SIMPLE CLEAN-UP OF METHAMPHETAMINE AND AMPHETAMINE IN HUMAN URINE BY DIRECT-IMMERSION SOLID PHASE MICRO EXTRACTION (DI-SPME)

Jpn. J. Forensic Toxicol. Vol.14, No.3, 1996

原　　報

SIMPLE CLEAN-UP OF METHAMPHETAMINE AND AMPHETAMINE IN HUMAN URINE BY DIRECT-IMMERSION SOLID PHASE MICRO EXTRACTION (DI-SPME)

Akira ISHII[a]*, Hiroshi SENO[a], Takeshi KUMAZAWA[b], Masanobu NISHIKAWA[a], Kanako WATANABE[c], Hideki HATTORI[c] and Osamu SUZUKI[a]

[a] *Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431−31, Japan*
[b] *Department of Legal Medicine, Showa University School of Medicine, 1−5−8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan*
[c] *Department of Legal Medicine, Aichi Medical University, Nagakute-cho, Aichi 480−11 Japan*

Received July 19, 1996
Accepted August 2, 1996

直浸固相ミクロ抽出 (DI-SPME) 法によるメタンフェタミン及びアンフェタミンのヒト尿からの迅速クリーンアップ

石井　晃[a]*, 妹尾　洋[a], 熊澤武志[b], 西川正信[a], 渡部加奈子[c], 服部秀樹[c], 鈴木　修[a]

[a] 浜松医科大学法医学教室 〒431−31 静岡県浜松市半田町3600
[b] 昭和大学医学部法医学教室　〒142 東京都品川区旗の台１−５−８
[c] 愛知医科大学法医学教室　〒480−11 愛知県愛知郡長久手町大字岩作字雁又21

Summary

An improved method for simple clean-up of methamphetamine and amphetamine in human urine by direct-immersion solid phase microextraction (DI-SPME) with a polydimethylsiloxane-divinylbenzene fiber is presented. Quantitation was made by capillary gas chromatography with nitrogen-phosphorus detection. The recoveries of methamphetamine and amphetamine from human urine were 37.5 and 17.9 %, respectively, which were several times higher than those by the headspace SPME method. Good linearity was obtained in the range of 12.5 to 200 ng / ml urine for both stimulants. Their detection limits were about 10 ng / ml.

Key words: Methamphetamine; Amphetamine; Gas chromatography (GC); Capillary GC; Solid phase micro extraction (SPME); Direct-immersion (DI) method ; Nitrogen-phsophorus detection (NPD)

*Correspondence should be addressed to Akira Ishii.
 0915-9606/96/$06.00

Introduction

Solid phase micro extraction (SPME), developed by Arthur and Pawliszyn [1], has recently been applied for extraction of methamphetamine and amphetamine from human urine [2] and whole blood [3] by Yashiki and his colleagues; they have exclusively used headspace SPME in spite of low recoveries of these stimulants. The direct-immersion (DI)-SPME has been applied for the analyses of caffeine in beverages [4], organic compounds in water [5,6], cocaine in human urine [7] and local anaesthetics in human blood [8]. In this paper, we have tested the DI-SPME method for extraction of methamphetamine and amphetamine from human urine using a new fiber and have got better recoveries.

Experimental

Materials

Methamphetamine-HCl was purchased from Dainippon Pharmaceutical (Osaka). Amphetamine sulfate was kindly donated by Dr. Yoshiko Yamamoto (Department of Legal Medicine, Kyoto University Faculty of Medicine). An SPME device and fibers, coated with 65 μm polydimethylsiloxane-divinylbenzene and with 100 μm polydimethylsiloxane, were purchased from Supelco, Inc. (Bellefonte, PA, USA) and an HP-5 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm) from Hewlett Packard (Wilmington, DE, USA). Other chemicals used were of analytical grade. Urine was obtained from a healthy subject.

SPME procedures

The polydimethylsiloxane-divinylbenzene coated fiber for SPME was pretreated in an injection port of a gas chromatography (GC) instrument at 250 ℃ for 30 min to remove contaminants on the fiber. A 1-ml aliquot of urine containing 100 ng each of methamphetamine-HCl and amphetamine sulfate was mixed with 1 ml of distilled water and 1.0 g Na_2CO_3 in a vial. The vials were sealed with silicone-septum caps. After heating at 65 ℃ for 5 min, the septum piercing needle of an SPME fiber holder was passed through the septum. The SPME fiber was exposed and immersed in the aqueous layer for 30 min at 65℃ with stirring. The fiber was retracted into the needle, pulled out from the vial, and then immediately inserted into the GC port.

The headspace SPME was also made for comparison under the conditions of Yashiki *et al.* [2] with the polydimethylsiloxane fiber.

GC conditions

GC analyses were carried out on a Hewlett Packard 6890 Series II instrument equipped with a nitrogen−phosphorus detection (NPD) system. The GC conditions were: column temperature, 60−280 ℃ (1 min hold at 60 ℃ and 10 ℃ / min); injection and detection temperatures, 260 and 280 ℃, respectively; helium flow rate, 3 ml/min. An SPME fiber was inserted in the splitless mode at a column tempreature of 60 ℃ and the splitter was opened after 1 min.

Results and discussion

Figure 1 shows gas chromatograms of the authentic metamphetamine-HCl and amphetamine sulfate (25 ng each on-column), drugs extracted from human urine by DI-SPME (the present method), drugs extracted by the headspace SPME (Yashiki's method) [2] and their blanks.

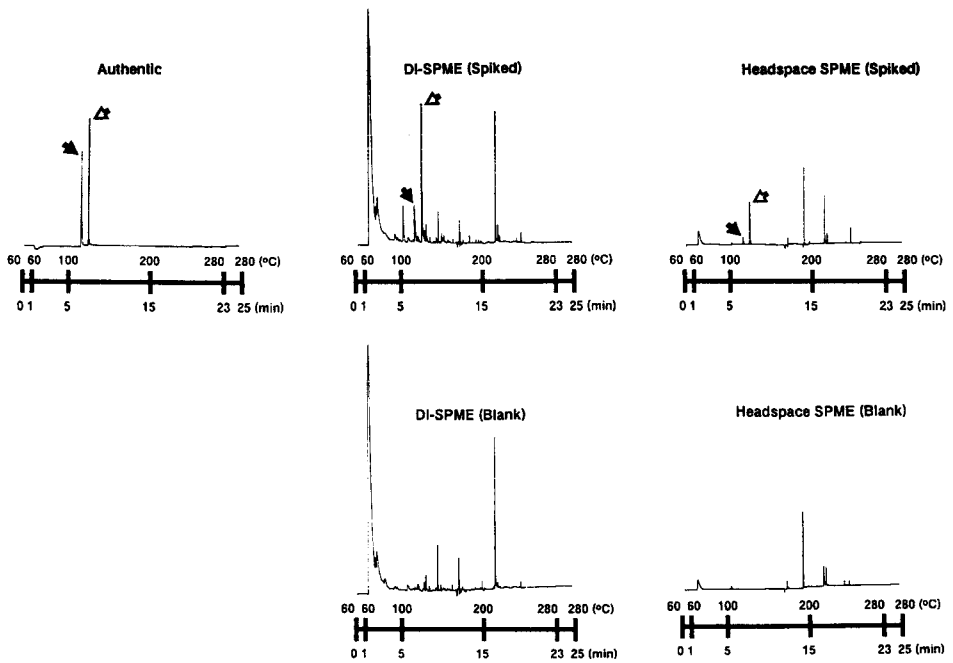

Fig. 1. Capillary GC-NPD for methamphetamine (open arrow) and amphetamine (filled arrow) extracted from human urine by use of the DI-SPME or headspace SPME. The mixture of methamphetamine-HCl and amphetamine sulfate (100 ng each) was added to 1 ml of human urine. The amount of the authentic methamphetamine-HCl and amphetamine sulfate was 25 ng each on column. The SPME fibers, coated with 65 μm polydimethylsiloxane-divinylbenzene and with 100 μm polydimethylsiloxane, were used for DI-SPME and for headspace SPME, respectively.

One hundred nanograms of methamphetamine-HCl and amphetamine sulfate had been added to 1 ml urine. The retention times of methamphetamine and amphetamine were 7.5 and 6.7 min, respectively. As shown in the figure, the amounts of drugs extracted by DI-SPME were several times larger than those extracted by headspace SPME, although more background noises were observed by the DI-SPME.

The recoveries of methamphetamine-HCl and amphetamine sulfate (100 ng each for 1-ml samples) were calculated by comparing the peak areas obtained from DI-SPME extracts of spiked urine samples with those obtained from the non-extracted authentic drugs (25 ng each on column) dissolved in methanol; the values for methamphetamine and amphetamine were 37.5 ± 5.0 and 17.9 ± 2.2 % (mean $\pm$ SD, $n = 5$), respectively.

The calibration curve for methamphetamine-HCl and amphetamine sulfate showed good linearities in the range of 12.5 to 200 ng/ml urine; their detection limits were as low as about 10 ng/ml without any derivatization.

In the present study, we have got satisfactory results for extraction of methamphetamine and amphetamine from human urine by DI-SPME. The DI-SPME method is recommendable for extracting stimulants from biological samples, because of its simplicity, rapidness and higher recovery. We are trying to extract them from human whole blood by DI-SPME.

Acknowledgement

This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture (No. 08770308).

References

1) Arthur, C. L. and Pawliszyn, J.: Solid phase microextraction with thermal disorbtion using fused silica optical fibers. Anal Chem, **62**, 2145−2148 (1990).

2) Yashiki, M., Kojima, T., Miyazaki, T., Nagasawa, N., Iwasaki, Y. and Hara, K.: Detection of amphetamines in urine using headspace-solid phase microextraction and chemical ionization selected ion minitoring. Forensic Sci Int, **76**, 169−177 (1995).

3) Nagasawa, N., Yashiki, M., Iwasaki, Y., Hara, K. and Kojima, T.: Rapid analysis of amphetamines in blood using head space-solid phase microextraction and selected ion monitoring. Forensic Sci Int, **78**, 95−102 (1996).

4) Hawthorne, S. B., Miller, D. J., Pawliszyn, J. and Arthur, C. L.: Solventless determination of caffeine in beverages using solid-phase microextraction with fused-silica fibers. J Chromatogr, **603**, 185−191 (1992).

5) Potter, D. W. and Pawliszyn, J.: Detection of substituted benzenes in water at the pg/ml level

using solid-phase microextraction and gas chromatography-ion trap mass spectrometry. J Chromatogr, **625**, 247−255 (1992).

6) Louch, D., Motlagh, S. and Pawliszyn, J.: Dynamics of organic compound extraction from water using liquid-coated fused silica fibers. Anal Chem, **64**, 1187−1199 (1992).

7) Kumazawa, T., Watanabe, K., Sato, K., Seno, H., Ishii, A. and Suzuki, O.: Detection of cocaine in human urine by solid-phase microextraction and capillary gas chromatography with nitrogen-phosphorus detection. Jpn J Forensic Toxicol, **15**, 207−210 (1995).

8) Kumazawa, T., Sato, K., Seno, H., Ishii, A. and Suzuki, O.: Extraction of local anaesthetics in human blood by direct immersion-solid phase microextraction (SPME). Chromatographia, in press (1996).