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Evaluation of the impact of matrix effect on quantification of pesticides in foods by gas chromatography–mass spectrometry using isotope-labeled internal standards

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ABSTRACT

The impact of the matrix effect in GC–MS quantification of pesticides in food using the corresponding isotope-labeled internal standards was evaluated. A spike-and-recovery study of nine target pesticides was first conducted using paste samples of corn, green soybean, carrot, and pumpkin. The observed analytical values using isotope-labeled internal standards were more accurate for most target pesticides than that obtained using the external calibration method, but were still biased from the spiked concentrations when a matrix-free calibration solution was used for calibration. The respective calibration curves for each target pesticide were also prepared using matrix-free calibration solutions and matrix-matched calibration solutions with blank soybean extract. The intensity ratio of the peaks of most target pesticides to that of the corresponding isotope-labeled internal standards was influenced by the presence of the matrix in the calibration solution; therefore, the observed slope varied. The ratio was also influenced by the type of injection method (splitless or on-column). These results indicated that matrix-matching of the calibration solution is required for very accurate quantification, even if isotope-labeled internal standards were used for calibration.

1. Introduction

Various pesticides are used to protect foods against pests and diseases [1]. However, high levels of residual pesticides in food may result in adverse effects on human health. Therefore, analysis of various residual pesticides in food is routinely performed worldwide, and ensuring the reliability of these results is crucial for controlling the risk associated with pesticide residues.

In general, analytical methods for determining the presence of pesticide residues in food require complex extraction of the target pesticides, multi-step clean-up of the extracts, and chromatographic analysis of the prepared sample solution [2–4]. In the last step, the observed detector response for the target pesticide may differ for the sample solution and a matrix-free calibration solution. Consequently, the observed analytical value may be biased from the "true" value due to the phenomenon termed the "matrix effect". In GC–MS measurements, the sample matrix can cause an enhancement in the detector response by increasing transfer of the target pesticide from the hot vaporizing injector. This may occur by (I) reduction of the thermal stress to labile compounds and (II) by masking active sites (such as silanols and metal ions) in the injector, column, and detector for the adsorption or decomposition of the target pesticide [5–9]. There are many strategies to eliminate this analytical bias: the most effective strategy is to use matrix-matched calibration solutions or analytical protectants [5].

An accurate analytical method must be utilized for characterization of certified reference materials (CRMs) or to determine the assigned values of proficiency testings (PTs) by measurement. Isotope-dilution mass spectrometry (IDMS) has been recognized as having the potential to be a primary method of measurement [10,11]. In this method, a known amount of an isotope-labeled congener of the target analyte, generally determined by weighing, is added to the sample prior to initiation of any pretreatment. After isotopic equilibration is achieved, the initial isotopic ratio between the target analyte and corresponding isotope-labeled congener does not change in principle during the following sample pretreatment and measurement processes. Consequently, accurate analytical results can be obtained by measuring the isotopic ratio of the resulting sample solution via mass spectrometry. Certain national metrology institutes (including our institute) have

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applied IDMS to the characterization of CRMs [12–17] and to provide assigned values of PTs [18–21] for analysis of pesticide residues in food.

In GC–MS quantification, the use of isotope-labeled congeners of the target analytes as the internal standard, including in the case of IDMS, is expected to offset the bias of the analytical results caused by matrix effects [22–25]. Ueno et al. [26] reported a spike-and-recovery study of 89 pesticides in vegetables and fruits by GC–MS after GPC and SPE cleanup. The ratios of the observed analytical concentrations to the spiked concentrations were remarkably good (94.6–108.6%) for the 14 target pesticides that were quantified using the isotope-labeled pesticides as internal standards. Furthermore, Yu and Xu [27] evaluated the matrix effect for 176 pesticides using on-line GPC-GC-MS, where different suppression effects were observed for chlorpyrifos and chlorpyrifos-d_{10}. This was because di-*n*-butyl phthalate co-dissolved in the sample solution was co-eluted with chlorpyrifos-d_{10} but not with chlorpyrifos; thus, the intensity of only the chlorpyrifos-d_{10} peak was significantly decreased. To the best of our knowledge, no comprehensive study concerning the matrix effect in GC–MS using isotope-labeled internal standards has been published for pesticide residue analysis, even though the trueness of the observed analytical results may be influenced by this effect.

The objective of the present study is to evaluate the impact of matrix effects on the quantification of pesticide residues in food via GC–MS using isotope-labeled internal standards. A spike-and-recovery study of nine target pesticides is conducted, demonstrating the adverse influence of matrix effects on the observed analytical values. By comparing the matrix-matched and matrix-free calibration solutions, we address the effectiveness of matrix matching of the calibration solution to achieve highly accurate quantification.

2. Experimental

2.1. Chemicals and materials

Nine pesticides, including 6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine (simazine), *O*,*O*-diethyl-*O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (diazinon), *O*,*O*-dimethyl-*O*-4-nitro-*m*-tolyl phosphorothioate (fenitrothion), *S*-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorodithioate (malathion), *O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos), *S*-4-chlorobenzyl diethyl(thiocarbamate) (thiobencarb), diisopropyl 1,3-dithiolan-2-ylidenemalonate (isoprothiolane), *O*,*O*-diethyl *O*-5-phenyl-1,2-oxazol-3-yl phosphorothioate (isoxathion), and 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether (etofenprox), were used as the target pesticides. High-purity standards of the target pesticides were obtained from Wako Pure Chemical Industries (Osaka, Japan), except for thiobencarb that was obtained from Kanto Chemical (Tokyo, Japan). High-purity standards of diazinon-d_{10}, fenitrothion-d_6, chlorpyrifos-d_{10}, isoprothiolane-d_4, isoxathion-d_{10}, and etofenprox-d_5 were obtained from Hayashi Pure Chemical Ind. (Osaka, Japan). High-purity standards of simazine-d_{10}, malathion-d_6, and thiobencarb-d_{10} were obtained from CDN Isotopes Inc. (Pointe-Claire, Canada). 2-Chloro-2′,6′-diethyl-*N*-(methoxymethyl) acetanilide (alachlor), which was used as a syringe spike, was obtained from GL Sciences (Tokyo, Japan). Acetonitrile, acetone, toluene, and anhydrous sodium sulfate, all of which were of Pesticide Residue and PCB Analysis grade, were obtained from Kanto Chemical. Reagent grade sodium chloride, dipotassium hydrogenphosphate, and potassium dihydrogen phosphate were also obtained from Kanto Chemical. The water used for sample preparation was prepared with a Millipore (San Jose, CA) Milli-Q Gradient system at an output of 18.2 MΩ cm.

Corn paste, green soybean paste, carrot paste, and pumpkin paste (used as the base materials) were kindly supplied by Hatano Research Institute, Food and Drug Safety Center (Hadano, Japan). These pastes were (or are intended to be) used for preparing PT samples in the External Quality Control for Food Hygiene [28].

2.2. Preparation of spike solution, syringe-spike solution, and calibration solutions

All solutions described below were prepared gravimetrically. High-purity standards of the target pesticides were individually dissolved with acetone. The pesticide mixture solution (62.5 mg/kg each) was then prepared by combining these solutions with addition of acetone. The high-purity standards of the isotope-labeled pesticides were also individually dissolved with acetone, and the isotope-labeled pesticide mixture solution (6.25 mg/kg each) was subsequently prepared by combining these solutions along with acetone. By combining the pesticide mixture solution and the isotope-labeled pesticide mixture solution, the spike solution was prepared so that the concentration of each target pesticide and isotope-labeled pesticide was 5.68 mg/kg. The syringe-spike solution (0.238 mg/kg) was prepared by dissolving alachlor in acetone. The calibration solution (Cal A) was then prepared by mixing the spike solution with the syringe-spike solution. The concentration of each target pesticide and isotope-labeled pesticide in this solution was 1.24 mg/kg, whereas the concentration of alachlor was 0.186 mg/kg. The other calibration solutions (Cals B1, B2, B3, B4, B5, and B6) were prepared by combining the pesticide mixture solution, the isotope-labeled pesticide mixture solution, and acetone. The concentration of the isotope-labeled pesticides in these solutions was constant at approximately 1.25 mg/kg, whereas that of the individual target pesticides ranged from 0.25 to 6.25 mg/kg.

2.3. Pretreatment of the sample

The pretreatment protocol applied in the present study is based on the Multiresidue Method for Agricultural Chemicals by GC/MS (Agricultural Products), which is a part of the Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food [2], and was partially modified. An outline of the employed protocol is as follows: the base material (5 g) was weighed, and the weighed spike solution (550 μL) was added to it. For corn paste or green soybean paste samples, water (20 mL) was added, and the sample was allowed to stand for 15 min. The sample was then homogenized with acetonitrile (50 mL) for 2 min using a Kinematica (Lucerne, Switzerland) Polytron PT 1200E homogenizer equipped with a PT-DA 12/2EC-E157 dispersing aggregate, and filtered with a cellulose filter (diameter: 60 mm; retentive particle size: 1 μm) obtained from Kiriyama Glass Works (Tokyo, Japan). The residue on the filter was re-extracted with acetonitrile (20 mL) for 2 min and the pesticide-containing filtrates were combined. An approximately 40 mL aliquot of the crude extract was fractionated in a separatory funnel (the ratio of the fractionated portion was obtained gravimetrically), and shaken with sodium chloride (10 g) and 0.5 mol/L phosphate buffer solution (pH 7.0, 20 mL) for 10 min.

In the case of carrot paste or pumpkin paste samples, the obtained upper (acetonitrile) layer was dehydrated with anhydrous sodium sulfate (approximately 10 g). The extract was concentrated and dried using a rotary evaporator and a nitrogen gas stream, respectively; an acetonitrile/toluene mixture (3:1, *v*/*v*; 2 mL) was then added.

In the case of corn paste or green soybean paste samples, a series of the obtained upper (acetonitrile) layer and acetonitrile (2 mL)