International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 2(C); February 2018; Page No. 9746-9754
DOI: http://dx.doi.org/10.24327/ijcar.2018.9754.1625

Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CLEANING METHODOLOGY FOR RESIDUAL DETERMINATION OF IGURATIMOD

Ravindra Bhaskar Nehete and Pushpendra Sharma

Sri Satya Sai University of Technology & Medical Sciences, Sehore

A R T I C L E I N F O

Article History:
Received 14th November, 2017
Received in revised form 5th December, 2017
Accepted 3rd January, 2018
Published online 28th February, 2018

Key words:

Iguratimod, Residual determination, validation, development, LOD & LOQ, recovery.

A B S T R A C T

Objective of this study was development and validation of simple and precise method for quantification of Iguratimod in the rinse samples to validate cleaning procedure. Analytical methodology was optimized to get suitable precision and recovery; detection wavelength for Iguratimod was selected as 257 nm. Proposed method was validated for parameters like precision, Linearity, accuracy, limit of detection and limit of quantification. Linearity was performed for concentration range 0.1 ppm to 15 ppm, squared correlation coefficient was observed as 0.999. Recovery was observed between 90-110%. LOD and LOQ for final analytical method were 0.2ppm and 0.5 ppm respectively.

Objectives: Cleaning validation is process to assure removal of residues of active drug manufactured by using the equipments. Complete residues are removed to predetermined level for ensuring the quality of next drug to be manufactured. In current scenario, manufacturing unit do not compromised with the contamination of previously manufactured drug. These are requirements for good manufacturing practices to prevent the drug form contamination. Simultaneous object is to quantify residual Iguratimod at lower level with accurate, precise methodology by using high-performance liquid chromatographic (HPLC).

Methods: The method was developed using the isocratic solvent system, for this, an isocratic condition of mobile phase comprising buffer (pH 2.5) and methanol in a ratio of 58:42, v/v at a flow rate of 2.0 mL/minute over Water symmetry C18, 150 × 3.9 mm, 5µm) column at 40°C temperature was maintained. Use acetonitrile as diluent. By using this chromatographic system Iguratimod is successfully eluted with accuracy and precision.

Results: Analytical method validation was successfully completed for routine analysis for quantification of Iguratimod as residue. Analytical method validated for precision, linearity, accuracy, specificity, detection limit and quantification limit, etc.

INTRODUCTION

Chemically, Iguratimod 3-Formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one is used as an anti-inflammatory drug for the treatment of rheumatoid arthritis. It has following structure,

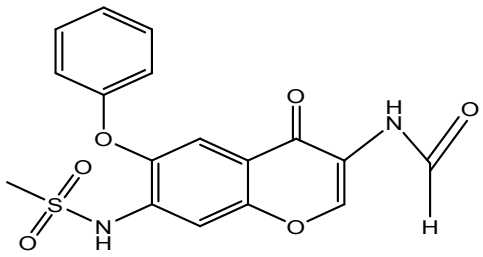

Fig 1 Structures of Iguratimod

Corresponding author:* **Ravindra Bhaskar Nehete
Sri Satya Sai University of Technology & Medical Sciences, Sehore

IUPAC name for Iguratimod is N-[(formylamino)-4-oxo-6-phenoxy-4Hchromen- 7-yl] methane sulfoanamide. Iguratimod was first Reported in product patent US4954518.[1] Its Therapeutic category is Anti-arthritic and novel immunomodulator.[2] Iguratimod is a nuclear factor NF-κB activation inhibitor used in the treatment of rheumatoid arthritis. It also suppressed inflammatory cytokine production in cultured human synovial cells induced by tumor necrosis factor (TNF)-α by inhibiting the activity of nuclear factor-κB. Several synthesis processes are reported for Iguratimod. [3-6]. Efficacy of a drug substance is critical for its safety assessment. It is compulsory to identify and characterize the possible impurities in active drug. This compound is aromatic heterocyclic compound; belong to class of organic compound known as chromones. These are compounds containing a benzopyran-4-one moiety.

Information used to establish an effective cleaning validation is based on the result of validated methodology. High degrees of confidence in results are required for human safety and are depend on the absence of residues remaining on equipment

during drug manufacturing. We will discuss about various aspects related to analytical method validation for cleaning validations.

Analytical method is key factor for establishing the cleanliness of equipments used in drug manufacturing, hence high level of confidence for results are required. And this confidence can be established by defining the usefulness of analytical methodology.

Proper cleaning method strategy includes development and validation of analytical method, which includes high level of confidence in results. Method validation demonstrates the reliability of results to the scientific staff, manufacturing personnel, regulatory agency, etc.

All analytical methods needs to revalidate and reasons may be change in instrumentation, change in analyte, change in manufacturing process, change in cleaning process, etc. Due to these reasons and also if there is possibility to affect the ability of analytical method for accurate determination of analyte, revalidation of analytical method is required.

Measuring the trace residue on surfaces is difficult task. After extraction of residue from the surface and residue to be recovered from the medium, then the residue van be quantified. Analysis of residue is quite difficult from routine analysis of drug substance or drug product. Because achieved accuracy and precision may have the wide limit. Hence sensitivity of this method is to be established for linearity, precision and accuracy.

Residue to be measure could be active drug, excipients, cleaning component, or process degradants, or mixture of all, etc. A suitable analytical technique can be selected. Method validation is reproducibility of developed methodology. During method development analyst gets information about limit establishment, precision, linearity, accuracy, etc. Understanding all these parameters during method development is the basic required for method validation.

Out of all the chromatographic techniques, more commonly used chromatographic technique is High Performance Liquid Chromatography (HPLC). Other techniques include Total Organic Carbon (TOC), Capillary electrophoresis, Atomic Absorption spectroscopy (AAS), Inductively Coupled Plasma (ICP), Titrations, Ultraviolet spectroscopy, Infrared spectroscopy, etc.

All these techniques require analytical method validation. ICH parameters for method validation are suitable for chromatographic techniques and also for non-chromatographic techniques.

Quantification of active drug at residual level for cleaning validation was determined by high performance liquid chromatography instrumental technique. Since literature did not cite such methodology for determination of Iguratimod, it was planned to develop a user friendly, accurate and precise HPLC based methodology.

HPLC Chromatographic technique is used in most of the cases for quantification of active drugs at trace level as this technique is specific, accurate, precise, and user friendly. Chromatographic technique gets rid of tedious extraction and isolation procedures. Chromatographic separation is multistage separation technique, in which the sample components are distributed in two phase's i.e. stationary phase and mobile phase. Stationary phase is either solid or a liquid supported on a solid or a gel. Stationary phase packed in column, spread as layer and distributed as a thin film, or applied by appropriate technique. Mobile phase may be either gas or liquid / supercritical fluid. Separation is based on adsorption, mass distribution (partition), or ion exchange; or due to differences in physicochemical properties of the molecules, like molecule size, mass of molecule, and volume of molecules. This type of chromatography is used for qualitative and quantitative analysis by using different techniques.

Selection of suitable mobile phase, diluent and wavelength

Development of an analytical method for assessment of residue content for drug substances as well as pharmaceutical dosage form is of utmost necessity. This is required in cleaning validation to avoid the contamination due to previous manufactured drug into the drug to be manufactured. There are no methods available for assessment of cleaning validation for Iguratimod. Very few methods by using high-performance liquid chromatography (HPLC) have been observed for determination of Iguratimod dosage form, but no method observed for Iguratimod determination at lower level which can be used for cleaning validation.

We put an effort to develop a cost-effective, rapid, and robust reversed phase (RP)-HPLC method with enough data of validation parameters. First, pKa of drugs was investigated and pKa of Iguratimod was 2.96. As a rule of thumb, pH of mobile phase is selected two units above or below the pKa value of drug. If we consider pKa of Iguratimod, then we cannot choose the pH above 5.0, which is damaging to silica beds of column. With respect to Iguratimod, we could choose the pH of mobile phase between pH 2.0 to 4.0. Therefore, we thought a pH of around 2.5, which will be nearby pKa, and at this pH, Iguratimod will remain ionized, which makes the retention time much shorter at with minimum organic concentration. Thus, we tried with different buffers having a pH between 2.0 to 5.0 with different ratios of methanol in isocratic condition. Method gave sharp peak of Iguratimod without co-elution or any interference. It was found that with increased pH, retention of Iguratimod was increase. Thus, finally, a pH of 2.5 was chosen so as to get sufficient retention of the peak. Optimized chromatographic parameters are summarized in Table 1. Typical chromatograms are shown in Fig 2 and Fig.3. This study was validated according to the guidelines of International Conference on Harmonization (ICH) and USP.

MATERIALS AND METHODS

Materials: Iguratimod standard, Triethyalmine (HPLC grade), Orthophosphoric acid (HPLC grade), Water (HPLC grade).

HPLC system: High-performance liquid chromatographic system, equipped with an auto sampler and UV–visible detector, was used for the analysis. Analytical column, waters symmetry C18, 150 × 3.9 mm, 5 µ was used to analyze the standard and samples.

Preparation of mobile phase: 1.0ml of Triethylamine was mixed in 1000 mL of water, adjusted pH to 2.5 ± 0.1 with diluted orthophosphoric acid. Then this buffer and HPLC grade methanol were mixed together at a ratio of 58:42, v/v. Finally, it was filtered through a 0.45-µm membrane filter and sonicated to degas.

Preparation of diluent: Use acetonitrile as such

Preparation of standard solutions: Dissolve 25 mg of Iguratimod in a 50mL volumetric flask; add about 30 ml diluent. Sonicate to dissolve and make up the volume up to the mark with diluent (Stock solution). Further dilute 10ml of this stock solution to 20ml with diluent. Dilute 1ml of resultant solution to 25ml with diluent. Thus, we got the test solution of Iguratimod of a concentration of 10ppm (µg/ml). Stock solution was further diluted to get required concentration over the range from detection limit to 150% of test concentration.

Chromatographic conditions: All analyses were done at 40°C temperature under isocratic condition. The mobile phase was run at a flow rate of 2.0 mL/minute for 10 minutes. Injection volume was 20µL for standard and samples. The column eluent was monitored with UV detection at 257 nm.

Table 1 Optimized chromatographic conditions

Chromatographic Conditions	
Instrument	: HPLC equipped with Pump, Injector, UV detector and Recorder.
Column	: Waters symmetry C18, 150 × 3.9 mm, 5µ
Flow rate	: 2.0 mL/minute.
Detector	: UV at 257nm.
Injection volume	: 20µL
Column oven temp	: 40°C
Run time	: 10 minutes
Approximate retention time for Iguratimod peak : About 4 minutes	

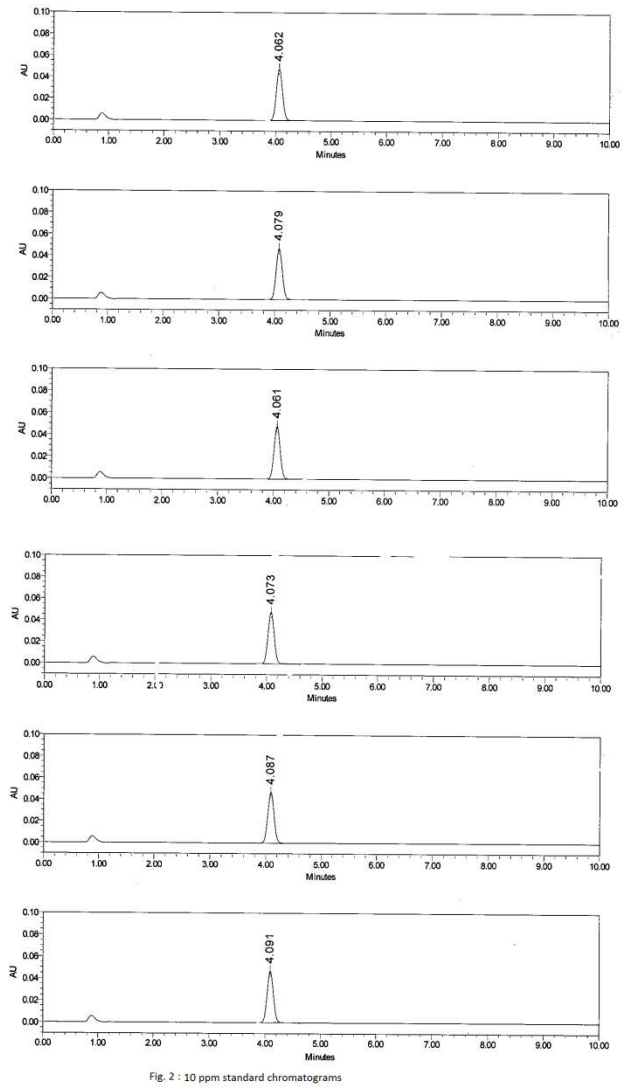

Fig. 2 : 10 ppm standard chromatograms

Method validation procedure

After successful completion of analytical method development and optimization, method validation to be initiated, demonstrates that method is suitable for its intended purposes. As per International Conference on Harmonization (ICH) guidelines, analytical method to be validated for system suitability, precision, specificity, linearity, limit of detection (LOD) and limit of quantification (LOQ), and recovery.

System suitability and precision parameter

System suitability and precision parameters are to be set for verification that analytical system is suitable and working properly with accuracy and precision. System suitability was carried out for freshly prepared 10ppm Iguratimod standard solution by injecting in six replicates. As acceptance criteria for check of %RSD, proves that methodology is accurate and precise. RSD limit for area response is not more than 10%, whereas RSD for retention time is not more than 1%. The values were recorded in Table 2.

Table 2 System suitability and precision parameters

System suitability and precision parameters		
Injection number	**Area of Iguratimod standard (10ppm)**	**Retention time (minutes)**
1	792911	4.062
2	799401	4.079
3	799161	4.061
4	799214	4.073
5	790615	4.087
6	795174	4.091
Average =	796079	4.076
SD =	3770.2302	0.0125
%RSD =	0.47	0.31
Acceptance criteria=	NMT 10.0%	NMT 1.0%

Iguratimod 10 ppm standard preparation

Dissolve 25 mg of Iguratimod in a 50mL volumetric flask; add about 30 ml diluent. Sonicate to dissolve and make up the volume up to the mark with diluent (Stock solution). Further dilute 10ml of this stock solution to 20ml with diluent. Dilute 1ml of resultant solution to 25ml with diluent. Thus, we got the standard solution of Iguratimod of a concentration of 10ppm (µg/ml). From the Table 2, it can be concluded that the system suitability and precision parameters meet the requirements of method validation.

Specificity

Specificity is access by measuring the ability of a method to determine the possible components in presence of analyte such as impurities, degradation products, and matrix components.

For cleaning method, residue of drug substance, drug product, excipients, etc are to be quantified. Demonstration for absence of interference must be performed initially. Also co-elution is major concern during development. Peak purity can be calculated by using the photodiode detector and is calculated by HPLC software.

During cleaning process, analyte component should not be affected. Recovery study can be performed during development, if satisfactory recovery is achieved for the methodology, method can be used. If not, method needs to be modified or new method to be developed.

Specificity parameter was carried out by using 10 ppm standard solution of Iguratimod and diluent blank. Based on the data tabulated in Table 2, Fig. 3, analytical method is specific, that is there is no interference of blank peaks with Iguratimod standard solution.

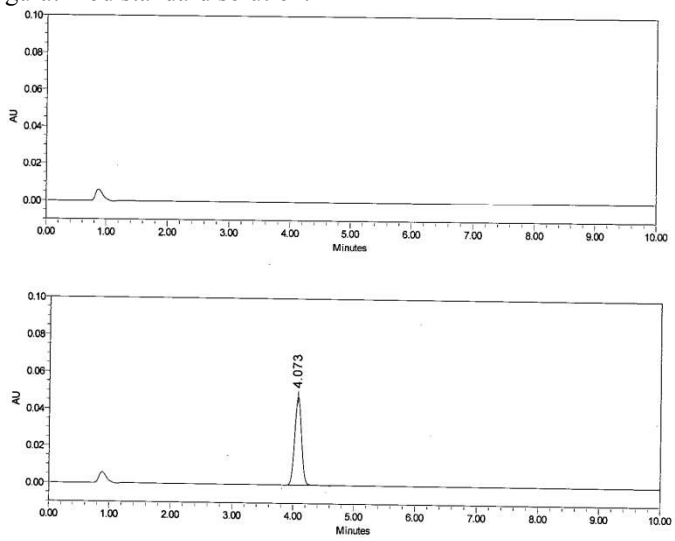

Fig 3 : Blank and standard chromatogram

Linearity and Range

Linearity access the ability of assay to get a proportionate response to change in analyte concentration.

Some type of detector produce nonlinear responses, like Gas chromatography with flame photometric detector, evaporative light scattering or mass spectrometers may have limited linear range as compared to Ultraviolet-Visible detector, Flame ionization detectors etc, however specific ranges could be established for these type of detectors. Upper and lower concentration of analyte which shows precision, linearity, accuracy can be stated as range.

As per ICH, validation of analytical procedures: text and methodology Q2(R1), range for assay of drug substances is 80 -120% with respect to test concentration, for content uniformity of drug product range is 70- 130%, for impurity detection range is from reporting level to 120% of specification limit.

For cleaning validation range will be much greater than specified in ICH guideline to monitor the amount of residue in monitoring sample. For lower range, process monitoring samples will not allow to warn the potential problems.

Key requirement of for range is accuracy, linearity and precision. There are several method ranges from simple observation to comprehensive statistical evaluations. Method choice will be normally vary to vary by pharmaceutical company policies. Specific methodology should be mentioned in protocol, if it nor mentioned in the standard operating procedure (SOPs). Criteria for linearity should be set prior to validation, e.g. For precision for each level in linearity, relative standard deviation (RSD) should be less than 3%, regression coefficient r^2 should be greater than 0.999 with respect to predefined range. The requirements of validation parameters and acceptance criteria may vary by type of method, instrument, analyte, etc. RSD may vary greatly based on concentration level of analyte. For linearity minimum five concentration levels are analyzed and minimally in duplicate. Commonly practice is triplicate analysis and also followed by most of the pharmaceutical companies.

For linearity, solution with different concentration are being prepared and injected. The series of solutions should encompass the range of results expected from analysis of actual samples. The y-intercept value is a good indication of bias. If the y-intercept is 0, no bias exists. Bias exists if the y-intercept deviates from 0.

Linearity of the analytical method is ability to obtain the results, which are well defined mathematical transformation, in proportion with analyte concentration within working concentration range. Optimized analytical method has been validated as per ICH guidelines, standard solution injected in the between the concentration range of 0.2 ppm to 15 ppm. Area response obtained for each concentration level was tabulated. Linearity was plotted for area response on Y axis and applied concentration on X-axis. Correlation coefficient and squared correlation coefficient was found to be 0.9999. Linearity plot and values are recorded in Table 3 and fig 4.

Regression analysis performed by plotting concentration (ppm) on X-axis and area response on Y-axis. Calculated standard error on Y axis (Steyx) and slope of the linearity curve generated with concentration area response from 3 and Fig.4.

Table 3 Linearity different levels of concentrations and LOD, LOQ prediction

Linearity & Lod, Loq Prediction		
Sr. No.	**Actual concentration (ppm)**	**Average area response**
1	0.10	8837
2	0.20	16825
3	0.50	39927
4	5.02	397128
5	8.04	645199
6	10.05	794207
7	12.06	952566
8	15.07	1188423
Correlation coefficient =		1.0000
Squared correlation coefficient =		0.9999
Standard error of Y Axis(STEYX) =		3905.0725
Slope =		78927.8952
Predicted LOD in ppm (STEYX x 3.3/Slope)=		0.1633
Predicted LOQ in ppm (STEYX x 10/Slope)=		0.4948

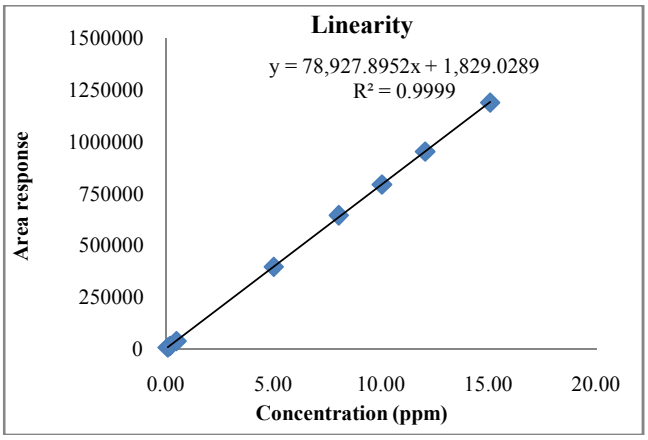

Fig 4 Linearity Plot for Actual concentration (ppm) Area response

From the Table 3, it is clear that observed area response of Iguratimod versus actual concentration in ppm of Iguratimod is linear in the required concentration range. Correlation coefficient and squared correlation coefficient calculated from the regular plot are >0.999. Hence, this analytical method is

linear for specified working concentration range for residual determination of Iguratimod.

Limits of Detection and Quantitation

Introduction of Limit of detection and quantitation was done by the American Chemical Society (ACS). Limit of detection of analytical method is the lowest amount of analyte determined but not necessarily quantified, whereas limit of quantitation is the lowest limit of analyte quantitatively determined with suitable accuracy and precision.

For assay test for drug substance or drug product, LOD and LOQ are not of matter of concern, because working concentration range is very much on higher side. For cleaning validations analyte concentration is on lower side and results are sometimes are reach upto to these lower level i.e. either LOD or LOQ. Hence LOD and LOQ determination and validation is required, also it needs to be defined in analytical procedure used for cleaning validation. General method for LOD and LOQ determination is by signal to noise ratio. Now a day's chromatographic software is available for determination of LOD and LOQ. LOD will be the three times concentration of the signal to noise ratio, where as LOQ will be the ten times concentration of the signal to noise ratio.

LOD and LOQ determination can be calculated by different methods; in regularity document no any specific method is recommended. Hence method for LOD and LOQ determination needs to be written in official document like standard test procedure or validation study protocol.

All literature based LOD and LOQ determination techniques assumes that, there is no variation in matrix which will give constant noise level throughout the experimentation. But in practical there is difference in matrix which impact on the recovery of residue from the surface of manufacturing equipments.

Limit of detection (LOD) is the lowest amount of analyte can be detected but not necessary to be quantified under specified analytical condition. Limit of quantification (LOQ) is the lowest amount of analyte quantified with acceptable precision, under the specified analytical conditions.

LOQ solution precision

From the Table 5 precision results, it can be concluded that the cleaning method validation is precise at LOQ at concentration 0.5 ppm and LOD at concentration 0.2 ppm level.

RSD for area response obtained from six replicate injections for Iguratimod at LOQ level should be not more than 10%.

Table 4 Limit of detection (LOD) establishment

LOD ESTABLISHMENT	
Sr. No.	**Area response for concentration 0.2 ppm**
1	17053
2	17593
3	17236
4	17665
5	18001
6	17256
Average area =	17467

Accuracy

Accuracy is generally referred as recovery or trueness of the results. Accuracy can be defined as closeness of analytical results to the true value. Accuracy is performed by evaluation of known concentrations of analyte across the analysis range. Results can be expressed in percentage.

Table 5 Limit of quantification (LOQ) establishment

LOQ ESTABLISHMENT	
Sr. No.	**Area response for concentration 0.5 ppm**
1	40955
2	39969
3	39658
4	40521
5	40329
6	42589
Average area =	40670
Standard Deviation (SD) =	1040.9431
% Relative standard deviation (RSD) =	2.56

Accuracy assures the analyte concentration in the matrix. Matrix can be inprocess samples, intermediate, finished product, etc.

In some cases recovery may be upto 100% but in some cases it may be upto 50%. For cleaning specific as well as nonspecific method are sued. For nonspecific methods (e.g. TOC) total response is to be assumed of analyte. Sampling generally done by indirect method e.g. Rinse solutions. But direct methods are mostly preferred like swabbing. This choice of selection of directs / indirect method is generally depends on accessibility of the surface to be sampled and solubility of the residue.

For specific method like HPLC, for rinse samples (Indirect sampling), interference of matrix need to be show over the working concentration range. Matrix components may be excipients, drug substance, drug product, degradation impurities, solvents, cleaning agents, etc. Recovery can be evaluated by spiking analyte in the sample containing all possible impurities. Solvent used in the experiments should be representative of those used to final rinse of equipment being cleaned.

Range is typically 25 to 150% of allowable limit of analyte. Also it needs to be documented the stability of analyte in the solution. For swan sample, the task becomes more difficult as it needs to evaluate the ability of swab to remove the analyte from the surface being cleaned, ability of swab to remove the analyte during solution preparation.orm swab, potential of interference from impurities, excipients, cleaning agents and swabbing material. Accuracy of method is generally established during method development to avoid the surprise during validation activity.

For cleaning validation experimentation, a coupon represents the surface area to be use for cleaning. Composition of coupon may be of size 5x5 inches with material stainless steel SS304 /SS316, Teflon, Tygon or other contact material as per requirements. Accuracy solution typically 100µl are to be applied on coupon surface and coupon allowed to dry. If the equipment is being heated during cleaning then the coupons can be placed in oven to simulate the temperature seen during cleaning process. Coupons during drying process needs to be keep on flat surface and protected from any contamination. Once surface is dried, analyte is to be recovered by rubbing the swab uniformly across the surface of cou7pon. Prior to swabbing, swab is to be dipping in the solvent that is to be used for extract the residue from the surface to be clean.

Excess solvents can be removed by squeezing the swab inside the vial, containing extraction solvent. Swab is rubbed along the surface of coupon from vertical side as well as horizontal

side. Periodical rotation of swab on both sides is required to clean then surface.

In some cases second swab may be used to clean the remaining analyte form the coupon surface. But the second dry swab generally picks up the solvent s well as residue left by first swab. Sometime second swab wet with same extraction solvents can be used to clean the remaining analyte stuck to the coupon surface. Any established swabbing pattern can be used and kept uniform during the recovery study as well as in actual sampling of the equipment. Insufficient training to the analyst is major source of errors. Sop should define the swabbing patter, swab quality, extraction solvent to be used drying activity with quantity of extraction solvents, etc.

Allow the swab to be immersed fully in the extraction solvent and may often allowed to be immersed for long period or sonicated to produce the highest recoveries. These studies are to be performing for samples are stable for long period.

Results are expressed as recovery and compared with the theoretical values. Blank study is to be performed to check the interference of present system, Ideal systems which do not have any interference. However blank values to be subtracted form the other results to adjust the possible interference is acceptable.

Acceptable % recovery in cleaning validation is source of debate. Recovery value can be used in calculating the observed value or factor to be applied for acceptance criteria.

For example, the % recovery cleaning method is 75% and acceptable limit is 5 ppm, then 5ppm x 0.75 = 3.75 will be the new limit. This factor should apply to acceptable limit only and not for analytical results.

surfaces. Recovery for spiked sample was performed by rinsing the surface with acetonitrile.

Selected three cleaned and dried 10 cm×10 cm surface area stainless steel plates. Spread 10 mL of the spiking solution on dried 10 cm×10 cm surface area steel plates, taking utmost care to avoid any spillage. Dry the plate at room temperature. Using 25 mL of accurately measured diluent recover the test sample from 10cm × 10cm surface area stainless steel plate, by gentle swirling. Filter the solution and inject into high-pressure liquid chromatography, performed the exercise in triplicate.

Finally recorded the area of the test sample in rinse recovery on stainless plate in Table 6 than calculate the % rinse recovery below formula. From the Table 6 results, it can be concluded that % of rinse recovery on SS plate is consistently above 90%. The values obtained above are in good agreement in terms reliability, suitability, and accuracy of the proposed method

Precision

Precision is measurement for series of results close to each other. Precision can be divided in three subparts; Repeatability, intermediate precision and reproducibility. Precision is expressed in percentage of relative standard deviation.

Repeatability is precision under same operating condition over a short time interval, e.g. Sample injected six times and area response evaluated for all six replicate injections with respect tom each other. This parameter measures precision of instrument, like integrator, data collector, integrator etc. For auto injector, need to take care for injector septa, so that it should not be damaged during analysis, otherwise this may introduce the error during precision study.

Table 6 % Rinse recovery results

Rinse recovery								
Sr. No.	**Level**	**Spiked concentration (ppm)**	**Area response**	**Recovered concentration (ppm)**	**% Recovery**	**Mean % Recovery**	**SD**	**% RSD**
1	LOQ level (0.5ppm)	0.5043	37521 36329 38589	0.4764 0.4613 0.4900	94.47 91.47 97.16	94.37	2.8464	3.02
2	50% level (5 ppm)	5.043	378137 367796 368670	4.8015 4.6702 4.6813	95.21 92.61 92.83	93.55	1.4418	1.54
3	100% level (10 ppm)	10.086	773705 776903 775692	9.8243 9.8649 9.8495	97.41 97.81 97.66	97.63	0.2021	0.21
4	150% level (15 ppm)	15.129	1172772 1181436 1183552	14.8915 15.0015 15.0284	98.43 99.16 99.34	98.98	0.4819	0.49

For low recovery, major source of reduced recovery is to be ascertained. Spike the swab material with extraction solvent and check for recovery. If recovery still on lower side, extraction solvents or alternate swabbing material to be used. If recoveries are found within acceptable limit, problem of low recovery may be due to removal of residue form coupon itself. Again alternative swabbing material or extraction solvents can be tried. Volatility of the extraction solvents should not be overlooked while performing the recovery.

To study of the reliability, suitability, and accuracy of the method recovery experiments were carried out for cleaning method validation for residual determination of Iguratimod.

The rinse recovery of the sampling method shall be established by spiking a solution of known concentration on both stainless

Along with area response, retention time measurement is also parameter of precision.

Intermediate precision is measure of intra-laboratory variations, e.g. Sample analysis on different day, by different analyst, by using different chemicals and reagents, by different analytical column (for HPLC, GC etc), different laboratory, etc. Intermediate precision measures the precision between analysts and instruments. Major problems are observed due to change in environmental conditions like temperature, humidity, etc. For cleaning validation by swab study, different make or lot number of swab may be used.

Reproducibility can be measured by analysis in different laboratories; it's a measure of precisions between different laboratories. During transfer of method from one laboratory to

another laboratory, due to different material of construction and cleaning procedures may be rise the concern for repeatability and intermediate precision. As per ICH guideline for reparability, minimum nine determinations over the working concentration range or six determinations at specification level are recommended.

Accuracy and repeatability of method generally changes with analyte concentration. As the determination of lower concentration level wrt to higher concentration level, significant variation will be observed for accuracy and precision.

experimentation by varying the chromatographic parameters in stepwise process, like mobile phase composition, mobile phase pH, flow rate of mobile phase, column oven temperature, injection volume etc.

There is no significant impact on chromatography by varying chromatographic parameters within allowable range, like flow rate by ±10%, mobile phase composition by ±10%, mobile phase pH by ±0.1, etc.

Table 7 System precision

System Precision				
No.	**Area**	**Average**	**Standard deviation**	**%Relative standard deviation (%RSD)**
Injection -1	792911	796079	3770.2302	0.47
Injection -2	799401			
Injection -3	799161			
Injection -4	799214			
Injection -5	790615			
Injection -6	795174			

Table 8 Method precision

Method Precision								
Sr. No.	**Level**	**Spiked concentration (ppm)**	**Area response**	**Recovered concentration (ppm)**	**% Recovery**	**Mean % Recovery**	**SD**	**% RSD**
1	Method Precision (Analyst-1)	10.086	787334	9.9515	98.67	94.61	2.3682	2.5
			760482	9.6121	95.30			
			754349	9.5346	94.53			
			742772	9.3882	93.08			
			731436	9.2449	91.66			
			753552	9.5245	94.43			
2	Method Precision (Analyst-2)	10.086	743705	9.8044	97.21	98.50	3.1318	3.18
			766903	10.1103	100.24			
			795692	10.4898	104.00			
			731237	9.6401	95.58			
			747196	9.8505	97.67			
			736700	9.7121	96.29			

Table 9 Intermediate precision (Cumulative results)

Precision (Overall)					
Analyst	**Test**	**% Recovery**	**Overall % Recovery**	**SD**	**%RSD (Cumulative)**
Analyst-1	Sample-1	98.67	96.56	3.3358	3.45
	Sample-2	95.30			
	Sample-3	94.53			
	Sample-4	93.08			
	Sample-5	91.66			
	Sample-6	94.43			
Analyst-2	Sample-1	97.21			
	Sample-2	100.24			
	Sample-3	104.00			
	Sample-4	95.58			
	Sample-5	97.67			
	Sample-6	96.29			

Ruggedness

Analytical method should be so rugged even it is used by variety of personnel. Method should not be affected by small changes occurred during day to day activity, e.g. generally chromatographic method in which column heater is not used, should not affected by change in laboratory temperature. If there is impact on chromatographic method, then it should be evaluated and defined. No any regulatory agency ask for ruggedness study and no need to incorporate in validation report, however it is assumed by regulatory agency that effect of various parameters have been studied during method development. Those parameters which are doing affect the chromatography are controlled and documented in the methodology. Ruggedness can be evaluated through

Summary

Analytical method validation report is the final output of analytical method validation activity. Analytical method validation report should content all the parameters studied including the pre-establishment criteria. Parameters required in method validation should be established during method development and then it should be defined thoroughly in method validation process. Understanding of analytical method validation not limited to analyst who perform the activity but also the personals who are involved in cleaning validation process. It is very much important to all the personnel to understand that changes in overall cleaning validation process may affect the method and lead to necessity of revalidation of analytical method.

Table 10 Summary and evaluation of results

Validation parameters	Acceptance criteria	Results
System suitability	The % of RSD for the area response of Iguratimod peak obtained from six replicate injections of system suitability should be NMT 5.0%	System suitability parameter meets the criteria % of RSD = 0.47%
Specificity	The peaks of blank should not interfere with Iguratimod peak	The peaks of blank do not interfere with Iguratimod peak Individual solutions Peak name — Retention time (in minutes) Blank — No peak System suitability solution — 4.062
Linearity	The correlation coefficient and the squared correlation coefficient between concentration and area response of Iguratimod should be NLT 0.995	The method is linear Correlation coefficient=1.0000 Squared correlation coefficient=0.9999
LOD/LOQ	The RSD for area response of Iguratimod from six replicates at LOQ level should be NMT 10.0%	The RSD for area response of Iguratimod from six replicates at LOQ level=2.56% LOQ in ppm = 0.50 ppm LOD in ppm = 0.20 ppm
Recovery study	Report the % rinse recovery if the % rinse recovery is less than 90.0% then incorporate recovery factor to analytical method	% Rinse recovery Level — Mean % recovery At LOQ level — 94.37 At 50% level — 93.55 At 100% level — 97.63 At 150% level — 98.98

RSD: Relative standard deviation, SD: Standard deviation, LOD: Limit of detection, LOQ: Limit of quantification.

For recovery study, report the % rinse recovery if the %rinse recovery is less than 90.0% then incorporates the recovery factor to the analytical method.

RESULTS

It is required to develop fast, cost effective, precise, and sensitive analytical cleaning method. The primary target in developing and validate this cleaning RP-HPLC method is to achieve the quantification at lower level. All residues are removed to predetermined levels to ensure the quality of the next product and also to prevent cross contamination and as a good manufacturing practices requirement. Based on the above-observed results, the developed cleaning method validation for Iguratimod method is valid and run successfully, the summary and evaluation of results are in below Table 10.

CONCLUSION

A validated reversed phase high-pressure liquid chromatography cleaning method has been developed for cleaning method validation for residual determination of Iguratimod cleaning samples. The proposed method is simple, rapid, accurate, precise, and specific. Its chromatographic run time of 10 minutes allow the analysis of a large number of cleaning samples in a short period. Therefore, it is suitable for the routine analysis of Iguratimod cleaning samples in active pharmaceutical ingredients bulk drugs.

References

1. Shuntaro Takano, Chosaku Yoshida, Takihiro Inaba, Keiichi Tanaka, Ryuko Takeno, Hideyoshi Nagaki, Tomoya Shimotori, 4H-1-benzopyran-4-one derivative or its salt, process for producing the same and pharmaceutical composition comprising the same as active ingredient., *Toyama Chemical Company*, Ltd., Tokyo, Japan US4954518, 4 Sept, 1990.
2. Wang Jinyi, Li Xudong, Lin Guoqiang, Zhang Zheng Gen, Wang Lin, Lu Wen bud Preparation of 3-(formamide)-7-(methylsulfonyl amine)-6-(phenoxy)-4H-1-(benzopyran)-4-ketone., *Jiangsu Yangtze River Pharmaceutical Group Co. Ltd.,* CN 1462748. *aceutical Group Co. Ltd.,* CN 1462748.
3. Takihiro Inaba, keiichi Tanaka, ruuko takeno, hideyoshi nagaki, Chosaku Yoshida, Shuntaro takano, Synthesis and Antiinflammatory Activity of 7-Methanesulfonylamino-6-phenoxychromones. Antiarthritic Effect of the 3-Formylamino Compound (T-614) in Chronic inflammatory disease models.*Chem. Pharma. Bull,* 2000; 48(1): 131-139.
4. Shanghai Huagong, 2008; 32(12): 22-24.
5. Wang Yan Xiang, Gao Hong, Cao Feng hua, Song Dan Qing, Synthesis of Iguratimod *Zhongguo Xinyao Zazhi,* 2006; 15(23): 2042-2044.
6. Huagong_Shikan, 2010; 24 (9): 267[1]).
7. International Conference on Harmonization (ICH) of Technical Requirements Guide Lines of Analytical Method Validation Procedures: Text and Methodology: Q2 (R1). Marcel Dekker; 1997. Available from: http://www.dekker.com.
8. US FDA, Guidelines for submitting samples and analytical data for method validation, Rockville, MD, Center for Drugs and Biologics Department of Health and Human Services; 1987.
9. International Conference on Harmonization (ICH), 2005. Harmonised Tripartite Guideline: Validation of Analytical Procedures: Methodology (Q2B), 2005.
10. United States Pharmacopeia (USP) 34 (NF 29), Chapter 兢621兜, Edition 2011.
11. Szepesi G. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. III. Method validation. *J Chromatogr*. 1989;464: 265-278.
12. Carr GP, Wahlich JC. A practical approach to method validation in pharmaceutical analysis. *J Pharm Biomed Anal.* 1990;86:613-618.

13. 13.General Chapter, Validation of compendial methods, United States Pharmacopeia, 26th Revision, National Formulary, 21st Edition, Rockville, MD, The United States Pharmacopoeial Convention, Inc, 2440; 2003.
14. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures, ICH-Q2A, Geneva; 1995.
15. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: *Methodology*, ICH-Q2B, Geneva; 1996.
16. US FDA Technical Review Guide: Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CDER), Rockville, MD; 1993.
17. US FDA, General principles of validation, Rockville, MD, Center for Drug Evaluation and Research (CDER); 1987.

How to cite this article:

Ravindra Bhaskar Nehete and Pushpendra Sharma (2018) 'Analytical Method Development and Validation of Cleaning Methodology for Residual Determination of Iguratimod', *International Journal of Current Advanced Research,* 07(2), pp. 9746-9754. DOI: http://dx.doi.org/10.24327/ijcar.2018.9754.1625

****