Cyn-mono-CBI (6), Glu (7), Gly (8), Tyr (9), Ala (10), Asp (11), Tau (12), Ala-Gln (13), Val (14), Met (15), nVal (16), Trp (17), Ile (18), Leu (19), Phe (20), Arg (21), Cyn-bis-CBI (22) and Lys (23).

4 Conclusions

Two in principle different approaches for on-capillary mixing of reactants, namely EMMA and TDLFP, were compared. The EMMA based method combined with sweeping pre-concentration, provides on average 5.3× higher sensitivity (2.1-13.1×), comparable precision in terms of migration times, but on average 2× better precision in peak areas. Finally, results obtained by EMMA are significantly less dependent on the sample matrix.

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P9 OPTIMIZATION OF GRADIENT SEPARATION CONDITIONS IN TWO-DIMENSIONAL LIQUID PHASE SYSTEMS

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Summary

The gradient separation conditions in two-dimensional liquid chromatography are usually used for improvement of the separation efficiency and for better utilization of the separation space. In the work, procedures for optimization of both, gradient two-dimensional liquid chromatography and liquid chromatography coupled to capillary electrophoresis were developed. The in-laboratory build software for processing of two-dimensional data was extended with the optimization protocols.

1 Introduction

The separation of complex samples of biological origin containing tens of even hundreds of analytes may be difficult or nearly impossible using a single separation method. The increased separation power is achieved using two-dimensional separations, which provide enhanced peak capacity with respect to the unidimensional methods. The proper optimization of such methods requires adjusting separation conditions in both dimensions, as well as conditions for efficient transfer of the fractions, with respect to the compatibility of both separation methods used. Under gradient separation conditions, orthogonality and peak capacity of the system can be significantly improved [1]. Due to the peak compression effects, the liquid chromatographic conditions with fast gradients provide higher efficiency with respect to the isocratic separation mode [2]. The protocols for optimization of the two-dimensional separations employing liquid chromatography in both separation dimensions and liquid chromatography in combination with capillary electrophoresis were developed in this work.

2 Experimental

The experimental data used in the study were obtained using two-dimensional methods described elsewhere [3]. The developed optimization protocols were incorporated into the software for processing of two-dimensional data, Eval2D, developed in our laboratory and programmed using Python object-oriented language (version 3.3.5.) with Matplotlib plotting library (version 1.3.1.). The mass spectrometric data in combination with pressure-driven and electrodriven separations were acquired using AB SCIEX QTrap 4500 mass spectrometer (SCIEX, Framingham, MA, USA) operated in ESI mode.

3 Results and Discussion

The mathematical models suitable for description of retention data were developed for hydrophilic interaction liquid chromatographic separation of oligomeric series of analytes [4]. The numerical integration together with the iteration process was used for the calculation of gradient retention times [5]. The gradient data were combined together for two-dimensional LCxLC system; under LCxCE conditions, the electrophoretic separations were selected with respect to the highest peak capacity and highest peak-production rate. The effects of the composition of first dimension eluate were compensated using gradient conditions in the second dimension for both, liquid chromatography and capillary electrophoresis. Thus, the protocol for optimization of changing composition of background electrolyte for the analysis of consecutive fractions transferred from LC was developed. Finally, the gradient optimization procedures were incorporated into the software developed in our laboratory for processing of experimental data obtained by two-dimensional pressure-driven and electrodriven separations, coupled to either single-channel, or multichannel detection techniques (i.e. in combination with mass spectrometry).

4 Conclusions

Two-dimensional liquid chromatography and liquid chromatography in combination with capillary electrophoresis are viable methods for analysis of highly complex samples. Optimized gradient conditions used in both dimensions significantly improve the separation performance of the method, increase compatibility of phase systems and utilization of separation space.

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P10 EVALUATION OF STATIONARY PHASES FOR HPLC BASED ON NANOFIBERS

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Summary

Due to their properties nanofibers have the potential to be a good sorbent in separation techniques. In this study, the performance parameters were compared for the set of chromatographic columns filled with polyacrylonitrile nanofibers processed in the different manner. This material has been used as non-treated and pulverized mat, as well stacked or rolled membrane. The advantages and challenges of the novel fillings were discussed.

1 Introduction

Nanomaterials are particles possessing at least one dimension in the nanometer range. In the case of nanofibers (NFs), the crucial dimension is the diameter that typically varies in the limit of tens to hundreds of nm. NFs are made from inorganic (oxides, metals, carbon), organic (synthetic polymers, biopolymers) materials or mixtures of them [1]. The leading technology of its production represents electrospinning based on