BIOAVAILABILITY AND PHARMACOKINETICS OF VASICINE IN WISTAR RATS

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Summary

To determine vasicine in rat blood plasma by HPLC and to study its bioavailability and pharmacokinetics.

Vasicine (>97% pure by HPLC) was purchased from SPIC Pharmaceuticals Division, Chennai, India. The standard plot was prepared by spiking the various concentrations of pure vasicine into the normal rat blood plasma followed by extraction and HPLC determination. A single oral dose of 0.065mg/Kg of pure vasicine was administered to all the rats. Plasma levels of vasicine were analysed by using HPLC. Vasicine was extracted from the rat blood plasma by adding 10N hydrochloric acid at pH 6.2 for the precipitation of plasma proteins followed by extraction into chloroform and the chloroform extract was evaporated to dryness to obtain the vasicine residue. The residue was reconstituted in methanol (1ml) and then injected into the HPLC system.

The standard plot was linear for various concentrations studied with linear regression coefficient R^2 of 0.9903. Accuracy was established by the percent recovery at all the concentrations of vasicine and the mean recovery was found to be 108.73%±7.845. The mean peak plasma vasicine concentration of 12.8 ng/ml was observed at 4 h.

The present study shows determination of pure vasicine in rat plasma by HPLC. The various pharmacokinetic parameters for vasicine were studied.

Key Words: Vasicine, plasma, HPLC, bioavailability, pharmacokinetics

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Introduction

Vasicine, a bioactive pyrralazoquinazoline alkaloid isolated from the medicinal herb *Adhatoda zeylanica* also known as *Justacia adhatoda* (Family-Acanthaceae) was reported to possess bronchodilatory and expectorant properties (1-3). Determination of vasicine by HPLC in herbal formulations has been reported earlier by other authors (4, 5). Vasicine is also determined in Adulsa syrup by TLC-UV spectrophotometric method (6). Pharmacokinetics of vasicine in healthy volunteers after intravenous administration has been studied (7). Various methods are available for the determination of vasicine from the formulations but no literature is available for bioavailability and pharmacokinetic studies for vasicine on oral administration in rats. Hence, the current experiment employs high performance liquid chromatographic method for the determination of vasicine concentration in rat blood plasma and its pharmacokinetic studies after the single oral dose of administration in Wistar rats.

Material and methods

Drugs and chemicals

The standard vasicine (>97% pure by HPLC) was purchased from SPIC Pharmaceuticals Division, Marai Malai Nagar, Chennai, India. Methanol (HPLC grade) and water (HPLC grade) were requisioned from Merck and Nice Chemicals respectively, while all other chemicals used in the experiment were of analytical grade.

Instruments and Chromatographic Conditions

Shimadzu HPLC of SCL-10AVP equipped with LC-10-ATVP pump and SPD-10-AVP UV detector were used (Shimadzu Corp., Japan). The column used was Luna 5µ-C18(2) of 250 x 4.6 mm id (Phenomenex, USA). Methanol and water in the ratio of 40:60 was used as a mobile phase. The flow rate of 0.7 ml/min and the λ_{max} at 298 nm was used (8).

Experimental Animals

Normal healthy albino rats of Wistar strain of either sex weighing between 150-200 g were selected for the experiment from an inbred colony maintained under the controlled conditions of temperature (23 ± 2°C), humidity (50±5%) and light (10 and 14 h of light and dark, respectively).

Typical chromatogram

The vasicine compound was dissolved in methanol in a volumetric flask to get the concentration of 100 µg/ml. Twenty microliters of this standard solution was injected into the HPLC system. Peak area value and retention time were recorded (Fig. 1).

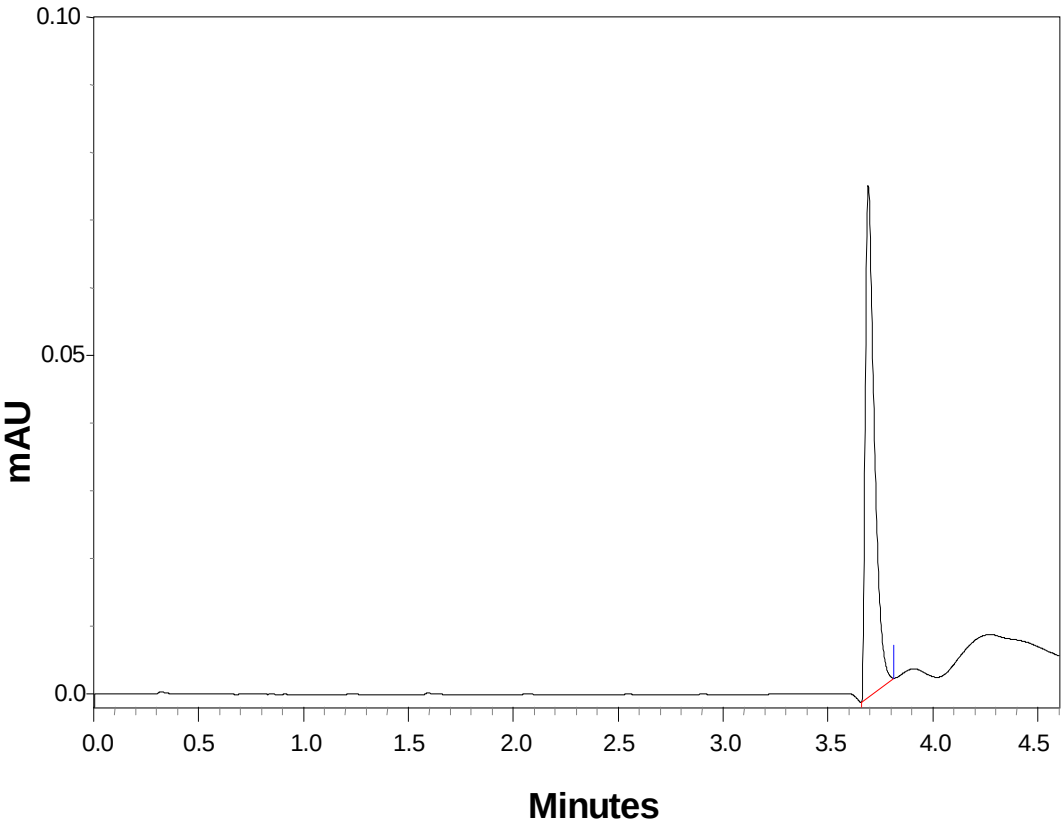

Figure 1: Typical chromatogram for vacisine.

Preparation of Standard Plot

The stock standard solution of vasicine at a concentration of 100 µg/ml was prepared in saline and stored at 4^0 C. Various concentrations of vasicine 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ng/ml were prepared from the stock in saline. From each of these concentrations 100 µl was spiked to 200 µl of normal healthy rat blood plasma in different tubes and added 10 µl of 10N Hydrochloric acid at room temperature to adjust the pH 6.2 for the precipitation of plasma proteins. Contents of the tubes were vortexed in a cyclo-mixer for ten seconds. From this mixture vasicine was then extracted by adding 5 ml of chloroform. After centrifuging for 10 min at 2000 rpm the organic layer was separated and evaporated under nitrogen at room temperature. The residue remaining was reconstituted in 100 µl of methanol. The HPLC system was adjusted for the chromatographic parameters and 20 µl of methanolic sample was injected into the

HPLC system. The experiment was carried out atleast three times to ascertain the accuracy of the method. A standard graph was plotted by taking concentration on abscissa and peak area on the ordinate.

Accuracy

Percentage recovery was calculated for 10-100 ng of vasicine after spiking into the normal rat blood plasma followed by extraction and HPLC detection. The results were expressed as percent recovery of vasicine as given in Table 1.

Table 1: The absolute recovery of vasicine by HPLC method after spiking into the normal rat blood plasma

Spiked vasicine concentration (ng)	Measured vasicine concentration (ng)	% Recovery of vasicine
10	12.28±0.42	122.8±5.23
20	20.60±0.38	103.0±4.68
30	32.97±0.41	109.9±6.11
40	42.08±0.62	105.2±4.38
50	55.60±0.45	111.2±5.06
60	68.40±0.55	114.0±4.80
70	75.53±0.62	107.9±7.18
80	84.48±0.56	105.6±5.66
90	96.03±0.66	106.7±7.68
100	101.00±0.76	101.0±6.83
Mean ± SEM		108.73±7.845

Selection of the vasicine dose

Extract obtained from 1 g of leaf powder is recommended as a single dose by Indian Pharmacopoeia. Each gm of the dried leaf powder contains 640-750 µg of vasicine collected from the different regions. We have considered 750 µg vasicine as the daily single human dose as an expectorant for therapeutic purpose. This human dose was converted for the use in rats (9) and was found to be 67.5 µg/kg body wt. of rat. In our study 65 µg/kg body wt. of rat was selected as oral dose for bioavailability studies.

Bioavailability and Pharmacokinetic study in rats

The study was approved by Institutional Animal Ethical Committee (IAEC), Kasturba Medical College, Manipal-576104, India (No. IAEC/KMC/80/2001-2002) prior to the experiment. Twenty four rats were divided into eight groups of three each and housed in polypropylene cages containing sterile paddy husk (procured locally) as bedding

throughout the experiment. Animals were fasted for 18-24 h before experiment but had free access to water. Vasicine dissolved in saline using ultrasonic bath was administered orally at 65 µg/kg body wt. to the animals of all groups. Blood samples were periodically collected from the retro-orbital plexus using heparinised capillaries at 0, 0.5, 1, 2, 4, 6, 12 or 24 h post-drug administration. The plasma was immediately separated from the blood by centrifugation (Sorvall Instruments RC5C, DuPont, Minnesota, USA) at 3000 rpm for 3 min and stored in deep freezer at -20°C in separate tubes until analysis.

Estimation of vasicine in rat plasma

Chromatogram of normal rat blood plasma without vasicine is presented in the (Fig. 2). Vasicine was extracted from 100 µl of plasma and analyzed by HPLC as described earlier. Vasicine concentration in plama samples were calculated from the standard plot. The limit of quantification of vasicine with this method was 10 ng/ml of plasma with in a day and between day variables were less (the coefficient of variation being less than 2% for inter day and 1% for intra day) and the mean percent drug recovered for the above concentration range was 106% indicating the method is reproducible. The mean plasma concentration vs time curve was plotted.

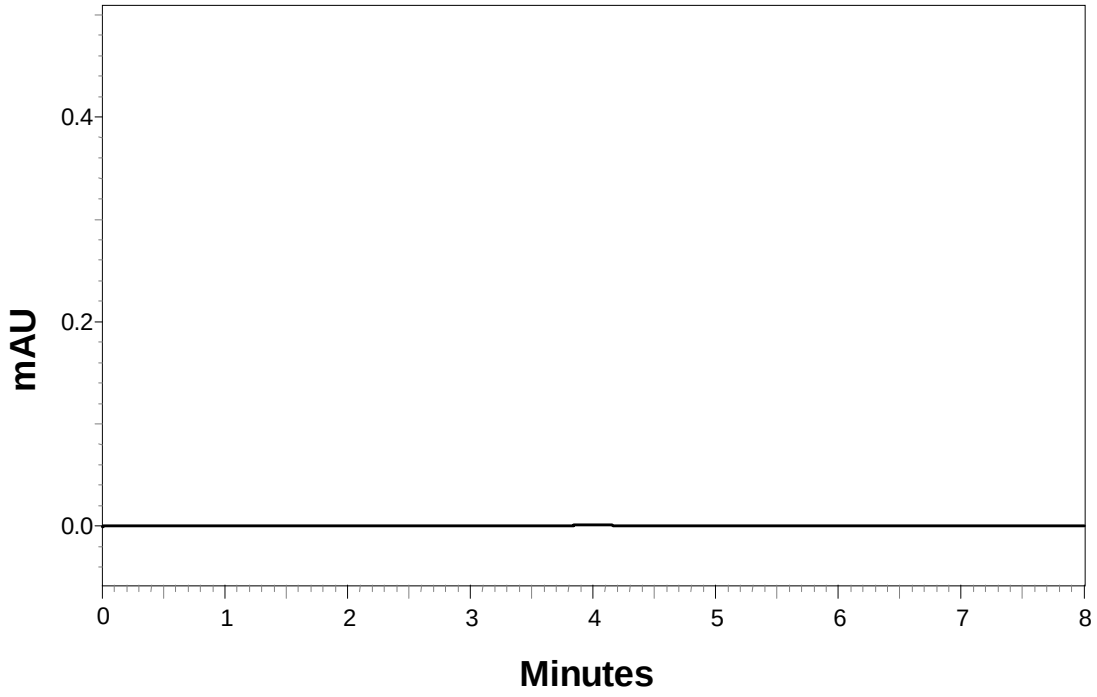

Figure 2: Chromatogram of normal rat blood plasma without vasicine.

Pharmacokinetic analysis

Peak concentrations (C_{max}) and time of its occurrence (t_{max}) are the observed values obtained by visual inspection of the data. The individual pharmacokinetic parameters were computed with PK Solutions 2.0 Version-Windows 2.0.6 (10) using curve stripping method, Conc = $A.e^{-\alpha t} + D.e^{-\beta t} + E.e^{-\lambda t}$ in which α, β and λ are the first-order rate constants for the absorption, distribution and elimination phases respectively. The total area under the curve (AUC_0-∞) was calculated by trapezoidal method with extrapolation to infinity by addition of last observed concentration divided by the terminal elimination rate constant.

Results

Standard plot was linear for the concentrations studied with linear regression coefficient R^2 of 0.9903. Accuracy was established by calculations of the percentage recovery for all the concentrations and the average recovery was found to be 108.73%±7.845, of vasicine.

Chromatogram showing the analysis of vasicine at T_{max} 4 h is presented in (Fig. 3). The mean plasma concentration-time profiles of vasicine after oral administration are shown in the Figure 4. The pharmacokinetic parameters calculated are given in Table 2. The mean concentration of maximum 12.8 ng/ml was achieved in T_{max} 4 hours.

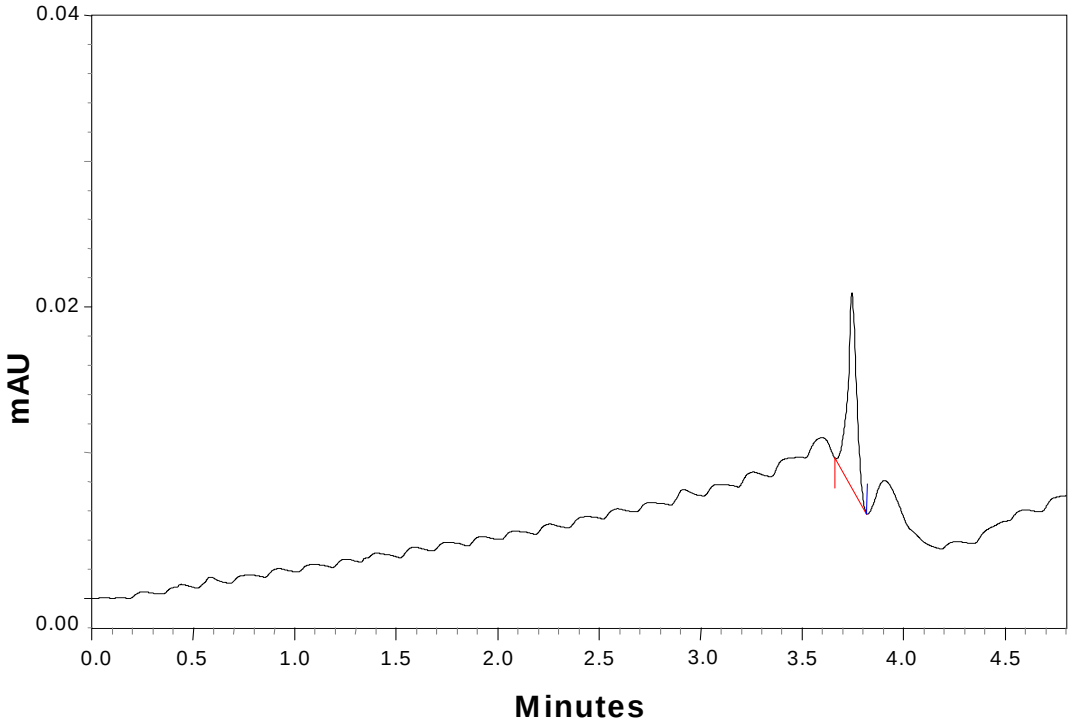

Figure 3: Chromatogram of normal rat blood plasma containing 12.812 ng/ml of vasicine measured at 4 h post-vasicine administration time in rats.

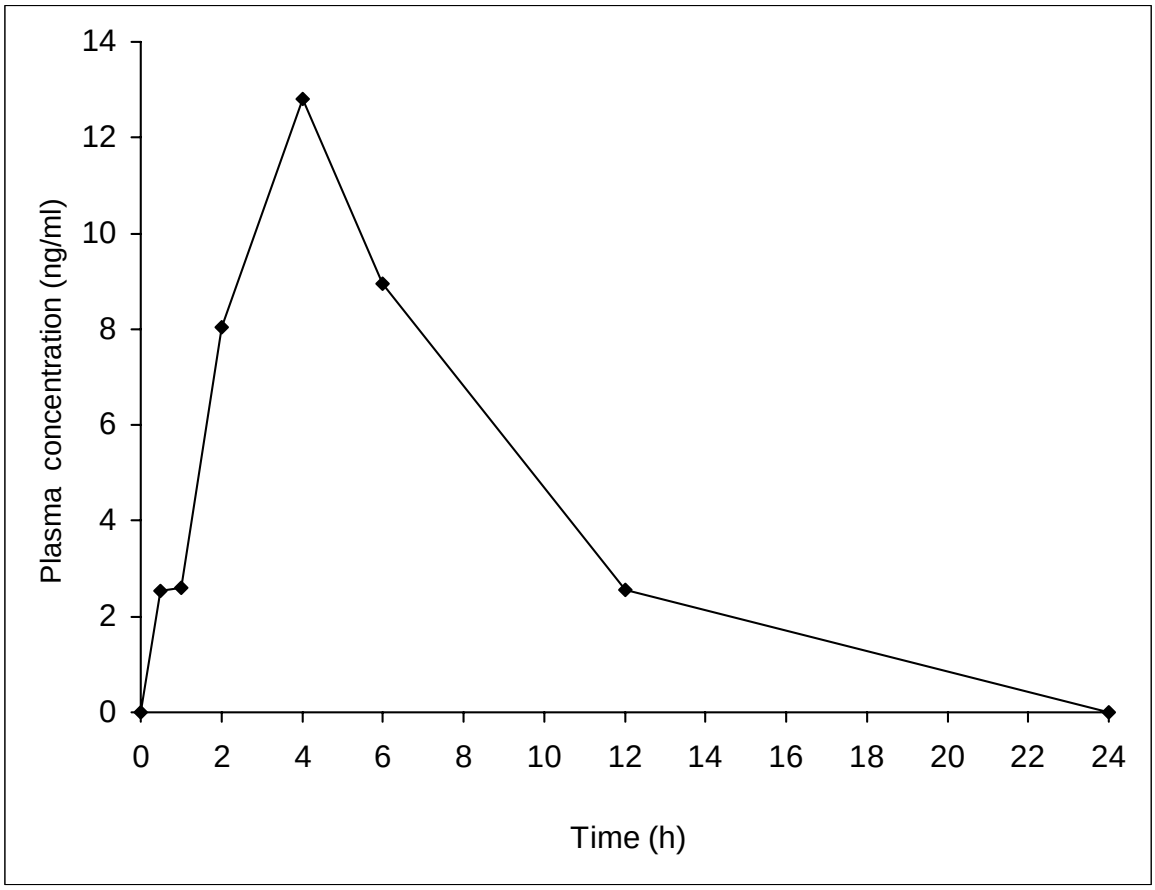

Figure 4: Concentration of vasicine in rat blood plasma obtained after various time intervals of post-vasicine oral administration in rats.

Table 2: Pharmacokinetic parameters of vasicine after the oral administration of single dose

Parameters	Vasicine determinations
C_{max} (ng/ml)	12.8±0.567
T_{max} (h)	4±0.340
AUC $_{(0-24)}$ (ng-hr/ml)	84.4±4.255
$t_{1/2}$ (absorption) (h)	0.641±0.0007
$t_{1/2}$ (elimination) (h)	3.308±0.0007
K_a (absorption) (h^{-1})	1.081±0.0035
K_e (elimination) (h^{-1})	0.209±0.0007
Clearance (ml/h)	770.097±1.421
Volume of distribution (ml)	3258.7±1.702

Values are mean ± SEM (n=3).

Discussion

Several chromatographic methods using HPLC have been reported for the analysis of vasicine either from the extract or marketed formulations but the biopharmaceutic analysis in plasma is not reported. The present study shows the determination of vasicine in rat plasma and its pharmacokinetics after the oral administration in rats. Further survey of literature did not reveal the therapeutic dose of the compound. In spite of these limitations, we have made an attempt to fix the oral dose for pure vasicine in rats and its determination from the plasma matrix. Therefore, we conclude that this method can easily and conveniently be employed for bioavailability studies of herbal formulations containing vasicine with the slight modification if necessary.

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References

1. The Wealth of India, (Publication and Information Directorate, CSIR, New Delhi) 1948, Vol I.
2. Nadakarni KM. Indian Materia Medica, (Popular Prakashan, Bombay) 1976, Vol I.
3. Atal CK. Chemistry and Pharmacology of Vasicine, (Director, RRL, Jammu Tawi) 1980, 41.
4. Parikh KM, Doshi VJ, Salunkhe JB, Kamath RP. High Performance Liquid Chromatographic determination of Vasicine and Vasicine from pharmaceutical formulations, Indian Drugs, 1989;27:64-6.
5. Francis M, Sane RT, Tipins S. Simultaneous HPLC method for determination of vasicine and glycyrrhizin from herbal preparations, Indian Drugs, 2003;40:712-15.
6. Gaitonde BV, Ulhas V, Bhat. TLC-Spectrophotometric determination of vasicine alkaloid from marketed formulation of Adhatoda vasica, Nees, Adli Tip Ders., 1988;4:15-7.
7. Amla V, Bano G, Johri RK, Zutshi U, Atal CK. Pharmacokinetics of vasicine in healthy Indian volunteers, Zhongguo Yaoli Xuebao, 1987;B(2):190-2.
8. Indian Herbal Pharmacopoeia, (IDMA and RRL (CSIR), Jammu Tawi) 1998.
9. Paget GE & Barnes JM. Evaluation of Drug Activities: Pharmacometrics, edited by Laurence DR, Bacharach AL (Academic Press, New York) 1964.
10. PK Solutions 2.0 (Version-Windows 2.0.6), Noncompartmental Pharmacokinetics Data Analysis, Pharmacokinetics and Metabolism Software, Summit Research Services, Open Field, CO, USA, 1999.