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Letter to the Editor

Simplified gas chromatographic analysis of ethanol in blood and tissue

Sir,

Quantitation of ethanol by gas and liquid chromatography has been previously reported [1–8]. Head-space analysis [1–3] of volatile organics is frequently used, since biological samples can be analyzed without extraction and separation techniques. However, a disadvantage of head-space and breathalyzer analysis of ethanol is that the liquid–air partition coefficient of ethanol varies depending upon the biological matrix [9,10] or in the case of breathalyzer analysis, the subject's pulmonary function [11–13]. We report a sensitive and specific method for quantitating ethanol in blood or tissue, which allows direct injection of the liquid sample with minimal sample preparation and rapid analysis time.

EXPERIMENTAL

A Hewlett-Packard Model 5830A gas chromatograph [14] equipped with a flame ionization detector was used. The column was coiled glass, 1.22 m × 4 mm I.D., packed with 5% Carbowax 20M on 100–120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The carrier gas was ultra-high-purity helium at a flow-rate of 30 ml/min. The detector purge was ultra-high-purity hydrogen at 30 ml/min mixed with dry air at 200 ml/min. Operating temperatures were: injection port, 150°C; column, 100°C; detector, 200°C. Before being connected to the detector, a new column was conditioned at 160°C for 24 h with a carrier flow-rate of 15–20 ml/min.

The following reagents were used as received from commercial sources: absolute ethyl alcohol and 1-butyl alcohol. Stock solutions of ethanol and 1-butanol were prepared daily by diluting 0.125 ml of each up to 25 ml in distilled water to yield concentrations of 3.95 mg/ml for ethanol and 4.05 mg/ml for 1-butanol. A 100-μl volume of 1-butanol stock solution (the internal standard) was added to a series of plastic 7-ml round-bottom tubes. Calibration standards for ethanol were prepared by adding 5, 10, 25, 50, 100, 200 and 300 μl of ethanol stock solution to consecutive tubes and sufficient water to bring the final volume to 400 μl per tube. Drug-free blood or brain homogenate was added to each of the calibration

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tubes; unknown blood or brain homogenate was added to tubes containing internal standard only.

Animal studies

Male CD-1 mice, six weeks old, received 2 g/kg ethanol (30%, v/v) by intraperitoneal injection. Mice were sacrificed at various time points, brains rapidly removed and placed in pre-weighed, pre-chilled test tubes containing 800 μl of ice-cold water with internal standard. The tubes were immediately capped and replaced on ice. The tubes were reweighed and the brains homogenized using a polytron homogenizer, setting 7, for two quick bursts. The homogenate was centrifuged at 2600 *g* for 10 min. A 2-μl aliquot of the supernatant was injected directly onto the chromatograph.

Human studies

A healthy male volunteer, age 28, weight 79 kg, ingested 60 ml of commercially available vodka (80 proof) following an overnight fast. Serial blood samples were drawn by venipuncture prior to dosage and at 0.5, 1, 1.5, 2, 2.5, 3 and 4 h postdose. At each time point, two 200-μl aliquots of blood were rapidly placed into separate 2-ml glass vials containing ice-cold internal standard. The vials were immediately capped, and shortly thereafter a 2-μl aliquot of each was injected directly onto the chromatograph.

Recovery and stability studies

To evaluate the possibility of losses during preparation and analysis, or to enzymatic and/or non-enzymatic conversion of ethanol, the following recovery studies were performed. To evaluate the effect of brain enzymes and/or brain homogenization on ethanol levels, a series of 1 mg/ml ethanol standards were prepared; half were analyzed immediately and the other half after a mouse brain had been added to each and processed as described above. A similar experiment was performed to estimate potential ethanol losses due to enzymes in whole blood. A series of freshly drawn, whole human blood samples were spiked with ethanol, 1 mg/ml, and analyzed along with a series of 1 mg/ml ethanol standards in water.

Ethanol loss during storage was evaluated as follows. The brains of ethanol-dosed mice (see above) were bisected and placed in separate test tubes containing ice-cold water and internal standard. One of the two samples was analyzed immediately, while the other was stored at $-20\,^{\circ}$C overnight before being homogenized and analyzed the next day.

RESULTS

Under the described conditions, the retention times for ethanol and 1-butanol were approximately 2.1 and 3.9 min, respectively (Fig. 1). The supernatant of blank brain homogenate, as well as ethanol-free whole blood, contained no interfering peaks. The relation between ethanol concentrations and peak-height ratio (versus internal standard) was linear at least to 3 mg/ml. Analysis of nine standard curves over four weeks showed that the correlation coefficient was always

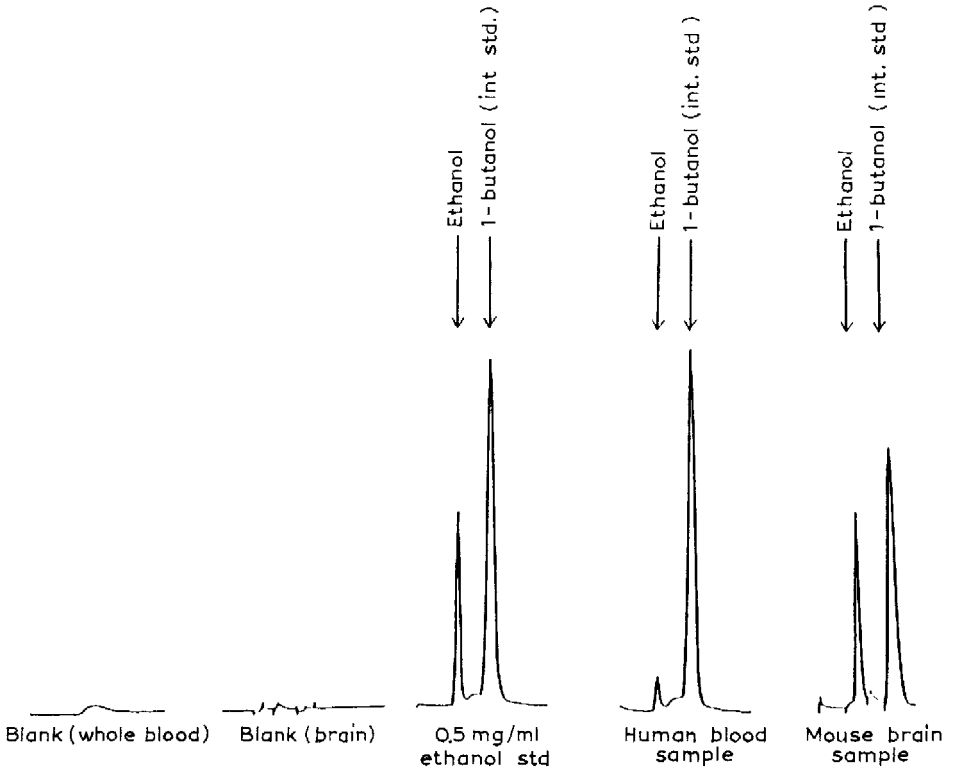

Fig. 1. Chromatograms of (left to right): blank sample of human whole blood; blank homogenate of mouse brain; calibration standard containing 0.49 mg/ml ethanol; actual human blood sample following ingestion of ethanol; mouse brain sample after 2 g/kg injection of ethanol. Retention times are: ethanol, 2.1 min; 1-butanol, 3.9 min.

greater than 0.99 with a coefficient of variation of 8.8%. The limit of detection was 0.01 mg/ml. Within-day coefficients of variation for identical samples ($n=6$) were 11.7% at 0.01 mg/ml, 11.8% at 0.05 mg/ml and 8.0% at 1 mg/ml. A freshly packed column was stable over a period of at least six weeks (300 or more injections).

The mean ($\pm$S.E., $n=4$ at each time point) ethanol levels in mouse brain were 1.60 ± 0.23 mg/g at 20 min, 1.42 ± 0.07 mg/g at 40 min, 1.89 ± 0.21 mg/g at 60 min, 1.35 ± 0.20 mg/g at 70 min, 1.12 ± 0.23 mg/g at 85 min and 1.10 ± 0.26 mg/g at 100 min. The blood ethanol levels for the human were 0.427 mg/ml at 0.5 h, 0.355 mg/ml at 1 h, 0.275 mg/ml at 1.5 h, 0.235 mg/ml at 2 h, 0.150 mg/ml at 2.5 h, 0.150 mg/ml at 2.5 h, 0.109 mg/ml at 3 h and 0.046 mg/ml at 4 h. These values are consistent with previously published results [3].

The recovery of ethanol from brain and from whole blood was essentially complete. The mean brain concentration for six half-brain samples analyzed immediately (1.42 mg/g) was nearly identical to the six samples analyzed after freezing and overnight storage (1.45 mg/g) (paired $t=0.02$, not significant).

DISCUSSION

This paper describes a reliable, selective method for the quantitation of ethanol in blood or brain using GC with flame ionization detection. The method produces blank samples that are free from interfering peaks in the areas corresponding to ethanol or internal standard. The method requires no extraction steps or use of reagents other than the internal standard. Based on recovery studies, ethanol losses due to evaporation, spontaneous oxidation and/or enzymatic conversion are negligible. Others report the use of thiourea to prevent oxidation and perchloric acid to prevent enzymatic conversion of ethanol [1,2,8,15]. However, many reported methods [1,2,15] are concerned with simultaneously monitoring acetaldehyde levels. Acetaldehyde concentrations are 50- to 1000-fold lower than the corresponding ethanol levels in rats [1,2] and approximately 2000-fold lower in humans [3]. Therefore negligible losses of ethanol due to conversion to acetaldehyde may cause appreciable changes in acetaldehyde levels. However, this is relevant only if acetaldehyde must be simultaneously quantitated.

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