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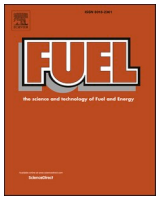

Full Length Article

A methodology to determine additive markers in fuel ethanol using gas chromatography-mass spectrometry

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A B S T R A C T

Ethanol has been increasingly utilized on a large scale as a biofuel. In Brazil, hydrated fuel ethanol is available in two types: regular (EHC) and a version known as additive ethanol (EHA), which contains multifunctional chemical compounds for enhanced performance. Each brand or distributor may select various additive packages for additive options. The detection of additives in fuels, such as gasoline and diesel, is widely discussed in scientific literature; however, methodologies capable of assessing the presence of these additives and correlating them with their origin are lacking. Thus, this study developed a new methodology to distinguish between EHA and EHC, and to identify fuel additives characteristic of the major brands available in the Brazilian market, using gas chromatography coupled with mass spectrometry (GC–MS) and a multivariate analysis-based approach. Overall, 198 commercial fuel ethanol samples were analyzed, 98 EHC and 99 EHA. A regression model built using 17 chemical compounds presented an accuracy of 97 %. Oleic acid, 2-ethylhexanol, and 1,4-diethylbenzene were identified as the main chemical markers. In summary, this study presents a rapid, robust, and highly accurate method for distinguishing between additive and regular hydrated fuel ethanol, representing a breakthrough in biofuel quality assurance. This advancement contributes significantly to ensuring the quality of biofuels, with direct impacts on environmental safety, preventing economic losses, and strengthening oversight and regulation in the sector.

1. Introduction

Fuel ethanol, or bioethanol, is a product of the fermentation of biomass, mainly sugar cane, corn and sugar beet [1], among other sources [2], and was first tested as a fuel in an internal combustion engine in 1826. Its combustion results in lower net CO_2 emissions and is an alternative to the use of fossil fuels. However, despite its use being in line with current environmental requirements, it has specific limitations, such as difficulties in starting and driving at low temperatures, requiring new engine technologies to overcome these obstacles [3–5].

Despite its limitations, the demand for bioethanol is on the rise, and it is currently the most utilized biofuel globally, accounting for 74 % of the total volumetric production in this industry [6]. In 2023, global ethanol production reached 29.5 billion gallons, with the United States and Brazil emerging as the predominant producers, contributing 53 % and 28 % to the global output, respectively. [7].

The use of bioethanol, like its production, is diverse and dependent on the locality and maturity of policies developed in each region. In the United States, commercially available blends are 10 % (E10), 15 % (E15), and 85 % (v/v) (E85) ethanol in gasoline [8]. In India, the current ethanol concentration in gasoline is 5 % (v/v) (E5), but the implementation of a 20 % (v/v) (E20) blend is planned for 2025. In China, the use of ethanol is not yet consolidated, with only some provinces utilizing a 5 % (v/v) ethanol blend in gasoline [9–12]. The use of gasoline blends, as seen in these countries, which incorporate other compounds such as ethanol and other oxygenates, can result in higher octane ratings, improved engine efficiency, and the production of a higher-quality fuel [11–16].

In Brazil, the Proálcool, 1975, a program to encourage the use of fuel ethanol, was responsible for increasing the production and use of fuel ethanol, making it competitive, and for reducing the local market's

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Nomenclature

EHC	Hydrated ethanol fuel
EHA	Additive ethanol fuel
CO_2	Carbon dioxide
GC–MS	Gas chromatography coupled to mass spectrometry
Proálcool	National Alcohol Program
RenovaBio	National biofuels policy
EHCP	Hydrous ethanol premium fuel
ANP	Brazilian National Agency of Petroleum, Natural Gas, and Biofuels
VOCs	Volatile organic compounds
PMQC-GO/DF	Fuel Quality Monitoring Program of Goiás State and Federal District
PODE1	Polyoxymethylene dimethyl ether
ETBE	Ethyl *tert*-butyl ether
DME	Dimethyl ether
DiEGME	Diethylene glycol monomethyl ether, DiEGME
HPLC	High-performance liquid chromatography
QC	Quality control
GCMS-QP	Gas chromatography coupled to quadrupole mass spectrometry
AOC	Automatic liquid sampler
EI	Electron impact ionization
NIST	National Institute of Standards and Technology
PCA	Principal component analysis
PLS-DA	Partial least squares discriminant analysis
VIPScores	Variable importance projection
MLR	Multiple linear regression

dependence on imported petroleum derivatives. [17,18]. In addition to Proálcool, the National Biofuels Policy (RenovaBio) was established in 2017 aiming to promote the expansion of biofuels in the local energy matrix, emphasizing the regularity of fuel supply and ensuring predictability for the fuel market. RenovaBio also seeks to induce gains in energy efficiency and to reduce greenhouse gas emissions in production, commercialization, and in the use of biofuels [19].

Last year, Brazilian Law 14.993 established an increase up to 35 % (v/v) of anhydrous ethanol in gasoline (currently at 27 %), provided its technical feasibility is confirmed [20].

In Brazil, fuel ethanol is commercialized as a) anhydrous ethanol, used as an additive in type A gasoline at a concentration of 27 % (v/v), which is called type C gasoline; and b) as a biofuel, named hydrated fuel ethanol, with ethanol content varying from 92.5 to 95.4 % (v/v) for common (EHC) and 95.5 to 97.7 % (v/v) for premium (EHCP) [21,22]. Beyond its standard form, hydrated ethanol can be sold at a premium price when enhanced with an additive package. This specialized variant is offered in the local market under the designation of additive-enhanced hydrated fuel ethanol (EHA).

According to the Brazilian National Agency of Petroleum, Natural Gas, and Biofuels (ANP), additives for automotive fuels are products composed of one or more components that confer additional benefits to the fuel [23]. Various compounds, such as oxygenated ethers, long-chain alcohols, and metallic oxides, are the focus of studies aimed at evaluating their functionalities when added to fuel. One example is the use of oxygenated ethers like polyoxymethylene dimethyl ether (PODE1), a compound that, when added in concentrations of up to 10 % in E10 gasoline/ethanol blends, reduces soot emissions and improves internal combustion [24,25].

Other ethers, such as ethyl *tert*-butyl ether (ETBE) and dimethyl ether (DME), due to their lower density and viscosity compared to biofuels, are used as additives to enhance the air–fuel mixture and reduce pollutant emissions [26]. More recent studies have explored the use of alcohols like n-pentanol as potential fuel additives. Because of properties such as lower polarity, anti-corrosive effects and good stability when mixed with diesel, for example, this long-chain alcohol has stood out as a promising additive [27]. In the aviation fuel field, property monitoring is rigorous, mainly due to the environmental conditions to which the fuel tank is exposed.

Therefore, maintaining characteristics such as freezing point, free water content, and oxidative stability, among others, is crucial for optimal engine performance. To address this, freezing point inhibitors like diethylene glycol monomethyl ether (DiEGME) and antioxidants are added to the fuel composition [28].

Confirming the inclusion of additives is advantageous for both producers and consumers. Verifying the use of additives serves as a tool for traceability and quality control for producers. For consumers, it ensures that the additive-enhanced product, often sold at higher prices, indeed contains the expected additives. Furthermore, identifying the chemical species present in commercials fuel additive packages is crucial for accurately estimating the environmental and social impacts associated with the use of this fuel.

In the literature, approaches are available for the discrimination and identification fuel additives applied to gasoline, which commonly involve well-established methods, such as ^{1}H NMR [29], IR Spectroscopy [30,31] and chromatography and thermogravimetry [32]. In addition to these, gas chromatography coupled with mass spectrometry (GC–MS) is another commonly used technique for determining additives present in diesel oil [33] and jet fuels [34].

Despite their importance, in Brazil, fuel additives are not subject to local registration requirements for commercialization, nor is there certification to confirm the presence of additives, details regarding the specific molecules used, or identification of fuels with additives, such as the EHA group. In ethanol fuels, EHA samples are commonly found in green and colorless varieties, but may assume various colors, except for orange and blue.

Although various approaches for additive characterization are well established for many types of fuels, there are still no analytical methodologies in the literature specifically adapted for fuel ethanol, such as that sold in Brazil. Furthermore, confirming the presence of additives is challenging, as their composition can vary according to each EHA brand and is subject to industrial confidentiality.

Thus, this study developed a new gas chromatography coupled with mass spectrometry (GC–MS) and multivariate-based method to identify the organic compounds present in hydrated fuel ethanol. In addition, our work determined which compounds discriminated between EHC and EHA and characterized the chemical markers/volatile organic compounds (VOCs) for the leading EHA brands within the biofuels market.

2. Materials and methods

2.1. Sample collection and preparation

All samples were from ANP's New Fuel Quality Monitoring Program of Goiás State and Federal District (New PMQC-GO/DF), which collects samples from various fuel retail stations. Each sample was stored in 1 L polyethylene containers. EHA samples were collected from October 2023 to January 2024, while EHC samples were randomly selected within the same period.

New PMQC-GO/DF approved all samples utilized in this study, ensuring quality control for chromatography analysis as transparent, homogeneous, and without particulate material [35].

A total of 197 samples of fuel ethanol were collected, comprising 98 common samples (EHC) and 99 additive-treated samples (EHA). Within

the EHA group, 60 samples originate from distributors designated here as "V" (EHA-V), 27 from distributor "S" (EHA-S), 8 from distributor "I" (EHA-I), and 5 from other distributors "U" (EHA-U).

In addition to the fuel ethanol commercial samples, BrQuímica© supplied a package of additives (here referred to as additive "A"), both with and without a green dye. To investigate the influence of this dye, commonly added to differentiate EHA samples, two samples of HPLC-grade ethanol (Honeywell®, Charlotte, North Carolina, USA; purity equal to or greater than 99.9 %) were prepared and doped with additive "A" 0.05 % (v/v), with and without the dye, as used by some fuel distributors.

For monitoring injections and normalization of peak areas, 1-dodecanol was chosen as an internal standard because this molecule exhibits good solubility in ethanol, offers a low-cost commercial internal standard, and does not elute alongside other peaks. Thus, 12.5 $mL.L^{-1}$ solution of 1-dodecanol (Sigma Aldrich, St. Louis, MO, USA; analytical grade) in HPLC-grade ethanol (Honeywell®, Charlotte, North Carolina, USA; purity equal to or greater than 99.9 %) was used as an internal standard.

The collected samples were stored in 100 mL amber high-density polyethylene containers at room temperature (25 °C). Subsequently, 1.4 mL of each sample and 100 μL of internal standard were transferred into 2.0 mL vials for chromatographic analysis.

A pooled quality control (QC) sample was prepared in a 100 mL high-density polyethylene container to evaluate instrumental precision. This pooled QC contained 0.4 mL from each of the 197 samples, a total volume of 78.8 mL.

The QC sample was distributed into 10 glass vials of 2.0 mL each, with 1.4 mL of the QC sample and 100 μL of the 1-dodecanol internal standard added per vial. HPLC-grade ethanol (Honeywell®, Charlotte, North Carolina, USA; purity $\geq$ 99.9 %) was used as the chromatographic blank. Sample analyses were performed in random order, while QCs and blanks were analyzed at the beginning and end of batches of 20 samples.

2.2. Chromatographic analysis

The instrumental analytical technique employed to analyze fuel ethanol samples was gas chromatography coupled with mass spectrometry (GC–MS). The equipment used for these analyses was the Shimadzu GCMS-QP2010 chromatograph, equipped with a Shimadzu AOC-5000 automatic sampler (Shimadzu, Kyoto, Japan) featuring a liquid injection accessory with a 10 μL syringe. Data acquisition was performed using LABSolutions GCMS software, version 4.50 SP1 (Shimadzu, Kyoto, Japan).

Samples of 2 μL were injected in split mode with a split ratio of 1:100. Ultra-pure helium 5.0 (99.999 % – White Martins, Goiânia, Goiás, Brazil) was used as the carrier gas at a flow rate of 1.30 $mL.min^{-1}$ and a linear velocity of 45.0 $cm{\cdot}s^{-1}$. Chromatographic separation of the compounds present in the fuel ethanol samples was carried out using an Agilent DuraBond DB-5MS column (30 m x 0.25 mm x 0.25 μm) with a stationary phase of 5 %-phenyl-methylpolysiloxane (Agilent Technologies, California, USA). The initial oven temperature was set at 40 °C for 3 min, increasing to 110 °C at a rate of 20 $°C.min^{-1}$, followed by an increase to 320 °C at a rate of 15 $°C.min^{-1}$, with isothermal heating for 3 min, totaling 23.50 min of chromatographic runtime.

Mass spectra were acquired in full-scan mode over the m/z range of 35–500, with a solvent cutoff time of 1.6 min, a scan speed of 1666 $u.s^{-1}$, and a scan cycle time of 0.3 s. The acquisition was executed using electron impact ionization (EI) at 70 eV.

The compounds in the analyzed samples were identified by comparing the acquired spectrum with those in the NIST17 Standard Spectral Libraries (National Institute of Standards and Technology, Gaithersburg, USA).

2.3. Data processing

Chromatogram files were converted from.qgb to.mzDATA using LABSolutions GCMS software version 4.50 SP1 (Shimadzu, Kyoto, Japan). Data processing was performed using MZmine version 3.9.0 [28], applying the parameters described in the Materials and Methods section of the Supplementary Material. After data processing, a feature list was generated, where variables represent both the mass-to-charge ratio and the retention time, and observations represent its feature intensities by sample. Feature intensities normalized to the intensity of the internal standard are referred to as *relative intensities*.

Statistical analyses were performed using R software version 4.4.0 within the RStudio free environment version 2024.04.2 Build 764 (Posit Software, Boston, Massachusetts, USA) and packages plotly version 4.10.4 [36], tidymodels version 1.2.0 [37] and mixOmics version 6.30.0 [38]. Feature relative intensities were autoscaled prior to both principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Model performances were evaluated based on the accuracies obtained through 100-fold cross-validations. The parameter used to assess the model accuracy was the confusion matrix. Based on the values of true positive, true negative, false positive, and false negative, the measures of accuracy, sensitivity, and specificity of the models were calculated. In addition, the most important features for discrimination between EHA and EHC were selected according to their VIPScores (Variable Importance Projection) [39] and using features and samples heatmap correlation from the EHA group.

The presence checking of the identified organic compounds in patents was conducted using the Web of Science (all databases) [40] and PatentScope platforms [41], with the available scope up to June 3, 2024. Multiple linear regression (MLR) was applied along with the evaluation of accuracy, sensitivity, and specificity to assess class discrimination performance of the selected VOCs.

3. Results and discussion

3.1. Additive and quality control samples

The study of techniques for distinguishing between EHC and EHA has not yet been reported in technical-scientific literature. The development of analytical methodologies capable of identifying fuel additives is crucial for ensuring quality control and market reliability for fuel retailers and consumers.

Chromatograms of both "A"-doped samples, Fig. 1S, having and not having a green dye, presented the same profiles indicating that the evaluated dye does not present any peak in the chromatographic analysis. In the overall, 65 compound annotations were identified from the organic classes of alcohols, acetates, esters, and both linear, branched, and aromatic hydrocarbons (Table S1).

Despite the chemical diversity of this additive package, not all compounds presented in Table S1 are responsible for optimizing fuel performance. Long-chain alcohols (e.g., 2-methyl-1-propanol and 3-methyl-1-butanol) and Aromatic hydrocarbons (e.g., 1,4-diethylbenzene and o-Cymene) serve multiple functions, while long-chain acid esters contribute to anticorrosive and improve lubricity properties (e.g., methyl ester of hexadecenoic acid) [42–44].

For instance, solvents (e.g. heptane and undecane) and fuel markers may be part of the package composition, however, they do not necessarily possess properties that enhance fuel quality and are commonly added to commercial samples of liquid fuels. Another example includes dyes and miscibility improvers, which can be used in additive packages and be considered fuel additives [33,35].

The verification of chromatographic precision during the analysis of fuel ethanol samples was conducted by evaluating the variation in peak areas in the QC samples (Fig. 2S). The QC chromatograms exhibit consistent patterns, demonstrating high reproducibility in chromatography analysis. In addition to the qualitative evaluation, coefficient of

variations for each peak area in the QC samples were calculated. Among the obtained values (Table 2 of the supplementary material – Table S2), 93.54 % showed a relative standard deviation below 30 %, indicating good chromatographic precision.

3.2. EHC and EHA samples

After the chromatographic analysis of 197 fuel ethanol samples (EHC and EHA), 62 features were annotated. The number of compounds in the EHA group ranged from 6 to 42, while in the EHC group, it ranged from 3 to 28. A Venn diagram (Fig. 1) was constructed to evaluate the distribution of compounds according to class and fuel brand suppliers. Annotations common to both groups accounted for 67.2 %, while 32.8 % were found exclusively in the EHA group, and none were exclusive to the EHC group (Fig. 2-A). Table 3S presents the occurrence of each compound and its relative frequency. The chemical composition of hydrated fuel ethanol is a result of many factors, such as fermentation by products, contamination during the production and transportation chain, markers, and additives [45]. However, the chemical variability of compounds in the EHA group suggests that these compounds may result from the additive process.

According to Fig. 1-B, 45.9 % of the samples belong to both the EHA-S and EHA-V brands since 86.9 % of the additive-enhanced samples are associated with these brands. On the other hand, no compound was found to be exclusively present in the EHA-I group nor the additive-enhanced samples from smaller brands (EHA-U).

Among the compounds found exclusively in the EHA-V and EHA-S brands 15 out of 28 VOCs exhibit a high relative frequency in the EHA-S brand, specifically VOC28 to VOC43, except for VOC34 (Table 3S). This result suggests that these compounds might be included in the additive composition of this brand.

The score plot for the first two principal components, Figs. 2 and 3, explained almost 50 % of the total data variance. EHC samples showed a high group similarity when compared to the EHA samples. This result indicates the use of different additives which consequently leads to more significant chemical variability in the EHA group as it can be seen in Fig. 2.

Upon analyzing Fig. 3, it is evident that a distinct group is formed concerning the samples EHA-V, EHA-I, and EHA-U, as well as the EHC group, which is predominantly composed of EHA-S samples, along with 6 EHA-V samples and one EHA-U sample. This behavior can be explained when analyzing the chromatograms of EHA samples, which reveal a unique peak pattern between 7 and 10 min (Fig. 4) exclusively to this group.

Although the VOCs from this region appear in 6 EHA-V samples, they cannot be used as markers of additives for this brand, as they are only present in 10 % of the samples in this group. On the other hand, this region is found in all the EHA-S samples, indicating that these VOCs can be used as marker additives for these samples. Furthermore, this peak pattern can also be identified in the additive package "A" (Fig. 1S).

3.3. Additive markers

Despite the identification of potential markers for the additive-enhanced samples in the EHA-S group, determining VOCs that represent other brands and, consequently, serve as additive markers for all EHA samples is challenging. Therefore, a multivariate classification model using PLS-DA was employed to identify compounds responsible for distinguishing between EHA and EHC samples.

Model accuracy of 96.86 % was obtained after cross-validation and a standard deviation of 0.37 % using four latent variables. The confusion matrix of PLS-DA cross-validation is presented in Table 4S. The high accuracy demonstrates that the proposed model exhibited robust classification capability for the EHA and EHC groups.

Given the robustness of the model, variables responsible for class discrimination were selected based on VIPScores $\geq$ 1.1. The selected VOC, along with their interaction with the EHA suppliers and their interaction with the EHC group, can be visualized in Fig. 5 using a heatmap based on scaled sample values.

Therefore, when observing the correlation of the selected compounds with each class, it is noted that 17 out of the 18 selected VOCs exhibit a high correlation (>1.0) with the EHA class and 1 VOC with the EHC group. The additive markers determination should consider only compounds that are predominantly correlated with the additive group, considering that an EHA has the same composition as the EHC group in addition to the additive package composition. This way, considering VOC10 correlation with the EHC class, this feature was excluded as a potential additive marker.

Table 1 presents selected VOCs, their identification, VIPScores, presence in additive package patents, potential functions reported in

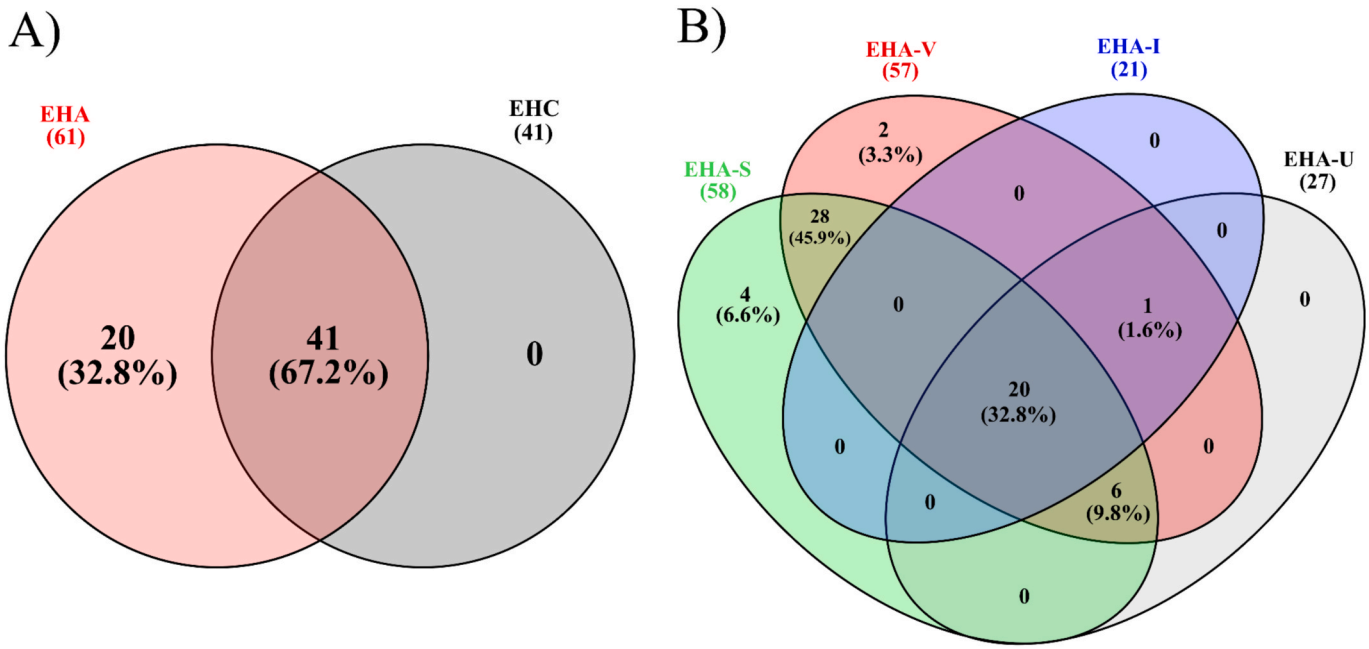

Fig. 1. Venn Diagram of VOCs for A) EHA and EHC groups; B) EHA group categorized by brands EHA-S, EHA-V, EHA-I, and EHA-U.

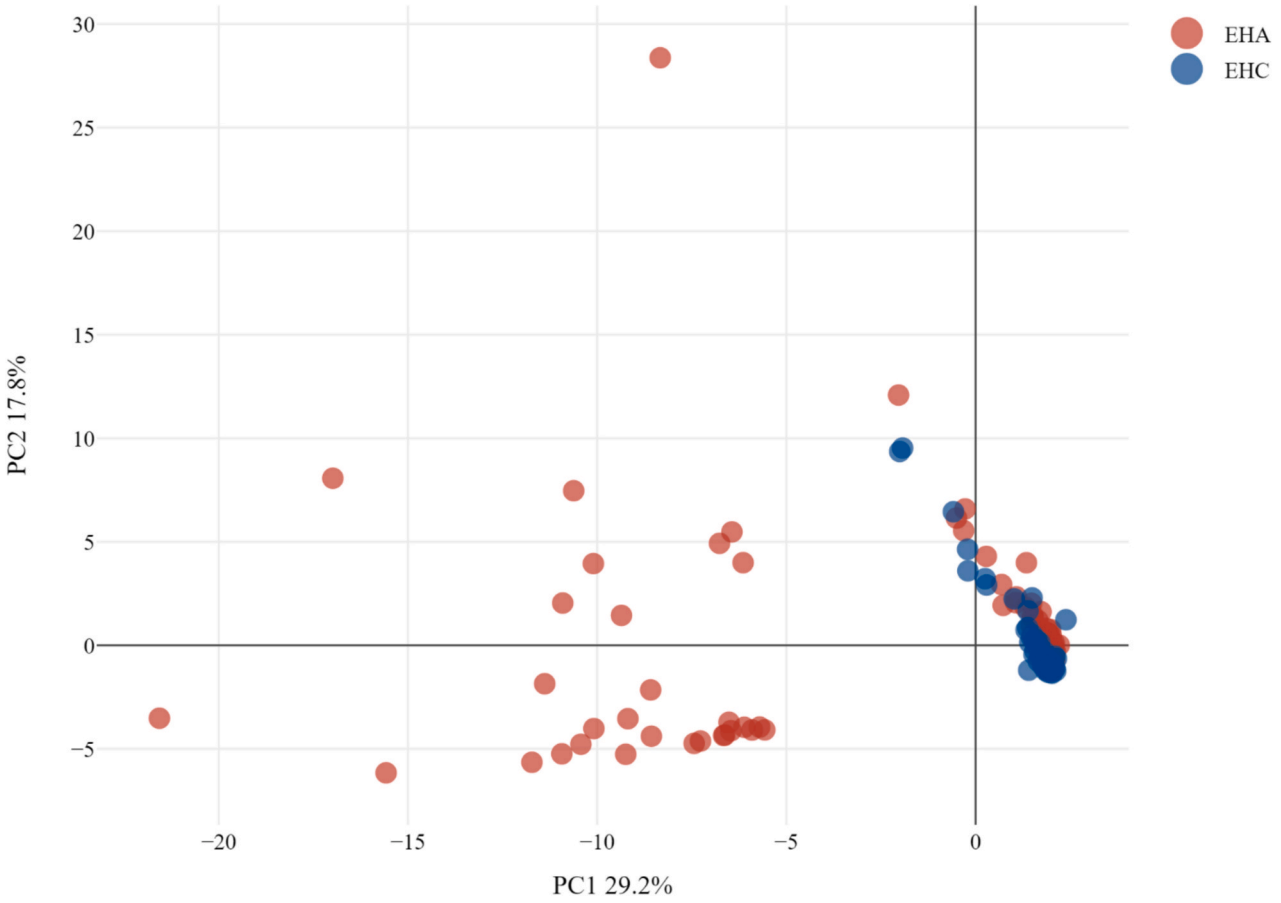

Fig. 2. Scores plot of PC1 vs. PC2 for EHC and EHA samples.

other fuels and correlation with distributor brands.

Of the 17 potential additive markers, Undecane (VOC34) and Naphthalene (VOC44) were not found in additive package patents for other fuels. However, these compounds may be part of the composition of exclusive fuel additives ethanol packages. Naphthalene, for instance, is an aromatic compound that, when added in small concentrations, may improve the fuel's lubricating properties [36].

The list of compounds identified in fuel additives patents consist of 3 branched alcohols (2-ethyl-1-hexanol, 3-methyl-1-butanol, 2-methyl-1-butanol), 1 ether (1,1-diethoxy-ethane), 1 long-chain carboxylic acid (oleic acid), and 12 aromatic hydrocarbons in their composition (naphthalene, 1-ethenyl-4-ethylbenzene, 4-ethenyl-1,2-dimethylbenzene, 1,2,3,4-tetramethylbenzene, 1,2,3,5-tetramethylbenzene, 1,2,4,5-tetramethylbenzene, 1,4-diethylbenzene, 2-ethyl-1,3-dimethylbenzene, 1-methyl-4-propylbenzene, 1-ethyl-2,4-dimethylbenzene, 2-ethyl-1,4-dimethylbenzene, 1-methyl-2-(1-methylethyl)benzene). The role of both branched alcohol and aromatic hydrocarbons may be related to improving the lubricating properties of fuel ethanol [43]. In addition to lubricants, light aromatics might act as antiknock agents in low-octane fuels, which are relevant for medium distillate fuels but do not apply to fuel ethanol due to its high-octane values.

Furthermore, long-chain carboxylic acids such as oleic acid (VOC59) can be used as lubricants and anticorrosive additives [43]. These additives are essential for improving the quality of commercial bioethanol, as the long-term use of alcohol-based fuels may lead to lubrication and corrosion issues [43,45,55] justifying the use of such additives in fuel ethanol.

The choice of oleic acid as an additive for ethanol is based on its good solubility in this fuel when compared to the ester of this fatty acid. Additionally, it is highly abundant in nature, making its use economically viable, and offering better oxidative stability than other fatty acids as it has only one unsaturation in its carbon chain.

The correlation between organic compounds and the leading brands of additive fuel on the market is a valuable parameter for quality control. This correlation enables, checking the presence of additives, making it possible to suppose the likely origin of the additive fuel.

According to Table 1 data and Fig. 5, VOC25, VOC34, VOC44, VOC11, and VOC13 presents positive correlation with the EHA-V class. The VOC identified as light aromatic hydrocarbons (VOC40, VOC41, VOC42, VOC37, VOC38, VOC29, VOC33, VOC28, VOC32, VOC31, and VOC36) also showed positive correlation exclusively with the EHA-S class. Oleic acid (VOC59), 2-methyl-1-butanol (VOC11), and 2-methyl-butanol (VOC13) presented a positive correlation with the EHA-I samples. For the EHA-U samples, the VOCs that were positively correlated were VOC25, VOC59, VOC34, VOC44, and VOC11.

As a result, a correlation was found for more than one additive marker for each brand within the additive class.

To create a marker for each main EHA suppliers, one additive marker was chosen for each brand. The selection of markers for the EHA-V and EHA-I classes was based on the compounds with the highest VIPScores: VOC25 for the EHA-V class and VOC59 for the EHA-I class.

For the EHA-S brand, 11 out of 17 VOCs chosen presents positive correlation with this brand. The retention times (Table 1) of the VOCs correlated with this brand reveals that they fall between 7.69 min and 8.72 min, which aligns with the compounds found in the characteristic region of the EHA-S brand (Fig. 1). Furthermore, the VIPScores for the VOCs selected for this brand are the lowest among those identified, with VOC40 showing the highest value (1.22) and VOC36 the lowest (1.11).

The selection of VOC marker for EHA-S was based on the peak having the highest intensity in the region exclusively present in samples of this brand (Fig. 5). Consequently, 1,4-diethylbenzene (VOC29) was chosen as the additive marker for the EHA-S samples. For the EHA-U samples,

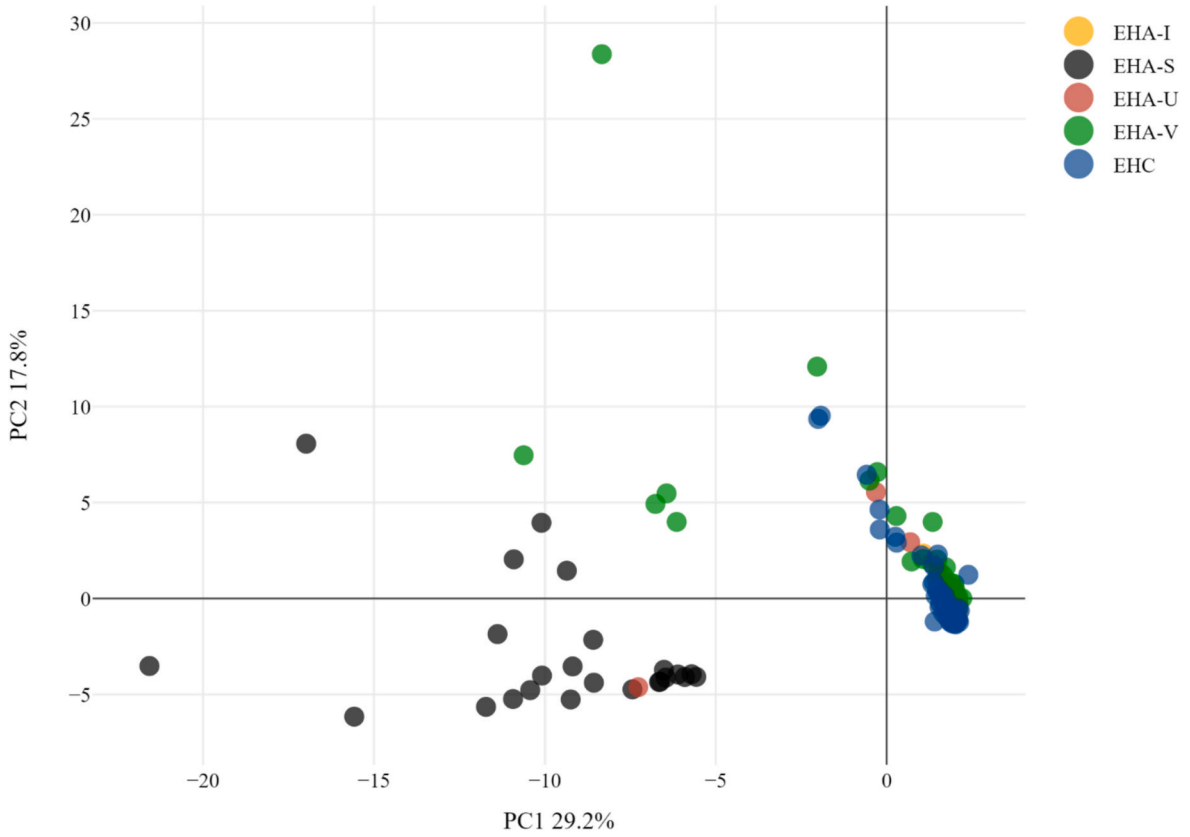

Fig. 3. Scores plot of PC1 vs. PC2 for EHC and EHA samples, showing EHA brands.

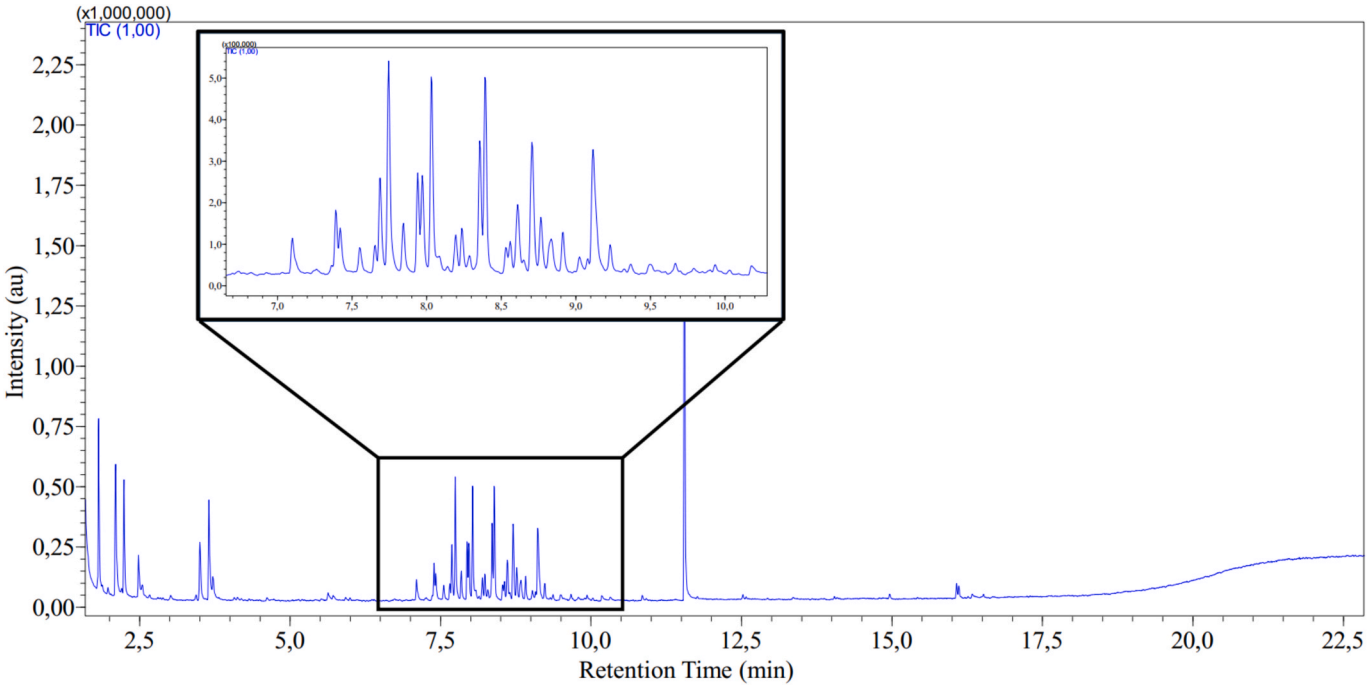

Fig. 4. Total ion chromatography (TIC) of HPLC grade ethanol sample with the additive package "A" at a concentration of 0.05% (v/v), highlighting the region between 7 and 10 min.

no additive marker was determined, as these represent various smaller brands, meaning there is no additive pattern that would justify identifying such a specific marker.

A multiple linear regression, MLR, model was employed to evaluate the discrimination performance of the three selected markers for EHA-V, EHA-S, and EHA-I brands in differentiating the EHA and EHC classes. The performance of the regression model and the confusion matrix for the sample predictions are provided in Table 2.

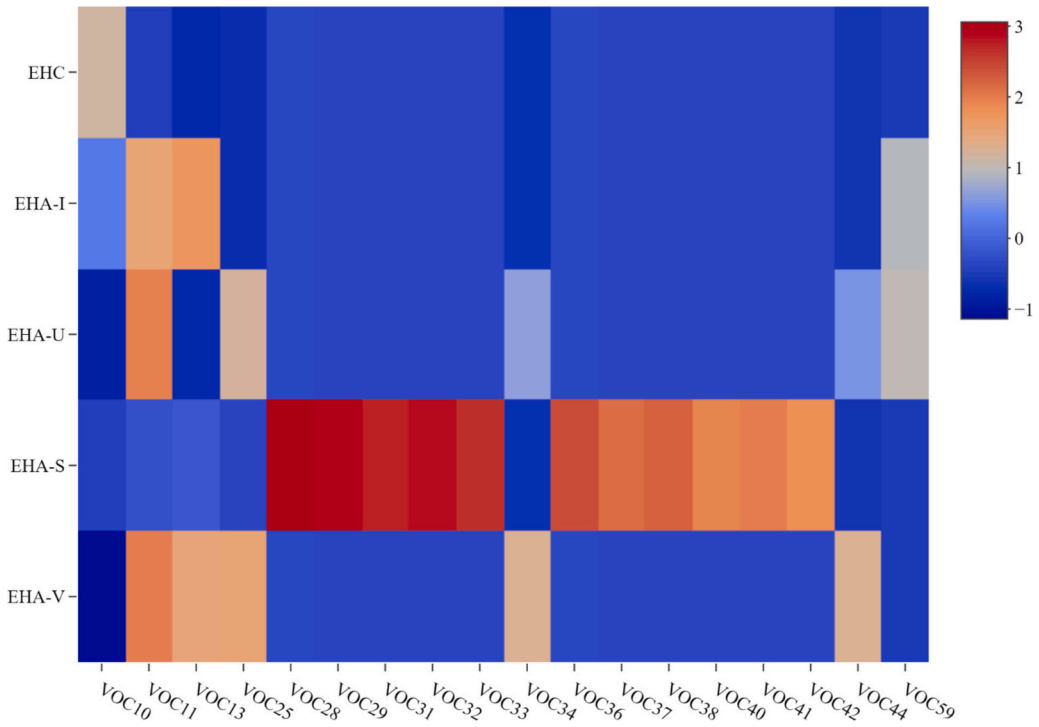

Fig. 5. Heatmap for selected VOCs and ethanol fuels classes: a) red color refers to high (>1) correlation and b) blue color refers to low (<1) correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
List of selected potential additive markers, including identification, VIPScore, patent presence, reported functions, brand correlation, and references.

ID	Compound[a]	VIP	RT	*m/z*	Present in fuel additive patents	Reported Function	Brands	References
VOC25	2-ethyl-1-hexanol	2.84	7.42	57.0	Yes	Multiple Functions	EHA-U, EHA-V	[46–49]
VOC59	Oleic Acid	2.18	16.33	69.0	Yes	Lubricant and anticorrosive	EHA-I, EHA-U	[43,50]
VOC34	Undecane	2.05	8.14	49.0	No	–	EHA-U, EHA-V	–
VOC44	Naphtalene	1.70	9.10	54.0	No	Lubricant	EHA-V, EHA-U	[36]
VOC11	3-methyl-1-butanol	1.31	3.65	54.0	Yes	Lubricant	EHA-I, EHA-U, EHA-V	[43,51]
VOC40	1-ethenyl-4-ethyl-benzene	1.22	8.61	117.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC41	4-ethenyl-1,2-dimethyl-benzene	1.21	8.70	117.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC42	1,2,4,5-tetramethyl-benzene	1.20	8.72	119.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC13	2-methyl-1-butanol	1.19	3.71	41.0	Yes	Lubricant	EHA-I, EHA-V	[43,51]
VOC37	1,2,3,4-tetramethyl-benzene	1.19	8.36	134.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC38	1,2,4,5-tetramethyl-benzene	1.18	8.39	117.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC29	1,4-diethyl-benzene	1.18	7.75	119.0	Yes	Lubricants and Anti knocking	EHA-S	[53]
VOC33	2-ethyl-1,4-diethyl-benzene	1.17	8.03	119.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC28	1-methyl-4-propyl-benzene	1.17	7.69	105.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC32	1-ethyl-2,4-dimethyl-benzene	1.17	7.97	119.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC31	2-ethyl-1,4-dimethyl-benzene	1.16	7.94	119.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]
VOC36	1-methyl-2-(1-methylethyl)-benzene	1.11	8.24	119.0	Yes	Lubricants and Anti knocking	EHA-S	[44,52–54]

[a] – The compound identification was realized comparing mass-spectrum obtained to NIST library mass-spectrum.

All 98 EHC samples were correctly classified by the model. For the additive group, 91 out of 98 samples were classified as EHA. The class discrimination model, using three additive markers, demonstrated high accuracy (>95 %) and emerged as a feasible analytical alternative for the quality control of additives in fuel ethanol.

GC–MS allowed the evaluation of VOCs that could serve as additive markers. By tracking the selected compounds, it was possible to monitor the presence of additives in hydrated fuel ethanol samples and determine the possible origin.

Monitoring these markers is crucial for guiding the development of

Table 2
MLR Performance and confusion matrix.

MLR Performance			
Prediction	Accuracy 95.94 %	Sensitivity 91.20 %	Specificity 100 %
Confusion Matrix			
True class	EHA		EHC
EHA	91		8
EHC	0		98

other methodologies for quality control of additives in bioethanol. This can be achieved through analytical techniques that target specific molecules, the development of colorimetric assays tailored to these molecules, or the creation of sensors to certify the presence of additive packages used in the production of additive hydrated ethanol. Furthermore, new classes of molecules, such as nano additives and polymeric additives, can be explored as additive markers through other analytical techniques.

In conclusion, the determination of markers and the confirmation of additive presence do not reflect the performance of additive use in combustion engines, requiring further studies to evaluate the efficacy and impacts of EHA usage. Nevertheless, determining additive markers in fuel ethanol through GC–MS combined with multivariate analysis emerges as a valuable tool for ensuring additive presence for the consumer and tracking throughout the production chain.

4. Conclusion

In this study, a GC–MS-based methodology was developed to explore the organic compounds present in commercial fuel ethanol samples through a multivariate approach, which is classified according to the presence of additives in the fuel. The variables responsible for class discrimination were selected based on VIPScores ≥ 1.1. The correlation between VOCs and the EHA and EHC groups was made using a heat map based on scaled sample values. It emerged that of the 18 VOCs highlighted, 17 showed a high correlation (>1.0) with the EHA class and 1 VOC with the EHC group. The classification model demonstrated an accuracy of 96.86 %. These were characterized, including their presence in additive package patents and their potential functions.

Three markers were chosen based on the top brands in the Brazilian market: 2-ethyl-1-hexanol, oleic acid, and 1,4-diethylbenzene. These markers achieved an accuracy of 95.94 % in differentiating between the EHA and EHC groups, successfully classifying 91 out of 99 EHA samples.

Therefore, the methodology developed here is pioneering in enabling the evaluation of additive presence in additive hydrated fuel ethanol. It addresses the gap created by the absence of a standard method for biofuel quality control. Future studies may include quantitative analyses to assess the concentration of used packages as well as the development of sensors for field application and tracking throughout the supply chain and production.

CRediT authorship contribution statement

Leonardo Silva Gonçalves: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dyovanna de Sousa Costa:** Methodology, Conceptualization. **Rhyan César Santos Dias:** Investigation. **Lilian Fernandes de Oliveira:** Methodology, Investigation. **Aline Moreira Aguiar:** Methodology, Investigation. **Dayane Cristina da Costa:** Validation, Methodology, Investigation. **João Marcos Gonçalves Barbosa:** Investigation. **Anselmo Elcana de Oliveira:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Data curation. **Nelson Roberto Antoniosi Filho:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2026.138334.

Data availability

Data will be made available on request.

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