RJC

RASĀYAN *J. Chem.*
Vol. 6 | No.4 | 315 - 319 | October- December| 2013
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ESTIMATION OF RAMELTEON IN BULK AND TABLET DOSAGE FORM BY HPLC

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ABSTRACT

A simple, accurate and precise reverse phase HPLC method was developed, described and validated for the determination of ramelteon in bulk and tablet dosage form. Chromatography was carried on an ODS column using a mixture of acetonitrile and 0.05Mphosphate buffer, pH 6.8 (in the ratio 40:60 v/v) as the mobile phase at a flow rate of 1.2 mL/min with detection at 285 nm by ultraviolet detector i.e. incorporated in HPLC. The retention time of the drug was found to be 7.0 min. The method validation proofs were carried out as per the ICH guidelines. The developed method was validated for linearity over a range of 500µg/mL to 1500µg/mL, with a correlation coefficient of 0.999, which shows the method is quite linear. Further precision, ruggedness, accuracy were validated. The %RSD for system precision was observed to be 0.7, whereas the method precision was observed to be 0.5. And for ruggedness the observations were found to be 0.5 and 0.4 respectively. The average recovery of 100.0% indicates the capability of the method, and finally no significant differences in % RSD values w.r.t retention time prove the robustness of the method. As per ICH guidelines, method validation results are in good agreement. The proposed approach is effective and can be applied for the estimation of ramelteon in bulk and tablet dosage form.

Keywords: Ramelteon, HPLC, Validation, Precision, Accuracy, Robustness.

INTRODUCTION

Ramelteon is freely soluble in ethanol, methanol, and dimethyl sulfoxide and slightly soluble in water and aqueous buffers pH 3 to 11. Chemically Ramelteon is (S)-N-[2-(1,6,7,8-tetrahydro-2H-indeno-[5,4-b]furan-8)- l-ethyl]propionamide (Figure-1) has a formula weight of 259.34 ($C_{16}H_{21}NO_2$) [1-2]. Ramelteon is orally active hypnotic drug for the treatment of transient and chronic insomnia in adults. Ramelteon has advantages over other hypnotic drugs in not causing rebound insomnia, withdrawal symptoms, or dependence which is common with the activation of BZP, opiate, or dopamine receptors. It acts at the melatonin (MT1 and MT2) receptors to promote sleep. Earlier publications have described chromatographic methods for determination of ramelteon in different analytical aspects[3-5]. So it is felt necessary to develop and validate analytical methods for its determination. This paper proposes RP-HPLC technique with UV detection for determination and its validation, useful for routine quality control of ramelteon in bulk and tablet dosage forms with the USP required limits [6-7].

EXPERIMENTAL

Chemicals and solvents

Ramelteon bulk drug were kind gift from Cellogen pharma, Navi Mumbai, and rozerem 8mg tablets was obtained from local pharmacy. Acetonitrile, methanol (HPLC grade) was obtained from Merck, Mumbai. Potassium hydrogen phosphate, triethyl amine and ortho phosphoric acid were of analytical grade and

were purchased from Qualigens, Mumbai. HPLC grade de-ionized water was used throughout the analysis.

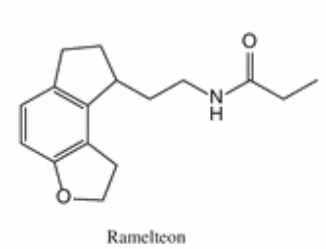

Fig.-1: Chemical structure of ramelteon

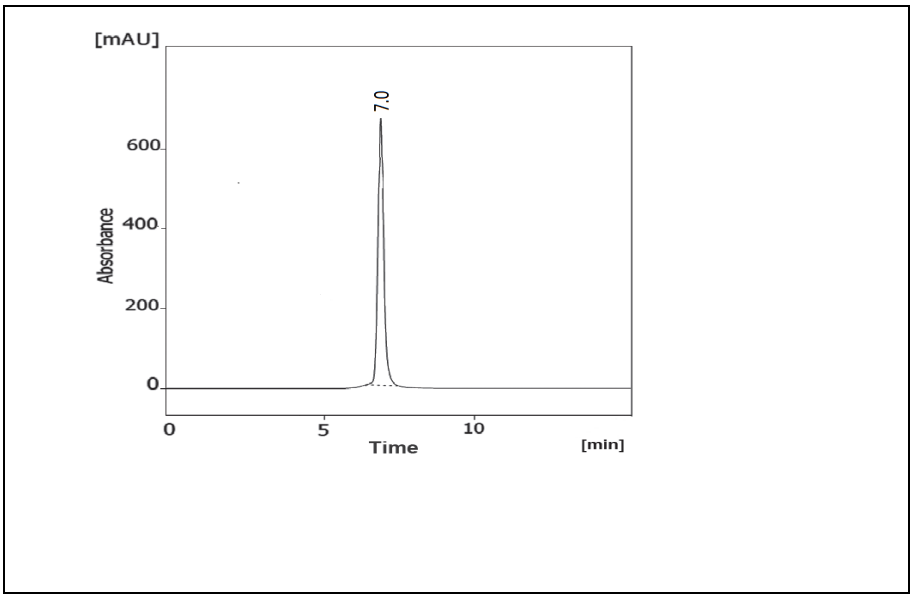

Fig.-2: standard chromatogram showing ramelteon peak

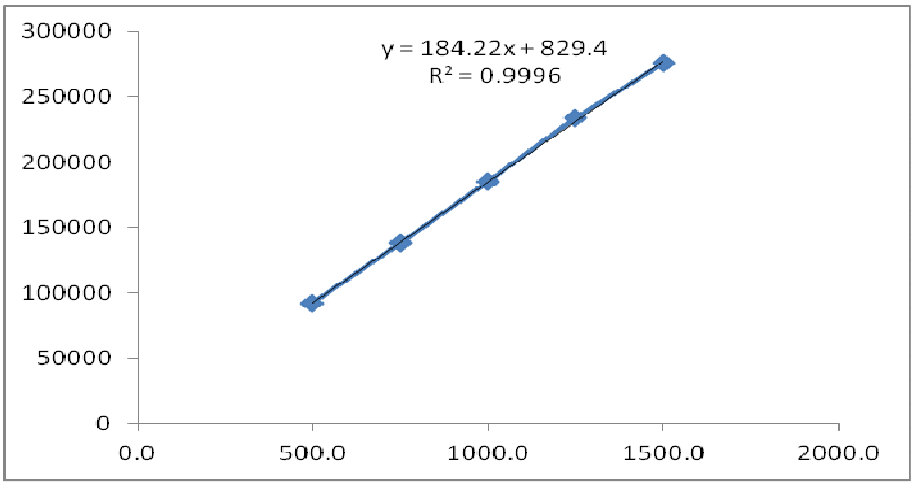

Fig.-3: Linearity graph for ramelteon

Chromatographic conditions

The HPLC system, make Shimadzu equipped with a LC-10ATVP solvent delivery module and SPD-10AVP UV detector was used for the complete method. Analysis was carried out by Hypersil ODS column, with dimension as 150x4.6 mm, 5 µm at 30°C temperature. The column outlet was monitored at 285 nm.Buffer was prepared by adding 8.5 gm of dipotassium hydrogen phosphate in 1.0 L of water in which 1.0ml triethylamine was added, then pH adjusted to 6.8 with ortho-phosphoric acid. The mobile phase consisted of acetonitrile: buffer (40:60 v/v) that was set at a flow rate and injection volume of 1.2 ml/min and 20µl respectively. Diluent was made up of methanol and water in 40:60 ratio. The mobile phase was degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system.

Table-1: System suitability parameters obtained from the proposed method

S. No.	Response	Theoretical plate	Tailing factor
Standard 1	185271	1954	0.80
Standard 2	184235	1995	0.79
Standard 3	190124	1975	0.80
Standard 4	184625	1980	0.80
Standard 5	184754	1997	0.81
Mean	185802	1980	0.80
Std Dev.	2444.4	17.4	0.0
% RSD	1.3	0.9	0.9

Table-2: system and method precision on intra and interday by two different analysts

S.No.	Analyst 1/Day 1		Analyst 2/Day2	
	System Precision	Method Precision	System Precision	Method Precision
1	184971	100.0	187452	99.8
2	185935	101.0	186548	99.9
3	183924	100.1	185721	100.0
4	185625	99.7	187561	99.0
5	186754	99.8	186549	99.8
6	187574	99.8	185241	99.9
Mean	185797	100.1	186512	99.7
Std.Dev	1289	0.5	919.5	0.4
% RSD	0.7	0.5	0.5	0.4

Preparation of solutions

Preparation of standard drug solution

The standard solution was prepared by dissolving ramelteon in diluent with intermittent shaking to get concentration equal to 1000 µg/ml.

Preparation of sample solution

Twenty tablets (8 mg/tablet) were crushed finely and powder equivalent to 100 mg taken in a 200ml volumetric flask containing 150.0 ml of diluent, sonicated at controlled temperature with intermittent shaking for 10 minutes, dissolved and makeup to 200ml. Further diluted by diluent to get concentration equal to 1000 µg/ml.

Both standard and sample solutions were injected as per the chromatographic conditions and chromatogram was recorded as shown in Figure 2. Retention time obtained was equal to 7.0 minute. Other results w.r.t. system suitability (Table1) is in good agreement that gives an idea about the capability of the proposed method.

Table-3: Accuracy results at three different concentration levels

		Added	Found	% Recovery
50 % of test conc.	1	499.5	500.0	100.1
	2	500.0	500.0	100.0
	3	500.1	500.0	100.0
			Mean	100.0
			Std.Dev.	0.1
			% RSD	0.1
100 % of test conc.	1	1000.0	1000.0	100.0
	2	998.5	999.5	100.1
	3	1001.0	1000.1	99.9
			Mean	100.0
			Std.Dev.	0.1
			% RSD	0.1
150 % of test conc.	1	1500.0	1510.0	100.7
	2	1499.7	1500.0	100.0
	3	1505.0	1500.0	99.7
			Mean	100.1
			Std.Dev.	0.5
			% RSD	0.5

RESULTS AND DISCUSSION

The standard ramelteon solution was further diluted in 10 mL volumetric flask to get various concentrations ranging from 500 to 1500 µg/mL of drug using diluent. From this each calibration standard solutions 20 µL was injected in to the HPLC system. The chromatograms were recorded. The concentration of the ramelteon is taken in X axis and peak response of standard solutions were taken in Y axis. The linearity graph was plotted.

The calibration graphs were found to be linear in the mentioned concentrations and the correlation coefficients for the regression line was 0.999 which is shown in Figure-3.

The limit of detection (LOD) and the limit of quantification (LOQ) were performed by measuring the analytical background response at lower concentrations levels. The values obtained for LOD & LOQ are 0.4µg/ml and 1.1µg/ml respectively.

Precision of the method was determined in terms of repeatability and intraday and interday precision. In order to measure system suitability, six consecutive injections were made with the standard solution. The response obtained was evaluated for % RSD. And method precision was evaluated by calculating assay for six different sample preparations. The data shows good precision of the system (% RSD=0.7) and method (% RSD=0.5) (Table-2). To study ruggedness, system and method precision was repeated by another analyst on another day with marketed tablets of the drug in similar operational and environmental conditions using developed method. The % RSD values of intra- and inter-day studies showed that the precision of the method was satisfactory (Table-2).

The accuracy of the HPLC assay method was evaluated by spiking known amount of drug to a sample solution of known concentration and analyzed the samples as per the method. 100.0 % recovery from

accuracy study and %RSD obtained at three different concentration level proves that the method has no interference from the additives used for the formulation which is shown in (Table-3).
Robustness was performed by small variation in the chromatographic conditions. %RSD for retention time (Table-4) found to be unaffected by small variations like ±2% variation in volume of mobile phase composition, ±0.1 mL/min in flow rate of mobile phase, ±0.1 variation in pH and ± 2nm variation in wavelength.

Table-4: % RSD values obtained for retention time under different chromatographic parameters for robustness

Organic composition +2 %	0.6
Organic composition -2 %	0.4
Flow rate + 0.1ml/min	0.8
Flow rate - 0.1ml/min	0.7
pH + 0.1	0.8
pH - 0.1	1.0
Wavelength + 2 nm	0.3
Wavelength - 2 nm	0.9

ACKNOWLEDGEMENTS

The authors are thankful to Department of Pharmaceutical Analysis, Jyothishmathi Institute of Pharmaceutical Sciences, Ramakrishna Colony, Thimmapur, Karimnagar, Department of Pharmaceutical Analysis, Sri Venkateswara College of Pharmacy, Etcherla, Srikakulam, and Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India for providing all necessary support.

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[RJC-1072/2013]