

Development of In Vitro Dissolution Test Method for Bilastine and Montelukast Fixed-Dose Combination Tablets

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ABSTRACT

Bilastine and montelukast are Biopharmaceutical Classification System (BCS) class II compounds with low bioavailability, especially when taken orally. It is challenging to develop a dissolution test. The purpose of this study is to select an in vitro dissolution test that would be useful for bilastine and montelukast solid oral dosage forms. Solid oral dosage forms containing bilastine and montelukast, for which in vivo data are available, were studied using different in vitro dissolution test conditions. Dissolution tests were performed under sink conditions in various non-biorelevant media with changes in various parameters like United States Pharmacopeia (USP) basket or paddle apparatus, rotation speed, volume of media, with different dissolution media. A novel high-performance liquid chromatography method was developed and validated to simultaneously estimate the dissolution profile. The optimal dissolution conditions for bilastine and montelukast are 900 mL 0.5% sodium lauryl sulphate (SLS) in water at 37 ± 0.5 °C and 75 rpm using the paddle apparatus.

KEYWORDS: Montelukast, bilastine, dissolution, fixed-dose combination

INTRODUCTION

Bilastine (BLS) is a new second generation H₁ antihistamine drug substance that reduces allergic rhinitis and urticaria, functioning as an antiallergenic agent (1). Montelukast sodium (MTK) is a potent, selective cysteinyl leukotriene receptor antagonist that inhibits bronchospasm (2). Combining BLS and MTK reduces severe acute respiratory syndrome (SARS) symptoms and improves long-term quality of life of patients with asthma (3). This combination drug product is currently sold in India in tablet form under the brand names of Billargic M (Synkem Pharmaceuticals), Antegy M (Intas Pharmaceuticals), and Bilamove M (Synkem Pharmaceuticals), containing 20 mg BLS and 10 mg MTK.

Official monographs of the *Indian Pharmacopoeia* and *British Pharmacopoeia* describe dissolution testing procedures for quality control of MTK drug products (4, 5). No official monograph exists for BLS. Various analytical methods have been developed for quality control testing

of BLS and MTK (6–9). However, an in vitro dissolution method has not been developed for BCS class II drugs with low solubility and high permeability, such as BLS and MTK solid oral dosage forms. The dissolution test studies the drug's gradual release into a dissolution media. It is crucial to evaluate several elements that may have an impact on the dissolution rate. For example, agitation speed affects the diffusion layer's thickness and reflects the gastrointestinal tract's peristaltic motions (10, 11).

This investigation aims to find the optimal dissolution conditions for release of BLS and MTK from solid oral dosage forms and develop a reverse-phase high-performance liquid chromatography (HPLC) method for simultaneous estimation of BLS and MTK content in dissolution samples.

METHODS

Chemicals

BLS and MTK working standards were received as gift

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samples from Synokem Pharmaceuticals, Haridwar, Uttarakhand, India. The marketed formulation of BLS + MTK combined tablets was procured from the local pharmacy. Methanol, acetonitrile, triethylamine, orthophosphoric acid were of HPLC grade from Merck. Sodium lauryl sulphate, ammonium acetate, glacial acetic acid, hydrochloric acid (HCl), potassium dihydrogen phosphate were of analytical grade.

Preparation of Standard Solution

Stock standard solutions of MTK and BLS (190 µg/mL and 380 µg/mL, respectively) were prepared by dissolving the appropriate amount of working standard in diluent. Working solutions of standard of MTK and BLS (11 µg/mL and 22 µg/mL, respectively) were prepared by adequately diluting the stock solution with respective dissolution media.

Analytical Method Development

An HPLC system (1260 Infinity II, Agilent) with a photo diode array detector was used for analysis. The initial method development was done by trial and error, injecting blank and standard solutions for peak detection and different trials with varying mobile phase buffer ratio, flow rate, and gradient. The reversed phase chromatographic conditions included a Zorbax eclipse plus C18 column (150 mm × 4.6 mm, 5 µm) as a stationary phase, 0.05 M ammonium acetate buffer (pH 5.2) using glacial acetic acid as mobile phase A, and a mixture of methanol and water (90:10 %v/v) as mobile phase B, at 1.2 mL/min in gradient mode of separation. The injection volume was 50 µL, and chromatograms were recorded at 280 nm using a column oven temperature of 25 °C. A homogenous mixture of methanol and ammonium acetate buffer (pH 5.2) (75:25 %v/v) was used as a diluent. The gradient conditions are given in Table 1.

Table 1. HPLC Gradient Elution Conditions

Time (mins)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	40.0	60.0
1.0	40.0	60.0
3.0	10.0	90.0
9.0	10.0	90.0
12.0	40.0	60.0
15.0	40.0	60.0

HPLC: High-performance liquid chromatography.

Analytical Method Validation Protocol

The analytical method was validated for specificity, repeatability, precision, linearity, recovery, and stability in aqueous solution according to International Council for Harmonization (ICH) guidelines (12).

Repeatability was determined by analyzing six replicates of same solution containing 11.1 µg/mL of MTK and 22.2 µg/mL of BLS at the 100% level.

Precision was evaluated by repeating the dissolution test with six replicates (method precision). In addition, intermediate precision was evaluated by repeating the dissolution test with six replicates on a different day by a different analyst with a different column on another HPLC system.

Linearity was evaluated using five different concentrations ranging from 5.5–16.6 µg/mL for MTK and 11.1–33.3 µg/mL for BLS, corresponding to 50%–150% of sample concentration.

Recovery was evaluated in triplicate at three different levels (50%, 100%, and 150%) of sample concentration using standard addition method.

Stability of MTK and BLS was evaluated using the standard solution over a 48-hour period while stored at room temperature (at 37 ± 0.5 °C). Sample solutions were prepared using the same dissolution media and conditions as those used as the dissolution test. The drug concentrations in samples at 0, 12, 24, and 48 hrs were measured and compared.

Dissolution Method Development

Preliminary tests were ran to select the dissolution media. Solubility of BLS and MTK were determined in 0.1 N HCl, purified water, and pH 6.8 phosphate buffer. Purified water, 0.1 N HCl, and 0.05 M phosphate buffer (pH 6.8) were selected for development trials, and varying concentrations of sodium lauryl sulphate (SLS) were incorporated (0.2%, 0.5%, and 1.0%) as a surfactant. Media volumes of 500 and 900 mL were evaluated for feasibility.

Six BLS and MTK fixed-dose combination tablets were weighed and transferred into individual bowls containing dissolution media maintained at 37 ± 0.5 °C. The dissolution tests were carried out using an Electrolab dissolution apparatus (EDT 08Lx) with auto sampling mechanism, fitted with the United States Pharmacopeia (USP) basket or paddle apparatus (apparatus 1 or 2, respectively), at 75 or 100 rpm. The dissolution test was performed using different dissolution media. Samples (10 mL) were collected at 10, 20, 30, 45, and 60 mins from the midway zone between the wall of the vessel and top of paddle, not less than 1 cm from the vessel wall. Each sample was filtered through a 0.45-µm polyvinylidene difluoride (PVDF) syringe filter. The first 5 mL of filtrate

was discarded to saturate the syringe filter, and the other 5 mL of filtrate was collected and analyzed.

RESULT AND DISCUSSION

HPLC Method Validation

Results of the HPLC method validation parameters are presented in Table 2.

Table 2. Method Validation Results

Parameters	Specifications	Montelukast	Bilastine
System suitability	NMT 2.0 %RSD	1.15	0.92
Method precision	NMT 2.0 %RSD	0.64	0.76
Intermediate precision	NMT 2.0 %RSD	1.60	1.77
Linearity	$R^2 > 0.99$	$R^2 = 0.9997$ (49.05x + 1.725)	$R^2 = 0.9992$ (40.652x + 275.09)
Recovery levels			
50%	95–105%	99.5%	99.1%
100%	95–105%	99.9%	100.2%
150%	95–105%	99.5%	99.4%

NMT: not more than; RSD: relative standard deviation.

System Suitability

A system suitability parameter was established by injecting five replicate injections of standard solution. The %RSD values for MTK and BLS were 1.15 and 0.92, respectively. The chromatographic parameters were within the ICH stated range, having retention times of 3.7 and 6.7 mins for MTK and BLS, respectively.

Specificity

The method was specific, with no interference of excipients and blank (dissolution media) at the retention time of analyte peaks.

Precision

The %RSD values for precision must be less than 2.0%, and the absolute difference between method precision and intermediate precision values should not exceed 3.0%. All values were within the acceptable range. The %RSD values for repeatability with MTK and BLS were 0.64 and 0.76, respectively.

Linearity

A linear relationship was obtained between mean peak area under the curve (AUC) and concentration of the drug in the range of 5.55–16.65 µg/mL for MTK and 11.1–33.3 µg/mL for BLS. The calibration curve of MTK and BLS was obtained by plotting the graph between mean peak AUC against concentration (µg/mL). The correlation coefficient (R^2) for MTK and BLS were 0.9997 and 0.9992, respectively.

Recovery

Recovery was evaluated in the range of 50%, 100%, and 150% of drug concentration MTK (5.56–16.67 µg/mL) and BLS (11.10–33.33 µg/mL). The recovery values were within the expected range of 95–105%.

Solution Stability

During this study, only a 0.8% and 0.6% change in the concentration of MTK and BLS was observed from the initial value following up to 24 hours of storage at room temperature (25 °C).

Filter Compatibility Study

A filter compatibility study was conducted to compare the percentage of drug release in sample solutions filtered through different syringe filters with that of the control solution, which was centrifuged. Based on the %RSD criteria for both BLS and MTK sample solutions, the 0.45-µm PVDF syringe filter, 0.22-µm PVDF syringe filter, and 0.45-µm nylon filter (SY25NN) were deemed suitable, as they exhibited a percentage deviation of drug release below 1.5% compared to the control solution. Consequently, the 0.45-µm PVDF syringe filter was used to filter sample solution throughout study.

Optimizing the Dissolution Method

Figure 1 shows the dissolution profiles of MTK and BLS in three different dissolution media. Water as a dissolution medium had the fastest drug release rate compared to others. Because BLS+MTK is a class II drug, the incorporation of surfactant plays a crucial role in the solubility of drugs during the dissolution test. Various concentrations of SLS in water were studied to optimize the concentration of SLS in the dissolution medium. The drug release profile at 60 minutes showed that 0.5% SLS in water is the most suitable medium for dissolution.

Figure 2 shows the dissolution profiles of MTK and BLS in apparatus 1 or 2 with different agitation speeds (75 and 100 rpm) and media volumes (500 and 900 mL). Using apparatus 1 (basket) at 75 and 100 rpm did not generate enough force for complete drug release from the tablet formulation after 60 min of dissolution. Apparatus 2 (paddle) was then used (also at 75 and 100 rpm) to maximize the rate of drug release along with discrimination power. A satisfactory outcome was achieved with apparatus 2 at 75 rpm, with a gradual increase in drug release over 60 min. Media volumes of 500 and 900 mL were tested to evaluate feasibility of the drug's release profile as a class 2 drug. The use of 900 mL was favorable to achieve the criteria of sink condition and better drug solubility.

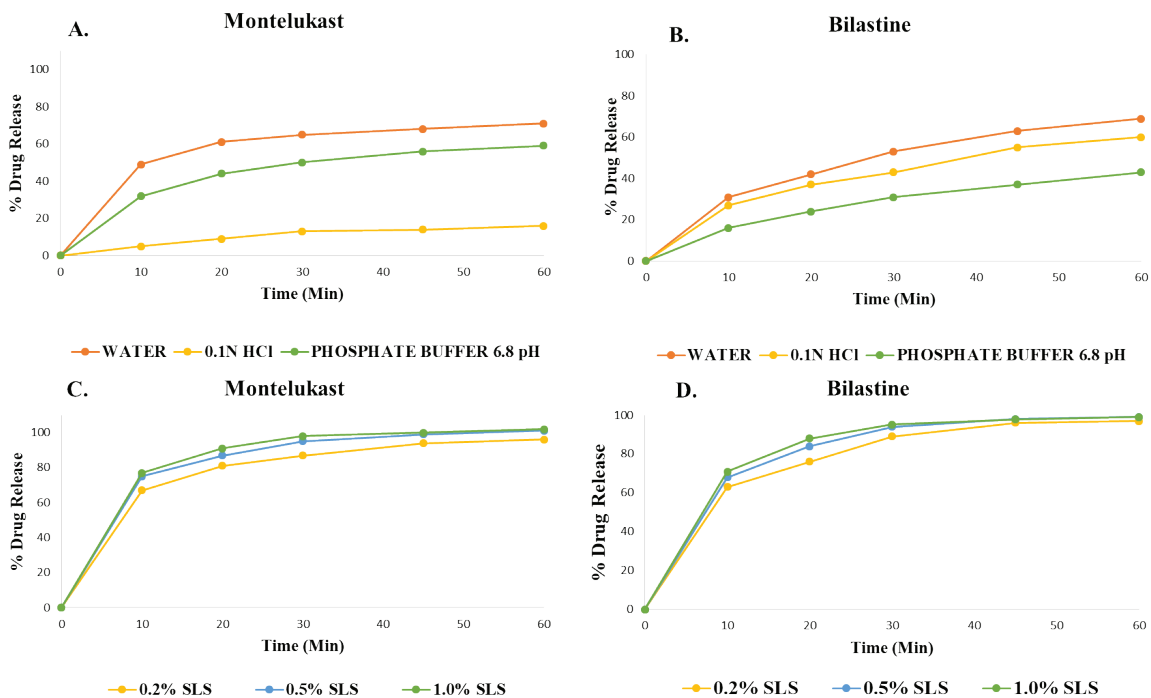


Figure 1. Dissolution profiles of montelukast and bilastine in different media (A and B) and sodium lauryl sulphate (SLS) concentrations (C and D).

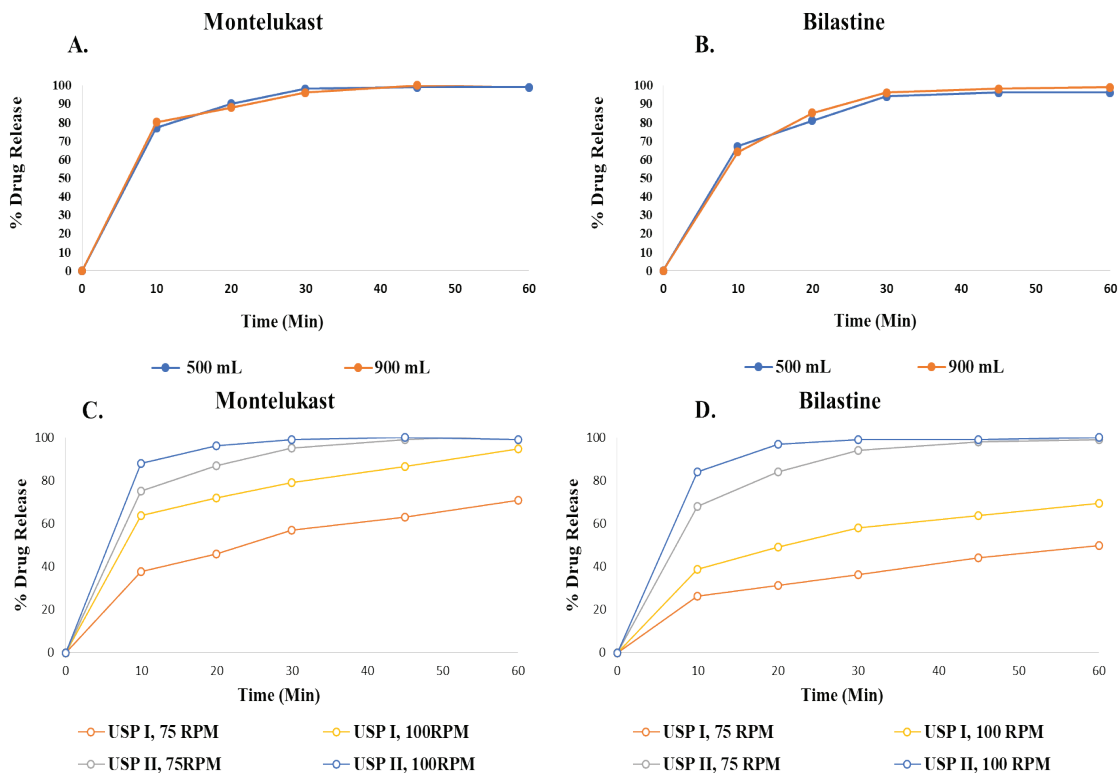


Figure 2. Dissolution profiles of montelukast and bilastine in different media volumes (A and B) and apparatus/stirring speeds (C and D). USP: United States Pharmacopeia.

The optimal dissolution conditions for BLS and MTK are 900 mL 0.5% SLS in water at 37 ± 0.5 °C using the paddle apparatus at 75 rpm.

CONCLUSION

The objective of this study was to develop and validate dissolution method for MTK and BLS fixed-dose combination tablets. Several factors were investigated to determine the optimal method. The most robust dissolution conditions were recorded using apparatus 2 (paddle) with 900 mL of 0.5% SLS surfactant in water as dissolution medium at 37 ± 0.5 °C and 75 rpm. MTK and BLS were found to be stable for 24 hrs, indicating good stability of the drug in dissolution medium. The relatively shorter run time (15 min) for both drugs facilitates rapid estimation of drug release in dissolution samples during routine analysis. The optimized dissolution test conditions proved to be adequate, reliable, and feasible, and all parameters evaluated in this study met the USP acceptance criteria. This method could be considered for future official pharmacopeial methods and for studies in the pharmaceutical industry where MTK and BLS dissolution is required.

ACKNOWLEDGEMENTS

We would like to sincerely thank Graduate School of Pharmacy, Gujarat Technological University for providing required laboratory support and infrastructure to carry out this work smoothly.

FUNDING

The authors disclosed no financial support related to this article.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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