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Setting up of recovery profiles: A tool to perform the compliance with recovery requirements for residue analysis

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Abstract

The use of the recovery term has presented some confusion in Analytical Chemistry. Recent IUPAC recommendations propose to distinguish between two terms: *recovery* or *recovery factor*, $\Re$, and *apparent recovery*, $\Re$*. *Apparent recovery* includes *recovery factor* and a new recovery term proposed in this paper, named *calibration recovery*, $\Re^C$, which depends of the type of systematic error due to the matrix effect (constant and/or proportional) and is related to the applied calibration methodology. This paper highlights the dependence of the calibration recovery on the sample analyte concentration and, for extension, of the apparent recovery, defines the recovery profile, and makes evident the need to determine a "fit for purpose" analyte concentration interval to comply with a regulated recovery requirements. An approach to estimate the calibration recovery and its associated uncertainty in relation to the above-mentioned dependence is presented. The usefulness of the proposed methodology has been shown in the quantification of a pesticide by GC-ECD for assessing dermal exposure.

Keywords: Recovery factor; Apparent recovery; Calibration recovery; Uncertainty; Recovery profile

1. Introduction

The estimation and use of the term *recovery* and the application of recovery studies in the field of Analytical Chemistry has presented different practices and even some confusion in different sectors. The absence of consistent strategies for the estimation and use of information derived from recovery studies implies a difficulty to make valid comparison between results produced in different laboratories and to verify the suitability of the data for the intended purpose. In this sense, some documents have been published with the aim to clarify and use properly the different terms and information derived from recovery studies [1,2]. In this context, it is possible to distinguish the application of recovery studies with two different aims: (i) to check the trueness of a certain analytical method with the purpose to estimate the full systematic error of the process (validation), and (ii) to estimate the recovery with the aim to obtain a correction factor for the results. The first approach is an essential component of the validation of the analytical process while the second one can be considered as a direct process calibration [3].

To establish the conventional true value for checking the trueness, the recovery studies can be carried out by using representative reference materials, that have been classified [4] as certified reference materials, standard reference materials and in-house reference materials. Among these last ones, s*piked materials* are widely used, being defined as a natural or artificial material which has a matrix that is similar to that of the sample and in which the analyte is not present; adding known quantities of the analyte to material blank. Matrix blank is referred to as material free of detectable levels of the analyte, which can be also called: sample blank, portion blank, extract blank, material blank or blank matrix [5,6].

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In addition, it can be also recommended that a typical test sample is analysed either in its original state and after the addition, and the difference between both results, expressed as a proportion of the mass added, is called "surrogate recovery" or sometimes "marginal recovery" [7]. On the other hand, the interpretation of the term recovery is not uniform, since it can be used to assess the apparent analyte concentration in analyte stability studies, the efficiency of an extraction process (or any sample treatment) or the method trueness.

The other use of the recovery information is related to the revision of experimental values with the double aim to either compensate analyte losses or degradations (mainly produced in the separation and/or digestion procedures previously applied to the measurement), and/or correct the matrix error in the analyte measurement (when the calibration is performed on a matrix that is dissimilar to the real sample matrix). This implies to perform recovery assays at different analyte concentrations into the analytical range. Although recent guidelines [1,6,8] have recommended to correct the analytical results using the mean recovery, if it is significantly different to 100%, at this moment the polemic on the appropriateness of this operation keeps on being a discussion subject in the analytical community.

Recovery is defined as the proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is recovered and presented for measurement [1]. However, recent IUPAC recommendations propose to distinguish between two different terms in order to avoid confusion caused by the use of the term recovery [2]: (i) the measurement of the yield of an analytical process in relation to pre-concentration or extraction stages (named properly *recovery* or *recovery factor*, $\Re$); or (ii) the ratio "experimental observed concentration/reference concentration", obtaining this experimental value from the analytical process using a calibration curve (named *apparent recovery*, $\Re^*$).

Apparent recovery is an estimation of the overall systematic error of the analytical process (method bias). From a very general point of view, let us consider that the global error in the result of an analytical method can be due to two causes that are not self excluding: (i) the measuring system does not receive the same analyte quantity initially present in the sample, and (ii) the measuring system yields a wrong measurement of the analyte quantity that it receives. Therefore, the apparent recovery includes the systematic error due to the "falling down" available analyte content in the analytical operations (the IUPAC *recovery factor*) and the systematic error due to the matrix effect (a no-considered component in the IUPAC document).

In this paper, this new recovery component is defined and called as *calibration recovery*, $\Re^C$. In addiction, a recovery formal model expressed as a function of the actual analyte concentration and of two coefficients (correction coefficients) related with the constant and proportional components of systematic matrix error is proposed. From this model, a methodology to calculate the apparent recovery and to distinguish between *recovery factor*, *calibration recovery*, *correction* (*calibration*) *factor* and *apparent recovery* is presented (all this terms are defined and clarified).

A comprehensive way to estimate the uncertainty associated with the recovery has already been published [9], which considers different approaches depending on how the analyte reference concentration is obtained: (i) from representative CRMs, (ii) from spiking studies, (iii) by comparison with a standard method, and (iv) from extraction monitoring studies when the previous three approaches are impractical. However, this way of uncertainty estimation is properly based to apply a methodological approach on recovery. In this paper, a novel expression to calculate the uncertainty associated to the calibration recovery, from a metrological point to view, is also presented.

Depending on the applied methodology, the estimated recovery, obtained in a traditional way, could be any of both previously defined, and the analyst must always clearly describe the designed protocol for recovery studies. The tolerable range of recovery values for trace analysis of organics (residue analysis) is regulated in standard documents, but the procedure to check the compliance is not always clearly specified. Generally, in quality control of pesticide multiresidue methods, a routine recovery within the range 60–140% may be considered as acceptable, but when violative residues are detected in a sample, data should be supported by a routine recovery within the range 70–110% [5]. In residue method validation, different ranges of mean recoveries are defined, depending on the sample analyte concentration; an example, for concentrations ranging from 0.1 to 1 mg/kg, a mean recovery range between 70 and 110% is recommended [6,10].

The recovery factor can be considered constant and independent of the amount of analyte present in the test sample. On the contrary, the calibration recovery could depend on the actual concentration of analyte, in function of the type of error (constant or proportional) due to the matrix. For this reason, a single recovery value is just representative of the tested analyte concentration and cannot be extrapolated to the whole range of the method application. The established model permits to define the actual analyte concentration interval, which complies with a specific requirement about the recovery range indicated in accepted guidelines or regulations.

As example of the application and usefulness of this methodology, the quantification of the pesticide procymidone by GC-ECD for assessing dermal exposure, using the "whole-body" method with Tyvek Pro-Tech coverall as sampling medium [11] has been carried out.

2. Theoretical aspects

2.1. Calibration recovery

In general, quantification systematic errors can be classified in two groups [12]: those that produce an analyte "falling down" in the entire analyte quantity before the measurement

(losses, co-reactions, degradations, . . .) and those that cause a measuring error at the quantification moment (matrix effects, matrix variations). The first group is linked with the IUPAC recovery factor, $\Re$, while the second one is related with the new component firstly defined in this paper and named by authors as *calibration recovery*, $\Re^{C}$. The IUPAC *apparent recovery*, $\Re^{*}$, considers all the method bias sources and includes both recovery components.

Formally, a basic expression could be written as:

$$\Re^{*} = \Re \times \Re^{C} \quad (1)$$

where each recovery term is expressed as:

$$\Re^{*} = \frac{C_{meas}}{C_{refer}}; \quad \Re = \frac{C_{availab}}{C_{refer}}; \quad \Re^{C} = \frac{C_{meas}}{C_{availab}} \quad (2)$$

C_{meas} and C_{refer} are the experimentally measured concentration (estimated from the calibration curve) and the reference (conventional true) concentration in the sample, respectively, and $C_{availab}$ is the analyte concentration which is available for measuring, that is, the actual analyte concentration in the test portion that is able to be measured in the analytical conditions. A similar decomposition of recovery terms has been recently reported [13] particularised to the extent of the matrix effect using different interfaces in HPLC with tandem MS/MS detection. An antecedent of a methodological recovery decomposition has already been offered [9]; the recovery for a particular sample is considered as comprising three components related to: (i) the error of the analytical method (recovery method), (ii) the difference in the recovery for a particular sample compared to the recovery observed for the material used in the recovery study, and (iii) the difference between the spiked sample, when it is used, and the real sample with incurred analyte.

Systematic errors produced by those sources could be removed or diminished by selecting a suitable calibration methodology, so if the calibration standards are subjected to the full analytical process (process calibration [3]), errors due to a possible analyte "falling down" can be compensated; whereas if a matrix-matched calibration (where sample representative standards are used as calibration standards) is established, matrix errors could be avoided [6,14].

It is possible to consider a *validation function* (bias function or recovery function) [2,15] as a function, which relates the measured analyte concentration with its corresponding reference (true) value. This function is, in fact, a direct calibration function because of the related variables are expressed in the same magnitude, and it is given by:

$$C_{meas} = \Delta_C + \Delta_P \times C_{refer} \quad (3)$$

The function features Δ_C and Δ_P represent the constant (translational bias) and proportional (rotational bias) components of the overall systematic error in the estimation of the analyte concentration.

From this function it is possible to obtain the apparent recovery, which depends, either on the values of these constant and proportional systematic components of the error (Δ_C and Δ_P, respectively) and on the reference concentration (C_{refer}):

$$\Re^{*} = \frac{C_{meas}}{C_{refer}} = \Delta_P + \Delta_C \times \frac{1}{C_{refer}} \quad (4)$$

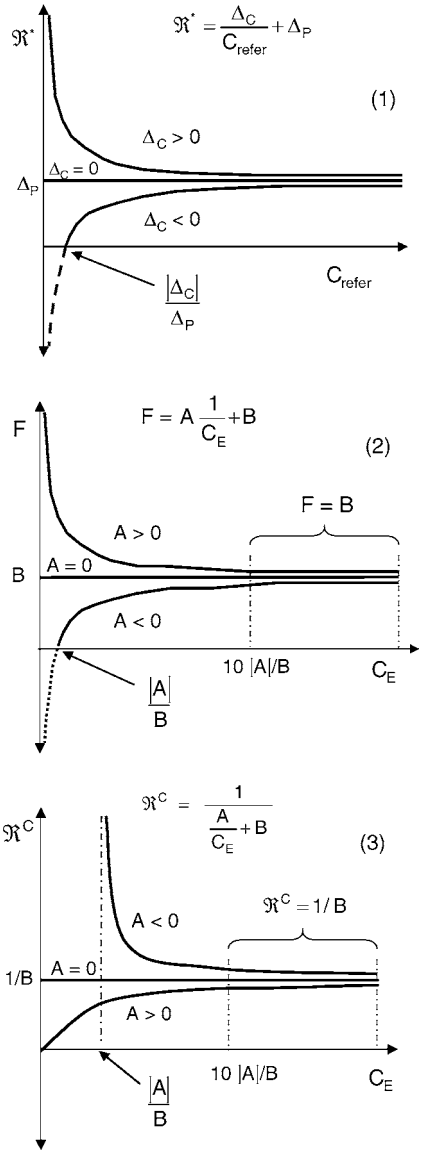

Fig. 1. Dependence (1) of the apparent recovery ($\Re^{*}$) with the reference analyte concentration (C_{refer}); and (2) of the correction factor (F) or (3) of the calibration recovery ($\Re^{C}$) with the estimated analyte concentration from EC (C_E) (see explanation in the text).

As can be seen in Fig. 1(1), by plotting the *apparent recovery* $\Re^{*}$ in function of C_{refer}, it is possible to obtain different experimental curves depending on Δ_C (positive, negative or zero). If $\Delta_C = 0$, the apparent recovery will be constant, that is, it will be independent on the measured analyte concentration and will take the value corresponding to the proportional component of the systematic error ($\Re^{*} = \Delta_P$). On the contrary, if $\Delta_C > 0$ or $\Delta_C < 0$, two different curves can be obtained, in which the apparent recovery will tend to take the value cor-

responding to the proportional component of the systematic error, for high concentration levels. However, for low concentration levels, if $\Delta_C > 0$ the apparent recovery will take higher values and if $\Delta_C < 0$, its value will be decreasing as C_{refer} decreases, taking a value of zero, when C_{refer} corresponds to a value numerically similar to $|\Delta_C|/\Delta_P$.

The apparent recovery can be identified as the inverse of the corresponding correction factor, mentioned above, applied to a measurement process. Each obtained recovery is representative of one selected concentration level and it cannot be extrapolated to other concentration values, since usually the recovery changes along the studied range in the analytical method.

2.2. Correction coefficients and correction factor

If an analytical process is affected by systematic matrix errors, we could considered two kind of calibration curves for the characterisation of such matrix effects [14]: (i) from substance reference material composed of the pure analyte dissolved in reagent blank, named external calibration (EC), and (ii) from matrix reference materials prepared by incorporating co-extractives to the standard solution, named matrix-matched calibration (MC). If both calibration curves are linear and a significant difference exits between their corresponding slopes and/or intercepts, a correction function (CF) could be established by the expression:

$$C_M = A + B \times C_E \tag{5}$$

where C_M and C_E are the experimental analyte concentrations estimated from MC and EC, respectively, and A and B are the coefficients of the model, named correction coefficients. This function is constructed by plotting C_M versus C_E and it can be applied to find the corrected concentration values from the concentration directly estimated from EC, in order to remove the matrix effect error, avoiding the need to calibrate in the presence of matrix in routine analysis.

The correction function has already been proposed by authors in a previous paper [16] and applied to obtain results free of systematic error when the analytical measuring process is disturbed from matrix effects. In this paper, a metrological approach of the calibration recovery is developed based on the relationship between this new feature and the coefficients of the correction function.

In effect, C_M can be written as the result to multiply C_E by a *correction* (or *calibration*) *factor*, F, which could be expressed, from the Eq. (5), as:

$$C_M = F \times C_E \Rightarrow F = B + A \times \frac{1}{C_E} \tag{6}$$

This correction factor is not a constant rather it depends of C_E, as can be seen in Fig. 1(2).

The *calibration recovery*, $\Re^C$, which represents the ratio of the analyte concentration measured before and after the application of the *correction function*, can be estimated as the inverse of the correction factor as:

$$\Re^C = \frac{1}{F} = \frac{C_E}{C_M} = \frac{1}{\frac{A}{C_E} + B} \tag{7}$$

Plotting $\Re^C$ versus C_E, different curves are obtained (Fig. 1(3)), which depend on the value of the coefficient A (positive, negative or zero). Three different situations can be considered:

(a) If $A = 0$, the calibration recovery is the inverse of the coefficient B ($\Re^C = 1/B$).
(b) If $A < 0$, the calibration recovery will spread to infinite when CE is $|A|/B$ and it will take values close to $1/B$ for high analyte concentrations.
(c) If $A > 0$, the calibration recovery will take values comprised between 0 for low concentration levels and $1/B$ for high analyte concentrations. This situation is less frequent than the previous ones.

As it can be seen in Fig. 1(3), for the last two cases, when the concentration C_E takes a value higher than 10-fold the ratio $|A|/B$ ($C_E > 10 \times |A|/B$), the calibration recovery remains constant and it will be the inverse of the coefficient B (similar to the first situation).

It is possible to establish a parallelism between C_E and C_M with C_{meas} and $C_{availab}$, respectively. In this sense, the different recovery terms can be expressed as:

$$\Re^* = \frac{C_E}{C_{refer}}; \quad \Re = \frac{C_M}{C_{refer}}; \quad \Re^C = \frac{C_E}{C_M} \tag{8}$$

as a consequence, a relationship between the correction coefficients and the terms of the Eq. (3) can be deduced. Starting of Eq. (1) and operating, the following expression can be obtained:

$$\Re^* = \frac{\Re}{B} - \frac{A}{B} \times \frac{1}{C_{refer}}. \tag{9}$$

The apparent recovery is a function of the reference analyte concentration (spiked concentration), that is, the method bias depends on the actual analyte concentration in sample. The apparent recovery can be only considered constant (equal to the ratio $\Re/B$) for any analyte concentration value, when the correction coefficient A is zero or when the ratio $A/\Re$ is negligible in relation to the actual analyte concentration (i.e., the second term of the subtraction in Eq. (9) is negligible in relation to the first one).

By comparing Eqs. (4) and (9), the coefficients A and B can be related to the constant and the proportional components of the overall systematic error, respectively, involved in Eq. (3). They can be expressed as:

$$\Delta_P = \frac{\Re}{B}; \quad \Delta_C = -\frac{A}{B}. \tag{10}$$

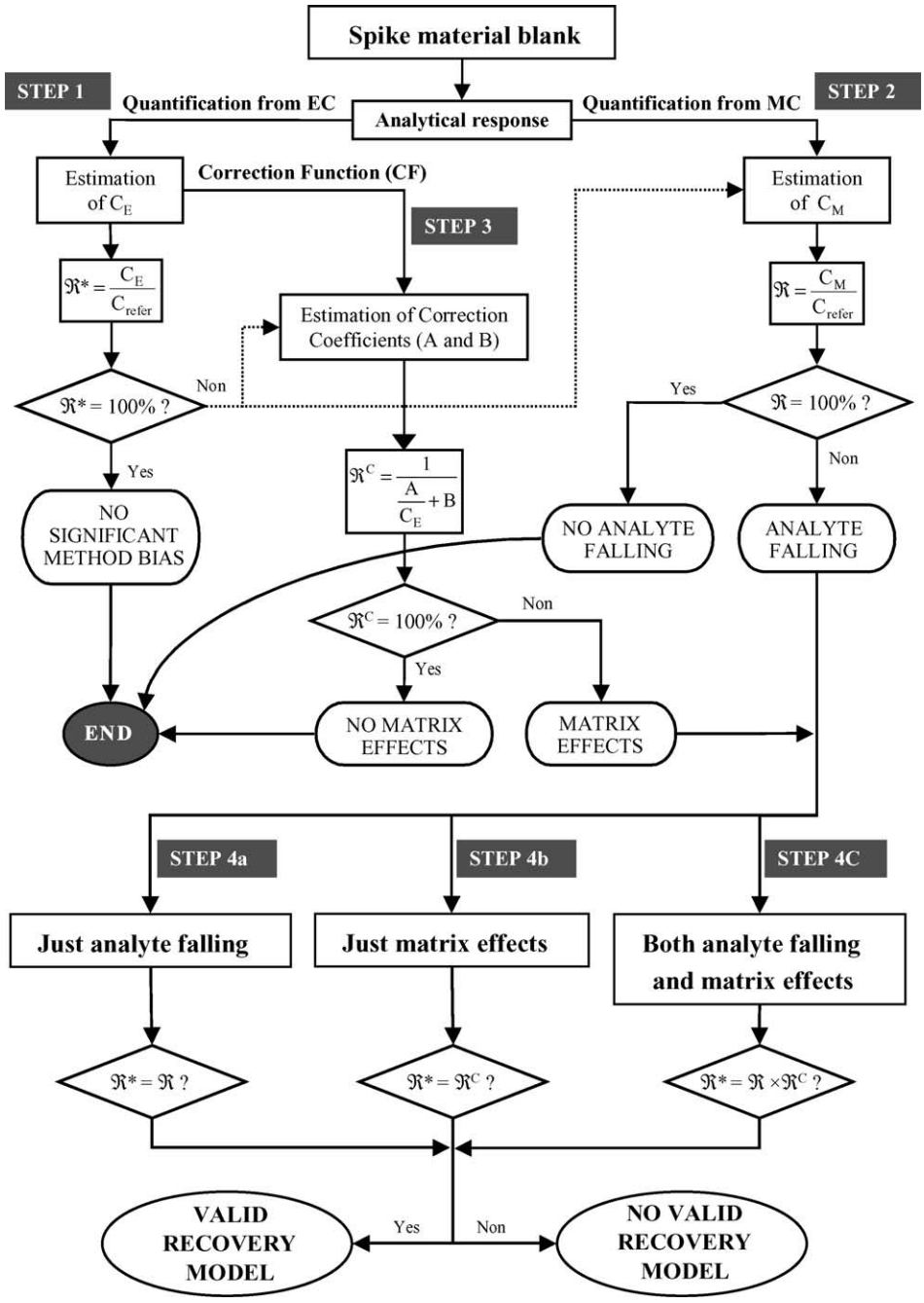

Fig. 2. Flow-chart diagram for the validation of the proposed recovery model (see explanation in the text to follow the process).

2.3. Evaluation of the different recoveries: validation of the recovery model

Following the flow-chart described in Fig. 2, the proposed model (Eqs. (1) and (9) can be validated. From a measured spiked material blank, and applying EC, MC and CF, it is possible to make a single estimation of each recovery term: apparent recovery, recovery factor and calibration recovery, and the occurrence of both analyte losses and/or matrix effects can be screened following three steps:

STEP 1 Applying EC, an apparent recovery is calculated from both C_E and C_{refer}, which represent the fraction of analyte estimated when using EC. The presence of method bias is checked by comparison of this recovery to 100%, using a conventional Student's *t*-test ($\alpha = 0.05$). If no significant difference exists, C_E estimates correctly C_{refer} (there are neither analyte "falling down" nor matrix effect) and the process is ended. However, if a significant bias is detected, it is necessary to estimate the recovery components.

STEP 2 To check the analyte "falling down", MC is applied to estimate C_M and a recovery factor ($\Re$) is obtained, which represents the ratio of analyte estimated when using MC, in relation to C_{refer}. A non-significant difference of $\Re$ with 100% (Student's *t*-test, $\alpha = 0.05$) implies that C_{refer} is correctly estimated from MC due to no analyte losses exist.

STEP 3 A calibration recovery ($\Re^C$) is calculated from Eq. (7) (by applying the correction coefficients). A non-significant difference of $\Re^C$ with 100% (Student's *t*-test, $\alpha = 0.05$) implies that a matrix effect is not disturbing the analyte quantification.

In order to validate the agreement between the calculated recovery values and the proposed model, and depending on the kind of systematic error found, such recovery values are compared using a suitable Student's *t*-test:

STEP 4a if there is analyte "falling down", $\Re^*$ and $\Re$ are compared;

STEP 4b if there is matrix effect, $\Re^*$ and $\Re^C$ are compared;

STEP 4c if both sources of error occur, $\Re^*$ is compared with the product of $\Re$ by $\Re^C$.

In the three cases, if there are not significant differences, the obtained recovery model is valid and can be applied.

2.4. Uncertainty of the calibration recovery

From a metrological point to view, to calculate the standard uncertainty associated to the calibration recovery, $u(\Re^C)$, it is necessary to know the uncertainties of the correction coefficients (*A* and *B*) and the measured (estimated) analyte concentration (C_{meas}) from the calibration curve applied. When the error propagation is properly applied on the Eq. (7), and replacing C_E by C_{meas}, the following expression is derived:

$$u^2(\Re^C) = (\Re^C)^4 \times \left[\frac{A^2}{C_{meas}^4}u^2(C_{meas}) + \frac{1}{C_{meas}^2}u^2(A) + u^2(B) + \frac{2}{C_{meas}}r_{A,B}u(A)u(B)\right] \quad (11)$$

where $u(A)$ and $u(B)$ are the standard uncertainties associated to the *A* and *B* estimations, respectively, and $r_{A,B}$ is the correlation coefficient between both coefficients. The methodology to calculate these uncertainty contributions has been established by us in a previous paper [16]. The $u(C_{meas})$ term has to be known by the analyst and must be previously established. Some examples have been recently published for GC methods in residue analysis [17–22] (the standard uncertainty values of habitually found in pesticide multiresidue analysis vary between 10 and 25%).

In the same way, if the calibration recovery is expressed as correction (or calibration factor) Eq. (6), the associated standard uncertainty can be obtained as follows:

$$u^2(F) = \frac{1}{C_{meas}^2} \times \left[\frac{A^2}{C_{meas}^2}u^2(C_{meas}) + u^2(A) + C_{meas}^2u^2(B) - 2r_{A,B}u(A)u(B)\right]. \quad (12)$$

2.5. Recovery profiles

When the recovery value is function of the analyte concentration in sample, it is necessary to have information about the recovery behaviour along the whole application method interval to evaluate the compliance with recovery regulation limits. In this sense, the conclusions derived from the traditional procedure considering only a few recovery values (one or two analyte concentration levels) could be erroneous because the selected studied recovery levels may not be representative of the analytical method. Therefore, we propose to establish previously, for example in the validation step, a "fit for purpose" analyte concentration range for which the recovery requirements are complied. With this purpose, the recovery model established (Eq. (9)) could be easily applied.

In Fig. 3, both apparent and calibration recoveries, theoretically obtained by applying Eq. (9), are plotting in function of the analyte reference (actual) concentration (arbitrarily selected between 0 and 10), as well as a recovery compliance interval (70–110%). In all selected cases, a value for the recovery factor of 80% has been considered, which implies a 20% of the analyte concentration simulates is "falling down" during the preliminary steps of the analytical process. As a consequence, different recovery profiles are obtained, corresponding to the values for both apparent recovery and the calibration recovery, for which its mutual separation depends on the value of the recovery factor. In the case of no analyte "falling down" occurs, $\Re = 100\%$, only one recovery profile, corresponding to the calibration recovery, should be obtained because of $\Re^* = \Re^C$ (the apparent recovery profile overlaps to the calibration recovery profile).

The curve profile indicates that the recovery depends on the analyte concentration and tends to have a constant value at high concentration values. The higher the coefficient *A* is, the higher the curvature degree will be, so, if $A = 0$, a horizontal straight line would be obtained. The height of the curves (or the straight line when $A = 0$) depends inversely on the quantity of the coefficient *B*.

Depending on the values of the correction coefficients (*A* and *B*) and of the recovery factor ($\Re$), different situations can be considered: $A < 0$ and $B > 1$ (Fig. 3(1)); $A > 0$ and $B > 1$ (Fig. 3(2)); $A > 0$ and $B < 1$ (Fig. 3(3)); $A < 0$ and $B < 1$ (Fig. 3(4)). When *A* takes a negative value (Fig. 3(1) and (4)), the recoveries decrease as the analyte concentration decreases, however, when *A* takes a positive value (Fig. 3(2) and (3)), the opposite situation occurs.

From the cut-points between the recovery profiles and the top and bottom extremes of the recovery compliance interval it is possible to deduce the "fit for purpose" analyte concentration range in which the analytical method complies with

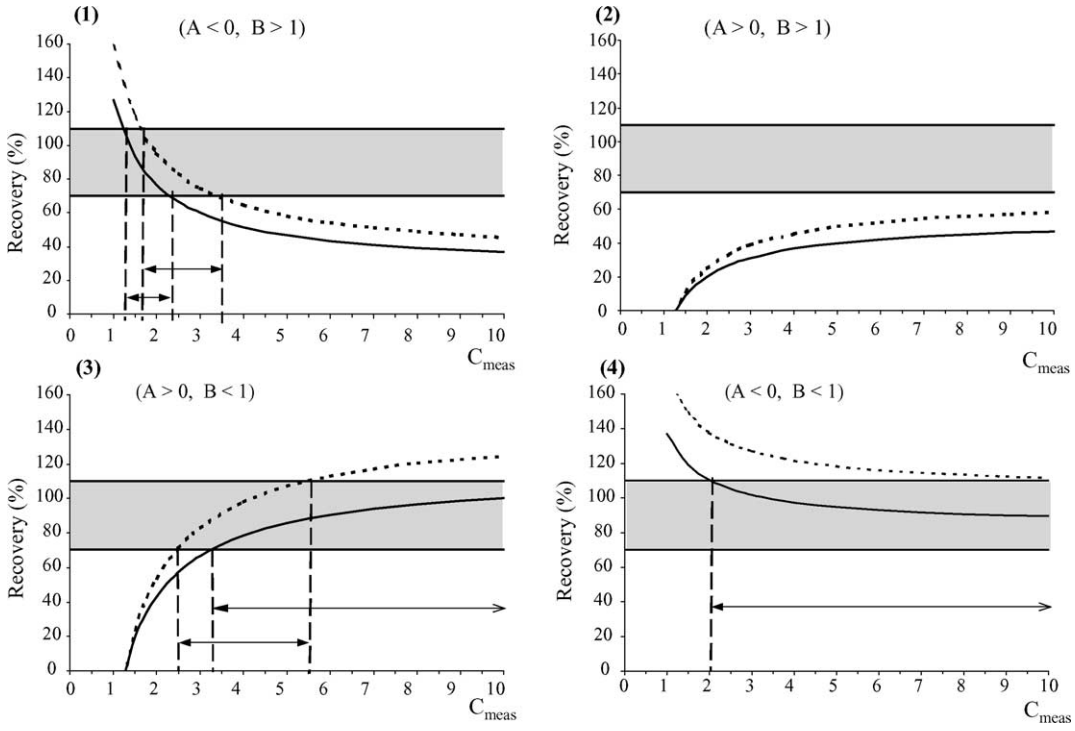

Fig. 3. Different examples of recovery profiles for apparent recovery (solid line) and the calibration recovery (dashed line) curves and recovery compliance bands in presence of matrix effects (correction coefficient values, (1): $A = -3$, $B = 3$; (2): $A = 1$, $B = 1.5$; (3): $A = 1$, $B = 0.7$; (4): $A = -0.5$, $B = 0.95$. Recovery factor value, $\Re = 0.8$; see further explanation in the text).

recovery requirements. In this sense, Fig. 3(2) shows an adverse situation because of the recovery profiles are out of the established interval and the analytical method does not comply with requirements for any analyte concentration. Therefore, in order to achieve the compliance it is necessary to decrease the matrix effect, for example, by improving the analyte clean-up step or applying a matrix-matched calibration.

Situations shown in Fig. 3(3) and (4) are seemingly satisfactory but it can be cautiously considered. In both cases, two different systematic errors are present, which are mutually compensated. The analyte "falling down" introduces a systematic error by defect ($\Re < 100\%$) while the matrix effect contributes with a systematic error by excess ($\Re^C > 100\%$), and, as a consequence, the apparent recovery complies the requirements. So to increase the assurance to compliance, it is necessary to decrease simultaneously both sources of errors.

The recovery profiles plotted in Fig. 3(1), that represents, in our experience, a common situation in the pesticide residue analysis, shown a small "fit for purpose" application interval since the horizontal curve portion is out of the compliance interval.

3. Application example of the proposed methodology

3.1. Equipment, chemicals, extraction procedure and analytical method

A Hewlett-Packard (Palo Alto, CA, USA) Model 5890 gas chromatograph equipped with an electron capture detector (ECD ^{63}Ni) was used in all the experiments. Fused silica HP-1 and HP-1701 capillary columns were used. Injections were performed with an autosampler HP 7673. An HP 3365 Chemstation software was used for instrument control and data treatment.

Disposable coveralls Tyvek Pro-Tech (DuPont Engineering Products S.A., L-2984 Luxembourg) was the personal protection equipment (PPE) used.

The characteristics of all used reagents and chemical (procymidone and dieldrin standard, *n*-hexane, placebo of commercial formulation of procymidone) were described in a previous article [16]. The experiences for the procymidone extraction and determination have been carried out following the methodology previously developed [23].

3.2. Establishment of the calibration curves

Three calibration curves were prepared as described in Ref. [16]: (i) a solvent external calibration (EC) from four standards of procymidone in *n*-hexane (solvent-blank); (ii) a "coverall matrix-matched calibration" (CMC) from calibration solutions with the same concentration levels but using a material blank extract of Tyvek Pro-Tech coverall to fill-up to the volume, and (iii) a "coverall and formulation matrix-matched calibration" (CFMC) from calibration solutions that include placebo formulation and surfactant solution, which are added to obtain a concentration equivalent to 1 and 0.1% (m/v), respectively, to obtain a similar concentration to the spray tank used in field applications, and material blank ex-

tract of Tyvek Pro-Tech coverall. These last solutions contain the same ingredients as the field samples.

In all cases, the calibration solutions contained 500, 750, 1000 and 1500 $\mu g\,l^{-1}$ of procymidone and 0.1 $\mu g\,l^{-1}$ dieldrin (internal standard).

3.3. Recovery study

Recoveries were assessed by spiking some pieces of Tyvek Pro-Tech (30 cm × 30 cm) at two concentration levels (750 and 1500 $\mu g\,l^{-1}$, respectively) with six replicates for each level. The spiked solution used was a standard pesticide solution (500 $mg\,l^{-1}$) prepared in water and containing placebo formulation in the same way as the calibration solutions.

3.4. Correction functions, calibration recovery model and calibration recovery uncertainty

Due to the presence of matrix effect, the results previously obtained [16] showed that the calibration curves present significant differences between them (EC from CMC and EC from CFMC). It is possible to establish two correction functions, which will allows us to convert the analytical results obtained from EC and CMC to the corresponding results that would be obtained applying CFMC (which are considered free of matrix error).

The correction functions are established by calculating the corresponding correction coefficients ($A_{EC\text{-}CFMC}$, $B_{EC\text{-}CFMC}$ and $A_{CMC\text{-}CFMC}$, $B_{CMC\text{-}CFMC}$) from the slopes and intercepts, of the regression curves (EC, CMC and CFMC) previously established. The found values were: $A_{EC\text{-}CFMC} = -172.6$; $B_{EC\text{-}CFMC} = 1.44$; $A_{CMC\text{-}CFMC} = 0$; and $B_{CMC\text{-}CFMC} = 1.14$, their associated uncertainties were: $u(A_{EC\text{-}CFMC}) = 44.53$; $u(B_{EC\text{-}CFMC}) = 0.054$; $u(A_{CMC\text{-}CFMC}) = 0$; and $u(B_{CMC\text{-}CFMC}) = 0.044$, and the correlation coefficient between A and B were 0.3037 for all cases (the way to calculate this features has been described in Ref. [15]).

The calibration recovery models corresponding to each established calibration are given by:

$$\Re^{C} = \frac{1}{\frac{-172.6}{C_{meas,EC}} + 1.46}; \quad \Re^{C} = \frac{1}{1.14} = 0.878 \qquad (13)$$

where $\Re^{C}{}_{EC}$ and $\Re^{C}{}_{CMC}$ are the calibration recoveries obtained when EC and CMC are applied, respectively. Table 1 shows the calculated values for $\Re^{C}{}_{EC}$ and $\Re^{C}{}_{CMC}$ and their uncertainties, and the experimental mean values for $\Re^{*}{}_{EC}$, $\Re^{*}{}_{CMC}$ and $\Re$, together with the standard deviation found from six replicates, for two analyte reference concentrations (750 and 1500 $\mu g\,l^{-1}$). As can be seen in Eq. (13), $\Re^{C}{}_{CMC}$ is constant (87.8%) and independent from the analyte concentration and its uncertainty takes a value of 3.4%.

Table 1
Estimated analyte concentrations

	$C_{refer} = 750\ \mu g\,l^{-1}$	$C_{refer} = 1500\ \mu g\,l^{-1}$
$C_{meas,EC}$ ($\mu g\,l^{-1}$)	630.8	1156.5
$\Re^{C}{}_{EC}$ (%)[a]	86.1	77.8
$u(\Re^{C}{}_{EC})$ (%)[b]	7.55	4.56
$\Re^{*}{}_{EC}$ (%)[c]	84.1	77.1
$s_{\Re^{*}\ EC}$ (%)	6.42	4.94
$C_{meas,CMC}$ ($\mu g\,l^{-1}$)	651.8	1288.5
$\Re^{C}{}_{CMC}$ (%)[a]	87.8	87.8
$u(\Re^{C}{}_{CMC})$ (%)[b]	3.4	3.4
$\Re^{*}{}_{CMC}$ (%)[d]	86.9	85.9
$s_{\Re^{*}\ CMC}$ (%)	7.51	4.07
$\Re$ (%)[e]	100.1	101.8
$s_{\Re}$ (%)	9.90	7.98

Estimated analyte concentrations obtained from EC, $C_{meas,EC}$, and CMC, $C_{meas,CMC}$, and the corresponding calibration recoveries, $\Re^{C}{}_{EC}$ and $\Re^{C}{}_{CMC}$, together with their associated uncertainties, $u(\Re^{C}{}_{EC})$ and $u(\Re^{C}{}_{CMC})$, corresponding to two different reference analyte concentrations. Mean values of the experimental apparent recovery ($\Re^{*}$) and experimental recovery factor ($\Re$) and their corresponding standard deviations ($s_{\Re^{*}}$, $s_{\Re}$) for spiked free-analyte samples at the same two reference analyte concentration levels, quantified by using different calibration curves (EC and CMC).

[a] By application of Eq. (13).

[b] By application of Eq. (11). For the estimation of the uncertainty, the values are calculated considering a constant relative uncertainty associated to the measured concentration equal to 5%.

[c] $\Re^{*}{}_{EC} = C_{meas,EC}/C_{refer}$.

[d] $\Re^{*}{}_{CMC} = C_{meas,CMC}/C_{refer}$.

[e] $\Re = C_{meas,CFMC}/C_{refer}$.

3.5. Validation of the model recovery

From recovery data included in Table 1, and following the steps described in the flow-chart in Fig. 2, both recovery models expressed in Eq. (13), have been validated by the different statistical comparisons. From these statistical comparisons it is possible to assure there is not analyte "falling down" (recovery factor is not significantly different from 100%), and the systematic errors are only due to the matrix effect. This effect decreases when a calibration containing coverall extract is used for quantification purposes, and the corresponding apparent recoveries increase.

3.6. Recovery profiles: delimitation of the "fit for purpose" analyte concentration range

Fig. 4(1) shows the calibration recovery profile when the quantification is carried out from the solvent external calibration curve (EC), as well as the corresponding uncertainty bands, calculated from the Eq. (11). In addition, the compliance intervals in connection with the recovery requirements for validation (70–110%) [6,10], quality control (60–140%) [5] have also been plotted. From the cut-points between the recovery curve and/or the uncertainty bands with the straight lines which limit those intervals, it is possible to obtain an analyte concentration range that is fitted for purpose.

There is an important contribution from the matrix effect, which yields to a curvilinear shape dependence due to the

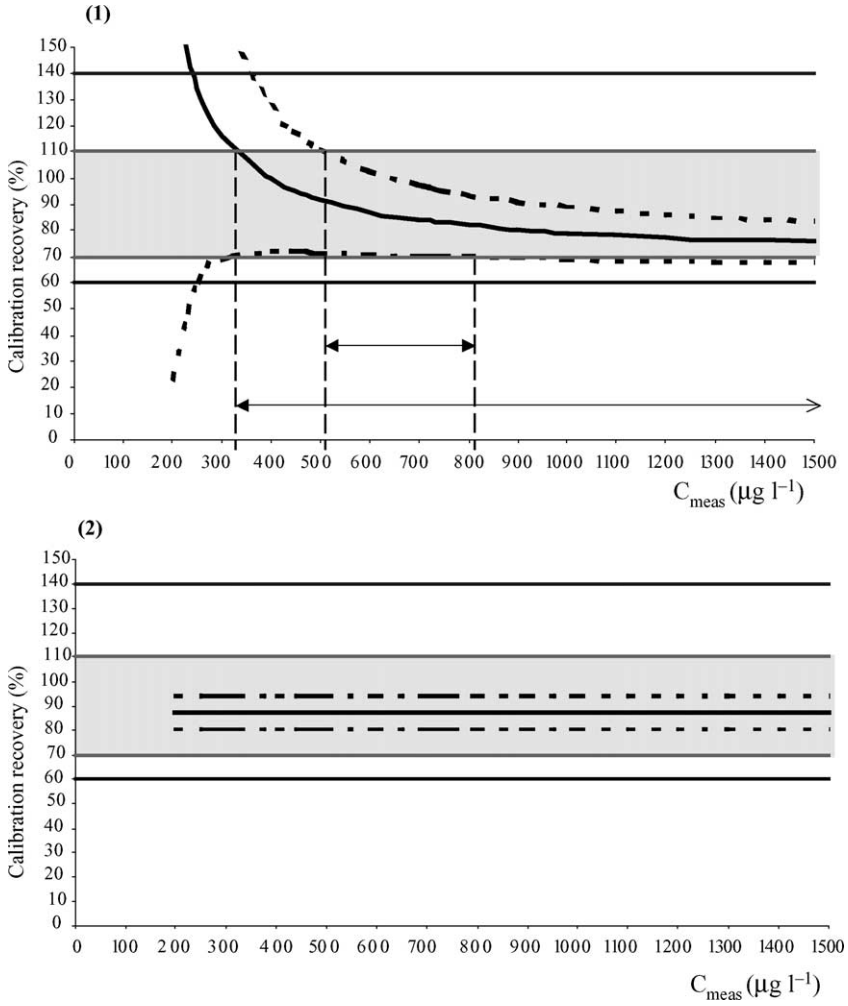

Fig. 4. Recovery profiles obtained from: (1) external solvent calibration (EC), and (2) coverall matrix-matched calibration (CMC) for the procymidone determination in coverall workers by GC-ECD to assess dermal exposure. Two compliance bands have been indicated, covering the ranges between 70 and 110% (for validation purposes) and between 60 and 140% (for routine quality control). Dashed curves represent the uncertainty bands on the calibration recovery.

high value of the correction coefficient $A_{EC\text{-}CFMC}$. However, when the analyte concentration increases, the calibration recovery profile tends to a constant value of approximately 75%, that is function of the correction coefficient $B_{EC\text{-}CFMC}$. Due to the calibration interval covers the analyte concentration range on 500–1500 µg l^{-1}, the analytical method complies with the routine quality control requirements. Nevertheless, in relation with the validation requirements, two situations can be considered: (i) if the recovery curve is just judged, the method is also fitted for purpose; but (ii) if the uncertainty bands are taken into account, the applicable analyte concentration range covers only the values between 500 and 800 µg l^{-1}, approximately. The use of the uncertainty interval is justified because it improves the assurance on the validation conclusions, since it removes the risk to obtain recoveries out of the compliance interval due to procedure errors.

If the analyst wishes to enlarge the applicable analyte concentration range it is necessary to reduce the systematic matrix error. For this purpose, it is possible to incorporate some matrix components to the calibration solution such as coverall blank extracts and to establish a "more closed matrix-matched calibration" (coverall matrix-matched calibration, CMC).

When the quantification is carried out from this CMC, the correction coefficient *A* becomes zero (the recovery curve is a straight line) and the correction coefficient *B* decreases (the recovery curve ascends to a value of 87.8%). In these conditions, the method is fitted for both quality control and validation purposes in the whole application interval (see Fig. 4(2)).

4. Conclusions

Recovery is a analytical feature very usual in Analytical Chemistry. Nevertheless, the lack of harmonisation in the meaning of the recovery term and how the recovery factor is calculated have created some confusions among the analysts, since it can be used to assess the apparent analyte concentration in analyte stability studies, the efficiency of an extraction

process (or any sample treatment) or the method trueness in validation.

In the current article, the authors define and justify a new term, called "calibration recovery" related with the matrix-effect error which usually disturbed the results in residue chromatographic analysis on real samples even when MS is used as hyphenated detection technique. This term would complete the recent IUPAC approach [2] since it allows the establishment of a formal relation between the IUPAC terms "recovery factor" and "apparent recovery" Eq. (1). The meaning of all these terms is clarified and related with different types of analytical systematic errors and with the appropriate steep of the analytical process.

From a metrological point to view, the "calibration recovery" is related to a correction factor and defined based on two correction coefficients which have been defined by authors in a preceding paper [21]. Therefore, to calculate the calibration recovery and to differentiate both recovery components (recovery factor and calibration recovery) is need to establish two calibrations: an external calibration (from standard in working solvent) and a matrix-matched calibration (from standard in free-analyte matrix). If it was necessary, the proposed model (Eqs. (1) and (9)) could be validated following the process described in Section 2.3).

In addition, the authors propose an approach to assess the compliance of the recovery values with the validation and/or quality control requirements for residue analysis, which takes in consideration the dependence of the recovery with the sample analyte content and the recovery uncertainty. By means of a sufficient but realistic number of experiments, the procedure, based on setting up the recovery profile, shows how a "fit for purpose" analyte concentration interval complying with regulated recovery requirements can be delimited.

The confidence in the conclusions obtained on this recovery compliance could only be established if the calibration recovery is recognised and formally characterized because, as it has been stated in the paper, it varies with the actual analyte concentration in the real sample.

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