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RAPID COMMUNICATION

Analysis of non-coloured phenolic compounds in red wines. A comparison of high-performance liquid chromatography and capillary zone electrophoresis

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Non-coloured phenolic compounds occurring in a Portuguese red wine were analysed qualitatively and quantitatively by HPLC and CZE. Both methods gave good agreements for most of the compounds detected, but CZE was less sensitive for the detection of flavonols, which were not fully resolved in the wine extract. 4-hydroxyphenethyl alcohol was separated and identified for the first time by CZE. It is concluded that CZE, while lacking the versatility of HPLC, may be regarded as a complementary analytical technique for these compounds.

INTRODUCTION

Phenolic compounds, such as phenolic acids, catechins and other flavonoids have an important role in wine quality; they contribute to sensory attributes and are also important in the colour chemistry of red wine during ageing (Singleton & Esau, 1969; Lea *et al.*, 1979). Their similar chemical characteristics, are the cause of many problems in the separation of these compounds. Many traditional techniques, such as paper chromatography, thin-layer chromatography and column chromatography, have now been superseded by high performance liquid chromatography (HPLC) (Lea *et al.*, 1979; Nagel *et al.*, 1979; Wulf & Nagel, 1976). Recently, capillary zone electrophoresis (CZE), a highly efficient separation technique, has been used to separate these compounds (Morin & Dreux, 1993; Bjergegaard *et al.*, 1992, Pietta *et al.*, 1991; Ferreres *et al.*, 1994; Seitz *et al.*, 1992; Ng *et al.*, 1992).

The aim of this work was to compare CZE analysis of non-coloured phenolic compounds from red wine, with HPLC analysis, which as far as we are aware, has not been done previously. For this work, we used a Portuguese red wine.

MATERIALS AND METHODS

Vinification

Wine was made from *Vitis vinifera* var. Roriz grapes, using standard vinification procedures, at Tua, Portugal. The wine was fermented in the presence of 150 mg litre^{-1} sulphur dioxide but the frequency of pumping-over the mash was doubled during fermentation, to produce a wine with an enhanced level of phenolics.

Extraction of wine phenolics

For each analytical technique, wine (5 ml) was extracted with diethyl ether (5 ml), for 3 min using a Whirlimixer. The ether layer was separated and evaporated to dryness using a nitrogen stream. For HPLC analysis, the residue was dissolved in a mixture of aqueous 0.6% perchloric acid containing 2% (v/v) methanol (1 ml) and for CZE, a separate extract was dissolved in methanol (0.5 ml).

HPLC analysis

Samples were filtered (0.45 μm) and analysed using a Hewlett Packard 1090 M Series II liquid chromatograph,

equipped with a diode array detector, an auto-injector (25 μl) and a ODS-Hypersil reversed phase column (100 × 2.1 mm, particle size 5 μm), at 40°C. Elution solvents were acidified water (0.6% perchloric acid) and methanol, with a flow rate of 0.3 ml min^{-1}, using a linear gradient starting at 2% methanol, increasing to 62% methanol after 60 min. Chromatograms were recorded at 280 nm. All analyses were done in duplicate and results expressed as the mean value.

CZE analysis

Separations were performed on a Beckman P/ACE System 5510 apparatus using a 50 cm × 75 μm I.D. fused silica capillary at 30°C with diode array detection at 280 nm. The running buffer was 0.1-M sodium borate (pH 9.5), with a voltage of 20 kV and an average current of 97 μA. Samples were injected by hydrodynamic injection for 2 sec.

The capillary was conditioned daily by washing with methanol for 5 min followed by freshly prepared 1-M sodium hydroxide (5 min), 0.1-M sodium hydroxide (5 min), distilled water (3 min) and fresh electrophoretic buffer (3 min). In order to optimise migration time and peak shape reproducibility, the capillary was flushed between analyses with 0.1-M sodium hydroxide (3 min) and distilled water (2 min). All analyses were performed in duplicate and the results expressed as mean values.

Identification of non-coloured phenolics

Phenolic compounds were identified and quantified by comparison of retention times and UV spectra using the following pure compounds: 4-hydroxyphenethyl alcohol (tyrosol), (−)epicatechin, (+)-catechin, syringic acid, *p*-coumaric acid, vanillic acid, caffeic acid, gallic acid, 3,4-dihydroxybenzoic acid, quercetin, myricetin and kaempferol (Sigma, St. Louis, MO, USA), (−)-epigallocatechin (Extrasynthese, Genay, France). *Cis*-coumaroyl tartaric acid (*cis*-COUTA), *trans*-coumaroyl tartaric acid (*trans* -COUTA), *cis*-caffeoyl tartaric acid (*cis*-CAFTA) and isorhamnetin were isolated in our laboratories.

RESULTS AND DISCUSSION

Gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxyphenethyl alcohol, *cis*-CAFTA, (+)-catechin, vanillic acid, syringic acid, *p*-coumaric acid and (−)-epicatechin separated well and were characterised by both HPLC and CZE (Figs 1 and 2). The order of elution differed according to technique; the separation of phenolics by

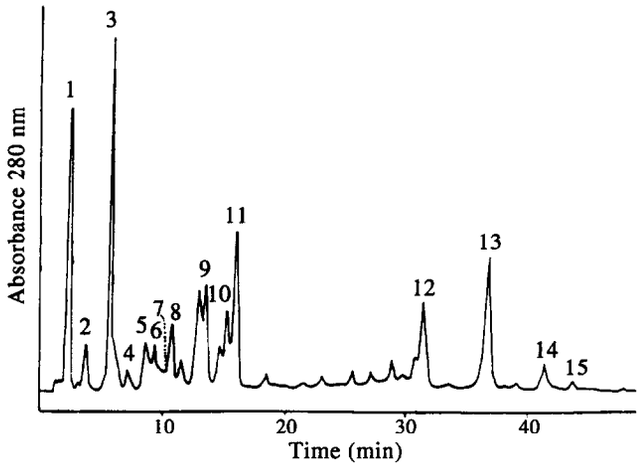

Fig. 1. HPLC chromatogram of red wine phenolic compounds. (1) Gallic acid, (2) 3,4-dihydroxybenzoic acid, (3) 4-hydroxyphenethyl alcohol, (4) *cis*-CAFTA, (5) (+)-catechin, (6) vanillic acid, (7) *trans*-COUTA, (8) caffeic acid, (9) syringic acid, (10) *p*-coumaric acid, (11) (−)-epicatechin, (12) myricetin, (13) quercetin, (14) kaempferol, and (15) isorhamnetin.

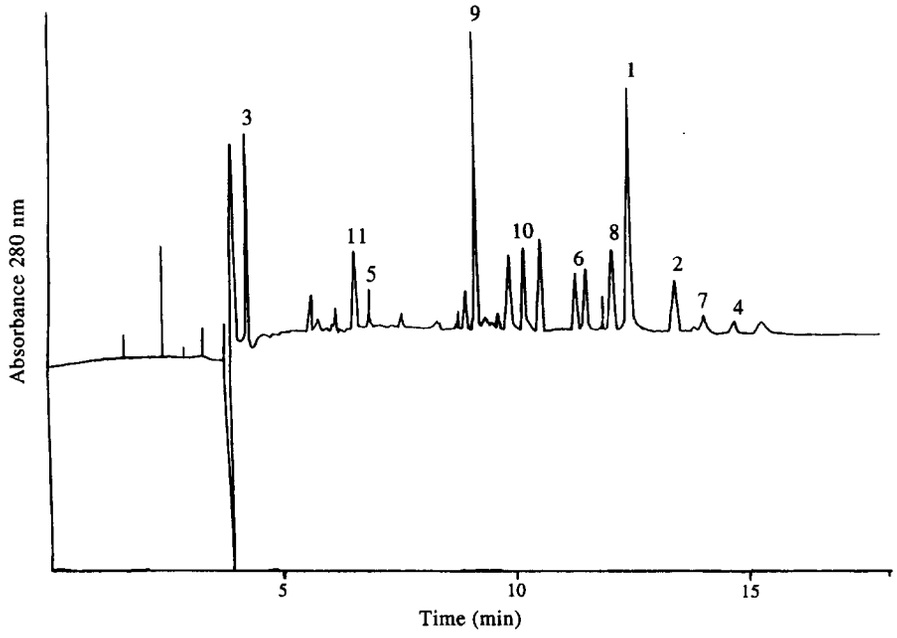

Fig. 2. CZE electropherogram of red wine phenolic compounds. Numbers as in Fig. 1.

Table 1. Comparation of quantitative determination of phenolic compounds in red wine by HPLC and CZE

Peak number	Compound	HPLC	(SD)	CZE	(SD)
1	Gallic acid	25.0	(0.62)	29.0	(2.84)
2	3,4-Dihydroxy benzoic acid	6.7	(0.27)	9.0	(0.75)
3	4-Hydroxyphenethyl alcohol	105.8	(5.81)	104.0	(0.18×10^{-7})
4	*cis*-CAFTA	2.1	(0.51)	NQ	
5	(+)-Catechin	1.7	(0.02)	6.4	(0.98×10^{-7})
6	Vanillic acid	8.0	(1.60)	8.2	(0.41)
7	*trans*-COUTA	NQ		0.5	(0.32×10^{-7})
8	Caffeic acid	5.4	(0.03)	26.0	(2.78)
9	Syringic acid	8.4	(0.27)	4.0	(0.50)
10	*p*-Coumaric acid	4.0	(0.30)	3.2	(0.29)
11	(−)-Epicatechin	7.7	(0.06)	21.2	(0.10×10^{-7})
12	Myricetin	27.0	(1.02)	-	
13	Quercetin	51.4	(1.25)	-	
14	Kaempferol	0.4	(0.02)	-	
15	Isorhamnetin	3.2	(0.90)	-	

Results are mean values of two determinations, expressed as mg litre^{-1}. (SD) Standard deviation. (NQ) Not quantified. (–) Not detected.

reversed phase HPLC depends mainly upon the polarity of the eluate and the eluent, while the rather more complex rationale for the order of separation of non-coloured wine phenols by CZE, has been discussed elsewhere (Gil *et al.*, 1995).

Trans-COUTA could not be separated satisfactorily from vanillic acid by HPLC under our conditions and caffeic acid was not well resolved from a closely eluting unidentified compound using CZE. Also, the flavonols, myricetin, quercetin, kaempferol and isorhamnetin detected and identified by HPLC, were not detected by CZE. This discrepancy may be due to a sensitivity effect with CZE. Although the detection wavelength used was not the optimum for these compounds (c. 260 nm or c. 360 nm), we do not consider this to be a major factor. The deficiency in detection is more likely to be resolved by replacing the standard detector cell with a capillary having a Z-shaped cell, a modification known to produce greatly enhanced sensitivity (Dr T. K. McGhie, personal communication).

Both techniques showed minor anomalies in quantitation for certain wine phenolics (Table 1). (+)-Catechin and (−)-epicatechin gave greater values in CZE analysis than HPLC, but standards of these compounds were not well duplicated by CZE, although the results of their quantitation in the wine extract gave good duplication. The reason for this discrepancy is not clear at present, but it may possibly be due to the pH used in this technique. Inconsistencies in the results for caffeic acid (CZE) and syringic acid (HPLC) may be due to co-elution problems.

The comparative merits of HPLC and CZE have been extensively discussed in recent publications, mostly with special reference to the analysis of pharmaceutical products (Altria & Rogan, 1994; Steuer *et al.*, 1990, Altria & Dave, 1993). Principally, however, it appears that for these applications HPLC is superior in terms of accuracy, sensitivity and precision. It can also be used successfully in a preparative mode. CZE, however, gives better separation efficiency and has both practical and economic advantages (e.g. use over a wide pH range, minimal set-up time and low operational cost).

The main practical observations noted during this work, were that CZE halved the analysis time, but required at least double the sample concentration to achieve comparable sensitivity with HPLC. HPLC is the more rugged method and is more versatile in terms of stationary phase, organic modifier and temperature. The lack, in CZE, of a technique equivalent to HPLC gradient elution, means that CZE cannot separate all the sample components in a comparable manner. The effect of high pH analytical conditions in CZE may also have implications in the analysis of certain wine phenolics.

Thus we conclude that while HPLC can be applied successfully to the analysis of non-coloured phenolics in wines, CZE may also have a future in this field as a complementary technique to HPLC, producing a better separation of *cis–trans*-isomers and, in our experience, better peak shapes. However, since migration-reproducibility in CZE depends on factors such as buffer ionic strength, age of the capillary, nature and frequency of previous capillary treatments, applied voltage and external capillary temperature, an exhaustive testing of the variables involved in this technique may produce results which compare more favourably with HPLC.

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