

Combination of Analyte Protectants To Overcome Matrix Effects in Routine GC Analysis of Pesticide Residues in Food Matrixes

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Analyte protectants were previously defined as compounds that strongly interact with active sites in the gas chromatographic (GC) system, thus decreasing degradation, adsorption, or both of coinjected analytes. In this study, we evaluated various combinations of promising analyte protectants for the volatility range of GC-amenable pesticides using GC/quadrupole mass spectrometry (MS) and 1- μ L hot splitless injection for sample introduction. A mixture of ethylglycerol, gulonolactone, and sorbitol (at 10, 1, and 1 mg/mL, respectively, in the injected samples) was found to be the most effective in minimizing losses of susceptible analytes and significantly improving their peak shapes (due to reduction of peak tailing). When added to final sample extracts and matrix-free calibration standards alike, these analyte protectants induced a similar response enhancement in both instances, resulting in effective equalization of the matrix-induced response enhancement effect even after a large number of fruit and vegetable extract injections. As compared to matrix-matched standardization, the analyte protectant approach offers a more convenient solution to the problems associated with calibration in routine GC/MS analysis of pesticide residues and possibly other susceptible analyte types in diverse samples. Moreover, the use of analyte protectants also substantially reduced another adverse matrix-related effect caused by gradual build-up of nonvolatile matrix components in the GC system, thus improving ruggedness and, consequently, reducing need for frequent maintenance.

In real-world trace gas chromatographic (GC) analysis, such as the analysis of pesticide residues in foods, coextracted matrix components may cause serious problems including inaccurate quantitation, decreased method ruggedness, low analyte detectability, and even reporting of false positive or negative results.¹ Matrix-induced response enhancement, first described by Erney

et al.,² is presumably the most discussed matrix effect negatively impacting quantitation accuracy of certain analytes in GC.¹ When a real sample is injected, the matrix components tend to block active sites (mainly free silanol groups) in the GC inlet and column, thus reducing losses of susceptible analytes caused by adsorption or degradation on these active sites. This phenomenon results in higher analyte signals in matrix-containing versus matrix-free solutions, thus precluding the convenient use of calibration standards in solvent only, which would lead to overestimations of the calculated concentrations in the analyzed samples.

Another potential problem associated with matrix injections involves gradual accumulation of nonvolatile matrix components in the GC system, resulting in formation of new active sites and gradual decrease in analyte responses. This effect, sometimes called matrix-induced diminishment,³ negatively impacts ruggedness (i.e., long-term repeatability of analyte peak intensities, shapes, and retention times), which is a highly important factor in routine GC analysis.⁴

In theory, elimination of active sites or matrix components would overcome the matrix-induced enhancement effect; however, absolute and permanent GC system deactivation or thorough sample cleanup is virtually impossible in practice.⁵ Careful optimization of injection and separation parameters (such as the injection technique, temperature, and volume; liner size and design; solvent expansion volume; column flow rate; column dimensions) can lower the number of active sites (due to a decreased surface area) or shorten the analyte interactions with them. This results in a reduction but rarely complete elimination of the effect. For instance, application of a pressure pulse or temperature programming during the injection (to reduce residence time or thermal degradation in the injection port) may serve as examples of this effort.^{6–9}

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Since an effective elimination of the sources of the matrix-induced response enhancement is not likely in practice, the analysts often try (or are required) to compensate for the effect using alternative calibration methods. The current compensation approaches include use of the following: (i) matrix-matched standards, (ii) standard addition method, and (iii) isotopically labeled internal standards (not feasible in multiresidue pesticide analysis due to their unavailability or prohibitive price). All of these techniques require extra labor and costs; moreover, they may still lead to quantitation inaccuracies because the extent of the effect depends on analyte concentration and matrix composition¹⁰ (problems in the case of standard addition and matrix-matching, respectively).

Matrix-matched standardization is thus far the most widely used approach despite its imperfections including a rather time-consuming and laborious preparation of matrix-matched standards and need for an appropriate blank material (ideally the same as the analyzed samples). The matrix-matching procedure becomes especially onerous when different commodity types are to be analyzed in one batch of samples, which is often the case in routine practice. For regulatory enforcement of pesticide residue limits in foods, the guidelines for residue monitoring in the European Union (EU) require the use of matrix-matched standards (or an alternative approach that provides equivalent or superior accuracy) for analytes affected by matrix effects.¹¹ In contrast, the federal regulatory agencies in the United States, the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA), do not permit matrix-matched standardization for the enforcement purposes.

All the above problems associated with the current compensation approaches call for a more convenient, effective, and widely acceptable solution. Erney and Poole first came up with an idea of compound additives ("masking reagents") that would prevent/minimize pesticide interactions with the active sites in the GC system.¹² However, they only studied eight different compounds and none of them provided a desirable effect. In an extensive study, we revisited this approach and evaluated the application of more than 90 compounds from different chemical groups.¹³ Our objective was to find compounds that would strongly interact with the active sites, thus providing strong enhancement of analyte responses (without undesired side effects, such as analyte degradation or GC system deterioration). We termed these compounds "analyte protectants" because they protect coinjected analytes against degradation, adsorption or both in the GC system. The concept idea is to add suitable analyte protectants to sample extracts as well as matrix-free (solvent) standards to induce an even response enhancement in both instances, resulting in effective equalization of the matrix-induced response enhancement effect.

In general, hydrogen-bonding capability and volatility (retention time coverage) of the analyte protectant were found to be the most

important factors in the enhancement effect. From the tested compounds, L-gulonic acid γ -lactone (gulonolactone) provided the highest overall enhancement, which can be explained by formation of several broad peaks of gulonolactone and its degradation products covering a very wide pesticide elution range. For that reason, gulonolactone can serve as a very effective single-compound additive to generally improve responses (peak shapes and intensities) of analytes, mainly those eluting in the middle region of the chromatogram. However, to effectively compensate for matrix-induced response enhancement in the GC multiresidue pesticide analysis, the used analyte protectants should induce very strong response enhancement throughout the *entire* range of analytes. Therefore, a suitable combination of several analyte protectants, covering the entire volatility range of GC-amenable pesticides, is more likely to meet the analytical need.

Thus, in this follow-up study, we evaluated various combinations of analyte protectants previously identified as promising for early-, middle-, and late-eluting analytes. Our objective was to find a combination that would effectively compensate for matrix-induced response enhancement in GC pesticide residue analysis in food matrixes. Furthermore, we wanted to study ruggedness of the approach to assess its applicability to routine real-world analysis.

EXPERIMENTAL SECTION

Chemicals and Materials. Pesticide reference standards, all 95% or higher purity, were obtained from the National Pesticide Standard Repository of the U.S. EPA (Fort Meade, MD), Dr. Ehrenstorfer GmbH (Augsburg, Germany), Ultra Scientific (North Kingstown, RI), and Chemservice (West Chester, PA). Stock solutions of 2000 $\mu\text{g}/\text{mL}$ for each pesticide and a 50 $\mu\text{g}/\text{mL}$ composite standard solution of 30 pesticides (see Table 1 for their list) were prepared in acetonitrile (MeCN) with addition of acetic acid (HAc) at 0.1% (v/v) to prevent degradation of certain base-sensitive analytes in MeCN.¹⁴ A composite internal standard (ISTD) stock solution (50 $\mu\text{g}/\text{mL}$) of heptachlor and triphenyl phosphate (TPP) was also prepared in MeCN containing 0.1% HAc. The 30 pesticides were chosen carefully based on their diverse properties (e.g., volatility, thermolability, polarity, pH sensitivity) and quality of their GC analysis, including those prone to degradation, adsorption, or both in the GC system and also nonproblematic pesticides for comparison purposes.

Table 2 lists compounds evaluated as analyte protectants in this study; all of them were 95% or better purity obtained from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI), Fisher Scientific (Fair Lawn, NJ), and Fluka (Buchs, Germany). Stock solutions of ethylglycerol (100 mg/mL) and poly(ethylene glycol) (10 mg/mL) were prepared in MeCN. Sorbitol (10 mg/mL), *meso*-erythritol (8.5 mg/mL), and triglycerol (10 mg/mL) were dissolved in 85:15 (v/v) MeCN/water; gulonolactone (20 mg/mL), caffeine (10 mg/mL), and 4,6-*O*-ethylidene- α -D-glucopyranose (10 mg/mL) in 80:20 (v/v) MeCN/water.

MeCN was a high purity grade solvent for pesticide residue analysis from Burdick & Jackson (Muckegon, MI), and glacial HAc was HPLC grade from Fisher Scientific. Ultrapure water was prepared by a Barnstead (Dubuque, IA) water purification system. Anhydrous MgSO_4 and NaCl were obtained from Aldrich and

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Table 1. List of Pesticides, Their Retention Times, and SIM Ions Used for Quantitation

pesticide	retention time (min)	quantitation ion (<i>m/z</i>)
dichlorvos	5.60	185
methamidophos	5.83	141
(<i>Z</i>)-mevinphos	6.94	192
(<i>E</i>)-mevinphos	6.99	192
acephate	7.22	136
<i>o</i> -phenylphenol	7.81	170
omethoate	8.66	156
dimethoate	10.07	93
diazinon	10.33	304
lindane	10.64	219
vinclozolin	11.90	285
heptachlor (ISTD)	12.21	272
metalaxyl	12.28	206
carbaryl	12.41	144
pirimiphos-methyl	12.43	290
chlorothalonil	12.46	266
methiocarb	12.87	168
dichlofluanid	12.90	224
chlorpyrifos	13.21	197
fenthion	13.28	278
cyprodinil	14.31	224
captan	15.16	79
thiabendazole	15.29	201
folpet	15.40	260
imazalil	16.42	215
TPP (ISTD)	18.47	326
<i>p,p'</i> -dicofol	18.56	251
endosulfan sulfate	18.58	272
<i>o,p'</i> -dicofol	19.26	251
phosalone	19.73	182
azinphos-methyl	19.78	132
<i>cis</i> -permethrin	20.32	183
<i>trans</i> -permethrin	20.45	183
coumaphos	20.88	362
deltamethrin	23.81	181

Table 2. Compounds Evaluated as Analyte Protectants and Approximate Pesticide Elution Regions for Which They Are the Most Effective

Compound	CAS No.	pesticide elution region (min)
3-ethoxy-1,2-propanediol (ethylglycerol)	1874-62-0	<10
1,2,3,4-butanetetrol (<i>meso</i> -erythritol)	149-32-6	<10
4,6- <i>O</i> -ethylidene- α -D-glucopyranose	13224-99-2	10-13
caffeine	58-08-2	12-13
L-gulonic acid γ -lactone (gulonolactone)	1128-23-0	8-15
poly(ethylene glycol) (PEG) 300	25322-68-3	8-20 ^a
triglycerol	20411-31-8	15-20
D-sorbitol (D-glucitol)	50-70-4	14-20

^a Scattered coverage.

primary secondary amine (PSA) sorbent was from Varian (Harbor City, CA). The MgSO₄ was heated for 5 h at 500 °C in a muffle furnace to remove moisture and phthalates. Fruit and vegetable samples were obtained from local organic food stores.

Preparation of Analyte Protectant Solutions for Evaluation Experiments. Individual analyte protectant solutions at 0.5, 1, 2.5, and 5 mg/mL were prepared in MeCN with 0.1% HAC containing 500 ng/mL concentrations of the 30 pesticides and 1000 ng/mL concentrations of the two ISTDs. In the case of ethylglycerol, concentration levels of 10 and 20 mg/mL were also

tested. For evaluations of analyte protectant mixtures, various compositions and concentrations of individual components were investigated. The idea was to cover the entire volatility range of GC-amenable pesticides; thus, analyte protectants effective for early-eluting analytes (ethylglycerol at 10 mg/mL or *meso*-erythritol at 1 or 2.5 mg/mL) were systematically combined with analyte protectants effective for middle- and late-eluting analytes (added at 0.5–2 mg/mL). Examples of promising analyte protectant combinations included the following: (1) *meso*-erythritol, gulonolactone, caffeine, and PEG (all at 1 mg/mL); (2) ethylglycerol, gulonolactone, and PEG (10, 1, and 2 mg/mL, respectively); and mainly (3) ethylglycerol, gulonolactone, and sorbitol (10, 1, and 1 mg/mL, respectively).

Preparation of Matrix Extracts. Matrix extracts were prepared by the QuEChERS method,¹⁵ according to which 10 g of a homogenized sample was extracted with 10 mL of MeCN in a 40-mL fluorinated ethylene propylene centrifuge tube for 1 min using a vortex mixer. Then, 4 g of anhydrous MgSO₄ and 1 g of NaCl were added, and the tube was vortexed immediately for 1 min and then centrifuged for 3 min at 5000 rpm (3450 rcf) using a Sorvall RT6000B centrifuge (Newtown, CT). For a mixed fruit extract, the composite sample was obtained by mixing together equal amounts of homogenized apple, green grape, peach, and plum blank matrixes (2.5 g each for 10-g sample). In the case of a mixed vegetable extract, equal amounts of cauliflower, cherry tomato, cucumber, and green pepper homogenized blank samples were used. Dispersive solid-phase extraction was employed for the extract cleanup using 25 mg of PSA and 150 mg of MgSO₄ per 1 mL of the extract (upper MeCN layer). The mixture was vortexed in a centrifuge tube for 30 s and then centrifuged for 1 min at 5000 rpm.

For the purpose of this study, the final extract was evaporated down to 50% using a Zymark Turbovap LV evaporator (Hopkinton, MA). Then analyte protectant, pesticide, and ISTD solutions were added, and the sample was diluted by an appropriate amount of MeCN to reconstitute the original concentration of matrix components in the final extract. This procedure was convenient in this study because we were exploring different combinations and amounts of various analyte protectants. For routine use, however, a small volume of a composite solution of analyte protectants (the optimal mixture) can be simply added to the final extracts and solvent standards without the need for evaporation and reconstitution steps.¹⁶

For the long-term performance study described below, two sets of pesticide solutions at 0, 10, 25, 50, 100, 225, and 500 ng/mL were prepared in MeCN and in mixed fruit and vegetable extracts (pesticide concentrations in ng/mL correspond to ng/g of the original sample). All solutions contained 1000 ng/mL heptachlor and TPP (ISTDs) and 0.1% HAC to prevent degradation of certain pesticides. Analyte protectants (ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL, respectively) were added to one of the duplicate set of samples. For example, the preparation of 500 ng/mL pesticide solution in fruit extract with analyte protectants involved evaporation of 1 mL of the cleaned extract down to 500 μ L, to which the following solutions were added: (1) 100

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μL of 5 $\mu\text{g}/\text{mL}$ working solution of 30 pesticides in MeCN; (2) 20 μL of 50 $\mu\text{g}/\text{mL}$ ISTD solution; (3) 10 μL of 10% HAC in MeCN; (4) 100 μL of ethylglycerol (100 mg/mL) in MeCN; (5) 50 μL of gulonolactone (20 mg/mL) in 80:20 (v/v) MeCN/water; (6) 100 μL of sorbitol (10 mg/mL) in 85:15 (v/v) MeCN/water; and (7) 120 μL of MeCN. For preparation of samples without analyte protectants, ethylglycerol, gulonolactone, and sorbitol solutions were substituted by 250 μL of MeCN. For pesticide solutions in solvent only, MeCN was used instead of matrix extracts.

GC/MS Conditions. The GC/MS analyses were conducted with a Hewlett-Packard (Agilent; Little Falls, DE) model 5890 Series II Plus GC coupled to a 5972 mass-selective detector. The system was equipped with a split/splitless injection inlet, an electronic pressure control, and a 7673A autosampler; Chemstation software was used for instrument control and data analysis. DB-5ms, DB-XLB, and DB-35ms (Agilent; Folsom, CA), and RTX-5ms SIL (Restek, Bellefonte, PA) capillary columns (all typically used in GC/MS pesticide residue analysis) of 30 m, 0.25-mm i.d., 0.25- μm film thickness were employed throughout the study. The chromatographic conditions were the following: He constant flow of 1 mL/min, inlet temperature 250 $^{\circ}\text{C}$, injection volume 1 μL (splitless), MS transfer line temperature 290 $^{\circ}\text{C}$, temperature program of 80 $^{\circ}\text{C}$ for 1.5 min, then a 20 $^{\circ}\text{C}/\text{min}$ ramp to 180 $^{\circ}\text{C}$ followed by a 5 $^{\circ}\text{C}/\text{min}$ ramp to 230 $^{\circ}\text{C}$, and a 25 $^{\circ}\text{C}/\text{min}$ ramp to 290 $^{\circ}\text{C}$ (held for 10 min). After each injection, the syringe was washed several times with an acetone/water (1:1, v/v) mixture (followed by MeCN) to carefully remove rather polar analyte protectants and prevent the plunger from sticking. The quadrupole was operated in selected ion monitoring (SIM) mode detecting 2–3 ions for each analyte (note: the use of analyte protectants in an ion trap or time-of-flight MS instruments could be problematic.). Table 1 gives quantitation ions and typical retention times (t_{R}) for the pesticides on the DB-XLB column, which was used in the long-term performance study described below.

Long-Term Performance Study: Sequence of Samples. To evaluate long-term effects of the use of analyte protectants in GC/MS analysis, the pesticide solutions in MeCN and matrix extracts containing the optimal mixture of analyte protectants (ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL, respectively) were repetitively analyzed in five sets of sample sequences, between which no GC system maintenance was performed (also, the column was extensively used in the analysis of pesticide residues in food samples and analyte protectant evaluations prior to this long-term performance study). Test injections of 500 ng/mL pesticide solution in MeCN without analyte protectants were also made throughout each sequence. The order of the injections in the sequences was as follows: (1) test injection, (2–7) solvent standards with analyte protectants (pesticides at 500, 225, 100, 50, 25, and 10 ng/mL in MeCN), (8) test injection, (9–15) fruit extracts with analyte protectants (pesticides at 500, 225, 100, 50, 25, 10, and 0 ng/mL in mixed fruit extract), (16) test injection, (17–22) solvent standards with analyte protectants (pesticides at 500, 225, 100, 50, 25, and 10 ng/mL in MeCN), (23) test injection, (24–30) vegetable extracts with analyte protectants (pesticides at 500, 225, 100, 50, 25, 10, and 0 ng/mL in mixed vegetable extracts). After the fifth set (150 samples), another test injection and series of solvent standards with analyte protectants were analyzed. Thus, altogether 157

samples were injected, including 136 samples with analyte protectants and 70 matrix extracts. Additionally, similar sequences of solvent standards and matrix extracts without the addition of analyte protectants were analyzed before and after the series of 157 samples for comparison purposes.

RESULTS AND DISCUSSION

Evaluation of Different Combinations of Analyte Protectants. As mentioned in the introduction, we previously evaluated more than 90 different potential analyte protectants to find the most effective ones for improving peak shapes and intensities of selected analytes representing the volatility range of GC-amenable pesticides (from dichlorvos to deltamethrin). The evaluations were based on comparing peak heights of susceptible pesticides injected in the tested single analyte protectant solutions in MeCN (with 0–25% water depending on the analyte protectant solubility in MeCN at the tested concentration level of 1 mg/mL) versus their peak heights obtained in MeCN only. Peak heights (normalized to pesticides unaffected by matrix effects) were selected as the best indication of the response enhancement because they reflect results of two effects: (1) increased analyte transfer to the detector due to reduced analyte degradation or irreversible retention in both the inlet and column and (2) decreased analyte peak tailing and broadening due to reduced interactions of analytes with the active sites mainly in the column.

A ranking system (values between 1 and 4) was devised to compare the enhancement effect for each susceptible pesticide caused by the compounds screened as potential analyte protectants. A ranking of 4 meant that the compound (relatively compared to the others) induced a very strong signal enhancement for a particular analyte, whereas a value of 1 indicated that there was no significant response enhancement versus injection in MeCN only. Rankings of 2 and 3 were assigned based on proportional splitting the enhancement extent difference between the 1 and 4 scores. This system allowed us to identify prospective analyte protectants and also correlate the enhancement effect for particular pesticides to elution profiles (volatility) of the protecting agents. On the basis of the summation of individual pesticide rankings, gulonolactone provided the highest overall enhancement, being most effective for analytes eluting in the middle region of the chromatogram.¹³

In this study, we evaluated various combinations of analyte protectants previously identified as promising for early-, middle-, and late-eluting analytes. Table 2 lists the tested compounds and indicates pesticide elution regions, in which these analyte protectants induce strong response enhancement (rankings 4 or 3 obtained in the previous study). Our basic idea was to compose a mixture that would cause as high as possible enhancement effects for all susceptible pesticides without distorting analyte peaks. At the same time, the concentration (coinjecting amount) of each analyte protectant and water content (added to MeCN to improve solubility of certain analyte protectants) in the injected sample should be as low as possible. As described in the Experimental Section, we varied mixture composition and concentration of individual components and in each case compared peak heights of susceptible analytes versus injections in MeCN using a similar ranking system as devised in the previous study.

Gulonolactone was demonstrated to be an invaluable analyte protectant, but as mentioned above, it needed additional help. Two

compounds were tested to increase peak heights for the early eluting analytes: 1,2,3,4-butanetetrol (*meso*-erythritol) and 3-ethoxy-1,2-propanediol (ethylglycerol). Sugars (pentoses and hexoses) can also serve as excellent protecting agents for early (to middle)-eluting pesticides,¹³ but we preferred to avoid their injections in routine analysis and chose to use their derivatives (mainly lactones and alcohols). *meso*-Erythritol is a highly effective analyte protectant for methamidophos even at relatively low concentrations of 0.5–1 mg/mL (with 1–2% water in MeCN), but other early-eluting (and also highly susceptible) analytes acephate and *o*-phenylphenol required much higher concentrations, resulting in a higher water content and consequently distortion of early-eluting peaks. Ethylglycerol, on the other hand, is readily soluble in MeCN and, thus, can be used at a concentration of (up to) 10 mg/mL, which induces strong signal enhancements of early-eluting susceptible analytes yet without peak distortions. The relatively high volatility of ethylglycerol decreases risk of GC system contamination potentially associated with injecting of such a high concentration in routine practice.

In the case of less volatile (later eluting) analyte protectants, we tried to keep the concentration as low as possible, sacrificing unsubstantial signal improvements. For instance, a gulonolactone concentration of 1 mg/mL was found to be optimal for most of the analytes because increasing its concentration to 2.5 or 5 mg/mL did not provide significant additional response enhancement for the majority of the susceptible pesticides tested in this study (note: a highly deteriorated column may require increased analyte protectant concentrations,¹³ but such a column would unlikely be used in pesticide residue analysis in routine practice). Similarly, no substantial effect was observed when caffeine or 4,6-*O*-ethylidene- α -D-glucopyranose (compounds effective as single-analyte protectants for middle-eluting pesticides) were used in combination with 1 mg/mL gulonolactone.

In the case of PEG, the GC injection resulted in formation of several peaks with a wide range of retention times, but compared to gulonolactone, these peaks were much sharper, providing a less effective (more scattered) coverage.¹³ A combination of PEG with gulonolactone and ethylglycerol (at 2, 1, and 10 mg/mL, respectively, in the injected sample) was very promising, but long-term injections of PEG solutions deteriorated column performance to such an extent that even cutting a large portion from the front part of the column did not recover it. Moreover, PEG did not improve responses of the late-eluting analytes to the same degree as triglycerol and mainly sorbitol, which was previously shown to work well for late eluters during the QuEChERS method development.¹⁵

From the tested combinations and concentrations of the individual components, the best overall results were achieved using the mixture of ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL, respectively, in the injected sample (resulting in 2.5% water in MeCN solutions), which corresponds to 10, 1, and 1 μ g introduced into the GC system using a 1- μ L splitless injection. Figure 1 schematically shows regions of influence of each component of this mixture on signals of susceptible analytes throughout the volatility range of the GC-amenable pesticides.

Evaluation of the Optimal Analyte Protectant Combination in a Long-Term Performance Study. To evaluate the effectiveness and long-term influences of the ethylglycerol, gulonolactone,

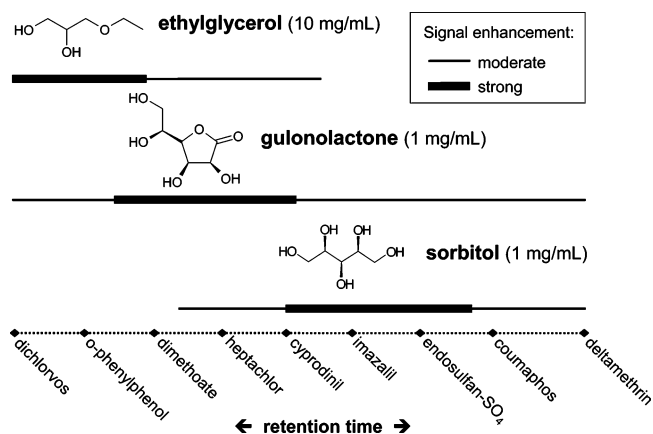


Figure 1. Schematic illustration of the effect of the optimal combination of analyte protectants (ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL, respectively, in the injected pesticide solutions in acetonitrile) on the signal enhancement of susceptible analytes throughout the elution range of GC-amenable pesticides.

and sorbitol mixture application in routine pesticide GC/MS analysis, the pesticide solutions in MeCN and matrix extracts containing this combination of analyte protectants were repetitively analyzed (together with test solutions without analyte protectants) as described in the Experimental Section. In total, 157 injections were made including 136 samples with analyte protectants and 11 test solutions. For comparison purposes, series of pesticide solutions in MeCN and matrix extracts *without* analyte protectants were also injected at the beginning and at the end of this relatively long sequence of samples. The main objective of this study was to determine how effectively the given analyte protectant mixture compensates for matrix-induced response enhancement in a real-world situation.

Generally, the matrix-induced response enhancement should be investigated when the response in matrix versus matrix-free standard exceeds the upper limit of the mean recovery requirement for quantitative methods. The EU criteria for the pesticide residue analysis require mean recovery within the range of 70–120% for the pesticide concentration range of 10–100 ng/g and 70–110% mean recoveries for concentrations of > 100 ng/g.¹¹ For our discussion, we decided to use response in solvent \pm 15% (corresponding to 0.85–1.15 matrix vs solvent response factors) as tolerance limits indicating no significant difference between analyte responses in solvent and matrix. The \pm 15% limit was set as a compromise, being more stringent than the recovery requirement for lower pesticide levels (\leq 100 ng/g), at which the extent of the enhancement effect is known to be larger as compared to higher analyte concentrations.¹⁰

Figure 2 compares peak shapes and intensities of three selected pesticides obtained in solvent standards and fruit extracts spiked at 100 ng/mL (corresponding to 100 ng/g sample) and analyzed without and with analyte protectants at the beginning of the sequence. The figure clearly demonstrates the beneficial effect of the analyte protectant addition, resulting in similar analyte responses in solvent and matrix solutions and reduced analyte tailing. The three pesticides were selected to represent different analyte susceptibility to matrix-induced response enhancement: lindane usually not susceptible, phosalone moderately susceptible, and *o*-phenylphenol very susceptible to the effect as indicated by the signal (peak height) enhancement factors in the figure.

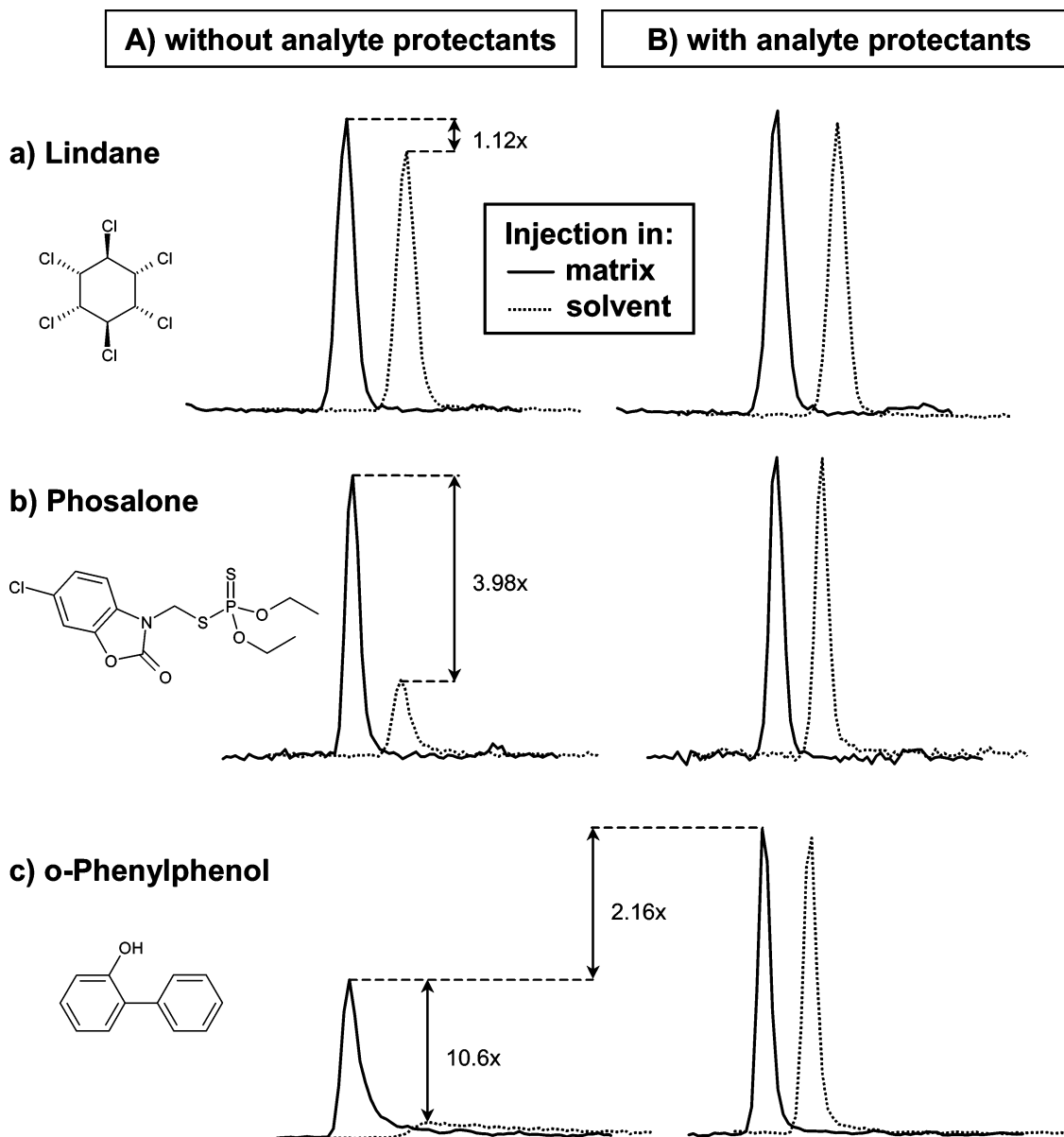


Figure 2. Comparison of peak shapes and intensities of 100 ng/mL lindane (m/z 219), phosalone (m/z 182), and *o*-phenylphenol (m/z 170) obtained by injection in matrix (mixed fruit extract) and solvent (acetonitrile) solutions (A) without and (B) with the addition of analyte protectants (ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL in the injected sample, respectively) at the beginning of the sequence described in the Experimental Section. The numbers demonstrate signal (peak height) enhancement factors (signal in matrix vs solvent) obtained without the use of analyte protectants and improvement in *o*-phenylphenol signal intensity in matrix with the use of analyte protectants.

Phosalone is an example of an analyte prone mainly to losses in the injection port, whereas *o*-phenylphenol is primarily notorious for tailing on columns typically used in the GC multiclass, multiresidue analysis of pesticides. This can be demonstrated by comparing peak height versus area enhancement factors obtained for these compounds in the example given in Figure 2.

Without the use of analyte protectants, *o*-phenylphenol gave almost an 11-fold taller peak in matrix than in solvent solution, whereas the peak area was only ~ 2 -fold larger (when carefully and consistently integrating the peaks including their ~ 1 -min tails). In the case of phosalone, the difference between peak height and area enhancement factors was much less pronounced: about 4- versus 3-fold increase, respectively. Thus, in both examples, the presence of matrix components in the injected samples leads to both reduced losses and tailing, the degree of each depending

on functional groups in the analyte structure.¹ The addition of the ethylglycerol, gulonolactone, and sorbitol mixture equalizes the responses in matrix extracts and solvent solutions by having a similar effect as matrix components or by interacting with the active sites to an even larger extent, thus potentially lowering detection limits in both solvent and matrix extracts. For example, the peak height of *o*-phenylphenol more than doubled in fruit extract with the addition of analyte protectants. Again, this effect was caused mainly by reducing *o*-phenylphenol interactions with the active sites in the column, resulting in a sharper, significantly less tailing peak (note: the area increase was only ~ 1.2 -fold in this case).

Figure 3 demonstrates the ruggedness (long-term repeatability of analyte peak intensities, shapes, and t_R) of the approach by overlaying peaks of the three pesticides obtained in MeCN

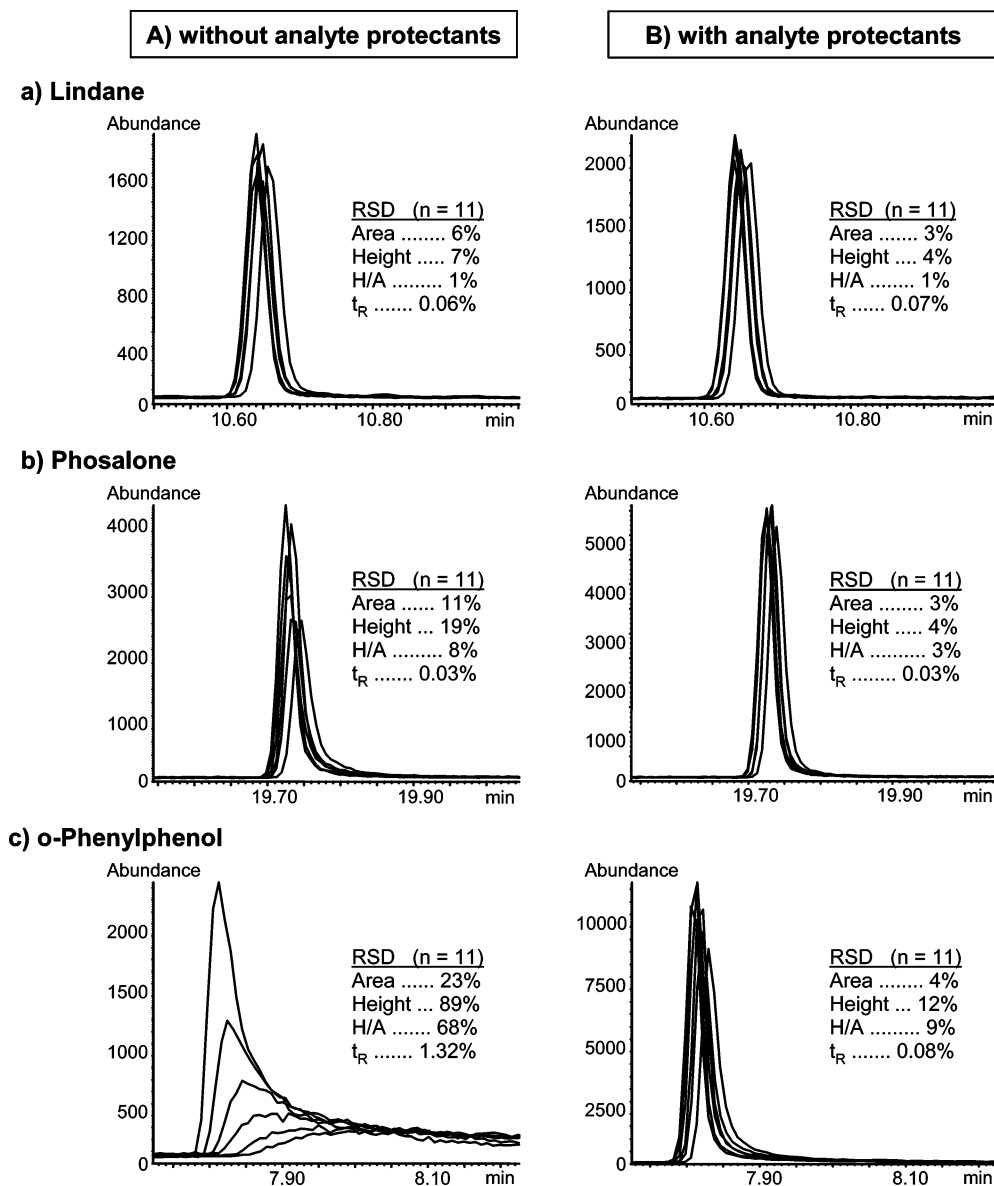


Figure 3. Overlay of extracted ion chromatograms of lindane (m/z 219), phosalone (m/z 182), and *o*-phenylphenol (m/z 170) obtained in the analysis of 500 ng/mL standard solutions in acetonitrile (A) without and (B) with the addition of analyte protectants (ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL in the injected sample, respectively) at the beginning and after each set of samples in the sequence described in the Experimental Section (i.e., after 30, 60, 90, 120, and 150 GC injections). RSDs of peak areas, heights, height-to-area ratios (H/A), and retention times (t_R) are provided for all 500 ng/mL acetonitrile standards with analyte protectants and test solutions (without analyte protectants) injected immediately before them throughout the sequence ($n = 11$).

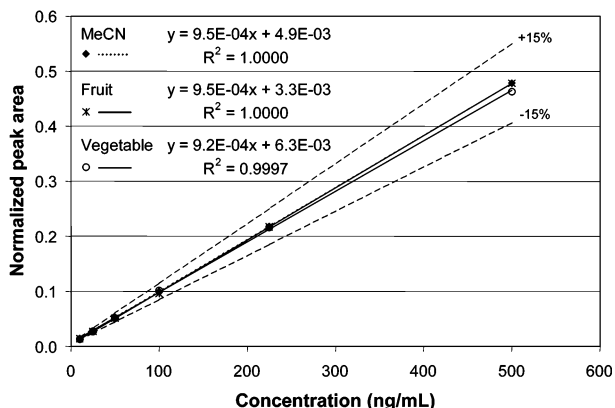
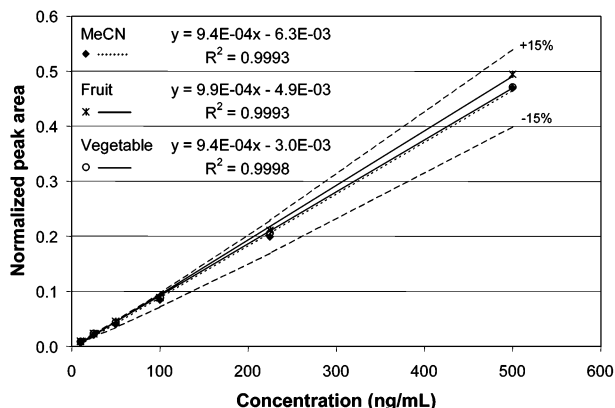
throughout the long sequence. Without the addition of analyte protectants, phosalone and especially *o*-phenylphenol signals were significantly deteriorating with the increasing number of injected samples. Whereas, the side-by-side injections of the same pesticide solution containing analyte protectants resulted in superior long-term signal repeatabilities as documented by the relative standard deviations (RSDs) obtained for peak areas, heights, height-to-area ratios (H/A), and t_R . Even for very susceptible analytes, such as *o*-phenylphenol, the addition of analyte protectants minimized matrix-induced response diminishment characterized by a gradual decrease in peak heights, which is usually accompanied by a gradual increase in t_R and peak broadening (indicated by decreasing H/A ratios).⁴ A practical outcome of this is the reduced need for frequent GC system maintenance.

Figure 3 also shows that, especially for analytes prone to tailing, the peak areas are more reproducible than peak heights, which is one of the reasons why peak areas are generally preferred for quantitation purposes in the GC pesticide multiresidue analysis. Figure 4 compares calibration curves based on peak areas (normalized to heptachlor—an ISTD not susceptible to matrix effects) of the three selected pesticides obtained in MeCN and mixed fruit and vegetable extracts without and with the ethylglycerol, gulonolactone, and sorbitol mixture. Without the analyte protectants, the injections of susceptible pesticides in MeCN resulted in nonlinear calibration curves with lower slopes and intercepts as compared to the situation in matrix extracts. This is a typical manifestation of the matrix-induced response enhancement effect, which would lead to significantly overestimated results

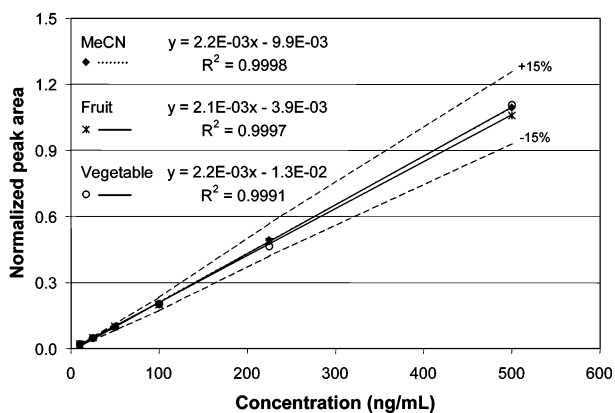
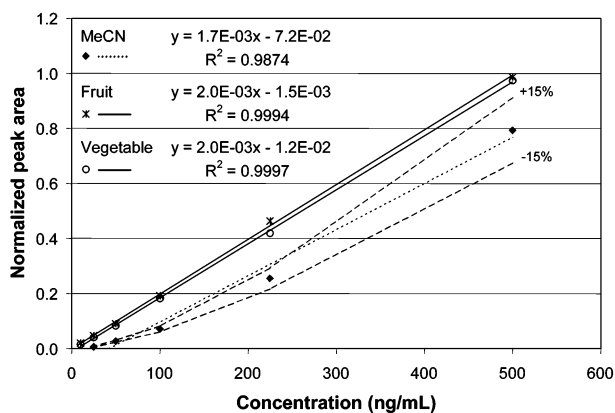
A) without analyte protectants

B) with analyte protectants

a) Lindane



b) Phosalone



c) o-Phenylphenol

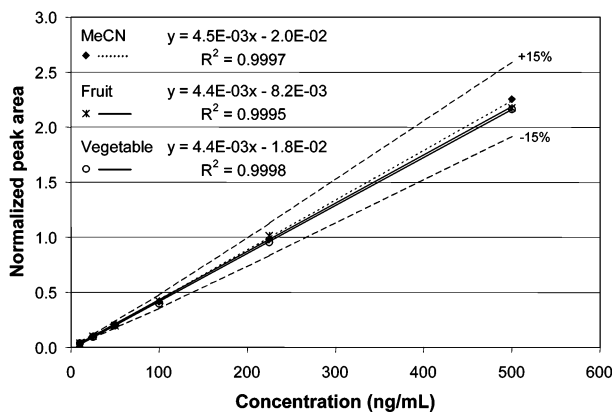
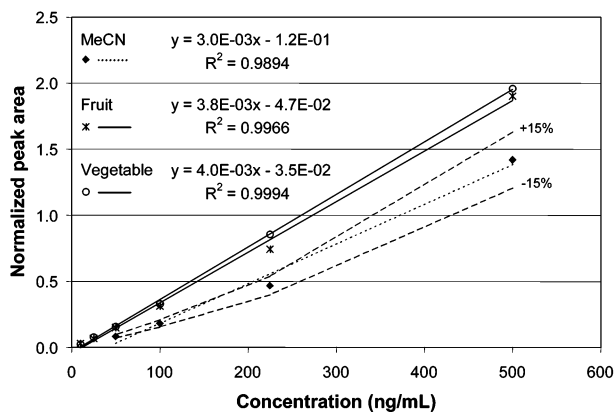


Figure 4. Comparison of calibration curves (based on peak areas normalized to ISTD heptachlor) of lindane, phosalone, and *o*-phenylphenol obtained by injection of solvent (MeCN) standards and spiked fruit and vegetable extracts (A) without and (B) with the addition of analyte protectants (ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL in the injected sample, respectively). Dashed lines denote $\pm 15\%$ peak area tolerance for values obtained in MeCN standards.

in the analyzed samples if solvent standards were used for calibration. The addition of analyte protectants practically eliminated the differences between calibrations obtained in matrix versus matrix-free solutions. Thus, in this case, solvent standards can be used for calculation of pesticide concentrations in matrix extracts as demonstrated in Figure 5.

The bars in Figure 5 represent calculated concentrations of all tested pesticides (except for captan and thiabendazole, which had matrix interferences) spiked at 50 ng/mL in fruit extracts and injected with the addition of analyte protectants at the beginning and at the end (in the first and fifth set of samples) of the long sequence. Solvent standards with analyte protectants

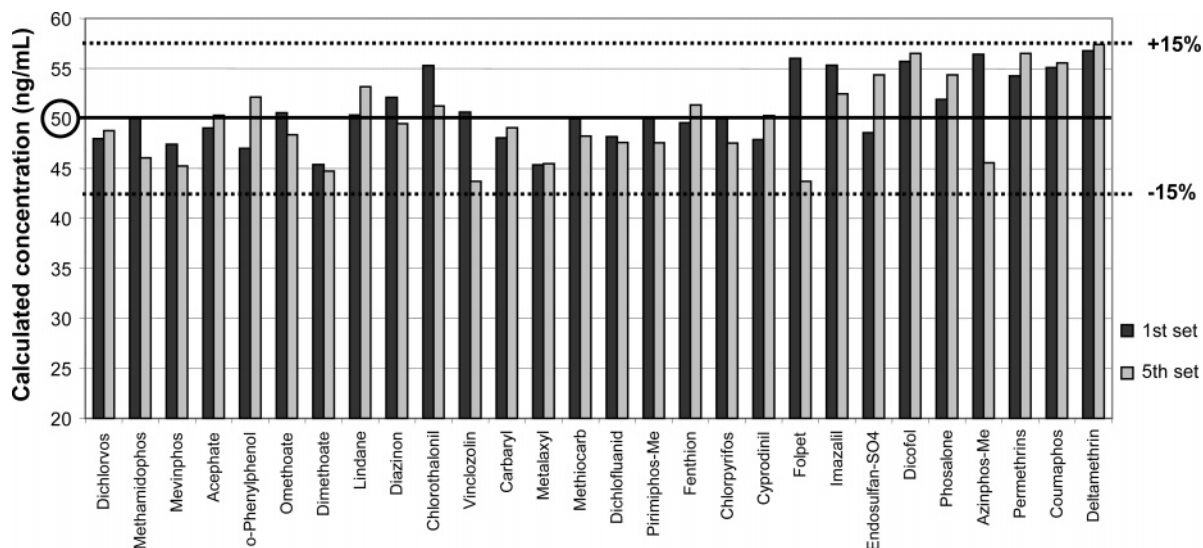


Figure 5. Calculated concentrations of pesticides in fruit extracts spiked at 50 ng/mL and injected at the beginning (first set) and at the end (fifth set) of the sequence of samples analyzed within the long-term performance study (see the Experimental Section for details). Analyte protectants ethylglycerol, gulonolactone, and sorbitol were added at 10, 1, and 1 mg/mL, respectively, to the fruit extracts and the solvent standards used for quantitation.

analyzed in the same sample sets were used for the calculation of the results. The figure shows that the addition of ethylglycerol, gulonolactone, and sorbitol (at 10, 1, and 1 mg/mL, respectively) effectively compensated for the matrix-induced enhancement effect even at 50 ng/mL and after more than 70 matrix injections. For the late-eluting analytes, the calculated values were generally higher than 50 ng/mL, but still within acceptable limits. The reduced coverage of the latest-eluting pesticides relates to the problem of finding a very effective analyte protectant that has sufficiently low volatility, but would not contaminate the GC system.¹³

CONCLUSIONS

In this study, various combinations of compounds, previously identified as potential analyte protectants, were evaluated. A mixture of ethylglycerol, gulonolactone, and sorbitol (10, 1, and 1 mg/mL, respectively, in MeCN or sample extracts) was found to be the most promising combination. The addition of these analyte protectants effectively compensated for the matrix-induced response enhancement even after more than 150 GC injections, including 130 samples with analyte protectants and 70 matrix

(mixed fruit or vegetable) extracts. Moreover, they also significantly reduced the matrix-induced response diminishment effect, resulting in improved ruggedness and, consequently, reduced need for frequent GC system maintenance. Thus, the simple addition of these analyte protectants to sample extracts and solvent standards can offer a convenient and effective solution to overcome adverse matrix effects in GC/MS analysis of pesticide residues and undoubtedly many other susceptible analytes.

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