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RESEARCH ARTICLE

Method Development and Validation of Montelukast in Bulk and Pharmaceutical Dosage form by RP-HPLC

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ABSTRACT

The present work describes a simple, precise and accurate HPLC method for estimation of montelukast sodium in bulk and in tablet dosage form. Montelukast sodium is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene (CysLT1) receptor. The separation was achieved by using Waters symmetry shield RP-C_8 column and acetonitrile: sodium di-hydrogen Phosphate dehydrate (pH 3.7) in proportion of 70:30 v/v as mobile phase, at a flow rate of 1.5 ml/min. Detection was carried out at 225 nm. The retention time of montelukast sodium was found to be 3.721 min. The limit of detection was found 0.1357 µg/ml and limit of quantification 0.4111 µg/ml. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity (1-30 µg/ml), accuracy, precision, robustness and specificity according to ICH guidelines. The method was statistically validated and RSD was found to be less than 2% indicating high degree of accuracy and precision of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining Montelukast in bulk or in pharmaceutical dosage forms.

KEYWORDS: Montelukast, High Performance Liquid Chromatography, Method development, Validation

INTRODUCTION:

Montelukast sodium is described chemically as [*R*-(*E*)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methyl ethyl) phenyl] propyl] thio] methyl] cyclopropaneacetic acid, monosodium salt (Fig.1). The empirical formula is $C_{35}H_{35}C_lNNaO_3S$, and its molecular weight is 608.18. It is used primarily for the treatment of Asthma in children and adults. It is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene (CysLT1) receptor. Literature survey reveals that liquid chromatography with fluorescence detector, stereoselective high performance liquid chromatography (HPLC) for montelukast and its S-enantiomer, column switching HPLC with fluorescence detector, semi-automated 96-well protein precipitation, HPLC with derivative spectroscopy, pressurized liquid extraction followed by HPLC and LC-MS methods have been reported for the estimation of montelukast sodium. The present study illustrates development and validation of a simple, accurate and precise procedure for determination of montelukast sodium by RP-HPLC in bulk and in tablet dosage form.

MATERIALS AND METHODS:

CHEMICALS & REAGENTS:

Montelukast tablets, claimed to contain 5mg, 10mg of Montelukast procured from Dr. Reddy's Lab., Hyderbad, A.P. India. The HPLC grade solvent used were of Merck (India) Ltd, Mumbai.

INSTRUMENTATION:

Quantitative HPLC was performed on Shimadzu HPLC with LC- 20AT pumps besides SPD-20A UV-Visible detector. Shimadzu spincrom-CFR software is used along with Waters symmetry shield RP-C_8 (150 mm × 3.9 mm), 5 µm column for the separation.

SELECTION OF MOBILE PHASE:

Selected Drugs were injected to the column with different mobile phases of different ratios with different flow rates till sharp peaks without any interference peaks containing spectra were obtained. The different mobile phases were containing one or the combinations of two of the following: sodium di-hydrogen phosphate dehydrate, Acetonitrile (HPLC grade) in the ratio 70:30 and pH adjusted to 3.7 with Ortho-phosphoric acid solutions.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

RP- HPLC analysis was performed by isocratic elution with flow rate of 1.5 ml/min. The mobile phase containing 300 ml of buffer (pH adjusted to 3.7 with Ortho-Phosphoric acid) with 700 ml of Acetonitrile to obtain well-resolved peak of

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Montelukast (R_t = 3.717 min) is as shown in Fig. 2. Waters symmetry shield RP-C_8 column as stationary phase, run time of 5 minutes and 40^{0}C were found to be suitable for the analysis. The drug shows reasonably good response at 225 nm.

PREPARATION OF MOBILE PHASE:

The mobile phase was prepared by mixing buffer and Acetonitrile in the ratio of 30:70. The mobile phase was sonicated for 10 minutes and then was filtered through a 0.45 µ filter.

PREPARATION OF STANDARD STOCK SOLUTION:

An accurately weighed quantity of 25 mg of Montelukast was transferred to 100 ml volumetric flask, which was then dissolved and made up to volume with mobile phase to give 250 µg/ml concentrations.

CALIBRATION CURVE:

Aliquots of standard stock solutions of the drug were taken in 10 ml volumetric flask and diluted up to the mark with mobile phase in such a way that final concentration of drugs were in the range of 5-30 µg/ml. Triplicate injections of solutions were injected using a 20 µl fixed loop system and chromatograms were recorded. Calibration curve was drawn by plotting peak area on y-axis respective concentrations of drug on x-axis. The linearity table of Montelukast is shown in table 1. The calibration curve is shown in the Fig. 3.

ANALYSIS OF THE MARKETED FORMULATIONS:

Twenty tablets were taken, weighed and crushed to form fine powder. Accurately weighed quantity of powder equivalent to 25 mg of Montelukast was dissolved in 100 ml of volumetric flask with the mobile phase. The flask was sonicated for 20 min. and the volume was made up to 100ml with mobile phase. Then the solution was filtered using Whatman filter paper no. 41 and from the filtrate, two ml of sample solutions were transferred into five different 50 ml volumetric flasks and the volume was made up to the mark with mobile phase to obtain 10 µg/ml of montelukast. The solution was injected under above chromatographic conditions and peak areas were measured. The results are shown in the Table 2.

VALIDATION OF THE METHOD:

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, specificity, limit of detection and limit of Quantitation and repeatability of measurement as per ICH guidelines Q_2B.

LINEARITY:

The linearity range was found 5-30µg/ml. The regression equation for Montelukast was found to be Y=46130x + 2320 and Correlation coefficient (r^2=0.999).

ACCURACY:

It was found out by recovery study using standard addition method. Known amounts of standard Montelukast was added to pre-analyzed samples at a level from 50 % up to 150% and then subjected to the proposed HPLC method. Results of recovery studies are shown in Table 3.

PRECISION:

Intra-day and inter-day precision of the assay samples containing Montelukast (10µg/ml) were analyzed five times in the same day (intraday), and for three consecutive days (inter-day). Precision was calculated as intra and inter-day Coefficient of variation or %RSD [% C.V. = (S.D./mean) x 100] as shown in the Table 4 and 5.

SPECIFICITY:

The peak purity of Montelukast was assessed by comparing the retention time (R_t) of standard Montelukast. Good correlation was also found between the retention time of standard and sample of montelukast.

RUGGEDNESS:

Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions, expressed as %RSD. These conditions include different laboratory conditions and different analysts as shown in the Table 6.

ROBUSTNESS:

By introducing small but deliberate changes in the mobile phase pH (±0.1), flow rate (± 1%), temperature (± 2^{0}C), detection wavelength (± 2.0 nm) and mobile phase composition (± 2%) robustness of the described method were studied. The robustness of the method was assessed for 10 µg/ml

concentration. The results of robustness are given in the Table 7 to 11.

SENSITIVITY:

The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve. LOD = (3.3 x S.D /S and LOQ = 10 x S.D/S) where, S.D is the standard deviation of the y-intercepts of regression line and 'S' is the average slope of the calibration curve.

RESULTS AND DISCUSSION:

OPTIMIZATIONS OF THE METHOD:

The method was chosen after several trials with various proportions of sodium di-hydrogen phosphate buffer and acetonitrile i.e. 40:60, 50:50, 30:70, 25:75, 35:65 and at different pH values i.e. 2, 2.45, 3, 3.45, 4, 4.5, 2.48. A mobile phase consisting of buffer (pH 3.7) and acetonitrile in the ratios of 30:70 was selected to achieve best chromatographic peak and sensitivity. System suitability was performed by injecting five replicate injections of working standard Solution (100µg/ml). The System suitability results (the mean of five replicate injections) are shown in Table 8. The modalities adopted in experimentation were successfully validated as per analytical procedures laid down in routine. The results of specificity studies indicates that no interference from excipients, impurities and assured that the peak response was due to a single component only. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The percentage of average recoveries was obtained in the range of 99 to 101. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The lower limit of detection (LOD) and the limit of quantitation (LOQ) were found to be 0.1357 and 0.4111 µg/ml respectively. This demonstrates that the developed HPLC method is linear, accurate, robust, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk.

Concentration (µg/ml)	Area Response	Statistical Analysis
5	224124	Slope = 46130, Intercept= 2320, R^2 = 0.999
10	468854	
15	704244	
20	929245	
25	1154224	
30	1379203	

Table No. 1: Linearity of Montelukast

Formulation (µg/mL)	Label claim (mg/tablet)	Amount found (mg/tablet)	% of drug found	C.I.	% RSD	SE
10	5	4.92	99.633	99.901 ± 0.7831	0.631	0.282
	5	4.98	100.100			
	5	4.88	98.940			
	5	5.06	100.306			
	5	4.95	100.526			

Table No. 2: Analysis of Marketed formulation (Emlukast Tablet) (*n=5)

% Level of recovery	Formulation (µg/ml)	Amount of pure drug added (µg/ml)	Amount of drug recovered (µg/ml)	C.I.	Statistical Analysis
50	10	5	4.98	99.840 ±1.2287	Mean = 4.992 SD = 0.0217 %RSD = 0.4343
	10	5	5.01		
	10	5	4.97		
	10	5	4.98		
	10	5	5.02		
100	10	10	9.98	99.860 ±1.0763	Mean = 9.986 SD = 0.0508 %RSD = 0.5086
	10	10	9.92		
	10	10	10.02		
	10	10	9.84		
	10	10	10.05		
150	10	15	14.95	99.800 ±0.9051	Mean = 14.970 SD = 0.0872 %RSD = 0.5824
	10	15	14.91		
	10	15	15.07		
	10	15	14.87		
	10	15	15.05		

Table No. 3: Accuracy data of the RP-HPLC Method for Montelukast

SD: Standard deviation, %RSD: Regression Standard deviation, %SE: Percent standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = $R \pm t_s/\sqrt{n}$, R: Mean percent result of analysis of Recovery study (n = 5). Theoretical 't' value at 95% confidence level for n – 1 degrees of freedom.

Sl. No	Concentration (µg/ml)	Area	Statistical Analysis
1	10	470364	Mean = 470176.4, SD = 401.6781, %RSD = 0.0854
2	10	469885	
3	10	469942	
4	10	470800	
5	10	469891	

Table No. 4: Intraday Precision data of the RP-HPLC Method for Montelukast.

Sl. No	Concentration (µg/ml)	Area	Statistical Analysis
1	10	469882	Mean = 470216.8, SD = 683.9735, % RSD = 0.1454
2	10	470564	
3	10	469541	
4	10	471242	
5	10	469855	

Table No. 5: Inter-day Precision data of the RP-HPLC Method for Montelukast

Analyte	*Analyst 1*		*Analyst 2*	
	Calc. Amt. (µg/ml)	*Statistical Analysis*	*Calc. Amt. (µg/ml)*	*Statistical Analysis*
Montelukast (10 µg/ml)	10.09	Mean = 9.972 SD = 0.092 %RSD = 0.923	9.95	Mean = 10.064 SD = 0.088 %RSD = 0.871
	9.96		10.09	
	9.88		10.19	
	10.04		10.03	
	9.89		10.07	

Table No. 6: Ruggedness data of the RP-HPLC Method for Montelukast

Analyte	*pH (+ 0.1Units)*		*pH (- 0.1Units)*	
	Calc. Amt. (µg/ml)	*Statistical Analysis*	*Calc. Amt. (µg/ml)*	*Statistical Analysis*
Montelukast (10 µg/ml)	10.06	Mean = 10.018 SD = 0.103 %RSD = 1.028	10.16	Mean = 10.101 SD = 0.069 %RSD = 0.682
	10.12		10.06	
	9.88		10.19	
	9.95		10.03	
	10.09		10.07	

Table No. 7: Robustness Data of the RP-HPLC Method at Different pH for Montelukast

Analyte	*Flow (+1%)*		*Flow (-1%)*	
	Calc. Amt. (µg/ml)	*Statistical Analysis*	*Calc. Amt. (µg/ml)*	*Statistical Analysis*
Montelukast (10 µg/ml)	10.04	Mean = 9.995 SD = 0.065 %RSD = 0.655	10.09	Mean = 9.964 SD = 0.118 %RSD = 1.182
	9.89		9.96	
	9.98		9.88	
	10.03		9.82	
	10.03		10.08	

Table No. 8: Robustness Data of the RP-HPLC Method at Different flow rate for Montelukast

Analyte	*Temperature +2^0 C*		*Temperature -2^0 C*	
	Calc. Amt. (µg/ml)	*Statistical Analysis*	*Calc. Amt. (µg/ml)*	*Statistical Analysis*
Montelukast (10 µg/ml)	9.96	Mean = 10.005 SD = 0.096 %RSD = 0.960	9.98	Mean = 9.984 SD = 0.107 %RSD = 1.074
	10.05		9.82	
	10.15		9.96	
	9.98		10.09	
	9.90		10.07	

Table No. 9: Robustness Data of the RP-HPLC Method at Different temperature for Montelukast

Analyte	*Wavelength +2 nm*		*Wavelength -2 nm*	
	Calc. Amt. (µg/ml)	*Statistical Analysis*	*Calc. Amt. (µg/ml)*	*Statistical Analysis*
Montelukast (10 µg/ml)	9.98	Mean = 10.025 SD = 0.085 %RSD = 0.851	9.99	Mean = 10.045 SD = 0.092 %RSD = 0.914
	9.98		10.07	
	10.05		10.06	
	10.17		9.93	
	9.96		10.18	

Table No. 10: Robustness Data of the RP-HPLC Method at Different wavelength for Montelukast

Analyte	*+2 % of Organic solvent in Mobile phase*		*-2 % of Organic solvent in Mobile phase*	
	Calc. Amt. (µg/ml)	*Statistical Analysis*	*Calc. Amt. (µg/ml)*	*Statistical Analysis*
Montelukast (10 µg/ml)	9.97	Mean = 10.001 SD = 0.055 %RSD = 0.546	9.98	Mean = 10.033 SD = 0.085 %RSD = 0.849
	10.07		10.09	
	10.05		10.05	
	9.99		10.13	
	9.94		9.92	

Table No. 11: Robustness Data of the RP-HPLC Method at Different mobile phase for Montelukast

Parameter	Results of Montelukast
Retention time	3.721
Theoretical plates	5270.13
Assymetric factor	1.10
Capacity factor	3617
Repeatability (%RSD)	0.0852
Limit of Detection (LOD)	0.1357 µg/ml
Limit of Quantitation (LOQ)	0.4111 µg/ml

Table No. 12: System Suitability

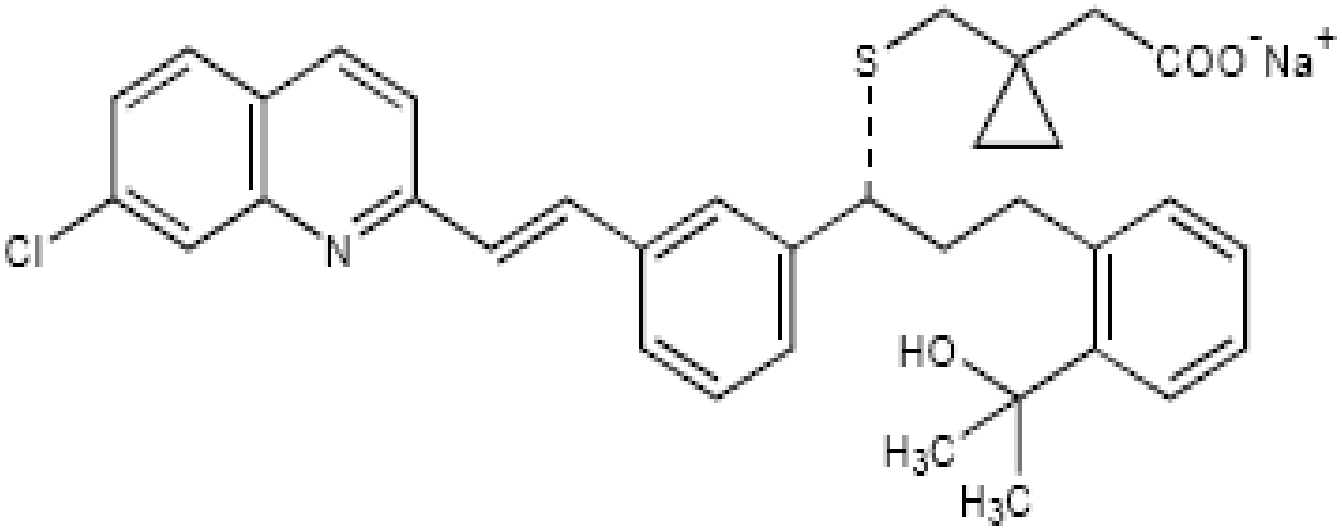

Figure No. 1: Montelukast structure

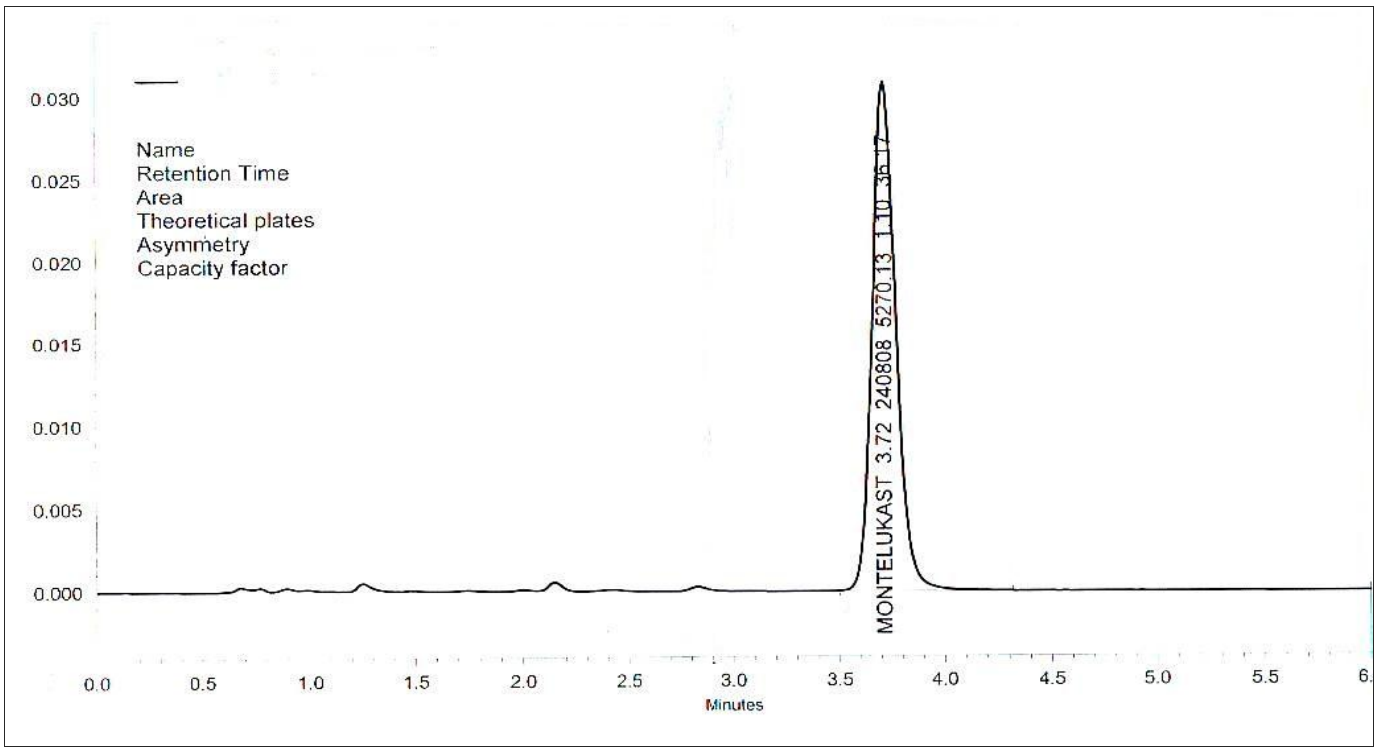

Figure No. 2: Typical Chromatogram of standard Montelukast

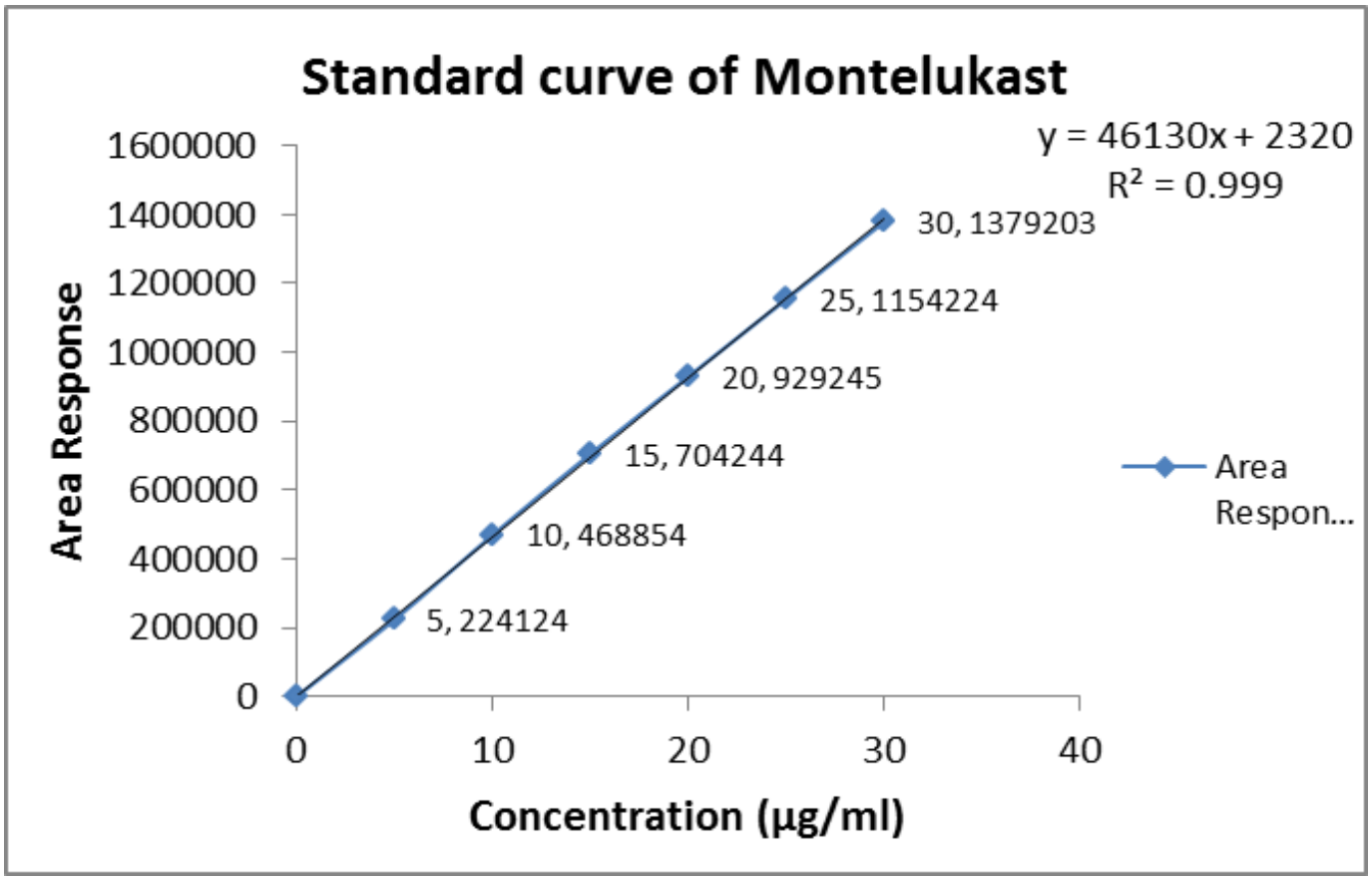

Figure No. 3: Calibration curve of Montelukast

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