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Analytical Method Development and Validation of Reslizumab In Bulk Drug and Pharmaceutical Dosage Form by Rp-Hplc Method

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ABSTRACT

A simple specific and accurate RP-HPLC method has been developed and validated for the estimation of Reslizumab in bulk drug and pharmaceutical dosage forms. The chromatographic conditions were viably created for the unit of Reslizumab by using Inertial - ODS C18 (250 x 4.6 mm,5µ), stream is 1.0 ml/min, convenient stage extent was Methanol: Water (75:25), recognizable proof wave length was 225 nm. Acetonitrile was used in this experiment. The results of the tablet analysis were validated with respect to accuracy (recovery), linearity, limit of detection (LOD) and Limit of quantitation (LOQ) were found to be satisfactory.

Keywords: Reslizumab; RP-HPLC; Acetonitrile; Acuracy; linearity; limit of detection (LOD) and Limit of quantitation (LOQ)

INTRODUCTION

Resilizumab is a high affinity IgG4/k humanised monoclonal antibody against IL-5 [1,2]. It is approved as additional therapy for severe eosinophilic asthma [3,4]. People with severe eosinophilic asthma have large number of eosinophils in sputum and peripheral blood.It is a phenotype of asthma which has reduced lung function, difficulty in symptom control and increased number of excerbations [5,6] so they do not respond adequately to corticosteroid .Here comes the role of additional therapies which specifically targets at molecular on IL-5 which is released by eosinophils in this subset of asthma patients [7,8]. The FDA approved reslizumabwith US trade name Cinqair [9]. Cinqair is approved for patients who have a history of severe asthma attacks (exacerbations) which are not controlled by current asthma medicines [10]. Reslizumab was initially developed by Chuan-Chu Chou at Schering-Plough and was previously known as SCH-55700. In 1993, Chou and his group at Schering-Plough were granted the patent for the design, cloning and expression of the reslizumab drug [11]. Reslizumab contains the complementary determining regions (CDRs) of the original rat anti human IL-5 antibody 39D10 grafted onto a human frame work which is produced in the murine myeloma NSO expression system. Its molecular weight ~147 kDa.

Reslizumab is available as a solution in 100-mg/10-mL (10 mg/mL) single-use vials. It appears clear to slightly opaque and colourless to slightly yellow. Population pharmacokinetic analyses indicate that the simultaneous use of leukotriene antagonists or cortico steroids does not interfere the pharmacokinetics of reslizumab [12].

Study to Evaluate the Efficacy and Safety of Reslizumab (3.0 mg/kg) in the Reduction of Clinical Asthma Exacerbations in Patients (12-75 Years of Age) With Eosinophilic Asthma.Reslizumab was first used for eosinophilic asthma in 2008. Furthermore, the patients receiving reslizumab showed improvements in airway function, and a general trend toward greater asthma control than those receiving placebo was observed [13]. These results led to the FDA approval for the maintenance treatment of severe asthma in patients aged 18 years and older, with an eosinophilic phenotype on March 23, 2016 [14]. Reslizumab is degraded by enzymatic proteolysis into small peptides and amino acids, as are other monoclonal antibodies [15] (Figure 1).

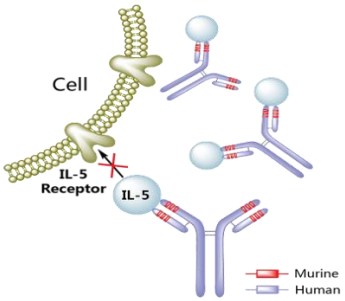

Figure 1: Resilizumab

MATERIALS AND METHODS

Pure samples of Reslizumab were obtained from Hetero drugs Pvt.Ltd. for the analytical development and validation of Reslizumab. HPLC grade Orthrophosphric acid, Acetonitrile and Methanol were procured from Merck. High pure water prepared by using Qualigens. Necessary PPE (personal protective equipment) was used during the analysis. Destruction of solid samples and disposition of solvents was done.

Instruments

- HPLC –Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator(FAST CLEAN)

Selection of wave length (λ max)

A solution of 100 µg/ml of Reslizumab was prepared in Qualigens water. The resulting solutions were scanned individually on HPLC PDA detector from 200nm to 400 nm and also in UV-Visible spectrophotometer. The optimal response for both of them was obtained at 225 nm. Hence the complete method was processed at the wavelength of 225 nm.

Preparation of standard solution

100 mg of Reslizumab was accurately weighed and transferred into a 100 ml clean dry volumetric flask and added methanol sonicated (30 minutes) to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000ppm (Stock solution). Further 4 ml of Reslizumab was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent to give a concentration of 40ppm.

Preparation of sample solution

10ml of Reslizumab was pipetted out from the above stock solution into a 100 ml clean dry volumetric flask and diluents was added it and was shaken by mechanical stirrer and sonicated for about 10 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent to give a concentration of 1000ppm and allowed to stand until the the residue settles before taking an aliquot for further dilution (stock solution). 4ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluents up to the mark to give the required concentration.

RESULTS AND DISCUSSION

Validation of the developed method

From the system suitability studies it was observed that % RSD of retention time was found to be 3.444nm for Reslizumab, % RSD of peak area was found to be 2102937.51for Reslizumab.A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated. From the Linearity data it was observed that the method was showing linearity in the concentration range of 40-50ppm for Reslizumab.

The standard solution was injected for six times and the area for all six injections was measured in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits. The % RSD of Reslizumab for repeatability and precision was found to be150%. Assay was performed in triplicate for various concentrations of Reslizumab and equivalent to 100, and 150 % of the standard amount was injected into the HPLC system The recoveries of pure drug from the analysed solution of formulation was 100.22% which shows that the method was accurate. The Chromatograms of Standard and sample are identical with nearly same Retention time. No interference due to Placebo and Sample at the retention time of analyse which shows that the method was specific. As the % RSD of retention time and asymmetry were within limits for variation in flow rate (0.1 ml). Hence the allowable flow rate should be within 0.1 ml to 1.2 ml/min (Table 1-3).

Method was optimized and the retention time was reported as 3. 444nm.The Chromatograms were recorded as table no.1-3 and fig no.1-4 for standard, sample respectively (Figure 2-5).

Table 1: Chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Water (75:25)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	6 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	225nm
Drug RT (min)	3.444min

Table 2: Data of Repeatability (Method precision)

	Injection	Peak Areas of Reslizumab	%Assay
Concentration 40ppm	1	2102877.32	100.22
	2	2102956.23	100.22
	3	2102997.12	100.23
	4	2103022.22	100.23
	5	2103075.84	100.23
	6	2103124.45	100.23
Statistical **Analysis**	**Mean**	2103008.86	100.23
	SD	87.4487	0.00418
	% RSD	0.00415	0.00417

Table 3: Data of Linearity

Concentration (ppm)	Average area	Statistical analysis	
0	0	slope	52296
20	1051491.45	Y-intercept	6339
30	1577236.65	correlation coefficient	0.999
40	2102982.87		
50	2628727.42		
60	3154473.86		
70	3649285.27		

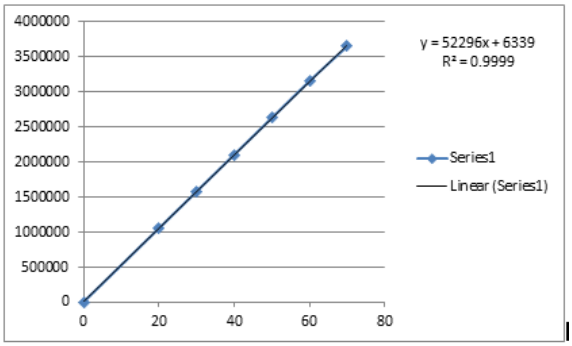

Figure 2: Linearity plot (concentration Vs response)

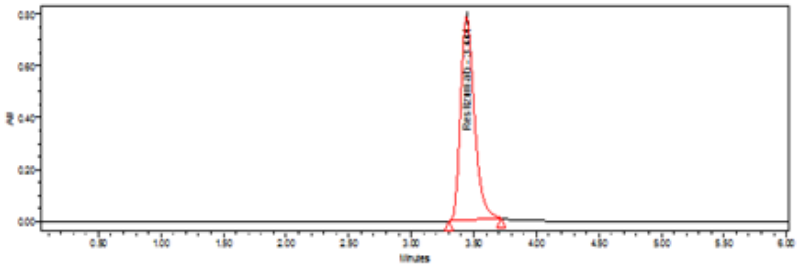

Figure 3: Standard chromatogram

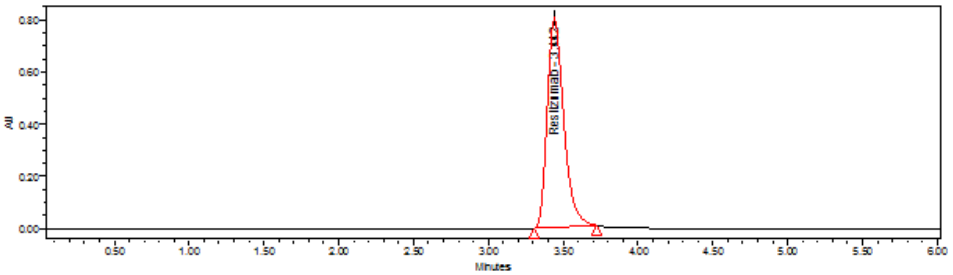

Figure 4: Repeatability chromosomes (Repeatable Chromatograms)

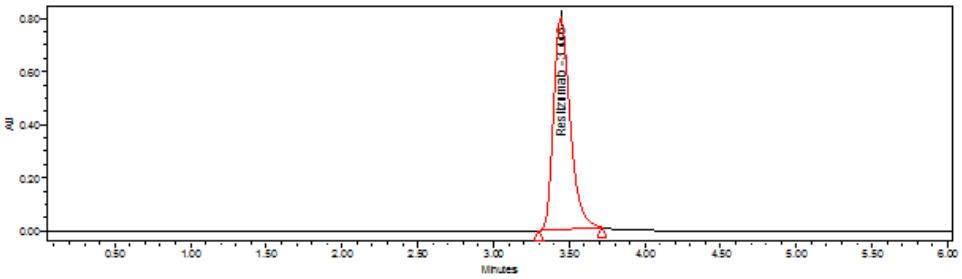

Figure 5: Chromatograms of Intermediate Precision

CONCLUSION

Good agreement was seen in the assay results of pharmaceutical formulation by developed and validated method. The method was validated for parameters such as system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Reslizumab in Bulk drug and pharmaceutical formulation.

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