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Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides☆

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Abstract

A common problem in gas chromatography (GC) applications is the analyte losses and/or peak tailing due to undesired interactions with active sites in the inlet and column. Analytes that give poor peak shapes or degrade have higher detection limits, are more difficult to identify and integrate, and are more prone to interferences than stable analytes that give narrow peaks. For susceptible analytes, significant peak quality improvements are obtained when matrix components are present because they fill active sites, thus reducing analyte interactions. This phenomenon is called "matrix-induced chromatographic response enhancement." Several approaches have been proposed to minimize peak distortion phenomena and compensate for matrix-induced effects, which is especially important for accurate quantitation, but each approach has serious limitations for routine multi-pesticide analysis. In this study, we demonstrate the feasibility of using "analyte protectants" to provide a more convenient and effective solution to the problem than other approaches developed thus far. The protecting agents are added to extracts and matrix-free standards alike to provide the chromatographic enhancement effect even for the most susceptible analytes in a very dirty GC system. In this study, we evaluated 93 different compounds to find the most suitable ones for improving chromatographic quality of the signal. Because hydrogen bonding has been shown to be an important factor in analyte interactions with active sites, we mainly focused on additives with strong hydrogen bonding capabilities. Dramatic peak enhancements were achieved using compounds containing multiple hydroxy groups, such as sugars and sugar derivatives, and gulonolactone appears to be the most effective protecting agent for the most pesticides that we tested. The benefits of using analyte protectants versus alternative procedures for overcoming matrix-induced effects in quantitation include: (a) simpler procedure; (b) easier integration of peaks; (c) lower detection limits; (d) better quantitation; (e) less maintenance of the GC inlet; and (e) lower cost. However, long-term influences on the performance of the chromatographic system have yet to be established.

Keyword: Pesticides

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1. Introduction

In pesticide residue analysis using gas chromatography (GC), the quantitation of certain important pesticides is adversely affected by a phenomenon commonly known as the "matrix-induced chromatographic response enhancement effect," which has been a subject of many studies in the last decade [1–16]. This effect is noted by improved chromatographic peak intensity and shape of affected compounds when they are injected in the presence of a complex matrix. When no matrix is present, poor peaks with low response result for the susceptible analytes. The accepted explanation is that matrix components mask active sites in the GC system, which leads to fewer of these sites being available to interact with analytes, thus leading to fewer losses and better peak shapes. The matrix-induced enhancement effect had been apparent for many years, particularly in the GC analysis of certain types of pesticides, but it was not until 1993 that Erney et al. gave the effect its name and proposed an explanation for its cause [1].

A variety of factors are involved in matrix-induced enhancement, which include: (1) number and type of active sites in the inlet and GC column; (2) chemical structure (hydrogen bonding character and thermolability) of the analytes; (3) analyte concentration; (4) injection temperature; (5) interaction time (a function of flow rate, pressure, injection volume, solvent expansion volume, column diameter, and retention time); and of course (6) the matrix type and concentration. Among these factors, the analyte and its concentration cannot be altered, but attempts have been made to reduce or eliminate the effect by varying each of the other aspects, mainly by addressing the injection process, as described below.

Accurate quantitation in the analysis of susceptible analytes depends on consistent compensation for the matrix-induced enhancement effect and/or overcoming it altogether. Ideally, the root cause of the effect (silanols, metal ions, and other active sites on surfaces in the GC system) would be eliminated to solve the problem. However, it is virtually impossible to make a suitably inert, inexpensive, and heat-tolerant surface for GC analysis to avoid this problem. Even the most stable column phase polymers break down over time at hot temperature which generates surface activity. In any event, nonvolatile matrix components will build up on potentially inert surfaces after repeated injections and form new active sites that pose the same problem. This is the reason that priming of the GC system with matrix is only a short-lived and usually ineffective remedy, which also increases the need for GC maintenance over time.

The use of cold, on-column injection is another possible approach to reduce the matrix-induced enhancement effect, but on-column techniques lead to more maintenance of the column and are impractical for routine applications of complex matrices. Programmable temperature vaporization (PTV) injection is generally preferred over on-column techniques, but this involves the use of a liner as in classical injection techniques. Pulsed-splitless injection works well to reduce residence time and minimize solvent expansion volume, but this approach can force nonvolatile matrix components farther into the column than desirable [8]. Furthermore, all of these injection techniques only reduce the effect and do not eliminate it [8,11,16].

Extensive cleanup of the extracts is an approach that could work in theory to eliminate the matrix components that cause the effect, but it is untenable in practice due to the wide polarity range of the pesticide analytes in multiclass, multiresidue methods and complexity of the matrices [7]. Moreover, the matrix-induced enhancement effect gives larger and higher quality peaks, thus it would serve the analysis better to take advantage of this phenomenon rather than eliminate it.

Since the source of the matrix-induced enhancement effect is not likely to be eliminated, the other ways to avoid its problems are to compensate for it. The method of standard additions may improve quantitation of susceptible compounds in matrix, but this approach requires much extra effort and still leads to inaccuracies because the matrix effect is concentration dependent. The use of deuterated internal standards for each pesticide susceptible to the effect would solve the problem, but such standards are not generally available, and would be prohibitively expensive if they were. Further limitations with using isotopically labeled internal standards are the restriction in the use of detection techniques other than mass spectrometry (MS) and the additional burden of developing analytical conditions for so many more compounds. The use of a matrix-induced enhancement calculation

factor to compensate for the effect during the calculation of results is not acceptable because the effect is too variable and the notion of using such a "fudge factor" is objectionable to most analytical chemists.

The most widely used approach to account for the phenomenon is the use of matrix-matched standardization [3]. This involves the preparation of calibration standards in blank extracts to provide the same amount of matrix-induced enhancement as in the sample extracts. This procedure works reasonably well, but the disadvantages include: need for enough blank matrix (ideally exactly the same as the samples) and its long-term storage; extra time, labor, and expense for preparing the blank extracts for calibration standards; greater amount of matrix material injected onto the column in a sequence which leads to greater GC maintenance; and greater potential for analyte degradation in the matrix solution.

In routine laboratories, the need for matrix-matching standardization creates a heavy burden. In a sequence of samples, there may be several different types of sample extracts, and it is too onerous for the analysts to prepare matrix-matched standards for each commodity. Therefore, routine monitoring laboratories often take short-cuts in their matrix-matching procedures by using blanks for one commodity to substitute for another. This procedure is more convenient, but it can lead to inaccuracies because the degree of the matrix-induced enhancement is commodity dependent (and sometimes sample dependent of the same matrix).

Another problem with the use of matrix-matched standards is that the two most prominent federal regulatory agencies in the US, the Environmental Protection Agency (EPA) and Food and Drug Administration (FDA), do not permit their use for enforcement purposes involving pesticide residues in food. These policies are in place because matrix-matching can be manipulated by unscrupulous or ill-informed analysts to give the desired results in an analysis. Of course, *not* using matrix-matched standards is well *established* to provide erroneously high results, so the policy ignores the reality of the problem. In Europe, the regulatory guidelines for pesticide analysis call for the use of matrix-matched standards unless matrix effects are demonstrated not to affect the signal [17]. For susceptible pesticides, such as methamidophos, acephate, omethoate, monocrotophos, and many others, the true concentrations and recoveries in the GC analysis are suspect unless matrix effects are taken into account. If standards in solvents are used as EPA and FDA policies infer, then calculated results for the susceptible pesticides are over-estimated.

EPA and FDA policies for pesticide analysis do not prohibit the use of additives to the extracts to overcome or generate the effect consistently. With this in mind, Erney and Poole studied compound additives to minimize the matrix-induced chromatographic response enhancement [2]. However, they only studied eight different "masking reagents," and none gave a suitable effect for the purpose of pesticide residue analysis. Their conclusion was "that because of the wide range of physical and chemical properties of pesticides and their matrices that are possible, it is unlikely that any single compound exists which is able to totally resolve the matrix-induced chromatographic response effect" [2].

In this study, we have revisited this concept and tested a larger number and wider variety of compounds that we have termed "analyte protectants." In a previous study concerning the development of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticides [18], we noted that the matrix-induced enhancement effect was significantly reduced after the dispersive-solid-phase extraction (dispersive-SPE) cleanup step using primary secondary amine (PSA) sorbent. We realized that if the types of compounds that protected the analytes from detrimental interactions could be added to the solution, and if these analyte protectants did not interfere in the analysis, then the approach could be very useful.

The aim of this study was to evaluate a variety of different additives that could be employed as analyte protectants in the GC analysis of susceptible pesticides and to demonstrate the effectiveness of these agents for improving the quality of the analysis. Furthermore, we wanted to develop a simple and inexpensive procedure that does not violate EPA and FDA guidelines for enforcement purposes and still provide accurate quantitation in the analysis of difficult GC-amenable pesticides. Additionally, we sought to further elucidate the mechanisms behind the matrix-induced chromatographic response enhancement effect.

Table 1
List of pesticides, their typical retention times, and SIM quantitation ions used to measure the effect of different analyte protectants in GC analysis

Code	Pesticide	Retention time (min)	Quantitation ion (*m/z*)
	Dichlorvos	5.3	185
a	Methamidophos	5.8	94
	Mevinphos	6.6	127
b	Acephate	7.3	136
c	*o*-Phenylphenol	7.3	170
d	Omethoate	9.1	156
	Diazinon	9.2	179
	Lindane	9.8	181
e	Dimethoate	10.1	93
	Vinclozolin	10.7	285
f	Chlorothalonil	11.5	266
	Metalaxyl	11.5	206
	Chlorpyrifos	12.1	197
	Dichlofluanid	12.3	224
g	Carbaryl	12.4	144
h	Methiocarb	12.5	168
	Fenthion	12.7	278
	Cyprodinil	13.4	224
	Captan	14.9	79
	Folpet	15.1	260
I	Imazalil	15.6	215
j	Thiabendazole	15.7	201
	Endosulfan sulfate	16.8	272
	Dicofol	17.4	251
k	Phosalone	18.1	182
	cis-Permethrin	18.5	183
	trans-Permethrin	18.7	183
l	Azinphos-methyl	18.8	160
m	Coumaphos	19.6	362
	Deltamethrin	23.1	181

The code numbers, which are used in Table 4, are given for the pesticides that are most indicative of the matrix-induced enhancement effect.

2. Experimental

2.1. Materials

Table 1 lists the pesticides used for analysis in this study, and Tables 2 and 3 give the compounds evaluated as analyte protectants. Table 2 covers the initial list of compounds evaluated in less rigorous experiments for the purposes of quantitation in the development of the QuEChERS method [18]. Pesticide reference standards were obtained from the National Pesticide Standard Repository of the US EPA (Fort Meade, MD, USA), Dr. Ehrenstorfer GmbH (Augsburg, Germany), Ultra Scientific (North Kingstown, RI, USA), and Chemservice (West Chester, PA, USA). Stock solutions of 2000 μg/ml for each pesticide and a working standard pesticide mixture of 50 μg/ml were prepared in MeCN. All compounds evaluated as analyte protectants in this study were 95% or better purity obtained from Sigma (St. Louis, MO, USA), Aldrich

Table 2
List of initial compounds evaluated as analyte protectants in preliminary experiments for the development of the QuEChERS method

Compound	MW	CAS no.	Code
Compounds with (multiple) hydroxy groups			
1,3-Propanediol	76	504-63-2	
1,2-Butanediol	90	584-03-2	D02
2-Methyl-1,3-propanediol	90	2163-42-0	
Ethoxyethanol	90	110-80-5	
D-Glycerol	92	56-81-5	B01
Neopentyl glycol	104	126-30-7	
3-Ethoxy-1,2-propanediol	120	1874-62-0	D05
D-Sorbitol	182	50-70-4	B06
D-Fructose	180	57-48-7	A08
D-Glucose	180	50-99-7	A06
Saccharose	342	57-50-1	
Stigmasterol	413	83-48-7	
Acidic compounds			
2-Methyl butyric acid	102	116-53-0	
Benzoic acid	122	65-85-0	
Cinnamic acid	148	621-82-9	
p-Coumaric acid	164	501-98-4	
Vanillic acid	168	121-34-6	
Capric acid	172	334-48-5	
Stearic acid	284	57-11-4	
Compounds with amino and carboxy groups			
Glycine	75	56-40-6	
Serine	105	56-45-1	
6-Aminohexanoic acid	131	60-32-2	
3,5-Diaminobenzoic acid	152	535-87-5	
Compounds with amino and hydroxy groups			
Diethanolamine (DEA)	105	111-42-2	
Triethanolamine (TEA)	149	102-71-6	
Basic compounds			
Thiourea	76	62-56-6	
Urea	60	57-13-6	
Imidazole	68	288-32-4	F01
1,6-Hexanediamine	116	124-09-4	
Aliphatic compounds			
Dodecane	170	112-40-3	
Docosane	310	629-27-0	
Squalene	411	111-02-4	

The codes given for some compounds refer to Table 3.

Table 3
List of compounds evaluated as analyte protectants in the study categorized into different chemical classes

Code	Compound name	CAS no.	%Water
Group A: Sugars			
A01	D-Ribose	50-69-1	2
A02	D-Arabinose	28697-53-2	9
A03	L-Arabinose	87-72-9	3
A04	D-Xylose	58-86-6	2
A05	D-Lyxose	1114-34-7	2
A06	D-Glucose	50-99-7	10
A07	D-Galactose	59-23-4	5
A08	D-Fructose	57-48-7	4
A09	L-Sorbose	87-79-6	8
Group B: Sugar alcohols			
B01	D-Glycerol	56-81-5	2
B02	*meso*-Erythritol (1,2,3,4-butanetetraol)	149-32-6	2
B03	1,4-Anhydroerythritol (tetrahydro-3,4-furandiol)	4358-64-9	–
B04	Ribitol (adonitol)	488-81-3	2
B05	Xylitol	87-99-0	2
B06	D-Sorbitol (D-glucitol)	50-70-4	6
B07	D-Mannitol	69-65-8	3
B08	D-Galactitol (D-dulcitol)	608-66-2	14
B09	*myo*-Inositol	87-89-8	25
B10	Quebrachitol	642-38-6	11
Group C: Other sugar derivatives			
C01	2-Deoxy-D-ribose	533-67-5	2
C02	1,6-Anhydro-α-D-glucopyranose	498-07-7	1
C03	1-*O*-Methyl-β-L-arabinopyranoside	3795-69-5	3
C04	1-*O*-Methyl-β-D-xylopyranoside	612-05-5	2
C05	1-*O*-Methyl-α-D-glucopyranoside	97-30-3	2
C06	4,6-*O*-Ethylidene-α-D-glucopyranose	13224-99-2	2
C07	1,2-*O*-Isopropylidene-α-D-glucofuranose	18549-40-1	2
C08	D-Ribonic acid γ-lactone	5336-08-3	–
C09	D-Gluconic acid δ-lactone	90-80-2	2
C10	L-Gulonic acid γ-lactone	1128-23-0	2
Group D: Diols			
D01	2,3-Butanediol	513-85-9	–
D02	1,2-Butanediol	584-03-2	–
D03	1,2,4-Butanetriol	3068-00-6	–
D04	3-Methoxy-1,2-propanediol	623-39-2	–
D05	3-Ethoxy-1,2-propanediol	1874-62-0	–
D06	3-Allyloxy-1,2-propanediol	123-34-2	–
D07	(*R*)-3-Benzyloxy-1,2-propanediol	56552-80-8	–
D08	Monomyristin	589-68-4	–
D09	1,6-Hexanediol	629-11-8	–
D10	1,9-Nonanediol	3937-56-2	–
D11	Pentaerythritol (2,2-bis(hydroxymethyl)-1,3-propanediol)	115-77-5	–
Group E: Poly-ethers			
E01	Diethylene glycol	111-46-6	–
E02	Diethylene glycol monomethyl ether	111-77-3	–
E03	Diethylene glycol dimethyl ether	111-96-6	–
E04	Diethylene glycol diethyl ether	112-36-7	–
E05	Diethylene glycol monohexyl ether	112-59-4	–
E06	Diethylene glycol stearyl ether (Brij 72)	9005-00-9	–

Table 3 (*Continued*)

Code	Compound name	CAS no.	%Water
E07	Diethylene glycol oleyl ether (Brij 92)	9004-98-2	–
E08	Triethylene glycol	112-27-6	–
E09	Triethylene glycol dimethyl ether	112-49-2	–
E10	Tetraethylene glycol	112-60-7	–
E11	Tetraethylene glycol dimethyl ether	143-24-8	–
E12	PEG (polyethylene glycol) 300	25322-68-3	–
E13	18-Crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane)	17455-13-9	–
E14	Triglycerol	20411-31-8	1
Group F: Basic compounds			
F01	Imidazole	288-32-4	–
F02	2-Thiouracil	156-82-1	1
F03	Caffeine	58-08-2	2
F04	Albendazole	54965-21-8	–
F05	Thiabendazole	148-79-8	–
F06	Imazalil	35554-44-0	–
Group G: Miscellaneous			
G01	5-(Hydroxymethyl)-2-furaldehyde (HMF)	67-47-0	0.25
G02	*N*-Glycylglycine	556-50-3	25
G03	Diacetin	102-62-5	–
G04	Triacetin	102-76-1	–
G05	Benzoin	119-53-9	–
G06	Propyl gallate	121-79-9	2
G07	Cyclamic acid	100-88-9	0.5
G08	Acesulfame K	55589-62-3	1

The percent water column indicates the amount of water needed to dissolve the compound to make a 1 mg/ml concentration in MeCN solution.

(Milwaukee, WI, USA), Fisher (Fair Lawn, NJ, USA), Fluka (Buchs, Germany), or other source. Working solutions of ≈10 mg/ml were prepared of each potential protecting agent in MeCN or MeCN:water solutions (known amounts of water were added to the MeCN solution which was sonicated until the compound dissolved). Anhydrous $MgSO_4$ and NaCl were obtained from Aldrich. The $MgSO_4$ was heated for 5 h at 500 °C in a muffle furnace to remove phthalates. Primary secondary amine sorbent was obtained from Varian (Harbor City, CA, USA); fruit and vegetable samples were obtained from local organic food stores. For the screening evaluation experiments, analyte protectant solutions typically of 10 mg/ml (in MeCN or MeCN:water) were added to pesticide standards in MeCN to yield final concentrations of 1 μg/ml for pesticides and 1 mg/ml for protecting agents. The percentage of water present in the final pesticide standard solutions after adding the potential protecting agents is given in Table 3. In a few cases, other protectant and pesticide concentrations were evaluated for the more promising analyte protectants, and fruit and vegetable matrix extracts were also used in some experiments.

2.2. Sample preparation

The QuEChERS method which was used to prepare extracts is presented elsewhere [18], but in brief, the procedure entails: (1) weigh 10 g previously chopped sample into a 40 ml Teflon centrifuge tube; (2) add 10 ml MeCN and shake the sample vigorously for 1 min using a vortex mixer; (3) add 4 g $MgSO_4$ and 1 g NaCl and vortex immediately for 1 min; (4) centrifuge the extracts for ≈3 min at 5000 rpm; (5) transfer a 1 ml aliquot of the upper layer into a 2 ml micro-centrifuge vial containing 25 mg PSA and 150 mg $MgSO_4$; (6) vortex for 30 s and centrifuge the extracts for ≈1 min at 5000 rpm; (7) transfer 0.5 ml of the extract into an autosampler vial for GC/MS analysis.

Analyte protectants were added or not depending on the experiment being conducted.

2.3. Analysis

All analyses were conducted with a Hewlett-Packard (Agilent; Little Falls, DE, USA) Model 5890 Series II Plus GC coupled to a 5972 mass selective detector. The system was equipped with a split/splitless injection inlet, electronic pressure control, and a 7673A autosampler; Chemstation software was used for instrument control and data analysis. The chromatographic conditions were: DB-35ms (Agilent; Folsom, CA, USA) capillary column of 30 m, 0.25 mm i.d., 0.25 μm film thickness, He constant flow of 1 ml/min, inlet temperature 250 °C, injection volume 1 μl (splitless), MS transfer line temperature 290 °C, temperature program of 95 °C for 1.5 min, then 20 °C/min ramp to 190 °C followed by a 5 °C/min ramp to 230 °C and a 25 °C ramp to 290 (held for 20 min). Total run time was 36.65 min. Full scan analysis (40–450 *m*/*z*) was used in experiments to determine the chromatographic and MS traits of the different compounds and selected ion monitoring (SIM) mode was used to measure the effect of the potential analyte protectants on the intensity and quality of the pesticide peaks. Table 1 gives typical retention times (t_R) and quantitation ions for the pesticides on the DB-35ms column. In follow-up experiments, the same GC method was also used with a 30 m, 0.25 mm i.d., 0.25 μm film thickness Rtx-5ms column (Restek; Bellefonte, PA, USA), and the SIM program was modified slightly to account for the small retention time differences. It should be noted that the GC inlet and columns were poorly maintained on purpose to better ascertain and show the effects of the analyte protectants.

3. Results and discussion

3.1. Features of analyte protectants

The main factors to consider in the search for a good analyte protecting agent (or combination of protectants) include: (1) hydrogen bonding ability; (2) volatility; and (3) practical aspects. The primary consideration is that the protecting agent(s) must give a strong matrix-induced enhancement effect. Previous observations have indicated that hydrogen bonding is a key factor in the phenomenon. Indeed, pesticides with hydroxy (–OH) and amino (R–NH–) groups, imidazoles and benzimidazoles (–N=), carbamates (–O–CO–NH–), urea derivatives (–NH–CO–NH–) and several organophosphate compounds (–P=O) are the most susceptible type of analytes to the effect [1–8]. These types of molecules interact with silanol groups and possibly metal ions on glass surfaces (liners, glass wool, etc.) as seen by the greater enhancement effect when nonsilanized glass is used versus deactivated surfaces. Moreover, cleanup of fruit and vegetable extracts with PSA sorbent resulted in increased tailing and degradation rates of target analytes during GC/MS, obviously due to the removal of compounds that provide good protection and peak enhancement [18]. In our QuEChERS experiments, we noticed that various organic acids (such as phenolic and fatty acids) and carbohydrates (such as fructose) were among the most prominent compounds removed. PSA contains primary and secondary amine groups and thus has the ability to interact via hydrogen bonding (and ionic interactions) which further supports the importance of hydrogen bonding in the effect. Effective analyte protecting agents are therefore likely to need multiple hydroxy and/or amino groups to be able to interact with active sites via hydrogen bonding.

Ideally, a low concentration of analyte protectants should give the full response enhancement effect, but this will depend on the number of active sites in the GC system to be filled and the affinity of the agents to these sites. Furthermore, the protecting agent must be filling the active sites in the liner and column at the same time that the pesticide of interest is most susceptible to interactions with these active sites. This means that volatility of the analyte protectant(s) should be similar to that of the analyte(s) being protected. Thus, for a wide range of analytes, the protecting agent(s) must also cover a wide volatility range.

Practical features of an ideal protecting agent for specific and general purposes include: (a) it should be unreactive with analytes in solution or the GC system and not induce degradation; (b) it should not deteriorate performance of the GC column or detector; (c) it should not interfere in the detection (thus it must have low intensity, low mass ions in its MS spectra); (d) it should not accumulate in the GC injector or elsewhere in the system; (e) it should be widely available,

inexpensive, and non-hazardous; and of course (f) it must be soluble in the solvent of interest. Given the fact that the most effective agent(s) will most likely possess multiple polar groups, relatively polar solvents such as MeCN, which are miscible with water, seem to be more appropriate. However, in order to avoid adverse chromatographic effects, not too much water co-solvent should be needed to achieve sufficient solubility of the protectant.

3.2. Initial investigations

In exploratory experiments, an initial list of 32 compounds was evaluated, and Table 2 lists the potential protectants divided into six groups: (1) compounds with multiple hydroxy groups (including sugars and sugar alcohols); (2) acidic compounds with carboxy groups (including fatty and phenolic acids); (3) amphoteric compounds with carboxy and amino groups (including amino acids); (4) compounds with amino and hydroxy groups; (5) basic compounds; and (6) aliphatic compounds.

Each pesticide/compound pair was evaluated in terms of peak heights, areas, retention times, and possible interferences in order to measure and compare the effects of the different prospective analyte protectants. Most agents were used at a concentration of 1 mg/ml, but several of the most volatile compounds were also injected at higher concentrations up to 20 mg/ml. Observations regarding the protective effect of the agents are discussed as follows.

3.2.1. Agents with (multiple) hydroxy groups

As expected, those compounds that act as strong hydrogen bond donors and acceptors, were the most effective at enhancing peak intensity and reducing the analyte peak tailing. Some of the agents themselves gave broadly eluting and tailing peaks, which protected pesticides over a broad volatility range. The most volatile of the agents tested (1,3-propanediol, 1,2-butanediol, 2-methyl-1,3-propanediol, and neopentyl glycol) only worked for the most volatile analyte, dichlorvos. The slightly less volatile compound, 3-ethoxy-1,2-propanediol, offered excellent protection for pesticides with short and intermediate retention times, but did not work well for the late-eluting ones. In the case of the much less volatile compound, sorbitol, only late-eluting pesticides were protected. Unlike sorbitol, fructose and glucose contain a reactive carbonyl group that causes them to readily degrade into several products in solution and during injection. These degradation products elute throughout the chromatogram, which serves to protect analytes over a broader volatility range. It should be noted that the degradation pattern of sugars may strongly depend on the pH of the extract. In the case of fructose, for example, we noticed very different GC degradation pattern, resulting in different analyte protection properties, when acidic versus neutral solutions were injected. Interestingly, the multi-hydroxy compounds tested did not interfere with the detection of the tested analytes, as will be discussed in Section 3.4. A disadvantage of sugars, however, is the potential formation of caramelization products that can contaminate the GC system over time.

3.2.2. Acidic agents

In the case of organic acids, the protection was best for analytes eluting close to the agent. For example, stearic acid worked well for analytes with retention times of ≈13–15 min (fenthion, cyprodinil, and imazalil), which co-eluted with the broad stearic acid peak, but it did not protect analytes that eluted earlier or later. Interestingly, base-sensitive analytes, such as captan, folpet, dichlofluanid, and chlorothalonil, were effectively protected irrespective of whether they co-eluted with the acidic agents or not. Later experiments determined that the source of this “protection” was the lower pH provided by the acidic agents which minimized degradation of the base-sensitive pesticides in solution.

3.2.3. Basic agents

In theory, compounds with basic functions should be very promising analyte protectants because amino groups are well known to strongly interact with acidic silanol groups. However, due to the higher pH of the solution, base-sensitive pesticides were partly or entirely lost in the presence of the bases. As in nearly all cases, certain analytes with t_R in close proximity to the agents were protected very well.

3.2.4. Amphoteric agents with amino and carboxy or amino and hydroxy functions

As in the case of basic agents, base-sensitive pesticides were negatively affected by these type of

compounds. An exception was 3,5-diaminobenzoic acid, in which the strength of the carboxylic acid group is greater than the basicity of the aniline groups. This compound gave the best protection of the basic pesticides, thiabendazole and imazalil. This can be attributed to co-elution effects and/or the affinity of the agent to the active sites that potentially interact with the basic analytes. A disadvantage of 3,5-diaminobenzoic acid is that it strongly and rapidly reacts with sugars. In an experiment where 3,5-diaminobenzoic acid and fructose were added at the same time for protection purposes, the solution turned dark, obviously because of Maillard-type reactions, and many of the analytes originally contained in the solution disappeared from the chromatogram.

3.2.5. Aliphatic compounds

In order to investigate the influence of high molecular weight compounds that do not form hydrogen bonds, a few aliphatic compounds were tested. Dodecane was included because it is frequently used as a "keeper" in pesticide residue analysis to reduce losses of highly volatile analytes during solvent evaporation steps. The late-eluting squalene was the only aliphatic compound that had a positive impact on any of the pesticides, and these were the late-eluting coumaphos and deltamethrin, which importantly demonstrates that analyte protection can also be provided by agents that do not form hydrogen bonds. The proposed mechanism of this protection process will be discussed in Section 3.8.

Figs. 1 and 2 show the degree of the effects observed with the co-injection of different compounds on the peak shapes and intensities of 1 μg/ml each of omethoate (code d from Table 1) and thiabendazole (code j), two pesticides with notorious susceptibility to the matrix-induced enhancement effect and severe peak tailing. The instrument conditions were so bad that there were almost no observable peaks for these pesticides when standards in MeCN solvent were injected without the use of protectants.

As can be seen in Fig. 1, 20 mg/ml of 3-ethoxy-1,2-propanediol (code D05), also known as 3-*O*-ethylglycerol, gave excellent results for omethoate (and other early-eluting pesticides not shown, such as methamidophos, acephate, mevinphos, *o*-phenylphenol and carbaryl). A chromatogram of a similarly fortified tomato extract from the QuEChERS method

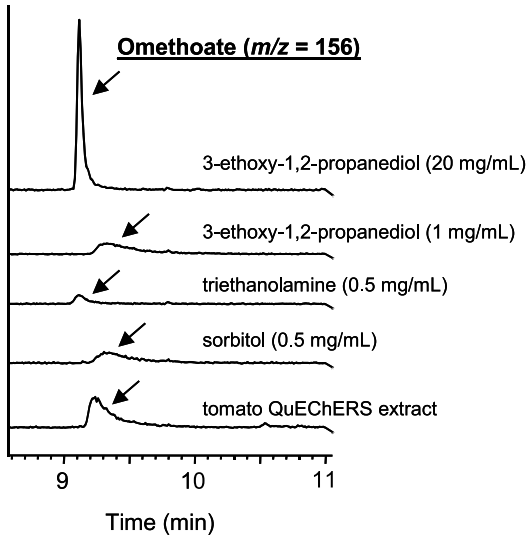


Fig. 1. Effect of different analyte protectants evaluated on the peak shape and intensity of 1 μg/ml omethoate.

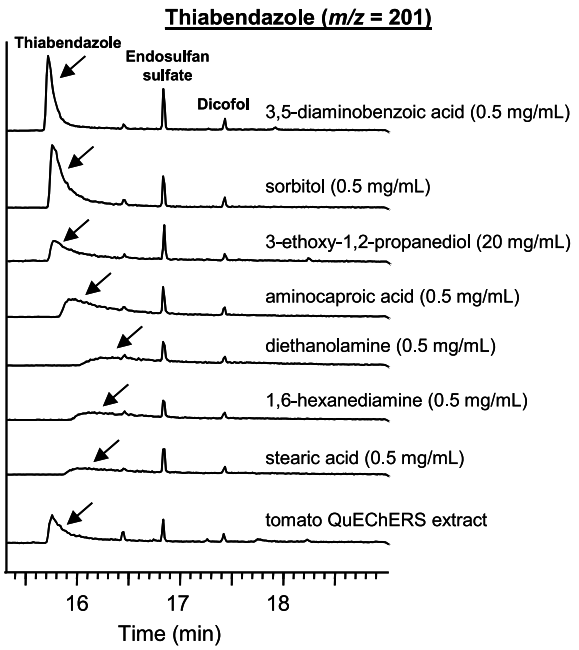


Fig. 2. Effect of different analyte protectants evaluated on the peak shape and intensity of 1 μg/ml thiabendazole.

after dispersive-SPE cleanup with PSA is provided for comparison. Other than 3-*O*-ethylglycerol at 20 mg/ml, none of the other 25 compounds evaluated gave an enhancement effect stronger than the tomato matrix for omethoate. A high concentration of 3-*O*-ethylglycerol was necessary because it did not work as well at 1 mg/ml concentration. In general, the injection of such a large amount of any compound would cause concern in capillary GC analysis, but we assumed that volatile protecting agents, such as 3-*O*-ethylglycerol, were not likely to contaminate the GC system.

In Fig. 2, a few of the same and other examples of potential analyte protectants are shown in the case of thiabendazole. Notable observations not only pertain to the degree of tailing that occurs for the thiabendazole peak, but also how much the t_R shifts depending on the analyte protecting effect of the different compounds. The sharp peaks at ≈16.8 min (endosulfan sulfate) and ≈17.4 min (dicofol) demonstrate how the peak shapes and t_R vary less significantly for those pesticides not strongly affected by the matrix-induced enhancement effect. The best enhancement for thiabendazole was achieved with 3,5-diaminobenzoic acid.

In a separate experiment, the effect of analyte protectant concentration on the degree of protection of various pesticides was tested. In Fig. 3, the effect of increasing concentrations of 3,5-diaminobenzoic acid on thiabendazole and imazalil and of 3-*O*-ethylglycerol on mevinphos and dichlorvos is presented. As the figure shows, the addition of 3,5-diaminobenzoic acid significantly improved the quality of thiabendazole and imazalil peaks, and initially caused them to shift to shorter t_R. However, as the concentration of 3,5-diaminobenzoic acid was increased, shifts to longer t_R were observed. Similar t_R anomalies at high agent concentrations also occurred for some other protectant/analyte combinations, usually when they co-eluted. We propose possible mechanisms to explain these types of effects in Section 3.8. Fig. 3b shows how 20 mg/ml 3-*O*-ethylglycerol caused a disturbance in the analyte focusing process and distorted the dichlorvos peak. The highest tested concentration of 3-*O*-ethylglycerol did not adversely affect the peak shapes of other pesticides, as the figure shows in the case of mevinphos (in which the higher concentration led to better resolution of the two isomers).

Unfortunately, none of the 32 initial compounds screened was able to serve by itself as a useful analyte protectant for all pesticide analytes evaluated. Instead, we chose a combination of 3-*O*-ethylglycerol and sorbitol to achieve our objectives in the development of the QuEChERS method [18], and Fig. 4 presents how this pair of protectants improved the quality and intensity of the peaks for many pesticides in the method.

In summation of the initial experiments, three main conclusions could be made: (1) compounds with multiple hydroxy groups generally provided better analyte protection than other types of compounds; (2) volatility (in terms of t_R) is a critical factor and protectants of a certain volatility better protect pesticide analytes of a similar volatility; and (3) greater concentration of the protecting agents generally leads to a larger enhancement effect. However, our initial experiments did not find the ideal analyte protectant(s), and further experiments were planned and conducted for a wider variety of compounds. We chose to investigate additional analyte protectants in order to better understand the mechanism of the protection process and to find a more ideal protecting agent or combination of agents.

3.3. Evaluation of 68 possible analyte protectants

As shown in Table 3, the 68 compounds for investigation consisted of (A) sugars; (B) sugar alcohols; (C) other sugar derivatives; (D) diols (including a triol and tetraol); (E) poly-ethers (including the mono-ether, diethylene glycol); (F) basic compounds; and (G) miscellaneous others. These were primarily selected on the basis of the preliminary results, but also we chose some compounds that were already available in the laboratory or those that could be ordered and delivered quickly. A wide variety of amine-containing compounds were not evaluated because as discussed earlier, strong bases lead to pesticide degradation in solution, which make the use of such compounds impractical for multiclass, multiresidue applications. The amount of water present in the final mixture to yield a 1 mg/ml solution of the compound in MeCN also appears in Table 3.

The experimental isolation of different parameters to study matrix-induced enhancement effects and the development of a measurable approach to quantify the results in an objective manner were challenging tasks. In our experiments, it was very easy to alter a

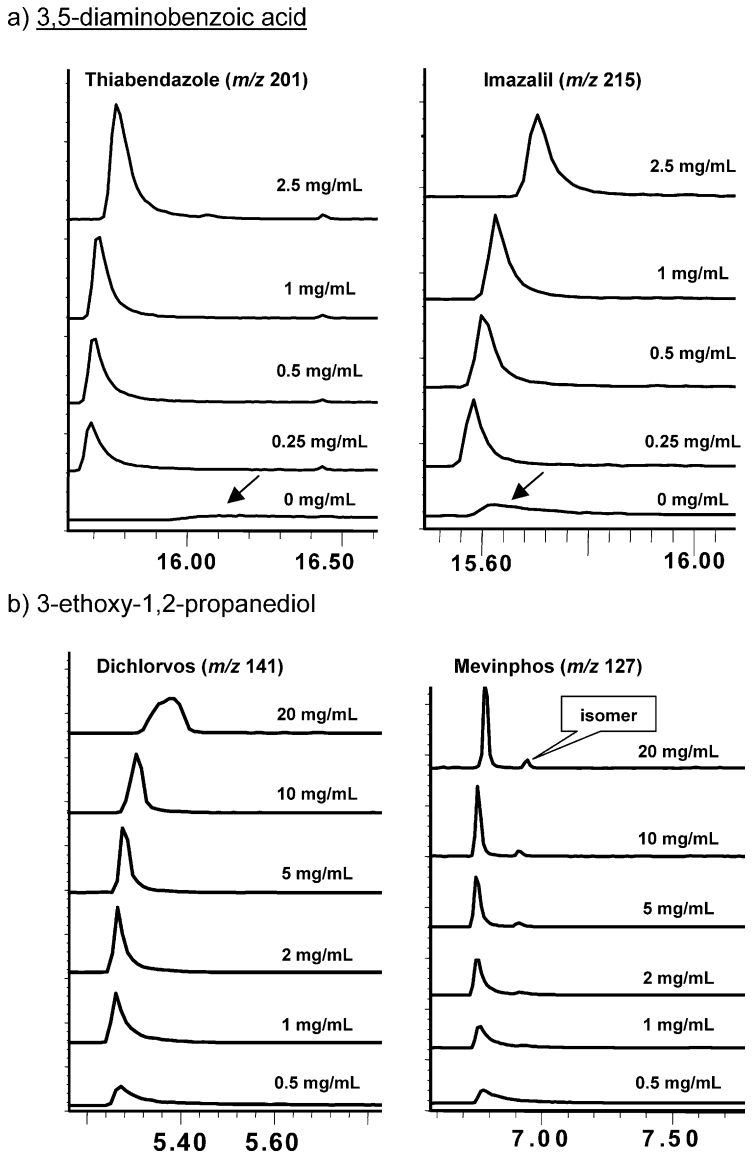


Fig. 3. Impact of the agent concentration on the peak shape, intensity and retention time of various pesticides: (a) 3,5-diaminobenzoic acid in the case of thiabendazole and imazalil and (b) 3-ethoxy-1,2-propanediol in the case of dichlorvos and mevinphos (each at 0.25 μg/ml).

single parameter, namely the protecting agent used, but an important uncontrollable parameter, the condition of the GC system, changes over time. To compensate for this, we placed standards in MeCN solvent, 3-*O*-ethylglycerol, and sorbitol in the sequence approximately every seven injections. GC/MS in the SIM mode was used to evaluate each pesticide/compound pair for a total of 110 injections. The sequence was repeated for each potential analyte protecting agent in full scan MS mode (scan range = 40–420 *m*/*z*) to determine the potential chromatographic and mass spectral interferences. A 20 min baking-out period at 290 °C was monitored at the end of each chromatographic analysis to check for late-eluting peaks, and we checked frequently for ghost peaks from previous injections. No verifiable

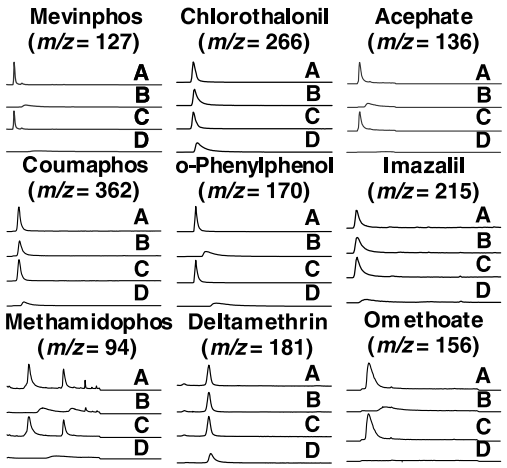


Fig. 4. Improvement of peak shapes and peak areas with the help of 10 mg/ml 3-*O*-ethylglycerol and 0.5 mg/ml sorbitol—GC/MS (SIM) chromatograms of a mixture of pesticides at 1 μg/ml in A: matrix extracts + analyte protectants; B: matrix extracts; C: solvent + analyte protectants; and D: pesticides in solvent only. The matrix consisted of a mixture of several fruits and vegetables which was extracted using the QuEChERS method [18].

ghost peaks (carry-over of compounds from previous injections) were observed for any of the compounds evaluated. The longest t_R for any significant peak in the GC/MS analyses was 21.7 min for Brij 72 and 92 (E06 and E07), which was shorter than the last pesticide to elute (23.1 min for deltamethrin).

The evaluation to measure the effects was performed in a number of different ways until we decided on the final systematic approach. In essence, we wanted to assess the two different undesirable mechanisms that occur during injection and analysis: (1) analyte losses due to degradation and/or irreversible retention (mainly occurring in the GC inlet); and (2) tailing of the analyte peaks due to retention on active sites (mainly occurring on the column). The latter effect could be measured from the peak height to peak area ratio (H/A) to indicate the degree of peak tailing. The overall enhancement effect however could be better measured from peak height. To compensate for fluctuations within the sequence, the peak heights were normalized to the sum of the peak heights for the three most unaffected pesticides (lindane, chlorpyrifos, and endosulfan sulfate), which only gave 10–15% RSD for their variability in peak heights among the 110 injections. Both the H/A and normalized peak height approaches were conducted to evaluate the results, but only the peak height evaluation is presented here.

Table 4 ranks and compares most of the compounds evaluated in this study, as listed by the code numbers given in Table 3 and coded pesticides from Table 1. The compounds are ordered according to overall results using a ranking system based on normalized peak height. A value between 1 and 4 was assigned for each of 13 pesticides (codes a–m) susceptible to the matrix-induced enhancement effect with a wide range of retention times. A ranking of 4 meant that the compound induced a strong effect (among the highest of all compounds evaluated for a particular pesticide). A ranking of 1 meant that the peak response was very poor, even to the point of giving a lower response than standards in MeCN alone. Rankings of 2 and 3 were based on splitting the signal range difference between the 1 and 4 scores, and no ranking ("–") indicates when an interferant occurred that did not allow integration to be possible. It is important to mention, that the ranking does not necessarily reflect the real potential of an agent to protect analytes since the effect is strongly dependent on the concentration of the agents and the activity of the system. Some of the sugars and derivatives tested are isomers and behaved similarly in terms of retention times, degradation products, and enhancement effects. In Table 4, we have therefore grouped all pentoses (codes A01–A05) and hexoses (codes A06–A09) using the average protection effects for ratings. For the sake of brevity, compounds that gave a total ranking less than 22 are not listed in the table.

As the table shows, compound C10, L-gulonic acid γ-lactone (gulonolactone), gave the highest summation of individual pesticide rankings among all compounds evaluated with a score of 50 out of a possible 52 (all of the selected pesticides except methamidophos and acephate gave rankings of 4). The other sugar lactones evaluated also gave high overall scores, and all of the sugars yielded among the highest overall scores. For comparison purposes, the initially chosen analyte protectants for the QuEChERS method, sorbitol (B06) and 3-ethoxy-1,2-propanediol (D05), gave significantly lower summation scores of 34 and 22, respectively (note that the concentration of sorbitol was twice as high as previously and the concentration of 3-*O*-ethylglycerol was 20-fold lower than the

Table 4
Evaluation and ranking of the compounds screened as analyte protecting agents

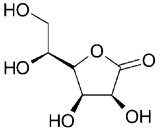
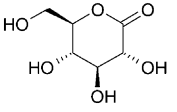
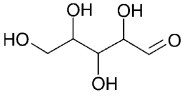
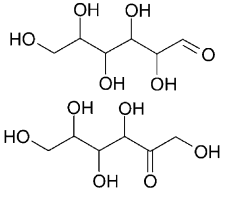
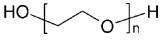
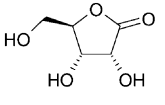
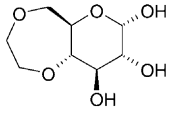
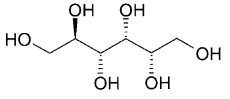
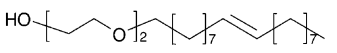
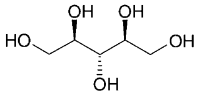
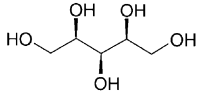
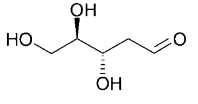
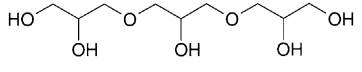
Code	Structure	MW	Retention time(s) of major component(s) (min)	MS base peak	Rating for enhancement effect on selected pesticides													
					a	b	c	d	e	f	g	h	i	j	k	l	m	Sum
C10		178	12.1 (10.9–13.3)[a] 6.7 (6.6–11.1) 16.9 (15.3–18.5)	73 (5) 55 (6) 44 (2)	3	3	4	4	4	4	4	4	4	4	4	4	4	50
C09		178	12.0 (10.8–13.3)[a] 6.7 (6.6–11.0) 16.9 (15.3–18.5)	73 (5) 55 (6) 44 (2)	3	2	4	3	3	4	4	4	4	4	3	4	3	45
A01–A05		150	7.6 (7.0–8.8)[a] 4.9 (5.2–5.6) 5.8 (5.6–6.2) 6.4 (6.3–7.0) 16.7 (15.3–18.4)	73 (1) 43 (0) 57 (2) 45 (0) 43 (4)	4	4	3	4	4	4	4	4	2	3	3	4	2	45
A06–A09		180	4.9 (4.7–5.4) 5.4 (5.3–9.0) 7.3 (7.1–8.6) 7.6 (7.5–8.6) 9.1 (9.0–10.7) 17.0 (15.8–18.6)	43 (0) 97 (2) 57 (2) 43 (1) 43 (3) 43 (4)	4	4	3	4	4	4	4	4	2	2	2	3	2	42
E12		300	5.1 (5.0–6.5) 7.1 (7.0–9.0) 10.1 (10.0–12.0) 14.1 (14.0–16.0) 16.7 (16.6–18.5) 18.6 (18.5–20.5)	45 (0) 45 (1) 45 (1) 45 (1) 45 (2) 45 (2)	3	2	4	1	4	2	2	3	4	4	3	4	2	38
C08		148	8.6 (6.9–10.8)	73 (1)	2	3	2	3	4	4	4	4	2	2	2	3	2	37

Table 4 (*Continued*)

Code	Structure	MW	Retention time(s) of major component(s) (min)	MS base peak	Rating for enhancement effect on selected pesticides													
					a	b	c	d	e	f	g	h	i	j	k	l	m	Sum
C06		206	9.0 (8.7–10.0)[a] 4.9 (4.8–6.3) 6.9 (6.7–7.1) 7.2 (7.1–7.4) 7.5 (7.4–8.0) 8.2 (8.0–10.0)	73 (0) 45 (1) 45 (3) 45 (1) 45 (4) 45 (4)	2	4	3	4	4	4	4	3	2	1	1	2	1	35
B06		182	16.2 (12.2–18.8)	73 (3)	1	1	1	1	3	3	2	3	3	4	4	4	4	34
E07		357	7.3 (7.2–8.7) 9.0 (8.9–11.0) 11.2 (11.1–11.9) 12.0 (11.9–12.4) 14.7 (14.6–15) 15.7 (15.6–16.0) 16.9 (16.8–17.8) 18.7 (18.6–20.0) 21.7 (21.6–23.0)	41 (8) 55 (10) 55 (9) 57 (8) 55 (12) 45 (10) 45 (10) 45 (9) 45 (9)	1	1	2	1	3	4	2	4	4	2	3	4	3	34
B04		152	10.9 (8.3–13.0)	74 (3)	2	1	2	1	3	3	2	3	4	4	3	3	2	33
B05		152	10.1 (7.9–12.0)	61 (2)	2	1	2	1	3	3	3	3	4	4	2	3	2	33
C01		134	6.2 (5.7–10.4)[a] 5.5 (5.4–5.8)	44 (5) 57 (1)	4	3	4	1	2	2	2	2	4	2	2	3	2	33
E14		240	16.5 (14.1–20.0)[a] 7.9 (7.7–10.0)	57 (3) 57 (6)	2	1	2	2	3	2	2	2	3	4	4	4	2	33

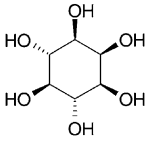
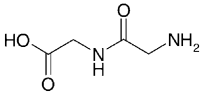
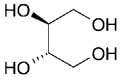
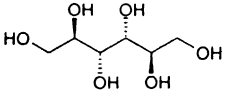
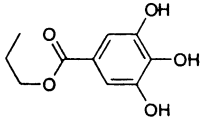
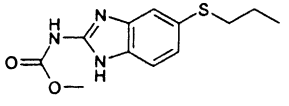
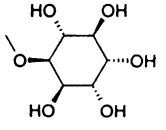
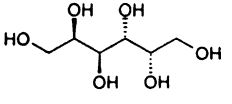
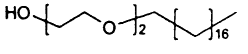
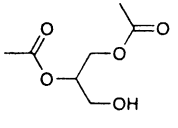
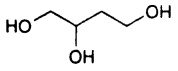
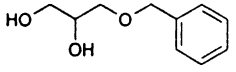
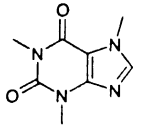
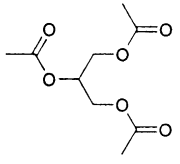
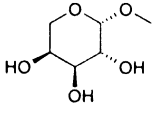
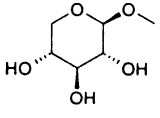
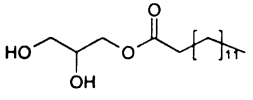
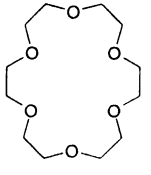
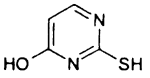
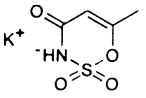
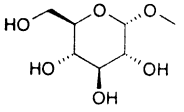
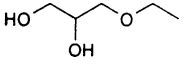
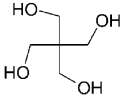
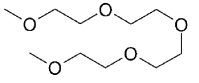
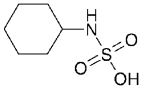
B09		180	16.5 (11.7–18.8)	73 (1)	1	1	1	2	3	3	2	3	3	3	2	4	4	32
G02		132	11.0 (9.9–12.8)	114 (1)	1	1	1	1	2	3	3	4	4	4	2	2	2	30
B02		122	6.1 (6.0–10.0)	61 (1)	4	2	3	2	3	3	2	3	2	1	1	2	1	29
B07		182	16.4 (12.9–18.0)	73 (2)	2	1	2	1	2	2	1	2	3	2	3	4	4	29
G06		212	14.2 (12.3–18.2)[a] 13.2 (11.8–20.0)	153 (4) 209 (9)	1	1	1	1	2	3	4	4	1	3	4	1	3	29
F04		265	20.0 (18.5–24.3)[a] <4.5 (<4.5–8.0) 18.1 (17.5–18.5)	165 (12) 44 (0) 178 (9)	2	1	2	1	1	1	2	2	4	4	4	3	1	28
B10		194	11.2 (8.8–17.2)	87 (4)	1	1	1	1	3	3	2	3	4	2	2	3	1	27
B08		182	15.5 (12.6–17.8)	73 (2)	1	1	1	1	2	2	1	2	2	2	3	4	4	26
E06		359	11.4 (11.2–12.4) 14.8 (14.7–15.8) 16.9 (16.8–18.0) 18.8 (18.7–19.8) 21.7 (21.6–22.7)	43 (9) 57 (7) 45 (8) 45 (8) 45 (7)	1	1	1	1	2	3	2	2	3	2	2	3	3	26
G03		176	5.3 (<4.5–20.0)	43 (1)	2	1	2	1	2	3	2	3	2	1	2	3	2	26

Table 4 (*Continued*)

Code	Structure	MW	Retention time(s) of major component(s) (min)	MS base peak	Rating for enhancement effect on selected pesticides													
					a	b	c	d	e	f	g	h	i	j	k	l	m	Sum
D03		106	5.1 (<4.5–8.0)	75 (1)	3	1	3	1	2	2	2	2	2	1	2	2	1	24
D07		182	7.8 (7.6–12.0)	91 (5)	2	1	4	1	2	2	2	2	2	2	1	2	1	24
F03		194	12.3 (11.8–14.2)	194 (5)	1	1	1	1	2	4	4	4	1	1	1	2	1	24
G04		218	5.8 (5.1–20.0)	43 (2)	2	1	3	1	2	3	2	2	1	1	2	2	2	24
C03		164	6.4 (5.8–11.8)	60 (0)	3	3	4	1	1	2	1	1	2	2	1	1	1	23
C04		164	6.7 (5.9–12.0)	60 (0)	4	3	4	1	2	2	1	1	1	1	1	1	1	23
D08		302	15.7 (15.3–20.0)[a] 14.8 (14.4–24.0)	43 (20) 117 (10)	1	1	2	1	2	2	1	1	4	–	2	3	3	23
E13		264	11.5 (11.2–12.7)	45 (3)	1	1	1	1	2	4	2	4	1	1	2	2	1	23

F02		128	12.0 (11.8–16.0)	128 (2)	1	1	1	1	2	2	4	3	2	2	1	2	1	23
G08		201	8.1 (7.7–16.3)	43 (2)	1	1	2	1	2	4	2	3	1	1	2	2	1	23
C05		194	9.2 (8.0–10.8)	60 (1)	1	2	1	2	4	2	1	1	3	1	1	2	1	22
D05		120	5.9 (5.5–10.0)	31 (1)	3	1	2	1	2	3	2	2	1	1	1	2	1	22
D11		136	7.6 (6.1–9.5)	57 (0)	1	2	2	1	3	3	2	2	1	1	1	2	1	22
E11		222	6.8 (6.7–12.2)[a] 5.7 (5.6–6.2)	59 (1) 45 (0)	3	1	2	1	1	2	2	2	1	1	2	2	2	22
G07		179	5.1 (<4.5–9.0)	56 (2)	1	1	2	–	2	3	2	3	1	1	2	2	2	22

Code refers to the list of compounds in Table 3, and the selected pesticides are marked in Table 1. The numbers assigned are based on a 4-point ranking scale in which the peak heights (normalized to the sum of lindane, chlorpyrifos, and endosulfan sulfate) were given point values in relation to the signal (highest signals = 4). The sum of all 13 values gives the overall enhancement effect for the range of most susceptible pesticides. Also given in the table are the MS base peak (in parentheses appear the number of ions of $m/z > 75$ and relative abundance >10%) and retention time(s) of major component(s) (with time window that the peak appears) in the GC/MS chromatogram.

[a] Peak of the original compound added.

amount used in the QuEChERS method). On the surface, it appears that gulonolactone could be used as the sole analyte protectant for the entire analytical range of pesticides, but further evaluations discussed below lead us to believe that a combination of analyte protectants still probably yield the best results.

3.4. Mass spectral interferences

To help estimate the potential of mass spectral interferences when using the various agents, the following indicative parameters have been included in Table 4: (1) the molecular weight (MW) of each agent; (2) the retention time ranges of all agents and/or of their major degradation products; and (3) the base peaks from the mass spectra, and in parentheses the number of ions with $m/z > 75$ that exceeded 10% relative abundance of the base peak. In many cases, several peaks appeared in the chromatogram due to degradation of the analyte protectant in the hot GC system and/or in solution. The chromatographic peak for the original compound added to the solution is denoted in the table when it could be determined through mass spectral library identification with NIST'98.

Interestingly, none of the most promising analyte protectants gave interferences in the SIM analysis (identification and integration) of the 30 selected pesticides using a quadrupole GC/MS instrument. This can be generally explained by the low mass fragments that occur from the protectants, which are less likely to interfere with the higher mass ions of pesticides. Overall, only isolated instances of interferences occurred for the 30 pesticides at the chosen ions in the experiments with the 93 protecting agents (only two instances in Table 4). In cases when an interference occurs, the generally broad, significantly tailing peak of an effective protecting agent could be easily distinguished from analyte peaks. When feasible, different ion(s) could be chosen for quantitation of the affected analyte. However, even if no interference occurs in the SIM mode, the identification of full-scan mass spectra with mass spectral libraries can still be adversely affected by the presence of the analyte protectants. Furthermore, we have not tested the concept on ion trap or time-of-flight instruments that may be prone to indirect MS interferences when a large co-elution occurs with the analyte, even though the targeted ions can be much different from those of the large co-elutant.

3.5. Effects of volatility

As concluded in the preliminary experiments, volatility (retention time coverage) of the protecting agent was an important factor in the enhancement effect, and the larger experiment more clearly presents this aspect. The pesticides a–m in Table 4 appear in order of t_R from left to right on the page, and by looking at the individual scores, it becomes apparent how the rankings correlate fairly well versus elution profile of the agents. Compounds C04 (1-*O*-methyl-β-D-xylopyronoside), C08 (ribonolactone), and B06 (sorbitol) serve as examples of the effect. From left to right, C04 gives rankings of 4, 3, 4, 1, 2, 2, 1, 1, 1, 1, 1, 1, 1, which indicates how it mainly protects only the early-eluting pesticides. In the case of C08, the order of the rankings is 2, 3, 2, 3, 4, 4, 4, 4, 2, 2, 2, 3, 2, which shows how the compound is very effective for pesticides with retention times in the middle of the chromatogram, but not at the beginning or end. In the case of B06, the scores 1, 1, 1, 1, 3, 3, 2, 3, 3, 4, 4, 4, 4 reflect how it protects only late-eluting compounds. On the other hand, the elution profile for gulonolactone (C10) and its degradation products covers almost the entire pesticide t_R range, which was the main reason that it gave the highest overall score.

An interesting example to show the effect of degradation products is the comparison between the result for gluconolactone (C09) and 1-*O*-methylglucopyranoside (C05), which on the surface have very similar structures. Dramatic differences occur in the enhancement effects on the susceptible pesticides as indicated by the ranking score of 45 for C09 and 22 for C05. The reason for this large disparity is linked to the greater stability of C05 in the solution and GC system. Thus, C05 generates a relatively narrow elution profile centered at 9.2 min in the chromatogram and has minimal impact, whereas C09 (and all of the good protecting agents) breaks down into a number of compounds to better mask and co-elute with a wider array of analytes of diverse volatility. This further demonstrates that masking of active sites in the inlet is not the only factor that is involved in the matrix-induced enhancement effect.

3.6. Effect of concentration

Concentration of the analyte protecting agents in the injected solution is another important factor in the response enhancement effect. To achieve routine application of the analyte protection concept in real-world pesticide analysis, a single concentration of analyte protectant solution would be added to both standards in solvent and matrix extracts to give a consistently maximized response to all analytes of interest. In theory, a limited number of active sites occurs in a particular GC system (liner + column combination), and their saturation can be reached if enough agent is present to mask all active sites. However, the masking effect is only temporary, and the degree of protection that the various analytes experience during the chromatographic run depends on the elution characteristics and concentration of the agent and, of course, on the local activity of the system.

To determine the effect of concentration, we conducted an experiment that measured peak heights of the different pesticides (at 0.5 μg/ml in MeCN) with respect to increasing concentration of gulonolactone (the best overall analyte protectant in Table 4). Fig. 5 shows the results of this experiment. The GC conditions were so poor that without the analyte protecting agent, no response was observed for some of the pesticides (that is why the 0 mg/ml bar cannot be seen for dimethoate and coumaphos). For carbaryl, thiabendazole, phosalone, and coumaphos (and most other pesticides with $t_R > 12$ min), maximized responses (a response plateau) were achieved at gulonolactone concentrations exceeding ≈10 mg/ml, while in the cases of fenthion and *o*-phenylphenol, this saturation effect was observed at lower concentrations. Unfortunately, at gulonolactone concentrations >10 mg/ml, peak splitting occurred for pesticides with $t_R < 10$ min (such as *o*-phenylphenol and dimethoate). This had a negative impact on the peak height of these analytes and explains the absence of a response plateau in their case in Fig. 5. The peak splitting is likely a result of droplet formation in the column due to the higher content of water and agent in the 10 and 20 mg/ml gulonolactone solutions [19,20].

3.7. Observations about certain pesticides

The overall rankings and main conclusions became clear when the data was compiled in Table 4, but several observations and notes were taken while all of the

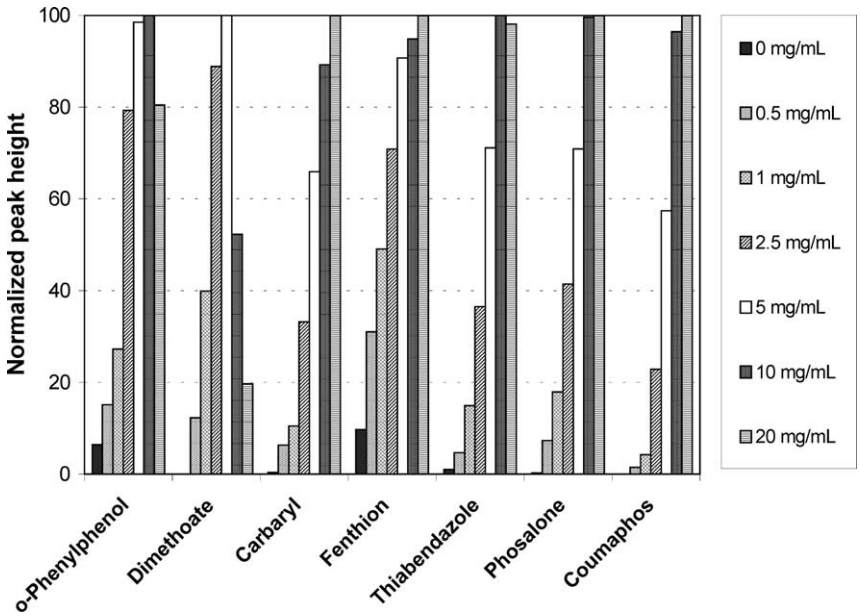


Fig. 5. Effect of concentration of gulonolactone (C10 in Table 3) in MeCN solution on the peak height of selected pesticides at 1 μg/ml (normalization was to the highest peak height for each pesticide in the series of injections).

pesticide peaks were being integrated because some interesting results for certain pesticide/compound pairs would have been missed otherwise. For example, deltamethrin sometimes gave two peaks, but only the pure *cis*-isomer was added to the solution. Some of the deltamethrin was converted to the *trans*-isomer either in solution or in the GC inlet when certain compounds were present in MeCN. This is not an uncommon event for deltamethrin in pesticide analysis with acetone and MeCN, and we are conducting further investigations to further characterize this effect.

Several of the 30 targeted pesticides were only slightly affected by any of the analyte protectants, if at all. Analytes that were not susceptible to the enhancement effect included lindane, chlorpyrifos, and endosulfan sulfate. For this reason, those pesticides served as excellent internal standards to normalize the signal against in the evaluation of the different compounds in the case of susceptible pesticides. Also, diazinon was only slightly affected by the analyte protectants.

Other pesticides were unaffected in nearly all cases except a curious enhancement would occur in a few spurious instances. For example, vinclozolin gave a very reproducible normalized peak height except when co-injected with benzoin (G05), which gave a 14-fold increase versus the solvent standard. In this case, the peak was exceptionally narrow and tall which means the effect was chromatographic rather than degradative. The t_R also shifted from 10.74 min in MeCN alone to 10.87 min in the presence of benzoin, which co-eluted with vinclozolin (benzoin $t_R \approx 10.8$ min).

Similar events happened for other pesticide/compound pairs. For instance, the co-elution of 18-crown-6 ether (E13) with metalaxyl at 11.5 min increased peak height of metalaxyl by a factor of 2 and shifted its t_R from 11.51 to 11.57 min. Caffeine (F03, t_R = 11.8–14.2) gave peak height enhancement effects on several pesticides that eluted at approximately 12 min, which included: carbaryl (14-fold enhancement), methiocarb (7.7-fold) fenthion (2.5-fold), and cyprodinil (2.8-fold). Interestingly, the competition of these pesticides with the high concentration of caffeine caused $\approx$7 s shifts to shorter t_R rather than delayed elution as in the previous examples.

Cyprodinil injected with 2-thiouracil (F02) and propyl gallate (G06) gave three times higher peaks for standards in analyte protectants versus standards in MeCN alone. Propyl gallate also worked exceptionally well in the case of folpet (10-fold significant improvement). Furthermore, folpet response was also improved by a factor of 4 with Brij 72 and 92 (E06 and E07) and the sugar lactones (C10, C09 and C08); these lactones worked very well for dicofol, too. Permethrin in combination with sorbitol (B06), Brij 72 and 92 (E06 and E07), and albendazole (F04) increased relative response factors by 1.8, 2.0, and 4.5 times, respectively.

3.8. Possible mechanisms

In the GC analysis of pesticides and other analytes in general, the optimal intensity and quality of the peaks relies on several facets in the process: (1) stability of the analytes in the sample solution; (2) complete transfer of the analytes from the injection port to the column; (3) minimization of peak distortion during focusing of the analyte at the front of the column and peak broadening (due to diffusion) during the separation process; and (4) elimination of peak tailing of the analyte due to strong interactions with the stationary phase (primarily with silanol groups in the case of polar pesticides). In consideration of these factors with respect to our results, we have postulated four mechanisms that may contribute to the analyte protecting process, in respective order: (1) the use of stabilizing compounds, such as acids, prevents the degradation of base-sensitive pesticides; (2) the transfer efficiency of the injection process is increased through the use of masking agents that fill the active sites in the liner and front of the column which act to reduce irreversible adsorption and degradation of analytes; (3) peak broadening is reduced due to the decreased diffusion (in both the gas and stationary phases) in the presence of the highly concentrated co-eluting agent which, presumably, increases the viscosity of the gas phase within the elution band and competes with a substantially smaller amount of the analyte in the partition process, such as in the vinclozolin/benzoin and similar examples described in Section 3.7.; and (4) tailing is minimized when active sites (e.g. silanol groups) on the stationary phase are being masked by a co-eluting compound with high affinity and/or high concentration. This latter case may entail physico-chemical interactions with the active sites (such as hydrogen bonding) or simply a physical blockage of analyte access to the active sites.

In addition to improved peak shapes and intensities, t_R shifts were observed in the case of certain analyte/agent combinations. Shifts to shorter t_R can be explained by reduced interactions of the analyte with the stationary phase and the active sites, whereas shifts to longer t_R are more difficult to understand. We conceptualize that the latter case can be attributed to the following two mechanisms: (1) the highly concentrated agent acts like a "keeper" to delay the vaporization of the affected analytes to some extent; and (2) interactions (such as hydrogen bonding) occur between the analyte and the co-eluting protecting agent, that temporarily modifies the stationary phase, resulting in a slightly increased analyte retention. In the first case, shifts to longer t_R should occur simultaneously for various analytes of similar volatility. This was observed in the case of 3-*O*-ethylglycerol at high concentrations, where peaks of several early-eluting analytes, such as dichlorvos (see Fig. 3b), methamidophos and acephate were shifted to longer t_R. The second proposed mechanism would only affect analytes, which physico-chemically interact with the agent. Such an effect was observed in the case of thiabendazole and imazalil when co-injected with 3,5-diaminobenzoic acid (see Fig. 3a). In this experiment, peaks of these two compounds shifted to longer t_R while other compounds, such as chlorpyrifos, which eluted even closer to the agent were not affected at all.

3.9. Quantitation using analyte protectants

A major advantage in the use of analyte protectants is the possibility to avoid matrix-related errors in GC analysis. Ideally, the analyte protectants should provide the same degree of protection (signal enhancement) regardless of whether the solution contains matrix components or not. In order to determine if this was the case, we fortified pure solvent and sample extracts (peach extracted using the QuEChERS method) with a mixture of the 30 pesticides at 1 μg/ml. These solutions were injected with and without the addition of 0.5 mg/ml of gulonolactone (C10), and the comparison was made between the relative response enhancement effects (or positive bias in a calculated concentration for a real sample using the calibration standards in solvent with and without the use of the analyte protectant). Peak areas were used in this experiment rather than peak heights.

As shown in Fig. 6, the errors due to matrix effects were dramatically reduced with the help of 0.5 mg/ml gulonolactone. Previously, a combination of 3-*O*-ethylglycerol and sorbitol was shown to work

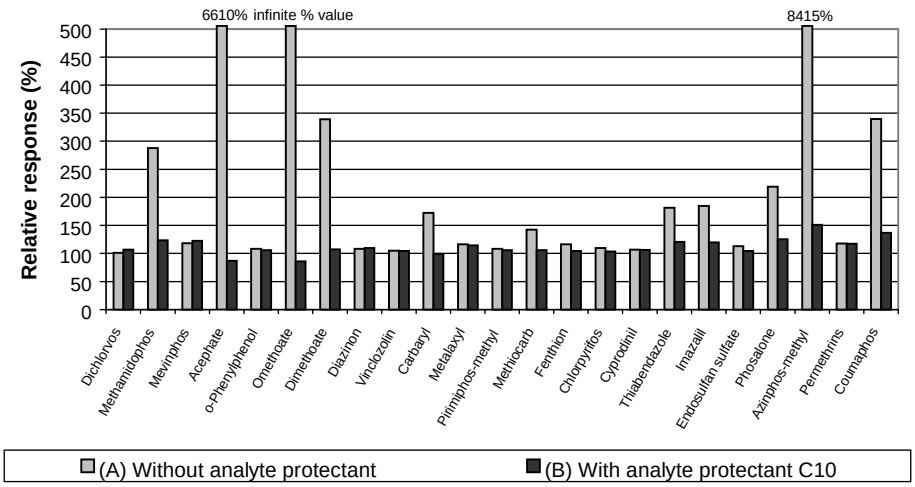


Fig. 6. Comparison of relative responses of selected pesticides at 0.5 μg/ml obtained (A) without and (B) with 0.5 mg/ml gulonolactone (C10) added as the analyte protectant. The relative responses were determined from the relationships: (A) peak area in peach matrix-matched standard/peak area in solvent standard × 100%, and (B) peak area in matrix + analyte protectant/peak area in solvent with analyte protectant × 100%. A value of 100% relative response is the correct one, and values >100% are overestimations due to the matrix-induced response effect.

similarly [18]. The use of analyte protectants is thus a good alternative to other approaches dealing with the problem of matrix-induced signal enhancements.

4. Conclusions

This report re-introduced the concept of reagent masking agents, or analyte protectants as we prefer to call them, to provide a consistently high matrix-induced enhancement effect for the analysis of pesticide residues. In the previous study [2], the authors only tested eight compounds and concluded that the concept was not viable. In this study, 93 different compounds were evaluated as analyte protectants, and the results demonstrate that the approach is feasible with the appropriate protecting agent(s). A variety of sugars and sugar lactones provided the best overall effect for the volatility range of GC-amenable pesticides tested (from dichlorvos to deltamethrin). Further work should be done to evaluate the performance of the best protecting agents in actual analyses of pesticide residues and other applications.

The advantages of using analyte protectants include: (1) intensity and shape of analyte peaks are improved; (2) cleanup of extracts can be conducted without concern for loss of matrix-induced enhancement; (3) peak identification and integration become easier and more accurate; (4) as a result, greater selectivity, lower detection limits, and greater confidence in the results may be achieved; (5) errors caused by matrix-induced enhancement effects can be eliminated without the need to perform inconvenient approaches, such as matrix-matching; (6) the policies of US federal agencies do not preclude the use of analyte protectants in regulatory analyses concerning pesticide residues in food; (7) the approach is very easy, fast, and inexpensive; and (8) less maintenance of the GC system is needed because even a very dirty system can provide good results with the use of analyte protectants.

The long-term influence of the analyte protectants on the GC/MS system still needs to be evaluated, but we have made approximately 1000 injections using analyte protectants in our GC/MS quadrupole instrument without conducting a source cleaning. The analyte protectants do not give interferences in the GC/MS SIM analyses of the 30 pesticides tested at our conditions, but indirect effects in GC/MS analyses using ion trap or time-of-flight instruments have not been investigated.

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