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RESEARCH PAPER

A High-Performance Liquid Chromatography Assay for Yohimbine HCl Analysis

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ABSTRACT

The analysis used yohimbine HCl solution prepared from commercially available yohimbine HCl powder. Stability-indicating high-performance liquid chromatographic (HPLC) assay procedures were established and utilized to analyze the concentration of the drug. The method proved to be a simple model since it does not contain a buffer system. The mobile phase used, a methanol:water 70:30 ratio, was similar to that suggested by the manufacturer for the storage of the column. Therefore, the solvent system saves analytical processing time since it does not require a change in the mobile phase before and after the analysis. The analytical method has been shown to be stability indicating. The assay method showed a retention time for yohimbine of 4.2 min; for caffeine, the internal standard, it was 2.3 min. The standard deviation and the coefficient of variation were under acceptable limits of 2% and were specifically 1.51% and 1.35% for within-day and between-day samples, respectively. The results showed that the degradation products obtained from stressing yohimbine HCl by heat and extremes in pH did not interfere with the yohimbine HCl peak, although the internal standard, caffeine, did show some interference due to having a retention time similar to the degradation products.

Key Words: *Caffeine; HPLC; Yohimbine HCl.*

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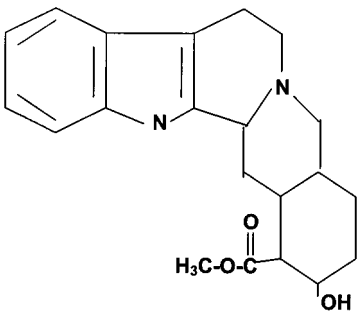
INTRODUCTION

The purpose of developing an assay method for yohimbine HCl was to provide an analytical method not documented previously in the literature. The USP accepts only those methods that have not been documented and that improve on the earlier methods that are either cumbersome or difficult to perform. The present method would be very useful in determining yohimbine concentrations for various stability and kinetic studies.

The specific objective of the study was to develop a stability-indicating assay method for the quantitative analysis of yohimbine. The analytical procedure should have the ability to determine distinctively the parent compound without interference from the degradation products. The drug solution is subjected to stress conditions such as extremes of pH and heat to test the stability of the assay. The precision of this assay procedure was evaluated in the following ways: The correlation coefficient of the calibration curve obtained from the assay procedure will not be less than 0.950; and the percentage relative standard deviation or the coefficient of variance of the readings obtained from 25 identical concentrations will be not more than 2.00% (1,2).

Yohimbine is an indole alkaloid available as either yohimbine HCl or yohimbine bark; it is commonly used for indications such as impotence, orthostatic hypotension, and diabetic neuropathy (3) in a dose of 15 mg/day. Yohimbine acts by antagonizing the alpha-2 inhibitory adrenergic receptors (3), and its mechanism of action can be divided into general and sexual mechanisms of action. Yohimbine can also inhibit platelet aggregation, and it is a calcium channel blocker. Yohimbine acts by increasing the adrenergic alpha-1 activity and decreasing the adrenergic alpha-2 activity.

Yohimbine is obtained from Yohimbehe bark (Structure 1) (4). Yohimbehe bark is derived from *Pausinystalia yohimba*, Pierre (Fam. Rubiaceae; syn. *Corynanthe yohimbe*, Schum), a tree indigenous to Cameroon and the French Congo. Yohimbine is a colorless, weakly basic, tertiary indole alkaloid with a pK_a (5) of 6–7.5. It gives a characteristic ultraviolet spectrum.



Structure 1. Structural formula of yohimbine (4).

When yohimbine is heated with concentrated potash solution, it is converted into potassium yohimbate, from which yohimbic acid $C_{20}H_{24}O_3N_2 \cdot H_2O$ is obtained on treatment with acetic acid. It crystallizes from water in lustrous prisms with a melting point of 269°C or 299°C (dry, dec.) and optical rotation $[\alpha]_D$ of +138.8° (pyridine) (4).

On esterification with methyl alcohol and its homologues, analysis confirmed that yohimbine is the methyl ester of yohimbic acid, $C_{20}H_{24}O_3N_2$. Yohimbine is readily soluble in chloroform and ethanol and is sparingly soluble in diethyl ether. The UV-Vis absorption spectrum of yohimbine shows absorbance in the range 279–282 nm (5).

ANALYSIS

It is necessary to have a stability-indicating assay procedure to analyze yohimbine quantitatively in the dosage form. The solution of yohimbine HCl in water has a pH of 7.3. Since the pH is not low or high enough to dissolve the column, the use of a buffer in the assay procedure can be neglected. Phosphate, acetate, and citrate buffers are known to be insoluble in methanol. This can result in the precipitation of the buffer salts within the column when the mobile phase contains a high concentration of methanol for the analysis. A mixture of 70% methanol in water was selected as the mobile phase with a flow rate of 1.0 ml/min. The peaks appeared to be distinct and resolved. However, the retention time was too short. The flow rate was then reduced to 0.6 ml/min, which gave an optimum retention time of around 4.2 min. The mobile phase had 70:30 methanol:water (v/v) and had a flow rate of 0.6 ml/min. Caffeine was used as an internal standard. The advantage of this method is that C18 columns are usually stored in 66% (v/v) methanol:water, which is very close to the concentration of methanol used in the analysis. This process avoids the mobile phase change before and after the analysis and saves analytical processing time.

METHODOLOGY

Reagents and Chemicals

All reagents used were USP, NF, or ACS grade. HPLC-grade methanol (lot M182KMNM) was obtained from Cuetin Scientific (Houston, TX); caffeine USP (lot

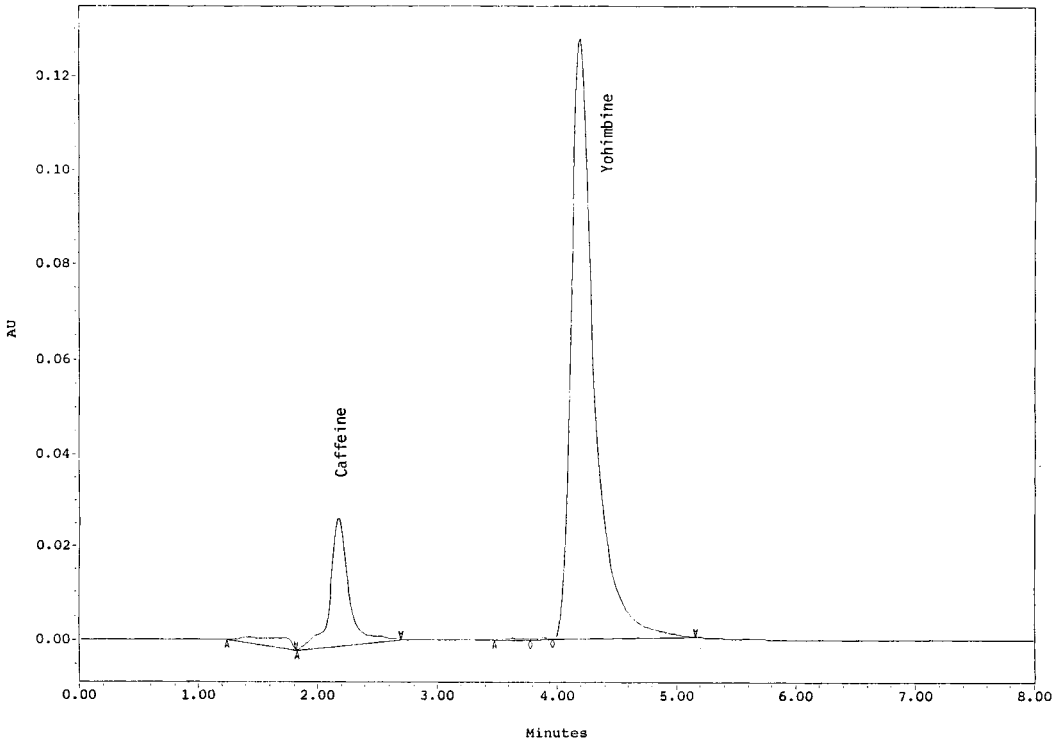


Figure 1. HPLC chromatogram of yohimbine HCl standard solution (60 μg/ml) with caffeine as the internal standard.

0710463) was obtained from Ruger Chemical Company, Incorporated (Elizabeth, NJ); and yohimbine HCl 99% (lot A01007001) was obtained from Acros Organic (division of Fisher Scientific, Pittsburgh, PA).

High-Performance Liquid Chromatographic Instrumentation and Condition

The HPLC system consisted of Waters 501 HPLC pumps with a 2200-μl loop injector. The detector used was a Waters 486 Tunable Detector. The sample processor was the Waters 712 WISP (Waters Intelligent Sample Processor). The software for acquiring and processing the data was Millennium® (version 2.1) obtained from Waters. The column was a Novopak™ C18 column, 4.6 mm × 150 mm with a particle size of 5 μm.

The flow rate of the mobile phase was set at 0.6 ml/minute. The column pressure varied between 1100 and 1300 psi. The detector was operated at 270 nm and 1 AUFS. The filter was set at 1. The analytical column was at room temperature for all the separations. The mobile phase for the HPLC assay of yohimbine HCl was prepared according to the following procedure. A 700-ml volume of methanol was transferred into a graduated cylinder. It was made up to a liter with distilled water. The solution was filtered through a 0.45-μm membrane filtration system (Millipore Corp., Milford, MA) to remove

Table 1

Data for the Calibration Plot for Yohimbine HCl

Concentration of yohimbine HCl (μg/ml)	7.92	11.88	19.80	27.72	35.64	59.40
Peak area for yohimbine HCl (AU)	181,132	325,881	493,647	867,646	999,529	1,827,828

The equation obtained from calibration plot: Peak area = 31,798 (Concentration) − 77,833; R^2 = 0.9935.

Table 2

Precision of the Yohimbine HCl Assay Peak Area

Day 1	Day 2	Day 3	Day 4	Day 5
1,629,497	1,763,921	1,748,572	1,776,569	1,773,704
1,701,881	1,715,756	1,713,086	1,715,836	1,734,019
1,696,231	1,741,616	1,738,795	1,710,155	1,761,626
1,689,949	1,716,340	1,716,112	1,694,269	1,730,354
1,687,693	1,692,873	1,717,480	1,741,945	1,710,360

Standard deviation between days is 23,144. Mean between days is 1,720,746. Coefficient of variation between days is 1.35%.

any particulate matter. The mobile phase was degassed with nitrogen before use.

The internal standard stock solution was prepared using 10 mg of caffeine quantitatively weighed on a Mettler balance and transferred into a 100-ml volumetric flask. The volume was made up to 100 ml with distilled water to give a final concentration of 100 μg/ml caffeine internal standard stock solution.

Calibration Curve and Assay Precision

Yohimbine stock solution was prepared by accurately weighing approximately 20 mg of yohimbine HCl and dissolving it in 100 ml of water in a volumetric flask to give a final concentration of approximately 0.2 mg/ml yohimbine HCl stock solution.

The calibration plot for the yohimbine HCl HPLC method was obtained using the following procedure. Yohimbine HCl stock solution in 3.0, 5.0, 7.0, 9.0, 15.0, and 25.0 ml portions was transferred to six 50-ml volumetric flasks. The volume was made up to 50 ml with reverse-osmotic (RO) water. About 5 ml of each of these solutions were filtered through a 0.2-μm filter. The internal standard, approximately 1 ml, was added to each of the six 5-ml portions. The sample processor was adjusted to withdraw 20 μl of each of these solutions. The internal standard helped confirm the proper working of the column throughout the analysis by showing the caffeine peak with similar retention times. The peak area for yohimbine HCl was recorded. Peak area was plotted on the *y* axis against the concentration of yohimbine HCl in the final dilution on the *x* axis. The equation obtained by linear regression would be used to calculate the yohimbine HCl concentration.

The precision of the HPLC assay procedure was tested by the following procedure. Precision of the assay was determined by injecting identical samples five times each day over a 5-day period. The detector response was compared, and the percentage relative standard deviation for the area under the curve (AUC) ratios was calculated for between-day and within-day variations.

Table 3

Mean and Standard Deviation for Results Within Day[a]

Day	Mean Within Days ($n = 5$)	Standard Deviation Within Days ($n = 5$)
1	1,681,050	29,348.88
2	1,726,101	27,283.07
3	1,726,809	15,867.18
4	1,727,755	32,238.55
5	1,742,013	25,449.65

[a]Mean square coefficient of variation within day is 1.51%.

Stability-Indicating Nature of the Assay

The stability indicating nature of the yohimbine HCl HPLC assay procedure was tested using the following procedure. The drug was subjected to extreme acidic and alkaline conditions while being heated to promote possible degradation. A 0.399-mg/ml stock solution of yohimbine HCl was prepared by dissolving 39.9 mg in 100 ml of RO water. To 15 ml of the stock solution in a 100-ml volumetric flask, 4 g of NaOH were added, and the volume was made up to 100 ml with water. The solution was then heated for 30 min to allow degradation. To 15 ml of the stock solution in another 100-ml volumetric flask, 8.3 ml of HCl (38% v/v) were added, and the volume was made up to 100 ml. The solution was then heated for 30 min to allow for degradation. Both solutions were cooled and transferred to WISP vials after filtering through a 0.2-μm filter. The solutions were then injected into the column. Later, they were spiked with caffeine, the internal standard, and then run through the column again.

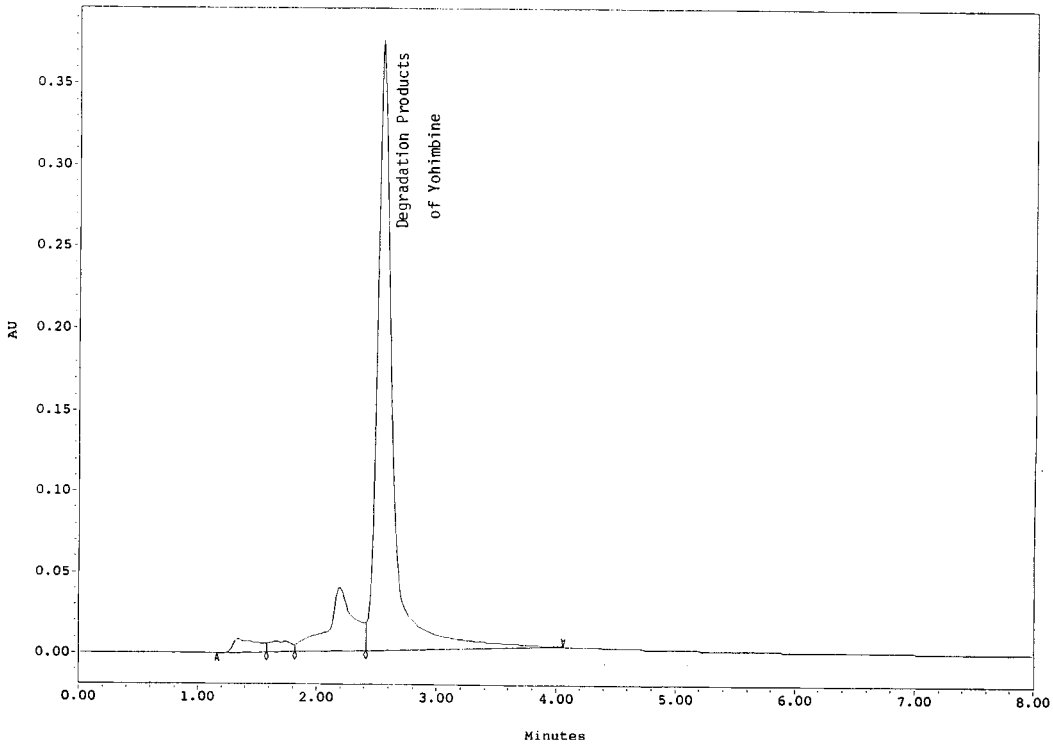


Figure 2. HPLC chromatogram of yohimbine HCl standard solution treated with 1 M NaOH and subjected to heat.

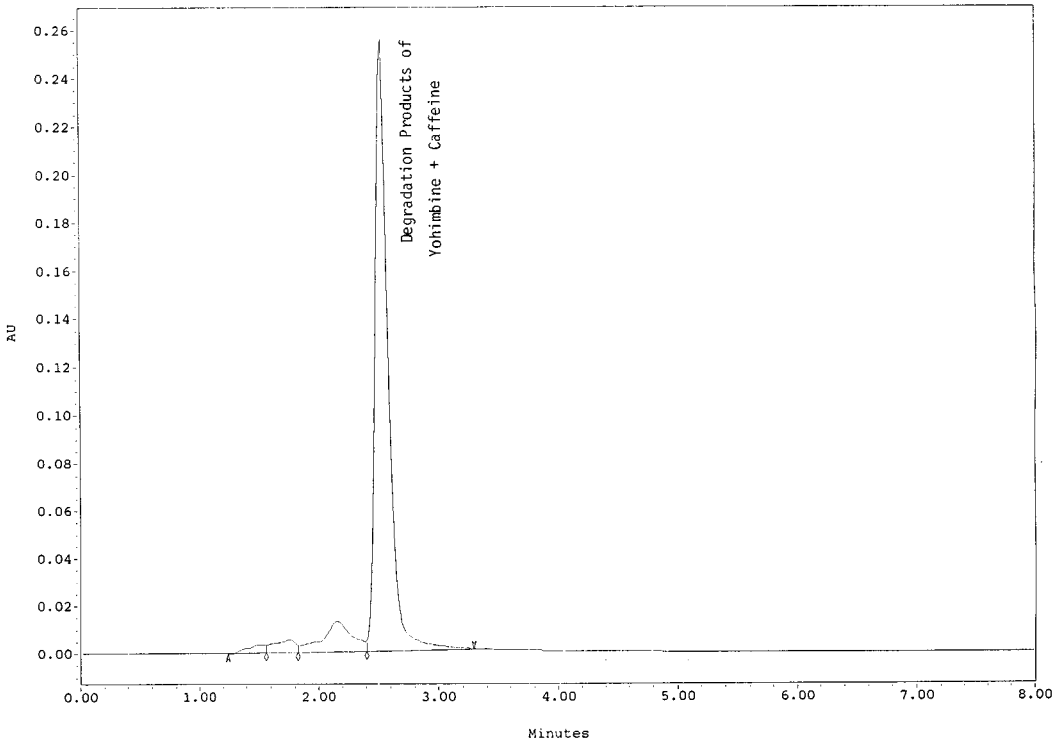


Figure 3. HPLC chromatogram of yohimbine HCl standard solution treated with 1 M NaOH and spiked with caffeine after heating.

Precision of the Sampling Assay

The precision of the HPLC sampling method was obtained using the following procedure. A single concentration of yohimbine HCl that fell between the concentrations used in the calibration plot was prepared from the stock standard. This solution was injected from five different vials, one injection from each vial, on one day, and this process was carried out for five days. This gave us 25 chromatograms of the same sample injected under identical instrument conditions. The precision of the sampling assay was performed by calculating the percentage standard deviation for the sample injected within day and between days. The area of the yohimbine HCl peak and its variance over the five samples in a day and over the samples between days was used to compute the standard deviation for the two cases.

RESULTS AND DISCUSSION

When a solution containing yohimbine HCl and caffeine was injected onto the chromatographic column, a clear separation of these compounds occurred (Fig. 1). The retention times for caffeine and yohimbine HCl were 2.3 min and 4.6 min, respectively.

The calibration plot providing the area under the curve for yohimbine HCl on the *y* axis against concentration of yohimbine HCl on the *x* axis showed an excellent coefficient of correlation of 0.9935. The data for the calibration plot are given in Table 1.

The percentage relative standard deviation of the area under the curve for within and between days was obtained by injecting an identical sample five times on each of 5 days; the results are presented in Tables 2 and 3, respectively. The percentage relative standard deviations for within day and between days were calculated and were found to be 1.51% and 1.35%, respectively.

The stability-indicating nature of the HPLC assay was determined by subjecting yohimbine HCl to extreme acidic or alkaline conditions and heating. The yohimbine HCl solution stressed under alkaline conditions showed zero potency for yohimbine HCl and a peak at 2.5 min (Fig. 2). This solution was spiked with the internal standard stock solution and again injected onto the HPLC column. This showed interference between the internal standard and the degradation peak (Fig. 3). The degradation products of yohimbine HCl are not known, so the interference of the degradation peak with the internal standard peak is possible. The yohimbine HCl solution stressed under acidic conditions showed a peak for the

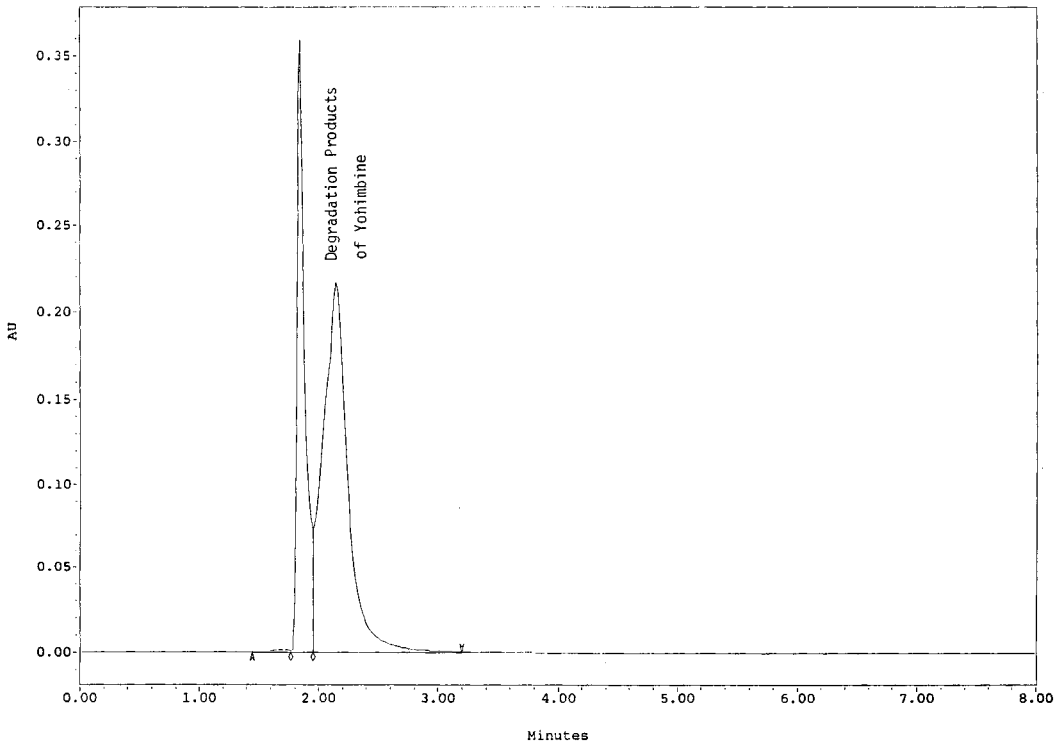


Figure 4. HPLC chromatogram of yohimbine HCl standard solution treated with 1 M HCl and subjected to heat.

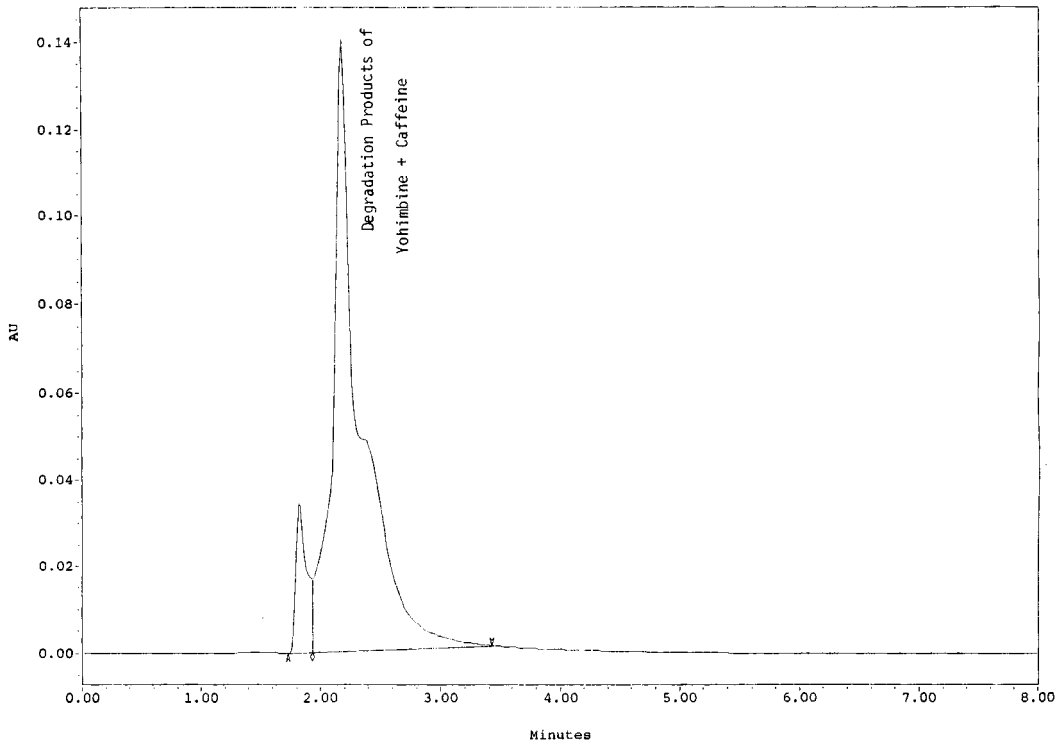


Figure 5. HPLC chromatogram of yohimbine HCl standard solution treated with 1 M HCl and spiked with caffeine after heating.

internal standard and a peak at 2.5 min (Fig. 4). The retention time for the degradation peak is similar to that of caffeine and not of yohimbine HCl (Fig. 5). The above results demonstrate that the HPLC assay procedure is stability indicating.

CONCLUSION

The investigation developed a modified stability-indicating HPLC procedure for the quantification of yohimbine HCl is dosage forms. This method proved to be a simple model since it does not contain a buffer system. The mobile phase used was nearly the same as that suggested by the manufacturer for the storage of the column. Therefore, the process saves analytical processing time since it does not require a change in the mobile phase before or after the analysis. It may also be possible to maximize column life by using the mobile phase suggested by the manufacturer to store the column. The calibration plot obtained using this method of analysis resulted in a coefficient of correlation of 0.9935. The percentage relative standard deviations for within day and between days for the HPLC assay were calculated to be 1.51% and 1.35%, respectively, demonstrating good precision of the assay. The analytical method was shown to be stability indicating through the analysis of samples stressed under either acidic or basic conditions with applied heat. The results showed that there is no interference of the degradation products with the peak for yohimbine HCl, though interference was observed with caffeine, the internal standard. The assay also showed that there is good separation between yohimbine HCl and its major degradation products and hence supports the selectivity of the method.

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