COMPARATIVE STUDY OF QUALITY CONTROL PARAMETERS OF DIFFERENT BRANDS OF ORAL MONTELUKAST TABLETS AVAILABLE IN BANGLADESH

A project submitted in partial fulfillment of the requirements of BRAC University for the degree of Bachelor of Pharmacy

by

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Dedicated to my Parents, Sister and Grandparents

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ABSTRACT

Quality control of pharmaceutical product emphasizes on various testing of the product which include both in-process and finished product quality control tests that are conducted prior to release of the drug in the market. In the present study the quality control parameters of ten different brands of Montelukast tablets available in Bangladesh were evaluated and compared to assess the quality of the tablets. The samples of the tablets were taken from five leading pharmaceutical companies of Bangladesh represented as A to E respectively, three medium ranked companies designated as F, G, H and two low ranked companies denoted as I and J respectively. Quality control tests such as weight variation, friability, hardness as well as disintegration tests were performed. *In vitro* dissolution study was carried out and analyzed by HPLC to determine the percentage release of drug after 30 minutes which may reflect *in vivo* performance of the drug. The weight variation results show that there was hardly any variation among the leading pharmaceutical companies (value ranging 0.17±0.01 gm to 0.2±0.01 gm) except for company B (0.35±0.01 gm) and the middle and lower ranked company showed slightly higher results. The tablets of all the ten companies showed acceptable values of hardness except for one low-ranked company J with a high value of 16.57±1.4 kg/cm^2. There is a marginal difference in the result of the friability test of the all the ten companies (all values less than 1% according to BP specification), signifying that the Montelukast tablets produced by the different companies of Bangladesh have sufficient mechanical strength to withstand the pressure due to processing, storage and shipment. Disintegration times of the tablets of leading companies were found to be within 3 minutes indicating a very good result except for company A (7.40±0.9 minutes). Company F and company I showed the highest disintegration times (9.4±1.17 minutes and 9.8±3.6 minutes respectively). Consequently, the percentage release of drug for company A, company F and company I are less compared to other companies as shown by the dissolution study. Nevertheless, all the companies showed greater than 90% dissolution of drug after 30 minutes, thus complying with the specifications of British Pharmacopeia and US FDA guidelines for INN drugs. Hence, it can be concluded that Montelukast tablets produced by the pharmaceutical companies in Bangladesh are of consistent quality with very little variation among them and complies with the specifications of British Pharmacopeia.

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LIST OF ABBREVIATIONS

LTRA- Leukotriene receptor antagonist

FDA-Food and Drug Administration

CysLT1-Cysteinyl Leukotriene 1

Cmax- Mean peak montelukast plasma concentration

Tmax-Time to reach mean plasma concentrarion

AUC-Area under curve

EIB- Exercise-Induced Bronchoconstriction

PKU- Phenylketonuria

CYP- Cytochrome P450

LABA-Long acting beta agonists

IVIVC- In vitro/in vivo correlation

BDM- Biorelevent dissolution media

SIF- Simulated intestinal fluid

USP-United States Pharmacopeia

SLS-Sodium lauryl sulfate

ICS- Inhaled corticosteroid

AHR- Airway hyperresponsiveness

MDA- Malondialdehyde

MPO- Myeloperoxidase

BUN- Blood urea nitrogen

IRD- Immune reconstitution diseases

NIRS- Near-infrared spectroscopy

BP – British Pharmacopeia

HPLC – High Performance Liquid Chromarography

Chapter-1

INTRODUCTION

1. INTRODUCTION

Montelukast is a leukotriene receptor antagonist (LTRA) used for the treatment of asthma and to relieve symptoms of seasonal allergies in children and adults (1-3). Leukotrienes are fatty compounds produced by the immune system which are responsible for asthma, bronchitis and constriction of airway (4). Leukotrienes constitute a group of locally acting hormones produced in living systems from arachidonic acid. Major leukotrienes are Leukotriene B4 (LTB4), Leukotriene C4 (LTC4), Leukotriene D4 (LTD4), and Leukotrine E4 (LTE4). Biosynthesis of these leukotrienes begins with the action of the enzyme 5-lipoxygenase on arachidonic acid to produce Leukotrine A4 (5). A leukotriene antagonist is any drug or substance that inhibit leukotrienes (6). A leukotriene antagonist is also known as leukast (6). Montelukast causes inhibition of airway cysteinyl leukotriene receptors by the inhibition of bronchoconstriction due to inhaled LTD4 in asthmatics (7). The structure of leukotrienes was elucidated in 1979 and their proposed implication in the etiology of respiratory diseases caused many laboratories to initiate programs to discover blockers of leukotrienes as new treatment for asthma. Merck Frost started two parallel programs, one to find an inhibitor of the key biosynthesizing enzyme 5-lipoxygenase and the other to find a selective blocker of the Leukotrine D4 receptor. These projects proceeded for more than 10 years with many failures and 6 compounds were brought into human clinical trials before Montelukast was identified (8). Montelukast received the UK license since 1998 as add-on therapy for the treatment of the patients 6 years or older with mild to moderate asthma who were inadequately controlled as required with short-acting ß-agonists and inhaled corticosteroids. It is also licensed for prophylaxis for asthma in which the predetermined component is exercise-induced bronchoconstriction. The license has recently been extended to include the 2 to 5 year age group (9). In August 3 2012, FDA has approved 10 generic drug manufacturers to start making generic versions of Singulair (Montelukast sodium). The available forms of Montelukast sodium in market are 10mg film-coated tablet and 5mg chewable tablet (10). Commonly available brand of Montelukast sodium is known as Singulair produced by Merck Sharpe & Dohme Ltd (11-12). Montelukast is highly bound to plasma protein and rapidly metabolized (13). The dose of the drug varies according to age, gender and clinical state of the person. Pregnant and person sensitive to Montelukast should avoid this drug (14).

1.1. AIM

In the pharmaceutical industry, total quality of the product must be ensured in order to avoid the product which does not meet the requirements and specifications mentioned in the Pharmacopoeias (United States Pharmacopeia, British Pharmacopeia). When the production process is running there is a chance for errors to occur, so it is necessary to control the error that may occur during production procedure and stringent quality control tests must be performed to determine the quality of the product. Quality control of pharmaceutical product emphasizes on various testing of the product to find out the defects that may occur during production. Therefore to assure the total quality of the product, both in process and finished product quality control tests are essential requirements of the manufacturing process which are conducted prior to release of the drug in the market. In-process control tests are tests that are performed before the manufacturing process is completed in order to comply with the specifications.

The aim of the study is to evaluate and compare the quality control parameters of oral Montelukast sodium tablets of top five leading pharmaceutical companies, three medium ranked pharmaceutical companies and two low ranked pharmaceutical companies marketed in Bangladesh in order to assess the quality and efficacy of oral Montelukast sodium tablets available in Bangladesh.

The quality control tests performed during the study are as follows:

(i) Weight variation test
(ii) Hardness test
(iii) Friability test
(iv) Disintegration test
(v) Dissolution test using USP Paddle II method and analyzed by HPLC

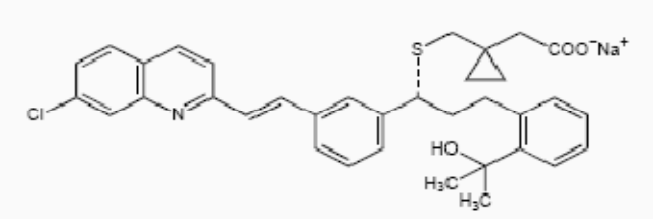

Figure-1. Structure of Montelukast

Commonly used brand name(s) –Singulair

Manufacturer –Merek Sharpe & Dohme Ltd.

Chemical name – [R]-1-[1-[3-[2-(7-chloro-2-quinolinyl) ethenyl] phenyl-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl] thio] methyl] cyclopropaneacetic acid, monosodium salt.

Category-antihistaminic (leukotriene receptor antagonist) (15)

COMPANY NAME	BRAND NAME	DOSAGE FORM
Square Pharmaceuticals Ltd	**MONTENE 10**	Montelukast sodium INN 10mg/tablet
Beximco Pharmaceuticals Ltd	**MONOCAST**	Montelukast sodium INN 10mg/tablet
Incepta Pharmaceuticals Ltd	**MONTAIR**	Montelukast sodium INN 4mg, 5mg & 10mg/tablet
Renata Ltd	**ODMON**	Montelukast sodium INN 5mg & 10mg/tablet
Eskayef Bangladesh Ltd	**LUMONA 10**	Montelukast sodium INN 10mg/tablet
ACME Laboratories Ltd	**MONAS**	Montelukast sodium INN 4mg, 5mg & 10mg/tablet
Opsonin Pharma Limited	**TRILOCK**	Montelukast sodium INN 4mg, 5mg & 10mg/tablet
ACI Limited	**REVERSAIR**	Montelukast sodium INN 10mg/tablet
Drug International Ltd	**M-KAST-10**	Montelukast sodium INN 10mg/tablet
Healthcare Pharmaceuticals Limited	**AERON**	Montelukast sodium INN 10mg/tablet

Table-1. Commercially available oral Montelukast sodium tablets in Bangladesh

1.2. THERAPEUTIC INDICATIONS

1.2.1. Asthma-Asthma is a common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasm (6). Common symptoms include wheezing, coughing, chest tightness, and shortness of breath (2).

Montelukast sodium is indicated for the prophylaxis and chronic treatment of asthma in adults and pediatric patients 2 years of age and older (14).

1.2.2. Exercise-Induced Bronchospasm- Exercise-induced bronchospasm is frequent in children and in young patients with mild asthma, and is often associated with other markers of uncontrolled asthma, such as symptoms induced by other nonspecific triggers or frequent exacerbations (16-17). In these cases, the patient should be managed according to general recommendations. Sometimes, however, bronchoconstriction is induced almost exclusively by exercise, particularly in elite athletes, thus representing a true clinical phenotype (18,19). In these patients, Montelukast has demonstrated greater efficacy than beta2-agonists, both as regular and occasional treatment, in preventing exercise-induced asthma, with the advantage of no loss of efficacy over time (20-22).

Another trigger of asthma attacks is aspirin and other related chemicals (often present in some food as additives or preservatives). Aspirin-sensitive patients often have severe asthma, and may have-greater activation of the leukotriene cascade, as demonstrated by high levels of urinary LTE4 (23). Some studies tried to assess whether aspirin-sensitive patients are particularly responsive to LTRA treatment, with some positive results (24). However, these data have not been confirmed by other studies.

1.2.3. Allergic Rhinitis- Allergic rhinitis is an allergic inflammatory reaction which occurs in nasal airway. It occurs when an allergen, such as pollen, dust, or animal dander (particles of shed skin and hair) is inhaled by an individual with a sensitized immune system (14). Allergic rhinitis is frequently associated with asthma both in allergic and nonallergic patients, and untreated upper airway disease represents a frequent cause of uncontrolled asthma (25). As Montelukast is effective on both upper and lower airways, its use might be particularly useful in patients with both asthma and rhinitis. A post hoc analysis of a subgroup of patients enrolled in a study comparing budesonide plus Montelukast with a doubling dose of budesonide showed that patients with asthma and rhinitis reported a greater improvement in symptoms and pulmonary function with budesonide plus Montelukast (26). After that, many other clinical and observational studies have confirmed that the addition of Montelukast to current treatment

induced a considerable and long-lasting improvement in asthma control in patients with both asthma and rhinitis (27-28).

Montelukast sodium is indicated for the relief of symptoms of seasonal and perennial allergic rhinitis in patients 2 years of age and older (14).

1.3. PHYSICAL PROPERTY

1.3.1. Description- Montelukast sodium is a hygroscopic, optically active, white or off white powder (18).

1.3.2. Solubility-Freely soluble in ethanol, methanol, water and in acetanilide it is partially insoluble (7).

1.4. MECHANISM OF ACTION

Montelukast is a selective leukotrine receptor antagonist of cysteinyl leukotriene CysLT1 receptor. The cysteinyl leukotnienes (LTC4, LTD4, LTE4) are produced form arachidonic acid metabolism that are released form mast cell, eosinophils and other cells. Cysteinyl leukotriene receptors are found in human airway. Cysteinyl leukotriene binds with this receptor. This binding is associated with the pathophysiology of asthma, including smooth muscle construction, airway edema, and altered cellular activity (factor that helps in asthma) and that is how Montelukast inhibits broncoconstriction. Montelukast inhibits physiologic action of LTD4 at the CysLT1 receptors without any agonist activity (7).

1.5. PHARMACOKINETICS

1.5.1. Absorption- Montelukast is rapidly absorbed following oral administration. After administration of the 10 mg film-coated tablet to fasted adults, the mean peak Montelukast plasma concentration (Cmax) is achieved in 3 to 4 hours (Tmax). The mean oral bioavailability is 64%. The oral bioavailability and Cmax are not influenced by a standard meal in the morning.

For the 5 mg chewable tablet, the mean Cmax is achieved in 2 to 2.5 hours after administration to adults in the fasted state. The mean oral bioavailability is 73% in the fasted state versus 63% when administered with a standard meal in the morning.

For the 4 mg chewable tablet, the mean Cmax is achieved 2 hours after administration in pediatric patients 2 to 5 years of age in the fasted state.

The 4 mg oral granule formulation is bioequivalent to the 4 mg chewable tablet when administered to adults in the fasted state.

The safety and efficacy of Montelukast sodium in patients with asthma were demonstrated in clinical trials in which the 10 mg film-coated tablet and 5 mg chewable tablet formulations were administered in the evening without regard to the time of food ingestion. The safety of montelukast sodium in patients with asthma was also demonstrated in clinical trials in which the 4 mg chewable tablet and 4 mg oral granule formulations were administered in the evening without regard to the time of food ingestion. The safety and efficacy of Montelukast sodium in patients with seasonal allergic rhinitis were demonstrated in clinical trials in which the 10 mg film-coated tablet was administered in the morning or evening without regard to the time of food ingestion.

The comparative pharmacokinetics of Montelukast when administered as two 5 mg chewable tablets versus one 10 mg film-coated tablet has not been evaluated (7, 17).

1.5.2. Distribution- Montelukast is more than 99% bound to plasma proteins. The steady state volume of distribution of Montelukast averages 8 to 11 liters. Studies in rats with radiolabeled Montelukast indicate minimal distribution across the blood-brain barrier. In addition, concentrations of radiolabeled material at 24 hours postdose were minimal in all other tissues (7, 17).

1.5.3. Metabolism- Montelukast is extensively metabolized. In studies with therapeutic doses, plasma concentrations of metabolites of Montelukast are undetectable at steady state in adults and pediatric patients (7, 16).

In vitro studies using human liver microsomes indicate that CYP3A4, 2C8, and 2C9 are involved in the metabolism of Montelukast. At clinically relevant concentrations, 2C8 appears to play a major role in the metabolism of Montelukast (7, 17).

1.5.4. Elimination-The plasma clearance of Montelukast averages 45 mL/min in healthy adults. Following an oral dose of radiolabeled Montelukast, 86% of the radioactivity was recovered in 5 day fecal collections and <0.2% was recovered in urine. Coupled with estimates of Montelukast oral bioavailability, this indicates that Montelukast and its metabolites are excreted almost exclusively via the bile (7, 18).

In several studies, the mean plasma half-life of Montelukast ranged from 2.7 to 5.5 hours in healthy young adults. The pharmacokinetics of Montelukast are nearly linear for oral doses up to 50 mg. During once-daily dosing with 10 mg Montelukast, there is little accumulation of the parent drug in plasma (14%).

1.6. PHARMACODYNAMICS

Montelukast causes inhibition of airway cysteinyl leukotriene receptors as demonstrated by the ability to inhibit bronchoconstriction due to inhaled LTD4 in asthmatics. Dose as low as 5 mg cause substantial blockage of LTD4-induced bronchoconstriction. In a placebo-controlled, crossover study (n=12), Montelukast sodium inhibited early- and late-phase bronchoconstriction due to antigen challenge by 75% and 57%, respectively.

The effect of Montelukast sodium on eosinophils in the peripheral blood was examined in clinical trials. In patients with asthma aged 2 years and older who received Montelukast sodium, a decrease in mean peripheral blood eosinophil counts ranging from 9% to 15% was noted, compared with placebo, over the double-blind treatment periods. In patients with seasonal allergic rhinitis aged 15 years and older who received Montelukast sodium, a mean increase of 0.2% in peripheral blood eosinophil counts was noted, compared with a mean increase of 12.5% in placebo-treated patients, over the double-blind treatment periods; this reflects a mean difference of 12.3% in favor of Montelukast sodium. The relationship between these observations and the clinical benefits of Montelukast noted in the clinical trials is not known (7, 14, 17).

1.7. DOSAGE AND ADMINISTRATION

1.7.1. Asthma

Montelukast sodium should be taken once daily in the evening. The following doses are recommended:

For adults and adolescents 15 years of age and older: one 10-mg tablet.

For pediatric patients 6 to 14 years of age: one 5-mg chewable tablet.

For pediatric patients 2 to 5 years of age: one 4-mg chewable tablet.

Safety and effectiveness in pediatric patients less than 12 months of age with asthma have not been established.

There have been no clinical trials in patients with asthma to evaluate the relative efficacy of morning versus evening dosing. The pharmacokinetics of Montelukast are similar whether dosed in the morning or evening. Efficacy has been demonstrated for asthma when Montelukast was administered in the evening without regard to time of food ingestion (14, 16).

1.7.2. Exercise-Induced Bronchoconstriction (EIB) in Patients 15 Years of Age and Older

For prevention of EIB, a single 10 mg dose of Montelukast should be taken at least 2 hours before exercise. An additional dose of Montelukast should not be taken within 24 hours of a previous dose. Patients already taking Montelukast sodium daily for another indication (including chronic asthma) should not take an additional dose to prevent EIB. All patients should have available for rescue a short-acting β-agonist. Safety and effectiveness in patients younger than 15 years of age have not been established. Daily administration of Montelukast sodium for the chronic treatment of asthma has not been established to prevent acute episodes of EIB (14, 17).

1.7.3. Allergic Rhinitis

For allergic rhinitis, Montelukast sodium should be taken once daily. Efficacy was demonstrated for seasonal allergic rhinitis when Montelukast was administered in the morning or the evening without regard to time of food ingestion. The time of administration may be individualized to suit patient needs.

The following doses for the treatment of symptoms of seasonal allergic rhinitis are recommended:

For adults and adolescents 15 years of age and older: one 10-mg tablet.

For pediatric patients 6 to 14 years of age: one 5-mg chewable tablet.

For pediatric patients 2 to 5 years of age: one 4-mg chewable tablet

Safety and effectiveness in pediatric patients younger than 2 years of age with seasonal allergic rhinitis have not been established.

The following doses for the treatment of symptoms of perennial allergic rhinitis are recommended:

For adults and adolescents 15 years of age and older: one 10-mg tablet

For pediatric patients 6 to 14 years of age: one 5-mg chewable tablet

For pediatric patients 2 to 5 years of age: one 4-mg chewable tablet

Safety and effectiveness in pediatric patients younger than 2 years of age with perennial allergic rhinitis have not been established (14).

1.7.4. Asthma and Allergic Rhinitis

Patients with both asthma and allergic rhinitis should take only one Montelukast sodium dose daily in the evening (14).

1.8. USE IN PREGNANCY

1.8.1. Pregnancy Category B

There are no adequate and well-controlled studies in pregnant women (7).

1.8.2. Teratogenic Effect

No teratogenicity was observed in rats and rabbits at doses approximately 100 and 110 times, respectively, the maximum recommended daily oral dose in adults based on AUCs.

During worldwide marketing experience, congenital limb defects have been rarely reported in the offspring of women being treated with SINGULAIR during pregnancy. Most of these women were also taking other asthma medications during their pregnancy. A causal relationship between these events and SINGULAIR has not been established (14, 17).

1.8.3. Nursing Mothers

Studies in rats have shown that Montelukast is excreted in milk. It is not known if Montelukast is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when SINGULAIR is given to a nursing mother (14).

1.9. CONTRAINDICATIONS

1.9.1. Acute Asthma

Montelukast sodium is not indicated for use in the reversal of bronchospasm in acute asthma attacks, including status asthmaticus. Patients should be advised to have appropriate rescue medication available. Therapy with Montelukast sodium can be continued during acute exacerbations of asthma (19). Patients who have exacerbations of asthma after exercise should have available for rescue a short-acting inhaled β-agonist (14).

1.9.2. Concomitant Corticosteroid Use

While the dose of inhaled corticosteroid may be reduced gradually under medical supervision, Montelukast sodium should not be abruptly substituted for inhaled or oral corticosteroids (14).

1.9.3. Aspirin Sensitivity

Patients with known aspirin sensitivity should continue avoidance of aspirin or non-steroidal anti-inflammatory agents while taking Montelukast sodium. Although Montelukast sodium is effective in improving airway function in asthmatics with documented aspirin sensitivity, it has not been shown to truncate bronchoconstrictor response to aspirin and other non-steroidal anti-inflammatory drugs in aspirin-sensitive asthmatic patients (14).

1.9.4. Neuropsychiatric Events

Neuropsychiatric events have been reported in adult, adolescent, and pediatric patients taking Montelukast sodium. Post-marketing reports with Montelukast sodium use include agitation, aggressive behavior or hostility, anxiousness, depression, disorientation, disturbance in attention, dream abnormalities, hallucinations, insomnia, irritability, memory impairment, restlessness, somnambulism, suicidal thinking and behavior (including suicide), and tremor. The clinical details of some post-marketing reports involving Montelukast sodium appear consistent with a drug-induced effect.

Patients and prescribers should be alert for neuropsychiatric events. Patients should be instructed to notify their prescriber if these changes occur. Prescribers should carefully evaluate the risks and benefits of continuing treatment with Montelukast sodium if such events occur (14).

1.9.5. Eosinophilic Conditions

Patients with asthma on therapy with Montelukast sodium may present with systemic eosinophilia, sometimes presenting with clinical features of vasculitis consistent with Churg-Strauss syndrome, a condition which is often treated with systemic corticosteroid therapy. These events usually, but not always, have been associated with the reduction of oral corticosteroid therapy. Physicians should be alert to eosinophilia, vasculitic rash, worsening pulmonary

symptoms, cardiac complications, and/or neuropathy presenting in their patients. A causal association between Montelukast sodium and these underlying conditions has not been established (14).

1.9.6. Other precautions

Before taking Montelukast, consulting with doctor or pharmacist is required if patients are allergic to it; or if they have any other allergies. This product may contain inactive ingredients, which can cause allergic reactions or other problems (14, 16).

Before using this drug, patient should tell to doctor or pharmacist about his medical history, especially of: liver disease (14).

Before having surgery, patients have to consult with doctor or dentist about all the products they use (including prescription drugs, nonprescription drugs, and herbal products) (14, 16, 17).

The chewable tablets may contain aspartame. If the patients have phenylketonuria (PKU) or any other condition that requires him to limit/avoid aspartame (or phenylalanine) in his diet, he should ask doctor or pharmacist about using this medication safely (18).

During pregnancy, this medication should be used only when clearly needed. Patient should discuss about the risks and benefits with his doctor (18).

1.10. SIDE EFFECTS

Serious side effects:

- Skin rash, bruising, severe tingling, numbness, pain, muscle weakness;
- Mood or behavior changes, anxiety, depression, suicidal tendency
- Tremors or shaking;
- Easy bruising, unusual bleeding (nose, mouth, vagina, or rectum), purple or red pinpoint spots under the skin;
- Severe sinus pain, swelling, or irritation; or
- Worsening asthma symptoms.

Less serious side effects may include:

- headache;
- stomach pain, heartburn, upset stomach, nausea, diarrhea;
- tooth pain;
- tired feeling;
- fever, stuffy nose, sore throat, cough, hoarseness; or
- mild rash (17)

1.11. DRUG INTERACTIONS

The Montelukast causes significant change in the pharmacokinetics of theophylline, warfarin, immunoreactive digoxin, terfenadine, fexofenadine, oral contraceptives containing norethindrone 1mg and ethinyl esterdiol 35mcg, prednisone, or prednisolone. Combination containing any of the following medications, depending on the amount percent, may also interact with this medication.

Phenobarbital (recent use results in significant decrease [approximately 40%] in bioavailability curve for montelukast, as a result of induction of hepatic metabolism; however no dosage adjustment is required (2).

Montelukast at a dose of 10 mg once daily dosed to pharmacokinetic steady state:

- Did not cause clinically significant changes in the kinetics of a single intravenous dose of theophylline (predominantly a cytochrome P450 1A2 substrate).
- Did not change the pharmacokinetic profile of warfarin (primarily a substrate of CYP 2C9, 3A4 and 1A2) or influence the effect of a single 30-mg oral dose of warfarin on prothrombin time or the INR (International Normalized Ratio).
- Did not change the pharmacokinetic profile or urinary excretion of immunoreactive digoxin.
- Did not change the plasma concentration profile of terfenadine (a substrate of CYP 3A4) or fexofenadine, its carboxylated metabolite, and did not prolong the QTc interval following co-administration with terfenadine 60 mg twice daily.

Montelukast at doses of ≥100 mg daily dosed to pharmacokinetic steady state:

- Did not significantly alter the plasma concentrations of either component of an oral contraceptive containing norethindrone 1 mg/ethinyl estradiol 35 mcg.
- Did not cause any clinically significant change in plasma profiles of prednisone or prednisolone following administration of either oral prednisone or intravenous prednisolone (16).

Thyroid Hormones, Sedative Hypnotics, Non-Steroidal Anti-Inflammatory Agents, Benzodiazepines, and Decongestants: Although additional specific interaction studies were not performed, Montelukast was used concomitantly with a wide range of commonly prescribed drugs in clinical studies without evidence of clinical adverse interactions. These medications included thyroid hormones, sedative hypnotics, non-steroidal anti-inflammatory agents, benzodiazepines, and decongestants.

Cytochrome P450 (CYP) Enzyme Inducers: Phenobarbital, which induces hepatic metabolism, decreased the area under the plasma concentration curve (AUC) of Montelukast approximately 40% following a single 10-mg dose of Montelukast. No dosage adjustment for Montelukast sodium is recommended. It is reasonable to employ appropriate clinical monitoring when potent CYP enzyme inducers, such as phenobarbital or rifampin, are co-administered with Montelukast sodium.

Montelukast is a potent inhibitor of CYP2C8 in vitro. However, data from a clinical drug-drug interaction study involving Montelukast and rosiglitazone (a probe substrate representative of drugs primarily metabolized by CYP2C8) in 12 healthy individuals demonstrated that the pharmacokinetics of rosiglitazone are not altered when the drugs are coadministered, indicating that Montelukast does not inhibit CYP2C8 in vivo. Therefore, Montelukast is not anticipated to alter the metabolism of drugs metabolized by this enzyme (e.g., paclitaxel, rosiglitazone, and repaglinide) (14).

1.12. RECENT STUDY ON MONTELUKAST SODIUM

The first demonstrations of the efficacy of Montelukast in asthma were obtained in the mid-1990s, when the results of both comparative studies of Montelukast versus placebo and studies of the protective effect of Montelukast on bronchoconstriction induced by exercise or other nonspecific stimuli were published (29,30). Montelukast improved symptoms, rescue medication use and pulmonary function, and reduced the rate of exacerbation and the level of blood eosinophils, in mild-to-moderate asthmatics not treated with ICS. Montelukast also protected against bronchoconstriction induced by exercise better than long-acting beta2-agonists (LABAs) (22). These data led to the introduction of Montelukast into the market at the end of the 1990s.

At the same time, the efficacy of Montelukast in rhinitis was evaluated in other studies, which showed that Montelukast was effective and well tolerated with additional benefits over antihistamines, although still less effective than intranasal corticosteroids (31). The following studies were conducted in an attempt to determine the place of Montelukast in asthma treatment.

Nayak A. published an excellent review in Expert Opinion on Pharmacotherapy journal entitled "**A review of montelukast in the treatment of asthma and allergic rhinitis**" (Expert Opinion Pharmacotherapy, 2004; 5(3): 679-86) (32). The review gives a description of Montelukast sodium (Singulair, Mreck) as a selective and orally-active leukoriene-receptor antagonist (LTRA) which inhibites the cysteinyl leukotriene 1 (CysLT 1) receptor. It is one of the effective and most tolerated preventative treatments for asthma and allergic rhinitis in adults and children. Similar inflammatory response to allergen challenge was found for both upper and lower airway. Leukotriene are inflammatory mediator substances that are known as the slow-reacting substances of anaphylaxis produced by a number of cell types including mast cells, eosinophils, basophils, macrophages, and monocytes. Synthesis of these mediators results from the cleavage of arachidonic acid in cell membranes and they exert their biological effects by binding and activating specific receptors. This causes a series of events that lead to contraction of the human airway smooth muscle, chemotaxis and increased vascular permeability. These effects have led to their important role in the diseases of asthma and allergic rhinitis. As these agents lead to the production of symptoms in patients that are asthmatic or allergic, the use of LTRAs, particularly

Montelukast, may seem appropriate. Clinical trials have shown that Montelukast is effective and safe in the patient with such diseases.

According to a study by Okumu A, DiMaso M, Lobenberg R on "**Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug**" (Pharma Res. 2008 Dec; 25(12):2778-85) (33).

A dissolution test method was developed that can predict the oral absorption of Montelukast sodium, and to establish an in vitro/in vivo correlation (IVIVC) using computer simulation. Using different media Drug solubility was measured. The dissolution behavior of Montelukast sodium 10mg film coated tablets was studied using the flow-through cell dissolution method following a dynamic pH change protocol, as well as in the USP Apparatus 2. Computer simulations were performed using GastroPlus. Biorelevent dissolution media (BDM) was prepared using bile salts and lecithin in buffers was used as the dissolution media, as well as the USP simulated intestinal fluid (SIF) pH 6.8 and blank FaSSIF pH 6.5. Dissolution tests in the USP Apparatus 2 were performed under a constant pH condition, while the pH range used in flow-through cells was 2.0-7.5. The in vitro data were used as input functions into GastroPlus to simulate the in vivo profiles of the drug. At low pH the solubility of the Montelukast sodium was low, but with the increase of pH the solubility also increased. No significant difference in the solubility was found in the pH range of 5.0 to 7.5 in blank buffers, but the solubility of the drug was higher in biorelevant media compared with the corresponding blank buffers at the same pH. Using the flow through cells, the dissolution rate was fast in simulated gastric fluid containing 0.1% SLS. The dissolution rate slowed down when the medium was changed to FaSSIF pH 6.5 and increased when the medium was changed to FaSSIF medium at pH 7.5. In the USP Apparatus 2, better dissolution was observed in FaSSIF compared with the USP buffers and blank FaSSIF with similar pH values. Dissolution was incomplete with less than 10% of the drug dissolved in the USP-SIF, and was practically nonexistent in blank FaSSIF .

A study was performed by Fey C, Thyroff-Friesinger, Jones S. et al on "**Bioequivalence of two formulations of montelukast sodium 4mg oral granules in healthy adults**" (34). The aim of the study to compare bioavailability, and characterize the pharmacokinetic profile and safety of Sandoz generic Montelukast 4mg oral granules relative to Singular mini (Merck, Sharp & Dohem). An open-label, randomized, single-dose, two-treatment, two-period, two-sequence, two

way crossover bioequivalence study was performed in healthy male volunteers aged 18-55 years, under fasting conditions. The duration of the clinical part of the trial was almost 11 days. The plasma level of Montelukast was quantified using a validated liquid chromatography tandem mass spectrometry method, and pharmacokinetic parameters calculated from the drug concentration –time profile using a non-compartmental model. A total of 40 subjects completed both study periods. The ratio test/reference of geometric least squares means was calculated for both study formulations of Montelukast for the In-transformed pharmacokinetic parameters; the 90% confidence intervals (CIs) were within the pre-defined limits of 80.00-125.00%: 92.2% (90% Cl:87.42-97.30%) for Cmax, 98.1% (90% Cl:94.49-101.81%) for AUC(0-t) and 97.6% (90%Cl:94.14-101.27%) for AUC(0-∞). Two study subjects each reported one mild adverse event: dyspepsia (possibly related to study medication) and throat pain (not consider related to study medication). Sandoz Montelukast 4mg oral granules are bioequivalent to Singulair 4mg mini oral granules, with a similar safety profile. This suggests that these two preparations can be considered interchangeable in clinical practice.

Mclvor A, Kaplan A, Koch C et al carried out a study on "**Montelukast as an alternative to low-dose inhaled corticosteroids in the management of mild asthma (the SIMPLE trial): An open-label effectiveness trial**" (Can Respir J. 2009 May-Jan; 16 (Suppl A): 11A-16A (35).

The objective of the study was to evaluate the effectiveness of Montelukast as monotherapy for patient with mild asthma who remains uncontrolled or unsatisfied while on inhaled corticosteroid (ICS) monotherapy.

The design of the study included a multicenter, open-label study. Patients (six years of age or older) had ICS therapy discontinued and were treated with orally administered Montelukast once daily for six weeks. The primary outcome measure was the rate at which asthma symptom control was achieved or maintained after six weeks of treatment. The secondary outcome measures were to compare compliance and physician satisfaction, and to further assess the safety and tolerability of montelukast.

Of the 534 patients enrolled, 481 (90.1%) completed the study. Mean (±SD) age was 27.8±19.0 years. The number of patients with uncontrolled symptoms decreased from 455 (85.2%) at baseline to 143 (26.8%) at week 6 ($P<0.001$), and mean Asthma Control Questionnaire score

decreased from 1.4±0.8 to 0.6±0.6 (P<0.001), representing a clinically significant improvement. Of the 79 patients with controlled asthma symptoms at baseline, 73.4% maintained asthma control at week 6. Compliance to asthma therapy increased from 41% at baseline for ICS to 88% at week 6 for Montelukast (P<0.001). Physician satisfaction with treatment increased from 43% to 85% (P<0.001) and patient satisfaction increased from 45% at baseline to 94% at week 6. No serious adverse effects were reported over the course of study. From the study they observed that Montelukast is an effective ad well-tolerated alternative to ICS in patients with mild asthma who are uncontrolled or unsatisfied with low-dose ICS therapy.

A research work was performed by Takeda K, Shiraishi Y, Matsubara S in the year 2010 on "**Effects of combination therapy with montelukast and carbocysteine in allergen-induced airway hyperresponsiveness and airway inflammation"** (36). According to them the selective cysteinyl LT receptor 1 antagonist, Montelukast, has been widely used in the treatment of asthma and has been shown to be effective through the suppression of Th2 cytokine production and airway inflammation.

S-carbocystine was originally introduced as a muciregulator to decrease mucus viscosity and improve mucus clearance, and has been used in the treatment of mucus-associated respiratory diseases such as chronic obstructive pulmonary disease for more than 30 years. Further S-carbocysteine has been shown to have an anti-oxidant effect and inhibitory activity on neutrophil chemotaxis. S-carbocysteine treatment reduced AHR (airway hyperresponsiveness) and inflammatory cell infiltration into the airways through increasing levels of Th1 cytokines.

Thus, S-carbocysteine and Montelukast have distinct activities in reducing allergen-induced airway inflammation and airway dysfunction. There are limitations with the use of either drug alone. Montelukast given alone is not enough to reduce use of inhaled corticosteroids in chilshood asthma patients and S-carbocysteine does not have clear evidence of efficacy in asthma. In this study, the potency of a combination of S-carbocysteine and Montelukast in allergen-induced AHR (airway hyperresponsiveness) and airway inflammation was examined in a secondary allergen challenge model where airway allergic inflammation was established before drug treatment was initiated in an attempt to more closely model the clinical situation.

The finding of the research work was the combination of S-carbocysteine and Montelukast demonstrated additive effects in the prevention of a allergen-induced airway inflammation and AHR (airway hyperresponsiveness) through complementary activities and as such, this combination may be beneficial in the treatment of asthmatics, especially those refractory to treatment with either drug alone and where the use of corticosteroids must be reduced.

Kose E, Beytur A, Dogan Z et al performed a study on "**The effects of montelukast against amikacin-induced acute renal damage**" (Eur Rev Med Pharmacol Sci 2012 Apr; 16(4):503-11) (37).

The objective of the study was to determine the therapeutic and protective effects of Montelukast against amikacin-induced acute renal damage.

35 Wister albino female rats were divided into 5 groups as follows:

Group I: Control

Group II: Control+Montelukast

Group III: Amikacin

Group IV: Amikacin+Montelukast

Group V: Montelukast+Amikacin

At the end of the experiment, the kidney tissues and the blood of rats were collected. Malondialdehyde (MDA), myeloperoxidase (MPO), and reduced glutathione (GSH) levels were determined for kidney tissues. Blood urea nitrogen (BUN), creatinine (Cr), TNF-alpha and IL-1beta levels were assessed in the serum. In addition the kidney tissues were examined histologically.

The MDA, MPO, BUN, and Cr levels of group III significantly increased when compared to groups I and II. These parameters of group IV decreased when compared to group III. In addition, GSH levels significantly increased when compared to the first three groups. MDA, BUN and Cr levels of group V did not reach significant level in comparison with the control group. The most significant histological damage was observed in the group III followed by the

groups IV and V. Immunohistochemically, group III showed a significantly increased apoptotic staining. In group IV, it was observed that Montelukast treatment reduced the expression of apoptotic cells.

A study was conducted by Hardwick C, White D, Morris E et al on "**Montelukast in the treatment of HIV associated immune reconstitution diseases**" (Sex Transm Infact. Dec 2006; 82(6): 513-514) (38).

According to the study the pathogenesis of immune reconstitution diseases (IRD) is not well understood and it can be difficult to manage. Leukotrienes exert proinflammatory effects, have an important role in the innate immune response, and are relatively deficient in HIV infection. They report a series of three patients with severe HIV associated IRD, who obtained clinically dramatic responses to treatment with montelukast. The first case is of IRD to secondary syphilis and resolve on restarting. Montelukast should be consider in HIV associated IRD as an alternative to steroids and where these are not effective. Leukotriene overactivity may be implicated in IRD.

Naser A, Natour S, Qaddomi A et al researched on "**Formulation and in vitro and in vivo evaluation of film-coated montulukast sodium tablets using Opadry yellow 20A82938 on an industrial scale**" (Drug Des Devel Ther.2013; 7: 83-91). (39) The purpose of the study was to formulate stable film-coated montelukast sodium (MS) tablets using Opadry yellow 20A82938 and to evaluate their in vitro and in vivo release profile.

Montelukast sodium core tablets were manufactured using a direct compression method. Opadry yellow 20A82938 aqueous coating dispersion was used as the film-coating material. Dissolution of the film-coated tablets was tested in 900 ml of 0.5% sodium lauryl sulfate solution and the bioequivalence of the tablets was tested by comparing them with a reference formulation-Singulair tablets. In vitro-in vivo correlation was evaluated. The stability of the obtained film-coated tablets was evaluated according to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines.

The efficiency of the film coating was determined by subjecting the coated tablets to gastric pH and drug release was analyzed using high-performance liquid chromatography. The coated tablets had no obvious defects. Montelukast sodium release met the study criterion of not less

than 80% dissolved after 30 minutes in 0.5% sodium lauryl sulfate solution. Statistical comparison of the main pharmacokinetic parameters clearly indicated no significant difference between test and reference in any of the calculated pharmacokinetic parameters. Level A correlation between in vitro drug release and in vivo absorption was found to be satisfactory.

The findings suggest that aqueous film coating with Opadry yellow 20A82938 is an easy, reproducible and economical approach for preparing stable montelukast sodium film-coated tablets without affecting the drug-release characteristics.

Ahmed B, Abdalla A. conducted a research on "**Comparison of FT-NIR Transmission and HPLC to Assay Montelukast in Its Pharmaceutical Tablets**" (American Journal of Analytical Chemistry, 2011, 2, 885-891) (40). They use near-infrared spectroscopy (NIRS) as an analytical technique. The goal of this study is to show the capacity of this new technique to assay the active ingredient in low-dosage tablets. NIR spectroscopy is a rapid, non-destructive technique and does not need any sample preparation. A prediction model was built by using a partial least square regression fit method. The NIR assay was performed by transmission. The results obtained by NIR spectroscopy were compared with the conventional HPLC method for Montelukast tablets produced by Sigma pharmaceutical corp. The study showed that Montelukast tablets can be individually analyzed by NIR with high accuracy. It was shown that the variability of this new technique is less important than that of the conventional method which is the HPLC with UV detection.

According to a study on "**A simple spectrophotometric assay of Montelukast in Pharmaceutical formulations**" (J. Chem. Pharm. Res., 2011, 3(6):23-27) by Srihari G, Nagaraja K, Rami N (41), a simple and sensitive spectrophotometric method was developed by them for the estimation of Montelukast by formation of ion pair complex with wool fast blue. The ion pair complex is formed by the interaction of drug with wool fast blue. Wool fast blue is insoluble in water and soluble in chloroform. The organic layer is extracted from chloroform and the absorbance of organic layer is measured at 585 nm against chloroform blank. Montelukast and wool fast blue was treated with chloroform in the pH 1.5 to form ion pair complex. The complex is extracted from the chloroform layer. The absorption spectral analysis shows that the

maximum of absorbance of Montelukast was found to be 585 nm. The absorbance of blue chloroform layer is measured at 585 nm against reagent blank.

Chapter-2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1. MATERIALS

The materials used throughout the study are listed in Table 2.

Serial no.	Materials
01.	Tablets of ten different Bangladeshi pharmaceutical company
02.	Syringe
03.	Filter paper
04.	Beaker
05.	Volumetric flask
06.	Measuring cylinder
07.	Filter funnel
08.	Mortar and pestle

Table-2. List of Materials Used for Study

2.2. REAGENTS

2.2.1. Reagents for disintegration test

The reagents used for disintegration test is shown in Table 3.

Serial No.	Reagents
01.	Distilled water

Table-3. Reagent for Disintegration Test

2.2.2. Reagents for dissolution test

The reagents used for dissolution test is shown in Table 4.

Serial No.	Reagents
01.	Acetonitrile
02.	Distilled water
03.	Glacial acetic acid
04.	Sodium lauryl sulfate
05.	Active drug(standard)
06.	Methanol
07.	Ammonium acetate

Table-4. List of Reagents for Dissolution Test

2.3. EQUIPMENTS

The equipments used throughout the study are listed in Table 5.

Name of the Equipment	Model	Manufacturer	Country of Origin
01. Analytical Balance	ATX Series Max Cap: 210g, Readability: 0.001g	OHAUS Corp. pine Brook,	USA
02. Hardness tester	Monsanto Hardness Tester. Model: EH-01. (Braking force tester USP-12)	Electrolab.	India
03. Friabilator.	EF-FRIABILATOR	Electrolab.	India

Name of the Equipment	Model	Manufacturer	Country of Origin
04. Disintegration machine	ED-2L	Electrolab.	India
05. Dissolution machine	UDT-804-8	LOGAN	USA
06. pH meter	Seven compact S220-K	Mettler-Toledo	Switzerland
07. HPLC	SPD-M20A, (Prominence Diode array detector). DGU-20A5R (degassing unit). LC-20AT, (prominence Liquid chromatography) SIL-20AHT, (prominence auto sampler). CTO-10ASVP, (column oven).	SHIMADZU	Japan

Table-5. List of Equipments Used for Study

Figure-2. Analytical Balance (ATX Series)

Figure-3. Monsanto Hardness Tester

Figure-4. EF- Friabilator

Figure-5. Disintegration machine (ED-2L)

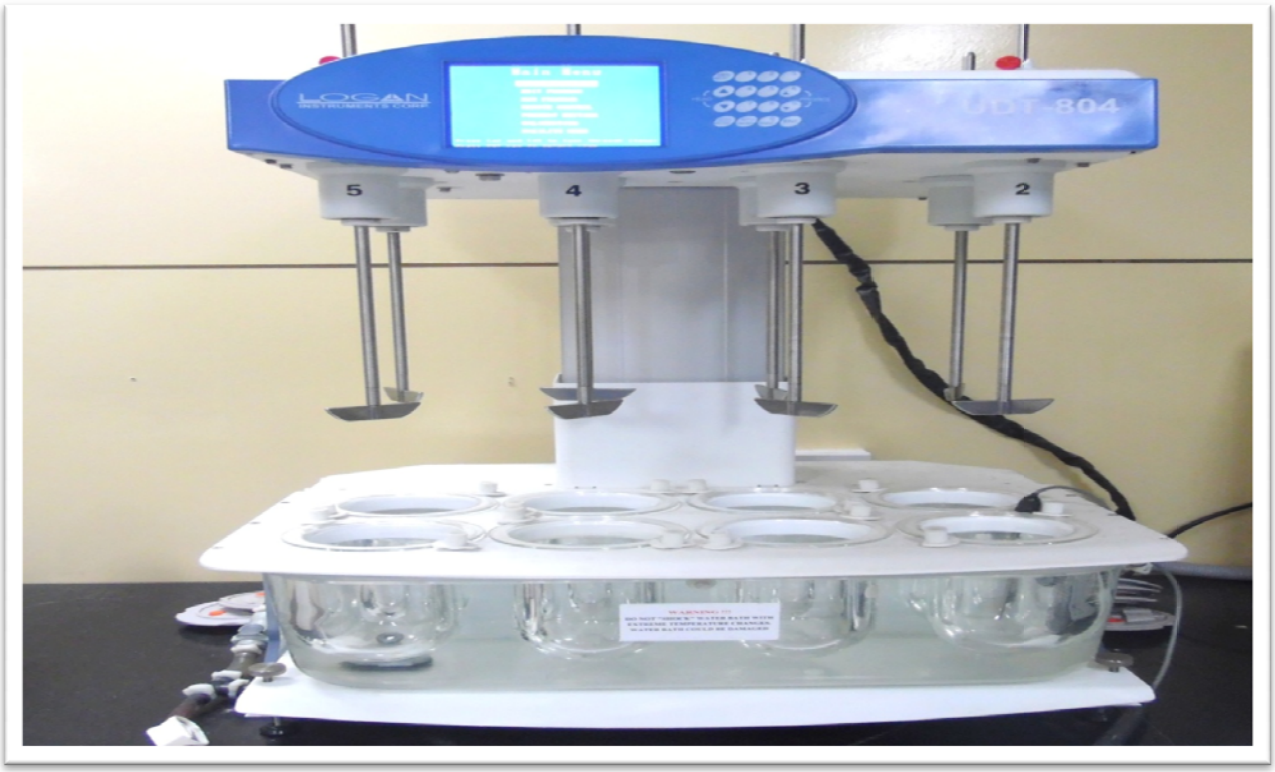

Figure-6. Dissolution Tester (UDT-804-8)

Figure-7. pH meter (Seven compact S220-K)

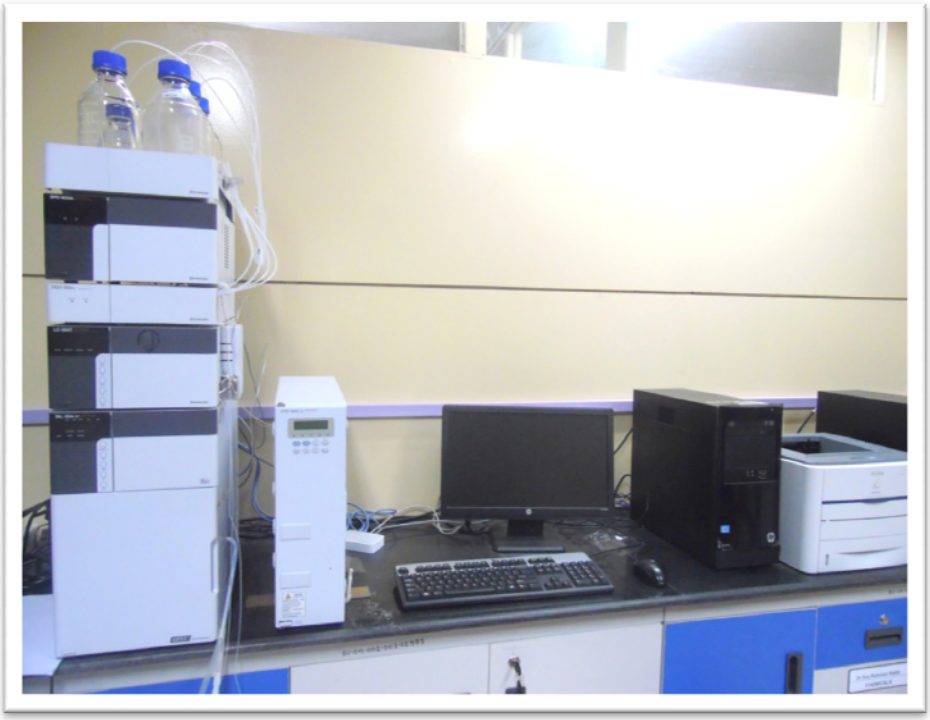

Figure-8. Shimadzu HPLC Prominence Liquid Chromatogram

2.4. METHODS

Samples of Montelukast sodium tablets from total ten different companies were taken from top ranked to middle and lower ranked pharmaceutical companies by considering their popularity and in terms of sales in the local market. The leading pharmaceutical companies have been represented as A-E respectively, the middle ranked companies are designated by F, G, H and the low ranked companies as I and J respectively. All the companies were from Bangladeshi pharmaceutical companies and the following quality control tests were performed.

2.4.1. Weight variation test

Weight variation test is performed to determine the uniformity of the tablet weights.

The weight of the tablet is the quantity of tablet granules that contain the labeled amount of therapeutic ingredient. After the tablets are prepared the weights are checked regularly to ensure the acclaimed weight of the tablet.

20 tablets were taken and weighed properly. Then the average weight was determined which was the standard weight of an individual tablet. Weight of each tablet was taken separately and observed whether the individual tablets were within the range or not.

2.4.2. Hardness test

Hardness test is done to find out the hardness of tablets by using hardness tester.

Too soft tablets can disintegrate while transportation. Too hard tablets could be a problem too as it can damage the teeth and will take more time to disintegrate within the body. An acceptable hardness is required and tablet strength testing is necessary for both research and development of new formulations and for quality control of the tablet formulation.

First of all the sliding scale of the hardness tester was adjusted by bringing it to zero. Then the tablet was placed vertically between the two jaws of the hardness tester. Force was applied by rotating the screw thread and spring of the tester until the tablet fractured. The reading form hardness tester was taken and was displayed in kilogram/cm^2 (kg/cm^2). The process was repeated

for about ten times with ten different tablets of the same variety. Then the average hardness of the tablet was calculated.

2.4.3. Friability test

Friability test is done to determine how well tablets will stand up to coating, packaging, shipping and other mechanical and processing conditions.

Friability is the measurement of the tendency of a tablet to crack, crumble or break when compressed. This tendency is usually confined to uncoated tablet and surfaces during handling or subsequent storage.

Weight of 10 tablets was taken and considered as initial weight and then they were placed in the section 1 of drum of the friability tester and rotated at 25 rpm for 4 minutes and the count was set to 100. Then the tablets were reweighed and considered as a final weight. Percentage of loss was counted. According to USP, percentage loss of weight should not be more than 1%.

% loss= (Initial weight-Final weight)/Initial weight×100

2.4.4. Disintegration test

The objective of disintegration test is to determine whether tablets or capsules disintegrate within the prescribed time when placed in a liquid medium in the experimental conditions.

First of all the disintegration tester was assembled. 600ml of distilled water was taken in each 1000ml beaker. The temperature was maintained at 37°C. In each of the 6 tubes one tablet was placed. The switch button was turned on and the time taken for the tablet to disintegrate was noted down.

Disintegration is considered to be achieved when there is:

- No residues remain on the screen, or
- If there is a residue, it consists of a soft mass having no palpably firm, unmoistened core, or

- Only fragments of coating (tablets) or only fragments of shell may adhere to the lower surface of the disc.

2.4.5. *In vitro* Dissolution test

Dissolution test is an *in vitro* technique of great importance in formulation and development of pharmaceutical dosage forms as it can be used to measure the percentage of drug release as a function of time which reflects either reproducibility of the product manufacturing process and can predict in certain cases, *in vivo* drug release.

2.4.5.1. Preparation of ammonium acetate buffer

3.85gm ammonium acetate was taken into 1000ml of distilled water. Then pH was adjusted to 3.5 using glacial acetic acid.

2.4.5.2. Preparation of 0.5% sodium lauryl sulfate solution (dissolution medium)

This solution served as a dissolution medium. For each liter of distilled water 5g of sodium lauryl sulfate was required. Sodium lauryl sulfate was poured slowly into the distilled water without shaking. It took some time to completely dissolve and make a clear solution.

2.4.5.3. Preparation of Stock Solution

12.5 mg of Montelukast sodium was taken in a 100 ml clean and dry volumetric flask containing 1 ml of methanol. When the drug is dissolved then dissolution media was added up to the mark to make the stock solution of standard (100 μg/ml).

2.4.5.4. Parameters of the Analytical method for dissolution

The parameters of the analytical method used for dissolution is summarized in Table 6.

Equipment	Shimadzu HPLC Prominence Liquid Chromatogram Integrated with PDA Detector
Column	C-18 column
Mobile phase	Ammonium acetate(pH=3.5): methanol=15:85
Diluting solution	Dissolution media
Temperature	Room temperature (RT)
Flow rate	1.5ml/min
Monitoring wavelength	254nm
Injection volume	10 µl
Retention time	10 minutes (approx).

Table-6. Parameters of the HPLC Analytical method used for dissolution

2.4.5.5. Preparation of Calibration curve

0.8, 0.9,1.0, 1.1 and 1.2 ml of 100 µg/ml of Montelukast sodium solution were taken into 5 different 10 ml volumetric flasks and dissolution media was added up to the mark to produce 10.0, 11.25, 12.5, 13.75 and 15 µg/ml Montelukast sodium solutions respectively. The solutions were filtered through 0.2µ disk filter and transferred into clean & dry HPLC vials. Then the solutions were injected consecutively into the HPLC machine and the chromatograms were recorded.

Concentration (µg/ml)	Peak Area
10.00	123568
11.25	147752
12.50	162814
13.75	174182
15.00	193305

Table-7. Peak Areas of Various Concentrations of Standard Solutions

Calibration Curve of Standard was constructed by plotting Peak Area versus Concentration using Microsoft Office Excel 2007 software. The calibration curve is shown in Figure-9 Linearity was observed in the concentration range from 10 – 15µg/ml with a correlation coefficient greater than 0.98.

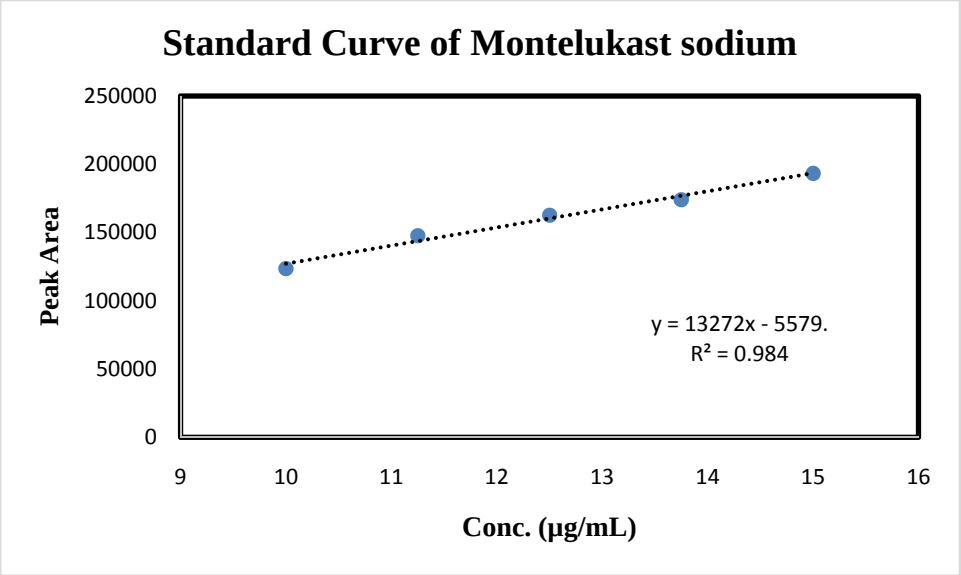

Figure-9. Standard Calibration curve for Montelukast sodium

2.4.5.6. Dissolution study

900ml of dissolution medium was placed into each vessel and the apparatus was assembled. Then the medium was allowed to equilibrate to a temperature of 37±0.5°C. One tablet was placed into each vessel, covered and the apparatus was operated at the specified rate. After 30 minutes, a definite volume of dissolution medium was withdrawn and filtered with 0.45µm filter paper. Then this solution was filtered through 0.2µ disk filter and placed into HPLC vials. After that, the solutions were injected consecutively into the HPLC machine and the chromatograms were recorded.

Dissolution media	0.5% SLS solution in distilled water
Apparatus	Dissolution machine
Starring speed	50rpm
Time	30 minute
Temperature	$37^{o}C \pm 0.5°C$

Table-8. Dissolution Specifications of Montelukast tablets

The peak areas of dissolution sample solutions were substituted in the equation of standard calibration curve in order to calculate the concentrations of Montelukast sodium in the sample test solutions.

The equation derived from the standard calibration curve is as follows -

$$y = 13272x - 5579.8$$

Where,

y = Peak area

x = Concentration in µg/ml

Finally, the percentage release of drug was calculated using the following equation -

% of dissolution of Montelukast sodium =

$$\frac{\text{Conc. of Montelukast sodium in sample (µg/ml)} \times 900 \text{ (ml)} \times Y \times 100}{100000 \text{ (µg)} \times 100}$$

Where, Y = Potency of Montelukast sodium (Working standard) = 99.9 %

Chapter-3

RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1. Weight variation test

The results of weight variation test (in grams) of the ten chosen pharmaceutical companies (A-J) of Bangladesh are as follows:

Sample No.	A	B	C	D	E	F	G	H	I	J
01.	0.178	0.337	0.170	0.205	0.153	0.277	0.297	0.243	0.185	0.313
02.	0.178	0.353	0.169	0.207	0.154	0.259	0.290	0.247	0.185	0.310
03.	0.177	0.353	0.171	0.206	0.149	0.253	0.300	0.245	0.189	0.315
04.	0.178	0.355	0.173	0.207	0.156	0.256	0.297	0.245	0.184	0.312
05.	0.175	0.360	0.173	0.207	0.153	0.287	0.299	0.247	0.183	0.312
06.	0.177	0.353	0.169	0.206	0.158	0.237	0.294	0.243	0.175	0.313
07.	0.177	0.355	0.168	0.207	0.155	0.246	0.300	0.243	0.189	0.311
08.	0.175	0.346	0.175	0.208	0.153	0.245	0.299	0.244	0.187	0.314
09.	0.177	0.350	0.171	0.208	0.157	0.250	0.305	0.245	0.183	0.317
10.	0.173	0.356	0.173	0.207	0.144	0.262	0.298	0.245	0.182	0.310
11.	0.173	0.352	0.169	0.209	0.153	0.263	0.301	0.247	0.183	0.317
12.	0.176	0.350	0.167	0.205	0.157	0.263	0.297	0.244	0.180	0.310
13.	0.175	0.336	0.168	0.206	0.151	0.260	0.295	0.245	0.188	0.319
14.	0.175	0.357	0.170	0.209	0.152	0.260	0.304	0.246	0.186	0.311
15.	0.173	0.356	0.169	0.211	0.151	0.258	0.300	0.250	0.183	0.306
16.	0.173	0.349	0.173	0.207	0.158	0.255	0.301	0.246	0.187	0.310
17.	0.177	0.354	0.170	0.205	0.143	0.253	0.296	0.247	0.191	0.312
18.	0.174	0.358	0.170	0.205	0.152	0.252	0.300	0.247	0.185	0.313
19.	0.176	0.349	0.171	0.204	0.158	0.252	0.305	0.244	0.186	0.308
20.	0.173	0.363	0.170	0.209	0.150	0.271	0.305	0.245	0.184	0.311
Mean± SD	0.17± 0.01	0.35± 0.01	0.17± 0.002	0.2± 0.001	0.15± 0.004	0.25± 0.01	0.3± 0.003	0.24± 0.001	0.184± 0.003	0.31± 0.003

Table-9. Results of weight variation test

Note : SD=Standard deviation

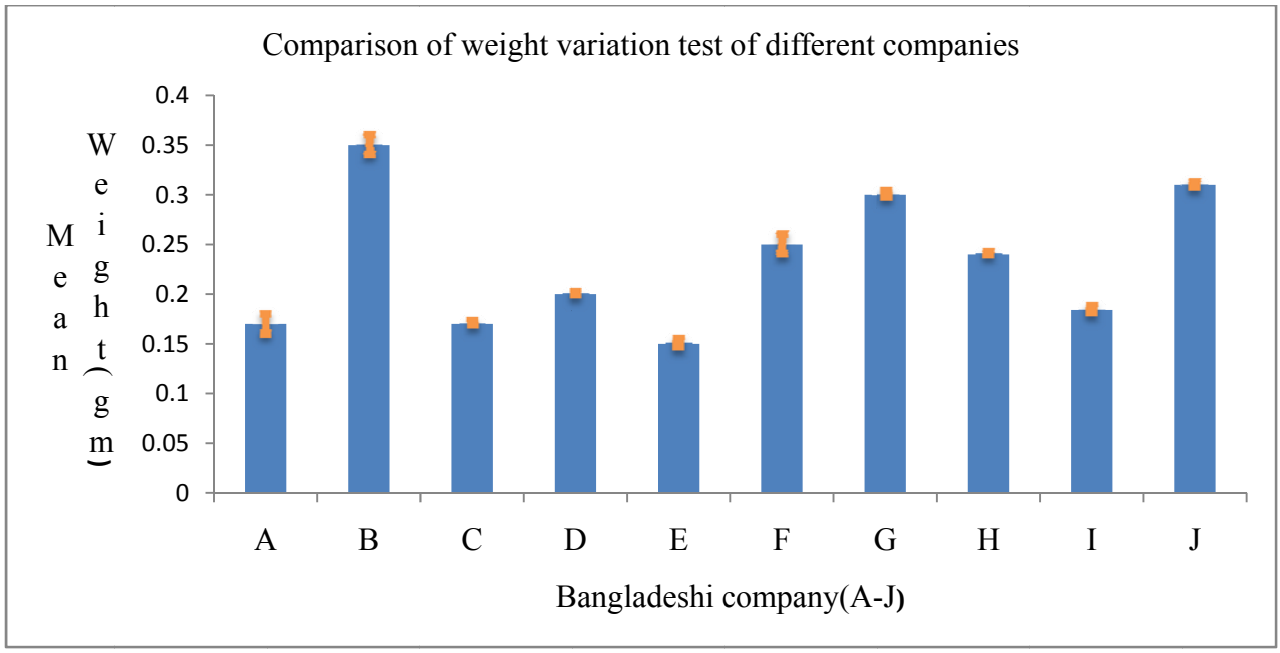

Figure-10. Comparison of weight variation test of different companies

3.1.2. Hardness test

The results of the hardness test (in kg/cm^2) of the ten chosen pharmaceutical company of Bangladesh are as follows:

Sample No.	A	B	C	D	E	F	G	H	I	J
01.	6.43	11.73	11.08	9.22	7.84	4.69	6.15	12.05	5.22	17.87
02.	7.16	10.55	8.9	8.49	8.21	6.39	6.71	13.75	6.59	17.55
03.	5.34	9.45	10.55	7.84	7.48	3.64	7.6	12.33	4.49	17.51
04.	4.97	7.88	8.61	9.26	8.45	9.46	6.87	12.86	5.26	16.38
05.	2.75	10.76	7.56	7.28	6.59	8.21	7.04	11.93	5.38	18.03
06..	6.83	10.31	9.83	8.25	6.51	6.79	6.75	12.78	6.71	14.35
07.	5.42	10.71	10.35	8.33	6.95	7.16	6.13	12.57	5.46	17.59
08.	5.3	5.5	11.4	7.6	8.25	6.23	5.58	11.64	4.97	14.76
09.	5.42	10.8	9.14	6.67	4.69	8.49	7.16	12.57	5.46	14.72
10.	6.63	12.57	10.67	7.4	8.21	8.53	7.28	12.33	4.49	16.94
Mean± SD	5.6± 1.2	10± 1.02	9.8± 1.2	8.03± 0.8	7.3± 1.1	6.9± 1.8	6.7± 0.6	12.48± 0.6	5.4± 0.7	16.57± 1.4

Table-10. Results of Hardness test

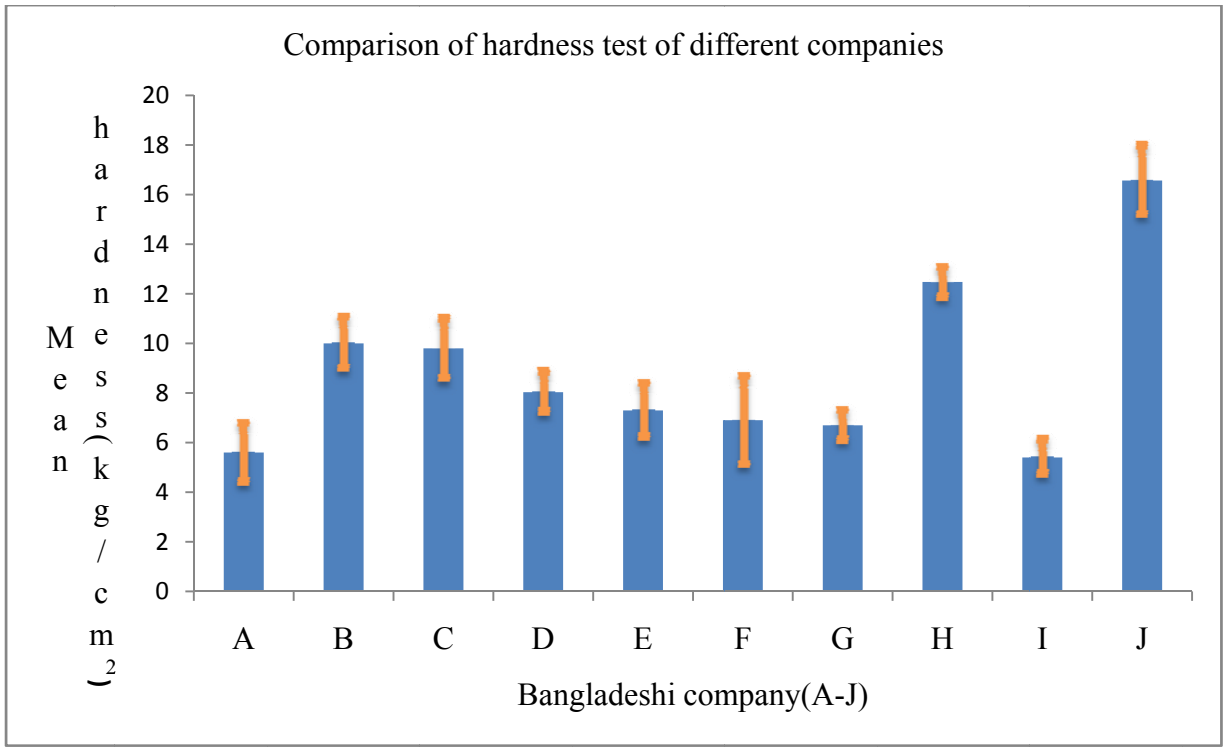

Figure-11. Comparison of hardness test of different companies

3.1.3. Friability test

The results of friability test of the ten chosen pharmaceutical company of Bangladesh are as follows:

	A	B	C	D	E	F	G	H	I	J
Initial weight of 10 tablets(gm)	1.8	3.546	1.698	2.075	1.556	2.548	2.979	2.453	1.837	3.123
Final weight of 10 tablets(gm)	1.798	3.542	1.693	2.069	1.554	2.541	2.972	2.446	1.835	3.119
% loss	0.11	0.11	0.3	0.28	0.12	0.27	0.23	0.28	0.11	0.12

Table-11. Results of Friability test

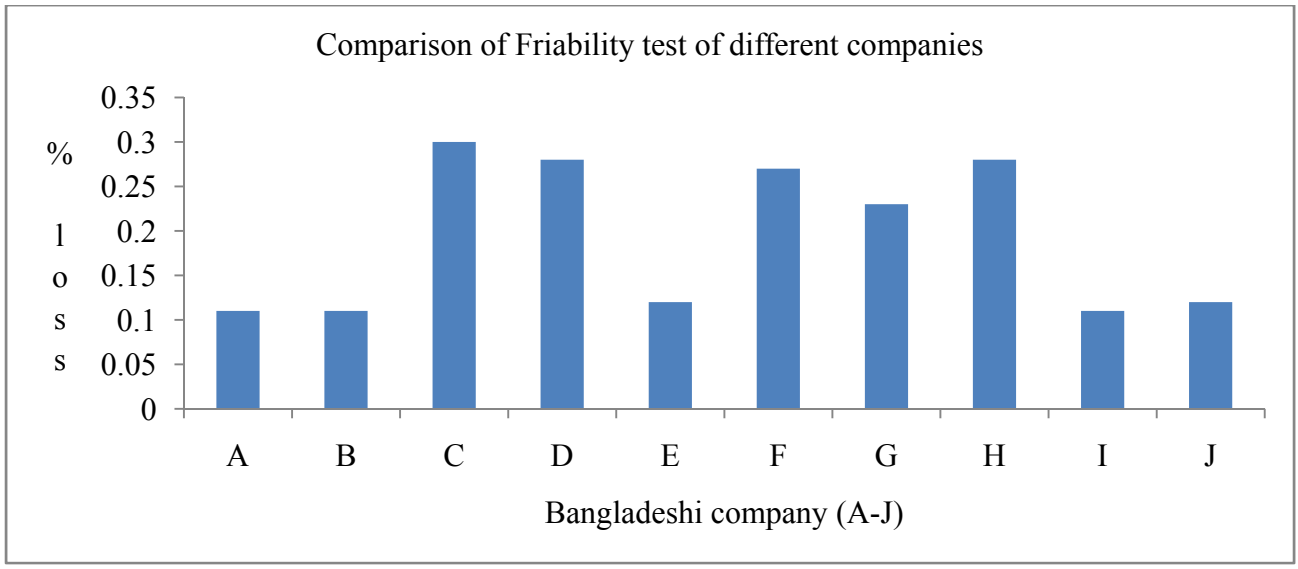

Figure-12. Comparison of Friability test of different companies

3.1.4. Disintegration test

The results of disintegration test (in minutes) of the ten chosen pharmaceutical company of Bangladesh are as follows:

Sample No.	A	B	C	D	E	F	G	H	I	J
01.	6.02	2.17	2.45	0.59	6.12	7.3	6.04	7.25	5.21	5.57
02.	6.50	2.2	3.8	0.59	7.05	7.5	6.15	7.3	6.25	5.57
03.	7.27	2.6	3.12	1.14	7.36	9.38	7.0	7.35	9.3	6.5
04.	8.07	3.15	3.25	1.44	7.56	9.34	7.0	7.44	10.44	6.25
05.	8.29	4.08	3.25	2.55	8.09	10.37	7.43	7.44	13.5	6.16
06.	8.36	4.08	4.2	3.11	8.3	10.35	8.21	7.5	14.1	6.4
Mean± SD	7.40± 0.9	3.05± 0.8	3.2± 0.6	1.57± 1.04	7.4± 0.78	9.4± 1.17	6.97± 0.8	7.38± 0.1	9.8± 3.6	6.1± 0.41

Table-12. Results of Disintegration test

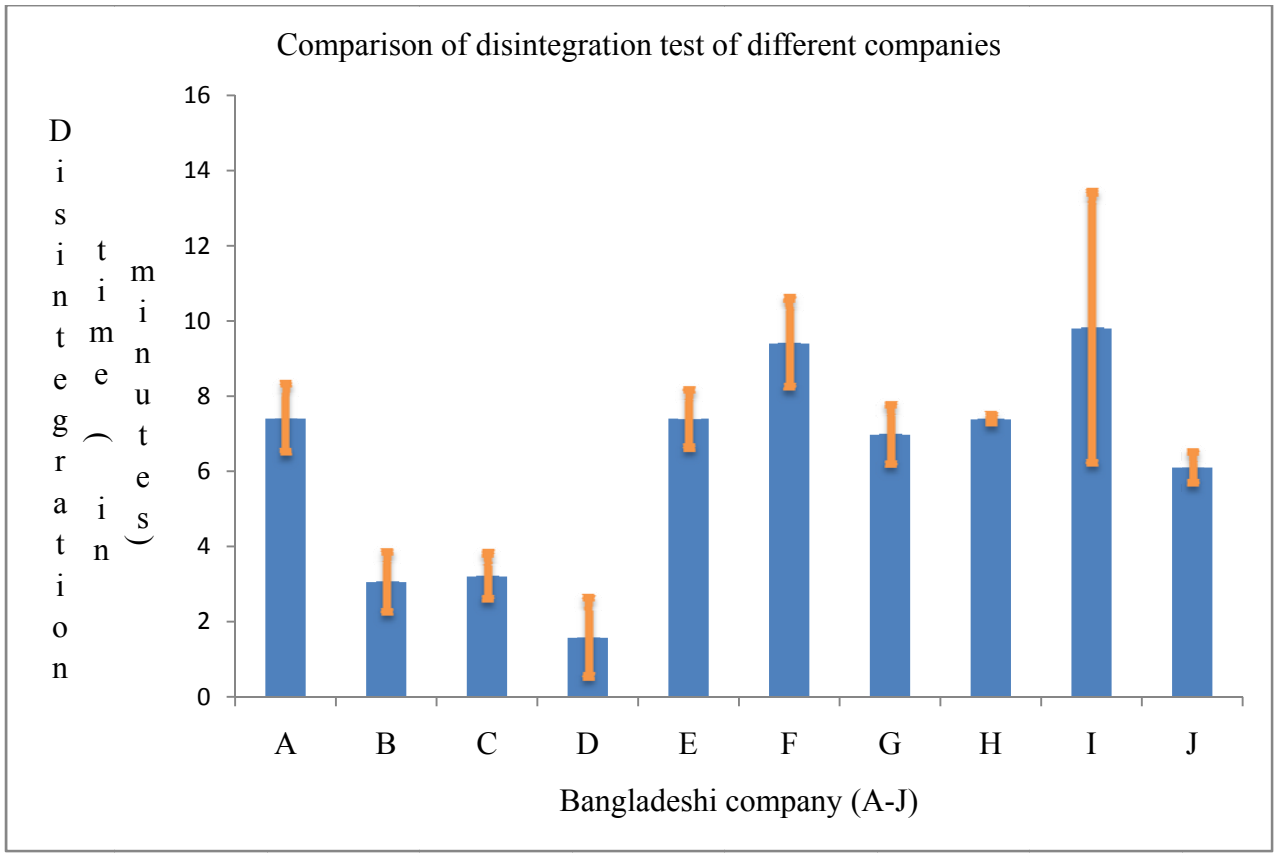

Figure-13. Comparison of disintegration test of different companies

3.1.5. Dissolution Test

The result of the dissolution test of the six samples for each company (Company A-J) has been shown in Tables 13-22 for the pharmaceutical companies A-J respectively and their corresponding chromatograms have been shown in Figures 14-23. Figure 24 highlights the differences in percentage of dissolution for all the ten pharmaceutical companies.

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.185	1.057	7605	6966	122297	9.6	86.6
02.	10.191	1.064	7612	7043	124520	9.8	88.1
03.	10.122	1.063	7627	7485	130430	10.2	92.1
04.	10.038	1.070	7700	7584	131335	10.3	92.8
05.	9.843	1.067	7857	8137	136847	10.7	96.5
06.	9.803	1.068	7806	8171	136960	10.7	96.6
Average	**10.030**	**1.065**	**7701.17**	**7564.33**	**130398**	**10.2**	**92.1**

Table-13. Quantitation of Montelukast sodium for market sample A

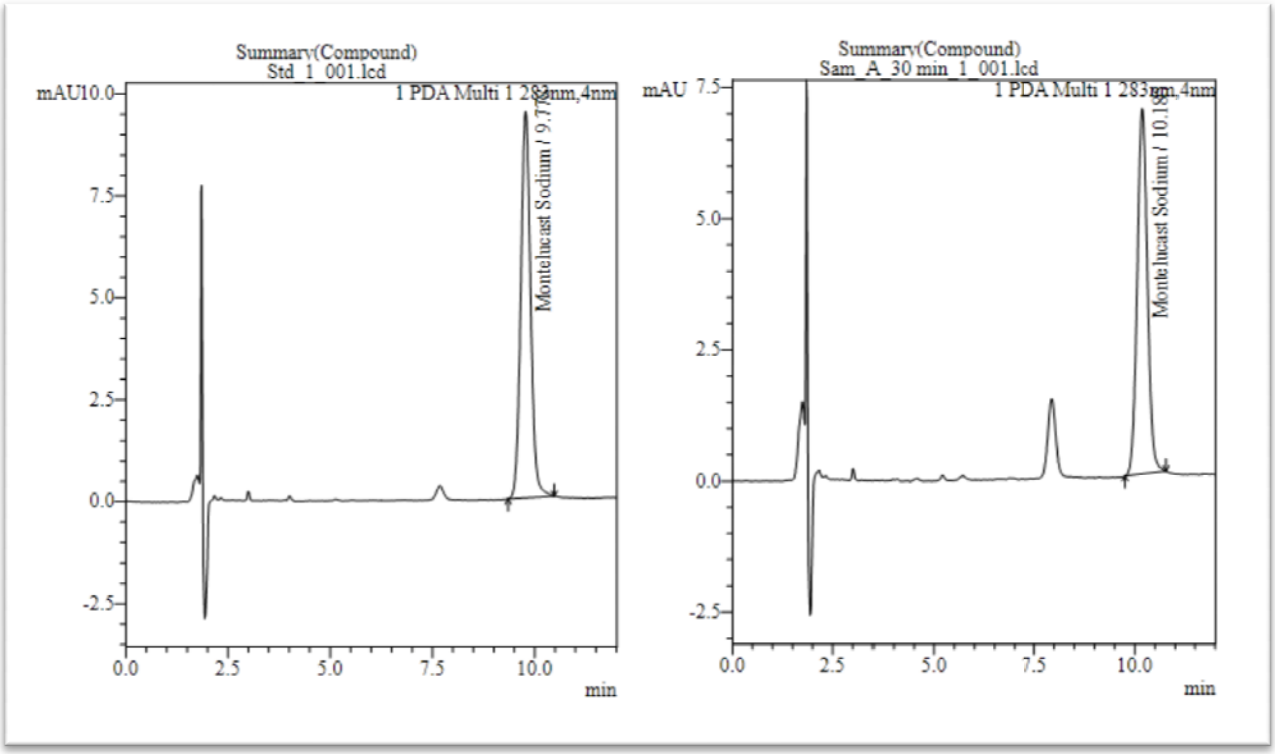

Figure-14. Chromatogram for standard and sample A

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.487	1.068	7642	8422	153400	12.0	107.7
02.	10.433	1.066	7548	8477	157141	12.3	110.2
03.	10.370	1.075	7552	8322	146248	11.4	102.9
04.	10.336	1.067	7629	8340	153332	12.0	107.7
05.	10.288	1.064	7517	8347	143430	11.2	100.9
06.	10.256	1.049	7550	8357	146674	11.5	103.1
Average	**10.362**	**1.064**	**7573**	**8377.5**	**150038**	**11.7**	**105.4**

Table-14. Quantitation of Montelukast sodium for market sample B

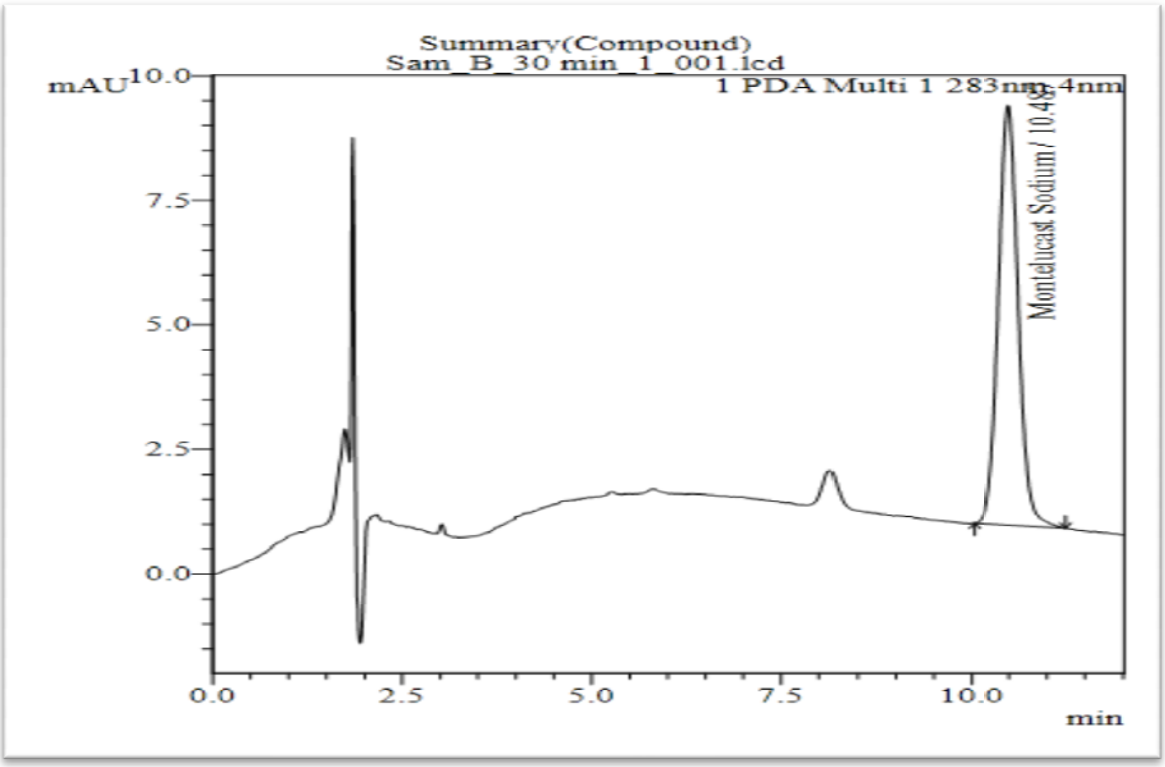

Figure-15. Chromatogram for sample B

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.184	1.044	7679	8386	145475	11.4	102.3
02.	9.750	1.061	7872	8536	152737	11.9	107.3
03.	10.263	1.051	7568	8352	147738	11.6	103.9
04.	10.347	1.054	7512	8522	153474	12.0	107.7
05.	10.443	1.052	7542	8470	148843	11.6	104.6
06.	10.670	1.055	7613	8530	151198	11.8	106.2
Average	**10.276**	**1.053**	**7631**	**8466**	**149911**	**11.7**	**105.3**

Table-15. Quantitation of Montelukast sodium for market sample C

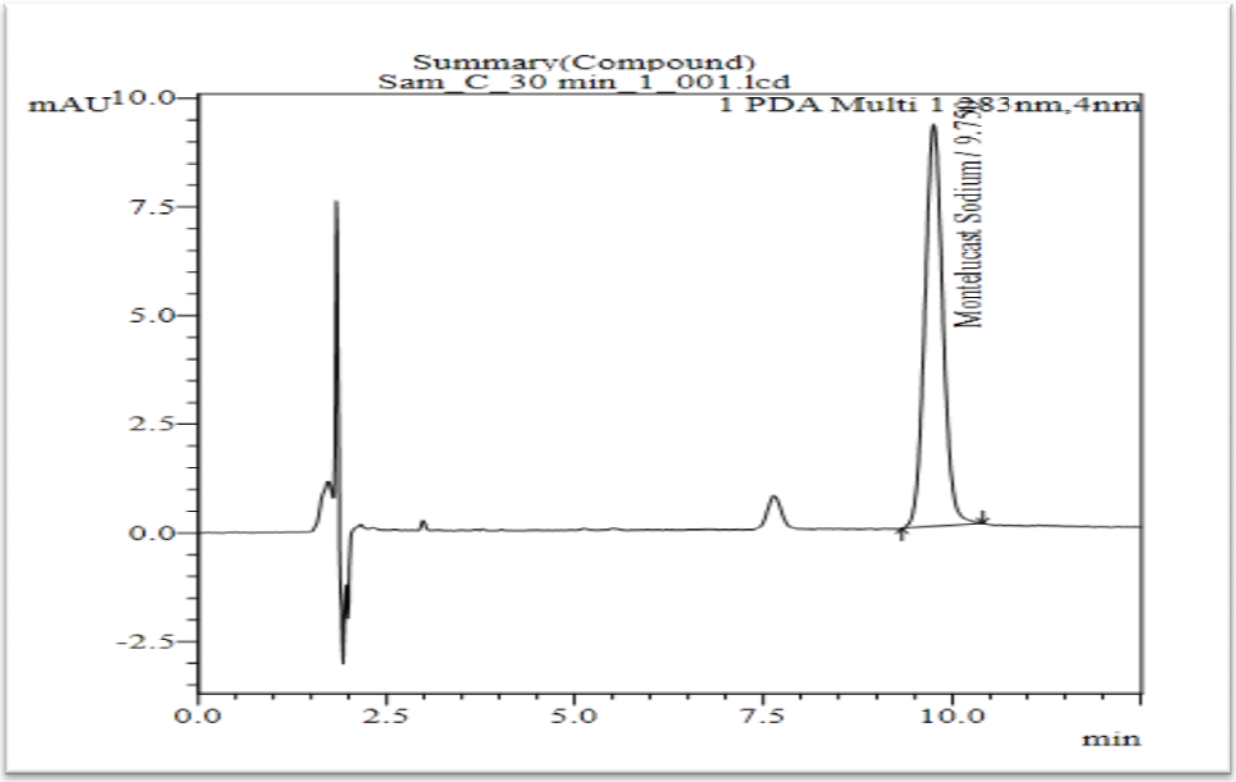

Figure-16. Chromatogram for sample C

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.386	1.082	7727	8740	156786	12.2	110.0
02.	10.358	1.076	7736	8718	155632	12.1	109.2
03.	10.078	1.084	7651	8914	155667	12.1	109.2
04.	10.075	1.084	7715	8927	155576	12.1	109.2
05.	10.155	1.075	7578	9002	158953	12.4	111.5
06.	10.205	1.080	7530	8969	160245	12.5	112.3
Average	**10.209**	**1.080**	**7656.17**	**8878.3**	**157143**	**12.3**	**110.2**

Table-16. Quantitation of Montelukast sodium for market sample D

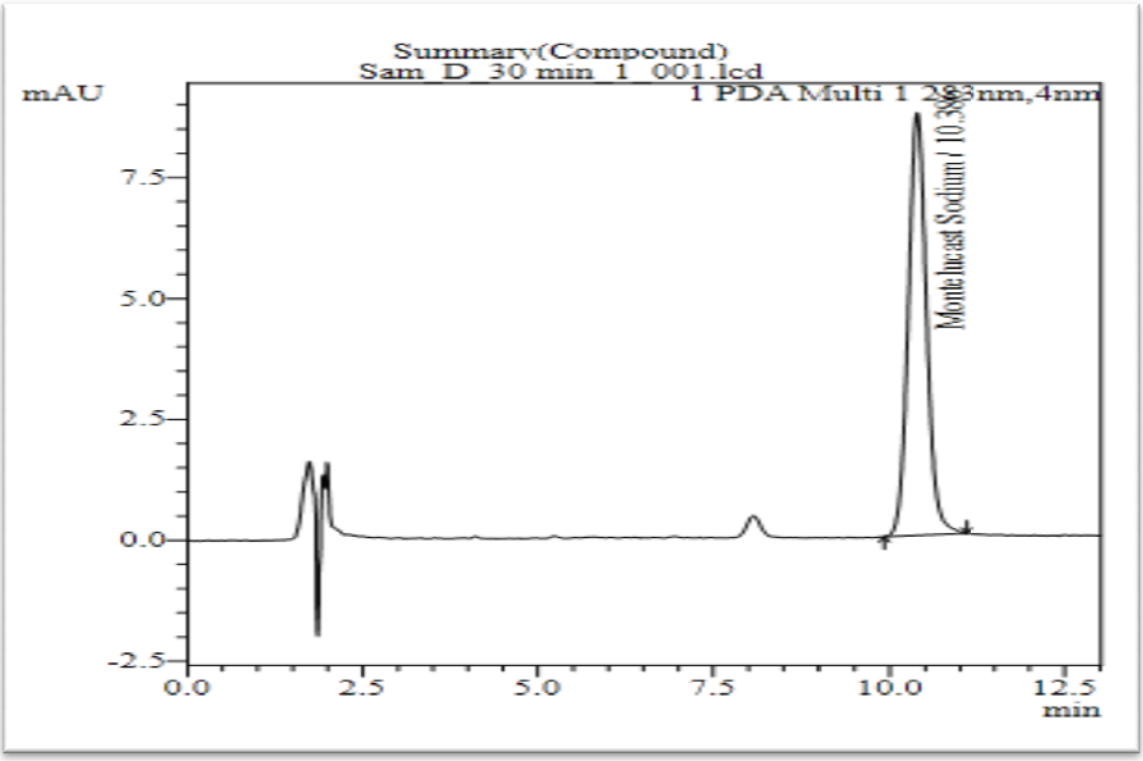

Figure-17. Chromatogram for sample D

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.023	1.087	7440	8689	153282	12.0	107.6
02.	10.048	1.084	7526	8739	153700	12.0	107.9
03.	10.024	1.082	7495	8740	153789	12.0	108.0
04.	9.974	1.078	7550	9233	159985	12.5	112.2
05.	9.994	1.072	7520	9200	159738	12.5	112.0
06.	9.724	1.084	7902	7441	122781	9.7	87.0
Average	**9.964**	**1.081**	**7572.1**	**8673.7**	**150546**	**11.8**	**105.8**

Table-17. Quantitation of Montelukast sodium for market sample E

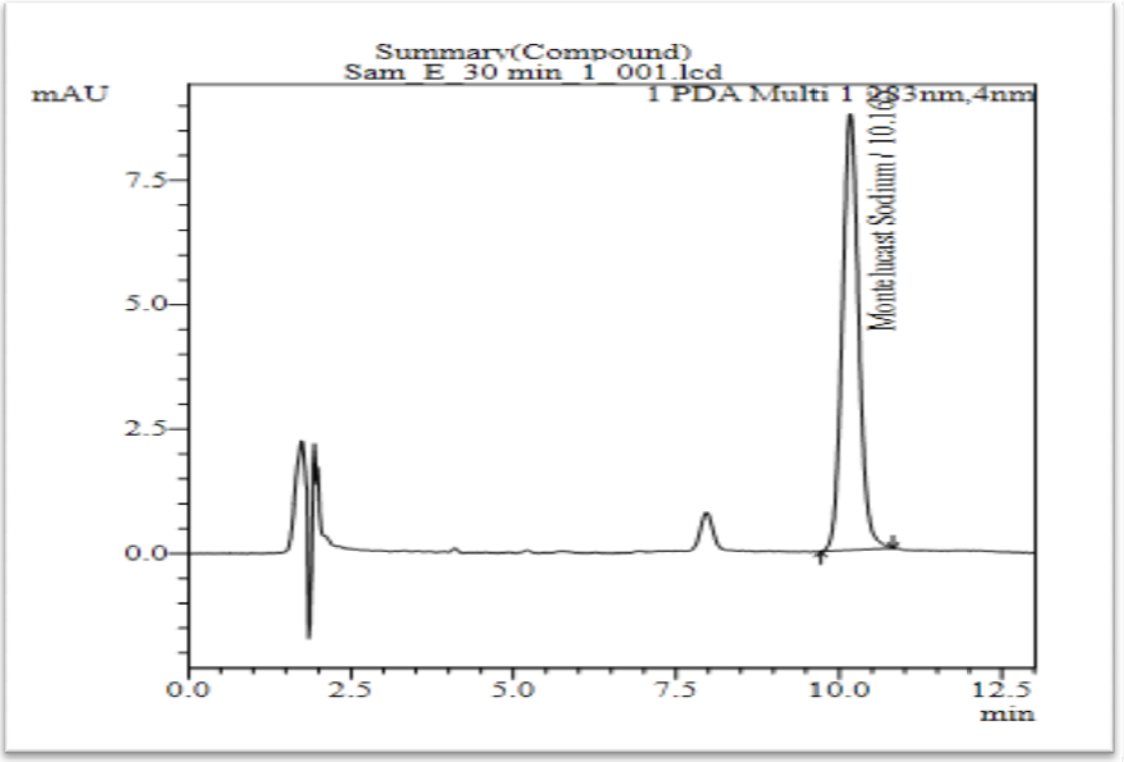

Figure-18. Chromatogram for sample E

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	9.632	1.081	7978	9302	151861	11.9	106.7
02.	9.566	1.094	7984	6853	111509	8.8	79.3
03.	9.514	1.089	8025	7386	119444	9.4	84.7
04.	9.483	1.092	7982	8011	128958	10.1	91.1
05.	9.443	1.084	8232	8532	130269	10.2	92.0
06.	9.564	1.091	8020	8007	125743	9.9	89.0
Average	**9.533**	**1.088**	**8036.8**	**8015.2**	**127964**	**10.1**	**90.5**

Table-18. Quantitation of Montelukast sodium for market sample F

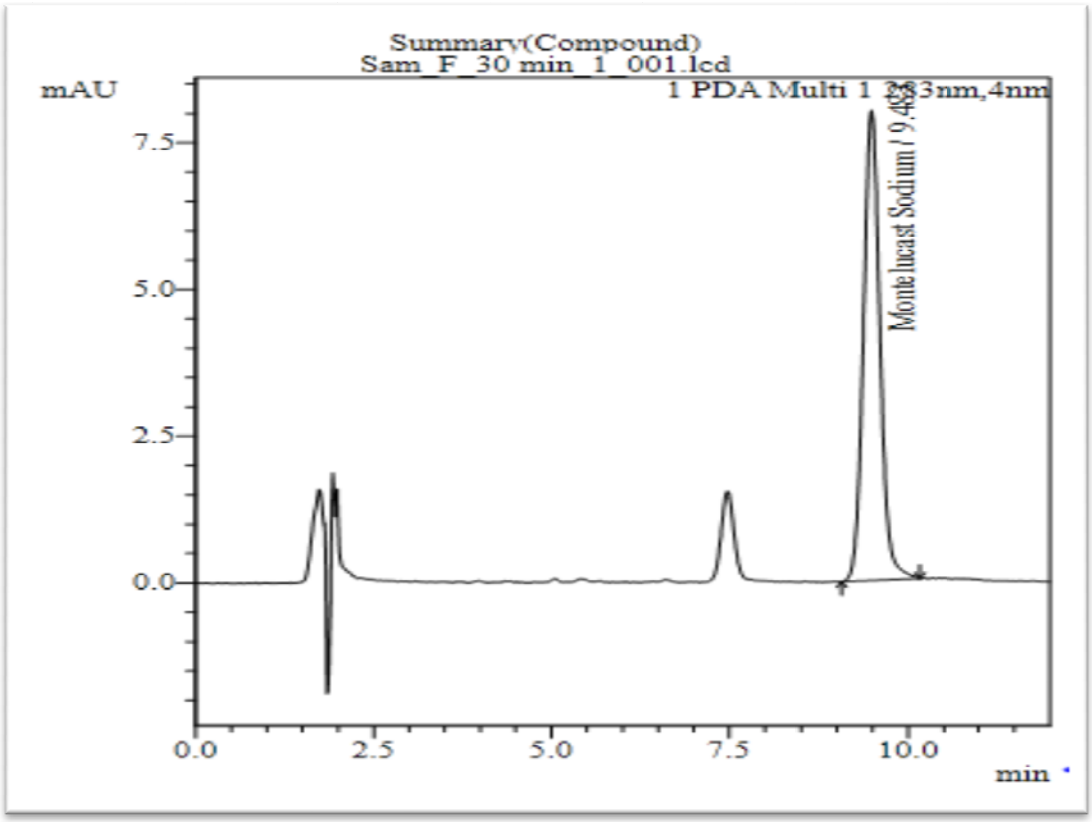

Figure-19. Chromatogram for sample F

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.163	1.061	7730	9551	166501	13.0	116.6
02.	10.049	1.050	7843	8804	149388	11.7	105.0
03.	9.844	1.059	7850	9764	163310	12.7	114.4
04.	9.791	1.068	7814	9990	167904	13.1	117.5
05.	10.031	1.054	7856	9995	168716	13.1	118.1
06.	9.992	1.063	7796	9320	158594	12.4	111.2
Average	**9.983**	**1.059**	**7814.9**	**9570.6**	**162402**	**12.7**	**113.8**

Table-19. Quantitation of Montelukast sodium for market sample G

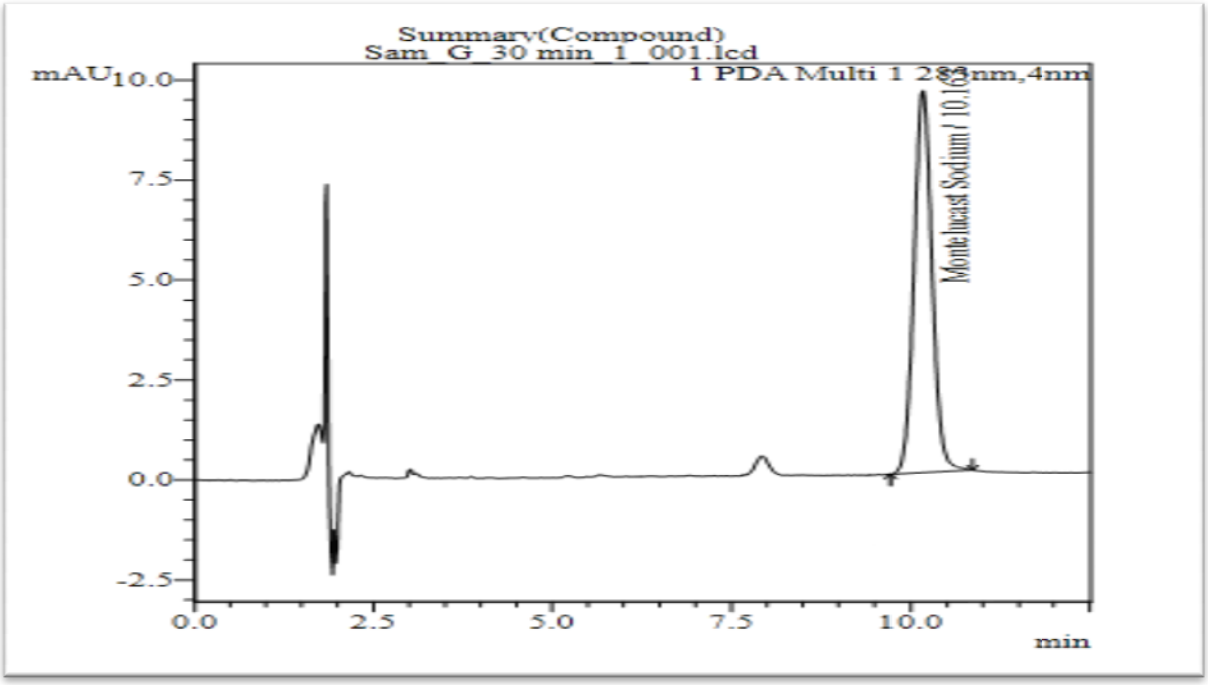

Figure-20. Chromatogram for sample G

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.517	1.046	7340	8071	148738	11.6	104.5
02.	10.623	1.052	7430	8488	157506	12.3	110.5
03.	10.459	1.056	7555	7647	138277	10.8	97.5
04.	10.308	1.057	7665	7468	132116	10.4	93.3
05.	10.355	1.055	7226	7886	143576	11.2	101.0
06.	10.469	1.053	7670	7540	133920	10.5	94.5
Average	**10.455**	**1.053**	**7481**	**7850**	**142356**	**11.1**	**100.2**

Table-20. Quantitation of Montelukast sodium for market sample H

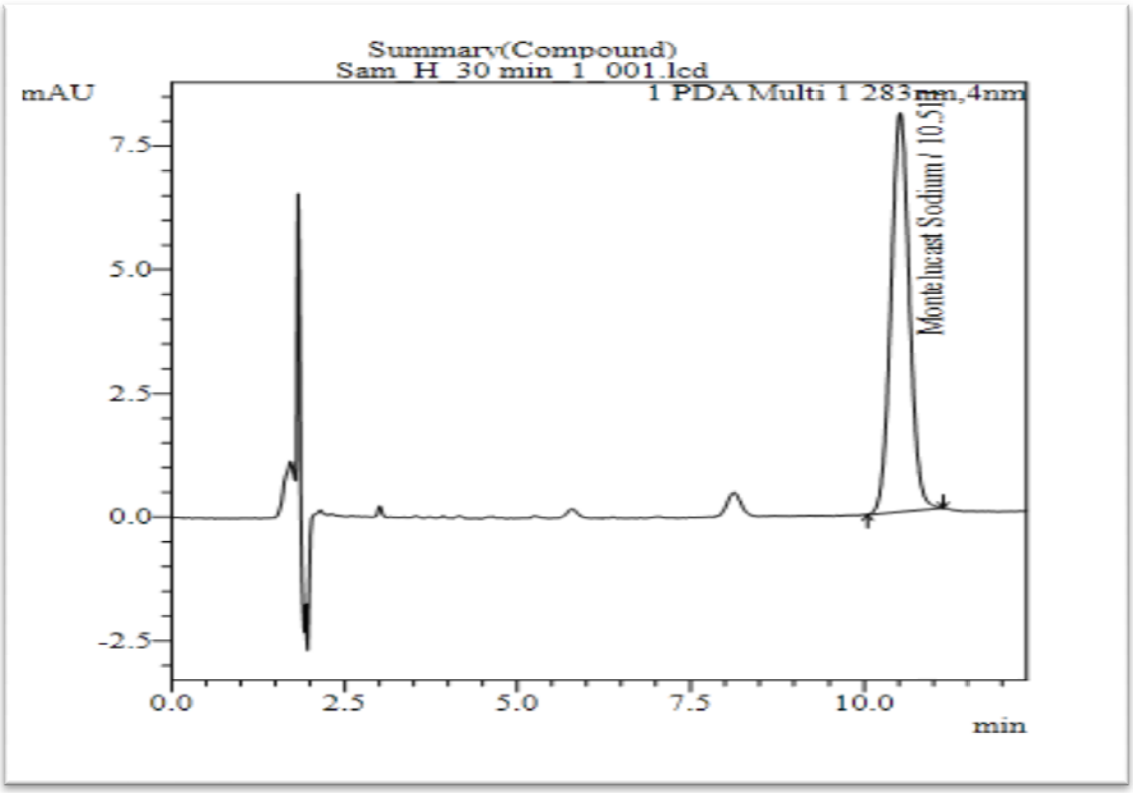

Figure-21. Chromatogram for sample H

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	9.470	1.078	8064	8077	128724	10.1	91.0
02.	9.473	1.087	8018	8089	129673	10.2	91.6
03.	9.463	1.088	7995	8638	138604	10.9	97.7
04.	9.461	1.081	8084	8674	137997	10.8	97.3
05.	9.461	1.088	8076	8731	139685	10.9	98.4
06.	9.437	1.090	7986	8699	139409	10.9	98.2
Average	**9.461**	**1.085**	**8037.2**	**8484.6**	**126636**	**10.6**	**95.7**

Table-21. Quantitation of Montelukast sodium for market sample I

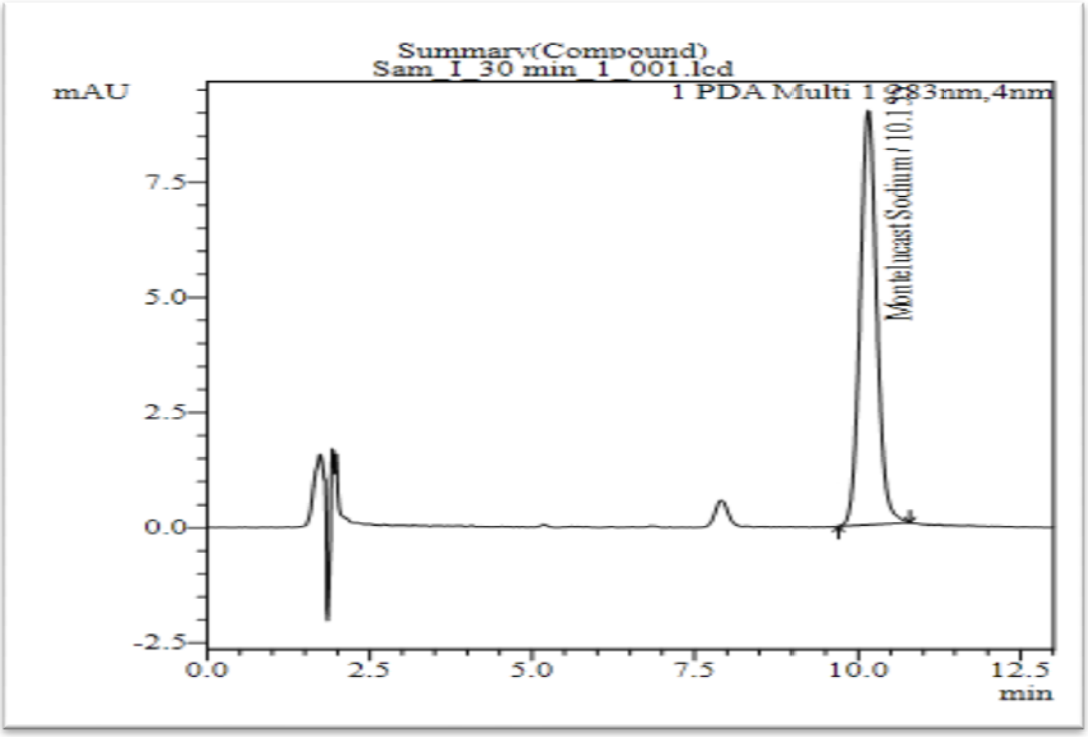

Figure-22. Chromatogram for sample I

Sample no.	Retention time	Tailing Factor	Theoretical Plate	Height	Peak Area	Concentration of Montelukast sodium in sample obtained from standard curve (µg/ml)	% of Dissolution
01.	10.190	1.064	7568	9502	168258	13.1	117.8
02.	10.206	1.062	7645	9081	160406	12.5	112.4
03.	10.223	1.048	7645	9507	166156	12.9	116.3
04.	10.223	1.049	7665	9130	159860	12.5	112.1
05.	10.213	1.052	7589	9423	163482	12.7	114.5
06.	10.198	1.057	7623	9116	161897	12.6	113.5
Average	**10.211**	**1.055**	**1622.5**	**9293.16**	**163670**	**12.7**	**114.4**

Table-22. Quantitation of Montelukast sodium for market sample J

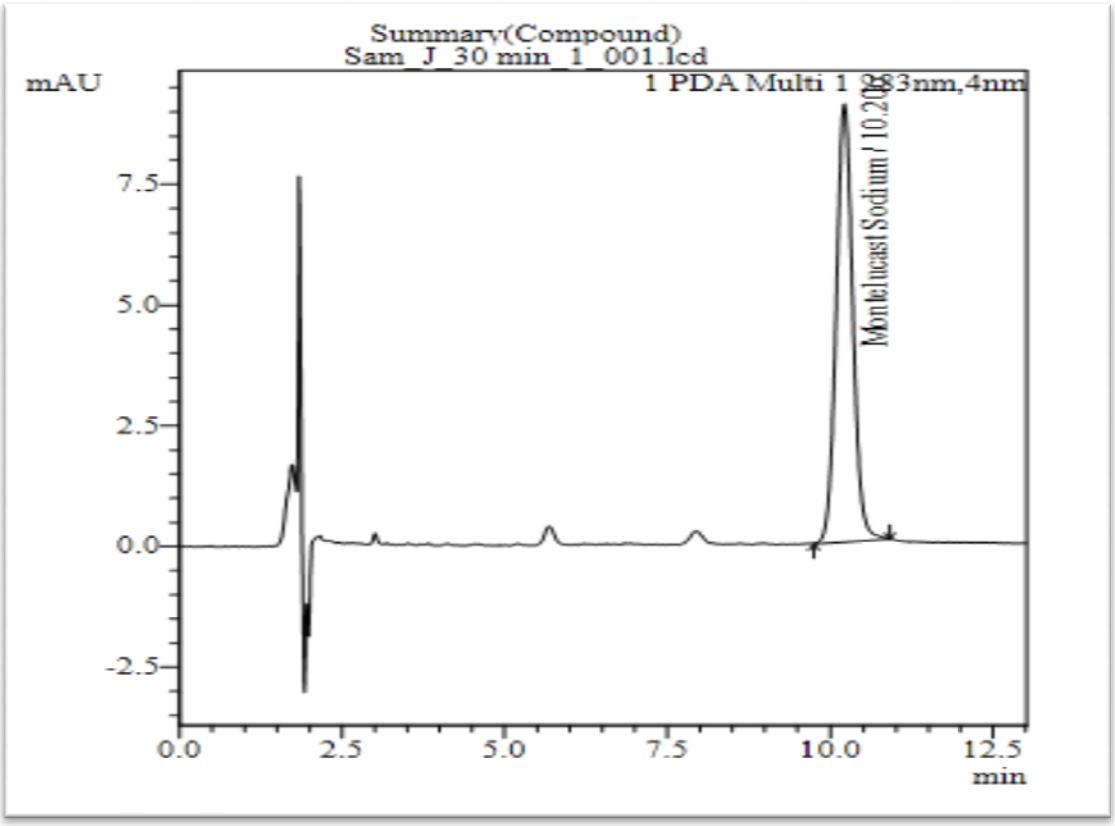

Figure-23. Chromatogram for sample J

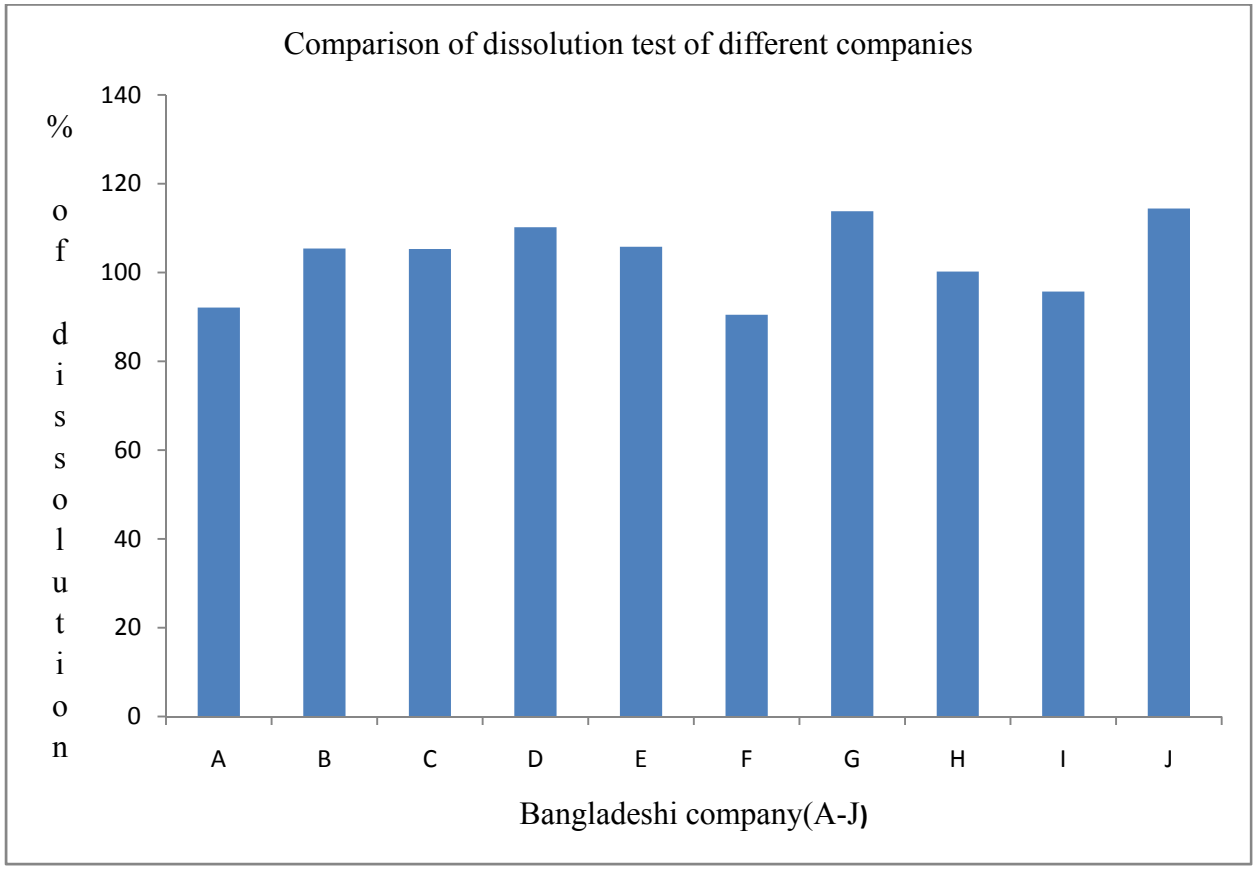

Figure-24. Comparison of dissolution test of different companies

3.2. DISCUSSION

3.2.1. Weight variation test

From the results of weight variation test of the ten different companies, it is apparent that the range of values is between 0.17±0.01 gm to 0.35±0.01 gm. Most of the leading companies have similar values ranging from 0.17±0.01 gm to 0.2±0.01gm except for one company B which represents a value of 0.35±0.01 gm. The middle ranked companies (F, G, H) showed weight variation results slightly higher than the leading companies ranging from 0.24±0.001 gm to 0.3±0.003 gm. The lower selling companies (I and J) showed varying results (0.184±0.003 gm and 0.31±0.003 gm respectively). Hence, it can be concluded that there was hardly any variation in the result of variation test among the leading companies selling Montelukast sodium in Bangladesh and the middle and lower ranked companies showed slightly different results.

3.2.2. Hardness test

The results of hardness test showed that the leading companies show different values ranging from 5.6±1.2 kg/cm^2 to 10±1.02 kg/cm^2. Company B, C, D showed very similar values. For Company E the value was closer to company B, C and D (7.3±1.1 kg/cm^2). However, for company A the value (5.6±1.2 kg/cm^2) differed greatly from the values of the other four dominating companies of Bangladesh. On the contrary the middle ranked companies F and G showed almost same values of hardness (6.9±1.8 kg/cm^2 and 6.7±0.6 kg/cm^2) respectively but the company H revealed twice the value (12.48±0.6 kg/cm^2) compared to company F and G. Among the lower ranked companies I and J, company I showed a good value of hardness (5.4±0.7 kg/cm^2) but company J showed a very high value of hardness (16.57±1.4 kg/cm^2) indicating that greater force will be required to break the Montelukast tablets of company J that may lead to slight higher disintegrating time which is undesirable. Therefore it can be pointed out that except for company H and J; the other companies gave acceptable values of hardness.

3.2.3. Friability test

The values of percentage of loss of Montelukast sodium for the ten Bangladeshi companies ranges from 0.11 % to 0.3 %. The percentage loss of values for the leading companies A, B and E were very similar (0.11% and 0.22%) and that of companies C and D were slightly higher compared to company A, B and E (0.3% and 0.28% respectively). The middle ranked companies

F, G and H showed similar values (0.27%, 0.23% and 0.28% respectively) as well and these values are slightly higher than the values shown by company A, B and E but almost same as company C and D. The low-ranked companies I and J demonstrated values as same as that found for the top-ranked companies A and E. Hence, it can be concluded there was hardly much difference in results of friability test of the ten companies, thus signifying that the tablets produced by the different companies of Bangladesh have sufficient mechanical strength to withstand the pressure due to processing, storage and shipment.

3.2.4. Disintegration test

The time taken for the Montelukast sodium tablets of the leading companies to disintegrate was found to be highly variable ranging from 1.57±1.04 minutes to 7.4±0.9 minutes. Company D showed the least time taken for disintegration (1.57±1.04 minutes). Company A and E showed equal time for disintegration (7.4±0.9 minutes and 7.4±0.78 minutes respectively). Similarly the tablets of company B and C has shown close values for disintegration (3.05±0.8 minutes and 3.2±0.6 minutes). The disintegration time of the middle-ranked companies F, G, H were slightly higher compared to the top companies with F taking the highest time for disintegration (9.4±1.17 minutes), compared to the other companies. The low-ranked companies I and J also demonstrated more time for disintegration and gave values that closely resemble that of the middle-ranked companies. Therefore, it can be concluded that tablets of company D (one of the leading company) has the lowest disintegration time and F (middle-ranked company) company and I (low-ranked company) revealed the highest disintegration time, indicating that company D has shown the best result in terms of disintegration time. Among the leading companies, company B and C has shown acceptable results but company A has shown a result similar to the middle and low-ranked company which was not expected.

3.2.5. Dissolution test

All the tablets were studied according to British Pharmacopeia and US FDA guidelines for INN drugs for *in vitro* dissolution of Montelukast tablets (42,43). Percentage of drug released in the dissolution medium was calculated following the analytical method proposed by Naga *et al.* (44) represented in Table-7. Quantification of the released drug content was performed by calibration curve method (45).

A simple high performance liquid chromatography method was used to determine the *in vitro* release of ten different brands of Montelukast sodium available in Bangladesh. Montelukast sodium was analyzed using Luna 5µ C-18 column (250×4.6mm i.d.). The mobile phase was ammonium acetate pH 3.5: methanol (15:85) at flow rate of 1.5ml/min. The retention time was approximately 10 minutes. Detection was carried out at 254nm at room temperature. The method was found to be linear within the range of 10-15mg/ml.

The concentration of each of the six samples for each company was calculated using the equation derived from the standard curve y=13272x-5579.8 where y denotes the peak area and x denotes the concentration (µg/ml). The average value for concentration of Montelukast sodium was considered for each pharmaceutical company (A-J) and it was put in the following equation to determine the percentage of drug released after 30 minutes.

% of dissolution of Montelukast sodium =

$$\frac{\text{Conc. of Montelukast sodium in sample (µg/ml)} \times 900\ \text{(ml)} \times Y \times 100}{100000\ (\mu g) \times 100}$$

where, Y = Potency of Montelukast sodium (Working standard) = 99.9 %

All the companies have good dissolution profiles showing greater than 90% of release of drug. The leading companies A-E showed consistent results for drug release. Company A demonstrated slightly lower release of drug (92.1%) compared to other four leading companies (B, C, D and E). Company B, C and E showed almost the same results for percentage of drug release whereas company D showed a slightly greater value. The middle ranked company F indicated lower drug release (90.5%) compared to company G (113.8%), and company H (100.2%) which are companies of the same rank. The low-ranked company I showed that there was 95.7% release of drug after 30 minutes and for company J % release of drug was 114.4%, clearly indicating a marked difference between these two companies. Overall, it can be concluded from the dissolution study that all these companies manufacturing Montelukast sodium shows acceptable dissolution profile and complies with the specifications of British Pharmacopeia. The high values of percentage release of drug for company D, company G and company J may be attributed to personal error during running of the

experiment or it may be assumed that the companies may have used greater proportion of active pharmaceutical ingredient in the dosage form to increase the shelf-life of the product.

CONCLUSION

The quality control parameters of ten different brands of Montelukast tablets available in Bangladesh were evaluated and compared to assess the quality of the tablets. Quality control tests such as weight variation, friability, hardness as well as disintegration tests were performed. *In vitro* dissolution study was carried out and analyzed by HPLC to determine the percentage release of drug after 30 minutes which may reflect *in vivo* performance of the drug. The weight variation test results showed that there was hardly any variation among the leading pharmaceutical companies (value ranging 0.17±0.01 gm to 0.2±0.01 gm) except for company B (0.35±0.01 gm) and the middle and lower ranked company showed slightly higher results. The tablets of all the ten companies showed acceptable values of hardness except for one low-ranked company J with a high value of 16.57±1.4kg/cm^2. There was a marginal difference in the result of the friability test of the all the ten companies (all values less than 1% according to BP specification), signifying that the Montelukast tablets produced by the different companies of Bangladesh have sufficient mechanical strength to withstand the pressure due to processing, storage and shipment. Disintegration times of the tablets of leading companies were found to be within 3 minutes indicating a very good result except for company A (7.40±0.9 minutes). Company F (middle-ranked company) and company I (low-ranked company) showed the highest disintegration times (9.4±1.17 minutes and 9.8±3.6 minutes respectively). Consequently, the percentage release of drug for company A, company F and company I are less compared to other companies as shown by the dissolution study. Nevertheless, all the companies showed greater than 90% dissolution of drug after 30 minutes, thus complying with the specifications of British Pharmacopeia and US FDA guidelines for INN drugs. Hence, it can be concluded that the Montelukast tablets produced by the pharmaceutical companies in Bangladesh are of consistent quality with very little variation among them and complies with the specifications of British Pharmacopeia.

REFERENCES

01. Wang K, Birring S, Taylor K et al. Montelukast for postinfectious cough in adults: a double-blind randomised placebo-controlled trail. *The Lancent Respiratory Medicine*. 2014; 35-43.
02. Karonen T, Neuvonen P.J, Backman J. T. CYP2C8 but not CYP3A4 is important in the pharmacokinetics of montelukast. *Br J Clin Pharmacol*. 2012; 73(2): 257–267
03. Raghavendra R, Abdul M, Panchal A. A novel approach to sustained Montelukast sodium release: Differentlly coated mini-tablets in HPMC capsules. *PG Department of Pharmaceutics*.2011; 1-3
04. Casale T.B. and Burks A.W. Hymenoptera-Sting Hypersensitivity. *N Engl J Med*. 2014; 370:1432-1439
05. Belley L, Serge L, Patrick R. Unsaturated hydroxyalkylquinoline acids as leukotriene antagonists. *Merck Frosst Canada Inc*. 1991; 16-18
06. NHLBI Guideline 2007, pp. 11–12
07. Singulair package insert (MSD-US), Revised 2/98, Rec 3/13/98
08. Available at: http://www.rjpbcs.com/pdf/2011_2%283%29/33.pdf.
09. Kenyon & Kenyon LLP, New York, NY, 10004, US.2002.
10. Felton P, Linda A, Stuart C. An update on pharmaceutical film coating for drug delivery. *Expert Opinion on Drug Delivery*. 2013; 10 (4): 421–35.
11. Shangraw R, Mitrevij A, Shah M. A new era of tablet disintegration. *Pharma Technol*. 1998; 4:49-47
12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12214893
13. Vanheek M, France C F and Compton D S. In vivo metabolism-based discovery of a potent cholesterol absorption inhibitor, SCH58235, in the rat and rhesusmonky through the identification of the active metabolites of SCH48431. *Pharmacol Exp Ther.* 1997; 283: 157-159.

14. U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894 National Institutes of Health, Health & Human Services. Last updated: 17 November 2014. Available at: http://www.nlm.nih.gov/medlineplus/druginfo/meds/a600014.html
15. Kanakadurga N, Prameela Rani A and Mrudula BS. New RP-HPLC methods for the analysis of Montelukast sodium in pharmaceutical dosage forms. *Journal of Pharmacy Research*; 2010; 3(4):803-808.
16. Available at: http://dailymed.nlm.nih.gov/dailymed/archives/fdaDrugInfo.cfm?archiveid=15499
17. Available at: http://www.rxlist.com/singulair-drug/overdosage-contraindications.htm
18. Available at: http://www.webmd.com/drugs/mono8277MONTELUKAST++ORAL.aspx?drugid=6478&drugname=Montelukast+Sodium+Oral
19. FDA label for Singulair® oral tablet dosage form of Montelukast sodium (equivalent to 10 mg base) approved on August 10, 2010. Available at www.fda.gov.
20. Raissy H.H, Harkins M, Kelly F, Kelly H.W. Pretreatment with albuterol versus montelukast for exercise-induced bronchospasm in children. *Pharmacotherapy*. 2008; 28: 287–294
21. Wenzel S.E. Asthma: Defining of the persistent adult phenotypes. *Lancet*. 2006; 368: 804–813
22. Villaran C, O'Neill S.J, Helbling A, van Noord J.A, et al. Montelukast versus salmeterol in patients with asthma and exercise-induced bronchoconstriction. *J Allergy Clin Immunol*. 1999; 104: 547–553
23. Gaber F, Daham K, Higashi A et al. Increased levels of cysteinyl-leukotrienes in saliva, induced sputum, urine and blood from patients with aspirin-intolerant asthma. *Thorax*. 2008; 63: 1076–1082
24. Dahlén S.E, Malmström K, Nizankowska E et al. Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist: A randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med*. 2002; 165: 9–14
25. Bousquet J, Gaugris S, Kocevar V.S et al. Increased risk of asthma attacks and emergency visits among asthma patients with allergic rhinitis: A subgroup analysis of the

investigation of montelukast as a partner agent for complementary therapy. *Clin Exp Allergy*. 2005; 35: 723–727

26. Price D.B, Swern A., Tozzi C.A et al. Effect of montelukast on lung function in asthma patients with allergic rhinitis: Analysis from the COMPACT trial. *Allergy*. 2006; 61: 737–742
27. Borderias L, Mincewicz G, Paggiaro P.L et al. Asthma control in patients with asthma and allergic rhinitis receiving add-on montelukast therapy for 12 months: A retrospective observational study. *Curr Med Res Opin*. 2007; 23: 721–730
28. Philip G, Nayak A.S, Berger W.E. et al. The effect of montelukast on rhinitis symptoms in patients with asthma and seasonal allergic rhinitis. *Curr Med Res Opin*. 2004; 20: 1549–1558
29. Leff J.A, Busse W.W, Pearlman D et al. Montelukast, a leukotriene receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *New Engl J Med*. 1998; 339: 147–152
30. Reiss T.F, Chervinsky P, Dockhorn R et al. Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma. A multicenter, randomized, double-blnd trial. *Arch Int Med*. 1998; 158: 1213–1220
31. Nayak A, Langdon R.B. Montelukast in the treatment of allergic rhinitis: An evidence-based review. *Drugs*. 2007; 67: 887–901
32. Nayak A. A review of montelukast in the treatment of asthma and allergic rhinitis. *Expert Opinion Pharmacotherapy* 2004; 5(3): 679-86
33. Okumu A, Dimaso M, Lobenberg R. Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug. *Pharma Res*. 2008; 25(12):2778-85.
34. Fey C, Thyroff U and Jones S et al. Bioequivalence of two formulations of montelukast sodium 4mg oral granules in healthy adults. *Clinical and Translational Allergy*. 2014; 4-7
35. Mclvor A, Kaplan A, Koch C et al. Montelukast as an alternative to low-dose inhaled corticosteroids in the management of mild asthma (the SIMPLE trial): An open-label effectiveness trial. *Can Respir J*. 2009; 16 (Suppl A): 11A-16A.

36. Takeda K, Shiraishi Y, Matsubara S. Effects of combination therapy with montelukast and carbocysteine in allergen-induced airway hyperresponsiveness and airway inflammation. *Br J Pharmacol.* 2010; 160(6): 1399-1407
37. Kose E, Beytur A, Dogan Z et al. The effects of montelukast against amikacin-induced acute renal damage. *Eur Rev Med Pharmacol Sci.* 2012; 16(4):503-11
38. Hardwick C, White D, Morris E et al. Montelukast in the treatment of HIV associated immune reconstitution diseases. *Sex Transm Infact*. 2006; 82(6): 513-514
39. Naser A, Natour S, Qaddomi A et al. Formulation and in vitro and in vivo evaluation of film-coated montulukast sodium tablets using Opadry yellow 20A82938 on an industrial scale. *Drug Des Devel Ther*.2013; 7: 83-91
40. Ahmed B, Abdalla A. Comparison of FT-NIR Transmission and HPLC to Assay Montelukast in Its Pharmaceutical Tablets. *American Journal of Analytical Chemistry*. 2011; 2: 885-891
41. Srihari G, Nagaraja K, Rami N. A simple spectrophotometric assay of Montelukast in Pharmaceutical formulations. *J. Chem. Pharm. Res*. 2011; 3(6):23-27
42. Available at:http://www.accessdata.fda.gov/scripts/eder/dissolution/dsp
43. British Pharmacopeia 2015 (Montelukast Preparations III: 859 -860).
44. Naga K, Swamy G, Lakshmana A. Development and validation of RP-HPLC method for the determination of Montelukast Sodium in bulk and in pharmaceutical formulation. *International Journal of Pharmaceutical, Biological and Chemical Sciences*. 2011; 1(1):12-16
45. Behera S, Ghanty S, Ahmad F et al. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. *J Anal Bioanal Techniques*. 2012, 3:151.Available at : http://omicsonline.org/uv-visible-spectrophotometric-method-development-and-validation-of-assay-of-paracetamol-tablet-formulation-2155-9872.1000151.php?aid=9714