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Measurement uncertainty of chloramphenicol in food products by LC-MS/MS

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Introduction

Chloramphenicol (CAP) is a broad-spectrum antibiotic used in both preventive and therapeutic veterinary medicine. Adverse reactions and side effects in humans have been demonstrated leading to aplastic anaemia (bone marrow depression), which has a high rate of mortality. Therefore, the use of CAP in all food-producing animals has never been permitted in the USA and has been banned in Europe since 1994. Furthermore, since the toxic effects of CAP are not dose dependant but more related to the hypersensitivity of certain individuals, a zero tolerance level (no Maximum Residue Limit) was set for this compound in foods (1). It was however observed that honey imported from China can be contaminated with CAP. This led our laboratories to develop a method for the control of food products containing honey. As the detection limit of the method had to be as low as possible because of the zero tolerance level for CAP, the best approach for this analyte was to use isotope dilution LC-MS/MS.

The notion of detection and quantification limits have now been replaced by those of decision limit (CCα) and detection capability (CCβ) as required by the European Union, which is currently revising the technical criteria that must be applied in the screening and confirmation of veterinary drug residues in foods (2).

CCα and CCβ criterias introduce an error probability to decide if a sample is contaminated with a given residue and then to detect, identify and quantify it.

The decision limit, CCα, is the limit above which a sample can be considered to be truly violative with an error probability of α. In the case of banned or unauthorized substances (α=1 %), the decision limit is the lowest concentration level at which a method can discriminate with a statistical certainty of 1-α whether the analyte is present.

The detection capability, CCβ, is the lowest content of the analyte that may be detected, identified and quantified in a sample with an error probability of β. In the case of banned or unauthorized substances (β=5 %) the detection capability is the lowest concentration level at which a method is able to truly detect contaminated samples with a statistical certainty of 1-β.

In accordance with the requirements of the ISO norm 17025, we estimated the uncertainty associated with the determination of CAP in food products containing honey (3). The approach applied was that suggested by the Eurachem guide (4). Using this approach, the main uncertainty sources were identified and their contribution to overall uncertainty evaluated. The measurement uncertainty estimation was also used for the determination of the decision limit CCα and the detection capability CCβ.

Methodology

The procedure used for the estimation of measurement uncertainty follows the recommendations given in the Eurachem guide. According to the guide it is divided into six steps:

1) Description of the method
2) Specification of the measurand
3) Identification of all uncertainty sources
4) Quantification of individual uncertainty components
5) Calculation of combined uncertainty
6) Expression of final expanded uncertainty

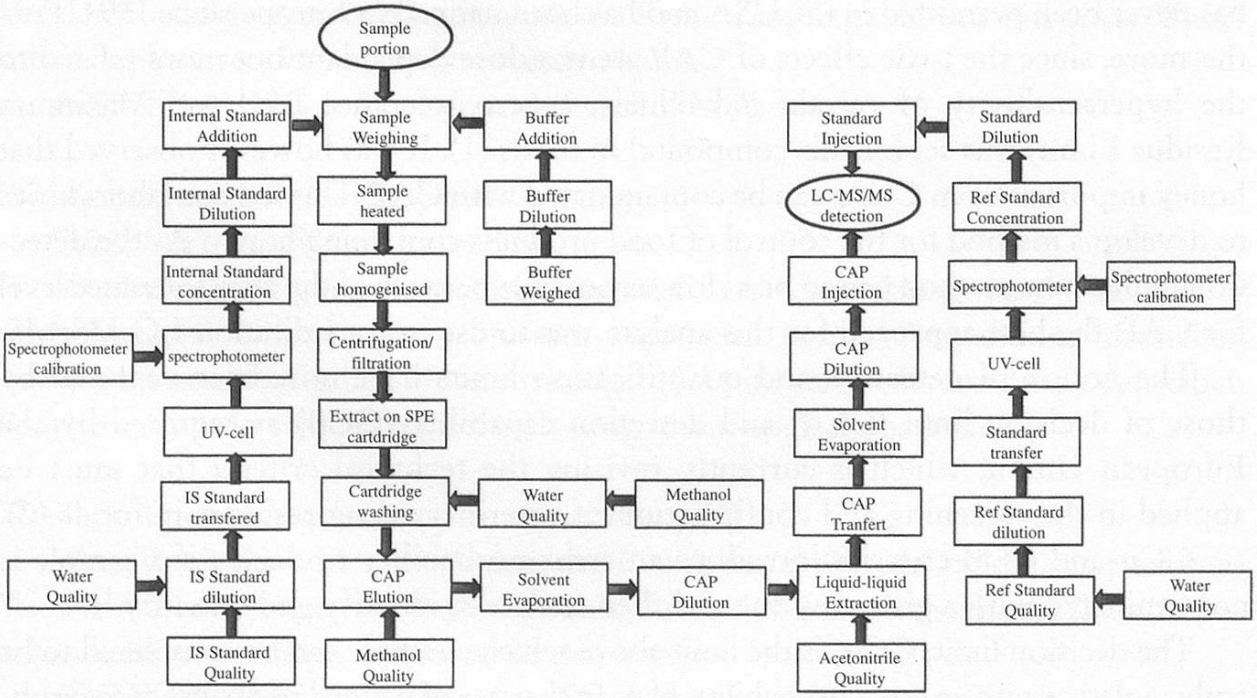

Figure 1 **Flow chart of the analytical procedure**

Step 1. Description of the method

The analytical method is published elsewhere (5, 6), however a summary is given here for the measurement uncertainty estimation procedure. A deuterated internal standard, d5-CAP, is added to the test portion, followed by the addition of a buffer solution. The sample is briefly heated and homogeneized with Ultra-Turrax to liberate CAP from possible binding matrix. The extract is filtered and purified onto an OASIS HLP cartridge and then by liquid-liquid partition in acetonitrile/dichloromethane. The final qualification and quantification are carried out by LC-MS/MS in multiple reaction monitoring (MRM) mode after negative spray ionization. A flow chart of the method is given in figure 1.

Step 2. Specification of the measurand

The relationship between the measurand (CAP) and the input quantities is given by the following equation:

$$\text{CAP (ng/100 g)} = \frac{\left(\frac{A_a}{A_{is}}\right) - I}{S} \cdot \frac{M_{is}}{M_a} \cdot \frac{1}{R}$$

Where:

A_a = peak area of CAP in the sample
A_{is} = peak area of d5-CAP in the sample
I = intercept of the regression line for the considered transition
S = slope of the regression line for the considered transition
M_{is} = mass of internal standard added to the test portion (ng)
M_a = mass of test portion (g)
R = correction factor for recovery

Step 3. Identification of uncertainty sources

The relevant uncertainty sources are shown in a cause and effect diagram. The measurand is represented by the central arrow and the major diagonal arrows represent the variables from the above equation (figure 2).

This diagram can be refined (figure 3) taking into account the following remarks:

1) The measurement uncertainty estimation is performed in repeatability conditions therefore the contributions to repeatability from all operations regarding sample preparation and chromatography (mass, volume of sample and peak area) can be included into one repeatability standard deviation of the whole analytical procedure, which is known from validation studies.
2) The uncertainty of the linear regression model is also grouped in one arrow, because it is estimated as a whole (see calibration model).
3) The extractibility of the native compound could not be established since no reference material was available. However it is expected that the heating step

followed by Ultra-Turrax homogeneization allowed maximum extractability of CAP from matrix.

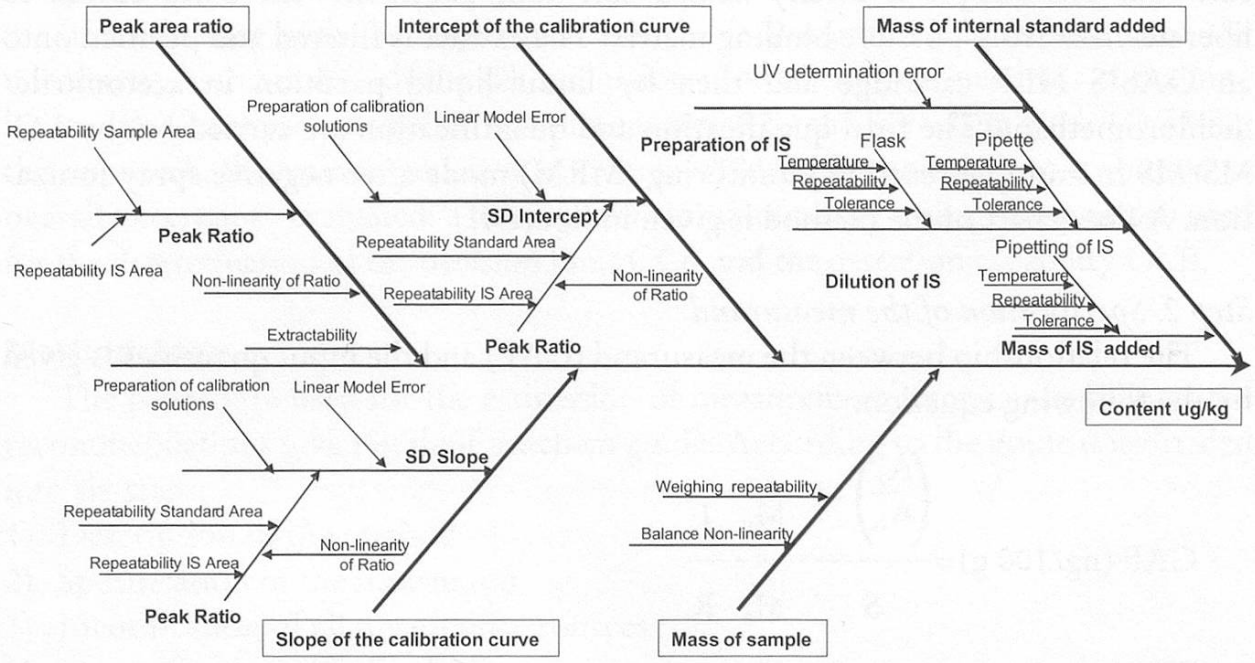

Figure 2 **Cause and effect diagram of CAP analysis**

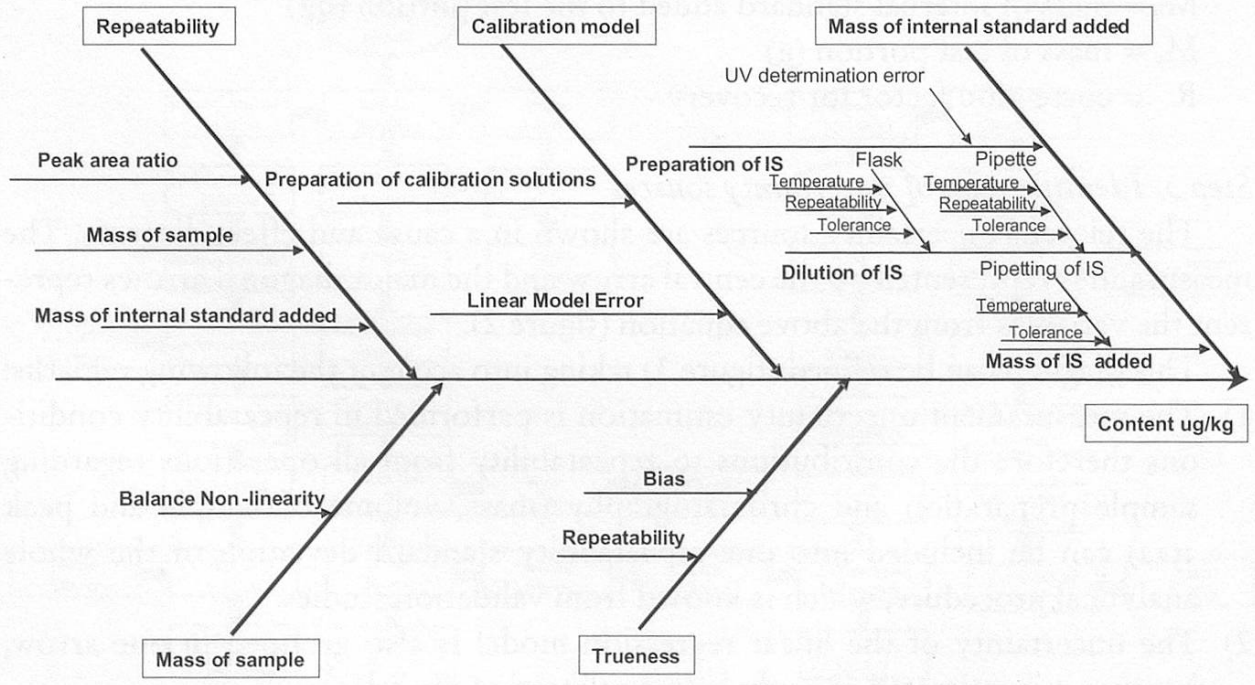

Figure 3 **Refined cause and effect diagram**

Step 4. Quantification of the individual uncertainty components

The remaining individual components are analysed and their respective uncertainties calculated. The quantification is based on the peak area ratios from two transition reactions of the compound. Two other transition reactions are monitored to check the correct isotopic ratio of the chlorine atoms (35 and 37) present in CAP. The following table summarises the transition reactions used for quantitation and qualification.

Table 1
Transition reactions analysed by LC-MS/MS

	35Cl transitions used for quantitation	*37Cl transitions used for confirmation*
CAP	321 -> 152	323 -> 152
	321 -> 257	323 -> 257
D5-CAP	326 -> 157	328 -> 157
	326 -> 262	328 -> 262

Repeatability

The standard deviation of repeatability (standard deviation of test results obtained under repeatability conditions) is known from validation data and it is shown in table 2.

Table 2
Repeatability values observed for CAP

	Transition 321 -> 152				*Transition 321 -> 257*			
Contamination level ug/kg	0.1	0.2	0.5	1.2*	0.1	0.2	0.5	1.2*
Number of replicates	6	6	6	6	6	6	6	6
Mean	0.11	0.21	0.51	1.11	0.10	0.21	0.50	1.26
SDr	0.02	0.03	0.04	0.06	0.01	0.02	0.04	0.06

* estimated value from native contaminated sample

Calibration model

A calibration curve consisting of the zero intercept and five calibration points, was established at the beginning of each experiment. CAP was quantified by means of two external calibration curves built from the calibration solutions. The area ratios $\left(\frac{A_a}{A_{is}}\right)$ of the two transitions were plotted against the concentration ratios, knowing that in each calibration solution the concentration of d5-CAP is the same.

The calibration model has two sources of uncertainty: the uncertainty that comes from the preparation of calibration solutions and the uncertainty of the calibration curve itself.

Preparation of standard solutions

Five calibration dilutions were prepared from a stock solution whose concentration was controlled with a spectrophotometer. The relationship used for the calculation of the stock solution was:

$$\text{Concentration (ng/µng)} = \frac{A \cdot M_w \cdot 1000}{\epsilon \cdot d}$$

A = absorbance of the stock solution
M_w = molecular weight of CAP (g/mol)
1000 = conversion factor from ug to ng
ϵ = molar absorptivity of CAP in water at 298 nm (l/mol .cm)
d = cell length (cm)

The cause and effect diagram related to this equation is shown in figure 4.

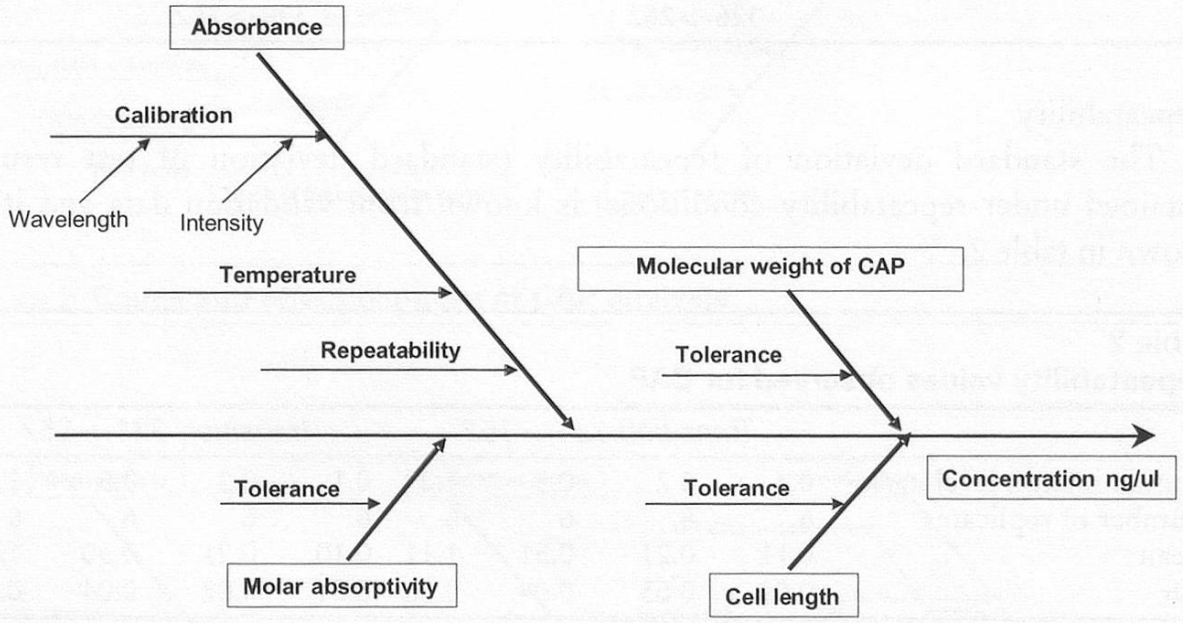

Figure 4 **Cause and effect related to equation 2**

The deviations in wavelength observed for the holmium spectrum are in a range of 0.2 and 0.6 nm. The differences in absorbance observed for the holmium spectrum between two analysis performed within an interval of one month are 1.2 % at 240.9 nm and 0.02 % at 536.5 nm.

The error on the absorbance measurement has been previously evaluated. Five measurements of an absorbing solution made the same day, by the same person and with the same instrument gave an average absorbance of 0.15588 with an SD of 0.0002 (RSD 0.12 %).

The value of the molar absorptivity coefficient is recognized internationally. Nevertheless according to our experiment the relative standard deviation on ϵ can

be estimated as 1%. The tolerance on the quartz cell is in the order of 0.1%. Taking into account all these considerations, the calculation of the uncertainty on stock solution concentration is summarized in table 3.

Table 3
Estimation of uncertainty of the concentration of stock solution (c_0)

$$C_0 = \frac{A \cdot M_w \cdot 1000}{\epsilon \cdot d}$$

	Symbol (x_i)	*Value*	*Standard uncertainty* $u(x_i)$	*Relative uncertainty* $\frac{u(x_i)}{x_i}$
Absorbance	A	0.6		0.012
Molar absorptivity	ϵ	298 L/mol cm	2.98	0.01
Cell length	d	1 cm	0.001	0.001

$$\frac{u(c_0)}{c_0} = \sqrt{(0.012)^2 + (0.01)^2 + (0.001)^2} = 0.016$$

The stock solution is then diluted and mixtures of CAP/d5-CAP are prepared. The dilution is performed by adding 500 µl of stock solution with a glass syringe in a volumetric flask of 100 ml. One example of calculation of the uncertainty on dilution is presented in table 4.

Finally, the uncertainties of the calibration solutions taking into account the dilution and the addition of d5-CAP are summarized in table 5. We can observe that the relative measurement uncertainty is constant for all the dilutions, and an average of 2.6 % of relative standard deviation can be taken at all levels.

Linear calibration curves

The study of the calibration curves showed that the relationship between the area ratios and concentration ratios is linear and that the least square model ax+b is adapted. However, the study of residual highlighted the heteroscedasticity of the data, as the standard deviation of the area ratios increased with the increase of concentration ratio. Therefore, the standard deviation (SD) of residuals was not constant. However, expressed as a relative standard deviation (RSD) the value is constant along the range calibrated that is from 0 to 2 µg/kg. To transform the relative standard deviation of residuals that was expressed in area ratio into the concentration ratio, the RSD was divided by the slope of the calibration curve. The values obtained for both transitions are 7 % for 321 -> 152 and 5 % for 321 -> 257.

Mass of internal standard added

The internal standard was added to the sample with an automatic pipette. The calculation of the uncertainty is summarized in table 6.

Table 4
Estimation of uncertainty for the dilution of CAP and d5-CAP standard solutions

$$\text{Dilution factor} = \frac{\text{Volume}_{\text{syringe}}}{\text{Volume}_{\text{flask}}}$$

	Value	*Standard uncertainty u(x)*	*Relative uncertainty* $\frac{u(x)}{x}$
Repeatability of 500 ul syringe filling	500 µl	1.14 µl[a)]	0.0023
Tolerance of syringe	500 µl	$\frac{5}{\sqrt{3}} = 2.88$ µl[b)]	0.0058
Temperature effect	500 µl	$\frac{2.5}{\sqrt{3}} = 1.44$ µl[c)]	0.0029
Repeatability of 100 ml flask filling	100000 µl	40[d)]	0.0004
Tolerance of 100 ml flask	100000 µl	$\frac{100}{\sqrt{3}} = 57.73$ µl	0.0006
Temperature effect	100000 µl	$\frac{500}{\sqrt{3}} = 288.68$ µl	0.0029

$$\frac{u(\text{Dilution factor})}{\text{Dilution factor}} = \sqrt{(0.0023)^2 + (0.0058)^2 + (0.0004)^2 + (0.0006)^2} = 0.0063$$

a) The relative standard deviation (repeatability) obtained by filling ten times a syringe with 500 µl of water is 1.14 µl.
b) The tolerance of the syringe declared by the supplier is ±1% given as an interval. The value must be divided by square root of 3 to be transformed into standard deviation.
c) The temperature interval in the laboratory was ±5°C. The volume expansion of organic solvents being 1×10^{-3}°C^{-1}, the temperature effect is then $\pm(500\times1\times1\times10^{-3}) = \pm2.5$ µl given as an interval. The value must be divided by square root of 3 to be transformed into standard deviation.
d) The tolerance of the 100 ml flask used is ±100 µl, a rectangular distribution is assumed. The repeatability observed by filling up to the mark 10 times a 250 ml flask was 0.07 ml. The temperature interval in the laboratory was ±5°C. The volume expansion of organic solvents beomg 1×10^{-3}°C^{-1}, the temperature effect is then $\pm(100000\times1\times1\times10^{-3}) = \pm500$ µl given as an interval. The value must be divided by square root of 3 to be transformed into standard deviation.
e) Temperature effects on solvent expansion of both syringe and flask are the same if the solvent transferred and solvent used for dilution are the same, therefore this contribution is not taken into account for the calculation of the uncertainty on dilution factor.

Table 5
Uncertainties of calibration solutions

CAP Concentration (ng/ml)	*d5-CAP Concentration (ng/ml)*	*CAP/d5-CAP concentration ratio*	*Standard uncertainty*	*Relative uncertainty (%)*
1	5	0.2	0.005	2.6
2	5	0.4	0.010	2.6
5	5	1	0.027	2.7
10	5	2	0.051	2.5
20	5	4	0.102	2.5

Table 6
Estimation of uncertainty on the mass of internal standard added to the sample ($C_{d5\text{-}CAP/sample}$)

$$C_{d5-CAP/sample} = \frac{C_{d5-CAP} \cdot V_{d5-CAP}}{Mass_{sample}}$$

	Value	*Standard uncertainty* $u(x)$	*Relative uncertainty* $\frac{u(x)}{x}$
Stock solution	10 ng/ml	0.18	0.018
Volume transfered	100 ml	0.48	0.0048
Mass of sample	2 g	0.0041	0.0021

$$\frac{u(C_{d5-CAP/sample})}{C_{d5-CAP/sample}} = \sqrt{(0.018)^2 + 2 \cdot (0.0048)^2 + (0.0021)^2} = 0.019$$

Mass of sample

The uncertainty of the mass of a sample portion was given as an interval of ±0.005 for the balance used. To transform it into standard deviation it has to be divided by square root of 3 (assuming rectangular distribution). This component uncertainty is counted twice, once for the tara and once for the sample

$$u(\text{sample mass}) = \sqrt{2 \cdot \left(\frac{0.005}{\sqrt{3}}\right)^2} = 0.0041 \text{ g}$$

Trueness/Recovery

Recovery (R) and its uncertainty (u(R)), were estimated by analysis of spiked samples. $\overline{R}_m$, the method recovery, is estimated by comparing the mean of n replicate analysis of spiked samples with the certified value $\overline{R}_m = \frac{\overline{c}_{obs}}{c_{spike}}$. The uncertainty associated with this estimate $u(\overline{R}_m)$ is composed of the uncertainty in the spiked sample value, $u(c_{spike})$, and the uncertainty in the observed value $u(c_{obs})$, it is calculated by the following equation, data and results are given in table 7.

$$u(\overline{R}_m) = \overline{R}_m \cdot \sqrt{\frac{1}{n}\left(\frac{u(\overline{c}_{obs})}{\overline{c}_{obs}}\right)^2 + \left(\frac{u(c_{spike})}{c_{spike}}\right)^2}$$

A significance test was used to determine whether the mean recovery was significantly different from unity. The statistic $t = \frac{\overline{R}_m - 1}{u(\overline{R}_m)}$ was compared to a critical value t_{crit} (two tailed t test at 95 % confidence level with n-1 degrees of freedom) and to the coverage factor (k=2) used for the final expression of uncertainty.

For all the spiked levels no correction were necessary, or implicitly R=1. The required standard uncertainty associated with R=1 is given in Table 7. In fact, the absolute recovery determined by spiking with ^{14}C-CAP is around 70 %, but the loss

of substance during the laboratory work is corrected with the internal standard added at the beginning of the analysis.

Table 7
Estimation of recovery uncertainty obtained from the replicate analysis of spiked samples

Transition	*Spiking level (µg/kg)*	*u(Rm)*	*t*	*n*	t_{crit}	*Correction*
321→152	0.1	0.060	0.39	18	2.11	No
	0.2	0.036	1.40	18	2.11	No
	0.5	0.031	1.88	18	2.11	No
321→257	0.1	0.064	0.16	18	2.11	No
	0.2	0.052	1.08	18	2.11	No
	0.5	0.049	0.46	18	2.11	No

n = number of replicates
t_{crit} = critical value for one tailed t-test n=18

Step 5. Calculation of the combined uncertainty

The combined uncertainties are calculated for the two different types of transitions by using the uncertainty budgets in table 8 and table 9. All the measurement uncertainties for the different spiking levels and incurred residue are summarized in table 10. The individual contributions to the combined uncertainty are shown in figures 5 and 6.

Table 8
Uncertainty budget for the transition 321→152

$$\text{CAP (ng/100 g)} = \frac{\left(\frac{A_a}{A_{is}}\right) - I}{S} \cdot \frac{M_{is}}{M_a} \cdot \frac{1}{R}$$

	Symbol (x)	*Value*	*Standard uncertainty u(x)*	*Relative uncertainty* $\frac{u(x)}{x}$
Repeatability	r	0.11 µg/kg	0.02 µg/kg	0.18
		0.21 µg/kg	0.03 µg/kg	0.14
		0.51 µg/kg	0.04 µg/kg	0.08
		1.11 µg/kg	0.06 µg/kg	0.05
Recovery	R	1.00	0.060	0.060
		1.00	0.036	0.036
		1.00	0.031	0.031
Calibration model				0.07
Concentration ratio CAP/d5-CAP	C_{ratio}	0.02 to 4.0	Depends on value	0.026
Mass of sample	M_s	2 g	0.005 g	0.0025
Addition of d5-CAP	M_{is}	0.5 µg/kg	0.0095 µg/kg	0.02

$$\frac{u(\text{CAP})}{\text{CAP}} = \sqrt{(0.18)^2+(0.06)^2+(0.07)^2+(0.026)^2+(0.0025)^2+(0.02)^2} = 0.19^*$$

*example for a concentration of 0.11 µg/kg

Table 9
Uncertainty budget for the transition 321→257

$$\text{CAP (ng/100 g)} = \frac{\left(\frac{A_a}{A_{is}}\right) - I}{S} \cdot \frac{M_{is}}{M_a} \cdot \frac{1}{R}$$

	Symbol	*Value*	*Standard uncertainty*	*Relative uncertainty*
	(x)		*u(x)*	$\frac{u(x)}{x}$
Repeatability	r	0.10 µg/kg	0.01 µg/kg	0.10
		0.21 µg/kg	0.02 µg/kg	0.095
		0.50 µg/kg	0.035 µg/kg	0.07
		1.26 µg/kg	0.05 µg/kg	0.04
Recovery	R	1.00	0.064	0.064
		1.00	0.052	0.052
		1.00	0.049	0.049
Calibration model				0.05
Concentration ratio CAP/d5-CAP	C_{ratio}	0.2 to 4	Depends on value	0.026
Mass of sample	M_s	2 g	0.005 g	0.0025
Addition of d5-CAP	M_{is}	0.5 µg/kg	0.0095 µg/kg	0.02

$$\frac{u(CAP)}{CAP} = \sqrt{(0.10)^2+(0.064)^2+(0.05)^2+(0.026)^2+(0.0025)^2+(0.02)^2} = 0.13^*$$

*example for a concentration of 0.10 µg/kg

Table 10
Summary of measurement uncertainty for the different levels

Transition	*Spiking level (µg/kg)*	*u(x) (µg/kg)*	$\frac{u(x)}{x}$	*U(x) (µg/kg)*
321→152	0.11	0.023	0.205	0.046
	0.21	0.034	0.164	0.068
	0.51	0.059	0.116	0.118
	1.11*	0.112	0.101	0.214
321→257	0.10	0.013	0.133	0.026
	0.21	0.026	0.124	0.052
	0.50	0.052	0.104	0.104
	1.26*	0.109	0.087	0.218

* recoveries obtained with the higher level of spiking were used for calculation of incurred residues
U(xi) is the expanded measurement uncertainty, that is the standard uncertainty multiplied by a coverage factor of two for a confidence level of 95%

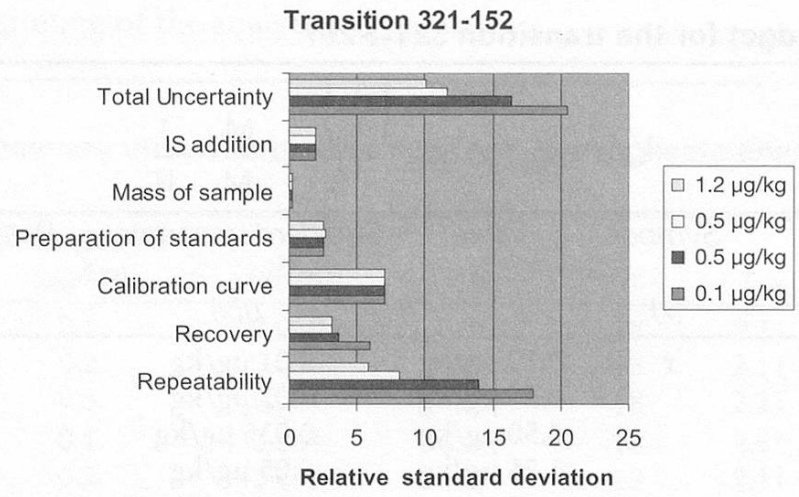

Figure 5 **Uncertainty budget for transition 321 -> 152**

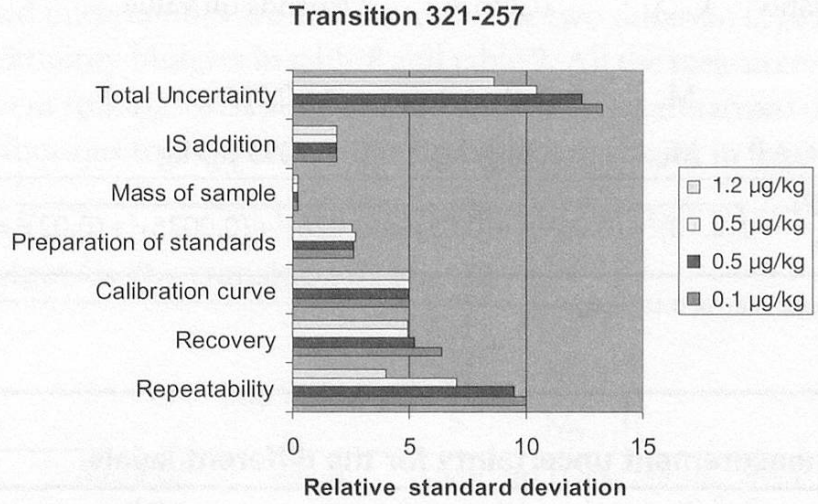

Figure 6 **Uncertainty budget for transition 321 -> 257**

Step 6. Expression of final expanded uncertainty

The final uncertainty is expressed as an interval or expanded measurement uncertainty U(x). To transform the standard uncertainty u(x) into U(x), it is multiplied by a coverage factor of 2 as suggested by the Eurachem guide for a confidence level of 95 %. The expression of the analytical result is then reported as Result $\pm 2 \times u(x)$ or Result $\pm U(x)$.

Intermediate reproducibility

The precision calculated with results obtained by three different operators at one week time intervals and on the same apparatus in the same laboratory yielded the values presented in table 11.

Table 11
Intermediate reproducibility values observed for CAP

	Transition 321 -> 152				*Transition 321 -> 257*			
Contamination level ug/kg	0.1	0.2	0.5	1.2*	0.1	0.2	0.5	1.2*
Number of replicates	18	18	18	18	18	18	18	18
Mean	0.10	0.19	0.47	1.11	0.10	0.21	0.52	1.26
SD_R	0.024	0.030	0.045	0.15	0.026	0.041	0.096	0.10
RSD_R	0.24	0.15	0.10	0.13	0.26	0.20	0.18	0.08

* estimated value from native contaminated sample

Calculation of CCα and CCβ

These limits have been estimated by spiking blank materials at different level (0.1, 0.2 and 0.5 ug/kg). After analysis, the signal ratio was plotted against the added concentration. The corresponding concentration at the y-intercept plus 2.33 times the standard deviation of intercept is an estimation of CCα. To estimate CCβ 1.64 standard intermediate reproducibility or standard measurement uncertainty is added to CCα. The results are summarized in table 12.

Table 12
Decision limits and detection capabilities calculated for the two transitions

	Transition	*Limit values in intermediate reproducibility conditions (µg/kg)*	*Limit values with measurement uncertainty* (µg/kg)*
CCα	321->152	0.03	0.03
			0.02
			0.04
	321->257	0.04	0.04
			0.03
			0.06
CCβ	321->152	0.04	0.04
			0.03
			0.06
	321->257	0.07	0.06
			0.05
			0.06

* three experiments were carried out in repeatability conditions

Discussion

The contribution of the different parameters to the whole measurement uncertainty depends on the concentration analysed. At low levels the precision (measured in repeatability conditions) is clearly the main contribution. However, for concentrations equal to or higher than 0.5 ug/kg, the recovery and uncertainty of the calibration are no longer negligible. The measurement uncertainty estimation obtained

for the transition 321->257 appears lower than for the transition 321->152. However, the precision measured in intermediate reproducibility conditions is higher for the second transition that is a consequence of the higher long-term variation of the instrument for this transition.

Nevertheless, the measurement uncertainties estimated are of the same order of magnitude as the intermediate reproducibility. This is of great importance regarding the estimation of the decision limit and detection capability, because that means that the calculation of these limits under repeatability conditions is possible. The standard measurement uncertainty u(x) used instead of SD_R in the calculation of CCα and CCβ gives the same values.

The measurement uncertainties estimated in this study were compared to the empirical Horwitz's formula (7, 8) from which the intermediate reproducibility could be estimated by $SD_R = 0.01C^{0.85}$, where C is the concentration expressed in mass fraction, in our case $C = 10^{-9}$ for ug/kg (see table 13). The Horwitz empirical approach tends to overestimate SD_R. One explanation could be that the precision of instruments that allow such low detections has been improved since the results used for Horwitz's approach were produced. Nevertheless, in all cases the Horwitz's formula gives values higher than the estimated measurement uncertainty from validation data. This implies that, to a first approximation, using Horwitz's empirical formula does not underestimate the measurement uncertainty.

Table 13
Decision limits and detection capabilities calculated for the two transitions

Transition	*Concentrations* (µg/kg)	*Measurement Uncertainty* u(x)	*Measured* SDR	*Horwitz' formula result* SDR
321->152	0.11	0.023	0.024	0.034
	0.21	0.034	0.030	0.059
	0.51	0.059	0.045	0.126
	1.11*	0.112	0.150	0.245
321->257	0.10	0.013	0.026	0.032
	0.21	0.026	0.041	0.059
	0.50	0.052	0.096	0.124
	1.26	0.109	1.100	0.272

Conclusions

The uncertainty in the chromatographic determination of CAP has been calculated. Individual sources of uncertainty have been identified, quantified and combined into a relative standard uncertainty for two transitions measured by LC-MS/MS.

The main contributions to the relative uncertainty for CAP in Food are the repeatability at low levels of contamination. At levels higher than 0.5 µg/kg, the contribution of the recovery and the calibration are no longer negligible. The

standard uncertainties are not constant over the analytical range. Even the relative standard uncertainties are not constant over the studied ranges.

The relative standard uncertainties obtained from the intermediate reproducibility (different operators, different times of analysis) were found comparable to the calculated relative uncertainties. This means that the intermediate reproducibility value can be used to estimate the uncertainty of the method, if validation data are not available.

The decision limit and detection capability were determined in intermediate reproducibility conditions and in repeatability conditions by the use of measurement uncertainty. The values obtained in both conditions are comparable. That means that the measurement uncertainty provides a first estimation of CCα and CCβ that should be confirmed with intermediate reproducibility data and or by spiking the samples at the calculated level.

By using Horwitz empirical approach, we can get a rapid estimation of the measurement uncertainty for a given level of contamination. This study shows that the results obtained with that formula are all above the measurement uncertainty estimated with validation data.

Abstract

One of the requirements of the new ISO norm 17025 for accredited test laboratories concerns the measurement uncertainty of results. This latter has been estimated for chloramphenicol for food products containing honey. As a consequence of the zero tolerance level of choramphenicol in food, the calculation addressed the content of chloramphenicol at the lowest detectable quantities. The measurement uncertainty value obtained in repeatability conditions was compared to intermediate reproducibility i.e. long term precision of the method. Since the values are not significantly different we can state that, for this method, intermediate reproducibility corresponded to an adequate estimation of measurement uncertainty. Furthermore, measurement uncertainty was used for the determination of both decision limit CCα and detection capability CCβ, criteria introduced by the European Union to replace detection limit. These limit values were not different from those calculated under intermediate reproducibility conditions as recommended by the european guidelines. Hence limits can be obtained before having carried out tests under intermediate reproducibility conditions, which needs more time. Moreover, the estimated measurement uncertainties were compared to the empirical approach by the Horwitz's formula. All the values calculated with this formula were higher than the values obtained from validation data. This means that using the Horwitz approach for a first measurement uncertainty estimation there is no risk of its underestimation for the chloramphenicol analysis.

Résumé

Une des exigences de la norme ISO 17025 relative aux laboratoires d'essais accrédités concerne l'estimation de l'incertitude de mesure qui doit être fournie avec un résultat. L'incertitude de mesure a donc été calculée pour l'analyse du chloramphenicol dans des aliments contenant du miel. Sachant que le chloroamphenicol est soumis à une tolérance zéro dans les produits, l'incertitude de mesure a été estimée à des concentrations proches de la limite de détection de la méthode d'analyse. Cette incertitude a été calculée en conditions de répétabilité puis comparée à la reproductibilité intermédaire ou fidélité du laboratoire. Les deux valeurs ne se sont pas avérées différentes. Cela signifie que la reproductibilité intermédiaire est une bonne estimation de l'incertitude avec pour avantage de pouvoir utiliser cette valeur en lieu et place d'un calcul fastidieux. Et vice versa, l'incertitude calculée en condition de répétabilité peut remplacer la reproductibilité intermédiaire pour l'évaluation des limites CCα et CCβ pour les laboratoires qui doivent évaluer ces limites sans disposer d'une trop grande quantité de valeurs en conditions de reproductibilité intermédiaire. Les incertitudes de mesures estimées dans cette étude ont été comparées aux résultats que l'on obtient avec l'approche empirique d'Horwitz. Les valeurs obtenues avec la formule d'Horwitz sont toutes plus élevées que celle calculées. Cela signifie que si pour une première approximation de l'incertitude de mesure on utilise l'approche proposée par Horwitz, il ne devrait pas y avoir de risque de sous estimation pour cette méthode.

Zusammenfassung

Eine Auflage der neuen ISO Norm 17025 bezüglich der Akkreditierung von analytischen Testlaboratorien betrifft eine Angabe der Messunsicherheit eines Messwertes. In der vorliegenden Studie wurde die Messunsicherheit rechnerisch ermittelt, die der HPLC-MS-Bestimmung von Chloramphenicol in Lebensmitteln anhaftet, die Honig enthalten. Angesichts der gesetzlichen Vorschrift, dass Chloramphenicol in Lebensmitteln nicht nachweisbar sein darf, wurde die Messunsicherheit im Bereich der unteren Bestimmungsgrenze ermittelt. Die unter Wiederholbedingungen bestimmte Messunsicherheit wurde mit der verglichen, die man unter sog. in-house oder Labor-Vergleichsbedingungen beobachtet. Da im Fall der vorliegenden Methode beide Ansätze zu sehr ähnlichen Ergebnissen führen, kann man festhalten, dass die Labor-Vergleichbarkeit für diese Methode eine angemessene Abschätzung der Messunsicherheit erlaubt und somit eine aufwendige Berechnung derselben ersetzen kann. Umgekehrt erlaubt es die unter Wiederholbedingungen errechnete Messunsicherheit, die sog. CCα und CCβ-Werte für die Nachweis- und Bestimmungsgrenze dieser Methode festzulegen, die sonst nur unter Hinzuziehung ausreichender Validierungsdaten ermittelt werden können. Dies kann sich als nützlich herausstellen, wenn und solange zu wenige Vergleichbarkeits-Daten verfügbar sind. Zuletzt wurden die so ermittelten Messunsicherheiten mit den Werten verglichen, die man mittels des empirischen Ansatzes nach der Horwitz-Formel

ableitet. Die nach dieser Formel erhaltenen Werte waren ausnahmslos höher als die von uns bestimmten. Dies bedeutet, dass die Horwitz-Formel für eine erste Abschätzung der Messunsicherheit in dem Sinne hinreichend ist, dass das Risiko einer Unterschätzung der Messunsicherheit dieser Methode äußerst gering ist.

Key words

Measurement uncertainty, validation, chloramphenicol, decision limit, detection capability

References

1 Chloramphenicol Summary report from the EMEA Committee for Veterinary Medicinal products. The European Agency for the Evaluation of Medicinal Products (1996)

2 *Council Directive 2002/657/EC:* Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities, L 221/8, (2002)

3 *ISO 17025:1999:* General requirements for the competence of testing and calibration laboratories.

4 *Eurachem:* Quantifiying Uncertainty in Analytical Measurement. 2nd Edition (2000)

5 *Mottier P., Parisod V., Gremaud E., Guy P.A. and Stadler H.R.:* Determination of antibiotic chloramphenicol in meat and seafood products by liquid chromatography-electrospray ionisation tandem mass spectrometry. J. Chrom. A **994**, 75–84 (2003)

6 *Verzegnassi L., Royer D., Mottier P. and Stadler R.H.:* Analysis of chloramphenicol in honeys of different geographical origin by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Food additives and contam. **20**, 335–242 (2003)

7 *Horwitz W.:* The certainty of uncertainty. J. AOAC International **86**, 109–111 (2003)

8 *Thompson M. and Lowthan P.J.:* The Horwitz funtion revisited. J. AOAC **80**, 676–679 (1997)

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