WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 14, 1229-1241. Review Article ISSN 2277–7105

A NEW METHOD DEVELOPMENT AND VALIDATION OF MIRABEGRON IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

Vidya H. Barhate*, Aishwarya S. Bhandari, H. V. Kamble and S. A. Waghmare

Student Takali haji, tal-shirur, Dist-Pune Shirur Maharashtra India.

Article Received on
04 June 2024,

Revised on 24 June 2024,
Accepted on 14 July 2024

DOI: 10.20959/wjpr202414-33290

***Corresponding Author**
Vidya H. Barhate
Student Takali haji, tal-shirur, Dist-Pune Shirur Maharashtra India.

INTRODUCTION

In general, the drugs may be new or partially modified in structure of the existing ones with combinations releasing into the market annually. Frequently, from its introduction into the market to the inclusion of pharmacopoeias, it is being delayed, so there is a lack of analytical methods for these drugs, and for such drugs, this can lead to the development of newer analytical methods.

Chromatography- An overview

To resolve a multi-component mixture into its individual components, chromatography, is a new, well-known and a primary tool of separation and it can be applied both quantitatively and qualitatively. Despite, some other methods like IR spectroscopy, Nuclear Magnetic resonance spectroscopy or Mass spectroscopy etc. are required for the final identification and confirmation.

Tswett. M, in 1806, in Warsaw, invented a new technique, while separating the plant pigments by a column of calcium carbonate, which acted as an adsorbent and the different substances get adsorbed to different extent and this give rise to the different coloured bands, at different positions on the column. In Greek, chroma means colour and graphos means writing. Hence, he termed the system of coloured bands as the chromatogram and the method as chromatography.

High Performance Liquid Chromatography (HPLC)

HPLC is used to figure out the amount of specific compound in a solution. It supports reliable quantitative range to allow the determination of substances in a single run. This method is

considered to be rapid, accurate, precise and specific and offers the ease of automation. It is because methods using HPLC have more advantages over the conventional methods.

Principle of HPLC

A mixture of sample is dissociated into components for its identification, quantification and purification by HPLC, due to the differences in their relative affinities for the mobile phase and stationary phase used. Especially, RP-HPLC, relies on the principle of hydropohobic interactions, as the more non-polar the material is, longer it will be retained. Due to their low affinities and polar nature, most of the drugs elute at a faster rate through the column and so they are separated and detected easily.

Types of HPLC

There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. **Normal Phase HPLC**
2. **Reverse Phase HPLC**
3. **Size-exclusion HPLC**
4. **Ion-Exchange HPLC**

Application of HPLC

The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

- **Pharmaceutical applications**

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

- **Environmental applications**

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

- **Applications in forensics**

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.

3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

- **Food and Flavour**

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

- **Applications in clinical tests**

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

UV-Vis spectroscopy

UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration. Since this spectroscopy technique relies on the use of light, let's first consider the properties of light.

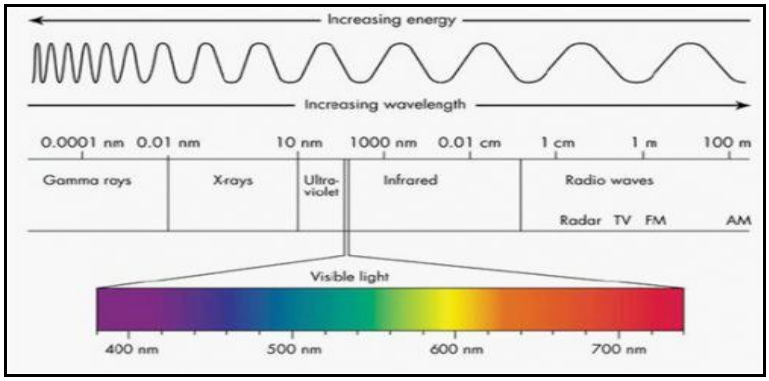

Figure 1.1: UV Radiations.

Origen and Characteristics of UV-Visible Spectrum

UV-VIS spectrum results from the interaction of electromagnetic radiation in the UV-Visible region with molecules, ions or complexes. It forms the basis of analysis of different substances such as, inorganic, organic and biomolecules. These determinations find

applications in research, industry, clinical laboratories and in the chemical analysis of environmental samples. It is therefore important to learn about the origin of the UV-VIS spectrum and its characteristics.

Radiation and Energy

Radiation is a form of transmitted energy Electromagnetic radiation is so-named because it has electric and magnetic fields that simultaneously oscillate in planes mutually perpendicular to each other and to the direction of propagation through space Electromagnetic radiation has the dual nature: its exhibits wave properties and particulate properties.

The nature of light

Light is a form of energy. Energy can be transferred from one point to another point either by particle motion or by wave motion. Accordingly, different theories on the nature of light have been proposed. The important theories are as follows: -

1. Particular like theory.
2. Electromagnetic wave theory.

Method development of drugs

The analyte is enough to know the information of the compound. One can select the most suitable HPLC method development by the physical and chemical characteristics and by a vast literature review. Information regarding the sample can be achieved by molecular weight, structure, functionality, Pka value, UV-spectra and solubility of the compound. By knowing whether the pure compound is organic soluble or water soluble, one can select the best mobile phase and column for HPLC method development. In many laboratories, typical detectors like Mass spectroscopy, UV-Visible detectors are used as they can detect a wide variety of compounds.

Analytical methods are intended to establish the identity, purity, physical characteristics and potency of the drugs. To support drug testing against specifications that arise during manufacturing and quality release operators, as well as during long-term stability studies and for safety and characterization studies or evolution of drug performance, methods are developed. upon column efficiency, selectivity and retention time. For chromatographic separation, mobile phase composition or strength plays a vital role.

Method validation of drugs

According to ISO definition, validation is defined as "Verification, where the specified requirements are adequate for an intended use." Method validation can be used for qualitative, semi-quantitative or quantitative methods.

The scientific soundness of the measurement or characterization and also to varying extents throughout the regulatory submission process, the validation of analytical method is required. The effort done in method development and optimisation leads to the effective development of HPLC method and its final performance. For the method development of samples in chromatographic separation, method validation is very important.

Method validation

This process consists of establishments of the performance characteristics and the limitation of the method.

Method performance parameters are determined using equipment that is

1. Within specification
2. Working correctly
3. Adequately calibrated

Method validation is required when

1. A new method is being developed
2. Revision of the established method
3. When established method are used in different laboratories and by different analysts etc.
4. Comparison of method
5. When quality control indicates method changes

Performance characteristics examined when carrying out method validation are

1. Accuracy
2. Precision
3. Specificity
4. Selectivity
5. Sensitivity
6. Limit of detection.
7. Limit of quantification

8. Linearity and Range
9. Ruggedness
10. Robustness
11. System suitability

1. Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentrations levels the specified range (i.e., three concentrations and three replicates of each concentration). Accuracy was tested (% Recovery and % RSD of individual measurements) by analysing samples at least in triplicate, at each level (80,100 and 120 % of label claim) is recommended. For each determination fresh samples were prepared and assay value is calculated.

2. Precision

The precision of an analytical procedure expresses the closeness of agreement between series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variations of a series of measurements.

$$Stanadard\ Deviation = \sqrt{\frac{\sum(x-\overline{x})^2}{n-1}}$$

Where,

$x = sample,$

$\bar{x} = mean\ value\ of\ samples,$

$n = number\ of\ samples$

$$Relative\ Standard\ Deviation = \frac{Standard\ Deviation}{\overline{x}} \times 100$$

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra –assay precision.

Intermediate precision

Intermediate precision expresses with in laboratories variations: different days, different analyst and different equipment.

Reproducibility

When the procedure is carried out by different analyst in different laboratories using different equipment, regents and laboratories setting. Reproducibility was determined by measuring repeatability and intermediate precision. Reproducibility is assessed by means of an inter-laboratory trial.

3. Specificity

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities. An ICH document defines specificity as the ability to assess unequivocally the analyte in the presence compounds that may be expected to products and matrix components. The definition has the following implications:

Identification test

Suitable identification tests should be able to discriminate compounds of closely related structure which are likely to be present. Ensure identity of an analyte, the analyte should have no interference from other extraneous components and be well resolved from them.

Purity test

To ensure that all the analytical procedures performed allow an accurate statement of the content of impurity of the content of impurity of an analyte i.e. related substances test, heavy metals, residual solvents etc.

Assay

To provide an exact result, this allows an accurate statement on the content or potency of the analyte in a sample.

4. Selectivity

It is a procedure to detect qualitatively the analyte in the presence of compounds that may be expected to be present in the sample matrix or the ability of a separative method to resolve different compounds. It is the measure of the relative method location or two peaks.

Determination of selectivity: Selectivity is determined be comparing the test results obtained on the analyte with or without addition of potentially interfering material. When

such components are either unidentified or unavailable a measure of selectivity can be obtained by determining the recovery of a standard addition of pure analyte to a material containing a constant level of the other compounds.

5. Sensitivity

Sensitivity is the capacity of the test procedure to record small variation in concentration. It is the slope of the calibration curve.

6. Limit of detection (LOD)

It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities as an exact value, under the stated experimental conditions. The detection limit is usually expressed as the concentration of analyte (percentage parts per million) in the sample.

Determination of detection limit

For instrumental and non- instrumental methods detection limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$LOD = \frac{3.3 \times Standard\ Deviation}{Slope}$$

7. Limit of quantification (LOQ)

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Quantification limit is expressed as the concentration of analyte (e.g: % ppm) in the sample.

8. Linearity and Range

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to concentration of analyte in samples. The range of an analytical is the intervals between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision accuracy and linearity.

9. Ruggedness

Degree of reproducibility of test results obtained by the analysis of the same samples under a variety of condition such as different laboratories, different analysts, different instruments

etc., normally expressed as the lack of influence on test results of operational and environmental variable of the analytical method.

10. Robustness

Robustness of an analytical method is measure of its capacity to remain unaffected small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Testing varying some or all condition:

- Solvent
- Column temperature
- pH of buffer in mobile phase
- Flow rate
- Wave length

AIM

A New Simple Method Development and Validation of Mirabegron in Bulk and Pharmaceutical Dosage Form by RP-HPLC.

OBJECTIVE

Literature Survey.

Selection of Drug.

Selection of Method and Instrument.

To developed sensitive, rapid, accurate, precise and desirable method for estimation of drug by RP-HPLC method.

To develop RP-HPLC method for Mirabegron.

To validate Novel Qualitative and Quantitative Analytical method development for estimation of Mirabegron in bulk and pharmaceutical formulation as per ICH guidelines.

Statistical analysis of the recovery data obtained from different techniques for Mirabegron.

MATERIAL

Details of marketed formulation

(50mg)Mirabegron 50 Mg Tablets IPCA Pharmaceuticals Ltd. Mumbai

Details of standard drug and manufacturer

1 Mirabegron Standard (98.94%)

List of Chemicals use in Research work

Sr. No. Chemicals Grade

1 Methanol HPLC

2 Disodium Hydrogen Phosphate GR

3 Potassium Dihydrogen Phosphate GR

14 Sodium Chloride GR

5 Ortho phosphoric acid GR

6 Hydrochloric acid GR

7 Sodium hydroxide GR

8 Hydrogen peroxide GR

9 Double distilled water GR

Method development

Chromatographic conditions

During mobile phase optimization Methanol: Acetonitrile: Potassium Dihydrogen Phosphate (pH adjusted to 6.0 with orthophosphoric acid) in the ratio of 30: 30: 40 was found to be satisfactory for Mirabegron. The low-pressure gradient mode at a flow rate of 1.0ml/min at ambient temperature was used. The mobile phase was degassed and filtered through membrane filter (0.22µ). The injection volume was 10 µL and the run time for the analysis was 10 mins. The detection was carried out at 252 nm using UV detector. Water: ACN (50:50) was used as a diluent in the study.

Preparation of standard stock solution

Accurately weighed quantity of Mirabegron was transferred into two separate 100ml volumetric flask containing 60ml Diluent. The content was dissolved by sonication for 5 mins. The volume was made up to the mark with methanol. It was further diluted appropriately using diluent to get desired concentrations.

Preparation of internal standard solution

Accurately weighed quantity of Mirabegron (100 mg) was transferred to 100ml of volumetric flask containing 30ml methanol. The content was dissolved by sonication for 10mins. The volume was made up to 100ml with water and aliquot was further appropriately diluted using diluent to get a concentration of 10µg/ml.

Preparation of sample Solution and Assay of marketed formulation

Accurately weighed about 403mg of tablet powder (equivalent to 150mg of IRB and 12.5mg of HCTZ) was transferred to 100 ml of volumetric flask containing 60ml of methanol. The

sample was dissolved by sonication for 15 mins and volume was made up to the mark with methanol. The resulting solution was filtered using membrane filter 0.22µ. The filtrate was further diluted appropriately with diluent to get 36 µg/ml of IRB and 3 µg/ml of HCTZ. This diluted sample was then analyzed by HPLC.

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Mirabegron is official in Indian Pharmacopoeia.

CONCLUSION

A very few analytical methods appeared in the literature for the determination of Mirabegron includes HPLC, HPTLC and UV- Visible spectrophotometric methods. In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical. In the present investigation HPLC method (Using Quality by Design) for the quantitative estimation of Mirabegron in bulk drug and per ICH guidelines pharmaceutical formulations has been developed. HPLC methods were validated as and results of linearity, precision, accuracy, Specificity, System suitability and robustness pass the limit. The HPLC method is more sensitive, accurate and precise compared to the previously reported method. There was no any interference of excipients in the recovery study. The low value of %RSD, molar extinction coefficient (L mol-1 cm-1) suggested that the developed methods are sensitive. The proposed high-performance liquid chromatographic method has also been evaluated over the accuracy, precision and robustness and proved to be convenient and effective for the quality control of Mirabegron. Developed method was found simple and cost effective for the quality control of Mirabegron.

SUMMARY

The contents of the thesis have been divided into Nine chapters and appropriate references have been placed after the 9th chapter.

Spectrophotometric method was developed for the estimation of Mirabegron in Pharmaceutical Formulation.

Designed of Experiment.

Optimized and Developed method for Spectrophotometry.

Spectrophotometric method was validated for Linearity, Accuracy, Interday & Intraday Precision, Specificity & Selectivity, Sensitivity, Robustness.

Optimized and Developed method for Chromatography.
Chromatographic method was validated for Linearity, Accuracy, Interday & Intraday Precision, Specificity & Selectivity, Sensitivity, Robustness.

All the developed methods were successfully applied to determine the drugs in Pharmaceutical preparation.

REFERENCE

1. Kar A. Pharmaceutical Drug Analysis. New age international limited publishers, 2005; 2: 452-474.
2. Sethi P.D. Quantitative Analysis of drugs in Pharmaceutical Formulations. CBS publishers and distributors, New Delhi, 1997; 3: 17-19.
3. J. Bassett, R. C. Denney, G.H. Jeffery and J. Mendham. Vogel's Text Book of Quantitative Inorganic Analysis. Longman Group Ltd: England, 1978; 4: 1-9.
4. Masoom, R. Zeid, A. AlOthman, a. Nafisur, R. Analytical techniques in pharmaceutical analysis: A review, Arabian Journal of Chemistry, 2013; 1878- 5352.
5. Panchumarthy, R., Naga, N. Pravallika, D. Navya, S. A Review on Step-by-Step Analytical Method Validation, IOSR Journal of Pharmacy, 2015; 5(10): 07-19.
6. Skoog D., Holler, J., Crouch, S., Principles of Instrumental Analysis. 6th ed. Belmont, CA: Thomson Brooks/Cole, 2007; 169–173.
7. Mendham, J., Denney, R.C., Barnes, J.D., Thomas, M.J. Vogel's Textbook of Quantitative Chemical Analysis. Publication of Pearson Education, 6: 172- 173, 301-308.
8. Christian, G., Analytical Chemistry. Publication of John Wiley and Sons, 2003; 6: 1-15, 92-114, 126-132, 604-619.
9. Sharma, B.K., Instrumental Methods of Chemical Analysis. Goel Publication Co.; Meerut, 2002; 25: 3, 6.
10. Chatwal, G.R., Anand S.K., Instrumental Methods of Chemical Analysis. Himalaya Publishing House; Delhi, 2007; 5: pp.2.150, 2.566-2.585, 2.624-2.630.
11. Sharma, Y.R., Introduction of Organic Spectroscopy. CBS Publishers and Distributors; New Delhi, 1991; 4: 5-25.
12. Beckett A.H., Stenlake J.B., Practical Pharmaceutical Chemistry. Publication of New Delhi, 2004; 4, 2: 275-300.

13. Lampman, Pavia. Kriz, Vyvyan, Spectroscopy. Cengage Learning, 4: 379-406.
14. P. S. Kalsi., Spectroscopy of organic compounds. New age International publisher, 6: 9-54.
15. G. Vidya sagar. Instrumental method of drug analysis. Pharma med press editor, 2012; 106.
16. The Merck Index, Merck & Co. Inc. White house station. NJ, 2006; 14: 4781, 491, 4935, 6528.
17. Sethi. PD. HPLC Quantitative analysis of pharmaceutical formulations, CBS Publishers and distributors. New Delhi, 2001; 1: 94-105.
18. Glajch. L., Kirkal. J, and Snyder R., Practical HPLC method development. John Wiley and sons. INC. New York, 1999; 2: 234-260.
19. Sethi, P.D. HPLC- High Performance Liquid Chromatography- Quantitative Analysis of Pharmaceutical Formulations. 1st ed. CBS Publishers and Distributors. New Delhi, 2001; 3-72, 116-120.
20. E. Sacco, R. Bientinesi, Therapeutic advances in urology, 2012; 4: 315-324.
21. K. Konishi, D. Tenmizu, S. Takusagawa, European journal of drug metabolism and pharmacokinetics, 2017; 43: 1-9.
22. T. Takasu, M. Ukai, S. Sato, T. Matsui, I. Nagase, T. Maruyama, M. Sasamata, K. Miyata, H. Uchida, O. Yamaguchi, Journal of Pharmacology and Experimental Therapeutics, 2007; 321: 642-647.
23. C. Von Pirquet, Munchen Med Wchnschr, 1906; 53: 1457-1458.