# Determination of Vinca Alkaloids in Periwinkle Plants Using HPLC-ECD

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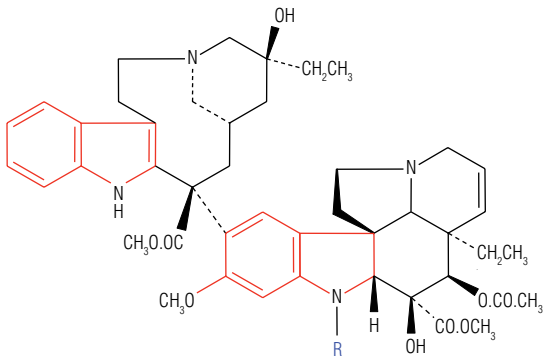
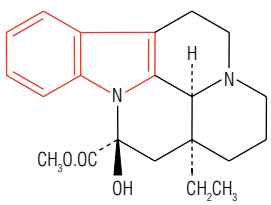
## Key Words
Periwinkle, HPLC-ECD, Alkaloids, Bis-Indole Derivatives, Neoplastic Diseases

## Goal
To develop an HPLC-ECD method capable of measuring low levels of the vinca alkaloids typically found in plant extracts

## Introduction
The vinca alkaloids consist of the bis-indole derivatives, vinblastine and vincristine isolated from the periwinkle plant, *Vinca rosea* Linn and the monomeric indole derivative vincamine, which is the major alkaloid of *Vinca minor* Linn (Figure 1). The vinca alkaloids are used as chemotherapeutic agents to treat a variety of neoplastic diseases including Hodgkin's disease, choriocarcinoma, acute and chronic leukemias, lymphosarcomas and a variety of other cancers. Their mechanism of action involves binding to tubulin with disruption of mitotic spindle formation eventually leading to inhibition of mitosis, metaphase arrest and cell death.[1]




Vinblastine (R = $CH_3$)
Vincristine (R = CHO)




Vincamine

Figure 1. The chemical structures of vinblastine, vincristine, and vincamine.

Vinca alkaloids have previously been measured using a variety of techniques including: radioassay and high-performance liquid chromatography with UV, fluoresence and electrochemical detection (ECD).[2–5] In general these approaches suffer from either a lack of sensitivity (HPLC techniques) or selectivity (radioimunoassay and HPLC techniques). Coulometric electrode array detection when coupled to gradient HPLC possesses both the selectivity and sensitivity required to measure the vinca alkaloids in natural products (e.g. periwinkle leaf extracts) and for the study of their pharmacokinetics and metabolism in vivo. Furthermore, this method is readily adaptable for the analysis of the ever broadening variety of synthetic analogs.[6]

## Materials and Methods
The gradient analytical system consisted of two pumps, an autosampler, and a twelve-channel Thermo Scientific™ Dionex™ CoulArray™ Coulometric Array Detector. Higher levels of analytes were also verified using an UV absorbance detector placed after the array.

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| LC Conditions | |
|---|---|
| Column: | Base-deactivated, 4.6 × 150 mm, 5 µm |
| Mobile Phase A: | 100 mM Sodium acetate; acetonitrile; methanol (85:10:5 v/v/v), final pH 6.2 with phosphoric acid |
| Mobile Phase B: | 100 mM Sodium acetate; acetonitrile; methanol (50:30:20 v/v/v), final pH 6.2 with phosphoric acid |
| Gradient Conditions: | Isocratic 25% B until 1.1 min. Linear increase of phase B from 25% to 100% over 19 min. Hold for 18 min Isocratic, 25% B for 3 min. |
| Flow Rate: | 1.0 mL/min |
| Temperature: | Ambient |
| Injection Volume: | 20 µL |
| **Detectors Conditions** | |
| Detector: | Model 5600A, CoulArray |
| Applied Potentials: | +200 to +950 mV vs Pd. in 75 mV |
| Detector Wavelength: | 274 nm |

## Results and Discussion

The vinca alkaloids were readily resolved both chromatographically and voltammetrically from the other alkaloids in under 41 min (Figure 2). The method was linear over at least four orders of magnitude and had limits of detection of <50 pg for all compounds examined (Figure 3).

The CoulArray detector is easily capable of measuring the low levels of the vinca alkaloids typically found in plant extracts. This can be done routinely because of the inherent stability of the coulometric electrode design. As analytes are identified both by retention time and voltammetric behavior across the array, compound misidentification and coelution can be minimized.[7,8] Furthermore, this method has greater sensitivity and selectivity than PDA-based approaches. This allows analysis in complex samples such as plant extracts and biologicals with a minimum of sample preparation. This same method can be used to measure a variety of other alkaloids such as yohimbine.

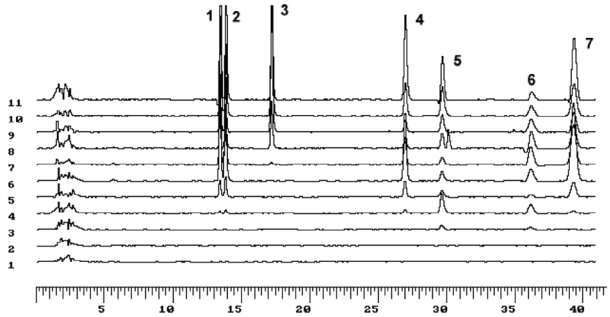


Figure 2. Chromatogram showing separation of vinca and other alkaloids (10 ng on column). 1-corynanthine; 2-yohimbine; 3-vincamine; 4-ajmalicine; 5-vincristine; 6-vinblastine; 7-tetrahydro-alistonine. The applied potentials were channel 1 to channel 11, +200 to +950 mV in +75 mV increments. The chromatogram is presented at a gain of 500 nA.

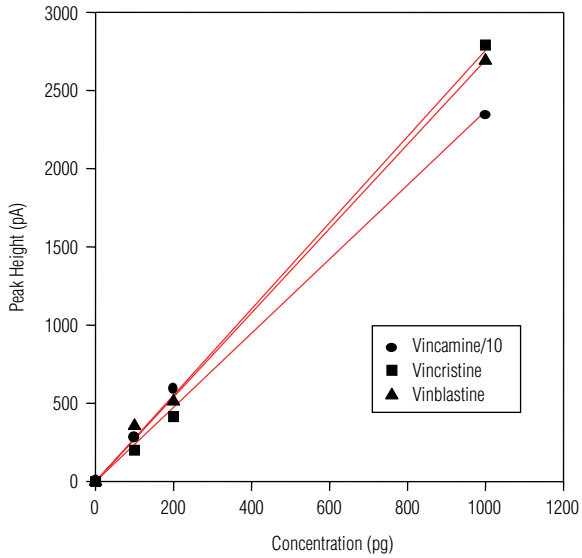


Figure 3. Linearity of the vinca alkaloids.

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