Determination of Volatile Alcohols and Acetone in Serum by Non-Polar Capillary Gas Chromatography after Direct Sample Injection

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In this method for detection and quantification of volatile alcohols by capillary gas chromatography, the serum sample is deproteinized, then directly injected into the gas chromatograph with 1-propanol as the internal standard. The capillary column is a 30-m bonded methylsilicone-coated, fused-silica column. With helium as the carrier gas, the injector inlet is set at a split ratio of 1/30 and the average linear velocity in the column is 25 cm/s. Injector and flame-ionization detector temperatures are 280 °C, oven temperature 35 °C. Chromatography time is <3 min.

Poisoning by toxic volatile alcohols is relatively uncommon, but early detection and quantification are essential to effective treatment. The current technique of choice is gas chromatography (GC) of serum or plasma, and many such methods have been published (1). With the recent advent of bonded-phase fused-silica capillary columns however, many clinical laboratories are abandoning packed-column GC in favor of capillary GC. Fused-silica columns are flexible and reasonably rugged, and the bonded phases coating the wall of these columns are much more resistant to abuse than are their non-bonded predecessors. Adding these new attributes to those characteristics of capillary columns in general—i.e., enhanced peak resolution, sensitivity, and speed of analysis—the reasons for the trend to this new technology become obvious. Because of the widespread (and usually well-founded) advice by gas chromatograph and capillary column manufacturers to install water traps in carrier-gas lines, it is not generally appreciated that one can actually inject aqueous samples into non-polar capillary columns under appropriately mild conditions. Thus, up to now, published reports on the use of capillary GC in analysis for volatile alcohols have only dealt with specialized methods and accessories such as headspace techniques (2), gas chromatography-mass spectrometry (3), which is beyond the budget of many laboratories, or cryogenic accessories (4), which would in effect be dedicated to this one clinical application. I report here an alternative: a simple, rapid, and reliable capillary GC method of analysis for volatile alcohols after direct injection of deproteinized serum. One case of massive methanol ingestion has already been detected with this procedure, resulting in the saving of both the patient's life and his sight.

Materials and Methods

Materials

Reagent-grade methanol, acetone, 1-propanol, 2-propanol (isopropanol), cupric sulfate pentahydrate, and sodium tungstate dihydrate were used as supplied. Ethanol (95%) was redistilled before use.

Methods

Sample preparation: In a 2-mL centrifuge tube, combine 200 μL of serum or standard solution with 200 μL of internal standard solution (1-propanol, 13.0 mmol/L, in de-ionized water), 200 μL of 200 mmol/L sodium tungstate, and 200 μL of 200 mmol/L cupric sulfate (5). Vortex-mix and centrifuge for 2–3 min at 9500 × g to sediment the precipitate. Then directly inject 1 μL of the aqueous supernate into the gas chromatograph.1

1 This sample preparation procedure works equally well with whole-blood, giving similar results, but because in this hospital only serum samples are routinely used, this report describes the results obtained for serum.

References

Gas-chromatographic analysis: We used a Hewlett-Packard (Avondale, PA) Model 5880 "Level Four" gas chromatograph equipped with a split/splitless capillary injector port and a flame-ionization detector. The wall-coated open tubular column was a 30-m Supelco (Bellefonte, PA) methylsilicone bonded phase (SPB-1) fused-silica column with an internal diameter of 250 μm and a film thickness of 250 nm. With helium as the carrier gas, the inlet pressure was set at 70 kPa to provide an average linear velocity at 35 °C of 25 cm/s. The split ratio was 1/30. Flow rates for hydrogen, make-up gas (helium), and air were 35, 35, and 350 mL/min, respectively. We ran the analyses isothermally at 35 °C, with injector and detector temperatures set at 280 °C. The split liner in the injector port contained a 10-mm column of 3% OV-1 on Gas Chrom Q, plugged at the top and bottom with silanized glass wool. This column served to trap nonvolatile impurities from the sample; it gradually blackened from the top down with use. After the analysis of several hundred samples about 70% of the column was discolored, and the liner was then emptied, cleaned, and refilled with fresh packing.

Conveniently, the instrument, through its "Keystroke Programming" feature, could be set up to run semi-automatically. The technologist merely runs the program and injects the sample when the "Ready for Injection" prompt is printed out. Accordingly, making the volatile-alcohols analysis available 24 h a day has become a simple matter.

Results

Figure 1 shows a typical chromatogram obtained with a mixture of volatile alcohols. The peaks are typically well shaped, and baseline or near-baseline resolution was obtained with all peaks. The analysis is so rapid that the chart speed must also be fast (5 cm/min). There is no solvent peak, because water is the injection solvent and flame-ionization detectors are insensitive to water. Thus a blank sample with no internal standard present produces a flat baseline.

Analytically, we could account quantitatively for all of these volatile compounds in serum as well as in distilled water. Detector response for these compounds was linear from zero up to concentrations known to be fatal (ethanol 10.0 g/L, all others 2.36 g/L), the curve passing through the origin. The method is very sensitive, easily capable of quantifying peaks representing concentrations of less than 1 mmol/L, but we set the lower reportable limit at 1 mmol/L to eliminate reporting of positives of negligible clinical significance. The information in Table 1 demonstrates that the procedure is highly reproducible. Further, in monitoring this procedure for several months, we did all ethanol determinations on patients' sera in parallel with the alcohol dehydrogenase-based (6) method used in the aca discrete analyzer (Du Pont Co., Clinical Systems Div., Wilmington DE). Results from the two methods agreed well (y = 1.020x − 0.537, where y = GC data, x = aca data; units, mmol/L; correlation coefficient = 0.991; n = 32).

Discussion

The major advantageous feature of this method is that deproteinized serum is directly injected into a general-purpose nonpolar capillary GC column to give a rapid, accurate, and reproducible result. However, a clean column is important. High-boiling impurities left over from other analyses can degrade column performance for the analysis of volatile alcohols. The column may be easily cleaned after such "dirty" analyses by making three splitless 5- to 10-μL injections of water, at 1-min intervals, at an oven temperature of 150 °C, then heating the column to 280 °C for 15–20 min. The analysis for volatile alcohols is quite "clean," not necessitating a column wash.

The only interference we have encountered is that methanol and acetaldehyde have the same retention time. However, the acetaldehyde peak appears only when the ethanol peak is present, and then it is only <1% of the ethanol peak (7), so it is not a notable problem. Indeed, we find that acetaldehyde concentrations very rarely exceed the reporting threshold of 1 mmol/L.

We also tested the procedure in a converted Hewlett-Packard Model 5880 gas chromatograph, an instrument that is not capable of reaching an oven temperature close to ambient unless the oven door is open. We made several runs, using a Hewlett-Packard 12-m crosslinked methylsilicone coated fused-silica column in this instrument, with the door left open, and with no screen or other provision for protecting the column from random air currents. The resulting chromatograms were as good as that shown in Figure 1.

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<th>Table 1. Precision of Capillary GC Analysis for Volatile Compounds in Serum</th>
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<td>Mean concn*, mmol/L</td>
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* For each volatile we analyzed four samples per day for five days, a total of 20 determinations.
Evidently this method can be used even with gas chromatography having relatively poor oven-temperature control, although an air current diffuser is probably advisable if such instruments were to be used routinely.

I thank Lorette Fournier for her excellent technical assistance with the precision study, and various other technologists in the department who, under the supervision of Mr. Naresh Rawal, performed the GC/Cos study.

References

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Silver-Stained Phenotyping of α₁-Antitrypsin in Dried Blood and Serum Specimens

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In this technique for determining the electrophoretic phenotype of α₁-antitrypsin in dried blood or serum specimens, the adsorbed material is eluted with a concentrated solution of dithiothreitol, focused on polyacrylamide thin-layer gel, and made visible with silver stain. With this staining technique all normal and pathological α₁-antitrypsin phenotypes can be detected. The procedure is relatively simple, inexpensive, and suitable for use in large-scale screening for α₁-antitrypsin deficiency in selected populations.

Additional Keyphrases: screening · genetic variants · electrophoresis, polyacrylamide gel · pediatric chemistry · heritable disorders

Subjects with congenital deficiency of α₁-antitrypsin (AT) in their serum are prone to lung and liver disorders (1). About half of those with homozygous deficiency of AT (electrophoretic ZZ phenotype, as compared with the MM phenotype of normal subjects) start to develop liver disorders during fetal life; the minority of those affected develop manifest cirrhosis during the first postnatal decade (2). Another 5 to 10% of those with the ZZ phenotype develop liver cirrhosis of unknown etiology after age 50. The clinical evolution of affected individuals who do not show clinical signs of liver involvement in childhood appears to be related to environmental conditions, or life style, or both. E.g., smokers frequently develop chronic obstructive lung disease and die at about age 40–50. Conversely, the life expectancy of non-smokers with this phenotype is fairly close to that of normal individuals (3). There are several other combinations of variant alleles (SZ, MZ, etc.) besides the ZZ phenotype, all associated with an intermediate quantitative deficiency of AT, but their clinical significance is still uncertain.

To assess the incidence of AT deficiency in the general population and to study the natural history of the related disease, screening has been done. Current screening tests involve dried blood specimens, from which AT is eluted, then semiquantitatively determined (4, 5). We describe here a relatively inexpensive technique for phenotype determination of AT in either dried blood or serum, and illustrate its advantages and results of its application.

Materials and Methods

Samples. No. 3 Whatman paper was used to adsorb specimens of fresh, nonheparinized blood or serum. Instructions for use of such strips were sent to 37 departments of pediatrics, centers of respiratory physiopathology, and clinical chemistry laboratories of northern Italy, where the incidence of AT deficiency is highest. The samples obtained from 30 subjects were mailed to our laboratory and a further 50 samples were obtained in our own laboratories from voluntary donors or patients who were carriers of AT deficiency. The blood or serum was eluted from the paper, either promptly upon arrival or after storage for a week at 4°C or a month at −18°C. For the elution, we placed a 0.5-cm sample-impregnated square of the filter paper in a conical test tube and added a few drops of N,N-dithiothreitol (DTT; cat. no. D-0632; Sigma Chemical Co., St. Louis, MO 63178), 20 g/L in doubly distilled water. The quantities of paper or solution are not critical, but the best results were obtained by using 0.5-cm paper squares with 100 μL of solution, and by incubating these at a constant temperature of 37°C for 1 h. After 30 min the paper at the bottom of the test-tube should be pressed to further release blood or serum into the solution.

Genetic typing of AT. We used the following gel composition: 2.5 mL of a 27.5 g/L solution of acrylamide (cat. no. 161-0100; Bio-Rad Laboratories, Richmond, CA); 2.5 mL of a 27 g/L solution of bisacrylamide (cat. no. 161-0200, Bio-Rad); 10 mL of doubly distilled water; 200 μL of Amphetamine pH 3.5–5 (cat. no. 1509-111; LKB, Bromma, Sweden); 100 μL of Amphetamine pH 4–6 (cat. no. 1609-116, LKB); and 1.4 g of

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