

Student Skill Development with the Real World: Analyzing *tert*-Butyl Alcohol Content in Gasoline Samples

Bruna F. Gonçalves, Gabriela Botelho, Maria J. Medeiros, and Michael J. Smith

ABSTRACT

In the activity described in this communication, the authors present a simple experiment that can be implemented with moderate operational costs and that allows students to acquire the manipulative skills necessary for chromatographic analysis of a familiar fuel that still plays a fundamental role in providing energy for transport of passengers and goods.

The use of gasoline formulations in a laboratory activity presents students with a motivating subject of study and an opportunity to apply analytical procedures to the characterization of controlled substances used in vehicle fuel formulations.

KEYWORDS

Upper-Division Undergraduate, Laboratory Instruction, Analytical Chemistry, Oxygenates, Gasoline, Chromatographic Techniques, Qualitative and Quantitative Analysis

INTRODUCTION

The analysis of gasoline has been the objective of several student experiments over the last two decades¹⁻¹², motivated by the increase in student interest that the use of everyday commodities contributes to practical activities. Experiments with fuels have quantified lead compounds in commercial formulations²⁻⁵ and characterized specific components or additives introduced to substitute organometallic compounds.⁸⁻¹⁰ Previous strategies have applied volumetric and spectroscopic methods^{2-4, 12}, however the most appropriate method for complex formulations is gas chromatography with suitable detectors.^{8,9}

Gasoline chemistry has supported the mechanical development of spark-initiated internal combustion engines (SIICEs) for over a hundred years and significant investment has been rewarded with lucrative commercial returns.¹³

Early fuel formulations were modified to avoid mechanical failure of engine components through “knocking” or “pinking” a process resulting from the ignition of pockets of compressed fuel before the spark-initiated combustion front reached the air-fuel charge. The increase of compression ratios and octane rating to improve power output and energy efficiency of SIICEs required further alterations to fuel formulations. In the 1970s, the introduction of catalytic converters resulted in significant reduction in emissions of CO, hydrocarbon and nitrous oxide in exhaust gases. This improvement was made possible by the substitution of tetramethyl (TML) and tetraethyl lead (TEL) by oxygenated additives that contributed anti-knock and octane boosting properties but did not poison heavy-metal-sensitive catalysts. This new formulation increased performance, improved running economy and reduced volatile organic compound emission.

Fuel additives developed during the last few decades include a range of substances that provide chemical stabilization, oxidation protection, octane enhancement, exhaust gas reduction, detergent action and coloration.¹³ These additives are compatible with the principal component in fuel lines and storage containers throughout the commercial distribution network. The principal “gasoline fraction” is often a mixture of about 200 hydrocarbons with carbon numbers from 4 to 12 and a boiling range between 35 and 200 °C. Octane boosters, as high percentage components, must blend well with the gasoline fraction and reduce carbon monoxide and unburned hydrocarbons in exhaust gas emission. In general, ethers are favored as they perform well under normal engine operating conditions¹³ however, these compounds share the toxicity and environmental consequences of some alcohols, also used as oxygenates.¹⁴⁻¹⁶ Oxygenate addition is permitted up to about 5-6 vol % in USA¹⁷ and 15 vol % in Europe.^{18, 19} In the past, methyl tert-butyl ether (MTBE), ethyl tert-butyl ether (ETBE), tert-amyl methyl ether (TAME), diisopropyl ether (DIPE) tert-butyl alcohol have all been chosen as oxygenates as they perform well under a wide range of engine operating conditions. However, the detection of MTBE and tert-butyl alcohol (TBA) in groundwater close to gasoline spillage sites, led to a major change in fuel formulation policy. Under spill-site conditions, MTBE can be biodegraded into TBA. TBA is toxic, difficult to eliminate due to its water-miscibility and suffers slow

biodegradation. TBA is used in synthesis of MTBE and other ether oxygenates and may be present as a contaminant. Under the Clean Air Act (USA, 2012) producers of fuel and additives are required to provide information regarding the emissions from their products and effects on public health. Largely as a response to these controls most fuel oxygenates in the USA are now based on ethanol.²⁰

Both TBA and MTBE have been detected in seawater, ground and surface water²¹ and in soils. Although the impact of these compounds on human health is not yet clear, strict limits have been imposed on their use in gasoline, and it is therefore essential to evaluate their concentration with accuracy. Evaluation of oxygenate content has been reported by GC^{8,22}, GC-MS⁹, 1H NMR²³ and FTIR.^{12,24} Gas chromatography is a widely-used quantitative and qualitative instrumental technique introduced in analytical chemistry courses and therefore an essential component of practical classes. In this experiment, students evaluate TBA content of commercial fuels and compare their results with current legislation.¹⁸ The study of real-world samples provides an important stimulus to student interest. This activity has been designed for mid-course students that have completed introductory courses on Organic, Inorganic and Physical Chemistry and are either attending lecture courses on analytical instrumentation or separation techniques. The content is intended to provide a practical experience to support these modules.

EXPERIMENTAL SECTION

Materials and instrumentation

Samples of different brands of gasoline from three suppliers (designated as A, B and C in Table 1) were collected in completely filled and sealed flasks. Samples were cooled, transported to the laboratory and analyzed, without pre-treatment, within 24 h. *Tert*-butyl alcohol with 99.5 % purity was obtained from Acros.

Table 1. Gasoline samples analyzed

Brands	
A	Unleaded 95 Unleaded 98 Unleaded 98 Premium
B	Unleaded 95

Analysis was performed using a CHROMPACK CP 9001 equipped with injector and flame ionization detector temperatures of 190 °C. Samples were characterized in duplicate by injecting 1 μ L samples onto a packed column (2 m x 3.2 mm x 2.1 mm I.D., 10 % Carbowax 20 M with 80/100). The column temperature was maintained at 50 °C for the first three minutes, then increased from 50 to 190 °C at 10 °C/min, and held at this end-temperature for 3 minutes. Data was acquired and stored using DataApex Clarity software installed on a desktop computer.

Preparation of calibration samples

The standard addition method was chosen because of the small amounts of compound present and because of lower detection limit of this method relative to other quantification methods. Six 1 mL solutions were prepared from each gasoline sample and spiked with different volumes of TBA (0, 10, 20, 30, 40 and 50 μ L). It is advisable to prepare each solution immediately before the injection and to use sealed vials with septa to reduce evaporation of volatile components.

Hazards

TBA and gasoline samples are volatile, flammable liquids. Preparation and manipulation should be carried out within a fume hood while using lab coats, gloves and safety spectacles. All chemicals should be disposed of using an appropriate waste container.

RESULTS

To identify the TBA peak in gasoline, prior to quantification, students prepared a standard solution with a small amount of pure TBA compound in acetone. This solution was analyzed using optimized instrumental conditions and the t_r of TBA was determined ($t_r = 6.18$ min). The commercial gasoline samples were characterized using the same experimental conditions and the TBA peak, shown in Figure 1, was identified from its t_r .

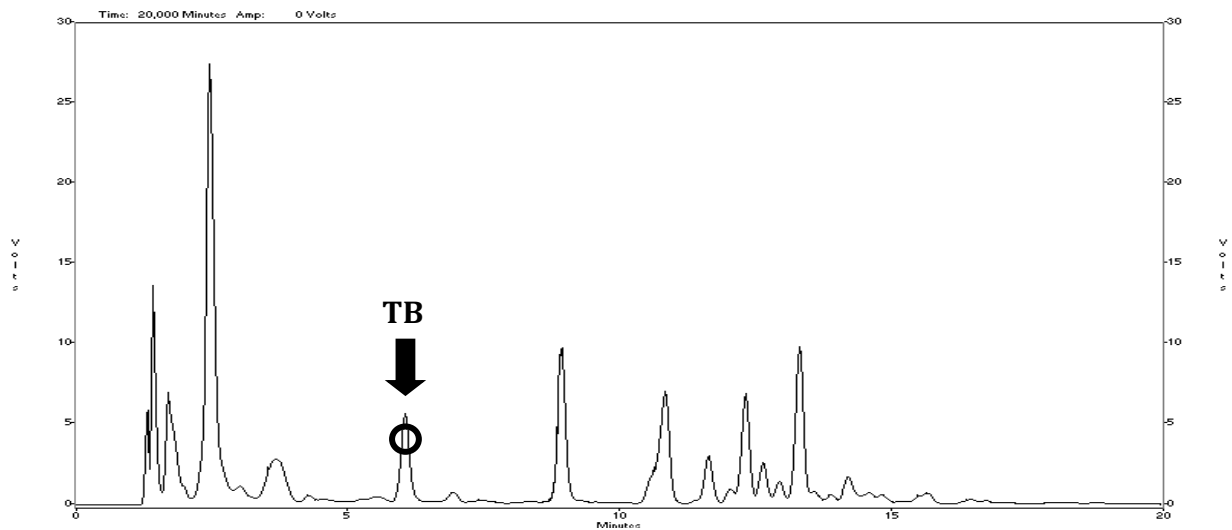


Figure 1. Chromatogram of TBA in gasoline 95 from brand A

Quantitative measurement of TBA using the standard addition method

A known amount of TBA was added to each gasoline sample in accordance with the standard addition procedure. The evaluation of areas under the TBA peaks (Figure 1) and baseline correction was carried out using the same software routines for all samples. TBA peak area (A_{TBA}) was plotted as a function of TBA spike concentration ($C_{TBA \text{ added}}$) in the samples (Figure 2). A linear relationship was observed, according to equation 1, with slope m and intercept b :

$$A_{TBA} = m C_{TBA \text{ added}} + b \quad (\text{equation 1})$$

The original concentration of TBA in the gasoline samples ($C_{TBA \text{ sample}}$) was calculated by extrapolation of the standard addition graph (Figure 2) to $y = 0$ using equation 2:

$$C_{TBA \text{ sample}} = b / m \quad (\text{equation 2})$$

The concentration of TBA was calculated from the slope and intercept of the plots shown in Figure 2.

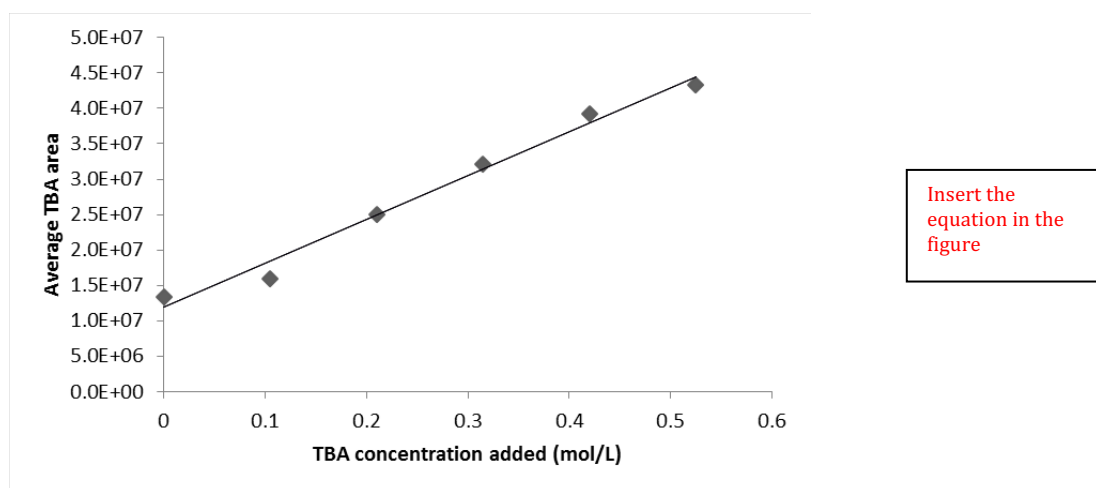


Figure 2. Standard addition plot of TBA in 98 Premium gasoline, brand A

The conversion of concentration of TBA from (mol/L) to % (v/v) was performed according to equation 3:

$$\% [TBA] = \frac{n \cdot M}{\rho \cdot 10^3} \times 100 \quad (\text{equation 3})$$

where n (mol) are the moles of TBA obtained by extrapolation, M (g/mol) is the molecular weight of TBA, ρ (g/cm³) is the density of TBA and 10³ (cm³) is the conversion factor. The quantification and statistical results obtained from the data plotted according to equation 1 are displayed in Table 2 (Supporting information).

Table 2. Quantification of TBA in gasoline samples

Straight line equation	r ^a	DL ^b (mol/dm ³)	QL ^c (mol/dm ³)	x _E ^d ± S _{xE} x t ^e (mol/dm ³)	% ± Δ%
y = [(55 ± 4) x + (16 ± 3)] x 10 ⁶	0.995	0.08	0.26	0.30 ± 0.17	2.8 ± 0.4
y = [(41 ± 4) x + (17 ± 2)] x 10 ⁶	0.996	0.08	0.26	0.40 ± 0.70	3.9 ± 0.5
y = [(62 ± 4) x + (12 ± 3)] x 10 ⁶	0.992	0.08	0.28	0.20 ± 0.10	1.8 ± 0.3
y = [(112 ± 8) x + (20 ± 5)] x 10 ⁶	0.995	0.08	0.26	0.17 ± 0.14	1.7 ± 0.3
y = [(46 ± 5) x + (17 ± 4)] x 10 ⁶	0.991	0.11	0.37	0.37 ± 0.32	3.5 ± 0.7
y = [(60 ± 2) x + (22 ± 2)] x 10 ⁶	0.998	0.05	0.16	0.37 ± 0.11	3.5 ± 0.3
y = [(46 ± 1) x + (17 ± 0.8)] x 10 ⁶	0.9995	0.03	0.10	0.36 ± 0.07	3.5 ± 0.2

^acorrelation coefficient; ^bDL-Detection limit; ^cQL-Quantification limit; ^d|x_E|-TBA concentration; ^eS_{xE} x t- TBA concentration error.

DISCUSSION

Optimization of the temperature program was carried out in preliminary experiments to establish the most favorable conditions used for component separation and characterization. Each sample was chromatographed only twice and the data obtained from the chromatograms were recorded in a table. Students identified TBA in samples using t_r of the compound.

From examination of the standard addition plot obtained from the TBA peak area (A_{TBA}) as a function of concentration of the TBA spike ($C_{TBA \text{ added}}$) (Figure 2), students confirmed the reproducibility of results. In all cases a good correlation coefficient (r) and similar detection (DL) and quantification limits (QL)²⁵ were obtained. The TBA concentration (X_E) was estimated by extrapolation of the standard addition plot to $y = 0$ where the t -student used was 2.776 for 97.5 % probability and 4 ($n-2$) degrees of freedom. The error in volume introduced with the addition of the 50 μL spike was considered to be insignificant and was not taken into account in calculations.^{26, 27}

The value of Student's t used was for 95% probability and $n-2$ degrees of freedom.²⁸

The average value for the concentration of TBA in gasoline obtained by the students was 0.25 mol/L with data ranging from 0.17 to 0.40 mol/L (Table 2). The percentages of TBA obtained after conversion of the concentration of TBA from (mol/L) to % (v/v) were all well below the legal limit of 15 % (v/v).^{18,19}

Students readily arrived at the conclusion that all of the gasoline samples contained TBA within the legal limit established by current legislation.

CONCLUSION

In this communication, the authors describe an experiment prepared for upper-division undergraduates. Student interest is sustained by real-world relevance of the content, with learning objectives that are appropriate for analytical/instrumental laboratory or Environmental Chemistry modules, and hands-on experience in the application of an important chromatographic technique. During this experiment students gain first-hand experience in the application of the standard addition method to analysis with GC equipment

and practice in the interpretation of chromatograms. The experiment is simple, convenient and provides accurate determination of TBA in gasoline. Instructors may use this experiment to encourage their students to consider the errors introduced by the volumetric dilution that occurs as a result of spike addition and the consequences on the analytical data. In many practical classes, due to unavoidable constraints on the duration of laboratory activities, student experiments are limited to a small number of replicas. This limitation naturally has consequences on the precision of the experimental data. Students should be aware that in “real-world” situations these restrictions can be removed.

The relationship between Chemistry and the Environment is almost always complicated by the many physical and chemical interactions involved. This experiment provides an opportunity to study the evolution of a familiar commercial product and how environmental impact has been minimized and the operational benefits maximized. For many students, this leads to a new appreciation of the rather tortuous evolutionary path that chemists must follow to attain the goal of environmentally-conscious production. (1 865 words)

ACKNOWLEDGEMENTS

The authors acknowledge support from the Foundation for Science and Technology (CQ/UM PEST-C/QUI/UIO686/2013 FCT, Portugal) and FEDER (European Fund for Regional Development)-COMPETE-QREN-EU through the Chemistry Research Centre of the University of Minho.

REFERENCES

- (1) Cassidy, R.F. Jr.; Schuerch, C. Gas chromatographic analysis of gasoline. A laboratory experiment. *J. Chem. Educ.* **1976**, *53*, 51-52.
- (2) Watt, S.L.; Martino, T.M.; Chamberlin, M.A.; Laswick, P.H. Simple titrimetric determination of lead in gasoline. *J. Chem. Educ.* **1977**, *54*, 262-263.
- (3) Coleman, M.F.M. The determination of lead in gasoline by atomic absorption spectrometry, *J. Chem. Educ.* **1985**, *62* (3), 261-262.

- (4) Bye, R. A simple extraction procedure for the determination of lead in gasoline by atomic absorption spectrometry, *J. Chem. Educ.*, **1987**, *64* (2), 188-189.
- (5) Wittcoff, H. Nonleaded gasoline: Its impact on the chemical industry, *J. Chem. Educ.*, **1987**, *64* (9), 773-776.
- (6) Tackett, S.L. Determination of methanol in gasoline by gas chromatography: A laboratory experiment, *J. Chem. Educ.*, **1987**, *64* (12), 1059-1060.
- (7) Kostecka, K.S.; Rabah, A.; Palmer, C.F.; GC/MS Analysis of the Aromatic Composition of Gasoline, *J. Chem. Educ.*, **1995**, *72* (9), 853-854.
- (8) Brazdil, L.C. Oxygenates in Gasoline: A Versatile Experiment Using Gas Chromatography. *J. Chem. Educ.*, **1996**, *73* (11), 1056-1058.
- (9) Quach, D.T.; Ciszkowski N.A.; Finlayson-Pitts, B.J.; A New GC-MS Experiment for the Undergraduate Instrumental Analysis Laboratory in Environmental Chemistry: Methyl-t-butyl Ether and Benzene in Gasoline. *J. Chem. Educ.*, **1998**, *75* (12), 1595-1598.
- (10) Donahue, C.J.; D'Amico, T.; Exline, J.A. Synthesis and Characterization of a Gasoline Oxygenate, Ethyl tert-Butyl Ether, *J. Chem. Educ.*, **2002**, *79* (6), 724-726.
- (11) Mueller, S.A.; Anderson, J.E.; Wallington, T.J.; Hammond, R.M. A Classroom Demonstration of Water-Induced Phase Separation of Alcohol-Gasoline Biofuel Blends, *J. Chem. Educ.*, **2009**, *86* (9), 1045-1048.
- (12) Conklin A.; Goldcamp, M.J.; Barrett, J. Determination of Ethanol in Gasoline by FT-IR Spectroscopy. *J. Chem. Educ.*, **2014**, *91* (6), 889-891.
- (13) Hamilton, B. Gasoline FAQ, <http://www.faqs.org/faqs/autos/gasoline-faq/part4/preamble.html>, Accessed on 24 May 2018.
- (14) Mezcuca, M.; Aguera, A.; Hernando, M. D.; Piedra, L.; Fernández-Alba, A. R. Determination of methyl tert-butyl ether and tert-butyl alcohol in seawater samples using purge-and-trap enrichment coupled to gas chromatography with atomic emission and mass spectrometric detection. *J. Chromatogr. A*. **2003**, *999*, 81-90.

- (15) Schmidt, T. C. Analysis of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) in ground and surface water. *Trends Anal. Chem.* **2003**, *22*, 10, 776–784.
- (16) Rosell, M.; Lacorte, S.; Barceló, D. Simultaneous determination of methyl tert-butyl ether, its degradation products and other gasoline additives in soil samples by closed-system purge-and-trap gas chromatography-mass spectrometry, *J. Chromatogr. A.* **2006**, *1132*, 28–38.
- (17) Clean Air Act, 2012. 42 USC § 7401, et seq.
- (18) Directive 2009/30/EC of the European Parliament and of the Council, 23 April, 2009.
- (19) European Standard BS EN 1601:2014, BS 2000-408:2014
- (20) Henley, M.; Letinski, D.J.; Carr, J.; Caro, M.L.; Dughtrey W.; White, R. Health assessment of gasoline and fuel oxygenate vapors: Generation and characterization of test materials, *Regulatory Toxicology and Pharmacology*, **2014**, *70*, S13–S17.
- (21) Achten, C.; Puttmann, W. Method for determination of methyl tert-butyl ether in gasoline by gas chromatography. *J. Chromatogr. A* **2001**, *910*, 377–383.
- (22) Eweis, J. B., Labolle, M., Benson, D.A., Fogg, G.E., Role of volatilization in changing TBA and MTBE concentrations. *Environ. Sci. Technol.* 2007, *41*, 6822–6827.
- (23) Meusinger, R. Qualitative and quantitative determination of oxygenates in gasolines using ¹H nuclear magnetic resonance spectroscopy. *Anal. Chim. Acta.* **1999**, *391*, 277–288.
- (24) Lob A.; Buenafe, R.; Abbas, N. M. Determination of oxygenates in gasoline by FTIR. *Fuel.* 1998, *77*, 15, 1861–1864.
- (25) Magnusson, B.; Ornemark, U. Eurachem Guide: The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics, 2nd ed, Eurachem, (2014). Available from <https://www.eurachem.org/index.php/publications/guides>. (accessed July 2018).
- (26) Bruce, G.R.; Gill, P.S. Estimates of Precision in a Standard Additions Standard. *J. Chem. Educ.*, **1999**, *76* (6) 805-807.

- (27) Bader, M. A Systematic Approach to Standard Addition Methods in Instrumental Analysis. *J. Chem. Educ.* **1980**, 57 (10), 703-706.
- (28) Miller, J.J.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed.; Prentice Hall: England, 2018.

Oxygenates in gasoline, analysis of a real-world commodity

Supporting information for Students and Instructors

EXPERIMENTAL GOAL

The practical objective of this experiment was to determine the concentration of *tert*-butyl alcohol (TBA) in commercial gasoline from three different local gas stations using Gas Chromatography with a Flame Ionization Detector (GC-FID). A standard solution of 1×10^{-3} M TBA sample in acetone was prepared and injected into the GC, under optimized experimental conditions, to determine the characteristic retention time (t_r). The commercial gasoline samples were then injected and analyzed, using the same experimental conditions, to determine the TBA content.

LEARNING OBJECTIVES

- Review GC-FID theory and recognize widespread applicability.
- Operate GC equipment and interpret experimental chromatograms.
- Calculate the analyte concentration in a sample using the standard addition method based on calibration data and peak areas.
- Identify the function of TBA in gasoline and justify the restrictions imposed by environmental legislation.
- Interpret the experimental results and confirm that the fuel respects current legislation.

CHEMICALS

TBA CAS No. 75-65-0

Acetone CAS No. 67-64-1

Gasoline	Brands	Samples
A	Unleaded 95	
	Unleaded 98	
	Unleaded Premium	98
B	Unleaded 95	
	Unleaded 98	
	Unleaded Premium	98
C	Unleaded 95	
	Unleaded 98	

INSTRUMENTATION

The GC-FID analyses were performed using a CHROMPACK CP 9001 gas chromatograph equipped with flame ionization detector (FID) at 190 °C. The

injector temperature was 190 °C and the nitrogen carrier gas pressure was set to 80 kPa. Analyses were carried out in duplicate by injecting approximately 1 μ L liquid samples of gasoline onto a packed column (2 m x 3.2 mm x 2.1 mm I.D., 10 % Carbowax 20 M with 80/100). The column temperature was maintained at 50 °C for the first 3 minutes, then increased from 50 to 190 °C at 10 °C/min, and held at this end-temperature for 3 minutes.

INTRODUCTION

As a result of the prohibition of the use of organo-metallic compounds of lead in gasoline, oxygenated compounds were developed as alternative octane-enhancing additives. Examples of common alcohol and ether oxygenates used in this application include methanol, ethanol, isopropanol, TBA, methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME) and *tert*-amyl ethyl ether (TAEE). Fuel oxygenates are used as additives in gasoline to increase octane rating, enhance fuel combustion efficiency and produce cleaner-burning gasolines. As a consequence of their mass production and physico-chemical properties, particularly their high volatility and water solubility, TBA and MTBE have frequently been detected in seawater, ground and surface waters and in soils. The use of MTBE as a gasoline additive was eventually reduced due to environmental and health concerns. The unknown effects of TBA on human health, makes it important to control its use in gasoline.

The formulation of commercial gasoline is subjected to strict restrictions, particularly with respect to the maximum total content of benzene, olefins, aromatics, oxygenates, sulfur and other compounds. Aromatic compounds, particularly benzene, have been analyzed by GC, ¹H NMR, GC-MS, HPLC and FTIR techniques. TBA and MTBE oxygenates have been quantified by GC, ¹H NMR and FTIR techniques. This experiment uses CG-FID to quantify TBA in commercial gasoline and to verify compliance of samples with current legislation.

The GC-FID technique is a very common analytical technique, widely used in the petrochemical industry to quantify the amount of specific organic compounds in a particular sample. Through chromatographic analysis of gasoline, and standard solutions of gasoline with different TBA concentrations, it is possible to identify the TBA peak of the chromatogram (by its t_r) and quantify the content. An example of a gasoline chromatogram is included in Figure S1.

The standard addition method has been used to quantify TBA in gasoline. With this method, known volumes of TBA were added to all the commercial gasoline samples. The areas under the peaks of TBA in the chromatogram of the gasoline samples were evaluated using the software provided by a specialist company to control, acquire and store data from the instrument. An example of typical data collected from the chromatogram is presented in Table S1.

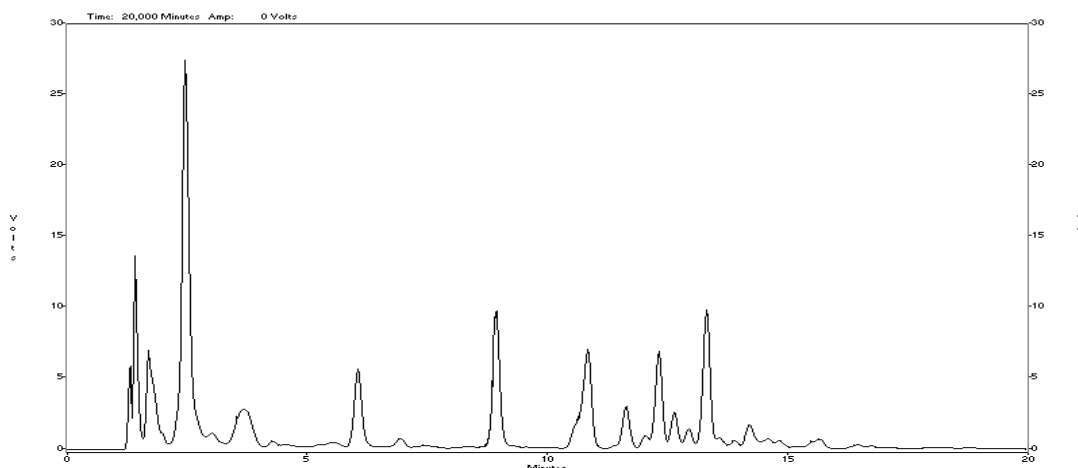


Figure S1. Chromatogram of a gasoline sample.

Table S1. Example database for data analysis of standard solutions. **Data are not real.**

A	B	C
TBA concentration added (mol/L)	TBA peak area (V/min)	TBA t_r (min)
0	200	3.1
1	300	3.2
2	400	3.1
3	500	3.2
4	600	3.2
5	700	3.1

The TBA peak area (A_{TBA} , column B) was plotted as a function of the concentration of TBA added ($C_{TBA \text{ added}}$, column A) to the gasoline samples, and the results are illustrated in figure S2.

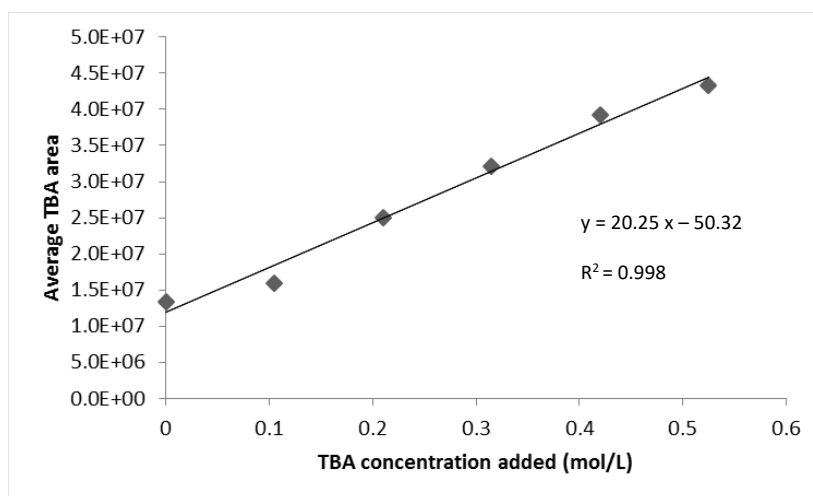


Figure S2. Standard addition plot obtained for the analysis of TBA in gasoline samples.

A linear relationship was obtained, according to equation 1, with slope m and intercept b :

$$A_{TBA} = m C_{TBA \text{ added}} + b \quad (\text{equation 1})$$

The original concentration of TBA in the gasoline samples was calculated by extrapolation of the standard addition plot (Figure S2) to $y = 0$ using equation 2:

$$C_{TBA \text{ sample}} = b / m \quad (\text{equation 2})$$

The conversion of concentration of TBA from mol/L to % (v/v) was performed using equation 3:

$$\% [TBA] = \frac{n * M}{\rho * 10^3} * 100$$

(equation 3)

where n (mol) are the moles of TBA obtained by the extrapolation, M (g/mol) is the molecular weight of TBA, ρ (g/cm³) is the density of TBA and 10^3 is the conversion factor.

EXPERIMENTAL PROCEDURE

Hazards:

TBA and gasoline samples are volatile and flammable liquids. All preparations should be performed in a fume hood and students should use protective gloves and lab-coats. All chemicals should be disposed of using an appropriate waste container.

Preparation of standards:

Six solutions, each with a total volume of 1 mL were prepared with each sample gasoline and TBA was added in different amounts using an auto-pipette ('spikes') (0, 10, 20, 30, 40 and 50 μ L), to each sample. The use of sealed vials with septa in the preparation of the standard solutions is essential to prevent evaporation, especially of the highly-volatile TBA component. It is also strongly recommended that each standard solution should be prepared immediately before the sample is injected into the GC.

GC-FID chromatogram of standards:

Before analyzing the samples, the GC-FID should be switched on and “purged” by heating the column oven to a high temperature, above 250 °C, for a period of at least 30 minutes to remove any residues left on the column by previous studies. Students should collect a background chromatogram to make sure that there are no remaining contaminants. The TBA standards should be injected using a 1 µL syringe.

After obtaining the chromatograms of the standards, the relevant peaks were identified, the retention time was recorded and the TBA peaks were analyzed, with baseline correction (using the same routines for all the samples). The area of TBA peak was determined using the GC-FID equipment software.

Each standard was analyzed once, with a single repetition, and the GC-FID column was purged before analyzing the next sample of gasoline. The data obtained was recorded using a worksheet as exemplified in Table S1.

Construction of the Standard Addition Calibration Curve:

After collecting the data from all the standards, the TBA peak area (column B) was plotted against the TBA concentration added (column A), using a x - y scatter plot. Appropriate software was used to obtain a linear trend line and record the equation of the line. This equation was then used to calculate the amount of TBA in each gasoline sample.

Analysis of Gasoline Samples:

All the gasoline samples should be injected into the GC-FID using the same conditions described in the analysis of the standards section. The TBA peak was identified using the retention time found in the analysis of the standards.

Calculation of the Percentage of TBA in Gasoline Samples:

The equation obtained from the analysis of the standard solutions of each gasoline sample was used to calculate the original concentration of TBA. The standard addition calibration curve should be extrapolated to $y=0$, for each gasoline sample, by using equation 2. The concentration units of TBA were converted from mol/L to % (v/v) using equation 3.

Waste:

All standards and samples must be eliminated using a waste bottle for disposal. The GC-FID syringe and needle should be washed with acetone, before subsequent use, and the washings should be eliminated to the solvent waste bottle.

Data Tables:**Standards (for one sample):**

TBA concentration added (mol/L)	TBA peak area (V/min)	Mean TBA peak area (V/min)	TBA t_R (min)

Standards (for all samples):

Mean TBA peak area (V/min)	Samples	TBA concentration added (mol/L)					
		0					
A1							
A2							
A3							
B1							
B2							
C3							
C3							

Gasoline samples:

Samples	Equation of calibration curve	Correlation coefficient of calibration curve	TBA concentration (mol/L)	% TBA (v/v)	Legislation % TBA limit (v/v)
A1					
A2					
A3					
B1					
B2					
C1					
C2					

INSTRUCTOR NOTES

Standards solutions of TBA/gasoline mixtures should be prepared immediately before injection into the GC-FID, due the volatility of the compound.

Gasoline samples should be collected in flasks completely filled to minimize air-space and sealed after filling. Samples should be cooled, transported to the laboratory and analyzed within 24 h without any pre-treatment.

TBA, acetone and gasoline are volatile, flammable, toxic and irritant liquids and should therefore be handled with suitable precautions. All transfers and standard preparations should be performed in a fume hood with personal protection equipment. Waste should be placed in an appropriate container for disposal.

This experiment provides students with an opportunity to practice their sample injection technique and to become familiar with the use of GC equipment and sample analysis. Although sample preparation is much simpler than many other GC experiments, students still need to prepare their laboratory activity with care, organize their injection sequence and deal with experimental data efficiently. In this respect the experiment is quite demanding.

In view of the increasing importance of chromatographic techniques in chemical analysis, practical experience with at least one of the instrumental methods of analysis should be provided, probably about the middle of the student's course. This experience is most useful after students have understood the basic concepts of Organic, Inorganic and Physical Chemistry and are able to appreciate the relevance of chromatographic separation and the nature of the processes involved.

BACKGROUND - OXYGENATES IN FUELS

Gasoline has fueled internal combustion motor transport and power generation for more than a century and during this extensive period of commercial exploitation various phases in the development of new fuel formulations have been registered.

Spark ignition internal combustion engines (SPICEs) fabricated at the beginning of the 20th century were unreliable and inefficient and manufacturers began to research ways to obtain reliable performance, power and economy. High compression designs were one of the strategies adopted, however the use of low octane fuel in these engines caused “knocking” or “pinking”. This potentially-damaging irregularity was caused by pockets of fuel-air mixtures formed in the low-grade fuel being ignited by compression, before the spark-ignited flame-front could consume the entire fuel charge. The most convenient response (at that time) was to add lead-based compounds, including tetra-ethyl and tetra-methyl lead, to the fuel formulation. While the resulting increase in fuel octane controlled pre-ignition, the consequences of lead residue emission were very harmful for the environment and human health. The effects of exhaust emissions were recognized and in 1975 catalytic converters were introduced to remove carbon monoxide, nitrogen oxides and unburned hydrocarbons from engine exhausts. As lead compounds poisoned these catalysts, gasoline formulation evolved to substitute organo-metalics with oxygen-containing additives that raised the fuel octane rating and lowered ozone and carbon monoxide levels, without damaging catalytic converters. Various ether-based oxygenates were developed including methyl-tertiary-butyl ether (MTBE), ethyl-tertiary butyl ether (ETBE), tertiary amyl methyl ether (TAME) and di-isopropyl ether (DIPE) and some alcohols such as methanol, ethanol and tertiary butyl alcohol (TBA), were added to gasoline formulations in many countries. Reformulated gasoline (RFG) was introduced by mandate of US Congress in the 1990 Clean Air Act. The RFG program was intended to provide a cleaner combustion, reducing smog-formation and the emission of toxic pollutants and required minimum fuel oxygen content of 2.1 percent by weight.

The benefits of using oxygenates in gasoline include increased octane rating, improved combustion efficiency and reduced emissions, partly through the substitution of aromatic components by less harmful oxygenates. From the late

70's to the early 2000's, oxygenates, particularly MTBE, were used in different proportions in many different geographical regions, with formulation adjustments to reduce volatile organic compound emission during summer and carbon monoxide emission during winter months. The concentrations of these oxygenates were typically up to 15% by volume (or 2 % by weight) in base gasoline formulations and about 11% by volume in RFG. At this stage MTBE was considered the refiner's oxygenate of choice and was present in about 90% of the USA gasoline supply. A period of increasing consumption of MTBE was brought to an abrupt end when reports of MTBE and TBA contamination of groundwater were confirmed in 1996 and MTBE was initially identified as a potential carcinogen.

The extensive use of MTBE in gasoline formulations led to widespread contamination of groundwater through spillage and leakage from underground storage facilities. In a large-scale survey, MTBE was found to be the second most detected volatile organic compound found in domestic and public water supplies. TBA is recognized as a contaminant of fuel-grade MTBE and in view of its high water solubility, low taste intensity and high odor threshold, low sorption on soil particles and resistance to air stripping, TBA is now recognized as an emerging contaminant that gives some cause for concern. The association of TBA with MTBE arises because of feedstock content (perhaps also of ETBE and industrial ethanol) but more importantly because TBA is a known product of environmental MTBE biodegradation.

In 1994, the US Environmental Protection Agency introduced a further rule (referred to as regulation 211, (b)) under the Clean Air Act (1). This addition to the Act required that further test data, regarding vapor emissions and their health and welfare effects, should be provided by fuel and additive manufacturers in order to maintain the registration of currently used additives, or to initiate new product registration. This amendment recognizes that the major source of human exposure to gasoline and oxygenates is in specific environments, for example, during vehicle refueling. Recent results of studies carried out on samples from gasoline and fuel oxygenated with various ether class additives, demonstrated that liberated vapor test materials caused minimal impact on reproductive parameters (2), cytogenetic activity (3), developmental toxicity (4), immunotoxicity (5) and endocrine disruption (6),

suggesting that initial concerns regarding the potential impact of fuels and oxygenates on human health are largely unfounded.

The results reported in a review of a large number of gasoline plumes in groundwater (7) confirm that benzene, MTBE and TBA show similar length and stability behavior. Quantitative estimation of human risk at low exposure from drinking water (8) has led to the conclusion that it is only necessary to emit advisories when drinking water content exceeds the 20 – 40 $\mu\text{g.L}^{-1}$ level. Recommendations are that this odour threshold level should be established as a practical Public Health Goal. TBA is a recognized MTBE metabolite often found in blood and urine of residents near contaminated sites. TBA concentrations in gasoline are lower than those of MTBE and therefore the advisory limits established for these substances in water may also be considered appropriate for blood and urine levels.

The global situation (9) regarding oxygenates is that four different oxygen-containing components are currently used in gasoline formulation: ethanol, MTBE, methanol and ETBE. In terms of volume, ethanol is the most popular choice and is used in most countries, to some extent. Ethanol is not an ideal oxygenate choice. Gasoline ethanol formulations are expensive and the presence of ethanol leads to higher toluene-xylene (TX) volatilization loss (10) causing an increase in ozone and smog formation (11). MTBE was the most common commercial oxygenate from the late 1970's until about 2006. MTBE is still produced in the United States, but the majority of the production volume is exported. This oxygenate is still used in Latin America, Europe, the Middle East, Africa and Asia. Methanol, produced from coal, has been adopted as the gasoline oxygenate of choice in China, motivated primarily by economic factors. The evolution of gasoline formulation over the last century provides an excellent example of how chemists have collaborated with engineers and environmental scientists to maintain technical performance, adjust to new operating characteristics (octane), develop alternative active components (oxygenates), substitute aggressive reagents (TEL and TML) and to minimize environmental damage. The development of gasoline and other fuels is by no means finalized. Given the improvement in our understanding of the complex interactions with the atmosphere and aqueous environment, it is inevitable that fuel compositions continue to evolve to meet future challenges.

IN-LABORATORY QUESTIONS

1. Compare the TBA content in each gasoline sample with the current legislation and state your conclusions regarding the compliance of the gasoline with the established legal limits.
2. Identify the most significant sources of error in the experimental technique.

POST-LABORATORY QUESTIONS

1. The use of GC-FID equipment, or indeed chromatographs with detectors that work on other principles, is very common in the analysis of a wide variety of substances. Search the literature to find examples of applications with thermal conductivity (TCD) and electron capture (ECD) detectors. Identify, and describe briefly, **one** example for each detector.
2. Currently the combination of GC with mass spectroscopy in GC-MS instruments is becoming more common in analytical laboratories. Why is GC-MS becoming the technique of choice for many samples? Identify the advantages of GC-MS over “traditional” GC analysis using the detectors identified in the previous question.
3. Detection of eluted components is certainly important, but separation of sample components by the chromatographic process is clearly fundamental for a successful analysis. Identify the parameters that may be adjusted to obtain the best possible resolution of components and the shortest analytical process time.
4. Oxygenates for inclusion in gasoline formulations have been proposed as part of a multi-faceted solution for environmental contamination by organometallic lead compounds and as a means of improving the energy efficiency and reducing emissions of IC motors, however the detection of significant levels of certain oxygenates in groundwater has alerted the scientific community to the hazards associated with spillage and leaks from storage containers. Why is MTBE a compound of particular concern?
5. A surprising conclusion that studies of spillage sites have reached is that in spite of the greater concentration of MTBE in gasoline, TBA has been found in higher concentration in groundwater and well-water. Identify three possible explanations for this observation.
6. In this experiment, the standard addition method is applied to the analysis of a volatile component of gasoline fuel samples. This analytical method assumes that the spike reference component does not suffer significant alteration under the injector and column conditions. What would be the consequence of partial degradation of this component? How might the stability of the compound be confirmed?

Bibliography

1. Clean Air Act, 42 USC § 7401 (2012); § 7545 (2013).
2. Gray, T.M., Steup, D., Roberts, L.G., G.W., Hoffman, G.M., Schreiner, C.A., Clark, C.R. Health assessment of gasoline and fuel oxygenates vapors: Reproductive toxicity assessment, *Regul. Toxicol. Pharmacol.* 70 (2S), (2014) S48-S57.
3. Roberts, L.G., Gray, T.M., Tyl, R.W., Trimmer, G.W., Parker, R., Murray, F.J., Schreiner, C.A., Clark, C.R., Clark, C.R., Health assessment of gasoline and fuel oxygenates vapors: developmental toxicity in mice, *Regul. Toxicol. Pharmacol.* 70 (2S), S58-S68.
4. Roberts, L.G., Gray, T.M., Marr, M.C., Tyl, R.W., Trimmer, G.W., Hoffman, G.M., Murray, F.J., Schreiner, C.A., Health assessment of gasoline and fuel oxygenates vapors: developmental toxicity in mice, *Regul. Toxicol. Pharmacol.* 70 (2S), S69-S79.
5. White Jr, K.L., Peachee, V.L., Armstrong, S.R., Twerdok, L.E., Clark, C.R., Schreiner, C.A. Health assessment of gasoline and fuel oxygenates vapors: Immunotoxicity evaluation, *Regul. Toxicol. Pharmacol.* 70 (2S), S43-S47.
6. Wallace, G. "Producers confident that MTBE is not an endocrine disruptor", Press Release, European Fuel Oxygenates Association (2014), <https://www.petrochemistry.eu/mediaroom/producers-confident-that-mtbe-is-not-an-endocrine-disruptor/>
7. Connor, J.A., Kamath, R., Walker, K.L., McHugh, T.E. Review of Quantitative Surveys of the length and stability of MTBE, TBA, and Benzene plumes in groundwater at UST sites, *Groundwater* 53, (2015) 195-206.
8. Health Canada (2006) Guidelines for Canadian drinking water quality: Guideline Technical Document - Methyl Tertiary butyl ether (MTBE). Water Quality and Health Bureau, HealthyEnvironments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.
9. Cagliari, J., Fedrizzi, F., Finotti, A.R., Teixeira C.E. Volatilization of monoaromatic compounds (benzene, toluene and xylenes (BTX)) from gasoline: effect of ethanol", *Environmental Toxicology and Chemistry*, 29 (2010) 808-812.
10. Cardona, C.A., Sanchez, O.J., Gutiérrez, L.F. Process Synthesis for Fuel Ethanol Production, CRC Press (2010), Boca Raton, USA.
11. IHS Markit report. Gasoline octane improvers / Oxygenates, August 2017. <https://ihsmarket.com/products/gasoline-octane-improvers-chemical-economics-handbook.html>.