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Analysis of low molecular mass phenolic compounds, furfural and 5-hydroxymethylfurfural in *Brandy de Jerez* by high-performance liquid chromatography–diode array detection with direct injection[1]

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Abstract

The polyphenolic composition of aged distillates originates principally from the ageing process in oak casks. It is accounted for by a narrow range of species. The relative simplicity of this wine industry product permits the analysis by HPLC with direct injection. The repeatability of the method has proved very high, in respect of both retention times and peak areas. The initial optimization of the elution gradient was completed by means of a solution comprising a mixture of standards and was definitely improved using real samples. A C_{18} column, 25 cm × 4 mm I.D., was used, with a particle size of 5 μm. Spectrophotometric detection, covering the UV–Vis range of 240–390 nm, was performed using a photodiode array detector.

1. Introduction

The characterisation of *Brandy de Jerez* has become extremely interesting for two main reasons. The first has to do with the possibility of differentiating the product analytically from all the other similar products on the market, thus protecting its authenticity. And a second reason, with a view to achieving the highest possible product quality in *Brandy de Jerez*, is the usefulness of an analytical procedure which could monitor the brandy during its elaboration process, when variables such as humidity and temperature in the cellar, oxygenation and exposure to light, the characteristics of the oak casks, alcoholic content of the spirit and so on, are exerting their effect on the product's final composition. The role of polyphenolic compounds in creating the flavour and aroma of the brandy has been fully and clearly proven by many authors [1,2].

Although phenols can normally be found in wine distillate, it is believed that the process of maturing in oak casks is the main cause of the polyphenolic content of brandy. The process of maderization of spirits is characterised by the diffusion of recoverable compounds from within the wood, compounds such as aromatic benzoic and cinnamic aldehydes, in particular [3]. It has been generally recognised that these compounds are the result of the degradation of the lignin and although the mechanisms by which they are released into solution are not well known, it is believed that they could have various origins: hydroalcoholysis and acidolysis, and/or hydrothermolysis during the fitting of the staves, or direct

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pyrolysis during the heating. The polysaccharide fraction of the wood is responsible for the enrichment of the brandy with volatile compounds. In addition, hydrolysis, extraction, and oxidation of tannins (which leads to colour changes) are taking place, together with evaporation.

Some authors have already dealt with the analysis of polyphenols in wine by direct injection [4,5].

The phenomena that take place during ageing involve only a narrow range of species and, hence, a relatively simple matrix; and, although various authors have worked with aged distillates of wine, performing an extraction and/or preconcentration of the sample prior to analysis by HPLC [6–8], the use of direct injection for the study of polyphenols of low molecular weight in *Brandy de Jerez* was investigated in this case.

2. Experimental

The standards were from Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), Eastman Kodak (Rochester, NY, USA) and Sigma (St. Louis, MO, USA). HPLC grade methanol was also obtained from Merck. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

All of the HPLC equipment used was from Waters (Milford, MA, USA) and comprises the Millenium 2010 Chromatographic Manager Software, two Model 510 high pressure pumps, a Model 717 automatic injector and a Model 996 diode array detector.

The column used in the study was a RP-C_{18} (Merck) LiCrospher 250 × 4 mm I.D., with particle size of 5 μm. The injection volume was always 50 μl. Prior to their injection in the HPLC, the samples were filtered through a membrane of 0.45 μm pore size. For the mobile phase, methanol–acetic acid–water (5:2:93) was used as eluent A and methanol–acetic acid–water (90:2:8) as eluent B.

Table 2
Elution gradient selected

Time (min)	%A	%B	Curve
0	100	0	–
20	90	10	7
60	25	75	6

Flow velocity = 1 ml/min.

The samples of brandy were supplied by the "Consejo Regulador de la Denominación Específica *Brandy de Jerez*".

3. Results and discussion

To optimize the chromatographic elution gradient, a mixture of standards of polyphenolic compounds characteristically found in brandy was used Table 1, in a synthetic matrix similar to that of the real sample (approx. 40% ethanol). Various tests were done starting with the gradient which Guillén et al. [9] proposed in their method for the analysis of low molecular mass polyphenols in samples of must. The gradient finally selected consisted of two stages completed in a period of 60 min Table 2. The separation achieved in the synthetic medium is that

Table 1
Composition of the mixed solution of standards

Standard	Key	Standard solution (mg/l)	k' (t_0 = 1.85 min)	α
Galic acid	Gc	12.60	5.50	–
5-Hydroxymethylfurfural	HMF	8.50	7.08	1.29
Furfural	F	1.00	10.39	1.47
p-Hydroxybenzaldehyde	pOHBde	6.64	18.07	1.74
Vanillic acid	Vc	7.20	19.15	1.06
Siringic acid	Sc	10.40	20.62	1.08
Vanilline	V	22.60	20.81	1.01
Siringaldehyde	Sde	13.60	21.92	1.05
Elagic acid	Ec	10.00	28.07	1.28

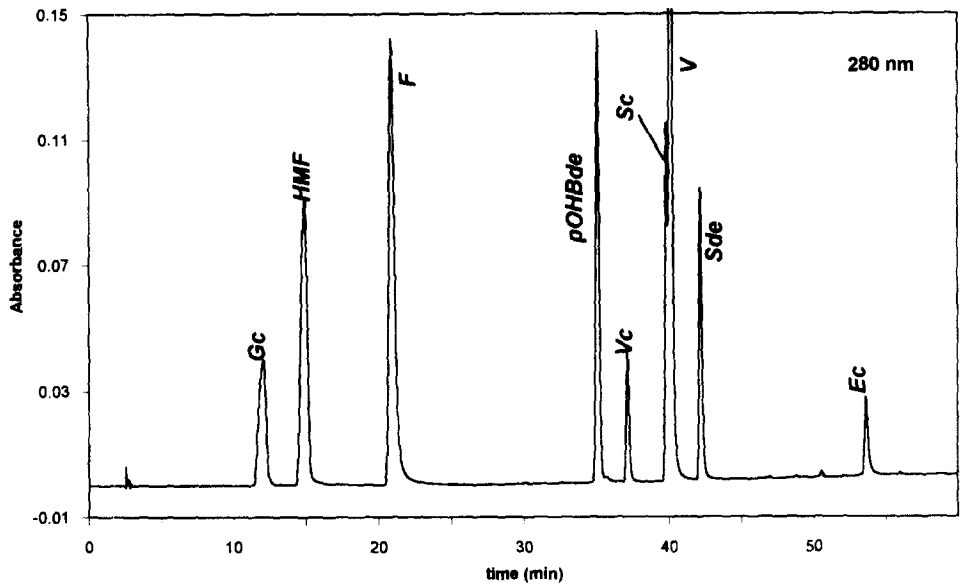

Fig. 1. Chromatogram (280 nm) of standard solution. Peak identification key is given in Table 1.

shown in Fig. 1. The parameters retention factor, k', selectivity, and concentration of each of the species under the experimental conditions proposed are presented in Table 1. The relative simplicity of the brandy matrix, even in those subjected to a long ageing time, allowed well-resolved chromatograms to be obtained. To illustrate this point, Fig. 2 shows the chromatogram at 280 nm of a *Brandy de Jerez*, aged in American oak casks and handled according to the traditional dynamic maturing system of

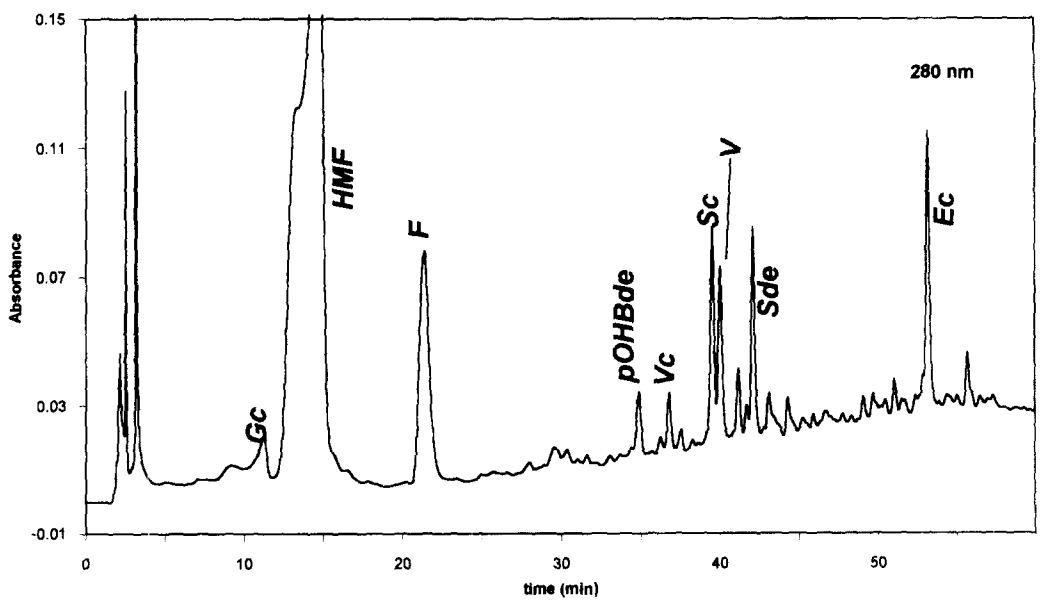

Fig. 2. Chromatogram (280nm) of *Brandy de Jerez*. Peak identification key is given in Table 1.

"soleras y criaderas". Fig. 3 is an application of the method to several brandies of Jerez with different ageing times.

The species in the real samples were identified by comparing their UV–Vis spectra in the 240–390 nm range with the library of spectra previously compiled by the authors. The results indicated a restricted series of compounds. The spectra of the principal peaks identified can be seen in Fig. 4.

In addition, it was studied whether the normal variations in alcoholic content between the different brandies will have any significant effect on the retention times of the chromatographic peaks. For this reason, a study was undertaken of 58 commercial samples with alcoholic content varying between 33.71 and 41.42 (% by volume). The retention times of all of the compounds studied were submitted to a statistical analysis; it was found that the standard deviation in every case was less than 0.4 Table 3.

An enormous advantage of direct injection is that, by reducing the manipulation of the sample to a minimum, the repeatibility of the method is virtually assured. To prove this, five experiments were performed on the same brandy. Quantification was done by areas of peaks, the nine compounds identified being selected for this study. The subsequent statistical treatment of the data showed that the relative standard deviation for each compound was less than

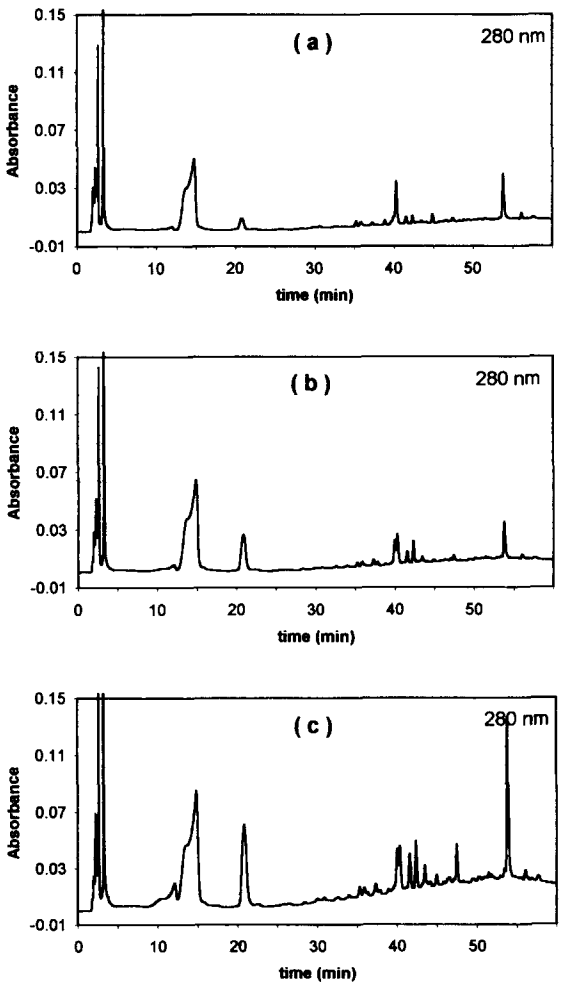

Fig. 3. Chromatograms (280 nm) of *Brandies de Jerez*:(a) "Solera", with an ageing of at least six months:(b) "Solera Reserva", with a minimum ageing of one year;(c) "Solera Gran Reserva", with an ageing of three or more years.

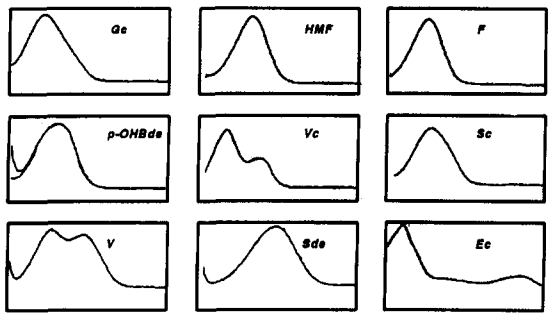

Fig. 4. Spectral comparison of library standards and chromatographic peaks in *Brandies de Jerez*. Peak identification key is given in Table 1.

Table 3
Statistical analysis of the retention times of all of the compounds studied (study undertaken of 58 samples)

Key	Retention time	S.D.
Gc	12.02	0.14
HMF	14.95	0.14
F	21.08	0.34
pOHBde	35.28	0.38
Vc	37.28	0.39
Sc	40.00	0.36
V	40.34	0.36
Sde	42.40	0.33
Ec	53.77	0.50

Table 4
Repeatibility study of the analytical method

	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	C.V.
Gc	738895	742552	742054	747644	740127	0.2
HMF	7289162	7268585	7245904	7295455	7225855	0.4
F	1068897	1065846	1065198	1066591	1056398	0.2
pOHBde	101891	110519	104768	107452	104645	0.8
Vc	194247	197548	200152	193474	190247	1.6
Sc	445042	441273	440317	439931	433047	0.2
V	535424	531823	540310	539176	524284	0.7
Sde	610763	613452	607156	611993	599534	0.4
Ec	1426521	1431654	1420889	1448232	1430682	0.6

1.0%, results which confirmed the high repeatibility of the method devised (Table 4).

4. Conclusions

By means of direct injection in HPLC and with the help of a photodiode array detector, a method of analysing polyphenols of low molecular weight in *Brandy de Jerez* has been confirmed as valid. This method allows a good separation of the main polyphenolic compounds, with the selected gradient, rapidly, accurately and at low cost; these advantages constitute a significant attraction.

5. Acknowledgements

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