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ORIGINAL

Enantiospecific HPLC and CE Methods for Separation and Determination of *S*-Darifenacin in Pharmaceutical Formulations

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Abstract Two separation techniques were developed for the determination of *S*-(−)darifenacin (DAR) in the presence of its *R*-(+) isomer: The first method is high performance liquid chromatography (HPLC) and the second is capillary electrophoresis (CE). Chiral separation for chromatographic HPLC method development was carried out for *S*-DAR on Daicel CROWNPAK CR (+) (5 μm, 4.0 × 150 mm) column which contains (3,3-diphenyl-1,1-binaphthyl)-crown-6 coated onto a 5.5 μm silica support. The mobile phase system was aqueous acidic 70 % $HClO_4$ (pH 2.5): methanol in the proportion of 90:10 v/v. This current mobile phase was delivered at flow rate 0.8 mL min^{-1} using UV detector adjusted at 286 nm. In CE method, the enantiomers were separated using 50 μm inner diameter fused-silica capillary cut to total lengths of 31.2 cm using 50 mM phosphate buffer as background electrolyte adjusted to pH 2.5 by triethanolamine. A wide range of cyclodextrins (CDs) were used such as highly sulfated α, γ CDs, hydroxyl propyl-β-CD and sulfobutyl ether-β-CD as chiral selectors. The effects of chiral additives regarding its concentration and content of organic modifier on the enantioseparation were investigated. Linear concentration ranges were from 2.5 to 50 and 40 to 300 μg mL^{-1} with detection limits 0.67 and 12.28 μg mL^{-1} for chromatographic HPLC and electrophoretic CE methods, respectively. The two methods were validated according to ICH guidelines with respect to linearity, accuracy, precision, LOQ, LOD and robustness. The suggested methods are suitable for separation and quantitation of *S*-DAR in tablets.

Keywords HPLC · Capillary electrophoresis · Chiral separation · Darifenacin

Introduction

Darifenacin hydrobromide (DAR), (*S*)-1-[2-(2,3-dihydro-5-benzofuranyl) ethyl]-α,α-diphenyl-3-pyrrolidine acetamide as shown in (Fig. 1) [1]. DAR is a potent and competitive muscarinic (M_3) receptor antagonist that is indicated for the treatment of overactive bladder disorder such as frequent or urgent urination, and incontinence (urine leakage). Overactive bladder is characterized by increased in frequency of micturition, urgency and urge is a ubiquitous complaint particularly with increasing age [2, 3]. DAR works by relaxing the involuntary smooth muscle found in the wall of urinary bladder by blocking acetylcholine, it helps the muscle of the bladder wall to be relaxed. In turn, this reduces the need to pass urine [4]. The recommended starting dose of DAR tablets is 7.5 mg once daily, the dose may be increased to 15 mg according to individual's response as early as 2 weeks after starting therapy. DAR is approximately 98 % bound to plasma proteins and metabolised by the liver which is mediated by cytochrome P_{450} enzymes CYP2D6 and CYP3A4. The elimination is 60 % by urine and 40 % through faeces with $t_{1/2}$ 13–19 h [5, 6]. Analytical enantioseparation

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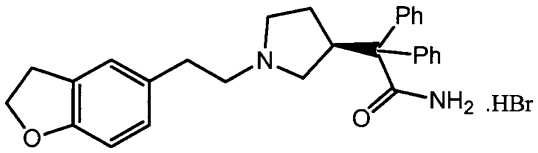

Fig. 1 Chemical structure of *S*-darifenacin

of racemic compounds is considered as one of the most attractive topics in the field of pharmaceutical analysis, therefore, optical purity requires highly efficient enantioresolution chromatograms [7]. It had been well known that the therapeutic effect is limited in most cases to one enantiomer (eutomer). Additionally, the pharmacological inactive eutomer may show undesirable side effects; in some cases antagonistic and even toxic effects [8, 9]. In general, the development of method(s) for the analysis of chiral molecules and recognition of its enantiomeric purity nowadays is crucial as the enantiomers have the same physical properties but differ in pharmacokinetic and pharmacological behavior which leads to discrimination and separation is a very difficult issue. The quantitative analysis of enantiomers and assessment of enantiomeric purity is extremely challenging as it is only separated under chiral environment [10]. HPLC is becoming more widely used as a powerful separation technique for the direct separation of chiral compounds. As advantage it could be used for the separation of many enantiomers. CE is a family of electrokinetic separation methods in which analytes can be separated according to ionic mobility. Additionally, analytes may be concentrated or focused by means of gradients in conductivity and pH. In last decades, many separation techniques had been reported describing the use of chiral stationary phases (CSPs) in conjunction with HPLC or chiral selectors with CE for separation and quantitation of the enantiomers. No published CE methods dedicated for the enantioseparation of *S*-DAR while chiral separation of 12 cathinone analogues were reported [11]. Furthermore, literature survey revealed only one HPLC method reported for the estimation of *S*-DAR in the presence of its *R*-isomer [12], HPLC [13–16] UPLC [17] and LC–MS–MS [18].

The aim of the present work is to develop and evaluate a novel direct HPLC method using CROWNPACK CR(+) for enantioseparation of acetamide containing compounds for the first time after application of crown ether for determination of thyroxine [19]. Furthermore, in CE method, to our knowledge, there are no reported CE methods have been applied for this application. This analytical study to explore different chiral selectors and select the best one which provides the most possible separation of DAR enantiomers.

Experimental

Materials and Reagents

All standard compounds were of analytical grade used without any further purification. *S*-DAR and DAR racemate were purchased from TLC PharmaChem Inc CAS No. 133099-07-7 and D-178 (China) with an estimated purity about 98.2–99.7 % w/w. Emselex® tablets manufactured by Novartis Pharmaceutical limited, UK claimed to contain 15 mg per each tablet. Methanol HPLC grade (Sigma-Aldrich, St Louis, USA). Perchloric acid 70 % (Fisher Scientific, Loughborough, UK). Sulfuric acid (Janssen Chimica, Belgium. Highly sulfated-α-CD and highly sulfated-γ-CD were purchased from Beckmanm coulter (Fullerton, CA, USA). Hydroxypropyl-β-CD and sulfobutylether-β-CD were purchased from Cyclolab R&D (Budapest, Hungary). Perchloric acid was prepared by weighing about 16.3 g of 70 % $HClO_4$ and diluted with double distilled water to 1.0 L to get pH 1.0 (stock A). To prepare $HClO_4$ with pH 2.5 or pH 1.5 transfer 100 or 316 mL of stock (A) and diluted to 1.0 L for each one; respectively, then filtered and degassed before use. Water was produced in house by the Milli Q System (Millipore, Milford, MA).

Instruments

1. Chromatographic analysis were performed using HPLC Agilent 1200 series (Agilent, Germany), comprised of vacuum degasser, thermostatic column compartment G1316A/G1316B, auto sampler and multiple wavelength detector SL, quatenary pump (Germany).
2. Electrophoretic experiments were performed by Beckman P/ACE MDQ Capillary Electrophoresis System equipped with a diode array detector (Beckman Coulter, Fullerton, CA, USA).
3. Ultrasonic sonicator, Bandeline Songrex (Sigma Aldrich, St. Louis, MO, USA).
4. Jenway 3305 pH/mv meter in conjunction with glass electrode (Fisher, USA) was used for pH measurements. Teflon filter, pore size 0.45 µm and 47 mm diameter, used for solvents (Millipore, Milford, MA, USA).

Chiral Stationary Phase

1. Chromatographic column, Daicel CROWNPAK CR (+) (5 µm, 4.0 × 150 mm), DAICEL Chemical industries, LTD, Japan was used.
2. Electrophoretic analysis were performed on 50 µm inner diameter (i.d.) fused-silica capillary cut to total

lengths 31.2 cm with an effective length 21.0 cm was employed (Beckman Coulter, Fullerton, CA, USA). Injections were performed using positive pressure mode of 0.5 psi for 3.5 s upon applying an electrical field 300 V cm^{-1} (≈9.5 kV). Detection wavelength was fixed at 220 nm.

Chromatographic HPLC Conditions

All separations were performed using crown ether as a CSP (Daicel CROWNPAK CR(+) column (5 μm, 4.0 × 150 mm) at room temperature. Elution was performed under isocratic conditions with mobile phase consisting of aqueous acidic 70 % $HClO_4$ (pH 2.5) with methanol in the proportion of (90:10 v/v), then filtered using 0.45 μm filter (Millipore, Milford, USA) and finally degassed by sonicator prior to use with a flow rate of 0.8 mL min^{-1}, UV detection at 286 nm and an injection volume 20 μL. The system allowed equilibrating with the mobile phase for 30 min before the first injection to obtain steady baseline and the column pressure stabilized. All determinations were performed at room temperature 25 °C.

Electrophoretic CE Conditions

Before first used, the new capillary was flushed with 1 M NaOH for 30 min at 40 °C, 0.1 M NaOH for 10 min at 25 °C, water for 30 min at 25 °C, and finally with the used BGE 50 mM phosphate buffer—triethanolamine (pH 2.5) for 10 min at 25 °C. Capillary preconditioning was performed at the beginning of each working day by rinsing with 1 M NaOH for 5 min, 0.1 M NaOH for 5 min, Milli-Q water for 5 min and finally the phosphate—TEA buffer for 5 min. Each preconditioning step was performed at a pressure of 60 psi during 15 min at temperature 25 °C. After each run capillary was washed with 0.1 N NaOH for 2 min, rinsed for 2 min with buffer and equilibrated for 1.0 min with chiral selector solution.

Stock Solutions

Standard stock solutions were freshly prepared by dissolving 25 mg of racemic DAR and *S*-DAR separately in 25 mL volumetric flasks (1.0 mg mL^{-1}). Stock solutions were diluted by mobile phase or phosphate buffer for HPLC and CE; respectively. Series of working solutions of *S*-DAR were prepared by the appropriate dilution to reach the concentration ranges of 2.5–50 and 40–300 μg mL^{-1} for HPLC and CE; methods, respectively.

Procedures

For HPLC Method

Triplicate injections each of 20 μL were applied for each concentration and chromatographed under the condition described above. The peak area for each concentration plotted against the corresponding concentration to obtain the calibration curve and regression equation was derived.

For CE Method

Triplicate 10 μL injections were applied using positive pressure of 0.6 psi for 3.5 s upon applying an electrical field 300 V cm^{-1} (≈9.5 kV). Peak area of each concentration was plotted against the corresponding concentration to obtain the calibration curve and regression equation was derived.

Sample Preparation

Samples were prepared by weighing ten Emselex® tablets and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg was transferred to 200 mL conical flask followed by 40 mL of mobile phase for HPLC, the solution was stirred for 30 min with magnetic stirrer, filtered using 0.45 μm filters (Millipore, Milford, MA, USA) into 50 mL volumetric flask and the volume was completed with the same solvent (1.0 mg mL^{-1}). For CE, the procedures were applied as mentioned above using phosphate buffer as a solvent; the concentrations of *S*-DAR were obtained from corresponding regression equation.

Results and Discussion

For HPLC Method

Crown ethers are macrocyclic polyether compounds that consist of a ring containing several ether groups. The repeating unit being ethylenoxy moiety (–CH_2CH_2O–) which capable of forming guest–host complexes with inorganic cations [20]. Optically active crown ethers have the ability to form enantioselective complexes with primary amines present in an ammonium cations which bear a chiral center directly adjacent to the amino groups [21]. Its chiral recognition is achieved through complex formation with an ammonium derived samples. So, it was developed for direct resolution of amino acids enantiomers [22]. The novelty of this method lies on that crown ether was successfully applied for determination of acetamide containing compounds for the first time. However, secondary amine

cannot form this type of complex with crown ethers due to the bulky substituent group, thus could not separated by this type of column [23]. Since the studied drug has NH_2 group in its structure (Fig. 1), therefore, it can be considered as recognition centre which offer a good chance for enantioseparation and resolution of the racemic form of the studied drug by CSP. The stability is dependent upon the spatial arrangement of each enantiomer in the formed complex. Since the *S*-enantiomer bear a phenyl ring at the optical active center in different plane (away from the viewer) compared to the naphthyl substituents of the CROWNPAK CR(+) column, the complex formed should be more stable, due to less steric hindrance is existed. However, this is not the case with the *R*-enantiomer, where the phenyl ring at the optical active center is considered to be in steric hindrance with the naphthyl substituents of the column, thus lead to form complex but less stable than the complex formed with *S*-enantiomer. The NH_2 group became protonized in the acidic mobile phase condition; thus, the more acidic mobile phase is, the more stronger hydrogen bonding formed with oxygen atoms on CSP, resulting in increase of capacity factor [24]. To shorten the retention time of analytes, the addition of organic modifier such as methanol was quite effective but it is not recommended to use mobile phase contains more than 15 % methanol for this column. In addition, the use of other organic modifier rather than methanol is not recommended for column's safety, because the column may to be over pressurized and lose its capacity. So, 70 % $HClO_4$ (pH 2.5) and methanol were used as a mobile phase in the proportion (90:10 v/v). The peaks achieved were well defined and resolved free from tailing with retention times of 2.40 and 2.91 min for *R*-DAR and *S*-DAR; respectively, the *S*-(−) enantiomer being preferentially retained on Crownpak CR(+) as shown in (Fig. 2).

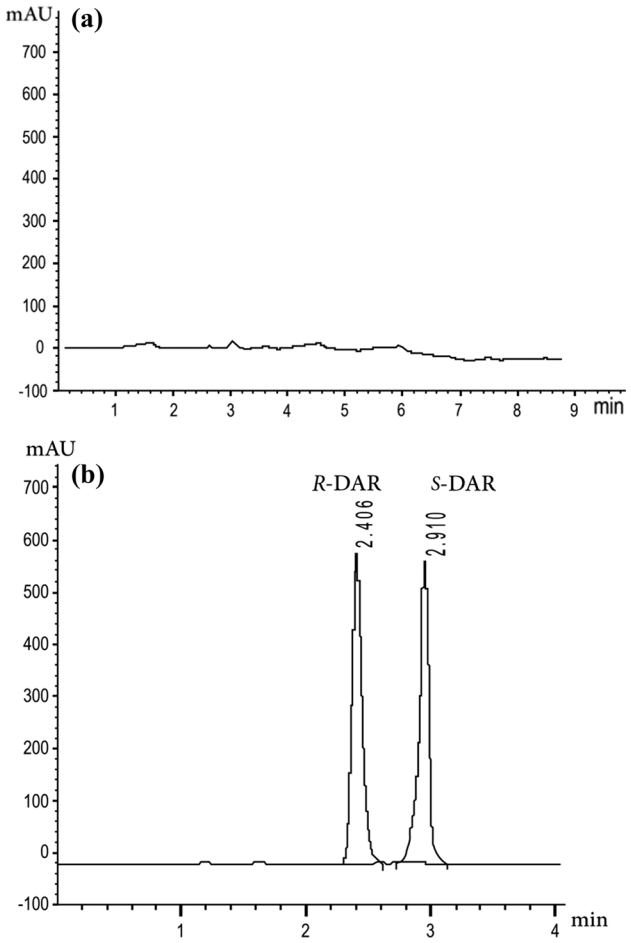

Fig. 2 Representative HPLC chromatogram for **a** blank, **b** racemic DAR (30 μg mL^{-1}) using chiral column, CROWNPAK CR (+) (5 μm, 4.0 × 150 mm)

For CE Method

DAR is considered a basic compound due to the presence of pyrolidine ring and NH_2 group in its chemical structure; it has relatively high pKa value about 9.2. For weak bases [1], ionoselective interaction complexes with the cyclodextrins could be performed at low pH; consequently an acidic background electrolyte (BGE) was suitable for its determination [25]. In the range between 2.5 and 5, a well-shaped peak was identified after the injection of DAR sample; as it is well established that at low acidic pH values the influence EOF is minimized, and the drug would migrate mostly through its own electrophoretic mobility. In the pH range between 6 and 8 DAR migrates very close or even with the EOF, its own electrophoretic mobility being very low. Under acidic conditions (pH <6.0) DAR was positively charged, but as the pH becomes more alkaline, the effective mobility decreases because DAR starts to deprotonated according to cumulative experience from previous studies, the most important parameters should be optimized in CE were the type of CDs, the concentration of the selected CD, the pH of the electrolyte and the percentage of organic modifier in the electrolyte [26, 27]. These parameters were considered to have the most influenced in chiral separation by CE, in addition, the separation depends on other factors such as the EOF, the ionic strength and the co-ion of BGE, the presence of additives such as triethanolamine and cellulose. These other parameters were not examined but were kept constant.

The highly sulfated CDs consist of (α- and γ-) are negatively charged have strong electrophoretic mobility toward the anode in the CE environment attached to sulfate groups, the number of sulfates per molecule is 11–13 for α- and γ-CD; respectively. If the enantiomeric samples interact with the HSCDs, analytes will be swept toward the anode regardless of their charge state. Under the above conditions, basic compounds (which are strongly cationic at very low

pH) will interact ionically with negatively charged sulfates in the hydrophobic cavity. At pH 2.5, Zwitterionic analytes will become positively charged and behave similarly such as basic compounds. Finally, our previous experience shows that the use of HSCDs at low pH could achieve the majority of chiral separations. The peaks achieved were well defined, resolved and free from tailing with retention times of 5.67 and 6.09 min for *R*-DAR and *S*-DAR; respectively, as shown in (Fig. 3). Mixtures of a HSCDs and a neutral CD could yield better results, but in a general strategy, the simplicity is important and thus, the use of two CDs mixtures were considered only when the use of HS-CDs alone does not lead to an acceptable separation [28].

Effect of Buffer Type, Strength and pH

The electrolyte system (BGE) is considered one of the key problem in the application of CE. The main purpose of BGE is to provide the transport of electric current and then separation of analytes. The BGE should primarily provide an appropriate migration of the analyte in a reasonable time with no peaks broadening and migration interferences. The most often used buffers as BGE in chiral separation with HS-CDs are phosphate and acetate buffers, usually in concentration range from (2.5 to 20 % m/v). DAR has high *p*Ka value, so it requires nearly acidic medium to be positively charged. The acidic pH is preferred for basic drugs as the analysis time would be relatively shorter and analytes will migrate after the EOF under these conditions. Better separation efficiency has been obtained with phosphate buffer more than basic borate buffer. Varying the pH of the BGE ranging from 2 to 5, produced significant difference in resolution efficiency. It was found that best resolutions were achieved at pH 2.5 as illustrated in (Fig. 4). It is clearly known that upon increasing the buffer ionic strength that would modulate the EOF and electrophoretic mobility of the analyte. Different buffer strengths were studied 10, 20, 40, 50, 80 and 100 mM of phosphate buffer (pH 2.5). Buffer concentrations ranged from 50 to 100 mM showed results in similar resolution for DAR enantiomers. Finally, based on these observations the optimum buffer strength is 50 mM phosphate buffer (pH 2.5) and selected for further analyses.

Type of Chiral Selector and its Concentration

Many types of native, derivatised and HS-CDs were initially tested in high and low concentrations added to the selected running buffers, and resolution of DAR enantiomers was examined. Native β-CDs at 5 and 10 mM did not produce any separation of the enantiomers. This may be due to the relatively large size of the cavity for β-CD which did not allow any stereoselective interaction with DAR. For HP-β-CD its concentration ranging from 1 to 10 mM was not efficient in separation of DAR enantiomers. This may be attributed to the substitution of secondary hydroxyl ring on the surface of the β-CD which could dramatically affect selectivity of the separation. HS-α-CD and HS-γ-CD are charged CDs have possessed an intrinsic electrophoretic mobility which allows separation of both charged and neutral chiral compounds. The best resolution was obtained using HS-γ-CD > HS-α-CD > SBE-β-CD > HP-β-CD for the analyte, that may be attributed to these negative charges on the highly sulfated CDs which favor interaction with the positively charged analytes may lead to increasing inclusion complex stability of enantiomers separation would be more efficiently. Subsequently, the CD concentration which affects the chiral separation was investigated as shown in (Fig. 5). The resolution markedly increased from 2.5 to 10 (% m/v) for HS-γ-CD and HS-α-CD; respectively; while in case of SBE-β-CD a gradual increase from 2.5 to 5 mM, ranging between 7.5 and 10 mM the resolution appeared to slightly decrease. This may be explained by considering the formed inclusion complexes between the analyte and the chiral selectors led to chiral discrimination ability results from the difference in the stability constant of each enantiomer upon complexation with the CDs. Finally, an acceptable retention time and satisfactory resolution were simultaneously obtained at the concentration level.

Effect of Applied Voltage

Different voltages ranging from 15 to 25 kV were examined to determine the most optimum voltage suitable for separation. Higher voltages more than 25 kV were not tested due to expected joule heat generated inside the capillary which may adversely affect the peak resolution and deteriorate the inner capillary wall. Voltages lower than 5 and/or 10 kV was found to produce very bad resolutions as a result of band broadening. It was found that best resolution obtained at 25 kV.

System Suitability

System suitability tests have been checked by injecting different concentrations of *S*-DAR working standard and separation factor was monitored throughout the validation process as shown in Table 1.

Validation

The suggested HPLC and CE methods were validated according to ICH guideline [29] with respect to all validation parameters such as; accuracy, precision, robustness, specificity, LOD and LOQ.

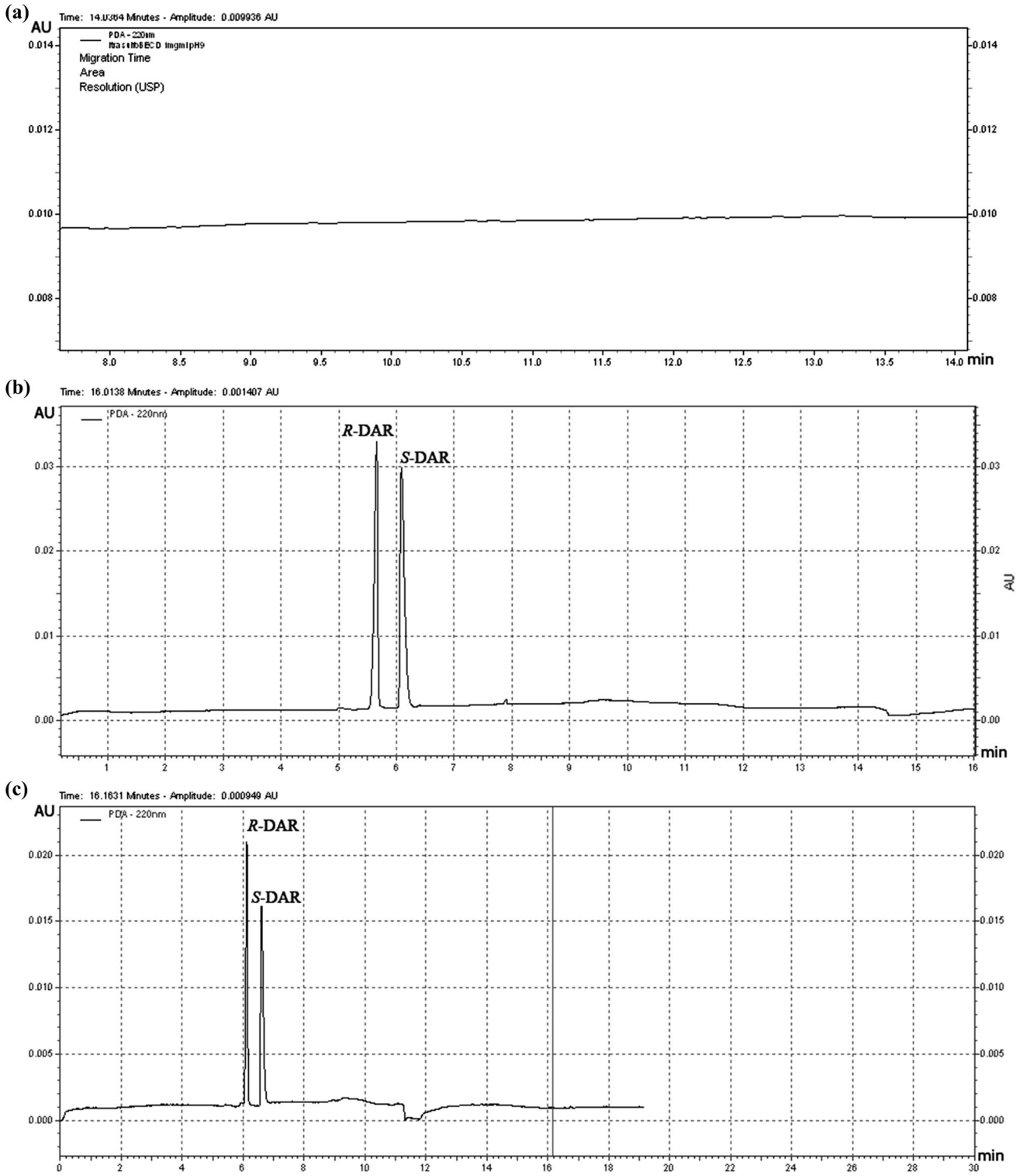

Fig. 3 Representative CE electropherogram for racemic DAR (200 μg mL^{-1}) under the following conditions: **a** blank, **b** HS-γ-CD (10 % m/v), **c** HS-α-CD (10 % m/v), **d** HP-CD (50 mM) and **e** sulfobutyl ether β-CD (5 mM) using 50 mM phosphate buffer (pH 2.5)

Linearity, Detection and Quantitation Limits

The linearity of the proposed methods was investigated by analyzing a series of different concentrations of *S*-DAR revealed a good linearity. LOD and LOQ were estimated a single to noise ratio of 3.3 and 10 σ/S where σ is the standard deviation of intercept, S is the slope derived from calibration curve, all results were summarized in Table 2.

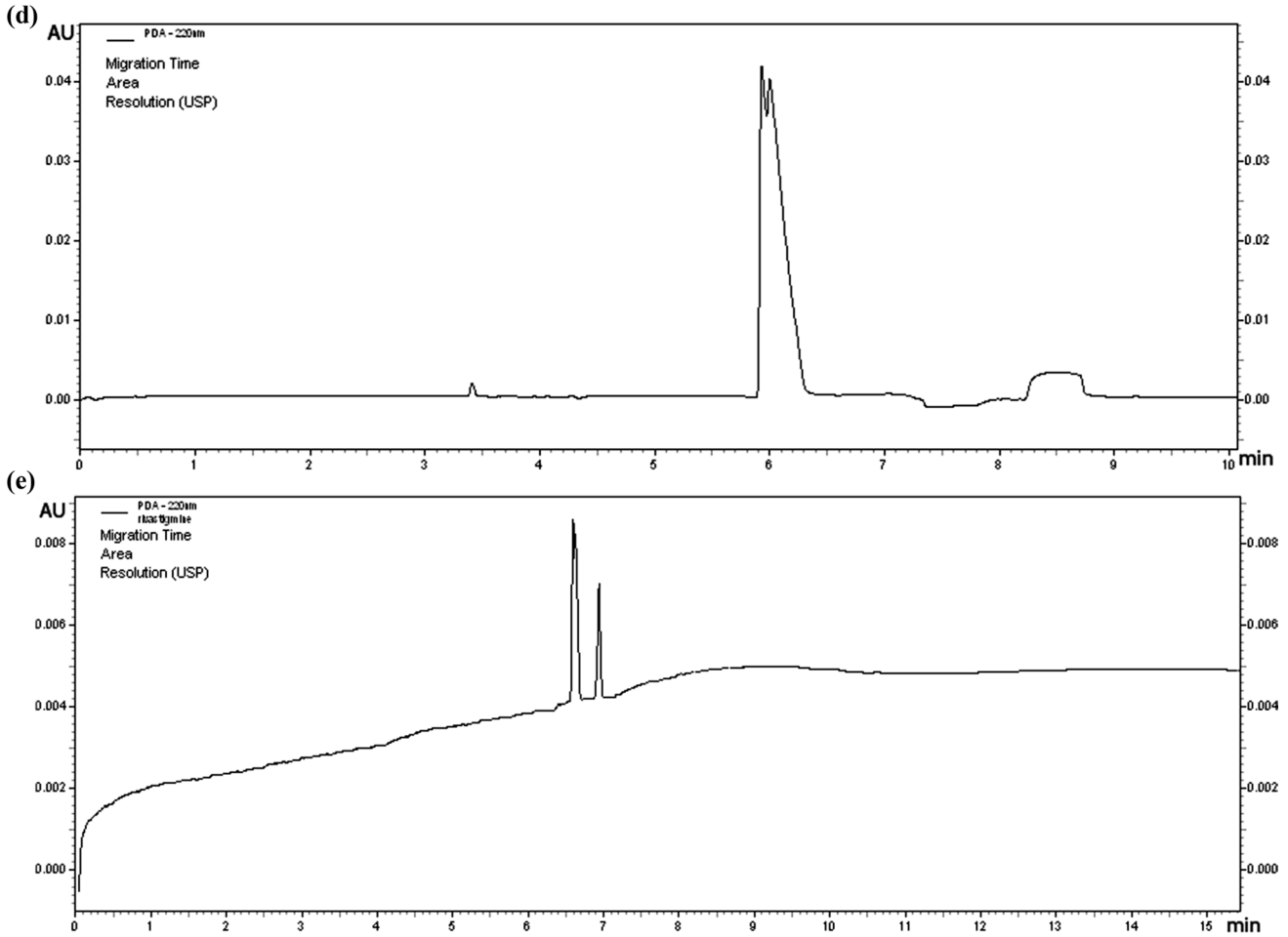

Fig. 3 continued

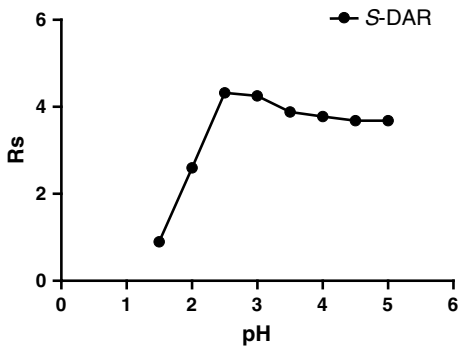

Fig. 4 Effects of the acidic pH on the chiral separation of racemic DAR (200 μg mL^{-1}) in CE method using BGE consist of HS-γ-CD (10 % m/v) in phosphate buffer (50 mM, pH 2.5)

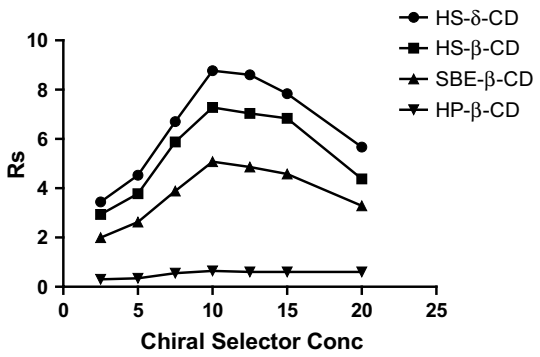

Fig. 5 The effect of chiral selector concentrations on the electrophoretic separation of racemic DAR (200 μg mL^{-1})

Accuracy

It was applied by adding different amounts of *S*-DAR to a solution of tablets analyzed previously. The average recovery was 99.25 % according to standard addition technique; the accuracy based on the mean % recovery of the measured concentrations compared with actual concentrations, these results showed that there was no interference from common excipients present in tablets, as shown in Table 3.

Table 1 Parameters for system suitability tests of the proposed chiral HPLC method

Parameter	Reference value	*S*-DAR
Flow rate (mL min^{-1})	–	0.8
Retention time (min)	–	2.91
Resolution (*R*)	$R > 2$	8.7
Selectivity factor (α)	>1	1.20
Column capacity (K')	0.1–10	7.34
Tailing factor (*T*)	≤2	0.71
Column efficiency (*N*)	≥2000	4477
HETP	*L*/*N*	0.03

HETP height equivalent theoretical plates

Table 2 Analytical parameters for *S*-DAR using HPLC and CE method

Parameter for *S*-DAR	HPLC	CE
Linear range (μg mL^{-1})	2.5–50	40–300
Intercept (a)	1.62	−433.07
Standard deviation of intercept (S_a)	21.41	103.80
Slope (*b*)	104.01	27.88
Standard deviation of slope (S_b)	0.77	0.53
Correlation coefficient (*r*)	0.9998	0.9993
Determination coefficient (r^2)	0.9996	0.9987
SD of residuals (Sy·x)	36.41	118.51
Limit of detection (LOD μg mL^{-1})	0.67	12.28
Limit of quantitation (LOQ μg mL^{-1})	2.05	37.23

Precision

The precision of the assay (within-assay and between-assay) was determined for *S*-DAR, the intraday precision was assessed by analyzing three concentrations in triplicate of each sample in the same day while the interday precision was assessed by analyzing the same sample, in triplicate, in three successive days. All results were satisfactory and the relative standard deviation (% RSD) were less than 1.1 % as shown in Table 3.

Robustness

The robustness of an analytical procedure was assessed by evaluating its capacity to remain unaffected by small, but deliberate variations such as organic modifier, flow rate, temperature and wavelength. The results indicate that the ability of the method to remain unaffected by small changes in parameters. This provided an indication for the reliability of the proposed methods during routine work.

Tablet Analysis

The suggested methods were applied successfully for the determination of *S*-DAR enantiomers in its Emselex® tablets. Six replicates determinations were made then mean % recovery and standard deviation was calculated. The accuracy was evaluated using standard addition technique through four different concentrations of the pure drug

Table 3 Evaluation of the accuracy and precision for the determination of *S*-DAR using the proposed chiral HPLC and CE methods

Parameters at three levels	*S*-DAR				
	Taken (μg mL^{-1}) (%)	Found (μg mL^{-1})[a]		% mean recovery	% RSD of assay, $n = 3$[a]
HPLC method					
Inter-day precision	50	10	9.88	98.88	0.42
	100	20	19.96	99.80	0.68
	200	40	39.91	98.77	0.69
Intra-day precision	50	10	9.82	98.20	0.71
	100	20	19.92	99.61	0.55
	200	40	39.98	99.95	1.01
CE method					
Inter-day precision	50	100	98.91	98.91	0.97
	100	200	197.99	98.99	0.91
	150	300	298.75	99.58	0.86
Intra-day precision	50	100	99.57	99.57	0.65
	100	200	198.22	99.11	0.91
	150	300	299.01	99.67	0.98

[a] The value is the mean of three determinations

Table 4 Application of standard addition technique in the determination of *S*-DAR in Emselex® Tablet using the proposed HPLC and CE methods

Method	Taken (μg mL^{-1})	% Found ± SD[a]	Pure added (μg mL^{-1})	% recovery[b]	Mean ± SD
HPLC	20	99.71 ± 0.76	10	99.61	98.73 ± 0.323
			20	99.45	
			30	99.10	
			40	98.9	
CE	200	98.99 ± 0.792	100	98.9	99.26 ± 0.528
			150	98.8	
			200	98.0	
			300	99.25	

[a] Average of five determinations

[b] Average of three determinations

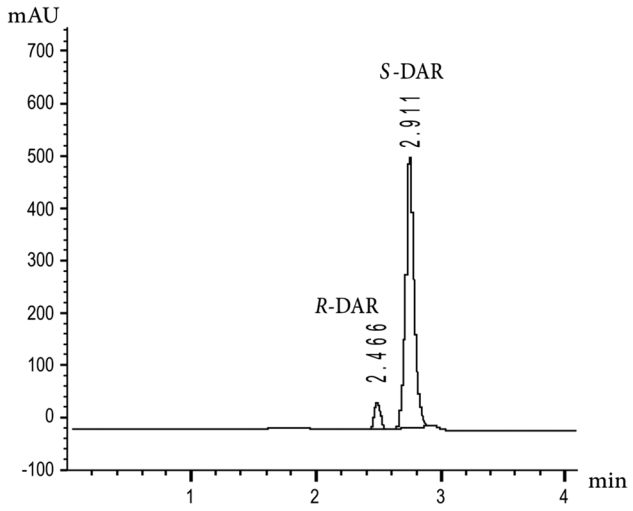

Fig. 6 HPLC chromatogram for *R*-DAR in Emselex® tablets (30 μg mL^{-1}) using chiral column, CROWNPAK CR (+) (5 μm, 4.0 × 150 mm)

added to the previously analyzed sample and the percentage recovery was calculated for each concentration. It was found that results were in good agreement with the labeled claim as shown in Table 4. The HPLC method is suitable for the detection of *R*-DAR enantiomer in tablet as shown in (Fig. 6) and was calculated by internal normalization formula ($AUC_{(R)}$/Total areas for *R* and *S* enantiomers) × 100. According to above equation, the percent of *R*-DAR enantiomer was found to be 1.5 % per each tablet.

Conclusions

The present work is presented for the first time for chiral enantiosepration of *S*-DAR based on HPLC and capillary electrophoresis methods. HSP-β and γ-CD were found to be the best chiral selector with the highest resolution in CE method while crown ether based CSP column was used in HPLC. The suggested methods were fully optimized and could be used for separation and quantitation of the *S*-DAR enantiomer in a reasonable time with a good resolution. Obviously, HPLC showed advantages on CE in terms of higher sensitivity, shorter running time, precise sample loading and fewer variables to be optimized. On other hand, CE was advantageous in its higher resolution and using less expensive chiral selector, solvents and running buffer. Both techniques have been successfully applied for the analysis of *S*-DAR in its commercial tablets.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

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