

## **EXTRACTION OF INDOLE ALKALOIDS FROM *CATHARANTHUS ROSEUS* BY USING SUPERCRITICAL CARBON DIOXIDE**

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### **Summary**

Indole alkaloids, particularly vindoline and catharanthine, were extracted from the leaves of *Catharanthus roseus* by supercritical extraction with CO<sub>2</sub>. The contents of vindoline and catharanthine in the extracts were determined by HPLC and identified by LC/MS. About 52 % (w/w) of the initial vindoline content, 1.5 mg vindoline/g dry wt leaves, was recovered after extracting this material for 10 h with the CO<sub>2</sub> flow rate of 400 ml/min at 40°C and 150 bar. Vindoline concentration in the extract was 67 % (w/w).

### **Introduction**

The medicinal plant *Catharanthus roseus*, Madagascan periwinkle, contains various indole alkaloids of which vindoline and catharanthine are the most important. Vindoline and catharanthine are well known as the precursors in the biosynthetic pathways of valuable dimeric indole alkaloids such as vinblastine and vincristine. These two dimeric alkaloids have been used for many years as chemotherapeutic agents in the treatment of acute leukemia and Hodgkin's disease (Svoboda *et al.*, 1959; Boder *et al.*, 1964; Miura *et al.*, 1988). However, bulk production of vindoline has not been successful by plant cell tissue cultures although catharanthine has been so produced (Drapeau *et al.*, 1987; Smith *et al.*, 1987; Tallevi and DiCosmo, 1988). In addition, as vindoline content in the intact plant is quite small, its isolation by column chromatography on a large scale is tedious and time-consuming. In this connection the objective of this study was to test the applicability of supercritical fluid extraction (SFE) process for selectively extracting vindoline from the leaves of *Catharanthus roseus* by using carbon dioxide as a supercritical solvent.

## Materials and Methods

### Sample Preparation

The leaves of *Catharanthus roseus* were dried for 24h in the oven maintained at 60°C and then powdered to 200-300 mesh size in order to increase the mass transfer efficiency between supercritical carbon dioxide and the sample particles.

### SFE Procedure

A continuous flow-through SFE system was used for the tests. Carbon dioxide was supplied from a gas cylinder and was directed to an electrically driven diaphragm-type compressor. It was filtered through 10  $\mu\text{m}$  filter-discs. After compression, the carbon dioxide was introduced into a surge vessel to dampen the fluctuations generated by the compressor. To maintain a constant pressure within the system, a back pressure regulator with an accuracy of  $\pm 1\%$  of the relief pressure range was employed. The extraction column, 100 $\text{cm}^3$ , was packed with 3-mm glass beads and about 7g powdered *Catharanthus roseus* in order to disperse the solvent flow and to minimize the pressure drop through the column less than 1 bar. A metal filter was inserted at the top of the column to prevent the entrainment of fine sample particles during the experiment. The temperature inside the air bath was controlled to within  $\pm 0.1^\circ\text{C}$  by using a proportional temperature controller. The carbon dioxide-solute mixture leaving the top of the extractor was expanded to atmospheric pressure through a micrometering valve into cold traps where the solute has condensed. The flow rate and volume of carbon dioxide were measured by a flow meter and a dry test meter. The dry test meter was equipped with gauges to measure flow temperature and pressure. The use of low solvent flow rates guarantees the attainment of equilibrium conditions at the extractor's outlet.

### Quantitative Analysis by HPLC

The crude alkaloid extract obtained from the SFE experiment was analysed by a Hitachi 655A-12 HPLC. The extract was dissolved in a small volume of methanol and filtered through a 0.5 $\mu\text{m}$  FH-type Millipore filter. This sample was then loaded onto a reversed-phase  $\mu$ -Bondapak  $\text{C}_{18}$  column (30 $\text{cm} \times 3.9\text{mm}$ ). The run was performed using 5mM  $(\text{NH}_4)_2\text{PO}_4$  (pH 7.3) / methanol / acetonitrile (3:4:3) with a flow rate of 1  $\text{mL}/\text{min}$ . The compounds were detected by absorbance at 298 nm. The quantitative analysis was determined by comparing the peak areas of the sample with those of authentic alkaloids.

### Identification by LC/MS

The contents of vindoline and catharanthine in the extracts were also checked by a Hewlett-Packard 5988A mass spectrometer equipped with a particle-beam LC/MS interface. The solvent for LC/MS was eluted with methanol / acetonitrile / 5 mM ammonium acetate (3:4:3) at a flow rate of 0.5  $\text{mL}/\text{min}$ . The other conditions for LC were the same as described in the quantitative analysis. Electron impact ionization at 300°C temperature and with 278  $\mu\text{A}$  current and 70 eV voltage was used for the MS.

## Results and Discussion

The extraction of vindoline and catharanthine from *Catharanthus roseus* was carried out at several temperature and pressure conditions by using carbon dioxide as a supercritical solvent and the contents of the extracts analysed by HPLC are summarized in Table 1.

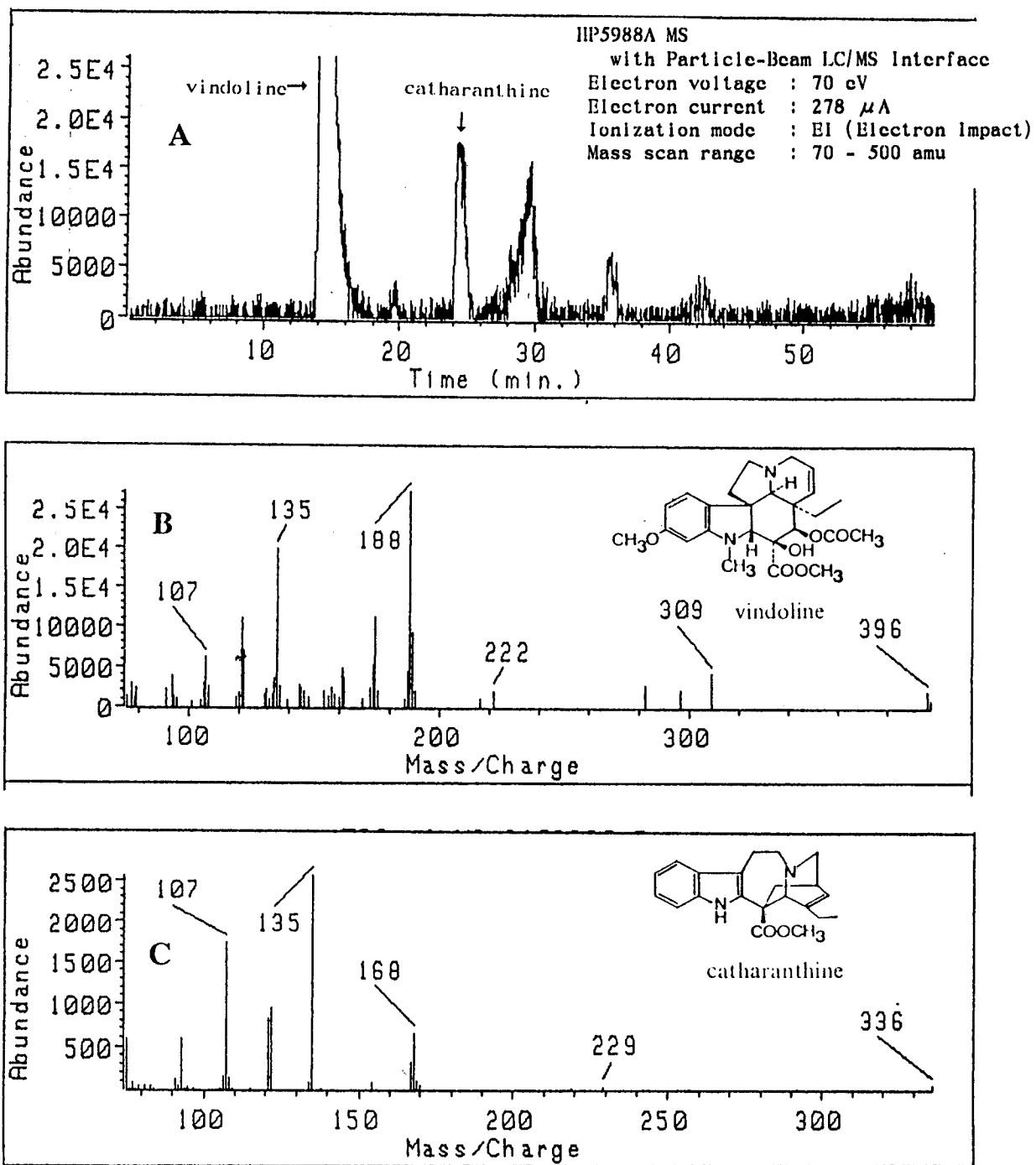


Figure 1. LC/MS analysis of vindoline and catharanthine extracted from leaves of *Catharanthus roseus* by using supercritical carbon dioxide.  
 A. Total ion chromatogram of crude alkaloids.  
 B. Mass spectrum of vindoline identified.  
 C. Mass spectrum of catharanthine identified.

**Table 1. Concentrations of vindoline and catharanthine in the extracts at five different temperature and pressure conditions.**

| T(°C) | P(bar) | Vindoline %<br>in extract | Catharanthine %<br>in extract |
|-------|--------|---------------------------|-------------------------------|
| 40    | 150    | 67.2                      | trace                         |
|       | 350    | 61.3                      | 5.8                           |
|       | 400    | 46.8                      | 10.6                          |
| 70    | 150    | 64.1                      | 5.5                           |
|       | 400    | 47.1                      | 8.2                           |

The qualitative identification of vindoline and catharanthine was confirmed by LC/MS (Figure 1). About 52 % (w/w) of the initial vindoline content, 1.5 mg vindoline/g dry wt leaves, was recovered after extracting this material for 10 h with the CO<sub>2</sub> flow rate of 400 ml/min at 40°C and 150 bar. Vindoline concentration in the extract was 67 % (w/w). The selectivity of SC-CO<sub>2</sub> for vindoline was decreased with pressure at constant temperature, whereas for catharanthine increased with pressure (Table 1). However, the effect of temperature on the selectivity was quite small for both indole alkaloids. The high selectivity of SC-CO<sub>2</sub> for vindoline, i.e., 67 % (w/w) at 40°C and 150 bar, shows that the SFE process would provide a potential route for bulk production of vindoline from *Catharanthus roseus*. However, in our opinion these overall results stated above are preliminary and further investigations are needed to resolve some essential problems such as the interaction between supercritical solvent and various alkaloids, the enhancement of both solvent power and selectivity by a small addition of cosolvents, the determination of optimal SFE conditions and finally many other factors related to commercial scale-up.

#### Acknowledgment

This work was supported by the Ministry of Science and Technology and the Korea Science and Engineering Foundation.

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