Journal of Chromatography A, 1428 (2016) 126–133

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Comparison of nano and conventional liquid chromatographic methods for the separation of (+)-catechin-ethyl-malvidin-3-glucoside diastereoisomers

Lukáš Kučera[a,b], Salvatore Fanali[b], Zeineb Aturki[b], Tomáš Pospíšil[c], Petr Bednář[a,*]

[a] Regional Centre of Advanced Technologies and Materials, Department of Analytical Chemistry, Faculty of Science, Palacký University, 17. listopadu 12, 779 00 Olomouc, Czech Republic
[b] Institute of Chemical Methodologies, Italian National Research Council, Area della Ricerca di Roma I, Via Salaria Km 29,300, Monterotondo, 00015 Rome, Italy
[c] Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Chemical Biology and Genetics, Faculty of Science, Palacký University, Šlechtitelů 27, 78371 Olomouc, Czech Republic

ARTICLE INFO

Article history:
Received 4 June 2015
Received in revised form
11 September 2015
Accepted 21 September 2015
Available online 26 September 2015

Keywords:
Nano-liquid chromatography
Capillary column
High-performance liquid chromatography
Anthocyanins
Bridged dyes

ABSTRACT

Nano-liquid chromatography and conventional HPLC were used for the separation of diastereomers of (+)-catechin-ethyl-malvidin-3-glucoside. Those bridged anthocyanin dyes were obtained by reaction of (+)-catechin with malvidin-3-glucoside in the presence of acetaldehyde. Both diastereomers were isolated with semipreparative chromatography and their structures were confirmed by nuclear magnetic resonance and mass spectrometry. In-laboratory prepared capillary columns packed with fully porous particles Chromosphere C18, dp = 3 μm, core–shell particles Kinetex C18, dp = 2.6 μm (100 μm i.d.) and monolithic column Chromolith CapRod (100 μm i.d.) were used for the separation of (+)-catechin, malvidin-3-glucoside and both diastereomers. Chromosphere C18 stationary phase provided the best chromatographic performance. Mobile phase containing water:acetonitrile (80:20) acidified with trifluoroacetic acid (0.1%, v/v/v) was used in an isocratic elution mode with a flow rate of 360 nL min^{-1}. Separation of studied compounds was achieved in less than 7 min under optimized conditions. The nano-liquid chromatographic method and a conventional HPLC one using the same fully porous particles (Chromosphere C18, 3 μm, 100 mm × 4.6 mm) were compared providing higher separation efficiency with the first analytical method and similar selectivity. A better peak symmetry and higher resolution of the studied diastereomers was achieved by conventional chromatography. Nevertheless, nano-liquid chromatography appeared to be useful for the separation of complex anthocyanin dyes and can be utilized for their analysis in plant and food micro-samples. The developed method was used for analysis of red wine grape pomace.

1. Introduction

Anthocyanins are water soluble natural dyes having a number of protection and regulatory functions in plant world. These compounds are found in relatively high concentrations in fruits (e.g. red grapes and various berries) as well as in foods produced from plants. Anthocyanins have many beneficial effects on human health [1,2]. Native anthocyanins show relatively low chemical stability influenced by many factors such as pH, temperature, their concentration in solution, presence of oxygen or enzymes, irradiation, etc. [3,4]. Anthocyanins can be stabilized by reaction with certain small molecules including aldehydes, ketones and organic acids resulting in more complex dyes (e.g. pyranoanthocyanins, compounds consisting of several covalently connected flavonoid units, etc.). Study of different spatial distribution of anthocyanins and related complex dyes in plant tissue and related plant food represents a challenging analytical task allowing elucidation of many functional processes in living plant as well as during storage of a food (effect of mechanical damage of tissue, microbial attack and many others). The most common methods for such purposes are based on direct surface scanning (imaging) by means of scanning microscopy methods and matrix assisted laser desorption ionization mass spectrometry [5,6]. On the other hand, since the plant and food material represents a complex mixture of chemically various compounds,

* Corresponding author.
E-mail address: petr.bednar@upol.cz (P. Bednář).

http://dx.doi.org/10.1016/j.chroma.2015.09.074
0021-9673/

chromatographic analysis of a scission of small part of tissue after extraction would be unavoidable in many cases.

The most common approach for the separation of anthocyanins combines HPLC coupled to photodiode-array (PDA) detection, where selective detection of anthocyanins is possible since they absorb in the visible range around 500–550 nm [7,8]. The hyphenation of HPLC with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) provides increased sensitivity and structural elucidation capabilities [9,10].

Miniaturization represents one of the main trends in modern analytical chemistry. Recently, great attention has been paid to the development of miniaturized versions of LC, like capillary-/nano-LC, that have become an important area of research, resulting attractive especially for industries, due to their reliability and reproducibility. The use of these methodologies provides numerous benefits, such as higher separation efficiency and resolution, lower sample and solvent consumption and in particular higher analysis speed, making these techniques environmentally friendly and cost-effective [11–13]. Capillary columns can be laboratory made by using small amounts of stationary phase making possible the use of expensive (e.g. chiral packing sorbents) and/or novel materials [11,14]. Furthermore, the small internal diameter of the columns leads to a decrease of sample dilution eluted from the column resulting in a signal to noise improvement [15]. Considering the low flow rate of miniaturized systems, they are particularly compatible with low flow ionization techniques like nanospray interfaces for efficient coupling with mass spectrometry [16]. A critical aspect related to nano-LC can be the low sample loading due to the small dimension of the capillary column which leads to loss of detection sensitivity. In order to increase the detection limit and to obtain benefit from the smaller dilution factors, different large-volume injection methods called on-column focusing procedures have been investigated [17]. Among them, the selection of the appropriate composition of the sample solvent in which a large sample volume dissolved in a solvent with a lower elution strength than the one present in the mobile phase is introduced in the column without compromising the chromatographic performance of the system [11,18] or a mobile phase composition programming [19]. Another approach is to use a trapping column technique, in which a pre concentration procedure as well as a clean up step of real samples can be performed [20]. NanoLC has a great applicability in proteomic [21,22]. As an example of successful application in plant research, a nanoLC-ESI-MS/MS analysis of proteins in excised spots from gel after two-dimensional electrophoretic purification and digestion of *Arabidopsis thaliana* leaves (i.e. analysis of small pieces of material) has been published [23].

The aim of the present work was to optimize a nano-liquid chromatographic method for the separation of catechin, malvidin-3-glucoside and pigments formed by their mutual condensation in the presence of acetaldehyde (following reaction described elsewhere [24]) and to compare it with a conventional chromatographic method. Pigments produced by linkage of anthocyanins with flavanols appear in significant content in different foods such as red wine vinegard [25] and red wine [26–28]. This study represents one of the initial steps in development of a comprehensive analytical platform for targeted monitoring of condensation of anthocyanins in micro-samples of plant material and related food.

2. Experimental

2.1. Chemicals

All used chemicals were of analytical reagent grade. Acetonitrile (ACN) and methanol (MeOH) were purchased from Carlo Erba (Milan, Italy) and VWR International (Radnor, PA, USA), acetaldehyde was from Merck Schuchardt (Hohenbrunn, Germany), glacial acetic acid (99.8% pure), formic acid (99% pure) and trifluoracetic acid (99% pure) were from Sigma–Aldrich (St. Louis, MO, USA) and Carlo Erba (Milan, Italy). Double distilled water was provided by Milli-Q water purification system (Millipore, Waters Milford, USA). Standards of (+)-catechin (Cat) and malvidin-3-glucoside (Mv3Glu) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of reaction mixture, chromatographic purification of reaction products and preparation of sample of red wine grape pomace extract

Reaction mixture was prepared by mixing of (+)-catechin and malvidin-3-glucoside (both 1 mmol) in 4 mL of water. Then 0.86 μL of formic acid, 2.24 μL of acetaldehyde and 1 mL of methanol were added and the reaction mixture was immediately well mixed. Reaction process and the yield of the product was controlled by chromatographic analysis using HPLC/DAD/ESI-MS system (see Section 2.3). The highest concentration of products were found after 8 days of reaction. Purification of reaction products was performed using HPLC system Smartline (Knauer, Germany) equipped with semi-preparative core–shell column Ascentis C18 (250 mm × 10 mm; 5 μm; shell layer 0.5 μm, Supelco, USA). Injection volume 1 mL was used for those experiments.

Red wine grape pomace was kindly provided by Department of Post-Harvest Technology of Horticultural Products, Faculty of Horticulture in Lednice, Mendel University in Brno. The crude material was lyophilized and stored in freezing box. 250 mg of lyophilized material was extracted using 1 mL of 0.1% formic acid in methanol for 10 min in ultrasonic bath at laboratory temperature. Then the formed mixture was centrifuged (1400 rpm for 5 min). 0.5 mL of the supernantant was separated and 8 times diluted with mobile phase (0.1% TFA in mixture 80:20 water:acetonitrile, v/v/v). This sample was spiked by mixture of studied compounds (final concentration of added analytes was roughly 10 mg L^{-1}).

2.3. Instrumentation

Nano-LC experiments were carried out using a laboratory made apparatus composed by SpectraSYSTEM P2000 conventional HPLC pump, a Spectra System UV1000 detector, all purchased from Thermo Separation Products (San Jose, CA, USA). A modified injector valve (Enantiosep GmbH, Münster, Germany) with a 50 μL loop and a nano-injector (100 nL) (VICI Valco, Houston, TX, USA) were used as reservoir for the mobile phase and for the introduction of the samples into the column, respectively. Mobile phase reservoir and nano-injector were connected each other via another T-piece using a fused silica capillary (50 μm × 11 cm). A split flow system was assembled to reduce the flow from μL/min to nL/min range. The pump was connected to a stainless steel tee (VICI Valco, Houston, TX, USA) using a polyetheretherketone (PEEK) tube (50 cm × 130 μm). The second entry of the T was coupled to the injection valve via a stainless steel tube of 7 cm × 500 μm i.d. and joined to the waste through a 50 cm × 50 μm i.d. fused silica capillary. The capillary chromatographic column was directly connected to the nano-injector. The separation of samples was performed in laboratory-made capillary columns (100 μm i.d., 375 o.d.) packed with stationary phases in our laboratory (packing procedure is described below) and on Chromolith CapRod column FR (150 mm × 100 μm i.d.) purchased from Merck (Merck, Darmstadt, Germany). Data were recorded using ChromQuest software (Thermo Fischer Scientific, Waltham, USA). The wavelength for UV detection was 205 nm.

Conventional high performance chromatography experiments were done using an HPLC Smartline (Knauer, Germany) equipped with columns Chromsphere C18 (100 × 4.6 mm, 3 μm, Agilent