

Validated RP-HPLC method for determination of related substances of montelukast from montelukast sodium chewable tablets

M. S. Charde*, P. Agrawal, A. S. Welankiwar and J. Kumar

Government College of Pharmacy, Kathora Naka, Amravati-444604, (M.S.) India – 444604

Abstract

The development of a RP-HPLC method for Montelukast in the presence of its impurity and degradation product generated from force degradation studies drug was exposed through various degradation stress conditions and found to be stable column used BDS Hypersil C18 (250 mm x 4.6mm) 5µm. Mobile phase was used in mixture of Buffer and Acetonitrile (30:70, v/v). The HPLC method was developed and validated with respect to linearity, precision, accuracy, ruggedness and specificity.

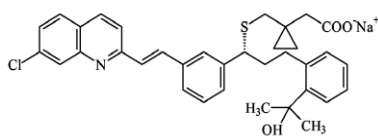
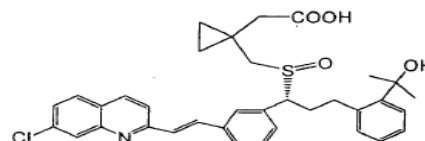
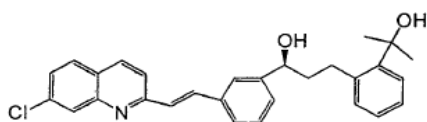
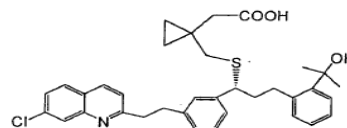
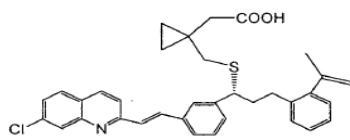
Keywords: Montelukast, High Pressure Liquid Chromatography, Validation

1. Introduction

Montelukast sodium¹⁻³ [Fig.1] is Antiasthmatic, Antiallergic, Antinflammatory and Cryoprotective Agent. Montelukast sodium is a selective and orally active Leucotriene receptor antagonist that inhibits the cysteinyl CysLT1 receptor which is used in respiratory disorder as leukotriene antagonist. Chemically it is Sodium Salt of 1-[[[(1R)-1[3-[(1E)-2-(7-chloro-2-quinolinyl) ethenyl]phenyl]-3-[2-(1-hydroxy-1-methyl ethyl)phenyl] propyl] thio]methyl]cyclopropane acetic acid. Montelukast sodium is a white to pale yellow hygroscopic powder and Well absorbed orally excreted by biliary only 1% in urine. Montelukast sodium is soluble in water and methanol; practically insoluble in Acetonitrile. Adverse effects of Montelukast sodium are Stomach pain, headache, nausea, dizziness, flu, cough, fever, stuffy nose. Impurities of Montelukast sodium are Sulfoxide impurity [Fig.2], Hydroxy compound [Fig.3], Dihydro impurity [Fig.4], Styrene impurity [Fig.5]. Chemically, Sulfoxide impurity is 1-[[[(1R)-1[3-[(1E)-2-(7-chloro-2-quinolinyl)ethenyl] phenyl]-3-[2-(1-hydroxy-methylethyl)phenyl]propyl]

sulfanyl]methyl)cyclopropane acetic acid, Hydroxyl impurity is [(E)-2-[3[(S)-[3-[2-(7-chloro-2-quinolinyl)-ethenyl] phenyl]-3-hydroxypropyl] phenyl]-2-propanol, Dihydro impurity is 1-[[[(1R)-1[3-[2-(7-chloro-2-quinolinyl)ethenyl] phenyl] - 3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]thio]methyl] cyclopropane acetic acid and Styrene impurity is 1-[[[(1R)-1[3-[(1E)-2-(7-chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1-(1methyl) etenyl)phenyl] propyl] thio] methyl] cyclopropane acetic acid.

Literature survey⁶⁻⁸ was carried out that no method has been reported for Development of Analytical Method and Validation for determination of Related Substances of Montelukast from Montelukast Sodium Chewable tablets. The present work is undertaken with an Objective to develop a new in-House, economical, simple, accurate, precise and reproducible Method for determination of Related Substances of Montelukast from Montelukast Sodium Chewable tablets by RP-HPLC method⁴⁻⁵ and its validation (a non Pharmacopoeial, non compendial method).

**Fig 1 : Structure of Montelukast Sodium****Fig 2 : Structure of Sulfoxide impurity****Fig 3 : Structure of Hydroxide impurity****Fig 4 : Structure of Dihydro impurity****Fig 5: Structure of Styrene impurity**

2. Methods and Materials

2.1 Chemicals and Reagents:

All the solvents and chemicals used were of HPLC and analytical grade. Milli Q water and 0.45 μ m Teflon filter was used throughout the experimental work. The gift drug samples of Montelukast Sodium were provided by Ajanta Pharmaceuticals, Kandivli, Mumbai. Chemicals and Reagents Used are Triethylamine (AR grade), Ortho Phosphoric Acid (AR grade), Sodium Dihydrogen orthophosphate dihydrate (AR grade), Acetonitrile, Water, Methanol.

2.2 Instrument:

The chromatographic separation performed using Jasco HPLC System with PDA detector, model 2080.31. Software used to monitor was Borwin and Quaternary pump is applied. Analytical balance is used, Make Sartorius (Model AB - 20.04). pH meter was also used, Labindia Make, Model pH System 362.

2.3 Preparation of Mobile Phase:

Buffer and Acetonitrile (30:70 v/v) mixed properly 300 mL of Buffer and 700 mL of Acetonitrile and sonicated for 5 minutes to degased.

2.3.1 Buffer Preparation:

Dissolved 3.9 gm of Sodium dihydrogen orthophosphate dihydrate in 1000 ml of HPLC water, 1.0 ml of triethylamine was added and pH was adjusted to 4.6 with diluted orthophosphoric acid. Solution was filtered through 0.45 μ nylon membrane filter.

2.3.2 Preparation of diluent:

Methanol of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the mixture of Water and Acetonitrile in the ratio 10:90.

2.3.3 Blank Preparation:

Use diluent as a blank.

2.4 Selection of Analytical Wavelength:

MONTE WS (20.8 mg) was weighed and transferred into 100 mL volumetric flask. Then 50 mL of diluents were added, sonicated to dissolve and diluted up to the mark with diluent and mixed (200 ppm of MONTE) and further diluted (1 ml of Preparation to 200 ml with diluent). The solutions were scanned in the range of 200-400 nm. The spectra was shown in the **Fig.6**

2.5 Sulphoxide impurity stock preparation (Solution A):

Weighed and transferred about 1 mg of Sulphoxide impurity standard into 10 mL volumetric flask. 5mL of diluent were added, sonicated to dissolve and diluted up to the mark with diluent and mixed (100 ppm of Sulphoxide impurity).

2.6 Hydroxy impurity stock preparation (Solution B):

Weighed and transferred about 1 mg of Hydroxy impurity standard into 10 mL volumetric flask. 5mL of diluent were added, sonicated to

dissolve and diluted up to the mark with diluent and mixed (100 ppm of Hydroxy impurity).

2.7 Dihydro impurity stock preparation (Solution C):

Weighed and transferred about 1 mg of Dihydro impurity standard into 10 mL volumetric flask. 5mL of diluent were added, sonicated to dissolve and diluted up to the mark with diluent and mixed (100 ppm of Dihydro impurity).

2.8 Styrene impurity stock preparation (Solution D):

Weighed and transferred about 1 mg of Styrene impurity standard into 10 mL volumetric flask. 5mL of diluent were added, sonicated to dissolve and diluted up to the mark with diluent and mixed (100 ppm of Styrene impurity).

2.9 Montelukast Sodium standard stock preparation:

Montelukast Sodium (20.8 mg) was weighed and transferred into 100 mL volumetric flask. Then 50 mL of diluents were added, sonicated to dissolve and diluted up to the mark with diluent and (200 ppm of MONTE).

2.10 Standard Preparation:

1 mL of Montelukast Sodium standard stock solution preparation were diluted to 200 mL with diluent.

2.10.1 Resolution preparation:

Solution A, solution B, solution C and solution D(0.2 mL of each) were pipette out and 0.1 mL Montelukast Sodium standard stock preparation was added in 20 mL volumetric flask and diluted up to the mark with diluent (1 ppm each). The chromatogram was shown in the **Fig.7**

2.10.2 Sample preparation:

Transferred 10 intact 5 mg Chewable tablets into 250 mL volumetric flask and added 125 mL of diluent and sonicated for 15 minutes with intermittent shaking. Maintain the water of sonicator at room temperature within sonication. Further diluted up to the mark with diluent and filtered through 0.45 μ membrane filter (200 ppm).

2.11 Optimization of Chromatographic Condition for Estimation of Drugs:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing Montelukast Sodium was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through 0.45 μ m Teflon filter.

Finally, the optimal composition of the mobile phase, Buffer & Acetonitrile (30:70, v/v) was selected. It gave high resolution of Montelukast Sodium with minimal tailing.

2.12 System Suitability Test:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate

injections of standard solutions. Resolution solution was injected and resolutions of the major adjacent peaks were checked. Standard preparation was injected in six replicates and % RSD for area of peak due to Montelukast was calculated.

2.13 Validation Parameters⁹⁻¹⁰

i) Specificity:

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interferences from blank, placebo and impurities.

Specificity by spiking impurities:

Placebo preparation:

Transferred 10 placebo tablets in 250 mL volumetric flask and proceed further as per the test preparation.

Impurity Preparation:

Dilute 1 mL of solution A, solution B, solution C, & solution D to separate 100 mL volumetric flask with diluent to produce 1 ppm of Sulphoxide impurity, Hydroxy impurity, Dihydro impurity, & Styrene impurity respectively.

Test preparation:

As per test preparation for 5 mg Chewable tablets described in the above preparation.

Spiked Test preparation:

Transferred 10 intact tablets into 250 mL volumetric flask. Added 125 mL of diluent and sonicated for 15 minutes with intermittent shaking. Maintained the water of sonicator at room temperature within sonication. Added 2.5 mL of each Solution A1, Solution B1, Solution C1 and Solution D1 and dilute upto the mark with diluent and filter through 0.45µ membrane filter.

Injected Blank preparation, Resolution preparation, Standard preparation in six replicates, Placebo preparation, Test preparation, Sulphoxide impurity preparation, Hydroxy impurity preparation, Dihydro impurity preparation, Styrene impurity preparation, Test spiked preparation and recorded the chromatograms. Calculated % RSD of the area of Montelukast from Standard preparation.

Limit of Quantitation (LOQ):

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a Test that can be quantitatively determined with suitable precision, accuracy respectively.

The limit for quantitation is established by standard deviation of response and the slope or by the signal to noise ratio whichever is appropriate.

a) LOQ by Linearity:

LOQ was Calculated by standard deviation of response and the slope from 0.2 ppm to 2.0 ppm of linearity. Prepare LOQ solution by subsequent dilution of respective impurity and Montelukast sodium working standard solutions. LOQ solution prepared by weighed and transferred accurately 0.920 mg of Sulphoxide impurity standard, 0.990 mg of Hydroxy impurity standard, 1.223 mg of Dihydro impurity standard, 1.229 mg of Styrene impurity standard in separate 20 mL volumetric flask and 20.8 mg of Montelukast Sodium working standard added

in 100mL Volumetric flask. Diluents was added to the flask and sonicated for 5 minutes. Pipetted out 0.026 mL of Sulphoxide Impurity, 0.026 mL of Hydroxy impurity, 0.026 mL of Dihydro impurity, 0.150 mL of Styrene impurity, 0.024 mL of Montelukast Sodium, in 100 mL volumetric and diluted upto the mark with diluent. Inject six replicates of LOQ solution. Calculate % RSD for peak area. Incorporate this LOQ area for linearity.

$$\text{LOQ} = \frac{10 \times \text{Std. Deviation}}{\text{Slope}}$$

b) LOQ by Signal to noise ratio:

LOQ solution (Concentration higher than LOD) was prepared by subsequent dilution of respective impurity and MONTE WS solution. Six replicates of LOQ were injected S/N ratio was determined from baseline noise. %RSD were calculated for peak area and these LOQ area was incorporated for linearity

ii) Limit of Detection (LOD):

The detection limit is the lowest amount of analyte in a Test that can be detected, but not necessarily quantified, under the stated experimental conditions. The limit for detection was established by standard deviation of response and the slope or by the signal to noise ratio whichever is appropriate.

a) LOD by Linearity:

LOD was Calculated by standard deviation of response and the slope from 0.2 ppm to 2.0 ppm of linearity. Prepare LOD solution (as per LOQ solution) by subsequent dilution of respective impurity and Montelukast Sodium working standard solutions. Inject three replicate injections of this solution. The limit for detection was established by signal to noise ratio.

$$\text{LOD} = \frac{3.3 \times \text{Std. Deviation}}{\text{Slope}}$$

b) LOD by signal to noise ratio:

Prepare LOD solution by subsequent dilution of respective impurity and Montelukast Sodium working standard solutions. Inject three replicate injections of this solution.

iii) Linearity and Range:

Linearity:

The ability of a method to produce results those are directly proportional to the concentration of the analyte in Tests within a given range. Linearity was performed at 8 levels, viz. LOQ, 20%, 50%, 75%, 100%, 125%, 150% and 200% w.r.t. Specification concentration of known impurity (1 ppm of each impurity) and LOQ, 50%, 125%, 188%, 250%, 313%, 375% and 500% w.r.t. Specification concentration of unknown impurity (0.4 ppm of Montelukast). Response factor is calculated by plotting the graph of Area vs. Concentration for 0.50 ppm to 2.00 ppm of linearity solution and by using following formula.

$$\text{Response Factor} = \frac{\text{Slope of Montelukast Standard}}{\text{Slope of Impurity}}$$

Range:

The range of analytical procedure is the interval between the upper & lower concentration of analyte in the Test for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy & linearity. The range is derived from the linearity studies.

iv) 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. Accuracy may often be expressed as percent recovery by assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method that is true for all practical purpose.

A. Accuracy (Recovery) for Known impurities:

Performed accuracy at 4 levels, viz. LOQ, 50%, 100% and 200% of specification concentration. All the known impurities were spiked in the Test and compare with the respective impurity standard. Calculated % recovery of each impurity by subtracting the area of known impurities present in the unspiked Test.

Impurity standard preparation:

Weighed and transferred about 5 mg of each Sulphoxide impurity, Hydroxy impurity, Dihydro impurity, Styrene impurity standard into separate 20 mL volumetric flasks. Added 5 mL of diluent sonicate to dissolve and dilute up to the mark with diluent to produce 250 ppm of each i.e solution A3, solution B3, solution C3, & solution D3 respectively. Pipetted out 0.1 mL each of Impurity stock solution in 25 mL volumetric flask and diluted upto the mark with diluent (1 ppm of each impurity).

Unspiked test preparation:

As specified under test preparation for 5mg Chewable tablet.

Level I – LOQ level:

Transferred 10 intact tablets into 250 mL volumetric flask. Added about 125 mL of diluent and volume of solution A3, solution B3, solution C3, and solution D3 so that the concentration of impurity should be of LOQ level after final dilution of Test. Sonicated for 15 minutes with intermittent shaking. Diluted upto the mark with diluent and filter through 0.45µ membrane filter.

Level II – 50% level:

Transferred 10 intact tablets into 250 mL volumetric flask. Added about 125 mL of diluent and 0.5 mL each of solution A3, solution B3, solution C3, and solution D3 in the flask. Sonicated for 15 minutes with intermittent shaking. Diluted upto the mark with diluent and filter through 0.45µ membrane filter.

Level III – 100% level:

Transferred 10 intact tablets into 250 mL volumetric flask. Added about 125 mL of diluent and 1.0 mL each of solution A3, solution B3, solution C3, and solution D3 in the flask. Sonicated for 15 minutes with intermittent shaking. Diluted upto the mark with diluent and filter through 0.45µ membrane filter.

Level IV – 200% level:

Transferred 10 intact tablets into 250 mL volumetric flask. Added about 125 mL of diluent and 0.2 mL each of solution A3, solution B3, solution C3, and solution D3 in the flask. Sonicated for 15 minutes with intermittent shaking. Diluted upto the mark with diluent and filter through 0.45µ membrane filter.

Each level prepared in triplicate. Injected Impurity standard preparation in six replicates, unspiked test preparation in duplicate and each preparation in single. Calculated the average area of respective impurity in unspiked test preparation and subtract from the spiked Test and then calculated the recovery. The result was shown in the **Table 2, 3 & 4.**

Calculation:

$$\text{ppm added (Actual)} = \frac{\text{WS} \times \text{DV} \times \text{PI} \times 1000}{20 \times 100}$$

$$\text{ppm recovered} = \frac{\text{AT} \times \text{WI} \times 0.1 \times 250 \times \text{PI} \times 1000}{\text{AS} \times 20 \times 25 \times 100}$$

$$\% \text{ recovery} = \frac{\text{ppm recovered} \times 100}{\text{ppm added (actual)}}$$

Where,

AT = Corrected peak response of individual impurity from test preparation.

AS = Mean peak response of individual impurity from Impurity standard preparation.

WS = Wt. of individual impurity std. taken in mg for impurity Standard stock preparation

DV = volume of Impurity Standard stock preparation spiked in mL

WI = Wt. of individual impurity standard taken in mg for Impurity standard preparation

PI = Potency of the individual impurity standard in % on as is basis

B. Accuracy (Recovery) for Unknown impurities:

Performed accuracy at 4 levels, viz. LOQ, 50%, 100% and 200% of specification concentration. Spiked Montelukast sodium in the Placebo and compared with the Montelukast sodium standard. Calculated % recovery of Montelukast.

Level I – LOQ level:

Transferred 10 intact placebo tablets into 250 mL volumetric flask. Added about 125 mL of diluent & volume of Montelukast sodium stock preparation was added so that the concentration of Montelukast sodium should be of LOQ level after final dilution of Test. Sonicated for 15 minutes with intermittent shaking. Maintained the water of sonicator at room temperature within sonication. Diluted upto the mark with diluent and filter through 0.45µ membrane filter.

Level II – 50% level:

Transferred 10 intact placebo tablets into 250 mL volumetric flask. Added about 125 mL of diluent and 0.5 mL Montelukast sodium stock preparation in the flask. Sonicated for 15 minutes with intermittent shaking. Maintained the water of sonicator at room temperature within sonication. Diluted upto the mark with diluent and filter through 0.45µ membrane filter.

Level III – 100% level:

Transferred 10 intact placebo tablets into 250 mL volumetric flask. Added about 125 mL of diluent and 1.0 mL Montelukast sodium stock preparation in the flask. Sonicated for 15 minutes with intermittent shaking. Maintained the water of sonicator at room temperature within sonication. Diluted upto the mark with diluent and filter through 0.45 μ membrane filter.

Level IV – 200% level:

Transferred 10 intact placebo tablets into 250 mL volumetric flask. Added about 125 mL of diluent and 2.0 mL Montelukast sodium stock preparation in the flask. Sonicated for 15 minutes with intermittent shaking. Maintained the water of sonicator at room temperature within sonication. Diluted upto the mark with diluent and filter through 0.45 μ membrane filter. The result was shown in the **Table 5**.

1. Precision:

The precision of an analytical method is the closeness of agreement (degree of scatter) between series of measurements obtained from multiple samplings of the same homogeneous Test under the prescribed conditions.

A. System Precision:

Injected Blank preparation, Resolution preparation in single and standard preparation in six replicate and calculated the % RSD for peak area.

B. Method Precision (Repeatability):

Repeatability expresses the precision under the same operating conditions over a short interval of time. Method Precision were established by carrying out related substances test on six Test preparations as described in above preparation. Individual known impurity value, individual unknown impurity value and total impurities were calculated.

C. Intermediate Precision (Ruggedness):

Intermediate precision expresses within-laboratory variation on a different day, by a different analyst, using different instrument, different Column and using same lot of test as specified under repeatability. Calculated individual known impurity value, individual unknown impurity value and total impurities. The result was shown in the **Table 6**.

2. 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 indications of its reliability during normal usage.

Following parameter were changed one by one and observe their effect on % of impurity.

- i) Change in Column temperature by $\pm 2^\circ\text{C}$ (i.e. 23°C and 27°C)
- ii) Change in the wavelength by ± 2 nm (i.e. 223 nm and 227 nm)
- iii) Change the pH of Buffer used in the mobile phase by ± 0.05 (i.e. 4.55 and 4.65) The procedure followed for method precision were repeated by using above changes in method one by one except the three Tests to be taken instead of six. Also individual known impurities, individual unknown and total impurities

were calculated. The result was shown in the **Table 7, 8, & 9**.

3. Solution Stability:

Standard preparation and Test preparation were prepared as described in the above preparation. Standard preparation and Test preparation were injected at initial and keep them in auto sampler of HPLC at 5°C . Standard preparation and Test preparation were Inject at different time interval viz, Initial, After 2 Hours and after 4 Hours, also injecting the fresh Standard preparation at each time interval where ever possible. Calculated the % impurity at every time interval.

4. Filter Paper Interference:

Filter paper interference was checked by filtering the Standard preparation by selected filter paper. Filtered about 10 mL of the Standard preparation with 0.45 μ nylon membrane filter paper. The filtrate was injected along with Unfiltered Standard preparation and % variation of filtered Standard preparation with Unfiltered Standard preparation was calculated.

3. Results and Discussion**3.1 Optimization of Chromatographic Condition for Estimation of Drug**

Instrument	: HPLC with gradient pump and DAD Detector
Column	: BDS Hypersil C18, 250 mm x 4.6mm, 5 μ or equivalent
Mobile phase	: Buffer : Acetonitrile (30:70)
Wavelength	: 225 nm
Flow rate	: Gradient programming
Injection volume	: 20 μL
Column temperature	: 25°C
Auto sampler temperature	: 5°C
Run time	: 45 minutes
Retention time	: about 15 minutes for Montelukast

The result was shown in the **Fig 10**.

The observations and result obtained for each parameter including Specificity, Limit Of Quantitation, Limit of Detection, Linearity, Accuracy (Recovery), Method Precision (Repeatability), Intermediate precision (Ruggedness) Robustness, Solution Stability and System Suitability. % recovery of Montelukast containing unknown impurity was found to be 103.6 with % RSD 2.17. Specificity of the method was demonstrated by analyzing Blank preparation, Placebo preparation, diluted standard preparation, Individual Known Impurity preparation, Test preparation and Test spiked preparation did not show any interference at the Retention time of Montelukast Sodium. The robustness of the method was evaluated by altering the variables such as different Column oven Temperature (23°C and 27°C), different Wavelength (223 nm and 227 nm) and different pH of Buffer (4.55 and 4.65). The data

obtained from individual condition shows that the method is robust including repeatability

Fig 6 : λ_{max} spectra of Montelukast

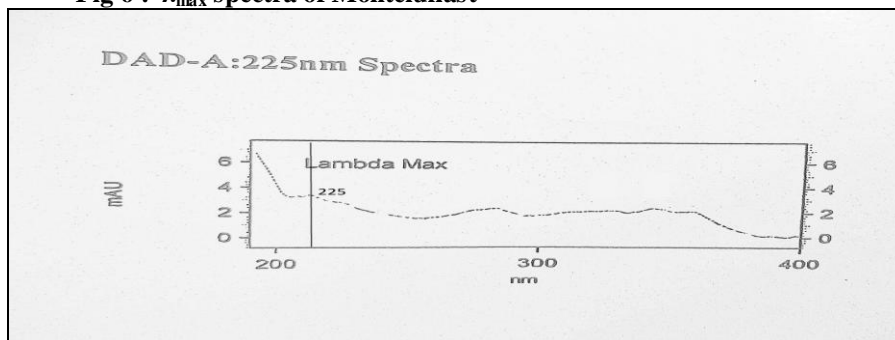


Fig 7 : Chromatogram of Montelukast resolution mixture

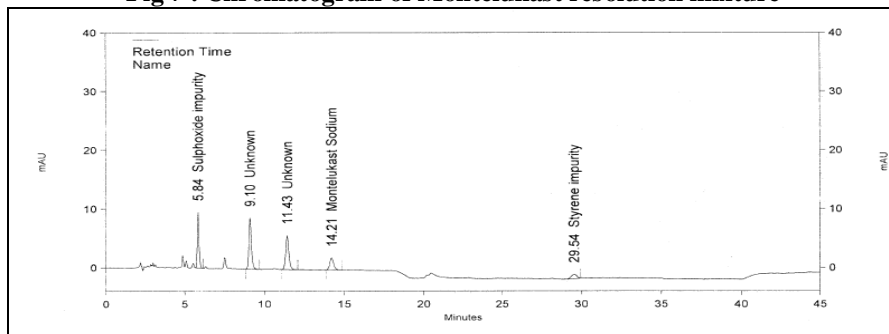


Fig 8 : Chromatogram for Diluent

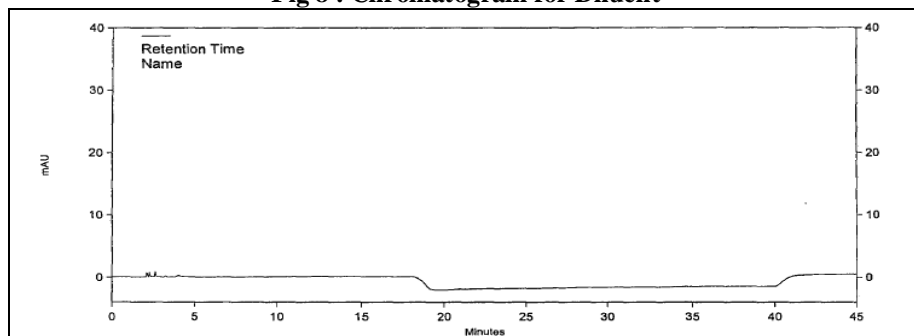


Fig 9 : Chromatogram for Placebo

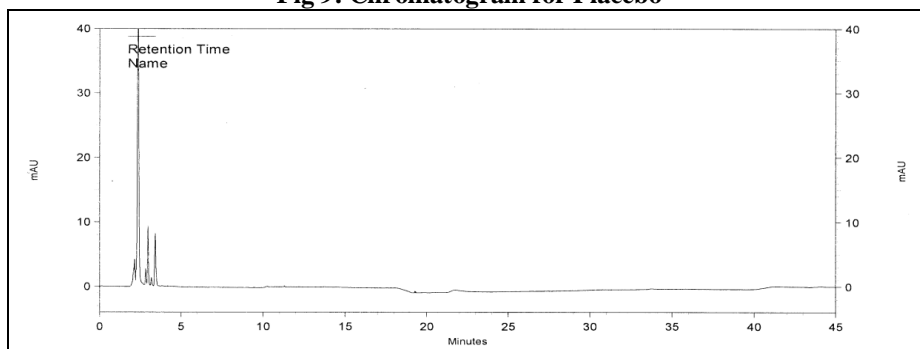


Fig 10: Chromatogram for Montelukast Sample

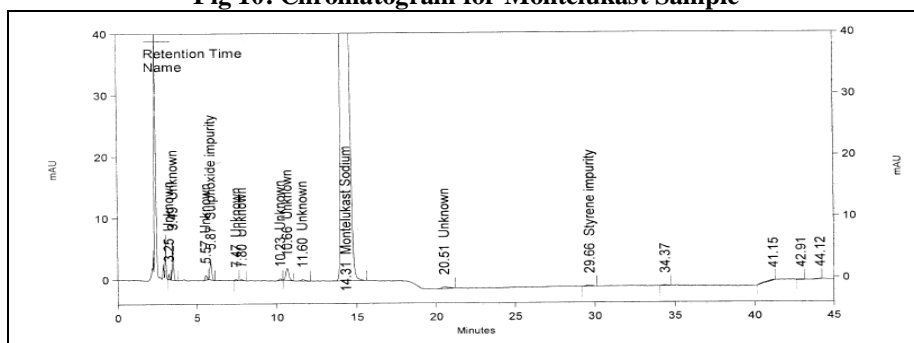


Fig 11 : Chromatogram for Sulphoxide impurity

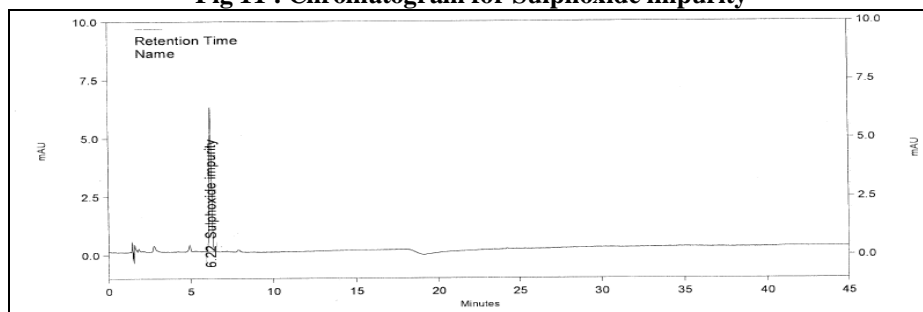


Fig 12: Chromatogram for Hydroxy impurity

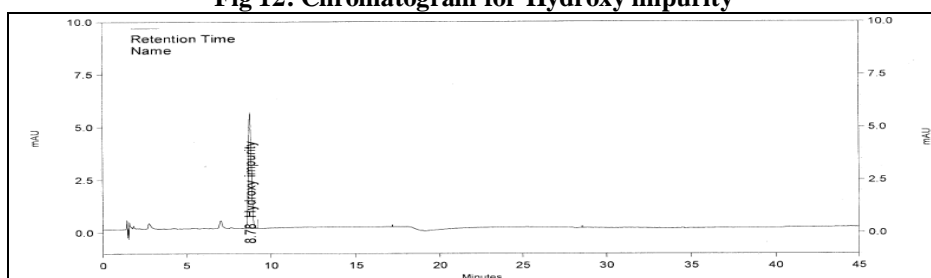


Fig 13: Chromatogram for Dihydro impurity

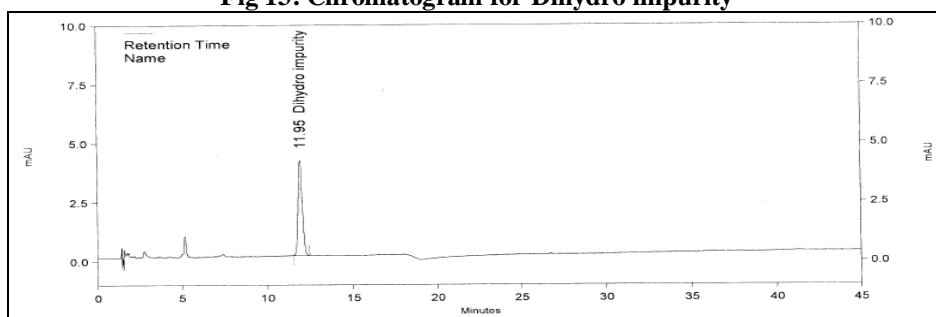


Fig 14 : Chromatograms of Styrene impurity

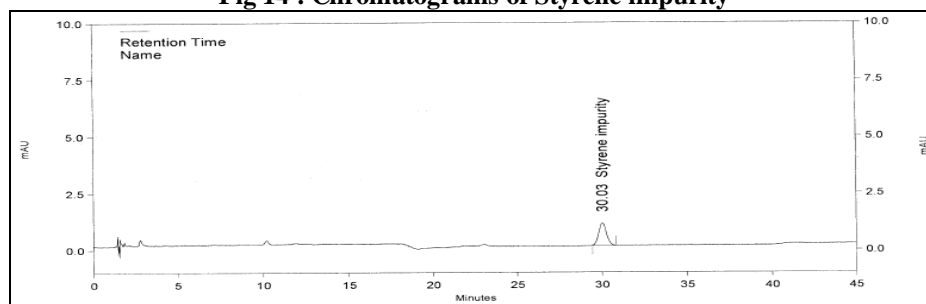


Fig 15: Chromatogram for Montelukast

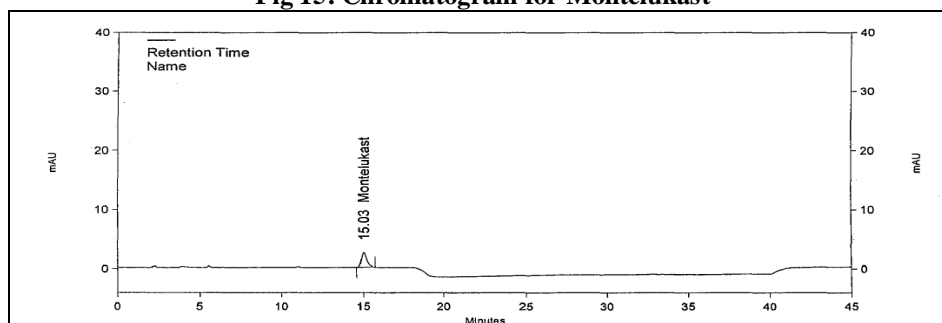


Table 1: Response Factor

Component Name	Relative Retention Time	Response Factor
Sulphoxide impurity	0.37	1.05
Hydroxy impurity	0.55	0.86
Dihydro impurity	0.77	0.73
Montelukast	1.00	1.00
Styrene impurity	2.02	1.89

Table 2: Accuracy (Recovery) for Known impurities:

Name of Compound	Resolution
Sulphoxide impurity	--
Hydroxy impurity	11.25
Dihydro impurity	6.44
Montelukast Sodium	6.22
Styrene impurity	23.87

Table 3 : Styrene impurity standard

Injection No.	Area	Asymmetry	Theoretical Plates
1	32242	1.23	23497
2	32035	1.22	23400
3	32297	1.14	23454
4	31444	1.12	23914
5	31575	1.13	23876
6	31444	1.19	23486
Mean	31840	1.17	23605
Std. Dev.	398.1	---	--
% RSD	1.25	---	---

Table 4 : Recovery for Styrene impurity

Accuracy Level	Sample No.	Actual Amount added in mg	Recovered Amount in mg	% Recovery	Mean % Recovery	Std. Dev.	% RSD
LOQ Level	1	63.113	57.325	90.8	96.5	4.91	5.09
	2	63.113	62.611	99.2			
	3	63.113	62.743	99.4			
Level I (50%)	1	210.375	198.049	94.1	94.4	0.42	0.44
	2	210.375	199.727	94.9			
	3	210.375	198.326	94.3			
Level II (100%)	1	420.750	400.802	95.3	95.2	0.75	0.79
	2	420.750	403.326	95.9			
	3	420.750	397.129	94.4			
Level III (200%)	1	841.500	799.794	95.0	95.5	0.55	0.58
	2	841.500	808.595	96.1			
	3	841.500	803.706	95.5			
				Overall Mean Recovery	95.4		
				Std. Dev.	2.27		
				% RSD	2.38		

Table 5: Accuracy (Recovery) For Unknown Impurity

Name of Impurity	mean % Recovery				Overall Mean % Recovery	STD DEV.	%RSD
	Level I LOQ	Level II 50%	Level III 100%	Level IV 150%			
Sulphoxide impurity	104.7	102.6	107.8	98.5	103.4	4.14	4.00
Hydroxide impurity	104.3	106.4	109.3	102.3	105.6	2.94	2.78
Dihydro impurity	99.8	102.6	103.9	104.5	102.7	2.62	2.55
Styrene impurity	104.8	102.7	108.4	103.0	104.7	2.53	2.42
Montelukast	104.5	103.7	101.8	104.2	103.6	2.25	2.17

Table 6: Precision

Type	Mean Area mVS	STD DEV.	% RSD
System Precision for Montelukast std	56472	362.99	0.64
Method Precision for montelukast std (Repeatability)	56472	362.99	0.64
Intermediate Precision(Ruggedness)	58516	631..56	1.08

Table 7: Robustness

Type	Resolution of impurities				
	Sulphoxide	Hydroxy	Dihydro	Montelukast	Styrene
Change in column oven temperature:23 ⁰ c	---	13.41	8.11	7.34	25.51
Change in column oven temperature:27 ⁰ c	---	13.23	7.78	7.06	26.28
Change in wavelength:223 nm	---	13.57	7.42	6.95	26.07
Change in wavelength:227 nm	---	13.80	7.56	7.09	26.01
Change in buffer Ph 4.55	---	13.78	7.64	7.14	26.02
Change in buffer Ph 4.65	---	14.37	6.83	6.85	26.04

Table 8: Robustness For Montelukast Sodium Standard

Type	Mean area	Std dev.	%RSD
Change in column oven temperature:23 ⁰ c	58887	309.65	0.53
Change in column oven temperature:27 ⁰ c	59271	693.26	1.17
Change in wavelength:223 nm	61164	389.37	0.64
Change in wavelength:227 nm	56069	134.51	0.24
Change in buffer pH 4.55	62099	246.05	0.04
Change in buffer pH 4.65	61535	407.02	0.66

Table 9: Robustness For % of Impurities

Type	Mean % of impurities test preparation (1,2 and 3)					% total impurities
	Sulphoxide impurity	Hydroxy impurity	Dihydro impurity	Styrene impurity	Individual % Unknown impurities	
Change in column oven temperature: 23 ⁰ c	0.166	NA	NA	Below quantitation limit	0.986	0.275
Change in column oven temperature: 27 ⁰ c	0.164	Below detection limit	Below quantitation limit	Below quantitation limit	0.093	0.280
Change in wavelength:223 nm	0.171	NA	0.021	Below quantitation limit	0.084	0.276
Change in wavelength: 227 nm	0.167	Below detection limit	NA	Below quantitation limit	0.086	0.280
Change in buffer Ph 4.55	0.164	NA	NA	Below quantitation limit	0.082	0.276
Change in buffer Ph 4.65	0.163	NA	NA	Below quantitation limit	0.085	0.277

4. Conclusion

Quantitative determination of the drugs by HPLC is very accurate & simple method as compare to the other analytical method. The method gave good resolution in Related substances separation substances of Montelukast from Montelukast Sodium in chewable tablet dosage form. The method has been shown to be specific for Montelukast Sodium and founded to be linear, precise and accurate across a suitable analytical range. Solutions have been shown to be stable for at least 24 hours on ambient storage condition. The method has been shown to be robust towards deliberate minor changes in the method parameters.

References

- Budhavari, S., Eds;In; The Merck Index, 12th Edn., Merck & Co Inc., Whitehouse Station, NJ, 1999, 6337.
- Prafit,K., Edn., In; Martindale, The complete drug reference ,33rd Edn., The Pharmaceutical press,London,1999,756.
- Morrow, J.D, and Roberts, L.J., In; Hardman, J.G. and Limbird, L.E. Eds., The Pharmacological Basis of Therapeutics, 10th Edn., McGraw Hill, New York,2001.677.
- Liu L, Cheng H, Zhao JJ, Rogers JD. Determination of montelukast and its S-enantiomer in human plasma by stereoselective high-performance liquid chromatography with column switching. *J Pharm Biomed Anal.* 1997; 15:631-638.
- Ochiai H, Uchiyama N, Takano T, Hara K, Kamei T. Determination of montelukast sodium in human plasma by column-switching high performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Appl.* 1998; 713:409-414.
- Radhakrishna, T., Narsaraju, A., Ramakrishna, M and Satyanarayana, A., *J. Pharma. Biomed. Anal.*, 2003, 31,359.
- Arsarra, I.A., Saudi pharm. J., 2004, 12,136.
- Smith, G.A., Rawls c.M. and Kunku R.L., *pharm . Res.*, 2004, 21, 1539.
- International Conferences on Harmonization Q3A (R2): Draft Revised Guidance on impurities in New Drug Substances, 2006.
- International Conferences on Harmonization Q2 (R1) Validation of analytical Procedures: text and methodology, 2005.