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Isotope ratio mass spectrometry – history and terminology in brief

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The history of isotope ratio mass spectrometry (IRMS) is briefly described. It is shown that the fundamental design of isotope ratio mass spectrometers has not changed since the 1940s. The basic findings concerning the natural variation of isotope abundances even date back to the 1930s. Recent improvements in the methodology mainly concern online coupling and analytical peripherals. The nature of isotopic scales necessitates a specific terminology which is unfamiliar to many analysts. However, corresponding guidelines exist that should be adopted by the anti-doping community. Currently, steroids represent the only group of compounds routinely analyzed by IRMS in doping-control. Suggestions are made in respect to a harmonized terminology concerning the nature and origins of steroids. Copyright © 2012 John Wiley & Sons, Ltd.

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History

Today, many scientists concerned with forensic and environmental analytics will appreciate the introduction of isotope ratio mass spectrometry (IRMS) as a most innovative tool. Hitherto unsolvable problems, such as reliable source assignments of given compounds, often can be adressed successfully. The breakthrough thus achieved for doping control can hardly be overestimated. If still challenging, $^{13}C/^{12}C$ analysis of urinary steroids by gas chromatography coupled to IRMS is now a standard procedure. However, it merely represents one out of a multitude of emerging applications of stable isotope analysis at natural abundance.

Strange as it may seem to many who have used quadrupole mass spectrometers for decades, IRMS is anything but new. The technique became a standard tool for the geo-sciences and related disciplines soon after World War II. For example, significant IRMS-based knowledge about the systematic variation of $^{13}C/^{12}C$ ratios in nature was available around 1950.[1] The fundamental depletion of ^{13}C in organic material was even recognized as early as 1939.[2]

This development essentially was brought forward by Alfred Nier.[3] Any scientist currently concerned with IRMS will immediately be familiar with Nier's mass spectrometer design from 1947.[4] In fact, significant development of the ion optics, source design, etc., has taken place in the meanwhile.[5] But ever since Nier's inventions, these instruments feature multiple collectors and the characteristic magnetic sector field. Even more striking, the fundamental design of isotope ratio mass spectrometers has not changed since 1940.[6] Images from Nier's original instrument are available from the National Museum of American History[7] where the pivotal impact for the Manhattan Project is stressed. Nier built his first mass spectrometer in 1936.[8] This instrument featured a 180° magnetic sector field analyzer and soon yielded most important isotopic data.[9] It also served to discover the above-mentioned depletion of ^{13}C in wood, oil, and other biogenic materials.[2] But on principle, the concept even dates back to 1918, when A. J. Dempster advocated the 180° design as 'A New Method of Positive Ray Analysis'.[10] Five years earlier, J. J. Thomson[11] had shown the usefulness of '[. . .] Positive Rays as a Method of Chemical Analysis'. Thomson's demonstration '[. . .] that [. . .] neon is [. . .] a mixture of two gases, one of which has an atomic weight about 20 and the other about 22' finally revealed the isotopy of the elements. According to Budzikiewicz and Grigsby[12] the corresponding apparatus '[. . .] could be called the first mass spectrometer' and had been proposed by Thomson in 1912.[13] In retrospect, this close coevolution of mass spectrometry and isotope analysis certainly does not come as a surprise. But the primordial predominance of isotope analytical applications is possibly less known.

So, IRMS is a well proven, rather traditional methodology. Why then is it perceived as an innovative and modern technique? The reason is probably given by the development of so-called continous flow interfaces. The term is motivated by the viscous flow of carrier gas which continously introduces bands of sample gas into the ion source. This principle facilitates online coupling of a variety of peripheral analytical devices. One consequence is that the signals are transient and appear as peaks. In order to validly calculate isotope ratios from these, dedicated algorithms have been developed.[14]

Prior to the invention of continous flow interfaces, samples were mostly prepared offline, viz. combusted to CO_2 in case of $^{13}C/^{12}C$ analysis. See de Groot's compilations[15,16] for a comprehensive review of both, online and offline techniques. In fact, the continous flow design comes at the cost of increased pressure in the ion source which gives rise to analytical error. This 'pressure effect' was discovered early[17] and has been invesigated systematically, for example, by Fallick and Baxter.[18] Nonetheless, continous flow techniques more or less have become the

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standard option. Mostly, the impairment of accuracy is readily accepted in favor of flexibility and a reduction of minimum required sample amounts.

The term 'continous-flow isotope ratio mass spectrometry' (CF-IRMS) was presumably introduced in 1988 when Preston and McMillan coupled an elemental analyzer to an isotope ratio mass spectrometer via a variable leak.[19] This system already represents an advanced and largely simplified design. It originates from Preston's and Owens' work from 1983.[20] Even earlier, gas chromatography (GC) had been exploited for compound specific isotope analysis (CSIA). To this end, Sano *et al.* employed on-line combustion to CO_2.[21] However, not an IRMS but a single detector mass spectrometer was employed. The signals for *m/z* 44 and *m/z* 45 were recorded and integrated alternately in 0.5 s intervals. Actually, the resulting lack of isotopic accuracy and precision was negligible because the authors were rather interested in the metabolism of ^{13}C labelled drugs. The motivation to incorporate the combustion step was to avoid deconvolution of the mass spectra. The conversion to CO_2 served to normalize the signals.

Thus, Sano *et al.* achieved significant progress for the detection of ^{13}C labelled compounds. Amongst other improvements, the extension to CSIA of ^{15}N labelled compounds was achieved by the design proposed by Matthews and Hayes in 1978.[22] But it took six more years until true integration of GC-combustion and IRMS (GC-C-IRMS) could be demonstrated by Barrie *et al.*[23] This finally allowed for the compound specific analysis of stable isotope ratios at natural abundances. The design featured an open-split interface to the IRMS. In general, it does not look very much different from what today represents state of the art.

The design which has probably been most successful in commercial terms was described by Brand in 1996.[24] One crucial problem in GC-C-IRMS is removal of excess solvent before it can enter the combustion reactor. To this end, the design proposed by Brand features a programmable temporary backflush of the furnace.

Today, commercial designs do well for numerous applications. But it must be noted that GC-C-IRMS does require much more attendance and maintenance than GC-MS. The peripherals are still very complex. The respective users must be aware that they will have to afford some development on their own. In fact, many improvements of the GC-IRMS methodology have been proposed more or less recently.[25–30] Most of these will probably not make it into commercial applications. But this clearly documents that technical development is far from complete.

Terminology

IRMS differs from all other mass spectrometric techniques in that it intrinsically yields amount ratios of isotopic ions. Relative differences between isotope ratios can be determined quite precisely. As an example, differences between $^{13}C/^{12}C$ ratios of, say 0.01111 and 0.01112 can be identified with ease. However, finding an accurate value for one of these ratios is surprisingly difficult. Actually, the corresponding uncertainty is likely to exceed several times the above-mentioned difference of 1×10^{-5}.

Therefore – at least at natural abundances – isotope ratios are typically expressed relative to a standard on so-called δ scales. Due to the relative nature of these scales, the value of the standard on principle may even be unknown. However, the *traceability* of any measurement to the respective standard must be ensured. Moreover, the standard has to be isotopically homogenous.

The $^{13}C/^{12}C$ ratio of a given sample is expressed by the relation $\delta^{13}C_{VPDB} = (R_{Sample}/R_{VPDB}) - 1$. Here, R pertains to the respective $^{13}C/^{12}C$ ratio where VPDB is the carbon-specific international standard. "VPDB" reads "*Vienna Pee-Dee-Belemnite*" and its $^{13}C/^{12}C$-ratio is defined by a homogenized calcite lot named NBS-19.[31,32] By definition, $^{13}C/^{12}C$ of NBS-19 is 1.00195 times that of VPDB. Note that VPDB doesn't exist physically! It is a *virtual standard*. Its name is a reminiscence to PDB (Pee-Dee-Belemnite), a Cretaceous fossil which formerly defined the ^{13}C scale.[1,17] Its $^{13}C/^{12}C$ ratio was calculated as 0.0112372 by Craig in 1957.[17] However, PDB has long been exhausted. VPDB was established to replace PDB and to resemble it as close as possible.

Evidently, the ratio of VPDB defines the zero point of the scale. $\delta^{13}C_{VPDB}$-values are finally multiplied by 1000 which removes insignificant digits. This operation also motivates the use of the (pseudo-)unit 'per mil'. On the VPDB scale, a $^{13}C/^{12}C$ ratio of 0.01111 is thus depleted in ^{13}C by ca. 0.9 per mil *vs* a value of 0.01112.

The relative nature of δ scales must always be kept in mind. As a most important consequence, divisions are not feasible. It follows immediately that measurement uncertainties must not be expressed as coefficients of variation. Due to the design of Nier-type mass spectrometers, the analytical error is largely independent of the measured isotope ratio anyway.

Recently, Coplen compiled guidelines concerning the use of essential equations, terms, and definitions related to stable isotope analytical work.[33] It is categorically recommended to adopt these. The definition and appropriate use of δ scales is also covered there. Alas, for several reasons δ scales are not SI conformable.[34] One of the reasons is that δ values actually express *molar ratios*. By definition, a molar ratio is dimensionless and has unit '1'. Moreover, there has also been some confusion whether the multiplication by 1000 is an innate feature of δ scales or not. However, the SI is clear in that constants must not be part of such definitions. In order to circumvent these problems, Brand and Coplen have most recently suggested to introduce the unit 'urey' (Ur) to express isotope ratios relative to international standards.[35] Accordingly, the problematic expression 'per mil' would translate to 'milliurey' (mUr) while the fundamental calculations stay unchanged.

Currently, CSIA in doping control exclusively is applied to urinary steroids. The purpose of this procedure is probably best termed *source assignment*. Source assignment of some specific matter of interest is a subject of high priority in many disciplines, in particular the environmental sciences. IRMS has become a standard tool here. If still based on offline sample combustion, compound specific – or rather fraction specific – $^{13}C/^{12}C$ analysis has been employed explicitly for this purpose at the latest in 1989.[36] When emphasis is on quantitation of the resepctive contributions, the term *source apportionment* is more appropriate.

In doping control, it is implicitly assumed that there are two distinct sources for urinary steroids: Endogenous steroids and/or synthetic steroids. There is a wealth of literature, which demonstrates that synthetic steroids betray their origin by depleted $^{13}C/^{12}C$ ratios.[37–46] Therefore, low $^{13}C/^{12}C$ ratios are often immediately associated with synthetic origin. However, theoretically and practically the situation is more complicated and this should fundamentlly be considered. First, the $^{13}C/^{12}C$ ratios observed for pharamaceutical steroids are anything but characteristic for synthetic products. Cawley *et al.* found $\delta^{13}C_{VPDB}$

values from −31.8 to −26.6 per mil for authentic testosterone pharmaceuticals.[47] Rather, these values are typical for bulk C-3 plant matter (see Hoefs, 1997[48]). However, little is known about the primordial sources and the chemical processing of steroid pharmaceuticals. Recently, some $^{13}C/^{12}C$ ratios of confiscated black-market steroids have been reported[49] which fall into the range of endogenous urinary steroids from Central Europeans.[50–52]

Terms and definitions for possible sources of urinary steroids are not very consistent. For instance, *endogenous steroid* is mostly used for a compound synthesized physiologically in an organism. But sometimes it is also used to classify pharmaceuticals with chemical structures identical to those of the physiological compounds (cf. WADA, 2004[53]). To avoid confusion, and for the sake of the denotation of *endogenous*, the term should be used exclusively to indicate physiological origin. By contrast, the term *synthetic steroid* should pertain to compounds that have been synthesized or processed chemically. When found in human urine, these must have been administered and their source is necessarily *exogenous*.

Probably, these definitions are not practiced because many do associate *synthetic steroids* with structures that are not found in nature at all, such as stanozolol or trenbolone. However, according to IUPAC, for this property the term *xenobiotic* has already been reserved. Therefore, it will probably be helpful to adopt this definition and to rather discriminate *xenobiotic steroids* from *steroids* as such. The term *steroids* would then exclusively pertain to those congeners found in nature. Possibly, the meaning can be rendered more precise by employing the term *natural steroids*. However, this could be even more confusing, due to possible combinations, such as 'synthetic natural steroids'.

As a guideline, it should be clearly indicated, whether any identifier pertains to the *structure* (natural vs. xenobiotic), to the *source* (physiological vs. synthetic), or to the *origin* (endogenous vs. exogenous). As indicated, neither synthetic nor xenobiotic steroids can be endogenous. Therefore, as a second guideline, the corresponding terminology should be as *parsimonious* as possible.

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