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# Mechanistic considerations of enantiorecognition on novel *Cinchona* alkaloid-based zwitterionic chiral stationary phases from the aspect of the separation of *trans*-paroxetine enantiomers as model compounds

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## ABSTRACT

The enantiomers of *trans*-paroxetine were separated on four chiral stationary phases (CSPs) based on chiral zwitterionic *Cinchona* alkaloids fused with (*R*,*R*)- or (*S*,*S*)-*trans*-2-aminocyclohexanesulfonic acid. The enantioseparations were carried out in polar-ionic or in hydro-organic mobile phases with MeOH/THF, MeCN/THF, MeCN/THF/$H_2O$ and MeOH/MeCN/THF containing organic acid and base additives, in the temperature range 0–50 °C. The effects of the mobile phase composition, the natures and concentrations of the additives and temperature on the separations were investigated. Thermodynamic parameters were calculated from plots of $\ln\alpha$ *vs* $1/T$. $\Delta(\Delta H^\circ)$ ranged between −3.0 and +1.5 kJ mol$^{-1}$, and $\Delta(\Delta S^\circ)$ between −8.8 and +5.9 J mol$^{-1}$ K$^{-1}$. The enantioseparation was generally enthalpically controlled, the retention factor and separation factor decreasing with increasing temperature, but entropically controlled separation was also observed. The elution sequences of the paroxetine enantiomers on the two pairs of pseudo-enantiomeric CSPs were investigated, and an attempt was made to explain the observed anomalies *in silico* in order to gain an insight into the underlying molecular recognition events between the four chiral selectors and the analyte enantiomers.

## 1. Introduction

The stereoselective analysis of pharmaceutical compounds is extremely important for the pharmaceutical industry since many drug compounds are chiral molecules whose stereoisomers usually possess quite different pharmacological and toxicological properties. Often only one isomer exhibits the required activity (that is the eutomer), while the other(s) (defined as distomer(s)) may possibly be inactive or even toxic. For this reason, the chiral purity of enantiomers and their analysis is essential.

Paroxetine contains two chiral carbon centres, and therefore has four stereoisomers, two in a *cis* and two in a *trans* configuration (Fig. 1). The active ingredient is *trans*-(−)-paroxetine [(3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy) methyl]-4-(4-fluorophenyl) piperidine], which is a well-known antidepressant drug of the most specific serotonin reuptake inhibitor type. It is used to treat depression, panic disorder, social anxiety/social phobia disorder, post-traumatic stress disorder, generalized anxiety disorder and obsessive-compulsive disorder [1–3].

Several methods of analysis have been reported for the non-stereoselective determination of paroxetine and its key intermediate (precursor) or metabolites in human serum, human plasma or pharmaceutical formulations. High-performance liquid chromatography (HPLC) has frequently been coupled to a diode array [4], ultraviolet (UV) [5], electrochemical [6], fluorescence (without [7] or with derivatization [8]) or mass spectrometric (MS) detection [9]; gas chromatography has been coupled to MS detection [10] or to ultra-HPLC [11]. Two of the important precursors in the synthesis of *trans*-(−)-paroxetine, *trans*-3-ethoxycarbonyl-4-(4′-fluorophenyl)-1-methylpiperidine-2,6-dione and paroxol, have earlier been analysed stereoselectively on polysaccharide-derived

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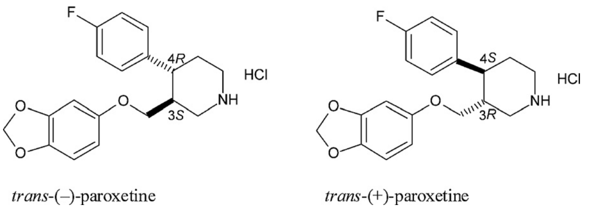

**Fig. 1.** Structures of *trans*-(−)- and *trans*-(+)-paroxetine.

chiral stationary phases (CSPs) by means of supercritical fluid chromatography as a powerful alternative technique to HPLC [12,13].

For the chromatographic resolution of the enantiomers of paroxetine, a number of diverse CSPs and "chiral" columns thereof have been investigated successfully. They include β- and γ-cyclodextrin [14], macrocyclic glycopeptide [14], Pirkle or "brush"-type optically active metal complexes (Ru adsorbed to porous spherical ceramics by cation exchange) [14], polysaccharides (amylose and cellulose) [14,15], glycoprotein (ovomucoid) [16] or tartardiamide-based (*O*,*O*′-bis(4-*tert*-butylbenzoyl)-*N*,*N*′-diallyl-L-tartardiamide) [17] columns. The present study involves the use of chiral ion exchangers as a promising alternative with a view to solving this analytical challenge.

In liquid chromatography, the retention and selectivity are strongly influenced not only by the mobile phase conditions, but also by temperature. This is especially true for chiral separations. The relationship between the selectivity and the column temperature is as follows:

$$\ln\alpha = -\frac{\Delta\left(\Delta H^{\circ}\right)}{RT} + \frac{\Delta\left(\Delta S^{\circ}\right)}{R} \qquad (1)$$

where α is the selectivity factor ($\alpha = k_2/k_1$) and $\Delta(\Delta H)^{\circ}$ and $\Delta(\Delta S)^{\circ}$ are the differences in standard enthalpy and standard entropy, respectively, for a given pair of enantiomers. In general, $\Delta(\Delta H^{\circ})$ is invariant with temperature, a plot of $R \ln \alpha$ *vs* $1/T$ having slope—$\Delta(\Delta H^{\circ})$ and intercept $\Delta(\Delta S^{\circ})$. It is important to note that there are both "achiral" and "chiral" contributions to the retention that can vary with a wide variety of experimental parameters [18–23]. In this study we did not process and quantify the thermodynamic coefficients for the chiral and nonchiral sites, *i.e.* the van't Hoff plots yield apparent enthalpy and entropy values, in which the respective contributions of the chiral and achiral interactions are combined. (Linear plots might be expected when the retention mechanism would not change with temperature, or the relative weights of the achiral and chiral interactions would not change dramatically.) As described by Fornstedt, for a more realistic approach, the contributions of the enantioselective and nonselective sites should be distinguished [24].The present work involves mechanistic considerations of enantiorecognition relating to the separation of *trans*-paroxetine enantiomers as model compounds (Fig. 1) on four newly developed *Cinchona* alkaloid quinine (QN) or quinidine (QD)-based zwitterionic ion-exchange-type selectors (SOs) chemically fused with (*R*,*R*)- and (*S*,*S*)-aminocyclohexanesulfonic acid (ACHSA) (Fig. 2). A polar-ionic mode (PIM) and a hydro-organic mobile phase were applied. The effects of the composition of the mobile phase, the natures of the additives, the amount of the counter-ion, the temperature and the combination of the chiral subunits of the ampholytic ion-exchangers (Fig. 2) on the retention and enantioselectivity are discussed. Computer modelling was carried out in an effort to achieve a better understanding of the underlying molecular recognition mechanism and the observed elution sequences of the enantiomers.

## 2. Materials and methods

### 2.1. Chemicals

Methanol (MeOH), acetonitrile (MeCN) and tetrahydrofuran (THF) of HPLC grade were purchased from VWR International (Arlington Heights, IL, USA). $NH_3$, ethylamine (EA), diethylamine (DEA), triethylamine (TEA), *n*-propylamine (PA), tri-*n*-propylamine (TPA), *n*-butylamine (BA), tri-*n*-butylamine (TBA) and formic acid (FA) of analytical reagent grade were from Sigma-Aldrich (St. Louis, MO, USA). Synthesis chemicals were purchased from ABCR G.m.b.H. (Karlsruhe, Germany) and LachNer (Neratovice, Czech Republic). The Milli-Q water was further purified by filtration on a 0.45 μm HV Millipore filter (Molsheim, France). All eluents were degassed in an ultrasonic bath, and helium gas was purged through them during the HPLC analyses.

Standard selectands (SAs) of *trans*-(−)-paroxetine and a *trans*-(+)-paroxetine (Fig. 1) were obtained from Gedeon Richter Ltd. (Budapest, Hungary). Analytes were dissolved in MeOH at a concentration of 1.0 mg ml$^{-1}$ or 5 mg ml$^{-1}$ and further diluted with mobile phases when appropriate.

### 2.2. Apparatus and chromatography

The analytical chromatographic measurements were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-2996 photodiode-array detector and an Empower 2Chromatography Manager data system (Waters Chromatography, Milford, MA, USA). This system was equipped with a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 20 μl loop. For thermostating of the columns, a Lauda Alpha RA 8 cooling thermostat with a temperature adjustment precision of ±0.1 °C (Lauda Dr. R. Wobser Gmbh & Co. Kg, Königshofen, Germany) was used. Nuclear magnetic resonance spectroscopy (NMR) measurements were carried out on an Agilent 400-MR DDR2 spectrometer. $CD_3OD$ was used as solvent and the signals of the solvent served as an internal standard, *J* values being given in Hz.

Of the four zwitterionic CSPs (Fig. 2 and Table 1), ZWIX(+)$^{TM}$ [QN-(*S*,*S*)-ACHSA] and ZWIX(−)$^{TM}$ [QD-(*R*,*R*)-ACHSA] are commercially available from Chiral Technologies Europe (CTE, Illkirch, France), while ZWIX(-A) [QD-(*S*,*S*)-ACHSA] was prepared as described in the Supplementary Material. Each of these three CSPs comprised 3 μm particles packed into 150 × 3.0 mm I.D. columns. ZWIX(+A) [QN-(*R*,*R*)-ACHSA] comprised 5 μm or 3 μm particles packed into 150 × 4.0 mm I.D. or 150 × 3 mm I.D., respectively. The synthesis of this CSP material was described previously [25]. All columns were provided by CTE (Illkirch, France).

All chromatographic experiments were carried out in isocratic mode at a flow rate of 0.6 ml min$^{-1}$, with UV detection at 295 nm. The void volume of the columns ($t_0$) was determined by injecting a solution of acetone in MeOH.

### 2.3. Molecular modelling methods

With the same protocol as used previously [26–28], a cubic box was built with a 30 Å side length. For a realistic reproduction of the stationary phase environment, 4mercaptopropyl-functionalized silanols (~1.97 μmol m$^{-2}$), 8 free silanols (~8.0 μmol m$^{-2}$) and 45 silicon atoms were considered for each grafted SO unit (~0.5 μmol m$^{-2}$), at the base of the box. All the silicon atoms and their bonded hydrogen atoms in the base layer were set frozen during the molecular dynamics. A custom solvent of MeOH/THF (80:20 v/v) was created in a house with the aid of the gromacs tools [29,30] and added to the simulation systems. All the (eight) simulations with the four CSP systems were performed in the canonical ensemble at 298 K. The temperature in the simulation cell was