Fast and Quantitative Determination of Wine Flavor Compounds Using Microextraction with Freon 113

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A fast and efficient method for analyzing wine volatiles has been developed. Twenty-six compounds can be quantified with a single-step sample treatment of only 1 h via microextraction with Freon 113. Precision, linearity, and accuracy of the method have been tested using different wines and synthetic mixtures. Detection limits are in the micrograms per liter range, and relative precision is better than 3%.

INTRODUCTION

The task of studying, monitoring, controlling, and standardizing the flavor of a foodstuff, and in particular that of wine, demands from the analytical laboratory a number of requisites difficult to deal with as they are often contradictory.

On one hand, the flavor of a wine is extremely complex, due to the great number of species which are found in it, the disparity of concentrations in which those species exist, and the interactions that take place among them and with other matrix components (Maarse and Visscher, 1989; Piggot and Findlay, 1984; Williams and Rosser, 1981). Therefore, to obtain objective information representing, though only partially, the subjective phenomenon of flavor, we need to get first of all quantitative data of a huge number of compounds which are, moreover, present in a wide range of concentrations.

On the other hand, controlling a process, standardizing the composition of a product, or correlating the aromatic composition with the sensory data requires analysis of a great number of samples as quickly as possible, particularly in the first case. It is necessary, therefore, to achieve an equilibrium between the quantity and the quality of the information obtained and the cost and time needed. This balance has not as yet been attained.

There is no doubt that the most complete information can be obtained by combining different chromatographic methods over a very concentrated flavor extract (LC/HRGC; HRGC/HRGC; HPLC/HRGC...), but this approach is unaffordable, due to the high cost and the large amount of time needed to make an extensive study. By contrast, the information can be obtained most quickly by using direct injection or by means of automatic headspace techniques, but these methods give little information about the relevant flavor compounds.

Traditionally, wine has been extracted continuously using a low boiling point solvent (Cobb and Bursey, 1976; Marais and Houtman, 1979; Usseglio Tomasset, 1983; Boison and Tomlinson, 1990). The extract is then dried, concentrated, and finally injected in the chromatograph by means of the split injection technique. However, there are several objections to this approach:

(1) The extraction times are long, and the solvents must be scrupulously purified.

(2) In general, the extracts can only be injected on split; so to reach the sensitivity required, they often have to be concentrated thousands of times. These concentration processes result in a significant loss of solutes due to coevaporation, as well as a certain loss in accuracy (Grob and Muller, 1987; Murray, 1979).

(3) Discrimination processes can take place during the concentration step and in the injection of a very dirty sample (Munari and Trestianu, 1981; Grob and Neukom, 1979).

Although all of these objections can be minimized through scrupulous work, they constitute, however, an important handicap.

In this work a method to analyze wine volatiles by means of microextraction with 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113) is presented. A microextraction is a single-step extraction with a very high liquid sample/solvent ratio. Thus, the extract obtained does not need to be concentrated, and the process can be carried out at room temperature.

Grob et al. (1975), Jennings (1981), Rhoades and Nulton (1980), and Kok et al. (1987) have proposed several analytical methods based on this idea, but their results are semiquantitative. Other microextraction methods are quantitative but cannot be applied to wine (Cacho et al., 1992). Yuvas et al. (1991) and Rapp (1993) have recently proposed a similar method based on the use of Freon 113 for the analysis of spirits. Freon 113 is a solvent with a higher extractive capacity than pentane (McDaniel et al., 1990) and with a relatively high boiling point, which allows the extract to be injected by splitless. On the basis of these ideas, a quick, quantitative, and easy to manage analytical method is presented which allows for the simultaneous quantification of at least 26 wine volatile compounds.

APPARATUS AND REAGENTS

Reagents. The pure chromatographic standards of analytical quality were obtained from Chemservice (West Chester, PA) and Merck (Darmstadt, Germany). Two standard solutions, one in ethanol and another in Freon 113, of these compounds were prepared. Their concentrations can be seen in Table I.

Synthetic wine solutions: ethanol, 8, 10, 10.5, 11, 11.5, 12, 12.5, 13, and 13.5% (v/v); tartaric acid, 6 g L^{-1}; pH 3.2.

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Table I. Calibrated Standard Solutions Used in This Study

compound	concentration, mg L^{-1} in ethanol	concentration, mg L^{-1} in Freon 113
ethyl butyrate	133.4	156.2
ethyl hexanoate	112.8	165.1
ethyl octanoate	170.9	248.9
ethyl decanoate	80.20	139.3
ethyl laurate	32.40	33.76
ethyl succinate	750.0	910.8
ethyl lactate	724.8	805.8
ethyl malonate	100.3	192.7
ethyl cinnamate	11.64	15.15
isobutyl acetate	194.9	240.0
isoamyl acetate	253.0	292.3
hexyl acetate	62.90	65.57
amyl acetate	5.09	6.63
1-butanol	39.30	40.92
1-pentanol	14.75	19.27
isoamyl alcohol	1601	1335
β-phenylethanol	1056	785.0
1-hexanol	597.6	935.2
1-heptanol	6.49	8.45
1-octanol	4.34	5.65
cis-3-hexenol	35.50	37.46
trans-2-hexenol	18.70	19.58
linalool	4.78	6.23
geraniol	5.03	6.55
nerol	4.84	6.30
hexanoic acid	84.5	138.1

1,1,2-Trichloro-1,2,2-trifluoroethane (Freon 113) of HPLC quality was obtained from Aldrich (Milwaukee, WI).

Apparatus. *Volumetric material*: Hamilton syringes of volumes between 1 and 500 μL previously calibrated.

Extraction tubes: centrifuge tubes with a conic bottom; 12 cm × 17 o.d.; screw-capped with Teflon septa.

Mechanical shaker: GFL 3016.

HP 5890 Series II gas chromatograph, fitted with split–splitless injector, and automatic sampler 7673 A were used.

Column: Supelcowax 10, 60 m × 0.32 mm i.d., 0.5-μm film thickness.

Chromatographic conditions: carrier, H_2; head pressure, 120 kPa; split flow, 27 mL min^{-1}; purge flow, 3 mL min^{-1}; splitless time, 3 min; injector and detector temperatures, 220 °C; initial column temperature, 40 °C, held for 5 min and then raised to 180 °C at 2 °C min^{-1}; makeup gas, N_2 at 30 mL min^{-1}; detector FID, H_2 at 30 mL min^{-1}; air, 300 mL min^{-1}; injected volume, 1 μL.

Data were recorded and processed in a NEC computer with Maxima from Waters program, version 3.3, 1990.

Mass spectrometry: HP 5890 Series II chromatograph, fitted with a 5971A electronic impact MS detector, was used. Signal was registered and processed with MS Chemstation Series II software fitted with the Wiley MS library.

Conditions: injected volume, 1 μL; mode, splitless (3 min); carrier, He at 50 kPa; initial temperature 40 °C, held for 5 min and then raised at 2 °C min^{-1} to 220 °C.

Column: Supelcowax 10, 60 m × 0.32 mm i.d., 0.5-μm film thickness.

METHODS

Proposed Methods. Introduce 10 mL of wine into the extraction tubes. Add 4.2 g of ammonium sulfate, 2 μL of a solution of 4-methyl-2-pentenol (400 mg L^{-1} in ethanol as internal standard) and 100 μL of Freon 113 as extracting agent. Cap the tubes and shake them until the salt dissolves. Then put the tubes to shake in an automatic shaker for 1 h at maximum speed. After this, centrifuge the tubes (3000 rpm for 15 min) and recover the organic phase with a 0.25-mL syringe, transfer it into a vial, and analyze it under the chromatographic conditions given before. After chromatographic analysis, the relative areas or heights of the calibrated peaks are interpolated from calibration graphs created with synthetic wine solutions having an alcohol content similar to that of the analyzed wine, as explained below.

Calibration Graph. (The calibration graph equations vary slightly but noticeably with the degree of alcohol in the wine. Thus, it is convenient to use several groups of calibration graphs covering the range of alcohol content of the wines to be studied.) By dilution of the standard ethanol solution given in Table I, prepare eight solutions containing 0.05, 0.2, 0.5, 1, 2, 3, 5, and 10 mL of the volatile solution in 100 mL of synthetic wine (with an alcohol content similar to that of the wines to be extracted). Extract them following the proposed method and create the calibration graphs with the chromatographic results.

Qualitative Analysis. Identification of the different compounds was performed by mass spectrometry and comparison of the retention times with those of the pure chemical standards.

Method Optimization. Analytical Characteristics of the Proposed Method. *Optimization of the Chromatographic Quantification.* (*a*) *Precision.* A wine extract was injected six times under optimum chromatographic conditions, and quantification was carried out on the basis of relative peak areas and heights and on absolute areas.

(*b*) *Linearity.* Linearity of injection was tested by injecting in triplicate five standard solutions in Freon 113 prepared from solution 2 in Table I. In separate 25-mL volumetric flasks, we added 25, 15, 10, 5, and 2 mL of solution 2. The same amount of internal standard was added to each flask and then brought to volume with Freon 113.

(*c*) *Accuracy.* To test possible injection matrix effects, a wine extract was diluted with pure solvent 10, 20, 30, and 50% and reinjected under the same previous conditions to look for possible changes in the relative areas and/or heights.

Extraction Optimization. A wine was extracted using different volumes, salts, salt amounts, and shaking times to look for the optimum extracting conditions.

Extraction Features. (*a*) *Precision of the Extraction.* Three different wines (white, 11.3% alcohol and pH 2.90; rosé, 12.5% alcohol and pH 3.15; and red, 13.2% alcohol and pH 3.40) were extracted six times, each one under the optimum conditions previously found.

(*b*) *Linearity and Accuracy of Extraction.* Using the same three wines, a standard addition experiment was performed in the following way: For each wine, two solutions were prepared, one of them containing 6 mL of the standard solution I and wine up to 500 mL and the other one 6 mL of pure ethanol and wine up to 500 mL. Then 0, 5, 10, 20, 30, 40, and 50 mL of the first of those solutions was, respectively, transferred to six 50-mL volumetric flasks and brought to volume with the second solution. This procedure was followed with each wine (white, rosé, and red). These last solutions were extracted and analyzed according to the proposed method.

A standard dilution experiment was equally carried out with the same three wines. Each wine was diluted 10, 20, 40, and 60% with a synthetic wine solution of similar alcohol content and pH and then extracted and analyzed following the proposed method.

To study the influence of the alcohol content on the extraction, nine calibration graphs were plotted, following the method explained before, with synthetic wine solutions of alcohol content between 8 and 13.5%.

(*c*) *Detection Limits.* A plot representing chromatographic height vs concentration in wine was made for each compound. The minimum detectable amount of solute was considered to be that whose height was 10 times higher than the background signal.

RESULTS AND DISCUSSION

Chromatographic Separation and Quantification. The whole chromatographic process (injection + separation + quantification) must be sensitive, efficient, and, above all, quantitative. In capillary gas chromatography, the key step to achieve these requisites is the chromatographic injection. Wine extracts contain a certain amount of nonvolatile material, and as it is not convenient to clean

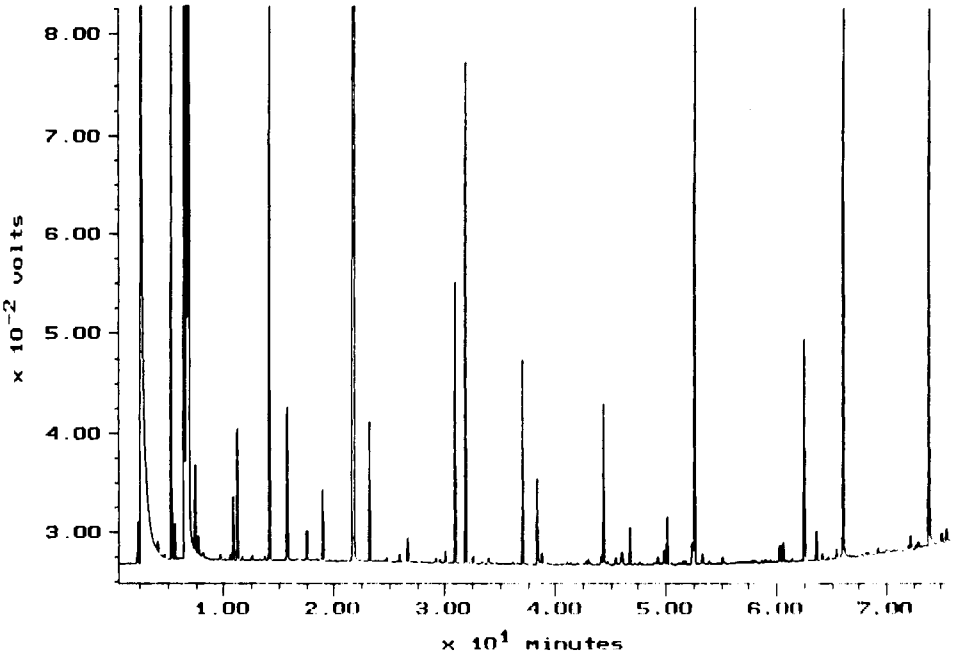

Figure 1. Chromatogram of a wine microextract obtained from a grenache red wine from Aragón. Split injection 1/20.

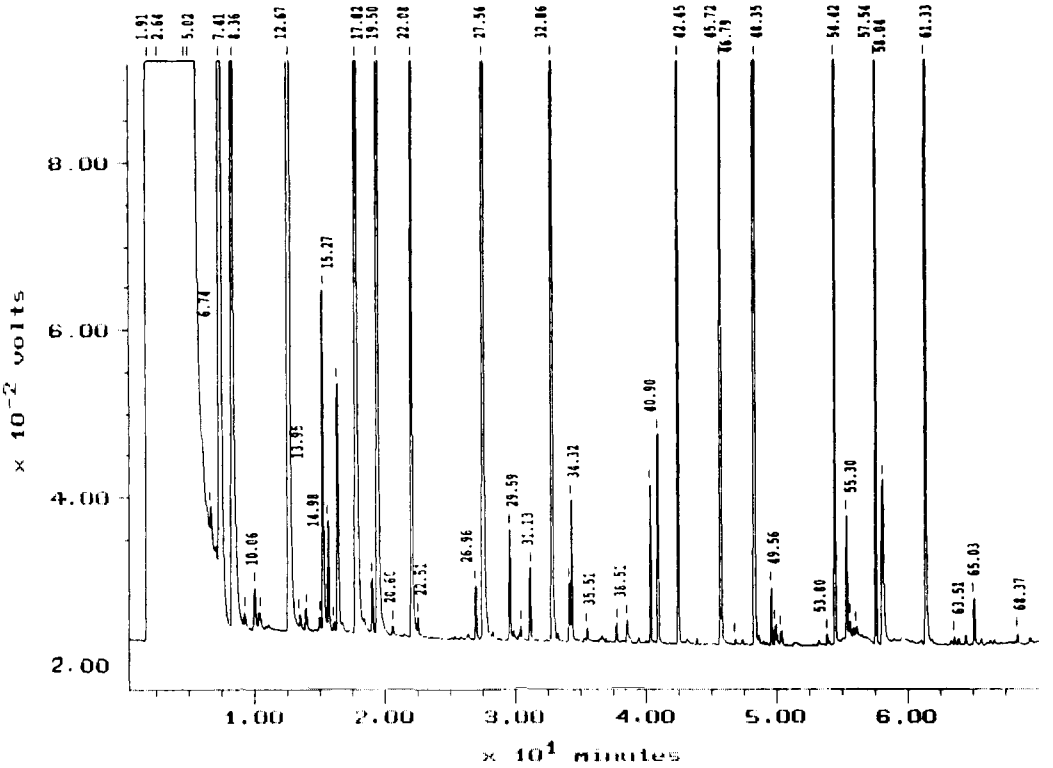

Figure 2. Calibration chromatogram obtained from the microextraction of a synthetic hydroalcoholic calibrated mixture.

up the wine extract to inject it by on-column techniques; a vaporizing injection technique must be used, and during vaporization some quantitative changes may occur.

Split injection is easy, and good peak resolution can be achieved; but it is the least sensitive technique, and discrimination may occur. Sensitivity can be improved by further concentrating the extract, but concentration of a very small volume of extract is problematic and makes the extract dirtier. Thereby, classical splitless injection has been chosen in this work. Besides, the boiling point of the solvent (49 °C) permits exploitation of the advantages of the solvent effect due to its recondensation inside the chromatographic column. Efficiency of this kind of injection is acceptable, and only the first peaks of the chromatogram show an effect of band broadening in time (Grob, 1982). Its only limitation is that some peaks from highly volatile compounds are lost because they are overlapped with solvent and ethanol peaks (for instance, ethyl propanoate and ethyl acetate). However, this loss is not very important, and the amount and quality of information generated are high. Figures 1–3 show the chromatograms obtained in (1) a split injection of a wine extract, (2) a splitless injection of a calibrated solution; and (3) a splitless injection of a wine extract.

Table II shows analytical data of this kind of injection for both real wine extracts and synthetic mixtures. Three

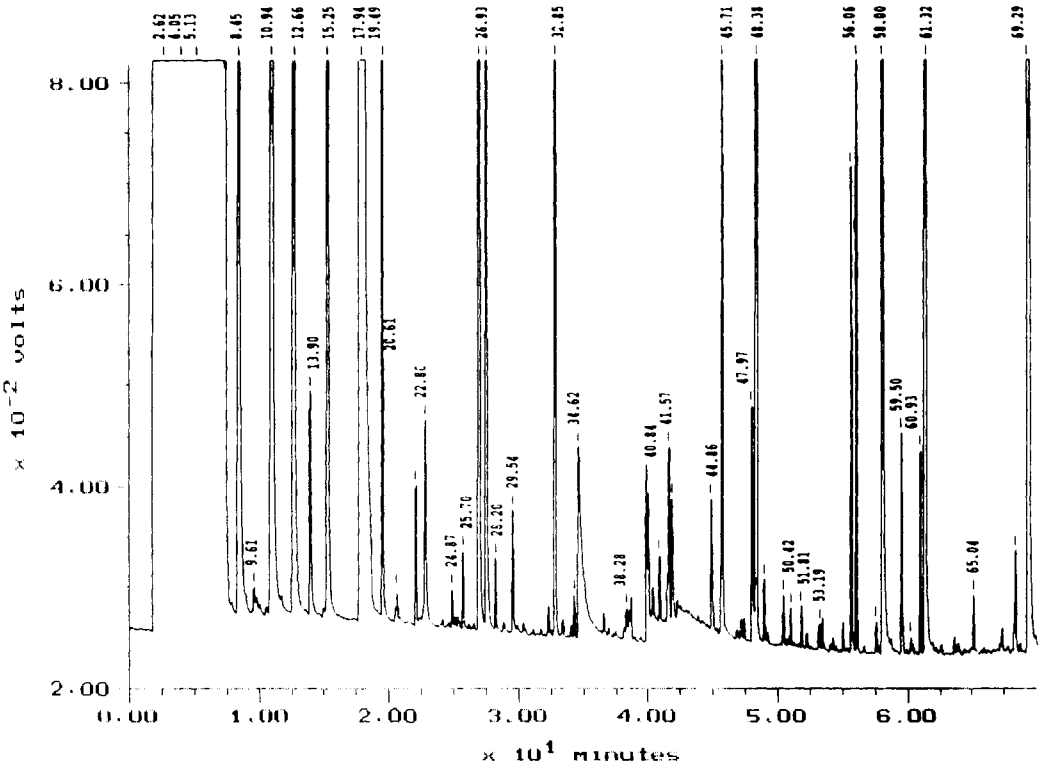

Figure 3. Chromatogram of a wine microextract obtained from a grenache red wine from Aragón. Splitless injection. Identification of some components (splitless injection): 7.41, ethyl isobutyrate and isobutyl acetate; 8.40, ethyl butyrate; 10.94, isobutanol; 12.66, isoamylacetate; 13.90, 1-butanol; 15.26, internal standard; 16.40, amyl acetate; 17.90, isoamyl alcohol; 19.50, ethyl hexanoate; 20.60, amyl alcohol; 22.07, hexyl acetate; 22.80, acetoine; 25.70, 4-methypentanol; 26.93, ethyl lactate; 27.56, 1-hexanol; 28.20, *trans*-3-hexenol; 29.57, *cis*-3-hexenol; 31.13, *trans*-2-hexenol; 32.85, ethyl octanoate; 34.32, 1-heptanol; 34.62, acetic acid; 39.97, 2,3-butanediol; 40.40, linalool; 40.91, 1-octanol; 41.57, isobutyric acid; 42.45, diethyl malonate; 44.90, butyric acid; 45.71, ethyl decanoate; 47.97, isovalerianic acid; 48.38, diethyl succinate; 48.92, α-terpineol; 50.35, methionol; 56.06, phenylethyl acetate; 57.56, ethyl laurate; 58.00, hexanoic acid; 59.50, benzyl alcohol; 61.33, β-phenylethanol; 67.82, γ-nonalactone; 69.29, octanoic acid.

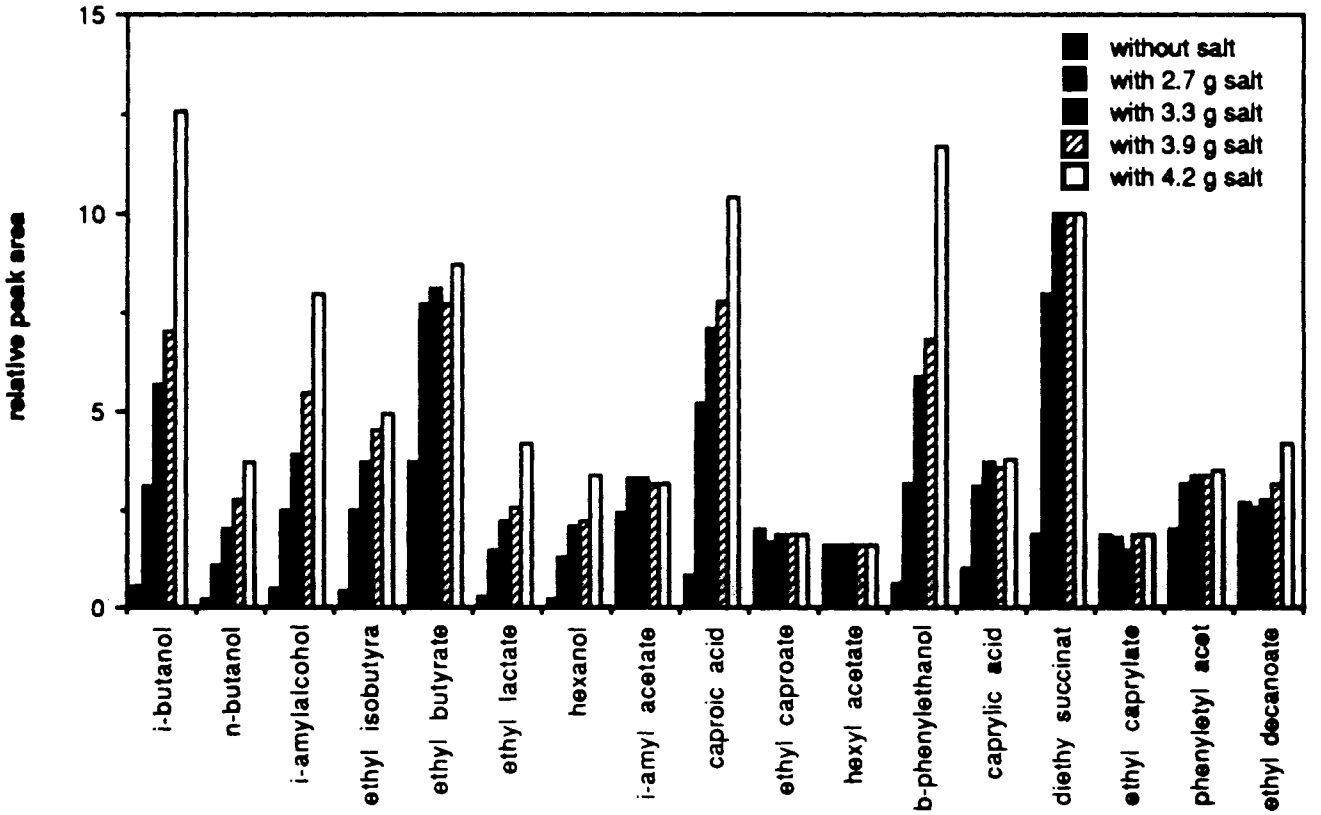

Figure 4. Salting out effect over the microextraction.

different calibrations were studied: (1) external standard based on areas (data shown as RSD 1 and r_1); (b) internal standard based on areas (data shown as RSD 2 and r_2); and (c) internal standard based on heights (data shown

Table II. Precision and Linearity of Injection, Retention Times, and Their Reproducibility[a]

compound	RSD 1, %	RSD 2, %	RSD 3, %	r_1	r_2	r_3	Rt, min	RSD, %
isobutyl acetate	9.7	1.1	2.2	0.99975	0.99953	0.99991	7.4	2.1
ethyl butyrate	11.9	2.7	2.6	0.99999	0.99997	0.99989	8.4	1.3
isobutanol	8.6	0.27	0.6				10.94	0.9
isoamyl acetate	9.0	1.1	0.95	0.99994	0.99999	0.99998	12.66	0.5
1-butanol	7.6	3.2	2.0	0.99982	0.99912	0.99976	13.90	0.6
amyl acetate				0.99618	0.99657	0.99940	15.76	0.3
isoamyl alcohol	6.5	2.5	3.1	0.99990	0.99995	0.99994	17.90	0.9
ethyl hexanoate	10.0	1.1	1.6	0.99997	1.0000	0.99998	19.50	0.8
pentanol	8.9	0.5	0.9	0.97485	0.97505	0.99780	20.60	0.6
hexyl acetate	9.4	0.78	1.3	0.99995	0.99994	0.99987	22.07	0.3
acetone	7.7	1.3	1.3				22.80	0.4
3-methylpentanol	8.2	2.9	2.1				24.97	0.1
4-methylpentanol	7.6	1.9	2.0				25.70	0.1
ethyl lactate	8.8	0.68	0.50	0.99990	0.99981	0.99971	26.93	0.1
1-hexanol	9	0.60	0.70	0.99989	0.99994	0.99994	27.56	0.2
cis-3-hexenol	9.0	2.9	1.2	0.99998	0.99992	–0.99973	29.57	0.2
trans-2-hexenol	11.0	2.9	1.2	0.99948	0.99984	1.0000	31.13	0.3
ethyl octanoate	9.4	0.8	1.3	0.99988	0.99996	0.99902	32.85	0.3
1-heptanol	10.4	0.96	0.95	0.99847	0.99870	0.99994	34.32	0.2
acetic acid	11.2	3.8	4.5				34.62	0.6
benzaldehyde	13.1	5.7	3.4				36.15	0.2
ethyl 3-hydroxybutyrate	9.3	0.47	0.81				36.57	0.7
propanoic acid	14.3	6.7	4.2				38.86	0.4
2,3-butanediol	12.1	4.8	5.6				39.97	0.5
linalool	9	1.11	1.08	0.99840	0.99838	0.99645	40.31	0.5
1-octanol				0.99981	0.99991	0.9992	40.91	0.4
isobutyric acid	12.6	4.3	4.6				41.57	0.2
diethyl malonate	12.0	3.6	0.90	0.99980	0.99976	0.99919	42.45	0.6
butyric acid	11.2	3.1	2.23				44.90	0.5
γ-butyrolactone	9.3	3.2	2.3				45.52	0.3
ethyl decanoate	9.0	0.41	0.68	0.99758	0.99967	0.99504	45.71	0.1
isovalerianic acid	12.3	8.3	6.2				47.97	0.1
diethyl succinate	13.0	4.7	0.51	0.99818	0.99926	0.99930	48.38	0.4
α-terpineol	9.0	0.41	0.49				49.12	0.2
methionol	9	0.25	0.46				50.35	0.1
ethyl phenylacetate	13.0	6.0	2.1				54.55	0.1
phenylethyl acetate		0.48	0.39				56.06	0.0
nerol		1.7	2.0	0.9984	0.99877	0.99812	56.52	0.2
ethyl laurate	9.8	0.81	0.62	0.99505	0.99959	0.99914	57.54	0.1
hexanoic acid	12.0	3.1	11.0	0.9984	0.99984	0.99576	58.00	0.1
isoamylacetamide	8.7	0.84	1.5				58.59	0.2
geraniol	9.7	0.69	0.56	0.9979	0.99899	0.99882	58.90	0.2
benzyl alcohol	10	1.1	0.7				59.50	0.1
β-phenylethanol	7.5	1.9	2.2	1.0000	0.9982	0.99789	61.32	0.0
γ-nonalactone	6.9	2.2	1.3				67.93	0.1
octanoic acid	8.9	1.6	4.2				69.29	0.1
ethyl cinnamate				0.99904	0.99975	0.99985	72,31	0.3

[a] RSD 1 and r_1 ae referred to absolute area calibration, RSD 2 and r_2 to relative areas, and RSD 3 and r_3 to relative altures calibration.

as RSD 3 and r_3). The results of the experiment lead to the following conclusions:

(*1*) Under the conditions used in this study, external standard calibration was the worst calibration method, showing a high degree of imprecision. This may be due to problems in the automatic sampler.

(*2*) Internal standard calibration based on areas is the most appropriate for the most concentrated compounds, for instance, ethyl octanoate or ethyl decanoate.

(*3*) On the contrary, internal standard calibration based on heights is the most appropriate for the most diluted compounds, for instance, *trans*-2-hexenol and *cis*-3-hexenol.

(*4*) For most of the compounds, precision (as RSD) and linearity (as r) are very good (RSD < 2.5%; $r > 0.999$).

(*5*) Retention times remain constant, allowing easy identification.

The experiments carried out with diluted extracts revealed that there were no significant changes in the relative areas for the quantified compounds, thus showing that, in the conditions given, there were no significant matrix effects.

Extraction Optimization. The experiments carried out with different salts showed that ammonium sulfate is the most appropriate salt for achieving good salting out effects. The other salts tested (magnesium sulfate, sodium chloride, and monosodium phosphate) showed more tendency of emulsion formation, and the recovery of the organic phase became problematic.

Both the amount of salt and the shaking time were critical to achieve a good extraction. The results of the extraction with different amounts of salt are presented in Figure 4. Over 10 mL of wine and 4.2 g of ammonium sulfate must be added, since lesser quantities might lead to poorer recoveries, and larger amounts could demixture the wine and form an ethanolic phase containing volatiles and polyphenols. Shaking time has a very strong influence on the extraction precision. One hour of shaking was required to reach a good extraction precision for all compounds studied.

Other important parameters to be considered are the organic solvent amount and the dimensions of the extracting tube. The volume of organic solvent was fixed at 100 μL to recover a volume suitable to be injected by the automatic sampler. The use of volumes smaller than 50 μL makes solvent recovery difficult and troublesome. The dimensions of the extracting tube were also critical because

Table III. Precision (as Average of RSD %) of the Microextraction over Three Different Wines

compound	average RSD %[a]	compound	average RSD %[a]
isobutyl acetate	1.6	1-octanol	1.7
ethyl butyrate	1.2	isobutyric acid	7.6
isobutanol	0.4	diethyl malonate	3.4
isoamyl acetate	0.5	butyric acid	5.3
1-butanol	0.4	furanone	0.43
amyl acetate	nd	ethyl decanoate	2.35
isoamyl alcohol	o.r.	diethyl malate	3.1
ethyl hexanoate	0.47	isovalerianic acid	>10
pentanol	2.4	diethyl succinate	0.48
hexyl acetate	0.54	α-terpineol	2.3
acetoine	1.4	methionol	4.4
3-methylpentanol	2.2	phenylethyl acetate	2.0
4-methylpentanol	2.1	ethyl phenylacetate	2.3
ethyl lactate	1.83	nerol	2.1
1-hexanol	0.77	ethyl laurate	1.15
cis-3-hexenol	1.04	hexanoic acid	1.1
trans-2-hexenol	1.17	isoamylacetamide	2.43
ethyl octanoate	1.97	geraniol	2.53
1-heptanol	3.1	benzyl alcohol	4.8
acetic acid	>10	β-phenylethanol	1.7
benzaldehyde	5.3	γ-nonalactone	4.9
ethyl 3-hydroxybutyrate	2.2	octanoic acid	2.2
2,3-butanediol	6.2	ethyl cinnamate	nd
linalool	2.1		

[a] nd, not detected. o.r., out of range.

Table IV. Average of Linear Correlation Coefficients and Slopes[a] of the Standard Addition Performed over Three Different Wines

compound	r	wine 1, m1	wine 2, m2	wine 3, m3
isobutyl acetate	0.9991	80.1	78.7	78.4
ethyl butyrate	0.9996	79.3	76.8	76.3
isoamyl acetate	0.9997	83.2	81.6	80.7
1-butanol	0.9968	85.8	94.1	97.6
amyl acetate	0.9999	124	123	121
isoamyl alcohol	0.9997	85.0	89.9	92.2
ethyl hexanoate	0.9996	107	106	106
pentanol	0.9978	76.7	74.8	74.3
hexyl acetate	0.9998	111	111	110
ethyl lactate	0.9966	15.1	15.0	15.2
1-hexanol	0.9990	82.4	82.1	80.9
cis-3-hexenol	0.9989	40.4	41.1	41.7
trans-2-hexenol	0.9986	38.9	38.2	37.3
ethyl octanoate	0.9999	115	112	111
1-heptanol	0.9999	89.8	83.5	80.2
linalool	0.9994	91.9	90.2	89.4
1-octanol	0.9990	78.9	76.7	76.0
diethyl malonate	0.9995	84.7	80.9	79.6
ethyl decanoate	0.9992	113	112	112
diethyl succinate	0.9999	105	101	99.8
nerol	0.9989	98.6	97.1	96.8
ethyl laurate	0.9996	109	106	104
hexanoic acid	0.9969	105	108	110
geraniol	0.9988	108	107	106
β-phenylethanol	0.9994	24.8	24.7	25.9
ethyl cinnamate	0.9993	32.5	33.6	33.7

[a] The graphs built were concentration in the extract vs added amount. The slopes represent the concentration factors (number of times that a compound is concentrated). $y = mx + b$, where $m1$ is the slope of wine 1, $m2$ is the slope of wine 2, and $m3$ is the slope of wine 3.

bigger tubes made emulsion formation easier and smaller ones made shaking less efficient.

Extraction Features. (*a*) *Precision.* The data obtained for the three wines are presented in Table III. The data given are the average RSD (percent) for the three wines, and each compound was calibrated according to the first part of the study (Chromatographic Separation and Quantification). As can be seen, extraction is very precise for almost all compounds studied (average RSD % is 2.8; only six compounds have a RSD % larger than 5, and it seems to be worse for those diluted compounds poorly eluted in the Supelcowax column. The high RSD values found in such compounds are in part due to a bad integration.

It might be concluded that microextraction can be as precise as classical extractions, or even better.

(*b*) *Linearity and Accuracy.* Standard addition experiments performed with the wines test whether the

Table V. Standard Dilution Expressed as Average of the Relative Areas to the Areas Found in the Wine

compound	wine content 100%	90%	80%	60%	40%
isobutyl acetate	100.0	89.9	80.6	60.4	40.7
ethyl butyrate	100.0	90.4	79.2	58.9	39.5
isobutanol	100.0	90.0	80.7	61.3	40.1
isoamyl acetate	100.0	89.3	79.9	60.4	41.2
1-butanol	100.0	88.7	79.0	58.4	38.2
amyl acetate	100.0	85.3	n.d.		
isoamyl alcohol	100.0	91.3	81.0	62.2	44.3
ethyl hexanoate	100.0	90.1	80.7	59.5	40.4
amyl alcohol	100.0	89.5	78.7	58.6	38.9
hexyl acetate	100.0	90.1	80.2	59.8	40.3
acetone	100.0	89.7	79.4	59.0	39.7
ethyl lactate	100.0	90.0	79.8	60.9	41.0
1-hexanol	100.0	89.8	80.1	60.6	42.2
cis-3-hexenol	100.0	90.2	80.3	60.0	40.1
trans-2-hexenol	100.0	88.5	77.1	n.d.	
ethyl octanoate	100.0	89.9	80.2	59.7	39.9
1-heptanol	100.0	88.9	79.2	60.5	39.0
isobutyric acid	100.0	91.1	80.3	59.8	40.2
linalool	100.0	89.9	81.2	60.6	n.d.
1-octanol	100.0	87.6	80.2	62.4	n.d.
ethyl 3-hydroxybutyrate	100.0	90.4	80.2	59.7	40.0
diethyl malonate	100.0	89.9	79.9	60.4	41.2
furanone	100.0	89.1	79.3	61.2	40.4
ethyl decanoate	100.0	90.1	80.0	60.1	39.8
butyric acid	100.0	90.9	82.3	63.1	45.3
diethyl succinate	100.0	90.0	80.5	58.7	38.9
isovalerianic acid	100.0	88.8	78.7	59.0	38.8
α-terpineol	100.0	89.5	79.6	61.2	n.d.
methionol	100.0	90.2	80.7	59.4	39.7
phenylethyl acetate	100.0	89.7	79.9	60.2	40.2
ethyl phenylacetate	100.0	90.0	80.1	59.6	40.3
nerol	100.0	82.6	n.d.		
ethyl laurate	100.0	89.8	80.6	60.0	39.5
hexanoic acid	100.0	91.3	78.7	60.5	41.3
isoamyl acetamide	100.0	89.9	79.2	60.5	41.4
geraniol	100.0	89.2	76.8	n.d.	
β-phenylethanol	100.0	90.1	80.9	61.2	40.1
ethyl cinnamate	100.0	89.6	79.9	60.8	40.9

Table VI. Ethanol Influence; Extraction Coefficients (as Slopes of the Straight Lines from the Graph Concentration in the Extract vs Concentration in Synthetic Wine) Found in Different Synthetic Wines

compound	8° [a]	10°	10.5°	11°	11.5°	12°	12.5°	13°	13.5°
isobutyl acetate	82.1	81.7	81.4	80.7	79.3	78.6	78.5	78.4	78.2
ethyl butyrate	81.4	80.3	80.0	79.4	78.7	77.8	77.0	76.5	76.2
isoamyl acetate	87.4	85.4	84.5	83.6	83.0	82.0	81.3	80.6	80.1
1-butanol	77.3	77.8	80.2	83.0	87.1	91.4	94.2	96.7	98.2
amyl acetate	133	128	126	125	124	123	122	122	121
isoamyl alcohol	79.6	80.0	81.9	83.6	86.4	88.4	90.7	91.7	92.7
ethyl hexanoate	112	108	108	107	107	106	106	106	105
amyl alcohol	79.4	78.7	78.0	77.3	76.8	76.1	74.9	74.0	73.3
hexyl acetate	120	115	114	112	111	111	110	110	110
ethyl lactate	14.6	14.7	14.8	14.9	14.9	14.9	15.0	15.0	15.1
1-hexanol	83.6	83.3	82.7	82.3	82.0	81.8	81.3	80.8	80.1
cis-3-hexenol	39.3	40.3	40.5	40.7	40.9	41.6	41.7	41.8	41.8
trans-2-hexenol	39.6	39.2	38.7	38.7	38.5	38.2	37.7	37.2	36.9
ethyl octanoate	119	117	117	116	115	114	114	112	111
1-heptanol	95.4	94.0	92.8	91.1	88.9	85.3	83.6	80.8	78.9
linalool	93.8	93.2	93.0	92.1	91.7	91.4	90.8	89.6	89.5
1-octanol	84.2	83.0	81.0	79.8	78.6	77.4	76.9	76.3	75.8
diethyl malonate	90.3	89.5	87.1	86.3	83.0	82.2	81.1	80.3	78.3
ethyl decanoate	114	114	113	113	113	112	112	112	112
diethyl succinate	112	111	108	105	103	102	101	100	99.1
nerol	100	99.7	99.2	98.7	98.4	97.6	97.3	97.0	96.7
ethyl laurate	117	116	113	111	108	107	106	104	102
hexanoic acid	95.4	97.7	99.0	103	107	107	108	109	110
geraniol	111	110	109	109	107	107	106	106	106
β-phenylethanol	23.5	23.5	23.9	24.1	24.7	24.8	25.4	25.9	26.2
ethyl cinnamate	31.9	32.2	32.3	32.9	32.9	33.1	33.7	33.6	33.6

[a] °, percent ethanol in the synthetic wine.

extraction is linear with different amounts of solutes, without changes in the matrix basic composition. Furthermore, it allows us to compare the slopes of the straight lines obtained among the different wines, to see whether there are matrix effects or not. The different straight standard addition lines have the same slopes, which means that extraction is not disturbed by the matrix composition, at least for the added compounds and in the studied interval. The selected interval for each compound was proportional to the amounts usually found of these compounds in wines.

Table IV summarizes these results. It can be observed that the slopes vary slightly in the interval studied. This indicates the following:

Extraction is linear in all cases studied, i.e., extraction efficiency is not dependent on the concentration of the solutes.

The slopes are similar but not equal for each compound in the different wines. This implies that if only an external calibration graph is used, the obtained data may be affected by a systematic error.

There can be one or several factors which disturb the extraction.

These factors affect different compounds in different way.

The standard dilution experiments study the following: the effect of dilution on wine and wine volatile behavior; the behavior of noncalibrated compounds; the influence of changing the concentration of the other wine components when only the alcohol content and pH are held constant.

Results are shown in Table V, and it can be concluded that there is no distortion of extraction for lower concentrations and that for other noncalibrated compounds the behavior seems to be linear, too. This means that the observed matrix effect in the standard addition experiment should be due to ethanol content or pH since it cannot be due to the other wine components.

Synthetic mixtures showed the same behavior as wines in the experiments performed. It can be clearly seen in Table VI that the observed matrix effect is due to ethanol.

Table VII. Concentrations (Micrograms per Liter) of the Calibrated Compounds Found in the Three Different Wines[a]

	white wine		rosé wine		red wine	
compound	D	SA	D	SA	D	SA
isobutyl acetate	99	102	128	132	107	108
ethyl butyrate	119	119	165	160	376	361
isoamyl acetate	2080	2100	2140	2118	3235	3247
1-butanol	177	180	723	724	1364	1349
amyl acetate	nd	nd	nd	nd	nd	nd
isoamyl alcohol			>100000			
ethyl hexanoate	493	497	544	536	802	813
pentanol	82	80	841	863	138	139
hexyl acetate	55	60	68	69	81	83
ethyl lactate	28410	28570	40460	40240	39670	39520
1-hexanol	1206	1176	2251	2270	2699	2680
cis-3-hexenol	199	199	240	237	99	98
trans-2-hexenol	5	5	<1	0.5	4	4
ethyl octanoate	1223	1230	803	812	981	963
1-heptanol	44	46	13.8	13.4	5.2	5.6
linalool	3.1	3.4	2.3	3.1	1.9	1.6
1-octanol	9.0	8.1	2.1	2.3	8.6	8.4
diethyl malonate	13.8	13.9	56.8	57.2	13.6	13.4
ethyl decanoate	53.2	51.6	375	356	143	150
diethyl succinate	3278	3174	6456	6398	7745	7651
nerol	2.3	3.0	nd	nd	nd	nd
ethyl laurate	143	149	14.8	15.1	61.3	67.2
geraniol	3.0	2.8	nd	nd	nd	nd
β-phenylethanol	43400	43200	26750	26890	34560	34520
ethyl cinnamate	nd	nd	nd	nd	73	76

[a] D, interpolated in the calibration graphs. SA, extrapolated in the standard addition lines. nd, not detected.

The concentration factors obtained with synthetic wines are similar to those obtained in wines of similar ethanol content. The behavior of each compound is particular, and it is very difficult to find an adequate internal standard for all compounds or for a given group of them. Thus, calibration graphs constructed with synthetic wines covering the usual range of alcohol content of the wines studied should be used. For each wine, the calibration graph corresponding to a synthetic wine of similar alcohol content must be used. For wines of similar alcohol content, a

Table VIII. Quantification Limits of the Calibrated Compounds

compound	quant limit, $\mu g\ L^{-1}$	compound	quant limit, $\mu g\ L^{-1}$
isobutyl acetate	2.5	ethyl octanoate	2.3
ethyl butyrate	2.4	1-heptanol	2.0
isoamyl acetate	2.2	linalool	1.6
1-butanol	1.4	1-octanol	1.1
amyl acetate	1.9	diethyl malonate	4.4
isoamyl alcohol	2.2	ethyl decanoate	1.5
ethyl hexanoate	1.2	diethyl succinate	3.0
pentanol	4.4	nerol	2.0
hexyl acetate	1.7	ethyl laurate	1.7
ethyl lactate	20.2	hexanoic acid	4.9
1-hexanol	2.1	geraniol	1.7
cis-3-hexenol	5.4	β-phenylethanol	7
trans-2-hexenol	6.7	ethyl cinnamate	3.4

calibration graph built with a synthetic wine of an intermediate alcohol content may be enough.

Table VII shows the results from three wines by both the standard addition and interpolation in the calibration graph built with a synthetic mixture its same alcohol content. It can be observed that the correlation of the results is quite good except for the very diluted compounds.

(c) *Estimated detection limits*, as explained under Methods, can be seen in Table VIII, and they are fairly good. It should be noted that, in all cases studied, detection limits are below the olfactory threshold of the different compounds.

Global Conclusion. The proposed method allows quantification of 26 wine flavor compounds with only a single-step sample treatment. One operator can process nearly 50 wine samples in an 8-h working day. The method can quantify compounds at very low concentrations. The results are precise and accurate. Therefore, this method is proposed as an interesting alternative for the analysis of wine flavor components. Further research is being done in our laboratories to inject larger volumes of extract in the chromatographic column to improve sensitivity even further.

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