Article

Low-Temperature Partitioning: A Simple Screening Method for Determining Diethylene Glycol in Beer by Gas Chromatography

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Diethylene glycol is an extremely toxic substance to humans. Recently, cases of beer contamination in Brazil have raised awareness of the need for developing simple screening methods to evaluate this type of compound. This research developed a liquid-liquid extraction with low-temperature partitioning technique to determine diethylene glycol in beer via gas chromatography. Employing a flame-ionization detector simplifies the method, lowers its cost and therefore, it can be used as screening step to assess the possibility of contamination. A gas chromatograph coupled to a mass spectrometer would be used only for a confirmatory analysis. The optimized method was validated for the main figures of merit, and it proved to be adequate, with good values of recovery rate (94-106%), limit of detection (3.0 mg L^{-1}), and quantification (10.0 mg L^{-1}). Accuracy, in terms of repeatability and intermediate accuracy, showed variation coefficients lower than or equal to 20%. This method was applied to 28 samples of beers marketed in Brazil, and diethylene glycol was found above the limit of detection in three of them (10.7%). These results were confirmed by a gas chromatograph coupled to a mass spectrometer, which showed the reliability of the screening method for determining diethylene glycol in beer samples.

Keywords: diethylene glycol, quality control, low-temperature partitioning, GC-FID, GC-MS
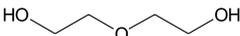
Introduction

Beer and wine are the fermented beverages most consumed worldwide, with consistently high production rates.[1] Considering the economic growth and the importance of the market, entrepreneurs have to be attentive to the product quality during manufacture so as to increase the value of their brands.[2]

Cooling is a relevant factor in brewing, both for physicochemical reasons of the process itself and for controlling the biological activity developed during fermentation.[3] It is common to use water, ethanol, propylene glycol, and glycerol, substances considered suitable for use in the cooling coil within the tanks, facilitating the cooling process.[3] Diethylene glycol (DEG, Table 1), which has physicochemical properties similar to glycols and is cheaper than those, has been improperly and unsafely used in coils.[4]

In Brazil, a likely beer-manufacturing incident was reported, in which DEG used in the cooling process caused the intoxication and death of dozens of people.[5] Caldeira *et al.*[4] analyzed some beer samples, including those considered contaminated, and found DEG in 8% of the samples. In that case, the cooling fluid had come into contact with the beer due to leakage from the coils. Continuous and acute oral ingestion of DEG has

Table 1. Diethylene glycol (DEG): structure and physicochemical properties

Compound	Molecular formula	Chemical structure	Chemical group	Molar mass / (g mol^{-1})	Boiling point / °C	Density / (g cm^{-3})
DEG	$C_4H_{10}O_3$	HO⌒⌒O⌒⌒OH	glycol	106.12	242-247	1.18

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significant adverse effects on consumers' health, including nephrotoxicity and lethality. That episode revealed the inappropriate use of the substance and showed that quality control related to DEG in beer samples is of utmost importance. Nonetheless, studies on the analysis of this contaminant in beer are still scarce, and it is essential to develop simple, efficient, and low-cost techniques that allow screening beers for DEG contamination.

To identify and quantify DEG in beers, an analyte-extraction step must be performed.[6] The most common extraction techniques used in beer analysis are solid-phase extraction,[7] solid-phase microextraction,[8,9] QuEChERS (quick, easy, cheap, effective, rugged, and safe)[10] and precipitation with organic solvents.[11] Liquid-liquid extraction with low-temperature partitioning (LLE/LTP) has proved to be an efficient tool for extracting several analytes from different matrices, such as water,[12] milk,[13] honey,[14] and urine.[15] de Paula *et al.*[16] successfully employed LLE/LTP and paper-spray mass spectrometry (PS-MS) to determine benzodiazepines in various beverages, including beer. LLE/LTP is based on the analyte partition between the aqueous and organic phases due to temperature reduction. In this technique, the single phase consisting of the sample and the organic solvent are separated by lowering the temperature. The aqueous phase is frozen and the organic solvent containing the analytes is easily removed. In general, this extraction process does not require further purification steps.[14]

The identification and quantification of DEG are performed by gas chromatography (GC)[4,17,18] or liquid chromatography.[19,20] Gas chromatography equipped with a flame-ionization detector (GC-FID) can be considered a robust and sensitive enough method to quantify DEG in beer samples,[17] in addition to being simple, reliable, versatile, and easy to operate.[21] In turn, despite being costly, the gas chromatography mass spectrometry (GC-MS) is a powerful method for identifying compounds due to the high specificity given by *m/z* ratio selection.[22]

In the present study, a simple and low-cost method was optimized and validated to determine DEG in beer. This method was later applied in beer samples marketed in Brazil.

Experimental

Reagents and solutions

Standard solutions of DEG and pentanol were purchased from Carlo Erba (São Paulo, Brazil) and Sigma-Aldrich (Saint Louis, USA), respectively. The solvents acetonitrile (ACN) (99.5%, high-performance liquid chromatography (HPLC) grade) and ethyl acetate (ACT) (99.5%), used for the extractions, were acquired from Sigma-Aldrich (Burlington, USA, CAS 75-05-8) and VETEC (São Paulo, Brazil, CAS 141-78-6), respectively. Salts, such as sodium sulfate (VETEC, São Paulo, Brazil, CAS 7757-82-6), sodium chloride (Êxodo Científica, São Paulo, Brazil, CAS 7647-14-5), and sodium thiosulfate (Neon, São Paulo, Brazil, CAS 10102-17-7) were also used in this experiment.

From the standard solutions of DEG and pentanol (internal standard, IS), stock solutions were prepared in ACN at 500 and 5000 mg L^{-1}, respectively. Working solutions were prepared by dilution of stock solutions with ACN and stored in amber glass flasks in a freezer at -20 ± 1 °C.

Samples

Beer samples of a specific brand (DEG-free) were purchased at supermarkets in the city of Viçosa, Brazilian state of Minas Gerais, and used for optimization and validation of the method. Preliminary tests confirmed the absence of DEG in those samples. Once optimized and validated, the method was applied to 28 authentic beer samples of different brands and styles, acquired from local markets of the Viçosa region. The samples were stored in a refrigerator (3 ± 1 °C) until testing. All experiments were performed in triplicate.

Equipment

The chromatographic analyses were performed with a gas chromatograph (GC-Shimadzu, model GC 2014 Plus, Kyoto, Japan) equipped with an autosampler (Shimadzu, model AOC-20i Plus, Kyoto, Japan) and a flame-ionization detector (FID). A gas chromatograph coupled to a mass spectrometer (Shimadzu, model GCMS-QP2020, Kyoto, Japan) and equipped with an autosampler (Shimadzu, model, AOC-20i, Kyoto, Japan) was employed to confirm the DEG identification.

Chromatographic conditions

The conditions in the gas chromatograph equipped with a split/splitless injection system and a flame-ionization detector for DEG separation and determination in beer samples are described as follows. Nitrogen (99.999%, White Martins, Rio de Janeiro, Brazil) was employed as the carrier gas. The temperature at both the injector and detector was kept at 250 °C. An aliquot of 1 μL was injected in a splitless mode with 60 s sampling. The separation was carried out in a capillary column NA-Wax (30 m × 0.25 mm × 0.25 μm film thickness). The column temperature program was optimized

by injecting the DEG and standard solutions. The oven temperature started at 75 °C maintaining it for 2 min, then it ramped to 130 °C at a 15 °C min⁻¹ rate, maintaining it for 5 min. After that, it was raised to 230 °C at 20 °C min⁻¹ and kept at this temperature for 5 min. In these conditions, the total run time was 20.67 min *per* sample.

The beer samples that were considered suspect of contamination by DEG, after analysis by GC-FID, were analyzed by GC-MS to confirm the results. Helium (99.999%, White Martins, Rio de Janeiro, Brazil) was employed as the carrier gas. The temperature at both the injector and detector was kept at 250 ℃. A volume of 1 μL was injected in a splitless mode with a 60 s sampling time. The separation was carried out in a capillary column NA-Wax (30 m × 0.25 mm × 0.25 μm film thickness). The oven temperature was set to start at 75 °C for 5 min, then increased to 130 °C at a 20 °C min⁻¹ rate and kept so for 5 min. Lastly, it was heated to 230 °C at a 20 °C min⁻¹ rate and held at this temperature for 15 min. The total run time of each sample was 32.75 min. The MS data were acquired in the scan mode, using to cover either a wide range of *m/z* ratios and electronic energy of 70 eV. A cut time of 5.0 min was set to prevent damage to the equipment. The detector interface and ionization source temperatures were set at 280 and 250 ℃, respectively.

DEG and the IS were identified by comparing the retention times of the peaks obtained from the sample extracts with those from the standard solutions. Regarding the GC-MS, the confirmation was also checked by the mass spectrum. The quantification employed the matrix superposition method, fortifying the beer samples free from the compounds with five DEG concentrations (10 to 50 mg L⁻¹) and the IS at 50 mg L⁻¹. The samples were subjected to the extraction method and analyzed via GC-FID and GC-MS.

Optimization of the LLE/LTP method

The LLE/LTP was optimized for DEG analysis in beer. In this method, 4.0 mL of a beer sample were put into contact with the extraction solution. After vortexing for 1 min, the mixture was placed in a freezer at −20 ℃ for 6 h to separate the phases by freezing/cooling the aqueous one. The organic extract was then collected, transferred to a vial, and analyzed with the GC-FID.

The method was optimized in two stages. The first one consisted of a univariate analysis of salt usage to promote the salting-out effect. After establishing the best extraction condition using salts, a factorial design 2^2 was applied to the method, considering the ratio between the extraction solvents (ACN:ACT).

Univariate analysis using salts for DEG extraction from beer samples

The effect of salt addition was assessed by varying the types of salts and their concentrations on the efficiency of DEG extraction by LLE/LTP. Beer samples and beer samples spiked with standard DEG solution and without added salt were used for comparison (assay A) (Table 2).

Table 2. Identification of assays (A to G) as a function of the addition of different salts to the beer samples at various concentrations

Assay	Salt	Salt concentration / (mol L⁻¹)	Ionic strength / (mol L⁻¹)
A	absent	absent	absent
B	Na_2SO_4	1.41	4.23
C	Na_2SO_4	4.22	12.66
D	NaCl	3.42	3.42
E	NaCl	10.27	10.27
F	$Na_2S_2O_3$	1.26	3.78
G	$Na_2S_2O_3$	3.79	11.37

The best conditions, i.e., the highest percentage of DEG extracted from beer samples, were used in further experimental steps.

Multivariate analysis of the effects of the salt and extraction solvent (ACN:ACT) on DEG extraction from beer samples

A factorial design 2^2 was applied to investigate the effects of adding salt and changing the polarity of the extraction solvent on DEG extraction from beer samples. The analyses were performed in triplicates, and the resulting data are shown in Table 3. The best conditions were appraised according to the chromatographic responses (areas) obtained in each trial.

Validation

The method LLE/LTP-GC/FID for determining DEG in beer samples was validated for the following figures of merit: selectivity, linearity, matrix effect, accuracy, precision, limit of detection, and limit of quantification. These parameters were estimated according to the validation protocol specified by the Ministry of Agriculture, Livestock, and Supply (MAPA)[23] and the National Health Regulatory Agency (ANVISA),[24] two public organs responsible for controlling the quality and safety of foodstuff in Brazil.

Selectivity

The selectivity of the LLE/LTP-GC/FID method was evaluated by contrasting the chromatograms obtained from the analysis of the beer extracts free from DEG and the IS (blank) with those fortified with these compounds.

Table 3. Factorial design 2^2 performed in triplicate to establish the best conditions for DEG extraction from beer samples

Assay	Factor (codified values)		Factor (real values)	
	ES	S	ES	S
1	+	−	ACN	no salt
2	−	−	ACN:ACT	no salt
3	+	+	ACN	salt
4	−	+	ACN:ACT	salt
5	+	−	ACN	no salt
6	−	−	ACN:ACT	no salt
7	+	+	ACN	salt
8	−	+	ACN:ACT	salt
9	+	−	ACN	no salt
10	−	−	ACN:ACT	no salt
11	+	+	ACN	salt
12	−	+	ACN:ACT	salt

ES: extraction solution; S: salt; ACN: acetonitrile; ACT: ethyl acetate; ACN:ACT: mixture of the two solvents at 3.25:0.75 (v/v). Data corresponding to the (−) the lowest and (+) highest values of the factorial design.

Matrix effect

The matrix effect was assessed by contrasting the calibration curves obtained by injecting the standard DEG solutions concocted with solvent or matrix extract at different DEG concentrations (10, 20, 30, 35, and 50 mg L^{-1}). The matrix extracts were prepared by applying the optimized extraction procedure to the analyte-free beer samples, as previously described. The slopes and intercepts of the calibration curves prepared with the solvent and those using the matrix extract were compared, as proposed by the Brazilian Ministry of Agriculture, Livestock, and Supply (2015).[23]

Limit of detection (LOD) and limit of quantification (LOQ)

The limits of detection and quantification were estimated by the signal-to-noise ratio method. The determination of the signal/noise ratio was performed by comparing the measured signals (area) of the blank sample with the samples with known low concentrations of the analyte, establishing the minimum concentration at which the analyte can be detected or quantified. To establish the LOD and LOQ, beer samples were fortified at increasing concentrations of DEG (from 0.25 to 50 mg L^{-1}). The limit of detection was estimated considering the signal-to-noise ratio of 3:1. The limit of quantification was estimated in an equivalent way but considering the signal-to-noise ratio of 10:1.

Method linearity

The linearity of the method response was tested by submitting beer samples to the LLE/LTP technique. The samples were previously fortified with the standard solutions of DEG at concentrations ranging from 10 to 50 mg L^{-1} and the IS at 50 mg L^{-1}. The curve prepared in the pure solvent showed similar linearity and good fits with R^2 of 0.9974.

Precision and accuracy

The precision of the LLE/LTP-GC/FID method was appraised as a function of repeatability and intermediate precision, whereas the accuracy was estimated by the recovery rates. Since there is no maximum limit of DEG allowed in beers, report limit was adopted to evaluate the accuracy and precision of the method.[23]

To investigate the repeatability of the method, samples were fortified at three concentrations (10, 30, and 50 mg L^{-1}), in triplicate, and submitted to LLE/LTP-GC-FID. These samples were handled by the same analyst, on the same day, using the same equipment. To study the intermediate precision, samples fortified at three concentrations (10, 30, and 50 mg L^{-1}) were submitted to the method on three different and non-consecutive days (days 1, 3, and 5). These analyses were performed by the same analyst, employing the same equipment.

The precision of the method was expressed by the coefficient of variation (CV), given by equation 1.

$$CV\ (\%) = \frac{\text{(estimate of the standard deviation of the obtained areas} \times 100)}{\text{average of the obtained areas}} \quad (1)$$

A recovery study was conducted to assess the accuracy.[24] Blank samples were fortified in triplicate and analyzed at three DEG concentrations (10, 30, and 50 mg L^{-1}). These samples were subjected to the LLE/LTP method and then analyzed with the GC-FID. Accuracy was assessed by the recovery percentage (R), according to equation 2, using an analytical curve plotted under the same conditions.

$$R\ (\%) = \frac{Ce \times 100}{Ct} \quad (2)$$

where, Ce: average of the relative concentrations obtained through the equations of the curve of the analyte, Ct: concentration at which the samples were fortified.

The samples were analyzed in triplicates for precision and accuracy studies.

Results and Discussion

Optimization of the LLE/LTP method

LLE/LTP was used to determine diethylene glycol in beer. In this technique, acetonitrile is placed in contact with an aqueous sample containing analyte(s) of interest, forming a homogeneous mixture. With the lowering of the

temperature of the system and, consequently, the reduction of solubility, acetonitrile becomes an effective extracting solvent, establishing a more effective transfer of matter. The efficiency LLE/LTP may be influenced by factors such as the addition of salt to the medium, the type of salt, the extraction solution of choice, and the ratio between the solvents, among others. Choosing an adequate set of parameters leads to the best sensitivity and precision of the method.[12]

Initially, the LLE/LTP efficiency was assessed univariately, approaching the salt added to the system. The addition of salt to the medium aimed to increase the extraction efficiency of the analyte (DEG) and to evaluate the salting-out effect on the system. Adding salt to the beer sample alters the physicochemical properties of the system formed by the sample and the extraction solvent, partitioning the analytes preferably to the organic phase (salting-out effect).[25] Based on the obtained areas, the results suggested that sodium chloride provided the lowest extraction efficiency (0.1174×10^5), whereas sodium sulfate was the ideal salt for extracting DEG (0.4864×10^5). The better performance of sodium sulfate in relation to sodium chloride may have occurred because the latter does not allow the aqueous phase to freeze completely, which otherwise could have enhanced the analyte partition to the organic phase. Freezing is an essential step of the technique, as it makes it possible to separate the phases and clean the extract.[14]

To better understand this behavior, the results were appraised considering the influence of the ions within the respective salts, i.e., in relation to the ionic strength of the medium. These results are depicted in Figure 1.

Considering the ionic strength (Figure 1) in relation to the areas obtained in the chromatographic analysis, it significantly influenced the behavior of DEG extraction. To better understand this behavior, the data were gathered in Table 4.

In DEG extraction, the negative effect caused by adding NaCl is quite pronounced compared to the area
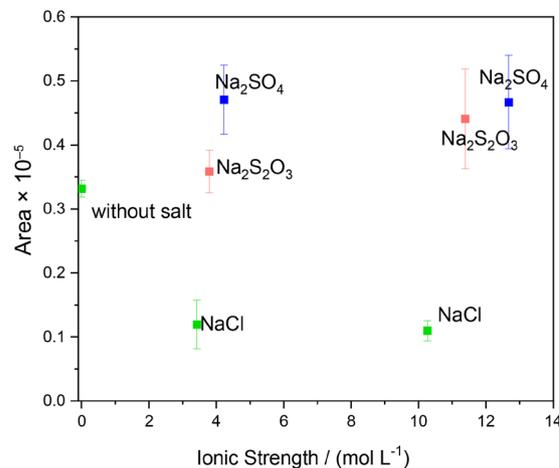
Figure 1. Effect of salt addition on DEG extraction from beer samples in relation to the ionic strength.

without salts (66% reduction, on average) (Table 4). In the presence of thiosulfate, the area increased linearly as the ion concentration augmented (equation 3).

$$\hat{A}.10^{-5} = (0.328 \pm 0.005) + (3.92 \pm 0.26).10^{-3}.c(S_2O_3^{2-}) \quad (3)$$
$$R^2 = 0.9913; s_{res} = 0.0007; rA = 0.18 \text{ mol L}^{-1}; df = 1$$

where, $\hat{A}$: area, c: concentration, R^2: determination coefficient, s_{res}: estimative of standard deviation of residues, rA: analytical resolution, df: freedom degree.

Despite having few points (degree of freedom equal to 1), the quality of the fit is enough to consider the phenomenon valid. The slope, therefore, is associated with the activity coefficient of the neutral species (salting-out). This is because the ionic strength is proportional to the salt concentration, as equation 4 demonstrates.

$$I = 3c(S_2O_3^{2-}) \quad (4)$$

where, I: ionic strength, and c: salt concentration.

Even though the linear effect was not observed, the ion sulfate showed a remarkable positive effect (41% increase,

Table 4. Data on DEG extraction using different salts at various concentrations

Assay	Salt	Concentration / (mol L^{-1})	Area average (× 10^{-5})	SD (× 10^{-5})	CV / %	Effect of salt addition / %
A	no salt, no fortification		0.332	0.013	4	0.0
B	Na$_2$SO$_4$	1.41	0.4.71	0.013	2	41.9
C	Na$_2$SO$_4$	4.22	0.467	0.076	16	40.7
D	NaCl	3.42	0119	0.011	9	−64.0
E	NaCl	10.27	0.11	0.017	15	−67.0
F	Na$_2$S$_2$O$_3$	1.26	0.359	0.012	3	8.1
G	Na$_2$S$_2$O$_3$	3.79	0.441	0.085	19	32.9

SD: standard deviation; CV: coefficient of variation.

on average) (Table 4), proving more suitable for DEG extraction by LLE/LTP. Regarding sodium sulfate, both concentrations were statistically equal (Table 4). However, the concentration of 0.6×10^6 mg L^{-1} was chosen to ensure the best precision of the results.

Subsequently, the best extraction conditions attained in the preliminary tests were evaluated in a factorial design 2^2. A simultaneous study was carried out to evaluate the polarity of the medium, altering the ratio of acetonitrile (ACN) and ethyl acetate (ACT) and the Na$_2$SO$_4$ presence or lack thereof. Utilizing a mixture of organic solvents is an alternative that may boost the process efficiency when salts are used in a liquid-liquid extraction method.[25]

Table 5 contains the variables used in the factorial design 2^2 and the average values of the peak areas for different proportions of the extraction solvents and the use or not of the salt. The average difference between the presence or absence of each variable was compared with the pooled standard deviation, obtained by the square root of the standard deviation values (S). The values were obtained using Origin Pro 2021.[26]

According to the results in Table 5, there was no significant difference in DEG extraction from beer at a 95% confidence level ($p < 0.05$). It means that decreasing the polarity of the extraction solution by adding ethyl acetate did not significantly interfere with the DEG extraction. Therefore, the optimized method consists in using 4.0 mL of acetonitrile as the extraction solvent and 4.0 mL of a beer sample containing sodium sulfate at 0.6 mg L^{-1}. This mixture is vortexed for 1 min, cooled down for 6 h in a freezer at −20 °C for phase separation, and then analyzed with a GC-FID.

Validation

Selectivity

To evaluate the selectivity of the LLE/LTP-GC/FID technique, the optimized method was applied to DEG-free beer samples. Subsequently, these samples were fortified with DEG and the IS and resubmitted to extraction and analysis. Once compared, the chromatograms of the samples showed no interference in the retention times of the analytes of interest, IS (t$_R$ = 4.911 min) and DEG (t$_R$ = 14.654 min), which emphasizes the selectivity of the method (Figure 2).
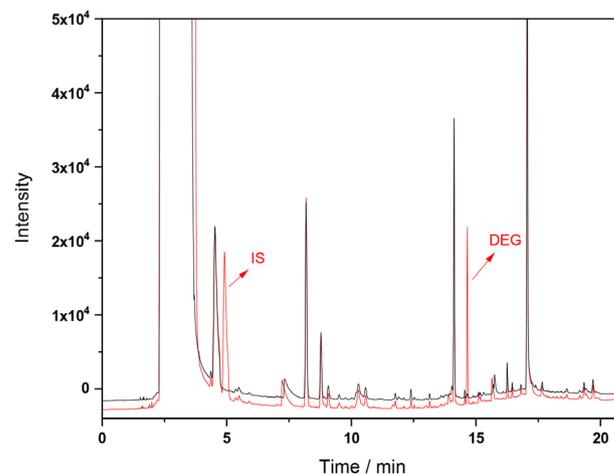
Figure 2. Chromatograms of beer sample extracts: DEG-free (black); and added of the IS (t$_R$ = 4.911 min) and DEG (t$_R$ = 14.654 min), both at 50 mg L^{-1} (red).

Matrix effect

The matrix effect on the chromatographic response of DEG was evaluated by comparing the slope and intercept values of the analytical curves with solvent or matrix extract by applying the *t*-test. The results are in Table 6.

This study verified that the correlation coefficients of both analytical curves were higher than 0.99. The responses of the averages at the different analyte concentrations were statistically equal, and no significant difference was observed when compared by the *T*-test at a 95% probability level ($p > 0.05$). Thus, the matrix did not interfere with the DEG chromatographic responses.

Table 5. Summary of the values obtained from the experimental design 2^2 and the codes of the factors studied for DEG

Assay	Area average (× 10^{-5})	SD (× 10^{-5})	CV / %	Code (ACN)	Code (sulfate)	Interaction
1	0.450	0.088	20	−1	−1	1
2	0.554	0.047	9	−1	1	−1
3	0.465	0.054	12	1	−1	−1
4	0.527	0.073	14	1	1	1
Average difference				−0.0030	0.0415	−0.0105
s-pooled				0.0779	0.0779	0.0779
p-value				0.97	0.63	0.90
				nsig	nsig	nsig

SD: standard deviation; CV: coefficient of variation; ACN: acetonitrile; nsig: non-significant.

Table 6. Comparison of the slope and intercept of the analytical curves for evaluating the matrix effect by the *t*-test

Blank equal to zero (matrix extract curve)		Blank equal to zero (solvent curve)		Equal slopes	
t calculated	1.32	*t* calculated	3.09	*t* calculated	2.33
p-value	0.279	*p*-value	0.054	*p*-value	0.102
	nsig		nsig		nsig

nsig: non-significant.

Limit of detection (LOD) and limit of quantification (LOQ)

Beer samples spiked with DEG at low levels were submitted to the extraction method and analysis to determine the DEG concentration that generated a signal of the required order. Values of LOD equal to 3.0 mg L^{-1} and LOQ of 10.0 mg L^{-1} were obtained. The performance of the proposed method regarding the LOD and LOQ was equal to or slightly better than those found in similar studies in the literature.[4,17]

Working range linearity

For the linearity study, the *y*-values had their variance analyzed for each *x*-value. The goal was to define the best regression model (OLS (ordinary least squares) or WLS (weighted least squares)) to be applied for obtaining the equation that predicted the results of *x* from any *y*-value. The variance was calculated with equation 5.

$$S_{y_i}^2 = \frac{1}{m-1}\sum_{j=1}^{m}\left(y_{ij} - \hat{y}_i\right)^2 \qquad (5)$$

where j represents the jth result of the set of replicas i, and m is the number of replicas of the analytic response *y* at each point. That is, j ranged from 1 to 5 (5 points of the curve), and m was equal to 3 (analyses performed in triplicates). The variances are presented in Table 7.

The Cochran test was applied to determine whether the variance of *y* could be considered constant. The null hypothesis (H0) stated that all variances were equal, while the alternative hypothesis (H1) assumed that at least one variance differed from the others. According to the statistical test, C was 0.515. The C$_{critical}$ value at a 5% significance level for the 5 points analyzed in triplicate was 0.6838. As C < C$_{critical}$, the null hypothesis (homoscedastic

Table 7. Variances of *y*-values for each concentration

DEG concentration / (mg L^{-1})	Variance
10	149.62
20	598.50
25	934.96
35	1833.16
50	3740.67

DEG: diethylene glycol.

data) is accepted. The quality of the linear fit was also verified and confirmed by the correlation coefficient (r) higher than 0.995.

As C < C$_{critical}$, the WLS could be used, i.e., the curve equation could be represented as y = a + bx. The equation of the curve is represented in Table 8. The coefficients of determination (r^2) for DEG were higher than 0.99, indicating good linearity of the method.[23]

Accuracy and precision

Precision was expressed in terms of repeatability and intermediate precision, and these criteria were condensed in the CV. In turn, accuracy was conveyed as the recovery rate. The results are presented in Table 8.

Considering the analytical procedures for assessing DEG in beer samples, the recovery values should have ranged, on average, from 90 to 107% at each level of fortification, with coefficients of variation lower than 20% (CV ⩽ 20%).[23] The values attained in this research (Table 8) comply with the requirements proposed by the MAPA[23] for validating analytical methods for foodstuff. Therefore, the LLE/LTP-GC-FID procedure was adequate for determining DEG in beer samples.

Few studies involving the determination of DEG in beer

Table 8. Method validation parameters: linear range, coefficient of determination (r^2), fortification level (FL), recovery rate (R), repeatability, and intermediate accuracy resulting from the LLE/LTP-GC-FID analytical method developed for DEG analysis in beer samples

Compound	Linear range / (mg L^{-1})	Linear equation	Coefficient of determination (r^2)	FL / (mg L^{-1})	Recovery rate (R) / %	Repeatability (CV) / %	Intermediate precision (CV) / %
				10	106 ± 0.10	11	17
DEG	10-50	*y* = 0.0128*x* + 0.0204	0.9914	30	94 ± 0.24	8	16
				50	104 ± 0.91	16	18

CV: coefficient of variation; DEG: diethylene glycol.

are found in the literature, which shows the importance of developing methods that allow detecting its presence in this type of sample. Recently, in 2021, a method was optimized and validated by Caldeira *et al.*[4] to determine ethylene glycol and diethylene glycol in beer by GC-MS. The results using GC-FID, presented in this work, were like those obtained by Caldeira *et al.*,[4] which shows the feasibility of its application for screening in beer quality control.

Application of the method to authentic samples

To demonstrate the applicability of the LLE/LTP-GC-FID method for quality control in beer, it was tested in samples of different brands, manufacturers, and lots commercially available in the Viçosa region.

Twenty-eight beer samples were analyzed, thirteen of them from the brewery where DEG had been detected, and the others from different brands. All beer samples unrelated to the brand under suspicion had negative results, proving that they were safe for consumption. DEG was detected only in three samples of those beers considered contaminated and, in two of them, the concentration was below the LOQ of the method. The DEG concentration in the third sample was 1567.10 mg L^{-1}. This result is in accordance with the data presented by Caldeira *et al.*,[4] who found DEG ranging from 1000 to 2000 mg L^{-1} in 1% of their samples. The contaminated samples were analyzed with the GC-MS to ratify the results. The presence of DEG was confirmed by the retention time, as well as by the NIST library and the mass spectrum (the most intense ion was $m/z = 45$). Figure 3 shows the chromatograms of the beer sample contaminated with DEG.

It is noteworthy that the positive results for DEG were found only in the brand under suspicion. This shows that the production of beer in Brazil is relatively safe, in view of this study. The contamination case is an isolated brewing fault, which could have been monitored by employing low-cost screening methods, such as the one described in this study.

Conclusions

The LLE/LTP-GC-FID method, once optimized and validated for extracting DEG from beer samples, proved to be a simple, effective, low-cost, and environmentally friendly technique. Thus, it is feasible and can help determine this contaminant in beer, with recovery rates ranging from 94 to 106%. Moreover, the compound extraction and extract clean-up take place simultaneously. The method was applied to 28 beer samples, and DEG was detected in three of them-only one was above the limit of quantification. The results revealed the importance of devising methods for beer quality control, as studies such as this are scarce, and DEG is commonly used in beverage manufacturing processes.

Acknowledgments

Author Contributions

All authors contributed to the work presented in this paper. Mariane M. Azevedo was responsible for the conceptualization, data curation, investigation, methodology, validation and writing; Liany D. L. Miranda for the data curation, formal analysis investigation, methodology, software, supervision, validation, visualization and writing; Maria Eliana L. R. de Queiroz for the conceptualization,
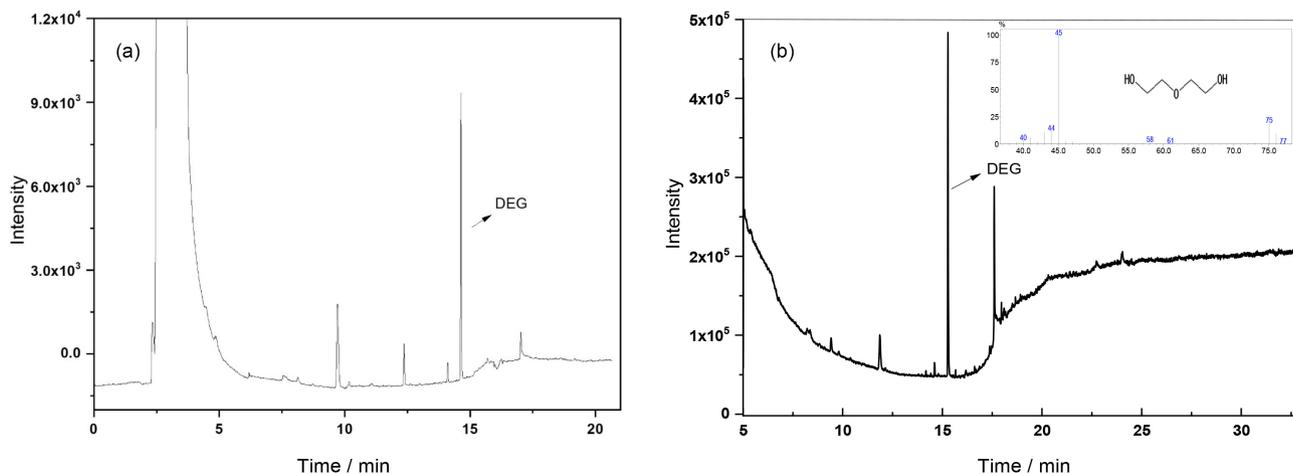
Figure 3. Chromatograms of the DEG-contaminated beer sample extracts analyzed by (a) GC-FID (t$_R$ = 14.654 min) and (b) GC-MS (t$_R$ =15.250 min), with mass spectrum (inset).

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