Talanta 82 (2010) 1358–1363

Contents lists available at ScienceDirect

Talanta

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

The analysis of café espresso using two-dimensional reversed phase–reversed phase high performance liquid chromatography with UV-absorbance and chemiluminescence detection

Mariam Mnatsakanyan[a], Paul G. Stevenson[a,b], Xavier A. Conlan[c], Paul S. Francis[c,d], Tiffany A. Goodie[d], Geoffrey P. McDermott[d], Neil W. Barnett[d], R. Andrew Shalliker[a,*]

[a] Australian Centre for Research on Separation Science (ACROSS), Nanoscale Organisation and Dynamics Group, and the School of Natural Sciences, University of Western Sydney, Parramatta, NSW 2050, Australia
[b] Centre for Complementary Medicine Research, University of Western Sydney, Campbelltown, NSW, Australia
[c] Institute for Technology Research and Innovation, Deakin University, Geelong, Victoria 3217, Australia
[d] 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
Antioxidants
Chemiluminescence detection
Coffee

ABSTRACT

In this study, an activity based screening technique combining two-dimensional liquid chromatography (2DHPLC) with UV-absorbance and chemiluminescence detection was applied to study "Ristretto", "Decaffeinatto" and "Volluto" espresso coffees. This technique, which coupled the separation power of 2DHPLC with the sensitivity and selectivity of the chemiluminescence detection, offers great potential for screening complex samples for antioxidant compounds. Detailed information regarding the complexity of the sample, and the variation between these three coffees could be obtained using this multidimensional-hyphenated method of analysis.

1. Introduction

Antioxidants have received growing interest as active agents in various natural products offering benefits to human health [1,2]. Sources of natural antioxidants range from marine sponges [3] to microbes [4] and plants [5] and they are considered by many to be revolutionising foods, medicines, and cosmetics [6–9] serving as either a substitute for synthetic compounds or as active ingredients for health fortifying purposes [10]. Thus finding new "unconventional sources" of functional molecules, and in particular antioxidants, could lead to new and important discoveries. Tulp et al. suggested that foods and beverages, primarily not known for their medicinal properties, could potentially be the next valuable source of natural compounds that require the attention of the scientific community [11].

Roasted and green coffee beans have extremely complex chemical compositions, containing large numbers of components with a wide range of properties and sizes; some of which (e.g. chlorogenic acids and related phenolic compounds, and Maillard reaction products (melanoidins)) [12–14] are strong antioxidants with beneficial physiological properties for human health [15]. The chemical profile becomes even more complex based on the geographical origin, roasting degree, and the type of coffee beans [16,17]. Coffee is an important source of natural antioxidants, because it is consumed worldwide, throughout almost every culture. Accordingly, analytical techniques that provide reliable separation and analysis of antioxidants from the complex coffee matrix could be of great importance.

Recently, much effort has been directed towards accelerating the screening and evaluation of antioxidant content in foods and plants. So far, modification of traditional batch type antioxidant assays into so-called high resolution screening techniques that combine detection with separation are showing the greatest promise to rapidly discover key antioxidant compounds [18–21]. These on-line screening assays, which substantially reduce discovery time and cost [22], are important hyphenated methods of analysis, but once the peak capacity of the separation process is exceeded, the ability of the hyphenated detector to provide unequivocal information about specific compounds decreases as the complexity of the sample increases. Hence, separation (according to information, i.e. the hyphenated mode of detection) must be transposed to the physical separation, that is, chromatographically. In recent times there has been a drive towards more powerful separations that incorporate multiple selectivity steps (i.e.

* Corresponding author. 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.2010.06.066

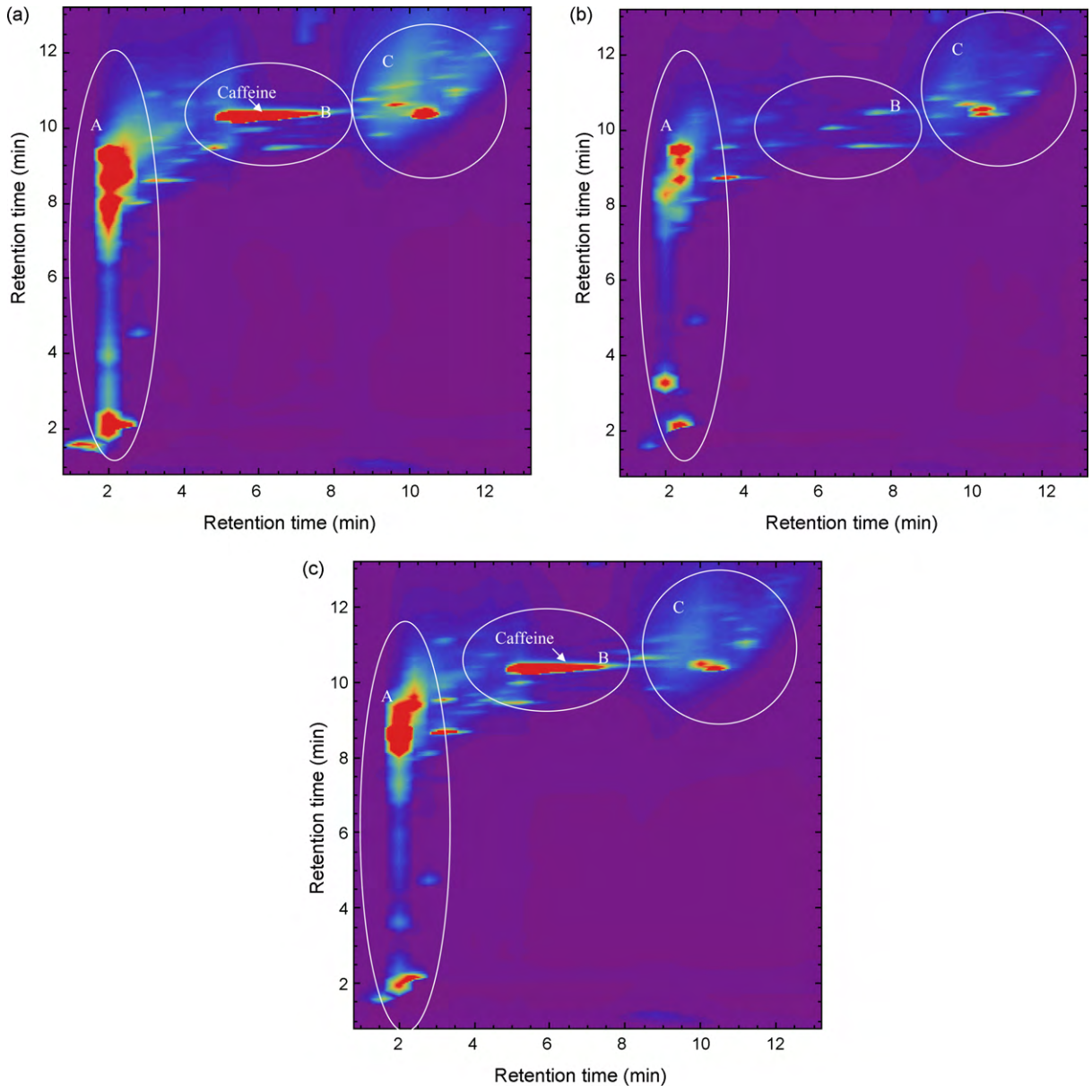

Fig. 1. Two-dimensional separations of (a) Ristretto, (b) Decaffeinato and (c) Volluto café espresso. First dimension Cyano and second dimension C18 phases. In both dimensions mobile phase was aqueous methanol, going from 100% water to 100% methanol. All conditions identical for each phase system.

multidimensional HPLC, more often referred to as two-dimensional HPLC (2DHPLC)) [23,24].

The combination of the powerful separation process with that of antioxidant detection should enable the rapid identification and subsequent targeted extraction of bioactive compounds from complex sample matrices [25]. Here we demonstrate the application of 2DHPLC using chemiluminescence detection in the search for antioxidants in espresso coffees. A comparative study was undertaken that tested three types of café espresso coffees, which included a decaffeinated variety. The two-dimensional chromatographic system consisted of a cyano stationary phase and a C18 stationary phase, both employing water/methanol gradient elution mobile phases. The chemiluminescence detection involved a post-column reaction with acidic potassium permanganate. This reagent provides highly sensitive detection of polyphenols and various other readily oxidisable compounds [26,27] and has previously been utilised to establish the total antioxidant capacity of teas and fruit juices (using flow-injection analysis methodology) [28] and to explore the relative reactivity of individual sample components after chromatographic separation [25,28].

2. Experimental

2.1. Chemicals and reagents

All mobile phases were prepared from HPLC grade solvents and were purchased from Lomb Scientific (Tarren Point, NSW, Australia). Sodium hexametaphosphate (crystals, +80 mesh) was purchased from Merck (Kilsyth, Victoria, Australia). Potassium permanganate was purchased from Chem-Supply (Gillman, SA, Australia). Milli-Q water (18.2 MΩ) was obtained in-house and filtered through a 0.2 μm filter.

2.2. Reagent preparation

The chemiluminescence reagent was prepared by dissolution of potassium permanganate (5×10^{-4} M) in a 1% (m/v) sodium hex-