Quantitation of Organophosphate Insecticides in Drinking Water Using Automated Online Sample Preparation and a 3-D Ion Trap Mass Spectrometer

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Overview

Purpose: A fast, automated, inexpensive method of analysis of organophosphates in water – saving time, money and resources.

Method: Automated online sample concentration LC/MS detection with dedicated MS/MS using a 3-D ion trap mass spectrometer.

Results: Organophosphates were detected down to levels as low as 0.005 ppb using automated online sample concentration and MS/MS detection on a 3-D ion trap MS.

Introduction

Organophosphates are irreversible acetylcholinesterase inhibitors and as such are highly toxic. While organophosphates degrade rapidly with exposure to sunlight, air, water and soil, residual amounts can still be detected in food and drinking water. Due to the toxicity of organophosphates and other effects such as delayed learning rates in children, an increased risk of Alzheimer's, and chronic fatigue symptoms, it is essential to be able to detect even low levels of these compounds. This presentation will demonstrate the use of automated online sample preparation coupled with a 3-D ion trap mass spectrometer for the detection of organophosphates in drinking water.

Method

Sample Preparation

Standards were diluted in methanol to concentrations 20X of final desired level. The resulting standards were added to water resulting in concentrations from 0.001 ppb to 100 ppb with a 5% methanol concentration. Samples were analyzed directly using volumes of 1 mL or 4 mL with no additional preparation.

LC Conditions

We used the Thermo Scientific EQuan MAX system consisting of a Thermo Scientific Accela 1250 UHPLC eluting pump, an Accela™ 600 loading high flow pump and an Open Accela Autosampler configured with a 5 mL injection loop and a 2.5 mL syringe (Figure 1). Solvents used for both pumps were A: water w/ 0.1% formic acid and B: methanol with 0.1% formic acid. We used a Thermo Scientific Hypersil GOLD aQ loading column (2.1 x 20 mm, 12µm) and the Hypersil GOLD™ aQ analytical column (2.1 x 200 mm, 1.9µm). Chromatographic conditions are as follows: loading pump 5% solvent B, 5 mL/minute during loading; eluting pump 250 µL/minute 70% B; hold two minutes for loading; followed by an 11-minute gradient to 80% B; finalized with a 2-minute flush at 100% B and re-equilibration at 70% B. The total run time was 20 minutes.

Mass Spectrometry

The EQuan MAX system was equipped with the Thermo Scientific LCQ Fleet ion trap mass spectrometer – source conditions: heated electrospray ionization (HESI) probe 400 °C; spray voltage 3 kV; capillary temp 180 °C; sheath gas 45; aux gas 5. Dedicated tandem mass spectrometry (MS/MS) experiments were performed using a timed mass list with a 3 amu isolation width and a normalized collision energy of 30 for all organophosphates (Figures 2A and 2B).

FIGURE 1. EQuan MAX automated online sample preparation system equipped with the LCQ Fleet mass spectrometer.

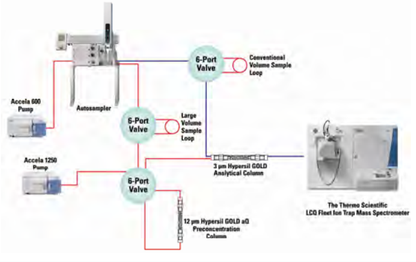


FIGURE 2A. LCQ Fleet Mass Spectrometer Method. Dedicated MS/MS Using Mass List. Simplifies method, only uses one segment.

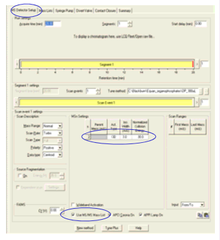
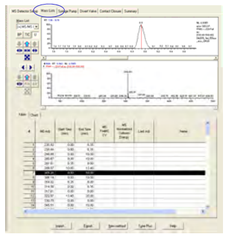


FIGURE 2B. Mass List Tab; simplifies MS/MS set up, user can import mass list with times, or import raw data file to choose mass and time windows.



Results

Organophosphates are traditionally analyzed by either gas chromatography (GC) or GC/MS techniques. These techniques require extensive sample preparation using techniques such as liquid-liquid extraction (LLE), solid phase extraction (SPE) and solid phase micro extraction. All of these techniques are time, labor and material intensive. An added disadvantage is that sample degradation can occur during the extraction process. We will demonstrate a method that eliminates the offline sample preparation and provides excellent detection limits for the analysis of organophosphates in water.

Initial analyses were performed in full scan to determine retention times and ionization species. All data shown is from 1 mL injections. The full scan data in Figure 3 depicts the elution profile of the 18 organophosphates analyzed. Due to the close elution of several of the organophosphates it was necessary to employ overlapping MS/MS scan windows which was achieved using the timed mass list shown in Figure 2 and the retention times determined from the full scan analysis shown in Figure 3.

After determining retention times and setting MS/MS windows, standards were run to generate calibration curves. Multiple fragment ions from the full scan MS/MS spectra were used for quantitation, where possible, providing added confidence in identification. An example of the full scan MS/MS spectra for the OP Diazinon is shown in Figure 4, illustrating the high quality of MS/MS spectra even at low levels of compound. Criterion for calibration curve acceptance was goodness of fit R^2 0.99 or greater, limits of detection (LODs) were determined by %difference and %RSD less than 20%; limits of quantitation (LOQs) were determined by %difference and %RSD less than 15% (Figure 5 and Table 1).

FIGURE 3. Full scan data displaying the reconstructed ion chromatograms for all 18 of the organophosphates analyzed.

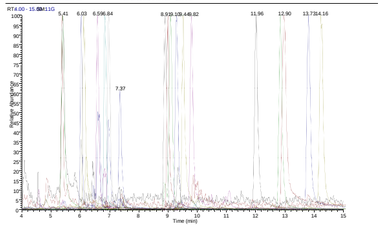


FIGURE 4. MS/MS Spectra for Diazinon showing two fragment ions for the LOD and a mid-level calibrator; Panel A: 0.005 ppb, Panel B: 5 ppb.

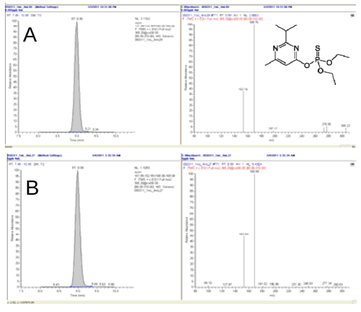
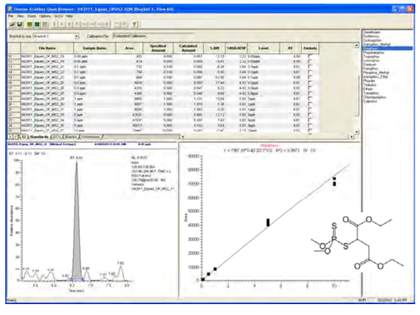


FIGURE 5. Representative calibration curve (malathion) with %Difference and %RSD. %Difference is [(Calculated - Specified amount)/Specified] x 100. %RSD is STD/mean x 100.



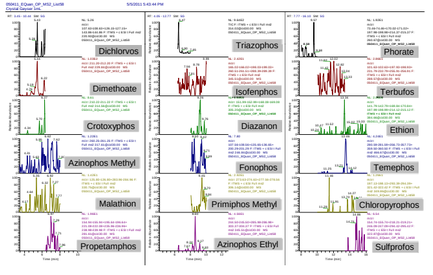
In addition to the calibration standards, six different water samples were analyzed. Three samples were from local water sources (DI, SF, SJ) and three samples were from various commercial bottled waters (AW, CG, KL). No organophosphates were detected in any of the water samples that were analyzed (Table 1). The chromatograms from sample CG for all of the analyzed organophosphates, are displayed in Figure 6.

To demonstrate the power of large volume injections, 4 mL injections were also made. Figure 7 contrasts the increase in detection limit comparing the 1 mL and 4 mL injections of a 0.05 ppb standard. Note that all of the compounds, except phorate are detected at in the 4 mL injection.

Table 1. Studied Organophosphates with corresponding LOD, LOQ and goodness of fit and tested water samples (DI- de-ionized water, SF- San Francisco water, SJ- San Jose water, AW- Arrowhead bottled water, CG- Crystal Geyser sparkling water, KL- Kirkland water)

Organophosphate	LOD (ppb)	LOQ (ppb)	R2	DI Water	SF Water	SJ water	AH Water	CG Water	KL Water
Dichlorvos	0.5	1	0.9966	ND	ND	ND	ND	ND	ND
Dimethoate	NA	NA	NA	NA	NA	NA	NA	NA	NA
Crotoxyphos	1	1	0.9773	ND	ND	ND	ND	ND	ND
Azinophos Methyl	0.05	0.1	0.9966	ND	ND	ND	ND	ND	ND
Malathion	0.05	0.1	0.997	ND	ND	ND	ND	ND	ND
Propetamphos	0.1	0.1	0.9927	ND	ND	ND	ND	ND	ND
Triazophos	0.05	0.05	0.9957	BDL	BDL	ND	BDL	ND	ND
Isefenphos	0.05	0.1	0.9967	ND	ND	ND	ND	ND	ND
Diazinon	0.005	0.005	0.9967	ND	ND	ND	ND	ND	ND
Fonophos	0.1	0.5	0.9979	ND	ND	ND	ND	ND	ND
Primiphos Methyl	0.05	0.1	0.9947	ND	ND	ND	ND	ND	ND
Azinophos Ethyl	NA	NA	NA	ND	ND	ND	ND	ND	ND
Phorate	NA	NA	NA	ND	ND	ND	ND	ND	ND
Terbufos	0.5	0.5	0.9978	ND	ND	ND	ND	ND	ND
Ethion	0.05	0.1	0.9994	ND	ND	ND	ND	ND	ND
Temephos	0.05	0.05	0.9971	ND	ND	ND	ND	ND	ND
chloropyrophos	0.1	0.1	0.9981	ND	ND	ND	ND	ND	ND
Sulprofos	0.5	0.5	0.9975	ND	ND	ND	ND	ND	ND

FIGURE 6. Representative chromatogram for one of the six water samples (CG) that were analyzed. No organophosphates were detected.

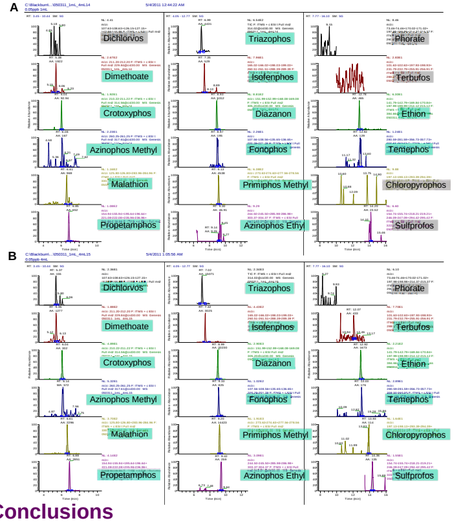


Discussion

Of the eighteen organophosphates that were analyzed some are so unstable that it was not possible to create complete calibration curves due to compound degradation, specifically phorate and crotoxyphos, both of which are known to have a short half-life in water[1] (Table 1). In addition, some of the organophosphates are only minimally soluble in water making it difficult to look at higher concentration standards or to detect at all as in the case of azinophos ethyl which is virtually insoluble in water and was poorly and inconsistently detected. The LC/MS analysis of organophosphates is complicated by the tendency of some species to form adducts in the presence of sodium or ammonium ions. While this could be advantageous, as it improves the ionization of the compounds, the resulting MS/MS fragmentation is the loss of either the sodium or the ammonium ions resulting in very poor or non-existent MS/MS spectra and reducing sensitivity. Therefore, in this study the [M+H]+ ion was used for all species analyzed.

In some cases, such as dimethoate, the fragmentation of the [M+H] resulted in a non-specific water loss and no additional fragmentation. Due to this lack of specificity dimethoate response was the same for all but the 50 ppb and 100 ppb standard making it impossible to quantitate or detect dimethoate with any certainty below those levels. This example illustrates the need for unique fragmentation ions for identification and quantification. However, even with the afore mentioned caveats, calibration curves and LODs were achieved for the majority of the compounds analyzed, with no additional sample preparation required, and excellent levels of sensitivity.

FIGURE 7. Detection limits, 1 mL versus 4 mL injection 0.05 ppb. Panel A: 1 mL injection. Panel B: 4 mL injection, grey labels are below detection limit and green labels are detected



Conclusions

- We have successfully demonstrated an automated online sample preparation system in conjunction with a 3-D ion trap mass spectrometer to provide a cost effective and time efficient method for monitoring organophosphates in water.
- The EQuan MAX system equipped with Accela pumps allows the loading of large volumes of samples (up to 20 mL) and enables the detection of lower compound concentrations.
- Advantages of using a 3-D ion trap MS for the analysis include versatility in data acquisition. Available MS^n provide more confident compound identification. Data acquisition is possible in a targeted approach through preset mass lists or in an non-targeted approach through data dependent experiments.
- Thirteen out of eighteen organophosphates were detected at the 0.05 ppb level in spiked water as compared to seventeen out of eighteen insecticides when a larger sample volume was loaded on to the preconcentration column (Figure 7).
- LODs as low as 0.005 ppb in spiked water is possible using LC/MS and the EQuan MAX system, however this is compound dependent, with 0.05 or greater ppb being the norm.
- No organophosphate insecticides were detected in the tap and bottled waters that were analyzed.

References

1. Hoffman, D.J., B.A. Rattner, G.A. Burton, Jr. and J. Cairns, Jr. (Editors). 2003. Handbook of Ecotoxicology, 2nd edition, CRC Press, Boca Raton, FL, 1290 pp.