ejpmr, 2018,5(6), 772-777

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Case Report

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PRANLUKAST HYDRATE IN ITS SYNTHETIC MIXTURE

Gajanan G. Kalyankar[1*], Priti. H. Vansiya[1], Kunjan B. Bodiwala[2], Sandesh R. Lodha[1], Pintu B. Prajapati[1], Shrikant V. Joshi[1]

[1]Maliba Pharmacy College, Gopal Vidaya Nagar, Tarsadi Mahuva Road, Tarsadi, Dist Surat, 394350.
[2]L.M.College of Pharmacy, Navrangpura, Opp. Gujarat University, Ahemedabad-380009.

***Corresponding Author: Dr. Gajanan G. Kalyankar**

Maliba Pharmacy College, Gopal Vidaya Nagar, Tarsadi Mahuva Road, Tarsadi, Dist Surat, 394350.

Article Received on 19/04/2018 **Article Revised on 10/05/2018** **Article Accepted on 01/06/2018**

ABSTRACT

Accurate, precise and robust UV spectrophotometric method has been developed and validated for the estimation of Pranlukast Hydrate in its synthetic mixture. UV-spectrophometric determination was carried out at an absorbance maxima of 262 nm using ethanol as solvent. Developed method has been validated for linearity range, precision, accuracy, limit of detection, and limit of quantification as per ICH Q2(R1) guidelines. Linear regression analysis data for the calibration plots showed a good linear relationship with R^2 of 0.9984 for Pranlukast hydrate in concentration range of 10-50 µg/ml. The intraday and interday precision of the method in terms of % RSD were found to be 0.66-1.21 % and 0.81-1.33 % respectively. LOD for Pranlukast hydrate was found to be 0.49µg/ml, while LOQ was found to be 1.51µg/ml. %Recovery of Pranlukast hydrate was found in range of 98-100.1 %. The method was applied to synthetic mixture for analysis of Pranlukast hydrate. The assay result was found to be in good agreement with amount of Pranlukast hydrate present in synthetic mixture.

KEYWORDS: Pranlukast hydrate, Spectrophotometric Method, Synthetic Mixture, Validation.

1 INTRODUCTION

Pranlukast hydrate chemically N-[4-Oxo-2-(1H-tetrazol-5-yl)-4H-chromen-8yl]-4(4-phenyl butyloxy) benzamide hemihydrates is an antiasthemic drug. That selectively binds and blocks the action of leukotriene receptors. Leukotrienes are powerful mediators of inflamation, cause leukocyte recruitment, stimulate bronchoconstriction and increase capillary permeability leading to pulmonary oedema. Metabolism of arachidonic acid via 5-lipoxygenase pathway yields the cysteinyl leukotrienes-LTC_4, LTD_4, and LTE_4 which activate cysteinyl leukotriene receptor to cause bronchoconstriction the effect of leukotrienes can be prevented either by inhibiting the leukotriene synthesis or by blocking their stimulatory effect on cys-LT receptors. Effect of leukotrienes on cys-LT receptors can be blocked by Pranlukast hydrate.[1-4] It is white to yellowish white and crystalline powder. It is soluble in dimethyl sulfoxide and in dimethyl formamide, ethanol (99.5%), practically insoluble in water, acetonitrile, diethyl ether, dichloromethane.[5,6] and structure of Pranlukast hydrate has been presented in Figure 1.

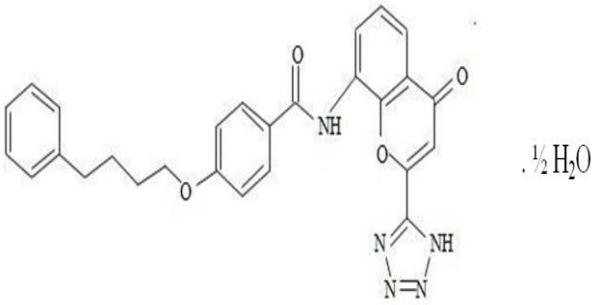

Figure 1: Chemical structure of Pranlukast Hydrate.

Literature review reveals that Pranlukast hydrate is official in Japanese Pharmacopoeia in which liquid chromatographic method has been used for assay of Pranlukast hydrate and estimation of related substances.[5] Marchese A. et al; have determined Pranlukast and its metabolites in human plasma by LC/MS/MS with PROSPEKT ™ on-line solid phase extraction.[7] The literature does not report UV-spectrophotometric method for routine quality control analysis of Pranlukast hydrate in its formulation. Therefore, it was thought of interest to develop a precise, accurate, robust, spectrophotometric method for the analysis of Pranlukast Hydrate in its synthetic mixture. The developed method was validated as per the

guidelines of International Conference on Harmonization [ICH Q2 (R1)].[8]

2 Experimental work

2.1 Instrumentation

A double beam UV-Visible spectrophotometer (Lab India, UV-Visible spectrophotometer 3092) was used for all absorbance measurements with matched quartz cells. Electronic balance (AUX- 220, Shimadzu) was used for weighing purpose.

2.2 Chemicals and Reagents

All chemicals and reagents used were of analytical or HPLC grade. Absolute Ethanol analytical grade from Loba chemie (99.5%). Pranlukast hydrate was provided by Cadila Pharmaceuticals Ltd., Ankleshwar.

2.3 Preparation of working standard solutions

An accurately weighed 10 mg of Pranlukast hydrate was transferred into 10 ml volumetric flask, dissolved in 8 ml ethanol, diluted up to the mark with the same to get stock solution having strength of 1000 µg/ml. Aliquot of 1 ml from standard stock solution was transferred into 10 ml volumetric flask and diluted up to the mark with ethanol to get solution having strength of 100 µg/ml.

2.4 Preparation of synthetic mixture and placebo

Synthetic mixture for Pranlukast hydrate was prepared by physically mixing commonly used excipients for tablet formulation with drug.All ingredients were accurately weighed and physically mixed in poly bag for 10 min to make synthetic mixture and placebo. Quantitative of each ingredient for synthetic mixture and placebo has been shown in table 1.

Table 1: List of ingredients for preparation of synthetic mixture and placebo.

Ingredient	Quantity to be taken (mg)	
	Synthetic Mixture	Placebo
Pranlukast Hydrate (API)	112.5	-
Lactose	-	112.5
Sodium lauryl sulfate	6	6
Poly vinyl pyrrolidone K_{30}	12	12
Talc	6	6
Silicon dioxide	6	6
Titanium dioxide	6	6
PVG 4000	12	12
MCC	q.s for 230	q.s for 230

2.5 Selection of wavelength of detection

The working standard solution of Pranlukast hydrate was scanned in the range of 200-400 nm keeping ethanol as blank and wavelength of absorbance maxima was determined from the spectrum. Figure: 2.

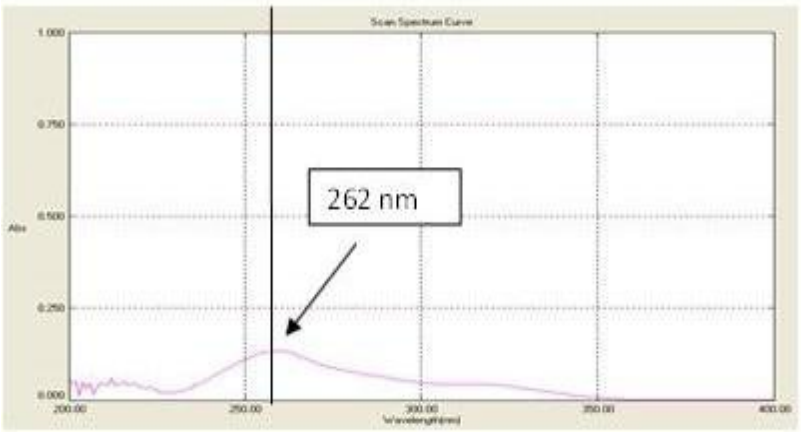

Figure 2: UV Spectrum of Pranlukast hydrate (10 µg/ml).

2.6 Preparation of Calibration Curve

Aliquots of 1ml, 2ml, 3ml, 4ml, and 5 ml from working standard solution were transferred into 10 ml volumetric flasks and diluted up to the mark with ethanol to get solutions having strength ranging from 10-50 µg/ml respectively. The absorbances were measured at 262 nm and calibration curve was plotted of absorbance against concentrations.

2.7 Solution Stability Study

The freshly prepared working standard solution of Pranlukast hydrate (100µg/ml) was stored at room temperature for 24 hour. The solution was analyzed immediately after preparation and after (24 hr) using optimized chromatographic conditions. Absorbances of Pranlukast hydrate obtained at 0 hour and 24 hour were compared to check the stability of solution.

2.8 Validation of Developed method

The method was validated as per ICH guidelines for validation of analytical procedures, Q2(R1) with respect to linearity, precision, LOD, LOQ, accuracy, and robustness.

2.8.1 Linearity and Range

From the working standard solution containing 100 µg/ml of Pranlukast hydrate, dilutions were made to prepare range of standard solutions having different concentrations of Pranlukast hydrate (10-50 µg/ml). The absorbances were measured at 262 nm. The five representative calibration curves for Pranlukast hydrate in range of 10-50 µg/ml were plotted and from average data of five calibration curves correlation coefficient of regression and regression line equations were computed.

2.8.2 Precision

Repeatability of method was determined by measuring absorbance of the 30 µg/ml standard solution seven times. In interday variation the sample was analyzed with concentrations range (10-50µg/ml) on five consecutive days. In intraday variation the sample was analyzed with concentrations range (10-50µg/ml) five times in a day. The % RSD of the results was used to evaluate the method precision.

2.8.3 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope and intercept of the linearity plot were determined. LOD and LOQ were calculated using the following formula:[8]

$$LOD = \frac{3.3 \times \text{Std.deviation of y intercept}}{\text{Mean of slope}}$$

$$LOQ = \frac{10 \times \text{Std.deviation of y intercept}}{\text{Mean of slope}}$$

2.8.4 Specificity

To check the interference between placebo and drug substance, specificity study was carried out. The solutions of placebo and synthetic mixture (30µg/ml) were scanned in the range 200-400 nm and compared to assess the interference between placebo and synthetic mixture.

2.8.5 Accuracy

The accuracy of the developed method was determined by recovery studies at three levels (80%, 100% and 120%) by standard addition method. Recovery study was carried out by spiking three different known amounts (8mg, 10mg and 12mg) of the standard drug to the placebo. Standard Pranlukast hydrate 8 mg, 10 mg and 12 mg was spiked in three different 10 ml volumetric flasks, respectively. All three flasks were filled to about 80 % with ethanol, sonicated for 30 minutes and diluted up to the mark with ethanol. These solutions were filtered through whatman filter paper individually. From each filtrate, 1 ml of each was diluted up to 10 ml with ethanol individually. Aliquot of 3 ml of each resulting solution was diluted up to 10 ml with ethanol. From calibration curve, the amount of Pranlukast hydrate recovered was calculated and % recovery was determined.

2.8.6 Robustness

Robustness was determined by the analysis of standard solutions (10-50 µg/ml) at different wavelengths (± 2 nm).The percentage RSD of the results was used to evaluate the method robustness. Spectrums were scanned at different scan speeds (slow, medium and fast) and scan intervals (0.5, 1 and 2).

2.9 Analysis of Synthetic mixture

Synthetic mixture was analyzed using developed method. Accurately weighed synthetic mixture equivalent to 10 mg Pranlukast hydrate was transferred to 10 ml volumetric flask, dissolved in 5 ml ethanol (99.5%) then sonicated for 30 min and diluted up to the mark with ethanol and filtered through Whatman filter. Aliquot of 1 ml from the filtrate was transferred into 10 ml volumetric flask and diluted up to the mark with ethanol. Aliquot 3 ml From above solution was transferred into 10 ml volumetric flask and diluted up to mark with ethanol. Absorbance of resulting solution was measured at 262 nm.

3 RESULTS AND DISCUSSION

3.1 Preparation of calibration curve

Calibration curve was prepared in range of 10-50 µg/ml for Pranlukast hydrate. Absorbance of Pranlukast hydrate increases linearly with concentration. Data of calibration curve is shown in Table 2.

Table 2: Data for calibration curve.

Concentration (10-50 µg/ml)	Absorbance
10	0.136
20	0.306
30	0.454
40	0.630
50	0.763

3.2 Method Validation

3.2.1 Linearity

Representative calibration curve of Pranlukast hydrate was obtained by plotting the mean absorbance of Pranlukast hydrate against concentration over the range 10-50 µg/ml (n=5) Figure.

3. It was found to be linear in the above mentioned range with Regression coefficient of 0.9984. The % RSD for each level of Pranlukast hydrate was found to be in range of 0.65-1.31%. The average linear regressed equation for calibration curve was y =0.0158x-0.0161. Linearity data is depicted in Table 3 and summary data depicted in Table 4.

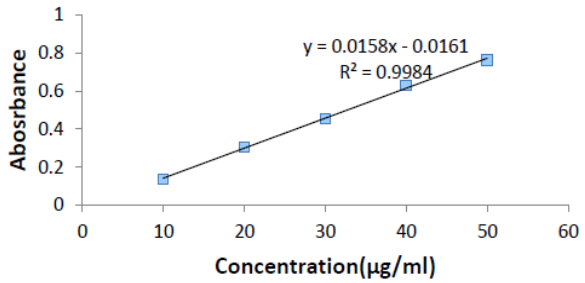

Figure 3: Linearity Curve for Pranlukast hydrate(10-50 ug/ml) in ethanol.

Table 3: Data for Linearity curve.

Concentration (µg/ml)	Absorbance (mean ± SD) (n=5)	% RSD
10	0.136 ± 0.0013	0.95
20	0.305 ± 0.0038	1.24
30	0.454 ± 0.0064	1.4
40	0.630 ± 0.0083	1.31
50	0.763 ± 0.005	0.65

Table 4: Summary of linearity data for estimation of Pranlukast hydrate.

Parameters	Results
Linearity range	10-50 µg/ml
Regression line equation	y =0.0158x-0.0161
Slope ± S.D. (n= 5)	0.015 ± 0.0004
Intercept ± S.D. (n= 5)	0.0148 ± 0.0023
Regression coefficient (R^2)	0.9984

3.2.2 Precision

3.2.2.1 Repeatability of sample measurement

Repeatability of the instrument was checked by measurement of absorbance (working standard solution, 30µg/ml) seven times. The % RSD for absorbance was found to be 0.61.% which is less than 2% hence method is repeatable. The data for repeatability of measurement is depicted in Table 5.

Table 5: Data for Repeatability for estimation of Pranlukast Hydrate.

Concentration (µg/ml)	Absorbance
30	0.462
30	0.469
30	0.469
30	0.468
30	0.469
30	0.470
30	0.471
Mean (n=7)	0.468
Standard Deviation	0.0029
% RSD	0.61

3.2.2.2 Intermediate precision

The % RSD for intra-day and inter-day precision of Pranlukast hydrate was found to be in range of 0.66-1.21 % and 0.81-1.33 % respectively. The data for intra-day and inter-day precision for Pranlukast hydrate is depicted in Table 6. % RSD is less than 2% hence method is precise for measurement of Pranlukast hydrate.

Table 6: Data for Intermediate precision for estimation of Pranlukast hydrate.

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	Absorbance Mean ± SD (n=5)	% RSD	Absorbance Mean ± SD (n=5)	% RSD
10	0.137 ± 0.0013	0.95	0.135 ± 0.0014	1.09
20	0.309 ± 0.0037	1.21	0.310 ± 0.0041	1.33
30	0.462 ± 0.0050	1.09	0.447 ± 0.0052	1.17
40	0.647 ± 0.0073	1.14	0.644 ± 0.0081	1.25
50	0.783 ± 0.0052	0.66	0.765 ± 0.0062	0.81

3.2.3 Limit of detection and limit of quantification

The limit of detection was found to be 0.49 µg/ml. Limit of Quantification was found to be 1.51µg/ml.

3.2.4 Specificity

An overlain spectrum of placebo and synthetic mixture is depicted in Figure 4. Placebo showed no absorbance in range of 200-400nm, this indicates excipient did not interfere for estimation of Pranlukast hydrate.

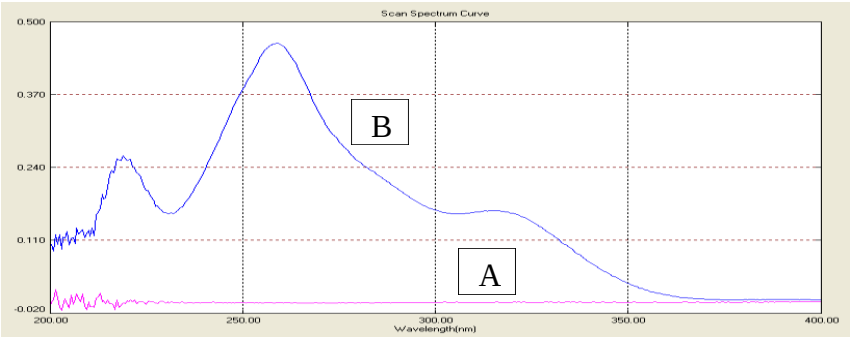

Figure 4: Overlain spectra of placebo (A) and Synthetic mixture (B) (30 µg/ml).

3.2.5 Accuracy

Accuracy was determined in terms of recovery study and the recoveries are done at three levels i.e.80%, 100% and 120%. The % recovery of Pranlukast hydrate was found to be in range of 98- 100.1%. The data for accuracy of method for Pranlukast hydrate is depicted in Table 7. All results are in the range of 98-102% hence is accurate in measurement of Pranlukast hydrate.

Table 7: Recovery data of Pranlukast Hydrate.

Amount added (mg)	Spiked level (%)	Concentration taken (µg/ml)	Amount found (mg) ± SD (n=3)	% Recovery
8	80	24	07.84 ± 0.081	98
10	100	30	10.01 ± 0.329	100.1
12	120	36	11.95 ± 0.514	99.58

3.2.6 Robustness

The % RSD for robustness of Pranlukast hydrate was found to be in range of 0.32-1.10 %. % RSD is less than 2% hence method is robust for measurement of Pranlukast hydrate. The data for robustness for Pranlukast Hydrate is depicted in Table 8. Overlain Spectrum for Pranlukast hydrate at different intervals and different scan speed (10 µg/ml) is shown in Figure 5 and 6.

Table 8: Robustness data of Pranlukast hydrate.

Concentration (µg/ml)	Absorbance			Absorbance Mean ± SD	% RSD
	260 nm	262 nm	264 nm		
10	0.135	0.136	0.138	0.136 ± 0.0015	1.10
20	0.312	0.315	0.318	0.315 ± 0.003	0.94
30	0.450	0.452	0.456	0.452 ± 0.0030	0.66
40	0.632	0.638	0.634	0.634 ± 0.0030	0.47
50	0.772	0.774	0.777	0.774 ± 0.0025	0.32

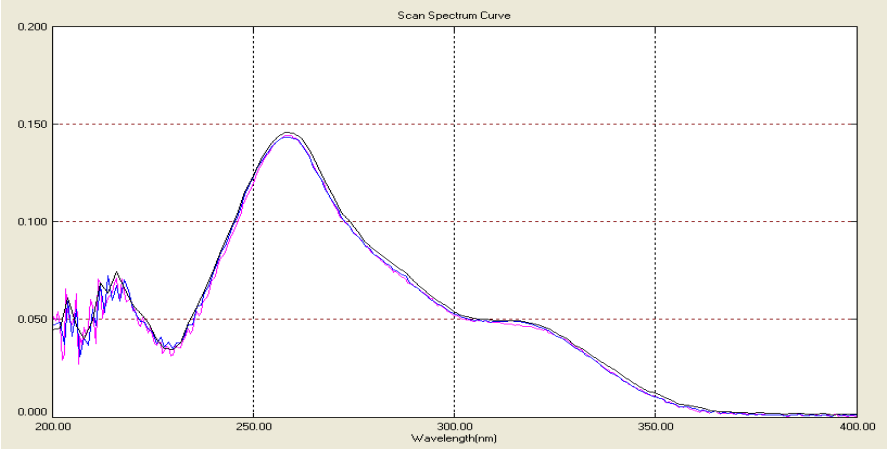

Figure 5: Overlain Spectrum for Pranlukast hydrate at different intervals (10 µg/ml)

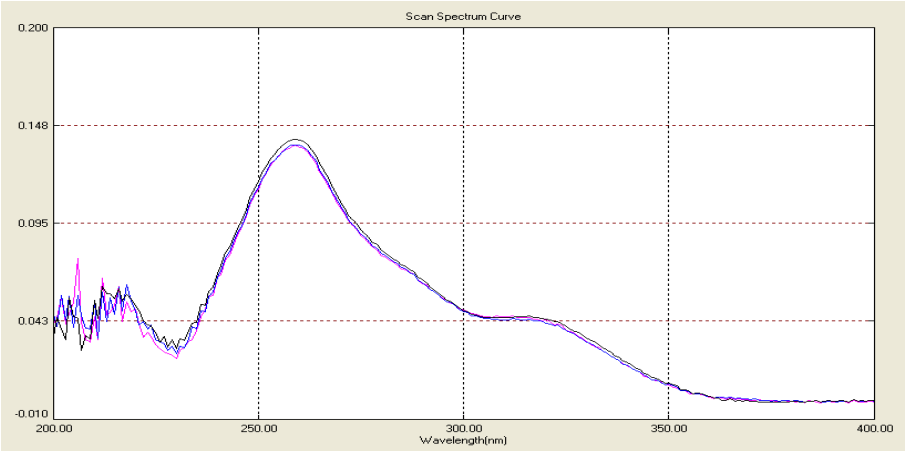

Figure 6: Overlain Spectrum for Pranlukast hydrate at different scan speed (10µg/ml).

3.3 Analysis of synthetic mixture by proposed method

Applicability of the proposed method was tested by analyzing synthetic mixture. The percentage of Pranlukast hydrate in synthetic mixture was calculated from the calibration curve of Pranlukast Hydrate. The assay value for synthetic mixture of Pranlukast hydrate was 100.35%. Assay result of synthetic mixture of Pranlukast hydrate is depicted in Table 9.

Table 9: Analysis of synthetic mixture.

Amount of drug in synthetic mixture (mg)	Amount of drug found in synthetic mixture (mg) (n=3) ± SD	Assay (%)
112.5	112.9 ± 0.282	100.35

The summary of validation results is depicted in Table 10.

Table 10: Summary of validation parameters.

Sr. No.	Parameters	Results
1	Linearity Range	10-50 (µg/ml)
2	Regression equation	Y=0.0158x-0.0161
3	Regression coefficient (r^2)	0.9984
4	Precision (RSD)	
	Repeatability (n=7)	0.61 %
	Intermediate precision	
	Intra-day precision (n=5)	0.66-1.21 %
	Inter-day precision (n=5)	0.81-1.33 %
5	Limit of detection(LOD)	0.49 (µg/ml)
6	Limit of quantification(LOQ)	1.51 (µg/ml)
7	Accuracy (n=3)	98-100.1 %
8	Robustness	0.32-1.10 %

4 CONCLUSION

The UV method has been developed for quantification of Pranlukast hydrate in synthetic mixture. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. Hence the proposed method can be routine used for the estimation of Pranlukast Hydrate and its pharmaceutical dosage form.

5 ACKNOWLEDGMENT

We wish to thank Principal, Maliba Pharmacy College for providing all the facilities for this research work. Also we express our gratitude to Cadila Pharmaceutical for providing Pranlukast hydrate API as gift sample for this research work.

REFERENCES:

1. Sharma H.L, Sharma K.K. Principles of Pharmacology. Second edition. Hyderabad; India: Paras Medical Publisher, 2011; 642-649.
2. Tripathi K.D, Essential of Medical Pharmacology. Sixth edition., New Delhi; India: Jaypee Brothers Medical Publishers, 2008; 211-227.
3. Waugh A, Grant A. Ross and Wilson Anatomy & Physiology in Health and Illness. 12th edition. China: Churchill Livingstone Elsevier, 2014; 262, 264.
4. Ohmoto K, et al. Ethynlindole compound. EP. Chemical Abstract, 2012.
5. Japanese Pharmacopoeia. Sixtieth edition., The Ministry of Health, Labour and Welfare, Supplement II, 2748-2749.
6. Ono Pharmaceutical Co., Ltd. 14th edition. 2010. March.
7. Marchese A, McHugh C, Kehler J, Bi H. Determination of Pranlukast and its metabolites in human plasma by LC/MS/MS with PROSPEKT™ on-line solid-phase extraction. Journal of Mass Spectrometry, 1998; 33(11): 1071-1079.
8. ICH Harmonized Tripartite Guideline. Validation of Analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization, Geneva: Switzerland, 2005.