ELSEVIER

Journal of Chromatography A, 730 (1996) 59–62

JOURNAL OF CHROMATOGRAPHY A

Determination of serotonin released from coffee wax by liquid chromatography

M. Kele, R. Ohmacht*

Department of Medical Chemistry, University Medical School, 7643 Pécs, Hungary

Abstract

A simple hydrolysis and extraction method was developed for the release of serotonin (5-hydroxytryptamine) from a coffee wax sample obtained from decaffeination of coffee beans. The recoverable amount of serotonin was determined by reversed-phase high-performance liquid chromatography with gradient elution and UV detection, using the standard addition method. Different type of basic deactivated chromatographic columns were used for the separation.

Keywords: Coffee; Serotonin; Pyridine; Phenol

1. Introduction

Serotonin (5-hydroxytryptamine) plays an important role in cerebral metabolism and it is a potential drug with different activities, e.g., vasoconstrictive, antihypertonic, antiallergic, antipsychotic, antitabac.

One of the main types of lipid compounds present in coffee is N-alkanoyl-5-hydroxytryptamines [1]. The decaffeination process leads to a reduction of about 75% in the N-alkanoyl-5-hydroxytryptamine content of coffee; in turn, the wax obtained by the decaffeination process (called coffee wax) contains a considerable amount of the above substances [2,3]. Therefore, employing an appropriate extraction method, coffee wax can be used as a cheap source of serotonin.

HPLC is widely used for serotonin determination in different samples, usually on reversed-phase (RP) or cation-exchange analytical columns, with electrochemical, fluorimetric or UV detection, depending on the amount of serotonin and the origin of the sample [1,2,4–10]. In contrast to literature data, a simple mobile phase system was chosen, water–acetonitrile mixtures containing 0.05% trifuoroacetic acid, and it was used in the gradient elution mode. We focused attention on the selection of the appropriate column. The effect of different RP stationary phases (so-called "basic deactivated" for the separation of basic compounds and non-deactivated) on the separation was studied.

The aim of this work was to determine the recovery of 5-hydroxytryptamine from a coffee wax sample after hydrolysis of the above-mentioned tryptamides and the determination of serotonin after extraction. For the quantification

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)01186-2

of serotonin a rapid HPLC separation method was developed.

2. Experimental

2.1. Apparatus

Chromatographic separations were performed using a Model 300 B pump, a Negretti Model 220 sample injection valve, a Model 250 B gradient former (all from Gynkotek, Germering, Germany) and an HP 1050 series variable-wavelength detector controlled by a Chemstation, software version A.00.33 (Hewlett-Packard, Waldbronn, Germany).

For peak purity measurements a Waters Model 991 photodiode-array detector was used (Waters, Milford, MA, USA).

Four different HPLC stationary phases were used: LiChrosorb RP-18, 5 μm, and LiChrosorb RP Select B, 5 μm (both from Merck Darmstadt, Germany), Hypersil BDS C_{18}, 5 μm (Shandon, Cheshire, UK), and a laboratory-made, silica-based, deactivated C_{18} phase, 5 μm. The phases were packed into 150 × 4 mm I.D. columns by BST (Budapest, Hungary).

2.2. Reagents and standard

Serotonin hydrochloride standard was of 99% purity. All chemicals were of analytical-reagent or HPLC grade.

2.3. Hydrolysis and extraction

A 4.0-g amount of coffee wax sample was suspended and dissolved partially in a mixture of 21 ml of potassium hydroxide (22%, w/w) and 22 ml of 2-butanol. The suspension was stirred in an inert gas atmosphere at 95°C for 20 h for the hydrolysis of the amide bonds. Subsequently the suspension was cooled and the pH was adjusted to ca. 4 with dilute hydrochloric acid. The aqueous phase was isolated in a separating funnel. To the remaining 2-butanolic phase 25 ml of water were added and was shaken vigorously. The immiscible solvents were separated with centrifugation at 4000 *g*. The supernatant was transferred into a separating funnel and the extraction was repeated three times. The aqueous phases were collected together and diluted with water to 250 cm^3. An aliquot of this solution was directly injected into the HPLC system. The hydrolysis and extraction method was similar to that in Ref. [9], modified and miniaturized for analytical purposes.

2.4. Chromatographic separation

The efficiency of the separation of basic substances was characterized on all columns by the separation of a pyridine–phenol mixture with water–acetonitrile (1:1) as eluent.

The separation of the serotonin-containing extracts was carried out using the gradient elution technique. Mobile phase A was acetonitrile–water (5:95, v/v) containing 0.05% trifluoroacetic acid and mobile phase B was acetonitrile–water (20:80, v/v) containing 0.05% trifluoroacetic acid. A linear gradient was applied from 100% A to 100% B in 5 min with a flow-rate of 2.0 ml min^{-1}. (For optimum analysis conditions on the LiChrosorb Select B column eluent A contained only 2% acetonitrile.) UV detection was carried out at 215 nm.

3. Results and discussion

Fig. 1 shows the chromatograms of the pyridine–phenol separations. On all "deactivated" packing materials pyridine eluted first, but a clear difference existed between the phases regarding relative retention and peak asymmetry. On the non-deactivated column the pyridine cannot be eluted in reasonable time. With a small influence of residual silanol groups, pyridine elutes before phenol.

Differences in the separation of a coffee wax sample also occurred on the different columns (Fig. 2). The Hypersil and laboratory-made packings behave similarly in the serotonin determination, although the specific surface areas of the two packings are different, 170 and 350 m^2

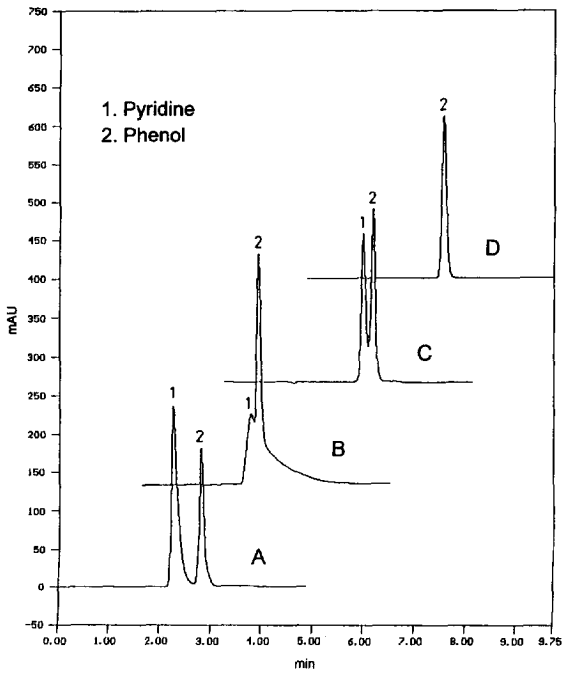

Fig. 1. Separation of pyridine and phenol with acetonitrile–water (1:1) eluent. Column: (A) laboratory-made; (B) Hypersil BDS; (C) LiChrosorb Select B; (D) LiChrosorb C_{18}.

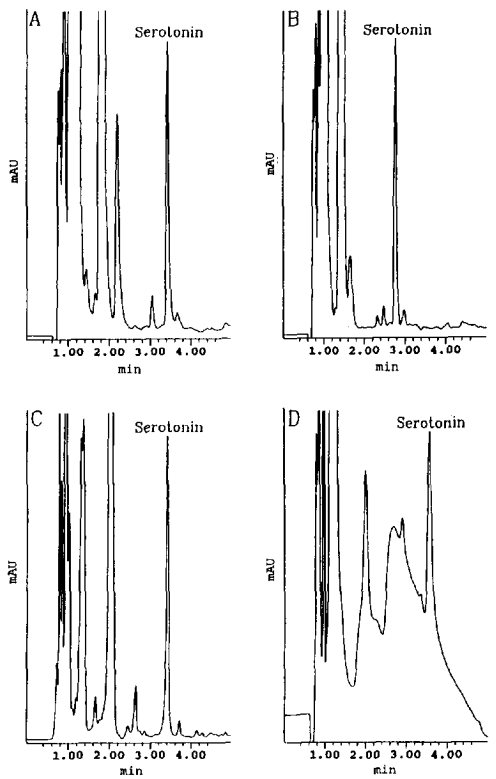

Fig. 2. Chromatogram of a coffee wax sample. Columns A–D as in Fig. 1. For conditions, see text.

g^{-1}, respectively, but the surface coverages are similar, 3.6 and 3.4 μmol m^{-2}, respectively). On the Select B column good separation can be achieved with modification of the mobile phase system. This packing is a C_8-modified silica material. The different selectivity of this phase can be explained by the different surface structure. A general-purpose, non-deactivated C_{18} column is far less suitable for the separation of coffee wax extract.

Under optimum mobile phase conditions on all deactivated columns the serotonin peak was completely resolved from the other components of the sample. Peak purity was determined using the three-dimensional peak spectrum surface measured with a diode-array detector. A 97% overlap between the serotonin peak of a pure serotonin standard and the serotonin peak of the coffee wax extract was found.

The determination of the serotonin content of the coffee wax sample was carried out with a standard addition method. The appropriate amount of standard serotonin was added to the coffee wax sample before hydrolysis. The recovery of the serotonin added to the coffee wax sample was 89.8% determined from six parallel measurements. In different measurements the hydrolysis and extraction procedure was carried out using only serotonin standard and coffee wax and serotonin standard, respectively. In both cases the same recovery was found. We believe that the recovery of the serotonin released from the coffee wax is similar.

The detection limit of the method was 2 ng of serotonin with a signal-to-noise ratio of 2. The serotonin calibration graph was found to be linear from 2 ng to 3.7 μg of standard.

The average serotonin content of the coffee wax sample determined from six measurements was 4.1 mg g^{-1}.

Acknowledgements

The authors thank József Kovács (Acropharma, Cologny, Switzerland) for kindly supplying the samples. This work was supported by the Hungarian National Scientific Research Fund (OTKA), project No. 458, and the US–Hungarian Joint Fund, project JFN0. 049/90, which is gratefully acknowledged.

References

[1] M.L. Battini, M. Careri, A. Casoli, A. Mangia and M.T. Lugari, Ann. Chim., 79 (1989) 369.
[2] A. Laganá, L. Curini, S. de Angelis Curtis and A. Marino, Chromatographia, 28 (1989) 593.
[3] U. Harms and J. Wurziger, Z. Lebensm.-Unters.-Forsch., 138 (1968) 75.
[4] E. Traiffort, P. Hubert and N. Tayeb, J. Chromatogr., 571 (1991) 231.
[5] P. Nebinger and M. Koel, J. Chromatogr., 427 (1988) 326.
[6] T.D. Crowe and D.W. Jacobsen, J. Liq. Chromatogr., 15 (1992) 221.
[7] F.C. Cheng, N.N. Lin, J.S. Kuo, L.J. Cheng, F.M. Chang and L.G. Chia, Electroanalysis, 6 (1994) 10.
[8] B. Takkenberg, E. Endert, H.E. Van Ingen and M. Ackermans, J. Chromatogr., 565 (1991) 430.
[9] Swiss Pat., 601227 (1978).
[10] C. Opper, G. Barka and W. Wesemann, Laborpraxis, 16 (1992) 346.