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A SIMPLE HPLC METHOD FOR THE ESTIMATION OF YOHIMBINE FROM YOHIMBE BARK

Yedugani Lingam*[1], Gajanan Patange[1], Dipak Gangadhar Ambhore[1], Atish Jayaprakas Salunke[1]

[1*]Bhoomi Nutraceuticals Private Limited, Research and Development Centre, B-3, Basmat Road- Nanded, MIDC Area, Basmat, Maharashtra, India.

ABSTRACT

A simple, rapid, selective and quantitative HPLC method has been developed for the determination of Yohimbine from Yohimbe bark. The mobile phase was Methanol: Acetonitrile: Phosphoric acid (0.4%) 37: 0.7: 62.3 (V/V), Flow rate 1.0ml/min. The retention time of pure Yohimbine was 8.662 minutes. The content of Yohimbine extract powder was 62% and reasonable pure sample purity was 95% respectively. The developed method was found to be simple, robust, rugged and economical for routine analyse the Yohimbine extract and bark raw material.

KEYWORDS

Yohimbine, HPLC, Yohimbine bark and Retention time.

Author for Correspondence:

Yedugani Lingam,

Bhoomi Nutraceuticals Private Limited,

Research and Development Centre,

B-3, Basmat Road- Nanded, MIDC Area,

Basmat, Maharashtra, India.

Email: bhoomi.npl1@gmail.com

INTRODUCTON

Yohimbine is an alkaloid derived from the African plant Pausinystalia yohimbe (Rubiaceae)[1] and its bark contains up to 6% of a mixture of alkaloids. The bark of the plant has been traditionally used in folk medicine as a general tonic, performance enhancer and as an aphrodisiac[2-6]. The pharmacological research on yohimbine (Figure No.1) have been started since 1949[7-10]. The development of a simple HPLC method with good resolution to such complex alkaloids is a real challenge.

In the present study, a simple method for the determination of Yohimbine by reverse phase High Performance Liquid Chromatography (HPLC) analytical procedure was established to analyse the crude and reasonably pure Yohimbine. There are major changes in our analytical method like

selecting a suitable column, flow rate, nm and mobile phase. We have achieved better resolution from previous methods. There are several reports on HPLC methods for the analysis of Yohimbine. However, our method was simple and gave better resolution with a strong peak to analyse the Yohimbine raw material and extract.

EXPERIMENTAL

MATERIAL AND METHODS

Yohimbine was isolated from dried Yohimbe bark. The plant raw material was obtained from the African market and the moisture content is 11.2%. Methanol, water, Acetonitrile (HPLC grade, Qualigens).

Preparation of Referance Solution

Weighed accurately 3mg of Yohimbine reference substance and transferred in to a 25ml volumetric flask. Methanol was added to the mark.

Sample Solution Preparation

Weighed the appropriate amount of Yohimbine extract sample and transferred in to a 25ml volumetric flask. Methanol was added to the mark and sonicate for 20 min. The solution was filtered using 0.5 micrometer micro porous membrane.

RESULTS AND DISCUSSION

The Yohimbine standard material was injected initially and the HPLC chromatogram is depicted in (Figure No.2). The retention time of Yohimbine standard is at 8.662 in our HPLC conditions. The HPLC conditions are depicted in (Table No.1). Our HPLC method is very suitable to analyse the Yohimbine raw material (bark) and HPLC chromatogram is depicted (Figure No.3) and the retention time of raw material was detected at 1.92. We have pulverized the Yohimbine bark raw material and extracted in methanol. The extracted material is subjected to acid base treatment followed by charcoal treatment and obtains a reasonable good-purity extract. The HPLC chromatography is depicted in (Figure No.4). Our method is suitable to analyse the crude and ram material and the pictorial representation is depicted in (Figure No.5).

Determination of λ max

The drug was scanned in Shimadzu,-Model-UV-1601PC UV Spectrophotometer (with methanol as blank). The λmax was found to be 226nm which matches with standard value of Yohimbine.

Calculation

The HPLC instrument was stabilised and a baseline was established. A sample was injected and the Yohibine content was calculated as below:

Assay of Yohimbine = A/A0 x C0 x V/M x D%

A- Peak area of the test sample

A0 - Peak area of the reference standard

C0 - The concentration of reference solution

V- Dilution volume of the test sample

M - Weight of test sample

D% - Content of reference standard

In the present study, an HPLC method was developed for the estimation of Yohimbine in crude and pure material. Yohimbine was extracted using methanol and TC-C18, 4.6x250mm column was used. Sample measurement was done at 226nm with a mobile phase of Methanol: Acetonitrile: Phosphoric acid (0.4%) 37: 0.7: 62.3 (V/V). This is a major modification of the method described by earlier authors[10].

This modification was attempted because of the strong peak and better resolution of the compound at 226nm. The results of the study aimed at the determination of the Yohimbine content in crude and pure material. The developed HPLC method for estimation of Yohimbine in various crude samples is a very sensitive, reliable and accurate one. Such a sensitive method is essential for determination of various pharmacokinetic parameters of this useful phytochemical. This method will be very useful while designing future clinical trials with Yohimbine in humans.

Since Yohimbine is freely soluble in methanol, the plant materials were extracted with methanol. The mobilephase is Methanol: Acetonitrile: Phosheric acid for HPLC. The first run was of blank to check errors from the mobile phase. In the present HPLC conditions, the reasonably pure Yohimbine material is eluting at retention time 8.662 and some polar impurities were obtained at differentretention times respectively. The moisture content of the isolated

drug was found to be 0.34% the Karl–Fischer method.

Table No.1: HPLC conditions

S.No	Item	Conditions
1	Column	TC-C18, 4.6x250mm
2	Mobile Phase	Methanol: Acetonitrile: Phosphoric acid (0.4%) 37: 0.7: 62.3 (V/V)
3	Flow Rate	1.0mL/min
4	Wavelength	226nm
5	Column Temperature	25^{o}C
6	Run Time	20 min
7	Sensitivity	0.01 AFUS
8	Injection Volume	10 microliters

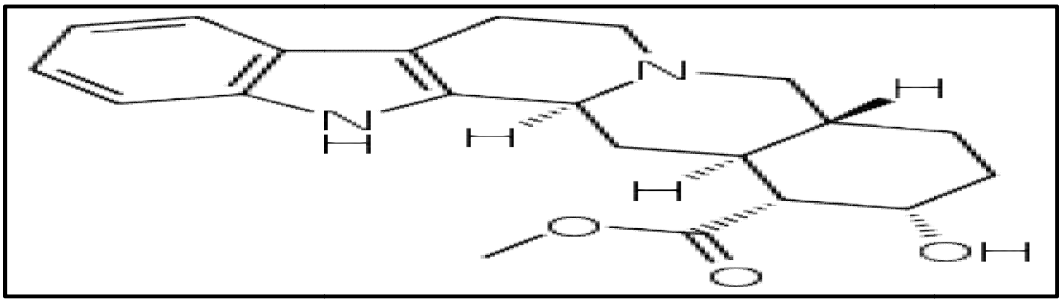
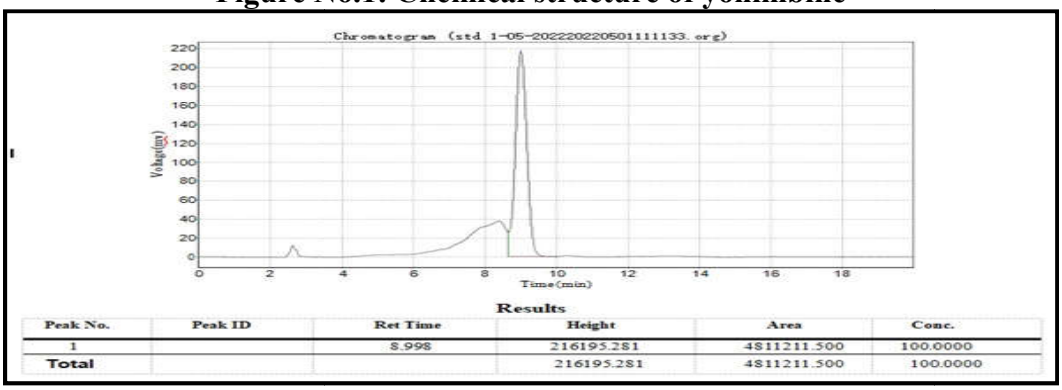


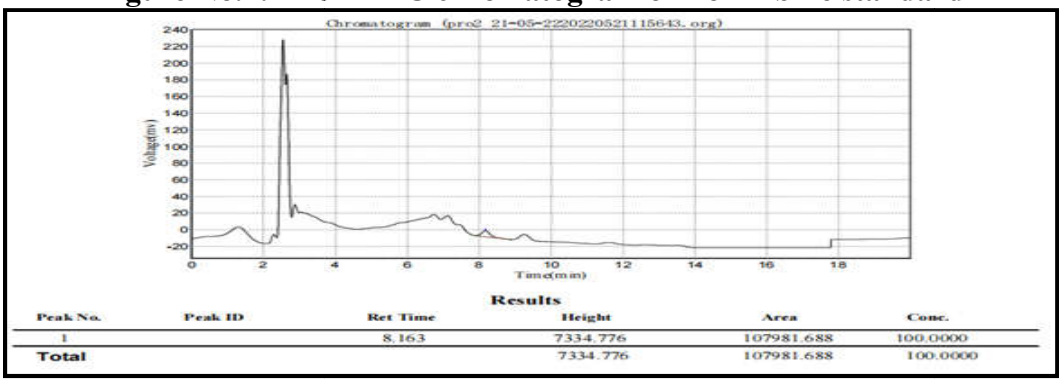
Figure No.1: Chemical structure of yohimbine



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		8.998	216195.281	4811211.500	100.0000
Total			216195.281	4811211.500	100.0000

Figure No.2: The HPLC chromatogram of Yohimbine standard



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		8.163	7334.776	107981.688	100.0000
Total			7334.776	107981.688	100.0000

Figure No.3: The HPLC chromatogram of Yohimbine bark (raw material)

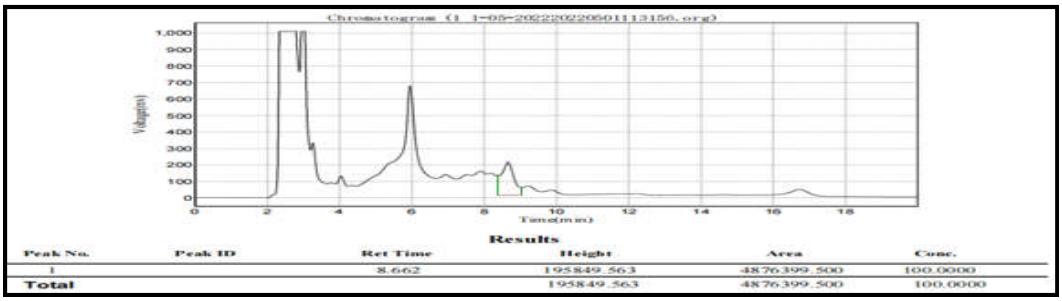


Figure No.4: The HPLC chromatogram of Yohimbine extract powder

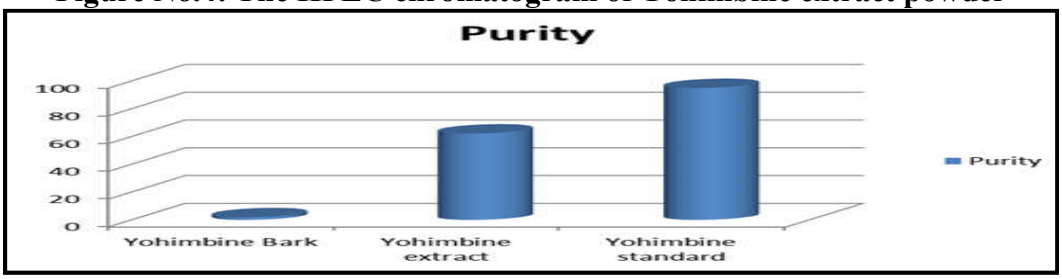


Figure No.5: The graph of Yohimbine content bark, extract and standard

CONCLUSION

The newly developed HPLC analytical method is a simple, specific and selective for the determination of Yohimbine in bulk. The developed method was found to be precise, better resolution, strong peak, robust, stable and economical for routine use in the herbal drug industry.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

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