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ESTIMATION OF RAMELTEON IN TABLET DOSAGE FORM BY HPLC

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ABSTRACT

A simple, accurate and precise reverse phase HPLC method validated for the determination of Ramelteon Tablet dosage form. Chromatography was carried on C18 column using a mixture of PHOSPHATE BUFFER and METHANOL, pH 4.0 (in the ratio 80:20 v/v) as the mobile phase at a flow rate of 1 ml /min with detection at 280 nm by ultraviolet detector i.e. incorporated in HPLC. The retention time of the drug was found to be 5,503 min. The method validation proofs were carried out as per the ICH guidelines. The developed method was validated for linearity over a range of 12µg/ml to 28µg/ml, with a correlation coefficient of 0.998, which shows the method is quite linear. Further precision, ruggedness, accuracy were validated. The %RSD for system precision was observed to be Less Than 2, whereas the method precision was observed to be 0.456. 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 with respective 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 tablet dosage form estimation of Ramelteon in tablet dosage form.

KEYWORDS

Ramelteon, HPLC, Validation, Precision, Accuracy and Robustness.

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INTRODUCTON

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 No.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

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10 mg of Ramelteon in 20ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100µg/ml of Ramelteon is prepared by diluting 1ml of Ramelteon to 10ml with mobile phase (10µg/ml). This solution is used for recording chromatogram

Preparation of sample solution

5tablets (each tablet contains 8mg of Ramelteon) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 100µg/ml were prepared by dissolving weight equivalent to 10 mg of Ramelteon dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of Ramelteon was made by adding 1ml of stock solution to10 ml of mobile phase.

Linearity mixed and range

Preparation of standard solution

Weigh accurately 10 mg of Ramelteon in 10 ml of volumetric flask and from this, 1ml dissolve in 10ml of mobile phase and make up the volume with mobile phase.

CHROMATOGRAPHIC CONDITIONS

The HPLC system, make Shimadzu equipped with a LC-20ATVP solvent delivery module and SPD-10AVP UV detector was used for the complete method. Analysis was carried out by INERTSIL Column C18 with dimension as 150x4.6 ID, 5 µm at 30°C temperature. The column outlet was monitored at 280nm. 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 Phosphate buffer: methanol (80:20 v/v) that was set at a flow rate and injection volume of 1.0ml/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 membrane filter before pumping into HPLC system.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.

Preparation of standard stock solution of RAMELTEON

10mg of RAMELTEON was weighed and transferred in to 10ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the Figure No.8.1 and the absorption curve shows characteristic absorption maxima at 280 nm for RAMELTEON.

Assay

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10 mg of RAMELTEON in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of RAMELTEON is prepared by diluting 1 ml of

RAMELTEON to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

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Table No.1: Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogenortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate buffer	AR Grade
Sodium dihydrogen phosphate	AR Grade

Table No.2: Drugs used

RAMELTEON	Chandra labs, Prashnathinagar, kukatpally, Hyd
RAMELTEON (100 MG) RAMELTEON-100mg	OBTAINED FROM LOCAL PHARMACY OBTAINED Obtained from local pharmacy

Table No.3: Linearity Preparations

S.No	Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)
1	Preparation 1	0.6	10	12
2	Preparation 2	0.8	10	16
3	Preparation 3	1	10	20
4	Preparation 4	1.2	10	24
5	Preparation 5	1.4	10	28

Table No.4: Optimized chromatographic conditions

Mobile phase	Phosphate buffer: Methanol (80:20)
pH	4.0
Column	INERTSIL column, C18 (150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature (20-25^{o}C)
Sample temperature	Room temperature (20-25^{o}C)
Wavelength	280 nm
Injection volume	20 µl
Run time	8 mins
Retention time	About 5.503 min for RAMELTEON

Table No.5: Assay results

RAMELTEON			
S.No		**Standard Area**	**Sample Area**
1	Injection-1	871.075	875.999
2	Injection-2	868.307	871.069
3	Injection-3	869.282	871.020
4	Injection-4	875.134	874.919
5	Injection-5	873.263	875.177
6	Average Area	871.4122	873.6368
7	Assay (% purity)	100.2553	

Observation:

The amount of RAMELTEON present in the taken dosage form was found to be 100.25%.

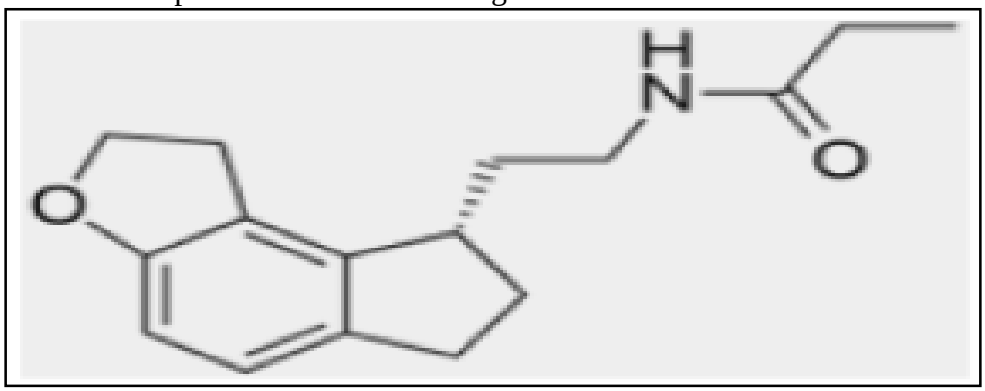
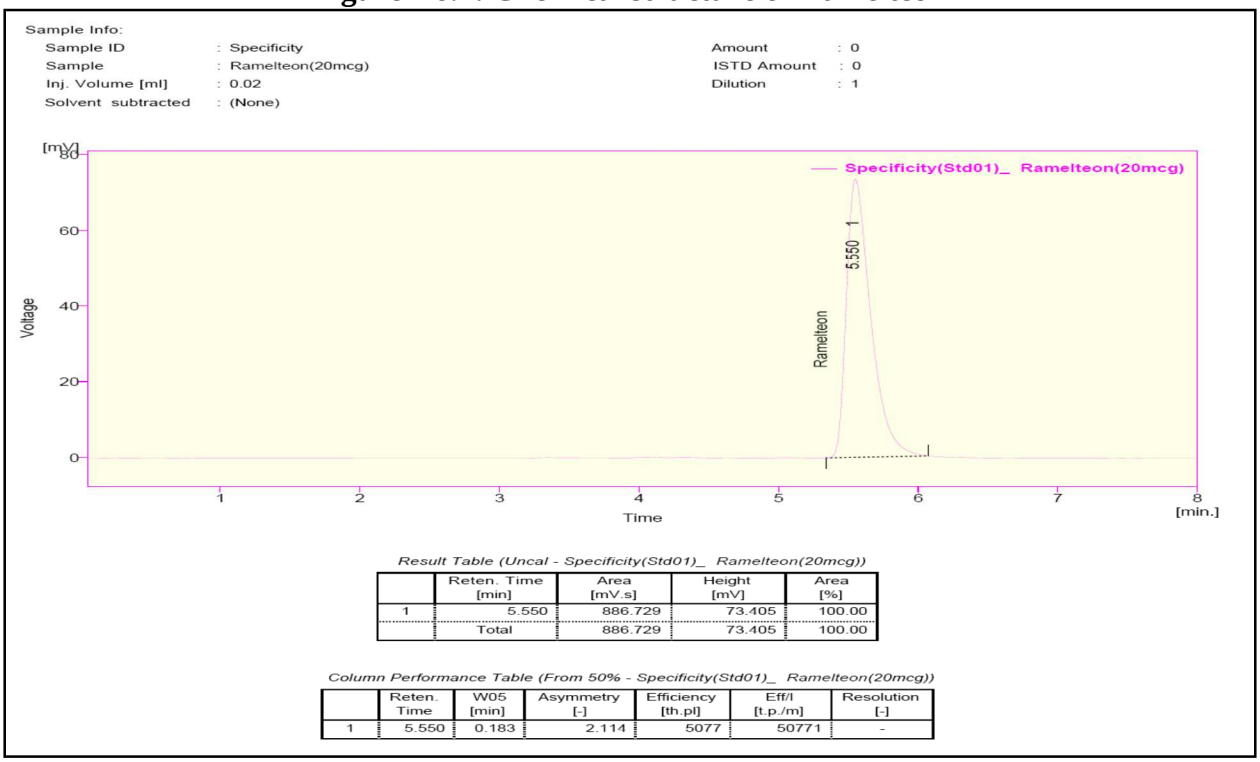


Figure No.1: Chemical structure of Ramelteon



Result Table (Uncal - Specificity(Std01)_ Ramelteon(20mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	5.550	886.729	73.405	100.00
	Total	886.729	73.405	100.00

Column Performance Table (From 50% - Specificity(Std01)_ Ramelteon(20mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	5.550	0.183	2.114	5077	50771	-

Figure No.2: Standard Chromatogram showing Ramelteon peak

It is observed that the diluents and excipients peaks are not interfering with the Ramelteon peaks.

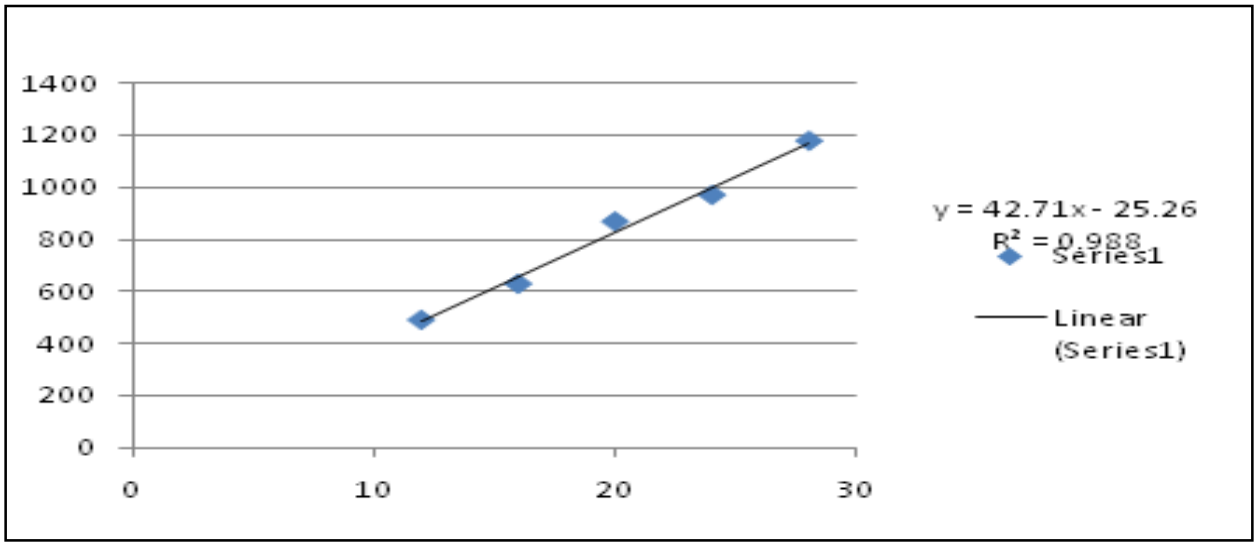


Figure No.3: Linearity graph of Ramelteon

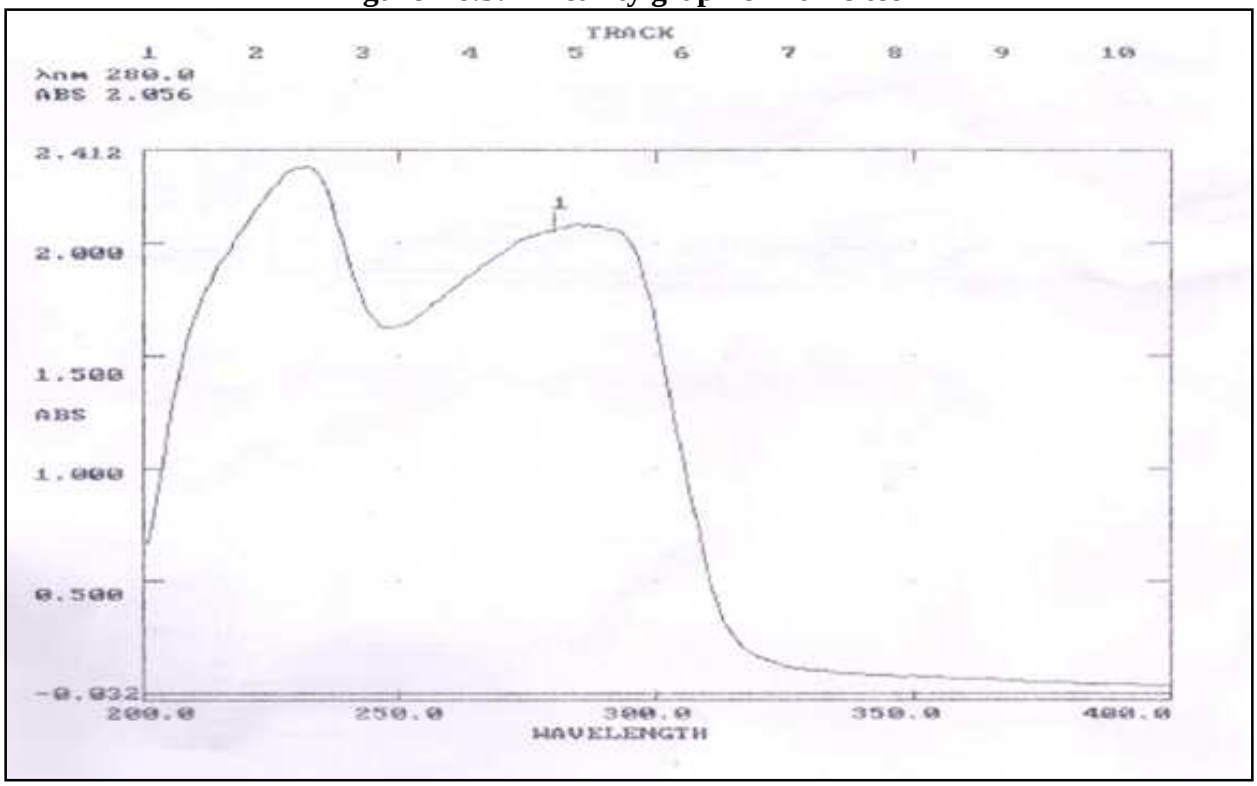


Figure No.4: UV-VIS spectrum of Ramelteon

Observation: λ_{max} was found to be 280 nm for RAMELTEON shown in the figure.

CONCLUSION

The results was concluded that the methods developed in the present investigation are simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the Ramelteon in tablet dosage form.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Methods of Analysis, http://www.pharmatutor.org/ pharma-analysis.
2. Douglas A, Skoog F, James H, Stanley R C. Liquid Chromatography, In *Instrumental Analysis, Cengage Learning India Pvt. Ltd, New Delhi,* 9th edition, 2007, 893-934.
3. Skoog, Holler, Crouch. Liquid Chromatography, In *Instrumental Analysis, Cengage Learning India, New Delhi,* 2011, 893-934.
4. Chatwal R G, Anand K S. High Performance Liquid Chromatography, In *Instrumental Methods of Chemical Analysis,* Himalaya Publishers, Mumbai, 5th edition, 2010, 2.570-2.629.
5. Sharma B K. High Performance Liquid Chromatography, In *Instrumental Methods Of Chemical Analysis, Goel Publishers, Meerut,* 24th edition, 2005, 295-300.
6. Alfonso R G, Ara H D M, Glen R H, Thomas M, Nicholas G P, Roger L S, Steve H W. Chromatography, In *Remington: The Science and Practice of Pharmacy, Lippincott Williams and Wilkins: Philadelphia,* 20th edition, 2000, 587-591.
7. Adsorption Chromatography- http://www.separationprocesses.com/ Adsorption/ AD_Chp05a.html.
8. Adsorption Chromatography - http://cemca.org/andcollege/ and cwebsite/ subject01/ CHEtext.pdf.
9. Types of Chromatography - http://www.separationprocesses.com/ Adsorption/ AD_Chp05a.html.
10. Partition Chromatography - http://media.rsc.or/ Modern %20 chemical %20 techniques/ MCT5 %20 Chromatography.pdf.
11. Ion Exchange Chromatography - http://www.gelifesciences.com/ webapp/ wcs/ stores/ servlet/ catalog/en/ GE Life Sciences-IN/ products/ion-exchange-chromatography-iex/.
12. Ion Exchange Chromatography- http://wolfson.huji.ac.il/ purification/PDF/ Ion Exchange/ AMERSHAM _ iIEX and Chromatofoc Manual.pdf.

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