Original Article

A Novel Validated Gas Chromatography-Flame Ionization Detector Method for Measuring Methanol and Ethanol in Blood Samples Using a Solvent Partitioning Extraction Technique

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ABSTRACT

Background: The measurement of blood alcohol levels is a routine analysis carried out in many forensic laboratories. Headspace and Solid-Phase Micro-Extraction techniques coupled with Gas Chromatography have emerged as the method of choice for alcohol detection in blood samples, which is too expensive and unavailable in most clinical laboratories in developing countries. **Aim:** The objective of this study was to validate and develop an efficient and sensitive Gas Chromatography-Flame Ionization Detector analytical method for detecting and quantifying methyl and ethyl alcohol in blood by solvent partitioning extraction technique without the need for Headspace techniques. **Methods:** Analysis was performed on sheep's whole blood using chloroform as the extracting solvent and dichloromethane as the internal standard. Linearity was achieved across a concentration range of 10 mg to 320 mg/dl for methanol and ethanol. The developed method was fully validated according to the guidelines of the Scientific Working Group for Forensic Toxicology. **Results:** The linearity of the method showed a correlation coefficient of 0.999 and 0.998 for whole-blood methanol and ethanol, respectively. The limit of quantification (LOQ) and limit of detection (LOD) were within the range of 7.2–10 mg/dl and 4.0–8.0 for methanol and ethanol in whole blood, respectively, and resulted in a precision of 1.17–6.5 and 0.79–2.98 and an accuracy of 1.07–5.27% and 0.37–3.6% for methanol and ethanol, respectively. **Conclusion:** This new method was a much more convenient and reliable process to measure methanol and ethanol in whole blood samples by reducing sample pretreatment effort and cost with no need for Headspace techniques.

Keywords: Methanol, Ethanol, GC-FID, Partition coefficient, Partition extraction etc.

I. INTRODUCTION:

Methanol and ethanol are important aliphatic alcohols in the field of chemistry, with chemical formulas CH3OH and C2H5OH, respectively. Their molecular weights are 32.04 g/mol and 46.07 g/mol. These versatile chemicals find application in various industries such as solvents, fuels, resins pharmaceuticals, antifreeze, pesticides, electronics, and methanol is utilized as a denaturant to make alcohol unfit for consumption (industrial ethanol) (Ertas et al., 2013; Kaneko et al., 1994).

Methyl alcohol intoxication is a severe and potentially deadly health condition. While methanol itself is not toxic, its metabolites are extremely toxic. This can pose a significant danger to individuals who consume methyl alcohol, as the resulting metabolites can cause severe harm to the body. It is crucial to seek immediate medical attention if you suspect methyl alcohol intoxication. The conversion of methanol occurs through the action of the alcohol dehydrogenase enzyme (ADH) in the liver. This process results in the formation of formaldehyde, which is further converted to formic acid by aldehyde dehydrogenase. Formaldehyde, a known carcinogenic compound, has been linked to age-related damage in brain neurons. Similarly, formic acid is associated with increased blood acidity, ocular toxicity, and optic nerve damage. It also disrupts energy production in mitochondria, leading to tissue damage in organs. The severity of these complications depends on the amount ingested. As little as 10 ml of methanol can result in permanent blindness, while consumption of 100 to 200 ml can be fatal in most instances. It is crucial to handle these substances with extreme caution due to their potential health risks (Kaneko et al., 1994; Portari et al., 2008). The analysis of methyl alcohol levels in the bloodstream is crucial for diagnosing cases of accidental or intentional methanol poisoning, which can result in a significant mortality rate even with intensive medical attention (Boswell and Dorman, 2015).

Ethyl alcohol is used more frequently and in enormous amounts around the world and it is a psychoactive compound. Alcohol consumption is linked with different crimes as, sexual abuse and Car road accidents so alcohol detection is very important issue in forensic medicine basically in post mortem analysis (Pawliszyn, 1977).

Hamajima et al. (2002) stated that ethanol consumption is a cause of cancer, and 10 g of ethanol per day is enough to increase the risk of breast cancer by 6–10%. Acetaldehyde is a metabolite of ethanol that forms DNA

adducts (Abraham et al., 2011); on the other hand, Kukoski et al. (2007) said that the evidence on the genotoxic carcinogenesis of ethanol is generally weak.

The analysis of blood alcohol concentration is a routine analysis performed in many forensic laboratories. The Headspace technique coupled with Gas Chromatography (HS-GC) has been considered the method of choice for methanol and ethanol detection and quantification for medico-legal analysis and scientific research objectives (Tiscione et al., 2011). Solid Phase Micro Extraction (SPME) is a solvent-free sample preparation technique created by Pawliszyn in the early 1990s that allows immediate sampling, extraction, pre-concentration, and introduction of analytes from a matrix in a single procedure (Pawliszyn et al., 1997). The SPME technique depends on immersing the coated silica fiber with a suitable stationary phase in the sample matrix, where the alcohol molecules are adsorbed on the fiber's surface and then thermal desorption occurs to alcohol molecules when they are transferred to the GC. SPME can be combined with the headspace system to eliminate interference (Ertas et al., 2013).

These methods measure the concentration of alcohol molecules in the head space (gas phase) over the sample (liquid phase) in sealed vials. In contrast, the partition coefficients (K) of methyl and ethyl alcohols between the aqueous phase (sample) and the gas phase at 37^{o} C are very high (2140 and 3330, respectively) (Kaneko et al. 1994). This means that there are 2140 and 3330 times the volumetric concentrations of ethanol and methanol in the sample than in the headspace at 37^{o} C. When the partition coefficient is below 1.0, the analyte has a preference for the headspace. Conversely, when the partition coefficient exceeds 1.0, the analyte shows a preference for the liquid phase. This means that it should be suitable to use headspace sampling to extract light hydrocarbons from aqueous solutions, but it is more difficult to extract alcohols from aqueous solutions (Bullock, 2009).

The sample matrix is a significant issue when considering the intermolecular interactions of the solutes and solvents. Although methanol and ethanol are volatile, when they are miscible with blood samples, the hydrogen bond formation decreases the volatile ability of alcohol molecules, leading to an increasing partition coefficient between the sample and the headspace, leading to a decrease in the concentration of alcohol in the headspace (Boswell and Dorman, 2015). To overcome this problem, they heat the sealed vials to increase the alcohol molecules in the

sample's headspace, which increases the intra-vial pressure and may cause the vials' explosion. On the other hand, this heating also increases other molecules in the headspace, such as H2O, which may increase noise and column bleeding (Kuhn, 2002). In addition, headspace and SPME instruments are too expensive and unavailable in most clinical laboratories in developing countries. Using direct injection, samples can be added to the gas chromatography system without the requirement for expensive sampling equipment or sample preparation. Contrarily, direct injection will result in smearing of the injector chamber and column and the usage of additional materials, such as inlet liners, even with dilution (Wasfi et al., 2004). Additionally, this method is linked to significant analyte loss (Portari et al., 2008) and equipment upkeep (Morris-Kukoski et al., 2007). Due to the lack of accuracy and precision, direct injection would not be preferred.

This research aimed to validate and develop an efficient and sensitive Gas Chromatography-Flame Ionization Detector (GC-FID) analytical method for detecting and quantifying methyl and ethyl alcohol in the blood by solvent partitioning extraction without the need for headspace techniques.

This research was applied as a patent with application No. EG/P/2022/27 at the Egyptian Academy of Scientific Research and Technology on January 11, 2022.

II. Materials and Methods:

Chemicals

Methanol, ethanol, dichloromethane, chloroform, and Ultra-pure water were of analytical grade and provided by Sigma-Aldrich. Sheep whole blood was purchased from Thermo Fisher Scientific.

Instrumentation

A Gas Chromatograph (GC) from Dani Master (Italia) with a Flame Ionization Detector (FID) is the tool used for the analysis. Chromatography was achieved on a Gs-BP 100% dimethylpolysiloxane capillary column (10 m x 0.53 mm ID, 2.65 µm film thickness, Supelco, Bellefonte, PA). Data acquisition and analyses were accomplished by the Clarity software package.

Preparation of solutions:

Preparation of methanol and ethanol Standard Solution

Methanol stock solution of concentration 4.0 g/dl was prepared by dissolving 5.05 ml of methanol with ultrapure water in a 100 ml volumetric flask. Ethanol stock solution of concentration 4.0 g/dl, was prepared by dissolving 5.07 ml of ethanol with ultrapure water in a 100 ml volumetric flask. Seven

standard solutions with concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 g/dl were prepared using stock solutions in a 10 ml volumetric flask filled with ultrapure water in a precise manner for accuracy and consistency.

Preparation of Internal Standard Solution

Internal standard (IS) stock solution of concentration 5.0 g/dl was prepared by dissolving 3.77 ml of dichloromethane in a 100 ml volumetric flask. To prepare 50 mg/dl of dichloromethane dissolve 100 ul of stock solution in a 10 ml volumetric flask. All stocks, working standards, and IS solutions were stored in tightened closed vials at 4°C until use.

Preparation of Calibration standard curve:

A sheep's whole blood (0.5 ml) was used to prepare calibrators by spiking 50 µl from the above-mentioned alcohol working standards to produce final concentrations equivalent to 5, 10, 20, 40, 80, 160, and 320 mg/dl. Similar dilutions were done using ultrapure water. They were vortexed for 2 minutes at 3000 rpm and then stored at 4ºC pending analysis. The calibration samples were generated in five duplicates, and the calibration curve was calculated using the peak areas verses nominal concentration of the samples. Blood blank and ultrapure water samples were also examined in each run.

Preparation of Samples for accuracy, precision, and recovery

By using the working standard solutions of methanol and ethanol of concentrations 3.2 g/dl, 1.6 g/dl, and 0.4 g/dl, three working dilutions of concentrations 320 mg/dl, 160 mg/dl, and 40 mg/dl were prepared by spiking 50 µl from the above-mentioned alcohol working standards into distilled water with a total volume of 0.5 ml. Similar dilutions were done using sheep's whole blood.

Preparation of Sample:

A closed tube containing 0.5 ml of blood and 50 µl of internal standard was used for the blood sample. A cap with a crimper and septa were used to seal the vial.

Preparation of samples for the determination of the partition coefficient:

For the detection of the partition coefficient of methanol and ethanol when extracted by chloroform from ultrapure water, we use two dilutions of methanol and ethanol with ultrapure water only. From the working standard solutions of methanol and ethanol at concentrations of 3.2 g/dl and 0.4 g/dl, two working dilutions at concentrations of 320 mg/dl and 40 mg/dl were prepared by spiking 50 µl of the above-mentioned alcohol

working standards into ultrapure water with a total volume of 0.5 ml.

Chromatographic Conditions:

GC conditions: The GC's cycle time was set to 5.00 minutes. The helium flow rate was kept at 5 ml/min. With a split flow of 5.0 ml/min, a split ratio of 1:1, and a septum purge flow of 5 ml/min, the injection port's temperature was kept at 200^{o} C up until the completion of the run, and the GC oven's initial temperature of 50°C was maintained. It took five minutes to complete the run.

Procedure for the determination of methanol and ethanol in prepared samples:

0.5 ml of chloroform was added to 0.5 of each tube of the previously mentioned prepared blood and water samples with 50 µl of IS, then the tubes were vortexed for 2 minutes and centrifuged at 3000 rpm for 5 minutes. About 100 µl of the lower organic layer was transferred into a vial for injecting 1.0 µl into the chromatographic system (Figure 1).

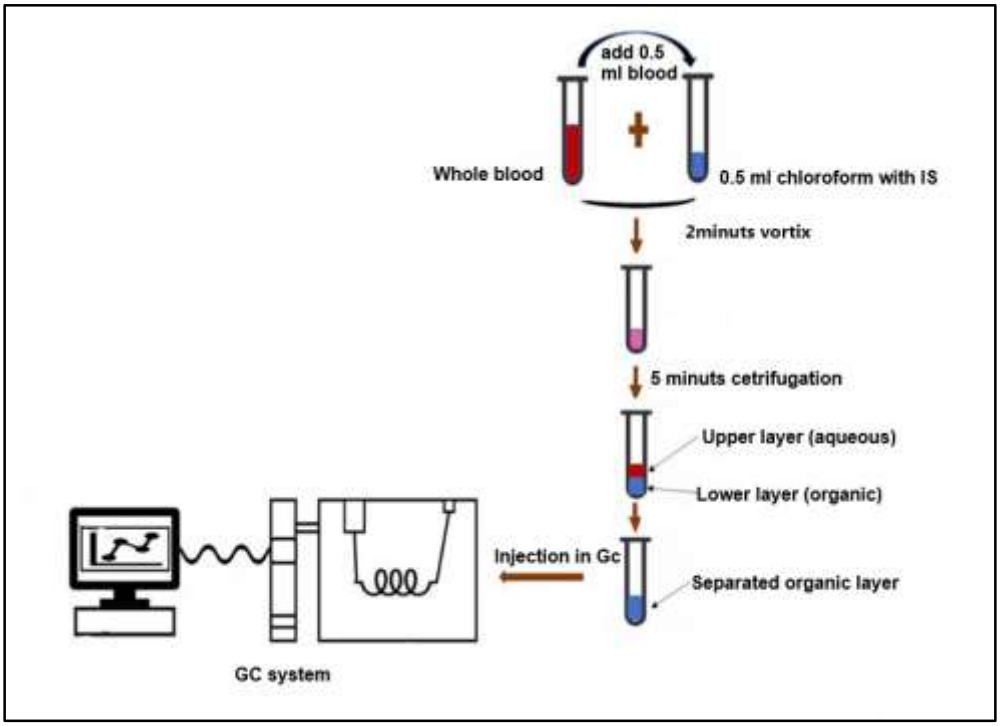

Figure 1: Schematic diagram of the GC-FID analytical method for the detection and quantification of methyl and ethyl alcohol in blood by solvent partitioning extraction.

Statistical analysis:

The mean and standard deviation were calculated using the Microsoft Excel 2010 program for completing validation calculations according to guidelines set forth by the Scientific Working Group for Forensic Toxicology (SWGTOX) (2013).

Ethics Considerations:

There was no work on animal or human subjects, so the ethical committee showed no indication of ethical approval, but approval from the Poison Control Center at Ain Shams University Hospitals administration to complete this work in the chromatographic lab was obtained.

III. RESULTS:

The method that was developed has undergone a comprehensive validation process to ensure its accuracy and reliability. This validation included assessments of specificity, linearity, accuracy, precision, recovery, carryover effect, detection limit, and quantification limit. The validation was conducted in accordance with the guidelines set forth by the Scientific Working Group for Forensic Toxicology (2013).

Specificity: Specificity is the possibility of evaluating the analyte in light of the existence of components that are expected to be present. Qualitative detection was done to match the results of individual standards of methanol, ethanol, and dichloromethane, which were processed with the developed method. By overlapping the graphs of negative blood, positive blood, and standard, no interference was shown in the retention times of either standard or internal standard.

The mixture of standard and internal standards in the sample matrix: Analytes methanol (0.35 min), ethanol (0.43 min), and dichloromethane (0.53 min) were detected in the spiked samples of the standard (water and whole blood) in addition to the chloroform peak (0.73 min) as extraction solvent at the end of the chromatogram, as shown in Figure 2.

Calibration curve and linearity: The peak areas of the produced calibration samples (blood and water) were plotted on linear calibration curves against the corresponding concentration ranges of methanol and ethanol standards, which ranged from 10 mg/dl to 320 mg/dl. According to Figure 3, each analyte in the sample matrix followed a linear graph with a regression coefficient ranging from 0.999 to 0.998 for ethanol and methanol, respectively, throughout the range of 10 mg/dl to 320 mg/dl.

Accuracy: As stated in Table 1, interday and intraday accuracy were computed as the

percentage accuracy bias of how closely the mean test concentration achieved by the procedure matched the actual concentration of analytes. The correctness of the matrix was assessed across three concentrations. The formula used to calculate percentage accuracy is as follows:

$$\%\text{Accuracy} = \frac{(\text{Calculated conc. Of analyte} - \text{Known conc.})}{\text{Calculated Conc. Of analyte}} \times 100$$

Precision: By examining 5 replicates of 3 serial concentrations (40, 160, and 320 mg/dl) on a single day, intraday precision was assessed. By examining five replicates of three consecutive concentrations on five distinct days, interday precision was conducted. The precisions so determined, shown as % CV (coefficient of variation), fell within the range of the coefficient of variation. In sample matrixes using dichloromethane as the internal standard, inter- and intraday precisions were represented as CV% for all matrices and discovered to be within the acceptable range of 1.87-6.84% for methanol and 0.37-4.5% for ethanol, as shown in Table 1.

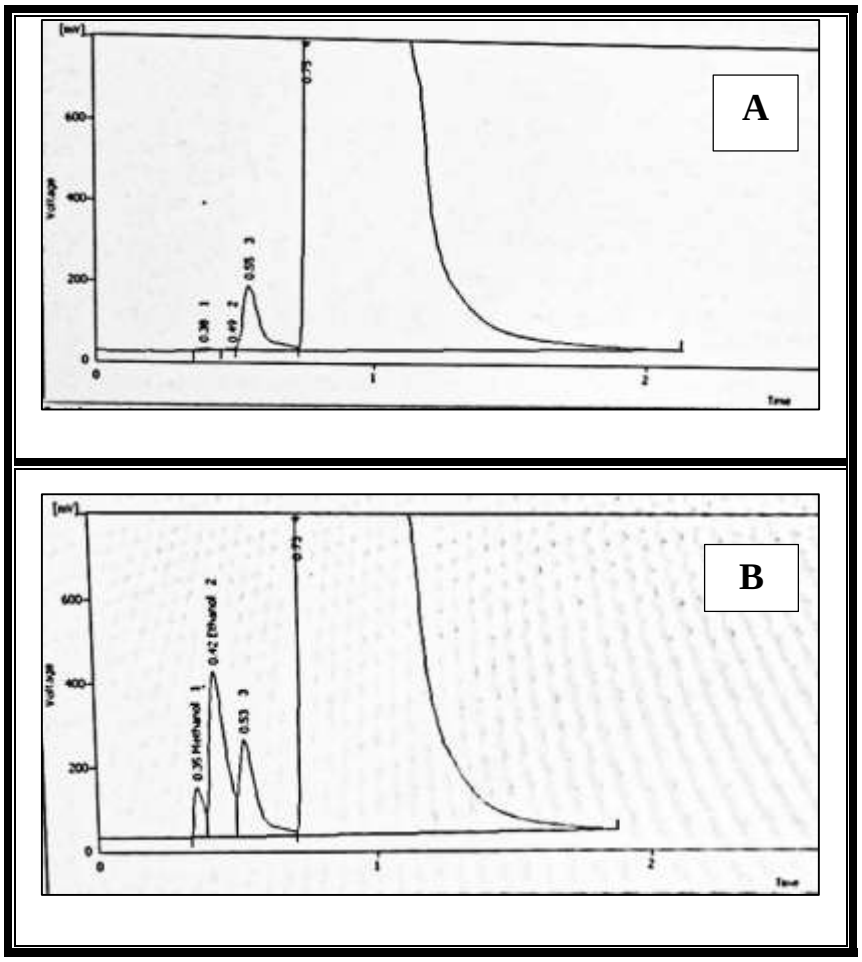

Figure 2: Gas chromatograms of methyl and ethyl alcohols using chloroform as an extraction solvent with internal standard dichloromethane A) Blank sample chromatogram showing internal standard and extraction solvent chloroform peaks B) Spiked sample chromatogram showing methanol, ethanol, and internal standard in addition to the chloroform peaks.

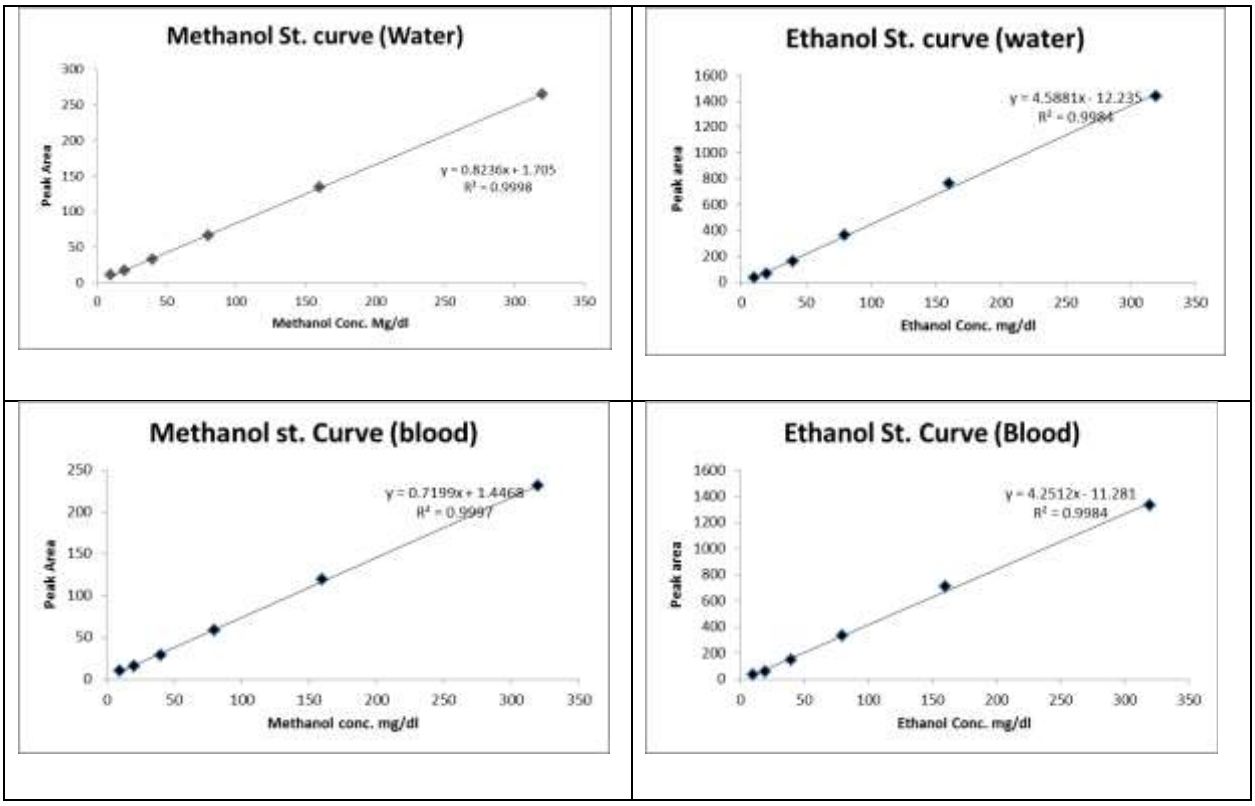

Figure 3: Calibration curves of methanol and ethanol in water and in blood

Table 1: Interday and intraday precision and accuracy of analyzed samples

Solvent	Matrix	Conc. mg/dl	Intraday			Interday		
			Measured Conc. ±SD	Precision (% CV)	Accuracy (%Bais)	Measured Conc. ±SD	Precision (%CV)	Accuracy (%Bais)
Methanol	Water	40	39.9±2.73	6.84	5.44	39.1±1.92	4.91	4.3
		160	160.7±4.38	2.72	2.1	158.7±2.95	2.95	2.34
		320	318.6±7.15	2.24	1.87	323.9±14.6	4.52	4.2
	Blood	40	39.4±2.56	6.5	5.27	38.68±2.49	6.43	5.7
		160	159±4.79	3.01	2.47	159.7±5.55	3.52	2.61
		320	318.3±3.74	1.17	1.07	317±9.32	2.94	2.49
Ethanol	Water	40	39.4±0.55	1.4	1.48	38.74±1.53	3.95	3.95
		160	167.3±1.46	0.87	4.5	166.8±2.48	1.49	4.22
		320	327.8±3.43	1.05	2.44	326.7±3.85	1.18	2.1
	Blood	40	38.88±1.16	2.98	3.6	39.5±1.8	4.56	3.65
		160	162.5±1.74	1.07	1.56	164.5±4.43	2.69	2.96
		320	316.7±2.52	0.79	0.37	316.5±2.25	0.71	1.08

CV: coefficient of variation; Conc. ± SD: Concentration ± Standard deviation;
*Results represent the mean of experiments which were repeated five times.

Recovery: The given matrix was spiked separately with three distinct concentrations, and the mean was computed for the five replicates of each concentration. As demonstrated in Table 2, recovery was estimated by contrasting the outcomes of samples that had been spiked with those of the pure standard at that concentration. The recovery % was determined using the following equation:

$$\text{Recovery \%} = \frac{\text{Measured concentration}}{\text{Known concentration}} \times 100$$

Table 2: Recovery % of spiked samples related to the pure standard of the corresponding concentration

Solvent	Matrix	Concentration (mg/dl)	Mean ± SD	Recovery %
Methanol	Water	40	39.9±2.73	99.75
		160	160.7±4.38	100.4
		320	318.6±7.15	99.56
	Blood	40	39.4±2.56	98.5
		160	159±4.79	99.37
		320	318.3±3.74	99.46
Ethanol	Water	40	39.4±0.55	98.5
		160	167.3±1.46	104.5
		320	327.8±3.43	102.4
	Blood	40	38.88±1.16	97.2
		160	162.5±1.74	101.5
		320	316.7±2.52	98.9

SD: Standard deviation.
*Results represent the mean of experiments which were repeated five times.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The calibrators of methanol and ethanol with the lowest concentration (10 mg/dl) were gradually diluted to find the lowest limit of detection and quantification, which helped evaluate the method's sensitivity. For determining LOD, levels needed to produce a ratio of signal to noise of 3 were deemed acceptable. The signal-to-noise ratio of 10 obtained by diluting the standard to the point when all chemicals are detected with crisp, symmetrical chromatographic peaks served as the basis for the LOQ estimation. Table 3 displays the LOD and LOQ results.

Table 3: LOQ and LOD of methanol and ethanol of the method in the sample matrix

Solvent	Matrix	LOD (mg/dl)	LOQ (mg/dl)
Methanol	Water	5.0	7.5
	Blood	7.2	10.0
Ethanol	Water	2.5	5.0
	Blood	4.0	8.0

LOD: Limit of Detection; LOQ Limit of Quantification.

***Partition coefficient calculation*:** The ratio of the concentration of solute molecules (methanol and ethanol) between the two phases (the aqueous phase and the organic phase) determines the partition coefficient (Weber et al., 1986). Three replicates of each of two distinct concentrations (40 mg/dl and 320 mg/dl) were examined to determine the partition coefficient (K), which was obtained using the equation:

$K = Conc_{aq.} / Conc_{org.}$……. (1)

$Conc_{aq.}$: is the concentration of methyl or ethyl alcohols in the water (aqueous) phase.
$Conc_{org.}$: is the concentration of methyl or ethyl alcohols in the chloroform (organic) phase.

As the compound concentrations in each phase are related to the peak area for each compound, we can use the peak area to express the K in the following equation:

$K = PA_{aq.} / PA_{org.}$……. (2)

$PA_{aq.}$: is the peak area of methyl or ethyl alcohols in the water (aqueous) phase.
$PA_{org.}$: is the peak area of methyl or ethyl alcohols in the chloroform (organic) phase.

Table (4): Partition coefficient for methanol and ethanol between water and chloroform

Solvent	Conc. Mg/dl	Peak area (Mean ± SD)		Partition Coefficient (K)
		Water	Chloroform	
Methanol	40	366.3 ± 5.13	32.8 ± 2.56	11.17
	320	2893 ± 11.13	256 ± 6.5	11.3
Ethanol	40	702 ± 8.08	160 ± 5.0	4.38
	320	6886.3 ± 14.57	1553 ± 8.0	4.43

Conc.: Concentration; SD: Standard deviation.
*Results represent the mean of experiments which were repeated three times.

***Carry-over Effect*:** Carryover is the occurrence of an analytical substance in a run after the injection of a blank that contains no analyte. A blank is injected right after an analyte concentration with a high concentration in order to estimate any carryover that may have occurred during the test run. It can be inferred that the procedure sees no carryover even with high concentrations of 320 mg/dl due to the next blank runs showing no carryover.

IV. DISCUSSION:

The minimal standards of practice for forensic toxicology analytical technique validation were released in a 2013 document by the Scientific Working Group for Forensic Toxicology (SWGTOX). The process of conducting a series of trials that accurately predict the effectiveness and dependability of an analytical technique or a change to an already-validated technique is known as validation. Establishing objective proof that a technique can function successfully at the level of its intended usage and identifying the method's limitations under typical operating settings are the two main objectives of validation. The presented study obtained acceptable results according to the SWGTOX standard; on the other hand, these results were in accordance with the previous studies as follows:

The method outlined allows for the rapid and sensitive GC-FID detection analysis of blood samples for the identification of methanol and ethanol in blood utilizing solvent

partitioning extraction, without the need for headspace methods. In Figure 3, all calibration curves for the methanol and ethanol sample matrices, which are certified for high linearity by SWGTOX, have correlation coefficients greater than 0.99 over the range of 10 mg/dl to 320 mg/dl. These findings concur with those of Ertas et al. (2013), who measured the amounts of methanol and ethanol in blood samples using a headspace-solid phase micoextraction and gas chromatography system and discovered that all correlation coefficient values had an average of 0.989.

The results for methanol and ethanol in the sample matrix showed a range of 1.07–5.7% and 0.37–4.22% for accuracy and 1.17–6.7% and 0.71–4.56% for precision, respectively, both intraday and interday. These outcomes are better than those of Bursova et al. (2015), who detected methanol, ethanol, and formic acid in serum using HS-GC-FID. They discovered that the ranges of accuracy and precision for methanol and ethanol in blood samples were 0.2–14.4% and 2.3–11.0% for accuracy, and 7.3–11.5% and 5.4–11.1% for precision, respectively.

The present investigation yielded recovery rates for methanol and ethanol of 98.5–100.4% and 97.2-104.5%, in that order. These findings were consistent with those of Krishna et al. (2020), who measured the amount of methanol in blood using HS-GC-FID and discovered that the range of methanol recovery was 93.48–99.39%. Additionally, Mihretu et al. (2020) discovered that when ethanol was detected in blood samples using HS-GC-FID, the recovery for ethanol ranged from 91 to 109%. Although the limit of detection (LOD) and quantitation (LOQ) in this method were set at 7.2 and 10.0 mg/dl for methanol and 4.0 and 8.0 for ethanol, respectively, they were significantly higher than those obtained by Mergen et al. (2010), who detected methanol and ethanol in blood using HS-GC-FID and reported the LOD and LOQ as 2.06 and 6.24 mg/dl for methanol and 1.48 and 4.48 mg/dl for ethanol, respectively. but these results are acceptable when compared with the recommended methanol and ethanol blood levels (20 mg/dl and 200 mg/dl, respectively) and should be satisfactory for emergency cases since antidote treatment is recommended (Bursova et al., 2015; Vale, 2016). This difference in sensitivity may be attributed to the difference in column specification, which can affect peak resolution, retention time, and sensitivity (Nilratnisakorn et al., 1999; Rood, 2005).

As indicated in Table 4, the values of K for methanol varied between 11.17 and 11.3,

while those for ethanol varied between 4.38 and 4.43. These results showed a high decrease in K values when compared with the value of K when calculated between aqueous and headspace, according to Kaneko et al. (1994), who calculated K for methanol and ethanol in aqueous and headspace at 37° C, which equals 2140 and 3330, respectively. As previously mentioned, this means that the measured phase in this method (organic solvent) captures more alcohol molecules than the headspace method, and this reflects on the sensitivity, which increased with the decrease in K value. On the other hand, due to intermolecular interactions and a stronger hydrogen bond between methanol and water than between ethanol and water, the partition coefficient for methanol is higher than that for ethanol.

V. CONCLUSION and RECOMMENDATIONS:

In this study, the solvent partition extraction method using the GC-FID system and chloroform as the extracting solvent, with dichloromethane as an internal standard, proved to be highly effective in detecting and quantifying methanol and ethanol in blood samples. This method offers numerous advantages, including convenience, affordability, time efficiency, reliability, and ease of execution. Notably, it does not necessitate expensive equipment that is typically required for conventional headspace alcohol detection via GC-FID or GC/MS analysis. Moreover, a mere 0.5 ml of sample was required for analysis, and by incorporating an internal standard, the entire analysis (1 analyte, 1 IS) could be completed within just 5 minutes. Method validation was conducted by the guidelines provided by the Scientific Working Group for Forensic Toxicology. The limits of detection and quantification achieved in the blood samples were suitable for routine analysis. Overall, this proposed methodology demonstrates remarkable competence in conducting quantitative and qualitative analyses of methanol and ethanol present in blood samples, as well as being suitable for research, clinical, and medicolegal samples.

VI. Funding sources:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors; it was totally funded by the author.

VII. Conflict of interest:

The author declare that there is no conflict of interest.

VIII. Acknowledgment:

The authors deeply appreciate the help of the Poison Control Center at Ain Shams

University Hospital administration to complete this work.

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طريقة جديدة محققة باستخدام جهاز كروماتوغرافيا الغازى ملحق بمقياس تأين اللهب لقياس الكحول الميثيل والكحول الإيثيلي في عينات الدم بإستخدام تقنية الإستخلاص بتجزئة المذيبات

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الخلفية: يعتبر قياس مستويات الكحول في الدم تحليل روتيني يتم إجراؤه في العديد من مختبرات الطب الشرعي. وتعتبر تقنيات مساحة الرأس والاستخلاص الدقيق للمرحلة الصلبة مقترنة بالكروماتوغرافيا الغازية الطريقة المفضلة للكشف عن الكحول في عينات الدم، ولكنها مكلفة للغاية وغير متوفرة في معظم المختبرات السريرية في البلدان النامية.

الهدف: الهدف من هذه الدراسة هو التحقق من صحة وتطوير طريقة تحليلية فعالة وحساسة بإستخدام جهاز الكرواتوجرافيا الغازية الملحق بكاشف اللهب المتأين (GC-FID) للكشف عن وقياس الكحول الميثيل والكحول الإيثيلي في الدم عن طريق تقنية الإستخلاص بتجزئة المذيبات دون الحاجة إلى تقنيات مساحة الرأس.

الطرق: تم إجراء التحليل على دم الأغنام الكامل باستخدام الكلوروفورم كمذيب للاستخلاص وثنائي كلورو ميثان كمعيار داخلي. تم تحقيق الخطية عبر نطاق تركيز يتراوح بين 10 مجم إلى 320 مجم/ديسيلتر بالنسبة للميثانول والإيثانول. تم التحقق من صحة الطريقة المطورة بالكامل وفقًا لإرشادات مجموعة العمل العلمية لعلم السموم الشرعي لعام 2013.

النتائج: أظهرت خطية الطريقة معامل ارتباط قدره 0.999 و 0.998 للميثانول والإيثانول في الدم الكامل، على التوالي. كان حد القياس الكمي (LOQ) وحد الكشف (LOD) في نطاق 7.2-10 ملغم/ديسيلتر و4.0-8.0 للميثانول والإيثانول في الدم الكامل، على التوالي. كما أظهرت هذه الطريقة إنضباط يتراوح قيمته بين 1.17-6.5 و0.79 -2.98 ودقة تتراوح بين 1.07-5.27% و0.37-36% للميثانول والإيثانول، على التوالي.

الاستنتاج: كانت هذه الطريقة الجديدة عملية و أكثر ملاءمة وموثوقية لقياس الميثانول والإيثانول في عينات الدم الكاملة عن طريق تقليل جهد المعالجة المسبقة للعينة والتكلفة دون الحاجة إلى تقنيات مساحة الرأس.

التوصيات: نوصى بمزيد من الدراسة لطريقة الإستخلاص بتجزئة المذيبات دون الحاجة إلى تقنيات مساحة الرأس لقياس تركيز الميثانول والايثانول بإستخدام جهاز الكرواتوجرافيا الغازية المقترن بكاشف اللهب المتأين لتوضيح مدى أهميتها في القياس الدقيق للميثانول والإيثانول في دم المرضى مع تقليل الوقت والجهد والتكلفة.