APPLICATION NOTE

Gas Chromatography

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GC-FID: Determination of Ethylene Glycol and Diethylene Glycol in Polyethylene Glycol 400 according to USP Monograph

Introduction

Polyethylene glycol (PEG) is a polyether compound derived from petroleum and one of the most popular polymeric materials with diverse industrial/biological/medical applications. PEG is routinely synthesized by polymerizing ethylene oxide, wherein ethylene oxide reacts with ethylene glycol (EG) in presence of a catalyst to form polymers of ethylene oxide.[1] The structure of PEG is commonly expressed as $H-(O-CH_2-CH_2)_n-OH$ where n represents the number of monomer units. PEG not only is soluble in both aqueous and organic solvents, but also has different physical properties based on the molecular weight (MW), and hence it is widely used in many industrial applications. More noticeably it has a predominant use as Food and Drug Administration (FDA) approved excipient in cosmetics and pharmaceutical products. PEG can be used as a permeation enhancer, thickener, solubilizer, coating agent for tablets capsules and as a surface modifier in various drug delivery formulations.[2] PEG with a molecular weight 400 (PEG 400) is a clear, colorless, viscous liquid that readily dissolves many hydrophobic drugs and hence it is widely used as a solvent for many oral and topical pharmaceutical products, specifically in ophthalmic solutions to treat dry eyes.[3]

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PEG is traditionally considered safe by the FDA, however depending on the synthetic route employed during the polymerization process, toxic impurities like ethylene oxide, 1,4-dioxane, ethylene glycol (EG) and diethylene glycol (DG) are commonly encountered.[4,5] Both EG and DG are considered toxic to human health and in some cases are found to be nephrotoxic. To detect and quantify these impurities United States Pharmacopoeia (USP) monograph for PEG specifies Gas Chromatography with Flame Ionization Detection (GC-FID) as the analytical method of choice.[6] Specifically, the monograph specifies determination of EG and DG impurities based on the molecular weight (MW) of the PEG sample under test. This application note will focus on PEG samples having a MW of less than 450 where USP specifies a GC-FID using a packed column as the analytical method of choice. USP recommends a stainless-steel (SS) packed column with a 12% G13 phase (D-Sorbitol, packed on a S1NS solid support), herein a custom made Restek packed column equivalent to it was utilized for this application.

This application note reports the results from the analysis of Ethylene Glycol and Diethylene Glycol in Polyethylene Glycol 400 sample according to USP requirements performed with the PerkinElmer GC 2400™ System equipped with a packed column injector and FID as detector, showing improved productivity and lab time optimization. PerkinElmer SimplicityChrom™ Chromatography Data System (CDS) Software manages the analytical workflow and it supports compliance with Title 21 of the Code of Federal Regulations (CFR), Part 11.

Instrumentation

The PerkinElmer GC 2400 System, coupled with a packed column injector and a 12% G13 SS packed column on S1NS support provides a robust sample introduction system for the analysis. The GC 2400 System was configured with a PerkinElmer AS 2400™ Liquid Sampler and a FID Detector enabling a reliable platform for quantifying EG and DG impurities in PEG. The PerkinElmer Simplicity Vision™, running on the detachable touchscreen or any PCs, enables real time monitoring, from any location on the same network, optimizing time and ultimately increasing productivity of the lab.

Figure 1: PerkinElmer GC 2400 System.

Experimental

A 1.5 m x 3 mm SS packed column with 12% G13 phase on S1NS support was installed in the packed column injector and conditioned according to standard practices. The GC conditions required for the analysis are listed in Table 1 and are as per USP monograph. Water was used as a solvent for standard and sample preparation. USP-grade EG and DG were purchased from Millipore Sigma to be used for standard preparation. Commercial grade PEG 400 was purchased from Millipore Sigma to be used as a sample.

Standard Preparation

A standard was prepared with the following solution:

- 0.50 mg/mL each of USP EG and USP DG, in water was prepared as stated in USP monograph.

Sample Preparation

A sample was prepared using commercial grade PEG 400 at 400 mg/mL in water as stated in the USP monograph.

Spiked Sample: The commercial grade PEG 400 sample was intentionally spiked with EG and DG standards to mimic a non-USP grade sample under test. A spiked sample was prepared at 400 mg/mL commercial grade PEG 400 in water and spiked with approximately 0.70 mg/mL each of EG and DG standard solutions in water. This will be used as spiked sample.

Table 1: Chromatography conditions.

GC Parameters		
Instrument	GC 2400 System	
Column	1.5 m x 3 mm SS packed column with 12% G13 on support S1NS	
GC Oven Parameters	Initial	Final
	140 °C	140 °C (40 min)
AS 2400 Liquid Sampler Parameters		
Syringe Size	5 µL (N6402556)	
Injection Volume	2.0 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	Packed Column Injector, Septum Flow: 3 mL/min	
Temperature	250 °C	
Carrier/mode	Helium/Constant Flow mode	
Flow Rate (mL/min)	50 mL/min	
FID Detector Parameters		
Type	FID with Packed Column Adapter (N6406057)	
Temperature	280 °C	
Hydrogen	30 mL/min	
Air	400 mL/min	
Makeup Gas	Nitrogen 25 mL/min	
Data rate	10 pt/sec	

Consumables

Product Description	Part Number
Restek 1.5 m x 3 mm Stainless Steel (SS) Capillary Packed Column with a 12% Sorbitol on Diato-WNAW 60/80 Support (equivalent to 12% G13 on S1NS support)	PN# PKC49347
FID Packed Column Adapter, Pkg. 1	N6406057
Advanced Green Inlet Septum, Pkg. 10	N9306218
5 µL Autosampler Syringe, Pkg. 1	N6402556
Graphite Vespel Column Ferrules 1/8 in I.D., Pkg. 10	09920133
2 mL Clear Glass 9 mm Screw Top Vial with Write-On Patch, Liquid Autosampler Vials, Pkg. 100	N9307801
9 mm Blue Screw Caps with PTFE/SIL Liner (Liquid Autosampler Caps), Pkg. 100	N9306202
Triple Filter (Hydrogen and Nitrogen), Pkg. 1	N9306110
Moisture/Hydrocarbon Trap (Air), Pkg. 1	N9306117
Triple Filter (Helium), Pkg. 1	N9306106
GC 2400 Packed Injector Liner, 3 mm I.D., Pkg. 1	N6406035

Data Acquisition

Instrument control and data analysis was done using SimplicityChrom CDS Software (version 2.0) which allows streamlined instrument setup, data acquisition, and processing.

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. The GC 2400 System's advanced Pneumatic Pressure Controller (PPC) specifically for packed column injectors is capable of highly repeatable separations, allowing reliable identification of compounds by RT. Table 2 shows the RT and % relative standard deviations (%RSD) of 0.08% for EG and 0.09% for DG were obtained, respectively. Peak response %RSD for EG and DG were less than 1%, further validating the precise and reproducible temperature and flow control of the GC 2400 System. Using SimplicityChrom CDS Software the system suitability (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.

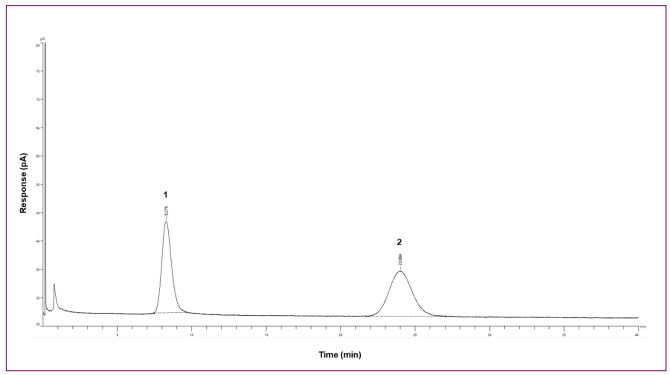

Figure 1: Standard Chromatogram containing 0.50 mg/mL of Ethylene Glycol and Diethylene Glycol in water. Peak 1: ethylene glycol (RT 8.279 min), Peak 2: diethylene glycol (RT 23.984 min).

Table 2: Retention time (RT) and Peak Response for Standard Solution.

	Ethylene Glycol RT (min)	Diethylene Glycol RT (min)	Ethylene Glycol Peak Response (pA)	Diethylene Glycol Peak Response (pA)
Trial 1	8.283	24.010	16.008	7.977
Trial 2	8.280	24.018	15.955	7.904
Trial 3	8.272	23.961	16.140	8.038
Trial 4	8.291	23.984	16.311	8.101
Trial 5	8.279	23.984	16.191	8.032
Trial 6	8.286	23.978	16.257	7.984
Avg. RSD	8.282 0.08%	23.989 0.09%	16.144 0.86%	8.006 0.84%

Sample Analysis

Commercial grade PEG 400 was used as a test sample. The sample was prepared at 400 mg/mL in water and then analyzed as per the test conditions specified in Table 1.

USP monograph specifies that if a peak is present in the sample at the RT of EG or DG, then it needs to be quantified based on standard peak response quantitation method, as presented in the following equations.

$$\%\ \text{Ethylene Glycol} = \frac{(\text{Peak Response of EG in SPL})}{(\text{Peak Response of EG in STD})} \times \frac{(\text{Concentration of EG in STD (mg/mL)})}{(\text{Concentration of PEG 400 in SPL (mg/mL)})} \times 100$$

EG: Ethylene Glycol, SPL: Sample; STD: Standard

$$\%\ \text{Diethylene Glycol} = \frac{\text{Peak Response of DG in SPL}}{\text{Peak Response of DG in STD}} \times \frac{\text{Concentration of DG in STD (mg/mL)}}{\text{Concentration of PEG 400 in SPL (mg/mL)}} \times 100$$

DG: Diethylene Glycol, SPL: Sample; STD: Standard

Sample Analysis

A chromatogram of commercial grade PEG 400 sample injection is presented in Figure 2. As seen, EG and DG detected in the PEG 400 sample are confirmed based on the RT of EG and DG peak to that of the standard solution. Table 3 shows the % content of each of these impurities in the sample calculated based on the formulas presented before. USP monograph states an acceptance criterion of not more than (NMT) 0.25% for the sum of % EG and % DG in the sample. From the results summarized in Table 3 the commercial PEG 400 meets this acceptance criteria as specified in USP.

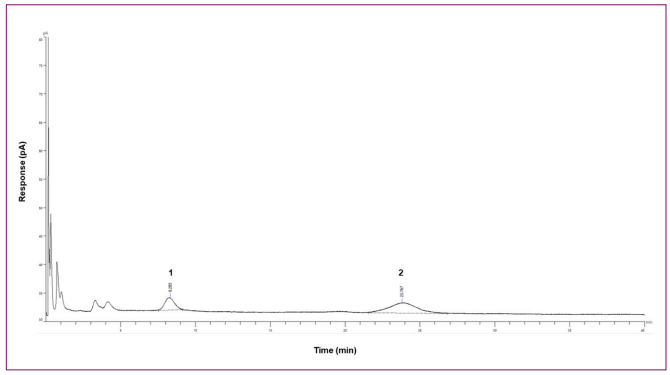

Figure 2: Sample Chromatogram of commercial PEG 400 sample solution at 400 mg/mL in water. Peak 1: ethylene glycol (RT 8.285 min), Peak 2: diethylene glycol (RT 23.767 min).

Table 3: Sample Results for PEG 400.

Analyte	Retention Time (min)	Sample Peak Response (pA)	PEG 400 Sample Concentration (mg/mL)	Standard Peak Response (pA)	Standard Concentration (mg/mL)	% Content
Ethylene Glycol	8.285	2.194	400.331	16.144*	0.495	0.02
Diethylene Glycol	23.767	1.800		8.006*	0.498	0.03
Sum of % Content of Ethylene Glycol and Diethylene Glycol in sample						0.05

** Average peak response of 6 consecutive standard injections (refer Table 2).*

Spiked Sample Analysis

To test the validity of the method, the commercial grade PEG 400 sample was intentionally spiked with EG and DG standards to mimic a non-USP grade sample under test. This spiked sample was analyzed as per the test conditions in Table 1 and then quantification of EG and DG was carried out as specified by USP and as explained prior. Table 4 presents the results for the content of % EG and % DG found in the spiked PEG 400 sample, and it fails to meet the USP requirement of NMT 0.25% for the sum of % EG and % DG found. The analysis shows that the new PerkinElmer GC 2400 System can carry out USP PEG analysis for limit of ethylene glycol and diethylene glycol, for PEG of MW less than 450.

Table 4: Sample Results for PEG 400 spiked sample.

Analyte	Retention Time (min)	Sample Peak Response (pA)	PEG 400 Sample Concentration (mg/mL)	Standard Peak Response (pA)	Standard Concentration (mg/mL)	% Content
Ethylene Glycol	8.254	24.280	400.814	16.144*	0.495	0.19
Diethylene Glycol	23.953	11.672		8.006*	0.498	0.18
Sum of % Content of Ethylene Glycol and Diethylene Glycol in sample						0.37

** Average peak response of 6 consecutive standard injections (refer Table 2).*

Conclusion

For the determination of the limit of ethylene glycol and diethylene glycol impurities, the PerkinElmer GC 2400 System, configured with a packed column injector and a FID Detector, delivers results according to USP PEG monograph. The retention time repeatability for this analysis was quite precise with %RSD of less than 0.1% and peak response repeatability of 1% or less demonstrating superior performance. SimplicityChrom CDS Software provides a practical, customizable user experience with multifunctionality and accessibility options. In addition, the detachable touchscreen provides versatility and portability which ultimately offers time optimization for busy lab environments.

References

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