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Evaluation of Octopamine hydrochloride in its bulk and tablet dosage forms by using RPHPLC method.

K. A. S. Raghava[1], S. Venkat Rao[2*], S. L. Nagaravindra. M[1] and J. Ananth Krishna[1]

[1] *School of Pharmaceutical sciences and Technology, JNTU, Kakinada -533003*
[2] *Chemrich fine chemicals Pvt ltd, Banjarahills, Road No-5. Hyderabad -34*

ABSTRACT

*A novel, precise, rapid, economical, and Accurate HPLC method for evaluation of Octopamine in bulk and formulations was developed and validated. The Chromatographic resolution of Octopamine was achieved by using Acetonitrile**:** 0.01M Potassium phosphate (Ortho phosphoric acid) Buffer , **(10: 90 V/V)** as a mobile phase UV detection at **273 nm** and **Sunfire C-18** column .flow rate 1ml/min. the extraction Recovery of octopamine from its formulation dosage form (tablets) was **>99.10%** And the calibration curve was linear **(r2 = 1)** over octopamine concentration ranging from **5 to 30 µ g /ml**. the method has an accuracy of **> 99%** and **LOD** and **LOQ** of **0.03423 µ g** /ml and **0.10680 µ g** /ml respectively. A result of the present method was validated statistically and by recovery studies were found to be satisfactory.*

Key words: Octopamine hydrochloride, RPHPLC, Biogenic amine, weight loss Product, Method validation.

INTRODUCTION

Octopamine was first discovered by Italian scientist **Vittorio Erspamer** in 1948 in the salivary glands of the octopus **[1]** and has since been found to act as a neurotransmitter, neurohormone and neuromodulator in invertebrates. It is widely used in energy-demanding behaviors by all insects, crustaceans (crabs, lobsters, crayfish), and spiders. Such behaviors include flying, egg-laying, and jumping. Octopamine is a biogenic amine that is mostly use to support fat loss. It is derived fromtyramine, a natural amino acid that may also be found in various foods such as tomatoes and liver.

This is also known as β, 4-dihydroxyphenethylamine, or norsynephrine. Heberlein et al., **[2]** have conducted studies of alcohol tolerance in fruit flies; they found that a mutation that caused octopamine deficiency also caused lower alcohol tolerance **[3], [4], [5]** and **[6].**

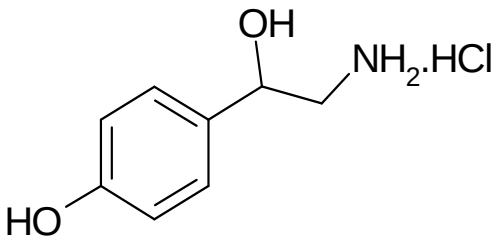

Figure-1: Chemical structure for Octapamine Hydrochloride

It is believed that octopamine helps to break down fat cells in the body, and it has thus been used in many fat-loss products. Another benefit of this ingredient is that it is less likely to lose its effectiveness over longer periods of time. This is significant, because most stimulants used in fat burner products may lose their effectiveness within the first few weeks. Research is still being conducted to support these claims. Octopamine has also been known to increase the body's metabolism, resulting in the burning of calories **[7].** It also supports the insulin secretion and sensitivity. This may also cause a reduction in the symptoms of diabetes patients. These hormones form part of the sympathetic nervous system of the body. There are few publications available on clinical and pharmacokinetics studies for octopamine as above mentioned. But none of them can explain the simple Reverse phase high performance liquid chromatography method to evaluate it in bulk and tablet dosage form. Herein the current work the author has validates various validation parameters and established a novel ideal method to estimate Octopamine in economic trends.

MATERIALS AND METHODS

Instruments

The apparatus used in this study were Waters- Alliance E2695 with empower 2 Software and UV-2489 detector with auto sampler HPLC, Micro processor –LP-1395 model pH meter , Biotechnics India -9L250H model Sonicator and Sartorius BSA 2245-CW balance was operated.

Materials methods

All the reagents and solvents used in this study were from Merck chemicals and few are of from Rankem. The drug standards and formulation samples were gifted by Chandra labs, Hyderabad.

Preparation of standard drug solution

20mg octopamine dissolved in 100ml of mobile phase (Acetonitrile and phosphate buffer pH~2.4), the obtained solution was sonicated for 10 minutes, and this solution is further diluted to six concentrations like **5 to 30 µ g /ml.**

Buffer preparation:

1.3609 gm Potassium Dihydrogen phosphate in 100ml of water, is subjected for sonication process for 5 minutes and passed through 0.45 Micron filters, finally pH adjusted with Orthophosporic acid.

RESULTS AND DISCUSSION

Method Validation

Selection of Mobile phase and Column:

The author tried different columns with various mobile phase ratios and the parameters like flow rate, UV, and temperature were kept same for all the trials as 1ml/min, 273 nm, and Ambient respectively and all the trails can see in the table-1

Table-1: selection of chromatographic conditions

TRAILS	Column	Mobile Phase Tried
1	INERTSIL ODS-3	METHANOL:WATER (80:20)
2	INERTSIL ODS-3	ACN :WATE (60:40)
3	INERTSIL ODS-3	0.01M POTASSIUM PHOSPHATE BUFFER : ACN (40:60)
4	INERTSIL ODS-3	0.01M POTASSIUM PHOSPHATE BUFFER : ACN (50:50)
5	**SUNFIRE C18**	**0.01M POTASSIUM PHOSPHATE BUFFER : ACN (90:10)**
6	SUNFIRE C18	0.01M POTASSIUM PHOSPHATE BUFFER : ACN (75: 25)

Table-2: Linearity range of Octapamine

Concentration µg/ml	Peak Area	Statical Analysis
5	395041	
10	804867	Slope: 80271
15	1206489	
20	1608420	Intercept: 601.9
25	2007477	
30	2402586	Correlation coefficient: 1

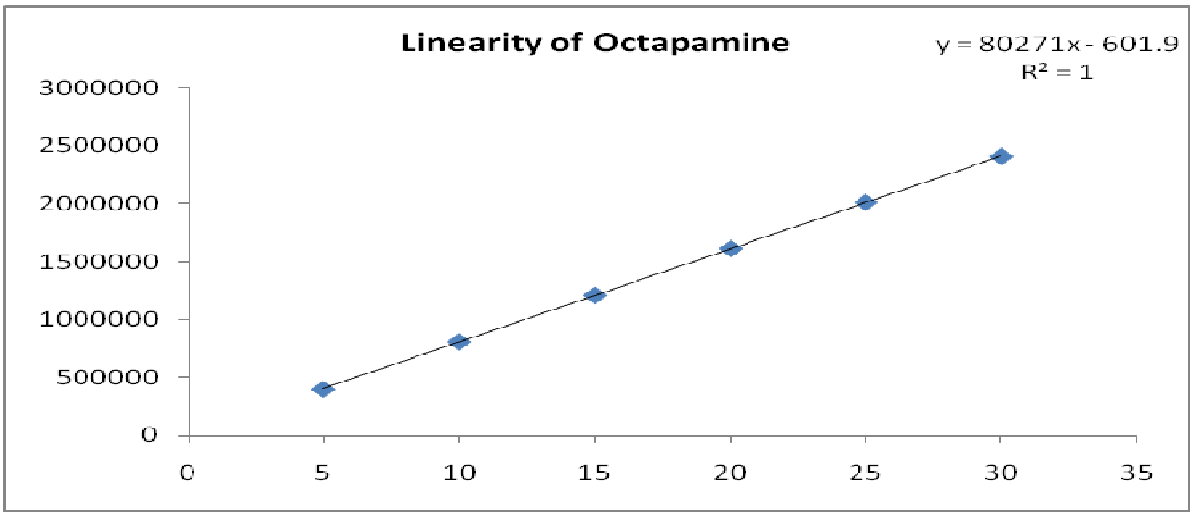

Figure -2: Graph for Linearity

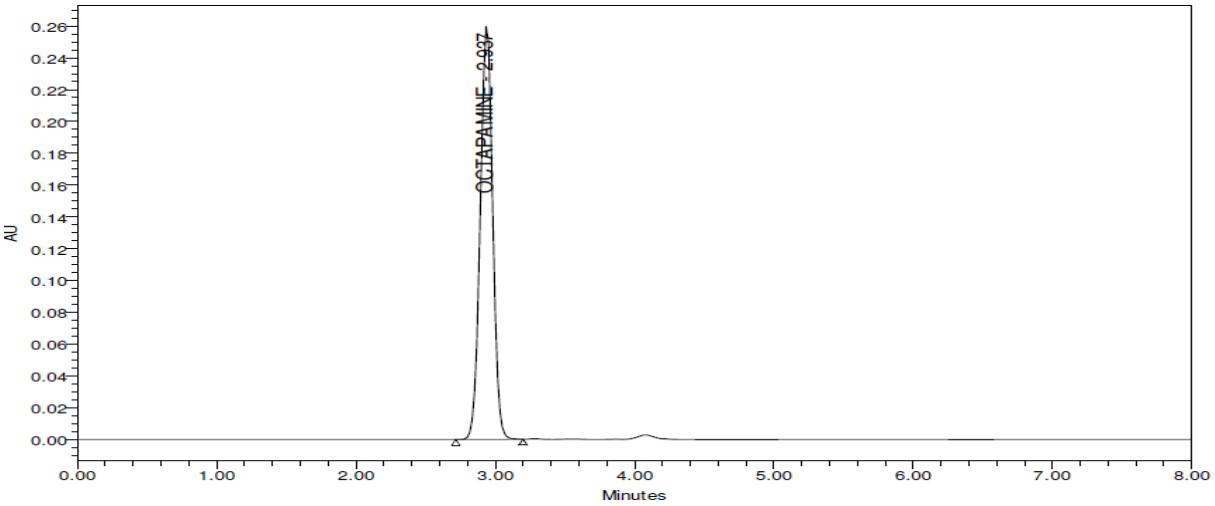

Figure-3: HPLC Standard Chromatogram of Octapamine Hydrochloride

Specificity and selectivity of the method was assessed by preparing a drug concentration of 100µg/ml from pure drug stock and commercial sample stock in selected mobile phase and analyzed. The HPLC chromatograms recorded for the drug matrix showed almost no other peaks within a retention time range of 2.937min as shown in the figure-3.

Table- 3: Accuracy studies

Accuracy	Amount Added	Amount Recovered	% Of Recovery
50%	50µg	49.55	99.1
100%	100µg	98.57	98.57
150%	150µg	147.35	98.23

**each value is the average of five determinations*

Recovery studies procedure

The finely powdered formulation dosage and accurately weighed sample of formulation equivalent to 200 mg Octapamine Hydrochloride was extracted with Acetonitrile in a 100ml volumetric flask using ultra sonicator. This solution was diluted with mobile phase, so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in five replicates and the obtained results; represented data was shown in table 4

Table -4: Amount of Bortezomib in formulation tablet By HPLC Method

S. No.	Formulation Injection Brand Name	Labeled amount in mg.	Amount Recovered in mg	% of Recovery
1	**Norden**	200mg	199.08mg	99.54%

**each value is the average of five determinations*

Table- 5: Precision studies

Concentration µg/ml	Peak area	% RSD
25	1610090	0.05

**each value is the average of five determinations*

Table-6: System suitability

S.No.	Parameters	Values
1	Theoretical Plates (N)	5509.000
2	LOD, µg/ml	0.03423
3	LOQ, µg/ml	0.10680

The method is linear in the concentration range 5 to 30 µ g/ml and its linearity curve can see in figure-2, intra day precision was studied by five replicate measurements at three different concentration levels over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evolution revealed that relative standard deviation (%RSD) of the drug at different concentration levels for five injections was less than 0.2. Precision and accuracy data were shown in table 5 and 3 respectively. For system suitability, five replicates of standard sample were injected and different parameters were studied (table 6). The tailing factor for Octapamine was always less than 2 .0, thus the HPLC method developed in this study is selective for Octapamine.

CONCLUSION

The results obtained from these studies are well fit into the standard specifications stipulated by the regulatory agencies. The method is able to reproduce the results consistently and the recovery studies of Octapamine are found to be **99.54%.** This indicates that commonly used excipients in pharmaceutical formulation were not interfering in the proposed method. The observation of **% C.C less than 2.0** for intra day measurements also indicates high degree of precision. In the present method, we have established a linearity range of **5-30 µg /mL**; this linearity range covers all the strengths of Bortezomib, hence this can be conveniently used in the pharmaceutical manufacturing and formulation environment.

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