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Chromatographic fingerprint analysis of yohimbe bark and related dietary supplements using UHPLC/UV/MS

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ABSTRACT

A practical ultra high-performance liquid chromatography (UHPLC) method was developed for fingerprint analysis of and determination of yohimbine in yohimbe barks and related dietary supplements. Good separation was achieved using a Waters Acquity BEH C_{18} column with gradient elution using 0.1% (v/v) aqueous ammonium hydroxide and 0.1% ammonium hydroxide in methanol as the mobile phases. The study is the first reported chromatographic method that separates corynanthine from yohimbine in yohimbe bark extract. The chromatographic fingerprint analysis was applied to the analysis of 18 yohimbe commercial dietary supplement samples. Quantitation of yohimbine, the traditional method for analysis of yohimbe barks, were also performed to evaluate the results of the fingerprint analysis. Wide variability was observed in fingerprints and yohimbine content among yohimbe dietary supplement samples. For most of the dietary supplements, the yohimbine content was not consistent with the label claims.

1. Introduction

Yohimbine is an active indole alkaloid with sexual stimulant and aphrodisiac effects found naturally in *Pausinystalia yohimbe* (K. Schumann, Yohimbe) [1]. It has been used as a prescription medicine for the treatment of erectile dysfunction in the United States as the hydrochloride form [2]. Commercial dietary supplements of yohimbe bark are widely used as over-the-counter dietary supplements for the treatment of sexual dysfunction and enhancement of sexual satisfaction [3]. Reported side effects for yohimbine are involved in elevated systolic blood pressure and heart rate as well as anxiety, headache, and increased urinary output [4–7]. The main active chemical present in yohimbe bark are indole alkaloids including yohimbine, α-yohimibine, β-yohimbine, ψ-yohimbine, allo-yohimbine, ajmalicine, tetrohydroaltonine, yohimbic acid, corynanthine and some other minor constituents with same skeleton but different substitutional groups [8,1]. Several analytical methods, including thin layer chromatography [9], high performance liquid chromatography [2,10], non-aqueous capillary electrophoresis [11], and high performance liquid chromatography tandem mass spectrometry [8,12] have been reported for the analysis of yohimbine in barks, dietary supplements, and bio-fluids. However, the methods reported to date are suitable for analysis of yohimbine in pharmaceutical preparations, they are not appropriate for the analysis of yohimbine barks or dietary supplements based on yohimbine barks due to the fact that these methods cannot resolve the yohimbine peak from the corynanthine peak in their chromatograms. The structures of yohimbine and corynanthine are shown in Fig. 1. Corynanthine not only has the same mass as yohimbine, its fragmentation pathway is also the same as yohimbine [8,10]. Thus, neither selected ion monitoring (SIM), selected reaction monitoring (SRM), multiple reaction monitoring (MRM), nor isotope-dilution mass spectrometry can differentiate between the two. All previous reports measured the combined peaks of yohimbine and corynanthine from yohimbe bark extract as the yohimbine peak, either knowingly or unknowingly [10]. In addition, these methods were either not fully compatible with mass spectrometry due to the use of triethylamine (2 mM) [10], hexanesulphonic acid (2.5 mM) [13], or phosphate buffer (39 mM) [13] and/or used toxic reagents [10] (chloroform and triethylamine).

For pharmaceutical preparations, targeted analysis, such as quantitation of yohimbine, is enough for quality assessment. For complex botanicals, such as yohimbe bark extracts, quantitation of a single or selected targeted compound usually does not provide the whole picture. For this reason, the chromatographic fingerprint technology was accepted by the WHO as a strategy for identification and quality evaluation of herbal medicines in 1991 [14].

In the presented work, an ultra high-performance liquid chromatography (UHPLC) method has been developed for qualitative (fingerprinting) and quantitative (yohimbine) analysis of yohimbe bark and related dietary supplements. The method is fully compatible with mass spectrometry and shows superiority in resolution,

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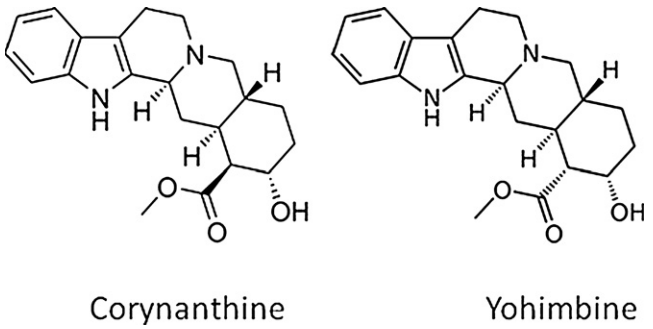

Fig. 1. The structures of corynanthine and yohimbine.

analytical time, and solvent consumption compared to previously reported methods. It is the first reported LC/UV/MS method that is capable of resolving corynanthine from yohimbine in yohimbe bark extracts. The method was successfully applied to the analysis of yohimbine in two yohimbe barks and 18 dietary supplements samples.

2. Experimental

2.1. Chemicals and reagents

Water, acetonitrile, methanol, and ammonium hydroxide (Assay 20–22% as NH_3) of Optima grade were purchased from Fisher Scientific (Pittsburgh, PA, USA). Formic acid of mass spectrometry grade was purchased from Sigma–Aldrich (St. Louis, MO, USA). Yohimbine hydrochloride was obtained from Sigma (purity > 98%, St. Louis, MO, USA). Corynanthine, α-yohimbine, yohimbic acid, ajmalincine and tetrohydroaltonine were purchased from ChromaDex (Irvine, CA, USA). All chemicals were stored in the refrigerator as required to ensure stability.

2.2. Standards solution

The yohimbine standard was accurately weighed and then dissolved in an appropriate volume of 1% NH_4OH–methanol to produce corresponding stock solutions. The working standard solution of yohimbine for the calibration curve was prepared by serial dilution of the stock solution with methanol in six concentration increments ranging from 0.11 to 214 μg/mL. Quality control (QC) samples were prepared from the stock solutions at 85.7 μg/mL, 21.4 μg/mL, and 10.7 μg/mL. All stock and working solutions were maintained at −20 °C.

2.3. Yohimbe bark samples and commercial dietary supplements

Authentic yohimbe (*Pausinystalia johimbe*) bark was purchased from American Herbal Pharmacopoeia (AHP, Certificate #374). Eighteen yohimbe dietary supplements were purchased from local stores and the internet. Five dietary supplements are in tablet form, seven are in capsule form, and six are in liquid form.

2.4. Chromatographic and mass spectrometric conditions

Samples were analyzed on an Agilent 1200 HPLC rapid resolution HPLC system (Agilent Technologies, Palo Alto, CA) consisting of a binary pump with a vacuum degasser, a thermostatted column compartment, a temperature-controlled well plate autosampler, and a diode array detector (DAD). A Waters Acquity BEH C_{18} (Waters Corporation, Milford, MA, USA, 1.7 μm particle size, 100 mm × 2.0 mm) UPLC reversed-phase column, in combination with an in-line column filter (Analytical Scientific Instruments, El Sobrante, CA), was used for the separation. Chromatographic separation was achieved using a gradient elution system consisting of (A) 0.1% NH_4OH in water (v/v) and (B) 0.1% NH_4OH in methanol (v/v). A gradient elution program was employed as follows: 20–35% B at 0–15 min, 35–65% B at 15–30 min, 65–70% B at 30–45 min, and then to 95% B at 50 min with a flow rate of 0.3 mL/min. The post-run time for re-equilibrating the system was set for 7 min. The DAD was set at 280 nm with a band width of 16 nm. The UV spectra were continuously recorded from 190 to 400 nm.

MS analysis was performed on an Orbitrap-XL high resolution mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA), equipped with electrospray ionization source; the interface was operated in positive ion mode over the range of m/z 100–700. The following conditions were used: sheath gas flow rate, 80 arb. units; aux gas flow rate, 10 arb. units; spray voltage, 4.50 kV; heated capillary temperature, 220 °C; capillary voltage, 34 V; tube lens offset, 25 V. The normalized collision energy was 30% for the MS/MS scan.

2.5. Sample preparation

Yohimbe bark samples were ground into fine powders using an IKA A11 grinder (IKA® Works, Inc., Wilmington, NC) with a knife blade. The contents of capsules of commercial dietary supplement samples were emptied into a bottle and thoroughly mixed. Tablets of commercial dietary supplement samples were ground into fine powders using a Retsch RM100 grinder (Retsch Inc., Newtown, PA). All the samples were passed through a 60-mesh sieve. Fifty mg of yohimbe bark or portions equivalent to 50 mg of yohimbe bark of each commercial sample (according to their labels) were carefully weighed into 15 mL centrifuge tubes and were moistened with 1 mL NH_4OH (20–22%). Ten milliliter MeOH then was added and sonicated for 20 min, and then centrifuged for 5 min at 5000 rpm. The supernatant was filtered through a 0.20 μm PVDF syringe filter (VWR Scientific, Seattle, WA). For liquid samples, the samples were diluted 10 times with methanol–water–concentrated ammonium hydroxide (60:40:1, v/v/v) and filtered. The injection volume for HPLC analysis is 2 μL.

2.6. Method evaluation

The limits of detection (LOD) and quantitation (LOQ) were defined as the lowest concentrations of analytes in a sample that can be detected and quantified. These LOD and LOQ limits were determined on the basis of signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively. The precision was evaluated by intra-day and inter-day percent relative standard deviation % (RSD). The intra-day and inter-day variability was evaluated by analyzing QC samples at three different concentration levels (estimated at 5.36 μg/mL, 21.42 μg/mL, and 85.68 μg/mL). The intra-day precision and accuracy was calculated by analyzing QC samples in six replicates at each concentration. The analyses for calculation of inter-day precision were performed in each concentration for five consecutive days.

2.7. Fingerprint and chemometric analysis

Characteristic peaks chromatographic fingerprinting (CPCF) was used in this study. The CPCF approach initially selects the chromatogram of an authentic sample as the reference fingerprint (RF, in this study, yohimbe bark from AHP was used). The most obvious or characteristic peak of the RF was selected as the reference peak (RP, the yohimbine peak in this case). The areas of all other peaks in the chromatogram were normalized against the area of the RP and the ratios of the peaks are entered into a peak table. Then chromatograms from each sample were compared to the RF one-by-one and the corresponding peaks are entered into the peak table. After