ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF MONTELUKAST SODIUM AND BILASTINE BY HPLC

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Abstract:
A HPLC method was developed and validated of Montelukast Sodium and Bilastine in bulk formulation. The chromatographic separation of drug was achieved on 29/05/2021. The mobile phase consisted of Acetonitrile and Buffer (70:30), The flow rate was adjusted to 1.5 ml/min. and %RSD as 0.0013. This proposed method is accurate, highly sensitive and precise which helps in cost reduction of analysis, hence we can use it for routine quality analysis in laboratories.

Keywords: Montelukast Sodium, Bilastine, HPLC, Validation, simultaneous estimation.

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INTRODUCTION:

A. Montelukast Sodium (MTS) is freely soluble in Methanol, Ethanol, Water and Acetonitrile. MTS is 1-[[[(1R)-1-[3-[(1E)-2-(7-Chloro-2-Quinolinyl) Ethynyl] Phenyl]-3-[2-(1-hydroxy-1-methyl-ethyl) Phenyl] Propyl] Thio] Methyl] cyclopropane acetic acid. Montelukast Sodium is a selective, Potent and Orally Active Antagonist of the Cysteinyl, CysTL1, Leukotriene receptor used for the treatment of Asthma in children's and adults. It is a practically insoluble in acetonitrile and freely soluble in ethanol, methanol, and water. Montelukast Sodium is a potent drug, selectively CystLT1 receptors antagonist. It is indicated for the prophylaxis and chronic treatment of asthma in adults and pediatric patients. Several analytical methods have been reported for the determination of montelukast sodium including derivative spectroscopic, by colorimetry, by fluorimetry[8], by TLC, by HPTLC, by simultaneous UV determination in combination drug formulation[11], by voltammetry, by HPLC, and by LCMS[7].

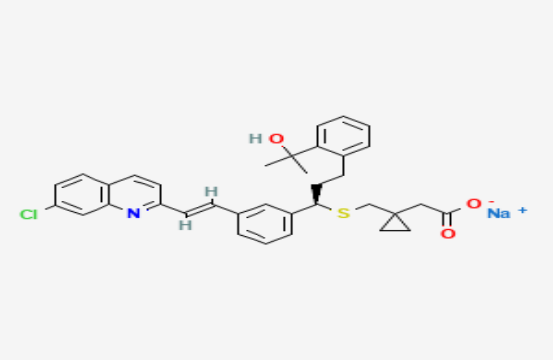


Figure:1

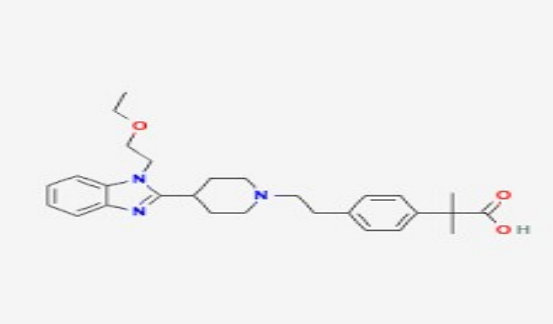


Figure:2

EXPERIMENTATION:

A. INSTRUMENTATION: RP-HPLC, Ultrasonic Liquid Processors.

B. MATERIALS:

Reference samples were gifted from Anil Enterprises PVT. LTD., Kaleamb, Himachal Pradesh.

B. Bilastine is chemically known as 2-[4-[2-[4-[1-(2-ethoxyethyl) benzimidazol-2-yl] piperidin-1-yl] ethyl] phenyl]-2-methylpropanoic acid. For symptomatic relief of nasal and non-nasal symptoms of seasonal rhinitis in patients 12 years of age and older and for symptomatic relief in chronic spontaneous urticaria in patients 18 years of age and older. Bilastine is a novel new generation antihistamine that is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action. Histamine plays a major role in the allergic reaction and is released by mast cell degranulation[4]. This histamine binds with H_1 receptors, activates the receptors and causes allergic reactions. Bilastine binds with H_1 receptor and prevents the activation of H_1 receptor by histamine. Thus, it acts as an antagonist for histamine. Bilastine shows no cardiotoxic, sedative side effects and undergoes minimal or no first pass metabolism [5]. It has less chance to undergo drug-drug interactions. Therefore, it is useful for treating patients suffering with renal/ hepatic dysfunction [6]. Bilastine, a piperidine class antihistamine medication used for the treatment of allergic rhinitis and chronic urticaria. From the review of literature, it was found that very few methods such as LC-MS/MS [7], HPLC-fluorescence [8] in biological sample, RP-HPLC [9], HILIC [10] and UV-spectrophotometry [11] are available for estimation of Bilastine. The aim and objective of the present work was to develop and validate as per ICH guidelines [12] a simple, fast, accurate, precise, economic and sensitive method for estimation of Bilastine using UV- spectrophotometry, in both bulk and pharmaceutical formulation, which can be used for routine analysis in QC laboratories.
Methanol for HPLC, Ammonium acetate, Triethylamine, Glacial Acetic Acid, Acetonitrile.

C. Preparation of mobile phase:

Mobile Phase A: Used Buffer (30%)
Mobile Phase B: Used Acetonitrile (70%)
Diluent: Used Methanol

D. Preparation of Buffer:

Added 7.7041 gm of Ammonium Acetate to 2000 ml of Water, Added 2ml of Triethylamine. Adjusted pH to 5.54 with Glacial Acetic Acid.

E. Preparation of Test solution:

1. MONTELUKAST SODIUM AND BILASTINE (1:2):
Took 100.07gm of Bilastine and 49.79gm of Montelukast Sodium into 100ml Volumetric Flask, added 70ml of diluent and sonicated to

dissolve the content. Diluted to volume with diluent and mix thoroughly, labeled as STKSMB.

a. Preparation of 200PPM of Bilastine and 100PPM of Montelukast Sodium:

Took 10ml STKSMB into 50ml Volumetric Flask, diluted to volume with diluent and mixed thoroughly.

b. Preparation of 100PPM of Bilastine and 50PPM of Montelukast Sodium:

Took 5ml STKSMB into 50ml Volumetric Flask, diluted to volume with diluent and mixed thoroughly.

c. Preparation of 80PPM of Bilastine and 40PPM of Montelukast Sodium:

Took 8ml STKSMB into 100ml Volumetric Flask, diluted to volume with diluent and mixed thoroughly.

d. Preparation of 40PPM of Bilastine and 20PPM of Montelukast Sodium:

Took 4ml STKSMB into 100ml Volumetric Flask, diluted to volume with diluent and mixed thoroughly.

e. Preparation of 20PPM of Bilastine and 10PPM of Montelukast Sodium:

Took 2ml STLSMB into 100ml Volumetric Flask, diluted to volume with diluent and mixed thoroughly.

f. Preparation of 10PPM of Bilastine and 5PPM of Montelukast Sodium:

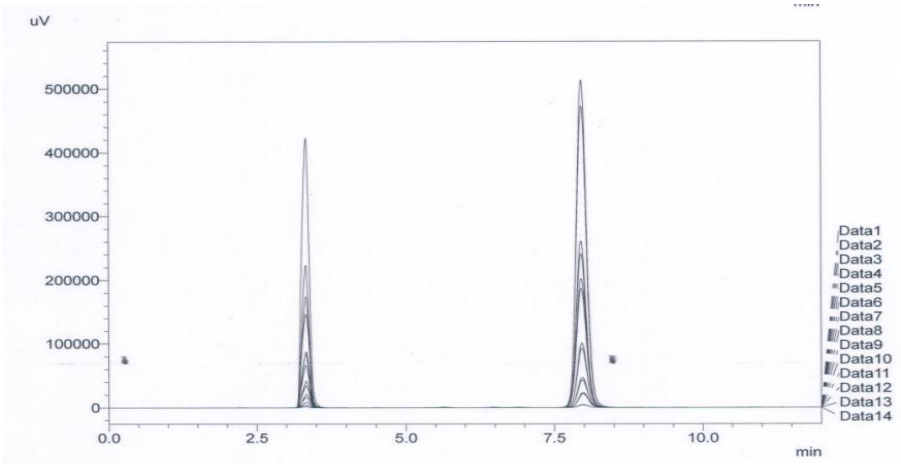
Took 10ml of 20PPM Bilastine and 10PPM Montelukast Sodium solution into 20ml Volumetric Flask, diluted to volume with diluent and mixed thoroughly.

g. Preparation of 2PPM of Bilastine and 1PPM of Montelukast Sodium:

Took 5ml of 20PPM Bilastine and 10PPM Montelukast Sodium solution into 50ml Volumetric Flask, diluted to the volume with diluent and mixed thoroughly

2. Graphs

a. Bilastine and Montelukast Sodium



Bilastine:

SNo.	Concentration	Retention time	Area	Theoretical Plate	Tailing Factor
1	2PPM	3.333	34729	2938	0.957
2	10PPM	3.342	177859	3010	0.991
3	20PPM	3.333	352518	3016	1.008
4	40PPM	3.333	746713	3015	0.998
5	80PPM	3.333	1490314	3019	0.967
6	100PPM	3.333	1910502	3019	0.954
7	200PPM	3.333	3663470	2980	0.970

Montelukast sodium:

SNo.	Concentration	Retention Time	Area	Theoretical Plate	Tailing Factor
1	1PPM	7.975	51372	9770	1.092
2	5PPM	7.983	260439	9854	1.065
3	10PPM	7.967	522134	9778	1.096
4	20PPM	7.967	1112534	9831	1.063
5	40PPM	7.958	2244460	9810	1.061
6	50PPM	7.950	2894898	9788	1.091
7	100PPM	7.958	5681805	9801	1.079

F. STUDY:

1. Linearity

Validation for linearity requires the preparation and analysis of a set of several independently prepared solutions. Linearity studies are important because they define the range of the method within which the results are obtained accurately and precisely. As an example, according to ICH guidelines, HPLC method linearity is normally based on five concentration levels between 70% and 130% of the nominal concentration.

2. Precision

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It is also termed as intra-assay precision. It is assessed by making six sample determinations at 100% concentration or by preparing three samples at three concentrations in triplicates covering the specified range for the procedure. Precision is measured by injecting a series of standards or analyzing series of samples from multiple samplings from a homogeneous lot.

3. Accuracy

The accuracy is the degree of closeness between the 'true' value of the sample and the value method obtain analytical evaluation. Accuracy is often determined by measuring samples with known concentrations and comparing the measured values with the 'true' values.

4. Robustness

The quality and ability to overcome the excess testing and adverse conditions. It can also be said that dependability on the method can be done to get proper results.

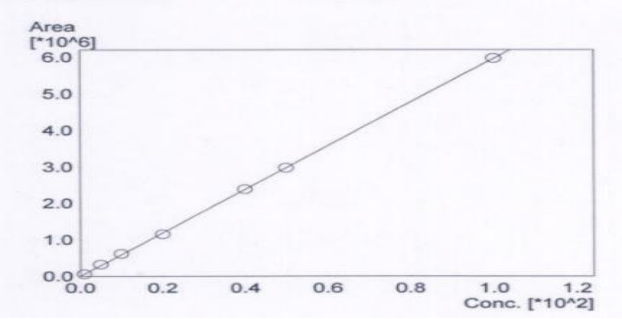
5. LOD and LOQ

Loss on Drying is an unspecific analytical technique removing not only water but all other volatile impurities like alcohol etc. LOD is calculated by =3.3*(SD/Slope) and LOQ= 10*(SD/Slope).

Discussion and Result:

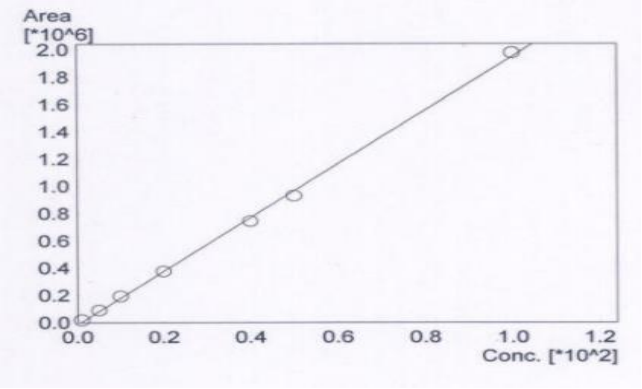
1. Linearity

Montelukast sodium



Range of Concentration	1-100PPM
Slope	57210.0
Y- Intercept	23118.3
Equation of Line	y=57210.0*x-23118.3
R^2	0.9997875
Mean Response Factor	5.458967+-0.04

Bilastine



Range of Concentration	2-200PPM
Slope	18448.9
Y-Intercept	5315.47
Equation of Line	y= 18448.9* x+5315.47
R^2	0.9994588
Mean Response Factor	1.821362+-0.04

2. Precision

Montelukast

SNo.	Concentration	Area	Retention Time	Tailing Factor
1.	5PPM	260432	7.987	1.095
2.	10PPM	522138	7.979	1.092
3.	40PPM	2244465	7.981	1.090
4.	50PPM	2894895	7.975	1.091

SNo.	Concentration	Area	Retention Time	Tailing Factor
1.	5PPM	260438	7.981	1.091
2.	10PPM	522135	7.978	1.090
3.	40PPM	2244469	7.975	1.095
4.	50PPM	2894890	7.987	1.092

SNo.	Concentration	Area	Retention Time	Tailing Factor
1.	5PPM	260432	7.978	1.090
2.	10PPM	522139	7.987	1.091
3.	40PPM	2244465	7.979	1.092
4.	50PPM	2894895	7.987	1.094

Bilastine

SNo.	Concentrations	Area	Retention Time	Tailing Factor
1.	10PPM	177850	3.334	0.992
2.	20PPM	352525	3.333	1.007
3.	80PPM	1490320	3.332	0.995
4.	100PPM	1910495	3.333	0.999

SNo.	Concentration	Area	Retention time	Tailing Factor
1.	10PPM	177857	3.333	1.002
2.	20PPM	352529	3.334	0.999
3.	80PPM	1490328	3.333	0.998
4.	100PPM	1910505	3.332	0.995

SNo.	Concentration	Area	Retention Time	Tailing Factor
1.	10PPM	177855	3.332	0.999
2.	20PPM	352530	3.333	1.001
3.	80PPM	1490320	3.334	0.995
4.	100PPM	1910500	3.333	0.997

3. Accuracy

Drugs	Label Claim (mg)	Amount added mg (%)	Total Amount mg	Actual Conc. Taken	Recover Conc.	Recovery (%)
Montelukast Sodium	10	5(50%)	15	5	5.002±0.005	100.04%
		10(100%)	20	10	10.03±0.002	100.3%
		15(150%)	25	20	20.10±0.004	100.5%
Bilastine	20	10(50%)	30	10	10.002±0.005	100.02%
		20(100%)	40	20	20.03±0.002	100.15%
		30(150%)	50	40	40.10±0.004	100.25%

4. Robustness:

Factors	levels	Retention Time		Area	
		Mont.	Bilastine	Mont.	Bilastine
A. Flow rate (ml/min.)					
1.4	-1	7.975	3.333	522130	352510
1.5	0	7.983	3.342	522139	352518
1.6	+1	7.980	3.339	522134	352512
Mean(n=3) 522134 352513			7.979±0.004		3.338±0.005
B. % of Acetonitrile in mobile phase					
69%	-1	7.983	3.332	522132	352512
70%	0	7.976	3.333	522134	352516
71%	+1	7.979	3.333	522138	352518
Mean(n=3) 522134 352515			7.979±0.005		3.332±0.006

5. LOD and LOQ:

LOD Montelukast = 0.0002, Bilastine = 0.0005
LOQ Montelukast = 0.002, Bilastine = 0.005

Conclusion:

A rapid method with easy, simple, precise, accurate and cost-effective method was developed and validated. It shows that %RSD is 0.0013 which is less than 2.

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