

Case Report

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Headspace-gas chromatography/mass spectrometry analysis of methanol in blood

Dilek Salkim Islek, Sibel Ramadanoglu

Forensic Science Institute of Istanbul University, Istanbul, Turkey

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Abstract

A headspace gas chromatographic-mass spectrometric (HS-GC/MS) method was developed for detection of ethanol and methanol in blood and validation was performed. The linearity ranges of the method were 0-2g/L for ethanol and methanol. The detection and quantitation of limit was 0.03g/L-0.05g/L respectively for ethanol and methanol. The range of recoveries was between 94%-120% for both ethanol and methanol. In our study case; 60-year-old woman was found unconscious at home, with a needle mark on her leg. A bottle of purple liquid was found next to the body. She was presented involving a transient loss of consciousness resulting from self-administered denaturated alcohol (methylated alcohol). Doctor demanded us to determine methanol and ethanol level in blood and purple liquid. The compounds were identified and quantified by headspace gas chromatography coupled to mass spectrometry.

Key-words: Methanol, ethanol, Headspace-Gas chromatography-mass spectrometry, blood

Introduction

Methanol, also known as methyl alcohol, wood alcohol or carbinol is the simplest alcohol, and is a light, volatile, colorless, flammable liquid with a distinctive odor that is very similar to but slightly sweeter than ethanol. Methanol is an alcohol toxic to mammals and is widely used as a solvent for extraction or in detergents, automotive antifreeze, fuel [1], and because of its toxic properties, it is frequently used as a denaturant additive for ethanol manufactured for industrial uses — this addition of methanol exempts industrial ethanol from liquor excise taxation. It is also used for producing biodiesel via transesterification reaction. Accidental intake of the compound will result in severe intoxication due to accumulation of highly toxic metabolites such as formaldehyde and formic acid [2,3]. Acute intoxication of methanol usually causes headache, vertigo, fatigue, nausea, vomiting, blurred vision, blindness and even death [4]. These symptoms result from the accumulation of toxic levels of formate in the bloodstream, and may progress to death by respiratory failure. The ester derivatives of methanol do not share this toxicity. Oral lethal dose of methanol for humans ranges from 340 µg to 1 mg/kg of body weight. If ingested, as little as 10 mL can cause permanent blindness by destruction of the optic nerve and 30 mL is potentially fatal, [5] although the usual fatal dose is typically 100–125 mL (4 fl oz). Toxic effects take hours to start and effective antidotes can often prevent permanent damage [5]. Because of its similarities to ethanol (the alcohol in beverages), it is difficult to differentiate between the two (such is the case with denaturated alcohol).

Methanol is toxic by two mechanisms. Firstly, methanol (whether it enters the body by ingestion, inhalation, or absorption through the skin) can be fatal due to its CNS depressant properties in the same manner as ethanol poisoning. Secondly, in a process of toxication, it is metabolised to formic acid (which is present as the formate ion) via formaldehyde in a process initiated by the enzyme alcohol dehydrogenase in the liver [6]. The reaction to formate proceeds completely, with no detectable formaldehyde remaining [7]. Formate is toxic because it inhibits mitochondrial cytochrome c oxidase, causing the symptoms of hypoxia at the cellular level, and also causing metabolic acidosis among a variety of other metabolic disturbances [8]. Fetal tissue will not tolerate methanol. Among the most important findings related to chronic methanol intoxication are extrapyramidal system findings and Parkinson syndrome [9].

Methanol poisoning can be treated with the antidotes ethanol or fomepizole [6,10,11]. Both of these drugs act to reduce the action of alcohol dehydrogenase on methanol by means of competitive inhibition, so that it is excreted by the kidneys rather than being transformed into toxic metabolites [6]. Further treatment may include giving sodium bicarbonate for metabolic acidosis and haemodialysis or haemodiafiltration can be used to remove methanol and formate from the blood [6]. Folinic acid or folic acid is also administered to enhance the metabolism of formate [6]. Methanol has a high toxicity in humans and monkeys, but not in rats or mice. This is because of the expression of methanol poisoning is related to the ability of an animal to metabolize formate to carbon dioxide and the rate of formate oxidation is related to hepatic tetrahydrofolate (H4folate) content and the activities of folate-dependent enzymes. Studies have shown that H4folate and total folate levels are lower in human liver than that found in rat or monkey liver [12].

*Corresponding Author: Dilek Salkim İşlek, Forensic Science Institute of Istanbul University, Istanbul, Turkey
E-mail: salkimdilek@gmail.com

Materials and methods

Samples and reagents

Methanol and ethanol free blood samples were used for recovery test and validation of the method. The case work samples were received in the form of whole blood and serum. Headspace vials were purchased from Agilent Technologies(Palo Alto,CA,USA). Liquid-chromatographic-grade solvents (purity above 99.5%), such as methanol, ethanol and n-butanol, were purchased from Merck. Standard solutions of the above authentic chemicals were made up with spiked methanol and ethanol free blood samples (2g/L). Methanol and ethanol free blood samples were spiked with methanol and ethanol at a concentration of 0.05, 0.1, 0.5, 1.0 and 2.0 g/L, and replicates were analyzed for both analytes, respectively.

Headspace-Gas Chromatographic-mass spectrometry (HS-GC-MS) analysis

Headspace Procedure

The samples were placed in 20 mL headspace vials by adding 1 mL of samples, standards or quality control samples and 0.5mL of internal Standard. Parameters of the instrument are shown in *Table 1*.

Table 1. Headspace Parameters

Analysis time(min)	11
Vial equilibration time(min)	10
Vial pressurize	0.20
Sample loop fill time(min)	0.20
Loop EQTime	0.05
Inject time	0.30
Vial mixing	High

Gas Chromatographic-mass spectrometry(GC-MS) analysis

The analysis of methanol and ethanol were conducted in an Agilent 5973 Mass Selective Detector interfaced to an Agilent 6890 gas chromatography with an Agilent 7694 headspace sampler, a HP 5MS column (25m x 0.25mm x 0.25 μ m). Flow rate of carrier gas helium was set at 1 mL/min. The temperatures at injector port and detector were set at 250°C, and split mode injection (split ratio was 50:1) was used. Oven temperature was controlled with a temperature elevation program during analysis, which was initially set at 37°C for 2 min, elevated to 40°C at the rate of 2°C/min and maintained for 3 min.

The MSD was operated in the electron impact (EI) mode and in sim mode. Extracted ion chromatograms were used to determine the analyte and IS peaks from the total ion chromatograms. Peak areas were used for quantitation.

Determination of ethanol and methanol content

Five point calibration curve covering the blood concentration range of 0-2 g/L were constructed. Ethanol and methanol standards, quality control samples and internal standard(n-buthanol, 1.6g/L) were prepared in

distilled water and in both analytes free blood from HPLC grade solvents. Quality control methanol and ethanol-free blood samples were spiked with methanol and ethanol at a concentration of 0.05, 0.1, 0.5, 1 and 2g/L and replicates were analyzed for both analytes, respectively.

Recovery test and validation of the method

Recovery of the methanol was determined by comparing the original content of methanol in the blood sample and the content with standard addition. In order to evaluate the validation of the HS-GC method, a range of standard methanol and ethanol concentrations (2.0, 1.5, 1.0, 0.5, 0.1 and 0.05 g/L) was determined 6 times a day (intra-day validation) and the same determination was repeated on 3 successive days (inter-day validation). Standard deviations and coefficients of variation were calculated for index of precision, and relative error of mean was for index of accuracy. The ions m/z 31 and 46 were selected for the quantification of ethanol, and the m/z ions 29 and 31 were selected for the quantification methanol.

The linearity ranges of the method were 0-2g/L for ethanol and methanol. The detection of limit was 0.03g/L for ethanol and methanol. The quantitation of limit was 0.05g/L for ethanol and methanol. The range of recoveries was between 94%-120% for both ethanol and methanol.

Case Summary

A 60-year-old woman was found unconscious in her home, lying in the living room. There was no evidence of violence and a needle mark was found on one leg. A bottle of denaturated alcohol (methylated spirit) was found near the body.

At the intensive care unit, specimens of whole blood and centrifuged blood serum were collected at arrival to the emergency clinic. Post-dialysis serum sample from the second day and whole blood samples from the third day were collected for toxicological analysis, and stored at 4°C until analysis. A sample of the purple liquid from the bottle found near the patient was also collected. A sample of whole blood was collected at the clinic a week after the incident and was sent for toxicological analysis for the control and confirmation of the treatment.

The purple liquid found in the bottle was diluted 100 times and tested for ethanol and methanol using the headspace-GC/MS method. The whole blood sample collected at the arrival was found to be un-fit for the analysis due to clotting and low volume, the centrifuged blood serum was preferred instead. Remaining specimens were also tested for methanol and ethanol, respectively.

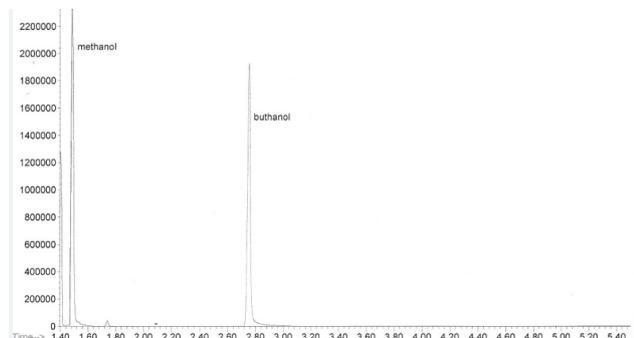
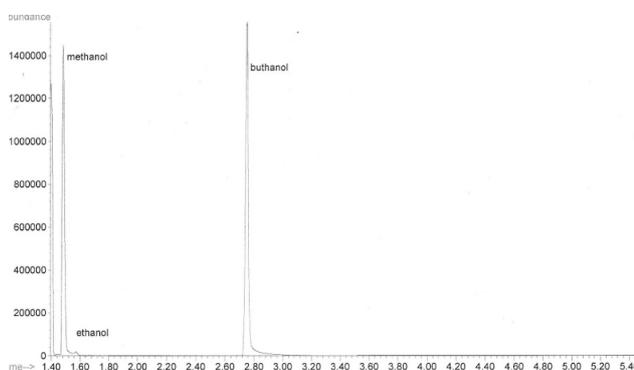
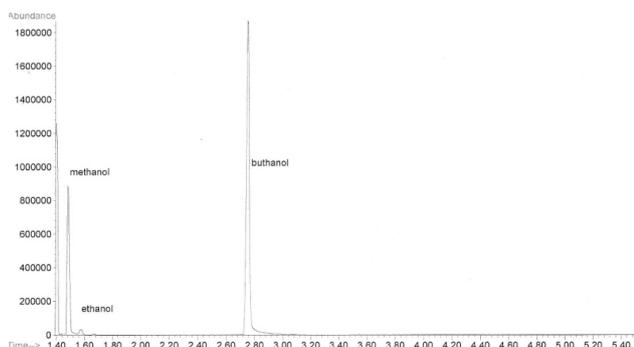
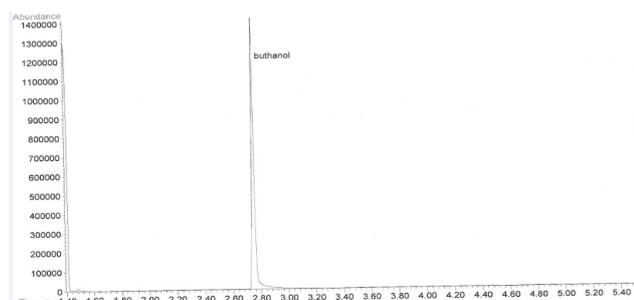
Results and Discussion

The five samples were analyzed, respectively. Ethanol and methanol quantitative analysis were recorded for each sample, as seen in *Table 2*.

Table 2. Case Work Ethanol and Methanol Quantitative Test Results*

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Ethanol	3.00 g/L	Not suitable	0.03 g/L	0.04 g/L	0.00 g/L	0.00 g/L
Methanol	453.00 g/L	for analysis	3.35 g/L	1.56 g/L	1.00 g/L	0.00 g/L

* The purple liquid from the bottle found near the patient (Sample 1), centrifuged blood serum (Sample 3) taken at the 1st day, post-dialysis serum sample (Sample 4) collected at the 2nd day, whole blood sample (Sample 5) from the 3rd day and whole blood sample (Sample 6) taken on the 7th day were tested for methanol and ethanol. Whole blood sample (Sample 2) taken at the 1st day was not suitable for the analysis, due to clotting and low volume.

**Figure 1.** The purple liquid of chromatogram**Figure 2.** Centrifuged blood serum of chromatogram**Figure 3.** Post-dialysis serum sample collected at the 2nd day**Figure 4.** Whole blood sample from the 3rd day of chromatogram

A method for quantitative ethanol and methanol analysis by HS-GC/MS using HP-5MS column was developed and validated. The analysis requires only 1ml of blood and is very simple and quick because the sample preparation only requires the addition of internal standard.

Conclusions

Methanol may be encountered in clinical and forensic cases. We have been able to extract ethanol and methanol in blood by headspace-Gas chromatography/Mass Spectrometry. This method for quantitative methanol analysis by HS-GC/MS using HP-5MS column was developed and validated. This study is advantages; easily available sample of blood and easily and rapid technique of headspace. Furthermore, this method was found suitable for clinical and toxicological analysis.

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