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Screening method for ethylene glycol and diethylene glycol in glycerin-containing products

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ABSTRACT

This paper describes a capillary gas chromatographic method with flame ionization detection for the identification/quantification of ethylene glycol (EG) and diethylene glycol (DEG) in glycerin. The validation study shows that the proposed method is specific, sensitive, precise, and accurate. The linear range of the method was 0.013–0.031 mg/mL for EG and 0.012–0.030 mg/mL for DEG. Wider ranges may be achievable but were not investigated. The limit of detection of EG and DEG were determined as 0.0018% and 0.0036% (w/w) respectively, and at this concentration the signal-to-noise ratios for EG and DEG were approximately 3:1. The method was also used to determine EG and DEG in toothpaste. The results were compared to those obtained by thin-layer chromatography (TLC) and showed greater sensitivity and specificity.

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1. Introduction

Glycerin is a colorless, odorless, viscous liquid with a sweet taste and is widely used in liquid pharmaceutical, cosmetic, and food products. It is produced in two different ways: natural glycerin (a main by-product of biodiesel and soap production) and synthetic glycerin. The quality of crude natural glycerin depends mostly on the manufacturing process regardless of source (tallow, vegetable, or a mixture of vegetable/tallow), and it is typically 80% [1] to 95% [2] pure. Further purification and refining steps can take glycerin to 99.5% purity, which is the major grade on the market for human consumption. Synthetic glycerin is petroleum-based, and its production involves several processing steps using petrochemical building blocks. Although both procedures can produce a highly refined and purified product, the market share for synthetic glycerin is not large. Dow Chemical, once the only US producer of synthetic glycerin, closed its glycerin plant in Freeport, Texas, because of “the flood of glycerin from biodiesel production” [3]. Dow Chemical still operates a glycerin plant in Germany.

According to the SRI Consulting research report on glycerin [2], in 2007 North America was the third largest producer and consumer of refined glycerin after Asia and Western Europe. Annual consumption of glycerin in the United States ranged between 400 and 450 million pounds in 2005–2008 [3]. Reported applications of glycerin included food products (24%); personal care products, including skin, hair, and soap products (23%), oral care products such as toothpaste and mouthwash (17%), and pharmaceuticals (only 7%) [4]. The other 29% was distributed among tobacco, polyether polyols for urethanes, alkyd resins, cellophane, explosives and miscellaneous plasticizer, humectants, and lubricant manufacturers [4].

The history of glycerin used for human consumption involves many tragic stories, including numerous deaths. Perhaps the most famous case was the 1937 Elixir Sulfanilamide disaster in the United States, which resulted in deaths of more than 100 Americans and ultimately led to the enactment of the Federal Food, Drug, and Cosmetic Act of 1938. Diethylene glycol (DEG) found in the elixir at a very high concentration was responsible for this tragedy [5]. Over the years additional outbreaks of DEG poisoning associated with pharmaceutical products occurred in different parts of the world [6,7]. The most recent incident involved the sale of counterfeit toothpaste [8]. In most cases, the poisonings are believed to have occurred because of counterfeit products made with DEG.

DEG, a known nephrotoxin and hepatotoxin, is used as an industrial solvent and antifreeze. Glycerin, DEG, and ethylene glycol (EG) have similar physical properties, including natural sweetness. This facilitates the adulteration of glycerin with less expensive, more toxic DEG. Complexities in the glycerin distribution system can include multiple brokers in international trade, which underscores the need for manufacturers to screen glycerin and its end products.

Because of the consequences of using poor-quality glycerin in the production of medicine and mouth care products, the US Food and Drug Administration (FDA) has taken several measures, including issuance of a “Guidance for Industry: Testing of Glycerin for

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Diethylene Glycol" in May 2007 [9]. This guidance recommends that manufacturers "perform a specific identity test that includes a limit test" for DEG in glycerin and glycerin-based raw materials to ensure the DEG content does not exceed 0.1%, a level that FDA identified in the guidance to be a "relevant safety limit", recognized in the *United States Pharmacopeia* (*USP*) monograph for glycerin [10]. Validated alternative procedures that demonstrate equivalent identification and sensitivity for DEG can be used. A thin-layer chromatographic (TLC) method published in the *Journal of AOAC International* [11] was presented as an example of an alternative method with a sensitivity of 0.05% for DEG. This method looked very attractive to many glycerin manufacturers and users because of its misleading simplicity, sensitivity, speed of analysis, and cost. The performance of the TLC method was evaluated in parallel with development and validation of a gas chromatographic (GC) method capable of detecting and quantitating trace amounts of EG and DEG. Even though EG was not mentioned in the FDA guideline [9], USP added this compound to the list of potential adulterants of glycerin because of its high toxicity. A minimal risk level of 0.8 mg/kg/day has been established for both acute-duration oral exposure (14 days and less) and intermediate-duration exposure (15–364 days) to EG [12].

The current USP Glycerin monograph includes a GC method for the limit of diethylene glycol and related compounds in which the limit of DEG is 0.1% [10]. A robust GC method that is sensitive enough to unambiguously quantitate 0.025% (w/w) DEG and EG in glycerin has been developed. This paper reports the approaches taken by USP to analyze DEG and EG in glycerin and glycerin-containing products.

2. Experimental

2.1. Chemicals

We used USP Glycerin Reference Standard (RS) (Rockville, MD, USA); ethylene glycol and diethylene glycol from Fluka (Buchs, Switzerland); glycerin from Spectrum Chemicals (New Brunswick, NJ, USA); 2,2,2-trichloroethanol and corn starch from Aldrich (Saint Louis, MO, USA); toluene, potassium permanganate, and chloroform from ACROS (Geel, Belgium); iodine, ammonium hydroxide, and acetone from Fisher (Pittsburg, PA, USA); methanol from Fisher and B&J Brand (Morristown, NJ, USA); and Milli-Q water. All reagents were of analytical grade.

2.2. Equipment

Two different GC systems were used. One GC system consisted of an Agilent 6890N (G1530N) gas chromatograph equipped with a flame ionization detector (FID) and an Agilent 7683 Series (G2613A) injector. The data on this system were acquired via GC Chemstation Rev. B.01.01 [164] SR1 software. The second system consisted of a Varian CP-3800 gas chromatograph equipped with an FID and a Varian CP-8400 injector. The data on this system were acquired via Varian WS Saturn GC/MS Workstation Version 6.40. We used an Agilent 5183-4647, low-pressure, deactivated, split liner with glass wool and a J&W Scientific DB-624, 0.53-mm × 30-m, 3-μm film G43 fused silica column, Part No. 125-1334E. TLC plates with silica gel 60 F254 coating (250 μm) were obtained from EMD, and we used a CAMAG Reprostar 3 VideoScan.

2.3. Preparation of solutions

2.3.1. Internal standard stock solution

A 1.0 mg/mL solution of 2,2,2-trichloroethanol (internal standard) was prepared in methanol.

2.3.2. Ethylene glycol stock standard solution

A 0.5 mg/mL solution of ethylene glycol was prepared in methanol.

2.3.3. Diethylene glycol stock standard solution

A 0.5 mg/mL solution of diethylene glycol was prepared in methanol.

2.3.4. Glycerin stock standard solution

A 50 mg/mL solution of glycerin was prepared in methanol.

2.3.5. Standard solution

A solution containing 0.025 mg/mL each of EG and DEG and 0.05 mg/mL of internal standard was prepared in methanol from EG, DEG, and internal standard stock solutions.

2.3.6. Resolution solution

A solution was prepared in methanol from USP Glycerin RS and spiked with EG, DEG, and internal standard stock solutions to give 50 mg/mL of glycerin, 0.025 mg/mL each of EG and DEG, and 0.05 mg/mL of internal standard.

2.3.7. Peak identification solution

A solution containing 0.025 mg/mL each of glycerin, EG, and DEG and 0.05 mg/mL of internal standard was prepared in methanol from glycerin, EG, DEG, and internal standard stock solutions.

2.3.8. Test solution

A solution was prepared in methanol from glycerin bulk and spiked with internal standard stock to give 50 mg/mL of glycerin and 0.05 mg/mL of internal standard.

2.3.9. Test solution from toothpaste samples

About a 1.0-g portion of a toothpaste sample was mixed with 5.0 mL water and 5.0 mL of acetonitrile and was vortexed. The solution was centrifuged at 2000 rpm for 30 min. A 5.0-mL portion of the clear supernatant liquid was mixed with 5.0 mL each of internal standard stock solution and methanol and was filtered through a 0.2-μm filter; we discarded the first 1.5 mL of filtrate.

2.4. Chromatographic conditions

We employed a DB-624, 30-m × 0.53-mm fused silica column coated with 3-μm G43 stationary phase, an FID detector set at 250°, a 1-μL injection (injector was maintained at 220°), and a gradient oven program. The oven was programmed to maintain 100° for 4 min, then to increase to 120° at a rate of 50° per minute, and to maintain 120° for 10 min, then again to increase to 220° at a rate of 50° per minute, and to maintain 220° for 6 min. A split injection system with a split ratio of about 10:1 was used. We used an Agilent low-pressure, deactivated split liner with glass wool. The carrier gas consisted of helium at a flow rate of 4.5 mL/min. The air flow was 375 mL/min, and the hydrogen flow and the make-up gas (nitrogen) flow were 40 mL/min.

3. Results and discussion

3.1. Method development

The TLC procedure developed by Kenyon et al. [11] was evaluated as a potential identification test for EG and DEG in glycerin. This procedure uses a developing solvent mixture of toluene–acetone–5 M ammonium hydroxide (5:85:10, v/v/v). Fifteen microliters of each test solution containing 400 mg/mL of glycerin separately spiked with 0.1% and 0.05% each of EG and

DEG were applied on TLC plates. Regardless of the use of different types of TLC plates (glass-backed or plastic-backed silica gel 60 F254 coating) and staining procedures (iodine starch and potassium permanganate staining) the DEG and EG spots were not visible at the 0.1% level in glycerin. Also, the method was not sufficiently specific to separate EG and DEG. The appearance of the TLC plates after development showed that the plates were overloaded with glycerin.

A modified TLC procedure that uses a solvent mixture of chloroform–acetone–5 M ammonium hydroxide (10:80:10, v/v/v) was reported by Kenyon et al. [11] and also was evaluated. This method showed better sensitivity than the earlier TLC procedure and is capable of detecting 0.1% DEG or EG (0.05% with ambiguity) in glycerin with potassium permanganate staining. The drawbacks of the TLC method include the analyst's techniques, the time dependence of the spot-detection process, the toxicity of chloroform, and the disposal of potassium permanganate. With this TLC procedure, a false negative test is possible at 0.1% of DEG or EG concentrations.

A GC method was developed as a sensitive and robust screening procedure. The method was based on the chromatographic conditions listed in the limit of diethylene glycol and related compounds in glycerin [10]. The original method was capable of quantitating DEG at 0.1% (w/w) in glycerin as well as estimating the amount of other related compounds based on total detectable area. The goal for the new GC method was to increase the method sensitivity and introduce a limit for another potential contaminant in glycerin, EG. We decided to retain some of the original chromatographic system settings unchanged, such as a DB-624, 30-m × 0.53-mm fused silica capillary column coated with 3-μm G43 stationary phase, helium as carrier gas, the injector and detector temperatures at 220° and 250°, respectively.

Improved sensitivity in chromatographic methods typically is accomplished by increasing the analyte amount injected on a column. This can be achieved either by increasing the analyte concentration in a test solution or by increasing the injection volume. Using more concentrated glycerin test solutions could be a problem because of the high viscosity of glycerin. Therefore, we decided to increase the injection volume to 1 μL. The limited capacity of GC liners to hold expanded solvent vapors led us to investigate the possibility of using organic solvents such as isopropyl alcohol and methanol instead of water. Better precision resulted when methanol was used as the solvent. Though better sensitivity was seen with splitless injections, the chromatography was difficult to interpret due to the presence of a large number of unknown peaks. A split injection with 10:1 ratio was chosen for the final chromatographic conditions, which gave signal-to-noise ratios of 46 and 25 for EG and DEG, respectively, for a solution containing 0.025% each of EG and DEG.

The responses of EG and DEG were on average 25% higher in the presence of glycerin matrix in either of the organic solvents. To improve the recovery results, we evaluated regular and pulse injection splitless modes. Unsuccessful attempts to correct the recovery issue also included the increase in the injector temperature. As an alternative to modifications of chromatographic conditions to improve EG and DEG recovery results, the use of an internal standard was proposed. 2,2,2-Trichloroethanol was a suitable candidate because it neither interfered with any of the analytes of interest, including glycerin, nor showed any interference from other unknown peaks that come from glycerin samples. GC analysis of a resolution solution showed that for EG, DEG, and internal standard the tailing factors were 1.3, 1.3, and 1.0, and the theoretical plates were 26,000, 53,000, and 46,000, respectively. The resolutions between the glycerin, DEG, EG, or internal standard were greater than 2.0. The chromatogram presented in Fig. 1(a) shows good chromatographic separation between the peaks of interest.

3.2. Method validation

3.2.1. Specificity

The specificity of the method was established by the absence of interference from solvent blank (methanol) and glycerin test solution without internal standard at the retention times of EG, DEG, or internal standard and from internal standard at the retention times of EG or DEG. A typical chromatogram of a test solution is presented in Fig. 1(b). Method specificity also was examined by exposing a portion of the test solution without internal standard to ultraviolet and white light and aging another portion of the same test solution at ambient temperature for 24 h. Absence of interferences showed the method is specific to EG and DEG.

3.2.2. Linearity

The linearity was investigated at five levels ranging from 50% to 120% (0.013–0.030 mg/mL) of the nominal EG and DEG concentration of 0.025 mg/mL. A single injection each of the linearity solution and of a solvent blank was made. The construction of a plot of the peak area ratios of EG or DEG to internal standard vs. concentration of EG or DEG, followed by regression analysis, showed the correlation coefficients were 0.9996 and 0.9990 for EG

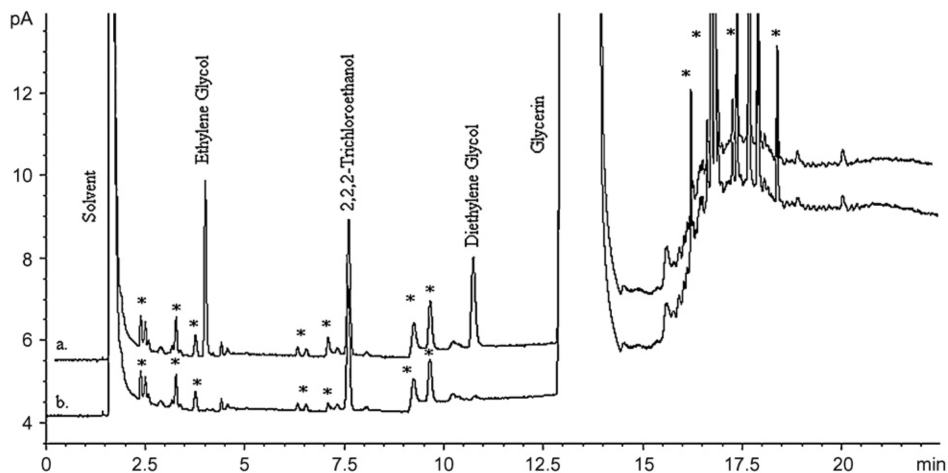

Fig. 1. An overlay chromatogram of (a) a resolution solution (50 mg/mL glycerin, 0.025 mg/mL of EG and DEG, and 0.05 mg/mL of internal standard) and (b) a test solution (50 mg/mL of glycerin and 0.050 mg/mL of internal standard). *Peaks from solvent and glycerin.

Table 1
Accuracy and precision results obtained by spiking glycerin with EG and DEG at 50%, 100%, and 120% of the nominal concentration of 0.025 mg/mL.

Analyte	Level	Preparation	Concentration added (mg/mL)	Concentration found (mg/mL)	% recovery	Average % recovery	% RSD
EG	50%	1	0.0123	0.0125	102.1	101.8	1.3
		2	0.0121	0.0125	102.9		
		3	0.0129	0.0129	100.3		
	100%	1	0.0246	0.0253	102.9	102.8	0.1
		2	0.0242	0.0249	102.7		
		3	0.0258	0.0265	102.7		
	120%	1	0.0295	0.0305	103.5	102.4	1.1
		2	0.0291	0.0297	102.3		
		3	0.0309	0.0313	101.3		
DEG	50%	1	0.0123	0.0133	106.8	104.0	4.0
		2	0.0121	0.0140	106.0		
		3	0.0129	0.0124	99.2		
	100%	1	0.0246	0.0260	104.3	103.3	1.2
		2	0.0242	0.0275	103.8		
		3	0.0258	0.0254	101.9		
	120%	1	0.0295	0.0316	105.8	104.1	2.3
		2	0.0291	0.0334	105.1		
		3	0.0309	0.0303	101.4		

and DEG, respectively. The percentages of *y*-intercept bias were 0.9% and 0.1% for EG and DEG, respectively. The results show good correlations between the peak area ratios and concentrations of both components.

3.2.3. Accuracy

The accuracy of the method was demonstrated by recovery studies at 50%, 100%, and 120% levels, in triplicate. The accuracy solutions were prepared by spiking glycerin test solutions with EG and DEG to obtain 50%, 100%, and 120% of the nominal EG and DEG concentration of 0.025 mg/mL. The individual recoveries and the average recoveries were found to be in the range of 99–107% for both EG and DEG (Table 1).

3.2.4. Precision

The instrumental precision of the method was determined by making five consecutive injections of a standard solution and calculating the response ratios of EG or DEG to internal standard. The relative standard deviations (RSDs) were lower than 3.0%.

The repeatability of the method was demonstrated by calculating the RSDs of triplicate preparations of accuracy samples at 50%, 100%, and 120% levels. The RSDs were less than 4.0% at all levels (Table 1).

Analyst and equipment variation was evaluated by analyzing two sets of six independent samples prepared by two analysts using the same glycerin bulk. One set of samples was prepared by Analyst 1 and analyzed on the Agilent chromatograph. The second set of samples was prepared by Analyst 2 and analyzed on Varian chromatographs on different days using the same column. The absence of EG and DEG in glycerin necessitated the test solutions be spiked with EG and DEG at 100% of the nominal concentration. The amounts of EG and DEG in each sample were calculated. The results obtained by Analyst 2 were compared to those obtained by Analyst 1 using the equivalence test at 90% confidence interval and were shown to be equivalent (Table 2).

Table 2
Analyst and equipment variation results obtained by spiking glycerin with EG and DEG, each at 0.05% level.

Analyte	Preparation	Analyst 1 instrument 1	Analyst 2 instrument 2
EG	1	101.98	99.25
	2	102.76	105.04
	3	102.87	101.54
	4	102.00	103.70
	5	103.54	105.53
	6	103.84	98.23
	Estimated ratios of means[a]	NA	101
	90% CI for the ratios	NA	97.1–101.7
	Satisfies an equivalence criterion	NA	Yes
DEG	1	103.33	96.31
	2	103.92	110.70
	3	102.16	102.85
	4	103.08	107.35
	5	104.17	115.97
	6	105.44	109.06
	Estimated ratios of means[a]	NA	102
	90% CI for the ratios	NA	98.2–108.2
	Satisfies an equivalence criterion	NA	Yes

[a] Acceptable range for ratio of means: 90.0–111.1%.

3.2.5. Range

The range of the method is 0.013–0.031 mg/mL of EG and 0.012–0.030 mg/mL of DEG, at which concentration range the linearity, accuracy, and method precision criteria were met.

3.2.6. Limit of quantitation

The limit of quantitation was set as 0.025%, at which concentration the signal-to-noise ratios for EG and DEG peaks were 46 and 25, respectively, and the RSDs for the signal-to-noise ratio of EG or DEG for 10 replicate injections were 3.4% and 4.2%, respectively.

3.2.7. Limit of detection

The limit of detection of EG and DEG were 0.0018% and 0.0036%, respectively, at which concentration the signal-to-noise ratios for EG and DEG were approximately 3:1.

3.2.8. Robustness

The robustness of the method was determined by analyzing the resolution solution with the following deliberate changes to the chromatographic conditions: carrier gas flow ±5%, injector temperature ±5%, detector temperature ±5%, and initial oven temperature ±5% (no change in hold time, temperature ramp ±0.5°/min using slower ramp with higher initial oven temperature and faster ramp with lower initial oven temperature). The resolutions between the peaks of interest were greater than 2.0 under all modified conditions, and the performance of the chromatographic system was not influenced by the variations of the operational parameters inside an accepted domain.

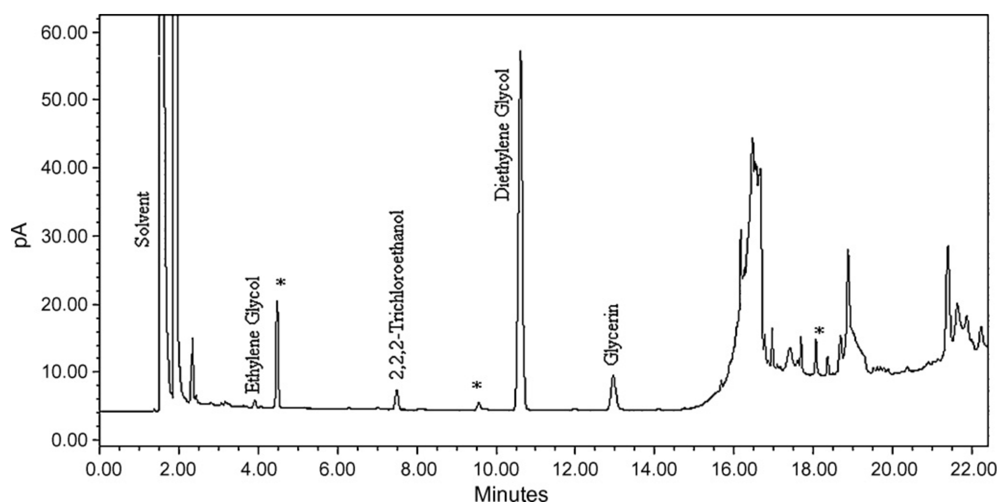

Fig. 2. A chromatogram of the toothpaste sample "8". *Peaks from sample matrix.

Table 3
Comparison of GC and TLC techniques.

Toothpaste sample	GC method		TLC method
	%EG (w/w)	%DEG (w/w)	EG/DEG detected
1	ND[a]	ND	ND
2	ND	0.12	ND
3	ND	1.86	yes
4	ND	0.07	ND
5	ND	0.21	ND
6	0.08	ND	ND
7	ND	5.17	yes
8	0.03	1.80	yes
9	ND	0.09	ND
10	ND	0.03	ND

[a] ND: not detected.

3.2.9. Analysis of glycerin-containing products

To demonstrate the applicability of the developed method in the analysis of healthcare products, the GC-FID method was applied to the analysis of commercially available toothpastes from different manufacturers. The samples were prepared following the procedure described in an FDA publication [13]. The samples were analyzed simultaneously using the TLC procedure and the GC method described herein. Fig. 2 depicts a chromatogram of the toothpaste sample "8" containing both EG and DEG. The results summarized in Table 3 show that GC method is more sensitive in detecting EG and DEG in samples. Only higher levels of DEG in toothpaste were identified by TLC.

4. Conclusion

The developed GC method was robust and sensitive enough to unambiguously quantitate 0.025% (w/w) DEG and EG in glycerin compared to the TLC method, which with some limitations was capable of detecting only 0.1% of the analytes. The accuracy of the TLC test depends on an analyst's techniques. Additionally, the transitory nature of the spot development process may give false negative results. The GC method is more efficient when dealing with a huge number of samples because it can be easily automated. It can be used for the assessment of identity and content of EG and DEG in glycerin as well as in glycerin-containing products such as toothpaste. The method was validated and showed satisfactory data for all the validation parameters tested.

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