Pharmaceutical Sciences Department[1] and Environmental Geochemistry Laboratory[2], Federal University of Rio Grande do Sul, Porto Alegre, Brazil

# Stability indicating method for sodium montelukast in pharmaceutical preparations by micellar electrokinetic capillary chromatography

J. Roman[1], M.D. Malesuik[1], A. Jablonski[2], M. Steppe[1]

*Received February 24, 2010, accepted March 24, 2010*

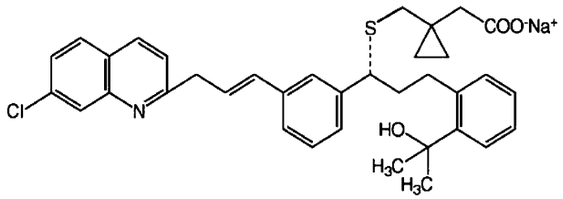
*Msc J. Roman, Pharmaceutical Sciences Department, Federal University of Rio Grande do Sul, Av. Ipiranga, 2752. Lab. 402, Porto Alegre-RS CEP, 90610-000, Brazil*
*Juliana@uri.com.br*

*Pharmazie 65: 645–649 (2010)* *doi: 10.1691/ph.2010.0055*

A simple, reliable micellar electrokinetic chromatography method (MEKC) for the determination of sodium montelukast in coated tablets was developed and validated. Successful results were obtained with 10 mmol $L^{-1}$ borate buffer and 30 mmol $L^{-1}$ sodium dodecyl sulfate at pH 9.4, injection time of 5.0 s, an applied voltage of 25 kV and a column temperature of 25 °C. The detector response for sodium montelukast was linear over the concentration range from 20 to 100 μg $mL^{-1}$ ($r$ = 0.9995). The intra and inter-day precision showed suitable results (RSD < 1.46%). The analytical method accuracy was 99.67% (RSD = 1.11%). The limits of detection and quantitation were 0.75 and 2.00 μg $mL^{-1}$ respectively. The method demonstrated robustness and showed to be viable for the sodium montelukast determination in pharmaceutical dosage form.

## 1. Introduction

Sodium montelukast is a potent and selective antagonist of the cysteinyl leukotriene (anti-LTs) receptor used for the treatment of asthma, which belongs to a styrylquinolines series with the following chemical name 2-[1-[1(R)-[3-[2(E)-(7-chloroquinolin-2-yl)vinyl]phenyl]-3[2-(1-hydroxy-1-methylethyl)phenyl] propylsulfanylmethyl] cyclopropyl] acetic acid sodium salt (Ramakrishnam et al. 2005; Migoya et al. 2004).




Sodium montelukast

Despite of being largely employed in the treatment of common asthma, there is no monograph of this drug in any pharmacopoeia. Besides, there are few methods reported in the literature for sodium montelukast determination in biological fluids (Liu et al. 1997; Ochiai et al. 1998; Spector 2001; Al-Rawithi et al. 2001; Kitchen et al. 2003; Smith et al. 2004; Alsarra et al. 2005; Papp et al. 2007), and pharmaceutical formulations (Al-Rawithi et al. 2001; Radhakrishna et al. 2002; Alsarra 2004; Hoang et al. 2007), stability (Al-Omari et al. 2007) by high performance liquid chromatography (HPLC), ultraviolet (UV) spectroscopy, voltammetry and spectrofluorometry. The determination of impurities was related for Shakalisava and Regan (2008) by capillary electrophoresis (CE), but, no CE method can be found in the literature for sodium montelukast determination in pharmaceutical dosage forms.

The aim of this study was to develop and validate a CE method for the assay of sodium montelukast in pharmaceutical formulations. For this purpose, the influence of buffer type, buffer concentration, pH, organic modifier, applied voltage and injection time was systemically investigated. Studies to validate this method were performed according to the evaluation of the validation parameters such as linearity, sensitivity, selectivity, accuracy, precision, and robustness according to the ICH Guidelines (ICH 2003; ICH 2005). The results obtained by the developed method were compared with those received by a the HPLC method from the literature (Shakalisava and Regan 2008).

## 2. Investigations, results and discussion

### *2.1. Method development*

The experimental conditions were chosen after testing the different parameters that influence the capillary electrophoresis analysis such as dissolution medium, buffer pH and concentration, applied potential and injection time.

The capillary electrophoresis free solution was tested firstly due to its easy implementation. The choice of the buffer system used as electrolyte solution has a major influence on the separation and should be made carefully. Phosphate buffer (30 mmol $L^{-1}$ and pH 6.8, 7.4 and 8.0) was evaluated as electrolyte solution. The results were unsatisfactory due to the appearance of large and asymmetric peaks.

Sodium tetraborate decahydrate solution was tested with concentrations ranging from to 10 to 30 mmol $L^{-1}$. Best results were obtained with a concentration of 10 mmol $L^{-1}$ and pH 9.4, but these conditions showed asymmetric peaks. To solve this problem, sodium dodecyl sulfate was added to the sodium tetraborate decahydrate solution in order to obtain a micellar system. The 10 mmol $L^{-1}$ of sodium tetraborate decahydrate and

30 mmol $L^{-1}$ sodium dodecyl sulfate mixture showed a good peak shape, short migration times, low peak width and higher efficiency.

The effect of the buffer pH was investigated within the range from 7.0 to 9.4 in the 10 mmol $L^{-1}$ of sodium tetraborate decahydrate and 30 mmol $L^{-1}$ sodium dodecyl sulfate mixture. 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 electrolyte solution in basic range allowed a complete ionization of the molecule. When the pH of the buffer solution increased, the sodium montelukast migration times decreased, improving the peak symmetry. So pH 9.4 was chosen as the optimum pH value of the running buffer.

The addition of organic modifiers to the running buffer was considered because they affect several parameters such as viscosity, dielectric constant, zeta potential, migration time, peak symmetry and resolution (Altria 1996). Also, organic solvents may be used as modifiers in electrolyte buffers to influence the mobilities of the analytes in a particular direction, which can improve the selectivity in a single system. Thus, methanol was added at various concentrations (5, 10 and 15%, v/v) to the running buffer of 10 mmol $L^{-1}$ of sodium tetraborate decahydrate and 30 mmol $L^{-1}$ sodium dodecyl sulfate at pH 9.4. The sodium montelukast migration times increased significantly with the addition of organic modifier, so no organic modifiers were added to the electrolyte solution.

The effect of the applied voltage was studied in the range 20–30 kV. Using 10 mmol $L^{-1}$ of sodium tetraborate decahydrate and 30 mmol $L^{-1}$ sodium dodecyl sulfate at pH 9.4 as running buffer, the increased applied voltage led to both shorter analysis times and sharper peaks. However, higher voltages also exhibited higher currents and increased Joule heating. To limit this heating inside the capillary, the voltage chosen was 25 kV.

In order to improve sensitivity, sample solutions were hydrodynamically injected at 50 mbar while the injection time ranged from 3 to 6 s. The peak area increased with increasing injection times. After 5 s, the peak shapes of sodium montelukast deformed, so 5 s was selected as the optimum injection time. The pressure used for injection was always 50 mbar.

Under these optimized conditions, the migration time of sodium montelukast was $5.03 \pm 0.04$ min.

### *2.2. Method validation*

Before applying an analytical method in the quality control, it is necessary to validated according to the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH 2003; ICH 2005) and USP 30 (USP 2007) guidelines.

The use of an internal standard is recommended for quantitative analysis to correct errors, which are introduced by variables injection, volume or voltage and to improve the injection precision (Mayer 2001; Pereira et al. 2005). However the internal standard is usually employed when the sample is taken through some type of pretreatment prior to injection, such as extraction or successive steps of sample preparation.

The external standardization method of calibration was performed to evaluate the linearity of proposed method. This procedure was chosen because the analysis of sodium montelukast samples on different days provided good repeatability (RSD < 1.00%).

#### *2.2.1. Specificity*

In order to verify the specificity of the method, forced degradation studies were performed (ICH 2003; USP 2007). The analysis of the excipients was evaluated and it was shown that the sodium montelukast peak did not suffer interference from any compound present in the formulation (Fig. a).

The hydrolysis study in alkaline, acid, and oxidative conditions showed degradation of sodium montelukast (Figs. b, c and d respectively) but no interference was observed in the sodium montelukast quantitation, showing the specificity of the method.

Preliminary stability investigations revealed that sodium montelukast undergoes 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 (Fig. e). Sodium montelukast was quantified in biological fluids using a liquid chromatographic method (Overbeek et al. 2004), and his impurities determinated by capillary electrophoresis (Shakalisava and Regan 2008) which suggested that the drug rotates to its geometric configuration *cis* isomer, in the presence of light.

The stress degradation study at high temperatures did not promote degradation of sodium montelukast (Fig. f).

The peak purity tool shows that the peaks were 100% pure. Thus, the specificity evaluation shows no interferences from the results of the stress test studies, diluents, impurities, and excipients, showing a high specificity degree of this method for sodium montelukast.

#### *2.2.2. Linearity*

Under the optimum analysis conditions, linearity was studied in the concentration range from 20 to 100 μg $mL^{-1}$ for sodium montelukast. A standard curve was constructed by plotting concentrations (μg $mL^{-1}$) versus absolute area (mV s). The slope and intercept (± RSD, n = 3) of calibration plot for this drug were 1.781 (± 0.006) and 0.582 (± 0.044), respectively. The correlation coefficient was r = 0.9995, indicating good linearity. The data were validated by means of the analysis of variance, which demonstrated significant linear regression ($F$ calculated = 17185.146 > $F$ critical = 4.6; $p < 0.05$) and no significant linearity deviation ($F$ calculated = 2.489 < $F$ critical = 2.96; $p < 0.05$).

#### *2.2.3. Sensitivity*

A signal to noise ratio of approximately 3:1 is generally considered to be acceptable for estimating the limit of detection (LOD), which is the lowest concentration that can be detected (ICH 2005). The calculated LOD values of sodium montelukast was 0.75 μg $mL^{-1}$ (RSD = 3.88%) ($n = 7$). The limit of quantitation (LOQ) is the lowest concentration of sodium montelukast on the standard curve that can be quantified with acceptable precision and accuracy (ICH 2005). The LOQ was found as 2.00 μg $mL^{-1}$ (RSD = 2.75%) ($n = 7$). These values are adequate to the purpose of the CE method.

#### *2.2.4. Precision*

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. The analysis precision was determined by calculating the relative standard deviation (RSD%). The R.S.D. values of intra-day and inter-day studies ranged from 0.43 to 1.46%, showing that the method precision was satisfactory.

#### *2.2.5. Accuracy*

The accuracy expresses the agreement between the accepted value and the value found. The mean recovery was found to be 99.67% for tablets with RSD of 1.11%. This value shows the good accuracy of the purposed method.

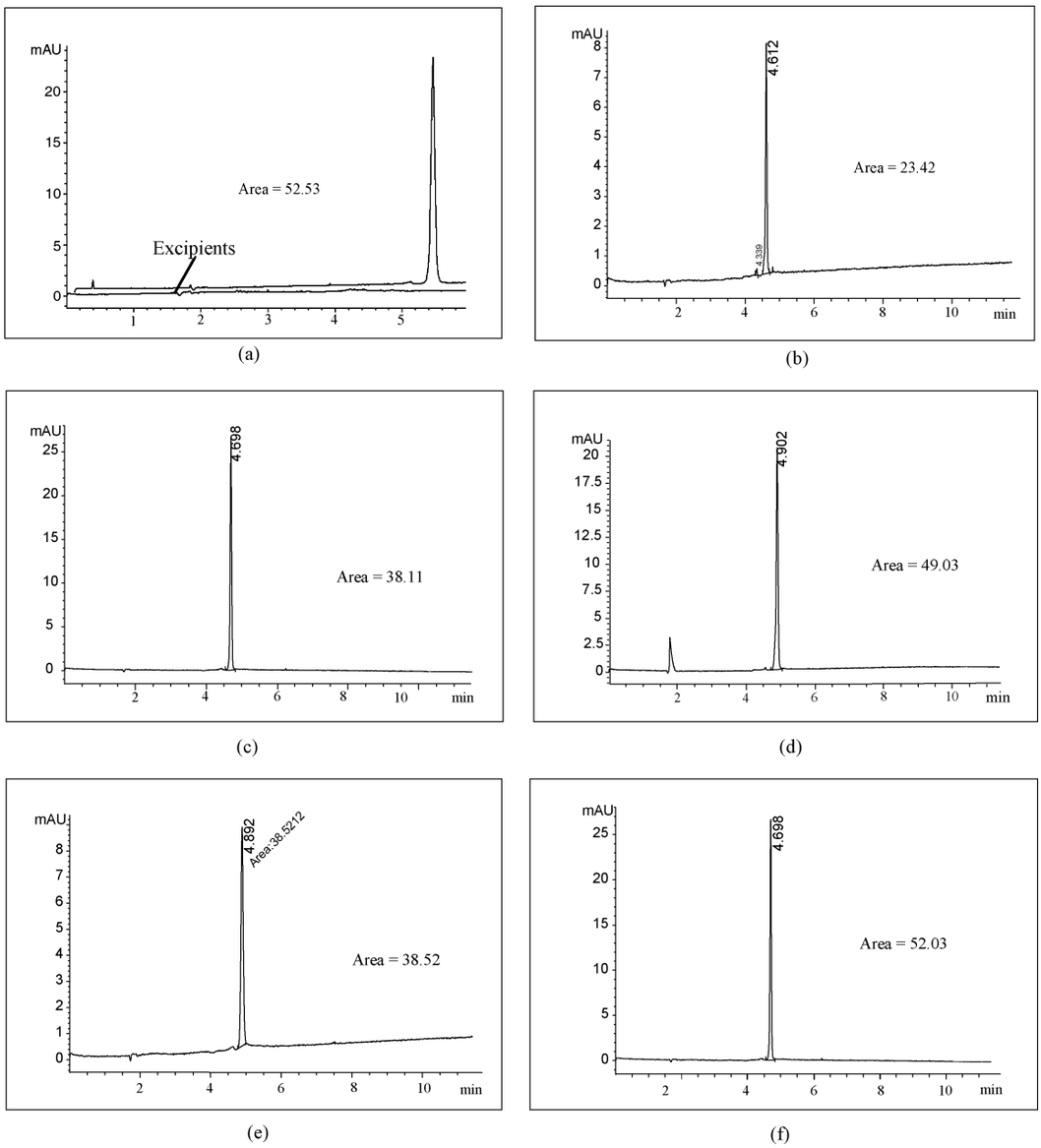



Fig.: Electropherograms of sodium montelukast. Overlapping of the electropherograms of the sample solution of sodium montelukast and the excipients solution (a); hydrolysis with 0.1 mol $mL^{-1}$ NaOH for 2 h (b); 0.1 mol $mL^{-1}$ HCl for 2 h (c); $H_2O_2$ 3.0% for 2 h (d); photodegradation (352 nm) for 15 min (e) and temperature for 80 °C for 8 h (f)

### *2.2.6. Robustness*

The robustness of the proposed method was examined by evaluating the influence of small variations of some of the most important procedure variables such as buffer pH (9.3 and 9.5), applied voltage (24 and 26 kV) and hydrodynamic injection (4 and 6 s) for 50 μg $mL^{-1}$ of sodium montelukast tablets solution through the CE method. Analyses were carried out in triplicate and only one parameter was changed in the experiments at a time. The migration time, the sodium montelukast content, symmetry and theoretical plates under the various conditions were not different when compared to the standard solution submitted to the same variables. None of these results affected the sodium montelukast assay significantly and the proposed method could be considered robust. Statistical evaluation of the robustness results through analysis of variance shows no significant variations for all parameters ($p < 0.05$).

The method shows good performance with respect to specificity, linearity, accuracy, precision and robustness and it offers a simple, fast, inexpensive and precise way for the sodium montelukast determination in pharmaceutical preparations. The proposed method was directly applied to the analysis of pharmaceutical dosage forms without the need for separation or complex sample preparation such as extraction steps prior to the drug analysis.

## *2.3. Comparison between MEKC and HPLC method*

The amount found by the HPLC method was $101.45 \pm 1.72$ (mean % ± SD, $n = 5$) of the label declaration, which is within the limits fixed by USP 30 (USP 2007). Due to the fact that sodium montelukast has no monograph available in pharmacopoeias, the acceptance criteria applied were those in the general notes of USP 30 (USP 2007). These suggest that 10% of deviation is allowed for tablets (uncoated and film-coated) containing 80 mg or less. According to these suggestions, the limits for tablets range from 90 to 110% of the label declaration.

**Table: Sodium montelukast content of Singulair® coated tablets determined by MEKC and HPLC (the declared amount was 10 mg)**

| | CE ($n = 5$) | HPLC ($n = 5$) |
|---|---|---|
| $\bar{X}$ as (%) | 100.74 | 101.45 |
| SD | 1.35 | 1.72 |
| RSD % | 1.32 | 1.69 |
| *t*-Test of significance (P = 0.05) | | 0.66 ($t_{Table} = 2.13$) |

$\bar{X}$ is the mean, SD is the standard deviation, and RSD is the relative standard deviation

In the CE method, the amount of sodium montelukast in the tablets, calculated from the peak areas as stated above, was $100.74 \pm 1.35\%$ of the label declaration, which is again within the limits stipulated. The tablets are therefore, in an agreement with official standards and offer several advantages such as lower LOQ and reagent consumption. Also, HPLC consumes a relatively large amount of organic solvent, which is expensive and harmful to the environment.

The t-test result showed that differences between results obtained through CE and HPLC methods were not significant. The results obtained from the application of the method to tablets are listed in the Table. Thus, both methods can be employed to the routine quality control of this drug in pharmaceutical dosage forms.

## 3. Experimental

### *3.1. Apparatus*

All CE experiments were performed using $HP^{3D}$ –CE (Hewlett-Packard Waldbronn, Germany) system equipped with a diode array detector (DAD) and a temperature control system. The CE instrument was controlled by the HP Chemstation Software (rev. A.06.03, Hewlett-Packard). For pH measurements, a pH meter (Digimed DM-20, São Paulo, Brazil) calibrated with standard buffers was employed. An UV chamber (100, 18, 17 cm) with mirrors and an UV fluorescent lamp (Black Light Blue Lamp-Orion UV-A, 30 W, 130 V, 1.26 × 10-3 watts/cm$^2$) emitting radiation at 352 nm was used. All HPLC experiments were carried out on 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, USA) $C_{18}$ column (150 mm × 4.6 mm, i.d., 5 μm particle size). The mobile phase consisted of acetonitrile:potassium dihydrogen phosphate (0.05 M) with pH adjusted to $3.5 \pm 0.1$ using phosphoric acid (70:30, % v/v). The flow rate was 2.0 ml/min. The analyte detection was performed at 280 nm. The temperature was 25 °C in the column oven. The quantitation was performed using the peak absolute area.

### *3.2. Chemicals and reagents*

Sodium montelukast used as reference substance (assigned purity, 100%) was purchased by Sequoia Research Products Ltd (Pang-Bourne, United Kingdom). Singulair® (manufactured by Merck Sharp & Dohme Pharmaceuticals Ltd, Northumberland – United Kingdom) coated tablets for oral administration (10 mg per tablet, excipients: microcrystalline cellulose, magnesium stearate, lactose, sodium croscarmelose, hydroxypropyl cellulose; coated excipients: iron oxide yellow, iron oxide red, titanium dioxide, carnauba wax, hydroxypropyl methylcellulose, hydroxypropyl cellulose) was purchased in the market. Liquid chromatography grade acetonitrile and methanol were purchased from Tedia (Fairfield, USA), phosphoric acid, sodium tetraborate decahydrate and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany) and sodium dodecyl sulfate was purchased from Synth (Diadema, Brazil). High purity water was prepared by using Millipore Milli-Q purification system (Billerica, USA). The solutions were filtered through 0.45 μm Millipore membrane filter (São Paulo, Brazil) before injection.

### *3.3. Capillary electrophoresis*

All analyses were carried out in a fused silica capillary with total length of 47 cm (39.5 cm of effective length) × 75 μm of internal diameter. The analyses were performed using 25 kV potential. The injection was hydrodynamic (50 mbar/5 s). The detection was conducted at 280 nm and the temperature was maintained at 25 °C.

The run buffer solution was prepared by dissolving an amount of 3.81 g of sodium tetraborate decahydrate and 8.65 g of sodium dodecyl sulfate in purified water, using ultrasonic bath. The solution was transferred into a 100 mL volumetric flask followed by marking up to volume using the same diluent. An aliquot of 10 mL was transferred into a 100 mL volumetric flask and diluted with water. The final concentration obtained was 10 mmol $L^{-1}$ of sodium tetraborate decahydrate and 30 mmol $L^{-1}$ of sodium dodecyl sulfate, pH 9.4.

The capillary was washed every day with 0.1 mol $L^{-1}$ NaOH for 30 min, followed by pure water for 15 min and run buffer solution for 15 min. Between each injection, the capillary was flushed for 2 min with 0.1 mol $L^{-1}$ NaOH, for 2 min with pure water and for 3 min with the electrolyte solution.

### *3.4. Standard preparation*

A stock solution of 1000 μg $mL^{-1}$ sodium montelukast reference substance was prepared using an amount equivalent to 50 mg that was transferred into a 50 mL volumetric flask with 40 mL of methanol. This flask was kept in ultrasonic bath (USC2850 Unique ultrasound) for 15 min, followed by marking up to volume using the same solvent.

### *3.5. Sample solutions preparation*

Twenty tablets were weighed and finely powdered. A quantity equivalent to 50 mg of sodium montelukast was transferred into a 50 mL volumetric flask with 40 mL of methanol. This flask was kept in ultrasonic bath for 15 min. The volume was completed using the same solvent and filtered with quantitative filter. An aliquot of 1 mL of this solution was diluted into a 20 mL volumetric flask with 10 mmol $L^{-1}$ of sodium tetraborate decahydrate solution to give a final concentration of 50 μg $mL^{-1}$.

### *3.6. Method validation*

The analytical method was validated with respect to parameters such as specificity, linearity, limit of detection, limit of quantitation, precision, accuracy, and robustness (ICH 2003; ICH 2005; USP 2007).

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