

# Two important limitations relating to the spiking of environmental samples with contaminants of emerging concern: How close to the real analyte concentrations are the reported recovered values?

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**Abstract** Occurrence and effects of contaminants of emerging concern pose a special challenge to environmental scientists. The investigation of these effects requires reliable, valid, and comparable analytical data. To this effect, two critical aspects are raised herein, concerning the limitations of the produced analytical data. The first relates to the inherent difficulty that exists in the analysis of environmental samples, which is related to the lack of knowledge (information), in many cases, of the form(s) of the contaminant in which is present in the sample. Thus, the produced analytical data can only refer to the amount of the free contaminant ignoring the amount in which it may be present in other forms; e.g., as in chelated and conjugated form. The other important aspect refers to the way with which the spiking procedure is generally performed to determine the recovery of the analytical method. Spiking environmental samples, in particular solid samples,

with standard solution followed by immediate extraction, as is the common practice, can lead to an overestimation of the recovery. This is so, because no time is given to the system to establish possible equilibria between the solid matter—inorganic and/or organic—and the contaminant. Therefore, the spiking procedure need to be reconsidered by including a study of the extractable amount of the contaminant versus the time elapsed between spiking and the extraction of the sample. This study can become an element of the validation package of the method.

**Keywords** Contaminants of emerging concern · Free and conjugated forms · Environmental samples · Recoveries · In-house analytical methods

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## First limitation

While investigating the fate of organic contaminants in the environment, unavoidably, a number of chemical analyses must be performed regarding the amount or the concentration of the parent compounds and/or their transformation products and/or their metabolites in samples taken from the relevant environmental receptors. To this effect, and in order to produce comparable results, international and national bodies developed and validated methods of analysis for a large number of organic and inorganic substances in various environmental compartments, e.g., air, water, wastewater, soil as well as in plant and animal materials.

However, the rapidly increasing requirement to study the fate of the newly identified contaminants of emerging concern in the environment urges the research laboratories (in the absence of standard or official methods) to develop in-house analytical methods to fit the purpose; i.e., to develop methods

for the specific analyte(s) of interest at the specific concentrations and for the specific matrices. These methods need to be properly validated. In many instances addressing the full validation package is not necessary for R&D laboratories (EURACHEM/CITAC 1998). Most often, for the R&D laboratories, the validation of an in-house method includes establishing a linear relationship between the signal/response versus the entire range of concentrations of the analyte of interest, the bias (recovery), the limits of detection and quantitation, the relative standard deviation of the measurements, and the interferences where this is applicable (NORMAN 2009). Although all the performance characteristics within the validation package are very important elements, the bias of the method is of paramount importance attracting special interest with regard to how it is determined.

Ideally the bias of a method, i.e., the difference of the measured amount of the analyte from the “true” value can be determined using the appropriate Certified Reference Material (CRM) having stated uncertainty at a stated level of confidence for each one of the analytes of interest or can be determined using a Reference Material (RM) (EURACHEM 2002); provided, that both materials have the same matrix as that in the samples which is to be analyzed by the in-house method and also the analytes are at the same range of concentrations as those in the sample. There are some thousands of CRMs and RMs available, produced by national/international bodies (e.g., NIST, IRMM) or by private companies, which cover a wide range of matrices and compounds (e.g., COMAR database). Unfortunately, despite the plethora of these materials, it is hardly possible to find the appropriate one particularly in the area of the organic contaminants of emerging concern in matrices like plant materials, soils, wastewater, and sludge from wastewater treatment plants, to name a few.

To overcome the deficiency of the appropriate matrix RM, spiking of blanks or samples with standard solution of analytes at concentrations expected to be present in the samples is used as a compromising solution (Fig. 1).

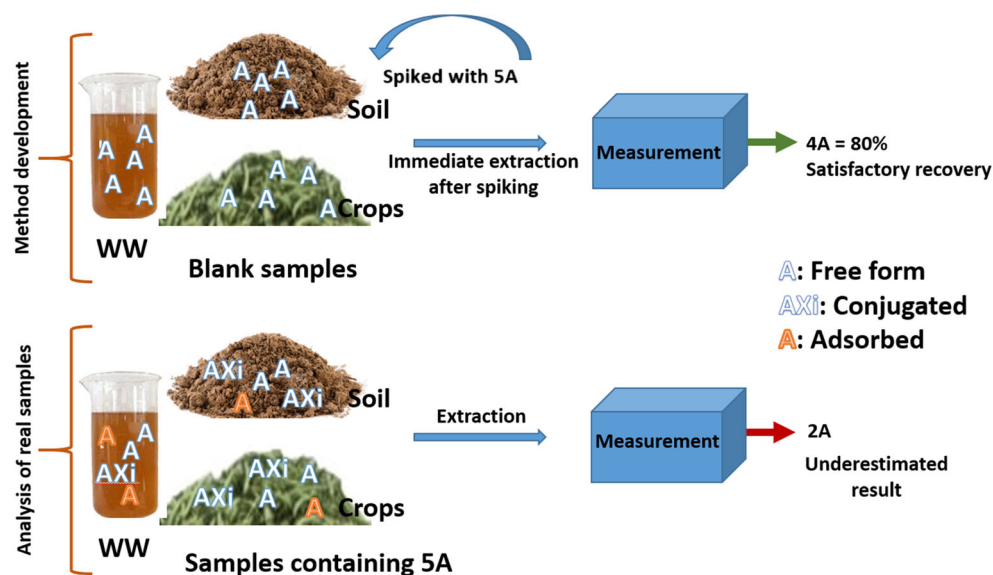
Of course, in these cases, it is assumed that the analyte in the real sample and the analyte in the standard solution are in the same form, which actually is not known a priori. Therefore, even if the recoveries obtained via spiking of samples can be very high, this does not mean that the measured content of the analyte in the real sample represents the actual amount of the analyte present, and thus, this can lead to an underestimation of the total amount of the analyte. In the real sample, the analyte can be present, for example, in the form of a conjugate with matrix substances, or bound to the organic and/or the inorganic constituent in the case of soil; it can thus behave in a completely different way than that of the free analyte in the standard. An example of such problem has been demonstrated by the observation that the concentration of some pharmaceuticals, e.g., carbamazepine and salicylic acid in the inflow stream of municipal sewage treatment plants was

found lower than that in the outflow stream. This paradox was thoroughly investigated and it was proved that, among other reasons (Göbel et al. 2005; Gulkowska et al. 2008), in the inflow stream, these pharmaceuticals are present also in the form of the glucuronide conjugates of the parent compounds, which obviously were not included in the determination of the free drugs. During the biological treatment, the glucuronides are broken down—due to the glucuronide activity of the activated sludge (Ternes et al. 1999; Vieno et al. 2006; Leclercq et al. 2008), and consequently, the free form of the pharmaceuticals is released; thus this can be determined, giving rise to higher concentrations compared to that found in the inflow. The possibility of having conjugates or chelates of the parent compound—apart of the free form—is expected to be pretty high in the analysis of organic contaminants of emerging concern in crop materials, in soils, in sludge from sewage treatment plants, and sediments. In these cases, the analyte can be bound to small or bigger organic molecules of the matrix, and therefore the applied isolation procedure (extraction, pre-concentration with SPE, etc) for the free compound may not be adequate for the conjugate too; thus leading to an underestimation of the present amount of the contaminant. These examples confirm the inherent difficulties that exist in the analysis of real environmental samples where there is no information regarding the form(s) of the contaminant present. In this regard, the chemical stability of conjugates during the extraction procedure and the knowledge related to which fraction of contaminant is extracted (total versus free forms) is of paramount importance.

## Second limitation

A second important aspect related to the spiking procedure—which deserves special attention—is the time elapsing between spiking the blank/samples and commencing the analysis. The general information that so far is given in the scientific publications is restricted only to the amount of the analyte or analytes spiked in a certain amount of blank/sample. No information is provided on how long the spiked blank/samples have been left before analysis and what conditions they are kept (temperature, darkness, etc.) before the analytical process is commenced. According to the current practice, the spiked samples are prepared and used just shortly before the analysis of the unknown samples. This practice leads to recoveries that cannot always be considered the same as those obtained from the real samples. This is acknowledged in an IUPAC technical report (Thompson et al. 1999) and also in the EURACHEM Guide (EURACHEM 2014) as an inherent problem, stating that this technique is giving an unrealistically high impression of the extraction efficiency. Out of the big number of relevant manuscripts studied, the authors managed to retrieve clear information on the time interval between spiking the samples

**Fig. 1** **a** Immediate extraction of spiked samples may lead to an overestimate of the recovery performance of the method and **b** the form(s) of the analyte present in real environmental samples may lead to an underestimate of the results



with the standards and the beginning of the extraction procedure only from two (Calderón-Preciado et al. 2009; Yang et al. 2010).

The importance of the spiked-to-extraction time is more pronounced in soil and sludge analysis where adsorption of the spiked substance(s) on the matrix is time depended; thus, in these cases, the extraction performance must be checked after the adsorption equilibrium is reached. As a consequence, harsher extraction regimes may be needed (ASE, microwave assisted methods, etc.) to extract the bound fraction of the analyte. Even with the application of the US EPA method 1694 (US EPA 2007) for the determination of pharmaceuticals in soil, Li et al. (2013) found that a small amount of carbamazepine (about 5%) was non extractable from soil spiked with  $^{14}\text{C}$ -labeled carbamazepine, while in another study, the same author (Li et al. 2014) found that up to about 70 to 93% of  $^{14}\text{C}$ -acetaminophen added in soil after 120 d was not extractable. These findings are only some examples showing that more careful evaluation is needed of the recoveries obtained after spiking complex matrices. A more complex problem is the one related to the multi-residue analysis where, based on the contact time of the spiked analytes with the matrix, some analytes may be adsorbed or form conjugates or chelates (Graouer-Bacart et al. 2013) faster than others. Consequently, we think that a new approach should be considered with the recovery experiments in order to reflect the real bias of the in-house methods.

To our opinion, when presenting the data on recoveries, which are measured after spiking samples with standard solutions of analytes, the time interval between spiking the samples and the analysis is essential to be reported along with the other performance characteristics of the method. A study of the recovery of each analyte versus the spike-to-extraction time is considered imperative and not just useful. The in-

house methods' recoveries can be considered satisfactory when (a) in the acceptable percentage range for the levels of the analytes measured and (b) remain constant with various spike-to-extraction times provided that the analyte is not degraded for any reason with time. This information (spike-to-extraction time) could be considered as an important element of the robustness of the method. However, for pesticides residues in food and feed, DG SANCO of the European Union (DG SANCO 2015) noted that spiking of samples would not simulate incurred residues even if the spiked sample is left standing for a certain time.

### Other considerations

It is essential to strongly underline here that other aspects related to the spiking procedure should be also considered. For example, in cases where the extraction is not applied to the whole spiked sample but sub-samples of it are taken for extraction, then the homogeneity of the spiked sample (most important in spiking solid samples like soil, sludge, or sediments) should be demonstrated and reported. Of course, the volume and the solvent of the spiking standard solution as well as the method (absolute or relative) used to determine the recovery must be also reported.

### Closing remarks

This is a first attempt to raise the concern of the scientific community on two critical issues that are related to the true-ness of the produced data in the domain of the environmental analysis by the R&D laboratories. The intention of the authors is to provoke an open discussion among the scientists working

in environmental analysis and in related fields aiming at reconsidering the standard procedures followed to date for the measurement of organic pollutants especially in environmental samples.

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