Application of cellulose 3,5-dichlorophenylcarbamate covalently immobilized on superficially porous silica for the separation of enantiomers in high-performance liquid chromatography

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Abstract

Our earlier studies have demonstrated the applicability of polysaccharide-based chiral selectors in combination with superficially porous (or core-shell) silica (SPS) particles for the preparation of highly efficient chiral stationary phases (CSP). In earlier studies, CSPs were prepared by coating (adsorption) of the chiral selector onto the surface of silica. In this study we report for the first time the CSP obtained by covalent immobilization of a chiral selector onto the surface of SPS particles. The applicability of this CSP for the separation of enantiomers in pure methanol and acetonitrile, as well as in n-hexane/2-propanol mobile phases is shown. The effect of the injected sample amount, mobile phase flow rate and detection frequency on separation performance were studied, as well as high efficiency separation of enantiomers with the analysis time less than 30 seconds was attempted.

Keywords:

Enantioseparations, chiral HPLC, chiral stationary phase based on superficially porous silica, covalently immobilized polysaccharide-based chiral stationary phase

1. Introduction

Polysaccharide derivatives represent one of the most successful groups of chiral selectors used for the liquid-phase separation of enantiomers [1,2]. Such separations have been well documented not only in high-performance liquid chromatography (HPLC) [1-4] but also in nano-liquid chromatography (nano-LC) [5-9], capillary electrochromatography (CEC) [5,6,8,9] and also with lab-on a chip devices [10]. Polysaccharide esters and carbamates in combination with a wide range of mobile phases, such as hydrocarbon-alcohol (so called normal phase) [1-3], aqueous-organic (so called reversed phase) [1,2,11-13], pure organic solvents (polar organic mode) [2,4] and also super- or sub-critical fluids [14,15], exhibit universal chiral recognition ability towards various groups of chiral analytes [1,2]. Their only limitation in terms of mobile phase compatibility is their solubility or swelling in some solvents of common use such as tetrahydrofurane, chlorinated solvents (e.g. chloroform, methylene chloride), acetone, ethylacetate and dimethylsulfoxide. This property of polysaccharide-based chiral selectors prohibits the use of the above-mentioned solvents as mobile phase components and also as sample solvent. Unfortunately, this is a significant limitation given the occasional need to use such solvents for improving sample solubility, especially in preparative and production scale separations. In order to address this solubility limitation of polysaccharide-based chiral selectors various strategies for the covalent immobilization of chiral selectors onto the surface of silica have been developed since 1987 [16-22], resulting useful CSPs commercialized by leading companies in this field.

Some potential advantages of SPS over fully porous silica as support for the preparation of CSPs for high-performance liquid-phase separations of enantiomers was

clearly demonstrated for the first time with polysaccharide-based chiral selectors [23] and these findings were supported in our follow-up studies [24-27]. Meanwhile, impressive results have been described in the literature on combining of other type-, mostly low-molecular weight, chiral selectors with SPS [28-40].

The present study describes for the first time the chromatographic behavior of a polysaccharide-based chiral selector covalently attached onto SPS surface.

2. Experimental

2.1 Materials

Noncommercial chiral sulfoxides, 2-benzylsulfinyl benzamide (**1**), 2-(3-bromobenzylsulfinyl) benzamide (**2**), 2-(benzylsulfinyl) N-methyl benzamide (**3**), 2-(2-methylbenzylsulfinyl) benzamide (**4**), 2-(3-methylbenzylsulfinyl) benzamide (**5**) 2-(4-methylbenzylsulfinyl) benzamide (**6**), 2-(benzylsulfinyl) N,N-dimethyl benzamide (**7**) used as chiral analytes were synthesized in our laboratory according to the method described in ref. [41]. *trans*-Stilbene oxide (**8**) was commercially available from Sigma-Aldrich (St. Louis, MO, USA). Etozoline (**9**) was provided by Prof. Gottfried Blaschke from the Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, Münster, Germany. The structures of studied chiral compounds which were used in racemic form are shown in Fig. 1. HPLC-grade acetonitrile, n-hexane, methanol and 2-propanol were supplied by Karl Roth (Karlsruhe, Germany). SPS particles with 3.6 μm nominal particle diameter and 50 nm nominal pore size were provided by Phenomenex Inc. (Torrance, CA, USA). The SPS particles were coated with cellulose-(3,5-dichlorophenylcarbamate) (CDCPC) made of Avicel cellulose (Fluka, Buchs,

Switzerland) and covalently immobilized with a proprietary technique. A slurry of the packing material was prepared in n-hexane/2-propanol mixture 9/1 (v/v), decanted two times and then packed at 600 bar in stainless-steel HPLC columns of 4.6x100 mm dimension. The empty column hardware was provided by Phenomenex Inc.

2.2. Instruments

HPLC separations of chiral test compounds dissolved in mobile phase at the concentration 0.2 mg/ml were performed on Agilent 1290 Infinity LC series u-HPLC system equipped with G4220A binary pump, G4226A automated liquid sampler, G1316C thermostated column compartment and G1315C diode array detector with 2 microliter cell. Rotary evaporator used in this study was Rotavapor R-210/R-215 from BUCHI Labortechnik GmbH (Essen, Germany) with temperature control and an ultrasonic bath Sonorex RK-100 was from Bandelin (Berlin, Germany). Elemental analysis (N, C) was performed using an Elemental Combustion System CHNS-O, Model ECS4010 by Costech Analytical Technologies Inc. (Valencia, CA, USA).

3. Results and Discussions

The immobilization of CDCPC on 3.6 μm SPS particles having 50 nm pores was successful. The load of chiral selector part of CSP was 2% (w/w) based on the ratio of chiral selector and silica used and was confirmed with elemental analysis of CSP. The selector could not be extracted with solvents considered aggressive towards coated CSPs such as dichloromethane, tetrahydrofurane, chloroform or ethyl acetate.

This material behaved well in column packing and exhibited reasonable operational pressure when switching between various mobile phases.

3.1. Enantiomer resolving ability of SPS-based CSPs in polar-organic mobile

Cellulose tris(3,5-dichlorophenylcarbamate) (CDCPC) is soluble in n-hexane/2-propanol mixtures. Therefore, the coated version of the CSP based on this cellulose-derivative cannot be used for the separation of enantiomers with alcohol-hydrocarbon type eluents. Coated CDCPC was extensively evaluated in combination with polar-organic and aqueous-organic mobile phases [42-44] and found to exhibit wide chiral recognition ability. Therefore, the covalently immobilized CDCPC onto SPS was first evaluated for the separation of enantiomers in acetonitrile and methanol as mobile phases.

Separation results of 7 chiral sulfoxides and etozoline in methanol as mobile phase are summarized in Table 1. While the enantiomers of etozoline and chiral sulfoxides were baseline resolved with good selectivity, with rather short analysis times and with acceptable peak efficiency, the enantiomers of *trans*-stilbene oxide were not separated by this CSP in methanol. The low chiral recognition ability of this CSP towards the enantiomers of *trans*-stilbene oxide can be explained with the low content of chiral selector (just about 2% w/w) compared to commercially available analogues based on fully porous silica (typically 16-25%, w/w).

For ethozoline, the enantioselectivity of this CSP in acetonitrile was lower compared to methanol. The opposite was observed for all studied sulfoxides, with higher enantioselectivity in acetonitrile compared to methanol. Although the plate numbers in acetonitrile were lower compared to methanol, due to the higher selectivity better

resolution values were achieved when the former solvent was used as mobile phase (Fig. 2). The lower enantioselectivity in methanol for chiral sulfoxides indicates that hydrogen-bonding type interactions may be involved in chiral recognition.

Kinetic plots were constructed (see example on Fig. 3) for the studied chiral compounds in the flow rate range 0.1-5.0 ml/min and the results fitted to the classical van Deemter equation:

$$H = A + \frac{B}{u} + C \cdot u \qquad (1)$$

A, B and C coefficients were calculated by fitting experimental results shown on Fig. 3 as a representative example to van Deemter equation (eq. 1) where H is height equivalent to a theoretical plate, **A** is eddy-diffusion coefficient, **B** is diffusion coefficient of the eluting species in the longitudinal direction, **C** is the resistance to mass transfer coefficient of the analyte between mobile and stationary phase and **u** is linear velocity of the mobile phase. The values of these coefficients are listed in Table 2. These numbers seem to be quite reasonable and indicate minor contribution of the mass transfer coefficient (C) to peak broadening on the columns packed with SPS-based CSP under this study as expected for chromatographic sorbents made with SPS particles.

3.2 Enantiomer resolving ability of SPS-based CSPs under normal-phase conditions

As already mentioned above, due to its solubility in alcohol-hydrocarbon mixtures CDCPC-based coated-type CSPs cannot be used in combination with such mobile phases for the separation of enantiomers. Successful covalent immobilization of CDCPC can overcome this problem [16,20,45-48].

Due to the high enantioselectivity of CDCPC chiral selector towards the studied chiral sulfoxides in n-hexane/2-propanol 9:1 (v/v) as mobile phase, the elution time of the second enantiomer was excessively long despite the low chiral selector content of the CSP (only 2% (w/w)), the relatively short column length (100 mm) and the high flow rate of 5.0 ml/min (Fig. 4). In contrast, *trans*-stilbene oxide could only be partially resolved under such conditions. Therefore, the separation of its enantiomers was studied in hexane/2-propanol 98:2 (v/v) as mobile phase. Under optimized experimental conditions (flow rate, injected amount, detection frequency) rather high plate numbers in the range of 95 000-113 000 per meter were generated within an analysis time just over 1 minute (Fig. 5). The effect of flow rate on column performance under such conditions is shown in Figure 5. The shape of the H/u curve is as expected of sorbents made with SPS particles, while its minimum is higher than what is typically observed with columns developed for achiral separations.

3.3 Fast separation of enantiomers

Achieving high-speed separations of enantiomers was always of interest to practitioners [49] but this became especially hot topic in recent years [10,26,27,29,30-40]. An insignificant increase in plate height at higher mobile phase flow rates was observed for the CSP made by immobilizing CDCPC on SPS as shown on Fig. 3 and insert on Fig. 5. Such small increase makes high-speed separations of enantiomers feasible as illustrated in Figs. 6a-i. It is noteworthy that analysis times are below 30 seconds in all these cases and the peak resolution is at minimum acceptable. With further optimization

of column dimensions, CSP and mobile phase conditions further reduction of the analysis time seems feasible.

3.4 Instrumental and experimental considerations for obtaining high chromatographic performance at high mobile phase flow rates

For obtaining highly efficient separations at high flow rates of the mobile phase several thermodynamic, kinetic and instrumental requirements need to be considered simultaneously. These requirements have been mentioned earlier by us [23-27,50], by Gasparrini, Cavazzini and co-workers [30,32-38] and by Armstrong and co-workers [28,29,31,39,40]. Some of these requirements are shortly discussed below.

Speed of detection is one of the critical issues in fast separations. As it is shown on Fig. 7a at a low flow-rate it does not matter whether the detector operates at 5 or 160 Hz. The separation process taking place in the column is correctly described by detection at both 5 and 160 Hz detector frequencies. However, for the same separation performed with 5 ml/min flow rate, the chromatogram recorded at 5 Hz detector frequency inadequately describes the separation process (Fig. 7b). The fitting of the data recorded at 5 Hz detector frequency to van Deemter equation (1) or Knox equation sometimes results in negative values for coefficients A, B or C, values that are physically meaningless.

Some data even collected at 160 Hz detection frequency did not fit well the typical van Deemter curve. We assumed the effect of temperature inside the column due to friction at higher flow rate of the mobile phase to be responsible for this negative effect and made an attempt to compensate for it by performing separations at higher

temperature (30 and 40°C). Unfortunately, no significant improvement of the shape of the curves was observed at higher temperature.

As shown in Fig. 8 quite surprisingly, even for the separation performed with 1ml/min flow rate perhaps at least 20-40 Hz detector frequency is required for the adequate description of the separation process primarily for a less retained analyte like *trans*-stilbene oxide enantiomers ($k<0.45$ under these particular separation conditions).

In line to earlier studies, plate numbers decreased with increasing sample volume for both concentrated and diluted samples of the analyte *trans*-stilbene oxide (Fig. 9).

4. Conclusions

This study reports for the first time the HPLC separation of enantiomers on a chiral stationary phase prepared by covalent immobilization of a polysaccharide derivative, namely cellulose (3,5-dichlorophenylcarbamate), onto the surface of superficially porous silica. Baseline separation of enantiomers were achieved for several chiral sulfoxides, *trans*-stilbene and etozoline in pure methanol, acetonitrile or n-hexane/2-propanol as mobile phases with analysis times less than 30 seconds. The effect of the mobile phase flow-rate on the separation performance was studied and van Deemter coefficients determined for several analytes. The effect of some experimental factors such as injected sample volume and especially, detector frequency on recorded results are also shortly discussed.

References

[1] J. Shen, Y. Okamoto, Efficient separation of enantiomers using stereoregular chiral polymers, Chem. Rev. 116 (2016) 1094-1138.

[2] B. Chankvetadze, Recent developments on polysaccharide-based chiral stationary phases for liquid-phase separation of enantiomers, J. Chromatogr. A 1269 (2012) 26-51.

[3] Y. Okamoto, M. Kawashima, K. Hatada, Chromatographic resolution: XI. Controlled chiral recognition of cellulose triphenylcarbamate derivatives supported on silica gel, J. Chromatogr. 363 (1986) 173-186.

[4] K.S.S. Dossou, P. Chiap, B. Chankvetadze, A.C. Servais, M. Fillet, J. Crommen, Enantiomer resolution of basic pharmaceuticals using cellulose tris(4-chloro-3-methylpnelycarbamate) as chiral stationary phase and polar organic mobile phases, J. Chromatogr. A, 1216 (2009) 7450-7455.

[5] S. Rocchi, S. Fanali, T. Farkas, B. Chankvetadze, Effect of content of coating percentage of chiral selector and pore size of core-shell type silica support on the performance of amylose tris(3,5-dimethylphenylcarbamate)-based chiral stationary phases in nano-liquid chromatography and capillary electrochromatography, J. Chromatogr. A 1363 (2014) 363-371.

[6] S. Fanali, G. D'Orazio, K. Lomsadze, B. Chankvetadze, Enantioseparations with cellulose(3-chloro-4-methlphenylcarbamate) in nano liquid chromatography and capillary electrochromatography, J. Chromatogr. B 875 (2008) 296-303.

[7] K. Si-Ahmed, Z. Aturki, B. Chankvetadze, S. Fanali, Evaluation of novel amylose and cellulose-based chiral stationary phases for the enantiomer separation of

flavanones by means of nano-liquid chromatography, Anal. Chim. Acta, 738 (2012) 85-94.

[8] M. Girod, B. Chankvetadze, G. Blaschke, Enantioseparations using non-aqueous capillary electrochromatography on cellulose and amylose tris(3,5-dimethylphenylcarbamate)s coated on silica gels of various pore and particle size, Electrophoresis, 22 (2001) 1282-1291.

[9] D. Albals, A. Hendrickx, L. Clincke, B. Chankvetadze, Y. Vander Heyden, D. Mangelings, A chiral separation strategy for acidic drugs in capillary electrochromatography using both chlorinated and non-chlorinated polysaccharide-based selectors, Electrophoresis 35 (2014) 2807-2818.

[10] S. Thürmann, C. Lotter, J. J. Heiland, B. Chankvetadze, D. Belder, Chip-based high-performance liquid chromatography for high-speed enantioseparations, Anal. Chem. 87 (2015) 5568-5576.

[11] K. Tachibana, A. Ohnishi, Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases, J. Chromatogr. A 906 (2001) 127-154.

[12] L. Peng, S. Jayapalan, B. Chankvetadze, T. Farkas, Reversed phase chiral HPLC and LC/MS analysis with tris(Chloromethylphenylcarbamate) derivatives of cellulose and amylose as chiral stationary phases, J. Chromatogr. A 1217 (2010) 6942-6955.

[13] I. Matarashvili, D. Ghughunishvili, L. Chankvetadze, N. Takaishvili, M. Tsintsadze, T. Khatiashvili, T. Farkas, B. Chankvetadze, Separation of enantiomers of chiral weak acids with polysaccharide-based chiral columns and

aqueous mobile phases in high-performance liquid chromatography: Typical reversed-phase behavior? J. Chromatogr. A 1483 (2017) 86-92.

[14] C. West, Enantioselective separations with supercritical fluids, Curr. Anal. Chem. 10 (2014) 99-120.

[15] C. West, M.-L. Konjaria, N. Shashviashvili, E. Lemasson, P. Bonnet, R. Kakava, A. Volonterio, B. Chankvetadze, Enantioseparation of novel chiral sulfoxides on chlorinated polysaccharide stationary phases in supercritical fluid chromatography, J. Chromatogr. A 1499 (2017) 174-182.

[16] Y. Okamoto, R. Aburatani, S. Miura, K. Hatada, Chiral stationary phases for HPLC: Cellulose tris(3,5-dimethylphenylcarbamate) and tris(3,5-dichlorophenylcarbamate) chemically bonded to silica gel, J. Liq. Chromatogr. 10 (1987) 1613-1628.

[17] J. Shen, T. Ikai, Y. Okamoto, Synthesis and application of immobilized polysaccharide-based chiral stationary phases for enantioseparation by high-performance liquid chromatography, J. Chromatogr. A 1363 (2014) 51-61.

[18] E. Francotte, D. Huynh, T. Zhang, Photochemically immobilized 4-methylbenzoyl cellulose as a powerful chiral stationary phase for enantioselective chromatography, Molecules 21 (2016) Article 1740.

[19] L. Oliveros, A. Senso, C. Minguillón, Benzoates of cellulose bonded on silica gel: Chiral discrimination ability as high-performance liquid chromatographic chiral stationary phases, Chirality 9 (1997) 145-149.

[20] B. Chankvetadze, T. Kubota, T. Ikai, C. Yamamoto, N. Tanaka, K. Nakanishi, Y. Okamoto, High-performance liquid chromatographic enantioseparations on

capillary columns containing crosslinked polysaccharide phenylcarbamate derivatives attached to monolithic silica, J. Sep. Sci. 29 (2006) 1988-1995.

[21] B. Chankvetadze, T. Ikai, C. Yamamoto, Y. Okamoto, High-performance liquid chromatographic enantioseparations on monolithic silica column containing covalently attached 3,5-dimethylphenylcarbamate derivative of cellulose, J. Chromatogr. A 1042 (2004) 55-60.

[22] B. Chankvetadze, T. Ikai, C. Yamamoto, Y. Okamoto, High-performance liquid chromatographic enantioseparations on monolithic silica column containing covalently attached 3,5-dimethylphenylcarbamate derivative of cellulose, J. Chromatogr. A 1042 (2004) 55-60.

[23] K. Lomsadze, G. Jibuti, T. Farkas, B. Chankvetadze, Comparative high-performance liquid chromatography enantioseparations on polysaccharide based chiral stationary phases prepared by coating totally porous and core-shell silica particles, J. Chromatogr. A, 1234 (2012) 50-55.

[24] S. Fanali, G. D´ Orazio, T. Farkas, B. Chankvetadze, Comparative separation of enantiomers with totally porous and coreshel polysaccharide-based chiral stationary phases in nano liquid chromatography and capillary electrochromatography, J. Chromatogr. A, 1269 (2012) 136-142.

[25] S. Rocchi, S. Fanali, T. Farkas, B. Chankvetadze, Effect of content of coating percentage of chiral selector and pore size of core-shell type silica support on the performance of amylose tris(3,5-dimethylphenylcarbamate)-based chiral stationary phases in nano-liquid chromatography and capillary electrochromatography, J. Chromatogr. A, 1363 (2014) 363-371.

[26] Q. Kharaishvili, G. Jibuti, T. Farkas, B. Chankvetadze, Further proof to the utility of polysaccharide-based chiral selectors in combination with superficially porous silica particles as effective chiral stationary phases for separation of enantiomers

in high-performance liquid chromatography J. Chromatogr. A, 1467 (2016) 163-168.

[27] L. Bezhitashvili, A. Bardavelidze, T. Ordjonikidze, T. Farkas, M. Chity, B. Chankvetadze, Effect of pore-size optimization on the performance of polysaccharide-based superficially porous chiral stationary phases for separation of enantiomers in high-performance liquid chromatography, J. Chromatogr. A, 1482 (2017) 32–38.

[28] M.D. Dolzan, D.A. Spudeit, Z.S. Breitbach, W.E. Barber, G.A. Micke, D.W. Armstrong, Comparison of superficially porous and fully porous silica supports used for a cyclofructan hydrophilic interaction liquid chromatographic stationary phase, J. Chromatogr. A 1365 (2014) 124-130.

[29] D.C. Patel, Z.S. Breitbach, M. F. Wahab, C.L. Barhate, D.W. Armstrong, Gone in seconds: Praxis, performance, and peculiarities of ultrafast chiral liquid chromatography with superficially porous particles, Anal. Chem. 87 (2015) 9137-9148.

[30] A. Cavazzini, N. Marchetti, R. Guzzinati, M. Pierini, A. Ciogli, D. Kotoni, I. D'Acquarica, C. Villani, F. Gasparrini, Enantioseparation by ultra-high-performance liquid chromatography, TrAC – Tr. Anal. Chem. 63 (2014) 95-103.

[31] D.C. Patel, M. Farooq Wahab, D.W. Armstrong, Z.S. Breitbach, Advances in high-throughput and high-efficiency chiral liquid chromatographic separations, J. Chromatogr. A, 1467 (2016) 2-18.

[32] O. H. Ismail, A. Ciogli, C. Villani, M. De Martino, M. Pierini, A. Cavazzini, D. S. Bell, F. Gasparrini, Ultra-fast high-efficiency enantioseparations by means of a teicoplanin-based chiral stationary phase made on sub-2μm totally porous silica particles of narrow size distribution, J. Chromatogr. A 1427 (2016) 55-68.

[33] M. Catani, O.H. Ismail, A. Cavazzini, A. Ciogli, C. Villani, L. Pasti, C. Bergantin, D. Cabooter, G. Desmet, F. Gasparrini, D. Bell, Rationale behind the

optimum efficiency of columns packed with new 1.9 μm fully porous particles of narrow particle size distribution, J. Chromatogr. A, 1454 (2016) 78-85.

[34] O.H. Ismail, L. Pasti, A. Ciogli, C. Villani, J. Kocergin, S. Anderson, F. Gasparrini, A. Cavazzini, M. Catani, Pirkle-type chiral stationary phase on core–shell and fully porous particles: Are superficially porous particles always the better choice toward ultrafast high-performance enantioseparations? J. Chromatogr. A, 1466 (2016) 96–104.

[35] M. Catani, O.H. Ismail, F. Gasparrini, M. Antonelli, L. Pasti, N. Marchetti, S. Felletti, A. Cavazzini, Recent advancements and future directions of superficially porous chiral stationary phases for ultrafast high-performance enantioseparations, Analyst 142 (2017) 555-566.

[36] O.H. Ismail, M. Antonelli, A. Ciogli, C. Villani, A. Cavazzini, M. Catani, S. Felletti, D.S. Bell, F. Gasparrini, Future perspectives in high efficient and ultrafast chiral liquid chromatography through zwitterionic teicoplanin-based 2-μm superficially porous particles, J. Chromatogr. A 1520 (2017) 91-102.

[37] A. Ciogli, O.H. Ismail, G. Mazzoccanti, C. Villani, F. Gasparrini, Enantioselective ultra high performance liquid and supercritical fluid chromatography: The race to the shortest chromatogram, J. Sep. Sci. 41 (2018) 1307-1318.

[38] M. Catani, S. Felletti, O.H. Ismail, F. Gasparrini, L. Pasti, N. Marchetti, C. De Luca, V. Costa, A. Cavazzini, New frontiers and cutting edge applications in ultra high performance liquid chromatography through latest generation superficially porous particles with particular emphasis to the field of chiral separations, Anal. Bioanal. Chem. 410 (l 2018) 2457-2465.

[39] C.L. Barhate, E.L Regalado, N.D. Contrella, J. Lee, J. Jo, A.A. Makarov, D.W. Armstrong, C.J. Welch, Ultrafast chiral chromatography as the cecond dimension in two-Dimensional liquid chromatography experiments, Anal. Chem. 89 (2017) 3545-3553.

[40] D.C. Patel, Z.S. Breitbach, J. Yu, K.A. Nguyen, D.W. Armstrong, Quinine bonded to superficially porous particles for high-efficiency and ultrafast liquid and supercritical fluid chromatography, Anal. Chim. Acta 963 (2017) 164-174.

[41] G. Pinna, M.C. Bellucci, L. Malpezzi, L. Pisani, S. Superchi, A. Volonterio, M. Zanda, An umpolung sulfoxide reagent for use as a functionalized benzyl carbanion equivalent, Tetrahedron 67 (2011) 5268-5281.

[42] B. Chankvetadze, C. Yamamoto, Y. Okamoto, HPLC Enantioseparation with cellulose tris(3,5-dichlorophenylcarbamate) in aqueous methanol as a mobile phase, Chem. Lett. 29 (2000) 352-353.

[43] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Enantioseparations using cellulose tris(3,5-dichlorophenylcarbamate) in high-performance liquid chromatography in common size and capillary columns: Potential for screening of chiral compounds. Combinatorial Chemistry and High Troughput Screening 3 (2000) 497-508.

[44] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Extremely high enantiomer recognition in HPLC separation of racemic 2-(benzylsulfinyl)benzamide using cellulose tris (3,5-dichlorophenylcarbamate) as a chiral stationary phase, Chem. Lett. 29 (2000) 1176-1177.

[45] T. Zhang, D. Nguyen, P. Franco, Y. Isobe, T. Michishita, T. Murakami, Cellulose tris(3,5-dichlorophenylcarbamate) immobilised on silica: A novel chiral stationary phase for resolution of enantiomers, J. Pharm. Biomed. Anal. 46 (2008) 882-891.

[46] C. Fanali, S. Fanali, B. Chankvetadze, HPLC separation of enantiomers of some flavanone derivatives using polysaccharide-based chiral selectors covalently immobilized on silica, Chromatographia 79 (2016) 119-124.

[47] N. Beridze, E. Tsutsqiridze, N. Takaishvili, A. Mskhiladze, T. Farkas, B. Chankvetadze, Comparison of chiral recognition ability of coated and covalently immobilized versions of two polysaccharide-based chiral selectors in high-performance liquid chromatography, Chromatographia 81 (2018) 611-621.

[48] J.M. Padro, S. Keunchkarian, State-of-the-art and recent developments of immobilized polysaccharide-based chiral stationary phases for enantioseparations by high-performance liquid chromatography (2013–2017), Microchem. Journ. 140 (2018) 142-157.

[49] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Very fast enantioseparations in HPLC using cellulose tris(3,5-dimethylphenylcarbamate) as chiral stationary phase, Chem. Lett. 32 (2003) 850-851.

[50] G. D´Orazio, R. Kakava, A. Volonterio, S. Fanali, B. Chankvetadze, An attempt for fast separation of enantiomers in nano-liquid chromatography and capillary electrochromatography, Electrophoresis 38 (2017) 1932-1938.

Legends to the figures:

Fig. 1 Chemical structures of studied chral analytes.

Fig. 2 Comparative separation of enantiomers of 2-benzylsulfinyl benzamide (a and b) and 2-(3-bromobenzylsulfinyl) benzamide (c and d) in methanol (a and c) and acetonitrile (b and d) at the mobile phase flow rate 5 ml/min. For other experimental conditions see subsection 2.2.

Fig. 3 The dependence of the height equivalent to the theoretical plate (H) on the linear flow rate of the mobile phase for both enantiomers of 2-(benzylsulfinyl)-N,N-dimethyl benzamide. The mobile phases were acetonitrile and methanol. Chromatograms were recorded at 250 nm and 160 Hz detector frequency.

Fig. 4 Separation of enantiomers of chiral sulfoxides with n-hexane/2-propanol = 90/10 (v/v) with a flow rate 5 ml/min as a mobile phase. Chromatograms were recorded at 200 nm and 160 Hz detector frequency.

Fig. 5 Separation of enantiomers of *trans*-stilbene oxide with n-hexane/2-propanol = 98/2 (v/v) with a flow rate 1 ml/min as a mobile phase. Chromatograms were recorded at 250 nm and 160 Hz detector frequency.

Fig. 6 Fast separation of enantiomers of 2-benzylsulfinyl benzamide (a), 2-(3-bromobenzylsulfinyl) benzamide (b), 2-(benzylsulfinyl)-N-methyl benzamide (c), 2-(benzylsulfinyl)-N,N-dimethyl benzamide (d), 2-(2-methylbenzylsulfinyl) benzamide (e), 2-(3-methylbenzylsulfinyl) benzamide (f), etozoline (g) and *trans*-stilbene oxide (i). The mobile phase was methanol in the cases a-g and n-hexane/2-propanol=98/2 (v/v) in the case (i) at the flow rate 5 ml/min in the cases

a-f and i and 5.5 ml/min in the case g. Chromatograms were recorded at 220 nm and 160 Hz detector frequency.

Fig. 7 The effect of detector frequency on the proper recording of chromatographic process at two different flow rates 0.1 ml/ min (a and b) and 5.0 ml/min (c and d). The analyte was 2-benzylsulfinyl benzamide. Chromatograms were recorded at 250 nm and detector frequency was 5 Hz (a and c) and 160 Hz (b and d).

Fig. 8 Dependence of theoretical plate numbers of *trans*-stilbene oxide enantiomers per meter (N/m) (a) and peak resolution (Rs) (b) on detector frequency at two different flow rates of the mobile phase n-hexane/2-propanol=98/2 (v/v), 1.0 ml/ min and 5.0 ml/min. Chromatograms were recorded at 250 nm.

Fig. 9 Dependence of theoretical plate numbers *trans*-stilbene oxide enantiomers per metre (N/m) on injected sample volume at two different concentrations, 1.0 mg/ml (a) and 0.1 mg/ml (b). The mobile phase was n-hexane/2-propanol=98/2 (v/v) with 1.0 ml/min flow rate. Chromatograms were recorded at 250 nm.

Figure 1
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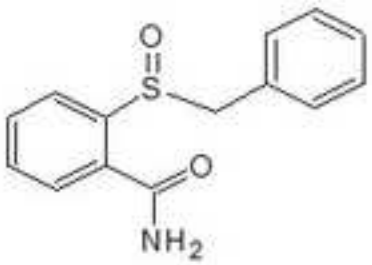
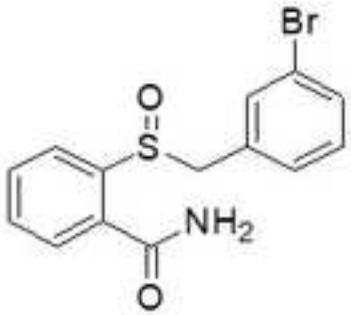
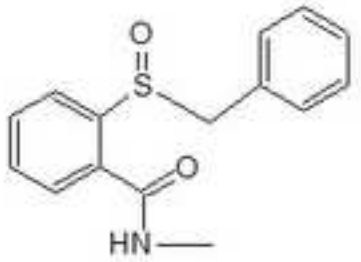


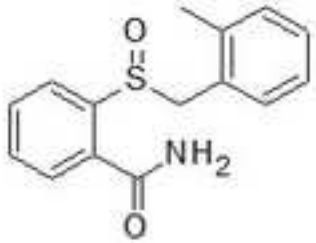
(1) 2-(Benzylsulfinyl) benzamide



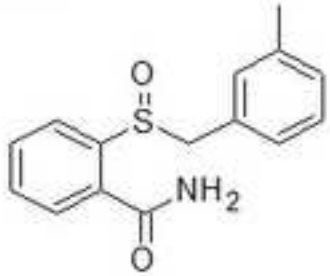
(2) 2-(3-Bromobenzylsulfinyl) benzamide



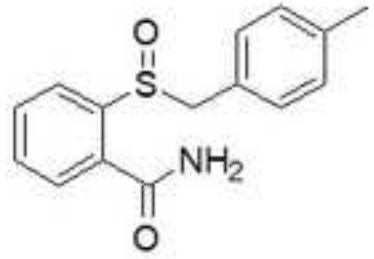
(3) 2-(Benzylsulfinyl) N-methyl benzamide



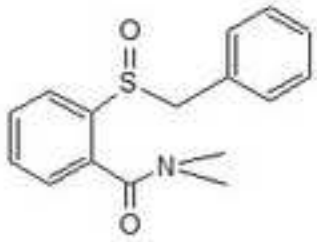
(4) 2-(2-Methylbenzylsulfinyl) benzamide



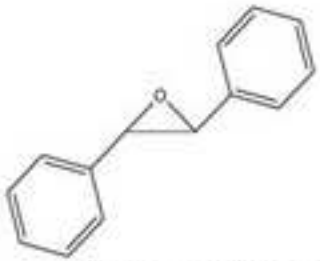
(5) 2-(3-Methylbenzylsulfinyl) benzamide



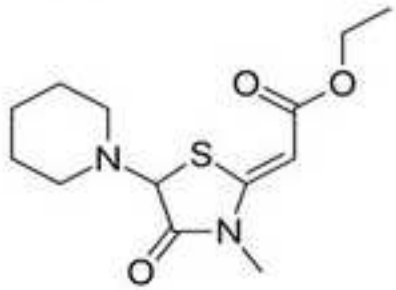
(6) 2-(4-Methylbenzylsulfinyl) benzamide



(7) 2-(Benzylsulfinyl) N,N-dimethyl benzamide



(8) *trans*-Stilbene Oxide



(9) Etozoline

Fig. 1

Figure 2
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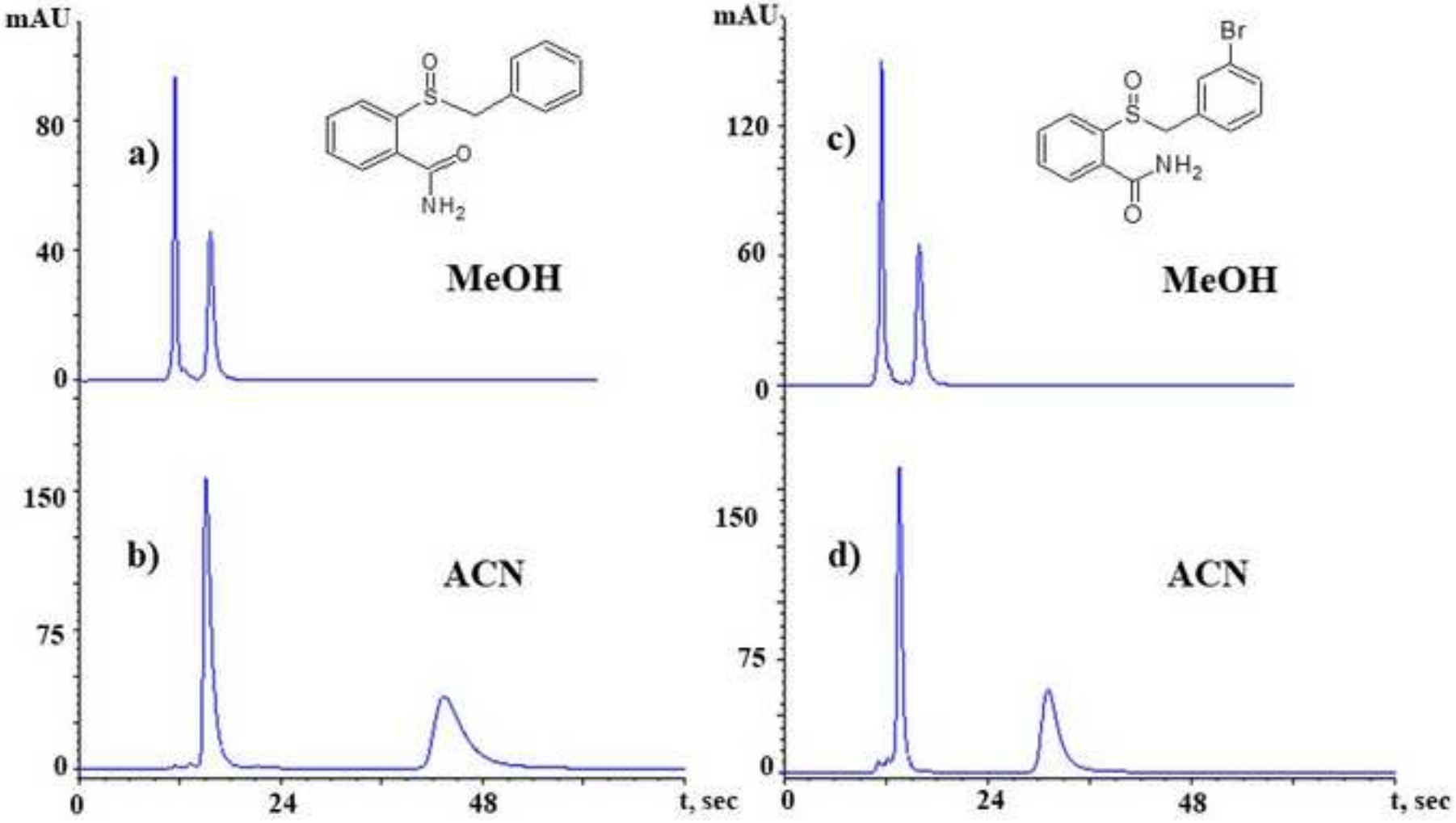

Fig. 2

Figure 3
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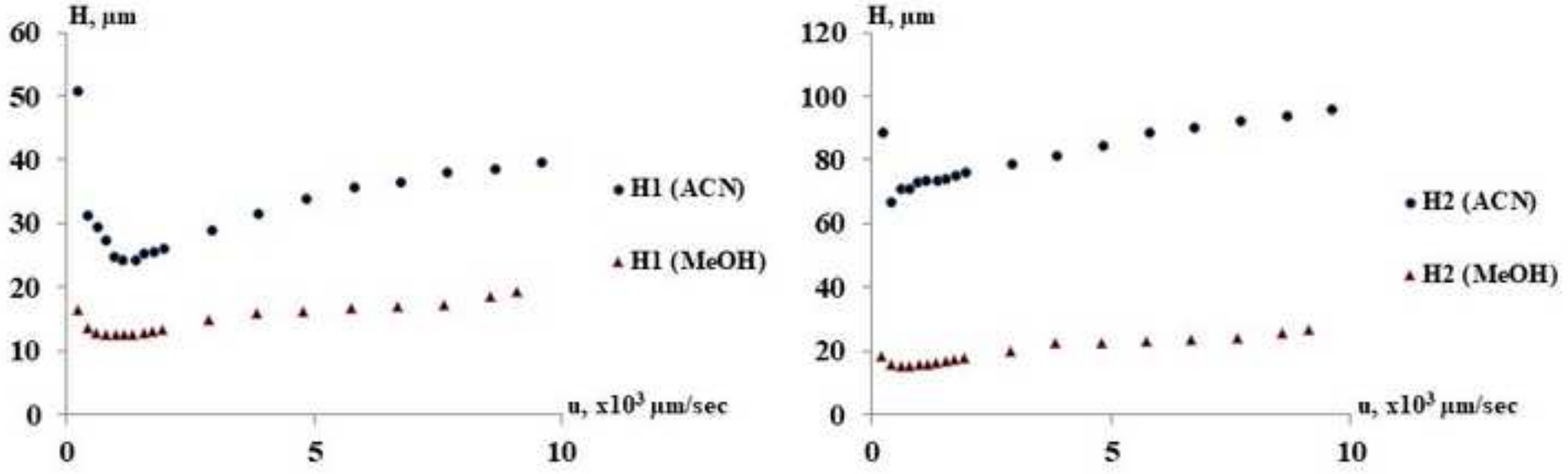

Fig. 3

Figure 4

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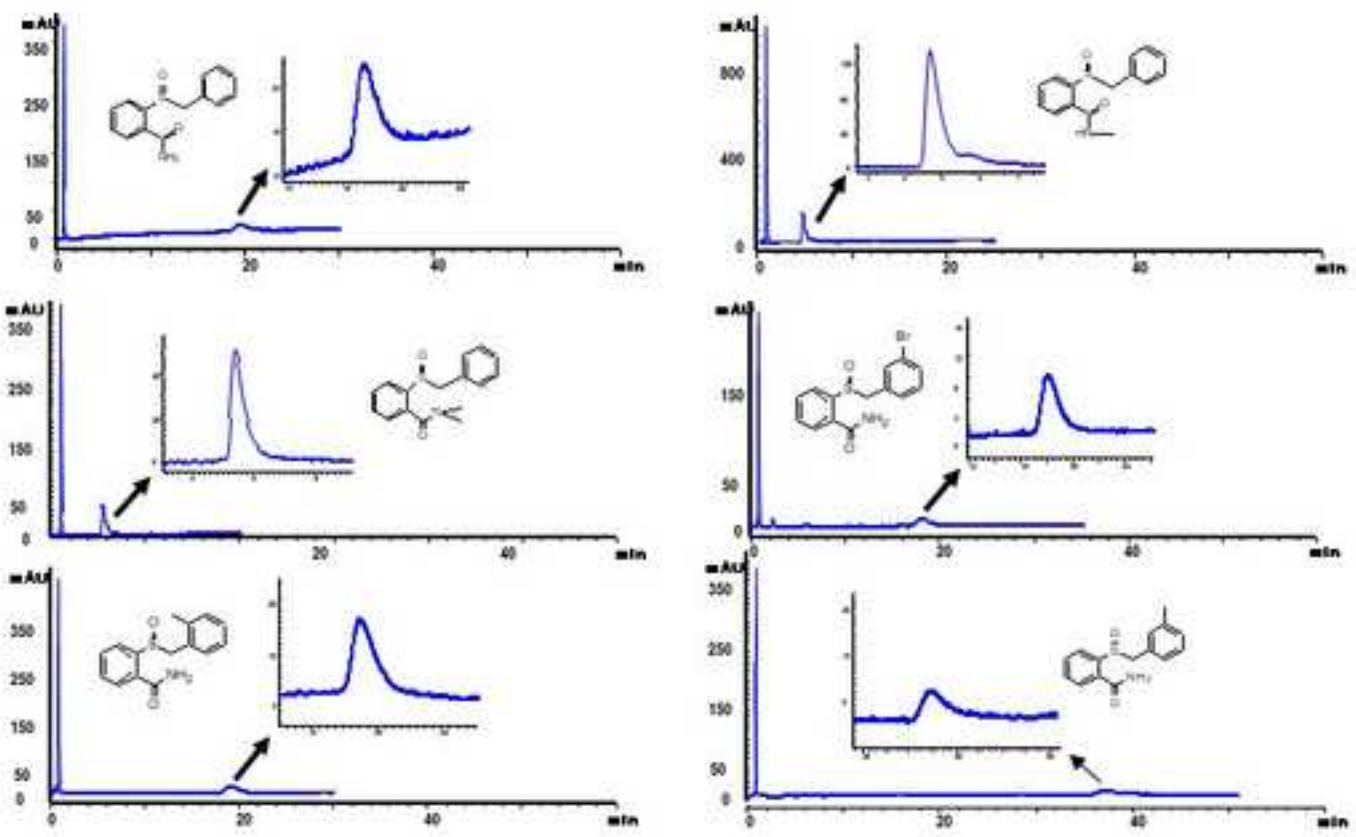

Fig. 4

Figure 5

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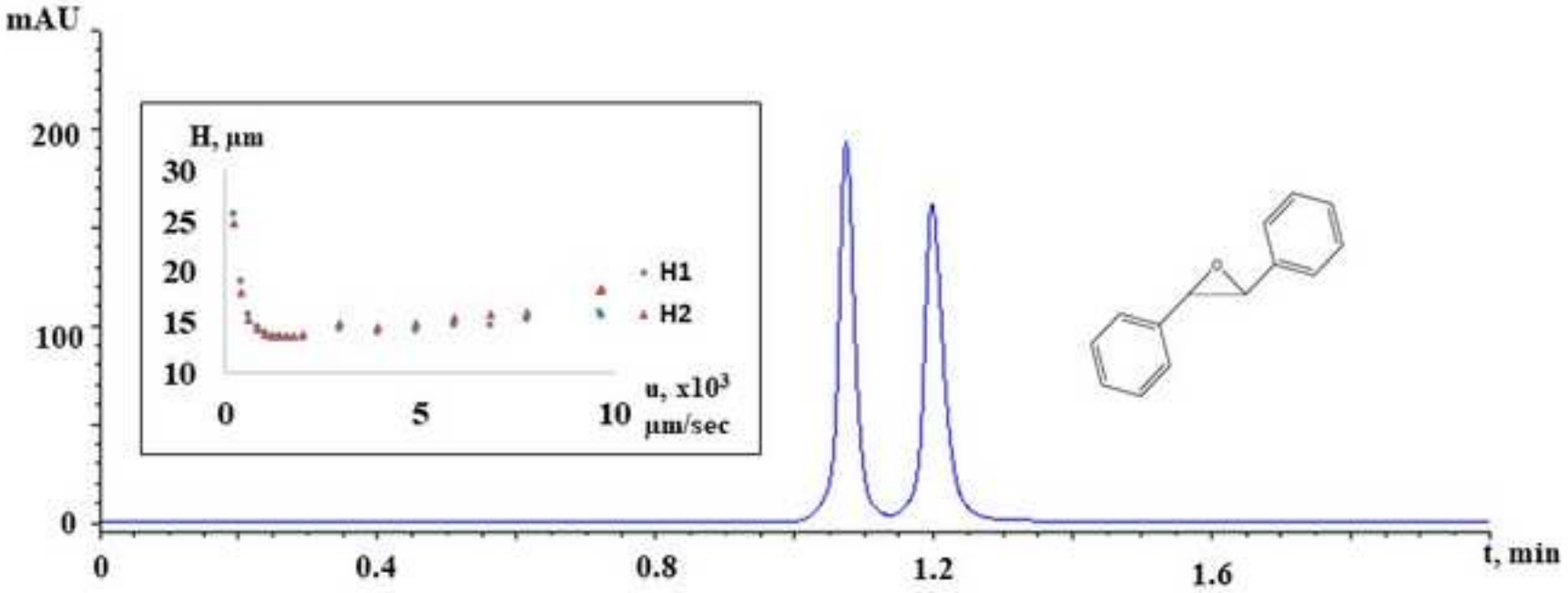

Fig. 5

Figure 6

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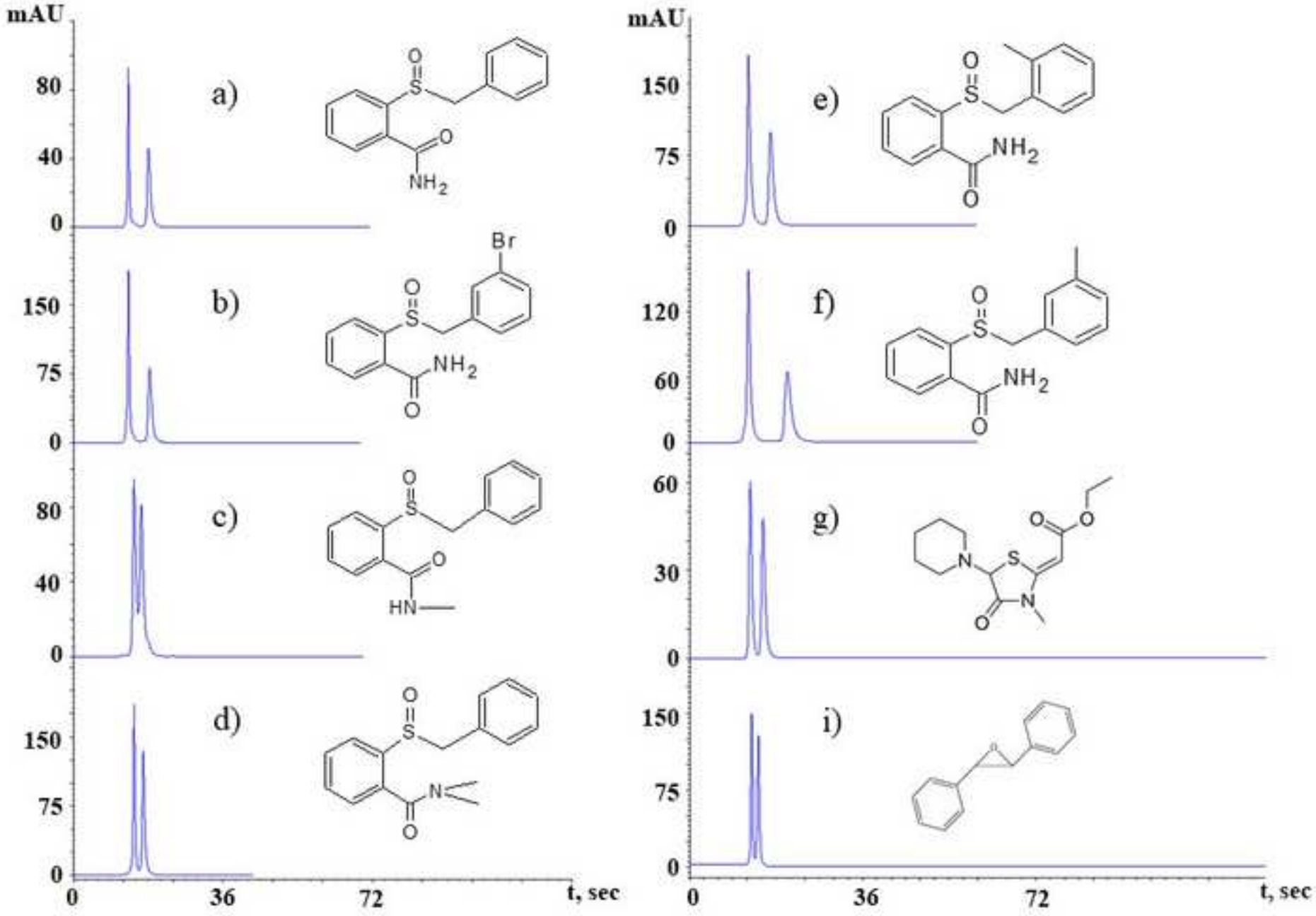

Fig. 6

Figure 7

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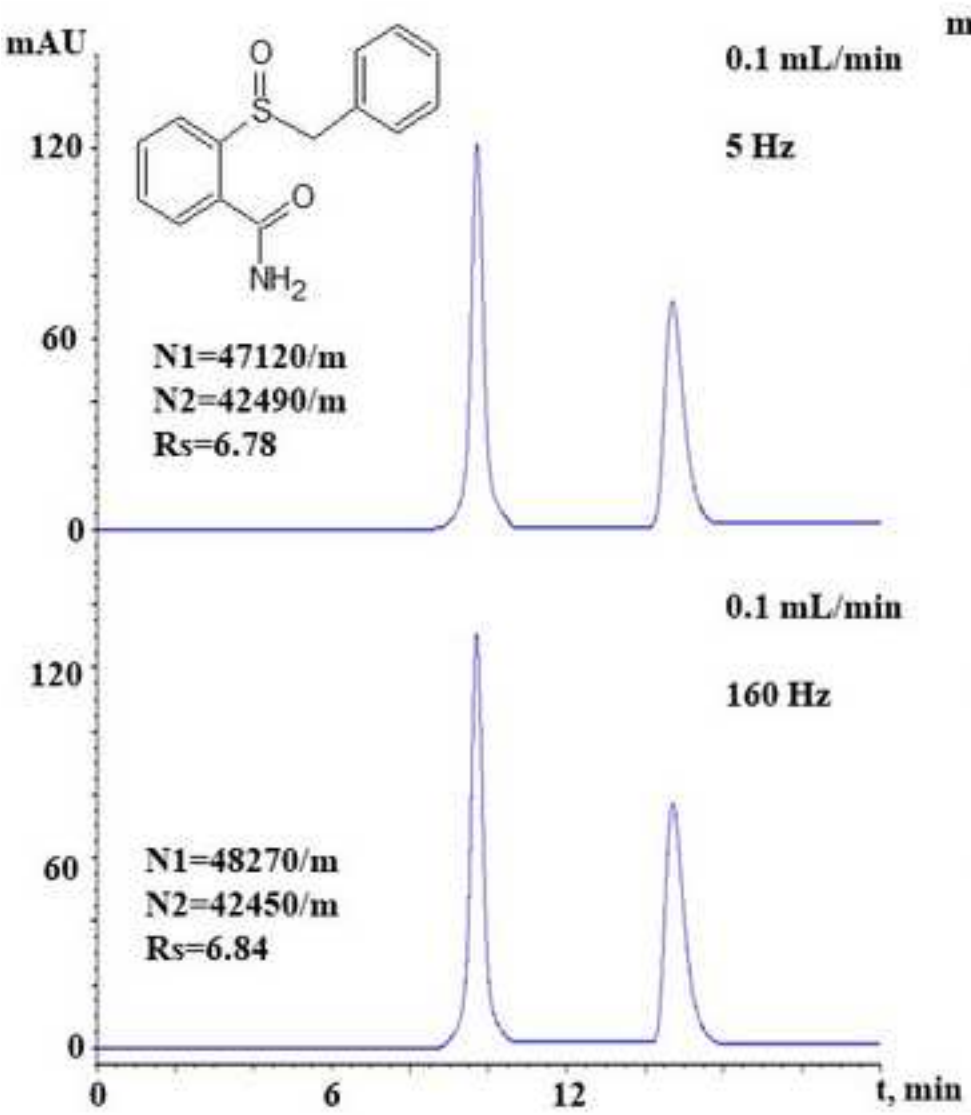

Fig. 7a

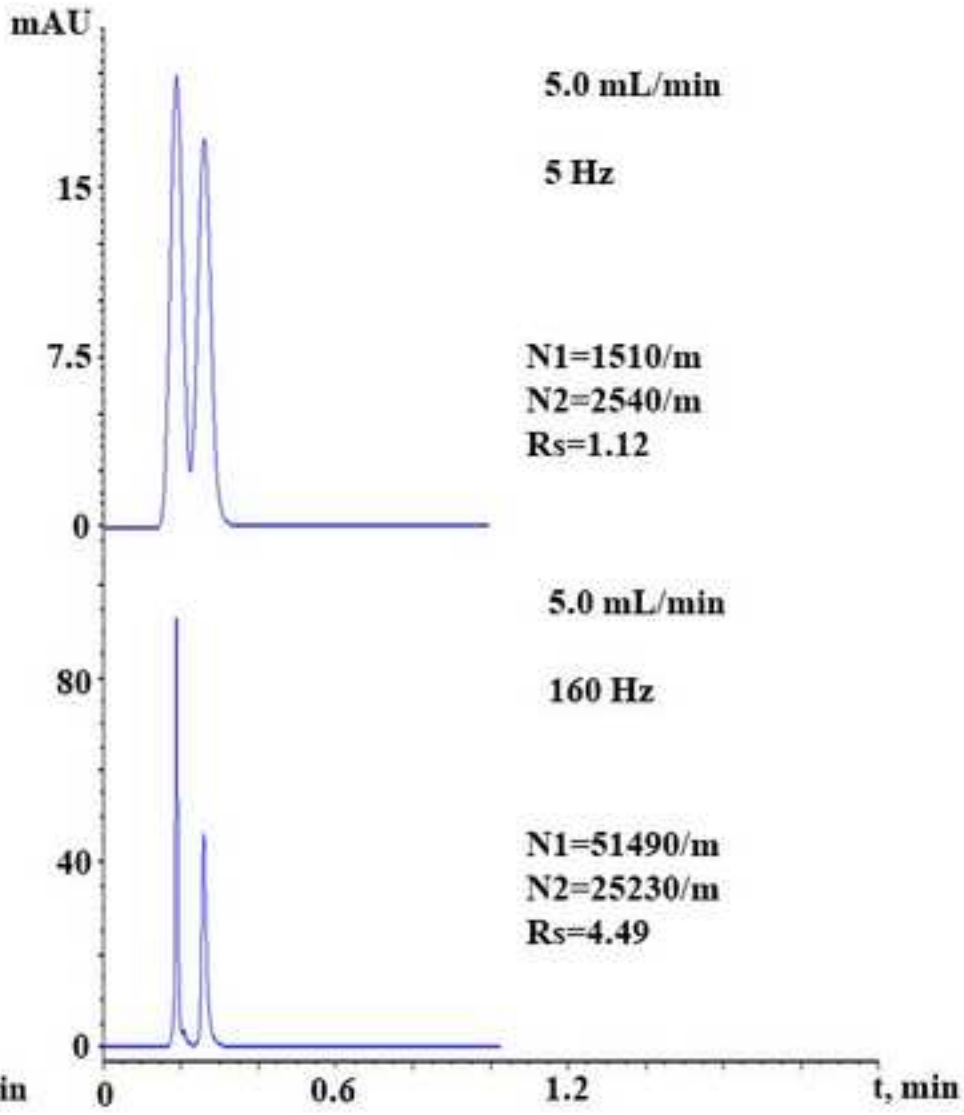

Fig. 7b

Figure 8
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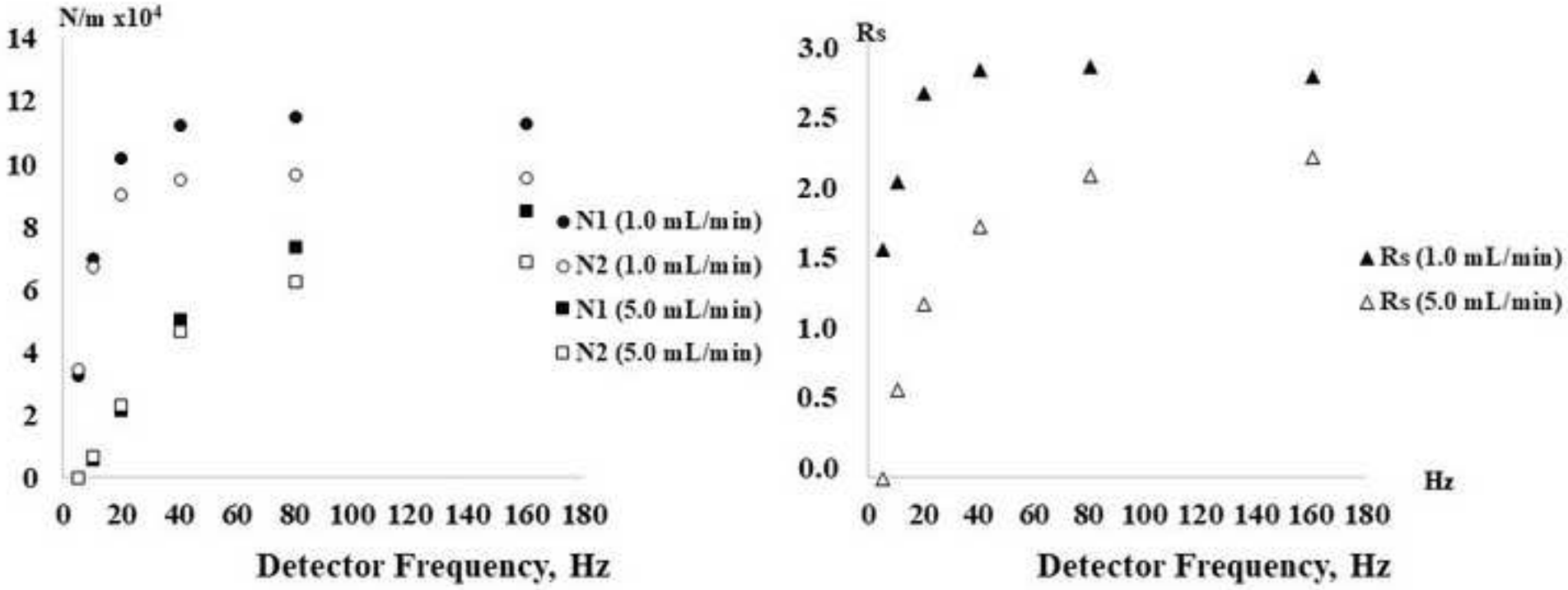

Fig. 8

Figure 9
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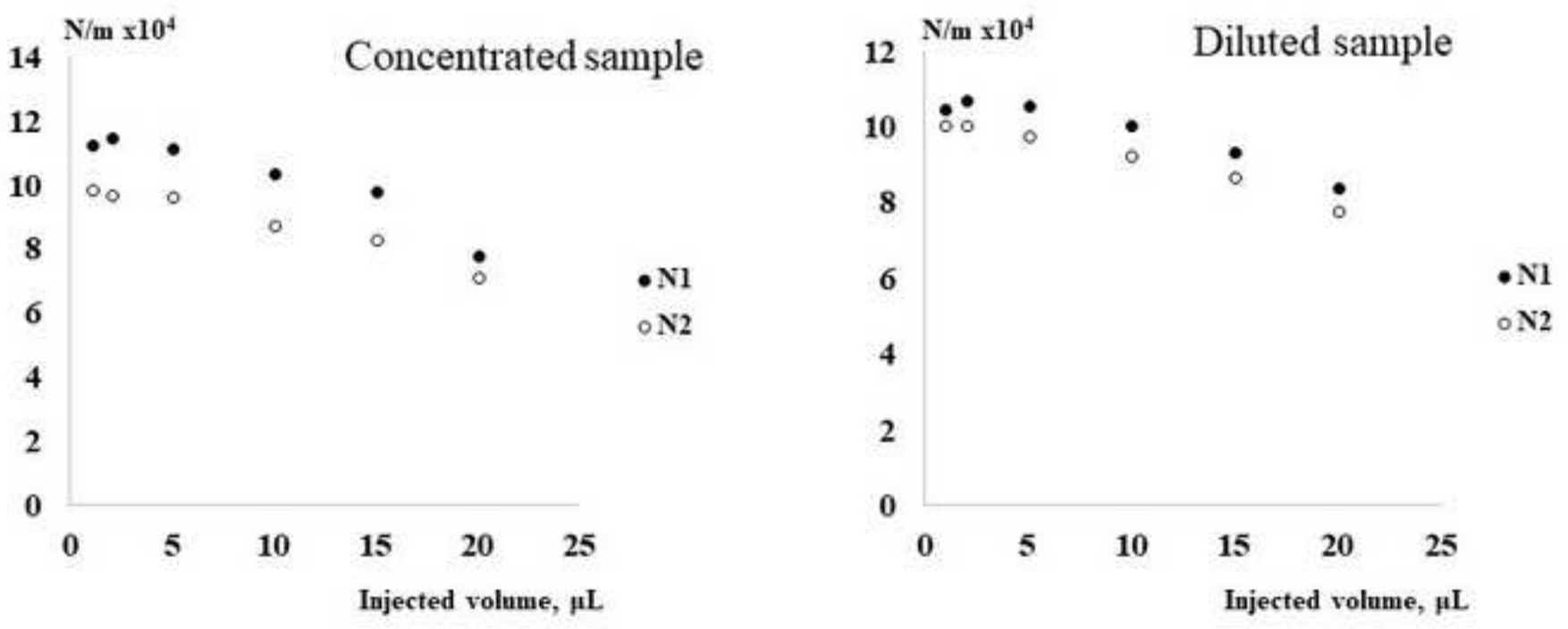

Fig. 9

Table 1 Separation results of studied chiral analytes on CDCPC containing SPS-packed column with methanol as a mobile phase (The flow rate was 1.0 ml/min. For other experimental conditions see the subsection 2.2).

Chiral compound	**t_1**	**t_2**	**k_1**	**k_2**	**α**	**N_1/m**	**N_2/m**	**Rs**
Etozoline	1.11	1.41	0.27	0.61	2.3	31820	30940	3.3
2-Benzylsulfinyl benzamide	0.96	1.42	0.09	0.62	7.0	108750	47830	7.9
2-(3-Bromobenzylsulfinyl) benzamide	0.97	1.46	0.10	0.67	6.5	78890	37890	7.2
2-(4-Methylbenzylsulfinyl) benzamide	0.98	2.38	0.11	1.72	15.3	80380	20020	11.0
2-(Benzylsulfinyl)-N,N-dimethyl benzamide	1.07	1.29	0.22	0.47	2.1	73600	54830	3.7
2-(Benzylsulfinyl) N-methyl benzamide	1.00	1.16	0.14	0.32	2.3	30950	27540	2.0
2-(2-Methylbenzylsulfinyl) benzamide	0.96	1.44	0.09	0.63	6.9	24690	23950	4.9
2-(3-Methylbenzylsulfinyl) benzamide	0.97	1.82	0.11	1.07	9.9	26940	19130	7.0

Table 2 van Deemter coefficients for separation of studied chiral analytes on CDCPC containing SPS-packed column with methanol, acetonitrile ~~as~~ and n-hexane/2-propanol as mobile phases (For experimental conditions see the subsection 2.2).

Chiral compound	Mobile phase	Peak number	**A, μm**	**B, μm^2/sec**	**C, sec,$^{-1}$**
2-(benzylsulfinyl) benzamide	MeOH	I	4.6	3773.8	0.0013
		II	14.7	2117.7	0.0024
2-(3-Bromobenzylsulfinyl) benzamide	MeOH	I	10.0	937.0	0.0011
	ACN	I	31.0	1791.2	0.0004
2-(Benzylsulfinyl)-N,N-dimethyl benzamide	MeOH	I	11.4	733.5	0.0009
		II	15.4	297.5	0.0013
	ACN	I	18.1	5839.2	0.0025
		II	66.7	3208.2	0.0034
trans-Stilbene oxide	HEX/IPA 98/2 (v/v)	I	11.2	2773.5	0.0005
		II	11.0	2606.2	0.0007