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Equivalent Method Development and Cross Validation of Pharmacopoeial Method of Montelukast Sodium

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this exploration research work article is to develop equivalent method and evaluate its equivalency (Cross validation) against pharmacopoeial method of Montelukast sodium for the evaluation and assessment of process related impurities i.e. Methyl MLK impurity in Montelukast sodium by HPLC method and its principles. The method mentioned in European Pharmacopoeia (EP) and United States Pharmacopoeia (USP) does not sufficiently separates impurity C and impurity D , as these impurities elutes under the main peak and the pharmacopoeial methods were also not able to detect the Methyl MLK impurity which is not listed in USP monograph. So our prime design of experiment is to develop of new high-performance liquid chromatographic (HPLC) method which eliminates the drawback of two pharmacopoeial methods and this proposed created strategy is fit for recognition and detection of Methyl MLK impurity and separation of all process related impurities of Montelukast sodium mentioned in EP and USP monographs. An efficient strategy screening and scouting in which various C-18 columns were tried and tested. LUNA C-18 column utilized in RP HPLC mode ended up being the phenomenal decision for the technique

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streamlining. The proportion of acetonitrile and trifluoroacetic acid (TFA) in water and in the mobile phase, column temperature, flow rate and diluents were considered as basic strategy boundary. The method developed equivalency was checked in terms of Specificity, LOD, Quantitation (LOQ), Precision, Linearity, Relative response factor (RRF), and Accuracy.

Keywords: RP-HPLC; montelukast sodium; HPLC (High Performance Liquid Chromatography); European Pharmacopoeia (EP); United States Pharmacopoeia (USP); process impurities methyl MLK.

1. INTRODUCTION

Montelukast sodium is a selective and orally active leukotriene receptor antagonist which is being utilized in the treatment of asthma. The action of leukotriene[1] D4 on the cysteinyl leukotriene CysLT1receptor was blocked by Montelukast sodium in the lungs and bronchial tubes (It has a place with astyrylquinolines series that repress the cysteinyl leukotriene CysLT1receptor [2].

Montelukast sodium is portrayed synthetically as 2-[1-[[(1R)-1-[3-[2-(7-chloroquinolin-2-yl)ethenyl] phenyl]-3-[2-(2-hydroxypropan-2-yl)acetic]cycl opropyl] sulfanylmethyl] propyl]phenyl acid} monosodium salt[2]. (Fig.1).

The pharmacopeial methods strategies couldn't give a sufficient separation of process impurity Methyl MLK from the main peak of Montelukast Sodium..It is often challenging to for the separation of such similar process impurity analytes, efficient method technique advancement and streamlining was important. To our knowledge, the case presented is the first that applies HPLC principles for the development and optimization of an impurity-profiling method for Montelukast sodium for the determination and separation of process related impurity. This carries numerous advantages to the new developed method. The design trial experiment supported method optimization encouraged a decrease of exploratory experimental work in comparison with the pharmacopoeial approach. During research and development of analytical method, the HPLC method was developed for the determination of process related impurities of Montelukast sodium[1].

2. AIM

Our main objective and aim of research work is to develop equivalent method and evaluate its equivalency (Cross validation) against pharmacopoeial method of Montelukast sodium for the evaluation and assessment of process related impurities i.e. Methyl MLK impurity in Montelukast sodium by HPLC method and its principles.

3. METHODS

Numerous analytical methods for quantitative evaluation and assessment of Montelukast sodium and its process-linked impurities in drug substance, active pharmaceutical ingredients (API) and in formulation have been reported in the literature.

In European Pharmacopoeia (EP) a monograph for control of Montelukast sodium drug substance is available, which recommends and specify quantitative determination of Montelukast sodium and its process method-related impurities by a HPLC method. The exactly similar technique is also recommended in the United States Pharmacopeia (USP) monograph [3].

3.1 Experimental Reagents, Materials and Reagents

Montelukast reference standard and its impurities were taken from Spectrum Labs (Hyderabad).An in-house Montelukast standard and drug substances were acquired from Unichem (Goa India).Milli-Q water, HPLC-grade acetonitrile, Trifluro-acetic acid (TFA) was bought from Merck (Darmstadt, Germany). All chemicals utilized were of pharmaceutical or special analytical grade.

As per the recommended monograph, the recorded and enumerated impurities are process-linked and these impurities are measured, controlled and monitored in the drug substance.

In some of the published available methods [4-6] depict assurance of impurity of Montelukast sodium. It isn't accounted and reported that the process-linked impurities may beconsidered

during the technique optimization and method development and advancement in these cases. The method selectivity technique for the process-linked impurities is therefore obscure and unidentified. In addition, other announced techniques depict assurance and determination of some process-linked impurities of Montelukast sodium, even not all pharmacopeial ones. Furthermore, none of methods makes reference to the Methyl MLK impurity which is process related impurity, which turned out as the most challenging impurity to be separated from Montelukast sodium.

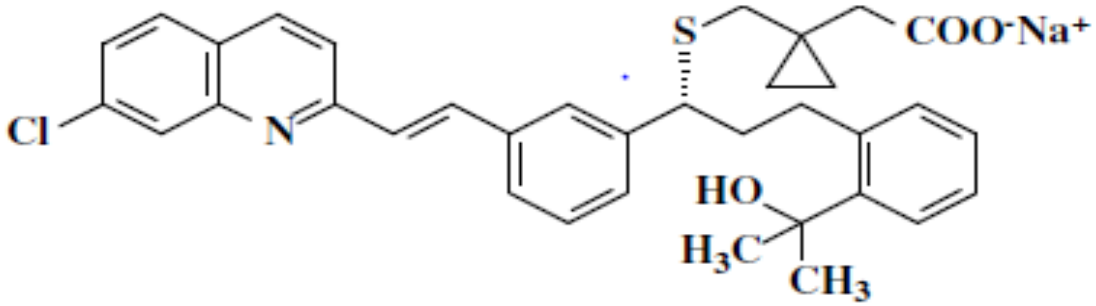
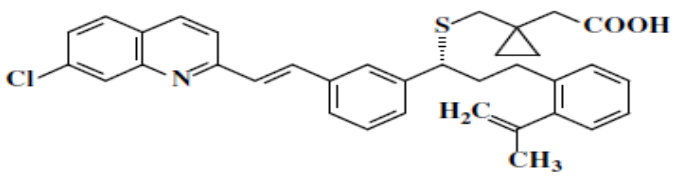
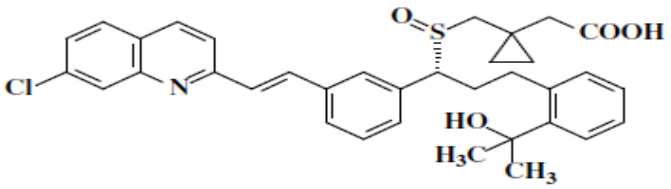
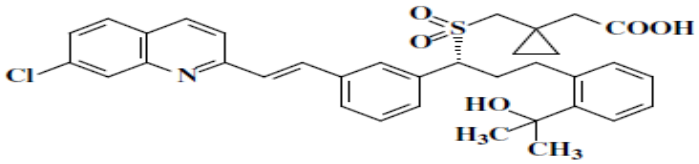
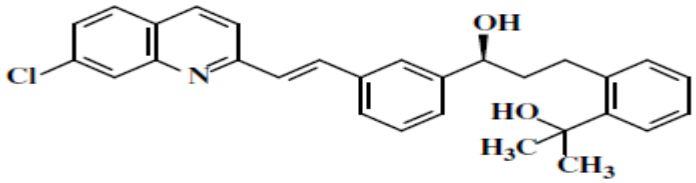
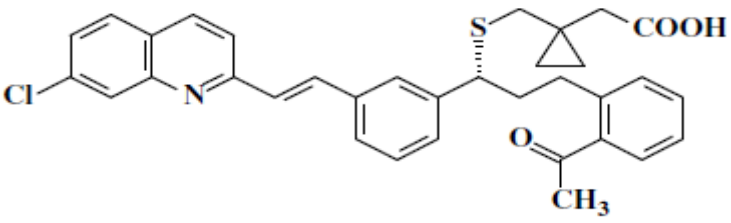
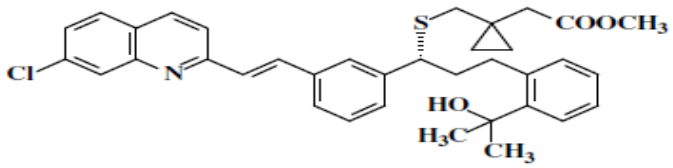
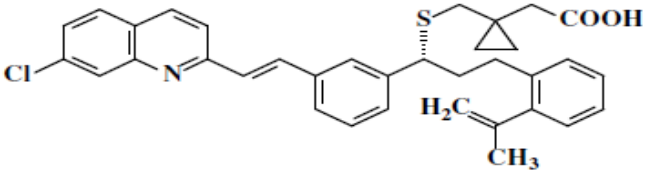
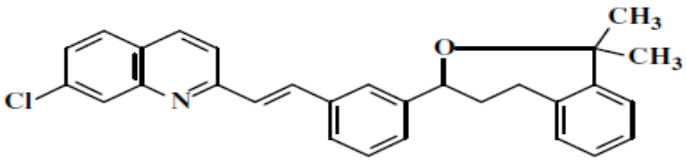
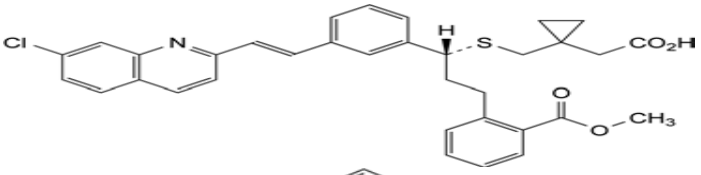
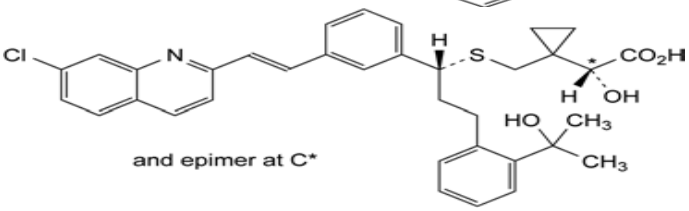
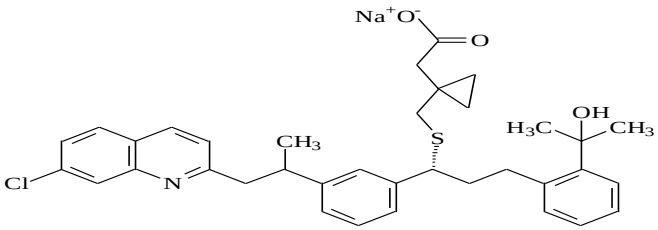
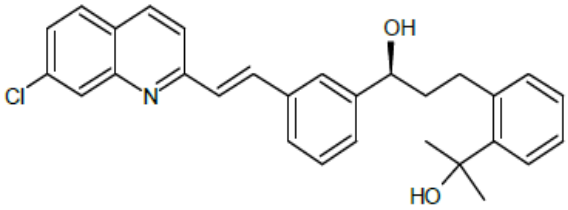


Fig. 1. Chemical structure of montelukast sodium [1]

Table 1. Structure of Montelukast sodium and its process-linked impurities [1] described in United States Pharmacopeia (USP) [1] and European Pharmacopeia (EP) [3]

Methyl styrene (Impurity-F)	
Sulfoxide (Impurity -A)	
Cis Isomer (Impurity -B)	
Michael Adducts 1 and 2 (Impurity-C)	
Impurity-D	

Methyl ketone (Impurity -E)	
Impurity- F	
Impurity - G	
Impurity –H	
Impurity –I	
MLK Methyl	
MKT	

The targeted goal of this research work articlewas to create and upgrade a particular and powerful logical technique for evaluating and determining all process-linked impurities of Montelukast sodium with much accuracy and repeatability.

Moreover, the strategy created has been checked in terms of linearity, sensitivity precision, accuracy, and the response factors for all process-linked impurities have been determined. Our cross validation research investigation started by testing the European Pharmacopoeial method [3,4], the proposed USP method process from Pharmacopeial Forum [6], to check and confirm whether these methods have the capability to isolate and separate all process-linked impurities of Montelukast Sodium that are expressed and indicated in European Pharmacopoeia and USP according to the standard criteria established in regulatory guidelines.

As per European Pharmacopoeia following impurities are the potential impurities of Montelukast Sodium API and the specification limit of these impurities are referenced underneath table:

The following two impurities are not part of specification and reported as unknown impurity with specification limit 0.10% and study shall be done for the verification of RT and presence of Montelukast sodium.

1. EP Impurity H
2. EP Impurity I
3. The specification limit for method suitability study has been considered as not more than 0.10% for EP impurity H and I in Montelukast sodium API.

As per USP following impurities are the potential impurities of Montelukast Sodium API and the specification limit of these impurities are mentioned below

3.2 Instrumentation

Theexploration technique and research method experiments were developed on a HPLC Alliance Waters 2695 system, comprising of a HPLC quaternary pump, auto sampler, and thermostat column compartment, using a UV/Visible detector Waters 2489. Instrument command and data acquisition information were performed utilizing Water's Empower 2 programming software.

3.3 Preparation of Solutions

3.3.1 Preparation of impurity marker solution preparation

Prepared solution containing 1mg/ml of Montelukast for peak identification CRS in diluents

3.3.2 Preparation of system suitability solution

Transferred 1 ml of impurity solution into a colorless glass vial, and expose to ambient light for approximately 20 minute to generate the impurity-G of Montelukast.

3.3.3 Preparation of IMP-I and IMP-H (about 0.10% of nominal concentration)

Prepare solution containing each 0.001 mg/ml in diluents.

Table 2. Specification limitsof potential impurities of montelukast sodium API between EP & current developed method

Impurity Name	Specification limit as per EP	Specification limit of proposed Developed Method
EP Impurity B (MLK-D)	NMT 0.30%	NMT 0.15%
EP Impurity C (MLK-SO)	NMT 0.20%	NMT 0.15%
Sum of EP Impurity D and E	NMT 0.15%	NMT 0.15%
EP Imp F (MLK-K)	NMT 0.15%	NMT 0.15%
EP Imp G (Cis MLK)	NMT 0.15%	NMT 0.15%
Any unspecified impurity	NMT 0.10%	NMT 0.10%
Total impurities	NMT 0.60%	NMT 0.50%

Table 3. Specification limitsof potential Impurities of montelukast Sodium API between USP & current developed method

Impurity Name	Specification limit as per USP	Specification limit of proposed Developed Method
Methyl styrene (Impurity F)	NMT 0.30%	NMT 0.15%
Sulfoxide(Impurity A)	NMT 0.20%	NMT 0.15%
Michael Adducts 1 and 2 (Impurity C+D)	NMT 0.15%	NMT 0.15%
Methyl ketone (Impurity E)	NMT 0.15%	NMT 0.15%
Cis isomer (Impurity B)	NMT 0.15%	NMT 0.15%
Any unspecified impurity	NMT 0.10%	NMT 0.10%
Total impurities	NMT 0.60%	NMT 0.50%

3.3.4 Preparation of Methyl MLK and MKT (about 0.10% of nominal concentration)

Prepare solution containing each 0.001 mg/mL in diluent.

3.3.5 Preparation of sample Solution

Prepare solution containing about 1.0 mg/ml of Montelukast Sodium in diluent.

3.3.6 Chromatographic conditions

Table 4. Mobile phase, diluent, column and chromatographic conditions of EP/USP method for the determination of impurity profile in Montelukast Sodium by HPLC

Column & packing	**Zorbax Phenyl Hexyl (50X4.6) mm, 1.8 µm.**		
Buffer Preparation	Eluent A: 1.5 ml of TFA dilute up to 1000 ml with H2O		
	Eluent B: 1.5 ml of TFA dilute up to 1000 ml with Acetonitrile		
	Time (min)	Eluent A (%)	Eluent B (%)
	0.0	60	40
Gradient of Eluent	3.0	60	40
	16.0	49	51
	16.1	60	40
Stop time	16 minutes		
Equilibration time	5 min		
Flow	1.2 ml/min		
Detector	238nm.		
Injection volume	10 µl.		
Diluent	90% Methanol: 10% H2O		
Column temperature	30°C		
Auto sampler temperature	5°C		

Table 5. Mobile phase, diluent, column and Chromatographic conditions of developed method used for the determination of impurity profile in Montelukast Sodium by HPLC

Column & packing	**LUNA C18(2) 100A 250x4.6mm, 5µm.**		
Buffer Preparation	Solution - I: 3 ml of TFA dilute up to 100 mL with H2O		
	Solution- II: 3 ml of TFA dilute up to 100 mL with Acetonitrile		
Eluent A	1ml of solution I to 2 liter of H2O		
Eluent B	1ml of solution II to 2 liter of Acetonitrile		
	Time (min)	Eluent A (%)	Eluent B (%)
	0.0	38	62
	10	35	65
Gradient of Eluent	20	35	65
	35	5	95
	40	5	95
Stop time	40 minutes		
Equilibration time	10 min		
Flow	1.5 ml/min		
Detector	225nm.		
Injection volume	20 µl.		
Diluent	80% Acetonitrile : 20% water		
Column temperature	25°C		
Auto sampler temperature	5°C		
Sample Concentration	1 mg/mL		

**TFA= Trifluroacetic acid*

4. RESULTS AND DISCUSSION

4.1 Methods Verification

In the concluding step of the developed research work for the determination and evaluation of process-linked impurities of Montelukast Sodium by HPLC was verified in terms of linearity limit of detection (LOD), and limit of quantification (LOQ), precision and accuracy. Method verification was performed as per ICH Q2 (R1) guidelines [7].

4.1.1 Precision

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 [8].

The precision of the proposed developed method was evaluated by injecting six replicates of sample solution of the drug substance [7]. Since MLK -impurity which is not listed and recorded in USP monograph hence needed to develop a precise method which is fit for the of detection of Methyl MLK impurity and efficiently separating as well as estimating all process related impurities of Montelukast sodium. Methyl MLK impurity is the conceivable impurity in Montelukast sodium, which was spiked to the sample at the 0.10% level (according to EP/USP monograph specification limit).

The pharmacopoeial method (USP/EP) as well as proposed developed method was found precise, accurate and linear over the range of4.9794 $x10^{-4}$– 2.6055 $x10^{-3}$ mg/ml (about 0.05%- 0.3 of the nominal concentration of MLK-Na sample in the method) for determination of Methyl MLK impurity which could be presented in developed method for Montelukast Sodium and limited as "Any" impurity with specification NMT 0.10%The intra-day RSD results obtained for the six replicates of an of impurity content is 1.7%. The result represents suitable precision of the proposed developed method.

4.2 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 [9].

The specificity of the proposed developed HPLC method for Montelukast sodium was conceded out in the presence of its potential impurities.

In order to verify and check the Specificity of the USP methods against the developed method MLK-Na 1mg/mL solution of Montelukast marker for peak identification (contains MLK-Na, Sulfoxide (Imp. A), Cis isomer (Imp. B) Michael adducts 1 and 2 (Imp. C and D), Methylketone (Imp.E), and Methyl styrene (Imp.F) was mixed with 1 mg/ml solution of Montelukast sample contains the MLK Methyl impurity:(1-[[[1-[3-[2-(7-chloro-2-quinolinyl)-1-methylethyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl)0phenyl]propyl]thio]methyl]-cyclopropaneacetic acid and injected to HPLC applying USP method while Zorbax Phenyl Hexyl 50*4.6 1.8 µ column(belongs to USP L11 group of column) was utilized.

Separation between main Montelukast Sodium peak and impurities peaks and separation between all impurities peaks were observed by both USP and proposed developed chromatographic methods.

Even though EP method for impurity determination by HPLC in Montelukast Sodium is good enough to detect-out impurity H and I. Primary evaluation of recovery of impurity H and I (100 % level), was observed to be satisfactory. The response of impurity H and I is almost same to that of the response of Montelukast sodium. However, internal sample of Montelukast sodium was observed to be free from the impurity H and I. Furthermore, any possible presence of impurity H and I in Montelukast Sodium will be integrated and captured under the head of “Any individual Impurity” controlled at the level of NMT 0.10 %.

4.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

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 [10].

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. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. [10]

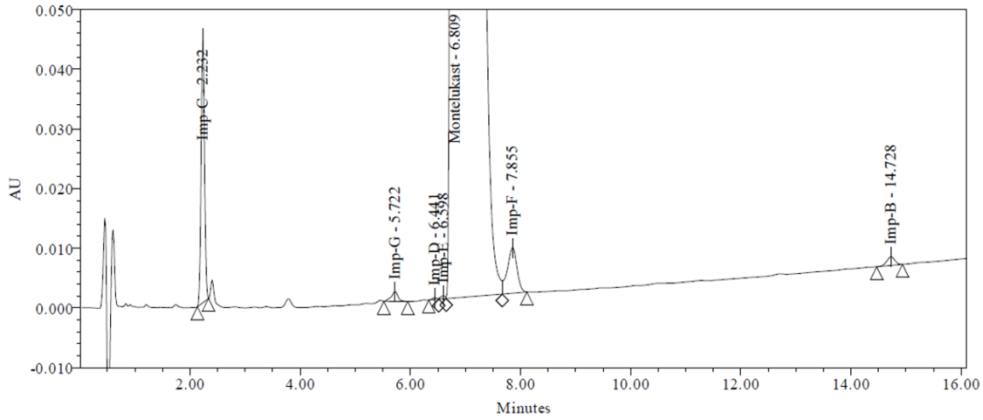


Fig. 2. Chromatogram of Specificity solution applying EP method

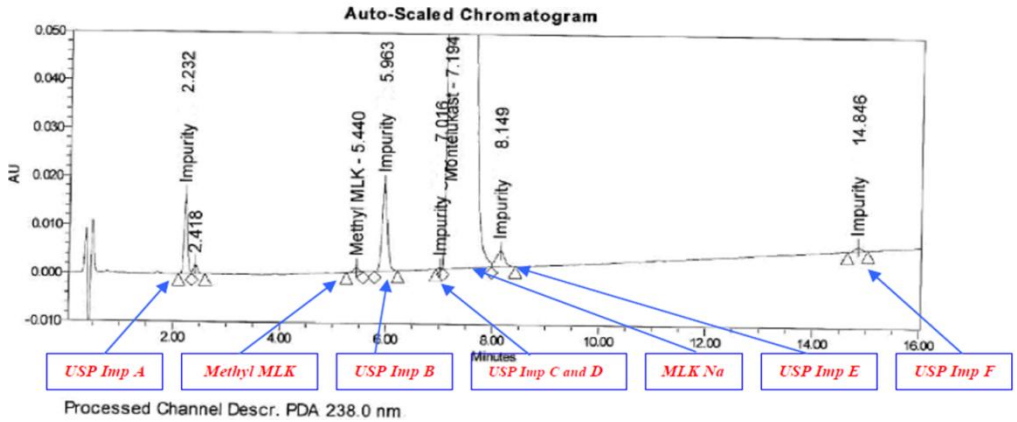


Fig. 3. Chromatogram of Specificity solution applying USP method

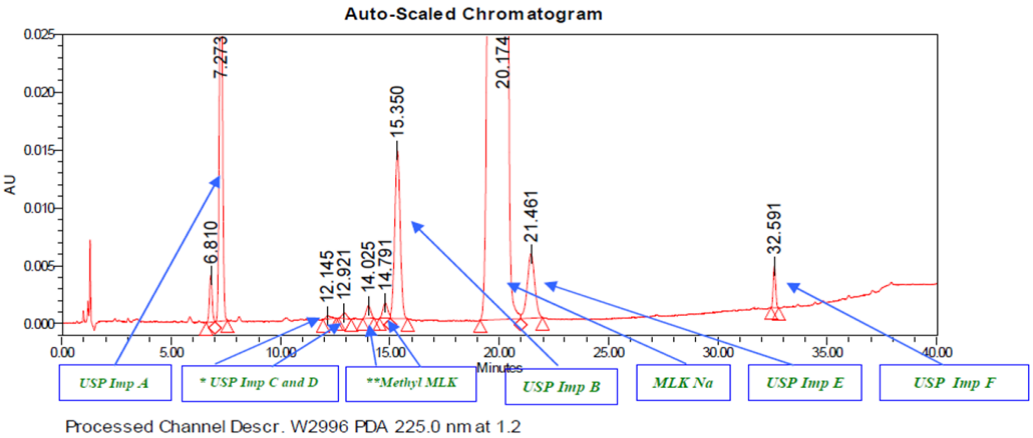


Fig. 4. Chromatogram of Specificity solution applying proposed developed method

Note: USP Impurity C and D are diastereomers and Methyl MLK is mixture of diastereomers and exhibit two peaks

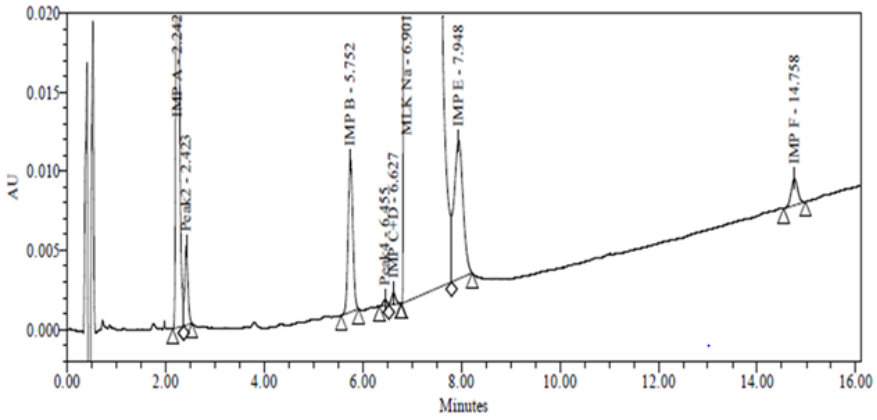


Fig. 5. Chromatogram for USP peak identification Solution (USP Method)

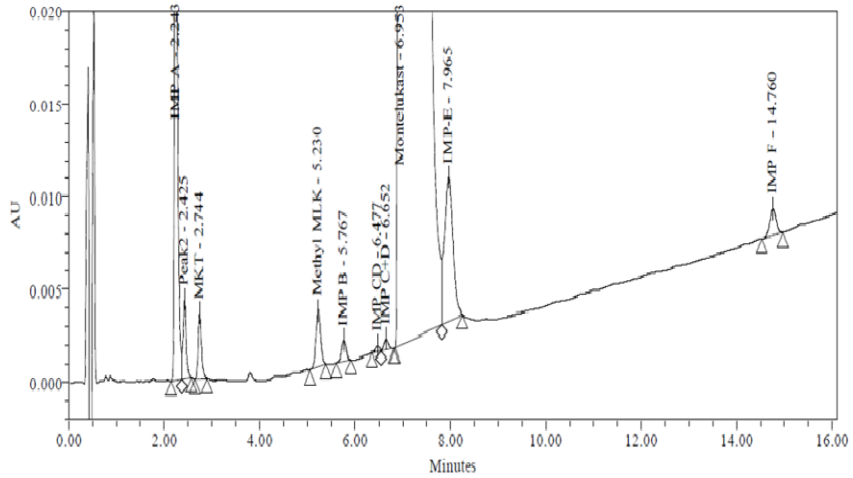


Fig. 6. Chromatogram for USP peak identification Solution (USP Method) spiked with MLK-Methyl and MKT impurity

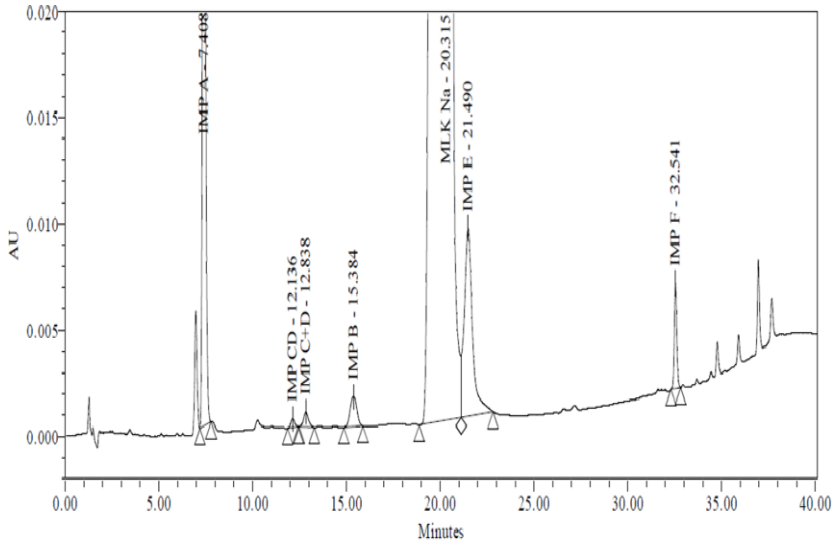


Fig. 7. Chromatogram for USP peak identification Solution (Proposed Method)

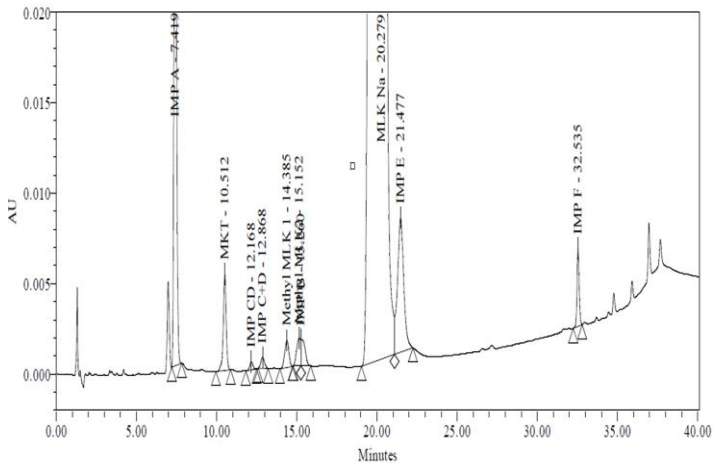
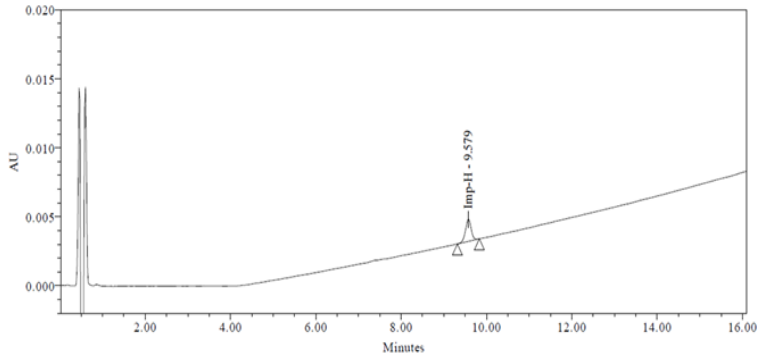


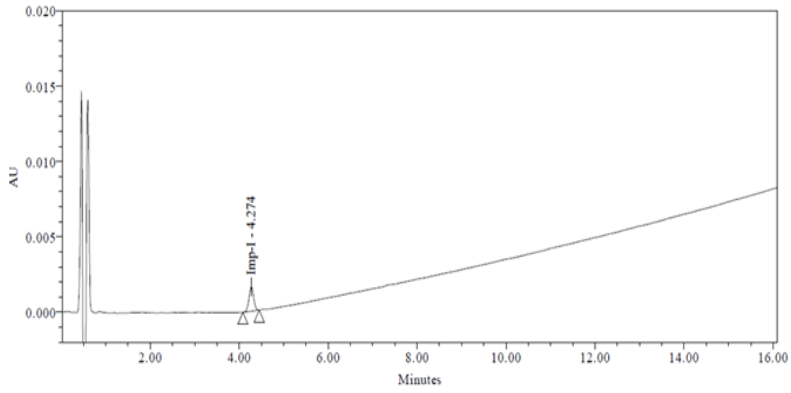
Fig. 8. Chromatogram for USP peak identification Solution (Proposed Method) spiked with MLK-Methyl and MKT impurity



Peak Results

	Name	RT	Area	% Area
1	Imp-H	9.58	14815	100.000

Fig. 9. Chromatogram for EP impurity-H



Peak Results

	Name	RT	Area	% Area
1	Imp-I	4.27	11916	100.000

Fig. 10. Chromatogram for EP impurity-I

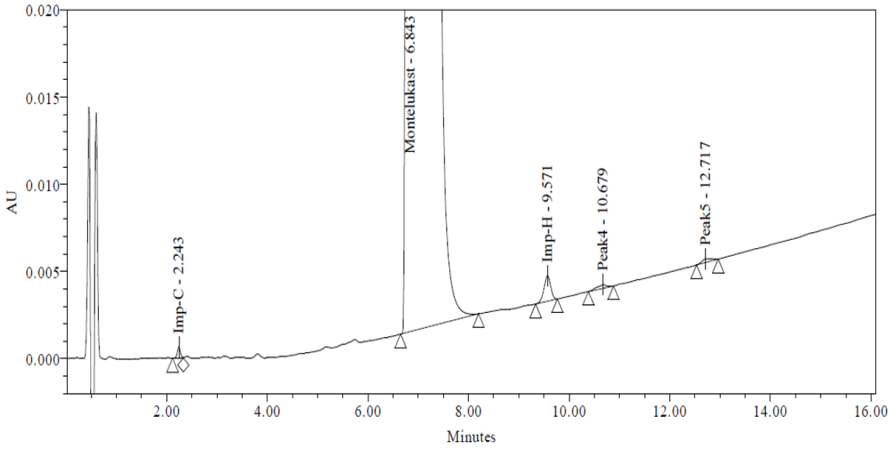


Fig. 11. Chromatogram for Montelukast Sodium Sample spiked with Impurity-H.by .(EP Method)

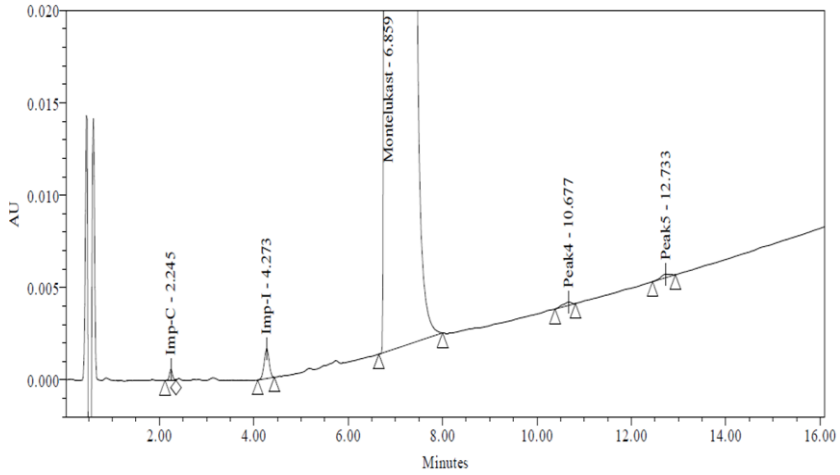


Fig. 12. Chromatogram for Montelukast Sodium Sample spiked with Impurity- I by (EP Method)

The Limit of Detection (LOD) of Montelukast Na was acquired by mathematical equation.

$$\text{LOD} = \frac{3 \text{ x standard deviation}}{\text{Slope MLK} - \text{Na}} = 0.2874 \text{ µg/ml}$$

The Limit of Quantitation (LOQ)of Montelukast Na was acquired by mathematical equation

$$\text{LOQ} = \frac{10 \text{ x standard deviation}}{\text{Slope MLK} - \text{Na}} = 0.4790 \text{ µg/ml}$$

The S/N ratio of LOD was found to be 7.5. By injecting six replicate injections LOQ was calculated and found to be 0.4790 µg/mL (0.05%) solution of Montelukast Na. The average S/N ratio was 27.17 with RSD of Montelukast Na peak area being 1.5%. The established and evaluated LOD and LOQ were 0.03% and 0.05%.

4.4 Quantitation Limit Verification of the USP Methods

A solution containing MLK-Na standard at concentration 0.4790µg/ml (about 0.05 % of the nominal concentration of a sample) was injected in six replicates.

4.5 Quantitation limit Verification of the USP Methods

A solution containing Methyl MLK standard at concentration 4.8794×10^{-4} mg/ml (about 0.05%

of the nominal concentration of a sample) was injected in six replicates.

The %RSD of Methyl MLK areas and average deviation of six replicates from the linear regression curve were calculated and summarized in the table:

4.6 Linearity for Methyl MLK impurity

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 [10].

Standard solutions of Methyl MLK impurity at concentration range of 4.9794 $x10^{-4}$ - 2.6055 $x10^{-3}$ mg/ml were made from two stock solutions and injected once. The acceptability of the method for quantitative determination of Methyl MLK over the concentration range of about0.05%- 0.3 of the nominal concentration of MLK-Na sample in the method was demonstrated by linear regression analysis. The results are summarized in following table:

Table 6. Summarized results of %RSD of MLK-Na areas of six replicates

Injection No.	Area	Average area	RSD %
1	4185	4183	1.5
2	4181		
3	4204		
4	4178		
5	4271		
6	4077		

The RSD of areas is < 10.0% and therefore QL of USP method is verified to be 0.05%

Table 7. Summarized results of %RSD of Methyl MLK areas of six replicates

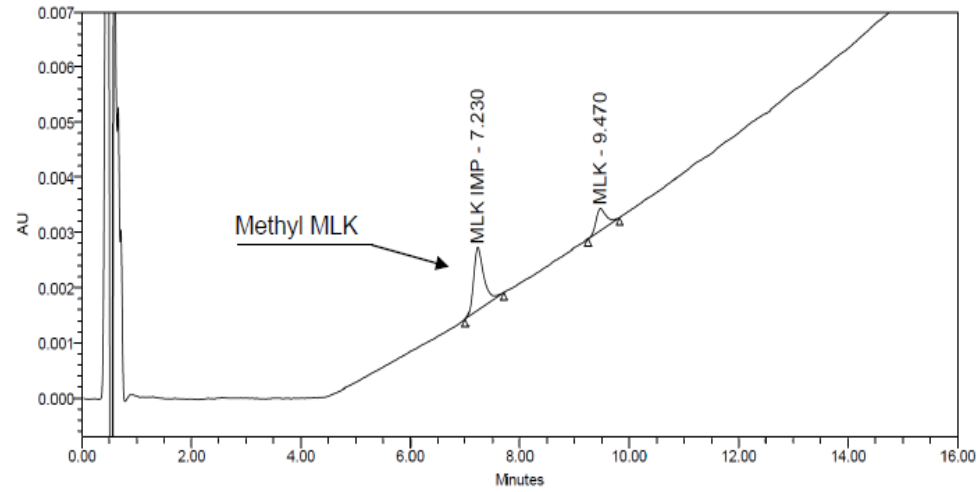
Injection No.	Area	Calculated concentration mg/mL	Accuracy %
1	14704	4.5847 $x10^{-4}$	93.96
2	1474	4.6449 $x10^{-4}$	95.20
3	15231	4.7714 $x10^{-4}$	97.79
4	15415	4.8365 $x10^{-4}$	99.12
5	14968	4.6782 $x10^{-4}$	95.88
6	15190	4.7568 $x10^{-4}$	97.49
Average	15063.7	**Average**	**96.6**
% RSD	**1.7**		

The RSD of areas is < 10.0% and average deviation from the linear regression curve is < 30.0% therefore QL of Methyl MLK impurity is 0.05%

Table 8. Summarized results Linearity parameter of validation for the proposed method

Concentration µg/mL	Measured Area
4.8794 $x10^{4}$	15190
7.8070 $x10^{4}$	24271
9.7588 $x10^{4}$	29912
1.5633 $x10^{3}$	44853
2.6055 $x10^{3}$	75663
Slope	28238742.9154
Y-Intercept	1757.2538
Correlation Coefficient (R^2)	**0.9991**

Statistics of linear regression analysis:
R^2 = 0.9991 > 0.990 Slope=28238742.9154 Intercept= 1757.2538
Calculated area at 1$x10^{-3}$ mg/ml (0.10%) is 29995.99674
Intercept value is 5.9 % of calculated area at 1$x10^{-3}$ mg/ml, less than 10.0%.



	Peak Name	RT	Area	% Area	RT Ratio	s/n
1	MLK IMP	7.230	14704	73.96	0.763	76.60
2	MLK	9.470	5177	26.04	1.000	27.17

Fig. 13. Typical QL chromatogram of Methyl MLK impurity at 4.8794x10^{-4}g/mL

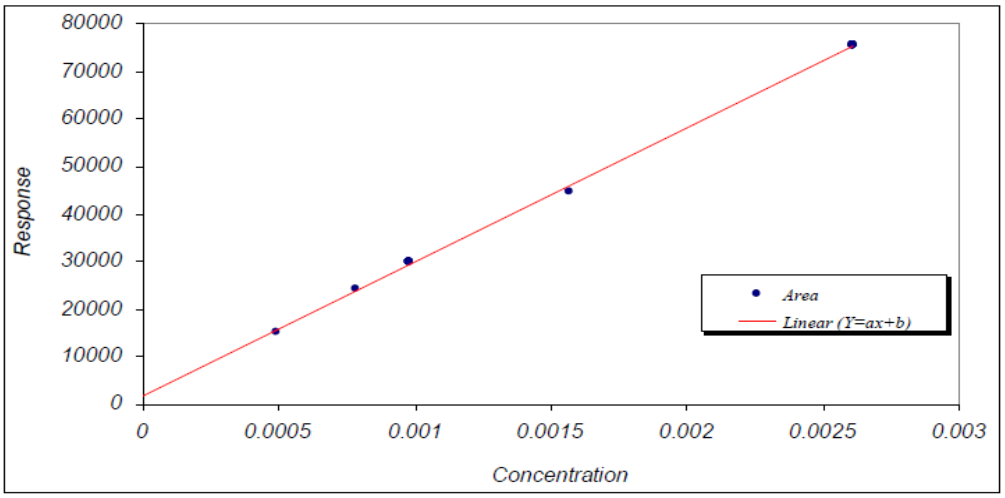


Fig. 14. Linearity graph of Methyl MLK impurity

4.7 Relative Response factor (RRF) for Methyl –MLK impurity

The relative response factor for Methyl –MLK was determined from the regression curve of Methyl MLK impurity presented in (paragraph 2.5.6) and regression curve of MLK –Na which presented in below table. Standard solutions of MLK-Na at concentration range of 4.7735x10^{-4} - 2.4705x10^{-3}mg/ml were prepared from two stock solutions and injected once. The results are summarized in following table:

4.8 Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) 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 [10].The range between the lowest and highest concentration of methyl MLK impurity which precise, linear and accurate are: 4.9794 x10^{-4} - 2.6055 x10^{-3} mg/ml: about 0.05%- 0.3% of the nominal concentration of MLK-Na sample in the method.

Table 9. Summarized results Linearity parameter for the proposed method

Concentration µg/mL	Measured Area
4.7735 x10^{-4}	5067
7.6376 x10^{-4}	9046
9.5470 x10^{-4}	11660
1.4823 x10^{-3}	17777
2.4705 x10^{-3}	30897
Slope	12831513.7359
Y-Intercept	889.5825
Correlation Coefficient (R^2)	**0.9993**

Statistics of linear regression analysis:
R^2 = 0.9993 > 0.990 Slope=12831513.7359 Intercept= -889.5825
Calculated area at 1x10^{-3} mg/ml (0.10%) is 11941.93121
Intercept value is 7.4 % of calculated area at 1x10^{-3} mg/ml, less than 10.0%.

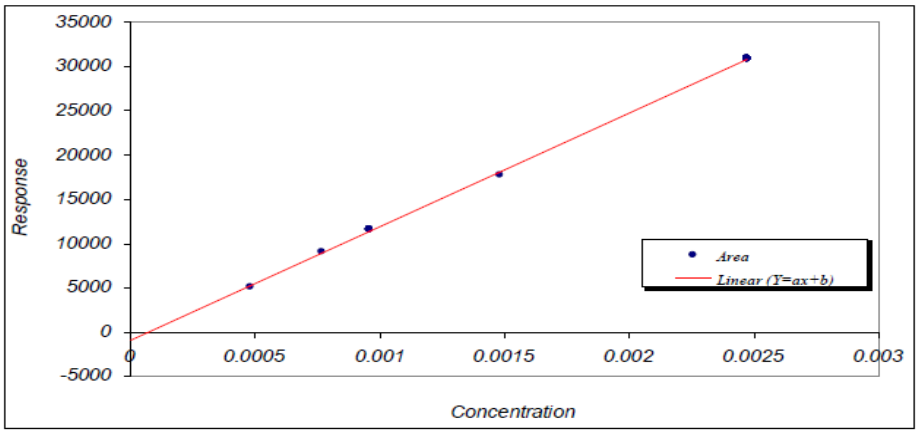


Fig. 15. Linearity Graph of MLK Na used for RRF calculation

$$\text{RRF of Methyl MLK} = \frac{\text{Slope Methyl MLK}}{\text{Slope MLK} - \text{Na}} = \frac{28238742.9154}{12831513.7359} = 2.20$$

4.9 Accuracy (Recovery of Methyl MLK impurity)

The proposed developed method strategy of accuracy was checked and verified by injecting three spiked sample solutions spiked with stock solution of Methyl MLK impurity explored at four different concentration levels (LOQ, specification limit, and 0.30% of specification limit) in three replicate. The designed specification limit for EP impurity A was 0.30%, whereas the specification limit for all other impurities was 0.15%. The recoveries are depicted in Table 10. The method was established and verified to be accurate.

4.9.1 Accuracy at concentration about QL level

Three series of injections were injected, unspiked MLK Na sample at the concentration of 1.0224 mg/ml, three replicates. MLK Na sample at the concentration of 1.0224 mg/ml was spiked with Methyl MLK standard at 0.5014µg/ml (about 0.05% of the nominal concentration of a sample respectively), and three replicates. Standard solution of the Methyl MLK, at concentration 0.5014µg/ml (about0.05% of the nominal concentration of a sample), three replicates. The results are summarized in the below table:

4.9.2 Accuracy at concentration about 0.10%

Three series of injections were injected, unspiked MLK Na sample at the concentration of 1.0224 mg/ml, three replicates. MLK Na sample at the concentration of 1.0224 mg/ml was spiked with Methyl MLK standard at 1.0636µg/ml (about 0.10% of the nominal concentration of a sample respectively), three replicates. Standard solution of the Methyl MLK, at concentration 1.0636

Table 10. Summarized results of accuracy at QL level

Impurity Name	Impurity Standard Solution		Spiked Solution		Main Analyte Standard Solution		Recovery %
	RT	**Area**	**RT**	**Area**	**RT**	**Area**	111.8
MLK-Methyl at concentration QL level (0.05%)	4.76	9174	4.74	20265	4.75	9945	
	4.77	9528	4.75	20811	4.75	10137	
	4.76	9256	4.75	20351	4.75	10076	
	Average	9319.33	Average	20475.67	Average	10052.67	
	SD	185.30	SD	293.57	SD	98.10	
	RSD	2.0	RSD	1.4	RSD	1.0	

Table 11. Summarized results of accuracy at 0.10%

Impurity Name	Impurity Standard Solution		Spiked Solution		Main Analyte Standard Solution		Recovery %
	RT	**Area**	**RT**	**Area**	**RT**	**Area**	107.0
MLK-Methyl at nominal concentration level (0.10%)	4.76	21229	4.75	31723	4.75	9945	
	4.76	19245	4.75	30645	4.75	10137	
	4.75	18921	4.75	31362	4.75	10076	
	Average	19798.33	Average	31243.33	Average	10052.67	
	SD	1249.54	SD	548.71	SD	98.10	
	RSD	6.3	RSD	1.8	RSD	1.0	

Table 12. Summarized Results of accuracy at 0.15%

Impurity Name	Impurity Standard Solution		Spiked Solution		Main Analyte Standard Solution		Recovery %
	RT	**Area**	**RT**	**Area**	**RT**	**Area**	104.7
MLK-Methyl at nominal concentration level (0.15%)	4.75	31700	4.75	41988	4.75	9945	
	4.75	29728	4.75	42927	4.75	10137	
	4.75	30593	4.74	4154	4.75	10076	
	Average	30673.67	Average	42154.33	Average	10052.67	
	SD	988.47	SD	704.39	SD	98.10	
	RSD	3.2	RSD	1.7	RSD	1.0	

Table 13. Summarized Results of accuracy at 0.30%

Impurity Name	**Impurity Standard Solution**		**Spiked Solution**		**Main Analyte Standard Solution**		**Recovery %**
	RT	**Area**	**RT**	**Area**	**RT**	**Area**	101.9
MLK-Methyl at nominal	4.75	63078	4.76	75731	4.75	9945	
concentration level	4.76	64294	4.76	73410	4.75	10137	
	4.76	63072	4.76	75123	4.75	10076	
(0.30%)	Average	63481.33	Average	74754.67	Average	10052.67	
	SD	703.80	SD	1203.54	SD	98.10	
	RSD	1.1	RSD	1.6	RSD	1.0	

µg/ml (about 0.10% of the nominal concentration of a sample), three replicates. The results are summarized in the below table:

4.9.3 Accuracy at concentration about 0.15%

Three series of injections were injected, unspiked MLK Na sample at the concentration of 1.0224 mg/ml, three replicates. MLK Na sample at the concentration of 1.6154 mg/ml was spiked with Methyl MLK standard of 1.6154 µg/ml (about 0.15% of the nominal concentration of a sample respectively), three replicates. Standard solution of the Methyl MLK, at concentration 1.6154 µg/ml (about 0.15% of the nominal concentration of a sample), three replicates. The results are summarized in the below table:

4.9.4 Accuracy at concentration about 0.30%

Three series of injections were injected, unspiked MLK Na sample at the concentration of 1.0224 mg/ml, three replicates. MLK Na sample at the concentration of 3.2307 mg/ml was spiked with Methyl MLK standard at 1.6154 µg/ml (about 0.30% of the nominal concentration of a sample respectively), three replicates. Standard solution of the Methyl MLK, at concentration 3.2307 µg/ml (about 0.30% of the nominal concentration of a sample), three replicates. The results are summarized in the below table:

Recoveries results of Methyl MLK investigated impurity area at four concentration levels is between 80.0%-120% for all four levels. The proposed developed method is suitable for the evaluation of Methyl MLK impurity.

Because not all the impurities were present in the analysed sample hence in one batch of MLK –Na sample were spiked and analysed by both method, and results are mentioned in below table.

The difference % of average impurity amount is maximum 25.0 % < 40.0%.

Separation between main Montelukast peak and impurities peaks and separation between all impurities peaks were observed by both developed method and EP method.

The results of developed method of Montelukast sodium obtained by applying EP Method passed the EP specification and similar results obtained by applying Developed method.

The difference of average impurities amount are less than 40% for each impurity between therefore Cross Validation analysis passes the acceptance criteria.

Table 14. Summarized results of chromatographic spiked MLK Na impurity method comparison of proposed method with EP method

MLK- Na Impurity Profile Comparative Results			
	Proposed Method	**EP Method**	**Difference**
EP Impurity B	<0.03	<0.05	NA*
EP Impurity C	<0.03	<0.05	NA
EP Impurity D and E	<0.03	<0.05	NA
EP Impurity F	<0.03	<0.05	NA
EP Impurity G	<0.03	<0.05	NA
Any Impurity	<0.03	<0.05	NA
Total	<0.03	<0.05	NA

**NA-Not Applicable*

Table 15. Summarized results of chromatographic impurity method comparison of proposed method with EP method

Spiked MLK-Na Impurity Profile Comparative Results			
	Proposed Method	**EP Method**	**Difference**
EP Impurity B	0.14	0.13	7.4
EP Impurity C	0.18	0.17	5.7
EP Impurity D and E	0.13	0.12	8.0
EP Impurity F	0.11	0.09	20.0
EP Impurity G	0.18	0.14	25.0
Methyl Impurity	0.08	0.09	11.8

Table 16. Chromatographic impurity method comparison of developed method with USP

MLK- Na Impurity Profile Comparative Results			
	Developed Method	**USP Method**	**Difference**
USP Impurity A	0.07	0.08	13.3
USP Impurity B	<0.05	<0.05	NA*
USP Impurity C and D	<0.05	<0.05	NA
USP Impurity E	<0.05	<0.05	NA
USP Impurity F	0.06	0.06	0.0
Any Impurity	<0.05	<0.05	NA
Total	0.13	0.14	7.4

**NA-Not Applicable*

The difference % of average impurity amount is maximum 25.0 % < 40.0%.

Separation between main Montelukast peak and impurities peaks and separation between all impurities peaks were observed by both developed method and USP method.

The results of developed method of Montelukast sodium obtained by applying USP Method passed the USP specification and similar results obtained by applying developed method.

The difference of average impurities amount are less than 60% for each impurity between 0.05% and 0.10 % therefore Cross Validation analysis passes the acceptance criteria.

5. FINAL CONCLUSION

In the presented research work as per our design of experiment, HPLC principles were effectively used for the development of simple and efficient new RP-HPLC method strategy designed for the assurance of process-linked impurities of Montelukast Sodium and validated as per ICH guidelines. The pharmacopeial methods strategies couldn't give a sufficient separation of process impurity Methyl MLK from the main peak of Montelukast Sodium. The new proposed developed method technique removes and eliminates these issues; it is able to do effectively extrication and determination of all process impurities listed by the EP monograph and by the proposed USP monograph in single one run.

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The method strategy developed was verified and validated in terms of Specificity, LOD, Quantitation (LOQ), Precision, Linearity, Relative response factor (RRF), and Accuracyand it was demonstrated to be excellent for its intended purpose. In addition, by means of HPLC method development approach, a robust and stout method was developed in spite of the specific separations involved.

The proposed developed method for Impurity profile was confirmed in terms of precision, sensitivity, accuracy, and linearity, and it was proven to be suitable and fit for its intended proposed purpose and found to be specific and suitable for testing of Montelukast Sodium. The USP and EP methods were successfully verified for specificity, detection limit and quantitation limit. The detection limit of USP method is 0.03% of the nominal concentration of sample required by the proposed developed method. The quantitation limit of USP method is 0.05% of the nominal concentration of sample required by the method. The USP method was found precise, accurate and linear over the range of4.9794×10^{-4}– 2.6055×10^{-3} mg/ml (about 0.05%- 0.30% of the nominal concentration of MLK-Na sample in the method) for determination of Methyl MLK impurity which could be presented in proposed developed Montelukast Sodium and limited as "Any" impurity with specification NMT 0.10%The QL for Methyl MLK is 0.05% of the nominal concentration of sample required by the proposed method. The Relative Response factor of Methyl MLK impurity is 2.20 and should be taken into account for further calculations.Every single measurable outcome results (Percentage difference, Percentage, % recovery Mean, RSD) were under the acceptance criteria.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement

of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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