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Comparison of the performance of chiral stationary phase for separation of fluoxetine enantiomers

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Abstract: Separation of fluoxetine enantiomers on five chiral stationary phases (chiralcel OD-H, chiralcel OJ-H, chiralpak AD-H, cyclobond I 2000 DM and kromasil CHI-TBB) was investigated. The optimal mobile phase compositions of fluoxetine separation on each column were hexane/isopropanol/diethyl amine (98/2/0.2, v/v/v), hexane/isopropanol/diethyl amine (99/1/0.1, v/v/v), hexane/isopropanol/diethyl amine (98/2/0.2, v/v/v), methanol/0.2% triethylamine acetic acid (TEAA) (25/75, v/v; pH 3.8) and hexane/isopropanol/diethyl amine (98/2/0.2, v/v/v), respectively. Experimental results demonstrated that baseline separation (R_S>1.5) of fluoxetine enantiomers was obtained on chiralcel OD-H, chiralpak AD-H, and cyclobond I 2000 DM while the best separation was obtained on the last one. The eluate orders of fluoxetine enantiomers on the columns were determined. The first eluate by chiralcel OJ-H and kromasil CHI-TBB is the *S*-enantiomer, while by chiralpak AD-H and cyclobond I 2000 DM is the *R*-enantiomer.

Key words: Fluoxetine, Chiral stationary phase, Enantiomeric separation, High performance liquid chromatography (HPLC)
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INTRODUCTION

Fluoxetine (Fig.1) is a selective serotonin-reuptake inhibitor used for treating depression and obsessive-compulsive disorders. So far the drug used is racemate, but the individual optical isomers do not have identical activity (Agranat and Cancer, 1999): *R*-isomer is effective for treating depression while *S*-isomer is for migraine headaches. Study on the separation of racemic fluoxetine hydrochloride is of important significance.

A few chromatographic methods for the separation of fluoxetine enantiomers have been reported. Indirect methods of GC (Torok-Both *et al*., 1992) and LC (Guo *et al.*, 2002; 2004; Wang *et al*., 2004; Eap *et al*., 1996; Potts and Parli, 1992) require the preparation of derivatives. The disadvantage of methods using chiral mobile phases (Piperaki *et al*., 1995) is the consumption of many chiral mobile phases. HPLC using chiral stationary phases (CSPs) is more effective (Gatti *et al*., 2003; Kaddoumi *et al*., 2001; Olsen *et al*., 1998; Wang and Yun, 1997; Bakhtiar and Tse, 2000; Shen *et al*., 2002; Yee *et al*., 2000; Piperaki and Poulou, 1993; Berthod *et al*., 1990; Yu *et al*., 2002). By using tris (3,5-dimethylphenyl carbamate) cellulose (chiralcel OD-H), Gatti *et al*.(2003) obtained a separation factor (α) of 1.10. Olsen *et al*.(1998) obtained a resolution of 1.4 by using ovomucoid (Ultron ES-OVM). A separation factor of 1.08 was obtained on urea derivative (Wang and Yun, 1997), and 1.09 on Teicoplanin (Bakhtiar and Tse,

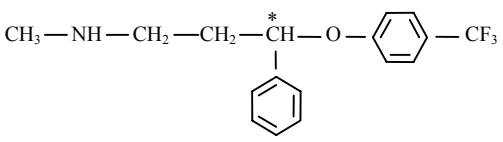

Fig.1 Structure of fluoxetine

2000) CSP. Furthermore, baseline separation was achieved by using α-acid glycoprotein (chiral AGP) or β-cyclodextrin (cyclobond I) (Piperaki and Poulou, 1993; Berthod *et al*., 1990).

The present work was aimed at studying the performance of four CSPs for the separation of fluoxetine enantiomers, i.e. chiralcel OJ-H, chiralpak AD-H, cyclobond I 2000 DM and kromasil CHI-TBB. The performance of chiralcel OD-H was also determined for comparison.

EXPERIMENTAL DETAILS

Reagents

HPLC-grade hexane, methanol, acetonitrile and isopropyl alcohol were from B & J Brand (Muskegon, MI). Ultra pure water was obtained by Milli-Q system (Millipore, Milford, MA). Acetic acid, diethylamine (DEA) and triethylamine (TEA) were filtered with 0.45 μm solvent filter and ultrasonically degassed. Samples of racemic fluoxetine hydrochloride were from Lijing Ltd. (Taizhou, China).

Apparatus

The chromatograph system consisted of a Waters 1525 Binary HPLC pump, a Waters 717 plus multiple autosampler and a Waters 2487 Dual λ UV detector (Waters, USA). The detection wavelength was 226 nm and the detector temperature was 20 °C. Chromatograms were recorded at 1 Hz, and peak-areas were determined by using a Waters Breeze data acquisition system.

Columns of chiralcel OD-H, chiracel OJ-H, chiralpak AD-H and cyclobond I 2000 DM were from chiral technologies (Exton, PA, USA). Kromasil CHI-TBB column was from Eka Chemicals AB (Bohus, Sweden). The columns were all of the same size of 25 cm×4.6 mm i.d., and the CSP particle size was 5 μm.

Dead volumes of the columns were determined with 1,3,5-tri-tert-butylbenzene for chiralcel OD-H, chiralcel OJ-H and chiralpak AD-H, toluene for cyclobond I 2000 DM, and trifluoroethene for kromasil CHI-TBB.

Sample preparation

Five milligrams of fluoxetine HCl was dissolved in 25 ml mobile phase. The injection size was 10 μl.

RESULTS AND DISCUSSION

Comparison of separation performance of different chiral columns

It is well known that the performance of a definite column is affected by the composition and the flow rate of the mobile phase. Experiments were carried out to find the optimal composition and flow rate for each column. The best separation results of the five columns at optimal conditions are listed in Table 1, the corresponding chromatograms are shown in Fig.2.

The performance of a stationary phase is usually evaluated by two criteria: resolution and capacity factor. The resolution should be acceptable if the enantiomers are baseline separated; while the capacity factor should be small so that the retention time is short and the mobile phase consumption is low. Fig.2 of the results shows that fluoxetine enantiomers are baseline separated by three of the CSPs studied: chiralcel OD-H, chiralpak AD-H and cyclobond I

Table 1 Comparison of performance of different chiral columns

No.	Stationary phase	Retention time (min)		Capacity factor		Separation factor (α)	Resolution (R_S)
		t_{R1}	t_{R2}	k_1'	k_2'		
1	Chiralcel OD-H	18.4	20.3	4.10	4.63	1.13	1.74
2	Chiralcel OJ-H	19.9	20.9	2.27	2.44	1.07	0.99
3	Chiralpak AD-H	19.2	21.4	2.18	2.54	1.16	1.79
4	Cyclobond I 2000 DM	16.4	19.2	3.32	4.05	1.22	2.30
5	Kromasil CHI-TBB	11.4	12.1	2.12	2.22	1.05	0.84

Mobile phase compositions of Nos. 1, 3 and 5 are hexane/isopropanol/DEA, 98/2/0.2 (v/v/v), of No. 2 are hexane/isopropanol/DEA, 99/1/0.1 (v/v/v), of No. 4 are methanol/0.2% TEAA (triethylamine acetic acid), 25/75 (v/v), pH 3.8; Flow rate of mobile phase of Nos. 1 and 4 is 0.8 ml/min, of Nos. 2, 3, and 5 is 0.5 ml/min; Column temperature of No. 1 is 15 °C, of Nos. 2 and 3 is 20 °C, of No. 4 is 30 °C, of No. 5 is 23 °C

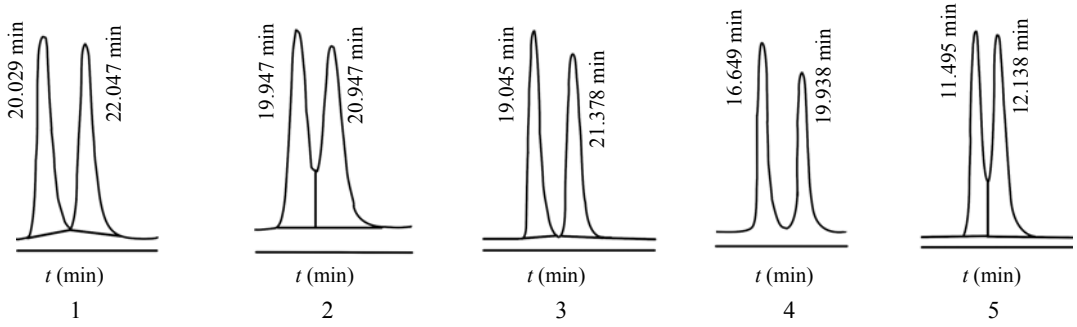

Fig.2 Chromatograms of fluoxetine of five columns
Chromatogram numbers are the same as in Table 1

2000 DM. Among them the best separation is obtained by cyclobond I 2000 DM (5-dimethyl-β-cyclodextrin), with a resolution of 2.30 (Table 1), much greater than that of all other CSPs reported in the literature. This is perhaps due to the hydrogen bond, dipole-dipole and π-π interactions, and especially due to the hydrophobic cavity of β-cyclodextrin affording a chiral environment. As compared to cyclobond I (β-cyclodextrin bonded CSP), the selectivity is probably enhanced by the addition of dimethyl groups.

The performance of chiralpak AD-H [tris (3,5-dimethylphenyl carbamate) amylose] is also satisfactory. Its capacity factor is much less than the other two CSPs while the resolution is good enough. In consequence, its mobile phase consumption is much less while the retention time is comparable to the other two CSPs. The performance of chiralcel OD-H [tris (3,5-dimethylphenyl carbamate) cellulose] is in-between that of the other two. Its resolution is similar to that of chiralpak AD-H while its capacity factor is similar to cyclobond I 2000 DM. Regarding the chemical nature of the CSPs, the resolution on cyclodextrin is the best, the mobile phase consumption by amylose is the least, and the performance of cellulose is somewhat in-between.

Fluoxetine enantiomers are not well separated by chiralcel OJ-H and kromasil CHI-TBB (0,0′-bis 4-tert-butylbenzoyl)-N,N′-diallyl-L-tartar diamide). The structures of chiralcel OJ-H [tris (4-dimethylphenyl carbamate) cellulose] and chiralcel OD-H are similar, differing only by one functional group, and provides an example of the significant effect of functional groups on separation performance.

Elution order of fluoxetine on different chiral columns

To the authors' knowledge, the elution order of fluoxetine enantiomers on chiralcel OJ-H, chiralpak AD-H, cyclobond I 2000 DM and kromasil CHI-TBB have never been reported. In the present work the elution order of three CSPs was examined.

Gatti *et al.*(2003) reported that the *S*-enantiomer of fluoxetine is eluted earlier than the *R*-enantiomer on chiralcel OD-H with hexane/isopropanol as mobile phase.

The first eluate of multiple injections on chiralcel OJ-H, cyclobond I 2000 DM and kromasil CHI-TBB were collected and evaporated. Then the samples were injected into a chiralcel OD-H column to obtain the chromatograms under the same conditions (mobile phase: hexane/isopropanol/DEA, 98/2/0.2 (v/v/v); flow rate: 0.5 ml/min; ambient temperature). Fig.3 of

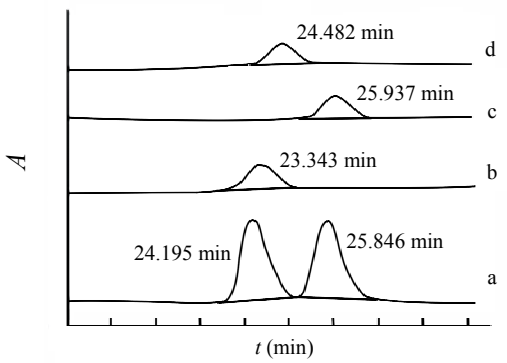

Fig.3 Comparison of chromatograms of fluoxetine enantiomers. (a) Fluoxetine enantiomers on column chiralcel OD-H; (b) First eluate from chiracel OJ-H; (c) First eluate from cyclobond I 2000 DM; (d) First eluate from kromasil CHI-TBB

chromatograms shows that the first eluate from chiralcel OJ-H and kromasil CHI-TBB is the *S*-enantiomer, and that from cyclobond I 2000 DM is the *R*-enantiomer.

CONCLUSION

Fluoxetine enantiomers were separated for the first time on four columns: chiralpak AD-H, chiralcel OJ-H, cyclobond I 2000 DM and kromasil CHI-TBB.

The resolution order of the studied CSPs for separation of fluoxetine enantiomers was: cyclobond I 2000 DM>chiralpak AD-H≈chiralcel OD-H> chiralcel OJ-H>kromasil CHI-TBB. Among them fluoxetine enantiomers are not resolved on the last two.

The first eluate by chiralcel OD-H, chiralcel OJ-H and kromasil CHI-TBB is the *S*-enantiomer, while by chiralpak AD-H and cyclobond I 2000 DM, it is the *R*-enantiomer.

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