

10th World Congress on **Pharmacology**

&

6th International Conference and Exhibition on**Advances in Chromatography & HPLC Techniques**

August 02-03, 2018 | Barcelona, Spain

Application of serially-coupled columns in chiral liquid chromatography**Tamara Alvarez-Segura, José Ramón Torres-Lapasió and María Celia García-Alvarez-Coque**
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Liquid chromatography with single columns often does not succeed in the analysis of complex samples, in terms of resolution and analysis time. A relatively simple solution to enhance chromatographic resolution is the modulation of the stationary phase through the serial coupling of columns. This can be implemented with any type of column using compatible elution conditions and conventional instruments. This review describes the key features of column coupling and published procedures, where two or more columns were coupled in series to solve separation problems in chiral liquid chromatography. In most reports, two chiral columns are coupled. However, coupling chiral and achiral stationary phases is also an active field, to analyze samples containing mixtures of chiral and achiral compounds. In all reports, the authors could not resolve their samples with single columns, whereas significant enhancement in chromatographic performance was obtained when the columns were combined. Particularly interesting is the reduction in the analysis time in the isocratic mode, which alleviates the general elution problem of liquid chromatography, and may represent a stimulus for the proposal of new procedures, especially in combination with mass spectrometric, electrochemical and refractometric detection. Developments proposed to make the serial coupling of columns useful in routine and research laboratories are outlined, including optimisation strategies that facilitate the selection of the appropriate column combination and elution conditions (solvent content, flow rate or temperature) in both isocratic and gradient modes. The availability of zero dead volume couplers, able to connect standard columns, and the commercialisation of short columns with multiple lengths, has expanded the possibilities of success.



Figure 1: Coupling of three columns in series.

Recent Publications

1. Alvarez-Segura T, García-Alvarez-Coque MC, Ortiz-Bolsico C and Torres-Lapasió JR (2015) Interpretative approaches to optimize serially-coupled columns in reversed-phase liquid chromatography. *Current Chromatography* 2:110-121.
2. Alvarez-Segura T, Torres-Lapasió JR, Ortiz-Bolsico C and García-Alvarez-Coque C (2016) Stationary phase modulation in liquid chromatography through the serial coupling of columns: A review. *Analytica Chimica Acta* 923:1-23.
3. Chu YQ and Wainer IW (1989) Determination of the enantiomers of verapamil and norverapamil in serum using coupled achiral-chiral high-performance liquid. *Journal of Chromatography B* 497:191-200.
4. Ferretti R, Gallinella B, La Torre F and Zanitti L (1998) Direct resolution of a new antifungal agent, voriconazole (UK-109,496) and its potential impurities, by use of coupled achiral-chiral high-performance liquid chromatography. *Chromatographia* 47:649-654.
5. Johnson DV and Wainer IW (1996) Enantioselective separation of cyclic chiral ketones and their corresponding diastereomeric alcohols by HPLC on chiral and chiral/chiral coupled stationary phases. *Chirality* 8:551-555.

Biography

Tamara Alvarez-Segura has completed her Degree in Chemistry in 2013 and Master degree in Experimental Techniques in Chemistry in 2014, both at the University of Valencia. She is now performing diverse research activities in the Department of Analytical Chemistry to get her PhD degree. She has received two pre-doctoral fellowships. During her Master studies, she began her collaboration with María Celia García-Alvarez-Coque and José Ramón Torres-Lapasió in the field of the modulation of the selectivity in HPLC, using serially coupled columns and other strategies to analyse complex samples. She has written 11 research articles and presented several communications in international meetings. The PhD period included a three-month stay (September to November 2017) in the Analytical Chemistry Department of the University of Barcelona (Spain), under the supervision of Prof Martí Rosés, working in the field of Hydrophilic Interaction Liquid Chromatography (HILIC).

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