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Autoanalyzer for continuous fractionation and quantitation of the polyphenols content in wines

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Abstract

A simple continuous flow autoanalyzer for the on-line fractionation of the polyphenols content in wines is proposed. The target compounds are isolated from the matrix by solid-phase extraction on an RP-C_{18} sorbent column, using selective solvents for the sequential elution of each polyphenol family. Moreover, evaporative light scattering detection (ELSD) is used for the first time for the on-line monitorization of the three polyphenol fractions present in the wine samples. Thus, a single sample injection is required to determine the global concentration of the three selected polyphenol fractions and the whole analysis is completed within a few minutes. Three calibration graphs were constructed for quantitative analysis of the global compounds concentration in every fraction and covered the range 5–300 mg l^{-1} (expressed as gallic acid). Average repeatability, expressed as relative standard deviation, was 4%. The proposed autonalyzer was applied to the analysis of a variety of commercial wine samples. The results obtained were compared with those provided by the Folin–Ciocalteau method, being similar in all instances.
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1. Introduction

The presence of wine in human culture goes back to 6000 years, carrying out very important social and religious functions [1,2]. The chemical composition of wine is too complex, containing more than 500 different compounds in a wide range of concentrations [3]. It has been proved that a moderate consumption of wine is related to a decrease in the risk of cardiovascular disease [4], what is summarized in the so-called French Paradox [5,6]. This positive influence may be due to various factors, mainly alcoholic and phenolic contents.

Phenols are characterised by the presence of hydroxyl groups in its structure, linked to aromatic rings. They are present in the grape (skin and seeds), but they may be produced by yeast metabolism and could be extracted from the oak barrels in which the wine is stored. They are also affected by the climatic conditions and vinification process. From the point of view of quality , this family of compounds affects directly to the sensorial properties of wine, as colour, astringency or bitterness [7]. Moreover, they are involved in a protective effect on cardiovascular [8] and neurodegenerative disease. Phenolic compounds have high antioxidant capacity [9] and they are excellent free radical scavengers [10]. Some researches have demonstrated that these type of compounds reduce the peroxide concentration in plasma, LDL oxidation and thrombosis risk. The determination of this group of compounds can help to identify variations in wine types and differences in winemaking and maturation processes as well.

Polyphenols can be divided into different families according to either the polarity or the molecular weight. Most of the methodologies proposed for the determination of phenolic compounds in wine involve liquid chromatographic [11–14] or electrophoretic [15,16] separation of the target analytes using diode array (DAD) or fluorimetric detection.

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To improve the resolution of the separation, a previous solid phase extraction (SPE) step is usually required. The main disadvantage of these methods is that they are tedious and time-consuming. In order to avoid these inconveniences, different fractionation models have been established in routine analysis as those proposed by Glories [17], Oszmianski et al. [18] and, Di Stefano and Cravero [19]. In these models the first step is the retention of analytes in a sorbent material, followed by a sequential elution with solvents of different polarity. Within the protective effects of phenolic compounds, synergetic properties have been demonstrated; in this manner, the quantification of total concentrations of each family could be interesting for further studies.

In this paper a fractionation method, proposed by Oszmianski et al., is automated, carrying out the determination of three families: (I) procyanidins, catechins and anthocyanin monomers, (II) flavonols and (III) anthocyanin polymers. The method uses a continuous flow manifold, which includes an on-line SPE step, directly coupled to an evaporative light scattering detector (ELSD). Its quasi-universal response provides a global signal for each of the polyphenol fractions and thus avoids chromatographic separation. For this purpose, dilute wine samples adjusted to pH 7 are pumped through an RP-C_{18} minicolumn. After a washing step with water, three sequential and selective elutions were performed, each fraction being sequentially monitored by the ELSD using a single sample aliquot.

2. Experimental

2.1. Reagents and samples

All reagents were of analytical grade or better. HPLC gradient grade organic solvents (acetonitrile, ethyl acetate, methanol and ethanol) were supplied by Scharlau (Barcelona, Spain). Gallic acid and sodium hydroxide from Sigma–Aldrich (Madrid, Spain) and Milli-Q ultrapure water (Millipore Corp., Madrid, Spain) were also used.

A total of 18 wine samples, commercially available were analyzed. Once opened, wine samples were transferred to two 100 ml amber glass bottles (no headspace volume was left in order to prevent analyte losses) and stored in the dark at 4 °C. Replicated analysis were carried out within a few days to avoid storage damage of the samples. Aliquots were filtered through a 0.45 μm nylon filter and diluted if necessary. Samples were adjusted manifold to pH 7 with sodium hydroxide prior to their injection into the flow.

Folin–Ciocalteu reagent and sodium carbonate decahydrate and anhydrous (all from Merck, Darmstadt, Germany) were also used. The sodium carbonate saturated solution for the Folin–Ciocalteu method was prepared as follows: 35 g of Na_2CO_3 was dissolved in 100 ml of water by heating at 70–80 °C; the solution was allowed to cool overnight and the supersaturated solution was seeded with crystals of $Na_2CO_3{\cdot}10H_2O$ and filtered through glass wool after crystallization.

2.2. Apparatus

The flow system consists of a Hewlett Packard 1050 high pressure quaternary pump for solvents [water, acetonitrile 16% (v/v) in water, ethyl acetate and methanol] delivery; a six port LC injection valve (Knauer 6332000) fitted with a 1 ml PTFE sample loop and a DDL 31 evaporative light scattering detector (Eurosep, Cergy-Pontoise, France) for monitoring of analytes. The temperature of the ELSD evaporation chamber was set at 65 °C and compressed air (at 2 bar) was used as nebulizing gas. Gain detector was set at 700, 550 and 650 V (depending on the polyphenol fraction being analyzed) and was changed during the analysis. The sample loop was filled by means of a syringe, using on-line filtration through a commercial nylon filter (0.45 μm pore size). PTFE tubing of 0.5 mm I.D. for coils, and standard connectors were also employed. The flow system was connected to the ELSD by means of a 50 cm × 0.1 mm I.D. PEEK tubing. For retention of analytes, a laboratory-made RP-C_{18} column was constructed by packing 40 mg of the sorbent into a 3 cm × 4 mm I.D. PTFE tube using small cotton beads to prevent material losses. Signals were acquired using a Radiometer (Copenhagen, Denmark) REC 80 Servograph recorder and peak height was used as analytical signal.

2.3. Official method

The standard method for polyphenols determination in wines was implemented in accordance with the AOAC's recommendation [20]. A working solution of 40 mg l^{-1} of gallic acid (in water) was used for the construction of the calibration curve. Different volumes of this solution were placed in a 5 ml volumetric flask, and 250 μl of Folin–Ciocalteu reagent and 1 ml of sodium carbonate saturated solution were added; the volumetric flasks were made up to the mark with Milli-Q water. The calibration curve was run for solutions containing 0–9 mg l^{-1} of gallic acid ($n = 12$). The product was monitored at 750 nm 30 min after sample preparation.

2.4. Autoanalyzer functioning

The autoanalyzer, based on the Oszmianski et al. fractionation model (Fig. 1), operates in a sequential fashion. Initially the loop of the injection valve (1 ml) was filled with the dilute wine sample adjusted to pH 7, while a distilled water stream at a flow rate of 1.4 ml min^{-1} was directly introduced into the ELSD to obtain the baseline. Then the injection valve was switched to the inject position and the sample, carried by a Milli-Q water stream, passed through the RP-C_{18} sorbent column at a flow rate of 1.4 ml min^{-1}. Polyphenols were quantitatively retained while other matrix components (phenolic acids and sugars) were driven to the detector; the aqueous stream was allowed to pass through the column for

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