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# Enantioseparation of pyroglutamide derivatives on polysaccharide based chiral stationary phases by high-performance liquid chromatography and supercritical fluid chromatography: A comparative study

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ABSTRACT

Analytical enantioseparation of three pyroglutamide derivatives with pharmacological activity against the purinergic receptor P2X7, was run in both high-performance liquid chromatography and supercritical fluid chromatography. Four polysaccharide based chiral stationary phases, namely amylose and cellulose tris (3,5-dimethylphenylcarbamate), amylose tris ((S)-α-methylbenzylcarbamate) and cellulose tris (4-methylbenzoate) with various mobile phases consisted of either heptane/alcohol (ethanol and 2-propanol) or carbon dioxide/alcohol (methanol or ethanol) mixtures, were investigated. After analytical screenings, the best conditions were transposed, for compound **1**, to semi-preparative scale. Each approach was fully validated to meet the International Conference on Harmonisation requirements and compared. Whereas the limits of detection and quantification were near six-fold better in HPLC than in SFC (respectively 0.20 and 0.66 μM *versus* 1.11 and 3.53 μM for one of the enantiomers), in terms of low solvent consumption (7.2 mL of EtOH *versus* 3.2 mL of EtOH plus 28.8 mL of toxic and inflammable heptane per injection in SFC and HPLC, respectively), time effective cost (9 min *versus* 40 min per injection in SFC and HPLC, respectively) and yields (98% *versus* 71% in SFC and HPLC, respectively), the latter method proved its ecological superiority.



## 1. Introduction

The development process of a drug candidate begins, most of the time, by the birth of a racemic molecule. As the two enantiomers may differ in their pharmacological profile [1], and to meet the United States Food and Drug Administration and the European Medical Agency guidelines [2], each of them must be generated enantiomerically pure. Asymmetric synthesis can be firstly considered but is neither time nor cost efficient. Moreover, both enantiomers are necessary for biological tests which would denote that the two synthetic routes must be developed. Therefore, chromatographic resolution seems to be the best way, and in this particular case high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) would be powerful tools thanks to their preparative mode furnishing the two enantiomers simultaneously [3–9]. For more than twenty years, HPLC using polysaccharide based chiral stationary phases (CSPs) has been extensively used for chiral drugs [10,11], mainly because of its wide range of applications due to numerous available CSPs, its sensitivity and its low limit of detection. The very abundant literature reporting considerable knowledge and advanced instrumentation has also favored this technique as the most popular one [12]. Taking advantages of improved instrumentation performance and of the transposition of these HPLC polysaccharide CSPs to SFC, the latter one gains a new interest [13,14] and a technological extend

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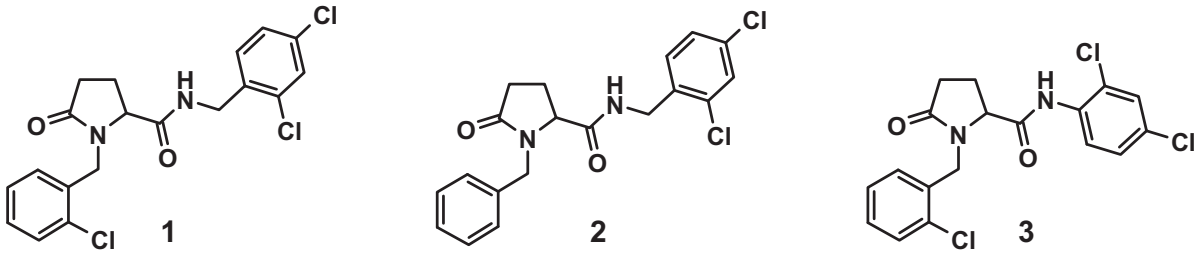


**Fig. 1.** Chemical structures of the pyroglutamide derivatives.

into Ultra-High Performance SFC (UHPSFC) [15]. The low viscosity and high diffusivity of carbon dioxide ($CO_2$) based mobile phase offer higher efficiencies, shorter run time and faster column equilibration. This reduced viscosity decreases the pressure drop across the column and mainly permits the use of higher flow rates, and also longer columns and column coupling [16,17]. Considering the preparative scale, same particle sizes can be used at analytical and preparative one as the pressure build-up in SFC is much lower than in HPLC. This is appreciable if we take into account the cost of preparative column. But the major advantage of SFC *versus* HPLC is the lower fraction volumes, reducing solvent consumption and thus time for solvent removal, minimizing global cost of enantiomer isolation. Therefore, these two techniques were employed to separate three pyroglutamide compounds **1**, **2** and **3** (Fig. 1) issued from a series of pyroglutamic acid derivatives synthesized and identified as P2X7 receptor antagonists with activity in the micromolar range. This antagonist activity P2X7 determination is an internal study and the corresponding pharmacological results will be discussed in detail in a paper currently in preparation. Indeed, focused on chronic inflammatory pathologies, the development of new P2X7 receptor antagonists was of great interest. This receptor is an ATP-gated ion channel, specifically expressed on immune system cells, which has largely been studied since the last decade [18]. It is now well-known that this receptor is involved in the release of pro-inflammatory cytokines, like IL-1β [19], and *in vitro* and *in vivo* studies have recently shown that it is a potent therapeutic target for the development of new therapeutic strategies for chronic inflammatory pathologies [20–22]. Thus, the identification of these new pyroglutamide derivatives as P2X7 receptor antagonists obtained as racemates, led to investigate the separation of each enantiomer by HPLC and SFC, in the aim to determine the best configuration for an optimal activity on this receptor. Based on these results, the evaluation of the anti-inflammatory efficacy should be carried out with the active enantiomers on animal models. To date, no data are available in the literature for the chiral separation of pyroglutamic acids either in HPLC or in SFC. After an analytical screening performed with both techniques on four polysaccharide CSPs, the best conditions were selected to proceed to the semi-preparative step. The aim of this work was to compare both approach abilities to generate individual enantiomers of compound **1** through running time, separated quantities, yield, solvent consumption and enantiomeric excess (*ee*). Each method was validated following the ICH

**Table 1**
Effect of mobile phase on retention times ($t_r$), selectivity ($\alpha$), resolution ($R_s$) of the three racemic mixtures on Chiralcel CSPs in HPLC.

| Compound | Mobile phase (v:v) | $t_{r1}$ (min) | $t_{r2}$ (min) | $\alpha$ | $R_s$ |
|---|---|---|---|---|---|
| *Chiralcel OD-H* | | | | | |
| **1** | Hept/EtOH 80:20 | 7.47 | – | 1.00 | – |
| | Hept/EtOH 90:10 | 14.01 | – | 1.00 | – |
| | Hept/IPA 80:20 | 10.26 | – | 1.00 | – |
| | Hept/IPA 90:10 | 28.57 | – | 1.00 | – |
| **2** | Hept/EtOH 80:20 | 7.04 | 7.84 | 1.14 | 1.16 |
| | Hept/EtOH 90:10 | 12.56 | 14.49 | 1.17 | 1.52 |
| | Hept/IPA 80:20 | 10.38 | 12.65 | 1.25 | 1.69 |
| | Hept/IPA 90:10 | 25.83 | 32.46 | 1.27 | 1.62 |
| **3** | Hept/EtOH 80:20 | 8.13 | 8.67 | 1.08 | 0.87 |
| | Hept/EtOH 90:10 | 14.82 | 16.01 | 1.09 | 1.02 |
| | Hept/IPA 80:20 | 12.64 | 15.09 | 1.21 | 1.68 |
| | Hept/IPA 90:10 | 28.82 | 35.84 | 1.25 | 1.97 |
| *Chiralcel OJ* | | | | | |
| **1** | Hept/EtOH 80:20 | 6.60 | 6.99 | 1.07 | <0.5 |
| | Hept/EtOH 90:10 | 11.29 | 12.61 | 1.23 | <0.5 |
| | Hept/IPA 80:20 | 8.86 | 9.98 | 1.14 | <0.5 |
| | Hept/IPA 90:10 | 21.94 | 26.18 | 1.20 | 0.93 |
| **2** | Hept/EtOH 80:20 | 6.56 | 6.92 | 1.06 | <0.5 |
| | Hept/EtOH 90:10 | 11.30 | 12.59 | 1.13 | <0.5 |
| | Hept/IPA 80:20 | 8.62 | 9.69 | 1.37 | <0.5 |
| | Hept/IPA 90:10 | 20.59 | 24.70 | 1.22 | 1.03 |
| **3** | Hept/EtOH 80:20 | 7.47 | 8.26 | 1.13 | <0.5 |
| | Hept/EtOH 90:10 | 13.33 | 15.48 | 1.17 | 1.32 |
| | Hept/IPA 80:20 | 10.64 | – | 1.00 | – |
| | Hept/IPA 90:10 | 24.30 | – | 1.00 | – |

*Conditions*: Flow rate: 0.8 mL min$^{-1}$; temperature: 25 °C; wavelength detection: 210 nm; concentration: 1 mM.