ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY SIMULTANEOUS ESTIMATION OF MONTELUKAST SODIUM AND BILASTINE BY UV SPECTROPHOTOMETRY

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Abstract

A simple, sensitive, specific UV Spectrophotometer method was developed and validated by simultaneous estimation of Montelukast Sodium and Bilastine. The optimum conditions were obtained, UV Spectra was achieved on 20/06/2021. The wavelength maxima of Bilastine and Montelukast Sodium were found to be 282 and 287 respectively. The linearity for this method was found to be in the range of 20-1 mcg and 10-0.5mcg for Bilastine and montelukast sodium respectively. The method showed highly sensitive with reproducibility in results. The calibration curve was drawn between absorption and concentration. The method showed the correlation coefficient(R) as 0.966 and 0.967 of Bilastine and Montelukast Sodium respectively. The regression equation was y= 0.159x-0.213 and y=0.094x-0.121 for Bilastine and Montelukast Sodium respectively. The proposed method may be suitable for the analytical method validation in bulk of Bilastine and Montelukast Sodium (2:1).

Keywords: Bilastine, Montelukast Sodium, UV Spectrophotometer, Simultaneous estimation, Analysis

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INTRODUCTION

A. Montelukast Sodium (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, monosodium salt is a white coloured powder and it is freely soluble in ethanol, methanol, and water. Molecular weight of Montelukast Sodium is 608.2 g/mol and formula is C35H35ClNO3S.Na. Montelukast Sodium is a potent drug, selectively CystLT1 receptors antagonist. It is indicated for the prophylaxis and chronic treatment of asthma in adults and paediatric 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, by voltammetry, by HPLC[8], and by LCMS[7]. (Figure: 1)

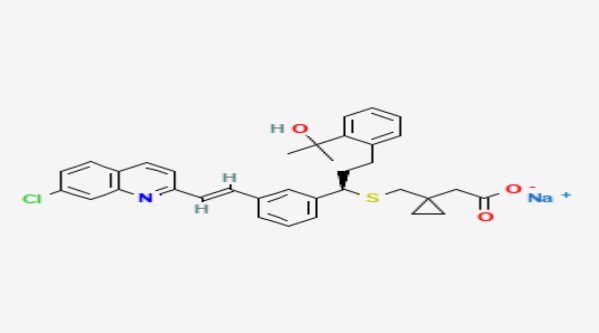


Figure:1

B. Bilastine is a highly selective new peripheral histamine H_1- receptor antagonist, chemically named as 2-[4-(2-{4-[1-(2-ethoxyethyl)-1H-1, 3-benzimidazol-2-yl] piperidin-1-yl} ethyl) phenyl]-2-methylpropanoic acid. Bilastine is a white crystalline powder having molecular formula $C_{28}H_{37}N_3O_3$, molecular mass of 463.61g/mole and melting point greater than 195^0 C [1]. It belongs to piperidine antihistamine class of drugs. It is a H_1 receptor inverse agonist like other antihistamines [2], used for treating allergic disorders such as rhino conjunctivitis and urticarial [3]. 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. (Figure:2)

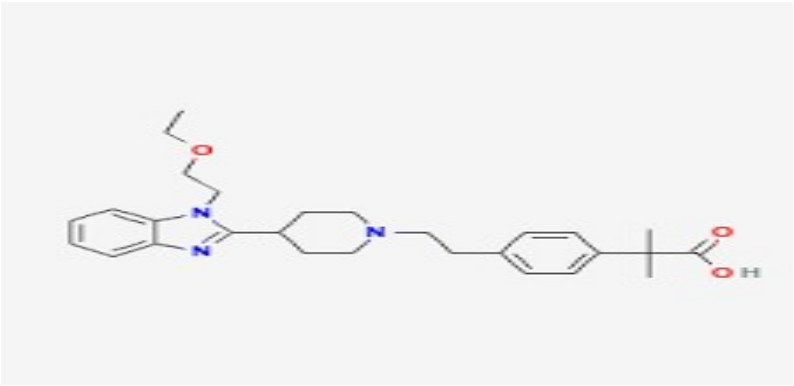


Figure:2

EXPERMENTATION:

INSTRUMENTS:

A double beam UV/ Visible spectrophotometer (Shimadzu 1700 Pharma spec), software used was UVProbe 2.71 lab solutions. Calibrated analytical balance (Shimadzu AY220), for sonication (Enertech Electronics pvt. Ltd.). All statistical calculations were done with the help of Microsoft Excel 2013.

CHEMICALS:

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

Methanol.

Method Development:

Preparation of standard stock solution:

Accurately weighed 100.74mg and 50.41mg of montelukast sodium and Bilastine and transferred to 100ml volumetric flask, then it was made up to level with the help of methanol as diluent. 5ml was pipetted out to 100ml volumetric flask and volume was makeup using methanol to 100ml to obtain 50PPM and 25PPM. Further dilution was done to obtain 25PPM and 12.5PPM using 5ml of the above solution, transferred to 100ml of volumetric flask, and levelled upto with methanol.

Preparation of standard working solution:

Dilution was made using 25PPM and 12.5PPM Solution such as 20ml of above diluted to 25ml using methanol to obtain 20:10PPM. 6ml of above solution was taken and 10ml methanol was added to get 15:7.5PPM. 4ml of above solution was diluted with methanol to obtain 10:5PPM. 1.6ml of the above solution was taken with 10ml of methanol to obtain 4:2PPM. 0.8ml of above solution was taken with 10ml of methanol to obtain 2:1PPM. 0.4ml of above solution was diluted with 10 ml of methanol to obtain 1:0.5PPM.

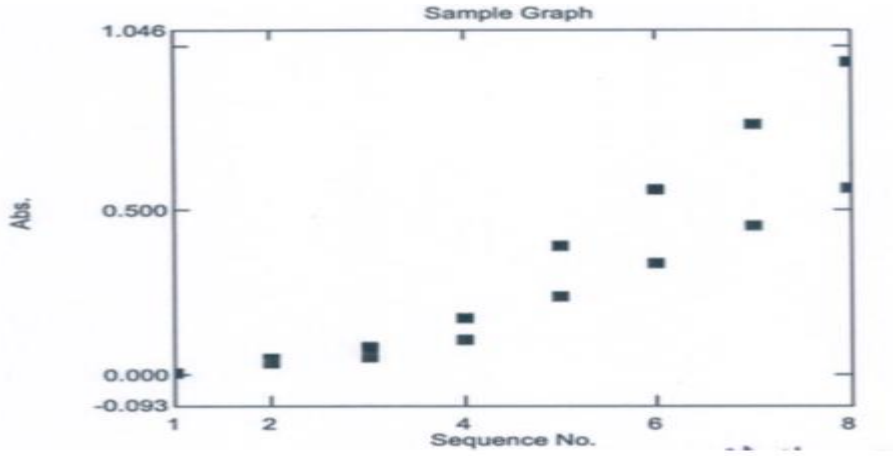
Simultaneous estimation equation:

It is also known as Vierordt's method which typically helps to estimate drugs in the combination of 2 or more then 2 in combined dosage form.

$C_X = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$ equation:1

$C_Y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$ equation:2

GRAPHS:



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[12], 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 analysing series of samples from multiple samplings from a homogeneous lot.

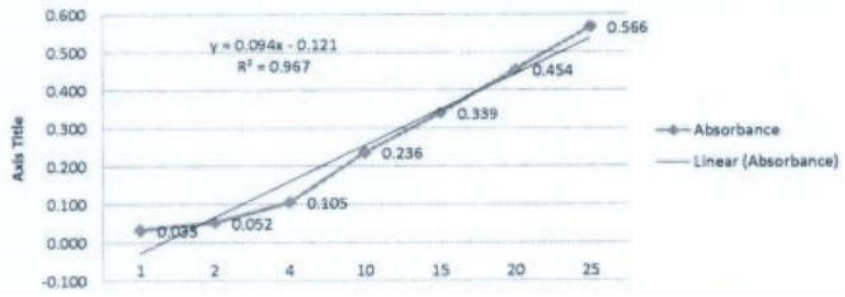
3. Accuracy:

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. 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).

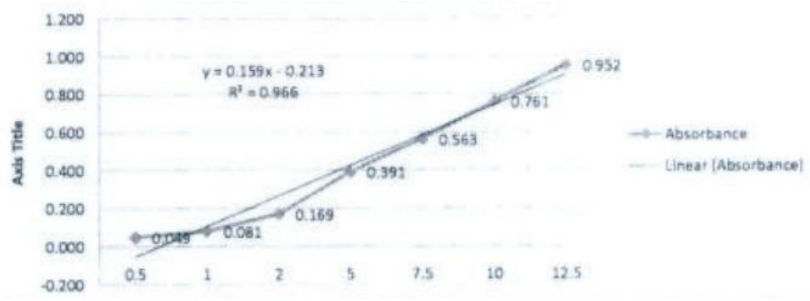
DISCUSION AND RESULTS:
1. Linearity curve:

Bilastine:



Range of linearity	1-25µg/ml
R^2	0.967
Equation of line	y= 0.094x-0.121
Slope	0.094
Y- Intercept	-0.121

Montelukast sodium:



Range of linearity	0.5-12.5µg/ml
R^2	0.966
Equation of line	y= 0.159x-0.213
Slope	0.159
Y- Intercept	-0.213

2. Precision:
Montelukast sodium:

Sno.	Concentration	Absorbance
1.	2	0.171
2.	5	0.395
3.	7.5	0.565
4.	10	0.765

1.

Sno.	Concentration	Absorbance
1.	2	0.170
2.	5	0.390
3.	7.5	0.562
4.	10	0.760

2.

Sno.	Concentration	Absorbance
1.	2	0.172
2.	5	0.394
3.	7.5	0.560
4.	10	0.762

Bilastine:

Sno.	Concentration	Absorbance
1.	4	0.107
2.	10	0.238
3.	15	0.338
4.	20	0.457

1.

Sno.	Concentration	Absorbance
1.	4	0.105
2.	10	0.236
3.	15	0.337
4.	20	0.456

2.

Sno.	Concentration	Absorbance
1.	4	0.106
2.	10	0.235
3.	15	0.338
4.	20	0.456

3. Accuracy:

Sno.	Drugs	Level of recovery %	Initial concentration	Added concentration	Drug Conc. Recovered ± SD	% Recovery
1.	Montelukast Sodium	80%	10	8	8.03± 0.098	100.1%
		100%	10	10	10.1± 0.069	100.2%
		120%	10	12	12.1± 0.150	100.2%
2.	Bilastine	80%	10	8	8.2± 0.960	100.2%
		100%	10	10	10.5± 1.20	100.5%
		120%	10	12	12.2± 0.68	100.2%

4. LOD and LOQ:

LOD
Montelukast sodium= 0.017
Bilastine= 0.029

LOQ
Montelukast sodium= 0.051
Bilastine= 0.087

5. Summary of Validation Parameters:

Parameters	Montelukast Sodium	Bilastine
Linearity(R^2)	0.966	0.967
Linearity Range	0.5-12.5µg/ml	1-25µg/ml
Precision (% RSD)	0.48	0.78
LOD	0.017	0.029
LOQ	0.051	0.087
Accuracy (% Recovery)	100.2%	100.3%
Assay 1	99.8%	100.1%
Assay2	100.1%	100%

Notes: 1. For assay1: Tablets used for the assay where Bilasure–M of Sun Pharmaceuticals Pvt. Ltd.
2.assay2: Tablets used for the assay where Bilanta-M of Ajanta Pharma Limited.

CONCLUSION:

A rapid method with easy, simple, precise, accurate and cost-effective method was developed and validated. It shows that %RSD is 0.48% and 0.78%

as of Montelukast sodium and Bilastine which is less than 2.

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