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A novel and simple dynamic coating capillary electrophoresis method for the chiral separation and quantification of mitiglinide enantiomers using hydroxyethyl cellulose as a dynamic coating agent

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A capillary electrophoretic method for the chiral separation and quantification of mitiglinide (MTG) enantiomers is described (less than 9.5 min) with resolution value R_s = 5.25 and with excellent peak shapes after performing the dynamically coating for the bare fused capillary. The study aims to develop and validate a novel and simple method for the separation and quantification of MTG enantiomers using CE after dynamic coating the capillary wall using the hydroxyethyl cellulose (HEC) coating agent. Dynamic coating procedure of the capillary inner surface is conducted via rapid flushes using 0.1 M sodium hydroxide, water, and aqueous solution containing HEC, and hydroxypropyl-γ-cyclodextrin (HP-γ-CD). Besides buffer was used for the dynamic coating process in addition to its use as the separation medium. When the dynamic coating was used, peak symmetry was improved. A bare fused-silica capillary was used throughout the separation after being coated using HEC dissolved in the background electrolyte (BGE) of 50 mM Na_2HPO_4 – 1 M H_3PO_4 solution; pH 8.5; containing 25 25 mg mL^{-1} of each HP-γ-CD and HEC. The dynamic coating procedure achieved an improvement in migration time as well as peak area precision. The adsorbed coating agent showed slight interactions with MTG, providing efficient separation with outstanding durability and reproducibility at slightly alkaline conditions (pH 8.5). Acceptable validation criteria for selectivity, linearity, precision, and accuracy were also studied. The newly developed method was effectively applied to the assay of enantiomers of MTG in pharmaceutical formulations. Additionally, it was proven to have the advantages of being simple, rapid, and accurate.

1. Introduction

Presently, there are around 285 million people suffering from diabetes around the world. This number is projected to reach 439 million by 2030 (Bergenstal and Gavin 2005; Blonde and Karter 2005). Because of such an increase, the World Health Organization (WHO) has declared diabetes mellitus as a worldwide epidemic, and as such, 14 November is considered as the "World Diabetes Day" (Vashist et al. 2011). Numerous new medications are present in the developmental stage for the cure of diabetes.

Mitiglinide (MTG), (2*S*)-benzyl-4-(cisperhydroisoindol-2-yl) butyric acid (Al Azzam and Muhammad 2015) (Fig. 1) (pK_a = 4.37) (Hadi et al. 2012; ChEMBL: Compound Record Card 2021) has chiral centers and thus act as enantiomers. Additionally, it is similar to other glinide group members, MTG works by promoting insulin secretion from pancreatic beta cells by sealing the ATP-sensitive $K^+[K(_{ATP})]$ channels. MTG is classified as a hydrophobic drug, which is used for the treatment of type 2 diabetes because it has low aqueous solubility, hence restricting its range of applications (Al Azzam and Muhammad 2015; Zhang et al. 2012). The (*S*)-configuration of the stereogenic C-atom in the succinyl moiety is needed for the insulin secretory effect (Liu et al. 2004).

(*R*)-Mitiglinide calcium (impurity) is one of the potential impurities formed in the synthesis by the inclusion of (*R*)-2-benzylsuccinic acid as one of the impurities in (*S*)-2-benzylsuccinic acid. It is caused by the presence of (*R*)-2-benzylsuccinic acid impurity in (*S*)-benzylsuccinic anhydride (Sastry et al. 2014). It would be of substantial benefit to obtain pure (*S*)-mitiglinide by developing the reaction conditions appropriate for the development of the required (*S*)-enantiomer, substantially free of the undesirable (*R*)-enantiomer (Drug Approvals International 2021).

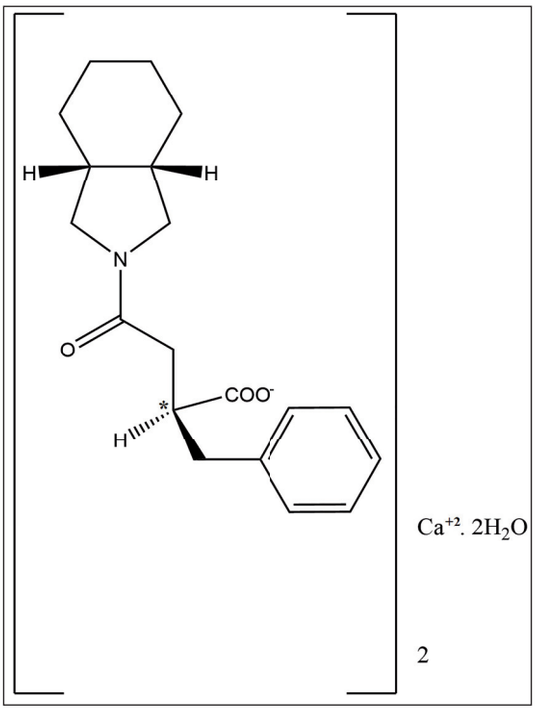

Fig. 1: Chemical structure of mitiglinide (MTG) (pK_a = 4.37). The asterisk indicates the chiral center position used in this study (Hadi et al. 2012; ChEMBL: Compound Record Card).

CE-based methods depend mainly on the use of inexpensive bare-fused silica capillaries; however, many published papers use coated capillaries which are advised for several reasons (Horvath and Dolnik 2001; Dolnik 2004; Znaleziona et al. 2008; Nowak et al. 2017; Lurie et al. 2004). One limitation with CE is the adsorption of analytes on the internal surface of the bare fused silica capillary. The latter is attributed to the apparent mobility of analytes, which is frequently less reproducible because of one run to another possibility in variations electroosmotic flow (EOF). CE method of separation of compounds with a strong positive charge is disrupted by electrostatic interactions between analytes and capillary walls (Bernal et al. 2008). Modifying the capillary wall can reduce the negative load of the silanol groups responsible for this undesirable adsorption effect. Commonly used strategies include the application of background electrolyte (BGE) components to the capillary wall (dynamic coating), examination at extreme pH, and use of permanently coated capillaries. The choice of BGE composition is significant; it is also important due to potential interactions between BGE analytes and ionic and nonionic species (chiral selectors, micelles, etc.) and between analytes and the inner surface of the capillary. The interactions can be electrostatic, hydrophobic, van der Waals, as well as hydrogen bonding (Znaleziona et al. 2008). The capillary coating is considered a potential tool for controlling or removing EOF, preventing excessive adsorption of analytes to the capillary inner wall surface, migration times reproducibility, resolution, and peak efficiency (Nowak et al. 2017). One of the earliest works on the use of coated capillaries to resolve such problems was published in 1985 (Hjerten 1985). These coatings are categorized into two diverse groups (Doherty et al. 2003; Lucy et al. 2008), that are covalently bonded coatings (Cifuentes et al. 1998) in addition to the physically adsorbed coatings or what is called static or dynamic (Znaleziona et al. 2008; Sanzgiri et al. 2006; Catai et al. 2005; Danger et al. 2007; Ullsten et al. 2008; Towns and Regnier 1990) and based on using of polymers or even small molecules (MacDonald and Lucy 2006; Wang and Lucy 2004, 2005). The creation of new CE coatings continues to be an important area of study. The key features of any physically adsorbed coating should consist of (Doherty et al. 2003; Lucy et al. 2008); (1) the ease of coating formation; (2) the probability of regeneration of the coatings layer; and (3) accessibility to the information related to the polymer coatings properties.

Dynamic capillary coating methods are easy and a cost-effective way to modify the EOF (Melanson et al. 2001). The vital benefit of dynamic coatings methods is that the process of coating can be conveniently prepared only by flushing the capillary with a coating solution which is either a polymer-based material or a low molecular mass material (Liu et al. 2010). Dynamic coating agents have the disadvantage of interfering with the analyte and causing CE separation to be compromised (Huhn et al. 2010). Since permanently coated capillaries and reagents for reversible dynamic coating are widely available commercially, this option is not straightforward (Nowak et al. 2017).

A large group of polymers that make up a physically adsorbed polymer layer has been used in CE. Examples include polybrene, poly(diallyldimethylammonium chloride), polyarginine, cationic agarosis, chitosan, poly(ethylene oxide) (Iiki and Yeung 1996), hydroxypropylmethyl cellulose, hydroxyethyl cellulose (HEC), and poly(*N*, *N*-dimethylacrylamide) (Verzola et al. 2000). For example, the introduction of low concentrations (0.004 – 0.4%) of cationic polymers to the BGE reverses the EOF. The magnitude is mainly dependent on both the form and concentration of the polymer (Znaleziona et al. 2008; Huhn et al. 2010).

HEC (Fig. 2) has strong biological compatibility, stable chemical structure, and good water solubility (Wang and Ye 2010). HEC is a very good cellulose derivative with excellent water retention and biocompatibility. It has many-OH groups located on its surface that permit it to be chemically modified by a variety of means. Furthermore, a hydrogen bond with a carbonyl group in pharmaceuticals is readily formed with the HEC free -OH group, which offers stabilization in solid-state. However, because of the high hydrophilicity, HEC matrices result in an early release of drugs and thus need to be altered to improve their applications in pharmaceuticals (Mahendra et al. 2015).

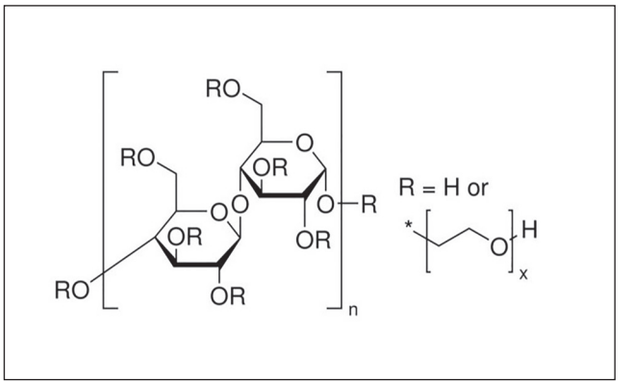

Fig. 2: Chemical structure of hydroxyethyl cellulose (HEC).

Several methods for the quantification of MTG in biological fluids (Liang et al. 2007; Lushan and Su 2006; Cai et al. 2008) and determining the pharmacokinetic properties of MTG (Liang et al. 2007; Zhang et al. 2008) have previously been reported using HPLC equipped with mass spectrometry (MS) (Zhang et al. 2008; Liang et al. 2007) or ultraviolet (UV) (Lushan and Su 2006) detections and UPLC coupled with MS detection (Cai et al. 2008).

Spectrophotometry (Kancharla et al. 2012) has also been identified as a method for analyzing MTG in pharmaceutical dosage forms. Nevertheless, only two reports on the stereoselective determination of MTG by HPLC were published using a chiral column and the separation was achieved within 25 min (Yu-ren et al. 2006) and the other is by derivatization of MTG with pre-column chiral derivatization reagent namely, S-(-)-1-(1-naphthyl) ethylamine. The diastereoisomers obtained were well separated using an Agilent Zorbax column with the following dimensions C18 (250 mm × 4.6 mm, 5 µm). The separation took place within 35 min (Jinzhao et al. 2009). HPLC methods utilized either one costly chiral column, or vast quantities of the chiral selector added to the mobile phase or pre-column derivatives that are time-intensive and time-consuming.

This paper illustrates the method development and validation for the separation and quantification of MTG enantiomers using CE after dynamically coating the capillary wall using the HEC coating agent. Despite this, only a few papers have looked at using dynamically coated capillaries in stereoselective separation studies.

To the best of our knowledge this is the first description of a stereoselective determination/quantification of MTG in pharmaceutical dosage forms using CE after the dynamic coating is applied to the capillary inner wall using the HEC coating agent. The coating step is deemed necessary (as direct separation without coating agents such as HEC could not be achieved) to overcome the adsorption problem

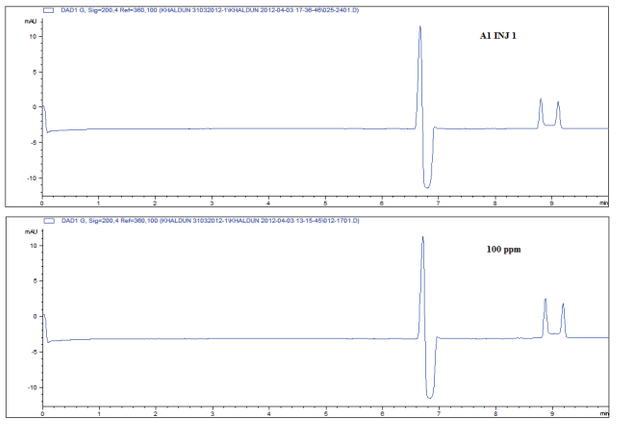

Fig. 3: Electropherograms after injecting MTG standard solution (A), and tablet solution (B). For CE conditions, please refer to Table 1.

and thus enhance peaks shape accordingly. Additionally, it is considered an alternative for the expensive coated capillaries available commercially. The process was further validated in compliance with the guidelines (ICH-Q2A 1995). The results point to certain distinct characteristics of dynamically coated capillaries that may be relevant in the context of other CE-based approaches as well. This approach not only removes the need for commercially coated capillaries but also offers excellent run-to-run reproducibility.

2. Investigations, results and discussion

In the current analysis, using the same BGE described earlier but on a dynamically coated capillaries, the same results were achieved in less than 9.5 min (Fig. 3) using the adopted electrophoretic conditions (Table 1). Highly reliable separation was achieved, as shown by the excellent run to other time precision of corrected peak areas and migration (Table 2).

Table 1: Adopted CE operating conditions

Background electrolyte (BGE)	50 mM Na_2HPO_4-1 M H_3PO_4 solution; pH 8.5; HP-γ-CD, 25 mg mL^{-1}, HEC, 25 mg mL^{-1}
Applied voltage	16 kV (normal polarity)
Sample injection	Hydrodynamic, 10 s
Capillary temperature	15 °C
Bare fused-silica capillary	50 µm i.d ×40 cm, (detection length, 8.5 cm from the outlet end of the capillary)
Detection wavelength	200 nm

Table 2: Intra- and inter-day precisions for the repeated introductions of different concentrations of racemic MTG standard solution

	RSD(%)[a]			
Factor conc. (µg mL^{-1})	Migration times		Corrected peak areas	
	MTG-E1[b]	MTG-E2[c]	MTG-E1	MTG-E2
Intra-day precision (n^d = 9)				
10	0.88	0.67	1.28	1.37
50	0.59	0.47	0.99	1.21
150	0.47	0.27	0.86	1.11
Inter-day precision (n^d = 54)				
10	1.46	1.23	2.43	2.22
50	1.04	0.87	2.01	1.72
150	1.02	1.15	1.24	0.99

[a] Relative standard deviation.
[b] Mitiglinide enantiomer 1.
[c] Mitiglinide enantiomer 2.
[d] Number of replicates.

Dynamic coating, it is said, prevents the capillary wall from interacting with the analyte of interest by covering it. The lack of interactions with silanol groups is attributed to these shifts in electrophoretic mobilities in this situation.

Preliminary capillary selection (based on the length and the internal diameter) at the beginning of the initial examination was done to select the proper capillaries that provided an appropriate baseline resolution between the two enantiomers. Several conditions were tested by comparing HEC concentrations along with various BGEs.

The results generated were compared to those produced using uncoated fused silica capillary, with the repeatability of all types of capillaries being considered. The use of coated capillaries has many advantages, involving faster separation, better resolution, and decreased adsorption between the analyte and the capillary wall.

HP-γ-CD (25 mg mL^{-1}) was added to the BGE to obtain stereoselective separations of MTG on both dynamically coated and uncoated capillaries. The MTG enantioresolution was first tried with an unmodified (uncoated) fused silica capillary using the BGE containing HP-γ-CD.

Research started by comparing the results of coated and uncoated capillary systems because the separation was poor, and the results on capillary coating were promising. The same capillary was then used for the analysis of the dynamic coating system.

2.1. Separation conditions optimization

Several derivatives of CDs, including HP-β-CD, HE-β-CD, M-β-CD, and HP-γ-CD, have been examined. Nonetheless, only HP-γ-CD could separate the MTG enantiomers, so it was utilized in subsequent studies.

Due to the similar characteristics of the enantiomers, enantiomeric resolution of multichiral center racemates is a difficult task. A versatile CE approach, on the other hand, may be able to address this problem. Considering these findings, researchers attempted to use CE to explain the enantiomeric resolution of multichiral center racemates. Also, the need for capillary electrophoresis in enantiomeric resolution of multichiral center racemates using various chiral selectors is deemed necessary (Ali et al. 2016).

The chemical structure of MTG possesses three chiral carbons. Also, it is reported in the literature that there may be more than one stereoisomer in the formulation as impurities. Also, a peak purity test was used to determine MTG enantiomers, and it was pure in all cases, indicating the absence of additional substances such as impurities coeluting at the same retention times. Since no other component was identified to be responsible for the peaks of MTG enantiomers. The Photo Diode Array (PDA), also known as the Diode Array Detector (DAD) can monitor the complete wavelength range in real time. Figure 6 shows the spectral absorbance profile analysis of MTG enantiomers. Other benefits of PDA include the possibility of finding an unknown peak in chromatograms using the spectral profile. It is well known that PDA is frequently used in the pharmaceutical sector to establish the target compound's peak purity.

The results were consistent with those obtained previously by our group (Al Azzam and Muhammad 2015) from a theoretical analysis of the host-guest inclusion complexation between the naturally occurring cyclodextrins (CDs), such as (α-CD, β-CD, and γ-CD) with MTG drug.

Our published paper (Al Azzam and Muhammad 2015) showed that the obtained complexes formed in the presence of γ-CD (Energy of complexation = –17.884 kcal/mol) and MTG-γ-CD inclusion complexes are energetically favored. However, the findings revealed that variations in intermolecular hydrogen bonding are the key explanation to produce more stable MTG-γ-CD complexes relative to MTG-α-CD or MTG-β-CD complexes (Al Azzam and Muhammad 2015).

Thus, we conclude that only the γ-CD was able to accommodate the asterisk carbon position (the chiral carbon investigated in our study, otherwise, β-CD will be the best choice for the other two chiral centers based on the cavity size) as shown in the energy minimized structures obtained from PM3 calculations (Fig. 2 in the work of Al Azzam and Muhammad 2015).

Additionally, the current method was utilized to calculate the MTG enantiomers in a pharmaceutical formulation, and the result was 10.13±0.72 mg, which matched the label claim of the tablets (Glufast) used in the investigation (stated that they contain 10 mg MTG active component). The latter indicates that the two enantiomers under investigation were caused by the chiral carbon highlighted by the asterisk (Fig. 1), rather than the other chiral centers in the MTG chemical structure.

pH is an important parameter to optimize since it affects capillary wall ionization (silanol group), which affects the degree of electroosmotic flow (EOF). pH plays an important role in assessing the degree of ionization of a particular analyte. Using a 100 mM buffer solution containing 20 mg mL^{-1} HP-γ-CD, the influence of pH (7.0 – 9.5) on the resolution was explored in this study. Figure 4 illustrates the outcomes. MTG is an acidic analyte (p*K*a = 4.37). Based on the p*K*a value of MTG which is 4.37, it is completely ionized at a pH value 2 – 3 units greater than the p*K*a value. Consequently, the pH of BGE should be adjusted to basic conditions (2 – 3 units higher than the p*K*a value) to attain it in the deprotonated form. At the optimized pH (8.5), the investigated analyte was deprotonated. Therefore, under basic conditions, the deprotonated analyte is moved to the detector (cathodic side).

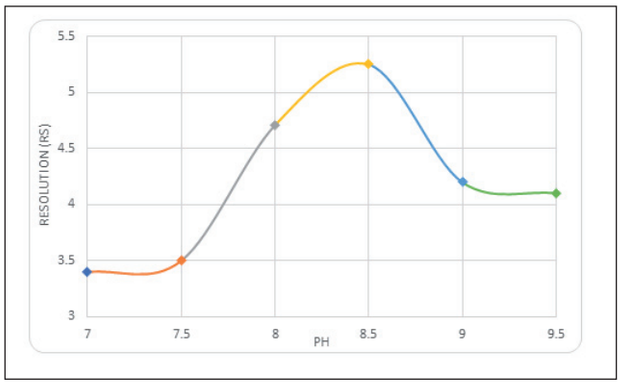

Fig. 4: Effect of pH on resolution parameter of MTG enantiomers (buffer concentration used, 50 mM Na_2HPO_4 – 1 M H_3PO_4; HP-γ-CD concentration, 20 mg mL^{-1}; HEC concentration, 25 mg mL^{-1}, temperature, 25 °C; and applied voltage, 15 kV; using normal polarity mode).

The effect of buffer concentration (Na_2HPO_4) (25 – 100 mM) on migration time and resolution has also been studied. Buffer concentration influences both the buffering capacity and the EOF. Buffering capability increases as the buffer concentration increases. This prevents depletion of the buffer and improves the reproducibility of the assay.

Furthermore, higher buffer concentrations lower the effective charges present on the capillary wall, lowering EOF. When using 50 mM, acceptable migration times and sharper peaks for MTG enantiomers were attained, and it was used in subsequent studies.

The effect of various concentrations of HP-γ-CD (10 – 50 mg mL^{-1}) on migration time and resolution was examined. Chiral separation was accomplished for all the concentration values tested (Fig. 5). Increased HP-γ-CD concentration increases migration time as well as resolution. This may be attributed to the chiral selector's advantageous complexation under these conditions. A compromise between resolution, peak shape, and analysis speed, 25 mg mL^{-1} HP-γ-CD was selected.

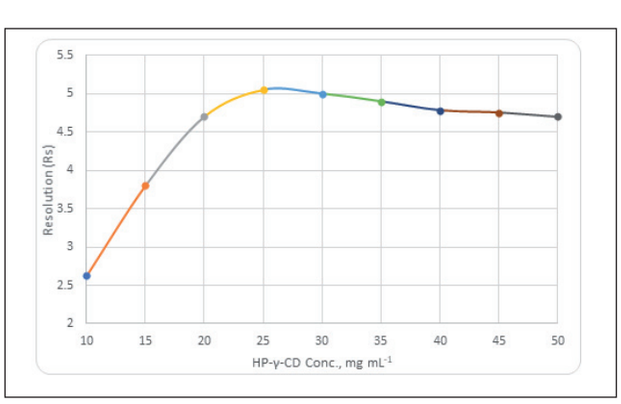

Fig. 5: Effect of HP-γ-CD concentration on resolution parameter of MTG enantiomers (50 mM Na_2HPO_4 – 1 M H_3PO_4 solution; pH 8.5; temperature 25 °C, HEC concentration, 25 mg mL^{-1}, and applied voltage, 15 kV; normal polarity).

The effect of the applied voltage in the range tested between 10 and 22 kV was also studied, as it is known that higher voltage results in faster migration times. Though, as the voltage is increased, the production of Joule heating will limit the theoretical improvement in resolution and efficiency. When operating at 16 kV, a resolution with a good peak shape and an acceptable migration time was attained.

The effect of temperature in the range examined between 15 and 30 °C was also investigated. The best findings were achieved when the samples were separated at 15 °C.

The effects of different HEC concentrations (10 – 50 mg mL^{-1}) on migration time and resolution were tested as well in presence of HP-γ-CD (as chiral separation could not be achieved without it).

For all the concentrations tested, chiral separation was achieved. Improved resolution and migration time are supported by improving HEC concentration (data not shown). This may be attributed to the chiral selector's advantageous nature in these circumstances. The compromise between resolution, peak shape, and migration time occurred when using 25 mg mL^{-1} HEC.

With EOF, the MTG enantiomers are more easily transported to the detector due to their slower mobility. With an increase in HEC concentration, a corresponding delay in migration time would be expected, as this increase reduces the EOF by increasing the viscosity of the solution and increasing the number of polymer fibers (HEC) to the migration of MTG enantiomers.

At 50 mbar, the impact of injection time was investigated in the tested range between 5 – 20 s. As expected, the peak area increased as the injection time increased. There was also a broadening of the peak. As a result, for the present analysis, a 10 second injection time was used.

The electrophoretic conditions used are given in Table 1, and the electropherogram for a standard MTG solution is shown in Fig. 3(A). The chosen electrophoretic procedures offer a good separation of MTG enantiomers (R_s = 5.25). Due to the unavailability of MTG enantiomer in our lab, the first and second MTG peaks are stated as MTG enantiomer 1 (MTG-E1) and MTG enantiomer 2 (MTG-E2), respectively.

2.2. *Pretreatment of the capillary*

Wall coating pre-treatment is a desirable approach to remove EOF and wall-analyte interactions in the capillary separation process. To obtain the best results for the coating, the capillary inner surface should be cleaned and enabled by the etching step before the coating procedure. The influence of each capillary preparation step on the column's final performance was thoroughly investigated. Optimal reproducibility of the coating involves the etching of columns with sodium hydroxide has been conducted in the preconditioning program (Horvath and Dolnik 2001).

The poor repeatability of migration times caused by EOF is an intrinsic disadvantage of capillary electrophoresis. Most researchers want to avoid this problem by using the relative migration times approach or various capillary coatings, all of which are considered expensive and difficult to compare. The results obtained indicate that dynamic coating is very effective at stabilizing migration times, with %RSD dropping from 2.96% (bare silica capillary) to less than 1.01% (after coating) (Table 3).

Table 3: %RSD of the migration time for the 1st MTG-enantiomer obtained by the developed method at different pH values of BGE with and without adding HEC to the BGE

pH	RSD[a], % (n^b = 6)	
		Without HEC
7.0	1.01	2.96
7.5	0.83	2.35
8.0	0.53	1.97
8.5	0.65	2.82
9.0	0.84	3.22
9.5	0.89	3.69

[a] Relative standard deviation.
[b] Number of replicates.
[c] Hydroxyethyl cellulose.

CE tests were carried out to assess the proposed method's ability to separate MTG enantiomers at various pH levels. Table 3 summarizes the %RSD values (n = 6) of t_m for the first enantiomer at pH 7.0 – 9.5. Satisfactorily low %RSD values of lower than 1.01% were attained from all pH values tested (with HEC), but %RSD values were quite high (3.69%) using bare fused capillary (without HEC). The developed method may be used for the study of MTG enantiomers in alkaline conditions (optimized pH 8.5). The addition of HEC to the BGE solution improved CE experiment reproducibility and capillary coating consistency in alkaline pH conditions.

2.3. Validation of the analytical method

2.3.1. Selectivity and peak purity

At the migration time of the MTG enantiomers, no interference from the composition of excipients could be observed (Fig. 3 (B)). Peak purity tests performed with a Diode Array Detector (CE-DAD) under the optimized electrophoretic conditions revealed that the two enantiomers were homogeneous and pure in all samples tested, indicating that no other peaks co-eluted with them. Furthermore, the FDA guidance stresses the value of well-separated peaks with $R_s > 2$ resolution between them for correct quantification. Both enantiomers followed this criterion and were also proven visually (Fig. 6).

2.3.2. Precision

Intra-day precision was determined by introducing MTG standard mixture at three concentrations namely, 10, 50, and 150 µg mL^{-1} nine times (same day). Both migration times and corrected peak areas had %RSD of less than 0.90 and 1.40%, respectively (Table 2). Inter-day precision was checked by the introduction of three standard concentrations (10, 50, and 150 µg mL^{-1}) nine times (over six successive days). Good accuracy as demonstrated by the %RSD values for migration time and corrected peak areas of less than 1.50 and 2.50 % were found, respectively (Table 2).

Upon using the dynamically coated capillary, the accuracy of the corrected peak area improved in general. Dynamic coating resulted in significant improvements in migration time and corrected peak area accuracy for MTG enantiomers, as seen in Table 2. The dynamic coating significantly enhanced the peak symmetry of the MTG enantiomers, and the reduced peak tailing is predicted to result in more reproducible peak integration.

2.3.3. Accuracy

The accuracy of the system was calculated by conducting recovery tests. Each sample was analyzed three times, with appropriate amounts of MTG tablets weighted and spiked with a known standard amount. Table 4 summarizes the findings of this study. The recovery ranged from 98.1 to 102.3%. The positive results show that this method can determine MTG enantiomers in pharmaceutical formulations.

Table 4: Recoveries obtained from the determination of MTG enantiomers when spiked with different levels of standard solution. Each concentration level carried out three replicates

Sample	Racemic MTG (µg mL^{-1})	Mean recovery (% ± SD[a])	
		MTG-E1[b]	MTG-E2[c]
1	10	99.1 ± 2.32	100.2 ± 2.21
2	50	98.1 ± 1.21	102.3 ± 1.42
3	100	100.3 ± 0.93	99.6 ± 1.30

[a] Standard deviation.
[b] Mitiglinide enantiomer 1.
[c] Mitiglinide enantiomer 2.

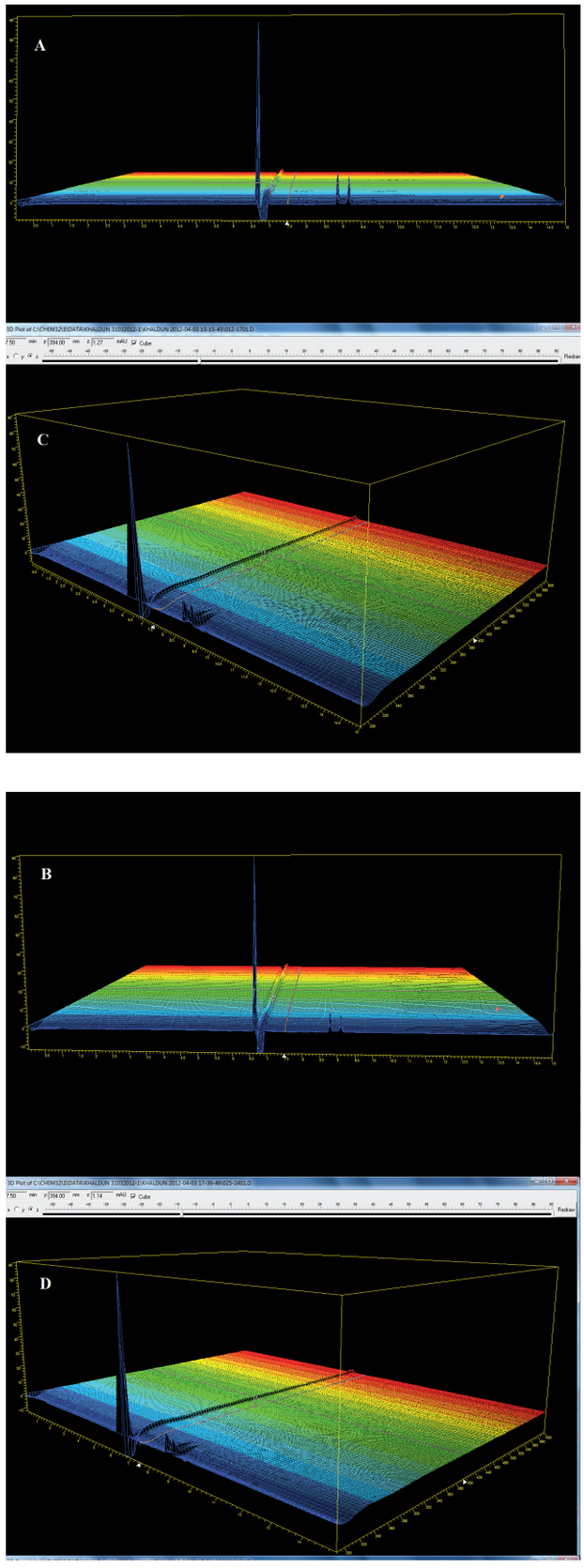

Fig. 6: Peak purity (method specificity) (view A & C for standard injection while B & D for tablet injection). The peak purity obtained upon using CE-DAD under the optimized electrophoretic conditions revealed that the two MTG-enantiomers were identical and pure in all samples tested, showing that no other peaks co-eluting with the enantiomers. (CE conditions in Table 1.) MTG concentration used 100 mg mL^{-1}.

2.3.4. Linearity

Plotting the corrected peak area (y) versus the analyte concentration (x) in µg mL^{-1} yielded the calibration curve. A total of eight standard solutions ranging from 1 – 150 µg mL^{-1} MTG standard were tested. The following summarizes the linear regression equations obtained:

MTG-E1: $y = 0.1891\ x + 0.0337$, $r^2 = 0.9996$

MTG-E2: $y = 0.1639\ x - 0.0243$, $r^2 = 0.9998$

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

The LOD values (MTG-E1 and MTG-E2 enantiomers) were 0.35 and 0.42 µg mL^{-1}, respectively.

The LOD was estimated as the quantity of sample presented to achieve a signal-to-noise ratio (S/N = 3) and the LOQ was determined as the quantity of sample introduced to achieve S/N = 10. MTG-E1 and MTG-E2 had LOQs of 0.94 and 1.00 µg mL^{-1}, respectively. Regression analysis was carried out on the plot. From the regression data, the correlation coefficients (r^2), the slope of the lines, the *Y*-intercepts, the line equations, accuracy, and precision were summarized in Table 5.

Table 5: Assay validation parameters of the developed method for the determination of MTG enantiomers

Parameter	MTG-E1[a]	MTG-E2[b]
Linearity range (µg mL^{-1})	1 – 150	1 – 150
Slope	0.1891	0.1639
Intercept	0.0337	– 0.0243
Regression coefficient (r^2)	0.9996	0.9998
Regression equation	y = 0.1891 x + 0.0337	y = 0.1639 x – 0.0243
LOQ[c] (µg mL^{-1})	0.94	1.00
LOD[d] (µg mL^{-1})	0.35	0.42
Accuracy (%)	98.1 – 100.3	99.6 – 102.3
Precision:		
Intra-day precision (RSD%, Migration times)	0.88	0.67
Intra-day precision (RSD%, Corrected peak area)	1.28	1.37
Inter-day precision (RSD%, Migration times)	1.46	1.23
Inter-day precision (RSD%, Corrected peak area)	2.43	2.22

[a] Mitiglinide enantiomer 1.
[b] Mitiglinide enantiomer 2.
[c] Limit of quantification.
[d] Limit of detection.

Table 6: Results for the determination of enantiomers of MTG in formulated tablets. The sample solution carried out three replicates

Sample no.	Manufacturer's claim (Mitiglinide) (mg)	MTG-E[a] ± SD[c]	MTG-E2[b] ± SD	Total MTG (mg ± SD)
1	10	5.21 ± 0.53	4.92 ± 0.34	10.13 ± 0.72

[a] Mitiglinide enantiomer 1.
[b] Mitiglinide enantiomer 2.
[c] Standard deviation.

2.4. Pharmaceutical formulation analysis

MTG enantiomers found in a pharmaceutical formulation were calculated using the validated method. Table 6 summarizes the findings of these determinations. The rational agreement was reached between the total value as indicated by the supplier and the newly developed CE method. Figure 3(B) shows a typical pharmaceutical formulation electropherogram.

As seen in Fig. 3, using HEC-coated capillary allows for a complete and rapid separation of MTG, which was not possible with bare silica capillaries due to adsorption issues. As a result, it can be inferred that using HEC-coated capillary inhibits MTG enantiomers from adsorbing.

The repeatability of the HEC coating in the simple BGE has been evaluated. The findings showed that the removal of the EOF tends to be a safer strategy to produce repeatable outcomes. The long-standing repeatability of the coating tests using 25 mg mL^{-1} HEC in the simple BGE was therefore investigated. Over six days, 54 injections were conducted (9 injections per day). The %RSD for migration time and corrected peak area (peak area/migration time) was found to be 0.87 – 1.46% and 0.99 – 2.43%, respectively, for n = 54. An extremely small difference in migration time was noticed. However, dynamic coating with HEC can provide relatively repeatable performance. It was also noted that capillaries can still be used after more than 54 injections.

2.5. Stability of dynamically coated capillary

CE tests for the separation of MTG at pH 8.5 were conducted. As we can see in Fig. 3(B) of the standard MTG electropherogram found by the developed method, two sharp peaks were observed between 8.5 and 9.5 min. The migration times of the two peaks of the MTG for both the developed method and our previous trials (uncoated capillary) have been investigated. The importance of migration times under the developed method barely changed across all CE runs (with HEC). The %RSD value of migration time over 54 runs was less than 1.5%. In the case of the use of HEC-free BGE, it was clear no separation at all occurred. The generation of EOF is demonstrated by a rise in the value of migration times. The method developed showed a significant increase in routine CE experiments in reproducibility turns. CE experiments were also performed on the separation of MTG by the suggested method at various pH levels (with and without the addition of HEC). The migration time %RSD values for the first MTG enantiomer at pH 7.0 – 9.5 were summarized in Table 3. The addition of HEC to BGE improved CE reproducibility and capillary coating stability under optimal pH conditions.

2.6. Electrophoretic performance of both uncoated and coated capillaries

There was a major difference in the time of analysis between the two methods, even though analogous operating conditions were utilized for both coated as well as uncoated capillaries. The migration times of the first MTG-enantiomer using coated and uncoated capillaries are presented in Table 3.

The migration times on the dynamically coated capillary were at least 25% shorter and, in some cases, almost 40% shorter. The dynamic coating technique indicates that the time of analysis could be shortened by using this sort of capillary coating. Another noteworthy feature was the major improvement in the peak shape with the capillary coating system. Switching from an uncoated to a coated capillary resulted in less peak skewing and improved baseline peak shape.

The HEC in the dynamically coated system's BGE can interact with the cationic MTG by an ion-pairing mechanism, preventing it from adhering to the capillary wall. It is important to note that this ion-pairing behavior can cause selective differences between uncoated and coated capillaries, so migration order between unmodified and coated capillaries should be tested, which can be determined by running one of the MGE enantiomers.

Since none of the MTG enantiomers are available in our lab, we will name them MTG-E1 and MTG-E2 as mentioned earlier. Improvement in peak shape, upon dynamic coating, for MTG enantiomers has been observed. The unique advantage of dynamically coated capillary methods over permanently coated capillary methods is that the coating may be eliminated to recover the unmodified capillary's original efficiency. The capillary was flushed with NaOH and HCl to eliminate the coating after compiling the data required for the dynamically coated system.

2.7. Dynamic coating effect on resolution

The decrease in the analysis time upon dynamic coating is not considered significant if the enantioresolution is unfavorably affected by such change. As seen in Fig. 7, the baseline separation increased in a favorable way when the dynamic coating was used. This change is attributed to the higher efficiency (higher theoretical plates) of the dynamically coated procedure conducted.

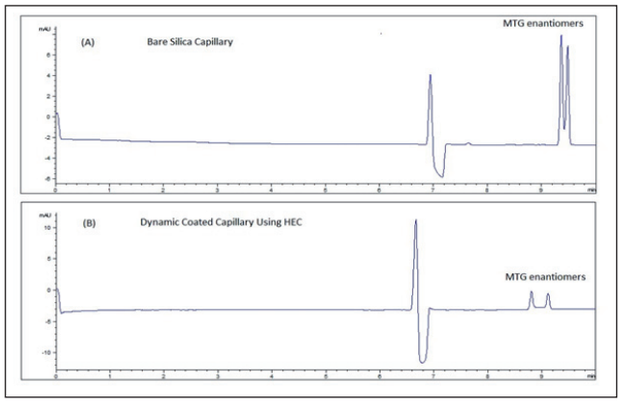

Fig. 7: Electropherograms obtained upon the injection of MTG standard using uncoated capillary (A) and the same capillary when the dynamic coating employed (B). Please refer to Table 1 for CE conditions (dynamic coating injection, electropherogram B) on details of the parameters used presented in the experimental part.

2.8. Conclusions

Significant differences in pharmacological activity, ((*R*)-mitiglinide calcium impurity which is considered as one of the potential impurities induced during synthesis), of various enantiomeric types of MTG, provide evidence for the necessity to establish effective analytical methods for their quantification.

As a result, using HP-γ-CD as a chiral selector and the dynamic coating agent HEC, a cost-effective and rapid CE process with a simple BGE and a normal polarity mode has been developed for the MTG stereoselective separation and quantification of MTG enantiomers. Since it does not require costly chiral columns or huge volumes of solvents as mobile phases, the developed method is favored over HPLC (Yu-ren et al. 2006; Jinzhao et al. 2009). It also has a faster analysis time (9.5 min). The dynamic coating is also less expensive, and easier to conduct under laboratory conditions. Sometimes even more effective than covalent bonding. Also, it is important that the coating can also be removed using NaOH, or HCl or regenerated by flushing the capillary with an appropriate BGE solution. This study shows that many additives are ideal for the successful separation of troublesome analytes, and many researchers are constantly working on inventing new reagents for use as additives, such as starch derivatives with a variety of functional groups, or ion liquids, etc. The results show that using HEC reduces or eliminates analyte adsorption and improves MTG enantiomer resolution as opposed to using an uncoated capillary.

The time of analysis for the coated capillary is better than the uncoated one. The proposed dynamic coating method is easy to produce/prepare, applied, easy to remove, and it does not interfere with chiral separation while also enhancing resolution. These coated capillaries are a relatively cost-effective alternative to permanently coated capillaries, which are labor-intensive and costly.

4. Experimental

4.1. Reagents and chemicals

MTG calcium hydrate reference standard (racemate) was offered by Hikma Pharmaceuticals (Amman, Jordan). Phosphoric acid (H_3PO_4) (85%), disodium hydrogen phosphate (Na_2HPO_4), and HEC were purchased from Sigma–Aldrich (St. Louis, USA). The commercial tablet pharmaceutical preparation (Glufast) (claimed to contain 10 mg MTG active ingredient) was obtained from KISSEI pharmaceutical CO., LTD and purchased from a local pharmacy. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD), 2-Hydroxyethyl-β-cyclodextrin (HE-β-CD), Methyl-β-cyclodextrin (M-β-CD), 2-Hydroxypropyl-γ-cyclodextrin (HP-γ-CD), methanol, and acetonitrile (HPLC grade) were obtained from Sigma–Aldrich (St. Louis, USA). Deionized water was generated by a Milli-Q unit (Millipore, Bedford, MA, USA), which was used for the preparation of solutions.

4.2. Electrophoretic conditions and instrumentation

HP^{3D}CE capillary zone electrophoresis system was used for the separation (Agilent Technologies, Waldbronn, Germany). The detector used was a Photo Diode Array (PDA). Agilent Technologies provided the bare (uncoated) fused-silica capillary 50 µm i.d × 40 cm (detection range, 8.5 cm from the outlet end of the capillary). ChemStation software was used to collect the data. The new capillary was conditioned by flushing it with 1 M NaOH solution for 30 min, then 0.1 M NaOH solution for 10 min, and water for 15 min. Between injections, it was preconditioned for 2 min with 0.1 M NaOH solution, followed by filtered water, then BGE, all for 2 min. Samples and standards were injected at 50 mbar hydrodynamically for 10 s using the following optimized conditions: voltage, 16 kV (normal polarity); capillary temperature, 15 °C; detector wavelength, 200 nm, and the BGE was 50 mM Na_2HPO_4 – 1 M H_3PO_4 solution, pH 8.5; containing HP-γ-CD, 25 mg mL^{-1}; HEC, 25 mg mL^{-1}. Finally, a 5-min wash of water was completed at the end of the day. A 0.2 µm regenerated cellulose membrane filter was used to purify all the standards, sample solutions, BGE, and NaOH solution.

4.3. Stock and standard solutions

Stock standard solution (100 µg mL^{-1}) of mitiglinide was made by dissolving in 1 mL methanol, and then diluted to the mark with water to achieve the desired concentration. Calibration levels were made by diluting the stock solution with water in a series of steps. When not in use, all solutions were refrigerated and kept in the dark.

4.4. Preparation of sample solutions

Twenty tablets were weighed, then ground, and mixed using pestle and mortar. A suitable amount of powder (equivalent to 20 mg of MTG) was dissolved in 1 mL methanol, sonicated for 5 min, and diluted to 250 mL with the aid of water. A membrane with a pore size of 0.2 µm was used to process the sample. For the separation, this solution was subjected to the CE system.

4.5. Capillary coating procedure

The use of dynamic coatings would greatly improve CE's routine performance and the consistency of migration time between different capillaries.

An easy, and simple dynamic coating procedure using HEC polymer (non-ionic) has been developed. The HEC polymer was immobilized on the capillary inner wall through hydrogen bonding to the silanol surface groups by flushing the capillary with BGE.

The process for coating the inner wall of the capillary was as simple as preconditioning the capillary for 2 min with 0.1 M NaOH, distilled water, and BGE, each for 2 min between runs. Excellent performance for the separation of MTG was achieved using the solution that contained HEC, 25 mg mL^{-1}.

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