

Short communication

Sensitive high-performance liquid chromatographic assay method for the determination of picoside I in plasma¹

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Abstract

Picoliv is a new hepatoprotective agent, being developed at the Central Drug Research Institute (CDRI, Lucknow, India). Picoside I is a major active constituent of picoliv. A sensitive high-performance liquid chromatographic assay method in plasma has been developed and validated for the determination of picoside I in plasma. The method consists of extraction of the drug, after protein precipitation with methanol, from rabbit plasma samples. Separation was achieved using a C₁₈ endcapped reversed-phase column coupled with a photodiode array detector and acetonitrile–0.1 M acetic acid (25:75) as mobile phase. The lower limit of quantification in plasma is 50 ng/ml. The standard curve was linear over the range of 50–500 ng/ml in rabbit plasma. The analytical recovery of picoside I added to plasma was >80%. The reproducibility was determined by the inter- and intra-assay precision which were <15%. © 1997 Elsevier Science B.V.

Keywords: Picoside I

1. Introduction

Picoliv [1] is a new hepatoprotective agent [2–5], undergoing phase 2 clinical trials. It is obtained from the ethanolic extract of the roots of *Picrorhiza kurroa*. It is a standardised irridiod–glycoside mixture having potent hepatoprotective action. Picoside I (6-*O*-cinnamoylcatalpol) (I, Fig. 1) [6–8] is one of the major active constituents of picoliv. Several methods have been reported for the estimation of picoside I in bulk drug samples and formulations [9–11]. This paper describes a simple and sensitive HPLC assay for the determination of picoside I in

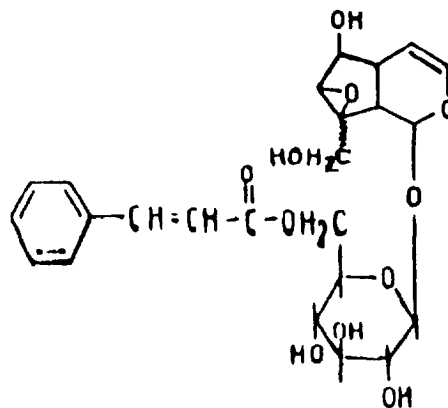


Fig. 1. Structure of picoside I.

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plasma used for bioavailability studies. The assay was used for quantitation of picroside I in rabbit plasma after oral administration of picroliv.

2. Experimental

2.1. Reagents and standards

Standard picroside I (>98%) was supplied by Dr. D.K. Kulshreshtha, Medicinal Chemistry Division of this Institute. HPLC-grade methanol and acetonitrile and glacial acetic acid of Analar grade were obtained from E. Merck (India) (Bombay, India).

2.2. Apparatus and chromatographic conditions

The HPLC system was equipped with a Perkin-Elmer 250 binary pump (Perkin-Elmer, Norwalk, CT, USA), a Model 7125 injector with a 20-ml loop (Rheodyne, Berkeley, CA, USA) and a Perkin-Elmer 235 diode array detector. Separation was achieved using a 25×0.4 cm (5 µm) C₁₈ endcapped cartridge analytical column coupled with a 4×4 mm guard column of the same material. Chromatograms were recorded on a G.P. 100 printer plotter (Perkin-Elmer).

The mobile phase consisted of acetonitrile–0.1 M acetic acid (25:75 v/v). It was filtered and degassed before use. Chromatography was performed at ambient temperature at a flow-rate of 1 ml/min. Effluents were monitored at 280 nm.

2.3. Plasma calibration standards

The stock solution of picroside I (100 µg/ml) was prepared by dissolving 5 mg of picroside I in 50 ml of water. Five plasma calibration standards were prepared by adding 25 µl each of (2–20 µg/ml) solutions of picroside I, prepared by a serial dilution method from stock solution, to drug-free plasma to obtain concentrations of 50, 100, 250, 375 and 500 ng/ml. The plasma standards were vortex mixed.

2.4. Extraction

Methanol (0.5 ml) was added to plasma (0.5 ml) by vortex mixing for 2 min and centrifuging for 10

Table 1
Recovery of picroside I

Conc. (ng/ml)	Extraction efficiency (%)	C.V. (%)
50.0	85.60	16.51
100.0	87.59	4.66
500.0	82.99	9.38

min at 3000 rpm. The resulting supernatant was transferred to another tube, methanol was evaporated off under nitrogen, and the residue was extracted with 3×0.5 ml of ethyl acetate. The ethyl acetate layers were combined and evaporated to dryness. The residue was reconstituted with 50 µl of methanol, and 20 µl were loaded onto the HPLC column.

2.5. Accuracy and precision

Picroside I was added to plasma ($n=3$) at three concentration levels. Aliquots (0.5 ml) of plasma, each spiked with 25 µl of (2, 4 and 20 µg/ml) aqueous solutions of picroside I, were processed as described above and concentrations were calculated from the standard curve in plasma. The accuracy of the method was calculated based on the difference between the mean calculated and added concentrations (%D.F.A.) while precision was determined by calculating the inter- and intra-day coefficient of variation (%C.V.).

2.6. Extraction efficiency

Plasma spiked with 50, 100 and 500 ng/ml of picroside I was analyzed according to the method described above. Absolute recovery of picroside I was calculated by comparing these peak heights with those obtained by direct injection of picroside I in mobile phase (Table 1).

3. Results and discussion

3.1. Chromatography

The HPLC method described herein provides a good separation of picroside I from the other indigenous plasma constituents. Fig. 2 shows a chromatogram of: (i) standard picroside I containing 4

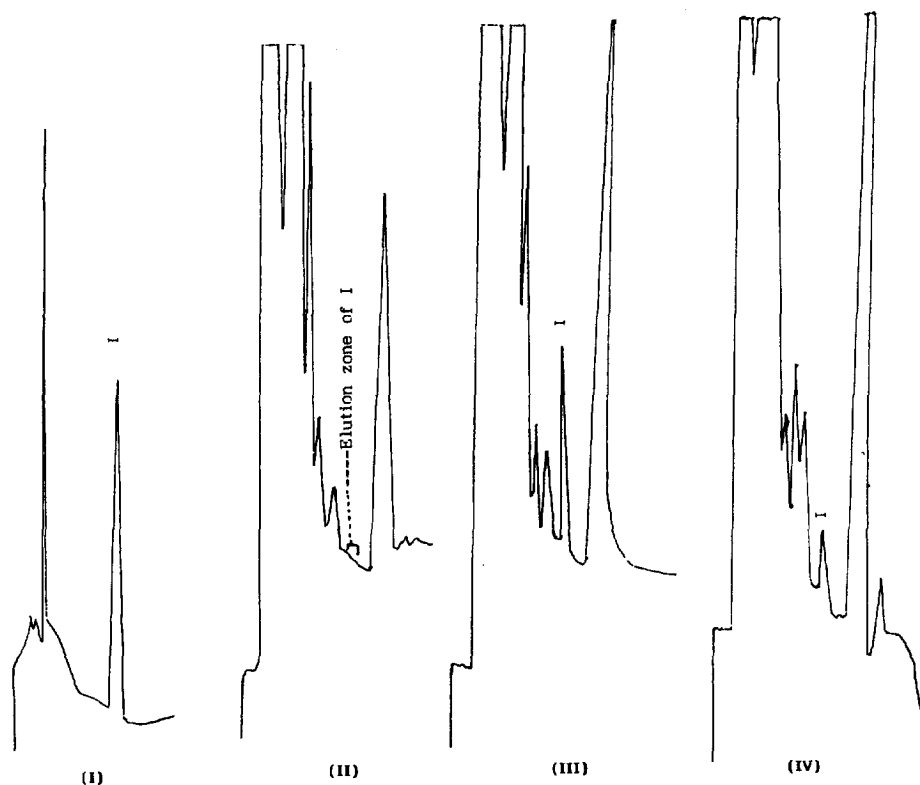


Fig. 2. Chromatogram of: (i) standard picoside I containing 4 $\mu\text{g/ml}$ in mobile phase; (ii) blank plasma from a rabbit; (iii) plasma containing 200 ng/ml of picoside I; and (iv) a plasma sample, after 5 h, from a rabbit administered a single 100-mg tablet of Picroliv.

$\mu\text{g/ml}$ in mobile phase; (ii) blank plasma from a rabbit; (iii) spiked plasma containing 200 ng/ml of picoside I; and (iv) a plasma sample, after 5 h, from a rabbit administered a single 100-mg tablet of Picroliv. Under the chromatographic conditions, the retention time of picoside I was about 8.5 min, other indigenous plasma constituents extracted did not interfere since they eluted either before or after the peak of interest.

The lower limit of quantification of picoside I was 50 ng per 0.5 ml of plasma. This method provides adequate sensitivity and specificity for monitoring plasma levels of picoside I.

3.2. Selectivity and peak purity

The photodiode array detector gave a peak purity index, which indicated a pure peak without any interference from endogenous substances of plasma.

The peak was identified by its retention time and absorption maxima at 280 nm.

3.3. Linearity and reproducibility

Linear least-squares regression analysis of the calibration graph demonstrated linearity in the range 50–500 ng/ml. A typical standard curve ($r=0.9974$) could be described by the equation

$$\text{Unknown conc. (ng/ml)} = 97.362 \times \text{peak height} - 23.273.$$

The reproducibility and accuracy of the method is calculated by inter- and intra-assay precision (%R.S.D.) of concentration found and by calculating % of mean deviation from actual concentration, respectively. Both of them were found to be well within the acceptable limits (Table 2) [12].

Table 2
Inter- and intra-assay precision

Conc. (ng/ml)	Inter-assay variation (n=3)			Intra-assay variation (n=3)		
	Conc. found (ng/ml)±S.D.	%C.V.	%D.F.A.	Conc found (ng/ml)±S.D.	%C.V.	%D.F.A.
50	55.40±5.86	10.59	10.80	55.40±5.86	10.59	10.80
100	110.26±3.28	2.98	10.26	106.94±7.62	7.13	6.94
500	510.48±6.62	1.3	2.1	500.46±11.64	2.33	0.09

3.4. Application of the method in pharmacokinetics

The assay method described here was applied to determine the concentration vs. time profile of picoside I in rabbits after a single oral dose (100 mg) of Picroliv. No interfering peaks were present in the elution region of picoside I. Further studies are in progress to evaluate the pharmacokinetic parameters of picoside I.

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