BIO Web of Conferences **3**, 02009 (2014)
DOI: 10.1051/bioconf/20140302009

Simultaneous determination of wine sugars, glycerol and organic acids $^{13}C/^{12}C$ isotopic ratio by ion chromatography-*co*-IRMS

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Abstract. Ion chromatography (IC) isotope ratio mass spectrometry (IRMS) coupling is possible using a liquid interface allowing chemical oxidation (*co*) of organic compounds. Synthetic solutions containing a mixture of glycerol, sugars (sucrose, glucose and fructose) and organic acids (gluconic, lactic, malic, tartaric, oxalic, fumaric, citric and isocitric) were elaborated to estimate analytical applications of two different IC systems. It appears that the use of carbonated solution in the eluting phase is a perturbation for ^{13}C isotope measurements as it creates a $\delta^{13}C$ deviation from the expected values. A sample of authentic sweet wine was analyzed by IC-*co*-IRMS using KOH gradient. Ratios of isotopic, ratios of organic acids and glycerol was found to be, in average, equal 1.01 ± 0.04 that is in accordance with our previous results.

1. Introduction

Stable isotope ratios are a valuable tool for authenticity studies in food product area. The main limitation of their application come from the necessity of a data bank elaborated from authentic samples. In wine area, each member state of the European Union and wine producer has to build its own data bank, on a yearly basis [1]. In order to avoid this data bank constitution step, some studies tried to determine, whether or not, $\delta^{13}C$ values of some intrinsic wines molecules could be characteristic of the matrix, acting as internal reference values. For this reason, coupling between isotope ratio mass spectrometry (IRMS) and separative techniques is wanted [2]. In wine area, two coupling with IRMS are developed, i.e., gas chromatography (GC) [3,4] and high performance liquid chromatography (HPLC) [5,6]. Coupling HPLC-IRMS is realized via a liquid interface that chemically oxidized (*co*) organic compounds. HPLC-co-IRMS allowed $\delta^{13}C$ simultaneous determination of glucose, fructose, glycerol and ethanol [6]. These results were computed in term of ratio of isotopic ratios precursors / fermentation products; This ratio appears to be constant and characteristics of an authentic wine.

It was interesting to follow this work with the study of wine organic acids $\delta^{13}C$ that cannot be analyzed using the coupling HPLC-*co*-IRMS. Therefore, the coupling ion chromatography (IC) – IRMS was used to study this compounds family [7]. Two IC systems using different separating conditions have been tested. Optimal conditions of separation and $\delta^{13}C$ measurements were applied to the analysis of a wine naturally sugared.

Table 1. System 1 gradient used for compound separation. Eluent A, water; B, NaOH-Na_2CO_3; C, Na_2CO_3.

Time (min)	Eluent A (%)	Eluent B (%)	Eluent C (%)
0.0	100	0	0
20.0	92	8	0
20.1	70	30	0
48.0	70	30	0
48.1	10	0	90
68.1	10	0	90
70.0	90	10	0
75.0	90	10	0

2. Material and method

2.1. Samples

Synthetic solutions were prepared from commercial products without any further purification. Final concentration of each compound was 0.5 gL^{-1}. Wine sample was firstly distillated to remove ethanol, diluted 10 times and filtered (0.45μ nylon filter) before analysis. 10 to 20 µL of solution were injected according to column model.

2.2. Instruments

Two ion chromatographic systems have been used: Methrom 850 Professionnel (System 1) and Dionex ICS2100 (System 2). In system 1, eluting solution was prepared using a ternary concentration gradient listed in Table 1 (water, NaOH-Na_2CO_3 (22 mM) and Na_2CO_3 (50 mM)) and an ASUP16 column. System 2 is based on the use of a KOH gradient and an IonPack AS15 column (full technical specifications in reference [7]).

Liquid interface, Liquiface© (Elementar), allows the chemical oxidation of the organic matter in carbon dioxide

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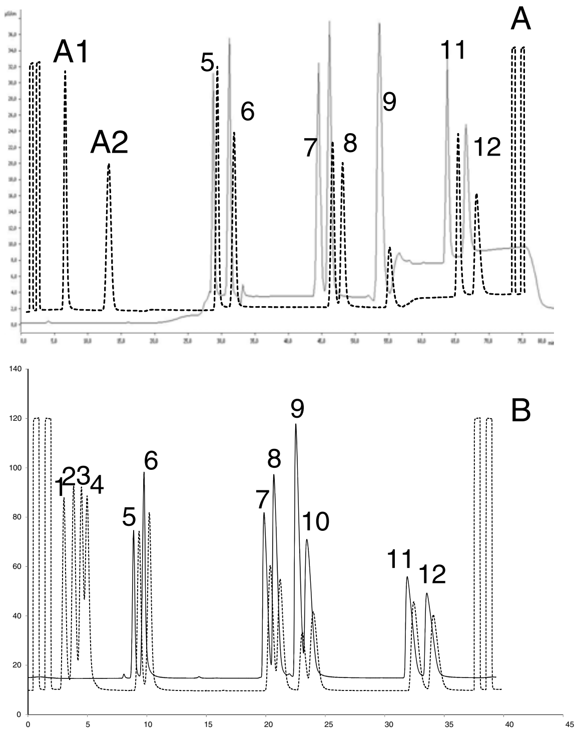

Figure 1. IC conductivity (solid line) and IC-co-IRMS $\delta^{13}C$ measurements (dashed line) obtained with system 1 (A) and system 2 (B). Composition: A1. mixture of sugars and glycerol, A2. ethanol; 1. glycerol, 2. sucrose, 3. glucose, 4. fructose, 5. gluconic, 6. lactic, 7. malic, 8. tartaric, 9. oxalic, 10. fumaric, 11. citric and 12. isocitric acid. *Fig 2.B extracted from J. Chromatography A ref. [7] with permission.*

using a mixture Persulfate/orthophosphoric acid (15% in mass / 2.5% in volume) at 93 °C. The system is coupled to IRMS (Isoprime, Elementar). The measurement of pure compound $\delta^{13}C$ value was performed using an elemental analyzer, model Vario MicroCube (Elementar). $\delta^{13}C$ values are provided against the international standard V-PDB (Vienna Pee Dee Belemnite) according to $\delta^{13}C = [(R_{sa}/R_{PDB})-1]x1000$ where R is the isotope ration $^{13}C/^{12}C$ of the sample (SA) and of the international standard (PDB).

3. Results and discussion

3.1. Optimization of chromatographic conditions

Until now, ion chromatography is used for carbohydrates or anions quantification according to the specific detection system, amperometric or conductimetric, respectively. The system liquid interface-IRMS acts as a "universal" detector for organic compounds as it allows simultaneous detection of sugars and organic acids. Two IC systems were tested using synthetic solutions based on a mixture of glycerol, 3 sugars and 8 organic acids. The chromatograms are presented in Fig. 1.

System 1 allows the detection of organic acids and ethanol but no separation between sugars and glycerol (Fig. 1A). Using system 2, organic acids and glycerol are well separated while a lower separation occurs between the three sugars (Fig 1B). Moreover, a co-elution is observed between sugars and ethanol, as a result a simultaneous analysis of these compounds is not possible in these conditions.

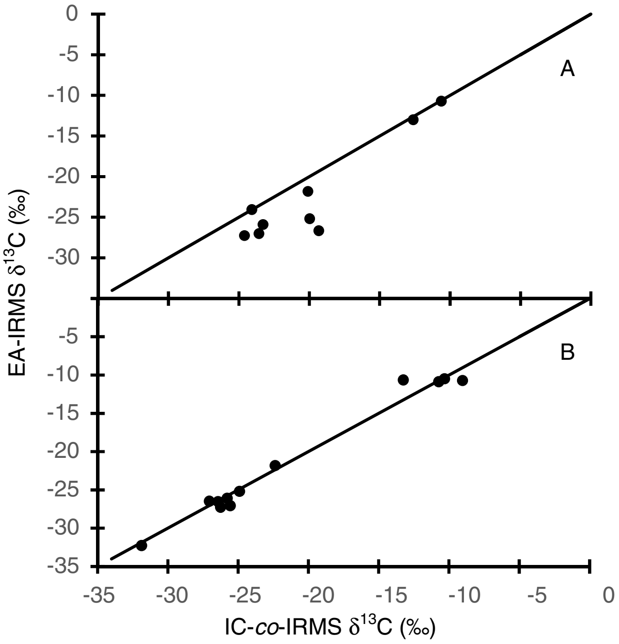

Figure 2. Correlation of EA-IRMS $\delta^{13}C$ with IC-co-IRMS $\delta^{13}C$ for all the studied compounds. (A) system 1 and (B) system 2 IC-*co*-IRMS data.

In order to estimate the accuracy of the measured $\delta^{13}C$ values, a study was realized to compare $\delta^{13}C$ of pure compounds, measured by EA-IRMS, and $\delta^{13}C$ value determined by IC-*co*-IRMS. Results are presented in Fig. 2 where some differences in compounds $\delta^{13}C$ values can be observed between the two systems: this is only the results of the use of different sample batches with different $\delta^{13}C$ values. System 2 presents an excellent correlation between the two measurements indicating that no isotopic fractionation occurs during chromatographic and chemical oxidation steps (Fig. 2B).

A deviation is observed in system 2 results (Fig. 2A). For many compounds, IC-co-IRMS $\delta^{13}C$ values are significantly different from the expected value. This deviation cannot be attributed to a technical isotope fractionation with respect to the good results obtained with system 2. Therefore, these differences have been attributed to carbonate presence in the eluent. This hypothesis seams verified with the study of the gap between expected and measured values in relation to the eluting gradient. The results presented in Fig. 3 show that the higher the eluent is concentrated in carbonate, the higher is the $\delta^{13}C$ gap. As an example, ethanol, point A2 in Fig. 3, is eluted with a low carbonate concentration; its $\delta^{13}C$ value is comparable to the expected ones. On the opposite, iso-citric acid, point 12 in Fig. 3, is eluted with an important carbonate concentration; its $\delta^{13}C$ value determine by IC-co-IRMS present a discrepancy higher than −6‰ regading the expected $\delta^{13}C$ value. Thus, despite the carbonate suppressor, it seems that a fraction of carbonate is not eliminated and is transferred to the

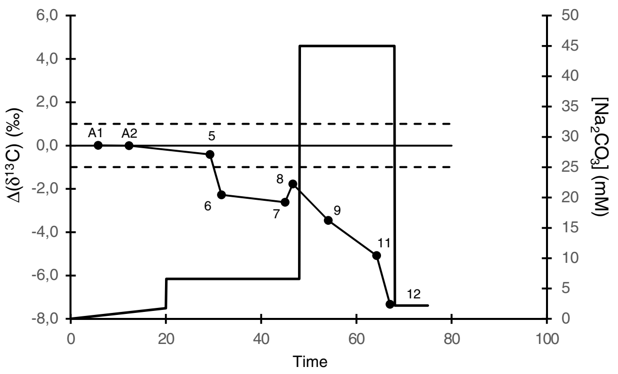

Figure 3. Evolution of δ^{13}C value deviation from the expected relative value along with carbonate gradient. Label information corresponds to the compounds described in Fig. 2. Solid line at 0‰is the expected value in the uncertainty domain (dashed lines).

interface. Oxidation reaction in the interface is performed under acidic conditions leading to a transformation of carbonate in CO_2 gas which is extracted with the CO_2 gas coming from the chemical oxidation of the organic molecules. These observations reinforce the fact that using organic compound in the eluent can impact the measured value, impact that can only be shown by an accuracy study. The second information is that for system 1, the carbonate suppressor needs to be reinforce in order to apply carbonated eluent to this coupling or it requires the use of another column type working with inorganic bases.

3.2. Application to a naturally sweet wine

System 2 was used to determine ^{13}C isotope ratio of an authentic sweet wine produced in the area of Bordeaux and used for the official European data bank [8]. The first step was a distillation to extract ethanol, then after dilution (1/10) of the residue, the solution was analyzed. The important difference in concentration between sugars and organic acid prevents from a simultaneous measurement of these compounds. Therefore, IRMS analysis sequence was optimized by setting an increase in the current trap in order to enhance smaller peaks intensity. This so-called “peak jump” is observable in Fig. 4 at around 410 s: an increase in the IRMS chromatogramm baseline is noticeable.

These conditions allow, for organic acids, a signal sufficiently intense to be in the IRMS linear domain. In Fig. 4, carbon 13 isotope ratio of glycerol (1), glucose (3) and fructose (4) are quantified in the first 6 minutes then δ^{13}C values of various organic acids, gluconic (5), lactic (6), malic (7) and tartaric (8). From these δ^{13}C values, ratio of isotope ratios have been computed ($R_{^{13}C_{A/B}} = \delta^{13}C_A/\delta^{13}C_B$). Considering the ratio of organic acids δ^{13}C and glycerol δ^{13}C, an average value of 1.01 ± 0.04 can be computed. This value, similar to the one obtained in a previous study [6], shows that the ratio of internal compound isotope ratio is characteristic of an authentic wine.

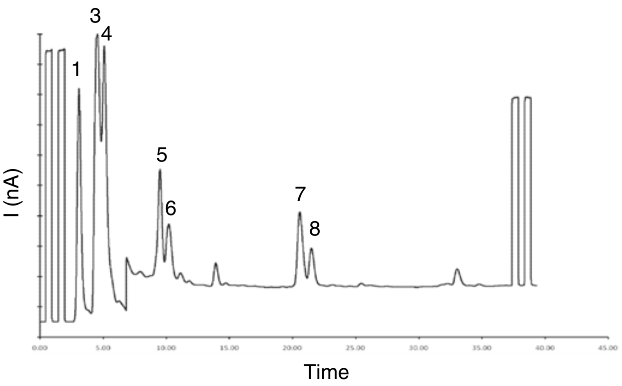

Figure 4. IC-co-IRMS chromatogram of a sweet wine. Peak identification: (1) glycerol, (3) glucose, (4) fructose, (5) gluconic, (6) lactic, (7) malic (8) tartaric acids.

4. Conclusion

This study presents some new developments in the coupling ion chromatography with IRMS. IC-*co*-IRMS allows the measurement of δ^{13}C values of glycerol, sugars and organic acids individually and in the same run. The use of organic solvents was warned to perturb isotopic measurement, the results presented in this study confirm this risk. Therefore, technical improvements need to be performed in the carbonate suppressor system if such elution conditions are mandatory. These preliminary results on sweet wine indicate that the ratio of isotope ratio can be considered as a wine authenticity parameter.

The authors would like to thank Veronique Henin and Jean-Luc Dumas (Metrohm), Audrey Brault and Nicolas Gautier (Dionex) for ion chromatography loan and Claudia Monsallier (Elementar) for helpful discussions.

References

[1] Regulation RCE 555/2000

[2] B. Medina, MH Salagoity, F. Guyon, J. Gaye, P. Hubert, F. Guillaume, in *New analytical approaches for verifying the orgine of food*. Ed. Brereton P.; Woodhead Publishing Limited, p 147 (2013)

[3] J. Jung, T. Jaufmann, U. Hener, A. Munch, M. Kreck, H. Dietrich, A. Mosandl, Eur. Food Res. Technol. **223**, 811 (2006)

[4] ME. Spitzke, C. Fauhl-Hassek, Eur. Food Res. Technol. **231**, 247 (2010)

[5] AI. Cabanero, JL. Recio, M. Rupérez, J. Agric. Food Chem. **58**, 722 (2010)

[6] F. Guyon, L. Gaillard, MH. Salagoity, B. Medina, Anal. Bioanal. Chem. **401**, 1551, (2011)

[7] F. Guyon, L. Gaillard, A. Brault, N. Gaultier, MH. Salagoity, B. Medina, J. Chromatogr. A **1332**, 62 (2013)

[8] F. Guyon, C. Douet, S. Colas, MH Salagoity, B. Medina , J. Agric. Food Chem. **54**, 9918 (2006)