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Quantitative analysis of 4-ethylphenol and 4-ethylguaiacol in red wine

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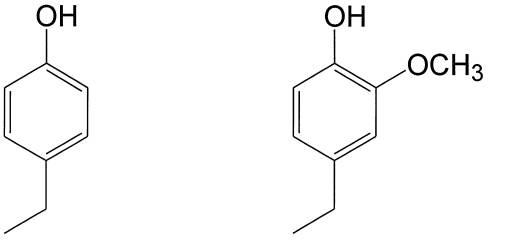
Abstract

2,3,5,6-[$^2H_4$]-4-Ethylphenol (d_4-4-ethylphenol) was synthesised for use as an internal standard in a new, rapid and accurate analytical method, employing gas chromatography–mass spectrometry to determine the concentration of the important aroma compounds 4-ethylphenol and 4-ethylguaiacol in red wine. The concentrations of both compounds in wine stored in 44 American and 47 French new and used oak barrels from several suppliers were measured. Wine stored in shaved and refired oak barrels contained up to 85% less 4-ethylphenol and 4-ethylguaiacol than wine stored in normal barrels of the same age that were not shaved. The concentration of 4-ethylphenol found in 61 bottled commercial Australian red wines of various ages ranged from 2 μg/l in a Merlot up to 2660 μg/l in a Shiraz, with a mean concentration of 795 μg/l. 4-Ethylguaiacol was also detected in every red wine analysed, ranging in concentration from 1 μg/l (in a Pinot Noir) up to 437 μg/l (in a Merlot) with a mean concentration of 99 μg/l.

Keywords: Wine; 4-Ethylphenol; 4-Ethylguaiacol

1. Introduction

The yeast *Brettanomyces/Dekkera* has been described as ''one of the most complex and controversial issues encountered in the making of red wine'' [1]. Among the aroma compounds produced in red wines by these yeasts are the volatile phenols, 4-ethylphenol and 4-ethylguaiacol (Fig. 1), which are formed from grape-derived *p*-coumaric acid and ferulic acid, respectively [2–5]. The aroma associated with 4-ethylphenol in red wine has been varyingly described as ''horsy'', ''leather'', ''medicinal'', ''smoky'', ''barnyard'', ''animal'' and ''sweaty saddle''-like [1,2,6,7]. 4-Ethylguaiacol in wine has a smoky, spicy, clove-like aroma [1,2,6,8–10]. At higher concentrations these odours can be undesirable, especially those from 4-ethylphenol [1,2,6]. While 4-ethylphenol and 4-ethylguaiacol are appar-



4-Ethylphenol **4-Ethylguaiacol**

Fig. 1. Structures of 4-ethylphenol and 4-ethylguaiacol.

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ently ubiquitous in red wine, the concentration found can vary considerably [2,3,11]. This paper describes a new method, which is both fast and accurate, for the analysis of 4-ethylphenol and 4-ethylguaiacol in wines to assist winemakers in determining how winemaking and storage processes affect the concentration of these compounds.

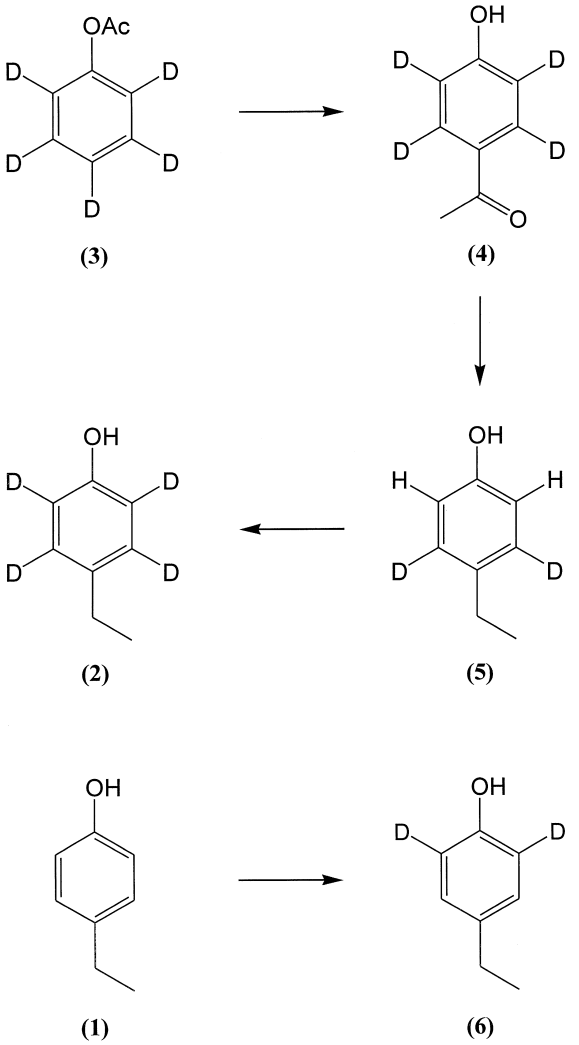
2. Experimental

Nuclear magnetic resonance (NMR) spectra were of deuterochloroform solutions and were obtained with a Jeol FX90Q spectrometer operating at 90 MHz.

2.1. Synthesis of 2,3,5,6-[$^2H_4$]-4-ethylphenol (Scheme 1)

Aluminium trichloride (15.73 g) was added to a carbon disulphide (15.7 ml) solution of [$^2H_5$]-phenylacetate (7.87 g, prepared in 96% yield from [$^2H_6$]-phenol, Aldrich, 98% $^2H_6$, in pyridine and acetic anhydride at room temperature), and the mixture was stirred at reflux for 70 min. The solvent was distilled off, and the residue was then stirred at 130°C for 3 h. The product was cooled, hydrochloric acid (6 *M*, 8 ml) then water (100 ml) were added and the mixture left to stand overnight. The aqueous solution was extracted with diethyl ether–pentane (1:4, 4×50 ml) to remove 2-hydroxyacetophenone. Sodium chloride (40 g) was then added to the aqueous solution, and the crude 4-hydroxyacetophenone (4.43 g) was recovered with diethyl ether (4×50 ml). The crude product was purified by flash chromatography under standard conditions [12] followed by recrystallization from diethyl ether–pentane (1:1) to give 2,3,5,6-[$^2H_4$]-4-hydroxyacetophenone (**4**, 3.72 g); NMR (δ) 2.55 (s); *m*/*z* 140 (M^+, 37%), 139 (6%), 126 (9%), 125 (100%), 124 (14%), 97 (26%), 96 (6%), 69 (16%).

Potassium hydroxide (2.93 g), followed by 2,3,5,6-[$^2H_4$]-4-hydroxyacetophenone (**4**, 1.22 g), then hydrazine hydrate (>99%, 1.27 ml) was added to diethylene glycol (15 ml) under nitrogen at room temperature. The reaction mixture was stirred for 15 min at room temperature, then at 130°C for 1 h. The temperature was further raised to 200°C over 30 min and maintained for 3 h. The reaction mixture was then cooled in an ice bath, acidified with hydrochloric acid (0.1 *M*) to pH<3. The volume was adjusted to 500 ml by the addition of water, and the product (**5**, 0.92 g) was recovered with *n*-pentane; NMR (δ) 1.20 (t, *J*=7 Hz, 3H), 2.58 (q, *J*=7 Hz, 2H), 6.73 (s, 2H); *m*/*z* 124 (M^+, 35%), 123 (5%), 110 (21%), 109 (100%), 108 (9%), 79 (9%), 78 (8%). A portion



Scheme 1. Formation of deuterium-labelled analogues of 4-ethylphenol (**1**).

(852 mg) of this product was placed in a 60-ml capacity thick-walled glass ampoule. Deuterium oxide (50 ml), then thionyl chloride (1.7 ml) were added, the ampoule was sealed, and the contents heated to 100°C for 5 days. The solution was neutralised with potassium carbonate (4.2 g), the product (830 mg) isolated with pentane and redistilled in vacuo to yield pure 2,3,5,6-[$^2H_4$]-4-ethylphenol (**2**) as a clear liquid; *m/z* 126 (M^+, 32%), 125 (4%), 112 (10%), 111 (100%), 110 (8%), 81 (4%), 80 (8%), 79 (5%). When unlabelled 4-ethylphenol (**1**) was treated with deuterium oxide and thionyl chloride under identical conditions, the $^2H_2$ analogue (**6**) was obtained; *m/z* 124 (M^+, 37%), 123 (5%), 110 (20%), 109 (100%), 108 (8%), 79 (8%), 78 (5%); NMR (δ) 1.20 (t, J=7 Hz, 3H), 2.58 (q, J=7 Hz, 2H), 7.02 (s, 2H); cf. unlabelled 4-ethylphenol (**1**), 1.20 (t, J=7 Hz, 3H), 2.58 (q, J=7 Hz, 2H), 6.73 (AA′ of AA′BB′, 2H), 7.05 (BB′ of AA′BB′, 2H).

2.2. Preparation of samples for analysis

A solution of [$^2H_4$]-4-ethylphenol (2.34 μg) in ethanol (100 μl) was added to the wine sample (5 ml) in a screw cap vial using a glass syringe (100 μl Hamilton). Diethyl ether–pentane (1:2, 2 ml) was added and the mixture was shaken briefly. A portion of the organic layer was then transferred to a vial ready for instrumental analysis.

2.3. Instrumental analyses

The organic extracts were analysed by gas chromatography–mass spectrometry (GC–MS) as described previously [13]. The unlabelled 4-ethylphenol and 4-ethylguaiacol used as standards were purchased from Aldrich. For quantification of 4-ethylphenol and 4-ethylguaiacol, mass spectra were recorded in the selective ion monitoring (SIM) mode. The ions monitored in SIM runs were: *m/z* 111 and 126 for [$^2H_4$]-4-ethylphenol (internal standard), *m/z* 107 and 122 for 4-ethylphenol and *m/z* 122, 137 and 152 for 4-ethylguaiacol. Selected fragment ions were monitored for 40 ms each. The ions used for quantitation were: *m/z* 126 for [$^2H_4$]-4-ethylphenol (internal standard), *m/z* 122 for 4-ethylphenol and *m/z* 152 for 4-ethylguaiacol. The other ions were used as qualifiers.

2.4. Validation

The method was validated by a series of duplicate standard additions (0 to 5000 μg/l 4-ethylphenol and 4-ethylguaiacol, $N=10\times2$ for both compounds) to model wine (10.0% ethanol, adjusted to pH 3.4 with potassium hydrogen tartrate and tartaric acid), white wine (1996 Chardonnay, 13.1% ethanol, pH 3.4) and red wine (1994 Shiraz, 12.6% ethanol, pH 3.4). The standard addition curves obtained were linear throughout the concentration range, with the following coefficients of determination and linear regression equations: $r^2=0.999$ for 4-ethylphenol in red ($y=1.07x+0.02$) and white ($y=1.07x$) wines, $r^2=1.000$ ($y=1.18x$) for 4-ethylphenol in model wine, $r^2=1.000$ ($y=1.21x+0.004$) for 4-ethylguaiacol in red wine and $r^2=0.999$ for 4-ethylguaiacol in white ($y=1.19x$) and model ($y=1.18x$) wines. Because all red wines apparently contain 4-ethylphenol (the lowest level of 4-ethylphenol found in a bulk red wine was 55 μg/l), the repeatability of the analysis at the lower end of the concentration range (10 μg/l) was determined by spiking seven replicate aliquots of model wine. The relative standard deviation (RSD) in the model wine was 2.73% for 4-ethylphenol and 1.27% for 4-ethylguaiacol. For seven replicate analyses of the bulk red wine (1995, 10.0% ethanol, pH 3.05, mean concentration of 4-ethylphenol and 4-ethylguaiacol of 55 μg/l and 10 μg/l, respectively), the RSD was 1.35% for 4-ethylphenol and 1.31% for 4-ethylguaiacol. The repeatability of the analysis was also determined at 2000 μg/l by spiking a separate bottled red wine in septuplicate, giving a RSD of 0.75% for 4-ethylphenol and 0.63% for 4-ethylguaiacol. The same red wine was also used for the standard addition curves above and contained 65 μg/l 4-ethylphenol and 23 μg/l 4-ethylguaiacol.

2.5. Investigation into deuterium exchange during chromatography

Separate solutions (diluted to ca. 0.5 mg/ml in dichloromethane) of [$^2H_4$]-4-ethylphenol (**2**) and unlabelled 4-ethylphenol (**1**) were analysed at the

start, middle and end of a 200 run sequence of wine extracts. They were injected with the injector block temperature set at 200°C, 220°C and 250°C on each occasion (GC conditions were as above). The ions monitored were *m/z* 118, 119, 120, 121, 122 for 4-ethylphenol (**1**) and *m/z* 122, 123, 124, 125 and 126 for [$^2H_4$]-4-ethylphenol (**2**).

2.6. Determination of 4-ethylphenol and 4-ethylguaiacol in red wine aged in French and American oak barrels of different ages

All new oak barrels used in the trial, were ''medium toast''. The main 1998 Shiraz red wine was pH 3.53, titratable acidity=6.7 g/l (expressed as tartaric acid), alcohol=13.7%, total SO_2 approx. 40 ppm, (no SO_2 added prefermentation). Details of other wines are given in the text or in Table 1.

3. Results and discussion

3.1. Synthesis of 2,3,5,6-[$^2H_4$]-4-ethylphenol (2)

2,3,5,6-[$^2H_4$]-4-Ethylphenol (**2**) was prepared from the acetate of polydeuterated phenol (**3**) via the Fries rearrangement followed by Wolff Kischner reduction (Scheme 1). The formation of deuterium-labelled 4-hydroxyacetophenone (**4**) was accompanied by a loss of slightly more than one deuterium ($^2H_4$:$^2H_3$, 4:1). Small singlets in the NMR spectrum of **4** at δ 6.92 and 7.87 in a ratio of 3:1 indicated that more deuterium was lost from the position *ortho* to the phenol group than from the *meta*-position. Reduction of the side chain of [$^2H_4$]-4-hydroxyacetophenone (**4**) to give labelled 4-ethylphenol (**5**) was accompanied by complete exchange of two of the deuterium atoms which were easily reintroduced by back-exchange with deuterium oxide under

Table 1
Determination of 4-ethylphenol and 4-ethylguaiacol in red wines aged in French and American oak barrels of different ages[a]

Oak origin	Previous barrel use	4-Ethylphenol		4-Ethylguaiacol	
		Concentration (μg/l)	RSD (%)	Concentration (μg/l)	RSD (%)
1998 Cabernet Sauvignon/Shiraz[b]					
American oak	New	201	6	24	4
American oak	Used once	391	8	35	10
1998 Shiraz[c]					
American oak	Used twice	563	5	31	3
American oak	Used 3 times	505	9	31	10
American oak	Used 4 times	555	11	31	13
American oak (re-shaved and fired 1999)	Used 4 times	95	29	5	30
1998 Shiraz[d]					
French oak	New	540	24	34	19
French oak	Used once	500	8	28	6
French oak	Used twice	499	15	33	14
French oak	Used 3 times	514	8	24	6
French oak (re-shaved and fired 1999)	Used 3 times	401	14	23	10

[a] Concentrations shown are the mean from the analysis of five barrels and are expressed in μg/l of red wine. All wines had matured in the barrels for approximately 8 months. Wine samples (5 ml) were taken and the internal standard added immediately on site.

[b] 1998 Cabernet Sauvignon/Shiraz, pH 3.52, titratable acidity=7.2 g/l (expressed as tartaric acid), alcohol=14.3%, total SO_2 approx 35 ppm (no SO_2 added pre fermentation).

[c] 1998 Shiraz, pH 3.55, titratable acidity=6.8 g/l (expressed as tartaric acid), alcohol=14.3%, total SO_2 approx 40 ppm (no SO_2 added pre fermentation).

[d] 1998 Shiraz, pH 3.53, titratable acidity=6.7 g/l (expressed as tartaric acid), alcohol=13.7%, total SO_2 approx 40 ppm (no SO_2 added pre fermentation).

strongly acidic conditions. The [$^2H_4$]-analogue (**2**) could not be prepared from unlabelled 4-ethylphenol (**1**) directly, as only two of the aromatic protons (presumably those *ortho* to the phenol group, as shown in Scheme 1) were exchangeable under strongly acidic conditions.

3.2. Investigation into deuterium exchange during sample preparation and chromatography

The ratios of the molecular ions, *m/z* 126, 125, 124, 123, 122 of [$^2H_4$]-4-ethylphenol (internal standard) remained the same before and after extraction; thus there was no back exchange into the wine during sample preparation. Indeed, during the synthesis, strong acid and high temperature for a prolonged period was required for exchange *ortho* to the phenol group, and the other hydrogens were not exchanged at all under these conditions. Solutions of [$^2H_4$]-4-ethylphenol and unlabelled 4-ethylphenol were also analysed separately at the start, middle and end of a 200 run sequence of wine extracts. They were injected with the injector block temperature set at 200°C, 220°C and 250°C on each occasion. For all the analyses there was no significant difference in the ratios of the ions monitored for either compound at all injection temperatures. Thus no significant deuterium exchange occurred during sample preparation, injection or chromatography.

3.3. The analytical method

Using [$^2H_4$]-4-ethylphenol as internal standard, 4-ethylphenol and 4-ethylguaiacol in wine could be quantified in pentane–diethyl ether (2:1) extracts at concentrations down to 1 μg/l and often lower. The internal standard [$^2H_4$]-4-ethylphenol differs from 4-ethylguaiacol in volatility and in solubility in non-polar solvents, as evidenced by experiments using solid-phase microextraction (SPME) for the analysis or employing pentane as an extracting solvent (data not shown). Nevertheless, under the conditions described in this paper [extraction with pentane–diethyl ether (2:1)], the similarity in behaviour between 4-ethylguaiacol and [$^2H_4$]-4-ethylphenol was such that the precision and accuracy for determining 4-ethylguaiacol were as good as those for 4-ethylphenol. Under conditions recently developed for oak lactone analysis [13], 4-ethylphenol could be determined with equal sensitivity by SPME of the headspace above the wine. However, because of differences in volatility between analyte and standard, the SPME method was less reliable for 4-ethylguaiacol.

The relative intensity of mass spectral fragments for fixed concentrations of [$^2H_4$]-4-ethylphenol, unlabelled 4-ethylphenol and 4-ethylguaiacol varied according to the instrumental operating conditions. It is therefore important to determine the relative molar ion response factors for standard solutions of all three compounds under the same instrumental conditions as employed for the analyses of each set of wine samples.

There are several published methods for determining volatile phenols in wine (e.g., Refs. [2,3,7,11,14]), but the advantages of the method described in this paper are that it is precise, accurate, has low detection limits, uses only 5 ml of wine, and sample preparation takes just a few minutes.

Furthermore the analyses for 4-ethylphenol and 4-ethylguaiacol can be combined with those for other oak volatile compounds, e.g., *cis*- and *trans*-oak lactone [13], vanillin [15], vanillyl ethyl ether [16], guaiacol and 4-methylguaiacol (unpublished data), i.e., the compounds analysed in this manner need not be of similar chemical structures, providing that isotopically labelled analogues are used as standards.

3.4. Determination of 4-ethylphenol and 4-ethylguaiacol in red wine aged in French and American oak barrels of different ages

The concentrations of 4-ethylphenol and 4-ethylguaiacol in red wine aged in new and used French and American oak barrels are shown in Table 1. Coopers were instructed to give all these barrels a medium toast at the time of firing. All the red wines were fermented on skins for 5–7 days and went through partial malolactic fermentation prior to going into the barrel. It is not certain that all the 4-ethylphenol was generated only in the barrel, as measurements of the wine were not taken prior to storage. Nevertheless, some differences between groups of barrels were observed.

A Cabernet Sauvignon/Shiraz red wine blend matured in new American oak had significantly less 4-ethylphenol and 4-ethylguaiacol (201 μg/l and 24 μg/l, respectively) than the same blend aged in barrels previously used once (391 μg/l and 35 μg/l, respectively). A Shiraz red wine stored in American oak barrels, previously used two to four times, had a mean 4-ethylphenol concentration of 541 μg/l, with no significant difference in the level of 4-ethylphenol between the barrels previously used two, three or four times. However, shaving and firing the barrels previously used four times resulted in a substantial decrease (of over 80%) in the concentration of 4-ethylphenol and 4-ethylguaiacol in the stored wine.

For the French oak, the previous usage of the barrels had no significant effect on the amount of 4-ethylphenol or 4-ethylguaiacol found in the wine. However shaving and refiring the barrels previously used three times resulted in a reduction of the mean 4-ethylphenol concentration by over 20% (from 514 μg/l to 401 μg/l).

The effects of shaving and refiring old French and American oak barrels can be attributed to a reduction of the microbiological load on the inner surface of the barrel, prior to use [2,3].

As a separate trial, the same 1998 Shiraz red wine was aged in 19 different types of new 300-l oak barrels of either French or American origin, made by 11 different cooperages. Two barrels of each type were analysed by the method. There were similar amounts of 4-ethylphenol (range 385–680 μg/l) and 4-ethylguaiacol (range 28–45 μg/l) found in all of the barrels. The mean 4-ethylphenol concentration was 496 μg/l with a 21% RSD. The mean 4-ethylguaiacol concentration was 31 μg/l with a 5% RSD. There was no significant difference in 4-ethylphenol and 4-ethylguaiacol content between wine aged in French compared to American oak, nor was there any significant difference between wines aged in fine or medium grained oak.

Chatonnet et al. [2], using the methodology of Boidron et al. [6], determined detection thresholds of 605 μg/l and 110 μg/l for 4-ethylphenol and 4-ethylguaiacol, respectively in a red wine. The aroma of the wines in all of the barrels are therefore unlikely to be strongly affected by these two compounds.

3.5. Analysis of 61 commercial Australian red wines

Sixty one bottles of different commercially available single variety Australian red wines were analysed by the method. The results are shown in Table 2. It must be stressed that these wines are not necessarily representative of Australian red wines as too few samples were selected and there may well be some regional biases (i.e., some regions over-represented and many regions not represented at all).

4-Ethylphenol was detected in every red wine. The concentrations found in the wines varied between 2 μg/l in a Merlot and 2660 μg/l in a Shiraz, with a mean concentration of 795 μg/l. (The highest level of 4-ethylphenol we have observed whilst analysing ''problem wines'' is 4500 μg/l). 4-Ethylguaiacol was also found in every red wine analysed, varying in concentration from 1 μg/l (in a Pinot Noir) up to 437 μg/l (in a Merlot) with a mean concentration of 99 μg/l. Within the wines of each variety, a wide range of concentrations was observed, consistent with the results of Chatonnet et al. [2,3] who have demonstrated the importance of winemaking practices on the formation of 4-ethylphenol and 4-ethylguaiacol. Although not enough wines were analysed to make a comprehensive investigation into the relationship between variety and 4-ethylphenol concentration, some trends were nevertheless observed. The mean concentration of 4-ethylphenol found in the Cabernet Sauvignon wines (1250 μg/l) was greater than that of Shiraz (605 μg/l) with 95% confidence and Pinot Noir (338 μg/l) with 99% confidence. Too few Merlots were analysed to make any significant comparisons, especially considering that the level of 4-ethylphenol found in the nine Merlots analysed ranged from 2 μg/l up to 2200 μg/l. There was no significant difference in 4-ethylguaiacol concentration between varieties.

The ratio of 4-ethylphenol to 4-ethylguaiacol also varied from wine to wine. The average ratio was approximately 10:1 for Cabernet Sauvignon, 9:1 for Shiraz, 8:1 for Merlot and 3.5:1 for Pinot Noir. The ratio difference between Shiraz and Pinot Noir was significant with 95% confidence.

Apart from a Merlot which had 2 μg/l of each compound, the lowest ratio of 4-ethylphenol to 4-

Table 2
Determination of 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) in 61 different bottles of commercial Australian red wines

Vintage	4-Ethylphenol (μg/l)	4-Ethylguaiacol (μg/l)	Ratio 4-EP/4-EG
Cabernet Sauvignon			
1986	2060	240	8.6
1992	267	45	5.9
1992	2450	141	17.4
1992	851	61	14.0
1993	2150	226	9.5
1994	594	47	12.6
1994	697	69	10.1
1994	1840	187	9.8
1994	1530	104	14.7
1994	683	72	9.5
1994	688	96	7.2
1994	518	45	11.5
1994	1130	145	7.8
1994	1870	115	16.3
1995	1130	295	3.8
1995	1240	134	9.3
1995	834	73	11.4
1997	1910	129	14.8
Mean	1250	124	10.1
Merlot			
1988	604	62	9.7
1991	2200	437	5.0
1994	1820	165	11.0
1995	528	61	8.7
1995	2100	232	9.1
1995	1280	113	11.3
1995	23	6	3.8
1996	2	2	1.0
1996	324	72	4.5
Mean	987	128	7.7
Pinot Noir			
1986	3	1	3.0
1991	51	21	2.4
1992	114	28	4.1
1994	1240	421	2.9
1995	58	13	4.5
1995	202	98	2.1
1995	1560	311	5.0
1995	32	23	1.4
1995	83	35	2.4
1995	193	121	1.6
1996	197	39	5.1
1996	498	126	4.0
1996	169	28	6.0
Mean	338	97	3.5

(*continued on next page*)

Table 2 (*continued*)

Vintage	4-Ethylphenol (μg/l)	4-Ethylguaiacol (μg/l)	Ratio 4-EP/4-EG
Shiraz			
1987	633	75	8.4
1987	275	75	3.7
1988	186	51	3.6
1989	82	12	6.8
1993	115	12	9.6
1994	1310	84	16.0
1994	282	66	4.3
1994	1580	99	16.0
1995	232	38	6.1
1995	407	67	6.1
1995	113	6	18.8
1995	709	31	22.9
1995	844	57	14.8
1995	258	29	8.9
1995	524	78	6.7
1995	283	28	10.1
1996	169	14	12.1
1996	572	44	13.0
1996	1390	161	8.6
1996	72	9	8.0
1996	2660	350	7.6
Mean	605	66	9.2
All varieties			
Mean	795	99	8.0

ethylguaiacol observed was 193:121=1.6:1 in a 1995 Pinot Noir. The highest ratio was 709:31=23:1 in a 1995 Shiraz.

Goldberg et al. [14] measured the concentration of *p*-coumaric acid, the precursor to 4-ethylphenol, in single-variety red wines from various countries. The range in *p*-coumaric acid concentration can be compared to that observed for 4-ethylphenol in the bottled red wines studied here. Among the Australian wines, *p*-coumaric acid was lowest in Pinot Noir as compared to Shiraz and Cabernet Sauvignon. In Californian wines, Pinot Noir was also equally lowest in *p*-coumaric acid (along with Zinfandel) and in South African wines, Pinot Noir had the lowest *p*-coumaric acid of the six varieties assayed. Indeed, Goldberg et al. [14] found Pinot Noir generally had low levels of *p*-coumaric acid across all varieties and countries.

Thus, although wine maturation conditions are paramount in determining the concentration of 4-ethylphenol and 4-ethylguaiacol to be found in commercial wines [2,3], genetic or cultural factors may also be influential. Further experiments are necessary to test this hypothesis.

4. Conclusions

The analytical method described here is fast, precise, accurate and reliable. Combined with automated instrumental analysis, the method has enabled our laboratory to measure 4-ethylphenol and 4-ethylguaiacol, along with other oak volatiles in the same analysis, in wines in large numbers. The method is now widely used in problem solving and is used in a commercial analytical service to support oak-barrel quality trials and evaluation of cellar practices throughout the Australian wine industry.

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