A validated high performance thin layer chromatography method for determination of yohimbine hydrochloride in pharmaceutical preparations

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ABSTRACT

Background: Yohimbine is an indole alkaloid used as a promising therapy for erectile dysfunction. A number of methods were reported for the analysis of yohimbine in the bark or in pharmaceutical preparations. **Materials and Method:** In the present work, a simple and sensitive high performance thin layer chromatographic method is developed for determination of yohimbine (occurring as yohimbine hydrochloride) in pharmaceutical preparations and validated according to International Conference of Harmonization (ICH) guidelines. The method employed thin layer chromatography aluminum sheets precoated with silica gel as the stationary phase and the mobile phase consisted of chloroform:methanol:ammonia (97:3:0.2), which gave compact bands of yohimbine hydrochloride. **Results:** Linear regression data for the calibration curves of standard yohimbine hydrochloride showed a good linear relationship over a concentration range of 80–1000 ng/spot with respect to the area and correlation coefficient (R^2) was 0.9965. The method was evaluated regarding accuracy, precision, selectivity, and robustness. Limits of detection and quantitation were recorded as 5 and 40 ng/spot, respectively. The proposed method efficiently separated yohimbine hydrochloride from other components even in complex mixture containing powdered plants. The amount of yohimbine hydrochloride ranged from 2.3 to 5.2 mg/tablet or capsule in preparations containing the pure alkaloid, while it varied from zero (0) to 1.5–1.8 mg/capsule in dietary supplements containing powdered yohimbe bark. **Conclusion:** We concluded that this method employing high performance thin layer chromatography (HPTLC) in quantitative determination of yohimbine hydrochloride in pharmaceutical preparations is efficient, simple, accurate, and validated.

Key words: Accuracy, dietary supplements, high performance thin layer chromatography, precision, selectivity, yohimbine

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INTRODUCTION

Yohimbine is an indole alkaloid obtained naturally from *Pausinystalia yohimbe*. It occurs also in *Rauwlfia serpentina* along with other active alkaloids. Many research articles have focused on the efficacy of yohimbine as a promising therapy for erectile dysfunction.[1-4] Yohimbine acts as a competitive antagonist selective for α-2 adrenergic receptor.[5] It should be administered with caution to patients with high blood pressure.[6] In addition, a slight anxiogenic action and increased frequency of urination could be noticed at relatively high doses.[7] Yohimbine is available in a number of pharmaceutical preparations including tablets and capsules; moreover, the powdered yohimbe bark is included in some dietary supplements. Since dietary supplements are considered as over the counter products; hence, these could be administered without doctor's prescription. For that reason, it is important to design a simple and reproducible method to estimate yohimbine in pharmaceutical preparations including these dietary supplements. A number of methods were reported for the analysis of yohimbine in the bark or in pharmaceutical preparations. These included ultraviolet (UV) spectrophotometry,[8] high performance liquid chromatography (HPLC),[9-11] gas chromatography,[12] nonaqueous capillary electrophoresis,[13] phosphorimetry,[14] and colorimetry.[15] In the present work, yohimbine is quantitatively determined in five different pharmaceutical preparations including dietary supplements using high

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performance thin layer chromatography (HPTLC). On reviewing the literature, thin layer chromatography was previously used in qualitative detection and separation of a mixture of alkaloids including yohimbine from different species belonging to family Apocynaceae.[16,17] Accordingly, HPTLC is used and validated for the first time in the present work for quantitative estimation of yohimbine in pharmaceutical preparations.

MATERIALS AND METHODS

Instrumentation

A Camag (Wilmington, USA) Linomat IV sample applicator was used to dispense the aliquots of the standard stock solution and the prepared samples. The plates were saturated in a twin trough chamber; slit dimension settings of length 6 and width 0.1 mm, monochromator band width 20 nm and scanning rate of 10 mm/s. Zones were quantified by using Camag TLC Scanner III densitometer (Wilmington, USA) controlled by CATS version 4 × software in the absorption mode using a deuterium source and a filter with wavelength of 280 nm.

Chemicals and pharmaceutical products

Standard yohimbine hydrochloride alkaloid was purchased from Sigma (Sigma-Aldrich, Germany), and certified to contain 99%. Solvents for extraction and development and precoated silica gel F_{254}, 0.25 mm were obtained from Merck Company (Darmstadt, Germany). The pharmaceutical preparations (tablets and capsules) were purchased from local markets of Egypt and Kingdom of Saudi Arabia.

Standard solution

The standard stock solution was prepared by weighing 100 mg of the authentic sample of yohimbine hydrochloride then quantitatively transferred to 100 mL volumetric flask and volume was adjusted with methanol, kept in the refrigerator and tightly closed. One milliliter of the stock solution was withdrawn and accurately transferred to 10 mL volumetric flask and volume was made up with methanol (stock solution A). From stock solution A, 1 mL was accurately withdrawn and transferred to 10 mL volumetric flask and volume was adjusted with methanol to give stock solution B. The calibration curve was constructed according to the requirements of the International Conference of Harmonization (ICH) guidelines.

Sample preparation

Five different products were used for this study. The products were selected so that they vary in composition and form to ensure validation of the method under different conditions. Two products (samples 1 and 2) were selected as tablets and capsules, respectively, containing mainly yohimbine hydrochloride together with lactose and sucrose (in sample 1) and vitamin E, nicotinic acid and caffeine (in sample 2). Three dietary supplements as capsules were chosen (samples 3, 4, and 5), each contains yohimbe bark powder together with royal jelly, ginseng, and bee pollen (in samples 3 and 4) and sample 5 contains royal jelly, ginseng, wheat germ oil, and garlic in addition to yohimbe bark powder. For extraction, 20 tablets or capsules were used from each product. The tablets (from sample 1) were finely ground by a mortar then sonnicated with methanol for 15 min, quantitatively transferred after filtration into 100 mL volumetric flash and volume was made up with methanol. The contents of 20 capsules from sample 2 were extracted by sonnication with methanol and treated as sample 1. The contents of the same number of capsules from samples 3 to 5 (containing yohimbe bark powder) were extracted by sonnication with acidulated methanol (prepared in a ratio of methanol:concentrated HCl (100:0.1 v/v)), then treated similarly as samples 1 and 2.

Calibration graph

Calibration curve was constructed according to requirement of ICH guidelines. Each concentration was applied to a plate (20 × 10 cm) in triplicates of 6 mm band length with a distance of 4 mm between each two bands. The distance from the plate side edge was 10 mm and from the bottom of the plate was also 10 mm. The application rate was 15 μL/s, the bands were developed using chloroform:methanol:ammonia (97:3:0.2) after saturation for 20 min. The development time was 15 min, the plates were air dried for 10 min showing Rf = 0.58 ± 0.01. Standard zones were quantified by linear densitometric scanning using Camag TLC scanner in the absorbance mode at λ 280 nm, the wavelength corresponding to the maximum sensitivity. Deuterium lamp was utilized as a source of radiation. Evaluation was done using linear regression analysis via peak areas. [Figure 1] illustrates the densitogram recorded for a 300 ng/spot of standard yohimbine hydrochloride at λ 280 nm.

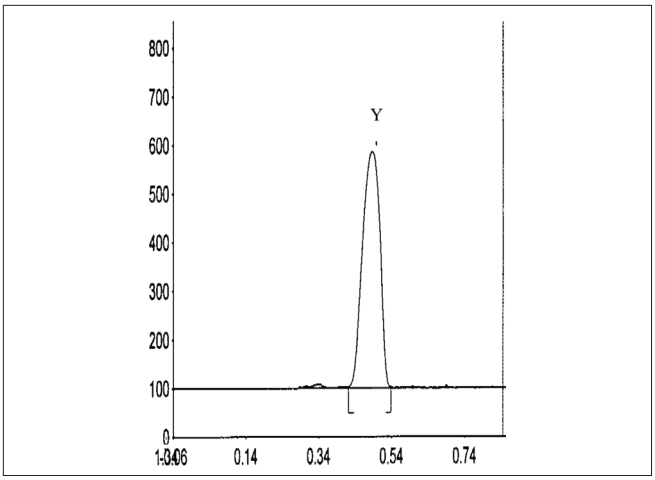

Figure 1: HPTLC scan densitogram showing the injection of 300 ng/spot of standard yohimbine hydrochloride [Y] scanned at λ 280 nm using chloroform: methanol: ammonia (97: 3: 0.2)

Sample assay

After preparation of the five samples as previously described, 6 µL of each sample and the standard solution were spotted in triplicates on a plate, developed under the same conditions as described for the standard yohimbine hydrochloride. After development and drying of the plates, the analyte was found to be completely separated from other components so the linear and compact zones were scanned at λ 280 nm and peak areas for yohimbine hydrochloride were recorded. [Figure 2] illustrates the densitograms for two representative samples 2 and 4.

RESULTS AND DISCUSSION

Linearity

The linearity of the HPTLC method was evaluated by analyzing a series of different concentrations of the standard yohimbine hydrochloride where each concentration was applied triplicate. Linear regression data for the calibration curves of standard yohimbine hydrochloride showed a good linear relationship over the concentration range of 80–1000 ng/spot with respect to the area. The correlation coefficient (R^2) was 0.9965 and linear regression equation was found to be: $y = 22292\ x + 7008.5$, where y is the spot area and x is the concentration of the analyte.

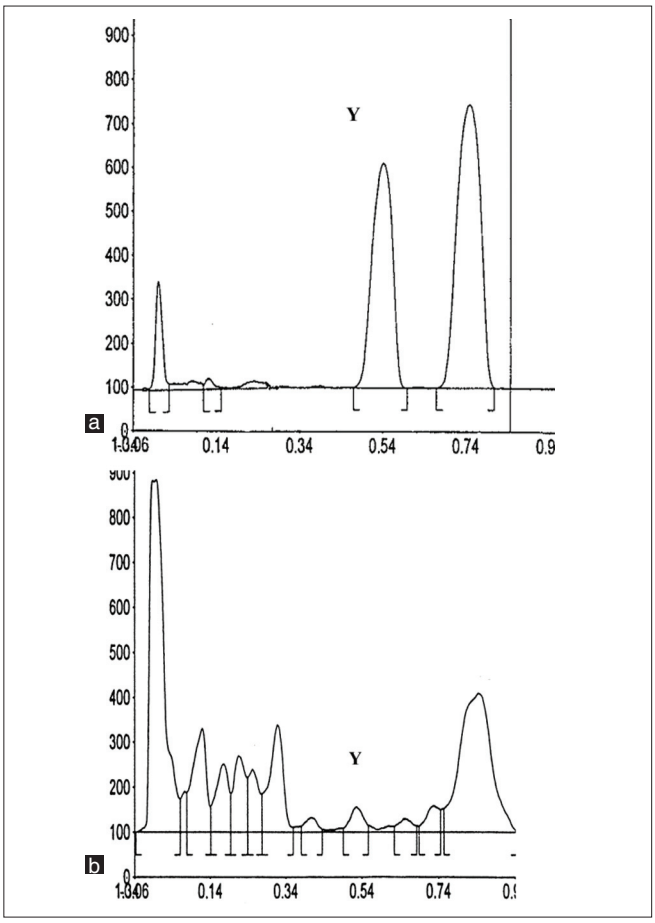

Figure 2: HPTLC scan densitograms showing the injection of 6 µL of two representative samples [2 (a) and 4 (b)] scanned at λ 280 nm using chloroform: methanol: ammonia (97: 3: 0.2)

Selectivity

Selective baseline separation was obtained between yohimbine hydrochloride and other components of the matrix in the samples 1 and 2. Moreover, good separation was noticed between yohimbine hydrochloride and the components of the complex mixtures in case of the dietary supplements (samples 3–5). The scan densitograms obtained from two different representative samples are presented in Figure 2.

System precision

The system precision was assessed by determination of six different concentrations of yohimbine hydrochloride each applied triplicate.

Method precision (repeatability)

Repeated analysis of a homogenous sample (sample 2) applied in triplicate using the same procedure was also performed and the low value of relative standard deviation (RSD)% (1.93) indicated precision of the method [Table 1].

Accuracy

Accuracy of the method was ascertained by spiking of a preanalyzed sample (sample 2) with known amount of standard yohimbine hydrochloride solution and then analyzing by the same method. Analysis was performed in triplicates and the average percentage recovery at each concentration level was evaluated. The results are illustrated in Table 2.

Limits of detection and quantitation

Limit of detection and limit of quantitation were validated based on signal to noise ratio where the minimum concentration at which the standard yohimbine hydrochloride solution can be reliably detected was recorded as 5 ng/spot (S/N = 3 of diluted standard) and minimum concentration at which the analyte can be reliably quantified (S/N = 10 of diluted standard) was found to be 40 ng/spot.

Robustness

Robustness of the method was studied by carrying out the

Table 1: Validation parameters of the HPTLC method for estimation of yohimbine hydrochloride

Parameter	Results
Linearity range (ng/spot)	80–1000
Correlation coefficient (R^2)	0.9965
Regression equation	$y = 22292\ x + 7008.5$
Limit of detection (ng/spot)	5
Limit of quantitation (ng/spot)	40
System precision (RSD, %)[a]	1.41
Method precision (RSD, %)[b]	1.93

RSD = Relative Standard Deviation, [a]$n = 6 \times 3$, [b]$n = 3 \times 3$

measurements under different conditions as slight changes in the composition of the developing mixture, changing the amount of developing mixture, time from spotting to chromatography and time from chromatography to scanning. Standard yohimbine hydrochloride solution was applied on plate in triplicates; the results are illustrated in Table 3.

Sample analysis

Five products were analyzed by the proposed method [Table 4]. Samples 1 and 2 contained an amount of pure yohimbine hydrochloride equals 2.3 ± 1.7 and 5.2 ± 1.6 mg/tablet or capsule, respectively. The amount of yohimbine hydrochloride in the dietary supplements (samples 3–5) varied from 1.5 ± 1.1 in sample 3 to 1.8 ± 1.9 in sample 4 while it was not detected in sample 5. This variation could be attributed to the age of the plant at which the bark was collected.[18] At the contrary of sample 5, which was devoid of the analyte; samples 3 and 4 contained slightly higher amount than expected as yohimbe bark is reported to contain 0.8–2.24% of yohimbine alkaloid. This may be due to the variation in age of the plant as previously mentioned or due to addition of trace amounts of pure yohimbine hydrochloride on the content of the dietary supplements.

Table 2: Recovery of yohimbine hydrochloride in sample 2 using the proposed method

Experiment number	Concentration (mg/mL)			
	Expected	Found + SD	Recovery (%)	RSD %
1 (sample)	0.05	0.051 ± 0.00109	97.7	2.12
2 (mixture 1)	0.275	0.269 ± 0.00354	98.1	1.32
3 (mixture 2)	0.375	0.364 ± 0.00635	97.2	1.74

Each result is an average of three measurements

CONCLUSIONS

In order to ensure the quality of dietary supplements, these products should be subjected to analysis of the active constituents especially in case of containing active principles that may cause severe side effects when abused. A recent study was conducted and

Table 3: Robustness testing data of the HPTLC method for determination of yohimbine Hydrochloride

Parameter	SD of concentration (0.2 mg/mL)	RSD %
Composition of mobile phase		
chloroform: methanol: ammonia system I: (97: 3: 0.2)	0.00284	1.32
System II: (98: 3: 0.2)		
System III: (98: 2: 0.2)		
Amount of mobile phase		
25 mL	0.00270	1.24
30 mL		
40 mL		
Time from spotting to chromatography		
5 min	0.00248	1.17
10 min		
15 min		
Time from chromatography to scanning		
10 min	0.00239	1.11
15 min		
20 min		

SD = Standard deviation, RSD = Relative standard deviation, Each result is an average of three measurements

Table 4: Amount of yohimbine hydrochloride determined in mg/tablet or capsule in the examined preparations compared with the concentrations illustrated on the product

Product number	Dosage form	Illustrated amount of yohimbine hydrochloride or yohimbe bark powder in mg/tablet or capsule	Calculated amount of yohimbine hydrochloride in mg/tablet or capsule ± RSD
1	Tablets	2 mg (yohimbine HCl)	2.3 ± 1.7
2	Hard capsules	5.4 mg (yohimbine HCl)	5.2 ± 1.6
3	Hard capsules	25 mg (yohimbe bark powder)	1.5 ± 1.1
4	Hard capsules	25 mg (yohimbe bark powder)	1.8 ± 1.9
5	Soft capsules	10 mg (yohimbe bark powder)	–

Each result is an average of three measurements, RSD = Relative standard deviation

proved that a substantial increase in the prevalence of adverse side effects associated with yohimbine herbal products.[19] Moreover, it is known that the toxicity of yohimbine could be potentiated upon coadministration of a number of drugs including antidepressants[20] and drugs that interfere with tissue binding of noradrenaline as imipramine.[21] Since the proposed HPTLC method used for analysis was found to be specific, reproducible, accurate, simple, rapid, and separated yohimbine from other constituents, it could be proposed for routine quality control process of herbal supplements containing yohimbine.

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