Stability assessment of organophosphorous pesticides during GCMS analysis in the presence of analyte protectants and frequently used organic solvents.

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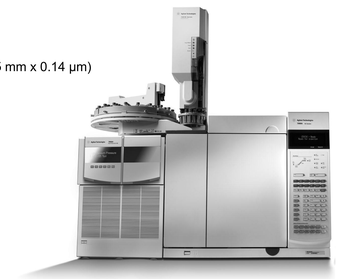
Overview

An endless battle when performing GCMS analysis is the assurance that the integrity of the analytes will not be compromised upon injection or while in solution. However, due to the effects of matrix induced chromatographic enhancement and analyte solvent stability, a wide variety of analytes affected are miscalculated and reported with skewed results. This study evaluates this effect and normalizes the recoveries between standards made in the company of analyte protectants and ones made in matrix. It also evaluates the stability of a number of organophosphorous pesticide residues under the presence of different solvents (nonane, methylene chloride, ethyl acetate and hexane) for GCMS analyses. An assortment of analyte protectants (d-sorbitol, olive oil, 3-ethoxy-1,2-propanol, glycerol and L-gulonic acid, γ-Lactone) are also taken into consideration on a diverse group of phosphorylated pesticides, particularly pesticides containing thioether (R-S-R') functionalities such as disulfoton, demeton-s, demeton-o and phorate. The matrix selected was prepared using a clients sample (a mixture of ground and waste water) sampled from Orange County California.

Methods and Equipment

Analytical instrument and parameters

GCMS system - Agilent 7000B tandem MS
Injector - Agilent mix mode injector (MMI)
Injection liner - 4 mm with gooseneck down
Autosampler - Agilent 7693
Analytical column - Two 15 m J&W DB-5 (15 m x 0.25 mm x 0.14 µm)
Data System - Agilent Mass Hunter
Injector Temperature - 250 °C
Oven Temperature - 60 °C for 1 min
40 °C to 170 °C hold 0
10 °C to 310 °C hold 1 min
Injection volume - 1 µL
Analysis type - MRM
Transfer line - 300 °C
Pressure - 13.842 psi
Flow - 1.088 mL/min
Average Velocity - 23.498 cm/sec



Reagents and Chemicals

Standards:

Organophosphorous Pesticide standards were obtained from CPI international. (P/N - C623-08)

Solvents:

Hexane - Fisher Scientific, Pesticide grade. P/N - H300-4

Methylene Chloride– B&J, high purity. P/N - CS299-200

MTBE - Fisher Scientific, HPLC grade. P/N - E127-4

Nonane - Sigma Aldrich 99% purity. P/N - N29406-100mL

Ethyl Acetate - B&J, pesticide grade. P/N - CAT100-4

Matrix - Client sample extracted with ethyl acetate.

Analyte protectants

3-ethoxy-1,2-propanol –CAS - 1874-62-0. Aldrich 98% purity P/N – 260428-1G

L-Gulonic acid γ-Lactone - CAS - 1128-23-0. Aldrich 95% purity P/N – 31,030-1-1G

Olive Oil - Carbonell Lot No. 82519B-45272

D-Sorbitol - CAS - 50-70-4 - Aldrich 99% purity P/N – 240850-5G

Solvent Determination

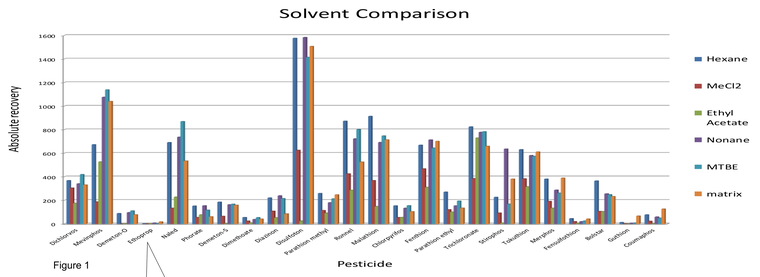


Figure 1

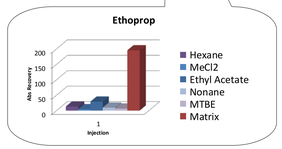


Figure 2

. 10 ppb standard was prepared in each solvent and injected into a GCMS system. The average recoveries were calculated and tabulated; represented in figure 1.

. Although the recoveries of most of analytes fluctuated between solvents. There were no extreme change observed for a few compounds such as ethroprop and dimethoate in different solvents. Figure 2.

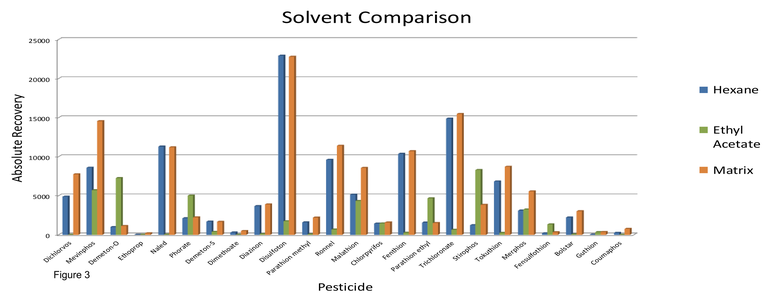


Figure 3

. Cleanup of figure 1 exemplifies a better understanding of the benefits of not only incorporating a matrix into the calibration but of choosing the right solvent for a particular analysis.

. Matrix was obtained by the extraction of analyte free sample into ethyl acetate.

. An increase in recovery of over 62% of the compounds was observed when hexane was used instead of ethyl acetate.

. The use of hexane show a significant improvement in recoveries over ethyl acetate.

Discussion

Traditionally, methylene chloride and ethyl acetate have been the solvents of choice for the analysis of organophosphorous pesticides via GCMS. However, as the study suggests, although not for the entire lists of compounds, hexane is a much better candidate for this type of analysis.

Further studies

. It was observed that the recoveries were most stable with solvents that have a low polarity index (P'). Traditional solvents such as methylene chloride and ethyl acetate covering polarity index of 3.1 and 4.4, respectively, were among the worst solvents to use.

Analyte Protectant

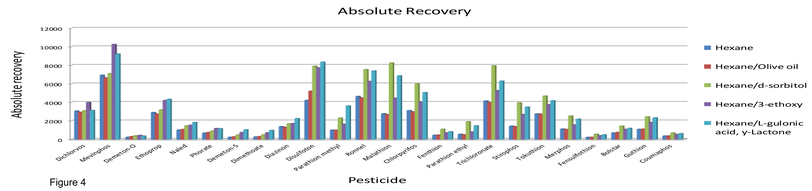


Figure 4

. 1000 ppm of each analyte protectant was added into 1 mL of hexane.

. 10 ppb pesticide standard was spiked.

. When using a protecting agent, significant recoveries was observed for 100% of the pesticides tested when compared to recoveries using pure solvent (hexane).

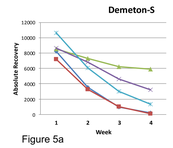


Figure 5a

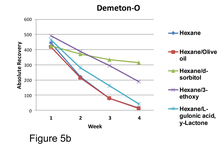


Figure 5b

Discussion

Since the primary focus is often placed on the sample preparation and analytical portion of the analysis. The link between the two and the possible interference is often forgotten and underdeveloped. Because sample preparation frequently brings more variables to the table, the need to normalize the response between a calibration and samples is essential and crucial to obtain accurate results. As presented in figure 1, matrix induced chromatographic enhancement plays one of the final roles on the integrity of each compound and accuracy of the reading, choosing the right solvent is detrimental for any GCMS analysis.

With this in mind, there are a number of approaches that can be taken to better control of the analyses. The effects of matrix induced chromatographic enhancement can be solved by the addition of one or possibly a mix of protectant compounds that are aliphatic or rich in hydroxyl groups (-OH)[1] and protect analytes of interest by adhering to active sites that present themselves in the liners and columns as the sample is introduced to the system. Alternatively, this can also be done by extracting blank samples with the matrix of interest and using this to make the standards. Although that process works well and has been confirmed by this and other studies, it tends to be costly, labor intensive and introduces more variables to the system.

Although solvents can improve the stability of these pesticides for a few injections, to prolong the reproducibly and reliability of these compounds, analyte protectors were found to be effective in preventing the degradation of a few analyte. As seen in figure 5a and 5b, lower analyte response was found with the absence of a protecting agent when standards were held and injected over a period of 4 weeks. However, when d-sorbitol is introduced along with the analytes, time tends to have a less drastic impact on the target compound than without it or with other protectants.

Further studies

. Study the effect when d-Sorbitol and 3-ethoxy-1,2-propanol are added at the same time to a standard.

. Use different concentrations of protectant to determine whether recoveries are dependent of the amount of analyte protectant.

Conclusion

. Hexane provided the best recoveries when compared to five other solvents.

. Commonly used solvents such as methylene chloride and ethyl acetate had adverse effects on the majority of compounds.

. d-Sorbitol (54%) and 3-ethoxy-1,2-propanol (33%) increased recoveries to early and late eluting compounds respectively.

. Although by selecting a better solvent and an analyte protectants significantly improved recoveries, a decrease in recovery for a group of analytes was observed when the sample was injected over a period of 4 week. Figure 5.

. In order to obtain accurate results, responses between calibration standard and samples in matrix have to be normalized. Since it was evident that matrix enhanced response, it was essential to determine what was causing this magnification and attempt to mimic its response. As seen by this study, using hexane as a solved and including a number of analyte protectants not only improves the recovery but it increases the stability of the compound in solution.

References

1.Michelangelo Anastassiades, Katreina Mastovska, Steven J. Lehotay, Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. Journal of Chromatography A 1015 (2003) 163-184

2.Cajka T, Mastovská K, Lehotay SJ, Hajslová J. Use of automated direct sample introduction with analyte protectants in the GC-MS analysis of pesticide residues.Journal of Separation Science 2005 Jun;28(9-10):1048-60.