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Multiple dual-mode countercurrent chromatography applied to chiral separations using a (*S*)-naproxen derivative as chiral selector

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ABSTRACT

Countercurrent chromatography (CCC) is a liquid–liquid chromatographic technique without a solid support. Several alternative elution modes can be applied to take advantage of the special nature of the liquid stationary phase. Among these dual-mode (DM) and multiple dual-mode (MDM) consist of switching alternatively between Reversed and Normal Phase operation during the experiment (once for DM and several times for MDM). In this paper, MDM has been applied to the chiral CCC separations of two racemic mixtures, (±)-*N*-(3,4-*cis*-3-decyl-1,2,3,4-tetrahydrophenanthren-4-yl)-3,5-dinitrobenzamide and *N*-(3,5-dinitrobenzoyl)-(±)-leucine, using (*S*)-naproxen *N*,*N*-diethylamide as chiral selector (CS). Although the behaviour of the two analytes differed, improved resolution factors were successfully obtained. Results are rationalized on the basis of the distinct partition behaviour of the CS/enantiomer complexes in the biphasic system.

1. Introduction

Nowadays, the significance of chirality in biological processes is well recognized. The strict regulations established by health authorities on the commercialisation of chiral drugs, has led to the requirement for developing analytical and preparative methods for enantioseparation. While several procedures have been indicated for this purpose [1], chromatographic techniques are the most widely used. However, research into improved or advantageous chiral processing technologies is ongoing.

Countercurrent chromatography (CCC) is a support-free liquid–liquid chromatographic technique in which the stationary and the mobile phase are composed by two immiscible solvents or solutions [2]. The instrumentation generates a centrifugal field that retains one of the liquid phases stationary, while the mobile phase is pumped through [3,4]. Most applications, non-chiral separations, are based on liquid–liquid partition between the two liquid phases. In those cases the solute retention depends only on the differences in distribution ratios of the compounds to be separated. When applying CCC to enantioseparation, a chiral environment has to be established to distinguish between the two enantiomers. Therefore, a chiral compound, the chiral selector (CS), is added to the stationary phase [5]. The CS has to be confined in the stationary phase while the racemate must partition between the two phases. The high loading capacity and lower solvent consumption for a given amount of product processed make CCC an advantageous alternative to HPLC for preparative separations. Although techniques such as peak-shaving and recycling, both common practices in preparative HPLC to improve the purity of the recovered compounds, can also be applied to CCC [6,7], the modest enantioselectivity of most CSs used up to now and the moderate efficiency of CCC constitute a limitation.

An additional advantage of CCC over other chromatographic technologies is the possibility to apply a variety of elution modes, which increase the potential of the technique in operation mode [8,9] and scale [10]. Some of these modes have been applied with the aim of improving enantioseparation. This is the case for pH-zone refining, a kind of displacement chromatography, which additionally produces an increase in the loading capacity of the technique [11–13].

Dual-mode (DM) is also an alternative eluting mode applicable to CCC. It consists of switching the liquid phase acting as mobile phase at a certain time within the experiment, as either phase can be used as the mobile phase with its respective pair becoming the stationary phase. By performing this operation the total

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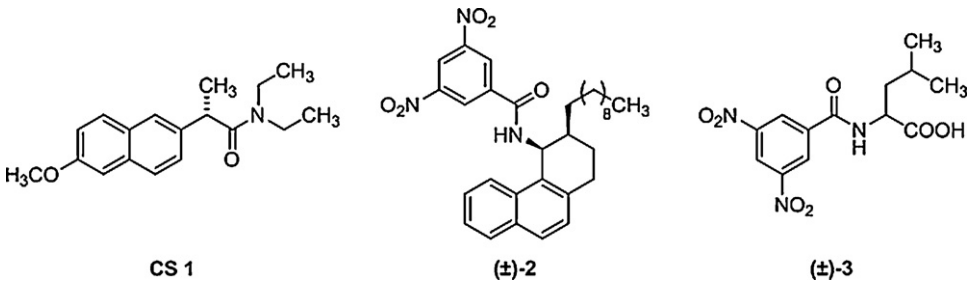

Fig. 1. Structure of the chiral selector derived from (*S*)-naproxen (**CS1**) and the racemic compounds (±)-**2** ((±)-*N*-(3,4-*cis*-3-decyl-1,2,3,4-tetrahydrophenanthren-4-yl)-3,5-dinitrobenzamide) and (±)-**3** (*N*-(3,5-dinitrobenzoyl)-(±)-leucine).

elution of the sample injected is ensured, while extremely high retention times for those analytes highly retained in the stationary phase are avoided. Various applications show the usefulness of this mode in reducing analysis time in preparative CCC and increasing the separation of compounds poorly separated in classical conditions [14]. DM has also been applied to enantioseparation [15] to promote the elution of a strongly retained enantiomer. However, in this case the enantiomer elutes with the CS, which has to be removed afterwards.

The extension of the DM methodology by performing several phase inversion cycles leads to what is known as multiple dual-mode (MDM) [7]. This eluting mode consists of a succession of mode changes between Normal and Reversed Phase where the flow direction is changed. For example, when the process starts in Reversed Phase, in which the lower aqueous phase flows from Head to Tail, it then switches to Normal Phase with the upper, organic phase flowing from Tail to Head and so on. To the best of our knowledge MDM has not been applied to enantioseparation before.

Here we describe the application of MDM to enantiomer separation aiming for the two isomers to elute from the same end of the column, thereby avoiding CS co-elution. Two different racemic mixtures were resolved using the diethylamide of (*S*)-naproxen (**CS1**) as CS (Fig. 1). Recently the preparative separation of (±)-*N*-(3,4-*cis*-3-decyl-1,2,3,4-tetrahydrophenanthren-4-yl)-3,5-dinitrobenzamide, (±)-**2**, by centrifugal partition chromatography (CPC) using **CS1** was achieved. The polar phase of a quaternary solvent system composed of a mixture of *n*-hexane–ethyl acetate–methanol–water (9:1:9:1, v/v/v/v) acted as stationary phase [16]. In this study MDM elution mode has been applied to improve this enantioseparation. The separation of the amino acid derived *N*-(3,5-dinitrobenzoyl)-(±)-leucine (±)-**3** using the same CS was undertaken to generalise the concept. The differences observed between the two cases have been rationalized on the basis of the distinct partition behaviour of the CS/enantiomer complexes.

2. Experimental

2.1. Reagents

Chiral selector, *N*,*N*-diethyl-(*S*)-naproxenamide and racemate (±)-**1** were prepared as reported previously [16]. *N*-(3,5-Dinitrobenzoyl)-(±)-leucine was purchased from Aldrich (Steinheim, Germany).

Solvents used for HPCCC separations were of analytical grade and for HPLC detection were of HPLC grade. The buffer solution was prepared from analytical monosodium and disodium hydrogen phosphate and MilliQ water.

2.2. Apparatus

HPCCC was performed on a Mini-DE centrifuge (Dynamic Extractions, Slough, UK). The apparatus is provided with a coil of 17.8 mL capacity made of a 0.8 mm bore tube. The rotational speed was controlled at 2100 rpm. The device was connected to an external cooling system which allowed the device to maintain a constant operating temperature of 25 °C. The inlet and outlet of the CCC device were connected to two solenoid valves that enabled the elution mode to be switched from Normal Phase (NP), where the upper less-dense organic liquid is the mobile phase (Tail to Head), to Reversed Phase (RP) mode, where the lower more dense aqueous liquid is the stationary phase (Head to Tail) and vice versa (Fig. 2).

The HPCCC separation setup consisted of two analytical HPLC pumps Knauer 501 (Berlin, Germany) and a spectrophotometer Knauer 2501 with a preparative cell operating at 230 nm (for analyte (±)-**2**) and 254 nm (for analyte (±)-**3**). In some experiments the two analytical Knauer pumps and the detector were replaced by a conventional HPLC system pump, autosampler, UV detector, and chromatography data station software; model HP 1100 (Agilent Technologies, Palo Alto, CA, USA). A manual injector equipped with a 0.178 mL (1% column volume) loop was used. Fractions of the

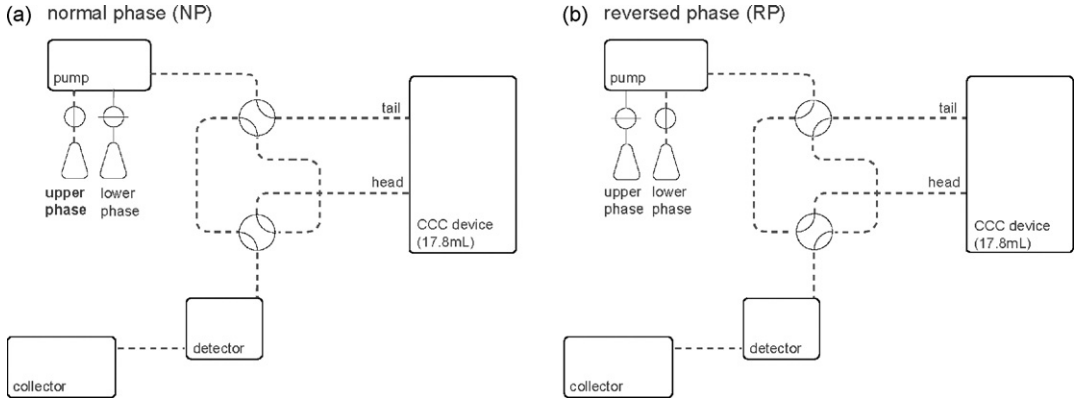

Fig. 2. Schematic setup of MDM operating system showing (a) Normal Phase elution mode (upper organic phase is the mobile phase) and (b) Reversed Phase elution mode (lower aqueous phase is the mobile phase).