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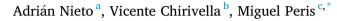
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Research article

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Measuring the effects of a hydrogen peroxide mouth rinse on breath alcohol values



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ABSTRACT

The high consumption of alcoholic drinks has become acceptable in many societies and is often promoted by commercials. Unfortunately, many people risk their lives by driving drunk. They even try to outsmart breathalyzer tests, for example, using a novel procedure based on the partial oxidation of expired breathed ethanol after rinsing the mouth with diluted hydrogen peroxide. To check the validity of this procedure, the different variables involved in the process were tested: the type of alcoholic beverage, the amount of ethanol swallowed, and the time elapsed between consumption and mouth rinsing. Our ultimate aim was to measure the effects of this process. If the mouth rinse succeeds in masking a drinker's true alcohol level, then further study of possible remedies is needed to prevent such fraud. However, if the rinsing proves to have no effect, then this work could help strengthen the integrity of the breathalyzer test and its ability to deter drivers from overdrinking. The final conclusion, after all the experiments, is that a reduction in the alcohol level is observed with the use of hydrogen peroxide as a mouthwash before performing a breathalyzer test.

1. Introduction

Alcohol is a type of drug that causes numerous problems, both social and health problems, within the sociocultural environment. Out of 100 fatal accidents, it has been calculated that alcohol is the cause of 30–50 (World Health Organization, 2019), most of which are traffic accidents. In this sense, it is mandatory to conduct breathalyzer tests for drivers, as well as any other person who is involved in an accident. This test determines the degree of alcohol, which represents the volume of alcohol in the blood and is measured in grams of alcohol per liter of blood (g L⁻¹) or rather its equivalent amount in exhaled air (Anderson and Hlastala, 2019). This last test is usually conducted since it can be carried out "*in situ*". Nevertheless, some authors (Brown, 1994) point out that if a person has used mouthwash the test results may be skewed. In this sense, Hair and co-workers (Hair et al., 2019) have recently developed a new procedure for optical ethanol sensing on the skin surface with camera-based quantification.

Alcohol can begin to be detected in the blood within 5 min of drinking (Schug, 2016) and its level increases rapidly (rising phase), a maximum being reached between 30 and 90 min afterward. Then, the curve appears to stabilize for a short period of time (plateau), after which the alcohol level begins to drop slowly (descending phase) until the complete

removal of alcohol from the blood (in certain cases, it can occur up to 19 h after the first drink).

The path of alcohol through the body begins when it is absorbed from the stomach, small intestine and colon and then passes into the blood. The higher the alcoholic strength of the beverage, the faster the assimilation is. On the other hand, if alcohol is mixed with carbonated drinks, they accelerate the absorption of alcohol by the body. After that, alcohol is uniformly and rapidly distributed throughout all tissues of the body, followed by its metabolization, with between 90 % and 98 % being almost exclusively oxidized in the liver and the rest through urine, sweat or breath. The last stage is the elimination of alcohol, which takes place at a constant rate of 8–10 g per hour, regardless of the alcohol level (Spanish Ministry of Health) (Alvarez and Del Río, 2001).

A common method used by police to determine the approximate concentration of ethanol in the blood consists of the fact that an equilibrium is formed in the lungs that relates this concentration to the concentration of ethanol vapor in the expired air. This air is introduced into the police breathalyzer at their request through the corresponding nozzle. The reading of the device is virtually instantaneous and allows the police to check whether the person is exceeding the legal limit established by the authorities.

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The objective of this study was thus to determine the "viability" of a method of deception based on causing the partial oxidation of ethanol (CH₃–CH₂–OH) present in the exhaled air just before passing through the nozzle of the breathalyzer. This can be carried out by rinsing with a mouthwash (H₂O₂, diluted hydrogen peroxide) available in pharmacies in such a way that a redox reaction occurs: ethanol is oxidized to ethanal (CH₃–CHO), whereas hydrogen peroxide is reduced to water (H₂O) (Petrucci et al., 2017).

This study focuses on verifying whether this system is effective in avoiding a positive breath test to raise awareness among the authorities of this possible fraud. If the aforementioned reaction takes place to an appreciable extent, then it would be necessary to warn about this illegality that could be committed in alcohol tests conducted on the roads. Alternatively, if the reaction hardly occurs, this fact will serve as a deterrent against drinking too much, since drivers will be well aware that this procedure will not "help" them if they have drunk alcohol and will be checked for drunk driving.

Two hypotheses have been established in this work, namely:

- Rinsing with hydrogen peroxide does not affect the results obtained by the breathalyzer
- The hydrogen peroxide rinse cannot cause the drunkenness test to indicate a negative result, when it really must be positive.

2. Materials and methods

2.1. Reagents

Three percent (v/v) neutral stabilized hydrogen peroxide was used in this study, which is utilized for mouthwash before the appearance of sores in the mouth or after dental extractions. Its repeated use in oral rinses is not recommended, as it can cause irritation of the oral mucosa ("hairy" tongue).

The amount of hydrogen peroxide (H_2O_2) used for each test was 10 mL, since this is the recommended volume for an oral rinse with any type of mouthwash.

2.2. Instrumentation

A Zaphir CDP 2000 Professional Breathalyzer was used with a measurement range of 0.00–2.00 g L^{-1} , response time of $<\!5$ s, and warm-up time of $<\!20$ s.

2.3. Alcoholic beverages used

Tests were carried out to obtain results with three types of alcoholic beverages with different alcohol contents: Beer ([ethanol] = 5.5 % v/v), wine ([ethanol] = 11.9 % v/v) and, finally, a distilled beverage (gin) ([ethanol] = 41.2 % v/v). All of them were purchased in local shops.

2.4. Experimental design

A data collection template is developed to obtain values to check if this procedure (mouth rinsing) causes partial oxidation of the ethanol present in exhaled air. Our experimental design (Montgomery, 2012) takes into account some factors that can influence a person's blood alcohol level. The factors considered are the following: the type of beverage, the amount of alcohol (mL) consumed, and the time in minutes elapsed between the ingestion of the alcoholic drink and the rinse with the hydrogen peroxide mouthwash.

To determine the number of individuals who would perform the test, the starting point was the idea of using a regression model to analyze the results. This model would quantify the effect of each type of drink, a quadratic effect of the amount of alcohol, and a cubic effect of the time to rinse, which means that 36 parameters have to be assessed, and therefore at least 36 observations are required. Since it is intended not to depend on non-significant effects to have sufficient power in the hypothesis tests, the number of tests should be greater. With gin as the limiting factor for the number of volunteers, five alcohol levels and four different times before rinsing were proposed, 20 candidates for this type of beverage being required. With not so many problems for beer and wine, 40 candidates were found for the experiments. This offers a total of 100 people for testing, and 63 residual degrees of freedom, which offers sufficient power in hypothesis testing.

In short, we decided to conduct the test with 100 people in such a way that 40 of them drank beer, 40 drank wine, and the remaining 20 drank distillate (gin). Each drinking group was divided into 4 parts, and each subgroup was assigned a waiting time until they performed the rinse, namely, 5, 10, 15, and 20 min. Finally, the range of the number of glasses that would lead to a similar interval in the amount of alcohol ingested was calculated according to the drink and the time to rinse, and the membership of each subgroup was drawn among the volunteers.

Each volunteer started their own "drink and wait" program. After the drinking quantities were achieved, the breathalyzer levels were measured in the exhaled air. Following the assigned waiting time, the rinses were then carried out, and the new alcohol levels in air were measured. Individuals who did not comply with the amounts of alcohol or with the waiting times that were originally assigned were noted, and they were reclassified into the corresponding group.

All experimental protocols were conducted according to the ethics guidelines and the Declaration of Helsinki, including informed consent obtained from each patient.

Ethical approval.- Research involving human subjects complied with all relevant national regulations and institution policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' institutional review board (ECER - *Ethical Commission of Experimental Research* of the Polytechnic University of Valencia). Informed consent was obtained from all individuals included in this study.

2.5. Method fundamentals

Hydrogen peroxide in the presence of a reducing agent can act as a strong oxidant, itself being reduced to water:

 $H_2O_2+2H^++2e^-\rightarrow 2H_2O\quad E=+1.77v$

In this case, this reducing agent is ethanol:

$$CH_3CHO + 2H^+ + 2e^- \rightarrow CH_3CH_2OH \quad E^o = +0.19v$$

Therefore:

Global reaction:
$$\begin{array}{l} H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \\ \frac{CH_3CH_2OH + 2e^- \rightarrow CH_3CHO + 2H^+}{CH_3CH_2OH + H_2O_2 \rightarrow CH_3CHO + 2H_2O} \end{array}$$

Ethanol is oxidized to ethanal, and hydrogen peroxide is reduced to water. According to the respective standard potentials:

$$\Delta E^{\circ} = E^{\circ}_{\text{oxidant}} - E^{\circ}_{\text{reductant}} = (+1.77) - (+0.19) = +1.58 \text{ v}$$

 $\Delta E^{\circ} > 0$, *i.e.*, $\Delta G^{\circ} < 0$ spontaneous reaction

In this way, the overall redox reaction will have a positive ΔE° , thus indicating a thermodynamically favorable process.

In view of the theoretical spontaneity of the reaction, it is then decided to carry out a more detailed study to check whether this redox reaction might occur in alcohol tests, under what conditions, and what factors influence the extension of this reaction. Considering that hydrogen peroxide (3–10 volumes), often used as a mouthwash (mouth rinse), is a fairly powerful oxidizing agent and that ethanol acts as a reducing agent, it can be inferred that it can be an effective method when facing a breathalyzer test. The ethanol content measured by the device would be lower than expected since part of it will be oxidized by the

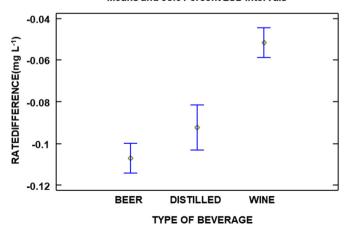
Table 1. ANOVA for the factors and interactions considered.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
ALCVOLUME	0.11796200	1	0.117962	103.56	0.0000
ALCVOLUME2	0.00030515	1	0.000305	0.27	0.6066
TIME2WM	0.00605589	1	0.006056	5.32	0.0244
TIME2WM2	0.00007733	1	0.000077	0.07	0.7953
TIME2WM3	0.00024460	1	0.000245	0.21	0.6447
BEVERAGE	0.06443264	2	0.032216	28.28	0.0000
BEVERAGE*ALCVOLUME	0.00321847	2	0.001609	1.41	0.2511
BEVERAGE*ALCVOLUME2	0.00160040	2	0.000800	0.70	0.4992
BEVERAGE*TIME2WM	0.00032981	2	0.000165	0.14	0.8655
BEVERAGE*TIME2WM2	0.00175531	2	0.000878	0.77	0.4671
BEVERAGE*TIME2WM3	0.00019453	2	0.000097	0.09	0.9183
ALCVOLUME*TIME2WM	0.00000137	1	0.000001	0.00	0.9725
ALCVOLUME*(TIME2WM2)	0.00017652	1	0.000177	0.15	0.6952
ALCVOLUME*(TIME2WM3)	0.00005485	1	0.000055	0.05	0.8270
(ALCVOLUME2)*TIME2WM	0.00067985	1	0.000680	0.60	0.4427
(ALCVOLUME2)*(TIME2WM2)	0.00144064	1	0.001441	1.26	0.2650
(ALCVOLUME2)*(TIME2WM3)	0.00003015	1	0.000030	0.03	0.8713
BEVERAGE*ALCVOLUME*TIME2WM	0.00122639	2	0.000613	0.54	0.5864
BEVERAGE*ALCVOLUME*(TIME2WM2)	0.00286278	2	0.001431	1.26	0.2916
BEVERAGE*ALCVOLUME*(TIME2WM3)	0.00046486	2	0.000232	0.20	0.8160
BEVERAGE*(ALCVOLUME2)*TIME2WM	0.00021419	2	0.000107	0.09	0.9104
BEVERAGE*(ALCVOLUME2)*(TIME2WM2)	0.00192936	2	0.000965	0.85	0.4336
BEVERAGE*(ALCVOLUME2)*(TIME2WM3)	0.00229883	2	0.001149	1.01	0.3704
MODEL	0.20755592	35	0.005930	5.21	0.0000
RESIDUAL	0.07176008	63	0.001139		
TOTAL (CORRECTED)	0.27931600	98			

hydrogen peroxide that remains in the mouth after rinsing. The problem arises, then, from the fact that the value obtained in the breathalyzer would not be representative because the blood will still have the actual alcohol content. Furthermore, this can be dangerous because the potential driver could think that if the test is negative, there is no reason to stop driving.

2.6. Data analysis

Following the procedure outlined above, a total of 100 results were obtained. Thereafter, the decrease in the alcohol level due to rinsing is calculated as the difference between the respective rates after and before



Means and 95.0 Percent LSD Intervals

Figure 1. LSD (Least Significant Difference) intervals for the three types of beverages.

mouth rinsing. After applying descriptive statistic techniques using the Statgraphics Centurion 18 program (Statpoint Technologies Inc., 2017), a clearly anomalous value was found, which was removed from the data set.

To determine whether the reduction in the alcohol level in air due to the use of rinsing is affected by the three selected factors, as well as their interactions, an analysis of variance (ANOVA) was performed; it was further complemented with a linear regression model to determine the existence of nonlinear effects on the quantitative factors (Draper and Smith, 1998).

Additionally, the influence of the selected factors on the probability of passing the breathalyzer test while drunk was determined, according to the limit established by Spanish legislation (0.25 mg L^{-1}). A logistic regression model (Kleinbaum et al., 2010) was used in this probability analysis.

3. Results and discussion

In the following sections, we present the results obtained for the two proposed analyses: the effect of the type of drink, the amount of alcohol swallowed, and the time elapsed between the first and second tests on the decrease in blood alcohol level after rinsing and (on the other hand) the

Table 2. Parameter estimation of the fitted regression model.				
Parameter	Estimate	Standard Error	T Statistic	P-Value
CONSTANT	-0.0286244	0.0101517	-2.81967	0.0059
WINE	0.0562037	0.00699831	8.03103	0.0000
ALCVOLUME	-0.00227123	0.000200666	-11.3185	0.0000
TIME2WM	0.00124011	0.00058325	2.1262	0.0361
DISTILLED*ALCVOLUME	0.0003433	0.000177104	1.93841	0.0556

Plot of RATEDIFFERENCE vs TIMEWR

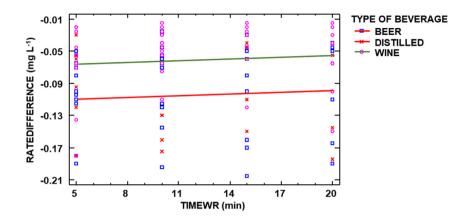


Figure 2. Relationship between the difference in the alcohol level and the time elapsed between ingestion and rinsing.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
WINE	0.0556777	1	0.0556777	57.56	0.0000
ALCVOLUME	0.124643	1	0.124643	128.87	0.0000
TIME2WM	0.00444155	1	0.00444155	4.59	0.0347
DISTILLED*ALCVOLUME	0.00363428	1	0.00363428	3.76	0.0556
Model	0.188397	4			

effect of these same factors on the probability of passing the breathalyzer test while being drunk.

3.1. The effect on the difference in the blood alcohol level

The difference in blood alcohol levels [(after mouth rinsing)-(before mouth rinsing)] measured in exhaled air is studied in this section. The differences are always negative, thus showing that blood alcohol levels always decrease after rinsing. The available variables are as follows: BEVERAGE (type of drink), ALCVOLUME (amount of alcohol swallowed), and TIMEMR (time elapsed between the first and second tests).

After performing an ANOVA on the difference in blood alcohol levels, all three factors were found to be significant. Given the possible existence of nonlinear relationships of the factors, a regression model is proposed that allows us to analyze the presence of a nonlinear relationship between (a) the difference in blood alcohol levels and the volume swallowed and (b) the difference in blood alcohol levels and the time elapsed until mouth rinsing. Moreover, these relationships could differ depending on the type of beverage chosen.

The following conclusions were then obtained:

3.1.1. Effect of the type of beverage

Generally, there was an effect of the type of drink on the difference in blood alcohol level, as indicated by a P-value of 0.000, which was less than 5 % (see Table 1).

Figure 1 shows the LSD (least significant difference) intervals for the types of beverages. Once we know that the effect of the type of drink exists, we observed that the difference in blood alcohol level due to the mouthwash is the same for beers and spirits, as shown by the overlap of their intervals, with an expected value close to -0.10 mg L^{-1} , and only the LSD interval of wine is clearly separated from the other two, so it can be inferred that wine has a different behavior, with an average value close to -0.05 mg L^{-1} . Here, in general, mouthwash works worse with wine than with the other beverages, since its decrease in the blood alcohol level is lower, at $0.0562037 \text{ mg L}^{-1}$ (according to Table 2).



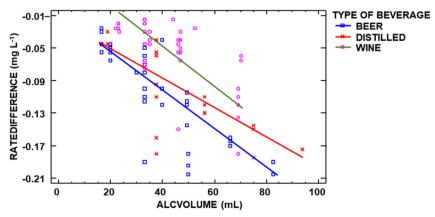


Figure 3. Relationship between the amount of alcohol ingested and the effect of the rinse.

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Table 4. Likelihood ratio test	for the	variables.
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Factor	Chi-Square	Df	P-Value
ALCVOLUME	5,27547	1	0,0216
ALCVOLUME2	6,98644	1	0,0082
TIMEMR	1,64965	1	0,1990
TIMEMR2	2,49786	1	0,1140
WINE	6,61516	1	0,0101
DISTILLED	0,0116362	1	0,9141

Table 5. Estimated logistic regression model.

Parameter	Estimate	Standard Error	Estimated Odds Ratio
CONSTANT	-7,30252	5,71376	
ALCVOLUME	0,342036	0,207411	1,40781
ALCVOLUME2	-0,00326404	0,00179996	0,996741
WINE	16,6485	100,002	1,6997E7

3.1.2. Effect of the time elapsed from ingestion to rinsing

Overall, the sooner the mouth rinse is performed, the greater the decrease in blood alcohol level. In Table 1, it is shown that the decrease is linear (proportional) with the time until mouth rinsing (TIMEWR) with a P-value of 0.0244, but there are no quadratic or cubic effects (TIMEWR^2 and TIMEWR^3) by their respective P-values of 0.7953 and 0.6447. In this case, the difference in blood alcohol levels decreased at a rate of 0.00124 mg L⁻¹ per minute elapsed before mouth rinsing, as summarized in Table 2. Figure 2 shows the relationship between the difference in the alcohol level and the time elapsed from the end of beverage ingestion to rinsing.

3.1.3. Effect of the volume of alcohol ingested

In general, the difference in blood alcohol level increases with increasing amount of alcohol swallowed, *i.e.*, the mouth rinse is more effective. The effect is proportional to the amount of alcohol, at least in the range of values studied, since as described in Table 1, the linear component (ALCVOLUME) is significant with a P-value of 0.0000, unlike the quadratic component (ALCVOLUME[^] 2) with a P-value of 0.6066.

Now, both beer and wine show the same decrease in the alcohol level with the amount of alcohol ingested, at a rate of 0.002271 mg L⁻¹ per mL of alcohol, as shown in Table 2. However, distilled beverage presents a different behavior, according to a P-value of 0.0556 observed in Table 3 (DISTILLED * ALCVOLUME). Here, the ratio was -0.00227123 + 0.0003433 = -0.00192793 mg per mL of distilled beverage (Table 2).

In summary, the greater the amount of alcohol ingested, the greater the effect of the rinse, although this effect is more important in beer and wine than in distillate, as shown in Figure 3.

3.2. Effect on the result of the breathalyzer test

The probability that a person that is considered legally drunk (alcohol in exhaled air over 0.25 mg L⁻¹) passes the breathalyzer test is now analyzed. This probability can be affected by the type of drink swallowed, the amount of alcohol consumed, and the time elapsed before mouth rinsing. For this analysis, the binary variable DRUNKBT has been defined, which identifies (value of 1) individuals who have ingested enough alcohol to test positive, as well as the binary variable PASSTEST, which indicates (value of 1) if a person has managed to pass the test despite still being drunk. The effect of the mouthwash on the breathalyzer test is, to say the least, worrying, since in our data, a total of 24 of the 32 people who were legally drunk managed to test negative.

3.2.1. Effect of the type of beverage

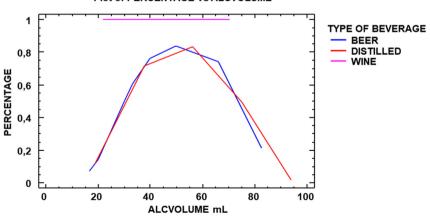
The type of beverage influences the probability of passing the breathalyzer test while being drunk. In the analysis, BEER was chosen as a reference, and WINE and DISTILLED appear in Table 4 to compare their effects. The variable that indicates distillates (DISTILLED) presents a P-value of 0.9141, which is greater than 0.05, so that beer and spirits have the same probability of passing the breathalyzer test while drunk. On the other hand, the P-value of wine (WINE) is 0.0101, which is less than 0.05, and therefore, the probability of passing the test is different if wine has been ingested instead of beer or distillate. Given that the estimation of the parameter of this variable in Table 5 is positive, it must be remarked that the probability of testing negative while drunk (with wine) is much higher than when beer or distillates have been ingested. In fact, the data show that all people who were legally drunk from drinking wine managed to pass the test, although in the data, there were no great differences in the blood alcohol level by the type of drink.

3.2.2. Effect of the time from ingestion to mouth rinsing

The time elapsed before mouth rinsing has no influence on the probability of passing the test while being drunk. This is deduced from Table 4, where there is neither a linear time effect (TIMEMR) (a P-value of 0.19990) nor a quadratic effect (TIMEMR2) (a P-value of 0.1140).

3.2.3. Effect of the volume of alcohol ingested

There is an effect of the volume of alcohol ingested on the probability of passing the breathalyzer test while being drunk. As seen in Table 4, the P-values of the linear (ALCVOLUME) and quadratic (ALCVOLUME²)



Plot of PERCENTAGE vs ALCVOLUME

Figure 4. Probability of passing the test while drunk vs. the amount of alcohol ingested.

components are 0.0216 and 0.0082, respectively. Since the estimates shown in Table 5 actually refer to the *oddratio* of the probability of passing the test while drunk, it would be better to look at Figure 4 to understand what is happening. In this figure, it can be noticed that for beer and distillate, the probability of passing the test while drunk increases with the amount of alcohol ingested until it reaches a maximum (approximately 50 mL) and then decreases. The exact value is difficult to determine because of the great variability in the data, which causes a person to be drunk with 18.75 mL of alcohol or sober with up to 56.25 mL of alcohol.

Despite these alarming results, if the breathalyzer limit imposed on professional drivers (0.15 mg L^{-1}) is considered, none of the 96 legally drunk individuals could test negative after mouth rinsing; therefore, a further tightening of the limit could avoid what we might call the "mouthwash trick".

4. Conclusions

Ultimately, we conclude that we can confirm the "feasibility" of this procedure for the objective pursued by those who may resort to this trick, since a reduction in the alcohol level is observed with the use of hydrogen peroxide as a mouthwash before performing a breathalyzer test. In general, the decrease in the alcohol level due to the use of mouthwash is greater for beer and distillate and much less for wine. In all cases, the sooner the mouth rinsing is done after drinking alcohol, the greater the effect; furthermore, this effect increases with increasing alcohol intake and is greater for beer and wine than for distillate.

On the other hand, it is observed with alarm that people who are legally drunk (>0.25 mg L⁻¹) are very likely to pass a breath test after rinsing with mouthwash. With similar blood alcohol levels and ingested amounts of alcohol in the three types of beverages, the probability of passing the test while drunk is very high in the case of wine and somewhat less with beer or spirits. This probability depends on the amount of alcohol ingested, with a maximum of approximately 50 mL.

Therefore, the present study (to avoid determining the actual blood alcohol level) is intended to alert of a possible fraud that could take place in breathalyzer tests performed on streets and roads, as well as to let the authorities know the "effectiveness" of H_2O_2 mouthwash for such situations. It is true that, in case of doubt regarding the goodness of the measurement of the breathalyzer, sometimes the possible offender is taken to undergo a blood test, which would reveal the real alcohol level in the blood (although somewhat lower due to the elapsed time) and would expose the fraud, but it is well known that for reasons of expediting the legal process, the result of the breathalyzer is accepted most of the time without further questions.

Declarations

Author contribution statement

Adrián Nieto: Performed the experiments; Analyzed and interpreted the data.

Vicente Chirivella: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Miguel Peris: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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