International Journal of Education and Science Research Review
IJESRR
E-ISSN 2348-6457
Volume-10, Issue-4 July-August-2023
P-ISSN 2349-1817
www.ijesrr.org
Email- editor@ijesrr.org

METHOD DEVELOPMENT AND VALIDATION FOR QUANTIFICATION OF ETHYLENE GLYCOL IN POLYETHYLENE GLYCOL 4000 BY GAS CHROMATOGRAPHY

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ABSTRACT

In order to ensure that breweries are following the rules, it was necessary to develop and test a new analytical method for the simultaneous detection of ethylene glycol and diethylene glycol in beer. Following the diluting of the samples with ethanol, gas chromatography in conjunction with mass spectrometry is used to determine the concentration of the compounds. There was no discernible departure from the acceptable range of merit data. The average analyte recoveries ranged from 91.9% to 108.9% of the original sample. We were successful in achieving repeatability as well as moderate precision (relative standard deviations of 10% or less). The quantification and detection limits for ethylene glycol and diethylene glycol that were obtained were excellent. The quantification limit for ethylene glycol was 10.0 mg.L1, and the detection limit for diethylene glycol was 5.0 mg.L1. Using the technology that is currently being discussed, it was possible to accurately determine the levels of ethylene glycol and diethylene glycol that were present in 701 different beer samples, which represented 67 different brands and 128 different labels..

Keywords: *Glycols, Beverage, Food contaminants, Gas chromatography, Mass spectrometry*

Introduction

Ethylene and diethylene glycol, abbreviated as EG and DEG, respectively, are examples of odourless, tasteless, and colourless liquids that are capable of attracting and holding onto moisture. These glycols are very easily dissolved in both water and polar organic solvents, so you won't have any trouble doing so. These substances are used extensively as humectants, solvents, antifreeze, and coolant agents in automotive antifreeze and coolant compositions, as well as anti-icing and deicing products for aeroplanes. These compositions are used in autos.

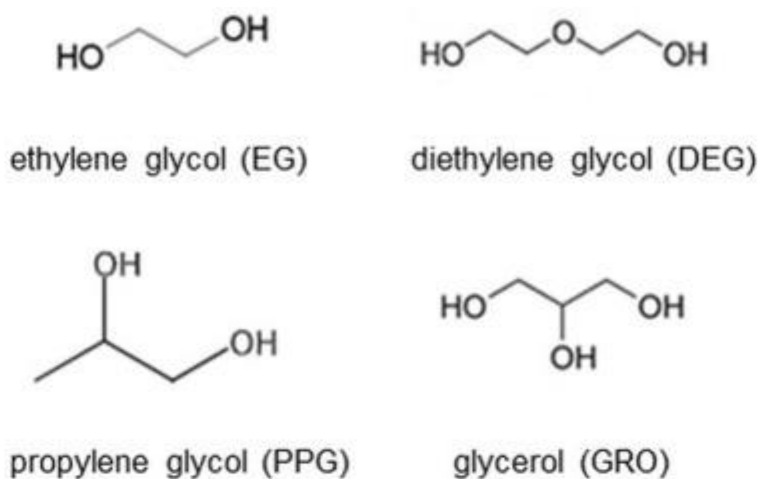

Fig. 1. Structures of the glycols ethylene, diethylene, propylene, and glycerol

In terms of their physicochemical properties, glycols such as glycerol (GRO) and propylene glycol (PPG), which are both non-toxic and more expensive than EG and DEG, are comparable to EG and DEG. See Fig. 1. As a consequence of this, they are frequently utilised as fillers in products that are based on GRO and PPG, particularly in the pharmaceutical industry. Things like toothpaste, injectable drugs, antipyretic medication, and sofa syrup are included in this category.

In contrast to PPG and GRO, which are both generally acknowledged to be free of health risks, the use of EG and DEG in food, cosmetics, or medications is prohibited because of the extreme toxicity of these two compounds. On many occasions, instances of human poisoning have been reported, most likely including DEG as the offending agent.

Several distinct analytical methods, including gas chromatography coupled to mass spectrometry (GC-MS), gas chromatography with flame ionisation detection (GC-FID), ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS), attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, direct analysis in real time-mass spectrometry (DART-MS) (Self, 2013

As was mentioned earlier, different analytical approaches are documented in the literature for the study of hazardous glycols in a variety of matrices. Despite this, gas chromatography–mass spectrometry (GC–MS) is the method of choice due to the physical–chemical characteristics of this class of compounds.

There have been less documented methods of analytical research concerning the detection of DEG in food. Because of the circumstances that surrounded the incorporation of DEG into wine in the 1980s, there are not many studies that examine this compound from the time period in question. Because of this, its already exquisite natural flavour was brought out even further. Only one of these research organisations made use of the GC-MS technology.

A more recent piece of research in this field includes a GC-FID analytical method for evaluating the quantities of coumarin, caffeine, diethylene glycol, and diethylene glycol monoethyl ether in a range of processed foods. These include soft drinks, fruit juices, snack foods, and foods intended for infant consumption. EG is outside the scope of this method's investigation. Because sample dilution happens in products that have a greater water content during the sample preparation process, the specified quantification limit values relate to the

concentration of analytes in the injected samples rather than the concentration of analytes in the original samples. This is because injected samples have a higher water content than original samples. Even though solvent-based calibration curves were used, the matrix effect in the various matrices to which the method was applied was not studied. This is despite the fact that the method was applied.

According to our best knowledge, there is no work that describes the simultaneous determination of EG and DEG in food that can be found in the literature that has been evaluated by peers. Only one of the studies that looked at non-food matrices used GC-MS, however four of the studies reported the simultaneous detection of both analytes. When evaluating more complicated matrices, the lower selectivity of GC-FID in compared to that of GC-MS can be a source of difficulty. The concentration ranges that are typically utilised when assessing hazardous glycols in matrices that do not involve food are also fairly broad. In the absence of a maximum permissible level for EG and DEG in food, an analytical method utilised in the food sector must have low limits of detection and quantification in order to be applicable. Excipients are significantly simpler to work with than food matrices, which can be messy and challenging to assess. In addition, excipients can be used in a wider variety of applications.

Glycols can only be used as a refrigerant in closed systems and at no stage in the brewing process can they come into direct contact with beer. This is a safety precaution that must be taken. As a result of this, it is sometimes necessary to verify the meal for the presence of glycols. Nonetheless, there was not a single piece of study that could be found in the academic literature that investigated the topic of EG or DEG determination in beer. In the current study, an analytical method was developed and then validated in order to test 701 samples of commercially available beer in Brazil for the presence of these two contaminants. The results of the study may be found below. In order to conduct GC-MS analysis and identify the levels of various analytes in beer samples, the samples are first diluted. In this study, our hypothesis was that our recently developed analytical method will accurately detect and quantify EG and DEG in beers. To test our hypothesis, we conducted an experiment.

Experimental

Equipment and experimental conditions

A Polymer Labs model 1000 evaporative light scattering detector from Varian, Amherst, United States of America was combined with a Waters 2695 Alliance system from Milford, Massachusetts, United States of America. The process of automating the system, collecting data, and analysing the results was carried out with the assistance of Waters Empower 2. The ELSD process was carried out with nitrogen serving as the carrier gas and a flow rate of one litre per minute. The evaporator temperature was set at 80 degrees Celsius, and the nebulizer temperature was set at 100 degrees Celsius.

A A 25 centimetre, 0.46 centimetre internal diameter, 5 millimetre Altima C-18 column that was made by Alltech in Deerfield, Illinois, USA was used for the purpose of separating PEG and M-PEG. In the column, the temperature was maintained at 55 degrees Celsius at all times. There was a flow of fluid at a rate of 1 millilitre per minute. Binary mixes with average molecular weights of M-PEG 200 to M-PEG 1000 have an isocratic composition of water:acetonitrile that is 50:50, whereas binary mixtures with average molecular weights of M-PEG 2000 and M-PEG 3000 have an isocratic composition of water:acetonitrile that is 55:45. Vinyl ether PEGs are separated by chromatography on a 25 cm Zorbax SB-18 column (Agilent Technologies, Wilmington, DE,

USA) with a 0.46 cm internal diameter and a 5 mm particle size under an isocratic mobile phase condition of a 60:40 combination of water and acetonitrile. The temperature of the separation is 30 degrees Celsius.

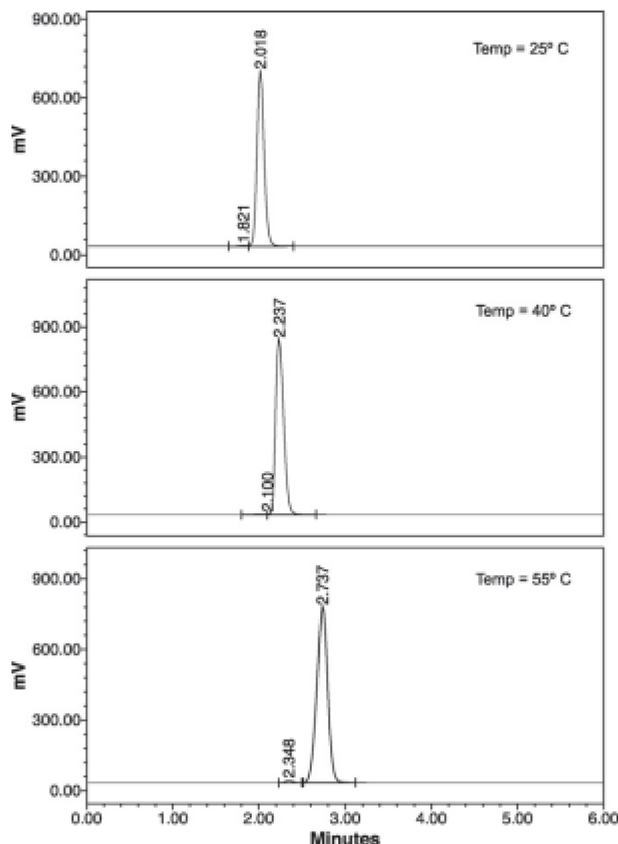

Fig. 2. The influence of column temperature on PEG and M-PEG 3000 separation

All of the samples were injected with the same volume, which was 20 mL, of a solution that was 1% (w/w) and comprised of a mixture of 50:50 acetonitrile and water. As a point of reference, the concentrations of PEG in the standard solutions ranged from 0.005% to 0.100% (w/w). When employed with typical 1% sample solutions, a calibration curve that consisted of six different standard solutions was sufficient for estimating the amount of PEG present in M-PEG or V-PEG at 0.5-10%.

The LC-MS equipment that was utilised was a Thermo Finnigan LCQ ion trap mass spectrometer. This instrument was utilised in conjunction with a Spectraphysics P4000 gradient pump, AS 3000 Autosampler, model 330 column heater, and Spectraphysics, which is situated in San Jose, California, USA. An atmospheric pressure chemical ionisation (APCI) source was utilised in order to carry out the ionisation process. The vaporizer temperature was set at 400 degrees Celsius, and the sheath gas was nitrogen. The temperature of the capillary was 185 degrees Celsius, and the discharge current was 3 milliamperes. Mass spectra in the positive ion mode were obtained and collected in the range of 300 to 2000 m/z.

Samples and reagents

Both pilot plant and commercial samples of Huntsman's M-PEG and V-PEG products are available for purchase from the company. PEG standards with average molecular weights of 400 Da, 600 Da, 1000 Da, 1450 Da, 2000 Da, 3400 Da, 6000 Da, and 8000 Da are all available from the company Aldrich in Milwaukee, Wisconsin,

United States. The liquid chromatographic grade acetonitrile that was utilised in this investigation was supplied by Fisher Scientific (located in Pittsburgh, Pennsylvania, United States). This water was of the same standard as Waters Milli-Q.

Results and discussion

Synthesis of M-PEGs, V-PEGs and PEG impurities

The key processes for the synthesis of M-PEG and V-PEG as well as the generation of PEG impurities are shown in their most basic forms in the following:

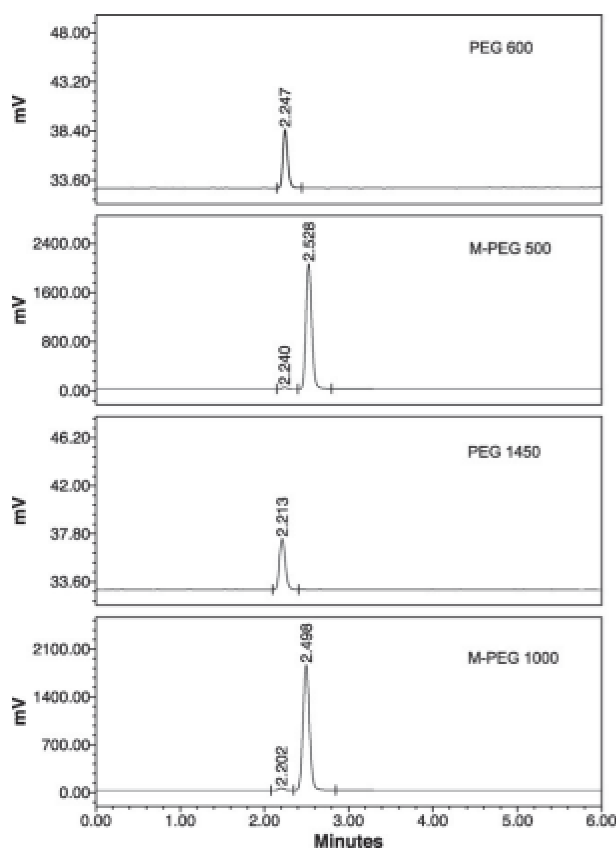

Fig. 3. M-PEG 500 and M-PEG 1000 are used to separate PEGs. There is 3.8% (w/w) of PEG in M-PEG 500, and 4.8% (w/w) in M-PEG 1000

A base catalyst such as potassium hydroxide is used to ethoxylate the R-OH molecule shown in Figure 1. This molecule represents a molecule that contains an alcoholic OH group and can either be an alcohol or a glycol. It is common knowledge that the synthesis of polymer molecules of diverse sizes and shapes, in addition to the propagation of functional groups (such as -OH), is the consequence of the addition of ethylene oxide (EO) monomer to the initiator molecule. This phenomenon is brought about as a direct outcome of the reaction. The culmination of this process yields a mixture of oligomers, each of which has its own molecular weight distribution. The population and the total number of oligomers can be approximately determined with the help of Poisson's distribution law.

Either methanol or diethylene glycol methyl ether (DEGME) can serve as the starting material for the synthesis of M-PEG. When attempting to purge any trace amounts of water that may be present, methanol creates a challenge for the purification process. Because of this, it is quite likely that an M-PEG will have considerable quantities of PEG impurities if methanol is used in the process, particularly if either the methanol itself or the reaction apparatus contains water. The examples of M-PEG synthesis presented in Table 1 can be carried out in either a single or a double addition step. DEGME was the starting point for all of the reactions, and the x and y values show, respectively, the amounts of ethylene oxide that were added into the reactor.

Ethoxylation of 22 moles of ethylene oxide with 4 moles of hydroxybutyl vinyl ether (HBVE) was required for the production of V-PEG 1100. In order to produce V-PEG 6000, V-PEG 1100 had an additional 111 moles of ethylene oxide added to it.

Before the process of ethoxylation can begin, the reactor must normally be filled with a very small amount of water (up to 0.1%). It is possible to incorporate it into the fundamental catalyst component itself or to add it to the reactive alcohol. Hydrolysis of ethylene oxide results in the production of ethylene glycol when it is carried out in the presence of water. It is also possible for difunctional glycols to be present as impurities in glycol ethers such as DEGME and HBVE. Glycols have two different reaction centres that they can undergo.

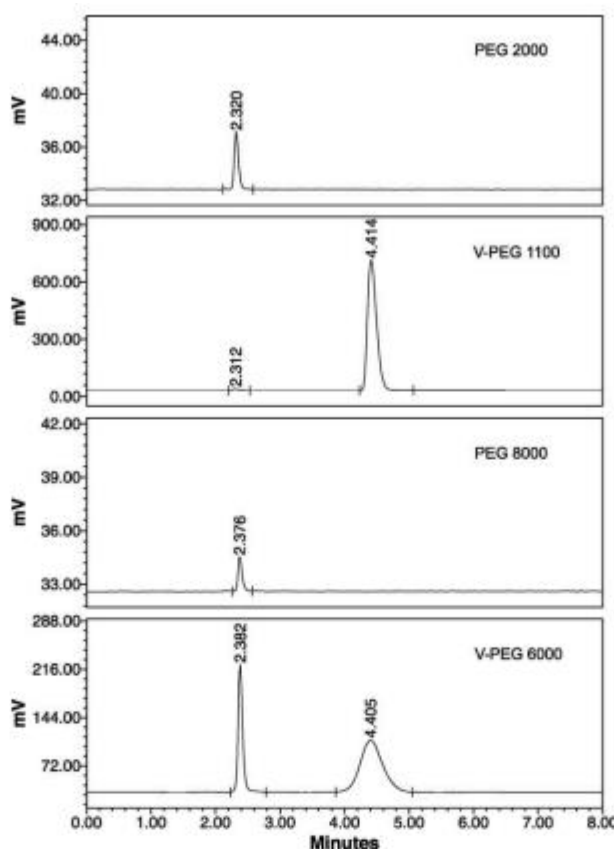

Fig. 4. V-PEG 1100 and V-PEG 6000 PEGs were isolated.

Therefore, assuming that the rate of reaction is the same, ought to incorporate ethylene oxide at a rate two times that of a molecule with a single reactive hydroxyl group, like an oligomer of monofunctional M-PEG or V-PEG. This is because an oligomer of monofunctional M-PEG or V-PEG contains several reactive hydroxyl groups. Therefore, it should come as no surprise that the production of the diol will call for roughly twice as

many moles of ethylene oxide as that of the monofunctional product. Impurities of difunctional ethoxylates, sometimes known as PEGs for short.

Separation of PEG from M-PEG or V-PEG

Experiments were carried out with samples that were known to include much higher-than-average quantities of PEG in addition to a range of reversed-phase columns for the purpose of separating PEG from monofunctional PEG. A comparable amount of work was put in to evaluating a number of other isocratic mobile phase formulations. Additionally, we investigated the effect that column temperature had on our capacity to differentiate between PEG and M-PEG. As was discussed in Section 2, the detector was installed in accordance with the instructions provided by the manufacturer. PEG standards were run simultaneously so that retention times could be compared and an educated decision could be made regarding which standards to utilise (see below).

The chromatograms for a number of different PEG standards are shown in Figure 2. Each of these chromatograms displays a single, distinct peak. These findings indicate the range of retention periods for various PEGs. They were obtained on an Altima C-18 column at 55 degrees Celsius and using an isocratic mobile phase composition of 45 percent water and 55 percent acetonitrile.It is important to note that the composition of the mobile phase that is detailed in this article is distinct from that which is frequently used to extract PEGs from M-PEG or V-PEG samples, as was covered in Section 2.

In the process of separating PEG from M-PEG, it has been found that temperature plays a significant part in the process. It has been discovered that high column temperatures provide the optimum resolution for distinguishing between PEG and M-PEG (for an example, see Figure 3).

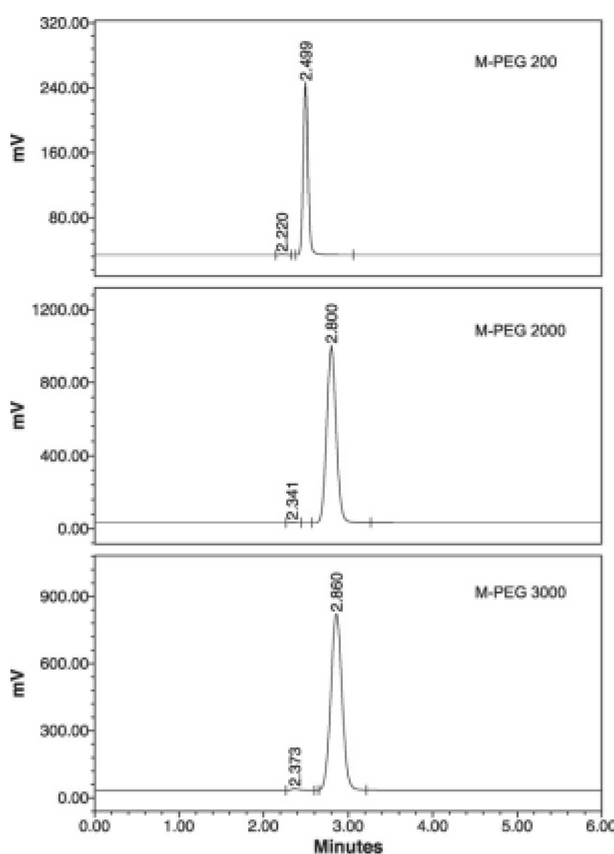

Fig. 5. Separation of PEGs from M-PEG 200, M-PEG 2000 and M-PEG 3000 samples

At a temperature of 55 degrees Celsius, M-PEG 3000 is manufactured. According to the information presented in Section 2, the mobile phase was a combination of water and acetonitrile with a ratio of 55:45. The PEG peaks are not well separated from the M-PEG peak when the temperature is set to 25 °C, and when the temperature is increased to 40 °C, they are only partially separated. The vertical lines, on the other hand, show where the peaks are located.

Conclusions

This study demonstrates strategies for doing quick reversed-phase liquid chromatography in order to examine monofunctional polyglycol ethers for the presence of polyethylene glycol impurities. By improving the parameters of the experiment, PEG and monofunctional PEG were able to be distinguished from one another as distinct, narrow peaks in less than six minutes. It was observed that the molecular weights of PEG impurities in various goods vary, and it was also established that detecting PEG impurities in various sample types allowed the use of an appropriate PEG standard for the quantitative detection of PEG. Both of these findings were made possible by the discovery that PEG impurities have different molecular weights in different products.

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