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Separation and identification of free phenolic acids in wines by high-performance liquid chromatography

G. P. CARTONI*, F. COCCIOLI and L. PONTELLI
Dipartimento di Chimica, Università "La Sapienza" di Roma, Piazzale Aldo Moro 5, 00185 Rome (Italy)
and
E. QUATTRUCCI
Ist. Naz. Nutr., Via Ardeatina 546, 00179, Rome (Italy)
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ABSTRACT

Free phenolic acids in Italian wines and a sherry were identified by high-performance liquid chromatography. The samples were concentrated and passed through a Sep-Pak C_{18} cartridge and the acids were recovered by elution with 2 ml of tetrahydrofuran. The separation was carried out by gradient elution on a reversed-phase column with methanol–water and phosphate buffer (pH 2.7). Detection was carried out 280 and 230 nm.

INTRODUCTION

The phenolic compounds in wine are very important as they contribute to the characteristic taste (astringency, bitterness), changing with age, and also possessing vitaminic and bacteriological effects [1]. These compounds are a heterogeneous group of substances consisting of several classes [2] (catechins, leucoanthocyanidins, flavonols, flavanol glicosides, high-molecular-weight tannins, hydrocinnamic acid–tartaric esters and their glucose esters [3], proanthocyanidins and anthocyanidins [4]). They include phenolic benzoic and phenolic cinnamic acids [5–7].

In this work the free phenolic acids in Italian wines and a sherry were identified by high-performance liquid chromatography (HPLC) with UV detection.

EXPERIMENTAL

Apparatus

An LKB two-pump gradient HPLC system was used. The LKB 2150 pump, based on the dual-piston principle, gives pulse-free flow from 5 ml/min to as low as 10 μl/min. The system employs a high-pressure mixing system (400 μl). A Jasco Model 100 VA variable-wavelength UV detector equipped with a 1-μl flow cell was used. A Rheodynemodel 7410 valve injector was fitted with an internal loop. A Perkin-Elmer

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Series 2 liquid chromatography with a Model LC 55 B variable-wavelength detector was also used.

Column 1 (165 mm × 4.6 mm I.D.) and column 2 (250 mm × 2.1 mm I.D.) both contained ODS_2 (5 μm). They were slurry-packed in the laboratory with Spherisorb obtained from Phase Separations (Norwalk, CT, U.S.A.) [8].

Reagents

Distilled water, stored in glass, was filtered and passed through a Norganic System Cartridge supplied by Millipore (Bedford, MA, U.S.A.) to remove organic substances.

The solvents used (methanol, acetic acid and tetrahydrofuran) were of HPLC grade (Carlo Erba, Milan, Italy). Phenolic acids were purchased from Fluka (Buchs, Switzerland). The cinnamic acids were in the *trans* form.

Phosphate buffer (pH 2.7) was prepared by adding 1.425 g of disodium hydrogenphosphate dihydrate to 4.58 ml of acetic and diluting to 1 l with distilled water. It was then filtered through a 0.45-μm Millipore filter. This stock solution was stored at 4°C.

Samples

The samples studied were a fino sherry (Tio Pepe), Verdicchio, Pinot Grigio, Fontana Candida and wine A (our production) white wines, Chianti red wine and a white vermouth.

Preparation of the wine sample

A 10-ml volume of wine sample was concentrated under vacuum to 3 ml at a temperature below 30°C fgor 20 min to remove ethanol, then transferred into a test-tube and 0.5 ml of phosphate buffer stock solution (pH 2.7) was added. This solution was passed through a Sep-Pak C_{18} cartridge that had previously been washed with methanol and subsequently with water, then the cartridge was washed with 2 ml of posphate buffer (pH 2.7) (1 ml of stock buffer solution diluted to 2 ml with distilled water). The phenolic acids were recovered by elution with 2 ml of tetrahydrofuran. The solvent was evaporated under nitrogen (below 30°C) and the residue was dissolved in 100 μl of methanol.

Preparation of standards

A 3-mg amount of each phenolic acid was dissolved in 3 ml of methanol. This solution was stored at −20°C in the dark for no longer than 2 months (gradual formation of *cis* isomers of cinnamic acids was observed on exposure to UV light) [9].

Under the elution conditions reported in Fig. 1, *trans* and *cis* isomers are well separated. The *cis* isomers are not present in nature.

Table I gives the absorption maxima for some of the phenolic acids [10]. The UV spectra were taken between 200 and 400 nm with the Perkin-Elmer LB 55 C detector by stopping the solvent flow and scanning the solute while stationary in the cell.

Each compound was injected in the isocratic mode at a mobile phase composition of methanol : water (buffer stock solution diluted 1:10) such as to obtain capacity factor, $k' > 2$.

TABLE I

ABSORPTION MAXIMA NM OF SOME PHENOLIC ACIDS

	λ_{max} (1) (nm)	λ_{max} (2) (nm)
Vanillic acid	221	260
Caffeic acid	295	315
Ferulic acid	321	
Sinapic acid	235	315
m-Coumaric acid	280	
o-Coumaric acid	276	325
p-Coumaric acid	309	
3,4,5-Trimethoxycinnamic acid	295	
3,5-Dimethoxybenzoic acid	249	297
Syringic acid	274	

RESULTS AND DISCUSSION

The chromatographic conditions in Table II and Fig. 1 were established through a preliminary study with a purified sample of wine.

Each phenolic acid was injected (as the single compound) at least twice. As the retention times obtained in gradient elution are not perfectly reproducible, identification of adjacent peaks was carried out by method of standard additions. The chromatogram of phenolic acids previously identified in wine was compared with each wine analysed at wavelengths of 280 and 320 nm. The recoveries of three phenolic acids added to distilled water, extracted and purified as described above were caffeic acid 81.1 ± 2%, ferulic acids 81.5 ± 2% and *o*-coumaric acid 86.4 ± 2%.

TABLE II

RETENTION TIMES OF THE PHENOLIC ACIDS INVESTIGATED

Systematic name	Trivial name	Retention time (min)
2,6-Dihydroxybenzoic acid	Resorcylic acid	6.0
2,4-Dihydroxybenzoic acid		10.0
3,4-Dihydroxybenzoic acid	Protocatechuid acid	10.1
3,4,5-Trihydroxybenzoic acid	Gallic acid	10.4
2,6-Dimethoxybenzoic acid		17.1
2-Hydroxybenzoic acid	Salicylic acid	22.0
4-Hydroxybenzoic acid		25.3
4-Hydroxy-3-methoxybenzoic acid	Vanillic acid	35.4
3,4-Dihydroxycinnamic acid	Caffeic acid	39.1
3,5-Dimethoxy-4-hydroxy acid	Syringic acid	39.1
trans-4-Hydroxycinnamic acid	*p*-Coumaric acid	46.4
trans-3-Hydroxycinnamic acid	*m*-Coumaric acid	54.7
4-Hydroxy-3-methoxycinnamic acid	Ferulic acid	54.7
3,4-Dimethoxybenzoic aicd	Veratric acid	57.1
3,5-Dimethoxy-4-hydroxycinnamic acid	Sinapic acid	59.5
trans-2-Hydroxycinnamic acid	*o*-Coumaric acid	64.4
3,5-Dimethoxybenzoic acid		68.5
3,4,5-Trimethoxycinnamic acid		73.6

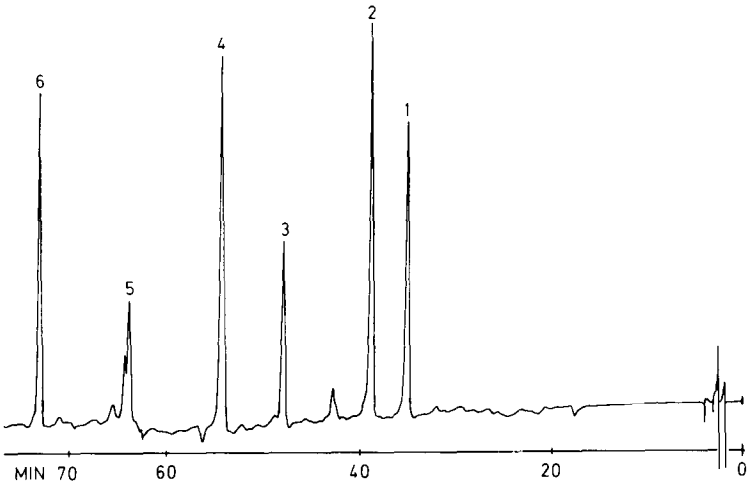

Fig. 1. Chromatogram of standard mixture detected at 280 nm. Peaks: 1 = vanillic acid; 2 = syringic acid; 3 = *p*-coumaric acid; 4 = *m*-coumaric acid; 5 = *o*-coumaric acid; 6 = 3,4,5-trimethoxycinnamic acid. Column, ODS_2 (250 mm × 2.1 mm I.D.); flow-rate 200 μl/min; mobile phase, (A) methanol–phosphate buffer (pH 2.7) (stock solution diluted 1:10) (95:5) and (B) phosphate buffer (pH 2.7) (stock solution diluted 1:10)–methanol (95:5) with the following gradient from B to A: 0 min, 0%; 36 min, 29%; 46 min, 29%; 72 min, 50%; and 80 min, 100% A.

Fig. 1 shows the elution of a standard mixture of phenolic acids (detected at 280 nm), prepared from the stock solution (1 μg/μl) of each phenolic acids in appropriate amounts according to the UV detector response.

Fig. 2 shows a chromatogram of a sample of Fontana Candida white wine and Fig. 3 that of a sample of white vermouth. Peaks K and F are unknown and are present in a larger amount in vermouth than in any of the other wines. The vermouth

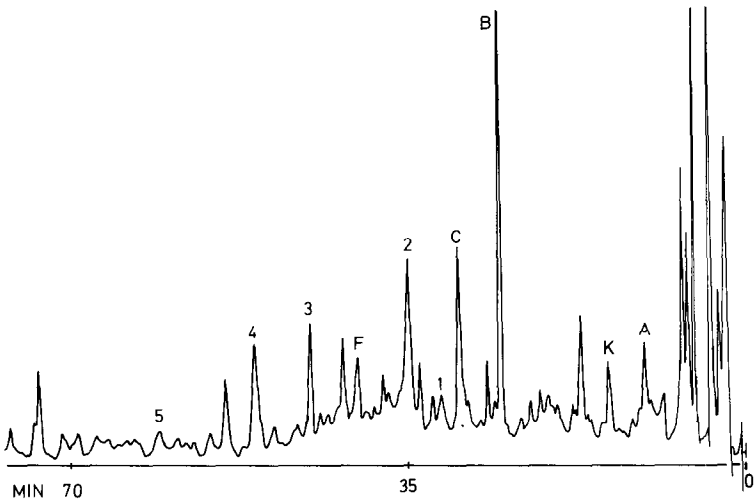

Fig. 2. Chromatogram of Fontana Candida white wine sample. Conditions as in Fig. 1 and peaks as in Table III.

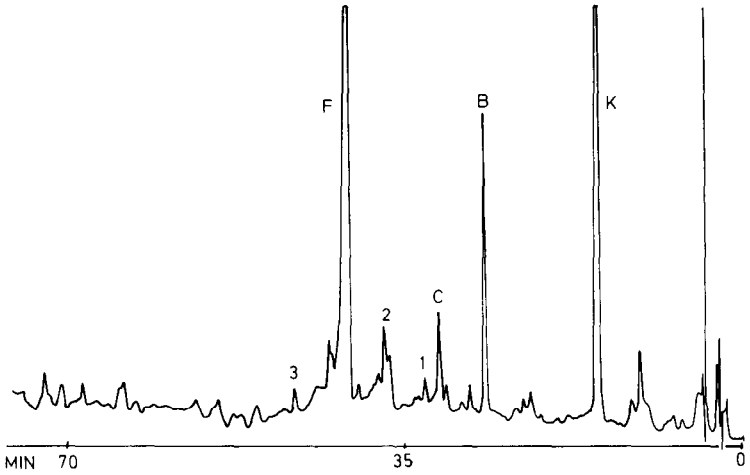

Fig. 3. Chromatogram of white vermouth sample. Conditions as in Fig. 1 and peaks as in Table III.

also contains substances from the extracts of aromatic herbs or plants, but it is not possible to establish the source of these two compounds.

Fig. 4 shows the chromatogram of the Fontana Candida whith wine at 320 nm and Fig. 5 that of the Chianti red wine. The chromatogram of red wine is more complex because larger amounts of flavonoids are present.

The chromatogram for vermouth in Fig. 6 shows that compound K does not absorb at 320 nm. Table III reports the free phenolic acids detected in the wines and vermouth at 280 and 320 nm.

Syringic acid is not resolved from caffeic acid but the former has maxima at 295

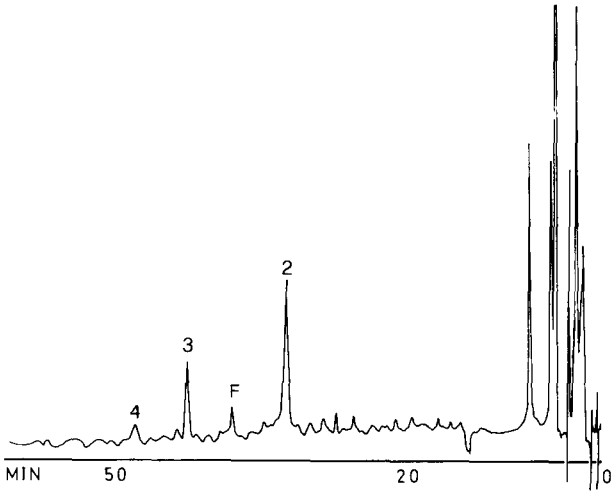

Fig. 4. Chromatogram of Fontana Candida white wine sample detected at 320 nm. Elution conditions as in Fig. 1 and peaks as in Table III.

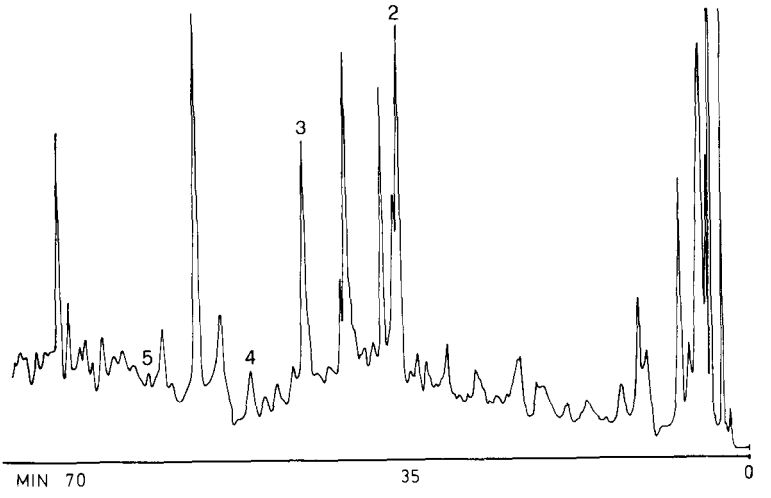

Fig. 5. Sample of Chianti detected at 320 nm. Elution conditions as in Fig. 1 and peaks as in Table III.

and 320 nm whereas former absorbs at 280 nm. Detection at 320 nm corresponds mainly to caffeic acids. Peaks C and F are characteristic of many wines but are unidentified; *M*-coumaric (absorption maximum at 280 nm) and ferulic acid (absorption maximum at 320 nm) are also not resolved.

The amounts of the acids are small and their determination is difficult, but their contribution to the conservation of wine and for the identification of hydroxycinnamic acid–tartaric acid esters are important.

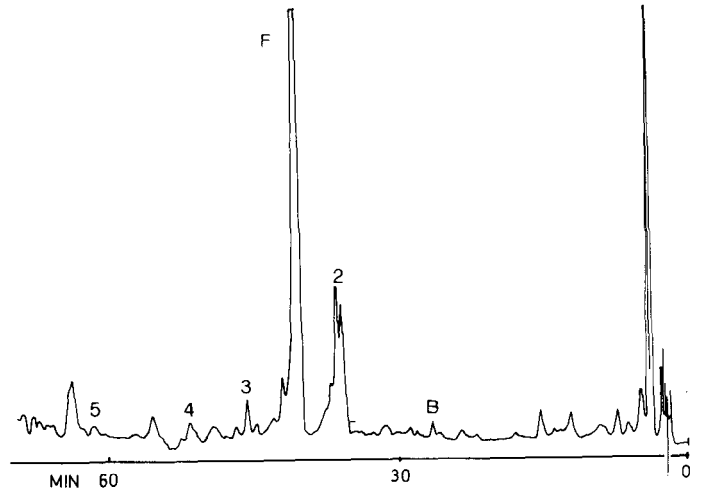

Fig. 6. Sample of vermouth detected at 320 nm. Elution conditions as in Fig. 1 and peaks as in Table III.

TABLE III

CHARACTERISTIC PEAKS OF WINES

+ = detected; − = not detected.

Wine	Gallic acid	4-Hydroxy-benzoic acid	Unknown	Vanillic acid	Syringic acid, caffeic acid	Unknown	*p*-Coumaric acid	*m*-Coumaric acid, ferulic acid
	A[a]	B[a]	C[a]	1[a]	2[a]	F[a]	3[a]	4[a]
Tio Pepe sherry	+	+	+	+	+	−	+	+
Verdicchio	+	+	+	+	+	+	+	+
Fontana Candida	+	+	+	+	+	+	+	+
Pinot Grigio	+	+	+	+	+	+	+	+
Wine A	+	+	+	+	+	+	+	+
Unbottled wine	−	+	−	+	+	+	+	+
Chianti	+	+	+	−	+	+	+	+
Vermouth	+	+	+	+	+	+	+	+

[a] Peak identification on chromatograms

REFERENCES

1 L. Paronetto, *Polifenoli e Tecnica Enologica*, Edagricole, Bologna, 1977.
2 M. L. R. Ramaraine and M. Tuchman, *J. Chromatogr. Sci.*, 24 (1985) 549–554
3 B. Y. Ong and C. W. Nagel, *J. Chromatogr.*, 157 (1978) 345–355.
4 S. M. Lunte, *J. Chromatogr.*, 384 (1987) 371–382.
5 G. Garcia Barroso, R. Cela Torrijos and J. A. Perez-Bustamante, *J. Chromatographia*, 17 (1983) 249–252.
6 D. A. Roston and P. Kissinger, *Anal. Chem.*, 53 (1981) 1695–1697.
7 F. Villeneuve, G. Abravanel, M. Moutonei and G. Albert, *J. Chromatogr.*, 234 (1982) 131–140.
8 M. Benincasa, G. P. Cartoni and F. Coccioli, *Ann. Chim. (Rome)*, 77 (1987) 801–811.
9 G. Kahnth, *Phytochemistry*, 6 (1967) 755–758.
10 M.-H. Salagoity-Auguste, C. Tricard and P. Sudraus, *J. Chromatogr.*, 392 (1987) 379–387.