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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF RAMOSETRON HCl

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ABSTRACT

A simple, specific, accurate, and stability indicating high-performance liquid chromatographic method was developed for the determination of **Ramosetron HCl**, using a Enamel C18 column and a mobile phase composed of Methanol:Water (50:50). The retention time of **Ramosetron HCl** found to be 3.59 min. Linearity was established for of **Ramosetron HCl** in the range of 200-600 ng/ ml. The percentage recoveries of was found to be in the range of 98.33-98.86 %. The drug was subjected to acid and base hydrolysis, oxidation, photolytic, and thermal degradation conditions. The degradation products of **Ramosetron HCl** were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for quantitative analysis of Ramosetron **HCl** in pure and tablet.

Key words: Ramosetron HCl, degradation products, stability - indicating method, HPLC.

INTRODUCTION

Ramosetron HCl (6R)-6-(1-methyl-1H-indole-3-carbonyl)-4,5,6,7-tetrahydro-1H-1,3-benzodiazole hydrochloride. It is a white crystalline, soluble in water and Methanol. Ramosetron is a 5-HT3 receptor antagonist. It exerts its antiemetic property by blocking of serotonin to 5-HT3 receptors present in the afferent vagal nerve-endings in the GI mucosa. Ramosetron HCl is not official in any pharmacopoeia. Several analytical methods that have been reported for the estimation of Ramosetron HCl in biological fluids include HPLC Method. The objective of the work was to develop simple, accurate, precise and economic Stability indicating RPHPLC method development and validation for the estimation of Ramosetron HCl in pharmaceutical dosage form.

MATERIALS AND METHOD

MATERIALS

Pure sample of Ramosetron HCl was obtained from Cadila Healthcare Ltd, Ahmedabad, Gujarat, India. The commercial pharmaceutical preparation Ibset containing 5µg of Ramosetron HCl were procured from local pharmacy. All reagents were obtained from Merck chem Ltd, India. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

METHODS

Preparation of Solution

- **Preparation of mobile phase**
  - ✓ Mobile Phase comprising Water: Methanol in the ratio of 50:50. Mobile phase was prepared by mixing 500mL Methanol and 500 ml Water
- **Preparation of Stock Solution**
  - ✓ Accurately weighed 10mg of Ramosetron HCl was transferred to volumetric flask and make up volume with Methanol and Water up to10ml (1000µg/ml).
- **Preparation of Sample Solution**
  - ✓ From the stock solution 0.1 ml was taken and diluted up to 10 ml with Methanol and Water (10 µg/ml).
- **Preparation Of Diluent**
  - ✓ Diluent was prepared by using 500 ml methanol, 500 ml water. This mixture was sonicated and filtered through 0.45 µm membrane filter and used as a diluents.

Table 1: Instruments and Apparatus Detail

Name	Company	Model No.
UV spectrophotometer	Shimadzu	UV-1800
HPLC	Shimadzu	LC 20 AT
FTIR spectrometer	Nicolet	6700
Weighing balance	Shimadzu	AUX 220
pH meter	Elico	LI 127

EXPERIMENTAL SECTION

Calibration curves of Ramosetron HCl

To prepare Calibration curve of Ramosetron stock solution (1000 µg/ml) and working standard of Ramosetron HCl (1 µg/ml) was used. The different concentrations of Ramosetron HCl (200, 300, 400, 500 and 600 ng/ml) were prepared.

Analysis of dosage form

Twenty tablets were weighed their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to one tablet content was transferred into a 10ml volumetric flask containing of Methanol and Water, mechanically shaken for 10 min, ultrasonicated for 5 min, and then diluted to volume with Methanol and Water.

RESULTS AND DISCUSSION

Forced degradation study

Acid Degradation: Take 1 ml of Ramosetron HCl from Standard stock solution into 10 ml of volumetric flask; add 1.0 ml of 1N HCl, kept at room temp. for 6 hr., after that neutralized with 1 ml of 1N NaOH solution, shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

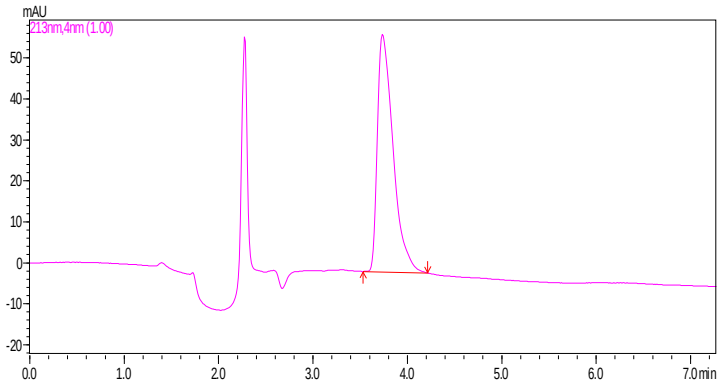

Fig 1: Chromatogram of Acid degradation

Alkali Degradation: Take 1ml of Ramosetron HCl from Standard stock solution into 10 ml of volumetric flask; add 1.0 ml of 0.1N NaOH, kept at room temp. for 10 min., after that neutralized with 1 ml of 1N HCl solution, shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

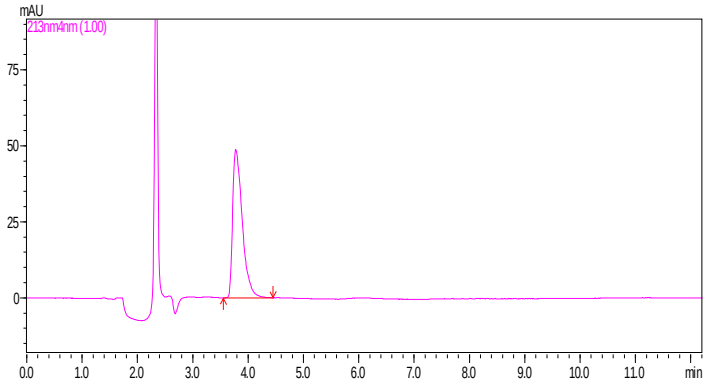

Fig 2: Chromatogram of Base Degradation

Oxidative Degradation: Take 1 ml of Ramosetron HCl from Standard stock solution into 10 ml of volumetric flask; add 1.0 ml of 3% H2O2 kept at room temp for 1 hr, shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

Photolytic Degradation: Take 1 ml of Ramosetron HCl from Standard stock solution in to 10 ml of volumetric flask and kept in U.V chamber 6 hr., after that shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

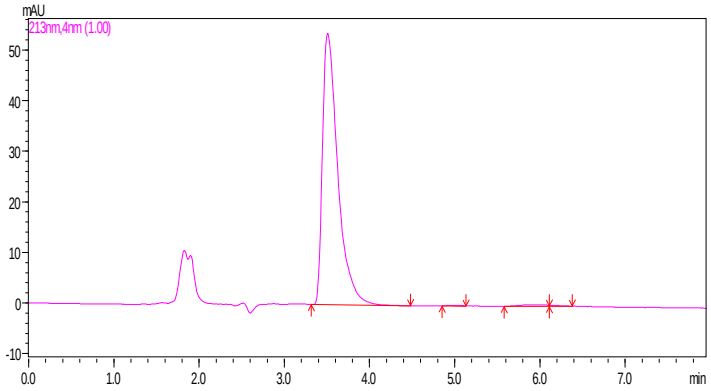

Fig 3: Chromatogram of Photolytic Degradation

Thermal Degradation: Take 1 ml of Ramosetron HCl from Standard stock solution into 10 ml of volumetric flask and kept in 70ºC for 24 hr., after that shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

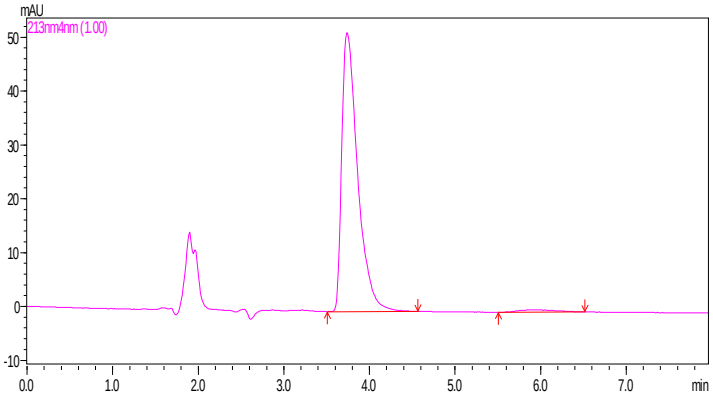

Fig 4: Chromatogram of Photolytic Degradation

Chromatographic conditions

Selection of Stationary Phase: On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with C18 bonded phase i.e. Thermo cyano C18 (25 X 4.6 mm) 5µ was selected.

Selection of Mobile Phase

After assessing the solubility of drug in different solvent as well on the basis of literature Survey; the mobile phase was selected is the mixture of Methanol:Water (50:50)

Selection of Detector and Detection Wavelength

UV detector was selected, as it is reliable and wavelength was set to 213nm.

Table 2: Result of System Suitability for Assay Method

Theoretical plate	2262.386
Tailing Factor	1.57
Retention time	3.59 min

Table 3: Summary of Force degradation study of Ramosetron HCl

Sr.No.	Condition	%Degradation	Remark
1	Acid Degradation (1N HCl)	15.52	Degradation observed
2	Alkali Degradation (0.1N NaOH)	25.27	Degradation observed
3	Peroxide Degradation (3% H2O2) v/v	4.32	Less Degradation Observed
4	Photolytic Degradation	15.17	Degradation observed
5	Thermal Degradation (70oC)	15.43	Degradation observed

Stability indicating assay: Degradation was observed for Ramosetron HCl sample during stress conditions like acid, alkaline, Oxidative, Photolytic, and in thermal condition. Graph shows test results confirmed Ramosetron HCl peak is homogeneous in all the stress

Sample Chromatogram

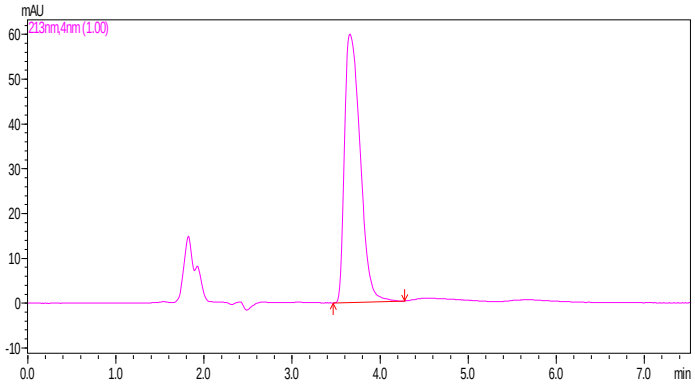

Fig 5: Chromatogram of Standard Ramosetron HCl

VALIDATION OF METHOD

Linearity, LOD & LOQ

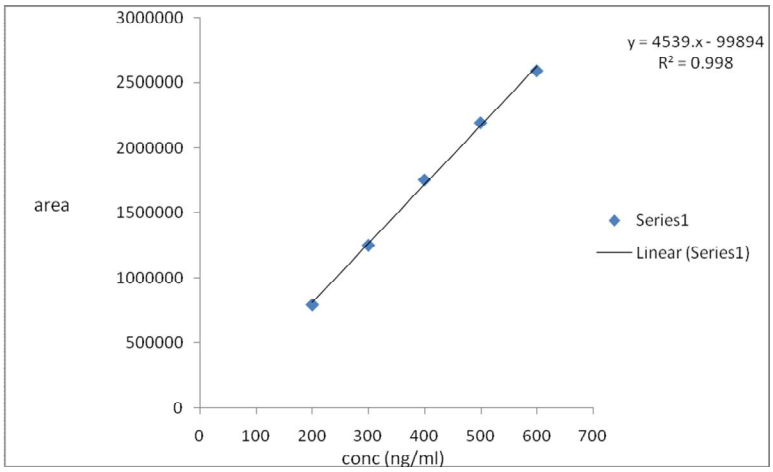

Fig 6: Calibration Curve of Ramosetron HCl (200 – 600ng) for Assay Method

ACCURACY

Table 5: Result of Accuracy for Assay Method

Drug	Test amount (ng/ml)	Spiked STD amount (ng/ml)	Total amount (ng/ml)(n=3)	Amount found (ng/ml)	% Recovery
Ramosetron HCl	200	160	360	354	98.33
		200	400	396	99
		240	440	435	98.86

PRECISION

Method Precision (Repeatability)

Table 6:- Result of Method Precision (Repeatability) for Assay Method

Concentration(ng/ml)	AREA
200	792395
200	806754
200	797865
200	789865
200	794537
200	798765
AVG OF AREA	796696.8
SD	5425.758
%RSD	0.681032

Table 7: Intraday Precision of Ramosetron HCl

Drug	Conc. (ng/ml)	Area			Average	SD	%RSD
		1	2	3			
Ramosetron HCl	300	1257561	1284362	1267653	1269859	11052.06	0.870338
	400	1795754	1774745	1753432	1774644	17278.03	0.973606
	500	2087695	2065421	2033526	2062214	22230.37	1.077985

Table 8: Interday Precision of Ramosetron HCl

Drug	Conc. (µg/ml)	Area			Average	SD	%RSD
		D1	D2	D3			
Ramosetron HCl	300	1250274	1285478	1267876	1267876	14371.97	1.133547
	400	1753776	1756987	1798654	1769806	20440.93	1.154982
	500	2192598	2195674	2134765	2174346	28015.92	1.288476

Table 9: Change Flow Rate of Ramosetron HCl

Drug	Flow Rate (ml/min)	Area			Average	SD	%RSD
Ramosetron HCl	**0.9(ml)**	806754	815436	809756	810648.7	3600.178	0.444111
	1.0(ml)	792395	795643	789654	792564	2447.918	0.308861
	1.1(ml)	776532	769854	769875	772087	3143.101	0.407092

Table 10: Change Mobile Phase Ratio of Ramosetron HCl

Drug	Ratioof Mobile Phase	Area			Average	SD	%RSD
Ramosetron HCl	**45:55**	796754	786598	793425	792259	4227.351	0.533582
	50:50	792395	798754	799564	796904.3	3205.681	0.402267
	55:45	789865	796543	793421	793276.3	2728.201	0.343916

Table 11: summery of Ramosetron HCl

Sr. No.	Validation Parameter	Ramosetron HCl
1	**System Suitability Parameter**	
	Retention time	3.59 min
	Resolution	---
	Tailing Factor	1.57
	Theoretical Plate	2262.386
2	**Linearity**	
	Regression Equation	y = 4539.x – 99894
	Regression Coefficient	0.998
3	**Range(ng/ml)**	200-600
4	**Accuracy (%Recovery)**	98.33-98.86

5	**Precision (%RSD)**	
	Repeatability	0.68
	Intraday	0.87-1.07
	Inter-day	1.13-1.28
6	**LOD (ng/ml)**	29.44
7	**LOQ (ng/ml)**	89.22

CONCLUSION

A simple, precise and accurate analytical simple and stability indicating RP-HPLC Methods has been developed for estimation of Ramosetron HCl drug in Tablet Formulation. The developed methods have been validated as per ICH guidelines, and it meets all the acceptance criteria given in ICH guidelines. The degraded product peaks were well resolved from the pure drug peak with significant. Difference in their retention time. The results of forced degradation within the acceptance Criteria So that simple assay method could be used for the routine analysis of Ramosetron HCl.

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REFERENCE

1. Tripathi KD, “Essential of medical pharmacology”,jaypee Brothers publication,fifth edition, pg no.661-663
2. “Ramosetron hydrochloride information from DrugsUpdate”,Feb 2013, http://www.drugsupdate.com/generic/view/1111
3. “Ramosetron hydrochloride”,Feb 2013 http://www.Curepluscureplus.org/i/111127/43.html .
4. http://www.chemicalbook.com/ChemicalProductProperty_EN_CB1397525.html.
5. The Merck Index. In An encyclopaedia of chemicals, drugs and biological; 14th Edn published by Merck Research Laboratories Division of Merck and co. Inc.USA,2006, pp1395
6. http://www. en.chembase.cn/substance-396167.html
7. “ Chemical Base” http://www. Curepluscureplus.org/i/111127/43.html

8. Antispasmodics & intestinal motility http://www.drug2day.com
9. http://www.genericpedia.com/generic/Ramosetron-Hcl/
10. Robinson JW, Skelly Frame EM, Frame GM. In Undergraduate Instrumental Analysis, 6th Edn, Marcel Dekker , 2005, pp 806.