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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR ESTIMATION OF IGURATIMOD IN FORMULATION

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ABSTRACT:

A simple, rapid and reproducible HPLC method was developed for determination of Iguratimod in the dosage forms. A SunQ C_{18} column (250 x 4.6 mm, 5 µ) and a mobile phase of Acetonitrile: Water (80:20 v/v) mixture was used for separation and quantification. Analyses were run at a flow-rate of 1 ml/minwith detection at 256 nm. Under these conditions, Iguratimod eluted at 3.20 min. The developed method was validated according to the ICH guidelines and found to be linear within the range 5– 30 µg/ml. The developed method was applied successfully for quality control assay of Iguratimodin their drug product and dissolution media.The mean % recovery was found to be 99.20 ± 0.98.

Keywords: High performance liquid chromatography, Iguratimod, Method validation

INTRODUCTION:

Iguratimod is an anti-inflammatory small molecule drug used for treatment of rheumatoid arthritis, together with methotrexate. TOYAMA chemical company were firstly developed this drug.[1] Iguratimod a nuclear factor NF-kB activation inhibitor used in the treatment of rheumatoid arthritis.[2] The structure is reported in Figure 1.

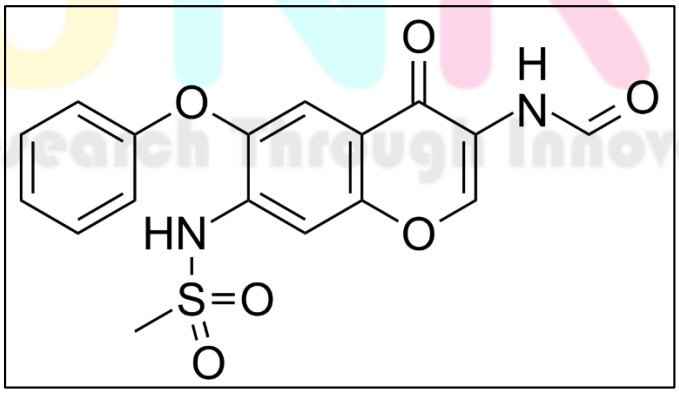

Figure 1: Structure of Iguratimod

From the literature survey few bioanalytical[2,3,4,5]and analytical[6,7,8,9] methods were found reported for determination of Iguratimod. The aim of this study to develop and validate an efficient HPLC method for determination of Iguratimod.

MATERIALS AND METHODS:

Materials:

Iguratimod was received as a gift sample from Lupin, Aurangabad. Other chemicals and reagents like Acetonitrile, Methanol,

Instrumentation:

Instrument which was used in this method is HPLC system comprising of Borwin-PDA software (version 1.50),HPLC pump-Model PU 2080 Plus, Rheodyne sample injection port – 20 µl loop, Column- SunQ C18 (250 x 4.6mm, 5µ), Detector- PDA MD 2010 Plus. Other instruments used were double beam UV spectrophotometer (Jasco, V 730) Electronic balance (Shimadzu, AY- 120), Water purification system (Lab Link, Xtra Pure)and Dissolution Apparatus (Electrolab, Type II- Paddle).

Preparation of Standard Stock Solution:

Accurately weighed 10 mg of Iguratimod was transferred to volumetric flask, and the volume was made up to 10 ml with acetonitrile, to get standard stock solution of Iguratimod (1000 µg/ml). One ml was further diluted to 10 ml with acetonitrile to get 100 µg/ml solution. Further dilutions were made with mobile phase to get of 5 - 30 µg/ml solutions for Iguratimod.

Detection wavelength:

A solution of 10 µg/ml in Acetonitrile was prepared from standard stock solution of Iguratimod (1000 µg/ml) and scanned over 200-400 nm in UV– Spectrophotometer. The maximum absorbance was shown at 256 nm. The spectrum for the drug is shown in the Figure 2.

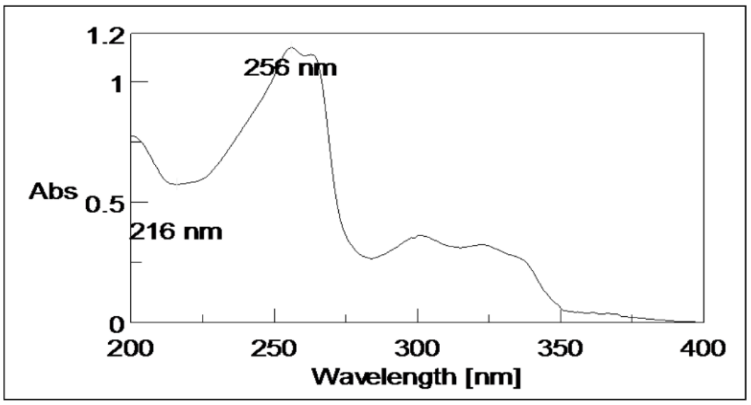

Figure 2: UV Spectrum of Iguratimod

Assay (Formulation analysis):

For sample solution, twenty tablets were weighed, average weight was determined and crushed into fine powder. Powder equivalent to 10 mg of Iguratimod (Label claim: 25mg Iguratimod per tablet) was accurately weighed and transferred into 10 ml volumetric flask and makeup volume with acetonitrile, shook well and filter it. One ml was further diluted to 10 ml with acetonitrile to get 100 µg/ml solution. Further 1 ml was diluted to 10 ml with mobile phase to get 10 µg/ml solution and injected. The procedure was repeated for six times. The concentration and % recovery was determined from linearity equation.

RESULTS AND DISCUSSION:

Optimized Chromatographic conditions:

Chromatographic separationand quantification was carried on SunQ C_{18} column (250 x 4.6 mm, 5 µ) using mobile phase Acetonitrile: Water (80:20 v/v)at a flow-rate of 1 ml/min with detection at 256 nm. Under these conditions, Iguratimod eluted at 3.20 min. Peak obtained for Iguratimod was good with excellent peak characteristics and it was eluted at 3.20 min. Plate count and tailing factor were satisfactory, so these optimized conditions were used and validated.Standard chromatogram of Iguratimod shown in Figure 3 with retention time 3.20 min.

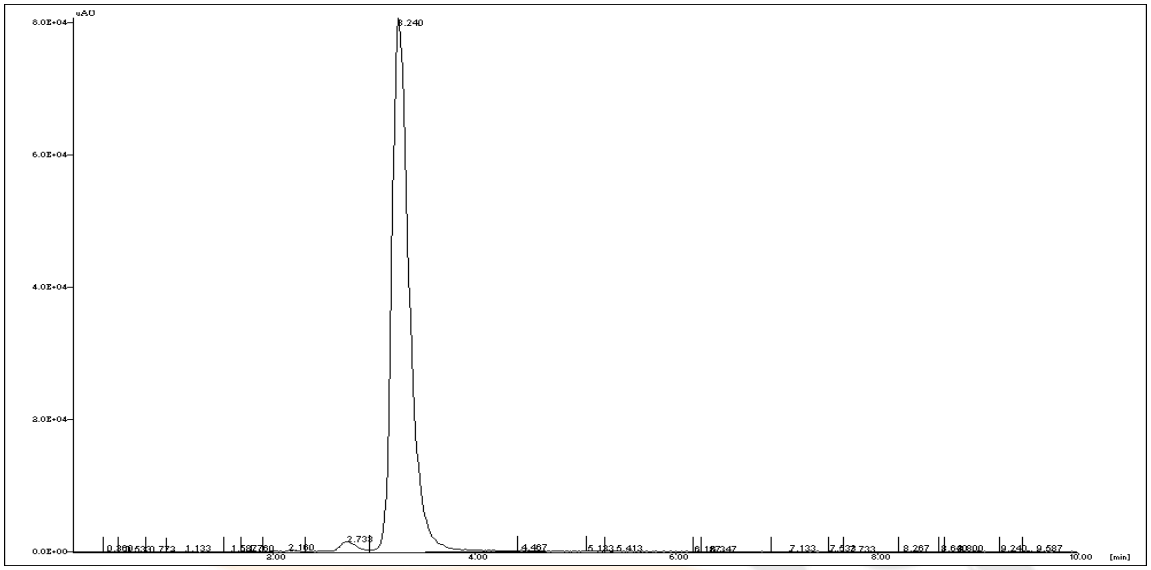

Figure 3: Standard Chromatogram of Iguratimod (10µg/ml)

Method validation:

Validation of analytical method involves linearity and range, precision, accuracy, assay, limit of detection (LOD) and limit of quantitation (LOQ). It was validated according to ICH Q2 (R1) guideline.[10]

Linearity and Range:

The linearity of the method was found to be within the concentration range of 5-30 µg/ml with R^2= 0.998 and the equation obtained isy = 96704x + 52857. The peak area was plotted against concentration to obtain the calibration curve as given in the Figure 4.

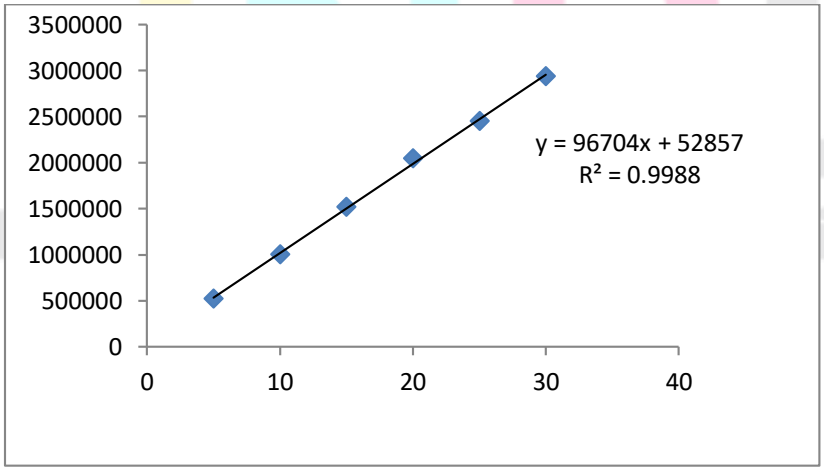

Figure 4: Calibration curve for Iguratimod (5-30 µg/ml)

Precision:

Precision was assessed in terms of Intra-day and inter-day precision at three different concentrations (10, 20, 30 µg/ml). In Intra-day studies triplicates of 3 concentrations were analysed on same day. Inter-day precision was

assessed by performing analysis on different days. The % RSD values less than 2 indicate precision of the method. Results are shown in Table 1 and Table 2.

Table 1: Results for intraday precision studies

Concentration(µg/ml)	Area	Amount Recovered(µg/ml)	%Recovery	S.D.	%RSD
10	996365	9.75	97.56	1.61	1.62
	1027020	10.07	100.73		
	1006922	9.86	98.65		
20	1964869	19.77	98.58	0.52	0.53
	1976980	19.89	99.48		
	1656789	19.68	98.44		
30	2987752	30.34	101.16	1.80	1.79
	2898824	29.42	98.09		
	2990934	30.38	101.27		

Table 2: Results for interday precision studies

Concentration (µg/ml)	Area	Amount Recovered (µg/ml)	%Recovery	S.D.	%RSD
10	999973	9.79	94.94	1.09	1.11
	1020687	10	100.08		
	1006306	9.85	98.59		
20	2000154	20.13	100.68	0.28	0.28
	2001043	20.14	100.72		
	2020236	20.24	101.20		
30	2989985	30.37	101.24	1.06	1.06
	2975886	29.50	100.75		
	2930673	29.75	99.19		

Assay:

Assay of formulation of the drug was performed and % drug content was found to be 99.20 ±0.98.Results are presented in Table 3.

Table 3: Results for assay study

Sr. No.	Concentration (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	Mean ± % RSD
1	10	1018901	9.99	99.89	99.20 ± 0.98
2	10	1002294	9.81	98.18	
3	10	1008987	9.88	98.87	
4	10	1002453	9.82	98.19	
5	10	1014171	9.94	99.40	
6	10	1026208	10.06	100.65	

Accuracy:

Accuracy of the method was determined by method of standard addition. Known amount of standard drug solution to be analyzed was added to the marketed formulation of Iguratimod at 50%, 100% and 150% level. The 3 replicates of 3 concentrations were evaluated to calculate % recovery. Results are presented in Table 4.

Table 4: Results for accuracy study

Sr. No	Level	Amount of marketed formulation added (µg/ml)	Amount of API added	Total amount of the drug (µg/ml)	Area	Amount recovered (Avg)	% recovery	%RSD
1	50%	10	5	15	1488337	14.72	98.15	0.74
2		10	5	15	1467584			
3		10	5	15	1474112			
4	100%	10	10	20	1960162	20.08	100.41	1.60
5		10	10	20	2020223			
6		10	10	20	2004631			
7	150%	10	15	25	2479871	25.08	100.43	0.07
8		10	15	25	2483208			
9		10	15	25	2479972			

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

The sensitivity of the method was determined from limit of detection (LOD) and limit of quantitation (LOQ) with the help of formula LOD = 3.3 σ / S and LOQ = 10 σ / S; where σ = standard deviation of the response (y-intercept), S = slope of the calibration curve of the analyte.The LOD and LOQ from equation were found to be 0.33 µg/ml and 1.01 µg/ml, respectively.

Robustness:

Robustness of the method was evaluated by small change in the optimized method parameters like change in detection wavelength, flow rate and composition of mobile phase. The effect on the result was examined and method was found to robust with % RSD less than 2. Results of robustness study given in Table 5.

Table 5: Results of robustness study

Sr.No.	Parameter	Condition	Area	%RSD
1	Change in detection wavelength(256±1nm)	255nm	979582	0.20
		256nm	983514	0.64
		257nm	989877	0.77
2	Change in Flow rate (1 ± 0.1 ml/min)	0.9ml/ min	1003330	1.66
		1 ml/ min	997224	0.69
		1.1 ml/ min	1018794	0.25
3	Varying mobile phase conc. (80:20 v/v±2)	82:18 v/v	988214	0.82
		80:20 v/v	981868	0.85
		78:22v/v	979972	0.99

CONCLUSION:

This work illustratea simple, sensitive and robust HPLC method developed for estimation of Iguratimod in bulk and dosage form as well as in its dissolution media was studied. The method was validated as per ICH Q2 (R1) guideline. The results obtained indicated that the developed method was found to be simple, accurate, reliable and sensitive for the analysis of Iguratimod in bulk and dosage form. For dissolution study tween was used to increase solubility of drug in dissolution media. It was seen that in given dissolution media the Iguratimod shows 96.4% release at the end of 30 min.

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