Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1121 (2006) 219–227
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Retention modelling in ternary solvent-strength gradient elution reversed-phase chromatography using 30 mm columns

Melvin R. Euerby [a,*], Federico Scannapieco [b], Hans-Jürgen Rieger [c], Imre Molnar [c]

[a] *AstraZeneca R&D Charnwood, Analytical Development, Pharmaceutical and Analytical R&D – Charnwood/Lund, Bakewell Road, Loughborough, Leicestershire LE11 5RH, UK*
[b] *University of Strathclyde, Department of Pharmaceutical Sciences, 27 Taylor Street, Glasgow G4 ONR, UK*
[c] *Institut für angewandte Chromatographie, Schneeglöckchenstrasse 47, D-10407 Berlin, Germany*

Received 22 September 2005; received in revised form 7 April 2006; accepted 10 April 2006
Available online 18 May 2006

Abstract

An optimization strategy for ternary solvent-strength gradient elution RP chromatography is described in which a two-dimensional model of gradient time (2 levels) against ternary proportions of organic modifiers (4 levels) was constructed. From the resolution surface the optimum ratio of organic modifiers could be selected. Excellent retention time and acceptable peak width and resolution simulations were obtained. The separation could be further optimized from the same input data by using a standard one-dimensional model in order to optimize for gradient slope, duration and shape. Excellent retention time and acceptable peak width and resolution simulations were obtained (<1, 2 and 6% error, respectively).

Keywords: Computer optimization/prediction; Ternary solvent-strength gradient chromatography; Rapid reversed-phase LC analysis; Short columns; Computer modelling software

1. Introduction

The use of computer simulation software to predict retention behaviour and to optimize chromatographic separations has now become a pivotal tool for the chromatographic method developer [1]. Commercially available computer modelling/prediction software packages, while originally designed for modelling analytical scale reversed-phase LC separations, have now been expanded into such areas as capillary electrophoresis [2], gas chromatography [3–6], ion pair chromatography [7], scale up from analytical to preparative scale separations [8], enantiomeric separations by chiral LC stationary phases [9], ion chromatography [10] and as an education tool [11].

In the RP-LC arena, the use of computer modelling packages has found greatest success not only in the separation of small molecule pharmaceuticals including synthesis impurities and degradation products of widely differing polarities [12–19], but also peptide/tryptic digests and protein mixtures [20–23], oligonucleotides [23], metabolites [18,24], complex mixtures of active compounds from plant origin [25–30], environmental pollutants [31–33] and robustness validation of LC methodologies for routine QC analysis [34].

One of the major reasons for the widespread use of these separation design packages resides in their excellent prediction accuracy for analyte retention and resolution [10,12,35,36] and the flexibility of the software, which can be used to model isocratic or gradient separations as a function of variables such as percentage organic, gradient time, gradient steps, pH, temperature, ion pairing reagent concentration or ionic strength in a continuous way.

The use of computer modelling is extremely attractive, as only limited input data is required in order to rapidly obtain accurate optimum separation conditions. In many cases the rate limiting stage of the process is peak tracking/assessment. The simplest way is to use peak areas. However, a more elegant approach is to use diode array UV spectrometry in conjunction with extracted ion mass spectrometry.

In addition to the one-dimensional modelling described above, some software packages can now accurately perform two-dimensional modelling, i.e. simultaneous variation of any two-separation variables for a chromatographic procedure. Examples include gradient time (t_G) versus pH, percentage organic (%B)

* Corresponding author. Tel.: +44 1509 64 4084; fax: +44 1509 64 5590.
E-mail address: mel.euerby@astrazeneca.com (M.R. Euerby).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.04.073

versus pH, t_G versus temperature and ionic strength versus temperature [1].

The modelling flexibility of these software programmes enables the chromatographer to develop their own/company method optimization strategies. A typical approach favoured by many chromatographers is to simultaneously model the selectivity of temperature and gradient steepness on a single selected RP column [37]. This two-dimensional approach has a much more pronounced effect on the separation selectivity than the additive effect of the two individual variables [38]. The t_G-temperature model is also the easiest to carry out automatically in an unattended mode.

The use of selectivity differences between the organic modifiers acetonitrile (MeCN), methanol (MeOH) and tetrahydrofuran (THF) in RP binary gradient chromatography is well documented [1,10]. Fig. 1 highlights the large selectivity differences that can be obtained using binary gradients of aqueous buffer and either MeCN, MeOH, THF, ethanol, 2-methoxyethanol or propan-2-ol in the separation of a multicomponent mixture containing 10 hydrophilic and lypophilic bases and two neutral compounds [39]. However, even with this comprehensive approach, certain separations can still remain intractable with binary gradient chromatography. Difficult separations can often be resolved when either ternary isocratic [35,39–44] or ternary gradient chromatography [45–47] is employed.

Numerous groups have produced theoretical descriptions of retention in linear binary gradient elution chromatography and these descriptions have been subsequently extended to include ternary gradients [45–47]. There are two types of ternary gradient chromatography defined by Jandera [49] as ternary solvent-strength and combined selectivity-solvent-strength as depicted in Fig. 2a and b. In the case of the former, the ratio of the concentrations of two organic modifiers is kept constant; whilst the sum of the two concentrations is changed (i.e. the solvent-strength increases, see Fig. 2a). In the latter case the ratio of the two organic modifiers and the sum of their concentrations changes simultaneously during gradient elution (see Fig. 2b).

This paper will deal exclusively with ternary solvent-strength gradient chromatography (where the ratio of the two organic modifiers is kept constant, see Fig. 2a); the effect of the sum of the two organic concentrations on the analyte retention is principally the same as in binary gradient chromatography. Therefore, a relationship similar to that employed in binary gradient chromatography can be modelled to describe the retention behaviour in ternary solvent-gradient chromatography [45].

Jandera [49,50] have previously reported a rational approach to the optimization of ternary solvent-strength gradient chromatography, however, to date there have been very few applications of its use. The strategy involved the selection of the appropriate ratio of the two organic modifiers in the ternary mobile phase composition followed by a gradient time/slope/shape optimization. Despite this elegant solution to the problem of "what two organic modifiers should be selected?" and "what will be their optimum ratio?" many workers still revert to a laborious trial and error solution to these problems.

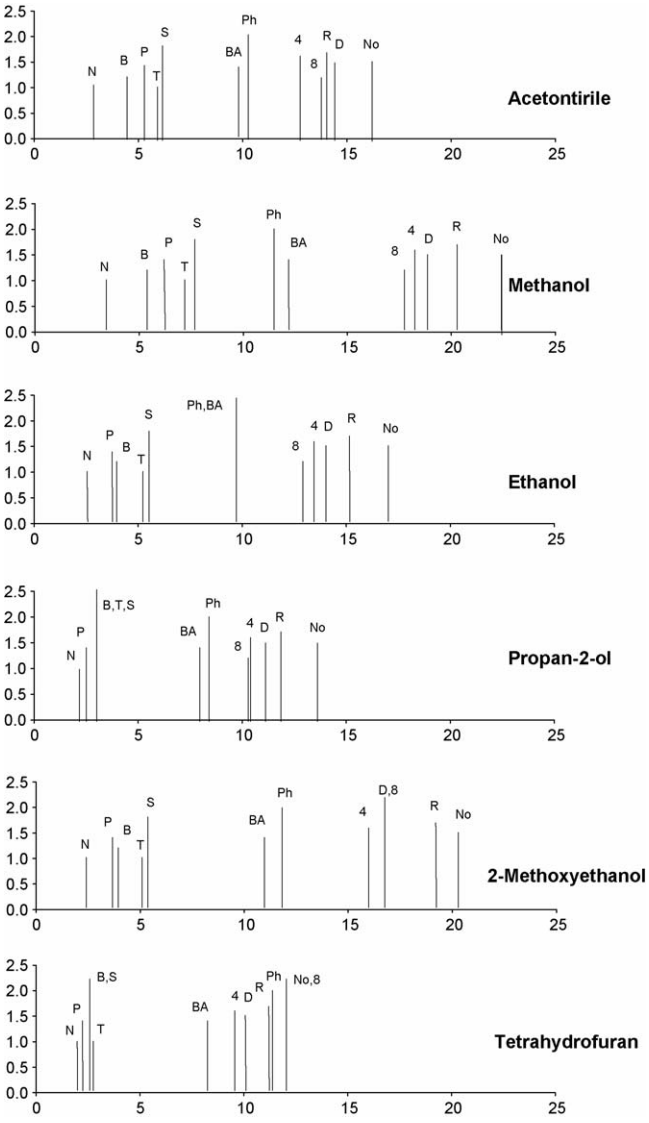

Fig. 1. The effect of organic modifier in binary gradient elution chromatography on the separation of 10 basic analytes and two neutral components. Hypersil GOLD C18 150 mm × 3 mm, 5 μm, 60 °C, 0.43 mL/min, t_G = 20 min, 5 min hold, gradient range 3.3–65% organic, 10 mM KH_2PO_4 pH 2.7 buffer. Peak identification, N, nicotine; B, benzylamine; P, procainamide; T, AR-D080301; S, salbutamol; BA, benzylalcohol; Ph, phenol; 4, AR12495; 8, AR-C68397; R, AR-R12924; D, diphenhydramine; No, nortripyline. For analyte structures see Ref. [48].

This paper seeks to evaluate the use of the "Jandera" approach to the optimization of ternary solvent-strength gradient chromatography using a commercially available chromatography modelling software package to rapidly and accurately predict and optimize the separation of an eight-component mixture using the increasingly popular approach of employing a short column (30 mm × 3 mm) operated at elevated temperature (60 °C) and high linear flow velocities (2 mL/min). The paper will discuss the applicability of simulation software to accurately model the retention, peak width behaviour and resolution using a two-dimensional model of % MeOH in MeCN versus gradient time. Once the appropriate ratio of MeOH in MeCN had been selected the model was used to optimize the duration,