RESEARCH PAPER

Accepted 16 October 2001
Revised 5 October 2001
Received 29 December 2000
Indian J. Pharm. Sci., 2002, 64(1): 44-47

Adaptation of Labat Test to the Assay of Piperine, alone and in Combination with Rifampicin, Isoniazid and Nimesulide

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A simple and rapid colorimetric method of assay of piperine is reported. It is an adaptation of Labat test for methylenedioxy group attached to an aromatic nucleus. The resultant blue green colour shows maximum absorbance at 656 nm and obeys Beer's law in a concentration range of 10-25 µg/ml. Results of analysis on different piperine samples are comparable with direct spectrophotometry and HPLC methods. The method is also suitable for assay of piperine in combination with rifampicin, isoniazid, and nimesulide.

Piperine, the principle pungent substance in pepper, is the major constituent of *Piper nigrum* and *P. longum*. It has diverse pharmacological activities such as CNS depressant, antipyretic, analgesic and anti-inflammatory activities[1] and antibacterial and antitumour activities[2]. Piperine causes enhanced bioavailability having the property of inhibiting hepatic and intestinal enzymes involved in the biotransformation of drugs[3]. It has shown pharmacokinetic synergistic effect on rifampicin[3], isoniazid[3] and nimesulide[4] resulting in reduced therapeutic dosage of the drugs.

Literature survey revealed a number of methods for estimating piperine, notably colorimetric methods using HNO_3[5], H_2SO_4 and aromatic aldehyde[6] and *p*-nitrophenyl diazonium fluoroborate[7], and direct spectrophotometric[8] and HPLC[9] methods. A colorimetric method of assay of piperine was reported[10] based on the reaction of formaldehyde, produced from piperine, with chromotropic acid reagent. Of all these methods the direct spectrophotometric and HPLC are more specific methods provided the samples are protected from photoisomerization.

Labat test is employed in the examination of plant products for the detection of methylenedioxy group attached to an aromatic ring system. We have adapted this test for estmation of piperine in solid samples as well as in extract besides combinations of piperine with other drugs and compared the results with those obtained by direct spectrophotometry and modified HPLC methods.

MATERIAL AND METHODS

A GBC Model 918 UV-Visible Spectrophotometer with matched quartz cuvettes was used for colorimetric and spectrophotometric determination.

The chemicals acetonitrile and water (HPLC grade) were purchased from Merck. Triethylamine and orthophosphoric acid were purchased from Thomas Baker while gallic acid was from Loba (all Analar grade).

Preparation of standard solution:

A standard sample of piperine was obtained by isolation from the fruits of *Piper nigrum* and its purity was assessed by t.l.c., m.p., UV, ^1H NMR, MS, DSC and HPLC.

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Presented at the 52 Indian Pharmaceutical Congress, Hyderabad, Dec 1-3, 2000.

A solution of piperine was prepared by dissolving 10 mg of standard piperine in 100 ml of methanol to get a working standard of 100 µg/ml.

Method of analysis:

Different aliquots of piperine solution (0.05 to 0.5 ml) were transferred to 10 ml stoppered test tubes. Gallic acid in methanol (0.1 ml) and concentrated. H_2SO_4 (5 ml) were added. The mixture was heated in a boiling water bath for 90-100 s, cooled to room temperature and the resulting blue green colour measured at 656 nm against a reagent blank in the UV-Visible spectrophotometer. In direct spectrophotometry the absorbance of samples containing 2-6 µg/ml in methanol was measured at 344 nm against a solvent blank.

HPLC method:

HPLC was performed in a system consisting of Model C-R7A, isocratic pump (LC-8A), Hychrome column (C-18, 250 x 4.6 mm, 5 µm) and a variable wavelength UV-Vis detector (SPD-10A) at 344 nm. The mobile phase was prepared by mixing 1ml of triethylamine in 500 ml of distilled water and the pH adjusted to 7.5 with dilute orthophosphoric acid. This solution was mixed with acetonitrile (1:1 v/v), filtered through a 0.45 µm particle size membrane and degassed under vacuum. The flow rate of the mobile phase was 1 ml/min. Samples (10 µl) containing 0.25 – 0.75 mg/ml were injected directly.

Recovery experiments:

Recovery experiments were performed by adding a known amount of piperine to known amount of rifampicin, isoniazid, and nimesulide (in the ratio of 1:5 to 1:20) and analyzing for piperine by the three methods.

RESULTS AND DISCUSSION

The blue-green colour showed an absorption maximum at 656 nm (fig. 1). The colour complex is stable for one hour and obeys Beer's Law in the range of 10-25 µg/ml of piperine. The molar absorptivity, Sandell's sensitivity, and correlation coefficient were found to be 2.77 x 10^4 l mol. cm, 0.036 µg cm, and 0.9994, respectively (Table 1). Linearity range, % relative standard deviation, and correlation coefficient were determined for HPLC (see Table 2 and fig. 2).

Piperine samples of different purity obtained from extracts as also the crude extracts were analyzed by the proposed method and the results compared with those obtained by the direct spectrophotometeric and HPLC

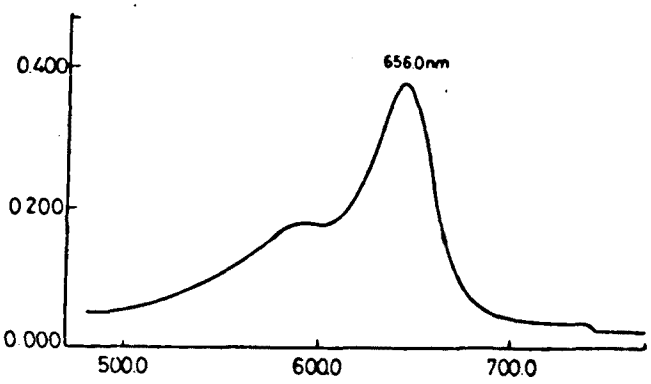

Fig. 1: Spectrum of piperine color complex.

TABLE 1: OPTICAL AND STATISTICAL PARAMETERS OF PIPERINE IN THE PROPOSED METHOD.

PARAMETER	VALUES
λmax (nm)	656
Beer's law limit (µg/ml)	10-25
Molar absorptivity (1.. mole. cm)	2.77 x 10^4
Sandell's sensitivity	
(µg/cm^2/0.001 absorbance unit)	0.036
Regression equation*	
Slope (b)	2.54 x 10^2
Intercept (a)	5.61 x 10^2
Relative standard deviation (%)**	0.103
% Range of error (0.05 level)	1.89
Correlation coefficient	0.9994

*Represents the equation Y = a + bx, where x is the concentration of piperine in µg/ml and Y is the absorbance at the corresponding λmax. **indicates that the value is an average of six replicates.

TABLE 2: EXPERIMENTAL PARAMETERS FOR THE ESTIMATION OF PIPERINE BY HPLC METHOD.

Parameter	Values*
Linearity range (µg/ml)	0.25 – 0.75
% Relative standard deviation	0.044
% Range of error	0.031
Correlation coefficient	0.9996

*Each value is an average of five determinations.

method (Table 3). The results obtained for solid samples by the three methods are comparable in all cases. Direct spectrophotometry is completely unsuitable for extracts whereas the proposed method and HPLC are suitable to some extent. The interference in the colorimeteric method was due to the presence of other components containing methylenedioxy group (like other piperinoids and lignans) present in the extracts.

The proposed method, as also the HPLC, is applicable for analyzing combinations of piperine with rifampicin, isonizaid, and nimesulide in the ratio of 1:5 to 1:20 without any interference (fig. 3), observed with the direct spectrophotometric method (due to obverlap of the absorption maxima). The results obtained for piperine added to the drugs in the ratio 1:10 are given in Table 4.

TABLE 3: ANALYSIS OF PIPERINE SAMPLES FROM *Piper nigrum* EXTRACTS.

Colorimetric	Direct Spectrophotometric	HPLC
95.3%	91.4%	94.11%
93.25%	91.23%	91.24%
92.3%	89.17%	90.40%
92.9%	93.0%	91.83%
81.22%	81.22%	82.22%

In the colorimetric method detection was made at 656 nm. In the direct spectrophotometric and HPLC methods detection was made at 344 nm.

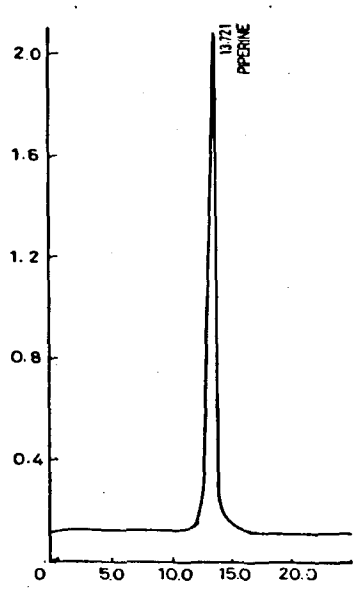

Fig. 2: HPLC chromatogram of piperine.

The proposed colorimetric method involves liberation of formaldehyde from the methylenedioxy group of piperine and conversion to paraformaldehyde which reacts with gallic acid in the presence of H_2SO_4 to produce the blue-green colour. We have observed that formaldehyde itself does not give the colour. The method is simple, precise and rapid and can be used for the routine analysis of piperine samples, alone and in combinations with drugs like rifampicin, isoniazid and nimesulide.

TABLE 4: RECOVERY OF PIPERINE ADDED TO RIFAMPICIN, ISONIAZID, AND NIMESULIDE.

Sample	Spectrophotometric method			Colorimetric method			HPLC method		
	Added (mg)	Found (mg)	% Recovery*	Added (mg)	Found (mg)	% Recovery*	Added (mg)	Found (mg)	% Recovery*
Rifampicin (50 mg)	5	4.6	92	5	4.85	97	5	4.95	99
Isoniazid (50 mg)	5	4.8	96	5	4.9	98	5	4.93	98.6
Nimesullde (50 mg)	5	4.8	96	5	4.88	97.5	5	4.95	99

*Values represent average of six experiments

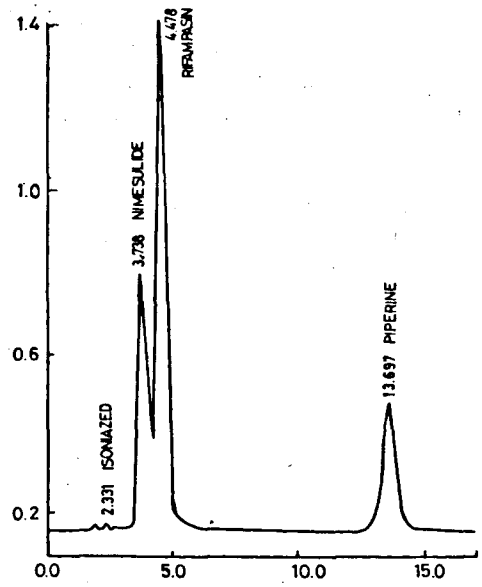

Fig. 3: HPLC chromatogram of piperine with isoniazed, rifampicin and nimesulide

ACKNOWLEDGEMENTS

The authors thank Mr. V.C. Nannapaneni, Chairman and Managing Director, Natco Pharma Ltd. for his interest in this work and encouragement and Ms. Veer Chemie and Aromatics Private Ltd., Hyderabad for the gift of a sample of isoniazid.

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