Simple analysis of naphthalene in human whole blood and urine by headspace capillary gas chromatography with large volume injection

SHORT COMMUNICATION

Simple analysis of naphthalene in human whole blood and urine by headspace capillary gas chromatography with large volume injection

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Received: 10 April 2009/Accepted: 24 April 2009

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Abstract Naphthalene is still being sold as a moth repellant; there is a possibility that accidental ingestion of such a product by a small infant causes serious poisoning. In this communication, a very simple method for analysis of naphthalene in human whole blood and urine by headspace gas chromatography (GC) has been presented. It neither needs solid-phase microextraction nor cryogenic trapping devices, but needs only a conventional capillary GC instrument with flame ionization detection. The advantage of the method is that as large as 5 ml of headspace vapor can be injected into a GC instrument in the splitless mode for sensitive detection. After heating a diluted whole blood or urine sample containing naphthalene and 1-methylnaphthalene (internal standard, IS) in a 7.0 ml vial at 80℃ for 30 min, 5 ml of the headspace vapor was drawn with a glass syringe and injected into the gas chromatograph. Before injection, the column was set at 50℃ to trap the analytes, and then the oven temperature was programmed up to 300℃. Sharp peaks were obtained for both analyte and IS, and only a few impurity peaks, which did not interfered with the test peaks, appeared especially for whole blood samples. The detection limits (signal-to-noise ratio ≧ 3) were about 0.05 and 0.01 μg/ml for whole blood and urine, respectively. Precision and linearity were also examined to confirm the reliability. Such a simple headspace GC technique with large volume injection can be probably applied also to other low-volatile compounds in biological matrices, and will be useful in forensic toxicological analysis.

Keywords Naphthalene・1-Methylnaphtalene・Gas chromatography・Headspace samplimg ・Large volume injection・Polycyclic aromatic hydrocarbon

Introducution

Naphthalene (naphthalin) is still being used as a moth repellant (insecticide); it is sold at supermarkets and local general stores. Because one package of the product usually contains as much as 1.8-5 g of naphthalene, there is a possibility of accidental ingestion of the compound by a small infant, resulting in serious poisoning and/or death [1,2].

Although many reports have been reported on analysis of polycyclic aromatic hydrocarbons including naphthalene in environmental samples [3-7], in human urine [8-10] and in human plasma [4,11] by gas chromatography (GC) or GC-mass spectrometry (MS), no reports are available on their analysis for human whole blood sample.

In this communication, we report a very simple method for analysis of naphthalene in human whole blood and urine by headspace capillary GC, which enables large volume injection of headspace vapor for sensitive detection without any special device.

Materials and methods

Materials

Naphthalene and 1-methylnaphthalene were purchased from Wako (Osaka, Japan); an Rtx-1 capillary column (30 m×0.32 mm i.d., film thickness 0.25 μm coated with 100% dimethylpolysiloxane stationary phase) from Restek (Bellefonte, PA, USA). Whole blood and urine samples were obtained from healthy volunteers. Other common chemicals used were of the highest purity commercially available.

Procedure

To a 7 ml vial, were added 0.5ml of whole blood or urine, 0.5 ml of distilled water, 10 μl methanolic solution containing various amounts of naphthalene and 10 μl methanolic solution containing 2.5 μg 1-methylnaphthalene (internal standard, IS). The vial was sealed with a silicone-septum cap and vertex-mixed for 10 s. After heating the vial at 80℃ for 30 min, 5-ml headspace (HS) vapor was drawn with a gas-tight syringe (10-ml volume with a 23 G needle), which had been preheated by placing it on the 80℃ heater, and injected into the GC inlet port in the splitless mode at an oven temperature of 50℃.

GC conditions

GC analysis was carried out on an HP 6890 Series gas chromatograph equipped with flame-ionization detection (FID) and with a cryogenic oven temperature device (Agilent, Palo Alto, CA, USA). The conditions were: column temperature, 50 to 300℃ (1-min hold at 50℃, 10℃/min from 50 to 200℃, 20℃/min from 200℃ to 300℃); injector and detector temperature, 250℃; helium flow rate, 3 ml/min. The 5-ml HS vapor was injected in the splitless mode, and the splitter was opened 1 min after completion of the injection. For GC quantitation, the peak areas of each compound were used.

Results

Optimization of conditions

Various conditions were tested for HS extraction of naphthalene and IS in whole blood and urine. The vials were heated at 50, 60, 80 and 90℃ for 15, 20 and 30 min; it was found the maximum extraction into the HS was attained for incubation at 80℃ for 30 min.

For analysis of whole blood, dilution with water was essential; 1-4 fold dilution with water was tested. Because the increase in extraction efficiency was not proportional to the dilution rate, we chose 2-fold dilution by mixing 0.5 ml whole blood with 0.5 ml water. Since the salting-out effect is often observed for HS extraction, we tried to add excess amounts of NaCl and Na_2CO_3, but neither of them did not show positive effects; thus we did not add any salt to the aqueous phase in the HS vial.

Various initial oven temperatures (10-100℃) were tested for trapping the vapor of naphthalene and IS. At temperatures at 10 and 30℃, the cryogenic oven device [12] had to be used. As shown in Fig. 1, at 10 and 30℃ of the initial oven temperature, each peak of naphthalene and IS appeared as a split shape. At 50℃, they appeared as higher, single and sharp peaks. At 80-100℃, the peak height of each peak became lower and broader according to the increase in the initial oven temperature. Therefore, we chose 50℃ as the initial oven temperature for trapping the target compounds in the HS vapor; this temperature was advantageous, because the cryogenic oven temperature device was not necessary.

Reliability of the method

Figure 2 shows gas chromatograms for naphthalene and IS spiked into human whole blood or urine (0.5 μg/ml each). Both naphthalene and IS appeared as excellent sharp peaks for both whole blood and urine; the retention times of naphthalene and IS were 8.6 and 10.4 min, respectively. There were no impurity peaks appearing at the same retention times as those of the test peaks (data not shown). There appeared some impurity peaks for whole blood, but no impurity peak appeared for urine.

The validation data are summarized in Table 1. Good linearity was found for naphthalene in the concentration range of 0.05-0.5 μg/ml for both whole blood and urine. The percent coefficients of variation showing precision were not greater than 9.70%. The extraction efficiencies were much lower for whole blood than for urine. The detection limit (signal-to-noise ratio≧3) was about 0.05 and 0.01 μg/ml for whole blood and urine, respectively.

Discussion

HS-GC or HS-GC-MS is widely used for analysis of volatile organic compounds and/or polycyclic aromatic hydrocarbons, because HS extraction gives very low background and much less impurity peaks with a very simple procedure. The only problem of the HS method is that relatively small volumes (usually less than 1.0 ml) of HS vapor should be injected into a GC port, when a middle-bore capillary column is used in the splitless mode. To collect target compound(s) in larger volumes of gas samples, various devices have been used, such as purge-and-trap [3], cryofocusing [13] and solid-phase microextraction (SPME) [8-10]. Recently, the

HS-SPME method seems to be gaining popularity to analyze volatile organic compounds [8-10].

About fifteen years ago, a microcomputer controlling cryogenic oven temperature below 0℃ has become available for modern types of GC instruments. It was originally designed for rapid cooling of an oven to reduce analysis time. We have used it to trap volatile organic compound(s) in gas samples, and named it "cryogenic oven trapping (COT)" [14]. This method has been applied to analyses of many volatile compounds as summarized in the previous report [12]. In COT, a vapor volume as large as 5 ml or more can be injected and introduced into GC column without loss of analytes; this results in sensitivity 10-50-fold higher than that of conventional splitless wide-bore capillary GC or split middle-bore capillary GC. COT also gives very sharp peaks for test compounds, probably because volatile organic compounds are trapped, at a cryogenic oven temperature, in a quite narrow zone at the head of a capillary column. Thus, the sensitivity of this method is comparable with that achieved by use of electron-capture detection or MS, and much higher than that by HS-SPME-GC [12].

During our studies on the COT method, we have realized that there are "gray-zone compounds", which are suitable for HS extraction, and can be trapped in a capillary GC column at 50-60℃ without using the COT device. The first example was HS-GC determination of amphetamines after in-matrix derivatization with heptafluoro-*n*-butyryl chloride coupled with large volume injection [15], which neither needed COT nor SPME, but only a conventional capillary GC instrument with FID. The present study on naphthalene analysis is the second trial on this line of experiments. There seem to be many other compounds that can be analyzed by usual capillary GC with large volume injection of HS vapor in the splitless mode.

We have also tried the large volume injection of HS vapor for GC-MS analysis. At the beginning, the MS instrument stopped automatically, because of the rapid decrease in vacuum of the ionization chamber. Secondly, the vacuum pump power was enhanced to maintain excellent vacuum of the ionization chamber, but the satisfactory linearity in analysis could not be obtained. Thus we have constructed a post-column switching device controlled by a microcomputer, which can eliminate the vapor gas before introduction of target compound(s) to MS (unpublished results). Such kinds of studied are now in progress in our laboratories.

In conclusion, we have presented very simple method for analysis of naphthalene in whole blood and urine by HS capillary GC with large volume injection without any special device. The present method is fully usable for analysis of naphthalene in whole blood and urine samples in actual forensic cases, because the blood concentration in a fatal naphthalene poisoning case was reported to be 0.55 μg/ml [2].

References

1. Watanabe K (2005) Naphthalene. In: Suzuki O, Watanabe K (eds) Drugs and poisons in humans: a handbook of practical analysis. Springer, Berlin Heidelberg New York, pp 437-441
2. Ijiri I, Shimosato K, Ohmae M, Tomita M (1987) A case report of death from naphthalene poisoning. Jpn J Legal Med 41:52-55
3. Hino T, Nakanishi S, Hobo T (1996) Development of a whole headspace injection method for the determination of volatile organic compounds in water. J Chromatogr A 746:83-90.
4. Singh AK, Spassova D, White T (1998) Quantitative analysis of polychlorinated biphenyls, organochlorine insecticides, polycyclic aromatic hydrocarbons, polychlorinated hydrocarbons and polynitrohydrocarbons in spiked samples of soil, water and plasma by selected-ion monitoring gas chromatography-mass spectrometry. J Chromatogr B 706: 231-244
5. Dugay A, Herrenknecht C, Czok M, Guyon F, Pages N (2002) New procedure for selective extraction of polycyclic aromatic hydrocarbons in plants for gas charomatographic-mass spectrometric analysis. J Chromatogr A 958: 1-7
6. Sakata SK, Taniguchi S, Rodrigues DF, Urano ME, Wandermüren MN, Pellizari VH, Comasseto JV (2004) Development of a static headspace gas chromatographic/mass spectrometric method to analyze the level of volatile contaminants biodegradation. J Chromatogr A 1048:67-71
7. Safarova VI, Sapelnikova SV, Djazhenko EV, Teplova GI, Shajdulina GF, Kudasheva FK (2004) Gas chromatography-mass spectrometry with headspace for the analysis of volatile organic compounds in waste water. J Chromatogr B 800:325-330

8. Waidyanatha S, Zheng Y, Rappaport SM (2003) Determination of polycyclic aromatic hydrocarbons in urine of coke oven workers by headspace solid phase microextraction and gas chromatography-mass spectrometry. Chem-Biol Interact 145:165-174
9. Campo L, Addario L, Buratti M, Scibetta L, Longhi O, Valla C, Cirla PE, Martinotti I, Foà V, Fustinoni S (2006) Biological monitoring of exposure to polycyclic aromatic hydrocarbons by determination of unmetabolized compounds in urine. Toxicol Lett 162:132-138
10. Campo L, Mercadante R, Rossella F, Fustinoni S (2009) Quantification of 13 priority polycyclic aromatic hydrocarbons in human urine by headspace solid-phase microextraction gas chromatography-isotope dilution mass spectrometry. Anal Chim Acta 631:196-205
11. Weistrand C, Jakobsson E, Norén K (1995) Liquid-gel partitioning using Lipidex in the determination of polychlorinated biphenyls, naphthalenes, dibenzo-*p*-dioxins and dibenzofurans in blood plasma. J Chromatogr B 669:207-217
12. Watanabe-Suzuki K, Ishii A, Suzuki O (2002) Cryogenic oven-trapping gas chromatography for analysis of volatile organic compounds in body fluids. Anal Bioanal Chem 273:75-80
13. Durury JA, Nycyk JA, Cooke RWI (1997) Pentane measurement in ventilated infants using a commercially available system. Free Radical Biol Med 22:895-900
14. Watanabe K, Seno H, Ishii A, Suzuki O (1997) Capillary gas chromatography with cryogenic oven temperature for headspace samples: analysis of

chloroform or methylene chloride in whole blood. Anal Chem 69:5178-5181

15. Watanabe K, Okamoto N, Yamagishi I, Nozawa H, Ishii A, Suzuki O (2003) Simple analysis of amphetamines in human whole blood and urine by headspace capillary gas chromatography with large volume injection. Chromatographia 58:455-458

Legends for Figures

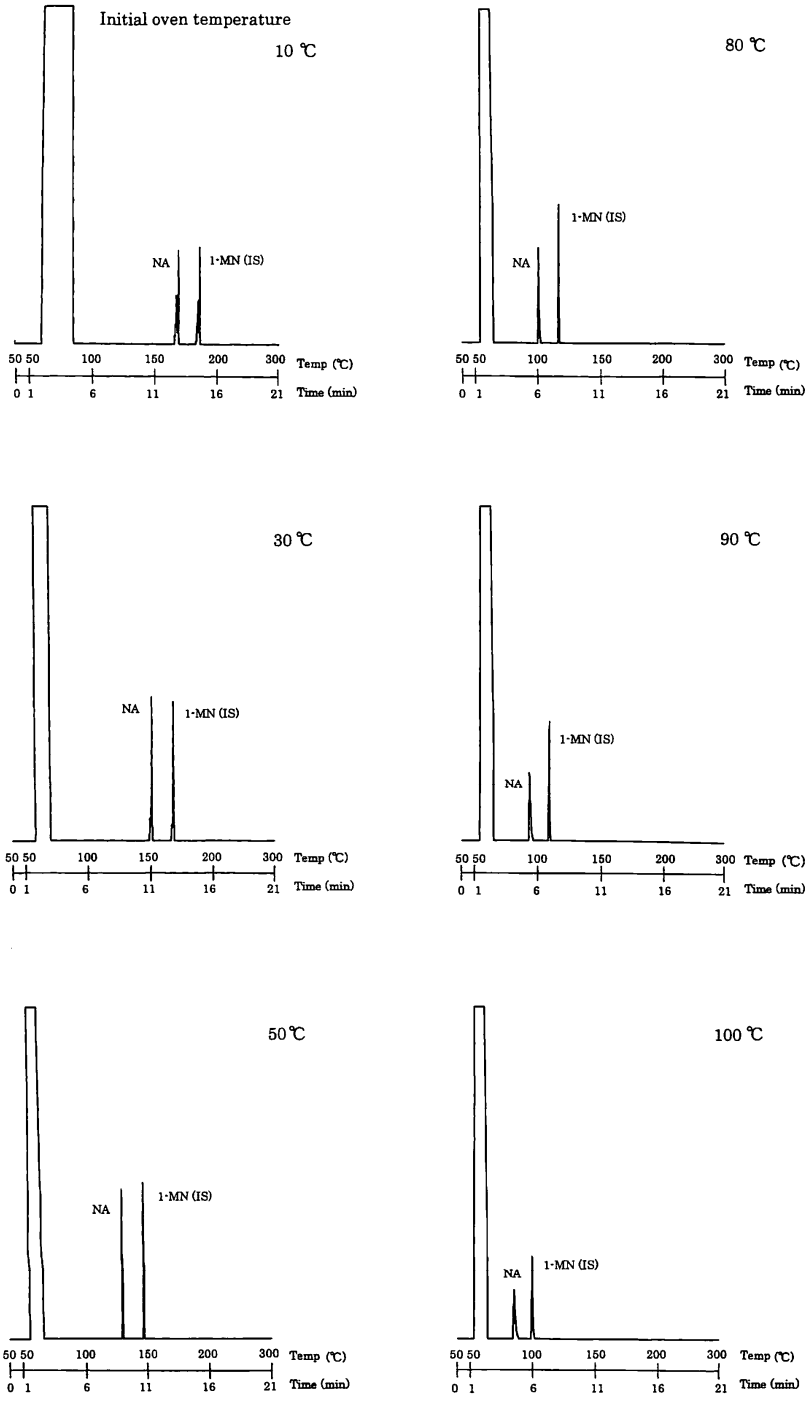

Fig. 1 Capillary GC chromatograms for naphthalene and internal standard 1-methylnaphthalene at different initial oven temperatures. Naphthalene and 1-methylnaphthalene (1 μ g each) dissolved in methanol was injected directly. The large front peaks are due to methanol used as solvent. NA:naphthalene ; 1-MN: 1-methylnaphthalene

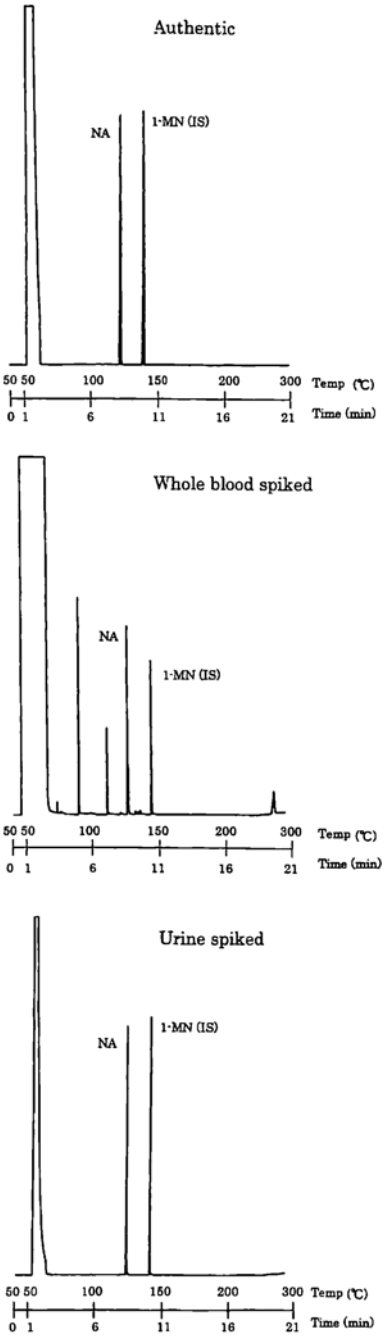

Fig. 2 Headspace capillary GC for naphthalene and internal standard 1-methylnaphthalene spiked into whole blood or urine. A 5.0-ml volume of the headspace vapor was injected into GC at 50℃ of the initial oven temperature. The amount of naphthalene and 1-methylnaphthalene spiked was 2.5 μg each in 0.5 ml whole blood or urine (5.0 μg/ml). The large front peaks are mainly due to methanol used as vehicle upon spiking

Table 1 Validation data for naphthalene spiked into human whole blood and urine obtained by the present method

Matrix	Regression equation[a] (range; correlation coefficient)	Spiked concentration (μg / ml)	Precision (%) (n=10)		Extraction[b] efficiency (%) (n=10)
			Intraday	Interday	
Whole blood	$y = 0.359\ x + 0.0316$	0.5	4.04	9.70	2.82±0.009
	(0.05−5.0 μg/ml; $r = 0.999$)	5.0	2.86	2.99	1.86±0.002
Urine	$y = 0.240\ x + 0.0122$	0.5	2.24	1.97	13.7±0.03
	(0.05−5.0 μg/ml; $r = 0.999$)	5.0	1.50	4.30	10.6±0.01

[a] The data were subjected to linear regression analysis of peak area ratios (y) of naphthalene to IS (1 μg per vial) against the spiked naphthalene concentrations (x) with 12 plots each

[b] The data were expressed as means±standard deviation; they were calculated by comparing peak areas obtained from the headspace gas of the spiked whole blood or urine samples with those obtained from non-extraced authentic naphthalene methanolic solution directly injected into the GC port