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Journal of Chromatography A, 1087 (2005) 112–123

JOURNAL OF CHROMATOGRAPHY A

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Phase system selectivity and two-dimensional separations in liquid column chromatography

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Abstract

Correlations between the separation selectivity in aqueous and non-aqueous reversed-phase systems and in normal-phase LC systems were investigated for samples containing different numbers of two repeat structural elements. Such samples are best separated in “orthogonal” two-dimensional chromatographic systems, showing selectivity for one type of the repeat structural element only in the first dimension and for the other structural element only in the second dimension. The number of resolved compounds improves as the degree of orthogonality of the separation systems increases with decreasing correlation between the selectivities for the sample structural distribution in the two dimensions. Orthogonal systems with non-correlated selectivities for each repeat structural element provide the highest number of separated peaks and regular arrangement of the peaks over the two-dimensional retention space according to the individual structural element distribution and the best use of the available peak capacity. Fully orthogonal systems are difficult to find in practice. Partially orthogonal system with correlated selectivities for one structural type distribution, but with one system non-distinguishing the distribution for the other structural element are still useful for the two-dimensional separations. The correlations between the selectivities for repeat regular structural increments were employed to evaluate the suitability of phase systems for two-dimensional HPLC separations. The selectivity correlation in various reversed-phase and normal-phase systems was evaluated for two sample types:

(1) Various RP columns show significantly inversely correlated selectivities for acyl lengths and numbers of double bonds distribution, but the differences in the double bond selectivity can be used for practical separations of triacylglycerols with the same equivalent carbon numbers.
(2) Synthetic EO–PO block (co)oligomers with two-dimensional distribution of oxyethylene and oxypropylene monomer units were separated according to the two distribution types using on-line two-dimensional reversed-phase–normal-phase LC with a C18 column in the first dimension and an aminopropyl silica column in the second dimension.

Keywords: Column liquid chromatography; Two-dimensional separations; Phase system selectivity; Peak capacity; Ethylene oxide–propylene oxide (co)oligomers; Triacylglycerols

1. Introduction

Although two-dimensional gas chromatography is much more frequently used and more elaborated technique for separation of complex sample mixtures [1,2], two-dimensional liquid chromatographic methods, whose development is more difficult, are gaining increasing attention in the recent years (often in connection with mass spectrometry), because of the demands on high-resolution LC separations raised by fast developing research in proteomics and metabolomics [3,4]. In two-dimensional (2D) systems, different chromatographic modes suiting particular separation problems are combined in each dimension to increase the number of separated compounds in complex samples [5].

The selectivity of separation in the individual modes is principally based on the differences in the size, polarity and shape of the sample molecules, on the acidity/basicity and on the specific charge of ionic compounds. Because of a mixed character of the forces controlling the retention mechanism in

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doi:10.1016/j.chroma.2005.01.061

real separation systems, the selectivity in various systems can be more or less correlated. Both reversed-phase and normal-phase separations are based on the differences in the polarities of the analytes, even though various selective interactions such as dipole–dipole or proton-donor/acceptor interactions may cause major selectivity differences for various isomers or homologous compounds in these phase systems. Molecular size is the basis of separation in size-exclusion chromatography, but it often contributes also to the retention and separation selectivity in reversed-phase or ion-exchange LC systems by hydrophobic, polar, or ionic interactions of the structural elements in large molecules [6,7].

Most often, synthetic polymers [8], peptide fragments or other biopolymers [3,4,9,10] are separated using size-exclusion chromatography for the separation according to the molar mass distribution in one dimension and reversed-phase [8–10] or ion-exchange [4] LC for the separation of species with different functionalities in the second one [2,4]. For separation of biopolymers, bioaffinity chromatography in the first dimension can be combined with reversed-phase LC in the second dimension [3]. Recently, applications of two-dimensional reversed-phase chromatography employing different stationary phases in each dimension (such as combination of silica- and zirconia-based columns) were reported [11–15]. “Pseudo-multidimensional” reversed-phase chromatography on a single column using sequential application of two or more selective gradient elution modes designed to elute only a certain class of compounds also increase the total peak capacity [16]. In so-called “comprehensive” two-dimensional liquid chromatography, every fraction from the first dimension is transferred on-line to the second dimension [17–20].

In multidimensional chromatography the number of resolved compounds considerably increases. However, based on the assumption that the retention of the sample components is a random process, the probability theory predicts that the number of peaks occurring in a chromatogram is much lower than the maximum peak capacity of a multi-dimensional separation system [21–25]. The number of singlets, overlapping doublets and triplets in poorly resolved chromatograms can be predicted by statistical overlap theory [26] and can be decoded to some extent using chemometric procedures [27,28].

Both the practical objective of the analysis and the sample type very often imply certain degree of structural relationship among the analytes of interest, for example, molar mass or end-group differences in polymers, different number of repeat units in homologues or homo-oligomers, equal structural units at different positions in the molecules in positional isomers, different numbers and annelation of aromatic rings in polyaromatic hydrocarbons, or different number and polarities of functional groups in various sample types of environmental pollutants, naturally occurring compounds, drugs and metabolites, etc. Giddings [9] called the regularity in the structural distribution of sample components “sample dimensionality”. A two-dimensional sample exhibits a disordered peak distribution in one-dimensional separation systems, but shows a more or less ordered distribution of peaks in two-dimensional separation systems [28,29]. The overlapping peak distribution in one-dimensional separation systems usually originates from dual distribution of the number of one or more repeat structural elements in the sample, both of which contribute more or less to the retention, depending on the sample character.

The best separation of “multidimensional samples” is accomplished in “orthogonal” separation systems with complementary techniques [30]. Each dimension should provide maximum separation selectivity for one type of distribution, but should be as far as possible insensitive to the differences in the other type of distribution [31].

Separation selectivity for repeat structural elements is a convenient measure of the extent to which structural differences in sample molecules affect the retention behaviour. It has important impact on the selection of suitable conditions for a particular separation problem, depends on both the stationary and the mobile phases and can be used for general characterization of separation systems to resolve sample compounds on the basis of various structural differences, such as size, polarity, shape, etc. [3,4,31].

In this work, correlation of selectivity for molecules differing in polarities (lipophilities) was investigated in aqueous and non-aqueous reversed-phase LC and in normal-phase LC. The approach was applied to triacyl glycerols in plant oil samples and to ethylene glycol–propylene glycol (EO–PO) (co)oligomers to develop orthogonal 2D LC separation methods by combination of reversed-phase and normal-phase systems.

Two-block (co)polymers are two-dimensional samples characterized by the distributions of the number of each monomer unit. EO–PO block (co)polymers are frequently used surfactants in washing machines, emulsifiers and solubilizers of flavors and fragrancies in cosmetic products. The oxypropylene chain $-[CH_2-CH(CH_3)-O]_m-$, i.e., $(PO)_m$, is significantly less polar than the oxyethylene chain $-[CH_2-CH_2-O]_n-$, i.e., $(EO)_n$. Two types of block copolymers were studied: (1) $(EO)_n-(PO)_m-(EO)_n$ (Slovanik) and (2) $(PO)_m-(EO)_n-(PO)_m$ (Novanik). Increasing average molecular mass of the industrial products enhances gelation and with increasing number of PO units, the wetting properties are improved. Consequently, molar mass distribution, chemical composition distribution and block sequence distribution are all important for the characterization of the industrial products of this type. Because of different polarities of EO and PO units, reversed-phase and normal-phase LC systems differ significantly in selectivities for the individual blocks [6,32].

Natural fat and oil samples contain saturated and unsaturated fatty acids, their esters or other derivatives and mono-, di- and tri-acylglycerols. These are typical multi-dimensional samples with acyl of different lengths (numbers of carbon atoms) in the first sample dimension, the number of double bonds in the second sample dimension and the positions of