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Application Note

# Determination of Glycols in Cough Syrup by GC-MS Using an Equity-1701 Column

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## Abstract

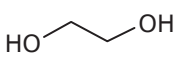
Gas chromatography coupled to mass spectrometry detection (GC-MS/MS) was used as an analytical tool to analyze glycols present in commercial cough syrup samples with an Equity-1701 capillary GC column 30 m × 0.25 mm, df 0.25 µm. The developed method is able to separate and quantify ethylene glycol, propylene glycol, diethylene glycol, glycerin, triethylene glycol, and sorbitol present in the commercial cough syrup preparations. For the quantification certified reference materials (CRMs) were used. The method is partially validated for establishing the performance characteristics.

**Keywords**: Cough syrup, adulterant, ethylene glycol, propylene glycol, diethylene glycol, triethylene glycol, sorbitol.

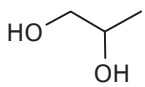
## Introduction

Glycols are chemical compounds characterized by two hydroxyl groups attached to separate carbon atoms, commonly referred to as aliphatic diols.[1] Various forms of glycols are utilized in industries for different purposes, such as liquid desiccants in air conditioning systems, antifreeze in car radiators[2], and are also used as additives in hydraulic and brake fluids. Ethylene glycol is recognized as the simplest type. Diethylene, triethylene, and propylene glycols are oligomers of ethylene glycol. Ethylene glycol is a potent cause of acute toxicity in humans, in contrast, propylene glycol is a generally recognized as safe additive for foods and medications.[3] However, when used in high doses or used for prolonged periods, propylene glycol toxicity can occur with reported adverse effects includes central nervous system (CNS) toxicity, hyperosmolarity, hemolysis, cardiac arrhythmia, seizures, agitation, and lactic acidosis.[4] Typically, colorless, and odorless, ethylene glycol and diethylene glycol have a sweet taste[5] and are often considered inexpensive adulterants, making them attractive for commercial sectors involved in drug formulation, including pediatric drugs.

However, when glycols are added to drug formulations beyond recommended limits, they can be toxic to human health, potentially leading to acute hepatotoxicity, renal failure, and even death in some cases. In recent times fatal incidences were reported e.g. from Indonesia and Gambia where of ethylene glycol and diethylene glycol were found in syrup medicines.[6,7,8] As a response to US Food and Drug Administration (US FDA) provided a guidance for high-risk drug components[9] and the United States Pharmacopoeia (USP) revised the monograph for propylene glycol that addresses the toxicity concerns by specifying the limits for ethylene glycol and diethylene glycol in formulation components such as propylene glycol to be not more than (NMT) 0.1% to ensure quality standards for pharmaceutical products.[10]




Ethylene Glycol (EG)

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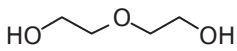
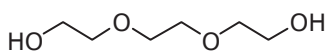
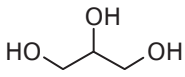


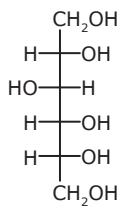

Diethylene Glycol (DEG)




Triethylene Glycol (TEG)




Glycerin (Gly)




Sorbitol (SOR)



MERCK

This study will present a method for the simultaneous determination of five glycols and sorbitol in a cough syrup sample using GC-MS/MS. Two commercially available cough syrups are analyzed unspiked and spiked. The developed method was partially validated as per the ICH recommended guidelines[11] and used for determination of minimum limit of detection and quantification of glycols.

The Equity-1701 column fulfils USP G46 requirements with 14% cyanopropylphenyl - 86% dimethylpolysiloxane and the max. temperature limit of this column is 280 °C. Even though this column is from the mid polar range, the Equity-1701 stationary phase along with its temperature characteristics was found capable for additional determination of sorbitol along with other glycol present in the samples. Since sorbitol is exhibiting a very high boiling point of 296 °C, in many cases, the reported method of choice for sorbitol is HPLC-RI/ELSD. Here it was included as additional analyte for the GC-MS/MS technique to further explore the capability of the developed method on the selected Equity-1701 phase.

## Experimental

### Standard preparation

The glycol standards were prepared as single component solutions of ethylene glycol (EG), propylene glycol (PG), diethylene glycol (DEG), glycerin (Gly), triethylene glycol (TEG), sorbitol (SOR) according to the following procedures using certified reference materials (CRMs) and methanol/water (95:5, v/v) as diluent.

- **Stock solutions glycols (500 µg/mL)**: Weigh and transfer about 10 mg of a glycol substance into a 20 mL volumetric flask, add about 10 mL of diluent and sonicate for 5 minutes, make up to volume with diluent and shake to mix thoroughly. The concentration of the glycol in the resulting solution is 500 µg/mL.
- **Stock solution sorbitol (1000 µg/mL)**: Weigh and transfer about 10 mg of sorbitol into a 10 mL volumetric flask, add about 10 mL of diluent and sonicate for 5 minutes, make up to volume with diluent and shake to mix thoroughly. The concentration of the sorbitol in the resulting solution is 1000 µg/mL.
- **Standard solution (SS, all glycols 20 µg/mL, sorbitol 200 µg/mL)**: Transfer 400 µL of each glycol stock solution and 2 mL of sorbitol stock solution into a 10 mL volumetric flask, add 5 mL of diluent and sonicate for 5 minutes. Then make up to volume with diluent and mix thoroughly.

#### Calibration Solutions

Calibration solutions were prepared as single-component solutions using the following dilution schemes:

Transfer of 100, 250, 500, 750, and 1000 µL from each of the above-mentioned glycol standard stock solutions of ethylene glycol, propylene glycol, diethylene glycol, glycerin, and triethylene glycol into 10 mL volumetric flask individually and to each individual flasks add 2, 2.5, 3, 3.5, and 4 mL of sorbitol stock solution. Make up to volume with diluent, sonicate for 5 minutes and mix thoroughly. Resulting solutions have 5.0, 12.5, 25.0, 37.5, and 50.0 µg/mL for each glycol and 200.0, 250, 300, 350, and 400 µg/L sorbitol respectively.

### Sample Preparation

Transfer 12 mg of commercially available cough syrup into a 10 mL volumetric flask, add 5 mL of diluent and sonicate for 5 minutes. Fill up to mark with diluent and mix thoroughly. Two commercially available syrups (A & B) were used for the assessments.

### Spiked Samples

- **Spiked sample 1 PG & Gly (20.8 mg/g)**: Weigh 12 mg of syrup sample A into a 10 mL volumetric flask and add 500 µL of each of PG and Gly stock solutions. Make up to mark with diluent, mix thoroughly and sonicate for 5 minutes. Solution contains spikes of 25 µg/mL of each glycol.
- **Spiked sample 2 PG & Gly (41.7 mg/g)**: Weigh 12 mg of syrup sample A to a 10 mL volumetric flask and add 1000 µL of each of PG and Gly stock solutions. Make up to mark with diluent, mix thoroughly and sonicate for 5 minutes. Solution contains spikes of 50 µg/mL of each glycol.
- **Spiked Sample 3 PG & Gly (20.8 mg/g)**: Weigh 12 mg of syrup sample B into a 10 mL volumetric flask and add 500 µL of each of PG and Gly stock solutions. Make up to mark with diluent, mix thoroughly and sonicate for 5 minutes. Solution contains spikes of 50 µg/mL of each glycol.
- **Spiked sample 4 PG & Gly (41.7 mg/g)**: Weigh and transfer 12 mg of commercially available sample B to a 10 mL volumetric flask, and add 1000 µL of each of PG and Gly stock solutions. Make up to mark with diluent, mix thoroughly and sonicate for 5 minutes. Solution contains spikes of 50 µg/mL of each glycol.

### GC-MS Analysis

The GC-MS analysis was performed on an intermediate polarity Equity-1701 column (**Table 1**). Depending upon the fragmentation recorded for each individual glycols, the selection of specific target ions and reference ions were selected for scanning at a defined retention time. Selection of the retention time was done as per the separation acquired by the developed method on Equity-1701 column (**Table 2**).

**Table 1.** GC -MS conditions used for glycol determination

| LC Conditions | | | | |
|---|---|---|---|---|
| Instrument: | Agilent 7000 Series Triple Quad GC-MS with Automatic Liquid Sampler (ALS) | | | |
| Column: | Equity-1701, 30 m × 0.25 mm I.D., df 0.25 µm (**28372-U**) | | | |
| Oven: | **Initial temp. (°C)** | **Temp. ramp (°C/min)** | **Final temp. (°C)** | **Hold time at final temp. (min)** |
| | 60 | - | 60 | 4 |
| | 60 | 10 | 150 | 4 |
| | 150 | 15 | 280 | 5 |
| Inj. temp.: | 250 °C | | | |
| Carrier gas: | Helium, 1.3 mL/min | | | |
| Detector: | MS/MS | | | |
| MSD interface: | 285 °C | | | |
| Injection: | 0.8 µL splitless | | | |
| Liner: | Spitless single taper | | | |
| Run time: | 30.667 min | | | |
| Samples: | Standards, unspiked and spiked samples all in methanol/water (95:5, v/v) | | | |
| **MS Conditions** | | | | |
| Quench gas: | Helium | | | |
| Solvent delay: | 4.5 minutes | | | |
| MS Source temperature: | 270 °C | | | |
| Quad Temperature: | 150 °C | | | |
| Electron energy: | 70 eV | | | |
| Dwell time: | 100 ms | | | |
| Quenching gas flow rate: | 2.25 mL/min | | | |
| MS measurement mode: | SIM | | | |
| MS mode: | Splitless | | | |
| Solvent delay: | 4.5 min | | | |
| Ion source: | EI | | | |
| Gain factor: | 10 | | | |
| Scan type: | MS1 SIM (see **Table 2**) | | | |

**Table 2.** MS SIM mode parameter (scan type MS1 SIM) used for glycol determination

| Compound | Retention time (min) | Target Ion m/z (Quantifier) | Reference Ion m/z (Qualifier) |
|---|---|---|---|
| Ethylene glycol | 5.6 | 43.0 | 62.0<br>61.1<br>46.1<br>44.0 |
| Propylene glycol | 6.2 | 45.1 | 76.1<br>61.0<br>57.0<br>43.1 |
| Diethylene glycol | 10.8 | 75 | 76.0<br>45.1<br>44.1<br>43.1 |
| Glycerin | 11.9 | 61 | 66.0<br>62.1<br>44.1<br>43.1 |

| Compound | Retention time (min) | Target Ion m/z (Quantifier) | Reference Ion m/z (Qualifier) |
|---|---|---|---|
| Triethylene Glycol | 15.1 | 89 | 87.0<br>75.0<br>58.0<br>45.1 |
| Sorbitol | 25.2 | 73 | 132.9<br>117.0<br>103.0<br>61.0 |

## Results & Discussion

The developed method applying an Equity-1701 (30 m × 0.25 mm I.D., df 0.25 µm) capillary GC column and splitless injection showed good retention and sufficient resolution of the analytes in scope. A representative chromatogram of an analyte mixture at 20 µg/mL each (sorbitol at 200 µg/mL) is shown in **Figure 1**.

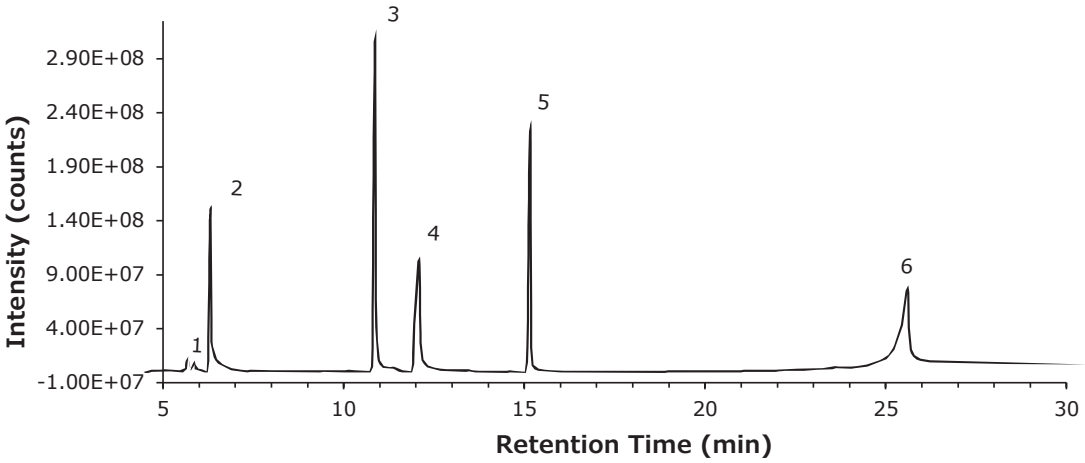



**Figure 1**. GC-MS chromatogram (SIM) for the standard solution (SS) mixture containing ethylene glycol (1), propylene glycol (2), diethylene glycol (3), glycerin (4), triethylene glycol (5), prepared in diluent at 20 µg/mL and sorbitol (6) at 200 µg/mL.

### Calibration

The quantification was based on an external calibration using single reference standard solutions as described in the experimental section. The GC-MS method linearity for ethylene glycol, propylene glycol, diethylene glycol, glycerin and triethylene was established in the concentration range of 5 to 50 µg/mL, and for sorbitol 200 to 400 µg/mL. In **Tables 3** & **4**, the experimental results including the linearity ($R^2$) and the Limits Of Detection (LOD) and Limits Of Quantification (LOQ) are summarized for all analytes. Based on the developed method the minimum concentrations of glycols that can be detected in the injected solutions as per the ICH guidelines[11] by using this method's linearity study is ranging for the five glycols from 3.33 µg/mL to 4.49 µg/mL, for sorbitol 50.12 µg/mL, and the LOQs ranged from of 10.09 µg/mL to 13.6 µg/mL, for sorbitol 126.58 µg/mL. Derived from the linearity study data the estimated potential LODs of glycols present in the original cough syrup samples are in the range of 2.78 mg/g to 3.74 mg/g, for sorbitol 41.74 mg/g, and the LOQs in the range of 8.41 mg/g to 11.33 mg/g, for sorbitol 126.58 mg/g.

**Table 3.** Calibration and linearity data for single component solutions of glycols in concentration range of 5.0 µg/mL to 50 µg/mL and sensitivity data LOD & LOQ for the injected solutions

| Conc (µg/mL) | Ethylene glycol (EG) Peak area | Propylene glycol (PG) Peak area | Diethylene glycol (DEG) Peak area | Glycerin (Gly) Peak area | Triethylene glycol (TEG) Peak area |
|---|---|---|---|---|---|
| 5.0 | 351528 | 26737103 | 33144877 | 3170740 | 26029651 |
| 12.5 | 4144163 | 110204479 | 201381021 | 102799986 | 122551897 |
| 25.0 | 15482559 | 340449089 | 554601754 | 354086381 | 404265938 |
| 37.5 | 24688538 | 526072468 | 967610390 | 573422008 | 697234333 |
| 50.0 | 34982955 | 711426370 | 1310399664 | 856726154 | 969786650 |
| **STEYX** | 906325 | 16718657 | 29236081 | 25861524 | 29214647 |
| **Slope** | 784013 | 15560508 | 28973976 | 19022370 | 21529257 |
| **$R^2$** | 0.997 | 0.997 | 0.997 | 0.995 | 0.995 |
| **LOD (µg/mL)** | 3.81 | 3.55 | 3.33 | 4.49 | 4.48 |
| **Derived LOD in cough syrup (mg/g)** | 3.18 | 2.96 | 2.78 | 3.74 | 3.73 |
| **LOQ (µg/mL)** | 11.56 | 10.74 | 10.09 | 13.6 | 13.57 |
| **Derived LOQ in cough syrup (mg/g)** | 9.63 | 8.95 | 8.41 | 11.33 | 11.31 |

**Table 4.** Linearity data for peak responses recorded for single component solution of sorbitol in concentration range of 200 µg/mL to 400 µg/mL and sensitivity data LOD & LOQ for the injected solution

| Conc (µg/mL) | Sorbitol (SOR) Peak Area |
|---|---|
| 200.0 | 14451452 |
| 250.0 | 116337377 |
| 300.0 | 390407327 |
| 350.0 | 685170975 |
| 400.0 | 1012463171 |
| **STEYX** | 77912996 |
| **Slope** | 5129714 |
| **$R^2$** | 0.973 |
| **LOD (µg/mL)** | 50.12 |
| **Derived LOD in cough syrup (mg/g)** | 41.77 |
| **LOQ (µg/mL)** | 151.90 |
| **Derived LOQ in cough syrup (mg/g)** | 126.58 |

## Reproducibility

The system repeatability was demonstrated by injecting five replicates of the standard solution (SS) containing mixture of glycol standards of ethylene glycol (EG), propylene glycol (PG), diethylene glycol (DG), glycerin (Gly), triethylene glycol (TG) and sorbitol (SOR) prepared in diluent (see **Figure 1** and for diluent blank **Figure 2**) The determined RSD ranged from 3.63–8.89% (**Table 5**).

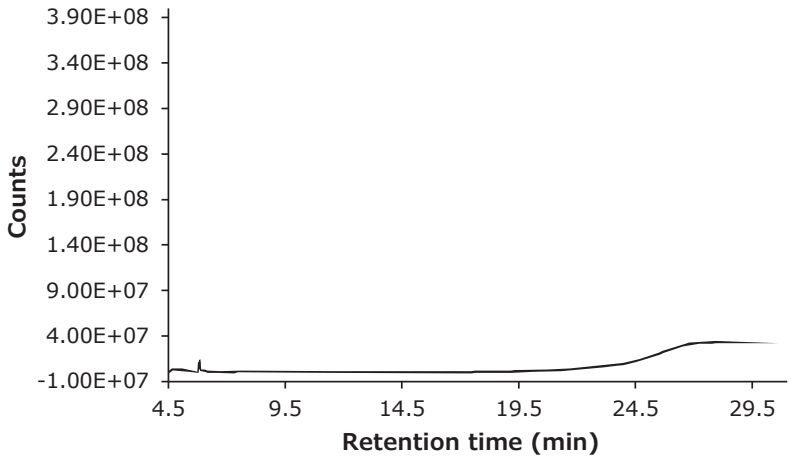



**Figure 2**. GC-MS chromatogram recorded for diluent (blank) containing methanol and water (95:5 v/v).

**Table 5.** Results of recovery determination using syrup sample A spiked with PG and Gly at 20.8 µg/g and 41.7 µg/g (conc in injected solution 25 and 50 µg/mL) of each component

| Standard | EG | PG | DEG | GLY | TEG | SOR |
|---|---|---|---|---|---|---|
| STD_1 | 7586692 | 294199962 | 493025336 | 233310311 | 333966927 | 254041862 |
| STD_2 | 7035251 | 272626245 | 421142413 | 228164215 | 303431809 | 254016947 |
| STD_3 | 7725775 | 252113903 | 433279452 | 260859790 | 335467316 | 258847989 |
| STD_4 | 7648240 | 238425134 | 384784659 | 211673646 | 268198599 | 277747053 |
| STD_5 | 7532348 | 265427921 | 406688698 | 228886113 | 300798479 | 250613678 |
| **Mean** | 7505661 | 264558633 | 429784111 | 232578815 | 316372626 | 259053506 |
| **Standard deviation** | 272646 | 21108538 | 38225347 | 17822033 | 16963196 | 10853630 |
| **% RSD** | 3.63 | 7.98 | 8.89 | 7.66 | 5.36 | 4.19 |

## Recovery Determination

For recovery determinations four solutions were prepared with two spike concentration levels 20.8 µg/g and 41.7 µg/g (25 and 50 µg/mL in the injection solutions) and two sets of compounds (**Table 6** & **7**) and 2 syrups used (A&B). For recovery calculation existing glycol background was subtracted.

**Table 6.** Results of recovery determination using syrup sample A spiked with PG and Gly at 20.8 µg/g and 41.7 µg/g (conc. in injected solution 25 and 50 µg/mL) of each component

| Compound | Concentration determined (µg/mL) | Recovery |
|---|---|---|
| **Spiked sample 1 (20.8 mg/g → 25 µg/mL)** | | |
| Propylene glycol | 20.5 | 82.0% |
| Glycerin | 40.4 | 80.8% |
| **Spiked sample 2 (41.7 mg/g → 50 µg/mL)** | | |
| Propylene glycol | 24.5 | 98.0% |
| Glycerin | 54.0 | 108.0% |

**Table 7.** Results of recovery determination using syrup sample B spiked with PG and Gly at 20.8 µg/g and 41.7 µg/g (conc. in injected solution 25 and 50 µg/mL) of each component

| Compound | Concentration determined (µg/mL) | Recovery |
|---|---|---|
| **Spiked sample 3 (20.8 mg/g → 25 µg/mL)** | | |
| Propylene glycol | 18.4 | 73.6% |
| Glycerin | 38.6 | 77.2% |
| **Spiked sample 4 (41.7 mg/g → 50 µg/mL)** | | |
| Propylene glycol | 21.6 | 86.4% |
| Glycerin | 56.4 | 112.8% |

### Original Sample Analysis

The method was applied for analyzing two different commercially available cough syrup samples (A & B) solutions (see **Figures 3** & **4**). **Table 8** shows the results for the analysis of glycols present in the two commercially available cough syrup samples (A & B).

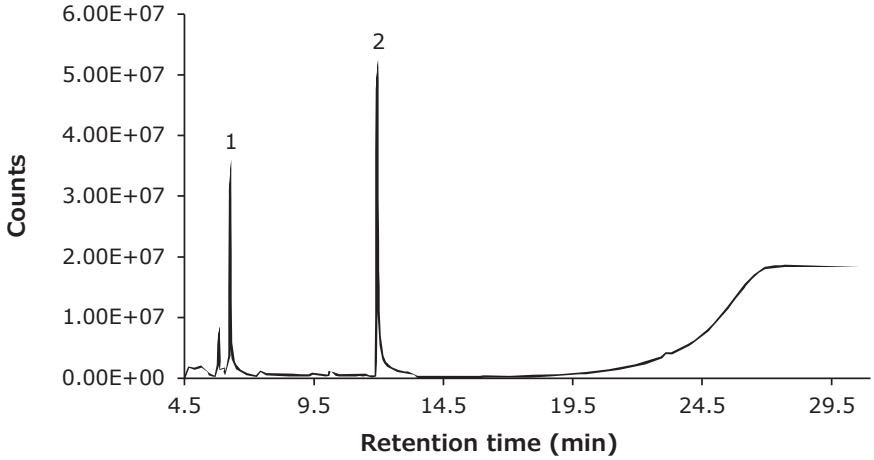



**Figure 3**. GC-MS chromatogram recorded for commercially available cough syrup sample A prepared in diluent, showing the presence of propylene glycol (1) and glycerin (2).

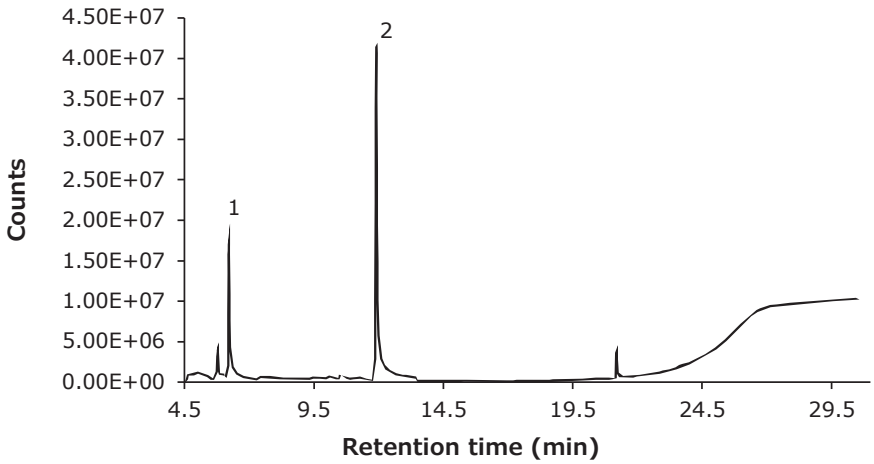



**Figure 4**. GC-MS chromatogram recorded for commercially available cough syrup sample B, prepared in diluent, showing the presence of propylene glycol (1), glycerin (2).

**Table 8.** Results of glycols present in two commercially available cough syrups samples represented in mg/g of sample

| Name | Retention Time (min) | Glycols in sample A (mg/g) | Glycols in sample B (mg/g) |
|---|---|---|---|
| Ethylene glycol | 5.602 | Not detected | Not detected |
| Propylene glycol | 6.216 | 7.23 | 5.73 |
| Diethylene glycol | 10.818 | Not detected | Not detected |
| Glycerin | 11.945 | 16.33 | 13.52 |
| Triethylene glycol | 15.117 | Not detected | Not detected |
| Sorbitol | 25.465 | Not detected | Not detected |

## Conclusion

This study presents a GC-MS/MS method for quantifying ethylene glycol, propylene glycol, diethylene glycol, triethylene glycol, glycerin and sorbitol present in formulations for patients such as cough syrup. The method development utilized an Equity-1701 30 m x 0.25 mm column with a film thickness of 0.25 µm. The % RSD for system repeatability with standard solution (SS) of the glycol mixture was well below 10% (3.63 to 8.89%). Derived from the external calibration data ($R^2$ ≥0.962) the estimated potential LODs of glycols present in the original cough syrup samples were for 5 compounds in the range of 2.78 mg/g to 3.74 mg/g, and 50.12 mg/g for sorbitol, and the LOQs were in the range of 8.41 mg/g to 11.33 mg/g, for sorbitol 126.5 mg/g. Analysis of the commercial sample A spiked with propylene glycol and glycerin showed recovery rates between 80.8% and 108 %. The second commercial sample B also spiked with propylene glycol and glycerin displayed recovery rates ranging from 73.6% to 112.8%.

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## Related Products

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| Propylene glycol pharmaceutical secondary standard; certified reference material | **PHR1051** |
| Diethylene glycol pharmaceutical secondary standard; certified reference material | **PHR1045** |
| Triethylene glycol pharmaceutical secondary standard; certified reference material | **PHR3826** |
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