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# Abstract

Effluent discharges from pulp mill, sewage, or pesticide run-off are released into aquatic environments as complex mixtures. This study uses two types of discharge compounds (an insecticide and a pulp mill condensate) to illustrate the LC/Q-TOF sensitivity, linear range, quantitative, and qualitative analysis functionalities.

## Introduction

Environment Canada is tasked with risk assessment and the evaluation of environmental impact of a variety of compounds [1, 2]. Effluent discharges from pulp mill, sewage, or pesticide run-off are released into aquatic environments as complex mixtures. Solid-phase extraction (SPE) or gel permeation chromatography (GPC) and GC/MS are typically used to characterize substances from the discharge. LC/MS has been used to identify difficult-to-analyze polar compounds. However, the potential for LC/MS to identify unknown polar compounds has yet to be fully realized.

This study uses two types of discharge compounds to illustrate the LC/Q-TOF sensitivity, linear range, quantitative, and qualitative analysis functionalities. Imidacloprid (an insecticide) is tricky to analyze by GC/MS. It has been used in Atlantic Canada (run-off from potato fields) and there appear to be some nontargeted toxicological effects by this insecticide. Accurate mass and quantitation capability from a Q-TOF are critical for the routine analysis of target compounds.

The other sample is a pulp mill condensate. Pulp mills are the largest users of fresh water in Canada. Bleached kraft pulp and paper mill final effluents are known sources of compounds that affect reproductive endocrine homeostasis in fish [3, 4]. Environment Canada is working closely with a pulp mill that has developed a reverse osmosis process to help meet its effluent regulatory requirements for toxicity. Manool, a terpenoid present in trees, is one of the compounds removed by reverse osmosis and is related to compounds suspected of causing the reproductive problems in fish. An analysis is needed to confirm its presence in the condensates treated by reverse osmosis. A Q-TOF is capable of screening and identifying impurities and degradation products.



## **Experimental**

#### Samples

Figures 1 and 2 show the two compounds used in this study. Calibration solutions (10, 20, 50, 100, and 200 ppb) of imidacloprid were made from a 10.11 ppm stock solution in methanol. An additional sample was a blind with an undisclosed concentration of imidacloprid to evaluate the quantitation ability of the Q-TOF.

Samples of manool included a standard solution in methanol and a pulp extract treated with reverse osmosis process.



Formula: C<sub>9</sub>H<sub>10</sub>CIN<sub>5</sub>O<sub>2</sub> 13826-41-3 255.05230





Formula: C<sub>20</sub>H<sub>34</sub>O CAS: 596-85-0 MW: 290.26095

Figure 2. Manool.

#### Instrument Parameters

All sample analyses were performed on an Agilent 1200 SL Rapid Resolution LC coupled to an Agilent 6520 Q-TOF.

All sample analyses were performed under Q-TOF autotune conditions. Mass accuracy, sensitivity, and resolution for all samples were measured without any changes to 6520 Q-TOF instrument parameters, except ion source conditions appropriate for the spray chamber type, LC flow, and sample thermal stability.

Mobile A	5 mM NH40Ac, pH 4
Mobile B	MeOH
LC column	ZORBAX XDB 2.1 × 50 mm, C-18,
	3.5-µm particle size
Flow rate	0.5 mL/min
Injection volume	5 µL
MS	Scanned at 2 scans/sec, 50 to
	1,100 <i>m/z</i>
Positive reference ions	<i>m/z</i> 121, 922
AutoMS/MS	2 scans/sec MS and 2 scans/
	sec MS/MS
Q-TOF parameters	Set by autotune
Drying gas	12 L/min N <sub>2</sub>
Drying gas temperature	300 °C (imidacloprid),
	150 °C (manool)
Nebulizer pressure	50 psi
ESI (+)	3 KV
Fragmentor	120 V

### **Results and Discussion**

#### Imidacloprid

Figure 3 is a "Batch-at-a-Glance" screen from the MassHunter software. All the samples analyzed are listed on the top half of the screen. The calibration curve and the corresponding quantitation ion for each sample are displayed on the bottom half of the screen. All calibration standards (10, 20, 50, 100, and 200 ppb) and samples were analyzed in triplicate during the batch to check precision and accuracy.

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		2000	Cal	20	1/18/2008 4:38 PM	20.0000	1.804	34910	63.47	m	20.2786	101.4	Good buildfution	
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	-	50pg	Cal	50	1/18/2008 5:13 PM	50.0000	1.005	78603	268.9		47.0347	94.2		
		50pg	Cal	50	1/18/2008 5:22 PM	50 0000	1.002	81609	46.55		48.9395	97.9		
	-	100pg	Cal	100	1/18/2008 5:30 PM	100.0000	1.804	166989	197.0		101.3415	101.3		
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Figure 3. "Batch-at-a-Glance" screen from the Mass Hunter software showing the quantitation and calibration results of imidacloprid.

The  $R^2$  for the calibration curve was 0.9984 and the accuracy for each standard was between 94 and 113%. The quantitation results (triplicate) of a sample with unknown concentration of imidacloprid were 305, 287, and 296 pg/µL. The results were very close to the actual amount of 303.3 pg/µL. The largest difference from the actual was less than 6% (287 versus 303.3). Table 1 shows the precision results for each calibration standard (three injections each). This demonstrates the good precision and accuracy of the LC/Q-TOF system.

 
 Table 1.
 Instrument Precision for Each Calibration Standard and the Unknown Sample (three injections for each level).

-		
Level	%RSD	
10	5.5	
20	1.3	
50	2.0	
100	0.7	
200	3.2	
(303.3)	3.1	

Table 2 shows that the mass accuracy for the 10-ppb imidacloprid standard was 1.76 ppm and 1.03 ppm for the 200-ppb standard. This illustrates that there is no trade-off between mass accuracy and dynamic range under the same autotune settings.

#### Manool

A standard solution of manool in methanol was analyzed by LC/Q-TOF. Multiple peaks were observed as shown in Figure 4. Several peaks are manool-related impurities (diterpenes). It is interesting to note that MH<sup>+</sup> of manool was not observed in the Q-TOF spectrum (Figure 5). The most significant ion was MH<sup>+</sup> – H<sub>2</sub>O. Other ions included MNH4<sup>+</sup> – H<sub>2</sub>O, MNH4<sup>+</sup>, and MNa<sup>+</sup>. The thermal neutral loss ions were confirmed by MS/MS analyses. Figures 6 and 7 show the MS1 and MS/MS spectra of ion m/z 308 and 290, respectively. The MS/MS spectra of both ions are very similar, suggesting similar precursor ions that differ by a loss of H<sub>2</sub>O (m/z 18).

 Table 2.
 Mass Accuracy of Measuring Different Concentrations of Imidacloprid

 (CqH10CIN502)

10	5111001115027			
	Exact mass	$Calculated MH^+$	$Measured\;MH^+$	Accuracy
10 ppb	255.052299	256.05958	256.06003	1.76 ppm
200 ppb	255.052299	256.05958	256.05984	1.03 ppm

A pulp mill condensate extract was also analyzed by LC/Q-TOF. Figure 8 is the overlay of the TIC and the largest 15 compounds (within m/z 200 to 600) found by molecular feature extractor. The major compounds were identified as: There were also unknowns with molecular formulae  $C_{20}H_{30}O$ ,  $C_{20}H_{30}O_3$ , and  $C_{20}H_{34}O_2$  that are  $C_{20}$ suspected diterpene analogues of manool. These data show that the reverse osmosis system removes these natural products from the condensates.

- C<sub>20</sub>H<sub>30</sub>O<sub>4</sub> (dihexyl phthalate)
- C<sub>20</sub>H<sub>34</sub>O (Manool)
- C<sub>22</sub>H<sub>34</sub>O<sub>4</sub> (diheptyl phthalate)
- C<sub>24</sub>H<sub>38</sub>O<sub>4</sub> (dioctyl phthalate)



Figure 4. Characterization of manool and impurities.

m/2 / Ion Formule Abundance 2020/2005 (MHH)/(H20) C03433 8509235 Best Formula III Jan Formula A Score V Crass Score Mass Call: Mass		$(No MH^{+})$
Rest Formula MI Ion Formula Score C Cross Score Mass Calc Mass		
C20H340 C20H33 100 100 290.26153 290.260	Difference (ppm) DBE 397 -1.96 4	$MH^+ - H_2O$
m/z ton Formula Abundance 230.2846 (M+10H4)+[H20] C20H36N 65733.1		
Best         Formula (M)         Ion Formula         Score         Closs Score         Mass         Calc Mass           (*)         C20H340         C20H36N         100         100         290.26134         290.260	Difference (ppm) DBE 397 -1.29 4	$MNH_4^+ - H_2$
m/z Ion Formula Abundance 308.25516 (M+NH4)+ C20H39N0 49932.9		
Best         Formula [M]         Ion Formula         Score         Closs Score         Mass         Calc Mass           [2]         C20H340         C20H39N0         100         100         290.26134         290.260	Difference (ppm) DBE 397 -1.29 4	MNH4 <sup>+</sup>
m/z / Ion Formula Abundance 313.25054 [M+Nal+ C20H34Na0 61352.9		
Best         Formula (M)         Ion Formula         Score         Closs Score         Mass         Calc Mass           C20H340         C20H34Na0         100         100         290.26132         290.260	Difference (ppm) DBE 197 -1.22 4	MNa <sup>+</sup>
S Spectrum Results		
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Figure 5. Characteristic ions of manool. This spectrum shows all of the different possibilities (for example, loss of water, adducts).



Figure 6. MS/MS of pulp sample confirming origin of thermal neutral loss ions in MS spectrum for m/z 308 (manool + NH<sub>4</sub>)<sup>+</sup>.



Figure 7. MS/MS of pulp sample confirming origin of thermal neutral loss ions in MS spectrum for m/z 290 (manool -H<sub>2</sub>O + NH<sub>4</sub>)<sup>+</sup>.



Figure 8. Molecular feature extractor extraction of the 15 largest m/z 200 to 600 compounds in a pulp extract.

### Conclusions

Excellent mass accuracy (< 2 ppm), instrument precision (%RSD < 6%), and quantitation results (quant accuracy < 6%) were obtained from the imidacloprid analysis.

Many manool-related compounds (terpenes) were identified by Q-TOF using formula search or molecular feature extractor followed by exact mass database search. The lower sprayer temperature and adding NH4OAc in mobile phase were critical to get molecular ions and MS/MS ions.

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