ORIGINAL ARTICLE

CHIRAL SEPARATION OF 16 BETA-BLOCKERS ON IMMOBILIZED POLYSACCHARIDE CHIRAL STATIONARY PHASES

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Abstract

Chiral separations method development is a tedious process, involving the use of high quantities of solvents and it is aquired over a long period of time. In order to simplify the process and reduce the time needed for selecting the optimal stationary phase for separating the enantiomers of interest, a normal phase gradient elution was developed using n-hexane/2-propanol as mobile phase with an alcohol gradient ranging from 20% to 50% (v/v). Four commercially available polysaccharide based stationary phases were tested on a set of 16 chiral beta-blockers. The effect of three basic additives was evaluated regarding their effect on selectivity. It was observed that depending on the stationary phase, the additives can have an important influence. This approach can supply a convenient set of data for future quantitative correlations between chemical structure of enantiomers and experimental chromatographic parameters. Moreover, the optimal mobile phase composition can be chosen for further development in isocratic mode.

Rezumat

Dezvoltarea de metode în domeniul separărilor chirale este un proces anevoios, care presupune utilizarea unor mari cantităţi de solvenţi şi care se desfăşoară pe o perioadă lungă de timp. Pentru a simplifica acest proces şi a reduce timpul necesar selectării fazei staţionare optime pentru separarea enantiomerilor de interes, a fost dezvoltată o procedură de eluţie în gradient în fază normală, utilizând n-hexan/2-propanol ca fază mobilă, cu un gradient al alcoolului între 20% şi 50% (v/v). Patru faze staţionare pe bază de polizaharide disponibile comercial au fost testate pe un set de 16 beta-blocante chirale. A fost evaluat efectul a trei aditivi bazici asupra selectivităţii observându-se că în funcţie de faza staţionară impactul acestora poate fi unul accentuat. Această abordare poate oferi un set de date potrivit pentru studii de corelare cantitativă între structura chimică a enantiomerilor şi parametrii cromatografici experimentali. Mai mult, compoziţia optimă a fazei mobile poate fi aleasă pentru o ulterioară optimizare în modul izocratic.

Keywords: beta-blockers, screening, chiral separations, HPLC

Introduction

In chiral chromatography classical approaches on method development prove to be costly and time consuming. Therefore, analysts have been concerned by understanding the chiral discrimination processes [10]. Apart from this, chemometric models in this field are of high interest especially in designing a new molecule. By foreseeing the chromatographic behaviour, the time needed for method development will be minimized and also the solvent use.

One way of predicting chromatographic separations is to create mathematical correlations between the analyte and the experimental results. Consequently, analyte molecular descriptors or characteristic properties have to be associated with chromatographic data. The chromatographic parameters for the chiral separations have to be identical for all analytes [6]. Most often isocratic elution mode is used for enantioseparations [1, 11, 13] and the mobile composition is finely tuned for achieving the best resolution. So, the resulted chromatographic databases are very heterogenic in terms of separation parameters. A robust approach for obtaining a homogenous database is to use gradient elution. This method has been previously proven to be a successful screening method [5].

Among the chiral stationary phases (CSPs), the polysaccharide ones are the most effective and versatile. Coated polysaccharide based CSPs were introduced as an alternative to the classical Pirkle type stationary phases. They provide very good enantioselectivity and chiral discrimination proprieties but their physical adsorption to the silica particles implies some solvent restrictions [7, 9]. The range of solvents resumes to polar solvents or non-polar solvents. Solvents with intermediate

polarities such as acetone, tetrahydrofuran, ethyl acetate, 1,4-dioxane or chlorinated solvents can dissolve the chiral selectors. To overcome these restrictions, recent advances were materialized into the development of immobilized polysaccharide CSPs. In this case the chiral selector is chemically bonded to the silica particles. These CSPs provide a good alternative for separating analytes that are insoluble or slightly soluble in water or common solvents and can be used with no restrictions regarding the used solvents [9].

Recently, important progress was made in characterizing the stability and selectivity of the immobilized CSPs in the chiral separation of some beta-blockers [7, 8, 15].

Given a CSP, the most important parameters that control the chiral discriminations are the mobile phase composition and the analyte's chemical structure and these two parameters are chosen to complement each other. Usually, normal phases are made of alkanes, (n-hexane or n-heptane), mixed with organic modifiers, such as alcohols (ethanol, 2-propanol) or other solvents (acetonitrile or methanol). Finally, small quantities of amines or acids (0.1-1%) are added to the previous mixture as additives, in order to finely adjust the selectivity [14].

Beta-blockers form a group of drugs generally used in the treatment of arterial hypertension, cardiac arrhythmias or ischemic cardiac disease [2, 12]. In case of beta-blockers with one chiral centre, the (-) enantiomer has a much higher affinity for the receptor than its antipode. Given that the two enantiomers have different pharmacodynamic and pharmacokinetic proprieties [3, 4], the administration of (+) enantiomers seems useless and may contribute to more intense adverse effects and more frequent drug interactions.

In the current study, we report an effective approach that can be used in a rational, targeted and cost effective manner to screen different stationary phases in order to resolve the enantiomers of a given racemate. Nonetheless, using the same procedure homogenous databases are created that can be further used for developing chiral separation algorithms. The test molecules were several representatives of the same pharmaceutical class of drugs (i.e. 16 beta-blockers). Given their different physical proprieties, a normal phase gradient elution mode was chosen in order to cover a wide mobile phase composition and have an unified analysis protocol [16].

Materials and Methods

Columns and chemicals

The four immobilized columns, Chiralpak IA, Chiralpak IB, Chiralpak IC and Chiralpak ID, were kindly supplied by Chiral Technologies (Illkirch, France). The chiral selectors were amylose tris (3,5-dimethylphenylcarbamate) for Chiralpak IA, cellulose tris (3,5-dimethylphenylcarbamate) for Chiralpak IB, cellulose tris (3,5-dichlorophenyl-carbamate) for Chiralpak IC and amylose tris (3-chlorophenylcarbamate) for Chiralpak ID. These chiral selectors are chemically bonded to silica particles.

All the columns had a size of 250 mm x 4.6 mm (I.D.) and silica particle size of 5 μm.

The mobile phases were prepared using HPLC-grade solvents. Acetonitrile and n-hexane were purchased from Sigma-Aldrich (Steinheim, Germany) and 2-propanol from Lach-Ner (Neratovice, Czech Republic). Diethylamine (DEA), ethylenediamine (EDA) and ethylamine (EA) were provided by Fluka (Buchs, Switzerland). Ultrapure water was produced using a RoDi Easypure water purification system (Barnstead, UK).

Beta-blocker racemic standards of sotalol, acebutolol, bisoprolol, carazolol, esmolol, betaxolol, labetalol, alprenolol, atenolol, carvedilol, pindolol, timolol, metoprolol and nadolol, with purity > 98% and R(+)-propranolol, S(-)-propranolol, R(+)-atenolol, S(-)-atenolol, S(-)-alprenolol, R(+)-alprenolol standards, with purity > 99%, were provided by Sigma-Aldrich (Steinheim, Germany). Racemic standards of propranolol and oxprenolol were kindly donated by Labormed S.A. Ammonium hydrocarbonate, of 99.8% purity was purchased from Riedel-de Haën (Seelze, Germany).

Instrumentation and chromatographic conditions

All the analyses were carried on an Agilent 1200 series chromatographic system equipped with a vacuum degasser, a quaternary pump, auto-sampler, thermostated column compartment and a DAD UV detector. The Chemstation software (Rev. B.03.01) was used for system control and data analysis.

The mobile phase was composed of a binary mixture of 2-propanol and n-hexane. A gradient elution was applied ranging from n-hexane/2-propanol – 80/20 to 50/50 (v/v). The additive was added to the 2-propanol as 0.1% of its volume.

In all analyses the flow was set to 1 mL/min, injection volume to 10 μL and the column temperature to 20^0C. The UV detection was performed simultaneously at 200, 220 and 228 nm. All mobile phases were filtered through a PTFE filtering membrane with pore size of 0.2 μm. The beta-blocker samples were dissolved in 2-propanol, at a concentration of 100 μg/mL.

Results and Discussion

Method development

The usual approach for method development in liquid chromatographic chiral separations is to use isocratic elution mode. This way the column is well

equilibrated and therefore higher resolutions are usually achieved. The disadvantages come from the fact that this elution mode is time consuming, accompanied by the use of large amounts of solvents.

By changing the polarity of the mobile phase, the gradient elution can offer quick insights regarding the enantioselectivity of the stationary phase, as well as the retention of the analyte. In this manner, the ideal composition of the mobile phase can be evaluated and further tested in isocratic mode.

The used mobile phase consisted of n-hexane and 2-propanol, with a gradient ranging from 20% to 50% alcohol. The percentage of alcohol was linearly increased over 20 minutes followed by an isocratic *plateau* of 5 minutes. The initial mobile phase composition was reached after another five minutes, followed by 10 minutes of column equilibration.

In these conditions we globally achieved baseline resolutions for 15 beta-blockers out of a total of 16 studied (see Table I). The highest values of resolution were recorded on Chiralpak ID column, while most baseline chiral separations accomplished with one mobile phase was on the same column using DEA as additive.

Very good results were also obtained on Chiralpak IA, in combination with EDA as additive. The chiral baseline chiral separation was achieved for 7 analytes. Isocratic tests were carried on the same column with a mobile phase composed of n-hexane/2-propanol 80/20 (v/v) and 0.1% EDA. It was found that the selectivity was the same, similar resolutions were achieved (data not shown), but for some slowly eluting analytes the retention time was up to 54 minutes (i.e. carvedilol). The viability of the gradient elution was proven once again, by offering similar resolution within a reasonable timeframe.

A high degree of complementarity has been observed between the four CSPs. The enantiomers of oxprenolol could be resolved in all chromatographic conditions, while for timolol no chiral resolution was achievable on any of the tested CSPs.

Table I

Chiral resolutions obtained with a gradient elution and three basic additives

	Chiralpak IA			**Chiralpak IB**			**Chiralpak IC**			**Chiralpak ID**		
	EDA	DEA	EA	EDA	DEA	EA	EDA	DEA	EA	EDA	DEA	EA
Acebutolol	-[a]	-	-	-	-	-	2.0	1.9	1.9	1.0	0.7	0.9
Alprenolol	3.9	1.6	3.3	3.4	1.7	-	-	-	-	-	-	0.6
Atenolol	-	-	-	0.6	0.4	-	1.1	1.0	0.8	1.0	0.6	0.7
Betaxolol	2.9	0.6	1.8	2.0	1.2	0.8	0.6	0.6	-	6.8	5.8	4.3
Bisoprolol	1.7	-	0.5	1.2	0.5	0.5	0.7	0.7	-	5.2	4.6	5.7
Carazolol	-	-	-	-	0.5	0.2	7.8	7.2	8.0	-	-	-
Carvedilol	10.9	3.7	10.6	0.8	1.0	1.2	-	-	-	-	-	-
Esmolol	2.1	-	-	1.3	-	-	-	-	-	7.4	5.8	6.3
Labetalol	-	-	0.9	-	-	-	1.5	1.3	1.4	-	-	-
Metoprolol	2.1	-	1.0	1.9	0.9	1.8	-	-	-	9.0	8.0	9.2
Nadolol	1.4	-	0.9	-	-	-	2.4	0.7	1.0	6.2	4.9	4.8
Oxprenolol	5.4	1.2	2.5	9.9	5.3	6.6	7.4	6.4	4.8	10.0	8.9	7.7
Pindolol	0.5	-	-	-	-	-	8.8	7.5	8.4	0.6	0.6	-
Propranolol	-	-	-	4.4	3.2	3.5	4.5	4.6	4.5	1.4	2.2	1.1
Sotalol	-	-	-	-	-	-	-	-	-	5.0	5.4	5.0
Timolol	-	-	-	-	-	-	-	-	-	-	-	-

[a] no chiral separation achieved

Additive type

The three additives were chosen in accordance to the analytes proprieties. Basic additives usually have a higher influence on the chromatographic behaviour of basic analytes than acidic ones.

It was found that the type of additive has a major influence on the enantioresolution of analytes obtained on Chiralpak IA, amylose based stationary phase. For this CSP ethylenediamine provides better results in terms of baseline separated analytes.

No significant differences were recorded testing the three additives on Chiralpak IB and IC columns, except in case of etylenediamine offering slightly better results. On Chiralpak ID the chiral resolution was similar for all the additives, which shows that the enantiospecific interaction with the stationary phase is more important in this case.

Given the different effects observed for the studied additives on the four CSPs, we can conclude that the underlying mechanisms of the enantioseparation of beta-blockers are different for the four stationary phases.

Conclusions

A novel gradient elution screening procedure was developed, which can be used for fast and targeted method development in chiral separations. At the

same time, homogenous databases are created for further use, especially in relation with computational chemistry (development of topological molecular descriptors) in order to create predictive models for enantioseparation.

The effect of three basic additives on chiral resolution was studied. Considering Chiralpak IA column, we can conclude that the additive of choice would be EDA, which provided much better separations compared to the other two additives. For the other columns, the separation of the beta-blocker enantiomers is more dependent on the chiral selector.

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References

1. Cavazzini A., Pasti L., Massi A., Marchetti N., Dondi F., Recent applications in chiral high performance liquid chromatography: A review. *Anal. Chim. Acta*, 2011; 706(2): 205-222.
2. Cozlea L.D., Fărcaș D.M., Vari C., Keresztesi A-A., Țifrea R., Cozlea L., Dogaru G., The efficiency of metoprolol tartrate in the treatment of pure arterial hypertension at patients with coronary artery disease and the risk factors associated. *Farmacia*, 2013; 61(4): 713-724.
3. Dasbiswas A., Dasbiswas D., Chirally pure S-metoprolol – place in therapy. *Indian Heart J.*, 2010; 62(2): 143-145.
4. Dasbiswas A., Shinde S., Dasbiswas D., S-metoprolol: the 2008 clinical review. *J. Indian Med. Assoc.*, 2008; 106(4): 259-262.
5. De la Puente M.L., White C.T., Rivera-Sagredo A., Reilly J., Burton K., Harvey G., Impact of normal-phase gradient elution in chiral chromatography: A novel, robust, efficient and rapid chiral screening procedure. *J. Chrom. A*, 2003; 983(1-2): 101-114.
6. Del Rio A., Gasteiger J., Simple method for the prediction of the separation of racemates with high-performance liquid chromatography on Whelk-O1 chiral stationary phase. *J. Chrom. A*, 2008; 1185(1): 49-58.
7. Ghanem A., Hoenen H., Aboul-Enein H.Y., Application and comparison of immobilized and coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases for the enantioselective separation of β-blockers enantiomers by liquid chromatography. *Talanta*, 2006; 68(3): 602-609.
8. Hu H., Jingyu J., Wonjae L., Comparative enantiomer separation of β-blockers on polysaccharide derived chiral stationary phases using high performance liquid chromatography with acid or base additive in the mobile phases. *Chinese J. Chromatogr.*, 2009; 27(4): 467-471.
9. Jin J.Y., Bae S.K., Lee W., Comparative studies between covalently immobilized and coated chiral stationary phases based on polysaccharide derivatives for enantiomer separation of N-protected alpha-amino acids and their ester derivatives. *Chirality*, 2009; 21(10): 871-877.
10. Lämmerhofer M., Chiral recognition by enantioselective liquid chromatography: mechanisms and modern chiral stationary phases. *J. Chrom. A*, 2010; 1217(6): 814-856.
11. Peluso P., Mamane V., Cossu S., Liquid chromatography enantioseparations of halogenated compounds on polysaccharide-based chiral stationary phases: role of halogen substituents in molecular recognition. *Chirality*, 2014; 27: 553-562.
12. Pop S.L., Mărginean C., Mirel S., Mureșan D., Pop Vornicescu D., Gherman R., Onacă E., Comparative study regarding celiprolol and metoprolol use in the treatment of essential arterial hypertension. *Farmacia*, 2014; 62(4): 674-682.
13. Ribeiro A.R., Maia A.S., Cass Q.B., Tiritan M.E., Enantioseparation of chiral pharmaceuticals in biomedical and environmental analyses by liquid chromatography: An overview. *J. Chrom. B*, 2014; 968: 8-21.
14. Zhang T., Franco P., Analytical and Preparative Potential of Immobilized Polysaccharide-derived Chiral Stationary Phases. In: Subramanian G., Chiral Separation Techniques: A Practical Approach, Wiley-VCH, Weinheim, 2007: 99-134.
15. Zhang T., Franco P., Nguyen D., Hamasaki R., Miyamoto S., Ohnishi A., Murakami T., Complementary enantiorecognition patterns and specific method optimization aspects on immobilized polysaccharide-derived chiral stationary phases. *J. Chrom. A*, 2012; 1269: 178-188.
16. Vlase L., Muntean D., Leucuta S.E., High-Throughput Determination of Ivabradine from Human Plasma by LC/MS/MS and Its Application to Pharmacokinetic Studies. *Studia Universitatis Babes-Bolyai Chemia*, 2009; 54(2): 43-51.