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DETECTION AND IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS IN BLOOD BY HEADSPACE GAS CHROMATOGRAPHY AS AN AID TO THE DIAGNOSIS OF SOLVENT ABUSE

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SUMMARY

A gas chromatographic method has been developed for the detection and identification of some volatile organic compounds in whole blood, plasma or serum. After incubation of the sample (200 μl) together with the internal standard solution in a sealed vial, a portion of the headspace is analysed using a 2-m glass column packed with 0.3% (w/w) Carbowax 20M on Carbopack C, 80–100 mesh. The column oven, after a 2-min isothermal period, is programmed from 35 to 175°C at 5°/min and held for 8 min. The effluent is monitored by both flame-ionisation and electron-capture detection, and peak assignment is by means of retention time and relative detector response.

The method has proved applicable to the detection of bromochlorodifluoromethane, *n*-butane, carbon tetrachloride, chlorobutanol, cryofluorane (Halon 114), dichlorodifluoromethane (Halon 12), ethyl acetate, halothane, isobutane, isopropanol, isopropyl nitrate, methyl ethyl ketone, propane, tetrachloroethylene, toluene, 1,1,1-trichloroethane, 2,2,2-trichloroethanol, trichloroethylene and trichlorofluoromethane (Halon 11) in blood specimens obtained from patients suspected of abusing these agents.

INTRODUCTION

The diagnosis of poisoning by volatile organic compounds such as solvents, aerosol propellants and anaesthetics is often aided by either the past medical history or circumstantial evidence such as the discovery of empty containers, the presence of traces of glue on the patient's clothing or the odour of chemicals on the breath. However, in the absence of such evidence, serious diagnostic problems may occur since the clinical features of acute poisoning with this type of compound are by no means definitive. These features range from ataxia and drowsiness to coma and res-

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piratory depression with in some instances direct cardio- or hepatotoxicity[1].

The introduction of gas chromatographic techniques for the detection, identification and measurement of ethanol[2,3] prompted the application of similar methods to compounds such as toluene, benzene, acetone, isopropanol and methyl ethyl ketone in blood specimens from poisoned patients[4,5]. More recently, some workers have used headspace analysis with packed columns operated isothermally and flame-ionisation detection (FID)[6–8] for the analysis of a variety of compounds, although others have used capillary columns[9], headspace mass spectrometry[10] or gas chromatography–mass spectrometry[11].

The system described in the present work was developed to facilitate the rapid detection and identification of a wide range of volatile substances in blood specimens at the concentrations attained in patients acutely poisoned with these agents. The method employs headspace sample preparation together with a programmed analysis using a column packed with 0.3% (w/w) Carbowax 20M on Carbopack C and split FID–electron-capture detection (ECD).

EXPERIMENTAL

Internal standard solution

The internal standards, 1,1,2-trichloroethane and ethylbenzene, were both obtained from BDH, Poole, Great Britain. Each batch of material was tested for the presence of interferences (notably 1,1,1-trichloroethane and toluene, respectively) by gas chromatography before use. Approximately 50 mg of each standard were weighed into 50-ml glass volumetric flasks containing outdated blood-bank whole blood. After thorough mixing, 1.0 ml of the stock 1,1,2-trichloroethane solution and 2.5 ml of the ethylbenzene solution were diluted to 100 ml with glass-distilled water containing sodium azide (approximately 100 mg) to give the working internal standard solution. This solution, if stored in 1-ml portions at −20°C, was found to be stable for at least 6 months.

Gas chromatography

The instrument used was a Perkin-Elmer F17 gas chromatograph fitted with a liquid carbon dioxide subambient accessory and dual-pen recorder. The injection port was fitted with a "septum swinger" (Perkin Elmer 045-0496), and the injection/detector block was maintained at 275°C. The column oven, after a 2-min isothermal period, was programmed at 5°/min from 35 to 175°C and held for 8 min. A 2 m × 2 mm I.D. glass column was packed with 0.3% (w/w) Carbowax 20M on Carbopack C, 80–100 mesh (Supelco, obtained from Atlas-Bioscan, Canvey Island, Great Britain), and was conditioned according to the manufacturer's recommendations. The carrier-gas (nitrogen) flow-rate was approximately 30 ml/min, and the column effluent was monitored using both FID and ECD with a stainless-steel splitter system giving a split ratio of 9:1. The hydrogen and oxygen (FID) inlet pressures were 10 and 14 p.s.i., respectively, and the ECD purge (nitrogen) flow-rate was 30 ml/min. The ECD was used at a pulse setting of 6 at range 1, attenuation 64, and the FID at range 1, attenuation 8.

Instrument calibration

A qualitative standard mixture (Table I) was prepared daily by adding the appropriate volume of the gaseous components to an evacuated 125-ml gas-sampling bulb (Supelco 2-2146). Air was admitted to atmospheric pressure and a portion (25 μl) of the mixture of the liquid and solid components (Table II) was added. The liquid–solid components mixture was stored in a glass-stoppered vessel at −20°C and was stable for at least 3 months.

The qualitative standard mixture was chromatographed daily prior to sample analyses. From 0.01 μl ("Nanojector", Precision Sampling, obtained from Atlas Bio-scan) to 1.0 μl (SGE 1.0-μl glass syringe) were injected onto the gas chromatographic

TABLE I

QUALITATIVE STANDARD MIXTURE

For details of preparation, see text.

	Volume added to gas sampling bulb (μl)
Bromochlorodifluoromethane	20*
Dichlorodifluoromethane	750*
Trichlorofluoromethane	20*
Liquid–solid stock mixture (see Table II)	25

* Volume of gas phase at atmospheric pressure.

TABLE II

LIQUID AND SOLID COMPONENTS STOCK MIXTURE

Compound	*Amount*
Acetone	20 ml
Methyl ethyl ketone	20 ml
Carbon tetrachloride	200 μl
Chlorobutanol	10 mg
Chloroform	1.5 ml
Ethanol	20 ml
Ethyl acetate	20 ml
Ethylbenzene	10 ml
n-Hexane	40 ml
Isopropyl nitrate	1 ml
Methanol	15 ml
Methyl isobutyl ketone	5 ml
Isopropanol	20 ml
Tetrachloroethylene	100 μl
Toluene	10 ml
1,1,1-Trichloroethane	1 ml
1,1,2-Trichloroethane	2 ml
2,2,2-Trichloroethanol	50 μl
Trichloroethylene	1 ml

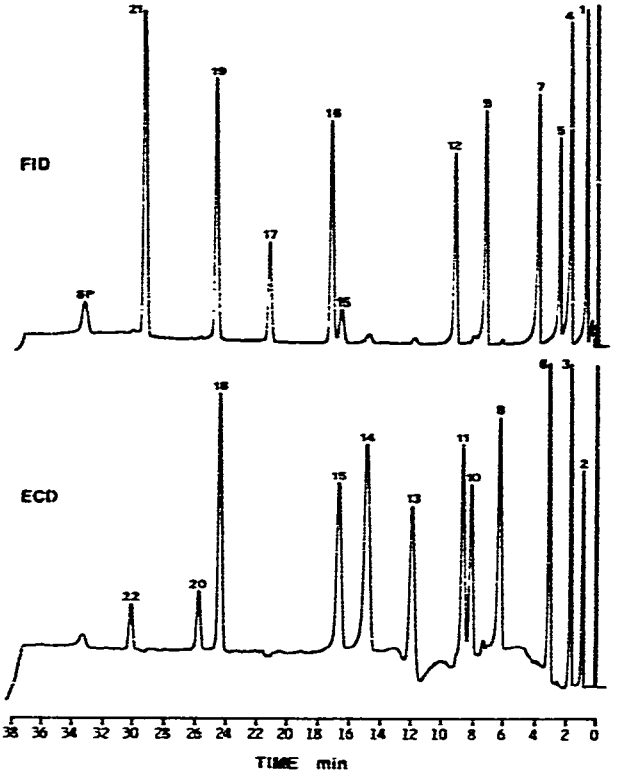

Fig. 1. Chromatogram obtained on analysis of the qualitative standard mixture; 0.75-μl injection. GC conditions: column 2 m × 2 mm I.D. glass packed with 0.3% (w/w) Carbowax 20M on Carbopack C (80–100 mesh); carrier gas flow 37 ml/min; temperature program: 2 min at 35°C, 5°/min to 175°C and held for 8 min. Peaks: 1 = methanol, 2 = dichlorodifluoromethane, 3 = bromochlorodifluoromethane, 4 = ethanol, 5 = acetone, 6 = trichlorofluoromethane, 7 = isopropanol, 8 = chloroform, 9 = methyl ethyl ketone, 10 = 1,1,1-trichloroethane, 11 = carbon tetrachloride, 12 = ethyl acetate, 13 = isopropyl nitrate, 14 = trichloroethylene, 15 = 1,1,2-trichloroethane, 16 = *n*-hexane, 17 = methyl isobutyl ketone, 18 = tetrachloroethylene, 19 = toluene, 20 = 2,2,2-trichloroethanol, 21 = ethylbenzene, 22 = chlorobutanol, SP = "Septum peak" (see text).

column, and the retention times of the components were measured manually from the injection point. The analysis of this mixture is illustrated in Fig. 1, and the retention times of these and some other compounds of interest are given in Table III.

Sample preparation

Internal standard solution (100 μl) was added using a disposable 1.0-ml plastic syringe to a 7-ml nitrogen-filled vial (Schubert, Portsmouth, Great Britain) sealed with a crimped-on teflon-silicone disc (Phase Separations, Queensferry, Great Britain). The vial was maintained at 65°C in a heating block. After 15 min, a portion (400 μl) of the headspace was taken using a warmed (approximately 40°C) gas-tight glass syringe (SGE) and injected onto the gas chromatographic column.

Subsequently, the sample (whole blood, plasma or serum) (200 μl) was added to the vial using a disposable syringe and, after 15 min, a further portion (400 μl) of

the headspace was taken for analysis. After sample injection, the gas-tight syringe was purged by removing the plunger and sucking ambient air through the barrel and needle of the syringe using a vacuum pump.

RESULTS AND DISCUSSION

The detection and identification of volatile organic compounds in specimens of body fluids poses unusual analytical problems in clinical and forensic toxicology. A wide variety of compounds may be encountered in such analyses ranging from, for example, the polyhalogenated hydrocarbon dichlorodifluoromethane (Halon 12, b.p. −30°C) to the aromatic hydrocarbon xylene (b.p. approximately 140°C), and simultaneous exposure to or abuse of more than one solvent is not infrequent. In addition, many compounds undergo transformation to metabolites which are usually more polar than the parent compound. The widespread occurrence of organic solvents in the environment (not least in that of analytical laboratories) must also be considered.

The aim of this study was thus to develop a practical method to assist in the diagnosis of solvent or aerosol abuse using readily available clinical specimens. It was clear that such a method should be capable of detecting the presence of a wide range of compounds and possess adequate selectivity and sensitivity. Such a technique should be simple, reliable and use easily available apparatus wherever possible, and should also permit the subsequent quantitative analysis of the compounds of interest.

Choice of gas chromatographic column and conditions

Initially, it was thought that a capillary system would prove ideal, but several disadvantages to the use of such systems became apparent. An inlet splitter system was needed in order to obtain reproducible retention times, and this had the effect of reducing the overall sensitivity of the method as compared to the system finally chosen even taking into account the enhanced peak height/peak area ratio obtainable with capillary columns. An additional problem lay in obtaining adequate retention of some of the more volatile compounds studied, for example the low molecular weight hydrocarbons and the halons. Some of these difficulties could probably be overcome by "trapping" the headspace components of interest using a solid adsorbent followed by desorption onto the chromatographic system, but only at additional cost and complexity. Another practical consideration was the much greater expected life of packed columns, especially when the repeated injection of 400-μl volumes of wet oxygen-containing headspace was contemplated.

Other packed columns considered included a range of Carbopack materials modified with different stationary phases and more conventional materials such as Tenax and Porasil. However, none of these materials gave such good resolution of the compounds of interest as the system chosen. An additional factor was that relatively polar materials such as ethanol and acetone gave rise to almost symmetrical peaks on the modified Carbopacks (*cf.* Fig. 1) in contrast to the results obtained on the other materials. However, where additional information or confirmation of compound identity was required, retention indices obtained on columns prepared using either OV-1 or Carbowax 20M were available from a variety of sources, and these data are also given in Table III.

TABLE III

RETENTION AND OTHER DATA OF THE COMPOUNDS STUDIED

Abbreviations: DNA = datum not available. NIM = not in Merck Index.

*Compound**	*Retention time (mm)***	*Electron capture response****	*Kovats retention index*§		*Boiling point (°C)*¶¶	*Molecular weight*	*Merck Index No.**
			OV-1	*Carbowax 20M*			
Methane	1.5	0	100	100	−161	16.0	5809
Ethane	1.5	0	200	200	−88	30.1	3649
Ethylene	1.5	0	DNA	DNA	−104	28.1	3728
Nitrous oxide	1.5	1	DNA	DNA	−88	44.0	6473
Cyclopropane	3.0	0	DNA	DNA	−33	42.1	2753
Propane	3.0	0	300	300	−42	44.1	7591
Propylene	3.0	0	310	DNA	−48	42.1	7638
Acetaldehyde	3.5	0	372	695	21	44.1	27
Ethylene oxide	3.5	0	400	680	11	44.1	3738
Methanol	3.5	0	491	883	65	32.0	5814
Vinyl chloride	4.0	2	440	505	−14	62.5	9645
Dichlorodifluoromethane (Halon 12)	4.5	2	305	DNA	−30	120.9	3038
Methyl formate	4.5	0	499	761	32	60.1	5942
Ethyl chloride	5.5	2	447	668	12	64.5	3713
Acetonitrile	7.0	0	455	1010	82	41.1	56
1-Chloro-2,2,2-trifluoroethane	7.5	1	375	665	7	118.5	NIM
Bromochlorodifluoromethane (BCF)	8.0	2	405	DNA	−4	165.4	NIM
Isobutane	8.0	0	370	DNA	−12	58.1	NIM
1-Butene	9.0	0	390	415	−6	56.1	1502
Methyl iodide	9.0	2	515	806	43	142.0	5955
Dichloromethane	9.5	2	515	917	40	84.9	5932
Ethanol	9.5	0	421	919	79	46.1	211
Cryofluorane (Halon 114)	10.0	2	361	DNA	4	170.9	2599
n-Butane	11.5	0	400	400	−1	58.1	1497
Acetone	12.5	1	469	819	57	58.1	52
Nitromethane	12.5	2	565	1154	101	61.0	6433
Carbon disulphide	13.5	1	524	745	47	76.1	1817
Trichlorofluoromethane (Halon 11)	15.0	2	484	605	24	137.4	9320

Acrylonitrile	16.0	0	590	1010	77	53.1	127
1,3-Dioxolane	17.0	0	635	935	74	74.1	NIM
Ethyl formate	18.0	0	545	920	53	74.1	3743
Methyl acetate	18.5	0	513	796	57	74.1	5884
Vinylidene chloride	18.5	2	515	715	32	97.0	9647
Methylal	19.5	0	505	740	42	76.1	5888
Propionitrile	19.5	0	580	1015	97	55.1	7617
Isopropanol	20.0	0	530	908	83	60.1	5069
Bromochloromethane	21.0	2	660	1060	68	129.4	NIM
n-Propyl chloride	21.0	1	570	740	47	78.5	NIM
1,1-Dichloroethane	22.5	2	563	881	57	99.0	3750
Tetrahydrofuran	25.0	0	638	872	66	72.1	8929
1,2-Epoxybutane	27.0	0	600	840	63	72.1	NIM
1,2-Dichloroethylene (mixed isomers)	27.5	2	556	846	55	97.0	85
n-Propanol	27.5	0	571	1033	97	60.1	7630
Ethyl iodide	29.0	2	680	880	72	156.0	3753
Diethyl ether	29.5	0	515	650	35	74.1	3742
Nitroethane	30.0	2	655	1161	115	75.1	6420
Chloroform	31.0	2	605	1024	61	119.4	2120
1,1,2-Trichlorofluoroethane	31.5	2	555	605	46	187.4	NIM
tert.-Butanol	32.0	0	512	875	82	74.1	1526
1,1,1,-Trichlorotrifluoroethane (Halon 113)	32.0	2	530	660	46	187.4	NIM
2,2,2-Trifluoroethanol	32.5	2	580	1135	77	100.0	NIM
1,2-Dichloroethane	35.0	2	631	1051	83	99.0	3733
Dibromomethane	36.5	2	765	1185	97	173.9	5930
Methyl ethyl ketone (MEK)	36.5	1	579	908	80	72.1	5941
Isopropyl formate	39.5	0	567	883	68	88.1	NIM
1,1,1-Trichloroethane	41.0	2	634	886	74	133.4	9316
Enflurane	41.5	2	462	840	57	184.5	3524
n-Pentane	41.5	0	500	500	36	72.2	6913
Epichlorohydrin	42.5	2	730	1205	118	92.5	3536
Halothane	42.5	2	533	858	50	197.4	4455
Carbon tetrachloride	43.0	2	659	886	77	153.8	1821
Cyclohexane	43.0	0	664	726	81	84.2	2728
Dioxane	45.5	0	687	1065	101	88.1	3300
sec.-Butanol	46.5	0	624	1014	100	74.1	1525
2-Chloroethanol	47.0	2	643	1358	129	80.5	3730
Ethyl acetate	47.0	0	596	870	77	88.1	3685
2-Methoxyethanol (methyl cellosolve)	47.0	0	616	1172	124	76.1	5913

(Continued on p. 430)

TABLE III *(continued)*

*Compound**	*Retention time (min)***	*Electron-capture response****	*Kovats retention index§*		*Boiling point (°C)¶¶*	*Molecular weight*	*Merck index No.**
			OV-1	*Carbowax 20M*			
n-Propyl formate	47.0	0	603	916	81	88.1	7647
2-Nitropropane	48.0	2	685	1109	120	89.1	6450
Methyl propionate	48.5	0	639	910	80	88.1	5982
Bromodichloromethane	51.0	2	715	1165	90	163.8	NIM
Isobutanol	51.0	0	619	1076	108	74.1	4981
Cyclohexene	57.0	0	705	811	83	82.1	2732
n-Butyl chloride	57.5	2	642	858	79	92.6	1546
Methyl isopropyl ketone	59.5	1	650	936	93	86.0	NIM
Isopropyl nitrate	60.0	2	693	970	101	105.1	NIM
Chloral hydrate (tailing peak)	62.5	2	695	1015	98	165.4	2033
Methyl cyclopropyl ketone	65.0	1	730	1095	114	84.1	NIM
1-Nitropropane	66.0	2	725	1220	132	89.1	6449
2-Methylbutan-2-ol	67.0	0	636	966	103	88.2	6934
Epibromohydrin	68.0	2	805	1330	135	137.0	NIM
Methylcyclopentane	68.0	0	650	775	72	84.2	NIM
n-Butyl nitrite	68.5	2	608	1220	78	103.1	1570
2-Methylpentane	71.0	0	610	704	72	84.2	NIM
Benzene	74.0	0	660	948	80	78.1	1069
Bromotrichloromethane	74.0	2	810	1070	105	198.3	NIM
n-Propyl iodide	74.0	2	785	975	103	170.0	7651
Trichloroethylene	74.0	2	710	964	87	131.4	9319
Isopropyl acetate	75.0	0	648	896	89	102.1	5066
1,2-Difluorotetrachloroethane (Halon 112)	75.5	2	730	870	93	203.8	NIM
Diethyl ketone	76.0	1	683	965	102	86.1	3102
1,1-Difluorotetrachloroethane	77.0	2	785	1010	92	203.8	NIM
Methyl *n*-propyl ketone	79.0	1	680	1071	102	86.1	5984
2-Mercaptoethanol	81.5	0	795	1855	157¶¶¶	78.1	5699
1,1,2-Trichloroethane	82.0	2	748	1240	113	133.4	9317
Diisopropyl ether	83.0	0	594	592	68	102.2	5073
2-Ethoxyethanol	84.0	0	701	1218	135	90.1	3678
Pyrrole	84.0	0	755	1472	130	67.1	7801
Methylcyclohexane	84.5	0	750	781	101	98.2	NIM
Ethylene glycol (tailing peak)	85.0	0	798	DNA	198	62.1	3735

n-Hexane	87.0	0	600	600	69	86.2	4563
n-Butanol	88.0	0	651	1128	117	74.1	1524
Methoxyflurane	88.0	2	701	1124	105	165.0	5866
N-Methylpyrrole	88.0	0	715	1139	115	81.1	NIM
2,3-Pentanedione	88.0	1	681	1044	108	100.1	NIM
Ethyl propionate	90.0	0	679	948	99	102.1	3790
1,2-Dibromoethane	90.5	2	830	1265	131	187.9	3732
n-Propyl acetate	91.5	0	696	950	102	102.1	7629
Methyl *n*-butyrate	93.0	0	723	980	102	102.1	5909
n-Butyl formate (multiple peaks)	97.5	0	701	1020	107	102.1	NIM
Methyl methacrylate	100.0	0	699	1008	100	86.1	5796
Methylpentynol	100.5	0	715	1275	121	98.1	5671
Isoamyl nitrite (amyl nitrite B.P.)	101.5	2	680	1185	98	117.2	5056
3-Methylbutan-1-ol	103.0	0	719	1159	132	88.2	5055
Cyclohexanone	105.0	1	855	1275	156	98.1	2731
Methyl isobutyl ketone	105.5	1	724	1010	117	100.2	5068
1,1,1,2-Tetrachloroethane	106.5	2	870	1255	131	167.9	NIM
Bromoform	107.0	2	469	926	149	252.8	1418
Pyridine (tailing peak)	107.0	0	695	1181	115	79.1	7752
Pentan-1-ol	109.5	0	766	1153	138	88.2	6916
2-Methylpentan-2-ol	111.0	0	725	1160	121	102.2	NIM
2-Methylhex-1-ene	112.0	0	725	740	92	98.0	NIM
Isopropyl propionate	112.5	0	745	948	110	116.2	NIM
Ethyl *n*-p opyl ketone	115.0	1	781	1055	125	100.2	NIM
Cyclohexanol	116.0	0	913	1375	161	100.2	2730
Acetylacetone	117.0	1	804	1230	141	100.1	73
n-Butyl iodide	117.0	2	840	1065	130	184.0	1562
Paraldehyde	117.0	0	786	1069	124	132.2	6832
Methyl *n*-butyl ketone	118.5	1	787	1173	127	100.2	5907
Isooctane	120.0	0	725	705	99	114.2	5051
Isobutyl acetate	120.0	0	753	984	118	116.2	4983
Tetrachloroethylene	121.5	2	789	1018	121	165.9	8907
1,2,3-Trichloropropane	122.0	2	910	1455	157	147.4	NIM
n-Amyl formate	124.0	0	772	1070	132	116.2	NIM
Toluene	124.0	0	756	1035	111	92.1	9225
1,1,2,2,-Tetrachloroethane	124.5	2	910	1500	147	167.9	8906
n-Heptane	125.5	0	700	700	98	100.2	4521
n-Butyl acetate	126.0	0	797	1078	125	116.2	1519
γ-Valerolactone	126.5	1	921	1617	218	100.1	NIM

(Continued on p. 432)

TABLE III *(continued)*

*Compound**	*Retention time (mm)***	*Electron-capture response****	*Kovats retention index*†		*Boiling point (°C)*††	*Molecular weight*	*Merck Index No.**
			OV-1	*Carbowax 20M*			
2,2,2-Trichloroethanol	127.5	2	857	1691	151	149.4	9318
1,3-Dichloropropan-2-ol	129.0	2	885	1765	174	129.0	3050
Diacetone alcohol	130.0	1	811	1316	168	116.2	2921
Allyl glycidyl ether	131.0	0	880	1325	154	114.1	NIM
Hexan-2-ol	132.0	0	786	1192	138	102.2	NIM
Chlorobenzene	136.5	1	860	1200	131	112.6	2095
1,1,1-Trichloropropan-2-ol	139.5	2	920	1650	162	163.4	9325
Acetonylacetone	141.0	1	894	1500	188	114.1	57
Cycloheptanone	141.0	1	1010	1495	180	112.2	2727
Di-*n*-propyl ketone	146.0	1	857	1131	144	114.2	3364
2-Ethoxyethyl acetate	146.5	0	872	1305	156	132.2	3679
Ethylbenzene	147.5	0	849	1120	136	106.2	3695
Chlorobutanol	149.0	2	949	1638	167	177.5	2103
2-Octyne	150.0	0	870	1040	138	110.2	NIM
Pyrrolidine	151.0	0	695	1022	89	71.1	7802
Caprylene	152.0	0	779	830	121	112.2	1763
Heptan-2-one	152.0	1	880	1275	152	114.2	4525
α-Pinene (from turpentine)	154.5	0	942	1035	156	136.2	7242
Heptanal	155.0	1	883	1186	153	114.2	4519
Bromobenzene	155.5	2	945	1365	156	157.0	1405
2-Ethylhexyl acetate	156.0	0	1144	1420	199	172.3	6570
Hexachloroethane	158.5	2	1085	1525	189	236.7	4545
Isoamyl acetate	159.0	0	907	1182	142	130.2	4958
n-Octane	159.0	0	800	800	126	114.2	6561

2,5-Dimethylfuran	161.5	1	697	931	94	96.1	NIM
Cumene	162.0	0	905	1162	152	120.2	2615
m-Xylene	166.0	0	863	1147	139	106.2	9743
Styrene	167.0	0	879	1272	145	104.1	8657
Benzaldehyde	171.0	0	947	1502	179	106.1	1057
p-Xylene	171.0	0	860	1140	138	106.2	9743
o-Xylene	172.5	0	884	1191	144	106.2	9743
Ethchlorvynol	176.0	2	1030	1445	173	144.6	3656
Di-*n*-butyl ether	177.0	0	811	969	142	130.2	1557
6-Methyl-5-hepten-2-one	180.0	0	975	DNA	73†	126.0	NIM
o-Chlorotoluene	186.0	1	985	1288	159	126.6	2160
m-Chlorotoluene	187.0	1	960	1291	162	126.6	2159
Octan-4-one	187.0	1	965	1250	163	128.2	NIM
Camphor	191.0	0	1136	1518	204	152.2	1734
Octan-3-one	192.0	1	928	1190	167	128.2	NIM
p-Chlorotoluene	197.0	1	955	1288	162	126.6	2161
Octan-2-one	201.0	1	991	1375	172	128.2	4586
2-Ethylhexan-1-ol	212.0	0	1037	1465	184	130.2	3746
1,4-Dichlorobenzene	213.0	2	994	1495	174	147.0	3030
Octan-1-ol	213.0	0	1083	1513	194	130.2	6563
2,6-Dimethylheptan-4-one (multiple peak)	218.0	0	999	1207	168	142.2	NIM
o-Tolualdehyde	266.0	0	1054	1632	201	120.0	9223
Nonan-5-one	290.0	1	1074	1330	188	142.2	NIM
Nonan-2-one	320.0	1	1093	1478	195	142.2	NIM

* Names of compounds based on those used in the Merck Index (9th Edition), Merck and Co., Rahway, NJ, U.S.A., 1976.

** Retention time (5 mm = 1 min) measured to the nearest 0.5 mm from the injection point on the Carbopack C/Carbowax 20M column system. See text for chromatographic conditions (retention times >180 mm obtained by increasing the final isothermal period).

*** Rated: 0 = nil, 1 = weak, 2 = strong.

† Compiled from various sources.

†† At 760 mmHg pressure, except:

††† 742 mm.

‡ 18 mm.

The temperature program used was influenced by the need to retain and resolve relatively volatile compounds such as butane, isobutane, BCF and the halons while completing the analysis of the remaining compounds in a reasonable time and maintaining adequate selectivity. A further consideration was the presence of a compound derived presumably from the septa used in the gas chromatograph which had a retention time of 33.2 min (166 mm) on this program (see figures), and was completely eluted under the conditions chosen. The amount of this compound (thought to contain silicon from mass spectrometric data) present in each analysis could be minimised by the use of the "septum-swinger" but it has proved impossible to eliminate it completely, either by the use of septum purge devices or by heat pretreatment of the septa.

The accurate reproduction of the program used in this work, especially at the low temperature end of the range, is obviously important in the use of the retention data (Table III), and the initial temperature quoted (35°C) has been checked by use of additional instruments. Once the temperatures attained during the program have been standardised, then the carrier-gas flow can be altered if necessary to adjust the retention times to those reported in Table III.

Since many of the commercially available solvents, anaesthetic agents and aerosol propellants show good ECD responses, it was felt that the use of a dual detection system using a split ratio of 9:1 FID/ECD would enhance the sensitivity of the method towards halogenated compounds while not appreciably reducing the sensitivity towards non-electron capturing substances. In practice, those compounds

TABLE IV

REPRODUCIBILITY OF THE RETENTION TIMES OF SOME COMPOUNDS IN THE STANDARD MIXTURE (TABLE I) OVER A 3-MONTH PERIOD

Compound	*Retention time (mm)**			
	n	*Mean*	*S.D.*	*C.V. (%)*
Dichlorodifluoromethane	26	4.3	0.42	9.8
Bromochlorodifluoromethane	26	8.2	0.45	5.5
Ethanol	30	9.5	0.36	3.8
Acetone	29	12.4	0.33	2.7
Trichlorofluoromethane	27	15.2	0.52	3.4
Isopropanol	28	20.1	0.50	2.5
Chloroform	30	31.0	0.62	2.0
Methyl ethyl ketone	27	36.7	0.62	1.7
1,1,1-Trichloroethane	28	40.7	0.68	1.7
Ethyl acetate	22	46.8	0.63	1.3
Isopropyl nitrate	11	60.1	1.03	1.7
Trichloroethylene	29	74.1	1.05	1.4
1,1,2-Trichloroethane	29	82.0	0.92	1.1
n-Hexane	18	86.7	1.54	1.8
Methyl isobutyl ketone	29	105.5	1.34	1.3
Tetrachloroethylene	30	121.5	1.53	1.3
Toluene	30	124.1	1.46	1.2
2,2,2-Trichloroethanol	30	127.7	1.63	1.3
Ethyl benzene	30	147.3	2.12	1.4

* 5 mm = 1 min.

responding fully to the ECD (Table III) only responded to the FID at much higher concentrations despite the split ratio. This was much less marked with some compounds, notably 1,1,2-trichloroethane (Fig. 1), but the relative response still provided a valuable adjunct to the retention time in the assignment of peak identity. Some compounds gave rise to a strong FID response accompanied by a relatively weak ECD response on the system used, and these compounds are also indicated in Table III.

The Carbopack C/Carbowax 20M column system has proved to be reproducible and reliable in routine use over a 12-month period. The inter-assay coefficients of variation (C.V.) of the retention times (all measured manually from the recorder trace) of some of the compounds present in the standard mixture are presented in Table IV. The coefficients were all 2.5% or less for those compounds eluting at retention times of 4 min (20 mm) or longer, although the variation was greater for those compounds eluting at shorter retention times. The standard mixture was chromatographed daily prior to sample analyses in order to ensure correct functioning of the system. However, where confirmation of the assignment of peaks occurring in sample chromatograms not present in the standard mixture was required, an appropriate volume (0.01-μl Pressure-Lok Mini-Injector or 0.1-μl SGE needle-in-plunger syringe) of vapour was injected separately.

Sample collection and storage

Blood was considered to be the specimen of choice for this work for several reasons. Firstly, blood specimens were most easily obtainable, especially from unconscious patients. Secondly, special containers were not required, in contrast to the custom-designed sample bags[12] or Tenax-filled tubes[13] needed when analysing samples of exhaled air. Finally, quantitative analyses carried out on blood were most likely to correlate with the clinical condition of the patient. In the event, most blood specimens sent for analysis during the evaluation of the present method were collected into plastic containers, transported at ambient temperature and stored at 4°C until the qualitative analysis was performed. The specimens were then stored at −20°C until a quantitative analysis was carried out if this was indicated. This procedure has proved satisfactory in routine use and, although no formal stability studies have been performed, repeat analyses of blood specimens containing BCF, Halons 11 and 12, propane, *n*-butane and isobutane, still gave rise to easily identifiable peaks after storage at −20°C for at least 3 months.

Nevertheless, it is prudent to recommend specimen collection into well-stoppered glass bottles [containing sodium fluoride (1%, to inhibit esterase activity) if possible] with a metal faced wad, and rapid (possibly refrigerated) transport and prompt analysis where the inhalation of very volatile materials is suspected. It is essential to ensure that samples of any products thought to have been abused which are to be analysed are packaged separately from biological specimens. It may be noted that many pressure sensitive adhesive tapes contain solvents such as toluene and thus contamination of specimens from this source is possible. It should also be noted that the earlier a clinical suspicion of solvent abuse is raised and specimens obtained and sent for analysis, the greater the likelihood of a positive finding since some inhaled agents may be excreted very rapidly[14].

Sample preparation

Headspace sample preparation was chosen since this required the use of only a small sample volume and a minimum of apparatus and introduced a minimum of contamination onto the chromatographic system. In practice, the only problem observed was that butyl rubber septum caps were found to absorb significant quantities of some solvents after relatively short (15 min) incubation times. The adoption of reusable PTFE faced disks obviated this problem, but at extra cost. This mode of sample preparation has the further advantage of ease of automation should this be contemplated, although the internal standard headspace analysis prior to sample addition would have to be omitted. In the early stages of experience with the technique, this would not be prudent, since by using this procedure any contributions to the sample chromatograms from the vial, cap, syringe or laboratory environment can be monitored.

Interpretation of sample chromatograms

With the exception of the peak derived from the septum used in the gas chromatographic system (see figures), no major peaks were represented on most "blank" chromatograms (Fig. 2). However, three compounds (ethanol, acetone and chloro-

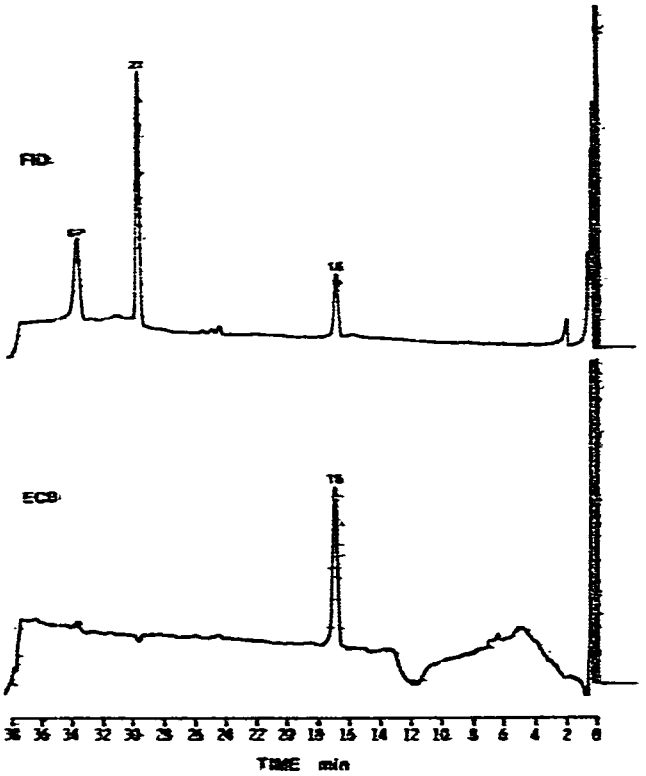

Fig. 2. Chromatogram obtained on analysis of a whole-blood specimen (200 μl) from a volunteer subject to which internal standard solution (200 μl) had been added: 400-μl headspace injection. Peaks: 15 = 1,1,2-trichloroethane and 21 = ethylbenzene (internal standards), SP = "Septum peak" (see text). For chromatographic conditions, see legend to Fig. 1.

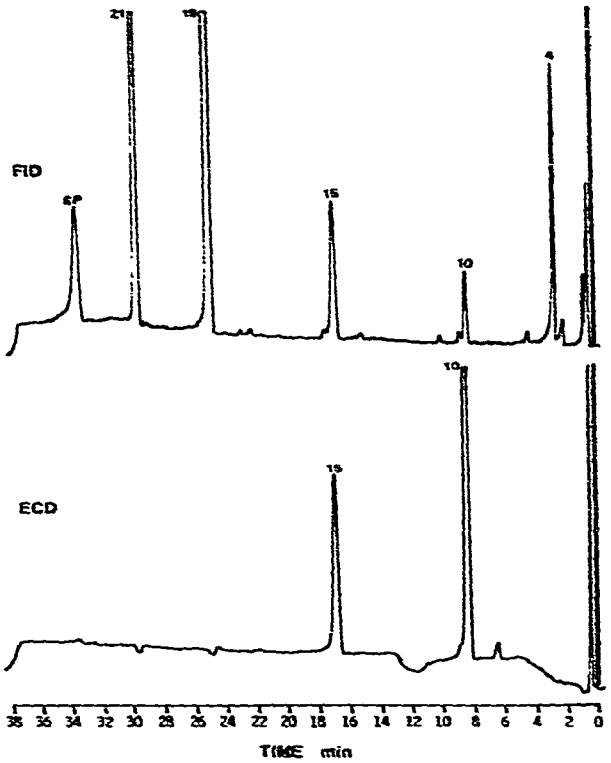

Fig. 3. Chromatogram obtained on analysis of a whole-blood specimen (200 μl) obtained from a patient suspected of abusing "solvents"; 400-μl headspace injection. Peaks: 10 = 1,1,1-trichloroethane, 19 = toluene. See legend to Fig. 1 for identification of remaining peaks and chromatographic conditions. The whole-blood 1,1,1-trichloroethane and toluene concentrations were found on subsequent analysis to be 3.4 and 11.2 mg/l, respectively.

form) were present in many samples, either alone or together with other compounds of interest (*cf.* Fig. 3). Both ethanol and acetone can be products of normal metabolism while ethanol is widely available in alcoholic beverages and both may also be produced by microbial action within the sample[15]. Chloroform (the identification of which has been confirmed by mass spectrometric analysis) was presumed to have arisen in many cases from contamination of the sample, possibly from within the laboratory.

Thus, only when the blood ethanol concentrations (measured by a separate gas chromatographic method) exceeded 0.1 g/l was the presence of this compound reported in clinical samples. Acetone and chloroform present more complex problems since both compounds may occur in preparations which may be abused by inhalation, and thus the presence of either compound was only reported when relatively high concentrations were present and there was circumstantial or other evidence that the compound of interest had been abused. This is illustrated by the case summarised in Fig. 4 where a relatively high concentration of chloroform was present together with tetrachloroethylene in a specimen obtained from a patient suspected of inhaling vapour from a dry-cleaning fluid, and also by the case summarised in Fig. 5 where the patient

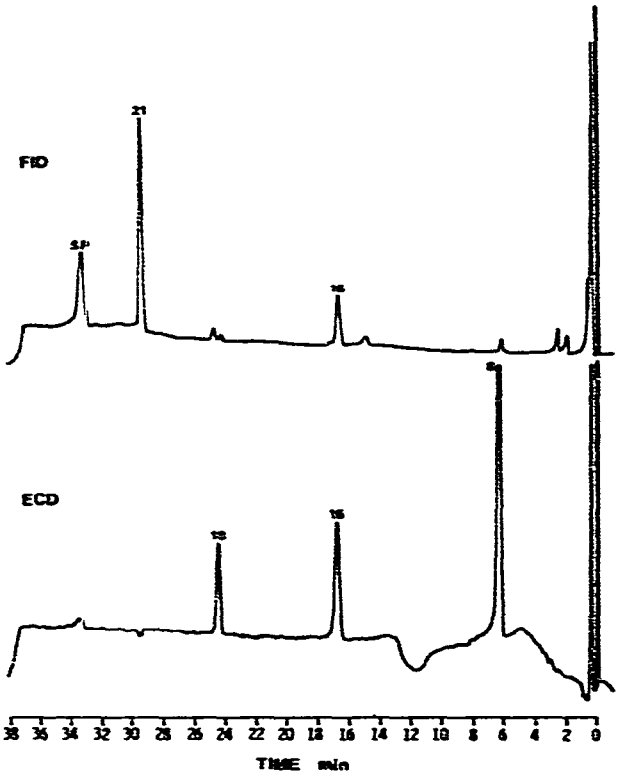

Fig. 4. Chromatogram obtained on analysis of a whole-blood specimen (200 μl) obtained from a patient suspected of inhaling vapour from a dry-cleaning fluid; 400-μl headspace injection. Peaks: 8 = chloroform, 18 = tetrachloroethylene. See legend to Fig. 1 for identification of remaining peaks and chromatographic conditions. The whole-blood tetrachloroethylene concentration was found on subsequent analysis to be 1.4 mg/l.

was suspected of abusing a de-greasing fluid containing trichloroethylene. (*N.B.* This latter case is also of interest since a high concentration of 2,2,2-trichloroethanol, presumably arising from the metabolism of trichloroethylene[16], was present.) The availability of more detailed quantitative information may assist in the interpretation of results where either acetone or chloroform is detected.

The presence of additional compounds derived from the abused agent provided useful corroboratory information in some instances. The presence of propane, isobutane and *n*-butane in a blood specimen from a patient who was suspected on abusing "butane" gas is illustrated in Fig. 6. Similarly, isopropyl nitrate, a stabiliser which has been added to commercial 1,1,1-trichloroethane, has been identified in blood specimens from patients suspected of abusing this latter agent, and methyl ethyl ketone has been identified together with toluene in specimens from patients who had inhaled the vapour from a preparation containing both compounds. When possible the direct analysis of an abused agent by either vapour phase infra-red spectrophotometry or gas chromatography can provide valuable information, and

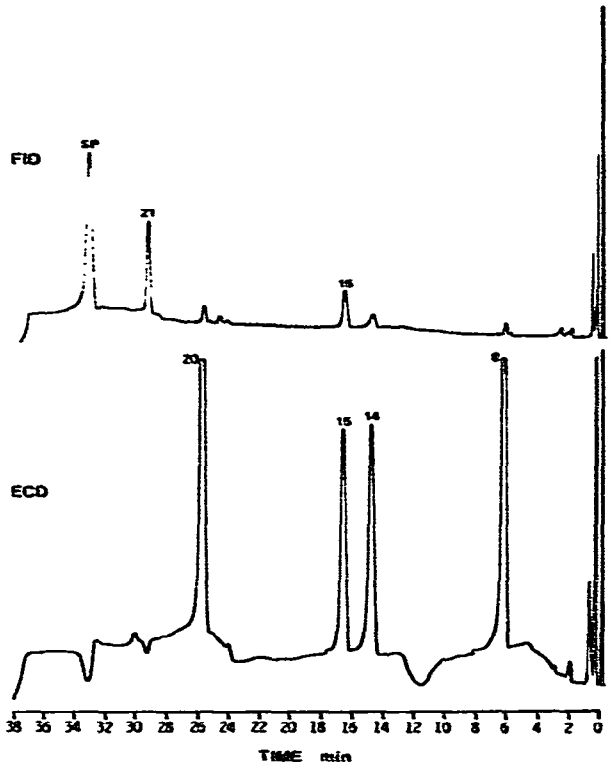

Fig. 5. Chromatogram obtained on analysis of a whole-blood specimen obtained from a patient suspected of inhaling vapour from a de-greasing fluid; 400-μl headspace injection. Peaks: 8 = chloroform, 14 = trichloroethylene, 20 = 2,2,2-trichloroethanol. See legend to Fig. 1 for identification of remaining peaks and chromatographic conditions. The whole-blood trichloroethylene and 2,2,2-trichloroethanol concentrations were found on subsequent analysis to be 9.5 and 77 mg/l, respectively.

the availability of mass spectrometric facilities for the confirmation or identification of unusual or unknown compounds may also be of value.

Limits of sensitivity

Detection limits for individual compounds depended on the appropriate detector response and to a certain extent on volatility both with respect to sample preparation and to elution time on the chromatographic system. The detection limits for some of the compounds encountered in the analysis of blood specimens were approximately 0.1 mg/l for toluene and the trichloro-compounds and 0.01 mg/l for the tetrachloro-compounds (Fig. 7). The availability of a series of clinical and forensic specimens from patients suspected of solvent abuse has proved crucial in the establishment of realistic detection limits for the technique, and it can be seen from the quantitative results summarised in Fig. 8 that in many of these specimens the compounds of interest were present at concentrations several-fold higher than these limits. There is much current interest in the analysis of volatile organic compounds in biological specimens both with respect to the monitoring of environmental pol-

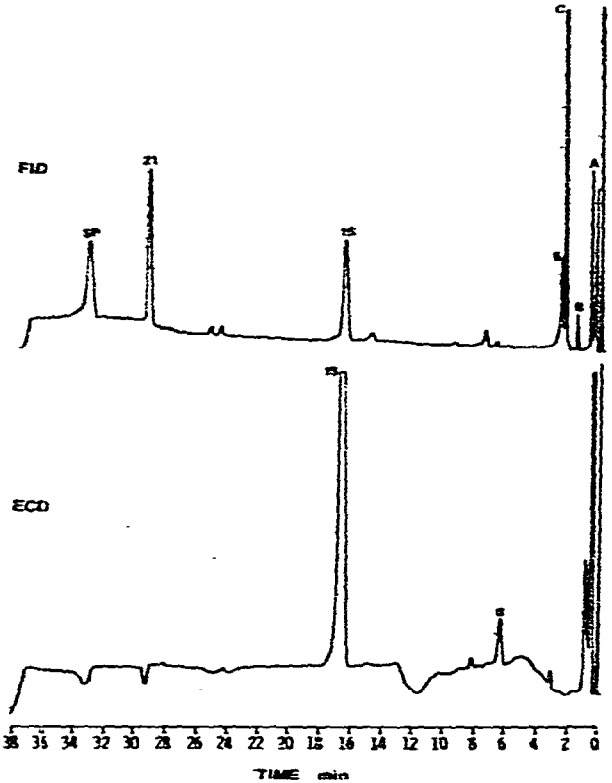

Fig. 6. Chromatogram obtained on analysis of a whole-blood specimen obtained from a patient suspected of inhaling "butane" from a cigarette-lighter refill: 400-μl headspace injection. Peaks: A = propane; B = isobutane, C = *n*-butane. See legend to Fig. 1 for identification of remaining peaks and chromatographic conditions.

lution[17] and to the possible use of such analyses in the diagnosis of disease[18,19]. However, it must be emphasised that the overall sensitivity of the present method is much less than that used in these other procedures, and thus the chromatograms obtained are normally free from interference from endogenous sample components (Figs. 2–7).

The method has proved applicable to the detection of bromochlorodifluoromethane, *n*-butane, carbon tetrachloride, chlorobutanol, cryofluorane, dichlorodifluoromethane, ethyl acetate, halothane, isobutane, isopropanol, isopropyl nitrate, methyl ethyl ketone, propane, tetrachloroethylene, toluene, 1,1,1-trichloroethane, 2,2,2-trichloroethanol, trichloroethylene and trichlorofluoromethane in specimens obtained from patients suspected of the abuse by inhalation or oral ingestion of these agents. In addition, cyclopropane, diethyl ether, paraldehyde and halothane have been detected in blood samples from patients treated with these compounds. However, we have no experience of the application of the technique where the vapours from complex mixtures such as petrol or paraffin have been inhaled. Chronic "petrol sniffing" has been diagnosed by the measurement of blood lead concentrations[20], and detection of the aromatic components could prove useful[21]. The measurement of

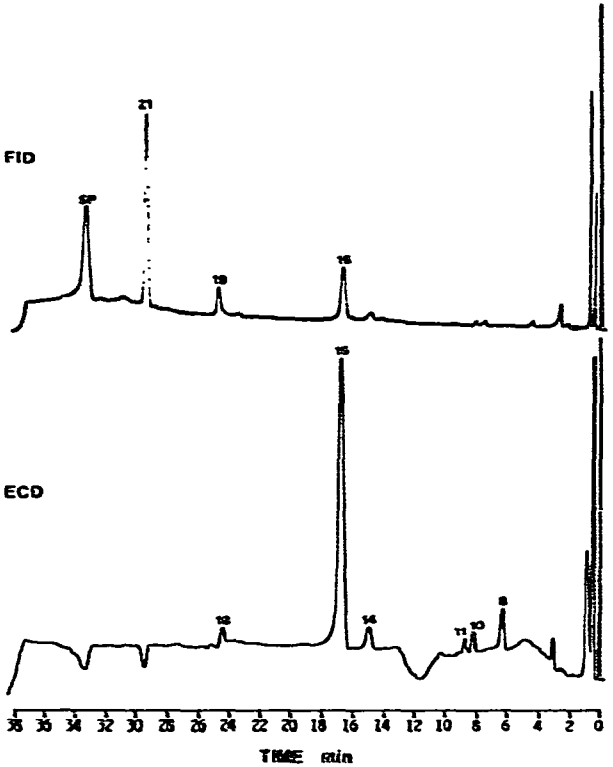

Fig. 7. Chromatogram obtained on analysis of a portion (200 μl) of a synthetic mixture prepared in expired blood-bank whole-blood; 400-μl headspace injection. Peaks: (blood concentrations in brackets), 10 = trichloroethane (105 μg/l), 11 = carbon tetrachloride (16 μg/l), 14 = trichloroethylene (114 μg/l), 18 = tetrachloroethylene (13 μg/l), 19 = toluene (87 μg/l). See legend to Fig. 1 for identification of remaining peaks and chromatographic conditions.

blood aluminium concentrations may provide useful diagnostic information where anti-perspirant aerosol preparations containing aluminum compounds may have been abused. On the other hand, it should be noted that the detection of esters such as ethyl acetate may be limited by blood non-specific esterase activity.

Application to saliva or urine specimens

In view of the lipophilicity and volatility of the compounds studied, it was thought unlikely that analyses of urine and/or saliva for the parent compound(s) would yield useful information. Indeed, the analysis of a number of such specimens taken at the same time as a blood specimen in which one or more of the compounds of interest were detected and identified confirmed this. However, it should be noted that in addition to the detection of metabolites containing the trichloro group[22], urine specimens may be useful in the identification and measurement of compounds such as trichloroacetic acid[23], hippuric[24] and benzoic acids and the cresols[25] (metabolites of toluene), and the toluric acids (metabolites of the xylenes[26]).

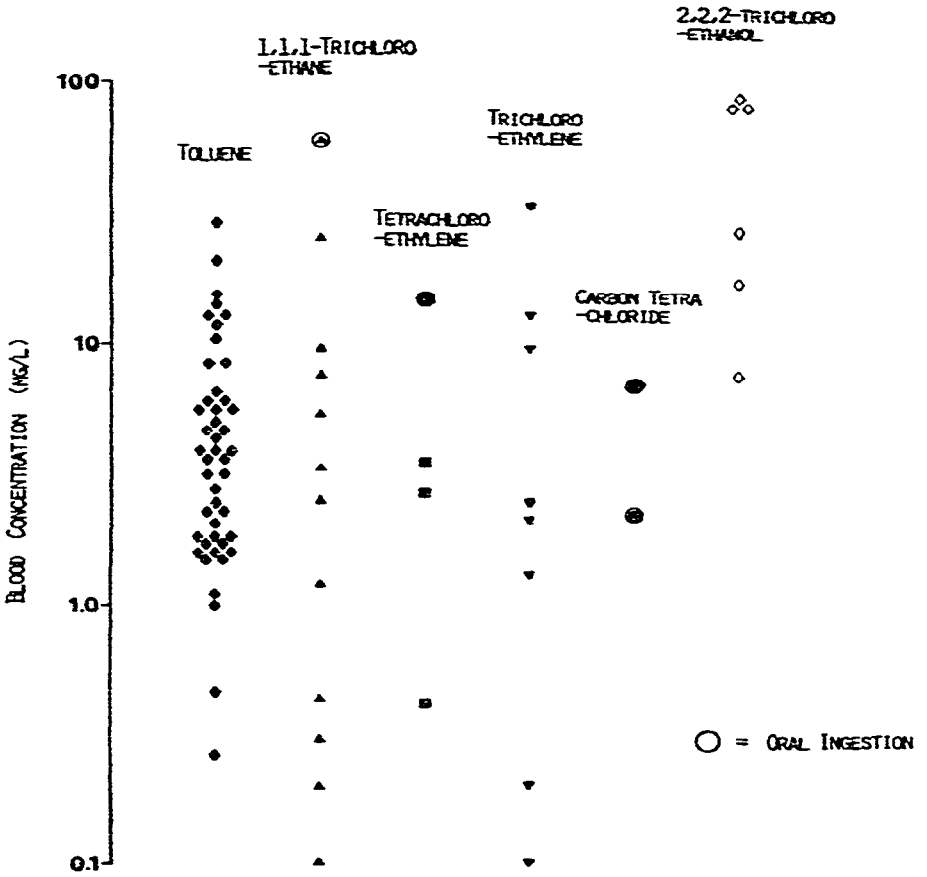

Fig. 8. Summary of quantitative results obtained on specimens analysed initially by the qualitative method.

Analysis of post-mortem tissue

Where forensic examination of tissue specimens was requested, it was found that a simple modification of the method gave satisfactory results. Tissue (approximately 200 mg) was incubated at 65°C for 30 min in a headspace analyser vial with internal standard solution (200 μl) containing a proteolytic enzyme (Subtilisin A; Novo, Windsor, Great Britain; 1 mg)[27]. Subsequently, a headspace sample (400 μl) was taken and analysed as described previously. Reagent blanks were performed in a separate vial.

Application to quantitative analyses

The method has been applied to the quantitative analysis of blood specimens containing some of the less volatile compounds studied (b.p. > 30°C), notably carbon tetrachloride, tetrachloroethylene, toluene, 1,1,1-trichloroethane and trichloroethylene, although it was found that a simple solvent-extraction technique[28] was suitable for the measurement of the blood 2,2,2-trichloroethanol concentrations attained following inhalation of trichloroethylene. Standard solutions at an appropri-

TABLE V

INTERNAL STANDARDS USED IN QUANTITATIVE ANALYSIS

For details of preparation, see text.

Analyte	*Internal standard*
Carbon tetrachloride	Bromodichloromethane
Chlorobutanol	1,1,1-Trichloropropan-2-ol
Halothane	Bromodichloromethane
Tetrachloroethylene	1,1,1,2-Tetrachloroethane
Toluene	Ethylbenzene
1,1,1-Trichloroethane	1,1,2-Trichloroethane
Trichloroethylene	1,1,2-Trichloroethane

ate range of concentrations were prepared gravimetrically in "volatile free" outdated blood-bank whole blood. After addition of the sample or standard and the internal standard solution (Table V) followed by equilibration for 30 min at 65°C, a portion of the headspace was analysed isothermally at an appropriate temperature. Calibration was by means of peak height ratios of analyte to internal standard on the appropriate detection system plotted against analyte concentration. When necessary, blood specimens were diluted with outdated blood-bank whole blood and re-analysed.

A summary of the results of quantitative analyses performed using specimens from patients suspected of abusing these compounds either by inhalation or oral ingestion is summarised in Fig. 8. All of these specimens had been analysed initially using the qualitative procedure. It should be emphasised that these results represent minimum values for the blood concentrations attained in these patients since it was not possible to take special precautions in the collection and transportation of the specimens, as discussed above.

The problems associated with the quantitative analysis of some of the more volatile compounds which have been detected using this procedure, for example butane and the halons, in biological specimens have been discussed[29] and include the need for precautions in the collection and transportation of specimens together with the difficulties inherent in preparing calibration solutions of these compounds.

CONCLUSIONS

The method described here has proved suitable for use in the detection and identification of a wide range of relatively volatile organic compounds in specimens obtained from patients suspected of the abuse of these agents. Only small specimens of blood (200 μl) or tissue (200 mg) are required, and special precautions in the collection and transportation of these specimens are not mandatory in the majority of cases although such precautions are desirable where very volatile materials are concerned. The method has proved to be reproducible and reliable in routine use and requires a minimum of apparatus, but may be readily adapted to automatic operation if required. Finally, the quantitative analysis of a number of the less volatile compounds studied has been accomplished using the same system together with the appropriate calibration solutions.

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