TECHNICAL NOTE

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Differentiation of Diethyl Ether/Acetone and Ethanol/Acetonitrile Solvent Pairs, and Other Common Volatiles by Dual Column Headspace Gas Chromatography

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ABSTRACT: Gas chromatography is the most widely used method for the analysis of ethanol and other low molecular weight volatiles for forensic applications. Many laboratories use only a single analytical method for this analyte. Concern over the possible misidentification of acetonitrile as ethanol, and our experience in a case where we misidentified diethyl ether as acetone using a single method approach, led us to develop and adopt the dual column method described herein. Two columns, 5% carbowax on 60/80 Carbopak B, and 0.8% THEED on 80/100 carbopak C, were used for the complementary analysis of 32 common volatile organic compounds.

KEYWORDS: toxicology, ethanol, diethyl ether, ethyl chloride, gas chromatography

One of the fundamental principles applied in forensic toxicology, is the use of a second complementary analytical technique for confirmation of identity. One area where this is frequently ignored however, is in blood volatiles analysis. In our experience, most laboratories performing blood volatiles testing for forensic purposes use a single column gas chromatography (GC) method, and rationalize this single method approach on the basis that there are very few volatile compounds likely to be found in biological fluids, and that these compounds are so well characterized that interference or misidentification can effectively be ruled out.

Recently however, authors have commented on the limited ability of some GC phases in widespread use to differentiate between some common volatiles. A report by Jones et al. [*1*] noted that using one of the stationary phase described here, 5% Carbowax 20M on Carbopak B, and another phase, 0.2% Carbowax 1500 on Carbopak C, acetonitrile and

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ethanol co-eluted, and could not be distinguished. A third phase, 15% Carbowax 20M on Chromasorb W was required to achieve the required separation. Another author [*2*] has confirmed the similar retention behavior of ethanol and acetonitrile on two phases; 5% Carbowax 20M on 80/120 Carbopak B (Supelco), and Carbowax 20% SP-2100 + 0.1% Carbowax 1500 on 100/120 Supelcoport (Supelco), although partial resolution was achieved with the latter phase. That author reported also that using a DB-wax megabore column (J& W Scientific), the acetonitrile interfered with the internal standard, n-propanol. Both authors concluded, based on their experience with the ethanol/acetonitrile solvent pair, that at least two independent methods of analysis were required for positive identification of ethanol. A further compound of concern is ethyl chloride (chloroethane), which has been mistaken for ethanol using 0.2% and 0.3% Carbowax columns [*3*].

In addition to the above considerations, we experienced a case in which a 15-year-old girl was abducted, and murdered by strangulation. At autopsy a blood sample was collected and analyzed routinely by GC (5% Carbowax 20M on 80/120 Carbopak B only) for alcohol [*4*]. Results showed the presence of "acetone" as indicated by comparison with a standard mixture (sample RT = 0.90 mins., acetone RT = 0.89 mins.). The acetone result was quantitated and reported as 0.05 g/100 mL, together with negative results for a blood drugs-of-abuse analysis.

In reviewing the autopsy findings and medical history, the medical examiner could find no indication of a prior diagnosis of diabetes. He did however note that police officers investigating the case had been told by the parents that they had smelled "ether" on returning home and finding the girl missing. Subsequent analysis of a diethyl ether standard on the GC system showed it to have a retention time indistinguishable from acetone on the 5% Carbowax 20M on Carbopak B system.

Headspace vapor from a second aliquot of blood from the deceased was analyzed by gas chromatography/mass spectrometry (GCMS) (HP 5970A). This analysis showed the presence of heptane in addition to diethyl ether, but no acetone.

This initial misidentification of diethyl ether as acetone was clearly unacceptable, and led to the investigation of a new GC stationary phase, and the development of the dual column protocol described below. During the development of this system it became clear that there are a number of possible misidentifications which can occur when a single GC method is used and we reiterate the conclusions of other workers in advocating the use of two separate methods.

Materials and Methods

Ethanol was 200° proof and was supplied by Midwest Grain Products, IL. n-Propanol was high purity solvent grade (Burdick and Jackson). Other solvents and chemicals were analytical grade or better and supplied by a variety of chemical suppliers. Water was deionized (Millipore, Milli-Q) and had a resistance of greater than 18M Ω.

Blood samples were collected in 10 mL tubes containing 20 mg of potassium oxalate and 25 mg of sodium fluoride as anticoagulants and enzyme inhibitor respectively (Vacutainer grey top, Becton Dickinson). Samples were shipped by regular mail and stored refrigerated or frozen until analysis.

The internal standard solution was prepared by dissolving n-propanol (150 μL) and NaCl (20 g) in water (1 L). Aliquots from the blood samples (200 μL) were removed and diluted with internal standard solution (2 mL), using a pipetter dilutor (Hamilton). The mixture was dispensed into a 10 mL vial capped with a septum and crimp-sealed.

Gas chromatography was performed on Perkin Elmer 2970 gas chromatographs equipped with flame ionization detectors, coupled to Hewlett Packard 23597 headspace autosamplers with 1 mL loops, using nitrogen as the carrier gas. Chromatograms were recorded on computing integrators (Spectra Physics SP4270) and results reported rounded to three

decimal places. Autosampler conditions were identical for both columns, requiring equilibration of samples for 3 min, pressurization of the sample for 10 s, and venting for 15 s. The bath temperature was 60°C, and the transfer loop was 65°C. Ethanol controls (0.100 g/100 mL, CAP) were run in an identical manner and constituted 10% of all analyses.

The first GC method employed a 6 ft glass column packed with 80/100 Carbopak C / 0.8% THEED (Supelco), operated at 67°C using nitrogen as carrier gas at a flow rate of 30 mL/min.

The second GC method employed a 6 ft glass column packed with 60/80 Carbopak B / 5% Carbowax 20M (Supelco). It was operated at 73°C using nitrogen as carrier gas at a flow rate of 30 mL/min.

The calibration of both instruments for ethanol was checked daily, at four points, 0.000, 0.079, 0.158, 0.316 g/100 mL, and was recalibrated if required. A reference mixture of methanol, acetone, ethanol, and isopropanol was run daily. Duplicate aliquots of each specimen were prepared. Each sample was analyzed once on each system, and the mean level rounded to two decimal places and reported.

The reproducibility of the retention time was evaluated for both systems over a period of seven days, by examining the retention data for n-propanol, the internal standard. Relative retention times were calculated with respect to n-propanol for all analytes. Under normal circumstances the run time was terminated at 4 min for the Carbowax system and 5 min for the THEED system, however for the purposes of evaluating the interference potential of a variety of other solvents, the run time in this study was extended to 30 min.

Thirty two common low molecular weight volatile organic compounds (listed in Tables 1 and 2) were diluted with water to a concentration at which they could be detected by both GC systems. With some immiscible solvents, methanol was added to assist with solubility.

Results and Discussion

Tables 1 and 2 show the retention behavior of the analytes that were tested on both systems. Table 1 is indexed by retention index on the THEED column, and Table 2 is indexed alphabetically. Both tables show both retention time, and retention index with respect to n-propanol. The selectivity of the THEED column was markedly different from that of the Carbowax column. The THEED phase is marketed for glycol analysis, and to our knowledge this is the first report of its routine use for forensic volatiles analysis. Figure 1 shows the lack of separation for ethanol/acetonitrile and diethyl ether/acetone on the Carbowax column and, their separation on THEED column. Figure 2 illustrates the difference in selectivity for the two columns, for all compounds eluting in under six minutes on both columns.

In terms of retention time reproducibility, both methods was extremely robust. The retention time for n-propanol on the Carbowax system for multiple injections (n = 150) over a period of five days, was 2.58 min. on every injection but three, for which the retention time was 2.59 minutes. Similarly on the THEED system the retention time for n-propanol for multiple injections (n = 155) over a period of five days, was 3.02 min. on every injection but five, for which the retention time was 3.01 (n = 2) or 3.03 (n = 3) minutes. This suggests excellent precision, both within day and between days for these particular instrument configurations. Some workers advocate the use of relative retention times in order to make inter-laboratory method comparisons more readily performed. Tables 1 and 2 includes both retention time and retention index data for all analytes.

Using the Carbowax column alone, it was noted that the following analyte groups differed in absolute retention time by less than 0.05 minutes and were considered indistinguishable: acetaldehyde/butane, diethyl ether/n-pentane/acetone/acrolein, acetonitrile/ethanol, dichloropropane/methylene chloride, 1,1,1, trichloroethane/diethylamine/isopropylamine, pyridine and n-butanol. Likewise, on the THEED system the following analyte groups were

TABLE 1—*Retention times (min.) and retention relative to ethanol for 32 volatile organic compounds.*

Compound	THEED		Carbowax	
	RT (mins.)	RRT ethanol	RT (mins.)	RRT ethanol
Propane	0.48	0.39	0.32	0.28
Acetaldehyde	0.55	0.45	0.46	0.40
Ethyl Chloride	0.67	0.54	0.58	0.50
Methanol	0.73	0.59	0.68	0.59
Butane	0.81	0.66	0.46	0.40
Methylene Chloride	0.87	0.71	1.24	1.08
Acetonitrile	0.88	0.72	1.13	0.98
Acrolein	1.05	0.85	0.90	0.78
Acetone	1.11	0.90	0.90	0.78
Ethanol	1.23	1.00	1.15	1.00
Formaldehyde	1.28	1.04	0.96	0.83
Diethyl Ether	1.73	1.41	0.89	0.77
Isopropylamine	1.82	1.48	2.39	2.08
2-propanol	2.14	1.74	1.68	1.46
Chloroform	2.25	1.83	2.98	2.59
n-Pentane	2.44	1.98	0.89	0.77
Methyl Ethyl Ketone	2.82	2.29	1.88	1.63
n-Propanol	3.02	2.46	2.58	2.24
1,1,1 Trichlorethane	3.05	2.48	2.34	2.03
Ethyl Acetate	3.47	2.82	2.16	1.88
Halothane	3.5	2.85	3.48	3.03
Isoflurane	4.18	3.40	4.34	3.77
Diethylamine	4.4	3.58	2.38	2.07
n-Butyl Chloride	4.82	3.92	2.25	1.96
1,2 Dichloropropane	5.28	4.29	1.22	1.06
1,2 Dibromoethane	10.29	8.37	10.32	8.97
1-Butanol	10.49	8.53	6.76	5.88
n-Heptane	12.51	10.17	5.46	4.75
Pyridine	13.2	10.73	6.74	5.86
Paraldehyde	27.76	22.57	12.27	10.67
Toluene	42.51	34.56	7.09	6.17
2-Furaldehyde	47.89	38.93	30.76	26.75

indistinguishable: methylene chloride/acetonitrile, n-propanol/1,1,1 trichloroethane, and ethyl acetate/halothane. There was no overlap of indistinguishable analyte groups between the two systems, meaning that with one exception all analytes could be distinguished when both GC systems were used. The only two compounds which could not be satisfactorily separated using this system were acetone and acrolein, an aquatic herbicide.

It was noted that ethyl chloride was well separated from ethanol on both columns. Laferty [*3*] suggests that discrimination between ethyl chloride and ethanol, eluting 0.02 minutes apart, can be made on a 0.3% Carbowax column by careful consideration of the chromatograms and judicious use of peak matching tolerence. The use of two columns with different selectivities however such as described here, would eliminate any possible confusion to a much higher degree of certainty.

In the homicide investigation described in the introductory section, duplicate analysis of both central and peripheral blood samples from the victim showed diethyl ether concentrations of 44 and 47 mg/L respectively. Concentrations of between 50 and 1500 mg/L are required for surgical anesthesia [*5*]. Diethyl ether and heptane are found together in carburetor fluid [*6*], which is a common source of this material for illicit drug manufacture.

TABLE 2—*32 volatile organic compounds and their retention behavior on THEED and Carbowax systems.*

Compound	THEED		Carbowax	
	RT (mins.)	RRT ethanol	RT (mins.)	RRT ethanol
Acetaldehyde	0.55	0.45	0.46	0.40
Acetone	1.11	0.90	0.90	0.78
Acetonitrile	0.88	0.72	1.13	0.98
Acrolein	1.05	0.85	0.90	0.78
Butane	0.81	0.66	0.46	0.40
1-Butanol	10.49	8.53	6.76	5.88
Chloroform	2.25	1.83	2.98	2.59
1,2 Dibromoethane	10.29	8.37	10.32	8.97
1,2 Dichloropropane	5.28	4.29	1.22	1.06
Diethyl Ether	1.73	1.41	0.89	0.77
Diethylamine	4.4	3.58	2.38	2.07
Ethanol	1.23	1.00	1.15	1.00
Ethyl Acetate	3.47	2.82	2.16	1.88
Ethyl Chloride	0.67	0.54	0.58	0.50
Formaldehyde	1.28	1.04	0.96	0.83
2-Furaldehyde	47.89	38.93	30.76	26.76
Halothane	3.5	2.85	3.48	3.03
n-Heptane	12.51	10.17	5.46	4.75
Isoflurane	4.18	3.40	4.34	3.77
2-Propanol	2.14	1.74	1.68	1.46
Isopropylamine	1.82	1.48	2.39	2.08
Methanol	0.73	0.59	0.68	0.59
Methyl Ethyl Ketone	2.82	2.29	1.88	1.63
Methylene Chloride	0.87	0.71	1.24	1.08
n-Butyl Chloride	4.82	3.92	2.25	1.96
n-Pentane	2.44	1.98	0.89	0.77
n-Propanol	3.02	2.46	2.58	2.24
Paraldehyde	27.76	22.57	12.27	10.67
Propane	0.48	0.39	0.32	0.28
Pyridine	13.2	10.73	6.74	5.86
Toluene	42.51	34.56	7.09	6.17
1,1,1 Trichlorethane	3.05	2.48	2.34	2.03

Conclusions

As illustrated with the described analyses, there are a variety of potential misidentifications for volatile solvents when either one of these systems is used alone. The same limitations can apply to all the popular phases in common use for forensic volatiles analysis. The most potentially serious of these misidentifications is ethanol/acetonitrile, because of the legal implications associated with ethanol ingestion, and the danger of misdiagnosing an acetonitrile poisoning. The second pair of solvents whose misidentification caused problems in the case discussed in the introduction, was acetone/diethyl ether. The ready availability of ether, its potential use as a deliriant or subduing agent, make its correct identification important in death investigation toxicology.

The otherwise general practice of complementary analysis for forensic applications is a good one, and the described illustrations show that volatiles analysis merits the same degree of care as do other, more complex, analyses.

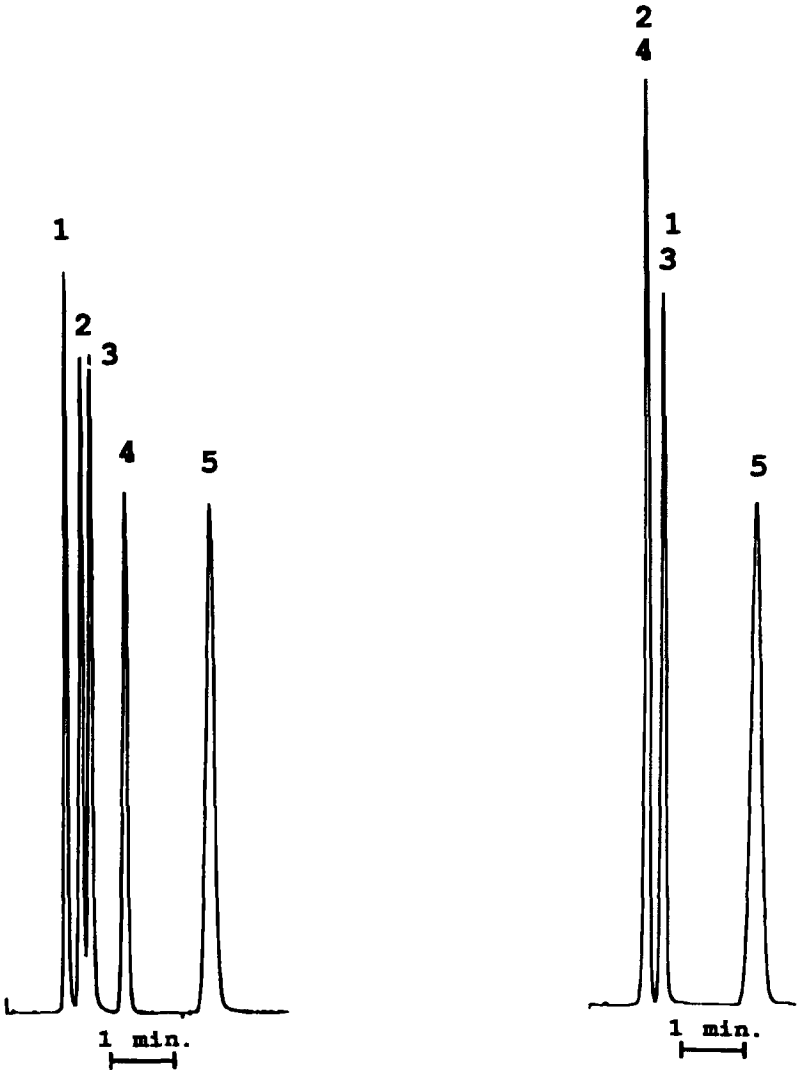

FIG. 1—*Mixture containing 1) acetonitrile, 2) acetone, 3) ethanol, 4) diethyl ether, and 5) n-propanol (internal standard) on* (a) *0.8% THEED on 80/100 Carbopak C, and* (b) *5% Carbowax 20M on 80/120 Carbopak B. For actual retention times see Table 1, and for remaining conditions see text.*

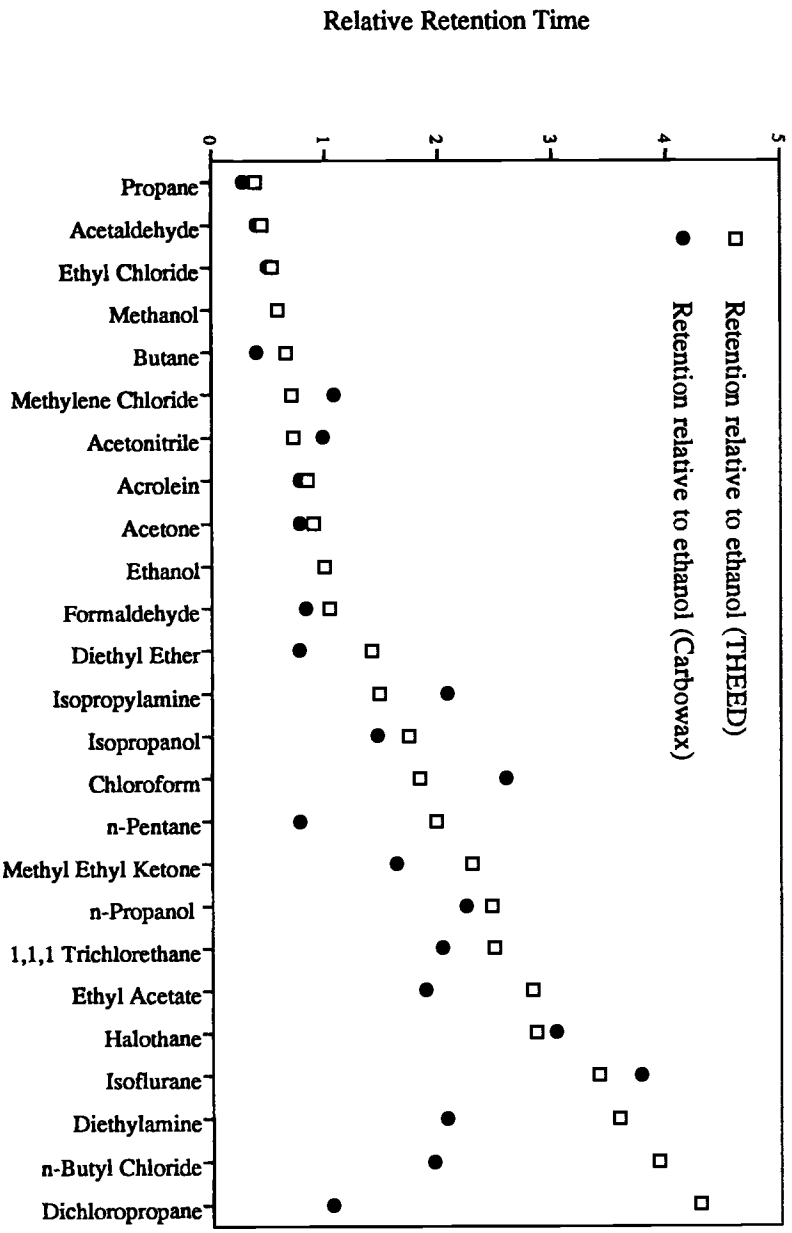

FIG. 2—*Gas chromatographic retention relative to ethanol for volatiles eluting in under six minutes. See Table 1 for actual retention time data.*

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