

# Estimation of the efficiencies and uncertainties of the extraction and cleanup steps of pesticide residue determination in cucumber using $^{14}\text{C}$ -carbaryl

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**Abstract** The estimation of the uncertainty of measurement has, nowadays, become an integral part of analytical results. The uncertainty and efficiency of extraction and cleanup are very important components of a pesticide residue analytical method. In this work, for a quick review of extraction and cleanup efficiency and to evaluate the individual analysis steps during method adaptation,  $^{14}\text{C}$ -carbaryl was applied at all fortification levels. Then, further analyses, such as ethylacetate extraction, filtration, evaporation and cleanup, were performed. The calibration of gel chromatograph column, performed with both  $^{14}\text{C}$ -carbaryl and fortification mixture (dichlorvos, malathion and chlorpyrifos), showed that pesticide fractions came through the column between the 8- and 23-mL fractions. The overall recovery of  $^{14}\text{C}$ -carbaryl after the extraction and cleanup step was 0.91, with the relative uncertainty of 0.084. Using the “bottom-up” approach, the uncertainty of extraction  $u_{\text{cEX}}$  and cleanup  $u_{\text{cGPC}}$  were 0.033 and 0.107, respectively. The combined standard uncertainty  $u_{\text{c}}$  associated with the described analytical method was 0.112. Similar values were obtained using the alternative “top-down” approach: uncertainty of extraction  $u_{\text{ct1}}$  was 0.039, uncertainty of cleanup  $u_{\text{ct2}}$  was 0.108 and the combined standard uncertainty  $u_{\text{cAV}}$  was 0.081. Both approaches showed that the uncertainty of cleanup was the main source of combined standard uncertainty.

**Keywords**  $^{14}\text{C}$ -pesticide · Uncertainty of extraction and cleanup · GPC calibration · Cleanup efficiency

## Introduction

Pesticide residue analytical methods entail extraction of the residues from the sample matrix, removal of the coextracted material and cleanup. Cleaning up of the extract prior to chromatographic analysis is commonly performed for a variety of reasons, which include: (1) sample matrix coextractants may have a deleterious effect on the gas chromatography (GC) capillary column, (2) sample matrix coextractants may interfere with the detection of pesticides at trace levels, (3) sample matrix coextractants may result in a sample-matrix-induced effect [1].

The most universally applicable cleanup is gel permeation chromatography (GPC). This cleanup method is a useful technique to isolate a group of target analytes with different chemical structures in the same elution fraction, and also to remove a large number of interfering high-molecular-weight matrix components [2]. The separation of compounds in GPC are based on their molecular size. The coextractive molecules, larger than the pore size of the gel, pass through the column faster than smaller pesticide molecules. The main components of a GPC system are ethylacetate/cyclohexane 1/1 v/v elution solvent and Bio-Beads<sup>®</sup> SX-3. To perform precise calibration of the GPC column is inevitable for obtaining clean eluate and high recoveries. If the eluent collection is started early, a substantial amount of the coextractives remain in the pesticide fraction. On the other hand, if the collection of pesticide fraction is delayed, the recoveries of early eluting compounds might be unacceptably low [3].

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The radiotracer technique (using  $^{14}\text{C}$ -labelled compound) is very useful tool for proving the quality assurance and quality control parameters, such as purity and/or purification of an active ingredient, homogeneity of the processed sample, extraction and cleanup efficiency and recovery. They should be within the required limits. The use of  $^{14}\text{C}$ -labelled pesticide is very advantageous in residue analysis, since we can precisely and quickly determine the analyte directly in the extract without using any chromatographic techniques. So the precision of the final results can be greatly improved. Generally,  $^{14}\text{C}$ -labelled pesticide is used for this aim and it can be traced in any analytical step by measuring its radioactivity with a liquid scintillation analyser. Unlabelled pesticides can also be used for this aim, but their applications take much longer and the estimated uncertainty of the procedure may be less precise [4–6].

A number of researches worked on extraction efficiency and reported that the best way for determining extraction efficiency is to use radiolabelled compounds [7–9]. The procedure was carried out as follows: (1) treat crops with radiolabelled pesticides at the field, (2) combust sample (collected from the field) for determining the total  $^{14}\text{C}$  residues, (3) extract sample with solvent based on the nature of compounds and crops, (4) calculate extraction efficiency as a percentage of the total applied  $^{14}\text{C}$  radioactivity.

The above mentioned efficiencies of the extraction and cleanup steps have vital importance in pesticide residue analysis, since they are a major source of bias (systematic error). The lower the extraction efficiency, the lower the recovery of analyte. Similarly, ineffective cleanup may result in not only lower recovery, but also matrix effects on the chromatographic system, which can cause inaccurate analytical results.

Another important quality control measure is to check repeatability as a component of precision. The repeatability of any analytical procedure is assessed by calculating the relative standard deviation (RSD%) of replicate measurements. The values and acceptable ranges for repeatability and recovery are set in many guidelines, such as the European Union Guide or Codex Guideline for a quantitative method to determine pesticide residues in foods [10, 11].

Besides common method validation parameters, residue laboratories should apply procedures for estimating the uncertainty of measurements [12, 13]. The information on uncertainty is needed in test reports whenever: (1) it is relevant to the validity of the test results, (2) a client's instructions so requires and/or (3) the uncertainty may affect compliance with a specification [14].

Several approaches were developed for the estimation of uncertainty related to analytical measurements [14–16] and two of the best known, namely, “bottom-up” and “top-down,” are described in the EURACHEM/CITAC document [17] and in the recently published ISO 21748:2004 [18], respectively. The first approach splits the analytical process into single steps, estimating the individual contribution of each one to the uncertainty of the final results. Subsequently, it is possible to decide which are more significant. The top-down approach may be more practical and gives guidance for the estimation of measurement uncertainty using the data obtained from in-house validation and/or interlaboratory studies.

The “bottom-up” and “top-down” concepts for the estimation of uncertainty were compared by Stepan et al. [14] in their intensive work. They reported that the main source of combined standard uncertainty was the uncertainty of extraction by using the “bottom-up” approach. The advantage of the “bottom-up” approach is the possibility of obtaining insight into the individual uncertainties and the identification of the most important ones. The latter approach is obviously important whenever further optimisation/upgrade of the method is planned. The “top-down” approach takes the combined sources of uncertainty directly into account and provides a relatively simple estimation of the uncertainty of measurement and seems to be a very effective solution in most cases, as it has characteristic features of the globally considered analytical method. From a practical point of view, it seems suitable to apply the “bottom-up” approach when a new method is implemented. Once the uncertainty is estimated and the important sources are known, the “top-down” approach represents a good compromise on the uncertainty calculation.

In fact, there are many potential sources of uncertainty which arise from individual phases of the described multiresidue method. Besides all gravimetric and volumetric steps, there are many other operations and factors (evaporation of sample extracts, temperature etc.) which contribute to the overall uncertainty. However, as mentioned above, detailed evaluation of all of these uncertainty sources is complicated and impractical. Therefore, the decision was made to evaluate the uncertainties of three basic analytical steps (extraction, cleanup and GC measurement) without further evaluation of the sources which are incorporated into them [14].

The purpose of our study was to determine the efficiency of extraction, the calibration of GPC and the efficiency of cleanup by using  $^{14}\text{C}$ -carbaryl. The methodology for the uncertainty assessment of extraction and cleanup was taken from Stepan et al. [14], who applied both “bottom-up” and “top-down” approaches.

## Materials and methods

### Chemicals and reagents

The standard dichlorvos, malathion and chlorpyrifos-ethyl, with purities of 97%, 99% and 99%, respectively, were obtained from Dr. Ehrenstorfer Laboratories GmbH, Germany.  $^{14}\text{C}$ -Carbaryl was supplied by the International Atomic Energy Agency (IAEA). The liquid scintillation cocktail was dioxane basis scintillator (0.05 g PO-POP + 7 g PPO + 100 g naphthalene in 1 L of dioxane) [6]. The solvents, such as ethylacetate (EtAc), cyclohexane and isooctane, were of analytical grade from Merck, with purities of 99%.

### Instrumentation

The following equipment were used to perform this study: a Waring blender (WB), 1-L stell containers (Waring Commercial Blender, USA), Ultra Turrax (T25 basic Ika-Werke), centrifuge (Beckman Model TJ-6 Centrifuge), centrifuge tube up to 50 mL capacity, rotary evaporator (Heidolph OB 2200), balance with a 0.0001-g digit, Vortex (Fisher Vortex cat. no: 12-812 Genie 2<sup>TM</sup>), a semi-automatic GPC system (equipped with a 20×1-cm glass column) operated with constant nitrogen pressure, Bio Beads S-X3 200–400 mesh (Bio-Rad Lab. cat. no. 152–2750), Packard 1550 Tri-Carb liquid scintillation analyser (liquid scintillation counter, LSC), HP 6890 GC Plus equipped with an autosampler (HP 7683) and a capillary column connected through a nitrogen phosphorus detection (NPD) system, polyethylene LSC vials and other basic glassware and equipment, such as measuring cylinders and Hamilton microsyringes.

### Experiment $E_1$

#### Homogenisation and fortification

To obtain homogenous material, a five-unit sample of cucumber was weighed [19] and processed with the WB at ambient temperature. Five grammes of  $\text{NaHCO}_3$  was added to the 30-g analytical portion and mixed. Fortification solution, including dichlorvos, malathion and chlorpyrifos-ethyl, was added to the sample based on fortification levels of 0.02, 0.2, 0.8 and 1 mg  $\text{kg}^{-1}$ , for  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$ , respectively. The replication numbers of analytical portions were seven for  $F_1$  and four for the other levels and the untreated sample ( $F_0$ ).

### Extraction

A 40-mL EtAc was added to the sample matrix and stirred. Then, 0.5 mL of  $^{14}\text{C}$ -carbaryl [ $1.2 \times (10^5)$ ] disintegration per minute, dpm) was added and the spatula was rinsed with the remaining 20 mL EtAc. Anhydrous  $\text{Na}_2\text{SO}_4$  was also added to the sample at the ratio of 1/1 *w/w*. Then, the mixture was extracted using an Ultra Turrax probe blender and centrifuged as indicated in the previous work [20]. The liquid part of the material in the tube was collected. Since the recovery was calculated based on weight, the mass of the sample matrix was recorded carefully before and after extraction and the centrifuge step.

#### Measurement of radioactivity for extraction repeatability

To check the repeatability and efficiency of extraction (expressed as recovery percent) and for further uncertainty assessment, three replicates of 0.5 mL of extract were pipetted to the LSC vials and weighed from each extract of each fortification level. Liquid scintillation cocktail was added to each vial, which was then radioassayed in the LSC to determine  $^{14}\text{C}$ -carbaryl radioactivity. The recovery of extraction for each sample was calculated as the ratio of the initially applied radioactivity.

### Experiment $E_2$

#### Filtration and evaporation

A 1/3 portions of the total extract volume from each extract, corresponding to a 10-g sample equivalent, was filtered through 60 g of anhydrous  $\text{Na}_2\text{SO}_4$  in a round-bottomed flask. Then, filter cake was washed three times with 20 mL of EtAc and then we waited for the complete removal of solvent. The filtrate was concentrated to 1–2 mL in a rotary evaporator, concentrated EtAc extract was transferred to a calibrated conical test tube and the evaporation was continued with a gentle  $\text{N}_2$  stream to 1 mL. For changing the solvent to EtAc/cyclohexane 1/1 *v/v*, 1 mL of EtAc/cyclohexane mixture was added to the tube and was finally evaporated to 0.8 mL. The final volume of extract was adjusted exactly to 1 mL, corresponding to a 10-g sample equivalent, for the GPC cleanup [21].

#### Calibration of the GPC column

As described in the previous work [22], the GPC column was filled with 8 g of Bio-Bead SX-3 gel. GPC calibration was

performed with both  $^{14}\text{C}$ -carbaryl and fortification mixture for finding the elution profile. A total of 28,324 dpm of  $^{14}\text{C}$ -carbaryl was injected into 250  $\mu\text{L}$  of EtAc/cyclohexane solution to the column in triplicate, which was then eluted with EtAc/cyclohexane at the 0.8  $\text{mL min}^{-1}$  flow rate. The eluent was collected in 1-mL increments until 30 mL. The collected fractions were radioassayed by using the LSC.

The calibration of the column was also performed with the fortification mixture in triplicate. An aliquot of 500  $\mu\text{L}$  from 0.001  $\text{mg mL}^{-1}$  of mixture was injected into the GPC and the pesticides were eluted with the eluting solvent. The collected fractions were subjected to  $\text{N}_2$  evaporation until dryness and then 1 mL of isooctane was added and evaporated again to nearly dryness. This procedure for changing the solvent was repeated twice. The final volume was adjusted with isooctane to 1 mL and analysed using a GC-NPD system [3, 21].

#### GC analysis of fractions

The GPC-eluted fractions were subjected to GC-NPD, simultaneously applying five-point calibration used at the following conditions: capillary column (30.0  $\text{m} \times 250 \mu\text{m}$  i.d.  $\times 0.25 \mu\text{m}$  nominal film thickness, HP 19091S-433, HP-5MS 5% phenyl methyl siloxane); carrier gas nitrogen 2.0  $\text{mL min}^{-1}$ , hydrogen 3.0  $\text{mL min}^{-1}$ ; air 60.0  $\text{mL min}^{-1}$ . The operating conditions were as follows; column temperature: 70–270  $^\circ\text{C}$ ; initial time 1 min at 70  $^\circ\text{C}$ ; rise (I): 20  $^\circ\text{C min}^{-1}$  to 160  $^\circ\text{C}$ —0 min, rise (II): 4  $^\circ\text{C min}^{-1}$  to 270  $^\circ\text{C}$ —10 min, total run time: 43 min; detector temperature: 300  $^\circ\text{C}$ , injector temperature: 200  $^\circ\text{C}$  (splitless), injection volume: 2  $\mu\text{L}$ .

#### GPC cleanup

The system was run by injecting 500  $\mu\text{L}$  of cucumber sample extract (corresponding to a 5-g sample equivalent), the pesticide fractions were collected based on our determined elution profile and the system was washed with 30 mL of solvent mixture. The combined collected eluent was evaporated to 1 mL with a rotary evaporator. After changing the solvent, the final volume was adjusted with isooctane to 4 mL, corresponding to a sample mass concentration of 1.25  $\text{g mL}^{-1}$ , for all of the samples, including fortification and  $F_0$  [23].

#### Measurement of radioactivity for the cleanup efficiency

The cleanup efficiency was calculated as the ratio between the mean radioactivity of the analytical portion determined

after the extraction step and the radioactivity of the same analytical portion measured after the cleanup step. For this aim, two replicates of 0.5 mL from clean extract were pipetted to the LSC vials and weighed for checking the cleanup efficiency and repeatability of the analysis. Liquid scintillation cocktail was added to the samples, which were subjected to liquid scintillation counting for the determination of the radioactivity.

A detailed schematic diagram of all of the analytical steps mentioned above is shown in Fig. 1. The uncertainty components associated with extraction and cleanup are shown in Fig. 2.

## Results and discussion

#### GPC calibration

The calibration of the GPC should be performed, since determining the laboratory's own elution profile is very important. Figure 3 shows the elution profiles of  $^{14}\text{C}$ -carbaryl. All radioactivity came through the column between the 8- and 18-mL fractions, but 80% of recovered  $^{14}\text{C}$ -carbaryl came through the 11–14-mL fractions at the 0.8  $\text{mL min}^{-1}$  flow rate of eluent.

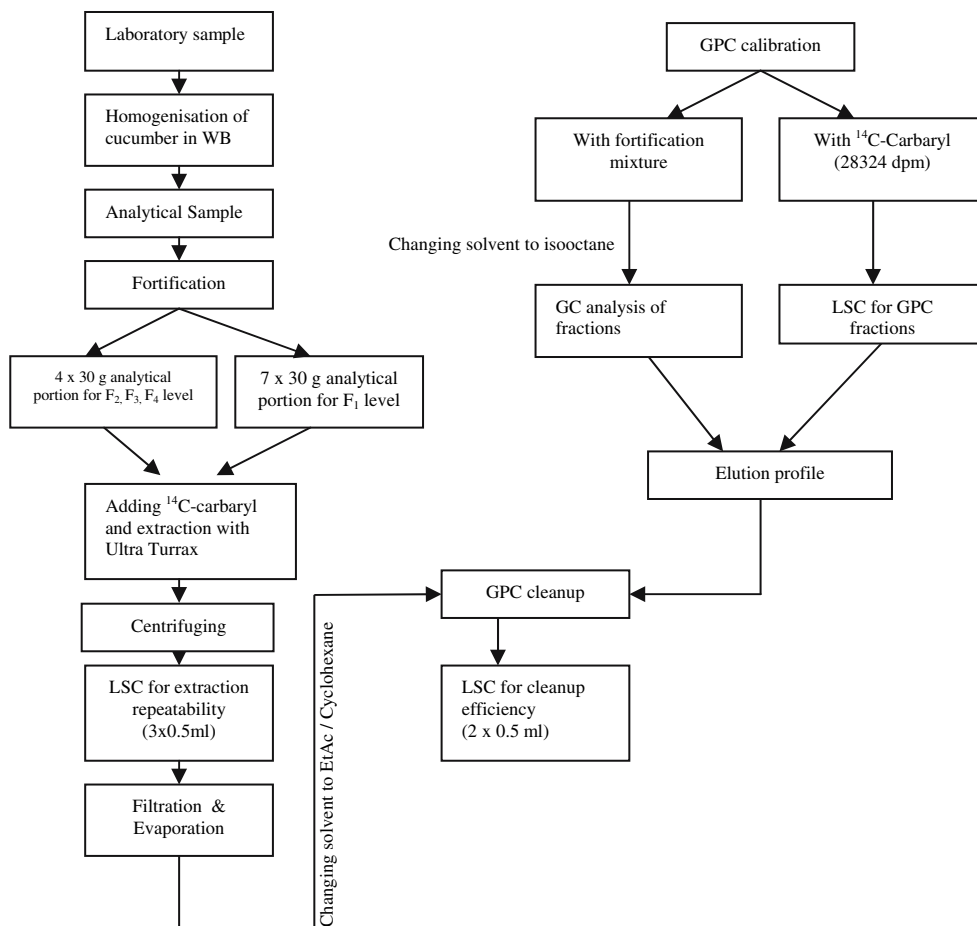
Concerning the calibration results with fortification mixture, the elution profiles of dichlorvos, malathion and chlorpyrifos on GPC are shown in Fig. 4. In the 11–15-mL fractions, malathion, chlorpyrifos and dichlorvos were eluted through the column in amounts of 63%, 79% and 100%, respectively, at the flow rate of 0.8  $\text{mL min}^{-1}$ . In other calculations, malathion, chlorpyrifos and dichlorvos were eluted in 8–23, 9–19 and 11–15 mL of fractions, respectively.

With the combination of both calibration results, it can be concluded that 8–23-mL fractions must be collected as pesticide fractions. The elution profiles showed that GPC was a very useful tool for the cleaning up of plant extracts. An earlier study showed that pesticide active ingredients were eluted in 10–20-mL fractions [22].

Performing precise calibration of the GPC column is inevitable for obtaining clean eluate and high recoveries. Each laboratory should use their own elution profile, which cannot be derived from the literature or from other laboratories. Eventually, the calibration of any cleanup system should become a routine internal quality control check [3, 4].

#### Recoveries of extraction and cleanup and their repeatabilities

In order to establish the uncertainty of extraction and cleanup, the recovered amounts of  $^{14}\text{C}$ -carbaryl obtained



**Fig. 1** Schematic diagram of the analytical procedure for the repeatability and efficiency of extraction and the cleanup efficiency (*GPC* gel permeation chromatography, *GC* gas chromatography, *LSC* liquid scintillation counter)

from each fortification level are summarised in Table 1. The recoveries ( $R$ ) were between 0.93–0.96 and 0.80–0.98 for extraction and cleanup, respectively. The repeatabilities of the recovery for extraction and cleanup, expressed as the relative uncertainty  $[u(R)/R]$ , were between 0.031–0.045 and 0.038–0.088, respectively.

The overall recovery ( $R$ ) of extraction ( $n = 19$ ) was 0.95, with a relative uncertainty  $[u(R)/R]$  of 0.036; the overall recovery ( $R$ ) of cleanup ( $n = 19$ ) was 0.87, with a relative uncertainty  $[u(R)/R]$  of 0.102 (Table 1). The table also shows the overall recovery of the method, along with the relative uncertainty. The extraction recovery was found to be within the required limit by using the radiotracer technique [10].

Since the recoveries were high and their relative uncertainties were quite small, GPC can be considered to be accurate and precise. A similar finding has been reported earlier, indicating that the GPC cleanup appeared to be an efficient cleanup technique. The average recoveries for tested insecticides in soybean were within the range 85–118% [2]. As in the case of the elution pattern,

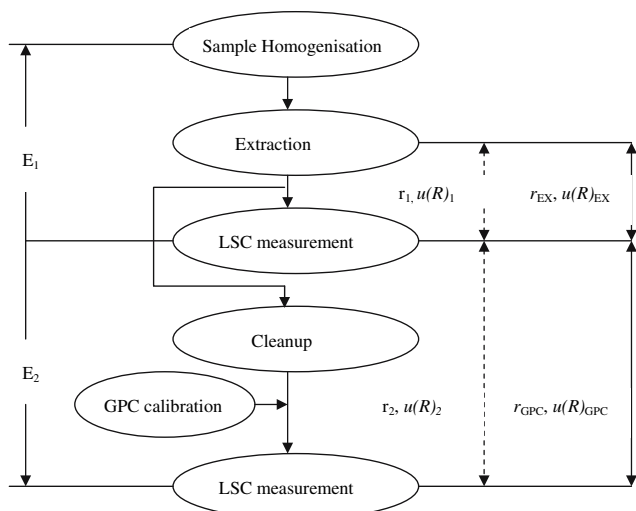
the cleanup and recovery efficiency cannot be derived from the literature. The cleanup efficiency should also become a routine internal quality control check [3, 4].

### Uncertainty assessment of extraction and cleanup

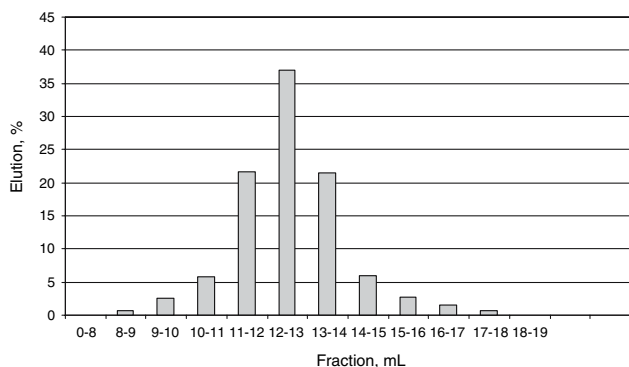
The strategy of this work aiming at the estimation of uncertainties associated with the measurement of pesticide residues in cucumber involved two basic steps: (1) quantification of uncertainty components, (2) calculation of the combined standard uncertainty. Two alternative approaches mentioned earlier were tested and compared in this study.

The “bottom-up” approach: random components of uncertainty

The random errors (as repeatability) of the extraction and cleanup steps were approximated by their relative



**Fig. 2** Uncertainty components associated with the analytical method  $E_{1-2}$ . The arrows show the phase of the analytical procedure with spiking by  $^{14}\text{C}$ -carbaryl.  $r_{1-2}$  are the repeatabilities obtained in experiments  $E_{1-2}$ .  $u(R)_{1-2}$  are the uncertainties of recovery obtained in experiments  $E_{1-2}$ .  $r_{\text{EX}}$ ,  $r_{\text{GPC}}$  are repeatabilities associated with the extraction and cleanup steps.  $u(R)_{\text{EX}}$ ,  $u(R)_{\text{GPC}}$  are the uncertainties of the recoveries of the extraction and cleanup steps individually (GPC gel permeation chromatography, LSC liquid scintillation counter)



**Fig. 3** GPC elution pattern of  $^{14}\text{C}$ -carbaryl

uncertainties, which were calculated from repeated determinations of  $^{14}\text{C}$ -carbaryl radioactivity ( $n = 57$  for extraction,  $n = 38$  for GPC), obtained from experiments  $E_{1-2}$  and shown in Fig. 2.

To determine the random components of uncertainty, which can be separately assigned to extraction ( $r_{\text{EX}}$ ) and cleanup ( $r_{\text{GPC}}$ ), Eqs. 1 and 2 were used. Since the extraction and cleanup uncertainties include the uncertainty of  $\text{LSC}_{\text{ex}}$  and  $\text{LSC}_{\text{GPC}}$ , respectively, the relative uncertainty figures obtained from LSC, which were calculated according to the LSC manual [24], were deduced from the following formulae:

$$r_{\text{EX}} = \sqrt{\left(r_1^2 - r_{\text{LSC}_{\text{ex}}}^2\right)} \tag{1}$$

$$r_{\text{GPC}} = \sqrt{\left(r_2^2 - r_{\text{LSC}_{\text{GPC}}}^2\right)} \tag{2}$$

The results are summarised in Table 2, Section A. The values of  $r_1$  and  $r_2$ , the repeatabilities of extraction recovery and cleanup recovery, were 0.036 and 0.102, respectively. Such results were within the required range [10]. Relatively good values of extraction ( $r_{\text{EX}}$ ) and GPC cleanup ( $r_{\text{GPC}}$ ) repeatability were obtained.

The “bottom-up” approach: systematic components of uncertainty

Systematic components of uncertainty were estimated on the basis of recoveries obtained in experiments  $E_{1-2}$  (Fig. 2). The uncertainties of these apparent recoveries [ $u(R)_{1-2}$ ] were derived from a rectangular distribution using Eq. 3:

$$u(R)_{1-2} = \frac{0.5(1 - R_{E_{1-2}})}{\sqrt{3}} \tag{3}$$

where  $R_{E_{1-2}}$  is the overall recovery of  $^{14}\text{C}$ -carbaryl in the respective experiments  $E_{1-2}$ .

Individual uncertainties associated with the recovery of extraction and the recovery of cleanup were calculated using Eqs. 4 and 5:

$$u(R)_{\text{EX}} = \sqrt{u(R)_1^2 - u(R)_{\text{LSC}_{\text{ex}}}^2} \tag{4}$$

$$u(R)_{\text{GPC}} = \sqrt{u(R)_2^2 - u(R)_{\text{LSC}_{\text{GPC}}}^2} \tag{5}$$

where  $u(R)_{\text{EX}}$  represents the uncertainty of the recovery of extraction and  $u(R)_{\text{GPC}}$  is the uncertainty of the recovery of GPC cleanup.

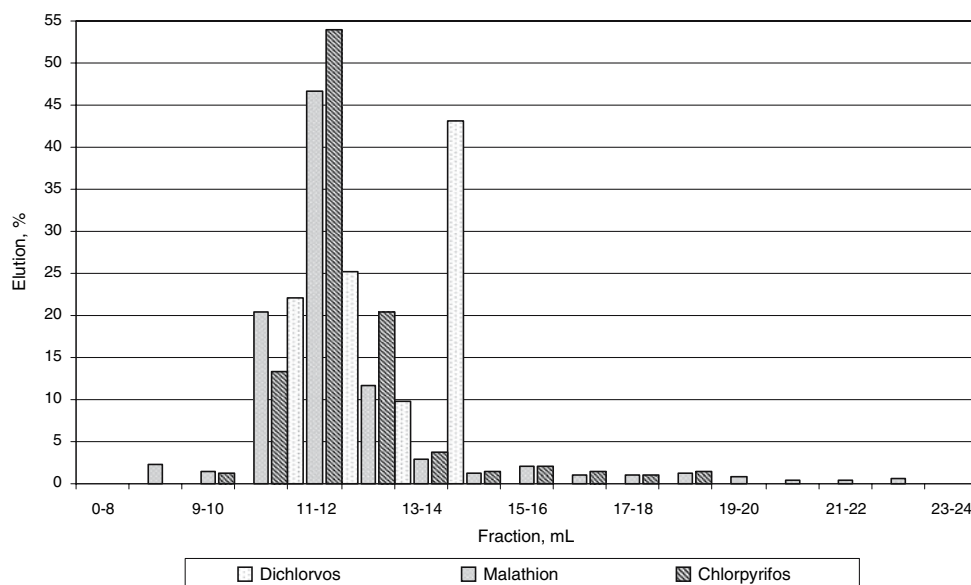
Recoveries determined in experiments  $E_{1-2}$  and systematic components of uncertainty calculated using Eqs. 4 and 5 are summarised in Table 2, Section B. Values of  $u(R)_1$  and  $u(R)_2$  (i.e. the uncertainties of recovery associated with experiments  $E_1$  and  $E_2$ ), were 0.014 and 0.037, respectively. The systematic components of extraction [ $u(R)_{\text{EX}}$ ] and GPC [ $u(R)_{\text{GPC}}$ ] uncertainties were 0.004 and 0.036, respectively.

#### Calculation of the combined standard uncertainty

Random and systematic components of uncertainty of each analytical step were used for the calculation of the combined uncertainty associated with extraction and cleanup:



**Fig. 4** GPC elution pattern of dichlorvos, malathion and chlorpyrifos



**Table 1** <sup>14</sup>C-Carbaryl recoveries (*R*) and the relative standard uncertainties [*u*(*R*)/*R*] at different levels related to each analytical step

Fortification level (mg kg <sup>-1</sup> )	Extraction		Cleanup		Total	
	<i>R</i>	<i>u</i> ( <i>R</i> )/ <i>R</i>	<i>R</i>	<i>u</i> ( <i>R</i> )/ <i>R</i>	<i>R</i>	<i>u</i> ( <i>R</i> )/ <i>R</i>
0.02 (F <sub>1</sub> /1–7)	0.96	0.031	0.83	0.038	0.89	0.081
0.2 (F <sub>2</sub> /1–4)	0.96	0.037	0.98	0.078	0.97	0.057
0.8 (F <sub>3</sub> /1–4)	0.94	0.045	0.80	0.086	0.87	0.103
1 (F <sub>4</sub> /1–4)	0.93	0.041	0.92	0.088	0.93	0.064
Overall	0.95 <sup>a</sup>	0.036	0.87 <sup>b</sup>	0.102	0.91 <sup>c</sup>	0.084

<sup>a</sup> Overall recovery of extraction (*n* = 19)

<sup>b</sup> Overall recovery of cleanup (*n* = 19)

<sup>c</sup> Overall recovery of the whole procedure (*n* = 38)

$$u_{cEX} = \sqrt{r_{EX}^2 + u(R)_{EX}^2} \tag{6}$$

$$u_{cGPC} = \sqrt{r_{GPC}^2 + u(R)_{GPC}^2} \tag{7}$$

where *u*<sub>cEX</sub> represents the combined uncertainty of extraction and *u*<sub>cGPC</sub> represents the combined uncertainty of GPC cleanup.

Combined uncertainties of each procedure were used for the calculation of the combined standard uncertainty *u*<sub>c</sub> associated with the analytical method employed for pesticide residue analysis:

$$u_c = \sqrt{u_{cEX}^2 + u_{cGPC}^2} \tag{8}$$

As shown in Table 2, Section C, the combined standard uncertainty for the whole procedure using the “bottom-up”

**Table 2** Uncertainty components of extraction and cleanup for <sup>14</sup>C-carbaryl by using the “bottom-up” approach

Section A: repeatabilities obtained in experiments <i>E</i> <sub>1–2</sub> and calculated repeatabilities of extraction and cleanup (random components)							
<i>r</i> <sub>1</sub>	<i>r</i> <sub>2</sub>	<i>r</i> <sub>LSCex</sub>	<i>r</i> <sub>LSCgpc</sub>	<i>r</i> <sub>EX</sub>	<i>r</i> <sub>GPC</sub>		
0.036	0.102	0.014	0.009	0.033	0.101		
Section B: recoveries of extraction and cleanup steps and associated uncertainties (systematic components)							
<i>R</i> <sub>E1</sub>	<i>R</i> <sub>E2</sub>	<i>u</i> ( <i>R</i> ) <sub>1</sub>	<i>u</i> ( <i>R</i> ) <sub>2</sub>	<i>u</i> ( <i>R</i> ) <sub>LSCex</sub>	<i>u</i> ( <i>R</i> ) <sub>LSCgpc</sub>	<i>u</i> ( <i>R</i> ) <sub>EX</sub>	<i>u</i> ( <i>R</i> ) <sub>GPC</sub>
0.95	0.87	0.014	0.037	0.014	0.009	0.004	0.036
Section C: summary of combined uncertainties obtained for <sup>14</sup> C-carbaryl using the “bottom-up” approach							
<i>u</i> <sub>cEX</sub>		<i>u</i> <sub>cGPC</sub>		Combined standard uncertainty <i>u</i> <sub>c</sub>			
0.033		0.107		0.112			

**Table 3** Summary of the uncertainties obtained for tested analytes using the “top-down” approach

Experiment 1 ( $E_1$ )			Experiment 2 ( $E_2$ )			Average combined standard uncertainty $u_{cAV}$
$r_{t1}$	$u(R)_{t1}$	$u_{cr1}$	$r_{t2}$	$u(R)_{t2}$	$u_{cr2}$	
0.036	0.014	0.039	0.102	0.037	0.108	0.081

approach was 0.112. The uncertainty of cleanup (both random and systematic components) represents an important source of the combined uncertainty.

The “top-down” approach

This part of our study was focussed on the experimental evaluation of uncertainty sources represented by the repeatability of radioactivity measurement of  $^{14}\text{C}$ -carbaryl and the uncertainty of recovery  $u(R)_t$  derived from a rectangular distribution using Eq. 9:

$$u(R)_t = \frac{0.5(1 - R_{t1-2})}{\sqrt{3}} \quad (9)$$

where  $R_t$  is the recovery of a particular analyte. In our case,  $R_{t1-2}$  is the overall recovery of  $^{14}\text{C}$ -carbaryl in the respective experiment  $E_{1-2}$ . This formula is very similar to Eq. 3.

Both uncertainty sources were combined to obtain the combined standard uncertainty  $u_{cm}$  using Eq. 10:

$$u_{cm} = \sqrt{(r_m^2 + u(R)_m^2)} \quad (10)$$

where  $r_m$  represents the repeatability,  $u(R)_m$  represents the uncertainty of recovery (series of experiments  $n = 1-2$ ).

The average of the combined standard uncertainties  $u_{cAV}$ , calculated as a quadratic mean (Eq. 11), was 0.081 (Table 3):

$$u_{cAV} = \sqrt{\frac{u_{cr1}^2 + u_{cr2}^2}{2}} \quad (11)$$

Comparing the data in Table 3, no significant differences were detected between the combined uncertainties of series 1 and 2. Also, the combined standard uncertainties were comparable to those obtained by the “bottom-up” approach (Table 2, Section C). The combined uncertainties were 0.112 and 0.081 for the “bottom-up” and “top-down” approaches, respectively. In both approaches, the uncertainty of cleanup was the main source of combined uncertainty of the analytical procedure.

## Conclusion

Our findings, obtained from the application of both “bottom-up” and “top-down” approaches, revealed that

the recovery and efficiency of extraction and cleanup could be determined precisely and quickly by using a radiotracer technique ( $^{14}\text{C}$ -carbaryl), which brings about a new aspect of the previously used methodology. We can also conclude that, although the “top-down” approach represents a simpler and more effective way of calculation of the combined standard uncertainty, the use of the “bottom-up” approach may be a better way, since the advantage of the latter is the possibility of obtaining insight into the individual uncertainties and the identification of the most critical ones from an internal quality control point of view. In fact, there is little difference between the two approaches.

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