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Uncertainties of gas chromatographic measurement of troublesome pesticide residues in apples employing conventional and mass spectrometric detectors

Radim Štěpán, Jana Hajšlová*, Vladimír Kocourek, Jana Tichá

Department of Food Chemistry and Analysis, Institute of Chemical Technology (ICT), Technická 5, Prague 6, 166 28, Czech Republic

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Abstract

In recent years the declaration of estimated uncertainty of measurement has become an integral part of analytical results. This study presents the assessment of results generated within the analysis of selected pesticides represented by carbamates, pyrethroides and azoles, residues of which may be found in treated apples. Multiresidue method used for analysis of spiked samples (residues at levels 0.040–0.163 mg/kg) consisted of (i) ethyl acetate extraction, (ii) GPC clean-up and (iii) identification/quantification of residues by GC. Procedures utilizing either conventional (electron-capture, nitrogen–phosphorus) or mass-selective detectors (quadrupole and ion trap analyzer) were evaluated. The results generated through alternative strategies of uncertainty estimation ("bottom-up", "top-down") were compared.

Using the "bottom-up" approach uncertainty of extraction which comprises two components—(i) repeatability of extraction and (ii) uncertainty of extraction recovery was shown to represent the main source of combined standard uncertainty (values of uncertainty of extraction for tested pesticides ranged from 4.6% to 21.6%). On the other hand, uncertainties associated with the GC calibration (uncertainties of weighing and diluting standards, uncertainties of purity of standards) were not so important (most of them did not exceed 2%). Combined standard uncertainties associated with the described analytical method ranged for individual compounds from 9.3% to 24.3%. Similar values of combined standard uncertainties were obtained using the alternative "top-down" approach.
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Keywords: Uncertainty of measurement; Bottom-up; Top-down; Pesticide residues; Plant matrices; Gas chromatography

1. Introduction

Wherever possible and practical the performance characteristics of analytical methods used for control of regulation limits, established to foods/feeds, should be evaluated through a collaborative trial conforming to an international protocol. Besides common performance characteristics (accuracy, ruggedness, sensitivity, linearity, limit of detection, etc. [1,2]) testing laboratories shall have and shall apply procedures for estimating uncertainty of measurements. By definition [3] uncertainty is "a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand". This clearly means that analytical result cannot be viewed only as a separate value. The information on uncertainty is needed in test reports whenever (i) it is relevant to the validity of the test results; (ii) a client's instructions so requires and/or (iii) the uncertainty may affect compliance with a specification. Mainly, the latter aspect is important in context of this study that is concerned with pesticide residues analysis aimed at control of compliance with maximum residue limits (MRL).

According to the EURACHEM/CITAC document [4] "bottom-up" approach can be used for estimation of combined standard uncertainty. This strategy splits the analytical process in single steps, estimating the individual contribution of each one to the uncertainty of the final results. Subsequently, it is possible to decide which are the more significant and which are negligible (and therefore do not deserve special attention). Alternatively, more practical "top-down" approach is recommended in the recently published ISO 21748:2004 [5] which gives a guidance for

* Corresponding author. Tel.: +42 224 353 185; fax: +42 224 353 185.
E-mail address: jana.hajslova@vscht.cz (J. Hajšlová).

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the estimation of measurement uncertainty using data obtained from interlaboratory studies performed in accordance with ISO 5725-2 (determination of repeatability and reproducibility of a standard measurement method) [6]. It does not describe the application of validation data in the absence of collaborative study results and the basic assumption is that all laboratories use strictly unified method. In specific areas, such as multiresidual analysis, the application of this attitude is also somewhat unrealistic. On the other hand, the "top-down" approach seems to be very effective solution in most cases as it applies a "black-box" model to estimate the uncertainty from characteristic features of the globally considered analytical method (bias, repeatability and reproducibility).

Different concepts can be combined to provide a more practical and understandable way of measurement uncertainty calculations, based mainly on already existing quality control and validation data, namely the use of CRM, participation in interlaboratory comparisons (proficiency test) and recovery tests. The (intermediate) reproducibility within-laboratory is combined with estimates of the method and laboratory bias where possible [7].

Several papers concerned with the estimation of uncertainty of organic contaminants measurement have been published during the recent decade [8–12]. The typical example of the uncertainty calculation using the "bottom-up" approach for the determination of nonylphenol in water by alternative procedures and GC–MS detection was described by Díaz et al. [12]. However, the estimation of all individual uncertainty contributions seems to be rather difficult for analytical methods employing complex operating procedures and involving many analytes.

Cuadros-Rodríguez et al. [8] applied the "bottom-up" approach in estimation of uncertainty associated with determination of organophosphorus and organochlorine pesticides contained in cucumber. Repeatability of determination of analytes in spiked samples and also uncertainty associated with the preparation of the calibration standard solutions (weighing, diluting) were identified as the most significant sources of combined uncertainty. The dependence of uncertainty value on the concentration of analyte in examined sample was documented in this study as well. In another study [9] "bottom-up" approach was applied to uncertainty estimation in analysis of organophosphorus pesticides in sweet peppers. Significant sources of uncertainty were identified on the basis of statistical comparison (*F*-test) between (i) combined uncertainty associated with gravimetric, volumetric and chromatographic quantification steps of analytical method (so-called "incomplete" estimation in this paper) and (ii) experimental dispersion of replicated analysis of spiked samples.

The aim of presented study was a critical assessment of two alternative approaches approved nowadays for estimation of combined uncertainty of measurement. Both "bottom-up" and "top-down" strategies were used for estimation of uncertainty associated with analysis of pesticide residues in apples. Carbamates, pyrethroides and azoles were selected as representatives of pesticides possessing wide range of physico-chemical properties hence differing in sources of uncertainty of measurement.

2. Experimental

2.1. Reagents and materials

(a) *Pesticide standards* (characteristics see in Table 1) were obtained from Dr. Ehrenstorffer (Germany). Stock solutions of individual pesticide standards in toluene were used for preparation of standard mixtures used for 4-point calibration. Concentrations relevant to the highest calibration point mixture D_1 are shown in

Table 1
Pesticide standards

Analyte	Purity of analytical standard (%)	Calibration mixture, D_1[a] (μg/ml, toluene)	Physico-chemical properties [14]		
			Molecular weight	Solubility in water (mg/l)	$\log K_{ow}$[b]
β-Cyfluthrin	97.0	0.079	434.3	2×10^{-3} (20 °C)	5.9
Bifenthrin	98.0	0.092	422.9	$<1 \times 10^{-3}$	>6
Bitertanol	98.0	0.108	337.4	3.8 (20 °C)	4.1
Carbaryl	99.5	0.221	201.2	120 (20 °C)	1.85
Chlorpropham	98.0	0.200	213.7	89 (25 °C)	–
Cypermethrin	91.0	0.107	416.3	4×10^{-3}	6.6
Deltamethrin	98.5	0.234	505.2	$<2 \times 10^{-4}$ (25 °C)	4.6
Fenarimol	99.5	0.197	331.2	13.7 (25 °C)	3.69
Fenoxycarb	98.5	0.163	301.3	7.9 (25 °C)	4.07
Fenvalerate	99.0	0.095	419.9	1×10^{-2} (25 °C)	5.01
Permethrin	97.5	0.205	391.3	6×10^{-3} (20 °C)	6.1
Prochloraz	97.5	0.325	376.7	34.4 (25 °C)	4.12
Propham	99.5	0.146	179.2	250 (20 °C)	–
Tebuconazole	98.0	0.133	307.8	36 (20 °C)	3.7

[a] See Section 2.1.
[b] K_{ow} = *n*-octanol–water partition coefficient.

Table 1. The other three solutions D_2, D_3, D_4 were prepared by dilution of D_1 (2×, 10×, 20×) by toluene, respectively. For experiments described in Section 2.4 spiking solutions in ethyl acetate—D_5 (conc. range 0.400–1.630 μg/ml) and ethyl acetate:cyclohexane (1:1, v/v)—D_6 (conc. range 0.020–0.089 μg/ml) were prepared.

(b) *Organic solvents* (for GC residue analysis): cyclohexane and toluene were purchased from Merck (Germany), ethyl acetate from Scharlau (Spain).
(c) *Sodium sulphate, anhydrous* obtained from Penta (Czech Republic) was dried at 500 °C for 6 h and then stored in a tightly closed glass container before use.
(d) *Apples:* fruit free of pesticide residues were obtained from organic farm.

2.2. Apparatus

(a) *Homogenizer* Ultra-Turrax (IKA, Germany) was used for sample preparation.
(b) *Vacuum rotary evaporator* Büchi Rotavapor (Büchi, Switzerland) was used for removing of organic solvents from extracts and sample fractions.
(c) *Automated high-performance gel permeation chromatography (HPGPC)* system Aspec (Gilson, France) equipped with 600 mm × 7.5 mm PLgel high-performance column (Polymer Laboratories, UK) was used for purification of crude apple extracts; conditions were as follows: ethyl acetate:cyclohexane (1:1) as a mobile phase, flow rate 1 ml/min.
(d) *Gas chromatograph* (i) HP 6890 Plus (Hewlett-Packard, USA) equipped with autosampler (HP 7683) and capillary column (see Section 2.3.3) connected through a Y-piece both to a nitrogen–phosphorus detection (NPD) system and an electron-capture detection (ECD) system;; (ii) HP 6890 (Hewlett-Packard, USA) equipped with a mass-selective detector (quadrupole) HP 5973 (Hewlett-Packard, USA) and (iii) the Trace 2000 (Thermo Quest, USA) equipped with CombiPal (CTC Analytics, Switzerland) autosampler and Polaris Q ion trap mass selection detector (Finnigan, USA) were used for identification/quantification of pesticide residues in purified extracts.

2.3. Analytical method

2.3.1. Extraction

Twenty-five grams of aliquot portion of homogenized apples was weighed into a glass beaker. Hundred milliliters of ethyl acetate and 75 g of anhydrous sodium sulphate were added and the mixture was homogenized (2 min) using Ultra-Turrax. The homogenate was then filtered through a layer of anhydrous sodium sulphate. The extraction beaker and filter cake were rinsed with 3 × 25 ml of ethyl acetate. Combined filtrates were vacuum evaporated and the residue was dissolved in ethyl acetate:cyclohexane (1:1, v/v) and made up to 50 ml.

2.3.2. GPC clean-up

Two milliliters of crude extract (0.5 g of original matrix in 1 ml) were automatically loaded onto gel column via the sample loop. The first portion of eluate was discarded into waste, the second one (15.0–31.0 ml) was collected. This pesticide fraction was vacuum concentrated and the residual solvent was removed with a gentle stream of nitrogen. Dry extract was dissolved in 1 ml of toluene and analyzed by gas chromatography.

2.3.3. Gas chromatography and data processing

All the gas chromatographic separations (regardless the instrument used) were carried out under the same conditions: injection (1 μl of sample or standard solution) was carried out by pulsed splitless technique (injector temperature 250 °C, injection pulse: 60 psi, injection pulse period: 2 min); capillary column DB5-MS (60 m × 0.25 mm, 0.25 μm, J&W Scientific, USA) was used for separation; temperature program: initial temperature 90 °C (2 min), 5 °C/min to 180 °C (0 min), 2 °C/min to 280 °C (5 min); helium was used as a carrier gas (programmed flow); detectors: (i) NPD: temperature 300 °C, hydrogen flow 3 ml/min, air flow 60 ml/min, nitrogen flow (make-up) 10 ml/min; (ii) ECD: temperature 300 °C, anode gas flow (nitrogen) 6 ml/min, make-up gas flow (nitrogen) 60 ml/min; (iii) MS: quadrupole analyzer, interface temperature 280 °C, ion source temperature 230 °C, (iv) MS: ion trap analyzer, interface temperature 275 °C, ion source temperature 200 °C, ionization technique employed in both mass-selective detectors for experiments: electron impact (EI), selected ions for identification/quantification: see Table 2.

All chromatographic data were processed using ChemStation (A.04.05, Hewlett-Packard, USA) or Xcalibur (Finnigan, USA) softwares.

2.4. Determination of uncertainties

Three sets of experiments shown in Fig. 1 and described below were carried out. Concentration levels of analytes in spiked samples injected onto GC column were identical in all experiments and corresponded to level D_2; 1 ml of these samples contained equivalent to 1 g of original apples. In each experiment $E_{1–3}$, six replicates of spiked samples were analyzed to obtain data for evaluation of repeatability and recovery [13].

2.4.1. Experiment E_1

2.5 ml of pesticide mixture in ethyl acetate (D_5, see Section 2.1) were added to the 25 g of apple homogenate placed in a glass beaker. Determination of target pesticides was carried out by procedures described in Sections 2.3.1–2.3.3.

Table 2
Experiments employing MS: monitored ions and MS–MS conditions (ions used for quantification are in bold)

Analyte	SIM experiments	MS–MS experiments		
	Selected ions (*m/z*)	Parent ion (*m/z*)	Daughter ion (*m/z*)	Excitation voltage (V)
β-Cyfluthrin	**163**, 206, 226	206	**150**	1.30
Bifenthrin	165, 166, **181**	181	**165**	1.00
Bitertanol	141, **170**, 171	170	**141**	1.30
Carbaryl	115, 116, **144**	144	**115**	1.00
Chlorpropham	**127**, 171, 213	127	**100**	1.50
Cypermethrin	163, **181**, 209	181	**152**	1.00
Deltamethrin	181, 209, **253**	181	**152**	1.50
Fenarimol	**219**, 251, 330	139	**111**	1.00
Fenoxycarb	116, 186, **255**	186	**157**	1.00
Fenvalerate	**167**, 225, 419	167	**125**	1.00
Permethrin	163, 165, **183**	183	**168**	1.50
Prochloraz	**180**, 266, 310	180	**138**	1.00
Propham	93, **137**, 179	179	**137**	1.00
Tebuconazole	125, **250**, 252	250	**163**	1.10

2.4.2. Experiment E_2

Four milliliters of ethyl acetate extract prepared from blank apples (see Section 2.3.1) were evaporated to dryness and the residue was then dissolved in 4 ml of standard solution in ethyl acetate:cyclohexane (1:1, v/v) (D_6, see Section 2.1). The sample was analyzed by procedures described in Sections 2.3.2–2.3.3.

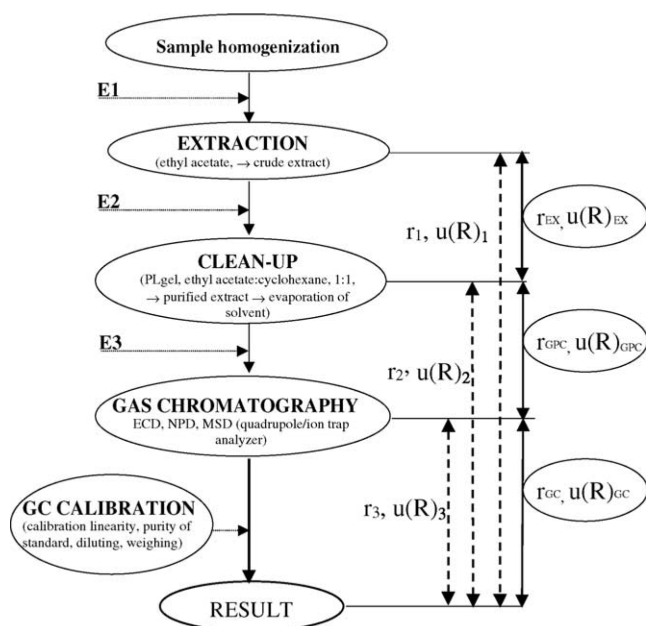

Fig. 1. Uncertainty components associated with the analytical method. E_{1-3} arrows show the phase of analytical procedure in which spiking by selected pesticides was carried out. r_{1-3} are repeatabilities (uncertainties type A, random errors) obtained in experiments E_{1-3}. $u(R)_{1-3}$ are uncertainties of recovery (uncertainties type B, systematic errors) obtained in experiments E_{1-3}. r_{EX}, r_{GPC}, r_{GC} are repeatabilities associated with extraction, clean-up and GC steps. $u(R)_{EX}$, $u(R)_{GPC}$, $u(R)_{GC}$ are uncertainties of recoveries of extraction, clean-up and GC steps, individually.

2.4.3. Experiment E_3

Purified extract (GPC eluate) prepared from blank apples by the procedures in Sections 2.3.1–2.3.2 was dissolved in 1 ml of calibration mixture in toluene (D_2) and analyzed by gas chromatography (see Section 2.3.3).

3. Results and discussion

General strategy of this study aiming at estimation of uncertainties associated with measurement of pesticide residues in apples involved three basic steps:

(i) specification of analytical procedure and identification of potential sources of uncertainty;
(ii) quantification of uncertainty components;
(iii) calculation of the combined standard uncertainty.

Two alternative approaches mentioned in Introduction were tested and compared in this study. The results are summarized below.

3.1. The "bottom-up" approach

Adopting this approach we considered the fact that under real-life conditions, uncertainty of each individual analytical step consists of its random and systematic component ("error"). Each of this type of component was quantified and incorporated into the combined standard uncertainty. In fact, there are many potential sources of uncertainty which arise from individual phases of described multiresidue method. Besides all gravimetric and volumetric steps (sample weighing, dilution of sample extracts, uncertainty of volume of GPC loop, etc.) there are many other operations and factors (evaporation of sample extracts, temperature, etc.) which contribute to the overall uncertainty. However, detailed exploration and evaluation of all these uncertainty

sources is complicated and impractical. Therefore the decision was made to evaluate uncertainties of three basic analytical steps (extraction, clean-up, GC measurement) without further evaluation of sources which are incorporated into them. In fact, this methodology represents a bottom-up strategy, where principal steps (extraction, clean-up and GC measurement) are considered as "black sub-box" treated in the "top-down" way—as there are uncertainty sources that cannot be easily quantified in an individual way. When considering the uncertainties associated with GC calibration, the "bottom-up" approach was fully adopted and therefore, uncertainties of weighing and diluting the pesticide standards together with uncertainties of purity of standards were evaluated and calculated. Uncertainty associated with calibration linearity is also discussed.

3.1.1. Uncertainty of extraction, clean-up and GC measurement

3.1.1.1. Random components ("random errors") of uncertainty. In the present study, the random errors of extraction, clean-up and GC determinative steps were approximated by relative standard deviations (R.S.D., %) which were calculated from repeated determinations of analytes ($n = 6$), obtained in experiments E_{1-3} shown in Fig. 1. It should be noted that repeatabilities r_{1-3} are not independent (r_1 includes r_2 and r_3, r_2 includes r_3).

To determine random components of uncertainty (expressed as repeatabilities), which can be separately assigned to extraction, clean-up and GC steps (r_{EX}, r_{GPC}, r_{GC}, see Fig. 1) following Eqs. (1)–(3) were used:

$$r_{EX}\,(\%) = \sqrt{r_1^2 - r_{GPC}^2 - r_{GC}^2} \quad (1)$$

$$r_{GPC}\,(\%) = \sqrt{r_2^2 - r_{GC}^2} \quad (2)$$

$$r_{GC}\,(\%) = \sqrt{r_3^2} = r_3 \quad (3)$$

The results are summarized in Table 3.

Values of r_1 repeatability (i.e. R.S.D. of whole procedure, see Table 3) ranged from 7.3% to 13.1% for most of compounds at spiking level 0.040–0.163 mg/kg. Such results are comparable with those reported in similar validation studies [15–18]. In presented study only prochloraz and tebuconazole showed higher r_1 repeatabilities (21.5% and 19.3%, respectively). In case of prochloraz calculation of repeatabilities associated with individual analytical steps enabled to identify extraction as the critical step as regards random error, since its repeatability represents the highest contribution to the overall repeatability r_1.

Relatively poor values of GC repeatability r_{GC} identified for prochloraz and tebuconazole (see Table 3) were probably due the tailing of their peaks what may result in inaccurate integration of their areas.

As shown in Fig. 2, rather high values of GC repeatability (compared to pyrethroides) were also obtained for carbamates. This is probably caused by difficulties occurring during transfer of these analytes from injector onto analytical column. Matrix induced chromatographic response enhancement ("matrix effects") reported by several authors [19–22] are mainly caused by thermodegradation of these compounds in hot GC injector.

Table 3
Repeatabilities obtained in experiments E_{1-2} and calculated repeatabilities of extraction, clean-up and GC steps

Pesticide	Detection	r_1 (%)	r_2 (%)	r_{EX} (%)	r_{GPC} (%)	$r_{GC} = r_3$[a] (%)
Pyrethroides						
β-Cyfluthrin	ECD	7.5	4.7	5.9	4.0	2.5
Bifenthrin	ECD	7.3	4.1	6.0	1.4	3.8
Cypermethrin	ECD	7.9	3.9	6.9	2.2	3.2
Deltamethrin	ECD	13.1	5.3	11.9	2.8	4.5
Fenvalerate	ECD	9.5	4.2	8.5	1.4	3.9
Permethrin	ECD	10.2	4.4	9.2	3.2	3.1
Carbamates						
Carbaryl	NPD	8.7	7.5	4.4	4.0	6.4
Chlorpropham	NPD	9.7	8.6	4.4	4.7	8.2
Fenoxycarb	NPD	8.5	7.2	4.5	2.4	6.8
Propham	NPD	9.8	8.1	5.4	3.1	7.9
Azoles						
Bitertanol	ECD	11.2	9.5	6.0	4.9	8.1
Fenarimol	ECD	12.8	5.7	11.5	2.8	4.9
Prochloraz	ECD	21.5	9.6	19.3	3.3	9.0
Tebuconazole	ECD	19.3	13.7	13.5	7.4	11.5

[a] See Eq. (3).

3.1.1.2. Systematic components ("systematic errors") of uncertainty. Systematic components of uncertainty were estimated on the basis of recoveries obtained in E_{1-3} experiments (see Section 2.4, Fig. 1). Responses of analytes in each batch of matrix containing samples were compared with those obtained in standard mixture in net solvent (toluene) containing the same concentration of respective analyte. Uncertainties of these apparent recoveries $[u(R)_{1-3}]$ were derived from rectangular distribution using Eq. (4)

$$u(R)_{1-3}\,(\%) = \frac{0.5 \times (100 - R_{E_{1-3}})}{\sqrt{3}} \quad (4)$$

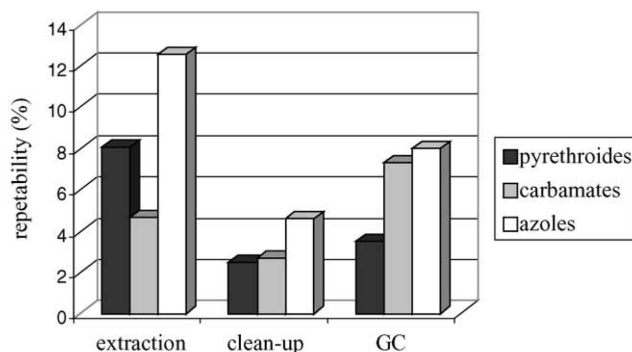

Fig. 2. Repeatabilities of extraction, clean-up and GC steps (average values for groups of pesticides).

where $R_{E_{1-3}}$ is recovery of particular analyte in respective experiment E_{1-3}.

Individual uncertainties associated with (i) recovery of extraction and (ii) recovery of clean-up were calculated using Eqs. (5)–(7)

$$u(R)_{EX}\,(\%) = \sqrt{u(R)_1^2 - u(R)_{GPC}^2 - u(R)_{GC}^2} \quad (5)$$

$$u(R)_{GPC}\,(\%) = \sqrt{u(R)_2^2 - u(R)_{GC}^2} \quad (6)$$

$$u(R)_{GC}\,(\%) = \sqrt{u(R)_3^2} = u(R)_3 \quad (7)$$

where $u(R)_{EX}$ represents uncertainty of recovery of extraction, $u(R)_{GPC}$ uncertainty of recovery of GPC clean-up and $u(R)_{GC}$ uncertainty of recovery of GC (resp. uncertainty associated with matrix effects).

Recoveries determined in experiments E_{1-3} and systematic components of uncertainty calculated using Eqs. (5)–(7) are summarized in Table 4.

As shown in Table 4, values of $u(R)_1$ uncertainty (i.e. uncertainty of recovery associated with the whole procedure—experiment E_1) ranged from 2.9% to 8.4% for most of compounds. The highest values of $u(R)_1$ and also $u(R)_{EX}$ (i.e. uncertainty of recovery associated with extraction) were obtained for prochloraz. This is probably due to partial ionization of this compound ($pK_a = 3.8$, [14]) hence its poorer extractability from acidic matrix. Our recent experience show possible solution of this problem in increasing the pH value of matrix before ethyl acetate extraction by adding Na_2CO_3 solution (recovery increases to 84% at pH = 7). Results of other studies indicate that using alternative extraction set-up may lead to better recoveries of prochloraz. For instance, Blasco et al. [23] achieved recoveries 80–101% for prochloraz extracted from oranges by matrix solid phase dispersion technique and dichlormethane as elution solvent, Gelsomino et al. [24] reported recovery 86% for spiked sample of melon extracted by acetone followed by liquid–liquid partitioning with dichloromethane.

Pyrethroides generally showed slightly lower recoveries of GPC clean-up (see Table 4), which lead to higher values of systematic component [$u(R)_{GPC}$] of GPC uncertainty. The reason is, that chromatographic band of this group of analytes on PLgel column is not completely separated from co-extractives (the molecular weight of pyrethroides is higher compared to other pesticides). To avoid unacceptable penetration of matrix components into fraction of pesticides, the front part of pyrethroides fraction eluted from the gel column has to be sacrificed.

It should be noted that rather worse recoveries could be obtained in analysis of incurred residues compared to spiked samples. Unfortunately, no such material (e.g. certified reference material or proficiency test sample) was available for our experiment.

3.1.2. Uncertainty sources associated with GC calibration

Besides of the above-discussed phases of the analytical process, there are also other potential sources of error that may contribute to the combined standard uncertainty. Within the "bottom-up" approach, uncertainties of several other operations and procedures potentially influencing the result of analysis were explored.

Attention was paid to weighing and diluting of the analytical standards as well as their purity.

3.1.2.1. Uncertainty of weighing. The random component (r_{BAL}) of this operation was calculated using Eq. (8)

$$r_{BAL}\,(\%) = \frac{S.D._w}{m_A} \times 100 \quad (8)$$

Table 4
Recoveries of extraction, clean-up and GC steps and associated uncertainties

Pesticide	Detection	R_{E_1} (%)	R_{E_2} (%)	R_{E_3} (%)	$u(R)_1$ (%)	$u(R)_2$ (%)	$u(R)_{EX}$ (%)	$u(R)_{GPC}$ (%)	$u(R)_{GC} = u(R)_3$[a] (%)
Pyrethroides									
β-Cyfluthrin	ECD	71	80	98	8.4	5.9	6.0	5.8	0.7
Bifenthrin	ECD	79	81	100	6.2	5.6	2.6	5.6	0.1
Cypermethrin	ECD	83	84	97	4.9	4.6	1.6	4.6	0.8
Deltamethrin	ECD	81	85	99	5.4	4.3	3.3	4.3	0.2
Fenvalerate	ECD	85	89	99	4.3	3.1	2.9	3.1	0.4
Permethrin	ECD	82	83	99	5.2	5.0	1.5	5.0	0.4
Carbamates									
Carbaryl	NPD	86	97	92	4.1	3.9	1.3	3.0	2.4
Chlorpropham	NPD	90	95	93	2.9	2.4	1.7	1.2	2.0
Fenoxycarb	NPD	87	99	97	3.6	1.4	3.4	1.1	0.9
Propham	NPD	73	95	96	7.8	1.5	7.6	0.7	1.3
Azoles									
Bitertanol	ECD	75	97	98	7.2	0.9	7.1	0.8	0.5
Fenarimol	ECD	77	94	96	6.7	1.8	6.4	1.4	1.1
Prochloraz	ECD	57	94	94	12.3	7.6	9.7	5.0	1.6
Tebuconazole	ECD	72	94	99	8.1	1.7	7.9	1.7	0.3

[a] See Eq. (7).

Table 5
Random and systematic components of uncertainty of weighing

Pesticide	r_{BAL} (%)	σ_{BAL} (%)
Pyrethroides		
β-Cyfluthrin	1.5	0.05
Bifenthrin	2.1	0.07
Cypermethrin	1.2	0.04
Deltamethrin	1.4	0.05
Fenvalerate	1.0	0.04
Permethrin	1.5	0.05
Carbamates		
Carbaryl	1.2	0.04
Chlorpropham	0.8	0.03
Fenoxycarb	1.9	0.07
Propham	0.7	0.03
Azoles		
Bitertanol	2.9	0.10
Fenarimol	3.1	0.11
Prochloraz	2.4	0.08
Tebuconazole	2.2	0.08

where S.D.$_w$ is standard deviation of repeated weighings ($n = 6$) of empty volumetric flask ($V = 10$ ml) which was used for preparation of stock solution of respective compound and m_A is the amount of pesticide standard used for preparation of stock solution ($V = 10$ ml). Values of m_A ranged for tested pesticides from 17.3 to 74.2 mg.

The systematic component (σ_{BAL}) of uncertainty of weighing was calculated for each compound according to Eq. (9)

$$\sigma_{BAL}\,(\%) = \frac{a}{m_A \times \sqrt{3}} \times 100 \tag{9}$$

where a is a weighing tolerance declared in the calibration certificate of balances (±0.033 mg). The results are summarized in Table 5.

3.1.2.2. Uncertainty of dilution. The random component (r_{DIL}) of uncertainty of dilution was calculated using Eq. (10)

$$r_{DIL}\,(\%) = \frac{\text{S.D.}_B}{m_B - m_C} \times 100 \tag{10}$$

where S.D.$_B$ is standard deviation of weighing the volumetric flask ($V = 10$ ml) which was repeatedly ($n = 6$) filled with toluene up to the mark, m_B is the average weight of equally processed volumetric flask and m_C is the average weight of empty volumetric flask ($V = 10$ ml, $n = 6$).

The confidence interval $\pm b$ of a volumetric flask supplied by manufacturer (±0.025 ml) was used for calculating the systematic component of uncertainty (σ_{DIL}); rectangular distribution was considered—Eq. (11):

$$\sigma_{DIL}\,(\%) = \frac{b}{V \times \sqrt{3}} \times 100 \tag{11}$$

where V is the volume of volumetric flask ($V = 10$ ml).

As shown in the above paragraphs, both components of uncertainty of dilution were dependent only on the specification of volumetric flask (volume and confidence interval provided by supplier) and therefore were the same for all tested compounds. Values of r_{DIL} and σ_{DIL}, resp. calculated using Eqs. (10) and (11) were 0.07% and 0.14%, respectively.

3.1.2.3. Uncertainty of purity of analytical standards. Since the uncertainty of standard purity was not declared in certificate provided by supplier, a decision was taken to replace it by the estimation given by Eq. (12) derived from rectangular distribution:

$$u_{STD}\,(\%) = \frac{0.5 \times (100 - y)}{\sqrt{3}} \tag{12}$$

where y (%) represents the purity of standard given in the manufacturer specification (see Table 1). It should be noted, that concentration of pesticides in stock and calibration solutions were not corrected for standard purity (its value for all standards was ≥97%, with exception of cypermethrin).

As shown in Table 6, uncertainties associated with purity of pesticide standards did not exceed 1% for most of compounds. Cypermethrin (see Table 1) represents example of analyte for which standard with purity only 91% was available. Accordingly, related uncertainty is relatively high. It should be noted that quality of analytical standard is given not only by their inherent purity but also by the quality of certificates obtained from suppliers.

3.1.2.4. Uncertainty of calibration linearity. Non-linearity of the calibration curve can be also considered as another source of uncertainty. Initially, we have proposed the estimation of its contribution based on the correlation coefficient.

Table 6
Uncertainties of purity of pesticide standards

Pesticide	u_{STD} (%)
Pyrethroides	
β-Cyfluthrin	0.87
Bifenthrin	0.58
Cypermethrin	2.60
Deltamethrin	0.43
Fenvalerate	0.29
Permethrin	0.72
Carbamates	
Carbaryl	0.15
Chlorpropham	0.59
Fenoxycarb	0.44
Propham	0.15
Azoles	
Bitertanol	0.58
Fenarimol	0.14
Prochloraz	0.72
Tebuconazole	0.58

Table 7
Summary of combined uncertainties obtained for tested pesticides using the "bottom-up" approach

Pesticide	Detection	u_{cEX} (%)	u_{cGPC} (%)	u_{cGC} (%)	u_{cBAL} (%)	u_{cDIL} (%)	u_{STD} (%)	Combined uncertainty, u_c (%)
Pyrethroides								
β-Cyfluthrin	ECD	8.4	7.1	2.6	1.5	0.16	0.87	11.4
Bifenthrin	ECD	6.6	5.8	3.8	2.1	0.16	0.58	9.8
Cypermethrin	ECD	7.0	5.1	3.3	1.2	0.16	2.60	9.7
Deltamethrin	ECD	12.4	5.1	4.5	1.4	0.16	0.43	14.2
Fenvalerate	ECD	9.0	3.4	4.0	1.0	0.16	0.29	10.5
Permethrin	ECD	9.3	5.9	3.1	1.5	0.16	0.72	11.6
Carbamates								
Carbaryl	NPD	4.6	5.0	6.8	1.2	0.16	0.15	9.7
Chlorpropham	NPD	4.7	2.8	8.5	0.8	0.16	0.59	10.2
Fenoxycarb	NPD	5.6	2.7	6.8	1.9	0.16	0.44	9.3
Propham	NPD	9.4	2.0	8.0	0.7	0.16	0.15	12.6
Azoles								
Bitertanol	ECD	9.3	5.0	8.2	2.9	0.16	0.58	13.7
Fenarimol	ECD	13.1	3.1	5.1	3.1	0.16	0.14	14.8
Prochloraz	ECD	21.6	6.0	9.1	2.4	0.16	0.72	24.3
Tebuconazole	ECD	15.7	7.6	11.5	2.2	0.16	0.58	21.0

It came out that this concept is not valid as the correlation coefficient is a poor measure of the curve-fit quality of heteroscedastic data [25]. As the contribution of the calibration non-linearity is apparently negligible (despite of the relatively wide range of concentrations), this source of uncertainty has been ignored.

3.1.3. Calculation of the combined standard uncertainty

Random and systematic components of uncertainty of each analytical step were used for calculation of combined uncertainty associated with analytical procedure (extraction, clean-up, gas chromatography, weighing, diluting)—see Eqs. (13)–(17)

$$u_{cEX}\,(\%) = \sqrt{r_{EX}^2 + u(R)_{EX}^2} \tag{13}$$

$$u_{cGPC}\,(\%) = \sqrt{r_{GPC}^2 + u(R)_{GPC}^2} \tag{14}$$

$$u_{cGC}\,(\%) = \sqrt{r_{GC}^2 + u(R)_{GC}^2} \tag{15}$$

$$u_{cBAL}\,(\%) = \sqrt{r_{BAL}^2 + \sigma_{BAL}^2} \tag{16}$$

$$u_{cDIL}\,(\%) = \sqrt{r_{DIL}^2 + \sigma_{DIL}^2} \tag{17}$$

where u_{cEX} represents combined uncertainty of extraction, u_{cGPC} represents combined uncertainty of GPC clean-up, u_{cGC} represents combined uncertainty of GC step, u_{cBAL} represents combined uncertainty of weighing, u_{cDIL} represents combined uncertainty of dilution.

Combined uncertainties of each procedure (together with uncertainty of purity of analytical standard, see Table 6) were used for calculation of combined standard uncertainty u_c associated with analytical method employed for pesticide residue analysis—see Eq. (18)

$$u_c\,(\%) = \sqrt{u_{cEX}^2 + u_{cGPC}^2 + u_{cGC}^2 + u_{cBAL}^2 + u_{cDIL}^2 + u_{STD}^2} \tag{18}$$

As shown in Table 7, summarizing all entries, u_c for target analytes ranged from 9.3% for fenoxycarb to 24.3% for prochloraz. As mentioned in Sections 3.1.1.1 and 3.1.1.2 and shown in Figs. 3–5, uncertainty of extraction (both random and systematic components) represents the most important source of the combined uncertainty (especially for pyrethroides and azoles). Uncertainty of clean-up is significant for pyrethroides, uncertainty of GC step for carbamates.

3.2. The "top-down" approach

This part of our study was focused on experimental evaluation of uncertainty sources represented by repeatability of analysis of spiked samples (expressed as relative standard deviation for individual analytes) and uncertainty of

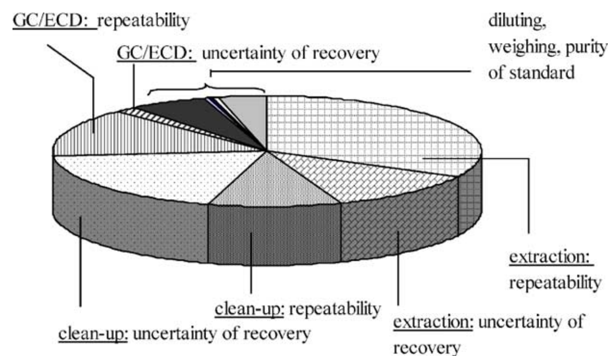

Fig. 3. Contribution of individual uncertainties to the total uncertainty (average values for pyrethroides).

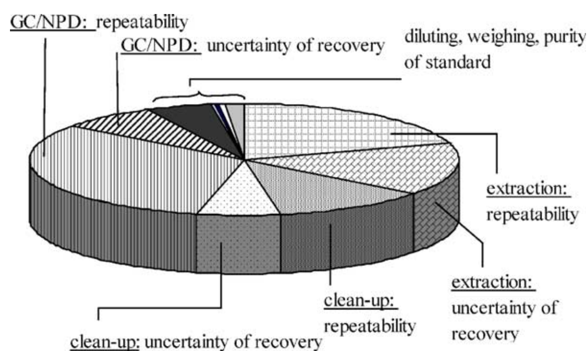

Fig. 4. Contribution of individual uncertainties to the total uncertainty (average values for carbamates).

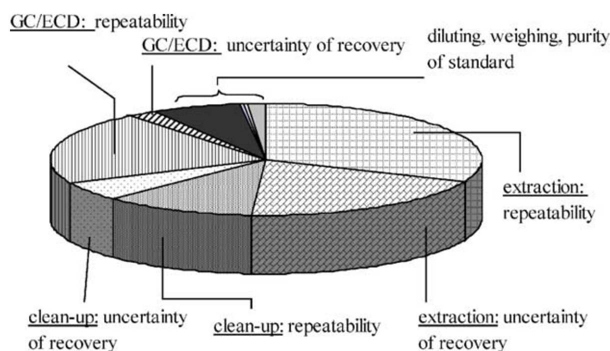

Fig. 5. Contribution of individual uncertainties to the total uncertainty (average values for azoles).

recovery $u(R)_t$ derived from rectangular distribution using Eq. (19)

$$u(R)_t\,(\%) = \frac{0.5 \times (100 - R_t)}{\sqrt{3}} \tag{19}$$

where R_t is a recovery of particular analyte.

In each experiment (realized as described in Section 2.4, experiment E_1) the series of six spiked samples (concentration level 0.040–0.163 mg/kg) was prepared and analyzed. Experiment was repeated in 4 months intervals during 1 year.

Both uncertainty sources were combined to obtain the combined standard uncertainty u_{ctn} using Eq. (20)

$$u_{ctn}\,(\%) = \sqrt{r_{tn}^2 + u(R)_{tn}^2} \tag{20}$$

where r_{tn} represents repeatability, $u(R)_{tn}$ represents uncertainty of recovery (series of experiment $n = 1$–3).

Average combined standard uncertainties u_{cAV} were calculated as a quadratic mean (21)

$$u_{cAV}\,(\%) = \sqrt{\frac{u_{ct1}^2 + u_{ct2}^2 + u_{ct3}^2}{3}} \tag{21}$$

Comparing the data in Table 8, no significant differences were detected between combined uncertainties of series 1–3 (there were not significant changes in performance of the method in a long-term period). Also combined standard uncertainties were comparable to those obtained by the "bottom-up" approach (see Table 7). In fact, "top-down" approach represents simpler and more effective way to calculation of combined standard uncertainty.

3.3. Repeatabilities of GC analysis using various detectors

For some compounds (carbamates, pyrethroides) the selection of detection/confirmation technique may lead to

Table 8
Summary of uncertainties obtained for tested analytes using the "top-down" approach

Pesticide	Detection	Series 1			Series 2			Series 3			Average combined uncertainty, u_{cAV} %)
		r_{t1} (%)	$u(R)_{t1}$ (%)	u_{ct1} (%)	r_{t2} (%)	$u(R)_{t2}$ (%)	u_{ct2} (%)	r_{t3} (%)	$u(R)_{t3}$ (%)	u_{ct3} (%)	
Pyrethroides											
β-Cyfluthrin	ECD	7.5	8.4	11.3	9.9	5.9	11.5	11.6	7.2	13.7	12.2
Bifenthrin	ECD	7.3	6.2	9.6	4.5	4.8	6.6	5.6	5.8	8.0	8.2
Cypermethrin	ECD	7.9	4.9	9.3	7.2	5.5	9.1	10.8	3.5	11.3	10.0
Deltamethrin	ECD	13.1	5.4	14.1	9.4	4.2	10.3	12.9	4.9	13.8	12.9
Fenvalerate	ECD	9.5	4.3	10.4	6.5	2.8	7.1	5.7	5.2	7.7	8.5
Permethrin	ECD	10.2	5.2	11.5	8.3	5.4	9.9	8.2	4.0	9.1	10.2
Carbamates											
Carbaryl	NPD	8.7	4.1	9.6	7.4	6.6	10.0	6.5	4.6	8.0	9.2
Chlorpropham	NPD	9.7	2.9	10.1	7.7	3.1	8.3	8.3	4.0	9.2	9.3
Fenoxycarb	NPD	8.5	3.6	9.2	7.2	3.1	7.9	9.1	5.5	10.6	9.3
Propham	NPD	9.8	7.8	12.5	8.9	3.4	9.6	14.0	4.9	14.8	12.5
Azoles											
Bitertanol	ECD	11.2	7.2	13.3	11.6	5.2	12.7	13.8	4.0	14.4	13.5
Fenarimol	ECD	12.8	6.7	14.4	9.3	9.0	13.0	11.6	5.5	12.8	13.4
Prochloraz	ECD	21.5	12.3	24.8	19.4	20.4	28.1	21.4	15.4	26.4	26.5
Tebuconazole	ECD	19.3	8.1	20.9	23.2	14.3	27.3	24.2	7.2	25.3	24.6

Table 9
Repeatabilities of GC measurement using various detectors (ND = not detected at studied concentration level, monitored ions and MS–MS conditions—see Table 2)

Pesticide	R.S.D. (%)				
	GC–ECD	GC–NPD	GC–MS		
			Quadrupole analyzer	Ion trap analyzer (mode SIM)	Ion trap analyzer (mode MS–MS)
Pyrethroides					
β-Cyfluthrin	2.5	ND	8.5	12.2	18.7
Bifenthrin	3.8	ND	4.8	6.0	7.2
Cypermethrin	3.2	ND	7.9	11.3	9.4
Deltamethrin	4.5	ND	8.1	11.0	9.9
Fenvalerate	3.9	ND	7.5	7.9	14.1
Permethrin	3.9	ND	6.2	7.2	5.8
Carbamates					
Carbaryl	ND	6.4	5.1	6.5	8.7
Chlorpropham	ND	8.2	3.0	3.4	10.0
Fenoxycarb	ND	6.8	4.6	5.7	8.4
Propham	ND	7.9	2.7	3.1	11.5
Azoles					
Bitertanol	8.1	ND	8.7	10.2	ND
Fenarimol	4.9	ND	4.9	5.2	ND
Prochloraz	9.0	ND	16.6	15.2	ND
Tebuconazole	11.5	ND	7.5	6.9	ND

SIM = selected ion monitoring.

changes in combined standard uncertainty. The tested detection techniques were conventional (ECD, NPD) and MS (employing quadrupole and/or ion trap as analyzers). In each system the sequence of six injections of matrix samples (prepared in experiment E_3, see Section 2.4) was carried out and relative standard deviations were calculated (concentration level of analytes: D_2, see Section 2.1) Results are summarized in Table 9.

One of the most pronounced differences in repeatability of measurement obtained by alternative detectors was noticed

Table 10
Detection limits

Pesticide	Detection limit (μg/ml)			
	ECD	MS		
		Quadrupole analyzer	Ion trap analyzer (mode SIM)	Ion trap analyzer (mode MS–MS)
Pyrethroides				
β-Cyfluthrin	0.005	0.020	0.025	0.035
Bifenthrin	0.001	0.015	0.020	0.020
Cypermethrin	0.005	0.020	0.025	0.020
Deltamethrin	0.002	0.020	0.030	0.030
Fenvalerate	0.005	0.020	0.030	0.030
Permethrin	0.003	0.020	0.025	0.020
	NPD	Quadrupole analyzer	Ion trap analyzer (mode SIM)	Ion trap analyzer (mode MS–MS)
Carbamates				
Carbaryl	0.010	0.005	0.012	0.015
Chlorpropham	0.020	0.003	0.008	0.025
Fenoxycarb	0.010	0.005	0.010	0.012
Propham	0.020	0.002	0.005	0.025
	ECD	Quadrupole analyzer	Ion trap analyzer (mode SIM)	Ion trap analyzer (mode MS–MS)
Azoles				
Bitertanol	0.010	0.010	0.015	0.085
Fenarimol	0.005	0.010	0.010	0.115
Prochloraz	0.030	0.050	0.050	0.200
Tebuconazole	0.010	0.020	0.020	0.095

for pyrethroides. Due to extensive fragmentation under electron impact conditions, only low intensity ions are available for selective detection. On this account relatively high detection limits [1] were obtained (see Table 10) and repeatability of peak intensity measurement was clearly worse than obtained by ECD.

On the other hand, for carbamates (carbaryl, fenoxycarb) repeatability remains quite comparable regardless of the detection technique. Relatively poor values of repeatabilities of propham and chlorpropham obtained in GC–NPD and GC–MS (mode MS–MS) systems are obviously due to the high limits of detection of these compounds.

Based on results summarized in Table 9 it can be concluded that repeatability achieved in the MS–MS mode is either similar or worse compared to SIM for the most of compounds. This is probably due to lower intensity of daughter ions even under optimized conditions—Table 2 (e.g. β-cyfluthrin, fenvalerate; in case of azoles the daughter ions were not detected at studied concentration level at all).

4. Conclusions

In the presented study, "bottom-up" and "top-down" approaches used for estimation of combined standard uncertainty were shown to provide comparable results. The advantage of the "bottom-up" approach is the possibility of getting insight into the individual uncertainties and identification of the most important ones. The latter aspect is obviously important whenever further optimisation/up-grade of the method in planned. On the other hand, the experimental evaluation especially in the case of multistep analytical methods as well as respective calculations are rather complicated and laborious. The "top-down" approach takes the combined sources of uncertainty directly into account and provides relatively simple estimation of uncertainty of measurement. However, specific suggestions for improvement of performance characteristics of respective method are not so straightforward.

As the main source of uncertainty extraction process (repeatability and uncertainty of recovery) was identified. Large differences in uncertainties of GC step depending on the type of detector should be also noted. For some pesticides better repeatabilities can be obtained by conventional detectors as compared to MS. This is mainly the case of compounds extensively fragmented under electron impact ionization (e.g. pyrethroides).

From practical point of view, it seems suitable to apply the "bottom-up" approach when new method is implemented. Once the uncertainty is estimated and the important sources are known the "top-down" approach represents a good compromise to uncertainty calculation, e.g. after revalidation of the analytical method.

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