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Development of stability-indicating HPLC-UV method and oxidative degradation kinetic study of montelukast in chewable tablet formulation

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Abstract: A simple and sensitive stability-indicating HPLC-UV method was developed and validated for the determination of montelukast in the development of chewable tablet formulation. Chromatographic separation was achieved using Atlantis® T3 3µm C18 (4.6mmID X 10cm) analytical column. The mobile phase was consisted of KH_2PO_4 (0.05mM)-ACN-TEA (450:550:1.33, v/v/v) adjusted to pH 2.0 with orthophosphoric acid. The analysis was run at a flow rate of 1.5 mL/min with detection wavelength at 255nm. Method validation was performed in accordance with ICH guideline. Stress degradation studies, comprising of acid and alkali hydrolysis (1M HCl and 1M NaOH), oxidative degradation (3% H_2O_2), photo degradation and heat degradation, were performed. The standard calibration curve was linear from 0.0025 - 0.375mg/mL. The LOD and LLOQ were 0.01µg/mL and 0.04µg/mL. Stress degradation result shows that montelukast sodium was sensitive to photo degradation, oxidation and acid hydrolysis. Oxidative degradation kinetic study of montelukast sodium followed first order reaction, with r^2=0.9877, apparent degradation rate constant, k= 0.1066 h^{-1}, $t_{1/2}$= 6.6151 hr and $t_{90\%}$ = 1.0118hr. In conclusion, HPLC-UV method was successfully developed and validated for determination of montelukast sodium in chewable tablet formulation.

Keywords: Montelukast, HPLC-UV, oxidation degradation kinetics, sulphoxide impurity.

INTRODUCTION

Montelukast is a leukotriene receptor antagonist commonly used in the treatment of chronic asthma, allergic rhinitis and as prophylaxis for exercise-induced asthma in children (Sweetman 2009; Martin 2011; Calapai *et al.*, 2014). It is used in the form of sodium salt, and is chemically known as 2-[1-[(R)-[3-[2(E)-(7chloroquinolin-2-yl)vinyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propylsulfanylmethyl] cyclopropyl] acetic acid sodium salt (Al Omari *et al.*, 2007). The molecular weight of montelukast sodium is 608.17 g/mol and the molecular formula is $C_{35}H_{35}ClNNaO_3S$. fig. 1 shows the molecular structure of montelukast sodium and its impurities.

Montelukast sodium is known to be sensitive to light and oxidizing agents, resulting in degradation of montelukast to its impurities. Radhakrishna *et al.* (2003) performed stress studies on montelukast sodium by exposing to heat and UV light to prove that the validated HPLC method was a stability-indicating method. Al Omari *et al.* (2007) worked on the effect of light source, temperature and solvent on the stability of montelukast sodium in solid and solution states. Roman *et al.* (2011) reported a stability-indicating method of montelukast in pharmaceutical dosage form and used the method to determine the photo degradation kinetic of montelukast. Quantification of montelukast in bulk and pharmaceutical dosage form using HPLC method (Singh *et al.,* 2010; Eldin *et al.,* 2011; Pankhaniya *et al.,* 2013; Ul-Hassan *et al.*, 2013) and photo degradation behaviour of montelukast sodium have been studied extensively.

Al Omari *et al.* (2007) and Emerce *et al.* (2015) showed in their studies that montelukast sulphoxide was the major degradant in montelukast solid dosage form when subjected to thermal testing during stability studies. It was stated in USP 40 (2017) that the acceptance criteria of oxidative impurities in both montelukast sodium and chewable tablets resulted from oxidation could not be more than 0.2% and 1.5%. Nevertheless, studies on oxidative degradation kinetic of montelukast sodium have hardly been reported.

In this study, a stability-indicating HPLC-UV method for the determination of montelukast in chewable tablet formulation was developed and validated. The method validation was performed in accordance with ICH guideline, comprising of specificity, linearity, accuracy, precision (repeatability and intermediate precision), robustness, system suitability and stability of chewable sample solution in autosampler. The HPLC-UV method demonstrated improved sensitivity with limit of detection at 0.01µg/mL and could resolve oxidative impurities (sulphoxide isomer and sulphoxide impurity). The run time was 35 min comparatively shorter than that reported by Bapatu *et al*. (2012) which was 45 min. In addition, the method was used in the oxidative degradation kinetic study of montelukast.

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MATERIALS AND METHODS

Montelukast sodium (potency 99.0%) was purchased from Afine Chemicals Limited, China. Montelukast sodium (potency 99.7%) working standard was purchased from Sigma Aldrich (M), Malaysia. Montelukast sulphoxide (mixture of diastereomers) impurity was purchased from CacheSyn Inc., Canada (catalog number: CSTM307). Magnesium stearate was purchased from Huzhou City Linghu Xinwang Chemical Co., Ltd, China. Mannitol was purchased from Qingdao Bright Moon Seaweed Group Co. Ltd, China. Inulin was purchased from Cosucra-Groupe Warcoing S.A, Belgium. Polyvinylpyrrolidone (PVP K-30) was purchased from Anhui Jinao Chemical Co. Ltd, China. Sodium starch glycolate (Explotab®) was purchased from JRS Pharma, USA. HPLC grades of acetonitrile and methanol were purchased from Merck KGaA, Germany. Triethylamine (≥ 99%), hydrochloric acid (37%w/w), sodium hydroxide pellets and potassium dihydrogen phosphate were purchased from Fisher Scientific, Belgium. Orthophosphoric acid (85%w/w) and hydrogen peroxide solution (35%w/v) were purchased from R&M Marketing, Essex, UK. All the chemicals and reagents used were of analytical grade. The materials were used as received.

Preparation of direct compressed chewable tablets

Mannitol-inulin-PVP co-processed material, sodium starch glycolate and montelukast sodium were sieved and blended for 5 min. The mannitol-inulin-PVP co-processed material was prepared using wet granulation method. Mannitol and inulin at a ratio of 65:35 were sieved through a 400µm sieve and mixed. The powder mixture was granulated with 0.5%w/w of PVP K-30 in ethanol for 30 minutes in a mixer (Kenwood, UK). The wet mass was sieved through a 1.25mm sieve before drying in the oven at 50ºC until loss on drying (LOD) was less than 2.0%. After drying, the co-processed granules were screened again using a 800µm sieve. After the addition of magnesium stearate, blending was continued for another 5 min. The powder mixtures were manually compressed using rotary tabletting machine equipped with 10 mm die and punches assembly (Manesty Machines Ltd, England). 50 tablets of 300mg each were prepared.

Instrumentation of HPLC system

The HPLC system (Shimadzu, Kyoto, Japan) was comprised of a degasser unit (DGU-20A), binary pump (LC-20AD), a controller (CBM-20A), a UV-Vis detector (SPD-20A), an auto-injector (SIL-20A HT) and a column oven unit (CTO-10AS VP). The computer software data system was Lab solution (version 5.30SP1). On the other hand, the UHPLC system (Shimadzu, Kyoto, Japan) was comprised of a degasser unit (DGU-20A5R), binary pump (LC-30AD), a controller (CBM-20A), UV-Vis detector (SPD-20A), auto-injector (SIL-30AC) and a column oven unit (CTO-20AC). The computer software data system was Lab solution (version 5.81). The UHPLC system was used for intermediate precision parameter (different equipment) in method validation.

Optimization of chromatographic conditions

Two chromatographic parameters namely, pH of mobile phase (potassium dihydrogen phosphate (0.05mM)-acetonitrile-triethylamine (450:550:1.33, v/v/v) adjusted to pH 2.0 and pH 6.7) and different analytical columns, Agilent Eclipse Plus C_{18} (4.6mm ID X 250mm, 5µm), Atlantis® T3 3µm C_{18} (4.6mmID X 10cm) and ACE 3 Phenyl (4.0mmID X 150mm, 3µm), were studied. The analysis was run at a flow rate of 1.5mL/min. The column temperature was set at 35˚C. The detection wavelength was 255nm. Sample volume of 15µL was injected into the system. The experiment was conducted in a dark room to avoid photo degradation of montelukast (Liu *et al.,* 1997; USP 40, 2017). The mobile phase was filtered using 0.45µm nylon membrane (Whatman filter 47mm, England) and degassed with ultrasonic vibrator (Elmasonic S100H, Germany) before use.

Preparation of system suitability solutions

System suitability solution 1 and montelukast standard solution (0.25mg/mL) were used for system suitability testing. System suitability solution 1 was prepared by diluting 1mL of the montelukast-cis isomer solution and 1mL of 0.25mg/mL of montelukast standard solution to 100mL with 70% methanol. Montelukast cis-isomer was obtained by exposing montelukast standard solution to UV lamp (365nm) for 24 hours to ensure maximum conversion (more than 90%) of montelukast sodium to montelukast cis-isomer (unpublished data). Montelukast standard solution (0.25mg/mL) was prepared from montelukast stock standard solution (1.25mg/mL of monteluakst) by dilution with 70% methanol. System suitability solution 1 was used to evaluate the resolution of montelukast and cis-isomer, while montelukast standard solution was used to evaluate theoretical plate (N), capacity factor (k') and relative standard deviation RSD (%) of the system (CDER, 1994).

Preparation of placebo and sample solution

294.8mg of placebo and 300mg of powderized chewable tablet (equivalent to 5mg of montelukast), were weighed and transferred into two 20mL volumetric flask. 70% methanol was added into the volumetric flasks and sonicated (Elmasonic S100H, Germany) for 30 min. After cooling down to room temperature (25±4ºC) and diluted to volume with 70% methanol, the placebo solution and sample solution were centrifuged at 4300.8*g* (8000rpm) for 15 min. The supernatants were filtered using syringe filter (0.45µm nylon membrane, Millipore Millex H-N, Merck KGaA, Germany). The first 5mL of the supernatant was discarded and the filtrate collected. The sample solution (0.25mg/mL of montelukast) was used

for the evaluation of precision (repeatability and intermediate precision), robustness and stability of chewable tablet sample solution in auto sampler. The percentage of montelukast in the chewable tablet was calculated using the following equation:

$$\text{Assay}(\%) = \frac{\frac{\text{Std wqt (mq)}}{10} \times \frac{1}{5}}{\frac{\text{Spt wqt (mq)}}{100} \times \frac{5}{20}} \times \frac{Splarea}{Meanstdarea} \times P(\%) \times \frac{586.18}{608.18} \quad (1)$$

$$\text{Percentage } deviation \text{ (\%)}: \frac{\text{y intercept}}{\text{Mean peak area of 100\% test concentration}} \text{ X100\%} \quad (2)$$

where P is the potency of the standard, 586.18 is the molecular weight of montelukast and 608.18 is the molecular weight of montelukast sodium.

Force degradation studies
Force degradation studies were performed on three sample solutions namely, stock standard solution (1.25mg/mL of montelukast), chewable tablet stock sample solution (1.25mg/mL of montelukast) and stock placebo solution. In acid and alkaline hydrolysis study, 1.0mL of 1.0M HCl or 1.0M NaOH prepared using 70% methanol was pipetted into three 5mL volumetric flasks containing 1.0mL of each sample solution. After intended storage period (0, 4 and 24 hours), the samples were neutralized immediately with either 1.0mL of 1.0M NaOH or 1.0M HCl and diluted to volume with 70% methanol. In oxidative degradation study, 0.43mL of 35% hydrogen peroxide was pipetted into three 5mL volumetric flasks containing 1.0mL of each sample solution. The sample solutions were diluted to volume and immediately injected into the system after intended storage time (0 and 2 hours) at room temperature. Photodegradation was performed on both solid and solution states. In solution state, sample solutions were diluted to volume with 70% methanol and exposed to UV light (365nm) (Spectroline CM-10A, USA) for 2 hours. In solid state, montelukast working standard, powderized chewable tablets and placebo were initially exposed to UV light for 2 hours before processing. In heat degradation, each sample solution was subjected to heating in water bath at temperature of 80˚C for 2 hours.

Linearity and accuracy
Seven calibration standards at concentrations of 0.0025, 0.0125, 0.125, 0.1875, 0.250, 0.3125 and 0.375mg/mL, were prepared by serial dilution of 1.25mg/mL montelukast stock standard solution. The regression coefficient and y intercept at 100% concentration (0.25mg/mL) was used to evaluate the linearity of the calibration curve (Eq 2). Accuracy was evaluated at three concentration levels, 80% (0.20mg/mL), 100% (0.25mg/mL) and 120% (0.30mg/mL), in three replicates. Accuracy was reported as percent recovery and was calculated using equations 3 and 4.

Percentage deviation of y intercept at 100% concentration

$$\text{Actual } conc \left(\frac{\text{mg}}{\text{mL}}\right): \frac{\text{Peak area} - \text{y intercept}}{\text{Slope}} \quad (3)$$

$$\text{Accuracy (\%)}: \frac{\textit{Actual} \text{ conc} \left(\frac{\text{mg}}{\text{mL}}\right)}{\textit{Theoretical} \text{ conc} \left(\frac{\text{mg}}{\text{mL}}\right)} \ X\ 100\% \quad (4)$$

Limit of detection and limit of quantification
The limit of detection (LOD) of montelukast was expressed as 3.3 (σ/s) and limit of quantification (LOQ) as 10(σ/s), where σ represents the standard deviation of the response and s represents the slope of the calibration curve (ICH, 1996).

Precision
Repeatability. System precision was evaluated by injecting the standard solution at six determinations at 100% of test concentration. Method precision was evaluated by injecting six samples prepared from homogenously powderized samples. The relative standard deviation (RSD (%)) was then calculated.

Intermediate precision
The intermediate precision was performed on three parameters namely, different day, different analyst and different equipment (HPLC and UHPLC). Relative standard deviation (RSD (%)) was used to evaluate the results obtained. The chromatographic separation for UHPLC was achieved using Shim-pack XR-ODS III (2.0 mmID X 15cm; 1.6µm) (Shimadzu, Japan). The analysis was run at a flow rate of 0.4mL/min. Injection volume was 5µL and the run time was 15 min.

Robustness
Two parameters were evaluated namely, column temperature (30º and 40ºC) and flow rate (1.3 and 1.7mL/min).

Autosampler stability of chewable tablet sample solution
The auto sampler stability of freshly prepared sample solutions after treatment was evaluated at 0, 6 and 24 hours. Relative standard deviation RSD (%) was determined.

Oxidative degradation kinetic study
The procedure was similar as described under force degradation studies (oxidation), except the standard solution of montelukast sodium was evaluated at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 hours. Standard solution without hydrogen peroxide was taken as zero hour sample. The degradation kinetics were determined by fitting, the remaining concentration of montelukast versus time (zero-order), logarithm of remaining concentration of drug versus time (first-order) and reciprocal remaining concentration of drug versus time (second order) curves. The reaction order was obtained by comparing the regression of coefficient (r^2) of the plots and selecting the

line of best fit. Apparent order degradation rate constant (k), $t_{1/2}$ and $t_{90\%}$ (time where 90% of original concentration of drug left) were obtained from the plots (Monkhouse 1984; Bindra *et al.*, 1994; Vaucher *et al.,* 2010; Souri *et al.*, 2013).

STATISTICAL ANALYSIS

Statistical analysis was performed using Microsoft Office Excel 2010.

RESULTS

Optimization of chromatographic conditions

The summary of the optimization of chromatographic condition of montelukast is presented in table 1.

System suitability

The system suitability results are presented in table 2. The resolution of cis-isomer and montelukast was more than 2.0. In addition, the results of tailing factor (T), theoretical plate count (N), capacity factor (k') and RSD (%) obtained for standard solution fulfilled the system suitability requirements.

Specificity

The chromatograms of solvent, placebo, standard solution and montelukast chewable sample solution are presented in fig. 2. Solvent and placebo did not contain any interference peak at the specific retention time of montelukast. The force degradation results are presented in table 3 and the corresponding chromatograms are given in fig. 3(a-f). Montelukast sodium degraded approximately 80% when exposed to acid hydrolysis for 24 hours but was relatively stable in alkaline condition with degradation of below 0.2%. Montelukast sodium degraded approximately 15% and 22% when exposed to oxidation for two hours in standard solution and chewable tablet sample solution. Approximately 88% of montelukast in standard solution and chewable tablet sample solution degraded after exposing to photo degradation in solution state. Photo degradation was observed to be more dominant when montelukast was in solution state as compared to solid state. Montelukast sodium was stable when exposed to heat with less than 0.5% degradation. The degradants or impurities peaks were well resolved from montelukast.

Linearity and accuracy

The standard calibration curve of montelukast was found to be linear from 0.0025 - 0.3750mg/mL, with regression of coefficient (r^2) of 1.000. The percentage deviation of y intercept from response at 100% concentration was -0.082%, indicating that the calibration curve was linear. The accuracy values obtained at three different concentration levels ranged between 99.073-100.171%, with RSD (%) of less than 1.0%. The accuracy results of the analytical method are presented in table 4.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LLOQ of montelukast were 0.01µg/mL and 0.04µg/mL, respectively.

Precision

The results of repeatability are presented in table 5a and 5b. The system precision and method precision show RSD (%) of montelukast assay of less than 2.0%, indicating that the system and sample preparation method was precise. As shown in table 6, the intermediate precision results showed RSD (%) of below 2.0%.

Robustness

The robustness study indicated that the analytical method could withstand variation in flow rate and column oven temperature, with RSD (%) of montelukast assay of less than 2.0% as shown in table 7.

Auto sampler stability of chewable tablet sample solution

The chewable montelukast sample solution was stable with difference of less than 2% for 24 hours in the auto sampler of HPLC system.

Oxidative degradation kinetic study

The montelukast remained and plots of oxidative degradation results are presented in table 8 and fig. 4. Approximately 53% of montelukast degraded after exposure of 7 hours to 3% of hydrogen peroxide. It could be observed that the oxidative degradation of montelukast followed first order kinetic reaction, with r^2 of 0.9877, apparent degradation rate constant (k) of 0.1066 h^{-1}, $t_{1/2}$= 6.6151hr and $t_{90\%}$ of 1.0118hr.

DISCUSSION

It could be observed from table 1 that montelukast and its impurity were eluted earlier with mobile phase at lower pH 2.0. The analyte retention time was further shortened after the addition of triethylamine (TEA). The retention time of montelukast sodium, an ionisable drug was affected by changes in pH of mobile phase. The pKa value of montelukast sodium is 4.7 (Roman *et al.,* 2011). At pH 6.7, montelukast and silanol group in the C18 column were ionized. The interaction between montelukast and silanol group resulted in a long retention time. When the mobile phase was adjusted to pH 2.0, montelukast was converted to non-ionized form, and hence eluted faster. The addition of triethylamine reduced the partition or interaction between montelukast and silanol group, leading to a shorter retention time. Among the three analytical columns, Atlantis® T3 C18 gave a relatively shorter retention time of 6.80 min and the cis-isomer and montelukast peaks were well resolved.

Table 1: Optimization of chromatographic condition for the quantification of montelukast in chewable tablet

No	Mobile phase	Analytical Columns	Retention time (mins)	Resolution (Rs)	Theoretical plate count (N)	Remarks
1	Potassium dihydrogen phosphate (0.05mM)-acetonitrile-triethylamine (450:550:1.33, v/v/v) adjusted to pH 6.7	Agilent Eclipse Plus C_{18} (4.6mm ID X 250mm; 5µm)	17.40	2.880	9394	Long retention time with impurity peak at 70 minutes.
2	Potassium dihydrogen phosphate (0.05mM)-acetonitrile-triethylamine (450:550:1.33, v/v/v) adjusted to pH 2.0	Agilent Eclipse Plus C_{18} (4.6mm ID X 250mm; 5µm)	9.31	2.767	11219	Shorter retention time with impurity peak at 35 minutes
3	Potassium dihydrogen phosphate (0.05mM)-acetonitrile (450:550, v/v) adjusted to pH 2.0	Agilent Eclipse Plus C_{18} (4.6mm ID X 250mm; 5µm)	13.04	2.842	10653	Slightly longer retention time with impurity peak at 52 minutes
4	Potassium dihydrogen phosphate (0.05mM)-acetonitrile-triethylamine (450:550:1.33, v/v/v) adjusted to pH 2.0	Atlantis® T3 C_{18} (4.6mmID X 100mm; 3µm)	6.80	2.601	9637	Shorter retention time with impurity peak at 26 minutes
5	Potassium dihydrogen phosphate (0.05mM)-acetonitrile-triethylamine (450:550:1.33, v/v/v) adjusted to pH 2.0	ACE 3 Phenyl (4.0mmID X 150mm; 3µm)	6.69	11.938	10409	Cis-isomer and sulphoxide impurity were not well resolved. In addition, the standard solution peak was distorted when stressed under acidic condition.

Table 2: System suitability results

Day	System suitability solution 1, n = 1	Standard Solution, n = 3			
	Resolution, Rs (NLT 2.0)	Tailing Factor, T (NMT 2.0)	Theoretical Plate Count, N (NLT 5000)	Capacity Factor, k'(NLT 1.5)	RSD (%) (NMT 1.0%)
1	2.375	1.047 ± 0.001	8933.667 ± 6.429	7.379 ± 0.010	0.266
2	2.361	1.048 ± 0.000	8890.667 ± 1.528	7.269 ± 0.011	0.068
3	2.385	1.046 ± 0.000	8902.333 ± 5.686	7.403 ± 0.008	0.349
4	2.389	1.045 ± 0.000	8892. 333 ± 10.263	7.446 ± 0.040	0.029
5	2.243	1.060 ± 0.001	8660.000 ± 6.557	6.726 ± 0.027	0.029

NLT: Not less than; NMT: Not more than

Force degradation study was conducted at early stage of method validation to ensure the specificity of analytical method, especially under extreme conditions. Montelukast showed degradation under oxidation, UV light and acid hydrolysis. It was reported that the main degradation product of montelukast sodium under UV light was cis-isomer (Radhakrishna *et al.*, 2003; Al Omari *et al.*, 2007; Roman *et al.*, 2011). Referring to the study conducted by Al Omari *et al.* (2007), montelukast degraded when exposed to UV light in the region of 200-400nm. The present work was in agreement with the findings of Al Omari *et al* (2007) that montelukast degraded more extensively when in solution state as compared to solid state. In acid hydrolysis,

Table 3: Force degradation results

Parameters	Percentage Remained (%)		Percentage Degraded (%)	Resolution (NLT 2.0)
	Control	After exposure		
(a) Standard Solution				
Acid and alkali hydrolysis				
1N HCl dissolved in 70% methanol for 24 hours	100.000	19.820	80.180	2.802
1N NaOH dissolved in 70% methanol for 24 hours	100.000	99.855	0.145	2.381
Oxidative degradation				
3% H_2O_2 for 2 hours	100.000	84.667	15.333	2.384
Photodegradation				
UV lamps-long wavelength 365nm for 2 hours	100.000	11.689	88.311	2.360
Heat degradation				
Heat at 80˚C for 2 hours	100.000	101.642	No degradation	2.389
Solid state				
UV lamps-long wavelength 365nm for 2 hours	100.000	93.040	6.960	2.390
(b) Chewable Tablet Sample Solution				
Acid and alkali hydrolysis				
1N HCl dissolved in 70% methanol for 24 hours	98.152	17.716	80.436	2.786
1N NaOH dissolved in 70% methanol for 24 hours	100.594	101.999	No degradation	2.381
Oxidative degradation				
3% H_2O_2 for 2 hours	102.976	81.415	21.561	2.383
Photodegradation				
UV lamps-long wavelength 365nm for 2 hours	100.702	12.032	88.670	2.360
Heat degradation				
Heat at 80˚C for 2 hours	102.976	102.722	0.254	2.386
Solid state				
UV lamps-long wavelength 365nm for 2 hours	100.702	86.199	14.503	2.383

Table 4: Accuracy results. Mean ± SD, n=3.-

Accuracy (%)	Actual weight of Montelukast sodium (mg)	Actual weight of Placebo (g)	Theoretical concentration (mg/mL)	Peak Area	Actual concentration (mg/mL)	Accuracy (%)	Mean± SD (%)	RSD (%)
80	4.163	0.2436	0.20014	3430883.753	0.20006	99.960	99.073 ± 0.768	0.775
	4.170	0.2434	0.20048	3391868.523	0.19778	98.653		
	4.167	0.2385	0.20034	3387772.331	0.19755	98.607		
100	5.219	0.2982	0.25091	4257830.405	0.24823	98.932	99.589 ± 0.571	0.573
	5.208	0.2969	0.25038	4289335.816	0.25006	99.872		
	5.203	0.2948	0.25014	4289089.491	0.25005	99.964		
120	6.252	0.3603	0.30058	5163910.685	0.30101	100.143	100.171 ± 0.180	0.180
	6.240	0.3573	0.30000	5165356.433	0.30109	100.363		
	6.249	0.3555	0.30043	5154299.005	0.30045	100.007		

RSD: Relative Standard Deviation

Table 5a: System precision. Mean ± SD, n = 6

Sample	Retention time (mins)	Peak Response (Area)
Standard 1	6.43	4399974.40
Standard 2	6.43	4398252.46
Standard 3	6.43	4394899.29
Standard 4	6.43	4428161.31
Standard 5	6.43	4429608.39
Standard 6	6.43	4412848.10
Mean	6.428	4410623.991
SD	0.003	15405.494
RSD (%)	0.047	0.349

SD: Standard Deviation; RSD: Relative Standard Deviation

Table 5b: Method precision. Mean ± SD, n=6

Sample	Retention time (mins)	Peak Response (Area)	Assay (%)
Sample 1	6.43	4337240.59	98.484
Sample 2	6.43	4287783.50	97.555
Sample 3	6.43	4238579.68	96.564
Sample 4	6.42	4231653.39	96.471
Sample 5	6.43	4304665.85	97.744
Sample 6	6.43	4301461.49	97.671
Mean	6.426	4283564.084	97.415
SD	0.003	40946.035	0.768
RSD (%)	0.047	0.956	0.788

SD: Standard Deviation; RSD: Relative Standard Deviation

Table 6: Intermediate precision. Mean ± SD, n = 6.

Sample	Assay Content (%)					
	Day		Analyst		Instruments	
	1	2	A	B	HPLC	UHPLC
Sample 1	98.484	98.082	98.082	98.412	98.082	95.968
Sample 2	97.555	97.834	97.834	98.631	97.834	97.244
Sample 3	96.564	97.847	97.847	98.045	97.847	94.943
Mean	97.728		98.142		96.986	
SD	0.649		0.318		1.26	
RSD (%)	0.664		0.324		1.299	
System suitability						
Resolution (Rs), between Cis-isomer and Montelukast > 2	2.385	2.243	2.243	2.389	2.243	3.559
Theoretical plate (N) > 5000	8907	8666	8666	8808	8666	12908
Tailing factor (T) < 2	1.046	1.059	1.059	1.053	1.059	1.144
Capacity factor (k') > 1.5	7.402	6.694	6.694	7.234	6.694	7.875
RSD(%) for replicate injection (< 1)	0.046	0.029	0.029	0.064	0.029	0.109

HPLC: High Performance Liquid Chromatography; UHPLC: Ultra-Fast High Performance Liquid Chromatography; SD: Standard Deviation; RSD: Relative Standard Deviation

two main degradants were observed at RRT of 2.74 and 3.84 (fig. 3(a)). On the other hand, in oxidative degradation, two main degradants were observed at RRT of 0.32 and 0.35 (fig. 3(c)). This RRT of two degradants (sulphoxide isomer and sulphoxide impurity) were identified by the injection of sulphoxide (mixture of diastereomers) reference standard solution spiked with montelukast. Both oxidative impurities were well resolved, with a resolution of more than 1.5. Bapatu *et al.* (2012) described the identification of sulphoxide isomer and sulphoxide impurity. However, the run time was relatively long at 45 min. The degradants generated under stress conditions did not interfere with the montelukast peak, indicating the specificity of this analytical method.

Table 7: Robustness. Mean ± SD, n = 9.

Sample	Assay Content (%)					
	Flow rate (mL/min)			Temperature (˚C)		
	1.3	1.5	1.7	30	35	40
Sample 1	98.350	98.082	97.822	100.439	98.082	97.212
Sample 2	98.527	97.834	97.087	98.745	97.834	97.351
Sample 3	98.486	97.847	97.180	98.243	97.847	97.031
Mean	97.913			98.087		
SD	0.520			1.033		
RSD (%)	0.531			1.053		
System suitability						
Resolution (Rs) between Cis-isomer and Montelukast > 2	2.298	2.243	2.242	2.262	2.243	2.56
Theoretical plate (N)> 5000	9068	8666	8252	8834	8666	8697
Tailing factor (T) < 2	1.057	1.059	1.061	1.044	1.059	1.058
Capacity factor (k') > 1.5	6.734	6.694	6.799	7.487	6.694	7.242
RSD(%) for replicate injection (< 1)	0.056	0.029	0.031	0.012	0.029	0.030

SD: Standard Deviation; RSD: Relative Standard Deviation

Table 8: Montelukast remained in oxidative degradation kinetic study. Mean (SD), n=3

Time (hrs)	Percentage remained (%)	Concentration remained (μg/ml)	Log Concentration remained	Reciprocal concentration remained $(\mu g/ml)^{-1}$
0	100.000 (0.073)	260.660 (0.191)	2.416 (0.306 X 10^{-3})	3.840 X 10^{-3} (0.577 X 10^{-5})
1	86.751 (1.623)	226.124 (4.232)	2.354 (8.091 X 10^{-3})	4.420 X 10^{-3} (0.000)
2	82.689 (0.946)	215.537 (2.467)	2.334 (4.951 X 10^{-3})	4.64 0X 10^{-3} (0.000)
3	72.785 (0.965)	189.722 (2.516)	2.278 (5.742 X 10^{-3})	5.270 X 10^{-3} (8.621 X 10^{-5})
4	68.548 (0.893)	178.676 (2.326)	2.252 (5.654 X 10^{-3})	5.600 X 10^{-3} (0.000)
5	59.503 (1.625)	155.101 (4.236)	2.191 (11.853 X 10^{-3})	6.450 X 10^{-3} (0.000)
6	55.871 (0.461)	145.634 (1.201)	2.163 (3.625 X 10^{-3})	6.870 X 10^{-3} (5.000 X 10^{-5})
7	47.015 (0.462)	122.551 (1.204)	2.088 (4.251 X 10^{-3})	8.160 X 10^{-3} (0.000)

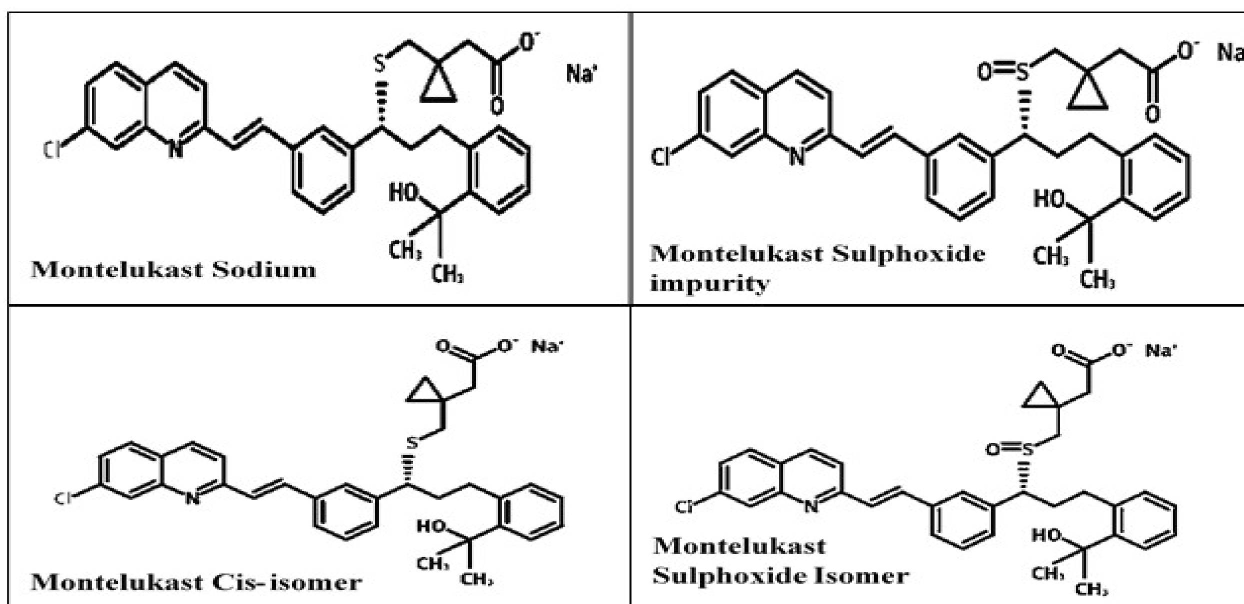

Fig. 1: Molecular structure of montelukast sodium and its impurities.

Fig. 2: Chromatograms of (A) Solvent; (B) Placebo; (C) Standard solution and (D) Montelukast chewable sample solution

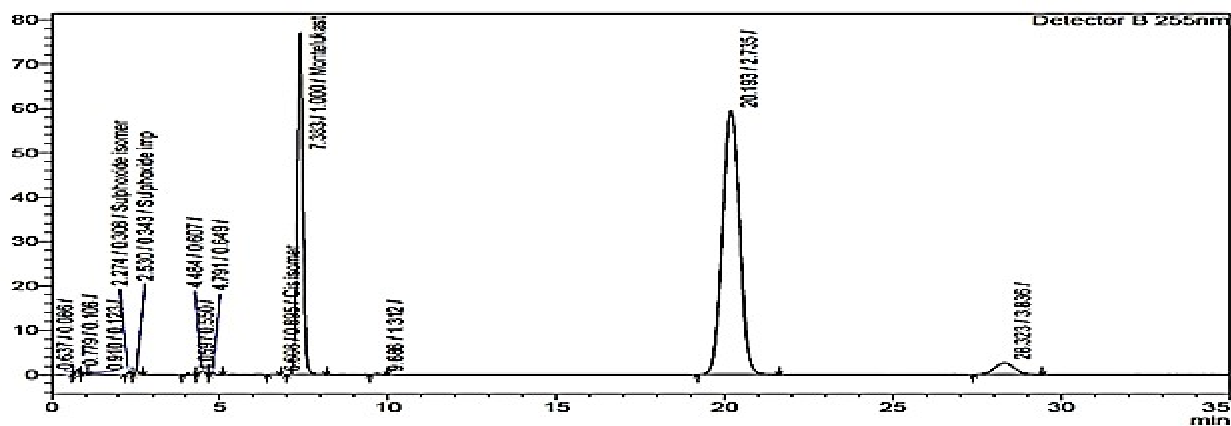

Fig. 3a: Standard solution of montelukast after exposure to acidic hydrolysis (1M HCl) for 24 hours

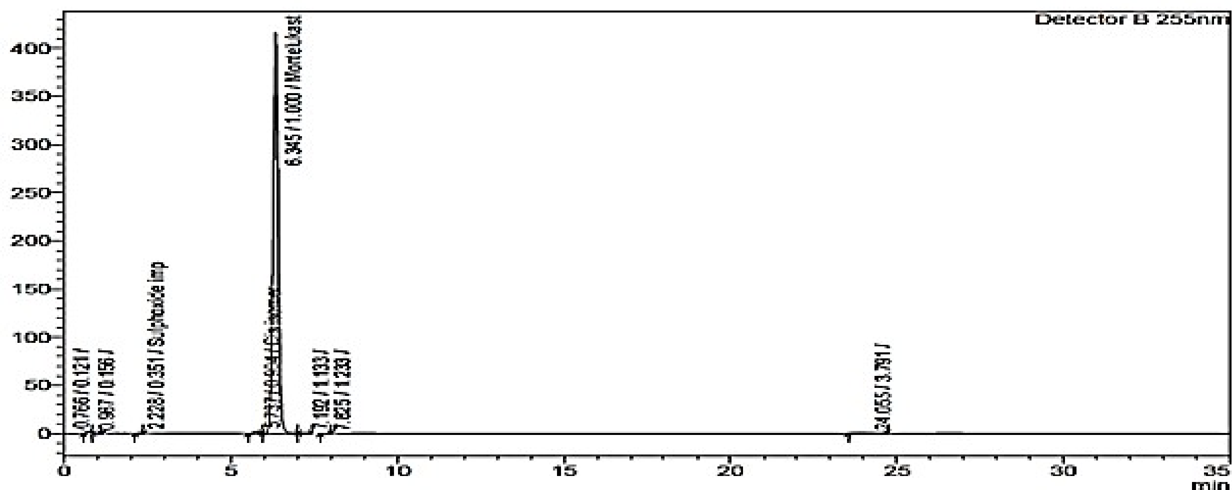

Fig. 3b: Standard solution of montelukast after exposure to alkaline hydrolysis (1M NaOH) for 24 hours

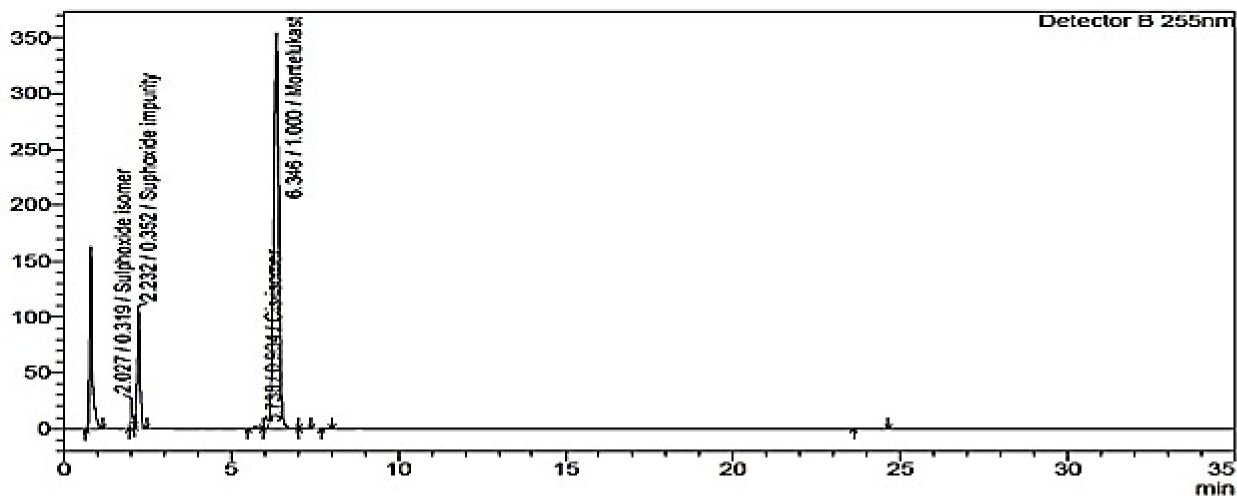

Fig. 3c: Standard solution of montelukast after exposure to 3% H_2O_2 for two hours

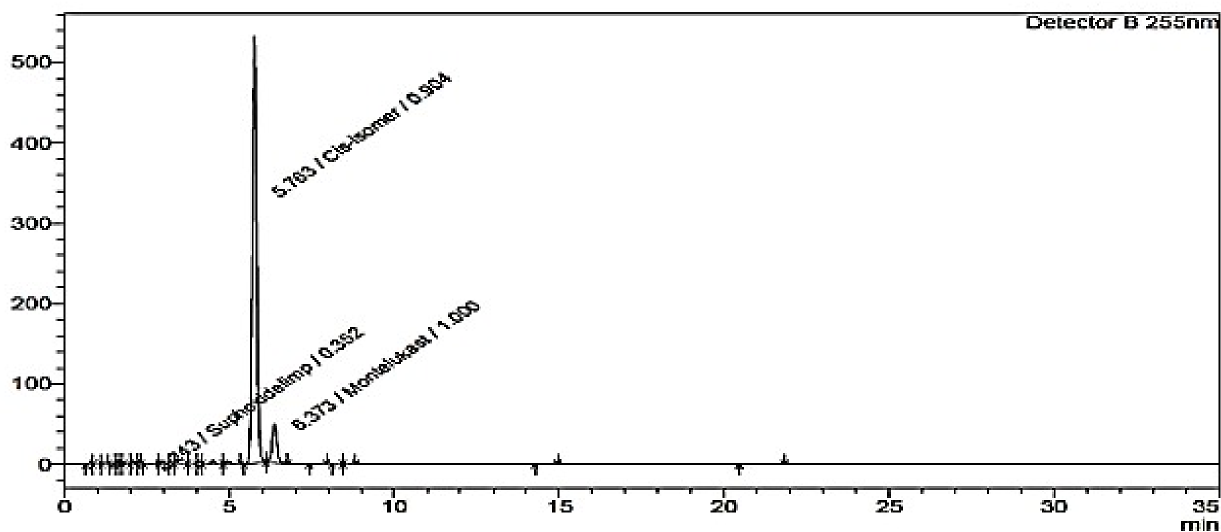

Fig. 3d: Standard solution of montelukast (solution state) after exposure for 2 hours at 365nm UV region

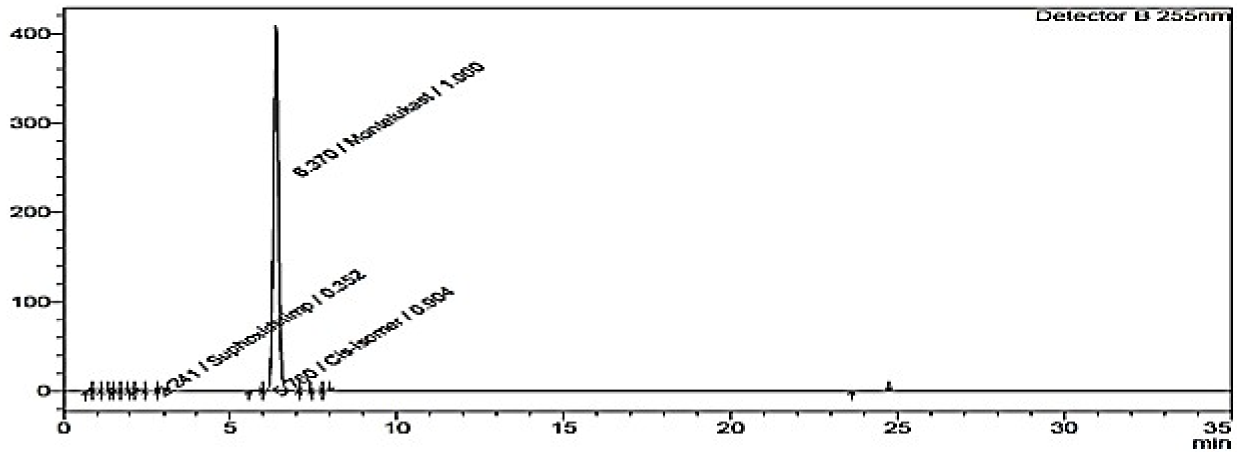

Fig. 3e: Standard solution of montelukast (solid state) after exposure for two hours at 365nm UV region

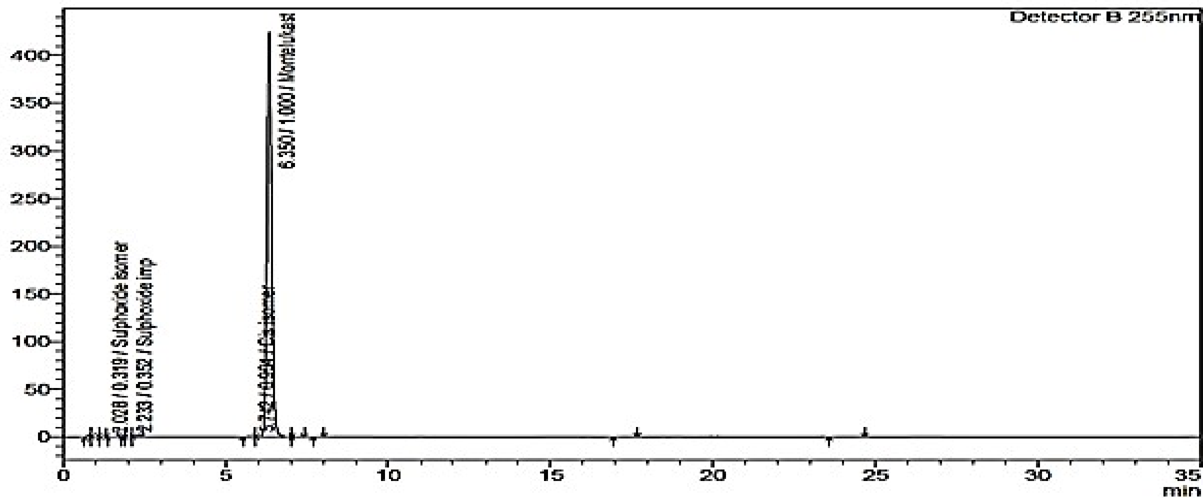

Fig. 3f: Standard solution of montelukast after exposure to heat at 80˚C for two hours

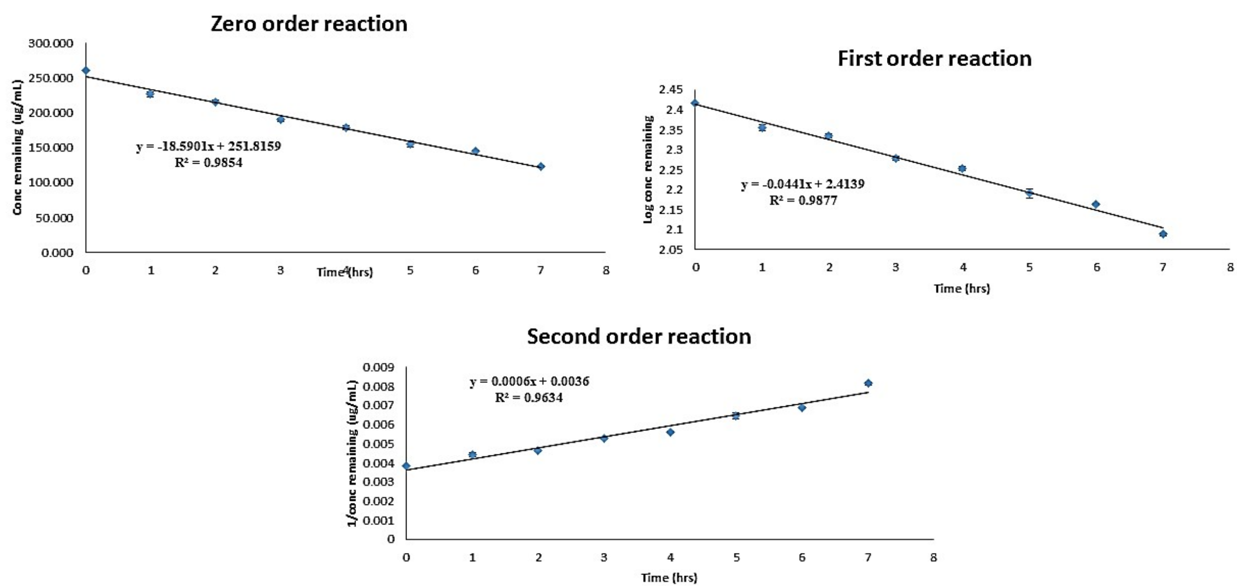

Fig. 4: Oxidation degradation kinetic of montelukast. The error bars indicate standard deviation

In intermediate precision evaluation, UHPLC was selected as an alternative instrument as it could shorten the sample run time and consume less solvent than HPLC system. Robustness should be evaluated during method validation to prevent the need of re-validation during later product development stage.

It was reported that there should be at least 50% degradation to calculate the kinetic parameters (Vaucher *et al.*, 2010). Based on the coefficient of determination values of montelukast remained, logarithm of montelukast concentration remained and reciprocal of montelukast concentration remained versus time plots, montelukast was found to follow first order kinetic reaction. The presence of sulphoxide impurity could be due to the oxidation of mercapto group in montelukast to its sulphoxide group (fig. 1). On the other hand, sulphoxide isomer (fig. 1) was believed to be the degradant, generated from both oxidation and presence of light or oxygen (Halama *et al*., 2011).

CONCLUSION

A specific, robust and more sensitive stability-indicating HPLC-UV method with LOD of 0.01µg/mL was successfully developed and validated. The specificity of the analytical method was demonstrated in force degradation study, where there was no interference to the montelukast peak. Two oxidative degradants were identified and the peaks were well resolved. The oxidative degradation of montelukast followed first order kinetic reaction with apparent degradation rate constant (k) of 0.1066 per hour, $t_{1/2}$= 6.6151hr and $t_{90\%}$ of 1.0118hr.

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