Journal of Chromatography A, 1217 (2010) 882–891

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Estimating uncertainty in analytical procedures based on chromatographic techniques

Piotr Konieczka, Jacek Namieśnik*

Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, 11/12 Narutowicza Str., 80-233 Gdańsk, Poland

ARTICLE INFO

Article history:
Available online 1 April 2009

Keywords:
Trace constituents
Chromatographic techniques
Uncertainty estimation
Sources of uncertainty

ABSTRACT

Chromatographic techniques are very frequently used in analytical procedures for the separation, determination and identification of a wide spectrum of analytes present in samples with complex and sometimes variable matrices. However, the estimation of uncertainty of the final results does not include the uncertainties associated with the actual chromatographic process. In effect, such results cannot always be treated as a reliable source of analytical information. In this paper we present the basic terms, sources of uncertainty, and methods of calculating the combined uncertainty that any presentation of final determinations should include.

Contents

1. Introduction 882
2. Factors influencing the uncertainty of analytical results 883
3. Approaches in the uncertainty evaluation of analytical results 884
4. Tools for estimating uncertainty 884
5. Procedure for estimating measurement uncertainty according to the Guide to the Expression of Uncertainty in Measurement 884
6. The main sources (elements) of uncertainty in chromatographic analysis 885
   6.1. Uncertainty associated with the amount of sample used for a determination 886
   6.2. Uncertainty associated with recovery (trueness) 886
   6.3. Uncertainty associated with repeatability 886
   6.4. Uncertainty associated with analyte concentration 887
   6.5. Uncertainty associated with calibration 887
   6.6. Combined uncertainty for chromatographic analysis 890
7. Case studies 890
8. Conclusions 890
   Abbreviations 890
   References 891

1. Introduction

In any type of scientific research, crucial decisions are based on analytical information, just as in other areas of life, such as medicine, law, health and safety at work and environmental management. Such information is based on analytical measurements, and the assumption is that they were obtained by reliable methods – hence the importance of quality assurance and quality control systems (QA/QC). Fig. 1 presents the tools of such a system that help assure the reliability of an analyst's work; they are applicable to both analytical procedures and analytical techniques, e.g. gas chromatography, liquid chromatography. One of these tools is the estimation of analytical measurement uncertainty.

Uncertainty is a basic characteristic of any measurement; uncertainty is always present, at every step of a procedure.

The terms used in QA/QC, including the terms of analytical measurement uncertainty, are very often misunderstood and confused. Therefore, in Table 1, we provide a list of all these terms together with their recommended definitions [1–3].

* Corresponding author.
E-mail addresses: piotr.konieczka@pg.gda.pl (P. Konieczka), chemanal@pg.gda.pl (J. Namieśnik).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.03.078

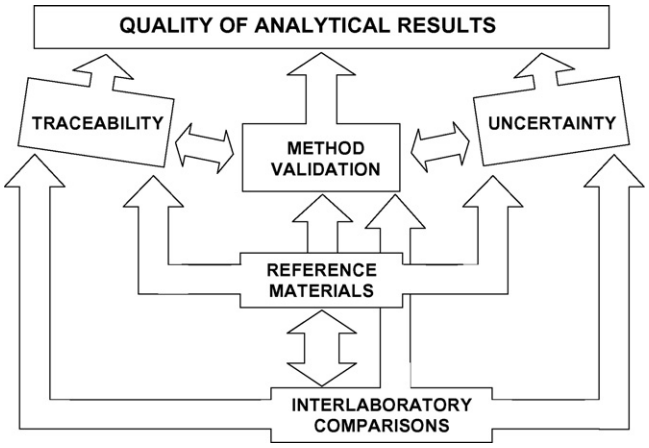

Fig. 1. The main parameters of the QA/QC system and the tools for its evaluation.

2. Factors influencing the uncertainty of analytical results

Estimation of uncertainty leads to better measurement reliability, renders data from inter-laboratory studies comparable, and helps to assess the statistical significance of the difference between the measurement and a relevant reference value.

Uncertainty of measurement is a component of uncertainty in all the individual steps of an analytical procedure [4–7]. Hence it is necessary to determine the sources and types of uncertainty for all these steps [8–10].

Table 2 lists the main sources of uncertainty during sample analysis with a relevant analytical procedure [11].

The term 'measurement' is inseparably associated with the term 'error of measurement'. According to the basic axiom of metrology, there is no such thing as an 'errorless measurement'. Measurements should always be performed with the awareness that their results are encumbered with errors.

There is always a difference, caused by various errors, between the value of a single measurement and the expected (true) value [12]. The effect of an error on the value of a measurement depends on the type of error. There are three main types:

(a) gross errors,
(b) systematic errors,
(c) random errors.

Depending on the manner of presentation, we can divide errors into:

(a) absolute errors, d_x, described by the formula:

$$d_{x_i} = x_i - \mu_x \quad (1)$$

(b) relative errors, ε_x, described by the formula:

$$\varepsilon_{x_i} = \frac{d_{x_i}}{\mu_x} \quad (2)$$

The sources of errors can be divided into:

(a) errors of method,
(b) instrument errors,
(c) personal errors.

Results with gross errors must be rejected from the measurement series using a relevant statistical test. But to do so requires parallel measurement series to be carried out; the use of just one measurement for the presentation of analytical results is not an option.

There are many known ways of detecting results with gross errors:

(a) confidence interval method,
(b) critical range method,
(c) Q-Dixon's test,
(d) Cochran's test,
(e) Grubbs's test,
(f) Hampel's test.

Each of them is applied in certain specific conditions. Detailed information about using an appropriate test are described in the book *Quality Assurance and Quality Control in the Analytical Chemical Laboratory: A Practical Approach* [13]. The systematic error is responsible for the accuracy of the final determination, and its value should be calculated during the validation of the analytical procedure. The result of the final determination can then be corrected using the calculated systematic error.

Random errors are the cause of uncertainty associated with the course of the analytical process and the plot of measurement results. This type of error should be regarded as a random variable (hence its name); thus, the final determination should always be treated as an approximation (estimate) of the true value.

Every analytical result is associated with uncertainty (for the sources of uncertainty – see Table 2). Therefore, the uncertainty of the result of a determination must be calculated and accompany its presentation. Moreover, an analytical result must be recorded not as one value, but according to the values of a continuous random

Table 1
Explanation of terms connected with the estimation of uncertainty in analytical results.

Term	Short definition	Symbol
Uncertainty of measurement	A non-negative parameter characterising the dispersion of the quantity values attributed to a measurand, based on the information used.	u
Definitional uncertainty	A component of the measurement uncertainty resulting from the finite amount of detail in the definition of a measurand.	–
Standard uncertainty	The uncertainty of the result x_i of a measurement expressed as a standard deviation.	$u_{(x_i)}$
Combined standard uncertainty	The standard measurement uncertainty obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model.	$u_{c_{(y)}}$
Expanded uncertainty	The product of the combined standard measurement uncertainty and a factor larger than unity.	U
Coverage factor	A number larger than one by which the combined standard measurement uncertainty is multiplied to obtain the expanded measurement uncertainty; the coverage factor is typically 2–3; for an approximately 95% level of confidence, $k = 2$.	k
Type A evaluation (of uncertainty)	The evaluation of a component of the measurement uncertainty by a statistical analysis of measured quantities obtained under defined measurement conditions.	A
Type B evaluation (of uncertainty)	The evaluation of a component of the measurement uncertainty determined by means other than a Type A evaluation of the measurement uncertainty.	B
Relative uncertainty	The standard measurement uncertainty divided by the absolute value of the measured quantity.	$u_{r(x_i)}$
Uncertainty budget	A statement of the measurement uncertainty, of the components of that measurement uncertainty, and of their calculation and combination.	–

Table 2
Possible sources of uncertainty in analysis.

Uncertainty sources	
Human factors	Factors related to equipment
• Erroneously or imprecisely defined value to be measured • Non-representative sample • Incorrect application of analytical procedure • Person-specific systematic reading error on analogue readouts • Lack of knowledge about all the external factors influencing the analytical result • Uncertainty associated with the calibration of the instrument	• Resolution of the measuring instrument • Uncertainty inherent in the standards and/or reference materials • Uncertainty of parameters determined as separate measurements and later used for calculating the final result, e.g. physiochemical constants • Approximations and assumptions related to the use of a particular instrument during analysis • Fluctuations during repeated measurements under seemingly identical external conditions

variable, as a confidence interval, i.e. the interval likely to include the expected value. Here is an example of the correct presentation of an analytical result:

$$C_{\text{PCB-28}} \pm U(k = 2) = 31.3 \pm 2.7\ \text{ng g}^{-1}$$

where PCB-28 is the analyte concentration (here an analyte from the PCB group – PCB-28 according to IUPAC) calculated as the mean of a series of parallel determinations; U is the expanded uncertainty of the measurement and k is the coverage factor (for $P = 95\%$, $k = 2$).

3. Approaches in the uncertainty evaluation of analytical results

At the beginning of the procedure for evaluating measurement uncertainty, a suitable approach needs to be selected. The various possible approaches are defined as follows [14–16]:

(a) *bottom-up*–based on the identification, quantification and combination of all individual sources of measurement uncertainty; the overall uncertainty is derived from the uncertainties of individual components; this approach, adopted by EURACHEM [2,17], is highly complex and thus demands a lot of time and effort;
(b) *fitness-for-purpose*–based on the definition of a single parameter called the fitness function, which takes the form of an algebraic expression and describes the relation between uncertainty and analyte content; calculating the uncertainty of the result of a measurement is very easy and less time-consuming than in the *bottom-up* approach;
(c) *top-down*–based on data obtained from inter-laboratory studies (precision);
(d) *validation-based*–based on inter- or intra-laboratory validation studies (precision, trueness, robustness);
(e) *robustness-based*–based on robustness tests from inter-laboratory studies.

4. Tools for estimating uncertainty

The correct estimation of uncertainty requires that analysts have an understanding of the whole analytical procedure. The most helpful tools here are [4,5]:

(a) *a flow diagram*, drawn on the basis of the information presented in detail in a standard operating procedure (*SOP*);
(b) *an Ishikawa, or cause-and-effect, or fishbone diagram*, showing the influence of parameters (sources of uncertainty) on a whole analytical procedure [18,19].

Figs. 2 and 3, respectively illustrate a flow diagram and an Ishikawa diagram of the method used for the determination of PCBs in sediment samples by application of pressurized liquid extraction (PLE) combined with GC–MS [20].

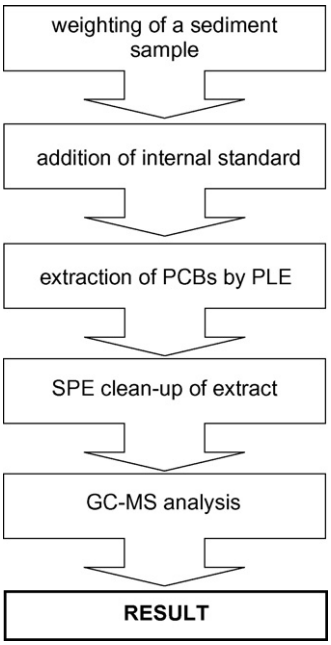

Fig. 2. Flow diagram of the procedure of PCBs determination in sediment samples by PLE-GC-IDMS method [20].

5. Procedure for estimating measurement uncertainty according to the Guide to the Expression of Uncertainty in Measurement

According to the Guide to the Expression of Uncertainty in Measurement (GUM) [2], the following conditions must be satisfied in order to determine the uncertainty of analytical results:

(1) The measurement procedure and the measurand must be defined.
(2) Modelling (usually mathematical modelling) must be applied to calculate the analytical result based on the measured parameters.
(3) Values must be assigned to all the possible parameters that could affect the final result of the analysis, and the standard uncertainty of each of them must be determined.
(4) The principles of uncertainty propagation must be applied when the standard uncertainty of an analytical result is being calculated.
(5) The final result of the analysis is presented as *result* ± *expanded uncertainty* (after using the appropriate k factor).

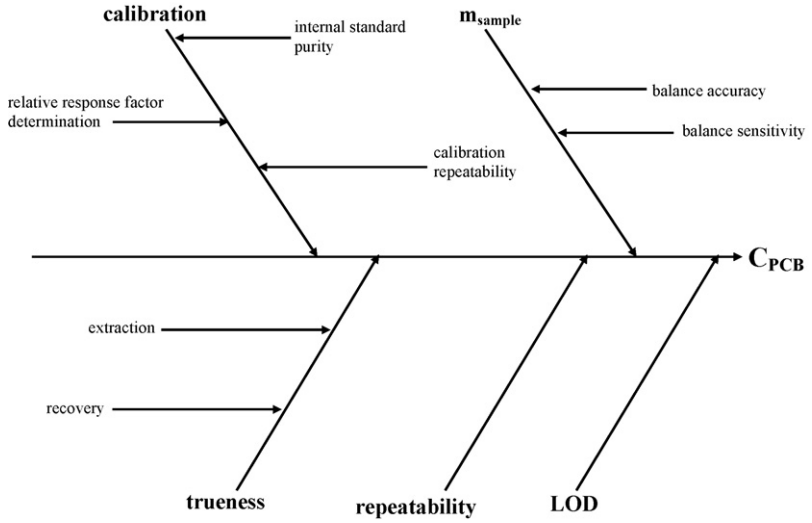

Fig. 3. Ishikawa diagram of the procedure of PCBs determination in sediment samples by PLE-GC-IDMS method [20].

Therefore, the final result of an analysis comprises the following:

(a) determination of the measured values and their units,
(b) the results with an expanded uncertainty value ($y \pm U$, along with units for y and U),
(c) the k factor, for which the expanded uncertainty has been calculated.

Estimating uncertainty is a necessary component of an analytical result, yet in our experience, producing measurements together with uncertainty values is still a serious problem in analytical laboratories, the underlying reasons being mainly:

(a) the lack of clearly and precisely written instructions and guidelines,
(b) the lack of adequate education in this field, even at university level.

Decisions taken in many fields of science and also in other areas of life are based on the results of analytical studies. The quality of such results is thus of the utmost importance.

Fig. 4 shows a flow-chart of the actions to be taken during an uncertainty estimation of the analytical result, according to the Guide to the Expression of Uncertainty in Measurement [2].

The final analytical result therefore consists of:

(a) the determination of measured values, including their units,
(b) the result and its total uncertainty ($y \pm U$, including units for y and U),
(c) the value of coefficient k for which the total uncertainty was calculated.

More detailed information on the theory of uncertainty of analytical results and practical approaches in the estimation of an expanded uncertainty can be found in the following works [13].

6. The main sources (elements) of uncertainty in chromatographic analysis

In a typical chromatographic analysis, the main elements of uncertainty are associated with:

(a) the amount of sample used for a determination,
(b) the recovery value of the analytical procedure, including the recovery of an analyte from a sample and the recovery associated with the accuracy of final determinations,
(c) the repeatability of determinations for a true sample (represented by the repeatability of signals),

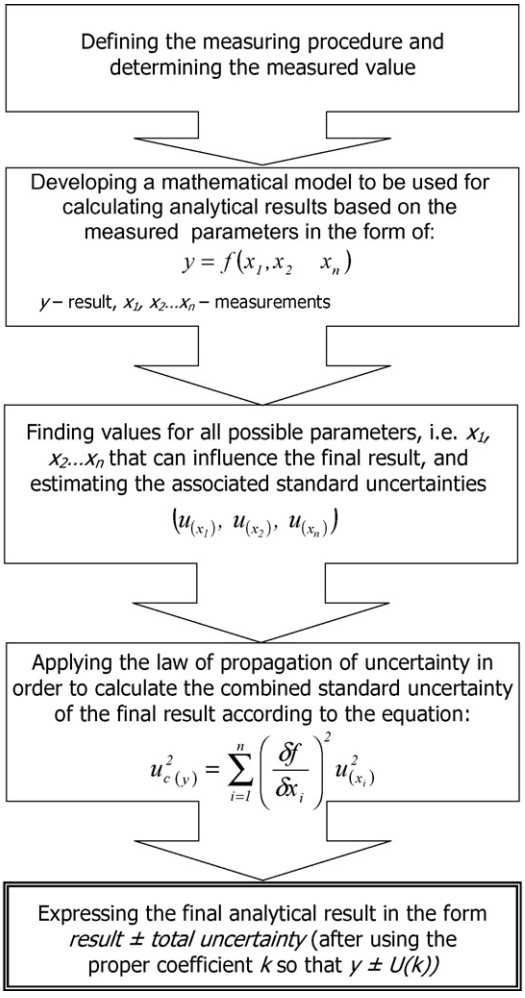

Fig. 4. Scheme of the procedure for estimating the total uncertainty of an analytical result [2].

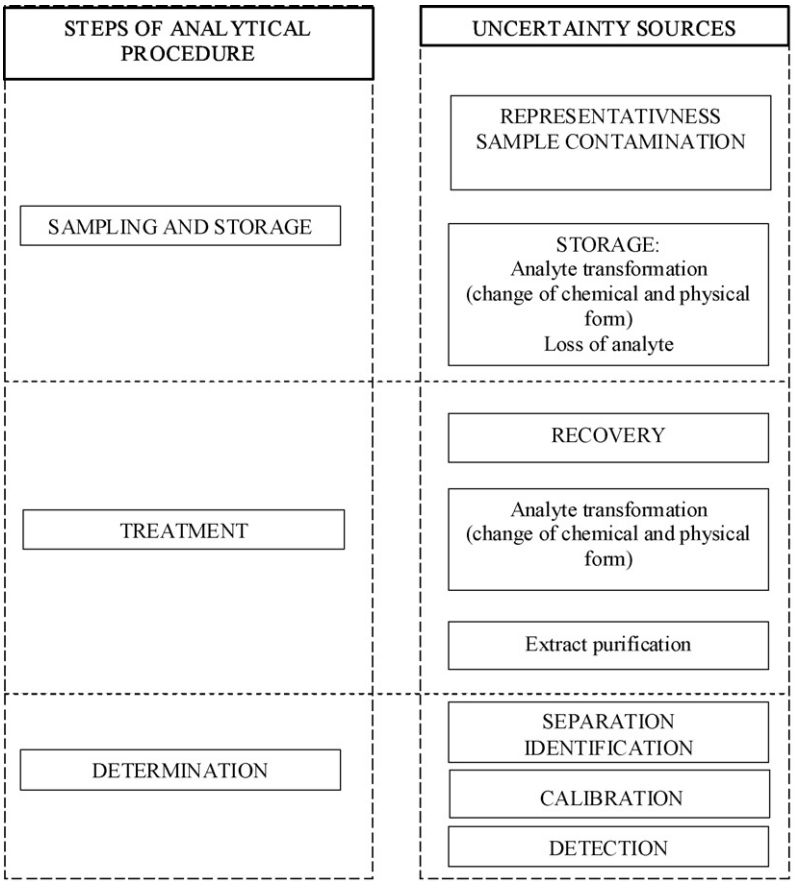

Fig. 5. Main sources of uncertainty associated with the individual steps of analytical procedures using chromatographic techniques.

(d) the concentration associated with the upper detection limit,
(e) calibration of the analytical instruments.

Fig. 5 presents the main sources of uncertainty associated with the individual steps of analytical procedures using chromatographic techniques.

Unfortunately, there are only a few original papers on the metrological characterisation of analytical procedures that use chromatographic techniques. In most cases, estimating the uncertainty is limited to calculating the standard deviation (SD) or relative standard deviation (RSD) on the basis of a series of results, in order to give final results in the form:

$$x_m \pm \frac{t\mathrm{SD}}{\sqrt{n}} \quad (3)$$

where SD – standard deviation, n – number of measurements.

6.1. Uncertainty associated with the amount of sample used for a determination

The uncertainty components inherent in the measurement of the weight and/or volume of a sample are usually small, because the measurements (gravimetric and volumetric) are made directly. As a result this source of uncertainty is very often not taken into account during construction of the uncertainty budget.

6.2. Uncertainty associated with recovery (trueness)

Recovery R is usually defined as the ratio of the determined content (concentration) to a reference value for the particular material tested. The recovery could be used to correct the determined value against an appropriate reference scale. When such a correction has been made, it is clear that any uncertainty in the recovery will contribute to uncertainties in the declared result.

In practice, measurements are usually made to ensure that recovery is likely to be reasonably close to unity; the assumption is then made that $R = 1$. The trueness of an analytical method can be assessed by calculating the proportional bias of the method in terms of apparent recovery. If the apparent recovery does not differ significantly from unity, then the analytical method does not have a significant bias. If this is the case, the bias is neglected and the uncertainty associated with the bias is included in the uncertainty budget of results [21].

To quantify the uncertainty, it is necessary to consider the degree to which a particular sample matrix under test is represented by the reference material employed and, where relevant, the extent to which spiking provides a representation of native analyte behaviour [22].

However, when assessing trueness there is always the probability of incorrectly concluding that the proportional bias is not significant. Therefore, the uncertainty of results may be underestimated [21].

Fig. 6 presents a schematic diagram of the application of different types of techniques for isolating and/or preconcentrating analytes prior to the application of chromatographic techniques.

6.3. Uncertainty associated with repeatability

The uncertainty associated with repeatability of measurements for true samples is frequently the main element of the uncertainty budget.

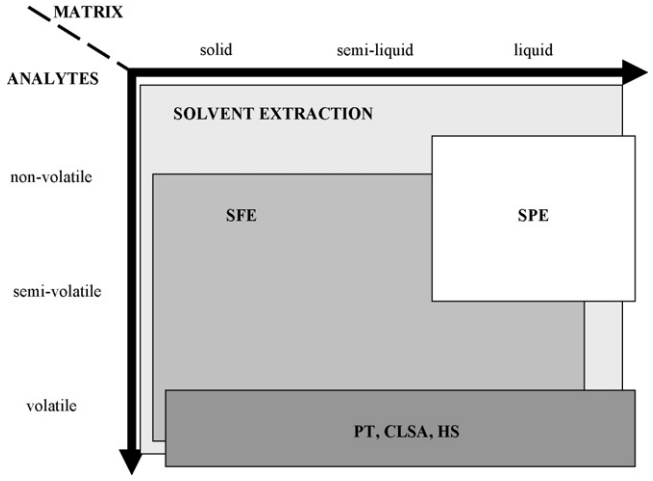

Fig. 6. The application of different types of techniques for isolating and/or preconcentrating analytes prior to the use of chromatographic techniques.

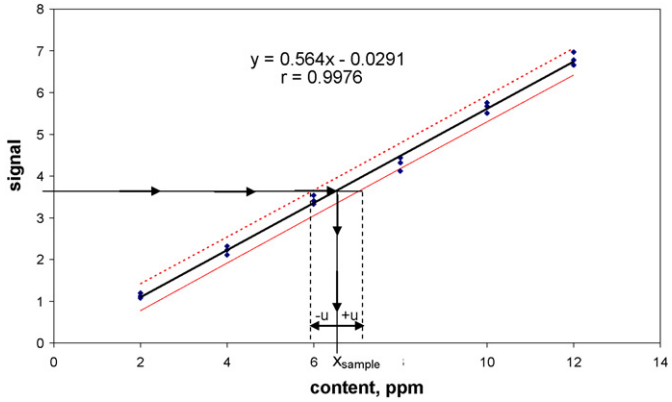

Fig. 7. A calibration curve together with marked confidence intervals and the uncertainty in the determination of analyte concentration in a sample.

In some cases, this value can be estimated as a confidence interval. The basic principle of uncertainty propagation is underlining the influence of the quantity with the highest value.

Therefore, if one of the parameters has a dominant influence over the uncertainty budget, calculation of uncertainty may be limited to a calculation based on the value of that parameter.

If that dominant parameter is the repeatability of measurements, then the expanded uncertainty can be calculated according to the following relation:

$$U = k\frac{\mathrm{SD}}{\sqrt{\mathrm{n}}} \tag{4}$$

6.4. Uncertainty associated with analyte concentration

A blank value should be taken into consideration when the final result of a determination (x_m) is calculated on the basis of a set of independent measurements. Table 3 provides basic information on different types of blanks.

According to the definition of the limit of detection, measurement uncertainty is 100% when the concentration level is equal to LOD. Therefore, the higher the concentration calculated from the LOD, the lower the uncertainty. The value of the relative standard uncertainty associated with the limit of detection may be presented as:

$$u_{(\mathrm{LOD})} = \frac{\mathrm{LOD}}{c_{\mathrm{det}}} \tag{5}$$

6.5. Uncertainty associated with calibration

The calibration step and its execution affect both the final result of a determination and the value of the combined measurement uncertainty.

The vast majority of analytical measurements involve a calibration step, which is associated with the relative (comparative) nature of measurements. During this step, a calibration curve technique is usually used, which is determined using linear regression. This step of the analytical procedure influences the combined uncertainty of the result of a determination for a real sample. The standard uncertainty due to this step should be included in the uncertainty budget.

Table 3
Short description of the different types of blanks that can influence the final result of the analysis.

Commonly used term	Alternative term	Application	Additional information
Laboratory blank			
System blank	Instrumentation blank	Serves to determine the basic line of the equipment (analytical instrument) in the absence of a sample.	The response of the analytical instrument is measured.
Solvent blank	Calibration blank	Serves to determine the level of contamination in the solvent. A calibration mixture not containing an analyte (zero calibration mixture) is analysed.	This blank concerns only solvents used at the sample dilution stage.
Reagent blank	Method blank	The level of this blank is used to detect and determine the level of contamination introduced to the sample during its preparation and final determinations.	This includes the blank covering all the chemical reagents applied at the sample preparation stage and in the final determinations.
Field blank			
Matrix blank	–	Serves to determine the qualitative and quantitative composition of contamination introduced into a sample during collection, transport, storage and final determinations.	Model research is performed, using a sample in an artificial matrix (with a composition similar to the composition of the real matrix).
Trap blank	Adsorbing medium blank	Helpful in the estimation of the level of contamination introduced to a sample as a result of the application of various media that may trap analytes from the sample (at the analyte isolation and enrichment stages)–filters, pipes, bulbs, containers.	Various media that may trap analytes are analysed.
Equipment blank	–	This blank is applied to determine the types and levels of contamination that may be introduced to a sample in contact with the sampling instrument. It also serves to assess the efficiency with which the instruments are washed and cleaned.	This blank is determined by the collection of samples of the water and solvent used for washing and cleaning the sampling instruments.

Table 4
Examples of uncertainty budget preparation of analytical procedure with application of chromatographic techniques.

Analyte(s)	Matrix	Procedure	Uncertainty sources taken into account during uncertainty budget construction	Ref.
TBT	Sediment	PLE-GC-IDMS	• Concentrations of standards • Determination of sediment sample mass • Relative standard deviation of response factor determined • Repeatability of results • Limit of detection	[26]
VOC	Human urine, blood	TLHS-DAI-GC-ECD	• Calibration step • Determination of enrichment factor • Repeatability of results • Lmit of detection	[27]
Ethanol	Tablets (pharmaceuticals)	HS-GC-FID	• Calibration step • Repeatability of results	[28]
VOC	Urine	TLHS–DAI–GC–ECD PV–DAI–GC–ECD HS–GC–ECD	• Calibration step • Determination of enrichment factor • Repeatability of results • Limit of detection	[29]
Triazines	Groundwater	SPE-HPLC	• Concentrations of standards • Determination of water sample mass/volume • Calibration step • Recovery determination	[30]
Pesticides, PCBs	Human serum	GC-ECD GC-MS-MS	• Determination of serum sample mass/volume • Calibration step • Repeatability of results • Recovery determination	[31]
1-Hydroxypyrene	Urine	SPE-HPLC-FLD	• Concentrations of standards • Determination of sample mass/volume • Calibration step • Repeatability of results • Recovery determination	[32]
Chloromethane	–	TD-GC-FID	• Sample mass determination • Calibration step • Repeatability of results	[33]
Ethene	–	TD-GC-FID	• Calibration step • Repeatability of results	[34]
Pesticide residues	Apples	GPC-GC-ECD, NPD, MS	• Concentrations of standards • Purity of standards • Calibration step • Repeatability of results • Recovery determination (extraction)	[16]
Pesticides	Water	SPME-GC-ECD	• Concentrations of standards • Calibration step • Repeatability of results • Recovery determination (trueness)	[35]
Pesticides	Cucumber	GC-NPD, ECD, MS	• Concentrations of standards • Calibration step • Repeatability of results (precision) • Recovery determination (extraction)	[36]
Total petroleum hydrocarbons	Soil	GC-FID	• Sampling • Calibration step • Repeatability of results (precision) • Recovery determination (extraction)	[37]
Benzene	Air	GC-FID	• Sampling • Calibration step • Repeatability of results (precision) • Preconcentration factor determination	[38]
Famoxadone	Grapes Wines	LLE-GC-ECD, MS	• Concentrations of standards • Calibration step • Repeatability of results (precision) • Recovery determination (accuracy)	[39]
Pesticides	Drinking water	SPE-GC-MS	• Calibration step • Repeatability of results (precision) • Recovery determination	[40]

Table 4 (*Continued*)

Analyte(s)	Matrix	Procedure	Uncertainty sources taken into account during uncertainty budget construction	Ref.
Carbon dioxide	Gases	GC-TCD	• Concentrations of standards • Calibration step • Repeatability of results (precision) • Limit of detection	[41]
Pesticides	Vegetables	GC-ECD	• Determination of sample (extract) volume • Concentrations of standards • Calibration step • Recovery determination	[42]
Ochratoxin A	Wines	HPLC-FLD	• Concentrations of standards • Calibration step • Repeatability of results (precision) • Recovery determination (accuracy)	[43]
Antibiotics	Water solutions	HPLC	• Repeatability of results (precision) • Recovery determination (recovery) • Procedure robusstness	[44]
Phenolic antioxidants	Edible oils	GC-FID HPLC-PDA	• Standards preparation • Repeatability of results (precision) • Recovery determination (bias) • Purity of standards	[45]
R-timolol	S-timolol maleate	LC-DAD	• Repeatability of results (precision) • Recovery determination (bias)	[46]
Carotenoids	Tomato	HPLC-PDA	• Standards preparation • Repeatability of results (precision) • Recovery determination (recovery)	[47]
Azoxystrobin, Kresoxim-methyl, Trifloxystrobin, Famoxadone, Pyraclostrobin, Fenamidone	Grapes Wines	HPLC-DAD GC-MS	• Concentrations of standards • Calibration step • Repeatability of results (precision) • Recovery determination (accuracy)	[48]
19-Norandrosterone	Urine	LC-MS-MS	• Standards preparation • Repeatability of results (precision) • Recovery determination (bias) • Purity of standards	[49,50]
PAHs	Smoke flavourings	HPLC-UV, DAD, FLD	• Repeatability of results (precision)	[51]

There are four sources of uncertainty due to the calibration step that can influence the standard uncertainty of a single measurement – $u_{(x\text{sample})}$ [8,23–25]:

(a) the repeatability with which the value of a signal y is read, both for standard samples (based on measurements for which the calibration curve is determined) and for study samples– $u_{(x\text{sample},y)}$,
(b) the uncertainty inherent in the determination of the reference value for standard samples – $u_{(x\text{sample},x\text{std}i)}$,
(c) the influence of the method of preparing the standard samples, usually the method of consecutive dilutions,
(d) the incorrect approximation of measurement points using a regression curve.

Using a calibration curve drawn on the basis of an appropriate equation, one can determine and identify the uncertainty of the regression curve by setting confidence intervals with the aid of a correlation described by the following equation:

$$\Delta y_i = Y \pm \mathrm{SD}_{xy} t_{(\alpha, f=n-2)} \sqrt{\frac{1}{n} + \frac{(x_i - x_m)^2}{Q_{xx}}} \quad (6)$$

Table 5
Calculated values of relative standard uncertainties, combined standard uncertainties and expanded uncertainties for the determination of PCBs in sediment samples [20].

Parameter	Value				
Analyte	PCB-28	PCB-101	PCB-105	PCB-153	PCB-170
Concentration (ng/g)	34.3	30.4	10.87	30.7	9.0
LOD (ng/g)	0.62	0.12	0.12	0.12	0.25
Repeatability–RSD (%)	2.5	2.2	2.3	3.8	4.2
Trueness – recovery ± $U(k=2)$ (%)	102.1 ± 6.8	99.9 ± 5.8	103.0 ± 6.2	98.2 ± 5.0	100.2 ± 8.4
Uncertainty					
Mass of sample – $u_{r(\text{sample})}$	0.0014	0.0014	0.0014	0.0014	0.0014
Calibration – $u_{r(\text{cal})}$	0.0069	0.0069	0.0069	0.0069	0.011
Recovery – $u_{r(\text{true})}$	0.033	0.029	0.030	0.025	0.042
Repeatability (for n repetitions) – $u_{r(\text{rep})}$	0.014	0.013	0.013	0.022	0.024
LOD – $u_{r(\text{LOD})}$	0.018	0.0039	0.011	0.0039	0.028
Combined uncertainty	4.1%	3.3%	3.5%	3.5%	5.7%
Expanded uncertainty ($k=2$)	8.2%	6.5%	7.1%	6.9%	11%
Result					
Concentration ± $U(k=2)$ (ng/g)	34.3 ± 2.8	30.4 ± 2.0	10.87 ± 0.77	30.7 ± 2.1	9.0 ± 1.0

where Δy_i – confidence interval of the calculated value of Y for a given value of x_i, Y – values calculated from the regression curve equation for given values of x_i, SD_{xy} – residual standard deviation, $t_{(\alpha, f=n-2)}$–Student's t-test parameter, n–the total number of standard samples used for plotting the calibration curve (number of points), x_i – calculated value of x for Δy_i, x_m – mean value of x (most frequently, x is the analyte concentration and is the mean of all the concentrations of a standard solution for which the measurement was made in order to plot a standard curve), Q_{xx} – a parameter calculated according to the relation:

$$Q_{xx} = \sum_{i=1}^{n} (x_i - x_m)^2 \tag{7}$$

The standard uncertainty for x_{sample} due to the uncertainty of the calibration and linear regression method, $u_{(sample,y)}$, can be calculated using the regression parameters determined from the following relationship:

$$u_{(xsample,y)} = \frac{SD_{xy}}{b}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(x_{sample} - x_m)^2}{Q_{xx}}} \tag{8}$$

where: $u_{(sample,y)}$ – standard uncertainty in the determination of the x_{sample} concentration as a result of the calibration correlation being applied, b – the direction coefficient of the calibration curve, p – the number of measurements (repetitions) carried out for a given sample.

Fig. 7 presents a calibration curve together with marked confidence intervals and the uncertainty in the determination of analyte concentration in a sample.

The uncertainty in the determination of analyte concentration in standard samples is usually significantly smaller than that associated with the calculation of analyte concentration based on the calibration function:

$$u_{(xsample,xstdi)} \ll u_{(xsample,y)} \tag{9}$$

Therefore, its value can be estimated only if the number of standard samples used at the calibration stage is taken into consideration. Since only one basic standard is usually used, after which the appropriate standard solutions are made up (consecutive dilutions), the standard uncertainty due to the application of standard solutions at the calibration step may be described by the following equation:

$$u_{(xsample,xstdi)} \approx \frac{u_{(xstdi)}}{n} \tag{10}$$

Such an uncertainty does not allow for that associated with the means of standard sample preparation. If each standard sample is prepared by consecutive dilutions, then the uncertainty budget must allow for the standard uncertainties associated with the standard sample preparation step. The standard uncertainty of a result associated with a given calibration technique normally requires only the value $u_{(sample,y)}$.

When internal standards are used in calibration, the main uncertainty element associated with this step of an analytical procedure is the uncertainty of the response coefficient. The value of this coefficient is usually calculated as the mean of the appropriately prepared standard solutions that contain known amounts of the analyte and the substance serving as the internal standard. The standard uncertainty is then given by the standard deviation of the measurement series divided by the square root of the number of repetitions:

$$u_{(cal)} = \frac{SD_{RRF}}{\sqrt{n}} \tag{11}$$

6.6. Combined uncertainty for chromatographic analysis

The combined uncertainty for chromatographic analysis includes the five afore mentioned elements. Therefore, the relative combined uncertainty of a measurement result can be described by the formula:

$$u_r = \sqrt{(u_{r(sample)})^2 + (u_{r(true)})^2 + (u_{r(cal)})^2 + \left(\frac{SD_{results}}{\sqrt{n}}\right)^2 + \left(\frac{LOD}{c_{det}}\right)^2} \tag{12}$$

7. Case studies

There is an urgent need to convince all chemists, including practising analysts using different chromatographic techniques in their work, that estimating the uncertainty of measurements will enhance the reliability of analytical information. We therefore present a number of case studies taken from the literature (see Table 4), in which chromatographic techniques are used for separating mixtures of analytes into individual components in samples with a complex matrix composition.

Additionally, the results of uncertainties estimation for determined contents of selected PCBs in sediment samples by PLE–GC–IDMS method is presented in Table 5 [20]. The calculation were done according to GUM [2] using the formula:

$$U = kc\sqrt{(u_{r(sample)})^2 + (u_{r(cal)})^2 + (u_{r(true)})^2 + (u_{r(rep)})^2 + (u_{r(LOD)})^2} \tag{13}$$

where: U – expanded uncertainty, k – coverage factor (usually two), c – average concentration of the analyte, $u_{r(sample)}$ – relative standard uncertainty of sediment sample mass determination, $u_{r(cal)}$ – relative standard uncertainty of calibration step, $u_{r(true)}$ – relative standard uncertainty of recovery determination, $u_{r(rep)}$ – relative standard uncertainty of repeatability, $u_{r(LOD)}$ – relative standard uncertainty of LOD determination.

8. Conclusions

Chromatographic techniques are frequently a very important aspect of the procedures applied to the analysis of samples with complex and sometimes variable matrices, in order to determine their trace constituents. Such procedures are usually labour-intensive and time-consuming, so everything should be done to ensure that the work of highly skilled staff is not in vain and that the resources used to purchase reagents are not wasted.

Extra effort must therefore be made if measurements of appropriately prepared samples are to supply reliable analytical information. Hence, more attention must be paid to the control and assurance of measurement quality.

This is especially important where chromatographic techniques are concerned; analysis of numerous articles published in chromatographic journals leads one to the conclusion that this is a pressing problem yet to be resolved.

We hope that the information presented here will facilitate the introduction of uncertainty estimation in chromatographic measurements on a much greater scale than is the case at present.

Abbreviations

CLSA	closed loop stripping analysis
DAI	direct aqueous injection
ECD	electron-capture detection
FID	flame ionization detection

FLD	fluorescence detection
GC	gas chromatography
GPC	gel permeation chromatography
GUM	guide to the expression of uncertainty in measurement
HPLC	high-performance liquid chromatography
HS	headspace
IDMS	isotope dilution mass spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JCGM	Joint Committee for Guides in Metrology
LC	liquid chromatography
LLE	liquid–liquid extraction
LOD	limit of detection
MS	mass spectrometry
NPD	nitrogen–phosphorus detection
PAH	polycyclic aromatic hydrocarbons
PCB	polichlorinated biphenyl
DAD	photodiode array detection
PLE	pressurized liquid extraction
PT	purge-and-trap
PV	pervaporation
QA/QC	quality assurance/quality control
RSD	relative standard deviation
SD	standard deviation
SFE	supercritical fluid extraction
SOP	standard operating procedure
SPE	solid-phase extraction
SPME	solid-phase microextraction
TBT	tributyltin
TCD	thermal conductivity detection
TD	thermal desorption
TLHS	thin-layer headspace
UV	ultraviolet
VOC	volatile organic compound

References

[1] International vocabulary of metrology — Basic and general concepts and associated terms (VIM), Joint Committee for Guides in Metrology, JCGM 200, 2008, http://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2008.pdf.
[2] Guide to the Expression of Uncertainty in Measurement (GUM), International Organisation for Standardisation, Geneva, 1993.
[3] A. Williams, Accred. Qual. Assur. 3 (1998) 92.
[4] A. Populaire, E. Campos Gimenez, Accred. Qual. Assur. 10 (2005) 485.
[5] S. Roy, A.-M. Fouillac, Trends Anal. Chem. 23 (2004) 185.
[6] V.R. Meyer, J. Chromatogr. A 1158 (2007) 15.
[7] R. Kadis, Accred. Qual. Assur. 3 (1998) 237.
[8] M.E. Conti, O.J. Muse, M. Mecozzi, Int. J. Risk. Assess. Manag. 5 (2005) 311.
[9] J.L. Love, Accred. Qual. Assur. 7 (2002) 95.
[10] P. Armishaw, Accred. Qual. Assur. 8 (2003) 218.
[11] P. Konieczka, Crit. Rev. Anal. Chem. 37 (2007) 173.
[12] E. Bulska, Metrologia, Malamut 2008, Warsaw (in Polish).
[13] P. Konieczka, J. Namieśnik, Quality Assurance and Quality Control in the Analytical Chemical Laboratory: A Practical Approach, CRC Press/Francis and Taylor, Boca Raton, FL, 2009.
[14] A. Sahuquillo, G. Rauret, in: A. Fajgelj, M. Belli, U. Sansone (Eds.), Combining and Reporting Analytical Results, Royal Society of Chemistry, Cambrige, 2007, p. 143.
[15] I. Taverniers, E. Van Bockstaele, M. De Loose, Trends Anal. Chem. 23 (2004) 480.
[16] R. Štepán, J. Hajšlová, V. Kocourek, J. Tichá, Anal. Chim. Acta 520 (2004) 245.
[17] S.L.R. Ellison, M. Rosslein, A. Williams, Quantifying Uncertainty in Analytical Measurements, EURACHEM/CITAC Guide, 2nd ed., 2000, http://www.measurementuncertainty.org/mu/QUAM2000-1.pdf.
[18] S.L.R. Ellison, V.J. Barwick, Analyst 123 (1998) 1387.
[19] A. Kufelnicki, S. Lis, G. Meinrath, Anal. Bioanal. Chem. 382 (2005) 1652.
[20] P. Konieczka, J. Namieśnik, Chem. Anal. (Warsaw) 53 (2008) 785.
[21] A. Maroto, R. Boque, J. Riu, F.X. Rius, Analyst 128 (2003) 373.
[22] V.J. Barwick, S.L.R. Ellison, Analyst 124 (1999) 981.
[23] K. Danzer, L.A. Currie, Pure Appl. Chem. 70 (1998) 993.
[24] P.L. Bonate, LC–GC 10 (1992) 531.
[25] J.N. Miller, Analyst 116 (1991) 3.
[26] P. Konieczka, B. Sejerøe-Olsen, T.P.J. Linsinger, H. Schimmel, Anal. Bioanal. Chem. 388 (2007) 975.
[27] Ż. Polkowska, K. Kozłowska, P. Konieczka, N. Jakubowska, T. Górecki, J. Namieśnik, Chem. Anal. 51 (2006) 109.
[28] M. Michulec, P. Konieczka, J. Namieśnik, Accred. Qual. Assur. 12 (2007) 257.
[29] N. Jakubowska, Ż. Polkowska, W. Kujawski, P. Konieczka, J. Namieśnik, Anal. Bioanal. Chem. 388 (2007) 691.
[30] T.P.J. Linsinger, M. Führer, W. Kandler, R. Schuhmacher, Analyst 126 (2001) 211.
[31] J.L. Martínez Vidal, M. Moreno Frías, A. Garrido Frenich, F. Olea-Serrano, N. Olea, Anal. Bioanal. Chem. 372 (2002) 766.
[32] Ž. Šaltienė, N. Jatulienė, M. Šalkauskas, D. Brukštienė, A. Ruzgytė, A. Tarasevičiūtė, J. Kalibatas, Accred. Qual. Assur. 10 (2005) 444–451.
[33] A. Świtaj-Zawadka, P. Konieczka, J. Szczygelska-Tao, J.F. Biernat, J. Wójcik, A. Przyjazny, J. Namieśnik, Chromatographia 60 (2004) 433.
[34] A. Naganowska-Nowak, P. Konieczka, J.F. Biernat, J. Szczygelska-Tao, A. Przyjazny, J. Namieśnik, Anal. Bioanal. Chem. 388 (2007) 1725.
[35] N. Ratola, L. Santos, P. Herbert, A. Alves, Anal. Chim. Acta 573–574 (2006) 202.
[36] L. Cuadros-Rodríguez, M.E. Hernández Torres, E. Almansa López, F.J. Egea González, F.J. Arrebola Liébanas, J.L. Martínez Vidal, Anal. Chim. Acta 454 (2002) 297.
[37] E. Saari, P. Perämäki, J. Jalonen, Chemom. Intell. Lab. Syst. 92 (2008) 3.
[38] H. Plaisance, T. Leonardis, M. Gerboles, Atmos. Environ. 42 (2008) 2555.
[39] S. de Melo Abreu, P. Caboni, P. Cabras, A. Alves, V.L. Garau, J. Chromatogr. A 1103 (2006) 362.
[40] J. Quintana, I. Martí, F. Ventura, J. Chromatogr. A 938 (2001) 3.
[41] I. Kuselman, B. Anisimov, L. Turovsky, A. Shenhar, Anal. Chim. Acta 318 (1996) 327.
[42] S. Yenisoy-Karakaş, Anal. Chim. Acta 571 (2006) 298.
[43] N. Ratola, L. Martins, A. Alves, Anal. Chim. Acta 513 (2004) 319.
[44] M.C. Gaudiano, A. Di Maggio, E. Antoniella, L. Valvo, P. Bertocchi, L. Manna, M. Bartolomei, S. Alimonti, A.L. Rodomonte, J. Pharm. Biomed. Anal. 48 (2008) 303.
[45] D.W.M. Sin, Y.C. Wong, C.Y. Mak, S.T. Sze, W.Y. Yao, J. Food Comp. Anal. 19 (2006) 784.
[46] R.D. Marini, P. Chiap, B. Boulanger, S. Rudaz, E. Rozet, J. Crommen, Ph. Hubert, Talanta 68 (2006) 1166.
[47] M. Graça Dias, M. Filomena, G.F.C. Camões, L. Oliveira, Food Chem. 109 (2008) 815.
[48] S. de Melo Abreu, P. Caboni, P. Cabras, V.L. Garau, A. Alves, Anal. Chim. Acta 573–574 (2006) 291.
[49] S.S.-C. Tai, B. Xu, L.T. Sniegoski, M.J. Welch, Anal. Chem. 78 (2006) 3393.
[50] S.S.-C. Tai, B. Xu, M.J. Welch, Accred. Qual. Assur. 12 (2007) 465.
[51] C. Brasseur, F. Brose, A. Pirlot, C. Douny, G. Eppe, G. Maghuin-Rogister, M.-L. Scippo, Accred. Qual. Assur. 12 (2007) 535.