World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 6, Issue 8, 2017 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TASIMELTEON BY RP-HPLC METHOD

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Received on: 19-07-2017; Revised and Accepted on: 11-08-2017

ABSTRACT

A new method was established for estimation of Tasimelteon by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tasimelteon by using Kromosil C_{18} 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.482 mins. The % purity of Tasimelteon was found to be 99.87%. The system suitability parameters for Tasimelteon such as theoretical plates and tailing factor were found to be4146, 1.23, the. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Tasimelteonwas found in concentration range of 30µg-150µgand correlation coefficient (r^2) was found to be 0.997, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilty.LOD value was 2.97 and LOQ value was 9.92.Hence the suggested RP-HPLC method can be used for routine analysis of Tasimelteon in API and Pharmaceutical dosage form.

KEYWORDS: *Kromosil C_{18}, Tasimelteon, RP-HPLC.*

INTRODUCTION

The chromatographywas discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma, and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin [1].

"*Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient [2].

"In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)" [3].

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Chromatographic Process: [4]

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

Types of Chromatography:

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also

called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography(CEC) [7-9].

Methods in Chromatography: [5]

1. According to nature of stationary and mobile phase:

- Solid- Liquid chromatography
- Liquid-Liquid chromatography
- Gas- Solid chromatography
- Gas -Liquid chromatography

2. According to principle of separation:

A. Adsorption chromatography:

- Gas Solid chromatography
- Thin layer chromatography
- Column chromatography
- High performance liquid chromatography
- Affinity phase chromatography
- Hydrophobic Interaction chromatography (HIC)

B. Partition chromatography:

- Gas liquid chromatography
- Paper partition chromatography
- Column partition chromatography

3. Based on modes of chromatography:

- Normal phase chromatography
- Reversed phase chromatography

4. Other types of chromatography:

- Size exclusion chromatography (SEC)
- Gel permeation chromatography
- Gel chromatography
- Gel Filtration
- Gel permeation chromatography
- Ion exchange chromatography
- Chiral chromatography

High Performance Liquid Chromatography (HPLC): [6]

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals [10-12].

MATERIALS AND METHODS

Materials:

Ortho phosphoric acid, Acetonitrile for HPLC, Tasimelteon KH_2PO_4, K_2HPO_4, Water and Methanol for HPLC.

Methodology:

HPLC Method Development:

The chromatographic method development for the estimation of Tasimelteon was optimized by several trials for various parameters as different column, flow rate and mobile phase; finally the following chromatographic method was selected for the separation and quantification of Tasimelteon in API and pharmaceutical dosage form by RP-HPLC method.

Table No. 1: Optimized chromatographic conditions for simultaneous estimations of Tasimelteon by RP-HPLC method

Column	Thermosil C_{18} 4.5×150 mm 5.0 µm
Column temperature	Ambient
Wavelength	265 nm
Mobile phase ratio	65:35% v/v methanol: water
Flow rate	0.8 min/ml
Auto sampler temperature	Ambient
Injection volume	20µl
Run time	6 minutes

Method Validation:

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity:

10 mg of Tasimelteon working standard was accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Accuracy:

10 mg of Tasimelteon standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.9 ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluents.

Precision:

Repeatability: 10 mg Tasimelteon working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 0.9 ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Intermediate Precision / Ruggedness:

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Limit of Detection (LOD):

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Limit of Quantification:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.8 ml/min to 1.2 ml/min. Standard solution 90 ppm of Tasimelteon prepared and analysed using the varied flow rates along with method flow rate.
b) The organic composition in the mobile phase was varied from 75% to 55% standard solution 90 µg/ml of Tasimelteon was prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

System Suitability:

10 mg of Tasimelteon standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 7 ml of

diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.9 ml Tasimelteon the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

RESULTS

Optimized chromatographic conditions for estimations of Tasimelteon by RP-HPLC method:

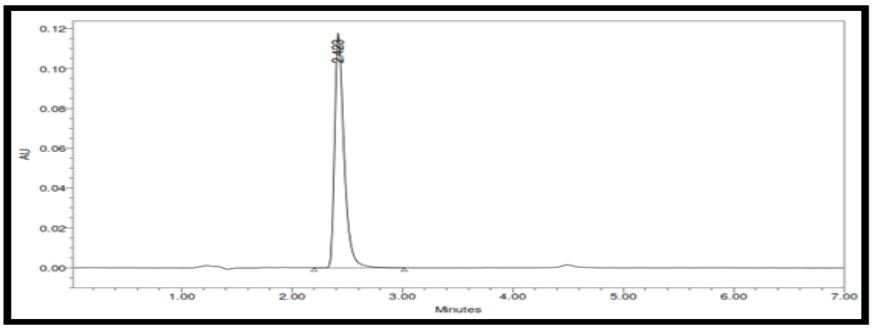

Fig. 1: Chromatogram showing trial injection

Observation: The separation was good; peak shape was good, still more trials were required to reduce the retention times of peaks

Validation Parameters:
Linearity:

Table No. 2: Showing the results for the linearity

Name	Rt	Area
Tasimelteon	**2.428**	**1608152**
Tasimelteon	**2.422**	**2592905**
Tasimelteon	**2.430**	**3778327**
Tasimelteon	**2.426**	**5170038**
Tasimelteon	**2.433**	**6249400**
Co efficient of correlation (R^2)		**0.997**

Tasimelteonr2= 0.997

Accuracy:

Table No. 3: Showing results for accuracy 50% of Tasimelteon

S. No.	Peak Name	RT	Area
1	**Tasimelteon**	2.431	1046104
2	**Tasimelteon**	2.429	1049450
3	**Tasimelteon**	2.430	1049306
	Mean		1048287
	Std. Dev.		1891.6
	% RSD		0.18

Table No. 4: Showing results for accuracy 100% of Tasimelteon

S. No.	Peak Name	RT	Area
1	**Tasimelteon**	2.433	1376694
2	**Tasimelteon**	2.433	1377029
3	**Tasimelteon**	2.436	1380876
	Mean		1378200
	Std. Dev.		2324.1
	% RSD		0.17

Table No. 5: Showing results for accuracy 150% of Tasimelteon

S. No.	Peak Name	RT	Area
1	**Tasimelteon**	2.439	1714604
2	**Tasimelteon**	2.429	1714196
3	**Tasimelteon**	2.441	1717641
	Mean		1715480
	Std. Dev.		1882.1
	% RSD		0.11

Table No. 6: Showing accuracy results for Tasimelteon

%Conc. (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1048287	5	5.14	100.2%	100.4%
100%	1378200	10	10.01	98.8%	
150%	1715480	15	15.2	96.5%	

Precision:

Table No. 7: Showing the results for precision

S. No.	Peak Name	RT	Area
1	**Tasimelteon**	2.423	693078
2	**Tasimelteon**	2.424	693338
3	**Tasimelteon**	2.424	695080
4	**Tasimelteon**	2.424	694843
5	**Tasimelteon**	2.423	695336
	Mean		694335
	Std. Dev.		1047.5
	% RSD		0.15

The Method precision study was performed for the %RSD of Tasimelteon was found to be 0.5 and (NMT 2).

Ruggedness (Intermediate Precision):

Table No. 8: Showing the results for id precision

S. No.	Peak Name	RT	Area
1	**Tasimelteon**	2.423	693877
2	**Tasimelteon**	2.424	696531
3	**Tasimelteon**	2.424	693977
4	**Tasimelteon**	2.424	695278
5	**Tasimelteon**	2.423	697676
	Mean		695468
	Std. Dev.		1642.7
	% RSD		0.24

Variation in Flow:

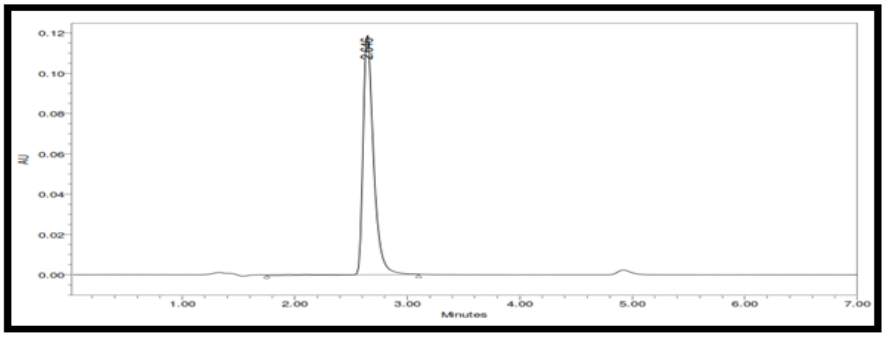

Fig. 2: Chromatogram showing less flow rate 0.8 ml/min

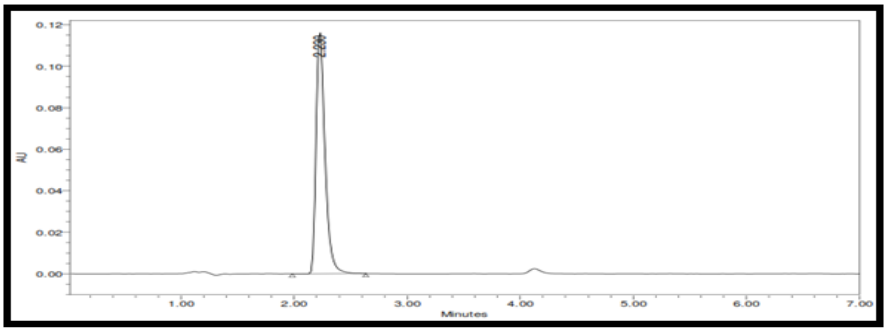

Fig.3 Chromatogram showing more flow rate 1.2 ml/min

Table No. 9: Showing system suitability results for Tasimelteon

S. No.	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	4352	1.1
2	**1**	**4024**	**1.2**
3	1.2	3730	1.2

Table No. 10: Showing system suitability results for Tasimelteon

S. No.	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	10 % less	4331	1.20
2	***Actual**	**4024**	**0.87**
3	10% more	3693	1.26

SUMMARY AND CONCLUSION

A new method was established for estimation of Tasimelteon by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tasimelteon by using Kromosil C_{18}4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.482 mins. The % purity of Tasimelteon was found to be 99.87%.The system suitability parameters for Tasimelteon such as theoretical plates and tailing factor were found to be4146, 1.23, the. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Tasimelteonwas found in concentration range of 30µg-150µgand correlation coefficient (r^2) was found to be 0.997, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilty.LOD value was 2.97 and LOQ value was 9.92.Hence the suggested RP-HPLC method can be used for routine analysis of Tasimelteon in API and Pharmaceutical dosage form.

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How to cite this article:

Gummadi Sridhar Babu, P.S. Malathi. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TASIMELTEON BY RP-HPLC METHOD. J Pharm Res 2017;6(8):130-134.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil