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The utility of volatile hydrocarbon analysis in cases of carbon monoxide poisoning

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Abstract A new approach to investigate the circumstances relating to carbon monoxide intoxication by analysing volatile hydrocarbons in the blood of cadavers is reported. Headspace gas chromatography/mass spectrometry was used to demonstrate the hydrocarbons. The results can be characterized into four categories depending on the compounds detected. In construction fire cases where no accelerants were found at the scene benzene, toluene and styrene were detected in the blood. In cases where gasoline was found in the fire debris surrounding the victim, high levels of benzene, toluene, ethylbenzene, xylene isomers, *n*-hexane and *n*-heptane were detected in the blood. In cases where kerosene was found in the fire debris around the victim, benzene, toluene, ethylbenzene, xylene isomers, C_9-aromatics(*n*-propylbenzene, trimethylbenzene isomers), *n*-octane, *n*-nonane and *n*-decane were detected in the blood. In cases where the victim was found inside a gasoline-fuelled automobile filled with exhaust gas, benzene, toluene, ethylbenzene, xylene isomers, C_9-aromatics were found, but no aliphatic hydrocarbons such as components of petroleum. The analyses of the combustion gases of inflammable materials, exhaust gas, gasoline vapours and kerosene vapours were also performed to evaluate the results of the blood analyses. Consequently, some compounds are proposed as indicators to discriminate between inhaled gases i.e. styrene in common combustion gas, *n*-hexane and *n*-heptane as well as benzene, toluene and C_9-aromatics in gasoline cases, *n*-nonane and *n*-octane as well as benzene, toluene and C_9-aromatics in kerosene cases, and benzene, toluene, C_9-aromatics but no aliphatic hydrocarbons in exhaust gas cases.

Key words Inhalation · Combustion gas · Petroleum accelerants · Exhaust gas · Volatile hydrocarbons

Introduction

In fire investigations, the examination of accelerants is usually performed by analysing the chromatographic profiles of fire debris samples [1–4]. Petroleum-based accelerants can be identified by gas chromatography or gas chromatography/mass spectrometry (GC/MS). However, when a victim is found dead at the scene of a fire, an examination can only provide some information on the cause of the fire and the circumstances surrounding the cadaver, but none on whether the victim was actually alive when the fire occurred and if petroleum fuel was used as an accelerant. In such cases there is no evidence as to whether the victim was in fact alive when the incident occurred except in the case of carbon monoxide intoxication. Kimura et al.[5] proposed a method to detect hydrocarbons in blood to elucidate the conditions prevailing before death. They also suggested the possibility of discriminating between gasoline and kerosene.

In this study, the blood of 47 victims found at the scenes of a fire and in automobiles filled with exhaust gas were analysed targeting 24 petroleum-related compounds by headspace capillary GC/MS. In addition, experimental combustion gases of inflammable materials, petroleum vapours and exhaust gas were also analysed to elucidate the results of the blood analyses. The results of the blood analyses could be partly summarized into four situations depending on the gas species and the characteristic indicators of the corresponding gases.

Materials and methods

Blood samples (cardiac blood) were collected at autopsy in cases relating to carbon monoxide intoxication. The intoxication case situations were distinguished into four groups depending on the species of gas inhaled 1. Construction fire cases where no evidence of accelerants was found at the scene of a fire (HbCO:15~78%).

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2. Cases where characteristic gasoline components were detected among the fire debris around the victim (HbCO:10~30%). 3. Cases where characteristic kerosene components were indicated among the fire debris collected around the victim (HbCO:8~54%). 4. Cases where the victim was found inside a gasoline-fuelled car filled with exhaust gas (HbCO:64~80%). The case information situations was provided by the Forensic Science Laboratory of the Fukuoka Prefectural Police Headquarters.

Inflammable materials such as newspaper, wood, (synthetic) carpets, (synthetic) curtains, gasoline and kerosene were obtained locally.

As target compounds or standards, benzene, toluene, ethylbenzene, *p*-xylene, *o*-xylene, styrene, C_9-aromatics (cumene, *n*-propylbenzene, 3-ethyltoluene, 2-ethyltoluene, mesitylene, pseudocumene, 1,2,3-trimethylbenzene), C_{10}-aromatics(*tert*-butylbenzene, *sec*-butylbenzene, *n*-butylbenzene), *n*-pentane, *n*-hexane, *n*-heptane, *n*-octane, *n*-nonane, *n*-decane, *n*-undecane and *n*-dodecane of petroleum-related compounds of GC analytical grade were used. As internal standards for a quantitative analysis, toluene-d_8 was purchased from Isotec (Miamisburg, Ohio) and indan from Wako Pure Chemical Industries, Osaka, Japan. Tetraethylene glycol dimethyl ether was purchased from Aldrich, Milwaukee, Wisc.

For the running standard solution 1 μl each of all target compounds and 1 μl each of two internal standards were dissolved in 10 ml of tetraethylene glycol dimethyl ether. The internal standard solution was 1 μl each of toluene-d_8 and indan dissolved in 10 ml of tetraethylene glycol dimethyl ether to give a concentration of 0.1 μl/ml. (The unit for quantitative results converted into μg/ml using specific gravity).

Blood samples were prepared by adding 1 ml to 1 ml of cold water in a 15 ml-glass vial with a silicon-rubber septum, which was then covered with the septum and sealed with an aluminum cap. The internal standard solution (1 μl) was injected through the septum into the vial. The vial was warmed and frequently shaken at 60° C for 20 min. Aliquots of 2 ml of the headspace were collected in a 2 ml-glass syringe and injected into the GC/MS instrument.

For the running standard sample, 2 ml of cold water was placed in a 15-ml vial as in the blood sample preparation. After sealing the vial, 1 μl of standard solution was injected, following which, the preparation was the same as for blood samples.

Combustion gases of inflammable materials were collected by placing in a petri dish and burning in a glass desiccator (2800 ml) under closed conditions. The combustion gas was collected through a septum with a glass syringe and subjected to GC/MS analysis.

The exhaust gas of a gasoline-fuelled automobile (1800 ml engine displacement) was collected directly into a 20 l-Tedlar bag while the engine was idling .

Analytical conditions. The GC/MS analysis was performed on a GC/MS QP-1000 (Shimadzu, Kyoto, Japan), operated in the positive electron impact mode (EI) with a DB-1 capillary column (2 × 30 m × 0.53 mm i.d., 5 mm film thickness, J & W Scientific, Folsom, Calif.). The GC conditions were: injection port temperature 100° C and the flow rate of the helium carrier gas 30 ml/min. The oven was maintained at an initial temperature of 40° C for 4 min, then programmed at 8° C/min to 200° C and finally kept at 200° C until termination. The separator temperature was set at 250° C and the ion source at 250° C. The MS conditions were: ionizing energy 20 eV, scan ranges 20–200 amu and a scan cycle every 2 s.

Selected ions for target compounds and internal standards were detected by constructing chromatograms of molecular ions/principal fragment ions as described in our previous report [6]: benzene =

Table 1 Hydrocarbon profiles of the inhaled combustion gases in the victim's blood

Compound	Construction fire (N = 20)		Petroleum				Exhaust gas (N = 10)	
	conc.	*F*	Gasoline (N = 7)		Kerosene (N = 10)		conc.	*F*
			conc.	*F*	conc.	*F*		
benzene	0.14–1.45	20	0.14–0.87	7	0.01–0.72	10	0.01–0.07	10
toluene	0.01–0.41	20	0.04–1.12	7	0.03–0.71	10	0.04–0.20	10
ethylbenzene	0.01–0.18	13	0.01–0.55	7	0.01–0.34	9	0.01–0.10	10
p-xylene	0.01–0.06	14	0.02–0.36	7	0.01–0.16	10	0.01–0.04	10
o-xylene	0.01–0.13	11	0.01–0.18	7	0.01–0.13	9	0.01–0.13	10
styrene	0.01–0.59	20	0.01–0.08	7	0.02–0.64	10	–	0
cumene	–	0	0.01–0.03	5	0.01–0.12	7	0.01	5
n-propylbenzene	–	0	0.01–0.07	7	0.01–0.04	8	0.01–0.02	8
3-ethyltoluene	–	0	0.01–0.09	6	0.01–0.17	10	0.01–0.07	7
mesitylene	–	0	0.02–0.05	6	0.01–0.07	10	0.01–0.02	4
2-ethyltoluene	–	0	0.02–0.05	6	0.01–0.09	7	0.01–0.02	9
pseudocumene	–	0	0.01–0.15	7	0.01–0.16	9	0.01–0.02	8
1.2.3-trimethylbenzene	–	0	0.02–0.08	6	0.01–0.08	7	0.01	2
tert-butylbenzene	–	0	–	0	–	0	–	0
sec-butylbenzene	–	0	–	0	–	0	–	0
n-butylbenzene	–	0	–	0	–	0	–	0
n-pentane	–	0	0.04–0.12	5	–	0	–	0
n-hexane	–	0	0.02–0.25	6	–	0	–	0
n-heptane	–	0	0.03–0.24	6	–	0	–	0
n-octane	–	0	–	0	0.01–0.37	9	–	0
n-nonane	–	0	–	0	0.06–1.00	10	–	0
n-decane	–	0	–	0	0.04–1.06	10	–	0
n-undecance	–	0	–	0	0.04–0.62	10	–	0
n-dodecane	–	0	–	0	0.01–0.97	7	–	0

conc. concentration range (μg/ml); *F* observed frequency; *N* number of cases; – not comfirmed/less than lower quantitative limits m/z 78, toluene = m/z 92 and 91, ethylbenzene and xylene isomers = m/z 106 and 91, styrene = m/z 104 and 78, ethyltoluene isomers, propylbenzne isomers and trimethylbenzene isomers = m/z 120, 105 and 91, butylbenzene isomers = m/z 134 and 91 and aliphatics = m/z 71 and 57.

Results

The analytical results of the volatile hydrocarbons detected in the blood of victims samples are shown in Table 1 according to the case situations. Although butylbenzene isomers were targeted as petroleum-related compounds [7–9], they were all below the lower detection limits. The calibration curves for quantitative analysis of hydrocarbons were linear from 0.01 to 1.0 μg/ml with correlation coefficients of 0.996–0.999. The detection limits using this method were 0.001 μg/ml for benzene and toluene, and 0.01 μg/ml for the remainder. The standard deviations ranged between 2.0 and 14.5% at the concentration of 0.1 μg/ml as described in our previous report [6]. The quantitative values were low in all the cases, but the qualitative profiles were quite different. The species of hydrocarbons detected were common in the corresponding cases. In construction fire cases, benzene, toluene and styrene were detected and benzene and styrene were detected at especially high levels. In gasoline cases, the target aromatics, *n*-pentane, *n*-hexane and *n*-heptane were detectable, and benzene and toluene were again at relatively high levels.

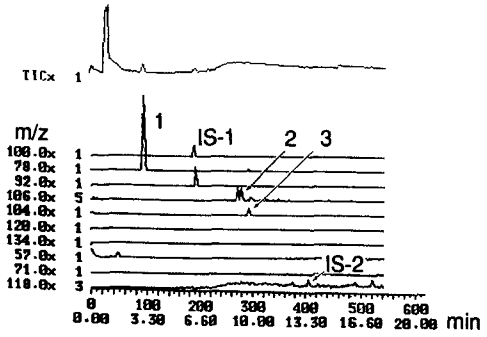

Fig. 1 Mass chromatograms of a construction fire case 1 = benzene, 2 = *p*-xylene, 3 = styrene, IS-1 = toluene-d_8, IS-2 = indan

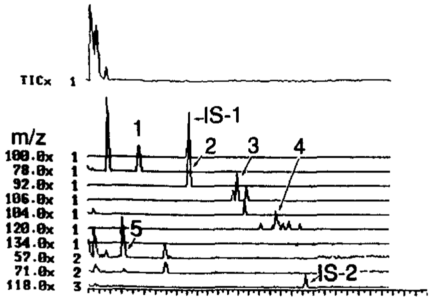

Fig. 2 Mass chromatograms of a gasoline fire case 1 = benzene, 2 = toluene, 3 = *p*-xylene, 3 = 3-ethyltoluene, 4 = *n*-hexane, IS-1 = toluene-d_8, IS-2 = indan

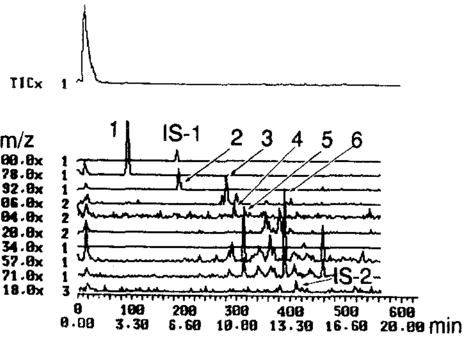

Fig. 3 Mass chromatograms of a kerosene fire case 1 = benzene, 2 = toluene, 3 = *p*-xylene, 4 = styrene, 5 = *n*-nonane, 6 = *n*-decane, S-1 = toluene-d_8, IS-2 = indan

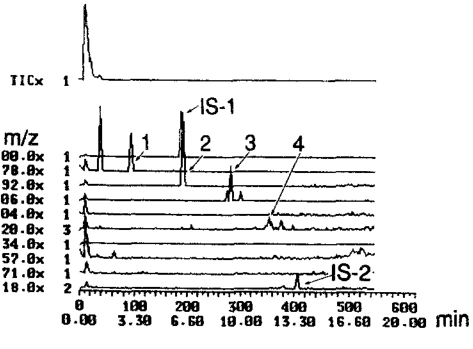

Fig. 4 Mass chromatograms of an exhaust gas inhalation case 1 = benzene, 2 = toluene, 3 = *p*-xylene, 4 = 3-ethyltoluene, IS-1 = toluene-d_8, IS-2 = indan

In kerosene cases, the target aromatics, *n*-octane, *n*-nonane, *n*-decane, *n*-undecane and *n*-dodecane were detected, with toluene, *n*-nonane and *n*-decane at especially high levels. In the exhaust gas cases, all the target aromatics other than styrene were detectable but at low levels. Typical mass chromatograms of each category are shown in Figs. 1–4.

Volatile hydrocarbons detected in exhaust gas and inflammable materials were qualitatively analysed to elucidate the origins of the components detected in the blood (Table 2). Benzene, toluene and styrene were commonly generated from inflammable materials. Although both petroleum vapours contained all the target compounds except styrene, in different quantitative profiles, those components with larger molecules were detected at decreased levels. In exhaust gas, petroleum-based combustion gas usually contained low concentrations of hydrocarbons because of a high combustion efficiency [10].

Discussion

The identification and quantitative analysis of some target compounds were performed basically in the same manner as in our previous report [6], although some analytical conditions were modified to obtain a superior chromatographic resolution. Each routine analysis was performed using a standard sample at a concentration of 0.1 nl/sam-

Table 2 Volatile hydrocarbons detected in inflammable materials and exhaust gas

Compound	Wood	Newspapers	Carpets	Curtains	Kerosene	Gasoline	Exhaust gas
benzene	+	+	+	+	+	+	+
toluene	+	+	+	+	+	+	+
ethylbenzene	+	–	+	+	+	+	+
p-xylene	+	–	+	+	+	+	+
o-xylene	+	–	+	+	+	+	+
styrene	+	+	+	+	–	–	–
cumene	–	–	–	–	+	+	+
n-propylbenzene	–	–	–	–	+	+	+
3-ethyltoluene	–	–	–	–	+	+	+
mesitylene	–	–	–	–	+	+	+
2-ethyltoluene	–	–	–	–	+	+	+
pseudocumene	–	–	–	–	+	+	+
1.2.3-trimethylbenzene	–	–	–	–	+	+	+
tert-butylbenzene	–	–	–	–	+	+	+
sec-butylbenzene	–	–	–	–	+	+	+
n-butylbenzene	–	–	–	–	+	+	+
n-pentane	–	–	–	–	–	+	+
n-hexane	–	–	–	–	+	+	+
n-heptane	–	–	–	–	+	+	–
n-octane	–	–	–	–	+	+	–
n-nonane	–	–	–	–	+	–	–
n-decane	–	–	–	–	+	–	–
n-undecance	–	–	–	–	+	–	–
n-dodecane	–	–	–	–	+	–	–

Table 3 Proportional indicators to characterize the inhaled gases in the victim's blood

Species of inhaled gas	Aromatics		Aliphatics		Styrene
	C6–C8	C9	C5–C7	C8–C12	
Construction fire	+	–	–	–	+
Gasoline	+	+	+	–	+
Kerosene	+	+	–	+	+
Automobile exhaust (gasoline fuelled)	+	+	–	–	–

+: at least one compound was detected

ple. The semi-quantitative value of the concentration of each compound was counted as more than 0.01 μg/ml based on the lower quantitative limit of the lower volatiles. Although individual profiles of target compounds in blood are variable, even in the same case group, the species of hydrocarbon detected seem to indicate the origin of the inhaled gases. From the results of the volatile hydrocarbons detected in the exhaust gas and inflammable materials, it is clear that styrene can be generated from many domestic or inflammable materials, but not from petroleum fuels. Therefore, when styrene was found in blood specimens, we suggest that the victim had inhaled material combustion gas from domestic after a fire. In kerosene and gasoline cases, styrene was also found in the blood specimens. We consider that the victims seemed to have inhaled evaporated petroleum components first and then combustion gas of inflammable materials after the fire had been started. The analytical results suggest that a discrimination between gasoline and kerosene can be performed by observing the differences in aliphatic components as described by Kimura et al. [5].

The inhaled gases were characterized as shown in Table 3. According to these results, we concluded the following; when aromatics ranging from benzene to xylene were detected in blood, the victim had inhaled some combustion gas in a construction fire. When both aromatics (C_6–C_9) and aliphatics were detected in blood, the victims had inhaled some petroleum fuel. The cases when aliphatics ranging from *n*-pentane to *n*-heptane were detected in the victims' blood were identified as gasoline vapour inhalation. The cases when aliphatics ranging from n-octane to n-dodecane were detected in the victims' blood were identified as kerosene vapour inhalation. The cases were aromatics ranging from benzene to C_9-aromatics without aliphatics were identified as exhaust gas inhalation. Styrene was detected in those victims who had inhaled the three kinds of fire combustion gases other than exhaust gas. Styrene was also found as a characteristic compound in fire combustion gas. It is clear that by detecting styrene in a victim's blood indicates fire-combustion gas inhalation. Each category of hydrocarbon profiles detected in a victim's blood differed as shown in Table 1. For this analysis, we used a conventional headspace method for sampling, but this method is insufficiently sensitive for less volatile compounds, so that improvements are required to perform a more sensitive analysis. Recently, a solid-phase micro-extraction (SPME) method has been developed which increases the volatility [11]. Headspace

SPME could provide highly sensitive results of a blood analysis, although the cut-off levels must be considered because petroleum-related compounds may be present in the atmosphere. For practical routine purposes, this method is sufficiently sensitive to demonstrate fire-related substances in the blood of a victim.

In conclusion combustion-/petroleum-related substances were detected in the blood samples of 47 victims by conventional headspace GC/MS. The results indicated four typical profiles of hydrocarbons depending on the case situations. Some indicators which characterize each situation are listed as follows: in construction fires, styrene as well as relatively high levels of benzene and toluene; in kerosene cases, *n*-nonane and *n*-decane as well as petroleum-related aromatics; in gasoline cases, *n*-hexane and *n*-heptane as well as petroleum-related aromatics and in exhaust gas cases, no special indicators were detected other than petroleum-related aromatics. When only a carboxyhemoglobin examination is performed in a carbon monoxide intoxication case, the cause of death can be determined but not the cause of the incident. We believe this analysis should be utilized to discriminate between the case situations related to carbon monoxide intoxication.

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