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Development and Validation of Stability Indicating Assay Method of Montelukast Tablet by RP-HPLC

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ABSTRACT

- **Background:** A simple, precise cost effective stability indicating assay method of Montelukast by RP-HPLC.
- **Material:** Chromatographic separation was achieved on a Meteoric core C18 column (100mm x 4.6 mm, 2.7µm) using a mobile phase 2 ml Trifluroacetic acid : mixture of acetonitrile 250ml and methanol 400ml (350:650) at a flow rate of 1.5ml per minute. Wavelength was detection at 255nm. The retention time of Montelukast was 4 minutes.
- **Results:** The method was found linear concentration the range of 120.39-802.57µg/ml, tablet analysis of % RSD 0.403, accuracy range 30%, 50%, 100%, and 200%, Precision %RSD 0.3. The proposed method was validated as per the ICH guidelines.
- **Conclusion:** The HPLC method was validated and demonstrated good linearity, precision, accuracy, specificity, robustness and stability indicating. The development HPLC method can be utilized for routine analysis stability studies for Montelukast.

KEYWORDS: *Montelukast tablet, RP-HPLC, Degradation studies, Validation, ICH guidelines.*

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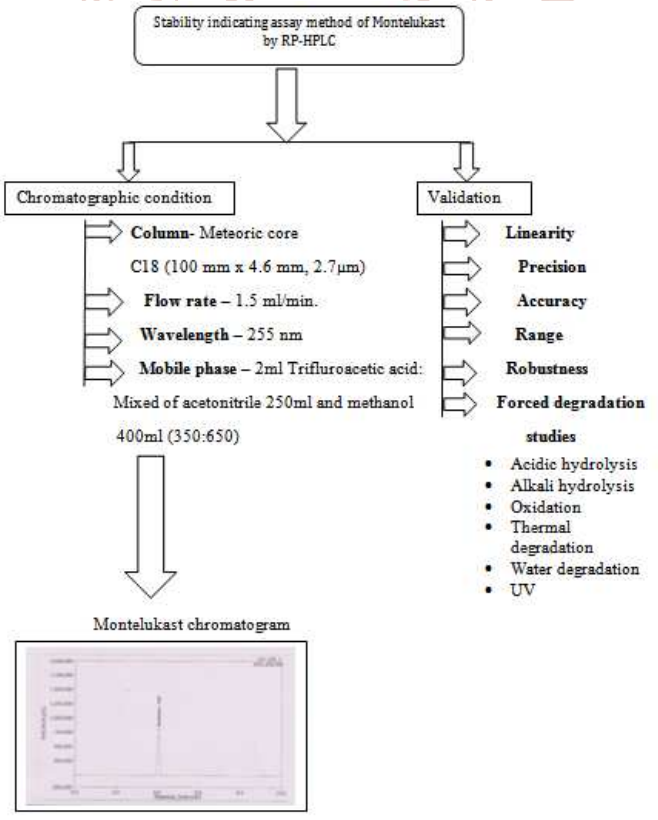
Graphical Abstract:



INTRODUCTION:

Montelukast (Fig 1) is chemically 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl]-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl}methyl] cylclopropyl] acetic acid [7]. Montelukast is a leukotriene receptor antagonist (LTRA) used for the treatment of asthma and to relieve symptoms of seasonal allergies in children and adults [3-4]. It is a potent selective inhibitor of leukotriene D4 (LTD4) at the cysteinyl leukotriene receptor cysLT1 [7-9]. It is also used as an alternative to anti-inflammatory medication in the management and exercise induced bronchospasm [10-12].

Literature survey that liquid chromatography with fluorescence detector, stereo selective high performance liquid chromatograph (HPLC) for Montelukast and its S-enantiomer[13], column switching HPLC with fluorescence detector[7-8], semi-automated 96-well protein precipitation, HPLC with derivative spectroscopy, pressurized liquid extraction followed by HPLC and LC-MS method have been reported for the estimation of Montelukast[15]. The present study illustrates development and validation of stability indicating assay method of Montelukast by RP-HPLC [16-20].

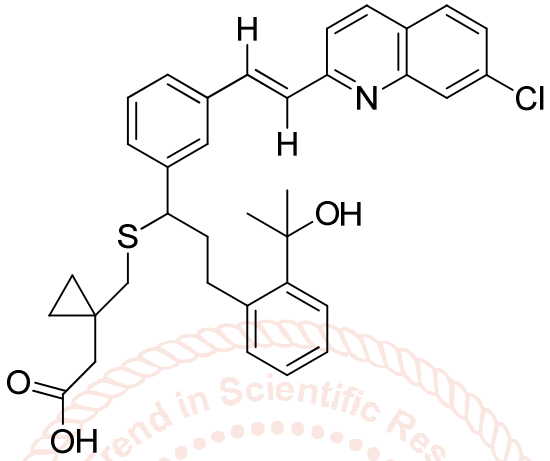


Figure: 1 Montelukast

Experimental:

Material and Methods:

Chemical and Reagents:

Montelukast working standards were procured for Alkem Laboratories Ltd, Taloja, Navi-Mumbai, Trifluroacetic acid, Acetonitrile, Methanol, and Water was used in HPLC Grade.

Instrumentation and chromatographic condition:

HPLC analysis was performed on Agilent HPLC system. Software was using chrome Leon. Separation was carried on column Meteoric Core C18 (100mm x 4.6mm, 2.7µm). The flow rate was 1.5 ml/min. UV detection was performed at 255 nm. HPLC Column oven temperature was 50ºC. Injection volume was 15µl. Sample compartment temperature was 5ºC. Retention time was Montelukast is about 4 minutes. Mobile phase 2ml of Trifluroacetic acid: Mixture of Acetonitrile 250 ml and methanol 400 ml (350:650). Mix and filter through 0.45µm nylon membrane filter and degas. Diluent mixed 250ml of Water milli-Q grade and 750ml of Methanol.

Preparation of Standard Solution:

Dissolve 42 mg of Montelukast reference standard or working standard in 100ml amber colored volumetric flask 70ml of Diluent, sonicate to dissolve and diluted to the mark with the diluent.

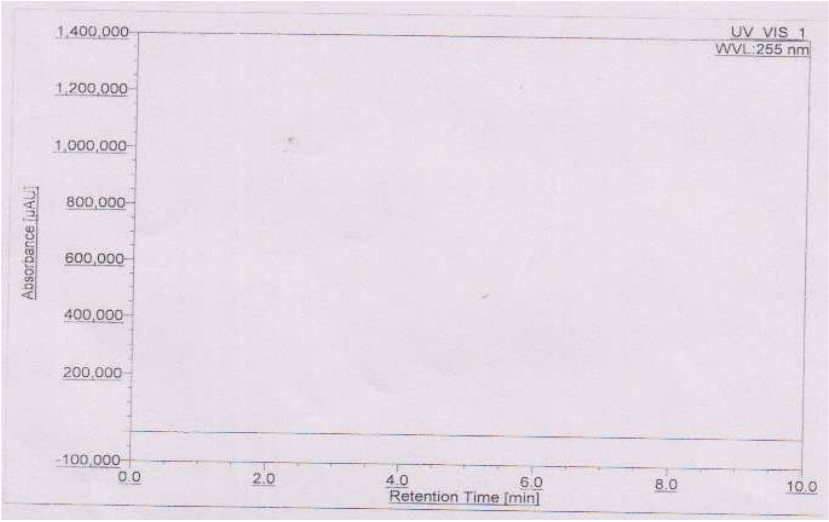


Figure: 2 chromatogram of blank

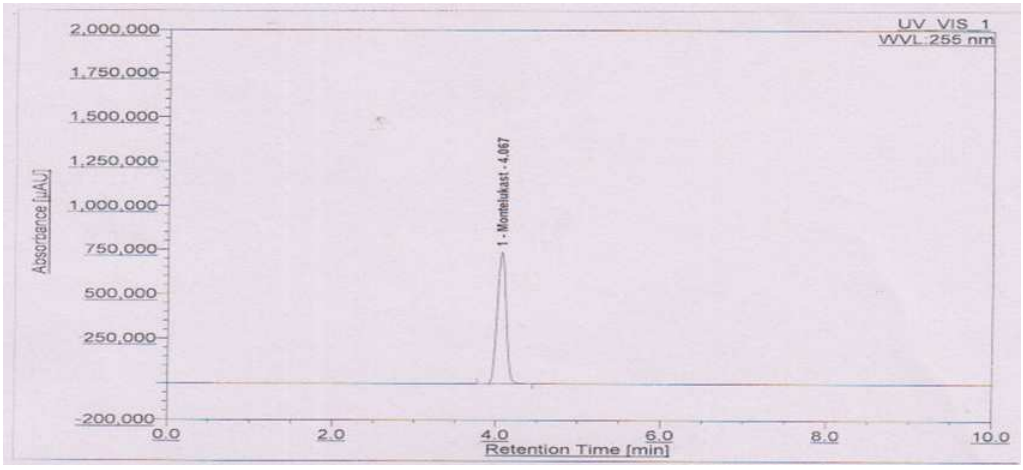


Figure: 3 chromatogram of standard

Preparation of Sample Solution:
Weight and transfer 10 tablets in 250ml amber colored volumetric flask. Add 180ml diluent and sonicate for 30 minutes in cool condition with intermittent shaking and make up the volume with dilute and stir for 30 minutes on magnetic stirrer. Centrifuge the solution at 4000 rpm for about 5 minutes. Then filter the supernatant solution through 0.45µm PTFE filter. Discard first few ml and use the filtrate.

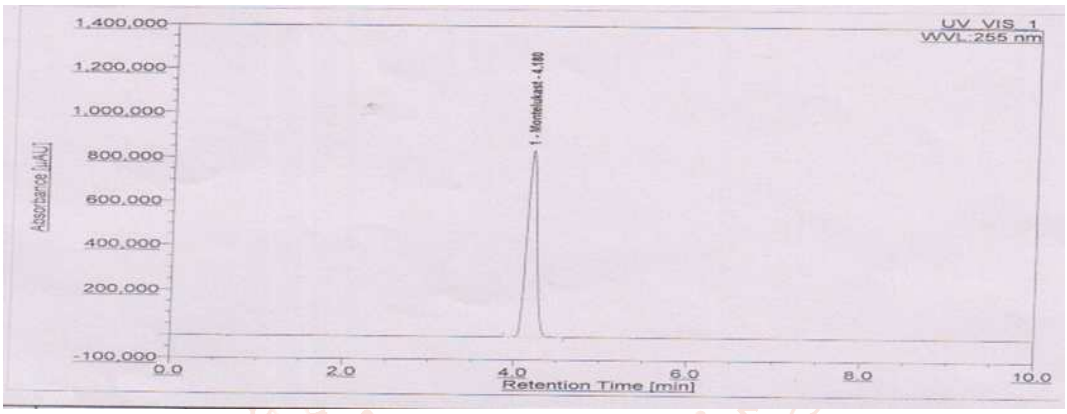


Figure: 4 chromatogram of sample

Results and Discussion:
Forced Degradation studies:
Acid hydrolysis:
An accurately weighed 2mg of pure drug was transferred to a clean and dried 100ml volumetric flask. To which 0.1M Hydrochloric acid and sonication for 10 min and neutralized with 5ml Sodium hydroxide and add 5ml diluent. Kept for 1hrs. 80ºC. From that make up to the mark with diluent, then injection into the HPLC system against a blank of diluent.

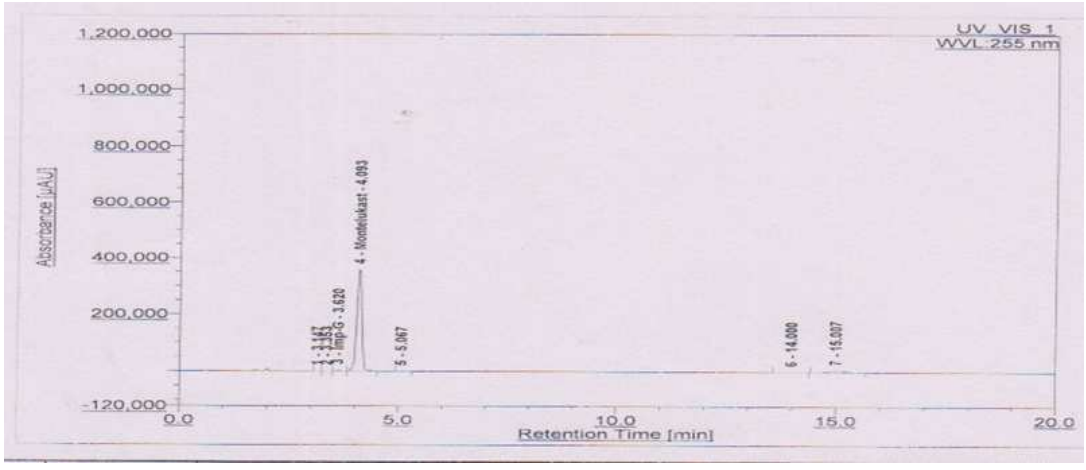


Figure: 5 chromatogram of acid hydrolysis

Alkali hydrolysis:
An accurately weighed 2mg of pure drug was transferred to clean and dried 100 ml volumetric flasks. To which 0.1M Sodium hydroxide was added and neutralized with 5ml Hydrochloric acid and add 5ml diluent. Kept for 1 hrs. at room temperature. From that make up to the mark with diluent, then injection into the HPLC system against a blank of diluent.

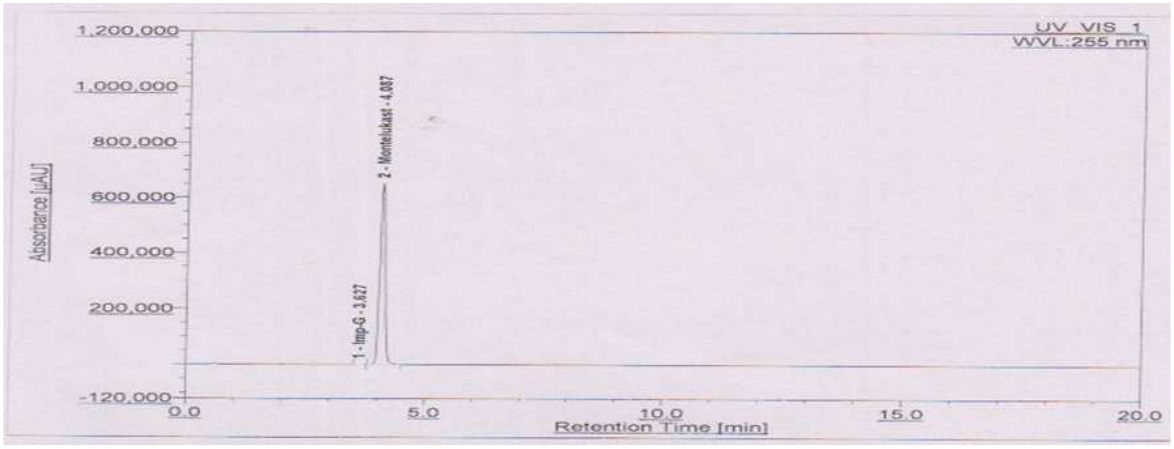


Figure: 6 chromatogram of alkali hydrolysis

Oxidation:

An accurately weighed 2mg of pure drug was transferred to clean and dried 100ml volumetric flasks. To which 10% hydrogen peroxide was added and adds 5ml diluents. Kept for 45 min. at room temperature. From the make up to the mark with diluent, then injection into the HPLC system against a blank of diluent.

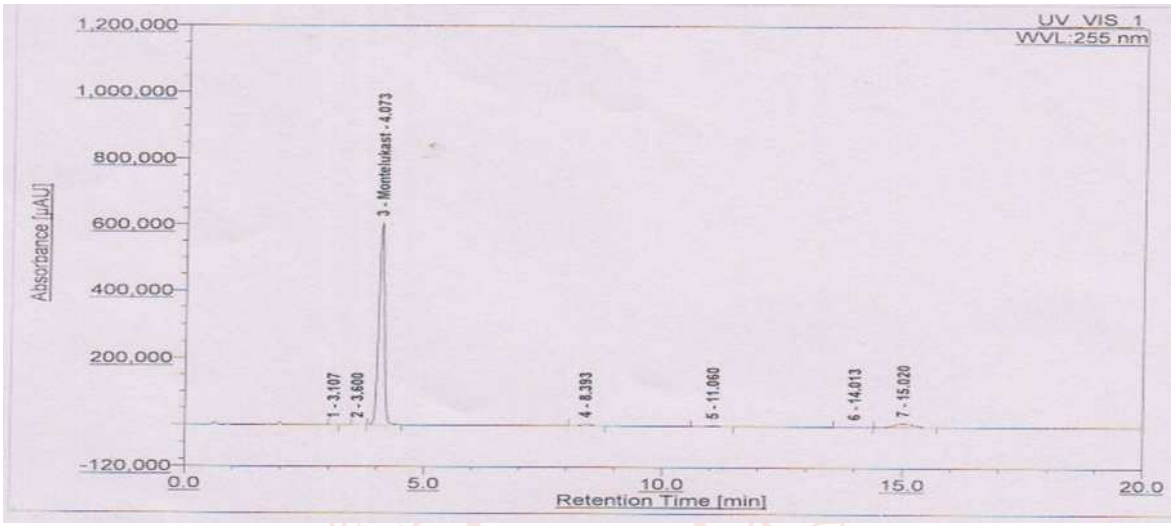


Figure: 7 chromatogram of oxidation

Thermal degradation:

An accurately weighed 2mg of pure drug was transferred to clean and dried 100ml volumetric flasks. To the make with diluent and was maintained at 70ºC for 3hrs.then injected into the HPLC system against a blank of diluent.

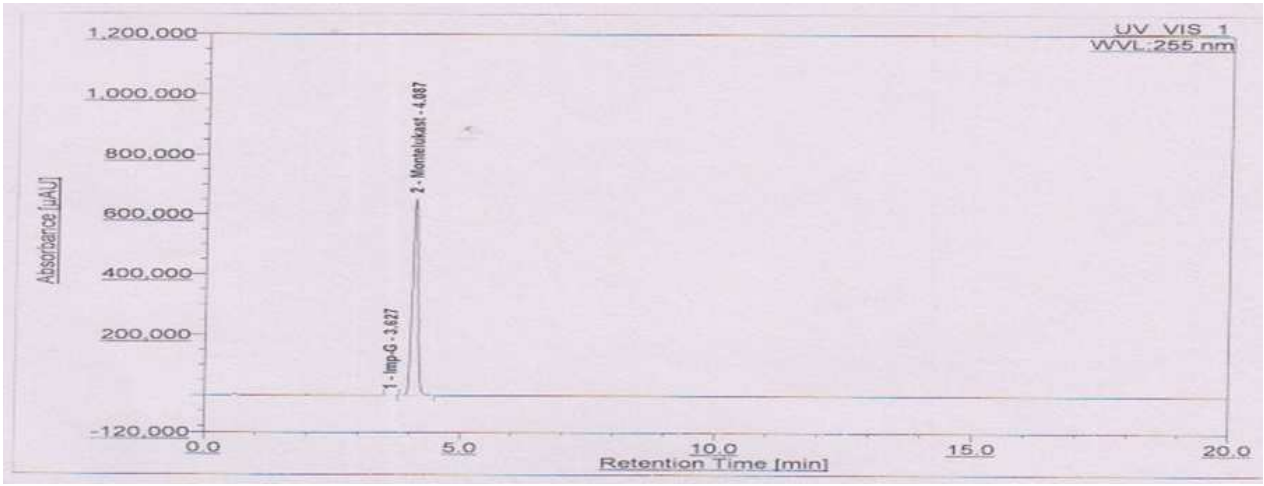


Figure: 8 chromatogram of thermal degradation

Water degradation:

An accurately weighed 2mg of pure drug was transferred to clean and dried 100ml volumetric flasks. To which 5ml water added and 5ml diluents. Kept for 1hrs. at 80ºC. From the make up to the mark with diluent, then injection into the HPLC system a blank of diluent.

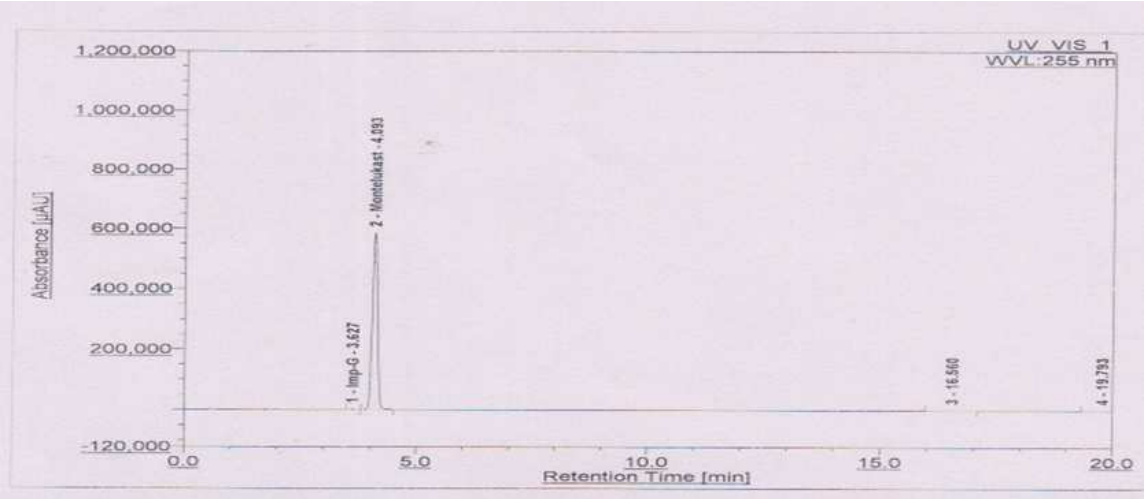


Figure: 9 chromatogram of water degradation

Table: 1 Tablet Analysis:

Sr. No.	Lable Claim (µg)	Area	Amount found in µg	% Found
1	10	6789133	2100.34	101.3
2	10	6678869	2100.13	101.1
3	10	6776027	2100.11	100.9
4	10	6759201	2100.24	102.0
5	10	6877585	2100.12	101.0
6	10	6857644	2100.12	101.0
			Average	101.0
			SD	0.407
			%RSD	0.403

Table: 2 Forced degradation data

Sr. No	Sample Identity	Sample taken(mg)	Area	% Concentration	Percentage Degradation
1	Untreated sample	1667.80	7011924	99.89	-
2	Acid hydrolysis	1677.10	6877219	97.42	2.5
3	Alkali hydrolysis	1680.61	6959422	98.85	1.1
4	Oxidation	1675.56	6980812	96.63	3.3
5	Thermal degradation	1682.78	6712591	98.96	1.0
6	Water degradation	1668.90	7011924	98.85	1.1
7	UV	1676.67	6827363	99.89	0.11

UV degradation:
An approximated weighed 2mg of pure drug was taken in a clean and dry Petridis. It was kept in a UV cabinet at 255nm wavelength for 2 hrs. Without interruption. Accurately weighted 2mg of the UV exposed drug was transferred to a clean and dried 100ml volumetric flask. First the UV exposed drug was dissolved in diluent and make up to the mark than injected into the HPLC system against a blank of diluent.

Method Validation:
The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in term of analytical parameters) to meet the requirements for the intended application of the method.

Specificity:
The specificity of an analytical method may be defined as the ability to unequivocally the analyte in presence of components that may be expected to be presence in the sample matrix.

Specificity was evaluated by preparation the analytical placebo and it was confirmed that the signal measured was caused only by the analyte.

A solution of analytical placebo containing all the tablets excipients except Montelukast was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of inactive ingredients, standard solution, and commercial pharmaceutical preparation were analyzed by the development method. The representative chromatograms did not show any other peaks, which confirmed the specificity of the method.

Peak purity of Montelukast was also evaluated for confirming the purity of the individual peak of Montelukast. In all the sample peak purity is more than acceptance limit (peak purity should be not less than 0.99).

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of detection response for Montelukast was established by analyzing serial dilution of a stock solution of the working standard. Six concentrations ranging from 120% to 802% of test concentration were preparation and analyzed. The final concentration of each solution in µg/ml was plotted against peak are response. Slope, correlation coefficient (R) and intercept were found to be 17245, 0.9996 and 37295.

Table: 3 linearity

Description	Observation
Linear range (µg/ml)	120.39-802.57µg/ml
Slope	17245
Intercept	37295
Correlation coefficient (r)	0.9996

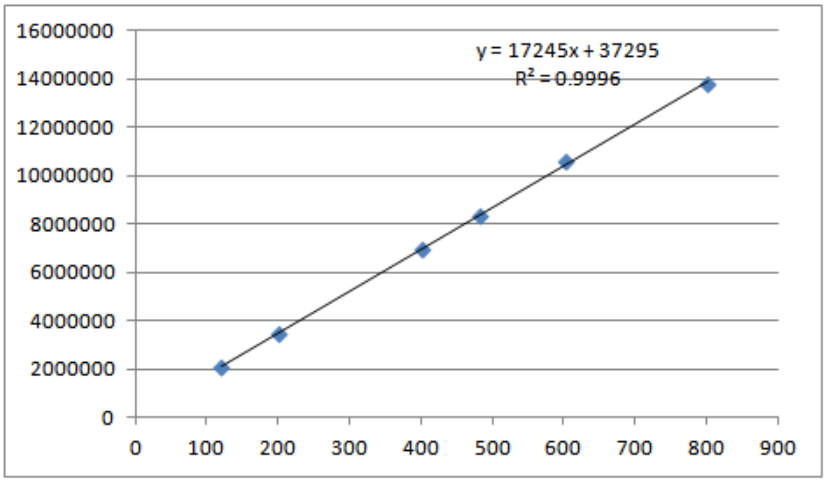


Figure:1 Linearity graph

Precision:

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

Six replicate samples were prepared and analyzed as per the sample preparation procedure. Assay of each replicate, the average of six replicates, and its standard deviation, %RSD and the 95% confidence interval were calculated. The results are shown in the table.

Table: 4 Precision

1. Respectability

Sr. No	Concentration (µg/ml)	Area	Concentration found(µg/ml)
1	120	6574818	98.93
2	120	6609557	99.34
3	120	6616175	99.50
4	120	6620041	99.47
5	120	6606518	99.40
6	120	6585762	99.03
Average			99.44
SD			0.310
%RSD			0.31

2. Reproducibility

Sr. No	Concentration (µg/ml)	Area	Concentration found(µg/ml)
1	120	6579442	97.93
2	120	6608578	99.24
3	120	6625968	98.58
4	120	6628050	99.40
5	120	6608437	98.60
6	120	6591024	97.94
Average			98.61
SD			0.286
%RSD			0.29

3. Intermediate Precision:

Sr. No	Concentration (µg/ml)	Area	Concentration found(µg/ml)
1	120	6570194	98.46
2	120	6610535	98.34
3	120	6606382	99.78
4	120	6612032	99.47
5	120	6603499	99.40
6	120	6580500	99.45
Average			99.15
SD			0.510
%RSD			0.51

Accuracy:

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

Recovery study was performed at 30%, 50%, 100% and 200% of target concentration by spiking placebo blend with the drug substance. Three replicates each were spiked at levels. Spiked sample were extracted and analyzed. The results are shown in table.

Table: 5 Accuracy

Level No.	Amount added in mg	% Recovery	SD	% RSD
30%	31.21	99.78	1.625	1.63
50%	51.47	98.33	0.323	0.33
100%	100.02	97.93	0.817	0.83
200%	200.79	99.13	2.187	2.21

Range:

The range of an analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The variation like flow rate of mobile phase, column temperature, does not have any significant effect on the method performance.

Table: 6 Robustness

Parameter		Variation	% conc.	SD	Percentage RSD
Flow rate (mL min^{-1})	(± 0.1 mL)	High Flow	105.3	1.405	1.334
		Low Flow	105.6	1.641	1.554
P^H	(± 0.2 unit)	High pH	103.3	1.732	1.677
		Low pH	103.1	1.209	1.173
Temperature	(± 5^0c)	High Temp.	104.9	1.572	1.499
		Low Temp.	104.8	0.822	0.784

Conclusion:

A simple, rapid, cost effective, accurate and development and validation of stability indicating assay method of Montelukast by RP-HPLC. The analytical condition and solvent system development provided good resolution between Montelukast and potential degradents within a short run time. The HPLC method was validated and demonstrated good linearity, accuracy, specificity, precision, robustness, and stability indication. Thus, the development HPLC method can be utilized for routine analysis stability studies for Montelukast Tablets.

Conflict of interest:

No any interest.

Acknowledgment:

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References:

[1] K. pallavi.; P. shinivasa babu,; validation UV spectroscopic method for estimation of Montelukast sodium from bulk and tablet formulation; international journal of advances in pharmacy, biology and chemistry vol-1 (4); 2012; 434-437.

[2] K. Naga Raju.; T. Gopala swamy.; A. Lakshmana Rao; Development and validation of RP-HPLC method for the determination of Montelukast sodium in bulk and in pharmaceutical formulation; International journal of pharmaceutical, chemical and biological sciences; 2011;12-16.

[3] N. Kanakadarga devi,; A. Prameela rani,; B. R. Madhavi,; B. S. Mrudula; New RP-HPLC method for the analysis of Montelukast sodium in pharmaceutical dosage forms; International journal of chemTech research; vol-2; 2010; 471-475.

[4] Mastanaiah Thummisetty,; Dr. jayapal reddy sama,; V. Surya ,; Narayana rao,; P. reddanna; stability indicating assay method for Montelukast sodium in pharmaceutical formulations by RP-HPLC; Journal of pharmaceutical sciences and research ;vol-3;2011;1373-1377.

[5] Shamkant s. patil,; shinde atul,; sunil bavaskar,; s.n mandrapkar,; pandrarang n.dhable,; bhanudas s. kuchekar; Development and statistical validation of spectrophotometry method for estimation of Montelukast in bulk and tablet dosage form; Jouranl of pharmacy research ;2009;714-716.

[6] K. vijiay sri,; P. Rohini,; G. Kamlakar reddy; Montelukast sodium oral thin film formulation and invitro evalution; Asian journal of pharmaceutical and clinical research; vol-5;2012;266-270.

[7] Nataraj K. S,; T. Venu baba,; M. Badrud duza; Method development and validation of Montelukast sodium in bulk and pharmaceutical dosage form by RP-HPLC; Journal of chemical and pharmaceutical science; vol-4;2011;55-57.

[8] Mahavir chhajed,; Deepshikha tiwari,; Amar malve,; Tunu godhwani,; Atika chhajed,; A.K.Shrivastava; formulation development and evaluation of Montelukast sodium orodisprsible tablet a new trending asthma treatment; International journal of pharmaceutical and research sciences;2012;127-139.

[9] N. Rshmitta,; T. Josephsunder raj,; chsrinivas,; N. Srinivas,; V. K. Ray,; Hement kumar,; K.mukkanti; A validation RP-HPLC method for the determination of impurities in Montelukast sodium; E-Journal of chemistry;2010;555-563.

[10] A. Patanik,; S. S. Panda,; S. Sahoo,; V. J. Patro; RP-HPLC method devlopment and validation for the determination and stability indication studies of Montelukast in bulk and its pharmaceutical formulation; E-Journal of chemistry;2012;35-42.

[11] Patel Nilam,; Patel Shrish,; Patel Dhara; devlopment and validation of a stability indicating HPTLC method for analysis of antiasthmatic drug; International Journal for pharmaceutical research scholars; vol-1;2012;8-16.

[12] Bonthu mohan Gandhi,; Atmakuri lakshmana rao,; Jangala venkateswara rao; method development and validation for simultaneous estimation of Montelukast sodium and desloratadine by RP-HPLC; American journal of analytical chemistry;2015;651-658.

[13] Juliana Roman,; Ana R. Breier and Marthin Steppe; Stability indicating LC method to determination of sodium Montelukast in pharmaceutical dosage form and its photo degradation kinetics; Journal of chromatographic science;vol49;2011;540-546.

[14] Ahmed B. eldin,; Abdalla A. Shalaby,; Maha Tohamy; Development validation of HPLC method for the determination of Montelukast and its degradation product in pharmaceutical formulation using an experimental design; Acta pharmaceutical sciences;2011;45-55.

[15] R. M.Singh,; P. K. Saini,; S. C.Mathur,; G. N. Singh,; B. Lal; Development and validation of RP-HPLC Method for estimation of Montelukast sodium in bulk and tablet dosage form; India Journal of pharmaceutical science;2010;1-4.

[16] Sethi P. D.; High performance liquid chromatography quantitative analysis of pharmaceutical formulation; CBS Publication and distribution Pvt Ltd;1st edition;vol-1;2010;59-63,11-14,101-103.

[17] Willard H. H,; Merritt L. L,; Dean J. A,; Settle F. A; Instrumental method of analysis CBS Publisher and distributors Delhi;7 edition;2001;2.5,592-600.

[18] Snyder L. R,; Kirkland J. J,; Glajch L. J; Practical HPLC method development; John wiley and sons; 2 edition;1997;1-15.

[19] Current Index of medical specialties (CIMS) (Indian IDR) (Drug index) ;Jan-March 2015; 453.

[20] Validation of analytical procedure text and methodology; ICH Harmonized tripartite guideline Q2 (R_1)2005.