DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF RAMELTEON IN BULK AND PHARMACEUTICAL DOSAGE FORM

Maaz Aaquil[1], Prof. Patel M. Siddik[2], Dr. Khan G.J[3], Abdul Bari[4], Ansari Mohd.Hammad[5], Mohd. Shoeb Nawaz[6], Shakih Mujeeb[7], Tamboli Saifali[8].

MAAZ AAQUIL Department of QUALITY ASSURANCE, JIIU's Ali Allana College of Pharmacy, Akkalkuwa. Dis: Nandurbar 425415. Maharashtra, India.

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 Methanol: water (0.1 % OPA), pH 2.7 (in the ratio 60:40 v/v) as the mobile phase at a flow rate of 0.7 ml /min with detection at 282 nm by ultraviolet detector i.e. incorporated in HPLC. The retention time of the drug was found to be 5.860 min. The method validation proofs were carried out as per the ICH guidelines. The developed method was validated for linearity over a range of 10µg/ml to 60µg/ml, with a correlation coefficient of 0.999, which shows the method is quite linear. Further precision, ruggedness, accuracy was 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.*

Keyword: Ramelteon, RP-HPLC Method Development, Anti-Anxiety, Validation.

INTRODUCTON:

Ramelteon is sparingly soluble in water, slightly soluble in HCL. 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, Ramelteon is used for the treatment of 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.(Fig no.01)

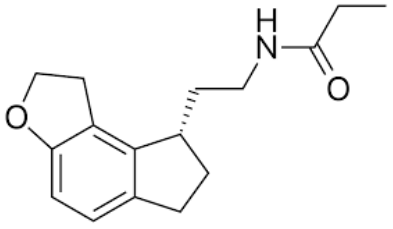


Figure no 01 : Chemical Structure of Ramelteon

Material and Method:

Table No.:01 Drug and Drug Supplier

Name of Drug	Drug Supplier
RAMELTEON (API)	Swapnroop drug and pharmaceutical, Aurangabad
RAMELTEON (8mg)	Obtained from local pharmacy

Table No.02: List of Reagent and chemical

Sr. No.	Name of chemicals	Manufacturer.
1.	Acetonitrile	(HPLC grade)
2.	Methanol	(HPLC grade)
3.	Ortophosphoric Acid	(HPLC grade)
4.	water	(HPLC grade)

Selection of formulation:

From the literature survey and market survey we selected Maxide formulation for work.

Marketed Preparation**:**

Table No.03: List of brand names of combined formulations of Ramelteon

Stock preparations:

Sr. No	Brand name	Formulation	Available strength	Address of manufacturer
1.	Rozerem	Tablet	Ramelteon 8 mg	Takeda Pharmaceutical

- Stock I : Standard Sample Preparation :Std. RAMELTEON 10 mg in 10 ml Methanol = 1000 µg/ml
- Stock II : Tab solution Preparation: Take 0.3 ML in 10 ml Methanol i.e. = 30 µg/ml Tab solution for injection

For Accuracy Solution Preparations: -

Take 10 µg/ml TAB SOLUTION FOR AUURACY,

- 80 % = 0.1 ml tab solution and add 8 µg/ml std rm and make up vol 10 ml with mobile phase
- 100 % =0.1 ml tab solution and add 10 µg/ml std rm and make up vol 10 ml with mobile phase
- 120 %= 0.1 ml tab solution and add 12 µg/ml std rm and make up vol 10 ml with mobile phase

Instruments and Equipment's

Table. 04: Instrument (HPLC) Details used during Method Development

SR. n	Name of Instrument	Company Name
1	HPLC Instrument	Agilent with auto sampler(DAD) (Chemstation software)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C_{18})	Agilent C_{18} (250mmX 4.6mm,5µm)
4	pH meter	VSI pH meter(VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance.
6	Sonicator	Ultrasonic electronic instrument

EXPERIMENTAL

Selection of Analytical Technique

- HPLC was selected as analytical technique for estimation of Ramelteon
- Instruments:

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector, DAD Detector. Equipped with Reverse Phase C_{18} (Agilent) with 250mm x4.6; (5µm), UV730D Absorbance detector and running chemstation 10.1 software.

a) Selection of stationary phase:

- The column used in this method C_{18} Agilent The configuration of the column is 4.6 x 250 mm, particle size 5 µm. C_{18} column gives high non polar retentively, symmetric peak shape, highly reproducible and stable ideal for HPLC method

b) Solubility Studies:

This study was carried out to find an ideal solvent in which drugs are sparingly soluble in water soluble. Various solvents were tried for checking solubility of Ramelteon. From solubility studies it was concluded that of Ramelteon is slightly soluble in HCL.

c) Chromatographic conditions :

- The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table No.05: chromatographic conditions (HPLC) details used during method Development.

1.	HPLC	Agilent Tech. Gradient System with Auto injector
2.	Software	chemstation 10.1
3.	Column	(Agilent) C18 column (4.6mm x 250mm
4.	Particle size packing	5 µm
5.	Stationary phase	C18 (Agilent)
6.	Mobile Phase	Methanol: water (0.1 % OPA) 60 : 40

7.	Detection Wavelength	280 nm
8.	Flow rate	0.7 ml/min
9.	Temperature	Ambient
10.	Sample size	20 µl
11.	pH	2.7
12.	Run Time	15 min
13.	Filter paper	0.45 µm

Study on the chromatographic conditions of Ramelteon:

Accurately weigh and transfer 5 mg Ramelteon working standard into 10 ml volumetric flask as about dilute Methanol prepared in completely and make volume up to the mark with the same solvent to get 500µg/ml standard (stock solution) and 15 min sonicate to dissolve it and from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol:(0.1%OPA) Water solvent. The resulting 10µg/ml of solution was subjected to chromatographic analyses using mobile phases of different strengths with chromatographic conditions mentioned below:

- Analytical column : Agilent C18 Column (250mm x 4.6mm), 5µm particle size.
- Injection volume : 20µl
- Flow rate : 0.7 ml/min
- Detection : 280nm
- Run Time : 10 min

METHOD DEVELOPMENT OF HPLC:

- List of Mobile Phase:

Table No.06: Selection of mobile Phase.

Sr .No.	Mobile Phase
1.	Methanol+ 0.1% (OPA)Water, (80+20% v/v) 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)
2.	Methanol+ 0.1% (OPA)Water, (70+30% v/v) 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)
3.	Methanol+ 0.1% (OPA)Water, (60+40% v/v) 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)
4.	Methanol+ 0.1% (OPA)Water, (50+50% v/v) 20Mcg C_{18} (Agilent) (4.6mm x 250mm)
5.	Methanol+ 0.1% (OPA)Water, (40+60% v/v) 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)

Preparation of standard stock solution: -

- Ramelteon standard stock solution: (Stock I)

An accurately weighed quantity, 5 mg of Ramelteon (RM) was dissolved in Methanol and water in a 100ml volumetric flask and volume made up to 10.0 ml to produce a solution of 100 µg/ml.

- Preparation of Stock Standard Solution :(Stock II)

Accurately weight and transfer 5 ml Ramelteon working standard into 10 ml volumetric flask as about diluents Methanol completely and make volume up to the mark with the same solvent to get 500µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol: Water (0.1%OPA) Water, prepared in (60 ml MEOH: 40ml WATER v/v)

Validation of method for analysis of Ramelteon:

- The developed method was validated as per ICH guidelines.

Linearity:

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range, The Result are shown in;**(Table No.07)**

- Determination:

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated.

- Acceptance Criteria:

The plot should be linear passing through the origin.
Correlation Coefficient should not be less than 0.999.

- **Preparation of standard stock solution for linearity:**

Average weight of tablet sample (equivalent to 10 mg of Ramelteon were weighed and transferred to 10 ml volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1 ml of this solution diluted up to 10 ml volumetric flask with diluents was added to make up the volume.

- **Preparation of linearity solution:**

A series of standard preparations of working standard of were prepared.

Table No.07: Table of linearity for RP-HPLC Method

Linearity of Ramelteon HPLC	
Sr .No.	**Concentration (µg/ml)**
1	10
2	20
3	30
4	40
5	60

Accuracy (recovery):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay, The RP-HPLC& UV Method Result are shown in; **(Table No:18)**

Preparation of standard stock solution:

10 mg of Ramelteon working standards were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume 0.1 ml of this solution diluted up to 10 ml with diluent.

Application of proposed method for analysis of tablet formulation:

Accuracy

The accuracy was determined by Ramelteon (equivalent to 10 mg (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. This powder containing 10 mg of Ramelteon were triturated and then subjected to chromatographic analysis using the described method. The resulting was analyzed in triplicates over three days. The % recovery of added drug was taken as a measure of accuracy**.**

The Result are shown in; **(Fig No:11)**

Repeatability:

Precision of the system was determined with the sample of RP-HPLC& UV Method for. Three replicates of sample solution containing 20 mg of Ramelteon were injected and peak areas were measured and %RSD was calculated. Is was repeated for five times result are shown in; **(Table No: 12) & (Fig No:)**

Application of proposed method for analysis of tablet formulation:

Average weight of tablet sample (equivalent to 10 mg of Ramelteon) was weighed and transferred to 10mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter 0.1 ml of this solution diluted up to 10 ml with diluent.

Precision:

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test

Robustness:

The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion and the flow rate was of Methanol : Water (0.1 % OPA) in the mobile phase composition (±1ml/ min^{-1}**)** and the change in detection wavelength (±1 ml/ min^{-1}**)** and the effect of the results were examined and it was performed using 20µg/ml solution of Ramelteon in triplicate. The Result are shown in; **(Table No: 31)**

Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

The result is shown in: **(chapter: 8)**

Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

$$QL = \frac{10\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.
The results are shown in **(chapter: 8)**

Analysis of marketed formulation

To determine the content of Ramelteon in marketed tablets (label claim 8mg of Ramelteon), 20 tablets powder weighed in 15.74gms and average weight of powder was calculated in 787gms.Tablets were triturated and powder equivalent to weighed in 6.55 mg The drug was extracted from the tablet powder with 10 ml Methanol . To ensure complete extraction it was sonicated for 15 min. 0.1mL of supernatant was then diluted up to 10 ml with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted**. (Fig No: 66)**.
Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of Ramelteon in the sample was calculated. The amount of Ramelteon per tablet was obtained from the regression equation of the calibration curve as described in analysis of Tablet formulation are shown in **(Table No.32).**

RESULTS AND DISCUSSION:

Preliminary studies on Ramelteon

Melting point

The procured reference standard of Ramelteon was found to melt in the range of 113^0C to 115^0C.

Solubility:

The drug was found to be

- Sparingly Soluble in Water.
- Slightly Soluble in HCL

UV Spectroscopy

Standard solutions were scanned in the range of 200-400nm ,against 10 ml methanol and volume make with water solvent system as reference Ramelteon in water was found to be selected wavelength is 280 nm .**(Figure No:19)**

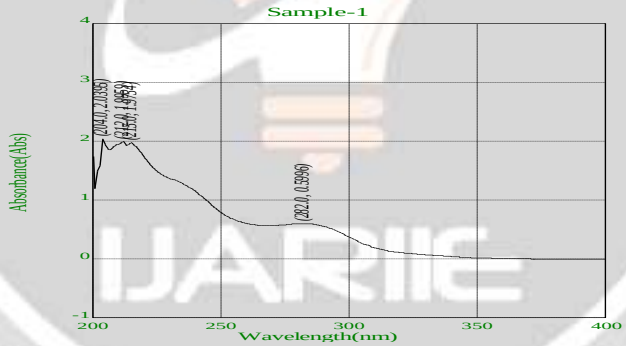


Fig No.02: UV Spectrum of Ramelteon

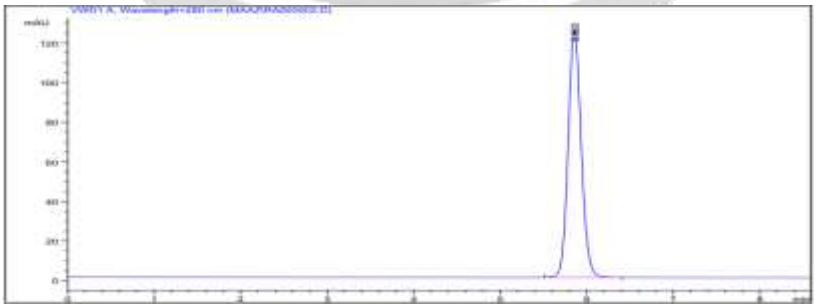


Fig No 03: Chromatogram of Final Trial

Table.No.15. Trial-3 of chromatogram of Ramelteon

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF

1	RM	5.860	1314.71423	100.00	6986.0	1.2273

Analytical of Method Validation:

1. Linearity:

From Ramelteon standard stock solution, different working standard solution (10-60µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographicsystemusing mixed volume loop injector Chromatograms were recorded. The area for each concentration was recorded (**Table No. 09)** The Calibration curves are shown in **[Fig. No.04]**

Fig.No.04. Chromatogram of linearity

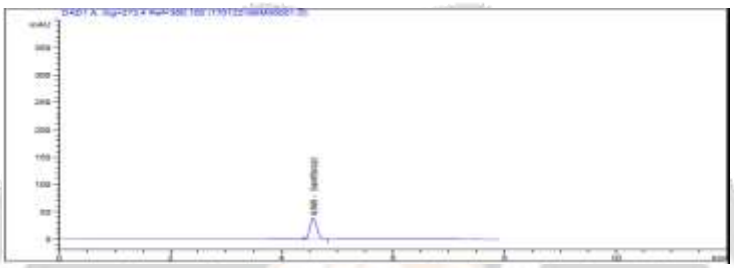


Table No 09. Linearity of Ramelteon

Sr. No.	Concentration µg/ml Ramelteon	Area Ramelteon
1	10	474.5999
2	20	928.3512
3	30	1366.1019
4	40	1787.9483
5	60	2705.7900

Fig.No.05. Calibration curve of Ramelteon for HPLC method

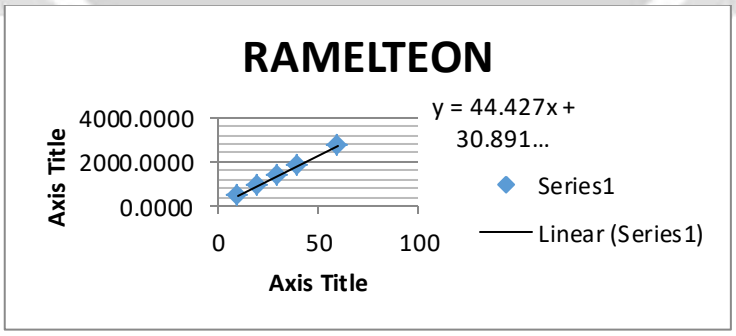


Table No 10. Regression equation data for Ramelteon

Regression Equation Data Y=mx+c	
Slope(m)	44.42
Intercept(c)	30.89
Correlation Coefficient	0.999

Linearity of Ramelteon was observed in the range of 10-60µg/ml Detection wavelength used was 280 nm. The plot should be linear passing through the origin; Correlation Coefficient should not be less than 0.999.that concluded. **(Table. No. 11)**

2. Accuracy:-

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analysed **.** Statistical validation of recovery studies shown in **Table No.11.Result For Accuracy**

METHOD	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	% Recovery Mean *± S.D.
RP-HPLC Method		80%	10	8	18.10±0.049	8.10±0.049	101.30±0.62
		100%	10	10	20.14±0.082	10.14±0.082	101.38±0.82
		120%	10	12	2.08±0.046	12.08±0.046	100.68±0.38

3. System suitability parameters :(Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Ramelteon system suitability parameters were studied. The result shown in below **(Table No.12)**

Table No.12: Repeatability studies on RP-HPLC for Ramelteon

Sr.No.	Concentration of Ramelteon (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	50	2714.094	60.44	100.74
2	50	2717.286	60.36	100.65
		Mean	60.40	
		SD	2.26	
		% RSD	0.08	

4.Precision:-

The method was established by analyzing various replicates standards of Ramelteon. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtaine for intraay is shown in **(Table No. 13)** respectively.

Table No .13: Result of Intraday and Inter day Precision studies on RP-HPLC Method for Ramelteon

METHOD	Drug	Conc· (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	% Amt. Found	Mean± SD	% Amt. Found
RP HPLC METHOD	RM	20	80.83±0.97	5.62	80.87±0.93	5.70
		30	267.33±1.15	17.74	271.33±1.18	17.82
		40	463.77±3.46	24.36	468.77±3.55	24.43

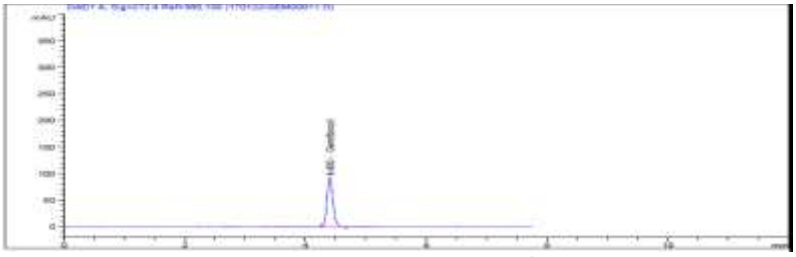


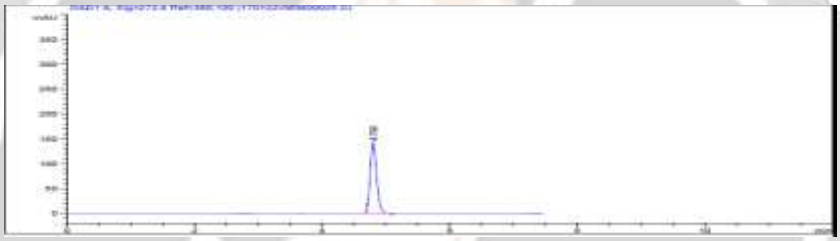
Fig No .06: Chromatogram of Precision

Intraday and Inter day Precision studies on RP-HPLC method for Ramelteons which shows the high precision %amount in between 5% to 24% indicates to analytical method that concluded.

5. Robustness:

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.The mobile phase composition was changed in (±1 ml/min^{-1}**)** proportion and the flow rate was varied by (±1ml/min^{-1}**)**, and wavelength change (±1 ml/min^{-1}**)** of optimized chromatographic condition. The results of robustness studies are shown in **(Table No.14**).Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

5.1 Flow Rate Change 0.6 ml



FigNo.07. Chromatogram of Flow rate change 0.6ml

1) Mobile phase composition Change : 59ml MEOH + 41ml Water

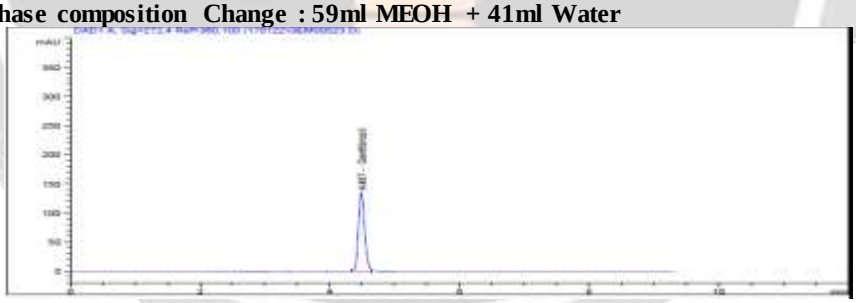


Fig No .08. Chromatogram of Mobile phase composition change: 59 ml MEOH + 41ml Water

Parameters	Conc.	Amount of detected(mean ±SD)	% RSD
Mobile phase composition-(59+41)	15	839.99±0.534	0.39
Mobile phase composition-(61+39)	15	344.55±0.94	0.27
Wavelength change281nm	15	653.7±399.41	61.10
Wavelength Change 283nm	25	620.06±438.13	70.66
Flow rate change(0.6ml)	20	748.25±504.86	67.47
Flow rate change(0.8ml)	20	56.06 ± 364.49	65.55s

Table No.14 Result of Robustness Study of Ramelteon

Robustness Study of Ramelteon:

The changes were did flow rate (±1 ml/ min^{-1}), PH of mobile phase composition (±1 ml/ min^{-1}), and Wavelength (±1 ml/ min^{-1}). %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded. (**Table No.14)**

6. Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

LOD= 3.3XAvd.SD/Slope
=3.3X6.90/44.42
=0.51232

Where, SD = Standard deviation of Y intercept
S = Slope

- The LOD of Ramelteon was found to be 0.5123 (µg/ml) analytical methods that concluded.

8. Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S
=10 X 6.90s / 44.42
= 1.55248 Where, SD = Standard deviation Y intercept S = Slope

The LOQ of Ramelteon was found to be 1.5524 s(µg/ml) analytical method that concluded.

Analysis of tablet formulation: -

Procedure:

Weigh 20 Ramelteon Tablet and calculated the average weigh 500 mg accurately weigh and transfer the sample equivalent to 31.25 mg Ramelteon into 10 ml volumetric flask. Add about 10ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.3ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (25 µg/ml). The simple chromatogram of test Ramelteon Shown in **(Fig No: 09)** the amounts of Ramelteon per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Label claim for %RSD Calculated, Result was shown in **(Table No. 15)**

Brand Name: ROZEREM 8 MG (Takeda pharmaceutical)

Total weight of 20-tab wt. = 500 mg

Avg. Weight = 0.025 mg. /Tab

Eq.wt for 10 mg= 10 X 25 / 8 = 31.25 mg

Take 0.3 ml in 10ml Methanol i.e. =30 µg/ml tab solution for injection

Sonicate 10 min i.e. 30 µg/ml Ramelteon

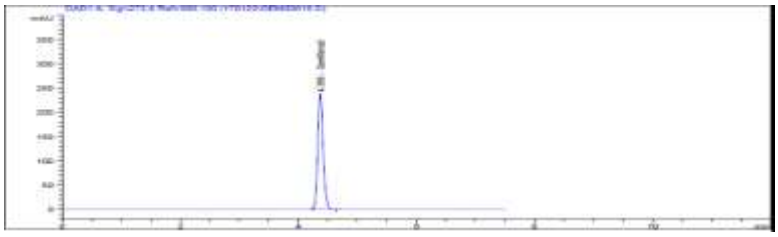


Fig No.09: Chromatogram for Marketed Formulation

Assay	Drug	Label Claimed	Amt.Found	% Label Claim	SD	% RSD
RP-HPLC Method	**RM**	30	30.39	100.71	2.78	0.404
		30	30.21	101.01	2.84	0.401

Table.15. Analysis of marketed formulation

Analysis of marketed formulation were also %Label Claim was found to be 100-101% Satisfactory are concluded. (Table No.15)

Table No.16: Result of linearity of Ramelteon

Parameter	RM
Concentration (µg/ml)	10-60(µg/ml)
Correlation Coefficient($r^{2)}$	0.999
Slope (m)	44.42
Intercept(c)	30.89

Table No.17: Optimized chromatographic conditions

1.	HPLC	Agilent Tech. Gradient System with Auto injector
2.	Software	chemstation 10.1
3.	Column	(Agilent) C18 column (4.6mm x 250mm
4.	Particle size packing	5 µm
5.	Stationary phase	C18 (Agilent)
6.	Mobile Phase	Methanol : water (0.1 % OPA)60 : 40
7.	Detection Wavelength	282 nm
8.	Flow rate	0.7 ml/min
9.	Temperature	Ambient
10.	Sample size	20 µl
11.	pH	2.7
13.	Filter paper	0.45 µm

Table no 18: Result of Recovery data for Ramelteon

% Level	% Mean±SD*
80%	101.30±0.62
100%	101.38±0.82
120%	100.68±0.38

Table no 19: result of precision

METHOD	Drug	Conc (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	% Amt. Found	Mean± SD	% Amt. Found
RP HPLC METHOD	RM	20	80.83±0.97	100.92	80.87±0.93	100.28
		30	267.33±1.15	100.75	271.33±1.18	100.83
		40	463.77±3.46	99.28	468.77±3.55	99.54

Conclusion: The result 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 the tablet dosage form.

Acknowledgement: The authors are sincerely thanks to Ali Allana College of Pharmacy, Akkalkuwa, Nandurbar, India for providing the facilities to complete this research work.

Conflict of Interest:

The author has declared no conflict of interest.

Abbreviations: RP-HPLC: Reverse Phase High Performance Liquid Chromatography: Ramelteon, H2O: Water,C2H3N:Methanol,ML:Micro litre,Mg:Miligram

Reference:

1. Ahmed M, Deepak BM, Shetty SA, Kuppast IJ, Anilkumar SM, MC Ravi. RP-HPLC method development and validation for estimation of macitentanintablet dosage form. J Pharm Pharm Sci 2014; 4:881.
2. Quality Assurance of Pharmaceuticals, A compendium of guidelines and related materials; WHO, Geneva; 1997. p. 119:24.
3. ICH Q2 (R1) Validation of Analytical Procedure; 2005. p. 14.
4. Ahmed M, Deepak BM, Shetty SA, Kuppast IJ, Anilkumar SM, Ravi MC. RP-HPLC method development and validation for estimation of macitentan in tablet dosage form. J Pharm Pharma Sci 2015; 4:887.
5. 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.
6. Skoog, Holler, Crouch. Liquid Chromatography, In Instrumental Analysis, Cengage Learning India, New Delhi, 2011, 893-934.
7. 5th edition, 2010, 2.570-2.629. 5. Sharma B K. High Performance Liquid Chromatography,
8. In Instrumental Methods of Chemical Analysis, Goel Publishers, Meerut, 24th edition, 2005, 295-300.
9. Alfonso R G, Ara H D M, Glen R H, Thomas M, Nicholas G P, Roger L S, Steve H W.
10. Chromatography, In Remington: The Science and Practice of Pharmacy, Lippincott Williams and Wilkins: Philadelphia, 20th edition, 2000, 587-591.
11. Adsorption Chromatographyhttp://www.separationprocesses.com/ Adsorption/ AD_Chp05a.html.
12. Adsorption Chromatography - http://cemca.org/andcollege/ and cwebsite/ subject01/ CHEtext.pdf.
13. https://pubchem.ncbi.nlm.nih.gov/compound/Ramelteon
14. https://reference.medscape.com/drug/rozerem-ramelteon-342928S