

# Application of a headspace mass spectrometry system to the differentiation and classification of wines according to their origin, variety and ageing

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## Abstract

The system based on coupling a headspace sampler to a mass spectrometer (HS-MS), considered a kind of electronic nose (e-nose), is an emerging technique in the field of food aroma analysis. The global mass spectrum this system provides is a fingerprint of each sample analysed that contains the information related to volatile composition of the sample. The use of chemometric techniques allows to compare the spectra of the samples and then, to classify them according to different properties. In this paper, we present the development of a method for wine analysis using a HS-MS system and multivariate analysis techniques. The method was successfully applied to differentiate and classify wines according to its origin, variety and ageing. The main advantages of the proposed methodology are the minimum sample preparation required and the speed of analysis (10 min/sample).

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## 1. Introduction

Aroma is an important factor in quality control and quality assurance of foods, but in wines, this factor is possibly the most important. Sensory analysis by a panel of trained judges is the most common technique for controlling the quality of wines. However, this technique requires a group of trained people that can only carry out a little number of analyses every day. Moreover, the subjectivity of the panellists affects the analysis in great measure.

In the last 20 years, there has been an increasing research in order to achieve a faster and more objective system for evaluating aromas, which has led to the development of the e-nose technology [1–5]. Most of the systems available in the market are based on the interaction of the volatile compounds in the headspace above the sample with a gas sen-

sor array. The sensor responses generated by these physical and/or chemical interactions are treated with chemometric techniques to differentiate and classify these complex mixtures of volatile compounds. Although a lot of food analysis applications have been made using this type of instrument, in wine research only a few studies have been done [6–9]. Furthermore, the small number of samples analysed in these studies, is not enough to prove the suitability of the technique for the differentiation and classification of wines. The poor results obtained by these instruments in wine analysis are because ethanol, the major component of the headspace of the sample, causes interference in gas sensors. To solve this problem, some research groups have tried to eliminate the ethanol of the samples, for instance, by using the pervaporation technique or a purge and trap system [10–12]. Even though these strategies enable to improve the results, the speed and the simplicity of the technique, two of the main advantages of the e-nose systems, are compromised.

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A few years ago, a new type of e-nose based on mass spectrometry (MS) was developed [13]. It involves injecting the headspace of the sample into the ionisation chamber of the mass spectrometer in which the analytes are fragmented. The result is a global mass spectrum for each sample analysed that is obtained from the time integral of all spectra. When several samples are analysed, a data matrix composed by the abundance of all mass-to-charge ratio ( $m/z$ ) monitored, is generated. The matrix is treated with multivariate analysis techniques to compare and classify the substances from their volatile composition. Chemical information about the kind of compounds responsible for the differences between samples can be obtained from the ion-fragmentation patterns and the results can be compared directly with the results from conventional GC–MS instruments. The MS-based electronic nose has already given good results in foodstuffs analysis [14–18]. In the field of alcoholic beverages, the suitability of the HS-MS e-nose has been proved for quantitative purposes—using multivariate calibration techniques—for determining 2,4,6-trichloroanisole in wines [19] and, also, for determining the ageing time of spirits in oak barrels [20]. The good results obtained in these studies are due to the fact that ethanol is not a problem in the analysis of alcoholic beverages with this instrument, because the ethanol interference can be easily avoided if the fragment ions corresponding to ethanol fragmentation are not selected in the mass spectrometric analysis. This fact is an important advantage of the instruments based on MS versus the instruments based on gas sensors.

In this study, we develop a simple, quick and automatable method for the differentiation and classification of wines according to different oenological parameters using an HS-MS electronic nose and multivariate analysis techniques.

## 2. Experimental

### 2.1. Samples

In the present work, three different studies related to differentiation and classification of wines according to their variety, origin and ageing have been carried out, by choosing suitable representative samples.

Samples from different Catalonian wine growing zones were chosen for the study of varietal wines. This study was carried out over three vintages (1999–2001), being the total amount of wines analyzed for the 3 years: 82 Cabernet sauvignon, 66 Tempraonillo and 61 Merlot wines. The samples chosen in the study of wine origin differentiation were from Priorat and Terra Alta, two very close Catalonian growing zones with very similar climatology. Most of the wines from both zones are blends of Garnatxa and Cariñena varieties. Samples of 12 red wines from Priorat and 13 red wines from Terra Alta were analyzed.

In the study of ageing differentiation, the wines chosen were from Ribera del Duero, a very important Spanish wine

growing zone where the commercial wines are classified according to their ageing process, by a regulatory council, into four different administrative categories: *Young* wines (red wines with an ageing time less than 12 months in casks), *Crianza* wines (red wines with an ageing of 12 months in casks), *Reserva* wines (red wines with an ageing time of 36 months between cask and bottle but with a minimum of 12 months in casks) and *Gran Reserva* wines (exceptional quality red wines with an ageing higher than 60 months and with at least 24 month ageing in casks followed by 36 month ageing in bottles). The total amount of samples analyzed in this study were: 54 *Young* wines, 34 *Crianza* wines, 33 *Reserva* wines and 10 *Gran reserva* wines.

The HS-MS analysis of the samples was always made in triplicate. However, the multivariate analysis was carried out with the average of the three mass spectra obtained with the triplicates of each sample. Before carrying out the chemometric analysis we examined the raw data and we removed some samples that we considered non-representatives because they were very clear outliers.

### 2.2. Apparatus

Wine analyses were carried out on a HS-MS electronic nose composed of a HP 7694 headspace sampler, a HP 6890 gas chromatograph and a HP 5973 quadrupole mass spectrometer with a diffusion pump from Hewlett-Packard (Waldbronn, Germany). The chromatographic column was only used to transfer the volatile compounds to the MS, not to resolve chromatographic peaks.

### 2.3. Procedure

In the optimised conditions, 5 mL of wine and 0.58 g of NaCl were placed in a 10 mL vial that was hermetically capped with a PTFE/silicone septum. To obtain the equilibrium of the volatile compounds between the liquid and the headspace, the sample was thermostated at 65 °C for 1 h under constant stirring. Afterwards, the headspace of the sample was introduced into the gas chromatograph injection port. The loop and transfer line temperatures were 90 and 105 °C, respectively, and the pressurisation and injection times were 0.30 and 0.60, respectively. Chromatographic injection was made in splitless mode for 1.6 min at 200 °C using an inlet of 1.5 mm i.d. We used a HP-5MS chromatographic column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with an oven temperature program of 70 °C (1 min), 70 °C/min, 180 °C (2.5 min). The carrier gas was helium with a flow-rate of 1.8 mL/min. The purpose of using these chromatographic conditions (oven temperature program and flow) was to transfer the volatile compounds to the MS in the shortest time (5 min). Mass spectra were recorded by electronic impact (EI) ionisation at 70 eV. The mass-to-charge ratio range ( $m/z$ ) used was 50–200. The ion source and mass quadrupole temperatures were 230 and 150 °C, respectively.

## 2.4. Chemometric treatment

The software we used for multivariate analysis was Pirouette 2.6 from Infometrix (Woodinville, WA, USA). The chemometric treatments carried out include the principal component analysis (PCA), that it is an unsupervised technique used to recognize natural groups of the samples, and soft independent modeling of class analogy (SIMCA) that is a supervised pattern recognition technique used to classify the samples. Before applying the chemometric treatments mass spectra were normalized to 100 and data matrix was mean-centered.

## 3. Results and discussion

The first part of the study was the optimisation of the parameters that could affect the response obtained in the HS-MS analysis of wines in order to achieve the highest response as well as to detect the parameters that are responsible of the transfer of the volatile compounds to MS system detector, to obtain the shortest analysis time. Once the optimal conditions were found, we carried out different studies related to differentiation and classification of wines according to different oenological parameters.

### 3.1. Optimisation

We performed the experiments using a commercial wine. Our criterion for obtaining the optimal conditions was to achieve the highest overall abundance of the mass range 50–200  $m/z$ . We selected this range because above 200  $m/z$  there are not ion fragments with a significant abundance. On the other hand, below 50  $m/z$  there are fragment ions corresponding to the ethanol fragmentation (MW 46) that could interfere in chemometric analysis, due to their high abundance (ethanol is a major compound of the headspace) in comparison with the rest of fragment ions. Moreover, in the signal below 50  $m/z$  the noise is higher.

The initial parameters studied were extraction time and extraction temperature because, as it is well-known, they affect the headspace composition. As these are closely related, we studied them simultaneously achieving a two-factor two-level factorial design with a central point [21]. We analysed three replicates of the sample in each experimental point studied. According to our experience in previous works [19], the temperature and time levels tested were 50 and 80 °C and 20 and 60 min, respectively. The best results were obtained at 65 °C for 40 min (the central point). By verifying more experimental points around the best one, we found that the 60 min response was slightly better than the 40 min one. Therefore, we chose 60 min as the extraction time.

Other important parameter that modifies the headspace composition is the ionic strength. Different amounts of sodium chloride were added to 5 mL of sample placed in 10 mL vials, which were shaken for 60 min at 65 °C. The

best results were obtained when the sodium chloride concentration was 2 M.

The criterion for optimising the transfer conditions was to achieve the shortest time in the transfer of the volatile compounds to the MS system because this step determines, in great measure, the run time analysis of the method. With the temperature program used, we were able to transfer all the compounds to the detector in only 5 min. However, the total run time analysis was 10 min because the gas chromatograph oven needs additional time to come back to the initial temperature conditions before injecting another sample. Therefore, the analysis time would be shorter if the transference of the volatiles to the MS detector was using an isotherm at high temperature. But this was not possible because we experimentally found that the signal obtained was very low at initial chromatographic temperatures higher than 80 °C. We cannot explain this fact, but we think that ethanol, that is a major component of the wine headspace, causes this upshot when it is introduced to the column at temperatures higher than its boiling point. Perhaps, when the initial oven temperature is below the ethanol boiling point, the response obtained is better due to the solvent effect. On the other hand, if we had used an uncoated deactivated retention gap as transfer line between the headspace autosampler and the detector, probably we would have shortened the run time analysis. However, we used an analytical capillary column because this configuration allows an easier change to switch from a HS-(GC)–MS e-nose to a conventional GC–MS.

### 3.2. Differentiation of wine varieties

One of the numerous factors that may affect the composition of wine aroma is grape variety, so wines made with Cabernet sauvignon, Merlot or Tempranillo grape varieties were analysed. The study was carried out over three vintages (1999–2001).

In 1999 vintage, 35 Cabernet sauvignon, 28 Tempranillo and 17 Merlot wines were analysed. The PCA was done on the covariance matrix because the differences between wines are caused by major components of wine aroma due to the low sensitivity of the static headspace technique to extract the minor components. Therefore, the results of the PCA on the correlation matrix were not better. In the first exploratory analysis carried out with all variables, we observed a slight differentiation among varieties. The cluster analysis carried out on the raw spectra and also on the PCs did not improve the results. However, a new PCA carried out with a selected group of variables (51–53, 57, 58, 67, 73–75, 77 and 108  $m/z$ ) allowed to find higher differentiation. In Fig. 1, where the scores' graphic is shown, a partial overlapping among groups can be observed. These overlapping can also be observed in the interclass distances obtained with the SIMCA classification method (Table 1). This parameter is a useful measure of class separation. The higher the interclass distance between two groups, the higher the difference between them. The small distances between Cabernet sauvignon and

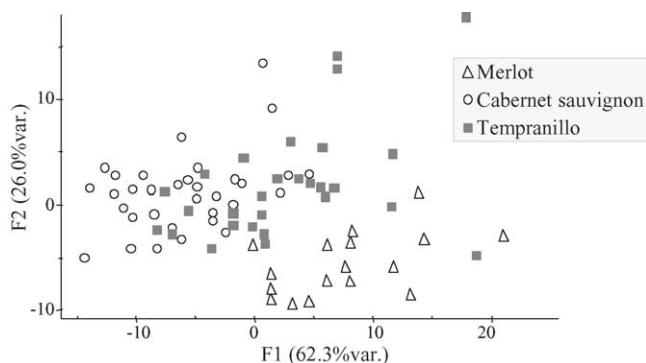


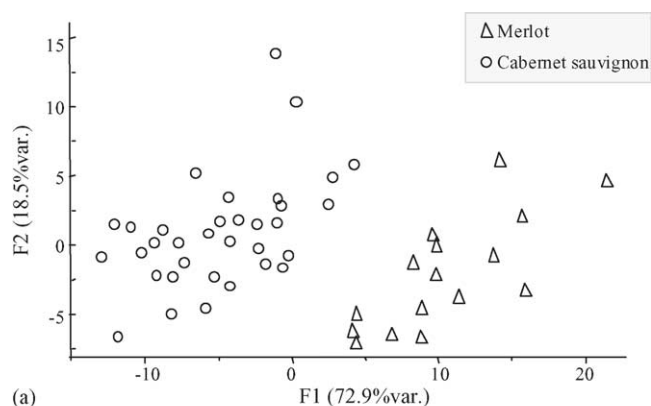
Fig. 1. Scores plot of PCA of Cabernet sauvignon, Merlot and Tempranillo wines of 1999 vintage.

Tempranillo wines as well as the distances between Merlot and Tempranillo wines, showed that each pair of these varietal wines are insufficiently distinguished from the aroma point of view. On the other hand, Cabernet sauvignon and Merlot wines are well-differentiated. The distance between this pair of varietal wines is larger than the distances between Cabernet sauvignon and Tempranillo wines or between Merlot and Tempranillo wines. These results are successful because by using an electronic nose, the differentiation of a significant number of wines according to an oenological parameter, such as variety, has been possible for the first time. In Fig. 2a and b are shown the scores and loadings graphics obtained considering only Merlot and Cabernet sauvignon wines. The loadings associated with each factor showed that fragment ions 58 and 73 are the variables which most contribute to factor 1. This factor, which accounts for 72.9% of the total variability of the samples, seems to be the most important for distinguishing Merlot from Cabernet sauvignon wines. This information can be useful for obtaining chemical information about the volatile compounds responsible of the differentiation between these two varieties of wines. For instance, the fragment ion 73 is characteristic of several organic acids present in wine aroma such as isobutyric, butyric, hexanoic and octanoic. As a conclusion, Merlot wines are richer than Cabernet sauvignon wines in some of these compounds. On the other hand, the origin of the fragment ion 58, whose abundance is higher in Cabernet sauvignon wines, may be caused by the fragmentation of 3-methylthio-1-propanol, a sulphur compound usually found in these kind of wines [22]. The possibility of obtaining additional chemical information is an important advantage of the e-nose based on MS versus the e-nose based on gas sensors.

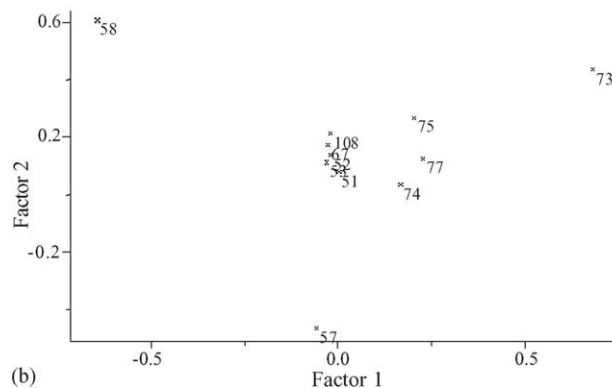
Table 1  
SIMCA models of varietal wines

	Vintage 1999	Vintage 2000	Vintage 2001
Cabernet sauvignon/Merlot	2.3	0.9	1.5
Merlot/Tempranillo	0.9	0.0	1.0
Cabernet sauvignon/Tempranillo	0.4	0.0	2.1

Interclass distances.



(a)



(b)

Fig. 2. PCA of Cabernet sauvignon and Merlot wines of 1999 vintage. (a) Scores plot and (b) loadings plot.

The results obtained in the analysis of 28 Merlot, 20 Tempranillo and 29 Cabernet sauvignon wines of the 2000 vintage were not as good as the 1999 vintage ones. The projection of the samples on different factors showed strong overlap of the three groups of wines. The selection of a set of variables (67–69, 71, 81–84  $m/z$ ) allowed a slight differentiation between Merlot and Cabernet sauvignon wines. These results are shown in Fig. 3 where it can be observed that the second

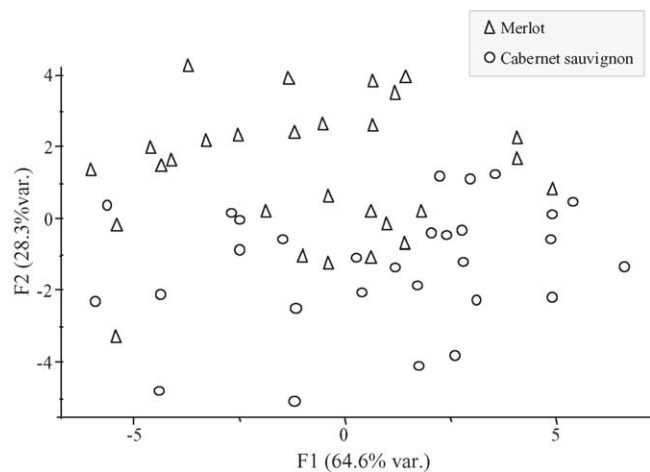


Fig. 3. Scores plot of PCA of Cabernet sauvignon and Merlot wines of 2000 vintage.

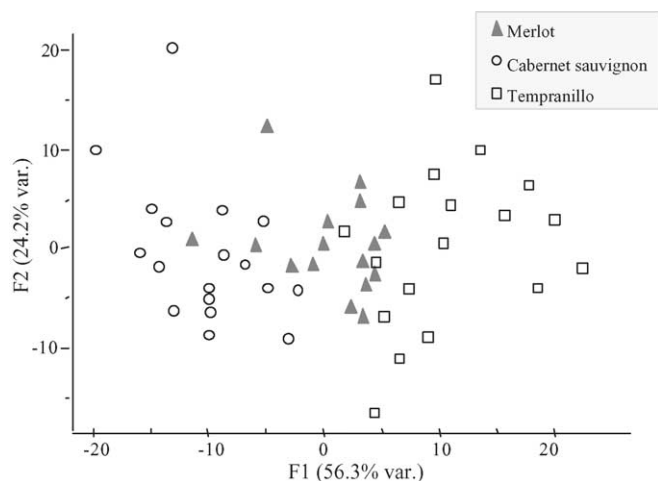


Fig. 4. Scores plot of PCA of Cabernet sauvignon, Merlot and Tempranillo wines of 2001 vintage.

factor is the most important for distinguishing both groups. Nevertheless, it only accounts for 28.3% of the total variance. The distance between Cabernet sauvignon and Merlot wines of vintage 2000 obtained with the SIMCA method (Table 1) also showed a very slight differentiation which was not sufficient for obtaining a clear classification of these varietal wines.

Although it was observed a partial overlap among the different varieties, in the 2001 vintage the differentiation of 16 Merlot, 18 Tempranillo and 18 Cabernet sauvignon wines was clearer than in the 2000 vintage. If we compare the graphic of scores showed in Fig. 4 with the same graphic obtained in the PCA of the 1999 vintage wines (Fig. 1), it can be observed that both plots are very similar but in 2001, the group in the middle is Merlot wines instead of Tempranillo wines. Therefore, as it can be observed in Fig. 4, the best differentiation obtained with 2001 vintage wines was between the Cabernet sauvignon and Tempranillo wines. The interclass distances detailed in Table 1, show that the three groups of wines are more differentiated in 2001 vintage than in 1999 vintage.

Although the results obtained in different vintages were different, the suitability of the method developed using the HS-MS technique to differentiate and classify wines according to their variety has been proved. Moreover, the high number of samples analysed ratify the results obtained because they are statistically representative. Besides grape variety, there are many other factors that may affect wine aroma composition and, some of them—for instance climatology—may be responsible of the aromatic differences among vintages.

### 3.3. Differentiation of wine origin

In the PCA carried out with the data matrix obtained in the HS-MS analysis of wines from Priorat and Terra Alta, we observed a slight differentiation between wines of different origin. In this first exploratory analysis, we found a group of

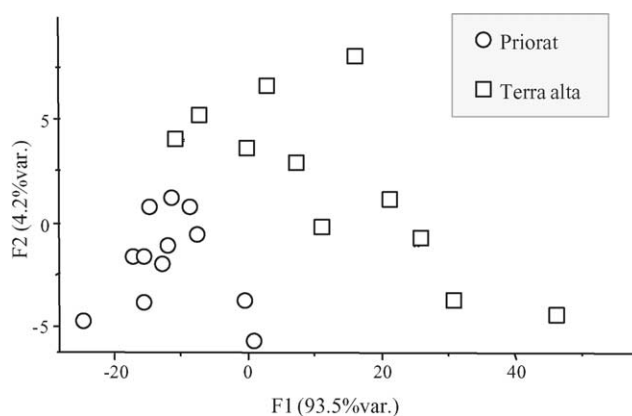


Fig. 5. Scores plot of PCA of red wines from different origin: Priorat and Terra Alta.

variables with an important influence on the differentiation (70, 73, 88, 101 and 115  $m/z$ ). Therefore, in order to achieve a better differentiation, we carried out another PCA but only using these fragment ions as independent variables. The projection of the samples on the first and second factors that, collectively account for 97.7% of the total variability of the samples, is shown in Fig. 5. As it can be observed, wines cluster according to their origin. The interclass distance obtained between Priorat and Terra Alta wines by applying the SIMCA classification method to the data matrix was 14.5. This high value corroborates the results observed in the PCA analysis since it indicates that the two classes are significantly different. These results are very promising because, as it has been previously explained, the zones studied are geographically very close and their wines are blends of the same varieties.

### 3.4. Differentiation of ageing

In Fig. 6 we show the scores' graphic of the PCA obtained in the analysis of 54 *Young* wines, 34 *Crianza* wines, 33 *Reserva* wines and 10 *Gran Reserva* wines from Ribera

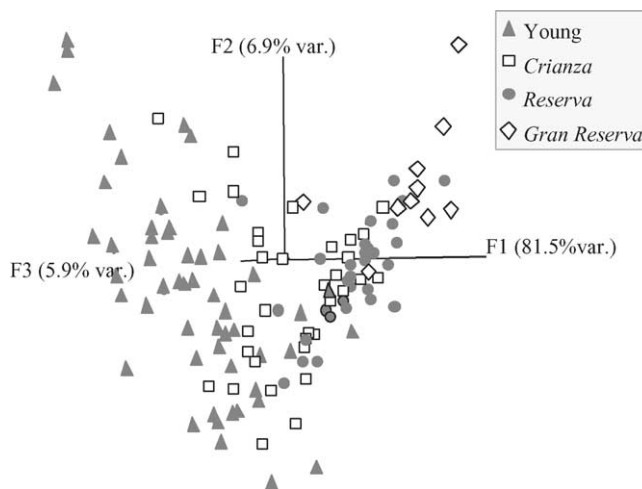


Fig. 6. Scores plot of PCA of wines with different ageing time from Ribera del Duero.

Table 2  
SIMCA model of Ribera del Duero wines

Classes	Interclass distance
<i>Young/Crianza</i>	1.0
<i>Young/Reserva</i>	1.7
<i>Young/Gran Reserva</i>	5.7
<i>Crianza/Reserva</i>	0.3
<i>Crianza/Gran Reserva</i>	3.8
<i>Reserva/Gran Reserva</i>	1.8

del Duero. The variables used in the multivariate analysis were the most important fragment ions in the differentiation of the four categories (69, 75, 87, 89, 97, 102, 103, 116 and 129  $m/z$ ). These fragment ions were found in a previous exploratory analysis. As it can be observed, although the four categories of wines are partially overlapped, there is a differentiation among them. This overlapping could be due to the small differences that exist between wines of different categories but similar ageing. For instance, a young wine and a *crianza* wine that have spent 11 and 12 months in cask, respectively, are very similar. However, they belong to different administrative wine categories. This fact is also shown in the results of the SIMCA classification method applied to the data matrix. In Table 2, where the interclass distances are shown, it can be observed that in most cases the distances are large, with the exception of the distances between *Young* and *Crianza* wines and between *Crianza* and *Reserva* wines. They are shorter because of the similar ageing of samples placed on the frontier ageing of these categories.

In order to know the suitability of a SIMCA model to classify Ribera del Duero wines in the different administrative categories, we divided the group of samples into two sets: the training set (composed of 89 wines to build the model) and the validation set (composed of 42 wines to study the prediction capacity of the model). The number of validation samples analysed belonging to each class and the prediction results obtained with the SIMCA model built are indicated in Table 3. We did not include any *Gran Reserva* wine because the number of samples of this class was small and we used all of them to build the model. Despite the fact that 13 wines were non-classified, the results were successful because 23 wines were correctly classified and only six wines were not. Four of these wrong classified samples corresponded to *Crianza* wines. Two of them were classified as young wines and the rest as *Reserva* wines. The prediction errors of the *Crianza* wines could be explained by the small interclass distances

Table 3  
Results of SIMCA model validation

Validation samples		Results of prediction		
Class	Number	Correct	Wrong	Non classified
<i>Young wines</i>	22	15	1	6
<i>Crianza wines</i>	15	6	4	5
<i>Reserva wines</i>	5	2	1	2
Total	42	23	6	13

between *Young* and *Crianza* wines and between *Crianza* and *Reserva* wines. In the evaluation of this results should be taken into account that, as it has been commented above, the differences among wines of different administrative categories, in some cases, are very small.

#### 4. Conclusions

The HS-MS electronic nose was successfully applied to the differentiation and classification of wines according to their origin, variety and ageing. The simplicity and speed in the sample preparation step and the speed of the HS-MS analysis (10 min/sample) of the method developed, offer a great number of advantages over sensory analysis in the authentication and in the quality control of wines. Moreover, as it was shown, it is also possible to achieve information about chemical composition of the responsible compounds of the differentiation, which may be of interest in wine aroma research.

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