A Gas/Liquid Chromatographic-Mass Spectrometric Method for the Rapid Screening of 250 Pesticides in Aqueous Matrices

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Introduction

Pesticide residues in food present a potentially serious and significant cause for concern [i]. Many pesticides have been associated with significant health effects to the nervous and endocrine systems and some have been deemed carcinogenic [ii]. There are many well-established techniques for pesticide analysis. However, commercial pesticide methods have traditionally only been available for specific pesticide families, such as chlorinated pesticides [iii]or herbicides [iv], and at detection limits ranging from 0.05 ppb to 1 ppm in aqueous matrices. Techniques that can quickly screen for the presence/absence of pesticide residues in food matrices are critical in ensuring the safety of food and water.

This paper outlines a combined Gas Chromatographic-High Resolution Mass Spectrometric (GC-HRMS) and Liquid Chromatographic Tandem Mass Spectrometric (LC-MS/MS) screening assay for 250 pesticides that was developed for use in water, and soda samples at screening levels ranging from 0.1-5 ppb. The pesticides selected have been identified by the European Union as being of concern and the target of possible legislation. The list encompasses a variety of pesticide classes and compound groupings.

Methods and Materials

The list of pesticides monitored using this method is shown in Table 2. A combination of GC-HRMS and LC-MS/MS was used to screen for the pesticides as some of the pesticides of interest were non-volatile, unstable at elevated temperatures, and not amenable to gas chromatography. Three liquid matrices were used in this study: water, cola and orange soft drinks.

For analysis by GC-HRMS, the samples were fortified with a surrogate solution consisting of carbofuran phenol-$^{13}C_6$ and carbaryl-$^{13}C_6$. The sample sizes used were 200 ml for water samples and 10 ml for soda samples. A negative control and a positive control sample were extracted and analysed with each sample batch to monitor batch performance and for use in quantitation. The samples were liquid-liquid extracted using either methylene chloride (for water) or heptane (for soda). The soda samples were degassed prior to extraction. The extracts were dried using sodium sulfate. Nonane was then added as a keeper solvent and the extracts were evaporated down to the nonane amount. An internal standard mix consisting of deuterated PAHs was added to a 20 µl portion of the extracts, and 2 µl was injected into the GC-HRMS. The positive control was spiked at the screening level of interest.

For analysis by LC-MS/MS, a 100 ml sample was fortified with a surrogate solution consisting of deuterated chlortoluron, and liquid-liquid extracted using ethyl acetate. A negative control, and a positive control sample were extracted and analysed with each sample batch. The extracts were dehydrated using sodium sulfate and evaporated to dryness using rotary evaporation and nitrogen blow down. The samples were then reconstituted in a solvent mixture consisting of 100 µl of methanol and 400 µl of water and analysed using LC-MS/MS.

	GC-HRMS	**LC-MS/MS**
Chromatograph	Hewlett Packard 5890 Series II	Hewlett Packard 1050 Series
Chromatographic Column	30 m DB5-MS	Phenomenex Aqua 5 µm C18 125 A
Mass spectrometer	VG 70-SE Series	Micromass VG Quattro II
Spectrometer Ionization mode	Electron Ionization	Positive Ion Atmospheric Pressure Chemical Ionization (APCI+)
Eluent/carrier	Helium	Methanol/water gradient containing 0.1% acetic acid
Data Acquisition	Scan 50-500	Multiple reaction monitoring (MRM)
Quantitation technique	Reconstructed ion chromatograms from 1-2 primary ions	MRM ion quantitation

Table 1: Details of instrumentation used in the screening process

The instrumentation and techniques used are summarized in Table 1. For the GC-MS analysis, the necessity to screen for 150+ analytes in a short time span

precluded the use of single ion monitoring techniques. Therefore, the mass spectrometer was operated in scanning mode.

Screening Procedure

GC-HRMS: A calibration standard was used to establish instrument response at the target reporting limit for the analysis and to set the retention time window for the analytes of interest. The ratio of the response from the target compound to its corresponding ^{2}H- labeled internal standard was used to calculate a response factor for each analyte. The standards used for quantification were spiked immediately prior to analysis. If the relative response (analyte area/internal standard area) for a sample was higher than the relative response for the positive control, the pesticide was tagged as being present above the screening level.

LC-MS/MS: Two calibration standards spiked at the required reporting limit were prepared in the matrix of the batch being analysed. The responses generated for the analytes were used in conjunction with the internal/surrogate standard response to yield response factors. The average response factors from the two calibration standards were used to calculate concentrations of the samples.

Results and Discussion

Chromatograms of 18 representative analytes of the 224 pesticides analysed using GC-HRMS are shown in Figure 1. Of this list, 198 pesticides in water had a detection limit of 0.1 ppb or less. In the soda lab control spikes, the detection limits were higher due to the smaller sample sizes used and the greater matrix interferences. 141 of the pesticides were detected at 0.2 ppb in the cola and 114 in the orange soda lab control spikes. As the data was acquired in scan mode, organic matrix interferences led to the increase in the overall noise level of the chromatograms.

Chromatograms of twenty of the pesticides screened using LC-MS/MS are shown in Figure 2. The sample is a water sample spiked at 0.1ppb for each of the analytes. The peak strength and signal to noise ratio indicate that lower detection limits can easily be achieved. Of the pesticides listed, aldicarb was not quantitated successfully from any of the matrices used in the study. This is likely due to the instability of the oxime group in the aldicarb under the analytical conditions.

Similar results were achieved in spiked soda matrices, though matrix interferences resulted in higher noise, hence lower signal/noise ratios (S/N). 23 out of the 26 pesticides were successfully detected in both the orange and cola matrices at a 0.1 ppb detection limit or less. A S/N of 5 or greater signified detection. For the orange soda samples, oxamyl and chlorobromuron had higher detection limits of 0.2 ppb. For the cola samples, oxamyl was the only pesticide showing reduced response and a detection limit of 0.2 ppb. Chlorobromuron was affected by matrix interferences and the oxamyl's oxime functional grouping contributed to decreased sensitivity.

Lab control spike recoveries were monitored for the analytes. Most of the analytes monitored using GC-MS were overestimated by 50-60% in all the test matrices. Recoveries were generally greater than 80%. Endosulfan alpha showed a recovery of only 27% in water and was not detected in the soda samples at a 0.5 ppb spiking level. Dicofol was also recovered at only 22% in the water samples. Therefore, some of the pesticides showed less than acceptable performance. This is not surprising given the large number, and chemical variety of the pesticides screened. While accuracy is critical for quantitative methods, a screening method only needs to demonstrate that a pesticide will be detected at the desired reporting level if present. Therefore, any result that is not an underestimation is considered acceptable.

Summary

Overall, this technique shows promise in quickly detecting the presence of a large number of pesticides in aqueous matrices. Setting the analysis up as a screen, instead of a quantitative technique enables the use of a single point calibration and accelerated run times. A set of 250 pesticides was successfully screened for quickly and efficiently using this technique. Further refinement is needed to reduce screening levels, especially in the cola and orange soda matrices. An extract cleanup step to remove certain bulk interferences could be implemented to reduce background noise.

References

[i] Pesticides in the Diets of Infants and Children (1993) National Academy Press, Washington DC
[ii] US EPA website http://www.epa.gov/pesticides/health/human.htm (2003)
[iii] Method 8081B (2000) EPA.No. SW-846
[iv] Method 8151A (1996) EPA.No. SW-846

GCMS List	DDE-o,p'	Oxadixyl	Etofenprox	TDE-p,p'
Aclonifen	DDE-p,p'	Oxydemeton-methyl	Etridiazole	Tebuconazole
Acrinathrin	DDT-o,p'	Paclobutrazole	Etrimfos	Tebufenpyrad
Alachlor	DDT-p,p'	Parathion	Famoxadone	Tecnazene
Aldrin	Deltamethrin	Parathion-methyl	Fenamiphos	Terbufos
Ametryn	Demeton-O	Penconazole	Fenarimol	Terbutryn
Atrazine	Demeton-S-methyl-sulphone	Pendimethalin	Fenazaquin	Terbutylazine
Azaconazole	Desmetryne	Permethrin-a	Fenchlorphos	Tetrachlorvinphos
Azinphos-ethyl	Dialifos	Permethrin-b	Fenhexamid	Tetraconazole
Azinphos-methyl	Flufenoxuron	Phenothrin	Fenitrothion	Tetradifon
Azoxystrobin	Fluoroglycofen-ethyl	Phenthoate	Fenoxycarb	Tetrahydrophthalimide
Benalaxyl	Fluquinconazole	Phenylphenol-2	Fenpiclonil	Tetramethrin
Bifenthrin	Flusilazole	Phorate	Fenpropidin	Thiometon
Bioallethrin	Flutolanil	Phosalone	Fenpropimorph	Tolclofos-methyl
Bioresmethrin	Flutriafol	Phosmet	Fenproprathrin	Tolyfluanid
Biphenyl	Fluvalinate-tau	Phosphamidon	Fenthion	Triadimenol
Bitertanol	Folpet	PiperonylButoxide	Fenvalerate	Triallate
Bromacil	Fonofos	Diazinon	Fipronil	Triazamate
Bromophos	Formothion	Dichlobenil	Fluazafop-butyl	Triazophos
Bromophos-ethyl	Fuberidazole	Dichlofenthion	Flucythrinate	Tricyclazole
Bromopropylate	Furalaxyl	Dichlofluanid	Fludioxinil	Trifloxystrobin
Bromuconazole	Heptachlor	Dichloran	Pirimicarb	Triflumizole
Bupirimate	Heptachlor_epoxide	Dichlorbenzamide	Pirimiphos-ethyl	Trifluralin
Buprofezin	Heptenophos	Dichlorvos	Pirimiphos-methyl	Vamidothion
Cadusafos	Hexachlorocyclohexane-A	Dicofol	Prochloraz	**LCMS List**
Captan	Hexachlorocyclohexane-B	Dieldrin	Procymidon	Acephate
Carbaryl	Hexachlorocyclohexane-D	Diethofencarb	Profenofos	Aldicarb
Carbofuran	Hexachlorocyclohexane-G	Diethyltoluamide	Prometryn	Aldicarb Sulfone
Carbofuran_phenol	Hexaconazole	Difenoconazole	Propachlor	Carbendazim
Carbophenothion	Indoxacarb	Diflufenican	Propargit	Chlorbromuron
Carbosulfan	Iprodione	Dimethenamid	Propazine	Chloroxuron
Carboxin	Isofenphos	Dimethipin	Propham	Chlortoluron
Chlofentezine	Kresoxim-methyl	Dimethoate	Propiconazole-a	Difenoxuron
Chlorbufam	Lenacil	Dimethomorph	Propiconazole-b	Diflubenzuron
Chlorfenapyr	Malathion	Diniconazole	Propoxur	Diuron
Chlorfenson	Mecarbam	Dioxathion	Propyzamide	Ethiophencarb Sulfone
Chlorfenvinphos	Mepanipyram	Diphenylamine	Prosulfocarb	Ethiophencarb Sulfoxide
Chloridazon(Pyrazon)	Mepronil	Disulfoton	Prothiophos	Imidacloprid
Chlorobenzilate	Metalaxyl	DMST	Pyrazophos	Isoproturon
ChlorothalDimethyl	Metamitron	Dodemorph(2-isomers)	Pyridaben	Linuron
Chlorothalonil	Metazachlor	Endosulfan_alpha	Pyridaphenthion	Methabenzthiazuron
Chlorpropham	Metconazole	Endosulfan_beta	Pyrifenox	Methamidophos
Chlorpropylate	Methacrifos	Endosulfan_sulfate	Pyrimethanil	Methiocarb
Chlorpyrifos	Methidathion	EPN	Pyriproxyfen	Methiocarb Sulfone
Chlorpyrifos-methyl	Methoxychlor	Epoxiconazole	Quinalphos	Methiocarb Sulfoxide
Coumaphos	Metolachlor	Eptam	Quinoxyphen	Methomyl
Cyanazine	Metribuzin	Esfenvalerate	Quintozene	Metoxuron
Cyfluthrin	Mevinphos	Ethion	Quizalofop-ethyl	Monocrotophos
Cyhalothrin-lambda	Myclobutanil	Ethiophencarb	Simazin	Monolinuron
Cypermethrin	Nitrofen	Ethofumazate	Spiroxamine	Omethoate
Cyproconazole	Nitrothal-isopropyl	Ethoprophos	Sulfotep	Oxamyl
Cyprodinil	Nuarimol	Ethoxyquin	TDE-o,p'	

Table 2: List of Pesticides studied using the pesticide screen method

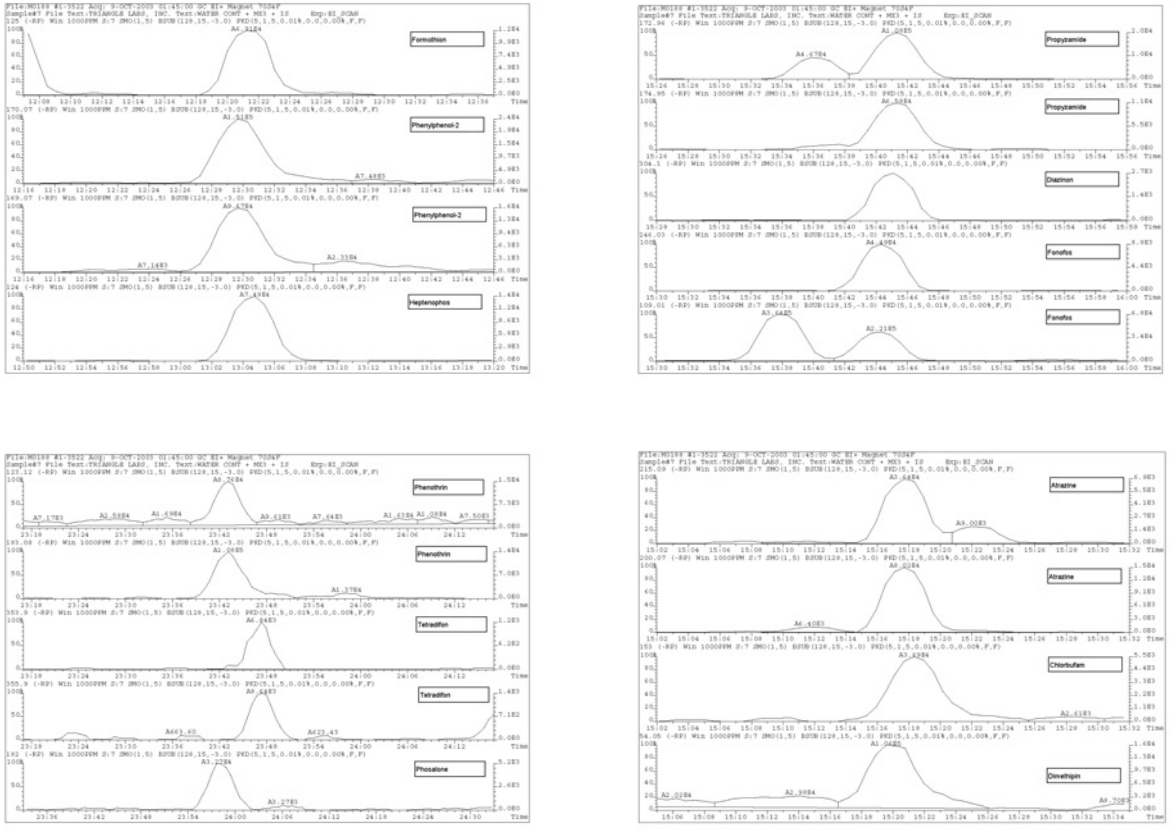

Figure 1: Chromatograms of select pesticides analyzed using GC-HRMS from water samples spiked at 0.1 ppb

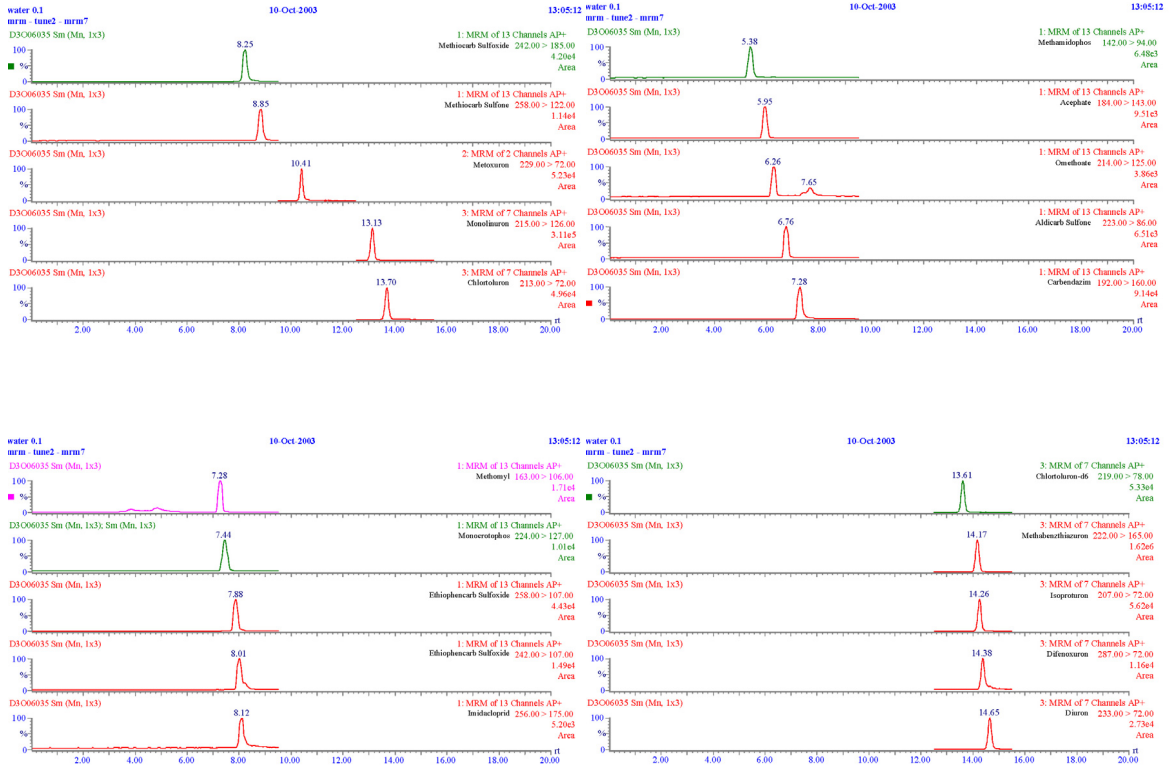

Figure 2: MRM Mass spectra of selected pesticides from a positive control spiked at 0.1 µg/ml analyzed using LC-MS/MS