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ORIGINAL ARTICLE **OPEN ACCESS**

Stability Indicating RP-HPLC Method Development and Validation of Few Bulk Drug Combinations and their Formulation

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ABSTRACT
A sensitive, exact, rapid, avaricious and robust RP-HPLC method was developed for the quantification of Bilastine(BT) and Montelukast(ML) withDAD detector. In this method, a reversed-phase Agilent C18 (250mm x 4.6ID, Particle size: 5 micron) column with a mobile phase of Methanol: (0.1% OPA, PH 2.7) (90:10; v/v) at 0.9ml/min flow rate was used to separate BT and ML with a detection of 282 nm.The volume injected was 20 µL. The retention time of BT and ML was obtained as 2.218min and 7.562min respectively. All necessary validation parameters and system suitability tests were carried out in details. The analytical curve was linear (r^2 = 0.999) over a wide concentration range of BT and ML (20 - 100 µg/ml) and (10 -50µg/ml).The BT and ML were exposed to different stress condition like thermal, photolytic, hydrolytic, and oxidative stress conditions and samples were analyzed by proposed method. The stressed sample demonstrated the specificity of assay method in presence of degradant products with no interferences was observed from its stress degradation products. The system shows adequate accuracy with relative standard deviation less than 2.0%. The method showed good duplicability and recovery with % RSD less than 2%. So, the proposed system was found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for practice analysis of Bilastine(BT) and Montelukast(ML) in bulk drug.
Keywords: *RP-HPLC estimation, DAD, Validation, Bilastine, Montelukast*

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INTRODUCTION

Analytical chemistry termed as science of determining the components of materials in terms of the elements or compound contained. The approach of this science is used to recognize the substances which may be present in a material and to determine the exact amounts of the identified substances. Analytical chemistry is important in nearly all aspects of chemistry. Analytical techniques proved in assuring and maintaining the quality of substance and are critical components of QA and QC.

Analytical method should be,

1. Most productive, economical and convenient,
2. As accurate and precise as required,
3. As simple as possible,
4. Most specific

Should be fully optimized before transfer for validation of its characteristics such as precision, accuracy, sensitivity etc [1-2].

Bilastine is a new drug in the category of Antihistamines, used for the treatment of allergic reactions like nasal congestion and urticaria. Chemically, it is 2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl) piperidin-1-yl)ethyl)phenyl]-2- methylpropionic acid. It has a molecular formula of C28H37N3O3 and molecular mass of 463.61 g/mol. Solubility studies show that it is slightly soluble in water, acetonitrile and soluble in methanol. It is a second-generation histamine H1 receptor antagonist, acts by binding to the receptor, preventing its activation thereby reduces the development of allergic symptoms.

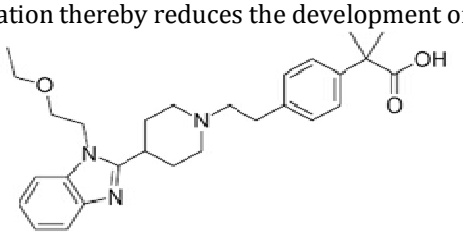

Figure 1: Chemical structure of Bilastine

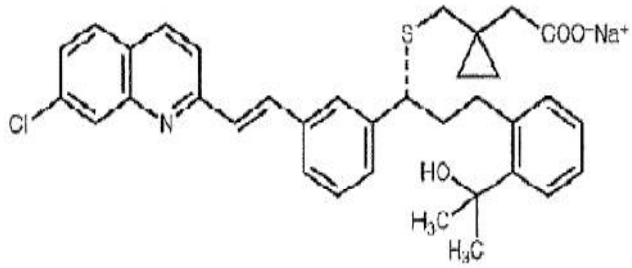

Figure 2: Chemical structure of Montelukast

Montelukast is a leukotriene receptor antagonist that demonstrates a marked affinity and selectivity to the cysteinyl leukotriene receptor type-1 in preference to many other crucial airway receptors like the prostanoid, cholinergic, or beta-adrenergic receptors [3, 4]. It is used as an alternative to anti – inflammatory medications in the management and chronic treatment of asthma and exercise-induced bronchospasm (EIB). Chemically Montelukast is 2-[1-[[(1R)-1- [3 - [(E) – 2 - (7-chloroquinolin–2–yl)ethenyl]phenyl]-3-[2-(2–hydroxypropan-2yl)phenyl]propyl]sulfanyl methyl] cyclopropyl] acetic acid. [5-7].

MATERIAL AND METHODS

Reagents and Chemicals:

Water, Methanol, Ortho-phosphoric acid,Sodium hydroxide, Hydrogen peroxide (H_2O_2), and Hydrochloric acid were used in the study.

Instrumentation:

Agilent (1100series) with Auto sampler and DAD detector with Chemstation software were used.

Chromatographic condition:

A High performance liquid chromatogram equipped with DAD detector, the purity determination performed on a Agilent C18 (250mm x 4.6ID, Particle size: 5 micron) at ambient temperature using mobile phase consisting of Methanol: 0.1% ortho phosphoric acid (90:10).

Preparation of standard solution BT and ML:

Weighed accurately about 20 mg of BT and 10 mg of MLstandard and transffered into 10mL of volumetric flask, added about 10 mL of diluent, shaked to dissolved and volume was made up to the mark with diluent. (concentration of BT and MLis 2000µg/ml and 1000 µg/ml)A-grade bulb pipette into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 20,40,60,80 and 100 µg/ml for BT 10,20,30,40, and 50 µg/ml for ML.

Preparation of Sample solution BT and ML:

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 20 mg of BT and 10 mg of ML was transferred into a 10.0 ml volumetric flask. Then 5.0 ml of diluent was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with diluent. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of the filtrate was transferred to a 10 ml volumetric flask and then the volume was made up to the mark with diluent to furnish a sample solution containing 80µg/ml of BT and 40 µg/mlof ML.

Method validation [8-11]

The developed method was validated following ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness.

Linearity:

Linearity is the ability of the analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical BT and ML transformation proportional to the concentration BT and ML of an analyte in samples within a given span at which the involved response is proportional to the analyte concentration.

Accuracy:

Accuracy is the nearness of a measured value to the true or accepted value. Accuracy designate the digression between the convey merit found and the true merit. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analyzed against the standard and blank solutions to ensure that no interference exists.

Precision:
The exactness of an logic method is the level of accord among single test results get when the method is pragmatic to many sampling of a similar sample. Precision is a measure of the dependability of the whole analytical process.

Limit of Detection (LOD):
The detection limit of an individual analyte procedure is the lowest amount of analyte which can be detected not necessarily quantified as an exact value. LOD was calculated using the following formula.

LOD = 3.3σ/S
Where σ is the standard deviation calculated from accuracy of the response and S is the slope from linearity

Limit of Quantification (LOQ)
The quantification limit of an individual analytical procedure is the lowest amount of analyte which can be quantitatively determined. LOQ was calculated using the following formula.

LOQ = 10.σ/S
Where σ is the standard deviation calculated from accuracy of the response and S is the slope from linearity.

System suitability parameter:
System suitability parameter is the evaluation of a composition of an analytical system to show that the performance of the system meets the standard required by the method. This parameter can be calculated experimentally to provide a quantities system suitability test report number of theoretical plates (efficacy) capacity factor, separation (relative retention), resolution, telling factor relative standard deviation (precision).

RESULTS AND DISCUSSION

Determination of λ max
UV absorption of 10 µg/mL solution of BTandMLin METHANOL was generated and absorbance was taken in the range of 200-400 nm.λmax ofBT and ML in Methanol was found to be 275nm and 281 nm respectively. This is essential since HPLC detection is basically UV based, thus a 10µg/mL solution of BT and ML in Methanol was used to get the following spectra.

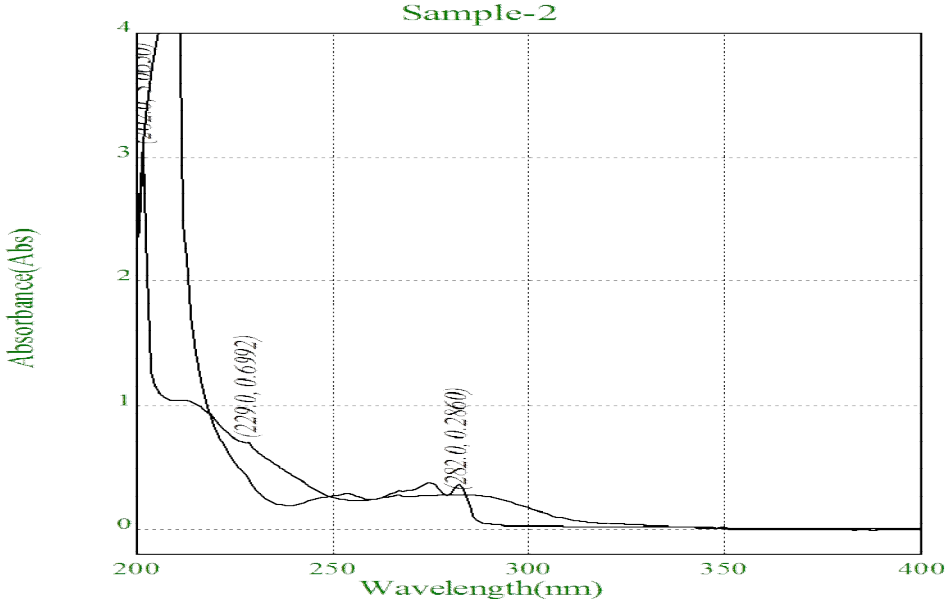

Figure 3: Iso-absorptive point of BT and ML

HPLC method development and optimization
Initially, pure drugs solution was chromategraphed using a mobile phase consisting of a mixture of 0.1%ortho-phosphoric acid in water (pH2.7) and Methanol in the ratio of (10:90) v/v at a flow rate of 0.9 ml/min. gives well- resolved peaks of drugs. Detection was carried out at 282 nm. The retention time under the optimized condition of Bilastine and Montelukast was found to be 2.218 min.&7.562 min. respectively. The total run time of the chromatogram was about 15 minutes. A typical chromatograph of a mixture of standard and sample BT and ML is summarized by Fig. 4 and Fig. 5 respectively.

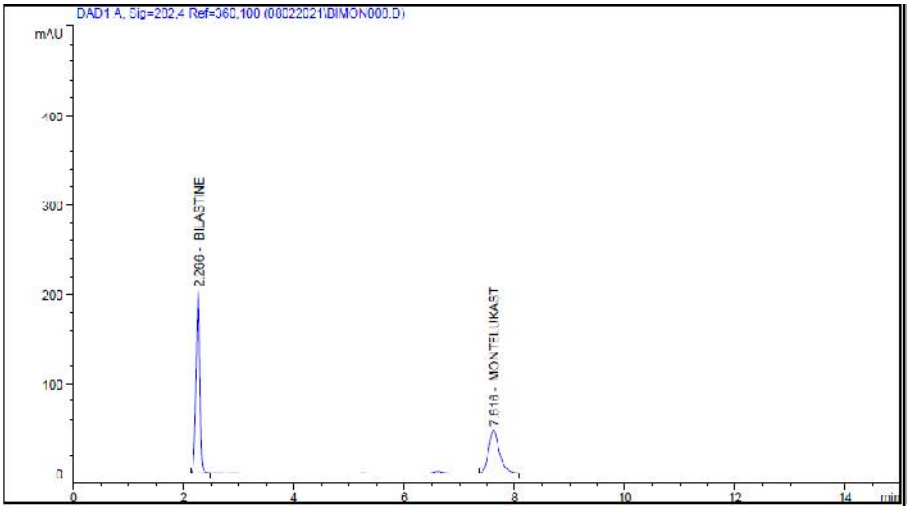

Figure 4: Chromatograph of mixture of standard BT and ML

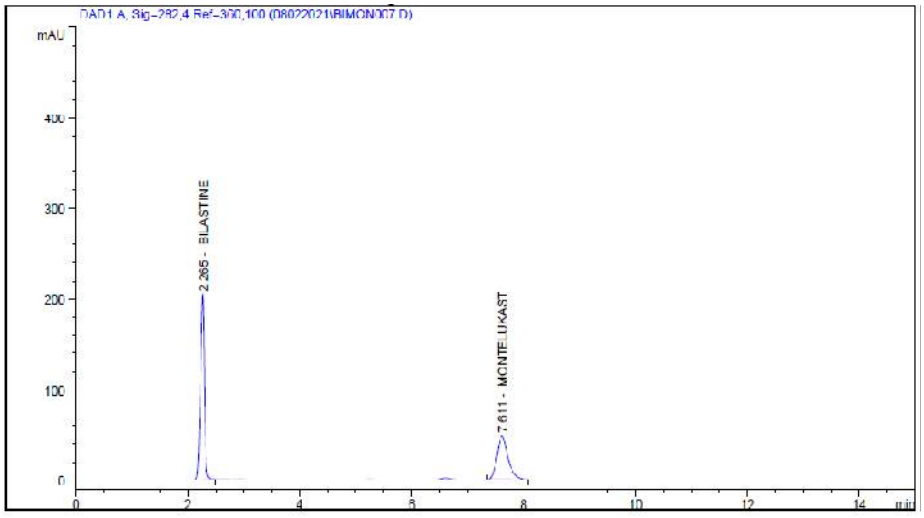

Figure 5: Chromatograph of mixture of sample BT and ML

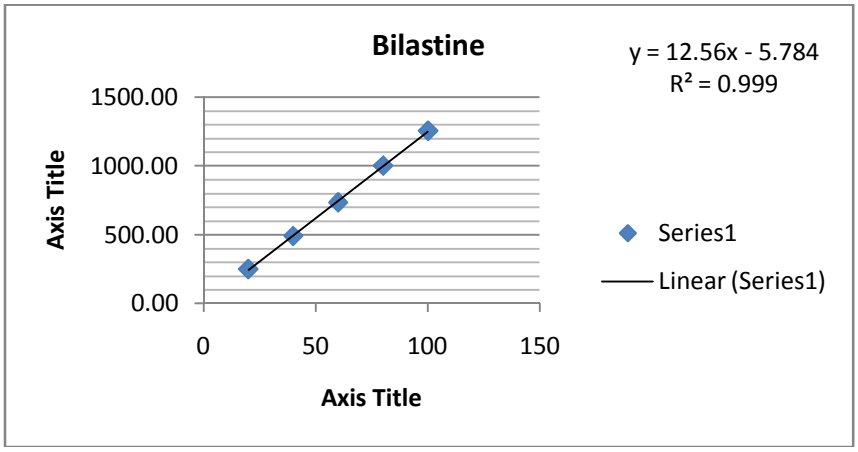

Figure 6: Linearity curve of BT

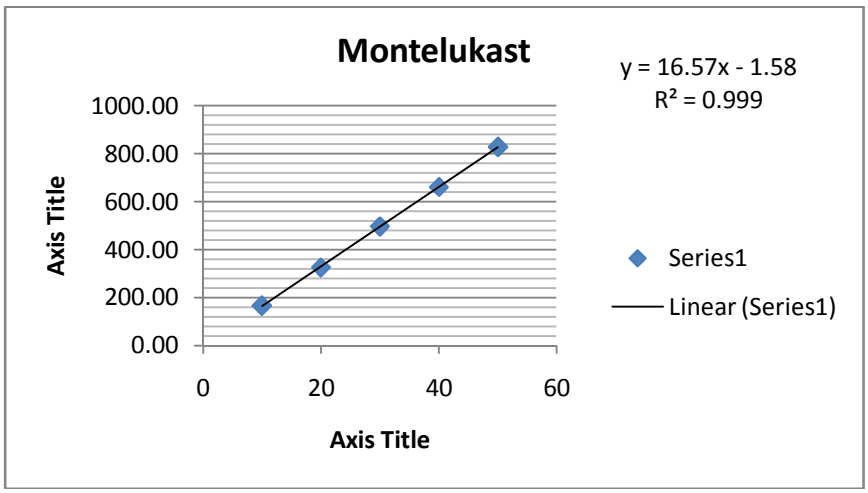

Figure 7: Linearity curve of ML

Validation of the method

System Suitability:

The suitability of the system was demonstrated by assessing various parameters. It was established by injecting two replicate injections of the standard solution. Theoretical plates were found to be 4009 and 6935, tailing factor of 0.73 and 0.75, and %RSD of peak area was 0.61 and 0.66 for both BT and ML respectively (Table 1). All the system suitability parameters were well within the limits, indicating that the system was well suitable for performing the analysis.

Table 1: System suitability results

Parameter	BT	ML
Theoretical Plate	5827	7866
Retention Time (Rt)	2.255	7.620
Tailing factor	1.14	0.76
% RSD	0.50	0.01

Rt: Retention time, %RSD: Percentage relative standard deviation

Linearity

Linearity was established by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range of 20-100 µg/ml for BT and 10-50 µg/ml for ML. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of BT and ML was shown in Fig. 4 and Fig.5respectively. The linear regression equation obtained was Y=12.568x+5.784 for BT and Y=16.572x+1.58 for ML with correlation coefficient 0.999and 0.999 respectively. The results of linearity are shown in Table 2.

Table 2: Linearity results of BT and ML

Parameter	BT	ML
Theoretical Plate	6067	7924
Retention Time (Rt)	2.234	7.564
Tailing factor	1.02	0.76
%RSD	0.09	0.77

Accuracy:

Accuracy was computed by recoveries studies. The mean percentage recoveries values for three levels were found to be between 99.51-101.05% and 99.35-100.29% for BT and ML respectively. The percentage of recoveries values within the limits, indicating the method developed was accurate. The results of recovery are shown in Table 3.

Table 3: Recovery Result

Drug	Level	Amount taken	Amount Found	% Recovery
BT	80	16	35.94	99.61
	100	20	40.19	100.95
	120	24	44.25	101.03
ML	80	8	18.02	100.25
	100	10	19.97	99.74
	120	12	21.92	99.37

***Average of three determinations**

Precision

The %RSD of intraday precision and interday precision were 0.05 and 0.28 for BT. The %RSD of intraday precision and interday precision were 0.18 and 0.60 for ML. The percentage RSD of system, method, and intermediate precision study was well within the limits (<2%), indicate that the method was precise.(Table 7).

LOD and LOQ:

The LOD was found to be 0.099µg/ml For BT and 0.28 µg/mlfor ML. The LOQ was found to be 0.30 µg/ml for BTand 0.85 µg/ml forML. The values of LOD and LOQ indicate that the method was greatly sensitive (Table 7).

Robustness

The robustness of the method was designed by changing the optimized condition adequately. To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between BT and ML was evaluated. On the assessment of the result it can be deduced that the variation in the changing wavelength, the flow rate does not affect the method significantly. %RSD <2% specifies that the developed method was robust. The results of robustness are shown in Table 4.

Table 4: Robustness results

Condition		**Bilastine**		**Montelukast**	
		SD	**%RSD**	**SD**	**%RSD**
Change in wavelength (210±1 nm)	**281nm**	**16.11**	**1.61**	**0.30**	**0.05**
	283nm	**16.30**	**1.63**	**0.03**	**0.01**
Change in flow rate (1.0±0.1 ml/min)	**0.8**	**7.09**	**0.62**	**5.37**	**0.74**
	1.0	**1.19**	**0.13**	**0.30**	**0.05**
Change in mobile phase (1.0±0.1 ml/min)	**89+11**	**0.76**	**0.07**	**0.44**	**0.07**
	91+09	**0.09**	**0.01**	**0.87**	**0.13**

***Average of three determinations, %RSD: Percentage relative standard deviation

Analysis of BT and MLfrom marketed tablets

The percentage assay of tablet formulation was found to be 98.71 and101.80% for BT and ML respectively. The results of tab assay are shown in Table-5

Table 5: Tab Assay results

Parameter	BT	ML
Theoretical Plate	5036	7709
Retention Time (Rt)	2.268	7.626
Tailing factor	1.27	0.76
% RSD	0.010	0.065

Rt: Retention time, %RSD: Percentage relative standard deviation

The stability of the drug solutions was observed for 1 h. In degradation studies, the drug was exposed to various stress conditions. From the chromatograms of stressed samples, it was found that no interference from degradants was observed at the retention time of BT and ML. Optimum degradation was observed in thepresence of acid and alkali. Substantial degradation was observed in the presence of water, light, and peroxide. minor degradation was observed in the presence of peroxide and thermal for BT and thermal for ML. The results of the percentage of degradation are presented in Table 6 and Fig. 8-12. Hence, the method was found to be specific.

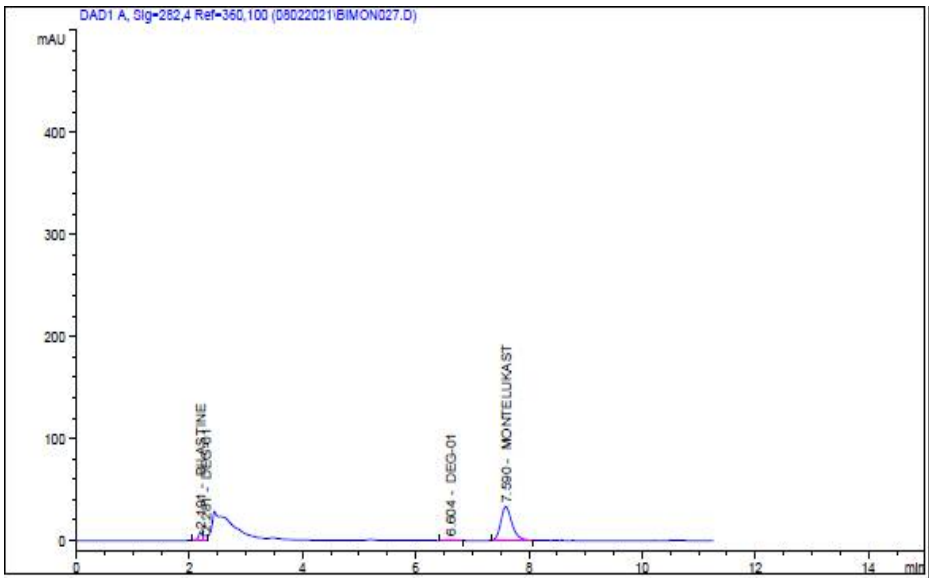

Figure. 8: Chromatogram of BT and ML degraded with acid hydrolysis

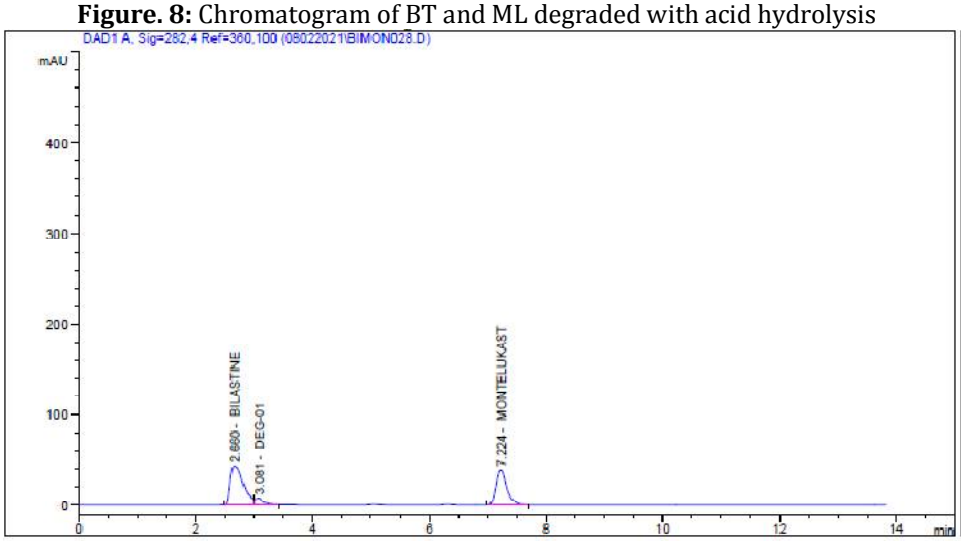

Figure. 9: Chromatogram of BT and ML degraded with alkali hydrolysis

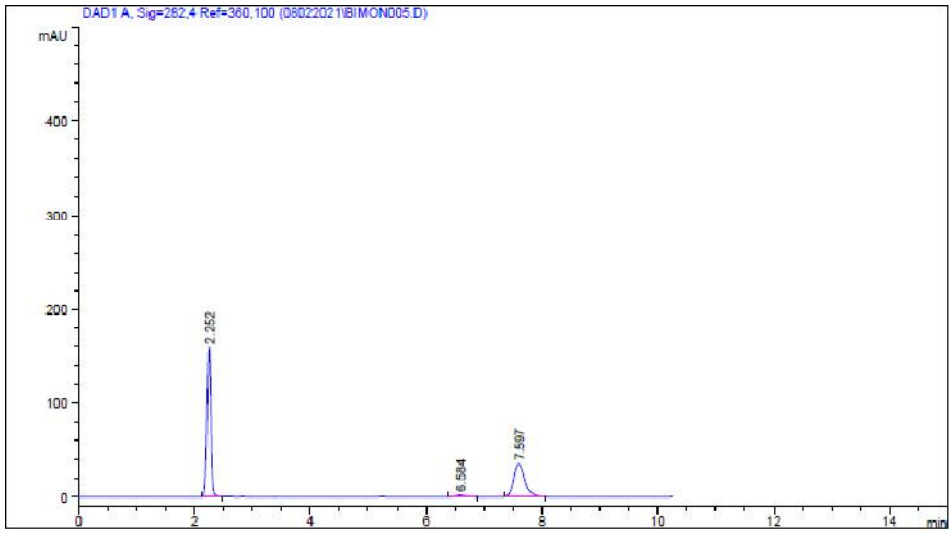

Figure. 10: Chromatogram of mixture of BT and ML degraded with neutral hydrolysis

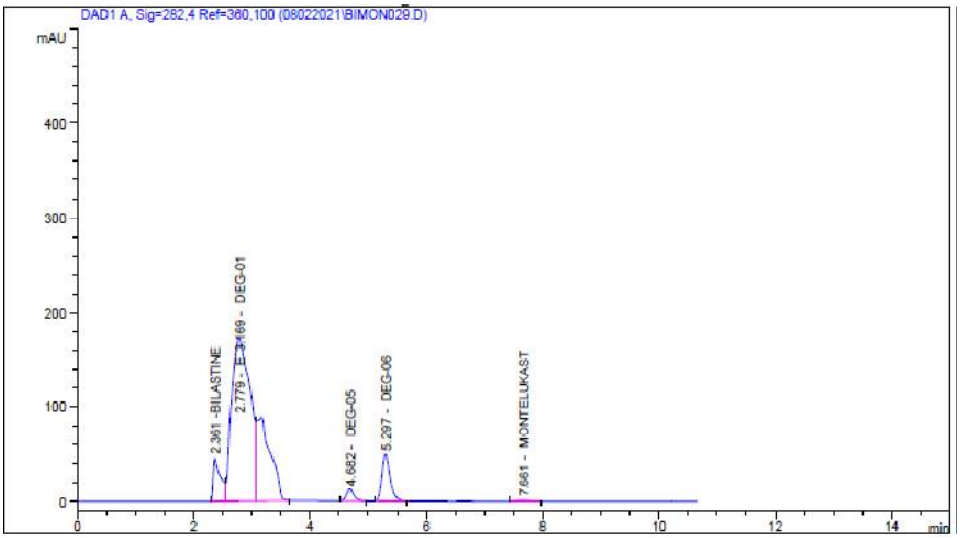

Figure. 11: Chromatogram of BT and ML degraded with oxidative hydrolysis

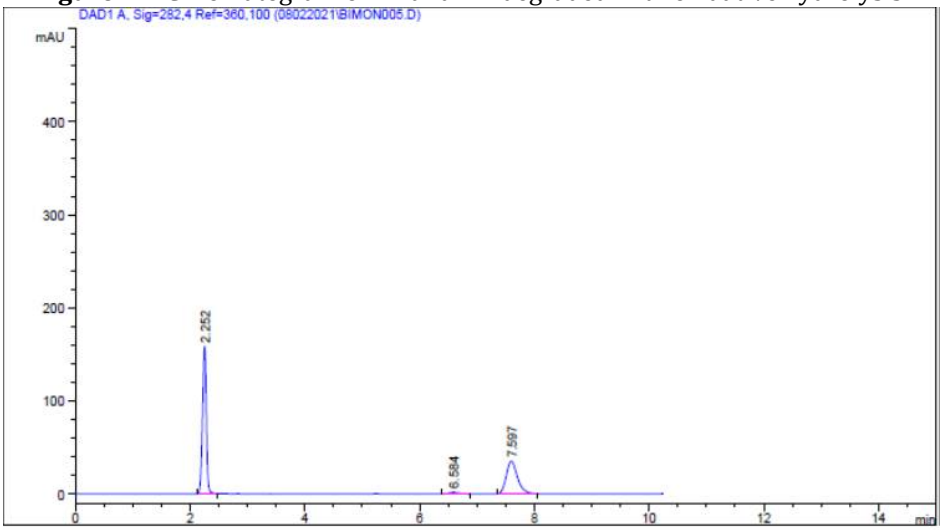

Figure. 12: Chromatogram of BT and ML degraded with exposed to direct sunlight

Table 6: Stability-indicating method data for BT and ML

Parameter	BT (% degradation)	ML(% degradation)
Acidic(0.1N HCL for 1hr)	92.57	7.41
Alkaline (0.1N NaH for 1hr)	6.88	9.10
Hydrolytic(HPLC waters for 1hr)	0.37	3.32
Oxidative(3% H2O2 for 1 hr)	17.26	96.83
Photo(sun light for 24 hr)	0.30	0.6

HPLC: High Performance Liquid Chromatography

Table 7: Summary of validation parameter

Parameter	BT	ML
Calibration Range (µg/ml)	20-100	10-50
Optimized wavelength (nm)	275nm	281nm
Retention Time	2.218	7.562
Precision (% RSD)	0.05-0.33	0.11-0.40
% Assay	99.21	99.68
LOD (µg/ml)	0.099	0.282
LOQ(µg/ml)	0.302	0.856

Average of five determinations, LOD: Limit of detection, LOQ: Limit of quantification

CONCLUSION

The method enables simple, rapid, accurate, precise, specific, economical, and sensitive analysis of BT and ML in combined bulk and tablet dosage form. This method was validated following ICH guidelines. The method can, therefore, be used for routine quality control analysis BT and ML in bulk and tablet dosage form.

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CONFLICT OF INTEREST STATEMENT

Authors declare that they have no conflict of interest exists in this investigation.

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