Research Article

Comparative study of Quality Control Parameters of Different Brands of Oral Montelukast Tablets Manufactured in Bangladesh

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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. The aim of the study was to evaluate and compare the quality control parameters of ten different brands of montelukast tablets manufactured in Bangladesh. The samples were taken from five leading pharmaceutical companies 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 and disintegration tests were performed. *In vitro* dissolution study was performed 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 (values ranging 0.17±0.01 gm to 0.21±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 company J. There was a marginal difference in the result of the friability test of the tablets (all values less than 1% according to British Pharmacopeia specification). Disintegration times of the tablets of leading companies were found to be within 3 minutes except for company A (7.4±0.9 minutes). Company F and I showed the highest disintegration times. Consequently, the percentage release of drug for company A, F and I were less compared to other companies. Nevertheless, all the companies showed greater than 90% release of drug after 30 minutes. Therefore, it can be concluded that montelukast tablets produced by the pharmaceutical companies in Bangladesh are of consistent quality with very little variation among them.

Keywords: Montelukast; Quality Control; Dissolution; HPLC; Bangladesh.

INTRODUCTION

According to World Health Organization (WHO), globally about 10-30% of pharmaceuticals are of poor quality, counterfeit, falsified or in a broad sense 'substandard'. Poor quality and substandard medicines lead to morbidity and increase the mortality rates of many diseases.[1]Substandard drugs are drug products that do not meet quality specifications set for them and they may either be genuine or counterfeit medicinal products. A counterfeit medicine can be defined as a medicine which is deliberately and fraudulently mislabelled with respect to identity and/or source [WHO].[2] There are several reports on the presence of substandard medicines on the market in both developed and developing countries.[3-7]

A fundamental quality attribute for all pharmaceutical preparations is the requirement of constant dose of drug between individual tablets. A small variation between individual preparations is acceptable and the limits for this variation are defined as standards in official pharmacopoeias known as the British Pharmacopoeia (BP) or the United States Pharmacopoeia (USP).There are various ways by which the quality of the medicines can be evaluated which includes either *in-vitro* or *in-vivo* methods. *In-vitro* methods are procedures that utilizes test apparatus and equipment in testing medicines without involving laboratory animals or humans whereas the *in-vivo* methods are usually more complex involving human subjects or laboratory animals mainly for assessing the bioequivalence of different brands. *In-vitro* methods can be used to assess the effect of physical and chemical properties of the drug, drug stability and large-scale production of the drug and drug product on the therapeutic performance of the drug. After laboratory testing, if a drug fails to meet the specifications enlisted in the official pharmacopoeias, then it is classified as a substandard drug.[8]

Montelukast is a leukotriene receptor antagonist (LTRA) used for the treatment of asthma and to relieve symptoms of seasonal allergies in children and adults.[9,10] Leukotrienes constitute a group of locally acting hormones produced in living systems from arachidonic acid that are responsible for asthma, bronchitis and constriction of airways. Major leukotrienes include Leukotriene B4 (LTB4), Leukotriene C4 (LTC4), Leukotriene D4 (LTD4), and Leukotrine E4 (LTE4).[11] It is used as an add-on therapy for the treatment of patients six years or older with mild to moderate asthma inadequately controlled on with required short-acting ß-agonists and inhaled corticosteroids and is also licensed for prophylaxis for asthma in which the predetermined component is exercise-induced bronchoconstriction.[9] In 2012, FDA approved generic drug makers to start making generic versions of Singulair (Montelukast sodium by Merck

Sharpe and Dohme Ltd) for the treatment of asthma and allergic rhinitis.[10-11]. It is usually administered once daily in a dose of 10 or 5 mg per tablet.[12] The mean oral bioavailability of montelukast when administered as a 10 mg immediate release film-coated tablet in adults was found to be 64 %.[13]

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 in Bangladesh in order to assess the quality of oral Montelukast sodium tablets manufactured in Bangladesh as well as to ensure the equivalence of these drug products.

MATERIALS AND METHODS

Materials

Montelukast sodium (standard) was supplied by Incepta Pharmaceuticals Limited, Bangladesh. In our study, ten conventional commercial Bangladeshi brands (selected according to ranking in terms of market shear) containing 10mg of active montelukast sodium was purchased from retail pharmacy store. The top ranked marketed samples are designated as A, B, C, D and E respectively. Similarly the middle ranked samples are represented as F, G and H respectively and lower ranked samples as I and J. In the study, sodium lauryl sulphate (SLS) was used as dissolution medium. Ammonium acetate of pH 3.5 and methanol were used as mobile phases for HPLC analysis. Glacial acetic acid was used to adjust the pH of ammonium acetate. All the solvents used were of analytical grade.

Weight Variation

For each brand, 10 tablets were randomly selected and individual weight was taken using an analytical balance (ATX Series Max Cap: 210g, Readability: 0.001g). The average weight was calculated with the standard deviations (SD).[14]

Hardness Test

The hardness test for randomly selected montelukast sodium tablets (10 tablets from each manufacturer) was determined by Monsanto hardness tester (Model: EH-0, Electrolab, India). The average crushing strength was determined.[15]

Friability test

The procedure for a standard tablet friability test applicable to manufactured tablets is outlined in USP 24/NF19.Individual weight of ten tablets from each commercial band was taken. Then each group of tablets was placed in a friabilator (EF-FRIABILATOR, Electrolab, India). The tablets were rotated for 1 minute at 100 rotations per minute. After rotation was complete, the tablets were collected and weighed again. The friability was calculated for each group of tablets by using the following equation: % loss= (Initial weight-Final weight)/Initial weight×100.[16]

Disintegration test

The test was performed by using disintegration machine (ED-2L, Electrolab, India). 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 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.[17]The disintegration time for each tablet was determined and the average time was calculated.

Preparation of standard curve

Accurately weighed montelukast sodium was dissolved in 0.5% SLS medium. Using stock solution, different concentration of solution was prepared to produce a standard calibration curve. The solutions were filtered through 0.2µ disk filter and transferred into clean and dry HPLC vials. Then the solutions were analyzed using HPLC machine (Shimadzu HPLC Prominence Liquid Chromatogram, Japan) and the chromatograms were recorded at 254nm. The standard curve was constructed by plotting Peak Area versus Concentration as shown in Figure 1. Linearity was observed in the concentration range from 10–15µg/ml with a correlation coefficient greater than 0.98.

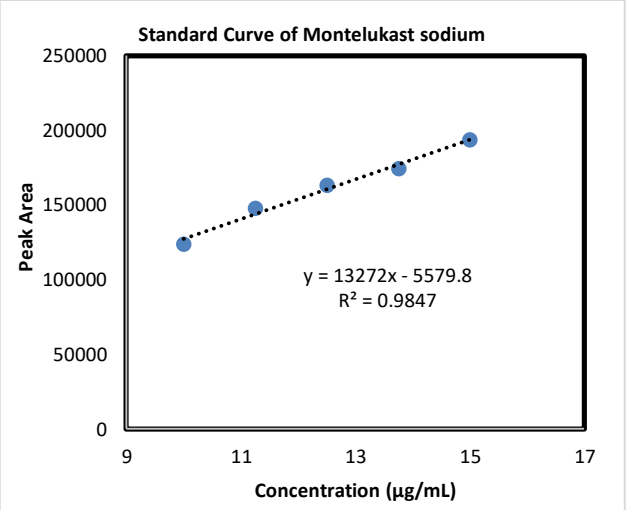

Figure 1: Standard curve of Montelukast sodium

In vitro dissolution study using HPLC

High Performance Liquid Chromatography (HPLC) was used to quantify the amount of montelukast released from the tablets of the ten different pharmaceutical companies of Bangladesh. *In vitro* dissolution study of montelukast sodium was conducted using USP Apparatus II (Paddle method).[18]

Ammonium acetate buffer was prepared and pH was adjusted by adding glacial acetic acid. A clear solution of 0.5% sodium lauryl sulphate was used as the dissolution medium. The next step was preparation of stock solution of montelukast sodium. 900ml of dissolution medium was placed into each vessel of the USP Paddle II type dissolution apparatus (Model: UDT-804-8, Logan, USA). 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 50 rpm. After 30 minutes, a definite volume of dissolution medium was withdrawn and filtered with 0.45µm filter paper. Then this solution was filtered again and placed into HPLC vials. Consequently, the solutions were injected consecutively into the HPLC machine and the chromatograms were recorded. Shimadzu HPLC Prominence Liquid Chromatogram was used to run the sample and ammonium acetate (pH=3.5) and methanol were used as mobile phase in a ratio of 15:85. The temperature was set at room temperature and flow rate was 1.5ml/min. The monitoring wavelength was maintained at 254nm and injecting volume was 10 µl. The retention time was approximately 10 minutes.

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 y = 13272x – 5579.8 whereby = peak area and x = concentration in µg/ml. The percentage release of montelukast from the tablets can be calculated using the following equation:

% Release of Montelukast sodium =

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

Where Y = potency of montelukast sodium (standard) = 99.9 %

RESULTS AND DISCUSSION

Weight Variation

According to USP, the acceptable range of variation in weight of each tablet (130mg or less) is ±10%. [16] The weights of the different brands of the montelukast are shown in Table 1. From the results of weight variation test of the ten different companies, it is apparent that the range of values was between 0.17±0.01 gm to 0.35±0.01 gm. Most of the leading companies have similar values ranging from 0.17±0.01gm to 0.21±0.01gm except for company B which showed 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 0.25±0.01 gm to 0.3±0.03 gm. The lower selling companies (I and J) revealed varying results (0.18±0.03 gm and 0.31±0.03 gm respectively). Therefore there was hardly any variation in the result of weight variation test among the leading companies selling montelukast sodium in Bangladesh but the middle and lower ranked companies showed slightly different results.

Table 1: Results of weight variation, hardness, friability and disintegration tests

Brand product of Montelukast sodium	Average weight (gm) (Mean ± SD)	Hardness (kg/cm^2) (Mean ± SD)	Friability (% loss)	Disintegration time (minute) (Mean ± SD)
A	0.17 ± 0.01	5.6 ± 1.2	0.11	7.4±0.9
B	0.35 ± 0.01	10.1 ± 1.1	0.11	3.1±0.8
C	0.17 ± 0.02	9.8 ± 1.2	0.32	3.2±0.6
D	0.21 ± 0.01	8.0 ± 0.8	0.28	1.6±1.0
E	0.15 ± 0.04	7.3 ± 1.1	0.12	7.4±0.8
F	0.26 ± 0.01	6.9 ± 1.8	0.27	9.4±1.1
G	0.30 ± 0.03	6.7 ± 0.6	0.23	6.8±0.8
H	0.25 ± 0.01	12.5 ± 0.6	0.28	7.4±0.1
I	0.18 ± 0.03	5.4 ± 0.7	0.11	9.8±3.6
J	0.31 ± 0.03	16.6 ± 1.4	0.12	6.1±0.4

Hardness Test

Tablet hardness is the force required to break the tablet in a diametric compression test. The hardness test or crushing strength test is often performed during developmental stability studies and as an in-process control during the tablet compression operation in manufacturing. Variation in tablet hardness can affect the in-vitro and in-vivo performance as it has a direct influence on tablet disintegration and dissolution. Too hard tablets will have a longer disintegration time which will eventually slow down the dissolution process and subsequent absorption of the drug whereas too soft tablets will not be able to withstand the mechanical pressure of packaging, shipping and transport. Orally tablets usually have a hardness of 4 to 8 or 10kg; however hypodermic tablets are much softer (3kg) and some

sustained release tablets are much harder (10-20kg).[19] The results of hardness test showed that the tablets of the leading companies have different values ranging from 5.6±1.2 kg/cm^2 to 10.1±1.1 kg/cm^2 (Table 1). Company B, C, D, E showed very similar values. However, company A had a value (5.6±1.2 kg/cm^2) that differed greatly from the values of the other four leading 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 showed twice the value (12.48±0.6kg/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 (16.6±1.4 kg/cm^2) indicating higher disintegrating time for the tablets produced which is undesirable. Therefore it can be suggested out that except for company H and J, all the other companies have acceptable values of hardness.

Friability test

Friability test is used as a quality control test for tablets as they are constantly subjected to abrasion and mechanical pressure during manufacturing process as well as packaging and transportation. Such stresses can lead to chipping and even breakage of tablets and therefore tablets must be able to withstand these stresses without damage to its appearance. The acceptable percentage of loss is usually less than 1%.[20] The values of percentage of loss of the montelukast sodium tablets of the ten companies ranged from 0.11 % to 0.32 % and thus meet the specification (Table 1). It is evident that there is 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 and are more or less equivalent.

Disintegration test

Disintegration test is a measure of the time required under a given set of conditions for a group of tablets to disintegrate into smaller particles and is useful as a quality assurance tool for conventional dosage forms.[21]The time taken for the montelukast sodium tablets of the leading companies to disintegrate was found to be highly variable ranging from 1.6±1.0 minutes to 7.4±0.9 minutes (Table 1). Company D showed the least time taken for disintegration (1.6±1.0 minutes). Company A and E showed equal time for disintegration (7.4±0.9 minutes and 7.4±0.8 minutes respectively). Similarly the tablets of company B and C has shown close values for disintegration time (3.1±0.8 minutes and 3.2±0.6 minutes). The disintegration time of the middle-ranked companies F, G, H are slightly higher compared to the top companies with F taking the highest time for disintegration (9.4±1.1minutes), compared to the other companies. The low-ranked companies I and J also demonstrated more time for disintegration and have values that closely resemble that of the middle-ranked companies. Therefore, it can be concluded that tablets of company D (leading company) has the lowest disintegration time and F (middle-ranked company) company and I (low-ranked company) reveals 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.

Table 2: Results of Dissolution test

Brand product of Montelukast sodium	Average Retention time (minute)	Average Peak Area	Average Concentration from standard curve (µg/ml)	Percentage Release of Drug (%)
MOS A	10.030	130398	10.2	92.1
MOS B	10.362	150038	11.7	105.4
MOS C	10.276	149911	11.7	105.3
MOS D	10.209	157143	12.3	110.2
MOS E	9.964	150546	11.8	105.8
MOS F	9.533	127964	10.1	90.5
MOS G	9.983	162402	12.7	113.8
MOS H	10.455	142356	11.1	100.2
MOS I	9.461	126636	10.6	95.7
MOS J	10.211	163670	12.7	114.4

***In vitro* Dissolution test**

In vitro dissolution test is well established not only as a quality control test to assess batch-to-batch consistency but also to predict *in vivo* drug release profiles for solid oral dosage forms and is an indicator of the bioavailability of the drug product.[22,23] HPLC was used to determine the *in vitro* release of ten different brands of Montelukast sodium according to US FDA guidelines and British

Pharmacopeia 2015.[24,25] Percentage of drug released in the dissolution medium was calculated following the analytical method proposed by Raju *et al.*[26] The method was found to be linear within the range of 10-15mg/ml. The average value of concentration of Montelukast sodium tablets of the pharmaceutical companies A-Jand the percentage of drug released after 30 minutes is shown in Table 2. 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%) as shown in 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 30minutes and for company J the percentage release of drug was 114.4%, clearly indicating a marked difference between these two companies. Therefore, it can be concluded from the dissolution study that all these companies manufacturing montelukast sodium in Bangladesh showed an acceptable dissolution profile and complies with the specifications of British Pharmacopeia 2015. 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. Chromatograms for the standard and companies A, H, I are shown in Figure 1,2,3 respectively.

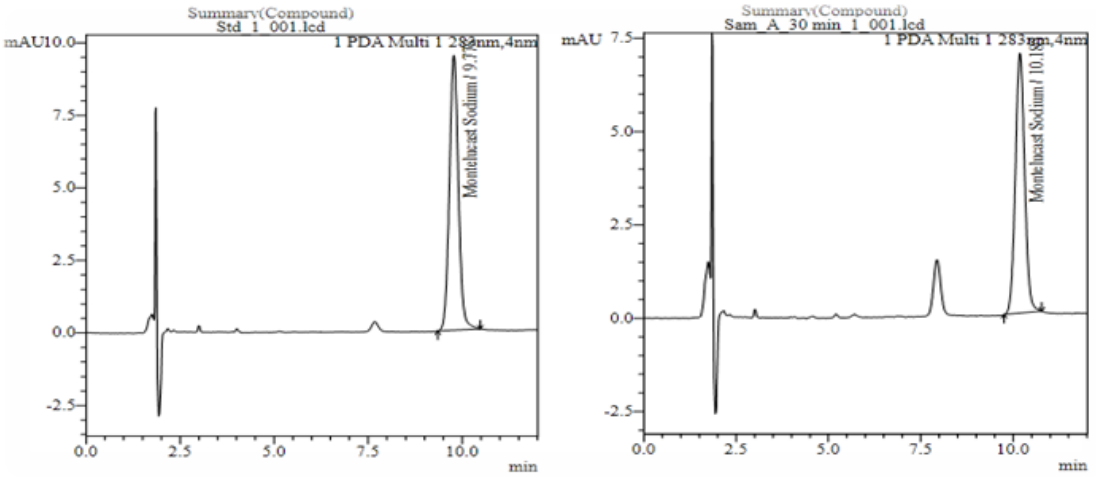

Figure 1: Chromatogram for standard and sample A

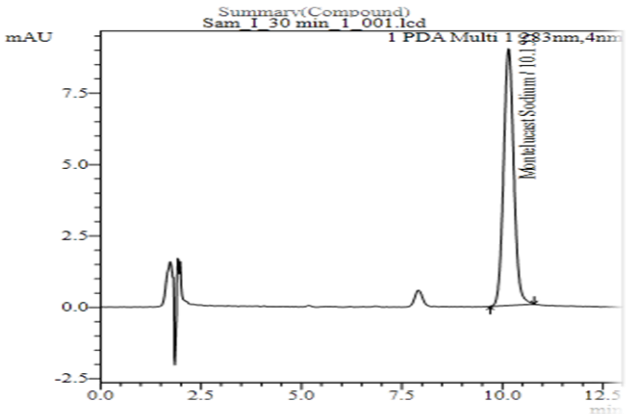

Figure 2: Chromatogram for sample H

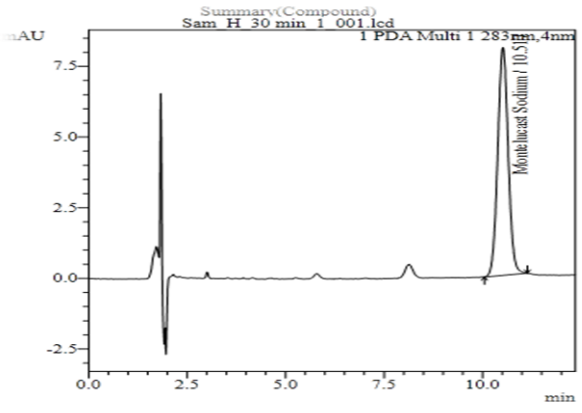

Figure 3: Chromatogram for sample I

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 and analyzed by HPLC to determine the percentage release of drug after 30 minutes which may reflect the in vivo performance of the drug. The weight variation results showed that there is hardly any variation among the leading pharmaceutical companies; however, 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.

There is a marginal difference in the result of the friability test of the all the ten companies (all values were less than 1% according to the specification of BP), 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 within 3 minutes indicating a very good result except for company A. Company F (middle-ranked company) and company I (low-ranked company) showed the highest disintegration times. Consequently, the percentage release of drug for company A, F and 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 2015. Therefore, 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. However, the tablets must be compared with international brands of montelukast sodium for further assessment of the quality of the tablets.

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REFERENCES

1. Ho NT, Desai D, Zaman MH. Rapid and specific drug quality testing assay for Artemisinin and its derivatives using a luminescent reaction and novel micro fluidic technology. Am J Trop Med Hyg, 92 (Suppl 6), 2015, 24–30.
2. World Health Organization. Sixty-First World Health Assembly, 2008. Available from: <http://apps.who.int/gb/ebwha/pdf_files/WHA61-REC1/A61_REC1-en.pdf>.
3. Johnston A, Holt DW. Substandard Drugs: a potential crisis for public health. Br J Clin Pharmacol, 78(2), 2014, 218–243.
4. Almuzaini T, Sammons H, Choonara I. Quality of medicines in Canada: a retrospective review of risk communication documents (2005-2013). BMJ Open, 4(10), 2014.
5. Veronin M. Packaging and labeling of pharmaceutical products obtained from the internet. J Med Internet Res, 13(1), 2011, e22.
6. Mackey TK, Liang BA, York P, et al. Counterfeit drug penetration into global legitimate medicine supply chains: a global assessment. Am J Trop Med Hyg, 92 (Suppl 6), 2015, 59–67.
7. Khurelbat D, Dorj G, Bayarsaikhan E, et al. Prevalence estimates of substandard drugs in Mongolia using a random sample survey. Springer plus, 2014, 3: 709.
8. Aulton ME. Pharmaceutics: The Science of Dosage Form.2nd ed. Leicester: Churchill Livingstone; 2002, 417-420.
9. Wang K, Birring S, and Taylor K, et al. Montelukast for post infectious cough in adults: a double-blind randomised placebo-controlled trail. The Lancent Respiratory Medicine, 2, 2014, 35-43.
10. Karonen T, Neuvonen PJ, Backman JT. CYP2C8 but not CYP3A4 is important in the pharmacokinetics of montelukast. Br J Clin Pharmacol, 73(2), 2012, 257–267.
11. Zaid AN, Mousa A, Ghazal N et al. Interchangeability and comparative effectiveness between generic and brand montelukast immediate release tablets after a single oral administration in healthy volunteers. Clin Transl Allergy, 5, 37, 2015.
12. Drugs @ FDA: FDA Approved Drug Products. [cited 2016]. Available from: <http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/020829s069,020830s071,021409s047lbl.pdf>
13. Singulair Drug Description (Merck & Co. Inc) Available from: <http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020829s051_020830s052_021409s028lbl.pdf>
14. Uniformity of Dosage Units -The United States Pharmacopeial Convention [cited 2011]. Available from: <http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/2011-02-25905UNIFORMITYOFDOSAGEUNITS.pdf>
15. United States Pharmacopeia and National Formulary (USP 32-NF 27). p. 726 Available from:<http://www.drugfuture.com/pharmacopoeia/usp32/pub/data/v32270/usp32nf27s0_c1217.html>
16. United States Pharmacopeia and National Formulary (USP 24 - NF 24). Available from: <http://www.pharmacopeia.cn/v29240/usp29nf24s0_c1216.html>
17. Lachman L, Liebermann HA. The Theory and Practice of Industrial Pharmacy. 3rd ed. CBS Publishers & Distributors Pvt. Limited, 2003, 255.
18. US Food and Drug Administration. Guidance for industry: statistical approaches to establishing bioequivalence. [cited 2001]. Available from: <https://www.fda.gov/downloads/drugs/guidances/ucm070244.pdf>
19. Felton LA. Remington: Essentials of Pharmaceutics. 1st ed. London: Pharmaceutical Press, 2012, 18.
20. Kim HB. Handbook of Stability Testing in Pharmaceutical Development: Regulations, Methodologies and Best Practices. 1st ed. Springer, 2009, 215.
21. Troy DB, Beringer P. Remington: The Science and Practice of Pharmacy. 21st ed. Philadelphia: Lippincott Williams and Wilkins; 2006, 916.
22. US FDA CDER. Guidance for Industry: Dissolution testing of immediate release solid oral dosage forms. [cited 1997] Available from: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070237.pdf>
23. Riley T, Christopher D, Arp J, et al. Challenges with Developing *In Vitro* Dissolution Tests for Orally Inhaled Products (OIPs). AAPS Pharm Sci Tech, 13(3), 2012, 978–989.
24. U.S Food & Drug Administration: Dissolution Methods. Available from URL: <http://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_getalldata.cfm>
25. British Pharmacopeia 2015 (Montelukast Preparations III p. 859 - 860).
26. Raju KN, Swamy G, Rao AL. Development and validation of RP-HPLC method for the determination of Montelukast Sodium in bulk and in pharmaceutical formulation. Int.J. Pharm.Chem.Biol.Sci, 1(1), 2011, 12-16.

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