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Rapid and sensitive method for the determination of acetaldehyde in fuel ethanol by high-performance liquid chromatography with UV–Vis detection

Abstract A high-performance liquid chromatography (HPLC) method for the determination of acetaldehyde in fuel ethanol was developed. Acetaldehyde was derivatized with 0.900 mL 2,4-dinitrophenylhydrazine (DNPHi) reagent and 50 μL phosphoric acid 1 mol L^{-1} at a controlled room temperature of 15°C for 20 min. The separation of acetaldehyde-DNPH (ADNPH) was carried out on a Shimadzu Shim-pack C$_{18}$ column, using methanol/LiCl$_{(aq)}$ 1.0 mM (80/20, v/v) as a mobile phase under isocratic elution and UV–Vis detection at 365 nm. The standard curve of ADNPH was linear in the range 3–300 mg L^{-1} per injection (20 μL) and the limit of detection (LOD) for acetaldehyde was 2.03 μg L^{-1}, with a correlation coefficient greater than 0.999 and a precision (relative standard deviation, RSD) of 5.6% ($n = 5$). Recovery studies were performed by fortifying fuel samples with acetaldehyde at various concentrations and the results were in the range 98.7–102%, with a coefficient of variation (CV) from 0.2% to 7.2%. Several fuel samples collected from various gas stations were analyzed and the method was successfully applied to the analysis of acetaldehyde in fuel ethanol samples.

Keywords High-performance liquid chromatography · UV–Vis detection · Isocratic elution · Acetaldehyde · 2,4-dinitrophenylhydrazine · Fuel ethanol

Introduction

To better balance Brazil's petroleum consumption with its crude oil production, intensive research was con-

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ducted from the late 1960s to the early 1970s, with the aim to identify an economically viable alternative to oil as a fuel source. Ethanol, extracted from sugarcane, was chosen as one of these alternatives. The objective of the National Alcohol Program (PROALCOOL), established in 1975, was to use ethanol as a fuel substitute for gasoline and to increase ethanol production for industrial uses.

Ethanol is a source of energy that can be used in various applications, such as neat automotive and aviation fuel, for blending with other fuels like gasoline and diesel oil, and as a feedstock for hydrogen generation in fuel cells and biodiesel production. When produced from biomass, such as what happens in Brazil, it can be classified as a renewable product; a plus in terms of sustainable development. Ethanol has also been classified as a clean fuel because of its environmentally friendly characteristics. Ethanol from biomass can help to mitigate the greenhouse effects and its use as a fuel or blending component can contribute to reduce air pollution.

Combustion of ethanol does not generate sulfur compounds, produces no particulates, and reduces emissions of carbon monoxide and unregulated toxins. Although emissions from ethanol-fueled engines are not well characterized, there is some concern about the formation of acetaldehyde and other aldehydes in unburnt fuel ethanol.

On the other hand, despite aldehyde emissions increasing with ethanol use, acetaldehyde is the preponderant specie that is considerably less toxic and photochemically reactive than formaldehyde, which is the main product of gasoline and diesel combustion.

Analytical methods available for the detection of acetaldehyde and carbonyl compounds in air [1–16], water [17–22], alcoholic beverages [23–25], and foods [26–29] are described in the literature. Various derivatization methods followed by gas chromatography (GC) [30–39] or high-performance liquid chromatography

(HPLC) [40–60] for its determination and other carbonyl compounds are also reported in the literature. These methods commonly involve derivatization of acetaldehyde with reagents such as 2,4-dinitrophenylhydrazine (DNPHi), 3-methyl-2-benzothiazoline hydrazone (MBTH), dansyl hydrazine (DNSH), and others. The principal method is based on the reaction of acetaldehyde with DNPHi and subsequent analysis of the hydrazone derivatives by HPLC or GC.

The purpose of the present work is to establish an accurate and sensible analytical method for acetaldehyde determination in fuel ethanol samples by HPLC with UV–Vis detection based on the DNPHi derivatization reaction.

Experimental

Reagents and chemicals

All chemicals used were of ACS (American Chemical Society) reagent grade or better. The acetaldehyde was from Merck (Darmstadt, Germany). All solvents used in the experiments, such as methanol, ethanol, and acetonitrile, were of HPLC grade from Mallinckrodt (Xalostoc, Mexico). Demineralized water was obtained from a Milli-Q water system (Millipore, USA). The DNPHi from Merck was purified by three successive recrystallizations from methanol. The H_3PO_4 was from Aldrich Chemical Company (Milwaukee, USA).

Standard of acetaldehyde-DNPH (ADNPH)

The ADNPH used as a control was prepared by the well known reaction [61] of carbonyl compounds with DNPHi, obtained as follows: DNPHi (0.4 g; ca. 2 mmol) was dissolved in concentrated phosphoric acid (2 mL) and demineralized water (3 mL). To this solution, the standard acetaldehyde (1 g), dissolved in ethanol (15 mL), were added. The reaction product was isolated via filtration and purified (twice) by recrystallization from absolute ethanol. Standard solutions were prepared by dissolving weighed amounts (1 g L^{-1}) of the pure ADNPH in acetonitrile. Portions of these solutions were then diluted with a suitable ethanol/water mixture (95:5 v/v) to minimize matrix effects and standard solutions for calibration purposes were prepared in the concentration range 3–300 mg L^{-1}.

Sample derivatization

A 0.4% solution of DNPHi was prepared by dissolving DNPHi (0.4 g; ca. 2 mmol) in acetonitrile (100 mL). In a volumetric flask, 0.900 mL of DNPHi solution, 4.0 mL of the sample (without previous concentration), and 50 μL of H_3PO_4 1.0 mol L^{-1} were introduced consecutively. The resulting solution was stirred at room temperature for 20 min. Samples derivatives were filtered (0.45 μm; Millipore, USA) and injected into the HPLC system.

Chromatographic apparatus

ProStar Varian HPLC apparatus was used in isocratic conditions with a mobile phase consisting of methanol/$LiCl_{(aq)}$ 1.0×10^{-3} M (80:20 v/v). A Rheodyne Model 7725 injection valve with a 20-μL sample loop was used. A reversed-phase column Shimadzu Shim-pack C_{18} (150×6.0 mm I.D.; 5 μm) was applied for all measurements with a guard column (Shimadzu Shim-pack C_{18}). Prior to use, mobile phase solvents were filtered through a 0.45-μm filter (Millipore Milex, USA) and degassed by an ultrasonic couple into HPLC system. An isocratic program was used for HPLC analysis as follows: wavelength detector set at 365 nm and flow rate was 1.0 mL min^1.

Spectrophotometric apparatus

An HP spectrophotometric model 8453 was employed for all measurements and a quartz spectrophotometer cuvet of the 1-cm optic way was also used.

Quantitative test procedure

The standard curve for ADNPH was obtained by linear regression plotting peak area versus concentration, and the correlation coefficient was very close to unity.

Results and discussion

Selection of wavelength

A solution of standard ADNPH derivative 50 mg L^{-1} in ethanol/water solution (95:5 v/v) was prepared and scanned at 200–600-nm wavelengths by the HPLC system with a UV–Vis detector under hydrodinamic conditions (Fig. 1a) and the conventional spectrophotometric method (Fig. 1b). Although three maximum bands are seen at 275 nm, 332 nm, and 367 nm at quiescent conditions, on the other hand, the solution of standard ADNPH presents the maximum peak absorption at 365 nm under hydrodynamic conditions. Therefore, 365 nm was selected as the best wavelength to detect ADNPH derivative in the chromatographic conditions.

Selection of the mobile phase

Regarding the mobile phase condition, different ratios of acetonitrile/$LiCl_{(aq)}$ 1.0 mM and methanol/$LiCl_{(aq)}$ 1.0 mM were tested. A concentration of 50 mg L^{-1} standard solution ADNPH derivative was injected into the HPLC system and the performance chromatograms
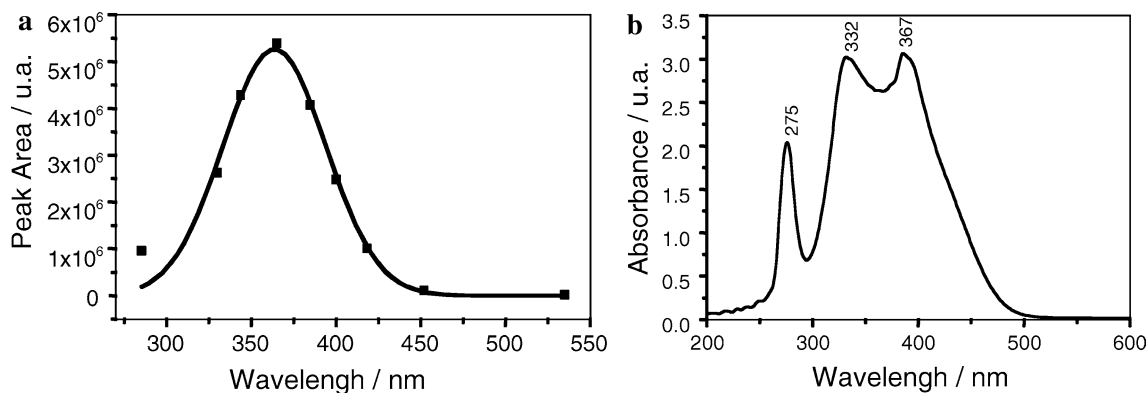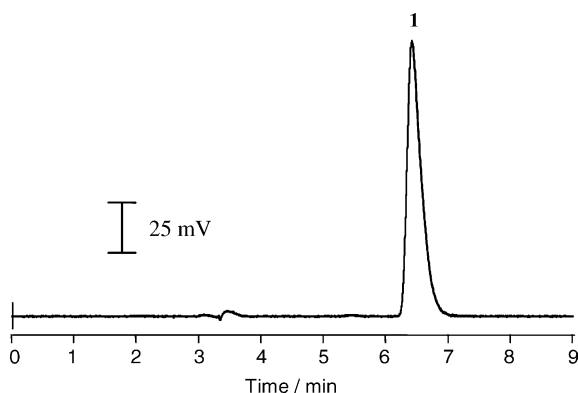
Fig. 1 a Standard ADNPH solution 50 mg L^{-1} in dynamic conditions by HPLC with a suitable ethanol/water solution (95:5 v/v) obtained with a Shimadzu Shim-pack C$_{18}$ column (150×6.0 mm I.D.; 5 μm) using a flow rate of 1.0 mL min^{-1}. **b** Mobile phase containing methanol/LiCl$_{(aq)}$ 1.0 mM (80:20 v/v) mixture and UV detection at 365 nm and static condition obtained in a suitable ethanol/water solution (95:5 v/v) by scan wavelength on a quartz spectrophotometer cuvet of 1-cm optic way

were compared. The HPLC chromatograms obtained for methanol/LiCl$_{(aq)}$ as a mobile phase leads to less background noise than that obtained for acetonitrile/LiCl$_{(aq)}$. The best condition was obtained for a methanol/LiCl$_{(aq)}$ 1.0 mM mixture in the proportion 80:20 v/v, which was chosen as the mobile phase for the detection and separation of acetaldehyde content in fuel ethanol samples. A typical chromatogram taken from standard ADNPH derivative solution with a retention time of 6.41 min can be seen in Fig. 2.

Optimum conditions of derivatization reaction

Phosphoric acid effect

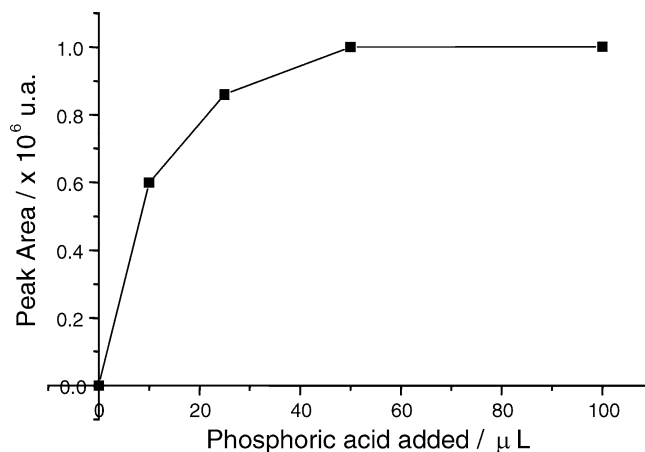According to the literature, the efficiency of the derivatization reaction from carbonyl compounds and DNPH can increase in the presence of phosphoric acid. The nucleophilic attack by the carbon of the carbonyl group in the protonated acetaldehyde to the DNPHi is easier in this acidic condition. In order to optimize this reaction condition, its effect was investigated by adding aliquots from 1 μL to 100 μL of phosphoric acid (1 mol L^{-1}) in the acetaldehyde solution containing DNPH 0.4% (m/v). The results obtained are shown in Fig. 3. It was observed that, in the presence of phosphoric acid from 10 μL to 50 μL, the peak area of the chromatograms increases markedly, and reaches a plateau for a concentration of ≥50 μL of phosphoric acid, indicating that a greater conversion of acetaldehyde content in the derivative form of ADNPH is occurring. Nevertheless, although stable peaks are obtained for phosphoric acid quantities of up to 100 μL, this condition needs to be avoided because there is an increase of background and noise level. Therefore, 50 μL of phosphoric acid is recommended for acetaldehyde derivatization.

Temperature and time effects

The effects of reaction duration and temperature on the acetaldehyde derivatization reaction were studied with

Fig. 2 HPLC chromatogram of the standard ADNPH solution (50 mg L^{-1}) on a Shimadzu Shim-pack C$_{18}$ column (150×6.0 mm I.D.; 5 μm) with a flow rate of 1.0 mL min^{-1}. Mobile phase containing methanol/LiCl$_{(aq)}$ 1.0 mM (80:20 v/v) mixture and UV detection at 365 nm. Identification of peaks: (1) ADNPH

Fig. 3 Effect of phosphoric acid added to the elution of standard ADNPH solution on a Shimadzu Shim-pack C$_{18}$ column (150×6.0 mm I.D.; 5 μm) with a flow rate of 1.0 μL min^{-1}. Mobile phase containing methanol/LiCl$_{(aq)}$ 1.0 mM (80:20 v/v) mixture and UV–Vis detection at 365 nm

the aim to minimize the time to completeness of the reaction and to avoid alteration of the acetaldehyde in the sample. The evaluation of temperature on the derivatization reaction was studied following the peak area of the chromatograms for generated ADNPH using reactions at an ambient temperature, 25°C, and a controlled temperature 15°C. For each temperature, aliquots were analyzed from reaction times of 5 min, 10 min, 20 min, and 30 min. The results are shown in Fig. 4. The acetaldehyde derivatization reaction reaches maximum signal after 20 min at 15°C. On the other hand, although maximum efficiency is obtained after a 20-min reaction at room temperature, smaller peak areas are observed than what is obtained at 15°C. In summary, the best condition for the direct derivatization of acetaldehyde with DNPHi can be obtained by using a reaction duration of 20 min at a controlled temperature of 15 °C.

Analytical curve

The analytical curve obtained for acetaldehyde using the best experimental conditions by HPLC with spectrofotometric detection based on peak area versus concentration was obtained for quantitative purposes and showed good linearity ($r^2 = 0.999$). The concentration and a linear relationship was obtained in the entire investigated range (3–300 mg L^{-1}), as shown in the Fig. 5. The limit of detection (LOD) determined at the lowest injected concentration, taken as a signal-to-noise ratio equal to 3:1, was 2.03 µg L^{-1} and the parameters of the analytical curve were as follows: peak area $= -2.75 \times 10^5 + 0.87 \times 10^5$ (ADNPH) and a precision (relative standard deviation, RSD) of 5.6% ($n = 5$).

Determination of acetaldehyde in fuel ethanol samples

Using the best experimental conditions as previously defined, several fuel ethanol samples from different gas

stations of Araraquara, SP, Brazil, were treated with DNPHi and phosphoric acid, and submitted to 20 min of reaction at 15°C. Aliquots of 20 µL of the yielding solution were analyzed by HPLC using a Shimadzu Shim-pack C$_{18}$ column under isocratic conditions of methanol/LiCl$_{(aq)}$ 1.0×10^3 mol L^{-1} (80:20, v/v) as a mobile phase coupled to a UV–Vis detector operating at a 365-nm wavelength, where the best separation was obtained.

Figure 6 shows the characteristic chromatographic separation acquired for typical commercial fuel ethanol samples on $t_r = 6.3$ min (identification peak 2). The retention times of the resultant HPLC peaks measured at 365 nm were recorded and compared to the standard ones for identification. The HPLC determination was completed in about 7 min, and no interferences were observed in the ADNPH retention time.

In addition, the method was also tested for several fuel ethanol samples, which were submitted to similar procedure. The relevant quantitative results are listed in Table 1. In general, the acetaldehyde content in fuel ethanol samples is significantly higher and the values point to high concentrations, ranging from 61.5 mg L^{-1} to 195 mg L^{-1}. These results indicates that acetaldehyde is a contaminant at high concentration in this kind of matrix and they can be used to suggest the need to develop new convertors during fuel ethanol manufacture.

Recovery test

Analytical recoveries were evaluated for ADNPH derivative using fuel ethanol samples spiked with standard derivative solution at a level of around 25% of the measured content and then performing five assays after each addition (Table 1). The recovery test was performed using the method established above, and a blank test (HPLC ethanol grade) was used as the control test. In each test, the retention time and peak of the sample solution were compared with the standard solution to

Fig. 4 Both the effect of reaction time and temperature on the formation of ADNPH in fuel ethanol on a Shimadzu Shim-pack C$_{18}$ column (150×6.0 mm I.D.; 5 µm) with a flow rate of 1.0 mL min^{-1}. Mobile phase containing methanol/LiCl$_{(aq)}$ 1.0 mM (80:20 v/v) mixture and UV–Vis detection at 365 nm
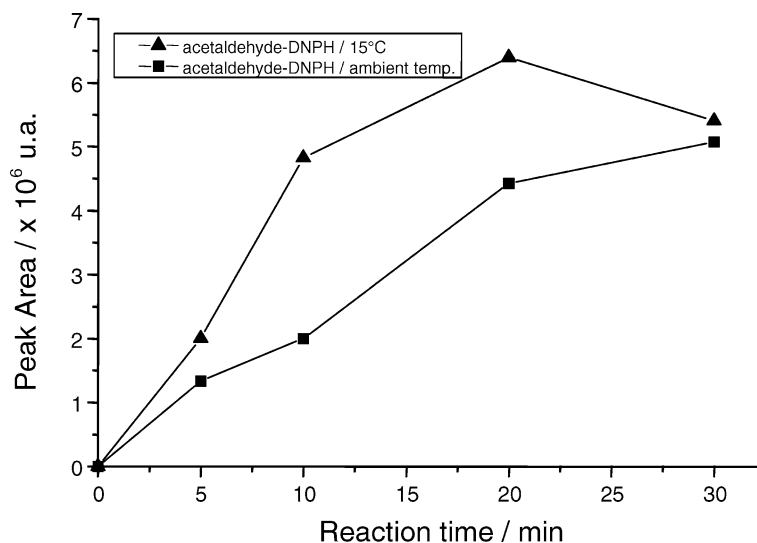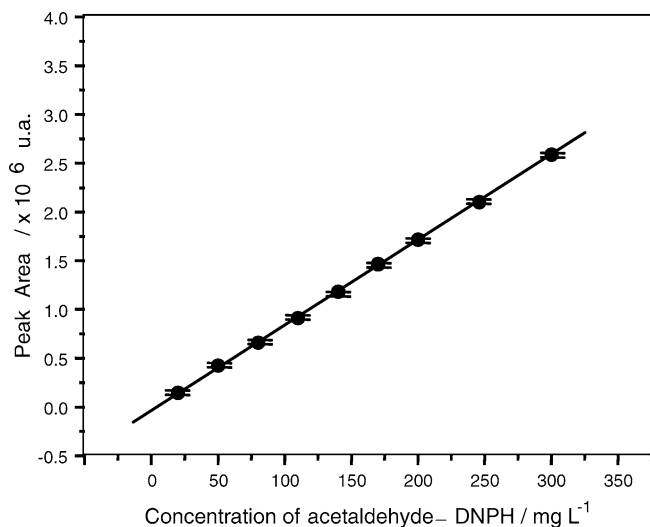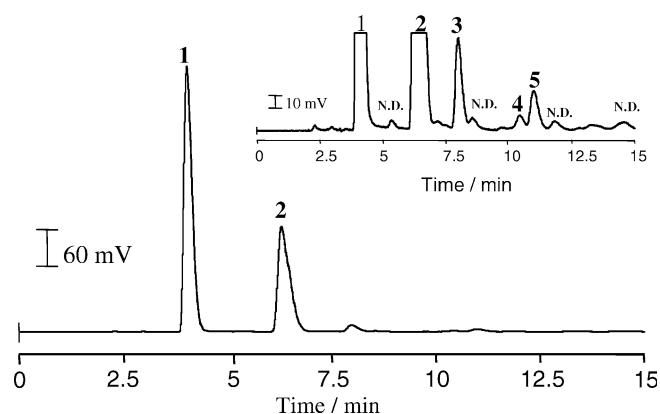
Fig. 5 Standard curve obtained from ADNPH solutions in the range 3–300 mg L^{-1} on a Shimadzu Shim-pack C_{18} column (150×6.0 mm I.D.; 5 μm) with a flow rate of 1.0 mL min^{-1}. Mobile phase containing methanol/LiCl$_{(aq)}$ 1.0 mM (80:20 v/v) mixture and UV–Vis detection at 365 nm

Table 1 Concentration of ADNPH solution determined in fuel ethanol samples and analytical recoveries added to its samples

Sample	Concentration present per mg L^{-1} (±SD)	Recovery (%)	CV (%)[a]
F.E.$_1$[b]	177 ± 2	99.5	6.0
F.E.$_2$	65.8 ± 1	99.5	0.6
F.E.$_3$	61.5 ± 1	100	0.2
F.E.$_4$	195 ± 3	98.7	7.2
F.E.$_5$	80.9 ± 1	102	0.5
F.E.$_6$	98.4 ± 1	98.8	0.5
F.E.$_7$	122 ± 2	100	1.6

$n = 5$, injections were performed in duplicate
[a]Coefficient of variation
[b]Fuel ethanol samples

identify and quantify, and calculate the recovery rate. As a result, the average recovery rate was 98.7% to 102%, with a coefficient of variation (CV) from 0.2% to 7.2%. The recovery rate of acetaldehyde was acceptable and had high reproducibility. Therefore, this method should fulfil the examination objective of acetaldehyde content in the fuel ethanol matrix.

Conclusion

The method proposed in this paper was successfully applied for acetaldehyde determination in fuel ethanol samples collected from various gas stations of Ara-raquara, SP, Brazil, with good levels of repeatability, simplicity, and accuracy. The legislation of Brazil does not impose limits for acetaldehyde content in fuel ethanol, but the presence of high amounts of this contaminant can be unhealthy for automotive fuel workers and can affect engine performance in combustion machinery. Our results indicates that a large quantity of acetaldehyde is present in this kind of sample from gas stations, and that this discovery could be used as a quality control parameter, since this information can be related to unsuitable conditions of production, transport, and storage of fuel.

Fig. 6 The HPLC chromatogram of the fuel ethanol sample solution on a Shimadzu Shim-pack C_{18} column (150×6.0 mm I.D.; 5 μm) with a flow rate of 1.0 mL min^{-1}. Mobile phase containing methanol/LiCl$_{(aq)}$ 1.0 mM (80:20 v/v) mixture and UV–Vis detection at 365 nm. Identification of peaks: (1) DNPHi; (2) ADNPH; (3) ketone–DNPH; (4) butyraldehyde–DNPH; (5) methyl ethyl ketone–DNPH; (N.D.) not detected

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