Determination of Yohimbine and Related Alkaloids Using HPLC-ECD

Paul Gamache and Ian Acworth
Thermo Fisher Scientific, Chelmsford, MA, USA

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 alkaloid 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 diastereoisomer, 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 α-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 antidiuretic (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.

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.
**Conditions**

Column: C18, 4.6 x 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.

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 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.

Figure 3. Linearity of yohimbine and related alkaloids.
References

Ordering Information

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