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Optimization and validation of an automated DHS–TD–GC–MS method for the determination of aromatic esters in sweet wines

Ana Marquez, Maria P. Serratosa, Julieta Merida, Luis Zea, Lourdes Moyano *

Department of Agricultural Chemistry, Faculty of Sciences, University of Cordoba, Edificio Marie Curie, Campus of Rabanales, E-14014 Cordoba, Spain

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ABSTRACT

A dynamic headspace sorptive extraction (DHS) combined with thermal desorption (TD) and coupled with gas chromatography–mass spectrometry (GC/MS) was developed for the determination of 11 esters which contribute to the fruity aroma in sweet wines. A full factorial (4 factors, 2 level) experiment design was used to optimize the extraction conditions and the results were evaluated by multiple linear regression (MLR) and principal component analysis (PCA). The esters showed optimal extraction using an extraction temperature of 30 °C during 20 min, and a subsequent purge volume of 300 mL and dry volume of 50 mL. Afterwards, quantification was achieved using calibration curves constructed for each ester with linear regression equations having correlation coefficients (R^2) ranging from 0.9894 to 0.9981. The proposed method was successfully validated and showed good intermediate precision, repeatability and accuracy values for all the monitored compounds. Finally, the method was applied to quantify esters, with fruity aromatic notes, of sweet white and red wines, elaborated with different winemaking processes.

1. Introduction

In recent years, the consumption of sweet wines has grown so much that in some countries the whole production is sold each year. The production process of these wines usually involves the grape dehydration to increase the sugar concentration. Different drying procedures exist depending on the climatic conditions of each production area. A detailed description of different grape hot-drying techniques has been described by different authors [1–6].

The flavor is one of the most important quality attributes of wine, which can determine consumer acceptance. It is the result of a wide variety of chemical compounds (alcohols, esters, acids, aldehydes, ketones, lactones, terpenes, and phenols), sufficiently volatile in the matrix to come easily to the vapor phase and reach the human senses with a specific aroma. The esters of fatty acids and the acetates of higher alcohols are the majority esters in sweet wines. These compounds contribute to the wine aroma with fruity odors [2,4,7]. Thus, an analysis of these volatile compounds, its identification and quantitative evaluation, can be an important source of information on wine quality. Furthermore, the characterization of the sweet wines is very important because of the high economic value of the wine-product for some Denominations of Origin or geographical regions in world wide areas. However, it is not an easy task due to the complexity of the sweet wine aroma, as mentioned above, and because many of the volatile compounds are present at $\mu g\,L^{-1}$ or even $ng\,L^{-1}$ concentrations [6,8–11]. Hence, it is very important to have analytical tools suitable for detecting these aroma compounds and thus be used for the selection and quality control of sweet wines.

Gas chromatography coupled with mass spectrometry (GC/MS) is the most extensively used technique for the analysis of the volatile wine aroma compounds with a high separation efficiency and sensitivity [12–14]. However, the extraction and concentration of aroma compounds is a controversial point in the analysis. Historically, liquid–liquid extractions with dichloromethane [15] or Freon-11 [9] were used. In recent years there have been excellent solvent-free extraction alternatives to conventional sample preparation techniques. Among them, stir bar sorptive extraction (SBSE), direct immersion solid-phase microextraction (DI-SPME) and monolithic material sorptive extraction (MMSE) are employed in the analysis of wines [14,16,17], but they constitute a more invasive way of sampling than other techniques based on headspace. Also, a headspace application could have the advantage that the results may reflect more in the actual sensory properties of the wine analyzed. In this sense, headspace techniques such as static headspace (HS), headspace sorptive extraction (HSSE) or

* Corresponding author. Tel.: +34 957 218612; fax: +34 957212 146.
E-mail address: qe1mocal@uco.es (L. Moyano).

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solid-phase microextraction (SPME) have been employed in the analysis of oenological products [11,13,18]. The HS–SPME technique is currently the most widely used and has been reported to be simple, fast, inexpensive and reproducible [12,19,20].

The dynamic headspace sampling (DHS) is a well consolidated technique used from more than 25 years. The sample is incubated at a fixed temperature under stirring, and the volatile compounds are evaporated to the headspace. These volatilized compounds are trapped in a tube with a sorbent material and are subsequently concentrated, purged by means of an inert gas and the water removed. Compounds with different chemical characteristics can be detected due to the wide variety of single and multiple sorbent materials. The main DHS advantages are a low sample manipulation, low detection limits and high sensitivity [21]. This technique has been utilized for the identification of volatile compounds in different matrixes, such as virgin olive oil [22], honey [23], kiwifruit tissue [24], vinegar [25] or sausage [26], but to our knowledge, it has not been applied for the analysis of esters with fruity aroma in sweet wines.

The aim of this study was the optimization and validation of a new and solvent-free method by dynamic headspace sampling–thermal desorption–gas chromatography/mass spectrometry (DHS–TD–GC–MS) for the quantification of esters in sweet wines, according to an experimental design. Under the optimized conditions, the fruity aroma esters of eight Spanish sweet white and red wines were quantified.

2. Materials and methods

2.1. Reagents and chemicals

Glucose and tartaric acid were supplied by Panreac (Barcelona, Spain), fructose was supplied by Probus (Barcelona, Spain), all of which were of analytical quality. Ethanol (purity $>99\%$) was supplied by Merck (Darmstadt, Germany). Milli-Q water was obtained from Milli-Q Plus water system (Millipore, Saint-Quentin-en-Yvelines, France). The standards of esters aroma compounds were obtained from Sigma-Aldrich (Munich, Germany); methyl butanoate (202 $\mu g\,L^{-1}$), ethyl isobutanoate (184 $\mu g\,L^{-1}$), isobutyl acetate (199 $\mu g\,L^{-1}$), ethyl butanoate (201 $\mu g\,L^{-1}$), butyl acetate (212 $\mu g\,L^{-1}$), isoamyl acetate (198 $\mu g\,L^{-1}$), ethyl hexanoate (188 $\mu g\,L^{-1}$), hexyl acetate (197 $\mu g\,L^{-1}$), ethyl heptanoate (191 $\mu g\,L^{-1}$), ethyl octanoate (205 $\mu g\,L^{-1}$) and ethyl decanoate (210 $\mu g\,L^{-1}$). They were selected on the basis of their previously reported presence in different sweet wine samples [2]. The internal standard (IS) used was γ-heptalactone (11.52 $mg\,L^{-1}$).

Table 1
Experimental design used for the method optimization.

Experiments	$T_{extract}$ (°C)	$t_{extract}$ (min)	V_{purge} (mL)	V_{dry} (mL)
E1	40	20	300	50
E2	40	20	300	100
E3	40	10	150	100
E4	30	10	300	50
E5	40	20	150	100
E6	30	10	300	100
E7	30	20	300	50
E8	30	10	150	50
E9	30	20	150	50
E10	30	20	300	100
E11	40	20	150	50
E12	30	20	150	100
E13	40	10	300	100
E14	40	10	150	50
E15	40	10	300	50
E16	30	10	150	100
E17	35	15	225	75
E18	35	15	225	75
E19	35	15	225	75
E20	35	15	225	75

2.2. Samples

Eight sweet wines from Andalusia (southern of Spain), three white wines and five red wines, with different characteristics (grape variety, sugar concentration and alcoholic degree) and a different elaboration (grape dehydration, winemaking conditions, and aging) were analyzed in order to test the suitability of the method and perform the comparative study. Three white wines (W1–W3) and two red wines (R1–R2) were elaborated with musts from sun-dried grapes and a subsequent fortification with wine alcohol. Two red wines (R3–R4) were elaborated with fortified musts from controlled chamber-dried grapes. The last red wine (R5) was obtained from grapes dehydrated *on-vine* and partial fermentation. Wines were kept at 4 °C until analysis to avoid losses of the volatiles esters. Each wine was analyzed by triplicate.

2.3. Experimental design and statistical analysis

A synthetic sweet wine containing 125 $g\,L^{-1}$ of glucose, 125 $g\,L^{-1}$ of fructose, 15% (v/v) of ethanol and pH adjusted at 3.7 with tartaric acid, was used for the optimization method. Among all the possible variables that might have some effect on the volatile esters recoveries in the DHS–TD–GC–MS method, four DHS parameters were systematically varied: extraction temperature ($T_{extract}$), extraction time ($t_{extract}$), purge volume (V_{purge}) and dry volume (V_{dry}). The DHS–TD–GC–MS conditions were optimized using an experimental design, based on a 2^4 factorial design with two levels (low and high) for each factor ($T_{extract}$, 30/40 °C; $t_{extract}$, 10/20 min; V_{purge}, 150/300 mL; V_{dry}, 50/100 mL). The experimental design was also completed with 4 replications of the central point to estimate the experimental error. Hence, the complete design consisted of 20 randomly performed experiments (Table 1). The response functions were the chromatographic peak area of the volatile esters primarily responsible for the fruity aroma of sweet wine.

The experiments were evaluated using a multiple linear regression (MLR) to obtain a response model for each compound. A principal component analysis (PCA) was performed, in order to highlight relationships among the responses and the experimental runs.

The statistical analyses were carried out by using a Statgraphics Computer Package v. 5.0 from Statistical Graphics Corp.

2.4. DHS–TD–GC–MS analysis

The volatile esters extraction was performed using a DHS system with a Gerstel MPS2 autosampler (Mülheim an der Ruhr, Denmark). The sorbent material used was Tenax TA (2,6-diphenylene oxide polymer, Gerstel) conditioned before use as recommended by the manufacturer. It was chosen because of the good results obtained by other authors in different matrices [21,22,24,26]. A volume of 5 mL of the sample was placed into a 20 mL glass vial, adding of 50 μL of IS. The extraction of the analytes was carried out using the optimized conditions obtained by means of the experimental design procedure. In particular, the sample was thermostated at 30 °C and stirred (500 rpm) for an extraction time of 20 min. Afterwards, the sample headspace was purged with a He flow of 25 $mL\,min^{-1}$ for a total purge volume of 300 mL and the analytes collected at 50 °C. In order to reduce the amount of aqueous vapor sampled, the tube was dried at 25 °C with a He flow of 10 $mL\,min^{-1}$ with a total volume of 50 mL.

Thermal desorption and cryofocusing of the volatile compounds were performed by means of a Thermo Desorption Unit (TDU) and a Cooling Injection System (CIS), respectively. The desorption of esters was carried out in a solvent venting mode with the following heating program: from 50 to 250 °C at 300 °C min^{-1}, with a final hold time of 3 min. Analytes were then cryofocused in the CIS injector cooled at 12 °C and successively desorbed from 12 to 250 °C at 12 °C s^{-1}, with a final hold time of 3 min. The temperature of the transfer line between the TDU and the CIS was kept constant at 280 °C.

Gas chromatography–mass spectrometry analyzes were performed with a 7890 Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5975C (Santa Clara, United States). An HP-MS capillary column was used (30 m × 0.25 mm × 0.25 μm film thickness, Agilent) with a split ratio of 1:30. The carrier gas was He, at a starting pressure of 7.5 psi with 2 min hold time, increased to 38.6 psi at 1.36 psi min^{-1} and a 5 min final hold time. The column oven temperature program was: initial temperature 40 °C for 2 min, then raised from 15 °C min^{-1} to 250 °C and held for 5 min. The interface was kept at 280 °C and the ionization mode was electron-impact (70 eV). For the quantitative determination the selective-ion monitoring (SIM) mode was used. Monitored ions are listed in Table 3. Esters were firstly identified by Wiley 7 N spectral library and then confirmed by means of standard.

2.5. Method validation

2.5.1. Calibration and detection limits

The calibration curves were created for the quantification of esters, using the optimized DHS–TD–GC–MS sampling conditions at seven concentration levels of the synthetic sweet wine used previously. All analyses were performed in triplicate. Linearity was evaluated graphically calculating response factor (relative area of peaks divided by their respective analyte concentrations). It was plotted as a function of the analyte concentrations and expressed by the squared regression coefficient (R^2).

The limit of detection (LOD) was defined as the lowest concentration of the calibration curve based on the signal-to-noise ratio of 3, and the limit of quantification (LOQ) on the signal-to-noise ratio of 10. LOD and LOQ were determined with data generated in the calibration plots according to Miller and Miller [27], as: LOD=($3S_a/b$) and LOQ=($10S_a/b$), where S_a is the standard deviation of the interception and b is the slope of the regression line.

2.6. Precision and accuracy

The repeatability was evaluated after the analysis on the same day of three different concentrations of the standard compounds in the synthetic sweet wine. The intermediate precision was determined by repeating the study during three different days. Each analysis was carried out three times.

The accuracy of the method was determined through the calculation of the deviation percent between the calculated value and the nominal value, which would be the value supposed to be if there were no errors [28–30].

3. Results and discussion

3.1. Extraction method optimization

The esters that are extracted with this method are from different types of families with different chemical characteristics: acetates (isobutyl, butyl, isoamyl, and hexyl), ethyl esters (isobutanoate, butanoate, hexanoate, heptanoate, octanoate, and decanoate) and

Table 2
Regression coefficients for dependent variables studied in the experimental design.

Coefficients	Methyl butanoate	Ethyl isobutanoate	Isobutyl acetate	Ethyl butanoate	Butyl acetate	Isoamyl acetate	Ethyl hexanoate	Hexyl acetate	Ethyl heptanoate	Ethyl octanoate	Ethyl decanoate
b_0	-9.73×10^7	2.73×10^7	1.78×10^7	-9.24×10^7	-7.43×10^7	-8.58×10^6	-6.50×10^7	-3.58×10^7	-2.77×10^7	-1.41×10^7	-1.85×10^7
b_1	$8.23 \times 10^{6***}$	$4.35 \times 10^{6****}$	$4.85 \times 10^{6****}$	7.78×10^6	5.93×10^6	4.58×10^6	6.51×10^6	4.36×10^6	4.93×10^6	3.60×10^6	3.25×10^6
b_2	6.91×10^7	6.14×10^6	4.09×10^6	8.10×10^6	6.28×10^6	7.13×10^6	8.18×10^6	6.51×10^6	7.23×10^6	5.64×10^6	5.15×10^6
b_3	$1.21 \times 10^{6**}$	$5.32 \times 10^{5***}$	$5.31 \times 10^{5**}$	8.21×10^5	4.97×10^5	3.35×10^5	$5.24 \times 10^{5*}$	$2.57 \times 10^{5***}$	$3.66 \times 10^{5*}$	$3.31 \times 10^{5*}$	$3.52 \times 10^{5***}$
b_4	1.37×10^6	-1.40×10^6	-1.32×10^6	-1.24×10^6	-6.90×10^5	-1.01×10^6	-5.91×10^5	-3.74×10^5	-3.51×10^5	-3.71×10^5	-8.00×10^5
b_{12}	$-2.31 \times 10^{5**}$	-1.91×10^5	-1.51×10^5	-2.13×10^5	-1.63×10^5	-1.76×10^5	-1.96×10^5	-1.49×10^5	-1.61×10^5	-1.19×10^5	-8.94×10^4
b_{13}	-4.32×10^4	$-2.58 \times 10^{4*}$	$-2.62 \times 10^{4***}$	$-2.81 \times 10^{4*}$	-1.74×10^4	-1.40×10^4	-1.71×10^4	-9.00×10^3	-1.26×10^4	-1.24×10^4	$-1.67 \times 10^{4*}$
b_{14}	9.00×10^3	7.58×10^3	4.98×10^3	2.18×10^3	-2.49×10^3	2.99×10^3	-2.64×10^3	-2.74×10^3	-3.20×10^3	2.27×10^3	1.84×10^4
b_{23}	6.75×10^2	2.37×10^2	3.64×10^3	-3.03×10^3	-1.11×10^3	-1.79×10^3	-5.22×10^2	-1.40×10^2	-92.15	1.60×10^3	1.23×10^3
b_{24}	2.80×10^4	2.22×10^4	2.23×10^4	1.43×10^4	4.56×10^3	5.39×10^3	$-8.16 \cdot 10^3$	-1.00×10^4	-1.64×10^4	$-1.91 \cdot 10^4$	-2.36×10^4
b_{34}	2.04×10^3	2.66×10^3	2.84×10^3	3.39×10^3	2.48×10^3	2.93×10^3	2.97×10^3	2.27×10^3	2.68×10^3	2.19×10^3	1.19×10^3

Subscripts: 1, extraction temperature; 2, extraction time; 3, purge volume; 4, dry volume.
* $p < 0.01$.
** $p < 0.005$.
*** $p < 0.001$.

methyl butanoate, and with fruity aromas of high interest in the aroma profile of sweet wines. In this way, it was necessary to compare different parameters which may affect the extracting of the compounds to establish the best condition for all esters studied.

The optimized variables were extraction temperature, extraction time, purge volume and dry volume. Essentially, the four factors chosen influence different aspects of the aroma compound extraction process and they were chosen according to the results obtained by Manzini et al. [21], optimizing a DHS procedure for the determination of furfurals in vinegars. All the DHS experiments were carried out on the synthetic sweet wine, according to the scheduled operations reported in Table 1. The experimental domain was defined taking into account instrumental and operative limits. These parameters are important in headspace techniques because it influences the headspace composition [21]. Also, the purge volume and the dry volume have to be carefully selected [26], because the first influences the trapping and the concentration of the analytes on the Tenax, while the dry volume is important to remove the water in order to preserve the chromatographic and MS systems.

The experimental design is used to evaluate the variable significance and the interaction among them. In this study, 20 experiments were randomly performed and the peak chromatographic areas of eleven volatile esters were the parameters used for the optimization. In order to evaluate the intermediate precision of the proposed design, four experimental runs of the center points were performed in four different days. Considering the peak areas of the investigated species, the intermediate precision was 8.6%. Repeatability was evaluated by considering the responses obtained for three replicates of the sample in the same day (one at the beginning, one at the middle and one at the end, respectively), using the experimental conditions of the center point. The repeatability was 4.9%.

The experiments were evaluated using a MLR analysis for each compound. The peak areas were the response functions (y) and were related to the controlled factors by a second-degree polynomial equation model, according to the four studied factors (1, extraction temperature; 2, extraction time; 3, purge volume; 4, dry volume):

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4$$

To confirm the significance of the factors, Student's t-test was applied to the results of the studied esters (Table 2). The purge volume and extraction temperature were shown to have the most statistically significant effects, both with positive influence. Therefore, the increase of these two variables goes up the response obtained. The results showed that the temperature was significant ($p < 0.001$) for ethyl isobutanoate and isobutyl acetate and for methyl butanoate ($p < 0.005$). The purge volume had a significant effect for eight compounds, with $p < 0.005$ for ethyl isobutanoate and decanoate and for hexyl acetate and with $p < 0.01$ for methyl butanoate, isobutyl acetate and the ethyl esters of 6, 7 and 8 carbons. The other factors studied (extraction time and dry volume) were not statistically significant in any case. In relation with the second order interactions, the regression coefficients to

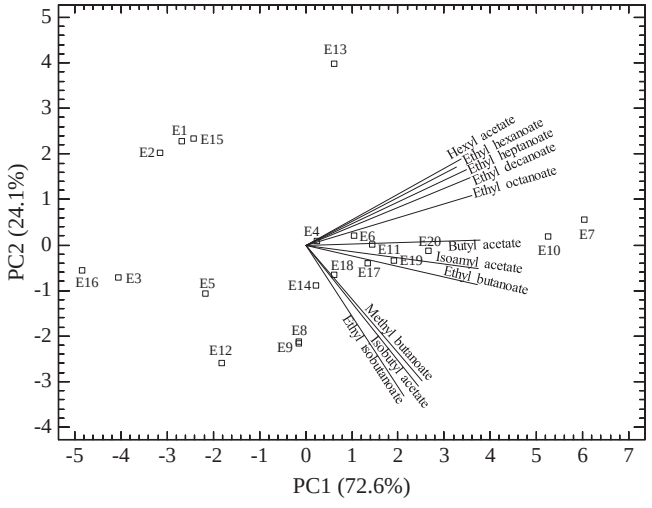

Fig. 1. Biplot of PC1 vs PC2 of the results obtained with design of the experimental design.

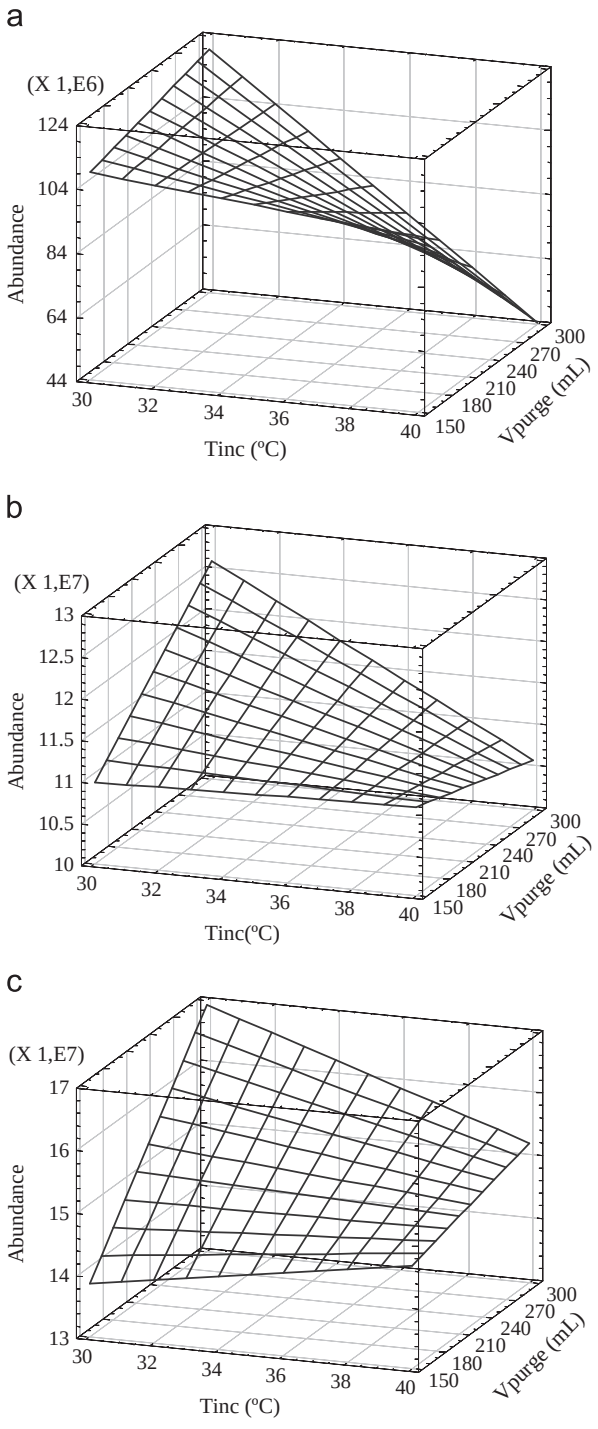

Fig. 2. Response surface model for methyl butanoate (a), isoamyl acetate (b) and ethyl decanoate (c) vs extraction temperature and purge volume.

interaction between temperature and purge volume were negative and significant for one acetate, isobutyl acetate ($p < 0.005$) and for three ethyl esters: isobutanoate, butanoate and decanoate ($p < 0.01$).

In order to visualize the relationships among the experiments and the analytical responses, the peak areas of esters were analyzed by PCA. The model was built with the two first PCs (explained 96.7% of the total variance) and the biplot obtained is reported in Fig. 1. According to the biplot, E7 and E10 experiments showed the highest positive score values on PC1, which explained the 72.6% of the variance. These two experiments were performed with the same extraction temperature (30 °C), extraction time (20 min) and purge volume (300 mL), and only differ for the dry volume, 50 mL and 100 mL, E7 and E10 respectively. Since the loadings of all the variables on PC1 are directly correlated and get positive values, the conditions planned in E7 experiment seem to correspond to a better performance with respect to the other runs settings.

On the other hand, three groups of esters were separated by PC2 (24.1% of variance): the first one (ethyl isobutanoate, isobutyl acetate and methyl butanoate), the second one (ethyl butanoate, isoamyl acetate and butyl acetate) and the last one (hexyl acetate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate and ethyl decanoate). Probably, the compounds belonging to the same group could show a

Table 3
Retention time (min), selected ions (m/z), calibration curve, regression coefficient, concentration range (μg L^{-1}), limit of detection (μg L^{-1}) and limit of quantification (μg L^{-1}) for the esters. The quantitative ions have been marked in bold.

	Compound	t_r	Selected ions	Linear regression equations	R^2	Concentration range	LOD	LOQ
1	Methyl butanoate	3.53	57/74/**102**	$y = 3.08 \times 10^4 x + 2.10 \times 10^6$	0.9894	51–1012	15	51
2	Ethyl isobutanoate	3.93	43/71/**116**	$y = 3.96 \times 10^4 x + 1.28 \times 10^6$	0.9922	46–460	9	29
3	Isobutyl acetate	4.10	43/56/**73**/101	$y = 4.55 \times 10^4 x + 1.71 \times 10^6$	0.9904	50–498	18	46
4	Ethyl butanoate	4.44	43/**71**/88/116	$y = 3.91 \times 10^4 x + 3.06 \times 10^6$	0.9896	50–1007	5	17
5	Butyl acetate	4.60	43/56/**87**	$y = 4.01 \times 10^4 x + 1.81 \times 10^6$	0.9934	53–529	10	34
6	Isoamyl acetate	5.36	43/70/**85**	$y = 3.56 \times 10^4 x + 3.07 \times 10^6$	0.9901	49–988	17	57
7	Ethyl hexanoate	6.81	43/88/**99**	$y = 4.55 \times 10^4 x + 1.71 \times 10^6$	0.9974	47–983	8	25
8	Hexyl acetate	6.96	**43/55/84**	$y = 1.88 \times 10^4 x + 1.44 \times 10^6$	0.9965	49–983	2	7
9	Ethyl heptanoate	7.87	**88/113**	$y = 9.24 \times 10^3 x + 9.76 \times 10^5$	0.9947	48–1436	7	24
10	Ethyl octanoate	8.88	88/140/**172**	$y = 5.63 \times 10^4 x + 4.93 \times 10^6$	0.9980	51–2051	12	41
11	Ethyl decanoate	10.67	88/157/**200**	$y = 5.71 \times 10^4 x + 3.49 \times 10^6$	0.9981	52–2097	4	15

Table 4
Precision and accuracy of esters with the proposed method.

	Compound	Concentration (μg L^{-1})	Repeatability (%)	Intermediate precision (%)	Accuracy (%)
1	Methyl butanoate	101	2.88	2.67	85
		506	4.76	6.67	110
		1012	8.18	8.51	97
2	Ethyl isobutanoate	92	2.32	3.58	91
		184	4.00	1.82	113
		460	4.50	6.59	98
3	Isobutyl acetate	100	2.44	3.46	92
		199	2.20	2.25	114
		498	5.01	6.26	98
4	Ethyl butanoate	101	1.54	3.84	80
		201	5.60	2.13	117
		504	10.05	7.25	112
5	Butyl acetate	106	1.94	3.52	96
		212	2.30	2.12	112
		529	6.01	5.59	99
6	Isoamyl acetate	99	1.69	4.95	79
		494	5.86	10.8	108
		988	1.62	8.48	97
7	Ethyl hexanoate	94	2.44	5.88	98
		470	1.34	4.25	106
		940	4.28	11.25	99
8	Hexyl acetate	98	2.44	12.05	98
		491	6.01	9.98	108
		982	2.22	11.52	98
9	Ethyl heptanoate	479	7.50	3.14	113
		957	2.68	11.54	102
		1436	13.44	9.68	98
10	Ethyl octanoate	103	5.42	8.18	96
		513	8.91	6.51	107
		1539	11.81	7.52	99
11	Ethyl decanoate	524	10.00	9.86	96
		1048	15.81	7.71	100
		1573	12.20	10.1	98

similar adsorbent behavior due to the individual characteristics of each ester, such as the molecular weight, molecular structure, etc. This would be consistent with the above, where the group with high PC1 values is formed with the longer chain esters.

Finally, to better observe the effects of the extraction temperature and time on the area of esters, a response surface model in a three-dimensional plane was plotted (Fig. 2). An ester of each group of compounds distinguished previously in the PCA has been chosen, methyl butanoate for the first group, isoamyl acetate for the second group and ethyl decanoate for the last one. For the model, the R^2 was 82.7%, 71.2% and 79.5% for ethyl isobutanoate, isoamyl acetate and ethyl decanoate respectively. The $R^2_{adjusted}$ for each one was 77.8%, 39.3% and 56.8% for the same compounds, where $R^2_{adjusted}$ was the explained variance corrected by the degrees of freedom.

For the three groups of esters, the analytical signal grown with the diminution of temperature and the increase of purge volume, meaning that the best conditions for the extraction were obtained at an extraction temperature of 30 °C and with 300 mL of purge volume. This result indicates that high purge volume would increase the extraction of the studied esters, but the high extraction temperature probably would increase the release of analytes from the Tenax to the headspace.

3.2. Method validation

3.2.1. Linearity, LOD and LOQ

For the method validation, the calibration curves for the 11 esters in the synthetic sweet wine were constructed using the optimized method, with an extraction temperature of 30 °C, an extraction time of 20 min, a purge volume of 300 mL and a dry volume of 50 mL. The linear regression equations and the correlation coefficients (R^2) are given in Table 3. R^2 values between 0.9894 and 0.9981 were obtained for this model, which indicates a good fit between the observed and the predicted response values and consequently a satisfactory linearity. LOD and LOQ obtained varied for the different compounds: the smallest LOD value (2 μg L^{-1}) was for hexyl acetate, LOD values between 4 and 9 μg L^{-1} were obtained for all the ethyl esters, excepted for ethyl octanoate, and LOD values $\geq$10 μg L^{-1} were for the remaining esters. The smallest LOQ values were for isoamyl acetate (57 μg L^{-1}) and hexyl acetate (7 μg L^{-1}), LOQ values between 15 and 34 μg L^{-1} were obtained for all the ethyl esters studied, excepted for ethyl octanoate. Finally, the above mentioned ester, methyl butanoate and isobutyl acetate shown LOQ values higher than 41 μg L^{-1}.

3.3. Precision and accuracy

The results for intraday and interday precision and accuracy are presented in Table 4. The precision of the method was evaluated studying repeatability and intermediate precision for the studied esters at three different concentrations. The two parameters changed depending on the compound and the concentration tested, but they did not exceed 15% in any case. So, the method could be considered precise for the compounds studied.

Finally, the accuracy results calculated for the three concentrations of all esters were within showed that 15% of the nominal value, which means that the method is considered accurate [30], excepting isoamyl acetate (99 μg L^{-1}) and ethyl butanoate (101 and 201 μg L^{-1}) which presented accuracy values of 21%, 20% and 17% respectively.

3.4. Wine analysis

To evaluate the reliability of the proposed method, the optimized and validated method described above was applied to 8 Spanish sweet wines by triplicate (24 sweet wine samples) of different origins, grape variety and winemaking. The chromatograms obtained by DHS–TD–GC–MS for two of them, a white type (Fig. 3a) and a red type (Fig. 3b) are shown. Esters quantification was based on the calibration curves obtained in the linearity experiments for each analyte.

Table 5 shows the average concentrations (n=3) for the 11 aromatic esters in each sweet wine sample, the odor descriptors and the odor threshold, defined this as the lowest concentration capable of producing a sensation [31]. Most of the wines analyzed exhibited significant concentrations of the 11 fruity esters studied. Except for methyl butanoate, all the quantified esters are included in the acetates and ethyl esters groups. Many studies have demonstrated that they are important odorants in wine [8,32–36].

Note that isobutanoate, butanoate, hexanoate, octanoate ethyl esters and isoamyl acetate showed concentrations above their odor threshold, and therefore are considered active odorant compounds and contribute greatly to fruity aroma of the sweet wines, already observed by other researchers [2]. The remaining esters contribute more weakly to the aroma profile of the wines, in any case depending on potential synergic effects enhancing specific odor sensations [37,38]. Also, quantitative differences between white and red sweet wines are observed, which can be a consequence of the different grape varieties (white and red grapes), drying process or/and winemaking. Ethyl isobutanoate with

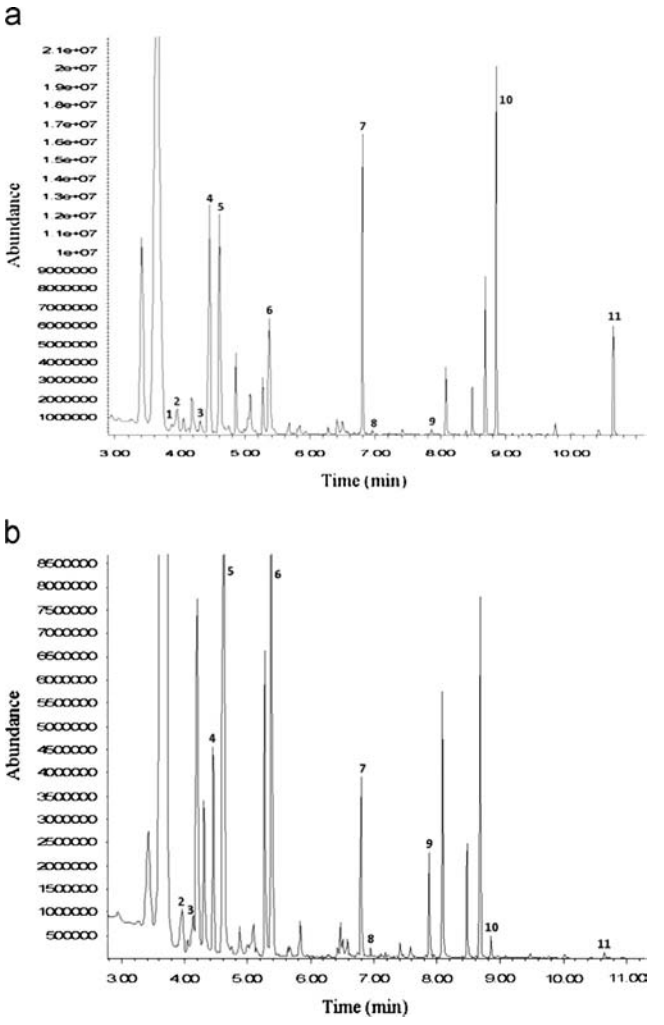

Fig. 3. DHS–TD–GC–MS chromatogram obtained for a sweet white wine (a) and a sweet red wine (b). Peaks correspond to: (1) methyl butanoate; (2) ethyl isobutanoate; (3) isobutyl acetate; (4) ethyl butanoate; (5) butyl acetate; (6) isoamyl acetate; (7) ethyl hexanoate; (8) hexyl acetate; (9) ethyl heptanoate; (10) ethyl octanoate; (11) ethyl decanoate.

Table 5
Odor descriptors, odor threshold ($\mu g\ L^{-1}$) and concentrations ($\mu g\ L^{-1}$) of fruity aroma esters in sweet white (W) and sweet red (R) wines.

	Compound	Odor descriptors	Odor threshold	Sweet white wines			Sweet red wines				
				W1	W2	W3	R1	R2	R3	R4	R5
1	Methyl butanoate	Strawberry	1000[a]	n.d.	n.d.	4 ± 1	n.q.	n.d.	n.d.	n.d.	n.d.
2	Ethyl isobutanoate	Strawberry, melon	15[b]	2180 ± 6	37 ± 2	15 ± 2	43 ± 7	31 ± 2	30 ± 1	n.q.	47 ± 4
3	Isobutyl acetate	Banana, apple	6140[a]	6 ± 1	7 ± 1	8 ± 2	n.q.	5 ± 1	n.q.	48 ± 1	20 ± 4
4	Ethyl butanoate	Pineapple, strawberry, banana	20[b]	516 ± 8	549 ± 6	61 ± 3	90 ± 3	785 ± 9	20 ± 1	118 ± 1	133 ± 2
5	Butyl acetate	Banana, ripe pear	4600[a]	960 ± 15	579 ± 11	9 ± 1	79 ± 2	579 ± 2	n.q.	13 ± 1	751 ± 28
6	Isoamyl acetate	Banana	30[b]	46 ± 5	287 ± 3	20 ± 2	13 ± 1	29 ± 2	6 ± 1	2923 ± 31	463 ± 3
7	Ethyl hexanoate	Banana, green apple	5[b]	214 ± 4	734 ± 11	6 ± 1	213 ± 3	215 ± 11	90 ± 6	886 ± 21	105 ± 8
8	Hexyl acetate	Apple, banana	1000[c]	n.q.	5 ± 1	n.q.	23 ± 2	30 ± 4	n.q.	8 ± 1	5 ± 1
9	Ethyl heptanoate	Ripe pear	300[d]	11 ± 1	18 ± 4	n.d.	16 ± 2	40 ± 5	n.q.	6 ± 1	17 ± 2
10	Ethyl octanoate	Pineapple, pear	2[b]	58 ± 1	302 ± 31	36 ± 2	11 ± 0.2	29 ± 3	4 ± 1	210 ± 5	8 ± 1
11	Ethyl decanoate	Apple, pear, banana	200[e]	n.q.	43 ± 2	8 ± 1	n.q.	n.q.	n.q.	13 ± 1	n.q.

Values are mean ± standard deviation ($n=3$). n.q.=not quantified; n.d.=not detected.

[a] [32].
[b] [10].
[c] [2].
[d] [33].
[e] [31].

strawberry and melon odor descriptors was the major ester quantified in the white sweet wines, particularly in the W1 wine sample ($2180 \pm 5.99\ \mu g\ L^{-1}$). This ester has a low odor threshold ($15\ \mu g\ L^{-1}$) therefore can be considered a potent odorant and a great contributor to fruity aroma to this wine. However, isoamyl acetate (banana odors) with an odor threshold of $30\ \mu g\ L^{-1}$ was the highest in red wines ($2923 \pm 31.2\ \mu g\ L^{-1}$, R4 sample). Furthermore, white and red wines exhibit different average concentrations for the remaining active odorant esters, between 61–549 $\mu g\ L^{-1}$ (W samples) and 20–785 $\mu g\ L^{-1}$ (R samples) for ethyl butanoate, 6–734 $\mu g\ L^{-1}$ (W samples) and 90–886 $\mu g\ L^{-1}$ (R samples) for ethyl hexanoate and 35–301 $\mu g\ L^{-1}$ (W samples) and 4–210 $\mu g\ L^{-1}$ (R samples) for ethyl octanoate. These esters presents low odor perception threshold (20, 5 and 2 $\mu g\ L^{-1,}$ respectively) and significantly contributed to W1, W2, *R*2 and R4 aroma profile with pineapple, strawberry, banana, green apple and pear odors. Taking to account the results of sweet wines studied, it is possible to confirm that isobutanoate, butanoate, hexanoate, octanoate ethyl esters and isoamyl acetate are the principal esters that greatest contributing to fruity aroma, in agreement with other studies [7,8,39–41].

4. Conclusions

The DHS extraction coupled with the GC/MS technique was successfully applied to the determination of aromatic esters in sweet wines. This method is rapid and simple, and it showed good intermediate precision and repeatability of the data, thanks to the high automation. The proposed experiment design and the statistical analyses showed that the optimal conditions were obtained at the lowest extraction temperature and dry volume, and the highest extraction time and purge volume. Calibration and validation were performed for the analyzed compounds and was demonstrated to be a linear, precise, accurate and sensitivity method. Therefore, this method could be a useful tool to determine active odorant esters and their impact on aroma profile of sweet wines.

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