Journal of Chromatography, 270 (1983) 293–299
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,077

SCREENING OF HALLUCINOGENIC MUSHROOMS WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND MULTIPLE DETECTION

A. L. CHRISTIANSEN* and K. E. RASMUSSEN
Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Oslo, Box 1068 Blindern, Oslo 3 (Norway)
(Received June 20th, 1983)

SUMMARY

A rapid, sensitive and specific method for the screening of hallucinogenic mushrooms has been developed. High-performance liquid chromatography with simultaneous use of ultraviolet, fluorescence and electrochemical detection was employed. Separation of the mushroom components was achieved on a silica column using an alkaline aqueous methanolic eluent. The use of detector response ratios for identification of hallucinogenic indole alkaloids has been evaluated.

INTRODUCTION

Recreational use of hallucinogenic mushrooms is an increasing problem in several countries. The hallucinogenic mushrooms, such as several *Psilocybe* species, contain one or more of the indole alkaloids psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine), psilocin (4-hydroxy-N,N-dimethyltryptamine) and baeocystin (4-phosphoryloxy-N-methyltryptamine)[1].

Several methods have been used previously for isolation and identification of the active components of hallucinogenic mushrooms. Paper chromatography[2,3], thin-layer chromatography[4,5] and classical liquid chromatography[6,7] have been employed in conjunction with colorimetric reagents as well as with ultraviolet (UV), infrared and mass spectroscopy. Today, analysis of hallucinogenic mushrooms is mainly carried out by high-performance liquid chromatography (HPLC). UV detection is most commonly employed, and fluorescence detection has been used for quantification of psilocybin[8,9]. Recently, separation on an HPLC column prior to mass spectroscopic identification of the isolated compounds has been reported[10].

In order to carry out a screening of hallucinogenic Norwegian mushrooms, a method was required that allowed determination of mushroom components to be made easily and with a great deal of specificity and sensitivity. Psilocybin and psilocin both exhibit native fluorescence[10], and they are electrochemically active. The purpose of the present investigation was to utilize HPLC with UV detection, fluorescence detection and electrochemical detection (ED) to provide qualitative and quantitative information concerning the eluted mushroom components. The use of detector re-

0021-9673/83/$03.00

sponse ratios of these three detectors as qualitative parameters for the identification of hallucinogenic indole alkaloids has been evaluated.

EXPERIMENTAL

Chemicals

Psilocybin and psilocin were supplied by Sandoz (Basel, Switzerland). All other chemicals were of analytical grade from E. Merck (Darmstadt, F.R.G.).

Individual reference standards of psilocybin and psilocin were dissolved in methanol and stored at 4°C and −20°C, respectively. Mushrooms were extracted with 10% 1 *M* ammonium nitrate in methanol as described elsewhere[9], and stored at 4°C.

The mobile phase consisted of methanol–water–1 *M* ammonium nitrate buffered to pH 9.6 with 2 *M* ammonia (220:70:10) containing the disodium salt of ethylenediaminetetraacetic acid (EDTA) (1 m*M*). The solvent mixture was filtered under vacuum and sonicated for 10 min before use. The flow-rate was 1.0 ml/min. All the measurements were carried out at ambient temperature.

Apparatus

Chromatography was performed with a Spectra-Physics Model 3500 liquid chromatograph equipped with a 25 cm × 4.6 mm I.D. stainless-steel column slurry-packed with small-particle silica (5-μm Partisil 5; Whatman, Maidstone, U.K.). Samples were introduced through a Valco 7000 p.s.i. rotary valve injector fitted with a 10-μl loop.

The eluent was split by means of a Valco tee as shown in Fig. 1, and passed to the UV and fluorescence detectors with the electrochemical detector connected downstream of the UV detector. UV detection at 254 nm was performed by a Spectra-Physics Model 225 UV detector. A Spectra-Physics Model FS 970 Spectrofluoro Monitor was used for fluorimetric detection, with excitation at 267 nm and a 320-nm emission filter. ED was accomplished using a Model LC-4 amperometric detector from Bioanalytical Systems (West Lafayette, IN, U.S.A.), equipped with a TL-5A

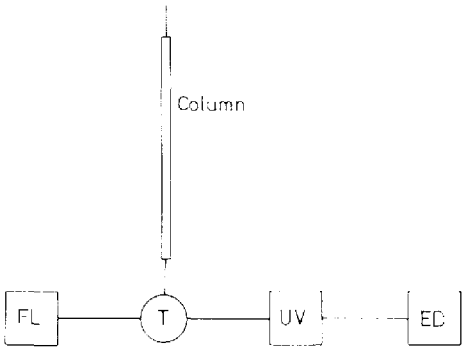

Fig. 1. Schematic diagram of the multiple detection system. T = a Valco stainless-steel 1/16 in. zero dead volume tee, joined to each detector with a length of 1/16 in. O.D., 0.029 in. I.D. stainless-steel tubing. UV = ultraviolet detector; FL = fluorescence detector; ED = electrochemical detector.

thin-layer detector cell. The glassy carbon working electrode was maintained at an applied potential of +0.65 V *versus* a silver–silver chloride reference electrode.

RESULTS AND DISCUSSION

Characteristics of the multidetection system

The hallucinogenic mushroom *Psilocybe semilanceata* and the non-hallucinogenic mushroom *Panaeolus rickenii* were analyzed with the apparatus as described above. The resulting UV, fluorescence and electrochemical chromatograms are shown in Figs. 2 and 3.

As seen in Fig. 2A, HPLC with UV detection at 254 nm produce a characteristic chromatogram of the mushroom extract. Retention time data indicate that *Psilocybe semilanceata* contains both baeocystin (peak 3) and psilocybin (peak 4). Owing to poor sensitivity, however, the method will fail to detect compounds present in small amounts, such as psilocin. The minimum detectable amounts, determined as twice the baseline noise, were found to be 10 ng for psilocybin and 7.5 ng for psilocin.

The great advantage of fluorescence detection is the selectivity of the method. Both psilocybin and baeocystin are known to exhibit native fluorescence at 335 nm on excitation at 267 nm[10]. As seen in Fig. 2B, the HPLC chromatogram after fluorescence detection is relatively simple. Under the applied conditions only psilocybin and baeocystin were adequately detected. The detectabilities of psilocybin and psilocin were 5 ng and 20 ng, respectively.

ED depends on the presence of oxidizable or reducible groups in the solute molecule. Many of the mushroom components extracted were electrochemically responsive under the applied conditions, and the resulting chromatograms add valuable data to the fingerprint of mushroom extracts. Fig. 2C shows the chromatogram of *Psilocybe semilanceata* when ED is employed. The greatest importance of ED is the high sensitivity obtained, especially for psilocin. This compound is easily discovered, even if present in trace amounts only.

Voltammograms of psilocybin and psilocin are shown in Fig. 4. The oxidation of psilocin (phenolic group) starts at a lower potential than the oxidation of psilocybin (indole N-oxidation). A potential of +0.65 V was chosen for investigation of mushroom extracts and analysis of psilocin. Under these conditions the detection limits for psilocybin and psilocin were 5 ng and 75 pg, respectively.

The aqueous methanolic solvent earlier used for the analysis of psilocybin[9], proved suitable for electrochemical detection. Addition of small amounts of EDTA to the mobile phase has been reported to improve the baseline stability[11]. Throughout this study a concentration of EDTA of 1 m*M* was used. However, recent investigations showed that the presence of EDTA in the mobile phase used in this study had no effect on the baseline stability and response.

Identification with the multidetection system

The simultaneous use of UV, fluorescence and electrochemical detectors to monitor the HPLC eluent provides qualitative information about the eluted compounds. The signals produced by the three-detector system can be used to calculate peak height ratios which can be used to confirm the identity of a peak.

To illustrate how such data can contribute to the identification of mushroom

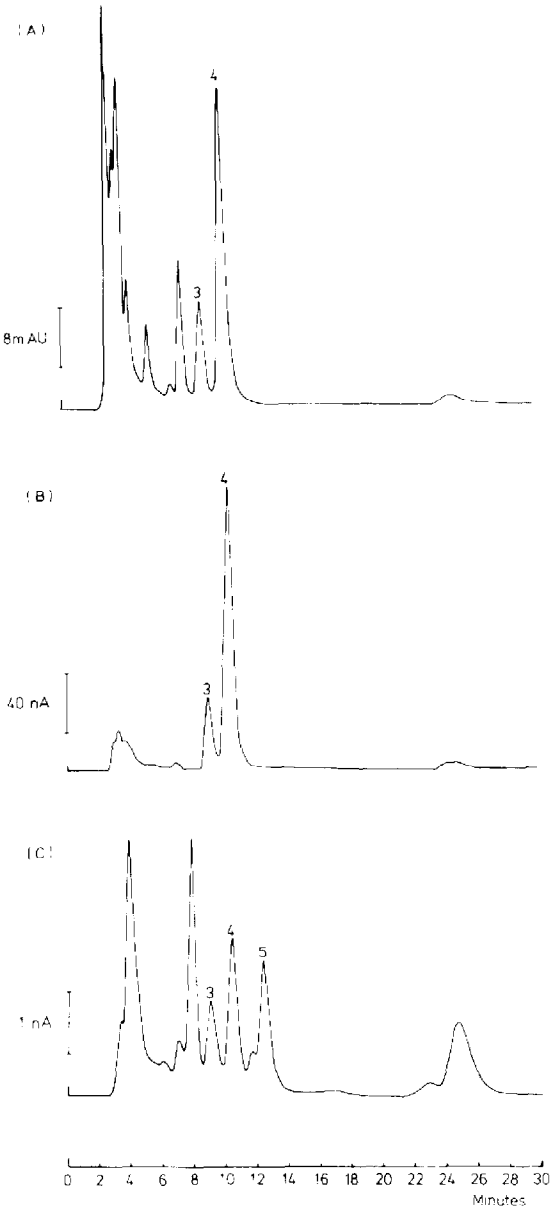

Fig. 2. Chromatograms of *Psilocybe semilanceata* after (A) UV detection (254 nm); (B) fluorescence detection (excitation, 267 nm; emission filter, 320 nm; sensitivity, 4.00); (C) electrochemical detection (+0.65 V). Peaks: 3 = baeocystin; 4 = psilocybin; 5 = psilocin.

components, the peak height ratios of peak 4 (psilocybin) in *Psilocybe semilanceata* (Fig. 2) and a reference standard were computed as shown in Table I. Also included are the peak height ratios of peak X in *Panaeolus rickenii* (Fig. 3), which have a retention time similar to that of psilocybin. The ratios are reported as the mean plus or minus the standard deviation after five injections of each sample. Detector settings are given in Fig. 2.

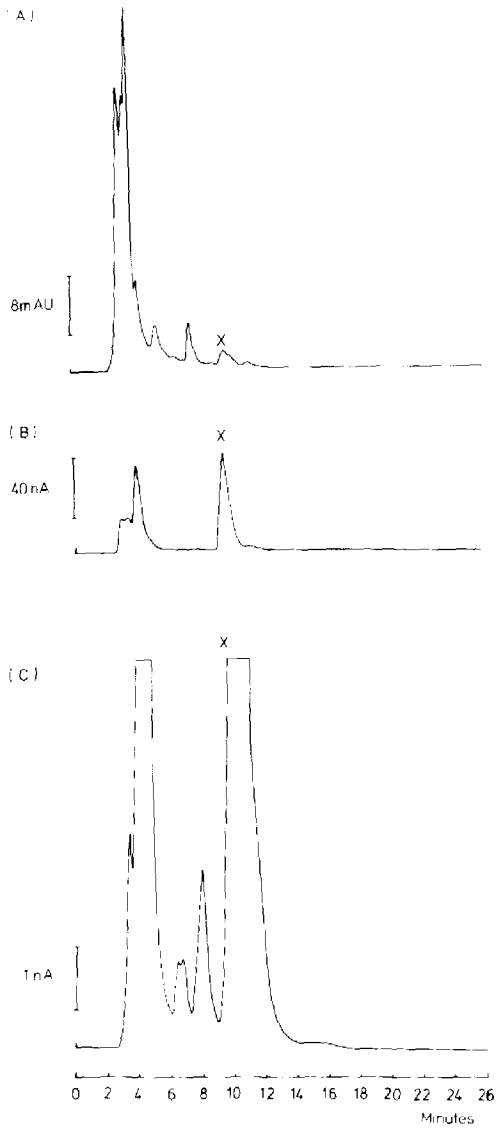

Fig. 3. Chromatograms of *Panaeolus rickenii* after (A) UV detection; (B) fluorescence detection; (C) electrochemical detection. Detector characteristics as in Fig. 2. Peak X = unknown.

The ratios UV/fluorescence, UV/electrochemical and fluorescence/electrochemical of psilocybin in *Psilocybe semilanceata* were similar to those of standard psilocybin. The similarity of the response ratios strongly indicates that the peak contains psilocybin and no other compounds. The identity of this peak was confirmed by mass spectroscopy as described earlier[10]. On the other hand, the response ratios of peak X in *Panaeolus rickenii* are different from those of standard psilocybin, indicating that this mushroom does not contain psilocybin.

The greater uncertainty of the response ratios of psilocybin where ED is em-

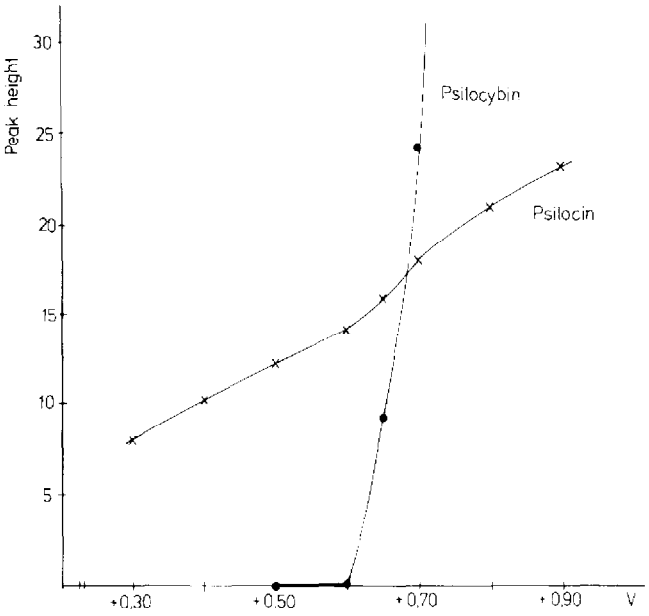

Fig. 4. Voltammogram of psilocybin standard (100 μg/ml) (●) and psilocin standard (1 μg/ml) (×).

ployed, is due to the reproducibility of the electrochemical detector for this compound. As can be seen in Fig. 4, the detector response of psilocybin greatly increases with increasing potential. Then, even small shifts in potential resulting from reference electrode drift or the internal resistance of the solution will have a large effect on the response.

The method of peak height ratios cannot be used for verification of the presence of psilocin in Norwegian *Psilocybe semilanceata*. This mushroom contains trace amounts of psilocin, and ED is the only method that will give a response. However, both the retention and the electrochemical behaviour of the compound give valuable information. Quantitative analysis of psilocin was based on peak height measurements after ED. The amount of psilocin in the *Psilocybe semilanceata* mushroom shown in Fig. 2, was found to be 0.002%.

TABLE I

PEAK HEIGHT RATIOS OF PSILOCYBIN STANDARD AND SIMILAR RETAINED COMPOUNDS IN *PSILOCYBE SEMILANCEATA* AND *PANAEOLUS RICKENII*

FL = fluorescence detection.

Compound	*Response ratio ± standard deviation*		
	UV/FL	*UV/ED*	*FL/ED*
Psilocybin	1.32 ± 0.04	1.70 ± 0.23	1.29 ± 0.14
Psilocybin in *Psilocybe semilanceata*	1.33 ± 0.03	1.82 ± 0.18	1.37 ± 0.12
Peak X in *Panaeolus rickenii*	0.15 ± 0.00	0.004 ± 0.000	0.025 ± 0.000

This method of multiple detection has been used in a screening of hallucinogenic Norwegian mushrooms. *Psilocybe semilanceata* has been found to contain hallucinogenic indole alkaloids[9]. During the screening program two other mushroom species were found to contain both psilocybin and psilocin[12].

CONCLUSIONS

HPLC with UV, fluorescence and electrochemical detection has proved suitable for the screening of hallucinogenic mushrooms. The simultaneous use of these three detectors remarkably shortens analysis time. Valuable qualitative information is gained from each run, and both psilocybin and psilocin can be analyzed with high sensitivity and specificity.

ACKNOWLEDGEMENT

We acknowledge the skilful technical assistance of Mr. Finn Tønnesen.

REFERENCES

1 L. Fishbein (Editor), *Chromatography of Environmental Hazards*, Vol. IV, Elsevier, Amsterdam, 1982, pp. 425–429.
2 A. Hofmann, R. Heim, A. Brack, H. Kobel, A. Frey, H. Ott, Th. Petrzilka and F. Troxler, *Helv. Chim. Acta*, 42 (1959) 1557.
3 R. G. Benedict, L. R. Brady, A. H. Smith and V. E. Tyler, Jr., *Lloydia*, 25 (1962) 156.
4 R. Heim, K. Genest, D. W. Hughes and G. Belec, *J. Forensic Sci. Soc.*, 6 (1966) 192.
5 D. B. Repke and D. T. Leslie, *J. Pharm. Sci.*, 66 (1977) 113.
6 A. Y. Leung, A. H. Smith and A. G. Paul, *J. Pharm. Sci.*, 54 (1965) 1576.
7 Y. Koike, K. Wada, G. Kusano, S. Nozoe and K. Yokoyama, *J. Nat. Prod.*, 44 (1981) 362.
8 B. M. Thomson, *J. Forensic Sci.*, 25 (1980) 779.
9 A. L. Christiansen, K. E. Rasmussen and F. Tønnesen, *J. Chromatogr.*, 210 (1981) 163.
10 A. L. Christiansen and K. E. Rasmussen, *J. Chromatogr.*, 244 (1982) 357.
11 L. Semerdjian-Rouquier, L. Bossi and B. Scatton, *J. Chromatogr.*, 218 (1981) 663.
12 A. L. Christiansen, K. E. Rasmussen and K. Høiland, *Planta Med.*, submitted for publication.