Discovery of Imidacloprid Metabolites in Onions Using Mass Profiler Professional

Application Note

Food

Authors

Imma Ferrer and E. Michael Thurman
Center for Environmental Mass Spectrometry
Department of Environmental Engineering
University of Colorado
Boulder, CO
USA

Jerry A. Zweigenbaum
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

Abstract

Mass Profiler Professional (MPP), a multivariate statistical program, was applied to complex mass spectral chromatograms from 18 combined samples to tease out differences that arise from the metabolism of the pesticide imidacloprid, when applied to onions. The purpose of MPP, in general, is to assist in analyzing the complex mass spectral data that arise when high resolution accurate mass profiling is used on samples containing thousands of accurate mass ions from both the target analytes and the sample matrix.

Agilent Technologies

Introduction

Imidacloprid is a neonicotinoid pesticide that was introduced to the market in the late 1990s for the control of homopteran pests, such as aphids, planthoppers, whiteflies, and certain beetles. Since then, it has become the most widely used agriculture insecticide in the world. Imidacloprid acts as an agonist of the nicotinoid acetylcholine receptor, which is highly specific to insects [1,2].

Imidacloprid is one of the most toxic insecticides to bees, and recent research suggests that widespread agricultural use of imidacloprid and other pesticides may be contributing to honey bee colony collapse. A decline of honey bee colonies in Europe and North America has been observed since 2006. Thus, imidacloprid is an important insecticide to investigate in plant fate and metabolism studies [3].

Although imidacloprid metabolism has been studied in a number of crops, only recently has it been studied in onions [4,5] using several accurate mass tools to identify seven new metabolites. This application note re-analyzes the same data set using Mass Profiler Professional to filter the data for another type of interpretation.

Experimental

Reagents and standards

A pesticide stock solution (approximately 1,000 µg/mL) was prepared in methanol and stored at –18 °C. From this solution, working standard solutions were prepared by dilution with acetonitrile and water, as described previously [4].

Instruments

This study was conducted using an Agilent 1290 Infinity LC System coupled to an Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system equipped with electrospray Jet Stream technology. The instrument run conditions are shown in reference [5].

Pesticide application

Onions (*Allium cepa L.*) were grown from certified seed in a greenhouse environment as previously described [4]. A 2.5 µg/mL amount of imidacloprid in 200 mL of water was applied to each plant.

Sample preparation

Water samples (leachate) were collected once a week for three weeks. A total of 36 samples each of soil and plants were collected in triplicate at 28, 38, and 53 days. Water samples were filtered, if necessary, and spiked with deuterated imidacloprid. Plant extractions were performed as previously described [4,5].

Data analysis

Mass Profiler Professional was used to interrrogate the samples as described below. First, the data was filtered by flags, keeping all entities in each sample that were present or marginal. Next, the data was filtered by variability, again keeping entities in each sample that had a coefficient of variation of 25% or less. This filtered data set was then processed by principal component analysis. Finally, the same filtered data was examined using find-by-similarities to discover new metabolites.

Results and Discussion

Principal component analysis

Principal component analysis reveals the data variation. This data set clearly shows differences in the samples treated with imidacloprid versus those not treated. Figure 1 displays these differences in a three dimensional graph. This graph accounts for 71% of the total variance in the data set. The separation between the treated and untreated samples is clearly seen in Figure 1, where the red points are treated samples, and the blue points are untreated samples. Interestingly, day 1 of the treated samples is quite different from day 10 and day 23. These are the three different sampling periods of the study. In addition, day 10 is different from days 1 and 23 of the untreated samples. One simple explanation for this variance for untreated samples is the senescence of the onion plant, which is a natural process of browning or wilting of the plant due to different water uptake and sun exposure in the greenhouse. These living organisms have variations in the natural organic compounds present in the plant, which are important for photosynthesis. These compounds are measured quite easily by accurate mass analysis.

A second explanation for the treated samples could be the stress that the plant receives after application of the insecticide imidacloprid. This stress or release of stress (we cannot be sure) changes the plant metabolism. This is reflected in the ion chromatograms measured in the extracted plant materials. Our previous reports [4,5] show that imidacloprid is primarily transformed from the insecticide to metabolites. It was found that the parent resides primarily in soil and the metabolites reside primarily in the onion plant.

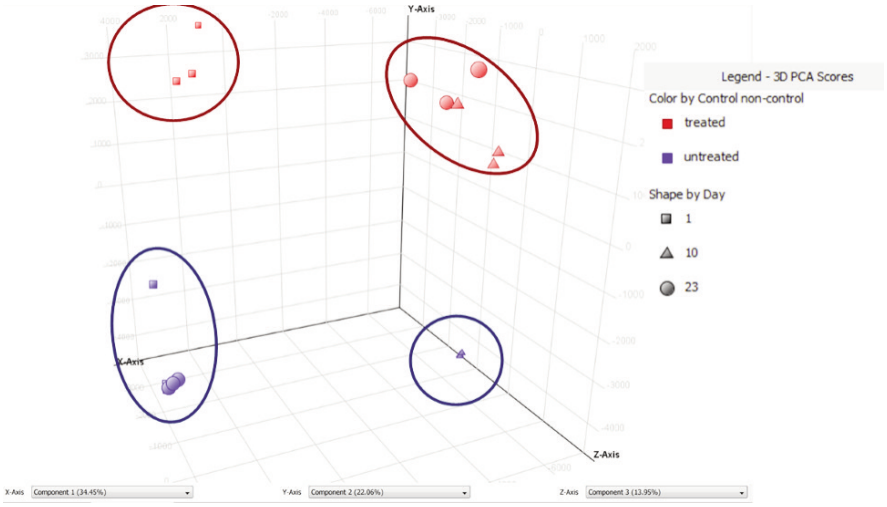


Figure 1. Principal component analysis of imidacloprid treated and untreated onion plants taken on three separate sampling days. Three individual plants were sampled on each day and each plant was only sampled once (18 separate plants in total).

Find similar entities

The second process used in Mass Profiler Professional is called **Find Similar Entities**, found in the analysis tab. To use this process, we applied the major guanidine metabolite previously found [4,5] at 6.4 minutes at a neutral mass of 210.0668. Figure 2 shows the five entities that have similar patterns to the guanidine metabolite (including this metabolite there are six) for both the treated and untreated sample set. The x-axis shows the nine untreated and the nine treated samples in this study. The y-axis shows the normalized data set. A zero value shows that all the samples are identical. A negative value shows that those samples are lower than the zero (median) value. A positive value shows that those samples are higher than the zero (median) value with respect to referenced entity at the mass of 210.0668. There was one treated sample, number 9, which was zero value. This means it was the same as the untreated samples. However, the soil showed the parent compound; therefore, the plant did not uptake the imidacloprid.

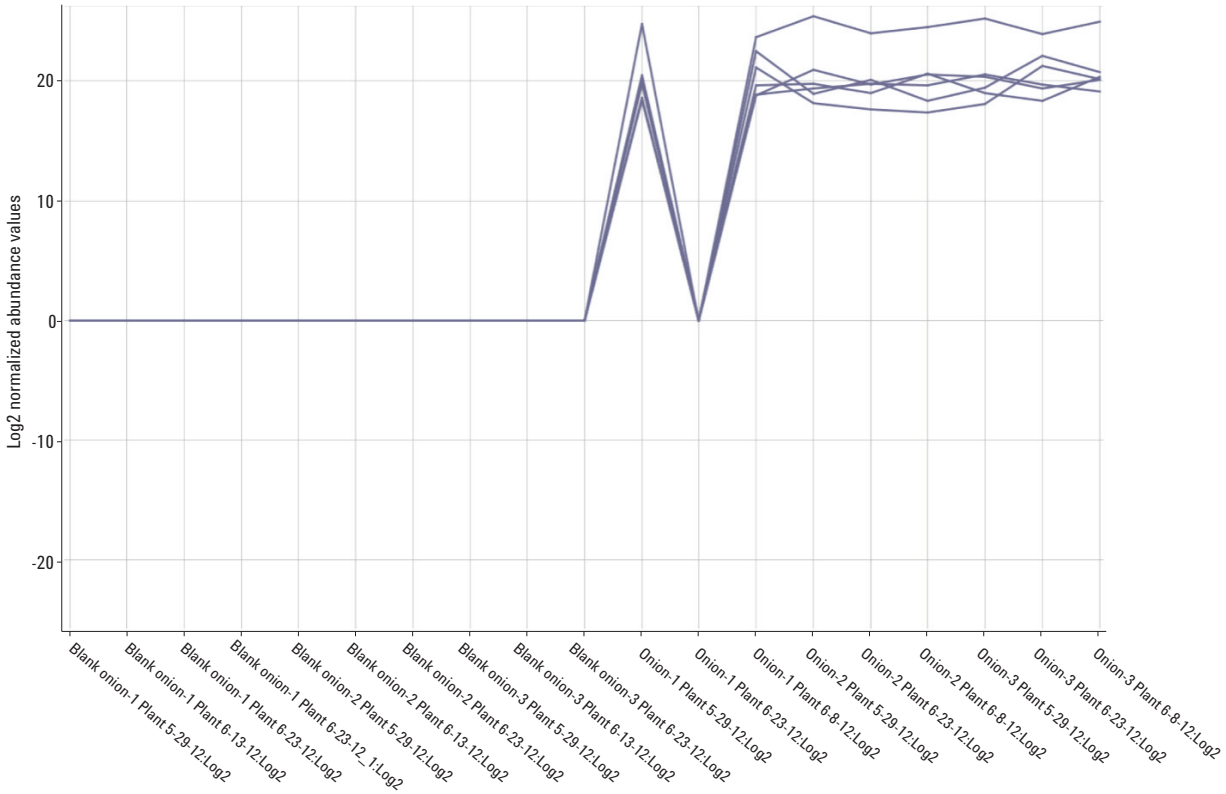
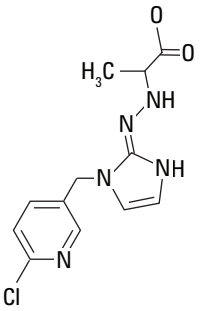


Figure 2. Plot of similar entities to the guanidine metabolite.

Figure 3 shows the list of six entities displayed as a positive value (+20) in Figure 2. A metabolite of imidacloprid not previously identified was found at a neutral mass of 295.0817 and a retention time of 11.01 minutes. The molecular formula given by the software was $C_{12}H_{14}ClN_5O_2$. From our previous experience with the metabolism of imidacloprid in onions [4,5], a putative structure is proposed in Figure 4. The guanidine metabolite, which was used for this correlation, is the basic structure that is proposed for conjugation. In this case, the amino acid, alanine, has the correct mass and formula for the new conjugated metabolite. The entity at neutral mass, 208.0516, at 5.93 minutes, was examined and found to be a fragment of a previously identified metabolite of imidacloprid-amine [5].

The remaining three entities did not contain chlorine in their molecular formulas and were not metabolites of imidacloprid.



Monoisotopic Mass: 295.0836 Da
Molecular Formula: $C_{12}H_{14}ClN_5O_2$

Figure 4. Putative structure of imidacloprid metabolite discovered using find similar entities in MPP.

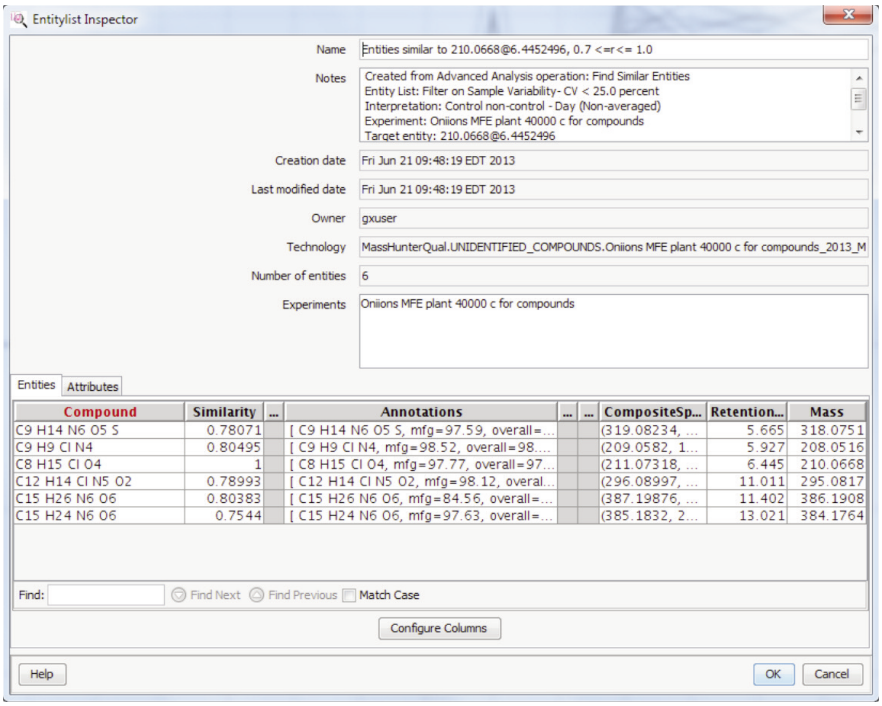


Figure 3. Screenshot of list of entities from find similar entities with the guanidine metabolite as the reference (entity with similarity of 1 in table).

Conclusions

The Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system and the Mass Profiler Professional are powerful tools for identifying and characterizing metabolites of pesticides in plants, such as onion. These tools enable detailed studies of the fate of the pesticide imidacloprid in plants. For example, a previously overlooked metabolite, the guanidine-alanine conjugate, was discovered using the Mass Profiler Professional tool set. Furthermore, this tool is useful for portraying changes in both the treated and untreated samples for future study.

References

1. S. Kagabu "Discovery of imidacloprid and further developments from strategic molecular designs" *J. Agric. Food Chem*. **59**, 2887-2896 (2011).
2. M. Tomizawa, J.E. Casida "Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors" *Annu. Rev. Entomol*. **48**, 339-364 (2003).
3. T. Iwasa, N. Motoyama, J. Ambrose, R.M. Roe "Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*" *Crop Protection* **23**, 371-378 (2004).
4. E.M. Thurman, *et al*. "Identification of imidacloprid metabolites in onion (*Allium cepa L.*) using high-resolution mass spectrometry and accurate mass tools" *Rapid Commun. Mass Spectrom.* **27**(17):1891-903 (2013).
5. E.M. Thurman, I. Ferrer, J.A. Zweigenbaum "Identification of imidacloprid metabolites in onions using high-resolution mass spectrometry and accurate mass tools" Agilent Technologies Publication 5991-3397EN (2013).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
February 11, 2014
5991-3931EN

Agilent Technologies