STABILITY INDICATING ISOCRATIC HPLC APPROACH TO QUANTIFY MONTELUKAST AND BILASTINE MIX IN BULK AND TABLET FORMULATION

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ABSTRACT

This paper describes an novel, rapid, simple, meticulous and unerring "stability indicating HPLC method" for the assessment of montelukast (MTT) and bilastine (BLE) mix in existence of their degradation products in tablet formulation. Zorbax column XDB-C18 was utilized to separate and analyse MTT and BLE mix. The isocratic mode elution of BLE and MTT mix was made with 0.1M disodium hydrogen phosphate buffer of pH 5.8 (55 % volume ratio) and methanol (45% volume ratio) at 1.0 mL min^{-1} flow stream. Strong linearity was recorded within the 2.5 - 20 µg mL^{-1} and 5 - 40 µg mL^{-1} concentration ranges, with coefficient determination values of 0.9994 and 0.9997, respectively, for MTT and BLE. The recovery percentiles of MTT and BLE were 99.593 % and 99.349 %, respectively. The precision displayed values less than 0.520 % for MTT and less than 0.745 % for BLE. The robustness too was shown in the chromatographic settings by minor variations. The specificity besides stability representing feature was evidenced since the degradation products arisen in stress situations did not interlope in the determination of MTT and BLE mix. Thus, this procedure can be applied for quality regulation analysis of BLE and MTT mix in formulations of tablets.

Keywords: Montelukast, Bilastine, Allergy treatment, Stability, HPLC, Analysis

INTRODUCTION

To enhance the therapeutic impact against diseases, commercial pharmaceutical formulations comprising more than one active components in pharmaceuticals including a constant matrix are used[1,2]. For the industrial production of medications, the evaluation of drug combinations seems to be very essential. We therefore need versatile, flexible and low-cost methods to achieve strongly precise as well as accurate results.

Montelukast (MTT) is a potent antagonist of the receptor for cysteinyl leukotriene and possess bronchodilating and anti-inflammatory properties[3]. MTT inhibits the cysteinyl leukotriene one receptor competitively and selectively, blocking the inflammatory facilitator leukotriene D4 from binding, which results in the suppression of inflammatory effects facilitated by leukotriene. MTT is before exercising to reduce bronchospasm during exercise, to alleviate sneezing, runny/ stuffy/itchy nose while allergic rhinitis hay fever and also to manage and stop wheezing and tightness of breath triggered by asthma[4,5].

Bilastine (BLE) is an effective antihistamine that has antiallergic effects and is highly specific for the histamine H1 receptor[6-9]. Mast cells go through degranulation during an allergic reaction, which activates histamine as well as other substances. Due to its ability to bind to and block histamine H1 receptor stimulation, BLE reduces the occurrence of allergic symptoms.

The formulation of MTT and BLE mix was used for allergic seasonal rhinoconjunctivitis and mild to severe asthma as an additive treatment[10]. The MTT and BLE mix was available as tablet dose formulation. The atom arrangements of MTT and BLE are provided in Fig. 1. So far one UV spectrophotometry-based method to quantify MTT and BLE mix in tablets was described by Raj et al[11]. BLE and MTT quantification in the Raj et al. method was performed by gauging absorption at 214 nm (BLE's λ_{max} value) and 281 nm (MTT's λ_{max} value), respectively in methanol. The current strategies of the International Harmonization Conference include the development, on

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the basis of the investigation of stability test samples, of stability-indicating analytical techniques appropriate for evaluating drugs. Stress tests are needed to assess the effect of temperature, light, oxidising agent, acid, and base on drug stability in solutions[12]. However, no "stability indicating HPLC method" was recorded to quantify the BLE and MTT mix in tablets. Using HPLC, this research paper aimed at establishing and validating a "stabilizing HPLC method" with UV detection appropriate for the BLE and MTT separation, quantification and stability studies.

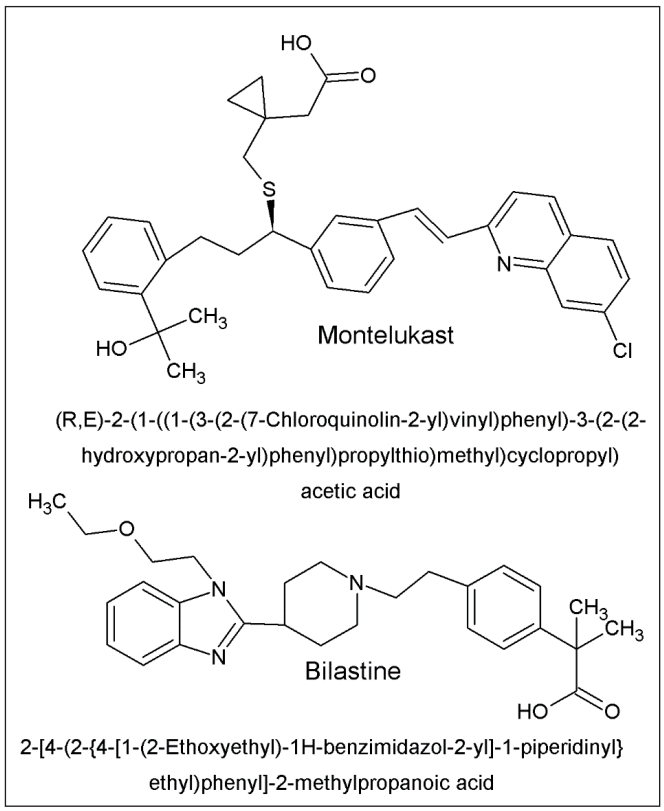


Fig. 1: MTT and BLE chemical structures and IUPAC names

MATERIALS AND METHODS

Liquid chromatography apparatus

Agilent 1100 series HPLC instrument with a Zorbax column XDB-C18 (250×4.6 mm; 5 µ id), UV detector G1314A, quaternary G1311A pumps, ColCom G1316A column thermostat and associated to PC computer heaped with station LC software were used in BLE and MTT separation, quantification and stability studies.

Materials

Reference standard of MTT was acquired from MSD Pharmaceuticals Pvt. Ltd., Mumbai, India. Reference standard of BLE was acquired from Cipla Limited, Hyderabad, India. Billargic M Tablets (Cipla Limited, Hyderabad, India) with labelled contents of BLE 20 mg and MTT 10 mg were used. Hydrochloric acid, sodium hydroxide and peroxide of AR grades were supplied by Rankem Chemicals Ltd, Maharashtra, India. HPLC class methanol including AR class disodium hydrogen phosphate was provided by Merck, Mumbai, India. AR class formic acid and HPLC class water were from Qualigens Fine Chemicals Limited, Maharashtra and Merck, India, respectively.

Conditions to assay BLE and MTT mix

The mobile phase to elute BLE and MTT mix was made of 0.1M disodium hydrogen phosphate buffer with pH 5.8 (55% volume ratio) and methanol (45% volume ratio) at an isocratic type flow velocity of 1.0 mL min^{-1}. Zorbax column XDB-C18 thermal reading was atmospheric. 20 µL of sample was interjaculated for analysis. The wavelength for analysing BLE and MTT mix was 223 nm.

BLE and MTT mix solutions

A proper amount of BLE (20 mg) and MTT (10 mg) was precisely weighed and diluted using methanol to 200 µg mL^{-1} (BLE) and 100 µg mL^{-1} (MTT) as a standard BLE and MTT solution. Working BLE and MTT solutions were made *via* sequential dilution process in mobile phase including calibration BLE and MTT standards. The concentrations of BLE and MTT in calibration standards were: MTT – 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0 µg mL^{-1} and BLE – 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 and 40.0 µg mL^{-1}.

BLE and MTT tablet solution

20 tablets of Billargic M were weighed and triturated with mortar pestle. The powder equivalent to 20 mg of BLE and 10 mg of MTT was incorporated in a 100 mL volumetric container. The volume was attuned up to mark (100 mL) using methanol to make stock BLE and MTT tablet solution that corresponds to 200 µg mL^{-1} (BLE) and 100 µg mL^{-1} (MTT). The components of the volumetric container were sonicated over 20 min. to fully dissolve the BLE and MTT contents. The solution was processed via a filter membrane paper. From this, 1.0 mL aliquot was placed into a 10 mL volumetric container and the cubic measure was attuned to mark (10 mL) with mobile phase compound. This BLE and MTT tablet solution with working concentrations of 200 µg mL^{-1} BLE and 100 µg mL^{-1} MTT in mixture was evaluated for assay determination.

Preparation of BLE and MTT calibration curves

20 µL of each calibration BLE and MTT solution was infused into Zorbax column XDB-C18. The

chromatograms and peak response areas of BLE and MTT were obtained at 223 nm. A graph was mapped as drug concentration against peak response area. The line equations of regression for BLE and MTT were also derived.

Analysis of BLE and MTT contents in tablets

20 µL of BLE and MTT tablet solution was infused into Zorbax column XDB-C18. The chromatograms and peak response areas of BLE and MTT were obtained at 223nm. The concentrations of BLE and MTT were evaluated using the relating linearity graph or line equation of regression.

Study of BLE and MTT degradation

Forced degradation experiments were done by rendering the BLE and MTT tablet sample to acidic (HCl), oxidative (H_2O_2), alkaline (NaOH), heat (60 ^{0}C) and photo (UV, 254 nm) degradations[13-15].

Acid, oxidative and alkaline studies were accomplished by adding 50 mL of hydrochloric acid (0.1 N), peroxide (3%) and sodium hydroxide (0.1N), respectively, to 10 mL of BLE and MTT tablet sample (200 µg mL^{-1} BLE and 100 µg mL^{-1} MTT). All solutions have been leftover for 24 h at room temperature. After accomplishment of degradation, volume was attuned to mark (100 mL) with mobile phase solution and was processed via a filter membrane paper.

During photolytic study, BLE and MTT tablet sample powder corresponding to 20 mg of BLE plus 10 mg of MTT was exposed to light (UV of 254 nm) for 6 h, whereas during thermal study powdered tablet formulation corresponding to 20 mg of BLE and 10 mg of MTT was exposed to heat chamber at 60 ^{0}C for 6 h. After accomplishment of degradation, sample was made as described in section "BLE and MTT tablet solution".

The suggested HPLC procedure was now applied to evaluate each sample and the percentage deterioration of BLE and MTT was determined.

RESULTS AND DISCUSSION

Method optimisation

Spectroscopic examination shows that BLE and MTT had an isobestic point of 223 nm in 200-400 nm scale range, as seen in Fig. 2. Consequently, at 223 nm, chromatographic identification and quantification of BLE and MTT was carried out.

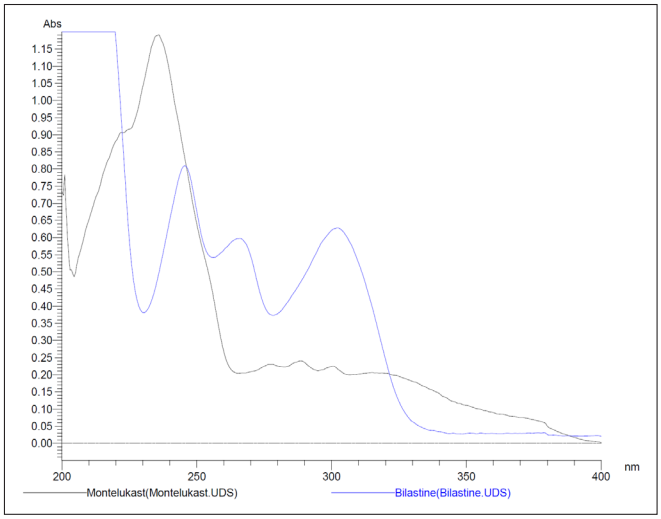


Fig. 2: UV scan of BLE and MTT

On various C18 columns, such as Waters, ProntoSIL Hypersorb ODS, Lichrospher ODS and Zorbax XDB, the column screening trails were conducted. Zorbax column XDB-C18 (250×4.6 mm; 5 µ particle size) was opted because of better partition of BLE and MTT with ample system suitability measures. Under quite a few trials (acetate buffer: methanol and disodium hydrogen phosphate buffer: methanol) of mobile phase screening regarding its component solvents ratio and pH, it was detected that mobile phase compiled of methanol (45% volume ratio): 0.1 M disodium hydrogen phosphate buffer (55% volume ratio) attuned to pH 5.8 using 0.1% formic acid, achieved the good baseline partition for BLE and MTT (Fig. 3). With said above conditions, MTT and BLE were separated and eluted at 3.7000 min and 5.2833 min. Table I shows all system suitability measures for

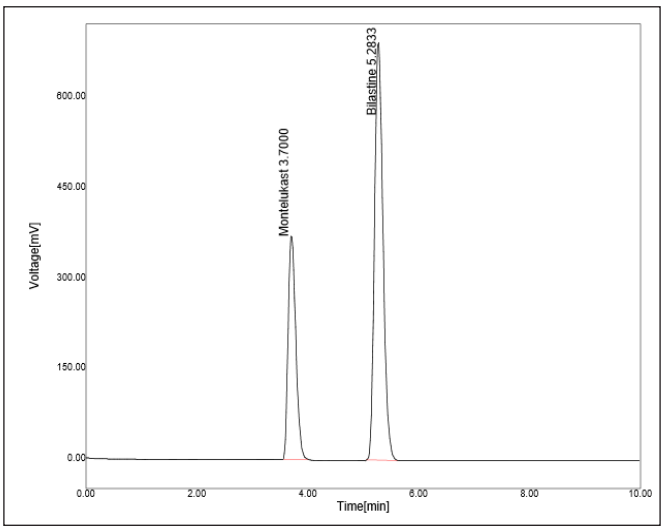


Fig. 3: BLE and MTT typical chromatogram with chosen assay conditions

the measurements of BLE and MTT mix with described above assay conditions.

Table I: BLE and MTT assay system suitability

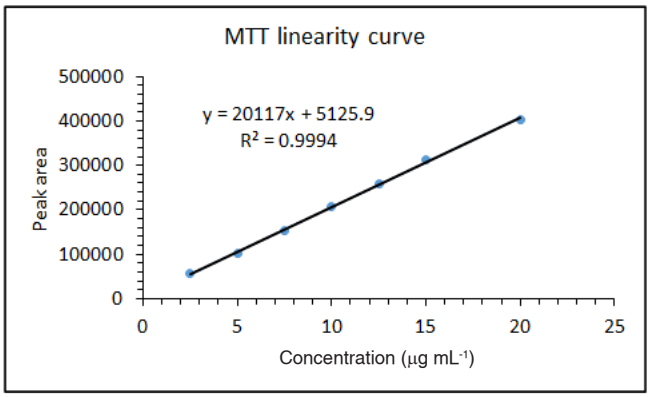
Parameter	Results	
Resolution	MTT	---
	BLE	5.07
Theoretical plates	MTT	3250
	BLE	5036
Tailing factor	MTT	1.13
	BLE	0.91
Response area	MTT	207269
	BLE	373751

METHOD VALIDATION

In compliance with the specifications of the ICH regulations the process, mentioned herein, was thoroughly validated[16].

Linearity

Using the HPLC approach suggested, the linearity scope for the calibration curves of MTT and BLE was obtained as 2.5 - 20 µg mL^{-1} and 5 - 40 µg mL^{-1}, respectively. The coefficient determination values (R^2) were revealed to be 0.9994 and 0.9997 for MTT and BLE, respectively. These findings showed that a strong linear correlation among the concentrations and HPLC peak areas was presented by the HPLC approach. In Fig. 4, the linearity curves and line equations of regression for BLE and MTT are given.



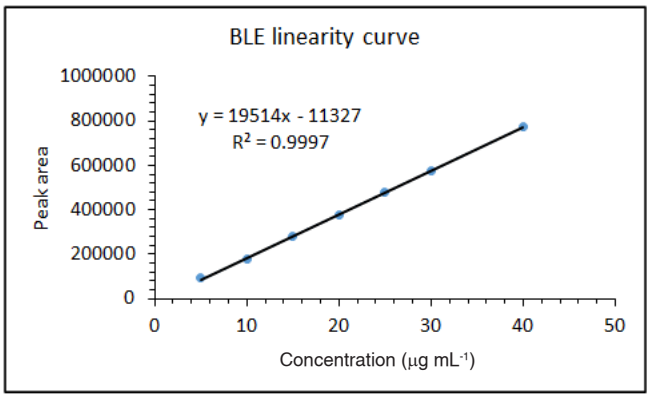


Fig. 4: Linearity curves and line equations of regression for BLE and MTT

Table II: BLE and MTT assay precision and ruggedness

Repeatability			Intermediate precision			Ruggedness		
Inj.	MTT	BLE	Day	MTT	BLE	Analyst	MTT	BLE
	Area response			Area response			Area response	
1	205010	367954	Day 1	204695	365348	1st analyst	205177	368242
2	203488	371640		202747	366955		204719	369942
3	205730	371677		203974	370326		205444	367794
4	205328	368968	Day 2	205009	366154	2nd analyst	206910	372396
5	204551	367473		204653	362437		205254	369378
6	203025	368346		204314	368644		207120	370833
Average[a]	204522	369343	Average[a]	204232	366644	Average[a]	205771	369764
SV[b]	1063.772	1859.281	SV[b]	809.1788	2730.29	SV[b]	995.076	1699.897
CSV[c] (%)	0.520	0.503	CSV[c] (%)	0.396	0.745	CSV[c] (%)	0.484	0.460

Inj. - sample injection number; [a] Average of six response areas; [b] Standard variation of six response areas; [c] Relative standard variation percentile

Table III: BLE and MTT assay accuracy

Level tested (%)	MTT			BLE		
	Spiked (µg mL^{-1})	Obtained (µg mL^{-1})	Recovery (%)	Spiked (µg mL^{-1})	Obtained (µg mL^{-1})	Recovery (%)
50	5	4.993	99.85	10	9.963	99.63
	5	4.967	99.33	10	9.944	99.44
	5	4.949	98.97	10	9.951	99.51
100	10	9.937	99.37	20	19.848	99.24
	10	9.972	99.72	20	19.776	98.88
	10	9.967	99.67	20	19.854	99.27
150	15	14.972	99.82	30	29.840	99.47
	15	14.978	99.86	30	29.791	99.30
	15	14.962	99.74	30	29.819	99.40
Statistical values	Average[a]		99.593	Average[a]		99.349
	SV[b]		0.302	SV[b]		0.215
	CSV[c] (%)		0.303	CSV[c] (%)		0.217

[a] Average of nine response areas; [b] Standard variation of nine response areas; [c] Relative standard variation percentile

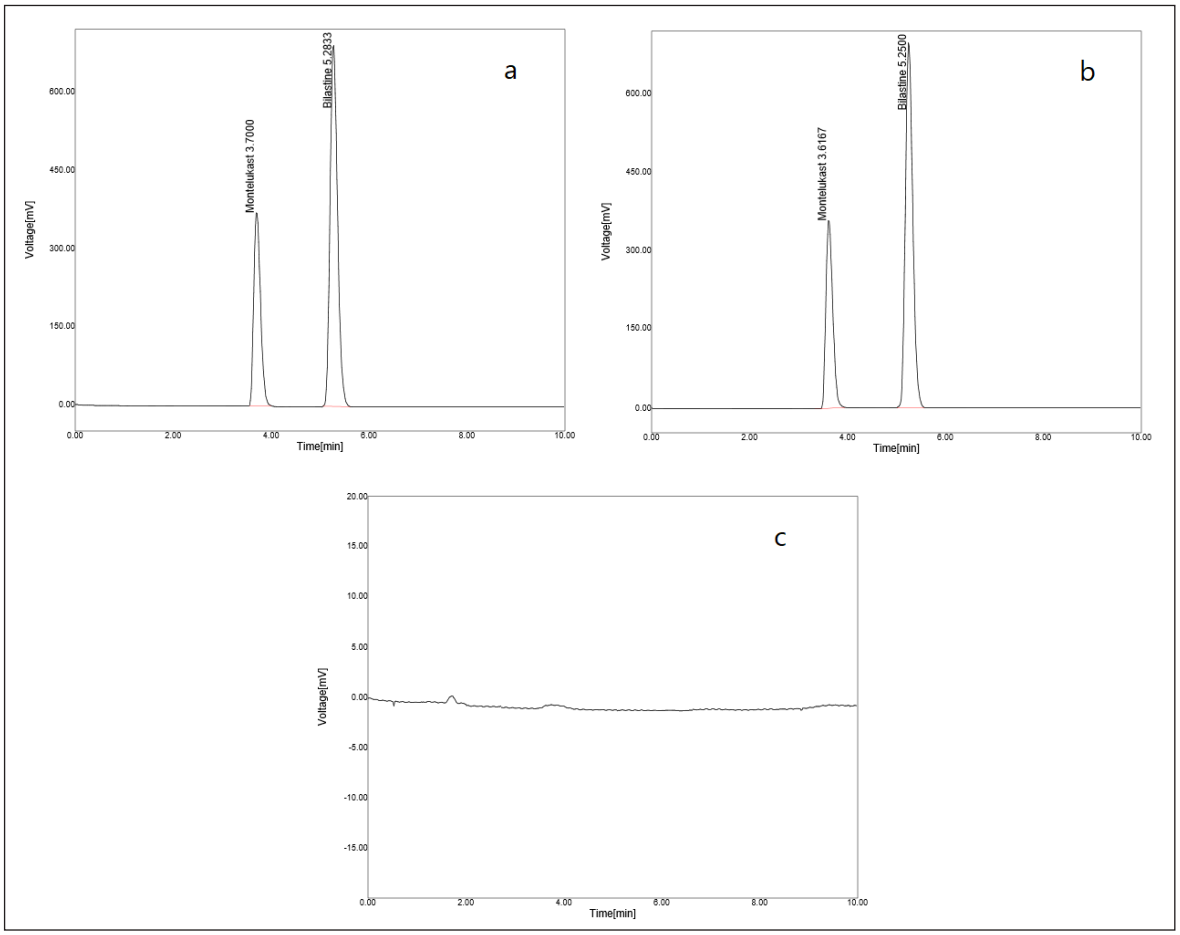


Fig. 5: Chromatograms of [a] Working BLE and MTT solution [b] Tablet formulation sample [c] Blank sample

Sensitivity

"Limit of detection" along with "Limit of quantification" are the two parameters of sensitivity criteria. The approach based on standard variance and slope of the linear graph was adopted to evaluate these parameters. The first parameter was stated to be 0.133 µg mL^{-1} and 0.287 µg mL^{-1}, while the second parameter was determined to be 0.402 µg mL^{-1} and 0.871 µg mL^{-1} for MTT and BLE, respectively, demonstrating that the suggested HPLC approach is extremely sensitive to the quantitation of the mix of MTT and BLE.

Selectivity

The selectivity of the HPLC process was assessed by infusing the blank, working (20 µg mL^{-1} BLE including 10 µg mL^{-1} MTT) and tablet (20 µg mL^{-1} BLE plus 10 µg mL^{-1}MTT) solutions separately into the Zorbax column XDBC18. Two pointed peaks were acquired at retention phases of 3.700 min (MTT) and 5.2833 min (BLE) in the chromatogram of working MTT and BLE solution (Fig. 5a), and at retention phases of 3.6167 min (MTT) and 5.2500 min (BLE) in chromatogram of tablet MTT and BLE solution (Fig. 5b). For blank solution such peaks were not seen (Fig. 5c). The selectivity studies also revealed that excipients presence did not demonstrate any conflict with the pointed and finely-resolved MTT and BLE peaks (Fig.5b).

Precision

Repeatability: The working mix (20 µg mL^{-1} BLE and 10 µg mL^{-1} MTT) was analysed intra-daily six times by using suggested HPLC method. The MTT and BLE were measured peak areas and their comparative standard variations were measured (Table II).

Intermediate precision: The suggested HPLC approach were repeated three times daily on two separate days for the evaluation of working (20 µg mL^{-1} BLE and 10 µg mL^{-1} MTT) solution. The MTT and BLE peak areas and their comparative standard variations were calculated (Table II).

The measures calculated for precision demonstrate that the suggested HPLC approach is extremely precise to the quantitation of the mix of MTT and BLE.

Ruggedness

The working solution (20 µg mL^{-1} BLE plus 10 µg mL^{-1} MTT) was analysed six times by using suggested HPLC method with two analysts. The MTT and BLE peak areas and their comparative standard variations were measured (Table II). The figures calculated for ruggedness demonstrate that the suggested HPLC approach is extremely rugged to quantify the mix of MTT and BLE.

Accuracy

The accuracy of suggested HPLC approach was tested as the percentages of recovery acquired when spiking tablet solution (20 µg mL^{-1} BLE and 10 µg mL^{-1} MTT) with specified MTT (5 µg mL^{-1}, 10 µg mL^{-1} including 15 µg mL^{-1}) along with BLE (10 µg mL^{-1}, 20 µg mL^{-1} plus 30 µg mL^{-1}) concentrations. The percentages for MTT and BLE recoveries have been determined as seen in Table III. Good recovery findings were achieved that showed the higher accuracy of the provided HPLC approach.

Specificity

The specificity of suggested HPLC approach was tested via degradation studies on tablet MTT and BLE solution. The BLE and MTT tablet sample was rendered to acidic (HCl), oxidative (H_2O_2), alkaline (NaOH), heat (60 ^{0}C) and photo (UV, 254 nm) degradations. A significant reduction in BLE and MTT peak areas with the concurrent development of new additional peaks were observed in conditions of acidic (HCl), oxidative (H_2O_2), alkaline (NaOH), heat (60 ^{0}C) and photo (UV, 254 nm) degradations (Table IV). The percentile degradation measurements indicated that

Table IV: BLE and MTT assay specificity

Condition	MTT			BLE		
	Area	Remained (%)	Degraded (%)	Area	Remained (%)	Degraded (%)
Acid	189754	91.55	8.45	355916	95.23	4.77
Peroxide	204629	98.73	1.27	350629	93.81	6.19
UV Light	199865	96.43	3.57	347850	93.07	6.93
Alkaline	195244	94.20	5.80	362969	97.12	2.88
Thermal	202439	97.67	2.33	351191	93.96	6.04

MTT was more vulnerable to acid and more stable in the degradation conditions of peroxide, while BLE was most stable in alkaline condition and less stable in the degradation condition of UV light. In all degradation cases, the additional peaks obtained after degradation were entirely resolved finely from the peaks of the BLE and MTT (Fig. 6), demonstrating that the suggested HPLC approach is extremely specific and stable for the quantitation of the mix of MTT and BLE.

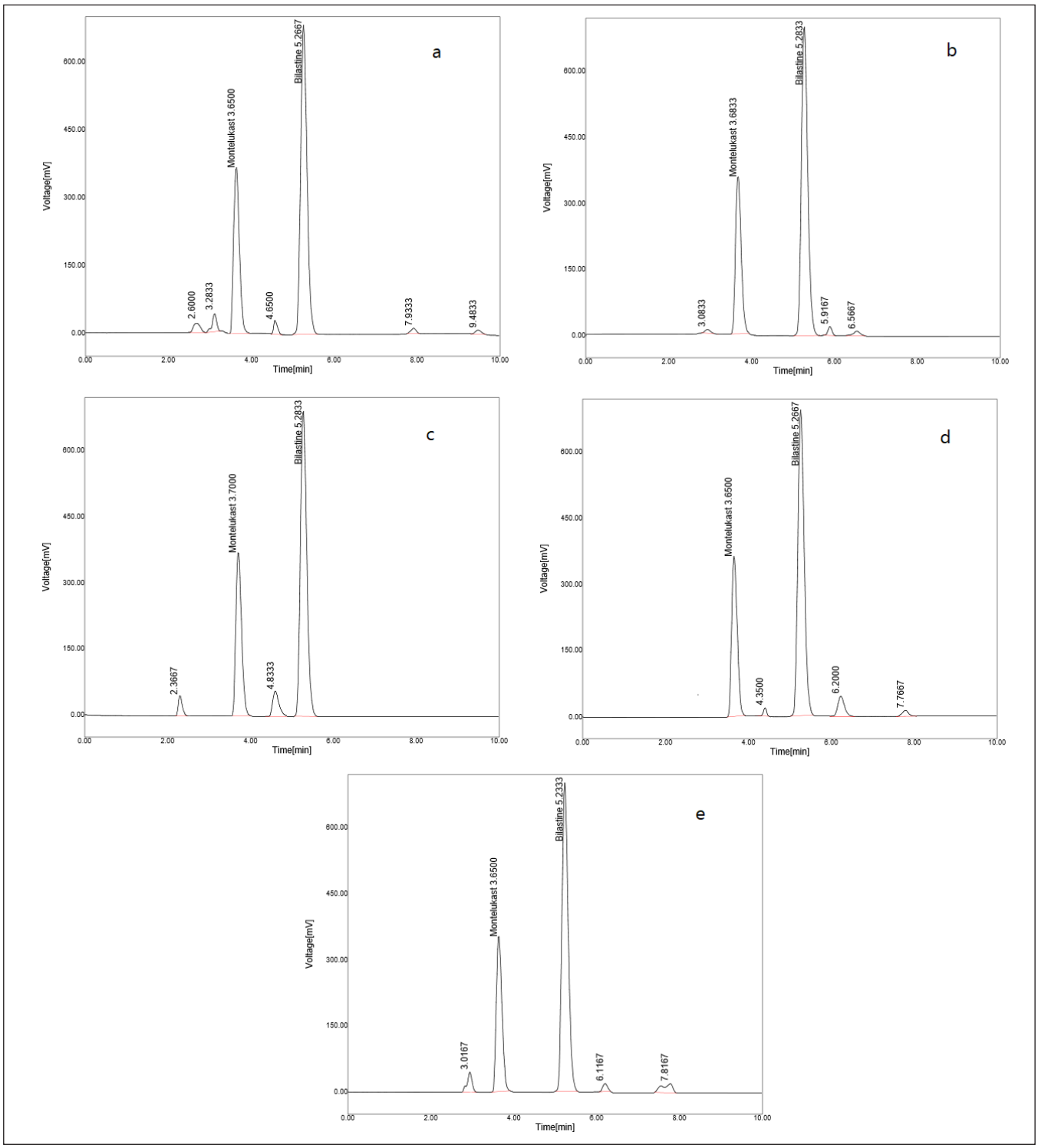


Fig. 6: Chromatograms of tablet BLE and MTT solutions after rendering to [a] acid [b] alkaline [c] peroxide [d] heat [e] light

Robustness

This parameter of the suggested HPLC approach was assessed by varying initial 5.8 buffer pH, the 223 nm wavelength, and the mobile phase to methanol ratio. The assessment of the findings was carried out by evaluating the percentage relative change in the MTT and BLE peak areas. The robustness findings summarized in Table V showed that even despite small modifications in the buffer pH, wavelength and methanol ratio, the suggested HPLC approach is sufficiently precise and reproducible.

Table V: BLE and MTT assay robustness measures

Condition	MTT	BLE
Mobile phase solvents ratio		
Optimized	207269	373751.1
Methanol: Buffer 50:50 (V/V)	205233	370624
Methanol: Buffer 40:60 (V/V)	205684	372676
Average[a]	206062	372350
SV[b]	1069.340	1588.778
CSV[c] (%)	0.519	0.427
Mobile phase pH		
Optimized	207269	373751.1
5.6	205804	375829
6.0	208006	372597
Average[a]	207026	374059
SV[b]	1120.877	1637.856
CSV[c] (%)	0.541	0.438
Wavelength		
Optimized	207269	373751.1
218	208674	370037
228	205757	375010
Average[a]	207233	372933
SV[b]	1458.827	2585.540
CSV[c] (%)	0.704	0.693

[a] Average of three response areas; [b] Standard variation of three response areas; [c] Relative standard variation percentile

Methodology application

Billargic M tablets were analyzed using the HPLC approach described above. The concentrations of MTT and BLE were estimated using the relating linearity graph or line equation of regression. The findings summarized in Table VI show that the suggested HPLC approach is sufficiently precise and accurate to quantify BLE and MTT mix in formulations of tablets.

Table VI: BLE and MTT assay accuracy measures

Tablet tested	MTT			BLE		
	Taken (µg mL^{-1})	Obtained (µg mL^{-1})	Retrieval (%)	Taken (µg mL^{-1})	Obtained (µg mL^{-1})	Re-trieval (%)
Billargic M	10	9.964	99.638	20	19.882	99.409
	10	9.859	98.587	20	19.823	99.117
	10	9.837	98.374	20	19.923	99.617
Statis-tical values	Average[a]		98.866	Average[a]		99.381
	SV[b]		0.677	SV[b]		0.251
	CSV[c] (%)		0.684	CSV[c] (%)		0.253

[a] Average of three recovery values; [b] Standard variation of three recovery values; [c] Relative standard variation percentile

CONCLUSION

The developed "stability indicating HPLC method" could be employed for simultaneous determination of BLE plus MTT mix in their tablet formulation (Billargic M) without interference with each other, excipients of formulation and degradation products. The "stability indicating HPLC method" developed presented satisfactory results, with higher sensitivity, preciseness, selectiveness, accuracy, and robustness; therefore, the same can be employed for the quality regulation investigation of BLE plus MTT in quality assurance/control laboratories.

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