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"Fit-for-purpose" development of analytical and (semi)preparative enantioselective high performance liquid and supercritical fluid chromatography for the access to a novel σ_1 receptor agonist

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ABSTRACT

A rapid and straightforward screening protocol of chiral stationary phases (CSPs) in HPLC and SFC resulted in three different methods "fit-for-purpose", i.e. analysis and scale-up to semi-preparative enantioselective chromatography. The efficient use of these three methods allowed expedited preparation of an important drug discovery target, (*R*/*S*)-**1**, a potent new sigma 1 (σ_1) receptor agonist. The approach taken resulted in significant savings of both time and labor for the isolation of enantiomers compared to the development of a stereo-selective synthesis.

The enantiomers of **1** have been isolated allowing studies of their chirooptical properties and an in-deep comparative examination of the pharmacological profile for the individual enantiomers.

1. Introduction

The sigma 1 receptor (σ_1R) has been intensively studied in an attempt to investigate its role as a therapeutic target in several pathologies [1], including neurodegenerative diseases, such as Parkinson's, Alzheimer's and amyotrophic lateral sclerosis [2], mood disorders [3,4] and pain [5]. In the last decade, our group designed and synthesized a large number of σ_1R ligands [6–8]. Among these, (*R*/*S*)-2-(4-phenylphenyl)-4-(1-piperidyl)butan-2-ol, (*R*/*S*)-**1** (Table 1) was recently identified as a potent σ_1R agonist [9]. Given that the stereoselectivity of the ligand binding to σ_1R remains one of the obscure, yet intriguing aspects of the activity of this protein, (*R*)- and (*S*)-**1** were prepared in amount suitable for evaluating their interaction with the biological target and their effect in promoting neurite outgrowth. As a result, (*S*)-**1** was found to be the best σ_1R ligand ($K_i\sigma_1$ = 4.7 nM, eudismic ratio = 8) and the only enantiomer effective in enhancing NGF-induced neurite outgrowth at the tested concentrations [9]. Unfortunately, during this study both enantiomers of **1** were obtained in minute amounts, only sufficient to support a preliminary in vitro biological investigation.

The work here presented is as a part of our ongoing efforts focused on the development of rapid and easy to use methods suitable for obtaining a quick access to the enantiomers of medicinal chemistry interest with high enantiomeric excess and amounts sufficient for biological investigations [10]. In the light of the above considerations, the aim of the present work was to develop a productive and robust system "fit-for-purpose" [11] suitable for isolating pure enantiomers of **1** in amounts sufficient to support an exhaustive biological investigation. It should be stressed that in medicinal chemistry and early phases of drug development high throughput of candidates rather than sophisticated analytical methods suitable for validation or fully optimized separations dedicated to production under GMP are the main focus. Therefore, a general applicable set of experimental conditions was developed and tested employing racemic **1**, for which neither a stereoselective synthesis, nor any other method for isolating the enantiomers had been described before.

Among the different approaches for the preparation of enantiopure compounds, (semi)-preparative enantioselective high performance liquid chromatography (HPLC) and (semi)-preparative enantioselective supercritical fluid chromatography using chiral stationary phases (CSPs) have been successfully employed for the

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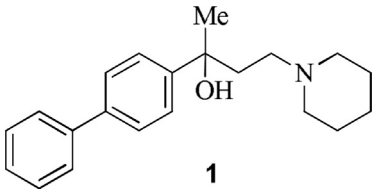
Table 1
Screening results for enantiomer separation of (*R*/*S*)-2-(4-phenylphenyl)-4-(1-piperidyl)butan-2-ol, (*R*/*S*)-**1**, via HPLC.



1

Eluent[a]	Cellulose based CSPs											
	Chiralpak IC[b]				Chiralcel OD-H				Chiralcel OJ-H			
	k_A	k_B	α	Rs	k_A	k_B	α	Rs	k_A	k_B	α	*Rs*
A	0.36		1	n.a.	0.34		1	n.a.	0.69	1.27	1.83	4.89
B	1.05		1	n.a.	0.19		1	n.a.	0.37	0.69	1.84	2.99
C	5.22	7.06	1.35	3.37	0.26		1	n.a.	0.69	1.05	1.52	2.95
D	4.22	5.02	1.19	2.54	0.25		1	n.a.	0.94	1.5	1.6	3.75
E	n.a.				0.44	0.71	1.59	1.61	n.a.			

Eluent[a]	Amylose based CSPs							
	Chiralpak IA[b]				Chiralpak AD-H			
	k_A	k_B	α	Rs	k_A	k_B	α	*Rs*
A	1.18		1	n.a	0.69	0.89	1.29	1.69
B	1.1		1	n.a	0.9		1	n.a
C	2.38	2.88	1.2	2.07	0.76	0.97	1.28	2
D	5.63	7.13	1.27	4.88	1.07	1.36	1.27	1.77
E	n.t.				n.t.			

[a] Mobile phases: A: MeOH; B: EtOH; C: *n*-Hp/EtOH (90/10, v/v); D: *n*-Hp/EtOH (95/5, v/v); E: *n*-Hp/IPA (98/2, v/v). All mobile phases contained 0.1% DEA. n.t. not tested; n.a.: not applicable.
[b] Mobile phase contained 0.3% TFA.

isolation of the enantiomers of a chiral molecule, being a viable route for straightforward and rapid access to both enantiomers with high optical purity and yields. Accordingly, a fast, pragmatic, and non-comprehensive column screening was the key driver for the rapid establishment of a resolution of **1** via enantioselective HPLC and supercritical fluid chromatography (SFC) on chiral stationary phases [12–14] at a (semi) preparative scale. The elution order of the two enantiomers could be switched by selection of suitable chromatographic conditions.

2. Materials and methods

2.1. Chemical and instruments

Solvents used as eluents (HPLC grade) were obtained from Aldrich (Italy). (*R*/*S*)-**1** was prepared by us, as already described [9].

HPLC measurements were carried out on a Jasco system (JASCO Europe, Cremella, LC, Italy) consisting of PU-2089 plus pump, AS-2055 plus autosampler and MD-2010 plus detector. Data acquisition and control were performed using the Jasco Borwin Software.

For all SFC runs an Investigator Analytical/(semi) preparative SFC system, Waters SpA (Milan, Italy) was employed. Data acquisition and control of the SFC systems were performed using the Waters SuperChrom Software Waters SpA (Milan, Italy).

Retention factors of first and second eluted enantiomer k_a and k_b, respectively, were calculated following IUPAC recommendations [15]; the dead time t_0 was considered to be equal to the peak of the solvent front for each particular run. Resolution was calculated according to Ph. Eur. 2.2.29 [16], enantioselectivity (α) was calculated according to: $\alpha = k_b/k_a$.

Optical rotations measurements were determined on a Jasco photoelectric polarimeter DIP 1000 system (JASCO Europe, Cremella, LC, Italy) with a 1 dm cell at the sodium D line (λ = 589 nm); sample concentration values c are given in g 10^{-2} mL^{-1}.

2.2. Chiral chromatographic resolution by HPLC

Analytical HPLC runs were performed using the commercially available Chiralcel OD-H (150 mm × 4.6 cm, 5 μm), Chiralcel OJ-H (150 mm × 4.6 cm, 5 μm), Chiralpak IC (250 mm × 4.6 cm, 5 μm), Chiralpak IA (150 mm × 4.6 cm, 5 μm) and Chiralpak AD-H (150 mm × 4.6 cm, 5 μm) columns (Daicel Industries Ltd., Tokyo, Japan). The mobile phase compositions as well as the chromatographic parameters are summarized in Table 1. Sample solutions of the analyte [0.5 mg mL^{-1} in ethanol (EtOH)] were filtered through 0.45 μm PTFE membranes (VWR International, Milan, Italy) before analysis. The injection volume was 10 μL, the flow rate was 1.0 mL min^{-1} and detection wavelength was 254 nm. All experiments were performed at room temperature (r.t.).

(Semi) preparative HPLC runs were carried out employing a Chiralcel OJ-H column (250 mm × 10 mm, 5 μm) (Daicel Industries Ltd., Tokyo, Japan), eluting with methanol (MeOH)/ diethylamine (DEA) (99.9/0.1; v/v) at a flow rate of 3 mL min^{-1}. Sample solutions of analytes (3 mg mL^{-1} in MeOH) were filtered before analysis. The injection volume was 1 mL and the UV detection at 254 nm (r.t). For the preparative HPLC runs the flow rate calculated from the linear scale-up (i.e. approx. 5 mL min^{-1}) led to a partial co-elution of an achiral impurity in the starting material; therefore the flow rate was reduced to 3 mL min^{-1}, for which no significant co-elution was observed.

The collected fractions were evaporated at reduced pressure. In process control was performed using an analytical Chiralcel OJ-H column.

2.3. Chiral chromatographic resolution by SFC

SFC analytical screening was carried out employing Chiralpak IA (250 mm × 4.6 cm, 5 μm) and Chiralpak IC (250 mm × 4.6 cm, 5 μm). A pilot screening was performed by gradient elution using carbon dioxide (CO_2) mixed with (i) polar modifiers (MeOH, EtOH