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Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF OCTOPAMINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop simple and cost effective Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the estimation of Octopamine.HCl in pharmaceutical dosage form. Methods: The estimation was carried out on SUNFIRE C_{18} (150*4.6mm, 5µ) using mobile phase consisting of phosphate buffer of pH (2.54) and Acetonitrile (90:10). The flow rate was 1.0 ml/min column effluents were monitored at 273 nm. The proposed method has been validated as per ICH Guidelines. Results: The retention time was 2.9 minute and the proposed method was linear in the concentration range of 5 to30 µg/ml with coefficient of correlation 0.9998. The % recoveries at 99%-101% were found to be 99.1%, 98.57%and 98.23% respectively. Conclusion: All the validation parameters were within the acceptance range. The developed method can successfully be applied for the routine estimation of the amount of octopamine in bulk and pharmaceutical dosage form. The proposed method can be used to determine the drug content in various pharmaceutical dosage forms.

Keywords: Octopamine.HCl, RP-HPLC, Validation, ICH guidelines

INTRODUCTION

Octopamine.HCl is a sympathomimetic agent and it stimulates lipolysis in mammalian adipocytes via activation of β_3 receptors. It is a invertebrate biogenic amine neurotransmitter, related to nor adrenaline, that is an adrenoceptor agonist. It has dual effect on glucose transport in adipocyteses. It inhibits transport via β_3 receptor activation but stimulates transport when oxidized by MAO. It also activates human α_{2A} receptors, inhibiting subsequent cAMP production. It binds to CYP3A4 (oxidation), with contribution from CYP2C8. Elimination half-life is approximately 12.4 hours and excreted in urine and faeces

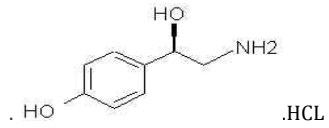

Fig-1: **Structure of Octopamine.HCl**

There are few other methods reported for estimation of Octopamine.HCl includes LC-mass spectroscopic[1], GC[2], HPLC[3-4], Ionic chromatography[5-6] and Radiochemical[7] methods are available for the determination of Octopamine.HCl. The aim of the present study is to develop a simple, precise, rapid and accurate RP-HPLC method for the determination of Octopamine.HCl in pharmaceutical dosage form.

EXPERIMENTAL

Materials and methods

Octopamine.HCl was a gift sample from the Dr.Reddy's laboratories, Hyderabad and the chemicals of HPLC grade Potassium dihydrogen orthophosphate, OrthoPhosphoric acid Acetonitrile and GR Grade Ammonium acetate, Methanol, and purified water were used for the preparation of buffer (pH 2.54). Reagents were obtained from E Merck (India). Mille Q Water (Millipore USA) was used throughout the procedure. A freshly prepared phosphate Buffer of pH 2.54 : Acetonitrile (90:10) V/V was used as a mobile phase. The solvents were filtered through 0.45 µ membrane filter and sonicated before use.

Instrument

A Waters high performance liquid chromatography (model 2695) of empower-2 software, dual absorbance UV/Vis detector, auto sampler, A Shimadzu UV-visible spectrophotometer – 1601 and a SUNFIRE C_{18} (150*4.6mm, 5µ) were used. A 20 µl Hamilton injection syringe was used for sample injection.

Buffer preparation

Weighed 1.3609 grams of KH_2PO_4 into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. Adjusted the pH to 2.54 with OrthoPhosphoric acid.

Mobile phase preparation

900 ml (90%) of the buffer and 100 mol of Acetonitrile (10%) were mixed. The solution was degassed in an ultrasonic water bath for 5 minutes and was filtered through 0.45 µ membrane filter.

Standard stock preparation

10 mg of Octopamine.HCl working standard was weighed accurately and transferred into a 100 ml clean dry volumetric flask, about 70 ml of diluent was added and sonicated to dissolve it completely. The volume was made up to the mark with the same solvent. From the above solution 5 ml was taken and added to 20 ml volumetric flask and the diluent was added up to the mark to get 25 ppm solution and sonicated for 5 min.

Sample preparation for Assay

capsules were taken and powder equivalent to 10 mg i.e. 12.8 mg of sample was taken into 100 mL volumetric flask .It was made to dissolve with mobile phase and made up to the mark with mobile phase to get the concentration of 100 µg/mL solution. From the above solution 5 mL was taken and added to 20mL volumetric flask and diluents was added up to the mark to get 25 ppm. The solution was degassed and filtered through membrane filter of pore size 0.45µ.Under UV-visible spectrophotometer in the range of 200 to 400 nm against Diluent as blank. The wave length 273 nm selected based on the maximum absorption occurred. Results shown in table 1.

Table1: Report for Assay

Compound	Standard area	Sample area	Standard weight	Sample weight	Average weight	Label claim	Standard purity
Octopamine.HCl	1613505.7	1613978	10 mg	12.8mg	255 mg	200mg	99.9%

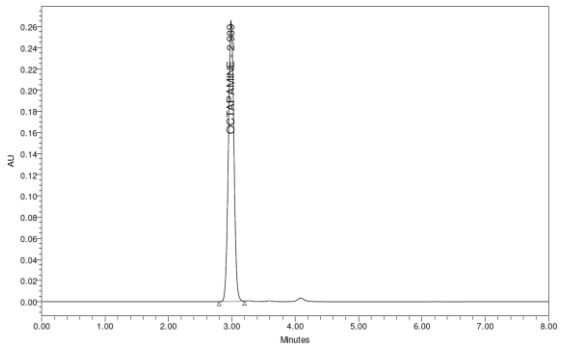

Fig2: A typical chromatogram of Octopamine.HCl

Method validation

System suitability

The system suitability studies were done with the 100ug/ml of standard drug. The % of RSD values are below 2%, theoretical plate count is above 4000 and tailing factor is less than 2, indicating that the method is suitable. The results of system suitability were shown in table-2.

Table2: System suitability results for octopamine

Parameter	Results	Acceptance criteria
Plate count	5101	NLT 1000
Tailing factor	1.15	NMT 2%

Specificity

Chromatogram of blank did not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. No interference was observed from the excipients and degradation products of degradation studies. Hence the method was found to be specific and stable. The results of specificity studies were shown in table 3.

Table 3: specificity results for octopamine HCL

	Octopamine.HCl	
Parameter	Results	Acceptance criteria
Tailing factor	1.15	NMT 2.0
% RSD	0.29	NMT 2%
Theoretical plate count	5101	NLT 1000

Linearity

Linearity of detector was found out by injecting five standard solutions with concentration ranging from 5-30µg/ml of the test concentration and a graph was plotted for concentration versus peak area. The results were shown in Table 4& fig-3

Table 4: Linearity results for Octopamine.HCl

S.No	Conc. Taken in µλµ/γ	R.T in min Octopamine.HCl	Peak area of Octopamine.HCl
1	5ppm	2.987	395041
2	10ppm	2.99	804867
3	15ppm	2.991	1206489
4	20ppm	2.994	1608420
5	25ppm	2.996	2007477
6	30ppm	2.995	2402586
7	Correlation	0.999	-

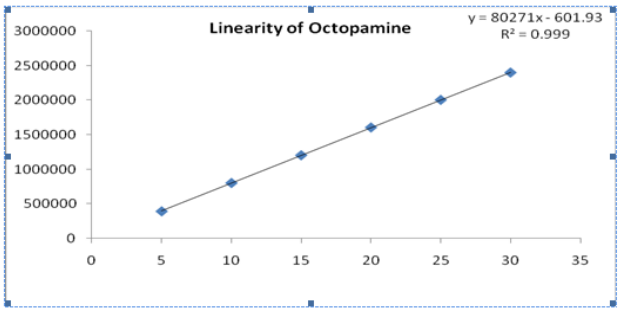

Fig 3: Linearity curve for Octopamine.HCl

Accuracy

Recovery studies are performed in the concentration range of 50, 100 and 150% keeping the average Weight constant and varying the quantity of active ingredient as per spike levels. Lower and higher Concentrations are prepared three times and triplicate at other levels to accurately quantify and to validate the accuracy. The results were as shown in Table-5

Table 5: Accuracy results for Octopamine.HCl

Inj.Sample	Spike Level	Mean Area(n)	Amount taken(mg)	Amount Obtained(µg)	% Recovery	Mean % Recovery
	50%	2406447	6.4	6.35	99.1%	
	100%	3195139	12.8	11.3	98.57%	
Octopamine.HCl	150%	3980147	20	17.35	98.23%	98.63%

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements. The precision of test method was determined by preparing six test preparations and the relative standard deviation of assay results was calculated and shown in table 6.

Table 6: Precision results of Octopamine.HCl

	Mean (n)		S.D	% RSD
Injection samples	Rt	Area	Area	Area
Octopamine.HCl	2.984	1610090	832.6694	0.005

Ruggedness

The ruggedness of method was determined by different instruments like Waters HPLC, Shimadzu HPLC by different operators using different columns.

Table-7: Results of Ruggedness

	Mean Retention time of Octopamine.HCl	Mean Area of Octopamine.HCl
AVG	2.98	1973711
SD	-	832.6
% RSD	-	0.05

Robustness

Effect of variation in organic phase proportion of the mobile phase, buffer pH and changing the flow rate, the system suitability parameters were checked by injecting system suitability preparation into HPLC system with 0.75ml/min, 0.80ml/min and 0.85ml/min and mobile phase ratio 85:15, 90:10 and 95:5 to get the robustness of the assay method. The results were as shown in table 8.

Table 8: Robustness of Octopamine.HCl.

Proposed variations		Octopamine.HCl USP Plate Count	USP Tailing
Variation in mobile phase composition (buffer : solvent)	1/3/1900 13:15	1623669	1.3
	1/3/1900 18:10	1610987	1.24
	1/3/1900 23:05	603513	1.27
Variation in flow rate	0.75ml/min	1716955	1.2
	0.80ml/min	1609431	1.32
	0.85ml/min	609583	1.14

Limit of detection and Limit of Quantification (LOD & LOQ)

The parameters LOD & LOQ were calculated on the basis of the height of the signal and the noise of response which were found to be 0.03 &0.1 respectively. The results were shown in table 9.

Table 9: LOD & LOQ results of octopamine HCL

Drugs	Linearity	Precision	Recovery	LOD	LOQ
Octopamine. HCl	Correlation is 1	0.05	99.54%	0.03	0.10
Acceptable criteria	NLT 0.999	%RSD NMT 2	98-103%	NMT 3.0	NMT10

Acid degradation

Capsule powder equivalent to average weight was transferred into 25ml volumetric flask. To that 10ml of 0.1N HCl was added and made up to the mark with methanol and sonicated for 60min. Then the solution was filtered through 0.45µm filter and diluted 5ml of the above solution to 25ml with methanol. The chromatogram was obtained as shown in figure 4.

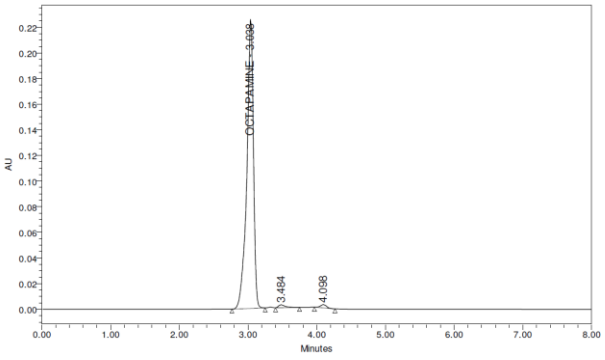

Figure 4: Acid degradation of Octopamine.HCl with 0.1 M HCl

Base degradation

Capsule powder equivalent to average weight was transferred into 25ml volumetric flask. To that 10mlof 0.1N NaOH was added and made up to the mark with methanol and sonicated for 60min.Then the solution was filtered through 0.45µm filter anddiluted5ml of the above solution to 25ml with methanol. The chromatogram was obtained as shown in figure 5.

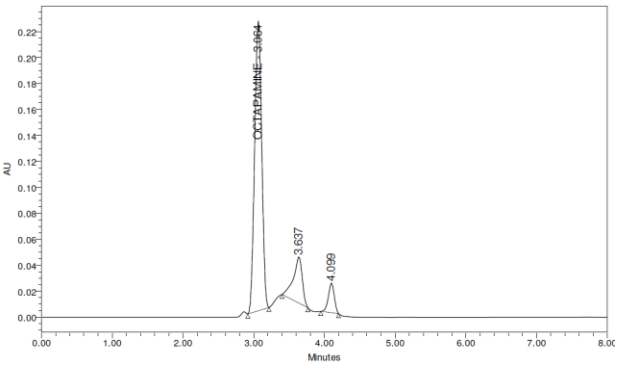

Figure 5: Base degradation of Octopamine.HCl with 0.1 M NaOH

Heat degradation

Capsule powder equivalent to average weight was transferred into 25ml volumetric flask and made up to the mark with methanol and kept at 60°C for60min. Then the solution was filtered through0.45µm filter and diluted5ml of the above solution to25ml with methanol. Light degradation Capsule powder equivalent to average weight was transferred into 25ml volumetric flask and made up to the mark with methanol and kept in U.V chamber for 60min. Then the solution was filtered through0.45µm filter and diluted 5ml of the above solution to25ml with methanol.

Peroxide degradation:

Capsule powder equivalent to average weight was transferred into 25ml volumetric flask. To that 2ml of sample shows the purity indicates the purity of the formulation. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise rapid and selective and can be employed successfully for the estimation of Octopamine.HCl in bulk and its dosage form.

RESULTS AND DISCUSSION

Method development

The selection of column and mobile phase was done by trial and error method. The drug is readily soluble in water, slightly soluble in methanol. Selection of mobile phase must be adapted based on solute retention and solute separation (solvent selectivity). Polar mobile phase gives low solute retention in normal HPLC and high solute retention in reverse phase HPLC.Most widely used solvents in Reverse Phase chromatography are Methanol and Acetonitrile. So that mobile phase comprising of phosphate buffer of pH (2.54) and Acetonitrile were mixed in the ratio of 90:10 and the retention time of elution is 2.9min with good peak symmetric properties. C_8 (OCTADECYL), C_{18} (OCTYL SILANE), CN (CYANOPROPYL), SUNFIRE C_{18} (150*4.6mm, 5µ) columns were tried, elution with good peak symmetry was observed with SUNFIRE C_{18} (150*4.6mm, 5µ) 0.5 mL/min, 1.0 mL/min and 1.0 mL/min flow rate were tried, 1.0 mL/min found to be suitable. The column temperature kept at 30^{0}C. Injection volume kept at 20 µL. On observation 3D spectra of sample in diode array detector response at 273 nm, it has shown good response, hence 273nm selected as the detection wavelength.

Method Validation

% Relative standard deviation (%RSD) of retention times and peak areas were less than 1, average of tailing factor <2 and theoretical plates >4000 hence method passes system suitability tests.. The standard and sample chromatograms were identical to each other, there was no interference of excipients in analysis of drugs that proves method is specific. The average amount of drugs found in six samples were 99.89% When analysis was performed by second analyst on second system the results were well under limit, that proves method precision. The method was linear in the range of 5-30 µg/ml. The mean % recoveries at 50%, 100% and 150% concentration level were found to be 99.1% 98.57% and 98.23% respectively.

The concentration and the correlation coefficient(r) for Calibration curve was found to be 0.999. The results of the robustness study indicates that the method is robust and is unaffected by small variations in the chromatographic conditions. The LOD and LOQ were calculated and were found to be 0.03 and 0.10µg/ml respectively. The forced degradation studies were performed and degradation found within limits .The assay of the Octopamine.HCl capsule dosage form (Octopamine.HCl 10mg) purity found to be 99.9%.

CONCLUSION

The results of study indicate that the proposed RP-HPLC method was simple, precise, highly accurate and rapid for the estimation of Octopamine.HCl in bulk and pharmaceutical dosage forms.

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