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Design of experiments for enantiomeric separation in supercritical fluid chromatography

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ABSTRACT

A new chiral melatoninergic ligand, potentially successor of Valdoxan®, presenting an improved pharmacological profile with regard to agomelatine, was chosen as a probe for a supercritical fluid chromatographic separation carried-out on an amylose *tris*[(*S*)-1-α-methylbenzylcarbamate] based stationary phase.

The goal of this work was to optimize simultaneously three factors identified to have a significant influence to obtain the best resolution in the shortest analysis time (*i.e.*, retention time of the second eluting enantiomer) for this chiral compound.

For this purpose a central circumscribed composite (CCC) design was developed with three factors: the flow-rate, the pressure outlet and the percentage of ethanol to optimize of two responses: shortest analysis time and best resolution. The optimal conditions obtained *via* the optimizer mode of the software (using the Nelder-Mead method) *i.e.*, CO_2/EtOH 86:14 (v:v), 104 bar, 3.2 mL min^{-1} at 35 °C lead to a resolution of 3.27 in less than 6 min. These conditions were transposed to a preparative scale where a concentrated methanolic solution of 40 mM was injected with a sample loop of 100 μL. This step allowed to separate an amount of around 65 mg of racemic melatonin ligand in only 3 h with impressive yields (97%) and enantiomeric excess (99.5%).

1. Introduction

Chirality is important in field of chemistry, and is of major interest in drug development since the two enantiomers interact differently with biological targets and thus one or the other enantiomer of a compound can have beneficial or disastrous results. Awareness of this phenomenon intensified when the teratogenic effects of thalidomide emerged in the 1960s. Chirality still remains, today, a challenge for analysts because the majority of pharmaceutically active molecules contain at least one chiral center [1]. Generally, chemical synthesis leads to racemic mixture, therefore it is important to separate efficiently the two (or more) enantiomers to investigate their pharmacological profile and this latter must be enlightened as soon as possible during the drug development process. Rapidly, high performance liquid chromatography (HPLC) has become the preferred methodology used in early pharmaceutical research and development [2,3] because it is known as the most rapid and efficient technique. Besides HPLC, supercritical fluid chromatography (SFC) has emerged and has established itself as a powerful analytical technique and as an attractive and green alternative in preparative chromatography [4–7]. Non toxic and non flammable pressurized carbon dioxide (CO_2) is the usual fluid used for SFC due to its high diffusivity and low viscosity, offering higher efficiencies, shorter run time and faster column equilibration. This reduced viscosity decreases the pressure drop across the column and permits the use of higher flow rates. Therefore SFC allows to obtain the two enantiomers faster and more efficiently with regard to HPLC [8]. Moreover, the lower fraction volume, reducing solvent consumption and thus time for solvent removal, minimizes global cost of enantiomer isolation [9]. SFC has benefited of the chiral stationary phases (CSPs) initially developed for HPLC *i.e.*, chemically derivatives amylose or cellulose, coated or immobilized chiral phases, and their chiral recognition mechanisms were largely studied by the team of C. West [10–14]. The chemical structure of these stationary phases mainly rule retention and separation [10–15]. The latter are also governed by the type and the fraction of solvent added to carbon dioxide. The pressure is known to influence retention whereas separation is scarcely affected by it. The last operating

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parameter that can be optimize in SFC is the temperature. However its effect is complex. Indeed temperature can act in two opposite way on the retention, at constant pressure an increase of the temperature enhances the coefficient of diffusion and the volatility of the solutes then decreases their retention. In the same time, the density of the carbon dioxide is reduced leading to a rise of the retention phenomena.

As each parameter variation is studied independently while the others are kept constant, the interaction effect of simultaneously changing two, three or more of these latter are not very well elucidated today. If design of experiment (DoE) is widely used for enantioseparation *via* dual cyclodextrins system in capillary electrophoresis [16–19], its publication is also reported, in a less extend, in chiral HPLC [20–24]. To the best of our knowledge and with regards to chiral SFC, only one very recent work pointed-out the usefulness of experimental design to particularly investigate the combined effects of operating parameters [25]. The aim of this article was therefore to optimize the enantioseparation of a melatoninergic ligand (Fig. 1) using an amylose *tris*[(*S*)-1-α-methylbenzylcarbamate] based CSP as chiral selector and to explore the combined effects which rule those separations. To achieve this goal, an univariate screening step was firstly performed to select the factor which might influence the retention and the resolution. Then a central circumscribed composite (CCC) design was implemented to evaluate optimal conditions. For compound **1** these optimized conditions were therefore transposed to a semi-preparative scale to obtain sufficient amount of each enantiomer. The two semi-preparative chromatographies carried out in HPLC [26] and in SFC were lastly compared in terms of spent time, solvent consumption and yield.

2. Material and methods

2.1. Chemicals

The synthesis of compound **1** and analogs (Fig. 1) was described previously [27]. For analytical screening, solutions of samples were prepared in methanol at 1.25 mM (0.34 g L^{-1}). For validation, solutions of samples were prepared in methanol at 1.00, 1.12, 1.20, 1.25, 1.37, 1.40 and 1.50 mM (between and 0.27 and 0.41 g L^{-1}). For preparative studies, assay samples were prepared in methanol at 40 mM (11 g L^{-1}). Solutions were always degassed by an ultrasonic bath prior to be used.

The methanol, ethanol, isopropanol, acetonitrile and *n*-heptane were HPLC grade and were purchased from VWR (Strasbourg, France). Carbon dioxide (CO_2) with purity of 99.995% was purchased from Air Liquide (Loos, France).

2.2. Supercritical fluid chromatography apparatus

The chiral analytical column used for this study, was *tris*[(*S*)-1-α-methylbenzylcarbamate] CSP (Chiralpak® AS-H) and was purchased from Chiral Technologies Europe (Illkirch, France). The column had dimensions 250 mm × 4.6 mm i.d. and was coated on a silica-gel support with 5 μm particle size. The chromatographic system used was an SFC-PICLAB hybrid 10–20 apparatus (PIC Solution, Avignon, France) equipped with an autosampler comprised a 48 vial plate and a 24 vial plate (model Alias, Emmen, The Netherlands), three model 40 P pumps: two for CO_2 and a third for the modifier (Knauer, Berlin, Germany), a column oven with a Valco ten-position column selection valve, and a Valco six-position solvent switching valve. The proportion of the modifier in the mobile

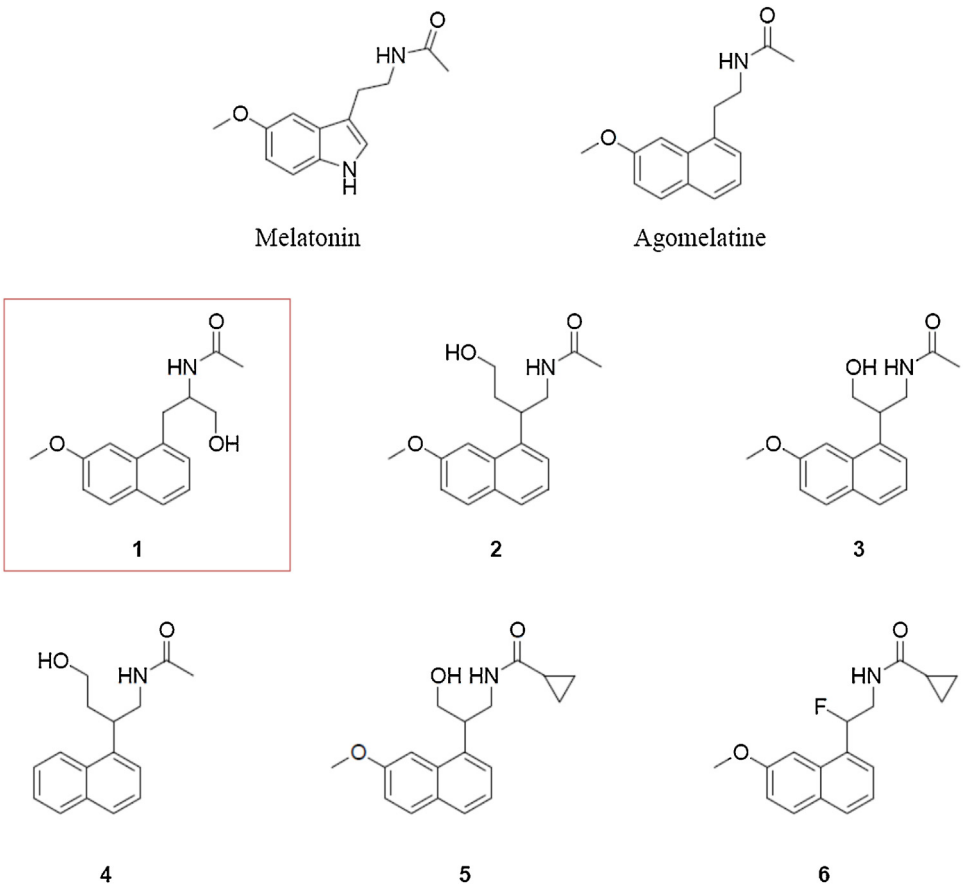

Fig. 1. Chemical structures of melatonin, agomelatine and naphtalen derivatives.