

Intensified Extraction of Ionized Natural Products by Ion Pair Centrifugal Partition Extraction

Mahmoud Hamzaoui¹, Jamila Hadj-Salem², Luc Marchal², Alain Foucault², Jean-Marc Nuzillard¹, Catherine Lavaud¹, Jean-Hugues Renault^{*1}

¹ Université de Reims Champagne-Ardenne, UMR CNRS 6229, Institut de Chimie Moléculaire de Reims, IFR 53 ;

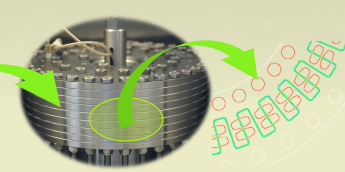
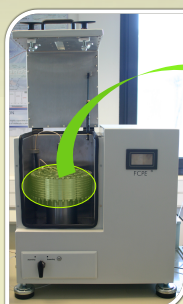
² Université de Nantes, UMR CNRS 6144, laboratoire GEPEA;

*UMR CNRS 6229 – Bât. 18, Campus Sciences Exactes et Naturelles, BP1039 – 51687 Reims Cedex 2 (France) – +33 326 91 34 03 – jh.renault@univ-reims.fr

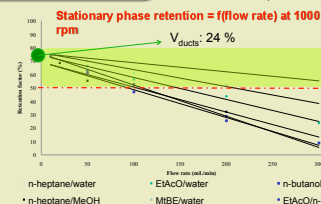
Introduction

The recently developed liquid-liquid extractor, named **Centrifugal Partition Extractor (CPE)**, fills a technological gap between classical liquid-liquid extractors and support-free liquid-liquid chromatographs (hydrostatic- or hydrodynamic-CCC). The design of this new lab-scale extractor, was inspired by CPC columns, but with **less cells of larger volume** (231 instead of 1000-2000) (see figure below). More precisely, the lab-scale CPE rotor consists of 7 circular partition disks engraved with 33 twin partition cells of 0.965 mL each connected in series by small ducts, resulting in an instrument of **231 cells with a total volume of 303 mL**, the dead volume (ducts) being around 25 %. In order to highlight the potentialities of CPE, the Ion-Pair mode has been evaluated for the extraction of ionized water-soluble heterosides.

The Centrifugal Partition Extractor



Centrifugal Partition Extractor (FCPE) developed by **Kromaton¹**: apparatus (303 mL), extraction rotor (7 partition disks) and detail of a partition disk engraved with twin partition cells (33 cells/disk).

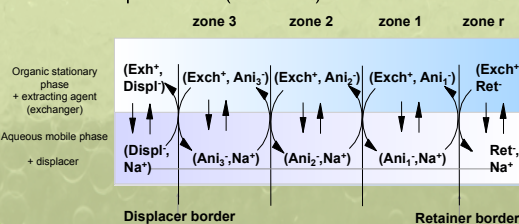


By considering only hydrodynamic aspects, **flow rates comprised between 10 mL/min and 300 mL/min** can be applied (depending on the biphasic system), allowing thus to reach high productivities.

Ion-pair centrifugal partition extraction

Case of anionic compounds of interest

In order to combine the advantages of a **strong ion-exchange centrifugal partition chromatographic^{2,6}** process and of **CPE**, a **lipophilic quaternary ammonium salt** has been selected as a strong anion-exchanger. Lipophilic ion pairs are generated by their association with hydrophilic anionic analytes that are thus captured by the organic stationary phase. After the **extraction step** in the organic stationary phase and the **"washing" step**, the **displacer is pumped** through the stationary phase in order to selectively release the analytes into the aqueous mobile phase as an isotachic compound train (see below).



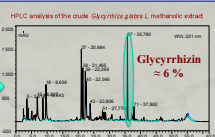
Preliminary studies

Glycyrrhizin Extraction

Glycyrrhiza glabra L.

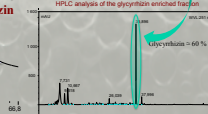
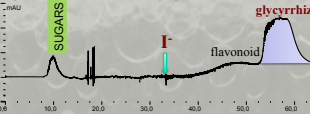


Methanolic solid-liquid extraction



EtOAc/n-BuOH/H₂O (3:2:5 v/v/v)
Exchanger: A136 4 mM
Displacer: 1 4 mM
Sample: 2 g of the crude extract
Flow rate: 10 mL/min
pH= 8.9
Enriched fraction: 88 mg

CPE effluent monitored by UV detection
Extraction and « washing » step Displacement step



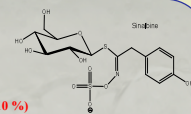
Sinabin Purification



White mustard
(*Sinapis alba*, Brassicaceae)



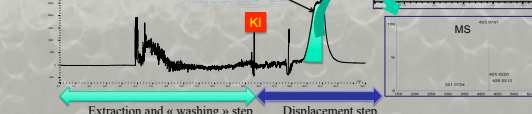
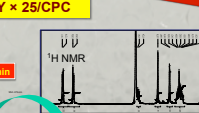
Sinabin crude extract (≈ 10 %)



CPE vs CPC
When chemical reactions involved in the displacement process replace the physical plates

Sinabin purification using CPC :
Flow rate : 2 mL/min
Duration time : 180 min
Purity : 95 % +

EtOAc/n-BuOH/H₂O (3:2:5 v/v/v)
Exchanger: A136 80 mM
Displacer: 1 60 mM
Sample: 3 g of the crude extract
Flow rate: 50 mL/min
Isolated sinabin: 380 mg
Purity: 95 % +



Examples

1. Kromaton, ZA Vernusson Pierre Martine, Atelier n° 3, 39 rue Cugnot, 49130 Sainte Gemmes sur Loire
2. Maciuk A., Renault J.-H.; Margraff R.; Trébucet P.; Zèches-Hanrot M.; Nuzillard J.-M., *Anal. Chem.*, **2004**, *76*, 6179-6186.
3. Toribio A.; Delannay E.; Richard B.; Plé K.; Zèches-Hanrot M.; Nuzillard J.-M.; Renault J.-H., *Journal of Chromatography A* **2007**, *1140*, 101-106.
4. Boudesocque L.; Forni L.; Giraud M.; McGarrity J.; Renault J.H., brevet OEB dépôt N° 10305656.0 **2010**
5. Toribio A.; Pinel B.; Boudesocque L.; Lafosse M.; Nuzillard J.-M.; De La Poype F.; Renault J.-H., *Journal of Separation Science* **2009**, *32*, 1801-1807.
6. Chevotot, L.; Collic-Jouault, S.; Foucault, A.; Ratiskol, J.; Sinquin, C., *Journal of Chromatography B: Biomedical Sciences and Applications* **1998**, *706*, 43-54.