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Discussion

Evaluation of the measurement uncertainty: Some common mistakes with a focus on the uncertainty from linear calibration

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ABSTRACT

The rational strategy in the evaluation of analytical measurement uncertainty is to combine the “whole method” performance data, such as precision and recovery, with the uncertainty contributions from sources not adequately covered by those data. This paper highlights some common mistakes in evaluating the uncertainty when pursuing that strategy, as revealed in current chromatographic literature. The list of the uncertainty components usually taken into account is discussed first and fallacies with the LOD- and recovery uncertainties are noted. Close attention is paid to the uncertainty arising from a linear calibration normally used. It is demonstrated that following a well-known formula for the standard deviation of an analytical result obtained from a straight line calibration leads to double counting the precision contribution to the uncertainty budget. Furthermore, the precision component itself is often estimated improperly, based on the number of replicates taken from the precision assessment experiment. As a result, the relative uncertainty from linear calibration is overestimated in the budget and may become the largest contribution to the combined uncertainty, which is clearly shown with an example calculation based on the literature data.

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1. Introduction

There is a general trend in modern analytical science and service, encouraged by laboratory accreditation requirements [1], towards providing analytical measurement results with their uncertainty, a key indicator of both reliability and fitness for purpose. In view of lack of a standardized procedure of the uncertainty evaluation, the task is far from being simple; it requires deep understanding of the measurement process and mastery of statistical tools, in which most analytical chemists do not take an active interest. Eventually it leads to incorrect evaluation, with the resulting uncertainty and the conclusions about the relative magnitudes of its constituents being questionable. This is particularly the case with regard to publications on measurement uncertainty in chromatographic analysis constituting perhaps the most part of the analytical uncertainty evaluations published.

The problem of the measurement uncertainty evaluation is complicated by the fact that there is no single way to do it, which would be universally recommended in all practical situations. Two principal approaches to measurement uncertainty are the *modelling* (or “bottom-up”) approach set forth in the basic *Guide to the expression of uncertainty in measurement* (GUM) [2] and the *empirical* (or “top-down”) approach that encompasses three “whole method” performance methodologies: single-laboratory validation, interlaboratory studies, and proficiency testing, as outlined in the EUROLAB report [3].

The modelling approach based on a thorough analysis of the measurement system, modelling, and propagating individual uncertainties through the measurement model relating the measurand to other quantities upon which it depends is rather difficult to implement in chemical analysis. Actually, an exhaustive model of a chemical measurement process is not always possible to build because of complexity of the process. Even if the model is considered known, the difficulty consists in evaluating independent uncertainty components each corresponding to an input quantity in the model.

On the other hand, the empirical approach that is based on the estimated “whole method” performance makes allowance for the all-inclusive variability at once, but immediately poses the question of sufficiency, the extent of coverage, and “extrapolation” from the specific conditions of the performance study to the actual conditions.

The helpful adaptation of the basic approach [2] to analytical chemical problems, made in the EURACHEM/CITAC Guide [4], allowed using the “whole method” performance parameters, such

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as precision and recovery, alongside with individual uncertainty components emerging from the model. The rational strategy is thus to combine the performance information, commonly obtained during in-house validation studies, with uncertainty contributions from sources not adequately covered in those studies. This is a kind of combination of the bottom-up uncertainty approach and in-house validation data.

With this strategy, however, the risk is run to take account of a source of uncertainty twice: once as an individual contribution and second one within the measure of the overall performance. Here we face a problem of double counting, an issue often overlooked in estimating the uncertainty of analytical measurement.

In the present paper, the problem of double counting is highlighted with regard to the uncertainty arising from a linear calibration function normally used. Calibration of the measurement system with reference materials is known to be an important point in instrumental analysis. However, is the uncertainty from calibration really the largest contribution to the combined uncertainty as suggested in many chromatography uncertainty evaluations published? As is seen from the discussion that follows, this finding is a consequence of inadequate evaluation rather than may reflect the reality.

2. The relevant uncertainty components

As may be seen from current publications [5–14],[1] the typical list of the component uncertainties to be taken into account in chromatographic analysis, following the combined bottom up/in-house validation approach, includes (as relative contributions): (i) the uncertainty associated with sample preparation, $u(\text{sample})_{\text{rel}}$, (ii) the uncertainty associated with calibration (including calibration standards uncertainty), $u(\text{calibr})_{\text{rel}}$, (iii) the uncertainty arising from random effects (precision), $u(\text{prec})_{\text{rel}}$, and (iv) the uncertainty associated with recovery, $u(\text{rec})_{\text{rel}}$, so that the expression for the combined standard uncertainty will run as follows:

$$(u_c)_{\text{rel}} = \sqrt{u(\text{sample})_{\text{rel}}{}^2 + u(\text{calibr})_{\text{rel}}{}^2 + u(\text{prec})_{\text{rel}}{}^2 + u(\text{rec})_{\text{rel}}{}^2} \quad (1)$$

Some authors [15–19] take also account of the *limit of detection* (LOD) as a separate source (an element) of uncertainty, quantified in relative form as the reciprocal of the analyte level: $u(\text{LOD})_{\text{rel}} = \frac{\text{LOD}}{c}$. The reasoning of the proponents of this view [15] such as "the uncertainty is 100% when the concentration level is equal to LOD" does not seem to be convincing enough to consider the LOD to be a source of uncertainty in analytical measurement. The LOD is not of direct relevance to the uncertainty evaluation. In fact, involving the term $\frac{\text{LOD}}{c}$ in Eq. (1) is an attempt to model the variation of the combined uncertainty with the level. The general mathematical expression proposed for this aim (termed *uncertainty* or *characteristic function* [20]) is based on a two-component model combining both constant (α) and proportional (βc) effects, which in case of the relative uncertainty takes the form: $\frac{u}{c} = \sqrt{\frac{\alpha^2}{c^2} + \beta^2}$. It is evident that the squared LOD-component suggested to be included in Eq. (1) acts as the first term in this model, intended merely to provide rapidly increasing values as concentrations fall towards zero. To sum it up, the dependence on the level (where a wide working range is covered) should be accounted for in the precision component $u(\text{prec})_{\text{rel}}$, without invoking the LOD.

Dictated by common sense, the constituents of the combined uncertainty on Eq. (1) are not objectionable, but their quantification in the evaluations cited rises questions.

For example, the recovery term $u(\text{rec})_{\text{rel}}$ reckons with the situation where the recovery factor or, more precisely, the "apparent recovery" [21], is (reciprocally) involved in the measurement equation so that the result of analysis is explicitly corrected for observed bias. Then the uncertainty associated with (corrected) recovery is given by [22]:

$$u(\text{rec})_{\text{rel}} = \sqrt{\frac{(s_{\text{rec}})^2_{\text{rel}}}{m} + (u_{\text{ref}})^2_{\text{rel}}} \quad (2)$$

where $(s_{\text{rec}})_{\text{rel}}$ is the relative standard deviation of the results during estimation of the recovery, m is the number of replicates performed, and $(u_{\text{ref}})_{\text{rel}}$ is the relative standard uncertainty of the reference value carried by a CRM or spiked material used for estimating the recovery.

Very often, however, even bad recoveries, such as 75–125% [23], are considered acceptable. (There must be technical and economic reasons that would justify a correction [24]; otherwise the correction is not applied.) In this case many authors simply omit the term $u(\text{rec})_{\text{rel}}$ considering that is all there is to it. However, an additional uncertainty contribution accounting for that uncorrected bias should be taken into consideration; a number of approaches for doing this was discussed in the literature (see the review articles [24,25]). As a matter of fact, the need for increasing the uncertainty to account for an estimated but uncorrected bias is ignored in papers on the uncertainty in chromatographic analysis.

It is generally assumed that the separate contributions in the uncertainty assessment budget expressed by Eq. (1) are not "overlapped". For instance, the uncertainty arising from random effects, if assessed under repeatability or even day-to-day precision conditions with calibration being unchanged, does not include the calibration uncertainty that needs therefore to be estimated separately. "Overlapping" may be difficult to avoid as is specifically the case with the contributions derived from precision and recovery studies. As is seen from Eq. (2), the uncertainty in the recovery estimate inevitably includes the precision of the observed mean value that is used in calculation of the recovery. Bearing this in mind, some authors [6,18,26,27] do not include the term $u(\text{prec})_{\text{rel}}$ into the budget in addition to the $u(\text{rec})_{\text{rel}}$ thus trying to avoid double counting.

In the following, we will focus mainly on the two uncertainty components in Eq. (1), $u(\text{calibr})_{\text{rel}}$ and $u(\text{prec})_{\text{rel}}$. Usually, it does not present any difficulty to quantify the component $u(\text{sample})_{\text{rel}}$ that incorporates contributions from the sample mass or volume measurement as well as from other operations during a sample preparation procedure (which are not covered by the precision/recovery experiment).

3. Calibration and its associated uncertainty

In a typical case of multipoint calibration, either external or internal, the linear calibration function $y = a + bx$ is used relating an analytical signal y to the analyte content x in the prepared sample solution, with the peak area or peak height, or the respective signal-ratio as the analytical signal. Initially, the least-squares regression is performed on a set of calibration data (x_i, y_i) to derive the parameters a and b of the best fit line. Thereafter the inverse of the calibration function

$$x_0 = \frac{y_0 - b}{a} \quad (3)$$

can be utilized to infer (or as they often say "to predict") the unknown analyte content x_0 corresponding to an observed response y_0.

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[1] Reports on quantitative analysis using chromatographic techniques with detailed uncertainty evaluations following the combined approach above, published from 2010 to 2016, have been taken into consideration only.

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