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GENERAL PAPER

K. Heydorn

The interpretation and use of stability data in the certification of reference materials

The author is a Member of the European Commission SM&T Scientific Evaluation Group on the Certification of Reference Materials

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K. Heydorn (✉)
Isotope Division, Risø National Laboratory, DK-4000 Roskilde, Denmark
Tel.: +45-4677-5357;
Fax: +45-4677-5347;
e-mail: heydorn@risoe.dk

Abstract Stability tests are carried out on candidate reference materials in order to ascertain that the certification values continue to be valid a reasonable time after completion of the certification analysis. These tests are also used for recommending storage conditions, as well as the duration of storage before certification values need be rechecked. BCR (Community Bureau of Reference) reference materials do not normally have an expiry date, but rely on stability monitoring throughout the lifetime of the certified material. The 1997 version of the BCR Guidelines for the production and certification of reference materials does, however, take into account the necessity of limiting the validity of a certification, when degradation of the material during storage cannot be ignored. This paper discusses an example of significant degradation taking place between the time of completion of the certification analysis and the issue of a formal certificate. Various options are presented together with an account of their influence on the certified values and their uncertainties.

Key words Certified reference materials · Stability · Validity

Introduction

Although the stability of a reference material is one of its most important characteristics, many producers of such materials still do not provide adequate information on this matter.

Stability tests should be carried out on candidate reference materials in order to ascertain that the certification values are valid for a reasonable time after completing the certification analysis. They also serve as a basis for recommendation of storage conditions, as well as the duration of storage before the certified values need be rechecked.

With the possible exception of corrosion-resistant alloys, glasses or ceramics, all materials are potentially unstable under normal conditions of storage if kept long enough.

Certified reference materials (CRMs) should therefore be (1) selected according to their expected stability – but the need for matrix-matching CRMs often overrules this consideration, (2) stored under conditions of guaranteed stability – but economic considerations make it unfeasible to store all materials at liquid-nitrogen temperature, (3) continually monitored during storage – but the skill and competence needed for these analyses may not be available to the vendor.

In all cases there is a need to predict how certification values change with time, as well as to estimate the uncertainty associated with such a prediction. The availability of CRMs with potentially unstable determinands in a matrix that is not inherently stable makes this part of a certification project increasingly important.

Materials and methods for stability tests

Stability tests are required for BCR (Community Bureau of Reference) reference materials [1] whenever the certified entity or the matrix material might suffer even slight degradation or change with temperature, time, exposure to air, humidity, light, or other conditions likely to be encountered in practice.

Instability caused by degradation of a biological matrix would lead to an apparent increase in the trace element concentrations with time, while the diffusion into the container wall of some elements in aqueous solution would result in a decrease of the certified concentration. In the case of vitamins in foods [2], both the matrix and vitamins could be degraded with time, and the resulting trend in concentration might be either positive or negative.

Stability tests are carried out at three different temperatures, typically at freezer temperature (−18 to −30 °C), refrigerator temperature (+4 °C) , and room temperature (18–25 °C), using one particular laboratory and a method of good reproducibility and in statistical control.

Such stability data were obtained over a period of almost 5 years for the certification project of vitamin C in milk powder (CRM 421) and Brussels sprouts (CRM 431). Results in Table 1 for CRM 431 were obtained by the same method and the same laboratory that made the homogeneity test, resulting in an experimentally determined reproducibility coefficient of variation of 3.5%.

Results in Table 2 for CRM 421 were obtained by another method used in the certification study, but with a reproducibility coefficient of variation of 5%.

Table 1 Determinations of vitamin C in g/kg in samples of CRM 431 (Brussels sprouts) stored at different temperatures [2]

Time (months)	Temperatures −30 °C	−18 °C	4 °C
0	4.83	4.83	4.83
3	5.23	5.18	4.99
6	5.03	5.10	4.74
18	4.99	4.76	4.70
24	4.85	4.62	4.26
30	4.51	4.57	4.16
39	4.67	4.62	4.28
45	4.67	–	–
58	4.39	–	–
Correlation	$r=-0.83$	$r=-0.81$	$r=-0.90$

Table 2 Determinations of vitamin C in g/kg in samples of CRM 421 (milk powder) stored at different temperatures [2]

Time (months)	Temperatures −18 °C	+4 °C	18 °C
0	0.748	0.748	0.748
6	0.727	0.743	0.673
12	0.755	0.736	0.674
18	0.671	0.641	0.586
24	0.769	0.635	0.537
39	0.744	–	–
52	0.743	–	–
58	0.680	–	–
Correlation	$r=-0.27$	$r=-0.90$	$r=-0.97$

Correlation coefficients clearly demonstrate a lack of stability for CRM 431 at all temperatures and for CRM 421 at least at the two higher temperatures.

Certification results and discussion

In cases where unmistakable instability has been observed, it is imperative to be able to predict the change of concentration with time under actual conditions of storage. Only then is it possible to certify values that are of any use to the analytical community.

Assuming that the decomposition of ascorbic acid takes place as a first-order reaction, the Arrhenius equation is well suited for this purpose.

The rate constant

$$k=\frac{\delta \ln(c)}{\delta t} \tag{1}$$

with c as the concentration of ascorbic acid and t as time, can be expressed as a function of temperature T

$$k=\mathrm{e}^{\alpha-\beta/RT} \tag{2}$$

where β is the activation energy and α is a constant.

Integration of Eq. 1 gives

$$\ln(c)=k\cdot t+\ln(c_0) \tag{3}$$

which means that k may be determined for each temperature by a linear regression of $\ln(c)$ on t.

In exactly the same way the parameters α and β can be determined by linear regression

$$\ln k=\alpha-B/T \tag{4}$$

of $\ln k$ on T^{-1}, using $\beta=R\cdot B$.

Based on the data in Table 1 we find $\alpha=-0.80$, and

$\beta=10.7$ kJ/mol

An analysis of variance is presented in Table 3 on the results for −30 °C using a regression slope determined by Eq. 4. The residual variance estimate corresponds to a coefficient of variation of 3.25%, in excellent agreement with the reproducibility of the analytical method. This means that the information content of these data is adequately represented by the assumed model for decomposition, and that the model therefore may be con-

Table 3 Analysis of variance for fit of regression line to stability data for CRM 431

Source	Sum of squares	*d.f.*	Variance estimate	$F_{1,7}$	P
Regression	0.3970	1	0.3970	16.72	0.005
Residual	0.1662	7	0.0237		
Total	0.5632	8			

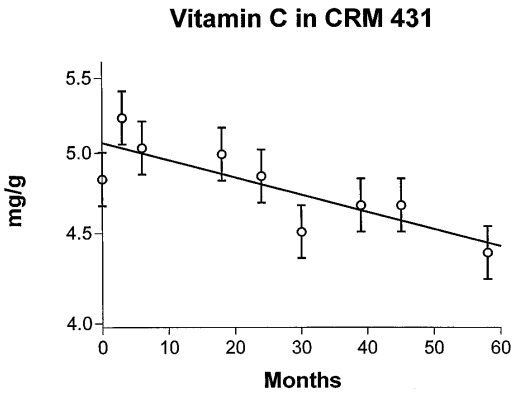

Fig. 1 Stability data for ascorbic acid in lyophilized Brussels sprouts powder stored for 5 years at −30 °C. The uncertainties of the experimental points are presented as the reproducibility coefficient of variation determined from the homogeneity test. The *line* represents the Arrhenius equation determined from stability data in Table 1

sidered well suited for predicting the concentration in a period after certification.

Individual results and their uncertainties are plotted on a logarithmic scale together with the trend line in Fig. 1.

Stability data for CRM 421 show significant instability only at temperatures above zero, and, with the modest number of data together with the relatively poorer precision of the measurements, the determination of the parameters in the Arrhenius equation becomes much more uncertain.

A comparison of stability data for CRM 431 and CRM 421 at the common temperature of −18 °C is nevertheless possible by comparing the squared deviations of the observations from the CRM 421 regression line with the deviations from a line with a slope corresponding to the CRM 431 data. This is done by the analysis of variance presented in Table 4, which shows that the difference between the slopes for the two materials at −18 °C is probably significant.

Individual results and their uncertainties for CRM 421 are shown in Fig. 2 on a logarithmic scale together with the trend line for CRM 431 corresponding to a temperature of −18 °C. This illustrates that the stability of the vitamin C content in milk powder is better than in Brussels sprouts, which again demonstrates that even for the same chemical species it is not permissible to infer stability for one material from observations made on another.

Certified values

The influence of the results of stability tests on the certified values depends upon whether the certificate carries a specified period of validity or remains valid until recalled. In either case there is little help to be found in the literature about how this is done either by BCR [1] or NIST (National Institute of Standards and Technology) [3].

BCR reference materials do not normally have an expiry date, but rely on stability monitoring throughout the lifetime of the certified material. The 1997 version of the BCR Guidelines for the production and certification of reference materials does, however, take into account the necessity for limiting the validity of a certification when degradation of the material during storage cannot be ignored.

The simplest solution is to certify the concentrations and their uncertainties with validity at the time when certification analysis took place. For the two reference materials CRM 421 and 431 these values are presented in the top row of Table 5. While this might be legally satisfactory, it is of little help to the user unless information is provided that enables the user to estimate the value and its uncertainty at the time of use.

A better solution is to present certified values that are valid at the time when the certificate is signed and the CRM becomes available to the users. This requires some correction of the original values, as well as their

Table 4 Analysis of variance for fit of regression line for CRM 431 to stability data for CRM 421

Source	Sum of squares	*d.f.*	Variance estimate	$F_{1,6}$	*P*
Slope difference	0.009495	1	0.0095	7.90	0.03
Residual	0.007212	6	0.0012		
Total[a]	0.016707	7			

[a] Sum of squared deviations from CRM 431 regression line

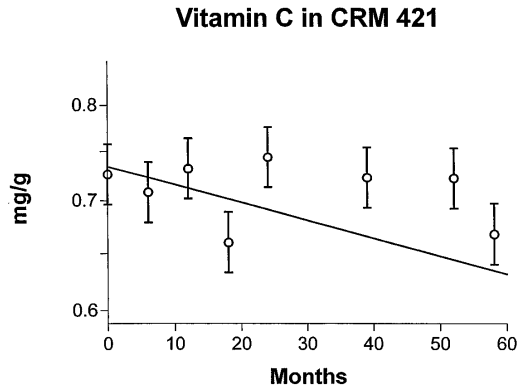

Fig. 2 Stability data for ascorbic acid in milk powder stored for 5 years at −18 °C. The uncertainties of the experimental points are presented as the residual coefficient of variation given in Table 4. The *line* represents the Arrhenius equation determined from stability data in Table 1

Table 5 Certified values for vitamin C for different applications of stability data with or without time limit

Certification dates	CRM 421		CRM 431	
	Value	Uncertainty	Value	Uncertainty
May 1994	769	12	4.83	0.24
March 1997	754	25	4.49	0.54
Valid until March 1999	744	58	4.36	0.34

uncertainties, in all cases where a significant change with time has been observed. In other cases where a change with time has not been definitely established but cannot be excluded, it has been proposed [4] that a correction be made anyway, while assuming an uncertainty of 100% of the correction. These values are presented in the second row in Table 5.

In all cases where a certificate is valid until recalled, information must be presented that states how the stability is being monitored during storage and on which criteria a date for recall is based. This is not required in the case where the certificate is valid for only a stated period.

In this case NIST seems to prefer extrapolation to the midpoint of the period of validity and to assume a uniform distribution of sales over the period of validity. In that case a standard uncertainty may be estimated as

$$\frac{b \cdot N}{2\sqrt{3}}$$

where b is the slope and N is the length of the period of validity.

The uncertainty of the estimated loss from the date of analysis to the certification date is estimated from the uncertainty of the slope of the logarithmic regression lines.

The combined standard uncertainty is multiplied by a coverage factor 2, and results are presented in the last row of Table 5.

For CRM 421 the uncertainty intervals are almost completely determined by the results of the stability test, and it makes a considerable difference whether the uncertainty is determined from the magnitude of the correction or from its uncertainty.

This also applies to CRM 431, but with the opposite result, because here the uncertainty of the slope is much less than the magnitude of the correction.

In the end, both certified values with 2 years validity have a total uncertainty of just below 8%, which would be satisfactory for most practical uses.

While any of the three methods of certification could be acceptable, as long as the implications are carefully described, the last method of limited validity would seem preferable from the point of view of the user who seeks traceability under conditions of well-defined uncertainty. The GUM (Guide to expression of Uncertainty Measurement) [5] requires analytical results to be corrected for all known sources of bias, and the uncertainty of the correction be taken into account. For certified values valid only at a particular point in time lack of stability makes this requirement impossible to fulfil.

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