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# Automated on-line solid-phase extraction–high-performance liquid chromatography–diode array detection of phenolic compounds in sherry wine

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**Abstract**

A new analytical method has been developed for sample preconcentration and analysis of phenolic compounds in sherry wine using on-line solid-phase extraction(SPE)–HPLC–diode array detection. The samples of wine were injected and adsorbed onto polystyrene divinylbenzene cartridges; a robotic semiflexible system was used to automate the SPE stage. Chromatographic separation was carried out in a Symmetry $C_{18}$ steel cartridge, with a two-step elution gradient. Peaks were identified by comparing their UV spectra with the library of spectra compiled by the authors.

*Keywords:* Wine; Sample preparation; Automation; Food analysis; Phenolic compounds

## 1. Introduction

The analysis of phenolic compounds in wine and musts is of considerable commercial importance; these compounds are known to play the major role in the browning process, a product deterioration causing significant losses to the wine producers.

One major problem in the analysis of these compounds is that a large number of them are involved under this heading and the level of each can vary considerably during the various stages of the wine process, from grape to bottled final product. Another problem is the high reactivity of these rapidly-evolving phenolic compounds.

Therefore, there is a need for an analytical method giving high powers of resolution, which implies a preconcentration stage to obtain easily-measurable analytical peaks, and requiring the minimum possible sample handling to avoid any evolution of the analytes.

The literature on the analysis of phenolic compounds by HPLC includes both isocratic elution [1,2] and elution by gradient [3–6]. Some authors have developed elution gradients for direct-injection HPLC as the method for analysing these compounds in wine [7–9], but most authors propose sample preparation prior to the HPLC injection, to preconcentrate the samples. Proposals have mostly been based on liquid–liquid extraction using diethyl ether [10,11] or ethyl acetate [12–14]. However, there has recently been noticeable growth in proposals for using solid-phase extraction (SPE) in the determination of phenolic compounds in different samples [15–21].

The success claimed for the SPE–HPLC technique lies in the numerous advantages it offers over others, such as high selectivity, speed and ease of automation.

This paper reports the development of a fully

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automated method for determining the phenolic compounds present in wines and musts, using solid phase extraction for the sample preconcentration stage. The SPE involves the use of a polymeric adsorbent, polystyrene–divinylbenzene, which retains the polar analytes strongest than $C_{18}$, the most commonly used adsorbent. The HPLC column used is a Symmetry $C_{18}$ containing a high charge of carbon. A two-stage elution gradient was optimized.

## 2. Experimental

### 2.1. Reagents and standards

Methanol (HPLC-gradient grade) and all other reagents were supplied by Merck (Darmstadt, Germany). Standards used for identification by standard addition method and to obtain the calibration curves were supplied by Fluka (Bunchs, Switzerland).

All the water used was purified in a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Before being used, all the solutions were filtered through 0.45 $\mu$m membranes (Millipore) and degasified in an ultrasonic bath.

### 2.2. Wine samples

Commercial-quality samples of wine were obtained directly from wine producers Osborne and Cia. Puerto Sta. María (Cádiz), Spain.

### 2.3. Chromatographic equipment and conditions

All the controls were carried out by HPLC; fractions collected were injected into a Waters chromatograph (Millipore), consisting of two Model M510 pumps, a Model M991 and a Millenium 2010 chromatographic control and data handling system.

The separation was performed using a Symmetry $C_{18}$ steel cartridge column (25 cm×4 mm I.D., particle size: 5 $\mu$m) with a $C_{18}$ Sentry precolumn (Waters, Milford, MA, USA). The chromatographic conditions employed were the following: flow-rate 0.8 ml/min.; detection by UV–Vis absorption with a photodiode array detector scanning between 230 and 390 nm; volume injected 20 $\mu$l; mobile phase methanol–water (10:90, v/v) as solvent A and methanol–water (90:10) as solvent B, both solvents at pH set-point 2.5 using sulfuric acid. A two-step elution gradient was employed (Table 1).

Table 1
Gradient elution programme

| $t$ (min) | %Dis. A | %Dis. B | Curve |
|---|---|---|---|
| 0 | 100 | 0 | — |
| 30 | 85 | 15 | 9 |
| 65 | 50 | 50 | 6 |
| 90 | 0 | 100 | 6 |

Peaks recorded for the samples were identified by comparison of their UV spectra with the library of spectra compiled by the authors [22] and by standard additions.

### 2.4. Sample preparation

An automatic, semi-flexible robotic system, namely a Benchmate Workstation (Zymark, Hopkinton, MA, USA), was used for the automated SPE sample preparation stage prior to the HPLC analysis. The "gravimetric confirmation" option of the equipment set to "on" for the sample processing; this permits control in the event of a fault in liquid handling.

The polymeric (polystyrenedivinylbenzene) adsorbent LiChrolut EN (Merck) was used, with 200 mg bed filling.

Table 2
Setup parameters for sample preconcentration step

| Parameters | Conditions |
|---|---|
| Adsorbent | EN (200 mg) |
| | Conditioned with 5 ml of methanol |
| | Conditioned with 3 ml of water |
| Sample | Sample volume: 5ml |
| | Addition 5 ml of water |
| | 9.8 ml of sample (solution 1:1 wine–water) is load in the cartridge |
| Wash | The cartridge is washed with 0.6 ml of water |
| Drying | The cartridge is dried for 150 s with He |
| Elution | 1 ml of THF |
| Flow rates | Condition flow: 0.25 ml/s |
| | Load flow: 0.01 ml/s |
| | Wash flow: 0.05 ml/s |
| | Elution flow: 0.05 ml/s |
| | Air flow: 0.05 ml/s |
| | Air factor: 0.6 |

Table 2 describes the automated sample preparation scheme devised by the authors.

## 3. Results and discussion

First the HPLC elution gradient was optimized as follows: a volume of 5 ml of sherry wine was passed through the LiChrolut EN SPE cartridge and eluted with 1 ml of methanol; this extract was then injected successively in volumes of 20 $\mu$l to test different phase compositions and gradients, in order to achieve the best resolution of the maximum number of peaks. The chromatographic conditions for this objective are those described in the above paragraph under this heading. The chromatogram obtained under these optimum conditions is shown in Fig. 1. The resolution of peaks is considered good, given the complexity of the sample.

Next the sample preconcentration stage of the SPE was optimized using the LiChrolut EN adsorbent in an automated system. The equipment set-up parameters, such as flow rates for conditioned samples, wash and elution, drying time, air factor, etc, the solvent volumes and compositions employed in the process were systematically modified to achieve the maximum recovery of phenolic compounds from the sample.

From the first of the series of different tests performed, the adsorbent demonstrated a high retention power, retaining a large number of phenolic compounds from the sample. Although if the wash volumes were increased, the recovery did not show any significant variation, indicating the strong interaction with the adsorbent. Initially, this was considered a positive result; however, there was an adverse factor, in that the use of methanol as the elution solvent produced poor recovery of low polar compounds, in contrast to the good recovery of high polar compounds. It was therefore deduced that there must be some compounds not being completely eluted from the adsorbent. This problem was eliminated by using the high elutropic solvent tetrahydrofuran (THF).

The best conditions for the extraction stage are shown in Table 2, while Fig. 2 shows the resulting chromatograms; Fig. 2a for the sample obtained without the extraction stage (by direct injection) and

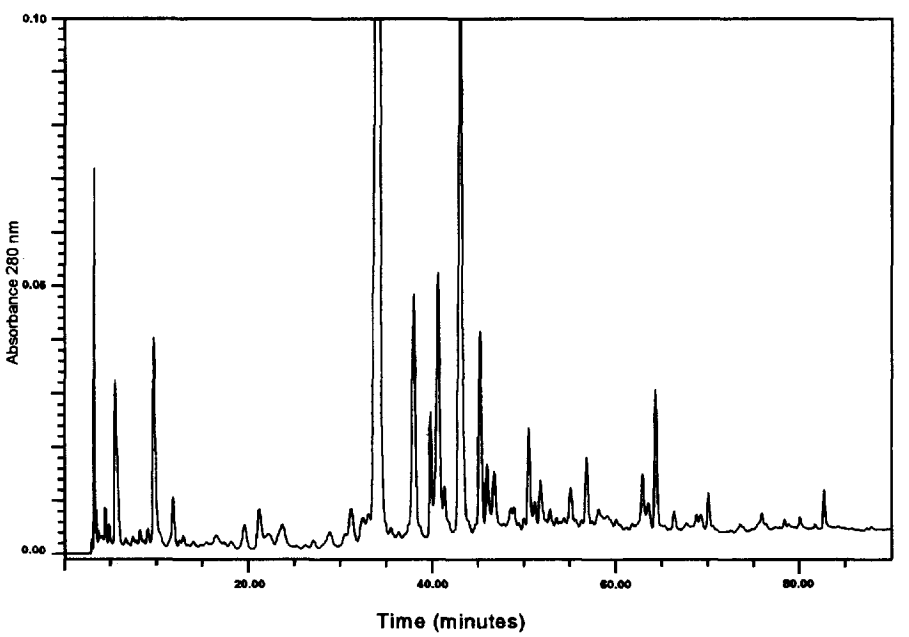


Fig. 1. Chromatogram (280 nm) obtained from an extract of wine with the best conditions and gradient founded.

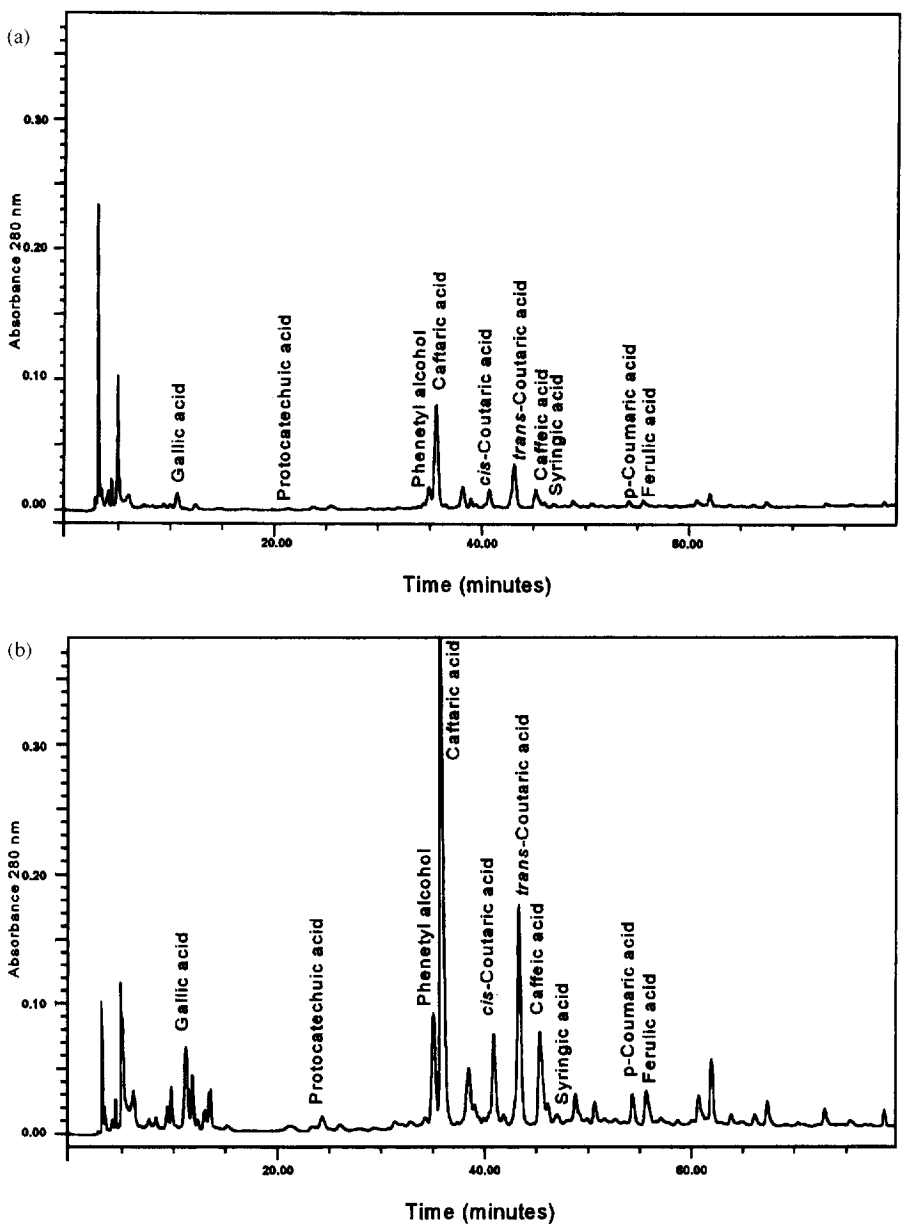


Fig. 2. (a), Chromatogram (280 nm) of 20 $\mu$l of "fino" sherry wine by direct injection; (b), Chromatogram (280 nm) of the fraction collected after the application of the automatic SPE to samples of "fino" sherry wine.

Fig. 2b for the same sample obtained by performing the extraction stage with 5 ml of the sample.

Following the optimization of both stages, the phenolic compounds extracted from the sample were identified by comparison of their UV spectra with the library of spectra maintained in the author's laboratory and by standards addition. The repeatability of the method was checked by processing six identical samples; results are given in Table 3. Those phenolic compounds recovered for which there are no com-

Table 3
Repeatability and recoveries for identified chromatographic peaks

| Compound | Concentration mg/l | | | | Recovery (%) | R.S.D. (%) |
|---|---|---|---|---|---|---|
| | Original in the sample | Theoretical in the extract | Added | Found | Recovery (%) | R.S.D. (%) |
| Gallic acid | 5.42 | 25.31 | 23.33 | 43.34 | 89.11 | 8.24 |
| Protocatechuic acid | 3.26 | 15.20 | 23.33 | 38.53 | 100.00 | 8.79 |
| Phenetyl alcohol | 30.06 | 140.27 | 23.33 | 123.84 | 75.70 | 3.11 |
| Caftaric acid | 21.64 | - | - | - | - | 8.27 |
| *cis*-Coutaric acid | 3.75 | - | - | - | - | 7.87 |
| *trans*-Coutaric acid | 7.38 | - | - | - | - | 7.20 |
| Caffeic acid | 5.79 | 27.04 | 23.33 | 43.07 | 85.51 | 10.63 |
| Siringic acid | 1.17 | 5.48 | 23.33 | 28.81 | 100.39 | 2.83 |
| *p*-Coumaric acid | 1.99 | 9.30 | 23.33 | 30.12 | 92.32 | 13.71 |
| Ferulic acid | 2.99 | 13.94 | 23.33 | 34.29 | 92.02 | 4.60 |

mercially-available standard (i.e., caftaric acid, *cis-p*-coumaric acid, *trans-p*-coumaric acid) were quantified by comparison with a calibration curve of the corresponding free acid; this method is feasible because molar extinction parameters do not vary greatly [23].

The recovery rates for those identified compounds having commercially-available standards were determined by adding known amounts of the same sample, and then extracting the compound by SPE. The results are given in Table 3.

It is therefore concluded that this method provides good recovery of phenolic compounds in sherry wine, good repeatability and has the added advantage of being fully automated. It thus represents an excellent technique for the analytical control of this type of wine.

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