Simultaneous quantification of 7 glycols in anti-freeze liquids by direct liquid injection gas-chromatography coupled with mass spectrometry

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Abstract

RATIONALE: Glycol-based antifreeze liquids, commonly composed of ethylene glycol or propylene glycol, have important uses in automotive cooling, but they should be handled with care due to their toxicity, ethylene glycol is highly toxic to humans and animals. A fast, accurate, precise and robust method was developed for simultaneous quantification of 7 most important glycols and their isomers. METHODS: Glycols were analyzed from diluted sample solution of coolants using gas-chromatography coupled with mass spectrometry in single ion monitoring mode. RESULTS: The method was developed and validated for 7 individual glycols (ethylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, propylene glycol, dipropylene glycol and tripropylene glycol). Limits of detection (1-2 μg/mL) and limit of quantification (10 μg/mL) obtained were appropriate. The present method was applied for determination of glycols in 10 different anti-freeze liquids commercially available on Romanian market, proving to be reliable. CONCLUSIONS: A method that requires only a two-step dilution of anti-freeze samples combined with direct liquid injection GC-MS was validated for the simultaneous quantification of 7 glycols (and their isomers) in 10 different types of anti-freeze liquids. The results obtained in the validation procedure proved that the GC-MS method is sensitive and precise for quantification of glycols.

INTRODUCTION

Glycol-based antifreeze liquids, commonly composed of ethylene glycol or propylene glycol, have several important uses in various applications:

- Automotive cooling: one of the primary uses of glycol-based antifreeze is in internal combustion engines. It helps regulate the temperature of the engine by preventing freezing and overheating. The antifreeze mixture is circulated through the engine and radiator, preventing coolant from freezing in cold temperatures while also raising the boiling point to prevent overheating.

- Heat transfer: glycol-based antifreeze is used as a heat transfer fluid in industrial processes. It efficiently carries heat away from equipment, such as HVAC systems, refrigeration units, and solar water heaters. The fluid's high heat capacity and low freezing point make it suitable for maintaining stable operating temperatures.

- Airplane de-icing: glycol-based antifreeze solutions are sprayed onto aircraft to remove ice and frost before take-off. These fluids effectively melt frozen deposits on the aircraft's surfaces, ensuring safe flight conditions.

- Refrigeration: in refrigeration systems, glycol-based antifreeze helps maintain consistent temperatures and prevents freezing of refrigerant lines and evaporators in low-temperature applications.

It is important to note that while glycol-based antifreeze liquids offer numerous benefits, they should be handled with care due to their toxicity. Proper disposal and management are essential to prevent environmental contamination. Ethylene glycol is highly toxic to humans and animals. Ingesting even small amounts can lead to severe health complications and potentially be fatal.

Ethylene glycol is metabolized in the body through a series of enzymatic reactions, ultimately forming toxic metabolites. These metabolites can cause damage to various organs, particularly the kidneys, central nervous system, and heart[1]

Ethylene glycol toxicity is a serious and potentially life-threatening condition. Immediate medical attention is necessary if there is suspicion of exposure or ingestion. Prevention, proper handling, and responsible disposal of ethylene glycol-containing products are key to avoiding accidental poisoning.

Several detection methods for glycols are present in the scientific literature. In surface and waste waters, traces of ethylene glycol, propylene glycol and diethylene glycol can be detected using GC-FID, with a limit of detection of 0.02 ppm glycol[2].

A method for the monitoring of workplace air quality and the presence of toxic glycols was presented by Giesen et al., using a sampling system, for a sample volume of 40L, with what they achieved a 0.5 mg/m3 limit of quantification[3]..

In biological samples, the most commonly used methods reported in literature for detecting glycols are based on gas chromatography using either flame ionization detector (FID) or mass spectrometry (MS), with derivatization. The limits of detection and quantification are in the sub-μg/mL range [4-5].

In antifreeze samples, only one method (spectrophotometric) was identified. Faizullah and Jabbar developed an extraction, pre-concentration method to detect EG in antifreeze samples using an indirect determination (Malaprade reaction) [6].

To our knowledge, this is the first developed and validated method for the simultaneous detection and quantification of glycols (and their isomers) in antifreeze samples using GC-MS direct injection technique. This method can be applied by laboratories involved in analysis of counterfeit products and, more important, by the manufacturers of antifreeze liquids, in order to assess the exact composition of their final products. The molecular structures of the glycols analyzed are presented in Figure 1.

EXPERIMENTAL

Reagents and materials

Ethylene glycol (EG), Diethylene glycol (DEG), Triethylene glycol (TEG), Tetraethylene glycol (TTEG), Propylene glycol (PG), Dipropylene glycol (DPG, mixture of isomers) and Tripropylene glycol (TPG, mixture of isomers) were purchased from CPA Chem. (Bulgaria). Ultrapure water was prepared in the laboratory using a Millipore Simplicity UV system.

Instrumentation

Gas chromatograph-mass spectrometry was carried out using a Perkin Elmer GC-MS (GC-Clarus 680, MS-Clarus SQ8T) equipped with split/splitless injector. The column used was a 30m x 0.25mm x 0.5 μm Elite WAX ETR (Perkin Elmer, USA) and Helium was selected as carrier gas at 1.5 mL/min constant flow. The temperature program used was as follows: initial temperature of 100°C (hold time 1min), then increased to 240°C, with a ramp of 10°C/min (hold time 10 min). The run time was 25.0 minutes. Injection of 1 μL solution was made in splitless mode, with a 20:1 split ratio. Injector, transfer line and source temperature were 250, 240 and 240°C.

The MS was operated in dual mode, a full-scan (m/z 30-240 amu) chromatogram was acquired, together also with one m/z ion per analyte in Single Ion Reaction (SIR). The m/z for each analyte monitored is presented in Table 1. For Dipropylene glycol we monitored and quantified both isomers present in the reference material and for Tripropylene glycol we also chosen the 2 isomers from the mixture (namely 1 and 2) for quantification.

Using a larger acquisition window for Tripropylene glycol can facilitate the quantification also for other minor isomers, if they are present in a real sample analyzed.

Preparation of standards and samples

Standard stock solution of each target analyte (1.0 mg/mL) were prepared in ultrapure water using individual certified reference materials for each glycol. Work solutions were then prepared for the calibration solutions.

A number of 10 different types of commercially available anti-freeze liquids were purchased from Romanian market. To establish the glycol profile for each type a sample solution was prepared as follows: 0.5 g of liquid was diluted first at 100 mL with ultrapure water. A 100x dilution was applied to the first solution to obtain a sample solution of anti-freeze of 50 µg/mL that was analyzed using the validated GC-MS method.

Method validation

The method validation was performed by evaluating specificity, linearity and calibration range, limit of detection (LOD) and limit of quantification (LOQ), accuracy, precision as specified in the ICH guidelines for analytical method validation and ASTM E202-18 Standard test methods for analysis of Ethylene glycols and propylene glycols[7].

RESULTS

Method validation

Specificity

The method specificity was demonstrated by injecting ultrapure water blank samples, reference solutions of each individual glycol and a mix solution of glycols (all at 50 µg/mL). No interference was observed at the retention time of target analytes. Overlayed chromatograms for each target analyte with individual solutions are presented in Figure 2.

Linearity and calibration range

The linearity of the method was determined using a 5-levels calibration curve for each analyte. The calibration points were 10, 25, 50, 75 and 100 µg/mL and 5 replicates per calibration point were injected in the GC-MS system in order to validate the linear range of each analyte. The calibration curves for all target analytes were found to be linear within the 10-100 µg/mL range and correlation coefficient (r2) for all 9 glycols were higher than 0.995. Linearity values are presented in Table 2 and the calibration curves are presented in **Figure**

Method sensitivity (LOQ and LOD)

The limit of quantification (10 µg/mL) was determined based on the signal-to-noise ratio for all target analytes. The acceptance criteria was an S/N ratio of not less than 10:1, for all analytes.

To establish the LOD concentration, serial dilutions were prepared from Calibration solution level 1 (10 µg/mL): 5, 2, 1 and 0.5 µg/mL of each target analyte. The LOD was defined as the lowest concentration for which the S/N ratio was not less than 3:1.

Limits of detection of 1 and 2 µg/mL were obtained for all target analytes. The results obtained for LOQ and LOD are presented in Table 3.

System Precision

The precision of the method was determined by GC-MS system precision, at three levels (low-10 µg/mL, mid-50 µg/mL and high concentration-100 µg/mL). 3 replicates at each level were prepared and injected. For all target analytes, the RSD% (based on peak area) were below 7.0% for all three levels tested, with a combined method RSD% of 2.22 %. The results obtained for system precision are presented in Table 4.

Robustness

According to validation guidelines, robustness is defined as a measure of method capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To validate this parameter, small variations in the instrumental method were introduced: change of carrier gas flow rate and split ratio of the flow. A summary of the changes introduced is presented below (Table 5).

Standard solutions of low level (10 µg/mL) and high level concentration (100 µg/mL) of target analytes were injected in triplicate using modified methods with small variations presented above. The RSD% for 6 injections (3 from System Precision and 3 from Robustness) was calculated. Deviation of peak areas for all target analytes were less than 10 %. These results proved that the method is robust. Results for this parameter are presented in **Table**

Stock solution stability

A stability study was conducted based on the stock solutions prepared in Day 1 of the validation and stored at 4°C. Calibration level 1 (10 µg/mL) and 5 (100 µg/mL) were prepared for 3 consecutive days and analyzed in triplicate using the method developed. The solutions proved to be stable, the concentrations were in the accepted interval of ± 20% of target concentration. The results are presented in Figure 3 and 4.

Real sample analysis

A number of 10 different ethylene glycol-based antifreeze liquids were purchased from Romanian market and analyzed using the validated GC-MS method. A short description of each one of the coolants analyzed is presented in Table 6. Each sample was prepared in triplicate and analyzed. According to the manufacturer, for 8/10 of samples analyzed, to reach an optimum coolant with a freezing point around 30-35°C, which corresponds to an ethylene glycol (EG) concentration of approximately 30%, a 1:1 dilution with distilled water was necessary. Antifreeze solution number 10 exhibited the highest EG concentration, measuring at 66.65%.

Antifreeze formulations designated as 'Ready to use liquids' by the manufacturer, namely, antifreeze numbers 1 and 7, contained EG concentrations of 30.23% and 35.75%, respectively.

Notably, the presence of diethylene glycol (DEG), Triethylene glycol (TEG), and Tetraethylene glycol (TTEG) was detected in all analyzed samples, with concentrations ranging from 5.04% to 10.62%.

Conversely, concentrations below the limit of quantification (LOQ) were observed in eight out of ten samples examined for DEG and in five out of ten samples examined for propylene glycol (PG).

Isomers of dipropylene glycol and tripropylene glycol were not detected in the analyzed samples. The results obtained for the analysis are presented in Table 7.

CONCLUSIONS

A method that requires only a two-step dilution of anti-freeze samples combined with direct liquid injection GC-MS was developed and validated for the simultaneous quantification of 7 glycols (and their isomers) in 10 different types of anti-freeze liquids from Romanian market. The results obtained in the validation procedure proved that the GC-MS method is reliable, sensitive and precise for quantification of glycols in real samples.

Acknowledgements

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Table 1. The m/z ion monitored and retention time for each glycol analyzed

Analyte name	**m/z ion monitored**	**Retention time (min)**
EG	31.0	6.94
DEG	45.0	10.70
TEG	45.0	14.10
TTEG	45.0	18.10
PG	45.0	6.54
DPG-isomer 1	59.0	9.79
DPG-isomer 2	59.0	9.73
TPG-isomer 1	59.0	11.99
TPG-isomer 2	59.0	12.10

Analyte	**Intercept**	**Slope**	**r^2**
EG	-2800	4370	0.9960
DEG	-5913	4617	0.9975
TEG	-15977	6666	0.9965
TTEG	-28427	6543	0.9956
PG	-9025	4638	0.9974
DPG-1	-1576	11533	0.9978
DPG-2	-10795	11341	0.9985
TPG-1	-2242	1715	0.9974
TPG-2	-2613	1766	0.9974

Table 2. Results for linearity validation

Table 3. Results obtained for LOQ and LOD validation

Analyte	**ΛOX (μγ/μΛ)**	**S/N-LOQ**	**ΛOΔ (μγ/μΛ)**	**S/N-LOD**
EG	10.0	11.47	1.00	4.64
DEG	10.0	10.23	1.00	3.60
TEG	10.0	11.92	2.00	5.36
TTEG	10.0	10.09	2.00	4.24
PG	10.0	11.21	2.00	4.12

Analyte	**ΛOX (μγ/μΛ)**	**S/N-LOQ**	**ΛOΔ (μγ/μΛ)**	**S/N-LOD**
DPG-isomer 1	10.0	12.10	1.00	3.35
DPG-isomer 2	10.0	12.42	1.00	4.02
TPG- isomer 1	10.0	10.65	2.00	4.70
TPG- isomer 2	10.0	10.48	2.00	4.52

Table 4. Results for system precision at 3 levels of concentration (low, mid and high-level)

Analyte	RSD% (n=3)	RSD% (n=3)	RSD% (n=3)	Method RSD% (n=9)
	Low-level (10 μg/mL)	Mid-level (50μg/mL)	High-level (100 μg/mL)	
EG	4.84	1.05	2.26	**2.22**
DEG	1.81	0.35	2.66	
TEG	2.14	0.10	2.75	
TTEG	2.96	3.67	1.72	
PG	6.53	1.98	2.85	
DPG-1	1.82	0.46	2.12	
DPG-2	3.41	0.82	1.88	
TPG-1	2.41	1.36	2.36	
TPG- 2	2.24	0.97	2.54	

Table 5. Analytical variations introduced in order to validate method's robustness

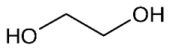
Parameter	Initial method (a)	Robustness method (b)
Flow rate (mL/min)	1.50	1.45
Split ratio	20:1	18:1

Table 6. Description of anti-freeze samples analyzed

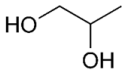
#	**Sample name**	**Type**	**Description**
1	Antifreeze 1 (green)	HT-12	Ready to use, -36°C
2	Antifreeze 2 (pink)	G12	Dilute 1:1 for -30°C
3	Antifreeze 3 (blue)	G11	Dilute 1:1 for -30°C
4	Antifreeze 4 (purple)	G13	Dilute 1:1 for -35°C
5	Antifreeze 5 (yellow-green)	D	Dilute 1:1 for -30°C
6	Antifreeze 6 (dark red)	G12++	Dilute 1:1 for -38°C
7	Antifreeze 7 (light red)	G12+	Ready to use, -35°C
8	Antifreeze 8 (pink)	G40	Dilute 1:1 for -37°C
9	Antifreeze 9 (pink)	G30	Dilute 1:1 for -36°C
10	Antifreeze 10 (blue-green)	LC-87	Dilute 1:1 for -38°C

Table 7. Glycols concentrations in real samples analyzed

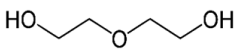
#	% (w/v)	% (w/v)	% (w/v)	% (w/v)	% (w/v)	% (w/v)	% (w/v)	% (w/v)	% (w/v)
	EG	DEG	TEG	TTEG	PG	DPG-1	DPG-2	TPG-1	TPG-2
1	**30.23**	n.d.	5.47	10.26	n.d.	n.d.	n.d.	n.d.	n.d.
2	**44.74**	<LOQ	5.75	10.62	n.d.	n.d.	n.d.	n.d.	n.d.
3	**50.26**	<LOQ	5.40	9.72	n.d.	n.d.	n.d.	n.d.	n.d.
4	**52.96**	<LOQ	5.35	9.20	<LOQ	n.d.	n.d.	n.d.	n.d.
5	**51.97**	<LOQ	5.04	8.95	<LOQ	n.d.	n.d.	n.d.	n.d.
6	**53.70**	<LOQ	5.16	8.52	<LOQ	n.d.	n.d.	n.d.	n.d.
7	**35.75**	n.d.	5.28	9.06	n.d.	n.d.	n.d.	n.d.	n.d.
8	**57.45**	<LOQ	4.98	8.43	n.d.	n.d.	n.d.	n.d.	n.d.
9	**56.31**	<LOQ	5.11	8.66	<LOQ	n.d.	n.d.	n.d.	n.d.
10	**66.65**	<LOQ	6.03	9.45	<LOQ	n.d.	n.d.	n.d.	n.d.



Ethylene glycol (EG)

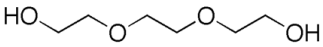


Propylene glycol (PG)

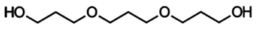


Diethylene glycol (DEG)

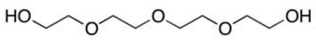
Dipropylene glycol (DPG) – mixture of isomers



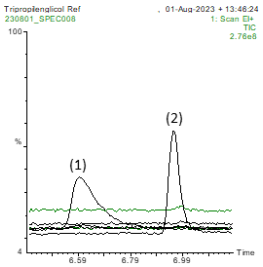
Triethylene glycol (TEG)



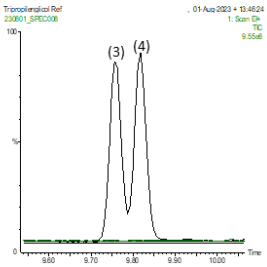
Tripropylene glycol (TPG) – mixture of isomers



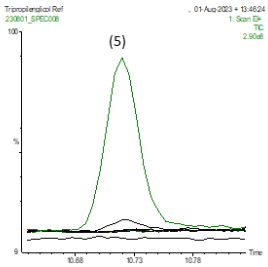
Tetraethylene glycol (TTEG)



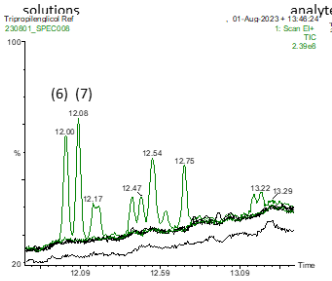
PG (1) and EG (2) peaks – Specificity - overlayed chromatograms with the other target analyte reference solutions



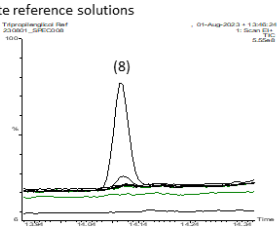
DPG isomer 2 (3) and DPG isomer 1 (4) peaks – Specificity - overlayed chromatograms with the other target analyte reference solutions



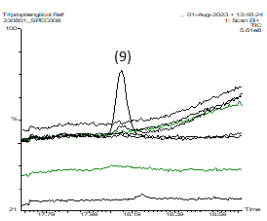
DEG peak – Specificity - overlayed chromatograms with the other target analyte reference solutions



TPG mixture of isomers – Specificity - overlayed chromatograms with the other target analyte reference solutions



TEG peak (8) – Specificity - overlayed chromatograms with the other target analyte reference solutions



TTEG peak (9) – Specificity - overlayed chromatograms with the other target analyte reference solutions

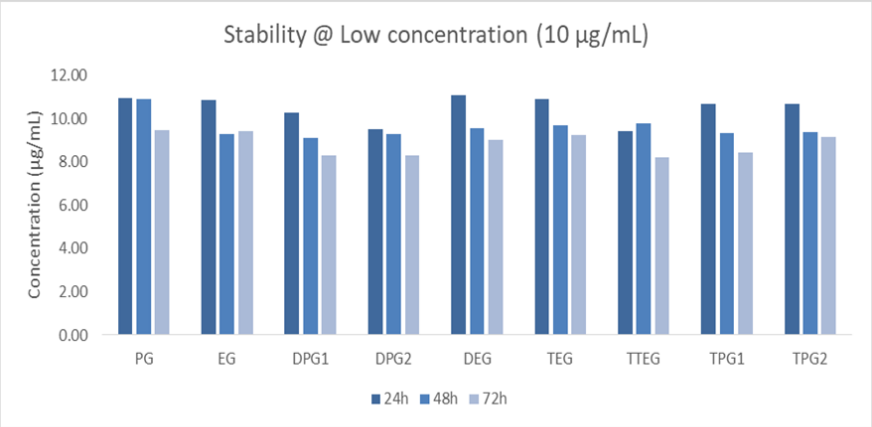


Figure 3. Stability of stock solution at low concentration of glycols

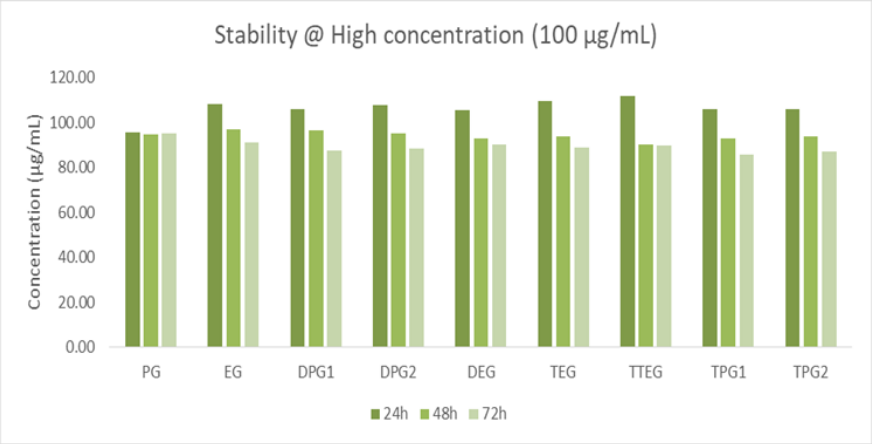


Figure 4. Stability of stock solution at low concentration of glycols