Automatic MS Data Analysis to reveal the metabolic pathways of pesticides in vegetables

Elisabeth Ortega-Carrasco[1], Rosalía López-Ruiz[2], Blanca Serra[1], Ismael Zamora[1], Roberto Romero-González[2], Antonia Garrido Frenich[2]

[1]Lead Molecular Design, Sant Cugat del Vallès (Barcelona); [2]Universidad de Almería, Almería.

International Food & Environmental Analysis Summit
Toledo, 03-04 April, 2019

Agilent

Poster number

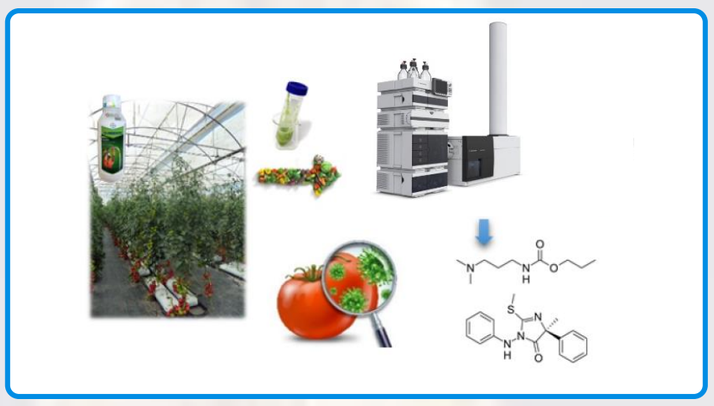
Introduction

Pesticides are used in order to reduce the production losses due to pest and maintain the food quality.

Their use has increased worldwide because of their rapid action. However, the presence of pesticide residues in fruits and vegetables, among other foods, has become a major concern for consumer health, especially in developing countries (Fantke et al., 2012; Skovgaard et al., 2017).

The active ingredients of some pesticides are absorbed by plants and they are converted by biotransformation process into metabolites. Metabolites are produced by biochemical reactions that naturally occour in the cell metabolism, and they depend on the molecular structure of the pesticide parent compound and other factors as type of plant, crop conditions and temperature (Fenner et al., 2013).

Advances in analytical techniques with increased sensitivity have led to the detection of a growing number of metabolites at low concentrations, being HPLC-HRMS the most used analytical method to perform this task. To help the processing and identification of the different compounds appearing in the sample, we use MassChemsite 2.0 (Molecular Discovery, Ltd.).



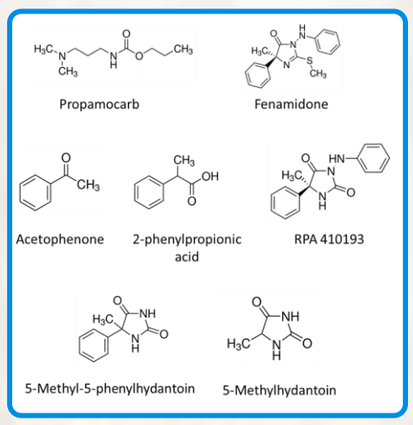
This work has been recently published in Chemosphere journal
https://doi.org/10.1016/j.chemosphere.2019.03.118

Experimental

Use of Consento® in vegetables

Consento® is a commercially available product formed by the mixture of two fungicides: propamocarb and fenamidone. This commercially available product has a good performance in controlling downy mildew and blight in tomato, cucumber and other crops.

Propamocarb
(propyl [3-(dimethylamino)propyl]carbamate) is a systemic fungicide with a protective action against phycomycetous diseases (Hu et al., 2007). Fenamidone ((S)-5-methyl-2-methylthio-5-phenyl-3-phenylamino-3,5-dihydroimidazole-4-one) is a systematic foliar fungicide used to control oomycete diseases such as early and late blights.
Both compounds degrade into a variety of metabolites:



Experimental

Sample preparation

The extraction procedure was the well-known QuEChERS method (Anastassiades et al, 2003): 1kg of sample fruits was crushed and homogenized as established by current regulations (SANTE/EU, 2017), storing it in the freezer at -21ºC. After, 10g of homogenized sample were placed in a 50mL plastic tube centrifuge tube and 10mL of ACN were added and shaken for 10 min in a rotatory shaker. After that, 1g of NaCl and 4g of $MgSO_4$ were added, and the mixture was shaken vigorously for 1 min in a vortex. Finally, the sample was centrifuged for 10 min at 5000rpm. Samples were collected after 1, 2, 3, 7, 9 11, 15, 21 and 23 days.

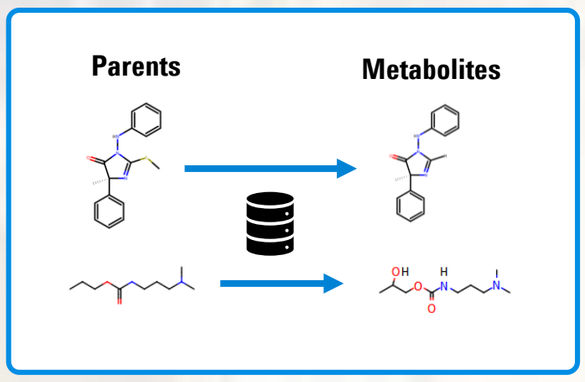
Injection preparation

For the UHPLC-HRMS analysis, 1mL of supernatant was collected and injected into the system. For the GC-HRMS analysis, 1mL of supernatant was dried under nitrogen current and redisolved in 500uL of EtOAc prior injection.

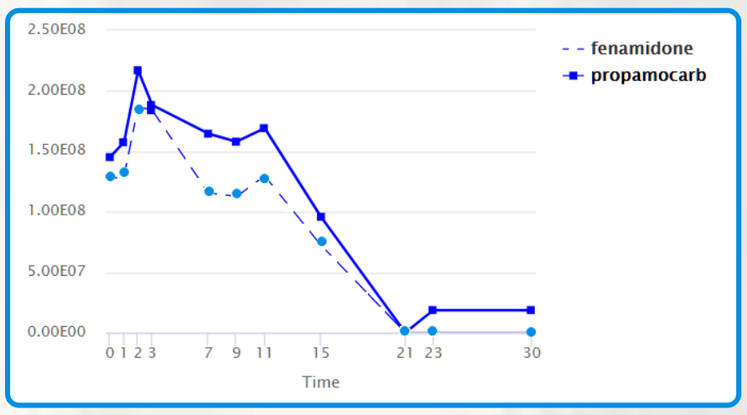
Results and Discussion

MassChemsite and WebChembase for automatic MS data analysis

The presence of propamocarb and fenamidone in cucumber samples was investigated using the Chemical Monitoring workflow of MassChemsite 2.0. This workflow is able to identify several parent compounds specified by the user and their corresponding derivatives (metabolites, degradants, etc.) using a editable reaction set.



MassChemsite is able to process raw data files and show the results individually. To consolidate the information of all the different timepoints together we used WebChembase (Molecular Discovery, Ltd, in development process) a webserver application. The aggrupation of the different timepoints together allows us to do a quick comparison on the areas of different compounds in the sample. As a example, in the next image there are plotted the LC area for each of the pesticides under study during the 30 days that lasts the analysis:



Targeted analysis of propamocarb

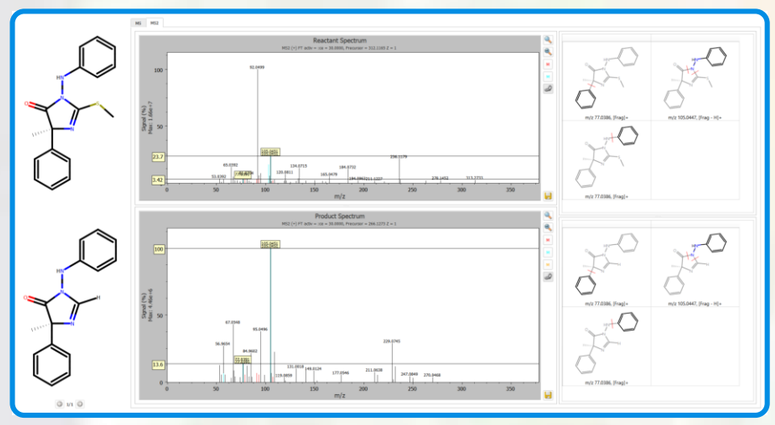
Propamocarb is a widely used pesticide. Its metabolites and degradation products are deeply studied (Brancato et al. 2017). One of those metabolites is the N-oxide propamocarb. MassChemsiie can speed up the structural identification of the expected metabolites.

Results and Discussion

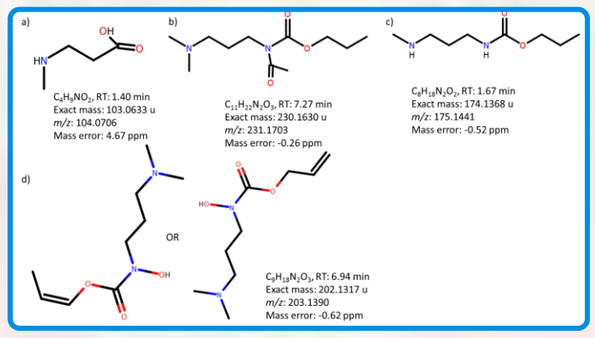
To perform a targeted analysis the user has to provide to MassChemsite the structure of the compound to find. Then a specialized algorithm will search the given compound into the MS/MS2 data. Last, the specified reactions will be applied in order to find derivatives of the targeted compound.

Untargeted analysis of propamocarb and fenamidone

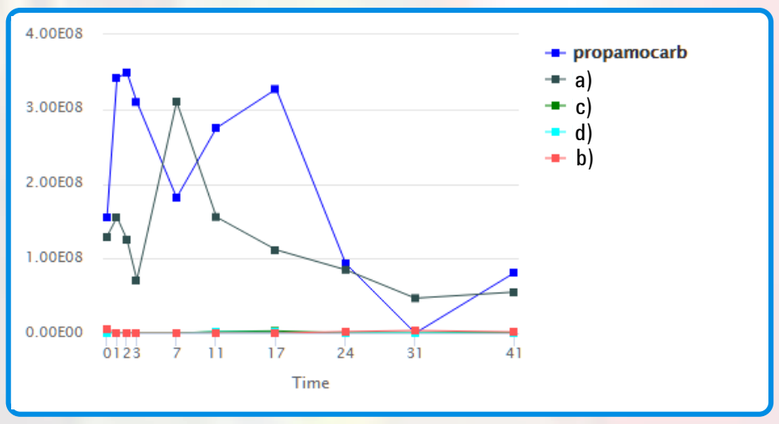
For fenamidone, an unexpected metabolite was found after 15 days, and corresponds to the carbon-sulfur group cleavage. The structure of that new metabolite was automatically elucidated by MassChemsite, using the fragmentation of the parent as template to build the product.



In the case of propamocarb, a total of 4 new metabolites were found:



From that set of newly found metabolites, only **a)** is present in high concentration and could be included in the MRL of propamocarb for tomatoes.



Conclusions

Here we presented our latest work in the identification of new metabolites for propamocarb and fenamidone using MassChemsite to process the data and WebChembase to consolidate different experiments and present the information in a comfortable way.
Additionally, an automatized procedure to find unexpected metabolites in samples was shown.