An Isocratic HPLC Method for the Analysis of Indole Alkaloids of *Catharanthus roseus*

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A simple isocratic reverse-phase column liquid chromatographic (RPLC) method for the determination of vindoline, catharanthine, vinblastine, vincristine and reserpine is described. The method can be applied to determine these alkaloids in plant extract as well as tissue culture of *Catharanthus roseus*.

Catharanthus roseus is a tropical perennial fast growing sub-shrub, originating in Medagascar and spreading to South-East Asia, India, Indonesia, Australia, North America and West Index[1]. The two clinically accepted alkaloids vincristine and vinblastine[2] are present in minor quantities in the leaves of the plant and require accurate method for their quantitation. High Performance Liquid Chromatography (HPLC) is the ultimate choice owing to its great sensitivity and accuracy. A number of isocratic as well as gradient HPLC methods[3-12] have been employed for the analysis of catharanthus alkaloids present in plant extracts but still the problem of reproducibility exist. Though the gradient technique is favoured as it is capable to give better resolution but the cost of additional instrumentation and time required for column equilibration after each analysis are the two major factors that make gradient technique less popular for rapid screening of a large number of plant samples to select high producing plants with respect to these alkaloids. To overcome this problem a simple reversed-phase isocratic HPLC method for the estimation of four major constituents of vinca alkaloids is described here which can be used easily in the rapid screening of a large number of plant extracts as well as cell culture samples.

Reserpine, vinblastine and vincristine were purchased from Sigma chemicals (U.S.A.), the later two as sulphate salts. Catharanthine and vindoline were generous gifts from Okayama University, Japan. Acetonitrile, methanol, triethylamine and acetic acid were of HPLC grade. Distilled water was prepared from deionised water, subjected to double distillation through quartz distillation apparatus and was filtered through a 0.45 μm filter before use. Ammonium acetate (AR Grade) was purchased from Glaxo, India and triethylamine from SISCO Research Laboratory, India. Stock solutions of vindoline, catharanthine, reserpine, vincristine and vinblastine contain 1 mg of each in 1 ml methanol. The calibration curve for each standard were plotted and were found linear in the range of 5 μg to 50 μg/ml.

A Water modular HPLC system consisting of a 501 pump, 484 variable wavelength absorbance detector, U6K injector, 730B integrater and a μ Bondapak C18 column (10 μm, 3.9 x 300 mm, Waters, U.S.A.) was used for analysis. The mobile phase was prepared from a mixture of methanol:acetonitrile:0.005M ammonium acetate (in water):triethylamine (1:2:2.5:0.02, v/v) and the pH of the mobile phase was adjusted to 8.5 with glacial acetic acid. The flow rate of the mobile phase was kept at 0.8 ml/min and the detection was done at λ max 254 nm.

Freshly harvested leaves cultivated at CIMAP farm, Bangalore were shade dried and powdered. Ethanol (90% 30 ml) was added to the leaf powder (5 g), left overnight and extracted with 90% ethanol (3 x 30 ml) at room temperature. The alcohol extract was filtered and concentrated in vaccuo at 40° to dryness, redissolved in alcohol (10 ml), diluted with water (10 ml) and then acidified with 3% HCl (10 ml). This was then extracted with hexane (3 x 30 ml) and the hexane extract was rejected. The aqueous portion was cooled to 10°, basified with ammonium hydroxide to pH 8.5 and extracted with chloroform

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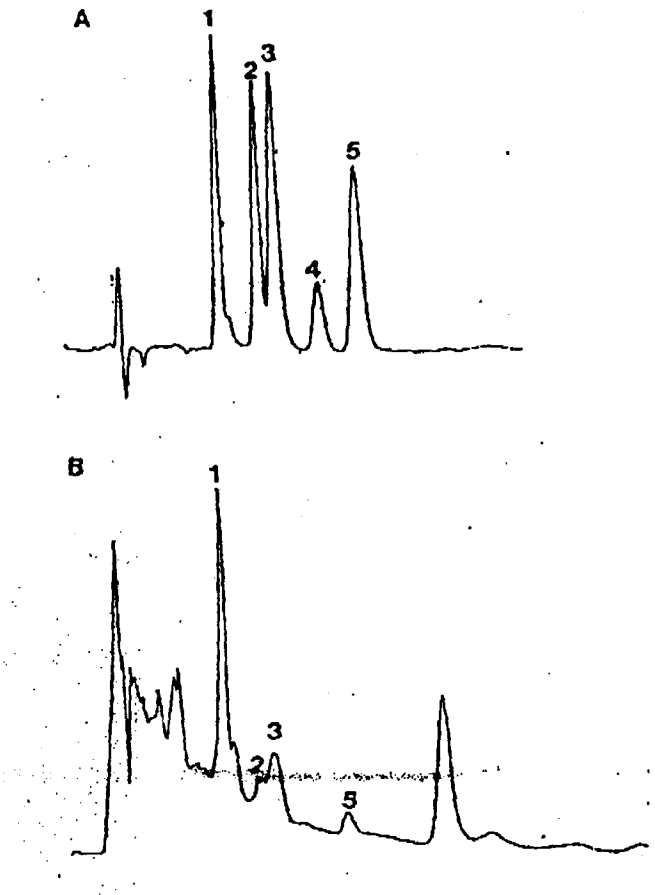

Fig. 1 : [A] HPLC chromatogram of standards : 1 Vindoline (t_R 8.93 min), 2. Catharanthine (t_R 11.21 min), 3. Vincristine (t_R 12.17 min), 4. Reserpine (t_R 14.60 min), 5. Vinblastine (t_R 16.87 min) [B] HPLC chromatogram of leaf extract under identical conditions.

(3 x 30 ml). The combined $CHCl_3$ extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness till constant weight for total alkaloid content. In case of cell cultures 8-10 week old *in vitro* grown multiple shoots of accession N-1 raised in tissue culture were dried and homogenised in 90% ethanol (10 ml/g f wt) and extracted following the procedures outlined above for plant leaves. 10 mg of the crude alkaloid was dissolved in 1.0 ml of methanol and (10 µl) was subjected to HPLC analysis.

The method reported is capable to resolve all the four constituents (Figure-A). The total analysis time for plant as well as cell culture sample was thirty minutes. The analytical data for all the four constituents present in crude leaf extract (Figure-B) and cell culture samples showed high reproducibility. The only limitations of this method is the high pH (8.5) of the mobile phase which may shorten the column life.

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