

Stability Indicating LC Method to Determination of Sodium Montelukast in Pharmaceutical Dosage Form and its Photodegradation Kinetics

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Abstract

Reversed-phase high performance liquid chromatography (LC) method is developed for the assay of sodium montelukast in coated tablets and its photodegradation kinetics. An isocratic LC separation is performed on a Zorbax XDB C18 column using a mobile phase of acetonitrile–methanol–water (pH 3.8) (75:10:15, v/v/v) at a flow rate of 0.8 mL/min and detection at 280 nm. The detector response for sodium montelukast is linear over the concentration range from 5–35 µg/mL ($r = 0.9999$). The specificity of the method is proved using stress conditions. The solutions are exposed to UV radiation (352 nm), alkaline and acid hydrolysis, oxidation, and temperature (80°C). The intra- and inter-day precision show suitable results (RSD < 0.49%). The accuracy of analytical method is 100.04% (RSD = 0.44%). Detection and quantification limits are 0.10 and 0.32 µg/mL respectively. The robustness of the method is assured after small changes in chromatographic conditions. The kinetic of photodegradation using a LC method is established and it can be described by zero-order kinetics. This developed method show to be viable for the determination of sodium montelukast in pharmaceutical dosage form and satisfactory in the determination of the kinetics of degradation.

Introduction

Sodium montelukast (Singulair), chemically known as [sodium 1-(1-(3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)-phenyl)(3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl)thio)-methylcyclo-propane) acetate] is a potent and selective leukotriene D4 receptor antagonist (anti-LTs) used in the prophylaxis and treatment of chronic asthma (1,2).

Antiasthma agents have been corticosteroids, beta (2)-agonists, and methylxanthines. Although these agents are safe and well tolerated when used properly, adverse effects may occur with use at higher dosages (3). Sodium montelukast and other

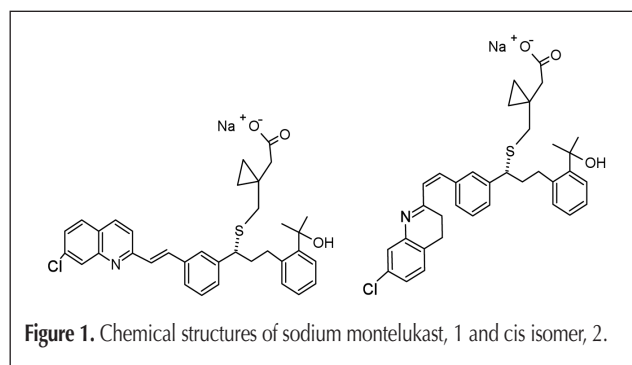
anti-LTs have been administered concomitantly with inhaled corticosteroids and demonstrate complementary effects (1,4). Recent clinical trial results suggest there may also be a role for anti-LTs as first-line therapy in children with mild asthma (5). It can be administered orally once daily and is the first leukotriene modifier approved by the US Food and Drug Administration for children from 2 to 12 years of age (2,3).

In solution, the compound is photosensitive requiring special handling precautions to protect specimens from light. Upon exposure to even very low levels of UV radiation, the montelukast readily rotates to cis isomer (6) (Figure 1).

The ICH emphasizes that the test of the drugs which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability-indicating testing methods (7,8).

Previously published analytical methods were described for the sodium montelukast determination in biological fluids (3,6,9–14), pharmaceutical formulation (12,15–18), stability (19) and impurities (20) by high performance liquid chromatography (HPLC), UV spectroscopy, voltammetry, spectrofluorometry, and capillary electrophoresis. There are no methods for sodium montelukast determination in pharmaceutical dosage form listed in any pharmacopoeia and its photodegradation kinetics was not evaluated.

Reversed-phase LC with photodiode array detection has become the technique of choice for this work, due to its compat-



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ibility with the samples, resolution, specificity, and sensitivity. There has been strong recent interest in techniques and apparatus for performing very fast high-performance liquid chromatography (HPLC) separations. Rapid and reliable analyses facilitate research studies leading to new products, solve important environmental problems, and produce new and better ways to attack health problems (21).

The previous HPLC methods for determination of sodium montelukast in tablets (15,17), require more complex procedure than the described in this paper. With this method, samples could be applied to HPLC without internal standard because the analysis of sodium montelukast samples on different days provided good repeatability. Besides the mobile phase was developed without the use of the buffer solutions causing less problems for the equipment and column.

The aim of this study was to develop and validate a rapid, sensitive, and selective HPLC method that has advantages for routine quality control analysis of sodium montelukast in tablets, like simple procedures such as the use of external standard and organic mobile phase, and the determination of its kinetics of photodegradation in methanol solution.

Experimental

Chemicals and reagents

Sodium montelukast used as reference substance (assigned purity, 100%) was purchased by Sequoia Research Products Ltd (Pang-Bourne, United Kingdom). Purity control was carried out by infrared absorption spectroscopy, and ¹H and ¹³C nuclear magnetic resonance spectroscopy.

Singulair (manufactured by Merck Sharp & Dohme Farmacêutica Ltda, Northumberland, United Kingdom) coated tablets for oral administration (10 mg per tablet, excipients: microcrystalline cellulose, magnesium stearate, lactose, sodium croscarmellose, hydroxypropyl cellulose; coated excipients: iron oxide yellow, iron oxide red, titanium dioxide, carnauba wax, hydroxypropyl methylcellulose, hydroxypropyl cellulose) was purchased in the market. Acetonitrile and methanol LC grade was purchased from Tedia (Fairfield, OH), the acetic acid was purchased from Merck (Darmstadt, Germany). High purity water was prepared by using Millipore Milli-Q purification system (Billerica, MA).

Apparatus and chromatographic conditions

The LC system consisted of an Agilent 1200 series, equipped with G1311A quaternary pump, G1322A Photodiode Array Detector, G1329A auto sampler, G1320B thermostat, G1322A degasser module, and data were acquired and processed by Chemstation software (Waldbronn, Germany).

The column used was a Zorbax Eclipse XDB (Palo Alto, CA) C18 column (150 mm × 4.6 mm, i.d., 5 μm particle size). The mobile phase was acetonitrile–water (pH 3.8, adjusted with acetic acid)–methanol (75:15:10, v/v/v). The flow rate was 0.8 mL/min and the samples injection volume was 20 μL. The detection was achieved with photodiode array detection at 280 nm. The temperature was at 25°C in the column oven. The quantita-

tion was performed using the absolute area of the peak.

The light source used for photodegradation kinetics was an UV fluorescent lamp model Ecolume, 30W, emitting radiation at 352 nm, fixed to a chamber in a horizontal position. The chamber was internally coated with mirrors, in order to distribute the light uniformly. The effect of light was studied exposing the methanol sample solutions in 1 cm quartz cells. The temperature was controlled into the chamber and was always ~ 30°C.

All solutions were filtered through a 0.45-μm Millipore membrane filter (São Paulo, Brazil) before injection.

Methods

Preparation of the standard solutions

A stock solution of 100 μg/mL sodium montelukast reference substance was prepared in a volumetric flask by dissolving 10 mg of drug in 100 mL of methanol. Aliquot of the 4 mL of this solution was transferred into 20-mL volumetric flask and the same solvent added to make up the volume in order to give a final concentration of 20 μg/mL.

Preparation of the sample solutions

Twenty tablets were weighed and finely powdered. A quantity equivalent to 10 mg of sodium montelukast was transferred to a 100 mL volumetric flask with 60 mL of methanol. This flask was kept in an ultrasonic bath for 20 min. The volume was completed with the same solvent and an aliquot of 4 mL of this solution was diluted in a 20-mL volumetric flask to give a final concentration of 20 μg/mL.

To avoid the influence of light, the preparation of samples and standard solutions was made with amber glass and low-light environment.

Method validation

The analytical method was validated with respect to parameters such as specificity, linearity, limit of detection, limit of quantification, precision, accuracy, and robustness (22,23,24).

Specificity

Forced degradation studies were performed to provide an indication of the method specificity (7,8). The interference of the excipients was also evaluated.

The hydrolysis study in alkaline and acidic conditions was carried out in 0.1 M NaOH and 0.1 M HCl respectively, at room temperature (24 ± 2°C) for 2 h and neutralized. An aliquot of each solution was diluted in methanol to give a final concentration of 20 μg/mL. The oxidative reaction was performed in 3% H₂O₂ at room temperature for 2 h. An aliquot of this solution was diluted in methanol to give a final concentration of 20 μg/mL. The stress degradation study in direct UV radiation (352 nm) was performed exposing the sodium montelukast in methanol solution (100 μg/mL) for 15 min in a photostability chamber. After this time this solution was diluted to 20 μg/mL with the same solvent. Samples protected from light, submitted to identical conditions, were used as a control. The stress degradation study in high temperature was performed exposing the sodium montelukast in

methanol solution (100 µg/mL) for 8 h in an oven (80°C). Peak purity test performed by photodiode array detector was used to show that the drug chromatography peak did not contain more than one substance.

Linearity

Stock solution (100 µg/mL) of sodium montelukast was prepared and aliquots were transferred to volumetric flasks to obtain the final concentration of 5, 10, 15, 20, 25, 30, and 35 µg/mL. Each one was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting three individual preparations of sodium montelukast and the relative standard deviation (RSD%) of the absolute area of the peak was evaluated.

Precision

The precision test was evaluated by analyzing six sample solution of sodium montelukast in the same day for intra-day precision (repeatability) and on three different days for inter-day precision (intermediate precision). The relative standard deviation (RSD%) was determined.

Accuracy

The accuracy was performed by the recovery test, which consisted of adding known amounts of reference substance to the samples solutions. Aliquots of 1.0, 2.0, and 3.0 mL of sodium montelukast standard solution (100 µg/mL) were transferred to 20 mL volumetric flasks respectively (corresponding to 25.0%, 50.0%, and 75.0% of nominal analytical concentrations), prepared as described in the "Samples preparation" section. Each solution was prepared in triplicate.

Robustness

Robustness testing was performed in order to evaluate the susceptibility of measurements due to deliberate variations in analytical conditions. Small changes in the chromatographic conditions were made, such as percentage of acetonitrile (73% and 77%), pH of aqueous phase (3.6 and 4.0), flow rate (0.7 and 0.9 mL/min) and column (with the same specification, but acquired from different supplier: ACE – 121-1546 (150 mm × 4.6 mm, i.d., 5 µm particle size) (Aberdeen, Scotland).

System suitability

System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such (24). To verify system suitability of the analytical method during the validation, five replicates injections of a standard solution were used. This solution was prepared with a final concentration of 20 µg/mL like was described under "Preparation of the standard solutions." Theoretical plates, tailing factor, and

retention factor were determined. Data were acquired and processed by Chemstation software (Waldbronn, Germany).

Photodegradation study

The kinetics of photodegradation of montelukast was evaluated in methanol. Twenty coated tablets were weighed and finely powdered. A quantity equivalent to 100 mg of sodium montelukast was transferred to a 200 mL volumetric flask with 120 mL of methanol. This flask was kept in an ultrasonic bath for 20 min and the volume was completed with the same solvent to give a final concentration of 500 µg/mL.

The stress degradation study was performed exposing the solutions contained in quartz cells in the chamber. The samples were positioned horizontally, to provide maximum area of exposure to the light source. Considering the UV absorption of sodium montelukast, the irradiation was carried out at 352 nm at different time intervals (0, 3, 6, 9, 12, 15, and 18 min). In order to evaluate the contribution of thermally induced change to the total change, protected samples, wrapped in aluminium foil, were used as dark controls. Three samples were analyzed for each time interval. After the time, the samples were diluted with methanol to give final concentration of 20 µg/mL. The samples were assayed by LC, using standard solution to the quantitation of the drug. All solutions were injected in triplicate. Placebo solutions were prepared at the same way, in order to verify the influence of the excipients in the degradation process.

Kinetics calculations

The degradation rate kinetics of sodium montelukast were determined by plotting concentration of the drug remaining versus time (zero-order process), logarithm of concentration of the drug versus time (first-order process), and reciprocal of concentration of the drug versus time (second-order process). The regression coefficient (r) was obtained, and the best fit observed indicates the reaction order. The kinetics parameters like apparent order degradation rate constant (k), and t_{90} (time where 90% of original concentration of the drug is left) were obtained.

Results and Discussion

Method development

The choice of the best dissolution medium for samples was accomplished prime. Considering the solubility of sodium montelukast, methanol, acetonitrile, water, and the mobile phase were tested. Methanol was chosen as the best diluent to the samples because it results in good analytical conditions.

The experimental conditions were chosen after testing different mobile phases. Acetonitrile and methanol are commonly used organic solvents in LC (23), and reversed-phase chromatography was performed with various mixtures of methanol–water, acetonitrile–water, and acetonitrile–water–methanol. The methanol–water and acetonitrile–water mixtures presented elevated retention time and the peak purity tool applied for sodium montelukast peak showed that it was not pure in these conditions. The pH values of aqueous phase were checked over a range (pH 3.0–8.0) before mixing with acetoni-

trile and methanol. Finally, a mobile phase consisting of a mixture of acetonitrile–water–methanol 75:15:10 (v/v/v), pH 3.8 was adopted, because it keeps the drug in a not ionized form allowing separate the degradation products and sodium montelukast satisfactorily. The net charge of the ion is dependent on the degree of ionization given by the pka value of the acid or basic functional group and the pH of the solution. The pka of sodium montelukast is 4.7 and the use of solution in acid range not allowed the ionization of the molecule.

Method validation

In order to verify the specificity of the method, forced degradation studies were performed (7,8). The analysis of the excipients was evaluated and it was proved that the peak at 6.8 min was not suffering interference of any compound from the formulation (Figure 2A).

	Intraday precision			Inter-day Precision*
	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	
Mean	101.13	101.76	100.77	101.22
RSD%	0.44	0.38	0.42	0.49

* Data expressed as mean of three days.

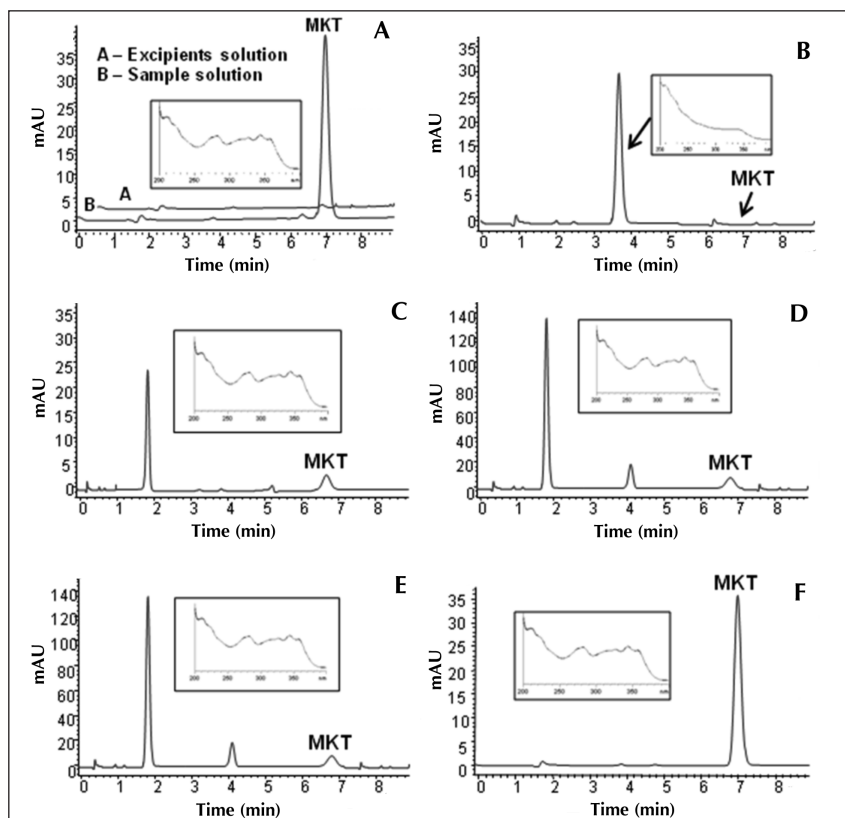


Figure 2. Chromatograms of sodium montelukast (MKT). Overlapping of the chromatograms of the tablet solution of sodium montelukast and the excipients solution (A); Hydrolysis with NaOH 0.1 N for 2 h (B); HCl 0.1 N for 2 h (C); H₂O₂ 3.0% for 2 h (D); photodegradation (352 nm) for 15 min (E); and temperature for 80°C for 8 h (F).

The hydrolysis study in alkaline, acid, and oxidative conditions showed extensive degradation of sodium montelukast (Figure 2B, 2C, and 2D, respectively). Several degradation products were formed and no interference was observed in quantitation of sodium montelukast, showing the specificity of the method.

Preliminary stability investigations revealed that sodium montelukast undergo degradation upon exposure to light and its photolability was established by forced degradation testing (stress testing). The exposure to UV radiation (352 nm) shows an important degradation with the presence of a majority peak (Figure 2E). This way, light is a critical factor in the development and validation of the LC method. Sodium montelukast was quantified in biological fluids using a liquid chromatographic method (6), which suggested that the drug rotates to its geometric configuration cis isomer, under light exposure. The stress degradation study in high temperature did not promote degradation of sodium montelukast (Figure 2F).

Thus, the specificity evaluation shows no interferences from the results of stress testing studies, diluents, impurities, and excipients, showing a high degree of specificity of this method for sodium montelukast. The tests with the photodiode array detector showed that there was no co-eluting peak interfering in the analysis of the drug. These results demonstrated that the developed and validated LC method is specific.

To assess the linearity, a standard curve for sodium montelukast was constructed by plotting concentrations ($\mu\text{g/mL}$) versus absolute area (mVs) and showed good linearity on the 5.0–

35.0 $\mu\text{g/mL}$ range. The slope and intercept (\pm RSD, $n = 3$) of calibration plot for this drug were 23.77 ± 0.07 and 7.45 ± 0.77 , respectively. The correlation coefficient was $r = 0.9999$, indicating good linearity. The data were validated by means of the analysis of variance, which demonstrated significant linear regression (F calculated = 122190.351 > F critical = 4.6; $p < 0.05$) and no significant linearity deviation (F calculated = 1.414 < F critical = 2.96; $p < 0.05$). The detection and quantitation limits determined were 0.1 and 0.32 $\mu\text{g/mL}$ (RSD = 0.852%), respectively. These low values indicated the high sensitivity of the purposed method. The experimental values obtained for the determination of the precision of the method are presented in Table I. The low relative standard deviation (RSD) of intra-day precision and inter-day precision showed the good precision of the method. The accuracy expresses the agreement between the accepted value and the value found. The mean recovery was found to be 100.40% for the tablets (Table II). This value shows the good accuracy of the purposed method.

Table III shows the results of the robustness of the method. At that rate, it was possible to demonstrate that the developed method was robust for the changes employed. Statistical evaluation of the robustness results by analysis of variance shows no significant variations for all parameters ($p < 0.05$).

The system suitability test of the chromatography system was performed during the validation run. It was verified through common parameters. The results were: theoretical plates ($N = 5755$, $RSD = 0.44\%$), tailing factor ($T = 1.022$, $RSD = 1.03\%$), and retention factor ($K = 2.6$, $RSD = 0.98\%$). The obtained values were in accordance with the literature (24).

Amount added	Concentration ($\mu\text{g/mL}$)			RSD (%)
	Amount found	n	% of recovery	
5	4.99	3	99.93	0.77
10	10.30	3	100.80	0.37
15	15.45	3	100.49	0.18
Mean			100.40	0.44

Chromatographic parameters	Retention time* ($\pm RSD\%$)	Area* ($\pm RSD\%$)	Asymmetry* ($\pm RSD\%$)	Theoretical plates* ($\pm RSD\%$)
Acetonitrile–methanol–water (pH 3.8) (75:10:15, v/v/v) Flow rate: 0.8 mL/min Zorbax Eclipse C18 column†	6.86 ± 0.03	500.53 ± 1.89	1.05 ± 0.02	5890 ± 1.09
(73:12:15, v/v/v)	5.89 ± 0.08	508.53 ± 5.60	1.06 ± 0.05	5792 ± 1.09
(77:08:15, v/v/v)	5.96 ± 0.03	507.53 ± 4.90	1.06 ± 0.04	6239 ± 1.08
Flow rate: 0.7 mL/min	7.91 ± 0.10	569.30 ± 5.69	1.07 ± 0.06	6448 ± 2.04
Flow rate: 0.9 mL/min	6.13 ± 0.16	441.10 ± 3.68	1.06 ± 0.11	5697 ± 3.06
pH 3.6	6.86 ± 0.09	503.17 ± 3.47	1.06 ± 0.05	6349 ± 1.07
pH 4.0	6.81 ± 0.05	502.35 ± 6.98	1.06 ± 0.06	6103 ± 2.06
Ace-121-1546 C18 column	6.46 ± 0.10	497.71 ± 4.68	1.11 ± 0.10	4631 ± 2.15

* Number of samples analysed is three.
† Work parameters

Time (min)	Measured concentration* ($\mu\text{g/mL}$)	RSD (%)
0	19.58 (97.92%)	0.53
3	18.52 (92.59%)	1.95
6	16.01 (80.08%)	0.49
9	12.96 (64.83%)	0.41
12	10.96 (54.80%)	0.39
15	9.24 (46.20%)	1.45
18	7.34 (36.72%)	0.70

*Each value is the mean of three analyses

Kinetics of photodegradation

In this work, the kinetics of photodegradation of sodium montelukast was carried out through the employment of stress conditions (25). The exposure to light was found to be an important adverse stability factor. The room light influence on sodium montelukast solutions was preliminary evaluated. Figure 3 shows the UV–vis spectra of sodium montelukast solution in methanol before and after exposure to daylight for different time intervals. It was observed that the drug undergoes quickly degradation by the time of exposure in the region 200–400 nm. The LC method was used to the determination of the drug in the degraded samples. The photodegradation profile of sodium montelukast was evaluated at different time intervals under radiation at 352 nm into a chamber. The effect of light on the residual concentration of sodium montelukast in degraded samples is shown in Table IV. It was observed that almost 65% of the drug degraded after 18 min of exposure to light. Typical chromatograms, showing the observed changes during the degradation in methanol solutions, in comparison to the initial sample,

are depicted in Figure 4. The majority degradation product at 5.7, can be observed for degradation in methanol solution. The differences between the spectrums obtained by the diode array detector for the sodium montelukast and the major degradation product are showed in Figure 5. In accordance with Al-Omari and his coworkers this peak attributed to a degradation product can be montelukast cis-isomer (19).

The kinetics of photodegradation was calculated, through the fall in drug concentration with the time. The concentration of remaining sodium montelukast was calculated at each time interval for the three replicates, in comparison with the mean concentration of the standard solution of the drug. The plots of concentration, logarithm of concentration, and reciprocal of concentration of drug remaining versus time, are shown in Figure 6. Through the evaluation of the correlation coefficients, it can be demonstrate that the degradation process of sodium montelukast in methanol solutions can be described by zero-order reaction

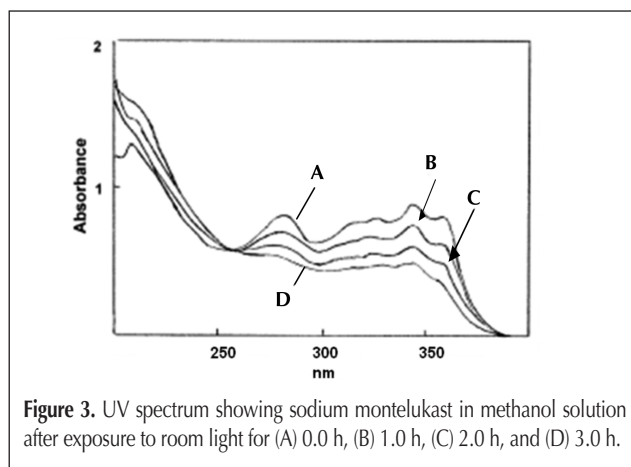


Figure 3. UV spectrum showing sodium montelukast in methanol solution after exposure to room light for (A) 0.0 h, (B) 1.0 h, (C) 2.0 h, and (D) 3.0 h.

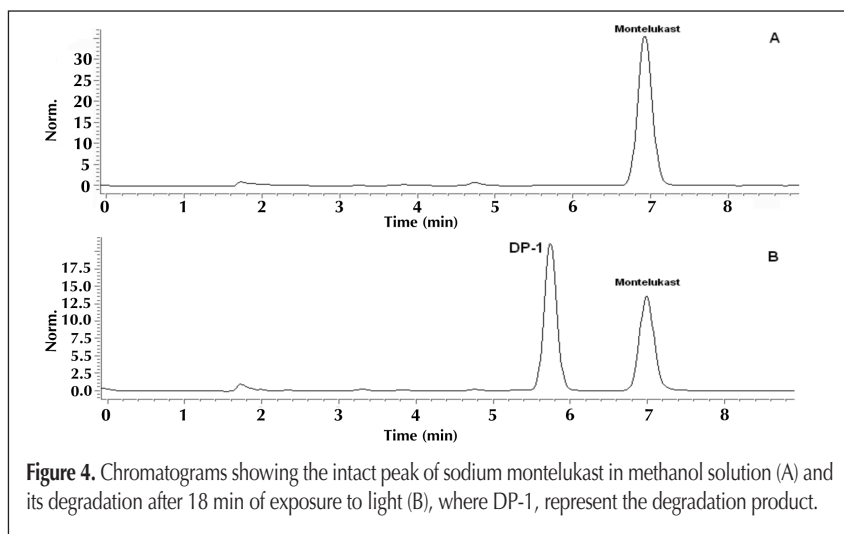


Figure 4. Chromatograms showing the intact peak of sodium montelukast in methanol solution (A) and its degradation after 18 min of exposure to light (B), where DP-1, represent the degradation product.

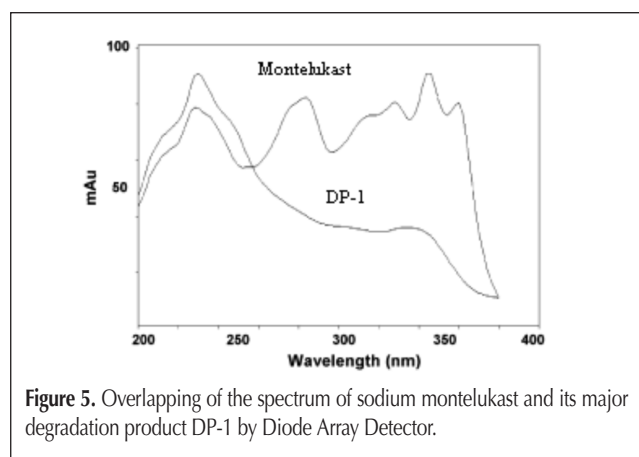


Figure 5. Overlapping of the spectrum of sodium montelukast and its major degradation product DP-1 by Diode Array Detector.

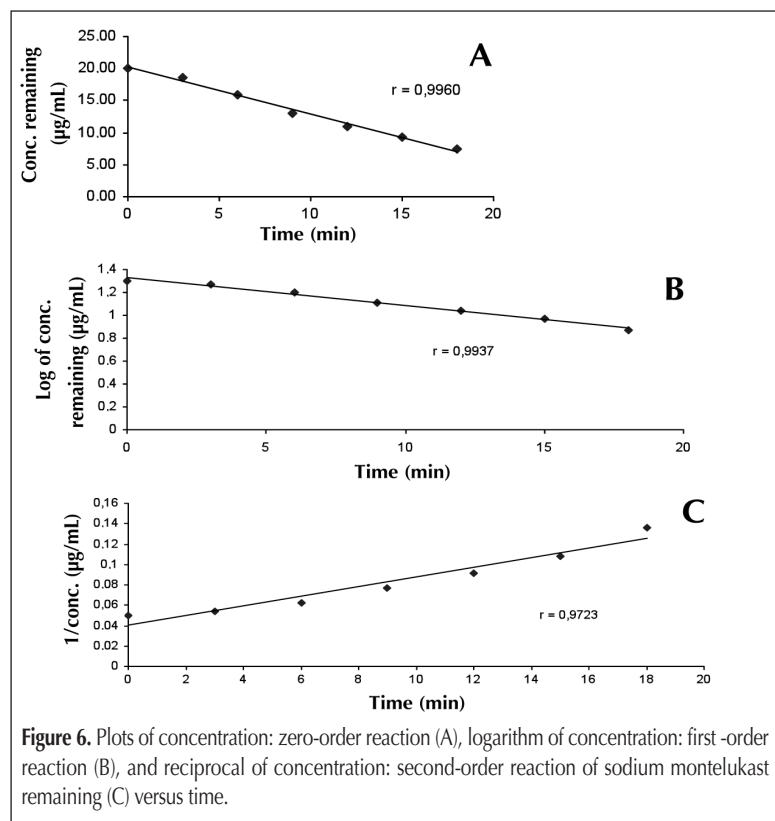


Figure 6. Plots of concentration: zero-order reaction (A), logarithm of concentration: first-order reaction (B), and reciprocal of concentration: second-order reaction of sodium montelukast remaining (C) versus time.

kinetics under the experimental conditions used in this study. In the zero-order reaction kinetics the concentration of the sodium montelukast decreases in proportion to the increased time of exposure to UV light. From the slopes of the straight lines it was possible to calculate the zero-order reaction degradation rate constant k , and the $t_{90\%}$, $0,6872 \text{ min}^{-1}$ and 2.90 min, respectively.

Considering the obtained results, future works will be done in order to elucidate the main photodegradation product of sodium montelukast using spectroscopic and chromatographic techniques.

Conclusion

The results indicated that the reversed phase LC assay presented linearity, precision, and accuracy. Besides, the developed method is specific, robust, and sensitive being an acceptable method for the routine quality control of sodium montelukast in the formulation studied. The kinetics of photodegradation of sodium montelukast in methanol solutions was determined. The stability-indicating LC method was satisfactorily employed in the quantitation of the drug in the presence of its degradation products. The photodegradation of sodium montelukast follow zero-order reaction kinetics. The kinetics parameters of degradation rate constant, and t_{90} were calculated.

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Manuscript received March 7, 2010;
revision received July 14, 2010.