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ScienceDirect
Journal of Chromatography A, 1137 (2006) 42–48
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Multidimensional liquid chromatography system with an innovative solvent evaporation interface

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Received 18 July 2006; received in revised form 28 September 2006; accepted 2 October 2006
Available online 7 November 2006

Abstract

An orthogonal two-dimensional liquid chromatographic (2D-LC) system was developed by using a vacuum-evaporation loop-type valve interface. Normal-phase liquid chromatography (NPLC) with a bonded CN phase column was used as the first dimension, and reversed-phase liquid chromatography (RPLC) with a C_{18} column was used as the second dimension. All the solvents in the loop of the interface were evaporated at 90 °C under vacuum conditions, leaving the analytes on the inner wall of the loop. The mobile phase of the second dimension dissolved the analytes in the loop and injected them onto the secondary column, allowing an on-line solvent exchange of a selected fraction from the first dimension to the second dimension. The chromatographic resolution of analytes on the two dimensions was maintained at their optimal condition. Sample loss due to evaporation in the interface was observed that depended on the boiling point of the compound. Separation of sixteen polycyclic aromatic hydrocarbon mixtures and a traditional Chinese medicine *Angelica dahurica* was demonstrated.

Keywords: Two-dimensional liquid chromatography; Traditional Chinese medicines; *Angelica dahurica*; Solvent exchange interface; Furancoumarin; Loop-valve interface; Band compression

1. Introduction

As the requirement for separation of complex samples, such as environmental, biological, and natural products samples, is more demanding now than ever before, the development of two-dimensional liquid chromatography (2D-LC) in order to improve separation capability of HPLC technology has been a hot topic in the field. According to the theory of multidimensional chromatography [1–3], the peak capacity of an ideal 2D system is the product of the peak capacities obtained on each of the two dimensions, and the resolution is the square root of the sum of the squares of the resolution of each dimension. Considering the differences in selectivity and separation efficiency, 2D-LC should provide much higher peak capacities and chromatographic resolution than one-dimensional LC.

The coupling of NPLC and RPLC should yield orthogonal selectivity and high separation ability if there was no incompatibility of mobile phases used by the two dimensions, no additional band dispersion in the second injection. However, there are several points [4] that should be considered in order to achieve the maximal separation efficiency and selectivity in the combination of NPLC and RPLC. They are:

(1) The conflict between the transferred volumes from the primary column and the maximal allowable injection volume of the secondary column, and peak band dispersion of the primary fractions on the head of the secondary column. The sample plugs are diluted when they migrate along the primary column, forming the injection bandwidth of the secondary column. Ideally, the primary bandwidths injected onto the secondary column shall be narrow enough to avoid deterioration of separation efficiency of the secondary column. A "refocusing" step is normally adopted to reduce the dispersion of bands and injection volumes on the secondary column.
(2) The introduction of large volumes of an incompatible solvent to the secondary column may yield broadened and distorted peaks. This problem is particularly serious on the

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doi:10.1016/j.chroma.2006.10.005

combination of NPLC and RPLC. Since the eluting strength of the primary eluent is always stronger than that of the mobile phase on the head of the secondary column, the peak dispersion of the primary fraction transferred to the secondary column is unavoidable. The problem may be solved by using a narrow bore or capillary column as the first dimension and a conventional column as the second dimension, sacrificing detection sensitivity as a result. For the coupling of identical dimensions of columns, the transferred volumes from the primary column were always larger than the maximal allowable injection volume of the secondary column. Therefore, an interface for the adaption of solvent volumes is required in order to maintain the optimum separation efficiency on the two columns.

(3) The limit of separation speed of the second dimension. A short packed column (3–5 cm) with fast gradient elution or a monolithic column (3–5 cm) [5] at high linear flow rate is normally used in the second dimension. However, the speed dilemma between the two dimensions is alleviated at the sacrifice of the secondary column efficiency by using the method.

Wise and coworkers [6] investigated the coupling of a normal phase NH_2 column and a reversed phase ODS column for the separation of polycyclic aromatic hydrocarbons (PAHs). Since the solvents of the two dimensions were immiscible, a trap column was developed as the interface of the system. The interface was subjected to an on-line solvent evaporation by N_2 to achieve solvent exchange. The complex instrumentation and the time required (about 30 min) for solvent evaporation restricted the application of the method.

One way to solve the problem of solvent immiscibility in 2D-LC was proposed by Dugo et al. [7]. They used a microbore column in the first dimension and a conventional column in the second dimension that permitted the injection of a relatively small volume in the secondary column, making the transfer of incompatible solvents from the primary to the second dimension possible.

The combination of normal (silica) and reversed (C_{18}) phase 2D-LC used for the analysis of alcohol ethoxylates was reported by Murphy et al. [8]. An aqueous solvent was used in the normal phase; therefore, the mobile phases used in the two dimensions were miscible, resulting in the easy injection of the entire first-dimension effluent onto the second-dimension column.

In 2D-LC, the sensitivity enhancement can be achieved if a solute band compression [9] at the head of the secondary column is realized during the transfer of the solute band from the primary column to the secondary column. Since the elution strength of the mobile phase in the NP separation is always stronger than that of the mobile phase at the head of the secondary column operated in RP mode, it is impossible to have a peak-focusing effect by taking advantage of weaker eluents in the first dimension and stronger eluents in the second dimension.

Both Murahashi [10] and Haefliger [11] used the interface of the parallel RP columns in the second dimension with the miscible solvents. In their work, high percentage of acetonitrile was used as the mobile phase in the first dimension that is a strong mobile phase in the second dimension as well. The eluted fractions from the first dimension cannot be loaded as such on a RP column. In order to solve the problem, the effluent from the primary column was diluted with large amount of water (to about 75%) before injection into the secondary column. The injection band was then focused at the head of the RP column.

In this study, a loop-interface was developed by using heating-vacuum to evaporate the solvent in the loop. The interface resolves the problems of solvent exchange, increases the concentrations of the analytes in fractions from first dimension, and reduces transferred volumes to the second dimension. An orthogonal 2D microcolumn LC system was constructed and evaluated, with NPLC as the first dimension and RPLC as the second dimension. PAHs and the hexane extract of *Angelica dahurica* were used as test samples.

2. Experimental

2.1. Instrumentation

An Ultra-Plus II liquid chromatograph (Micro-Tech Scientific, Vista, CA, USA), consisting of quaternary pumps, two micro-mixers, a ten-port switching valve (Valve III) and a six-port valve (Valve II), and a system controller, was used in this study. An additional six-port two-position manual valve (Model C2-2006) was purchased from Valco (Schenkon, Switzerland) and was used as injection valve (Valve I). The UV detectors used in the primary and second dimension were a CE-1575 on-column detector (Jasco, Japan) and a UVIS Linear 200 detector (Thermo Finnigan, CA, USA), respectively. Chrom Perfect for Windows software, from Justice Innovations (Mountain View, CA, USA), was used for data acquisition. The identification of major peaks of *A. dahurica* extract separation was determined by standards, UV spectra (photodiode array detector, Waters CapLC 2996) and MS (Q-TOF micro, Waters), respectively.

A laboratory-made heating tape for heating interface loop was constructed. The temperature of the loop was monitored by using a thin film Pt-100 element (Juchheim, Germany) and was controlled by using a Model REX-C100 temperature controller (RKC Instrument, Japan). A vacuum gauge was purchased from electronic department of Perking University (Beijing, China), and a Model 2XZ-2 vacuum pump was purchased from Shanghai Vacuum Pump (Shanghai, China).

2.2. Chromatographic conditions

A 200 mm × 0.53 mm I.D., fused silica column packed with 5 μm CN particles (Macherey-Nagel, Germany), was dry-packed according to the procedure described by Guan et al. [12] and used as the first dimension in normal-phase mode. A C_{18} 150 mm × 0.32 mm I.D., 5 μm particle size column (Micro-Tech Scientific, USA) was used as the second dimension in reversed-phase mode. Mobile phases used were hexane in the first dimension, and water (solvent A)/acetonitrile (solvent B) in the second dimension operated in a linear gradient