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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF IGURATIMOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The objective of the work was to develop and validate stability indicating HPTLC method for the estimation of Iguratimod.

Methods: The method employed HPTLC aluminium pre-covered silica gel 60 GF_{254} plates (10 cm × 10 cm with 250 µm layer thickness) as stationary phase while the solvent system was n-Hexane: Ethyl Acetate (5:5 v/v) with densitometric scanning at 256 nm. Sample was applied as a band of 8 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) using a linomat 5 applicator (Camag, Switzerland). Migration distance was 80 mm. Further the sample was subjected for stress conditions under acid and base hydrolysis, oxidation, thermal, neutral and photolytic conditions. Method validation done according to ICH Q2 (R1) guidelines.

Results: Retention factor (Rf) of the drug was 0.41±0.02. The linearity of the method was found to be within the concentration range of 200-1200 ng/band with R^2= 0.983. Limit of detection and limit of quantification were found to be 34.69 and 105.12 ng/band respectively. The % mean recovery was found to be 100.38±0.83. Stress results showed that there is degradation in acid and base conditions but two degradant peaks were observed only under alkaline stress condition

Conclusion: The developed method found to be accurate, simple and precise. Method is successfully employed for quantification of the drug under various stress conditions.

Keywords: High-performance thin layer chromatography, Iguratimod, Method validation, Stress degradation

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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. Iguratimod can inhibit nuclear factor-kappa B (NF-κB) activation by interfering with NF-κB translocation from the cytoplasm to the nucleus without affecting the degradation of Ikappa Balpa in lipopolysaccharide-stimulated THP-1 cells (human monocytic leukemia cell line) [2]. The structure is reported in fig. 1. From the literature survey few bioanalytical methods for determination of Iguratimod in human and rat plasma [2-5] and HPLC as well as LC-MS analytical methods [6-9] were found reported. Although reports give methods for estimation of impurities as well as related substances in Iguratimod but forced degradation studies are not detailed [6, 9]. Only one report estimates it by HPLC [7] but no reports found related to forced degradation stability study with estimation of Iguratimod by High Performance Thin Layer Chromatography.

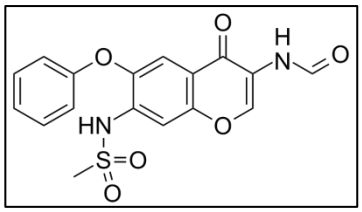

Fig. 1: Structure of iguratimod

The HPTLC methods are fast, consumes less solvent and are sensitive. The rational of present work was development and validation of stability indicating HPTLC method for determination of Iguratimod and study degradation behaviour of molecule.

MATERIALS AND METHODS

Materials

Iguratimod was received as a gift sample from Lupin, Aurangabad. Other chemicals and reagents like Ethyl Acetate, Acetonitrile, Methanol, n-Hexane, Hydrochloric acid, Sodium Hydroxide, Hydrogen Peroxide (All AR grade), are procured from LOBA CHEMIE PVT. LTD., Mumbai.

Instrumentation

Instrument which was used in this method is HPTLC system (CAMAG) comprising of TLC Scanner III, Linomat 5 applicator, Software [WINCATS (version 1.4.3)], Microliter syringes [Hamilton (100 µl)], TLC plates (Merck's aluminium TLC plate (precoated with silica gel 60 F_{254}) and twin trough glass chamber. Others instruments used UV-Visible spectrophotometer [JASCO (Model-V730)], Electronic balance [Shimadzu (Model AY-120)], Sonicator [PRAMA (Model SM15 US)], Hot air oven [BIOMEDICA], Photo-stability chamber (Newtronic, Model-IC DAC version 1.2).

Preparation of stock solution

Standard stock solution (1000 µg/ml) of Iguratimod was prepared by dissolving 10 mg of drug in 10 ml of Acetonitrile.

Detection of wavelength

From the standard stock solution (1000 µg/ml) further dilutions were made using acetonitrile which gives concentration 10 µg/ml which was scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed maximum absorbance at wavelength of 256 nm which is used for detection and quantitation. The spectrum for the drug is shown in the fig. 2.

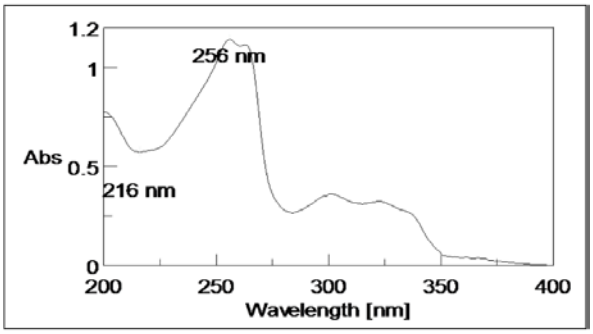

Fig. 2: UV spectrum of iguratimod

Chromatographic conditions

The partition was performed on pre-covered silica gel 60 F_{254} plates (10 cm × 10 cm with 250µm layer thickness) using n-Hexane: Ethyl acetate (5: 5 v/v) as mobile phase. Samples were applied with help of Camag 100 µl sample syringe (Hamilton, Switzerland) using Linomat 5 applicator (Camag, Switzerland).10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 20 min saturation conditions and 10 ml of mobile phase was used per run. The plates were developed at distance of 80 mm. Densitometric scanning was done using Camag TLC scanner at 256 nm, operated by win CATS software (version 1.4.3), a slit dimensions were 6.00 × 0.45 mm. Deuterium lamp was used as a radiation source. The Rf value was found to be 0.41±0.02. Densitogram of solution having concentration 1000 ng/band is shown in fig. 3.

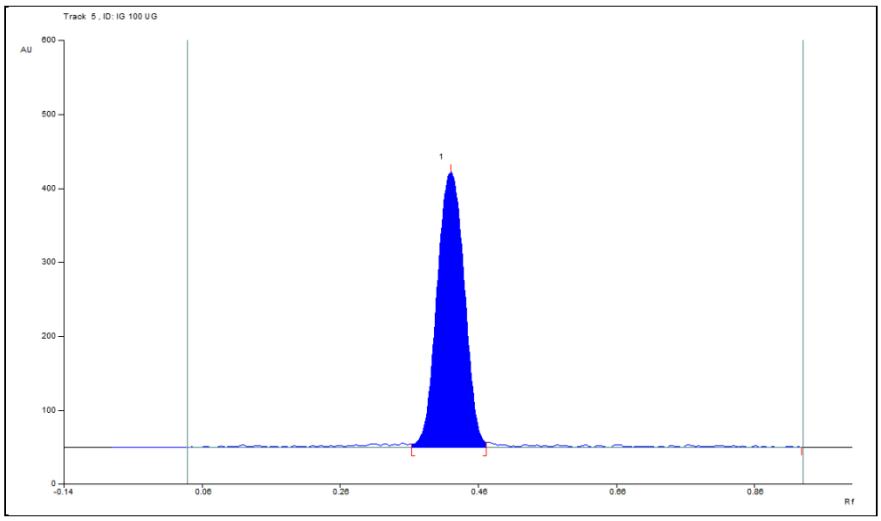

Fig. 3: Densitogram of iguratimod (1000ng/band; Rf= 0.41±0.02)

Forced degradation studies

In order to evaluate the stability indicating property of the developed method, forced degradation studies were carried out in accordance with ICH guidelines Q1A (R2) [10-12].

Degradation under acid hydrolysis condition

To 1 ml standard stock solution ofIguratimod (1000 µg/ml) 1 ml of 0.1 N HCl was added and volume was made up to 10 ml with acetonitrile to get 100 µg/ml of solution. Immediately 4 µl volume was applied on TLC plate and densitogram was recorded.

Degradation under alkali hydrolysis condition

1 ml standard stock solution of Iguratimod (1000 µg/ml) was mixed with 1 ml of 0.1 N NaOH was added and kept aside for 1 h at room temperature. After that volume was made up to 10 ml with acetonitrile to get 100 µg/ml. Then 4 µl solution was spotted on TLC plate and densitogram was recorded.

Degradation under oxidation condition

To 1 ml standard stock solution of Iguratimod (1000 µg/ml), 1 ml of 30% H_2O_2 was added and after that volume was made up to 10 ml with acetonitrile to get 100 µg/ml of solution. Immediately 4 µl solution was spotted on TLC plate and densitogram was recorded.

Degradation under dry heat

About 30 mg of drug sample was kept in hot air oven (100 0^C) for 2 h. After exposure 10 mg of drug weighed and dissolved into 10 ml of acetonitrile to give 1000 µg/ml. From this solution 1 ml was diluted to 10 ml of acetonitrile to get 100 µg/ml, the 4 µl solution was then applied on TLC plate.

Photo-degradation using UV light

Drug was exposed to fluorescence light (NLT 1.2 million Lux-H) followed by exposing drug to UV light (illumination of NLT 200watt hr/m2). After exposure weighed accurately 10 mg of drug and 100 µg/ml solution of Iguratimod was prepared in acetonitrile.4 µl of solution was then applied on TLC plate and densitogram was recorded.

Method validation

As per International Conference on Harmonization Q2 (R1) guidelines method validation was performed [13-15].

Specificity

Specificity of the process is determined from the peak purity.

Linearity and range

A 100 µg/ml solution was prepared from the standard stock solution of Iguratimod 1000 µg/ml. From this solution, 2 µl, 4 µl, 6 µl, 8 µl, 10 µl and 12 µl of solution was applied on the TLC plate to give bands having concentrations ranging from 200-1200 ng/band.

Precision

Precision was assessed in terms of Intra-day and inter-day precision with three different concentrations (600,800,1000ng/band). In Intra-day studies triplicates of 3 concentration were analysed on same day. Inter-day precision was assessed by performing analysis on different days. The obtained peak area was used to calculate % RSD.

Assay (formulation analysis)

For sample solution, twenty tablets were weighed, average weight was determined and the tablets were crushed into fine powder. Powder equivalent to 10 mg of Iguratimod (Label claim: 25 mg Iguratimod per tablet) was accurately weighed and transferred into 10 ml volumetric flask and makeup volume with acetonitrile, shook well and filter it. Then 1 ml solution was pipette out and diluted to 10 ml with acetonitrile to get the solution of 100 µg/ml. 4µl volume was applied on TLC plate to get concentration 400 ng/band. The procedure was repeated for six times. The concentration and % recovery were determined from linearity equation.

Accuracy

Accuracy of the method was determined by calculating recovery of the Iguratimod. The recovery studies were carried by spiking the standard drug solution to tablet solution at 3 different levels of 50,100 and 150% of assay concentration. % Recovery was calculated from linear equation.

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.

Robustness

Robustness of the method was evaluated by small change in the optimised method parameters like change in composition of mobile phase, chamber saturation time, detection wavelength. Time from development to scanning was also changed.

RESULTS AND DISCUSSION

Forced degradation studies

The Iguratimod was found degraded mainly under acid, alkali, oxidation and thermal conditions but degradant peaks were observed only under alkaline condition. The summary of results is presented in table 1 and densitogram of Iguratimod subjected to alkaline stress is presented in fig. 4. Spectral scanning of standard drug and degradation product obtained in the base degradation shows dissimilarity in spectrum indicating different nature of degradant (fig. 5).

Table 1: Summary of forced degradation studies

S. No.	Parameter	Condition	%Recovery	%Degradation
1	Acid	0.1 N HCl, Immediate application	77.95	22.04
2	Base	0.1 N NaOH at RT for 1 h	74.39	25.60
3	Oxidation	30 % v/v H_2O_2, Immediate application	85.49	14.50
4	Thermal	100 °C, 2 h.	80.51	19.48
5	UV	200 Watt hours/square Meter	94.70	5.29
6	Fluorescent	1.2 million luxhours	100	-

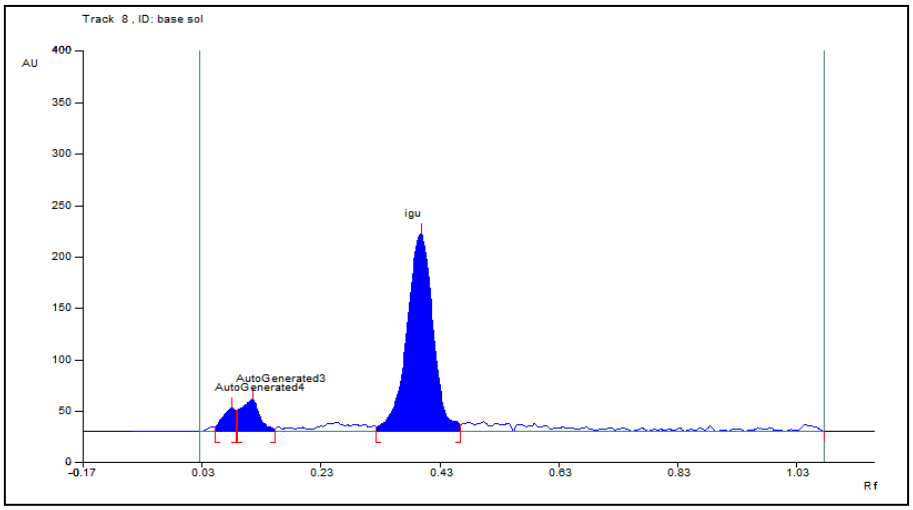

Fig. 4: Degradation under base hydrolysis with degradation product

As per literature survey, two HPLC papers reported degradation under acid and alkali hydrolysis with degradation peaks [6, 7]. We also got major degradation in acid and alkali with more percent of degradation as compared to reported method [7], which may be due to sensitive nature of HPTLC method. Degradant peaks were observed in base degradation only by HPTLC method, of which spectral scanning was also done.

Method validation

Specificity

No other peak observed at Rf value of drug as well as recorded peak purity values exceeded 0.999, indicating no interference with any other peak of degradation product, impurity or matrix at Rf value of analyte.

Linearity and range

The linearity of the method was found to be within the concentration range of 200-1200 ng/band with R^2= 0.983 and the equation obtained is y=9.7384x+3143.3. The peak area was plotted against concentration to obtain the calibration curve as given in the fig. 3 and 3D densitogram is given in fig. 4.

Precision

Precision studies for Iguratimodis shown in table 2a and table 2b. The % RSD values less than 2 indicate precision of the method.

Assay

Assay of formulation of the drug was performed and % drug content was found to be 99.48±0.64 (SD).

Accuracy

The recovery studies were carried by spiking the standard drug to tablet solution at 3 different levels 50,100 and 150% of 400 ng/band sample concentration.% recovery was calculated. The results are shown in table 4.

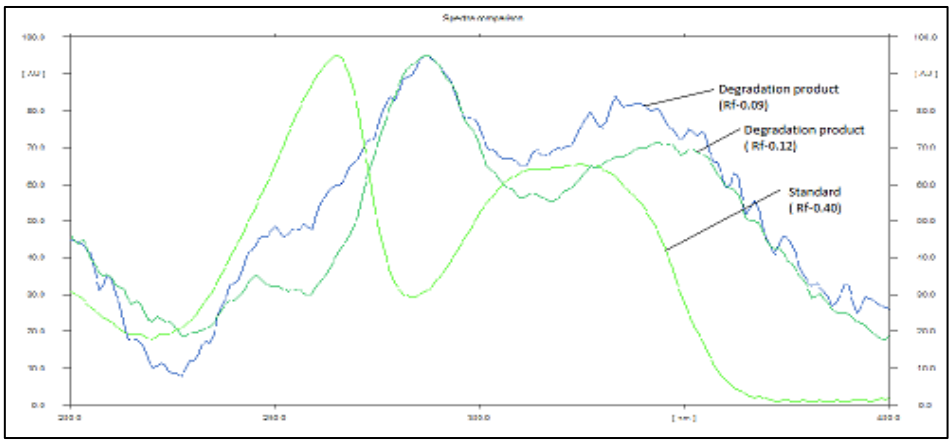

Fig. 5: Spectral scanning of standard drug and degradation product obtained in the base degradation

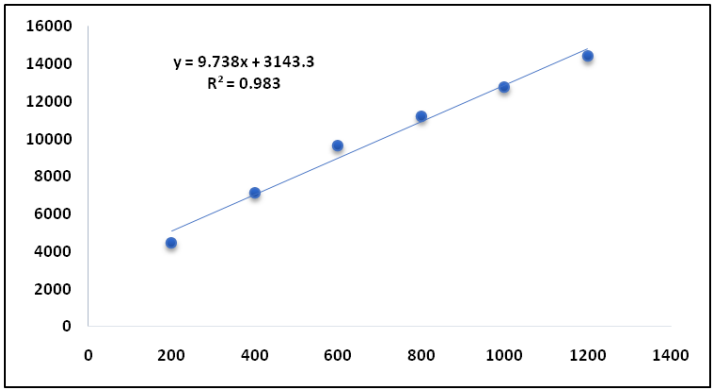

Fig. 6: Calibration curve for iguratimod (200-1200 ng/band)

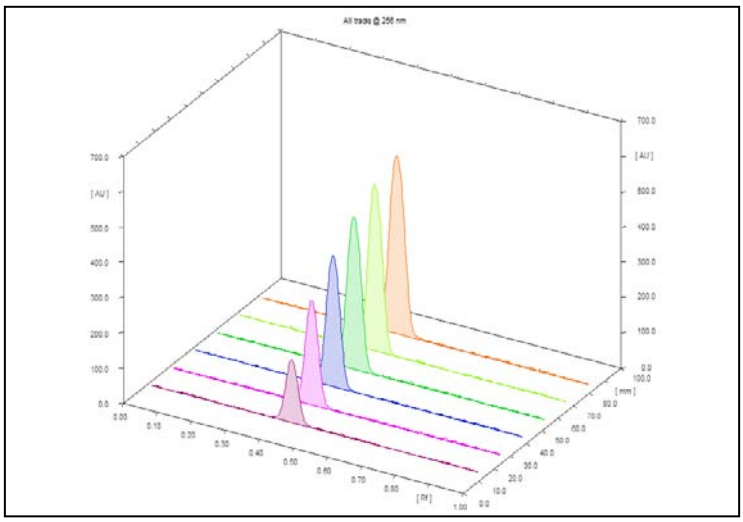

Fig. 7: 3D densitogram of iguratimod (200-1200ng/band)

Table 2(a): Results for intraday precision studies

Concentration (ng/band)	Area	Amount recovered (ng/band)	%Recovery	Mean±% RSD*
600	9013.4	602.83	100.47	100.79±0.39
	9058.3	607.44	101.24	
	9024.0	603.92	100.65	
800	11010.3	807.89	100.98	101.57±0.57
	11055.0	812.48	101.56	
	11101.0	817.21	102.15	
1000	12950.6	1007.15	100.71	100.73±0.34
	12986.9	1010.87	101.08	
	12918.4	1003.84	100.38	

*n= 3×3

Table 2(b): Results for interday precision studies

Concentration (ng/band)	Area	Amount recovered (ng/band)	%Recovery	mean± % RSD*
600	9010.1	602.49	100.14	100.61±0.21
	9018.5	603.35	100.56	
	9035.0	605.05	100.84	
800	11102.1	817.32	102.16	101.41±0.66
	11021.7	809.06	101.13	
	11005.0	807.35	100.91	
1000	12850.6	996.87	99.68	99.82±0.93
	12780.0	989.62	98.96	
	12960.0	1008.11	100.81	

*n= 3×3

Table 3: Results for assay study

S. No.	Concentration (ng/band)	Area	Amount recovered (ng/band)	% Recovery	mean±% RSD*
1	400	7028	398.95	99.73	99.48±0.64
2	400	7001	396.23	99.05	
3	400	7041	400.28	100.07	
4	400	6989	394.94	98.73	
5	400	6999	395.97	98.99	
6	400	7050	401.21	100.30	

*n= 6

Table 4: Results for accuracy study

S. No.	Level	Sample concentration (ng/band)	Standard concentration (ng/band)	Area	Conc. (ng/band)	% Recovery	mean±% RSD*
1		400	200	9010.0	600	100.4	
2	50%	400	200	9080.3	600	100.6	101.3±0.86
3		400	200	9110.2	600	102.1	
1		400	400	10860.9	800	99.0	
2	100%	400	400	10944.8	800	100.1	99.1±0.96
3		400	400	10796.5	800	98.2	
1		400	800	12866.3	1000	99.8	
2	150%	400	800	12999.3	1000	101.2	100.6±0.69
3		400	800	12960.0	1000	100.8	

*n= 3×3

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

The LOD and LOQ from equation were found to be 34.692 ng/band and 105.127 ng/band, respectively.

Robustness

The effect on the result was examined and method was found to robust with % RSD less than 2.

Table 3: Robustness for Iguratimod

S. No.	Parameter	Condition	%RSD
1.	Change in detection wavelength (256±1 nm)	255 nm	1.99
		256 nm	0.98
		257 nm	1.76
2.	Chamber saturation time (20±2 min)	18 min	0.92
		20 min	1.82
		22 min	1.39
3.	Varying mobile phase conc. (5:5 v/v±0.5)	4.5:5.5 v/v	1.53
		5:5 v/v	1.46
		5.5:4.5v/v	1.17
4.	Time from development to scanning	Immediate	1.09
		After 30 min	1.06
		After 60 min	1.02
		After 90 min	1.38

CONCLUSION

A simple, rapid and sensitive HPTLC method was developed for determination of Iguratimod in bulk and dosage form. The method validated according to ICH Q2(R1) guidelines. The results obtained which shows that Iguratimod degrade mainly under acid and alkali which may be due to cleavage of amide linkage in the molecule. It is also oxidation and thermal susceptible thus needs protective storage conditions.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

SVG designed the work. MSJ contributed for the analysis and data collection parts of the work. SVG and MSJ contributed to the interpretation of the results.

CONFLICT OF INTERESTS

Declared none

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