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Development And Validation Of UV Spectrophotometric Method For The Simultaneous Estimation Of Montelukast Sodium And Bilastine In Their Combined Dosage Form

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ABSTRACT: Aim: Development and validation of UV spectroscopic methods for the simultaneous estimation of Montelukast sodium and Bilastine.
Methods: All the methods validated for linearity, precision, repeatability, accuracy, LOD and LOQ. The method proposed from this study for the quantification of MON and BIL involves Absorbance ratio method, Area under Curve method and First Order Derivative method.
Results: The validated methods were found to be linear in concentration ranges of 5-25 g/ml for MON (Montelukast sodium) and 10-50 g/ml for BIL (Bilastine). The approach was discovered to be precise with RSD of 2% for interday, intraday, and repeatability. RSD 2.0 was used to validate the method's accuracy and precision. The described method was used to quantify and detect MON (Montelukast sodium) and BIL (Bilastine) in tablet dose from. This demonstrated that this approach can accurately assess medicines in solid dosage forms.
Conclusion: The proposed UV Visible Spectrophotometric method was validated according to ICH guidelines and results and statistical parameters demonstrated that the developed method is sensitive, reliable, accurate and simpler for the estimation of MON and BIL in combined dosage form.
KEYWORDS: Bilastine; Montelukast sodium; Spectrophotometric method; Validation.

I. INTRODUCTION

Method development is the process of demonstrating that an analytical technique can be used to determine the amount of an API present in a particular compounded dosage form. Analytical method development and validation are crucial steps that must be taken and are rigorously tested. To show that an analytical method is appropriate for its intended function, it must be validated before it may be utilized in medication development or production. [1, 2]

The analysis of the literature led to the conclusion that Bilastine has been tested using UV and HPLC both alone and in combination with other medications. Methods like UV, HPLC, and HPTLC have been described for the measurement of Montelukast Sodium, either alone or in combination with other medications. [3-6] However, as of this writing, there are no known methods for combining bilastine and montelukast sodium. Therefore, there was room to create a spectrophotometric approach and validate it for the combination of Montelukast Sodium and Bilastine. According to ICH requirements, various UV spectroscopic techniques were developed and verified. Methods include the first-order derivative method, the area under the curve method, and the absorbance ratio method.

1.1 INTRODUCTION TO ALLERGIC RHINITIS

Allergic rhinitis (AR) is a chronic inflammatory disease. AR is an immunoglobulin E−mediated inflammatory reaction in the nasal mucosa caused by inhaled allergens, such as pollen, mold, or animal dander. Allergen exposure leads to the allergens cross-linking with immunoglobulin E antibodies bound to mucosal mast cells and subsequent release of inflammatory mediators, such as histamine, prostaglandins, and leukotrienes. This mediators then develops AR symptoms like

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sneezing, nasal pruritus (itching), upper airway obstruction (congestion or blockage), rhinorrhea (clear nasal discharge), and itchy or watery eyes.

- Allergic Rhinitis is part of a systemic inflammatory process and is associated with other inflammatory disorders, including asthma, rhinosinusitis, and allergic conjunctivitis.
- Asthma is a disease that affects your lungs. It is one of the most common longterm diseases of children, but adults can have asthma, too. Asthma causes wheezing, breathlessness, chest tightness, and coughing at night or early in the morning. [7, 8]

1.2 DRUG PROFILE OF BILASTINE

An innovative new generation antihistamine with a quick onset and protracted duration of action, bilastine is highly selective for the H1 histamine receptor. Bilastine is an antiallergenic that works to lessen allergy symptoms like urticaria and nasal congestion. Typically, tablets are utilized. It has the chemical name 2-[4-[2-[4-[1-(2-ethoxyethyl)benzimidazol-2-yl]piperidin-1-yl]ethyl]phenyl] and is a white crystalline powder.acid 2-methylpropanoic. [9, 10]
For quality control and stability testing of Itraconaz ole in pharmaceutical formulations, limited methods have been published, because the drug is not yet o For quality control and stability testing of Itraconaz ole in pharmaceutical formulations, limited
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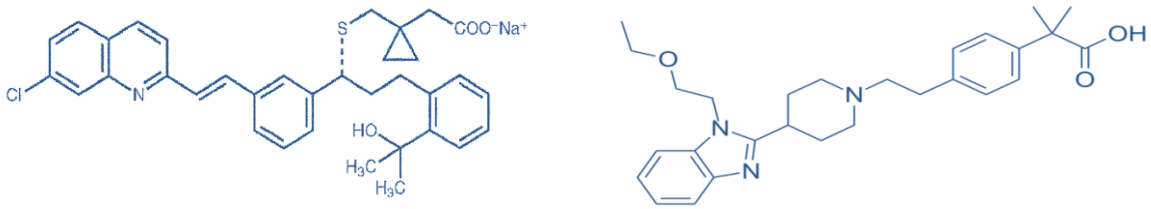
For quality control and stability testing of Bilastine in pharmaceutical formulations, limited method have been published, because the drug is not official in any pharmacopoeia.

1.3 DRUG PROFILE OF MONTELUKAST SODIUM

Montelukast Sodium is the monosodium salt of montelukast, an orally accessible selective cysteinyl leukotriene receptor antagonist having anti-inflammatory and bronchodilating properties. It's a powder that ranges from white to pale yellow. The chemical name of montelukast sodium is sodium 2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanylmethyl]cyclopropyl]acetate. Montelukast Sodium is used to control and prevent symptoms caused by asthma (such as wheezing and shortness of breath) and in allergic rhinitis. [11, 12]



A **B**

Fig. 1 Chemical structure – (A) Montelukast sodium (B) Bilastine

II. EXPERIMENTAL CONDITIONS:

2.1 REAGENTS AND MATERALS:

- Bilastine and Montelukast sodium was received as gift sample from Cipla Pharmaceuticals.
- Methanol (UV Grade – Thomas Baker, Mumbai)
- Marketed formulations (BILASURE-M) was procured from local market.

2.2 INSTRUMENT:

- Double beam UV- Visible spectrophotometer (Shimadzu-1800, Software –UV Probe, Version 2.42) having matched quartz cells of light path 1 cm.
- Electronic analytical balance (REPTECH)
- Ultrasonicator (Athena Technology)
- Volumetric flask – 10, 25,50 ml (Borosil)
- Pipettes- 1, 5, 10 ml (Borosil)

2.3 SELECTION OF APPROPRIATE SOLVENT

Common solvent for both drugs was found to be methanol as per solubility study. Therefore methanol was selected as solvent for UV methods. In Methanol both drugs Bilastine and Montelukast Sodium gives linear spectra at their measured wavelength. So Methanol is the preferred solvent.

2.4 PREPARATION OF STANDARD

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SOLUTION

2.4.1 Preparation of BIL and MON standard stock solution (1000 µg/ml):

10 mg of BIL and 10 mg of MON was weighed and transferred to two separate 10 ml volumetric flasks. It was then dissolved in methanol and the volume was made up to the mark with the methanol to give solution containing 1000 µg/ml for BIL and 1000 µg/ml for MON.

2.4.2 Preparation of BIL and MON working stock solution (100 µg/ml):

Aliquot of 2.5 ml from above standard stock solutions was pipetted out into 25 ml of volumetric flask each for BIL and MON and volume was made upto the mark with methanol to give a solution containing /100 µg/ml for BIL and 100 µg/ml MON.

2.4.3 Preparation of sample solution:

5 ml of Sample (Marketed Formulation) was taken into 100 ml of volumetric flask. Methanol was added and sonicated for 2-3 mins and volume was made up to mark with methanol. Solution was filtered through Whatmann filter paper no. 42. Thus, resulting solution gave 5000 µg/ml of MON and 1000 µg/ml of BIL. From the above solution, 2.5 ml was pipette out and transferred to 50 ml volumetric flask and volume was made upto mark with methanol in order to give a solution containing MON (250 µg/ml) + BIL (500 µg/ml). From the above solution, 1.0 ml was pipette out and transferred to 10 ml of volumetric flask and volume was made upto mark with methanol to give a solution containing MON (15 µg/ml) + BIL (30 µg/ml). This solution was used for assay i.e. estimation of MON and BIL in Marketed formulation.

III. METHODS

3.1 METHOD A: ABSORBANCE RATIO METHOD:

3.1.1 Selection of wavelength for Estimation of BIL and MON

The standard solution of BIL and MON was diluted with methanol individually to get the concentration of 10µg/ml and 5µg/ml of BIL and MON respectively. Both drugs were scanned in the UV range 400 - 200 nm. Two wavelengths **277 nm (λ max) for MON and 244.6 nm (Iso-absorptive point)** were selected. Overlain of spectra are represented in the figures below

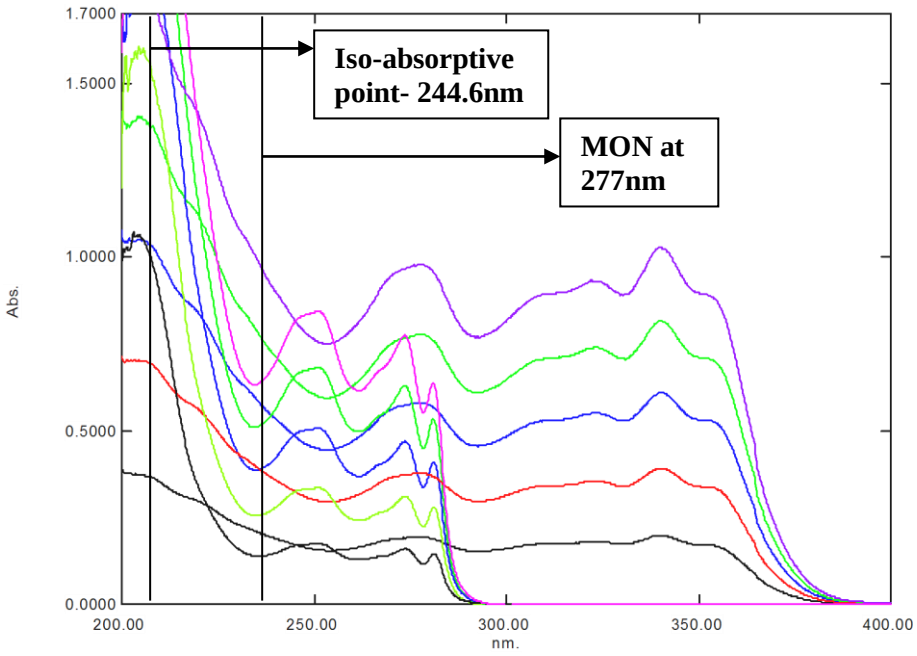

Fig. 2- Overlay spectra of BIL (10-50µg/ml) and MON (5-25µg/ml)

3.2 METHOD B: AREA UNDER CURVE METHOD:

The standard solution of BIL and MON was diluted with methanol individually to get the concentration of 10µg/ml and 5µg/ml of BIL and MON respectively. Both drugs were scanned in the UV range 400 - 200 nm. Area was selected for BIL is 244-257 nm and for MON it is 268-282nm.

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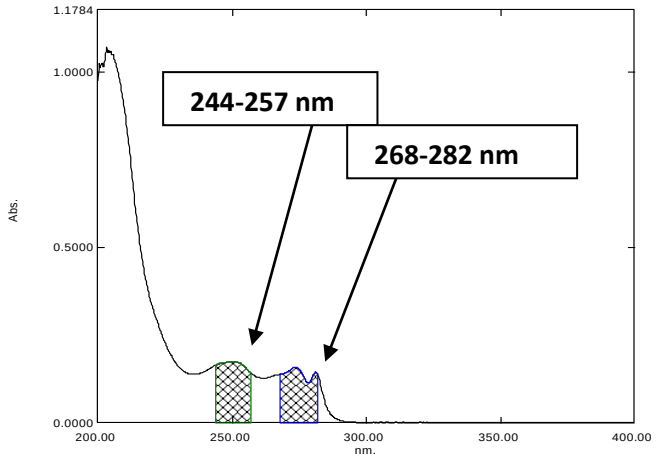

Fig. 3 AUC Spectra of Bilastine 10µg/ml in wavelength range 244-257 nm, 268-282 nm.

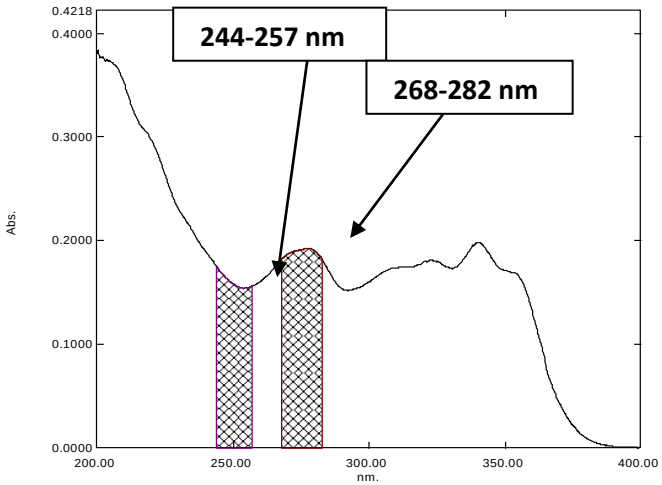

Fig. 4 AUC Spectra of Montelukast Sodium 5µg/ml in wavelength range 244-257 nm, 268-282nm

3.3 METHOD C: FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

To determine wavelength for estimation, standard spectra of BIL and MON were scanned from 200 nm – 400 nm against methanol. Zero Crossing Points were obtained at 261.60 nm and 277.60 nm for estimation of BIL and MON respectively since adequate absorbance is produced at these wavelengths.

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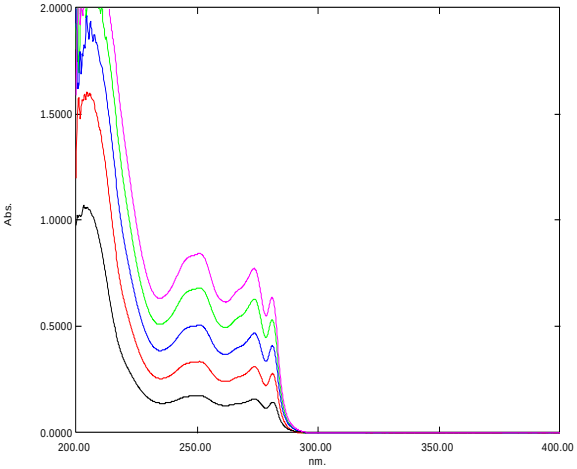

Fig. 5 Spectra of BIL (10-50 µg/ml) at 251 nm

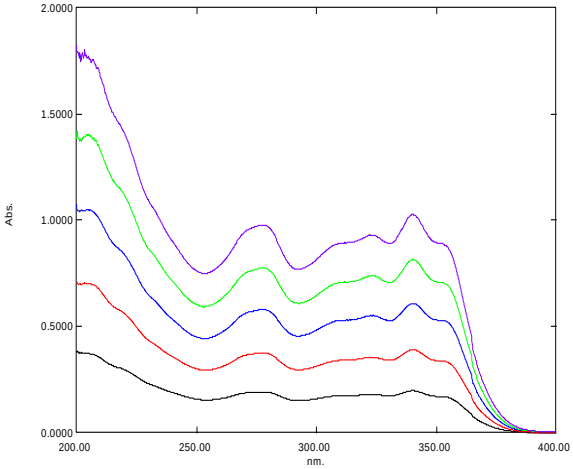

Fig. 6 Spectra of MON (5-25 µg/ml) at 277 nm

IV. VALIDATION PARAMETERS OF DEVELOPED METHOD:

4.1 Linearity: The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 10-50µg/ml for BIL and 5-25µg/ml MON for (n=5). The calibration curve of dA/dλ absorbance vs. concentration was plotted and correlation coefficient and regression line equations for BIL and MON were calculated.

4.2 Precision

➢ **Repeatability(n=6)**

Aliquots of 1 ml of standard working solution of BIL (100 µg/ml) and 0.5 ml of standard working solution of MON (100 µg/ml) were taken into two separate sequences of 10 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 10µg/ml and 5µg/ml of BIL and MON. Solution were analyzed six times (n=6) and % R.S.D. was calculated.

➢ **Intraday and Interday Precision(n=3)**

Aliquots of 2, 3 and 4 ml of standard working solution of BIL (100 µg/ml) were taken into series of 10 ml volumetric flask. Aliquots of 1, 1.5 and 2 ml of standard working solution of MON (100 µg/ml) were taken into series of 10 ml volumetric flask. Using methanol, volume was made up to mark to give a solution containing 20, 30 and 40 µg/ml of BIL and 10, 15 and 20 µg/ml of MON. Solution were analyzed for three times (n=3) on the same day within short interval of time and % R.S.D. was calculated.

4.3 Recovery study

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to pre analysed sample solution at three different concentration level (80%, 100% and 120%) within the range of linearity for both the drugs. The basic concentration of the drugs standard solution was 10 µg/ml of Montelukast Sodium and 20 µg/ml of Bilastine for all two methods.

4.4 LOD and LOQ

The **LOD (Limit of Detection)** was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula; LOD = 3.3 × S.D./Slope

The **LOQ (Limit of Quantitation)** was estimated from the set of 5calibration curves that were used to determine linearity of the method. The LOQ was calculated by using the formula: LOQ = 10 × S.D./Slope

Where, S.D. = Standard deviation of the Y – intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

V. RESULTS AND DISCUSSION:

Because most active chemicals show absorbance in the UV area, spectrophotometric procedures are the most simple, rapid, and applicable in all laboratories. Because no method for simultaneous analysis of the two medicines has previously been published, the new methods can be employed for routine analysis in their synthetic mixture. The linearity ranges for Montelukast Sodium are 5-25 g/ml and 10-50 g/ml for Bilastine.

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The proposed approach was validated in accordance with the ICH guidelines. The method's accuracy was determined by computing the mean percentage recovery at three levels: 80,100, and 120%. At their respective wavelengths, both medicines had good regression values.

5.1 METHOD A: ABSORBANCE RATIO METHOD

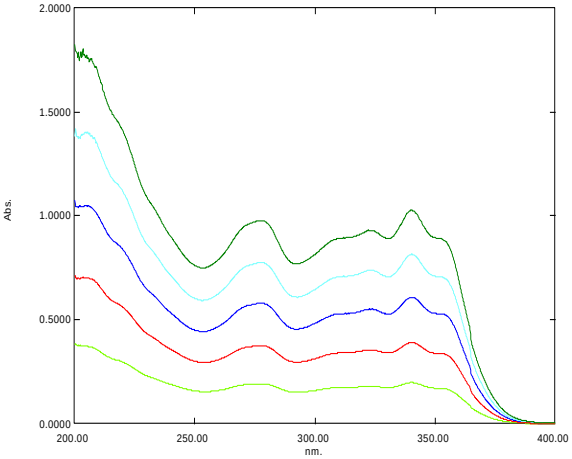

Fig. 7 Spectra of MON (5-25 µg/ml) at 277 nm

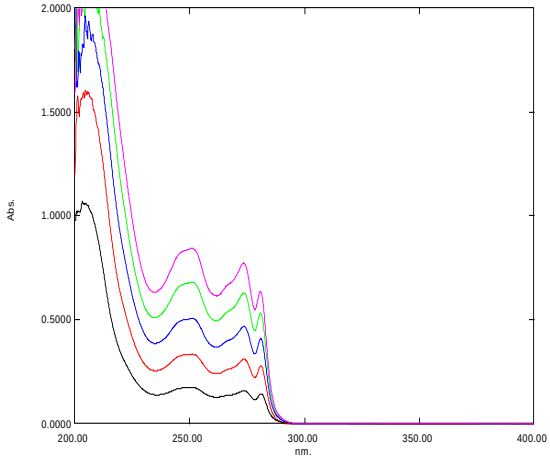

Fig. 8 Spectra of BIL (10-50 µg/ml) at 251 nm

5.1.1 METHOD VALIDATION:

1. Linearity:

Table No. 1 Linearity data for BIL at 244.6 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1.	10	0.15886 ± 0.0021	1.3917
2.	20	0.3254 ± 0.0045	1.4015
3.	30	0.4854 ± 0.0043	0.8864
4.	40	0.6576 ± 0.0053	0.8097
5.	50	0.8056 ± 0.0021	0.2632

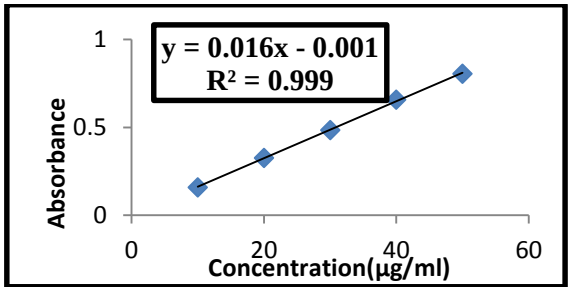

Fig. 9 Calibration curve for BIL at 244.6 nm

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Table No. 2 Linearity data for MON at 244.6 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1.	5	0.1384 ± 0.0213	1.2206
2.	10	0.3232 ± 0.0268	0.8322
3.	15	0.4834 ± 0.0091	0.8917
4.	20	0.6522 ± 0.0011	0.1796
5.	25	0.8222 ± 0.0048	0.1804

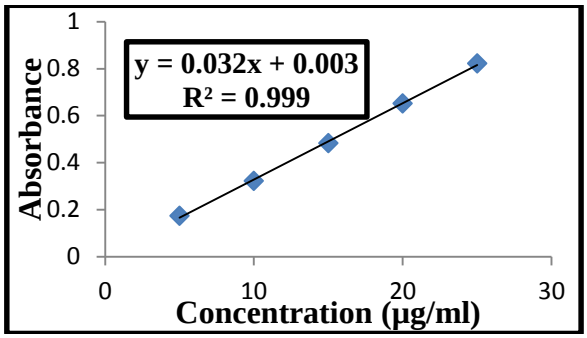

Fig. 10 Calibration curve for MON at 244.6 nm

2. Precision

a. Repeatability: The data for repeatability for BIL and MON at 244.6 nm and 277 nm.

Table No. 3 Repeatability data of BIL and MON

Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
BIL (244.6 nm)	30	0.4873 ± 0.0137	0.2835
MON (244.6 nm)	15	0.4733 ± 0.0137	0.2035

Table No. 4 Repeatability data of BIL and MON

Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
BIL (277 nm)	30	0.3723 ± 0.0015	0.0066
MON (277 nm)	15	0.5966 ± 0.0011	0.0172

b. Intra day Precision: The data for intraday precision for BIL and MON at 244.6 nm and 277 nm.

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Table No. 5 Intraday precision data of BIL at 244.6 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	20	0.3233 ± 0.0015	0.4739
2	30	0.4833 ± 0.0020	0.4715
3	40	0.6516 ± 0.0057	0.8874

Table No. 6 Intraday precision data of MON at 244.6 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	10	0.3227 ± 0.0020	0.6514
2	15	0.4833 ± 0.0037	0.7007
3	20	0.6133 ± 0.0015	0.1728

Table No. 7 Intraday precision data of BIL at 277 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	20	0.2533 ± 0.0015	0.0226
2	30	0.3702 ± 0.0001	0.0701
3	40	0.4953 ± 0.0015	0.0088

Table No. 8 Intraday precision data of MON at 277 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	10	0.3763 ± 0.0002	0.0315
2	15	0.5937 ± 0.0002	0.0993
3	20	0.7737 ± 0.0015	0.0197

c. Interday precision: The data for interday precision for MON and BIL at 244.6 nm and 277 nm.

Table No. 9 Interday precision data of BIL at 244.6 nm

Sr. No.	Concentration(µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	20	0.3467 ± 0.0050	1.4394
2	30	0.4899 ± 0.0583	1.1909
3	40	0.6733 ± 0.0080	1.2003

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Table No. 10 Interday precision data of MON at 244.6 nm

Sr. No.	Concentration(µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	10	0.3271 ± 0.0346	1.0536
2	15	0.4961± 0.0436	0.8881
3	20	0.6593 ± 0.0015	0.3168

Table No. 11 Interday precision data of BIL at 277 nm

Sr. No.	Concentration(µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	20	0.2463 ± 0.0002	0.8120
2	30	0.37057 ± 0.0030	0.0244
3	40	0.496 ± 0.00264	0.0534

Table No. 12 Interday precision data of MON at 277 nm

Sr. No.	Concentration(µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	10	0.3768 ± 0.0006	0.0022
2	15	0.5767 ± 0.0021	0.3591
3	20	0.7758 ± 0.0036	0.648

3. Accuracy:

Table No. 13 Determination of Accuracy of BIL and MON (244.6 nm)

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total Amount (µg/ml)	Amount of sample found (µg/ml)	% Recovey
BIL	0%	20	-	20	19.6	98%
	80%	20	16	36	35.95	99.86%
	100%	20	20	40	40.76	100.15%
	120%	20	24	44	44.51	100.90%
MON	0%	10	-	10	9.8	98%
	80%	10	8	18	17.77	98.72%
	100%	10	10	20	20.16	100.80%
	120%	10	12	22	22.41	100.86%

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4. Analysis of Marketed formulation (sample: Tablets):

Table No. 14 Determination of Assay of BIL and MON

	Actual Concentration (µg/ml)		Amount obtained Mean ± S.D. (µg/ml)		%BIL ± S.D.(n=5)	%MON ± S.D. (n=5)
	BIL	MON	BIL	MON		
BILASURE M TABLETS	30	15	30.15	14.89	100.5 ± 0.1580	99.26 ± 0.1324

5.2 METHOD B: AREA UNDER CURVE METHOD:

5.2.1 METHOD VALIDATION:

1. Linearity: The linearity range for BIL and MON was found to be in the range of 10-50 µg/ml and 5-25µg/ml.

Table No. 15 Linearity data for BIL at 244-257 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	10	2.0076 ± 0.0334	0.0834
2.	20	4.1238 ± 0.0782	0.9706
3.	30	6.041 ± 0.04519	0.7812
4.	40	8.4418 ± 0.0474	0.5154
5.	50	10.5062 ± 0.0115	0.1943

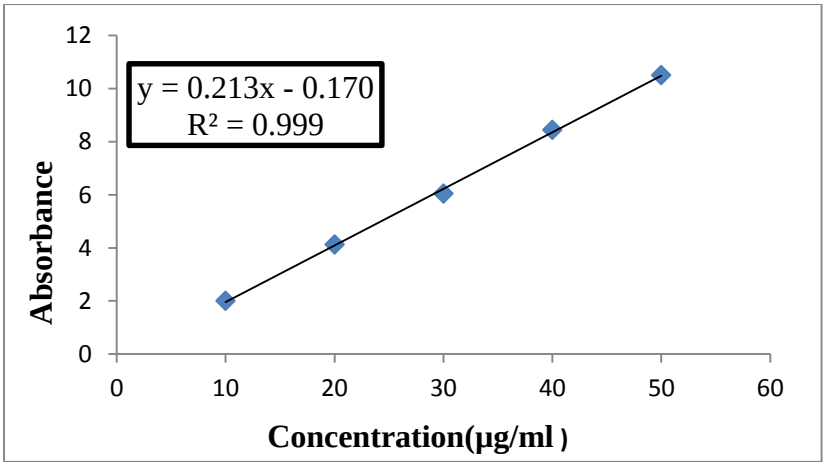

Fig. 11 Calibration curve for BIL at 244- 257 nm

Table No. 16 Linearity data for MON at 244-257 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	5	2.0872 ± 0.0311	0.1921
2.	10	3.9402 ± 0.0621	0.1900
3.	15	5.9578 ± 0.0129	0.5523

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4.	**20**	7.8951 ± 0.0712	0.4018
5.	**25**	10.0322 ± 0.0239	0.0379

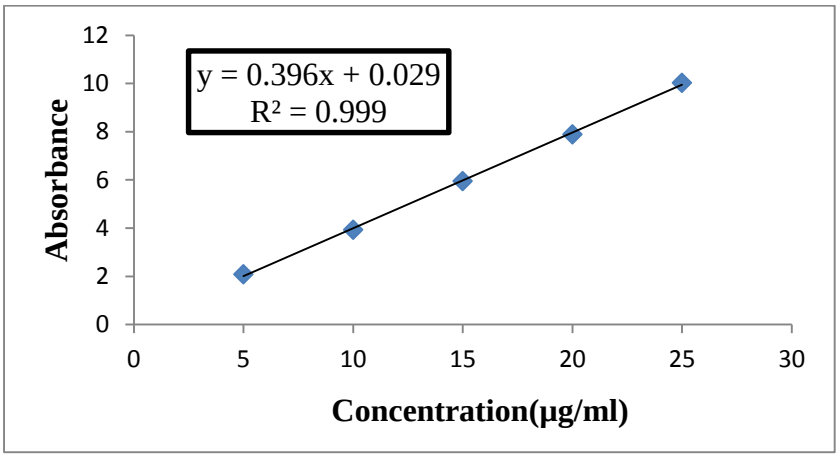

Fig. 12 Calibration curve for MON at 244- 257 nm

Table No. 17 Linearity data for BIL at 268-282 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	**10**	1.9864 ± 0.0896	0.4112
2.	**20**	3.8616 ± 0.0794	0.8245
3.	**30**	5.7584 ± 0.0999	0.3723
4.	**40**	7.6442 ± 0.0703	0.6529
5.	**50**	9.31342 ± 0.0658	0.7056

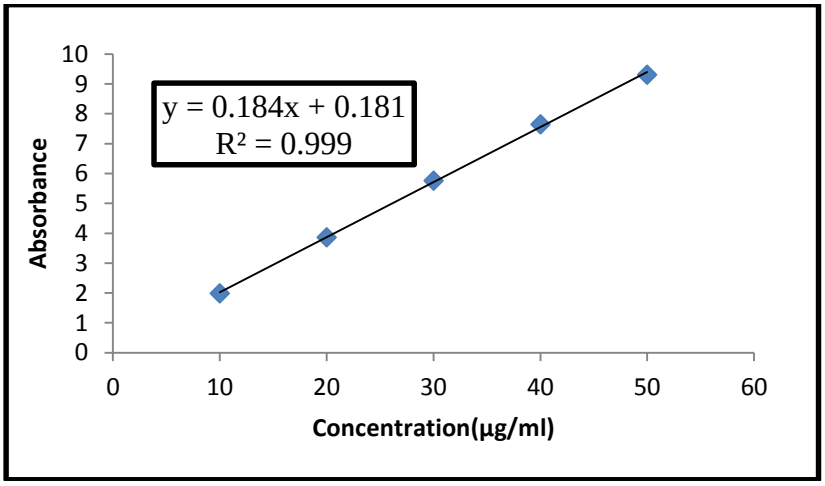

Fig. 13 Calibration curve for BIL at 268- 282 nm

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Table No. 18 Linearity data for MON at 268- 282nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	5	2.0811 ± 0.2775	0.3344
2.	10	5.0127 ± 0.0126	0.2461
3.	15	7.9826 ± ±0.0699	0.0751
4.	20	10.7972 ± 0.1342	1.6887
5.	25	13.1915 ± 0.5377	0.4758

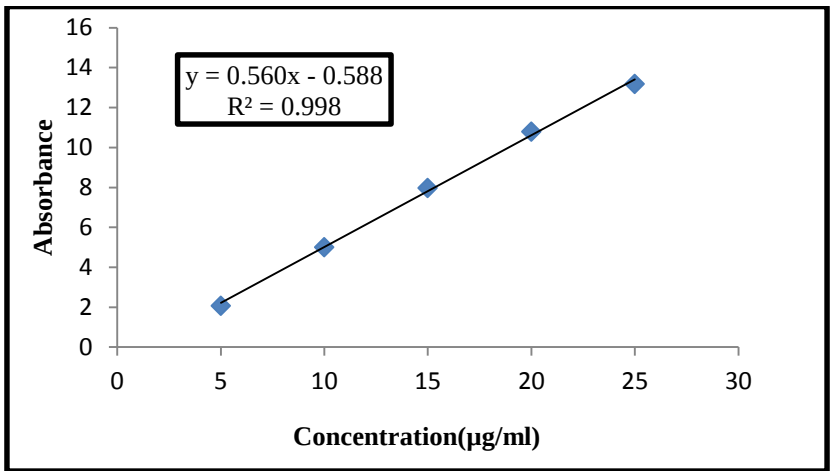

Fig. 14 Calibration curve for MON at 268- 282nm

2. Precision

a. Repeatability: The data for repeatability for BIL and MON at 244- 257 nm and 268 -282 nm

Table No. 19 Repeatability data of BIL and MON

Drugs	Concentration (µg/ml)	Mean AUC ± S.D. (n=6)	%R.S.D.
BIL (244-257 nm)	30	6.0307 ± 0.3426	0.5829
MON (268-272 nm)	15	7.9083 ± 0.0421	0.6827

b. Intraday precision: The data for intraday precision for BIL and MON at 244- 257 nm and 268 -282nm.

Table No. 20 Intraday precision data of BIL at 244-257 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1.	20	4.1031± 0.0036	0.0888
2.	30	6.0081 ± 0.0011	0.0164
3.	40	8.4836 ± 0.0101	0.1204

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Table No. 6.36 Intraday precision data of MON at 268-282 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1.	**10**	5.0161± 0.0129	0.2565
2.	**15**	7.9051± 0.0866	1.0956
3.	**20**	10.8221 ± 0.1473	1.3612

c. Interday precision:

Table No. 21 Interday precision data of BIL at 244-257 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1.	**20**	4.1111 ± 0.0094	0.2295
2.	**30**	6.0387 ± 0.0513	0.8501
3.	**40**	8.5211 ± 0.0546	0.6407

Table No. 22 Interday precision data of MON at 268-282 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1.	**10**	5.0437 ± 0.0337	0.6667
2.	**15**	7.5977 ± 0.1117	1.4706
3.	**20**	10.785 ± 0.1531	1.4194

3. Accuracy:

Table No. 23 Determination of Accuracy of BIL (244-257 nm)

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total Amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
BIL	0%	20	-	20	19.98	99.90%
	80%	20	16	36	35.59	98.86%
	100%	20	20	40	40.31	100.77%
	120%	20	24	44	44.54	101.22%

Table No. 24 Determination of Accuracy of MON (268-282 nm)

Drugs	Level	Amount of sample (µg/ml)	Amountof std. spiked (µg/ml)	Total Amount (µg/ml)	Amoumt of samplefound (µg/ml)	% Recovery
MON	0%	10	-	10	9.98	99.80%
	80%	10	8	18	17.87	99.27%
	100%	10	10	20	19.95	99.75%
	120%	10	12	22	22.09	100.40%

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- **Analysis of Marketed formulation (sample: Tablets):**

Table No. 25 Determination of Assay of BIL and MON

	Actual Concentration (µg/ml)		Amount obtained Mean ± S.D. (µg/ml)		%BIL ± S.D. (n=5)	%MON ± S.D. (n=5)
	BIL	MON	BIL	MON		
BILASURE M TABLETS	30	15	30.11 ± 0.0823	14.99 ± 0.0433	100.36 ± 0.4451	99.93± 0.2201

5.3 METHOD C: FIRST ORDER DERIVATIVE METHOD

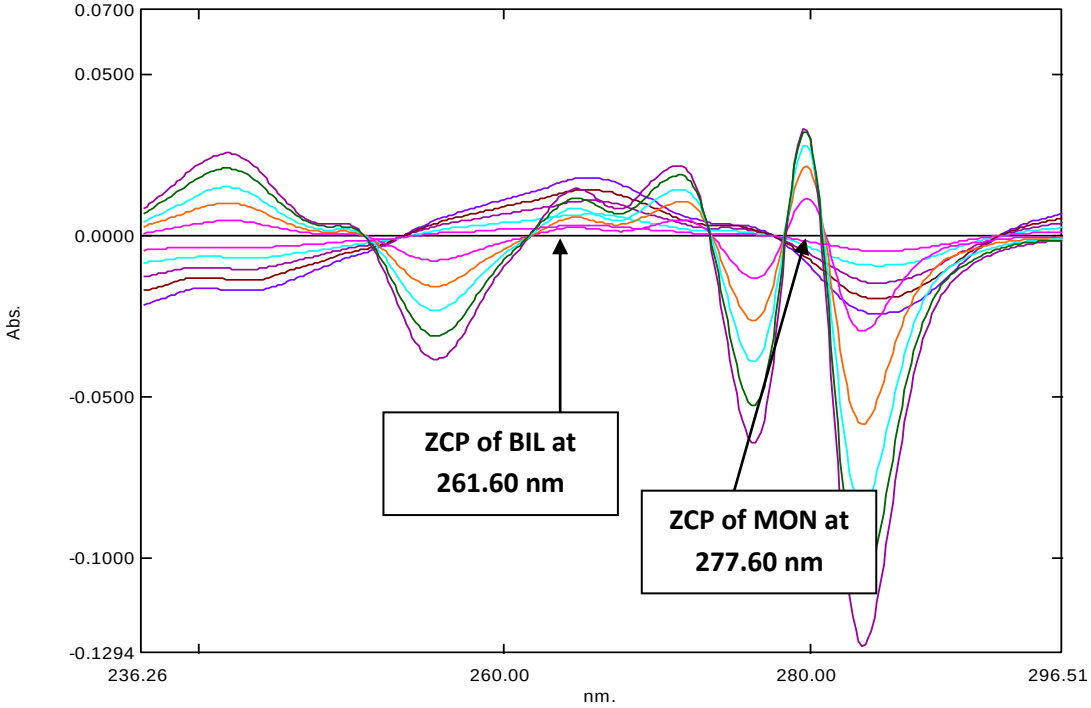

Fig. 15 Overlay First Order Spectra of BIL and MON

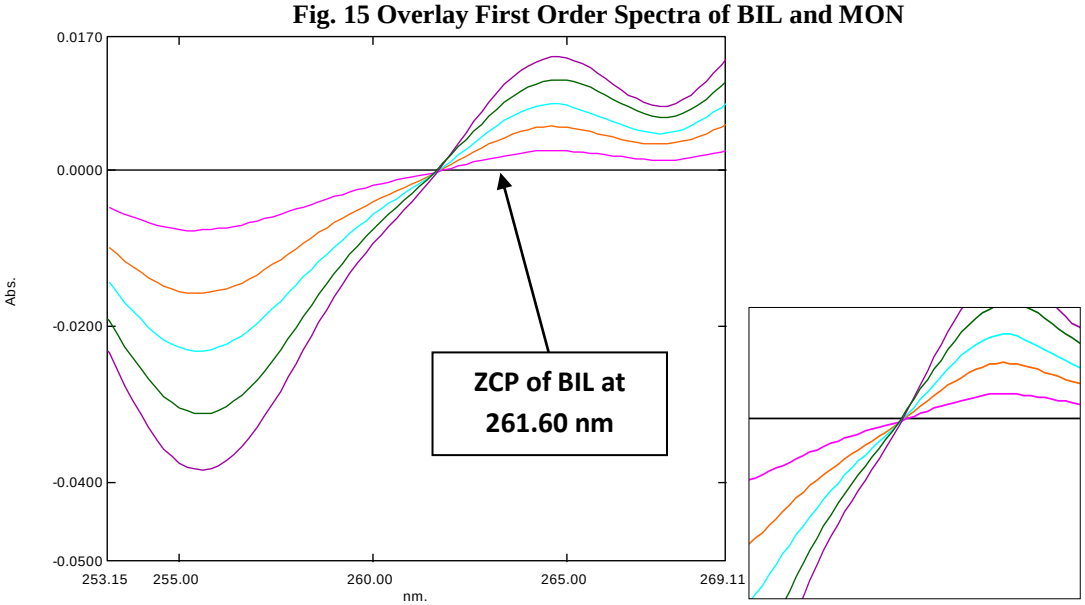

Fig. 16 First Order Overlay spectra of BIL (10-50 µg/ml)

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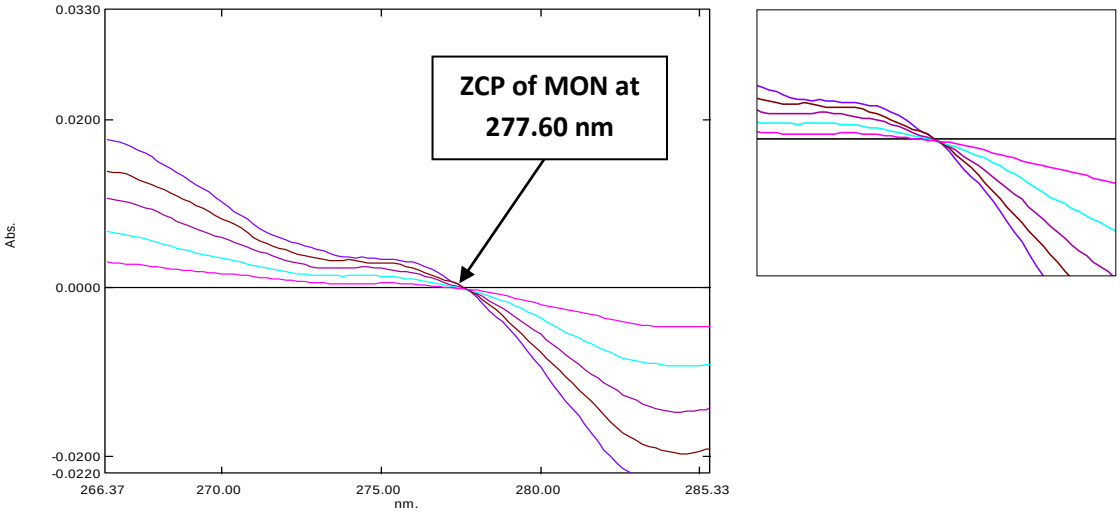

Fig. 17 First Order Overlay spectra of MON (5-25 µg/ml)

5.3.1 METHOD VALIDATION:

1. Linearity

The linearity range for BIL and MON was found to be in the range of 10-50 µg/ml and 5-25 µg/ml. Linearity data for BIL at **277.60 nm** and for MON at **261.60** nm are depicted in table below.

Table No. 26 Linearity data for BIL at 277.60 nm (ZCP of MON)

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1.	**10**	0.0084 ± 0.0017	0.3733
2.	**20**	0.0165 ± 0.0022	0.7427
3.	**30**	0.0234 ± 0.0073	0.7419
4.	**40**	0.0324 ± 0.0014	0.3575
5.	**50**	0.0396 ± 0.0041	0.6187

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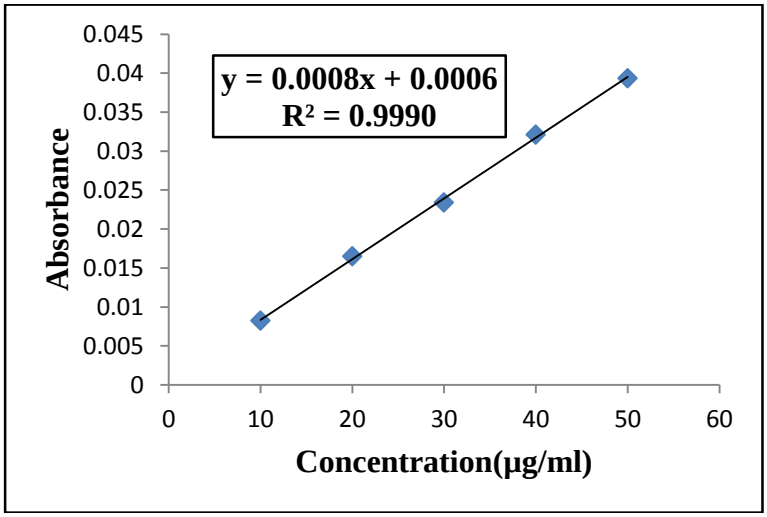

Fig. 18 Calibration curve for BIL at 277.60 nm

Table No. 27 Linearity data for MON at 261.60 nm (ZCP of BIL)

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1.	5	0.0022 ± 0.0001	0.4141
2.	10	0.0047 ± 0.0070	0.7988
3.	15	0.0078 ± 0.0054	0.6778
4.	20	0.0056 ± 0.0003	0.3167
5.	25	0.0132 ± 0.0075	0.7112

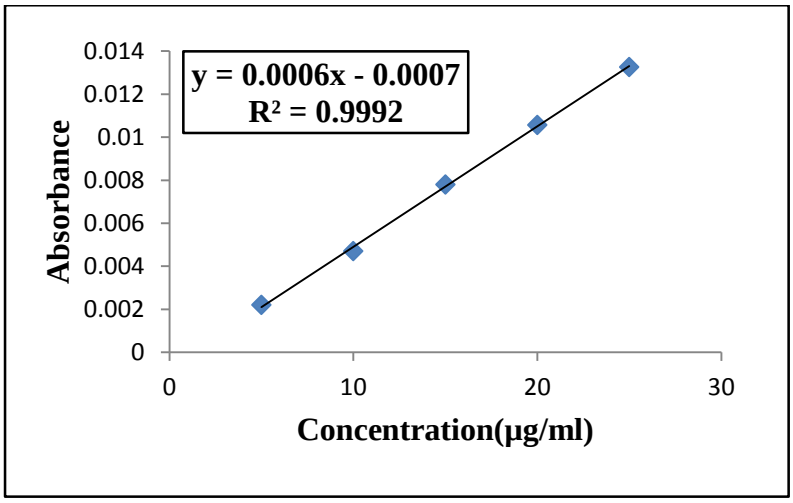

Fig. 19 Calibration curve for MON at 261.60 nm

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2. Precision

a. Repeatability: The data for repeatability for BIL and MON at 277.60 nm and 261.60 nm.

Table No. 28 Repeatability data of BIL and MON

Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
BIL	30	0.0233 ± 0.0074	0.3838
MON	15	0.0066 ± 0.0029	0.4471

b. Intraday Precision: The data for intraday precision for BIL and MON at 277.60 nm and 261.60 nm.

Table No. 29 Intraday precision data of BIL at 277.60 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	20	0.0044 ± 0.0004	0.0146
2	30	0.0083 ± 0.0710	0.0383
3	40	0.0323 ± 0.0053	0.0653

Table No. 30 Intraday precision data of MON at 261.60 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	10	0.0046 ± 0.0011	0.0014
2	15	0.0076 ± 0.0015	0.0033
3	20	0.0105 ± 0.0023	0.0053

c. Interday Precision: The data for interday precision for BIL and MON at 277.60 nm and 261.60 nm.

Table No. 31 Intraday precision data of BIL at 277.60 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	20	0.0163 ± 0.0031	1.4223
2	30	0.0233 ± 0.0011	0.4218
3	40	0.0323 ± 0.0073	0.5624

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Table No. 32 Interday precision data of MON at 261.60 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
1	10	0.0053 ± 0.0030	1.6246
2	15	0.0086 ± 0.0025	1.7156
3	20	0.120 ± 0.0099	1.9904

3. Accuracy: Accuracy of the proposed method was assured by performing recovery study from Marketed Formulation at three levels by standard addition method. Percentage recovery for BIL and MON at 277.60 nm and 261.60 nm was obtained respectively. The results are depicted in table no. 6.23 and 6.24. Recovery was found be in the limit of 98-102 %.

Table No. 33 Determination of Accuracy of BIL and MON at 277.60nm and 261.60nm

Drugs	Level	Amount of sample (µg/ml)	Amountof std. spiked (µg/ml)	Total Amount (µg/ml)	Amount of samplefound (µg/ml)	% Recovery
BIL	0%	20	-	20	19.91	99.55%
	80%	20	16	36	35.28	98%
	100%	20	20	40	39.66	99.15%
	120%	20	24	44	44.78	101.77%
MON	0%	10	-	10	9.86	98.60%
	80%	10	8	18	17.71	98.38%
	100%	10	10	20	20.13	100.65%
	120%	10	12	22	22.43	101.95%

- **Analysis of Marketed formulation (sample: Tablets):** Suitability of the method was tested by analyzing the Marketed Formulation. The results are depicted in table no. 34

Table No. 34 Determination of Assay of BIL and MON

BILASURE M TABLETS	Actual Concentration (µg/ml)		Amount obtained Mean ± S.D. (µg/ml)		%BIL ± S.D.(n=5)	%MON ± S.D.(n=5)
	BIL	MON	BIL	MON		
	30	15	29.99 ± 0.0224	15.01 ± 0.0431	99.96 ± 0.0552	100.06 ± 0.00541

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5. SUMMARY OF VALIDATION PARAMETERS:

Table No. 35 Summary of Absorbance ratio Method

Parameters	BIL	MON
Wavelength (nm)	277 nm	277 nm
Linearity (µg/ml) (n=5)	10-50µg/ml	5-25µg/ml
Regression Equation(y = mx + c)	y = 0.012x - 0.005	y = 0.039x + 0.010
Regression coefficient (R^2)	0.999	0.999
Correlation Coefficient (r)	0.9995	0.9995
Repeatability (%R.S.D.) (n=6)	0.0066	0.0172
Intraday precision(%R.S.D) (n=3)	0.0226 – 0.0088	0.0315 – 0.0197
Interday precision (%R.S.D.) (n=3)	0.8120 – 0.0534	0.0022 – 0.0648
LOD (µg/ml) (n=5)	0.1422 µg/ml	0.1831µg/ml
LOQ (µg/ml) (n=5)	0.3846 µg/ml	0.5549 µg/ml
% Recovery (n=3)	98 – 100.9	98 – 101.86
Assay (%) ± S.D. (n=5)	100.5 ± 0.1580	99.26 ± 0.1324

Table No. 36 SUMMARY OF AREA UNDER CURVE METHOD

Parameters	BIL	MON
Wavelength (nm)	244 – 257 nm	268 – 282 nm
Linearity (µg/ml) (n=5)	10-50µg/ml	5-25µg/ml
RegressionEquation(y = mx + c)	y = 0.2132x - 0.1705	y = 0.5601x - 0.5886

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Regression coefficient (R^2)	0.999	0.998
Correlation Coefficient (r)	0.9994	0.9989
Repeatability (%R.S.D.) (n=6)	0.5829	0.6827
Intraday precision(%R.S.D) (n=3)	0.0888 – 0.1204	0.2565 – 1.3612
Interday precision (%R.S.D.) (n=3)	0.2295 – 0.6407	0.6667 – 1.4194
LOD (µg/ml) (n=5)	0.4269	0.1393
LOQ (µg/ml) (n=5)	0.6939	0.4228
% Recovery (n=3)	99.90 – 101.22%	99.80 – 100.40%
Assay (%) ± S.D. (n=5)	100.36 ± 0.4451	99.93± 0.2201

Table No. 37 SUMMARY OF FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

Parameters	BIL	MON
Wavelength (nm)	277.60 nm	261.60 nm
Linearity (µg/ml) (n=5)	10-50µg/ml	5-25µg/ml
Regression Equation(y = mx + c)	y = 0.0008x + 0.0006	y = 0.0006x - 0.0007
Regression coefficient (R^2)	0.9990	0.9992
Correlation Coefficient (r)	0.9990	0.9992
Repeatability (%R.S.D.) (n=6)	0.3838	0.4471

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Intraday precision(%R.S.D) (n=3)	0.60606 – 0.47537	0.41494 – 0.94138
Interday precision (%R.S.D.) (n=3)	1.4226 – 0.5362	0.4725 – 1.2192
LOD (µg/ml) (n=5)	0.2844µg/ml	0.3229 µg/ml
LOQ (µg/ml) (n=5)	0.3011 µg/ml	0.5866 µg/ml
% Recovery (n=3)	99.55 – 101.77	98.60 – 101.95
Assay (%) ± S.D. (n=5)	99.96 ± 0.0552	100.06 ± 0.00541

CONCLUSION:

The developed Absorption ratio method, Area under curve and First-order derivativemethod was found to be simple, precise and accurate & it was validated as per ICH Q2 (R1) guideline.

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REFERENCES:

[1]. Beckett AH., Stenlake JB. Practical Pharmaceutical Chemistry; 4th Edn; Part II, CBS Publisher and Distributors, New Delhi, 2002, pp 279-300.

[2]. Chatwal GR., Anand SH. Instrumental Methods of Chemical Analysis; 5th Edn; Himalaya Publishing House, New Delhi, 2002, pp 2.167-2.172.

[3]. Andressa Tassinari da Silva, Gabriela Rossi Brabo, Isadora Dias Marques a, Lisiane Bajerski, Marcelo Donadel Malesuik, Clésio Soldateli Paim, " UV Spectrophotometric method for quantitative determination of Bilastine using experimental design for robustness." Drug Anal Res. **2017**, 01, 38-43.

[4]. V. Amarendra Chowdary, Anusha Kota, Syed Muneer, "Method Development And Validation Of Rp-Hplc Method For the Estimation of Bilastine in Pharmaceutical Dosage Form." W. Pharmacy and Pharm. Sci. **2017,** 6(8), 2297-2315.

[5]. Radia Ouarezki, Saliha Guermouche, Moulay-Hassane Guermouche, "Degradation kinetics of Bilastine determined by RP-HPLC method and identifcation of its degradation product in oxidative condition." Slovak Academy of Sciences. **2019**.

[6]. Rambabu Katta, N. N. V. V. S. S. Narayana Murty, Ramasrinivas, G. N. Rao, "Stability Indicating Method Development and Validation for the Determination of Bilastine And its impurities by Uplc Method." IJPSR. **2020**, 11(3), 1312-1321.

[7]. Russell May J, William K. Dolen, "Management of Allergic Rhinitis: A Review for the Community Pharmacist." Clinical Therapeutics. **2017**, 1(1), 1-10.

[8]. "Allergic Rhinitis", December 2020. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2846743/

[9]. Drug profile, "Bilastine", May 2021. https://go.drugbank.com/drugs/DB11591

[10]. Drug profile, "Bilastine", May 2021.

[11]. https://pubchem.ncbi.nlm.nih.gov/compound/Bilastine

[12]. Drug profile, "Montelukast sodium", May 2021. https://pubchem.ncbi.nlm.nih.gov/compound/Montelukastsodium

[13]. Drug profile, "Montelukast sodium", May 2021. https://go.drugbank.com/drugs/DB00471