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Original Paper

Determination of montelukast sodium by capillary electrophoresis

This work verifies the potential of CE in the analysis of significant impurities of montelukast sodium – an active ingredient for the treatment of bronchial asthma. Using 20 mM borate buffer pH 9.2 with 10 mM SDS and 10 mM (2-hydroxypropyl)-γ-CD (2HP-γ-CD) it was possible to separate montelukast and several impurities, including its *cis*-isomer, after exposure to light and oxygen. The obtained method surpasses a chromatographic method for montelukast sodium in terms of time of analysis (9 min of CE analysis *vs.* 35 min HPLC) and efficiency (CE offered over 900 000 theoretical plates for montelukast). Good repeatability of the method was supported by the low % RSD for the migration time of montelukast (0.53%). For the first time, the capillary electrophoretic method was employed for temporal study of the degradation of montelukast. The results showed that degradation of montelukast and the formation of the *cis*-isomer mainly occurred during the first 2 days of exposure, and occurred to a higher degree when there was no contact with the air (oxygen) in the exposed sample.

Keywords: Capillary electrophoresis / Impurity / Montelukast sodium

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1 Introduction

Montelukast sodium was developed by Merck [1] as a therapeutic agent for the treatment of bronchial asthma. Montelukast sodium is a potent and selective leukotriene D-4 (cysLT-1) receptor antagonist [2, 3]. The chemical structure of the compound is presented in Fig. 1. Montelukast sodium is very sensitive to the exposure to light or moisture. Light exposure is reported to cause isomerisation (formation of the *cis*-isomer). Exposure to oxygen causes formation of sulphoxide impurities at elevated levels. In the case of prolonged exposure to the atmosphere the compound picks up moisture and degrades. According to the literature provided by Merck, montelukast degrades at 60°C.

To monitor the manufacturing process a HPLC method is being employed. The chromatographic methods are known to be quite lengthy, consume large volumes of organic solvents and use expensive HPLC columns. Radhakrishna *et al*. [4] published the only up-to-date HPLC method for the separation of impurities of montelukast using high – low chromatography technique. The separation of impurities of montelukast was performed in approximately 35 min *per* single run, which would be quite a time-consuming procedure in the busy environment of the pharmaceutical industry. A high performance TLC method for the determination of montelukast sodium in bulk drug and pharmaceutical preparations was reported by Sane *et al*. [5]. Each analysis required approximately 45 min, which is even longer than the reported [4] HPLC method. Obviously the lengthy analysis time in HPLC requires larger quantities of mobile phase, the waste from which can add to the cost of analysis.

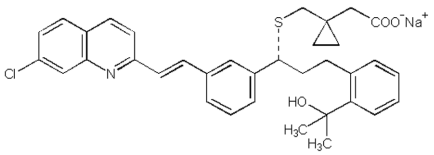

Figure 1. Chemical structure of montelukast sodium.

Even though a CE method has not been developed yet, this technique has proved to be very advantageous in many pharmaceutical separations. The many ways to vary parameters of the run buffer lead to the possibility of adapting each composition for the specific case whether charged or neutral compounds, structurally related or optical isomers have to be separated. The high efficiency of the separation, shorter analysis time, small volumes of samples and buffers required – all provide

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the reasons why this method should be investigated in the determination of montelukast sodium and its related impurities.

2 Materials and methods

2.1 HPLC instrumentation

HPLC separations were performed on a Hewlett Packard series 1050 system with a Model 78953C variable wavelength detector. The instrument was operated using Agilent ChemStation software version A.09.03 (Agilent Technologies, Palo Alto, CA, USA). A Rheodyne injection valve and an Alltech BRAVA BDS column (25 cm × 4.6 mm, particle size 5 μm) were used. The mobile phase flow was maintained at 1 mL/min. Separations were performed at 20°C. UV detection was at 225 nm unless otherwise stated.

2.2 CE instrumentation

CE separations were performed using an Agilent CE system. The PDA detector range was 190 – 600 nm. The CE instrument was operated using Agilent ChemStation software. The fused-silica capillaries (Composite Metal Services, The Chase, Hallow, Worcs. WR2 6LD) were 64 cm long (56 cm to the detector) with 50 μm id unless otherwise stated.

2.3 Reagents

All compounds investigated in the separations had a purity of 99% and were used without further purification. Montelukast sodium was kindly supplied by Merck, Sharp & Dohme, Ireland (Ballydine, Kilsheelan, Tipperary, Ireland). NaH_2PO_4, HCl, NaOH, SDS, (2-hydroxypropyl)-α-CD (2HP-α-CD) (MS ~0.6), (2-hydroxypropyl)-β-CD (2HP-β-CD) (DS ~4 – 10), (2-hydroxypropyl)-γ-CD (2HP-γ-CD) (MS ~0.6), γ-CD (γ-CD), KBr were purchased from Sigma – Aldrich, Dublin, Ireland. Methanol (HPLC grade) and ACN (HPLC grade) were from Lab-Scan, Dublin, Ireland. Boric acid was from Riedel-de Haën, Seelze, Germany. Buffers were prepared using distilled water and adjusted using 1 M and 0.1 M NaOH and 0.1 M HCl.

2.4 Standards

Stock solutions of 1 mM or 10 mM montelukast sodium were prepared in methanol for CE experiments. For HPLC experiment, 8.2 mM stock solution of montelukast sodium was prepared in water/ACN = 20:80 v/v. All were kept in amber flasks. Samples of montelukast for the analysis were exposed to light and air (oxygen) for 4 days unless otherwise stated.

2.5 Procedure

All procedures with montelukast sodium were performed in a dark room. All buffers were filtered through a 0.2 μm filter before use. Separations were carried out at 30 kV electrophoretic voltage and temperature 20°C. Injections were hydrodynamic at 50 mbar for 2 s. Conditioning between the runs was performed with 0.1 M NaOH for 1 min, followed by MeOH for 1 min and run buffer for 2 min. All separations were repeated at least three times.

3 Results and discussion

3.1 HPLC

In order to be able to observe impurities it is necessary to inject large sample concentrations. While this paper's main focus was the development of a CE method, it was first desirable to detect the impurities in the HPLC separation and compare the results with the literature. In the work of Radhakrishna *et al*. [4] the impurities of montelukast sodium were determined by an HPLC method. A high – low chromatography technique was employed in this case. The method reported by Radhakrishna *et al*. [4] was applied to the sample of montelukast sodium under investigation. The resultant chromatogram is shown in Fig. 2. The elution time of montelukast (peak 10) was in accordance with the chromatogram in the work of Radhakrishna *et al*. [4]. Peak 9 on the chromatogram was proposed to be a *cis*-isomer of montelukast sodium as it corresponded to the identified peak in the work of Radhakrishna *et al*. [4]. The obtained chromatogram did not fully correspond to the one from the literature. This could be due to the specifity of the analysed sample of montelukast sodium. The chromatographic analysis of the sample under the investigation showed at least six peaks of different intensities (peaks 3, 5, 6, 7, 9 and 11), which could represent the potential impurities, although a number of less significant peaks were present on the chromatogram.

3.2 CE

Common drawbacks of CE analysis are poor sensitivity where UV detection is used and poor reproducibility of the method. The former could strongly affect the aim of this study to develop a separation of montelukast and impurities, as the impurities in montelukast sodium are present at elevated levels. It was thought that intentional exposure of the montelukast sodium sample to light and air (oxygen) would cause an increase in the concentration of the potential impurities, which would be desirable especially for the method development. Reproduci-

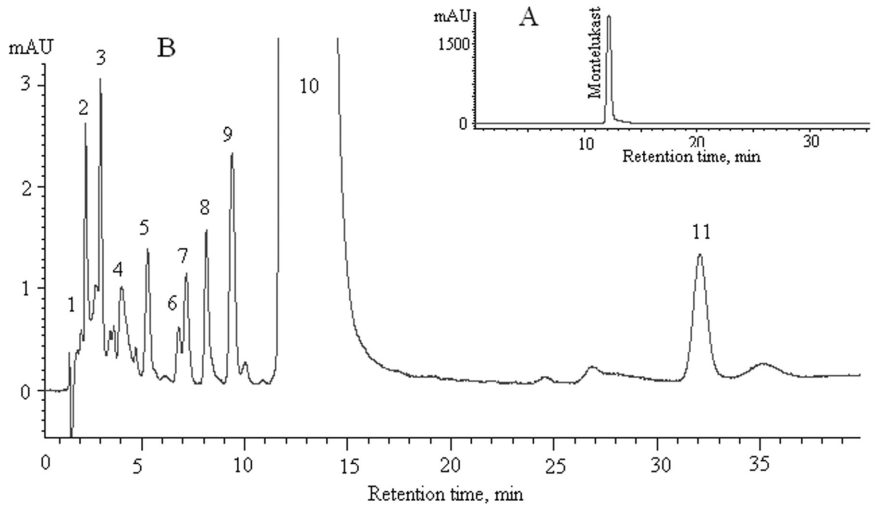

Figure 2. (A) High–low chromatogram of montelukast. (B) Expanded high–low chromatogram of montelukast. Chromatographic conditions: a Symmetry C_{18}, 250mm × 4.6 mm, 5 μm column; mobile phase 0.025 M NaH_2PO_4 pH 3.7/ACN = 20:80 v/v, flow rate 1 mL/min; injection 10 μL; detection at 200 nm. Peak identification: 1–9, 11 – not identified, 10 – montelukast. Concentration of montelukast = 8.2 mM.

bility is clearly important for valid pharmaceutical analysis.

Several modes of CE were tested in order to separate impurities of montelukast sodium. CZE was found to be unsuitable for such a challenging problem and only a single peak was obtained in this separation. Several additional peaks were observed on the electropherogram for the separation of montelukast sodium when employing micellar EKC (MEKC). Varying the concentration of SDS in the range of 2.5–50 mM, the resolution of the obtained peaks was found to be optimum with 6 mM SDS in the run buffer. Although the separation was improved, it was not the optimum as the montelukast peak and the impurities were not completely resolved.

3.3 CD-modified MEKC (CD-MEKC)

It is known that one of the main impurities in montelukast sodium is its *cis*-isomer. The addition of CD to the run buffer should have a positive effect on this separation and help to resolve compounds of such close structured similarity. A range of CDs was investigated in this study. The electropherograms illustrating the separations are shown in Fig. 3. A concentration of 5 mM of each CD was added to the run buffer containing 20 mM borate at pH 9.2, with 6 mM SDS. This composition was optimised in advance. It can be seen that the separation with 2HP-α-CD or 2HP-β-CD was not successful, as it resulted in coelution of the analyte and impurities. The addition of 2HP-γ-CD improved the separation and several additional peaks could be noted in Fig. 3(C). These results pointed to the fact that the size of the cavity of the employed CDs played a key role in the separation. The cavity of 2HP-α-CD and 2HP-β-CD was not large enough for the molecule of montelukast or its *cis*-isomer to penetrate the CD and form the inclusion complex. The diameter of the cavity of γ-CD is reported to be 10 Å [6]. This is likely to have allowed the formation of the inclusion complex of the molecule of the analyte and CD to occur.

Interestingly, Meras *et al*. [7] studied the complexation of montelukast and heptakis-(1,6-di-*O*-methyl) substitutes of β-CD using fluorimetry. Their investigation showed that a complex between montelukast and CD was formed. The association constant of the inclusion complex was found to be 959 M^{-1}. The CD investigated in this work and in the work of Meras *et al*. [7] are substitutes of β-CD and thus the cavity of both CDs should be similar in size (seven glucose units in the ring).

There can be several reasons for the difference in the behaviour of CDs. Firstly, the difference in the nature of functional groups of β-CDs and the degree of CD substitution can make significant impact on the results [8]. Secondly, the concentration of CD can have an effect. In this study 5 mM 2HP-β-CD was used with 1 mM montelukast (before the sample was exposed). In the work of Meras *et al*. [7], it was demonstrated that greater than 1000 times CD – montelukast molar concentration ratio did show inclusion. CD was employed in the concentration 6.9 mM while the investigated level of montelukast was 1.8 (M [7]. Such a low concentration of montelukast could not be investigated in this work as the objective was to study the impurities. Thirdly, the solvent employed in the study of Meras *et al*. [7] consisted of ethanol, acetate buffer and water. At such conditions montelukast is in its protonated form; according to the results of this paper, the pK_a of montelukast is 2.9. For the capillary electrophoretic analysis montelukast has a negative charge, as the borate buffer of pH 9.2 was used. The hydrophobic inclusion is less likely to occur while the guest molecule was charged.

One major peak and one peak of a lower intensity can be seen in the electropherogram in Fig. 3 with 2HP-γ-CD. One of the peaks is montelukast. The second peak is

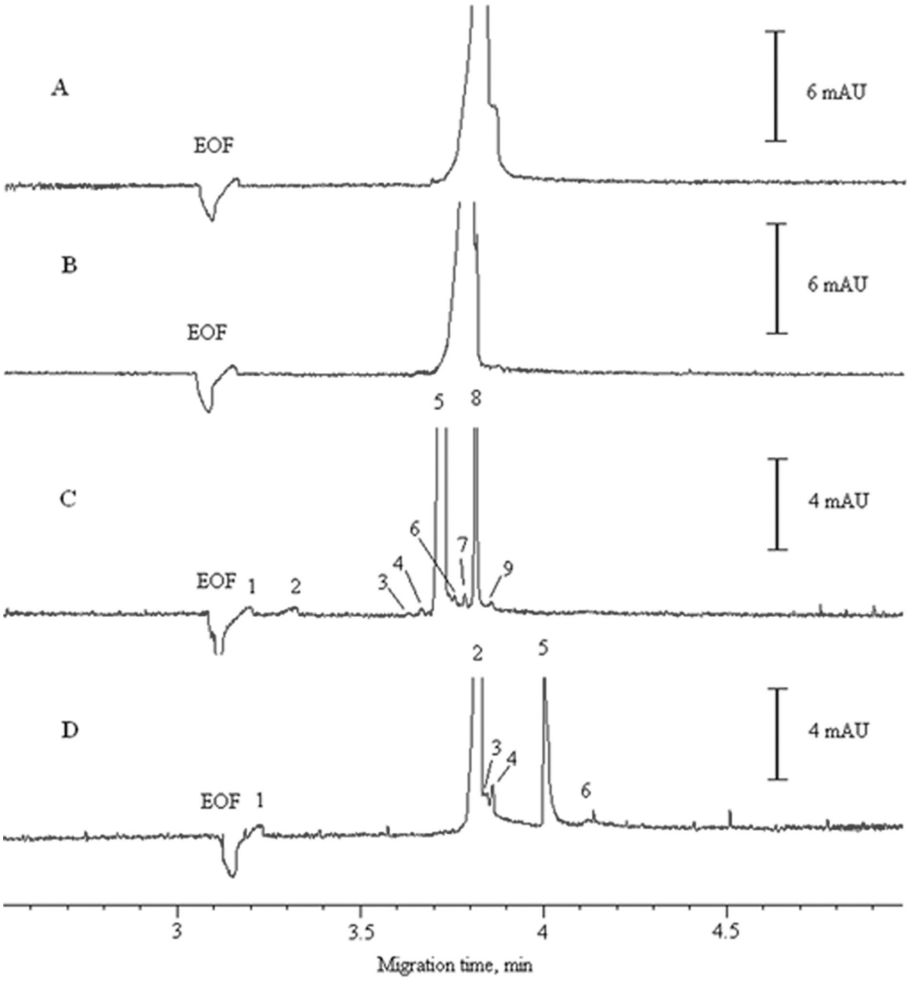

Figure 3. Electropherograms of CD-MEKC separation of MK. Separation condition: 20 mM borate buffer pH 9.2 6 mM SDS, 5 mM of (A) 2HP-α-CD, (B) 2HP-β-CD, (C) 2HP-γ-CD, (D) γ-CD, 30 kV, 20°C, hydrodynamic injection = 2 s, detection at 225 nm, capillary 64 cm (56 cm), 50 μm id, 1 mM MK in methanol exposed.

likely its *cis*-isomer as the sample was exposed to light and air for 4 days. There were other minor peaks separated with the investigated conditions. Peaks 1 and 2 on the electropherogram of the separation with 2HP-γ-CD are due to buffer constituents. Their presence was observed on the electropherogram of the blank injection. The number of the peaks (6) matching the impurities formed after the exposure of the montelukast to light and air corresponded to the number of impurities obtained in the separation by HPLC method (Fig. 2).

3.4 Identification of montelukast

In order to identify the peak due to montelukast in the separation with 2HP-γ-CD, the effective mobility of a fresh montelukast sample was compared to the effective mobility of two main peaks in the separation of the sample exposed to daylight and air over a period of time. The results are presented in Table 1.

The negative values of the mobility of montelukast and its *cis*-isomer mean that the molecules migrated against EOF. It can be seen that the mobility of peak 8 in the separation with 2HP-γ-CD (Fig. 3(C)) corresponded to the effective mobility of montelukast in the fresh sample. The effective mobility of montelukast was consistent while the sample was exposed to light and air for up to 15 days. It has to be noted that the area of montelukast peak 8 in Fig. 3(C) is smaller than the area of peak 5, which is suppos-

Table 1. Effective mobility of montelukast and *cis*-isomer in the fresh samples and exposed in time

	Peak 5[a)]		Peak 8[a)]	
Sample MK (1 mM)	Effective mobility ($cm^2 \cdot V^{-1} \cdot s^{-1}$)	% RSD	Effective mobility ($cm^2 \cdot V^{-1} \cdot s^{-1}$)	% RSD
Fresh	–	–	-11.89×10^{-5}	0.54
Exposed, 1 day	-9.95×10^{-5}	0.30	-11.25×10^{-5}	0.52
Exposed, 2 days	-10.46×10^{-5}	0.72	-11.83×10^{-5}	0.69
Exposed, 3 days	-10.53×10^{-5}	0.32	-11.82×10^{-5}	0.28
Exposed, 4 days	-10.54×10^{-5}	0.40	-11.77×10^{-5}	0.57
Exposed, 15 days	-10.23×10^{-5}	0.39	-11.34×10^{-5}	0.28

Separation conditions: 20 mM borate buffer pH 9.2, 6 mM SDS, 5 mM 2HP-γ-CD, $n = 3$.

[a)] The numeration of the peaks corresponds to Fig. 3(C).

Table 2. Purity of montelukast peak established by the peak-area ratio method of standard solutions and exposed sample at various wavelengths

Wavelength ratio λ_1/λ_2	Peak-area ratio Fresh MK[a)]	Peak-area ratio MK exposed[a)]
214/200	0.71	0.71
225/214	0.79	0.78
254/225	0.71	0.70

Separation conditions: 20 mM borate buffer pH 9.2, 10 mM SDS, 10 mM 2HP-γ-CD.

[a)] Mean from three determinations with % RSD < 5%.

edly its *cis*-isomer. This sample was exposed to daylight and air for approximately 4 days, when most of the montelukast was converted to the isomer.

3.5 Optimisation and validation of CD-MEKC separation

Since the aim of this research was to separate the montelukast from its impurities, only the peak of montelukast was considered when choosing the parameters of separation. In order to optimise the content of the separation buffer, the effect of the concentration of CD and SDS on the separation of montelukast peak was studied. From the investigated range of the concentration of 2HP-γ-CD (3 – 20 mM), the efficiency and resolution of montelukast peak were found to be optimum at 10 mM. In order to optimise the concentration of the surfactant in the CD-MEKC system, the range of 3 – 15 mM SDS was studied. The resolution of montelukast with peaks 7 and 9 (as in Fig. 3(C)) was not affected significantly by the changes in the concentration of SDS. However, a definite increase in the efficiency of the montelukast peak was observed when increasing the SDS concentration up to 10 mM SDS where the maximum efficiency was over 900 000 theoretical plates. Thus, 10 mM 2HP-γ-CD and 10 mM SDS were chosen as optimum for the determination of montelukast and its impurities.

The purity of the montelukast peak was established by the peak-area ratio method for fresh standard and the sample of montelukast was exposed to light and air for 4 days at different wavelengths. The results are demonstrated in Table 2. The developed method has yielded consistent purity of montelukast peak with 2HP-γ-CD in the run buffer.

The calibration graph for montelukast sodium was established with the peak area *versus* the concentration. The obtained linear regression equation was $y = 166.71x - 6.2014$, $R^2 = 0.9941$, $n = 3$. The data indicate good linearity of the method over the studied concentration of montelukast 0.01 mM – 1 mM. RSDs of the migration time and peak area of 1 mM fresh sample of montelukast sodium were 0.53 and 4.50%, respectively. The latter indicates the good reproducibility of the CE method in relation to the montelukast peak.

The developed method only allows the determination of montelukast from its impurity in the samples that have been exposed to light or air, which increases the concentration of the impurity in the sample. No impurity could be detected at their elevated levels in the fresh stock of montelukast. Further improvements of the LODs are necessary in order to be able to employ the developed method for the purity test of montelukast sodium.

3.6 Temporal study of the degradation of montelukast

The developed method was employed in the investigation of the degradation of montelukast sodium under exposure to air and light. One series of 1 mM solutions of montelukast sodium were exposed to light and air for 1, 2, 6 and 8 days. Before capillary electrophoretic analysis the solvent level of each sample was adjusted to obtain the same concentration. The second series of 1 mM solutions of montelukast were kept in air tight vials and exposed to light only for 1, 2, 6, and 8 days. It has to be mentioned that the samples were exposed to indoor light for 24 h. The changes in the peak areas of montelukast to its *cis*-isomer are presented in Fig. 4.

It can be seen that the most significant decrease in the concentration of montelukast occurs during the first 2 days of the sample being exposed to light and air. The concentration of montelukast in the sample that was exposed only to light decreased faster. The concentration of the sample exposed to both light and air approached 0.5 mM after 2 days. The peak area of *cis*-isomer of montelukast gradually increased during all period of exposure. Noticeably, the peak area of *cis*-isomer from the sample, which was in contact only with light, became more intense than from the sample where both light and air were involved. These results from Fig. 4 pointed to the fact that degradation of montelukast and the formation of *cis*-isomer were to a higher degree when there was no contact with the air (oxygen) in the exposed sample. It was thought that the presence of oxygen induced some other processes in the sample, which slowed down the formation of *cis*-isomer.

The developed method for the determination of montelukast and its impurities allowed to obtain valuable information about the stability of montelukast and different rates of the conversion to its *cis*-isomer. These data have never been obtained before by CE or other analytical techniques.

3.7 Comparison of HPLC and CE methods

The comparison with the HPLC method published in the literature [4] of the developed CE method for the determi-

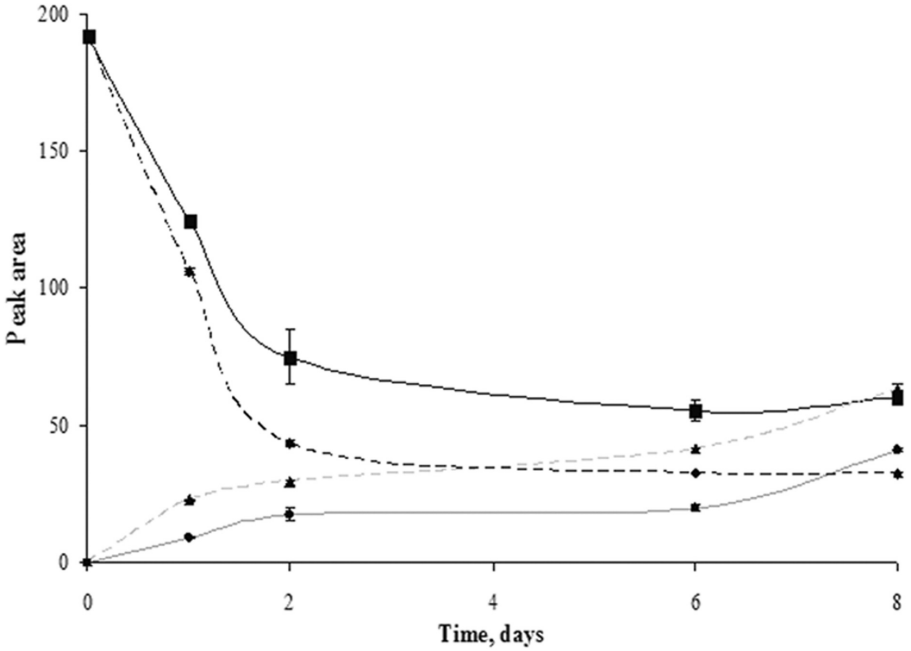

Figure 4. Effect of light and air on the peak area of montelukast and its *cis*-isomer. Separation condition: 20 mM borate buffer pH 9.2, 10 mM SDS, 10 mM 2HP-γ-CD, 30 kV, 20°C, hydrodynamic injection = 2 s, detection at 225 nm, capillary 64 cm (56 cm), 50 μm id, 1 mM MK in methanol exposed to light and air: −■− MK and −●− *cis*-MK; exposed to light only −◆− MK and −▲− *cis*-M.

Table 3. Comparison of the parameters of the HPLC and CE methods for the separation of the impurity in montelukast sodium

	HPLC	CE
t_r/t_m of MK	12.15 min	4.15 min
Complete time of one analysis	33 min	9 min
Resolution of MK peak	9.4 and 12.2	1.72 and 2.72
Efficiency of MK peak	3544 ± 10	936 859 ± 34 080
Repeatability (% RSD of t_r/t_m)	0.23%	0.53%

nation of montelukast and its impurity is presented in Table 3. It can be seen that one CE analysis required only 9 min, four of which were for conditioning the capillary prior to the analysis. The HPLC analysis took over 30 min. The montelukast peak had an acceptable resolution from its impurities in both methods though its resolution was less in the HPLC method. The extremely high efficiency of CE separation is noted in Table 3. The repeatability of the migration time was very good for both methods.

4 Concluding remarks

The aim of this work was to develop the CE method for the separation of the related impurities in montelukast sodium. At the present stage of developing the method, CE has showed good potential and many advantages over HPLC method for the determination of montelukast sodium. The potentials of CE for the determination of montelukast are clear from this study and the advantages of the CE method for the determination of montelukast sodium to date are:

(i) The full analysis time is less than 9 min, which is significantly less than the 35 min of HPLC method [4].

(ii) A high efficiency of the montelukast sodium peak over 900 000 theoretical plates was recorded with the achieved method.

(iii) The impurities were not identified but their quantities were in accordance with HPLC results obtained with the method [4].

Further experiments using MS would be desirable to identify the peaks of the impurities.

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