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Comparison of capillary electrophoresis and reversed-phase liquid chromatography for determination of the enantiomeric purity of an M3 antagonist

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Abstract

The chiral separation of an M3 antagonist was investigated using capillary electrophoresis (CE) with various sulfated cyclodextrins and by reversed-phase liquid chromatography with derivatized cellulose, derivatized amylose, and two protein stationary phases. Operational parameters for each technique, such as the concentration of the chiral selectors, background electrolyte (or mobile phase) pH and type, organic modifiers, injection mode and temperature were varied in order to achieve a desired elution order and to meet a 0.1% limit of quantitation (LOQ) criteria. Based on the advantages and disadvantages of each technique, a practical CE method using sulfated γ-cyclodextrin was selected. The method was validated in terms of linearity, LOQ, accuracy, ruggedness and precision.

Keywords: Enantiomer separation; Validation; Pharmaceutical analysis; Cyclodextrins

1. Introduction

The chiral analysis of pharmaceutical compounds is a very important field of application, especially in the pharmaceutical industry. In the past decades, reversed-phase high-performance liquid chromatography (RP-HPLC) and capillary electrophoresis (CE) have been widely used for enantiomeric separations. The applications of both techniques have been extensively covered in recent publications [1–9]. Protein columns, such as α_1-acid glycoprotein (AGP) and ovomucoid (OVM), and polysaccharide derivative-based columns, such as OD-R and OJ-R, have been widely used for RP-HPLC chiral separations [1–5]. CE has also been established as a tool for enantiomeric analysis. Cyclodextrins (CDs) are the most widely used chiral selectors in CE. Recently, charged CDs have provided additional alternatives towards the development of fast, simple and efficient CE enantiomeric separation methods. The first application utilizing charged CDs was reported by Terabe [10]. Subsequently, Williams and Vigh developed a charged resolving agent migration model (CHARM) for CE enantiomeric separations using negatively charged CDs [11]. Others, using Wren and Rowe's model, described the CE enantiomeric separation behaviors using randomly substituted charged CDs [12].

In this paper, the separation behaviors of an M3 antagonist (*RR* configuration) (Fig. 1) and its enantiomer (*SS* configuration) using RP-HPLC and CE are compared. Various operational parameters such as

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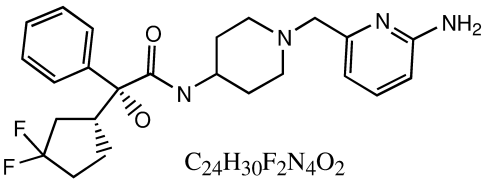

Fig. 1. Structure of an M3 antagonist. FW=Formula mass.

temperature, type of stationary phase and chiral selectors, mobile phase pH, ionic strength, organic modifiers and additives are evaluated in order to understand the separation mechanisms.

2. Experimental

2.1. Reagents

Both enantiomers of the M3 antagonist were synthesized by Merck Process Research (Merck, Rahway, NJ, USA). All organic solvents used in the study were HPLC grade and purchased from Fisher Scientific (Fairlawn, NJ, USA). Phosphoric acid (85%), sodium hydroxide (50%), citric acid, and triethylamine (TEA) were obtained from Aldrich (Milwaukee, WI, USA). Water used in the study was deionized water purified through a Millipore deionization system (Milford, MA, USA). Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD), heptakis-(2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) and heptakis-(6-sulfato)-β-cyclodextrin (HS-β-CD) were purchased from Regis Technologies (Morton Grove, IL, USA). Randomly sulfated-β-cyclodextrins (S-β-CDs) were purchased from Aldrich. Native neutral β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD) and α-cyclodextrin (α-CD) were purchased from Sigma (St. Louis, MO, USA). Highly sulfated γ-cyclodextrin (HS-γ-cyclodextrin, 20%, w/v, aqueous solution) was purchased from Beckman (Fullerton, CA, USA). Phosphate buffers (50 m*M*, pH 2.5–8.5), 1 *M* and 0.1 *M* sodium hydroxide (NaOH) were purchased from Hewlett-Packard (Wilmington, DE, USA).

2.2. Instrumentation

In the RP-HPLC mode, a system consisting of Shimadzu (Princeton, NJ, USA) SCL-10AS pumps, an APD-10AV UV–Vis detector, and an SCL-10A sample controller with a 10 μl sample loop was used. In the CE mode, a Hewlett-Packard ^{3}D capillary electrophoresis (HP ^{3}D, HPCE) system was used. All chromatograms and electropherograms were recorded and processed by a Perkin-Elmer (PE) (Norwalk, CT, USA) Nelson data system using TurboChrom software (version 6.1).

2.3. Chromatographic and capillary columns

The columns used in the reversed-phase mode were Chiralcel OD-R (25 cm×4.6 mm I.D. with 10 μm particles), Chiralcel OJ-R (25 cm×4.6 mm I.D. with 5 μm particles), Chiral AGP (15 cm×4.0 mm I.D. with 5 μm particles), and an Ultron ES-OVM chiral column (15 cm×4.6 mm I.D. with 5 μm particles). The OD-R and OJ-R columns were purchased from Chiral Technologies (Exton, PA, USA). The AGP column was purchased from Regis Technologies. The Ultron ES-OVM column was purchased from Hewlett-Packard. The capillary used in CE was fused-silica [64.5 cm (56 cm effective length)×75 μm I.D.] purchased from Hewlett-Packard.

2.4. Chromatographic conditions

All LC separations, unless specified otherwise, were performed at an ambient temperature of 25 °C. The mobile phases were isocratically pump-mixed at specified compositions. The flow-rate was 0.6 ml/min for the OJ-R and OD-R columns and 1.0 ml/min for the AGP and OVM columns. The injection volume was 10 μl and detection was by UV at 205 nm in all cases. The retention factor k for the two bands was determined as $k=(t_R-t_0)/t_0$, where t_R and t_0 were retention times of retained and unretained compounds, respectively. In RP-HPLC, the t_0 was determined by the solvent peak [5].

2.5. Electrophoretic separation conditions

In CE, unless specified otherwise, the background electrolyte (BGE) used was 25 m*M* phosphate buffer (pH 2.5) containing 2% HS-γ-CD and 1% acetonitrile. A diode array detector was used to collect UV

absorbance at 200 nm. The capillary was maintained at 20 °C. Prior to each CE run, the capillary was flushed with the running buffer for 10 min. Samples were injected hydrodynamically at 50 mbar for 3 s followed by 4 s injection of the running buffer. A voltage of negative 12.5 kV was applied.

2.6. Solutions

Except for the diluent study, all samples were dissolved in an acetonitrile–water (10:90, v/v) diluent. The targeted concentration of the major enantiomer was 0.4 mg/ml for the LC analysis and 0.8 mg/ml for the CE analysis. All CE solutions were filtered through a 0.45 μm membrane using a syringeless filter device (Waters, Milford, MA, USA).

3. Results and discussion

3.1. Separation in RP-HPLC mode

3.1.1. Selection of columns

Two types of chiral stationary phases (CSPs), protein-based (AGP and OVM) columns and polysaccharide-based (OD-R and OJ-R) columns were selected for the study. With a mobile phase that consisted of acetonitrile–phosphate buffer (pH_{app} = 7.0) at various ratios (v/v), all four columns gave baseline separations of the enantiomeric pair of the study compound. It is typically desirable to have the minor enantiomer elute in front of the major enantiomer. If the desired elution order is not achieved, the tail of the major enantiomer can cause interference towards the detection of low levels of the minor enantiomer. As summarized in Table 1, the elution order of M3 antagonist and its enantiomer was reversed from OJ-R to OD-R, and also reversed from OVM to AGP. Both OD-R and AGP columns gave the desired elution order, but the minor analyte cannot be quantitatively detected at the 0.05% level using an OD-R column due to the peak broadness. Therefore, the AGP column was selected for further separation optimization.

3.1.2. Effect of mobile phase pH_{app}

The pH_{app} of the mobile phase is expected to influence the retention and enantioselectivity for separation of the M3 antagonist due to the potential electrostatic interactions between the AGP CSP and solute [3]. AGP has a net negative charge when the pH_{app} of the mobile phase is above its isoelectric point of 2.7. On the other hand, the M3 antagonist has a pK_a 7.5 and possesses a positive charge when the pH_{app} of the mobile phase is below 7.5. Experiments were performed through variation of the pH_{app} of mobile phase (A) over a range from 3.0 to 7.5 in the presence of acetonitrile. The retention factors k of both enantiomers increased as the pH_{app} of the mobile phase increased with increasing enantioselectivity α. At a pH_{app} below 4.0, co-elution of the enantiomers occurred. At a pH_{app} above 7.0, both k and α were increased but the peaks became broader perhaps due to greater silanol interactions. A compromise choice for the mobile phase pH_{app} is around 6.3.

Table 1
HPLC column selection for enantiomeric separation of an M3 antagonist

Column screened	Separation achieved	Elution order	Limit of quantitation (%)
Protein	OVM	*RR*/*SS*	0.5
(OVM, AGP)	AGP	*SS*/*RR*	0.05
Polysaccharide	OD-R	*SS*/*RR*	0.2
(OD-R, OJ-R, AD-R)	OJ-R	*RR*/*SS*	>0.1

Enantiomers: *RR*, major; *SS*, minor.
HPLC conditions: mobile phase A, 20 m*M* sodium phosphate buffer (pH 7.0); B, acetonitrile; injection volume 10 μl; detection wavelength 205 nm.
For protein columns, mobile phase ratio was A–B (88:12) and flow-rate was 1.0 ml/min.
For polysaccharide columns, mobile phase ratio was A–B (85:15) and flow-rate was 0.6 ml/min.

3.1.3. Effect of organic solvents

The effect of the acetonitrile concentration was investigated at a mobile phase pH_{app} of 6.3. The retention factors (k) for both enantiomers decreased as the ratio of the acetonitrile (ACN) in the mobile phase increased. This can be attributed to a decrease in hydrophobic interaction between each enantiomer and the AGP CSP as the mobile phase becomes less polar. The linear trend of the log k vs. % ACN also supports the above explanation. The best separation was achieved at 12% of acetonitrile concentration. Retention factor was not changed significantly utilizing methanol or isopropanol (IPA) as the organic solvents, suggesting that hydrogen bonding is not a major contributor to the separation. The signal-to-noise (S/N) ratio was much higher when acetonitrile was used as solvent due to its lower UV cut-off. Therefore, acetonitrile was selected as a suitable organic solvent.

3.1.4. Effect of temperature

The investigation of separation as a function of temperature revealed increasing separation selectivity with decreasing temperature. However, the peak efficiency decreased as the temperature changed from 50 to 5 °C. Room temperature (25 °C) was a good compromise in this case. Quantitatively, the fact that the increase in retention factors (k) with decreasing temperatures was linear ($R^2>0.99$) with negative slope reflected an enthalpically driven separation process.

3.1.5. Effect of phosphate buffer concentration

The concentration of the phosphate buffer was varied from 10 to 45 m*M* since higher buffer concentrations (>50 m*M*) may adversely affect the operation of the HPLC system from the practical standpoint. No significant changes were observed in terms of the retention behavior in the buffer range studied. Therefore, a buffer concentration of 20 m*M* was selected.

3.1.6. Optimum conditions and validation

Based on the results discussed above, it was concluded that the AGP column with a 20 m*M* phosphate buffer (pH 6.3)–acetonitrile (88:12, v/v) as mobile phase was the optimum system. The chromatograms obtained under the optimized conditions are shown in Fig. 2. The method was validated in terms of limit of quantitation (LOQ),

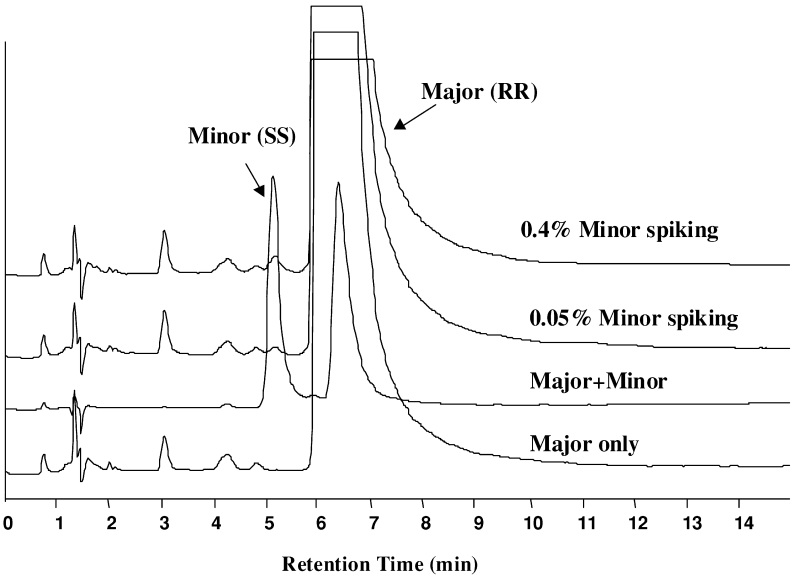

Fig. 2. HPLC chromatograms obtained under optimized LC conditions. HPLC conditions: column type, chiral AGP, 150×4.0 mm, 5 μm; temperature, 25 °C; mobile phase A, 20 m*M* phosphate buffer (pH 6.3); mobile phase B, acetonitrile; isocratic run with the ratio of the mobile phase A and B at A–B (88:12); flow-rate, 1.0 ml/min; injection volume, 10 μl; detection wavelength, 205 nm.

linearity, precision and the solution stability. However, a dramatic column-to-column variation was observed during a robustness study. Suitable separations were achieved using the AGP columns made from 1993 to 1997, however, the separations were not satisfactory using columns made from 1999 to 2000. Therefore, a CE technique was explored.

3.2. Separation in CE mode

3.2.1. Selection of CDs

The pK_a of the M3 antagonist compound and its enantiomer is 7.5 and thus they are fully protonated under the low pH (2.5) BGE conditions. An initial separation attempt was made using 25 m*M* phosphate buffer (pH 2.5) containing 2–5% of various neutral CDs and sulfated β-cyclodextins, such as α, β, and γ neutral CDs, HDAS-β-CD, HDMS-β-CD (single isomer type), HS-β-CD (randomly sulfated type). No satisfactory enantiomeric separation was readily achieved using these CDs. Therefore, the utilization of highly sulfated γ-CD (HS-γ-CD) was attempted since the M3 antagonist compound has a benzyl ring and a difluorinated pentyl ring in close vicinity of its chiral center. The larger size of HS-γ-CD hydrophobic core may afford better inclusion. As expected, baseline enantiomeric separation was readily achieved using 2% of HS-γ-CD in phosphate buffer, pH 2.5. Therefore, HS-γ-CD was selected for further investigations.

3.2.2. Effect of HS-γ-CD concentration on the separation

The effect of the HS-γ-CD concentration on the separation was investigated by varying the concentration from 0.0 to 3.0%. When the HS-γ-CD was not present in the BGE solution, a single analyte peak was observed at a positive polarity setting. When the HS-γ-CD concentration was only 0.01%, no analyte peak was detected at positive voltage setting up to 60 min, but a very broad single peak was observed in the negative polarity setting. This observation indicated that the analyte was complexed even at low CD concentrations, although the extent of complexation was not large enough for enantioselectivity to be exhibited. Williams and Vigh [11] developed a ''charged resolving agent migration'' model (CHARM) for CE enantiomeric separations using charged CDs. However, it cannot be applied directly in this study since the γ-CD used for the method development is randomly substituted and the binding constants between the chiral resolving agent and the enantiomers could not be determined due to dramatically increased ion strength. The plot of selectivity ($\alpha=\mu_2^{app}/\mu_1^{app}$, where the $\mu_2^{app}$and μ_1^{app} are the apparent mobility for the second and first eluted peaks, respectively) vs. HS-γ-CD concentration is displayed in Fig. 3. The selectivity reached its limiting value (~1.03) as the HS-γ-CD concentration increased to 1.0%, and leveled off as HS-γ-CD concentrations increased from 1.0 to 3.0%. Therefore, a concentration of 2.0% was selected since it is in the middle of a plateau region of the α–[CD] plot and gives a reasonable resolution. Equivalent separations were obtained with variation of the HS-γ-CD concentration within ±0.5 weight percent units. This ruggedness indicates that changes caused by batch to batch HS-γ-CD variability could be minimized.

3.2.3. Effect of BGE pH on the separation

Based on the CHARM model, by measuring mobility over a wide pH range, one can determine whether the un-ionized (type I), the ionized (II), or both forms (III) of the analyte associate selectively with the chiral selector [11]. The enantiomeric separation of the M3 antagonist is characteristic of type III cases since it was resolved at both pH 2.5

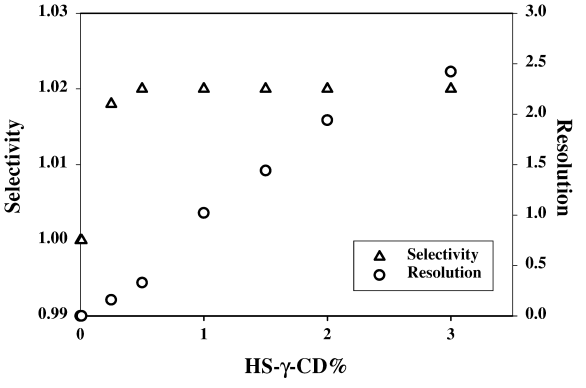

Fig. 3. Effects of HS-γ-CD concentrations on the separation selectivity and resolution for the M3 antagonist. CE conditions: background electrolyte, 25 m*M* phosphate buffer, pH 2.5 containing 0 to 3.0% (w/v) HS-γ-CD; capillary type, fused-silica, 64.5 c×75 μm I.D.; capillary temperature, 20 °C; detection wavelength, 200 nm; applied voltage, −10 kV.

and 8.5, where the compound is fully ionized to be cationic or deprotonated to the neutral form, respectively.

As shown in Fig. 4, the apparent elution orders were reversed by simply reversing the applied polarity and changing the pH of the BGE. In the pH 2.5 buffer, the effective mobility of both enantiomers becomes anionic upon forming a complex with HS-γ-CD at 2.0% (w/v). Therefore the more strongly bound enantiomer elutes first at the reversed polarity setting. At pH 8.5, the effective mobility of enantiomer: HS-γ-CD complexes are still anionic, but the electroosmotic flow (EOF) is significantly increased. With a normal polarity, both anionic peaks were dragged by the EOF. The stronger-binding complex peak carries more negative charge, hence it elutes later and the elution order was reversed.

For an enantiomeric analysis by LC, it is typically desired to have the minor enantiomer eluting first in order to achieve an adequate limit of detection. However, for CE chiral separations, due to the inherent high efficiency, this preference may not be necessary since in most cases, the peaks are well resolved. In this study, the lower pH condition was selected for the final method because it provided reproducible migration times due to suppression of the EOF. Under this condition, the elution order was favorable, but it was not a major concern.

3.2.4. Effect of organic modifiers on the separation

The addition of organic modifiers to the BGE can alter several parameters, such as the EOF, the viscosity, the conductivity of the BGE and the binding constant of the CD–enantiomer complexes. It is recognized that organic modifiers compete with analyte for the relatively hydrophobic cavity of CDs, therefore the binding between the analyte and the CD is reduced [13,14]. As shown in Fig. 5, the enantiomeric separation behaviors of the analytes were varied with different organic modifiers. At 10% of each modifier, the migration times of the analyte peaks were increased in the order of ACN<MeOH< EtOH<IPA. The current was lower with the addition of organic modifiers since addition of organic modifiers changed buffer viscosity and the dielectric constant. In addition, the detection sensitivity was reduced using methanol, ethanol, or isopropanol instead of acetonitrile.

To achieve reasonable running current and detection sensitivity, acetonitrile was chosen as the organic modifier. Different amount of acetonitrile was added to the BGE. The current reduced significantly with the addition of acetonitrile even at 1% level, but the migration times of the analyte increased and the resolution decreased as acetonitrile increased. The BGE containing 1% acetonitrile was a good compromise for both resolution and the current.

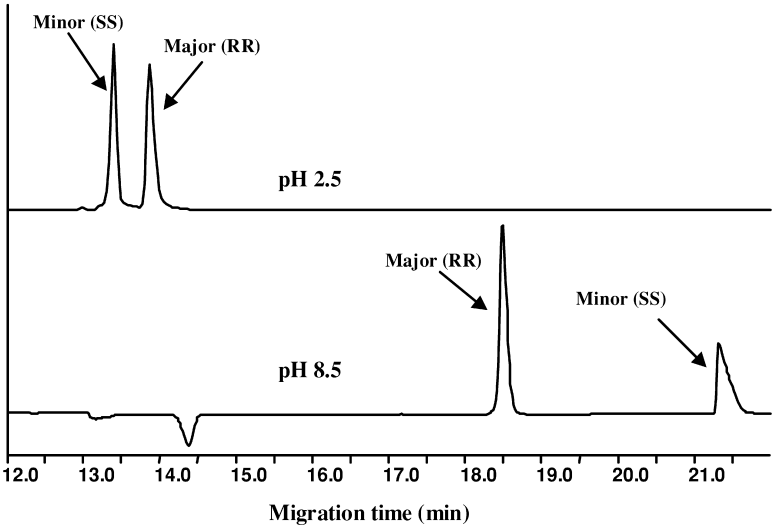

Fig. 4. Effects of the pH on the separation and the elution order of the M3 antagonist enantiomers. CE conditions: background electrolyte, 25 m*M* phosphate buffer containing 2% HS-γ-CD; applied voltage, −12.5 kV (pH 2.5) and +15 kV (pH 8.5). Other conditions as in Fig. 3.

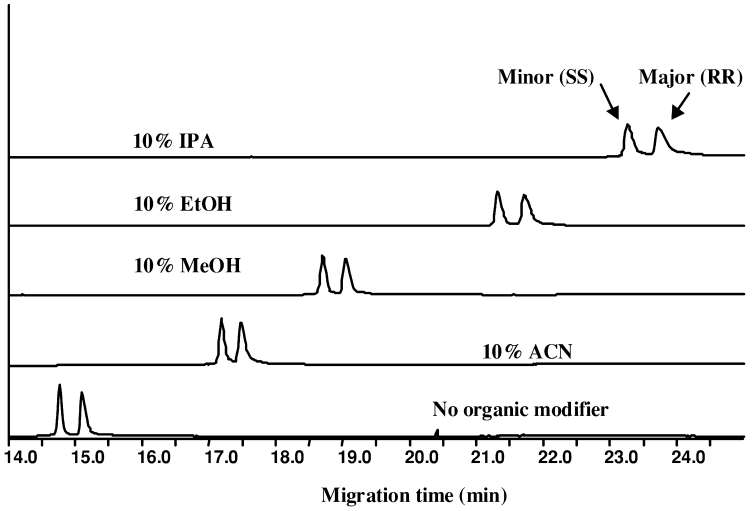

Fig. 5. Electropherograms of the M3 antagonist using selected organic modifiers. Background electrolyte: 25 m*M* phosphate buffer, pH 2.5 containing 2% HS-γ-CD and 10% specified organic modifier. Other conditions as in Fig. 3 except the applied voltage was −12.5 kV.

3.2.5. Effect of the buffer type on the separation

One of the major concerns for selection of a buffer system is the UV cut-off. Small detection path lengths gives rise to detection sensitivity issues, and thus BGE with low background UV absorbance is preferred to enhance sensitivity. Several buffer systems have been used for BGE preparation, including phosphate, citrate, triethylamine and methanol. The signal-to-noise ratio results are listed in Table 2. The results clearly demonstrated that the phosphate buffer is the best choice in this case in order to achieve a desired UV detection.

3.3. Optimum CE method and validation

Based on the results discussed above, the optimum CE conditions were selected. The running buffer was 25 m*M* phosphate buffer (pH 2.5) containing 2% HS-γ-CD and 1% acetonitrile, a negative 12.5 kV voltage was applied, the capillary temperature was maintained at 20 °C, and the UV wavelength was set at 200 nm. Samples were injected hydrodynamically at 50 mbar for 3 s.

3.3.1. Injection precision

To measure the injection precision, a 0.8 mg/ml solution of the major enantiomer was spiked with approximately 0.27% of the minor enantiomer. The spiked solution was injected six times consecutively. The relative standard deviation (RSD) of the area percent was 0.01% for the major enantiomer and 2.2% for the minor enantiomer.

Table 2
Effect of background electrolyte (BGE) type on the signal-to-noise (S/N) ratio

BGE type	S/N Ratio (analyte level=0.1%)	
	γ=200 nm	γ=220 nm
Sodium phosphate (pH 2.5)	47	14
Triethylamine–phosphate (pH 2.5)	21	8
Triethylamine–acetate (pH 3.0)	7	2
Sodium phosphate with 10% methanol (pH 2.5)	–	2
Triethylamine–citrate (pH 3.5)	7	6

Conditions: concentration of each background electrolyte=25 m*M*, containing 2% HS-γ-CD; capillary, fused-silica, 64.5 cm×75 μm I.D.; capillary temperature, 20 °C; applied voltage: −12.5 kV.

3.3.2. Linearity

Seven solutions of different concentration between 0.05 and 120% of the target concentration (0.8 mg/ml) were prepared. Each solution was injected in duplicate. The detector response was found to be linear over the entire range, with $R^2>0.999$, and the percent intercept was 0.02.

3.3.3. Ruggedness

The ruggedness of the method was evaluated by assaying a mixture of M3 antagonist and its enantiomer using three lots of HS-γ-CD solutions and two lots of capillaries. As shown in Table 3, the chiral separation was achieved on all analyses with consistent resolutions and migration times.

The ruggedness of the method was also evaluated by demonstrating solution stability. The solution used for the precision study was stored at ambient temperature. The sample was injected repeatedly within 24-h time period. The RSD of the area percent is 0.01% for the major enantiomer and 2.2% for the minor enantiomer, therefore, the solution is stable for at least 24 h at room temperature.

3.3.4. Limit of detection and limit of quantitation

The standard solution at 0.05% target concentration yielded a peak with a signal-to-noise ratio of 11. Therefore, the limit of detection (LOD) was determined to be 0.05% of the target concentration.

The LOQ was determined based on the satisfaction of three criteria: (1) the S/N ratio of peak for the LOQ solution was greater than 10, (2) the percent difference of response factor values at the LOQ and 5×LOQ was less than 20, and (3) the RSD of area counts for three consecutive injections of the LOQ solution was less than 15% [15,16]. The LOQ was found to be 0.1% of the target concentration based on satisfaction of these three criteria.

3.3.5. Recovery and accuracy

A 0.8 mg/ml major enantiomer solution was spiked with approximately 0.05%, 0.1%, and 0.5% of its minor enantiomer. As shown in Fig. 6, satisfactory chiral separation was achieved at all spiked levels. Each spiking solution was analyzed in duplicate with reproducible CE results. Average recoveries at 0.05%, 0.1%, and 0.5% spiking levels were greater than 90%.

The accuracy of CE method was confirmed by the LC method with the AGP column. A solution spiked with approximately 0.1% minor enantiomer was analyzed by both CE and LC methods. As shown in Table 4, area percents of major and minor enantiomer by CE and LC are comparable.

3.4. Comparison of CE and HPLC

Table 5 summarizes the validation results obtained from the CE method with HS-γ-CD and the HPLC method with AGP column. Both CE and LC validation results fully comply with the USP requirements, except for the large variation of the separation selectivity observed from column to column during LC validation.

In general, the method sensitivity is a potential disadvantage of the CE technique. However, this is also a challenge for the LC chiral separation since the peak efficiencies for the commonly used chiral

Table 3
Evaluation of the lot to lot variability of the capillary and HS-γ-CD

Capillary lot No.	HS-γ-CD lot No.	t_{SS} (min)	t_{RR} (min)	Resolution
1	A	13.71	13.99	2.8
	B	13.69	13.94	2.4
	C	13.83	14.12	2.6
2	B	13.68	13.93	2.3
	C	13.86	14.13	2.6
3	D	14.14	14.43	2.4

Conditions: background electrolyte, 25 m*M* phosphate buffer, pH 2.5 containing 2% HS-γ-CD and 1% acetonitrile; detection wavelength, 200 nm; sample solution, a 0.2 mg/ml racemic mixture. Other parameters as in Table 2.

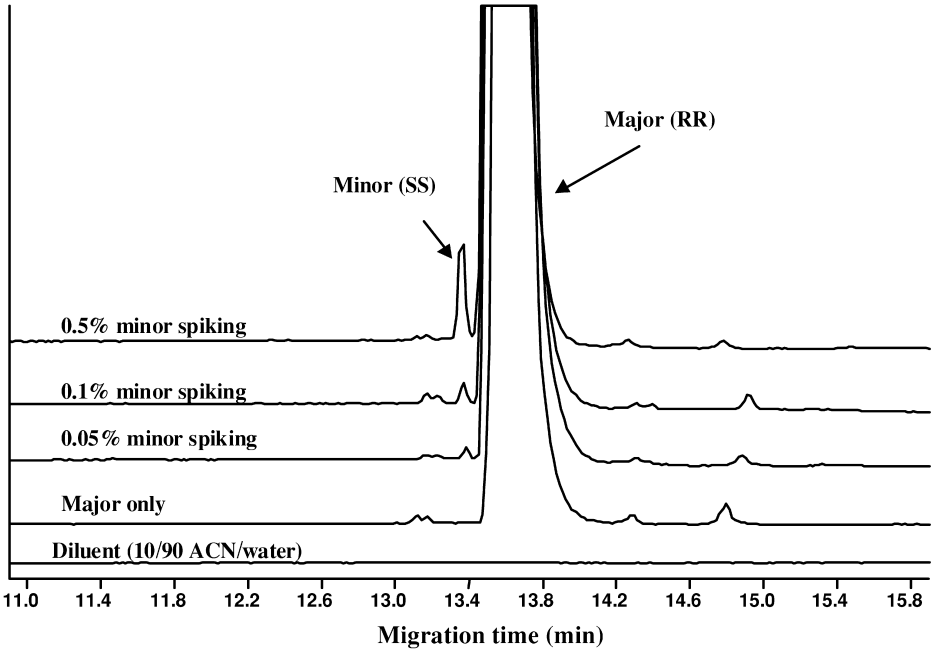

Fig. 6. Electropherograms of the pure major (*RR*) isomer and the major isomer spiked with the minor (*SS*) isomer. CE conditions: background electrolyte, 25 m*M* phosphate buffer, pH 2.5 containing 2% HS-γ-CD and 1% acetonitrile; capillary type, fused-silica, 64.5 cm×75 μm I.D.; capillary temperature, 20 °C; detection wavelength, 200 nm; applied voltage, −12.5 kV.

columns are low. As shown in Table 5, the peak efficiency obtained by CE is about 100 times higher than the LC, it would enhance the sensitivity in consequence. A important factor to achieve suitable method sensitivity is setting the UV wavelength as low as possible whether by LC or CE. Therefore, the UV cut-off of the mobile phase in LC and BGE in CE should be as low as possible.

In addition, poor precision is another disadvantage associated with the use of CE. Since the enantiomeric impurity is determined based on area percent, this problem is not a major concern. However, some CE-specific related parameters should be carefully controlled.

Migration time variation is a major concern for the CE separation. But the data shown in this paper indicate this problem can be well controlled as long as the operational parameters and compositional parameters are optimized.

4. Conclusions

The enantiomeric separations of an M3 antagonist have been achieved by RP-HPLC and CE techniques. CE was found to be superior to the RP-HPLC technique in terms of peak efficiency and method ruggedness for this application. Therefore, the enantiomeric purity of the M3 antagonist can be

Table 4
Comparison of CE and HPLC results

Method	Area percent of major (*RR*)	Area percent of minor (*SS*)
CE	99.91	0.09
HPLC	99.91	0.09

Conditions as in Table 3 for CE and as in Fig. 2 for HPLC except the sample solution was *RR* isomer spiked with 0.1% *SS* isomer.

Table 5
Comparison of validation of CE and HPLC methods

Parameter	CE	HPLC
Injection precision ($n=6$)	2.2% RSD for *SS*	3.9% RSD for *SS*
	0.01% RSD for *RR*	0.01% RSD for *RR*
Migration or retention time variations	<1 min	<2 min
Solution stability (24 h)	2.2% RSD for *SS*	3.6% RSD for *SS*
	0.01% RSD for *RR*	0.01% RSD for *RR*
Linearity (0.05–125%)	0.999 R^2	1.000 R^2
Recovery (0.1% *SS* spiking)	88.9%	90.0%
Limit of detection	0.05%	0.05%
Limit of quantitation	0.1%	0.1%
Robustness	Pass	Fail
System suitability by Turbochrom		
Theoretical plates	380 000 for *SS*	2300 for *SS*
(based on USP)	215 000 for *RR*	1300 for *RR*
Tailing factor	1.2 for *SS*	1.9 for *SS*
	1.5 for *RR*	2.3 for *SS*
Resolution	2.4	2.3

Enantiomers: *RR*, major; *SS*, minor.
RSD: Relative standard deviation.
Conditions as in Table 3 for CE and as in Fig. 2 for HPLC except that a system suitability solution was a 0.2 mg/ml racemic mixture.

quantitatively determined using a simple, fast, accurate, precise, rugged and sensitive chiral CE method.

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