# Determination of Yohimbine and Related Alkaloids Using HPLC-ECD

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## Key Words
Alkaloids, HPLC-ECD, Yohimbine, Vinca

## Goal
To develop a sensitive and selective HPLC-ECD method capable of measuring the low levels of yohimbine and related alkaloids typically found in plant extracts.

## Introduction
Yohimbine is an indolealkylamine alkoloid with chemical similarity to reserpine. Yohimbine has been isolated from *Cornanthe johimbe, Rubiaceae* and related trees, as well as *Rauwolfia serpentina*. Both yohimbine and a diastereo-isomer, corynanthine, are α2-adrenoceptor antagonists of limited duration.[1] Yohimbine has limited effect on smooth muscle, but it readily passes through the blood-brain barrier and produces a complex pattern of responses at doses lower than required to produce a-adrenergic blockade. Yohimbine has been reported to produce central excitation (e.g., elevated blood pressure, increased heart rate, increased motor activity, tremor, irritability, vomiting, nausea and sweating) and to act as an anti-diuretec (through its ability to release antidiuretic hormone).[2] Some evidence also suggests that yohimbine can act as an aphrodisiac,[3] although this effect may be limited to individuals with vascular diseases.[4,5]

A variety of techniques have been used to measure yohimbines including: GC-MS and HPLC with UV, fluorescence or amperometric detection.[6–8] In general these approaches suffer from a lack of sensitivity, selectivity or require extensive sample preparation and derivatization before analysis. A gradient HPLC coulometric array method was developed capable of measuring yohimbine, related alkaloids and the vinca alkaloids simultaneously.

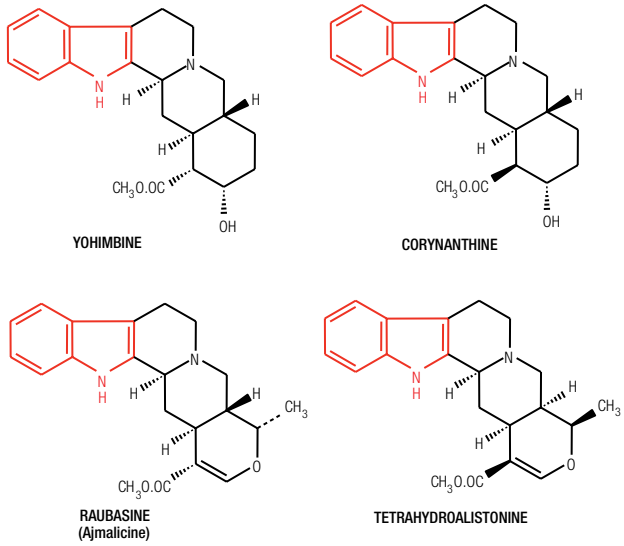


Figure 1. The structure of Yohimbine, Corynanthine, Raubasine and Tetrahydroalistonine.

This approach offers low picogram sensitivity and uses voltammetric resolution to correctly identify each analyte and check for possible coelutions. This method will have application to the measurement of these alkaloids in natural products (e.g., tree bark) and for the study of their pharmacokinetics *in vivo*.

## 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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| Conditions | |
|---|---|
| Column: | C18, 4.6 × 150mm, 5µm |
| 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 minutes. 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 |
| **Detector Conditions** | |
| Detector: | Model 5600A, CoulArray |
| Applied Potentials: | +200 to + 950 mV vs. Pd. in 75 mV |
| Wavelength: | 274 nm |

## Results and Discussion

Yohimbine and related 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.

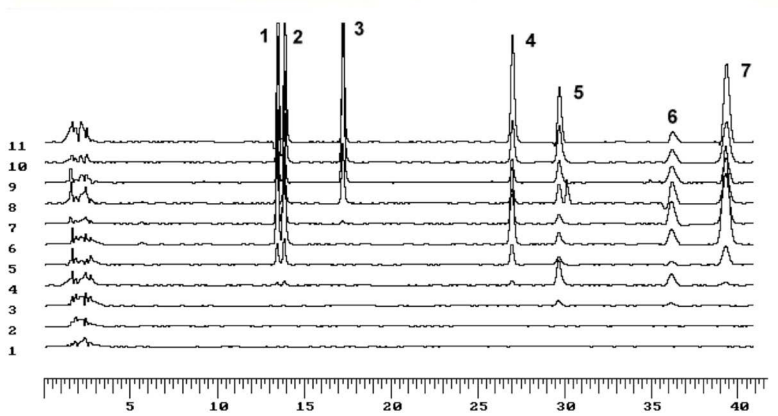


Figure 2. Chromatogram showing separation of vinca and other alkaloids (10 ng on column). 1 - corynanthine; 2 - yohimbine; 3 - vincamine; 4 - ajmalicine; 5 - vincristine; 6 - vinblastine; 7 - tetrahydro-alistonine. The applied potentials were channel 1 to channel 11, +200 to +950mV in +75mV increments.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

The CoulArray detector is easily capable of measuring the low levels of yohimbine and related 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.[9,10] This approach allows analysis in complex samples such as plant extracts and biologicals with minimal sample preparation. Furthermore, this method can be used to simultaneously measure a variety of other alkaloids such as the vinca alkaloids.

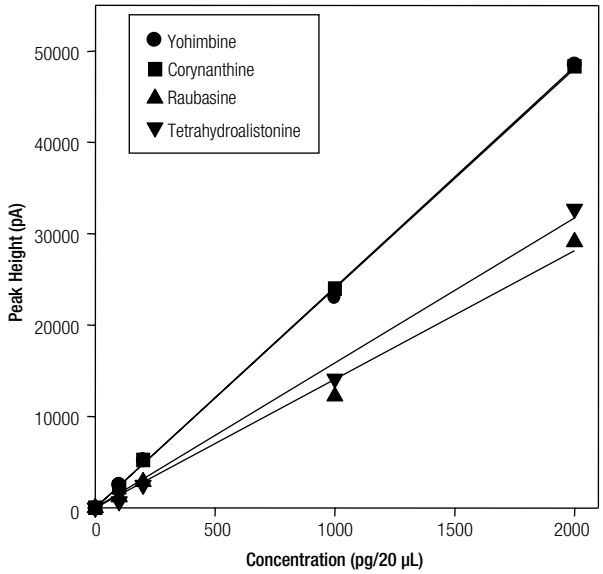


Figure 3. Linearity of yohimbine and related alkaloids.

## References


1. Goldberg, M. R., and Robertson, D. (1983). Yohimbine: A pharmacological probe for the alpha 2-adrenoreceptor. *Pharmacol. Rev.*, **35**, 143-180.
2. Goodman Gilman, A., Goodman, L. S., and Gilman, A. (Eds.). (1980). *The Pharmacolgical Basis of Therapeurics*. Sixth edition. MacMillan Publishing Co., Inc. New York.
3. Rosen, R. C., and Ashton, A. K. (1993). Prosexual drugs: Empirical status of "new aphrodisiacs." *Arch. Sex. Behav.*, **22**, 521-543.
4. DeWire, D. M. (1996). Evaluation and treatment of erectile dysfunction. *Am. Fam. Physician*, **53**, 2101-2108.
5. Montorsi, F., Guazzoni, G., Rigatti, P., and Poxxa, G. (1995). Pharmacological management of erectile dysfunction. *Drugs*, **50**, 465-79.
6. Diquet, B., Doare, L., and Gaudel, G. (1984). New method for the determination of yohimbine in biological fluids by high-performance liquid chromatography with amperometric detection. *J. Chromatogr.*, **311**, 449-455.
7. Reimer, G., Suarez, A., and Chui, Y. C. (1993). A liquid chromatographic procedure for the analysis of yohimbine in equine serum and urine. *J. Anal. Toxicol.*, **17**, 178-181.
8. Ylinen, M., Suhonen, P., Naaranlehti, T., Lapinjoki, S. P,m and Huhtikangas, A. (1990). Gas chromato-graphic-mass spectrometric analysis of major indole alkaloids of Catharanthus roseus. *J. Chromatogr.*, **505**, 229-234
9. Acworth, I., and Gamache, P. (1996). The coulometric electrode array for use in HPLC analysis. Part 1: Theory. *American Lab.*, **5**, 33-38.
10. Svendsen, C. (1993). Multi-electrode array detectors in high-performance liquid chromatography: A new dimension in electrochemical analysis. *Analyst*, **118**, 123-129


## Ordering Information

| Description | Part Number |
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| WPS-3000TBRS Biocompatible Rapid Separation | 5841.0020 |
| CoulArray, Model 5600A - 12 channel | 70-4329 |
| CoulArray Organizer with Temp. Control | 70-4340T |
| Accessory Kit, CoulArray Detector to Thermo Scientific™ Dionex™ UltiMate™ 3000 System | 70-9191 |





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