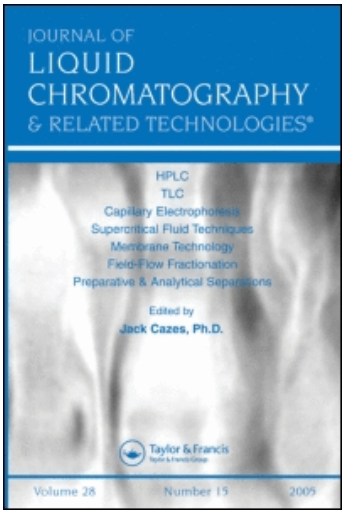

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Use of Validation Data for Fast and Simple Estimation of Measurement Uncertainty in Liquid Chromatography Methods

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Abstract: Measurement uncertainty has become a key task in the process of analytical methods validation. This paper summarizes the measurement uncertainty estimations for liquid chromatography methods carried out at the Nestlé Research Center between 2002 and 2004. These estimations are compared with validation data on one hand and intermediate reproducibility on the other hand (16 different methods and 48 different combinations of analyte and matrix). Based on these data, rules were established in order to make measurement uncertainty estimations for liquid chromatography methods faster and simpler when validation data are available.

Keywords: Uncertainty, Reproducibility, Repeatability, Trueness, Statistics, Liquid chromatography

INTRODUCTION

As stated in ISO/IEC 17025,[1] "calibration and testing laboratories shall have and shall apply procedures for estimating uncertainty in measurement." Measurement uncertainty estimation has then become essential when aiming to get a laboratory accreditation. A methodology, the bottom-up approach, for estimating and expressing the uncertainty associated to one laboratory result is given in ISO[2] and Eurachem[3] guides. This methodology is based on a 4-step approach:[4] specification of the measure and identification

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of the uncertainty sources, quantification of the uncertainty sources, and calculation of the combined uncertainty.

Since 2002, Nestlé is actively involved in the process of estimating measurement uncertainty,[5–8] following the approach described in the Eurachem guide. This work has taken a large amount of time and effort but has, in return, provided a large amount of data that can now be used to analyse the contributions of the different uncertainty sources. Indeed, previously published studies show that the contributions of some uncertainty sources were sometimes so low that they could easily be neglected.[9,10]

This paper summarizes the measurement uncertainty estimations of liquid chromatography methods carried out at the Nestlé Research Center. In the frame of method validation, 48 estimations have been calculated using the bottom-up approach. These estimations have been compared to validation data and to the intermediate reproducibility of the method. Relying on this high number of collected data, it is possible to establish a methodology that simplifies and speeds up the process of measurement uncertainty estimation. Contrary to other published approaches,[11,12] this methodology does not try to detect the key parameters of the method that influence the uncertainty: it is based on the data obtained through method validation.

EXPERIMENTAL

Measurement Uncertainty in Liquid Chromatography Methods

Identification of the Uncertainty Sources

Measurement uncertainty estimation, following the Eurachem approach,[3] first leads to the design of a cause and effect diagrams that lists all the potential uncertainty sources in liquid chromatography methods. A typical example is shown in Figure 1.

This diagram is then refined in order to simplify the presentation and avoid possible duplications in uncertainty sources. For example, measurements made in replicates are grouped under the term repeatability (see Figure 2).

Following this refinement, one can establish the list of the main uncertainty sources in a typical liquid chromatography method. They are:

Repeatability

Trueness/recovery

Measurement of the peak area

Concentration of standard

Mass of sample

Sample dilution factor

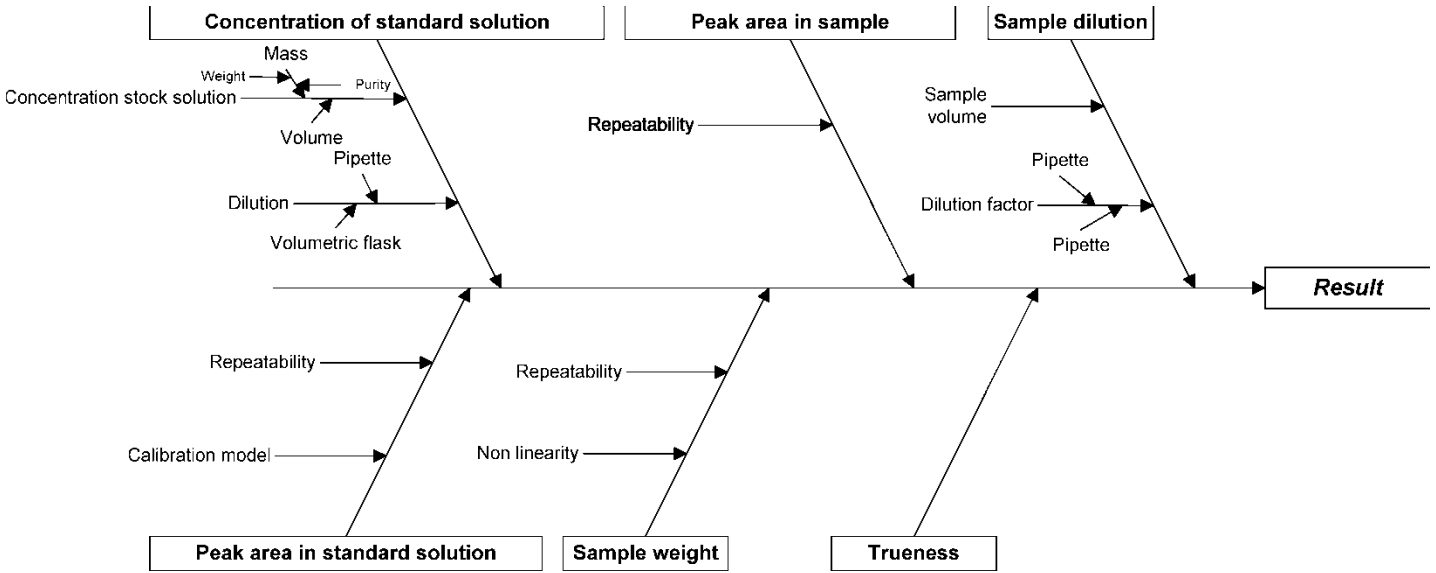


Figure 1. Cause and effect diagram for a typical liquid chromatography method.

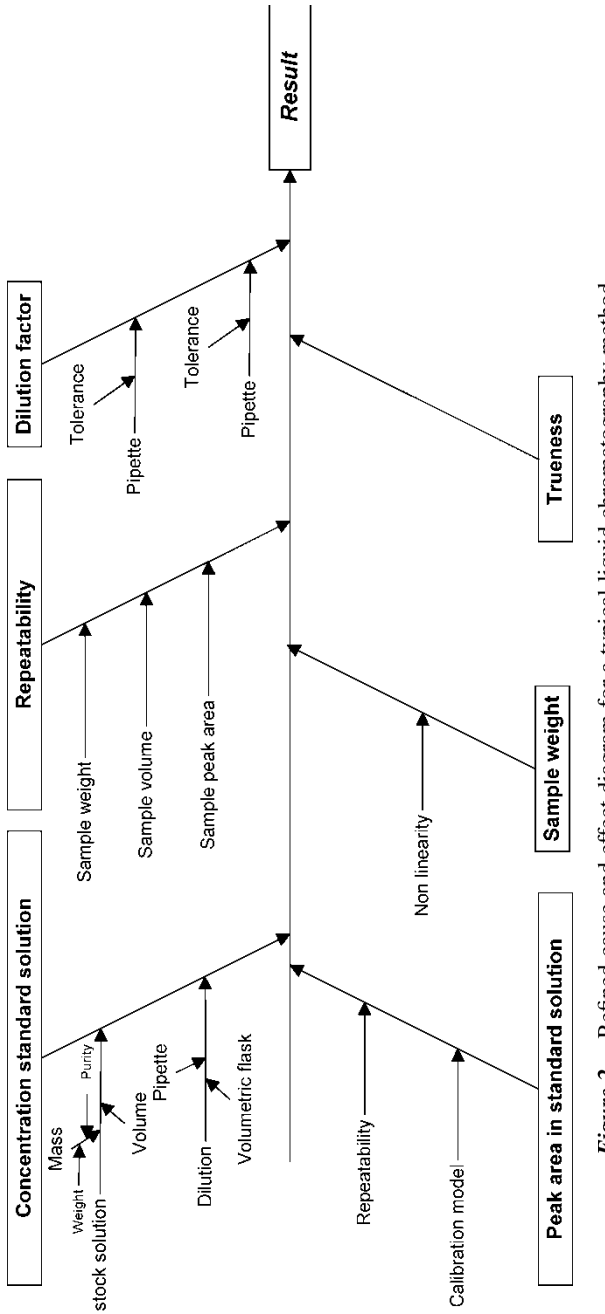


Figure 2. Refined cause and effect diagram for a typical liquid chromatography method.

The next step in the process of measurement uncertainty estimation is the quantification of the uncertainty associated to each of these potential causes under the form of a standard deviation. These uncertainties are then combined and added according to the rules of errors propagation.

Measurement uncertainty data are usually summarized in an uncertainty budget table. A graphical representation of this table is more readable and allows easier determination of the main contributions to the final uncertainty (see Figure 3).

Measurement Uncertainty and Validation Data

Using the validation data and the corresponding 48 measurement uncertainty estimations, the following contributions from the main validation parameters to the final uncertainty have been calculated:

Contribution of the repeatability to the total uncertainty, as a proportion.

Contribution of the repeatability and trueness to the total uncertainty, as a proportion.

Contribution of the repeatability, trueness, measurement of peak area of the standard solution to the total uncertainty, as a proportion.

Contribution of the repeatability, trueness, measurement of peak area, and concentration of the standard solution to the total uncertainty, as a proportion.

For each contribution, 3 statistics have been calculated: median, low, and high bounds of the 95% robust confidence interval.

It can be seen, from Figure 4, that the contributions of repeatability, recovery, and calibration parameters represent 99% of the final uncertainty. This confirms the fact that the contributions of other uncertainty sources (mass of sample, sample dilution, etc.) can be neglected in the final uncertainty budget, and that data coming from intra-laboratory validation can be used to estimate the measurement uncertainty of a liquid chromatography method.

Measurement Uncertainty and Intermediate Reproducibility

For 23 methods studied in the measurement uncertainty and validation data section, it has been possible to collect the value of the intermediate reproducibility. As stated in references [10] and [13], the standard deviation of reproducibility coming from validation in one single laboratory can be used as an estimation of measurement uncertainty.

The data corresponding to this comparison are presented in Table 1 and summarized in Figure 5.

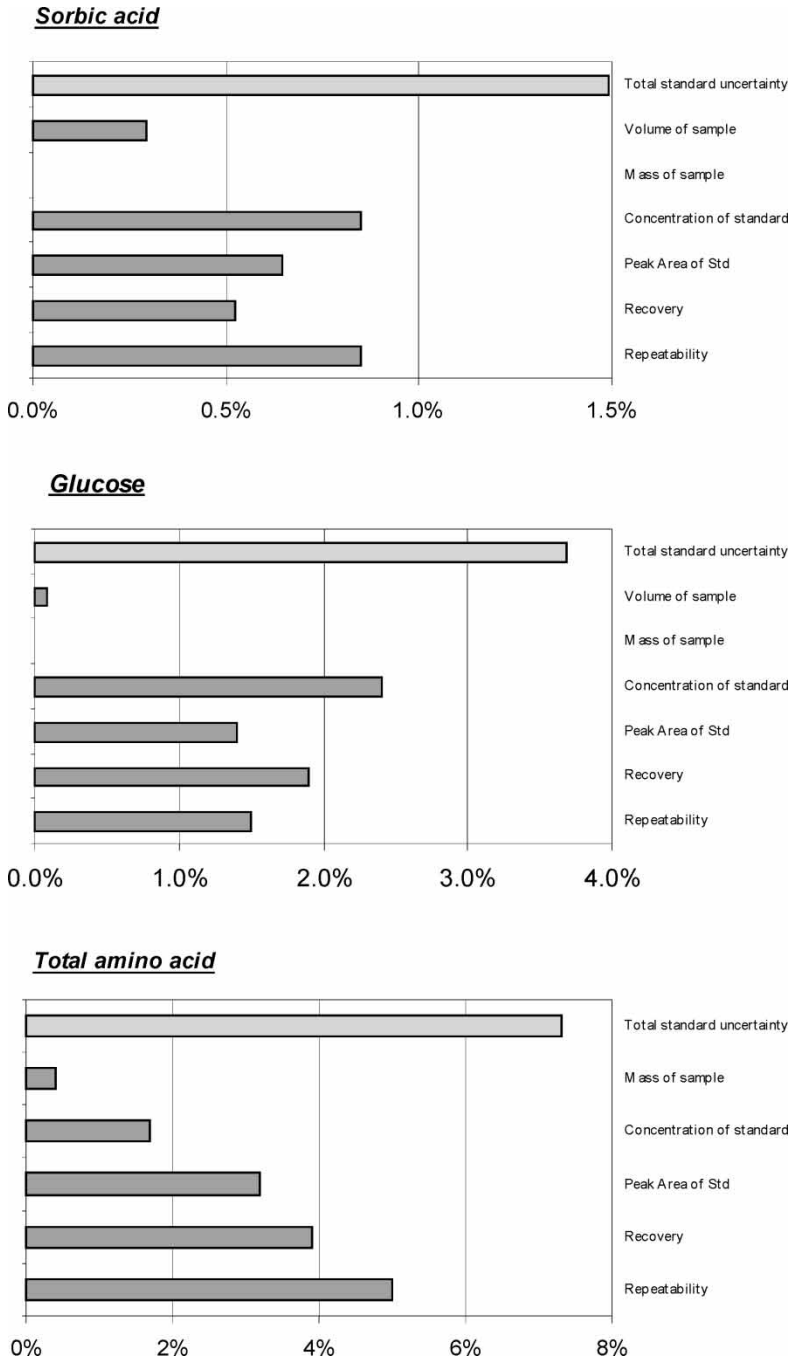
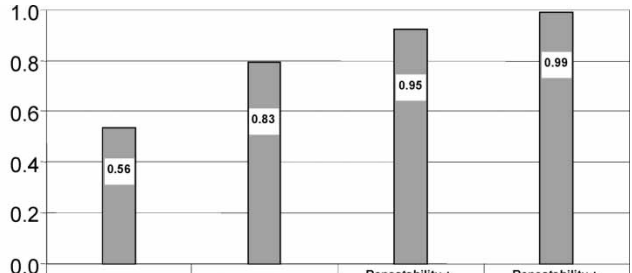


Figure 3. Uncertainty budgets for liquid chromatography methods.



	Repeatability	Repeatability + Recovery	Repeatability + Recovery + Peak area of standard	Repeatability + Recovery + Peak area std+ Std concentration
Max 95% robust confidence interval	0.63	0.86	0.96	1
Median contribution to total uncertainty	0.5608	0.8266	0.9461	0.9995
Mini 95% robust confidence interval	0.48	0.78	0.92	0.99

Figure 4. Contribution of the uncertainty sources to the total uncertainty for liquid chromatography methods.

Table 1. Liquid chromatography methods, comparison between relative standard deviation of intermediate reproducibility [RSD(iR)] and combined relative standard uncertainty [RSD(u)]

Analyte	Matrix	RSD(iR)	RSD(u)	$\frac{\text{RSD(u)}}{\text{RSD(iR)}}$
Sorbic acid	Cocktail sauce	0.008	0.015	1.88
Methyl paraben	Cocktail sauce	0.018	0.027	1.50
Sunset yellow	Pasta snack	0.023	0.039	1.70
Quinoline yellow	Pasta snack	0.043	0.045	1.05
Azorubine	Pasta snack	0.074	0.052	0.70
Erythrosine	Chocolate candy shells	0.097	0.088	0.91
Brilliant blue	Chocolate candy shells	0.049	0.033	0.67
Glucose	Infant cereal	0.055	0.037	0.67
Sucrose	Infant cereal	0.009	0.022	2.44
Maltose	Infant cereal	0.045	0.042	0.93
Lactose	Infant cereal	0.015	0.030	2.00
Free mannitol	Soluble coffee	0.123	0.144	1.17
Free galactose	Soluble coffee	0.029	0.045	1.55
Free glucose	Soluble coffee	0.102	0.144	1.41
Free sucrose	Soluble coffee	0.128	0.079	0.62
Free mannose	Soluble coffee	0.049	0.074	1.51
Free fructose	Soluble coffee	0.164	0.147	0.90
Vitamin A	Milk based product (wet mixing)	0.047	0.050	1.06
Vitamin K1	Milk based infant formula	0.108	0.104	0.96
Theobromine	Milk chocolate	0.060	0.044	0.73
Caffeine	Milk chocolate	0.194	0.095	0.49
Theobromine	Dark chocolate	0.048	0.044	0.92
Caffeine	Dark chocolate	0.098	0.076	0.78

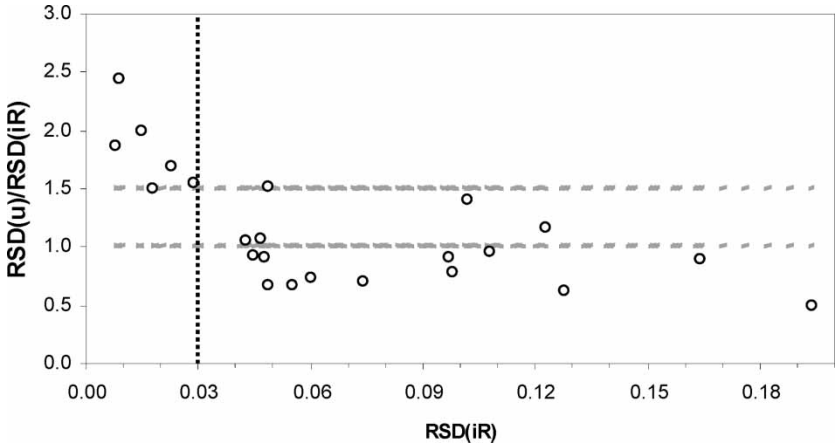


Figure 5. If RSD(iR) $>$ 3%, RSD(iR) can be considered as a reasonable estimation of RSD(u).

To increase the readability of this comparison, the ratio between the relative standard deviation of intermediate reproducibility [RSD(iR)] and the relative standard uncertainty [RSD(u)] of the method has been calculated.

As presented in Figure 5, two different situations can occur:

RSD(iR) $>$ 3%: in that case, RSD(iR) is, in average, equal to RSD(u) and can then be considered as a reasonable estimation of the measurement uncertainty.

RSD(iR) $<$ 3%: in that particular situation, not all the elements of the final uncertainty are taken into account in the intermediate reproducibility and it is then not possible to use only RSD(iR) to have an estimation of RSD(u).

CONCLUSION

The aim of this article was to summarize the set of measurement uncertainty estimations concerning liquid chromatographic methods carried out at the Nestle Research Center (16 methods covering 48 combinations of analytes and matrices). This work has been performed to simplify the process of measurement uncertainty determination based on sufficient data for the establishment of reliable rules. Based on these data, it is possible to derive the following conclusions:

The combination of the uncertainty associated to recovery, repeatability, peak area of standard, and concentration of standard is a reliable estimation of the measurement uncertainty in liquid chromatographic methods.

If the relative standard deviation of intermediate reproducibility is higher than 3%, this can be considered as a good estimation of the relative standard uncertainty of the method.

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