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Advanced (Multi-Dimensional) Separations for Practical Applications

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1. Introduction

The questions which analytical chemists in industry are confronted with are increasingly complex. For example, the shift from small-molecule drugs to large-molecule biopharmaceuticals poses a whole new string of questions. Molecules are much more complex, with larger numbers of different structures present (e.g. sequence variants and chemical modifications such as glycosylation and other proteoforms), supramolecular complexes and agglomerates may greatly affect activity and stability of the product. As a result, new methods are needed to study the heterogeneity of (bio)macromolecules and their molecular and higher-order distributions.

Methods for the characterization of higher-order structures of intact macromolecules with applications in chemical, biopharmaceutical, and biotechnological fields are emerging. In this context, non-destructive ("native") liquid-phase separation techniques, such as asymmetrical flow-field flow fractionation (AF4) with triple detection and on-line coupled to high-resolution MS, have been developed for an accurate quantitation of the various species present (1). Additionally, methods based on hydrophilic-interaction liquid chromatography (HILIC) coupled to high resolution MS have been developed to characterize the glycoform profile of a complex biotechnological product (2).

Much attention is also focussed on multi-dimensional-separation techniques, which feature a much greater separation power (higher peak capacity) than conventional one-dimensional liquid chromatography. Key examples include the separations of biofluids (3), protein digests (4), and polymers (5,6) "ISSN": "1520-6882", "PMID": "22928586", "abstract": "Online comprehensive two-di-

mensional liquid chromatography (LC × LC used in formulations. The principles, development and application of high-resolution comprehensive two-dimensional liquid chromatography (LC×LC) and its combination with (high-resolution) mass spectrometry (LC×LC-MS) have been described in several recent reviews (7,8).

Eventually, spatial separations are highly promising (3–5). In that case, peaks are not separated in time (through elution from a column), but in space (by reaching different positions in a separation medium (packed bed or monolith). Efficient spatial separations require new hardware devices, with effective flow control and confinement, stationary phases and detection techniques. If these can be developed successfully spatial 3D-LC promises extremely high separation power (peak capacities in the order of one million).

2. Results

A good example of the value of high-resolution LC×LC-MS methods is provided in *Figure 1*. A comprehensive combination of hydrophilic-interaction liquid chromatography in the first dimension, reversed-phase liquid chromatography in the second dimension and high-resolution mass spectrometry as on-line detection system (HILIC×RPLC-HRMS) is used to characterize polysorbates, which are frequently used in pharmaceutical formulations. HILIC is preferred in the first dimension, because it requires a relatively long equilibration time. Separation is according to the degree of ethoxylation (number of -CH₂-CH₂-O- units in the molecule. In the RPLC dimension components are separated based on hydrophobicity.

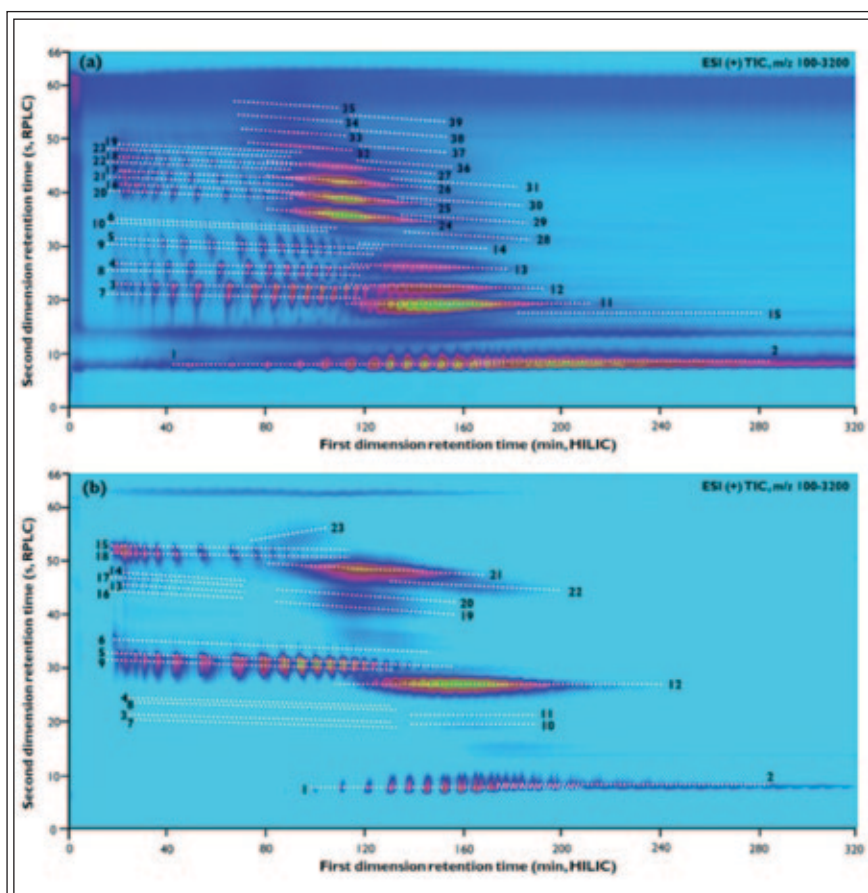


Figure 1 HILIC×RPLC-HRMS separation polysorbates, (a) Tween-20 and (b) Tween-80. Degree of ethoxylation is resolved in the first-dimension separation by hydrophilic-interaction liquid chromatography, while the second-dimension reversed-phase LC separation resolves the chemical species based on hydrophobicity. Reprinted from 9. See the reference for additional details.

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