SIMPLE EXTRACTION OF 1-PHENYLETHYLAMINE IN HUMAN URINE BY HEADSPACE SOLID PHASE MICROEXTRACTION (SPME)

Jpn. J. Forensic Toxicol. Vol.15, No.3, 1997

原　　報

SIMPLE EXTRACTION OF 1-PHENYLETHYLAMINE IN HUMAN URINE BY HEADSPACE SOLID PHASE MICROEXTRACTION (SPME)

Akira ISHII[a]*, Hiroshi SENO[a], Fuyu GUAN[a], Kanako WATANABE[a], Takeshi KUMAZAWA[b], 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-gun, Aichi 480−11, Japan*

Received June 9, 1997
Accepted June 23, 1997

ヘッドスペース固相ミクロ抽出 (SPME) 法による1-フェニルエチルアミンのヒト尿からの簡便抽出法

石井　晃[a]*, 妹尾　洋[a], 関　福玉[a], 渡部加奈子[a], 熊沢武志[b], 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21

Summary

A method for simple clean-up of 1-phenylethylamine (1-PEA, α-phenylethylamine, α-methylbenzylamine), a possible abused drug, in human urine by headspace solid phase microextraction (SPME) with a polydimethylsiloxane-divinylbenzene fiber, is presented. Quantitation was made by capillary gas chromatography with nitrogen-phosphorus detection and β-methylphenethylamine was used as internal standard (IS). The recoveries of 1-PEA and IS were 4.75 to 8.93% and 13.4%, respectively. The calibration curve for 1-PEA was linear in the range of 25−500 ng/ml urine. The detection limit was about 20 ng/ml urine.

Key words: 1-Phenylethylamine; α-Phenylethylamine; α-Methylbenzylamine; Gas chromatography (GC); Capillary GC; Solid phase microextraction (SPME); Nitrogen-phosphorus detection (NPD)

*Correspondence should be addressed to Akira Ishii.

Introduction

1-Phenylethylamine (1-PEA, α-phenylethylamine or α-methylbenzylamine) is a two-carbon homologue of amphetamine. Recently, large amounts of 1-PEA have been seized in Europe [1−4]. The seized powder contained 1-PEA, amphetamine and caffeine [1−3]. It is yet unclear whether the 1-PEA powder was deliberately produced as a new designer drug ; 1-PEA has some stimulating action on the central nervous system like amphetamines [5]. Solid phase microextraction (SPME) [6] has been applied to amphetamines in human urine [7, 8] or human blood [9]. In the present study, we have established a simple clean-up by headspace SPME for 1-PEA in human urine.

Experimental

Materials

1-PEA and β-methylphenylethylamine (internal standard, IS) were purchased from Sigma (St. Louis, MO, USA). Their chemical structures are shown in Fig. 1. Other chemicals were of analytical grade. An SPME device and fibers, coated with 65 μm polydimethylsiloxane-divinylbenzene were purchased from Supelco, Inc. (Bellefonte, PA, USA) and an Rtx®-5 Amine fused silica capillary column (30 m×0.32 mm i.d., film thickness 1.0 μm) from Restek (Bellefonte, PA, USA). Urine was obtained from a healthy subject.

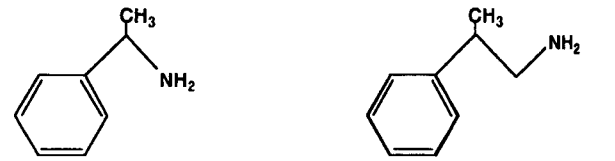

Fig. 1. Chemical structures of 1-phenylethylamine (1-PEA) and β-methylphenylethylamine (internal standard).

SPME procedure

The polydimethylsiloxane-divinylbenzene 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 50 or 250 ng of 1-PEA and 200 ng of IS was mixed with 100 μl of 10 M NaOH solution and 0.5 g K_2CO_3 in a vial. The vials were sealed with silicone-septum caps. After heating at 90℃ for 5 min, the septum piercing needle of an SPME fiber holder was passed through the septum, and the SPME fiber was exposed in the headspace at 90℃ for 30 min. The fiber was retracted into the needle, pulled out from the vial, and then immediately inserted into the GC port.

GC conditions

GC analyses were carried out on a Hewlett Packard 6890 Series instrument equipped with nitrogen-phosphorus detection (NPD). The GC conditions were: column temperature, 60 to 280℃ (1 min hold at 60℃ and 20℃/min); injection and detection temperatures, 240 and 280℃, respectively; helium flow rate, 2.5 ml / min. An SPME fiber was inserted in the splitless mode at a column temperature of 60℃ and the splitter was opened after 1 min.

Results and discussion

Figure 2 shows gas chromatograms for the authentic 1-PEA and IS (62.5 and 50 ng each on-column, respectively) dissolved in methanol, the blank of human urine, and the spiked urine (1 ml), to which 250 ng of 1-PEA and 200 ng of IS had been added. The retention times of 1-PEA and IS were 5.9 and 6.8 min, respectively.

The recoveries of 1-PEA (50 or 250 ng each for 1-ml samples) and IS (200 ng each for 1-ml samples) were calculated by comparing the peak areas obtained from SPME extracts of spiked human urine samples with those obtained from the non-extracted authentic drugs (12.5 or 62.5 ng each on-column for 1-PEA and 50 ng each on-column for IS) dissolved in methanol; the values for 1-PEA were 4.75±0.76 % at 50 ng/ml urine and 8.93±1.08 % at 250 ng/ml urine (mean±SD, n=4), and that for IS was 13.4±2.7 % (mean±SD, n=8).

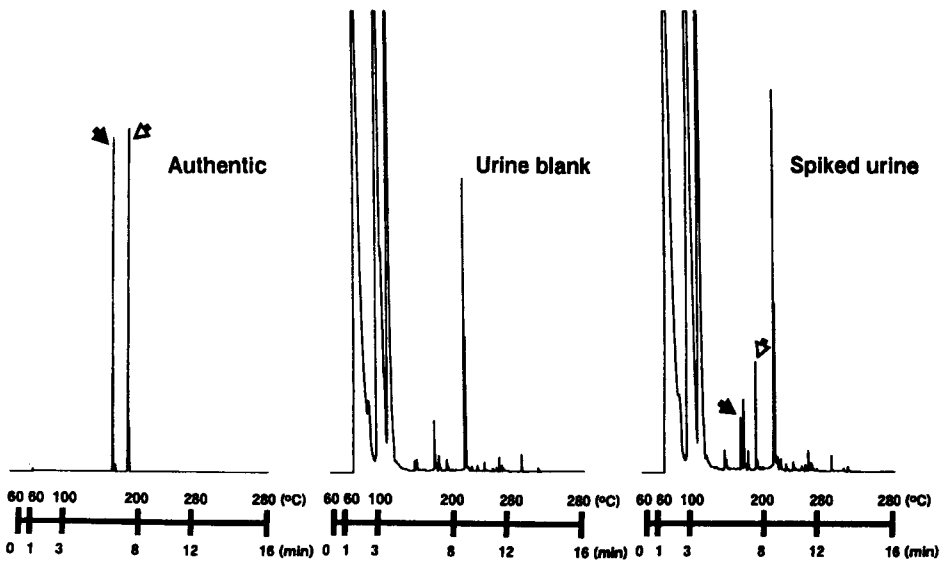

Fig. 2. Capillary GC-NPD for 1-PEA (filled arrow) and IS (open arrow). The amounts of the authentic 1-PEA and IS were 62.5 and 50 ng on-column, respectively. The drugs were extracted from 1 ml of human urine samples containing 250 ng of 1-PEA and 200 ng of IS.

The calibration curve for 1-PEA showed good linearity in the range of 25 to 500 ng / ml urine; the detection limit was about 20 ng/ml (signal-to-noise ratio=3).

Meyer *et al.* [2] reported a case of a couple of drug users found dead, whose urine contained 12 and 28 μg 1-PEA/ml, respectively. We have got satisfactory results for extraction of 1-PEA in human urine by headspace SPME in this study. There are many psychotropic drugs and natural compounds based on the 2-phenylethylamine (2-PEA) skeleton. Shulgin and Shulgin [10] have reported the production of 178 stimulants and hallucinogens based on 2-PEA. In the United States, *N*-methyl-1-phenylethylamine was identified from the powder produced at a clandestine laboratory [11]. In the near future, the different derivatives of phenylethylamines having stimulatory and/or hallucinogenic effects may appear, and we should be ready for their detection and identification. Since 1-PEA had been found in putrefied visceral tissues [12], the cut-off concentration of 1-PEA in putrefied specimen should be determined in further studies.

Acknowledgment

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture (No. 09307008). Guan Fuyu is also supported by a fellowship from the same Ministry.

References

1) Meyer, E., Van Bocxlaer, J., Lambert, W., Thienport, L. and De Leenheer, A.: α-Phenylethylamine identified in judical samples. Forensic Sci Int, **76**, 159−160 (1995).

2) Meyer, E., Van Bocxlaer, J. F., Lambert, W. E., Thienport, L. and De Leenheer, A. P.: Identification of α-phenylethylamine in judical samples. J Anal Toxicol, **20**, 116−120 (1996).

3) King, L. A., Poortman-van der Meer, A. J. and Huizer, H.: 1-Phenylethylamines: a new series of illicit drugs? Forensic Sci Int, **77**, 141−149 (1996).

4) Van Bocxlaer, J. F., Lambert, W. E., Theinpont, L. and De Leenheer, A. P.: Quantitative determination of amphetamine and α-phenylethylamine enantiomers in judical samples using capillary gas chromatography. J Anal Toxicol, **21**, 5−11 (1997).

5) Grana, E. and Lilla, L.: The inhibition of amine oxidase and the central stimulating action of the steroisomeric amphetamines and 1-phenethylamines. Br J Pharmacol, **14**, 501−504 (1959).

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

7) 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 monitoring. Forensic Sci Int, **76**, 169−177 (1995).

8) Ishii, A., Seno, H., Kumazawa, T., Nishikawa, M., Watanabe, K. and Suzuki, O.: Simple

clean-up of methamphetamine and amphetamine in human urine by direct-immersion solid phase micro extraction (DI-SPME). Jpn J Forensic Toxicol, **14**, 228−232 (1996).

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

10) Shulgin, A. and Shulgin, A.: PIHKAL (Phenylethylamines I Have Known And Loved)-A Chemical Love Story. Transform Press, Berkeley, CA, 1991.

11) Clark, C. C.: The identification of *N*-methyl-1-phenylethylamine. Microgram, **26**, 90−95 (1993).

12) Stevens, H. M. and Evans, P. D.: Identification tests for bases formed during the putrefaction of visceral material. Acta Pharmacol Toxicol, **32**, 525−552 (1973).