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Development of a Rotatory and Continuous Liquid–Liquid Extraction Technique for Phenolic Compounds in Wine

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A liquid–liquid technique for extracting phenolic compounds of low molecular mass from wine, by means of a rotary system with diethyl ether, permitting the simultaneous extraction of up to 12 samples, is described. This technique permits the simultaneous extraction of several samples with good recoveries and high reproducibility, while at the same time minimizing the appearance of analytical artifacts during the process of extraction.

Keywords: *Wine analysis; liquid–liquid extraction; polyphenol*

Introduction

It is generally accepted that polyphenolic compounds of low molecular mass are involved in the phenomena of browning and destabilization of white wines in general and of 'fino' sherry wines in particular[1–3] and as a result there is a need for analytical control of these species. Given the very complex nature of the samples, and the extreme variability of the concentrations in which these substances occur in wine (varying from mg l^{-1} levels in red wines down to μg l^{-1} levels in some white wines such as dry sherry wine), a process of preconcentration and separation of the species of interest, prior to their analysis by HPLC,[4–7] is consequently necessary. Both the chemical instability and the rapid evolution (oxidation, isomerization, polymerization, *etc.*), of these species lead to the appearance of undesirable analytical artifacts during the sample preparation stage.[8]

Various methods have been proposed for the preconcentration and separation of polyphenolic compounds in musts and wines: precipitation, demixing, extraction through adsorbent solids[5,7] and, in particular, methods of extraction with various solvents,[9,10] primarily with ethyl acetate and diethyl ether.[9,11–15]

Barroso *et al.*[16] applied the method of extraction in stages to musts, and found that an emulsion formed which made it difficult to separate the two phases and hence opted to apply differential extraction. Subsequently, Barroso *et al.*[8] also undertook a systematic study of both differential extraction and conventional extraction in stages with diethyl ether and acetate in solutions of standards of phenolic acids. They concluded that these techniques suffered from three basic defects: low yields for many of the species, low reproducibility and degradation of the samples, with the consequent appearance of analytical artifacts.

Baldi *et al.*[15] extracted polyphenols by fractionation by means of liquid–liquid extraction using ethyl acetate at two different pH levels (2 and 6). Paris and Nothis[17] and Gomes[12] fractionated polyphenolic compounds by means of extraction with diethyl ether, ethyl acetate and butanol.

Bengoechea *et al.*[11] extracted polyphenols from must and wine by means of three successive extractions with 10 ml of diethyl ether, followed by another three extractions with 10 ml of ethyl acetate. Le Bon *et al.*[13] also used ethyl acetate (at pH 2) to extract flavonoids.

Even though the recoveries are better with ethyl acetate than diethyl ether,[8] ethyl acetate is less convenient because of its higher boiling-point, which results in more degradation of the samples through having to concentrate them at higher temperatures.

However, although there have been numerous studies aimed at selecting the most suitable extractant for phenolic compounds, hardly any data exist on the quantitativeness of the extractions, the influence of factors such as the ionic force of the medium or the optimum methodology for performing the separation in practice and for minimizing the production of analytical artifacts during the separation.

In this paper, results are presented of a systematic study of rotary and continuous extraction with diethyl ether, as an alternative to conventional extraction in stages and to differential extraction. The study was based on a system originally designed by Mascré,[10] who described an automatic system to perform extraction operations with immiscible liquid phases, which we have subsequently redesigned. A total of 30 polyphenolic species of low molecular mass (16 phenolic acids and 14 aldehydes) whose presence has been detected in wine were included in the study.

Experimental

The extraction device consists of an electric motor geared for low revolutions per minute, to which a series of metallic rods provided with clamps to hold the glass extraction ampoules are attached, which make it possible to perform up to 12 extractions simultaneously (Fig. 1). Positioned on each of the arms and fixed by clamps are two glass ampoules, each with an approximate volume of 150 ml, joined by a glass tube of 30 cm × 1 cm id. Variables such as the number of extraction stages, rotation speed, phase volumes ratios and the effect of the ionic strength of the medium were studied and optimized.

For each complete rotation, the extracting agent flows completely through the body of the extractor twice. We therefore use the term 'stage of extraction' to each half revolution of the body of the extractor.

The following reagents were used for the extraction and determination of polyphenolic compounds: diethyl ether, anhydrous sodium sulfate and acetic acid (Merck, Darmstadt, Germany), methanol and sodium chloride (Panreac, Barcelona, Spain). The internal standards of polyphenolic compounds were mostly obtained from Fluka (Buchs, Switzerland). Working solutions (mixtures of four or five components) were prepared

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with these standards for studying the variables of the extraction method and were injected into the liquid chromatograph followed by isocratic elution. The concentrations of these standards are given in Table 1.

Isocratic elution of the working solutions was carried out using as mobile phase methanol–water–acetic acid, the concentration of methanol (15, 20, 25 or 30%) being adjusted for each of the working solutions by pumping a suitable mixture of the other two solvents to give proportions of 10:88:2 and 90:8:2. The utilization of several concentrations of methanol for the isocratic elution of each of the working solutions was carried out with the aim of obtaining good resolution of the chromatographic peaks of each working solution and also in order to carry out separations in the shortest possible time. Gradient elution with methanol–water–acetic acid (10:88:2 and 90:8:2) was used for the selection of the experimental conditions for the extraction of samples of wine.[18] To study the reproducibility of both the injection and the extraction method, a new solution of 24 standards was prepared and injected into the liquid chromatograph by means of a new gradient of polarity

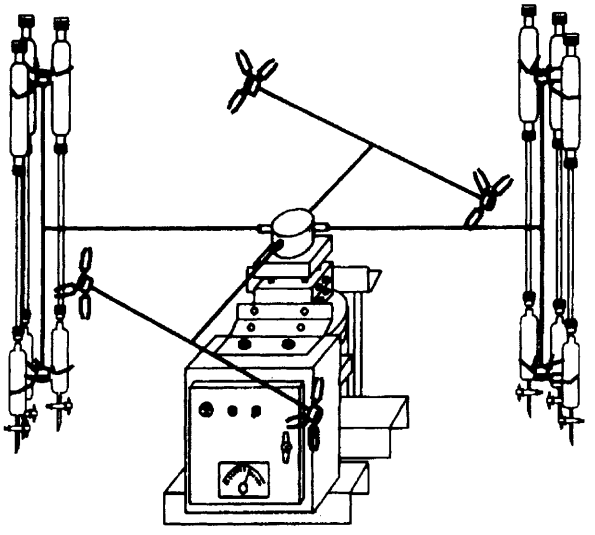

Fig. 1 Continuous rotary extractor.

Table 1 Concentrations of standards

Standard	Concentration (ppm)	Standard	Concentration (ppm)
Gallic acid	34.0	Protocatequialdehyde	24.4
Gentisic acid	255.2	*p*-Hydroxybenzaldehyde	11.6
m-Hydroxybenzoic acid	55.2	Vanillin	16.0
p-Coumaric acid	46.0	Syringaldehyde	14.8
Protocatechuic acid	48.4	*o*-Vanillin	64.0
p-Hydroxybenzoic acid	48.0	2,5-Dihydroxybenzaldehyde	117.6
Syringic acid	21.2	Isovanillin	36.4
Veratric acid	44.0	Veratraldehyde	50.0
Sinapic acid	48.0	2,4-Dimethoxybenzaldehyde	28.8
Caffeic acid	21.2	*m*-Hydroxybenzaldehyde	64.4
m-Coumaric acid	22.4	Salicylaldehyde	125.2
Vanillic acid	28.8	Anisaldehyde	20.0
Ferulic acid	28.0	3,4,5-Trimethoxybenzaldehyde	37.6
o-Coumaric acid	30.8	3,5-Dimethoxybenzaldehyde	28.4
3,5-Dimethoxybenzoic acid	20.0		
3,4,5-Trimethoxycinnamic acid	51.2		

recently devised by our group[19] utilizing the two solvents previously mentioned, which offers a better resolution of chromatographic peaks. The equipment used (Waters, Milford, MA, USA), included an automatic injector (Model 715 UltraWISP), a chromatograph with Model M-45 and 510 pumps and Milenium 2010 software.

The matrix solutions used for the extraction of the solutions of standards consisted of ethanol–water mixture (15% v/v), the pH of which was adjusted by the addition of tartaric acid.

The procedure followed for selecting the variables of the extraction method involved placing in the body of the extractor (Fig. 1) the exact volume of the matrix solution, plus 2 ml of each of the partial solutions of standards. The appropriate volume of diethyl ether was then added and finally this was topped up with distilled water until all the air was eliminated from the body of the extractor. The system was rotated until completion of the pre-set number of stages. The extract and the washing liquids were collected through the same funnel and decanted, separating the aqueous from the organic phase, and anhydrous sodium sulfate was added. It was allowed to dry for 1 h, filtered and concentrated in a Turbovap (Zymark, Hopkinton, MA, USA) under a flow of nitrogen at room temperature to a volume of approximately 0.5 ml. The extract was collected, the concentrating funnel was washed and the collected liquors were diluted to a final volume of 5 ml using methanol–water (HPLC quality) (1:1 v/v). Subsequently, the extracts of each partial solution were injected into the chromatograph under isocratic conditions, varying the percentage of methanol according to the different solutions of standards with the aim of achieving a good separation between the chromatographic peaks in a relatively short period of time (about 15–20 min).

In order to study the influence of the ionic force, the corresponding matrix solution, once prepared, was saturated with sodium chloride and the procedure was followed exactly as described before. The wine samples were subjected to the same extraction method as the standard solutions. All the experiments were performed in duplicate and the extracts obtained were injected in triplicate in all instances, except for the experiments to study reproducibility, in which six injections per test were made.

Results and Discussion

The distribution of a solute between two unmixed phases is an equilibrium phenomenon that follows the distribution law, where the constant of this equilibrium is taken as the ratio of the activities (or concentrations) of the solutes between the two phases. For this reason, to optimize a new liquid–liquid extraction technique, one must bear in mind not only the variables that could modify the equilibrium constant, but also those that influence the distribution kinetics of the solutes between the two phases. In relation to the equilibrium, the variables to consider are temperature, volume ratios (or extractant volume) and ionic force of the medium. In relation to the kinetics of the transfer of material between the two phases, in all partition equilibria one must ensure that equilibrium is attained, and consequently the variables to be considered in our technique would be time of extraction and rotation speed of the extractor.

Concerning the temperature, given that the extraction process is carried out at room temperature, which is virtually constant (around 25 °C), their effect on the equilibrium will be minimal. Concerning the time of extraction and rotation speed, which are dependent variables, we consider another variable that is independent, namely the number of stages (one stage = half a rotation).

In this study of the rotary method of extraction, our first objective was the selection of a suitable number of stages, for

which the volume of extracting agent and rotation speed were set at 60 ml and 1 rpm, respectively, given that in experiments performed previously, it was found that this volume and speed were sufficient to obtain good extraction yields, allowing for the extractant to pass completley from one end of the extractor body to the other. Hence the extraction test was performed in 200, 300 and 400 stages (Table 2) and it was concluded that equilibrium had been reached with 200 stages, the yields not being significantly improved with a larger numbers of stages. The convenience of carrying out a short number of extraction stages in order to reach the partition equilibrium led us to consider that, under these operational conditions, rapid transfer of material between the two phases takes place, owing on the one hand to the great similarity of the phenolic compounds to the extracting agent used and on the other to the suitability of the experimental device used for the extraction of these compounds. Further, it was noted that, for some of the compounds studied, an increase from 200 to 400 stages reduced the extraction yields, probably as a result of losses or degradation of the samples owing to the longer extraction time, which could be explained on the basis of the reactivity of these species, which have very reactive acidic and aldehydic groups.

The next step was to select the volume of extractant, for which the number of stages, already optimized at 200, and the speed of rotation, 1 rpm, were pre-set. It was found that the extraction yield (Table 2) increased considerably for nearly all the species studied with an increase in extractant volume from 60 to 100 ml, and that, in general, this increase was much greater on passing from 60 to 80 ml than on passing from 80 to 100 ml.

This can be explained by taking into account that an increase in the organic-to-aqueous phase ratio brings about an increase in the amount of species extracted into the organic phase as derived from the constancy of the equilibrium ratio. However, such an increase of the extractant volume may influence the mass transfer kinetics between the two phases in such a way that under these conditions (80 and 100 ml extractant volumes) the partition equilibrium may not have been attained for some of the polyphenolic species under study. Such a situation may arise either because a longer contact time is needed since the amount of matter to be transferred of the aqueous phase from the organic one is greater or, alternatively, from the point of view of the extraction device, because the imposed rotation speed does not allow the solvent to circulate completely from one end to the other through the extraction body. For this reason, in order to ensure attainment of the distribution equilibrium for all compounds investigated, two alternatives may be considered: first, to increase the number of extraction stages to guarantee attainment of equilibrium at the end of the extraction process even though in each of the individual stages the extraction may not attain its maximum value; and second, to reduce the turning speed of the extraction device in order to ensure maximum extraction for each of the individual extraction stages. In this latter option, maximum extraction could be obtained by the 200 extraction stages selected previously. Both options led to the same result, bringing about an increase in the extraction yield but with a longer time for the analysis, with a consequent greater possibility of degradation of the samples. We are therefore faced with the situation that it is essential to find a compromise between increasing the extraction yield and increasing the duration of the extraction process.

From all this, we can conclude that the optimum phase ratio is found with between 80 and 100 ml of extracting agent and that, even though the yields are better with 100 ml than with 80 ml, there is also an increase in the analytical artifacts that appear.

Once the volume of extractant had been selected, the next step was the selection of the rotation speed. Three rotation speeds were tried, 1, 0.8 and 0.6 rpm, having pre-set the number of stages at 200, and the volume of extracting agent at 80 ml. This series of experiments (Table 2) showed that, in general, there is a small increase in the recovery as the rotation speed is reduced from 1 to 0.6 rpm, although for some species there is a slight decrease in yield (and also an increase in the analytical artifacts seen), probably as a result of the degradation of the samples with the longer extraction time. For this reason, it was decided to adopt 0.8 rpm as the optimum rotation speed, given that the extraction time was not excessively long (2 h 5 min) and the yields were in general higher than those obtained at 1 rpm. Table 2 shows the yields obtained under these conditions.

Next to be studied was the influence of the ionic strength, for which a series of experiments identical with the previous one were performed, except that the matrix solution was saturated with sodium chloride.

The dissolution of an ionic salt such as sodium chloride in a polar solvent such as water lowers the effective number of water molecules available to effect dissolution of the different polyphenolic species in solution as derived from ionic solvation, hence a displacement of the partition equilibrium can be expected in the sense of increasing concentration of such compounds in the organic phase with increasing ionic strength. Such an effect was confirmed experimentally, the results obtained being coincident with those in previous experiments except that the yields obtained were generally considerably higher. The results obtained are given in Table 3.

Once we had completed the study to optimize the extraction conditions (200 stages, 80 ml of diethyl ether, 0.8 rpm and

Table 2 Extraction recovery (%) in the absence of NaCl

Number of stages	200	300	400	200	200	200	200
Extractant volume/ml	60	60	60	80	100	80	80
Rotation speed (rev min^{-1})	1	1	1	1	1	0.8	0.6
Gallic acid	5.3	4.9	4.1	8.4	13.6	8.7	16.3
Gentisic acid	63.4	64.9	65.3	65.9	79.6	83.1	77.7
m-Hydroxybenzoic acid	53.8	50.6	52.9	72.9	80.1	68.4	79.7
p-Coumaric acid	34.3	31.8	34.1	36.3	45.3	27.5	34.1
Protocatechuic acid	24.3	20.8	24.4	40.7	44.5	37.2	44.9
p-Hydroxybenzoic acid	45.9	40.9	43.5	72.3	71.8	73.2	76.2
Syringic acid	16.5	14.6	14.0	36.7	38.1	35.0	36.6
Veratric acid	36.4	33.1	37.4	73.3	62.6	67.4	58.0
Sinapic acid	12.2	8.4	12.5	23.6	24.1	22.2	21.9
Caffeic acid	39.5	35.2	35.9	26.9	60.3	27.9	36.3
m-Coumaric acid	82.2	76.7	78.9	74.0	92.7	83.1	85.4
Vanillic acid	44.5	45.8	46.4	65.4	65.4	64.4	60.8
Ferulic acid	47.6	40.6	40.4	57.5	67.6	41.2	40.8
o-Coumaric acid	77.8	63.6	65.6	87.9	84.3	40.2	51.5
3,5-Dimethoxybenzoic acid	59.9	71.1	66.7	86.2	96.6	91.5	83.4
3,4,5-Trimethoxycinnamic acid	46.7	40.2	39.2	70.3	72.3	60.5	57.1
Protocatequialdehyde	49.6	43.8	47.6	57.5	66.3	49.6	66.3
p-Hydroxybenzaldehyde	69.9	66.4	69.8	78.7	81.7	69.9	81.7
Vanillin	53.3	53.4	50.8	66.3	75.8	53.3	75.8
Syringaldehyde	31.9	27.6	30.1	38.7	49.8	31.9	49.8
o-Vanillin	78.8	70.9	73.2	93.1	89.6	78.8	89.6
2,5-Hydroxybenzaldehyde	67.3	69.9	67.7	74.6	82.2	66.0	80.3
Isovanillin	38.7	38.9	38.7	51.1	60.8	46.1	47.6
Veratraldehyde	44.0	44.7	44.7	57.7	67.1	57.7	48.4
2,4-Dimethoxy-Benzaldehyde	79.0	76.6	77.9	88.5	94.6	67.8	68.8
m-Hydroxybenzaldehyde	85.9	89.1	86.1	97.8	101.2	96.4	87.1
Salicylaldehyde	88.2	80.2	95.3	95.1	99.9	93.1	82.4
Anisaldehyde	79.8	76.6	80.3	88.3	92.1	83.7	79.3
3,4,5-Trimethoxy-benzaldehyde	55.5	55.0	56.3	68.6	76.3	55.4	61.9
3,5-Dimethoxy-benzaldehyde	93.9	86.9	95.5	95.3	98.8	91.7	81.9

saturation with sodium chloride) with the phenolic acid and aldehyde standards, we proceeded with the adaptation of the method to real wine samples.

Table 3 Extraction recovery (%) with NaCl-saturated solutions

Number of stages	200	300	400	200	200	200	200
Extractant volume/ml	60	60	60	80	100	80	80
Rotation speed (rpm)	1	1	1	1	1	0.8	0.6
Gallic acid	16.4	20.4	19.3	30.3	21.9	46.0	53.1
Gentisic acid	54.2	53.6	56.7	81.2	63.6	92.7	85.5
m-Hydroxybenzoic acid	81.0	81.9	80.9	92.2	99.0	93.4	96.0
p-Coumaric acid	39.2	40.3	41.1	46.0	44.1	33.2	44.8
Protocatechuic acid	79.8	86.0	81.7	80.1	77.7	82.2	90.8
p-Hydroxybenzoic acid	85.0	88.4	92.1	89.5	85.9	94.8	94.5
Syringic acid	92.0	86.4	88.9	77.7	82.0	85.9	93.0
Veratric acid	71.3	70.8	70.9	81.9	91.8	97.8	89.7
Sinapic acid	47.6	49.5	46.8	31.9	35.0	38.7	35.3
Caffeic acid	57.1	53.8	56.5	64.4	76.7	44.3	46.9
m-Coumaric acid	86.5	95.8	98.5	100.5	97.6	88.5	99.1
Vanillic acid	96.6	96.8	102.6	99.9	95.8	92.9	94.1
Ferulic acid	80.6	74.6	73.8	62.3	85.0	63.6	53.4
o-Coumaric acid	96.8	93.7	97.7	95.2	98.3	45.6	64.0
3,5-Dimethoxybenzoic acid	78.6	96.1	85.9	89.2	100.3	76.1	80.7
3,4,5-Trimethoxy-cinnamic acid	63.4	59.2	64.5	74.1	81.6	79.2	70.7
Protocatequialdehyde	86.8	83.9	74.6	62.2	70.0	50.0	47.9
p-Hydroxy-benzaldehyde	93.0	93.1	86.5	85.6	89.0	77.0	68.4
Vanillin	85.1	84.2	76.4	69.9	78.9	64.1	59.2
Syringaldehyde	78.2	75.1	63.2	49.7	68.7	49.1	46.5
o-Vanillin	93.4	85.8	64.6	63.8	67.1	54.3	63.4
2,5-Dihydroxy-benzaldehyde	18.9	15.8	25.0	46.4	52.7	48.0	85.7
Isovanillin	63.8	65.5	61.6	77.7	81.1	77.8	88.5
Veratraldehyde	81.7	81.9	83.3	73.0	89.9	88.8	75.9
2,4-Dimethoxy-benzaldehyde	77.5	81.3	77.7	41.3	96.4	94.9	65.7
m-Hydroxy-benzaldehyde	85.5	93.0	86.4	98.4	102.4	102.3	96.8
Salicylaldehyde	49.3	55.7	49.4	48.5	80.5	85.8	80.4
Anisaldehyde	79.8	80.6	77.6	87.2	93.2	94.3	91.5
3,4,5-Trimethoxy-benzaldehyde	75.8	78.7	73.9	73.3	82.9	86.2	68.5
3,5-Dimethoxy-benzaldehyde	69.7	65.3	65.5	87.0	96.2	97.7	85.7

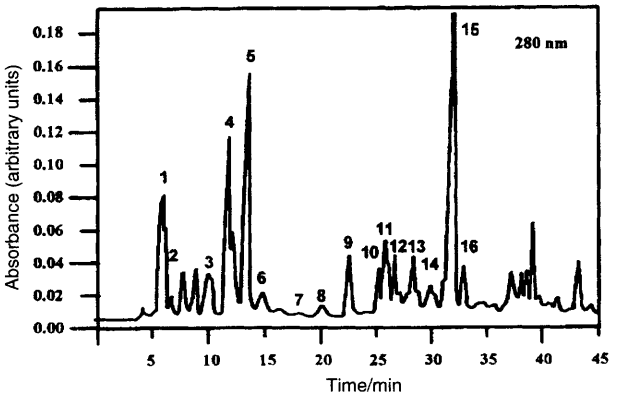

Fig. 2 Chromatogram corresponding to the rotary extraction of 100 ml of 'fino' sherry wine. Peaks: 1, gallic acid; 2, 5-hydroxymethylfurfural; 3, protocatechuic acid; 4, *trans*-caffeoyltartaric acid; 5, *cis*-*p*-coumaroyltartaric acid; 6, catechin; 7, *p*-hydroxybenzaldehyde; 8, chlorogenic acid; 9, caffeic acid; 10, syringic acid; 11, epigallocatechin gallate; 12, isovanillin; 13, *cis*-*p*-coumaric acid; 14, *trans*-*p*-coumaric acid; 15, *cis*-ferulic acid; and 16, *trans*-ferulic acid.

After a series of experiments with various volume ratios, dilution with water, different ionic strengths, *etc.*, in the sample of wine to be extracted, it was concluded that the optimum conditions for performing the extraction consist of extracting a volume of 100 ml of wine diluted 1 : 1 (v/v) with distilled water and brought to saturation with sodium chloride, extracting under the conditions described previously. Fig. 2 shows a chromatogram obtained from the rotary extraction of 100 ml of wine carried out according to the described conditions, injecting a volume of 20 μl with a gradient of polarity,[18] and with detection at 280 nm and at a sensitivity of 0.2 a.u.f.s.

As differential extraction had proved to be a much more convenient technique than conventional extraction[8] in its different alternatives (by stages, carrying out a preliminary sample preconcentration, *etc.*) for the extraction of low molecular mass phenolic compounds from wine samples, owing essentially to its good reproducibility and high yields, it was decided to carry out a correlation of the two extraction techniques. Fig. 3 shows the chromatogram obtained from the differential extraction (100 ml of the same wine, with 125 ml of diethyl ether and an extraction time of 3 h, injecting under identical conditions as before) and it can be seen that the peaks are smaller with differential extraction than with rotary

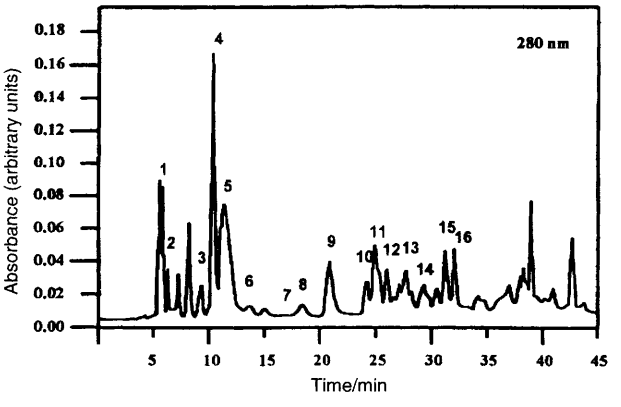

Fig. 3 Chromatogram corresponding to the differential extraction of 100 ml of 'fino' sherry wine. Peaks as in Fig. 2.

Table 4 Reproducibility and extraction recovery for standards

Standard	Extraction recovery (%)	s_r (%)
Gallic acid	40.7	6.11
Gentisic acid	89.9	4.53
m-Hydroxybenzoic acid	83.5	4.84
p-Coumaric acid	90.5	4.76
Protocatechuic acid	75.2	5.37
p-Hydroxybenzoic acid	90.9	5.01
Syringic acid	82.3	4.87
Veratric + ferulic acids	83.9	2.34
Sinapic acid	81.8	4.67
Caffeic acid	82.2	4.89
Vanillic acid	91.0	4.48
o-Coumaric acid	92.8	4.41
3,4,5-Trimethoxycinnamic acid	92.3	4.61
Protocatequialdehyde	84.8	1.52
p-Hydroxybenzaldehyde	88.5	5.20
Vanillin	81.5	5.57
Syringialdehyde	78.5	0.56
o-Vanillin	81.6	7.07
Veratraldehyde	81.0	4.91
3,5-Dimethoxybenzaldehyde	88.3	5.99
Catechin	68.5	4.76
Epicatechin	69.2	5.59
3,4,5-Trimethoxybenzaldehyde	83.9	4.85

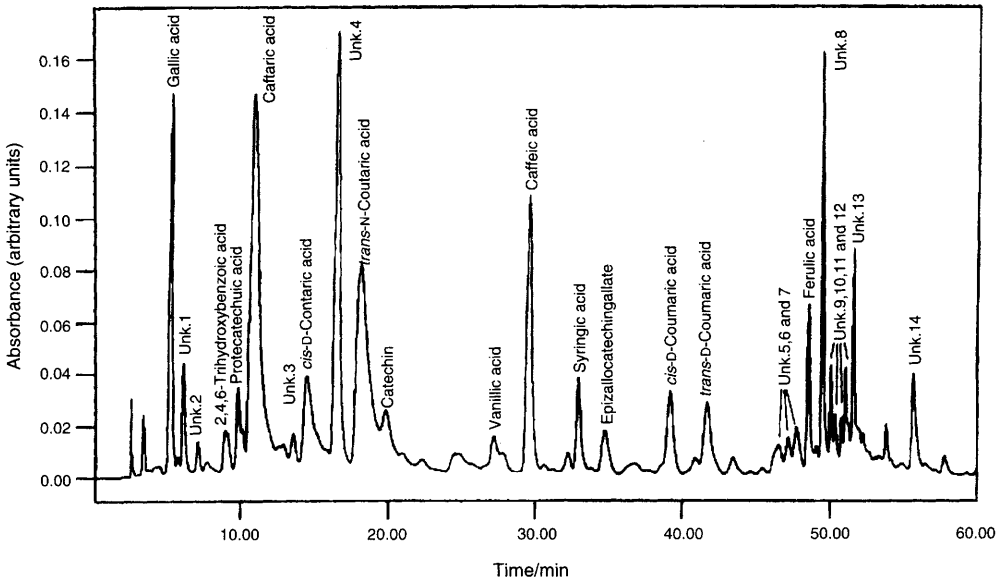

Fig. 4 Chromatogram corresponding to the extraction in the reproducibility study.

Table 5 Reproducibility for dry wine samples (%)

Compound	s_r (%) (areas)	Compound	s_r (%) (areas)
Gallic acid	7.12	Epigallocatechin gallate	12.32
Unknown 1	16.96	*cis-p*-Coumaric acid	6.92
Unknown 2	8.19	*trans-p*-Coumaric acid	10.89
2,4,6-Trihydroxy-benzoic acid	10.51	Unknown 5	14.46
Protocatechuic acid	7.74	Unknown 6	13.03
Caftaric acid	10.23	Unknown 7	10.41
Unknown 3	11.83	Ferulic acid	9.52
cis-p-Coutaric acid	9.22	Unknown 8	9.15
Unknown 4	7.24	Unknown 9	8.86
trans-p-Coutaric acid	9.35	Unknown 10	10.35
Catechin	8.03	Unknown 11	12.11
Vanillic acid	8.31	Unknown 12	9.46
Caffeic acid	8.89	Unknown 13	8.13
Syringic acid	11.81	Unknown 14	10.62

extraction. Further, taking into account that differential extraction requires a larger volume of extracting agent, prolonged heating of the extract at high temperature and with a longer extraction time, plus the impossibility of performing several extractions simultaneously, it is concluded that the rotary method is superior to the differential method. Fig. 4 shows one of the chromatograms corresponding to the extraction in the reproducibility study with dry wine samples.

Finally, we studied the reproducibility of this method of extraction under the conditions previously selected for the extraction of real samples of wine. New solutions of standards were prepared and, under the conditions described previously, six parallel extractions of the phenolic acid and aldehyde standards were performed (with peak-area measurement) and the relative standard deviation (s_r) for each of the species being studied was calculated (Table 4).

With respect to the extraction yields obtained, these are in general fairly high (above 70%), except for gallic acid (40%). With respect to the reproducibility of the extraction method, the s_r values fall mostly within the range 4–6% (with the exception of one of the standards), whereas those obtained in the study of reproducibility with dry wine samples (Table 5) are mostly between 8 and 10%.

The low s_r values obtained can be explained by taking into account that the experimental design used allows for the exact fixation of all the parameters related to the technique used (number of stages, extractant volume, rotation speed and ionic strength), further allowing for minimum manipulation of the extracts (drying, concentration and diluting to volume). Another advantage derives from the fact that no heating is applied to the samples, thereby reducing the possibility of their degradation.

Conclusions

A rotary liquid–liquid method for the extraction of phenolic compounds of low molecular mass from samples of wine was developed and optimized for practical use and has advantages over differential liquid–liquid extraction, such as higher reproducibility, very rapid production of extracts and the possibility of the simultaneous extraction of several samples (up to 12), while minimizing possibility of the appearance of analytical artifacts, as no heating is needed during the extraction process (as is the case with differential extraction). Further, the volume of wine needed to perform the rotary extraction is half that used in differential extraction but comparable peak areas and heights are obtained.

References

1 Barroso, C. G., Cela, R., Cobos, M. J. M., Brú, E. R., and Pérez-Bustamante, J. A., *Bull. Liaison–Groupe Polyphenols*, 1988, **14**, 332.
2 Barroso, C. G., Lopez-Sanchez, L., Otero, J. C., Cela, R., and Pérez-Bustamante, J. A., *Z. Lebensm.-Unters.-Forsch.*, 1989, **189**, 322.
3 Lee, C. Y., and Jaworski, A., *Bull. Liaison–Groupe Polyphenols*, 1986, **13**, 476.
4 Di Stefano, R., and García Moruno, E., *Vignevini*, 1986, **11**, 37.
5 Ferreres, F., Tomás-Barberan, F. A., Gil, M. I., and Tomás-Lorente, F., *J. Sci. Food Agric.*, 1991, **56**, 49.
6 Guillén, D. A., PhD Thesis, Universidad de Cádiz, 1994.
7 Oszmianski, J., Ramos, T., and Bourzeix, M., *Am. J. Enol. Vitic.*, 1988, **39**, 259.
8 Barroso, C. G., Pérez-Bustamante, J. A., Guillén, D. A., and Viseras, C., *Quím. Anal.*, 1986, **5**, 312.
9 Campos, M., Cavaleiro, C., Paranhos, A., Proença de Cunha, A., and Rauter, A., *Bull. Liaison–Groupe Polyphenols*, 1992, **16**, 59.
10 Mascré, M. H., *Ann. Pharm. Fr.*, 1957, **15**, 421.
11 Bengoechea, L., Gomez-Cordovés, C., and Hernandez, T., *Bull. Liaison–Groupe Polyphenols*, 1992, **16**, 88.
12 Gomes, E. T., *Bull. Liaison–Groupe Polyphenols*, 1992, **16**, 90.

13 Le Bon, A. M., Ziegler, L., Amiot, M. J., and Suschetet, M., *Bull. Liaison–Groupe Polyphenols*, 1992, **16**, 90.
14 Salagoity-Auguste, M. H., and Bertrand, A., *J. Sci. Food Agric.*, 1984, **35**, 1241.
15 Baldi, A., Romani, A., Mulinacci, N., Bambagiotti, M., and Vincieri, F. F., *Bull. Liaison–Groupe Polyphenols*, 1992, **16**, 60.
16 Barroso, C. G., Cela, R., and Pérez-Bustamante, J. A., *Ann. Bromat.*, 1985, **37**, 143.
17 Paris, R. R., and Nothis, A., *Plantes Méd. Phytothér.*, 1970, **4**, 63.
18 Barroso, C. G., Cela, R., and Pérez-Bustamante, J. A., *Chromatographia*, 1983, **17**, 249.
19 Guillén, D. A., Barroso, C. G., and Pérez-Bustamante, J. A., *J. Chromatogr.*, 1993, **655**, 227.

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