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Sensitive high-performance liquid chromatographic method for the determination of 2-phenylethylamine in human urine

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Abstract

A new sensitive high-performance liquid chromatographic (HPLC) method with fluorescence detection was developed for the determination of 2-phenylethylamine (PEA) in human urine. The analytical procedure involved a simple extraction of the analyte from urine, followed by precolumn derivatisation of the sample with *o*-phthalaldehyde. The HPLC separation was performed under isocratic conditions using an Erbasil S C_{18} (250 × 4.0 mm I.D., particle size 3 μm) reversed-phase column. The limit of quantitation was 0.5 ng of PEA/ml of urine. The method showed good linearity, accuracy and precision data in the concentration range 0.5–200 ng/ml of urine. The method was successfully applied to the determination of PEA urinary excretion in Parkinsonian patients after oral administration of the monoamine oxidase B (MAO-B) inhibitor, selegiline.

Keywords: 2-Phenylethylamine

1. Introduction

2-Phenylethylamine (PEA) is a biogenic amine which has been found in low concentrations in the human brain [1,2], and is also present in human urine [3]. The turnover of PEA is extremely rapid in the brain. Thus, a half-life of 0.4 min for the endogenous pool of PEA has been estimated in the rat brain [4]. The existence of a modulatory role for PEA in the catecholamine synapses, especially in the dopaminergic ones, has been suggested [5]. In line with this hypothesis, changes in the levels of PEA and/or in its metabolite, phenylacetic acid, in biological fluids have been reported in affective disorders, such as depression and schizophrenia [6–8]. Increased PEA was observed in human brain and urine [9,10] after treatment with the monoamine oxidase B (MAO-B) inhibitor, selegiline (L-deprenyl). It is commonly accepted that PEA is preferentially metabolized by MAO type B [11]. For this reason, PEA urinary excretion is usually taken as an index of MAO-B inhibition [12,13].

Several methods using mass spectrometry have been developed for the determination of very low levels of PEA in biological samples [14–17]. However, these assays require tedious purification procedures and/or complex preanalytical derivatisations. For PEA analyses in human urine, some HPLC methods were also set up using fluorescence or chemiluminescence detection after derivatisation with dansyl chloride, 4-fluoro-7-nitrobenzoxadiazole or naphthalene-2,3-dialdehyde (NDA) [18]; a limitation of these methods, however, is the long time or high temperature necessary for the derivatisation reaction. In addition, purification of the reagents or extraction of degradation products after derivatisa-

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tion is required to minimise interfering peaks and to obtain low quantitation limits. The detailed procedure for a sensitive and simple assay for PEA determination in human urine by HPLC with fluorescence detection after precolumn derivatisation with *o*-phthalaldehyde (OPA), is reported here. OPA easily reacts with primary amine groups [19] and affords stable derivatives. The method proved to be sensitive and specific and allowed the determination of PEA levels in samples of human urine.

2. Experimental

2.1. Apparatus

The HPLC system consisted of an Isochrom pump with a 200-μl loop, a FP 821 fluorescence detector (17-μl flow cell), a 4270 integrator and a Winner 386 data aquisition system, with "LABNET" software. All these instruments were supplied by Thermo Separation Products (San José, CA, USA) except for the detector which was purchased from Jasco (Tokyo, Japan).

2.2. Reagents and materials

2-Phenylethylamine hydrochloride was supplied by Sigma (St. Louis, MO, USA) and OPA reagent solution by Pierce (Rockford, IL, USA). All the other reagents and solvents were of analytical reagent grade from Carlo Erba Reagents (Milan, Italy). PEA stock standard solution was prepared by dissolving a weighed amount of the compound in water; this stock solution was stable for at least 1 month when stored at +4°C in the dark. Working standard solutions were prepared daily by diluting the stock standard solution in water or in 0.1 *M* HCl.

The pool of human urine used for the validation was prepared with urine samples collected from four male volunteers.

2.3. HPLC conditions

The chromatographic separation was performed using an Erbasil S C_{18} column (250 × 4.0 mm I.D., particle size 3 μm; Carlo Erba Reagents). The mobile phase was H_2O-CH_3OH-THF (33:55:12, v/v); the mixture was prepared daily and degassed under vacuum. The flow-rate of the mobile phase was 0.5 ml/min and the column was kept at room temperature. The fluorescence detector was set at an excitation wavelength of 340 nm and an emission wavelength of 455 nm and sent a 1 V signal to the integrator.

2.4. Sample extraction and derivatisation

Urine (1.0 ml) was placed into a 10-ml conical glass tube and mixed with 1.0 ml of 0.5 *M* borate buffer (pH 11). After addition of 2.5 ml of diethyl ether, the tubes were shaken using a vortex-mixer for 5 min and centrifuged at 1200 *g* for 5 min at room temperature. Then, the organic phase was collected. This extraction step was repeated, and the combined organic phase was back-extracted with 200 μl of 0.1 *M* HCl after vortexing for 2 min. After centrifugation at 1200 *g* for 5 min, the organic phase was discharged and the aqueous phase was subjected to the derivatisation reaction by adding 50 μl of OPA reagent solution; after 1 min at room temperature, 200 μl of the reaction mixture were injected onto the HPLC system.

2.5. Validation of the method

The pool of human urine later used for the validation was first assayed to ascertain the absence of interfering peaks with retention times close to that of derivatised PEA. For preparation and determination of calibration and quality control samples, the standard addition method [20] was employed. One milliliter samples obtained from the pool of human urine were assayed in triplicate on each day of analysis in order to determine the peak height (counts) of endogenous PEA; standard and quality control (QC) samples were prepared by spiking 1.0 ml of the same human urine with known amounts of PEA. Then, the mean peak height of endogenous PEA was substracted from the peak-height values of PEA measured in standard and QC samples, in order to obtain the peak height values of the spiked PEA.

Human urine samples spiked with different amounts of PEA (in the range 0.5–200.0 ng/ml) were assayed on five different days to evaluate the linearity of the method; precision and accuracy were

determined by assaying human samples spiked with low, medium and high concentrations of PEA (1.0, 12.0 and 100.0 ng/ml, respectively) on three different days.

All chromatograms were evaluated by peak-height measurement. The PEA concentration in QC samples was calculated using the calibration graph generated on each day by least-squares linear regression (weighting factor $1/y$) of the net peak height of the analyte against the amount added to 1 ml of urine. To evaluate the extraction recovery, the net peak height of extracted spiked urine samples was compared to that obtained with unextracted standard solutions injected directly onto the HPLC system.

2.6. Clinical study

Six de novo parkinson patients, five men and one woman (aged 48–66 years), received selegiline (10 mg) once a day for eight days, then selegiline (10 mg) and cabergoline (1 mg) for 22 days, and then cabergoline alone (1 mg) for 22 days. The 24 h urine was collected on days −1, 8, 30 and 52. Urine was collected in 1-l polyethylene containers, previously stored at 4°C and containing 0.5 g of Na_2EDTA, 0.5 g of sodium bisulfite and 0.5 g of semicarbazide hydrochloride. Upon completion of urine collection, samples were kept at −80°C until assayed.

3. Results and discussion

3.1. Derivatisation

The conditions for the derivatisation reaction with OPA were those previously applied to the amino acid analyses [21]. In preliminary studies, these conditions were tested for PEA and proved to be optimal. Since unreacted OPA is not fluorescent, procedures to remove traces of excess reagent or degradation products, after derivatisation, are not required. PEA derivative proved to be stable at room temperature for at least 60 min.

3.2. Linearity, precision and accuracy

The retention time of the PEA derivative obtained under the conditions described in Section 2 was about 21 min (Fig. 1).

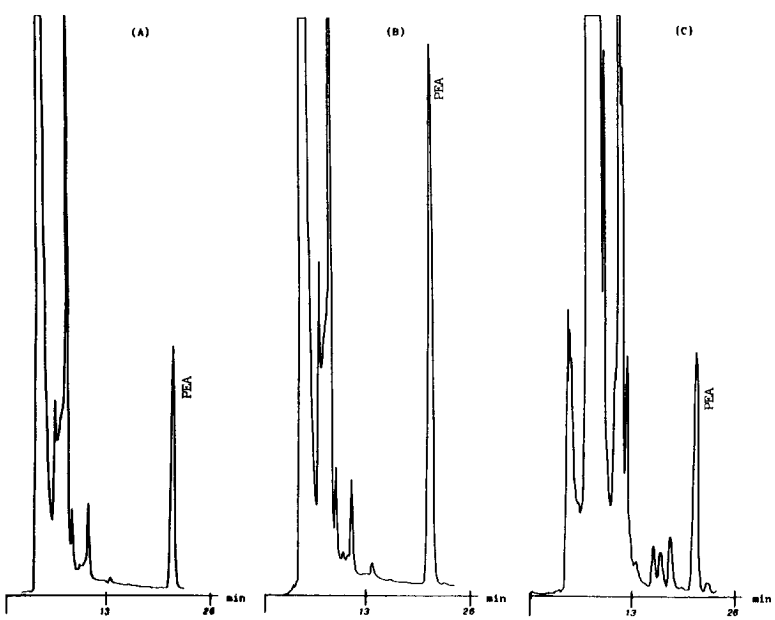

Fig. 1. Typical chromatograms obtained from standard urine before (A) and after (B) addition of 4 ng of PEA and from urine of a parkinsonian patient before selegiline administration (C).

Table 1
Back-calculated concentrations and parameters of the standard curves for PEA in human urine

Validation day	Concentration (ng/ml)					Standard curve parameters[b]		
	0.50 ng/ml[a]	2.00 ng/ml[a]	10.00 ng/ml[a]	50.00 ng/ml[a]	200.00 ng/ml[a]	Slope	y-intercept	r
1	0.47	1.94	10.73	49.92	199.48	15 098	2776	0.9999
2	0.43	2.24	9.90	51.33	198.67	13 404	2138	0.9999
3	0.49	2.13	9.62	50.12	200.16	14 524	2124	0.9996
4	0.54	2.13	9.49	45.71	205.26	14 415	495	0.9988
5	0.53	2.01	9.79	47.49	202.80	15 433	1707	0.9996
Mean	0.49	2.09	9.91	48.91	201.27	14 575	1848	0.9996
S.D.	0.04	0.12	0.49	2.27	2.71	776		
C.V. (%)	8.16	5.74	4.94	4.64	1.35	5.32		

[a]Standard concentration.
[b]Weighting factor $1/y$.

The calibration curves showed correlation coefficients ranging from 0.9988 to 0.9999; the mean calibration curve obtained was described by the equation $y = 14575x + 1848$ (slope C.V. = 5.32%, $n=5$), where y is the net peak height and x is the amount of analyte (ng) added to 1 ml of urine. When submitted to Student's t-test the y-intercept values were not significantly different from zero ($P>0.05$).

Back-calculated concentrations and standard curve parameters for PEA in human urine are reported in Table 1.

Intra-day precision (expressed as C.V.) ranged from 2.91 to 17.20% ($n=3$) and inter-day precision was better than 12.74% ($n=9$). Accuracy, expressed as the ratio of found/added amount of PEA, ranged from 92.8 to 113.2%; the pooled accuracy (inter-day) ranged from 102.1 to 104.0%.

The accuracy and the precision data obtained during the validation of the method are reported in Table 2.

3.3. Limit of quantitation

To evaluate the limit of quantitation (LOQ) of the analyte in urine, the signal-to-noise ratio and the precision for the lowest concentration of standard samples were considered. The LOQ of PEA was 0.5 ng/ml of urine; at this concentration the signal-to-noise ratio was better that 5:1 and the C.V. of replicate determinations was 8.2% ($n=5$).

Table 2
Accuracy and precision of the method used for the determination of PEA in human urine

Quality control sample ($n=3$) (ng/ml)	Day	Accuracy			Precision		
		Mean found (ng/ml)	Recovery (%)	S.D.	C.V. (intra-day) (%)	Pooled C.V. (inter-day) (%)	Pooled recovery (inter-day) (%)
1.0	1	0.93	92.8	0.16	17.20		
	2	1.00	100.3	0.10	10.00		
	3	1.13	113.2	0.04	3.54	12.74	102.1
12.0	1	13.03	108.6	0.71	5.45		
	2	11.93	99.4	1.75	14.67		
	3	12.00	99.9	0.56	4.67	9.10	102.6
100.0	1	99.71	99.7	3.67	3.68		
	2	101.84	101.8	5.24	5.14		
	3	110.56	110.6	3.22	2.91	5.89	104.0

3.4. Extraction recovery

For the calculation of the extraction efficiency, spiked urine samples were analysed on three different days; the calculation was:

$$\frac{A_{SA} - A}{A_{ES}} \times 100\%$$

where A_{SA} is the peak height after addition of known amounts of standard, A is the peak height without the addition of standard and A_{ES} is the peak height of the respective unextracted standard.

The resulting mean extraction recovery, evaluated on three different days in the concentration range of 0.5–200.0 ng/ml of added PEA, ranged from 63 to 74%.

3.5. Application

The method was successfully applied to the analysis of samples obtained in a clinical study [22], in which the urinary excretion of PEA was determined in parkinsonian patients to whom selegiline and cabergoline (a dopamine-D_2 receptor agonist) were given alone or concomitantly. The method proved to be sensitive and specific; no chromatographic interference due to amphetamine, which is the main selegiline metabolite and has a primary amine group potentially reactive with OPA, was detected. Using the procedure described in Section 2, a 3.5-fold increase in PEA excretion was observed after administration of the MAO-B inhibitor, selegiline, for eight days.

4. Conclusion

An HPLC method with fluorescence detection has been developed for the evaluation of PEA urinary excretion. This method showed good linearity, accuracy and precision in the concentration range of 0.5–200.0 ng/ml of urine. The assay utilises a simple and rapid derivatisation reaction with OPA that forms a stable PEA derivative. Taking advantage of these properties, further studies could be carried out in order to assess the possible full automation of the derivatisation and chromatographic analysis procedure. The method was successfully applied to evaluate PEA urinary excretion in patients with Parkinson's disease, during treatment with selegiline and/or cabergoline. Thus, this method could be advantageously used for clinical investigation during the administration of drug when changes in PEA urinary excretion might occur.

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References

[1] E.E. Inwang, A.D. Mosnaim and H.C. Sabelli, J. Neurochem., 20 (1973) 1469.
[2] S.R. Philips, B. Rozdilsky and A.A. Boulton, Biol. Psychiatry, 13 (1978) 51.
[3] G.P. Reynolds and D.O. Gray, Clin. Chim. Acta, 70 (1976) 213.
[4] D.A. Durden and S.R. Philips, J. Neurochem., 34 (1980) 1725.
[5] I.A. Paterson, A.V. Juorio and A.A. Boulton, J. Neurochem., 55 (1990) 1827.
[6] E. Fischer, H. Spatz, J.M. Saavedra, H. Reggiani, A.H. Mirò and B. Heller, Biol. Psychiatry, 5 (1972) 139.
[7] M. Sandler, C.R.J. Ruthven, B.L. Goodwin and A. Coppen, Clin. Chim. Acta, 93 (1979) 169.
[8] F. Karoum, S. Potkin, L.W. Chuang, D.L. Murphy, M.R. Liebowitz and R.J. Wyatt, Biol. Psychiatry, 19 (1984) 165.
[9] J.D. Elsworth, V. Glover, G.P. Reynolds, M. Sandler, A.J. Lees, P. Phuapradit, K.M. Shaw, G.M. Stern and P. Kumar, Physiopharmacology, 57 (1978) 33.
[10] G.P. Reynolds, P. Riederer and M. Sandler, Biochem. Soc. Trans., 7 (1979) 143.
[11] H. Yang and N.H. Neff, J. Pharmacol. Exp. Ther., 187 (1973) 365.
[12] C. Thorton, C.J. Doré, J.D. Elsworth, M. Herbert and G.M. Stern, Psychopharmacology, 70 (1980) 163.
[13] C.T. Dollery, D.S. Davies, M. Barrow, S.M. Murray and M. Strolin Benedetti, in K.F. Tipton, P. Dostert and M. Strolin Benedetti (Editors), Monoamine Oxidase and Disease — Prospects for Therapy with Reversible Inhibitors, Academic Press, London, 1984, p. 83.

[14] D.A. Durden, S.R. Philips and A.A. Boulton, Can. J. Biochem., 51 (1973) 995.
[15] J. Willner, H.F. LeFevre and E. Costa, J. Neurochem., 23 (1974) 857.
[16] F. Karoum, H. Nasrallah, S. Potkin, L. Chuang, J. Mayer-Schwing, I. Phillips and R.J. Wyatt, J. Neurochem., 33 (1979) 201.
[17] O. Suzuki and H. Hattori, Biomed. Mass Spectrom., 10 (1983) 430.
[18] K. Hayakawa, K. Hasegawa, N. Imaizumi, O.S. Wong and M. Miyazaki, J. Chromatogr., 464 (1989) 343.
[19] M. Roth, Anal. Chem., 43 (1971), 880.
[20] F. Cheng, J. Kuo, Y. Shihi, J. Laùi, D. Ni and L. Chia, J. Chomatogr., 615 (1993) 225.
[21] V. Barkholt and A.L. Jensen, Anal. Biochem., 177 (1989) 318.
[22] P. Dostert, G. Dordain, M. Strolin Benedetti, D. Vernay, R. La Croix, D. Defford and A. Dubini, Recent Advances in the Treatment of Neurodegenerative Disorders and Cognitive Disfunction, CINP Meeting (President's workshop), Capri, Italy, May 1–3 1993, Book of Abstracts, p. 140.