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Determination of the enantiomeric purity of 5,6-dihydroxy-2-aminotetralin by high-performance capillary electrophoresis with crown ether as chiral selector

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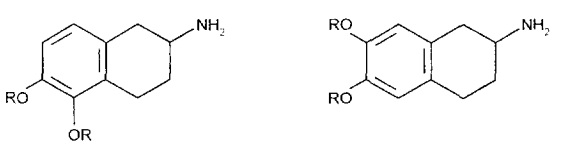
Abstract

5,6-Dihydroxy-2-aminotetralin was resolved into its enantiomers by high-performance capillary electrophoresis. The separation was performed with a background electrolyte containing 18-crown-6 tetracarboxylic acid as chiral selector. The chiral recognition is based on a primary interaction between the hydrogens of the amino group and the oxygens of the crown ether and a secondary interaction between the catechol and the carboxylates of the crown ether. The resolution factor was 1.42 and the detection limit of the enantiomer present as impurity was 0.3%. Data on the optimization and the validation of the assay are also reported.

1. Introduction

Dihydroxy-2-aminotetralins (ADTNs) (Fig. 1) are racemic dopamine receptor agonists of high potency. Their pharmacological dopaminergic activity resides on the *R*-enantiomer for 5,6-ADTN and on the *S*-enantiomer for 6,7-ADTN. The fact that the active enantiomers have opposite configuration has raised some speculation on the structural features required for the interactions of these agonist with the dopamine receptor [1–3]. Thus an analytical assay which can determine their enantiomeric purity is highly desirable for both the ADTN isomers and the corresponding 5,6- and 6,7-dimethoxy precursors.



R = H	5,6-ADTN	R = H	6,7-ADTN
R = OCH_3	5,6-ADMTN	R = OCH_3	6,7-ADMTN

Fig. 1. Structures of 5,6- and 6,7-dihydroxy-2-aminotetralin (ADTN) and of 5,6- and 6,7-dimethoxy-2-aminotetralin (ADMTN).

We have recently developed a direct chiral HPLC assay for the enantiomers of 5,6- and 6,7-ADTN [4]. The high resolution allowed small amounts ($<0.1\%$) of the distomer present as impurity in the eutomer to be determined, the only drawback being the high cost of the HPLC column.

High-performance capillary electrophoresis (HPCE) is an attractive alternative for chiral

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separations compared to the direct chiral HPLC assay, which is the most effective of the HPLC approaches. In fact many different types of expensive chiral phases are needed for the separation of a relatively narrow range of racemic compounds, while one single inexpensive silica capillary can be subsequently filled with background electrolytes containing different chiral selectors. In addition the small volume of a capillary electrophoresis system, which requires only few milliliters of electrolyte for multiple runs, allows the use of expensive or commercially unavailable chiral selectors.

Many examples of chiral separations by HPCE have been published over the last few years, most of them employing cyclodextrins and their derivatives. Fanali and co-workers reported the optical isomer resolution of epinephrine and related compounds [5], tryptophan and derivatives [6,7] and propranolol and terbutaline [8]. Novotny and coworkers showed the enantioseparation of basic drugs such as verapamil, fluoxetine, bupivacaine and some β-blockers [9] and other pharmaceuticals [10]. Another set of racemic basic drugs, most of them belonging to the cardiovascular or CNS-active compounds, was separated by Nielsen [11], while Peterson [12,13], Francotte et al. [14] and Rawjee et al. [15] reported the enantioseparation of ophthalmic drugs, non-steroidal aromatase inhibitors and barbiturates, and non-steroidal antiinflammatory drugs, respectively. Other examples of chiral selectors used in HPCE are bile acids [16,17], chiral surfactants [18], cyclodextrins incorporated into a gel matrix [19,20], cyclodextrins coated on a capillary wall [21], chiral functionalized micelles [22] and maltodextrins [23].

A more recent approach is the use of crown ethers, first reported by Kuhn and co-workers [24,25]. Since the chiral HPLC assay for the ADTNs was based on a column filled with a chiral crown ether stationary phase coated on silica gel, we investigated the addition of 18-crown-6 tetracarboxylic acid ($18C6H_4$) as a chiral selector to the background electrolyte for the separation of the enantiomers of 5,6- and 6,7-ADTN and of their dimethoxy precursors 5,6- and 6,7-ADMTN. This approach was recently reported also by Walbroehl and Wagner [26] for the enantioseparation of unsubstituted and monosubstituted aminotetralins. The present paper describes the results of the investigation and optimization of the parameters influencing the resolution between the enantiomers of 5,6-ADTN as well as the validation of the quantitative analysis.

2. Experimental

2.1. Chemicals

18-Crown-6 tetracarboxylic acid and tris(hydroxymethyl)-aminomethane (Tris) were of analytical grade and obtained from Merck (Darmstadt, Germany). Citric acid (RPE-ACS grade) was from Carlo Erba (Milan, Italy). All solutions were prepared in Milli-Q water (Millipore, Bedford, MA, USA), filtered through a 0.22-μm cellulose filter from Hewlett-Packard (Cernusco S/N, Italy) and degassed by sonication.

2.2. Instrumentation

A Beckman P/ACE 2100 capillary electrophoresis system equipped with a filter UV detector set at 280 nm was used. Separations were performed in unmodified silica capillary (57 cm × 75 μm I.D., 50 cm to detector) mounted on a liquid-cooled cartridge (Beckman). Data acquisition and processing was performed using the Beckman System Gold software installed on a Hewlett-Packard Vectra QS/20 personal computer.

2.3. Running conditions

Injections were made at the anodic site using the electrokinetic technique applying a 5 kV potential for 15 s. Separations were carried out at 25°C using the constant voltage mode in the range 5–15 kV; the observed current at 10 kV was 40 A.

Running buffer was prepared by dissolving 10

m*M* Tris-base in water, adjusting the pH to 2.2 with citric acid and adding the desired amount (10–50 m*M*) of 18C6H$_4$. The sample solutions were prepared by dissolving each solute in running buffer without the addition of 18C6H$_4$ at a concentration of approx. 0.5 mg/ml.

Conditioning for each experiment was done by rinsing the capillary with running buffer for 2 min while the daily conditioning before the beginning of a set of experiments was with 0.1 *M* sodium hydroxide, water and hydrochloric acid (2 min each).

2.4. Calculation of the resolution parameters

The resolution parameters were calculated as follows:

$$\text{separationfactor}(\alpha) = MT_2/MT_1 \quad (1)$$

$$\text{resolutionfactor}(R) = (MT_2 - MT_1)/0.88(W_2 + W_1) \quad (2)$$

where MT_1, MT_2, W_1 and W_2 are the migration times and the bandwidths at half-height of the chromatographic peaks. The suffixes 1 and 2 refer to the first and the last eluting enantiomer, respectively.

3. Results and discussion

18-Crown-6 tetracarboxylic acid is a macrocyclic polyether ring consisting of six oxygen atoms joined by methylene bridges. According to Kuhn and co-workers [24,25,27], the primary interaction between 18C6H$_4$ and 5,6-ADTN is by hydrogen bonding between the hydrogens of the primary amine group attached to the chiral centre and the oxygens of the ring system. This interaction occurs also for 6,7-ADTN and for their dimethoxy precursors 5,6- and 6,7-ADMTN. In fact, the addition of 18C6H$_4$ as chiral selector to the carrier electrolyte increased the migration time of both the ADTNs and the ADMTNs (Table 1) because the electrophoretic mobility of the analyte–chiral selector complex is lower than that of the plain analyte. This is due to the higher mass and the less positive charge of the complex, since at the pH of the buffer electrolyte 18C6H$_4$ is slightly negatively charged and hence migrates to the anodic site. The measured pK_a is 3.6 which corresponds to a 4% ionization at pH 2.2.

In addition a secondary lateral interaction, which is responsible for the chiral discrimination, takes place. This enantiorecognition was postulated to occur by either a steric hindrance mechanism or by electrostatic interaction between the carboxylate groups of 18C6H$_4$ and the guest molecule [25,27] and is very sensitive to the type and the position of the substituents on the tetralin ring [26]. In fact 5,6-ADTN, but not its positional isomer 6,7-ADTN or the dimethoxy analogues, could be resolved into its enantiomers: only for 5,6-ADTN did the spatial arrangement lead to a difference in the stability constant of the analyte–ligand complex of the individual enantiomers large enough to obtain enantiorecognition.

A kinetic model for chiral recognition in HPCE was described by Wren and Rowe [28–31]. According to this model, an optimum chiral

Table 1
Effect of the addition of 18-crown-6 tetracaboxylic acid on the migration time of 2-aminotetralins

18C6H$_4$ Concentration (m*M*)	Migration time[a]			
	5,6-ADTN	6,7-ADTN	5,6-ADMTN	6,7ADMTN
0	9.27	9.56	9.50	10.18
30	32.50	34.68	45.39	48.57

Applied potential = 10 kV. See Experimental for the other running conditions.
[a] Migration times are in min.

selector concentration exists that depends on the affinity of the analyte for the chiral selector and hence varies from case to case. The influence of the amount of $18C6H_4$ on the resolution at fixed applied potentials was therefore investigated. The results are shown in Table 2 and, as predicted by the model, we found an initial increase in resolution from 10 to 30 m*M* followed by a decrease when the chiral selector concentration was further increased to 50 m*M*. We also found an increase in resolution raising the applied voltage up to 10 kV. At higher values, the increase in peak efficiency of the enantiomers that is obtained by increasing the electric field across the capillary is overwhelmed by the decreased separation.

All experiments were carried out at pH 2.2, as recommended in the fundamental work by Kuhn et al. [25]. Since a more recent work showed the influence of pH on the resolution of D,L-histidine [27], we also investigated this parameter. At pH 2.0, a dramatic loss of resolution ($R_s < 0.4$) was observed, while slightly increasing the pH to 2.5 the migration time of both enantiomers shifted to more than 40 min with an increase in the separation factor and resolution. This favourable result was unfortunately associated with a severe distortion in peak shape which prevented its use for trace analysis. The reason of this distortion is unclear.

Temperature is another parameter which greatly influences resolution in capillary electrophoresis using a crown ether as chiral selector [24]. The result of the dependence of migration times and resolution on capillary temperature is shown in Fig. 2. As expected retention times and resolution decreased when temperature was increased, the former due to the lower viscosity of the electrolyte, and the latter to the enhanced band broadening resulting from higher diffusion. Therefore a strict control of the temperature is essential in order to obtain high resolution.

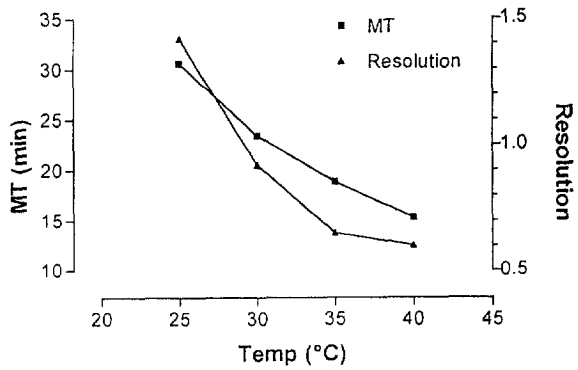

Fig. 2. Dependence of the migration time (MT) and resolution of the enantiomers of 5,6-ADTN on capillary temperature (voltage = 10 kV, $[18C6H_4] = 30$ m*M*). See Experimental for other electrophoretic conditions.

An electropherogram of a racemic mixture under optimized conditions is shown in Fig. 3. The enantiomeric migration order was determined by HPCE runs of the individual enantiomers under similar conditions. Thus, the peak with lower migration time was identified as the *R*-enantiomer.

Most papers about chiral separations by HPCE include chromatograms of a racemic mixture showing the resolution between the two

Table 2
Resolution parameters for the separation of 5,6-ADTN as a function of the amount of the chiral selector

Applied voltage (kV)	10 m*M*			30 m*M*			50 m*M*		
	R_s	MT[a]	α	R	MT	α	R	MT	α
5.0	0.966	49.3	1.025	1.382	76.1	1.025	1.059	80.7	1.024
7.5	0.967	29.8	1.022	1.382	45.0	1.024	1.136	55.5	1.021
10	1.136	23.8	1.023	1.420	32.5	1.025	1.263	39.9	1.020
15	1.003	14.7	1.022	1.308	19.1	1.021	0.941	21.4	1.019

Applied potential = 10 kV. See Experimental for the other running conditions.
[a] Migration time in min. of the first eluting enantiomer.

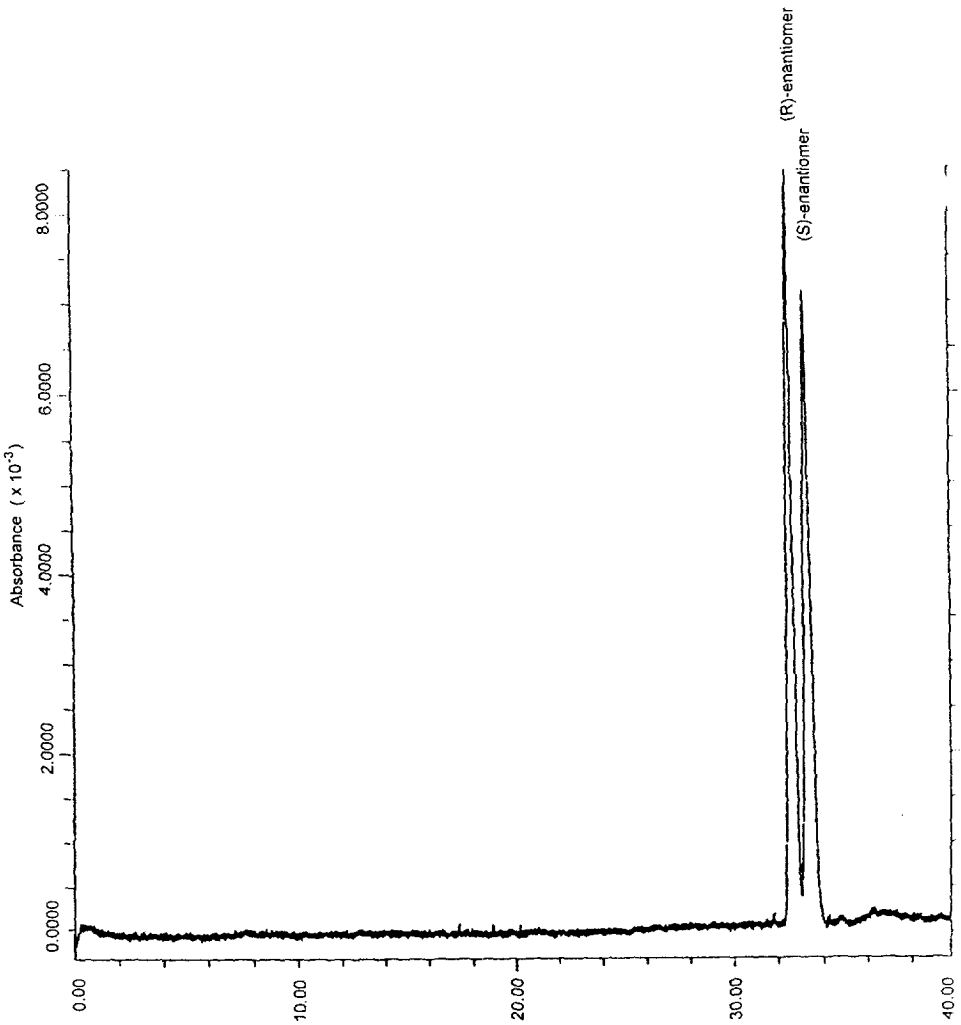

Fig. 3. Separation of the enantiomers of 5,6-ADTN under optimized conditions (voltage = 10 kV, [18C6H$_4$] = 30 m*M*, *T* = 25°C). See Experimental for other electrophoretic conditions. Time scale in min.

enantiomers, but only few show quantitative data on the determination of the enantiomer present as impurity [32–34].

Since we were interested not only in good resolution but also in the accurate determination of a small amount of distomer in the eutomer, we validated the method for both enantiomers by spiking one enantiomer with increasing amounts (range 0.5–5.0%) of the other present as impurity. The results are reported in Table 3 showing the good accuracy (expressed as bias, %) and precision (from the C.V. value) of the assay that allows quantitation down to 0.5% of the enantiomer present as impurity.

Quantitation was determined from peak areas (normalized to their respective migration times) of the enantiomers since there was no difference in their response factor. This assumption was experimentally verified from the fact that the normalized peak-area ratio of an authentic racemic mixture was 1.002 (*R*/*S* ratio, C.V. = 2%, $n = 12$). In addition the linearity of the response for both enantiomers was checked in the same range (0.5–5.0%) in the presence of

Table 3
Validation data for the determination of the enantiomeric purity of 5,6-ADTN

Concentration added (%)	$R(+)$ in $S(-)$			$S(-)$ in $R(+)$		
	Found[a] (%)	Bias (%)	C.V. (%)	Found[a] (%)	Bias (%)	C.V. (%)
0.5	0.39 ± 0.03	−22.0	6.7	0.58 ± 0.02	+ 16.0	3.8
1.0	1.00 ± 0.08	0.0	7.8	1.01 ± 0.07	+ 1.0	6.8
2.0	2.05 ± 0.14	+2.5	6.7	2.12 ± 0.07	+ 5.8	3.1
5.0	4.70 ± 0.13	−6.0	2.7	5.10 ± 0.06	+ 1.9	1.2

[a] Mean ± standard deviation ($n = 4$).

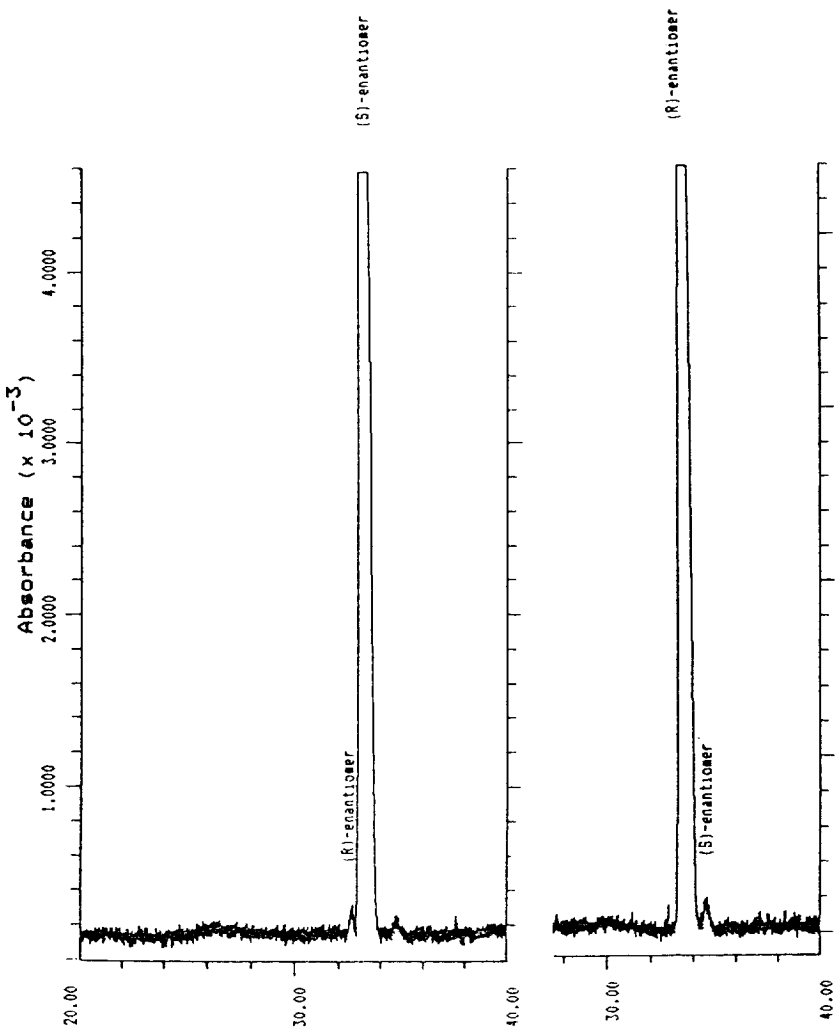

Fig. 4. Separation and trace amount analysis of (*S*)-5,6-ADTN (0.5%) in (*R*)-5,6-ADTN (left electropherogram) and of (*R*)-5,6-ADTN (0.6%) in (*S*)-5,6-ADTN (right electropherogram) under optimized conditions (voltage = 10 kV, $[18C6H_4] = 30$ m*M*, $T = 25$°C). See Experimental for other electrophoretic conditions. Time scale in min.

the corresponding amount (99.5–95.0%) of the other enantiomer. The regression analysis gave straight lines with slope 0.0506 ($r = 0.9991$) for the *R*-enantiomer and slope 0.0503 ($r = 0.9996$) for the *S*-enantiomer thus confirming their equal detector response.

This assay has been successfully employed for the determination of the enantiomeric purity of synthetic batches of both (*R*)- and (*S*)-5,6-ADTN. The electropherograms of a batch of a *R*-enantiomer containing 0.5% of the *S*-enantiomer and of a batch of *S*-enantiomer containing 0.6% of the *R*-enantiomer are shown in Fig. 4 showing a detection limit of about 0.3% (at a signal-to-noise ratio of 2).

Since we were involved in a research project where both ADTNs and ADMTNs are used as chiral building blocks for the synthesis of N,N-disubstituted aminotetralins acting on the dopaminergic system, we evaluated the use of other chiral selectors that could be able to perform the enantioseparation of 6,7-ADTN and of 5,6- and 6,7-ADMTN, which was unsuccessful using the system described here. The result of that study will be published elsewhere [35].

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