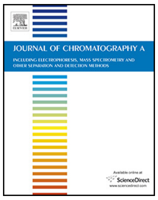
Journal of Chromatography A, 1496 (2017) 68–79

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma



Trapping multiple dual mode centrifugal partition chromatography for the separation of intermediately-eluting components: Operating parameter selection[☆]

Johannes Goll, Raena Morley, Mirjana Minceva[*]

Biothermodynamics, TUM School of Life Sciences Weihenstephan, Technische Universität München, Gregor-Mendel-Str. 4, 85354 Freising, Germany

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form 16 March 2017
Accepted 17 March 2017
Available online 20 March 2017

Keywords:
Countercurrent chromatography
Centrifugal partition chromatography
Liquid-liquid chromatography
Ternary paraben separation
Trapping multiple dual mode

ABSTRACT

The preparative separation of intermediately-eluting components in liquid-liquid chromatography is commonly performed with isocratic batch injections, a technique which often leads to low yield and/or purity as a result of peak overlap. Two-column trapping multiple dual mode centrifugal partition chromatography, an alternative discontinuous method for the separation of a mixture into three product fractions (early-, intermediately-, and late-eluting components) at full recovery, is presented in this work. A mathematical shortcut method based on equilibrium theory assumptions is derived for the determination of the key operating parameters (i.e., step durations and number of steps). The feasibility of the technique and the accompanying short-cut method is demonstrated by proof-of-concept experiments for the separation of two paraben model mixtures.

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1. Introduction

Liquid-liquid chromatography (LLC) has proven itself to be an effective technique for the separation of natural product compounds from complex mixtures [1]. The term LLC encompasses both countercurrent chromatography (CCC) with hydrodynamic units and centrifugal partition chromatography (CPC) with hydrostatic ones. LLC employs the two phases of a liquid-liquid biphasic system as the stationary and mobile phases. During a chromatographic run, the liquid stationary phase is held in place by means of the column geometry and the application of a centrifugal field; the mobile phase is then pumped through it. Separation is achieved as a result of the differing partition behavior of the mixture components between the two phases.

Working with a liquid stationary phase presents several advantages over conventional liquid chromatography techniques with a solid stationary phase, including high loading capacity and the absence of irreversible stationary phase adsorption. Additionally, the presence of a liquid stationary phase allows for high operational flexibility. Either one of the two phases of the selected biphasic system may be used as the stationary phase, and the roles of the phases may even be switched during operation. This key feature of LLC has led to the development of several unique operating modes not otherwise realizable with conventional chromatography techniques. Examples include batch processes such as elution-extrusion [2] and dual-mode [3], as well as continuous processes for binary separations [4,5]. It is often possible to run a variety of operating modes on a single LLC device, allowing the operator to select the mode best suited to the separation task at hand.

A common separation task in preparative chromatography is the isolation of an intermediately-eluting target component from a complex mixture. When the mixture has already undergone one or more preliminary separation steps of lower selectivity, the remaining impurity compounds often exhibit retention behavior similar to that of the target component (i.e., possess low separation factors). When working with isocratic batch injections, peak overlap resulting in decreased productivity and yield is often encountered, especially at the high column loadings desired for preparative separations. To improve target recovery, low purity cut fractions may be reinjected as part of an offline [6] or online [7–9] multidimensional (heart-cutting) separation strategy. However, these additional chromatographic runs add time and complexity to the process. Online selection and reinjection of cut fractions is difficult to automate and often involves complex equipment setups.

Continuous separation techniques in LLC, such as intermittent counter-current extraction (ICcE) [4,10–13] and sequential cen-

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[☆] Selected paper from the 9th International Counter-current Chromatography Conference (CCC 2016), 1–3 August 2016, Chicago, IL, USA.

[*] Corresponding author.
E-mail address: mirjana.minceva@tum.de (M. Minceva).

http://dx.doi.org/10.1016/j.chroma.2017.03.039

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trifugal partition chromatography (sCPC) [14–17], exhibit high selectivity and can be used to process large volumes of feed. However, they yield only two product fractions. The product streams are collected sequentially at opposite ends of a two-column setup during continuous feed injection between the neighboring columns. To separate a feed mixture into three fractions with ICcE/sCPC, two consecutive process steps must be conducted, where the product stream from the first process step containing the intermediately-eluting target component serves as the feed mixture in the second process step.

An alternative for ternary separations is a form of dual mode operation in which the intermediately-eluting target component remains "trapped" inside the column while the impurities are alternately eluted from opposite column ends [18]. This discontinuous LLC method for the recovery of intermediately-eluting compounds was presented in [4] and [19]. In both studies, a two-column CCC set-up with feed introduction between the two columns was used. Multiple elution cycles were performed, each consisting of two steps of alternating mobile phase and flow direction. During these cycles, the neighboring impurity compounds eluted intermittently from the two ends of the device. Meanwhile, the majority of the intermediately-eluting target component remained "trapped" inside the unit. After "washing away" the impurities in this manner, the purified target component could be obtained. In [4], the target component was a bioactive terpenoid (triptolide) from a Chinese herbal medicine present at only 2% in the crude extract. Injection of the crude extract was performed continuously over several cycles. At the end of the process, triptolide was obtained at 98% purity with a yield of 75%. This operating mode was designated as a form of ICcE.

In [19], the target component was Coomassie Brilliant Blue G-250, a dye used for staining in the analysis and quantification of proteins. Multiple injections of the impure sample were made, always at the start of a new cycle. This study referred to the technique as trapping multiple dual mode (MDM). Solubility limits in one phase were considered during the separation design. A mathematical model developed in [20] based on the peak (band front) positions of the mixture components within the column was used to determine the step durations in the two elution modes. A highly pure product fraction was achieved but at a low yield of 37%. The mathematical model applied in [19] approximates solute position inside of the column as the band front position under ideal conditions. However, it does not take into account the wide solute bands encountered at high volume loadings. Therefore, after the elution mode switch, the distance traveled by the solute is calculated relative to the band front position of the previous step. This band "front", relative to the new flow direction, is now effectively the back end of the solute band. This will cause elution of the solute band to occur earlier than predicted, a factor which likely contributed to the separation's low yield.

In both [4,19], it was acknowledged that separation performance could be improved through adjustment of the operating parameters, such as step durations and flow rates. The high target component purities obtained in these two studies clearly demonstrate the effectiveness of this type of "trapping" operating mode for the isolation of intermediately-eluting components. However, an approach for accurate prediction of the process operating parameters to simultaneously satisfy high purity and yield requirements is lacking in the literature.

This work presents a modification of the operating modes described in [4,20] for the separation of intermediately-eluting components using a two-column hydrostatic CPC device. To the authors' knowledge, this is the first report of such a separation using CPC technology. In this method, the feed mixture is introduced during a single column loading cycle without simultaneous mobile phase flow, rather than continuously or repeatedly. For clarity, and to distinguish it from the previous studies using hydrodynamic (CCC) columns, the technique investigated in this work will be referred to as trapping multiple dual mode centrifugal partition chromatography (trapping MDM CPC). However, the presented technique and design methodology will be applicable to separations on both CPC and CCC instruments.

The objectives of this study were to demonstrate the feasibility of the trapping MDM CPC technique and to establish a method for determination of the key operating parameters (i.e., step durations, number of steps). A mathematical short-cut method was derived for determination of the operating parameters allowing for the complete separation of an intermediately-eluting component from a multicomponent mixture under ideal conditions. Proof-of-concept of the trapping MDM CPC technique and short-cut method was then demonstrated experimentally. To show the flexibility of the technique and short-cut method, two trapping MDM CPC experiments with different ternary feed mixtures were performed. The model feed mixtures contained parabens differing only in their alkyl chain lengths. The liquid-liquid biphasic system was ARIZONA N (*n*-heptane/ethyl acetate/methanol/water 1/1/1/1 v/v/v/v).

In a subsequent publication [21], the presented mathematical short-cut method is implemented as part of a simulation-based strategy for throughput maximization in trapping MDM CPC. This throughput maximization strategy involves selection of the maximum feed concentration, maximum flow rate, and step durations during column loading and separation.

2. Theory

The isolation of an intermediately-eluting target component from a complex mixture can be viewed as a pseudo-ternary separation. When performing the same separation using an isocratic batch injection, two product stream cuts would be required to obtain the target component fraction: one between the early-eluting components and the target component, followed by one between the target component and the remaining late-eluting components. In trapping MDM CPC, three distinct product streams analogous to these three cut fractions are obtained. The early- and late-eluting components elute from opposite ends of the unit while the intermediately-eluting target component remains trapped inside. The target component is then recovered at the end of the process.

Trapping MDM CPC is a cyclic, discontinuous, non-steady state process. One cycle consists of two steps in two different elution modes: descending (Des) mode and ascending (As) mode. In Des mode, the lower phase is used as the mobile phase. The roles of the phases and the mobile phase flow direction are reversed in As mode. The lower phase is the stationary phase while the upper phase is pumped through the columns as the mobile phase.

For the following explanation of the concept of trapping MDM CPC, the simplified case of a three-component feed mixture of compounds A, B, and C is considered. In this example, B is the intermediately-eluting target compound to be trapped within the columns and later recovered. Partition coefficients (K_k^{Des}, K_k^{As}) are defined for the process as the concentration of a component k in the stationary phase divided by its concentration in the mobile phase. Since the roles of the upper and lower phases are reversed from one mode to the other, two elution mode-specific definitions for the partition coefficients are given in Eqs. (1) and (2).

$$K_k^{Des} = \frac{c_k^S}{c_k^M} = \frac{c_k^U}{c_k^L} \tag{1}$$

$$K_k^{As} = \frac{c_k^S}{c_k^M} = \frac{c_k^L}{c_k^U} \tag{2}$$

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