



Using Analyte Protectants and Solvent Selection to Maximize the Stability of Organophosphorus Pesticides during GC/MS Analysis

Application Note

Authors

Eduardo Morales
Weck Laboratories, Inc.
Industry, CA
USA

Anthony Macherone
Agilent Technologies, Inc.
Wilmington, DE
USA

Abstract

Several solvents and analyte protectants were evaluated for their ability to stabilize and maximize the recoveries of a number of organophosphorus pesticide residues during GC/MS analysis. Hexane provided the best analyte stability for a wide range of pesticides, and d-sorbitol offered the most benefit to recovery as an analyte protectant.

Introduction

When performing gas chromatography/mass spectrometry (GC/MS) analysis, ensuring that the integrity of the analytes will not be compromised upon injection or while in solution is an endless battle. However, due to matrix effects and the instability of certain analytes in some solvents, many analytes are reported with skewed results.

The quantitation of pesticides can be adversely affected by a phenomenon known as the matrix-induced chromatographic response enhancement effect [1]. This effect is noted by improved chromatographic peak intensity and shape when affected compounds are injected in the presence of a complex matrix. Matrix components mask potentially active sites in the GC flow path, leading to fewer of these sites being available to interact with analytes, and thus fewer losses of analytes and better peak shapes.

One approach to compensate for the effect of matrix is to use analyte protectants which bind to potential active sites in the GC flow path and inhibit their interference in the analysis. In this way, the benefit of matrix-induced chromatographic enhancement (larger and sharper peaks) can be attained without the risk of matrix interferences.



Agilent Technologies

Some pesticides are also not stable in some of the common extraction solvents. For example, certain organophosphorus pesticides stored in ethyl acetate solutions were seen to degrade over long periods of time and elevated temperatures [2, 3].

This application note evaluates the stability of a number of organophosphorus pesticide residues in several solvents, resulting in the selection of an optimal solvent. It also evaluates the positive effect of several analyte protectants on the recoveries of organophosphorus pesticides during GC/MS analysis. Hexane provided the best analyte stability for a wide range of organophosphorus pesticides, and d-sorbitol provided the most consistent improvement in recovery as an analyte protectant.

Experimental

Standards and Reagents

Organophosphorus pesticide standards were obtained from CPI International.

Solvent	Source
Hexane	Fisher Scientific, pesticide grade
Methylene chloride	Burdick & Jackson, high purity
Methyl tertiary butyl ether	Fisher Scientific, HPLC grade
Nonane	Sigma-Aldrich 99% purity
Ethyl acetate	Burdick & Jackson, pesticide grade

Analyte protectant	Source
3-Ethoxy-1,2-propanol	CAS - 1874-62-0, Sigma-Aldrich 98% purity
L-Gulonic acid γ -Lactone	CAS - 1128-23-0, Sigma-Aldrich 95% purity
Olive Oil	Carbonell, Lot No. 82519B-45272
D-Sorbitol	CAS - 50-70-4, Sigma-Aldrich 99% purity

In addition, a client sample of a mixture of ground and waste water was used to test the effect of matrix.

Instruments

The experiments were performed on an Agilent 7890A Series GC equipped with a multimode inlet (MMI) and an Agilent 7693A autosampler, coupled to an Agilent 7000B Triple Quadrupole GC/MS in EI mode. Backflush was performed using two 15 m \times 0.25 mm, 0.25 μ m columns connected using the Agilent Purged Ultimate Union (p/n G1472A) and electronic pneumatic control (EPC) module. Table 1 lists the instrument conditions.

Table 1. Agilent 7890A/7000B Gas Chromatograph and Triple Quadrupole Mass Spectrometer Conditions

GC run conditions	
Analytical columns	Columns 1 and 2: Agilent J&W HP-5ms Ultra Inert, 15 m \times 0.25 mm, 0.25 μ m (p/n 19091S-431UI)
Injection volume	1 μ L
Injection mode	MMI
Inlet temperature	65 $^{\circ}$ C hold for 0.2 minute, then to 270 $^{\circ}$ C at 600 $^{\circ}$ C/min
Gas saver	On, 20 mL/min after 3 minutes
Purge flow	1 mL/min
Vent flow	20 mL/min at 1 minute
Vent pressure	13.842 psi
Average velocity	23.498 cm/s
Cryo	On
Cryo use temperature	200 $^{\circ}$ C
Fault detection	On
Timeout detection	On 10 minutes
Oven temperature	60 $^{\circ}$ C for 1 minute 40 $^{\circ}$ C/min to 170 $^{\circ}$ C, hold for 0 minutes 10 $^{\circ}$ C/min to 31 $^{\circ}$ C, hold for 1 minute
Carrier gas	Helium in constant flow mode Column 1: 1.088 mL/min Column 2: 1.188 mL/min
Transfer line temp	300 $^{\circ}$ C
Run Time	22.3 minutes

GC backflush conditions	
Run time	4 minutes
Oven temperature	310 $^{\circ}$ C
Column flow	Column 1: -36341 mL/min; Column 2: 4 mL/min

MS conditions	
Tune	AutoTune FC-43
Acquisition parameters	Multiple reaction monitoring (MRM)
Collision gas	Constant flow, 1.5 mL/min
Quench gas	Constant flow, 2.25 mL/min
Solvent delay	4 mL/min
MS temperatures	Source 300 $^{\circ}$ C; Quadrupole 150 $^{\circ}$ C

Sample Preparation

The matrix used for comparison was provided by an Agilent customer, and consisted of a mixture of ground and waste water from Orange County California. It was extracted per EPA method 3535, using a Horizon 4790 automated extractor. The sample was extracted on a C-18 disk (UCT- ECUNI525) and eluted with two aliquots of methylene chloride and ethyl acetate. It was then concentrated to 1 mL under a gentle stream of nitrogen.

For the recovery study, organophosphorus pesticide standards were dissolved in the appropriate solvents at 10 parts per billion (ppb). For the analyte protectant study, the organophosphorus pesticide standards were dissolved in 1 mL of hexane at 10 ppb, and the appropriate protectant was added at 1,000 parts per million (ppm).

Analysis Parameters

Table 2 lists the analysis parameters.

Table 2. Agilent 7000B Triple Quadrupole GC/MS Analysis Parameters

Compound name	Precursor ion	Product ion	CE (V)	Compound name	Precursor ion	Product ion	CE (V)
Bolstar	322.1	155.9	5	Guthion	160.1	132.1	5
Bolstar	322.1	97	25	Guthion	160.1	77.1	20
Chlorpyrifos	313.8	258	14	Malathion	173.1	117.1	5
Chlorpyrifos	196.9	168.9	15	Malathion	173.1	99	15
Coumaphos	362	334	6	Merphos	201.9	112.9	10
Coumaphos	362	109	15	Merphos	168.9	57.1	5
Demeton-O	171	114.9	5	Mevinphos	192	127	10
Demeton-O	171	96.9	25	Mevinphos	127	109	10
Demeton-S	169.9	114	5	Naled	144.9	108.9	10
Demeton-S	88.1	59	15	Naled	108.9	79	5
Diazinon	304	179	15	Parathion ethyl	291	109	10
Diazinon	179.1	121.1	40	Parathion ethyl	291	81	10
Dichlorvos	185	109	15	Parathion methyl	263	246	2
Dichlorvos	185	93	15	Parathion methyl	263	109	10
Dimethoate	229	87	10	Phorate	260	75.2	10
Dimethoate	143	111	10	Phorate	231	175	10
Disulfoton	274	88.1	5	Ronnel	287	272	12
Disulfoton	88	60	5	Ronnel	285	270	12
Ethoprop	158	114	5	Stirophos	333	109	18
Ethoprop	158	97.1	15	Stirophos	331	109	25
Fensulfothion	293	141	10	Tokuthion	309	239	15
Fensulfothion	293	125	10	Tokuthion	267	239	5
Fenthion	278	168	18	Trichloronate	297	223	34
Fenthion	278	151	12	Trichloronate	296.8	269.2	16

Results and Discussion

Organophosphorus Pesticide Stability in Solvents

The recoveries of organophosphorus pesticides analyzed by GC/MS varied significantly with the solvent used, and some solvents provided better results than using extracted blank matrix as the solvent (Figure 1). While methylene chloride and ethyl acetate have often been the solvents of choice for the analysis of organophosphorus pesticides, recoveries using these solvents were most often lower than those obtained

with other solvents or extracted matrix. Hexane provided the highest recoveries with every pesticide tested, including those obtained in extracted blank matrix. In general, recoveries were highest with solvents having a low polarity index, while methylene chloride and ethyl acetate, with polarity indexes of 3.1 and 4.4 respectively, gave the lowest recovery values. The relative recoveries using hexane, extracted matrix, and ethyl acetate illustrate the significant advantage of using hexane and extracted matrix, rather than ethyl acetate (Figure 2).

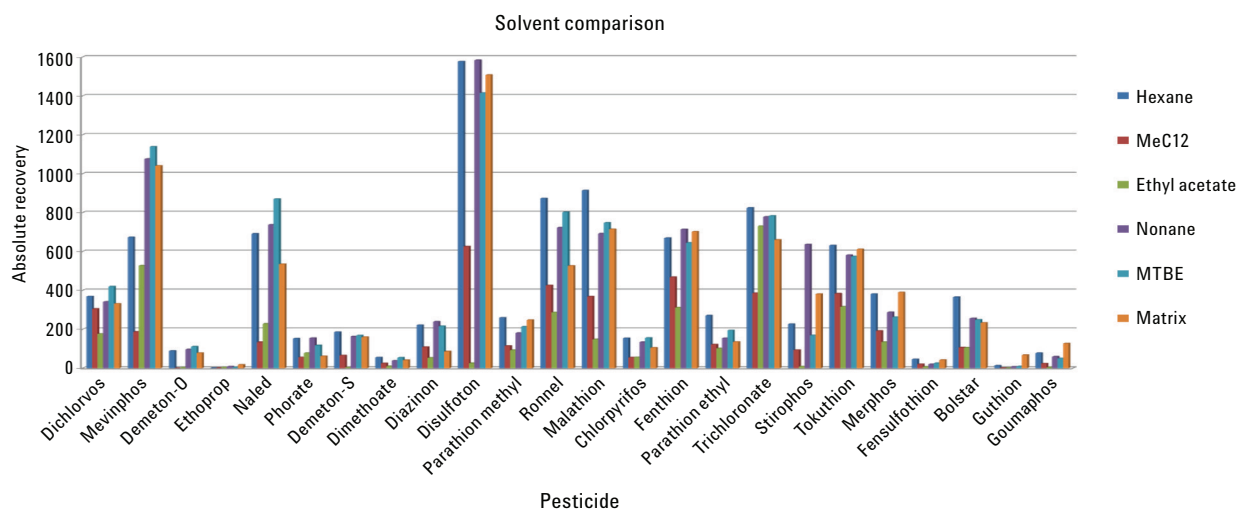


Figure 1. Absolute recoveries for 24 organophosphorus pesticides in various solvents.

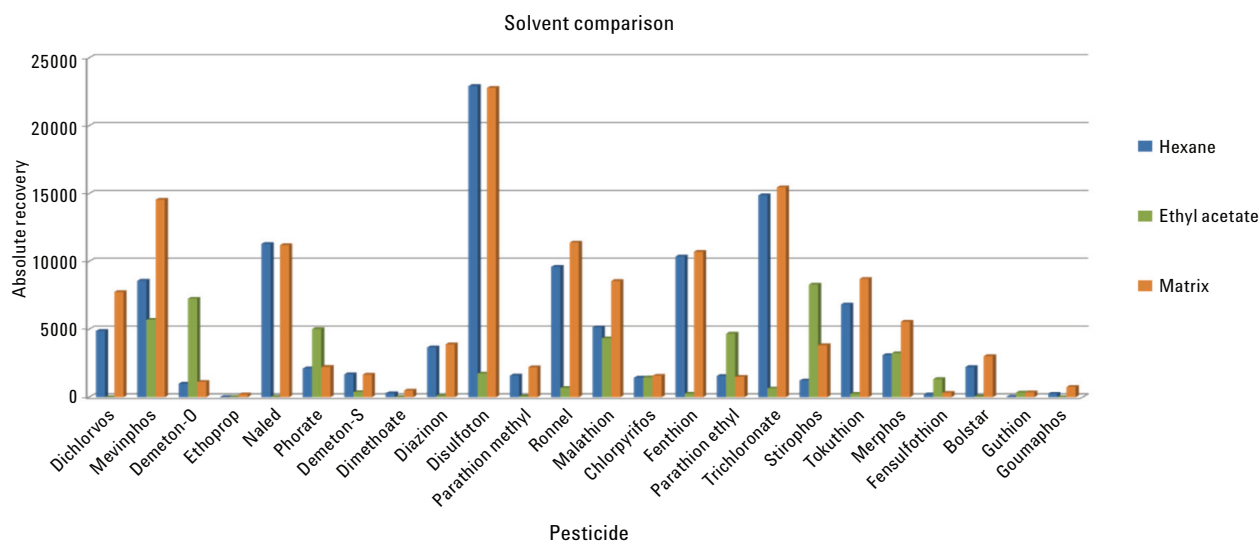


Figure 2. Comparison of recoveries using hexane, ethyl acetate and extracted matrix.

The Benefit of Analyte Protectants

Active sites may occur within the GC flow path as free silanols within poorly or nondeactivated glass liners, poorly or nonuniformly coated open tubular columns that expose the fused silica, or even some hardware interfaces. The use of analyte protectants that are generally rich in polar hydroxyl groups has been shown to mitigate these effects [1]. Although the mechanisms are not fully understood, it is postulated that reversible hydrogen bonding between the active site and the analyte protectant or Langmuir binding is responsible for the effect. While extracted blank matrix can also be used for this purpose, it can be time-consuming and costly, while introducing more variables and possible interferences.

With the exception of olive oil, the recovery of every pesticide tested (except dichlorvos) was improved significantly by the addition of each protectant studied (Figure 3). However, d-sorbitol generated the highest recoveries for more than half of the pesticides tested.

Analyte protectants can also be used to increase the stability of organophosphorus pesticides. When some pesticide standards were stored in 2 mL amber vials in hexane over a period of four weeks, their recoveries were seen to decrease dramatically. However, with the exception of olive oil, when stored in the presence of an analyte protectant, stability over time was improved dramatically (Figure 4). The greatest increase in recovery was observed when the pesticides were stored in the presence of d-sorbitol.

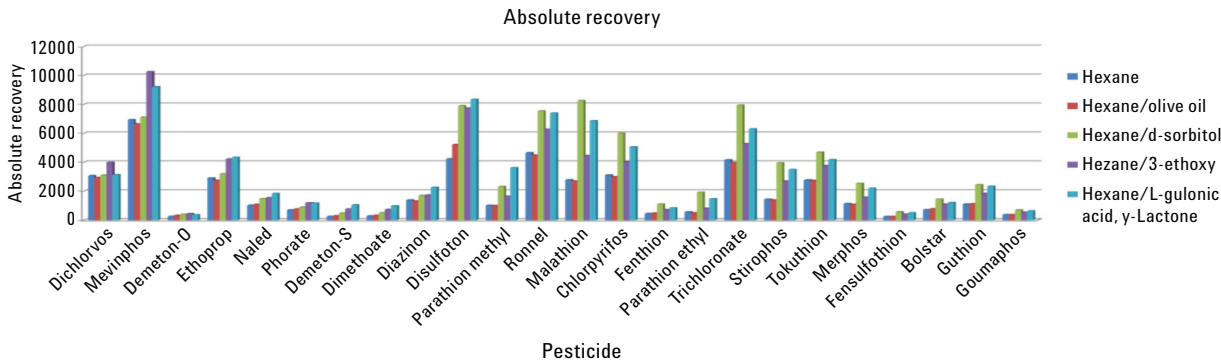


Figure 3. Comparison of recoveries using hexane as solvent, as well as several different analyte protectants.

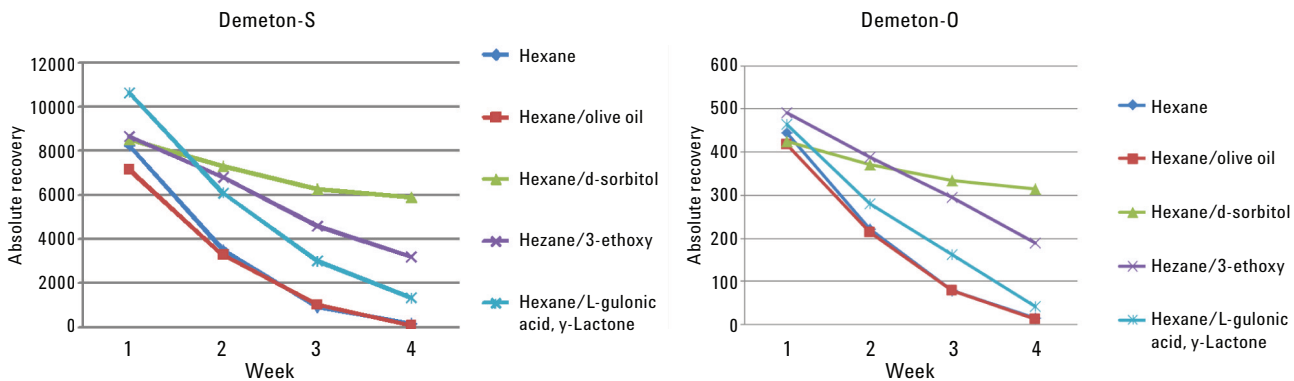


Figure 4. The effect of analyte protectants on the stability of demeton-S (A.) and demeton-O (B.) stored over four weeks. All tested protectants improved stability, with the exception of olive oil, and the presence of d-sorbitol improved their stability the most.

Conclusions

The choice of solvent can impact GC/MS analysis results for the organophosphorus pesticides, and hexane provided the best recoveries for the solvents tested. Commonly used solvents such as methylene chloride and ethyl acetate had adverse effects on the majority of compounds.

When used as analyte protectants, 3-ethoxy-1,2-propanol (33%) and d-Sorbitol (54%) increased recoveries of early and late eluting compounds respectively. Some analyte protectants, notably d-sorbitol, can also prolong the stability of some organophosphorus pesticides, when stored for a four week period in hexane. These solvent and protectant effects should be taken into account when using calibration curves to quantitate organophosphorus pesticides in complex matrices.

References

1. M. Anastassiades, K. Mastovská, S. J. Lehotay "Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides." *J. Chromatogr. A.* **10**, 163-84 (2003).
2. V. Kocourek, J. Hajslova, K. Holadova, J. Poustka "Stability of pesticides in plant extracts used as calibrants in the gas chromatographic analysis of residues." *J. Chromatogr. A* **800**, 297-304 (1998).
3. S.L. Reynolds, et al., Intercomparison Study of Two Multi-Residue Methods for the Enforcement of EU MRLs for Pesticides in Fruit, Vegetables and Grain (SMT4-CT95-2030), Phase I, Progress Report EUR 17870 EN, European Commission, Brussels, 1997.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc., 2013
Printed in the USA
October 25, 2013
5991-1808EN



Agilent Technologies