Journal of Chromatography A, 1427 (2016) 90–95

Contents lists available at ScienceDirect

Journal of Chromatography A

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

# Switching solvent and enhancing analyte concentrations in small effluent fractions using in-column focusing☆

H.C. van de Ven[a,b,*], A.F.G. Gargano[a,b], Sj. van der Wal[a], P.J. Schoenmakers[a]

[a] University of Amsterdam, Faculty of Science, Science Park 904, 1098 XH Amsterdam, The Netherlands
[b] TA-COAST, Science Park 904, Amsterdam, The Netherlands

ARTICLE INFO

*Article history:*
Received 7 September 2015
Received in revised form
23 November 2015
Accepted 24 November 2015
Available online 30 November 2015

*Keywords:*
Modulation
Peak compression
Solvent switch
Temperature

ABSTRACT

A novel approach to achieve solvent switching and focusing of sub-column-volume analyte fractions in liquid chromatography is presented. By altering the temperature between loading and elution in back-flush mode, solvent transfer of analytes and focusing occurs, provided that the analytes exhibit temperature dependent retention on a given trap column. When retention on the trap decreases with increasing temperature, which is almost always the case, the loading of the trap-column takes place at a higher temperature than the elution. This principle is demonstrated using three small aromatic molecules (toluene, p-xylene and benzophenone) on a capillary monolithic column. On this column, the analytes show a traditional van't Hoff dependence on temperature with enthalpy effects of, −15, −16 and −18 kJ mol$^{-1}$, respectively, for a mobile phase of 25% acetonitrile in water. The column was loaded at 110 °C, cooled in an ice bath and eluted in back-flush mode at 0 °C. When operated in this way, the analytes are transferred from the loading solvent to the elution solvent, achieving solvent switching. Substantial focusing can also be obtained if the desorption solvent is stronger than the loading solvent.


## 1. Introduction

Comprehensive two-dimensional liquid chromatography (LC × LC) is the separation approach of choice for detailed analysis of complex, non-volatile mixtures. This is because LC × LC provides higher separation power (*i.e.* peak capacities) than one-dimensional LC (1D-LC) within a reasonable time [1] and because LC × LC provides additional selectivity. When the sample dimensions match the separation dimensions [2] LC × LC also yields structured, readily interpretable chromatograms [3]. A standard interface between the first ($^1$D) and second ($^2$D) separation dimensions consists of a switching valve equipped with two collection loops. Both dimensions are run continuously, as the two loops are alternately switched between collecting a $^1$D fraction and injecting it into the $^2$D [4,5]. Following common nomenclature from comprehensive two-dimensional gas chromatography (GC × GC) [6,7] this process is called modulation. In LC × LC operational drawbacks that can be attributed to the collecting interface often hamper full realization of the method's resolving power. Factors commonly mentioned in literature as contributors to modulation-induced decrease in efficiency of LC × LC analyses are (1) asymmetry of collection loops [5], (2) lengthy modulation cycles [8], (3) viscosity mismatch [9,10], (4) solvent immiscibility or incompatibility [11], (5) $^1$D eluent being too weak or too strong an injection solvent in $^2$D and (6) large $^2$D injection volumes [12–14]. Two different ("asymmetrical") collection loops can cause significant differences in consecutive (odd and even) $^2$D runs [5]. Different loops may also lead to differences in band broadening and peak shapes. A poorly connected or partially blocked loop may lead to peak distortion in the odd or even series of chromatograms. Long modulation cycles can compromise the separation obtained in the first dimension due to under-sampling and can result in relatively large $^2$D injection volumes, which may add significantly to the second-dimension band broadening [15,16]. Incompatibility of the $^1$D effluent and the $^2$D mobile phase can give rise to several problems. Viscosity mismatch of eluents can cause viscous fingering, which may distort the solute band profiles [17]. The $^1$D elution solvent can be a strong injection solvent for the $^2$D, which can cause fronting, tailing, band broadening and other peak distortions [18]. Peak splitting [13] and breakthrough of analyte peaks [12] have been reported as dramatic consequences of solvent incompatibility. Finally, the first-dimension solvent may be incompatible with the detection method envisaged after the $^2$D separation [15]. Overall, these

☆ Selected paper from 42nd International Symposium on High Performance Liquid Phase Separations and Related Techniques, 21–25 June 2015, Geneva, Switzerland.

* Corresponding author at: University of Amsterdam, Faculty of Science, Science Park 904, 1098 XH Amsterdam, The Netherlands.
*E-mail address:* rikkid6@gmail.com (H.C. van de Ven).

http://dx.doi.org/10.1016/j.chroma.2015.11.082
0021-9673/

effects combine to diminish the potential of LC × LC analysis [15]. To combat these disadvantages and achieve the full resolution potential of two orthogonal separation mechanisms, an ideal modulation interface should realize a change of solvent and focus the $^{1}$D elution band, while re-injecting in a relative short time without any discrimination between the sample components [19].

Modulation in comprehensive two-dimensional gas chromatography (GC × GC) has been studied in depth and is routinely employed in a (near-) universal and robust way [20]. In GC × GC the dominant modulation principle is based on effects of temperature on retention, trapping the compounds from the $^{1}$D by rapid local cooling and re-injecting into the $^{2}$D by rapid heating. Analogously, in LC × LC, modulation based on thermal effects on retention has not yet been demonstrated. However, temperature effects have been used for trap and release purposes in LC [21–25]. In LC × LC diluting make-up flows between separation dimensions are more commonly employed to mitigate the aforementioned drawbacks at the expense of higher complexity and possibly reduced sensitivity [26,27]. The most-promising LC × LC modulation interface consists of a trapping column between the dimensions to trap the analytes, focus them and change solvents [28]. The use of such a packed-loop interface requires loading of multiple trap-column volumes (possibly resulting in loss of separation achieved by the first dimension), expert know-how, time-consuming optimization, high retention factors and miniaturization [29–31]. Packed-loops have, however, proven their value in LC × LC modulation [32–39]. Other modulation principles, such as eluent-evaporation approaches, have been explored, but these have found little acceptance, due to limited applicability, high complexity or lengthy time-cycles [40,41].

To best carry out solvent switching and focusing desired for LC × LC modulation, a miniaturized packed loop interface should be operated in back-flush elution mode. Back-flush elution has been shown to have benefits over the more traditional forward flush elution mode both in cycle time and focusing [30,31]. In the context of LC × LC modulation, miniaturized packed loops require high retention factors if multiple column volumes of $^{1}$D effluent are to be loaded. This may lead to effective concentration and solvent switching, but modulation times may be longer, low-retained analytes may be lost and contaminants from the eluent will be focussed together with the analytes. The system may also be difficult to generalize and optimize in case the effluent that needs to be modulated arises from a (first-dimension) gradient-elution separation. Loading only a single trap-column volume or less cannot result in analyte solvent transfer or focusing when performed in isothermal back-flush mode. The analytes would elute in the loading solvent and never transcend the loading-elution solvent boundary. A chromatographic column (or trap) is completely filled (*i.e.* one trap-column volume) with a solution containing (parts of) one or more analyte bands (*e.g.* the effluent from a previous separation stage). This is illustrated schematically in Fig. 1A where isothermal loading (a–d) and back-flush elution (d–g) take place without any solvent switching or focusing. Therefore, there is a need to increase the retention of analytes whilst on column, still in the loading solvent, if solvent change of single or sub-trap-column volume is desired. An effective way to achieve this is by varying the temperature. The current trend of using temperature to increase trapping efficiency on trap-columns relies on cold-traps that are heated up at the time of elution. For sub-column volumes, however, this approach fails. In

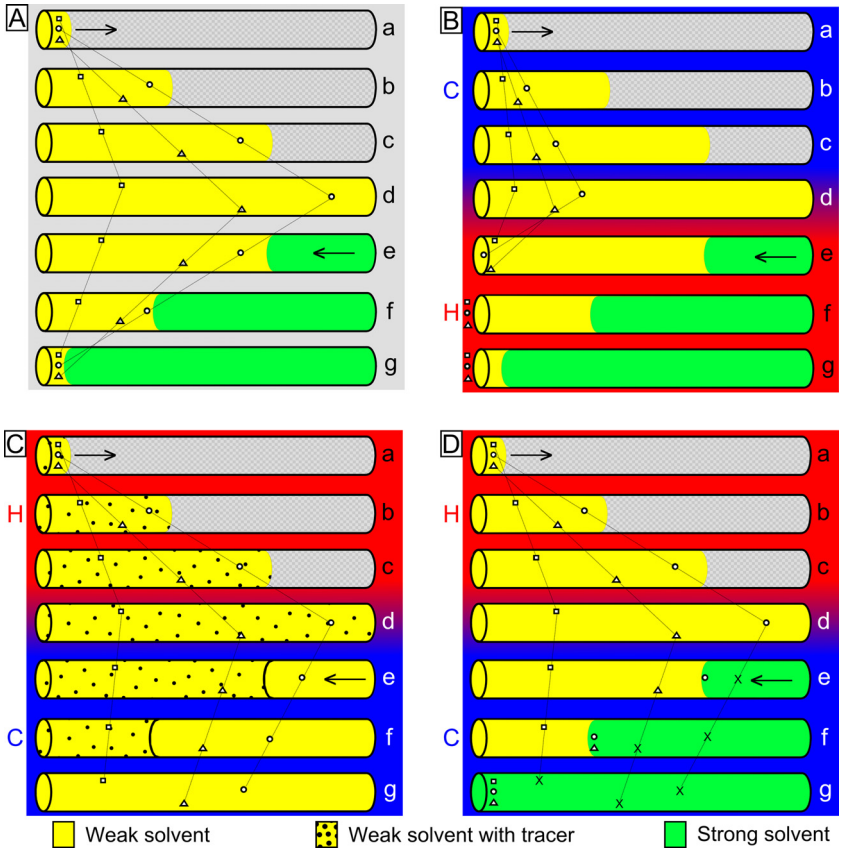


**Fig. 1.** Loading and elution schemes. Schematic representation of loading (a–d) and back-flush elution (d–g) of an analyte plug composed of three analytes (□, ◯, △) with different retention. The analyte plug is injected (a) and loaded with one trap volume of mobile phase (the loading solvent). Fig. A illustrates a constant-temperature process. In figures B, C and D a temperature change takes place (d). A high column temperature is illustrated with a red backdrop (H) and a low temperature is shown in blue (C). In D, X marks the imaginary position of analytes, had they not been swept up. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)