ELSEVIER

Journal of Chromatography B, 718 (1998) 285–289

JOURNAL OF CHROMATOGRAPHY B

Short communication

Sensitive determination of xylenes in whole blood by capillary gas chromatography with cryogenic trapping

H. Hattori[a,*], M. Iwai[a], S. Kurono[a], T. Yamada[a], K. Watanabe-Suzuki[b], A. Ishii[b], H. Seno[b], O. Suzuki[b]

[a]*Department of Legal Medicine, Aichi Medical University, Nagakute-cho, Aichi-gun, Aichi 480-1195, Japan*
[b]*Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-3192, Japan*

Received 24 February 1998; received in revised form 18 May 1998; accepted 19 May 1998

Abstract

A new and sensitive method for measurement of *o*-, *m*- and *p*-xylenes in human whole blood by capillary gas chromatography (GC) with cryogenic trapping is presented. After heating 0.5 ml of whole blood and 0.5 ml of distilled water containing the xylenes and aniline (internal standard, I.S.) in a 4.0-ml vial at 100°C for 30 min, 2 ml of the headspace vapor was drawn into a glass syringe. All vapor was introduced through the GC port into an AT-Wax middle-bore capillary column in the splitless mode at an oven temperature of 5°C to trap the entire analytes, and the oven temperature was then programmed up to 180°C. The present conditions gave sharp peaks for xylenes and aniline (I.S.), and low background noises for whole blood samples; the peaks of *p*- and *m*-xylenes showed about 90% separation with the AT-Wax column. As much as 41.0–46.3% of xylenes, which had been spiked to whole blood could be recovered. The calibration curves showed linearity in the range of 0.1–0.5 μg/0.5 ml of whole blood. The detection limit was estimated to be about 10 ng/0.5 ml. The coefficients of intra-day and inter-day variations for xylenes were not greater than 9.38%. The data for actual detection of xylenes in post-mortem blood of self-ignition suicide cases by the present method were also presented.

Keywords: Cryogenic trapping; Xylenes; Aniline

1. Introduction

Xylenes are widely used as solvents in industries and laboratories. Their large scale production may lead to environmental pollution or damages to human health. Xylenes are also contained in gasoline and kerosene [1], and are sometimes required to be detected for forensic identification of accelerants in arson, fire or self-ignition cases [2,3]. A suicidal case due to inhalation of gasoline vapor was also reported [4]. Xylenes are usually analyzed by capillary gas chromatography (GC) [5–7] and GC–mass spectrometry (MS) [2–4,8–10].

Purge-and-trap sample concentration seems to be the most sensitive technique to extract volatile organic compounds, because a large volume of water or solid sample can be used for analysis [5,8]. However, this technique is not suitable for biological samples, such as blood and tissue homogenates, as it causes serious bubbling. Recently, a microcomputer-

*Corresponding author.

0378-4347/98/$ – see front matter
PII: S0378-4347(98)00254-0

controlled device for cooling of oven temperature down to or below 0°C has become available for new types of GC instruments. It had been originally designed for rapid cooling of oven temperature to reduce analysis time. In our recent report [11], we presented a new GC method for sensitive determination of chloroform and methylene chloride in human whole blood by trapping them at low oven temperature for headspace samples using the above device; this method allowed us to inject large volumes of headspace vapor without any loss, resulting in much higher sensitivity. In this study, we have extended this line of experiments to xylene isomers in human whole blood and have optimized conditions to establish their assay method.

2. Experimental

2.1. Materials

Xylene isomers (*o*-, *m*- and *p*-) were purchased from Wako, Osaka, Japan; human whole blood used for spiked tests was obtained from healthy subjects; post-mortem whole blood was also obtained at forensic autopsies or medical examinations from male and female victims (46–73 years of age), who died of fires by self-ignition using gasoline or kerosene.

2.2. Procedure

Stock solutions of xylene isomers (25 μg/ml) and I.S. (10 mg/ml) were prepared by dissolving them in methanol. To a 4-ml screw vial containing 0.5 ml of whole blood and 0.5 ml of distilled water, was added 30 μl (20 and 10 μl, respectively) of methanolic solution containing 500 ng or less of xylenes and 100 μg of I.S. (aniline) for spiked test samples; for actual determinations of xylenes in forensic cases, only methanolic solution of I.S. was added to a vial containing 0.5 ml each of whole blood and water. The vial was rapidly sealed with a PTFE/silicone-septum cap and heated at 100°C on an aluminum block heater. After 30 min of heating, the needle of a Hamilton gastight syringe (5-ml volume) (Hamilton, Reno, NV, USA) was passed through the septum. A 2.0- or 2.5-ml volume of the headspace vapor was drawn into the syringe and injected into the GC port in the splitless mode at an oven temperature of 5°C.

2.3. GC conditions

GC analyses were carried out on an HP 6890 Series gas chromatograph equipped with flame ionization detection (FID) and with a cryogenic oven temperature device (Hewlett-Packard, Palo Alto, CA, USA). An electrically operated solenoid valve introduces liquid carbon dioxide at a rate appropriate to cool the oven to desired temperatures. The GC column used was an AT-Wax fused-silica capillary column (30 m×0.32 mm I.D., film thickness 0.5 μm, Alltech, Deerfield, IL, USA). The GC conditions were: column temperature 5°C (1 min hold) to 180°C (4 min hold) at 15°C/min; injection temperature 180°C; detection temperature 250°C; and helium flow-rate 2.2 ml/min. The vapor samples were injected in the splitless mode at an oven temperature of 5°C and the splitter was opened 0.5 min after completion of the injection.

3. Results and discussion

3.1. Analytical conditions

Various conditions for the headspace extraction of xylene isomers from whole blood were tested. We heated the vials at 60–120°C for 15–90 min; it was found that optimal extraction into the headspace was attained at 30 min and 100°C.

We tested various initial oven temperatures for trapping and separation of xylene vapor as shown in Fig. 1. The xylenes and I.S. could be completely trapped even at 20°C. However, the separation of *p*- and *m*-xylenes was not good at the temperature; it became better down to 5°C. At an oven temperature of 5°C, about 90% separation between *p*- and *m*-xylenes could be achieved; no further improvement on separation between them was observed down to −10°C; thus we adopted 5°C as the initial oven temperature.

In previous reports [2–4,7,9,10], *p*- and *m*-xylenes appeared as a single peak with non-polar and slightly polar polysiloxane capillary columns; even with polyethylene glycol or special capillary columns,

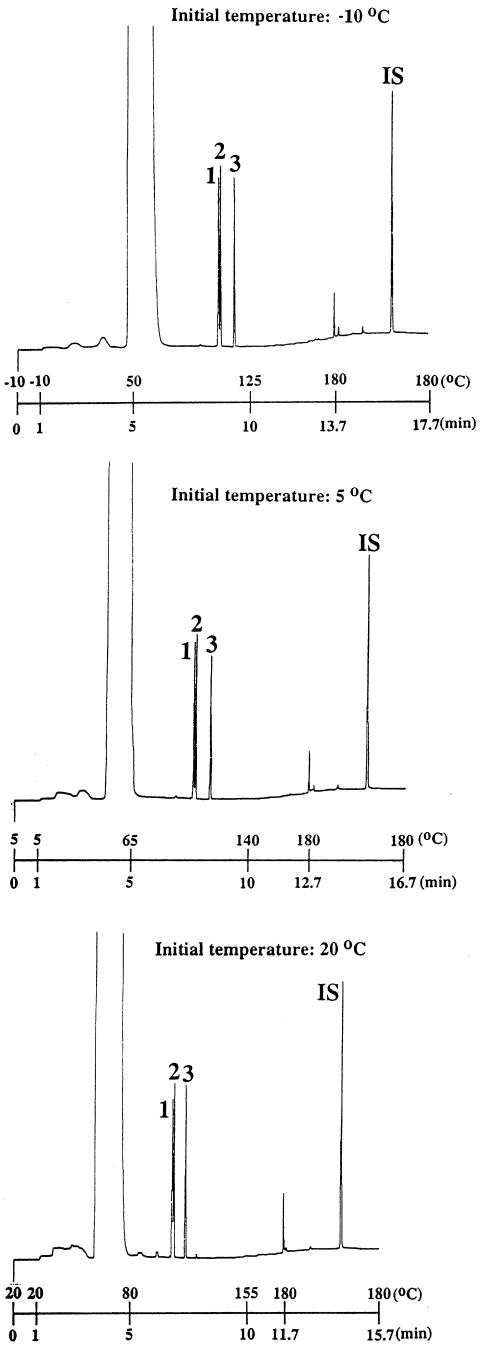

Fig. 1. Headspace capillary GC for xylene isomers and aniline (I.S.) as a function of various initial oven temperatures. Keys:1=*p*-xylene; 2=*m*-xylene; 3=*o*-xylene; I.S.=internal standard aniline. The amounts of the xylenes and I.S. added to 0.5 ml of whole blood were 0.2 and 100 μg, respectively.

complete separation of the isomers could not be attained [5,6,8]. We have tested various capillary columns including Carbowax and AT-Wax; the latter column showed the best separation of *p*- and *m*-xylenes. Thus, we used the AT-Wax (polyethylene glycol) capillary column in the present study. According to the manufacturer's note, the temperature range should be 40–280°C. In our experience, however, this column was found to be resistant to low temperature at 5°C; it could be used for at least three months with good reproducibility.

We have tested various compounds for I.S., such as ethylbenzene, pentane, butyl acetate and aniline. Some of these compounds are listed as environmental pollutants by the US Environmental Protection Agency (EPA), and are possible for concomitance with xylenes at analyses. We have selected aniline as I.S. in view of retention time, stability and its absence in gasoline, kerosene and thinner solvents, although aniline is much less volatile than the xylenes.

3.2. Reliability of the method

Fig. 2 shows gas chromatograms for headspace extracts from 0.5 ml of human whole blood in the presence (0.2 μg each for xylenes and 100 μg of I.S. aniline) and absence of the compounds. The backgrounds gave a few and small impurity peaks; no interfering peaks appeared around the test peaks.

The net recovery of xylene isomers and I.S. was determined by adding 0.2 μg each of xylenes and 100 μg aniline to 0.5 ml of whole blood. The peak area of each compound spiked to blood (after cryogenic trapping of headspace prior to GC analysis) was compared with the peak area obtained by direct GC injection of each authentic compound. The recovery was 46.3±3.9%, $n=5$ for *o*-xylene, 44.0±3.7%, $n=5$ for *m*-xylene, 41.0±3.0%, $n=5$ for *p*-xylene and 0.12±0.01%, $n=5$ for I.S. aniline. In spite of the low recovery of I.S., it gave stable and sharp peaks (Figs. 1 and 2). Since 2.0 ml of headspace vapor was drawn from a 4.0-ml vial, the above data mean that the recovery of xylenes is excellent.

Calibration curves for xylene isomers in human whole blood were drawn by plotting five concentrations against I.S. They were liner in the range of

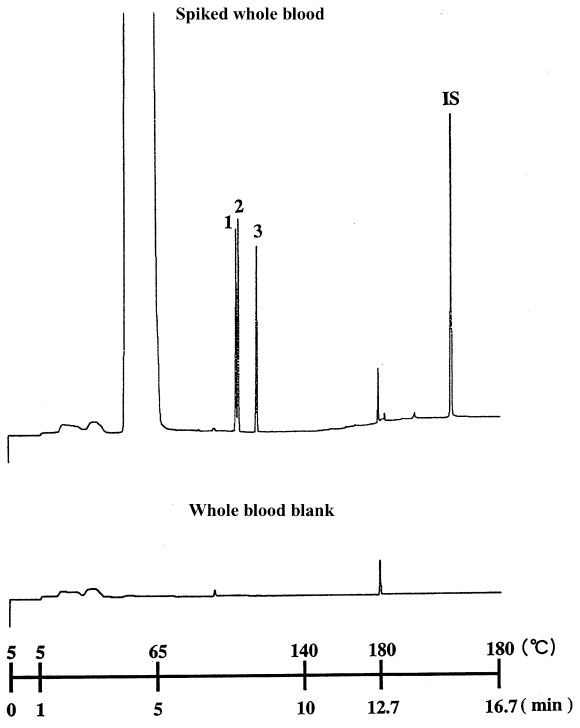

Fig. 2. Capillary GC chromatograms with cryogenic trapping at 5°C of oven temperature for headspace extracts from whole blood in the presence (upper panel) and absence (lower panel) of 0.2 μg each of xylenes and 100 μg of I.S. in 0.5 ml. For key numbers, see Fig. 1. The big front peak of the upper panel is due to methanol used as vehicle for dissolving the authentic xylenes and I.S.

0.1–0.5 μg/0.5 ml blood. The equations and r values for the curves were: $y=4.3x+0.048$ and $r=0.998$ for *o*-xylene [y, peak area ratio of compound to I.S.; x, spiking concentrations (μg) of xylenes per 0.5 ml blood]; $y=4.6x+0.095$ and $r=0.998$ for *m*-xylene; and $y=4.4x+0.058$ and $r=0.998$ for *p*-xylene.

The detection limit (signal-to-noise ratio=3) for three xylene isomers was estimated to be about 10 ng/0.5 ml of blood. Our GC method with FID gave sensitivity comparable to that by GC–MS [2], because of the cryogenic trapping of entire headspace vapor without any loss. Upon coupling our cryogenic method with selected ion monitoring in MS, sensitivity and selectivity would be improved.

To check reproducibility of the present method, we added 0.2 μg of each compound to 0.5 ml of whole blood and determined each concentration. The coefficients of intra-day variation for *o*-, *m*- and *p*-xylenes were 8.01, 9.29 and 9.38%, respectively ($n=5$ each); those of inter-day variation of the isomers on five consecutive days were 9.34, 8.78 and 8.83%, respectively.

3.3. Actual measurement of xylenes

The present method was actually applied to measurement of xylenes in whole blood samples obtained at forensic autopsies or medical examinations from victims of both sexes, who were estimated to have died of fires by self-ignition using gasoline or kerosene. The chromatograms for the four cases are presented in Fig. 3. For the gasoline cases, the concentrations of *o*-, *m*- and *p*-xylenes were 154, 250 and 59.3 ng/ml for case 1, and 84.7, 98.0 and 46.5 ng/ml for case 2, respectively. For the kerosene cases, only *o*-xylene was measurable; its concentrations were 24.2 ng/ml for case 3 and 66 ng/ml for case 4. These results are in agreement with the fact that gasoline is more enriched in aromatic hydrocarbons than kerosene [1]. The characteristic profile with three peaks due to xylene isomers seems to be good suggestion especially for the presence of gasoline.

In connection with xylenes in gasoline and kerosene, it should be mentioned that aliphatic hydrocarbons with carbon numbers of 5–8 and of 8–9 are also useful for identification of both petroleum fluids. However, quite different types of columns should be used for detection of aliphatic hydrocarbons [12], and their identities should be confirmed by MS connected to GC, because of appearance of many interfering impurities especially in fire cases.

4. Conclusions

To our knowledge, this is the first report dealing with GC with cryogenic trapping for xylenes in biological samples. It is recommended for use in forensic and environmental toxicology, because it is simple and requires no special GC operation, and gives high sensitivity and good separation of the compounds.

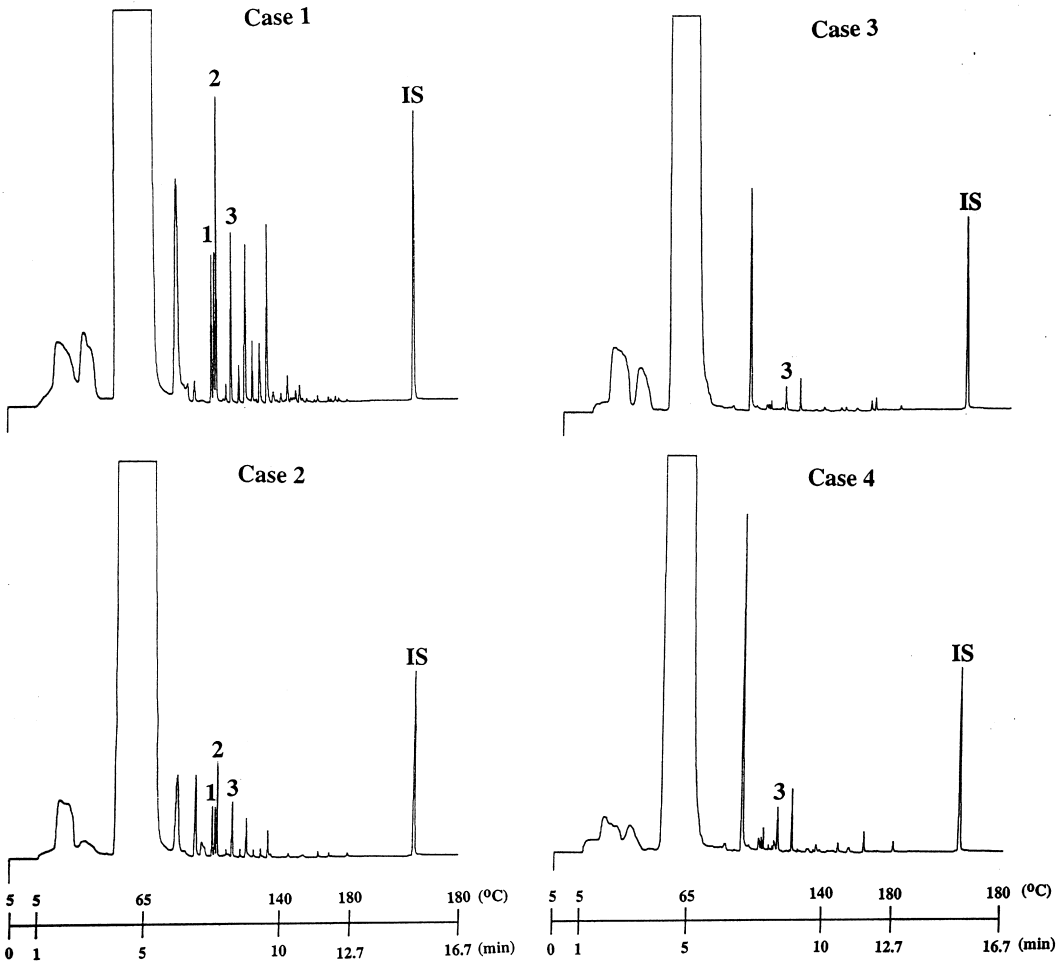

Fig. 3. Capillary GC chromatograms with cryogenic trapping for post-mortem whole blood (0.5 ml) of victims of both sexes, who died of fires by self-ignition using gasoline (cases 1 and 2) and kerosene (cases 3 and 4). Case 1: 47-year-old male; case 2: 59-year-old male; case 3: 73-year-old female; case 4: 46-year-old male. For key numbers, see Fig. 1.

References

[1] W. Bertsch, G. Holzer, in: J. Yinon (Ed.), Forensic Applications of Mass Spectrometry, CRC Press, Boca Raton, FL, 1995, p. 129.
[2] M. Morinaga, K. Hara, M. Kageura, Y. Hieda, M. Takamoto, S. Kashimura, Z. Rechtsmed. 103 (1990) 567.
[3] Y. Iwasaki, M. Yashiki, N. Nagasawa, T. Miyazaki, T. Kojima, Jpn. J. Forensic Toxicol. 13 (1995) 189.
[4] J. Nishigami, T. Ohshima, T. Kondo, M. Ohtsuji, Jpn. J. Forensic Toxicol. 12 (1994) 33.
[5] H. Kessels, W. Hoogerwerf, J. Lips, J. Chromatogr. Sci. 30 (1992) 247.
[6] R. Otson, P. Kumarathasan, Chemosphere 30 (1995) 1109.
[7] J.R. Almirall, J. Bruna, K.G. Furton, Sci. Justice 36 (1996) 283.
[8] R. Otson, C. Chan, Int. J. Environ. Anal. Chem. 30 (1987) 275.
[9] E.D. Pellizzari, L.A. Wallace, S.M. Gordon, J. Expo. Anal. Env. Epid. 2 (1992) 341.
[10] A. Górna-Binkul, R. Keymeulen, H. Van Langenhove, B. Buszewski, J. Chromatogr. A 734 (1996) 297.
[11] K. Watanabe, H. Seno, A. Ishii, O. Suzuki, T. Kumazawa, Anal. Chem. 69 (1997) 5178.
[12] T. Nagata, K. Kimura, in: H. Brandenberger, R.A.A. Maes (Eds.), Analytical Toxicology for Clinical, Forensic and Pharmaceutical Chemists, Walter de Gruyter, Berlin, 1997, p. 177.