Journal of Chromatography A, 1372 (2014) 72–80

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Using of molecularly imprinted polymers for determination of gallic and protocatechuic acids in red wines by high performance liquid chromatography

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ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form 15 October 2014
Accepted 24 October 2014
Available online 31 October 2014

Keywords:
Molecularly imprinted polymer
Sorption capacities
Solid-phase extraction
Phenolic acids
Red wine

ABSTRACT

The sorption capacities of gallic- and protocatechuic acid-molecularly imprinted polymers (GA-MIP and PCA-MIP, respectively) and non-imprinted polymer (NIP) have been determined on the piston columns by the frontal analyses (FAs). Mobile phases consisted of MeOH, MeOH/H_2O (1:1), 12.5% EtOH or ACN. Solutes concentrations used in FAs were 1 μg/mL and 50 μg/mL. All sorption capacities were depended on analyte and solvent used. Results obtained from the FAs have shown that both imprinted polymers almost always were preferentially recognized PCA molecule. Only in MeOH, the GA-MIP had ability to recognize its template molecule positively. Surprisingly, in some cases, also the NIP exhibited higher sorption capacities than the MIPs for their templates, e.g. in ACN for GA or in MeOH for PCA. This behaviour indicates that in some solvents, the low affinity sites of the blank polymer can act as strong interacting sites. In the next, prepared MIPs were successfully used as the SPE-sorbents for the extraction and purification of chosen phenolic acids from red wine samples. The recoveries both of MIPs were the highest for PCA, what was in agreement with the experiments carried out in 12.5% EtOH during FAs. Prepared MIP-beads allowed the purification of chosen red wine samples with satisfactory selectivities and high recoveries. The linearity of the method was in the range from 10 μg/mL to 70 μg/mL and 0.1 μg/mL to 4.5 μg/mL for GA and PCA, respectively, with the determination coefficients ranging from 0.996–0.999. The LODs (S/N = 3) ranged from 0.1 μg/mL to 0.4 μg/mL. The RSDs for the recoveries varied from 4.0% to 8.1%. The PAs-MIPs and corresponding NIP were also characterized by attenuated total reflectance analysis Fourier transform infrared spectroscopy (ATR-FTIR) and scanning electron analysis (SEM).

1. Introduction

In the last years, determination of phenolic compounds have become of great interest due to their wide-ranging biological significance and potential utility. They represent the richest and the most widespread class of the plant natural products. There is no doubt that an antioxidant and antimicrobial properties of these compounds belong to the most significant. Phenolic compounds are commonly presented in the plants and in widely available plant foodstuffs [1,2]. The large family of these phytochemicals, among which are simple phenols, like phenolic acids (derivatives of hydroxybenzoic and hydroxycinnamic acids), coumarins or polyphenols, like tannins and flavonoids, is characterized by at least one aromatic group and one or more attached hydroxyl groups [3–8]. Phenolic compounds are acting as enzyme inhibitors, antioxidants, antiviral, anti-inflammatory or antiallergic agents. They have preventive effect against many known illnesses such as cancer, Alzheimer, Parkinson, diabetes mellitus, rheumatism, atherosclerosis or those connected to the cardiovascular system [6–8]. These incredible properties to prevent the human diseases being linked to their chemical structure and redox properties. The number and position of hydroxyl groups is associated with their antioxidant abilities - the higher number of hydroxyl groups in the molecule leads to higher antioxidant activity [8]. Phenolic compounds are presented in the most plant kingdom as esters, glycosides or complexes and only rarely occur in the free forms, mainly in processed food [2].

Molecularly imprinted polymers (MIPs) are highly crosslinked and porous materials with specific molecular recognition ability for particular target molecule or group of structurally related target molecules. In the presence of porogen and crosslinking monomer, the specific analyte, called template, is self-assembled, forming a

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http://dx.doi.org/10.1016/j.chroma.2014.10.070
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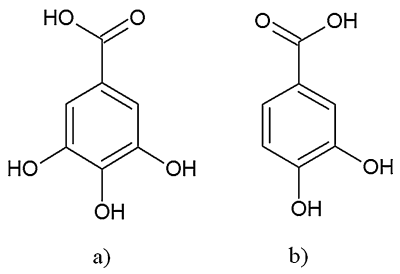

Fig. 1. Structures of studied phenolic acids – (a) GA and (b) PCA.

complex with the functional monomer. After polymerization process, the template is removed from the polymer-matrix by a simple solvent extraction, leaving three-dimensional cavities complementary in shape, size and functionality to the molecules of interest. Those "frozen memory sites" are afterwards able to selectively recognize the template even in the complex matrices. In this commonly used approach, the complex between the template molecule and the functional monomer is formed by interactions like hydrogen or ionic bonds, hydrophobic effect or van der Waals forces [9,10]. The high selectivity, ease of preparation, high stability and possibility of work with various chemicals in a wide pH-range makes that MIPs are excellent polymeric materials for various applications. The most common applications of the MIPs are sorbents for solid-phase extraction (MISPE) or stationary phases for chromatography [11–13]. The extraction, purification and determination of phenolics in the natural products very often is realized by the HPLC method coupled with SPE or LLE procedures [14,15].

In our work we would like to present two MIPs specific for the two structurally similar phenolic acids (PAs), derivatives of hydroxybenzoic acid–gallic acid (GA) and protocatechuic acid (PCA) (Fig. 1). Prepared MIPs were then successfully used as the MISPE material for selective isolation of the target molecules from the real samples and its purification from the interfering compounds.

Prior to starting the sample preparation by the SPE procedure, the sorption capacities of investigated polymers were carried out in the different solvents. The sorption capacity characterizes the maximum amount of a compound that can be loaded and retained on the MIP at given conditions. It should be kept in mind that each sorbent, depending on the solvent used, nature of the compound and volume of the sample, possess its own sorption capacity. Therefore, to ensure a reproducibility of the results, the loaded sample volumes should not exceed this capacity [16]. The determination of sorption capacities in different solvents is also gaining importance in the case of investigation on the selectivity of the system and the analyte behaviour in various solvents during the sorption processes on the MIP-material. Hence, in our research, we took into account the measurements in the mobile phases with different chemical properties. In our experiments, the sorption capacities were calculated from the breakthrough curves using frontal analyses (FAs). Then, the chosen MISPE-procedure was testified to be applicable for the purification of selected PAs, GA and PCA, respectively, in the chosen red wines.

2. Experimental

2.1. Chemicals and columns

GA and PCA were purchased from Sigma–Aldrich (Steinheim, Germany). Methanol (MeOH), ethanol (EtOH) and acetonitrile (ACN) (gradient grade) were obtained from J.T. Baker (Deventer, Netherlands), acetic acid (HAc), formic acid and acetone (p.a.) were purchased from MIKROCHEM (Pezinok, Slovakia), methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA) and azobisisobutyronitrile (AIBN) for synthesis were obtained from MERCK (Darmstadt, Germany), piston columns ECO^{PLUS}, 125 mm × 5 mm were delivered from KronLab (Dinslaken, Germany). GraceSmart RP18 column, 5 μm, 150 mm × 4.6 mm was purchased from AZ Chrom (Bratislava, Slovakia).

2.2. Wine samples

Red wines, Cabernet Sauvignon (CS) (Château Topol'čianky 2012, Topol'čianky, Slovak Republic), St. Laurent (SL) (Matyšák 2012, Pezinok, Slovak Republic) and Cuvée (C) (Vitis 2012, Pezinok, Slovak Republic) were purchased in local stores.

2.3. Preparation of the polymers

Polymers were prepared by a bulk polymerization according to the Zhang et al. [17] method and like in our previous experiments [18]. MAA as the functional monomer (1.8 mmol) and MeOH as the porogen (3.0 ml) in the presence of GA or PCA (0.3 mmol) as the templates was mixed together in the glass test tubes. Then EDMA as a cross-linker monomer (9.0 mmol) and AIBN (20 mg) as an initiator were added. The polymerization of PAs-MIPs was allowed to proceed in a water bath at 60 °C for 24 h. Afterwards, the prepared polymers were grounded and passed through 40 μm sieve. The fine particles were removed by the flotation process in acetone. The Soxhlet extraction of the dried particles (24 h, 150 ml MeOH/HAc (9:1, v/v)) was used in order to purify the GA-MIP and PCA-MIP from the individual templates. The corresponding control polymer (NIP) was prepared in the same manner like the PAs-MIPs with the only difference – the templates were not presence in the polymerization mixture.

2.4. Preparation of the piston columns

Piston columns were filled with definite amount each of the PAs-MIPs or the NIP (200 mg). Then, they were washed with MeOH for 24 h in order to elute all remaining residues. The flow rate of the mobile phase was gradually growing up to precise pack of particles of the polymers. The sorbents in the columns were pressed by the pistons as long as the resistance was not felt.

2.5. Apparatus

The breakthrough curves were measured using an Agilent Technologies 1260 Infinity system (Waldbronn, Germany) consisting of a degasser, a diode-array detector (DAD) in combination with WATERS 510 HPLC pump (Vienna, Austria) and an Agilent Technologies Chemstation. For the analysis of red wine samples there was used the same system from Agilent Technologies consisting of a pump with a degasser, a diode-array detector (DAD), 20 μL injector and an Agilent Technologies Chemstation. DAD detection was used in the range of 200–400 nm and the chromatograms were acquired at wavelengths of 220 (for GA) and 254 nm (for PCA).

IR spectra of grounded polymers and templates were measured by ATR (attenuated total reflectance analysis) technique on FTIR Spectrometer Nicolet 5700 in the 4000–400 cm^{-1} region.

The scanning electron microscopy (SEM) studies of the samples were performed using a scanning electron microscope JEOL JSM-500F. The samples were prepared by application of particles on the polyurethane adhesive and then were sputtered with Au using a sputter Coater BALZERS SCD 050 (working pressure cca 0.05 mbar, sputtering current 37 mA/430 V, working distance 20 mm, sputtering time cca 60 s). SEM images of the samples were obtained using program "PC SEM" at accelerating voltage 10 kV and magnification 200,000×. The pictures were taken by the same conditions by