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A simple, rapid and simultaneous analysis of complex volatile hydrocarbon mixtures in blood using gas chromatography/mass spectrometry with a wide-bore capillary column

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Summary. A screening method for detecting volatile hydrocarbons in blood has been developed using gas chromatography/mass spectrometry with a wide-bore capillary column and a headspace method. Toluene-d_8 and indan were used as the internal standards for quantitative analysis. Hydrocarbons with retention indices from 600 to 1200 were simultaneously and quantitatively detected in relatively low concentrations (0.01 μg/ml) in reconstructed ion chromatography. This method could prove useful in forensic cases in which urgent examination of complex hydrocarbon mixtures, e.g. petroleum components, is required.

Key word: Volatile hydrocarbons in the blood, screening method

Zusammenfassung. Es wird über eine Screeningmethode zum Nachweis flüchtiger Kohlenwasserstoffe im Blut mittels Gaschromatographie/Massenspektrometrie berichtet, wobei wide-bore Kapillaren und die Headspacetechnik eingesetzt wurden. Toluol-d_8 und Indan dienten als interne Standards bei der quantitativen Analyse. Kohlenwasserstoffe mit Retentionsindizes zwischen 600 und 1200 konnten mit der Ionenchromatographie simultan und quantitativ noch in relativ geringen Konzentrationsbereichen bis 0.01 μg/ml bestimmt werden. In der Praxis ist die Methode nützlich, wenn es in forensischen Fällen darum geht, komplexe Kohlenwasserstoffgemische (wie z.B. Petroleum) rasch zu analysieren.

Schlüsselwort: flüchtige Kohlenwasserstoffe im Blut, Screeningmethode

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Introduction

Volatile hydrocarbons in fuel oils, such as liquefied petroleum gas, gasoline and kerosene, are sometimes detected in the blood of burned human bodies. This is useful evidence that the victims have inhaled vapours of petroleum products shortly before death, as reported by Nagata et al. [1–3]. Gas chromatography/mass spectrometry (GC/MS) has been used for determining small amounts of hydrocarbon mixtures in biological materials, but two steps are needed to separate the petroleum products from blood: the headspace method for analysis of highly volatile compounds, and pentane extraction for less volatile compounds, such as trimethylbenzenes. However, possible impurities in the extracting solvent should also be taken into consideration.

Recently, capillary columns have become popular in gas chromatographic analyses of volatile compounds in biological materials [4–9]. In most reports, narrow-bore columns with diameters of less than 0.3 mm were used to increase the sensitivity of trace analysis. However, large volumes of headspace samples cannot be introduced into the columns without condensation systems and split systems. A wide-bore capillary column with a larger inner diameter of 0.53 mm has a relatively large sample capacity and can be used to analyse larger volumes from headspace samples [10].

Consequently, reconstructed ion chromatography (mass chromatography) with a wide-bore capillary column was used to evaluate the performance of the headspace method. This proved to be a simple and rapid method for the simultaneous analysis of complex hydrocarbon mixtures in blood.

Materials and methods

Materials. The standards, benzene, toluene, ethylbenzene, *p*-xylene, *m*-xylene, *o*-xylene, styrene, isopropylbenzene, *n*-propylbenzene, mesitylene, pseudocumene, 1,2,3-trimethylbenzene, 2-ethyltoluene, 3-ethyltoluene, 4-ethyltoluene, *n*-butylbenzene, *sec*-butylbenzene, *tert*-butylbenzene, *n*-hexane, *n*-heptane, *n*-octane, *n*-nonane, *n*-decane, *n*-undecane and *n*-dodecane, were all of analytical grade. The internal standard toluene-d_8 was purchased from E. Merck (Darmstadt, FRG), and indan, from Nacalai Tesque (Kyoto, Japan). For the purge-and-trap method, the adsorbent, Tenax TA (60/80 mesh) and the dehydrating agent, Chromosorb G (80/100 mesh), were obtained from Gasukuro Kogyo (Tokyo, Japan). Ethanol was distilled and used as the standard solution solvent.

Internal standard solution. To obtain the internal standard solution, 10 mg each of toluene-d_8 and indan were dissolved together in 100 ml ethanol.

Sample preparation. A 1-g aliquot of blood was placed in a 10-ml vial containing 1 ml cold tap-water, and 1 µl of internal standard solution was then added. The vial was sealed with a silicon rubber stopper and heated at 60°C for 20 min. After cooling to room temperature, 2 ml headspace vapour was sampled into a glass syringe and analysed in the GC/MS.

Conditions of GC/MS. A Shimadzu gas chromatograph-mass spectrometer QP-1000 was used. The wide-bore capillary column was a 15 m × 0.53 mm i.d. fused silica DB-5 (J & W Scientific, Calif., USA) with a film thickness of 1.5 µm. The temperatures of the ion source, the separator and the injection port were 250°C, 250°C and 100°C, respectively. The temperature of the column oven was programmed from 40°C to 90°C at a rate of 4°C/min. Ionization energy was 20 eV. Mass spectra were recorded every 2 s from m/z 20 to m/z 200. The carrier gas was helium with a flowrate of 10 ml/min. Reconstructed ion chromatography was performed after mass spectra had been collected.

Collection of retention indices. Standards were analysed on a Shimadzu gas chromatograph GC-7AG equipped with a flame ion detector, and their retention indices were calculated according to Newton and Foery [11]. The temperature of both the injection and the detector block was 100°C. The other chromatographic conditions were the same as for GC/MS.

Purge-and-trap method. A 1-g aliquot of blood was placed in a 50-ml glass tube containing 1 ml cold tapwater. Volatile substances were purged using a nitrogen stream at a flow rate of 500 ml/min at 40°C and collected in an adsorbent column (1-ml glass tube packed with 150 mg Tenax TA) via a dehydrating agent column (1-ml glass tube packed with 100 mg Chromosorb G). The adsorbent column was fixed to a heating sample inlet system (Shimadzu FLS-1). The temperature was programmed from room temperature to 220°C in 35 s and kept at 220°C until the end of the analysis. Finally, the volatile substances in the column were purged and introduced into a gas chromatograph by helium flow.

Results and discussion

Gas chromatographic retention indices and principal mass spectrometric ions of 25 standards and 2 internal standards are shown in Table 1. Reconstructed ion chromatography was used to identify a compound and confirm the intensity ratios of the principal ions to the base ion. On the reconstructed ion chromatogram, *m*-xylene and 3-ethyltoluene were indistinguishable from *p*-xylene and 4-ethyltoluene, respectively. An ion chromatogram of a blood sample spiked with standards is shown in Fig. 1, together with a control blood sample with only internal standards. The identifiable lower limits in blood were 0.001 μg/g for toluene, all the xylenes and ethylbenzene, and 0.01 μg/g for the remainder. Benzene was identified by tracing a single ion at m/z 78, because the other ion intensities were not sufficient to allow detection at lower concentrations. The lower detection limit for benzene was also 0.001 μg/g. There were no interfering background peaks derived from the control blood samples on the ion chromatograms. The purge-and-trap method was introduced in an attempt to enrich peak intensity in ion chromatography. This method not only resulted in a more than 10-fold increase in high peak intensities, but also, unfortunately, in broader peaks of some compounds than were obtained with a simple headspace method. In the purge-and-trap method and solvent extractions, the interference of contaminants in solvents and/or adsorbents should be considered. However, the headspace method was sufficiently sensitive to prove the existence of hydrocarbon inhalation in burned bodies devoid of any foreign pullutants.

For simultaneous quantitative analysis of alkylbenzenes, standard curves were prepared from blood samples with 0.01, 0.05, 0.10, 0.50 and 1.0 μg/g each of all standards, except for *p*-xylene and 4-ethyltoluene, which could not be distinguished from *m*-xylene and 3-ethyltoluene, respectively. Linear relationships were observed between peak height ratios of the standards to the corresponding internal standards and blood concentrations of the standards. Extracted ions for quantitative analysis, the curve formulas and the selected internal standards for each compound are shown in Table 2. Assay reproducibility was also checked by preparing five series of samples, each with 1 g blood 0.05 μg of each standard. The coefficients of variation (cv) are also shown in Table 2. Internal standards were selected to obtain smaller cv values. Should there be a demand for higher reproducibility and sensitivity, a selected ion monitoring technique could be used instead, although mass spectra cannot be obtained. Our study proves that

Table 1. Identification of hydrocarbons

Compound	RI	Principal ions (intensity)			
n-Hexane	600	57 (100)	43 (65)	56 (57)	42 (43)
Benzene	654	78 (100)	79 (7)	77 (5)	76 (3)
n-Heptane	700	43 (100)	71 (70)	57 (62)	56 (43)
Toluene	759	92 (100)	91 (48)	93 (8)	
n-Octane	800	43 (100)	57 (63)	85 (32)	56 (50)
Ethylbenzene	855	106 (100)	91 (97)	92 (9)	107 (9)
m-Xylene	863	106 (100)	91 (60)	105 (11)	107 (5)
p-Xylene	863	106 (100)	91 (60)	105 (11)	107 (5)
o-Xylene	888	106 (100)	91 (60)	105 (22)	107 (10)
Styrene	888	104 (100)	78 (16)	103 (15)	105 (15)
n-Nonane	900	57 (100)	43 (81)	85 (40)	71 (28)
Isopropylbenzene	920	105 (100)	120 (69)	91 (3)	79 (3)
n-Propylbenzene	948	91 (100)	120 (75)	92 (12)	105 (7)
3-Ethyltoluene	957	105 (100)	120 (86)	106 (10)	121 (9)
4-Ethyltoluene	957	105 (100)	120 (86)	106 (10)	121 (9)
Mesitylene	963	120 (100)	105 (68)	121 (12)	106 (6)
2-Ethyltoluene	974	105 (100)	120 (86)	121 (12)	106 (11)
tert-Butylbenzene	988	119 (100)	105 (60)	134 (34)	91 (17)
Pseudocumene	988	120 (100)	105 (67)	91 (19)	106 (6)
n-Decane	1000	57 (100)	43 (54)	71 (42)	85 (31)
sec-Butylbenzene	1006	105 (100)	134 (47)	106 (10)	91 (8)
1,2,3-Trimethylbenzene	1017	120 (100)	105 (74)	121 (10)	119 (6)
n-Butylbenzene	1051	92 (100)	134 (97)	91 (97)	105 (13)
n-Undecane	1100	57 (100)	71 (49)	43 (48)	85 (37)
n-Dodecane	1200	57 (100)	71 (54)	43 (41)	85 (37)
Toluene-d_8	759	100 (100)	98 (43)	99 (10)	101 (10)
Indan	1028	118 (100)	117 (90)	116 (22)	119 (18)

Figures underlined are numbers of ions extracted for reconstructed ion chromatography

quantitative analysis and confirmation of mass spectra can be performed simultaneously in a single simple procedure.

For simultaneous quantitative and qualitative analysis, care was taken to maintain reproducibility within the cv values, as shown in Table 2. When humid air at 60°C was injected into a gas chromatograph, water particles condensed on the column wall at 40°C and interfered with the analysis. For this reason, the headspace sample was cooled to room temperature after incubation at 60°C. After one measurement series had been carried out the column was heated from 90°C to 200°C at a rate of 30°C/min by temperature-programming. Since many compounds with various degrees of volatility were analysed simultaneously, the use of two internal standards should have been considered sooner. At first only toluene-d_8 was used as the internal standard, which was useful for the more highly volatile substances but not for the less highly volatile ones. This inadequacy was

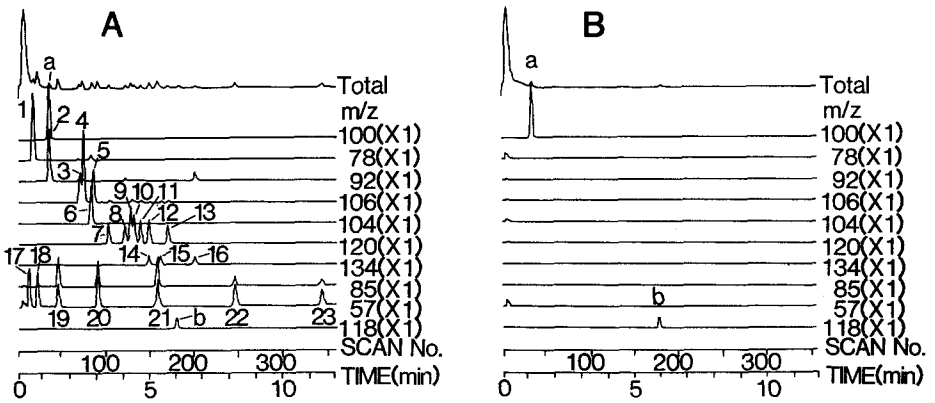

Fig. 1A, B. Reconstructed ion chromatograms for headspace samples of blood spiked with hydrocarbons: **A** 25 standards and 2 internal standards; **B** 2 internal standards (*a*, toluene-d_8, *b*, indan). *1*, Benzene; *2*, toluene; *3*, ethylbenzene; *4*, *m*- and *p*-xylenes; *5*, *o*-xylene; *6*, styrene; *7*, cumene; *8*, *n*-propylbenzene; *9*, 3- and 4-ethyltoluene; *10*, mesitylene; *11*, 2-ethyltoluene; *12*, pseudocumene; *13*, 1,2,3-trimethylbenzene; *14*, *tert*-butylbenzene; *15*, *sec*-butylbenzene; *16*, *n*-butylbenzene; *17*, *n*-hexane; *18*, *n*-heptane; *19*, *n*-octane; *20*, *n*-nonane; *21*, *n*-decane; *22*, *n*-undecane; *23*, *n*-dodecane

Table 2. Standard curves of aromatic hydrocarbons determined in blood by reconstructed ion chromatography

Compound	Extracted ion m/z	Standard curve	*r*	cv IS (%)
Benzene	78	y = 14.00x − 0.34	0.997	5 a
Toluene	92	y = 13.52x − 0.21	0.997	7 a
Ethylbenzene	106	y = 5.82x − 0.03	0.993	13 a
m-Xylene	106	y = 5.95x − 0.29	0.997	15 a
o-Xylene	106	y = 5.56x + 0.45	0.979	14 a
Styrene	104	y = 5.59x + 0.03	0.994	14 a
Cumene	120	y = 29.32x − 0.29	0.995	7 b
n-Propylbenzene	120	y = 37.11x − 1.18	0.997	19 b
3-Ethyltoluene	120	y = 27.60x + 0.15	0.996	17 b
Mesitylene	120	y = 48.08x − 1.19	0.996	16 b
2-Ethyltoluene	120	y = 34.48x − 0.71	0.995	12 b
tert-Butylbenzene	134	y = 16.90x − 0.33	0.998	16 b
Pseudocumene	120	y = 40.63x − 1.90	0.994	16 b
sec-Butylbenzene	134	y = 18.12x − 0.19	0.998	20 b
1,2,3-Trimethylbenzene	120	y = 20.95x + 0.35	0.998	14 b
n-Butylbenzene	134	y = 16.41x − 0.54	0.989	17 b

r = correlation coefficient
cv = coefficient of variation ($n = 5$), IS = Internal standard
y = peak height ratio of analyte to IS
x = concentration of analyte in blood (μg/g)
a, toluene-d_8; b, indan. Extracted ions for IS were m/z 100 for a and m/z 118 for b

eventually overcome by introducing indan as the second, satisfactory internal standard.

Conclusion

In this method, hydrocarbons with retention indices from 600 to 1200 can be simultaneously and quantitatively detected in sufficient levels to warrant its use in practical forensic cases. Therefore this method is recommended as a routine examination to prove ante-mortem exposure to varpours of petroleum products, such as gasoline and kerosene.

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