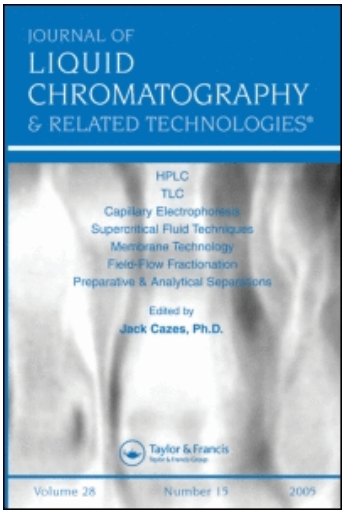


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Assay of Yohimbine in Human Plasma Using High Performance Liquid Chromatography with Electrochemical Detection

Michael R. Goldberg[a]; Loretta Speier[a]; David Robertson[a]
[a] Division of Clinical Pharmacology, Vanderbilt Medical School, Nashville, TN

To cite this Article Goldberg, Michael R. , Speier, Loretta and Robertson, David(1984) 'Assay of Yohimbine in Human Plasma Using High Performance Liquid Chromatography with Electrochemical Detection', Journal of Liquid Chromatography & Related Technologies, 7: 5, 1003 — 1012
To link to this Article: DOI: 10.1080/01483918408074022
URL: http://dx.doi.org/10.1080/01483918408074022

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(5), 1003-1012 (1984)

ASSAY OF YOHIMBINE IN HUMAN PLASMA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

Michael R. Goldberg, Loretta Speier and David Robertson*
Division of Clinical Pharmacology
Vanderbilt Medical School
Nashville, TN 37232

ABSTRACT

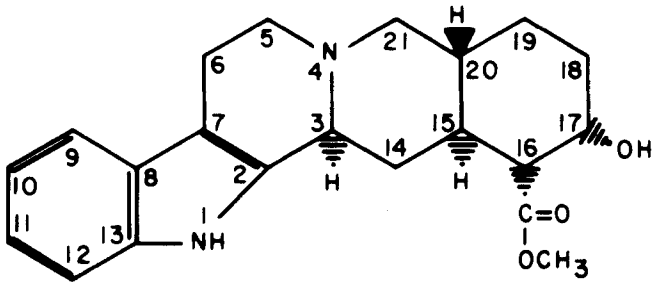
Yohimbine is a selective α_2 adrenoreceptor antagonist used in the study of α_2 adrenoreceptors in man. In order to better improve administration regimens for the study of yohimbine in man, we have developed an assay for the determination of yohimbine in plasma utilizing reverse phase high performance liquid chromatography with electrochemical detection. Using a C_{18} column and a methanol:acetate (60:40) mobile phase, we detected yohimbine in plasma following a simple chloroform extraction. Reserpiline was used as an internal standard. The assay was linear over a concentration range of 50-250 ng/ml in spiked plasma and had a lower limit of sensitivity of 10 ng/ml. It was used to detect yohimbine in plasma sampled from 4 volunteers during an infusion of the alkaloid.

*Author to whom correspondence should be addressed.

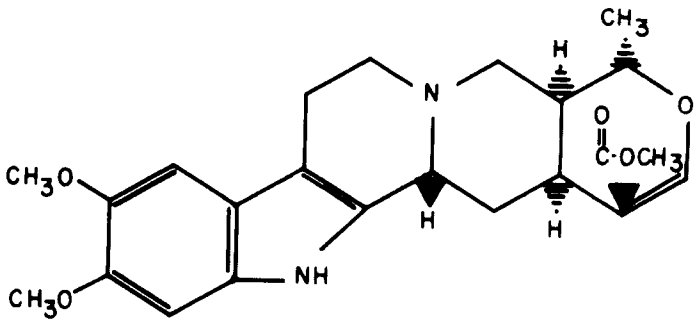
 0148-3919/84/0705-1003$3.50/0

INTRODUCTION

Yohimbine (Figure 1) is an α_2 adrenoreceptor antagonist which has been a useful probe for the study of the physiology and pharmacology of α_2 adrenoreceptors (1). α_2 receptors are important regulators of central sympathetic outflow and may also be involved in such disparate functions as regulation of catecholamine release at the sympathetic noradrenergic terminal, mediation of vascular smooth muscle contraction and platelet aggregation, and in the regulation of intermediary metabolism. In studying yohimbine in man we have noted that the alkaloid raises blood pressure at doses of 16 to 125 μg/kg (2). Based on the time course of these effects and the kinetics of reserpine, a chemically similar alkaloid (3), we designed an infusion regimen which was estimated to produce steady-state levels of about 50 ng/ml plasma (about 10^{-7}M) and used this regimen to study the influence of yohimbine on plasma catecholamines (2) and vasoconstrictor responses to epinephrine and phenylephrine (4). Although the chosen regimen (125 μg/kg bolus, 1 μg/kg/min infusion) elicited the anticipated pharmacologic effects(2,4), it was felt that future studies of the influence of yohimbine on other aspects of sympathetic function, required more rigorous design of regimens to achieve steady-state levels and verification of these levels. Accordingly, we have developed an analytical method for the determination of yohimbine in human plasma using high performance liquid chromatography with electrochemical detection and



Yohimbine



Reserpiline

Figure 1. Chemical structures of yohimbine and the internal standard for yohimbine, reserpiline.

reserpiline as an internal standard (Figure 1). We have applied this method to the analysis of samples during infusions at the empirically chosen rate.

MATERIALS

Reagents

Methanol and chloroform were purchased from Burdick and Jackson Laboratories, Inc. Muskegon, Michigan. Sodium

acetate was purchased from Sigma Chemical Co. St. Louis, Mo. Glacial acetic acid and ammonium hydroxide were from Fisher Scientific Co. Yohimbine HCl was purchased from Sigma Chemical Co. (St. Louis, Mo.). The internal standard, reserpiline, was purchased from K and K Rare and Fine Chemicals, Plainview, N.Y.

Standards

A stock solution of yohimbine HCl (100 μg/ml) was prepared daily and diluted in methanol. A stock solution of reserpiline (30 μg/ml) was prepared in methanol and stored in the refrigerator.

Instrumentation

Chromatography was performed with a model PM-30A solvent delivery system and a Rheodyne manual injector (both purchased from Bioanalytical Systems, Inc., West Lafayette, IN). A Waters Associates C_{18} μBondapak column (300 mm x 3.9 mm I.D.) was used for chromatographic separation of yohimbine and reserpiline from plasma constituents. A model LC4B amperometric detector (Bioanalytical Systems) with a glassy (vitreous) carbon electrode was used to detect yohimbine and reserpiline in extracts of plasma. A strip chart recorder (10 mv input) was used.

METHODS

Sample Extraction

Blood samples were collected in EDTA and centrifuged to separate the plasma which was stored frozen at -20° C

for periods of 1 to 3 months. To 3 ml of plasma 30 μg of the internal standard, reserpiline, was added. In dropwise fashion, 4 N NH_4OH was added to adjust the pH to 9 (usually about 100 μl). Three ml of chloroform were added to the plasma in 20 ml polyethylene tubes which were gently inverted 20 times. The samples were centrifuged for 5 minutes at 1100 G. The organic layer was removed using a pipette and saved and the plasma was extracted two more times. The 9 mls of chloroform containing yohimbine were evaporated to dryness with a stream of air at room temperature. The residue was redissolved in 1 ml of chloroform and 0.5 ml of 0.1 N acetic acid was added and vigorously vortexed. This mixture was centrifuged and the acid layer removed for injection onto the column in volumes of 50-100 μl. Using [^{3}H] yohimbine, this extraction resulted in a recovery of about 75%. In preliminary studies it was noted that extracted samples could be stored overnite prior to injection onto the chromatographic column without alteration of the peak-height ratio.

Chromatographic Conditions

The chromatographic system was operated at room temperature. The mobile phase consisted of a 60% methanol and 40% acetate buffer (0.4 M, pH 6.0) and was carefully degassed prior to use. The flow rate of the mobile phase was 1.5 ml/min. An applied potential of 950 mV across the glassy carbon electrode was used. The mobile phase was not recycled.

Quantitation

Peak height ratios of yohimbine to reserpiline were plotted against concentration. A least-squares regression analysis of this line was used to calculate yohimbine concentration in samples.

RESULTS AND DISCUSSION

Figure 2 shows a typical chromatogram of blank plasma and plasma containing 105 ng/ml yohimbine. Yohimbine elutes approximately 4 minutes after the injection while the internal standard appears at 8 minutes. No peaks are found in blank plasma which interfere with yohimbine or the internal standard. Figure 3 shows a typical standard curve. Current techniques limit sensitivity to 10 ng/ml. Three clinical samples were assayed in triplicate and produced levels of 57 ± 8 (S.D.), 80 ± 10 and 185 ± 23 ng/ml.

Four normal volunteers were given yohimbine at the previously cited regimen (125 μg/kg bolus, 1 μg/kg/min infusion) and plasma was sampled 10,20,30,60,90 and 120 minutes after administration of the bolus. Results of this study are shown in Figure 4. Levels peaked rapidly after the infusion was begun and administration of the initial bolus (175 ± 38 ng/ml at the 10 minute sample point) and reached an approximate steady state by the 30 minute sample point. At this time, levels were 66 ± 14 ng/ml. After 2 hours of infusion, levels had fallen to 46 ± 14 ng/ml.

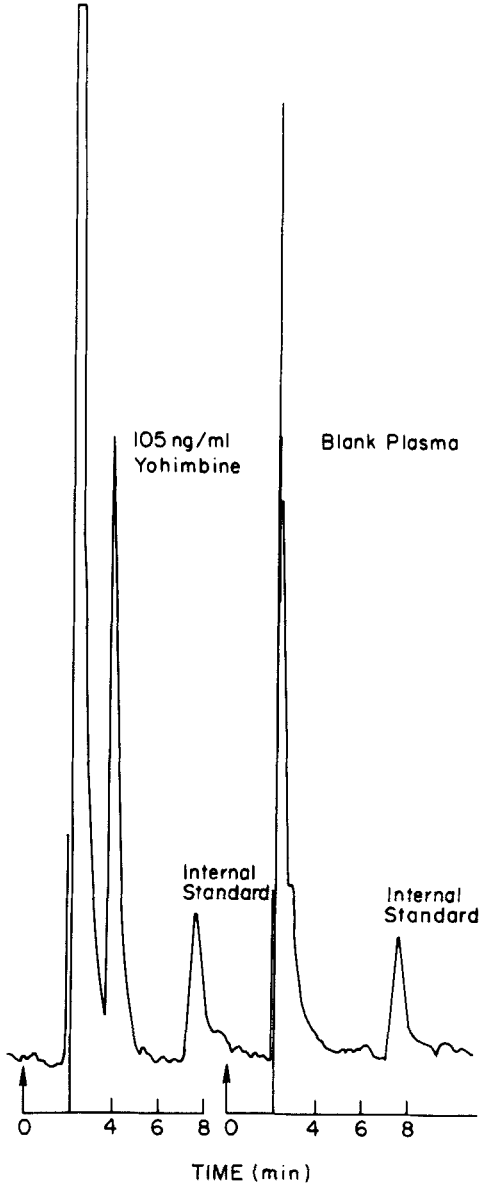


Figure 2. Chromatograms of samples of the same plasma before (right) and after administration of yohimbine intravenously. The sample was injected on the column at each arrow. 30 μg internal standard was added to the plasma

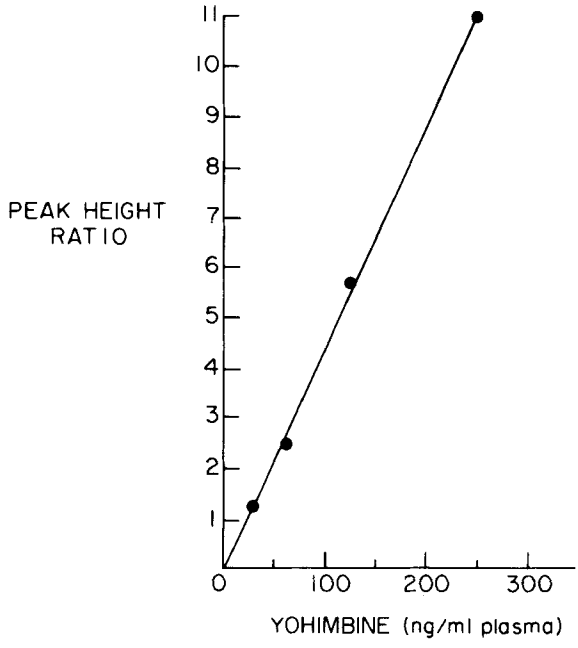


Figure 3. Standard curve determined in plasma relating known concentration of yohimbine to the ratio between the heights of the yohimbine and internal standard peaks (peak height ratio). The curve was linear from 25 to 250 ng/ml yohimbine.

These data show that the electrochemical detector can be used to measure yohimbine in human plasma. The methods employed appear generally applicable to the study of yohimbine in man and will prove useful in evaluating the effects of steady-state levels of yohimbine on autonomic function and in monitoring oral therapy with yohimbine in conditions which may include impotence (5) and autonomic dysfunction (6).

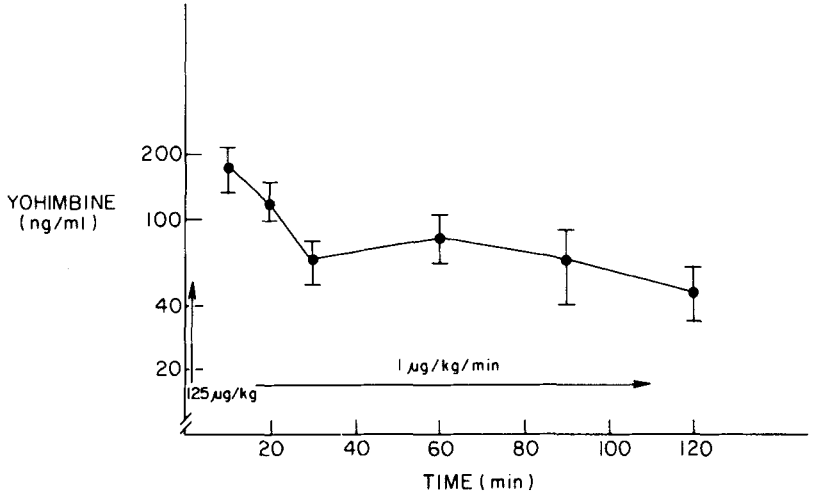


Figure 4. Plasma levels of yohimbine (mean ± SE) in 4 volunteers given a bolus of yohimbine HCl (125 µg/kg) followed by an infusion at a rate of 1 µg/kg/min.

ACKNOWLEDGEMENTS

This work was supported by grants 5-M01RR00095, GM31304, and HL14192 from the National Institutes of Health and by a grant from the Hrafn Sveinbjarnarsson Foundation. Dr. Goldberg was Clinical Associate Physician, Elliot V. Newman Clinical Research Center during the period of these investigations. His current address is Lilly Research Laboratories, 307 E. McCarty Street, Indianapolis, Indiana 46285. Dr. Robertson is recipient of a Research Career Development Award from the NIH GM00494.

Ms. Betty Campbell and Mrs. Carolyn Rork helped with preparation of the manuscript.

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