Talanta 82 (2010) 1349–1357

Contents lists available at ScienceDirect

Talanta

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

The assessment of π–π selective stationary phases for two-dimensional HPLC analysis of foods: Application to the analysis of coffee

Mariam Mnatsakanyan[a], Paul G. Stevenson[a,c], David Shock[a], Xavier A. Conlan[d], Tiffany A. Goodie[e], Kylie N. Spencer[e], Neil W. Barnett[e], Paul S. Francis[d,e], R. Andrew Shalliker[a,b,*]

[a] Australian Centre for Research on Separation Science (ACROSS), University of Western Sydney Node, School of Natural Sciences, Parramatta, NSW, Australia
[b] Nanoscale Organisation and Dynamics Group, University of Western Sydney, Parramatta, NSW, Australia
[c] Centre for Complementary Medicine Research, University of Western Sydney, Parramatta, NSW, Australia
[d] Institute for Technology Research and Innovation, Deakin University, Geelong, Victoria 3217, Australia
[e] School of Life and Environmental Sciences, Deakin University, Geelong, Victoria 3217, Australia

ARTICLE INFO

Article history:
Received 20 May 2010
Received in revised form 28 June 2010
Accepted 28 June 2010
Available online 24 July 2010

Keywords:
Two-dimensional HPLC
Natural Products
Selectivity
Coffee

ABSTRACT

Differences between alkyl, dipole–dipole, hydrogen bonding, and π–π selective surfaces represented by non-resonance and resonance π-stationary phases have been assessed for the separation of 'Ristretto' café espresso by employing 2DHPLC techniques with C18 phase selectivity detection. Geometric approach to factor analysis (GAFA) was used to measure the detected peaks (N), spreading angle (β), *correlation*, practical peak capacity (n_p) and percentage usage of the separations space, as an assessment of selectivity differences between regional quadrants of the two-dimensional separation plane. Although all tested systems were correlated to some degree to the C18 dimension, regional measurement of separation divergence revealed that performance of specific systems was better for certain sample components. The results illustrate that because of the complexity of the 'real' sample obtaining a truly orthogonal two-dimensional system for complex samples of natural origin may be practically impossible.

1. Introduction

Due to the complexity of natural products, designing methods for analysis and characterization can be a formidable task. Natural products are known for the range and novelty of their chemical diversity, often containing thousands of components, many with physical and chemical similarities, often present as low abundant species. It is because of this chemical complexity that two-dimensional high performance liquid chromatography (2DHPLC) has been attracting more interest in the field of natural product chemistry. In some cases 2DHPLC has shown potential for obtaining good separation of highly complex samples [1], including natural products [1–5], although, many two-dimensional separations employed in the field have not undergone a rigorous test of orthogonality, and few report the practical peak capacity of the system.

The principal advantage of two-dimensional separation is that it provides, relative to one dimensional, a greatly enhanced peak capacity [6], provided each of the dimensions offer divergent retention behaviour. In principle, the maximal theoretical peak capacity in 2DHPLC that employs orthogonal dimensions is equal to the product of the peak capacities of each respective one-dimensional separation, but the overall peak capacity reduces as a function of correlation between the systems [7–9]. In order to fully utilise the power of a two-dimensional separation, design of the system should be undertaken with due consideration to the nature of the sample [6]. That is, each of the dimensions within the separation system should ideally be selective towards a specific sample attribute. This ensures ordered displacement of sample components across the 2D space. In practice, a system comprising '*n*' dimensions for '*n*' sample attributes is impossible since multi-dimensional separations are largely limited to two-dimensions. Therefore, fully non-correlated selectivity for each dimension in a two-dimensional system is rarely found [10], especially for complex samples of natural origin. Thus it is important that in order to maximise system peak capacity, the operating conditions in the two-dimensional system should be carefully measured and subsequently optimised. Such studies require firstly a degree of understanding with respect to the behaviour of the solutes in each dimension. That is, selectivity studies must be undertaken with respect to both the stationary phase and also the mobile phase. These studies must be undertaken practically, since it is largely impossible to predict selectivity behaviour. Furthermore, due care

* Corresponding author at: Australian Centre for Research on Separation Science (ACROSS), University of Western Sydney Node, School of Natural Sciences, Victoria Road, North Parramatta, Parramatta, NSW 2150, Australia. Tel.: +61 2 9685 9951; fax: +61 2 9685 9915.
E-mail address: r.shalliker@uws.edu.au (R.A. Shalliker).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.06.065

must be paid to the types of compounds used to determine the 'selectivity' of the phase, as it must match that of the sample for an accurate measure of performance [11,12]. Thus, the sample itself should be used to test the selectivity changes, and herein lies the problem. Samples of natural origin are largely too complex for selectivity testing to be undertaken in a unidimensional sense, unless hyphenated methods of detection are employed that can track specific sample components as a function of the selectivity change. In complex samples, this tracking process is tedious, and to be effective, a large number of representative sample components must be tested. If the number is too small, or their distribution does not reflect that of the actual sample separation, then their reliability as a measure of selectivity performance may be questionable [11].

The most reliable approach to measuring selectivity differences is to employ the sample itself, as then selectivity changes are truly reflected in the separation of the natural product. However, for complex samples utilisation of the natural product itself during the design phase of separation is far from straight forward, as there are usually multitudes of components that co-elute, and changes in selectivity are likely to go unnoticed as one complex chromatogram looks very similar to another. Carr and co-workers [13], however, introduced the concept that a 2DHPLC system could in fact be utilised as a separation process as the first dimension, and then the second dimension serve as a selectivity detector. In that way, changes that are made to the first dimension can be assessed in the retention distribution in the second dimension. The relative change in selectivity of the different first dimensions can therefore be gauged. Selectivity in RP chromatography has been extensively studied. Cyano and phenyl phases showed little selectivity advantage over C18 columns in RP separations when initially investigated [14]. Further studies proved that there is an alternative selectivity for cyano and phenyl phases and theories on the interaction mechanisms have been put forth [15–17]. More recent studies have investigated the different selectivity observed between a phenyl phase using π–π interaction and a cyano phase using non-π-resonance or a dipole–dipole interaction [18]. Even the configuration of the phenyl phase induces changes in selectivity [19]. Fluoro-substituted columns have also shown alternative selectivity to alkyl and phenyl phases [20]. In the majority of these selectivity studies, a finite number of test analytes are used to characterize the selectivity. The current study will investigate selectivity with respect to the behaviour of a complex sample derived from natural origin containing a multitude of components. In this study the focus is on general selectivity differences rather than specific functional differences. To illustrate this process we assessed the separation of 'Ristretto' espresso on a number of π-selective stationary phases, employing 2DHPLC techniques with selectivity detection, with the view of maximising the separation power for extended studies on the analysis of coffee.

2. Experimental

2.1. Chemicals and samples

All solvents were of HPLC grade. Acetonitrile, methanol, tetrahydrofuran were from Lomb Scientific (Tarren Point, NSW, Australia). Milli-Q water (18.2 MΩ) was obtained in-house and used through all the experiments. Espresso 'Ristretto' coffee was obtained from the local market (Nespresso Australia, North Sydney, NSW, Australia). The coffee brews were home made using an 'espresso' coffee-making machine (Nespresso DēLonghi, Nestle Nespresso, SA, Australia). Coffee brews for analysis were prepared using a 30 mL shot and were diluted 1/4 in water prior to analysis. All samples prior to injection into the LC system were filtered through 0.45-μm pore filter.

2.2. Chromatography columns

All chromatography columns were supplied by Phenomenex (Lane Cove, NSW, Australia). Five different functionalities were tested: Luna 100 Å Cyano (CN), SphereClone ODS, Luna Phenylhexyl (PH), Synergi Hydro-RP 80 Å (C18 with polar end-capping) and a Luna pentafluorophenyl (PFP). All column formats were 150 mm × 4.6 mm, packed with 5 μm particles.

2.3. Chromatographic instrumentation

All chromatographic experiments were conducted using a Waters 600E Multi Solvent Delivery LC System equipped with Waters 717 plus auto injector, Waters 600E pumps, Waters 2487 series UV/VIS detectors and Waters 600E system controller. The chromatographic interface between the first and second dimensions consisted of two electronically controlled, two-position six-port switching valves fitted with micro-electric valve actuators.

2.4. Chromatographic separation

2.4.1. First-dimensional separations

First-dimensional separations were performed on either of the CN, C18 with polar end-capping, PH or PFP columns. Selectivity studies were undertaken in aqueous solvents of methanol, acetonitrile and THF, however, in this work we report only the results derived from the aqueous-methanol system as it yielded the greatest separation performance. Since the second dimension was to serve as the 'detector' (assessing the changes that result from the first dimension), the same mobile phase conditions that were employed in the first dimension were also used in the second dimension. All separations, in both dimensions, were operated under linear gradient conditions, starting with 100% aqueous mobile phase and finishing with 100% methanol mobile phase, at a gradient rate of 10% per min. All flow rates were 1 mL/min and injection volumes were 100 μL into the first dimension. Mobile phases were unbuffered for all experiments, despite the fact that coffee is known to contain a high number of carboxylic acids. Initial experiments were undertaken using acetate buffered mobile phases; however, the separation performance was essentially the same, perhaps even reduced (results not shown). Non-buffered mobile phases enhanced our ability to undertake mass spectral analysis in the negative ion mode and also reduced one further aspect of solvent mismatch between the respective first and second dimensions: That of pH shock in the second dimension.

2.4.2. Second-dimensional separations

The second dimension was conducted on the SphereClone C18 column, using gradient elution with an initial mobile phase of 100% water, running to a final mobile phase of 100% methanol, at a gradient rate of 10% min. The flow rate was 1 mL/min. The transfer volume from the first dimension to the second dimension was 200 μL. UV absorbance detection was set at 280 nm.

2.4.3. Operation

A 'comprehensive' or more precisely an 'incremental heart cutting' approach was used to express the two-dimensional peak displacement, by which a 200 μL heart-cut section was transferred to the second dimension, with subsequent second dimension