

Validation of the method for the determination of some wine volatile compounds

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ABSTRACT

Wine aroma is influenced by a number of volatile compounds. This article describes the validation of the method for 26 volatile compounds found in wine. Volatile compounds were determined with discontinuous liquid-liquid extraction and GC-MS detection. It was determined, that the method is linear with square correlation coefficient ranging from 0.961 to 0.999. Limits of quantitative determination range from 0.52 µg/L to 14.8 µg/L. Recoveries range from 71.1% to 105.7% except for two compounds with lower recoveries. Measurement uncertainty ranges from 5.0% to 28.9%. According to the validation, the method is suitable for the determination of at least 24 volatile compounds common to wine. A practical method application was presented on Zelen wine variety from two different production procedures.

Key words: wine, aroma, volatile compounds, GC-MS

IZVLEČEK

VALIDACIJA METODE ZA DOLOČANJE NEKATERIH Hlapnih spojin v vinu

Na aromo vina vplivajo številne hlapne spojine. Ta članek opisuje validacijo metode za 26 hlapnih spojin, ki jih najdemo v vinu. Hlapne spojine so bile določene z diskontinuirano ekstrakcijo tekoče-tekoče in GC-MS detekcijo. Določili smo, da je metoda linearna, z razponom kvadrata korelacijskega koeficienta od 0,961 do 0,999. Meje kvantitativne določitve imajo razpon od 0,52 µg/L do 14,8 µg/L. Izkoristki imajo razpon od 71,1% do 105,7%, razen za dve spojini, katerih izkoristek je nižji. Merilna negotovost ima razpon od 5,0% do 28,9%. Z ozirom na validacijo lahko potrdimo primernost metode za določanje vsaj 24 hlapnih spojin značilnih za vino. Praktični prikaz uporabe metode smo predstavili na vinih sorte Zelen iz dveh različnih postopkov pridelave.

Ključne besede: vino, aroma, hlapne spojine, GC-MS

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1 INTRODUCTION

Wine aroma, a very important sensory parameter is produced by a complex balance of several volatiles. More than 800 volatile compounds such as alcohols, esters, phenols, monoterpenes, norisoprenoides, lactones, aldehydes and ketones have been identified in wine (Selli *et al.*, 2004; Tamborra *et al.*, 2004).

The wine aroma is complex due to a large number of compounds present and their different chemical nature with a wide range of polarity, volatility, solubility and pH values. Therefore the sample preparation and particularly the extraction and concentration of volatile

compound are an important factor in their determination (Cabredo Pinillos *et al.*, 2004).

Appropriate extraction of wine volatile compounds must be performed before their detection. Exceptionally so called major wine volatile compounds present in mg/l, like acetaldehyde, ethyl acetate, methanol and higher alcohols are detected directly without previous extraction where samples are only diluted and deacidified prior to analysis (Peinado *et al.*, 2004; Lukić *et al.*, 2008).

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Extraction of minor volatile compounds, present in wine in $\mu\text{g/L}$, is done today mostly in three different ways. The first is discontinuous or continuous liquid-liquid extraction (LLE) of wine with organic solvent. Both discontinuous and continuous liquid-liquid extractions are suitable to measure volatiles, but to perform a second one special apparatus must be provided and main disadvantages, like time consuming process and large volumes of solvents, are not avoided (Cabredo Pinillos *et al.*, 2004). As solvents mainly dichloromethane (Selli *et al.*, 2003) or mixture of pentane: dichloromethane = 60:40 (Pérez-Coello *et al.*, 2003; Izquierdo *et al.*, 2008) are used. The second approach is solid phase extraction (SPE) using Sep Pack C_{18} cartridges (Tamborra *et al.*, 2004) or LiChrolut EN resins (Loscos *et al.*, 2010; Sáenz-Navajas *et al.*, 2010). The third approach is Solid Phase Micro Extraction (SPME) with different fibers used: carbowax-divinylbenzene (Lambropoulos and Roussis *et al.*, 2007; Antalick *et al.*, 2010), polydimethylsiloxane (Nasi *et al.*, 2008; Antalick *et al.*, 2010), polydimethylsiloxane/divinylbenzene (Nasi *et al.*, 2008; Antalick *et al.*, 2010), carboxen/polydimethylsiloxane (Nasi *et al.*, 2008; Antalick *et al.*, 2010) or divinylbenzene/carboxen/polydimethylsiloxane (Nasi *et al.*, 2008; Antalick *et al.*, 2010).

Detection of volatile compounds is performed by gas chromatograph (GC) coupled with Flame Ionisation Detector (FID) (Pérez-Coello *et al.*, 2003; Selli *et al.*, 2003; Selli *et al.*, 2004; Tamborra *et al.*, 2004; Selli *et al.*, 2006; Loscos *et al.*, 2010) or mass spectrometer (MS) (Pérez-Coello *et al.*, 2003; Selli *et al.*, 2003; Selli

et al., 2004; Tamborra *et al.*, 2004; Selli *et al.*, 2006; Lambropoulos and Roussis *et al.*, 2007; Izquierdo Cañas *et al.*, 2008; Nasi *et al.*, 2008; Rodríguez-Bencomo *et al.*, 2008; Loscos *et al.*, 2010; Antalick *et al.*, 2010; Sáenz-Navajas *et al.*, 2010). Quantification can be done with both detectors, while unequivocal identification only by MS.

On Agricultural institute of Slovenia we decided to introduce discontinuous liquid-liquid extraction method with dichloromethane, chosen as the most effective organic solvent for this type of extraction (Cabredo Pinillos *et al.*, 2004). The extraction was performed with intention to determine 26 minor volatile compounds with possible sensorial effect in wines (Schneider *et al.*, 1998; Selli *et al.*, 2003; Selli *et al.*, 2006; Lukić *et al.*, 2008). Liquid-liquid extraction is actually the oldest but still the reference technique for the extraction of volatile compounds in wine (Ortega *et al.*, 2002). 3-octanol and 4-nonanol were used as internal standards because of their high recovery (Cabredo Pinillos *et al.*, 2004; Selli *et al.*, 2006). By this procedure we achieved concentration factor 100. To enable qualitative and quantitative evaluation at the same time, MS was used for detection. After introduction, method was validated. Method was finally applied to real wine samples (variety Zelen) deriving from an experiment, where two different winemaking procedures were confronted.

2 MATERIALS AND METHODS

2.1 Materials

Chemicals:

Dichloromethane (Sigma-Aldrich) and ethanol absolute (Merck) with HPLC grade were used like solvents in our experiment, together with ultrapure water from the Milli-Q system. Similarly only the volatile compounds (Merck, Sigma-Aldrich, Fluka, SAFC) with the highest available purity on market (minimum of 95 %) were used with the exception of 4-vinylphenol (SAFC) only sold like 10 % solution.

Preparation of solutions:

Stock solutions in pure dichloromethane of individual volatiles were prepared in 50 ml volumetric flasks with concentrations ranging from 1.8 – 2.5 g/L. From 26 stock solutions one mix solution of all 26 volatiles was prepared in 200 mL volumetric flask. All other solutions used to determine linearity, limits of detection and limits of quantification were prepared from this mix solution with proper dilutions.

Internal standards 3-octanol and 4-nonanol for those dichloromethane solutions were prepared in 100 mL volumetric flask with dissolving them in quantity of 1.1 – 1.2

g/L in dichloromethane. They were added using 0.05 mL Hamilton syringe to 10 mL of dichloromethane solutions and mixed before determination.

Preparation of model wine solution:

First a mix stock solution of all volatiles in 100 % pure ethanol was prepared, with individual volatiles concentrations in range of 0.8 – 1.2 g/L. Stock solution was adequately diluted to model solution (mix) using 12 %vol ethanol in water to concentrations similar to ones determined in wines in average, to 0.04 – 0.07 mg/L, in 3000 ml volumetric flask. The pH was then adjusted to pH 3.2 with tartaric acid addition. Model wine solution was finally dispensed in twenty 125 mL flasks and they were stored in dark at 7 °C before extraction.

Internal standards 3-octanol and 4-nonanol used in our model wine solution were prepared in 100 mL volumetric flask with dissolving them in quantity of 0.04 – 0.06 g in ethanol absolute. They were added using 0.05 mL Hamilton syringe to model wine solution only during extraction process as described below.

2.2 Procedure

Liquid-liquid extraction of volatile compounds:

100 mL of model wine solution was transferred into 250 mL Erlenmeyer flask and cooled to 0 °C in an ice bath under nitrogen. 29 µg of 3-octanol and 23 µg of 4-nonanol were added as internal standards using 0.05 mL Hamilton syringe from corresponding ethanol solutions. Dichloromethane (40 mL) was added and the mixture was stirred at 350 min⁻¹ for 20 minutes (Moio et al., 1995). Then the mixture was centrifuged at 5°C (RFC = 8500, 10 minutes) and organic phase was

recovered. The aqueous phase was re-extracted twice in the same way described above. Finally organic phases were combined and dried over sodium sulphate. They were concentrated to a final volume of 1 mL with Vigreux distillation column and nitrogen gas flow prior to GC-MS analysis (Schneider et al., 1998).

The same procedure was used for the extraction of wine samples (Moio et al., 1995; Schneider et al., 1998; Selli et al., 2006).

2.3 Determination

Chromatographic conditions of GC (HP 6890)-MS (HP 5973) system:

Liner	Agilent 5062-3587
Injector temperature	200 °C
Injection type	Pulsed Splitless
Precolumn	2 m * 0.25 mm
Column	Varian, CP-WAX 57CB, 50 m x 0.25 mm ID
Temperature gradient	40 °C; 12 min 5 °C/min; from 40 °C to 200 °C 200 °C; 20 min
Ion source temperature	230 °C
Auxiliary temperature	200 °C
Detector temperature	150 °C
Carrier gas	Helium 6.0; constant flow 1.0 ml/min
Injection volume	1 µl
Detection	Selective Ion Monitoring (T, Q1, Q2, Q3): 1,6-Heptadien-4-ol (71,43) 1-Hexanol (56, 43, 55, 69) 2-Phenylethyl acetate (104, 43, 91) 3-Octanol (59, 83, 101) 4-Ethylguaiacol (2-Methoxy-4-ethylphenol) (137, 152) 4-Nonanol (55, 73, 83, 101) 4-Vinylguaiacol (2-Methoxy-4-vinylphenol) (150, 135, 107, 77) 4-Vinylphenol (120, 91) Benzaldehyde (77, 105, 106) Benzyl alcohol (79, 108, 107) <i>cis</i> -3-hexen-1-ol (67, 41, 82) Diethyl succinate (101, 129) Ethyl butyrate (Ethyl butanoate) (71, 43, 88) Ethyl cinnamate (131, 103, 176) Ethyl decanoate (Ethyl caprate) (88, 101, 155) Ethyl dodecanoate (Ethyl laurate) (88, 101) Ethyl hexadecanoate (Ethyl palmitate) (88, 101) Ethyl hexanoate (88, 99) Ethyl lactate (45, 75) Ethyl octanoate (Ethyl caprylate) (88, 101, 57) Geraniol (69, 93, 123) Hexyl acetate (43, 56) Isoamyl acetate (70, 43, 55) Nerol (69, 84, 93) <i>n</i> -Hexaldehyde (Capronaldehyde) (56, 44, 57) <i>trans</i> -2-hexen-1-ol (55, 69, 83) β-Ionone (177, 43) γ-Butyrolactone (42, 56, 86)

2.4 Aromatic compounds determination in wines from two winemaking procedures - preparation of wine samples

Healthy grapes of Zelen variety (40 kg) were manually harvested in 2008 at the ripeness stage corresponding to wines containing approximately 12 % vol ethanol. Grapes were divided in two equal parts. First half of grapes (Z_c = control without skin contact) was immediately destemmed, crushed and pressed up to 150 kPa using a small water press (Lancman VS-A 55, Slovenia). The juice was sulphited with 30 mg/L of sulphur dioxide, left to settle at 6°C for 12 hours, racked and divided in three glass laboratory fermentor vessels with 1.6 L juice each. The vessels were heated to 17°C, inoculated with 0.2 g/L of dried *Saccharomyces cerevisiae* (CM, Lallemand), supplemented with 0.2 g/L complex yeast nutrient (Fermaid E,

Lallemand) and fermented at 17°C. After alcoholic fermentations (residual sugars <2.5 g/L) and when most of the lees had settled, the wines were racked, 50 mg/L of sulphur dioxide was added and the wines were stored at 10°C. The second half of grapes (Z_p = freezing of the pomace) was destemmed and crushed. The pomace was equally divided in three plastic vessels, frozen overnight at -20°C, defrozen at 20°C and pressed up to 150 kPa. The juice from the individual plastic vessels was sulphited with 30 mg/L of sulphur dioxide, left to settle at 6°C for 12 hours, racked and poured in 3 glass laboratory fermentor vessels with 1.6 L juice each. The remaining procedure to obtain wines was the same as described previously. In this way, two different types of Zelen wines (Z_c , Z_p) in three repetitions were obtained.

3 RESULTS AND DISCUSSION

3.1 Linearity, limits of detection, limits of quantification

Linearity was verified by using the solutions of volatile compounds in dichloromethane (five repetitions for one concentration level, three to eight concentration levels for the calibration curve). Linearity and range were determined by linear regression, using the F test. Linear model is fit and remains linear over the range presented in Table 1. Limits of detection (LD) and limits of

quantification (LOQ) were calculated from the calibration curve and are presented in Table 1.

Linearity was verified for wider range also and is presented in Table 2.

Concentration factor for wine samples was due to extraction 100, so realistic linearity range, LDs and LOQs are 100-times lower.

Table 1: Linearity, limits of detection, limits of quantification

	linearity (mg/L)	R ²	LD (mg/L)	LOQ (mg/L)
1,6-Heptadien-4-ol	0.12 - 3.35	0.999	0.062	0.206
1-Hexanol	0.19 - 3.35	0.999	0.067	0.224
2-Phenylethyl acetate	0.0168 - 3.35	0.999	0.030	0.099
4-Ethylguaiaicol	0.0058 - 3.35	0.996	0.051	0.171
4-Vinylguaiaicol	0.05 - 3.35	0.998	0.062	0.208
4-Vinylphenol	0.05 - 3.35	0.998	0.061	0.203
Benzaldehyde	0.0111-1.67	0.993	0.041	0.136
Benzylalcohol	0.0116 - 1.67	0.994	0.038	0.126
cis-3-Hexen-1-ol	1.67 - 10.13	0.996	0.352	1.174
Diethyl succinate	0.022 - 3.35	0.996	0.066	0.218
Ethyl butyrate	0.0167 - 1.67	0.999	0.016	0.052
Ethyl cinnamate	0.05 - 3.35	0.998	0.056	0.186
Ethyl decanoate	0.05 - 1.67	0.995	0.056	0.186
Ethyl dodecanoate	0.01 - 3.35	0.996	0.065	0.216
Ethyl hexadecanoate	0.0139 - 1.67	0.995	0.034	0.115
Ethyl hexanoate	0.0092 - 3.35	0.996	0.053	0.176
Ethyl lactate	0.0099 - 1.67	0.991	0.044	0.148
Ethyl octanoate	0.1 - 9.51	0.992	0.444	1.480
Geraniol	0.011 - 3.35	0.996	0.065	0.216
Hexyl acetate	0.0092 - 1.67	0.990	0.048	0.159
Isoamyl acetate	0.05 - 3.35	0.998	0.059	0.196
Nerol	0.0058 - 3.35	0.996	0.056	0.187
n-Hexaldehyde	0.06 - 3.35	0.983	0.175	0.582
trans-2-Hexen-1-ol	0.05 - 3.35	0.982	0.179	0.597
β-Ionone	0.009 - 3.35	0.995	0.074	0.248
γ-Butyrolactone	0.024 - 3.35	0.997	0.055	0.183

Table 2: Linearity, wider range

	linearity (mg/L)	R ²
1,6-Heptadien-4-ol	0.12 - 11.92	0.961
1-Hexanol	0.19 - 19.4	0.988
2-Phenylethyl acetate	0.0168 - 33.53	0.995
4-Ethylguaiaicol	0.0058 - 11.5	0.987
4-Vinylguaiaicol	0.05 - 9.36	0.978
4-Vinylphenol	0.05 - 9.08	0.977
Benzaldehyde	0.0111-22.18	0.994
Benzylalcohol	0.0116 - 23.14	0.994
<i>cis</i> -3-Hexen-1-ol	1.67 - 10.13	0.996
Diethyl succinate	0.022 - 21.62	0.993
Ethyl butyrate	0.0167 - 33.45	0.999
Ethyl cinnamate	0.05 - 10.72	0.983
Ethyl decanoate	0.05 - 9.55	0.977
Ethyl dodecanoate	0.01 - 10.08	0.978
Ethyl hexadecanoate	0.0139 - 27.7	0.973
Ethyl hexanoate	0.0092 - 18.45	0.989
Ethyl lactate	0.0099 - 10.79	0.986
Ethyl octanoate	0.1 - 9.51	0.992
Geraniol	0.011 - 10.8	0.985
Hexyl acetate	0.0092 - 18.31	0.989
Isoamyl acetate	0.05 - 10.79	0.983
Nerol	0.0058 - 11.57	0.988
n-Hexaldehyde	0.06 - 11.31	0.998
<i>trans</i> -2-Hexen-1-ol	0.05 - 3.35	0.982
β -Ionone	0.009 - 9.32	0.980
γ -Butyrolactone	0.024 - 24.06	0.994

3.2 Trueness

Trueness was verified by checking the recoveries. Two parallel extracts of model wine solution were prepared each day for ten days and injected once respectively. The average of recoveries was calculated. The results are given in Table 3.

3.3 Precision

For the determination of precision (ISO 5725), i.e. repeatability and reproducibility, extracts of model wine solution was analysed (the same as for recovery evaluation). Within the period of 10 days two parallel extracts were prepared each day. Each was injected once. Then standard deviation of repeatability of the level and standard deviation of reproducibility of the level were both calculated. The results are given in Table 4.

Table 3: Recoveries for model wine solution

	conc. in model wine solution (mg/L)	recovery (%)	RSD (%)
1,6-Heptadien-4-ol	0.0435	84.4	3.1
1-Hexanol	0.0596	98.3	4.9
2-Phenylethyl acetate	0.0614	91.9	2.5
3-Octanol	0.2930	85.9	3.1
4-Ethylguaiaicol	0.0543	92.3	4.2
4-Nonanol	0.2300	87.1	2.8
4-Vinylguaiaicol	0.0692	95.0	8.9
4-Vinylphenol	0.0414	98.2	2.6
Benzaldehyde	0.0713	95.0	3.0
Benzylalcohol	0.0672	94.2	4.6
<i>cis</i> -3-Hexen-1-ol	0.0566	84.0	3.0
Diethyl succinate	0.0498	91.3	2.7
Ethyl butyrate	0.0599	77.5	3.8
Ethyl cinnamate	0.0697	95.1	2.9
Ethyl decanoate	0.0500	81.9	15.2
Ethyl dodecanoate	0.0624	67.8	8.9
Ethyl hexadecanoate	0.0524	27.9	9.5
Ethyl hexanoate	0.0588	76.7	3.4
Ethyl lactate	0.0709	79.4	3.5
Ethyl octanoate	0.0573	71.1	7.4
Geraniol	0.0495	105.7	3.0
Hexyl acetate	0.0593	80.6	4.0
Isoamyl acetate	0.0604	78.0	3.8
Nerol	0.0540	96.5	2.4
<i>n</i> -Hexaldehyde	0.0433	82.0	3.3
<i>trans</i> -2-Hexen-1-ol	0.0548	102.4	8.2
β -Ionone	0.0564	89.2	2.6
γ -Butyrolactone	0.0609	88.1	3.1

Table 4: Standard deviation of repeatability and reproducibility of the method, in mg/L

	conc. in model wine solution (mg/L)	means of the levels (mg/L)	standard deviation of repeatability (sr)	standard deviation of reproducibility (sR)
1,6-Heptadien-4-ol	0.0435	0.0367	0.0007	0.0011
1-Hexanol	0.0596	0.0583	0.0011	0.0029
2-Phenylethyl acetate	0.0614	0.0563	0.0012	0.0014
4-Ethylguaiaicol	0.0543	0.0501	0.0020	0.0024
4-Vinylguaiaicol	0.0692	0.0659	0.0055	0.0056
4-Vinylphenol	0.0414	0.0406	0.0009	0.0010
Benzaldehyde	0.0713	0.0676	0.0013	0.0021
Benzylalcohol	0.0672	0.0632	0.0014	0.0028
<i>cis</i> -3-Hexen-1-ol	0.0566	0.0475	0.0010	0.0014
Diethyl succinate	0.0498	0.0455	0.0012	0.0012
Ethyl butyrate	0.0599	0.0463	0.0010	0.0018
Ethyl cinnamate	0.0697	0.0662	0.0014	0.0019
Ethyl decanoate	0.0500	0.0404	0.0010	0.0064
Ethyl dodecanoate	0.0624	0.0419	0.0008	0.0039
Ethyl hexadecanoate	0.0524	0.0147	0.0012	0.0014
Ethyl hexanoate	0.0588	0.0449	0.0010	0.0016
Ethyl lactate	0.0709	0.0562	0.0015	0.0020
Ethyl octanoate	0.0573	0.0405	0.0009	0.0031
Geraniol	0.0495	0.0524	0.0014	0.0015
Hexyl acetate	0.0593	0.0476	0.0011	0.0019
Isoamyl acetate	0.0604	0.0470	0.0012	0.0017
Nerol	0.0540	0.0521	0.0012	0.0012
<i>n</i> -Hexaldehyde	0.0433	0.0354	0.0007	0.0012
<i>trans</i> -2-Hexen-1-ol	0.0548	0.0559	0.0044	0.0050
β -Ionone	0.0564	0.0503	0.0010	0.0013
γ -Butyrolactone	0.0609	0.0536	0.0012	0.0016

3.4 Uncertainty of repeatability and uncertainty of reproducibility

Uncertainty of repeatability and uncertainty of reproducibility were calculated by multiplying standard deviation of repeatability and standard deviation of

reproducibility by Student's t factor for 9 degrees of freedom and 95% confidence level ($t_{95;9} = 2.262$).

$$U_r = t_{95;9} \times s_r ; U_R = t_{95;9} \times s_R$$

The results are presented in Table 5.

Table 5: Uncertainty of repeatability and reproducibility of the method, in mg/L

	conc. in model wine solution (mg/L)	uncertainty of repeatability (U_r)	uncertainty of reproducibility (U_R)
1,6-Heptadien-4-ol	0.0435	0.0016	0.0026
1-Hexanol	0.0596	0.0025	0.0066
2-Phenylethyl acetate	0.0614	0.0026	0.0031
4-Ethylguaiaicol	0.0543	0.0046	0.0055
4-Vinylguaiaicol	0.0692	0.0124	0.0127
4-Vinylphenol	0.0414	0.0021	0.0023
Benzaldehyde	0.0713	0.0029	0.0047
Benzylalcohol	0.0672	0.0031	0.0064
<i>cis</i> -3-Hexen-1-ol	0.0566	0.0022	0.0032
Diethyl succinate	0.0498	0.0026	0.0027
Ethyl butyrate	0.0599	0.0022	0.0040
Ethyl cinnamate	0.0697	0.0031	0.0042
Ethyl decanoate	0.0500	0.0023	0.0144
Ethyl dodecanoate	0.0624	0.0017	0.0088
Ethyl hexadecanoate	0.0524	0.0027	0.0031
Ethyl hexanoate	0.0588	0.0023	0.0036
Ethyl lactate	0.0709	0.0034	0.0044
Ethyl octanoate	0.0573	0.0021	0.0071
Geraniol	0.0495	0.0031	0.0034
Hexyl acetate	0.0593	0.0025	0.0044
Isoamyl acetate	0.0604	0.0028	0.0040
Nerol	0.0540	0.0027	0.0028
n-Hexaldehyde	0.0433	0.0016	0.0026
trans-2-Hexen-1-ol	0.0548	0.0100	0.0113
β -Ionone	0.0564	0.0022	0.0029
γ -Butyrolactone	0.0609	0.0028	0.0036

3.5 Aromatic compounds determination in wines from two winemaking procedures

To determine volatile compound in real wine samples, the method proposed in this article was applied and results are presented in Table 6. Results are in correlation with previously observed aromatics content

in wines and differences due to two winemaking procedures are comparable to other skin contact procedures (Moio et al., 1995; Ortega et al., 2002; Selli et al., 2003; Selli et al., 2006; Rodriguez-Bencomo et al., 2008).

Table 6: Concentrations of individual aromatic compounds in the Zelen wines produced by two different procedures (Zc - control without skin contact, Zp - freezing of pomace), in µg/L.

	Zc wine	Zp wine
1,6-Heptadien-4-ol	17 ± 1 ^b	12 ± 1 ^a
1-Hexanol	1349 ± 67 ^b	1118 ± 18 ^a
2-Phenylethyl acetate	466 ± 49 ^b	253 ± 12 ^a
4-Ethylguaiacol	-	-
4-Vinylguaiacol	516 ± 8 ^a	908 ± 53 ^b
4-Vinylphenol	117 ± 5 ^a	354 ± 23 ^b
Benzaldehyde	2 ± 0 ^a	9 ± 2 ^b
Benzylalcohol	20 ± 3 ^a	90 ± 9 ^b
<i>cis</i> -3-Hexen-1-ol	21 ± 1 ^b	18 ± 0 ^a
Diethyl succinate	112 ± 16 ^a	129 ± 8 ^a
Ethyl butyrate	366 ± 10 ^b	217 ± 11 ^a
Ethyl cinnamate	-	-
Ethyl decanoate	593 ± 6 ^b	443 ± 9 ^a
Ethyl dodecanoate*	37 ± 2 ^a	38 ± 4 ^a
Ethyl hexadecanoate*	6 ± 1 ^a	8 ± 1 ^a
Ethyl hexanoate	570 ± 16 ^b	409 ± 0 ^a
Ethyl lactate	4276 ± 475 ^a	6350 ± 328 ^b
Ethyl octanoate	1239 ± 56 ^b	933 ± 25 ^a
Geraniol	-	-
Hexyl acetate	275 ± 40 ^b	46 ± 5 ^a
Isoamyl acetate	3260 ± 423 ^b	1291 ± 97 ^a
Nerol	-	-
<i>n</i> -Hexaldehyde	4 ± 1 ^b	- ^a
<i>trans</i> -2-Hexen-1-ol	-	-
β-Ionone	-	-
γ-Butyrolactone	2501 ± 60 ^a	2569 ± 62 ^a

Values are the mean value ± error at 95 % confidence level (n = 3).

Significant differences between procedures are indicated a, b at $p \leq 0.05$.

- = not detected

* = volatile compounds with low recoveries

4 CONCLUSIONS

According to the validation, the method is suitable for the determination of at least 24 volatile compounds in wine (the ones with recoveries >70%). The system is linear with R^2 higher than 0.96. Limits of detection range from 0.16 µg/L for ethyl butyrate to 4.44 µg/L for ethyl octanoate. Limits of quantitative determination range from 0.52 µg/L for ethyl butyrate to 14.8 µg/L for

ethyl octanoate. Recoveries range from 71.1% (ethyl octanoate) to 105.7% (geraniol), except for ethyl dodecanoate (67.8%) and ethyl hexadecanoate (27.9%). Uncertainty of reproducibility ranges from 5.0% for 2-phenylethyl acetate to 28.9% for ethyl decanoate. A practical application was checked and presented for Zelen wines from two different winemaking procedures.

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6 REFERENCES

- Antalick, G., Perello, M.C. and de Revel G. 2010. Development, validation and application of a specific method for the quantitative determination of wine esters by headspace-solid-phase microextraction-gas chromatography-mass spectrometry. *Food Chem.*, 121, 1236-1245.
- Cabredo Pinillos, S., Cedron Fernandez, T. and Saent Barrio, C. 2004. Comparison of different extraction methods applied to volatile compounds in wine samples previous to the determination by gas chromatography. *Anal. Lett.*, 37, 3063–3084.
- ISO 5725: *Accuracy (trueness and precision) of measurement methods and results - Part2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*, 1994, pp. 1-42.
- Izquierdo Cañas, P.M., García Romero, E., Gómez Alonso, S. and Palop Herreros, M.L.L. 2008. Changes in the aromatic composition of Tempranillo wines during spontaneous malolactic fermentation. *J. Food Compos. Anal.*, 21, 724-730.
- Lambropoulos, I. and Roussis, I. G. 2007. Inhibition of the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium by caffeic acid and gallic acid. *Food Res. Int.*, 40, 176-181.
- Loscos, N., Hernández-Orte, P., Cacho, J. and Ferreira, V. 2010. Evolution of the aroma composition of wines supplemented with grape flavour precursors from different varieties during accelerated wine ageing. *Food Chem.*, 120, 205-216.
- Lukić, I., Plavša, T., Sladonija, B., Radeka, S. and Perusic, D. 2008. Aroma compounds as markers of wine quality in the case of Malvazija Istarska young wine. *J. Food Quality*, 31, 717– 735.
- Moio, L., Chambellant, E., Lasschaeve, I., Issanchau, S., Schlich, P. and Etievant, P. X. 1995. Production of representative wine extracts for chemical and olfactory analyses. *It. J. Food Sci.*, 7, 265 – 278.
- Nasi, A., Ferranti, P., Amato, S. and Chianese, L. 2008. Identification of free and bound volatile compounds as typicalness and authenticity markers of non-aromatic grapes and wines through a combined use of mass spectrometric techniques. *Food Chem.*, 110, 762-768.
- Ortega Heras, M., Gonzales san Jose, M. L. and Belatran, S. 2002. Aroma composition of wine studied by different extraction methods. *Anal. Chim. Acta*, 458, 85- 93.
- Peinado, R.A., Moreno, J.A., Munoz, D., Medina, M. and Moreno, J. 2004. Gas chromatographic quantification of major volatile compounds and polyols in wine by direct injection. *J. Agr. Food Chem.*, 52, 6389 – 6393.
- Pérez-Coello, M.S., González-Viñas, M.A., García-Romero, E., Diaz-Maroto, M.C. and Cabezudo, M.D. 2003. Influence of storage temperature on the volatile compounds of young white wines. *Food Control*, 14, 301-306.
- Rodriguez-Bencomo, J.J., Méndez-Siverio, J.J., Pérez-Trujillo, J.P. and Cacho, J. 2008. Effect of skin contact on bound aroma and free volatiles of Listán blanco wine. *Food Chem.*, 110, 214-225.
- Sáenz-Navajas, M.-P., Campo, E., Fernández-Zurbano, P. and Valentin, D. 2010. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chem.*, 121, 1139-1149.
- Schneider, R., Baumes, R. L., Bayonove, C.L. and Razungles, A. 1998. Volatile compounds involved in the aroma of sweet fortified wines (Vins Doux Naturels) from Grenache Noir. *J. Agr. Food Chem.*, 46, 3230 - 3237.
- Selli, S., Cabaroglu, T., Canbas, A., Erten, H. and Nurgel, C. 2003. Effect of skin contact on the aroma composition of the musts of *Vitisvinifera* L. cv. Muscat of Bornova and Narince grown in Turkey. *Food Chem.*, 81, 341-347.
- Selli, S., Cabaroglu, T., Canbas, A., Erten, H., Nurgel, C., Lepoutre, J.P. and Gunata, Z. 2004. Volatile composition of red wine from cv. Kalecik Karasu grown in central Anatolia. *Food Chem.*, 85, 207-213.
- Selli, S., Canbas, A., Cabaroglu, T., Erten, H. and Gunata, Z. 2006. Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment. *Food Chem.*, 94, 319-326.
- Tamborra, P., Martino, N. and Esti, M. 2004. Laboratory tests on glycosidase preparations in wine. *Anal. Chim. Acta*, 513, 299-303.