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Matrix interference evaluation employing GC and LC coupled to triple quadrupole tandem mass spectrometry

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ABSTRACT

Gas and liquid chromatography coupled to triple quadrupole tandem mass spectrometry are currently the most powerful tools employed for the routine analysis of pesticide residues in food control laboratories. However, whatever the multiresidue extraction method, there will be a residual matrix effect making it difficult to identify/quantify some specific compounds in certain cases. Two main effects stand out: (i) co-elution with isobaric matrix interferents, which can be a major drawback for unequivocal identification, and therefore false negative detections, and (ii) signal suppression/enhancement, commonly called the “matrix effect”, which may cause serious problems including inaccurate quantitation, low analyte detectability and increased method uncertainty. The aim of this analytical study is to provide a framework for evaluating the maximum expected errors associated with the matrix effects. The worst-case study contrived to give an estimation of the extreme errors caused by matrix effects when extraction/determination protocols are applied in routine multiresidue analysis. Twenty-five different blank matrices extracted with the four most common extraction methods used in routine analysis (citrate QuEChERS with/without PSA clean-up, ethyl acetate and the Dutch mini-Luke “NL” methods) were evaluated by both GC-QqQ-MS/MS and LC-QqQ-MS/MS. The results showed that the presence of matrix compounds with isobaric transitions to target pesticides was higher in GC than under LC in the experimental conditions tested.

In a second study, the number of “potential” false negatives was evaluated. For that, ten matrices with higher percentages of natural interfering components were checked. Additionally, the results showed that for more than 90% of the cases, pesticide quantification was not affected by matrix-matched standard calibration when an interferent was kept constant along the calibration curve. The error in quantification depended on the concentration level. In a third study, the “matrix effect” was evaluated for each commodity/extraction method. Results showed 44% of cases with suppression/enhancement for LC and 93% of cases with enhancement for GC.

1. Introduction

The analysis of fruit and vegetables is an important issue in laboratories assessing food safety for consumers. To fulfill this goal, laboratories analyzing official samples must implement Analytical Quality Control (AQC) procedures [1]. According to the literature, traditional sample preparation methods for determining pesticide residues in fruit and vegetables are based on multiresidue extraction methods (MRMs) with the aim of extracting as many compounds as possible over a wide range of polarities [2]. In light of this, the MRMs commonly applied by laboratories are citrate QuEChERS (with/without clean-up), ethyl acetate and the Dutch mini-Luke (NL) method. Polar or apolar compounds can be extracted according to the solvent polarities utilized in the extraction method. Citrate QuEChERS uses acetonitrile as the extraction solvent, which can extract polar compounds more easily, while the ethyl acetate extraction method employs ethyl acetate, which is able to extract apolar compounds more easily. The NL-method can extract polar and apolar compounds as it uses acetone as the extraction solvent and subsequently, petroleum ether and dichloromethane as the partition solvents. However, the major drawback of MRMs is that they also extract organic compounds from the matrix along with the analytes of interest, thus generating interferences [3].

Both gas and liquid chromatography techniques have been traditionally applied to identify and quantify target pesticides in a wide range of matrices, mostly when coupled to tandem mass spectrometry

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(GC-MS/MS and LC-MS/MS). The most common MS systems employed for the routine analysis of pesticide residues in fruit and vegetables have been triple quadrupole (QqQ) and ion trap (IT), due to the selectivity, sensitivity and yield of this technique [4,5]. Even with the high selectivity demonstrated by this technique over the years, it is still possible to find both endogenous and exogenous interferents [6–10]. From the outset, we found it useful to maintain the distinction between: (a) whether an interferent is present in the retention time range of the target pesticide and has the same transition and (b) whether the interfering component chromatographically co-elutes with the target pesticide and affects the ionization efficiency of the target compound in the MS detector (LC); or whether the interfering component deactivates the active sites in the inject port and column (GC). Clause (a) profoundly affects unequivocal identification and thus, it can suppose a "potential" false negative; or a false positive if it is present at both transitions. Clause (b) is commonly referred to as the "matrix effect" and it covers cases where suppression or enhancement in the ion source leads to an increase or a decrease in detector responses (LC), and cases where an improvement in mass transference of the analyte to the detector leads to an increase in the detector response (GC). The "matrix effect" can be a significant problem as it may severely compromise quantitative analysis of the compounds; this is because it detrimentally affects accuracy, reproducibility, and sensitivity [11,12].

Current methods to eliminate or reduce matrix effects include optimizing sample preparation to remove interfering compounds from the samples [13–15], changing chromatographic conditions to avoid coelution of analytes and interfering compounds [16], changing mass spectrometric conditions to reduce the occurrence of matrix effects in the ion source [16], dilution of the sample [17] or using chemical treatment measures [18] or calibration techniques such as matrix-matched standards [1] and the standard-addition method [1], which corrects them. However, in certain cases, the best matrix component removal, or the most effective matrix effect correction can be more labor intensive and/or increase the overall uncertainty.

Accordingly, our aim was to carry out the chemical evaluation of co-extracted compounds as interfering components in pesticide analysis in some of the most relevant fruit and vegetable matrices. For this, twenty-five different matrices and four of the most common extraction methods used in routine analysis were evaluated by both GC-QqQ-MS/MS and LC-QqQ-MS/MS. The most demanding requirements regarding mass spectrometric confirmation currently set by EU AQC procedures were taken into account. In addition, we also investigated the possible problems reported on interfering components in the identification/quantification of the target pesticide and the number of "potential" false negatives. Finally, the signal suppression/enhancement was calculated, including in samples where matrix effects are highly relevant, and both identification and quantification errors were evaluated to determine to what extent they affect the determination of a total of 160 and 220 pesticides analyzed by LC-MS/MS and GC-MS/MS, respectively.

2. Experimental

2.1. Reagents and materials

All high-purity pesticide standards were obtained from Dr. Ehrenstorfer (Ausburg, Germany), Sigma-Aldrich (Steinheim, Germany) and Riedel-de-Haën (Seelze, Germany), and were stored at −30 °C. Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile and were stored in amber screw-capped glass vials in the dark at −20 °C. Standard mix solutions at 10 mg/L were prepared in acetonitrile, and were used for calibration after the appropriate dilution in acetonitrile:water (LC) or ethyl acetate (GC). Carbendazim-d$_3$ and lindane-d$_6$ were used as injection internal standards for LC and GC, respectively, at a 50 µg/L concentration level in the vial.

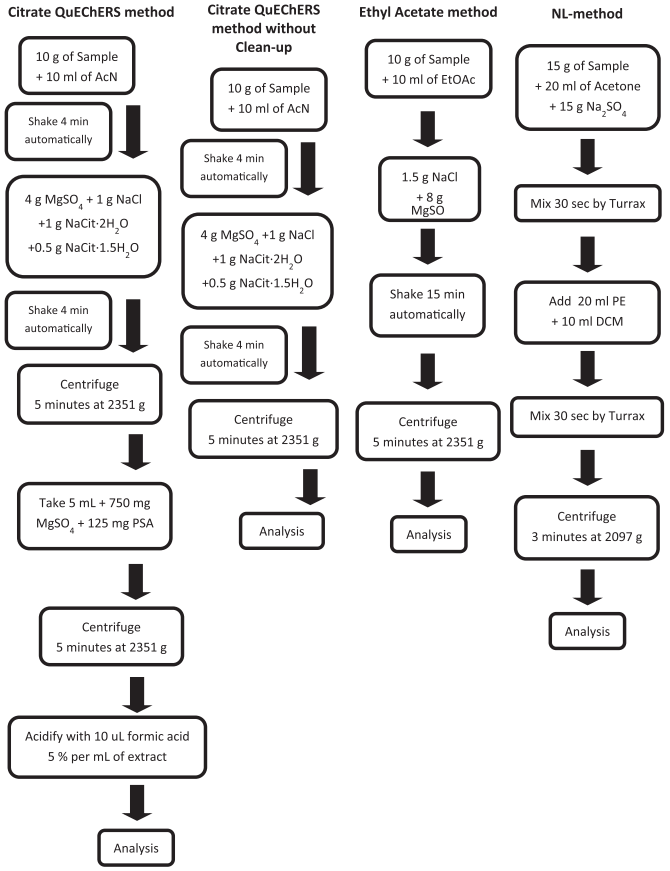

Fig. 1. Scheme for the four selected multiresidue methods applied to matrices. ACN: acetonitrile; EtOAc: ethyl acetate; PE: petroleum ether; DCM: dichloromethane.

Ultra gradient HPLC-grade acetonitrile and acetone were obtained from Sigma-Aldrich. Dichloromethane and HPLC-grade ethyl acetate were purchased from Fluka (Steinheim, Germany). Petroleum ether was supplied by Riedel-de-Haën. Anhydrous magnesium sulphate, sodium chloride, sodium hydrogenocitrate sesquihydrate and formic acid were purchased from Sigma Aldrich. Anhydrous sodium sulphate was obtained from J.T. Baker (Deventer, Holland). Tri-sodium citrate dihydrate was supplied by Merck KGaA (Darmstadt, Germany) and PSA from Supelco (Bellefonte, PA, USA). A Milli-Q-Plus ultrapure water system from Milli-pore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses by LC.

2.2. Extraction procedures

There are many different extraction procedures used in laboratories. In this study, we evaluated the four MRMs most commonly used in routine analysis (citrate QuEChERS with/without clean-up, ethyl acetate and Dutch mini-Luke "NL") by European laboratories for fruit and vegetable analysis. A summary of these can be seen in Fig. 1. Once the samples were ready to be injected into the vial, the injection internal standard was added in all cases.

2.2.1. Citrate QuEChERS method *[19]*

After extraction (see Fig. 1), 50 µL of the extracts in acetonitrile were diluted with 200 µL of Milli-Q water prior to injection by LC-QqQ-MS/MS; while for GC-QqQ-MS/MS analysis, 50 µL of the extracts were evaporated to dryness with a nitrogen stream and recomposed with the same volume of ethyl acetate.