ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Development and validation of analytical method by RP-HPLC estimation of Goserelin acetate in biodegradable microspheres

Suhas Marutirao Kakade*[1,2], Dehghan Mohamed Hassan[1]

[1]Department of Pharmaceutics, Y. B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad-431001, Maharashtra, India
[2]Wockhardt Research Centre, Aurangabad-431001, Maharashtra, India

Article History:

Received on: 21.03.2018
Revised on: 11.06.2018
Accepted on: 15.06.2018

Keywords:

RP-HPLC,
ICH,
Goserelin acetate,
Microspheres,
Validation

ABSTRACT

A simple, rapid, accurate and sensitive method was developed for quantitative analysis of Goserelin acetate in biodegradable microspheres formulation using reversed-phase high-performance liquid chromatography (RP-HPLC). The analysis is carried out using a reversed-phase C18 column with UV-Vis detection at 220nm. The isocratic mobile phase was phosphate buffer (pH7.4): acetonitrile in the ratio of 70:30 v/v at a flow rate of 1mL/min and column temperature maintained at 35°C. The developed method was validated according to the International Conference on Harmonisation (ICH) guideline with respect to system suitability, accuracy, precision, specificity, linearity and robustness. The linearity in the range of 2.5-90µg/mL presents a correlation of coefficient 0.999. The presence of components of the microspheres did not interfere in the results of the analysis. The method showed adequate precision, with a relative standard deviation (RSD) ≤ 2.0. The method was found to be suitable for routine quality control assay for encapsulated Goserelin acetate in biodegradable microspheres formulation was developed and validated.

* Corresponding Author

Name: Mr. Suhas Marutitao Kakade
Phone: +91-9028217999
Email: suhaspharma@yahoo.com

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v9i3.1544

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) analogues now offer the possibility of a medical castration, which reversible compared with the definitive castration produced by surgery or radiation. These analogues are a class of compound widely used in human medicine to treat prostate cancer, endometriosis and breast cancer. GnRH analogues are closely related to the naturally occurring decapeptide that is produced in the hypothalamus (Jean-Pierre G *et al.,* 1992).

The modification of the peptide at residues 6 and 10 leads to highly potent agonists with a marked and prolonged effect compared to the natural hormone (Bajusz S *et al.,* 1988).

Continuous administration of agonist analogues in supraphysiologic doses produces a decrease of pituitary luteinizing hormone-releasing factor (LHRH) receptors (receptor downregulation), which leads to inhibition of gonadotrophin synthesis and release and subsequently inhibits ovarian hormone production (Bambino TH *et al.,* 1980).

Goserelin acetate is a luteinizing hormone-releasing hormone agonist (LHRHa). It is a synthetic analogue of gonadotropin-releasing hormone or luteinizing hormone-releasing hormone. Initial or intermittent administration of Goserelin acetate stimulates the release of gonadotropins, pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH), from the anterior pituitary (Anderson J *et al.,* 2008, Miller K *et al.,* 2009 and Kaufmann M *et al.,* 1989).

Long term extended use of Goserelin acetate is associated with an early phase of increased LH and

FSH levels, followed by their suppression (Boccon-Gibod L *et al.,* 2011). Chemical structure of Goserelin acetate is shown in Figure 1.

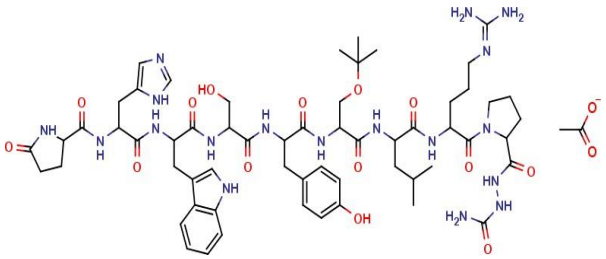

Figure 1: Schematic diagram shows the chemical structure of Goserelin acetate

Goserelin acetate biodegradable microspheres for subcutaneous administration for treating prostate and breast cancer effectively in order to improve patient compliance and convenience (Duque LF *et al.,* 2012).

The primary goal of this study was the development and validation of a simple and RP-HPLC method for estimation of this molecule from possible pharmaceutical dosage forms derived from microspheres systems. All of these present methods, however, are not employed for the determination of Goserelin acetate in a possible pharmaceutical dosage form and usually employed to quantify illegal substances in biological fluids.

In order to achieve this purpose, the analytical method proposed has been used for investigating drug loading and *in-vitro* release profiles of Goserelin acetate biodegradable microspheres formulation.

MATERIALS AND METHODS

Materials

Goserelin acetate was purchased from Hemmo Pharmaceutical Private Limited, Mumbai India. Acetonitrile (HPLC grade) was purchased from Merck (Germany). Potassium dihydrogen phosphate and Disodium hydrogen phosphate were purchased from Ranbaxy Fine Chemicals Ltd., India.

Instrumentation and chromatographic system

The Agilent 1200 series HPLC system used consisted of a quaternary pump (G1311A), autosampler (G1329A), solvent degasser (G1314B), was used for analysis. The results were analysed using LC-solution software. Analytical RP C18 column [octadecylsilane (ODS), 150×4.6mm, 5µ, Phenomenex Inc., Japan] was used for method development and its validation. The quantification wavelength of Goserelin acetate was set at 220nm.

Preparation of mobile phase

Accurately weighed and transferred 13.8g of Sodium dihydrogen phosphate monohydrate, 26.8g Di-sodium hydrogen phosphate heptahydrate and 8g Sodium chloride to a 1000mL volumetric flask, 300mL water was added, and the volume was made up to 1000mL with water. The pH was adjusted to 7.4. Buffer and acetonitrile were mixed in the ratio of 70:30 v/v. The solution was filtered through a 0.45µm membrane filter and degassed.

Preparation of standard solution

Accurately weigh and transfer 10mg of Goserelin acetate working standard into a 100mL volumetric flask add about 30mL of acetonitrile and sonicate to dissolve it completely and make the volume up to the mark with the phosphate buffer. Further pipette 3mL of the above stock solution into a 10mL volumetric flask and dilute up to the mark with phosphate buffer.

Sample preparation

Accurately weigh appropriate amount of the microspheres were completely dissolved in acetonitrile and suitably diluted with phosphate buffer pH7.4 (acetonitrile: phosphate buffer 30:70v/v). The samples were filtered through 0.2µm PTFE microfilter before analysis using a corrective and validated RP-HPLC method. The concentration of the Goserelin acetate was calculated based on a linear regression equation of the calibration curve.

Table 1: Typical parameters verified in method validation

Sr. No.	Validation Parameter
1	Accuracy
2	Precision
3	Specificity
4	Detection Limit
5	Quantitation Limit
6	Linearity
7	Range
8	Robustness

Method validation

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements of the intended analytical applications. Typical parameters verified invalidation of analytical method are listed in Table 1 (Central for Drug Evaluation and Research 1994, John H 2006, Joseph S *et al.,* 2012, Ahuja S *et al.,* 2007, and Lister AS *et al.,* 2005)/ ICH Q2 (R1) is considered the primary reference for recommendations and definitions on validations characteristics for analytical procedures (ICH-Q2A 1995, ICH Q2 R1, 2005and CH-Q2B, 1996).

System suitability

System suitability test to ensure adequate performance of the chromatographic system. Parameters

including retention time (RT), a number of theoretical plates (N) and tailing factor (T) were evaluated for replicate injections of the sample solution. To determine precision system Goserelin acetate standard solution was prepared and injected six times into the HPLC system. The mean, SD and %RSD for peak areas of Goserelin acetate were calculated.

Accuracy

Accuracy was determined by the standard addition method. The recovery studies were conducted at three different concentration of standard Goserelin acetate added to the sample solution with a known content of Goserelin acetate. The samples were prepared according to the "sample preparation", and the Goserelin acetate was spiked with 10, 20 and 30mg of the reference standard. The recovery experiments were performed in triplicate for each concentration, and the accuracy of the method was expressed as the per cent recovery and grand mean recovery. The accuracy of an analytical procedure is the closeness of the test results obtained by that procedure to the true value and found value, was evaluated as %bias for Goserelin acetate according to the following equation:

Precision

The precision of an analytical procedure is the closeness of agreement among individual test results when the procedure is repeatedly applied to multiple samplings of the same homogenous sample. It is usually expressed as the standard deviation or coefficient of variation of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical procedure under normal operating conditions. Reproducibility expresses the precision between different laboratories, as in a collaborative study. Intermediate precision expresses within-laboratory variation, as on different days, different analysts, different equipment etc. Repeatability expresses the precision under the same operating condition within a laboratory over a short period. The precision of the assay method was assessed with respect to repeatability and reproducibility. The precision of the proposed method was checked by intra and inter day repeatability of responses after replicating injections and expressed as %RSD among responses using the formula.

Specificity

The specificity is the ability to assess the analyte unequivocally in the presence of components which may be expected to be present, such as impurities, degradation products and matrix components. The placebo solutions containing excipients without Goserelin acetate were prepared. To evaluate the specificity of the method blank, placebo and test solution were injected.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected but not necessarily quantitated under the stated experimental conditions. The lower limit of quantitation (LOQ) is the lowest amount of analyte in the sample that may be measured with precision and accuracy. LOD and LOQ were determined from the calibration curve. The LOD (k=3.3) and LOQ (k=10) of the proposed method were calculated using the following equation (Holgado MA *et al.,* 2009):

$$A=k\sigma/S$$

Where A is LOD or LOQ, σ is the standard deviation of the response, and S is the slope of the calibration curve.

Linearity

The calibration curves were constructed with different concentrations of Goserelin acetate ranging from 2.5 to 90µg/mL. The calibration curves were constructed by plotting the ratio of the mean peak area of either Goserelin acetate versus the concentration. The linearity was assessed by linear regression analysis, which was calculated by the least square method (Holgado MA *et al.,* 2009 & Neves JD *et al.,* 2010).

Robustness

Robustness is an indication of the reliability of the analytical method during normal usage. The effect of the following deliberate changes in chromatographic conditions was monitored: flow rate±10%, the ratio in the mobile phase to ±2mL, temperature±5°C and detector wavelength±2 (Holgado MA *et al.,* 2009 & Neves JD *et al.,* 2010).

RESULTS AND DISCUSSION

Method Validation

System suitability

The results (Mean±%RSD of six replicates) of the chromatographic parameters are shown in Table 2, indicating the excellent performance of the system.

Table 2: Evaluation of the accuracy of the proposed method for quantitation of Goserelin acetate

The amount present in the MS formula (µg/mL)	The added amount of Reference standard Goserelin acetate (µg)	Detected amount (µg/mL)	Per cent Recovery (%)	% RSD
29.7	10	39.93	100.57	0.08
	20	50.66	101.93	0.14
	30	61.65	100.26	0.06
Grand mean of Recovery (%)			100.92	

Table 3: Intraday and interday precision of HPLC method

Sr. No.	Concentration (µg/mL)	Standard solution (% of the amount found)		Sample solution (% of the amount found)	
		Intraday precision	Interday precision	Intraday precision	Interday precision
1	30.0	100.3	100.1	100.2	100.1
2	30.0	99.99	100.2	100.1	100.1
3	30.0	100.1	100.1	99.98	100.1
4	30.0	99.99	100.1	100.2	100.2
5	30.0	100.1	100.2	100.3	100.1
6	30.0	100.2	100.1	101.1	100.1
Mean		100.1	100.1	100.3	100.1
SD		0.110	0.047	0.365	0.037
%RSD		0.11	0.05	0.36	0.04

Table 4: Robustness of the method

Parameter		Theoretical Amount of Goserelin acetate (µg)	Amount of Goserelin acetate detected (Mean ± SD)*	%RSD
Change in mobile phase composition	Buffer: Acetonitrile 68: 32 v/v	30	30.09±0.07	0.22
	Buffer : Acetonitrile 70: 30 v/v	30	30.04±0.03	0.12
	Buffer : Acetonitrile 72 : 28 v/v	30	30.08±0.03	0.10
Change in column temperature	25°C	30	30.07±0.08	0.26
	30°C	30	30.04±0.05	0.16
	35°C	30	30.08±0.07	0.22
Change in flow rate	0.9 mL/min	30	30.13±0.14	0.45
	1.0 mL/min	30	30.04±0.04	0.15
	1.1 mL/min	30	30.08±30.07	0.22

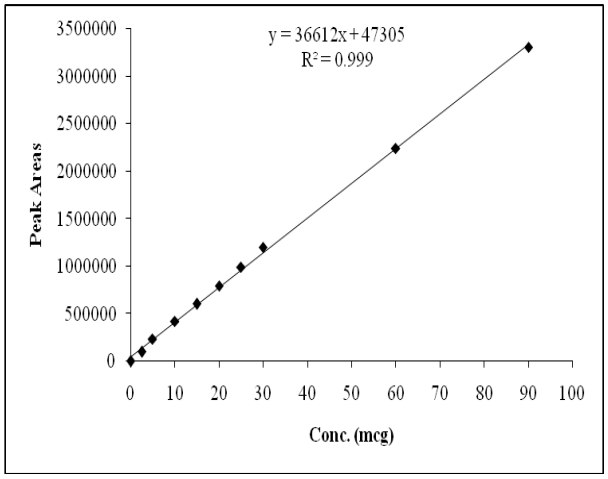

Figure 2: Linearity plot Goserelin acetate

Table 5: Chromatographic characteristics of system suitability solution

Parameter	Value (Mean±%RSD)*
Tailing factor	1.09±0.32
Theoretical plate	10684±131
Retention time	13.94±0.05

*Mean and % Relative Standard Deviation of six replicates

Linearity

The linearity of the method was found to be linear over a wide concentration range of 2.5-90µg/mL with a regression equation of "Y=36612X+47305" that showed a good correlation coefficient of 0.999 (figure 2). Besides, we had also plotted a lower

range concentration of 0.2-0.8µg/mL with a regression equation of "Y=39432X+13182" and found the excellent correlation coefficient of 0.999.

Accuracy

The Goserelin acetate content from biodegradable based microspheres was confirmed by spiking their peaks areas with three concentration levels of standard Goserelin acetate. The percentage of recovery was found to be within acceptable limit between 100.26% to 101.93% with a relative standard deviation of less than 0.2% as illustrated in Table 3 which implies high accuracy of the method.

$$\% \text{ Accuracy} = \frac{\text{Observed Concentration}}{\text{Nominal Concentration}}$$

Precision

The sample solution of Goserelin acetate microsphere was prepared and injected six times into the HPLC system. The mean, SD and % RSD for assay of Goserelin acetate was calculated. The % RSD for assay of Goserelin acetate peak from six replicate injections of standard solution was less than2%. Results for intraday and interday precision for quantification of Goserelin acetate in Goserelin acetate microspheres are shown in Table 4.

$$\% \text{ RSD} = \frac{\text{Standard deviation} \times \text{Mean}}{100}$$

Specificity

The specificity test demonstrated that the used biodegradable polymer and excipients did not interfere with the main peak of the Goserelin acetate. Thus, the HPLC method is used to quantify Goserelin acetate in the developed formulations. There was no interference between the peaks of Goserelin acetate as shown in figure 3. No peak was eluted at the retention time of Goserelin acetate in the blank. The results showed that the developed method was selective for determination of Goserelin acetate in biodegradable microspheres formulations.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Goserelin acetate by the proposed method were found 0.035µg/mL and 0.108µg/mL, respectively. The LOQ value shows that the method could be applied to the lower concentration of analytes. LOQ was good enough for the determination of the drug in the microspheres formulations containing 3.6mg of Goserelin acetate which is the usual dose of Goserelin acetate in currently marketed dosage forms.

Robustness

Robustness of the method was determined to base on the percentage recovery and relative standard deviation values obtained for different changes in the method analysis such as flow rate, temperature and mobile phase composition. Robustness of the current method, the effect of flow rate was studied at 0.9 and 1.1mL/min instead of 1.0mL/min. The effect of column temperature was studied at 25°C and 35°C instead of 30°C. The effect of mobile phase composition was assessed at (Buffer:Acetonitrile = 68:32v/v) and (Buffer:Acetonitrile = 72:28v/v) instead of (Buffer:Acetonitrile = 70:30v/v). The %RSD of robustness testing under these conditions was calculated in all cases is given in Table 5 and their tailing factor, plate count obtained within the limit. The deliberate changes in the flow rate, column temperature and mobile phase composition ratio did not affect the recovery of the drug which indicated the robustness of the method.

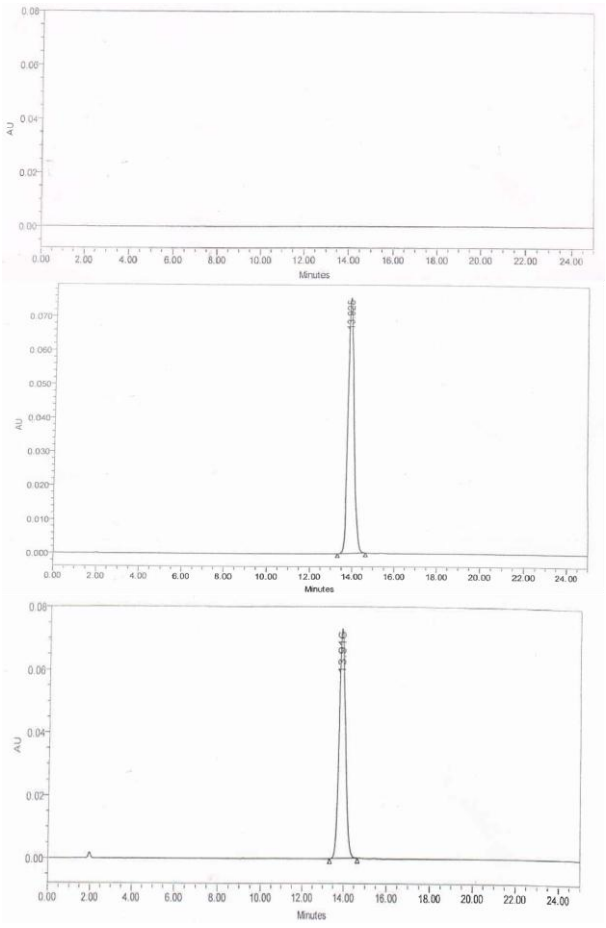

Figure 3: Chromatograms of blank biodegradable microspheres (A), Chromatograms of Goserelin acetate (B) and Chromatograms of Goserelin acetate in biodegradable microspheres (C).

Estimation of formulations

The assay values of Goserelin acetate in biodegradable microspheres formulations ranged from

99.89% to 101.24%, which a standard deviation of not more than 0.39%. The assay values for the formulations were the same as mentioned in the label claim. The results of the assay indicate that the method is selective for the assay of Goserelin acetate without interference from the polymer and excipients used in the dosage form. The estimated drug content with low values of standard deviation established the precision of the proposed method.

CONCLUSION

The reversed phase HPLC method depicted rapid, sensitive and high precision and accuracy technique to quantify Goserelin acetate from biodegradable microspheres was developed and validated. It should be used as a routine quality control analysis for such dosage form.

Acknowledgement

The authors wish to thank Dr. Vinod S Mannur, KLEU College of Pharmacy, Belgaum, Karnataka, India and Prof. Avinash M. Bhagwat, YSPM's Yashoda Technical Campus, Satara, Maharashtra, India for providing support and enthusiasm to complete this work.

REFERENCES

Ahuja S, Rasmussen H. 2007. HPLC Method Development for pharmaceuticals. Vol 8. Elsevier Publications. 441-449.

Anderson J, Abrahamsson PA, Crawford D, Miller K, Tomball B. 2008. Management of advanced prostate cancer can we improve on androgen deprivation therapy. BJU Int., 101:1497–1501.

Bajusz S, Kovacs M, Gazdag M, Boker L. 1988. Highly potent antagonists of Luteinizing hormone-releasing hormone free of edematogenic effects. Medical Sci., 85:1637-1641.

Bambino TH, Schreiber JR, Hsueh AJ. Gonadotropin-releasing hormone and its agonist inhibit testicular luteinizing hormone receptor and steroidogenesis in immature and adult hypophysectomized rats. Endocrinology 1980;107(4):908-17.

Boccon-Gibson, L., Meulen, E., Persson, B. 2011. An update on the use of gonadotropin-releasing hormone antagonists in prostate cancer. Ther. Adv. Urol. 3(3): 127-140.

Center for Drug Evaluation and Research, 1994. Reviewers Guidance, Validation of Chromatographic Methods.

Duque, L.F., Lathuile, L., Limousin, M.F., Klijnstra, E., Steendam, R., Veldhuis, G.J. 2012. Biodegradable monodisperse microspheres with sustained release of goserelin acetate for the treatment of prostate and breast cancer. Poster 136. The 39th Annual Meeting & Exposition of the Controlled Release Society; Quebec City, Canada.

Holgado, M.A., Cozar-Bernal, M.J., Salas, S., Arias, J.L., Alvarez-Fuentes, J., Fernandez Arevalo, M. 2009. Protein-loaded PLGA microparticles engineered by flow focusing: physicochemical characterisation and protein detection by reversed-phase HPLC. Int. J. Pharmaceutics. 380(1-2): 147–154.

ICH Harmonised Tripartite Guideline, 2005. Validation of a9nalytical procedures: Text and Methodology-Q2(R1).

ICH-Q2A, 1995. Proceedings of the International Conference on Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures. Geneva.

ICH-Q2B, 1996. Validation of analytical procedures," in Proceedings of the Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Geneva, Switzerland.

Jean-Pierre, G., Eliane, P., Claude, K. 1992. Precursor and deaminated forms of both luteinizing hormone-releasing hormone (LHRH) and (hydroxyproline) LHRH are present in the rat hypothalamus. Biochem. and Biophysical Res. Commun. 189(3): 1368-1373.

John, H. 2006. Method Validation in Pharmaceutical Analysis. Wiley Publication. 195-211.

Joseph, S., Lloyd, R. 2012. Practical HPLC Method Development. 2ed. Wiley Interscience Publication. 684-705.

Kaufmann, M., Jonat, W., Kleeberg, U., Maass, H. 1989. Goserelin, a depot gonadotropin-releasing hormone agonist in the treatment of premenopausal patients with metastatic breast cancer. J. Clin. Oncol. 7: 1113-1119.

Lister, A.S. 2005. Validation of HPLC methods in pharmaceutical analysis. Vol. 6. Separation Sci. Tech.191–217.

Miller, K., Anderson, J., Abrahamsson, P.A. 2009. Treatment of prostate cancer with hormonal therapy in Europe. BJU Int. 103(2): 2–6.

Neves, J.D., Sarmento, B., Amiji, M.M., Bahia. M.F. 2010. Development and validation of a rapid reversed-phase HPLC method for the determination of the non-nucleoside reverse transcriptase inhibitor dapivirine from polymeric nanoparticles. J. Pharma. Biom. Anal. 52(2): 167-172.