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Proficiency test on the determination of pesticide residues in grapes with multi-residue methods

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ABSTRACT

This manuscript presents the results of the International Measurement Evaluation Programme 37 (IMEP-37) study, a proficiency test (PT) which was organised to assess the world-wide performance of food control laboratories on the determination of pesticide residues in grapes. This PT supports the implementation of Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin. Eighty-one participants reported results, forty from EU Member States and forty-one from outside the EU. The test item was a grape sample spiked with 20 selected pesticides. The results of the participants were rated with *z*- and zeta (ζ-) scores in accordance with ISO 13528 and ISO 17043. The standard deviation for the proficiency assessment, $\hat{\sigma}$, of this PT was set at 25% for the 20 measured pesticides based on previous experience with similar measurands. The results reported to IMEP-37 showed that the participants performed satisfactorily, ranging from 81% (carbendazim) to 97% (azoxystrobin, penconazole, pyrimethanil) of the participating laboratories. However, only 30% of the participants managed to analyze all pesticides satisfactorily. Overall, the performance of the participants in this PT was good but there is room for improvement in the development of multi-residue methods for the simultaneous analysis of a large number of pesticides with an increased accuracy.

1. Introduction

According to Regulation (EC) No 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin [1], official controls to check compliance with maximum residue levels (MRL) of pesticides are needed. Indeed, regulatory compliance remains one of the most important drivers behind pesticide residue analysis. Before food products can enter a particular market, requirements for MRL must be met for a variety of pesticides [2]. For this reason there is a need for reliable and sensitive analytical methods that are able to quantify a large number of compounds at the low limits set by legislation [3]. Pesticide residue analysis remains a challenging area in food analysis because of the large number of target analytes with different chemical structures and the wide diversity of food matrices [4]. Multi-residue methods provide the tools to the analyst to measure these compounds [5–7]. Gas chromatography (GC) used to be the technique of choice but it has the drawback of being unsuitable for a number of pesticides because of their thermal instability and polarity [5]. During the past decade the advances in instrumental analysis led to simple preparation procedures coupled to liquid chromatography tandem mass spectrometry (LC–MS/MS) [8–10]. These new techniques allowed the analysis of many traditionally "difficult-to-analyse" pesticides [11]. Nowadays both GC and LC are complementary techniques for the coverage of the full range of pesticides. One example of simple sample preparation procedures is the so-called quick, easy, cheap, effective, rugged and safe (QuEChERS) method of pesticide analysis [12]. The QuEChERS method involves an acetonitrile partitioning and dispersive solid-phase extraction (d-SPE) which allows the simultaneous analysis of a large number of pesticides in a variety of food matrices [13–15]. It offers a good alternative to traditional techniques like liquid–liquid and solid phase extractions. In order to further increase the quality of multi-residue methods for the analysis of pesticides in the European Union, a guidance document of the Directorate-General for Health and Food Safety (DG SANTE) on analytical quality control and validation procedures for pesticide residues analysis in food and feed has been published [16]. Moreover the European Union

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Reference Laboratory for Pesticide Residues in Fruit and Vegetables (EURL-FV) in Almería organises PTs for pesticide residues for control laboratories in the European Union on a yearly basis as stipulated under Regulation (EC) No 882/2004 [17,18].

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the International Measurement Evaluation Programme (IMEP). It organises interlaboratory comparisons (ILCs) in support to EU policies. This work presents the outcome of IMEP-37, a PT organised for the determination of 20 pesticides in grapes in support to Regulation (EC) No 396/2005. The aim of the study was to investigate the performance of control laboratories world-wide and more specifically to compare the performance of laboratories located in EU Member States and laboratories outside the EU. The study included 15 fungicides (azoxystrobin, carbendazim, cyprodinil, difenoconazole, fenhexamid, fludioxonil, iprodione, kresoxim methyl, myclobutanil, penconazole, pyraclostrobin, pyrimethanil, quinoxyfen, tebuconazole and triadimenol), 4 insecticides (imidacloprid, indoxacarb, lambda-cyhalothrin, methoxyfenozide) and 1 acaracide (chlorpyrifos) spiked into grapes (*Vitis vinifera*). The pesticides selected were those typically found in grapes, explaining the large amount of fungicides in this study as fungi are of major concern during grape cultivation. The 20 pesticides that were selected for this PT study are all included in the EU coordinated monitoring programme of 2013–2015 [19]. Typical GC and LC amenable compounds were chosen in order to check the performance in both systems.

2. Materials and methods

2.1. Announcement of the study

The announcement of the PT study was done on the IMEP website and via the European Cooperation for Accreditation (EA), the Asia Pacific Laboratory Accreditation Cooperation (APLAC) and the InterAmerican Accreditation Cooperation (IAAC).

2.2. Preparation and evaluation of the test item

The test items (one treated and one blank) were prepared by the EURL-FV in Almeria, Spain. Eighty kilograms of seedless grapes *Sugraone*, organically grown in Almeria (southeast of Spain), were contaminated using a nebuliser. A first group of pesticides was added as commercial pesticide formulations dissolved in water (azoxystrobin, carbendazim, cyprodinil, difenoconazole, fenhexamid, fludioxonil, imidacloprid, indoxacarb, iprodione, kresoxim methyl, methoxyfenozide, myclobutanil, penconazole, pyrimethanil, tebuconazole, lambda-cyhalothrin and triadimenol). The purpose of using commercial pesticide formulations dissolved in water was to avoid the use of organic solvents in order to reproduce the difficulties in the extraction step as much as possible. These pesticide formulations could be dissolved or suspended in water before their application. However, not all pesticides were available as formulations dissolved in water. Therefore a second group was spiked in the form of analytical standards dissolved in acetonitrile/water (1:1, v/v; chlorpyrifos, pyraclostrobin and quinoxyfen). The concentrations of the spiked pesticides ranged from 0.031 mg kg^{-1} (lambda-cyhalothrin) to 0.332 mg kg^{-1} (difenoconazole). Table 1 provides details about the pesticides included in IMEP-37.

After spiking all the pesticides, a portion of the contaminated grapes was analysed to check if the residue levels present were close to the target levels or whether any additional spraying was necessary. When the residue levels in the grapes were close to the target ones, the entire batch of grapes was frozen and processed using cryogenic milling. The frozen minced grapes were mixed in a constantly-spinning container for 3 h. 210–250 g portions of the well-mixed homogenate were weighed out into screw-capped polyethylene plastic bottles, sealed and stored at −20 °C until shipment of the test item. The grapes of the blank test item were organically grown in the same field as the grapes of the test item. A homogenate was prepared in the same way as the contaminated test item described above, but without addition of pesticides.

Test items were dispatched to the participants with dry ice in order to keep the test items frozen during transport. A 'confirmation of receipt' form was sent together with the test item. This form had to be returned by the participant to the PT organiser confirming that the test item package had arrived under good conditions.

2.3. Homogeneity and stability

Homogeneity and stability studies were performed by the EURL-FV. The homogeneity of the test item was evaluated according to the test proposed by IUPAC [20]. IMEP always includes an uncertainty contribution related to homogeneity, u_{bb}, in the uncertainty associated to the assigned value (u_{ref}) as recommended by ISO 17043 and following the approaches described in ISO 13528 and ISO Guide 35 [21–23]. The stability of the test item was checked by analysing it at two different time intervals at $t = 0$ and $t = 6$ weeks. The material proved to be adequately stable for the twenty pesticides during six weeks that elapsed between the dispatch of the samples and the deadline for reporting.

The contribution from homogeneity (u_{bb}) and stability (u_{st}) to the uncertainty of the reference value (u_{ref}) was calculated using softCRM [24]. The raw data of the homogeneity and stability studies can be found in the report to participants [25].

2.4. Assigned values and their uncertainties

The assigned values (X_{ref}) used to benchmark the laboratories taking part in IMEP-37 were established independently from the results reported by the participants. The assigned values were determined by the following five expert laboratories, selected based on their good performance in past PTs on the determination of pesticides in food matrices organised by the EURL-FV of Almeria:

- Austrian Agency for Health and Food Safety (Ages), Austria.
- Laboratoire du SCL de Montpellier (SCL), France.
- European Union Reference Laboratory for Pesticide Residues in Fruit and Vegetables (EURL-FV), Almeria, Spain.
- European Union Reference Laboratory for Pesticide Residues in Fruit and Vegetables (EURL-FV) – Laboratorio Agroalimentario de Valencia, Spain.
- National Food Agency (NFA), Sweden.

Each expert laboratory received two bottles of test item to be analysed on two different days (one bottle/day) performing three independent replicates per bottle. The mean of the independent means provided by the expert laboratories was used to derive the assigned value (X_{ref}) of the different measurands according to the ISO Guide 35 [23]. The associated uncertainties (u_{ref}) of the assigned values were calculated combining the uncertainty of the characterisation (u_{char}) with the contributions from homogeneity (u_{bb}) and stability (u_{st}) in compliance with ISO Guide 35 [23] using Eq. (1):

$$u_{ref} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{st}^2} \quad (1)$$