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Rigorous evaluation of chemical measurement uncertainty: Liquid chromatographic analysis methods using detector response factor calibration

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Abstract

Chemical measurement methods are designed to promote accurate knowledge of a measurand or system. As such, these methods often allow elicitation of latent sources of variability and correlation in experimental data. They typically implement measurement equations that support quantification of effects associated with calibration standards and other known or observed parametric variables. Additionally, multiple samples and calibrants are usually analyzed to assess accuracy of the measurement procedure and repeatability by the analyst. Thus, a realistic assessment of uncertainty for most chemical measurement methods is not purely bottom-up (based on the measurement equation) or top-down (based on the experimental design), but inherently contains elements of both. Confidence in results must be rigorously evaluated for the sources of variability in all of the bottom-up and top-down elements. This type of analysis presents unique challenges due to various statistical correlations among the outputs of measurement equations. One approach is to use a Bayesian hierarchical (BH) model which is intrinsically rigorous, thus making it a straightforward method for use with complex experimental designs, particularly when correlations among data are numerous and difficult to elucidate or explicitly quantify. In simpler cases, careful analysis using GUM Supplement 1 (MC) methods augmented with random effects meta analysis yields similar results to a full BH model analysis. In this article we describe both approaches to rigorous uncertainty evaluation using as examples measurements of 25-hydroxyvitamin D₃ in solution reference materials via liquid chromatography with UV absorbance detection (LC-UV) and liquid chromatography mass spectrometric detection using isotope dilution (LC-IDMS).

1. Introduction

In a previous publication [5] we described a chemical purity assessment via quantitative Nuclear Magnetic Resonance spectroscopy, for which uncertainty evaluation is particularly suited to a Bayesian analysis using a hierarchical model. This approach is able to combine top-down and bottom-up elements of the uncertainty in a natural and straightforward manner. In this paper, we consider examples for two other common organic chemical analytical techniques, liquid chromatography with UV absorbance detection (LC-UV) and liquid chromatography with mass spectrometric detection using isotope dilution (LC-IDMS). The analyses described herein were performed to determine the mass fraction of 25-hydroxyvitamin D₃ (25(OH)D₃) in a methanolic reference standard solution. This reference standard and associated mass fraction value are used for instrumental calibration of

analytical methods for vitamin D metabolites. Compared to [5], the simpler experimental designs and less complicated measurement equations of these examples make it possible to carry out the uncertainty evaluations using standard GUM Supplement 1 Monte Carlo analysis [2]. This GUM approach may be preferred because user-friendly computational systems are readily available, including freely-accessible systems [3]. For the LC-IDMS example we show agreement amongst results from both a GUM-type analysis based on the measurement equation and a Bayesian analysis based on a hierarchical statistical model. The Bayesian analysis is much simpler, particularly when the experimental design is more complex with respect to calibration hierarchy, as in [5], or there are a greater number of experimental factors and assumptions that induce statistical correlations. While systems enabling Bayesian analysis are readily and freely available [8], the implementation of novel models requires more user effort.

First, consider the mass fraction (w) determination of chemical species (A) in a solution via LC-UV. Using an externally calibrated response factor approach, the measurement equation is

$$w_A = R_{A_s} \times RF, \quad (1)$$

where

$$RF = \frac{w_{A_c}}{R_{A_c}} \times P_{A_c}, \quad (2)$$

with

R_{A_s} = peak area response of analyte species, A, in unknown sample solution

RF = UV absorbance-response factor of A

w_{A_c} = known mass fraction of A (from chemical reference material) in calibration solution

R_{A_c} = peak area response of A in calibration solution

P_{A_c} = known mass purity of A neat chemical reference material in calibration solution.

The determination of w_A requires two separate experiments; a calibration experiment to estimate the response factor RF , and subsequent measurement of the unknown amount of analyte A. Usually, at least some of the input quantities of both (1) and (2) have associated uncertainties. For evaluation of a single calibration solution, one could use standard GUM [1] or GUM Supplement 1 Monte Carlo analysis [2, 3] to propagate the uncertainties through (2) and obtain an uncertainty or a probability distribution for RF . For measurement of a single sample solution with unknown amount of A, one could then similarly propagate the results of RF and an uncertainty of R_{A_s} through (1) to obtain uncertainty or a probability

distribution for the measurand w_A . In most cases however, there will be multiple (n) samples of unknown A, thus instead of using (1), the measurement equations are

$$w_{A_i} = R_{A_{Si}} \times RF, \quad i=1, \dots, n \quad (3)$$

and (2). It becomes necessary to aggregate different values w_{A_i} and to compute the uncertainty of the final consensus estimate. There are various forms that this estimate could take. One possibility is a weighted average. Another, generally more preferable possibility because it accounts for uncertainty in the individual estimates as well as between-sample uncertainty, is to apply a random effects model [4] to produce the consensus. In this calculation it is important to realize that the w_{A_i} are positively correlated due to multiplication by the term RF , and that ignoring this correlation results in underestimating the final uncertainty. The correlation is easy to compute. Denoting the mean value of $R_{A_{Si}}$ by $\mu_{A_{Si}}$, the variance of $R_{A_{Si}}$ by $\sigma_{A_{Si}}^2$, the value of RF by μ_{RF} , and the variance of RF by σ_{RF}^2 , the correlation between the mass fraction for replicate i (w_{A_i}) and replicate j (w_{A_j}) is

$$\rho(w_{A_i}, w_{A_j}) = \frac{\mu_{A_{Si}} \mu_{A_{Sj}} \sigma_{RF}^2}{\sqrt{(\sigma_{RF}^2 \sigma_{A_{Si}}^2 + \mu_{RF}^2 \sigma_{A_{Si}}^2 + \mu_{A_{Si}}^2 \sigma_{RF}^2)(\sigma_{RF}^2 \sigma_{A_{Sj}}^2 + \mu_{RF}^2 \sigma_{A_{Sj}}^2 + \mu_{A_{Sj}}^2 \sigma_{RF}^2)}} \quad (4)$$

This correlation is positive so long as the mean values of the peak area responses are positive and the variances are all non-zero. Appendix 1 shows the derivation of (4) in detail. (It is interesting to note that the denominator is a square root of the product of the variances of

w_{A_i} and w_{A_j} which the usual GUM procedure would approximate as $\mu_{RF}^2 \sigma_{A_{Si}}^2 + \mu_{A_{Si}}^2 \sigma_{RF}^2$ and $\mu_{RF}^2 \sigma_{A_{Sj}}^2 + \mu_{A_{Sj}}^2 \sigma_{RF}^2$. The additional terms could be added as a second order contribution to the Taylor approximation.)

In most cases, chemists use multiple (I) calibrants from which response factors (RF_i , $i = 1, \dots, I$) are derived. Generally, these have been averaged to obtain

$$\overline{RF} = \frac{1}{I} \sum_{i=1}^I RF_i \quad (5)$$

which is used in equation (3) instead of RF , and thus the variance of $\overline{RF}$ ($\sigma_{\overline{RF}}^2$) is necessary for computation of (w_{A_i} , w_{A_j}). In some cases, P_{AC} in expression (2) is the same for all response factor determinations, and so introduces a positive correlation amongst the RF_i that needs to be accounted during calculation of $\sigma_{\overline{RF}}^2$. Therefore, it is clear that a careful determination of the correlations introduced in the two measurement equations and the

experimental design is necessary for a complete evaluation of uncertainty when using a GUM or GUM Supplement 1 analysis.

In [5] we described an example of uncertainty evaluation of a complex chemical experiment that was evaluated using a Bayesian hierarchical (BH) model; that is, an observation equation [6] instead of the usual GUM-style analysis. The advantage of the observation equation approach is that the various correlations among the variables of the measurement equation and experimental design are a natural part of the statistical model, and so do not need to be directly calculated. A possible disadvantage of this approach is that calculations generally need to be performed using Markov Chain Monte Carlo, and so need proficiency with specialized software such as OpenBUGS [8]. In the current application, the experimental design and measurement equations are relatively simple, and so it is possible to calculate the necessary correlations in a Monte Carlo analysis as in [2]. Section 2 illustrates this for the mass fraction determination of 25-(OH)D₃ (i.e., species A) in a methanolic solution by LC-UV.

As a second example, consider an alternate approach to the determination of 25-(OH)D₃ mass fraction via LC-IDMS, using an isotopically-enriched internal standard (IS) for experimental response factor determination. The corresponding measurement equations for the determination of mass fraction (w) of chemical species A by this approach are:

$$w_A = \frac{w_{IS} \times R_{AS}}{R_{IS}} \div RRF, \quad (6)$$

$$RRF = \frac{w_{Ac} \times R_{ISc}}{w_{ISc} \times R_{Ac}} \times P_{Ac} \quad (7)$$

where,

w_{IS} = known mass fraction of the isotopically-labeled internal standard species IS added to the sample from an isotopically-enriched chemical reference material

R_{AS} = peak area response of analyte species A in sample

R_{IS} = peak area response of analyte species IS in sample

RRF = relative mass detection response factor of A and IS

w_{Ac} = known mass fraction of A (from chemical reference material) in calibration solution

w_{ISc} = known mass fraction of IS in calibration solution

R_{Ac} = peak area response of A in calibration solution

R_{ISc} = peak area response of IS in calibration solution

P_{AC} = known mass purity of A neat chemical reference material in calibration solution.

As in the previous example using an external standard calibration approach, the RRF and the w_A are estimated through separate experiments. Multiple samples of unknown amount of A will produce mass fraction results that are positively correlated due to multiplication by RRF in (6). In this procedure there is also an additional correlation due to the use of IS from the same chemical source (the isotopically-enriched reference material) that is implemented in both experiments to determine RRF and w_A . Section 3 shows uncertainty analysis of this type for the determination of mass fraction of 25-(OH)D₃ by LC-IDMS.

Conclusions are provided in section 4, and various code used to compute these analyses are provided in Appendix 2.

2. Mass fraction determination of 25-(OH)D₃ in reference solution by LC-UV

For this experiment, three individually-prepared calibrants are used for calculation of RF . The value of w_{Ac} for each of the gravimetrically-prepared calibrants is derived from the known mass fraction of a stock reference solution for species A (w_{RAi}), determined by purity assessment and gravimetric dilution of a neat chemical standard, the mass of stock reference solution (m_{RAi}), the total mass of the calibration solution (m_{ti}), and the associated uncertainties. This is calculated using the following measurement equation:

$$w_{Aci} = \frac{w_{RAi} \times m_{RAi}}{m_{ti}}, i=1, \dots, 3. \quad (8)$$

Applying (8), using a Monte Carlo method as in [2] with Gaussian distributions with the given means and uncertainties from Table 1, results in means and standard deviations for w_{Aci} given in Table 2.

In the RF portion of the experiment, 5 measurements of each calibrant were made to determine the peak area of A, R_{Aci} . These are given in Table 3.

A modification of equation (2), that is,

$$RF_{ij} = \frac{w_{Aci}}{R_{Acij}} \times P_{AC}, i=1, \dots, 3, j=1, \dots, 5, \quad (9)$$

describes the measurement equation for the determination of the response factor from each measurement. Again, expressing all inputs as Gaussian random variables with the means and standard deviations given in Tables 2 and 3, and treating known P_{AC} as a Gaussian random variable with mean 0.9526 and standard deviation of 0.0062, produces the distributions of the RF_{ij} . Use of the same P_{AC} in all 15 calculations elicits a positive correlation between all results. Further, there is a separate source of positive correlation among the five RF_{ij} for each i , due to the common w_{Aci} . Since the five R_{Acij} are from replicate measurements of the same sample, no information is lost by averaging these values and computing the standard

deviations (SD) for each calibrant (Table 3 gives Mean and $SE = \frac{SD}{\sqrt{5}}$). In statistics, this is called “reduction by sufficiency” because the averages and standard deviations are sufficient statistics [7]. We let $\bar{R}_{Ac_i} = \frac{1}{5} \sum_{j=1}^5 R_{Ac_{ij}}$, and then define

$$RF_i = \frac{w_{Ac_i}}{\bar{R}_{Ac_i}} \times P_{Ac_i}, i=1, \dots, 3. \quad (10)$$

An application of a GUM Supplement 1-type Monte Carlo analysis (MC, code in Appendix 2) to equation (10), using Gaussian distributions based on Table 2 and the Mean and SE values in Table 3, results in the RF_i values and uncertainties given in Table 4.

The Monte Carlo analysis also produces correlations among the RF_i values. These are

$$RF = \begin{bmatrix} 1 & 0.486 & 0.574 \\ 0.486 & 1 & 0.503 \\ 0.574 & 0.503 & 1 \end{bmatrix}.$$

As mentioned in the Introduction, the usual approach is to average the three RF_i values to obtain $\overline{RF} = 45.91$ to use in expression (3). The uncertainty of $\overline{RF}$ obtained by parametric bootstrap Monte Carlo analysis via the NIST Uncertainty Machine [3] is 0.33. Note that this calculation only accounts for the uncertainty in each calibrant RF_i value; it does not include the between-calibrant variability. To include this element of uncertainty, it is best to apply a multivariate version of the random effects model. That is, to define a multivariate Gaussian distribution for the vector of the three RF_i as

$$\begin{bmatrix} RF_1 \\ RF_2 \\ RF_3 \end{bmatrix} \sim Normal \left(\begin{bmatrix} RFm_1 \\ RFm_2 \\ RFm_3 \end{bmatrix}, V_{RF} \right),$$

where the RF_i are the mean values in Table 4, and V_{RF} is computed from the correlation

$$s_{RF} = \begin{bmatrix} 0.388 & 0. & 0. \\ 0. & 0.424 & 0. \\ 0. & 0. & 0.374 \end{bmatrix}.$$

matrix RF and the matrix of standard uncertainties

Further we let $RFm_i \sim Normal(\mu_{RF}, \sigma_{RF}^2)$, $i = 1, \dots, 3$, which defines the random effects. For ease of computation we use a Bayesian version of the random effects model, see for example [4], which requires prior distributions for the consensus value μ_{RF} and the variance between response factors, σ_{RF}^2 . We use non-informative distributions $\mu_{RF} \sim Normal(0, large)$, and $\sigma_{RF}^2 \sim Uniform(0, large)$. Evaluated using the OpenBUGS [8] software, with the code in Appendix 2, we obtain a consensus value $\widehat{RF} = 45.84$, with uncertainty of 0.39. A non-

Bayesian solution can be similarly obtained using a multivariate meta-analysis approach [9,10].

The final step of the calculation is to apply measurement equation (3) to replicated measurements of the sample peak areas $R_{A_{Si}}$ given in Table 5:

As with the RF estimate described above, if expression (3) is evaluated using Monte Carlo analysis applied to each line of Table 5 individually, the outputs w_{Ai} will be positively correlated due to common multiplication by the Gaussian random variable $\widehat{RF}$. Since the 20 measurements in Table 5 are just measurement replicates, it is simplest and sufficient to compute a consensus value for the peak area $\hat{R}_{A_s}$ and define

$$w_A = \hat{R}_{A_s} \times \widehat{RF}. \quad (11)$$

Since in this case uncertainties are expressed for each $R_{A_{Si}}$, the best consensus value is not a simple or weighted average, but again an output from a random effects model which accounts for both individual peak area uncertainties and the between-replicate uncertainty.

The statistical model is $R_{A_{Si}} \sim \text{Normal} \left(m_{R_{Si}}, \sigma_{A_{Si}}^2 \right)$, $m_{R_{Si}} \sim \text{Normal} \left(\mu_{A_s}, \sigma_{A_s}^2 \right)$. Evaluated using the code in Appendix 2, this results in posterior mean $\hat{R}_{A_s} = 17.83$ and uncertainty of 0.04. Finally, evaluation of expression (11) using the NIST Uncertainty Machine [3] yields $w_A = 817.33$ ng/g with standard uncertainty of 7.02 ng/g. For comparison, the result obtained ignoring the correlations among the RF_i is 817.33 ng/g with standard uncertainty of 4.49 ng/g.

In summary, the application of the random effects model to values of RF_i to obtain $\widehat{RF}$, and to values of $R_{A_{Si}}$ to obtain $\hat{R}_{A_s}$, before applying a GUM Monte Carlo analysis to the two

measurement equations $RF_i = \frac{w_{A_{Ci}}}{R_{A_{Ci}}} \times P_{A_C}$ and $w_A = \hat{R}_{A_s} \times \widehat{RF}$, takes the above method “beyond the GUM”. It tacks a top-down uncertainty evaluation method to a bottom up standard GUM Monte Carlo analysis, and as long as careful attention is given to the positive correlations that occur as a result of multiplication by a common random variable, the results will be similar to those obtained by an MCMC analysis of a BH model.

3. Mass fraction determination of 25-(OH)D₃ in reference solution by LC-MS using isotopically-enriched internal standards

3.1 Monte Carlo analysis

A second experiment used five calibrants to estimate a relative mass response detection factor, RRF , for 25(OH)D₃ (A) and an isotopically-enriched internal standard, [2H₆]-25(OH)D₃ (IS). Similar to the analysis in section 2, there were preliminary experiments and calculations needed to determine the w_{A_C} and, additionally, w_{IS_C} . The concentration of the internal standard, c_{IS} , was computed from the gravimetric IS solution preparation data in Tables 6 and 7:

Table 6

Weighing data^I from preparation of [²H₆] 25(OH)D₃ in methanol stock solution.

Mass (mg) of Neat [² H ₆]-25(OH)D ₃ , m_{IS}	u (mg) $\sigma_{m_{IS}}$	Mass of container (g), m_{B1}	u (g) $\sigma_{m_{B1}}$	Mass of container + stock solution (g), m_{B1s}	u (g) $\sigma_{m_{B1s}}$
1.3948	0.0005	354.96	0.10	743.98	0.10

^IUncertainty of weighing values estimated from repeatability of balance indication and analyst experience

Table 7

Weighing data^I from preparation of IS solution by dilution of stock [²H₆] 25(OH)D₃ in methanol solution.

mass of container (g), m_{B2}	u (g) $\sigma_{m_{B2}}$	mass of container + stock soln. (g) m_{B2s}	u (g) $\sigma_{m_{B2s}}$	mass of container + IS soln. (g) m_{B2IS}	u (g) $\sigma_{m_{B2IS}}$
159.97	0.10	210.66	0.10	238.43	0.10

^IUncertainty of weighing values estimated from repeatability of balance indication and analyst experience

as

$$c_{IS} = 10^6 \frac{m_{IS}}{m_{B1s} - m_{B1}} \times \frac{m_{B2s} - m_{B2}}{m_{B2IS} - m_{B2}}. \quad (12)$$

Evaluating expression (12) using Monte Carlo analysis (code in Appendix 2), for which all distributions are Gaussian with means and uncertainties consistent with the data in Tables 6 and 7, we obtain a mean value of c_{IS} as 2316.35 ng/g with uncertainty of 5.81 ng/g. To obtain distributions of w_{Ac} and w_{ISc} , we also need the following data (Table 8) that corresponds to the gravimetric preparation of calibration solutions containing a known amount of A and a known amount of IS.

We define

$$w_{ISc_i} = c_{IS} \times m_{ISS_i}, \quad (13)$$

$$w_{Ac_i} = w_{RA_i} \times m_{RA_i}, \quad i=1, \dots, 5. \quad (14)$$

Monte Carlo analysis with Gaussian distributions for c_{IS} , m_{ISS_i} , w_{RA_i} and m_{RA_i} using the means and standard uncertainties in Table 8 results in the means and standard uncertainties of w_{Ac_i} and w_{ISc_i} given in Table 9.

Note that based on (13), the five w_{ISc_i} are correlated due to the multiplication by the common random variable c_{IS} . In fact, these correlations are very close to 1. To calculate the

relative response factors RRF_i according to expression (7) we use the replicated measurements of the peak area ratios $\frac{R_{ISc}}{R_{Ac}}$ given in Table 10.

As in section 2, to eliminate unnecessary correlation in the RRF_i , it is simplest and sufficient to first aggregate the $\left(\frac{R_{ISc}}{R_{Ac}}\right)_{ij}$ for each calibrant i by computing a consensus value determined from corresponding replicate calibrant measurements. Applying the random effects model: $\left(\frac{R_{ISc}}{R_{Ac}}\right)_{ij} \sim Normal\left(p_{rcij}, \sigma_{rcij}^2\right)$, $p_{rcij} \sim Normal\left(\mu_{rci}, \sigma_{prci}^2\right)$, we obtain the mean values and uncertainties given as $\widehat{RC}_i$ in Table 10. Next, compute the RRF_i using Monte Carlo analysis with Gaussian distributions as:

$$RRF_i = \frac{w_{Ac_i}}{w_{ISc_i}} \times \widehat{RC}_i \times P_{Ac}, i=1, \dots, 5. \quad (15)$$

From these calculations, we obtain the means and standard uncertainties shown in Table 11.

Due to the correlation between the w_{ISc} and multiplication by the random variable P_{Ac} , the RRF_i are again positively correlated, this time more greatly so. The correlation matrix is:

$$RRF = \begin{bmatrix} 1 & 0.969 & 0.965 & 0.968 & 0.964 \\ 0.969 & 1 & 0.962 & 0.965 & 0.961 \\ 0.965 & 0.962 & 1 & 0.961 & 0.957 \\ 0.968 & 0.965 & 0.961 & 1 & 0.96 \\ 0.964 & 0.961 & 0.957 & 0.96 & 1 \end{bmatrix}.$$

Applying the random effects model, $\begin{bmatrix} RRF_1 \\ \vdots \\ RRF_5 \end{bmatrix} \sim Normal\left(\begin{bmatrix} RRFm_1 \\ \vdots \\ RRFm_5 \end{bmatrix}, V_{RRF}\right)$ with V_{RRF} computed as in section 2, and $RRFm_i \sim Normal(\mu_{RRF}, \sigma_{RRF}^2)$, $i=1, \dots, 5$, $\mu_{RRF} \sim Normal(0, \text{large value})$, and $\sigma_{RRF}^2 \sim Uniform(0, \text{large})$, we obtain a consensus value $\widehat{RRF}=5.98$, with uncertainty of 0.08. (For comparison, ignoring the correlations and applying the NIST Uncertainty Machine yields $\widehat{RRF}=5.99$, with uncertainty of 0.02.) We also note that $\widehat{RRF}$ has correlation of 0.3 with c_{IS} .

The final step in the evaluation is to apply MC to equation (6) for the measurement of 20 samples with unknown amounts of species A. From each of these measurements we obtain

ratios of IS mass to Sample mass (mr_{S_i}) and sample A peak area to IS peak area ($\frac{R_{AS_i}}{R_{IS_{S_i}}}$) given in Table 12. With this, we can compute the unknown mass fraction of species A in each sample solution (w_{A_i}) according to:

$$w_{A_i} = \frac{w_{IS_{S_i}} \times R_{AS_i}}{R_{IS_{S_i}}} \div \widehat{RRF}, \text{ where } w_{IS_{S_i}} = c_{IS} \times mr_{S_i}, \text{ or}$$

$$w_{A_i} = \frac{c_{IS} \times mr_{S_i} \times R_{AS_i}}{R_{IS_{S_i}}} \div \widehat{RRF}.$$

Once again, to avoid unnecessary correlations it is simplest and sufficient to calculate a

consensus value $\widehat{RS}$ for the 20 sample replicate values of $\frac{mr_{S_i} \times R_{AS_i}}{R_{IS_{S_i}}}$. In this case, since no uncertainties were assessed for the individual values, they are simply averaged as 2.121 with uncertainty of 0.020. The measurand, mass fraction of 25(OH)D₃ in methanol, is finally evaluated using MC as

$$w_A = \widehat{RS} \times c_{IS} \div \widehat{RRF} \quad (16)$$

resulting in 821.8 ng/g with standard uncertainty of 13.1 ng/g. For comparison, ignoring all correlations, the result is 819.8 ng/g with standard uncertainty of 8.5 ng/g.

To summarize the procedure, we first computed the response factors as

$RRF_i = \frac{w_{Ac_i}}{w_{IS_{c_i}}} \times \widehat{RC}_i \times P_{Ac}$ and combined them using random effects model, thus accounting for correlations to calculate a consensus $\widehat{RRF}$. We then computed the mass fraction of 25(OH)D₃ in methanol as $w_A = \widehat{RS} \times c_{IS} \div \widehat{RRF}$, using the correlation of 0.3 between c_{IS} and $\widehat{RRF}$. All calculations except for the random effects model, were done using the standard MC approach (code in Appendix 2).

As this procedure is somewhat complicated and could be made more complicated still by a more complex experimental design, it is useful to consider how this analysis could be done using a Bayesian observation equation model [5, 6].

3.2 Bayesian hierarchical model analysis

The first steps of this analysis are identical to the Monte Carlo analysis of section 3.1. That is, we obtain the distribution of

$$c_{IS} = 10^6 \frac{m_{IS}}{m_{B1s} - m_{B1}} \times \frac{m_{B2s} - m_{B2}}{m_{B2IS} - m_{B2}}$$

by defining Gaussian distributions for all of the input variables based on Tables 6 and 7. We also define distributions for c_{IS} , m_{ISS} , w_{RA_i} and m_{RA_i} using the means and standard uncertainties in Table 8. The main difference between the two approaches is that here we define a BH model for the RRF_i as an observation equation that both computes the RRF_i and combines them, rather than first computing the RRF_i from the measurement equation (15) and subsequently combining them externally using the random effects model. The advantage

of this approach is that the correlations among the RRF_i due to P_{AC} and c_{IS} are automatically incorporated during calculation of the consensus value. With MC, these correlations need to be computed and then specified for the random effects models.

We let

$$\left(\frac{R_{ISc}}{R_{Ac}}\right)_{ijij} \sim Normal\left(\frac{m_{ISS_i} \times c_{IS} \times RRF_i}{P_{AC} \times w_{RA_i} \times m_{RA_i}}, \sigma_{rcij}^2\right), i=1, \dots, 5, j=1, \dots, 3 \text{ or } 7.$$

$$RRF_i \sim Normal(\mu_{RRF}, \sigma_{RRF}^2),$$

$$\mu_{RRF} \sim Normal(0, large),$$

$$\sigma_{RRF} \sim Gamma(large, large).$$

Markov Chain Monte Carlo analysis (MCMC) using OpenBUGS (code in Appendix 2) leads to a posterior mean of μ_{RRF} of 5.98, with posterior standard deviation of 0.09. These are very similar to results obtained in section 3.1, with the posterior standard deviation being slightly larger.

The remaining analysis is again similar to that of section 3.1. We assume a Gaussian

distribution for the 20 values of $\frac{m_{rS_i} \times R_{AS_i}}{R_{IS S_i}}$ defined as

$$\frac{m_{rS_i} \times R_{AS_i}}{R_{IS S_i}} \sim Normal(\mu_{RS}, \sigma_{RS}^2), i=1, \dots, 20$$

$$\mu_{RS} \sim Normal(0, large),$$

$$\sigma_{RS}^2 \sim Gamma(large, large),$$

Finally, we define

$$w_A = \mu_{RS} \times c_{IS} \div \mu_{RRF}.$$

MCMC evaluation using OpenBUGS and the code provided in Appendix 2 yields posterior mean of w_A as 821.0 ng/g with posterior standard deviation of 14.9 ng/g.

Figure 1 compares three probability distributions; the posterior distribution of w_A obtained by the MCMC analysis of this section in blue, the probability distribution obtained in the previous section in green, and in red, a probability distribution obtained using an MC analysis ignoring all correlations. It is clear that the two procedures presented in this section and in 3.1 yield very similar results but ignoring the correlations causes an underestimation of the uncertainty in w_A .

4. Conclusions

This article delineates GUM Supplement 1 Monte Carlo (MC) analysis and Bayesian hierarchical (BH) model approaches developed for evaluation of example data sets for LC-UV and LC-IDMS chemical measurement methods. It shows that a careful MC based on measurement equations, augmented with a random effects meta analysis for combining of the results from various replicates of the experiments, can produce results similar to a BH of an observation model. This indicates that rigorous uncertainty evaluations of complex measurement procedures may be achieved using either an MC method with prudent elucidation of correlations or with a BH observation model. It is important to note that agreement between results of MC analysis and MCMC Bayesian analysis such as obtained here for the specific sets of measurement equations and assumptions is not universally attainable, as was shown in [11, 12]. The BH approach is intrinsically rigorous, thus making it a more straightforward method for use with complex experimental designs, particularly when correlations among data are numerous and difficult to elucidate or explicitly quantify. This capability is critical for promoting metrological soundness and confidence in chemical measurements that are inherently influenced by both bottom-up and top-down elements of uncertainty.

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Appendix 1. Derivation of the correlation between two random variables formed as products with a common multiplier random variable

Let $Z_1 = X \times Y_1$ and $Z_2 = X \times Y_2$ where the random variable X is independent of each of two mutually independent variables Y_1 and Y_2 . We are interested in the correlation between Z_1 and Z_2 . Let $E(.) = \mu$ denote the expected value and $Var(.) = \sigma^2$ denote the variance.

First note that $E(X^2) = \sigma_X^2 + \mu_X^2$ and $E(Y_i^2) = \sigma_{Y_i}^2 + \mu_{Y_i}^2$. Also, since X and each Y_i are independent random variables, $E(Z_i) = \mu_X \times \mu_{Y_i}$.

For $i = 1, 2$, $Var(Z_i) = E(Z_i^2) - E(Z_i)^2$. Because X and Y_i are independent, $Var(Z_i) = E(X^2 \times Y_i^2) - (\mu_X \times \mu_{Y_i})^2 = E(X^2)E(Y_i^2) - (\mu_X \times \mu_{Y_i})^2$. Therefore, from above, we get

$$Var(Z_i) = (\sigma_X^2 + \mu_X^2)(\sigma_{Y_i}^2 + \mu_{Y_i}^2) - \mu_X^2 \times \mu_{Y_i}^2 = \sigma_X^2 \times \sigma_{Y_i}^2 + \mu_X^2 \times \sigma_{Y_i}^2 + \mu_{Y_i}^2 \times \sigma_X^2.$$

The covariance of Z_1 and Z_2 is $Cov(Z_1, Z_2) = E(Z_1 \times Z_2) - E(Z_1) \times E(Z_2)$.

Since $Z_1 \times Z_2 = X^2 \times Y_1 \times Y_2$ where X^2 and $(Y_1 \times Y_2)$ are independent random variables, and since $E(X^2) = \sigma_X^2 + \mu_X^2$ we get $E(Z_1 \times Z_2) = \mu_{Y_1} \times \mu_{Y_2} \times (\sigma_X^2 + \mu_X^2)$. Thus,

$$\begin{aligned} Cov(Z_1, Z_2) &= E(Z_1 \times Z_2) - E(Z_1) \times E(Z_2) = \mu_{Y_1} \times \mu_{Y_2} \times (\sigma_X^2 + \mu_X^2) - \mu_X^2 \times \mu_{Y_1} \times \mu_{Y_2} \\ &= \sigma_X^2 \times \mu_{Y_1} \times \mu_{Y_2}. \end{aligned}$$

Note that this covariance is positive for any positive μ_{Y_1} and μ_{Y_2} .

We can compute the correlation $(Z_1, Z_2) = \frac{Cov(Z_1, Z_2)}{\sqrt{Var(Z_1) Var(Z_2)}}$.

Appendix 2. OpenBUGS code

Section 2

Monte Carlo analysis for RF_1 :

```
{purity~dnorm(0.9526,26014)
mfm~dnorm(0,1.0E-4)
mfunc~dgamma(1.0E-4,1.0E-4)
# Calculation of external calibrant mass fraction  $W_{A_{Ci}}$  (wac)
for(i in 1:3){stcprec[i]<-1/(stcunc[i]*stcunc[i])
               cc[i]~dnorm(stcconc[i],stcprec[i])
               calmassprec[i]<-1/(calmassunc[i]*calmassunc[i])
               cm[i]~dnorm(calmass[i],calmassprec[i])}
```

```

totmassprec[i]<-1/(totmassunc[i]*totmassunc[i])
tm[i]~dnorm(totmass[i],totmassprec[i])
wac[i]<-cc[i]*cm[i]/tm[i]}
#Calculation of the average  $R_{AC}$  (mean)
for(i in 1:15){cala[i]~dnorm(mean[caln[i]],sigr[caln[i]])
}
# Calculation of response factors in Table 4
for(i in 1:3){mean[i]~dnorm(0,1.0E-5)
sigr[i]~dgamma(1.0E-5,1.0E-5)
top[i]<-wac[i]/mean[i]
rrf[i]<-top[i] *purity}
# Calculation of the consensus value  $\hat{R}_{AS}$  (mfm)
for(i in 1:20){spprec[i]<-1/(spaunc[i]*spaunc[i])
mf[i]~dnorm(mfm,mfunc)
spa[i]~dnorm(mf[i],spprec[i])}
}

```

Initial values:

```
list(sigr=c(1,1,1), mfunc=1)
```

Data:

stconc[]	stcunc[]	calmass[]	calmassunc[]	totmass[]
totmassunc[]	cal[]			
2506.53	3.375859243		3.303 0.0001	9.35615
0.001	1			
2479.92	3.714038423		3.33212 0.0001	9.39519
0.001	2			
2459.11	4.096651353		3.35376 0.0001	9.42234
0.001	3			
END				
cala[]	caln[]	rep[]		
18.231	1	1		
18.572	1	2		
18.34	1	3		
18.222	1	4		
18.507	1	5		
18.437	2	1		
18.159	2	2		
18.085	2	3		
17.9	2	4		
18.007	2	5		

18.41	3	1
18.08	3	2
18.22	3	3
18.269	3	4
18.422	3	5

END

spa[]	spaunc[]
18.187	0.090935
17.801	0.089005
17.843	0.089215
17.771	0.088855
17.618	0.08809
17.74	0.0887
17.857	0.089285
17.862	0.08931
17.384	0.08692
17.637	0.088185
17.876	0.08938
18.118	0.09059
17.765	0.088825
17.938	0.08969
17.786	0.08893
17.88	0.0894
17.798	0.08899
17.995	0.089975
17.706	0.08853
18.012	0.09006

END

Random effects for RF :

```
{# random effects model to compute  $\mu_{RF}$  (murf)
  rrf[1,1:3]~dmnorm(rfm[],tau[,])
for(i in 1:3){rfm[i]~dnorm(murf,rfsig)}
murf~dnorm(0,1.0E-5)
rfsig~dgamma(1.0E-5,1.0E-5)}
```

Initial values:

```
list(rfsig=1)
```

Data:

```
list(tau=structure(.Data=c( 10.7411,-2.47335,-4.91593,-2.47335,7.32981,-2.913
97,-4.91593,-2.91397,11.8205),.Dim=c(3,3)),rrf=structure(.Data=c(45.88,46.23,
45.61),.Dim=c(1,3)))
```

Section 3.1

Monte Carlo analysis code:

```
{purity~dnorm(0.9526,26014)
for(i in 1:5){bprec[i]<-1/(bunc[i]*bunc[i])
               dprec[i]<-1/(dunc[i]*dunc[i])
               fprec[i]<-1/(func[i]*func[i])

  # b is stock concentration
  br[i]~dnorm(b[i],bprec[i])

  # d is mass stock
  dr[i]~dnorm(d[i],dprec[i])

  # f is total mass
  fr[i]~dnorm(f[i],fprec[i])

  # calmass is calibrant mass
  calmass[i]<-br[i]*dr[i]*purity

  # massis is internal standard mass
  # massisc is internal standard concentration
  massis[i]<-massisc*fr[i]
}

# T1b is mass 2H25(OH)D3
T1b~dnorm(1.3948,4000000)

# T1d is mass bottle in Table 1
T1d~dnorm(354.96,100)

#T1f is mass bottle and solution
T1f~dnorm(743.98,100)

# T2b is mass of bottle in Table 2
T2b~dnorm(159.9743,100)

# T2d is mass of bottle + bulk solution
T2d~dnorm(210.66,100)

# T2h is mass of bottle, bulk soln and ethanol
T2h~dnorm(238.43,100)

# massisc is internal standard concentration
massisc<-T1b/(T1f-T1d)*(T2d-T2b)/(T2h-T2b)*1000000

#calculation of the response factors
for(i in 1:5){ meanr[i]~dnorm(0,1.0E-5)
               rrf[i]<- massis[i]/meanr[i]/calmass[i]
}

# calculation of  $\widehat{RC}_i$  (meanr)
```



```

for(i in 1:27){ratprec[i]<-1/(ratunc[i]*ratunc[i])
               rat[i]~dnorm(meanr[calrf[i]],ratprec[i])}
# calculation of  $\widehat{RS}$  respm~dnorm(0,1.0E-5)respsig~dgamma(1.0E-5,1.0E-5)for(i
in 1:20){resp[i]<-col9[i]*col10[i]/col11[i]/col7[i]}for(i in 1:20)
{resp[i]~dnorm(respm, respsig)}}

```

Initial values:

```
list(respsig=1)
```

Data:

b[]	bunc[]	d[]	dunc[]	f[]
func[]	cal[]			
2506.545739		3.375859243	0.78406	0.0001
4.27654	0.0001	1		
2479.91191		3.714038423	0.79303	0.0001
4.69381	0.0001	2		
2459.14576		4.096651353	0.78071	0.0001
4.71618	0.0001	3		
2479.91191		3.714038423	0.78157	0.0001
5.02064	0.0001	4		
2459.14576		4.096651353	0.7692	0.0001
4.95239	0.0001	5		
END				
calrf[]	rat[]	ratunc[]		
1	0.977272727	0.000977273		
1	0.922594142	0.000922594		
1	0.879182156	0.000879182		
1	0.864027539	0.000864028		
1	0.837979094	0.000837979		
1	0.87037037	0.00087037		
1	0.850364964	0.000850365		
2	1.070038911	0.001070039		
2	1.015053763	0.001015054		
2	0.962671906	0.000962672		
2	0.933572711	0.000933573		
2	0.950850662	0.000950851		
2	0.968325792	0.000968326		
2	0.93220339	0.000932203		
3	1.073059361	0.001073059		
3	1.044345898	0.001044346		
3	1.021052632	0.001021053		

3 0.965048544 0.000965049
 3 0.977653631 0.000977654
 3 1.021052632 0.001021053
 3 0.938202247 0.000938202
 4 1.051282051 0.001051282
 4 1.005154639 0.001005155
 4 1.031128405 0.001031128
 5 1.055214724 0.001055215
 5 1.140540541 0.001140541
 5 1.053658537 0.001053659

END

col17[]	col19[]	col110[]	col111[]
219.65	443.68	56000	52400
233.47	439.52	58400	50300
225.42	457.41	45700	45400
223.44	446.47	45100	46100
222.76	446.43	20400	18800
220.29	438.29	18300	16500
221.57	459.74	55600	54200
223.82	450.31	56100	52100
221.43	443.42	46100	45800
225.28	440.12	58300	53500
223.88	464.77	46800	43400
223.05	455.55	51300	49000
218.35	455.7	54400	52700
223	451.27	58100	53700
220.96	448.36	48000	48000
220.43	442.21	46000	46200
221.24	452.44	21100	19300
222.67	440.2	18300	17400
220.34	458.83	44400	45100
224.29	448.02	53900	50000

END

Random effects for *RRF*:

```
{rrf[1,1:5]~dmnorm(rfm[, ],tau[, ])
for(i in 1:5){rfm[i]~dnorm(murf,rfsig)}
murf~dnorm(0,1.0E-5)
rfsig~dgamma(1.0E-5,1.0E-5)}
```

Initial values:

```
list(rfsig=1)
```

Data:

```
list(tau=structure(.Data=c(10632.3,-3004.47,-2427.64,-2836.23,-2316.21,-3004.
47,9297.11,-1990.36,-2328.4,-1898.17,-2427.64,-1990.36,7922.14,-1877.,-1526.5
8,-2836.23,-2328.4,-1877.,8914.37,-1789.79,-2316.21,-1898.17,-1526.58,-1789.7
9,7636.11),.Dim=c(5,5)),rrf=structure(.Data=c(6.001,5.97,5.96,6.13,5.89),.Dim
=c(1,5)))
```

Section 3.2

Bayesian hierarchical model:

```
{purity~dnorm(0.9526,26014)
rrfa~dnorm(0,1.0E-4)
sig~dgamma(1.0E-4,1.0E-4)
for(i in 1:5){bprec[i]<-1/(bunc[i]*bunc[i])
               dprec[i]<-1/(dunc[i]*dunc[i])
               fprec[i]<-1/(func[i]*func[i])

  # b is stock concentration
               br[i]~dnorm(b[i],bprec[i])

  # d is mass stock
               dr[i]~dnorm(d[i],dprec[i])

  # f is total mass
               fr[i]~dnorm(f[i],fprec[i])

  # calmass is calibrant mass
               calmass[i]<-br[i]*dr[i]*purity

  # massis is internal standard mass
  # massisc is internal standard concentration
               massis[i]<-massisc*fr[i]
               massrat[i]<-log(massis[i])-log(calmass[i])
               cut.massrat[i]<-cut(massrat[i])}

# T1b is mass 2H25(OH)D3
T1b~dnorm(1.3948,4000000)
# T1d is mass bottle in Table 1
T1d~dnorm(354.96,100)
#T1f is mass bottle and solution
T1f~dnorm(743.98,100)
# T2b is mass of bottle in Table 2
T2b~dnorm(159.9743,100)
# T2d is mass of bottle + bulk solution
```

```

T2d~dnorm(210.66,100)
# T2h is mass of bottle, bulk soln and ethanol
T2h~dnorm(238.43,100)
# massisc is internal standard concentration
massisc<-T1b/(T1f-T1d)*(T2d-T2b)/(T2h-T2b)*1000000
fffe<-exp(rrfa)
# hierarchical model for response factors using a log transformation
(consensus is 1/cut.fffe)
for(i in 1:5){ rrf[i]~dnorm(rrfa,siga)
               sigr[i]~dgamma(1.0E-5,1.0E-5)
               rrfe[i]<-exp(rrf[i])
             }
for(i in 1:27){ratprec[i]<-1/(ratunc[i]*ratunc[i])
               mean[i]<-cut.massrat[calrf[i]]+rrf[calrf[i]]
               lnrat[i]<-log(rat[i])
               lnrat[i]~dnorm(mean[i],sigr[calrf[i]])
             }
cut.fffe<-cut(fffe)
#calculation of  $\widehat{RS}$ 
(respm)respm~dnorm(0,1.0E-5)respsig~dgamma(1.0E-5,1.0E-5)for(i in 1:20)
{resp[i]<-col9[i]*col10[i]/col11[i]/col7[i]}for(i in 1:20)
{resp[i]~dnorm(respm, respsig)}#calculation of the measurand  $W_{Amfr}$ <-
respm*massisc*cut.fffe}

Data same as that above.

Initial values:

list(siga=1,sigal=1,sigr=c(1,1,1,1,1), sigr1=c(1,1,1,1,1),mfunc=1,mfunc1=1,
respsig=1)

```

Figure 1.

The posterior distribution of w_A obtained by the MCMC analysis (blue), the probability distribution obtained in section 3.1 (green), a probability distribution obtained by MC ignoring all correlations (red). The three means are indicated by the colored points.

Table 1

Weighing data for gravimetric preparation of calibration solutions from stock solutions of reference material [A].

Calibrant	[A] Stock soln., w_{RA_i} (uncertainty, $\sigma_{w_{RA_i}}$)(ng/g)	Stock soln. mass (g), m_{RA_i} (uncertainty, σ_{RA_i}) ^I	Total Calibrant mass (g), m_{ti} (uncertainty, σ_{mi}) ^I
1	2506.5 (3.4)	3.30300 (0.00010)	9.35615 (0.00010)
2	2479.9 (3.7)	3.33212 (0.00010)	9.39519 (0.00010)
3	2459.1 (4.1)	3.35376 (0.00010)	9.42234 (0.00010)

^IUncertainty of weighing values estimated from repeatability of balance indication and analyst experience

Table 2

External calibrant mass fraction (ng/g) means and standard uncertainties.

Calibrant	$w_{A_{ci}}(\text{ng/g})$	$\sigma_{A_{ci}}(\text{ng/g})$
1	884.9	1.2
2	879.5	1.3
3	875.3	1.5

Table 3

Species A LC-UV peak area integrals determined from replicate measurement of three external standard calibrants.

Calibrant	$R_{A_{cij}}$
1	18.231
1	18.572
1	18.340
1	18.222
1	18.507
Mean (SE)	18.37 (0.07)
2	18.437
2	18.159
2	18.085
2	17.900
2	18.007
Mean (SE)	18.12 (0.09)
3	18.410
3	18.080
3	18.220
3	18.269
3	18.422
Mean (SE)	18.28 (0.06)

Table 4

Mean values and standard uncertainties of three response factors determined via MC analysis of equation (1).

Calibrant	Mean RF_i	uncertainty
1	45.87	0.39
2	46.23	0.44
3	45.61	0.37

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Table 5

Sample solution LC-UV peak areas for A and associated uncertainties.

Sample peak area of A, $R_{A_{Si}}$	uncertainty of R_{A_S} , $\sigma_{A_{Si}}$
18.187	0.091
17.801	0.089
17.843	0.089
17.771	0.089
17.618	0.088
17.740	0.089
17.857	0.089
17.862	0.089
17.384	0.087
17.637	0.089
17.876	0.089
18.118	0.091
17.765	0.089
17.938	0.090
17.786	0.089
17.880	0.089
17.798	0.089
17.995	0.090
17.706	0.089
18.012	0.090

Table 8

Preparation of calibrant solutions (A–E) for LC-IDMS analysis of A, using reference solutions of known [A] (Table 1) and [IS] (Table 7).

Calibrant	Mean Stock [A] w_{RA_i} (ng/g) (uncertainty, $\sigma_{w_{RA_i}}$)	Mean Stock A mass (g), m_{RA_i} (uncertainty, $\sigma_{m_{RA_i}}$)	Mean IS Soln. mass (g), m_{ISS_i} (uncertainty, $\sigma_{m_{IS_i}}$)
1	2506.6 (3.4)	0.78406 (0.00010)	4.27654 (0.00010)
2	2479.9 (3.7)	0.79303 (0.00010)	4.69381 (0.00010)
3	2459.2 (4.1)	0.78071 (0.00010)	4.71618 (0.00010)
4	2479.9 (3.7)	0.78157 (0.00010)	5.02064 (0.00010)
5	2459.2 (4.1)	0.76920 (0.00010)	4.95239 (0.00010)

Table 9

Means and standard uncertainties of $w_{A_{ci}}$ and $w_{IS_{ci}}$ determined using MC analysis.

Calibrant	$w_{A_{ci}}(\text{ng})$ mean	$w_{A_{ci}}(\text{ng})$ uncertainty	$w_{IS_{ci}}(\text{ng})$ mean	$w_{IS_{ci}}(\text{ng})$ uncertainty
1	1965.3	2.7	9905.6	25.0
2	1966.7	2.9	10872.0	27.5
3	1919.9	3.2	10923.9	27.6
4	1938.2	2.9	11629.1	29.4
5	1891.6	3.1	11471.0	28.0

Table 10

Peak area ratios determined for each replicate measurement of calibrant solutions (1–5).

Calibrant	peak area ratio $\left(\frac{R_{IS_c}}{R_{Ac}}\right)_{ij}$	uncertainty σ_{reij}
1	0.9773	0.0010
1	0.9226	0.0009
1	0.8792	0.0009
1	0.8640	0.0009
1	0.8380	0.0008
1	0.8704	0.0009
1	0.8504	0.0009
$\widehat{RC}_1$	0.8818	0.0003
2	1.0700	0.0011
2	1.0151	0.0010
2	0.9627	0.0010
2	0.9336	0.0009
2	0.9509	0.0010
2	0.9683	0.0010
2	0.9322	0.0009
$\widehat{RC}_2$	0.9720	0.0004
3	1.0731	0.0011
3	1.0443	0.0010
3	1.0211	0.0010
3	0.9650	0.0010
3	0.9777	0.0010
3	1.0211	0.0010
3	0.9382	0.0009
$\widehat{RC}_3$	1.0019	0.0004
4	1.0513	0.0011
4	1.0052	0.0010
4	1.0311	0.0010
$\widehat{RC}_4$	1.0285	0.0006
5	1.0552	0.0011
5	1.1405	0.0011
5	1.0537	0.0011
$\widehat{RC}_5$	1.0802	0.0006

Table 11

Means and standard uncertainties of RRF_i calculated via MC analysis.

Calibrant	RRF_i mean	RRF_i uncertainty
1	6.001	0.05
2	5.970	0.05
3	5.962	0.05
4	6.134	0.05
5	5.893	0.05

Table 12

Ratios of IS mass to Sample mass and Sample A peak area to IS peak area for each measured sample solution of A (25(OH)D₃) in methanol.

mr_{S_i}	$\frac{R_{A_{S_i}}}{R_{IS_{S_i}}}$
2.01994	1.069
1.88256	1.161
2.02915	1.007
1.99817	0.978
2.00409	1.085
1.98961	1.109
2.07492	1.026
2.01193	1.077
2.00253	1.007
1.95366	1.090
2.07598	1.078
2.04237	1.047
2.08702	1.032
2.02363	1.082
2.02915	1.000
2.00612	0.996
2.04502	1.093
1.97692	1.052
2.08237	0.984
1.99750	1.078