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Note

Determination of piperine in biological tissues by thin-layer chromatography and ultraviolet absorption densitometry

B. GANESH BHAT and N. CHANDRASEKHARA*

Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore-570 013 (India)

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Piperine is the active principle of black pepper (*Piper nigrum* L.), which is one of the most commonly used spices and which is valued for its pungency and aroma. Methods available for the determination of piperine in pepper have been reviewed recently [1, 2]. These are based on: (1) formation of colour with chromotropic acid [3], (2) spectrophotometry [4, 5], (3) estimation of nitrogen and multiplying the nitrogen content by the appropriate factor [6], and (4) the colorimetric determination of piperidine after alkaline hydrolysis of piperine [7]. Examination of the above methods revealed that they were not suitable for tissues because of high blanks and/or low sensitivity. As a part of metabolic studies on black pepper and piperine in progress in this laboratory, it became necessary to standardize a method for the determination of piperine in biological tissues. In the suggested procedure, the tissue extract is subjected to thin-layer chromatography (TLC) in 35% acetone in petroleum ether (60—80°C) by which piperine is separated from interfering material. It is then quantitated by UV absorption densitometry.

EXPERIMENTAL

Materials

Piperine (Sigma, St. Louis, MO, U.S.A.) was used as the authentic sample at a concentration of 2 mg/ml in chloroform. Solvents were distilled before use. Rat serum, liver, stomach, small intestine, caecum and large intestine were used as important representative tissues for spiking with piperine.

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Animals

Male albino rats of Wistar strain (120—140 g body weight) were maintained on a 18% casein diet [8] for two weeks. After an overnight fast, but with no restriction on water, six rats were administered, by gavage, 15 mg per 100 g body weight of piperine in 1.0 ml of refined peanut oil. Another six rats were administered 1.0 ml of oil only and served as controls. Rats were sacrificed 2 h later under ether anaesthesia; the gastrointestinal tract was removed and the contents were thoroughly washed out with 0.9% sodium chloride.

Extraction

Various concentrations of piperine were added to 1 g of each tissue, homogenized and then extracted according to the method of Folch et al. [9]. Portions of 1 g of the intestinal tissue from control and piperine-administered rats were similarly extracted. All tubes were covered with black paper since piperine in solution isomerizes to isopiperine, chavicine and isochavicine on exposure to light [10]. Each extract was made up to 20 ml, concentrated by flash evaporation under nitrogen, and suitable aliquots were taken for piperine determination. Total lipid in the extracts was determined by gravimetry.

Spectrophotometric method

Extracts were suitably diluted with chloroform—methanol (2:1) and covered with black paper. Estimation of piperine in extracts was made as described by Fagen et al. [4], by determining the absorbance at 345 nm.

Thin-layer chromatography

TLC plates were prepared by coating 20 × 20 cm glass plates with a 0.3-mm layer of silica gel G (Glaxo Labs., Bombay, India) using a Camag automatic TLC coater. The plates were air-dried and activated at 100°C for 1 h before use. Authentic piperine and different tissue extracts containing piperine were applied to the TLC plates and run in a solvent system of petroleum ether (60—80°C)—acetone (65:35, v/v) in the dark at 24 ± 1°C. After the plates were air-dried, the piperine spots were visulaized under UV light and R_F values were determined.

UV absorption densitometry

The chromatoplates were scanned in the direction of solvent development using an automatic Camag TLC scanner Model 2, mounted on a fluorometer Model III (Turner Assoc.), attached to a W + W Recorder 1100 (Scientific Instruments, Switzerland). Scanning conditions were: lamp, No. 110-850 (emission 310—390 nm); primary filter, 110-811 (can pass 365 nm); secondary filter, 110-823 (10% neutral density); plate speed 2 cm/min; chart speed, 1 cm/min. Quantitation was by calculation of areas after triangulation.

RESULTS AND DISCUSSION

Chromatography

Attempts at quantitative determination of piperine in tissue extracts using available methods were not successful because of high blanks and low sensitivi-

ty in the chromotropic—sulphuric acid [3] and phosphoric acid [11] procedures and low recovery of added piperine in the spectrophotometric procedure [4]. TLC followed by chromotropic—sulphuric acid spray followed by densitometry as described by Gunner [12] was also not suitable because of the coloured background of lipid spots. In order to eliminate interference from tissue components, TLC separation of piperine was resorted to. TLC on silica gel G using the mobile phase 35% acetone in petroleum ether (60—80°C) yielded the best separation of piperine with an R_F value of 0.60 ± 0.009 (S.E.M., n = 15). Quantitation was by UV absorption densitometry.

Sensitivity, linearity and reproducibility

Linear calibration curves were obtained when the concentration of piperine spotted was in the 1—4 μg range. The reproducibility was evaluated by making a series of eight chromtograms of a given concentration of piperine and determining the relative standard deviation of peak areas. The relative standard deviation ranged from 2.0% to 7.3% for different concentrations of piperine (Table I).

TABLE I

LINEARITY, SENSITIVITY AND REPRODUCIBILITY OF THE SUGGESTED METHOD

	Peak area (cm^2) corresponding to piperine			
	1 μg	2 μg	3 μg	4 μg
1	2.88	5.20	7.45	9.94
2	2.56	4.95	7.50	9.31
3	2.76	4.86	7.30	9.99
4	2.48	4.95	6.00	9.80
5	2.80	5.01	7.20	9.00
6	2.60	5.01	7.44	9.84
7	2.52	5.00	7.68	9.95
8	2.48	5.00	7.48	—
Average	2.64	5.00	7.26	9.69
S.E.M.	±0.055	±0.034	±0.186	±0.144
Coefficient of variation (%)	5.9	2.0	7.36	3.98

Non-interference by high lipid content

Fig. 1 represents the densitometric scans of chromatograms of pure piperine, tissue extract containing piperine (piperine administered and spiked) and a control tissue extract. Under these experimental conditions, the intestinal tissue contained 45 ± 12 μg/g piperine.

Data on the effect of adding different amounts of tissue lipids to known amounts of piperine are given in Table II for the spectrophotometric method as well as for the TLC—UV absorption densitometric method. From the above it is seen that tissue lipids did not interfere in the suggested procedure even when their concentration was 60 times that of piperine.

In all tissues except the caecum, the proposed method was comparable or

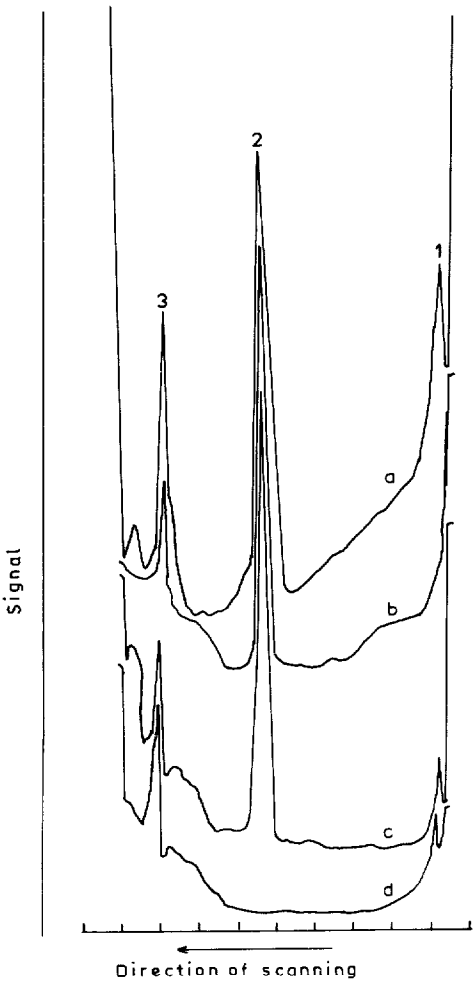

Fig. 1. Densitometric scans of chromatoplates spotted with (a) tissue extract (small intestine) containing piperine 2 h after administration, (b) piperine, (c) tissue extract spiked with piperine, and (d) control tissue extract. Peaks: 1 = origin, 2 = piperine, 3 = solvent front.

TABLE II

NON-INTERFERENCE BY TISSUE LIPIDS IN THE DETERMINATION OF PIPERINE

Tissue	Lipid/ piperine	Recovery* (%)	
		Spectrophotometric method	Suggested method
Serum	3	97	99
Liver	27	149	99
Stomach	23	92	99
Small intestine	58	90	97
Caecum	34	98	96
Large intestine	43	93	95

*Average of duplicates.

superior to the spectrophotometric method. The interference by non-piperine-absorbing materials in the spectrophotometric procedure is clearly seen in the case of liver. Since, in metabolic studies, it would not be possible to have a tissue blank, the suitability of the proposed method is evident.

Recovery

Known amounts of piperine were added to tissues which were then extracted by the method of Folch et al. [9]. These extracts were then taken through the procedure for piperine determination as mentioned above. As indicated in Table III, the recovery of added piperine was in the range 95—100%.

The method worked out has the advantage that the chromatoplate may be scanned directly, is more sensitive than other published procedures [3, 4, 7, 11, 12] and 0.01 μmol can be detected per gram tissue.

TABLE III

RECOVERY OF PIPERINE ADDED TO DIFFERENT TISSUES

Piperine added (mg)	Recovery (%) in tissues*					
	Serum	Liver	Stomach	Small intestine	Caecum	Large intestine
0.5	100	97	97	99	100	99
1.0	99	99	99	97	96	95
2.0	100	98	100	98	100	96
5.0	100	98	97	100	98	95

*Average of duplicates.

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REFERENCES

1 B. Labruyere, J. Agr. Food Chem., 14 (1966) 469.
2 V.S. Govindarajan, CRC Crit. Rev. Food Sci. Nutr., 9 (1977) 115.
3 A.L. Leonard, Anal. Chem., 28 (1956) 1621.
4 H.T. Fagen, E.P. Kolene and R.V. Hussong, J. Agr. Food Chem., 3 (1955) 860.
5 S. Narasimhan and D. Rajalakshmi, J. Food Sci. Technol., 18 (1981) 11.
6 Official Methods of Analysis, Association of Official Analytical Chemists, Washington, DC, 13th ed., 1980, p. 498.
7 M.L. Shankaranarayana, S. Nagalakshmi and C.P. Natarajan, Flavour Ind., 1 (1970) 173.
8 R. Vijayalaxmi and N. Chandrasekhara, Toxicology, 16 (1980) 259.
9 J. Folch, M. Lees and G.H. Sloane-Stanley, J. Biol. Chem., 226 (1957) 497.
10 M. Verzele, P. Mussche and S.A. Qureshi, J. Chromatogr., 172 (1979) 493.
11 D. Graham, J. Food Sci., 30 (1965) 651.
12 S.W. Gunner, J. Chromatogr., 40 (1969) 85.