# Detection of ethanol in Brazilian gasoline station attendants



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|---|---|
| Complete List of Authors: | Borille, Bruna; Federal University of Rio Grande do Sul, College of Pharmacy<br>Fiorentin, Taís; Federal University of Rio Grande do Sul, College of Pharmacy<br>Coppe, Bruna; Federal University of Rio Grande do Sul, College of Pharmacy<br>Comiran, Eloisa; Federal University of Rio Grande do Sul, College of Pharmacy<br>Jacques, Ana Laura; Federal University of Rio Grande do Sul, College of Pharmacy<br>Sousa, Tanara; Federal University of Rio Grande do Sul, Center for Drug and Alcohol Research<br>Pasa, Graciela; Federal University of Rio Grande do Sul, Center for Drug and Alcohol Research<br>Pechansky, Flavio; Federal University of Rio Grande do Sul, Center for Drug and Alcohol Research<br>Castro, Stela; Federal University of Rio Grande do Sul, Department of Statistics<br>Limberger, Renata; Federal University of Rio Grande do Sul, College of Pharmacy |

# *Detection of ethanol in Brazilian gasoline station attendants*

**Bruna Tassi Borille[a*]; Taís Regina Fiorentin[a]; Bruna Claudia Coppe[a]; Eloisa Comiran[a]; Ana Laura Bemvenuti Jacques[a]; Tanara Rosângela Vieira Sousa[b]; Graciela Gema Pasa[b]; Flavio Pechansky[b]; Stela Maris de Jezus Castro[c]; Renata Pereira Limberger[a];**

[a]Graduate Program in Pharmaceutical Sciences, College of Pharmacy, Federal University of Rio Grande do Sul, 2752 Ipiranga Avenue, Santana, 90610-000, Porto Alegre, Rio Grande do Sul, Brazil.

[b]Center for Drug and Alcohol Research - Hospital de Clinicas de Porto Alegre - UFRGS / Federal University of Rio Grande do Sul - Rua Prof. Alvaro Alvim 400, 90420-020, Porto Alegre, Rio Grande do Sul, Brazil

[c]Department of Statistics, Federal University of Rio Grande do Sul, 9500 Bento Gonçalves Avenue, 91509-200, Porto Alegre, Rio Grande do Sul, Brazil

*Corresponding author: College of Pharmacy, Federal University of Rio Grande do Sul, 2752 Ipiranga Avenue, Santana, 90610-000, Porto Alegre, Rio Grande do Sul, Brazil, Tel.: +55 51 33085762; Fax: +55 51 33085437. E-mail address: bruhhtb@gmail.com

**Abstract**

In Brazil, gasoline station attendants are regularly exposed to the ethanol contained in fuel, as well as the one used as gasoline additive. This study aimed to assess the potential exposure of these workers to fuels, using breathalyzer and oral fluid (OF) analysis by headspace gas chromatography/mass spectrometry (HS-GC/MS). Attendants of 26 gasoline stations were invited to respond a questionnaire covering the main features of the study population and the profile of drinking and driving behavior, followed by a breath test and OF collection with the Quantisal™ device. All OF samples were analyzed by HS-GC/MS. Ethanol was found in 100% of the OF samples whereas 72.83% had concentrations above the quantification limit of the method (0.00125 g/dL). Regarding the breath tests, only one exhaled air sample (0.62%) had a positive result (0.03 mg/L). The positive results in OF samples and negative results in exhaled air may be explained by the higher sensitivity of OF analysis by HS-GC/MS, when compared to the breathalyzer.

**Keywords:** Ethanol, oral fluid, alcohol concentration in exhaled air, blood alcohol concentration, gasoline station attendants.

## 1. Introduction

Road traffic injuries are a major cause of death globally and the leading cause of death for young people aged 15-29 years [1,2]. Still, according to the World Health Organization (WHO) it is estimated that more than one million people die annually in traffic-related accidents. Driving under the influence (DUI) of alcohol or drugs is a powerful predictor of traffic deaths [1,2].

To reduce the number of traffic accidents related to DUI of alcohol and drugs, the legal blood alcohol concentration (BAC) limit was introduced in Norway, in 1936 [3]. Currently in Norway, the legal BAC limit corresponds about of 0.02 g/dL [4]. Brazil introduced the legislation limits corresponding to a BAC of 0.08 g/dL, in 1989, and in 2008 the “zero” limit was introduced with suspension of driving privileges for a BAC above 0.02 g/dL [3]. Today, the legislation determined a limit of breath alcohol concentration (BrAC) of 0.05 mg/L and “zero” tolerance for BAC. In cases that the BAC rate is higher than 0.06 g/dL or for BrAC of 0.34 mg/L, more severe punishments, including prison, are applied [5].

The conversion values obtained in BrAC to the equivalent BAC are based on the principles of Henry's Law, which establishes the relationship of BAC and BrAC as 1/2000, i.e., 2 liters of exhaled air contains approximately the same amount of ethanol than a milliliter of blood [6].

A major concern regarding the use of breathalyzers is their potential inability to accurately report interfering volatile organic components (e.g., cross-reacting), and supply falsely increased ethanol reading [7,8]. Breath, for instance, is a rather complex mixture in which ethanol and acetaldehyde may be found. The origin of ethanol may greatly vary, it may be influenced by the environment, considering acetaldehyde is a product of its metabolism [9]. Acetone, methane, and isoprene are produced endogenously and, in high concentrations, they may appear in the breath [10]. Furthermore, ketone bodies may be produced in prolonged fasting, specific diets, and in diabetic individuals [7,9,11]. There may also be residual presence of ethanol in the mouth when recently consumed, which can alter blood/breath ratio, leading to falsely elevated results in the reading equipment [7,11]. Here, we highlight workers directly exposed to ethanol during the workday, as the case of gasoline station attendants, who are the focus of this research study.

It is crucial to confirm the positive results found in the breath analyzer through reliable and standardized laboratory techniques, thus obtaining indisputable results. A recommended technique for confirmatory analysis of volatile substances in biological matrices is gas chromatography (GC) and headspace (HS) [11,13], combined with mass spectrometry (MS) [13]. Analysis by GC/MS allows the unequivocal identification of analytes [12,13].

Ethanol is the main component in alcoholic beverages. As a chemical compound of low molecular weight ($CH_3CH_2OH$), it is polar, highly soluble in water [14,15], it does not bind to plasma proteins, and it is easily distributed to all tissues in the body, thus becoming a suitable compound for analysis in biological samples such as blood, urine, oral fluid, and sweat [15,16].

In pharmacokinetic studies with ethanol, it was shown that there are large individual variations in its absorption, distribution, and elimination [17]. This variability may occur due to factors such as gender, age, race, consumption of alcoholic beverages associated or not with food intake, exercise, alcoholism, and the use of some medications [18,19].

Oral fluid (OF) has been used worldwide for monitoring the consumption of alcohol and drugs [20-26]. The term "oral fluid" refers to an organic matrix consisting of pure saliva secreted by salivary glands, and a mixture of particles and fluids found in the oral cavity [26,28]. This biological matrix presents several advantages over the blood, which is usually employed for confirmatory analysis of ethanol concentration in drivers, such as the fact that the collection is noninvasive, easy to apply, fast, and predictive, making it difficult for sample tampering by the giver [16,24-26,29].

Differences in substance concentrations in different matrices may be explained by the fact that the transfer of substances from blood to OF occurs by passive diffusion, primarily [29,33]. Furthermore, pH differences in the biological matrix, chemical properties of substances, and substance-protein binding also influence substance concentrations [21,30,31]. Nevertheless, many scientific studies have shown high correlations for ethanol between OF and blood [32,33,34,35], also ethanol concentration in OF reflects positive correlations with exhaled air [36].

Gubala and Zuba (2002) performed a controlled study of ethanol intake and measured its concentration in OF and plasma after ingestion of alcohol, finding an average OF/plasma ratio of 1.08 [20]. Jones (1993) found a mean OF/plasma ratio of 1.094, and this ratio remained the same during absorption, distribution, and elimination phases [37].

Seeking to verify the correlation of ethanol concentration in exhaled air, OF, and urine, Bueno et al (2014) observed a linear association between the concentrations of OF/exhaled air and OF/urine, using Pearson's correlation [36]. The test showed that OF was significantly correlated with urine (0.93 - female; 0.91 - male) and exhaled air (0.88 - female; 0.96 - male) [36].

Thus, this study aimed to assess the potential interference of ethanol to which gas station attendants are exposed during the workday, in the breath analyzer test, since this test is applied on traffic, and gas station attendants are submitted to it.

## 2. Materials and methods

### *2.1 Chemicals, materials, and equipment*

Ethanol and *n*-propanol were purchased from Tedia Company (Fairfield, OH, USA). Quantisal™ OF collection devices, filters, and preservative buffer solutions were donated by Immunalysis Corporation (Pomona, CA, USA). HS vials and aluminum screw caps with PTFE/silicone septum were obtained from Agilent Technologies (Agilent J&W Scientific, Folsom, CA, USA). GC/MS analyses were performed on a GC 7890A coupled with mass detector 5975C (Agilent Technologies, CA, USA), equipped with an automatic HS auto-sampler (CTC Analytics Combipal, Basel, Switzerland) and ZB-BAC1 column (30 m x 0.32 mm x 1.80 μm), from Zebron (Phenomenex). Alco-Sensor IV breathalyzers with disposable mouthpieces were lent by the State Traffic Department of Rio Grande do Sul, Brazil.

### *2.2 Ethical issue*

Ethical approval was granted by the ethical advisory board of the Federal University of Rio Grande do Sul, RS, Brazil, deliberation number 23834513.4.0000.5347.

### *2.3 Sampling of exhaled air and oral fluid*

The sample size calculation for the comparison of two paired groups was performed from the null hypothesis, which postulates that the proportion of subjects with the presence of ethanol in exhaled air, detected by breathalyzer is equal to the proportion of subjects with the presence of ethanol in OF, detected by HS-GC/MS. The calculation was performed at a significance level of 5%, correlation between measures of 0.79, and magnitude of effect of 5%. For these parameters, 162 sampling units had a statistical nominal power equal to at least 80% to detect differences in proportions.

Paired exhaled air and OF samples were collected from gasoline station attendants at the end of their shifts. The study population consisted of gasoline station attendants who, after undergoing a questionnaire, had not drunk alcohol in the last 12 hours. The questionnaire was applied with the aid of ODK Collect app installed on tablets, followed by the application of the breathalyzer. Oral fluid samples were obtained using Quantisal™ OF collection devices, which contained a collection pad with an indicator that turns blue when 1 mL of OF has been collected. After collecting 1 mL of OF the collection pad was transferred to a plastic transport tube with 3 mL of buffering solution, with a final specimen volume of 4 mL. A cylindrical filter was used to separate oral fluid solution from the pad. All samples were transferred to the laboratory using a Styrofoam box with monitored temperature at approximately 5 °C, then they were frozen and stored no more than 2 days at about - 10 °C until analysis.

### *2.4 Oral fluid sample analysis*

Oral fluid samples were analyzed with the headspace gas chromatography/mass spectrometry (HS-GC/MS) technique using a previously published validated method [38]. An aliquot of 1 mL of OF sample with buffering solution was transferred to a HS vial, followed by the addition of 50 µL *n*-propanol 0.2 g/dL (internal standard). The HS vials were sealed with PTFE/silicone septa and aluminum screw caps, and were placed into the vial rack of the automatic sampler, operating in the HS mode. The HS vial was transported from the rack to the heater/stirrer and incubated for 7 min at 90 °C, with a stirring of 500 rpm. Finally, 1000 µL of gas phase was injected in GC/MS.

The injector was maintained at 200 °C and operated in split mode 25/1. The oven temperature was programmed starting at 40 °C for 3 min, with an increase of 5 °C/min to 70 °C, for 1 min. The total run was 10 min. The post-run temperature was maintained at 200 °C for 3 min. Helium ultra pure was used as carrier gas at the flow rate of 1.4 mL/min. Temperatures of the interface, ion source, and quadrupole were 220 °C, 230 °C, and 150 °C, respectively. The MS system was operated in electron impact ionization mode at 70 eV, and in selected-ion monitoring (SIM). The ions monitored were m/z 31, 45, 46 for ethanol, and m/z 31, 59, 60 for *n*-propanol.

### *2.5 Data analysis*

For data management and organization, the Microsoft Office Excel™ software was used and statistical analyzes were performed using the SAS software (v. 9.4).

Categorical variables were described by absolute frequency and relative frequency, and compared between groups of gender using Fisher's exact test. Quantitative variables were described by mean and standard deviation and compared between groups by Mann-Whitney test.

## 3. Results

### *3.1 Profile of gasoline station attendants*

Paired samples of exhaled air and OF ($n$ =162) were collected from gasoline station attendants in 26 gasoline stations located in cities from Southern Brazil. One volunteer was excluded for failing to satisfactorily provide oral fluid and both men and women work, on average, 8 hours per day.

Table 3.1 summarizes overall features of our study population. On average, the population consisted of young (31.7 ± 10.7 years old), mostly male (70.99%) individuals, and both men and women were overweight considering their body mass index (BMI). Diabetes was reported by 4.3% of the participants, and 45.8% of the total has a relative with the disease.

**Table 3.1** Main features of the study population.

| | **Total ($n$ = 162)** | **Male ($n$ = 115)** | **Female ($n$ = 47)** | **Significance** |
|---|---|---|---|---|
| **Age[a]** | 31.7 ± 10.7 | 32.1 ± 11.5 | 30.8 + 8.4 | 0.9882[d] |
| **Height[a]** | 1.70 ± 0.09 | 1.73 ± 0.07 | 1.61 + 0.07 | < 0.0001[d] |
| **Weight[a]** | 76.6 ± 14.6 | 79.0 ± 14.1 | 70.0 + 14.2 | 0.0006[d] |
| **BMI[a]** | 26.6 ± 4.90 | 26.2 ± 4.29 | 27.5 + 6.09 | 0.2763[d] |
| **Diabetes[b]** | 7 (4.3%) | 2 (28.6%) | 5 (71.4%) | 1.0000[e] |
| **Relatives with diabetes[b,c]** | 71 (45.8%) | 27 (38%) | 44 (2%) | 0.0326[e] |

[a]*Mean*±SD

[b]*n* (*percentage*)

[c] ($n$ = 155)

[d] Mann-Whitney test

[e] Fisher's exact test

The gasoline station attendants' behavior about drinking and driving, in this study, are shown in Table 3.2. Over half of the gasoline station attendants (63.3%) drive a car or a motorcycle every day. In the last year, 128 gasoline station attendants (79%) drank alcoholic beverages in any quantity. From the total subjects who drank alcohol and drove (87), 29.9% had consumed alcoholic beverages immediately before driving. When asked if they had suffered traffic accidents, 58 subjects answered yes. Regarding the group of gasoline station attendants who have suffered accidents and drive (50), 94% (47) were driving at the time of the accident. From these 47 subjects, 3 (6.38%) were DUI of alcohol at the time of the accident.

**Table 3.2** Profile of drinking and driving behavior.

| | | | | |
|---|---|---|---|---|
| **Drive** (*n* = 162) | Yes | 103 (63.3%) | Car | 78 (75.7%) |
| | | | Motorcycle | 25 (24.3%) |
| | No | 59 (36.4%) | | |
| **In the last year** | | | | |
| Consumption of any alcoholic beverage in any quantity (n = 162) | | | | |
| | Yes | 128 (79%) | 5 or more times a week | 7 (5.5%) |
| | | | 1 to 4 times a week | 20 (15.6%) |
| | | | 1 to 3 times a month | 52 (40.6%) |
| | | | Less than one time per month | 49 (32.3%) |
| | No | 34 (21%) | | |
| Doses* of alcohol ingested in a normal day of consumption (n = 128) | | | | |
| | | | 1 to 5 doses | 85 (66.4%) |
| | | | 6 to 12 doses | 31 (24.2%) |
| | | | >13 doses | 12 (9.4%) |
| Drives after work (n = 103) | | | | |
| | | | Always | 54 (52.4%) |
| | | | Almost always | 14 (13.6%) |
| | | | Almost never | 15 (14.6%) |
| | | | Never | 20 (19.4%) |
| Consumed alcohol immediately before driving (n = 87) | | | | |
| | Yes | 26 (29.9%) | | |

| | |
|---|---|
| No | 61 (70.1%) |
| **Has suffered a traffic accident (n = 162)** | |
| Yes | 58 (35.8%) |
| No | 104 (64.2%) |
| **Drives and Was the driver at the time of traffic accident (n = 50)** | |
| Yes | 47 (94%) |
| No | 3 (6%) |
| **Drives, Was the driver at the time of traffic accident, and Was under the influence of alcohol at the time of the accident (*n* = 47)** | |
| Yes | 3 (6.38%) |
| No | 44 (93.62%) |

*Dose: Beer – 350 mL; Wine – 90 mL; distillates – 50 mL.

### *3.2 Exhaled air and oral fluid*

From 162 exhaled air samples, only one showed positive result (0.03 mg/L) for breathalyzer, while in OF samples, all subjects, that is, 162 gasoline station attendants had ethanol concentrations within the limits of detection of the method (0.0005 g/dL).

From the 162 subjects with positive ethanol in OF sample, 118 (72.83%) had ethanol concentrations above the limit of quantification (0.00125 g/dL) of the method, but below the lower limit of quantification (0.005 g/dL) of the calibration curve, except for one subject who showed ethanol concentration of 0.005 g/dL in the OF sample.

The exhaled air positive sample is from the same subject that showed ethanol concentration of 0.005 g/dL in the OF sample. Since only one gasoline station attendant had quantitative level for ethanol in OF, from all gasoline stations, and only one exhaled air sample showed positive results as well, the results did not show variability, therefore it was not possible to make statistical tests for paired groups.

From 162 positives for ethanol in OF samples, when analyzed by age, the highest proportion of positive ethanol in OF samples were subjects aged 25-36 years (35.19%), followed by 18-25 years (33.95%) (Figure 3.1).

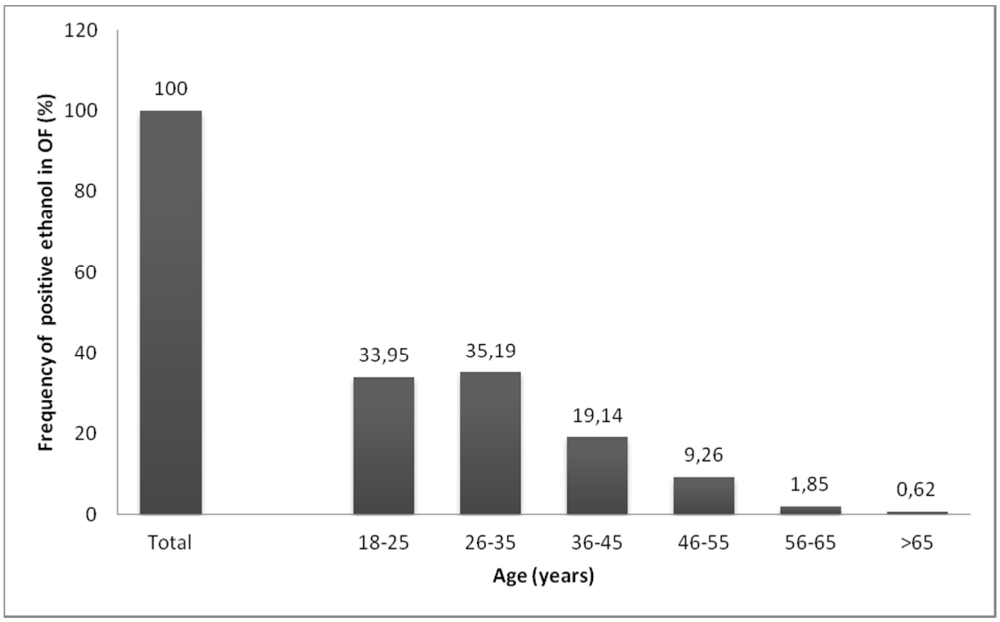


**Figure 3.1** Frequency of positive ethanol in OF (%) vs. Age.

Figure 3.2 shows an example of chromatogram obtained with the HS-GC/MS analysis for positive ethanol in OF sample, spiked with internal standard (*n*-propanol).

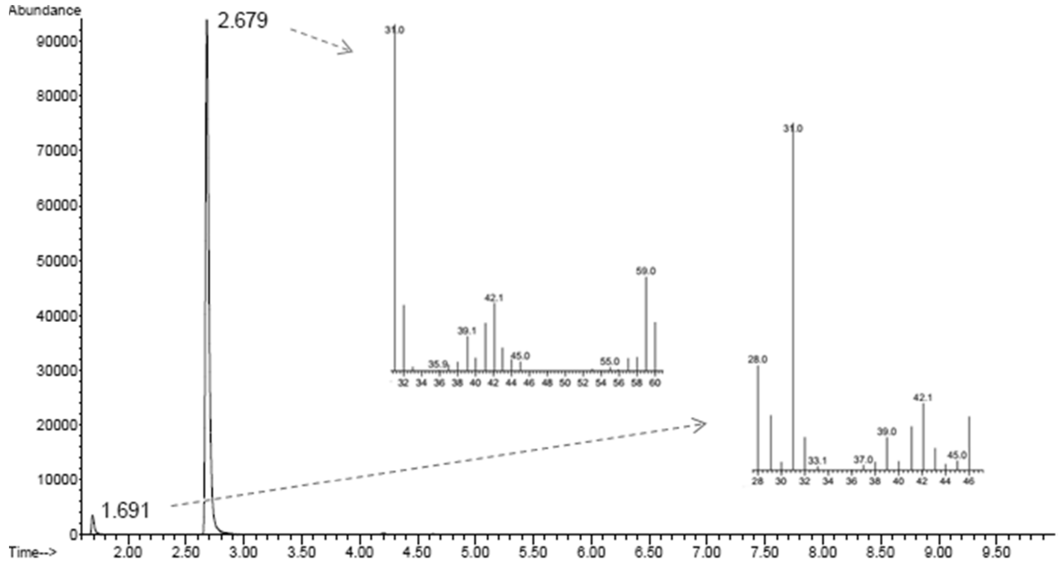


**Figure 3.2** Chromatogram obtained with HS-GC/MS analysis for ethanol (1.691 min) in OF sample, spiked with internal standard (n-propanol) (2.679 min).

## 4. Discussion

The definition and enforcement of BAC limits of 0.05 g/dL may lead to significant reductions in accidents related to alcohol consumption worldwide. Since 2008 there has been an advance in laws on DUI, and currently, 66% of the world's population applies a BAC limit of 0.05 g/dL or less. This limit is justified considering that above this concentration the risk of accidents significantly increases [2,3].

Oral fluid proved to be a great biological matrix to be applied in on-site collection approaches. The findings through the application of OF in this study corroborates with the previously mentioned advantages of this matrix, such as easy and quick collection, the lack of constraint, the low potential for tampering by the subjects, and the lack of need for specialized professionals.

Although the most widely used technique for confirmation of blood alcohol concentration in forensic laboratories is currently through headspace gas chromatography coupled with flame ionization detector (HS-GC/FID), it allows the occurrence of false positives with alcohols and other volatile compounds, such as methanol and other solvent results. Thus, the use of a HS-GC/MS showed the technique may be considered the "gold standard" for confirmation of unquestionable results.

With the results described, it may be inferred that OF analysis by HS-GC/MS shows more positive results than breathalyzer for ethanol analysis, when in very low concentrations, that is, it seems to be more sensitive. While only one breathalyzer showed positive ethanol results (0.62%), 100% (162 subjects) of OF analysis by HS-GC/MS showed positive ethanol results. These positive samples, 72.83% (118 samples) were above the quantification limit of the method, however, below the lower quantification limit of its calibration curve, and were considered semiquantitative samples.

Although one sample has tested positive for the breathalyzer, the test reading was below the limit recommended for maximum permissible error, generating uncertainty about the test, thus demonstrating the importance of conducting confirmatory analysis for breathalyzer, and the need for confirmatory sample to be collected in the nearest possible period from testing. Therefore, positive results in OF samples and negative results in exhaled air samples may be explained by the fact that the method of analyzing OF by HS-GC/MS is more sensitive than by the breathalyzer based on electrochemical cell sensor.

In Brazil the fuels used and sold legally are: the ethanol fuel, gasoline, which is added in a percentage of 25% ethyl alcohol, and diesel, which has no alcohol in the composition. Exposure to ethanol in fuels is probably responsible for 100% of positive results in OF samples. Despite the low concentrations found in OF of gasoline station attendants, ethanol concentration, in this matrix, depends on ethanol concentration in the blood. However, in some traffic legislations the limit allowed for ethanol in the blood of drivers is equal to zero (e.g., in Brazil) so that any level of ethanol detected may ultimately generate legal complications for the driver.

An advantage compared to OF and blood is that OF can be collected at the location of approach, immediately after the breathalyzer. Rather for blood, it is required to move the subject to the laboratory, and depending on the delay, it is possible that either the period of detection passes or a very distinct concentration is found over the ethanol concentration in the subject when they were approached.

Due to the discussion of the inability for breathalyzer test report in the presence of interfering volatile organic compounds [7,8], such as ketone bodies produced in prolonged fasting, specific diets, and in diabetic subjects [7,8,11,39], the population was stratified and assessed. Although 4.3% gasoline station attendants have reported being diabetic, and 45.8% reported that a close relative has the disease, nevertheless any interference was observed in this study.

Whereas all population worked, on average, 8 hours per day, the frequency of positive ethanol in OF was higher among 26-35 year-old subjects, which is the same age group of the population majorly involved in traffic accidents, as demonstrated in several studies, including in Brazil [4,40-44]. According to the World Health Organization, traffic accidents are the second cause of death among persons aged 15 to 29 years [2].

Because women have more fat and less body water per kg of body weight than men, they eliminate alcohol at a faster rate. Men and women generally have similar alcohol elimination rates when results are expressed as grams per hour. It is possible that a given oral dose of alcohol may produce a higher BAC in women than in men due to first-pass metabolism [19,45]. Although described in the literature, differences in elimination rate between gasoline station attendants were not observed since the exposure time for both genders was the same and all individuals, both male and female, showed positive results for ethanol in OF samples.

## 5. Conclusions

The present study allowed the analysis of the presence of ethanol in gasoline station attendants by the breathalyzer, while qualitatively and quantitatively analyzing OF by HS-GC/MS.

The results presented here demonstrate that the breathalyzer is less effective when compared to OF analysis by HS-GC/MS, showing the importance of confirmatory analysis for the breathalyzer. Although it was not possible to quantify all OF samples, the breathalyzer showed negative results while the OF samples by HS-GC/MS showed positive results.

As shown in several studies, this work proved that OF samples are an increasingly promising sample for forensic analysis, particularly for analyzes where the collection of the biological matrix is required at the site of approach.

The results of this study show the need for further research, both regarding exposure to ethanol and gasoline by gasoline station attendants, and to the effectiveness of the tests performed with breathalyzers, especially when it comes to traffic laws using this test to assess drivers in traffic, also considering the tolerance limit equal to zero for BAC, such as the Brazilian law.

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