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Short Communication

Determination of urinary β-phenylethylamine as its N-benzenesulphonamide derivative by gas chromatography with flame photometric detection

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ABSTRACT

A selective and sensitive method for the determination of urinary β-phenylethylamine (PEA) by gas chromatography (GC) has been developed. After extraction of the urine sample with *n*-pentane, PEA was converted into its N-benzenesulphonamide derivative and then determined by GC with flame photometric detection using a DB-1301 capillary column. By using this method, nanogram amounts of PEA in urine could be accurately and precisely determined without any influence from coexisting substances. Analytical results for the determination of PEA in urine samples from normal subjects are presented.

INTRODUCTION

β-Phenylethylamine (PEA) is a biogenic monoamine occurring naturally in tissues and body fluids, and is considered to play the part of a neuromodulator in the central nervous system. Abnormalities in the urinary excretion of PEA have been reported in neurological diseases such as schizophrenia [1,2], depression [3,4], phenylketonuria [5] and Parkinson's disease [6].

The determination of PEA in urine samples has been carried out by fluorimetric assay [7], thin-layer chromatography (TLC) [3], gas chromatography–mass spectrometry (GC–MS) [8,9], GC [10–12] and high-performance liquid chromatography (HPLC) [13]. However, the fluorimetric assay requires time-consuming preliminary clean-up of the sample by solvent extraction and TLC requires time-consuming separation. The GC–MS method has picogram sensitivity, but it requires expensive equipment. Some of the GC and HPLC methods lack sensitivity or specificity and require complicated preliminary clean-up of the sample.

A selective and sensitive method has been developed for the determination of primary amines by GC with flame photometric detection (FPD) in which these compounds are analysed as their N-benzenesulphonamide derivatives [14]. This paper reports the extension of this work to the determination of PEA in urine samples.

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EXPERIMENTAL

Chemicals

PEA and 3-phenyl-1-propylamine (PPA) as an internal standard (I.S.) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), and each was dissolved in water to make a stock solution at a concentration of 1 mg/ml. Benzenesulphonyl chloride (BSC) was obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical-reagent grade.

Preparation of urine samples

Urine samples from healthy volunteers were collected under hydrochloric acid and the creatinine concentrations were determined by the method of Folin [15]. A 4-ml aliquot of the urine sample was pipetted into a 10-ml Pyrex glass tube with a PTFE-lined screw-cap. After the addition of 0.1 ml of 1 μg/ml I.S. solution and 0.5 ml of 50% potassium hydroxide, the solution (pH 14) was saturated with sodium chloride and then extracted twice with 3 ml of *n*-pentane. The pooled pentane extracts were back-extracted into 0.45 ml of 0.05 *M* hydrochloric acid solution and the aqueous layer (pH 1–2) was used for the analysis as a urine extract.

Derivatization procedure

The urine extract was made alkaline by the addition of 0.05 ml of 50% potassium hydroxide and 20 μl of BSC were added. The mixture was then shaken at 300 rpm (up and down) for 15 min at room temperature. To the reaction mixture were added 1.5 ml of 65% potassium hydroxide solution containing 30% methanol, and the mixture (pH > 14) was extracted three times with 3 ml of *n*-hexane to remove excess reagent and N-benzenesulphonamide derivatives of secondary amines. After the hexane extracts were discarded, 3.5 ml of 15% hydrochloric acid were added to the aqueous layer, and the mixture (pH 6–8) was extracted twice with 3 ml of diethyl ether. After the solvent had been evaporated to dryness at 80°C, the residue was dissolved in 0.1 ml of ethyl acetate, and 1 μl of this solution was injected into the gas chromatograph.

Gas chromatography

The GC analysis was carried out with a Shimadzu 14A gas chromatograph equipped with a flame ionization detector and a flame photometric detector (S-filter). A fused-silica capillary column (15 m × 0.53 mm I.D., 1.0 μm film thickness) of cross-linked DB-1301 (J & W, Folsom, CA, USA) was used. The operating conditions were as follows: column temperature, programmed at 10°C/min from 200 to 280°C and isothermal at 280°C for 2 min; injection and detector temperature, 290°C; nitrogen flow-rate, 10 ml/min.

Gas chromatography–mass spectrometry

A Hewlett-Packard 5890A gas chromatograph was operated in conjunction with a VG Analytical 70-SE mass spectrometer and a VG 11-250J mass data system. The GC column was of the same type as used for GC analysis, with an ionizing voltage of 40 eV, an ion-source temperature of 240°C and a helium flow-rate of 8 ml/min.

RESULTS AND DISCUSSION

PEA could be conveniently converted into its N-benzenesulphonamide derivative as described previously [14]. The benzenesulphonylation of PEA proceeded rapidly and quantitatively in an aqueous alkaline medium at room temperature. After benzenesulphonylation, primary amines such as PEA were completely separated from secondary amines by extraction with hexane in an alkaline medium containing methanol. The benzenesulphonamide derivative of PEA remained in the aqueous layer was quantitatively extracted by diethyl ether under aqueous acidic conditions. As the ether extracts contained methanol, the solvent was evaporated at 80°C. The derivative could be prepared within 40 min. The structure of the derivative was confirmed by GC–MS. The mass spectrum of the PEA derivative is shown in Fig. 1. A molecular ion peak (M^+) with postulated *m/z* 261 and the prominent fragment ion peaks, *m/z* 170 [$M^+ - 91$ ($CH_2-C_6H_5$)], *m/z* 141 ($SO_2-C_6H_5$), *m/z* 91 ($CH_2-C_6H_5$) and *m/z* 77 (C_6H_5) were observed, and these peaks were

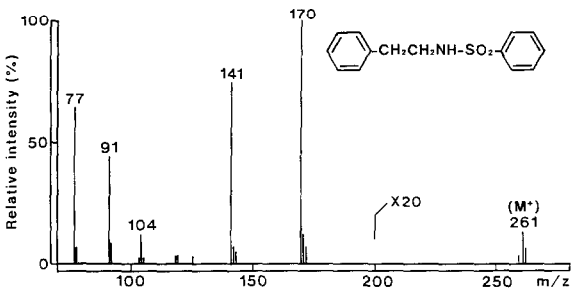

Fig. 1. GC–MS spectrum obtained from the N-benzenesulphonamide derivative of PEA.

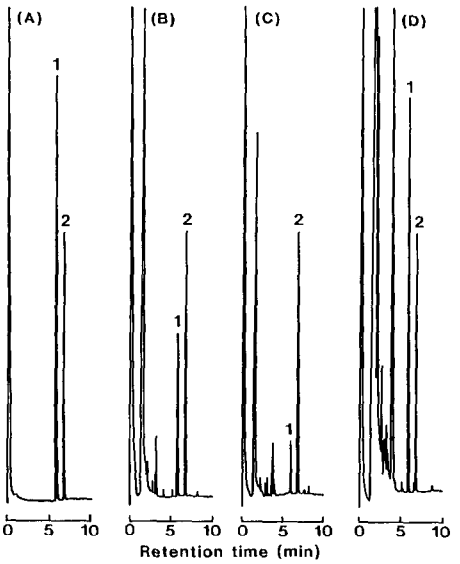

Fig. 2. Gas chromatograms obtained from the standard solutions and several urine samples. (A) Standard (containing 50 ng of PEA); (B)–(D) urine samples. GC conditions as given under Experimental; attenuation 10 × 16. Peaks: 1 = PEA; 2 = PPA (I.S.).

useful for structure elucidation. The derivative was found to be very stable under normal laboratory conditions, and no thermal decomposition was observed during GC analysis. As shown in Fig. 2A, the PEA derivative was eluted as single peak and provided an excellent response in the flame photometric detector. The minimum detectable amount of PEA required to give a signal three times as high as the noise under our instrumental conditions was *ca.* 50 pg as an injection amount, which was twenty times more sensitive than that obtained by GC with flame ionization detection. To test the linearity of the calibration graph, various amounts of PEA ranging from 10 to 200 ng were derivatized and aliquots representing 0.1–2 ng of PEA were injected. A linear relationship was obtained from both logarithmic plots, and the regression line was log $y = 1.364\log x - 2.559$ ($r = 0.9998$, $n = 15$), where y is the peak-height ratio and x is the amount of PEA.

The method developed was successfully applied to urine samples. PEA in urine was quantitatively extracted from alkaline medium into the *n*-pentane layer and then back-extracted into 0.05 *M* hydrochloric acid solution. By this treatment, low-molecular-mass amines and amino acids were removed, and PEA in urine was recovered at amounts greater than 97%. Fig. 2B–D shows the typical chromatograms obtained from several urine samples. PEA in the urine samples could be detected without any influence from coexistent substances. The quantitation limit of

TABLE 1

RECOVERIES OF PEA ADDED TO URINE SAMPLES

Sample	Added (ng/ml)	Found (mean ± S.D., $n = 4$) (ng/ml)		Recovery (%)
		No added PEA	Added PEA	
A	12.5	14.1 ± 0.2	25.9 ± 0.1	94.6
B	12.5	33.7 ± 0.2	46.0 ± 0.2	98.8
C	12.5	23.7 ± 0.1	36.1 ± 0.4	99.0

TABLE II

URINARY EXCRETION OF PEA IN NORMAL SUBJECTS

Subject No.	Age (years)	Sex[a]	Concentration of PEA (mean ± S.D., n = 3) (ng/mg of creatinine)
1	22	M	3.38 ± 0.02
2	22	M	15.61 ± 0.07
3	22	M	12.61 ± 0.05
4	24	M	6.79 ± 0.04
5	26	M	4.01 ± 0.04
6	21	F	10.06 ± 0.06
7	21	F	4.13 ± 0.02
8	21	F	4.17 ± 0.03
9	21	F	6.13 ± 0.03
10	21	F	5.33 ± 0.01

[a] M = male; F = female.

urinary PEA was *ca.* 2.5 ng/ml. As shown in Table I, the overall recoveries of PEA added to urine samples were 94–99%, and the relative standard deviations were less than 1.4%, indicating that this method is accurate and precise. The urinary levels of PEA in five men and five women were 8.39 ± 5.40 and 5.96 ± 2.44 ng/mg of creatinine, respectively (Table II), and no statistical difference was found between the sexes.

In conclusion, a convenient and reliable method for the determination of PEA in urine samples has been established. The method is based on the isolation of PEA by *n*-pentane extraction, and subsequent determination by GC–FPD as the N-benzenesulphonamide derivative of PEA. This method is selective and sensitive, and urine samples can be analysed without any interference from other coexistent substances. We believe that this method provides a useful tool in clinical and biochemical research.

REFERENCES

1 E. Fischer, H. Spatz, J. M. Saavedra, H. Reggiani, A. H. Miro and B. Heller, *Biol. Psychiatry*, 5 (1972) 139.
2 S. G. Potkin, F. Karoum, L. Chuang, H. E. Cannon-Spoor, I. Phillips and R. J. Wyatt, *Science*, 206 (1979) 470.
3 A. A. Boulton and J. Milward, *J. Chromatogr.*, 57 (1971) 287.
4 M. Matsuura, N. Ishii, J. Nishikawa, H. Hirasawa, S. Hanamura, M. Nakagawara and T. Kariya, *Folia Psychiatr. Neurol. Jpn.*, 34 (1980) 193.
5 G. P. Reynolds, J. W. T. Seakins and D. O. Gray, *Clin. Chim. Acta*, 83 (1978) 33.
6 B. Heller and E. Fischer, *Arzneim.-Forsch.*, 23 (1973) 884.
7 O. Suzuki and K. Yagi, *Clin. Chim. Acta*, 78 (1977) 401.
8 F. Karoum, H. Nasrallah, S. Potkin, L. Chuang, J. Moyer-Schwing and R. J. Wyatt, *J. Neurochem.*, 33 (1979) 201.
9 S. R. Philips, B. Rozdilsky and A. A. Boulton, *Biol. Psychiatry*, 13 (1978) 51.
10 J. W. Schweitzer, *Biol. Psychiatry*, 10 (1975) 277.
11 G. P. Reynolds and D. O. Gray, *Clin. Chim. Acta*, 70 (1976) 213.
12 G. B. Baker, T. S. Rao and R. T. Coutts, *J. Chromatogr.*, 381 (1986) 211.
13 M. Tsuji, K. Ohi, C. Taga, T. Myojin and S. Takahashi, *Anal. Biochem.*, 153 (1986) 116.
14 S. Ohrui, H. Kataoka, Y. Miyamoto, K. Ohtsuka and M. Makita, *Bunseki Kagaku*, 40 (1991) 119.
15 O. Folin, *Z. Physiol. Chem.*, 41 (1904) 223.