High-Performance Liquid Chromatographic Determination of Yohimbine and Strychnine in Dosage Forms

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An ion-pairing high-performance liquid chromatographic method was developed for the determination of yohimbine and strychnine in dosage forms. The mobile phase consists of methanol–water–acetic acid–triethylamine (50 : 50 : 1 : 0.3) with 1 mM sodium hexanesulfonate as the counter-ion. The analysis was carried out using an octadecyl silica (5 μm, 100 mm × 6 mm i.d.) column. Detection was done spectrophotometrically at 254 nm. Calibration graphs were rectilinear over the concentration ranges of 2—20 μg/ml for strychnine sulfate and 5—50 μg/ml for yohimbine HCl, with minimum detection limits of 0.1 and 0.4 μg/ml, respectively. For the determination of yohimbine alone, high-performance liquid chromatography/fluorometric analysis ($Ex_{270\,nm}$, $Em_{360\,nm}$) was carried out with the same mobile phase. The calibration graph was rectilinear over the concentration range of 5—40 ng/ml with a minimum detection limit of 2 ng/ml.

The proposed methods were applied to dosage forms containing yohimbine and/or a mixture of yohimbine and strychnine. The results obtained compared favorably with those found with alternative methods.

Keywords yohimbine; strychnine; ion-pairing; HPLC; dosage form; fluorometric detection

Strychnine is the main alkaloid obtained from the seeds of *Strychnos nux-vomica*. It is widely prescribed, and several methods have been described for its determination, based on titrimetry[2,3] spectrophotometry,[4,5] thin-layer chromatography (TLC),[6] gas chromatography (GC)[7] and high-performance liquid chromatography (HPLC).[8,9] Yohimbine is also well known for its aphrodisiac activity, and recently, its α_2 adrenergic properties have been utilized in the treatment of orthostatic hypotension.[10] Various methods have been reported for the determination of yohimbine, including spectrophotometry,[11] fluorometry,[12] anodic voltammetry,[13] TLC,[14] GC[15] and HPLC.[16,17]

Yohimbine and strychnine are frequently dispensed together in aphrodisiac and nerve tonic preparations. Salama and Belal[18] in 1986 described a spectrophotometric method for the simultaneous determination of yohimbine and strychnine, based on the use of the Vierordt equation. This method is tedious, requiring numerous calculations to get numerical factors.

In this study, we report a simple, rapid and reliable method for the analysis of mixtures of the two alkaloids in dosage forms using HPLC/spectrophotometric detection. Yohimbine alone could be determined in the presence of other ingredients using HPLC/fluorometric detection.

Experimental

Apparatus A Jasco Intelligence HPLC pump, model 880-Pu, equipped with a Rheodyne sampling valve (model 7125) and a 100 μl loop, was used. An ERC-ODS-1262 column (5 μm, 100 × 6 mm i.d.; Erma Optical Works, Ltd., Tokyo, Japan) was employed. A Hitachi 633 HPLC detector and Hitachi F-1000 HPLC fluorescence detector were utilized for the determination of both yohimbine and strychnine and strychnine alone, respectively.

Chemicals Strychnine sulfate, reagent grade was obtained from E. Merck (Darmstadt, Germany). Yohimbine HCl, reagent grade was from Sigma Chem. Co. (St. Louis, MO, U.S.A.). Sodium hexanesulfonate was obtained from Aldrich Chem. Co. (Milwaukee, WI, U.S.A.). Pharmaceutical preparations containing yohimbine or mixtures of yohimbine and strychnine were obtained from commercial sources or kindly provided by several pharmaceutical companies.

Solutions The mobile phase consisted of methanol–water–acetic acid–triethylamine (50 : 50 : 1 : 0.3, pH 4.5) containing 1 mM sodium hexanesulfonate; it was filtered and degassed directly before use.

HPLC Conditions For the analysis of mixtures of yohimbine and strychnine, the flow rate was 1.0 ml/min and the detection wavelength was 254 nm at 0.04 AUFS. For the determination of yohimbine alone, the flow rate was 1.0 ml/min and detection was done fluorometrically with $Ex_{270\,nm}$ and Em_{360nm}.

Application of the Proposed Methods to Dosage Forms Preparations containing yohimbine and/or strychnine salts: Weigh and pulverize 20 tablets or empty the contents of 10 capsules. An accurately weighed quantity of the powder containing 50 mg of yohimbine HCl (and the accompanying amount of strychnine) is extracted with 3 × 30 ml of water. The extract is filtered into a 100 ml volumetric flask, and the residue is washed with water. The combined filtrates and the washings are adjusted to 100 ml with water. The solution is filtered through a 0.45 μm filter and then chromatographed.

Tablets or Capsules Containing Yohimbine Extract Weigh and pulverize 20 tablets or empty the contents of 10 capsules. A weighed quantity of the powder equivalent to 5.0 mg of yohimbine HCl is extracted with 3 × 30 ml of 2 N HCl solution, and processed as described above.

Results and Discussion

Figure 1A shows a typical chromatogram of a mixture of yohimbine and strychnine obtained under the described conditions. The addition of the counter ion sodium hexanesulfonate was essential to increase the retention time of yohimbine, thus permitting the good separation of the two peaks. To confirm the identity of the two drugs in formulations, another solvent system consisting of 0.5 M phos-

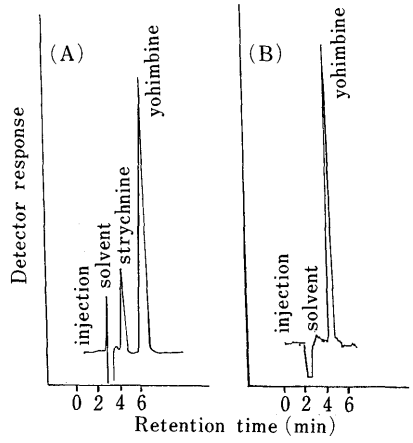

Fig. 1. HPLC Chromatograms of Strychnine and Yohimbine

(A) Typical chromatogram of strychnine sulfate (5 μg/ml) and yohimbine HCl (50 μg/ml), with UV detection at 254 nm, 0.04 AUFS. (B) Typical chromatogram of yohimbine HCl (40 ng/ml) with fluorometric detection ($Ex_{270\,nm}$, $Em_{360\,nm}$).

TABLE I. Application of the Proposed and Reference Methods to the Analysis of Tablets Containing Yohimbine and Strychnine

Preparation (per tablet)	% recovery			
	Yohimbine HCl		Strychnine sulfate	
	HPLC	SPM[a]	HPLC	SPM
Yohimbine HCl 5.0 mg + strychnine nitrate 0.5 mg + ginseng extract 150 mg	98.8 ±0.52	99.0 ±0.40	101.2 ±0.25	101.0 ±0.45
Yohimbine HCl 3.0 mg + strychnine sulfate 0.5 mg + hambi extract 20 mg	99.3 ±0.43	99.8 ±0.29	98.7 ±0.49	98.9 ±0.55
Yohimbine HCl 4.5 mg + strychnine HCl 1.5 mg + zinc phosphide 4 mg	99.5 ±0.25	99.8 ±0.38	98.9 ±0.45	99.1 ±0.60

a) Spectrophotometric method. Each result is the mean±S.D. from 4 separate determinations.

TABLE II. Application of the Proposed Method to the Determination of Yohimbine in Dosage Forms

Preparation (per tablet)	Label claim (mg)	Amount found (mg)	% recovery Mean±S.D.
Yohimbine HCl 5.0 mg + strychnine nitrate 0.5 mg + ginseng extract 150 mg	5.0	5.050	101.0±0.42
Yohimbine HCl 6.0 mg + garana extract 25 mg	6.0	5.866	97.8±0.37
Yohimbine extract 78.7 mg + muira extract 20 mg + damiane extract 16 mg + colae extract 20 mg + ginseng extract 40 mg + methyl testosterone 0.3 mg	3.0[a]	2.550	85.0±0.25
Yohimbine HCl 3.0 mg + strychnine sulfate 0.05 mg	3.0	2.985	99.8±0.51

Each result is the average of 4 separate determinations. *a*) Yohimbine extract is equal to 3.0 mg of yohimbine HCl.

phate buffer (pH 3)–methanol (45:55) was used. The retention times of yohimbine HCl and strychnine sulfate were 3.8 and 5.2 min, respectively. The detector wavelength (254 nm) coincides with the wavelength of maximum absorption of strychnine, which is the minor component in the preparation; and yohimbine (the major component) has an appreciable absorbance at this wavelength ($A^{1\%}_{1cm}$ at 254 nm = 116). The detection limits of strychnine and yohimbine were 0.1 and 0.4 μg/ml, respectively. Thus, a mixture of the two compounds in the medicinally recommended ratio could be accurately analyzed.

Figure 1B shows a typical chromatogram of yohimbine with HPLC/fluorometric detection. The calibration curve was rectilinear over the concentration range of 5—40 ng/ml. The linear regression equation for the curve can be expressed by the equation: $Y=0.33265X-0.02793$ with $r=0.999667$ (Y, peak height (cm), X, the amount injected (ng/ml)).

The proposed methods were applied to the determination of mixtures of yohimbine and strychnine and preparations containing only yohimbine. The results obtained (Tables I and II) were in good agreement with those found by the two-component spectrophotometric method.[18] Statistical analysis of the results obtained by both methods showed no significant difference in accuracy or precision as revealed by Student's t-test and the variance ratio F-test.[19] The proposed methods are simple, time-saving and readily adaptable to unit-dose analysis, and are recommended for routine analysis.

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References and Notes

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