Analyzing Compounds of Environmental Interest Using an LC/Q-TOF Part 3: Imidacloprid and Manool

Application

Environmental

Authors

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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.

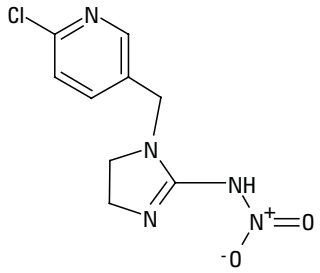
The other sample is a pulp mill condensate. Pulp mills are the largest users of fresh water in Canada. Bleached kraft pulp and paper mill final effluents are known sources of compounds that affect reproductive endocrine homeostasis in fish [3, 4]. Environment Canada is working closely with a pulp mill 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

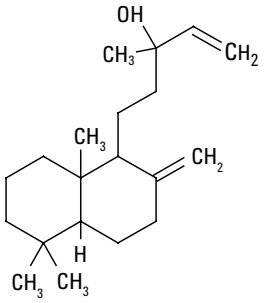
Figures 1 and 2 show the two compounds used in this study. Calibration solutions (10, 20, 50, 100, and 200 ppb) of imidacloprid were made from a 10.11 ppm stock solution in methanol. An additional sample was a blind with an undisclosed concentration of imidacloprid to evaluate the quantitation ability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

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



Formula: $C_9H_{10}ClN_5O_2$
CAS: 13826-41-3
MW: 255.05230

Figure 1. Imidacloprid.



Formula: $C_{20}H_{34}O$
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 NH_4OAc, 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 *m/z*
Positive reference ions	*m/z* 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_2
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.

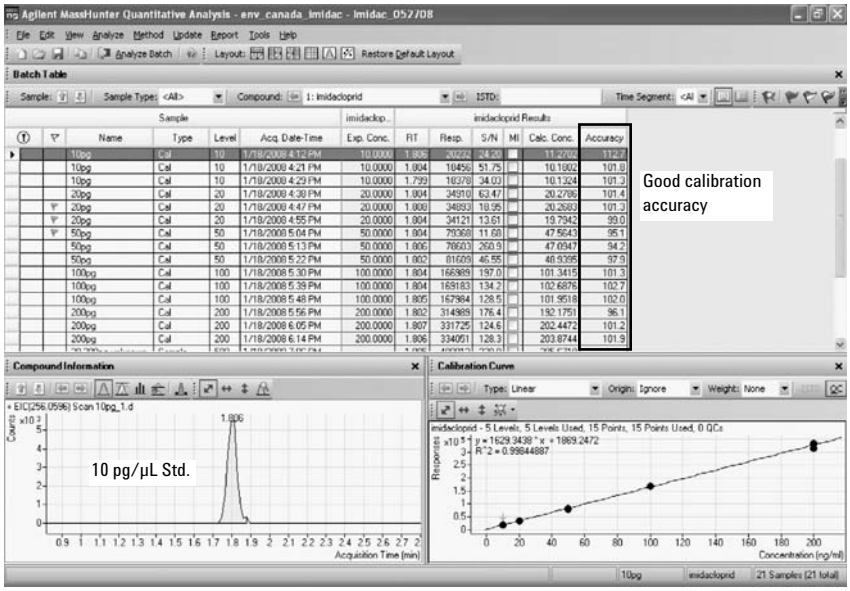


Figure 3. "Batch-at-a-Glance" screen from the Mass Hunter software showing the quantitation and calibration results of imidacloprid.

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^+ of manool was not observed in the Q-TOF spectrum (Figure 5). The most significant ion was $MH^+ - H_2O$. Other ions included $MNH_4^+ - H_2O$, MNH_4^+, and MNa^+. 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_20 (*m/z* 18).

Table 2. Mass Accuracy of Measuring Different Concentrations of Imidacloprid ($C_9H_{10}ClN_5O_2$)

	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:

- $C_{20}H_{30}O_4$ (dihexyl phthalate)
- $C_{20}H_{34}O$ (Manool)
- $C_{22}H_{34}O_4$ (diheptyl phthalate)
- $C_{24}H_{38}O_4$ (dioctyl phthalate)

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.

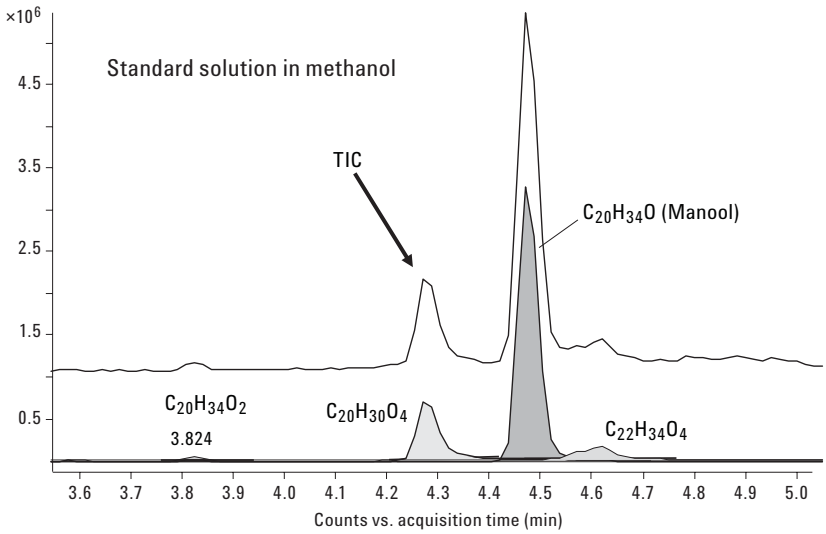


Figure 4. Characterization of manool and impurities.

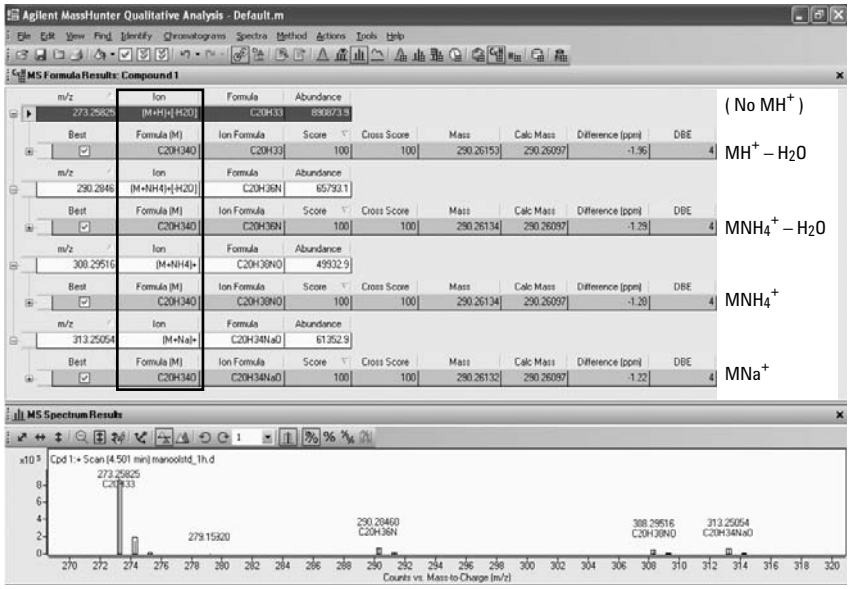


Figure 5. Characteristic ions of manool. This spectrum shows all of the different possibilities (for example, loss of water, adducts).

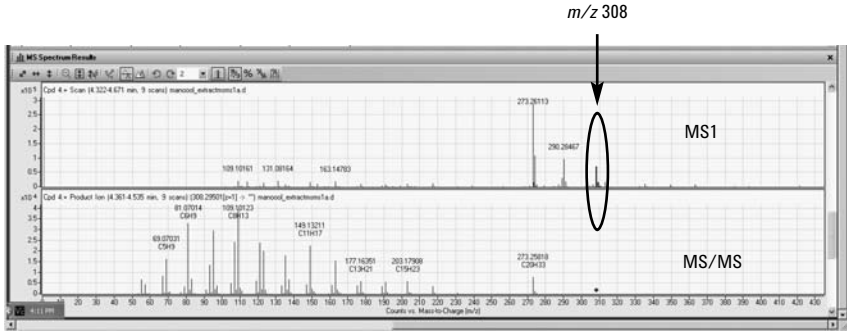


Figure 6. MS/MS of pulp sample confirming origin of thermal neutral loss ions in MS spectrum for *m/z* 308 (manool + NH_4)$^+$.

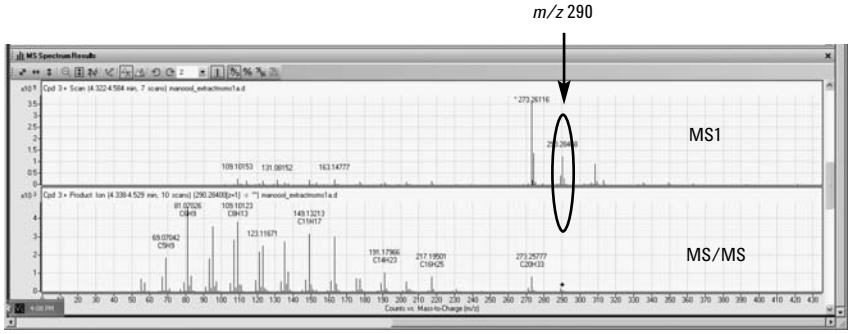


Figure 7. MS/MS of pulp sample confirming origin of thermal neutral loss ions in MS spectrum for *m/z* 290 (manool -H_2O + NH_4)$^+$.

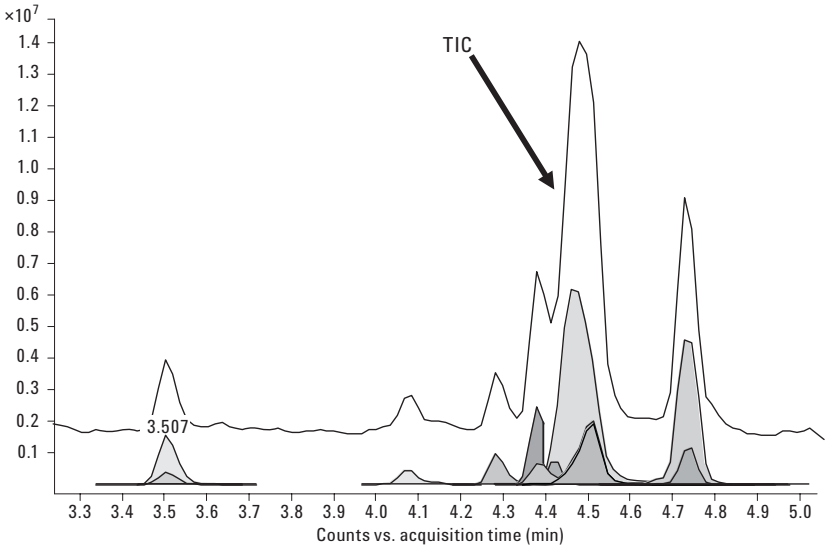


Figure 8. Molecular feature extractor extraction of the 15 largest *m/z* 200 to 600 compounds in a pulp extract.

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 NH_4OAc in mobile phase were critical to get molecular ions and MS/MS ions.

References

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2. Jim Lau, Chin-Kai Meng, Jennifer Gushue, Robert J. Letcher, and Shaogang Chu, "Analyzing Compounds of Environmental Interest Using an LC/Q-TOF – Part 2: Fluorotelomer Unsaturated Acids," Agilent Technologies publication 5989-9132EN, August 2008.
3. L. Mark Hewitt et al., "Isolation of Compounds from Bleached Kraft Mill Recovery Condensates Associated with Reduced Levels of Testosterone in Mummichog (*Fundulus heteroclitus*)," *Environmental Toxicology and Chemistry*, Vol. 21, No. 7, pp. 1359–1367, 2002.
4. L. Mark Hewitt et al., "Altered Reproduction in Fish Exposed to Pulp and Paper Mill Effluents: Roles of Individual Compounds and Mill Operating Conditions," *Environmental Toxicology and Chemistry*, Vol. 27, No. 3, pp. 682–697, 2008.

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