Journal of Chromatography A, 1229 (2012) 180–189

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Approaches to find complementary separation conditions for resolving complex mixtures by high-performance liquid chromatography

C. Ortiz-Bolsico, J.R. Torres-Lapasió*, M.C. García-Álvarez-Coque

Departament de Química Analítica, Universitat de València, c/Dr. Moliner 50, 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 24 November 2011
Received in revised form 12 January 2012
Accepted 13 January 2012
Available online 20 January 2012

Keywords:
Complex mixtures
Chromatographic optimisation
Complementary separation conditions
Comprehensive search
Genetic Algorithms
Peak count: Local search

ABSTRACT

Chromatographic problems are usually addressed trying to find out a single experimental condition aimed to resolve all compounds in the sample. However, very often, the chromatographic system is not able to provide full resolution. When a separation fails, the usual choice is introducing a drastic change in the chromatographic system (e.g. column, solvent, pH). There are, however, other possibilities that take advantage of the gathered information in the failed separation, without the need of new experiments, based on the concept of complementary separations (e.g. isocratic mobile phases, gradients, columns, chromatographic modes). One separation condition will focus on the resolution of some compounds in the sample, while the other compounds will be resolved using a second (or subsequent) condition(s). Complementary separations, being a simple and attractive idea, present, however, challenges in terms of computation volume and complexity of the required algorithms. This work describes in detail different approaches that have been developed up-to-date for this purpose, and introduces a new approach based on the peak count concept that is benefited of the best features of the previous approaches: high reliability in finding the solution, accessibility to analysts without specialised programming skills and short computation time.

1. Introduction

Chromatographic analyses demand finding experimental conditions to separate the compounds of interest. This task is often arduous and discouraging, and involves several objectives that are opposed each other (e.g. high resolution, short analysis time, and low economic and environmental costs). When the analyst is faced with a new sample, he/she ignores the full potential of the separation system. The optimised conditions may not be also the best that the system can offer. Fortunately, nowadays, a rigorous evaluation of the system potential is possible using numerical methods, which efficiently and reliably explore the separation performance of a chromatographic system. In spite of the literature available in this field [1–15], new challenges are continuously arising.

The success of the separation is determined by the chromatographic system: the instrumentation features, and the combination of column, modifier(s), experimental conditions, and incidentally, pre-conditioning steps used to change the nature of column or analytes. All these elements must be combined properly to reach an acceptable separation performance. In chromatography, more than in other fields, the experimental factors that can be modified to change the analytical behaviour are numerous, and the quality of the separations may vary drastically when the factors are changed.

The aim of the analysis should be clearly defined before starting the optimisation process. This may vary considerably depending on the problem. In most cases, the analyst is interested in the separation of all compounds in the sample. In others, the aim is less ambitious, focusing the attention on only a few compounds, or even, on a single compound [16]. The problem is most usually addressed trying to find a single experimental condition able to get the resolution of all compounds in the sample. However, very often, the chromatographic system will not be able to achieve full resolution. When a separation fails, the usual choice is introducing a drastic change in the chromatographic system (e.g. column, solvent, pH). There are, however, other possibilities that take advantage of the gathered information in the failed separation, without the need of new experiments.

In 2000, an optimisation strategy was proposed to achieve the chromatographic separation of complex samples to get full resolution, based on the concept of complementary situations [17]. One separation condition (e.g. an isocratic mobile phase, gradient, column) would allow the resolution of some compounds in the sample, while the other compounds would be resolved using a second (or subsequent) condition(s). The idea is attractive, but demanding in computation terms. A substantial reduction in the calculation effort was achieved through the application of natural

* Corresponding author. Tel.: +34 96354 4014; fax: +34 96354 4436.
E-mail address: jrtorres@uv.es (J.R. Torres-Lapasió).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2012.01.034

computation [17,18]. Recently, a simple approach more accessible to analysts was proposed to find complementary separations conditions (CSCs) [19,20]. This work describes in detail the different approaches that have been developed up-to-date for this purpose. A new approach, based on the peak count concept and assisted by local search, is also reported. The approach is benefited of the best features of the previous approaches.

2. Theory

2.1. Total, partial and specific optimisation strategies

Once the retention and peak profile behaviour for each compound in the target mixture has been appropriately modelled, three optimisation levels can be defined, which have been called total, partial and specific strategies, according to the number of compounds to be resolved in a sample [16]. The way of structuring the calculation is next described in detail for each type of strategy. In all cases, the reduction of the chromatographic information related to the resolution is performed in two consecutive steps. In the first step, a descriptor that measures the success in the separation of each pair of compounds (pair resolution), or of an individual compound from the remaining in the sample (elementary resolution), is obtained. We will only refer here to the descriptors that measure the elementary resolution associated with each compound. In the second step, the elementary resolutions are combined in a descriptor that measures the global resolution in the chromatogram.

The calculations are best outlined in matrix terms. The elementary resolutions are thus arranged in a matrix, **P**. Each column in **P** corresponds to a given compound, and each row is associated with a certain separation condition from a set of hypothetical conditions, whose performance is being investigated (namely, the conditions grid) (Fig. 1a). For each condition in the grid, simulated chromatograms are calculated and from them the elementary resolutions. The global resolution associated with each experimental condition is obtained by multiplying the elements in the corresponding row in **P** (Fig. 1b). If the process is extended to all conditions in the matrix, a global resolution vector is obtained. The element with a maximal value points out the optimal separation condition. On the other hand, the maximal value in each matrix column represents the maximal separation that can be expected for each compound, which is called the "limiting resolution" (Fig. 1c). The limiting resolution values for the different compounds in a sample are of great interest to establish the operative limits of the chromatographic system.

Instead of solving the *n* compounds in the sample using a single separation condition, *n* separation problems can be outlined, each aimed at solving a different compound. This approach is possible provided that the selected resolution function allows an independent evaluation of the contributions of each compound. Similarly to the calculation of the global resolution for a given experimental condition through the product of elementary values, for each matrix row (Fig. 1b), the elementary limiting resolutions can be multiplied as well, resulting in a combined resolution, the "global limiting resolution" (Fig. 1c). This indicates the maximal system performance and can be used to calculate the degree of success by dividing the global resolution associated with the selected conditions (those offering maximal resolution, or any other selected as satisfactory) by the global limiting resolution. The expectancies in getting a substantial improvement in the results, or the magnitude of the expected improvement, can be also evaluated.

The analyst interest is focused, sometimes, to resolve only some compounds in the sample. For this purpose, partial optimisation strategies should be applied, which classify the eluted compounds in two categories: the analytes (whose elementary resolution is optimised) and the interferences (whose resolution is not optimised, but should be taken into account). In this case, only the columns in the elementary resolution matrix that include the compounds of interest are considered, which gives rise to the calculation of partial resolutions (Fig. 1d).

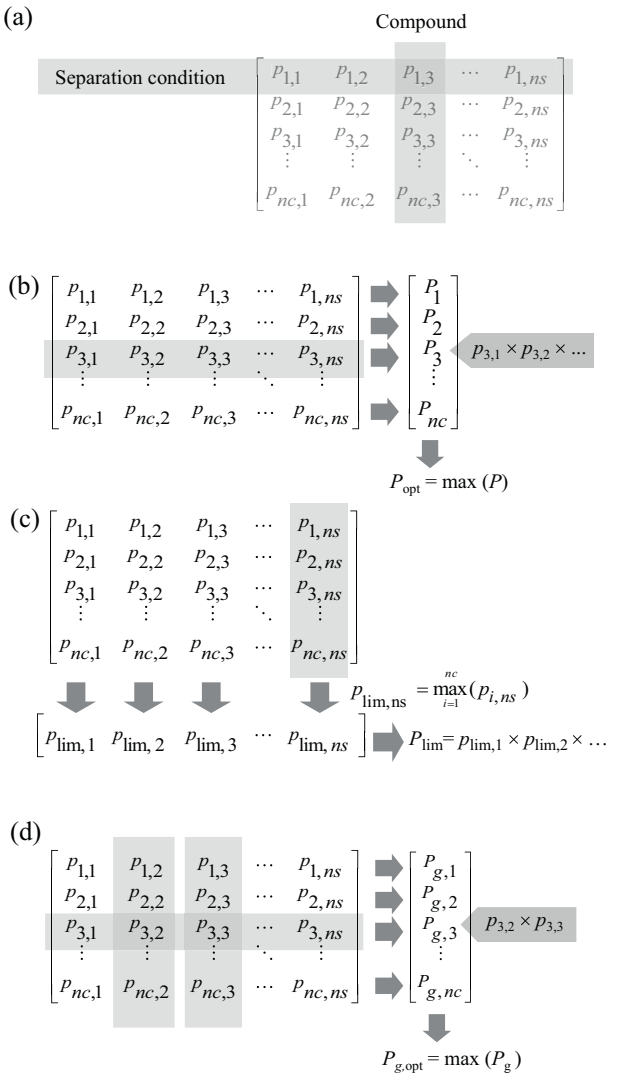

Fig. 1. Matrix of elementary peak purities: (a) each column corresponds to a compound and each row is associated with a certain separation condition; (b) Calculation of the global resolution associated with each separation condition and selection of the optimal value; (c) Calculation of the limiting elementary peak purities and global limiting purity; (d) Optimisation of the resolution of a group of two compounds.

2.2. A mixed strategy: complementary separation conditions

As the complexity of the sample increases, a single experimental condition is unlikely to provide an acceptable separation of all compounds in a sample. A possible solution is the use of a combination of two or more experimental conditions with complementary behaviour. The simplest and most frequent case, which will be taken as example in this work, is the optimisation of complementary mobile phases, using the same solvent system. It should be noted that the concept of complementarity goes beyond the optimisation of isocratic mobile phases: it is possible to optimise gradients, combinations of solvents, chromatographic columns, separation techniques and so on. Hence, the term CSCs used throughout this work.