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DEVELOPMENT & VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF RELATED SUBSTANCE OF BILASTINE & MONTELUKAST IN SUSPENSION DOSAGE FORM

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ABSTRACT

A simple, precise and accurate newly developed and validated method for Stability Indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for Simultaneous Estimation of Montelukast Sodium and Bilastine from its suspension dosage form. A reverse phase high performance liquid chromatographic method was developed for the determination of Related substance for Montelukast Sodium and Bilastine in suspension Dosage Form. The separation was achieved by Hypersil BDS C18 5µ column (150 x 4.6 mm) Column and Buffer: Acetonitrile as mobile phase, at a flow rate of 1.0 ml/min. Detection was carried out at 215 nm. Retention time of Montelukast Sodium and Bilastine was found to be 20 min. and 12 min. respectively. Sulfoxide impurity & styrene impurity are known impurities in Montelukast sodium while N-Oxide impurity & Hydroxy impurity are known impurities in Bilastine. The RS method has been validated for linearity, accuracy and precision. Developed method was found to be accurate, precise and rapid for Simultaneous Related substance detection in Montelukast Sodium and Bilastine in suspension dosage form. The method was validated according to ICH guidelines Q2 (R1).

KEYWORDS: Analytical Method Development, Analytical Method Validation (AMV), High Performance Liquid Chromatography (HPLC), Stability Indicating Method, Bilastine, Montelukast Sodium.

INTRODUCTION

Montelukast

The medication is a member of the leukotriene receptor antagonist (LTRA) category of drugs. Although capable of demonstrating effectiveness, the use of such LTRAs like

montelukast is typically in addition to or complementary with the use of inhaled corticosteroids or other agents in asthma step therapy. Regardless, in 2008-2009, there were FDA-led investigations into the possibility of montelukast to elicit neuropsychiatric effects like agitation, hallucinations, suicidal behaviour, and others in individuals who used the medication.

Structure

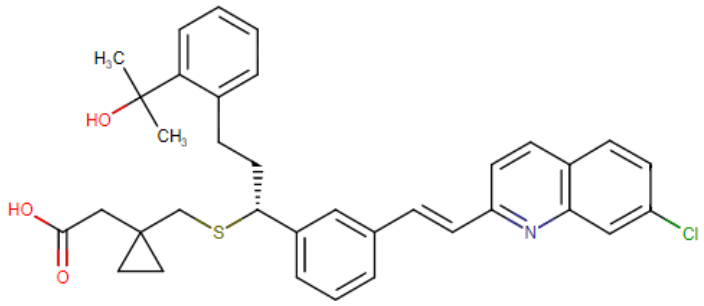

Fig 1: Cemical structure of Montelukast.

Molecular formula: $C_{35}H_{36}CINO_3S$

Molecular Weight: 586.2

Solubility: Montelukast (sodium salt) is soluble in organic solvents such as ethanol, Dimethylsulfoxide, and dimethyl formamide, which should be purged with an inert gas. The solubility of montelukast (sodium salt) in these solvents is approximately 30 mg/ml. It is also soluble in water at a concentration of 10 mg/ml.

Pharmacology

Montelukast is in the leukotriene receptor antagonist family of medications. It works by blocking the action of leukotriene D4 in the lungs resulting in decreased inflammation and relaxation of smooth muscle.

Montelukast functions as a leukotriene receptor antagonist (cysteinyl leukotriene receptors) and consequently opposes the function of these inflammatory mediators; leukotrienes are produced by the immune system and serve to promote bronchoconstriction, inflammation, microvascular permeability, and mucus secretion in asthma and COPD. Leukotriene receptor antagonists are sometimes colloquially referred to as leukasts.

Uses: Montelukast is used regularly to prevent the wheezing and shortness of breath caused by asthma and decrease the number of asthma attacks and coughing caused by asthma in adults and children 12 months of age and older. Montelukast is also used before exercise to prevent breathing problems during exercise (bronchospasm). Montelukast is also used to prevent bronchospasm (breathing difficulties) during exercise in adults and children 6 years of age and older. Montelukast is also used to treat the symptoms of seasonal (occurs only at certain times of the year), allergic rhinitis (a condition associated with sneezing and stuffy, runny or itchy nose) in adults and children 2 years of age and older, and perennial (occurs all year round) allergic rhinitis in adults and children 6 months of age and older. Montelukast should be used to treat seasonal or perennial allergic rhinitis only in adults and children who cannot be treated with other medications. Montelukast is in a class of medications called leukotriene receptor antagonists (LTRAs). It works by blocking the action of substances in the body that cause the symptoms of asthma and allergic rhinitis.

Bilastine

Bilastine is a novel new-generation antihistamine that is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action.

Structure

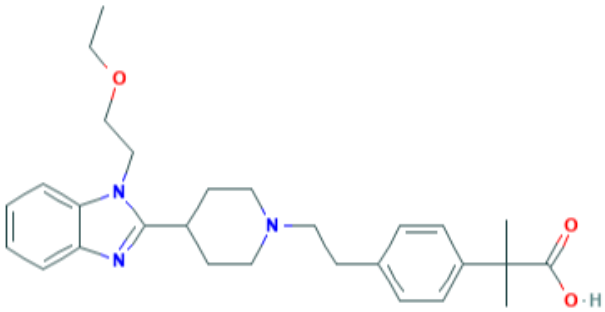

Fig 2: Chemical structure of Bilastine.

Molecular formula: $C_{28}H_{37}N_3O_3$

Molecular Weight: 463.6

Solubility: Soluble in chloroform, slightly soluble in methanol and insoluble in Acetonitrile.

Pharmacology: Bilastine is an antiallergenic and acts to reduce allergic symptoms such as nasal congestion and urticarial.

Mechanism of action: Bilastine is a selective histamine H1 receptor antagonist (Ki = 64nM) Label. During allergic response mast cells undergo degranulation which releases

histamine and other subastances. By binding to and preventing activation of the H1 receptor, bilastine reduces the development of allergic symptoms due to the release of histamine from mast cells.

Uses: Bilastine has been effective in the treatment of diseases of allergies, including rhinoconjuctivitis. Additionally, bilastine has been shown to improve quality of life, and all nasal and eye symptoms related to allergic rhinitis.

REVIEW OF LITERATURE

Sr. no.	Method	Description	Ref. no.
1.	Bilastine detection method	Stationary phase - C18 or C8 Mobile phase – inorganic salt buffer solution containing an ion pair reagent and an organic solvent Column temperature- 35-45^{o}C Flow rate- 0.8-1.2 ml/min Detection- 254nm	[23]
2.	RPHPLC method for simultaneous estimation of montelukast sodium & Bilastine	Stationary phase- C18 5µ 250 x 4.6mm Mobile phase- Acetonitrile : water Flow rate- 1 ml/min Detection- 215 nm	[24]
3.	Determination of Bilastine & its impurities by HILIC method	Stationary phase- Luna HILIC (100 mm x 4.6 mm, 5 µm particle size) Mobile phase- Acetonitrile-aqueous phase (50mMammonium acetate, pH adjusted to 5.3 with glacial acetic acid) (90.5:9.5, v/v) Column temperature- 30^{o}C Flow rate- 1 ml/min Detection- 275 nm	[25]
4.	Determination of Bilastine by UV spectrophotometric method	Solvent- 0.1M HCl Detection- 210 nm	[26]
5.	A new stability indicating RP-HPLC method for determination in bulk & pharmaceutical formulation	Stationary phase- Phenomenex Gemini C18 (150 x 4.60mm) 5 µm particle size Mobile phase- formic acid: methanol (50:50v/v) Flow rate- 0.8 ml/min Detection- 282 nm	[27]
6	Analytical method development & validation for estimation of Bilastine & Montelukast sodium in their combined dosage from by derivative UV-spectroscopy & RP-HPLC method	Column- C18 hyper chrome ODS-BP 250 x 6 mm, 5 µm Mobile phase- Acetonitrile: potassium dihydrogen orthophosphate (80:20v/v) pH 3.5 adjusted with OPA Flow rate- 1.2 ml/min Detector- 285 nm	[28]

7	Analytical method development of montelukast & Fexofinadine combination in pharmaceutical dosage form by HPLC method	Column- BDS hypersil C18 (250 x 4.6mm) 5 µm Detector- 230 nm Injection volume- 20 µL Flow rate- 1 ml/min Temperature- 40^{o}C Mobile phase- buffer : methanol (30:70)	[29]
8	Development & validation of a RP-HPLC method for estimation of montelukast sodium in bulk & tablet dosage form	Column- C18 Mobile phase- acetonitrile: 1mM sodium acetate adjusted to pH 6.3 with acetic acid in proportion of 90:10v/v Flow rate- 1.5 ml/min Detector- 285nm Retention time of montelukast- 3.4 min	[30]
9	Stability indicating HPLC method Simultaneous Determination of Montelukast and Fexofenadine Hydrochloride	Stationary phase- Lichrospher 100, RP-18e column (250 x 4.6mm, 5 µm Mobile phase- methanol : 0.1%OPA (90:10v/v) pH adjusted to 6.8 Detector- 226 nm	[31]

Official methods

Sr. no.	Official In	Method	Description	Ref. no.
1.	IP 2018	Montelukast sodium: Chromatographic method	Column: Hypersil ODS Octadecylsilane (15cm×4.6 mm, 5µm) Mobile phase A : Dissolve 3.85g of ammonium acetate in 1000 ml of water, add 1ml of triethylamine, adjusted to pH 5.5 with glacial acetic acid Mobile phase B: Methanol Wavelength: 240nm Flow rate: 1ml/min Injection volume: 20µl	[32]

SOLUBILITY TEST: Solubility of drug substance in different solvents/diluents.

For Bilastine: Solubility of Bilastine was checked in chloroform, methanol, Acetonitrile and following are the outcomes:

Result: Soluble in chloroform, slightly soluble in methanol and insoluble in acetonitrile.

For Montelukast: Solubility of Montelukast was checked in ethanol, methanol, water, Acetonitrile and following are the outcomes:

Result: Freely soluble in ethanol (95%), in methanol and in water, practically insoluble in acetonitrile.

WAVELENGTH SELECTION: To finalize the UV wavelength of drug substance for its detection in HPLC.

For Bilastine

Observation

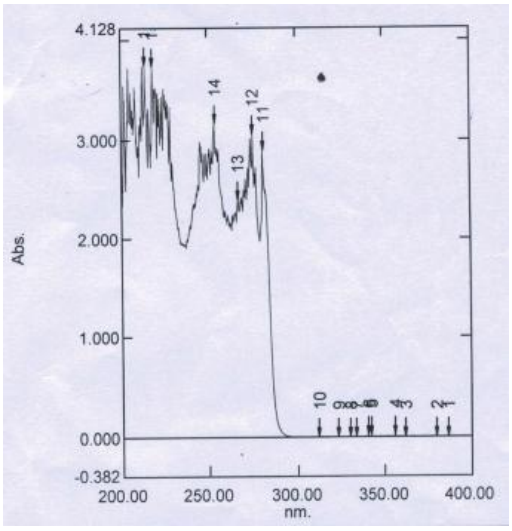

Fig 3: UV-Spectrum of Bilastine.

For Montelukast

Observation

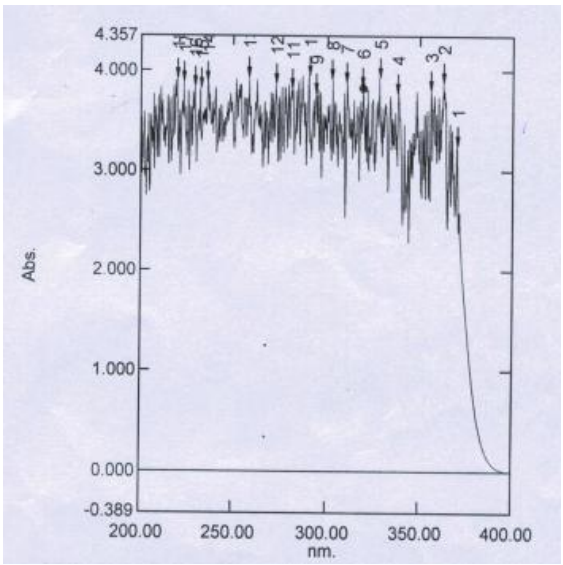

Fig 4: UV-Spectrum of Montelukast sodium.

METHOD

For Bilastine

Mobile Phase: Buffer (pH-4.5): Acetonitrile (70:30).

Chromatographic conditions

Standard Column type	:	Hypersil BDS, C18, 150mm x 4.6 mm, 5µm.
Flow rate	:	1.0ml/min.

Detector parameter	:	At UV wavelength 215nm
Injection volume	:	20µl
Column oven temperature	:	40°C
Run Time	:	30 minutes
Sampler Temperature	:	15°C
Bilastine Retention Time	:	About 10 Minute
Relative Retention Time of Hydroxy Impurity	:	About 0.60
Related Retention Time of N-Oxide Impurity	:	About 0.75

Test solution: Take weight sample (equivalent to 10mg of Bilastine) into a diluent and sonicate for 5 minutes with intermediate shaking dissolve and make up the volume with diluent. Filter through 0.45 µ syringe filter.

Reference stock solution (A): For Hydroxy impurity

Reference stock solution (B): For N-Oxide impurity

Reference stock solution (C): For Bilastine Standard

Take Bilastine in 100ml of volumetric flask, add 50ml diluent, sonicate approx. 5 min to dissolve, make up to the mark with diluent.

Reference Solution (Mixed): Dilute 1ml of each reference stock solution (A), (B) and (C) to 100ml with diluent by manual shaking.

Procedure: Separately inject equal volume of solution as per sequence of injection into the chromatogram and record the peak responses for major peaks and check for the system suitability requirements.

Sequence of injection

1) Blank (1)
2) Placebo (1)
3) Reference solution (mixed) (1….5)
4) Test solution (1)

System suitability requirements

The test is not valid unless,

1) The relative standard deviation for the peak area response for replicate injections of reference solution (mixed) is not more than 5.0%.
2) The number of theoretical plates should be not less than 1500 in standard solution.

3) Tailing factor of standard injection should be not more than 2.0.

For Montelukast

Mobile Phase (A): Take 1000ml water in suitable container and dissolve 1 ml Triethylamine with manual stirring. Adjust pH of the solution to 4.5 ±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter.

Mobile Phase (B): Acetonitrile.

Chromatographic conditions

Standard Column type	:	Hypersil BDS, C18, 150mm x 4.6 mm, 5µm.
Flow rate	:	1.0ml/min.
Detector parameter	:	At UV wavelength 215nm
Injection volume	:	20µl
Column oven temperature	:	40°C
Sampler Temperature	:	15°C
Montelukast Retention Time	:	About 20 Minute
Relative Retention Time of Sulfoxide Impurity*	:	About 0.50
Related Retention Time of Styrene Impurity	:	About 1.50

Note:*Sulfoxide Impurity elutes as Sulfoxide Impurity 1 (Montelukast Sulfone) & Sulfoxide Impurity 2 (Montelukast Sulfoxide Impurity).

Gradient Programming		
Time (In Minutes)	**M.P. (A) Concentration**	**M.P. (B) Concentration**
0	45	55
2	45	55
18	30	70
25	20	70
27	20	80
35	20	80
40	45	55
45	45	55

Test solution: Take weight amount of Sample by weight (eq. 4 mg Montelukast) into a 50ml volumetric flask, add 25ml diluent and sonicate for 5 minutes with intermediate shaking. Dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Reference stock solution (A): For Styrene impurity

Reference stock solution (B): For Sulfoxide impurity

Reference stock solution (C): For Montelukast Standard

Take weighed amount of Montelukast Sodium in 100ml volumetric flask, add 50ml diluent, sonicate approx. 5 min to dissolve, make up to the mark with diluent. Further dilute by taking 5ml of the solution in 50ml volumetric flask, make up to the mark with Diluent.

Reference Solution (Mixed): Dilute 2ml of each reference stock solution (A), (B) and (C) to 50ml with diluent by manual shaking.

Procedure: Separately inject equal volume of solution as per sequence of injection into the chromatogram and record the peak responses for major peaks and check for the system suitability requirements.

System suitability requirements

The test is not valid unless,

1) The relative standard deviation for the peak area response for Montelukast replicate injections of reference solution (mixed) is not more than 5.0%.
2) The number of theoretical plates should be not less than 1500 in standard solution.
3) Tailing factor of standard injection should be not more than 2.0.

METHOD VALIDATION STUDY

FOR BILASTINE

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Procedure: Prepare and inject blank, Placebo, Placebo with Montelukast (triplicate preparation), standard and test solution as per analytical method. Evaluate the system suitability and %impurities as per analytical method. Prepare individual impurity preparation (for identification) and spiked sample preparation (at respective specification level) as below and inject in single. Check interference from placebo and impurities if any and record the observation.

Individual Impurity solution preparation at respective specification level

Prepare individual impurity solution for all known impurities to obtain a final concentration of 20 ppm for each impurity.

Spiked sample preparation

Accurately weigh sample (equivalent to 10mg of Bilastine) into a 30ml diluent and sonicate for 5 minutes with intermediate shaking dissolve. Add to it appropriate volume of impurities stock solution (to obtain final concentration for each impurity as 1ppm) and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Acceptance criteria

There should be no interference of any peak due to empty capsule shell, placebo and blank with the main analyte and known impurities in sample preparation. Purity angle should be less than purity threshold.

System Suitability

Prepare the Standard solution as per the proposed test method and inject into the High Pressure Liquid Chromatography system by following the instrumental condition as per the test method. Record the system suitability parameters observed.

Acceptance criteria

1) The relative standard deviation for the peak area response for replicate injections of reference solution (mixed) is not more than 5.0%.
2) The number of theoretical plates should be not less than 1500 in standard solution.
3) Tailing factor of standard injection should be not more than 2.0.

Limit of Determination (LOD) and Limit of Quantification (LOQ)

Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Procedure

The detection and quantitation level for known and unknown impurity in Bilastine in Bilastine and Montelukast Oral Suspension will be established in term of Bilastine.

Prepare solutions of Bilastine standard and known impurities at different levels of test concentration and inject in single. Plot a graph between concentrations of analyte (ppm) (on-x-axis) Vs peak area of analyte (on-y-axis) from the linearity data. Determine the LOD & LOQ Using on standard deviation of intercept and slop of the linearity data.

Reference Solution (Mixed): Dilute 2ml of each reference stock solution hydroxy, N-oxide and 1ml of reference Stock solution Bilastine standard to 20ml with diluent by manual shaking.

Solutions for LOD and LOQ as per Detection

$$\textbf{Limit of detection} = \frac{3.3\sigma}{S}$$

$$\textbf{Limit of quantitation} = \frac{10\,\sigma}{S}$$

Where,

σ --- Standard deviation of Intercept of regression line.

S --- Slop of regression line

Prepare solutions for LOD & LOQ determination as in following table

Level	% with respect to test concentration	Concentration in ppm	Stock Solutions to be taken (ml)	Dilute to volume with diluent (ml)
1	0.05	0.1	0.1	10
2	0.15	0.3	0.3	10
3	0.30	0.6	0.6	10
4	0.40	0.8	0.8	10
5	0.50	1.0	1.0	10
6	0.60	1.2	1.2	10
7	0.75	1.5	1.5	10

Acceptance criteria: The % RSD of six replicate injection of LOD should not be more than 33.0% and for LOQ should not be more than 10.0%.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity of detector Response

Prepare the series of Standard concentrations ranging from LOQ, 50% to 150% of the targeted concentration of test sample of Bilastine and Montelukast Oral Suspension. Inject each of the linearity solution into the High Pressure Liquid Chromatography system.

Linearity Level	Stock Reference Solution (Mixed)) (ml)	Diluted to (ml)	ppm
At LOQ Level	**As Per Found**	**-**	**-**
At 50%	2.5	10	5
At 80%	4.0	10	8
At 90%	4.5	10	9
At 100%	5.0	10	10
At 110%	5.5	10	11
At 120%	6.0	10	12
At 150%	7.5	10	15

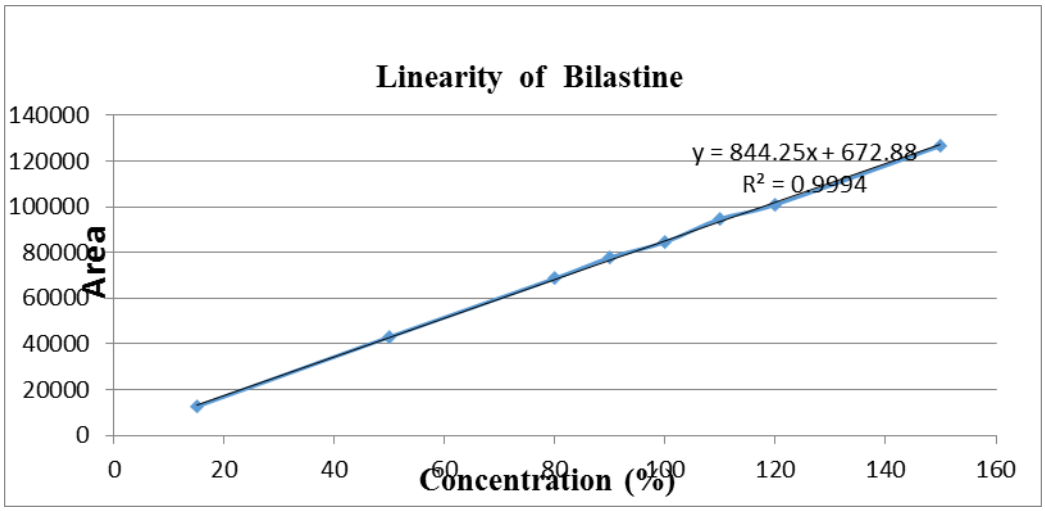

Fig 5: Linearity of Detector response For Bilastine.

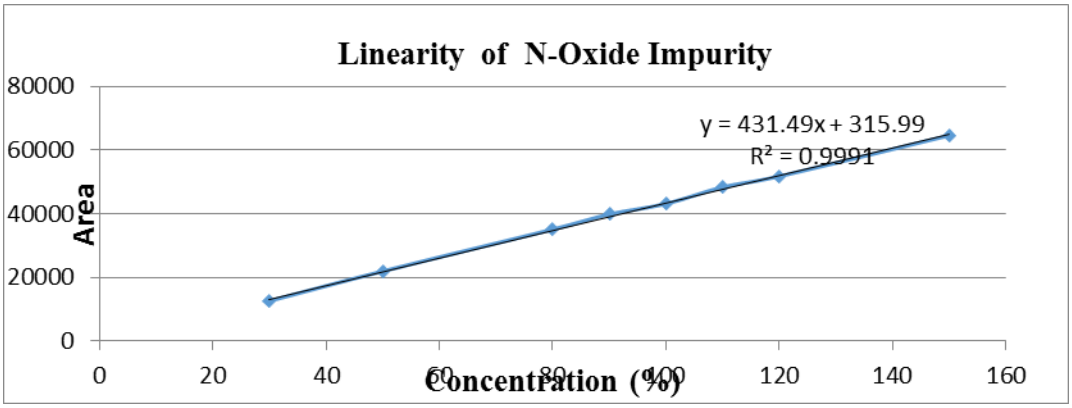

Fig 6: Linearity of Detector response For N-Oxide impurity.

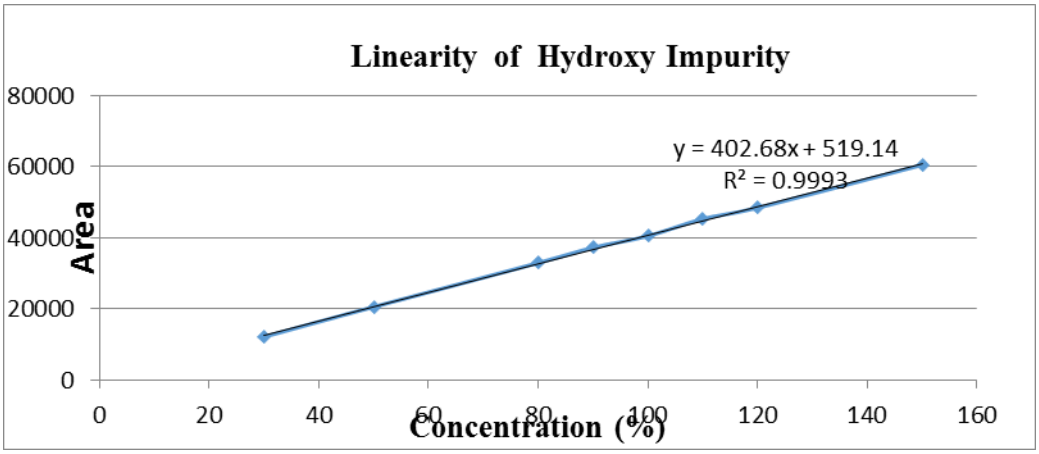

Fig 7: Linearity of Detector response For Hydroxy impurity.

Procedure: Separately inject Standard preparation and Linearity preparations into the chromatograph, record the chromatograms and measure the peak responses for the major peaks. Check the system suitability.

Acceptance Criteria: The correlation coefficient should be NLT 0.99 and Statistical Y intercept should be ± 3.

Precision Studies

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

System Precision

Prepare the Reference solution (mixed) proposed in test method and inject into the High Pressure Liquid Chromatography system in replicates. Evaluate the % RSD for the area responses and record the observations.

Acceptance criteria: The relative standard deviation for the peak area response for six replicate injections of reference solution (mixed) is not more than 5.0%.

Method Precision

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Procedure: Prepare and inject blank, Reference Solution (mixed), placebo with Montelukast preparation and standard preparation as per analytical method. Check for the system suitability. Prepare test sample as per analytical method in six replicates and inject each in single. Calculate % impurities.

Acceptance criteria: The %RSD of Known, Single maximum unknown impurity and total impurities should not be more than limit specified below as per the result observed.

Result Observed % RSD

≤ 0.10 % 15.00%

> 0.10 % 10.00%

> 1.0% 5.00%

Note: If results are below LOQ, % RSD needs not to be determined

Ruggedness (Intermediate precision)

Intermediate precision expresses within-laboratories variations: on different days, by different analysts, on different equipment, with different lot of column and with same lot of sample.

Procedure

Prepare and inject blank, Reference Solution (mixed), Placebo preparation and Standard preparation as per analytical method. Check for the system suitability. Prepare test sample as per analytical method in six replicates and inject each in single. Calculate % impurities.

Acceptance criteria: The RSD of Known, Single maximum unknown impurity and total impurities should not be more than limit specified below as per the result observed.

Result Observed % RSD

≤ 0.10 % 15.00%

> 0.10 % 10.00%

> 1.0% 5.00%

Note: If results are below LOQ, % RSD needs not to be determined

Acceptance criteria

If initial results are between LOQ to 0.10% then the % Variation Should be NMT 80%

If initial results are between 0.11% to 0.25% then the % Variation Should be NMT 60%

If initial results are between 0.26% to 1.0% then the % Variation Should be NMT 40%

If initial results are between 1.10% to 10.0% then the % Variation Should be NMT 15%

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Prepare the solution concentrations in the range of LOQ, 50%, 100% and 150% of specification limit each level is prepared in triplicate. Inject each preparation into the High Pressure Liquid Chromatography system.

Procedure: Inject Standard preparation and sample preparations of recovery solutions into the chromatograph and measure the peak responses for the major peaks. Check the system suitability described under 5.0.

Recovery Solution Preparation

Recovery at LOQ Level: Weigh & Transfer accurately about 5.7g of placebo and add LOQ Level (As per Found) into a 30ml diluent and sonicate for 5 minutes with intermediate shaking (maintain the sonicator temperature not more than 10°C by adding ice) dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Recovery at 50% Level: Weigh & Transfer accurately about 5.7g of placebo and add 5ml into a 30ml diluent and sonicate for 5 minutes with intermediate shaking (maintain the sonicator temperature not more than 10°C by adding ice) dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Recovery at 100% Level: Weigh & Transfer accurately about 5.7g of placebo and add 10ml into a 30ml diluent and sonicate for 5 minutes with intermediate shaking (maintain the sonicator temperature not more than 10°C by adding ice) dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Recovery at 150% Level: Weigh & Transfer accurately about 5.7g of placebo and add 15ml into a 30ml diluent and sonicate for 5 minutes with intermediate shaking (maintain the sonicator temperature not more than 10°C by adding ice) dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Acceptance criteria

1) The overall % RSD for % recovery for all spike levels should be NMT 5.0%.
2) The % recovery at each spike level shall be NLT 80.0% and NMT 120.0% of the added amount.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Procedure

Prepare and inject blank, placebo solution, standard solution and test preparation as per as such analytical method and with the following mentioned variations in analytical method. Check for system suitability and calculate % impurities. Compare the results obtained in as such method and method with varied parameters as per the acceptance criteria.

Change in flow rate of Mobile phase by ± 0.1ml/min (±10%)

Analyze the sample as per analytical method keeping all conditions same except flow rate of mobile phase. Use flow rate 0.9ml/min (-10%) and 1.1 ml/min. (+10%).

Change in Column Oven Temperature of mobile phase A by 2°C.

Analyze the sample as per analytical method keeping all conditions same except column temperature. Use column temperature as 42°C (+2°C) and 38°C (-2°C)

Acceptance criteria

For as such method and method with varied parameters:

If initial results are between LOQ to 0.10% then the % Variation Should be NMT 80%

If initial results are between 0.11% to 0.25% then the % Variation Should be NMT 60%

If initial results are between 0.26% to 1.0% then the % Variation Should be NMT 40%

If initial results are between 1.10% to 10.0% then the % Variation Should be NMT 15%.

Stability of standard and Sample solutions

Prepare and inject blank, placebo solution, standard solution and test preparation as per as such analytical method. Check for system suitability and % impurities. Keep the standard and sample at (Sampler Temperature) and inject at different time interval up to 36 hours to check the stability of standard and sample solution keeping the same mobile phase prepared at

initial. Compare the results obtained at initial interval with the results of solution analyzed at different time interval as per the acceptance criteria.

Procedure: Inject both the solutions prepared into the chromatograph and measure the peak response for the major peaks.

Acceptance criteria: The RSD of Area and Retention time of Initial Standard solution stability Upto 36 Hrs. should be NMT 5.0%.

Variance on samples results
If initial results are between LOQ to 0.10% then the % Variation Should be NMT 80%
If initial results are between 0.11% to 0.25% then the % Variation Should be NMT 60%
If initial results are between 0.26% to 1.0% then the % Variation Should be NMT 40%
If initial results are between 1.10% to 10.0% then the % Variation Should be NMT 15%.

FOR MONTELUKAST

System Suitability

Prepare the Standard solution as per the proposed test method and inject into the High Pressure Liquid Chromatography system as per the test method. Record the system suitability parameters observed.

Acceptance criteria

1) The relative standard deviation for the peak area response for replicate injections of reference solution (mixed) is not more than 5.0%.
2) The number of theoretical plates should be not less than 1500 in standard solution.
3) Tailing factor of standard injection should be not more than 2.0.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Procedure

Prepare and inject blank, system suitability solution, Placebo, Placebo with Bilastine (triplicate preparation), standard and test solution as per analytical method. Evaluate the system suitability and %impurities as per analytical method. Prepare individual impurity

preparation (for identification) and spiked sample preparation (at respective specification level) as below and inject in single. Check interference from placebo and impurities if any and record the observation.

Individual Impurity solution preparation at respective specification level

Prepare individual impurity solution for known impurities Styrene Impurity 0.8ppm and Sulfoxide impurity 1.6ppm to obtain a final concentration.

Spiked sample preparation

Accurately weigh sample (equivalent to 4mg of Montelukast) into a 50ml clean, dry volumetric flask and add about 25ml of diluent and sonicate to dissolve. Add to it appropriate volume of impurities stock solution (to obtain final concentration for Styrene Impurity 0.8ppm and Sulfoxide impurity 1.6ppm). Make up to volume with diluent. Filter through 0.45µ or finer porosity membrane filter.

Acceptance criteria

There should be no interference of any peak due to placebo and blank with the main analyte and known impurities in sample preparation. Purity angle should be Not More than Purity Threshold.

Limit of Determination (LOD) and Limit of Quantification (LOQ)

Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Procedure

The detection and quantitation level for known and unknown impurity in Montelukast in Bilastine and Montelukast Suspension will be established in term of Montelukast.

Prepare solutions of Montelukast standard and known impurities at different levels of test concentration and inject in single. Plot a graph between concentrations of analyte (ppm) (on-

x-axis) Vs peak area of analyte (on-y-axis) from the linearity data. Determine the LOD & LOQ.

Using on residual standard deviation and slop of the linearity data, using the following formula.

Reference Solution (Mixed): Dilute 2ml of each reference stock solution styrene, sulfoxide impurity and montelukast standard to 50ml with diluent by manual shaking.

Solutions for LOD and LOQ as per Detection

$$\text{Limit of detection} = \frac{3.3\sigma}{S}$$

$$\text{Limit of quantitation} = \frac{10\,\sigma}{S}$$

Where,

σ --- Residual standard deviation of regression line

S --- Slop of regression line.

Prepare solutions for LOD & LOQ determination as in following table

Montelukast

Level	% with respect to test concentration (Montelukast)	Concentration in ppm	Stock Solutions to be taken (ml)	Dilute to volume with diluent (ml)
1	0.05	0.04	0.5	10
2	0.15	0.12	1.5	10
3	0.30	0.24	3.0	10
4	0.40	0.32	4.0	10
5	0.50	0.40	5.0	10
6	0.60	0.48	6.0	10
7	0.75	0.60	7.5	10

Sulfoxide Impurity

Level	% with respect to test concentration (Montelukast)	Concentration in ppm	Stock Solutions to be taken (ml)	Dilute to volume with diluent (ml)
1	0.20	0.16	0.5	10
2	0.60	0.48	1.5	10
3	1.20	0.96	3.0	10
4	1.60	1.28	4.0	10
5	2.00	1.60	5.0	10

6	2.40	1.92	6.0	10
7	3.00	2.40	7.5	10

Styrene Impurity

Level	% with respect to test concentration (Montelukast)	Concentration in ppm	Stock Solutions to be taken (ml)	Dilute to volume with diluent (ml)
1	0.10	0.08	0.5	10
2	0.30	0.24	1.5	10
3	0.60	0.48	3.0	10
4	0.80	0.64	4.0	10
5	1.00	0.80	5.0	10
6	1.20	0.96	6.0	10
7	1.50	1.20	7.5	10

Acceptance criteria: The % RSD of six replicate injection of LOD should not be more than 33.0% and for LOQ should not be more than 10.0%.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity of detector Response

Prepare the series of Standard concentrations ranging from LOQ, 50% to 150% of the targeted concentration of test sample of Bilastine and Montelukast Suspension. Inject each of the linearity solution into the High Pressure Liquid Chromatography system by following the Test method.

Reference Solution (Mixed): Dilute 2ml of each reference stock solution styrene, sulfoxide impurity and montelukast standard to 50ml with diluent by manual shaking.

Linearity Level	Stock Reference Solution (Mixed) (ml)	Diluted to (ml)
At LOQ Level	**As Per Found**	**-**
At 50%	2.5	10
At 80%	4.0	10
At 90%	4.5	10
At 100%	5	10
At 110%	5.5	10
At 120%	6.0	10
At 150%	7.5	10

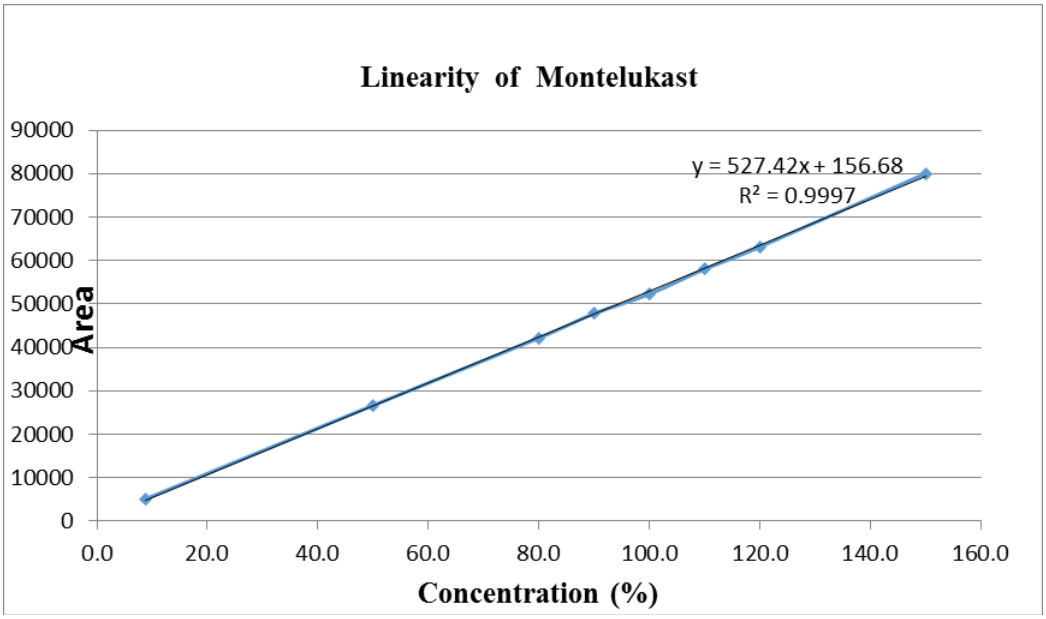

Fig 8: Linearity of Detector response For Montelukast.

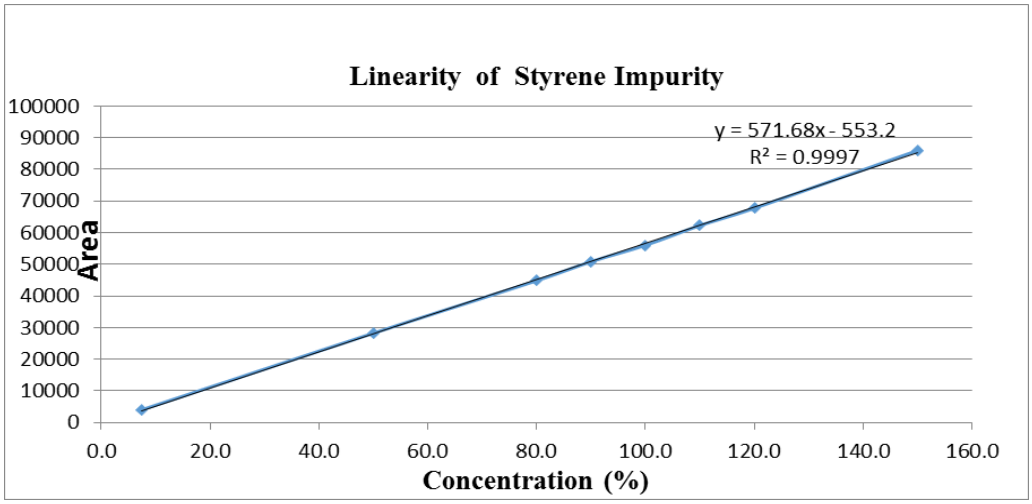

Fig 9: Linearity of Detector response For Styrene impurity.

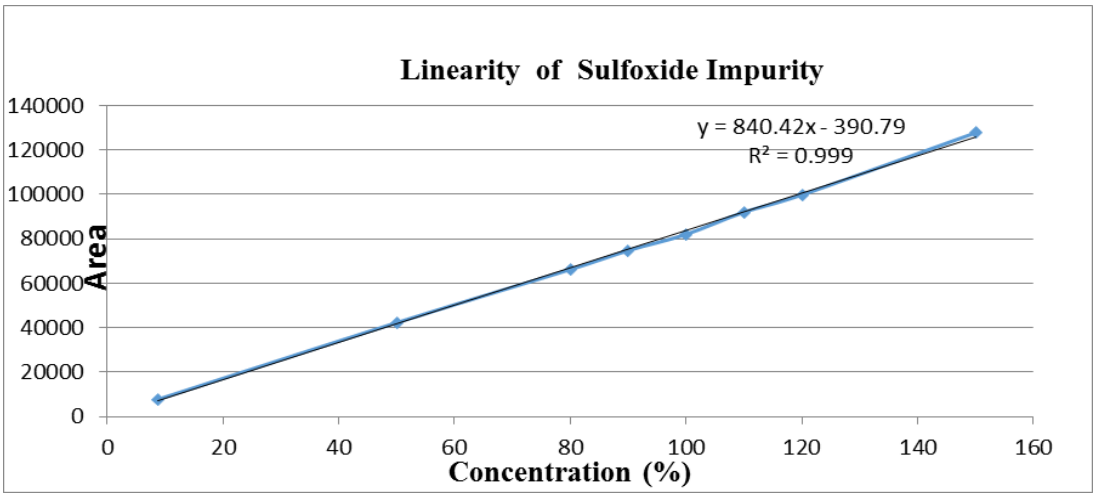

Fig 10: Linearity of Detector response For Sulfoxide impurity.

Procedure: Separately inject Standard preparation and Linearity preparations into the chromatograph, record the chromatograms and measure the peak responses for the major peaks.

Acceptance Criteria: The correlation coefficient should be NLT 0.99 and Statistical Y intercept should be ± 3.

Precision Studies

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

System Precision

Prepare the Reference solution (mixed) proposed in test method and inject into the High Pressure Liquid Chromatography system in replicates. Evaluate the % RSD for the area responses and record the observations.

Acceptance criteria: The relative standard deviation for the peak area response for six replicate injections of reference solution (mixed) is not more than 5.0%.

Method Precision

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Procedure: Prepare and inject blank, Reference Solution (mixed), placebo with Bilastine preparation and standard preparation as per analytical method. Check for the system suitability. Prepare test sample as per analytical method in six replicates and inject each in single. Calculate % impurities.

Acceptance criteria: The %RSD of Known, Single maximum unknown impurity and total impurities should not be more than limit specified below as per the result observed.

Result Observed % RSD

≤ 0.10 % 15.00%

> 0.10 % 10.00%

> 1.0% 5.00%

Note: If results are below LOQ, % RSD needs not to be determined

Ruggedness (Intermediate precision)

Intermediate precision expresses within-laboratories variations: on different days, by different analysts, on different equipment, with different lot of column and with same lot of sample.

Procedure: Prepare and inject blank, Reference Solution (mixed), Placebo placebo with Bilastine preparation and Standard preparation as per analytical method. Check for the system suitability. Prepare test sample as per analytical method in six replicates and inject each in single. Calculate % impurities.

Acceptance criteria: The RSD of Known, Single maximum unknown impurity and total impurities should not be more than limit specified below as per the result observed.

Result Observed % RSD

≤ 0.10 % 15.00%

> 0.10 % 10.00%

> 1.0% 5.00%

Note: If results are below LOQ, % RSD needs not to be determined.

Acceptance criteria

If initial results are between LOQ to 0.10% then the % Variation Should be NMT 80%

If initial results are between 0.11% to 0.25% then the % Variation Should be NMT 60%

If initial results are between 0.26% to 1.0% then the % Variation Should be NMT 40%

If initial results are between 1.10% to 10.0% then the % Variation Should be NMT 15%

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Prepare the solution concentrations in the range of LOQ, 100% and 150% of specification limit each level is prepared in triplicate. Inject each preparation into the High Pressure Liquid Chromatography system.

Procedure: Inject Standard preparation and sample preparations of recovery solutions into the chromatograph and measure the peak responses for the major peaks. Check the system suitability described under 5.0.

Accuracy Stock Solution Preparation

Reference Solution (Mixed): Dilute 2ml of each reference stock solution styrene, sulfoxide impurity and montelukast standard to 50ml with diluent by manual shaking.

Recovery Solution Preparation

Recovery at LOQ Level: Weigh & Transfer accurately about 5.7g of placebo and add LOQ Level (As per Found) into a 30ml diluent and sonicate for 5 minutes with intermediate shaking (maintain the sonicator temperature not more than 10°C by adding ice) dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Recovery at 100% Level: Weigh & Transfer accurately about 5.7g of placebo and add 2.0ml of Reference Stock Solution (A), 2.0ml of Reference Stock Solution (A) and 1.0ml of Reference Stock Solution (C) into a 30ml diluent and sonicate for 5 minutes with intermediate shaking (maintain the sonicator temperature not more than 10°C by adding ice) dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Recovery at 150% Level: Weigh & Transfer accurately about 5.7g of placebo and add 3.0ml of Reference Stock Solution (A), 3.0ml of Reference Stock Solution (A) and 1.5ml of Reference Stock Solution (C) into a 30ml diluent and sonicate for 5 minutes with intermediate shaking (maintain the sonicator temperature not more than 10°C by adding ice) dissolve and make up the volume up to 50ml with diluent. Filter through 0.45 µ syringe filter.

Acceptance criteria

1) The overall % RSD for % recovery for all spike levels should be NMT 10.0%.
2) The % recovery at each spike level shall be NLT 80.0% and NMT 120.0% of the added amount.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Procedure: Prepare and inject blank, placebo solution, standard solution and test preparation as per as such analytical method and with the following mentioned variations in analytical method. Check for system suitability and calculate % impurities. Compare the results obtained in as such method and method with varied parameters as per the acceptance criteria.

Change in flow rate of Mobile phase by ± 0.1ml/min (±10%)

Analyze the sample as per analytical method keeping all conditions same except flow rate of mobile phase. Use flow rate 0.9ml/min (-10%) and 1.1 ml/min. (+10%).

Change in Column Oven Temperature by ±2°C absolute

Analyze the sample as per analytical method keeping all conditions same except ratio of mobile phase A & mobile phase B. Use 38°C and 42°C.

Acceptance criteria

For as such method and method with varied parameters:

If impurity is between LOQ to 0.10% then the difference should be NMT 80%.

If impurity is between 0.11% to 0.25% then the difference should be NMT 60%.

If impurity is between 0.26% to 1.0% then the difference should be NMT 40%.

If impurity is more than 1.0% then the difference should be NMT 15%.

Filter Interference

Take the sample prepared under precision study or prepare the fresh sample and filter through different filters.

Procedure: Inject the Blank and Standard solution as per preparation and check the system suitability. Then inject Sample solutions prepared with different filters into the chromatograph and measure the peak response.

Acceptance criteria

If initial results are between LOQ to 0.10% then the % Variation Should be NMT 80%

If initial results are between 0.11% to 0.25% then the % Variation Should be NMT 60%

If initial results are between 0.26% to 1.0% then the % Variation Should be NMT 40%

If initial results are between 1.10% to 10.0% then the % Variation Should be NMT 15%

Stability of standard and Sample solutions

Prepare and inject blank, placebo solution, standard solution and test preparation as per as such analytical method. Check for system suitability and % impurities. Keep the standard and sample at room temperature (Sampler Temperature) and inject at different time interval up to 36 hours to check the stability of standard and sample solution keeping the same mobile phase prepared at initial. Compare the results obtained at initial interval with the results of solution analyzed at different time interval as per the acceptance criteria.

Procedure: Inject both the solutions prepared into the chromatograph and measure the peak response for the major peaks.

Acceptance criteria: The RSD of Area and Retention time of Initial Standard solution stability Upto 36 Hrs. should be NMT 10.0%.

If initial results are between LOQ to 0.10% then the % Variation Should be NMT 80%

If initial results are between 0.11% to 0.25% then the % Variation Should be NMT 60%

If initial results are between 0.26% to 1.0% then the % Variation Should be NMT 40%

If initial results are between 1.10% to 10.0% then the % Variation Should be NMT 15%.

CONCLUSION

The Analytical method for determination of Related Substance (Impurity) of Montelukast Sodium Eq. to Montelukast and Bilastine in Bilastine and Montelukast Suspension. The Impurity of Montelukast and Bilastine is validated by HPLC for the analytical performance parameter viz. interference of placebo, system precision, Method precision, Intermediate Precision, Accuracy & Recovery, LOD-LOQ, linearity & range, Robustness, Solution stability Upto 24Hr. for Montelukast and Upto 36Hr for Bilastine.

So, it is concluded that, this Analytical method is suitable and validation for Related Substance (Impurity) of Montelukast Sodium Eq. to Montelukast and Bilastine in Bilastine and Montelukast Suspension by High Performance Liquid Chromatography.

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