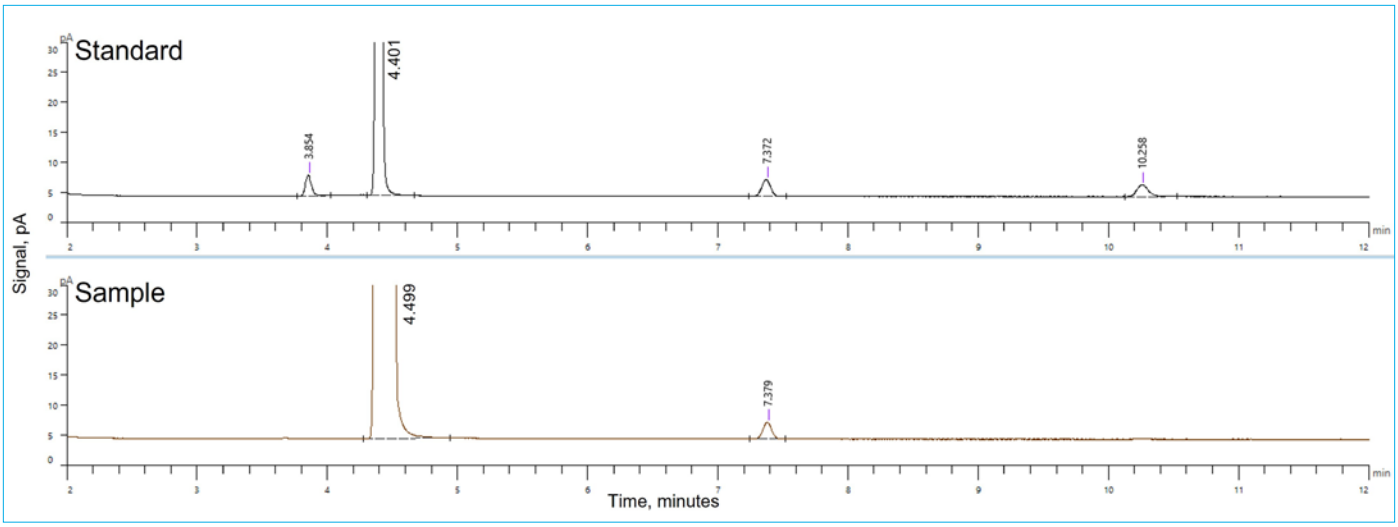

Figure 2: Overlay Chromatogram of Standard solution and commercial USP-grade propylene glycol sample solution in methanol. Analyte Peak in standard solution ethylene glycol (RT 3.854 min), propylene glycol (RT 4.401 min), 2,2,2-trichloroethanol as the internal standard (RT 7.372min) and diethylene glycol (RT 10.258 min). Analyte Peak in commercial USP-grade propylene glycol Sample Solution propylene glycol (RT 4.449 min) and 2,2,2-trichloroethanol as the internal standard (RT 7.379 min).

Spiked Sample Analysis

To test the validity of the method, the commercial USP-grade propylene glycol sample was intentionally spiked with ethylene glycol and diethylene glycol standards to mimic a real life non-USP grade sample under test. This spiked sample was analyzed as per the test conditions in Table 1 and then quantification of ethylene glycol and diethylene glycol was carried out by peak area ratio method as specified by USP and as explained prior.

Figure 3 represents an overlay of chromatograms of the spiked propylene glycol sample and standard solution. Table 5 presents the results for limit of ethylene glycol and diethylene glycol by peak area ratio calculations. Table 4 presented the peak area ratio of ethylene glycol and diethylene glycol with respect to the internal standard in the standard solution and, based on the results in Table 5, the spiked propylene glycol sample meets the specifications for limit of ethylene glycol but failed to meet the specifications for diethylene glycol. Nonetheless, the analysis shows that the GC 2400 System performs the USP propylene glycol analysis for limit of ethylene glycol and diethylene glycol.

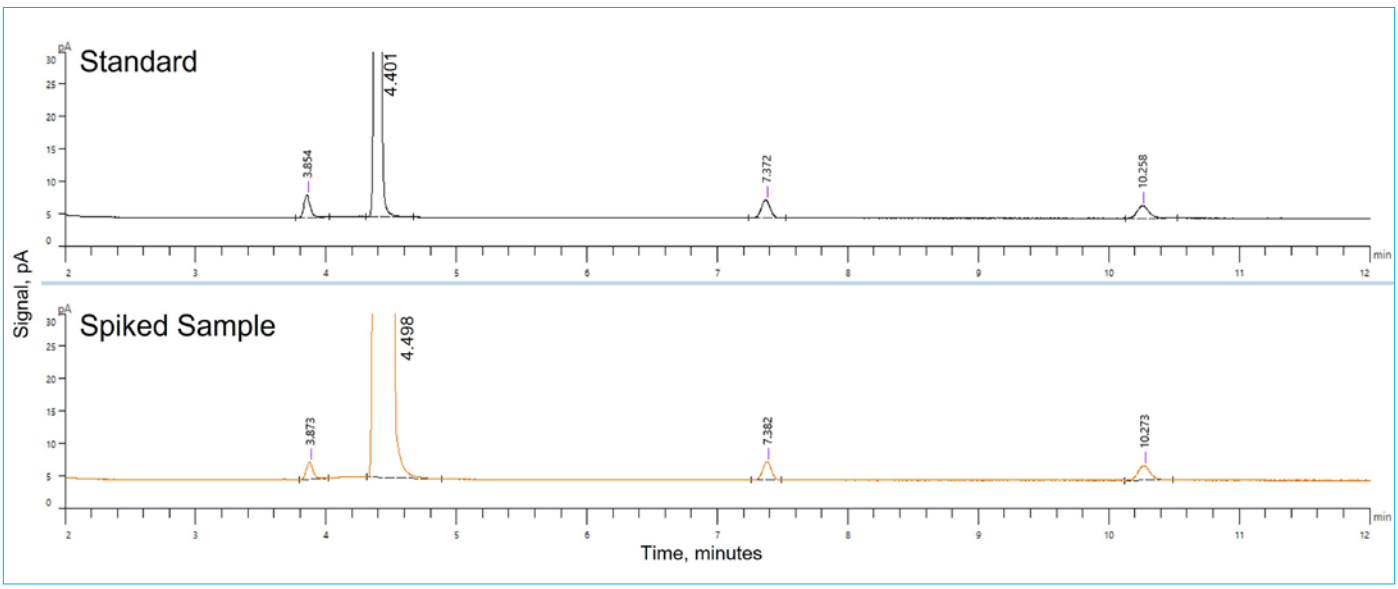

Figure 3: Overlay Chromatogram of Standard solution and Spiked Sample solution in methanol. Analyte Peak in standard solution ethylene glycol (RT 3.854 min), propylene glycol (RT 4.401 min), 2,2,2-trichloroethanol as the internal standard (RT 7.372min) and diethylene glycol (RT 10.258 min). Analyte Peak in Spiked Sample solution ethylene glycol (RT 3.873 min), propylene glycol (RT 4.498 min), 2,2,2-trichloroethanol as the internal standard (RT 7.382min) and diethylene glycol (RT 10.273 min).

Table 5: Peak Area Ratio results for Ethylene Glycol and Diethylene Glycol, in spiked sample.

Sample #	Ethylene Glycol Peak Area	Diethylene Glycol Peak Area	Internal Standard (2,2,2-trichloroethanol) Peak Area	Peak Area Ratio (Ethylene Glycol/Internal Standard)	Peak Area Ratio (Diethylene Glycol/ Internal Standard)
Sample 1	9.795	15.591	13.631	0.72	1.14
Sample 2	9.605	14.996	13.222	0.73	1.13
Sample 3	9.641	14.754	13.275	0.73	1.11
Avg	9.680	15.114	13.376	0.72	1.13
RSD	1.039%	2.850%	1.663%	0.62%	1.47%
Avg Peak Area Ratio in Standard Solution (Refer Table 4)				0.91	0.93

Conclusion

For the limit of ethylene glycol and diethylene glycol, the PerkinElmer GC 2400 System meets the USP propylene glycol monograph requirements. The retention time reproducibility for this analysis was quite precise with an average %RSD of 0.02% to 0.04% and average peak area reproducibility of about 1% or less demonstrating superior performance. Further, a resolution requirement of NLT 5 between ethylene glycol and propylene glycol was largely improved by 40% of that specified under USP monograph for propylene glycol: limit of ethylene glycol and diethylene glycol.

SimplicityChrom CDS Software supports compliance with 21 CFR Part 11 data requirements, and provides a practical, customizable user experience, adapting to different user-proficiency levels. In addition, the detachable touchscreen provides versatility and portability which ultimately offers time optimization for busy lab environments.

References

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