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Improving peak shapes with counter gradients in two-dimensional high performance liquid chromatography

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ABSTRACT

To achieve the greatest peak capacity in two-dimensional high performance liquid chromatography (2D-HPLC) a gradient should be operated in both separation dimensions. However, it is known that when an injection solvent that is stronger than the initial mobile phase composition is deleterious to peak performance, thus causing problems when cutting a portion from one gradient into another. This was overcome when coupling hydrophilic interaction with reversed phase chromatography by introducing a counter gradient that changed the solvent strength of the second dimension injection. It was found that an injection solvent composition of 20% acetonitrile in water gave acceptable results in one-dimensional simulations with an initial composition of 5% acetonitrile. When this was transferred to a 2D-HPLC separation of standards it was found that a marked improvement in peak shape was gained for the moderately retained analytes (phenol and dimethyl phthalate), some improvement for the weakly retained caffeine and very little change for the strongly retained *n*-propylbenzene and anthracene which already displayed good chromatographic profiles. This effect was transferred when applied to a 2D-HPLC separation of a coffee extract where the indecipherable retention profile was transformed to a successful application multidimensional chromatography with peaks occupying 71% of the separation space according to the geometric approach to factor analysis.

1. Introduction

Two-dimensional high performance liquid chromatography (2D-HPLC) separates samples that contain many compounds [1]. However, to achieve the maximum peak capacity each separation dimension must have unique retention mechanisms so the entire separation space is utilised, otherwise the benefit of 2D-HPLC is redundant [1,2]. Coupling normal phase (NP-) and reversed phase (RP-)HPLC will most likely provide the most divergent retention mechanisms, however, immiscibility between the mobile phases in each dimension presents a substantial barrier to this coupling [3]. Wei et al. presented strategies to overcome these solvent problems, however, many of them are not practical as they require specialist equipment and solvents, long treatment times and loss of resolution [4]. A solution to this is to operate both dimensions in the same mode of chromatography, e.g. RP-HPLC × RP-HPLC, though separations where the injection plug is miscible with the mobile phase stream are still not immune to solvent mismatch problems [5–9]. The effect of injection volume, injection mass and injection solvent strength on the shape of chromatographic peaks has been reported by several authors [10–12]. It has been found that injection volume and injection solvent strength both negatively influence the band shape; injection volumes of 1.25 μL can be used without excessive deleterious effects on separation performance. For larger injection volumes peak shape can be enhanced by taking advantage of on-column focusing that requires the solvent environment within the injection plug be weaker than the initial mobile phase composition, which allows the solutes to be trapped in a tight band at the stationary phase head [13]. For on-column focusing to be effective in 2D-HPLC the strongest solvent that is eluted from the first dimension gradient must be weaker than the weakest solvent in the second dimension. In RP × RP separations this is unlikely, and impossible when coupling hydrophilic interaction chromatography (HILIC) with RP-HPLC.

A 2D-HPLC separation of peptides using a HILIC stationary phase coupled with RP-HPLC has been shown by Gilar and co-workers [3] to achieve a high degree of orthogonality; separations in the HILIC mode have similar retention mechanisms to those of NP-HPLC but operate within a reversed phase solvent system. Ruta et al. [14] recently reported on the influence the strength of the solute plug had on the retention profile of polar compounds in HILIC mode. It was found that a maximum of 10% water could be used in the solute mixture before peak shape was significantly altered, and that

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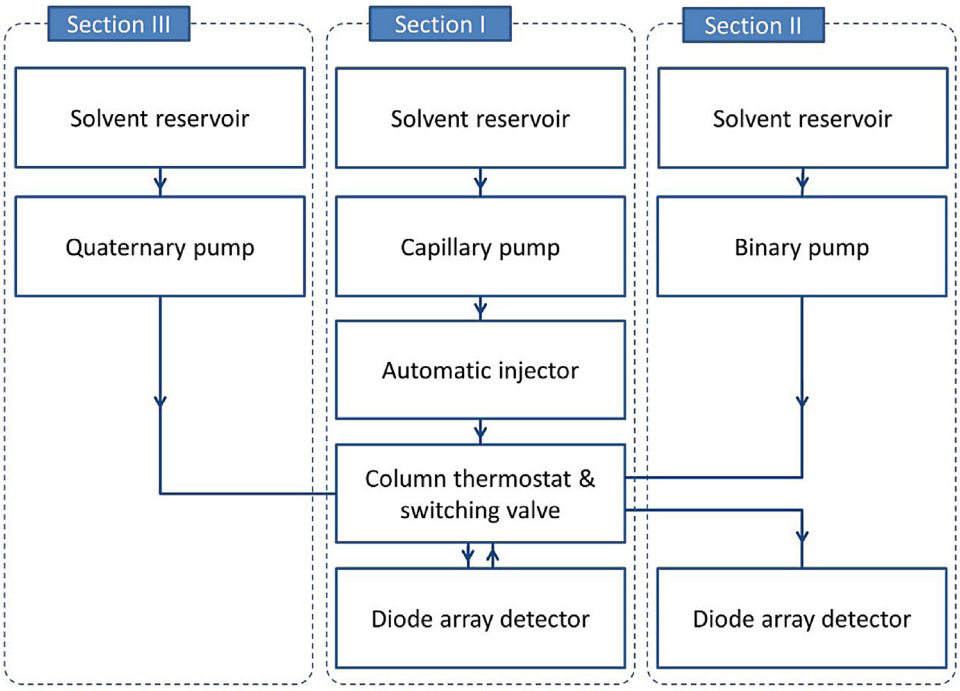

Fig. 1. Schematic of the 2D-HPLC system used in this work with the introduction of a third pump to control the counter gradient. Section I represents the one-dimensional analysis and the first dimension in 2D-HPLC, the second dimension by Section II and the counter gradient by Section III. For the 2D-HPLC separations both columns were housed in the column thermostat.

acetonitrile (ACN) or ACN with up to 50% isopropyl alcohol be used as the organic component.

Unlike traditional NP-HPLC, the mobile phase used in a HILIC separation, generally aqueous acetonitrile, is compatible with RP-HPLC and the elution order is generally reversed [15]. However, with such extreme differences in the retention mechanism incompatibilities between the two dimensions are inevitable [10]. For example, when a strong solvent plug is injected into a weak solvent mobile phase stream the solute will not immediately enter into the bulk phase, instead, the solute will *slowly* transfer from mobile to stationary phase resulting in broad peaks that was observed by Jandera et al. [16] who performed a HILIC × RP separation of ethylene oxide-propylene oxide (co)oligomers. They found that on a HILIC column when their samples that were dissolved in equal amounts of ACN and water they did not fully enter into the stationary phase that resulted in artefact peaks. Horváth and co-workers [17] found that small changes in the solvent strength of the solute plug eluting from a HILIC dimension onto a C18 column also had broadening effects on peaks to a point where apparently multiple peaks were observed.

This work aims to improve the peak shapes of 2D-HPLC separations when transferring a larger volume of eluent to the second dimension after dilution with a counter gradient that was introduced by a third HPLC pump. It was predicted that when the solvent strength of the fraction (i.e. the second dimension injection solvent) is decreased to a level close to that of the initial second dimension the negative effects causing poor peak shapes will be overcome resulting in an efficient separation with peaks conforming to a Gaussian profile.

2. Experimental

2.1. Chemicals and reagents

Chemical standards thiourea, caffeine, phenol, dimethyl phthalate, *n*-propylbenzene, anthracene were obtained from Sigma-Aldrich Pty Ltd (Castle Hill, NSW, Australia), HPLC grade acetonitrile (Ajax Finechem Pty. Ltd., Taren Point, NSW, Australia), and HPLC grade water was prepared by filtering deionised water (Continental Water Systems, Victoria, Australia) through a 0.45 μm filter (Sigma-Aldrich Pty Ltd). Trifluroacetic acid (Sigma-Aldrich Pty Ltd) was added to all mobile phases prior to analysis at a concentration of 0.1% v/v. A stock solution of the standard mixture was prepared by dissolving 10 mg (thiourea, caffeine, phenol, and anthracene) or 10 μL (dimethyl phthalate and *n*-propylbenzene) in 100 mL of ACN. This stock solution was then diluted with an appropriate amount of deionised water to prepare solutions of known injection solvent compositions. The 2D-HPLC separation of a real extract was done with 5 g Ristretto brand Nespresso coffee (Nespresso, North Sydney, NSW, Australia) extracted with 30 mL hot water by a Delonghi Nespresso Lattissima coffee machine (model number EN520W). The extract was filtered with a 0.45 μm syringe filter and made to a concentration of 95% acetonitrile prior to analysis.

2.2. Instrumentation

An Agilent 1260 Infinity chromatograph (Agilent Technologies, Mulgrave, Vic, Australia) was used for all separations incorporating three pumps (quaternary pump, capillary pump, and 1290 Infinity binary pump) with on-line degassers, an auto-sampler, a 1290 Infinity thermostated column compartment incorporating a 8 port 2 position switching valve (2D-LC valve kit), and a diode array UV detector in each dimension. The system was configured according to Fig. 1 so that:

- Single dimension separations were completed with the modules located within Section I,
- Multidimensional separations with Section I in the first dimension and Section II in the second, and
- The counter gradient was introduced by the pump located in Section III.