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ACCREDITATION OF STABLE ISOTOPE RATIO METHODS: GUIDELINES FOR THE IMPLEMENTATION OF A QUALITY SYSTEM ACCORDING TO ISO/IEC 17025:2005

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Abstract– Several types of isotopic analysis, carried out using SNIF-^{2}H-NMR and/or IRMS, are now standards or validated methods for testing the authenticity of food products. This study will offer guidelines on implementing a quality system for the accreditation of methods for stable isotope ratio analysis, based on ISO standard 17025. The focus will be on determining repeatability, linearity and uncertainty. Moreover, issues related to accuracy, reference materials, proficiency tests and some unclear areas of official standards will be tackled, and suggestions to improve the effectiveness of this analysis will be provided.

Keywords: accreditation; ISO 17025:2005; stable isotope; official methods; official standards

1. INTRODUCTION

The implementation of stable isotope analysis in the official authenticity control of food began in 1990 and several types of isotopic methods are nowadays standards or validated methods for testing the authenticity of food products.

These methods, carried out using SNIF-2H-NMR (Site-Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance) and/or IRMS (Isotope Ratio Mass Spectrometry), are based on measurement of the stable isotope ratios (i.e. ^{2}H/^{1}H, ^{13}C/^{12}C, ^{18}O/^{16}O, ^{15}N/^{14}N, ^{34}S/^{32}S) of a product or specific components, such as an ingredient or target molecules in the product. They provide information on its botanical and geographical origin.

Public and private laboratories that wish to apply these methodologies for control purposes need to demonstrate the reliability of the analytical data through accreditation, which is a process confirming and certifying competency, authority or credibility.

The general requirements for accreditation are reported in ISO/IEC 17025 [1]. Since 2004, confirmation of the competence and credibility of laboratories following ISO/IEC 17011 [2] has been tested by independent organisations (e.g. Accredia in Italy or DAkkS in Germany).

Accreditation, initially applied only to some sectors (e.g. steel working), is today mandatory in chemistry labs, not only public but in some cases also private. As reported in Directive 89/397/EEC [3], which affects public labs, the official control of foodstuffs means: “inspection by the competent authorities” for the purpose of “guaranteeing fair commercial transactions or protecting consumer interests”.Furthermore member states have to ensure that official laboratories comply with the general criteria for the operation of testing laboratories reported in EN 45001 (now ISO 17025) [1,4]. In the case of private labs, accreditation is mandatory only for laboratories testing wines and oils with PGI, PDO and TSG [5], although it is desirable for any kind of laboratory if the analysis is needed to support the issuing of an official assurance (export certificate). Compliance with ISO 17025 prescriptions can avoid the repetition of analysis in the country of destination; the so-called “One test, one stop”.

The standard provides for management requirements, which include not only the independence of the laboratory or the definition of a «Quality Policy» with internal inspection visits, but also thorough documentary controls and management of activities that do not comply. Control, revision and the issuing of internal and external documents are therefore regulated and strictly managed. Deviation from the requirements can lead to "non conformity" (may cause suspension of the accreditation), "observation" (must be solved) or "comment" (some suggestions to improve the quality of the laboratory).

For chemical laboratories, the standards cover general technical requirements such as guaranteeing the technical competence of staff, specification of the lab's environmental conditions, choice of a general scheme test report (with id number for sample, data and method). In addition, it requires:

1. Method validation. To test the ability of the lab to apply a method two approaches are foreseen: verification (for regulated methods in which repeatability and reproducibility are reported) or validation (unregulated methods).

2. Traceability of the data from acquisition to the test certificate. For this purpose it is mandatory to specify the equipment information (e.g. technical file, software, calibration program).

This study will offer guidelines on implementing a quality system for the accreditation of methods for stable isotope ratio analysis, based on ISO standard 17025. The focus will be on determining reproducibility, repeatability, linearity and uncertainty. Moreover, issues related to accuracy, reference materials, proficiency tests and some unclear areas of official standards will be tackled, and suggestions to improve the effectiveness of this analysis will be provided.

2. EXPERIMENTAL PROTOCOL

2.1 Methods

Currently standard methods recognised by ENV, EU Regulations and OIV are available for wine, fruit juice, cheese and olive oil. These methods can be used for other similar commodities (e.g. the methods for cheese can be applied to meat or butter, and those for fruit juice to any food containing sugar).

2.2 Instrumental platforms

In routine analysis, isotopic ratios are determined using mass spectrometry as well as nuclear magnetic resonance.

For $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$ ratios, Isotope Ratio Mass Spectrometry (IRMS) is applied. It is interfaced with an equilibration system for $^{18}O/^{16}O$ in food water, with an elemental analyser for $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ in organic matrices and with a pyroliser for $^{18}O/^{16}O$ and $^{2}H/^{1}H$ analysis in organic matrices.

^{2}H-SNIF-NMR (^{2}H-Site-specific Natural Isotope Fractionation - Nuclear Magnetic Resonance) is used for site-specific $^{2}H/^{1}H$ analysis of ethanol.

Stable isotope ratios (e.g. $^{18}O/^{16}O$ in water) may be analysed using Cavity Ring Down laser Spectroscopy (CRDS), but its application to complex matrices such as food has been demonstrated in only a few cases [6].

2.3 Data expression and reference materials

Isotope ratios measured using IRMS reflect deviations of the sample ratio from the international standard ratio (VPDB: Vienna – Pee Dee Belemnite for $\delta^{13}C$, Air-N_2 for $\delta^{15}N$ and VCDT: Vienna - Canyon Diablo Troilite for $\delta^{34}S$, VSMOW (Vienna Standard Mean Ocean Water) for $\delta^{18}O$ and $\delta^{2}H$). The results are reported in delta notation [7] and calculated according to the following general equation [8]:

$$\delta i\, E = \frac{(i\, RSA - i\, RREF)}{i\, RREF}$$

where i is the mass number of the heavier isotope of element E (for example ^{13}C);

RSA is the respective isotope ratio of a sample (such as, for C: number of ^{13}C atoms/number of ^{12}C atoms or as an approximation of $^{13}C/^{12}C$);

RREF is the relevant internationally recognised reference material.

The delta values are multiplied by 1000 and are expressed in units "per mil" (‰).

Using ^{2}H-SNIF-NMR, the $^{2}H/^{1}H$ or D/H ratio in ethanol is analysed site-specifically in methyl, $(D/H)_{I}$ and methylene, $(D/H)_{II}$, sites of ethanol. $(D/H)_{I}$ and $(D/H)_{II}$ are measured by comparing the height of the decoupled NMR peak with that of the internal reference material (usually tetramethylurea) and the D/H ratios are conventionally expressed in parts per million (ppm).

3. RESULTS AND DISCUSSION

Repeatability (r) is "the variation in measurements taken by a single person or instrument on the same item, under the same conditions, and in a short period of time". A specific standard [9] requires a minimum number of 10 repetitions, control of normality (e.g. Shapiro-Wilks 5%) and the presence of an outlier (e.g. Huber 5%) and calculation of standard deviation (SD) and t student following the equation:

r = SD*tstudent* radq(2)

The values should cover the whole range of application. For example, in the case of $\delta^{13}C$, if the lab's working range is between -30‰ and -10‰ a minimum of three points should be explored (e.g. -10‰, -23‰ and -30‰). Repeatability must be recalculated periodically. Every five years is advisable for isotopic methods.

The SD of repeatability is generally 0.1‰ for $\delta^{13}C$ and $\delta^{15}N$ in organic compounds and for $\delta^{18}O$ in water, 0.2‰ for $\delta^{34}S$ and $\delta^{18}O$ in organic compounds and 1‰ for $\delta^{2}H$.

Reproducibility (R) is "the ability of an entire analysis of an experiment or study to be duplicated, either by the same researcher or by someone else working independently". Several repetitions for the same sample over time are prescribed by the standard [9]. As best practice in the lab, an internal reference material (e.g. wine, protein or water) should be chosen and considered as a sample to be analysed once a month. In this case again, normality and outliers are checked and 'R' is computed as for 'r'. Moreover, the values of the internal reference material are used to build control charts, which are graphs of test results in relation to time or sequences of measurements, with limits within which results are expected to lie when the analysis is in a state of statistical control.

Inter-laboratory reproducibility is computed using the average SD of inter-laboratory collaborative studies or the target SD of Proficiency Tests. As an accreditation requirement, the laboratory must indeed participate in proficiency tests that comply with the ISO / IUPAC / AOAC International Harmonised Protocol for Proficiency Testing of analytical laboratories. There are two internationally recognised schemes for isotopic analysis of food. These are FIT-PTS (Food analysis using Isotopic Techniques – Proficiency Testing Scheme) organised by Eurofins Scientific and with external evaluation of the data by a scientific committee of experts in the field of food analysis proficiency testing. The second is FIRMS-PTS, organised by the FIRMS (Forensic Isotope Ratio Mass Spectrometry) Network in collaboration with the UK Laboratory of the Government Chemist (LGC) Standards Proficiency Testing. There is also a further proficiency test, called KPT organised by Agroisolab (DE). A wide range of matrices is considered and a large number of laboratories are involved.

In the last few years, different ways of calculating uncertainty have been proposed [10]. Calculation of combined uncertainty is very difficult. All the components that could have an effect on uncertainty have to be considered (e.g. uncertainty of the instrument, of extraction or due to the dexterity of different technicians) [11]. The use of reproducibility from QC data is widespread but is unsatisfactory due to the absence of accuracy input. The use of "Nordtest" [12-13] seems to be the best way to calculate uncertainty. The test combines QC data with the results of the Proficiency Test (PT), which could be considered the best tool to ensure the quality of the data. Dunn et al. (2015) [14] recently published guidance for calculating measurement uncertainty of stable isotope ratio delta values.

To comply with ISO 17025, specification of the different technical steps in each method is mandatory. The choice of whether to use single, double or more measurements, data expressions (e.g. mean or median), the number of significant digits, the position of standards in the analytical run (e.g. every 10 samples) or correction of linearity is the task of the lab. In our specifications, the result is the mean of two repetitions and it is expressed with one significant digit for C, N, O and S and without any digit for H. To normalise the data, ideally two reference materials or calibrated working standards with isotope values covering the variability range of the samples should be used for each element, as suggested by IUPAC [15].

A big drawback for data normalisation is the lack ofcertified reference materials particularly for some isotopic analysis such as $^2H/^1H$. In the case of the protein matrix, due to the presence of exchangeable hydrogen, correct measurement would require matrix-matched protein standards, currently unavailable.

4. CONCLUSIONS

The increasing demand by private and public customers for food controls through the application of stable isotope analysis requires accreditation of laboratories. This goal, albeit demanding, is the only way to certify the competency, authority and credibility of a stable isotope lab.

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