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Comparison of the miniaturised techniques capillary electrochromatography and capillary liquid chromatography for the chiral separation of chlorthalidone

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Abstract

The aim of this study was to compare the miniaturised techniques, capillary electrochromatography (CEC) and capillary liquid chromatography (CLC), for the chiral separation of chlorthalidone. In both cases, hydroxypropyl-β-cyclodextrin was used as a chiral selector in the mobile phase, while an achiral stationary phase was used. Earlier, this separation was already optimised in CEC. Now, the separation was optimised in CLC. The influence of the organic modifier content and the cyclodextrin concentration on the separation was studied by means of a central composite design. Optimal separation conditions were determined, after response modelling, from the response surface contour plots. When these conditions were compared with those of the CEC optimisation, we can see the potential of using CLC as a chiral separation technique since less chiral selector was used, faster separations were obtained and better repeatability was observed in comparison with its electrical-driven counterpart.

Keywords: Capillary electrochromatography; Capillary liquid chromatography; Chiral separation; Chlorthalidone; Hydroxypropyl-β-cyclodextrin

1. Introduction

High-performance liquid chromatography (HPLC) is a well-established separation technique widely used in different fields. Various applications in bio-analysis and pharmaceutical analysis have been reported [1–6]. Among the applications in pharmaceutical analysis, enantiomeric separations with HPLC have been studied thoroughly. Two approaches of direct chiral separations can be applied in HPLC, being the use of chiral stationary phases (CSPs) [7–13] or adding the selector to the mobile phase and using an achiral stationary phase [14–17]. However, the latter is nowadays less popular because of the high selector consumption. Here, miniaturised techniques may be the alternatives, as they will consume less sample and mobile phase

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during analysis. They include capillary electrophoresis (CE), capillary electrochromatography (CEC) and capillary liquid chromatography (CLC).

In CE, separations are obtained due to differences in charges and ionic radius. Since no stationary phase is used in the capillary, chiral selectors are added to the background electrolyte or running buffer, in order to separate enantiomers [18,19].

CEC combines both electromigration and chromatography: it uses capillary columns filled with stationary phase, but a voltage is applied, as in CE, to perform separations. Chiral separations can be performed with the two above-mentioned approaches [20]. Highly efficient separations can be obtained with these two electromigration techniques, but they are also characterised by some disadvantages, such as lack of reproducibility and the need of internal standards to make quantitative analysis possible.

CLC can be considered as the miniaturised version of HPLC. Instead of conventional columns, capillary ones with internal diameters between 100 μm and 1 mm are used. These capillary columns are commercially available and are known to give robust results, which is an advantage of the technique over CEC. CLC possesses a better sensitivity than conventional wide-bore HPLC [21,22]. Chiral separations can be obtained with the two above-mentioned approaches, but less mobile phase will be consumed [23–27].

Earlier, the chiral separation of chlorthalidone enantiomers in CEC with hydroxypropyl-β-cyclodextrin (HP-β-CD) as chiral mobile phase additive using an achiral stationary phase was described [28] and optimised [29]. The most influential factors, the concentrations of chiral selector and acetonitrile in the mobile phase, were varied according to an experimental design and the separation (resolution and analysis time) was modelled and optimised [29]. However, CEC has some disadvantages as separation technique. As already mentioned, users are practically obliged to pack their own columns to make the technique cost-effective. The fragility of these self-made columns affects merely the robustness of the used method, due to frequent breakage of the column. It was also seen that in CEC long “conditioning” times might be required. Besides these disadvantages, CEC has also advantages such as low sample and solvent consumption and high efficiencies [20]. Low sample and solvent consumption are perhaps the most interesting advantages, as higher efficiencies are not always sought in separations and were also not exhibited in Ref. [29]. CLC also possesses these advantages and might overcome the disadvantages exhibited by CEC when performing chiral separations on achiral stationary phases with selectors as mobile phase additives.

It was the aim to study the separation optimised with CEC in Ref. [29] by means of CLC, which allows comparing both techniques. The comparison of the results will focus on the ability to separate and on the conditions needed to obtain a baseline resolution. The procedure applied in CEC will be followed, i.e. when a separation is found from preliminary experiments, a central composite design [30] is executed to study the influence of the concentrations of organic modifier and chiral selector on the separation. Models for resolution and analysis time will be built and the conditions for a baseline separation will be predicted.

2. Experimental

2.1. Capillary electrochromatography

CEC experiments were performed on a Beckman P/ACE MDQ instrument (Fullerton, CA, USA) under conditions as indicated in Ref. [29]. The CEC column was a 100 μm ID fused-silica capillary (Composite Metal Services Ltd., Ilkley, UK), packed with Hypersil ODS 120–5 μm stationary phase. A phosphate buffer (pH 6.5; 1 mM) was used as background electrolyte. This solution was mixed with a given amount of acetonitrile (ACN). The required concentration of HP-β-CD was dissolved in this mobile phase. Injection of chlorthalidone was done at 10 kV for 45 s. Analyses were performed at 25 kV with 4.8 bar (70 psi) pressure on both vials during analysis. The temperature was kept at 20 °C, except when temperature effects were studied.

The indicated chiral selector concentration in these experiments represents the concentration in the total mobile phase (i.e. aqueous and organic phase), while further in the CLC experiments the indicated concentration is the one in the aqueous solution, before on-line mixing with ACN. More details related to the experimental section can be found in [29].

2.2. Capillary liquid chromatography

The CLC experiments were performed on an Agilent 1100 Series Capillary LC System (Agilent Technologies, Palo Alto, CA). The system includes a micro-vacuum degasser, a thermostated (10 °C) micro-autosampler, a thermostatted column compartment, a capillary pump and a diode-array capillary detector with a 500 nl flow cell. Instrument control was done by the ChemStation software (2000–2001 Agilent Technologies).

A Zorbax 300SB-C18 capillary column (Agilent) with an internal diameter of 300 μm and a length of 15 cm packed with 5 μm C18 particles was used.

Chiral separation of the enantiomers was performed isocratically with a flow rate of 5 μl/min. Preliminary and experimental design experiments were performed at 20 °C. Experiments at optimal conditions were done at 10, 15, 20 and 30 °C to study the influence of the temperature on the separation and the racemisation of the enantiomers.

The injection volume was 0.1 μl. For the preliminary experiments, a concentration of 1 mg/ml chlorthalidone dissolved in acetonitrile was used. The experimental design experiments used a 0.5 mg/ml concentration and the optimum experiments were performed with 0.25 mg/ml. These different dilutions were applied since sample overloading was observed in some cases during optimisation. UV absorbance was measured at 220 nm.

2.3. Chemicals and reagents

Capillary electrochromatography: For the chemicals used we refer to Ref. [29].

Capillary liquid chromatography: A stock solution of phosphate buffer (pH 6.5) was prepared by dissolving 1 mM disodium hydrogenphosphate (Na_2HPO_4) in water and adjusting the pH by means of diluted orthophosphoric acid 85% (v/v), both from Merck. The required cyclodextrin concentration was dissolved in the phosphate buffer, mixed on-line with the required amount of ACN (HPLC grade, Scharlau, Barcelona, Spain) and degassed.

2.4. Experimental design

A central composite design [30] for two factors, i.e. the chiral selector concentration and the amount of ACN in the mobile phase, was performed. It requires nine experiments, which were started and ended with the centre point. Four injections were performed at the conditions of each design experiment. All data (i.e. analysis times or resolutions of the four injections) were used to model both responses. Experimental design creation and modelling were done using the Nemrod software (LPRAI, Marseille, France).

2.5. Responses

The instrumental software mentioned above was used for data acquisition and treatment. When no separation occurred the retention time of the peak was used as analysis time. When separation of the enantiomers was seen, the retention time of the second eluting peak was taken. Resolution values between the two enantiomers were calculated according to the equation of the United States Pharmacopeia [31].

3. Results and discussion

3.1. Capillary electrochromatography

Optimal conditions were found after modelling resolution and analysis time from the results of a central composite design [29]. The concentration of ACN and HP-β-CD in the mobile phase were varied. Optimal mobile phase conditions were found with a phosphate buffer (pH 6.5; 1 mM), 33 mM HP-β-CD and 16% (v/v) ACN. These conditions gave a baseline resolution of the compound within 32 min.

While the separation was fast in comparison with results of [28], it seemed interesting to examine the same separation in CLC to compare the possibilities and the robustness of the technique.

In CLC, the flow is driven by pressure, instead of application of an electrical field as driving force in CEC. Attempting the chiral separation of chlorthalidone with CLC will reveal the difference between pressure-driven and electrical-driven separations.

No separation is expected in CE, as a neutral compound and a neutral chiral selector are used. Even if selector and analyte interact, neutral complexes will be formed that cannot be separated. Therefore, CE as miniaturised separation technique will not be considered for this particular separation. The presence of a

stationary phase in the capillary seems necessary to separate both enantiomers as separation was observed in CEC [28,29]. Apparently, the chromatographic partition between mobile and stationary phase greatly contributes to the separation. It can therefore be stated that the CEC separation of chlorthalidone mainly follows the chromatographic principle and less the electrophoretic one. However, this statement needs to be confirmed.

3.2. Capillary liquid chromatography

Preliminary experiments were performed to define an experimental domain (Table 1). Based on these results, the domain was chosen between 5 and 35 mM HP-β-CD and between 0 and 25% (v/v) ACN since a full or partial separation occurred in this domain. Temperature was not included in the design as a factor because it is known that chlorthalidone enantiomerises. Its influence was investigated separately afterwards.

When comparing the CLC domain with the CEC one tested, it was seen that the same HP-β-CD concentrations are used. Regarding the organic modifier content, a larger area will be investigated, since the preliminary experiments showed that less ACN induced a better separation. With CLC, it seems possible to elute the compound within a reasonable time at lower organic modifier contents, whereas in CEC this was not possible: a large increase in retention times was observed.

3.2.1. Experimental design

The experimental conditions and the results of four successive injections are given in Table 2. Very good repeatability was observed for the replicated injections of all design experiments. When the first and the last design experiment were compared, i.e. replicates at the centre point conditions, it was also seen that the time-different intermediate precision is good.

At conditions with the highest HP-β-CD concentrations, the viscosity of the mobile phase was increased, which can result in a flow and pressure perturbation. These instrumental problems are better to be avoided in routine use but were taken into account to model resolution and analysis time. Second-order polynomial models were built from the results of the experimental design. Contour plots of the models can be seen in Fig. 1a for resolution and Fig. 1b for analysis time.

Analysis of the residuals and analysis of the variance were used to evaluate the model validity. No abnormal observations were made, indicating that an acceptable model was obtained. The coefficients of

Table 1
Results of the preliminary experiments

[HP-β-CD]	ACN fraction, % (v/v)	t_{R1} (min)	t_{R2} (min)	Resolution	Separation
10 mM	0	10.85	12.69	2.28	Baseline
	5	10.54	12.22	2.00	Baseline
	10	12.19	13.31	1.04	Incomplete
	15	10.18	10.62	0.37	Beginning
	25	4.11	4.37	0.54	Beginning
20 mM	0	6.39	7.60	2.25	Baseline
	5	8.94	10.49	1.75	Baseline
	10	10.48	11.60	1.33	Incomplete
	15	8.92	9.39/9.97[a]	0.53/0.60[a]	Beginning
	25	4.04	4.32	0.63	Beginning
	35	3.17	3.29	0.38	Beginning
30 mM	0	7.13	8.19	1.88	Baseline
	5	6.85	7.85	1.82	Baseline
	10	7.75	8.67	1.38	Incomplete
	15	7.70	8.20/8.85[a]	0.60/0.72[a]	Beginning

Conditions: Zorbax 300SB-C18 column, 0.3 mm × 150 mm; mobile phase: phosphate buffer (pH 6.5; 1 mM) with indicated concentration of selector, mixed on-line with indicated fraction ACN; flow 5 μl/min; chlorthalidone 1 mg/ml in ACN; detection at 220 nm. Separation: "beginning" indicates $R_s < 1$, "incomplete" $1 < R_s < 1.5$, and "baseline" $R_s > 1.5$.

[a] Three peaks observed.

Table 2
Experimental design conditions and the results of four successive injections, sample conc. 0.5 mg/ml, other conditions as in Table 1

Factor levels		Experimental conditions		Responses		
HP-β-CD (mM)	%ACN	HP-β-CD (mM)	%ACN	t_{R1} (min)	t_{R2} (min)	Resolution
0	0	20	12.5	10.17	11.08	0.76
				10.08	11.00	0.79
				10.03	10.93	0.76
				9.97	10.87	0.77
−1	−1	9.4	3.7	13.19	15.25	1.95
				12.75	14.77	2.01
				12.44	14.44	2.05
				12.20	14.16	2.01
−1	1	9.4	21.3	5.67	6.04	0.58
				5.41	5.75	0.56
				5.34	5.69	0.58
				5.31	5.65	0.58
1	−1	30.6	3.7	6.19	7.23	1.98
				6.13	7.15	2.00
				6.01	7.01	1.97
				5.80	6.78	1.88
1	1	30.6	21.3	5.26	5.26	0.00
				5.13	5.13	0.00
				5.10	5.10	0.00
				5.08	5.08	0.00
−1.414	0	5	12.5	18.65	19.25	0.48
				17.78	18.31	0.41
				17.53	18.05	0.41
				17.38	17.86	0.37
1.414	0	35	12.5	7.71	8.58	1.07
				7.64	8.50	1.11
				7.55	8.39	1.08
				7.49	8.30	1.02
0	−1.414	20	0	6.73	8.10	2.39
				6.63	8.00	2.40
				6.41	7.74	2.30
				6.46	7.79	2.35
0	1.414	20	25	4.57	4.84	0.50
				4.19	4.39	0.47
				4.13	4.38	0.53
				4.11	4.33	0.51
0	0	20	12.5	10.17	11.12	0.78
				10.09	11.04	0.82
				10.07	11.00	0.82
				10.07	11.02	0.75

multiple determination (R^2) of resolution and analysis time were 0.932 and 0.918, respectively, also an indication for a good model. Further, predicted and measured results were in good agreement as will be discussed later.

In the resolution plot, the highest resolutions are obtained at the lowest ACN concentrations in the mobile phase. The domain with predicted baseline resolution is highlighted in Fig. 1a. In this domain, resolutions at a given ACN content

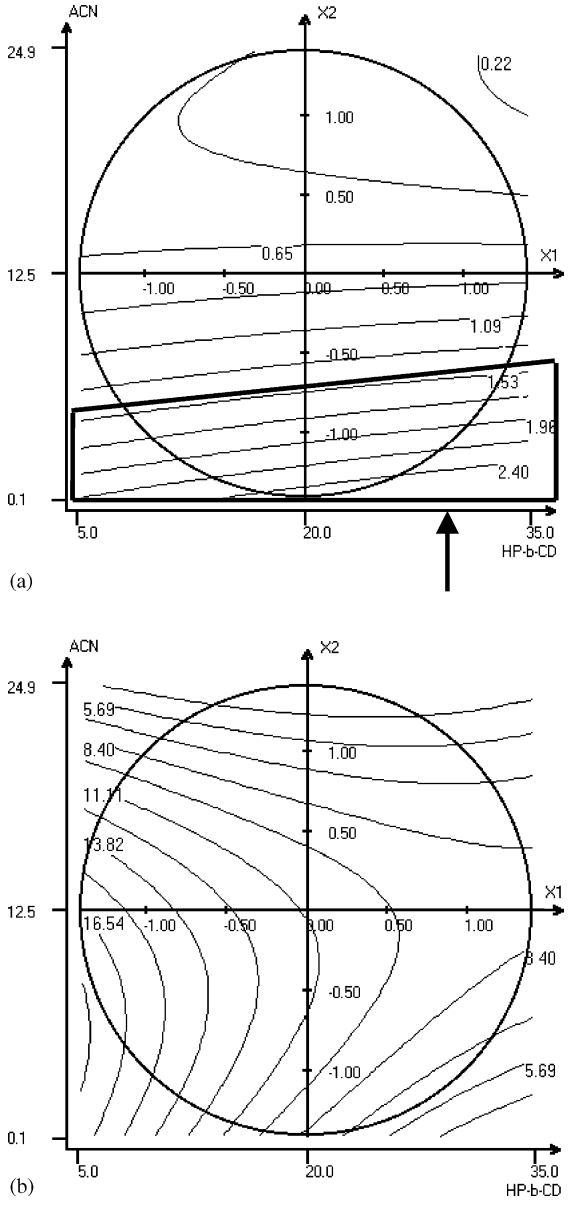

Fig. 1. Contour plots of the models describing (a) resolution and (b) retention time as a function of the HP-β-CD concentration and the ACN content. The area in (a) delimited by the square indicates the domain with predicted baseline resolution.

remain relatively constant as a function of selector concentration.

In the previous CEC experiments [29], 20 min analysis time was taken as maximum when selecting the optimum. For the CLC experiments, retention times are below 20 min in the whole experimental domain and therefore analysis time was not further taken into account to define an optimum.

3.2.2. Optimum

Only resolution is to be considered and the optimum can therefore be determined from the resolution contour plot. Highest resolutions are achieved at the lowest concentrations of ACN. Therefore this factor was chosen equal to 0% (v/v) for the optimum, i.e. a mobile phase without organic modifier will be used.

Instrumental problems were observed at selector concentrations above 30 mM. For this reason, the optimum will be chosen left of the arrow in Fig. 1a. Since baseline resolutions are achieved at all these CD concentrations, the retention times plot was consulted briefly anyway. Retention times are relatively high at lower CD concentrations (above 15 min below 10 mM) Therefore, optimal separation conditions are considered to be situated at CD concentrations above 10 mM.

Three concentrations were tested: 10, 15 and 20 mM. The results of six injections at three different temperatures can be seen in Table 3. They all gave resolutions above 2.00, which is a satisfying result. All measured resolutions were in accordance with the values predicted by the model, which was a confirmation of its applicability. For analysis time, a somewhat less good prediction was observed in comparison with the resolutions. The lowest retention times were obtained at 20 mM HP-β-CD, which was to be expected from the contour plots. At 10 mM, analysis time was not high either, all analyses were finished within 12.5 min. However, the selector consumption is lower, thus this concentration was chosen.

The mobile phase selected to baseline separate chlorthalidone enantiomers by means of capillary liquid chromatography is thus 10 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM).

3.2.3. Temperature effects

Table 3 also shows the results of injections performed at different temperatures. They were made to evaluate the effect of temperature on the enantiomerisation of the compound, since it is known that chlorthalidone enantiomerises [32]. Such enantiomerisation, which is the transition of one enantiomer into the other during analysis, can be seen as a

Table 3
Average values of injections done with 10, 15 and 20 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM), sample conc. 0.25 mg/ml, other conditions as in Table 1

[HP-β-CD] (mM)	Temperature (°C)	t_{R1} ± %RSD (min)	t_{R2} ± %RSD (min)	R_s ± %RSD	Area 1 ± %RSD ($\times 10^4$ mAU s)	Area 2 ± %RSD ($\times 10^4$ mAU s)
10	10	9.48 ± 1.80 ($n = 8$)	12.30 ± 1.81 ($n = 8$)	2.47 ± 1.87 ($n = 8$)	1.726 ± 2.67 ($n = 8$)	1.595 ± 3.08 ($n = 8$)
	15	9.10 ± 0.95 ($n = 8$)	11.46 ± 0.80 ($n = 8$)	2.36 ± 1.71 ($n = 8$)	1.672 ± 2.46 ($n = 8$)	1.549 ± 2.89 ($n = 8$)
	20	9.04 ± 0.76	11.09 ± 0.79	2.26 ± 0.24	1.650 ± 1.05	1.469 ± 1.66
15	10	7.58 ± 5.08	9.74 ± 5.05	2.64 ± 1.05	1.367 ± 4.05	1.254 ± 4.22
	15	7.18 ± 0.76	8.97 ± 0.70	2.48 ± 1.18	1.377 ± 4.82	1.276 ± 4.26
	20	7.08 ± 0.60	8.64 ± 0.58	2.27 ± 0.72	1.845 ± 0.78	1.624 ± 1.92
20	10	6.28 ± 2.92	7.90 ± 2.88	2.58 ± 1.98	1.274 ± 1.66	1.165 ± 1.38
	15	5.96 ± 1.58	7.32 ± 1.39	2.41 ± 3.21	1.198 ± 4.49	1.103 ± 6.23
	20	6.25 ± 1.01	7.53 ± 1.04	2.47 ± 1.61	1.208 ± 1.90	1.041 ± 1.90
	30	6.25 ± 1.04	7.26 ± 1.11	2.01 ± 2.34	1.395 ± 2.02	1.170 ± 2.96

Number of injections $n = 6$, unless otherwise indicated.

typical plateau between the two separated peaks. This was confirmed in previous analyses at 20 °C since a plateau was observed (see Fig. 2). Enantiomerisation can largely affect the repeatability of the method, but in this case it did not seem a problem (see also next section). However, quantitative analysis will be incorrect, since it is doubtful that the peak area due to an enantiomer can be correctly integrated.

The effect of temperature for the mobile phase with 20 mM HP-β-CD is displayed in Fig. 2. A higher temperature increases the enantiomerisation. At 10 °C, no enantiomerisation is seen anymore, since a complete return to the baseline was observed. Therefore, the lowest temperature seems most suitable from a quantitative point of view.

3.2.4. Repeatability of injections

At the chosen optimum (i.e. 10 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM)), RSD values of 0.79% for analysis time, 0.24% for resolution and 1.05 and 1.66% for the first and second peak area, respectively, were calculated from six injections at 20 °C. These values are quite low, indicating that the injections are very repeatable. Table 4 compares the RSD for both CEC and CLC at the optimal separation conditions.

In CEC, the enantiomerisation rate was not constant and therefore affected the repeatability of the injections strongly. At the conditions it occurred, it was impossible to obtain variations below 5% between successive injections.

Quantitative analysis for chlorthalidone in CLC will however not be preferred at 20 °C, due to the occurring enantiomerisation. Though peak areas give sufficient repeatability, it is impossible to determine the unbiased peak area by integration.

The RSD values for the results at 10 and 15 °C increased with decreasing temperature. For example, for analysis time RSD was 1.81% at 10 °C, in comparison

Table 4
Results of six injections done at optimal conditions with CEC and CLC

	Analysis time (min)		Resolution	
	CEC	CLC	CEC	CLC
	29.2	11.24	1.81	2.25
	28.7	11.13	1.77	2.26
	29.0	11.10	1.76	2.25
	29.4	11.05	1.79	2.26
	29.7	10.98	1.80	2.25
	29.8	11.06	1.76	2.26
%RSD	1.46	0.79	1.20	0.24

CEC conditions: 100 μm capillary column, 20 cm packed with Hypersil ODS, 31.2 cm total length; mobile phase: 33 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM)-acetonitrile (84:16, v/v); injection 10 kV 45 s; analysed at 25 kV. CLC conditions: 10 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM), other conditions as in Table 1.

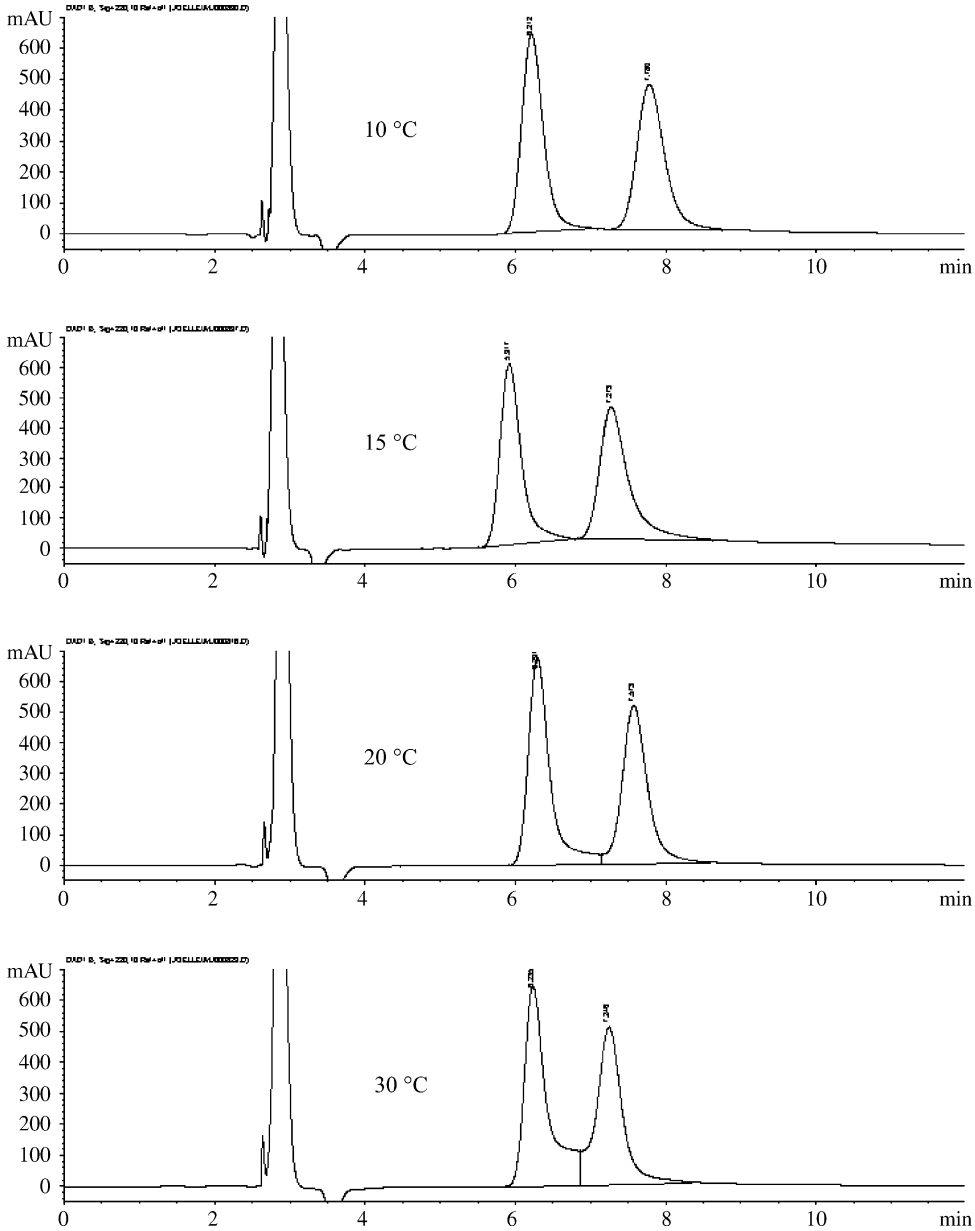

Fig. 2. CLC chromatograms of the separation of chlorthalidone enantiomers with at four different temperatures. Conditions: 20 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM), Zorbax 300SB-C18 capillary column, flow 5 μl/min, chlorthalidone 0.25 mg/ml, detection at 220 nm. The first peak is the ACN solvent peak.

with 0.79% at 20 °C, indicating a lower repeatability at lower temperatures. This can be explained by the long column conditioning that is needed at lower temperatures and the practical limitations of the instrument at lower temperatures. Calculating the RSD of the peak areas at 10 °C gives values of 2.67 and 3.08% for the first and second peak, which is considerably higher than at 20 °C (see above). Thus, to

assay chlorthalidone, at higher temperatures precision is better but biased peak areas can be expected, while at lower temperatures the enantiomerisation is reduced but precision is less good.

3.3. Comparison between the two techniques

It can be concluded that in CEC, for this particular separation, the chromatographic retention will

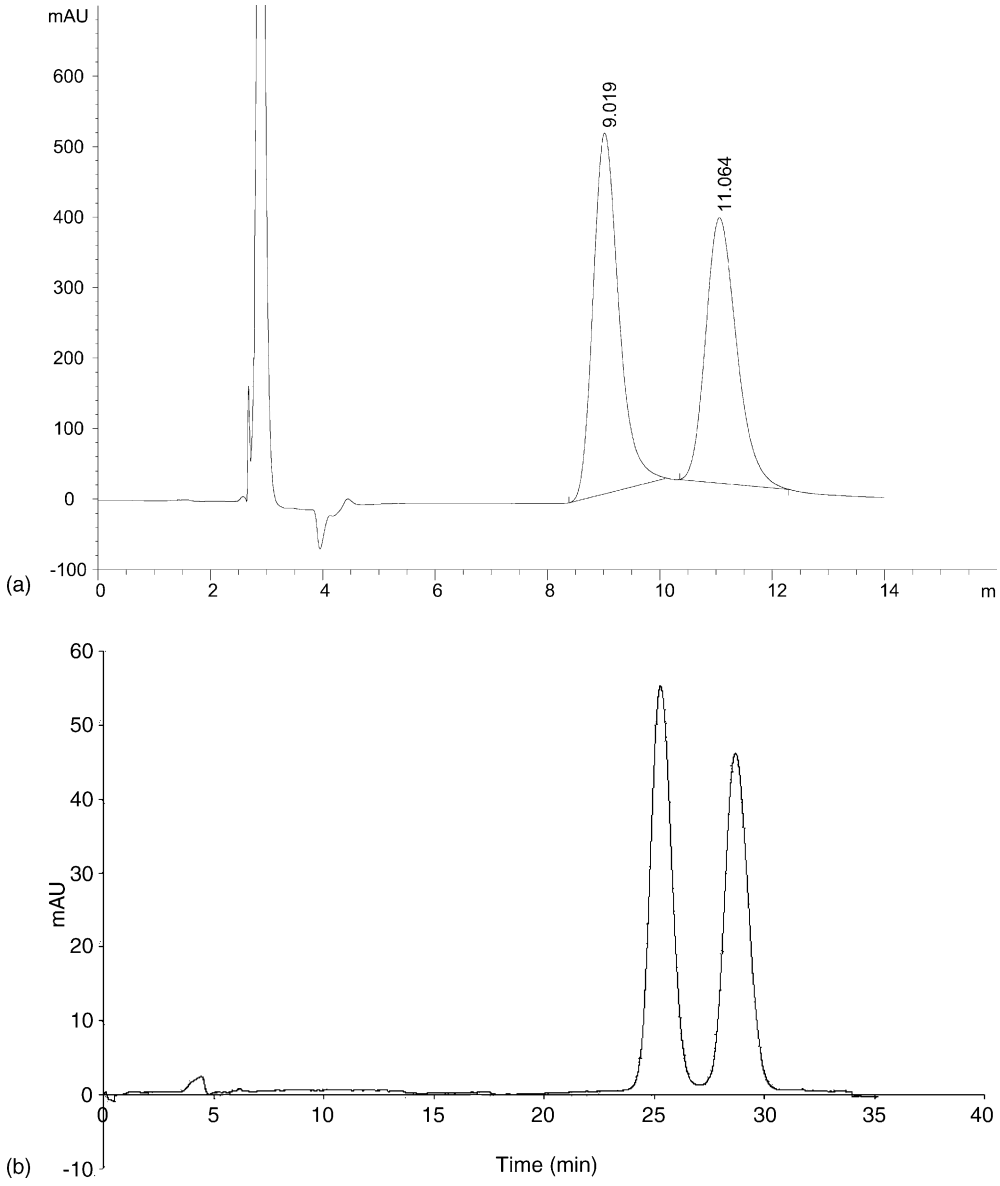

Fig. 3. Separation of chlorthalidone at optimal conditions with (a) CLC and (b) CEC. Conditions CLC: 10 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM), other conditions as in Fig. 2. Conditions CEC: capillary: 100 μm diameter; 31.2 cm total length; 20 cm packed with Hypersil ODS; mobile phase: 33 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM)-acetonitrile (84:16, v/v); injection 10 kV 45 s; analysed at 25 kV.

dominate as a separation mechanism. The presence of the achiral stationary phase is necessary to separate the complexes between selector and enantiomers. This points out the potential of both CEC and CLC when separating uncharged chiral substances with neutral selectors.

When CEC and CLC experiments are compared, a higher resolution in CLC at the same experimental conditions can usually be observed.

In CEC, at centre point conditions, results on two different new columns were repeatable, but the runs on an older column always took longer than on a new one. Also, considerable time was needed to equilibrate the system before obtaining repeatable injections. All these minor disadvantages of CEC resulted eventually in some time loss during analysis compared with CLC, since the latter technique gave highly repeatable injections from the start. The area of acceptable conditions during method optimisation was far more restricted in CEC than in CLC. Another disadvantage of CEC was the frequent breaking of columns requiring the preparation of a new one.

When the CLC optimum is compared with the one of CEC (33 mM HP-β-CD in phosphate buffer (pH 6.5; 1 mM)), a large reduction in the chiral selector consumption is possible. Only one third of the amount needed in CEC is used. The resulting analysis time is about three times shorter in CLC (about 12 min versus 32 min), while the resolutions are higher. A chromatogram in CLC and an electrochromatogram in CEC at the optima are displayed in Fig. 3.

Repeatability of retention times and resolutions at 20 °C were better for CLC than for CEC. At lower temperatures, they are comparable. The peak area RSDs indicated repeatable injections with CLC and the potential of using the technique for quantitative analysis.

In CEC, temperature studies showed that, in general, enantiomerisation was not present. The absence of the enantiomerisation in most experiments was the only advantage of CEC over CLC.

However, when one wants to add a chiral selector to the mobile phase, it can be recommended to use CLC rather than CEC since faster, more economic and more repeatable analyses are possible. All the above indicates that the pressure-driven separation offers great advantage to the electrical-driven one when following this direct chiral separation approach.

4. Conclusion

Two miniaturised techniques, CEC and CLC, were compared for the chiral separation of racemic chlorthalidone. This separation was earlier already optimised by means of CEC. It was now investigated using CLC, when a neutral chiral selector, HP-β-CD, was added to the mobile phase. A separation of the compound occurred using this chiral selector and an achiral stationary phase. The influence of the selector concentration and the organic modifier content in the mobile phase was studied by means of a central composite design. Contour plots showed a large area of conditions that gave baseline resolution between the enantiomers. The finally selected optimum conditions consisted of a mobile phase with 10 mM HP-β-CD in a phosphate buffer (pH 6.5; 1 mM) and a low analysis temperature. These conditions gave a separation with a resolution value of 2.5 in 12 min. Enantiomerisation of chlorthalidone was seen. It did not affect repeatability of the results, but for quantitative purposes, it is better to be avoided. Enantiomerisation is inhibited at the lowest temperature possible, here 10 °C.

The CLC and CEC results were compared and it could be concluded that the CLC approach is more advantageous for chiral separations when adding a chiral selector to the mobile phase and using an achiral stationary phase. The comparison of the two techniques also revealed better repeatability, economisation of the chiral selector, and more efficient and faster separations for CLC.

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