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SIMULTANEOUS ESTIMATION OF MONTELUKAST AND DOXOFYLLINE BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN API AND MARKETED FORMULATION

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Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Montelukast and Doxofylline, in its pure form as well as in tablet dosage form. Chromatography was carried out on X bridge C18 (4.6×150mm) 5µcolumn using a mixture of Methanol: Phosphate Buffer pH3 (60:40v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 260nm. The retention time of the Montelukast and Doxofylline was 2.6, 3.8±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Montelukast and 20-100µg/ml of Doxofylline respectively. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Montelukast and Doxofylline, RP-HPLC, Validation, Precision.*

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INTRODUCTION:

Pharmaceutical analysis is traditionally defined as analytical chemistry dealing with drugs both as bulk drug substances and as pharmaceutical products (formulations).

The Purpose of Pharmaceutical Analysis is to identify substances, purify them, separate them, quantify them, determine the molecular structures of chemical compounds that make up pharmaceuticals, and determine how these compounds are combined to make up a pharmaceutical product.

Specifically, it relates to the analysis of raw materials and pharmaceutical formulations, entails the determination of ingredients, impurities, excipients, and uniformity, solubility, and dissolution rate to identify active components, contaminants, and impurities.

Depending on the dosage form and the compound, the sample may be singular or combination. The substance utilized for pharmaceutical purposes is animals, plants, microbes, minerals, and a wide variety of synthetic chemicals.

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also, quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Based upon the determination type, there are mainly two types of analytical methods. They are as follows:

Qualitative analysis: This method is used for the identification of the chemical compounds.

Quantitative analysis: This method is used for the determination of the amount of the sample.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Substance quality and its specifications are based on substance analysis, and that knowledge is later used for quality control (QC) of the substance during full-scale production. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

- Manufacturing industries require both qualitative and quantitative analysis to ensure that their raw materials meet certain specifications, and to check the quality of final product. Raw materials are to be checked to ensure that the essential components are present within the predetermined range of composition and there are not any unusual substances present which might upset the manufacturing process or it may appear as a harmful impurity in the final product.
- In the development of new products which contains mixtures other then the pure material, it is necessary to ascertain composition of mixture which shows the optimum characteristics for which the material has been developed.
- Geographical surveys require analysis to determine the composition of soil sample and numerous rock samples collected from the field.
- Most of the industrial processes give rise to pollutants which may cause health related problems. So quantitative analysis of air, water and soil sample should be carried out to

determine the level of pollution and to establish the safe limits for pollutants.

Different methods of analysis:

The following techniques are available for separation and analysis of components of interest.

Spectral methods:

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods:

Electro analytical methods involved in the measurement of current voltage or resistance as a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.

Chromatographic methods:

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques:

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

MATERIALS AND METHODS:

Montelukast and Doxofylline Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Triethylamine from Merck.

Hplc method development:

Trails :

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Montelukast and Doxofylline working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 2.25ml of the above Montelukast and Doxofylline and 0.45ml of the Clonidine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and water in proportion 65:35 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Validation:

Preparation of mobile phase:

Preparation of mobile phase:

Accurately measured 650ml (65%) of HPLC Methanol and 350ml of Water (35%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Mobile phase	:	Methanol: Phosphate Buffer pH3 (60:40v/v)
Column	:	X bridge (4.6×150mm, 5 µ)
Flow rate	:	1.0 ml/min
Wavelength	:	260 nm
Column temp	:	Ambient
Injection Volume	:	10 µl
Run time	:	8 min

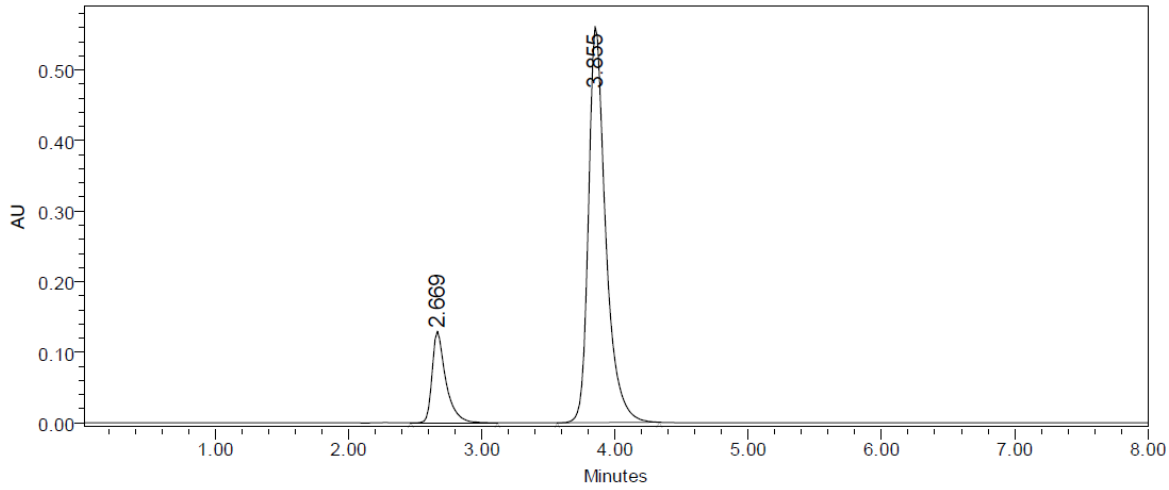

Figure-: Chromatogram for Trail 7

Table-: Peak Results for Trail 7

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Doxofylline	2.669	917817	128673		1.5	3553.0
2	Montelukast	3.855	5040175	562208	1.6	1.4	4676.7

Observation:

This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.

From the above chromatogram it was observed that the Montelukast and Doxofylline peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Retention time of Doxofylline– 2.669min

Retention time of Montelukast –3.855min

System suitability:

Table: Results of system suitability parameters for Doxofylline and Montelukast

S.No.	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Doxofylline	2.669	918738	128688		1.5	3548.3
2	Montelukast	3.855	5040175	562208	1.8	1.4	4676.7

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Validation parameters:

Assay (Standard):

Table: Showing assay standard results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Doxofylline	2.669	918297	128681		1.5	3551	1
2	Montelukast	3.855	5041295	562208	1.7	1.4	4676	1
3	Doxofylline	2.669	918483	128626		1.5	3549	2
4	Montelukast	3.855	5040175	562163	1.7	1.4	4593	2
5	Doxofylline	2.654	918216	128722		1.5	3596	3
6	Montelukast	3.849	5040153	562480	1.7	1.4	4619	3

Assay (Sample):

Table-: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Doxofylline	2.669	918297	128681		1.6	3551.1	1
2	Montelukast	3.855	50401745	562208	1.7	1.4	4676	1
3	Doxofylline	2.651	919584	128701		1.5	3548.8	2
4	Montelukast	3.849	15041295	562208	1.7	1.4	4676	2
5	Doxofylline	2.621	918297	128681		1.5	3551.1	3
6	Montelukast	3.840	5040216	562208	1.7	1.4	4676	3

Precision:

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table-: Results of method precession for Doxofylline:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Doxofylline	2.669	918297	128681	3551	1.6
2	Doxofylline	2.659	918355	128713	3547	1.6
3	Doxofylline	2.671	918248	128615	3575	1.6
4	Doxofylline	2.669	918637	128646	3563	1.6
5	Doxofylline	2.669	919579	128653	3713	1.6
Mean			918623.2			
Std. Dev			555.1704			
% RSD			0.060435			

Table: Results of method precision for Montelukast:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Montelukast	3.855	5040175	562208	4676	1.4	1.7
2	Montelukast	3.842	5046152	562218	4764	1.4	1.7
3	Montelukast	3.850	5053142	561437	4513	1.4	1.7
4	Montelukast	3.845	5076520	562149	4156	1.4	1.7
5	Montelukast	3.855	5063148	571543	4952	1.4	1.7
Mean			5055827				
Std. Dev			14383.99				
% RSD			0.284503				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision/Ruggedness:

Table-: Results of Intermediate precision for Doxofylline

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Doxofylline	2.669	918297	128676	3685	1.5
2	Doxofylline	2.529	908295	128458	3563	1.5
3	Doxofylline	2.669	907193	128476	3578	1.5
4	Doxofylline	2.569	909292	128622	3568	1.5
5	Doxofylline	2.569	908297	128633	3547	1.5
6	Doxofylline	2.669	908459	128418	3551	1.5
Mean			909972.8			
Std. Dev			4132.317			
% RSD			0.454116			

Table-: Results of Intermediate precision for Montelukast:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Montelukast	3.845	4940175	562183	4679	1.4	1.7
2	Montelukast	3.795	4951175	562494	4676	1.4	1.7
3	Montelukast	3.855	4942176	562199	4625	1.4	1.7
4	Montelukast	3.840	4840173	563542	4685	1.4	1.7
5	Montelukast	3.855	4950177	562185	4676	1.4	1.7
6	Montelukast	3.855	4942313	562488	4622	1.4	1.7
Mean			4927698				
Std. Dev			43117.6				
% RSD			0.875005				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy:

Table-: Accuracy (recovery) data for Doxofylline

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	577154	7.6	7.46	98%	98.8%
100%	918738	16	14.93	99.2%	
150%	1288228	22.6	22.48	99.3%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table-: Accuracy (recovery) data for Montelukast

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	3120598	30	29.9	98%	99.1%
100%	5040175	60	59.8	99.9%	
150%	7087907	90	89.9	99.6%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

Linearity:

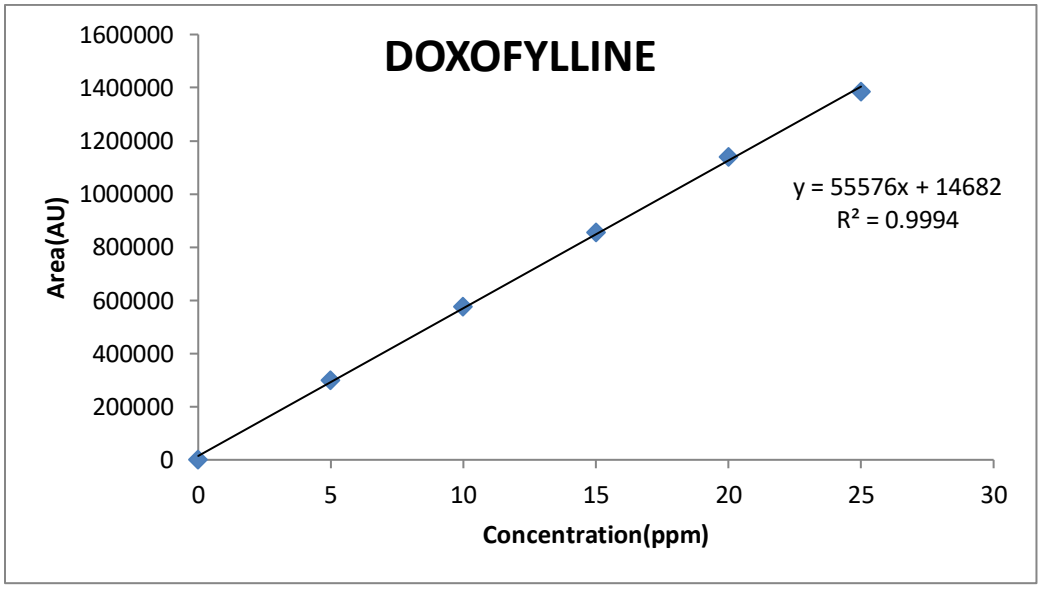

Figure: Calibration graph for Doxofylline

Linearity Results: (for Doxofylline)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	5	300011
2	II	10	575362
3	III	15	856267
4	IV	20	1139179
5	V	25	1385478
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.999

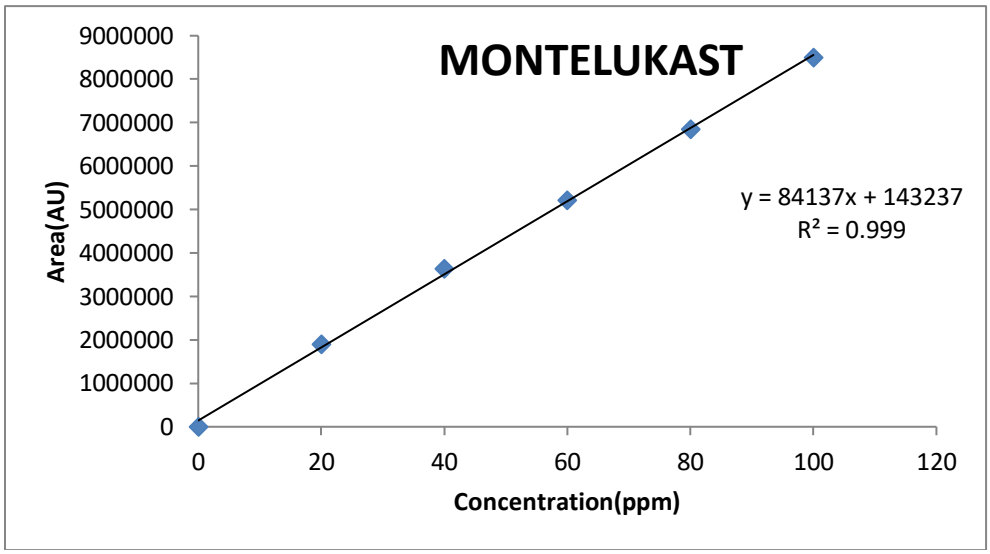

Figure: calibration graph for Montelukast

Linearity Results: (for Montelukast)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	20	1903923
2	II	40	3637045
3	III	60	5210175
4	IV	80	6856371
5	V	100	8493148
Correlation Coefficient			0.999

Acceptance Criteria:

- Correlation coefficient should be not less than 0.99.

Robustness:

System suitability results for Doxofylline:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4818.3	1.5
2	*Actual	3551.3	1.5
3	10% more	4722.8	1.5

System suitability results for Montelukast:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5835.2	1.4
2	*Actual	4676.6	1.4
3	10% more	5236.6	1.4

***** Results for actual mobile phase have been considered from Assay standard.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Montelukast and Doxofylline was done by RP-HPLC. The Phosphate buffer was p^H 3 and the mobile phase was optimized with consists of Methanol: Phosphate buffer (pH-3) mixed in the ratio of 60:40 % v/ v. An Xbridge column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Montelukast and Doxofylline were found to be from 5-25µg/ml, 20-100µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-99% of Montelukast and Doxofylline. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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