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Thiooxamide chiral stationary phase for liquid chromatography

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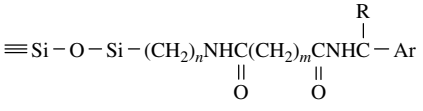
A new chiral stationary phase was prepared by reaction of aminopropylsilica with *N*-(α-phenylethyl)chloroacetamide in the presence of sulfur and used for the high performance liquid-chromatographic separation of 1,1′-binaphthyl-2,2′-diol and Tröger's base enantiomers.

The HPLC separation of optical isomers on chiral stationary phases (CSPs) is a dynamically growing research area.

The majority of CSPs are prepared by the attachment of relatively small chiral selectors[1] to the surface of silica gel microparticles. To achieve discrimination between enantiomers, a minimum of three points of interaction are needed. Hydrogen bonding, π–π interactions and steric hindrances are the most important interactions in chiral chromatography. To vary π–π interactions between an analyte and a chiral selector, different π-donating or π-accepting functional groups (NO_2, CN, F) may be introduced into an aromatic system.[2,3] Steric effects depend on the bulkiness and position of substituents in the selector or on the rigidity of selector molecule structure. The presence of functional groups with hydrogen donor–acceptor properties, such as hydroxyl, amino and carboxyl are required for hydrogen bonding. Owing to the conformational rigidity and polar nature of the urea functionality, many CSPs contain urea groups as the fragments of chiral selectors.[4]

One of the most successful class of chiral selectors attached to silica to produce efficient CSPs are chiral diamides. The CSPs



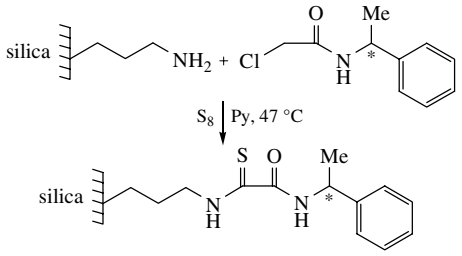
where *n* and *m* are the numbers of methylene chains, R is a bulky substituent at the asymmetric carbon, and Ar is an aromatic system, are selective for the separation of enantiomers.[4,5] The use of thiooxamide groups instead of urea groups might provide interesting chiral selectivity to CSP. In this study, a new chiral selector *N*-(*O*)-propyl-*N*-(*S*)-(1-phenylethyl)thiooxamide was used for the preparation of a CSP. Enantioselective properties were demonstrated on examples of the resolution of 1,1′-binaphthyl-2,2′-diol (BD) and a Tröger's base (TB). The effects of polar additives to the eluent, column temperature and mobile phase flow rate on the chiral recognition of enantiomers were considered.

The HPLC system consisted of a LC-10AT vp pump, an SPD-10AVvp UV detector, a Rheodyne 7725i injector equipped with a 20 μl loop, and a SCL-10AVvp system controller (Shimadzu, Germany). γ-Aminopropylsilica Silasorb SPH Amin (5 μm; pore diameter of 10 nm; surface area of 300 $m^2\ g^{-1}$) from Lachema, Czech Republic, was used. Sulfur, pyridine, triethylamine (all of reagent grade) and the optical isomers of (*R*),(*S*)-1,1′-binaphthyl-2,2′-diol (BD) from Merck; (*R*),(*S*)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) and 2,8-dimethyl-6*H*,12*H*,5,11-methanodibenzo[*b*,*f*][1,5]diazocine (TB) from Aldrich were used. HPLC-grade hexane, propan-2-ol, 2-methylpropan-2-ol and ethyl acetate (Reakhim, Russia) were used for chromatographic separations. *N*-(α-Phenylethyl)chloroacetamide (PCA) was synthesised in accordance with a standard procedure.[6]

A new reaction[7] was used to prepare a CSP with thiooxamide groups involved in the chiral selector molecule. The synthesis of CSP was performed in accordance with Scheme 1.†

The enantioselectivity of the thiooxamide CSP was evaluated for the separation of racemates of BD, TFAE and TB and other substances including alkyl aryl carbinols, amino acid derivatives and β-blockers.

Initially, the mixtures of hexane with propan-2-ol were used as a mobile phase. Only partial resolution was observed for the



Scheme 1 Synthesis of a CSP.

enantiomers of both BD and TB (Table 1). Racemic mixtures of the other substances were not resolved. In case of BD, the elution order was determined from the retention of individual enantiomers and *S*-(–)-BD was eluted first. Unfortunately, we had no individual enantiomers of TB and the elution order was not determined. Comparison of the chromatographic results for BD and TB shows that the retention of BD was greater even though a higher concentration of the polar modifier was used. This phenomenon can be explained by a matrix effect. Although, the synthesis of CSP was provided with an excess of the chiral modifier, some amino groups of aminopropylsilica could not have been involved in the reaction. Unreacted amino groups on the CSP surface are responsible for the attraction of these groups with hydroxyl groups of BD. At the same time, the attraction between primary amino groups of CSP and *tert*-amino groups of TB molecules seemed to be impossible.

The other polar additives to hexane such as 2-methylpropan-2-ol, ethyl acetate and a mixture of them have been checked as mobile phases for the separation of BD and TB enantiomers (Table 1). These additives have a small effect on the enantioselectivity of separation of BD enantiomers, but the peak resolution *Rs* was better with 2-methylpropan-2-ol. For TB the improvement in both enantioselectivity α and peak resolution *Rs* was noted for ethyl acetate–hexane mixtures as compared with alcohol-containing eluents. In accordance with the theory,

Table 1 Effects of polar additives to hexane on the separation of 1,1′-binaphthyl-2,2′-diol and Tröger's base racemates. Mobile phase flow rate, 1 ml min^{-1}; T = 22 °C.

Polar additive	C (vol%)	α	k_1	*Rs*
1,1′-Binaphthyl-2,2′-diol				
Propan-2-ol	5	1.040	14.04	0.25
	10	1.041	5.54	0.16
	20	1.037	3.04	0.10
2-Methylpropan-2-ol	4	1.049	11.57	0.54
	7.5	1.047	6.98	0.40
	10	1.046	6.14	0.39
Ethyl acetate	10	—	no elution	—
Ethyl acetate/ 2-methylpropan-2-ol	5/4	1.046	5.49	0.46
Tröger's base				
Propan-2-ol	2.5	1.044	2.70	0.28
	10	1.036	1.04	0.15
2-Methylpropan-2-ol	7.5	1.040	1.84	0.29
	10	1.033	1.21	0.14
Ethyl acetate	10	1.074	6.80	0.95
Ethyl acetate/ 2-methylpropan-2-ol	5/4	1.039	4.15	0.27

† *Synthesis of a CSP.* 1.6 g of sulfur powder was placed in a three-necked flask with a mixture of 50 ml of pyridine and 10 ml of triethylamine. The mixture was heated at 47 °C for 10 min, and then γ-aminopropylsilica was added. Then, 6.8 g of PCA was added to the obtained suspension with vigorous stirring and careful temperature control for 4 h. The sorbent was filtered off and triplicate washed with 20 ml of each warm pyridine, hexane and diethyl ether and dried under vacuum at room temperature. The sorbents were characterised by elemental analysis: γ-aminopropylsilica (C, 3.75%; H, 1.21%), CSP (C, 7.3%; H, 1.4%; S, 1.2%). The prepared CSP was slurry packed to a stainless steel chromatographic column (4.6×250 mm). The column efficiency evaluated from the chromatographic peak of toluene was about 80000 theoretical plates per metre.

Table 2 Effects of column temperature and flow rate on the chromatographic separation of a 1,1′-binaphthyl-2,2′-diol racemic mixture.

Temperature/°C	Flow rate/ ml min^{-1}	k_1	α	*Rs*
22	1.0	5.49	1.047	0.46
	0.5	5.45	1.050	0.62
	0.25	5.41	1.048	0.68
0	0.5	5.56	1.049	0.74
	0.25	5.57	1.050	0.82

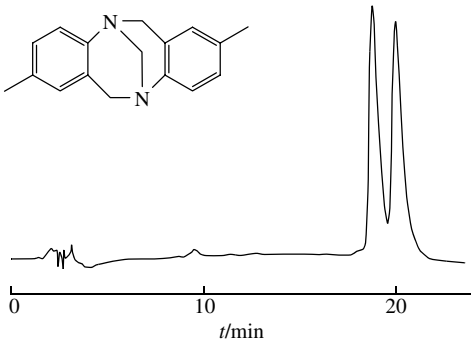


Figure 1 Chromatographic separation of (*R*) and (*S*) TB on the CSP. Mobile phase: hexane–ethyl acetate, 90:10 (v/v); flow rate, 1 ml min^{-1}; T = 22 °C; UV detector at 254 nm.

the decrease of the concentration of a polar additive in hexane lead to an improvement of peak resolution in all cases because of an increase of the retention factor related coefficient (k/k + 1) in *Rs* value.

The interesting improvement of column efficiency was found with a decrease of flow rate from 1.0 to 0.25 ml min^{-1} (Table 2). Correspondingly, the better peak resolution of enantiomers of BD was observed at a flow rate of 0.25 ml min^{-1}, which is obviously far from an optimum of the Van Deemter curve expected for the chromatographic column of an internal diameter of 4.6 mm packed with 5 μm silica. The possible explanation could be connected with slow kinetics of chiral recognition. The resolution can be affected by varying the column temperature. Thus, a few chromatograms were obtained at a lower temperature of 0 °C. The results show a slight improvement in enantioselectivity (Table 2). This is in agreement with the results of Gasparrini *et al.*,[8] who noted an increase of the enantioselectivity of chiral-phase chromatography with decreasing column temperature. The chromatograms of BD and TB obtained under optimum conditions are presented in Figures 1 and 2.

The CSP obtained has an aromatic ring and a thiooxamide functionality close to the asymmetric carbon, the methyl group at the same carbon atom is responsible for a steric hindrance effect (Scheme 1). Thus, it corresponds to the requirements of a

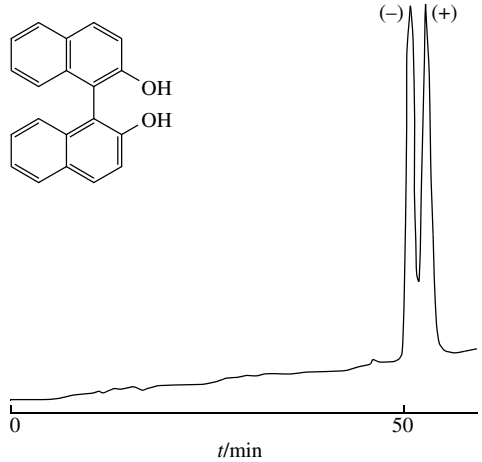


Figure 2 Chromatographic separation of (*R*) and (*S*) BD. Mobile phase: hexane–2-methylpropan-2-ol–ethyl acetate, 95:4:5; flow rate, 0.25 ml min^{-1}; T = 0 °C; UV detector at 254 nm.

three-point interactions model of Dalgliesh,[9] which are necessary for the chiral recognition of enantiomers. From the side of a chiral selectand, there are two polar functional groups in both BD (hydroxyl groups) and TB (*tert*-amine groups) molecules. To evaluate the contributions of hydrogen bonding forces to chiral recognition, the separation of enantiomers of the methoxy derivatives of BD was undertaken. No enantioselectivity was obtained for these substances. Thus, the presence of both hydroxyl groups in the BD molecule is important for enantiorecognition.

Thus, new CSP containing thiooxamide functionalities in chiral selector molecules was synthesised. This CSP demonstrated enantioselectivity for BD and TB. The nature of polar additives, the temperature and the velocity of a mobile phase affect the optic isomer peak resolution. Hydrogen bonding interactions are important for BD molecule chiral recognition on the CSP described. The optimization of the mobile phase composition can be considered as an important means of improving the enantioselectivity and efficiency of chiral separations.

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