Short communication

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Evaluation of polysaccharide-based chiral stationary phases in quality control of (*S*)-mirtazapine

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ABSTRACT: High-performance liquid chromatographic methods were developed for separation of the enantiomers of mirtazapine and its four process-related substances. The direct separations were achieved on chiral stationary phases containing amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak® AD-H), cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel® OD-H) and cellulose tris(4-methylbenzoate) (Chiralcel® OJ-H). The experimental data were utilized to discuss the effects of the mobile phase composition, the nature of the alcoholic modifier and the specific structural features of the analytes on retention and separation. The elution sequence was determined under the optimized separation conditions.

Keywords: mirtazapine; quality control; enantioseparation; coated polysaccharides phases; liquid chromatography

Introduction

Mirtazapine (MTZ) is a tetracyclic antidepressant that finds widespread use as a racemate in the treatment of patients with severe depression (Rao and Raju, 2009). The biological effects of MTZ have been comprehensively reviewed over the past two decades (Holm and Markham, 1999). It has a unique pharmacological profile combining dual action on both the noradrenergic and serotonergic neurotransmitter systems by blocking the α_2- adrenergic receptor and selectively antagonizing 5-HT_2 and 5-HT_3 receptors (Davis and Wilde, 1996). Investigations have revealed that the (*S*)-enantiomer of MTZ has better potential in the treatment of insomnia and the climacteric symptoms associated with the menopause (Kooyman *et al.*, 1994) because of its high receptor binding affinity (Kelder *et al.*, 1997). Thus, the development of single enantiomers as a new active pharmaceutical ingredient is of great importance because biological systems interact with and metabolize them differently.

The economic interests are obvious and an essential driving force in the development of advanced technology for the separation of chiral drugs. While studying the synthetic process (van der Linden *et al.*, 2008) in our laboratory (Fig. 1), we needed to develop a chiral HPLC method for quality control of (*S*)-MTZ. Several chiral assays have been reported in the literature for MTZ and its metabolites, mirtazapine-*N*-oxide, 8-hydroxymirtazapine and desmethylmirtazapine (Lanchote *et al.*, 2010; Aturki *et al.*, 2007; Meineke *et al.*, 2006; Zhai *et al.*, 2005; Fanali *et al.*, 2005; Mandrioli *et al.*, 2004; Paus *et al.*, 2004; Dodd *et al.*, 2000), but none of these can be used for quality control of (*S*)-MTZ.

HPLC on chiral stationary phases (CSPs) is an effective tool for resolution of chiral drugs. A broad spectrum of CSPs is necessary to meet the need for enantiomeric separations of diverse compounds. The most applicable CSPs are based on the linear derivatized polysaccharides, which may separate more than 80% of the racemates. The present paper describes a normal-phase HPLC enantioseparation of MTZ and four of its synthetic intermediates {**I**, 1-methyl-3-phenylpiperazine; **II**, 2-(4-methyl-2-phenylpiperazino)-3-pyridyl cyanide; **III**, 2-(4-methyl-2-phenylpiperazino)nicotinic acid; and **IV**, [2-(4-methyl-2-phenylpiperazino)-3-pyridyl]methanol} on polysaccharide-based chiral stationary phases (Fig. 1). The separation perfomances were compared, and the effect of the mobile phase composition, the nature of the alcoholic modifier and the specific structural features of the analytes on the retention and separation was discussed. To the best of the authors' knowledge there have been no published reports on the quality control of (*S*)-MTZ so far in the literature.

Experimental section

Chemicals and reagents

All the reagents and chemicals used were of analytical grade unless stated otherwise. Diethylamine (DEA), HPLC-grade *n*-hexane, ethanol (EtOH), 1-propanol (PrOH), *n*-butanol (BuOH) and 2-propanol (IPA; Merck, Mumbai, India) were used. MTZ and its process-related substances (**I–IV**; racemates and enantiopure forms) were obtained from the organic chemistry laboratory of IICT (Hyderabad, India). The racemates of MTZ and its process-related substances (**I–IV**) were more than 99% pure by HPLC, whereas the *S*- forms of MTZ and **I–IV** were more than 98% pure.

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Abbreviations used: BuOH, *n*-butanol; CSP, chiral stationary phase; DEA, diethylamine; EtOH, ethanol; IPA, 2-propanol; MTZ, mirtazapine; MP, mobile phase; PrOH, 1-propanol.

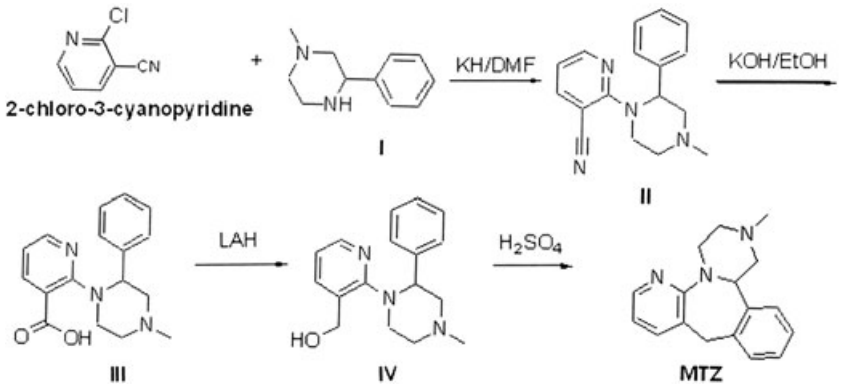

Figure 1. Chemical process in synthesis of mirtazapine (KH, Potassium hydride; DMF, N,N-dimethylformamide; KOH, Potassium hydroxide; LAH, Lithium aluminium hydride; H_2SO_4, Sulfuric acid).

Instruments

An HPLC system consisting of two LC-20AT pumps, an SPD-M20A diode array detector (PDA), a SIL-20AC auto sampler, a DGU-20A3 degasser, a column oven CTO-20A and a CBM-20A communications bus module (all from Shimadzu, Kyoto, Japan) was used. The chromatographic and the integrated data were recorded using an HP-Vectra (Hewlett Packard, Waldron, Germany) computer system using LC-Solution data-acquiring software (Shimadzu, Kyoto, Japan). A Chiralcel® OJ-H (250 × 4.6 mm i.d.; particle size 5 mm) column, Chiralcel® OD-H (250 × 4.6 mm i.d.; particle size 5 mm) column and Chiralpak® AD-H (250 × 4.6 mm i.d.; particle size 5 mm) column (Daicel Chemical Industries Ltd, Tokyo, Japan) were used for separation. A polarimetric detector (IBZ Messtechnik GmbH, Hannover, Germany; optical rotation range, 250; average, 10; offset, 50) was used to assign the order of elution of the enantiomers.

Procedures

The λ_{max} values of the studied analytes were 210 nm for **I**, 268 nm for **II** and **III**, 245 nm for **IV** and 292 nm for MTZ; hence, the chromatograms were recorded at 210 nm using a PDA detector. Analytes were dissolved in a minimum quantity of EtOH and further diluted with mobile phase. The injection volume was 20 µL and before injection the solution was filtered through a 0.45 µm nylon filter.

Results and discussion

Effect of organic modifier

The separations of the enantiomers of MTZ and its process-related substances **I–IV** (Fig. 1) on three different CSPs with *n*-hexane containing different compositions of alcoholic modifiers (EtOH, IPA, PrOH or BuOH) as mobile phases were evaluated. The separation data including the retention, separation and resolution factors are given in Table 1.

The nature of the alcoholic modifier exerted a considerable effect not only on retention but also on the enantioselectivity and resolution, i.e. the ratio of the chiral and achiral interactions

Table 1. Chromatographic data, retention factor (k') and resolution (R_s) for analytes on cellulose- and amylase-based chiral stationary phases with variation of the type and content of the alcoholic modifier

Analyte	MP	Chiralpak AD-H			Chiralcel OD-H			Chiralcel OJ-H		
		k'_1	k'_2	R_s	k'_1	k'_2	R_s	k'_1	k'_2	R_s
I	A	0.680	0.808	1.530	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	B	0.830	0.987	1.823	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	C	1.143	1.375	2.175	0.303	0.415	0.967	n.r.	n.r.	n.r.
	D	n.r.	n.r.	n.r.	0.436	0.553	1.307	0.561	0.656	0.484
	E	n.r.	n.r.	n.r.	0.552	0.707	1.482	0.663	0.783	0.562
	F	n.r.	n.r.	n.r.	0.781	1.012	1.811	0.855	0.979	0.485
	G	n.r.	n.r.	n.r.	0.343	0.408	0.799	n.r.	n.r.	n.r.
	H	n.r.	n.r.	n.r.	0.478	0.567	1.218	n.r.	n.r.	n.r.
	I	n.r.	n.r.	n.r.	0.541	0.638	1.297	n.r.	n.r.	n.r.
	J	0.738	0.864	1.301	0.241	0.299	0.915	n.r.	n.r.	n.r.
	K	1.239	1.472	1.898	0.535	0.622	1.018	n.r.	n.r.	n.r.
	L	1.132	1.326	1.478	0.949	1.107	1.267	n.r.	n.r.	n.r.
II	A	n.r.	n.r.	n.r.	0.404	0.545	1.692	0.840	1.467	5.988
	B	0.714	0.743	0.172	0.628	0.851	2.369	0.952	1.668	6.560
	C	0.944	0.988	0.434	0.696	0.932	2.383	1.182	2.136	7.877
	D	n.r.	n.r.	n.r.	0.511	0.730	2.402	0.999	1.830	4.846
	E	n.r.	n.r.	n.r.	0.673	0.960	2.748	1.169	2.209	5.651
	F	1.238	1.285	0.265	0.885	1.282	3.189	1.331	2.649	7.247
	G	n.r.	n.r.	n.r.	0.420	0.571	1.836	0.824	1.858	5.868
	H	n.r.	n.r.	n.r.	0.606	0.820	2.325	0.926	2.251	6.770
	I	n.r.	n.r.	n.r.	0.692	0.936	2.477	1.252	3.134	7.715
	J	n.r.	n.r.	n.r.	0.329	0.448	1.546	n.r.	n.r.	n.r.

Table 1. *(Continued)*

Analyte	MP	Chiralpak AD-H			Chiralcel OD-H			Chiralcel OJ-H		
		k'_1	k'_2	R_s	k'_1	k'_2	R_s	k'_1	k'_2	R_s
	K	n.r.	n.r.	n.r.	0.617	0.824	1.972	n.r.	n.r.	n.r.
	L	n.r.	n.r.	n.r.	1.085	1.426	2.897	n.r.	n.r.	n.r.
III	A	0.692	0.788	1.050	n.e.	n.e.	n.e.	0.648	0.765	1.123
	B	1.143	1.296	1.337	n.e.	n.e.	n.e.	0.945	1.125	1.566
	C	1.978	2.238	2.039	n.e.	n.e.	n.e.	1.773	2.118	1.926
	D	1.224	1.353	0.779	n.e.	n.e.	n.e.	1.763	2.181	1.339
	E	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	1.704	2.118	1.401
	F	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	3.179	3.922	1.423
	G	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	0.831	0.953	0.435
	H	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	1.336	1.565	0.674
	I	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	2.588	3.161	1.165
	J	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	1.075	1.263	0.615
	K	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	1.754	2.082	0.829
	L	n.r.	n.r.	n.r.	n.e.	n.e.	n.e.	3.202	3.866	1.260
IV	A	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.311	0.465	1.993
	B	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.433	0.653	2.619
	C	n.r.	n.r.	n.r.	0.886	0.972	0.387	0.744	1.158	3.875
	D	0.724	0.915	2.405	0.740	0.854	0.591	0.403	0.967	3.695
	E	1.123	1.438	3.251	0.809	0.935	0.550	0.569	1.396	4.527
	F	2.068	2.719	4.649	1.642	1.913	0.762	1.029	2.619	6.212
	G	0.695	0.851	2.023	n.r.	n.r.	n.r.	0.272	0.399	1.536
	H	1.111	1.372	2.712	n.r.	n.r.	n.r.	0.415	0.586	1.844
	I	2.097	2.629	3.740	n.r.	n.r.	n.r.	0.711	0.917	1.727
	J	1.373	1.580	0.838	n.r.	n.r.	n.r.	0.296	0.369	0.966
	K	0.368	0.440	0.455	n.r.	n.r.	n.r.	0.478	0.596	1.192
	L	2.252	2.602	0.832	n.r.	n.r.	n.r.	0.774	0.950	1.579
MTZ	A	0.908	1.128	2.978	0.566	0.694	1.484	n.r.	n.r.	n.r.
	B	1.119	1.414	3.188	0.405	0.532	1.581	n.r.	n.r.	n.r.
	C	1.373	1.797	3.994	0.701	0.826	1.415	0.609	0.654	0.367
	D	0.981	1.046	0.552	0.662	0.943	3.072	n.r.	n.r.	n.r.
	E	1.272	1.359	0.582	0.954	1.369	3.339	n.r.	n.r.	n.r.
	F	1.675	1.794	0.713	0.888	1.303	3.312	0.812	0.889	0.454
	G	0.699	0.831	1.408	0.610	0.779	1.914	n.r.	n.r.	n.r.
	H	0.878	1.036	1.646	0.369	0.517	1.774	n.r.	n.r.	n.r.
	I	1.260	1.511	1.953	0.403	0.563	1.840	n.r.	n.r.	n.r.
	J	n.r.	n.r.	n.r.	0.276	0.366	1.030	n.r.	n.r.	n.r.
	K	n.r.	n.r.	n.r.	0.363	0.447	0.967	0.542	0.582	0.177
	L	n.r.	n.r.	n.r.	0.294	0.381	0.934	0.690	0.751	0.394

Chromatographic conditions – mobile phase (MP): A, *n*-hexane/EtOH/DEA, 80:20:0.1; B, *n*-hexane/EtOH/DEA, 85:15:0.1; C, *n*-hexane/EtOH/DEA, 90:10:0.1; D, *n*-hexane/IPA/DEA, 80:20:0.1; E, *n*-hexane/IPA/DEA, 85:15:0.1; F, *n*-hexane/IPA/DEA, 90:10:0.1; G, *n*-hexane/PrOH/DEA, 80:20:0.1; H, *n*-hexane/PrOH/DEA, 85:15:0.1; I, *n*-hexane/PrOH/DEA, 90:10:0.1; J, *n*-hexane/BuOH/DEA, 80:20:0.1; K, *n*-hexane/BuOH/DEA, 85:15:0.1; L, *n*-hexane/BuOH/DEA, 90:10:0.1 (v/v/v); flow rate 1.0 mL/min, temperature ambient, detection 210 nm (n.r., not resolved; ne, not retained).

between the CSP and the analytes depended on the nature and concentration of the alcohol (Wang and Wenslow, 2003). The changes caused in the structure of CSPs by the different alcohols may affect the chiral selectivity of the CSP, depending on the size and structure of the analyte. The influence of the nature of the alcohol on the resolution was also investigated. Sometimes, alcohols with bulky and branched side-chains, such as IPA, PrOH and BuOH, resulted in higher resolution.

Chiralpak® AD-H and Chiralcel® OD-H columns exhibited very limited or no selectivity at all for **II** with mobile phase PrOH/*n*-hexane, 90:10 modified with 0.1% DEA. However, Chiralcel® OJ-H afforded good resolution ($R_s = 7.7$) to the same enantiomeric pair.

Another example of enantioselective specificity of the CSP is shown for the resolution of analyte **I**. Using a mobile phase composed of hexane/EtOH, 90:10 modified with 0.1% DEA, the enantiomers could be only partially resolved on Chiralcel® OD-H (Table 1) and were not resolved on Chiralcel® OJ-H. Nevertheless, complete resolution was achieved on Chiralpak® AD-H under exactly the same chromatographic conditions.

The nature of CSPs

The structures and stereochemistries of these CSPs differ significantly (Fig. 2). The packing composition for Chiralpak®

AD-H is amylose tris(3,5-dimethylphenylcarbamate), that for Chiralcel® OD-H is cellulose tris(3,5-dimethylphenylcarbamate) and that for Chiralcel® OJ-H is cellulose tris(4-methylbenzoate), each one coated on 5 µm silica-gel. Chiral discrimination studies of polysaccharide derivatives, particularly at a molecular level, are of great importance and interest from the viewpoint of better understanding the chiral separations. There are number of interaction sites with a different affinities on chiral polymers, and it is difficult to determine their precise structures both in the solid state and in solution. The comprehensive elucidation of the chiral recognition mechanism on polymeric CSPs is cumbersome (Aburatani *et al.*, 1990).

The high-ordered helical structures of the phenylcarbamates of cellulose and amylose are responsible for the chiral recognition. The glucose residues are regularly arranged along the helical axis, and a chiral helical groove surrounded by polar carbamate groups exists along the main chains. The polar carbamate groups are preferably located inside, while hydrophobic aromatic groups are located outside the polymer chain. Such an ordered structure seems to begin at a rather low degree of polymerization for the amylose derivatives, but may begin at a higher degree for the cellulose derivatives (Zhang *et al.*, 2008). The polar carbamate groups on the phenylcarbamates of the polysaccharides can probably interact with a racemate via hydrogen bonding on NH and C=O groups, and a dipole–dipole interaction on C=O. The π–π interaction between the phenyl groups of the phenylcarbamates and an aromatic group of a racemate may also play some role in the chiral discrimination. All the above-mentioned interactions could happen, based on the analyte structures and CSP.

A structure–retention relationship was observed on CSPs for some analytes. Derivatives containing the carboxyl (**IV**) or hydroxyl (**II**) group, the dominant chiral recognition and retention interactions are from hydrogen bonding. Differences in chromatographic behavior of analytes with the same ring number (i.e. **II–IV**) point to the importance of steric, π–π or polar interactions in the enantiomeric separation.

Separation of enantiomers of MTZ and its process-related substances

Figure 3 represents the optimized chromatograms of all the analytes. Since the receptor binding affinities of the enantiomers of MTZ are different, the (*S*)-enantiomer being the more potent α_2 -adrenoceptor antagonist of the two, it is a basic task to develop a chiral HPLC method for quality control of (*S*)-MTZ. Table 2 shows the chromatographic data for analytes under optimized separation conditions. Unfortunately it was not possible to separate all the analytes in one chromatographic run.

Conclusions

The present study demonstrates that the developed methods are quite successful for the direct enantioseparation of MTZ and

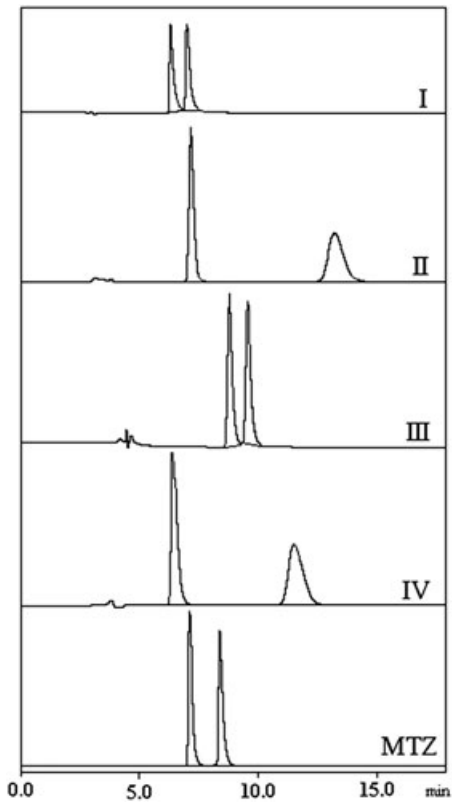

Figure 3. Chromatograms showing the enantioseparation of MTZ and process-related substances **I–IV** under optimized separation conditions. Chromatographic conditions – mobile phase (MP): *n*-hexane/EtOH/DEA, 90/10/0.1 for analytes **I**, **III** and MTZ; *n*-hexane/IPA/DEA, 90/10/0.1 for analyte **IV**; and *n*-hexane/PrOH/DEA, 90/10/0.1 for analyte **II**; flow rate, 1.0 mL/min; temperature, ambient; λ_{max}, 210 nm.

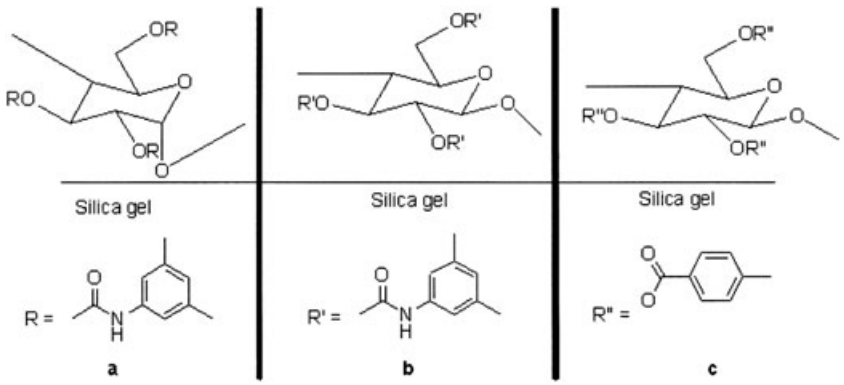

Figure 2. Packing material of (a) amylose tris (3,5-dimethylphenylcarbamate) [Chiralpak® AD-H], (b) cellulose tris (3,5-dimethylphenylcarbamate) [Chiralcel® OD-H] and (c) cellulose tris (4-methylbenzoate) [Chiralcel® OJ-H].

Table 2. Chromatographic retention time (RT), retention factor (k'), separation factor (α), resolution (R_s) and tailing factor (T_f) for analytes under optimized separation conditions

Analyte	Column	MP	RT_1	RT_2	k'_1	k'_2	T_{f1}	T_{f2}	α	R_s	Elution order
I	AD-H	C	6.40	7.09	1.143	1.375	2.871	2.545	1.204	2.175	*R*-(–) < *S*-(+)
II	OJ-H	I	7.27	13.34	1.252	3.134	1.350	1.264	2.502	7.715	*R*-(–) < *S*-(+)
III	AD-H	C	8.87	9.64	1.978	2.238	1.555	1.487	1.131	2.039	*R*-(–) < *S*-(+)
IV	OJ-H	F	6.51	11.61	1.029	2.619	1.616	1.365	2.545	6.212	*R*-(–) < *S*-(+)
MTZ	AD-H	C	7.10	8.37	1.373	1.797	1.747	2.071	1.309	3.994	*S*-(+) < *R*-(–)

Chromatographic conditions – mobile phase (MP): *n*-hexane/EtOH/DEA, 90/10/0.1 for analytes **I**, **III** and MTZ; *n*-hexane/IPA/DEA, 90/10/0.1 for analyte **IV**; and *n*-hexane/PrOH/DEA, 90/10/0.1 for analyte **II**; flow rate, 1.0 mL/min; temperature, ambient; λ_{max}, 210 nm; and polarimetric detector in series with PDA.

its process related substaces (**I–IV**). The direct separations were performed on commercially available CSPs. By variation of the chromatographic parameters, the separations of the enantiomers were optimized; as a result, baseline resolutions were achieved for all the investigated analytes in at least one chromatographic system. The elution sequences were determined in all cases. The current study will be useful in the quality control of (*S*)-MTZ.

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