

CHAPTER 30

## Isotope Ratio Mass Spectrometry: Precision from Transient Signals

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### 30.1 Introduction

Isotope ratio mass spectrometry has undergone dramatic changes in recent years. The major emphasis driving those changes always has been to study the variation of stable isotopes in life science elements (C, H, N, S, O) with ever higher precision from ever smaller samples. The year 1988 marked a major breakpoint. On the occasion of the 11th International Mass Spectrometry Conference held in Bordeaux, France the two leading manufacturers of isotope ratio mass spectrometers introduced devices that allowed a direct coupling of a Gas Chromatograph (GC) with an isotope ratio mass spectrometer (IRMS) [1]. During the nine years that have passed since the principles of this *isotope ratio monitoring* (irm) [2] approach have entered almost every laboratory dealing with high precision isotope measurements of the elements mentioned above. In many cases the new technology has surpassed the classical principles of isotope determination, mainly due to its elegance as opposed to the typically rather tedious and laborious sample preparation techniques that prevailed before 1988. The major reason for the rapid success, however, is that precision adequate for studying the variation of isotope ratios in nature is available for a sample size of less than one nanomole element. This is an improvement of more than three orders of magnitude.

Contrary to what we learn in school, isotope ratios are not constant on earth. Everyone knows for instance that  $^{13}\text{C}$  is 1.1% of the total carbon. There are, however, subtle but very significant variations of this whole earth value. Marine carbonates are "heavier" than marine oils, some plants (C-3) are lighter than others (C-4) depending on the  $^{13}\text{C}$  discrimination during the photosynthetic cycle. It is noteworthy that Europeans are lighter than Americans due to the fact that American food largely derives from maize, a C-4 plant whereas European diet is based more on plants utilizing the C-3 photosynthetic cycle. Also sea food is heavier than other, making for instance Japanese people heavier than Europeans but lighter than Americans.

Before starting to dwell more on the features and principles that are responsible for high precision from transient signals, a brief analytical example from doping laboratories that control drug abuse in sports shall illustrate the requirements for the experimental setup and form a bridge to further examples from other areas that use isotope ratio monitoring.

### 30.2 Detection of testosterone abuse in sports

Testosterone is a potent performance enhancement drug in human and equestrian sports. Its use is considered unethical due to the non-fairness to fellow athletes, potential long term health problems and because of the money involved in modern sports.

The currently established method of detection is a measurement of the testosterone/epi-testosterone ratio (T/E) using GCMS or LCMS techniques. If the T/E ratio is  $>6$  the athlete is probably positive. However, the method suffers from a lack of specificity. Some people have artificially high T/E values. Not all causes of  $\text{T/E} > 6$  are due to testosterone abuse. In addition, the time window for detecting drug abuse is no more than 5 hours after ingestion.

Hence, a more reliable test is urgently needed. It should allow rapid screening of a large number of urine samples during sports events. It should also allow for a larger detection time window, preferably  $>24$  hours.

The measurement of the isotope ratio of testosterone in urine either alone or in conjunction with other members of the biosynthetic pathway provides such an opportunity:

Figure 30.1 shows T/E results obtained from a volunteer who took a small dose of commercially available testosterone. The limited time window of the T/E is clearly visible. The fast decay reflects the regulatory action of the body to the high concentration of testosterone. The graph also shows the  $^{13}\text{C}$  isotope ratios expressed as Delta per mil (‰) versus the international standard PDB. The Delta values show a pronounced rise of about two per mil over baseline which extends over a time window of 12 hours. Another metabolite of testosterone,  $5\beta$ -Andros-

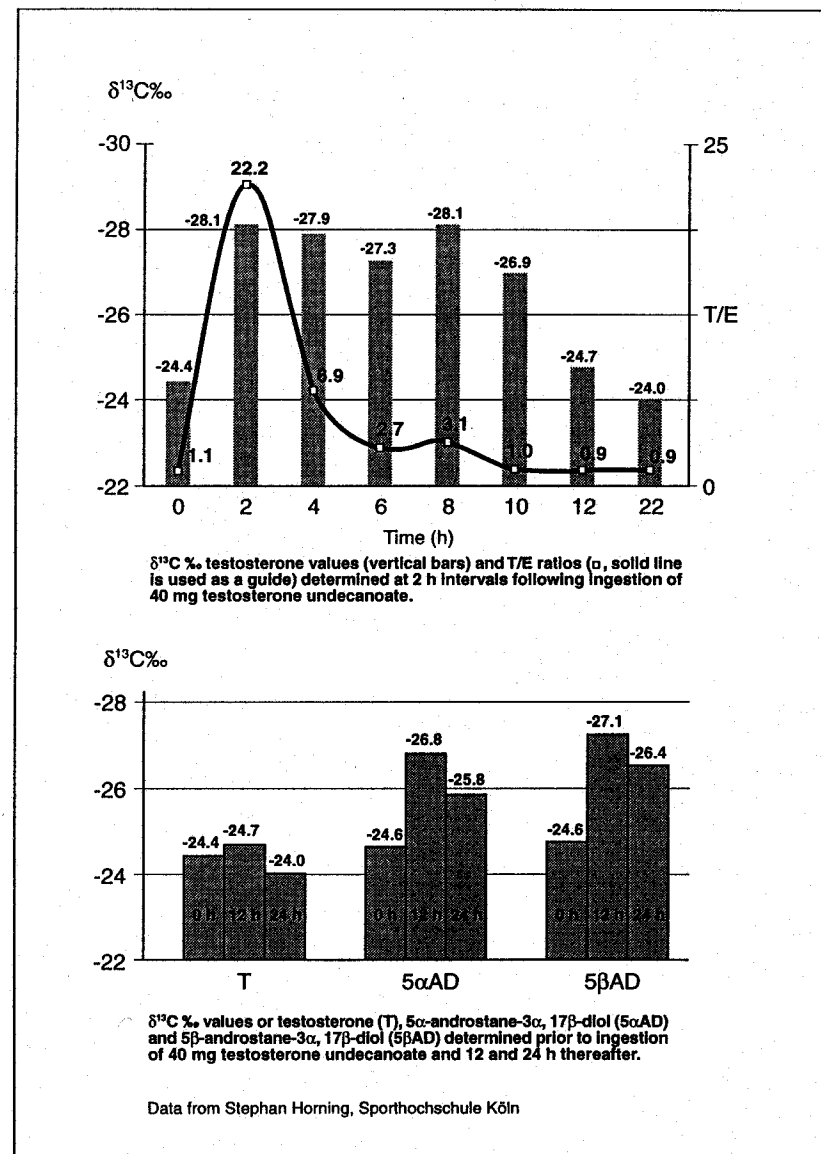


Figure 30.1

Table 30.1: Testosterone case study, quantitative assessment.

|   |                                    |
|---|------------------------------------|
| Maximum amount of urine available per analysis  | 10 mL (5 $\mu$ L preferred)        |
| Testosterone concentration  | >50 ng/mL                          |
| Testosterone available per analysis   | 500 ng                             |
| Testosterone (C <sub>19</sub> H <sub>28</sub> O <sub>2</sub> ) carbon content                                   | 80%                                |
| Carbon available per analysis   | 400 ng C<br>= 33 nmol Carbon total |
| 5 separate injections, 1 $\mu$ L each on column, thus 10 $\mu$ L solution is required with 3.3 nmol C/ $\mu$ L. |                                    |
| Conclusion:   |                                    |
| From 1 to 5 nmol C, injected on column, a precision of 0.1% should be achieved.                                 |                                    |

tanediol (5 $\beta$ AD) provides a possibility to detect testosterone abuse even 24 hours after ingestion.

The data are from the Sporthochschule Köln (S. Horning), one of the IOC drug laboratories, measured in collaboration with the application lab at Finnigan MAT (A. Hilker).

The reason for the ability to detect testosterone abuse via isotope ratio measurement is the fact that commercially available testosterone has an isotope value between -28‰ and -29‰. Human testosterone is close to -25‰ (central Europe) or higher, depending on the feeding habits of the individual.

Table 30.1 shows the amount of material available from a standard urine sample. From the quantitative assessment it is learned that for a required precision of 0.1‰ only 1 to 5 nmol carbon must be sufficient.

### 30.3 Requirements and characteristics of an isotope ratio mass spectrometer for handling transient signals

Contrary to the classical gas comparison technique with a dual inlet system [3], measurement is not made on clean gas samples. The gaseous samples in irm techniques always are entrained in a helium carrier gas stream. Thus, the mass spec-

trometer must be able to cope with the high helium load and, at the same time maintain or exceed classical performance.

#### 30.3.1 Sensitivity

In order to achieve maximum precision sample utilization is of utmost importance. There are two basic aspects of sensitivity, the mass spectrometer and the open space ratio.

Modern mass spectrometers have a basic sensitivity of better than 2,000 molecules per ion, i.e., out of 2,000 neutrals entering the EI ion source, one ion reaches the MS-detector. This is achieved by optimizing the source for gases that are pumped out with relative ease (closed source design) and allowing a large angle ion beam to pass through the entrance slit into the mass spectrometer. The ion optics of the mass spectrometer in general has double direction focusing properties so that the height of the ion beam is approximately equal at the entrance and at the exit. No ions are lost to the flight tube walls in the vertical (y) direction.

The maximum helium load into the mass spectrometer is limited by space charge in the (rather closed) ion source, but 0.3 to 0.5 mL/min are possible. In order to have the best sample utilization, the flow through the GC should be low (1.5 to 2 mL/min). In the coupling device, the addition of make up gas should be avoided.

Ultimately, precision is limited by the counting statistics (Poisson statistics). Specifically, the maximum precision (std deviation of a repeated measurement of certain quantity) is given by

$$1 \sigma = N^{-0.5}$$

with N being the number of ions reaching the Faraday cup. As an example we have an ion current ( $m/z$  44) of  $10^{-9}$  A as observed for a sample containing about 5 ng carbon. Then the minor  $m/z$  45 ion current, which almost exclusively limits precision, is  $10^{-11}$  A or  $6 \times 10^7$  ions/sec. Assuming a transient base to base peak width of 10 seconds this gives about  $3 \times 10^8$  ions per peak. Thus, maximum precision is  $6 \times 10^{-5}$  or 0.06%.

In practice, other sources of error like amplifier noise, ion source variations, isobaric interferences and noise from background radiation have similar contributions to the limits of precision. As a rule of thumb precision corresponding to 10 times the statistical limit can be achieved at the counting rates involved (0.24% in the example given above).

#### 30.3.2 Linearity

In the classical dual inlet technology ion currents were balanced in order to cope with instrumental dependence of the measured ratio upon ion current intensity. In

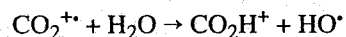
isotope ratio monitoring this luxury can no longer prevail. Complex chromatograms exhibit all intensities, very small peaks that just distinguish from the baseline to the maximum intensity that the detector can tolerate. Thus, ratio dependence upon signal intensity must be made as small as possible.

Two major sources contribute to non-linearities:

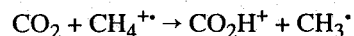
- space charge in the ion source and
- ion molecule reactions in the ion source producing isobaric interferences.

Due to the presence of both space charge effects and magnets for collimating the electrons inside the EI ion source, ions travel in an almost field free region at thermal velocities describing radii depending upon their mass (cyclotron motion). This causes mass dependent extraction properties to change with the space charge and, hence, intensity. This effect is more pronounced without helium since the relative change is larger than when the space charge is large but dominated by a constant amount of He<sup>+</sup> ions.

Before acceleration ions can suffer from ion molecule reactions, the most prominent being



and



Both reactions produce an isobaric interference on the minor isotope channel,  $m/z$  45. The first reaction is common in mass spectrometers because water is almost always present. The latter reaction represents a class of reactions with protonating agents. When <sup>13</sup>C in CH<sub>4</sub> must be analyzed great care must be taken that it is reacted quantitatively to CO<sub>2</sub> and no CH<sub>4</sub> enters the ion source together with the CO<sub>2</sub>.

Linearity is of utmost importance for irm techniques, thus manufacturers of mass spectrometers take great care to achieve good numbers. A typical specification is that linearity is <0.02%*nA* difference in the  $m/z$  44 signal.

### 30.3.3 Signal detection and digitization

The signals from the Faraday cups must be collected quantitatively because no information must be lost. In most isotope mass spectrometers this is achieved with a continuous and simultaneous observation of the ion currents of interest, slicing the observation times into 1/4 or 1/8 seconds intervals.

Digitization is achieved with Voltage-to-Frequency converters that either have a high base frequency or that rely on the detection of fractional counts through inverse frequency counting. Fast data transfer to the host computer is usually made

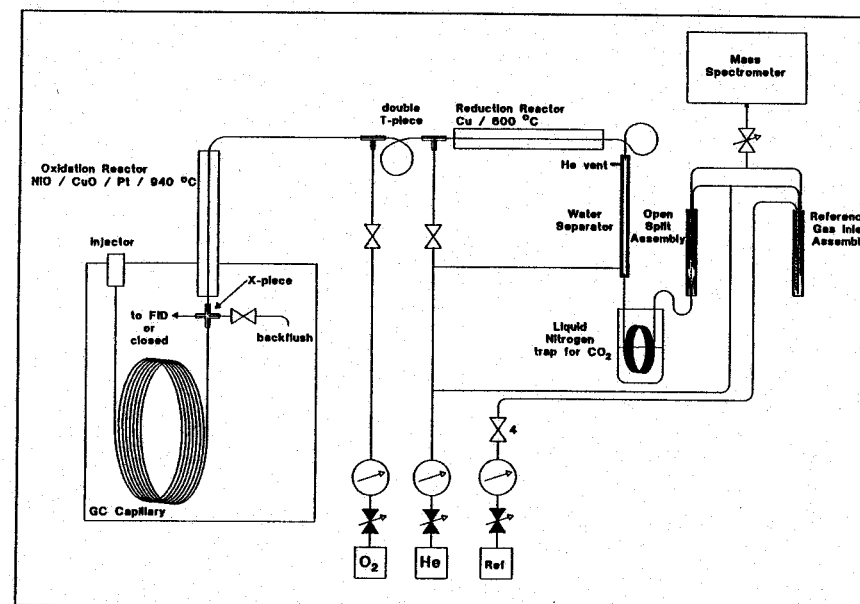


Figure 30.2 Setup for combustion of organic compounds eluting from a GC [4]. For a functional description please see the text.

via the IEEE 488 protocol, via an ethernet connection or with fast host bus extenders.

### 30.3.4 Stability

Whereas the classical technique allows a comparison of sample and reference gases in a seconds time frame, frequent standardization in long GC runs is not always possible. Thus the demand for high stability of the mass spectrometer response has increased. A good irm instrument should not drift by more than 0.2% in the <sup>13</sup>C/<sup>12</sup>C ratio per hour (i.e., admitting a pulse of CO<sub>2</sub> gas at the beginning of a run and another one from the same source for comparison one hour later). More frequent pulses of reference gas should be admitted whenever possible from an analytical point of view in order to follow and monitor instrumental performance throughout the analysis.

### 30.4 The GC combustion interface

Figure 30.2 shows a schematic layout of an interface for coupling the exhaust of GC to an isotope ratio mass spectrometer [4]. From the column, the gas flow

through an X-piece that is part of the backflush system for the solvent. The combustion reactor, operated at about 950°C, is a 0.5 mm i.d. alumina tube of 300 mm length. The reactor is filled with NiO and CuO to provide the oxygen source for quantitative combustion. The combustion reactor is followed by a reduction furnace kept at 600°C. Here, oxygen released from the combustion furnace is scavenged and nitrous oxides are reduced to N<sub>2</sub>. In between the two reactors helium can be introduced. With the backflush valve open, this reverts the flow path in the oxidation reactor. Oxygen might be added to this backflush stream of helium for reoxidation of the reactor. Reaction water is withheld in a continuous water separator made of Nafion™, a hygroscopic membrane that is flushed on the outside with dry helium. If nitrogen isotopes shall be measured, CO<sub>2</sub> is withheld in a loop of fused silica tubing immersed in liquid nitrogen.

Connection to the mass spectrometer is made via an open split arrangement which is basically a small sniffing capillary sitting in a larger diameter capillary at the exit of the combustion interface. The flow through the interface is identical with the GC flow (typically 1.5 to 2 mL/min). The sniffing capillary allows about 0.4 mL/min He to enter the mass spectrometer. Sample utilization thus is roughly 20 to 30%.

Reference gas is admitted to the mass spectrometer in much the same way (Fig. 30.3). Three fused silica capillaries are immersed into a 1 mm i.d. glass tube closed at the end. One of the capillaries provides a constant He flow of 10 mL/min, the second a constant flow of reference gas of 0.5 mL/min. The third capillary is a sniffing capillary that provides a small flow of about 0.02 mL/min into the mass spectrometer. The reference capillary has two positions so that the mixing point of helium and reference gas is either upstream or downstream from the sniffing point. Moving the reference capillary thus generates reference gas peaks of rectangular shape. Other means of injecting reference gas pulses into the MS include a 6-port valco valve with a sampling loop, admittance from the changeover valve (if present) or simply using peaks eluting from the GC as reference.

Important features and characteristics of the interface include

- quantitative combustion of the GC effluent
- efficient sample utilization
- efficient removal of water from the combusted effluent stream
- quantitative removal of CO<sub>2</sub> when N<sub>2</sub> shall be analysed (CO<sub>2</sub><sup>+</sup> generates CO<sup>+</sup> in the ion source which has an apparent isotope ratio of more than 400‰ vs N<sub>2</sub>!)
- isotopic integrity of the whole system
- full GC resolution without using make up gas for maximum sensitivity.

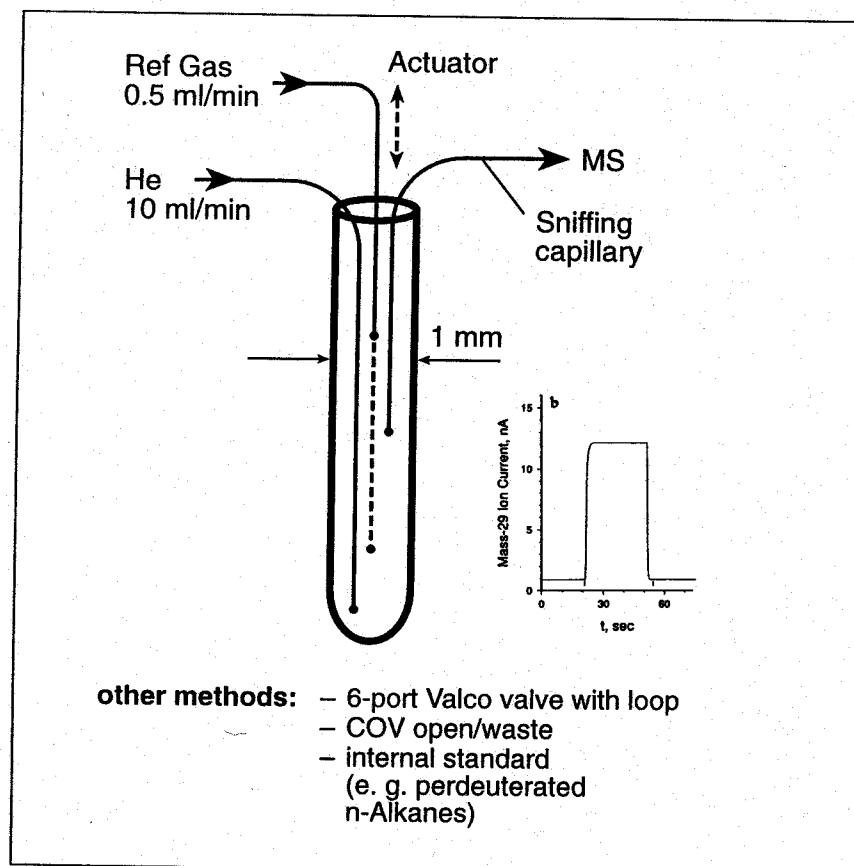


Figure 30.3 Moving fused silica capillaries in a helium stream is a simple method for reference gas introduction.

### 30.5 The irm-GCMS data

A typical example of a GC run from the alkane fraction of a crude oil on an irm GCMS system is shown in Figure 30.4. The lower trace shows the intensity variations at the mass 44 channel, it very much resembles an FID trace. (It can be viewed upon as a mass selective FID trace). There are two more such chromatograms, one for the mass 45, the other for the mass 46 channel.

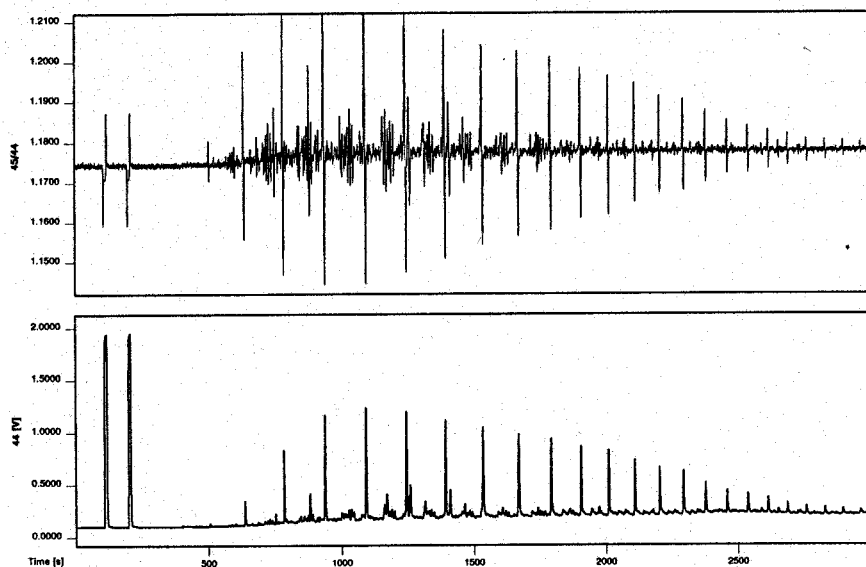


Figure 30.4 The  $\text{CO}_2^+$  signal generated by an irm-GC/MS system. The lower trace shows the "total ion current" of a series of *n*-alkanes derived from crude oil following GC separation and combustion. The trace can be looked at as a mass selective FID trace. The first two peaks are reference gas pulses for standardization.

The upper trace shows the instantaneous ratio of the 45/44 traces. Each peak swings forth and back, sometimes by more than  $\pm 50\%$ . This is due to isotopic separation in the GC column. It is not observed on the first two peaks which are reference gas pulses.

Figure 30.5 is a zoom into the chromatogram, this time showing all three ion current traces simultaneously. Close inspection of the middle (45) trace reveals a small offset in time, the peak here preceding the other two by about 100 msec. This gives rise to the large isotope swing in the upper ratio trace.

From Figure 30.5 it is clear that high precision isotopic information at the 0.1‰ level cannot be deduced from partial inspection of GC peaks. All of the peak, the peak boundaries carefully selected and background quantitatively corrected for, must be integrated from foot to foot before ratios may be calculated and compared to each other.

The 46 ion current is monitored because it contains the  $^{18}\text{O}$  information which in turn is used to correct the 45 trace for  $^{17}\text{O}$  contribution. Thus clean  $^{13}\text{C}$  information becomes available.

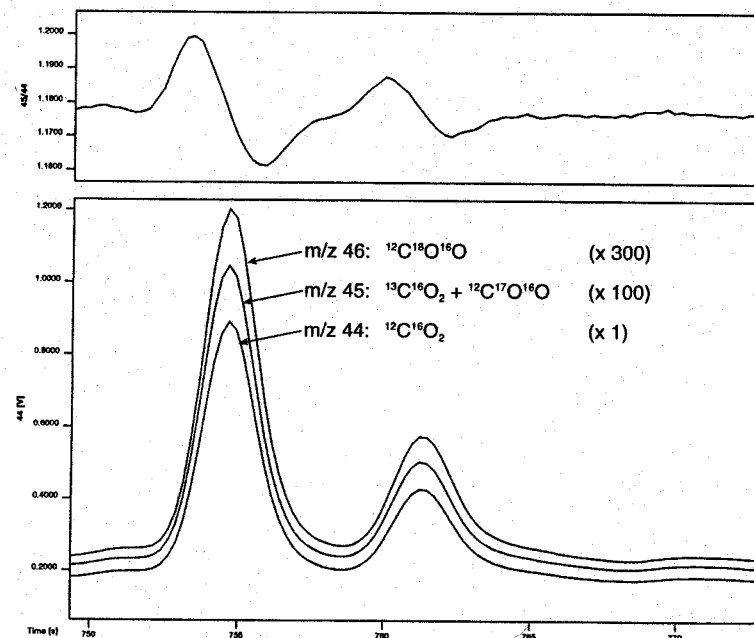


Figure 30.5 Expanded portion of an irm-GC/MS trace similar to Figure 30.4. The lower trace displays all three ion currents (44, 45 and 46) that have been recorded. A close look at the middle trace reveals that the peaks here precede those in the other two traces by about 0.1 seconds. This gives rise to the S-shapes shown in the upper trace.

## 30.6 Irm techniques in various scientific disciplines

### 30.6.1 Biogeochemistry

This area triggered the development of irm-GCMS to a large degree. Fossils are the eloquent remains of life in the past, so are fossil molecules. The isotopic signature of individual fossil compounds has become available with the development of irm techniques.

One example from this area is the discovery of a dramatic change of the properties of the world oceans around 550 million years before present by Logan Hayes *et al.* [5].

Revealed through an evaluation of systematic differences in the *n*-heptadecan isotope signal versus that of its neighboring peak, pristane (Fig. 30.6), it was concluded that the biogeochemical cycles were completely reorganized around 55

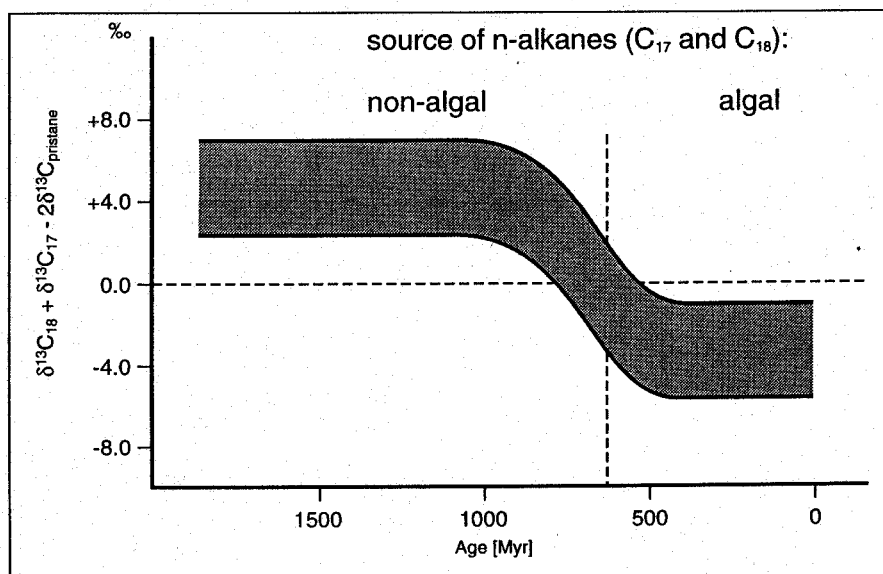


Figure 30.6 Carbon isotope relation between *n*-alkanes and pristane in sediments.

Myrs before present. Proterozoic sediments were mainly derived from sulfate reducing bacteria rather than primary producers whereas phanerozoic sediments are more and more dominated by the preservation of algal-lipid skeletons derived from rapidly sinking faecal pellets and organic remains of larger metazoans (Fig. 30.7). These for the first time in the history of life had developed muscular, unidirectional guts. As a consequence the whole oxygen budget of the oceans changed and made them inhabitable to the deep sea floors. It is suggested that this was one of the major causes for the rapid evolution of multicellular life which is known as the "Cambrian radiation" and happened over the rather short time span of about 10 million years. It led to the animal diversity found in the Ediacara and in the Burgess shale fossils.

### 30.6.2 Food control

The first coupling of a GC with a dedicated isotope ratio mass spectrometer reported in the literature was accomplished in the laboratories of Pernod-Ricard, a leading manufacturer of beverages [6]. The research group of Jacques Bricout and Josef Koziat used a small diameter quartz tube filled with  $Co_3O_4$  as combustion reactor. A backflush mechanism integrated with an open split helped to keep the solvent out of the combustion tube. The mass spectrometer was an old VG 602 modified by A. Barrie for fast data acquisition. The aim of their work was quality

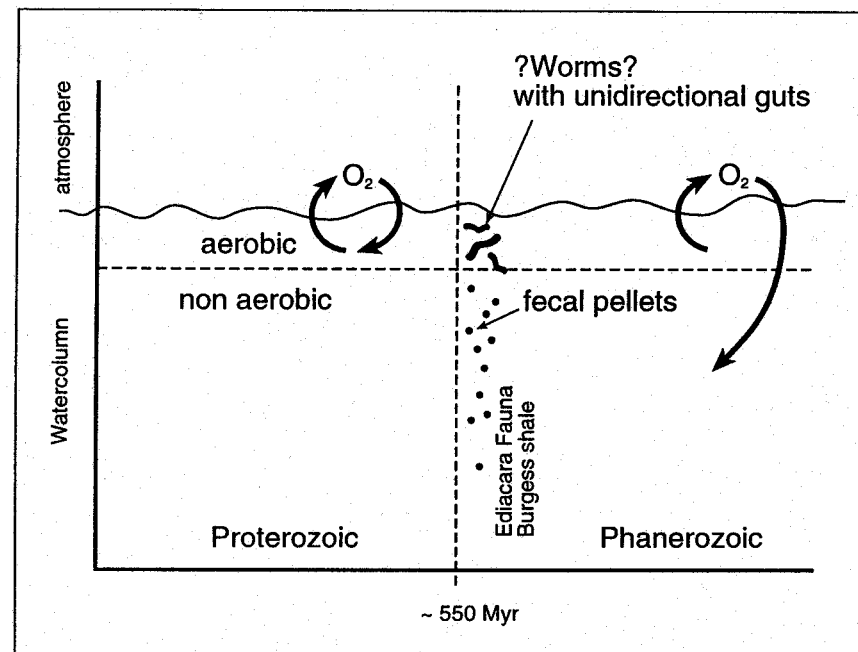


Figure 30.7 A scenario for the rapid development of multicellular life around 550 Myr before present. For more details see reference [5].

control of raw material entering the production of their beverages. The report precision was better than 1‰ on 5 to 8 nmol carbon samples. Their open split allowed the mass spectrometer to work at a constant load of gas irrespective of the pressure fluctuations generated by the combustion process. The open split coupling is now common to all commercial irm systems.

A more recent example of food adulteration control was published by Woodbury, Evershed *et al.* [7] They report the measurement of individual fatty acids extracted from various vegetable oils. The relative concentrations of the fatty acids are shown to be different between a test maize oil and the test adulterant, rapeseed oil. Thus, isotope mixing is not a mere straight line but follows a mixing curve. Using this method the isotopic analysis of these three fatty acids is sufficient to detect as little as 5% of a C3 adulterant oil to maize oil (and *vice versa*).

### 30.6.3 Archeology

There are only few reports of using irm-GCMS to tackle analytical problems in archeology or archeometry. A recent paper by A.W. Scott and R.P. Evershed [8

investigates the possibility to gather information on feeding habits of ancient communities. In particular the authors extracted cholesterol from human skeletons and measured their  $\delta^{13}\text{C}$  isotopic compositions.

Figure 30.5 of their paper shows the  $\delta^{13}\text{C}$  values from a large number of different skeletons. The remains are all from the same location, a burial site at Barton-on-Humber in the mid-east of the UK. The  $\delta^{13}\text{C}$  values range from  $-26\text{‰}$  to  $-20\text{‰}$ , reflecting individual food preferences: some preferring mainly C-3 derived food (vegetables, meat from beef, sheep or pigs), others feeding more on seafood.

Irm technology can thus be used to increase specificity of isotope ratio studies in the assessment of paleodiet.

### 30.6.4 Metabolic studies

The very first paper describing a post-GC combustion device coupled to an (organic) mass spectrometer was published by a Japanese group in 1976 (M. Sano, Y. Yotsui *et al.* [9]). The experimental setup is, of course, far from modern: The oxidation was achieved using CuO (Pyrolyser) and coupling was made with an "Enricher", a device similar to the then popular Biemann-Watson separator. Isotope ratios were measured by peak jumping in a multiple ion detection (m.i.d.) scheme.

The subject of their study was the fate of acetylsalicylic acid (Aspirin<sup>TM</sup>) in the human body. Figure 30.4 of the paper shows the chromatogram of a urine extract sample together with a ratio variance line. The latter corresponds to the ratio trace in modern irm systems. It is a very sensitive indicator for the label. The extracted information was only qualitative but it allowed the detection of all metabolites derived from a 100 mg Aspirin ingestion in a crude 24 hour urine fraction.

In conclusion, irm techniques can be used as a metabolite detector based on what characterizes the metabolic compound for the analyst: the label. They can replace the  $^{14}\text{C}$  techniques that are used in drug evaluation studies.

## 30.7 Other developments and recent additions to irm technology

### 30.7.1 Bulk combustion isotope ratio monitoring (ConFlo, ANCA)

The marriage between a conventional, commercially available elemental analyser (EA) for quantitative analysis of the carbon, nitrogen, hydrogen and sulfur content of organic matter and an isotope ratio mass spectrometer has become a widespread routine tool for isotope ratio analysis (Fig. 30.8).

This technique has largely replaced the traditional Kjeldahl and sealed tube techniques for carbon and nitrogen isotope analysis. It became popular in the mid

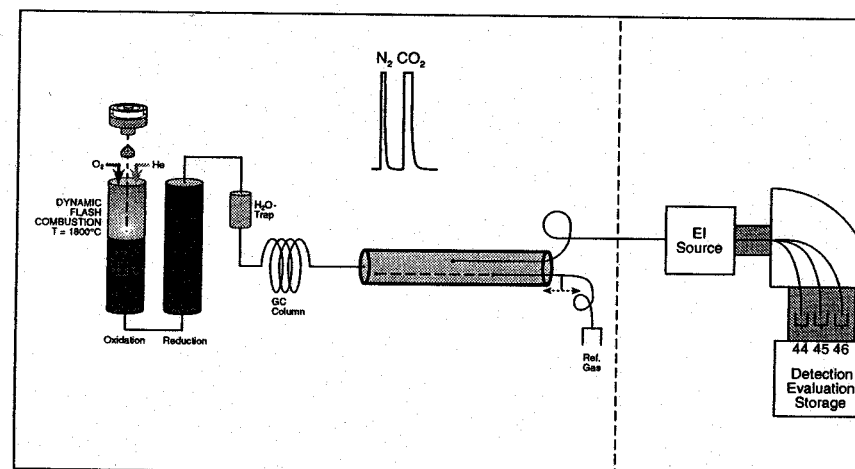


Figure 30.8 Principle setup of the coupling between an elemental CHN analyser and isotope ratio mass spectrometer.

80's when all leading isotope mass spectrometer companies introduced similar devices.

Bulk sample material is weighed into silver or tin boats and placed into autosampler. With a proper lock device that excludes nitrogen from air, the samples fall into the hot zone of a combustion reactor. The reactor is continuously flushed with helium, temporarily enriched with oxygen for quantitative combustion. Tin provides a flash temperature of about  $1800^{\circ}\text{C}$  in order to quantitatively burn large amounts of sample in a short period of time. The gaseous reaction products are swept through a reduction furnace that scrubs excess oxygen and converts nitrous oxides to molecular nitrogen. Water is trapped in a conventional  $\text{Mg}(\text{ClO}_4)_2$  trap, followed by an isothermal GC that separates nitrogen, carbon as  $\text{SO}_2$ , if present. An open split that may also serve to add reference gas pulses couples the exhaust of the EA to the IRMS. Routinely, 0.1 to 0.2‰ precision can be achieved from bulk samples as small as 5 to 10  $\mu\text{g}$  element.

The technique has more recently been extended to measuring  $^{34}\text{S}$  values from the original samples despite their unusually high carbon and low sulfur content.

### 30.7.2 $\delta^{18}\text{O}$ analysis from bulk samples

It was again Josef Koziol from Pernod-Ricard who pioneered the hitherto very difficult evaluation of organic oxygen isotope ratios [10]. J. Koziol replaced the combustion furnace of an EA with a high temperature ( $1300^{\circ}\text{C}$ ) pyrolysis unit. The reactor is lined with a vitreous carbon tube in order to avoid contact between the

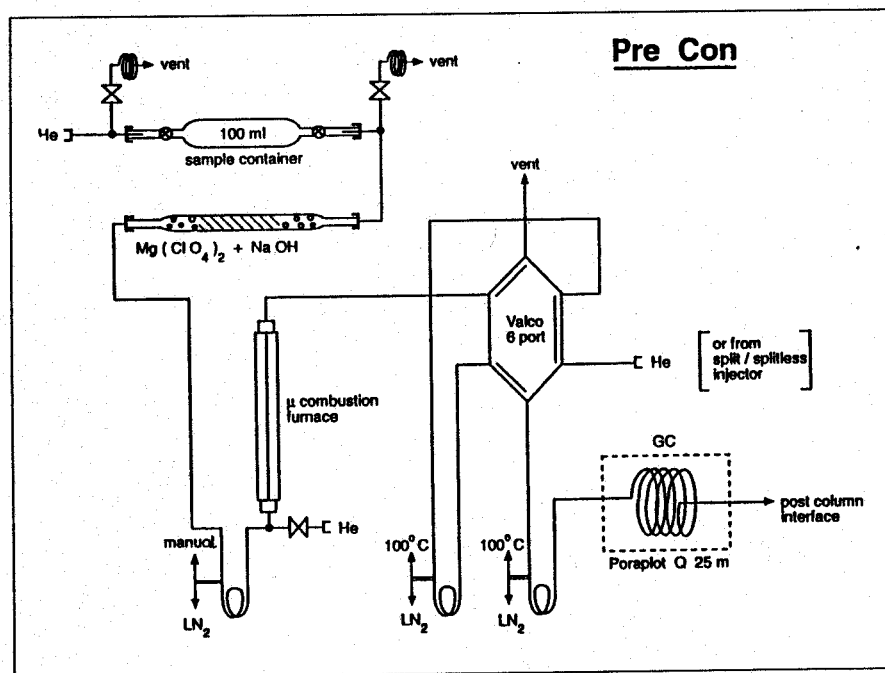


Figure 30.9 Layout of the pre-GC concentration device ("PreCon").

pyrolysis product, CO, and the reactor wall ( $\text{Al}_2\text{O}_3$ ). The reaction takes place in a graphite crucible placed at the center of the reactor. Excess carbon provides a source of carbon for water samples and for conversion of  $\text{CO}_2$  that might have been formed during pyrolysis to CO. The gaseous pyrolysis products are swept through a GC for separating  $\text{N}_2$  and CO.

$\delta^{18}\text{O}$  values are measured from the  $m/z$  30 and 28 ion currents. Two reference gas pulses provide the means for standardization. The measurement regime always has one sample of n-hexadecane (to scrub any oxygen that has been left from previous samples) followed by 5 identical samples. The first of those samples is discarded due to memory, from the last 4 samples the isotope value is established.

A precision of 0.1 to 0.7‰ from 2 to 14 mg size organic samples like Vanillin, sugar and cellulose has been achieved. Excellent accuracy was obtained for international water standards like GISP (Greenland Ice Standard Precipitation,  $\delta^{18}\text{O} = -189.7$ ) when measured against VSMOW (Vienna Standard Mean Ocean Water,  $\delta^{18}\text{O} = 0.0$ ‰). The technique allows the measurement of  $\delta^{18}\text{O}$  values from any organic sample or water. Even carbonates have been measured with good precision, however with lower accuracy (>1‰).

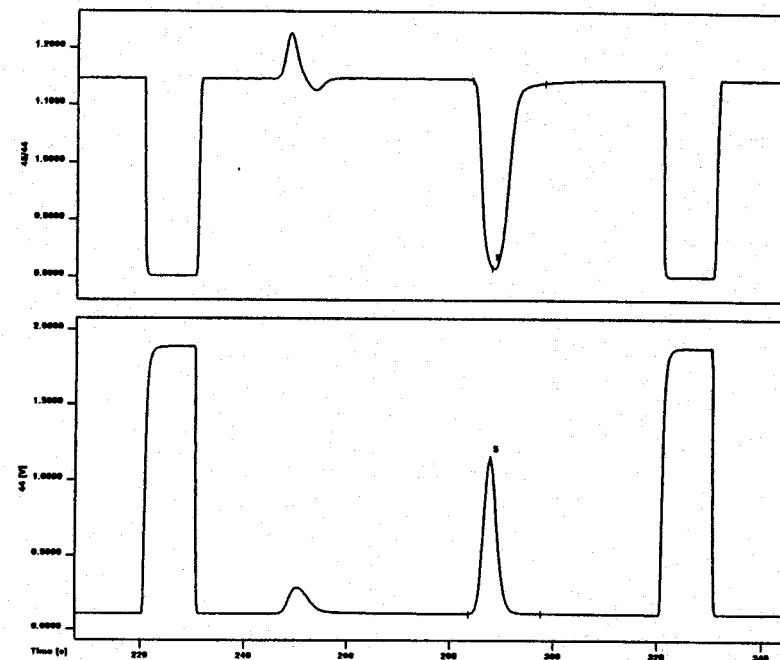


Figure 30.10 Isotope ratio monitoring gas chromatogram from  $\text{N}_2\text{O}$  sampling. In contrast to Figure 30.4, the reference peaks are non-calibrated  $\text{N}_2\text{O}$  gas.

### 30.7.3 Trace gas analysis in modern and archived air

Ambient air contains about 1.7 ppm methane and 0.3 ppm  $\text{N}_2\text{O}$ . Preindustrial values are less than half those values. Classical isotope techniques have been used to study sources and sinks of these greenhouse gases that also influence ozone chemistry in the stratosphere. The required large amount of air per analysis precludes more wide spread routine analysis of the isotope ratios of methane and nitrous oxide. An alternative approach termed "PreCon" has been developed recently [1].

A schematic layout of the device is shown in Figure 30.9. Using this setup, 10 mL of air is sufficient for  $\text{N}_2\text{O}$ ; 25 mL of air is necessary for  $\text{CH}_4$  measurement. With a He piston technique,  $\text{N}_2\text{O}$  is concentrated in a loop trap at  $-196^\circ\text{C}$  after scavenging  $\text{CO}_2$  from the sample using an ascarite trap. From this sampling trap the gas is transported to a second trap at the column head for excellent GC performance. Methane measurement requires conversion to  $\text{CO}_2$ . This is done after removing condensables from the sample stream at  $-196^\circ\text{C}$  in a combustion reactor filled with NiO at  $950^\circ$  to  $1000^\circ\text{C}$ . Figure 30.10 shows a typical chromatogram for  $\text{N}_2\text{O}$ .

( $m/z$  44, 45 and 46, as in  $\text{CO}_2$ ). The first peak is  $\text{CO}_2$  that escaped the ascarite trap. From the peak area it is concluded that the ascarite trap removes  $\text{CO}_2$  with an efficiency of 99.99%.

Using this approach, precision of better than 0.2 per mil has been achieved from clean air samples.

A similar preconcentration scheme for studying non-methane hydrocarbons (NMHC's) has been taken by Rudolph *et al.* [12].

Due to the very low concentrations of these VOC's in ambient air (<100 ppt to a few hundred ppb) the sample size was between 5 and 10 liters per air sample analysed. After removal of water  $\text{CO}_2$  concentration was done at  $-196^\circ\text{C}$  for all of the compounds simultaneously. VOC's were separated on a Poraplot Q column followed by a combustion interface coupled to the IRMS in the usual way. Depending on the size of the peaks (0.1 to 40 ng carbon), external precision ranged from 0.2 to 3.6‰. The isotope values of NMHC's from air samples exhibit a rather large range, varying considerably from one sampling site to the next. Thus, such measurement provide a valuable tool for identifying the source of this class of compounds in air.

### 30.7.4 Laser ablation isoprobe

Another dimension of information has recently been added to stable isotope analysis by Z. Sharp and T. Cerling [13], marrying a laser ablation system with an isotope ratio mass spectrometer through an open split interface:

Using off the shelf imaging equipment they were able to obtain isotopic information from specific locations in complex matrices. The principle setup of their experiment is rather straightforward: A 20 msec focused 20 W pulse from a  $\text{CO}_2$  laser hits the sample in a chamber that is flushed with helium. The design of the chamber with a total volume of about 5 mL allows to direct the gaseous products released from the laser shot to a GC for chromatographic separation. The intensity of the laser pulse is optimized for maximal  $\text{CO}_2$  and minimal CO production from a carbonate surface. The exhaust of the GC is directly coupled to the MS with an open split where also reference gas pulses are introduced. In another setup a Valco sport valve provides a loop that can be immersed in liquid nitrogen for concentrating small samples (small spots) prior to GC separation.

First results obtained with this arrangement from various carbonates are reported as a comparison with the accepted values. The data for  $^{13}\text{C}$  exhibit a rather good accuracy within  $\pm 0.5\text{‰}$  except for Siderite which has an offset of more than 2‰. The  $^{18}\text{O}$  data show a larger deviation of the measured versus the true values. Obviously each type of carbonate requires a different offset correction. External reproducibility for each type of carbonate is always better than 0.5‰, mostly better than 0.3‰. Further refinement of the experimental conditions, possibly use of a different type of laser (UV) may help to improve the data, in particular to

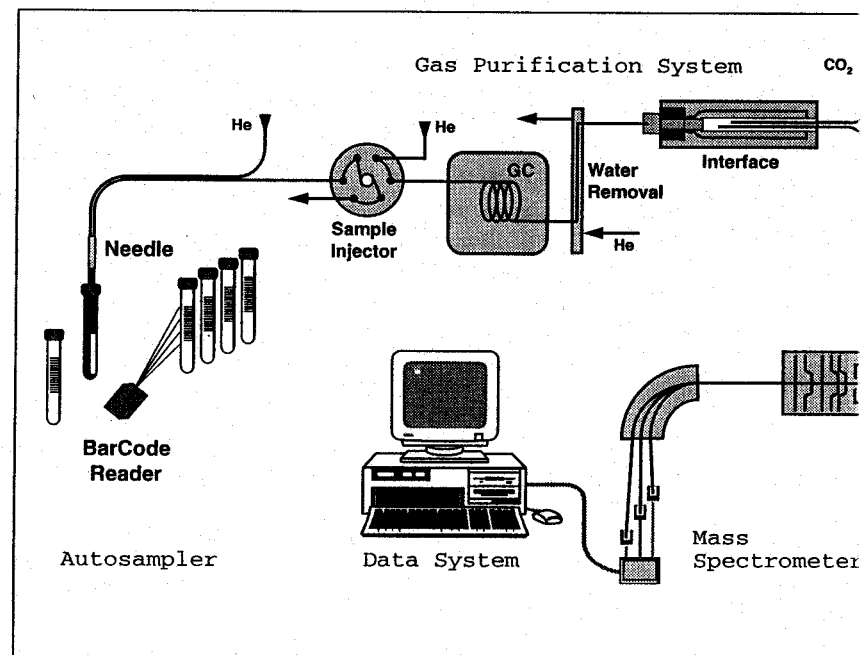


Figure 30.11 Experimental setup for measuring  $\delta^{13}\text{C}$  values in human breath  $\text{CO}_2$ , in particular for testing *helicobacter pylorii* infection.

lower the observed offsets in the  $^{18}\text{O}$  data. The method as is is suitable for screening. It allows very fast measurements without sample pretreatment. High precision  $^{18}\text{O}$  work for paleoclimate studies still requires the traditional methods, i.e., phosphoric acid digestion followed by dual inlet IRMS.

The system can also be used for other samples when the laser pulse is combined with gas phase chemistry followed by GC separation.

### 30.7.5 $^{13}\text{C}$ urea breath test

In medical diagnosis the  $^{13}\text{C}$  urea breath test is becoming an increasingly important analytical tool with over 100 instruments sold per year. The background is widespread stomach infection of the earth population (infection rate 20 to 80 varying from country to country) with a bacterium named *Helicobacter pylori*. This little bug is able to survive the harsh conditions in the stomach without being digested by hiding in the mucous membrane. It produces urease which is able to alter the pH value locally by cleavage of urea. *Helicobacter pylorii* was discovered

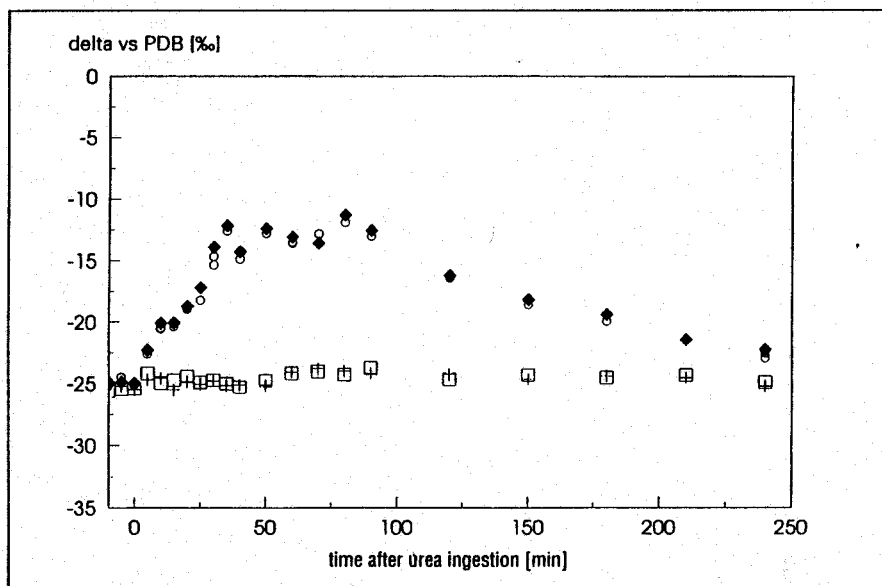


Figure 30.12 Isotope ratio recording of a full exhalation cycle after the intake of 75 mg of  $^{13}\text{C}$ -labelled urea, by a 35 year old lactating woman (positive) and a 42 year old man (negative).

in the early 80's by a group in Perth, Australia. It took a long time to get the gastro enterological community convinced that it is there and that it is very dangerous. It is now recognized as the major, if only cause for gastritis, gastric ulcers and, ultimately, cancer in stomach and duodenum. Thus, it must be detected and eradicated.

The presence of urease provides an excellent handle for a non-invasive analysis and diagnosis. Suspected patients suffering from stomach aches are administered a dose of 100 mg  $^{13}\text{C}$ -urea which in the case of an infection is converted to ammonia and  $^{13}\text{CO}_2$ . The labelled carbon dioxide appears in the breath within a short period of time, peaking at about 30 minutes. Figure 30.11 shows a typical commercially available experimental setup to detect the label in human breath. Analysis is made from vacutainers filled with 10 mL STP breath. A portion of the gas is swept into a loop from where GC separation is initiated. Water is removed with Nafion<sup>TM</sup> continuously. Coupling to the small, dedicated mass spectrometer is again done with the usual open split incorporating also the reference injection.

Figure 30.12 shows two full exhalation curves, one from a healthy subject, the other one from an infected lactating woman. Two samples per time were taken in

order to establish precision. The natural variation is about 1‰ depending on variation of the  $^{13}\text{C}$  isotope ratio in the diet. The rise over baseline must be more than 3‰ for a positive result. Positive patients are treated with a combination therapy of acid blocker and an antibiotic. If therapy is successful people are healed. Reinfection or recurrence is highly improbable.

Such dedicated, fully automated mass spectrometer systems today are relatively inexpensive, compared to the more research oriented general IRMS systems. The  $^{13}\text{C}$  urea breath test may initiate a ubiquitous presence of breath test mass spectrometry in hospitals and medical service laboratories world wide.

### 30.7.6 Irm-LCMS

It is an experimental challenge to extend isotope ratio monitoring techniques to non-volatile organics that typically are separated with liquid chromatography (LC). The first such system was reported in the literature by Caimi and Brecht [14] using a moving wire as a transport mechanism from the HPLC effluent to isotope ratio mass spectrometer. The experimental setup was based on an old interface from Pye Unicam intended for producing FID traces from LC effluents. Since this setup had some inherent deficiencies we have created our own system [15] that uses similar principles (Fig. 30.13).

The interface system employs a low-carbon nickel wire which is used once. From a feed spool it passes through a cleaning and oxidation furnace where organic residues and carbon inside the wire are burned off. The oxide layer provides a large, rough surface for depositing the HPLC effluent. It also serves as an oxygen source during combustion. The HPLC effluent stream is deposited onto the wire at a linear speed of 60 mm/sec. Coating capacity is up to 100  $\mu\text{L}$  liquid per minute. The solvent (water and organic solvent) is evaporated gently from the wire surface in a long, temperature regulated zone. The temperature of this zone can be ramped during analysis in order to optimize evaporation conditions for gradient elution. The wire then enters a short combustion tube at 850°C where  $\text{CO}_2$  is generated. Reaction water is scavenged in a Nafion<sup>TM</sup> dryer in front of the open split which provides the connection to the mass spectrometer.

A first chromatogram is shown in Figure 30.14 with the separation of the amino acids. The background is rather high in comparison with irm-GCMS. The major contribution is from carbon in the Ni wire. The variation on the ratio trace is much smaller than in the GC experiments, in particular the typical S-shape is not observed. There are small hints (inflections) to isotopic separation on the column which will probably become more pronounced with better separation. Standardization is made in the usual fashion.

First results exhibit reproducibilities of better than 0.5‰ from single (local) injections. With LC separation, precision drops to values around 2‰. Obvious noise from some unknown source of noise contributes to the signal. This needs improvement.

However, performance is adequate in particular for metabolite studies where replacement of the  $^{14}\text{C}$  techniques is urgently needed.

### 30.8 Irm for deuterium/hydrogen determination

An open challenge is isotope ratio monitoring of D/H ratios in naturally occurring water and organic matter samples, both in bulk analysis and from chromatographic effluents.

There are two major issues that have hampered progress up to now and need to be resolved:

1. Quantitative, unfractionated yield of hydrogen gas from any compound of interest, irrespective of its chemical nature.

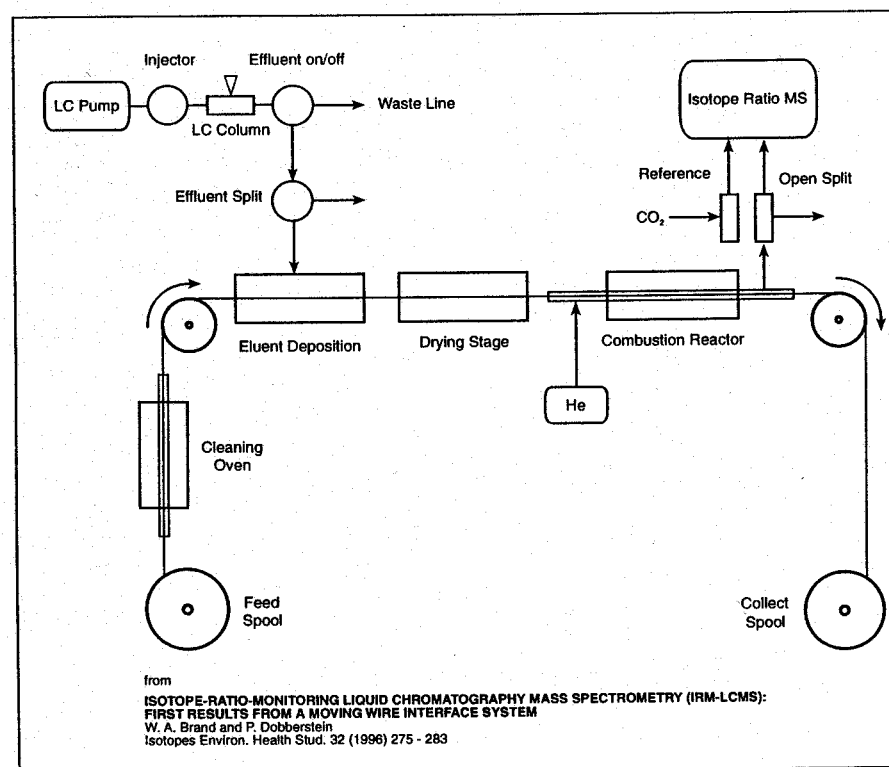


Figure 30.13 Moving wire interface for coupling a liquid chromatography system to an IRMS.

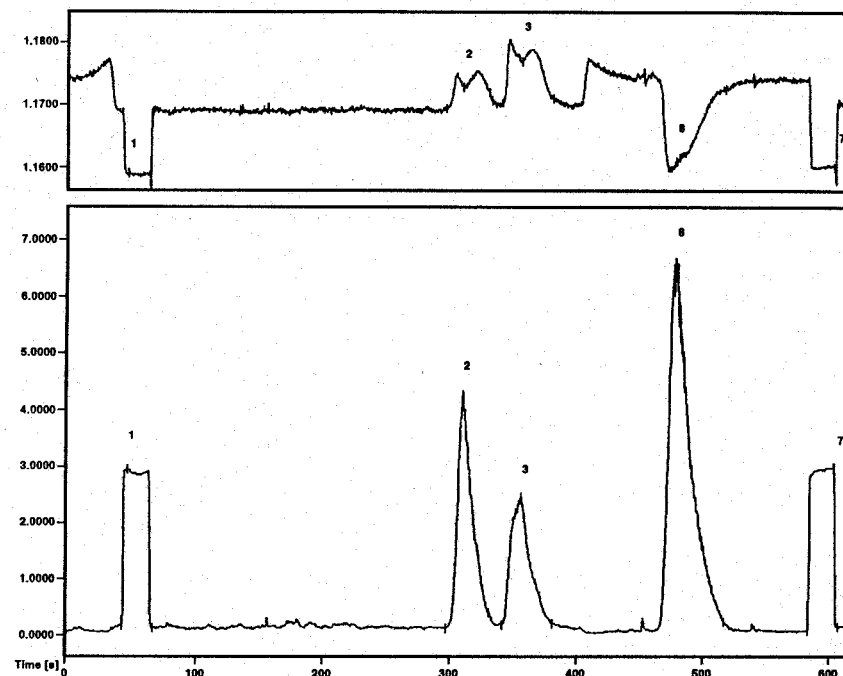


Figure 30.14 HPLC separation of ASP (2), Pro (3), and DOPA (8), each 20 nmol/m, peak (1) and (7) are from  $\text{CO}_2$  reference ( $-40.0\text{‰}$ ).

2. Mass spectrometric measurement of small variations of a small  $m/z$  ( $= \text{DH}^+$ ) signal in the presence of a large, neighboring helium  $m/z$  4 ion current.

The second problem has partly been overcome by the construction of a large dispersion gas isotope mass spectrometer [16]. Due to multiple collisions in the ion source and along the flight path light ions like  $\text{He}^+$  can suffer a large loss translational energy thus creating a tail in the mass spectrum over a wide  $m/z$  range. This tail in standard IRMS systems is large enough to saturate the mass detection channel with full helium load.  $\text{He}^+$  ion currents usually are in excess 100 nA whereas the  $\text{DH}^+$  current is of the order of 1 pA, more than 5 orders magnitude less. A desired precision of 1‰ means that changes of 1 fA need to be measured reliably. Thus, a total of more than 8 orders of magnitude on the neighboring masses 3 and 4 needs to be dealt with in a reliable fashion.

The approach of using a larger dispersion tries to minimize the portion of the energy deficient  $m/z$  4 ions that hit the mass 3 Faraday cup, but it cannot eliminate the tail *in toto*. Another means of discriminating such ions is to place an energy filter directly in front of the mass 3 bucket.

Current state of the art is to allow a reduced helium flux of 50  $\mu\text{L}/\text{min}$  or less into the ion source and use a favourite ratio of  $\text{H}_2$  and He (1:200) for measuring the isotope ratios (DH/HH) on the fly. Further development is necessary to allow for the fluxes of 300 to 400  $\mu\text{L}/\text{min}$  and hydrogen contents of  $10^{-4}$  in helium that are typical for capillary GC effluents.

The first problem mentioned, quantitative and fractionation free production of hydrogen gas from any sample, has been addressed in a number of ways. The most promising technique seems to be a pyrolysis reaction at temperatures in excess of  $1300^\circ\text{C}$  with the presence of carbon and, possibly, a catalyst like platinum. Precision of better than 5‰ seems to be attainable. With further care in injection procedures and better correction for  $\text{H}_3^+$  contribution 2 to 3‰ precision should be routinely achievable. This is confirmed in the paper by Begley and Scrimgeour [16] who used catalysed pyrolysis (nickelized carbon and Ni wool) at  $1050^\circ\text{C}$  for reduction of water samples. Reported precision was about 4‰ for water samples. This kind of precision is adequate for a number of applications because hydrogen, due to the large relative mass difference, shows the largest isotope effects resulting in the largest scale of isotope ratios in nature (from 0‰ for ocean water to less than -400‰ for antarctic precipitation).

### 30.9 Summary and conclusion

Isotope ratio monitoring is a vivid, precise and reliable technique rapidly expanding into a wide variety of scientific disciplines and industrial applications. It is still a novel tool for studying faint traces left by some process we are interested in, the process being of historical, geological, forensic, medical or simply scientific nature. It is an art in every discipline to read the books written in the ever changing alphabet provided by high precision isotope data.

### Acknowledgements

The numerous people involved in making irm-techniques possible is impossible to mention here. The technique is a lucid example of a successful joint effort between industry and science.

The contribution of John Hayes and his group at Indiana University has been outstanding. The very basic papers of this group are a must-read for every novice in the field.

The group at Pernod-Ricard (Jacques Bricout and Jo Koziat) provided excellent collaboration for the development team at Finnigan, Bremen. Within Finnigan Karleugen Habfast was the enthusiastic engine driving irm techniques to where they are today.

### References

1. a) P.A. Freedman, E.C.P. Guillyon, and E.J. Jumeau. *Int. Lab.* **7/8** (1988) 22.  
b) W.A. Brand, K. Habfast, and M. Ricci. On-line Combustion and High Precision Isotope Ratio Monitoring of Organic Compounds, in *Adv. Mass Spectrom.* **11B**, P. Langevialle, Heyden & Son, London, 1989, 1800.
2. a) D. E. Matthews and J. M. Hayes. *Anal. Chem.* **50** (1978) 1465-1473.  
b) W.A. Brand. High Precision Isotope Ratio Monitoring Techniques in Mass Spectrometry, *J. Mass Spectrom.* **31** (1996) 225.
3. C.R. McKinney, J.M. McCrea, S. Epstein, H.A. Allen, and H.C. Urey. *Rev. Sci. Instr.* **21** (1950) 724-730.
4. W.A. Brand, A.R. Tegtmeier, and A. Hilker. *Org. Geochem.* **21** (1994) 585.
5. G.A. Logan, J.M. Hayes, G.B. Hieshima, and R.E. Summons. Terminal Proterozoic reorganization of biogeochemical cycles, *Nature* **376** (1995) 53.
6. A. Barrie, J. Bricout, and J. Koziat. *Biomed. Mass Spectrom.* **11** (1984), 583.
7. S.E. Woodbury, R.P. Evershed, J.B. Rossel, R.E. Griffith, and P. Farnell. *Anal. Chem.* **67** (1995) 2685.
8. A.W. Scott and R.P. Evershed.  $\delta^{13}\text{C}$  Analysis of Cholesterol Preserved in Archaeological Bones and Teeth, *Anal. Chem.* **68** (1996) 4402.
9. M. Sano, Y. Yotsui, H. Abe, and S. Sasaki. A new technique for the detection of metabolites labelled by the isotope  $^{13}\text{C}$  using mass fragmentography, *Biomed. Mass Spectrom.* **3** (1976) 1.
10. J. Koziat. Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter, *J. Mass Spectrom.* **32** (1997) 103.
11. W.A. Brand. PreCon: a fully automated interface for the pre-GC concentration of trace gases in air for isotopic analysis, *Isotopes Environ. Health Stud.* **31** (1995) 2.
12. J. Rudolph, D.C. Lowe, R.J. Martin, and T.S. Clarkson. A novel method for compound specific determination of  $\delta^{13}\text{C}$  in volatile organic compounds at ppt levels in ambient air, *Am. Geophys. Res. Lett.*, submitted for publication, 1997.
13. Z.D. Sharp and T.E. Cerling. A laser GC IRMS technique for *in situ* stable isotope analysis of carbonates and phosphates, *Geochim. Cosmochim. Acta* **60** (1996) 2909.
14. R.J. Caimi and J.T. Brenna. *Anal. Chem.* **23** (1993) 3497.

15. W.A. Brand and P. Dobberstein. *Isotopes Environ. Health Stud.* **32** (1996) 275.
16. I.S. Begley and C.M. Scrimgeour. *Rap. Comm. Mass Spectrom.* **10** (1996) 969.