Application Note

Pharma & Biopharma

Agilent

Trusted Answers

# Estimation of Ethylene Glycol and Diethylene Glycol in Propylene Glycol, Glycerin, and Syrup Samples with the Agilent 8890 GC


**Author**

Praveen Arya and Vivek Dhyani
Agilent Technologies, Inc.



## Abstract

This application note demonstrates the use of the Agilent 8890 GC system coupled with an FID detector to detect and quantify ethylene glycol and diethylene glycol in propylene glycol, glycerin, and syrup samples.

The method provides high confidence in results for routine analysis in the pharma industry, whether in production, processing, storage, or commercial testing of syrup samples, or for academic purposes.


## Introduction

Propylene glycol and glycerin are commonly used in medicinal syrups as excipients[5]. The solubility of active ingredients is enhanced by their use during formulation[5]. These excipients should be tested for any contamination by ethylene glycol (EG) and diethylene glycol (DEG) as mentioned in regulations such as Indian Pharmacopeia and USP-NF monographs. Some USP-NF monographs include, as part of the applicable identity testing, a limit test for DEG and EG. The relevant safety limit for DEG and EG is not more than (NMT) 0.10%, as recognized by the applicable USP-NF monograph[3].

In this application note, we have shared the results of experiments using an Agilent 8890 GC to identify and quantify EG and DEG in matrices like propylene glycol, glycerin, and syrup samples.

# Experimental

## Instrument parameters

**Table 1.** Method Parameters

| Parameter | Value |
|---|---|
| Inlet Temperature | 250 °C |
| Inlet Liner | Ultra Inert, low pressure drop, split liner, 4 mm ID (p/n 5190-2295) |
| Inlet Septa | Inlet septa, long life, 11 mm (p/n 8010-0239) |
| Injection Volume | 0.5 µL |
| Column | Agilent J&W DB-624 GC column, 30 m × 0.53 mm × 3.00 µm, 7 inch cage, (p/n: 125-1334 |
| Column Flow | Helium, 2.5 mL/min |
| Split Ratio | 10:1 |
| Oven Program | 70 °C for 1 min |
| | 6 °C/min to 150 °C, hold 3 min |
| | 25 °C /min to 245 °C, hold 12 min |
| FID Temperature | 250 °C |
| FID H2 Flow | 40 mL/min |
| FID Air Flow | 300 mL/min |
| FID Make Up Gas | Nitrogen, 25 mL/min |

## Standard calibration

Standard solution-1 was prepared by weighing 100 mg each of EG and DEG in a 100 mL volumetric flask and making up the volume to 100 mL with methanol, with thorough mixing. This solution has a concentration of 1,000 µg/mL for EG and DEG.

IS solution-1 was prepared by weighing 100 mg of 1,3-propanediol in a 100 mL volumetric flask and making up the volume to 100 mL with methanol, with thorough mixing. This solution has a concentration of 1,000 µg/mL for 1,3-propanediol.

Using the above standards, the calibration standards were prepared as presented in Table 2.

## Sample preparation

500 mg of sample was added to a 10 mL volumetric flask. 500 µL of IS solution was added and the volume was made up to 10 mL with methanol, with thorough shaking.

# Results and discussion

## Standard chromatograms

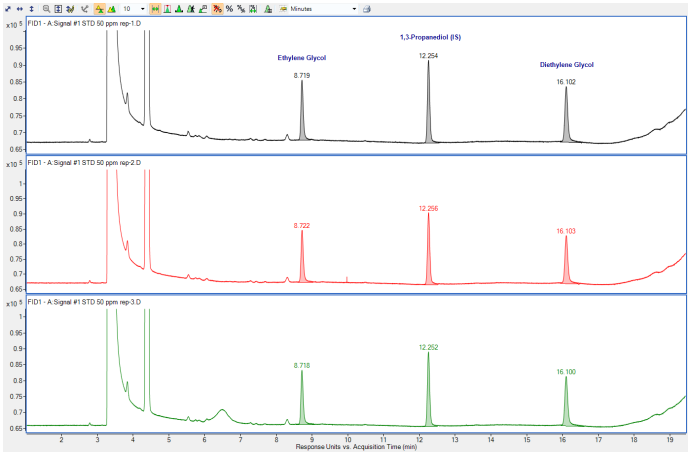


**Figure 1.** Three replicates of 50 µg/mL EG and DEG standards with IS using methanol as diluent.

Figure 1 shows the overlay of chromatograms obtained from three replicate injections of EG, DEG, and IS (1,3-propanediol) at 50 µg/mL concentration level. The peak shapes, resolution, and repeatability were found to be good. Figures 2 and 3 show the six-point internal-standard-based linearity for calibration standards of EG and DEG, respectively. Good $r^2$ values were obtained (0.997 for EG and 0.998 for DEG). Table 3 shows the repeatability in terms of internal standard RSD for RT and area for six calibration standards.

**Table 2.** Calibration standards.

| | Standard Solution-1, 1,000 µg/mL (mL) | IS Working Solution, 1,000 µg/mL (mL) | Make Up Volume with Methanol (mL) | Final Concentration for EG and DEG (µg/mL) | Final Concentration for 1,3-propanediol (µg/mL) |
|---|---|---|---|---|---|
| Calibration Standard-6 | 5 | 0.5 | 10 | 500 | 50 |
| Calibration Standard-5 | 2.5 | 0.5 | 10 | 250 | 50 |
| Calibration Standard-4 | 1 | 0.5 | 10 | 100 | 50 |
| Calibration Standard-3 | 0.5 | 0.5 | 10 | 50 | 50 |
| Calibration Standard-2 | 0.25 | 0.5 | 10 | 25 | 50 |
| Calibration Standard-1 | 0.1 | 0.5 | 10 | 10 | 50 |

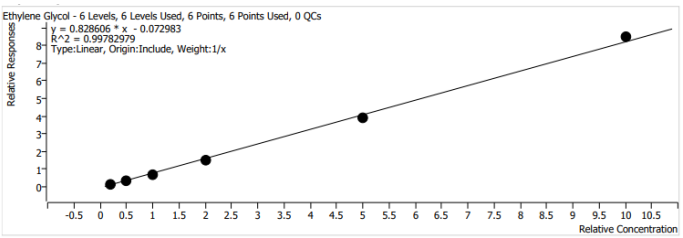


**Figure 2.** Six-point calibration of EG for 10, 25, 50, 100, 250, and 500 µg/mL, respectively.

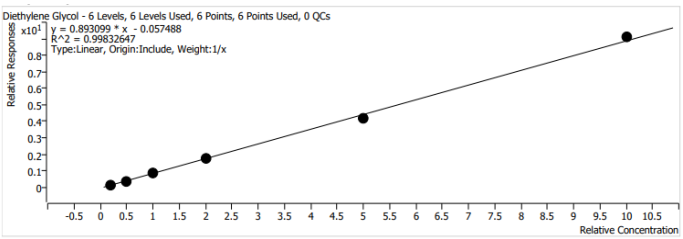


**Figure 3.** Six-point calibration of DEG for 10, 25, 50, 100, 250, and 500 µg/mL, respectively.

**Table 3.** %RSD for RT and area for internal standard in six-point calibration standards.

| | IS RT (min) | IS Area (Counts) |
|---|---|---|
| STD-1_10 µg/mL | 12.249 | 113107 |
| STD-2_25 µg/mL | 12.252 | 109688 |
| STD-3_50 µg/mL | 12.255 | 106848 |
| STD-4_100 µg/mL | 12.255 | 110376 |
| STD-5_250 µg/mL | 12.244 | 117225 |
| STD-6_500 µg/mL | 12.255 | 111914 |
| Mean | 12.252 | 111526.333 |
| SD | 0.0045 | 3513.21 |
| %RSD | **0.036** | **3.15** |

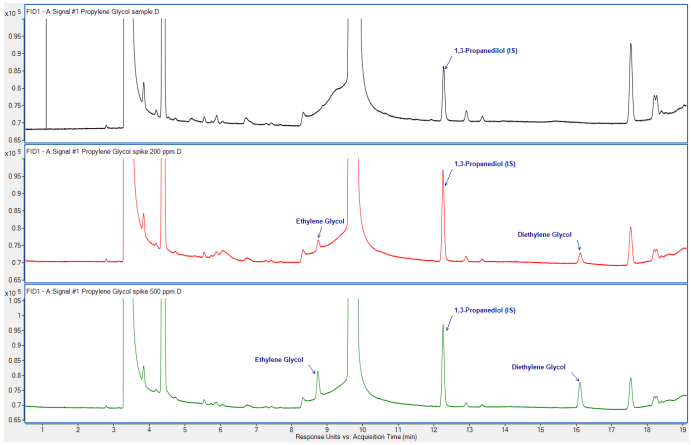


**Figure 4.** Chromatograms of (from top to bottom) blank propylene glycol sample, and 200 and 500 µg/mL spikes.

Figure 4 shows chromatograms obtained from injection of propylene glycol, and propylene glycol spiked with 200 and 500 µg/mL of EG and DEG, respectively. These chromatograms show good resolution of the compounds of interest from matrix peaks. Tables 4 and 5 show the recovery and repeatability (in terms of %RSD) for propylene glycol sample spiked with 200 and 500 µg/mL of EG and DEG, respectively. Excellent recovery and repeatability were achieved for this experiment with propylene glycol sample.

**Table 4.** Recovery and repeatability of EG and DEG in 200 µg/mL spiked propylene glycol sample.

| Spiked Sample | EG Results | | DEG Results | |
|---|---|---|---|---|
| | Calculated Amount (µg/mL) | Recovery (%) | Calculated Amount (µg/mL) | Recovery (%) |
| 200 µg/mL spike-1 | 204.70 | 102.35 | 222.05 | 111.025 |
| 200 µg/mL spike-2 | 203.22 | 101.61 | 220.96 | 110.48 |
| 200 µg/mL spike-3 | 204.86 | 102.43 | 214.37 | 107.185 |
| Mean | 204.26 | **102.13** | 219.13 | **109.56** |
| SD | 0.904 | | 4.155 | |
| %RSD | **0.443** | | **1.896** | |

**Table 5.** Recovery and repeatability of EG and DEG in 500 µg/mL spiked propylene glycol sample.

| Spiked Sample | EG Results | | DEG Results | |
|---|---|---|---|---|
| | Calculated Amount (µg/mL) | Recovery (%) | Calculated Amount (µg/mL) | Recovery (%) |
| 500 µg/mL spike-1 | 456.42 | 91.284 | 501.87 | 100.374 |
| 500 µg/mL spike-2 | 478.86 | 95.772 | 502.44 | 100.488 |
| 500 µg/mL spike-3 | 477.91 | 95.582 | 499.81 | 99.962 |
| Mean | 471.06 | **94.21** | 501.37 | **100.27** |
| SD | 12.690 | | 1.384 | |
| %RSD | **2.694** | | **0.276** | |

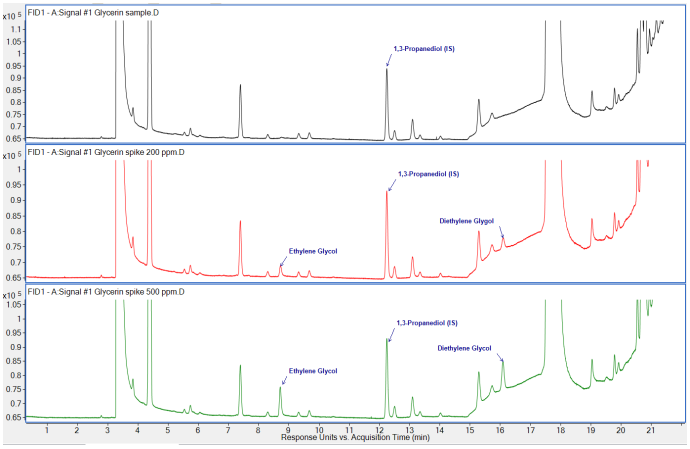


**Figure 5.** Chromatograms of (from top to bottom) blank glycerin sample, and 200 and 500 µg/mL spikes.

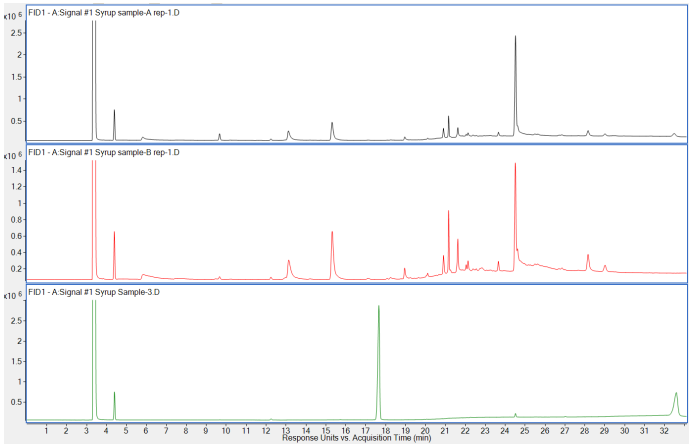


**Figure 6.** Chromatograms of three different brands of syrup samples.

**Table 6.** Recovery and repeatability of EG and DEG in 200 µg/mL spiked glycerin sample.

| Spiked Sample | EG Results | | DEG Results | |
|---|---|---|---|---|
| | Calculated Amount (µg/mL) | Recovery (%) | Calculated Amount (µg/mL) | Recovery (%) |
| 200 µg/mL spike-1 | 243.15 | 121.575 | 239.51 | 119.755 |
| 200 µg/mL spike-2 | 241.32 | 120.66 | 244.1 | 122.05 |
| 200 µg/mL spike-3 | 244.47 | 122.235 | 232.55 | 116.275 |
| Mean | 242.98 | 121.49 | 238.72 | 119.36 |
| SD | 1.582 | | 5.815 | |
| %RSD | 0.651 | | 2.436 | |

**Table 7.** Recovery and repeatability of EG and DEG in 500 µg/mL spiked glycerin sample.

| Spiked Sample | EG Results | | DEG Results | |
|---|---|---|---|---|
| | Calculated Amount (µg/mL) | Recovery (%) | Calculated Amount (µg/mL) | Recovery (%) |
| 500 µg/mL spike-1 | 530.25 | 106.05 | 532.81 | 106.562 |
| 500 µg/mL spike-2 | 536.04 | 107.208 | 547.87 | 109.574 |
| 500 µg/mL spike-3 | 540.16 | 108.032 | 547.6 | 109.52 |
| Mean | 535.48 | 107.10 | 542.76 | 108.55 |
| SD | 4.978 | | 8.618 | |
| %RSD | 0.930 | | 1.588 | |

Figure 5 shows chromatograms obtained from injection of glycerin, and glycerin spiked with 200 ppm and 500 µg/mL of EG and DEG, respectively. Compounds of interest are well resolved from matrix peaks. Tables 6 and 7 show the recovery and repeatability (in terms of %RSD) for glycerin sample spiked with 200 and 500 µg/mL of EG and DEG, respectively. Excellent recovery and repeatability were achieved for this experiment with glycerin sample.

**Table 8.** Recovery and repeatability of EG and DEG in 200 µg/mL spiked syrup sample.

| Spiked Sample | EG Results | | DEG Results | |
|---|---|---|---|---|
| | Calculated Amount (µg/mL) | Recovery (%) | Calculated Amount (µg/mL) | Recovery (%) |
| 200 µg/mL spike-1 | 208.04 | 104.02 | 185.17 | 92.585 |
| 200 µg/mL spike-2 | 214.14 | 107.07 | 187.7 | 93.85 |
| 200 µg/mL spike-3 | 210.9 | 105.45 | 181.68 | 90.84 |
| Mean | 211.03 | 105.51 | 184.85 | 92.43 |
| SD | 3.052 | | 3.023 | |
| %RSD | 1.446 | | 1.635 | |

**Table 9.** Recovery and repeatability of EG and DEG in 500 µg/mL spiked syrup sample.

| Spiked Sample | EG Results | | DEG Results | |
|---|---|---|---|---|
| | Calculated Amount (µg/mL) | Recovery (%) | Calculated Amount (µg/mL) | Recovery (%) |
| 500 µg/mL spike-1 | 433.18 | 86.636 | 389.61 | 77.922 |
| 500 µg/mL spike-2 | 429.64 | 85.928 | 392.47 | 78.494 |
| 500 µg/mL spike-3 | 431.82 | 86.364 | 385.83 | 77.166 |
| Mean | 431.55 | 86.31 | 389.30 | 77.86 |
| SD | 1.786 | | 3.331 | |
| %RSD | 0.414 | | 0.856 | |

Figure 6 shows the overlay of chromatograms obtained from injection of three different brands of syrup samples. No peak of EG and DEG was found in any of these samples. Tables 8 and 9 show the recovery and repeatability (in terms of %RSD) for one of the syrup samples spiked with 200 and 500 µg/mL of EG and DEG, respectively. Excellent recovery and repeatability were achieved for this experiment with syrup sample.

## Conclusion

An accurate and rugged method was developed for analysis of ethylene glycol and diethylene glycol in propylene glycol, glycerin, and syrup samples. The LOQ of the method is demonstrated at the 200 µg/mL level in samples. Repeatable results were found for replicates of spiked samples at 200 and 500 µg/mL levels. Good recovery was found at 200 and 500 µg/mL spiked concentration of EG and DEG in different samples. Thus, the method demonstrated in this study proves its usefulness for the routine analysis of excipients and syrup samples for EG and DEG content under the established regulatory limits mentioned in the USP monograph for glycerin[3].

## References


1) *Baffi, P.; Elneser, S.; Baffi, M.; De Melin, M. Quantitative Determination of Diethylene Glycol Contamination in Pharmaceutical Products. J. AOAC Int. 2000, 83 (4), 793–801.*
2) *U.S. Food & Drug Administration. Lab Method for Gas Chromatography-Mass Spectrometry (GC-MS) Screening Procedure for the Presence of Diethylene Glycol and Ethylene Glycol in Toothpaste. https://www.fda.gov/food/laboratory-methods-food/lab-method-gas-chromatography-mass-spectrometry-gc-ms-screening-procedure-presence-diethylene-glycol (accessed 2023-12-01).*
3) *U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Testing of Glycerin, Propylene Glycol, Maltitol Solution, Hydrogenated Starch Hydrolysate, Sorbitol Solution, and other High-Risk Drug Components for Diethylene Glycol and Ethylene Glycol: Guidance for Industry. https://www.fda.gov/media/167974/download (accessed 2023-12-01).*
4) *Pharmacopeia. cn USP Method for Glycerol Analysis. http://www.pharmacopeia.cn/v29240/usp29nf24s0_m35420.html#:~:text=Add%2010%20mL%20of%20a,blank%2C%20using%20a%20pH%20meter (accessed 2023-12-01).*
5) *Eccles, R. What is the Role of Over 100 Excipients in Over the Counter (OTC) Cough Medicines? Lung. 2020, 198 (5), 727–734. DOI: 10.1007/s00408-020-00390-x.*

www.agilent.com/chem

DE87274344


Published in the India, February, 2024
5994-7159EN

Agilent
Trusted Answers