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Development of an analytical method for the determination of polyphenolic compounds in vegetable origin samples by liquid chromatography and pulsed amperometric detection at a glassy carbon electrode

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ABSTRACT

A sensitive and accurate method for the determination of polyphenolic compounds in artichoke bract extracts and olive mill wastewaters by liquid chromatography coupled with pulsed amperometric detection at a glassy carbon working electrode was developed. Preliminary experiments were carried out by cyclic voltammetry to investigate the electrochemical behavior of polyphenols under different mobile phase compositions, and to test the detection and cleaning electrode potentials. Chromatographic separations were performed by using a core-shell C_{18} column, eluted with acetic acid and acetonitrile, by combined concave-linear binary gradients. Under the optimized experimental conditions, a good column efficiency and peak symmetry were observed, also for stereo and positional isomeric compounds. The developed three-step potential waveform for pulsed amperometric detection was successfully applied for the sensitive chromatographic determination of polyphenols in artichoke extracts and olive mill wastewaters. Linearity, precision and sensitivity of the proposed method have been evaluated. A wide linear range of response (up to 20 mg/L) has been obtained for all the investigated compounds. Detection and quantification limits in the vegetable origin sample extracts were in the range 0.004–0.6 mg/L and 0.01–2 mg/L, respectively, while the injection-to-injection repeatability ($n = 6$) ranged from 5 to 13%. The obtained results confirmed the excellent sensitivity of the electrochemical detection, and its suitability for the determination of electroactive polyphenolic compounds at low concentration levels.

1. Introduction

Phenolic compounds represent one of the most numerous and ubiquitous groups of plant metabolites and are an integral part of both human and animal diets. They are a heterogeneous family of chemical compounds comprising, among others, phenolic acids, flavonoids, tannins, stilbenes, coumarins and lignans. Polyphenols are synthesized by plants during the growth and in response to stress conditions, such as infection, wounding, UV radiation, etc. [1]. Traditionally, their relevance has been mainly related to the organoleptic properties, such as color (e.g., anthocyanins and curcumin), astringency (tannins), bitterness (flavanols) and taste [1,2]. Nevertheless, in the last decades they are increasingly being recognized for their nutritional value, since they can reduce the risk of chronic disease and, in general, have a positive influence on health, showing anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immune modulating, antimicrobial, vasodilatory, and analgesic effects. Therefore, there is an increasing demand of polyphenols from low cost materials (e.g., vegetable by-products) that are very important in food technology, since they represent an economically attractive resource of high-value components. The use of vegetable by-products as a natural source of antioxidant compounds could be very appreciated to avoid oxidation during food processing and storage, in replacement of synthetic additives. As a result, specific analytical methods for the characterization and quantification of polyphenolic compounds,

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and then for their extraction from vegetable and food-industrial by-products, are essential to obtain innovative products, representing a challenge for an eco-innovation.

Despite a great number of investigations [3–5], the separation and quantification of different phenolic compounds remain a challenging task, in particular the simultaneous determination of isomers, since stereo, structural and substitution isomerisms are common phenomena among polyphenols.

Colorimetric assays, including the Folin-Ciocalteu (F-C) and Prussian-Blue methods for total polyphenols quantification [6], are widely used as screening tools, mainly due to their simplicity and high sensitivity. However, these methods are not selective and give only an estimation of the phenolic content, since no separation and quantitative determination of each compound is provided.

The recent scientific activity for multi-analyte polyphenol determination is generally focused on liquid chromatography (LC) coupled with DAD or [7,8] mass spectrometry (MS) [9–11], which assure low detection limits, direct information concerning the molecular structure of the analyte and their metabolites, and the possibility to perform determination of multiple analytes from different chemical groups in the same run. On the other hand, LC–MS methods suffer from disadvantages, such as the need of an extensive sample cleanup to avoid matrix effects, expensive laboratory equipments and instrumentations, high analysis costs and trained operators.

Polyphenols are well known to be electroactive due to the presence of hydroxyl groups as substituents of aromatic rings that undergo electrochemical oxidation reactions [12]. Therefore, the amperometric detection following LC separation can be considered a useful technique providing good results in terms of sensitivity, selectivity, instrumental costs and simplicity. Quite recently, electroanalytical techniques based on voltammetric approaches and their coupling to flow-injection analysis, high-performance liquid chromatography or capillary electrophoresis for the analysis of polyphenols in wine have been reviewed [13]. In addition, several strategies for the determination of phenolic compounds in tea, alcoholic beverages, and pharmaceutical formulations have been reported including amperometric biosensors [14,15], the anodic detection at chemically modified electrodes [16,17] or expanded graphite-epoxy composite, and rotating spectral graphite disk electrodes [18]. However, these approaches refer to the determination of total phenolic content or to a limited number of polyphenols.

Among the various amperometric methods, constant potential detection (DC) is the simplest, but limitations due to the electrode poisoning from sample matrices and analyte oxidation products are generally observed [19]. Consequently, to get a reproducible electrochemical signal over time, the fouled electrode surface need to be periodically cleaned, prior to each measurement session. In pulsed amperometric detection (PAD) the electrode surface is renewed within a pulsed potential waveform that continuously cleans and reactivates the working electrode with a repeated sequence performed at a frequency of 0.5–2.0 Hz. Several potential waveforms have been proposed for the electrochemical detection of a wide range of analytes (carbohydrates, nitrogen, sulphur compounds, etc.) at gold or platinum working electrodes [20–22]. Compared to noble-metal electrodes, glassy carbon electrode (GCE) is very resistant to fouling, even though poisoning phenomena at constant potential occur, owing to the adsorption of the oxidation products at the electrode surface, which causes a decrease of sensitivity and a time-dependent deterioration of the response. In the literature, various activation/polishing strategies are reported [23] to obtain sensitive and stable electrode responses at GCE. Very recently in alternative to the off-line preactivation or in situ laser irradiation, the development of new approaches based on well-performing potential waveforms has been proposed for a sensitive and reproducible detection of arylethanolaminic and phenolic moiety based compounds [24]. The potential-time profile was designed to prevent the carbon electrode fouling following repeated analyses, assuring a reproducible and sensitive quantitative determination without the need of a mechanical polishing. The electrochemical characterization studies of carbon electrode surfaces by cyclic voltammetry and flow injection analysis has suggested the formation of an oxygen-rich surface film, consisting of quinone functionalities that appear to be likely candidates as mediators of electrons between the electrode and the electroactive species [24].

When the electrochemical detection is coupled with liquid chromatography, the proper choice of the mobile phase is a fundamental aspect to enhance the electrode performance and sensitivity. On the other hand, the selection and optimization of the eluent composition is a critical factor in achieving good chromatographic behavior as peak shape and resolution. Retention behaviors of phenolic compounds in LC by reversed phase column have received far less attention, and poor resolution and efficiency were generally obtained for polyphenol isomers, unless the selective detection in multiple-stage mass spectrometry is applied [25,26].

In this work, a new method based on LC coupled with pulsed amperometric detection at a glassy carbon working electrode for the determination of phenolic compounds is described. Chromatographic separations were performed by customized combined concave-linear binary gradients applied to a core-shell C_{18} column. The experimental parameters used for the electrochemical detection under chromatographic conditions were tested by evaluating linearity, repeatability and sensitivity. Finally, the method feasibility was demonstrated by the analysis of artichoke extracts and olive-mill wastewaters (OMWW).

2. Materials and methods

2.1. Chemicals

Chemical standards of caffeic acid (3,4-dihydroxycinnamic acid, >98%), chlorogenic acid (3-(3,4-dihydroxycinnamoyl)quinic acid, >98%), cynarin (1,3-di-O-caffeoylquinic acid, >98%), ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid, 99%), *p*-coumaric acid (trans-4-hydroxycinnamic acid, ≥98%), syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid, ≥95%), catechol (1,2-dihydroxybenzene, ≥99%), 3-hydroxytyrosol (2-(3,4-dihydroxyphenyl)ethanol, ≥98%), oleuropein (≥98%), tyrosol (2-(4-hydroxyphenyl)ethanol, 98%) and verbascoside (≥99%) were supplied by Sigma-Aldrich; apigetrin (apigenin 7-glucoside, 99%) and cynaroside (luteolin 7-glucoside, 90%) were supplied by LabService Analytica (Anzola Emilia, BO, Italy); neochlorogenic acid (trans-5-O-caffeoylquinic acid, 99%), cryptochlorogenic acid (4-O-caffeoylquinic acid, 96%), 1,5-di-O-caffeoylquinic acid (>99%), 3,4-di-O-caffeoylquinic acid (>96%), 3,5-di-O-caffeoylquinic acid (≥98%), 4,5-di-O-caffeoylquinic acid (>92%) were purchased from PhytoLab (Vestenbergsgreuth, Germany). The Folin reagent (Sigma–Aldrich, Madrid, Spain) and sodium carbonate (Panreac, Barcelona, Spain) were used for the measurement of the Folin-Ciocalteu total polyphenol index. Water and acetonitrile of LC grade were purchased from J.T. Baker-Holland (Devender, The Netherlands). All the solutions and extracts were filtered through 0.22 μm nitrocellulose membranes (Phenomenex, Torrance, CA, USA) and degassed in an ultrasonic bath before use. Polyphenol standard stock solutions were prepared in methanol at a concentration of 1000 mg/L, sonicated for 10 min and kept in freezer at −20 °C no longer than 2 weeks. Working standard solutions were prepared just before use by diluting stock solutions in water and stored at 4 °C between injections.