A NEW RP-PSLC-UV METHOD DEVELOPMENT AND VALIDATION FOR THE CONCURRENT ESTIMATION OF BILASTINE AND MONTELUKAST

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Abstract

A new RP-PSLC-UV method was developed and validated for the concurrent estimation of Bilastine and Montelukast by RP-PSLC-UV. The fingerprint region was obtained and compared with the standard. The stock solution of 10 ug/ml for each drug Bilastine and Montelukast was prepared by using Methyl alcohol and Cyanomethane in the ratio of 1:9. The chromatographic conditions were optimized to achieve efficient separation. The results showed that the established RP-HPLC-UV method had adequate sensitivity to the concentration of Bilastine and Montelukast.

The coefficient of regression (r2) was 0.9991 for Montelukast and 0.9994 for Bilastine. The developed method showed a linearity between 700-35ng/ml for Montelukast and 438-44ng/ml for Bilastine. This research developed and validated a simple selective responsive rapid rugged and reproducible method for the simultaneous estimation of Montelukast and Bilastine by the ICH Guidelines.

Keyword- Montelukast, Bilastine, RP- PSLC-UV, Simultaneous.

INTRODUCTION

Bilastine + Montelukast is a combination available as FDC in Indian market to treat the allergic symptoms effectively. Bilastine is an antiallergenic and acts to reduce allergic symptoms such as nasal congestion and urticaria. Bilastine is a selective histamine H1 receptor antagonist (Ki = 64nM) Label. During allergic response mast cells undergo degranulation which releases histamine and other subastances. By binding to and preventing activation of the H1 receptor, bilastine reduces the development of allergic symptoms due to the release of histamine from mast cells.

Montelukast is used to control and prevent symptoms caused by asthma (such as wheezing and shortness of breath). It is also used before exercise to prevent breathing problems during exercise (bronchospasm) and used to relieve symptoms of hay fever and allergic rhinitis (such as sneezing, stuffy/runny/itchy nose). It works by blocking certain natural substances (leukotrienes) that may cause or worsen asthma and allergies. It helps make breathing easier by reducing swelling (inflammation) in the airways.

For the quantification of montelukast and bilastine (1-7) many methods are available from UV spectroscopy to LCMS analytical to bioanalytical individually and no methods are available for the concurrent estimation by HPLC.

MATERIALS AND METHODS

Solvents, Chemicals, Utilities & Accessories used

Acetonitrile of HPLC grade Ammonium Formate of Analytical grade and Formic acid by SD Fine Chemicals Ltd and Aqua PSLC grade from Milli-Q RO system was used. The Working standard of Montelukast and Bilastine was obtained from Pure and Healthcare Private Limited, Raipur, Haridwar. Analytical columns Hibar® 250-4.6 Purospher® STAR RP-18e(150 x 4.4mm i.d., 5µ).

Instruments Used

- Systronics - pH meter.
- Shimadzu 1700 UV-Visible spectrophotometer
- Shimadzu FTIR 8400S series
- Ultra Sonicator
- Shimadzu single pan digital balance (BL 220 H)
- Shimadzu gradient HPLC LC-10 AT-VP system with Lab solution data station.

Preliminary studies

Solubility studies:

The solubility of Montelukast and Bilastine was determined by using different solvents and it was found to be freely soluble in methanol, insoluble in acetonitrile and water.

Selection of wavelength:

10 ug/ml solutions of working standards of Montelukast and Bilastine were prepared by dissolving them in methanol. The UV spectrum was obtained by scanning in the range of 200-400nm. The wavelength of each compound was determined and the isobestic point was calculated. The λ_{max} was measured and the isobestic point was found to be 275 nm.

Table 1 - Wavelength Maxima of drugs and Selection of Isobestic point

Drug name	**λ_{max}**
Montelukast	280 nm
Bilastine	272nm
ISOBESTIC POINT	**275nm**

OPTIMISATION OF CHROMATOGRAPHIC CONDITIONS

Mobile phase preparation:

The eluent was prepared by dissolving accurately weighed 0.315 g of Ammonium formate in 500 ml Millipore water (10 milli molar strength and pH made to 4.5 with 1% Formic acid) and Cyanomethane. The eluent was sonicated and used for the separation of samples in the HPLC system.

Preparation of standard (1 mg/ml) and working stock solution(10ug/ml):

The stock solution of Montelukast (1.0mg/ml) and Bilastine (1.0mg/ml) was prepared individually by accurately weighing 0.01g and dissolved in Methyl alcohol and made up to 10ml with Cyanomethane. The working standard solution was prepared from the standard stock solution individually, labeled, and kept for storage at 2-8℃.

Preparation of stock calibration curve samples (CC):

The calibration range was selected based on the Cmax value of the individual drugs. This determines the concentration range. The stock solution of 10 ug/ml for each drug Bilastine and Montelukast was prepared by using Methyl alcohol and Cyanomethane in a ratio of 1:9. The following calibration curve solutions 700,630,560,480,420,350,280,210,140,70,35,25,10 and 5 ng/ml for Montelukast and 390,350,306,260,220,175,130,87 and 43 ng/ml for Bilastine were prepared from the standard stock solution. The containers were labeled and stored at 2-8℃ until analysis.

RESULT AND DISCUSSION

An new RP-PSLC-UV method has been developed and validated for the concurrent estimation of Montelukast and Bilastine by using HPLC System. The drugs were subjected to preliminary studies such as solubility, and absorption maximum detection. Chromatographic conditions were optimized to achieve efficient separation. Following PSLC conditions can be used for the simultaneous of Montelukast and Bilastine.

Chromatographic conditions:

Table 2 - Optimised PSLC Conditions

Stationary phase	Hibar® 250-4.6 Purospher® STAR RP-18e(150x4.4mm i.d., 5µ)
Mobile Phase	Cyanomethane and 10 mM Ammonium formate buffer (pH-4.5)
Column temperature	Ambient temperature
Elution mode	**Gradient**: 40:60v/v% (0- 3 min) & 80:20v/v% (3.01- 15 min)
Flow rate	1.0 ml/min
Detection wavelength	275nm
Injection volume	20µl
Retention time	Bilastine-2.5 min & Montelukast-10.4 min

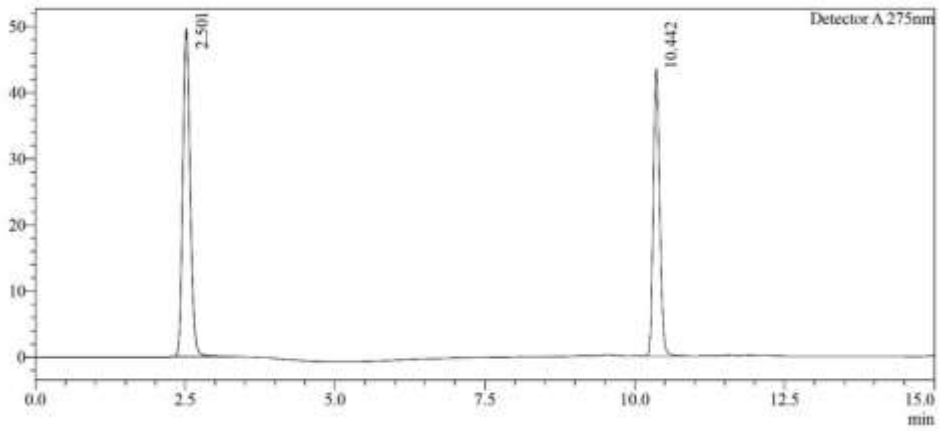

Fig 1- Typical chromatogram of Bilastine and Montelukast

Validation of the developed PSLC methods:

It is an integral part of any good analytical practice. Validation of the developed PSLC methods: The major aim of the validation process is to confirm that the analytical procedure employed for a specific test is suitable for its intended use. The validation parameters with results are discussed below.

Specificity/ Selectivity

The specificity and selectivity of the developed PSLC method was evaluated by the analysis of a solution containing Montelukast and Bilastine. There were no interferences observed at the retention time of both the drugs from the diluent solution.

Ruggedness & Robustness:

The ruggedness and robustness of the developed method were studied evaluated by carrying out experiments by changing conditions like columns, reagents, instruments, detection wavelength, and data systems. It was observed that there were no marked changes in the chromatograms demonstrating that the HPLC method has developed is rugged and robust.

LOD& LOQ

The LOD and LOQ were evaluated and tabulated in the Table-3.

Table 3 -LOD and LOQ for Bilastine and Montelukast

Drug name	Limit of detection	Limit of quantification
Bilastine	1.5ng/ml	5 ng/ml
Montelukast	2.5ng/ml	8 ng/ml

Linearity

The data of linearity studies evaluated between 44-438 ng/ml for Bilastine and 35-700 ng/ml for Montelukast based on their Cmax and the calibration curve was plotted against Concentration Vs Peak area are given below (Table-4). The correlation coefficient (r2) of Bilastine was 0.9994 and of Montelukast was 0.9991, demonstrating that the established RP-HPLC-UV method has adequate sensitivity to the concentration of Bilastine and Montelukast.

Table 4- Linearity studies of Montelukast & Bilastine

Bilastine		Montelukast	
Concentration (ng/ml)	**Peak area**	**Concentration (ng/ml)**	**Peak area**
438	194556	700	187165
394	176895	630	172342
350	154678	560	150151
306	134567	480	132528
263	117899	420	114356
219	98765	350	100345
175	82345	280	81236
88	43856	140	45673
44	25467	70	25078
		35	14334

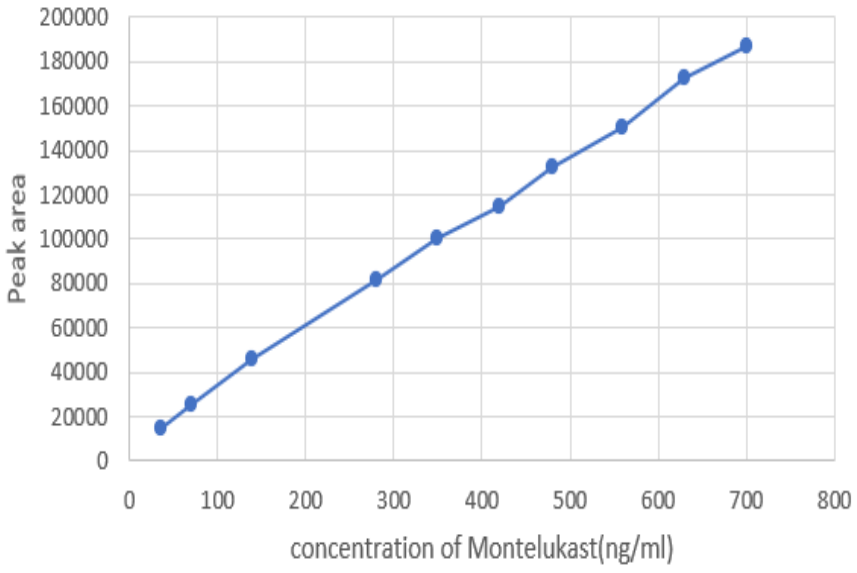

Figure - Calibration graph of Montelukast

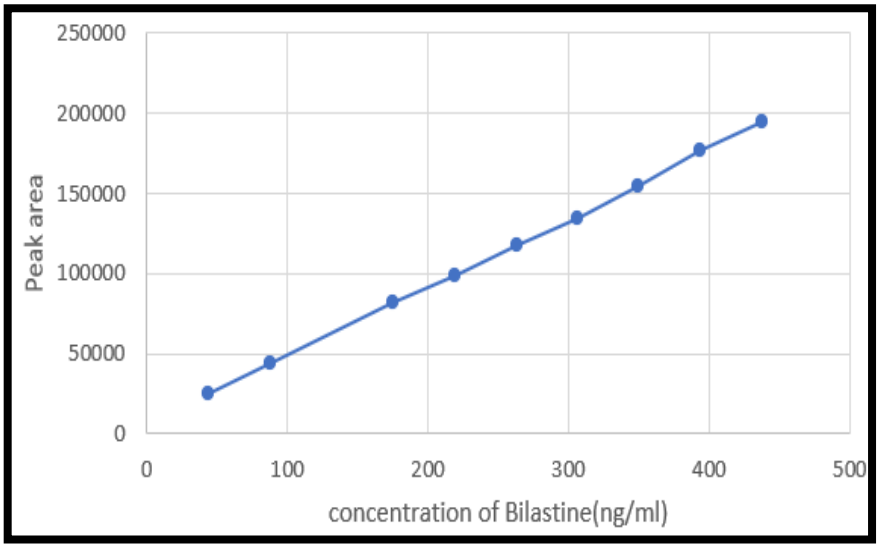

Figure - Calibration graph of Bilastine

Accuracy

The accuracy of the optimized method was determined by relative and absolute recovery experiments. The assay studies were carried out 6 times and the coefficient of variation was calculated (Table 5 and 5A).

Table 5-Accuracy result for Bilastine

Concentration (ng/ml)	Mean(ng/ml) ±SD	Accuracy (%)	C V (%)
LQC (44)	42±1.5	99.2	0.02
MQC (263)	258±4.8	100.2	0.01
HQC (438)	430±10.5	99.5	0.03

Table 5A-Accuracy result for Montelukast

Concentration (ng/ml)	Mean(ng/ml) ±SD	Accuracy (%)	CV (%)
LQC (35)	32±2.4	99.8	0.01
MQC (350)	346±5.6	99.4	0.02
HQC (700)	694±7.9	100.3	0.02

Precision

The precision of the established method was inspected by injecting six individual preparations by measuring the intra-day precision of concentration vs peak areas for Montelukast and Bilastine by injections (n=6) of 3 different concentration levels (35,350, and 700ng/ml) for Montelukast, and (44,263, 438 ng/ml) for Bilastine**.** The intermediate precision was evaluated by performing six consecutive injections and found that the method is precise (Table 6 and 6A).

Table 6- Precision values of Blastine

Intra-Day Precision			
Concentration (ng/ml)	**Mean(ng/ml) ±SD**	**Accuracy (%)**	**C V (%)**
LQC (44)	40±3.5	100.2	0.03
MQC (263)	258±5.5	99.5	0.01
HQC (438)	431±9.3	100.6	0.02
Inter-day Precision			
Concentration (ng/ml)	**Mean(ng/ml) ±SD**	**Accuracy (%)**	**C V (%)**
LQC (44)	39±2.5	99.5	0.02
MQC (263)	256±6.3	99.2	0.1
HQC (438)	430±10.5	100.3	0.01

Table 6A -Precision values for Montelukast

Intra-Day Precision			
Concentration (ng/ml)	**Mean(ng/ml) ±SD**	**Accuracy (%)**	**C V (%)**
LQC (35)	32±2.6	99.3	0.03
MQC (350)	345±4.1	100.4	0.03
HQC (700)	691±8.9	99.7	0.01
Inter-day Precision			
Concentration (ng/ml)	**Mean(ng/ml) ±SD**	**Accuracy (%)**	**C V (%)**
LQC (35)	30±4.3	100.3	0.01
MQC (350)	341±7.6	99.8	0.03
HQC (700)	688±11.9	99.5	0.02

System suitability parameters

System suitability parameters were studied according to the specifications of guidelines (Table 7).

Table 7- Validation Parameter and System suitability Studies

Validation parameters/ System suitability	**Observation**	
	Bilastine	**Montelukast**
Linearity and Range	44-438 ng/ml	35-700 ng/ml
Coefficient of regression(r2)	0.9994	0.9991
LOD	1.5ng/ml	2.5ng/ml
LOQ	5ng/ml	8 ng/ml
Number of theoretical plates(N)	6750	6500
Tailing factor	0.7	0.8
Asymmetric factor	1	1

CONCLUSION

The purpose of this research is to develop a new RP-PSLC-UV method and validate a simple selective responsive rapid rugged and reproducible method for the concurrent estimation of Montelukast and Bilastine by the ICH Guidelines. PSLC with UV detector was selected for the experiment and the influencing parameters such as chromatographic conditions, column selection, and composition of mobile phase were optimized to get efficient separation. Chromatographic condition-It can be concluded that the best separation with the good resolution was achieved by using the C18 column as the stationary phase and Cyanomethane:10mM Ammonium formate as the mobile phase -initially 40:60 (up to 3min), then 80:20 (up to 15 min). The flow rate is fixed as 1 ml/min with an injection volume of 20ul in gradient conditions.

Validation parameters:

The developed method showed a linear response over the concentration range of 700-35ng/ml for Montelukast and 438-44ng/ml for bilastine. The coefficient of regression (r2) was found to be 0.9991 for montelukast and 0.9994 for Bilastine. For montelukast, LOD and LOQ were found to be 2.5ng/ml and 8 ng/ml respectively. LOD was 1.5ng/ml and LOQ was 5ng/ml for Bilastine. The accuracy and precision were found to be within the specified limits according to the ICH guidelines. It can be summarized that the developed method was simple accurate precise and sensitive. It can be utilized to quantify the drugs Montelukast and Bilastine in API. It has further applications in bioavailability, bioequivalence and pharmacokinetic studies.

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