Turk J Pharm Sci 11(3), 323-328, 2014

Original article

Determination of Ramelteon in Tablet Dosage Form by Novel Validated Spectrophotometric Methods

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As per ICH guidelines three UV spectrophotometric methods viz. linear regression equation (LRE), standard absortivity (SA) and first order derivative (^{1}D) method were developed and validate. The Lambert- Beer law was followed in range of 10-50 µg/mL. The results of all validation parameters were found to be within acceptable limits (relative standard deviation was less than 2%). The drug content in tablet dosage forms was determined by validated methods as 100.24-100.43%, 99.70-100.35% and 100.14-100.26%, respectively with acceptable standard deviation. These validated spectrophotometric methods may be successfully applied for assay, dissolution studies, bio-equivalence studies as well as routine analysis in pharmaceutical industries.

Key words: Ramelteon, Spectrophotometric methods, Tablets

Valide Edilmiş Yeni Spektrofotometrik Yöntemlerle Tablet Formülasyonlarında Ramelteon Tayini

ICH kılavuzlarına göre lineer regresyon eşitliği (LRE), standart absorptivite (SA) ve birinci dereceden türev (^{1}D) şeklinde üç UV spektrofotometrik yöntem geliştirilmiş ve valide edilmiştir. Lambert-Beer yasası 10-50 µg/mL aralığında izlenmiştir. Tüm validasyon parametrelerin sonuçları kabul edilebilir sınırlar (relatif standart sapma % 2'den az) içinde bulunmuştur. Valide edilmiş metotlarla tablet formülasyonlarındaki ramelteon içeriği sırasıyla 100.24-100.43%, 99.70-100.35% ve 100.14-100.26% şeklinde kabul edilebilir standart sapma değerleri ile tayin edilmiştir. Bu valide edilmiş spektrofotometrik yöntemler başarıyla analiz, çözünürlük ve biyoeşdeğerlik çalışmaları ile ilaç endüstrisinde rutin analizler için de uygulanabilir.

Anahtar kelimeler: Ramelteon, Spektrofotometrik yöntem, Tabletler

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INTRODUCTION

Ramelteon (RMT) is a novel melatonin receptor agonist that is used for clinical treatment of insomnia (1). Chemically, it is designated as (*S*)-N-(2-(1,6,7,8-tetrahydro-2H-indeno-(5,4-b)furan-8-yl)ethyl) propionamide (Fig. 1). It is an orally active hypnotic and highly selective melatonin receptor agonist (2, 3). The enantiomeric separation of RMT was achieved on Chiralpak AD-H using a mobile phase system consisting of n-hexane, ethanol and methanesulfonic acid (4). Gradient reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was reported for purity analysis (5).

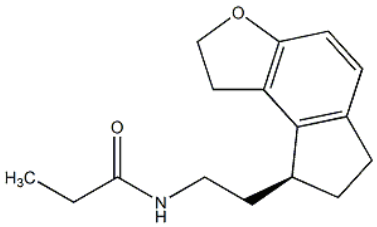

Figure 1. Structure of ramelteon

As per author's knowledge, there was no report available on spectrophotometric determination of RMT and the present research work was aimed to establish the validated spectrophotometric methods for determination of RMT in dosage form which may be applied for routine analysis in different matrix of RMT. All three spectrophotometric methods are applicable for determination of single analyte; which are simple, cost effective with acceptable accuracy and precision.

EXPERIMENTAL

Instruments, reagent and chemicals

Instruments, reagents and chemicals Ultraviolet spectrophotometer (1700 series Shimadzu, Japan) with 1 cm matched quartz cells were used for the measurement of absorbance. Shimadzu- Ax-200 electronic balance was used for weighing and class volumetric glasswares were used. Ramelteon working standard (RMT WS) was procured from Ranbaxy Laboratories Ltd. Gurgaon, New Delhi, as a gift sample. Analytical grade methanol and sodium hydroxide were procured from Merck Specialities Private Limited, Mumbai, India. RMT tablets (Ramitax^{TM}; film coated tablets, Ranbaxy Laboratories Limited, New Delhi, India) were purchased from local market. Distilled water was prepared in-house by distillation assembly.

Linear regression equation (LRE) method

About 50 mg of RMT WS was accurately weighed and dissolved in 10 mL methanol and volume was made upto 50 mL with distilled water to prepare stock A (1000 μg/mL). Aliquots of the stock A was diluted with 20% aqueous methanol to get concentration of 10, 20, 30, 40 and 50 μg/mL. These dilutions were scanned against 20% aqueous methanol as blank in the range of 200-400 nm to get UV spectra (Fig. 2). The absorbance of the dilutions was recorded at 287 nm. The calibration graph was plotted concentration vs. absorbance and regression equation was determined with correlation coefficient.

Standard absorptivity (SA) method

Five serial dilutions of RMT WS (10, 20, 30, 40, and 50 μg/mL) were prepared in triplicates and the absorbances were observed at 287 nm against 20% aqueous methanol as blank. The standard absorptivity A (1%, 1cm) and molar extinction coefficient ε were calculated by using above observed absorbances. The standard absorptivity and molar extinction coefficient would be used to determine the drug content of dosage forms.

First order derivative (^1D) method

The interference of one analyte in absorbance of another analyte may be nullified in the derivative mode; the first order derivative mode of Gaussian UV spectra was used to develop the method. Standard serial dilutions (10, 20, 30, 40, and 50 μg/mL) of RMT WS were scanned to get UV spectra and the spectra were converted into first order derivative mode (Fig. 3). The absorbance was observed at 295.50 nm in the derivative mode and linear regression equation was calculated.

Validation of methods

As per ICH guidelines (6), standard serial dilutions (10, 20, 30, 40 and 50 µg/mL) of RMT WS in triplicate were used to validate all three methods (LRE, SA and ^{1}D methods) for linearity, accuracy (by recovery studies, standard addition to pre-analysed samples), repeatability (within day), intermediate precision (days and analyst variation) and robustness (temperature variation: 35°C, 30°C and 25°C) and statistical parameters were calculated for them.

Delhi, India) were finely powdered; a quantity equivalent to 50 mg of RMT was dissolved in 10 mL methanol and volume was made upto 50 mL with distilled water. The solution was filtered through Whatman filter paper no. 41 to give stock I. Aliquots of stock I were diluted to obtain sample concentrations (20, 30 and 40 µg/mL) in the range of linearity. The absorbance values of these sample dilutions were observed in a multipoint calibration curve of quantitative mode at the selected wavelength to obtain test sample

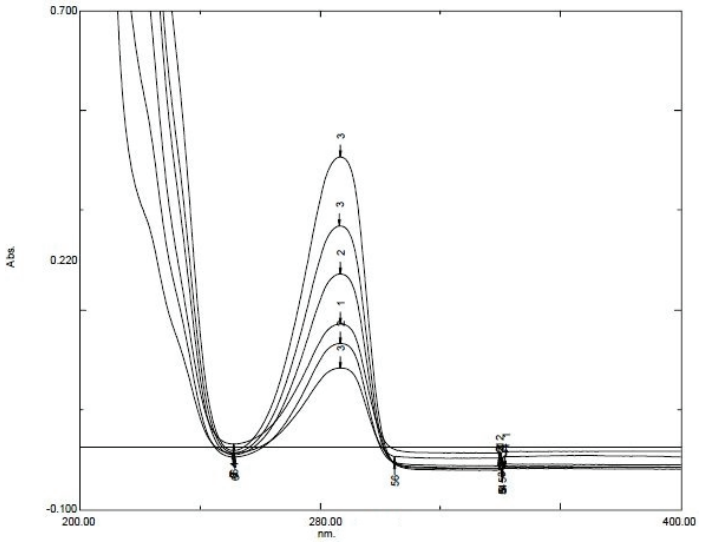

Figure 2. UV spectra of RMT in 20% aqueous methanol

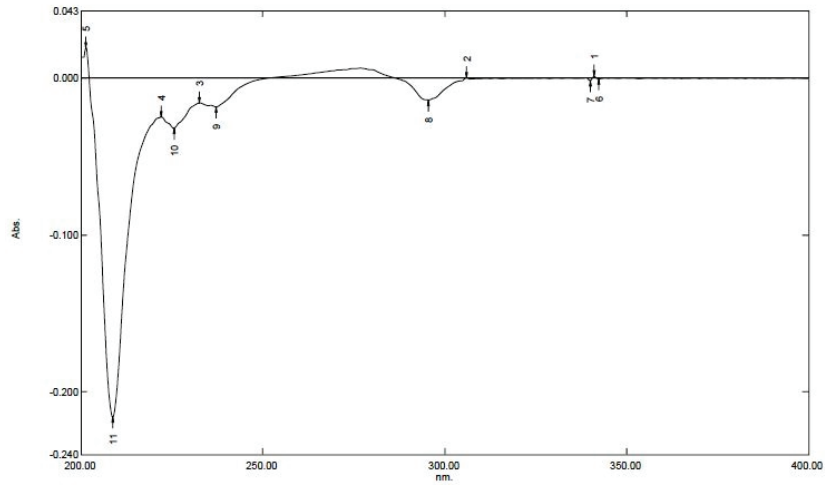

Figure 3. First order derivative of UV spectrum of RMT in 20% aqueous methanol

Analysis of dosage form

Twenty RMT tablets (Ramitax^{TM}, 8 mg, Batch No. 2497822, Mfd. 03/2013, Exp. 02/2015, Ranbaxy Laboratories Limited, New

concentration (LRE method); while the absorbance was observed in derivative mode at 295.50 nm for ^{1}D method determination.

Absorbance observed in LRE method was also used for SA method.

RESULTS AND DISCUSSION

Method development

Methanol and acetonitrile are the solvents for the method development as RMT is soluble in these solvents while insoluble in water. Methanol was chosen for analysis due to cost effectiveness over acetonitrile. Different concentrations of methanol were used and optimized 20% aqueous methanol with appropriate shape of Gaussian UV spectra and reproducibility (Fig. 2). The absorbances of standard dilutions were used for linear regression equation; which was as: $Y = 0.106 x + 0.010$ with R^2 (correlation coefficient) = 0.999 (Table 1). This linear regression equation method may be applied for routine analysis. Standard absorptivity A (1%, 1cm) and molar extinction coefficient (ε) were calculated from absorbances of five serial dilutions in triplicates. These values were found to be A (1%, 1 cm) = 111.38 dl/g/cm; ε = 2871.43 per Mol/cm (Table 2) and used for determination of RMT by using single absorbance. To nullify the interference of the other analytes (degradation products and other impurities) in determination of RMT in different matrix, the first order derivative mode of spectrophotometric method was used.

All three methods were validated as per ICH guidelines to assure the reliability and reproducibility of the methods. The linearity for all three methods (LRE, SA and ^{1}D) over the concentration 10-50 μg/mL was assured as 100.03%, 99.89% and 99.85% respectively with acceptable standard deviation (Table 4). The recovery method was adopted to assure accuracy of the methods which were found to be 99.95%, 100.93% and 100.54% respectively. Repeatability and intermediate precision were used to study of precision for the developed methods. The repeatability was found to be in between 99.98 - 100.22%, while the inter-day and analysts-to-analysts precision was assured in between 99.89 - 100.95%. The temperature variation (25, 30 and 35°C) was studied on the determination of RMT; the all methods have been proved to be robust with acceptable limits of the standard deviation. The developed methods were reproducible in the limit of acceptability as the percent relative standard deviations (% RSD) for all validation parameters were found to be far away from unit two.

Dosage form analysis

Validated all three methods were applied for the determination of drug content in tablet dosage form, where the drug was assayed at three levels vis. 20, 30 and 40 μg/mL in six batches. The drug content was determined in between 99.70 – 100.43% by all the methods with 0.38 – 0.70 standard deviation (Table 5). From the one way ANOVA (analysis of variance) analysis at concentration 20, 30 and 40 μg/mL, the calculated F values (0.551, 0.149 and 0.545) were less than tabulated F

Table 1. Calibration graph of RMT in 20% aqueous methanol

Conc. (μg/mL)	Absorbance at 286 nm					
	I	**II**	**III**	**IV**	**V**	**VI**
10	0.116	0.115	0.115	0.117	0.116	0.117
20	0.228	0.224	0.229	0.224	0.229	0.225
30	0.327	0.331	0.329	0.328	0.332	0.326
40	0.436	0.433	0.433	0.438	0.437	0.435
50	0.549	0.546	0.545	0.542	0.542	0.548
Regression Equation* $Y = 0.106 x + 0.010$; $R^2 = 0.999$						

* mean of above six replicates

Method validation

for 2 degree of freedom for numerator and 15 degree of freedom denominator at 5% level 3.682. The F ratio values have proved that no significant difference between three methods at different concentrations. Thus, all the methods are almost equally applicable with accuracy, precision and reproducibility.

CONCLUSION

Three validated spectrophotometric methods vis.linear regression equation (LRE), standard absortivity (SA) and first order derivative

Table 2. Standard absorptivity A (1%, 1cm) and molar extinction coefficient (ε)

Conc. (µg/mL)	Absorbance at 286 nm			Standard Absorptivity (A (1%, 1cm) = A/bc)		
	I	II	III	I	II	III
10	0.116	0.115	0.117	116.00	115.00	117.00
20	0.228	0.229	0.225	114.00	114.50	112.50
30	0.327	0.329	0.326	109.00	109.67	108.67
40	0.436	0.433	0.435	109.00	108.25	108.75
50	0.549	0.545	0.548	109.80	109.00	109.60
A (1%, 1 cm)* = 111.38 dl/g/cm; ε ** = 2871.43 per Mol/cm						

* Mean of 15 above standard absorbtivities determination

**Molar extinction coefficient ε = A (1%, 1cm) x Molecular weight/10.

The UV spectra were derivatized (where, $\Delta\lambda = 1$), where four negative peaks were observed but the absorbance at 295.50 nm was reproduced. The regression equation for the first order derivative method was found to be $Y = 0.013 x + 0.001$; $R^2 = 0.999$ (Table 3).

Table 3. Calibration graph of RMT in 20% aqueous methanol for first derivative method

Conc. (µg/mL)	Absorbance* at 295.50 nm in first order derivative mode					
	I	II	III	IV	V	VI
10	0.014	0.012	0.013	0.014	0.015	0.014
20	0.028	0.031	0.027	0.029	0.027	0.029
30	0.045	0.042	0.041	0.041	0.039	0.041
40	0.054	0.055	0.053	0.055	0.052	0.053
50	0.068	0.066	0.067	0.071	0.066	0.069
Regression Equation $Y = 0.013 x + 0.001$; $R^2 = 0.999$						

* All absorbance are in negative value

Table 4. Results of validation parameters for all three methods

Validation parameter	% Found (mean)* ± SD		
	LRE method	SA method	^{1}D method
Linearity	100.03 ± 0.032	99.89 ± 0.054	99.85 ± 0.047
Accuracy	99.95 ± 0.034	100.93 ± 0.039	100.54 ± 0.073
Precision			
I. Repeatability	100.11 ± 0.012	100.22 ± 0.061	99.98 ± 0.043
II. Intermediate precision			
a. Days	100.65 ± 0.058	99.99 ± 0.028	99.95 ± 0.082
b. Analysts	99.89 ± 0.099	100.95 ± 0.085	100.76 ± 0.083
Robustness			
a.35°C	100.54 ± 0.023	100.61 ± 0.078	100.53 ± 0.059
b. 30°C	99.90 ± 0.094	99.94 ± 0.087	100.06 ± 0.092
c. 25°C	100.66 ± 0.082	100.96 ± 0.079	99.93 ± 0.078

* mean of six dilutions in three replicates, SD = standard deviation

Table 5. Analysis of RMT in tablets

Batch ↓	Determined % of drug content by validated methods								
	LRE method			SA method			^{1}D method		
Conc. (µg/mL) →	20	30	40	20	30	40	20	30	40
I	100.89	100.58	99.88	100.65	99.84	100.11	100.12	100.78	99.92
II	99.89	100.29	100.98	99.89	100.67	100.01	100.95	99.39	100.91
III	100.57	99.98	100.87	99.99	99.87	99.91	99.92	100.94	99.89
IV	100.56	100.76	99.49	100.82	100.72	99.09	99.43	99.67	100.96
V	99.87	99.78	100.29	100.93	100.54	100.03	100.49	100.92	99.62
VI	100.79	100.56	99.93	99.82	99.23	99.07	99.93	99.83	99.96
Mean	100.43	100.33	100.24	100.35	100.15	99.70	100.14	100.26	100.21
SD	0.44	0.38	0.59	0.50	0.59	0.49	0.52	0.70	0.57
Conc. level (µg/mL)		20			30			40	
F value for ANOVA		0.551			0.149			0.545	

(^{1}D) method were successfully applied for the determination of RMT in tablet dosage form. In comparison of chromatography and electrochemistry, the developed methods are fast, cost-effective with acceptable accuracy and precision. These methods may be useful for analysis of RMT in bulk drugs, different dosage forms, dissolution studies, bioequivalence studies, degradation studies and in routine pharmaceutical industries.

ACKNOWLEDGEMENTS

One of the authors, Pawan Kumar Basniwal, is earnestly indebted to the Science and Engineering Research Board (SERB), DST, New Delhi, for the financial support for this research work under Fast Track Scheme for Young Scientists. The authors are highly thankful to the Head of the School of Pharmaceutical Sciences, RGPV, Bhopal, and the Principal of the LBS College of Pharmacy, Jaipur, for providing the experimental facilities for this research work.

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Received: 26.12.2013
Accepted: 20.02.2014