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Short communication

Liquid chromatographic method for determination of piperine in rat plasma: application to pharmacokinetics

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Abstract

Piperine, a major alkaloid of *Piper longum* and *Piper nigrum* has been reported to have several pharmacological/toxicological effects. Though a number of methods for analysis of this omnipresent food component in pepper fruits are available, its analysis in body fluids has been largely neglected. A high-performance liquid chromatography method for the analysis of piperine in rat plasma is presented in this communication. Analysis was performed using a Symmetry® C_{18} column (250×4.6 mm) by isocratic elution with 25 m*M* KH_2PO_4 (pH 4.5)–acetonitrile (35:65) and UV detection at 340 nm. The calibration plot was linear over the range studied (2–2000 ng) with correlation coefficient of 0.9984. Limit of detection and limit of quantitation were 1 ng/ml and 3 ng/ml, respectively. Good overall recovery (85.5±6%) was obtained with 4 ml ethyl acetate and extraction time of 3 min. Intra- and inter-assay coefficient of variation was found to be less than 7.5%. Plasma concentration–time profile of piperine in a conscious rat implanted with jugular vein cannula was obtained using this method. The method is simple, sensitive and reproducible.
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Keywords: Piperine

1. Introduction

Piperine (Fig. 1), a major alkaloid of black and long peppers, possesses several pharmacological actions [1–3]. In addition, it has been reported to enhance the bioavailability of several drugs [4,5]. Recently, it has been patented as bioavailability enhancer [6], non-nicotine smoking cessation aid [7] and as an important ingredient of incapacitating composition [8]. HPLC as well as gas chromatographic methods for analysis of piperine in peppers, pepper extract and oleoresin have been reported [9–11]. However, there is no sensitive and reproducible method for quantitation of piperine in body fluids. A HPTLC method has been reported for its analysis in body fluids with manually prepared silica gel plates [12]. Piperine could be detected in human milk and serum by using a complex HPLC method (isocratic plus gradient elution with mixture of four solvents) developed for quantitation of curcuminoids

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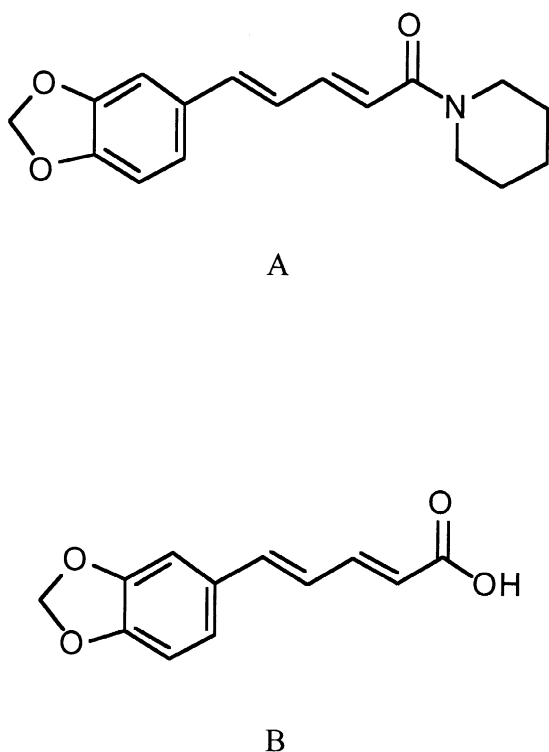

Fig. 1. Chemical structures of piperine (A) and piperic acid (B).

[13]. However, the method does not describe quantitation of piperine in these fluids. This communication describes a simple, rapid, sensitive and reproducible HPLC method for analysis of piperine in rat plasma.

2. Experimental

2.1. Materials

Analytical grade potassium dihydrogenphosphate, anhydrous sodium sulphate were from Ranbaxy (Mohali, Punjab, India) and orthophosphoric acid (analytical grade) was from Qualigens (Mumbai, Maharashtra, India). HPLC grade acetonitrile and ethyl acetate were from Merck (Mumbai, Maharashtra, India). Piperine (purity >99%, HPLC) was isolated from pepper oleoresin as described earlier [1] while piperic acid (purity >99%, HPLC) was obtained by alkaline hydrolysis of piperine. Pentobarbitone Na was purchased from Sigma–Aldrich (St. Louis, USA)

2.2. Chromatography

A Shimadzu (Japan) HPLC system with LC-10A pump, SPD-M10A photodiode array detector set at 340 nm was used. The system was fitted with a Rheodyne injector and samples were injected manually. Data were collected and analysed using computer software Class LC 10A ver 3.1 (Shimadzu, Japan). Separation was achieved using a Waters (Massachusetts, USA) Symmetry® C_{18} column (250×4.6 mm, 5 μm) preceded by a guard column of the same material, 10×3.2 mm. The solvent system, 25 m*M* KH_2PO_4 (pH 4.5, adjusted with orthophosphoric acid)–acetonitrile (35:65), was pumped isocratically at 1 ml/min.

2.3. Sample preparation

Plasma and bile samples (0.1 ml each) were extracted with 4 ml of ethyl acetate for 3 min in amber coloured tubes and centrifuged (6000 *g*, 5 min). Ethyl acetate layer was removed and dried over anhydrous sodium sulphate. The ethyl acetate was then evaporated under a stream of nitrogen at 50 °C. The residue was reconstituted in 0.1 ml of solvent system and 20–50 μl injected into the HPLC system. During all the operations, samples were protected from light due to susceptibility of piperine to photoisomerisation. A standard plot was prepared by injecting 2–2000 ng of piperine dissolved in acetonitrile. Recovery, intra-assay and inter-assay studies (3 days) were performed by spiking 0.1 ml of plasma and bile samples with 1, 50 and 200 ng of piperine ($n=4$ for each concentration) and extracting the samples with ethyl acetate as described above.

2.4. Plasma concentration–time profile of piperine and biliary excretion

The study was performed in conscious rats ($n=5$) implanted with jugular vein cannula. Piperine (20 mg/kg p.o.) was administered as a solution prepared in a 1:7:30 mixture of ethanol, propylene glycol and water, respectively. Blood samples (~0.3 ml) were collected in microcentrifuge tubes at 0, 0.083, 0.25,

0.5, 1, 2, 4, 6 and 8 h. The blood volume withdrawn was replaced with equal volume of heparinised saline. Plasma was immediately separated by centrifugation and analysed the same day. Bile was collected from a pentobarbitone Na anaesthetised rat by common bile duct cannulation. Bile collected before the administration of 100 mg/kg i.p. piperine served as control.

3. Results and discussion

Blank plasma gave no interfering peak at the retention time of piperine (Fig. 2) making the analysis very simple. The calibration plot was linear over the range studied (2–2000 ng). The equation for the straight line was $y = 0.0001(x) - 0.7348$ ($r^2 = 0.9984$). The solvent system of 25 mM KH_2PO_4 (pH 4.5)–acetonitrile (35:65) was found to be optimal for analysis of piperine in plasma. Good overall recovery (85.5±6%) was obtained with 1×4 ml of ethyl acetate with extraction time of 3 min. Lower extraction volume of ethyl acetate as well as decreased extraction time resulted in low and variable recoveries. Limit of detection and limit of quantitation were 1 ng/ml and 3 ng/ml, respectively. Ethyl acetate-extracted blank bile sample did not give any interfering peaks at the retention time of either piperine or piperic acid (Fig. 3). Intra- and inter-day assay variation was less than 7.5%.

The plasma concentration–time profile of piperine in rats ($n = 5$) is presented in Fig. 4. Piperine could be detected in plasma from 0.25 to 8 h after administration with maximum plasma concentration (2.83 μg/ml) at 0.5 h post dosing.

Piperine is a major alkaloid of peppers which are widely used as spice. It has received attention due to its potential to increase bioavailability of drugs when co-administered. Considering its omnipresence in food as well as its effect on drug metabolising enzymes and gastrointestinal tract, development of a simple method for its determination in body fluid is of obvious significance.

Piperine, a lipophilic molecule, could be extracted from plasma using ethyl acetate. For separation of piperine from endogenous plasma components as

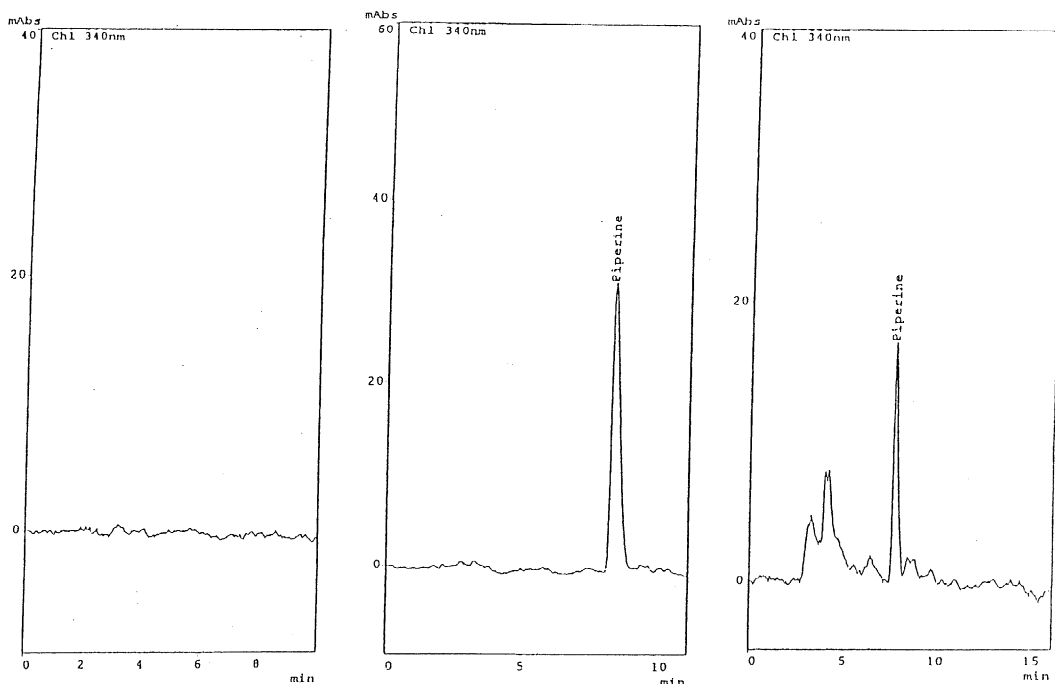

Fig. 2. Chromatograms of blank plasma (left panel), plasma spiked with 20 ng of piperine (middle panel) and a plasma sample obtained from a rat treated with 20 mg/kg p.o. piperine (right panel).

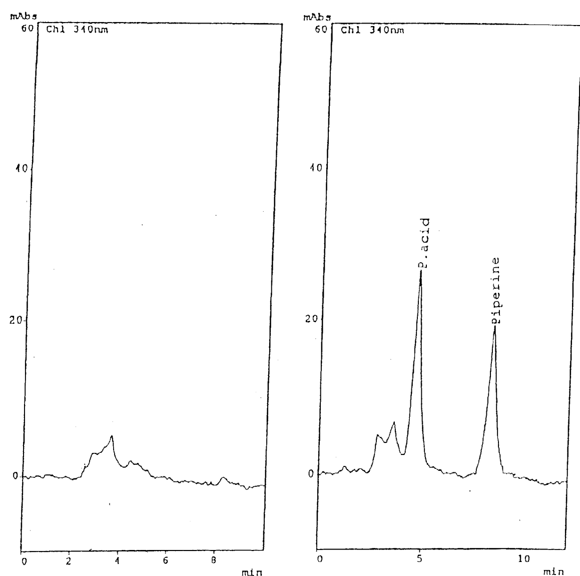

Fig. 3. Chromatograms of blank bile (left panel) and a bile sample spiked with 20 ng each of piperine and piperic acid (right panel).

well as to obtain a sharp peak, a balance of organic and aqueous phase was obtained. Use of more than 65% of organic solvent in the mobile phase resulted in the merger of piperine peak with plasma components; whereas, increasing the aqueous phase proportion to more than 35% broadened the peak. Monitoring of samples at 340 nm (λ_{max} for piperine) helped in reducing the interference from endogenous plasma components which absorb poorly at this wavelength.

The method was used for studying the plasma concentration–time profile of piperine in rats. Piperine is rapidly absorbed through the gastrointestinal tract and could be detected in plasma as early as 15 min after administration. In the previous report on pharmacokinetics of piperine [14], it could not be detected in serum up to 3 h after oral administration to rats due to low sensitivity of the HPTLC method used for analysis (limit of quantitation 4 μg/ml). Thus, complete and realistic pharmacokinetic results could be obtained using the method described here.

Piperic acid (Fig. 1), a metabolite of piperine, has been previously reported to be excreted in bile after administration of piperine [15]. Piperine itself was detected in faeces after intraperitoneal administration and it was suggested to be due to biliary excretion [14]. Hence, it was thought pertinent to see if this method could be used for analysis of piperic acid as well as piperine if both the entities happen to be excreted in bile. As can be seen in Fig. 3, the method could be easily applied to the analysis of both these compounds in bile. However, in the present studies, these compounds could not be detected in bile up to 6 h after administration, questioning the earlier studies. So far, piperine has not been directly detected in bile and its detection in faeces by some researchers could be due to incomplete systemic absorption from the suspension formulation used and may not be the result of biliary excretion.

In conclusion, a simple, rapid, sensitive and reproducible method for analysis of piperine in rat plasma is developed which could be used to obtain a complete plasma-concentration time profile of piperine in rats.

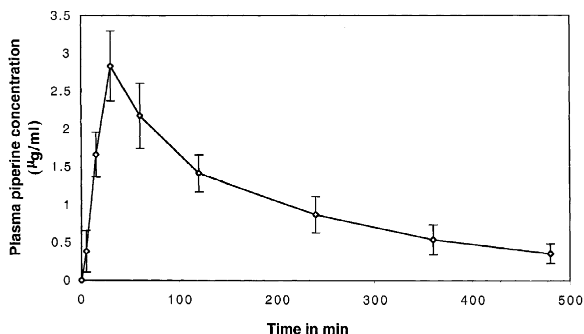

Fig. 4. Plasma concentration–time profile of piperine (20 mg/kg p.o.) in rats ($n=5$).

Acknowledgements

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References

[1] S. Bajad, K.L. Bedi, A.K. Singla, R.K. Johri, Planta Med. 67 (2001) 176.
[2] S. Bajad, K.L. Bedi, A.K. Singla, R.K. Johri, Planta Med. 67 (2001) 284.
[3] L. Zhixiu, J.R.S. Hoult, D.C. Benett, A. Raman, Planta Med. 65 (1999) 600.

[4] C.K. Atal, U. Zutshi, P.G. Rao, J. Ethnopharmacol. 4 (1981) 229.
[5] G. Bano, R.K. Raina, U. Zutshi, K.L. Bedi, R.K. Johri, S.C. Sharma, Eur. J. Clin. Pharmacol. 41 (1991) 615.
[6] M. Majeed, V. Badmaev, US Pat. No. 5744161 (1998).
[7] F.M. Behm, J.E. Rose, US Pat. No. 5893371 (1999).
[8] J. Fedida, US Pat. No. 5821450 (1998).
[9] M. Verzele, G. Redant, S.A. Qureshi, P. Sandra, J. Chromatogr. 199 (1980) 105.
[10] D.E. Games, N.J. Alcock, J.V.D. Greef, L.M. Nyssen, H. Moarse, M.C.T. Noever, J. Chromatogr. 294 (1984) 269.
[11] M. Verzele, S.A. Qureshi, Chromatographia 13 (1980) 241.
[12] G.B. Bhat, N. Chandrashekhara, J. Chromatogr. 338 (1985) 259.
[13] F. Khachik, C.J. Smith, J.C. Smith, Anal. Chem. 69 (1997) 1873.
[14] G.B. Bhat, N. Chandrashekhara, Toxicology 40 (1986) 83.
[15] G.B. Bhat, N. Chandrashekhara, Toxicology 44 (1987) 99.