Journal of Chromatography, 534 (1990) 287–290
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5495

Letter to the Editor

Determination of psilocin in rat urine by high-performance liquid chromatography with electrochemical detection

Sir,

Psilocin was isolated as one of active substances of the hallucinogenic mushrooms of the genus *Psilocybe* [1]. A number of mainly chromatographic methods have been described for the determination of psilocybin, psilocin and the other tryptamine derivatives. Thin-layer chromatography [2–4] is usually used for simple qualitative detection of the compounds of interest. Current methods for the quantification of tryptamine derivatives are high-performance liquid chromatography (HPLC) on silica gel [5–7], strongly acidic cation exchanger [8], or widely used reversed-phase HPLC [9–11]. UV spectrophotometry at 224, 254, or 267 nm or in the diode-array mode is a standard detection technique for tryptamines [5,7,12–14] A very suitable method is the electrochemical detection (ED), especially for the sensitive determination of hydroxy derivatives of tryptamine [11,12,15,16].

For metabolic studies, we suggest a new method for fast and sensitive determination of psilocin in rat urine without pretreatment.

EXPERIMENTAL

Chemicals

The standard substance 4-hydroxy-N,N-dimethyltryptamine (psilocin) was obtained from Sandoz (Basel, Switzerland). The other chemicals were of reagent grade from Lachema (Brno, Czechoslovakia).

Sample preparation

The samples of rat urine were centrifuged for 5 min at 1000 *g*. A 10-μl volume of the supernatant was injected on to the chromatographic column.

0378-4347/90/$03.50

Instrumentation

The high-performance liquid chromatograph consisted of an LC-3B high-pressure pump (Perkin-Elmer, Norwalk, CT, U.S.A.), and a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA, U.S.A.) with 10-μl sample loop. A μBondapak C_{18} (300 mm × 3.9 mm I.D.) column (Waters, Milford, MA, U.S.A.) was eluted with a mobile phase containing citrate–phosphate buffer (13.7 g of citric acid monohydrate, 4.7 g of potassium dihydrogenphosphate and 1 l of water; pH 2.8) and ethanol. The column was protected by a GuardPak C_{18} cartridge (Waters). The optimal flow-rate was 1.0 ml/min. An electrochemical detector with a three-electrode cell (glassy carbon working electrode) (Model EDLC, Laboratory Instruments, Prague, Czechoslovakia) was used to monitor the column effluent. The chromatographic data were evaluated using Baseline 810 software (Waters).

RESULTS AND DISCUSSION

As a result of previous investigations, we used a mixture of acid buffer with ethanol as the mobile phase for separation [15]. A high concentration of buffer is necessary to eliminate ohmic polarization of electrode system. The concentration of organic modifier was then optimized. We examined three concentrations in the range 5–25% (v/v) of ethanol. For the optimization we used the method described previously [16]. Two requirements were pursued: minimal analysis time and perfect separation of psilocin from the other, naturally occurring components of the rat urine. The best concentration of ethanol was found to be 10% (v/v). Fig. 1 shows the chromatographic separation of psilocin under the chosen conditions: there are no interferences.

We used ED for the quantification of very low concentrations of psilocin. It is known from the literature that a detection limit up to 50 times lower for ED of hydroxytryptamines may be achieved compared with the spectrophotometric detection [16]. The half-wave potential of +0.5 V *versus* saturated silver chloride electrode for psilocin was assessed from hydrodynamic voltammograms. Quantification was performed at the potential of the limiting diffusion current (of *ca.* +0.65 V). The slope of the calibration line was 12.8 nA/ng, with a correlation coefficient of 0.9948. The detection limit at a signal-to-noise ratio of 2 was 0.29 μg of psilocin in 1 ml of rat urine. According to our unpublished results, the spectrophotometric detection limit was over 10 μg/ml psilocin.

The method developed was tested using spiked rat urine (4.04 μg/ml of psilocin). A concentration of 3.96 μg/ml (*i.e.* 98%) psilocin with a relative standard deviation of 1.42% was found ($n = 7$). A major feature of the described analytical procedure is the elimination of the preconcentration step without a decrease in the determination sensitivity and selectivity.

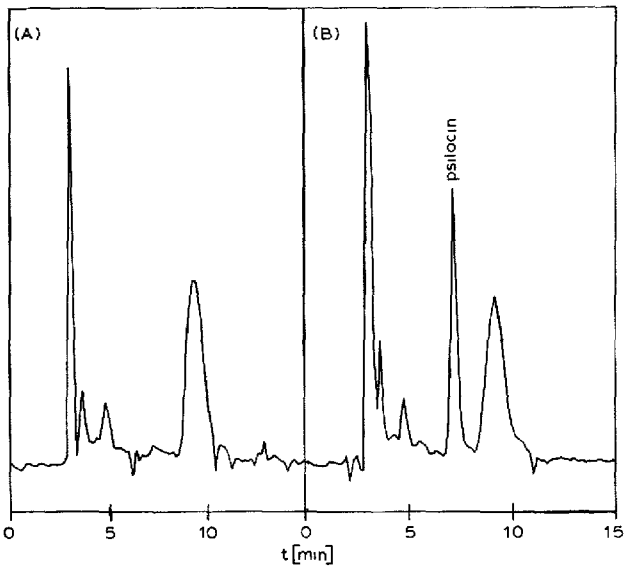

Fig 1. HPLC traces of (A) untreated rat urine and (B) rat urine spiked with 4 04 μg/ml psilocin. Injection volume, 10 μl, working electrode potential, +0 65 V (Ag/AgCl).

ACKNOWLEDGEMENT

We thank Dr. Dana Baudisova, Institute of Toxicology, Faculty of Medicine (Prague, Czechoslovakia) for providing us with the biological material.

Institute of Microbiology, Czechoslovak Academy of Sciences, Videnska 1083, CS-142 20 Prague 4 (Czechoslovakia)

ROMAN KYSILKA

1 A Hofmann and F. Troxler, *Experientia*, 15 (1959) 101.
2 D. B. Repke, D T Leslie and G Guzmán, *Lloydia*, 40 (1977) 566.
3 Y Koike, K. Wada, G Kusano, S. Nozoe and K Yokoyama, *J. Nat. Prod.*, 44 (1981) 362
4 S. Saupe, *Mycologia*, 73 (1981) 781.
5 P. C White, *J Chromatogr*, 169 (1979) 453.
6 A. L. Christiansen and K. E Rasmussen, *J. Chromatogr.*, 244 (1982) 357
7 S M Sottolano and I S Lurie, *J Forensic Sci.*, 28 (1983) 929
8 M Perkal, G. L Blackman, A L Ottrey and L. K Turner, *J. Chromatogr.*, 196 (1980) 180
9 B R Sitaram, R Talomsin, G. L Blackman, W R McLeod and G N Vaughan, *J. Chromatogr*, 275 (1983) 21
10 M. Wurst, M. Semerdžieva and J. Vokoun, *J. Chromatogr.*, 286 (1984) 229
11 R Kysilka and M. Wurst, *J Chromatogr*, 464 (1989) 434.
12 A. L. Christiansen and K E Rasmussen, *J Chromatogr.*, 270 (1983) 293

13 R Vanhaelen-Fastre and M Vanhaelen, *J Chromatogr*, 312 (1984) 467.
14 S. Borner and R. Brenneisen, *J Chromatogr*, 408 (1987) 402.
15 R. Kysilka, M Wurst, V Pacáková, K Štulík and L Haškovec, *J. Chromatogr*, 320 (1985) 414.
16 R. Kysilka and M Wurst, *J Chromatogr*. 446 (1988) 315

(First received May 11th, 1990; revised manuscript received July 23rd, 1990)