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Short communication

Headspace gas chromatography with capillary column for urine alcohol determination

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Abstract

A headspace gas chromatographic method using a fused-silica capillary column Poraplot Q has been developed and validated for the detection and quantification of ethanol in urine. Under optimized conditions, ethanol was properly separated from acetaldehyde, acetone, isopropanol, methanol and *n*-propanol. Limits of detection (LODs) and quantification (LOQs) were 0.008 and 0.010 g/l, respectively. The precision studies within-run and between-run, using spiked urine samples (0.08, 0.8 and 2.0 g/l) showed maximum coefficients of variation 5.9 and 6.5%, respectively. Results of ethanol recovery varied from 91.6±0.8 to 103.3±1.8% over the concentration range from 0.01 to 3.20 g/l. The method was appropriate for the detection of ethanol in urine samples. This matrix can be used for monitoring alcohol abuse in the workplace and used in alcohol rehabilitation programs. © 1997 Elsevier Science B.V.

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1. Introduction

Gas chromatography (GC) has been used for more than 30 years in order to determine ethanol in biological samples and presently is considered as a reference method [1–7]. Direct injection and headspace analysis are the most common techniques for this purpose, but headspace analysis shows clear advantages in extending column life and preventing injector contamination. For this reason, headspace GC is the chosen technique in laboratories with heavy routine workloads [4–10].

In 1992, Tagliaro and Lubli [7] reviewed chromatographic conditions of several methods for ethanol determination described in the literature giving information on the different columns adopted: out of 55 methods described, only three used capillary columns.

Since the use of capillary columns has spread in GC due to clear advantages in efficiency and resolution, in the present work a method for ethanol determination in human urine using a fused-silica capillary column Poraplot Q was developed.

The choice of this biological fluid is justified because urine is the typical biological sample in the workplace drug abuse testing and rehabilitation programs [7,11,12].

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2. Experimental

2.1. Chemicals

Ethanol aqueous working solutions were prepared from 4% and 9.6% (v/v) ethanol standard solutions (Sigma, St. Louis, MO, USA). The internal standard was prepared from an aqueous stock solution containing 20 g/l reagent grade *n*-propanol (Merck, Darmstadt, Germany). Acetaldehyde, acetone, isopropanol and methanol were from Merck.

2.2. Instrumentation

GC was performed on a 6890 Hewlett-Packard (HP) gas chromatograph (Hewlett-Packard, Wilmington, USA) fitted with Poraplot Q fused-silica capillary column (10 m×0.32 mm I.D.) (Chrompack, Netherlands). The oven temperature was isothermal at 100°C and the injector and the flame ionization detection (FID) system were at 250°C. Helium was used as carrier gas at 2.6 ml/min and N_2 was used as auxiliary gas. Air and H_2 were set for best FID function. The split rate was 1/5. A HP 3395 integrator was used with attenuation and threshold settings of 0 and peak width 0.04 min, respectively.

2.3. Analytical procedure

In a 10 ml glass vial 1.0 ml urine and 1.0 ml of internal standard were mixed with 2 g of anhydrous sodium sulfate. The vial was sealed with a rubber cap and an aluminium crimp seal and incubated for 30 min at 70°C. The upper gas phase was homogenized three times by pulling and pushing the vapor phase using the injection syringe. After this, a homogenized 250 μl gas aliquot was withdrawn through the rubber cap with a 250 μl gas-tight SGE syringe (Sydney, Australia) and injected directly into the gas chromatograph. (This syringe was equilibrated at 50°C, in order to prevent internal condensation on the walls).

Linearity and calibration curves were performed in water and urine spiked with ethanol standard to obtain concentrations of 0.01, 0.03, 0.07, 0.13, 0.32, 0.64, 1.60 and 3.20 g/l. The precision within-run and between-run and recovery tests were performed using urine samples in six replicates at concentrations of 0.08, 0.8 and 2.0 g/l for three days. Specificity was studied with acetaldehyde, acetone, ethanol, isopropanol, methanol and *n*-propanol in urine at the following concentrations: 0.04, 0.08, 0.10, 0.08 g/l.

3. Results and discussion

Under optimized conditions, ethanol was properly separated from acetaldehyde, acetone, isopropanol and methanol which are potential interferents in ethanol and *n*-propanol separation. Fig. 1 shows a chromatogram of a urine sample spiked with acetaldehyde and acetone (0.04 g/l), ethanol, isopropanol and *n*-propanol (0.08 g/l) and methanol (0.10 g/l). The results of absolute and relative retention times of these compounds in Table 1 demonstrate the appropriate selectivity of Poraplot Q column for ethanol determination.

The present method showed good linearity in a concentration range of 0.01 to 3.20 g/l ($y=5.261x-0.1473$; $r=0.9990$) which permits to control light use

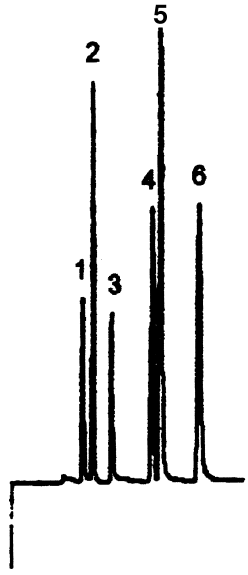

Fig. 1. Chromatographic profile of a blank urine sample spiked with (1) methanol 0.10 g/l, (2) acetaldehyde 0.04 g/l, (3) ethanol 0.08 g/l, (4) acetone 0.04 g/l, (5) isopropanol 0.08 g/l and (6) *n*-propanol 0.08 g/l.

Table 1
Results of absolute and relative retention times of the tested volatile substances using headspace gas chromatography

Elution order	Compound	Retention time (mean±S.D., $n=10$) (min)	Relative retention time
1	Methanol	1.929±0.0012	0.38
2	Acetaldehyde	2.212±0.0040	0.44
3	Ethanol	2.718±0.0024	0.54
4	Acetone	3.801±0.0027	0.75
5	Isopropanol	4.022±0.0042	0.79
6	*n*-Propanol	5.063±0.0076	1.00

of alcohol up to acute intoxication. Calibration curve in urine showed the following straight line equation: $y=4.848x-0.06536$, $r=0.9999$.

The limit of detection was 0.008 g/l of ethanol determined by progressive dilution method. The limit of quantification was 0.010 g/l with coefficient of variation (C.V.) of 4.3%, and average and standard deviation of 0.0129±0.0005 g/l for ten replicates.

Ethanol recovery in urine was calculated for different concentrations, comparing area ratios of ethanol and the internal standard obtained in the calibration curve and those obtained in the linearity study. A 100% recovery value was attributed to the aqueous solutions. Results varied from 91.60±0.81 to 103.28±1.79% (Table 2). C.V.s obtained in the precision study within-run and between-run showed maximum values of 5.9 and 6.5%, respectively, as is shown in Tables 3 and 4. The reproducibility study was performed by two different analysts, and showed C.V.s not superior than 5.3%, which confirms the above results.

Salt addition to the matrix has often been used to enhance the concentration of volatile components in the vapor phase for headspace analysis [12]. Sodium sulfate was chosen for this purpose due to its size, charge density and the resultant effects on water structure [13].

The proposed method was tested in urine samples of ten healthy male volunteers ranging from 19–30 years old. They ingested a single dose of whisky (0.68 g ethanol per kilogram of body weight). Table 5 shows the concentration of ethanol in urine sam-

Table 2
Recovery of ethanol from spiked urine samples

Concentration (g/l)	Recovery (mean±S.D., $n=3$) (%)
0.01	100.99±1.04
0.03	95.13±1.90
0.07	96.93±1.55
0.13	98.62±0.93
0.32	103.28±1.79
0.64	97.76±2.13
1.60	94.31±1.53
3.20	91.60±0.81

Table 3
Coefficients of variation within-run of urinary ethanol concentrations in spiked samples

Concentration (g/l)	n	x	S.D.	C.V. (%)
0.08	6	0.3895	0.0229	5.9
0.08	6	0.3522	0.0065	1.8
0.08	6	0.3511	0.0116	3.3
0.8	6	4.0101	0.2077	5.2
0.8	6	3.8158	0.0993	2.6
0.8	6	3.7748	0.1395	3.7
2.0	6	10.2857	0.5217	5.1
2.0	6	10.0703	0.5908	5.9
2.0	6	9.9377	0.4229	4.3

n=Number of analysed samples.
x=Mean of area ratio ethanol/*n*-propanol.
S.D.=Standard deviation.
C.V.=Coefficient of variation.

Table 4
Coefficients of variation between-run of urinary ethanol concentrations in spiked samples

Concentration (g/l)	n	x	S.D.	C.V. (%)
0.08	18	0.3650	0.0238	6.5
0.8	18	3.8669	0.1802	4.7
2.0	18	10.0979	0.5085	5.0

n=Number of analysed samples.
x=Mean of area ratio ethanol/*n*-propanol.
S.D.=Standard deviation.
C.V.=Coefficient of variation.

Table 5
Concentration of ethanol in urine samples of ten volunteers, according to the collect time

Time (h)	Concentration of ethanol (g/l)									
	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.57	1.11	1.17	0.85	0.84	1.21	1.28	1.44	1.02	1.23
2	0.96	1.04	1.32	1.00	1.22	1.10	1.17	1.24	1.17	1.43
3	0.83	1.02	1.07	0.75	1.01	0.94	0.90	0.87	0.99	1.12
4	0.68	0.69	0.81	0.50	0.69	0.68	0.71	0.53	0.67	0.83
5	0.39	0.50	0.60	0.24	0.40	0.43	0.49	0.32	0.15	0.73
6	0.11	0.28	0.25	0.05	0.18	0.15	0.26	0.15	0.00	0.36
7	0.03	0.07	0.08	0.00	0.00	0.04	0.07	0.03	0.00	0.11
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

ples of these volunteers, collected in a period of 12 h. Fig. 2 shows chromatographic profiles of urine samples collected before and 5 h after administration of ethanol.

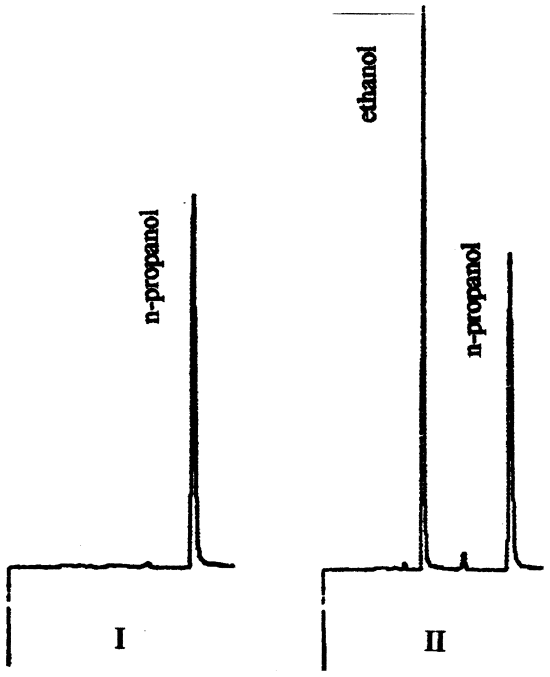

Fig. 2. Chromatographic profile of (I) a blank urine sample spiked with *n*-propanol (internal standard) and (II) urine collected 5 h after the administration of ethanol (0.68 g/kg bw). The measured concentration of ethanol was 0.39 g/l.

Since the method was effective for the determination of ethanol in urine during several hours after a moderate dose ingestion of alcohol when no intensive pharmacological effects was observed, it suggests its usefulness for monitoring alcohol abuse in workplace.

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