Screening and Identification of Unknown Contaminants in Untreated Tap Water Using a Hybrid Triple Quadrupole Linear Ion Trap LC-MS/MS System

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Introduction

Protection of our drinking water resources from contaminants is a major responsibility for both government and water producing bodies. The response taken to a potential drinking water emergency will depend upon both the composition and the nature of the identified contaminant(s). Furthermore it is essential that there is a high degree of confidence in the correct and rapid identification of the problem before remedial action is taken. To date it has been a necessity to employ a combination of multiple analytical techniques to meet this end.

Screening Using Accurate Mass Measurements and MS/MS

One method of detecting contaminants is the use of accurate mass as a way to predict the formula and identity of a contaminant. In this approach the mass spectrometer has to be accurately calibrated because the greater the error the more potential contaminants would be a match for the detected peak, as <2ppm mass error is ideal.

In this example two structural related but different pesticides (Prometryn and Terbutryn) produce the same molecular ion because they have identical molecular formulae. In the environment there are hundreds of compound with the same mass (Figure 2). Thus, a complete identification of unknown contaminants by accurate mass alone may not yield to a complete answer as this does not provide any structural information. In the example above separation of these two pesticides by HPLC was not clear-cut as they eluted with very similar retention times (Figure 3). However, Prometryn and Terbutryn have different MS/MS fragmentation patterns (Figure 2). Therefore product ion spectra are essential for confident identification of unknown contaminants.

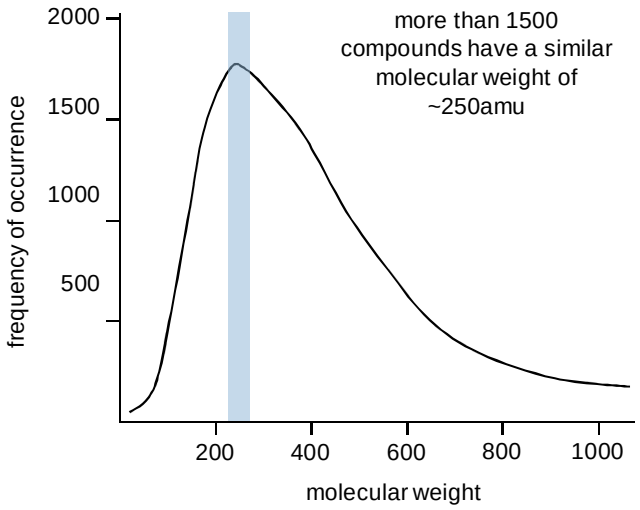

Figure 1. Abundance of compounds over molecular weight range of 100-1000 amu

Table 1. Comparison of sensitivities between the General Unknown Screening (GUS) and Multi Target Screening (MTS) approaches

			Multi Target Screening			General Unknown Screening		
Compound Name	**Compound Class**	**Polarity**	**MRM**	**Intensity at 1 µg/mL**	**~LOD (µg/mL)**	**Q3 Mass**	**Intensity at 10 µg/mL**	**~LOD (µg/mL)**
Brodifacoum	Rat poison	Negative	521.0/79.0	5.80E+04	0.05	521.0	7.70E+05	5.00
Chlorophacinone	Rat poison	Negative	373.0/201.1	1.23E+04	0.20	373.0	3.40E+05	15.0
Difenacoum	Rat poison	Negative	443.1/135.0	1.40E+04	0.25	443.1	1.80E+06	1.25
Difethialone	Rat poison	Negative	537.0/79.0	6.00E+04	0.07	537.0	1.40E+06	5.00
Flocoumafen	Rat poison	Negative	541.1/161.0	1.30E+04	0.12	541.1	1.40E+06	2.00
Warfarin	Rat poison	Negative	307.0/161.1	1.80E+04	0.20	307.0	1.80E+05	40.0
Endothal	Rat poison	Negative	185.0/141.0	6.00E+03	2.0	185.0	-	100
DNOC	Cresol	Negative	197.0/137.1	5.00E+04	0.10	197.0	2.00E+06	1.25
Azinphos-ethyl	Organo-phosphorus	Positive	346.0/160.1	5.13E+03	1.00	346.0	6.50E+04	200
Demeton-S-methyl	Organo-phosphorus	Positive	231.0/89.0	1.00E+04	0.50	231.0	2.30E+05	20.0
Dichlorvos	Organo-phosphorus	Positive	221.0/127.0	9.33E+02	10.0	221.0	4.00E+04	200
Disulfoton	Organo-phosphorus	Positive	275.1/89.0	2.00E+03	5.00	275.1	2.00E+04	2000
Propetamphos	Organo-phosphorus	Positive	282.1/156.0	2.20E+03	2.50	282.1	5.20E+04	200
Tebupirimfos	Organo-phosphorus	Positive	319.0/153.1	1.90E+04	0.50	319.0	2.90E+05	20.0
Parathion-ethyl	Organo-phosphorus	Positive	292.1/236.0	4.73E+03	2.00	292.1	1.00E+04	500
Parathion-methyl	Organo-phosphorus	Positive	281.1/264.3	5.00E+02	10.0	264.1	2.00E+04	400

General Unknown Screening and Multi Target Screening

There are two possible approaches of screening methods. The first would to screen for a complete unknown. This General Unknown Screening (GUS) would use a single ‘universal’ survey scan over a defined mass range and could either be a Time-of-Flight (TOF), quadrupole or ion trap scan. This survey scan can be used to trigger automatically the acquisition of a product ion spectrum if a signal of a detected compound is above a defined threshold. Finally, this spectrum can be searched against a mass spectral library for identification. Comparison of Total Ion Chromatograms (TIC) of unknown samples to that of the control reveal compounds that are either unique to the sample or those that are present at significantly higher concentrations than in the control.

The other approach is often called Multi Target Screening (MTS). In this approach a predefined list of compounds is looked for in a Single Ion Monitoring (SIM) or Multiple Reaction Monitoring (MRM) experiment. MRM mode is generally preferred because of higher selectivity and sensitivity. Once a compound is detected above a defined threshold a product ion scan is collected and compared against a library. Dynamic exclusion of compounds where MS/MS spectra are already acquired allows the data collection of co-eluting compounds (Figure 4).

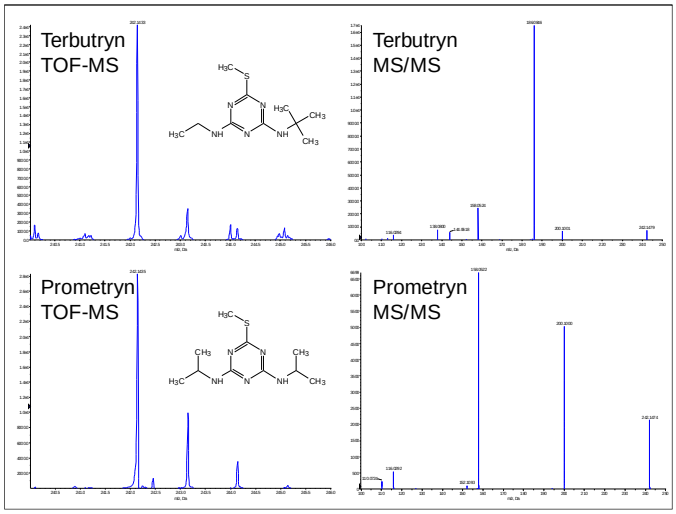

Figure 1. Accurate mass measurement of Prometryn (top) and Terbutryn (bottom) using a Quadrupole quadrupole-Time-of-Flight system in MS mode and MS/MS spectra of both pesticides

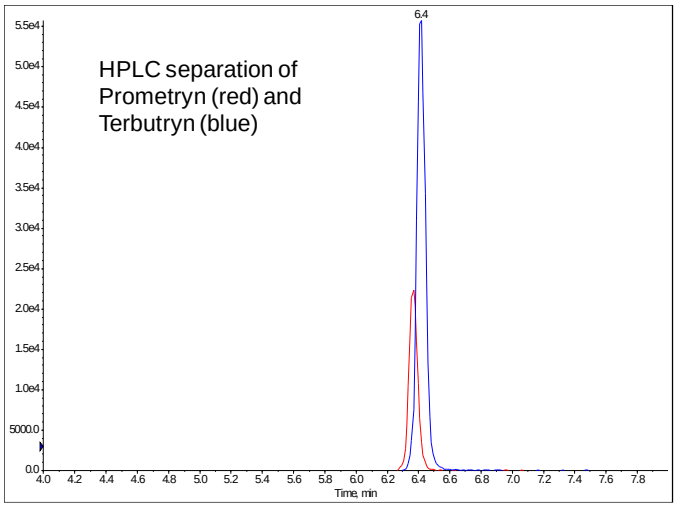

Figure 3. HPLC analysis of Prometryn and Terbutryn on a standard C18 reverse phase column, both compounds elute with a retention time difference of less than 6s

The technology that lends itself to this application is the hybrid triple quadrupole linear ion trap technology (QTRAP® LC/MS/MS systems). It allows the use of any triple quadrupole scan, including MRM, to trigger the acquisition of Linear Ion Trap MS/MS spectra by Enhanced Product Ion scanning. Enhanced Product Ion scan spectra give maximum sensitivity for library searching with a complete pattern characteristic for Collision Induced Dissociation (CID).

Experimental

In order to maximize sensitivity two injections, one in positive and the other in negative polarity, for both the GUS and MTS approach, were done. Additionally, this allowed the mobile phase to be optimum for either polarity.

HPLC

A Shimadzu HPLC system with binary LC10ADvp binary gradient pump and SIL-HT autosampler was used for all HPLC separations. The mobile phase used in positive mode was:

A: H_2O + 2 mM NH_4CH_3COO

B: CH_3OH + 0.1% HCOOH

The mobile phase used in negative mode was:

A: H_2O

B: CH_3OH + 0.1% NH_4OH

HPLC separation for Multi Target Screening was performed on a C18 monolithic column (Merck). Samples were analyzed using a rapid gradient over 1.5 minutes at a flow rate of 1200 µL/min (without splitting of the flow prior to the mass spectrometer). Injection volumes of 50 or 100 µL were used for analysis.

An ACE C18 (50 mm 5 µm HICHROM) column was used for HPLC separation for General Unknown Screening. The HPLC flow was set at 1200 µL/min with a gradient used from 25% B to 100% B over 16 minutes. An injection volume of 50 µL was used.

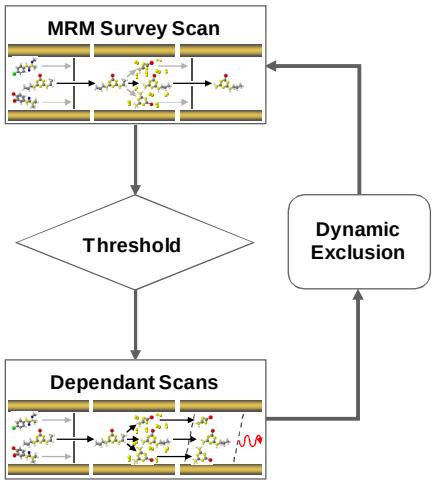

Figure 4. Experimental setup of a Multi Target Screening (MTS) approach

MS/MS

A 4000 QTRAP® LC/MS/MS system was used for both MTS and GUS experiments which triggered dependant Enhanced Product Ion scanning (mass range of 50 to 750 amu at 4000 amu/s) with a Collision Energy (CE) of 35 V and Collision Energy Spread (CES) of 20 V. The MTS survey scan used MRM transitions which have been optimized for each targeted analyte while the GUS screen used a Q3 scan with a mass range of 90 to 750 amu and a Declustering Potential (DP) of 60 V.

The source and gas settings for both MTS and GUS experiments were the same (Table 2)

Table 2. Ion source and gas parameters

Parameter	Value
Curtain gas	25 psi
Gas 1	50 psi
Gas 2	60 psi
CAD	10
Temperature	650°C
IonSpray™ source voltage	-4500 V
	+5500 V

Results and Discussion

Figure 5 and 6 present data obtained for an injection of 100 ng/mL Terbuthylazine and MCPP in both mineral and tap water, using the MRM to EPI MTS approach. The LINAC® collision cell of the 4000 QTRAP® system allows the simultaneous monitoring of up to hundreds of MRM transitions (contaminants) in a single sample injection. These MRM transitions triggered Enhanced Product Ion scan spectra in a cycle time of approximately 2.5 s without loss in sensitivity and full spectral quality.

Mineral water typically contains high levels of sodium, which may affect sensitivity due to adduct formation. However, Figure 5 and 6 indicate that there is nearly no effect on S/N to detect Terbuthylazine and MCPP in these water samples.

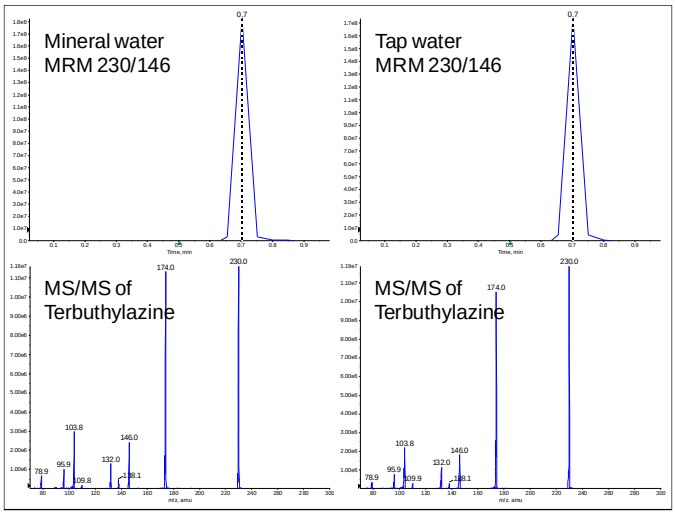

Figure 5. 100 ng/mL Terbuthylazine spiked into mineral and tap water analyzed in positive polarity MRM and EPI

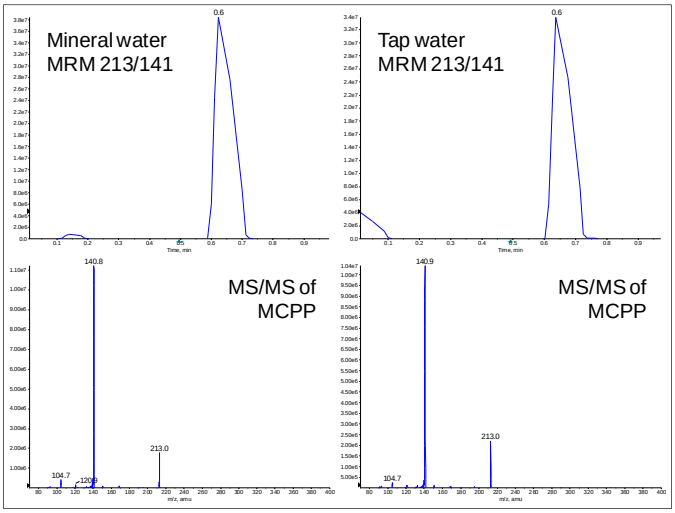

Figure 6. 100 ng/mL MCPP spiked into mineral and tap water analyzed in negative polarity MRM and EPI

The GUS approach shows the comparison of a blank control sample to a sample that has been spiked with 0.1 µg/L of a compound to be identified (Figure 7). The presence of the compound with m/z=350 amu is detected in the sample by comparing the two Q3 scan chromatograms. Acquisition of an Enhanced Product Ion scan spectrum followed by library searching allows to identification of Chlorpyrifos.

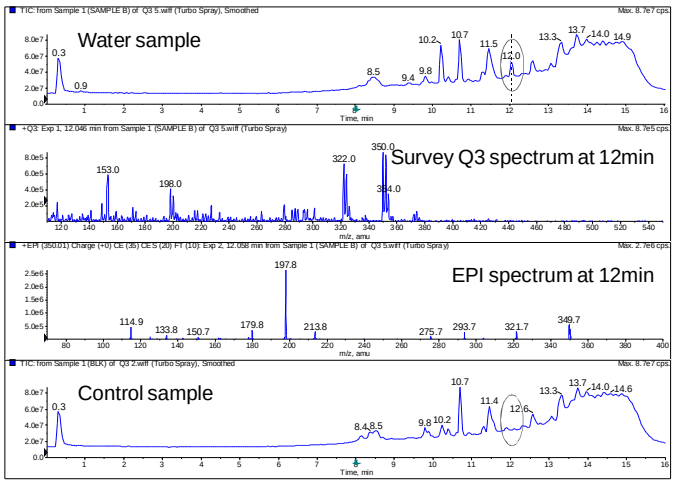

Figure 7. Comparison of a water sample to a blank control water with resulting Q3 scan and EPI spectrum of Chlorpyrifos detected and identified by library searching

In order to compare the relative sensitivities of both approaches, GUS and MTS, over 70 compounds were tested including compounds such as organophosphorus pesticides and rat poisons. Limits of Detection (LOD) were determined to be the triggering threshold of both approaches. In the GUS method the LOD was set at 500,000 cps of the parent ion in Q3 scan (background noise was generally lower than 500,000 cps). For the MTS approach LOD was 5000 cps in MRM which was determined as 2-3 times the background level of the most intense MRM trace. The chromatographic conditions of MTS were applied for this comparison work. Examples of results for 16 different compounds are given in Table 1 highlighting the higher sensitivity of the MTS approach. An average of 2 orders of magnitude comparing LOD of both approaches was found.

Summary

The 4000 QTRAP® LC/MS/MS system allows Multi Target Screening (MTS) and General Unknown Screening (GUS) of water samples to identify emerging contaminants. The MTS approach is the most rapid and sensitive method to screen for and detect the presence of targeted organic contaminants in water. More than 2000 targeted compounds can be screened in less than 20 minutes at low and sub µg/L level using the described procedure and multiple sample injections. The GUS method is an alternative to identify unknown compounds as it does not rely on any knowledge of the analytes. Here, a sample control comparison will detect unknown contaminants. In both approaches automatically generated Enhanced Product Ion spectra can be searched against a comprehensive mass spectral library and the fragmentation information can be used for identification and identification. However, the GUS approach is lower in sensitive and requires significantly longer run times.

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