

# The determination and quantitation of ten pesticides in drinking water by use of Solid Phase Extraction and ESI-LCMS

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## INTRODUCTION

Carbamates, chlorotriazines and phenylureas are among the most popular pesticides for home and industrial use. Pesticides are agents of chemical or biological nature that control undesirable plants & animals either by killing them or by preventing them from engaging in destructive behavior.

Carbamates interfere with the nervous system, and in the case of high-level exposure (poisoning) of humans and/or animals can cause grave illness and even death. Over use of these pesticides can place dangerous concentrations into the ground water supply. This high level exposure, or the suspicion there of, often necessitates the compulsory testing for these compounds by Federal, State, Local and even private entities.

This work demonstrates a process whereby a workable LC/MS method is developed, switching over from several EPA approved methods using HPLC only, by means of experimenting with various mobile phases and ESI conditions.

## EXISTING METHOD

The compounds of interest for this study were: Aldicarb Sulfoxide, Aldicarb Sulfone, Aldicarb, Cyanazine, Carbofuran, Chlorotoluron, Isoproturon, terbutylazine, Pyriproxyfen & Oxamyl.

Several EPA methods are required for complete testing of these ten selected pesticides. For instance, the approved method for Carbamates, specifies post column derivatization with fluorescent detection. After separation via HPLC, analytes are derivatized in line, yielding highly fluorescing boluses, which can then be detected and quantified by an HPLC UV Detector. This, post column derivatization methodology may or may not be compatible with other pesticides. Therefore in order to detect and report the presence and concentration of several pesticides (or for that matter, other classes of compounds) it is desirable to have a single LC/MS method.

## PROCESS TO THE NEW METHOD

The process whereby a suitable LC/MS method was created uses the following steps:

- 1) Solvent optimization** - using store bought standards and 50/50 (water/organic) m.p under flow injection with no column
- 2) ESI optimization** - using store bought standards and 50/50 (water/organic) m.p. under flow injection with no column
- 3) Chromatography** via optimized mobile phase, ionization and full scan detection by Electro spray Mass Spectrometry
- 4) Single Ion Monitoring** leading to much lower detection limits
- 5) Solid phase extraction** of real samples
- 6) Quantitation** and qualification of results

## INSTRUMENTATION – LC-ESI-MS

For LC-ESI-MS, the system included the following components. The liquid chromatograph used was an Agilent Model 1100 modular system with binary pumping system, vacuum degasser, column compartment, 100 vial autosampler with refrigerated chiller and variable wavelength detector. The HPLC column used was an Agilent Technologies Zorbax SB C18 150 x 0.5 mm. The mass spectrometer used was an Agilent 1100 LC/MSD G1946A, upgraded by CSS Analytical to "D" electronics, software and LAN communication, with ESI spray chamber.

## PROCESS #1 – SOLVENT OPTIMIZATION

Solvent optimization occurred using store bought standards, 50/50 (water/organic) mobile phase under flow conditions without a column. Various solvents (water, MeOH, ACN) and additives (Acetic, Formic) were used in order to determine which mobile phase produced maximum signal intensity for each compound class. Modern pesticides are designed to be chemically labile and as such possess a desirable characteristic: a short persistence in the environment. This chemical instability is reflected in the determination of the optimum solvent for Electro spray Ionization. Each of the pesticides demonstrated an increased instability with the addition of an acidic additive to the solvent system with only two of the compounds performing slightly better in an acidic medium (a 10% and 20% increase in abundance for pyriproxyfen and terbutylazine, respectively). Both of these compounds performed better in the MeOH/water with 0.1% formic acid. Generally, acidic additives are responsible for providing H<sup>+</sup> to protonate the molecules of interest. However, in this case H<sup>+</sup> present in the solution is aiding in the acid catalyzed breakdown of the pesticides. Conventional practice for enhancing ESI ionization actually reduces the abundance of molecular ions available. The absence of an acidic additive actually enhances the ease of detection as was discovered when the samples were analyzed in MeOH/ water.

## PROCESS #2 – ESI OPTIMIZATION

Once the optimum mobile phase is determined, various ESI parameters are adjusted in order to increase signal. Again, this optimization procedure should be conducted using 50/50 (water/organic) mobile phase under flow injection conditions. Typical of most ESI systems: adjust the flow rate of the drying gas, the pressure of the spray gas, drying gas temperature, capillary exit voltage (fragmentor voltage) and other parameters as needed.

Adjust the spray conditions using the single compound found to have the least abundance during Process #1. To be certain that you have optimized the spray conditions to all compounds, inject all compounds and compare against results of Process #1.

The optimum drying gas temperature can vary well be different for each compound. Run each compound separately (using flow injection) at various temperatures and at various percent of organic. If you know something about the retention times of each compound under generic conditions, try to match the mobile phase composition to the expected composition at elution time. Drying efficiency is generally a function of the organic nature of the spray - the more organic in the spray the faster it will evaporate and at a lower drying gas temperature. Generally, use as low a drying temperature as possible in order to avoid thermal degradation of the compound of interest. Sometimes, for some compounds, higher temperatures will actually increase ionization (molecules in the gas phase) so each compound will need to be evaluated over a range of temperatures.

Fragmentor voltage can also vary depending on compound. By and large, it varies by molecular weight. Test each compound individually.

As might be expected, multiple variables require tedious experimentation and data logging. A matrix of parameters and results may be needed in order to evaluate the best conditions.

## PROCESS #3 – CHROMATOGRAPHY

Method development begins by working out the chromatography with an LC/MS analytical column, which can often differ from columns used by typical HPLC methods. Typical HPLC methods use the 4 mm ID columns and sometimes 2 mm ID columns. LC/MS columns are much smaller in ID and often shorter – and getting smaller and smaller all the time.

Generally, start with a gradient program and 10% organic solvent and slowly move to 90% organic over say, 40 minutes. If all of your compounds elute in less than 40 minutes, use the traditional methods for HPLC method optimization: start at a higher percent organic in order to take time away from the front of the run and increase the gradient ramp rate to remove time on the back end of the run. Steps may need to be added for co-eluting peaks. If you already have a workable HPLC method, there are many conversion algorithms out there to convert from larger, longer columns to smaller, shorter columns. Contact a column manufacturer if you need assistance.

Create a 100 ml stock solution by placing 0.01 mg/ml of standard for each compound into a volumetric flask and filled to the mark with Water/Methanol make-up mixture. This concentration should be more than sufficient for the ESI/Quad to detect all compounds in full scan mode. Start with a simple mobile phase of 90/10 : H<sub>2</sub>O/MeOH and ramp it to 10/90 : H<sub>2</sub>O/MeOH as a gradient, with your column of choice. The adjust the gradient until all the compounds are separated with base line resolution. The final run time for our experiment was optimized to 25 minutes, including internal standard.

## PROCESS #4 – SIM (Single Ion Monitoring) & LOD (Limit of Detection)

Dilute the stock solution containing all compounds and internal standards, serially until full scan can no longer detect all of the compounds. Determine the base ions (and secondary ions), tie them to retention times. Build a SIM Table. Adjust your mass spec method – convert from scan to sim. With additional dilutions, LOD can be determined.

## PROCESS #5 – SOLID PHASE EXTRACTION of Real Samples

Perform extractions using a suitable vacuum manifold and SPE cartridges. Condition the cartridges according to instructions provided by the manufacturer by filling each reservoir with 5-ml acetonitrile/MeOH (1:1). Rinse with 3-ml of MeOH. Slowly pass the sample through the SPE cartridge by adjusting the vacuum controller for each sample in the manifold to a flow rate of about 10-ml/min. After all the samples have passed through the cartridges the sample is ready to be collected. Remove the adapter and reservoir from the cartridge. Set up the vacuum manifold with clean collection vessels and needles. Elute the target compounds with 6-ml of acetonitrile/MeOH (1:1). Pulse the vacuum pump to start the flow, then let the acetonitrile/MeOH drip through under gravity alone at a flow rate of approximately 1-ml/min. The samples are now ready for analysis, transfer to suitable vials.

## PROCESS #6 – QUANTITATION

To establish linearity a sequence containing a minimum of five calibration solutions for initial calibration (ICAL) will need to be employed. Use peak areas ( or peak heights when appropriate) to calculate the calibration factor for each compound, the mean calibration factor, standard deviation and percent relative standard deviation ( RSD) calculation.

## CONCLUSIONS

Carbamate insecticides act by a mechanism similar to organophosphate pesticides, but have a shorter half-life and have varying degrees of toxicity. High exposure to organophosphate and carbamate pesticides has been associated with neurobehavioral development in children. Malnourished populations may be particularly vulnerable to neurobehavioral effects of pesticide exposure. The effects include disrupt function of nervous system, mainly the brain. Complications include headache, dizziness, weakness, shaking, nausea, excitability & disorientation.

Thus it is important to determine the presence and concentration of these devastating elements in the soil and ground water. Doing so quickly, easily and cost effectively will prevent potential harm to humans and animals.

By combining the net results of several HPLC methods into one LC/MS method, one can leverage the "quick" and "easy" attributes of LC/MS and further take advantage of the unambiguous nature of detection by molecular weight, "mass spectrometry", versus that of HPLC alone. The "cost effective" benefits of LC/MS are realized by simplified sample prep, elimination of pre and/or post column derivatization, and by elimination of duplicate sample preparation and run on confirmation columns.

The methodology suggested herein can be used for other compounds and classes. To obtain a re-print of this paper, please contact the presenting author: [Snienmann@cssco.com](mailto:Snienmann@cssco.com).