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Fast analysis of important wine volatile compounds Development and validation of a new method based on gas chromatographic–flame ionisation detection analysis of dichloromethane microextracts

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Abstract

A method for the simultaneous determination of major (10–200 mg/l) and minor (0.1–10 mg/l) volatile compounds from wine has been optimised and validated. A 3-ml volume of wine is diluted with water (7 ml), salted with 4.5 g of ammonium sulfate and extracted with 0.2 ml of dichloromethane. The extract is injected in the split mode in a GC system, separated on a Carbowax 20M capillary column and detected by flame ionisation detection. Volatiles from wine are divided into four groups according to their behaviour in the extraction, and a specific internal standard has been selected for each group. The method allows satisfactory determination of more than 30 volatile compounds of wine. Compounds analysed include acetaldehyde, diacetyl, acetoine (3-hydroxy butanone), fusel alcohols and their acetates, and fatty acids and their ethyl esters. The linear dynamic range of the method covers the normal range of occurrence of analytes in wine and extends from at least one magnitude order to more than two, with typical r^2 between 0.9938 and 0.9998. Reproducibility ranges from 3.1 to 10% (as RSD) with 5.5% as the average. The analysis of spiked samples has shown that matrix effects do not significantly affect method performance.

Keywords: Wine; Food analysis; Volatile organic compounds

1. Introduction

The gas chromatographic analysis of volatile compounds in wine is a very important tool useful for wine classification [1–3], for quality control [4–7] or for understanding wine sensory properties [8,9]. The complete analysis of wine aroma is, however, extremely complex and expensive. The best example of such analysis can be found in the work by Guth [8] who, by using isotopomers, complex sample preparation schemes, and more than eight gas chromatography–mass spectrometry (GC–MS) runs, was able to accurately quantify 43 odor-active compounds from wine. There is no need to carry out such a complex analysis to gain a big deal of information. A single GC–flame ionisation detection (FID) chromatogram from a wine extract can provide quantitative data on compounds formed in

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different stages of wine making, and through different chemical pathways, which potentially would constitute a smart way to control the winemaking process. There is a logical interest, therefore, to develop robust, fast, cheap, and simple quantitative methods able to quantify some of the most important wine volatile constituents.

There are several methods described in the literature that partially fulfil these requisites, all of them use a single isolation–concentration step. The liquid microextraction method described by Ferreira et al. [10] made it possible to quantify more than 20 compounds, but it failed in the extraction and quantitation of some of the most polar volatiles of wine, such as acetaldehyde, acetoine (3-hydroxy butanone), diacetyl or butyric acid. This constitutes an important drawback since these compounds have remarkable sensory and biochemical properties and are very good markers of the wine microbiological state [4]. Similar criticism can also be made to other liquid microextraction strategies [11]. An interesting alternative consisting of the direct injection of wine in a programmed temperature vaporiser (PTV) injector has been proposed by Villen et al. [12], but it requires a PTV injector, a frequent cleaning of the chromatographic inlets, and it was not very sensitive. Several useful solid-phase microextraction (SPME)-based methods have also been proposed, but very often they have been developed to quantify a very narrow range of analytes [13–18]. In other cases, the proposed SPME strategies also fail in the analysis of the most polar compounds of wine [19,20].

Because of these reasons, the main target of our research was to develop a fast and simple method able to quantitate many of the most important wine volatiles in a single chromatographic run. The targeted analytes are acetaldehyde, acetoine, diacetyl, fusel alcohols and their acetates, short-chain fatty acids and their ethyl esters and some miscellaneous compounds. All of them are volatile compounds with remarkable sensory properties and are at concentrations high enough to be analysed in a single GC–FID run. The analysis, therefore, can provide information about wine microbiological state, as stated before; about part of the fruity, lactic, alcoholic, green, oxidised, or fatty odor nuances of wine [21–24]; about yeast redox state [25]; about yeast amino acid metabolism [26] and about the geographical and varietal origin of the wine [3]. Additional method requisites are the use of normal GC–FID equipment, and a reasonable degree of accuracy. The selected strategy has been a liquid–liquid microextraction with dichloromethane and the use of several internal standards to correct for matrix effects and to improve the method figures of merit. The results of that research are presented in this paper.

2. Material and methods

2.1. Reagents, samples and standards

Solvents: dichloromethane HPLC quality from Fisher Scientific (Loughborough, UK), absolute ethanol (analytical-reagent grade) from Riedel-de Häen (Seelze, Germany). Water was purified through a Milli-Q system (Millipore, Bedford, MA, USA). Reagents: solid anhydrous ammonium sulfate and tartaric acid (ACS-ISO quality) from Panreac (Barcelona, Spain). Standards: the source and grade can be seen in Table 1. Samples: all wine samples were from Spain: Ref ''W1'' (semi-dry young white 13.5%, v/v, ethanol); Ref ''W2'' (dry young white 11.0%, v/v, ethanol); Ref ''Rs'' (young rosé wine 12.5%, v/v, ethanol); Ref ''Rd'' (aged red wine 13%, v/v, ethanol). Standard solutions: internal standard solution A, 2-ethylhexanol in ethanol (1000 μg/ml); internal standard solution B, 2-butanol, acetone, ethyl 2-hydroxypentanoate, pentanoic acid, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone and 2-octanol at 140 μg/ml in ethanol; internal standard solution C: 2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone and 2-octanol at 140 μg/ml in ethanol. Standard solutions: exact masses (≈0.1 g) of the chemical standard compounds were dissolved in absolute ethanol. Synthetic wine samples: standard solutions were diluted with water and/or alcohol (adjusting final alcohol content to 12%, v/v) at concentrations typically found in wine. All solutions were added with 5 g/l tartaric acid and the pH was adjusted to 3.2 with 1 *M* NaOH.

2.2. Gas chromatography

A Hewlett-Packard 5890 Series II gas chromato-

Table 1
Chemical standards used in this work

Analyte	Supplier	Purity (%)
1-Propanol	Merck	99.0
1-Butanol	Panreac	99.5
2-Butanol	Merck	99.0
1-Hexanol	Sigma	99.0
Isobutanol	Merck	99.0
Isoamyl alcohol	Aldrich	99.0
cis-3-Hexen-1-ol	Aldrich	98.0
4-Methyl-2-pentanol	Polyscience	98.0
2-Octanol	PolyScience	98.0
2-Ethylhexanol	Chemservice	99.0
Benzyl alcohol	Aldrich	99.0
β-Phenylethanol	Fluka	99.0
Ethyl acetate	PolyScience	99.5
Propyl acetate	Chemservice	97.0
Isobutyl acetate	Chemservice	99.0
Isoamyl acetate	Chemservice	99.0
Hexyl acetate	Chemservice	99.0
2-Phenylethyl acetate	Chemservice	98.5
Ethyl propanoate	Fluka	99.0
Ethyl butyrate	Aldrich	99.0
Ethyl hexanoate	PolyScience	99.5
Ethyl octanoate	PolyScience	99.5
Ethyl decanoate	Polyscience	99.5
Ethyl isobutyrate	Aldrich	99.0
Ethyl 3-methylbutyrate	Aldrich	95.0
Ethyl 2-hydroxy valerate	Fluka	99.0
Ethyl 3-hydroxybutyrate	Aldrich	99.0
Ethyl lactate	Aldrich	99.0
Diethyl succinate	Fluka	99.0
Acetic acid	Panreac	99.5
Propanoic acid	PolyScience	99.5
Butyric acid	PolyScience	99.5
Isobutyric acid	Aldrich	99.0
Pentanoic acid	PolyScience	99.5
Hexanoic acid	PolyScience	99.5
Octanoic acid	Fluka	98.5
Decanoic acid	PolyScience	99.5
Isovaleric acid	Aldrich	99.0
γ-Butyrolactone	Aldrich	99.0
4-Hydroxy-4-methyl-2-pentanone	Aldrich	99.0
Acetone	Panreac	99.5
Methionol	Aldrich	98.0
Acetoine	Aldrich	98.0
Acetaldehyde	Aldrich	99.0
Diacetyl	Aldrich	99.0
Furfural	Chemservice	99.0

graph was used. The column (50 m×0.32 mm and 0.5 μm film thickness) was a DB-20 from J&W Scientific (Folsom, CA, USA). The column was preceded by a 2 m×0.53 mm uncoated pre-column. The temperature program was as follows: 40°C for 5 min, then raised at 3°C/min up to 200°C. Carrier gas was H_2 at 3 ml/min. Injection: 3 μl in split mode. Split flow was 30 ml/min. Detection was by FID.

2.3. Proposed method

In 15-ml screw-capped centrifuge tubes, containing 4.5 g of $(NH_4)_2SO_4$, 3 ml of wine, 7 ml of water, 15 μl of internal standard solution C and 0.2 ml of dichloromethane were added. The tube was shaken for 1 h and then centrifuged at 2500 rpm for 10 min. Once the phases had been separated, the dichloromethane phase was recovered with a 0.5-ml syringe and transfered to a 0.3-ml vial. The extract was injected into the gas chromatograph under the conditions listed above. The relative response areas for each of individual wine volatile compounds to the appropriate internal standard were calculated (see Table 3) and interpolated in the corresponding calibration graphs built as described below.

2.4. Calibration graphs

Synthetic wines containing known amounts of the volatile compounds, 12% (v/v) ethanol, 5 g/l tartaric acid and pH adjusted to 3.2 with 1 *M* sodium hydroxide, were extracted and analysed following the proposed procedure. The range of concentrations tested can be seen in Table 3.

2.5. Method development and validation

Initial tests to determine the best extraction conditions allowing for a clean phase separation and maximum concentration were carried out. In these experiments the wine samples (eight different wines were considered in this study) were progressively diluted with water (3:1; 2:1; 4:3; 1:1; 3:4; 1:2; 1:3) and added with different masses of ammonium sulfate. These solutions were extracted with different volumes of dichloromethane by shaking for 1 h. After that, the solutions were centrifuged at 2500 rpm and the volume of separated organic phase measured. Among the extraction conditions that did not provoke appreciable solvent emulsion, the one with minimum water proportion and maximum mass of salt was chosen.

The following experiments were carried out with the four wines described in Section 2.1.

Extraction recovery (%) and internal standard selection: 3-ml volumes of the wines were spiked with known amounts of wine volatile compounds and with internal standards from solution B. These volumes were extracted as indicated in the proposed method. The remaining hydroalcoholic solutions were spiked with 20 μl of the internal standard solution A, and re-extracted with 0.5 ml of dichloromethane. These latter extracts were analysed by GC under the conditions described above. The relative areas of analytes to the internal standard in solution A (2-ethylhexanol) were compared with those obtained in the analysis of a reference. Reference: 3 ml of wine (added with exactly the same amount of wine volatile compounds, water, salt and internal standard solutions A and B) was extracted with 0.5 ml of dichloromethane. The recovery was estimated as:

Recovery (%) =

(relative area − relative area reference)/

relative area reference · 100%

This study was conducted in duplicate for each of the four wines.

Linearity: method linearity was studied as described in Section 2.4, but using the internal standard solution B instead of C.

Repeatability and reproducibility: the four wines selected for the study and a synthetic wine containing known amount of wine volatile compounds were extracted in triplicate on 3 different days. Data from the 45 analyses (5 wines × 3 replicates × 3 days) were processed as follows. The three standard deviations of the 3 triplicates (one triplicate per day) for each wine were combined (square root of the arithmetic mean of the variances) to obtain method repeatability for each sample. The standard deviation of the three mean values for each wine (one per day) multiplied by the square root of 3 was taken as the reproducibility value (if this value is bigger than repeatability, if not, this last figure was taken also as reproducibility).

Existence of matrix effects: the wine referenced as Rd was selected for the study. The wine was spiked with known amounts of volatile compounds and, both samples, the original wine and the spiked sample, were extracted following the proposed procedure in triplicate. The average increments of relative areas were interpolated in the calibration graphs built with synthetic solutions.

3. Results and discussion

3.1. Method development

A previous comparative study of the ability of different solvent and sorbent systems to extract volatile compounds from alcoholic solutions [27] showed that dichloromethane was the most efficient solvent, and for this reason was selected for this study. Another important conclusion of that work is the need of using important amounts of salt to improve the extraction efficiency of compounds with Lewis acid properties, such as alcohols and acids. Unfortunately, the optimal addition of salt to wine makes the wine have a density too close to that of dichloromethane, which in turn, makes phase separation difficult. Because of this, several experiments were carried out to determine the best extraction conditions (volumes of wine, water and solvent, and amount of salt) allowing for a clean and fast extraction free of emulsion problems. The selected conditions (3 ml of wine plus 7 ml of water and 4.5 g of salt) have been found to be a reasonable compromise between extraction efficiency, concentration and extraction cleanliness. No emulsions problems were observed in the analysis of these different wines.

Four different wines were selected for the method development in order to take into account differences in matrix structure (e.g., alcohol content, pH, ionic strength, protein content, polyphenol composition). The four wines were spiked with known amounts of 44 volatile compounds (37 normal wine constituents and seven compounds previously selected as potential internal standards). The recoveries of those 44 compounds were calculated and are shown in Table 2. As expected, the range of recoveries is very wide. Compounds highly soluble in water, such as acetic and propanoic acids, *n*-propanol, acetoine, and acetaldehyde, are poorly extracted. Compounds with polar functions – such as alcohols and acids – with

Table 2
Recoveries of different compounds in four different wines

Compound	Recovery (%)			
	Wine W1	Wine W2	Wine Rs	Wine Rd
Potential internal standards				
2-Butanol	30	29	27	27
4-Methyl-2-pentanol	89	87	89	87
4-Hydroxy-4-methyl-2-pentanone	44	40	40	40
2-Octanol	98	97	98	97
Acetone	34	37	32	37
Ethyl 2-hydroxyvalerate	94	92	92	90
Pentanoic acid	56	56	56	55
Analytes				
Acetaldehyde	15	14	13	12
Diacetyl	26	29	27	27
1-Propanol	12	10	10	9
1-Butanol	35	33	30	32
Isobutanol	34	32	31	31
Isoamyl alcohol	68	67	67	66
Ethyl acetate	91	89	90	88
Isobutyl acetate	99	98	100	98
Isoamyl acetate	98	98	98	97
Hexyl acetate	96	95	95	95
Ethyl propanoate	98	98	99	98
Ethyl butyrate	99	98	100	98
Ethyl isobutyrate	100	99	99	98
Ethyl 3-methylbutyrate	99	100	99	98
1-Hexanol	94	91	93	90
cis-3-Hexenol	84	83	84	82
Acetic acid	8	6	4	4
Propanoic acid	12	13	11	10
Butyric acid	22	22	18	19
Isobutyric acid	24	16	21	20
Isovaleric acid	52	51	51	51
Ethyl lactate	41	38	38	39
Ethyl 3-hydroxybutyrate	65	62	63	62
γ-Butyrolactone	28	21	23	24
Methionol	37	33	37	37
Benzyl alcohol	85	82	84	82
Ethyl hexanoate	97	96	97	96
Ethyl octanoate	93	94	92	92
Ethyl decanoate	87	91	87	89
Phenylethyl acetate	96	95	96	94
Diethyl succinate	99	97	98	96
Hexanoic acid	86	85	86	84
Octanoic acid	98	97	98	96
Decanoic acid	95	94	94	93
β-Phenylethanol	84	81	83	81
Acetoine	6	7	7	7
Furfural	49	47	40	43

W1, W2, Rs, Rd are the codes of the four different wines used in the study.

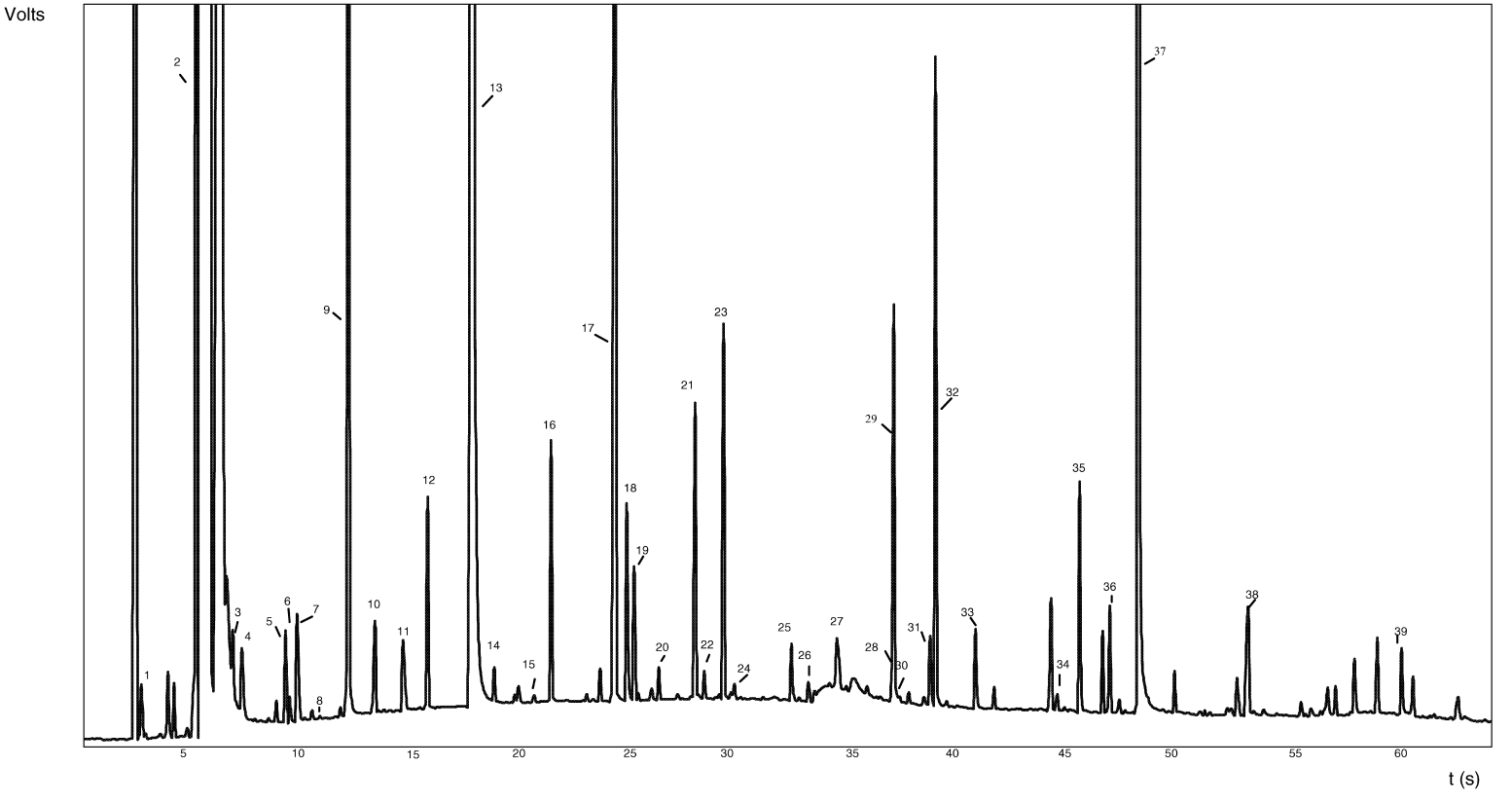

Fig. 1. Chromatogram of a wine extract obtained from an aged red wine from Spain. Peak identification: 1, acetaldehyde; 2, ethyl acetate; 3, ethyl isobutyrate; 4, diacetyl; 5, 2-butanol (internal standard); 6, ethyl butyrate; 7, 1-propanol; 8, ethyl 3-methylbutyrate; 9, isobutanol; 10, isoamyl acetate; 11, 1-butanol; 12, 4-methyl-2-pentanol (internal standard); 13, isoamyl alcohol; 14, ethyl hexanoate; 15, hexyl acetate; 16, acetoine; 17, ethyl lactate; 18, 1-hexanol; 19, 4-hydroxy-4-methyl-2-pentanone (internal standard); 20, *cis*-3-hexenol; 21, 2-octanol (internal standard); 22, ethyl octanoate; 23, acetic acid; 24, furfural; 25, ethyl 3-hydroxybutyrate; 26, propanoic acid; 27, isobutyric acid; 28, butyric acid; 29, γ-butyrolactone; 30, ethyl decanoate; 31, isovaleric acid; 32, diethyl succinate; 33, methionol; 34, 2-phenylethyl acetate; 35, hexanoic acid; 36, benzyl alcohol; 37, β-phenylethanol; 38, octanoic acid; 39, decanoic acid. Note: The apparent co-elution of compounds 28–30 is due to the poor quality of the printout.

less than six carbon atoms are partially extracted with recoveries ranging from 20 to 70%, and non-polar compounds or polar compounds with six or more carbon atoms are very well extracted with recoveries ranging from 80 to 100%. The general behaviour of the extraction can be considered as satisfactory in comparison to results obtained with other solvents. A comparison of the results obtained with the different wines shows an, in general, good agreement between the figures obtained for the four different wines for each compound. In addition, some of the differences observed in the table must be attributed to the imprecision of the experimental design. In spite of this, it is also clear that a reasonable accuracy in the analysis of the polar compounds will be obtained only if a good internal standard is selected to account for differences due to the matrix composition.

Internal standards were selected attending to precision and linearity criteria among the seven previously selected. In a first experiment, four wines and a synthetic solution were extracted repeatedly in different days, and the extracts injected also repeatedly on different days using a nested analysis of variance (ANOVA) design. In a second experiment, synthetic wines containing known amounts of the targeted volatiles were extracted and analysed following the procedure to check linearity. In these two experiments, the seven internal standards were added so that seven sets of results were calculated for each compound. The internal standard giving best results for each compound was selected. It was found finally that four internal standards are enough to get good data. A typical chromatogram obtained following the whole procedure can be seen in Fig. 1.

3.2. Method validation

Table 3 summarises method linearity data. As explained before, seven calibration graphs were built for each compound (one for each one of the seven potential internal standards assayed), but only data for the best of the seven are given. Data in the table clearly show that linearity is satisfactory in almost all cases, with the coefficient of correlation (r^2) ranging from 0.9938 (ethyl acetate) to 0.9998 (ethyl lactate). Linearity holds at least for one order of magnitude, and in most cases it holds for at least two, which ensures that the normal concentration range of nearly all the compounds present in Table 3 [28] is comprised in the linear range of the method. The slope of the straight calibration lines is a measure of method sensitivity and depends on both extraction efficiency and detector response for each compound. In this sense, the worst sensitivities are obtained for acetoine and propanoic acid, which are poorly recovered by the extraction (see Table 2) and, in addition, do not have very high FID responses.

Repeatability and reproducibility data are given in Table 4. Repeatability is the average standard deviation of a mean obtained from several replicate samples analyzed in the same batch. Reproducibility is the average standard deviation of the mean obtained from the analysis of several replicate samples analysed in different days. These data have been calculated in the four wines selected for the validation study and in a synthetic wine and, thereby, give an estimation of both figures of merit at different concentrations and in slightly different chemical environments. The study of the relationship of both parameters with the concentration reveals that at relatively high concentrations, the method shows, in general, a fixed relative standard deviation, and therefore, absolute repeatability or reproducibility increase with the concentration of analyte. At low analyte concentrations, however, it is the absolute values of repeatability and reproducibility, which remain constant with concentration and, therefore, relative standard deviation increases exponentially with decreasing concentration. This effect is clear in the case of acetaldehyde, for instance, which has a particularly poor FID response. In the case of some of the most concentrated compounds, however, which is observed at high concentrations is a fixed value of S (the standard deviation between measurements made on different days, i.e. reproducibility), as observed for isoamyl alcohol or for ethyl acetate. These observations are summarised in the two last columns of Table 4. The column headed by QL (quantification limit) gives the concentration at which the method estimated RSD will be 10%, and below which, higher RSD values are expected. The column headed by RSD gives the expected RSD of a measurement of the analyte at a concentration above its QL. In those cases in which a fixed S is observed, this value is given in Table 4.

Table 3
Method linearity data

Compound	Intercept	Slope	r^2	Range (mg/l)	n	I.S.[a]
Acetaldehyde	−0.1804	0.1155	0.9992	2–400	10	2B
Diacetyl	−0.0034	0.4754	0.9948	0.1–4	6	2B
1-Butanol	0.4608	1.2852	0.9996	2–220	10	2B
Isobutanol	0.9920	1.2416	0.9990	2–240	10	2B
Isoamyl alcohol	−0.8736	2.9417	0.9992	5–250	6	2B
Ethyl acetate	1.5354	0.4245	0.9938	5–110	6	4M
Isobutyl acetate	−0.0039	0.5745	0.9960	0.023–1	9	4M
Isoamyl acetate	−0.0135	0.7227	0.9964	0.05–5	10	4M
Hexyl acetate	−0.0104	0.9476	0.9956	0.020–1	9	4M
Ethyl propanoate	−0.0241	0.6006	0.9954	0.09–0.9	6	4M
Ethyl butyrate	−0.0026	0.6825	0.9974	0.04–4	10	4M
Ethyl isobutyrate	−0.0116	0.6463	0.9960	0.04–0.9	8	4M
Ethyl 3-methylbutyrate	−0.0041	0.7201	0.9956	0.02–0.9	9	4M
1-Hexanol	0.0249	1.0088	0.9994	0.250–25	10	4M
cis-3-Hexenol	−0.0045	0.9144	0.9994	0.02–1	9	4M
Propanoic acid	−0.0099	0.0497	0.9958	0.6–6	5	4O
Butyric acid	−0.0278	0.2672	0.9990	0.2–6	5	4O
Isobutyric acid	−0.0246	0.3059	0.9994	0.2–6	5	4O
Isovaleric acid	−0.0699	0.9150	0.9992	0.1–10	10	4O
Ethyl lactate	0.1157	0.4585	0.9998	2.5–250	10	4O
Ethyl 3-hydroxybutyrate	0.0004	0.9201	0.9994	0.04–1	8	4O
γ-Butyrolactone	0.0334	0.4486	0.9994	0.2–20	10	4O
Methionol	0.0010	0.5080	0.9992	0.1–3	8	4O
Benzyl alcohol	0.0069	1.6726	0.9994	0.02–1	9	4O
Ethyl hexanoate	−0.0105	0.8328	0.9978	0.05–3	8	2O
Ethyl octanoate	−0.0586	0.9457	0.9946	0.04–4	10	2O
Ethyl decanoate	−0.1075	0.8615	0.9980	0.14–1.4	5	2O
Phenylethyl acetate	−0.0181	1.4119	0.9996	0.023–1.2	9	2O
Diethyl succinate	−0.0606	0.6052	0.9998	0.23–23	10	2O
Hexanoic acid	−0.0476	0.7143	0.9996	0.09–9	10	2O
Octanoic acid	−0.0413	0.8012	0.9994	0.09–9	10	2O
Decanoic acid	−0.0336	0.9092	0.9982	0.08–8	10	2O
β-Phenylethanol	1.3825	0.9583	0.9996	3.5–70	6	2O
Acetoine	−0.0125	0.0475	0.9978	0.6–40	9	2O
Furfural	−0.0029	0.4587	0.9992	0.025–1.45	9	2O

[a] Internal standard. 2B: 2-butanol; 4M: 4-methyl-2-pentanol; 4O: 4-hydroxy-4-methyl-2-pentanone; 2O: 2-octanol.

It can be seen in Table 4, that QL ranges from 0.1 to 2.5 mg/l. For three compounds out of the 34 present in the table, QLs are higher than the mean concentration of these compounds in the four wines. This clearly indicates that the method is not suitable for the quantification of isobutyl acetate, ethyl 3-methylbutyrate and phenylethyl acetate because of the low concentration at which these compounds are present in the wine. For the rest of compounds, the sensitivity and precision given by the method is satisfactory. In five cases (isobutanol, isoamyl alcohol, ethyl acetate, ethyl lactate and β-phenylethanol), QLs could not be determined because they are well below the normal concentration of these compounds in wine. With regards to the overall precision of the method, the last column of Table 4 shows that RSD ranges from 3.1 to 10%, with an average value of 5.5%, which can be considered satisfactory for the purpose of the analysis.

Although recovery data in Table 2 showed a good agreement of the recoveries of each compound observed in the different wines, an experiment to verify the presence of matrix effects was carried out. In this case, volumes of wine spiked or not with known amounts of analytes were analyzed following the procedure. The measured relative area increments

Table 4
Repeatability and reproducibility data for four wines and a synthetic wine (All data are expressed in mg/l)

	Synthetic wine			White wine 1 (W1)			White wine 2 (W2)			Rosé wine (Rs)			Red wine (Rd)			QL[c]	RSD[d] (%)
	Mean	Srt[a]	Srd[b]	Mean	Srt[a]	Srd[b]	Mean	Srt[a]	Srd[b]	Mean	Srt[a]	Srd[b]	Mean	Srt[a]	Srd[b]		
Acetaldehyde	75.5	2.57	2.57	3.59	0.26	0.26	2.73	0.25	0.25	3.33	0.18	0.25	26.0	1.07	1.07	2.5	3.7
Diacetyl	1.17	0.10	0.10	0.40	0.01	0.10	<0.1			7.60	0.22	0.46	2.01	0.08	0.15	1.0	6.1
Isobutanol	10.6	0.20	0.20	21.9	0.26	0.86	28.2	0.30	0.92	55.8	1.37	1.70	28.3	0.10	0.89	–	3.1
Isoamyl alcohol	24.5	1.54	1.54	72.3	3.00	3.26	94.5	5.24	5.24	178	1.90	5.33	133	2.75	5.75	–	S=5.6
Ethyl acetate	46.4	2.75	2.75	79.9	1.91	3.76	73.0	0.60	3.85	52.3	2.10	3.25	120	1.20	3.82	–	S=3.7
Isobutyl acetate	0.62	0.03	0.03	<0.02			<0.02			0.06			0.05			<0.3	
Isoamyl acetate	1.37	0.07	0.07	0.10	0.00	0.01	0.47	0.02	0.02	0.54	0.02	0.02	0.77	0.03	0.04	0.1	5.1
Hexyl acetate	0.62	0.04	0.04	0.28	0.02	0.03	0.17	0.00	0.01	0.05	0.00	0.00	0.06	0.00	0.01	0.1	6.5
Ethyl propanoate	0.29	0.04	0.04	0.15	0.00	0.01	0.19	0.03	0.03	0.12	0.01	0.02	0.33	0.04	0.04	0.1	
Ethyl butyrate	1.16	0.05	0.05	0.25	0.01	0.02	0.40	0.03	0.03	0.18	0.01	0.02	0.24	0.01	0.02	0.2	4.3
Ethyl isobutyrate	0.43	0.06	0.07	0.20	0.01	0.01	0.11	0.01	0.02	0.07	0.00	0.01	0.08	0.01	0.01	0.15	
Ethyl 3-methylbutyrate	1.01	0.05	0.08	0.03			<0.02			<0.02			<0.02			<0.8	
1-Hexanol	4.07	0.08	0.08	0.88	0.06	0.08	1.13	0.09	0.09	1.14	0.04	0.05	1.31	0.01	0.09	0.7	S=0.08
cis-3-Hexenol	0.95	0.01	0.01	0.11	0.00	0.01	0.08	0.01	0.01	0.14	0.02	0.01	0.15	0.01	0.01	0.1	S=0.01
Propanoic acid	2.02	0.28	0.28	1.10	0.07	0.07	<0.6			1.74	0.05	0.07	4.27	0.46	0.46	0.7	10
Butyric acid	1.65	0.07	0.07	1.55	0.08	0.08	2.13	0.10	0.10	0.77	0.03	0.05	1.97	0.09	0.09	0.5	4.5
Isobutyric acid	1.61	0.07	0.07	1.56	0.07	0.07	1.06	0.04	0.04	2.49	0.14	0.14	1.81	0.10	0.10	0.4	5.0
Isovaleric acid	1.77	0.02	0.02	0.68	0.03	0.03	0.51	0.01	0.02	1.49	0.05	0.07	1.40	0.03	0.05	0.2	4.0
Ethyl lactate	7.63	0.24	0.24	205	7.84	8.01	59.6	3.20	5.37	61.7	1.09	3.09	109	3.44	3.70	–	5.3
Ethyl 3-hydroxybutyrate	0.98	0.03	0.03	0.19	0.01	0.01	0.25	0.02	0.02	0.42	0.02	0.02	0.93	0.02	0.04	0.1	5.3
γ-Butyrolactone	3.03	0.12	0.12	5.99	0.16	0.29	9.23	0.72	0.72	13.4	0.73	0.73	12.5	0.26	0.88	1.0	5.9
Methionol	2.63	0.12	0.12	0.30	0.01	0.03	0.54	0.01	0.03	2.19	0.11	0.16	2.90	0.11	0.11	0.3	S=0.12
Benzyl alcohol	0.95	0.04	0.05	0.08	0.00	0.00	0.27	0.01	0.01	0.69	0.02	0.02	0.85	0.02	0.10	0.1	3.9
Ethyl hexanoate	1.08	0.04	0.04	0.61	0.02	0.02	0.77	0.02	0.02	0.16	0.01	0.01	0.24	0.01	0.03	0.1	3.2
Ethyl octanoate	0.84	0.06	0.06	0.53	0.01	0.01	0.66	0.05	0.05	0.15	0.00	0.01	0.20	0.04	0.06	0.1	6.1
Ethyl decanoate	1.02	0.08	0.08	0.30	0.02	0.02	0.27	0.01	0.01	0.18	0.00	0.08	0.21	0.01	0.01	0.2	6.4
Phenylethyl acetate	0.48	0.01	0.01	<0.02			<0.02			<0.02			0.09	0.00	0.01	0.1	
Diethyl succinate	3.06	0.06	0.06	4.85	0.29	0.30	4.75	0.22	0.22	2.62	0.06	0.10	7.11	0.09	0.40	1	4.7
Hexanoic acid	1.53	0.07	0.07	4.78	0.21	0.21	5.45	0.35	0.35	1.21	0.05	0.07	2.05	0.02	0.33	0.7	8.6
Octanoic acid	1.73	0.05	0.07	6.58	0.10	0.10	6.46	0.19	0.48	1.09	0.05	0.05	2.38	0.04	0.10	0.5	4.7
Decanoic acid	1.48	0.04	0.06	1.43	0.01	0.06	1.09	0.05	0.07	0.19	0.01	0.01	0.46	0.04	0.04	0.2	5.8
β-Phenylethanol	11.3	0.65	0.65	7.20	0.48	0.48	12.3	0.71	1.57	34.5	0.83	2.71	43.2	1.05	2.70	–	7.2
Acetoine	3.34	0.15	0.15	29.0	2.99	3.11	2.38	0.13	0.30	16.8	0.94	1.00	30.7	1.58	1.96	2.5	7.2
Furfural	0.88	0.03	0.03	0.27	0.02	0.02	1.36	0.07	0.12	0.03	0.01	0.01	0.12	0.00	0.00	0.1	7.3

[a] Srt: Repeatability.
[b] Srd: Reproducibility.
[c] QL, Concentration for which the estimated reproducibility is 10%.
[d] Relative standard deviation (%) at concentrations higher than QL, or S (in mg/l) if this is constant.

were interpolated in the calibration graphs shown in Table 3. The results of the experiment can be seen in Table 5. In most cases, the degree of agreement between the real amount added and that determined by interpolation is satisfactory, and only for diacetyl differences are higher than 20%.

In conclusion, the proposed method allows for a fast and cheap quantitative determination of more than 30 volatiles in wine. Among these volatiles are some important analytes, markers for the microbiological state of wine, for the wine sensory characteristics, or are markers of wine origin (both geographic and varietal). The analytical characteristics – linearity, precision and accuracy – of the method are satisfactory. All these characteristics make the method useful for wine quality control and classification, and to give information which could be used in the control of winemaking processes.

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

	mg/l (added)	mg/l (calculated)
Acetaldehyde	68.0	67.0
Diacetyl	1.10	0.82
Isobutanol	12.2	11.8
Isoamyl alcohol	24.6	23.2
Ethyl acetate	41.7	46.4
Isobutyl acetate	0.68	0.70
Isoamyl acetate	1.44	1.27
Hexyl acetate	0.63	0.53
Ethyl propanoate	0.91	0.98
Ethyl butyrate	1.18	1.12
Ethyl isobutyrate	0.96	0.94
Ethyl 3-methylbutyrate	0.97	0.88
1-Hexanol	4.69	4.02
cis-3-Hexenol	1.20	1.00
Propanoic acid	2.71	2.79
Butyric acid	2.01	2.40
Isobutyric acid	1.98	2.05
Isovaleric acid	2.07	2.29
Ethyl lactate	9.75	9.97
Ethyl 3-hydroxybutyrate	1.10	1.12
γ-Butyrolactone	3.87	3.64
Methionol	28.3	27.1
Benzyl alcohol	1.18	1.11
Ethyl hexanoate	1.47	1.11
Ethyl octanoate	1.07	1.05
Ethyl decanoate	1.04	1.20
Phenylethyl acetate	0.70	0.57
Diethyl succinate	4.42	3.90
Hexanoic acid	2.34	2.34
Octanoic acid	2.48	2.19
Decanoic acid	2.06	1.90
β-Phenylethanol	16.48	17.81
Acetoine	5.33	5.96
Furfural	1.34	1.11

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References

[1] M.D. Cabezudo, M.C. Polo, M. Herraiz, G. Reglero, M. González-Raurich, I. Cáceres, P. Martín-Alvarez, in: Proceedings of the 4th International Flavor Conference, Rhodes, The Shelf Life of Foods and Beverages, 23–26 July 1985, p. 186.
[2] M. Forcen, A. Berna, A. Mulet, Lebensm.-Wiss. Technol. 26 (1993) 54.
[3] V. Ferreira, P. Fernandez, J.F. Cacho, Food Sci. Technol. 29 (1996) 251.
[4] Y. Shimazu, M. Uehara, M. Watanabe, Agric. Biol. Chem. 49 (1985) 2147.
[5] M.H. Laurent, T. Henick-Kling, T.E. Acree, Wein Wiss. 49 (1994) 3.
[6] P. Chatonnet, D. Dubourdieu, J.N. Boidron, M. Pons, J. Sci. Food Agric. 60 (1992) 165.
[7] A.P. Pollnitz, K.H. Pardon, M.A. Sefton, Aust. Grapegrower Winemaker June/July (2000) 45.
[8] H. Guth, J. Agric. Food Chem. 45 (1997) 3027.
[9] V. Ferreira, R. Lopez, J.F. Cacho, J. Sci. Food Agric. 80 (2000) 1659.
[10] V. Ferreira, A. Rapp, J.F. Cacho, H. Hastrich, I. Yavas, J. Agric. Food Chem. 41 (1993) 1413.
[11] C. SaenzBarrio, T. CedronFernandez, Chromatographia 51 (2000) 221.
[12] J. Villen, F.J. Senorans, G. Reglero, M. Herraiz, J. Agric. Food Chem. 43 (1995) 717.
[13] C. Fischer, U. Fischer, J. Agric. Food Chem. 45 (1997) 1995.
[14] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 808 (1998) 211.
[15] Y. Hayasaka, E.J. Bartowsky, J. Agric. Food Chem. 47 (1999) 612.
[16] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 849 (1999) 293.
[17] A.P. Pollnitz, G.P. Jones, M.A. Sefton, J. Chromatogr. A 857 (1999) 239.
[18] V. Bellavia, M. Natangelo, R. Fanelli, D. Rotilio, J. Agric. Food Chem. 48 (2000) 1239.
[19] D. De la Calle Garcia, M. Reichenbächer, K. Danzer, C. Hurlbeck, C. Bartzsch, K.-H. Feller, Fresenius J. Anal. Chem. 360 (1998) 784.
[20] S. Francioli, M. Guerra, E. Lopez-Tamames, J.M. Guadayoi, J. Caixach, Am. J. Enol. Vitic. 50 (1999) 404.
[21] R.F. Simpson, Vitis 17 (1978) 274.
[22] T. Shinohara, Bull. Off. Int. Vigne Vin 57 (1984) 606.
[23] P.X. Etievant, in: H. Maarse (Ed.), Volatile Compounds in Foods and Beverages, Marcel Dekker, New York, 1991, p. 483.
[24] V. Ferreira, C. Peña, A. Escudero, P. Fernandez, J. Cacho, J. Sci. Food Agric. 67 (1995) 381.
[25] D. Quain, J. Inst. Brewing 95 (1988) 315.
[26] L. Nykänen, Am. J. Enol. Vitic. 37 (1986) 84.
[27] V. Ferreira, L. Ortega, A. Escudero, J. Cacho, J. Chromatogr. Sci. 38 (2000) 469.
[28] H. Maarse, C.A. Vischer, Volatile Compounds in Food, Alcoholic Beverages, Qualitative and Quantitative Data, TNO-CIVO, Food Analysis Institute, Zeist, 1989.