# Analysis of Some Highly Volatile Compounds of Wine by Means of Purge and Cold Trapping Injector Capillary Gas Chromatography. Application to the Differentiation of Rias Baixas Spanish White Wines

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An optimized method of purge and cold trapping injection–capillary gas chromatography was applied to the analysis of some volatile constituents of Galician (northwestern Spain) white wines with certified brand of origin "Rias Baixas" and other white wines produced in neighbor regions in order to obtain their volatile profile. Identification of the chromatographic peaks was performed by coupling an ion trap detector to the chromatograph. Characterization of Rias Baixas wines was performed applying nonsupervised and supervised multivariate techniques of analysis (cluster analysis, principal components analysis, K-nearest neighbors). Thus, multivariate profiles of the wines were obtained, and certain differences between Rias Baixas wines and other Galician wines could be observed. Some atypical wines in both categories were pointed out by the exploratory and classification methods.

**Keywords:** *Purge and cold trapping injector; wine volatiles; multivariate characterization; capillary gas chromatography–mass spectrometry (ITD)*

## INTRODUCTION

Wine is a complex mixture of many organic and inorganic compounds. The composition is very dependent on many factors. Among them, the grape cultivar and the geographical origin certainly have a great influence. In certain vitivinicole regions, the production of quality wines has special economical importance as in Galicia (northwestern Spain). To achieve a certified brand of origin (denominación de origen controlada, DOC), wines must be elaborated with grapes from controlled vineyards and submitted to a few rules related to the alcoholic content and the acidity. Finally, wines are judged by their organoleptic characteristics.

In recent years, characterization of wines by means of different analytical parameters and multivariate statistical techniques has received increasing attention (Kwan and Kowalski, 1980; Scarponi et al., 1982; Borszecki et al., 1983; Moret et al., 1984; Médina and Van Zeller, 1984; Latorre et al., 1992). It has been recognized that the aroma constituents of the wine have a leading contribution to its varietal character (Rapp, 1972; Noble et al., 1980). White wines produced in Galicia under the DOC "Rias Baixas" have recently attracted international attention gaining increasingly wide markets over the world. Made up departing from 70 to 100% vs Albariño grapes, they are consumed usually as young wines, mostly along the production year. Their very fine taste and, especially, characteristic flavor have been probably the main reasons for their acceptance. Thus, it has to be expected that the aroma components of these wines would play an important role in their characterization and differentiation. However, several high-volatility compounds appear at very low concentration in wines, and some reliable and sensitive techniques of preconcentration are therefore needed. Of special interest are the compounds of high and medium volatility eluting close to ethanol in the chromatograms. Solvent extraction and different headspace techniques have been described and compared (Rapp et al., 1980; Wyllie et al., 1988; Etievant et al., 1986; Cela-Torrijos et al., 1994). Recently, cryogenic techniques have attracted great attention because of their potential for enriching compounds found in low concentration in complex mixtures like wine (Badings, 1984; Badings and De Jong, 1985; Badings et al., 1985; Darriet and Dubourdieu, 1993).

In this work the technique of purge and cold trap injection, based upon the theories and developments of Badings (1983) with some modifications and optimized for the analysis of wines, was applied to the analysis of Rias Baixas wines with certified brand of origin and other white wines produced in neighboring Galician regions. Differentiation of Rias Baixas certified wines was carried out by comparing their volatile profile to that obtained for the other white wines.

## EXPERIMENTAL PROCEDURES

**Samples.** Twenty-two wines were analyzed. Fourteen of these were Rias Baixas DOC wines collected in this restricted geographical area. All these wines were fermented at controlled low temperature (16–18 °C) in stainless steel tanks. Although each wine was produced in a different cellar, all of them were elaborated exclusively or in high proportion from cv. Albariño grapes (samples coded as A01 to A14). All these samples were kindly supplied and certified by the DOC Council. For differentiating purposes, Rias Baixas wines were considered class 1. The remaining eight samples came from different origins: one cv. Albariño wine produced outside the Rias Baixas geographical area (B01), one monovarietal cv. Treixadura wine (B02), one experimental sample from the Ribeiro DOC (B03), a commercial wine with unknown blending (B04), and four commercial wines from Ribeiro DOC (B05–B08). All these samples were blends of several grape varieties produced in Galicia but did not contain (except sample B01) any proportion of the Albariño variety. All non-Rias Baixas wines were considered as class 2.

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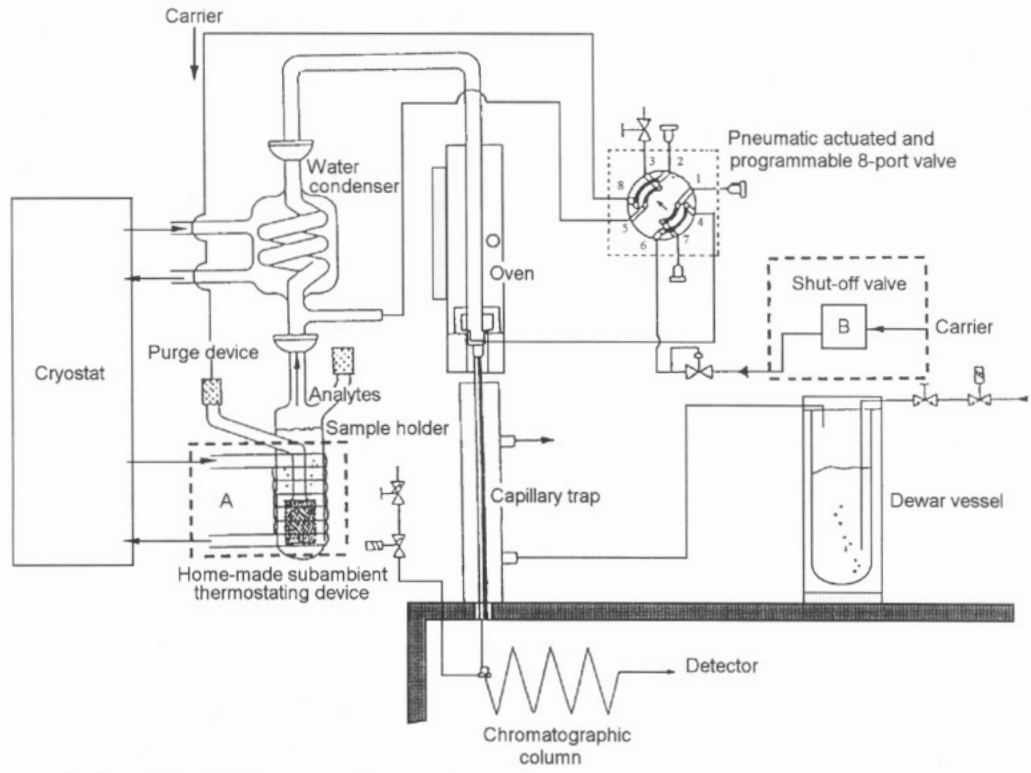


**Figure 1.** Schematic view of the PCTI system showing the two main modifications introduced: A; sample refrigeration device; B, stopped purge flow valve.

**Table 1. Optimized Experimental Conditions for the Purge and Cold Trapping Injector**

| | temperature (°C) | | |
|---|---|---|---|
| | precool (1 min) | purge (5 min) | injection (5 min) |
| oven compartment | | 50 | |
| condenser | 0 | 0 | 0 |
| capillary trap | −120 | −120 | −120 |

**Purge and Cold Trap Injector Injection System.** The purge and cold trap injector (PCTI) injection unit was supplied by Chrompack (Middelburg, The Netherlands), and it is shown schematically in Figure 1. The home-made modifications introduced on the commercial device (García-Martin, 1993) are pointed out in the scheme. These modifications allow the purge process at subambient (10−15 °C) temperature and increased repeatability of the purge process operating parameters by means of the introduction of a cooling device and a shut-off programmable purge gas valve not provided in the original commercial device. Main parts in this figure are referred to by these names in the following paragraphs. The operating conditions of the PCTI/GC system were the following: sample, 8 mL in a 10 mL nominal volume vial kept at 30 °C; nitrogen flows, purge = 10 mL/min and cleanup = 20 mL/min. Other operating conditions are described in Table 1.

**Gas Chromatography.** A Hewlett-Packard model 5890 Series II gas chromatograph equipped with a flame ionization detector (hydrogen, 40 mL/min; air, 400 mL/min) was used in the study. All the experiments were carried out using a BP20 [poly(ethylene glycol)] Scientific Glass Engineering (SGE, Ringwood Victoria, Australia) 30 m × 0.22 mm i.d. capillary column with a phase thickness of 0.25 μm. The chromatographic conditions were 5 min at 40 °C and a ramp of 5 °C/min up to 150 °C, maintaining this temperature for 3 min. The carrier gas (nitrogen; Carburos Metálicos, Spain) flow rate was 1.04 mL/min. Data acquisition and reprocessing were carried out by means of a Waters 840/SIM data station running waters expert software (Millipore Co., Milford, MA).

**Gas Chromatography−Mass Spectrometry.** A Varian (Walnut Creek, CA) Saturn 3 gas chromatograph−ion trap detector (ITD) operating at EI mode (70 eV) was used to identify the extracted compounds. The gas chromatographic operating conditions were the same as described in the previous paragraph. Helium (Carburos Metalicos, Spain) was used as carrier gas. The ITD processes were controlled by the automatic gain control (AGC) scan function. Some parameters were manually tuned to achieve the optimal conditions of sensitivity: electron multiplier voltage was set to 200 mV over the autotuned actual value; filament current, 80 μA; target, 65 000; ionization time, 25 ms; manifold temperature, 170 °C. Data acquisition was carried out on a Compaq 486/50 microcomputer equipped with the Saturn 4 Software and the NIST90 spectral library.

## RESULTS AND DISCUSSION

The operating conditions of the PCTI GC method were previously optimized for the extraction and quantization of some of the most volatile compounds in wine (García-Martín, 1993; García-Martin et al., 1993). The volatile compounds are purged from wine and carried to be trapped into a capillary trap at −120 °C before they are injected. The technique allows the compounds in very low concentration to be preconcentrated and cryofocused into the column head. Therefore, chromatographic sensitivity and resolution are highly improved. Twenty-eight compounds of high and medium-high volatility were detected and measured by area normalization in white Galician wines. From the inspection of the chromatograms, it was apparent that some peaks remain approximately constant irrespective of the wine sample considered; 19 of the 28 compounds detected and measured were selected and considered as variables for further processing, and 18 of these 19 compounds were positively identified by GC−MS. Results, expressed as percentage of the total area in the original chromatograms, obtained for the Rias Baixas and non-Rias Baixas wines are shown in Table 2.

The multiple box−whisker plots were obtained for each variable by means of the Statgraphics V. 6.0 package (Manugistics Inc., Rockville, MD). These plots allow facile study of the characteristics of the distribu-

**Table 2. Volatile Compounds Determined by Purge and Cold Trapping Injection–GC in White Wines from Rias Baixas DOC[a]**

| | | Rias Baixas wines (14 samples) | | | | non-Rias Baixas wines (8 samples) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | compound | mean | min | max | STD | mean | min | max | STD |
| 1 | acetaldehyde | 1.04 | 0.16 | 3.08 | 0.86 | 5.17 | 1.31 | 11.2 | 4.25 |
| 2 | ethyl formate | 0.22 | 0.05 | 0.51 | 0.11 | 0.22 | 0.04 | 0.58 | 0.18 |
| 3 | ethyl acetate | 50 | 41 | 62 | 5.45 | 54 | 46 | 67 | 7.13 |
| 4 | methanol | 2.14 | 1.24 | 3.13 | 0.56 | 1.52 | 1.06 | 2.14 | 0.39 |
| 5 | 3-methylbutanal | 0.22 | 0.11 | 0.48 | 0.10 | 0.07 | 0 | 0.17 | 0.06 |
| 6 | ethyl isobutyrate | 0.37 | 0.04 | 1.89 | 0.49 | 0.26 | 0 | 1.41 | 0.47 |
| 7 | ethyl butyrate | 1.31 | 0.62 | 1.56 | 0.24 | 0.66 | 0.21 | 1.29 | 0.38 |
| 8 | ethyl isovalerate | 0.74 | 0.36 | 2.12 | 0.50 | 1.41 | 0.09 | 4.10 | 1.31 |
| 9 | unknown | 0.06 | 0 | 0.14 | 0.04 | 0.28 | 0 | 0.89 | 0.29 |
| 10 | isobutanol | 2.13 | 0.44 | 5.26 | 1.13 | 3.05 | 1.01 | 7.12 | 2.11 |
| 11 | isoamyl acetate | 13.7 | 2.95 | 28 | 5.94 | 5.30 | 1.57 | 9.82 | 2.84 |
| 12 | ethyl 2-butenoate | 0.06 | 0 | 0.09 | 0.02 | 0.05 | 0 | 0.09 | 0.04 |
| 13 | isoamyl alcohols | 2.45 | 1.55 | 4.05 | 0.64 | 3.90 | 1.33 | 6.98 | 1.81 |
| 14 | ethyl hexanoate | 11.7 | 9.39 | 15.5 | 1.85 | 11.6 | 4.75 | 20 | 5.01 |
| 15 | hexyl acetate | 1.02 | 0.23 | 2.32 | 0.52 | 0.56 | 0 | 1.19 | 0.46 |
| 16 | hexanol | 0.13 | 0.05 | 0.23 | 0.05 | 0.11 | 0.06 | 0.20 | 0.05 |
| 17 | ethyl octanoate | 0.77 | 0.47 | 1.17 | 0.20 | 0.28 | 0.09 | 0.51 | 0.18 |
| 18 | furfural | 0.70 | 0.37 | 1.19 | 0.24 | 1.87 | 0.23 | 6.09 | 2.06 |
| 19 | acetic acid | 0.17 | 0.09 | 0.23 | 0.04 | 0.11 | 0 | 0.26 | 0.08 |

[a] All results are in percentage of total area.

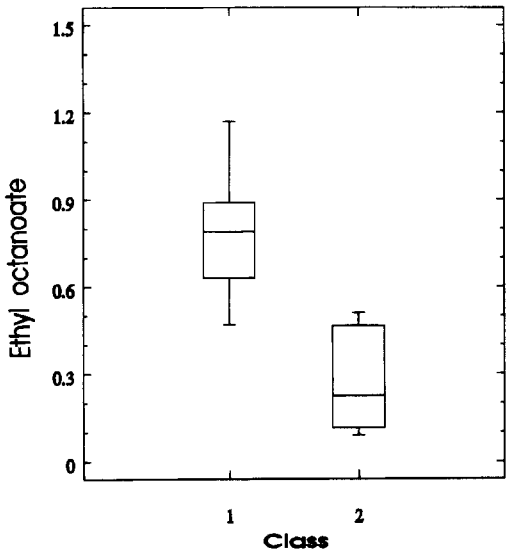


**Figure 2.** Box–whisker plot of Galician white wines by their content in ethyl octanoate: class 1, Rias Baixas wines with certified brand of origin; class 2, non-Rias Baixas wines.

tions of the variables. When wines are divided into classes, the separation between these classes based upon one single variable may also be possible. Except for methanol, 3-methylbutanal, and ethyl butyrate, the non-Rias Baixas samples (class 2) were observed to show greater dispersion than the Rias Baixas samples (class 1). The heterogeneous origin of non-Rias Baixas sample wines could explain these results. However, a good separation between classes was not observed with any of the variables. Best results would be obtained with ethyl octanoate (Figure 2).

Pirouette V 1.0 package (Infometrix Inc., Seattle, WA) was used in further data treatment. The first multivariate approach to the data was made by cluster analysis. Data were autoscaled (mean, 0; variance, 1). Using the flexible method of linkage (PIROUETTE, 1994) three clusters of objects could be observed within 40% of the similarity scale (Figure 3A). The first cluster was formed by all Rias Baixas wines excluding sample A06 and two non-Rias Baixas wines (samples B01 and B02). The other samples of non-Rias Baixas wines were distributed in the two remaining clusters, one of them

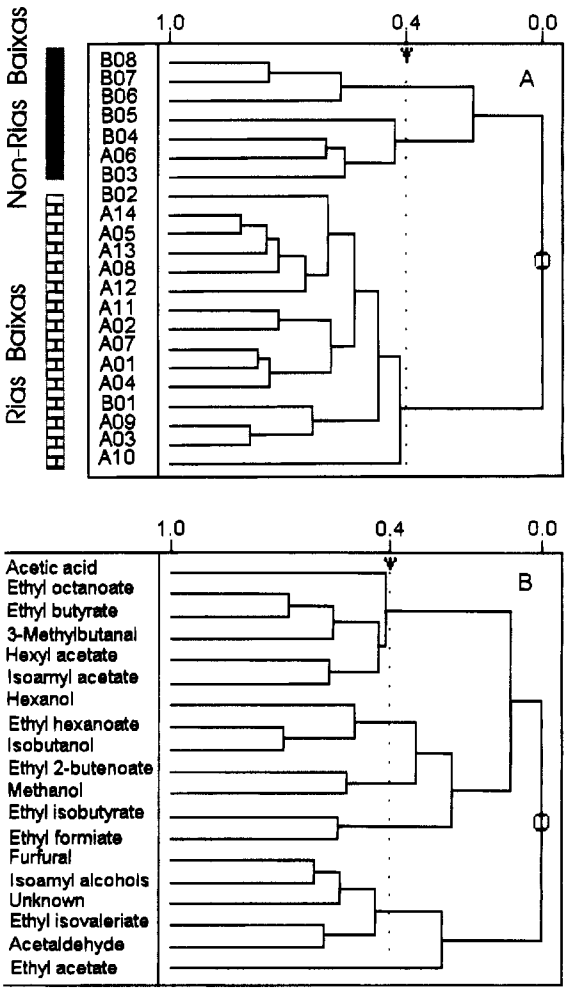


**Figure 3.** Dendrogram of cluster analysis of wines of Rias Baixas origin (samples AX) and non-Rias Baixas origin (samples BX): A, clustering of samples; B, clustering of variables.

with the Ribeiro commercial wines (samples B06–B08) and the second one, more heterogeneous, with samples B03–B05 and the Rias Baixas A06. At the same conditions of analysis, six clusters of variables were observed (Figure 3B).

By principal components analysis, with four factors

**Table 3. Loadings of the First Three Principal Components**

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| acetaldehyde | −0.2924 | −0.0832 | −0.3189 |
| ethyl formate | 0.1362 | 0.2128 | −0.3163 |
| ethyl acetate | −0.0894 | −0.1227 | 0.4112 |
| methanol | 0.1868 | 0.1374 | 0.1885 |
| 3-methylbutanal | 0.3170 | −0.1022 | −0.0992 |
| ethyl isobutyrate | 0.0969 | 0.1389 | −0.4876 |
| ethyl butyrate | 0.3404 | 0.0507 | 0.0733 |
| ethyl isovalerate | −0.2602 | −0.1985 | −0.0171 |
| unknown | −0.3119 | −0.0208 | 0.1240 |
| isobutanol | −0.1736 | 0.4183 | 0.1025 |
| isoamyl acetate | 0.2828 | −0.1602 | −0.1727 |
| ethyl 2-butenoate | 0.1143 | 0.3508 | 0.2099 |
| isoamyl alcohols | −0.2956 | 0.1224 | 0.0247 |
| ethyl hexanoate | −0.0925 | 0.4187 | 0.0717 |
| hexyl acetate | 0.2216 | −0.2174 | 0.0393 |
| hexanol | 0.0036 | 0.3416 | 0.2068 |
| ethyl octanoate | 0.3284 | 0.0438 | 0.2617 |
| furfural | −0.2761 | −0.2542 | 0.1176 |
| acetic acid | 0.1002 | −0.3117 | 0.3290 |

70% of the initial variance was retained (PC1, 32%; PC2, 19%; PC3, 11%; PC4, 8%). The contribution of the variables to the first three components is shown in Table 3. Ethyl butyrate, ethyl octanoate, 3-methylbutanal, and isoamyl acetate were the variables that contributed mostly to the positive first axis. The negative part of this axis was mostly influenced by an unidentified compound, the sum of the isoamyl alcohols, acetaldehyde, furfural, and ethyl isovaleriate. The second component is mainly built up with the contribution of isobutanol, ethyl hexanoate, ethyl 2-butenoate, and hexanol for the positive axis and acetic acid for the negative axis. The variables which influence most of the construction of the third component were ethyl isobutyrate and ethyl acetate.

Some of these compounds (ethyl octanoate, isoamyl acetate, and hexyl acetate) are usually recognized as contributing to the good quality of wine aroma. On the contrary, some others like isoamyl alcohols, acetaldehyde, and ethyl 3-methylbutyrate when present in high amounts impair negative features to wine flavor. Despite quantitative differences among samples, the Rias Baixas wines were characterized by their higher content in some of the esters responsible for the good aroma quality and, especially, by their low content in those characterizing poor aroma quality and off flavors. Therefore, the positive and negative axes of the two first-principal components could be associated with the quality of wine aroma.

In the space formed by the three first components (62% variance accounted) three groups of samples could be observed (Figure 4). The first axis ($x$) was highly responsible for the separation between the Rias Baixas samples (positive axis) and the non-Rias Baixas samples (negative axis). Sample B01 (which in fact is a vs Albariño wine although produced out of the delimited DOC area) and sample B02 were both included among the Rias Baixas wines as was also observed in the cluster analysis. The second axis ($y$) allowed to differentiate two groups of non-Rias Baixas wines, also confirming the results obtained by cluster analysis. Among the Rias Baixas wines, sample A06 was pointed out as an outlier by the third component ($z$ axis). This behavior may be explained by its low content in isoamyl acetate and hexyl acetate. Sample A06 was also characterized by its high content in isobutanol and hexanol. Therefore, by nonsupervised techniques of multivariate analysis, it was possible to establish differences between

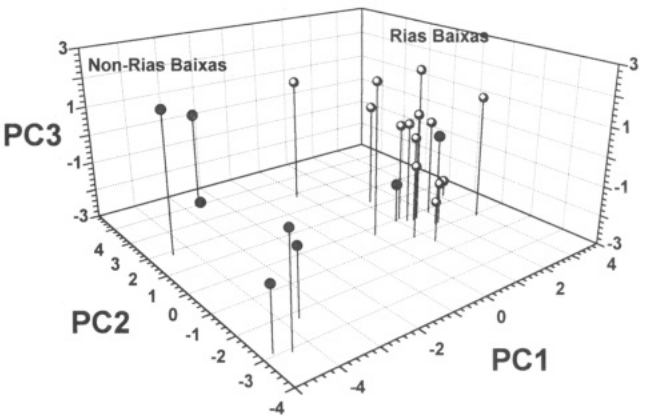


**Figure 4.** Principal components projection of Galician wine samples (62% variance accounted): Rias Baixas samples (A*X*) and non-Rias Baixas samples (B*X*).

Rias Baixas and non-Rias Baixas wines with respect to the volatile compounds extracted and concentrated by purge and cold trapping. The presence of some outliers was also pointed out.

Results were confirmed by the KNN classification technique. The minimum number of misclassified samples was obtained by using $K = 4$. In these conditions, samples B01, B02, and B04 were included in class 1. The misclassification of samples B01 and B02 has to be expected from the previous results. On the other hand, sample B04 appearing in fact as an outlier was assigned by the KNN used algorithm to class 1.

## CONCLUSION

The PCTI injection technique lead to the extraction and enrichment of the most volatile components of Galician white wine samples. Some of these compounds were positively identified by coupling an ion trap mass spectrometer tuned for the optimal conditions of sensitivity. The quantified compounds were the parameters used for the multivariate characterization of Rias Baixas certified wines. Differentiation of these wines from other Galician wines was possible, although two samples of non-Rias Baixas wines were considered as Rias Baixas. This behavior could be due to the predominance of the varietal character of the wines over the geographical origin.

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