RELEVANCY OF SPECIATION ANALYSIS FOR GEOGRAPHICAL ORIGIN DISCRIMINATION OF RED WINES

Véronique Vacchina[1], *Bernard Médina* [2], *Olivier Donard* [3], *Fabienne Séby* [1]

[1] UT2A, Pau, France- veronique.vacchina@univ-pau.fr
[2] Société des Experts Chimiques de France, Paris, France- bernard.pi.medina@gmail.com
[3] IPREM, Pau, France- olivier.donard@univ-pau.fr

Abstract–Wine traceability has become of primary importance for consumers as well as for producers. Indeed, because of its high market price, it's a product highly subject to fraud. Traceability analysis are, among others, performed by trace element analysis. Indeed, their content may change depending on the soils on which the vine has grown and also as a function of the agricultural practices. But in addition, these different origins may also impact these trace elements speciation. Therefore, the objective of our work was to check if the speciation analysis could be more discriminant than the single total element content for geographical discrimination purpose. In a first step, the development and the validation of the speciation methods of different elements (As, Se,…) in red wine will be described. Then, the application of these methods to the analysis of red wines from different geographical origin will be presented. Finally, the statistical treatment of these data will allow to the conclusion about the relevancy of using speciation analysis to discriminate the geographical origin of red wines.

Keywords:*geographical authentication, speciation, red wines*

1. INTRODUCTION

Authentication of the geographical origin of wine is of primary importance for both consumers and producers. Indeed, because of its high market price, wine is very attractive to fraud. It is therefore one of the most studied food commodities for authentication [1]. This kind of analysis is generally performed by microbial fingerprinting, sensory analysis, isotopic ratios determination, untargeted analysis and trace elements determination.

Regarding these trace elements, their presence can be endogenous or exogenous [2]. The endogenous ones are related to the type of soil the vine has grown on whereas the exogenous ones are associated with the external contamination such as the use of fertilizers or with the wine-making process. These different sources of elements may lead to differences in their speciation in the wine as already suggested for As [3]. However the potential relation between speciation and geographical origin has never been addressed. It was the objective of this study. The paper describes therefore the contribution of As, Se, Sb and Sn speciation for the geographical authentication of red wines.

2. EXPERIMENTAL

2.1. Samples

Samples of red wines were collected worldwide with provenance information. Briefly: France (n = 37, including 19 Bordeaux wines), United States (n = 26, including 15 Californian wines), Chili (n = 1), Spain (n = 1), Greece (n = 1), Slovenia (n = 4), South Africa (n = 1) and China (n = 15).

2.2. Total element determination

Samples were digested by HNO_3 69% on a hot plate (DigiPrep, SCP Sciences). The As, Se, Sb and Sn concentrations were determined by ICP MS (7500cx, Agilent) by the method of the standard addition.

2.3. As, Se and Sb speciation by HPLC – ICP MS

The HPLC separation was performed using a Model 1100 HPLC pump (Agilent).

The exit of the column (Hamilton PRP-X100) was directly connected to the ICP MS (7500cx, Agilent). For As and Sb speciation, the red wines were diluted two times by ultrapure water. For Se speciation, the red wines were subjected to proteolytic digestion. In both cases, the extracts were filtered at 0.45 µm prior to injection. For As speciation, elution was performed by a gradient from 20 to 50 mM ammonium carbonate, for Se speciation by 5 mM ammonium citrate (pH 4.9) and for Sb speciation by a gradient from 20 mM EDTA/2 mM KHP to 50 mM phosphate. The flow rate and the volume injected were set at 1 mL/min and 100 µL, respectively.

2.4. Sn speciation by GC – ICP MS

The GC separation was performed using a Trace GC Ultra GC (Thermo Fisher) coupled to a X Series 2 ICP MS (Thermo Fisher) using a 95%methyl 5%phenyl-polysiloxane capillary column. 3 mL of red wines were buffered at pH 4.5. Isooctane and $NaBEt_4$ were then added and the solution was vigorously shacked. The organic layer was recovered and analysed. The temperature gradient ranged from 80 to 280°C.

3. RESULTS AND DISCUSSION

3.1. Analytical strategy

The choice of the elements to be speciated was first of all purely analytical. Elements for which speciation methods, and not fractionation methods, existed (even if not already applied in wine) were selected. Among them, the elements for which one single species could be measured or was expected to be present were discarded. The elements selected for potential further speciation analysis were therefore: As, Se, Sb, Sn, Pb and Hg. The total content of these six elements was then compared to the sensitivity of the speciation method to evaluate the relevancy of performing the speciation analysis on the whole set of samples. The results presented here only deal with As, Se, Sb and Sn as **Pb and Hg are still in progress analysis.**

3.2. Total element content

The results obtained are shown Table 1. Concentrations of a few µg/L were obtained for As and Se. For Sb and Sn, even if concentrations up to several dozens of µg/L were obtained, in a significant number of samples the elements could not be quantified.

Table 1. Total element concentration range

	As	Se	Sb	Sn
concentration range (µg/L)	0.33 - 55.7	0.49 - 8.07	< 0.1 – 15.8	< 0.1 – 37.4

3.3. As speciation

As is usually present in wine as a consequence if the use of As-containing herbicides or pesticides [4] or because the vine has grown on a soil naturally containing As and has accumulated it [5].Therefore the expected As species in wine could be As(III), As(V), MMA and DMA. These species could be measured by HPLC – ICP MS. Sensitivity of the speciation method was evaluated at the sub µg/L level for each species which made it relevant for authentication purpose. Inorganic As were the most abundant forms found, accounting for 32 – 100% of the total As depending on the samples. The remaining As was present in the form of methylated species (mainly DMA but also MMA from time to time). A typical chromatogram is shown Fig. 1.

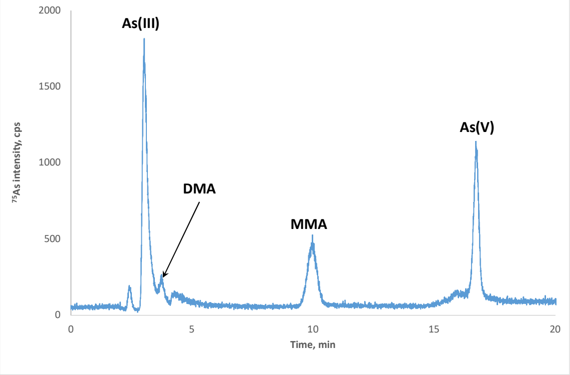

Fig. 1. Typical HPLC – ICP MS chromatogram obtained for As speciation in a red wine

3.4. Sb speciation

Sb speciation had never been addressed in wines but methods have already been published for Sb(III), Sb(V) and TMSb speciation [6].

When applying the HPLC – ICP MS method to the red wines, sensitivity was estimated in the range of the µg/L whereas the three quarters of the wines had a total Sb concentration lower than this value. Sb speciation for authentication purpose was therefore not investigated furthermore.

3.5. Se speciation

Se is present in the wine if the vine has grown on a Se-containing soil. However, during the vinification process, yeasts are used [7]. They may metabolize inorganic Se into SeMet. Both inorganic Se and SeMet were therefore investigated. The Se species could be measured by HPLC – ICP MS after proteolytic digestion. Sensitivity of the speciation method was at the sub µg/L level which again made it relevant for authentication purpose. Se(VI) and SeMet were found in all the samples investigated. A typical chromatogram is shown Fig. 2.

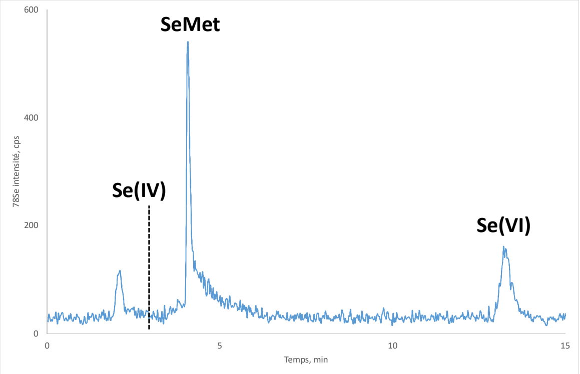

Fig. 2. Typical HPLC – ICP MS chromatogram obtained for Se speciation in a red wine

3.6. Sn speciation

Sn in wine can come from the soils the vine has grown on but also from the use of TBT-containing pesticides [8]. However if organotin compounds largely originate from anthropogenic activities, chemical and biochemical methylation reaction can convert inorganic tin compounds into methyltin forms [9]. Therefore the expected Sn species could be Sn^{4+}, the butyltin and the methyltin species. These species could be measured by GC – ICP MS.

However sensitivity for the Sn^{4+} detection was not low enough (1 µg/L) and co-elution of the TMT with the isooactane made these species unusable for authentication purpose. The other organic forms were. A typical chromatogram is shown Fig. 3.

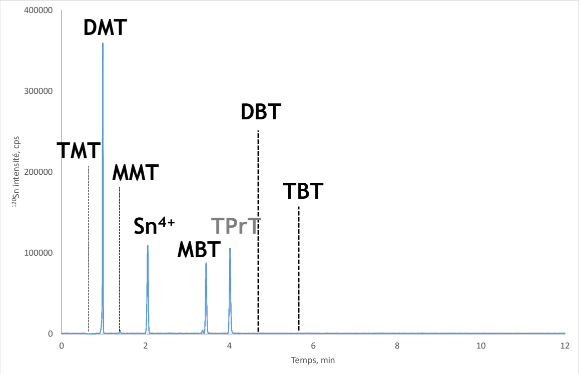

Fig. 3. Typical GC – ICP MS chromatogram obtained for Sn speciation in a red wine

Methyltin (DMT and MMT) species were found in nearly all the samples investigated and MBT was found in about half of the samples.

3.7. Validation of the speciation methods

In the absence of Certified Reference Material for trace elements speciation in wine, the validation was performed on a mixture of red wines from different origins. The figures of merit are presented Table 2. Both repeatability and intermediate precision were below 10 %. Accuracy was evaluated by checking the recovery levels at two different levels (the low one doubling the natural concentration of the species and the second one five times higher). It was in the range 86 – 107%. These figures of merit were consistent with the use of these methods for authentication purpose.

Table 2. Figures of merit of speciation methods in red wines by HPLC or GC – ICP MS for all the species investigated

	Repeat	Int. Prec.	Acc. (low)	Acc. (high)	LOD (µg/L)
As(III)	0.9%	2.3%	105%	93%	0.3
As(V)	2.7%	7.0%	103%	94%	0.2
MMA	0.5%	3.6%	107%	100%	0.3
DMA	1.4%	3.4%	102%	97%	0.1
Se(VI)	1.1%	5.2%	93%	104%	0.2
SeMet	1.1%	7.5%	97%	102%	0.1
MBT	4.9%	N/A$^{(*)}$	93%	85%	0.02
DBT	4.4%	N/A$^{(*)}$	101%	99%	0.01
TBT	6.0%	N/A$^{(*)}$	96%	93%	0.01
MMT	4.5%	N/A$^{(*)}$	97%	86%	0.01
DMT	5.1%	N/A$^{(*)}$	104%	90%	0.006

$^{(*)}$: in progress

3.8. Statistical treatment

In a first approach, the boxplots corresponding to the total elements concentration in the whole set of samples were built to check the presence of outliers. They were removed for the statistical treatment. The PCA representation obtained from the single total concentrations data, the single speciation data and the association of both is shown Fig. 4. Note that only the groups still containing more than 4 samples after the outliers removal were used for statistical relevancy. The Chinese samples, even if statistically relevant according to this criteria, were not included in the statistical treatment because too much dispersed. This might be due to the size of the China country that would require a cutting as a function of the different regions. The total concentration of the 4 elements investigated was clearly not discriminant of the geographical origin. A better discrimination could be obtained from the speciation data, but the dispersion of the Californian samples had a negative impact on it. The best discrimination could be achieved by combining both total and speciation data. Even if this discrimination was not comprehensive, the association of both parameters was clearly more discriminant than the single parameters.

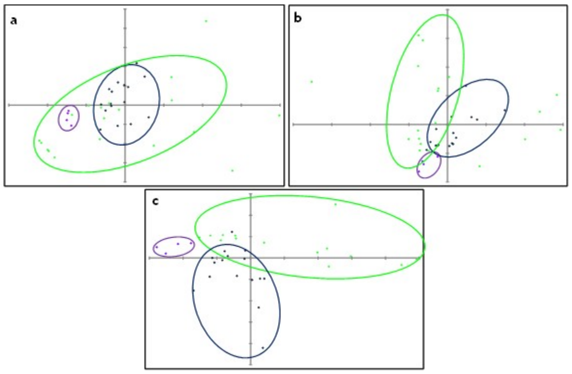

Fig. 4.PCA representation of the data. (a) from total data. (b) from speciation data. (c) from both total and speciation data. ◆ : Bordeaux. ◆ : California. ◆ : Slovenia.

4. CONCLUSIONS

The speciation methods developed and validated for the 4 elements investigated present figures of merit consistent with their use for authentication purpose. Even if only an incomplete discrimination of the samples can be achieved, the contribution of speciation data clearly improves the discrimination power. It has however to be pointed out that the study should be completed with the analysis of additional samples to improve the statistical relevancy and of other elements (Pb, Hg) to check if the model can be improved.

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