Multi-target screening approach for pesticide fraud detection using liquid chromatography high resolution mass spectrometry

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Abstract

The Ministry of Agriculture and Livestock (MAPA) is one of the Brazilian authorities responsible for approval and control of agrochemicals. It is estimated that 20% of agrochemicals consumed in Brazil are of illegal origin (falsification, theft, smuggling). It represents more than US$ 2 billion annually.

Since 2020 MAPA has a special program – Agricultural Defense Surveillance Program for International Borders (Vigifronteira), in cooperation with other Brazilian Authorities (Federal Police, Brazilian Institute of the Environment and Renewable Natural Resources –IBAMA). Many task forces has been done by Vigifronteira, and it demands laboratory analysis to confirm the active ingredient of suspicious products.

In this context, it was developed a fast and effective method for confirming the identity of pesticides and detecting fraud in products in order to protect the pesticide market and the final consumer.

Method Development

The method was developed using a pool of standards and the conditions were optimized aiming to identify each of them. The method was optimized for 347 compounds. Then, pesticides products were tested to evaluate the influence of the matrix in the identifications.
A small aliquot of the product was diluted in an appropriate solvent, usually water or isopropanol. A second dilution was made in water resulting in a final concentration between 100 to 500 ppb.

LC-MS/MS conditions:

Equipment: LC-MS/MS Q-Exactive (Thermo Scientific) with an ESI source in positive and negative modes.

Column: InfinityLab Poroshell 120 EC-C18 (3.0 mm x 3.0 mm, 2.7 µm).

Mobile phase: water and methanol (95:5) with 0.1 formic acid ammonium formiate 5mM (A) and water and methanol (5:95) with ammonium formiate 5mM (B)

Data Acquisition: PRM for 347 compounds

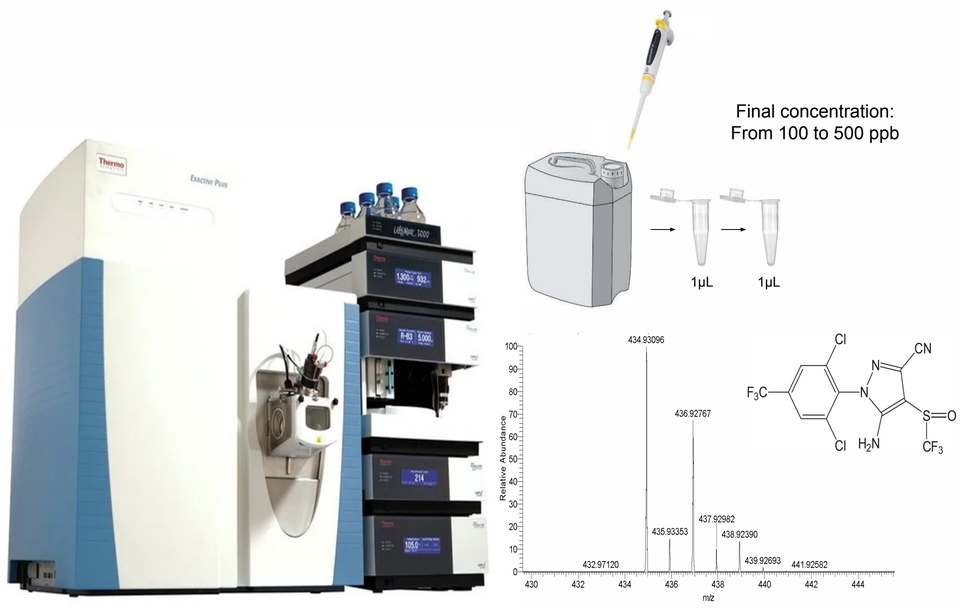


Figure 1: Equipment used in the method, workflow for the analysis and an example of mass spectrum for fipronil showing the characteristic isotopic distribution.

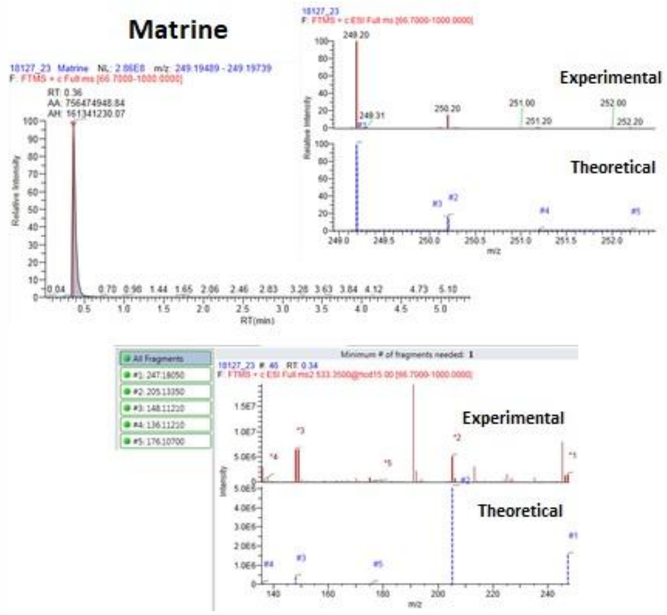
Qualitative Validation Plan

The validation of the qualitative method was conducted using the following parameters:

- Specificity: Analysis of a spiked pesticide product using a pool of standards aiming the identification of the compounds in the presence of different matrix.
- External validation: Blind analysis of thirty pesticide products aiming full identification. This experiment should be repeated by different analysts.
- Robustness: Analysis of the samples from external validation experiment using different solvents for initial solubilization.

Routine Analysis

During routine, samples received were initially evaluated about declared concentration and composition (in case it is available). At this point it is important to check if the compounds are in the PRM list and made the correct dilution.
The first confirmation point is given by liquid chromatography verifying the comparison between retention time of the sample and that one obtained for the standard. The second point check the exact mass (5 ppm tolerance) and the expected isotopic distribution.



The third confirmation point is given by the MS/MS experiment and the identification of at least two fragments with exact mass (5 ppm tolerance) gives the identity of the majoritarian compounds of the product.

Figure 2: Example of result for a sample containing the compound matrine. The figure shows chromatogram with the retention time, isotopic distribution and MS/MS spectrum and the identified fragments

Conclusion

The developed method was fast and efficient to identify compounds in pesticide products from falsification and smuggling giving to Agricultural Defense Surveillance a reliable result.
The confirmation of the identity of this compounds is important to protect the market, avoid misuse preventing damage to the environment and for the human health.

References

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