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Development of a validated capillary electrophoresis method for enantiomeric purity testing of dexchlorpheniramine maleate

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Abstract

A capillary zone electrophoresis method has been developed for the detection of 0.1% of (*R*)-levochlorpheniramine maleate in samples of (*S*)-dexchlorpheniramine maleate. Using 1.5 m*M* carboxymethyl-β-cyclodextrin in an acidic background electrolyte, resolution values of more than 10 were obtained. Under these conditions the *R*-enantiomer is migrating in front of the bulk *S*-enantiomer. The assay was validated for linearity (2–10 μg/ml; R^2=0.9992), selectivity [(*RS*)-pheniramine maleate and (*RS*)-brompheniramine maleate], limit of detection (0.25 μg/ml), limit of quantification (0.75 μg/ml), analytical precision (intra- and inter-day variability), repeatability of the method (RSD=5.0%) and accuracy. In samples of dexchlorpheniramine maleate from two different manufacturers, concentrations of, respectively, 0.15% and 1.95% (m/m) of levochlorpheniramine maleate were detected. The method was compared to the HPLC method described in the European Pharmacopoeia III monograph.

Keywords: Enantiomer separation; Pharmaceutical analysis; Chlorpheniramine maleate; Dexchlorpheniramine maleate

1. Introduction

Many pharmaceutical compounds possess a chiral centre that is responsible for the optical activity of the drug. The pharmacological activity of such compounds is mostly restricted to one of the enantiomers [1]. The use of single-enantiomer forms can often lead to an improvement in the efficacy of the drug or the suppression of side-effects related to the other enantiomer [2]. According to the ICH guidelines [3], with regard to chiral drug substances that are developed as single enantiomers, the control of the antipode enantiomer should be considered in the same manner as for other impurities. For the control of chiral drug purity, analytical methods are required with high-resolution power and high efficiencies [4]. Chromatographic techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas chromatography (GC) are frequently used for chiral separations [1]. During the last decade, capillary electrophoresis (CE) has proven to be an attractive alternative because it offers good selectivity and high separation efficiency in combination with short analysis times, low operational costs and fast method development [5,6].

(*RS*)-Chlorpheniramine maleate (RS-CPM) (Fig. 1) is a highly potent and widely used antihistaminic

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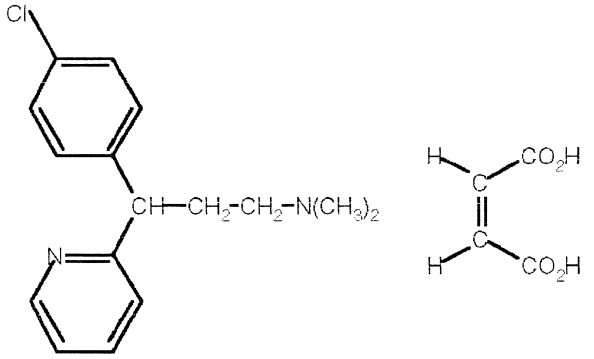

Fig. 1. Structure of (*RS*)-chlorpheniramine maleate [8].

drug. The activity is predominantly attributed to the dextrorotary *S*-enantiomer [7]. The European Pharmacopoeia III describes an HPLC method for the determination of the enantiomeric purity of dexchlorpheniramine maleate (S-CPM) [8], allowing the presence of 2% (m/m) of *R*-enantiomer in the tested sample. This HPLC method has some drawbacks. First of all, a normal-phase HPLC procedure is used, implying a preliminary extraction of the water-soluble maleate. Secondly, a quite low resolution ($R_s \geq$ 1.5) between the two enantiomers is specified. Thirdly, the impurity is migrating after the principal peak of the *S*-enantiomer, which can lead to masking of the minor impurity, especially when the resolution between the two peaks is low.

A number of CE methods have been reported for the qualitative chiral analysis of RS-CPM. Wu et al. [7] developed a capillary zone electrophoresis (CZE) method for the quantitative chiral analysis of S-CPM in tablets, but no reports are available on the enantiomeric purity testing of S-CPM by CE. Various chiral selectors such as cyclodextrins (CDs), polysaccharides and proteins have been used for the chiral separation of RS-CPM. Many authors describe the enantioseparation of RS-CPM with neutral CDs [4,5,9–11]. Several groups [5,10] have also shown that carboxymethyl-β-cyclodextrin (CM-CD), an ionisable CD, is a very effective chiral selector for the CE enantioseparation of RS-CPM, with high resolution values at low CD concentrations. Stalcup and Gahm [12] used sulphated-β-CD to separate this chiral drug in reversed polarity mode. Jin and Li [13] also used sulphated-β-CD to investigate the enantiomeric resolution of some racemic antihistamines. They noted that, due to co-migration, (*RS*)-pheniramine maleate, RS-CPM and (*RS*)-brompheniramine maleate could not be separated in one run. Mixtures of neutral and anionic CDs have also been used [14,15]. Baseline separations can also be obtained using polysaccharides such as heparin [16–18], dextran sulphate [17] and maltooligosaccharides [19]. Several authors used proteins as chiral selectors for the enantiomeric separation of RS-CPM. Tanaka and Terabe [20] and Ishihama et al. [21] used ovomucoid and Schmid et al. [22] carried out the separation with iron-free human serum transferrin.

For HPLC, different chiral stationary phases (CSPs) have been developed and used for the optical resolution of RS-CPM. Sakurai et al. [23] have developed an ovomucoid-conjugated column for this purpose. Also ovoglycoprotein from chicken egg whites bound to aminopropyl-silica gels has been used as CSP [24]. Stalcup and Gahm [25] have introduced a sulphated β-CD bonded CSP and this was used to resolve the enantiomers of several antihistamines, including RS-CPM. Fried et al. [26] have achieved enantioselective separation of RS-CPM on a β-CD CSP. Hiep et al. [27] separated the enantiomers on an amylose AD column.

In the present work, a CE method is developed, able to detect 0.1% (m/m) of *R*-enantiomer in samples of S-CPM. No extraction of the sample is necessary. The resolution between the two peaks is at least 5, so that small variations in the operational conditions will not greatly affect the separation. Moreover the impurity migrates before the principal peak. The developed method is then compared to the HPLC method described in the European Pharmacopoeia.

2. Experimental

2.1. Chemicals

(*RS*)-Chlorpheniramine maleate (RS-CPM), dexchlorpheniramine maleate (S-CPM), (*RS*)-brompheniramine maleate (RS-BPM), (*RS*)-pheniramine maleate (RS-PM) and (1*R*,2*S*)-ephedrine·HCl are purchased from Sigma (St Louis, MO, USA). A sample of the enantiomeric compound S-CPM is also kindly provided by Schering-Plough Avondale

(Lucerne, Switzerland). Hydroxypropyl-β-cyclodextrin (HP-CD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-CD) are purchased from Aldrich (Gillingham, UK), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-CD) from Sigma and carboxymethyl-β-cyclodextrin (CM-CD) from Cyclolab (Budapest, Hungary). The water used for preparing solutions is obtained from a Seralpur Pro 90 CN purification system (Seral, Germany). Orthophosphoric acid (85%), triethanolamine (TEA), diethylamine, concentrated ammonia and methylene chloride are purchased from Merck (Darmstadt, Germany). Hexane and 2-propanol are both obtained from Acros Organics (Geel, Belgium). All reagents are analytical reagent-grade.

2.2. CE apparatus and conditions

A Beckman (Palo Alto, CA, USA) P/ACE 2100 system equipped with a UV detector and a temperature control system is used. All separations are carried out in an uncoated fused-silica capillary (Beckman) of 37 cm (30 cm to detector)×75 μm I.D. The CE instrument is controlled by the chromatography software System Gold 7.11 (Beckman). Integration of the electropherograms is done by Kroma 2000 (Kontron Instruments, Milan, Italy). On-line UV detection is performed at 214 nm (range 0.01) and the applied voltage is 15 kV. The capillary is temperature controlled at 25 °C by liquid cooling. Sample solutions are introduced by pressure for 5 s, followed by a 1-s injection of water. In between runs, the capillary is flushed for 2 min with water and for 3 min with run buffer. The separation buffer consists of 0.1 *M* orthophosphoric acid, adjusted to pH 3.0 with TEA. The buffer is filtered through a 0.2-μm membrane. The appropriate amount of CD is dissolved in the buffer solution. Standard stock solution of RS-CPM is prepared in water at a concentration of 100 μg/ml and diluted with water to the concentration needed. Sample solutions are prepared at a concentration of 0.25 or 1 mg/ml S-CPM in water, depending on the amount of impurity that is expected.

2.3. HPLC apparatus and conditions

The LC system consists of a Varian 2010 pump equipped with a Varian 2550 UV detector (Walnut Creek, CA, USA) and a Merck–Hitachi D-2000 integrator (Darmstadt, Germany). The separations are performed on a Chiralpak AD column (25 cm× 0.46 cm) (Chiral Technologies-EuropeSARL, Illkirch, France). The mobile phase consists of hexane–isopropanol–diethylamine (98:2:0.3). UV detection was performed at 254 nm. 100.0 mg of test substance is dissolved in water. A few drops of concentrated ammonia are added to obtain an alkaline reaction. This solution is shaken with 5 ml of methylene chloride. The lower, methylene chloride layer is evaporated to an oily residue. The residue is dissolved in isopropanol and diluted with the same solvent to 10.0 ml. Before injection the solution is 10 times diluted with mobile phase.

3. Results and discussion

3.1. Method development

3.1.1. Type and concentration of selector

Three neutral CDs and one chargeable CD were tested as chiral selectors for the enantioseparation of RS-CPM. The optimal CD concentration was determined by the linear regression method previously developed by Van Eeckhaut et al. [28]. TM-CD concentrations of 0.5–4 m*M* were not able to separate the RS-CPM enantiomers. According to the model, enantioseparation only occurs at quite high CD concentrations. Chankvetadze et al. [9] found that the enantiomers of RS-CPM were nearly baseline resolved at a concentration as high as 56 m*M* TM-CD, which is in agreement with our assumption. With DM-CD the maximum velocity difference Δv between the two enantiomers was already reached in the low concentration range tested, but no complete resolution of the enantiomers was achieved (R_s=0.5 at 3.5 m*M* DM-CD). The HP-CD concentration at which maximal Δv occurred, was 15 m*M*. The observed resolution between the two CPM enantiomers was below 3, which is still lower than our prerequisite value of 5. CM-CD was chosen among the ionisable and ionised CDs since its effectiveness for the enantioseparation of RS-CPM was already shown by Fillet et al. [5], who also noticed the low CM-CD concentrations at

which the enantiomers are already resolved. Wu et al. [7] also demonstrated that RS-CPM, RS-PM and RS-BPM could be separated with CM-CD. It appears that this was not possible with sulphated CD [13]. Our experiments also show that CM-CD exhibits high resolution values even in the low concentration range tested. This high enantioselectivity of CM-CD is probably, at least partly, related to a slight deprotonation of the carboxyl groups at pH 3. The cationic compounds will more strongly interact with the chiral selector and also CM-CD will migrate towards the anode, which should increase the difference in electrophoretic mobility between the free and the complexed fraction of the enantiomers [5,29]. A concentration of 1.5 m*M* CMCD was chosen because the resolution between the two enantiomers was more than 10 and the migration time of the latest peak was less than 15 min. Under these conditions R-CPM is the first migrating peak.

3.1.2. Improvement of peak area reproducibility

As was also shown by Chevigné [30], a significant improvement in the repeatability occurred when, after the injection of the sample, water is injected during 1 s. This injection prevents the loss of sample due to the movement from sample to buffer, contamination of the inlet buffer by backward movement of the sample out off the capillary and the loss of sample when applying voltage. The peak area reproducibility also increased when the capillary outlet was placed into buffer during the introduction of sample instead of using an empty vial. These adjustments clearly improved the reproducibility of the peak area (data not shown).

3.1.3. The use of an internal standard (IS)

(1*R*,2*S*)-ephedrine·HCl was chosen as IS to correct for injection variability. Despite the addition of the IS, no improvement in the precision was found. The sample (2–10 μg/ml) and IS (2.5 μg/ml) concentrations are both quite low, which leads to doubling of the integration errors [6]. Therefore, the IS was no longer used in the calculations.

3.2. Method validation

3.2.1. Selectivity

Selectivity is evaluated by injecting a test mixture containing RS-CPM, S-CPM and two analogues, RS-PM and RS-BPM (Fig. 2). The three antihistaminic drugs are well separated and they are also separated into their enantiomers. Because the sample is also spiked with S-CPM, it can be clearly observed from the electropherogram that S-CPM mi-

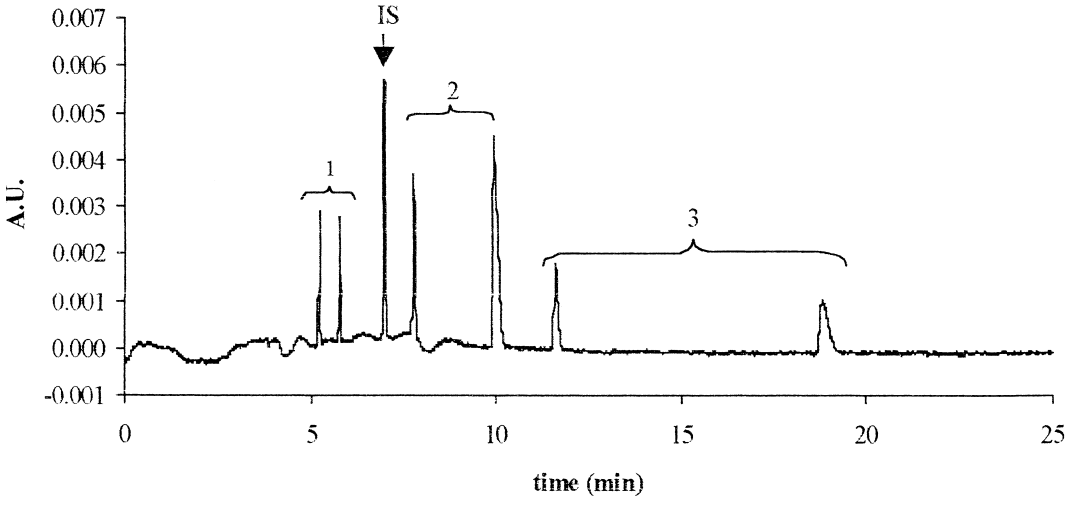

Fig. 2. Electropherogram of the separation of a mixture of (*RS*)-pheniramine maleate (1), (1*R*,2*S*)-ephedrine·HCl (IS), (*RS*)-chlorpheniramine maleate (2) and (*RS*)-brompheniramine maleate (3). Experimental conditions: capillary: 37 cm (30 cm effective length)×75 μm I.D.; separation solution: 100 m*M* TEA–phosphate buffer pH 3.0+1.5 m*M* CM–CD; detection: 214 nm; applied voltage: 15 kV; injection: 5 s (pressure); temperature: 25 °C.

grates after R-CPM (the peak area of S-CPM is twice as large as the peak area of R-CPM).

The monograph of S-CPM in the European Pharmacopoeia [8] mentions as related substances, besides R-CPM, also RS-PM. This compound is separated from both R- and S-CPM in this CE method.

3.2.2. Limit of detection and limit of quantification

The limit of detection (LOD) of R-CPM is determined as three times the noise of the baseline, which in this case equals 0.25 μg/ml. The limit of quantification (LOQ), which is calculated as nine times the noise, is 0.75 μg/ml (RSD=6.6%, $n=3$).

3.2.3. Linearity

Good linearity was obtained for R-CPM over the concentration range 2–10 μg/ml ($y=(6.752\pm0.150)x+(0.4643\pm0.5559)$, $n=3$). The correlation coefficient is 0.9992±0.0006 (RSD=2.6%).

3.2.4. Analytical precision

Intra-day precision was determined by comparing the peak area for six repeated injections of three standard concentrations (2, 6 and 10 μg/ml). The inter-day precision of standard curves in the range 2–10 μg/ml was determined on nine different days. The results of intra-day and inter-day precision are summarised in Table 1.

3.2.5. Repeatability of the method

Six replicate assays of two S-CPM samples from different manufacturers were performed to assess the method precision. For sample 1, which contained a low amount of enantiomeric impurity, 1 mg/ml of S-CPM was injected. For sample 2, the injection of 0.25 mg/ml was enough to assess the amount of impurity in this sample. For both samples, a RSD of approximately 5% was found (Table 2). Typical electropherograms of samples 1 and 2 are shown in Fig. 3a and 3b, respectively.

3.2.6. Accuracy

Accuracy was assessed through a standard addition method; 1 mg/ml of S-CPM was spiked with, respectively 2, 6 and 10 μg/ml of R-CPM, corresponding to 0.2, 0.6 and 1.0% (m/m) of impurity. All samples were made in triplicate. A linear relationship was found between the added and calculated concentrations ($y=1.008x+0.0014$; $R^2=0.9959$). In Table 3 the results of the accuracy testing are shown; 100% lies within the 95% confidence interval (C.I.: 98.9–104.9).

3.3. Comparison of the CE method with HPLC method of the European Pharmacopoeia

Before achieving the comparison between the CE and HPLC method, some modifications were made to the procedure of the HPLC method. The extraction procedure was slightly modified in order to obtain more efficient peaks. Instead of 10.0 mg test substance, 100.0 mg was used to perform the test. Afterwards the sample was diluted 10 times with mobile phase. The results of the HPLC and the CE method are given in Table 4. The amount of enantio-

Table 1
Analytical precision

Concentration (μg/ml)	Peak area (mean±SD)	RSD (%)
Intra-day precision (n=6)		
2	15.90±0.28	1.8
6	46.56±0.91	2.0
10	75.27±1.04	1.4
Inter-day precision (n=9)		
2	15.84±0.66	4.2
4	31.67±1.06	3.3
6	46.72±2.51	5.4
8	62.89±2.28	3.6
10	77.57±3.33	4.3

Experimental conditions: see Fig. 2.

Table 2
Repeatability of the method

Sample no.	Sample 1 (% m/m R-CPM)	Sample 2 (% m/m R-CPM)
1	0.141	1.77
2	0.163	1.92
3	0.161	2.01
4	0.157	2.01
5	0.152	1.96
6	0.152	2.03
Mean±SD	0.154±0.008	1.95±0.10
RSD (%)	5.1	5.0

Experimental conditions: see Fig. 2.

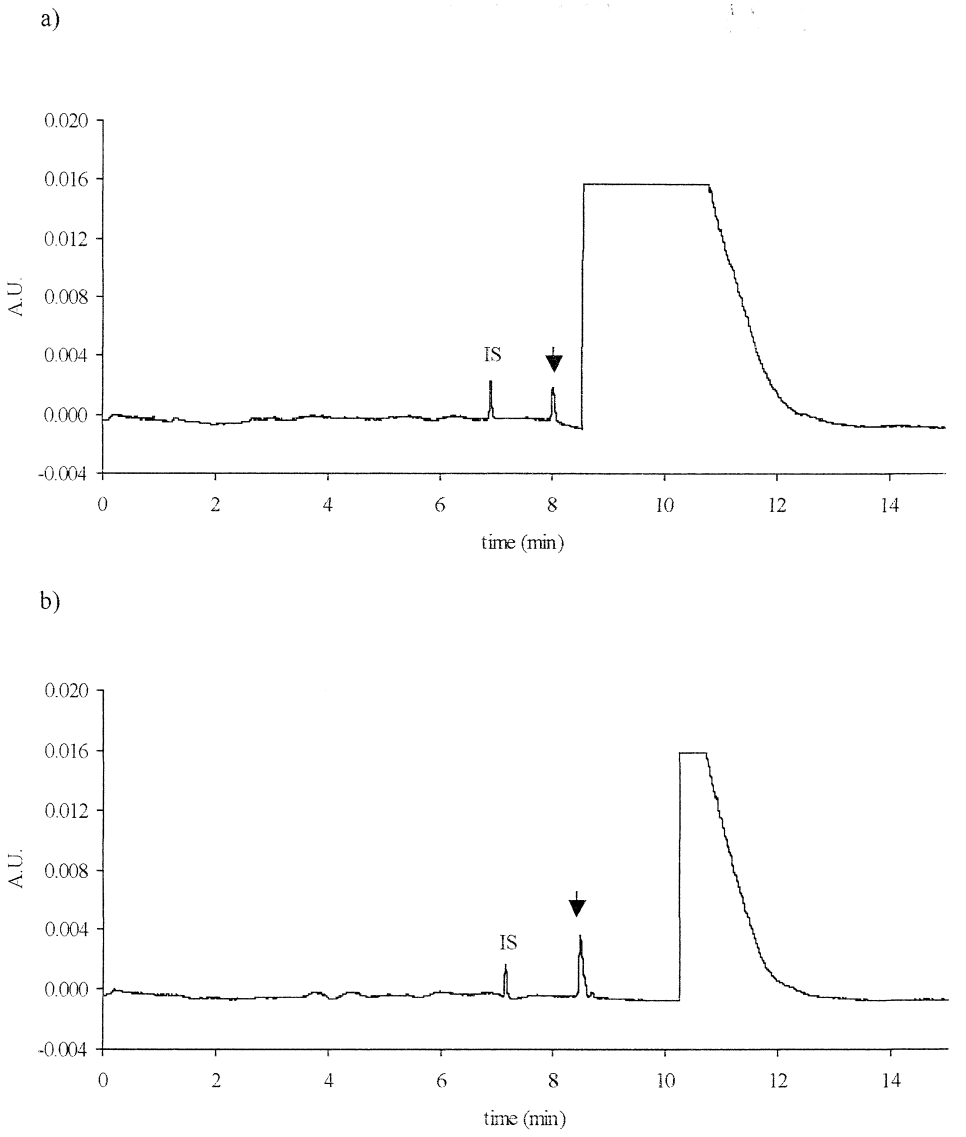

Fig. 3. Typical electropherogram of sample 1 containing 0.15% (m/m) R-CPM (a) and of sample 2 containing 1.95% (m/m) R-CPM (b). Experimental conditions: see Fig. 2; the arrow indicates R-CPM.

meric impurity present in S-CPM from sample 1 was too low to be detected with the HPLC method. The result for sample 1 obtained with the developed CE method is in agreement with the amount of 0.2% notified in the certificate of analysis.

The total duration of the analysis with CE (including rinsing the capillary) is 20 min. The runtime for HPLC is 30 min, but this does not include the extraction procedure.

4. Conclusion

A CE method has been developed which is able to detect 0.1% (m/m) of R-CPM in samples of S-CPM. The impurity elutes before the active enantiomer. All samples are dissolved in water, which means that there is no need for extraction. The method was validated for selectivity, LOD, LOQ, linearity, precision of injection, repeatability of the method and

Table 3
Accuracy

Concentration added (μg/ml)	Concentration found (μg/ml)	(%)
2.06	2.20	106.8
2.06	2.15	104.4
2.06	2.19	106.3
6.18	6.23	100.8
6.18	5.93	96.0
6.18	6.04	97.7
10.30	10.73	104.2
10.30	10.63	103.2
10.30	10.10	98.1
Mean±SD		101.9±3.95
CI (%)		98.9–104.9

Experimental conditions: see Fig. 2.

Table 4
Comparison between HPLC and CE (%, m/m R-CPM)

Sample	HPLC	CE
Sample 1	N.D.	0.15
Sample 2	1.90	1.95

Experimental conditions: see Fig. 2; N.D.=not detectable.

accuracy. The assay was tested on two different samples of S-CPM, which both contained very different amounts of enantiomeric impurity.

The results were compared to those obtained with the HPLC method described in the European Pharmacopoeia. For sample 2, the results are in agreement. Sample 1, which only contained 0.15% (m/m) of enantiomeric impurity, could not be analysed with the HPLC method because it was not sensitive enough. The results obtained with the CE method are comparable with the amount given in the certificate of analysis from the manufacturer.

With reference to the HPLC method of the European Pharmacopoeia, the following merits of the proposed CE method may be highlighted: (1) no need for expensive HPLC column; (2) no need for preliminary extraction, which makes the method cumbersome; (3) the impurity is eluting in front of the test substance with a higher resolution; (4) a lower LOD can be achieved; (5) faster analysis time.

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