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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST AND DOXYFYLLINE BY RP-HPLC

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Abstract:

The development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Montelukast and Doxofylline in pharmaceutical formulations were carried out. The method was optimized using a Waters HPLC system equipped with an auto-sampler and PDA detector (996 model). A Phenomenex Luna C18 column (4.6×250mm, 5µm) was employed for the separation of both drugs. The mobile phase consisted of acetonitrile and phosphate buffer (pH 4.6) in a 45:55 (v/v) ratio, with a flow rate of 1.0 ml/min. The wavelength for detection was set at 245 nm, and the injection volume was 10 µL. The column temperature was maintained at 35°C, and the total run time was 7 minutes. The method was validated as per ICH guidelines, and the results demonstrated excellent linearity, precision, accuracy, and robustness. The proposed method offers a simple, rapid, and reliable approach for the simultaneous estimation of Montelukast and Doxofylline in tablet dosage forms, and can be effectively used for quality control in pharmaceutical industries.

Keywords: *Montelukast and Doxofylline, RP-HPLC, Simultaneous Estimation, Pharmaceutical Dosage Forms, Waters HPLC.*

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INTRODUCTION:

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Analytical techniques There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. *Chromatographic techniques* provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called *hyphenation*, provides a powerful means of separating and identifying unknown compounds.

Analytical methods:

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

Method development forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;
- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;

- Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;
- The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factors.

Chromatography [2]:
Introduction:
The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.
" *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient.

"In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

Chromatographic Process [4]:
Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture component. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

High Performance Liquid Chromatography (HPLC) [6]:
The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics,

environmental matrices, forensic samples, and industrial chemicals.

Components of HPLC:
- Solvent
- Solvent Delivery System (Pumps)
- Injector
- Column
- Detectors
- Recorder (Data Collection)

Solvent:
- Compatible with the instrument (pumps, seals, fittings, detector etc).
- Compatible with the stationary phase.
- Readily available (often use liters/day) and of adequate purity.
- Spectroscopic and trace-composition usually.
- Not too compressible (causes pump/flow problems).
- Free of gases (which cause compressibility problems).
- The polarity index is a measure of the relative polarity of a solvent. It is used for identifying suitable mobile phase solvents.
- Increasing eluent strength or polarity index values mean increasing solvent polarity, and
- The analyte(s) and samples must be mobile phase and stationary phase compatible.

Pumps:
- It drives the mobile phase from the reservoir to the column.
- Because of the small particles used in modern HPLC, modern pumps need to operate reliably and precisely at pressures of 10,000 p.s.i. or at least 6,000 p.s.i.
- To operate at these pressures and remain sensibly inert to the wide variety of solvents used, HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats.
- For analytical purposes HPLC pumps should have flow rates that range from 0 to 10 ml/min., but for preparative HPLC, flow rates in excess of 100 ml/min may be required.
- The level of constancy is required because most HPLC detectors are flow sensitive and errors in quantization will result from changes in flow rate.

TYPES:
- Syringe pumps
- Reciprocating pumps
- Pneumatic pumps

Other errors are added to this due to capillary action and the small dimensions/cavities inside the injector.

Injectors:

❖ There are three types of injectors, they are
1. Septum injectors
2. Stop flow injectors
3. Rheodyne injectors

There are two modes of sample injection in LC, they are
1. Load position
2. Inject position

Columns:
- The column is one of the most important components of the LC chromatograph because the separation of the sample components is achieved when those components pass through the column.
- The Liquid Chromatography apparatus is made out of stainless steel tubes with a diameter of 3 to 5mm and a length ranging from 10 to 30cm.
- Normally, columns are filled with silica gel because its particle shape, surface properties and pore structure help to get a good separation.

MATERIALS AND METHODS:

Montelukast-Provided by Sura pharma labs, Doxofylline-Provided by Sura pharma labs
Water and Methanol for HPLC- LICHROSOLV (MERCK), Acetonitrile for HPLC-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 0.1ml of the above Montelukast and 0.3ml of the Doxofylline 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 and Water: Acetonitrile and Methanol: Phosphate

Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportion 45:55 v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH : 4.6
Mobile phase : Acetonitrile: Phosphate Buffer (45:55 v/v)
Flow rate : 1ml/min
Wavelength : 245 nm
Injection volume : 10 µl
Run time : 7 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.6):
Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase:
Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 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:

Trail 5 (Optimized Chromatogram):
Column : Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size
Column temperature : 38˚C
Wavelength : 248nm
Mobile phase ratio : Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 7minutes

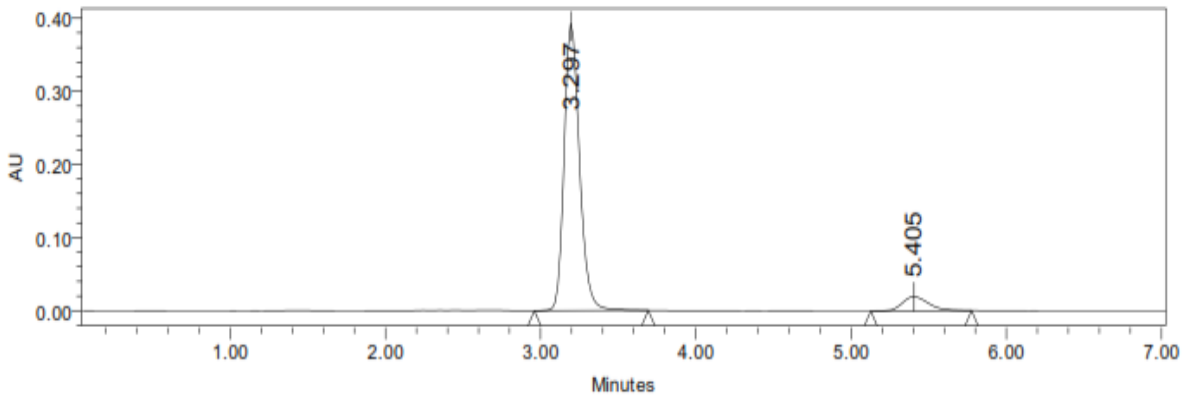

Figure-: Optimized Chromatogram (Standard)

Table-: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Montelukast	3.297	859856	42569	1.24	7896	
2	Doxofylline	5.405	5698	3652	1.36	6582	6.8

Observation: 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.

Optimized Chromatogram (Sample):

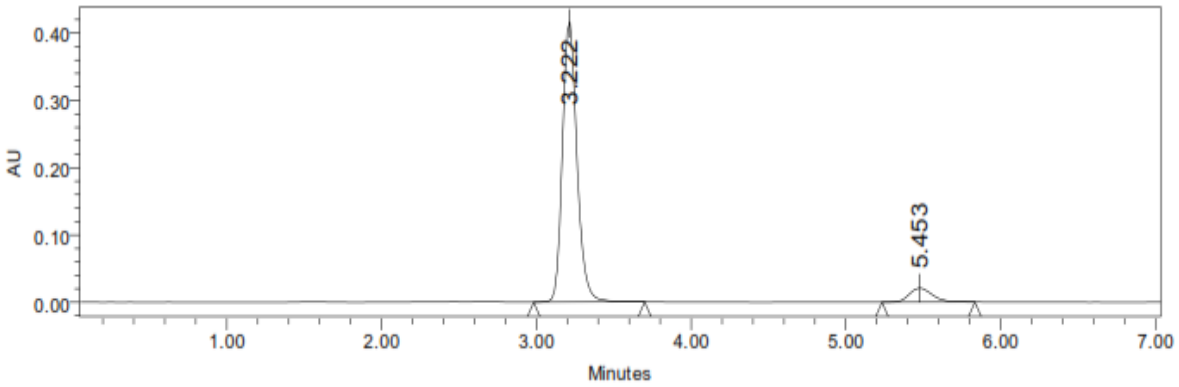

Figure-: Optimized Chromatogram (Sample)

Table-: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Montelukast	3.222	865898	43659	1.26	7985	
2	Doxofylline	5.453	5789	3785	1.38	6659	7.0

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.

System Suitability:

Table-: Results of system Suitability for Montelukast

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Montelukast	3.200	859865	42568	7895	1.24
2	Montelukast	3.248	859788	42587	7859	1.24
3	Montelukast	3.299	857984	42659	7869	1.24
4	Montelukast	3.297	854879	42875	7849	1.24
5	Montelukast	3.297	857896	42487	7859	1.23
Mean			858082.4			
Std. Dev.			2024.409			
% RSD			0.235922			

Acceptance Criteria:

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

Table-: Results of System Suitability for Doxofylline

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Doxofylline	5.413	5689	3659	6583	1.36
2	Doxofylline	5.484	5687	3648	6592	1.37
3	Doxofylline	5.405	5682	3698	6549	1.37
4	Doxofylline	5.405	5649	3675	6571	1.36
5	Doxofylline	5.409	5674	3649	6529	1.36
Mean			5676.2			
Std. Dev.			16.2696			
% RSD			0.286628			

Acceptance Criteria:

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

Assay (Standard):

Table-: Peak Results for Assay Standard

Montelukast

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Montelukast	3.211	859785	42598	1.25	7856
2	Montelukast	3.222	859865	42895	1.24	7859
3	Montelukast	3.254	857849	42578	1.25	7869

Doxofylline

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Doxofylline	5.414	5699	3685	1.36	6598	6.9
2	Doxofylline	5.453	5687	3659	1.37	6537	6.9
3	Doxofylline	5.424	5689	3649	1.36	6582	7.0

Assay (Sample):

Table-: Peak Results for Assay sample

Montelukast

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Montelukast	3.297	865985	43659	1.26	7985
2	Montelukast	3.294	865798	43875	1.26	7925
3	Montelukast	3.295	865456	43659	1.27	7946

Doxofylline

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Doxofylline	5.435	5789	3659	1.37	6659	6.9
2	Doxofylline	5.417	5798	3684	1.38	6689	7.0
3	Doxofylline	5.434	5749	3695	1.38	6648	6.9

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Montelukast and Doxofylline in pharmaceutical dosage form was found to be 99.82%.

LINEARITY: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Montelukast

Concentration µg/ml	Average Peak Area
30	545894
40	725985
50	897856
60	1068594
70	1245698

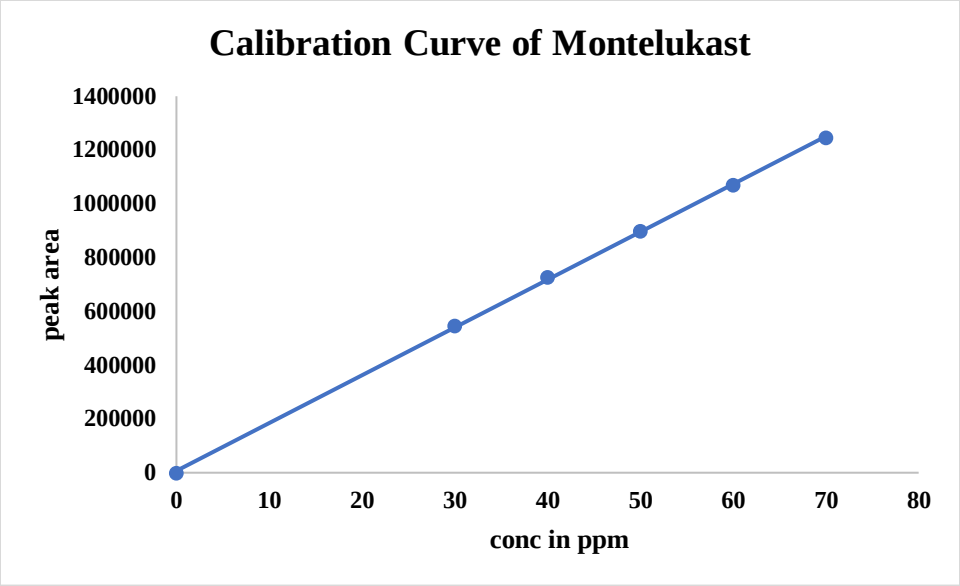

Fig-: Calibration Curve of Montelukast

Doxofylline:

Concentration µg/ml	Average Peak Area
10	2038
20	3859
30	5698
40	7489
50	9218

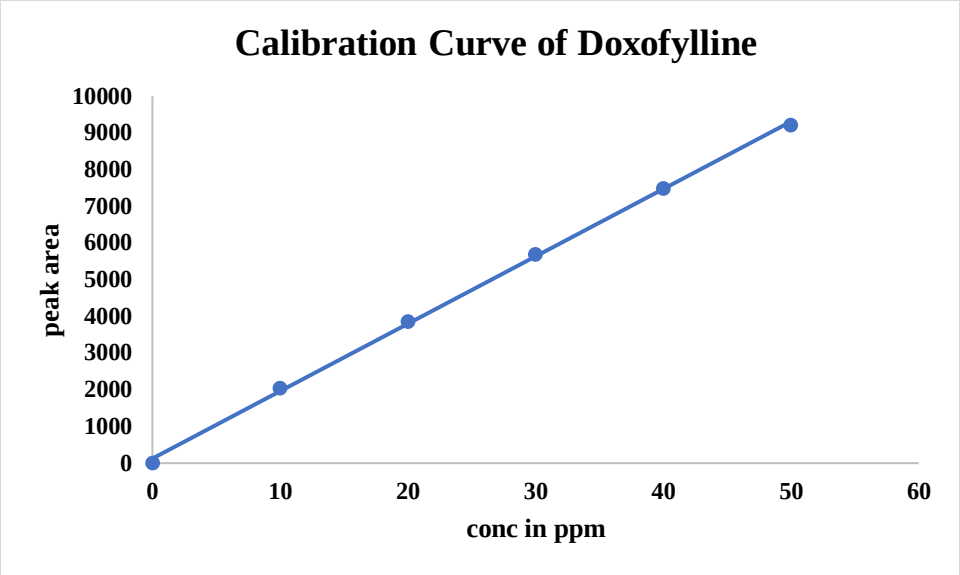

Fig-: Calibration Curve of Doxofylline

Precision:

REPEATABILITY

Table-: Results of Repeatability for Montelukast:

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Montelukast	3.213	859856	42659	7859	1.24
2	Montelukast	3.253	857985	42598	7869	1.24
3	Montelukast	3.297	856984	42587	7846	1.25
4	Montelukast	3.215	856987	42569	7819	1.25
5	Montelukast	3.254	859878	42894	7856	1.24
Mean			858338			
Std.dev			1454.222			
%RSD			0.169423			

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.

Table-: Results of repeatability for Doxofylline:

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Doxofylline	5.441	5697	3659	6592	1.36
2	Doxofylline	5.442	5689	3648	6539	1.36
3	Doxofylline	5.409	5698	3692	6584	1.37
4	Doxofylline	5.520	5639	3648	6579	1.36
5	Doxofylline	5.424	5688	3689	6549	1.36
Mean			5682.2			
Std.dev			24.57031			
%RSD			0.432408			

Intermediate precision:
Day 1:

Table-: Results of Intermediate precision for Montelukast

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Montelukast	3.211	868956	43659	7985	1.26
2	Montelukast	3.211	869857	43985	7954	1.27
3	Montelukast	3.210	865983	43879	7946	1.26
4	Montelukast	3.212	866587	43865	7963	1.27
5	Montelukast	3.211	864256	43875	7964	1.26
6	Montelukast	3.297	868974	43562	7942	1.26
Mean			867435.5			
Std. Dev.			2167.095			
% RSD			0.249828			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision for Doxofylline

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Doxofylline	5.411	5785	3789	6659	1.37
2	Doxofylline	5.410	5798	3758	6625	1.38
3	Doxofylline	5.420	5766	3746	6649	1.38
4	Doxofylline	5.423	5746	3795	6675	1.37
5	Doxofylline	5.419	5782	3761	6653	1.38
6	Doxofylline	5.409	5786	3752	6627	1.37
Mean			5777.167			
Std. Dev.			18.40018			
% RSD			0.318498			

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

DAY 2:

Table-: Results of Intermediate precision Day 2 for Montelukast

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Montelukast	3.211	845985	44585	8025	1.27
2	Montelukast	3.233	847895	44895	8069	1.28
3	Montelukast	3.244	848985	44758	8046	1.27
4	Montelukast	3.297	847859	44548	8094	1.28
5	Montelukast	3.297	845984	44865	8042	1.28
6	Montelukast	3.202	847898	44254	8076	1.27
Mean			847434.3			
Std. Dev.			1201.345			
% RSD			0.141763			

Acceptance Criteria: %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Doxofylline

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Doxofylline	5.411	5898	3986	6852	1.39
2	Doxofylline	5.410	5884	3955	6864	1.39
3	Doxofylline	5.420	5863	3956	6829	1.40
4	Doxofylline	5.405	5845	3945	6874	1.39
5	Doxofylline	5.409	5896	3925	6829	1.39
6	Doxofylline	5.463	5874	3962	6825	1.40
Mean			5876.667			
Std. Dev.			20.39281			
% RSD			0.347013			

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

ACCURACY:

Table-: The accuracy results for Montelukast

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	451144.3	25	24.998	99.992%	100.1873%
100%	897248.3	50	50.104	100.208%	
150%	1344562	75	75.278	100.362%	

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-: The accuracy Results for Doxofylline

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2895	15	15.084	100.560%	100.748%
100%	5685.333	30	30.282	100.940%	
150%	8449	45	45.335	100.744%	

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.

Robustness

Table-: Results for Robustness

Montelukast

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	859856	3.297	7896	1.24
Less Flow rate of 0.9mL/min	915847	3.639	7251	1.20
More Flow rate of 1.1mL/min	842564	2.859	7415	1.21
Less organic phase (about 5 % decrease in organic phase)	825498	3.460	7365	1.23
More organic phase (about 5 % Increase in organic phase)	814578	3.022	7258	1.22

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table-: Results for Robustness

Doxofylline

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.1mL/min	5698	5.405	6582	1.36
Less Flow rate of 0.9mL/min	6452	6.250	6785	1.32
More Flow rate of 0.8mL/min	5254	4.863	6365	1.34
Less organic phase (about 5 % decrease in organic phase)	5487	6.196	6254	1.38
More organic phase (about 5 % Increase in organic phase)	5369	5.010	6298	1.33

Acceptance Criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION:

SUMMARY:

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Montelukast and Doxofylline in pharmaceutical dosage forms. The method utilized a Waters HPLC system with an auto-sampler and PDA detector. The separation was achieved using a Phenomenex Luna C18 column (4.6×250 mm, 5 µm particle size) with a mobile phase consisting of acetonitrile and phosphate buffer (pH 4.6) in a 45:55 (v/v) ratio. The flow rate was 1.0 ml/min, and the detection was performed at 245 nm with an injection volume of 10 µL. The optimized chromatographic conditions provided a total run time of 7 minutes, making the method both efficient and rapid. The method was validated in accordance with ICH guidelines, and it exhibited satisfactory results for linearity, precision, accuracy, specificity, and robustness.

CONCLUSION:

The developed RP-HPLC method for the simultaneous estimation of Montelukast and Doxofylline is precise, accurate, and reliable. The method offers a significant advantage due to its rapid analysis time (7 minutes), making it suitable for routine quality control in pharmaceutical formulations. Validation studies confirmed the method's robustness and reproducibility, ensuring its applicability in both the development and quality control of pharmaceutical products. The method also provides an efficient approach for simultaneous drug analysis, thereby contributing to effective monitoring and ensuring the therapeutic efficacy of the combined formulation of Montelukast and Doxofylline.

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