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REGULAR ARTICLE

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Enantioselective separation of (±)-β-hydroxy-1,2,3-triazoles by supercritical fluid chromatography and high-performance liquid chromatography

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Abstract

This paper reports the enantioseparation of β-hydroxy-1,2,3-triazole derivatives, which present a broad range of biological properties, by supercritical fluid chromatography (SFC) and high-performance liquid chromatography techniques (HPLC). Polysaccharide-based chiral columns (cellulose and amylose) were used to evaluate the separation in SFC and HPLC. Time of analyses, consumption of solvent, and parameter optimization were reduced using SFC technique. The columns based on cellulose chiral stationary phase using 2-propanol and ethanol as modifiers showed the best results for the enantioresolution of the (±)-β-hydroxy-1,2,3-triazoles by SFC analyses. These techniques were applied to evaluate the selectivity of biocatalytic reduction of β-keto-1,2,3-triazoles by marine-derived fungus *Penicillium citrinum* CBMAI 1186 to obtain the (±)-β-hydroxy-1,2,3-triazoles.

KEYWORDS

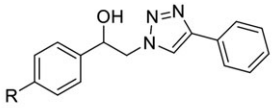
biocatalysis, enantiomeric separation, HPLC, SFC, triazoles

1 | INTRODUCTION

High-performance liquid chromatography (HPLC) is the major separation technique used for the qualitative and quantitative analysis of chiral compounds.[1,2] The widespread use of HPLC may be attributed to advances on the development of chiral stationary phases (CSPs), which improved the separation of racemic compounds.[3,4] Nevertheless, several limitations may be disadvantageous to the development of chiral methods by HPLC, such as long equi-libration time and long analysis time and the use of toxic and flammable solvents. Another drawback for enantiomeric purity determination is the effect of peak broadening caused by diffusion processes in HPLC, which can affect the quality of the separation contributing to low efficiencies.[3,5]

Supercritical fluid chromatography (SFC) has emerged as an alternative technique to HPLC for routine applications in enantioresolution of chiral compounds, mainly in the pharmaceutical industry for purification and enantioseparation of drugs and their impurities, improving the resolution and reducing analysis time.[6] SFC is considered as an environmental friendly technique due to reduced consumption of toxic solvents and additives.[7,8] Carbon dioxide and polar modifiers (e.g., alcohols and acetonitrile) are used as mobile phases coupled with a wide variety of stationary phases. The high diffusivity, low viscosity, and solvating power afforded by the use of CO_2 in the mobile phase is responsible for the success of SFC on the chiral separations.[8,9]

The polysaccharides-based derivatives (e.g., cellulose and amylose) CSPs are extensively used for HPLC as well as SFC enantioseparations due to versatility, durability, and sample loadability.[1,10] Comparative studies of enantioselective separations have been showed that both SFC and HPLC provide good selectivity; however, the advantage of SFC over HPLC occurs in terms of flow rate,



R = H (**1**), OCH_3 (**2**), Cl (**3**), Br (**4**), F (**5**), NO_2 (**6**)

FIGURE 1 (±)-β-Hydroxy-1,2,3-triazole derivatives evaluated at the study of enantioselective separation by SFC and HPLC

resolution, time of analysis, consumption of solvent, and high throughput.[1,8,9,11] Thus, the SFC has been used not only for purification purpose, but also for method development and screening of chiral and achiral compounds in different matrices.[12,13]

Therefore, in this work these two techniques were employed for the chiral separation of β-hydroxy-1,2,3-triazoles,[14] which are an important class of heterocyclic compounds obtained only by synthetic methodologies. These compounds have been extensively studied as potential targets for drug discovery since they possess a broad range of biological properties, including antimicrobial, antiviral, antiepileptic, anti-HIV and activities against several neglected diseases.[15]

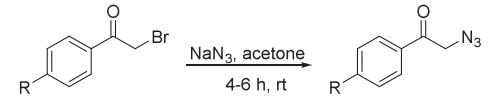
Racemic β-hydroxy-1,2,3-triazole derivatives (Figure 1) were obtained through the reduction of β-keto-1,2,3-triazoles using $NaBH_4$. The racemic compounds were used as analytical standard to evaluate the enantioselective bioreduction of β-keto-1,2,3-triazoles by marine-derived fungus[16] *Penicillium citrinum* CBMAI 1186.

CSP-base enantioselective separation has been an extremely useful tool in biocatalytic studies.[17] The asymmetric synthesis of chiral alcohols by biocatalysis consists in a well-known methodology to obtain enantiomerically pure compounds. Thus, based on the HPLC method previously established to monitor the bioreduction of β-keto-1,2,3-triazole by *P. citrinum* CBMAI 1186, we evaluated the potential of SFC technique on the enantioselective separation for these compounds.

2 | MATERIALS AND METHODS

2.1 | Chemical and reagents

Sodium borohydride (97%) was purchased from Vetec (Duque de Caxias, Brazil) and sodium azide from Merck (São Paulo, Brazil). The 2-bromo-1-phenylethanone 98%, 2-bromo-1-(4-chlorophenyl)ethanone 98%, 2-bromo-1-(4-bromophenyl)ethanone 98%, phenylacetylene 98%, and (+)-sodium L-ascorbate 98% were purchased from Sigma-Aldrich (São Paulo, Brazil). Ethyl acetate (EtOAc), hexane, and acetone were purchased from Synth (São Paulo, Brazil). Hexane, 2-propanol (IPA), acetonitrile (ACN), and ethanol (EtOH) were purchased from Panreac



2-bromo-1-phenyl-ethanone derivatives	2-azido-1-phenyl-ethanone derivatives
R = H	R = H, 89%
OCH_3	OCH_3, 91%
Cl	Cl, 79%
Br	Br, 66%
F	F, 83%
NO_2	NO_2, 56%

SCHEME 1 Synthesis of 2-azido-1-phenylethanone derivatives with the respective percentage of yield

(Barcelona, Spain). All reagents and solvents were used without further purification. Salts used in the preparation of artificial seawater were purchased from Synth, Merck, or Vetec (Brazil). Malt extract was purchased from Acumedia (Indaituba, Brazil), and Agar was purchased from Himedia (Mumbai, India).

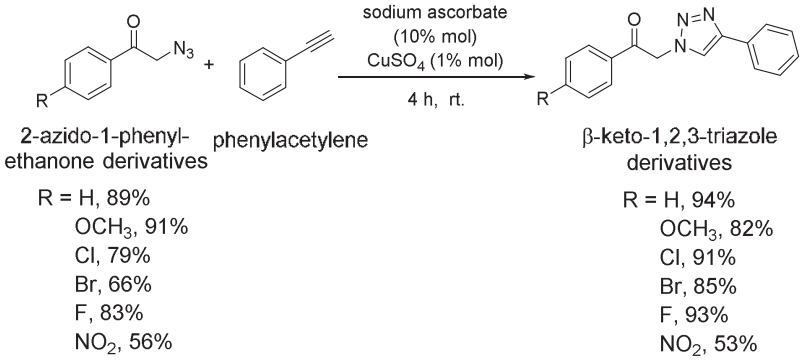
The racemic standards of β-hydroxy-1,2,3-triazole were prepared as described below by the following steps (Schemes 1, 2, and 3).

2.2 | Synthesis of 2-azido-1-phenylethanone derivatives

The 2-azido-1-phenylethanones were synthetized by reaction of 2-bromo-1-phenylethanone derivatives (5.00 mmol) and sodium azide (10.0 mmol) in round-bottomed flask (100 mL) containing acetone (30 mL), followed by stirring at room temperature for 4 to 6 hours (Scheme 1). The reactions were monitored by TLC and extracted with EtOAc (3 × 20.0 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The products were purified by column chromatography on silica gel and eluted with mixtures of hexane and EtOAc (7:3). The 2-azido-1-phenylethanones were characterized by spectroscopic analyses ^{1}H NMR, ^{13}C NMR, and IR.[16]

2.3 | Synthesis of β-keto-1,2,3-triazoles

The β-keto-1,2,3-triazoles were synthetized by the reaction of 2-azido-1-phenylethanones derivatives (1.24 mmol), phenylacetylene (1.86 mmol), (+)-sodium L-ascorbate (10% mol), and $CuSO_4.5H_2O$ (1% mol) in round-bottomed flask (50.0 mL) containing distilled water (20.0 mL), followed by stirring at room temperature for 4 hours (Scheme 2). The reactions were monitored by TLC and extracted with EtOAc (3 × 20.0 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The products were purified by column chromatography



SCHEME 2 Synthesis of β-keto-1,2,3-triazoles with the respective percentage of yield

on silica gel and eluted with mixtures of hexane and EtOAc (2:1). The β-keto-1,2,3-triazoles were characterized by spectroscopic analyses ^{1}H NMR, ^{13}C NMR, and IR.[16]

2.4 | Synthesis of (±)-β-hydroxy-1,2,3-triazoles

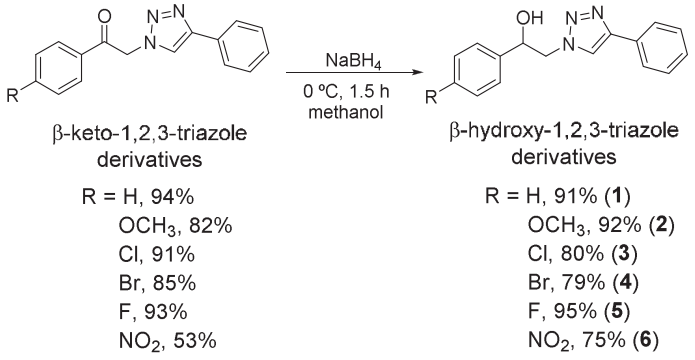
The (±)-β-hydroxy-1,2,3-triazoles **1** to **6** were synthetized by the addition of β-keto-1,2,3-ketotriazoles (50 mg) in round-bottomed flask (50 mL) containing distilled methanol (20 mL) and sodium borohydride (1.5 equivalent) on ice bath (T = 0°C), followed by stirring for 1.5 hours (Scheme 3). The reactions were monitored by TLC, and the solvent was evaporated under pressure and the residue extracted with EtOAc (3 × 10.0 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The products were purified by column chromatography on silica gel and eluted with mixtures of dichloromethane hexane and methanol (85:15:5). The (±)-β-hydroxy-1,2,3-triazoles **1** to **6** were characterized by spectroscopic analyses ^{1}H NMR, ^{13}C NMR, and IR.[16]

2.5 | Marine-derived fungus *Penicillium citrinum* CBMAI 1186

The isolation of marine-derived fungus *P. citrinum* CBMAI 1186 was previously described in Ferreira et al.[18] The procedures for the preparation of culture medium composition and fungus cultivation were described elsewhere.[18]

2.6 | Bioreduction of (±)-β-keto-1,2,3-triazoles by the marine-derived fungus *P. citrinum* CBMAI 1186

The biocatalytic reductions were performed with 2 g of wet mycelium from *P. citrinum* CBMAI 1186 and (±)-β-keto-1,2,3-triazoles (20 mg) dissolved in dimethylsulfoxide (300 μL), in 95 mL of phosphate buffer solution (Na_2HPO_4/KH_2PO_4, pH 5, 0.07 M) sterilized in autoclave (121°C, 1.5 kPa) and 5 mL of methanol (5% v/v) sterilized in ultraviolet light (20 min). The mixtures were incubated in orbital shaker for 12 days (32°C, 130 rpm). After 12 days of incubation, the reaction was filtered in a Buchner funnel and the mycelial mass obtained was transferred to a 250-mL Erlenmeyer flask and suspended in 60 mL of distilled water and EtOAc (1:1). This biphasic mixture was submitted to magnetic stirring for 30 min, filtered again together with the medium obtained in the previous filtration and extracted with EtOAc (3 × 25.0 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The products were purified by column chromatography on silica gel and eluted with a mixture of dichloromethane, hexane, and methanol (85:15:5). The aliquot of purified sample was suspended in 1.5 mL of 2-propanol or methanol.



SCHEME 3 Synthesis of (±)-β-hydroxy-1,2,3-triazoles with the respective percentage of yield

3 | INSTRUMENTATION

SFC

A Waters ACQUITY UPC^2 system, equipped with an ACQUITY UPC^2 binary solvent manager, ACQUITY UPC^2 sample manager, Automatic Back-Pressure Regulator (ABPR), and an ACQUITY photodiode array (PDA) detector was used to perform the SFC experiments. Data acquisition was performed using the Waters Empower^{TM} 3 software. The injector was equipped with a 10 μL loop. Peaks were registered at a wavelength of 190 to 800 nm using compensated mode (380-480 nm). Three columns were used to evaluate the separation of the (±)-β-hydroxy-1,2,3-triazole derivatives: ACQUITY UPC^2 Trefoil **AMY 1** (Amylose tris-[3,5-dimethylphenylcarbamate]–2.5 μm, 3.0 mm × 150 mm), ACQUITY UPC^2 Trefoil **CEL 1** (Cellulose tris-[3,5-dimethylphenylcarbamate]–2.5 μm, 3.0 mm × 150 mm), and ACQUITY UPC^2 Trefoil **CEL 2** (Cellulose tris-[3-chloro-4-methylphenylcarbamate]–2.5 μm, 3.0 mm × 150 mm). EtOH, IPA, and ACN were used as the co-solvent. The SFC binary pump was set up to initial pump with 20% of co-solvent at a flow rate of 1.0 ml min^{-1}. The gradient elution was linear from 20% to 60% of co-solvent in 6 minutes and then from 60% to 0% of co-solvent in 2 minutes. The density of the subcritical fluid was regulated *via* column temperature of 35°C and automated backpressure regulator (ABPR) setting of 13.8 (MPa), with injection volume of 2 μL.

HPLC

The HPLC analyses were performed on a Shimadzu Prominence equipped with a LC-20AT pump, a SIL-20A$_{HT}$ injector system with a 100-μL loop, and SPD-M20A diode array detector with a CBM-20A interface. Data acquisition was performed using the LC Solution software. The chiral columns used were CHIRACEL OD-H (cellulose tris [3,5-dimethylphenylcarbamate]–5.0 μm, 0.46 cm × 250 mm) for (±)-β-hydroxy-1,2,3-triazole **1** and CHIRALPAK AS-H (amylose tris [(*S*)-α-methylbenzylcarbamate]–5.0 μm, 0.46 cm × 250 mm) for (±)-β-hydroxy-1,2,3-triazoles **2** to **6**. The mobile phase was hexane:2-propanol (v:v) with flow rate of 0.5 mL min^{-1} ((±)-β-hydroxy-1,2,3-triazole **1–5**) and 0.6 mL min^{-1} ((±)-β-hydroxy-1,2,3-triazole **6**), oven temperature at 40°C and injection volume of 10 μL. All the solutions were prepared using 1 mg of each compound dissolved in methanol or 2-propanol.

3.1 | Data analysis

The separation parameters such as retention factor (k) (Equation 1), selectivity (or separation factor) (α) (Equation 2) and resolution (R_s) (Equation 3) were calculated using the following equations[6,19]:

$$k' = \frac{t_R - t_0}{t_0}, \tag{1}$$

$$a = \frac{k_2}{k_1}, \tag{2}$$

$$R_S = 2\frac{t_2 - t_1}{w_1 + w_2}. \tag{3}$$

4 | RESULTS AND DISCUSSION

4.1 | Enantiomeric separation of (±)-β-hydroxy-1,2,3-triazoles by HPLC

The polysaccharide-based CSPs columns have been widely used for screening of molecules under different mobile phase conditions. In general, the mechanisms evolved on the enantiodiscrimination of the enantiomers by polysaccharide-based CSPs are based on the spatial effects of cavities built by the polysaccharide, steric effects, π-π interactions, hydrogen bonding and dipole-dipole.[20,21]

Two polysaccharide-based CSPs, OD-H (cellulose tris(3,5-dimethylphenylcarbamate) and AS-H (amylose tris[(*S*)-α-methylbenzylcarbamate]) were used to improve the separation of (±)-β-hydroxy-1,2,3-triazole derivatives by HPLC. Table 1 shows the results of retention factor,

TABLE 1 Retention factor (k), selectivity (α), and resolution (R_s) for the (±)-β-hydroxy-1,2,3-triazoles **1-6** by HPLC analyses

Compounds	t_R, min	Mobile Phase (v:v)	k_1	k_2	α	R_s
1	65	85:15	8.22	8.78	1.07	0.61
2	35	75:25	3.15	3.80	1.21	1.85
3	57	90:10	6.52	7.18	1.10	1.06
4	65	90:10	7.21	8.03	1.13	1.18
5	60	90:10	6.02	6.66	1.11	0.91
6	60	80:20	3.90	6.74	1.75	4.33

t_a = analysis time; k_1 = retention factor for the peak 1 racemic compound; k_2 = retention factor for the peak 2 of the racemic compound. CHIRACEL OD-H and CHIRALPAK AS-H were used on the enantioseparation of compounds **1** and **2-6**, respectively. Hexane:2-propanol (v:v) was used as mobile phase at different compositions. A flow rate of 0.5 mL min^{-1} for the compounds **1-5** and 0.6 mL min^{-1} for the **6.**

selectivity and resolution previously established for (±)-β-hydroxy-1,2,3-triazoles **1** to **6**.

The determination of the exact mechanisms behind the enantiomeric separation is a difficult task. π-π Interactions between the phenyl moieties of (±)-β-hydroxy-1,2,3-triazole derivatives and the aromatic ring of the chiral selector from AS-H and OD-H CSP can be evolved on the chiral discrimination of the enantiomers.

Partial separation (R_S = 0.61-1.18) was observed for the compound **1** (85:15, v:v) and compounds **3**, **4**, and **5** (90:10, v:v) on CSP OD-H and AS-H, respectively, and complete resolution was achieved for the compounds **2** and **6** ($R_s > 1.5$). Besides the π-π interactions, the groups R═OCH_3 and NO_2, present in the compounds **2** and **6**, respectively, a hydrogen bond may be formed with ─NH group at AS–H chiral column. Moreover, the groups OCH_3 and NO_2 can interact by dipole-dipole bond with the C═O present at the AS–H CSP contributing to increase the selectivity for these compounds.[19]

4.2 | Enantiomeric separation of (±)-β-hydroxy-1,2,3-triazoles by SFC

Polysaccharide-based CSPs used on the SFC analyses were amylose derivative (**AMY 1)** and cellulose derivatives (**CEL 1** and **CEL 2)**. Table 2 shows the results obtained on the enantioseparation of β-hydroxy-1,2,3-triazole derivatives **1** to **6** by SFC. The eluent used in these columns were mixtures of carbon dioxide-co-solvent (acetonitrile, 2-propanol or ethanol). The retention time for all compounds was decreased when SFC was used.

From the results shown on Table 2 for SFC analyses, it can be observed that changes on the nature of co-solvent has different effects on CSPs columns. The improvement on the enantioselectivity using polysaccharide-based CSPs and co-solvent with different proton-acceptor characters by SFC was brightening discussed by Khater and West.[22] Based in a series of chemometric studies considering the type of based-CSPs and nature of co-solvent, the authors[22] highlighted that when the percentage of modifier is high (above 15%-20%) the elution can be more affected by interactions between analyte-mobile phases than the analyte-stationary phases. Better selectivity and resolution were achieved for all compounds (**1** to **6**) on cellulose derivatives based-columns **CEL 1** and **CEL 2** using 2-propanol or ethanol as co-solvent in comparison with **AMY 1**. The selectivity of the compounds with lower proton-donor capability demonstrated less difference in the presence of co-solvents (EtOH and IPA) when compared to the compounds with proton-donor character (**3** to **6**). On both, the enantioseparation was driven by hydrogen interactions.

The Figure 2 presents the chromatogram for the enantiomeric separation of (±)-β-hydroxy-1,2,3-triazole **2** at **CEL 1** using different co-solvents, illustrating the better separation in presence of EtOH ($R_s = 6.77$, $\alpha = 1.32$).

The retention factors in SFC were smaller than in HPLC for all compounds. This is an indicative that the

TABLE 2 Retention factor (k), selectivity (α), and resolution (R_s) for the (±)-β-hydroxy-1,2,3-triazoles by SFC analyses

Column	Compounds	Mobile Phase (v:v)	k_1	k_2	α	R_s
	1	ACN	1.92	1.99	1.03	1.09
		IPA	1.09	1.16	1.06	1.23
		EtOH	0.64	0.68	1.06	0.95
	2	ACN	2.09	2.22	1.06	0.69
		IPA	1.39	1.45	1.05	0.95
		EtOH	0.83	0.92	1.11	1.66
AMY 1	**3**	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	n.d.	n.d.	n.d.	n.d.
		EtOH	n.d.	n.d.	n.d.	n.d.
	4	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	n.d.	n.d.	n.d.	n.d.
		EtOH	n.d.	n.d.	n.d.	n.d.
	5	ACN	1.74	1.78	1.03	0.83
		IPA	0.96	1.00	1.04	0.94
		EtOH	n.d.	n.d.	n.d.	n.d.

(Continues)

TABLE 2 (Continued)

Column	Compounds	Mobile Phase (v:v)	k_1	k_2	α	R_s
	6	ACN	1.31	1.35	1.03	1.25
		IPA	1.46	1.60	1.10	2.18
		EtOH	0.95	1.05	1.10	1.83
	1	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	0.84	1.13	1.35	6.95
		EtOH	1.27	1.76	1.38	7.42
	2	ACN	2.66	2.80	1.05	0.67
		IPA	0.95	1.21	1.28	4.57
		EtOH	1.34	1.89	1.41	8.41
CEL 1	**3**	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	0.97	1.14	1.17	3.53
		EtOH	1.49	2.02	1.36	8.10
	4	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	1.14	1.31	1.15	3.37
		EtOH	1.78	2.32	1.31	6.93
	5	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	0.62	0.79	1.26	3.86
		EtOH	0.93	1.33	1.43	6.10
	6	ACN	2.44	2.74	1.13	1.30
		IPA	1.17	1.22	1.05	1.03
		EtOH	1.54	2.04	1.32	6.77
	1	ACN	...	...	...	...
		IPA	0.74	0.97	1.31	3.90
		EtOH	0.50	0.66	1.32	3.03
	2	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	1.01	1.20	1.19	2.39
		EtOH	0.74	0.88	1.19	2.49
CEL 2	**3**	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	0.78	1.04	1.33	3.59
		EtOH	0.55	0.72	1.31	3.14
	4	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	0.95	1.23	1.30	4.31
		EtOH	0.71	0.90	1.27	3.22
	5	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	0.50	0.70	1.40	3.74
		EtOH	0.29	0.42	1.45	2.92
	6	ACN	n.d.	n.d.	n.d.	n.d.
		IPA	0.99	1.16	1.17	2.50
		EtOH	0.73	0.89	1.22	2.78

k_1 = retention factor for the peak 1 of racemic compound; k_2 = retention factor for the peak 2 of the racemic compound. SFC conditions: Gradient elution was linear from 20% to 60% of co-solvent in 6 min and then from 60% to 0% of co-solvent in 2 min. Column temperature = 35°C and ABPR setting of 13.8 (MPa).

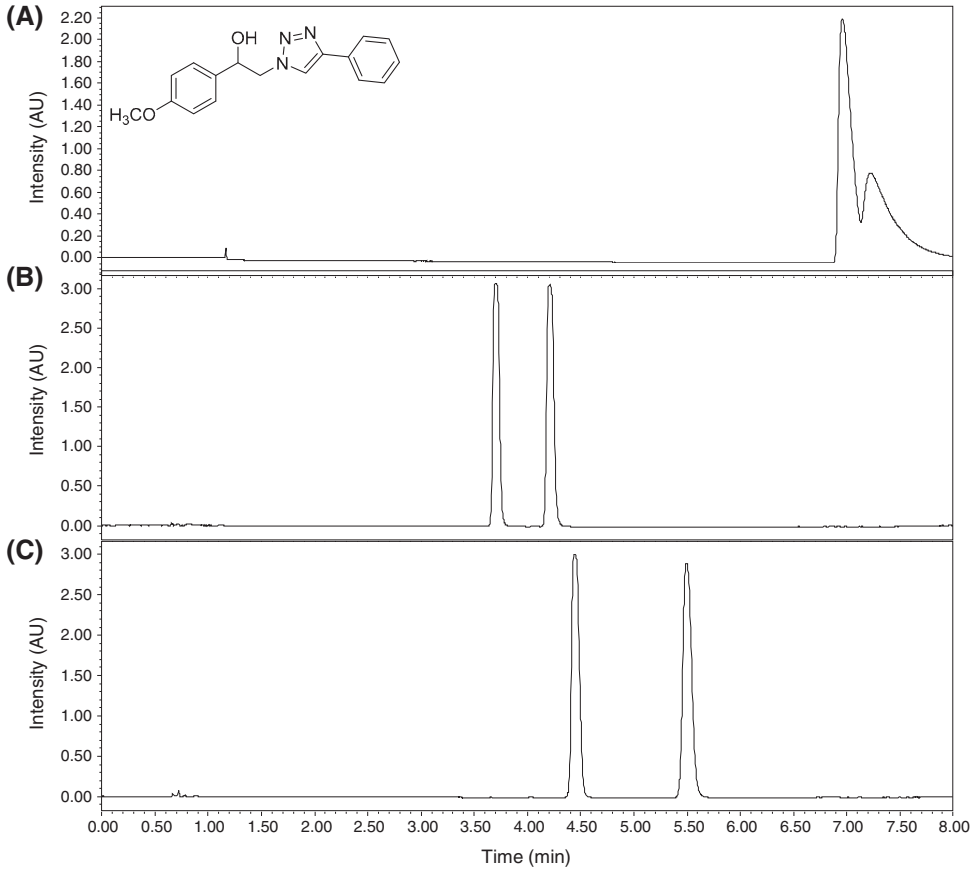


FIGURE 2 Chromatograms of (±)-β-hydroxy-1,2,3-triazole **2** obtained at column **CEL 1** in different mobile phases. A, ACN, B, IPA, and C, EtOH

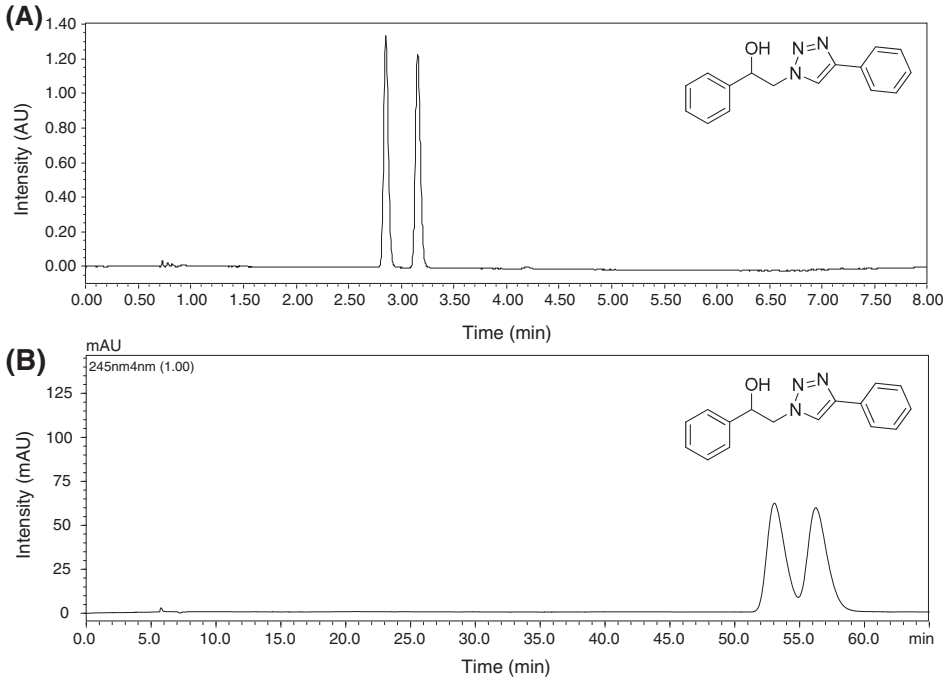


FIGURE 3 Chromatograms of (±)-β-hydroxy-1,2,3-triazole **1** obtained by A, SFC at column **CEL 2** using EtOH as modifier and B, HPLC at column OD-H using hexane:2-propanol (85:15) as mobile phase, $\lambda = 245$ nm

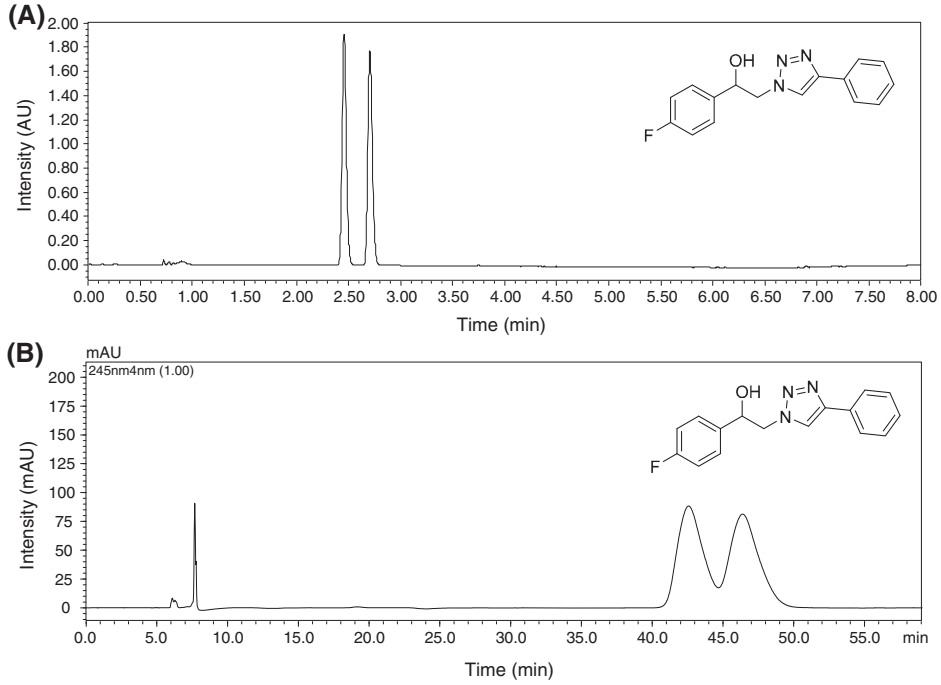


FIGURE 4 Chromatograms of (±)-β-hydroxy-1,2,3-triazole **5** obtained by A, SFC at column **CEL 2** using EtOH as modifier and B, HPLC at column AS-H using hexane:2-propanol (90:10) as mobile phase, λ = 245 nm

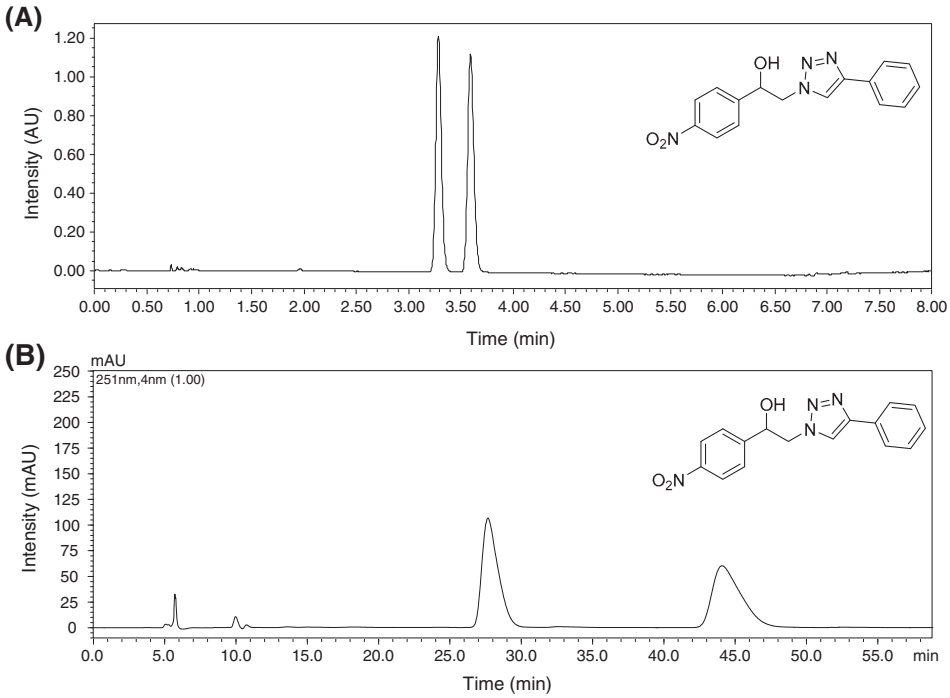


FIGURE 5 Chromatograms of (±)-β-hydroxy-1,2,3-triazole **6** obtained by A, SFC at column **CEL 2** using EtOH as modifier and B, HPLC at column AS-H using hexane:2-propanol (80:20) as mobile phase, λ = 251 nm

elution strength of the carbon dioxide-IPA and carbon dioxide-EtOH is higher than the hexane:IPA (v:v). These facts emphasize that the solute-mobile phase and mobile phase-stationary phase are not equivalent for both techniques.[2] Thereby, the separation of compound **1** was achieved in the SFC analysis, with good R_S, using **CEL 1** column.

No differences were observed by the use of modifier IPA or EtOH on the **CEL 2** columns for the selectivity and resolution of the enantiomers.

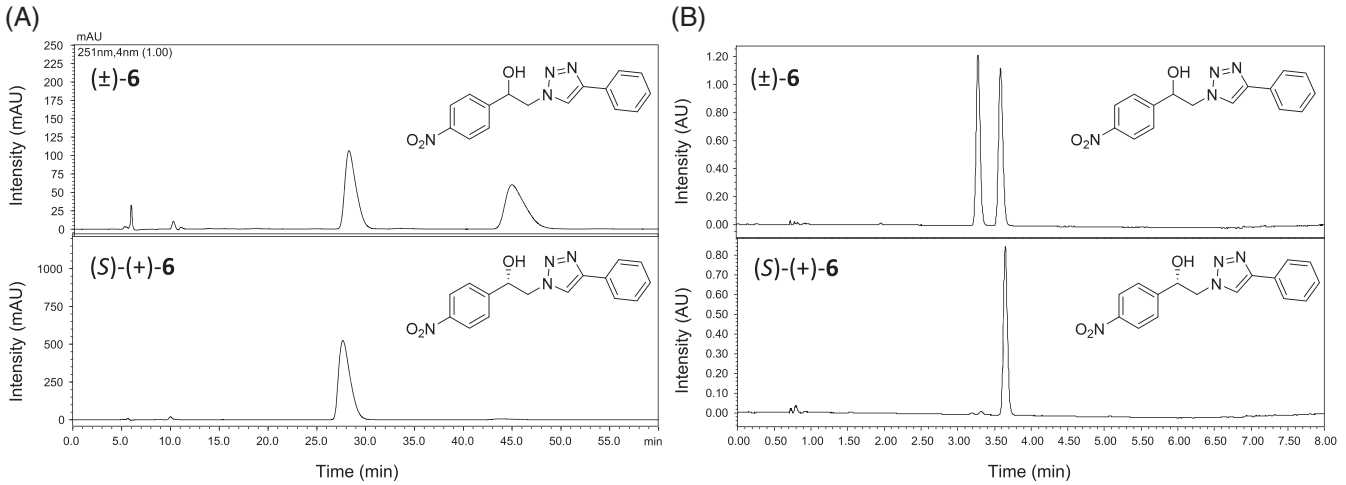


FIGURE 6 Chromatograms of (±)-β-hydroxy-1,2,3-triazole **6** and (*S*)-(+)-β-hydroxy-1,2,3-triazole **6** obtained by biotransformation with *P. citrinum* CBMAI 1186 by A, SFC at column **CEL 2** using EtOH as modifier and B, HPLC at column AS-H using hexane:2-propanol (80:20) as mobile phase, λ = 251 nm

Figures 3–5 present the chromatograms obtained for the (±)-β-hydroxy-1,2,3-triazoles **1**, **5**, and **6** by SFC and HPLC.

Figure 6 shows the chromatograms of the standard racemic mixture and the product of biotransformation by *P. citrinum* CBMAI 1186 using both techniques, HPLC and SFC.

The biotransformation reaction for the by *P. citrinum* CBMAI 1186 presented high selectivity towards the formation of (*S*)-(+)-β-hydroxy-1,2,3-triazole **6** with 96% of enantiomeric excess. It is noteworthy that the elution order for (*S*)-(+)-β-hydroxy-1,2,3-triazole **6** was inverted for the AS–H in HPLC and the **CEL 2** for SFC.

5 | CONCLUSION

The SFC technique provided a rapid and efficient enantioseparation for the hydroxy-1,2,3-triazole derivatives when compared to HPLC. The time of analyses, consumption of solvent, and parameter optimization can be minimized by the use of SFC. The superiority of cellulose derivatives CSP columns (**CEL 1** and **CEL 2**), using 2-propanol or ethanol as modifier, on the enantioseparation of the compounds was established by the resolution results.

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