Talanta 82 (2010) 426–431

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Application of an immobilised amylose-based chiral stationary phase to the development of new monoamine oxidase B inhibitors

Maria Luisa Sanna[a], Elias Maccioni[b], Sara Vigo[b], Cristina Faggi[c], Roberto Cirilli[a,*]

[a] Istituto Superiore di Sanità, Dipartimento del Farmaco, Viale Regina Elena 299, 00161 Rome, Italy
[b] Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Cagliari, Cagliari, Italy
[c] Università degli studi di Firenze, Dipartimento di Chimica Organica, Sesto Fiorentino, Firenze, Italy

ARTICLE INFO

Article history:
Received 19 February 2010
Received in revised form 8 April 2010
Accepted 16 April 2010
Available online 24 April 2010

Keywords:
Chiralpak IA
Enantioselective HPLC
Enantiomers
MAO-inhibitors
Absolute configuration
Chiroptical properties

ABSTRACT

A direct HPLC enantioseparation of three new chiral oxadiazoline derivatives endowed with potential MAO-B inhibitory activity was accomplished on the immobilised Chiralpak IA chiral stationary phase. Multi-mg amounts of enantiomers with high enantiomeric purity (ee ≥ 98%) were rapidly collected using pure dichloromethane as eluent. The absolute configuration and chiroptical properties of the enantiomers isolated at semipreparative scale were exhaustively determined.

1. Introduction

Monoamine oxidases (MAOs) are key role enzymes which catalyze the oxidative deamination of both endogenous and exogenous amines [1]. According to chemical structure, localization, physiological function, and substrates/inhibitors selectivity, two different enzymatic isoforms, named MAO-A and MAO-B, have been described and fully characterized [2–6]. Both MAO isoforms are important in the metabolism of monoamine neurotransmitters and, as a result, MAO inhibitors (MAOi) are studied for the treatment of several psychiatric and neurological diseases. In particular, MAO-B inhibitors are coadjuvant in the treatment of both Parkinson's (PD) [7] and Alzheimer's diseases (AD) [8], while MAO-A inhibitors are used as antidepressant and antianxiety drugs [9].

Recently the design, synthesis, and HPLC enantioseparation of efficient and selective MAO-B inhibitors have been reported by some of us [10–13].

Pursuing on our research on the design of new and even more efficient scaffolds for the selective inhibition of the B isoform of MAO, we have synthesised a series of 3-acetyl-2,5-diaryl-2,3-dihydro-1,3,4-oxadiazoles which can be considered isosters of previously reported highly efficient 3,5-diaryl-4,5-dihydropyrazole-1-carbothioamides [14]. The chemical structure of three representative molecules of this new family of potential MAO-B inhibitors is shown in Fig. 1. In a preliminary investigation the compounds **1**–**3** exhibited promising biological properties [14]. Chirality has already been demonstrated to play an important role in determining the inhibitory effect and isoform selectivity of similar compounds. In view of further studies pointed to check the *in vitro* biological behavior of each of the two enantiomers, an efficient method to produce enantiopure forms of the novel leads **1**–**3** was mandatory.

Enantiomers of new chiral molecules must be considered as two distinct compounds and tested separately for their biological effects. The availability of enantiopure forms and the knowledge of their absolute configuration may uniquely contribute to get informations about the groups and the forces involved in the biological molecular recognition events.

Although a large number of approaches have been used to produce single enantiomers, enantioselective HPLC has taken a leading role in this area and it has become the focus of intensive researches. Great efforts have been devoted to design new selectors with increased enantioselectivity that allowed to quickly isolate both enantiomers of heterogeneous classes of building blocks, intermediates or final products of chiral drugs in high enantiomeric purity and in quantities suitable for comparative biological evaluations, synthetic applications and stereochemical characterization studies.

* Corresponding author. Tel.: +39 06 49902407.
E-mail address: rcirilli@iss.it (R. Cirilli).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.04.039

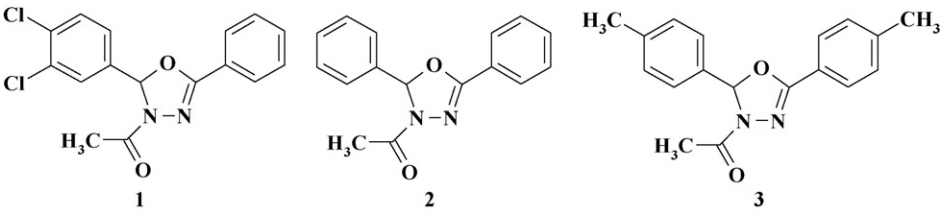

Fig. 1. Chemical structure of the compounds **1–3**.

Among the commercially available CSPs for HPLC, the esters or carbamate derivatives of cellulose and amylose appear to be the most widely used selectors for the enantioseparation of a wide large of chiral compounds including drugs. These polymeric selectors are coated [15,16] or immobilised on μm-particles of silica matrix [17–20]. The first type of CSP has been prepared by coating the selector onto macroporous silica gel and can be used with a restricted number of solvents, usually n-hexane, alcohols and acetonitrile in normal phase and polar organic modes and acetonitrile–water mixture in reversed-phase conditions. The immobilised-type CSPs break this limitation as a result of the anchoring of the polymeric selector onto silica support [21].

Then, the preference of immobilised CSPs on the coated ones lies in their inherent stability and flexibility of use [22–25]. The freedom of choosing any type of solvents in the preparation of mobile phase allows to define new enantioselective conditions which are forbidden for coated-type CSPs [26–28]. The advantage of this approach is particularly useful in the multi-mg enantioseparation of compounds with a limited solubility in n-hexane, alcohols or acetonitrile [29], which are the only solvents compatible with semipreparative columns based on the coated polysaccharide derivatives.

In this work the chiral recognition ability of the immobilised Chiralpak IA CSP towards the compounds **1–3** was evaluated using polar organic conditions. Method optimization for semipreparative enantioseparation of the investigated analytes and their stereochemical characterisation was discussed.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). The dichloromethane used was stabilized with amylene. HPLC enantioseparations were performed by using stainless-steel Chiralpak IA (250 mm × 4.6 mm i.d. and 250 × 10 mm i.d.) columns (Chiral Technologies Europe, Illkirch, France). All chemicals solvents for synthesis and spectral grade solvents were purchased from Aldrich (Italy) and used without further purification.

2.2. Instruments and chromatographic conditions

The analytical HPLC apparatus consisted of a PerkinElmer (Norwalk, CT) 200 lc pump equipped with a Rheodyne (Cotati, CA) injector, a 20-μl sample loop, a HPLC Dionex CC-100 oven (Sunnyvale, CA) and a Jasco (Jasco, Tokyo, Japan) Model CD 2095 Plus UV/CD detector. For semipreparative separations a PerkinElmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 1000 μl sample loop, a PerkinElmer LC 101 oven and Waters 484 detector (Waters Corporation, Milford, MA, USA) were used. The signal was acquired and processed by Clarity software (DataApex, Prague, The Czech Republic).

Mobile phases were filtered and degassed by sonication shortly before using. Experimental conditions for analytical enantioseparations are indicated in Table 1.

Table 1
Retention factor (k_1) for the first eluting enantiomer, enantioseparation (α) and resolution (R_s) factors of **1–3** in polar organic conditions.

Compound	Mobile phase	k_1 (AC)[a]	a	R_s
1	Methanol[b]	0.77 (*R*)	1.75	5.50
	Ethanol[c]	0.58 (*R*)	1.63	5.10
	Acetone[b]	0.19 (*R*)	1.00	–
	Ethyl acetate[b]	0.18 (*R*)	1.67	2.03
	Chloroform[c]	0.04 (*R*)	3.07	2.55
	Dichloromethane[b]	0.13 (*R*)	3.43	5.35
2	Methanol	0.47 (*R*)	1.58	3.61
	Ethanol	0.42 (*R*)	1.56	3.63
	Acetone	0.15 (*R*)	1.00	–
	Ethyl acetate	0.19 (*R*)	1.62	1.53
	Chloroform	0.04 (*R*)	2.55	1.25
	Dichloromethane	0.13 (*R*)	3.13	4.27
3	Methanol	0.73 (*R*)	1.57	4.33
	Ethanol	0.51 (*R*)	1.27	1.86
	Acetone	0.18 (*R*)	1.00	–
	Ethyl acetate	0.24 (*R*)	1.52	2.04
	Chloroform	0.04 (*R*)	2.96	1.29
	Dichloromethane	0.17 (*R*)	2.55	3.92

Column, Chiralpak IA (250 mm × 4.6 mm i.d.).
[a] Absolute configuration of the first-eluted enantiomer.
[b] Flow-rate: 1 ml min^{-1}; temperature, 25 °C; detection, UV at 250, 280 (ethyl acetate), 330 (acetone).
[c] Flow-rate: 0.5 ml min^{-1}; temperature, 25 °C; detection, UV at 250, 280 (ethyl acetate), 330 (acetone).

In analytical separations, fresh standard solutions were prepared shortly before use by dissolving about 1 mg of each analyte in 10 ml of mobile phase. The injection volume was of 20 μl. After the semipreparative separation, the collected fractions were pooled, evaporated and analyzed by a chiral analytical column to determine their enantiomeric excess (ee).

The column hold-up time (t_0 = 3.0 min for 250 mm × 4.6 mm i.d. column) was determined from the elution of an unretained marker (toluene), using methanol as eluent, delivered at a flow-rate of 1.0 ml min^{-1}.

Specific rotations of the enantiomers of **1–3** in chloroform were measured at 589 nm by a PerkinElmer (Norwalk, CT, USA) polarimeter model 241 equipped with a Na lamp. The volume of the cell was 1 ml and the optical path 10 cm. The system was set at a temperature of 20 °C using a Neslab RTE (Manasquan, New Jersey, USA) 740 cryostat.

Melting points (uncorrected) were determined automatically on a Electrothermal 9001. ^{1}H NMR spectra were recorded onVarian Unity 600,^{13}C NMR spectra on Bruker Avance TM 300; deuterated chloroform ($CDCl_3$) was used as the solvent.

Chemical shifts are expressed as δ units (parts per million) using TMS as an internal standard. Coupling constants J are valued in Hertz (Hz). Elemental analysis for C, H, and N were recorded on a PerkinElmer 240 B microanalyzer and the analytical results were within ±0.4% of the theoretical values for all compounds.

All reactions were monitored by TLC performed on silica gel Plates 0.2 mm thick (60 F254 Merck); spots were visualised by UV light.

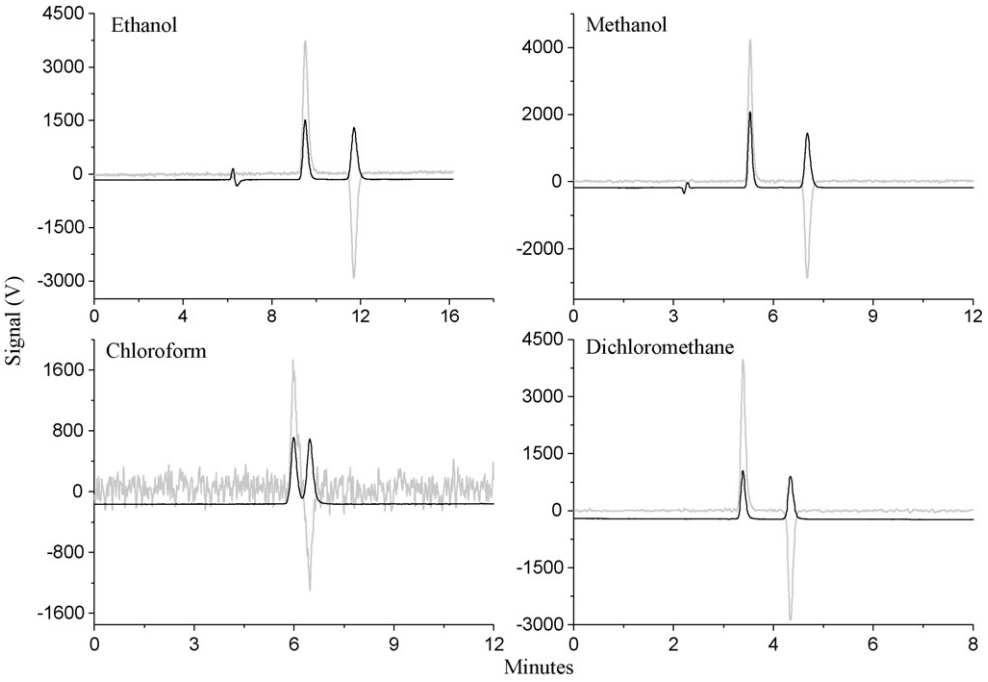

Fig. 2. HPLC chromatograms of the compound **1**. Column, Chiralpak IA (250 mm × 4.6 mm i.d.); eluent: ethanol, methanol, chloroform and dichloromethane; detector, UV (black) and CD (grey) at 250 nm; see Table 1 for other conditions.

2.3. Enantiomeric elution order determination

The enantiomeric elution order on the Chiralpak IA CSP was established by analysing samples enriched by the (*S*)-enantiomer.

2.4. General procedure for the synthesis of 3-acetyl-2,5-diaryl-2,3-dihydro-1,3,4-oxadiazoles (**1**–**3**)

N′-arylidene-benzohydrazide (0.003 mol) and acetic anhydride (0.003 mol, 6 ml) are refluxed under vigorous stirring for a period ranging from 15 min to 2 h. The reaction suspension is monitored by TLC (mobile phase: chloroform–methanol 20:1). Generally the complete solubilisation of the suspension together with the formation of an intense orange coloration indicates the end of the reaction.

The solution is then poured into ice water (100 g) and vigorously stirred. A precipitate is formed which is washed with $NaHCO_3$ (10% water solution) to remove the acetic acid. The obtained solid is further purified by crystallisation with an appropriate solvent.

According to the following procedure the compounds **1**–**3** were synthesized.

2.4.1. 3-Acetyl-2-(3,4-dichlorophenyl)-5-phenyl-2,3-dihydro-1,3,4-oxadiazole (**1**)

$C_{16}H_{12}Cl_2N_2O_2$. White crystals, m.p. 99–100 °C (ethanol), yield 43%.

^{1}H NMR (600 MHz, $CDCl_3$): δ_H 2.36 (3H, s, CH_3, $COCH_3$), 7.02 (1H, s, CH, C_2H-oxadiaz.), 7.34 (1H, dd, *J* 8.3/2.0, CH, 3,4-di-Cl– phenyl), 7.46 (3H, cum, CH, 3,4-di-Cl– phenyl, phenyl), 7.52 (1H, t, *J* 7.4, CH, phenyl), 7.56 (1H, d, *J* 2.1, CH, 3,4-di-Cl– phenyl), 7.88 (2H, d, *J* 7.2, CH, phenyl).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 21.43 (–CH_3, Acyl), 90.83 (>CH–, C_2H–oxadiaz.), 124.10 (>C=, phenyl), 126.03 (=CH–, 3,4-di-Cl–phenyl), 127.01 (=CH–, phenyl), 128.63 (=CH–, 3,4-di-Cl– phenyl), 128.79 (=CH–, phenyl), 130.83 (=CH–, 3,4-di-Cl– phenyl), 131.90 (=CH–, phenyl), 133.11 (>C=, 3,4-di-Cl– phenyl), 134.16 (>C=, 3,4-di-Cl– phenyl), 136.51 (>C=, 3,4-di-Cl– phenyl), 155.67 (>C=, CON), 168.12 (>C=O, $COCH_3$).

2.4.2. 3-Acetyl-2,5-diphenyl-2,3-dihydro-1,3,4-oxadiazole (**2**)

$C_{16}H_{14}N_2O_2$. Pale yellow crystals, m.p. 97–100 °C (isopropyl ether), yield 65%.

^{1}H NMR (600 MHz, $CDCl_3$): δ_H 2.36 (3H, s, CH_3, $COCH_3$), 7.1 (s, 1H, C_2H-oxadiaz.), 7.39–7.41 (3H, m, CH, phenyl), 7.44 (2H, t, *J* 7.7, CH, phenyl), 7.47 −7.52 (3H, m, CH, phenyl), 7.97 (2H, d, *J* 8.5, CH, phenyl).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 21.55 (–CH_3, Acyl), 92.36 (>CH–, C_2H–oxadiaz.), 123.96 (=CH–, phenyl), 124.63 (>C=, phenyl), 126.63 (=CH–, phenyl), 127.08 (=CH–, phenyl), 128.77 (=CH–, phenyl), 128.84 (=CH–, phenyl), 131.71 (=CH–, phenyl), 136.50 (>C=, phenyl), 155.86 (>C=, CON), 167.93 (>C=O, $COCH_3$).

2.4.3. 3-Acetyl-2,5-di(4-methylphenyl)-2,3-dihydro-1,3,4-oxadiazole (3)

$C_{18}H_{18}N_2O_2$. White crystals, m.p. 136–137 °C (isopropyl ether), yield 62%.

^{1}H NMR (600 MHz, $CDCl_3$): δ_H 2.34 (6H, cum, CH_3, $COCH_3$, 4-CH_3– phenyl), 2.41 (3H, s, CH_3, 4-CH_3– phenyl), 7.03 (1H, s, CH, C_2H-oxadiaz.), 7.19 (d, 2H, *J* 8.0, CH, 4-CH_3– phenyl), 7.24 (2H, d, *J* 8.1, CH, 4-CH_3– phenyl), 7.37 (2H, d, *J* 8.1, CH, 4-CH_3– phenyl), 7.8 (2H, d *J* 8.3, CH, 4-CH_3– phenyl).

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 21.31 (–CH_3, Acyl), 21.46 (–CH_3, 4-CH_3–phenyl), 21.64 (–CH_3, 4-CH_3– phenyl), 92.15 (>CH–, C_2H-oxadiaz.), 121.76 (>C=, 4-CH_3–phenyl), 126.51 (=CH–, 4-CH_3–phenyl), 126.96 (=CH–, 4-Cl– phenyl), 128.38 (=CH–, 4-CH_3–phenyl), 129.41 (=CH–,4-CH_3– phenyl), 133.68 (>C=, 4-Cl-phenyl), 142. 13 (>C=, 4-CH_3-phenyl), 155.94 (>C=, CON), 159.87 (>C=, 4-Cl-phenyl), 167.66 (>C=O, $COCH_3$).

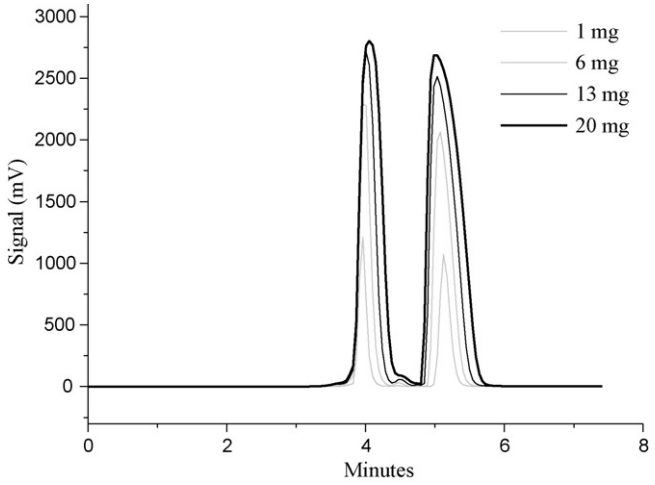

Fig. 3. Loading study of the compound **1**. Column: Chiralpak IA (250 mm × 10 mm i.d.); eluent, dichloromethane; flow-rate: 4.0 ml min^{-1}; detector, UV at 350 nm; temperature, 25 °C.

2.5. X-ray diffraction analysis of (+)-*1*

Colourless needle shaped crystals of (+)-**1** suitable for collection were obtained by crystallization from ethanol/water and X-ray analysis were carried out with a Goniometer Oxford Diffraction KM4 Xcalibur2 (Oxford Diffraction, Yarnton, UK) at room temperature.

Cu/Kα radiation (40 mA/−40 kV), monochromated by an Oxford Diffraction Enhance ULTRA assembly, and an Oxford Diffraction Excalibur PX Ultra CCD as detector, were used for cell parameters determination and data collection.

The integrated intensities, measured using the ω scan mode, were corrected for Lorentz and polarization effects [30]. Direct methods of SIR97 [31] were used to resolve the structure and it was refined using the full-matrix least squares on F^2 provided by SHELXL97 [32].

Multi-scan symmetry-related measurement was used as the experimental absorption correction type. The non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined as isotropic and all of them were assigned in calculated positions except H2 (hydrogen on chiral C2) which density was found in the Fourier difference map.

Crystal data $C_{16}H_{12}N_2Cl_2O_2$ M = 350.21, Monoclinic, space group *P*21, $a = 9.0956(1)$, $b = 6.552(1)$, $c = 13.870(1)$ Å, $\beta = 95.982(4)$, $V = 822.0(2)$ Å^3, $Z = 2$, $D_c = 1.415$, $\mu = 3.643$ mm^{-1}, $F(0\,0\,0) = 362.3040$ reflections were collected with a $4.89 < \theta < 69.78$ range with a completeness to theta 95.7%; 2307 were independent, the parameters were 213 and the final *R* index was 0.0446 for reflections having $I > 2\sigma I$, and 0.0689 for all data. Refinement of the absolute structure Flack parameter give expected value for the right configuration (0.04).

The X-ray CIF file for this structure has been deposited at the Cambridge Crystallographic Data Center and allocated with the deposition number CCDC 758787.

Copies of the data can be obtained, free of charge, from CCDC, 12 Union Road, Cambridge, CB2 1EZ UK (e-mail: deposit@ccdc.cam.ac.uk; internet://www.ccdc.cam.ac.uk).

3. Results and discussion

3.1. Analytical and semipreparative enantioseparation

As already mentioned, the preliminary *in vitro* biological and stereochemical characterisation of new chiral molecules requires both enantiomers in amounts of several tens of mg. The use of chiral pool or prochiral substrates activated by optically active auxiliaries or catalysts provide only one of the two enantioenriched forms. Moreover, the asymmetric synthetic approach needs laborious work to set the many parameters (temperature, solvent, concentration, etc.) that influence the success of the reaction in terms of enantioselectivity and yield. In the initial steps of drug development, the direct HPLC resolution procedure presents the advantage over competing techniques for rapidly accessing both enantiopure forms of a drug candidate compound. Following the identification of an appropriate enantioselective

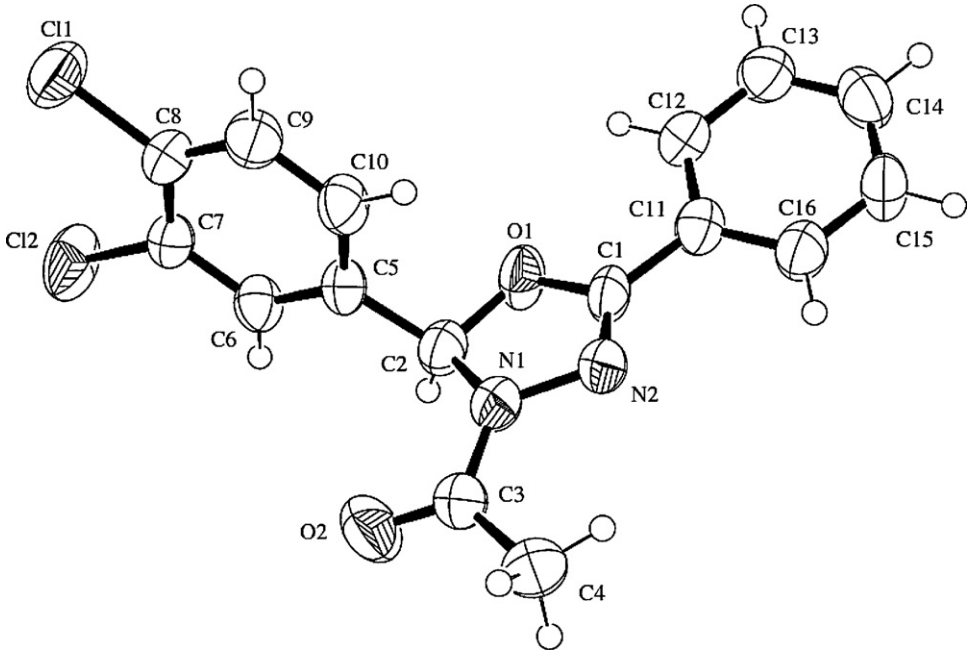

Fig. 4. An ORTEP view of the molecular structure of (*R*)-(+)-**1** enantiomer.

Table 2
Chromatographic and polarimetric analysis of the pooled fractions containing the first (F1) and second (F2) eluted enantiomers of **1–3**.

Compound	A[a]/V[b]	F1[c]		F2[c]	
		e.e. (%)	$[\alpha]_D^{20}$	e.e. (%)	$[\alpha]_D^{20}$
1	20/1.0	>99.0	+32 ($c = 0.1$, $CHCl_3$)	>99.0	−31 ($c = 0.1$, $CHCl_3$)
2	25/1.0	>99.0	+113 ($c = 0.1$, $CHCl_3$)	99.0	−109 ($c = 0.1$, $CHCl_3$)
3	5/0.5	99.0	+70 ($c = 0.1$, $CHCl_3$)	98.0	−66 ($c = 0.1$, $CHCl_3$)

Column: Chiralpak IA (250 mm × 10 mm i.d.); eluent: dichloromethane; flow-rate: 4.0 ml min^{-1}; detector: UV at 335 nm; temperature: 25 °C.
[a] Amount of sample (in mg) resolved in a single semipreparative run.
[b] Volume of sample (in ml).
[c] Enantiomeric purity and polarimetric data for the pooled fractions containing the less (F1) and more (F2) retained enantiomers.

CSP/eluent analytical system, the process can be easily scaled-up at semipreparative level using columns with larger internal diameter (i.d.). However, the productivity of the enantioseparation can be strongly limited by an inadequate discrimination ability of the chiral selector or a low dissolving power of the mobile phase.

In this study, we evaluated the enantioselective ability of the immobilised amylose-based Chiralpak IA CSP towards the compounds **1**–**3** using pure ethanol, methanol, ethyl acetate, acetone, dichloromethane and chloroform as eluents.

The enantioseparation results are listed in Table 1. In all the cases, the separation factors and peak efficiency were high enough to observe very good enantioseparations. Chiralpak IA CSP exhibited somewhat similar chiral recognition ability in alcohols and ethyl acetate. A better enantioselectivity could be attained using chlorinate eluents, which are prohibited with the corresponding coated-type Chirapak AD CSP which contains the same amylose tris(3,5-dimethylphenylcarbamate) selector. A typical example of this trend is showed in Fig. 2. The compounds **1** could be resolved with separation factors of 1.75 and 1.63 using pure methanol and ethanol, respectively. Such values increased to 3.07 and 3.43 in chloroform and dichloromethane. The similar effect was observed for the other two compounds. The α-values for the compound **2** were very close to that observed for **1** and superior with respect to those of the compound **3**. The elution order of the enantiomers was the same in each condition investigated with (*R*) eluting before (*S*). The chiral recognition ability was completely worse using acetone as eluent.

Pure dichloromethane not only led to the best enantioselectivity but also revealed the highest dissolving power for the samples **1**–**3**. The sample solubility was practically null in n-hexane and low in alcohols (about 1 mg/ml).

So, based on the chromatographic results of the analytical screening and the sample solubility evaluations, pure dichloromethane was identified as the most suitable eluent for the semipreparative enantioseparation of **1**–**3** on the Chiralpak IA CSP.

Table 2 shows the enantioselective HPLC and polarimetric analysis for the isolated enantiomers. All the racemic compounds were resolved into their enantiomers with high yields (>90%) and enantiomeric purity (ee ≥ 98%). The enantiomers of all compounds could be separated in one run within 8 min.

In Fig. 3 is showed a loading study for the compound **1**. The amount of racemic sample injected onto a 1-cm i.d. IA column was progressively increased from 1 to 20 mg. As showed by corresponding chromatograms, a complete separation of two enantiomeric peaks was demonstrated also in the maximum sample loading condition. The excellent performance of the Chiralpak IA CSP made possible the production of about 55 mg/h for each enantiomer with ee > 99%.

Polarimetric analysis indicated that the first eluting enantiomer collected on the semipreparative scale was dextrorotatory in ethanol solution at the wavelength of 589 nm.

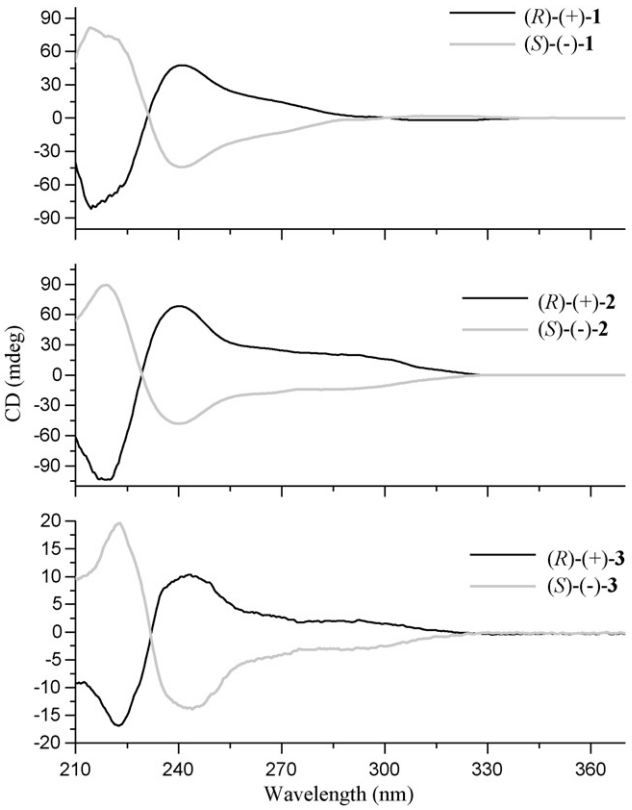

Fig. 5. CD spectra of the enantiomers of **1**–**3** in ethanol.

3.2. Absolute configuration assignment

The assignment of the absolute configuration of chiral bioactive compounds is a matter of great importance to comprehend the mechanisms involved in the different interactions of enantiomers with the biological target. A new class of chiral agents with inhibitory activity against MAO should be subject to additional studies to evaluate the influence of stereochemistry on the affinities for the single MAO isoforms and MAO-B/MAO-A selectivity [11,12].

Providing individual enantiomers, the stereochemical assignment may be performed by single-crystal X-ray diffraction technique. We were able to obtain suitable crystals for crystallographic analysis only in the case of the first eluting enantiomer of the compound **1**. The molecular structure of (+)-**1** as observed in the crystal structure is shown in Fig. 4. The absolute configuration of the stereogenic centre of the 2,3-dihydro-1,3,4-oxadiazole nucleus was determined to be (*R*). Known the absolute configuration of (+)-**1** the stereochemistry of the enantiomers of the other two compounds was indirectly achieved by CD spectroscopy. As showed in Fig. 5 the nature of substituents in both aromatic rings of **1**–**3** did not substantially influence the CD behavior, since all

three compounds displayed very similar CD curves. So, on the basis of the of X-ray and CD results all first-eluted enantiomers on the Chiralpak IA CSP had the (*R*) configuration and the more retained ones the (*S*) configuration.

4. Conclusions

The immobilised Chiralpak IA CSP revealed high chiral recognition ability towards new oxadiazoline derivatives endowed with potential MAO-B inhibitor activity. Due to its universal solvent compatibility and high loading capacity Chiralpak IA could be stably used for rapid and efficient enantioseparations of the racemic samples. Pure dichloromethane, which cannot be used with coated-type CSPs, was selected as eluent for multi-mg enantioseparations. Quantities ranging from 5 to 25 mg of racemic compounds were resolved in less than 8 min and the ee values of the collected fractions were ≥98%. The results from crystallographic and CD analysis of the isolated enantiomers provided to assign the (*R*) configuration to the first collected enantiomer and (*S*) configuration to the second collected one.

The contents of this article shows once again how the Chiralpak IA CSP finds a valid application to solve some critical aspects related to chirality of new biologically active agents.

References

[1] J. Wouters, Curr. Med. Chem. 5 (1998) 137–162.
[2] A.W.J. Bach, N.C. Lan, D.L. Johnson, C.W. Abell, M.E. Bembenek, S.W. Kwan, P.H. Seeburg, J.C. Shih, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 4934–4938.
[3] C.W. Abell, S.W. Kwan, Prog. Nucl. Acid Res. Mol. Biol. 65 (2001) 129–156.
[4] L. De Colibus, M. Li, C. Binda, A. Lustig, D.E. Edmondson, A. Mattevi, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 12684–12689.
[5] S.Y. Son, J. Mat, Y. Kondou, M. Yoshimura, E. Yamashita, T. Tsukihara, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 5739–5744.
[6] C. Binda, P. Newton-Vinson, F. Hubalek, D.E. Edmondson, A. Mattevi, Nat. Struct. Biol. 9 (2002) 22–26.
[7] A.M. Cesura, A. Pletscher, Prog. Drug Res. 38 (1992) 171–297.
[8] J. Saura, J.M. Luque, A.M. Cesura, M. Da Prada, Neuroscience 62 (1994) 15–30.
[9] G. Alper, F.K. Girgin, M. Ozgonul, G. Mentes, B. Erzos, Eur. Neuropsychopharmacol. 9 (1999) 247–252.
[10] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, O. Befani, P. Turini, S. Alcaro, F. Ortuso, M.C. Cardia, S. Distinto, J. Med. Chem. 50 (2007) 707–712.
[11] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, O. Befani, P. Turini, S. Alcaro, F. Ortuso, R. Cirilli, F. La Torre, M.C. Cardia, S. Distinto, J. Med. Chem. 48 (2005) 7113–7122.
[12] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, S. Carradori, S. Alcaro, F. Ortuso, M. Yáñez, F. Orallo, R. Cirilli, R. Ferretti, F. La Torre, J. Med. Chem. 51 (2008) 4874–4880.
[13] R. Cirilli, A. Simonelli, R. Ferretti, A. Bolasco, P. Chimenti, D. Secci, E. Maccioni, F. La Torre, J. Chromatogr. A 1101 (2006) 198–203.
[14] S. Vigo, S. Alcaro, A. Bolasco, C. Cardia, S. Distinto, E. Maccioni, F. Orallo, F. Ortuso, M.L. Sanna, D. Secci, M. Yáñez, Data Presented at the XIX National Meeting on Medicinal Chemistry, Verona, 14–18 September, 2008, p. 228 (Book of abstracts).
[15] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. Engl. 37 (1998) 1021–1043.
[16] E. Yashima, J. Chromatogr. A 906 (2001) 105–125.
[17] T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara, H. Kurosawa, J. Chromatogr. A 1075 (2005) 65–75.
[18] T. Zhang, D. Nguyen, P. Franco, Y. Isobe, T. Michishita, T. Murakami, J. Pharm. Biomed. Anal. 46 (2008) 882–891.
[19] I. Ali, H.Y. Aboul-Enein, Curr. Pharm. Anal. 3 (2007) 71–82.
[20] T. Ikai, Y. Okamoto, Chem. Rev. 109 (2009) 6077–6101.
[21] X. Chen, C. Yamamoto, Y. Okamoto, Pure Appl. Chem. 79 (2007) 1561–1573.
[22] P. Franco, T. Zhang, J. Chromatogr. B 875 (2008) 48–56.
[23] R. Cirilli, A. Simonelli, R. Ferretti, A. Bolasco, P. Chimenti, D. Secci, E. Maccioni, F. La Torre, J. Chromatogr. A 1101 (2006) 198–2203.
[24] R. Cirilli, S. Fiore, F. La Torre, E. Maccioni, D. Secci, M.L. Sanna, C. Faggi, Chirality 22 (2010) 56–62.
[25] S. Rapposelli, V. Calderone, R. Cirilli, M. Digiacomo, C. Faggi, F. La Torre, M. Manganaro, A. Martelli, L. Testai, J. Med. Chem. 52 (2009) 1477–1480.
[26] T. Zhang, D. Nguyen, P. Franco, J. Chromatogr. A 1191 (2008) 214–222.
[27] R. Cirilli, R. Ferretti, F. La Torre, A. Borioni, V. Fares, M. Camalli, C. Faggi, D. Rotili, A. Mai, Chirality 21 (2009) 604–612.
[28] M. Biava, R. Cirilli, V. Fares, R. Ferretti, B. Gallinella, F. La Torre, G. Poce, G.C. Porretta, S. Supino, C. Villani, Chirality 20 (2008) 775–780.
[29] T. Zhang, M. Schaeffer, P. Franco, J. Chromatogr. A 1083 (2005) 96–101.
[30] N. Walker, D. Stuart, Acta Crystallogr. A 39 (1983) 158–166.
[31] A. Altomare, M.C. Burla, M. Camalli, G.L. Cascarano, R. Caliandro, C. Giacovazzo, A. Guagliardi, A.G.G. Moliterni, G. Polidori, R. Spagna, J. Appl. Cryst. 32 (1999) 115–119.
[32] G.M. Sheldrick, SHELXL97: Program for Crystal Structure Refinement, Institut für Anorganische Chemie de Universitat Göttingen, Göttingen, Germany, 1997.