Isotope Ratio Mass Spectrometric Method for the On-line Determination of Oxygen-18 in Organic Matter

J. Koziet*
Pernod-Ricard Research Centre, 120 Avenue Marechal Foch, F-94015 Creteil, France

A method for the on-line determination of oxygen-18, at a naturally occurring level, in organic material is presented. After pyrolysis of the samples to form carbon monoxide, which is performed at 1300 °C in a vitreous carbon tube, the pyrolysis products are transported by a stream of helium gas. Using an open split, a small part of the effluent is transferred to the ion source of an isotope ratio mass spectrometer. The ratio is obtained from a measurement of the ion current intensities at *m/z* 30 and 28 ($^{12}C^{18}O$ and $^{12}C^{16}O$). The method was tested with the secondary water standard GISP (Greenland Ice Sheet Precipitation) and the carbonate standard NBS 19. The values obtained were −24.8‰ and 27.3‰ *vs.* VSMOW (Vienna Standard Mean Ocean Water) (IAEA reference values are −24.8‰ and 28.7‰ *vs.* VSMOW). The potential of the method was demonstrated by measuring the ^{18}O content of samples of beet and cane sucrose and also samples of vanillin extracted from vanilla pods or of synthetic origin.

J. Mass Spectrom. **32**, 103–108 (1997)

No. of Figures: 3 No. of Tables: 4 No. of Refs: 19

KEYWORDS: oxygen-18; organic matter; biological samples; on-line determination; isotope ratio mass spectrometry

INTRODUCTION

The permanent search for new analytical criteria to establish the genuineness of natural food products, particularly in the area of aroma and fruit juices, remains a major challenge for the food industry. For this purpose, numerous analytical and statistical methods have been proposed, including the isotopic determination of the natural variability of carbon-13 and deuterium in organic material. Generally these determinations are performed by isotope ratio mass spectrometry (IRMS) or by nuclear magnetic resonance (NMR) spectrometry of deuterium in order to detect the addition of sugar to fruit juices and wines.

Although organic matter contains oxygen-18 in large amounts, it has been scarcely used for food authentication. Techniques for oxygen-18 measurement in organic molecules using pyrolysis hitherto were experimentally difficult and complex. In contrast, the determination of oxygen-18 in water is simpler and is widely used for providing the addition of water to fruit juices.[1]

It is well established that determination of $^{18}O/^{16}O$ ratios in minerals or organic material can be very useful in tracing its genesis. For example, in rain water the $^{18}O/^{16}O$ ratio depends on the condensation temperature and consequently its value is related to the climate.[2,3] In geological studies, the $^{18}O/^{16}O$ ratio of a carbonate indicates the temperature at which the carbonate was precipitated.[4] The $^{18}O/^{16}O$ ratio in cellulose allows the reconstruction of the climatic conditions present during biosynthesis in a plant.[5] Hence it is obvious that studying the $^{18}O/^{16}O$ ratio in food or food constituents can provide an interesting parameter for establishing authenticity.

Mass spectrometric methods for oxygen isotope ratio determination are traditionally based on a final step involving CO_2 gas, followed by the measurement of *m/z* 46 and 44 ions originating from $[^{12}C^{16}O^{18}O]^+$ and $[^{12}C^{16}O_2]^+$, respectively.

For water the oxygen-18 content is obtained after an exchange reaction of the water sample with CO_2 gas of known isotopic composition under known time and temperature conditions. Then the measurement of the CO_2 after equilibration allows the determination, after correction using fractionation factors, of the oxygen content of water.[6]

In carbonates, the determination of oxygen-18 is carried out by treating the sample with phosphoric acid, measuring the liberated CO_2 and applying an accepted fractionation correction.[7,8]

When applied to organic compounds, oxygen-18 determination is carried out either following the reaction of the sample with $HgCl_2$ at 360–550 °C[9] or after pyrolysis at 1000–1200 °C in a quartz or nickel furnace.[10,11] Because the latter usually results in a mixture of CO and CO_2, a second step is necessary in order to convert CO into CO_2 either by oxidation with I_2O_5 [12] or by subjecting the product gas to an electric discharge[11] or to a nickel-catalysed reaction.[13] In these cases, complete conversion of CO into CO_2 is achieved,

* Correspondence to: J. Koziet.

CCC 1076–5174/97/010103–06
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Received 2 April 1996
Accepted 21 October 1996

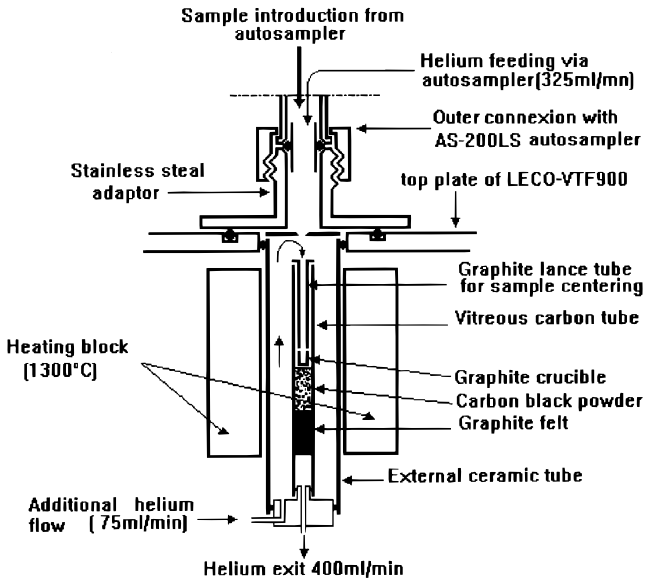

Figure 1. Schematic diagram of the pyrolysis system. External ceramic tube, length 310 mm, external diameter 35 mm; pyrolysis vitreous carbon tube, length 295 mm, internal diameter 20 mm; graphite crucible, length 25 mm, external diameter 16 mm.

according to the reaction

$$2CO \rightarrow CO_2 + C$$

The procedures described above are experimentally tedious, time consuming and require skillful manipulations. In addition, they are difficult to automate. In the case of oxidation using I_2O_5, one of the oxygen atoms in the product CO_2 is added with a different isotopic composition. For this, corrections have to be carried out.

Furthermore, when using quartz, silica (SiO_2) or ceramics (Al_2O_3) in the furnace, memory effects are introduced. These are due to exchange processes between the sample gas (CO and CO_2 formed by pyrolysis) and oxide constituting the wall of the furnace.[14]

We have succeeded in reducing these memory effects by using a vitreous carbon furnace tube. This material is virtually free of oxygen. In addition, to avoid the transformation step of CO to CO_2, we performed the isotopic measurements on CO gas by integrating the ion current signals at m/z 30 and 28 originating from $^{12}C^{18}O$ and $^{12}C^{16}O$, respectively. The same approach has been used earlier by Schmidt *et al.*[15] and Brand *et al.*[16] However, in the former case the system was applied exclusively to volatile organic compounds and in the latter only results for water samples were reported. Finally, in order to reduce considerably the time of each analysis, we applied an on-line technique using helium as a carrier for transporting the sample CO into a Finnigan MAT Delta S mass spectrometer.

EXPERIMENTAL

Reagents and vessel

The following were used: silver capsules for liquids (2 × 5 mm, 75–80 mg; Leco, ref. D9999); silver capsules for solids (6 × 4 mm, 30 mg; Leco, ref. D2006); high-purity helium, 99.995% (Carboxyque-France), as the carrier gas for the pyrolysis system; graphite felt square (Leco, ref. 502–228); carbon black powder (Leco, ref. 502–196); and a graphite crucible (Leco, ref. 502–230–110). The crucible was changed every 100 samples.

International isotopic standards

The standards used were VSMOW water (Vienna Standard Mean Ocean Water), SLAP water (Standard Light Antartic Precipitation), GISP water (Greenland Ice Sheet Precipitation) and NBS 19 calcium carbonate, white marble. These standards are available at the Analytical Quality Control Services (AQCS) of the International Atomic Energy Agency (IAEA), Vienna, Austria.

Apparatus and procedure

Prior to analysis, the samples are packed in either of two types of silver capsules used for solid or liquid samples. Solid samples are simply wrapped tightly into the preformed capsule. For liquids the capsule must be crimped carefully: the top of the silver capsule is sealed just above the liquid using a pair of cutting pliers. Thus, introduction of air during sample handling is minimized. In general, sample handling procedures must be carried out very carefully to avoid contamination of the samples and the silver capsules. The latter may be oxidized and must be clean and preferably conditioned by heating at 350–400 °C for 1 h in a quartz tube using a flow of dry air to decompose the silver oxide. Then the silver capsules are kept under an inert atmosphere, e.g. argon, in tightly closed vials or inside an appropriate desiccator.

The pyrolysis unit used (Fig. 1) is a commercially available pyrolysis system (Leco VTF900), fitted with a Carlo Erba AS-200LS autosampler using a laboratory-made stainless-steel adaptor. The vitreous carbon

pyrolysis tube is continuously flushed with helium (75 ml min^{-1}) on the outside to protect it from oxidation. A larger stream of helium (about 325 ml min^{-1}) is added through the autosampler. Thus, the total helium flow transporting the pyrolysis gas through the furnace is about 400 ml min^{-1}.

After packing, the samples are placed into the autosampler of the pyrolysis system. Prior to the pyrolysis reaction, each sample in the autosampler is purged with helium to eliminate all traces of water, oxygen or nitrogen. The reaction is started by dropping the samples into a graphite crucible which is positioned on a layer of carbon black powder (2.25 g) retained by a graphite felt plug. The crucible is positioned in the middle of the furnace. The pyrolysis takes place at 1300 °C. The gaseous pyrolysis products are carried to a gas chromatographic (GC) column (Fisons, molecular sieve 5A, 1.5 m × 1/4 in i.d.) for separation of the pyrolysis gases (H_2, N_2, CO). By using an open split arrangement, about 0.1% of the product gases are admitted to the Finnigan MAT Delta S mass spectrometer for isotopic measurement.

RESULTS AND DISCUSSION

Determination of the efficiency of the system

It is very difficult in an on-line system to determine directly the yield of the pyrolysis. However, because of the different isopic effects in the formation of CO_2 and CO during pyrolysis of organic matter, it is mportant to ensure total conversion of CO_2 into CO. This was studied by monitoring the ion current intensity at m/z 44 (I_{44}) following pyrolysis of a constant quantity of beet sucrose (2 mg) at various temperatures. The corresponding values reported in Table 1 show that at 1300 °C there is no CO_2 left.

Table 1. Formation of CO_2 according to the temperature during pyrolysis of a fixed quantity of beet sucrose (~2 mg)

Pyrolysis temperature (°C)	Ion current intensity at m/z 44 (I_{44}) (pA)
1000	258
1100	60
1200	12
1300	0

Complementary tests performed under identical conditions showed that replacing the powdered carbon by nickelized carbon allowed the pyrolysis temperature to be lowered to 1100 °C. However, the isotopic results were less accurate, possibly owing to memory effects and/or incomplete pyrolysis.

Figure 2(B) displays the chromatogram observed when recording the ion current intensities at m/z 28 during pyrolysis of anacetanilide sample. It demonstrates a good separation of the carbon monoxide and nitrogen peaks. This separation is essential for accurate isotope ratio determination. Isobaric interference from $[^{14}N_2]^+$ at m/z 28 would destroy the isotopic information on $[CO]^+$. Furthermore, Fig. 2(B) demonstrates the possibilityb of measuring both the oxygen and the nitrogen isotope ratios by simultaneously monitoring the ion currents at m/z 28, 29 and 30 and calculation of the $[^{12}C\,^{18}O]/[^{12}C\,^{16}O]$ and $[^{14}N\,^{15}N]/[^{14}N_2]$ ratios during elution of the respective representative peaks.

Calculation procedure

Figure 2 represents simultaneous recordings of the m/z 28 ion current and the instantaneous m/z 30/28 ratio

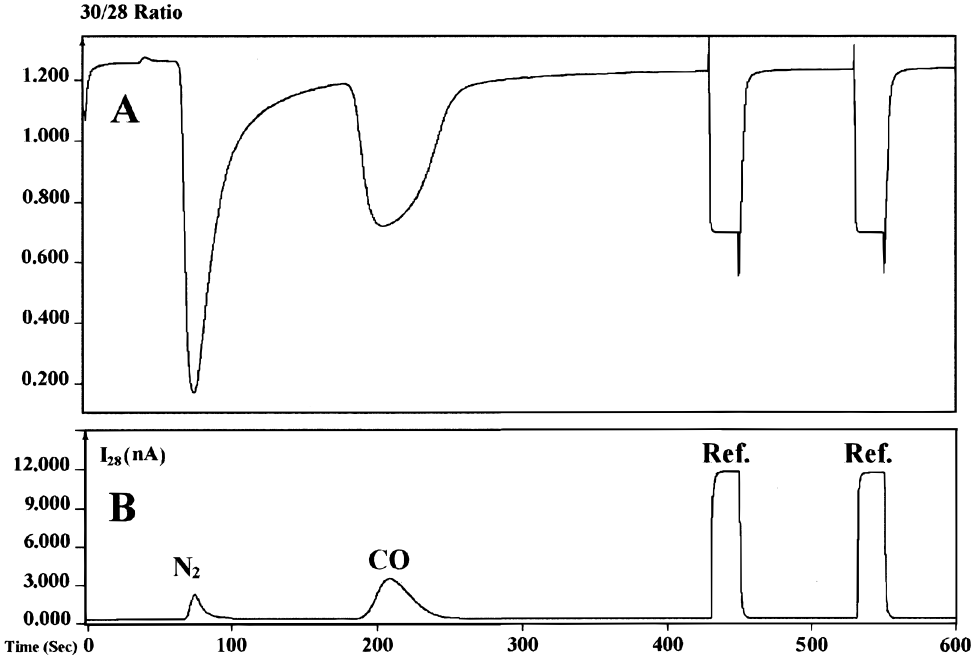

Figure 2. Chromatogram of pyrolysis products from a sample of acetanilide. (A) Ratio trace of the m/z 30/28 ion currents; (B) ion current intensity at m/z 28. Ref.: introduction of reference (CO gas).

obtained by pyrolysis of an acetanilide sample. As a result of isotopic separation of the GC column, the ratio changes along the CO peak. Therefore, it is necessary to integrate the intensities of each of the channels at m/z 28 and 30 separately prior to calculation.

At the end of the chromatogram two peaks are found that correspond to the injection of CO gas from the bellows inlet system. They provide the reference for the measurement. The mass spectrometer software calculates their isotope ratio and the final result for the sample in the δ notation, given in ‰ deviation from the reference:

$$\delta_e = \left(\frac{R_{\text{sample}}}{R_{\text{Reference}}} - 1\right) \times 1000$$

In practice, a series of standardization runs are performed to calibrate the reference CO gas, which will subsequently be used as a standard during the measurement. The calibration is done by comparing the reference CO gas with the international standard VSMOW. Using the ratio determined from VSMOW as the reference, the δ measured fo the CO reference gas is converted to a VSMOW scale value. For subsequent runs we use this value for calculation of the results expressed directly *vs.* VSMOW, according to the equation

$$\delta_{e/\text{VSMOW}} = \delta_e + \delta_{\text{Ref}} + \frac{\delta_e \times \delta_{\text{Ref}}}{1000}$$

where $\delta_{e/\text{VSMOW}}$ is the $^{18}O/^{16}O$ ratio in the delta notation using VSMOW as the reference (assigned value = 0.0‰).

Accuracy and reproducibility of the system: Applications

To evaluate the overall memory effect of the system, a series of five successive runs were carried out with samples of GISP water immediately after a series of five runs with VSMOW water. The results are given in Table 2 and show that the memory effect is limited to the first new water sample.

Other tests have demonstrated that, in case of cane sugar, with sample amounts between 2 and 14 mg, no significant effect on the measured oxygen-18 values is observed in this range. Furthermore, similar attempts conducted on compounds with different oxygen contents (water, vanillin) showed that an adequate sample amount is between 2 and 3 mg. This sample amount was adopted for the overall results presented here.

Each determination included five successive runs. As a result of the observed memory effect, the result of the first run was always discarded and the following four were used for calculating the final mean vlue for the sample.

Table 2. Results obtained from a series of GISP water samples analysed immediately after a series of five runs of VSMOW water analysis

Run No.	Determined value ($\delta^{18}O$‰ VSMOW)
1	−23.3
2	−24.5
3	−24.5
4	−24.9
5	−24.7

Five compounds with accurately known oxygen isotopic compositions were used for testing the reproducibility of the method (Table 3): VSMOW water, which is utilized for calibration purposes and which does not appear in Table 3; GISP water; laboratory water, its oxygen isotope ratio having been established previously in-house using the conventional method of CO_2–H_2O equilibration; standard calcium carbonate NBS 19; and laboratory calcium carbonate, the oxygen-18 content of which was established previously in-house using the conventional H_3PO_4 method.

Between each determination, which included five successive runs, a sample of hexadecane was pyrolysed to clean out the system. This procedure allows one to check the system and to verify the absence of external oxygen, which would show up as a CO peak. In general, in this case a small CO peak is observed (area ~1.5 nAs), which is neglegible compared with the CO peak of 750–900 nAs obtained by the pyrolysis of 2 mg of sucrose.

For water samples we can observe in Table 3 that the results are accurate within calculated experimental standard error when compared with the accepted value. The precision obtained is acceptable for most of our studies in organic matter. The carbonates exhibit good precision but the results determined by pyrolysis differ significantly from the accepted values. It must be borne in mind that the accepted carbonate values come from the transitional reaction with phosphoric acid at 25 °C involving a fractionation at equilibrium of more than 10‰.[17] The pyrolysis monitors only one or two of the three carbonate oxygen atoms. Thus, an unknown (non-equilibrium) fractionation factor will be involved in the reaction. Hence direct comparison of the values cannot be made before validation of the fractionation laws involved in pyrolysis.

Table 3. Precision and accuracy of results obtained from compounds of accurately known ^{18}O content (δ‰ VSMOW)

Sample	Measured values	Mean ± σ	Accepted value
GISP	−24.6, −24.6, −25.0, −24.8	−24.8 ± 0.2	−24.8
Lab. water	−7.9, −7.6, −7.7, −7.4	−7.6 ± 0.2	−7.5
NBS 19	27.0, 27.2, 27.4, 27.6	27.3 ± 0.2	28.7
Lab. carbonate	12.1, 12.3, 12.2, 12.2	12.2 ± 0.1	11.3

Table 4. Precision of results obtained from samples of cane and beet sugars and of vanillins extracted from vanillas from various origins and comparison with literature values

Sample	Measured values ($\delta^{18}O$‰ VSMOW)	Mean ± σ
Beet sugar	30.1, 30.0, 30.2, 30.6	30.2 ± 0.3
Cane sugar	34.1, 33.9, 34.3, 34.5	34.2 ± 0.3
Vanillin (Réunion)	9.3, 8.5, 8.6, 8.3	8.7 ± 0.4
Vanillin (Comores)	8.4, 8.3, 8.4, 8.1	8.3 ± 0.1
Vanillin (Madagascar)	10.7, 10.6, 10.4	10.6 ± 0.2
Vanillin (Lignine)	6.4, 6.8, 6.7, 6.1	6.5 ± 0.3
Vanillin (Gaïacol)	−2.7, −2.5, −3.1, −2.7	−2.8 ± 0.3
IAEA-C3 (Cellulose)	31.0, 30.6, 31.9, 32.0	31.4 ± 0.7
Values cited in literature:		
Beet sugars[13]		25–35
Natural vanillins[19]		11.8 and 12.5
IAEA-C3 cellulose[18]		31.3–32.7

At present, there is no established international standard available for oxygen isotopes in organic compounds. The IAEA proposes two reference materials for intercomparison purposes, one, IAEA-C3 cellulose,[18] was measured in this work and the second is IAEA-CH6 sucrose, previously referenced as ANU sucrose. However, accurate values of the oxygen-18 content of these two materials have not yet been sufficiently validated. However, we find our results satisfactory enough to apply this method to the determination of oxygen-18 in organic matter. Table 4 shows the results obtained on beet and cane sugars and vanillins of various origins. These results confirm the precision in the case of organic matter.

The values obtained are close to those cited in the literature. For instance, Donner *et al.*[13] measured beet sugars using the $HgCl_2$ method. Their values are in good agreement with our determinations. For vanillin, Brenninkmeijer and Mook[19] using a nickel furnace reported results similar to ours.

Furthermore, to check the long-term reproducibility, a sample of beet sugar was measured repeatedly over a period of 10 months. The results are presented in Fig. 3 and satisfy our application needs.

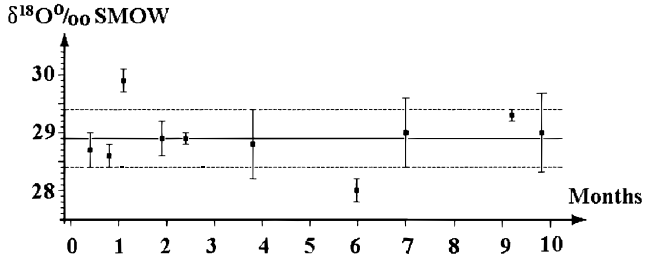

Figure 3. Long-term reproducibility of ten measurements of the same beet sugar sample performed over a period of 10 months. Solid line, mean of the ten determinations; dashed lines, limits of the calculated standard deviation for the ten determinations (σ_{10} = 0.5). Squares with error bars represent individual determinations with the corresponding precisions.

CONCLUSION

This pyrolysis method presented here for oxygen-18 determination is rapid and particularly suitable for the determination of oxygen in organic compounds. Oxygen is an element present in a large variety of compounds in nature and an easy determination of its isotopes could be particularly useful in a number of fields. The proposed technique will be developed further for the examination of the authenticity of food subtances, particularly in the field of beverages and flavours. The technique might also be of value in other areas such as geochemistry and in metabolic studies.

Acknowledgements

We thank Dr W. Brand, Finnigan MAT, Bremen, for helpful discussions and E. Falou for technical assistance.

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