Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 799 (2004) 331–336
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Rapid and sensitive static headspace gas chromatography–mass spectrometry method for the analysis of ethanol and abused inhalants in blood

Ibrahim A. Wasfi*, Ahmed Hassan Al-Awadhi, Zainat Naser Al-Hatali, Fatima Juma Al-Rayami, Nawal Abdulla Al Katheeri

Camelracing Laboratory, Forensic Science Laboratory, P.O. Box 253, Abu Dhabi, United Arab Emirates

Received 25 April 2003; received in revised form 22 September 2003; accepted 6 November 2003

Abstract

A sensitive and specific method using static headspace gas chromatography coupled with mass spectrometry (GC/MS) has been developed for the quantitative determination of ethanol in biological fluids using *n*-propanol as internal standard. Gas chromatography was performed in isothermal mode with a GC run time of 2.6 min. The quantification was performed using scan mode abstracting a quantitative ion and a qualifier ion for ethanol and for the internal standard. The method was linear (r^2, 0.999, in the concentration range of 5–200 mg/dl), specific (no interference from methanol acetaldehyde, acetone or from endogenous materials), sensitive (limit of quantification and limit of detection of 0.2 and 0.02 mg/dl, respectively) and robust (less than 5% inter- and intra-assay coefficient of variation). A slightly modified method was also developed for the quantification of five commonly abused inhalants (dichloromethane, ethyl acetate, benzene, toluene and xylene) in blood. The method used a gradient GC program with a run time of 8 min. The quantification was performed using scan mode and integrating the area under the peak using trichloroethane as an internal standard. Without optimization, the method was linear (from 5 to 100 mg/l) and sensitive.

Keywords: Ethanol; Dichloromethane; Ethyl acetate; Benzene; Toluene; Xylene

1. Introduction

Alcohol is a widely used substance worldwide and it results in a high rate of traffic fatalities. Forensic laboratories are required to develop fast and more precise methods for quantification of blood ethanol. The determination of ethanol in blood is probably the single greatest application of static headspace gas chromatography (GC). Although diluted blood samples may be directly injected in a GC, problems arise from contamination of the injector and column.

Now a days, headspace coupled to packed or capillary GC with flame ionization detection (FID) is the technique of choice for measuring ethanol concentration in biological sample [1–5]. Solid-phase micro extraction methods for the analysis of blood ethanol and volatiles are also reported [6,7]. However, as these methods use FID, confirmation analysis my be performed by injecting a single sample and splitting the exist into two different columns. Unequivocal confirmation, however, is done by mass spectrometry (MS).

There are few methods on the determination of ethanol in biological samples by gas chromatography–mass spectrometry (GC/MS). For example one method determined ethanol concentration in breath air by GC/MS using deuterated ethanol [8]. Another report investigated the presence of low-molecular-weight organic volatiles in the blood of drunk drivers by use of headspace GC and ion trap mass spectrometry [9].

The aim of the present study is to develop a quick, simple and sensitive method for the quantification of blood ethanol and for identification of organic volatiles in biological samples.

* Corresponding author. Fax: +971-2-446-3470.
E-mail address: iawasfi@emirates.net.ae (I.A. Wasfi).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.11.003

2. Materials and methods

2.1. Materials

Acetone, methanol, ethanol, acetaldehyde, benzene, ethyl acetate, dichloromethane, toluene, xylene and trichloroethane were obtained from Merck (Gernsheim, Germany). Headspace vials were purchased from Agilent Technologies (Palo Alto, CA, USA).

2.2. Analysis of ethanol

Ethanol analysis was performed by static headspace analysis. Five-point calibration curve covering the blood concentration range of 5–200 mg/dl was constructed. Ethanol standards, quality control samples and internal standard (*n*-propanol, 80 mg/dl) were prepared in distilled water and in ethanol-free blood from HPLC grade solvents. Quality control ethanol-free blood samples were spiked with ethanol at a concentration of 20, 50 and 150 mg/dl and replicates were analyzed for ethanol. Stability study was performed in ethanol-free blood samples spiked with ethanol at a concentration of 50 and 100 mg/dl and replicates were analyzed for ethanol for three consecutive days. A mixture of ethanol, *n*-propanol, methanol acetaldehyde and acetone at a concentration of 100 mg/dl each was used to evaluate the resolution of the method.

2.3. Headspace procedure

The samples were placed in 20 ml headspace vials by adding 1.0 ml of samples, standards or quality control samples and 1 ml of internal standard. The samples were sealed using crimptop vial caps with septa and were placed in the headspace rack. Parameters of the instrument are shown in Table 1. The headspace parameters for analysis of abused inhalants were similar to that of ethanol except that the equilibration time of the samples was 15 min.

2.4. Capillary gas chromatography–mass spectrometry (GC/MS) analysis

GC/MS analysis was carried out using an Agilent (Palo Alto, CA, USA) 5973 Mass Selective Detector interfaced to an Agilent (Palo Alto, CA, USA) 6890 gas chromatography with an Agilent (Palo Alto, CA, USA) 7694 headspace sampler. Injections were made in the split mode onto an Agilent (Palo Alto, CA, USA) DB-ALC1 column (30 m × 0.32 mm i.d. and 1.8 μm film thickness). The column temperature program was isothermal at 70 °C. Helium was used as the carrier gas. Data were acquired in a scan mode in the mass range of 20–120 *m/z* at 11.9 scans/s. The injector and transfer line temperatures were 200 °C and the split ratio was 50:1. To evaluate the effect of splitting ratio, a set of ethanol standards at a concentration of 50 mg/dl was analyzed using a split ratio of 25:1. For evaluation of carry over effect, blank samples were analyzed after 200 mg/dl ethanol standards.

For the quantification of ethanol, the ions *m/z* 31 (quantification ion) and *m/z* 46 (qualifier ion the ratio of which should be ±20% of the quantitative ion) and the *m/z* ions 31 (quantitative) and *m/z* 60 (qualifier) for the internal standard (*n*-propanol) were abstracted from the total ion chromatogram and the ratios of peak areas of ethanol to *n*-propanol were calculated.

Table 1
Head space conditions

Parameter	
Instrument	Agilent 7694 head space samples
Vial equilibration time (min)	10
Vial mixing	Low
Vial pressurize (min)	0.15
Sample loop fill time (min)	0.30
Inject time (min)	0.30
Oven temperature (°C)	70
Sample loop temperature (°C)	80
Transfer line temperature (°C)	90

2.5. Application of the method on authentic samples

Thirty-seven authentic blood samples were analyzed by this method and by a validated in-house head space GC method with a flame ionization detector. In the latter method, a Perkin-Elmer (Norwalk, CT, USA) auto system XL GC equipped with Perkin-Elmer HS 40 XL HS auto sampler was used with a packed column (3 ft × 1/8 in. o.d. SS packed with Porapak Q 80/100, Norwalk, CT, USA). The column temperature was kept isocratically at 200 °C and the run time was 5 min. The detector and injector temperatures were kept at 200 °C. The head space conditions and sample treatment were similar to that of the GC/MS method.

2.6. Screening of abused inhalants

For the identification of abused inhalants, a mixture of five common inhalants: dichloromethane, ethyl acetate, benzene, toluene and xylene were prepared in methanol (10–100 mg/l). Trichloroethane was used as an internal standard at a concentration of 50 mg/l. For evaluation of carry over effect, blank samples were analyzed after 100 mg/l of the mixture standards. The GC temperature program was gradient. The initial column temperature was 70 °C and it was programmed to rise at 20 °C/min to 200 °C and was held at 200 °C for 5 min. Other GC parameters were similar to that for ethanol analysis. Data were acquired in a scan mode in the mass range of 20–200 *m/z* at 7.4 scans/s. The injector and transfer line temperatures were 200 °C and the split ratio was 10:1. Solvents-free blood samples were spiked with the inhalants mixture at a concentration of 50 mg/l each and replicates were analyzed by the method to quantify the respective inhalant. For the quantification of the inhalants, the area under the peak of each compound

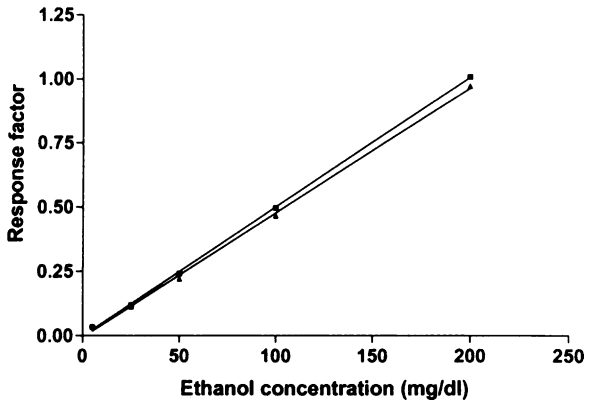

Fig. 1. Calibration curves ($n = 3$, 5–200 mg/dl) of ethanol in blood (■) ($y = 0.005021x - 0.001935$; $r^2 = 0.9996$) and ethanol in water (▲) ($Y = 0.004858x - 0.00424$; $r^2 = 0.9988$).

was integrated and the ratios of these peak areas to that of the internal standard (trichloroethane) were calculated.

3. Results and discussion

Under the chromatographic conditions used, there was no interference with the analyte by any endogenous materials present in blood. There were no blank effects. The method for ethanol was linear in the concentration range used (Fig. 1). Correlation coefficients above 0.99 were easily obtained. The results of the linear regression line of ethanol prepared in water and blood are shown in Table 2. The slopes and the intercepts of the water and blood prepared standards were not significantly different from each other. Under the described conditions, ethanol and *n*-propanol (the internal standard), were well separated from methanol, acetaldehyde and acetone which are potential interferants in ethanol analysis (Fig. 2). Table 3 shows the accuracy ($n = 10$) and the intra-day coefficient of variation ($n = 10$) and inter-day coefficient of variation ($n = 25$) evaluated on four consecutive days at concentrations of 5, 50, 100 and 150 mg/dl. The method proved to be sensitive, robust and reproducible. Sensitivity was reflected by the limit of quantification (S:N = 10) and limit of detection (S:N = 3) which were 0.20 and 0.02 mg/dl, respectively. The method will therefore be useful in forensic cases when blood ethanol levels are very low and back calculation is required to estimate the ethanol levels in previous time. There was no carry over effect when blank samples were analyzed after 200 mg/dl ethanol standards (data not shown). Decreasing the split ratio from 50:1 to 25:1 increased the area count of ethanol standard (50 mg/dl) and *n*-propanol by 50.6 and 49.2%, respectively (Table 4). Ethanol-free blood spiked with ethanol at a concentration of 20, 50 and 150 mg/dl ($n = 5$) and analyzed by the method gave reproducible results (mean ± S.D.) 19.8 ± 0.56, 51.1 ± 0.76 and 152.3 ± .0.93 mg/dl, respectively. There was small alcohol decreases of blood ethanol after 3 days being less than 2% of the original alcohol concentrations. It is known that the addition of salt such as sodium sulphate, to aqueous solutions can increase the sensitivity of static head space analysis by decreasing the solubility of organic compounds in water and hence, lowering the partition coefficients [10]. This salting out effect lowers the solubility of a solute by increasing the overall ionic strength of the solution. The effect is more pronounced for polar compounds that have a greater affinity for water. Because the method reported here is very sensitive, it is not necessary to add salt to the samples, and hence the overall throughput of the method will be increased. Indeed the sensitivity of our method can simply be increased by decreasing the split ratio (Table 4).

Table 3
The accuracy, intra-day coefficient of variation and inter-day coefficient of variation of four concentration (mg/dl) of ethanol measured by static headspace GC/MS

	5	50	100	150
Accuracy ($n = 10$, %)	–	–	–	–
Mean	97.8	100.3	101.9	101.6
S.D.	2.28	0.44	0.74	1.89
Lower 95% CI	96.2	100.0	101.4	99.4
Upper 95% CI	99.4	100.6	102.4	100.9
Coefficient of variation (%)	2.33	0.44	0.73	1.24
Intra-day coefficient of variation ($n = 10$, %)	2.33	0.44	0.73	1.24
Inter-day coefficient of variation ($n = 25$, %)	2.73	1.16	1.08	1.91

Measured over four consecutive days. S.D., standard deviation; CI, confidence interval.

Table 2
Linearity of the static headspace GC/MS procedure for the analysis of ethanol in the concentration range 5–200 mg/dl

Parameter	Water prepared standard	Blood prepared standard
Slope (mean ± S.E.)	0.004858 ± 0.000097	0.005021 ± 0.00005848
Y-intercept (response ratio)	−0.00424 ± 0.0099	−0.001935 ± 0.00603
X-intercept (mg/dl)	0.8729	0.3853
Slope 95% CI	0.004551 to 0.005165	0.004835 to 0.005207
Y-intercept (response ratio)	−0.0359 to 0.02742	−0.02112 to 0.01725
r^2	0.9988	0.9996
Sy.x	0.015	0.0164
LOD (mg/dl)	0.02	0.02
LOQ (mg/dl)	0.2	0.2

Sy.x, standard deviation of the regression line; S.E., standard error; LOD, limit of detection (S:N = 3); LOQ, limit of quantification (S:N = 10).

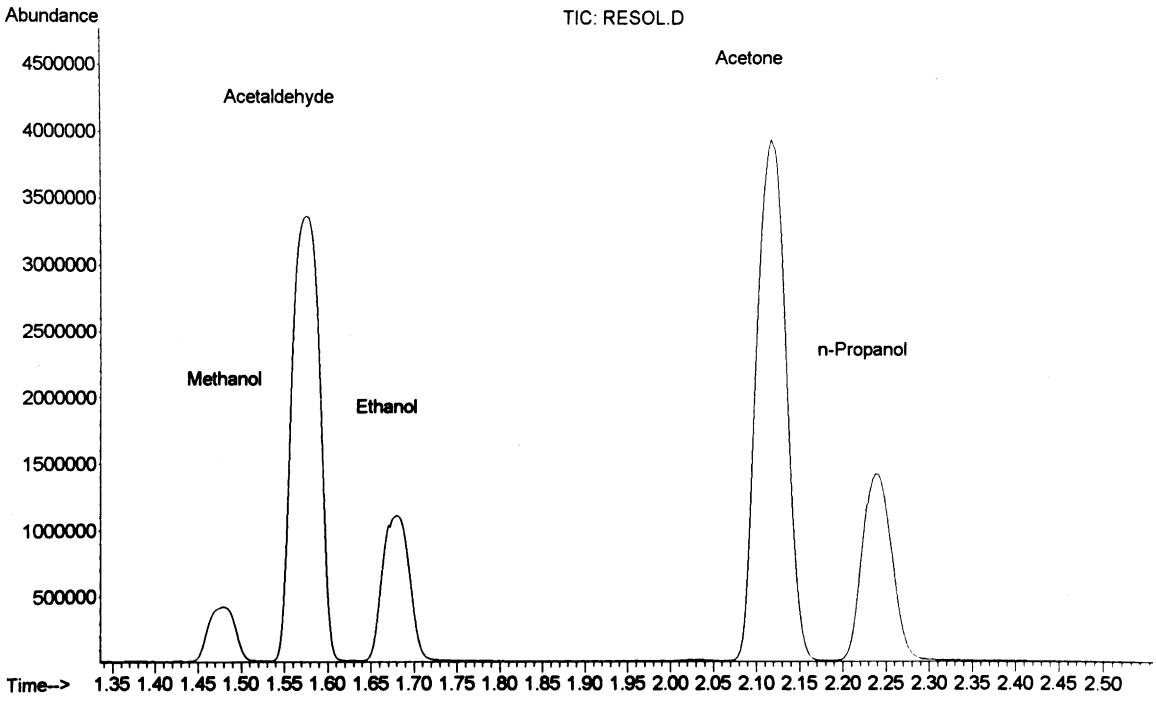

Fig. 2. Total ion chromatogram of methanol, acetaldehyde, ethanol, acetone and *n*-propanol at a concentration of 100 mg/dl each.

Table 4
The effect of modifying the split ratio on the integrated area response of ethanol (50 mg/dl) and *n*-propanol (80 mg/dl)

	Ethanol ($n = 5$)			*n*-Propanol ($n = 5$)		
	50:1	25:1	Increase (%)	50:1	25:1	Increase (%)
Mean	1907000	2878000	50.66	6896000	10290000	49.22
S.D.	10400	21760	1.527	47260	74690	1.392
Lower 95% CI	1894000	2851000	48.76	6838000	10200000	47.49
Upper 95% CI	1920000	2905000	52.56	6955000	10380000	50.95
Coefficient of variation (%)	0.55	0.76	3.02	0.69	0.73	2.83

S.D.: standard deviation; CI: confidence interval.

The calibration curve of the abused inhalants was linear over the concentrations used (10–100 mg/l) and the r^2 was above 0.99 for most compounds (Table 5, Fig. 3) and the different compounds were well resolved from each other (Fig. 4). There was no carry over effect when blank samples were injected after 100 mg/l standards (data not shown). We used only five compounds because these are what we commonly encounter. The method, however, will easily accommodate other inhalants and if the need arises, the GC temperature program could be modified to allow for late eluting compounds. We did not validate the inhalant method, as we are not required to quantify abused inhalants in blood.

Table 5
Linearity of the static headspace GC/MS procedure of abused inhalants in the concentration range of 10–100 mg/l

Parameter	Dichloromethane	Ethyl acetate	Benzene	Toluene	*p*-Xylene	*o*-Xylene
Slope (mean ± S.E.)	0.0139 ± 0.0002	0.0081 ± 0.0002	0.0306 ± 0.0016	0.0369 ± 0.0027	0.0162 ± 0.0002	0.0327 ± 0.0015
Y-intercept (response ratio)	0.0205 ± 0.0087	0.0267 ± 0.0131	0.229 ± 0.0945	0.3702 ± 0.1548	0.0154 ± 0.0093	0.1815 ± 0.0839
X-intercept (mg/l)	−1.487	−3.277	−7.499	−10.04	−0.9529	−5.557
Slope 95% CI	0.0132 to 0.0145	0.0072 to 0.0091	0.0235 to 0.0376	0.0253 to 0.0485	0.0155 to 0.0169	0.0264 to 0.0389
Y-intercept (response ratio)	−0.0172 to 0.0581	−0.0298 to 0.0831	−0.1777 to 0.6360	−0.2957 to 1.036	−0.0245 to 0.0553	−0.1794 to 0.5424
r^2	0.9998	0.9984	0.9942	0.9895	0.9998	0.9960
Sy.x	0.0104	0.0156	0.1123	0.1839	0.0110	0.0997

Sy.x, standard deviation of the regression line; S.E., standard error.

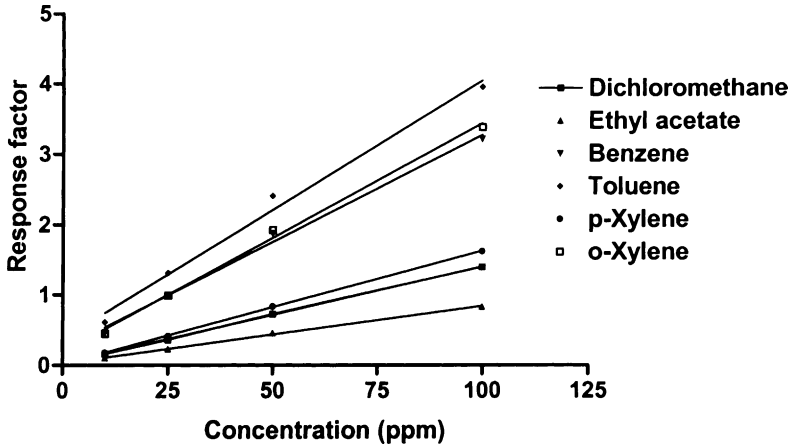

Fig. 3. Calibration curves ($n = 3$, 10–100 mg/l) for dichloromethane, ethyl acetate, benzene, toluene, *p*- and *o*-xylene. For details of the regression equations (see Table 5).

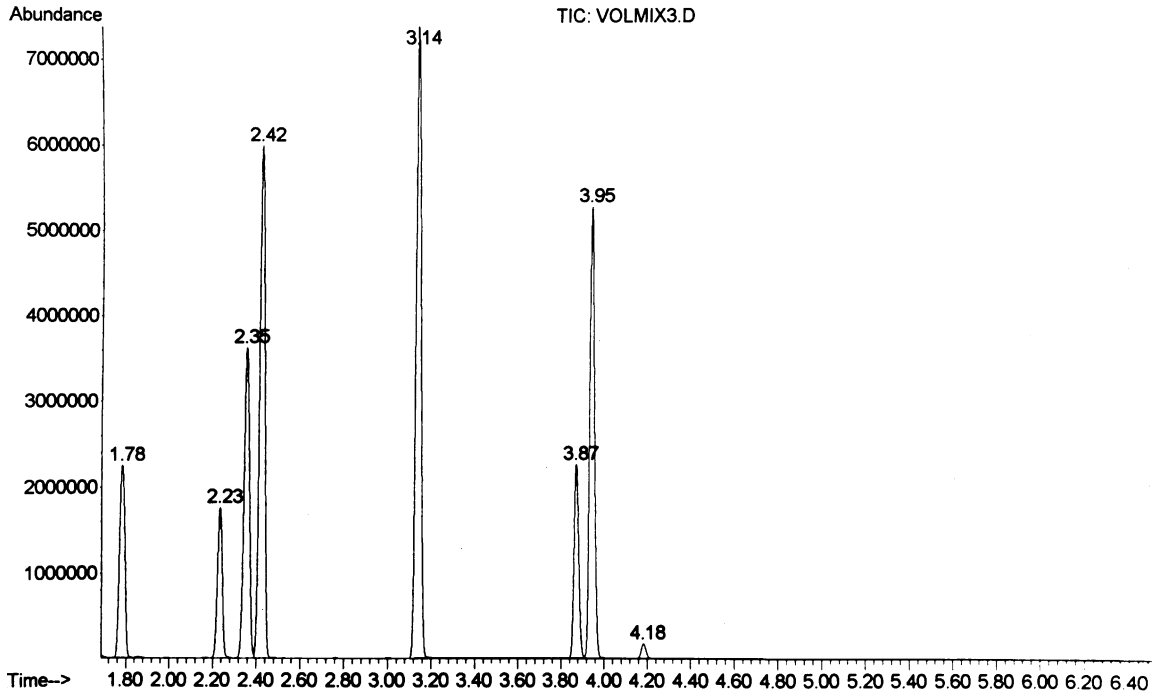

Fig. 4. Total ion chromatograms of a blood sample spiked with five abused inhalants at a concentration of 50 mg/l showing excellent resolution of the different compounds. Dichloromethane (1.78 min), ethyl acetate (2.23 min), trichloroethane (internal standard, 2.35 min), benzene (2.42 min), toluene (3.14 min), *p*-xylene (3.87 min) and *o*-xylene (3.95 min).

Table 6
The concentration of six non-polar inhalants in spiked blood samples ($n = 4$) at a concentration of 50 mg/l

	Dichloromethane	Ethyl acetate	Benzene	Toluene	*p*-Xylene	*o*-Xylene
Mean	47.38	48.20	59.85	59.03	42.90	46.88
S.D.	0.6946	5.763	7.165	7.620	5.052	6.037
Lower 95% CI	46.27	39.03	48.45	46.90	34.86	37.27
Upper 95% CI	48.48	57.37	71.25	71.15	50.94	56.48
Coefficient of variation (%)	1.47	11.96	11.97	12.91	11.78	12.88

S.D., standard deviation; CI, confidence interval.

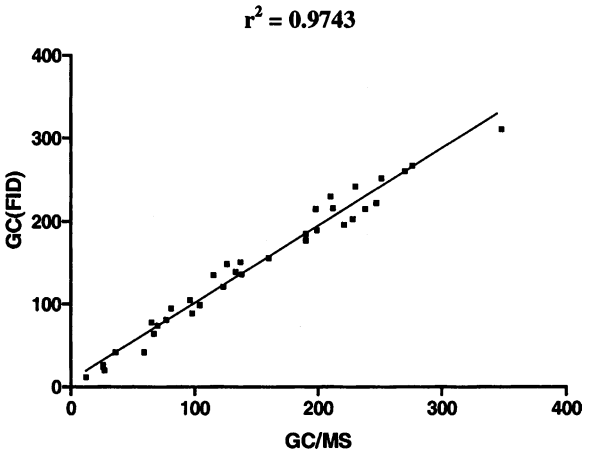

Fig. 5. The correlation of the present GC/MS head space method with a traditional GC head space method using flame ionization detector in analyzing authentic blood samples, $n = 37$. The scale in mg/dl.

However, solvent free blood spiked with the inhalant mixture at a concentration of 50 mg/l each gave reproducible results (Table 6). The concentration of non polar inhalants in blood are generally low in the mg/l range [11,12] or even lower in occupational exposures [13–15], but the area responses we observed for the lowest levels of the calibration curves were high enough to allow for detection and quantification of μg/l concentrations. This may further be improved by optimizing the method.

Application of the GC/MS method for the quantification for blood ethanol in authentic samples ($n = 37$) in comparison with a traditional GC head space method using a FID and a packed column gave acceptable results where the r^2 was 0.9743 (Fig. 5).

Few reports utilized mass spectrometry for the quantification of blood ethanol and abused inhalants [13,14]. This may be due to the prohibitive cost of these instruments in the past. However, with affordable bench tops GC/MS instruments now a days, more laboratories are expected to shift to these instruments. Clearly, such instruments are advantageous to use as a second column for confirmation would not be necessary and unequivocal confirmation of compounds will be obtained. This is of particular importance in forensic samples suspected for presence of abused inhalants.

Acknowledgements

Supported by H.E. General Sheikh Seif bin Zayed Al-Nahyan, under Secretary, Ministry of Interior, General Mohammad Khamis, Deputy Director, Abu Dhabi Police and Lt. Colonel Naser Salem Al-Nuaamy, Director of the Office of H.E. under Secretary, Ministry of Interior.

References

[1] D.J. Brown, W.C. Long, J. Anal. Toxicol. 12 (1988) 279.
[2] A.W. Jones, J. Forensic Sci. 37 (1992) 1208.
[3] M.T. Watts, O.L. McDonald, Am. J. Clin. Pathol. 93 (1990) 357.
[4] T. Macchia, et al., J. Anal. Toxicol. 19 (1995) 241.
[5] C.L. Correa, R.C. Pedroso, J. Chromatogr. B Biomed. Sci. Appl. 704 (1997) 365.
[6] D. Zuba, et al., J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 773 (2002) 75.
[7] B.S. De Martinis, et al., Forensic Sci. Int. 128 (2002) 115.
[8] A.W. Jones, et al., Phamacol. Biochem. Behav. 18 (1983) 267.
[9] J. Schuberth, Biol. Mass Spectrom. 22 (1991) 699.
[10] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925.
[11] N. Barbera, et al., J. Forensic Sci. 43 (1998) 1250.
[12] J.P. Goulle, et al., J. Anal. Toxicol. 23 (1999) 380.
[13] F. Brugnone, et al., Sci. Total Environ. 235 (1999) 247.
[14] R.L. Moolenaar, et al., Int. Arch. Occup. Environ. Health 69 (1997) 139.
[15] L. Perbellini, et al., Int. Arch. Occup. Environ. Health 76 (2003) 461.