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Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection

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Abstract

A new method for the quantitative determination of important wine odorants has been developed. The wine (50 ml) is extracted in a 200 mg solid-phase extraction (SPE) cartridge filled with Lichrolut-EN resins from Merck. The elution is carried out with 1.3 ml of dichloromethane. These extracts are directly analyzed by GC-Ion Trap-MS without further concentration. Twenty-seven important wine odorants, such as volatile phenols, vanillin derivatives, aliphatic lactones, nor-isoprenoids, minor esters and terpenols, can be quantitatively determined in a single gas chromatography–mass spectrometry (GC–MS) run. The recoveries in the SPE isolation are in good agreement with those expected from the calculation of breakthrough volumes from solid–liquid distribution coefficients and are higher than 90%, except for guaiacol, vanillin, 2,6-dimethoxyphenol and 4-vinylphenol. In most cases, precision is below 10%. Method linearity is satisfactory, with r^2 higher than 0.99 in all cases. The analysis of spiked samples has shown that there is good agreement between the real mass of compound added to the wine and that determined by analysis. In all cases detection limits are below the odor detection threshold of the compounds, and the calibrated interval covers the natural range of occurrence of the compounds in wine.

Keywords: Wine; Food analysis; Flavours; Aroma compounds; Phenols; Cinnamic esters; Vanillin; Nor-isoprenoid; Terpenol

1. Introduction

Understanding the chemical nature of wine aroma demands the quantitative determination of quite a large number of different odor-active compounds [1]. These compounds are very diverse from a chemical point of view and, consequently, the analytical problems found in wine flavor analysis are also very diverse, which requires the design of a global analytical strategy. An example of such strategy is the scheme of separation and analysis developed by Guth [2,3], which can be applied to the analysis of the aroma of a wide range of products, but whose application in routine analysis is problematic and very expensive. A more efficient strategy should classify the odor-active molecules of wine attending to their analytical accessibility into the following categories:

- Category 1: Compounds easily accessible from the analytical point of view. This group comprises

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all the compounds present at relatively high concentration ($C>0.1$ mg/l) which can be determined after a single isolation step and a GC–flame ionization detection (FID) analysis. Acetaldehyde, higher alcohols and some of their acetates, fatty acids and their ethyl esters are typical examples.

- Category 2: Compounds of intermediate analytical accessibility. The analysis of these compounds is possible after a powerful isolation-preconcentration step and further GC–MS. Generally speaking, these are compounds with a reasonably good chromatographic behavior present at concentrations between 0.1 μg/l and 0.1 mg/l. Compounds in this group are volatile phenols, some lactones, vanillin derivatives, some minor esters, and some nor-isoprenoids, such as β-damascenone and β-ionone.
- Category 3: Compounds of very difficult analytical accessibility. This is a heterogeneous group formed by compounds whose analysis is very difficult due to different reasons, such as bad chromatographic behavior and poor chemical stability [4], extremely low concentrations [5], or very poor analytical properties. Volatile sulfur compounds [6,7], aldehydes [8], alkyl methoxypyrazines [9,10], furaneol and sotolon [3,11,12], and some aromatic thiols [5,13] are well-known examples of this category. In general, the analysis of these compounds requires the development of specific methods of isolation, or detection, or the use of chemical derivatives.

An optimum strategy, therefore, should be able to analyze in single runs all the compounds classified in categories 1 and 2. A recently published method covers the GC–FID analysis of compounds of the first category [14]. On the other hand, some of the compounds in group 2 can be satisfactorily analyzed by a method that couples a demixture by salting out with a micro-extraction [15]. Unfortunately, that method is time-consuming and some important polar compounds, such as vanillin, are not very well extracted. Consequently, an alternative method should be developed for the analysis of compounds of that second category.

Among all the different isolation and preconcentration possibilities, solid-phase extraction (SPE) represents a good choice. A previous study has shown that polymeric sorbents provide highest solid–liquid distribution coefficients [16], and new styrene–divinylbenzene sorbents, not tested in such study, still show a better behavior [17]. The main aim of the present work is, therefore, the development and validation of a SPE–GC–MS method for the quantitative determination of a wide range of wine odor-active compounds present at concentrations in the 0.1–100 μg/l range.

2. Material and methods

2.1. Reagents, samples and standards

Dichloromethane, HPLC quality was from Fisher Scientific (Loughborough, UK), Methanol was LiChrosolv quality from Merck (Darmstadt, Germany), absolute ethanol, ACS quality, was purchased from Panreac (Barcelona, Spain), pure water was obtained from a Mili-Q purification system (Millipore, USA).

LiChrolut EN resins, prepacked in 200 mg cartridges (3 ml total volume) were obtained from Merck (Darmstadt, Germany). The chemical standards were purchased from Aldrich, Fluka, Sigma, Lancaster, PolyScience, Chemservice and Firmenich (see Table 1).

The Internal Standard solution contained 4-hydroxy-4-methyl-2-pentanone and 2-octanol, both at 300 μg per g of dichloromethane. The BHA (3-*tert*.-butyl-4-hydroxyanisole) solution contained 10 mg of this compound per g of ethanol.

Three wine samples were used in the development and validation of the method: a 5-year-old red wine (wine 1), a young white wine (wine 2), and a rosé wine (wine 3). Fifty-seven different Spanish red wines were analyzed following the proposed procedure.

2.2. SPE equipment

A VAC ELUT 20 station from Varian was used.

2.3. Gas chromatography–mass spectrometry

A Star 3400CX gas chromatograph fitted to a Saturn 4 electronic impact ion trap mass spectrometer from Varian was used. The column was a DB-

Table 1
Chemical standards and MS fragments used for quantitative analysis

Analyte	Supplier	Purity (%)	Quantitative fragments m/z
2,6-Dimethoxyphenol	Aldrich	99.0	154
2-Phenylethyl acetate	Chemservice	98.5	104
4-Ethylguaiacol	Lancaster	98.0	137
4-Ethylphenol	Aldrich	99.0	107+122
4-Propylguaiacol	Lancaster	98.0	137+166
4-Vinylguaiacol	Lancaster	97.0	135+150
4-Vinylphenol	Lancaster	10.0	120
5-Methylfurfural	Fluka	97.0	109
Acetovanillone	Aldrich	98.0	151+166
Butyl acetate	Fluka	99.0	56+61
Ethyl 2-methylbutyrate	Aldrich	99.0	57+74
Ethyl benzoate	Fluka	99.0	105
Ethyl cinnamate	Fluka	98.0	131
Ethyl dihydrocinnamate	Fluka	98.0	104
Ethyl vanillate	Lancaster	97.0	151+196
Eugenol	Aldrich	99.0	164
Furfural	Chemservice	99.0	95
Furfuryl alcohol	Fluka	98.0	98
Guaiacol	Aldrich	98.0	109+124
Linalool	Aldrich	97.0	93+121+136
Methy vanillate	Lancaster	99.0	151+182
o-Cresol	Aldrich	99.0	108
Vanillin	Polyscience	99.0	151+152
Whiskylactone	Aldrich	98.0	99
α-Terpineol	Fluka	99.0	121+136
β-Citronellol	Aldrich	98.0	123
β-Damascenone	Firmenich	99.0	121
β-Ionone	Sigma	98.0	177
γ-Nonalactone	Aldrich	97.0	85
3-*tert*.-Butyl-4-hydroxyanisole	Fluka	98.0	Not applicable

WAXetr from J&W (Folsom, USA), 60 m×0.25 mm with 0.5 μm film thickness, and was preceded by a 3 m×0.32 mm uncoated (deactivated, intermediate polarity) precolumn. The carrier was He at 1 ml/min. The chromatographic oven was initially 40 °C for 5 min, and then was raised to 230 °C at 2 °C/min. A 1093 SPI (septum-equipped programmable injector) injector from Varian was used. The initial temperature of this injector was 30 °C for 0.6 min, and was then raised to 230 °C at 200 °C/min. Then, 3 μl of sample were injected. A 35–220 m/z mass range was recorded, and the extracted ion chromatograms described in Table 1 were taken for quantitation by peak area. Ion peaks described in Table 1 were taken for quantitation.

2.4. Determination of the distribution coefficients of solid–liquid systems

An exact weight of the sorbent (0.12 g) was placed inside a glass vial, together with a 50 ml volume of wine fortified with 2 mg/l of some selected compounds. The vials were shaken softly for 24 h. After this, 10 ml of the extracted wine was transferred to a 15 ml centrifuge tube containing 3.3 g $(NH_4)_2SO_4$, plus 20 μl of the internal standard solution and 0.5 ml of dichloromethane. The tubes were closed, shaken gently for 45 min, centrifuged, and the organic phases analyzed by GC–MS. Relative areas were compared to those obtained in the direct analysis of dichloromethane extracts (10 ml

wine, plus 3.3 g $(NH_4)_2SO_4$, plus 20 μl of the internal standard solution and 0.5 ml of dichloromethane from the fortified wines. All the experiments were duplicated.

Phase ratio and holdup volumes were directly measured by weighing the chromatographic beds before (m_0) and after (m_1) the addition of the necessary amount of mobile phase (whose density, ρ, was calculated) to form the bed, and after (m_2) the expulsion of interstitial liquid by a flow of air.

Volume of mobile phase in pores: $(m_2 - m_0)/\rho$

$\approx V_p$ (pore volume)

Volume of interstitial mobile phase: $(m_1 - m_2)/\rho$

$\approx V_M$ (holdup volume)

And the phase ratio, ϕ, is then $\phi = m_0/(V_M + V_p)$

The number of plates, N, was estimated from the breakthrough curves built in frontal elution experiments as described below.

2.5. Proposed method

2.5.1. Cartridge conditioning

The cartridges with the sorbent were placed in the extraction system and rinsed with 4 ml of dichloromethane, 4 ml of methanol and, finally, with 4 ml of a water–ethanol mixture (12%, v/v).

2.5.2. Sample loading

Fifty milliliters of wine, containing 25 μl of BHA solution, were passed through the SPE cartridge at around 2 ml/min. After this, the sorbent was dried by letting air pass through it (−0.6 Bar, 10 min).

Table 2
Properties of the Lichrolut-EN resins

Compound	K	Estimated V_b[a]	Recovery (%)	SD (%)
Furfuryl alcohol	35	2	17	8
Furfural	36	2	20	12
Isoamyl alcohol	88	6	n.d.	
β-Phenylethanol	441	28	n.d.	
Guaiacol	498	32	58	6
Vanillin	587	38	58	7
2,6-Dimethoxyphenol	663	43	72	5
4-Vinyl-phenol	770	49	81	4
Methyl furfural	954	61	100	4
Butyl acetate	2167	139	102	3
γ-Nonalactone	2871	184	92	9
Acetovanillone	2930	188	95	4
Ethyl 2-methylbutyrate	3235	208	103	4
4-Ethyl guaiacol	3289	211	107	6
4-Vinylguaiacol	3391	218	96	3
4-Ethylphenol	3430	220	106	5
4-Propyl guaiacol	4089	262	109	3
cis-Whiskylactone	4240	272	99	3
2-Phenylethyl acetate	5280	339	98	3
α-Terpineol	7728	496	106	4
Eugenol	9508	610	96	5
Ethyl cinnamate	15504	994	109	5
Ethyl benzoate	21498	1379	107	4
β-Ionone	40000	2565	103	4
Ethyl dihydrocinnamate	56940	3651	104	3
Linalool	n.d.		96	3
o-Cresol	n.d.		97	5
Methyl vanillate	n.d.		102	6
β-Damascenone	60000	3848	100	2

Solid–liquid distribution coefficients, estimated breakthrough volumes and recoveries in the SPE of 50 ml of wine.
[a] Phase ratio 0.54; approximate number of plates 7; hold up volume, 0.18 ml. n.d. not determined.

2.5.3. Elution

Analytes were recovered by elution with 1.3 ml of dichloromethane. Twenty-five microliters of the S.I. solution were added over the eluted sample. The mixture was then hermetically capped and stored at −25 °C until the GC–MS anlysis.

2.5.4. Calibration

Calibration graphs were prepared by the GC–MS analysis of dichloromethane solutions containing known amounts of the standards and of the internal standards.

2.6. Method validation

Recovery of the SPE process was determined by the analysis (extraction with 5+5 ml of dichloromethane, and further concentration) of the three wine samples before and after the SPE. The reproducibility of the method was determined by replicate analysis of wines 1 and 2 on 3 different days. The existence of matrix effects was checked by the replicate analysis of wines 1, 2 and 3 and of those wines spiked with known amounts of analytes.

3. Results and discussion

3.1. SPE method development

Among all the possible sorbents for the SPE extraction, Lichrolut-EN resins have been selected because in previous studies they have shown to have an excellent ability for the extraction of neutral

Table 3
Reproducibility data for three analyses of two wines

	Wine 1		Wine 2	
	Mean (μg/l)	RSD(%)	Mean (μg/l)	RSD(%)
2,6-Dimethoxyphenol	38.19	2.9	tr	
2-Phenylethyl acetate	16.12	2.5	115.40	8.1
4-Ethylguaiacol	27.76	2.6	0.13	9.1
4-Ethylphenol	239.20	1.3	0.7	3.5
4-Propylguaiacol	0.45	5.1	tr	
4-Vinylguaiacol	43.99	14.0	320.61	11.7
4-Vinylphenol	27.95	17.2	141.51	7.9
5-Methylfurfural	3.51	1.7	0.66	22.9
Acetovanillone	42.28	6.0	36.10	3.2
Butyl acetate	1.86	9.5	3.78	7.9
cis-Whiskylactone	126.12	2.7	0.29	32.7
Ethyl 2-methylbutyrate	26.09	11.3	8.38	8.1
Ethyl benzoate	0.06	23.2	tr	
Ethyl cinnamate	1.20	5.0	0.75	7.1
Ethyl dihydrocinnamate	0.85	1.1	0.23	8.2
Ethyl vanillate	118.91	5.2	2.50	3.7
Eugenol	17.62	1.7	1.38	1.8
Furfural	28.27	3.6	26.30	17.3
Furfuryl alcohol	16.02	51.8	1.32	58.7
Guaiacol	6.59	2.6	0.37	10.2
Linalool	2.65	8.1	16.69	0.7
Methy vanillate	9.40	9.4	15.82	3.0
o-Cresol	1.14	6.6	tr	
Vanillin	34.90	20.6	1.43	10.7
α-Terpineol	9.27	0.3	23.79	1.7
β-Citronellol	1.65	29.8	1.37	6.7
β-Damascenone	1.06	3.3	3.01	0.9
β-Ionone	0.11	4.5	tr	
γ-Nonalactone	8.96	5.1	2.79	9.0

tr: traces.

compounds from wine [1,18]. The solid–liquid distribution coefficients of important wine odorants between wine and these resins have been measured, and those coefficients were used to calculate approximated breakthrough volumes using the Lövkist–Jonhsson model [10,19]. Both sets of values can be seen in Table 2. The table shows that there are big differences between compounds. While less polar compounds, such as eugenol, ethyl cinnamate or β-damascenone, are very well extracted from wine by the polymeric resins, some other more polar compounds, such as furfuryl alcohol or furfural, show very low solid–liquid distribution coefficients. Accordingly, the expected breakthrough volumes are very small for these last compounds and very large for the less polar ones. From a practical point of view, this means that it is not possible to design a single extraction procedure for all the compounds in the table. Fortunately, furfural, isoamyl alcohol and β-phenylethanol can be analyzed by GC–FID [14] and their low expected breakthrough volumes are not limiting in the present method. Furfuryl alcohol will require a specific extraction procedure and, therefore, will have to be determined with some other polar compounds, such as furaneol or sotolon.

Leaving aside these compounds, the limiting breakthrough volumes for the SPE operation are those of some volatile phenols, such as guaiacol, vanillin and 4-vinylphenol. According to data in Table 2, the breakthrough volume of guaiacol is 32 ml for a 200 mg cartridge. If this volume is selected as the sample loading volume, a concentration factor of about 25 would be achieved in the SPE step, since the minimum elution volume for this cartridge is about 1.3 ml. It would be desirable, however, to achieve a higher concentration factor in the SPE

Table 4
Method linearity data

Analyte	Intercept	Slope	r^2	Range (μg/l)	n	IS[a]
2,6-Dimethoxyphenol	−0.0009807	0.01188	0.9984	2.8–49	5	2O
2-Phenylethyl acetate	0.003035	0.01758	0.9995	15–620	4	2O
4-Ethylguaiacol	0.001680	0.01567	0.9987	11–290	4	2O
4-Ethylphenol	−0.02064	0.02950	0.9975	9–588	6	2O
4-Propylguaiacol	−0.0002082	0.03386	0.9971	0.4–20	4	2O
4-Vinylguaiacol	−0.001237	0.005967	0.9988	8–564	6	4H
4-Vinylphenol	−0.01423	0.01325	0.9997	27–299	4	4H
5-Methylfurfural	0.0007771	0.007892	0.9999	3–160	5	2O
Acetovanillone	−0.002168	0.02090	0.9968	5–131	4	2O
Butyl acetate	−0.00008070	0.008173	0.9999	0.6–16	5	2O
cis-Whiskylactone	0.0001369	0.006623	0.9994	8–542	4	2O
Ethyl 2-methylbutyrate	0.0002433	0.009257	0.9997	2.9–42	4	2O
Ethyl benzoate	0.00007429	0.03853	0.9991	0.1–4.0	4	4H
Ethyl cinnamate	−0.0001457	0.01446	0.9993	0.5–8.9	4	2O
Ethyl dihydrocinnamate	−0.00004022	0.01512	0.9998	0.5–8.1	4	2O
Ethyl vanillate	−0.006188	0.02281	0.9971	5–274	5	2O
Eugenol	0.000003575	0.009288	0.9999	0.6–16	4	2O
Furfural	0.0005110	0.008057	0.9996	5–361	6	2O
Furfuryl alcohol	0.0005588	0.004697	0.9994	0.3–20	4	4H
Guaiacol	0.00005692	0.03787	0.9999	0.5–27	5	4H
Linalool	−0.00005998	0.01525	0.9997	2–43	5	4H
Methy vanillate	−0.002695	0.02131	0.9965	4.9–133	4	2O
o-Cresol	−0.00009515	0.006653	0.9998	0.8–4.4	4	2O
Vanillin	−0.001517	0.01658	0.9961	5–140	4	2O
α-Terpineol	−0.0003930	0.007935	0.9987	2–37	5	2O
β-Citronellol	−0.00008067	0.001362	0.9941	1–30	4	2O
β-Damascenone	−0.00005312	0.01076	0.9905	0.5–8.7	4	2O
β-Ionone	−0.00003357	0.01728	0.9915	0.1–1.0	4	2O
γ-Nonalactone	−0.0005952	0.01432	0.9998	2.7–48	4	2O

[a] Internal standard, 2O, 2-octanal; 4H, 4-hydroxy-4-methyl-2-pentanone.

process, so that an additional concentration step by solvent evaporation becomes unnecessary. Consequently, the selected sample loading volume has been 50 ml, although this will mean lower analytical precision in the determination of the aforementioned volatile phenols.

The recoveries obtained in the SPE process can be seen in Table 2 and are in good agreement with the breakthrough volumes given in the table. Recoveries for nearly all the compounds with breakthrough volumes larger than 50 are nearly total. On the contrary, recoveries for furfural and furfuryl alcohol are very low, while in the cases of guaiacol, vanillin, 2,6-dimethoxyphenol and 4-vinylphenol, recoveries range between 58 and 81. After these results, the calibration is carried out by the analysis of dichloromethane solutions containing known amounts of the target compounds. For compounds whose recoveries are lower than 90%, the value of concentration obtained by the direct interpolation of the relative peak area in the corresponding calibration graph is thereafter corrected by the corresponding recovery.

3.2. Selection of quantitative *m*/*z* fragments

The pattern of fragmentation in the ion trap is less stable than that obtained in normal quadrupole instruments, even though the automatic gain control (AGC) keeps the number of ions inside the trap at a steady level. Such instability can cause the mass spectrum of a molecule to be deformed with changes in the mass of analyte. In our experience this means

Table 5
Degree of agreement between real mass of analyte added to wine and mass added determined by the analysis of the spiked and non-spiked samples

Analyte	μg per 50 ml (added)	μg per 50 ml (calculated)	SD ($n=3$)	DL[a] (μg/l)
2,6-Dimethoxyphenol	0.41	0.43	0.07	0.62
2-Phenylethyl acetate	8.29	10.28	1.3	0.29
4-Ethylguaiacol	7.21	9.86	1.5	0.035
4-Ethylphenol	5.75	6.12	0.5	0.54
4-Propylguaiacol	0.42	0.57	0.08	0.24
4-Vinylguaiacol	5.51	5.26	0.7	0.83
4-Vinylphenol	2.83	2.81	0.2	1.0
5-Methylfurfural	5.67	5.83	0.4	0.059
Acetovanillone	4.81	4.38	0.8	0.84
Butyl acetate	0.34	0.37	0.3	0.64
cis-Whiskylactone	5.30	5.25	0.4	0.13
Ethyl 2-methylbutyrate	0.42	0.49	0.3	0.072
Ethyl benzoate	5.65	6.07	0.5	0.042
Ethyl cinnamate	4.96	5.97	0.9	0.36
Ethyl dihydrocinnamate	4.54	5.74	0.9	0.21
Ethyl vanillate	4.86	4.15	0.3	0.17
Eugenol	0.58	0.55	0.2	0.074
Furfural	5.23	7.03	2.1	0.50
Furfuryl alcohol	4.19	7.72	3.2	0.067
Guaiacol	0.45	0.47	0.05	0.026
Linalool	0.60	0.56	0.08	0.17
Methyl vanillate	4.91	5.49	0.5	0.49
o-Cresol	0.47	0.75	0.2	0.43
Vanillin	5.15	5.00	0.4	0.57
α-Terpineol	0.51	0.54	0.3	0.25
β-Citronellol	0.51	0.62	0.7	0.44
β-Damascenone	4.86	6.13	1.2	0.20
β-Ionone	0.30	0.31	0.04	0.089
γ-Nonalactone	0.40	0.36	0.02	1.1

[a] DL, detection limits for the overall method.

that particular attention should be paid to the ion fragments selected for quantitation. In particular, the relationship between the intensity of the ions and the concentration of analyte becomes a criterion as important as sensitivity. For instance, in the case of butyl acetate m/z fragment 61 is the most sensitive and selective. However, its relative intensity changes from 10% at 2.4 μg/l level to 20% at 41.4 μg/l level. This problem is overcome if the calibration is done with the sum of fragments 56 and 61 m/z ($r^2=0.9997$ against 0.9953 in the case of 61 m/z fragment). Accordingly, both criteria have been taken into account to select the ions shown in Table 1.

3.3. Method validation

The reproducibility of the method is given in Table 3. Those values have been obtained by the replicate analysis of two different samples in different days. In most of the cases, the RSD is below 10%, and in nearly half below 5%, which can be considered to be satisfactory for the levels at which the compounds are found in wine. Some of the poor RSD figures shown in Table 3 are simply caused by the low concentration at which the compounds were found in those wines. This is the case of 5-methylfurfural and *cis*-whiskylactone in wine 2, or of ethyl benzoate in wine 1. In some other cases, such as furfuryl alcohol, furfural and vanillin, the poor and erratic recoveries in the extraction should be blamed for the low reproducibility. And finally, in the cases of 4-vinylguaiacol and 4-vinylphenol, the relatively low precision is caused by the presence of interfering compounds.

The linearity obtained in the analysis of dichloro-

Table 6
Average, maximum and minimum concentrations and odour thresholds (μg/l) of the different odour-active compounds found in aged red wines

Analyte	Average ($n=57$) (μg/l)	Minimum (μg/l)	Maximum (μg/l)	Odor threshold[a] (μg/l)
2,6-Dimethoxyphenol	31	13	56	570
2-Phenylethyl acetate	22	7	65	250 [3]
4-Ethylguaiacol	76	0.53	420	33 [20]
4-Ethylphenol	390	8.6	1500	440 [21]
4-Propylguaiacol	2.7	0.24	13	–
4-Vinylguaiacol	67	5.4	236	40 [3]
4-Vinylphenol	36	8.1	98	180 [21]
5-Methylfurfural	13	0.15	51	20000 [22]
Acetovanillone	67	30	160	1000
Butyl acetate	3.1	1.5	7.8	1880 [22]
cis-Whiskylactone	210	46	520	67 [22]
Ethyl 2-methylbutyrate	15	6.7	37	18 [20]
Ethyl benzoate	0.61	0.043	5.9	570 [20]
Ethyl cinnamate	1.3	0.66	6.2	1.1 [20]
Ethyl dihydrocinnamate	0.85	0.40	2.7	1.6 [20]
Ethyl vanillate	160	71	380	990
Eugenol	29	4.2	73	6.0 [20]
Guaiacol	5.8	2.6	13	9.5 [20]
Linalool	4.1	0.57	11	25 [20]
Methy vanillate	17	4.4	50	3000
o-Cresol	2.2	0.99	5.2	31 [22]
Vanillin	59	9.6	140	200 [3]
α-Terpineol	12	4.0	33	250 [20]
β-Citronellol	2.4	0.57	5.4	100 [3]
β-Damascenone	1.5	0.32	3.4	0.05 [3]
β-Ionone	0.47	0.10	2.0	0.09 [20]
γ-Nonalactone	10	1.7	23	30 [23]

[a] Between brackets, the reference from which the value has been taken. In the cases in which this value has been determined in this work there is no bracket. In these last cases, orthonasal threshold values in a 10% hydroalchoholic solution at pH 3.2 are given.

methane solutions can be seen in Table 4. In all studied cases, the relationship between the signal (ion peak area normalized by one of the internal standards) and the concentration is linear, and the squared regression coefficients are higher than 0.99. The calibrated intervals range from 1 to 1.7 orders of magnitude and cover the normal range of occurrence of most compounds in wine.

The existence of matrix effects was checked by the analysis of samples spiked with known amounts of analytes. The increments of area (normalized to the corresponding internal standard) were interpolated in the calibration graphs shown in Table 4 and, when necessary, were corrected by the figure of recovery given in Table 2. The agreement between the real amount added and that determined was

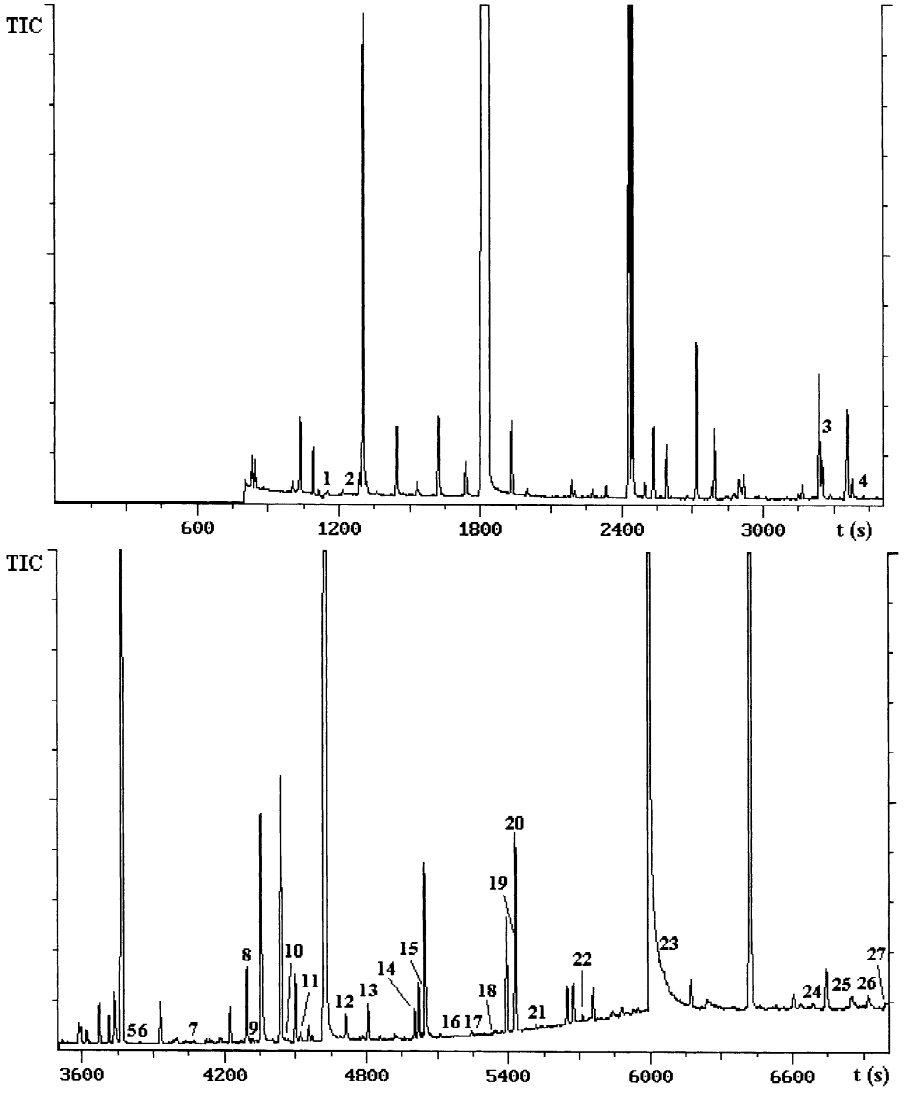

Fig. 1. Reconstructed ion chromatogram from a 5-year-old red wine. Peak identification: 1, ethyl 2-methylbutyrate; 2, butyl acetate; 3, linalool; 4, 5-methylfurfural; 5, furfuryl alcohol; 6, α-terpineol; 7, β-citronellol; 8, 2-phenylethyl acetate; 9, β-damascenone; 10, guaiacol; 11, ethyl dihydrocinnamate; 12, β-ionone; 13, *cis*-whiskylactone; 14, 4-ethylguaiacol; 15, γ-nonalactone; 16, *o*-cresol; 17, 4-propylguaiacol; 18, ethyl cinnamate; 19, eugenol; 20, 4-ethylphenol; 21, 4-vinylguaiacol; 22, 2,6-dimethoxypenol; 23, 4-vinylphenol; 24, vanillin; 25, methyl vanillate; 26, ethyl vanillate; 27, acetovanillone. TIC, total ion current.

satisfactory in nearly all cases (Table 5), and the standard deviations obtained were not very different from those expected, which indicates that the matrix effect is not significant at the level of precision of the method. Method detection limits were estimated by the analysis of real samples and the figure obtained corresponds to the concentration at which the signal-to-noise ratio becomes 3. The detection limit depends mainly on the selectivity achieved on both the chromatographic separation and the MS detection, and ranges from 0.026 μg/l (guaiacol) to 1.1 μg/l (γ-nonalactone). In most cases, this detection limit is well below the odor threshold of the compound, which guarantees that the method can be used to determine these odor active compounds of wine.

3.4. Wine analysis

The method has been applied to the analysis of 57 Spanish oak-aged wines. A summary of the results obtained in the analysis is given in Table 6. A typical GC–MS chromatogram can be seen in Fig. 1. As is shown in the table, in most cases the concentration of the volatile compounds lies into the calibrated intervals of the method. Some of the compounds present in the table are important aromas of the wines, and their concentration is well above the corresponding flavor threshold. Among them appear 4-ethylguaiacol, 4-ethylphenol, 4-vinylguaicol, guaiacol, eugenol, *cis*-whiskylactone, ethyl 2-methylbutyrate, ethyl cinnamate, ethyl dihydrocinnamate, linalool, β-damascenone and β-ionone.

From a practical point of view, the method shows a series of advantages over other methods for the analysis of wine aroma. Some advantages derive from the simplicity of the semi-automated SPE isolation, which makes it possible to achieve in a single step the desired concentration, minimizing the handling of the samples. Another set of advantages is due to the particular sensitivity of ion trap MS. It has been observed that retention times change slightly from one run to another due to the high temperatures to which the chromatographic column must be exposed to ensure a clean baseline. This drift is a minor problem in a system that records full scans along the whole chromatogram.

In summary, the proposed method makes it possible to determine a wide range of wine aromas present at minor and trace concentration after a single SPE isolation process. The analytical properties of the method are satisfactory for the purpose of the study of wine aroma.

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