Optimization of Liquid Chromatography in Aldehyde Analysis: Sustainable Alternatives for Vehicle Emission Control

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ABSTRACT

Vehicle Emission Control is one of the most discussed topics in the automotive industry in the 21st century - accompanied by significant investments - to ensure that pollutant emission levels from motor vehicles comply with Air Quality Standards (Proconve - Brazilian Motor Vehicle Emission Control Program). Liquid chromatography techniques for separating and quantifying aldehydes in vehicle emission gases follow the analysis conditions outlined in ABNT NBR 12026/2021, where acetonitrile is used as the organic agent in the mobile phase of HPLC (High-Performance Liquid Chromatography). The cost and availability of acetonitrile, combined with its high toxicity, have driven efforts to validate aldehyde analysis methods that do not rely on this solvent.

The present study aims to validate new analysis conditions, simultaneously seeking to improve the economic viability of the process with potential monthly savings of up to 66% while ensuring equivalent test results. In this study, methanol was used as the organic solvent in the mobile phase, and the validation method was applied in tests using E22 (22% ethanol, Gasool A22), E61 (61% ethanol, Gasool A11H50), and EHR (100% ethanol) as fuels for motor vehicles.

INTRODUCTION

The growing concern regarding the impacts caused by atmospheric pollutants has directed efforts toward controlling emissions from motor vehicles. According to data from the Greenhouse Gas Emissions Estimates System (SEEG), in 2023, motor vehicles emitted approximately 223.8 million tons of CO_2, representing around 44% of the GHG emissions from the energy and industrial processes sectors in the country [1].

In this context, the main objective of this article is to evaluate alternative methodologies to the current model for analyzing aldehydes in vehicle exhaust gases, aiming to investigate the hypothesis of improving the economic feasibility of the process while ensuring equivalent test results. For this purpose, an experimental method is applied to test the replacement of the commonly used solvent in the mobile phase of liquid chromatography. In this study, methanol is employed as the organic solvent in the mobile phase, and the validation method was conducted using tests with E22, E61 and EHR as fuels in motor vehicles. The results obtained were compared in terms of mass in µg per sample, retention time, and repeatability.

Regarding the research problem, the focus lies on the potential impacts associated with this change, considering the need to revisit certain concepts related to chromatographic results and the potential effects on equipment used according to the method applied. This study suggests that replacing acetonitrile with methanol under the operational conditions adopted and in compliance with the ABNT NBR 12026:2021 Standard will not compromise the effectiveness of chromatographic analyses of aldehydes present in vehicle exhaust gases. It is expected that methanol will offer equivalent performance in terms of resolution, sensitivity, and reproducibility, while also providing economic and environmental advantages.

The justification for this study stems from the fact that acetonitrile, although widely used in chromatographic analyses, presents (i) high cost; (ii) strict regulatory control; and (iii) unstable supply. These factors may hinder its adoption in Brazilian laboratories, and this study proposes to investigate a viable alternative. These aspects make its replacement an attractive option, especially in contexts where economic feasibility is increasingly relevant for industry [2]. Alternatively, methanol is considered more accessible as it does not require special licenses and is widely available in the domestic market. Its physicochemical properties are compatible with the methodology described in ABNT NBR 12026, and initiatives for its domestic production, including from renewable sources, further reinforce its attractiveness as an alternative solvent [3,4,5].

LITERATURE REVIEW

The Proconve (Vehicle Emissions Control Program) was established by the National Environmental Council in 1986 to reduce atmospheric pollutant emissions from

vehicles. In 1993, the determinations of the Conama Resolution were intensified, establishing limits on emissions of certain chemical compounds such as carbon monoxide, nitrogen oxides, hydrocarbons, and aldehydes [6]. Accordingly, technical standards were developed by relevant entities to standardize and ensure quality in procedures. Among them is the ABNT NBR 12026 Standard, which specifies procedures for the determination of aldehydes in vehicle exhaust gases using liquid chromatography.

Also in this context, High-Performance Liquid Chromatography (HPLC) has advanced significantly in recent years. It was developed to identify and quantify chemical components in samples. HPLC-based methods are suitable for a variety of organic compounds that exhibit high polarity and low volatility or high thermal instability [7]. A key characteristic is that the mobile phase must dissolve the sample without chemical interaction. Additionally, it must be of high purity to allow for sensitive analyses, since impurities may interfere with UV detection of the analyte [8].

According to the ABNT NBR 12026:2021 Standard, acetonitrile, ethanol, or methanol may be used as solvents for chromatographic analysis. Aldehydes and ketones present in vehicle exhaust gases during each phase of a dynamometer test are absorbed into an absorbing solution, forming carbonyl derivatives using the impinger method (reference method). These derivatives are separated, identified, and quantified by HPLC [3].

Using HPLC to solve a given problem requires the correct combination of operational parameters: type of column packing and mobile phase, column length and diameter, mobile phase flow rate, separation temperature, sample size, etc.

Methanol and acetonitrile exhibit several favorable properties for chromatographic applications, such as complete miscibility with water, excellent UV transmission (λ = 195 nm for acetonitrile and 205 nm for methanol), low viscosity in aqueous solutions, high purity as required by the standard, and low chemical reactivity with various sample species and instrument or HPLC column surfaces [9].

An important parameter to assess before their integration is the solvent polarity. The relative dielectric constant is a measure of solvent polarity. Solvents with a relative dielectric constant below 15 are considered nonpolar. The commonly used rule "like dissolves like" implies that polar solvents preferentially dissolve polar solutes, and nonpolar solvents dissolve nonpolar solutes [10].

Polar solvents can also be classified as protic or aprotic, according to IUPAC. Protic polar solvents (e.g., water, methanol) can stabilize ions through proton donation, forming hydrogen bonds, and through donation of non-bonding electron pairs. Aprotic polar solvents (e.g., acetonitrile, acetone) lack hydrogen atoms with weak chemical bonds and thus cannot form hydrogen bonds [11,12].

The selection of the most appropriate solvent must also consider additional characteristics such as cost, boiling point, flammability, density, toxicity, and environmental impact. As a general rule, among the possible solvents, one should choose the least flammable, least toxic, and with the lowest environmental impact.

According to the Safety Data Sheet (SDS), acetonitrile has a higher viscosity than methanol, with values of 0.34 and 0.55, respectively. In this same context, acetonitrile has a stronger elution power than methanol due to being more hydrophobic, which reduces its interaction with the stationary phase and facilitates compound elution. However, methanol forms hydrogen bonds with the stationary phase and water, which prolongs the retention of analytes [13].

CHEMICAL DETERMINATION OF ALDEHYDES

The specific reaction between carbonyl compounds and 2,4-dinitrophenylhydrazine (2,4-DNPH) resulting in the formation of 2,4-dinitrophenylhydrazone (2,4-DNPHZ) is a qualitative technique used for identification and quantification in organic analyses [14].

The quantification of aldehydes via liquid chromatography with ultraviolet-visible detection is complex. Aldehydes do not absorb UV radiation, requiring a derivatization reaction for quantification. Furthermore, due to their low molecular weight and high polarity, they are not easily retained in reverse-phase chromatographic columns [15].

To derivatize aldehydes, the common reagent used is 2,4-dinitrophenylhydrazine (2,4-DNPH). The carbonyl group in the aldehyde reacts with the amino group in DNPH under acidic conditions, forming the 2,4-DNPHZ derivative [15].

In the first step of the hydrazone formation mechanism (Figure 1), the amine interacts with the carbonyl compound. The alkoxide ion gains a proton while the ammonium ion loses one, resulting in a neutral tetrahedral intermediate. This intermediate, known as a carbinolamine, is in equilibrium with two protonated forms. Protonation may occur on either oxygen atom. The elimination of water from the protonated oxygen intermediate yields a protonated hydrazone, which loses a proton to become the hydrazone. Hydrazone formation is a reversible process. In acidic aqueous solutions, hydrazone derivatives are hydrolyzed back into the original carbonyl compound and DNPH, establishing an equilibrium state [14].

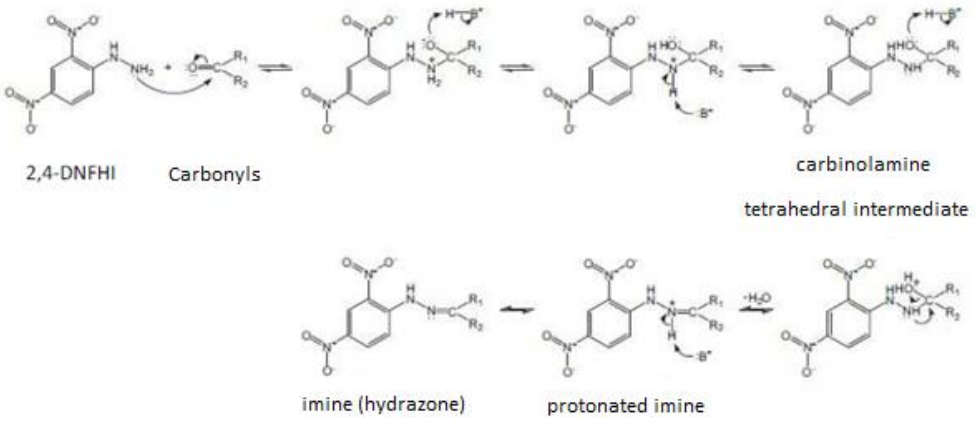

Figure 1. Mechanism of formation of 2,4-dinitrophenylhydrazone from carbonyl compounds.
Source: UCHIYAMA; INABA; KUNUGITA, 2011.

METHODOLOGY

Samples were obtained from tests carried out at the Vehicle Emissions Laboratory (LEV) of Horse Powertrain. The vehicles were subjected to chassis dynamometer tests, where the exhaust gases were directed, through a gas sampler, to a system of impingers containing absorption solution, allowing the capture of aldehydes present in the emissions.

CHROMATOGRAPHIC ANALYSES

For the instrumental analyses, a High-Performance Liquid Chromatograph (HPLC), model 1260 Infinity II from Agilent Technologies, was used. It was equipped with a ZORBAX Eclipse Plus C18 column (4.6 x 250 mm, 5 µm), a UV detector set at 365 nm, and a quaternary pump. In all analyses, the main acquisition parameters were kept constant: 10 µL sample injection volume, 1 mL/min mobile phase flow rate, and 40°C column temperature.

The reagents used in this study are listed in Table 1. Except for water, which was purified using a Milli-Q Ultra-Purification System, all other reagents were used without any modifications to the manufacturer's specifications.

Table 1. Reagents used.

Reagents	Supplier	Purity
Formaldehyde	SIGMA	0.999
Acetaldehyde	SIGMA	0.999
Acetonitrile	MERCK	0.999
2,4-DNPH	ÉXODO CIENTÍFICA	0.999
Water	-	Ultrapure

The reagents used in this study had specific functions within the analytical procedure. Standard solutions of formaldehyde and acetaldehyde were employed in the construction of calibration curves to evaluate the chromatographic response. Acetonitrile was used both as a component of the mobile phase in the conventional method and in the preparation of the absorption solution, which consists of a mixture of DNPH (2,4-dinitrophenylhydrazine) in acetonitrile. Ultrapure water was used as the second component of the mobile phase in both proposed methods.

To validate the proposed method, tests were performed to ensure that the modified method would meet analytical quality requirements. The linearity of the calibration curve, limits of detection and quantification, recovery, and method reproducibility were evaluated.

CALIBRATION CURVE

According to Figueiredo (2012), linearity refers to the ability of an analytical approach to demonstrate that the results obtained are directly proportional to the analyte concentration in the sample, within a given range. Therefore, two calibration curves were constructed, differing only in the mobile phase composition.

With this adaptation, it is considered that Method 1 represents the conditions adopted by the laboratory according to the recommendations of ABNT NBR 12026:2021, using acetonitrile and water in the mobile phase, while Method 2 uses a methanol:water ratio, as shown in Table 2, since for both methods the flow rate was 1 mL/min.

Table 2. Elution adaptation used.

Method	Ratio (%)
1	59:41:00
2	69:31:00

After establishing the calibration curves, samples from fifteen tests were analyzed (five for each fuel type: E22, E61, and EHR). Each sample was injected using both

chromatographic methods, enabling a direct comparison between the use of acetonitrile and methanol in the mobile phase.

IMPINGER AND FLASK CONDITIONING

In addition to replacing the solvent in the mobile phase, tests were conducted by substituting acetonitrile with methanol in two additional steps: the conditioning of impingers and volumetric flasks. Before sample collection, impingers are usually conditioned with acetonitrile before the absorption solution is added. In this study, methanol was tested as an alternative at this stage. Similarly, after collection, samples are transferred to volumetric flasks previously conditioned with acetonitrile, and methanol was also assessed for its viability as a substitute in this step.

RESULTS AND DISCUSSION

This section presents and discusses the experimental results in a segmented manner, addressing separately the replacement of the mobile phase, impinger conditioning, and volumetric flask conditioning.

MOBILE PHASE REPLACEMENT

Table 3 presents the standard deviations obtained using acetonitrile:water and methanol:water in the mobile phase, with tests 1 to 5 corresponding to fuel E100, tests 6 to 10 to E61, and tests 11 to 15 to E22.

Table 3. Standard deviations of formaldehyde and acetaldehyde values (µg/sample), and NMOG value derived from comparison.

Test	SD Formaldehyde	SD Acetaldehyde	SD NMOG
1	1.181	0.170	0.0007
2	0.064	0.283	0.0000
3	0.071	0.580	0.0000
4	0.424	0.120	0.0000
5	0.156	0.926	0.0000
6	0.141	1.146	0.0007
7	0.113	0.255	0.0000
8	0.007	0.523	0.0000
9	0.071	0.014	0.0000
10	0.219	0.007	0.0000
11	0.071	0.651	0.0000
12	0.085	0.389	0.0000
13	0.191	0.085	0.0000
14	0.523	0.007	0.0000
15	0.898	1.011	0.0007

To compare the methods, variables such as mass per sample (µg/sample), and the NMOG value were evaluated. To simplify data presentation and highlight result consistency, the corresponding standard deviations for each variable were provided.

The term NMOG (Non-Methane Organic Gases) refers to volatile organic gases present in vehicle emissions excluding methane. NMOG includes a wide range of substances such as aldehydes, ketones, and non-methane hydrocarbons emitted during fuel combustion [17,18].

It was observed that, although the standard deviation of aldehydes was slightly above what is typically considered acceptable, it did not significantly influence the NMOG. The standard deviation (σ) indicates the dispersion of the data in relation to the mean. Low values suggest good repeatability and precision, which are desirable in analytical methods. Generally, values below 10% of the mean are considered acceptable, though interpretation should consider the experimental context [19].

According to Heftmann, methanol has a lower elution strength than acetonitrile. Therefore, an increase in retention time would be expected. However, by adjusting the mobile phase ratio, this effect was minimized, resulting in retention times compatible with the original method.

IMPINGER CONDITIONING

This section presents the results from tests in which impingers were conditioned with methanol, evaluating the entire process from preparation of the absorption solution through to sample preparation for chromatographic injection. To ensure a proper comparison between the methods, the tests were conducted using standard vehicles routinely used in the laboratory under equivalent operating conditions.

Test 1 was performed with impingers conditioned with acetonitrile, while tests 2 and 3 were conditioned with methanol, keeping the absorption solution in contact with residual methanol for 4 and 24 hours, respectively. The results are shown in Table 4, which highlights the influence of methanol exposure time on formaldehyde quantification.

Table 4. Results in µg/sample and respective standard deviation.

Time	Test	Acetaldehyde (µg ± SD)	Formaldehyde (µg ± SD)
-	1	44.74 ± -	4.07 ± -
4 h	2	42.84 ± 1.343	4.65 ± 0.410
24 h	3	39.14 ± 3.959	15.50 ± 8.082

It is noted that the standard deviation for the sample exposed for over 24 hours was greater than for the one exposed for a shorter duration. The higher reactivity of formaldehyde compared to acetaldehyde can be explained by its simpler structure, lacking alkyl groups near the carbonyl. This makes it more electrophilic and, therefore, more susceptible to methanol attack in acidic medium,

favoring hemiacetal formation. Additionally, its lower molecular weight (30.03 g/mol) indicates a less stabilized structure compared to acetaldehyde (44.05 g/mol) due to steric and inductive effects [21,22].

The variation observed in formaldehyde results when impingers were pre-rinsed with methanol prior to the addition of the absorption solution may be related to the formation of unstable hemiacetals. In acidic media—such as the DNPH solution in perchloric acid—formaldehyde can react with residual methanol, forming compounds that may decompose during analysis and release formaldehyde again, artificially increasing its concentration. This behavior is typical of short-chain, highly reactive aldehydes such as formaldehyde, and was not observed with acetaldehyde, supporting this interpretation [21,23].

VOLUMETRIC FLASK CONDITIONING

As part of the new method validation process, the possibility of replacing acetonitrile in the conditioning of volumetric flasks used for sample dilution was also evaluated. Methanol conditioning was carried out following the same criteria as before. The results obtained in table 5 showed that the substitution did not significantly alter the concentrations of the quantified analytes.

Table 5. Standard deviation of mass and NMOG.

Compound	SD (µg/sample)	SD NMOG
Formaldehyde	0.919	0.0000
Acetaldehyde	0.028	
Formaldehyde	1.576	0.0000
Acetaldehyde	0.183	
Formaldehyde	2.856	0.0007
Acetaldehyde	0.289	

The data in Table 5 show that the use of methanol in volumetric flask conditioning yielded results equivalent to those obtained with acetonitrile in terms of both precision and accuracy, as the standard deviation related to NMOG was effectively zero. These findings support the feasibility of replacing the solvent at this step as well, contributing to cost reduction and lower handling risks associated with acetonitrile, without compromising analytical quality.

COST ANALYSIS

To implement the proposed change in the method, no initial investment was required, as the equipment, operator, and reagents were already in use for these procedures. It is important to note that only the costs of the replaced reagent were considered, since other expenses - such as labor and electricity - are constant and not affected by the solvent change.

Given that acetonitrile substitution was not considered feasible for impinger conditioning, only the replacement in volumetric flask conditioning and the mobile phase of the HPLC were taken into account, based on the assumption of five tests conducted per day. Table 6 presents the current costs for analyses conducted in the chemical lab at Horse and the projected costs if acetonitrile were replaced by methanol.

Table 6. Total costs and impact of proposed savings.

TOTAL COSTS (BRL)	
ACETONITRILE	
Monthly Total	R$ 7,955.27
Annual Total	R$ 95,463.18
METHANOL	
Monthly Total	R$ 2,666.65
Annual Total	R$ 31,999.83
SAVINGS	
Monthly Savings	R$ 5,288.61
Annual Savings	R$ 63,463.35

To calculate the savings generated by replacing acetonitrile with methanol in the mobile phase and flask conditioning, the average monthly expenses with each solvent were considered. The average monthly cost of acetonitrile was R$7,955.27, whereas methanol cost R$2,666.65. The savings were calculated using Equation 1:

((previous cost – current cost) / previous cost) * 100%

Thus,

((7,955.27 – 2,666.65) / 7,955.27) * 100% = 66.48%

Based on the monthly average consumption, replacing acetonitrile with methanol resulted in approximately 66.5% savings in solvent-related costs. This reduction represents a significant financial impact without compromising the analytical performance of the method.

CONCLUSION

According to the results obtained, it is concluded that the partial replacement of acetonitrile with methanol in the analysis of aldehydes in vehicle emission gases is technically feasible and advantageous. The substitution was effective in the mobile phase of HPLC and in the conditioning of volumetric flasks, maintaining analytical performance comparable to the traditional method. However, replacement in the impingers proved inadequate due to instability observed in quantifications, especially for formaldehyde.

From an economic standpoint, the new procedure resulted in an approximate 66.5% reduction in solvent costs, without the need for additional investment in equipment or training. Furthermore, the use of methanol is supported by the ABNT NBR 12026 standard, which

reinforces the applicability of this approach in the laboratory setting.

Considering the challenges associated with acetonitrile - such as high cost, toxicity, and limited availability - this study contributes to the modernization and sustainability of analytical practices in vehicle emissions laboratories. Future research could explore the complete replacement of the solvent, assessing strategies to mitigate the effects observed in the impingers. Therefore, this proposal represents progress both economically and in terms of occupational safety and environmental responsibility.

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