THE DETERMINATION OF BLOOD ALCOHOL LEVELS BY MODIFIED HEAD SPACE GAS CHROMATOGRAPHY-MASS SPECTROMETRY IN DRUNKEN DRIVERS

ALKOLLÜ SÜRÜCÜLERİN KAN ALKOL DÜZEYLERİNİN MODİFİYE HEAD SPACE GAZ KROMATOGRAFİ-KÜTLE SPEKTROMETRESİ YÖNTEMİ İLE TAYİNİ

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ABSTRACT

Purpose: *This study describes the analysis of ethanol blood samples from drivers involved in traffic accidents using the head space gas chromatography-mass spectrometry (GC/MS) method.* ***Methods:*** *We surveyed the emergency cases in three major hospitals in Ankara. Blood samples taken from patients who had been involved in traffic accidents were analyzed by modified head space GC/MS. With the developed method, for methanol, ethanol and isopropyl alcohol, optimization of instrument conditions, limit of detection (LOD), limit of quantitation (LOQ) and recovery of alcohols in blood samples were calculated. This method was applied to 100 drivers' blood samples.* ***Results:*** *LOD values for methanol, ethanol and isopropyl alcohol were 2 mg/dl, and the LOQ for methanol and ethanol was 5 mg/dl. Recovery of methanol, ethanol and isopropyl alcohol from blood samples was 97.37%, 97.90% and 98.70%, respectively. In 39 blood samples from 100 drivers, measurable alcohol results were obtained. Twelve blood samples had levels above 50 mg/dl (76.55-239.27 mg/dl), eight had levels between 25 and 50 mg/dl, eleven had levels between 10 and 25 mg/dl, and eight had levels below 10 mg/dl (6.42-9.77 mg/dl).* ***Conclusion:*** *In Turkey the legal alcohol limit in traffic was accepted as 50 mg/dl. This limit is now considered to be high enough to influence the driver's performance negatively. Therefore, a more reliable and sensitive GC/MS method was developed. By this modified method, we studied blood samples taken from 100 drivers admitted to the emergency departments of 3 major hospitals in Ankara.*

Key Words: *Head Space, GC/MS, Blood Alcohol, Traffic Accidents, Drivers.*

ÖZET

Amaç: *Bu çalışmada trafik kazalarına karışmış sürücülerin kan alkol düzeylerinin modifiye head space gaz kromatografi-kütle spektrometresi yöntemi ile tayin edilmesi amaçlanmıştır.* ***Metod:*** *Ankaranın üç önemli hastanesinin acil servislerine trafik kazasına karışması sonucu getirilen sürücülerin kan örnekleri alınarak modifiye head space GC/MS yöntemi ile tayin edilmiştir. Bu amaçla metanol, etanol ve izopropil alkol için head space yöntemi modifiye edilmiş, bu alkoller için tanımlanabilirlik (LOD), tayin edilebilirlik (LOQ) değerleri ile yüzde verim hesaplanmıştır. Geliştirilen analiz yöntemi 100 sürücü kan örneğine uygulanmıştır.* ***Bulgular:*** *Metanol, etanol ve izopropil alkol için LOD değerleri 2 mg/dl,iç standard olarak izopropil alkol kullanıldığında metanol ve etanolün LOQ değeri 5 mg/dl olarak saptanmıştır. Bu alkollerin kandan geri alım yüzdesi ise metanol, etanol ve izopropil alkol için sırasıyla % 97,37, % 97,90 ve % 98,7 olarak belirlenmiştir. Bu metod 100 sürücü kan örneğine uygulanmış ve 39 adedinde ölçülebilir etanol saptanmıştır. On iki kan örneğinde alkol düzeyi 50 mg/dl düzeyinin üzerinde (76,55-239,27 mg/dl), 8 örnekte 25 - 50 mg/dl, 11 örnekte 10 - 25 mg/dl, ve 8 örnekte 10 mg/dl düzeyinin altında (6,42-9,77 mg/dl) kan alkol düzeyi ölçülmüştür.* ***Sonuç:*** *Türkiye'de yasal alkol limiti 50 mg/dl olarak kabul edilmiştir. Günümüzde bu alkol düzeyinin sürücülerin performanslarını olumsuz yönde etkileyebilecek derecede yüksek olarak değerlendirilmektedir. Bu nedenle biz alkol ölçümü için güvenilir ve hassas GC/MS yöntemi geliştirmiş ve 100 sürücü kan örneğine uygulamış bulunmaktayız.*

Anahtar Kelimeler: *Head Space, GC/MS, Kan Alkolü, Trafik Kazaları, Sürücüler.*

INTRODUCTION

Every year, many people are reported to die in road accidents in Turkey (1,2). Road traffic injuries are a huge public health and development

problem. Over a million people lose their lives in traffic accidents every year and 20 to 50 million injuries are reported. Road traffic injuries in particular are on the rise and the situation is expected to get worse. By 2020, road traffic injuries are forecast to rise from the ninth to the second leading cause of lives lost in developing countries. It is estimated that by 2020, road traffic injuries will account for about 2.3 million deaths a year, almost double the current burden of mortality of 1.2 million deaths each year. Without appropriate action, by 2020, road traffic injuries are predicted to be the third leading contributor to the global burden of disease and injury (3,4). Accepted legal blood alcohol levels were considered high in many countries. Scientific studies indicated that lower blood alcohol levels were also impairing the driver's performance negatively. Therefore, many countries have set their legal alcohol limits in traffic below the 50 mg/dl concentration. These low alcohol levels need more sensitive and reliable determination methods and techniques. In this study, our aim was to develop a reliable, convenient, and sensitive blood alcohol determination method by the modifying head space GC/MS technique, and to apply this method to drivers involved in traffic accidents.

MATERIALS AND METHODS

A. Subjects: The subjects were chosen from the Emergency Department of Gazi University Hospital, Ministry of Health Numune Hospital, and Ankara Hospital from among drivers involved in traffic accidents. The subjects were asked to fill in a questionnaire containing personal information (name, age, educational status, details of the accidents etc.). Ethics committee approval was obtained from the three hospitals prior to the study.

B. Biological samples and storage: Blood samples not exceeding 5 ml were drawn from the antecubital region veins of drivers into glass tubes containing 1% sodium flouride, and sealed with parafilm. The samples were stored in the refrigerator at +4°C until analysis. They were collected from 5 May through 31 December 2003, and were analyzed at the Department of Analytical Toxicology, Gulhane Military Medical Faculty.

C. Determination of head space heating conditions: Standard solutions of 250 mg/dl ethanol, methanol and isopropyl alcohol (as an internal standard) were prepared in water as a 1 ml final volume in a head space vial (Agilent, part number: 5182-0838, USA). The vials were heated for 10, 20, 40, and 60 minutes at 70 °C in an oven (Nüve FN400), with occasional shaking. At the end of the heating period, 8 µl of evaporated samples were taken with a gas-tight 10 µl injector (Hamilton Bonaduz AG, Switzerland). To prevent the condensation of vaporized alcohols during the injection process, the glass body of the injector was also heated at the same temperature as the vials. At the end of the incubation period, sampling from the vial and injecting into the GC/MS (Shimadzu, GC-170, GCMS-QP5050A, Japan) was performed as soon as possible.

D. Determination of blood alcohol recovery: The same set of alcohol standard concentrations were prepared in alcohol-free blood samples, which were incubated and injected into the GC/MS as described above. The obtained GC/MS abundance values of each alcohol were compared with prior standards prepared in water.

E. Determination of LOD and LOQ values of alcohol standards in blood samples: Methanol and ethanol standard solutions were prepared in alcohol-free blood, at 1, 2, 5, 10 and 20 mg/dl concentrations. Isopropyl alcohol was added at 50 mg/dl concentration as an internal standard. These concentrations of alcohol standard solutions were chosen from the results obtained in prior experiments.

F. Determination of drivers' blood alcohol levels: Standard solutions of ethanol were prepared in alcohol-free blood samples at 10, 25, 50, 100, 150 and 250 mg/dl concentrations, and isopropyl alcohol at 250 mg/dl concentration. Since we had no knowledge about the drivers' alcohol consumption, ethanol concentrations were set as described above. Twenty mg/dl was below the legal alcohol limit, and 250 mg/dl was extremely high for measuring excess alcohol consumption. Isopropyl alcohol as an internal standard gave the optimal GC/MS abundance value for this set of ethanol standards. From these standards, 1 ml of each was pipetted into a 10 ml head space vial, heated at 70 °C for 15 minutes (considered an optimal incubation period), and injected into GC/MS in an 8 µl volume as

Table-1: GC/MS abundance values of 250 mg/dL Methyl, Ethyl and Isopropyl alcohols.

Alcohols		GC/MS Abundances (Mean ± SD) 10 min.	20 min.	40 min.	60 min.
In water	Methyl	5765102±18232	6351317±389055	6147973±214441	5270020±26477
	Isopropyl	17448440±2463	18339850±1117287	19712662±721846	17591542±79116
	Ethyl	9146706±1234	10376576±481903	10506182±310674	9059623±56473
In blood	Methyl	6223252±99048	5591236±85227	5339896±64831	5620337±62053
	Isopropyl	18259096±203434	18211845±168200	17246759±88434	1820476±60242
	Ethyl	10245418±137526	9532157±71221	8828433±128891	9397169±66973

described above. The calibration curve of ethanol was obtained using GC/MS abundance values (r^2= 0.9915). To measure the driver's blood ethanol, a 1 ml sample was taken and 50 µl of isopropyl alcohol was added (final concentration 250 mg/dl), and the resulting mixture was treated as described above. Alcohol levels of the blood samples were calculated using a standard calibration curve.

G. GC/MS operation conditions: Gas chromatography conditions: Carrier gas helium 1 ml/min, total gas flow 7.2 ml/min, column inlet pressure 48.9 kPa, split mode 5, injection port temperature 200 °C, sampling time 1 min, capillary column INNOWAX ZB-WAX (Zebron capillary GC column, Pnenomex, Germany, 30 m x 0.25 mm, film thickness 0.5 mm) were used. GC oven temperature program: 40 °C/2 min, 50 °C/min to 90 °C/ 5 min, 20 °C/min to 150 °C/3 min were set. Mass spectrometry conditions: Acquisition mode scan, solvent cut time 2 min, detector voltage 1.5 kV, threshold 9000, interval 0.5 sec, start time 2.50 min, start m/z 20, end m/z 120, scan speed 250 were set. Under these GC/MS program conditions, methanol, isopropyl alcohol and ethanol peaks were obtained, and identified by NIST 07 and WILEY 229 libraries, which were present in our GC/MS software.

H. Statistical analysis: Differences between incubation time period abundance values for each alcohol injection for water and blood samples were analyzed. Differences between the measurements at the different incubation periods for methanol or ethanol in water and blood were determined by Friedman test, and post-hoc tests for the measurements at the different incubation periods were compared in pairs using the Wilcoxon Signed Ranks test. The water levels of alcohols were compared with the blood levels at the same incubation period by Mann-Whitney U test.

RESULTS

Head space incubation period results for methanol, ethanol and isopropyl alcohol are given in Table 1 and Fig. 1. The GC/MS abundance values from each time period for alcohols were similar. Thus, we decided that 10-20 minutes was the optimal time period for the evaporation and condensation of alcohols in blood samples. From each incubation period, similar abundance values were obtained for each alcohol standard.

We wanted to determine LOD and LOQ values for methanol and ethanol in this research. Detection limits for these alcohols were 2 mg/dl (LOD) and 5 mg/dl (LOQ) using isopropyl alcohol as an internal standard. Their separation with the column and GC/MS conditions used were also satisfactory. Alcohol peaks did not cover either of them (Fig. 2). Because of retention time values, isopropyl alcohol (rt.: 3.925 min) was also a suitable internal standard for both methanol (rt.: 3.600 min) and ethanol (rt.: 4.025 min).

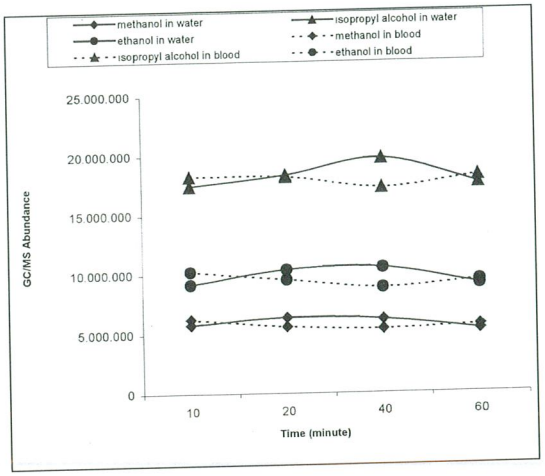

Fig. 1: GC/MS chromatogram of standard solutions of methanol, isopropyl alcohol and ethanol (250 mg/dL) from the 20 minute incubation period in blood sample.

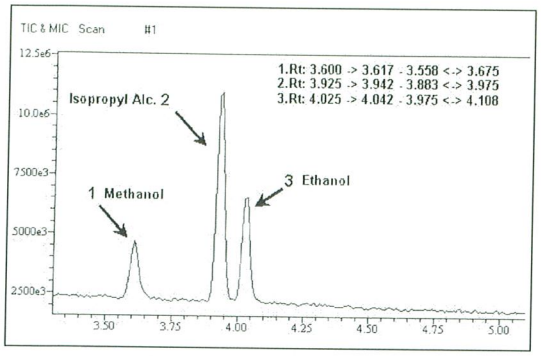

Fig. 2: GC/MS abundance values versus heat incubation period for 250 mg/dL concentration of alcohol standards in water and blood (mean ± SD from 3 injections).

Although multiple comparisons indicate that there was a difference between the measurements at the different incubation periods for methanol or ethanol (in water $x^2=8.20$, $p=0.042$ for methanol and $x^2=8.20$, $p=0.042$ for ethanol; in blood $x^2=8.20$, $p=0.042$ for methanol and $x^2=9.00$, $p=0.029$ for ethanol, Friedman test), post-hoc tests did not reveal a significant difference as the measurements at the different incubation periods were compared in pairs ($p>0.05$ for all comparisons; Wilcoxon Signed Ranks test). As the water levels were compared with the blood levels at the same incubation period, no significant difference was observed for methanol, ethanol, or isoprophyl alcohol ($Z=-1.964$, $p>0.05$ for all measurements, Mann-Whitney U test). Recovery values for methanol, ethanol and isopropyl alcohol were 97.37%, 97.90% and 98.7%, respectively. This indicates that alcohols in blood or other samples (i.e. cologne or alcoholic beverages) could be analyzed using our method.

We applied this modified head space GC/MS method to the drivers' blood samples. In 39 blood samples of 100 drivers, measurable alcohol results were obtained (Table 2). The highest blood alcohol level was 279.27 mg/dl, and the lowest was 6.42 mg/dl. The rest of the drivers' blood samples contained no ethyl alcohol, including trace amounts (neither LOQ nor LOD values).

Table-2: Blood alcohol levels of drivers.

Alcohol Concentration (mg/dl)	Drivers	%
>50	25-50	10-25
<10	12	8
11	8	31
20	29	20
Total	39	100

DISCUSSION

According to the WHO and the World Bank Reports, estimated deaths caused by traffic accidents will have increased 80% in developing countries and decreased 30% in developed countries by the year 2020. This means that preventive measures may be effective in decreasing traffic accidents, injuries and deaths. Traffic accidents are a major problem in spite of the mandatory alcohol limit, seat belt use and spontaneous strict controls in traffic. Since traffic accidents cause loss of life and economic losses, new education programs, physical improvement of the roads, and new legislative measures have to be implemented. It was reported that 95% of the accidents were the driver's fault (5,6). There are many factors including alcohol and drug usage, and physical and mental conditions that might affect the driver's performance in traffic. Scientific studies have indicated that lower blood alcohol levels also impaired the driver's performance. Thus, many countries re-evaluated the legal alcohol limits in traffic, and defined their alcohol limits lower than the previous limits (3,5,7).

One preventive measure for reducing traffic accidents is spontaneous road traffic controls. As a result, an 85% increase was noted in first time temporary suspension of driver's licence cases because of drunk driving in 2003 in Ankara. On the other hand, 15% and 80% reductions were observed in the second and third time temporary suspension of driver's licence cases, respectively (8).

In 2003, 21,996 cases were reported to the 112 Emergency Service admission in Ankara, of which 59% were medical and 22% were traffic accident cases. Nationwide, among the 461,460 cases, 68% medical and 14% traffic accident cases were reported (9). According to this report, traffic accidents were the second most common cases in the Emergency Departments.

In Turkey the legal alcohol limit in traffic has been accepted as 50 mg/dl since 1983. At that time, this limit was lower than that in many Europen countries and the USA (6). According to recent thinking, the alcohol level has to be reduced in Turkey (10,11).

The first comprehensive blood alcohol determination study in traffic in Turkey was performed in 1981. Blood alcohol levels of drivers were determined using GC. Ninety-three drivers' blood samples were analyzed. The risk of being in a traffic accident among drivers was higher as the blood alcohol levels increased, especially above 80 mg/dl (12). This study was the main scientific research conducted in Turkey presented to the legislative authorities, and in 1983 the blood alcohol limit was set at 50 mg/dl.

Similar research was presented in 1991 by Saygi and Vural(13). In that study, a survey of blood alcohol effect on drivers (n: 366) was carried out in Ankara and the relation to driving offences and accidents was determined. The incidence of detection of alcohol was greater among traffic offenders (82%) compared with those who were involved in traffic accidents (52%). Nearly 65% of the drivers who were involved in traffic accidents or offences had blood alcohol levels greater than or equal to 100 mg/dl.

In the present study, we surveyed the emergency cases in 3 major hospitals in Ankara. Among the forensic cases, traffic accidents accounted for 44%. We observed that 39 out of 100 drivers referred to the emergency departments had alcohol in their blood. Twelve blood samples had alcohol above the permissible level (76.55-239.27 mg/dl), 8 contained 25-50 mg/dl, 11 contained 10-25 mg/dl and 8 contained less than 10 mg/dl (6.42-9.77 mg/dl) (Table 2). Alcohol levels exceeded the legal limit in 31% of the drivers. On the other hand, if we accepted 25 mg/dl as the legal alcohol limit, 51% of the driver's blood level would have been above the legal limit.

From the toxicological point of view, not only ethanol, but also other toxic alcohols are important factors affecting the human physical and menthal status. An important toxic alcohol is methanol (7,14). Since it is considerably less expensive than normal alcoholic beverages, the alcoholic derelict may consume it (14). In adults, death may follow the ingestion of 20-250 ml and acute toxicity presents some 8-24 hours after exposure (15,16). Four milliliters have been reported to cause blindness (15,17). Because of its alcohol content, alcoholics may also consume cologne samples as an alcoholic beverage. In spite of state regulation, methyl alcohol was determined to range from 20% to 88% in 27 cologne samples collected in Ankara (18). Early determination of methanol in blood samples allows effective antidotal treatment.

With conventional alcohol determination methods, such as the breath analyzer and immunoassay techniques (i.e. EIA, FPIA), discrimination of ethanol and other toxic alcohols is not possible. In addition, determination of low blood alcohol levels requires more specific and sensitive analytical methods (7,19). If the accepted alcohol level in drivers' blood is lowered, sophisticated analytical methods will be needed to decide if the measured alcohol concentration is above or below the legal limit. In this study, we developed a sensitive and reliable alcohol determination method. Without a head space unit, an ordinary CG/MS instrument is capable of measuring ethanol, methanol and other toxic alcohols. Since the head space unit makes the GC or GC/MS instrument extremely expensive, our head space method contributes to the effectiveness of toxicology laboratories that already use GC or GC/MS.

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