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Compensation of matrix effects in gas chromatography–mass spectrometry analysis of pesticides using a combination of matrix matching and multiple isotopically labeled internal standards

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ABSTRACT

In the multi-residue analysis of pesticides using GC–MS, the quantitative results are adversely affected by a phenomenon known as the matrix effect. Although the use of matrix-matched standards is considered to be one of the most practical solutions to this problem, complete removal of the matrix effect is difficult in complex food matrices owing to their inconsistency. As a result, residual matrix effects can introduce analytical errors. To compensate for residual matrix effects, we have developed a novel method that employs multiple isotopically labeled internal standards (ILIS). The matrix effects of ILIS and pesticides were evaluated in spiked matrix extracts of various agricultural commodities, and the obtained data were subjected to simple statistical analysis. Based on the similarities between the patterns of variation in the analytical response, a total of 32 isotopically labeled compounds were assigned to 338 pesticides as internal standards. It was found that by utilizing multiple ILIS, residual matrix effects could be effectively compensated. The developed method exhibited superior quantitative performance compared with the common single-internal-standard method. The proposed method is more feasible for regulatory purposes than that using only predetermined correction factors and is considered to be promising for practical applications.

1. Introduction

Monitoring residual pesticides in foodstuffs is essential not only for the protection of consumer health but also for the promotion of confidence in international and national food trading systems. Proper evaluation of the compliance of food with the maximum residue limits supports the legal framework needed for an efficient control of food safety and an effective implementation of fair trade practices. This goal relies on the ability of the analytical community to establish accurate and reliable methods for pesticide residue analysis that satisfy the requirements of national authorities. Considering the large number of possible pesticide residues in foodstuffs, the development of multi-residue methods is necessary for efficient monitoring.

Currently, the most widely used analytical techniques for multi-residue analysis of pesticides in modern analytical laboratories are gas and liquid chromatography coupled to mass spectrometry (GC–MS and LC–MS, respectively). These powerful tools have enabled the detection of hundreds of pesticides at trace levels in complicated food matrices. Since their introduction in the field of pesticide analysis, the GC–MS and LC–MS analytical methods in combination with various extraction and cleanup procedures have been intensively studied in many laboratories worldwide [1–6].

Although GC–MS and LC–MS analyses are widely accepted as being sufficiently reliable for regulatory purposes, it is known that their quantitative performance is often vulnerable to a phenomenon known as the "matrix effect" [7,8]. In GC–MS analysis, matrix effects are related to the blocking of active sites by co-extracted matrix components during the transport of analytes from the injector to the detector [9,10]. The reduction in the number of active sites and fewer losses of analytes in the GC system lead to increased analyte signals in the presence of the matrix relative to standards in solvents, thus resulting in inaccurate quantifications. The degree of matrix effect can be influenced by a variety of factors, which include the analyte concentration, the chemical properties of the analyte, the concentration of the matrix in sample extracts, and the operating conditions of the GC–MS system [11–14].

To date, numerous strategies have been proposed to diminish matrix effects. In the field of pesticide residue analysis, the following are often cited as means to avoid matrix effects: (1)

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the method of standard additions [15,16], (2) reduction of matrix components by extensive cleanup [17], (3) compensation of the calculated results by a predetermined correction factor [18,19], (4) use of isotopically labeled internal standards (ILIS) [20], (5) matrix-matched calibration, and (6) use of analyte protectants [21,22]. It is known that each of these strategies has its own drawbacks or limitations for application in multi-residue analysis. The implementation of (1), (2), and (3) not only involves extra cost and effort but can also introduce additional sources of analytical error. Approach (4) can effectively compensate for matrix effects, but its application is limited owing to the lack of commercial availability or prohibitive cost of the reagents required and the complexity of the analysis. At present, the matrix-matched calibration method is generally recognized as the most practical approach for compensating matrix effects. In the field of pesticide residue analysis, the matrix-matched calibration method, often combined with the use of several analyte protectants, has become a common practice in many laboratories.

The main drawback of matrix matching is the difficulty in preparing appropriate matrix extracts for the calibration standards. It is known that matrix effects can vary from sample to sample even within the same type of food [12]. Although the use of the exact same blank matrix as the sample can provide ideal matrix matching for calibration, it is not feasible in real-world routine analysis, and compromises must be made. Considering that the "residual" matrix effects are complicated and often unpredictable, the inconsistency of matrix effects among diverse samples can severely affect the quality of matrix-matched results, especially when food matrices are highly complex.

In order to remedy the imperfections of matrix-matched calibration, we developed a novel method that involves the use of multiple internal standards (IS). Although ideally the IS should have a similar structure to the target pesticide, it is known that the use of IS with matrix matching generally improves the quantitative performance in most cases, even if the IS are not isotopic labels for each target pesticide [12,23]. Considering that pesticides are very diverse in terms of chemical properties, it is reasonable to assume that the use of multiple IS can improve the performance of the method in multi-residue analysis. Nevertheless, at present, in the field of multi-residue analysis, the use of a single or a few IS is more common than the use of multiple IS. The main reason for the lack of acceptance of multiple IS is that the selection of appropriate combinations of IS and pesticides is highly complex and poses an analytical problem. The inappropriate assignment of IS can introduce additional error and may degrade the analytical performance, potentially making it even worse than the single-IS method.

In the multi-residue analysis of pesticides, several studies have used multiple ILIS to improve the quality of the GC–MS analysis [24,25]. In these studies, combinations of ILIS and pesticides are determined by investigating which patterns of variation are shared between the ILIS and the pesticide or by classifying ILIS and pesticides based on the similarity of their specific chemical properties. The limitation of these approaches is that they lack clarity and rely too much on analytical expertise. Considering that routine monitoring involves a large number of target pesticide/commodity combinations, the above approach becomes more difficult to achieve as the number of ILIS and pesticides increases.

At present, even a complex QSAR model [26] cannot correctly predict sample-to-sample or day-to-day variations of matrix effect in real world analysis. Therefore, in this paper, we present a new method that utilizes information obtained by the GC–MS analysis of spiked pesticides in various agricultural products. In our method, the use of ILIS of pesticides, not the mere use of multiple IS, is necessary to judge the quality of the obtained data. By applying simple statistical methods, the appropriate combinations of IS and pesticides are determined from the obtained data, and the difficult manual selection of combinations of IS and pesticides can be avoided. The quantitative performance of our method is compared with that of the common single-IS method, and the advantages, limitations, and possible applications of our method are discussed.

2. Materials and methods

2.1. Chemicals and materials

Food samples were collected from local markets, and all samples were verified to be free of the pesticides of interest. Pesticide-residue-grade acetonitrile (MeCN) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Pesticide standards (Table 1) were purchased from Hayashi Pure Chemical Industries (Osaka, Japan), Wako Pure Chemical Industries, Chem Service Inc. (West Chester, USA), Fluka (Buchs, Switzerland), and Riedel-de-Haën (Seelze, Germany). High-purity ILIS (Table 1) were purchased from Hayashi Pure Chemical Industries, Dr. Ehrenstorfer (Ausburg, Germany), C/D/N Isotopes (Pointe-Claire, Canada) and Fluka. A composite mixture of all of the pesticides (1 μg/mL) and a composite mixture of all of the ILIS (2.5 μg/mL) were prepared in MeCN. The working standard solutions containing 0.02, 0.05, 0.1, 0.2, and 0.4 μg/mL of pesticides and the working solution of ILIS with a concentration of 0.25 μg/mL were freshly prepared by diluting the composite mixture solutions with MeCN.

Polypropylene centrifuge tubes, 50 and 15 mL in volume for the initial extraction and dispersive solid-phase extraction steps, respectively, were purchased from Restek (Pennsylvania, USA). Reagent grade sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were purchased from Wako Pure Chemical Industries. Primary and secondary amine (PSA) sorbent, C18 sorbent, and graphitized carbon black (GCB) sorbent were purchased from Agilent Technologies (Folsom, USA). 3-Ethoxy-1,2-propanediol, l-gulonic acid γ-lactone, d-sorbitol, and shikimic acid (all ≥95% purity) were purchased from Sigma–Aldrich and employed as analyte protectants [21]. The analyte protectant stock solution was prepared in 0.5% formic acid in 4/1 (v/v) MeCN/water with the following concentrations of protectants: 10 mg/L ethylglycerol, 1 mg/L l-gulonic acid γ-lactone, 1 mg/L d-sorbitol and 0.5 mg/L shikimic acid.

2.2. Preparation of matrix extracts

About 500 g of food samples was finely ground using an MX-V100 mill (National, Osaka, Japan), except for peanuts, cashew nuts, and almonds, which were comminuted using a Grindomix GM 200 mill (Retch, Haan, Germany) after being frozen and mixed with dry ice. The comminuted samples were stored at −30 °C until use. The sample preparation method used in this study is based on a modified version of QuEChERS [3,12], which is optimized for cereal grain samples and fattier types of food. The blank matrix extracts were prepared by the following procedure: 2.5 g of homogenized sample was weighed into a 50 mL centrifuge tube to which 10 mL of water and 10 mL of MeCN were then added. The tube was capped tightly, vortexed for a few seconds to fully disperse the sample in the solvent, and then shaken for 60 min on a bioshaker (BR-21UM, TAITEC Co, Saitama, Japan). Next, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl was added, and the tube was immediately hand shaken for 1 min. The tube was then centrifuged at 5000 rpm for 10 min. Subsequently, a 4 mL aliquot of the upper layer was transferred to a 15 mL centrifuge tube containing 0.6 g of PSA, 0.2 g of C18, 0.03 g of GCB, and 0.6 g of anhydrous $MgSO_4$. The tube was vortexed for 0.5 min and then centrifuged at 12000 rpm for 5 min. A 200 μL aliquot of the supernatant was transferred to a glass tube to which 17.5 μL of the analyte protectant solution, 20 μL of the ILIS solution, and 12.5 μL