Analytical Methods

PAPER

Chiral separation of perindopril erbumine enantiomers using high performance liquid chromatography and capillary electrophoresis

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Two separation methods were developed for the determination of *S*- and *R*-perindopril *tert*-butylamine (erbumine salt) (PER): high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The HPLC method uses a chiral stationary phase (CSP), ChiraDex column constituting β-cyclodextrin chemically bonded to spherical silica gel particles. The mobile phase consisted of phosphate buffer (50 mM, pH 3.0) and acetonitrile (45 : 55 v/v). The flow rate was 1.0 mL min^{-1} and the detection wavelength was 210 nm. In CE, 2-hydroxylpropyl-β-cyclodextrin (10 mM) was used as a chiral selector. It was added to the background buffer composed of phosphate buffer (100 mM, pH 7.0) and methanol (15% v/v). The applied voltage was 15 kV and the detection was carried out using a diode array detector. All factors affecting the chromatographic or electrophoretic separations were studied and optimized. The linear concentrations ranged from 5–150 and 25–800 μg mL^{-1} with detection limits of 2.3 and 14.7 μg mL^{-1} for HPLC and CE methods, respectively. The methods were validated according to ICH and USP guidelines. The suggested methods were applied for the determination of *S*-PER in bulk powder and commercial tablets containing PER erbumine racemate.

1. Introduction

Perindopril erbumine (PER) is an angiotensin-converting enzyme (ACE) inhibitor. It is used for the treatment of hypertension, heart failure and stable coronary artery disease. PER induced vasodilatation occurs as a result of the combined effects of decreasing vasoconstriction caused by decreasing levels of angiotensin II and increased bradykinin level that is a potent vasodilator.[1] About half the drugs in use are chiral compounds. It is well known that the pharmacological effect is restricted in most cases to one of the enantiomers (eutomer). In addition, the pharmacologically inactive enantiomer (distomer) can show unwanted side effects; in some cases antagonistic and even toxic effects are observed.[2,3] For example, *S*-PER, (2*S*,3*aS*,7*aS*)-1-[(*S*)-*N*-[(*S*)-1-carbethoxybutyl]alanyl]hexahydro-2-indole carboxylic acid is the active form (Fig. 1). *R*-PER, (2*S*,3*aS*,7*aS*)-1-[(*R*)-*N*-[(*S*)-1-carbethoxybutyl]alanyl]hexahydro-2-indole carboxylic acid, is a by-product formed during the synthesis of *S*-PER. It does not have the same therapeutic effects as *S*-PER.[4] Therefore, chiral separation is essential for several drugs including PER racemate. HPLC and CE methods are considered the most important techniques in chiral separation.[5] Generally, to achieve enantioselective resolution, an interaction between a chiral selector and the analyte under investigation is used. Cyclodextrins (CD) are widely used as chiral selectors in both HPLC and CE.[6,7] In HPLC, the chiral selector is either added to a mobile phase or fixed to a solid support forming the chiral stationary phase (CSP). While in CE, the chiral selector is added to the background electrolyte (BGE). The formation of an inclusion complex between the hydrophobic cavity of CD and the non-polar part of the analyte is considered the predominant driving force for chiral recognition and separation. Chiral HPLC methods were reported for the analysis of stereoisomers of octahydroindole-2-carboxylic acid, an intermediate in the synthesis of perindopril erbumine.[8,9] Literature survey revealed that all the reported techniques for estimation of PER enantiomers are electrochemical methods[10–15] or flow injection systems using electrochemical detectors.[16,17] Herein we report the first HPLC and/or CE methods dedicated for the chiral separation of *S*-PER and application of these methods in the analysis of *S*-PER in commercial tablets. The separation of PER enantiomers was investigated by chiral HPLC and CE techniques. Some validation parameters were examined in order to access the validity of the suggested method for quality control.

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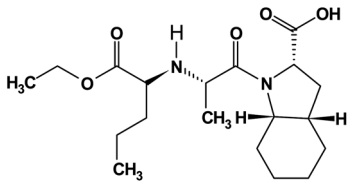

Fig. 1 Chemical structure of *S*-perindopril (*S*-PER).

2. Materials and methods

2.1. Apparatus

All chromatographic analyses were carried out on an Agilent 1200 series HPLC (Agilent, Waldbronn, Germany), equipped with a vacuum degasser, auto sampler, thermostated column compartment, multiple wavelength detector and quaternary pump. The separation was performed on a ChiraDex chiral column (5 μm, 4 × 250 mm) (Agilent technologies Inc, CA, USA) which is a β-cyclodextrin bonded to porous silica particles. Chromatographic peaks were electronically integrated and recorded using chemstation software, V. B.03.01 (Agilent, Germany).

The electrophoretic experiments were performed on an Agilent capillary electrophoresis system (G 1600A model, Agilent technologies, Waldron, Germany) consisting of fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 64.5 cm total length and effective length of 56 cm with 50 μm internal diameter. The capillary temperature was set at 25 °C connected to a UV diode-array detector (DAD).

An ultrasonic bath, Bandelin Songrex (Sigma-Aldrich, St. Louis, MO, USA), was used for solvent degassing. A Jenway 3305 pH/mv meter with a double junction glass electrode (Fisher, USA) was used for pH measurements. A Teflon disposable filter, pore size 0.45 μm and 47 mm diameter, was used for solvents (Millipore, Milford, MA, USA).

2.2. Reagents

All standard compounds were of analytical grade and were used without further purification. Racemic (*R*,*S*) PER and *S*-PER were provided by ADWIA Pharmaceuticals Co., (El-Oubor City, Cairo, Egypt) with an overall general estimated purity of 99.2% w/w. Coversyl® tablets (B.no.15444) manufactured by Servier Egypt Industries Limited, (6^{th} of October, City, Giza, Egypt) under the license of Servier – Ireland, Industries Ltd. Coversyl® tablets are labeled to contain five mg PER erbumine per tablet.

Acetonitrile, HPLC grade, was purchased from Prolabo (Paris, France). Methanol, HPLC grade, was purchased from Fisher Scientific (Loughborough, UK). Hydroxypropyl-β-cyclodextrin (HB-β-CD) was purchased from Sigma, (St. Louis, MO, USA). HPLC grade potassium dihydrogen phosphate, phosphoric acid, sodium hydroxide and hydrochloric acid were purchased from E. Merck, (Darmstadt, Germany). Ultra pure Milli-Q water (Millipore, Bedford, MA, USA) was used for the preparation of buffer and other related aqueous solutions.

2.3. Chromatographic conditions

The mobile phase consists of 50 mM phosphate buffer of pH 3.0 and acetonitrile in the ratio of 45 : 55 v/v. The mobile phase was filtered with a 0.45 μm membrane filter (Millipore, Milford, USA) and degassed by ultrasonic vibration prior to use. The samples were also filtered through 0.45 μm disposable filters and 20.0 μL was injected into the HPLC system. The separation was performed on a ChiraDex chiral column using 1.0 mL min^{-1} flow rate. The column was equilibrated with the mobile phase for at least 30 min until a steady baseline was obtained and the column pressure was stabilized. All determinations were performed at room temperature using UV detection at 210 nm.

2.4. Electrophoretic conditions

A new capillary was flushed with 1 M sodium hydroxide for 40 min at 40 °C, 0.1 M sodium hydroxide for 10 min at 30 °C, water for 30 min at 30 °C, and finally with running buffer for 10 min at 30 °C. Preconditioning flushing between runs was performed with 1.0 M hydrochloric acid for 5 min, water for 5 min, 0.1 M sodium hydroxide for 5 min, water for 5 min, and finally with running buffer for 5 min. The separation was performed using a background electrolyte containing phosphate buffer (100 mM, pH 7) and hydroxyl-propyl-β-cyclodextrin (10 mM). The sample was injected with the aid of applied voltage and pressure at the sample inlet of 15 kV and 50 mbar for 10 seconds. All separations were carried out at 15 °C using a circulating coolant to maintain the constant temperature inside the capillary cartridge. The run voltage was 15 kV and detection was carried out using a diode-array detector at 210 nm.

2.5. Preparation of standard drug solutions

Stock standard solutions of racemic PER erbumine and *S*-PER were prepared daily by dissolving 25 mg of each in 25 mL volumetric flasks (1.0 mg mL^{-1}) using the mobile phase and 20% aqueous methanol for HPLC and CE methods, respectively. Series of working solutions of PER erbumine were prepared by the appropriate dilution of the stock solutions with corresponding solvent to reach a concentration range of 5–150 and 25–800 μg mL^{-1} of *S*-PER for HPLC and CE, respectively.

2.6. Preparation of sample solutions

Twenty Coversyl® tablets were weighed and finely powdered. A portion of the powder equivalent to 50 mg PER erbumine was weighed accurately and then dissolved in 50 mL of mobile phase and 20% aqueous methanol for HPLC and CE, respectively. The sample solution was then filtered through 0.45 μm disposable filters.

3. Results and discussion

3.1. HPLC method development and optimization

Chromatographic separation of a racemic PER described in this work was carried out on a ChiraDex column, as a chiral stationary phase (CSP) in which β-cyclodextrin is chemically

bonded to spherical silica particles. One major advantage of ChiraDex is the use of very simple solvents as a mobile phase. The most successful separations were observed with ChiraDex when a mobile phase containing methanol, water, phosphate buffer or triethyl ammonium acetate was used.

3.1.1. Organic modifier in the mobile phase. The choice of the organic solvent influenced the enantioselectivity of ChiraDex. The increase in the polarity of the organic solvent (methanol > ethanol > acetonitrile) improved the separation of the enantiomers. Different mobile phases containing two organic modifiers, acetonitrile and methanol, in the range of 10–80% v/v in phosphate buffer (50 mM, pH 3.0) were attempted. Chiral separation was observed with acetonitrile, but not with methanol because of the greater solvent strength of acetonitrile and its higher affinity for the cyclodextrin cavity. Increasing the acetonitrile percentage up to 45% improved the chromatograhic resolution. However, further increase in acetonitrile percentage resulted in poor resolution.

The effect of pH was studied over a range from 2–5, at pH lower than 3.0, it was not recommended by the manufacturer to use the column in highly acidic medium at pH 1.0 or 2.0 but upon using pH 2.0 it was observed there was not a great difference rather than pH 3.0, while at pH higher than 3.0 a forked peak formed and with great tailing for the *R*-enantiomer. So, the optimum pH was 3.0. Different flow rates (0.7–1.2 mL min^{-1}) were tested. Good resolution was obtained using flow rate 1.0 min^{-1}. The influence of temperature was also examined. During the change from 20 °C to 30 °C, the selectivity was improved. It might be due to the positive influence of change of entropy on separation. However, further increase in the temperature worsened the separation. Simultaneous detection of *S*-PER and *R*-PER was carried out at 210 nm. Higher wavelengths (230, 238 and 280 nm) gave low detector response. Under the optimum HPLC conditions, satisfactory baseline separations of *S*- and *R*-PER were obtained using cyclodextrin based CSP and the mobile phase consists of acetonitrile and 50 mM phosphate buffer (pH 3.0) in the ratio of 45 : 55 v/v. The analytes' peaks were well defined, resolved and free from tailing. The tailing factors were less than two for all peaks. The retention times were 2.23 and 6.05 min, for *R*-PER and *S*-PER, respectively (Fig. 2).

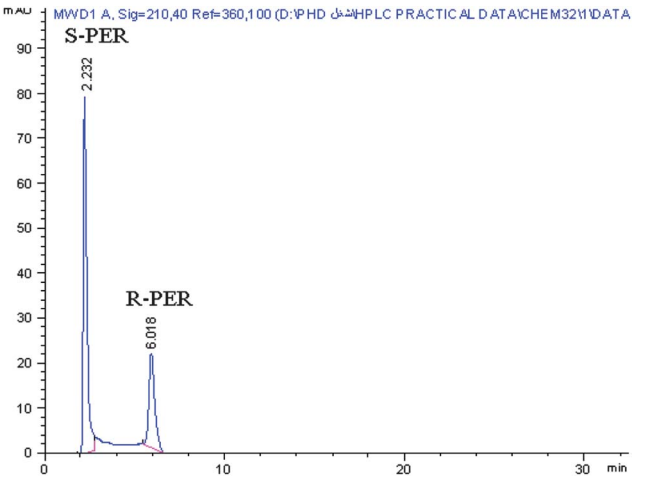

Fig. 2 Representative HPLC chromatogram for racemic PER (100 μg mL^{-1}) using chiral column, ChiraDex chiral column (5 μm, 4 × 250 mm), the mobile phase consists of 50 mM potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile (45 : 55 v/v), flow rate 1.0 mL min^{-1} at 25 °C. UV detection at 210 nm.

3.2. CE method development and optimization

The chiral separation of PER was studied using various types of native and derivatized β-CD. All the parameters affecting the efficiency of separation through its effect on electro-osmotic flow (EOF) were studied and optimized. These parameters were buffer system composition (type, pH and ionic strength), effects of HP-β-CD, organic modifier as well as analytical voltage and pressure applied during sample injection.

3.2.1. Buffer type, pH and strength. Phosphate and borate buffers were most often used as BGE in chiral separation with β-CDs. Usually they were used in the concentration range from 10 to 100 mM. Separations of enantiomers exhibiting acidic properties like PER erbumine were separated using BGEs with high pH values. Alkaline or neutral conditions were preferred for acidic drugs because the analyte will be negatively charged, hence, the analysis time will be relatively shorter and the analyte will migrate after the EOF. In the proposed method, efficient separation was obtained with phosphate buffer rather than borate buffers.

Upon variation of the pH of the BGE from 3.0 to 9.0, a significant difference in the resolution efficiency was observed and the best results were achieved at pH 7.0. Increasing the buffer ionic strength modulated the EOF and electrophoretic mobility of the analyte. The studied buffer strengths were 10, 15, 20, 40, 60, 80 and 100 mM of phosphate buffer (pH 7.0). Buffer concentrations from 20 to 100 mM resulted in a similar resolution for PER erbumine enantiomers with a gradual decrease in migration times. Based on these observations, an optimum buffer strength of 100 mM was selected for further analyses.

3.2.2. Type and concentration of the chiral selector. Several types of native and derivatized β-CD were added in different concentrations to the run buffers, and the resolution of PER erbumine enantiomers was examined. β-CD at 5 and 10 mM did not produce resolution of the enantiomers. This could be due to

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

Parameter	Reference value	*S*-PER
Flow rate (mL min^{-1})	—	1.0
Retention time (min)	—	2.23
Resolution (*R*)	$R > 2$	6.01
Selectivity factor (α)	>1	1.16
K′ (column capacity)	0.1–10	1.39
Symmetry	—	0.82
Tailing factor (*T*)	≤2	1.02
N (column efficiency)	≥2000	2866
HETP[a]	$=L/N$	0.013

[a] HETP is the height equivalent theoretical plates.

the large size of the cavity of the β-CD which did not allow enantioselective interaction with PER erbumine. However, the use of HP-β-CD was very efficient in separating PER erbumine enantiomers. This might be attributed to ability of the substitution at the secondary hydroxyl rim on the surface of the β-CD to dramatically affect the selectivity of separation. It provided additional interaction points with the analyte. HP-β-CD at concentrations of 1 to 10 mM was examined. Baseline separation of the enantiomers was achieved using a concentration of 10 mM HP-β-CD. An increase in the concentration to 20 mM resulted in less efficient separation of the PER erbumine enantiomers.

3.2.3. Type and percentage of the organic modifier. Water miscible organic solvents like methanol and acetonitrile were tested to modify the viscosity of the medium and to reduce the adsorption of the analytes to the capillary wall. Best results were obtained with methanol which can be explained by its ability to reduce the EOF, hence increasing the separation efficiency. The effect of methanol percentage in the BGE was studied in the range of 5–20% v/v. Further increase in the methanol concentration prolonged the migration time and broadened the peaks. Therefore, 20% methanol was chosen for the enantiomer separation as it gave the best resolution efficiency.

3.2.4. Effect of temperature and applied voltage. Electrophoretic separation was performed between 15 and 30 °C. The resolution was slightly decreased upon increasing the temperature above 15 °C. Consequently all electrophoretic measurements were carried out at 15 °C.

The electrophoretic separation was directly proportional to the applied voltage. Analysis at different voltages (15, 20 and 25 kV) was carried out to determine the optimum voltage for separation. Voltages higher than 25 kV were not tested due to the expected Joule heat generation inside the capillary which adversely affect the peak shapes and deteriorate the inner capillary wall. Lower voltages (5 and 10 kV) were found to produce bad resolutions as a result of band broadening. The best resolution was obtained at 15 kV. According to the optimization process, the following conditions were recommended for the CE analysis; 10 mM HP-β-CD was used as a chiral selector, 100 mM phosphate buffer at pH 7.0 as BGE, 15 kV applied voltage and the temperature was controlled at 15 °C. Under these conditions a good separation of PER erbumine enantiomers was achieved and the migration order was *R*-PER followed by *S*-PER (Fig. 3).

3.3. System suitability

U.S Pharmacopoeia (USP) states that system suitability tests are an integral part of liquid chromatographic methods.[18] System suitability was used to verify the efficiency (N), selectivity factor (α), capacity factor (K'), and resolution (R) of the chromatographic system. These parameters were calculated for HPLC and are presented in Table 1.

3.4. Validation of the proposed methods

The suggested analytical procedure was subjected to validation procedures according to ICH guideline.[19,20] The following validation parameters were examined; precision, robustness, accuracy, specificity, LOD and LOQ (Fig. 4).

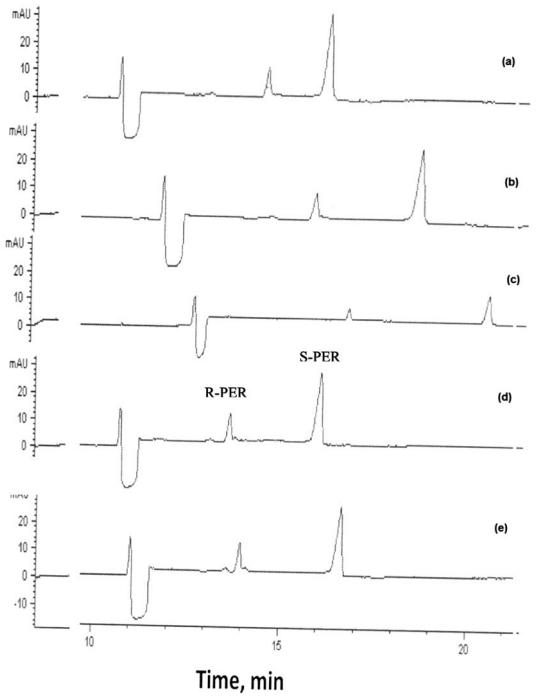

Fig. 3 The effect of HP-β-CD concentration on the electrophoretic separation of racemic PER (350 μg mL^{-1}) at (a) 1 (b) 3 (c) 5 (d) 7 and (e) 10 mM; under the following conditions: BGE consists of phosphate buffer (100 mM, pH 7), voltage 15 kV, temperature 20 °C and injection pressure 50 mbar for 10 s.

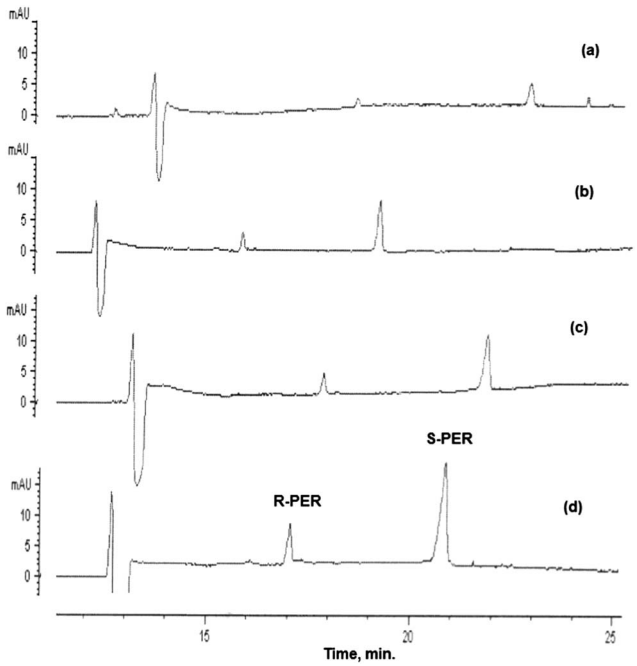

Fig. 4 Typical electropherograms show the effect of methanol concentration on the separation of racemic PER (350 μg mL^{-1}) at (a) 5 (b) 10 (c) 15 and (d) 20%; under the following conditions: BGE consists of HP-β-CD (5 mM) in phosphate buffer (100 mM, pH 7), voltage 15 kV, temperature 20 °C and injection pressure 50 mbar for 10 s.

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

Parameter for *S*-PER	HPLC	CE
Linear range (μg mL^{-1})	5–150	25–800
a (intercept)	20.94	0.127
S_a (standard deviation of intercept)	2.79	0.262
b (slope)	4.01	0.059
S_b (standard deviation of slope)	0.038	0.0006
r (correlation coefficient)	0.9996	0.9997
r^2 (determination coefficient)	0.9991	0.9994
SD of residuals (Sy.x)	5.51	0.462
LOD (limit of detection μg mL^{-1})	2.29	14.65
LOQ (limit of quantitation μg mL^{-1})	6.95	44.40

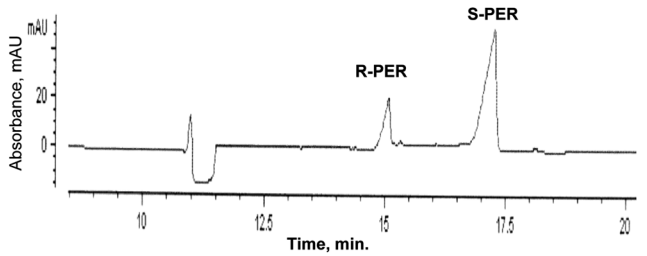

Fig. 5 Typical CE electropherograms of racemic PER (100 μg mL^{-1}) under the following conditions: BGE consists of 5 mM HP-β-CD in phosphate buffer (100 mM, pH 7), voltage 15 kV, and injection pressure 50 mbar for 10 s.

Linearity, detection and quantitation limits. Calibration plots were constructed using the optimal conditions. The characteristic parameters and necessary statistical data of the regression equation, limit of quantization (LOQ) and limit of detection (LOD) are summarized in Table 2. These limits were calculated mathematically using following formula; LOD or LOQ = $n\sigma/S$, where σ is the standard deviation of intercept, S is the slope of the calibration graph and n is a numerical value (3.3 for LOD and 10 for LOQ). The obtained LOD values for *S*-PER were 2.3 and 14.7 μg mL^{-1}, while the values of LOQ were 5.0 and 25.0 μg mL^{-1} for HPLC and CE methods respectively (Table 2). It is clear that the sensitivity of the HPLC method is five times higher than the CE method (Fig. 5).

Precision. The intermediate precision and repeatability of the HPLC and CE methods was investigated at the inter- and intra-day precision level respectively. The calculated relative standard deviation values were all below 2% indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis are summarized in Table 3.

Table 4 Application of standard addition technique in the determination of *S*-PER in Coversyl® tablet using the proposed HPLC and CE methods

Method	Taken μg mL^{-1}	% Found[a] ± SD	Pure added μg mL^{-1}	% Recovery[b]	Mean ± SD
HPLC	40	98.65 ± 1.19	10	98.52	99.46 ± 0.84
			20	99.61	
			30	99.19	
			40	100.52	
CE	400	98.93 ± 1.02	100	99.25	99.75 ± 0.56
			200	100.30	
			300	99.30	
			400	100.18	

[a] Average of five determinations. [b] Average of three determinations.

Accuracy. Standard addition technique was applied to examine the accuracy of both methods. Different amounts of *S*-PER were added to a previously analyzed tablet solution. The mean % recoveries were 99.46 and 99.75 for HPLC and CE methods, respectively (Table 4). This result showed that there was no interference from the common encountered recipients which are normally present in tablets.

3.5. Analysis of commercial tablets

The proposed methods were applied for determination of PER erbumine enantiomers in commercial COVERAM® tablets. Five replicates determinations were made and the mean % recovery and standard deviation were calculated. The accuracy of the methods was evaluated by applying the standard addition technique. Four different concentrations of the pure drug were added to the previously analyzed sample and the recovery was calculated for each concentration. The results were in good agreement with the labeled claim as shown in Table 4. For CE quantification, the peak areas (AUC) were divided by the corresponding migration time (t) to give the corrected area in order to compensate for the shift in migration time from run to run. This treatment reduced the variation of the responses. The percentage of the *R* enantiomer in PER

Table 3 Evaluation of the inter- and intra-day precision for the determination of *S*-PER using the proposed chiral HPLC and CE methods

		Inter-day precision			Intra-day precision		
Method	Taken μg mL^{-1}	Found	% Recovery[a]	RSD	Found	% Recovery[a]	RSD
HPLC	40	39.51	98.77	0.62	39.54	98.85	1.29
	60	59.43	99.05	0.86	50.81	101.62	0.75
	100	100.42	100.42	1.22	60.48	100.8	0.81
CE	50	49.92	99.84	1.40	49.785	99.57	0.51
	100	101.74	101.74	1.41	99.76	99.76	0.51
	200	200.96	100.49	0.37	196.58	98.29	0.84

[a] The value is the mean of three determinations.

erbumine was calculated by internal normalization from corrected peak areas (peak area/migration time) at a concentration level of 400 μg mL^{-1}, and applying eqn (1).[21] According to this equation, the percentage of PER *R*-enantiomer was calculated. It was found to be 27.24% per tablet.

$$\%R\text{-PER} = \left(\frac{\text{AUC}_R/\text{mig. time}_R}{\text{AUC}_S/\text{mig. time}_S}\right) \times 100 \quad (1)$$

3.6. Comparison between HPLC and CE chiral methods

The time required for CE method development was shorter because it did not require extensive equilibration times compared with the chiral HPLC method. It was possible to test several buffer systems in CE within one hour. Another major advantage in CE is the low consumption of solvents and buffer additives. About 20 determinations could be carried out using only 10 mL of buffer. In addition, CE used chiral selectors which were relatively less expensive than the HPLC columns having chiral stationary phases.

One of the principal limitations of CE was the sensitivity. The calculated LOQ for the chiral CE and HPLC methods for *S*-PER, were 25.0 and LOQ 5.0 μg mL^{-1} respectively. The HPLC method was five times more sensitive than CE method. The migration times for the separated enantiomers using the CE method were about 14.7 and 17 min for *R*- and *S*-PER respectively. In the HPLC method, the retention times were 6.0 and 2.2 min for *R*- and *S*-PER respectively. Consequently, the analysis time was shorter for the HPLC method.

4. Conclusions

This work presented for the first time the chiral separation method for PER erbumine analysis based on capillary electrophoresis and HPLC. HP-β-cyclodextrin was used as a chiral selector in CE while β-cyclodextrin based chiral column was used in HPLC. The proposed methods were optimized and could be used for separation of the two enantiomers of PER erbumine in a reasonable time with good resolution. HPLC showed obvious advantages on CE in terms of shorter running time, higher sensitivity, and precise sample loading and fewer variables to be optimized. In contrast, CE was advantageous in its higher resolution and use of less expensive chiral selector and running buffer. Both techniques were applied successfully for the analysis of the *S*-PER content in commercial tablets containing PER erbumine racemate.

References

1 A. C. Moffat, M. D. Osselton and B. Widdop, *Clarke's Analysis of Drugs and Poisons*, Pharmaceutical Press, London, UK 2004, electronic version.
2 W. H. De Camp, *J. Pharm. Biomed. Anal.*, 1993, **11**, 1167–1172.
3 J. Tomaszewski and M. M. Rumore, *Drug Dev. Ind. Pharm.*, 1994, **20**, 119–139.
4 M. Vincent and P. Shiavi, *Modelling, synthesis and pharmacological study of perindopril (S9490): an angiotensin I converting enzyme inhibitor*, VCH Publishers Inc/Lavoisier-Tech & Doc, Paris, France, 1991, vol. 5, pp. 98–100.
5 G. Gübitz and M. G. Schmid, *Biopharm. Drug Dispos.*, 2001, **22**, 291–336.
6 E. L. Izake, *J. Pharm. Sci.*, 2007, **96**, 1659–1676.
7 Y. Xiao, S.-C. Ng, T. T. Y. Tan and Y. Wang, *J. Chromatogr. A*, 2012.
8 J. Z. Wang, S. Zeng, G. Y. Hu and D. H. Wang, *J. Chromatogr. A*, 2009, **1216**, 3044–3047.
9 S. Sun, Y. Jia, N. Zeng and F. Li, *Chromatographia*, 2006, **63**, 331–335.
10 R. I. Stefan, J. K. van Staden and H. Y. Aboul-Enein, *Chirality*, 1999, **11**, 631–634.
11 J. F. van Staden, R. I. Stefan and H. Y. Aboul-Enein, *Fresenius. J. Anal. Chem.*, 2000, **367**, 178–180.
12 H. Y. Aboul-Enein, R. I. Stefan and G. L. Radu, *Prep. Biochem. Biotechnol.*, 1999, **29**, 55–61.
13 K. I. Ozoemena, R.-I. Stefan, J. F. v. Staden and H. Y. Aboul-Enein, *Sens. Actuators, B*, 2005, **105**, 425–429.
14 K. I. Ozoemena, R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Instrum. Sci. Technol.*, 2004, **32**, 371–378.
15 K. I. Ozoemena, R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Talanta*, 2004, **62**, 681–685.
16 R.-I. Stefan, J. F. van Staden, L. V. Mulaudzi and H. Y. Aboul-Enein, *Anal. Chim. Acta*, 2002, **467**, 189–195.
17 R.-I. Stefan, J. K. F. van Staden and H. Y. Aboul-Enein, *Sens. Actuators, B*, 1999, **54**, 261–265.
18 *The United States Pharmacopoeia XXVIIII and NF XXV*, American Pharmaceutical Association, Washington, DC 2007, electronic version.
19 ICH Harmonized Tripartie Guideline Q2A: "Text on validation of analytical procedures", Incorporated in November 2005, http://www.ich.org.
20 ICH Harmonized Tripartie Guideline Q2B (R1): "Validation of Analytical Procedures: Text and Methodology", Incorporated in November 2005, http://www.ich.org.
21 C. E. Sanger-van de Griend and K. Groningsson, *J. Pharm. Biomed. Anal.*, 1996, **14**, 295–304.