Accepted Manuscript

Title: "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

Author: Daniela Rossi Annamaria Marra Marta Rui Stefania Brambilla Markus Juza Simona Collina

PII: S0731-7085(15)30216-8
DOI: http://dx.doi.org/doi:10.1016/j.jpba.2015.10.047
Reference: PBA 10324

To appear in: *Journal of Pharmaceutical and Biomedical Analysis*

Received date: 29-7-2015
Revised date: 30-10-2015
Accepted date: 31-10-2015

Please cite this article as: Daniela Rossi, Annamaria Marra, Marta Rui, Stefania Brambilla, Markus Juza, Simona Collina, "Fit-for-purpose" Development of Analytical and (Semi)preparative Enantioselective High Performance Liquid and Supercritical Fluid Chromatography for the Access to a Novel *rmsigma*1 Receptor Agonist, Journal of Pharmaceutical and Biomedical Analysis http://dx.doi.org/10.1016/j.jpba.2015.10.047

"Fit-for-purpose" Development of Analytical and (Semi)preparative Enantioselective High Performance Liquid and Supercritical Fluid Chromatography for the ~~First Time~~ Access to a Novel σ_1 Receptor Agonist

Daniela Rossi[1#], Annamaria Marra[1#], Marta Rui[1], Stefania Brambilla[2], Markus Juza[3], Simona Collina[1]

[1] *Department of Drug Sciences, Medicinal Chemistry and Pharmaceutical Technology Section, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy*

[2] *NicOx Research Institute, Via Ariosto 21, 20091 Bresso (MI), Italy*

[3] *Corden Pharma Switzerland LLC, Eichenweg 1, 4410 Liestal, Switzerland*

[#] These Authors contributed equally to this work.

Corresponding authors at:

- Department of Drug Sciences, Medicinal Chemistry and Pharmaceutical Technology Section, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy. Tel: +39-0382987379; Fax: +39-0382422975
  E-mail address: simona.collina@unipv.it (S. Collina)
- Corden Pharma Switzerland LLC, Eichenweg 1, 4410 Liestal, Switzerland. Tel: +41-61-9065909; *E-mail address*: Markus.Juza@cordenpharma.com (M. Juza)

KEY WORDS: amylose and cellulose derived CSPs, chiral resolution, elution order, enantioselective HPLC and SFC, sigma 1 (σ_1) receptor agonist.

Highlights

- Efficient screening for enantioselective analytical and semi-preparative HPLC and SFC results in methods “fit-for-purpose”
- ~~First~~ Analytical enantiomer separation of (*R*/*S*)-2-(4-phenylphenyl)-4-(1-piperidyl)butan-2-ol by HPLC or SFC
- Successful scale-up in enantioselective HPLC and SFC resulting in sufficient amounts for determination of chirooptical properties and assignment of absolute configuration in less than two weeks

Graphical abstract

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 [6,7,8]. 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 ~~were evaluated~~. 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 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

81 phases ~~(CSPs)~~ [12,13,14] at a (semi)preparative scale. The elution order of the two enantiomers
82 could be switched by selection of suitable chromatographic conditions.
83

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 x 4.6 cm, 5 µm), Chiralcel OJ-H (150 mm x 4.6 cm, 5 µm), Chiralpak IC (250 mm x 4.6 cm, 5 µm), Chiralpak IA (150 mm x 4.6 cm, 5 µm) and Chiralpak AD-H (150 mm x 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.

Please insert Table 1

2.3 Chiral chromatographic resolution by SFC

SFC analytical screening was carried out employing Chiralpak IA (250 mm x 4.6 cm, 5 µm) and Chiralpak IC (250 mm x 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 or isopropanol (IPA) added with 0.1% DEA) or ii) mixtures of n-heptane (n-Hp) and alcohols (IPA or EtOH) added with 0.1% DEA. ~~and,~~ Successively, isocratic runs were performed. Results are summarized in Table 2. Sample solutions were prepared by dissolving the analyte at 1 mg mL^{-1} in IPA. The injection volume was 10 µL, the flow rate 4 mL min^{-1} and the detection wavelength was 254 nm. All experiments were performed at 40°C.

The (semi)preparative runs were carried out employing either a Chiralpak IA (250 mm x 10 mm, 5 µm) eluting with 70% of CO2 and 30% of n-Hp/EtOH/DEA (9/1/0.1, v/v/v) at a flow rate of 10 mL min-1, or a Chiralpak IC column (250 mm x 10 mm, 5 µm), eluting with 75% CO2 and 25% of n-Hp/IPA/DEA (9/1/0.1, v/v/v) at a flow rate of 8 mL min-1. Sample solutions of analytes (10 mg mL-1 in IPA) were filtered before analysis. For the preparative SFC runs the flow rate calculated from the linear scale-up (approx. 20 mL min-1) was out of the operating range of the instrument; however, it could be increased to 8 mL min-1on Chiralpak IA, due to the partial co-elution of the two enantiomers, and even to 10 mL min-1 on Chiralpak IC for which the two enantiomers were separated better. Fraction collection was performed according to the UV profile; analytical in process control of collected fractions was performed using the Chiralpak IA column eluting with 70% of CO2 and 30% of a mixture of n-Hp/EtOH/DEA (90/10/0.1, v/v/v). The collected fractions were evaporated under reduced pressure.

Please insert Table 2

3. Results and Discussion

The synthesis of racemic 1 and analogous biphenylyl-alkylamines has been reported four decades ago [17]. However, no stereo-selective synthesis or enantioselective chromatographic method for obtaining the single enantiomers in g scale has been described ever before. In order to obtain both enantiomers of 1 in amounts sufficient for an exhaustive biological investigation, preparative enantioselective HPLC and SFC separations were developed, scaled-up and the obtained results compared. The design of experiments followed the general strategy recently outlined by analytical development groups working at Pfizer and Vertex focusing on methods "fit-for-purpose" in early stages of drug development [11]. "Fit-for-purpose" means that "the method used is sufficient to answer the question *at the time of need*, but will probably change as the development progresses" [11]. In view of the good solubility of 1 in alcohols only normal phase and polar organic solvent chromatography were tested [18].

3.1 Analytical screening and development of a scalable enantiomer separation of 1

For HPLC the screening started with a standard protocol for cellulose and amylose derived CSPs [19] which was applied to Chiralpak IC, Chiralcel OD-H and Chiralcel OJ-H (all cellulose derivatives) as well as to Chiralpak IA and Chiralpak AD-H (amylose derivatives). We intentionally narrowed our screening to some of the most versatile ~~promising~~ CSPs available in our laboratories; elution conditions in the screening included alcohols (methanol, ethanol and 2-propanol) and mixtures of *n*-heptane and polar modifiers (ethanol or 2-propanol). Results of the screening protocol are reported in Table 1 as retention factor ~~capacity factor~~ (k), selectivity (α) and resolution (R_s) factors.

The retention times of 1-enantiomers on Chiralpak IC and IA with non-polar eluent compositions were quite long and do not give grounds for a productive scale-up; with polar eluents no separation was observed. Enantiomer separation of 1 on Chiralcel OD-H could only be achieved when using a mobile phase with very high alkane content, while the results on Chiralcel OJ-H turned out to be quite promising for further scale-up. Interestingly Chiralpak IA (the immobilized version of Chiralpak AD-H) shows significantly longer retention times in comparison to its non-immobilized analogue employing alkane-based mobile phases, while retention behavior and enantioselectivity with methanol and ethanol as mobile phase are very similar and do not allow enantiomer separation of 1.

Using pure methanol as eluent (with 0.1% DEA) relatively short retention times (3.4 min for the first eluted enantiomer and 4.6 min for the second), high enantioselectivity and good resolution (α = 1.8, R_s = 3.9 at r.t.) could be observed on Chiralcel OJ-H (Fig. 1A).

Accordingly, these experimental conditions are suitable for the scale-up to (semi)preparative scale. In view of these results no further attempts were made to extend the screening under HPLC conditions.

Simultaneously, we tested enantioselective SFC for the enantiomer separation of 1, which is considered as one of the most rapid and efficient methods for obtaining directly both enantiomers in high optical purity [20,21,22,23]. Recently, the advantages of enantioselective SFC over HPLC in analytical [24,25] and preparative separations [26] have been reported ~~reviewed~~ by several authors. Due to lower viscosities SFC allows running chromatographic separations at faster flow rates [27] and often gives the opportunity to use less solvent in the final fraction. Therefore a straightforward and fast screening [28,29] of suitable chiral stationary phases and polar modifiers (MeOH, EtOH and IPA; all with 0.1% DEA) under gradient conditions (5% to 45%) was performed. First scouting experiments on two columns (Chiralpak IA and Chiralpak IC) using the aforementioned solvents resulted in five enantiomer separations of 1 (Table 2) under 10 minutes. Only the use of EtOH as polar CO_2 modifier did not result in chiral resolution of 1 on Chiralpak IA. Also in this case the screening was not broadened considering the high success rate of the first experiments.

In a second step, the optimization of selectivity and resolution was performed under isocratic conditions, excluding unpromising experiments from the screening matrix (e.g. experiments with pure EtOH as polar modifier on Chiralpak IA). We included also mixtures of n-heptane with IPA and EtOH in the screening and, at the first glance ~~surprisingly~~, an excellent separation on Chiralpak IA was discovered with 30 % *n*-heptane/EtOH (90/10, v/v) in CO_2 (Fig. 1B and Table 2, α = 1.25, R_s = 2.56). However, as our screening under HPLC conditions (Table 1) had shown, Chiralpak IA shows good enantioselectivity employing various ratios of *n*-Hp/EtOH, even though retention times were relatively long compared to other conditions. In view of the relatively high content of modifier it can be assumed that the separation is no longer under supercritical conditions, but subcritical conditions, in which compressed CO_2 ~~is no longer a fluid~~ is no longer a supercritical fluid, but a liquid [30,31]. Retention times are significantly reduced in comparison to the HPLC conditions due to the fourfold higher flow rate. In a similar way also the separation conditions on Chiralcel IC were optimized. The best conditions in regard to enantioselectivity and resolution were found using 25 % IPA/ *n*-heptane (90/10, v/v) in CO_2 (Fig. 1C and Table 2, α = 1.39, R_s = 4.25).

Please insert Figure 1

3.2 Preparation of 1 enantiomers through HPLC and SFC systems

Preparative resolution of enantiomers using HPLC and SFC is a powerful technique for rapid generation of enantiomers in pharmaceutical discovery [26]. Employing a HPLC system, among the most important prerequisites for an economic and productive preparative enantiomer separation are retention times as short as possible, a high solubility of the racemate and the enantiomers in the eluent/injection solvent and the use of a mobile phase consisting of a pure low-cost solvent, facilitating workup and re-use of mobile phase. As previously discussed, using a Chiralcel OJ-H and pure methanol as eluent (with 0.1% DEA), relatively short retention times (3.4 min for the first eluted enantiomer and 4.6 min for the second), high enantioselectivity and good resolution (α = 1.8, R_s = ~~3.9~~ 4.9 at r.t.) could be observed (Fig. 1A). Accordingly, these experimental conditions were selected for the scale-up to (semi)preparative scale [32]. Based on scale-up calculations [33,34] the enantiomer separation was transferred to a Chiralcel OJ-H column with an ID of 10 mm on which a maximum of 3.0 mg could be separated in one run within 16 minutes. 21 mg (*R/S*)-1 have been processed in 7 cycles affording 8.7 mg of the first (yield: 43.3%; ee = 99.9 %; $[\alpha]_D^{20}$ + 24.0) and 9.1 mg of the second eluted enantiomer (yield: 45.5 %; ee = 99.9 %; $[\alpha]_D^{20}$ - 24.0) at an overall yield of 88.8 % (Table 3). Therefore, using the available (semi)preparative set-up per day 270 mg racemic 1 can be processed using enantioselective HPLC on Chiralcel OJ-H. Based on these experiments a specific productivity [35,36] of 27 g racemate separated per 24 h on 1 kg of CSP can be assumed.

Regarding SFC technique, both separations which gave rise to the best resolutions were scaled-up employing (semi)preparative columns with an inner diameter of 10 mm and 250 mm length packed with 5 µm CSPs optimized [37]. Starting from an injection volume of 50 µL and a flow rate of 5 mL min^{-1}, as suggested by literature [38], gradual steps of both parameters were performed. The best profiles were obtained 1) on Chiralpak IA injecting 50 µL per run and eluting at a flow rate of 10 mL min^{-1}, 2) on Chiralpak IC injecting 50 µL per run and eluting at a flow rate of 8 mL min^{-1}. In detail, using Chiralpak IA 20 mg (*R/S*)-1 could be processed in 40 cycles of 10 min each (Fig. 2A). 9.1 mg of the (*S*) enantiomer (first eluted, yield: 45.5%; ee = 99.9 %; $[\alpha]_D^{20}$ + 24.0) and 8.2 mg of the (*R*) enantiomer (second eluted yield: 41.0 %; ee = 94.5 %; $[\alpha]_D^{20}$ – 23.1) at an overall yield of 86.5 % (Table 3). On Chiralpak IC 20 mg (*R/S*)-1 has been processed in 40 cycles of 11 min each (Fig. 2B). 9.6 mg of the (*R*) enantiomer (first eluted, yield: 48.0%; ee = 99.1 %; $[\alpha]_D^{20}$ – 23.9) and 9.5 mg of the (*S*) enantiomer (second eluted, yield: 47.5 %; ee = 98.9 %; $[\alpha]_D^{20}$ + 23.8) at an overall yield of 95.5 % (Table 3). In summary, using the available (semi)preparative set-up per day 72 mg

racemic 1 can be separated using enantioselective SFC on Chiralpak IA. Based on these experiments a specific productivity of 7.2 g racemate separated per 24 h on 1 kg of CSP can be assumed [35]. On Chiralpak IC in SFC 64.8 mg of racemic 1 can be separated/24 h. The specific productivity estimated is in the range of 6.5 g per kg CSP/24 h. The specific productivities observed are at least two orders of magnitude under those observed for commercial processes [36], ~~however, the objective of our development work was to obtain the previously never before described enantiomers in the quickest possible way with the tools at hand employing methods "fit-for-purpose~~". Actually, productivity of SFC separation could have been improved further performing a full optimization of the process (*i.e.* by using mobile phase composition ensuring higher solubility of the analytes - some portion of dichloromethane for example- or stacked injections instead of batch injections). Actually, the process was not fully optimized in preparative scale, mainly due to the limited amount of the molecule available, and also considering that i) the optimization might have taken one or two days, by which the compound was already isolated in high yield and enantiomeric excess, and, more importantly, ii) the objective of our development work was to obtain the enantiomers in the quickest possible way with the tools at hand employing methods "fit-for-purpose".

Our experiments show that the elution order [39] for the enantiomers of 1 is *S* before *R* on Chiralcel OJ-H in HPLC as well as on Chiralpak IA in SFC and *R* before *S* on Chiralpak IC in SFC, which allows to choose which enantiomer will be eluted as first peak (cf. Figure 2 and Table 3).

Please insert Figure 2 and Table 3

4. Conclusions

A systematic and pragmatic screening protocol for enantioselective HPLC was established for 1, which led to a fast and easy-to-use chiral HPLC separation suitable for a (semi)preparative scale-up. Overall time frame for screening, linear scale-up and isolation of *R*- and *S*-1 was less than two weeks.

As a result of a first standard screening, it was found that Chiralcel OJ-H and a mixture of methanol/diethylamine (99.9/0.1, v/v) lead to relatively short retention times, high enantioselectivity and good resolution (α = 1.8, R_s = 3.9). The (+)-(*S*)-1 enantiomer elutes as the first peak on Chiralcel OJ-H. The developed method proved to be suitable for obtaining a quick access to the desired enantiomers with enantiomeric excess as high as 99.9% and amounts sufficient for preliminary biological assays.

A rapid screening protocol under SFC-conditions run in parallel made it possible to identify another number of promising conditions for the enantiomer separation of 1. The protocol under SFC condition revealed an inversion of elution order of the enantiomers on Chiralcel IC using CO_2 with 25 % of the polar modifier IPA/n-heptane/diethylamine (90/10/0.1, v/v/v) as eluent.

Scale-up to (semi)preparative SFC allowed assessing productivities and recoveries under HPLC and SFC conditions. Even though recoveries and yields in (semi)preparative HPLC and SFC are in the same range and compounds with high enantiomeric excess were obtained through both technologies, the specific productivity of SFC method is almost 4 times lower that the specific productivity observed in (semi)preparative HPLC.

Employing the SFC system, the bottle neck is the injection volume possible for each run (50 µL), which turned out to be very limited. The eluent consumption on Chiralpak IA and Chiralpak IC is 3.3 and 2.6 times higher, respectively. However, under the consideration that the eluents in SFC consisted of 70 or 75 % CO_2, the organic solvent use for Chiralpak IA is equal to the amount of solvent used in (semi)preparative HPLC on Chiralcel OJ-H and one third lower on Chiralcel IC.

In summary, enantioselective (semi)preparative HPLC proved to be superior in the case of 1 in terms of specific productivity compared to SFC in our laboratory. The (-)-(*R*)-1 and (+)-(*S*)-1 enantiomers were obtained ~~for the first time~~ with an ee >99% and therefore can be used for an in-deep comparative examination of the pharmacological profile for the individual enantiomers of the new σ1 receptor agonist 1.

The recovery of the enantiomers after chromatography was in the range of 41 to 48 %, equivalent to 82 up to 96% of the theoretically possible yield of the individual enantiomers.

(Semi)preparative enantioselective chromatography for compounds of interest in medicinal chemistry proves to be a straightforward, productive and robust methodology for the quick access to the desired amounts of pure enantiomers even at low specific productivities. It remains one of the most versatile and cost effective tools for fast isolation of desired enantiomers from a racemic mixture.

Analytical and semi-preparative enantiomer separations have been developed “fit-for-purpose” using a limited number of CSPs and sub-optimal equipment for scale-up. In case larger amounts of the desired enantiomers will be required an intensified and broader screening of CSPs and mobile phases will be employed, for which ample protocols exist.

Compound 1 is one in a series of more than twenty structurally related compounds that have recently been screened and successfully separated employing the “fit-for-purpose”-protocol developed by the authors.

323 References

324

[1] S. Collina, R. Gaggeri, A. Marra, A. Bassi, S. Negrinotti, F. Negri, D. Rossi, Sigma receptor modulators: a patent review, Exp. Opin. Ther. Patent 23 (2013) 597-613.

[2] M. Peviani, F. Salvaneschi, L. Bontempi, A. Petese, A. Manzo, D. Rossi, M. Salmona, S. Collina, P. Bigini, D. Curti, Neuroprotective effects of the Sigma-1 receptor (S1R) agonist PRE-084, in a mouse model of motor neuron disease not linked to SOD1 mutation, Neurobiol. Dis. 62 (2014) 218-232.

[3] S.K. Kulkarni, A. Dhir, Effects of (+) SKF 10,047, a sigma-1 receptor agonist, on anxiety, tested in two laboratory models in mice, Expert Rev. Neurother. 9 (2009) 1021-1034.

[4] Bermack, J.E., Debonnel, G.J., Roles of sigma-1 receptors in Alzheimer's disease, J. Pharmacol. Sci. 97 (2005) 317–336.

[5] S. Collina, G. Loddo, M. Urbano, L. Linati, A. Callegari, F. Ortuso, S. Alcaro, C. Laggner, T. Langer, O. Prezzavent, G. Ronsisvalle, O. Azzolina, Design, synthesis and SAR analysis of novel selective sigma1 ligands, Bioorg. Med. Chem. 15 (2007) 771–783.

[6] D. Rossi, M. Urbano, A. Pedrali, M. Serra, D. Zampieri, M.G. Mamolo, C. Laggner, C. Zanette, C. Florio, D. Shepmann, B. Wünsch, O. Azzolina, S. Collina, Design, synthesis and SAR analysis of novel selective sigma1 ligands (Part 2), Bioorg. Med. Chem. 18 (2010) 1204–1212.

[7] D. Rossi, A. Pedrali, M. Urbano, R. Gaggeri, M. Serra, L. Fernandez, M. Fernandez, J. Caballero, S. Rosinsvalle, O. Prezzavento, D. Shepmann, B. Wünsch, M. Peviani, D. Curti, O. Azzolina, S. Collina, Identification of a potent and selective σ□ receptor agonist potentiating NGF-induced neurite outgrowth in PC12 cells., Bioorg. Med. Chem. 19 (2011) 6210–6224.

[8] D. Rossi, A. Marra, P. Picconi, M. Serra, L. Catenacci, M. Sorrenti, E. Laurini, M. Fermeglia, S. Price, S. Brambilla,N. Almirante, M. Peviani, D. Curti, S. Collina, Identification of RC-33 as a potent and selective σ1 receptor agonist potentiating NGF-induced neurite outgrowth in PC12 cells. Part 2: g-scale synthesis, physicochemical characterization and in vitro metabolic stability, Bioorg. Med. Chem. 21 (2013) 2577–2586.

[9] D. Rossi, A. Marra, M. Rui, E. Laurini, M. Fermeglia, S. Pricl, D. Schepmann, B. Wuensch, M. Peviani, D. Curti, S. Collina, A step forward in the sigma enigma: A role

for chirality in the sigma1 receptor-ligand interaction?, Med. Chem. Comm. 6 (2015) 138–146.

[10] R. Gaggeri, D. Rossi, S. Collina, B. Mannucci, M. Baierl, M., Juza, Quick development of an analytical enantioselective high performance liquid chromatography separation and preparative scale-up for the flavonoid Naringenin, J. Chromatogr. A 1218 (2011) 5414–5422.

[11] R. DePianta, K. Douville, B. Nickerson, R. Borjas, Chiral Screening Methods for Pharmaceutical Analysis and Purification in an Industrial Laboratory, in: S. Ahuja (Ed.), Chiral Separation Methods for Pharmaceutical and Biotechnological Products, John Wiley & Sons, Hoboken NJ, 2011, p 209-250.

[12] Jr. Leonard, W.R. Henderson, D.W. Miller, R.A. Spencer, G.A. Sudah, O.S. Biba, M., C.J. Welch, Strategic use of preparative chiral chromatography for the synthesis of a preclinical pharmaceutical candidate, Chirality 19 (2007) 693–700.

[13] L. Miller, M. Potter, Preparative chromatographic resolution of racemates using HPLC and SFC in a pharmaceutical discovery environment, J. Chromatogr. B 875 (2008) 230–236.

[14] K. De Klerck, D. Mangelings, Y. Vander Heyden, Supercritical fluid chromatography for the enantioseparation of pharmaceuticals, J. Pharm. Biomed. Anal. 69 (2012) 77–92.

[15] L.S. Ettre, Nomenclature for Chromatography, Pure Appl. Chem. 65 (1993) 819–872.

[16] European Pharmacopoeia, 2.2.29. LIQUID CHROMATOGRAPHY, 8th ed., EDQM, European Pharmacopoeia, Council of Europe, B.P. 907, 67029 Strasbourg, France, July 2013.

[17] DE2523565 (1975); FR2288518 (1976); DE2450989 (1976).

[18] A.A. Younesa, H. Ates, D. Mangelings, Y. Vander Heyden, A separation strategy combining three HPLC modes and polysaccharide-based chiral stationary phases, J. Pharm. Biomed. Anal. 75 (2013) 74–85.

[19] M. Juza, Optimization of Chiral Separations in HPLC, in: S. Kromidas (Ed.), HPLC Made to Measure - A Practical Handbook for Optimization, Wiley-VCH, Weinheim, 2006, p 427.

[20] C. Roussel, A. Del Rio, J. Pierrot-Sanders, P. Piras, N. Vanthuyne, Chiral liquid chromatography contribution to the determination of the absolute configuration of enantiomers, J. Chromatogr. A 1037 (2004) 311–328.

[21] K.W. Phinney, R.W., Stringham, Chiral Separations Using Supercritical Fluid Chromatography, in: G. Subramanian (Ed.), Chiral Separation Techniques, Wiley-VCH, Weinheim, 2007, pp. 148–153.

[22] K.L.Williams, L.C. Sander, Enantiomer separations on chiral stationary phases in supercritical fluid chromatography, J. Chromatogr. A 785 (1997) 149–158.

[23] C. West, Enantioselective Separations with Supercritical Fluids - Review, Curr. Anal. Chem. 10 (2014) 99-120.

[24] W. Ren-Qi, O. Teng-Teng, T. Weihua, N. Siu-Choon, Recent advances in pharmaceutical separations with supercritical fluid chromatography using chiral stationary phases, TrAC 37 (2012) 83–100.

[25] D. Baudelet, N. Schifano-Faux, A. Ghinet, X. Dezitter, F. Barbotin, P. Gautret, B. Rigo, P. Chavatte, R. Millet, C. Furman, C. Vaccher, E. Lipka, Enantioseparation of pyroglutamide derivatives on polysaccharide based chiral stationary phases by high-performance liquid chromatography and supercritical fluid chromatography: a comparative study, J. Chromatogr. A 1363 (2014) 257–269.

[26] J.C. Welch, Chiral Chromatography in Support of Pharmaceutical Process Research, in: G. Cox (Ed.), Preparative Enantioselective Chromatography, Blackwell Publishing Ltd., London, 2005, pp. 10–18.

[27] E. Lesellier, C. West, The many faces of packed column supercritical fluid chromatography – A critical review, J. Chromatogr. A 1389 (2015) 49–64.

[28] C. Hamann, M. Wong, I. Aliagas, D.F. Ortwine, J. Pease, D.E., Schmidt Jr., J. Victorino, The evaluation of 25 chiral stationary phases and the utilization of sub-2.0μm coated polysaccharide chiral stationary phases via supercritical fluid chromatography, J. Chromatogr. A 1305 (2013) 310–319.

[29] M. Maftouh, C. Granier-Loyaux, E. Chavana, J. Marini, A. Pradines, Y. Vander Heyden, C. Piccard, Screening approach for chiral separation of pharmaceuticals. Part III. Supercritical fluid chromatography for analysis and purification in drug discovery, J. Chromatogr. A 1088 (2005) 67–81.

[30] R.W. Stringham, K.G. Lynam, C.C. Grasso, Application of subcritical fluid chromatography to rapid chiral method development, Anal. Chem. 66 (1994) 1949–1954.

[31] G. Terfloth, Enantioseparations in super- and subcritical fluid chromatography, J. Chromatogr. A 906 (2001) 301–307.

[32] S. Golshan-Shirazi, G. Guiochon, Theory of Optimization of the Experimental Conditions of Preparative Elution Chromatography: Optimization of the Column Efficiency, Anal. Chem. 61 (1989) 1368-1382.

[33] H. Colin, Large-Scale High Performance Preparative Liquid Chromatography, in: G. Ganetsos, P.E. Parker (Eds.), Preparative and Production Scale Chromatography Marcel Dekker, Inc., New York, 1993, p 15.

[34] A.S. Rathore, A. Velayudhan, An Overview of Scale-Up in Preparative Chromatography, in: A.S. Rathore, A. Velayudhan (Eds.), Scale-Up and Optimization in Preparative Chromatography, Marcel Dekker, Inc., New York, 2003, pp. 1–32.

[35] M. Schulte, R. Ditz, R.M., Devant, J.N., Kinkel, F. Charton, Comparison of the specific productivity of different chiral stationary phases used for simulated moving-bed chromatography, J. Chromatogr. A, 769 (1997) 93–100.

[36] L. Miller, Use of dichloromethane for preparative supercritical fluid chromatographic enantioseparations, J. Chromatogr. A. 1363 (2014) 323–330.

[37] A. Tarafder, C. Hudalla, P. Iraneta, J.K. Fountain, A scaling rule in supercritical fluid chromatography. I. Theory for isocratic systems, J. Chromatogr. A 1362 (2014) 278–293.

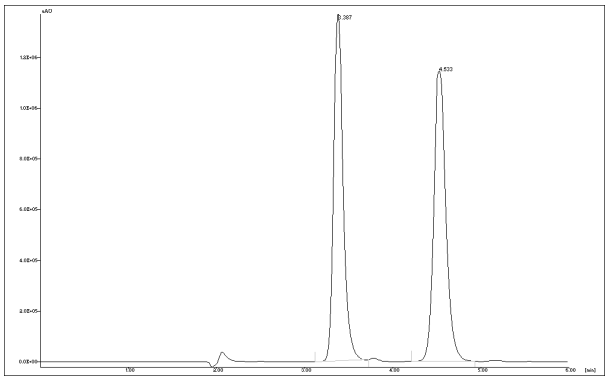
[38] A. Rajendran, Design of preparative-supercritical fluid chromatography, J. Chromatogr. A 1250 (2012) 227–249.

[39] M. Okamoto, Reversal of elution order during the chiral separation in high performance liquid chromatography, J. Pharm. Biomed. Anal 27 (2002) 401–407.

Figure legends

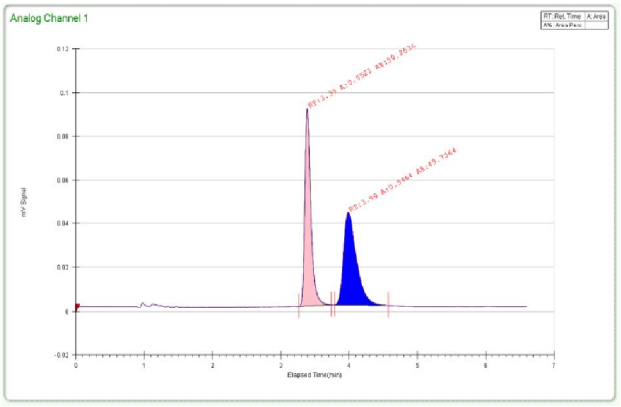
Figure 1. Analytical enantiomer separation of (R/S)-1 on A) Chiralcel OJ-H (4.6 mm x 150 mm, d_p = 5µm), t_{R1}: 3.4 min; t_{R2}: 4.6 min at r.t; B) Chiralpak IA (25 cm x 0.46 cm, dp = 5 µm), t_{R1}: 3.39 min; t_{R2}: 3.99 min at 40°C; C) Chiralpak IC (25 cm x 0.46 cm, dp = 5 µm), t_{R1}: 2.82 min; t_{R2}: 3.53 at 40°C For all: Injection volume 10 µl, detection at 254 nm, eluent composition and flow rates see text in Figure.

A



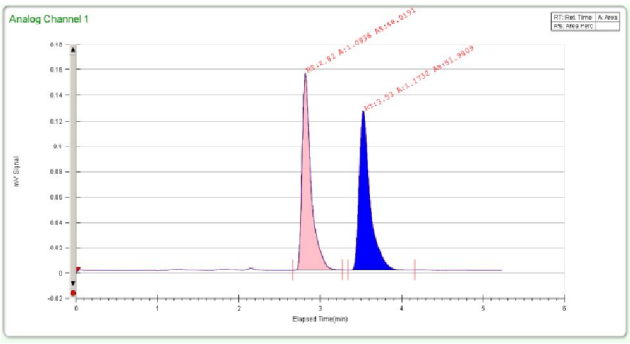
System: **HPLC**
Column: Chiralcel OJ-H
Eluent: MeOH/DEA (99.9/0.1, v/v)
Flow rate: 1.0 mL min^{-1}
α = 1.83; R_s = 4.89

B



System: **SFC**
Column: Chiralpak IA
Eluent: 70% CO_2, 30% n-Hp/EtOH/DEA (9/1/0.1, v/v/v)
Flow rate: 4.0 mL min^{-1}
α = 1.25; R_s = 2.56

C



System: **SFC**
Column: Chiralpak IC
Eluent: 70% CO_2, 25% IPA/n-Hp/DEA (9/1/0.1, v/v/v)
Flow rate: 4.0 mL min^{-1}
α = 1.39, R_s = 4.25

Figure 2. (Semi)preparative enantiomer separation of 1 by SFC and final analysis.
Left: (Semi)preparative enantiomer separation of racemic 1 A) on Chiralpak IA (10.0 mm x 250 mm, d_p = 5µm), eluting with 70% of CO_2 and 30% of n-Hp/EtOH/DEA (9/1/0.1%, v/v/v), flow rate 10 mL min^{-1}, t_{R1}: 6.5 min, t_{R2}: 7.25 min (α = 1.16, R_s = 0.93), injection volume 0.05 mL (c = 10 mg mL^{-1} in IPA); and B) on Chiralpak IC (10.0 mm x 250 mm, d_p = 5µm), eluting with 75% of CO_2 and 25% of IPA/n-Hp/DEA (9/1/0.1%, v/v/v), flow rate 8 $mLmin^{-1}$, t_{R1}: 7.1 min; t_{R2}: 9.2 min (α = 1.45, R_s = 2.25). In both cases the injection volume

was 0.05 mL (c = 10 mg mL^{-1} in IPA), detection at 254 nm at 40°C. Cut-points for fraction collection are indicated in the chromatogram with horizontal dashes (⊓).

Right: Analytical enantioselective analysis of first and second collected fraction on Chiralpak IA (4.6. mm ID x 250 mm, dp = 5 µm), eluting with 70% of CO_2 and 30% n-Hp/EtOH/DEA (90/10/0.1, v/v/v), flow rate 4 mL min^{-1} at 40°C. Analytes were detected at 254 nm. A) t_{R1}: 3.21 min; t_{R2}: 3.71 min at 40°C; B) t_{R1}: 3.33 min (second eluted enantiomer on Chiralpak IC) t_{R2}: 3.95 min (first eluted enantiomer on Chiralpak IC).

Figure 2

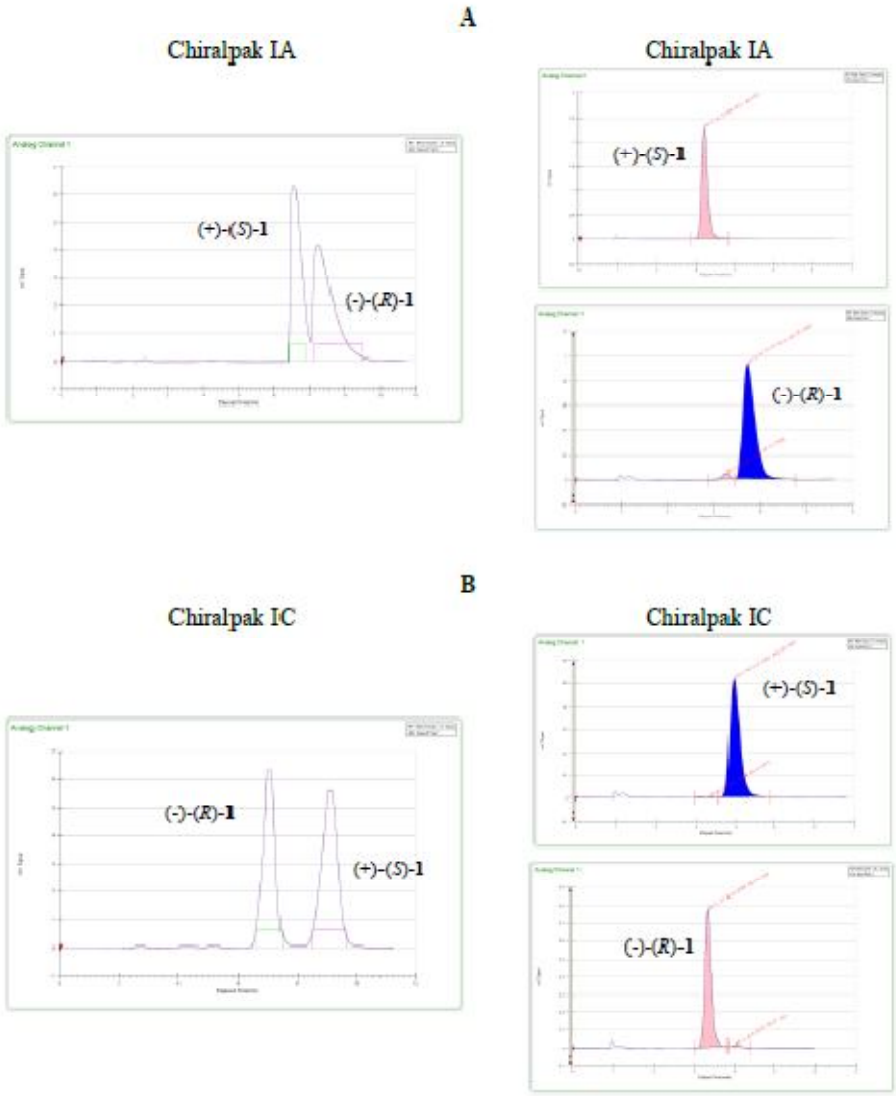
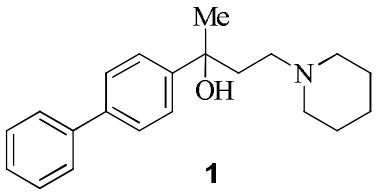


Table 1

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	1.50	1.60	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	1.20	2.07	0.76	0.97	1.28	2.0
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.

Table 2

Screening results for enantiomer separation of (*R/S*)-**1** *via* SFC.

		Chiral Stationary Phase							
	Per-centage	**Chiralpak IA**				**Chiralpak IC**			
Organic modifier[a]	**[%]**	k_A	k_B	**α**	**Rs**	k_A	k_B	**α**	**Rs**
MeOH	**5-45**[b]	4.9	5.5	1.12	1.48	6.4	6.9	1.08	1.57
	10	6.6	8.3	1.26	1.18	n.t.		**10**	6.6	8.3
	20	2.4	3.2	1.33	1.89	4.2	5.0	1.19	2.07
	30	n.t.				1.9	2.2	1.16	1.18
EtOH	**5-45**[b]	4.2	1.0	n.a.	4.9	5.5	1.12	1.74	4.2
IPA	**5-45**[b]	3.9	4.3	1.10	1.77	5.1	5.9	1.16	2.07
	15	2.2	2.9	1.32	2.95	n.t.			
	20	1.4	1.8	1.29	1.95	2.4	3.4	1.42	1.11
	25	0.9	1.2	1.33	2.12	n.t.			
	30	n.t.				1.2	1.6	1.33	0.89
IPA/*n*-Hp (9/1, v/v)	**15**	1.4	1.9	1.36	2.66	n.t.			
	25	n.t.				**1.8**	**2.5**	**1.39**	**4.25**
	30	n.t.				1.3	1.8	1.39	2.70
IPA/*n*-Hp (8/2, v/v)	**15**	2.5	3.3	1.32	1.77	n.t.			
***n*-Hp/EtOH (9/1, v/v)**	**30**	**2.4**	**3.0**	**1.25**	**2.56**	n.t.			

[a] All modifiers contained 0.1% DEA; [b] Gradient conditions: linear decrease from 95 to 55 % of CO_2 from the time 0 to 10.25 minutes; isocratic at 55% CO_2 for 2 minutes; return to the

initial conditions (95% CO_2) in 15 seconds, equilibration of the system from 12.40 min to 18 min at 95% CO_2; n.t. not tested.

Table 3 Conditions and isolated amounts of (+)-(*S*)-**1** and (-)-(*R*)-**1** obtained by (semi)preparative enantioselective SFC or HPLC starting from racemic **1**.

System	(Semi) preparative CSP	Amount of (*R/S*)-1 separated [mg]	n° cycles	Vol. Inj (µL)	Specific Productivity [kkd][d]	Isolated amount [mg]	ee [%]	Yield [%]	$[\alpha]_D^{20a}$
HPLC	Chiralcel OJ-H	21	7	1mL[b]	0.0270	8.7	99.9	43.3	+ 24.0
						9.1	99.9	45.5	- 24.0
SFC	Chiralpak IA	20	40	50µL[c]	0.0072	9.1	99.9	45.5	+ 24.0
						8.2	94.5	41.0	- 23.1
	Chiralpak IC	20	40	50µL[c]	0.0065	9.6	99.1	48.0	- 23.9
						9.5	98.9	47.5	+ 23.8

[a] c = 0.50 % in MeOH

[b] c = 3 mg mL^{-1} in MeOH

[c] c = 10 mg mL^{-1} in IPA

[d] kkd = kg racemate separated per kg CSP per day