



SZENT ISTVÁN UNIVERSITY
FACULTY OF FOOD SCIENCE

DEVELOPMENT OF PESTICIDE
ANALYTICAL METHODS

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Introduction

Detoxification processes in plants may go together with the formation of pesticide metabolites that are bioavailable and bioaccessible for higher animals and humans. As these novel metabolites possess unknown toxicity, they should be considered as potentially harmful compounds. For authorized pesticides and for some of their metabolites (e.g., dimethoate, omethoate) MRL values have been set; however, foods may also contain previously undetected or unknown pesticide residues and metabolites that can be the result of food processing steps. Therefore, the continuous development of pesticide analytical methods is a must to provide high food safety levels, which hopefully triggers the re-evaluation and lowering of MRL values, and even the introduction of novel pesticide metabolites to monitor.

Theses

1. I was able to set up UPC² based analytical system with ESI-MS detection to study the chromatographic behavior of target pesticides. I noticed that increasing temperature decreases the elution strength of CO₂ mobile phase - this phenomenon was enhanced for most of the target analytes with the addition of CH₃CN as coeluent. However, its use resulted in opposite effects for certain analytes, i.e., dimethoate, piperonyl-butoxide, tebufenpyrad, prochloraz, as the eluotropic strength of the mobile phase was enhanced. The observation indicates that the elution characteristics of CH₃CN dominate over those of supercritical CO₂ in the case of dimethoate, piperonyl-butoxide, tebufenpyrad and prochloraz.

2. I developed a novel sample preparation method and a related HPLC-MS/MS procedure for a special processed food matrix, i.e, a cake sample containing 12 m/m % fat that was exposed to a previous heat treatment. The overall method could be used for the quantitative monitoring and mass balance determination

of a postharvest fungicide, imazalil, from the raw material (citrus peel) until the end cake product.

3. I was the first to describe the heat treatment based degradation of imazalil in a processed food matrix with the help of an HPLC-ESI-QTOF-MS instrumental setup.

4. I developed and validated a new analytical method for fruit matrices with HILIC-ESI-TOF-MS and HILIC-ESI-MS/MS instrumental setups. This method could be used to detect and quantify higher number of polar pesticides (17 in positive ion mode and 9 in negative ion mode) than the QuPPE version 7.1 offered that was available at the time of thesis preparation.

5. I proved that the HILIC method I had validated for the quantification of polar pesticides are suitable for high oil containing food matrices, e.g., olive is olive oil.

Applicability of ultra performance convergence chromatography, a new generation of supercritical fluid chromatography, for the analysis of pesticide residues

Monitoring and controlling a wide variety of pesticide residues is an essential part of food safety. In the field of multiresidue pesticide analysis, HPLC and GC-based mass spectrometry (MS) methods are the preferred choices and they are considered complementary to each other. In the latest years, there has been a remarkable increase in the number of LC-MS applications; nevertheless, there are compounds such as quintozone, aldrin, folpet, and heptachlor that are typically GC-amenable and LC separation cannot provide satisfactory chromatographic results. In our study, an ultra-performance convergent chromatography (UPC²), a new generation supercritical fluid chromatography (SCF), coupled to ESI-MS/MS system was applied to separate a set of pesticides to investigate their chromatographic behavior under various UPC² conditions. The 30 components involved in the study

were representatively selected based on LC and GC compatibility, $pK_{o/w}$ values and compatibility with the EN 15662:2008 sample preparation method. Chromatographic parameters such as the capacity factor and the peak shape of tested pesticides were compared to the corresponding data obtained from LC-MS and GC-MS runs. Based on these results, an attempt was made to estimate the similarity of the chromatographic behavior of the tested pesticides in the UPC² system with the one observed with GC and LC separations. It can be concluded that separation mechanisms relevant to the studied compounds in the UPC² system do not resemble to what is known in LC or GC systems, which indicates that UPC² can be considered as an alternative chromatographic approach with not yet fully established separation mechanisms. Furthermore, our findings indicate that using CO₂ in the UPC² mobile phase has a significant influence on ESI-MS ionization. That is, the number of detected components highly depends on the mobile phase composition used. In case methanol was used as a mobile phase modifier (co-eluent) in CO₂, lower ion suppression was observed compared to the case

when acetonitrile was used. An interesting observation was also made regarding the eluotropic strength of acetonitrile and methanol acting as modifiers of the CO₂ mobile phase of the UPC² system: acetonitrile that is considered the stronger eluent in RP-LC separations compared to methanol turned out to be weaker when the same compounds were separated in an UPC² system with CO₂ as the main constituent of the mobile phase.

Follow-up of the fate of imazalil from post-harvest lemon surface treatment to a baking experiment

Imazalil is one of the most widespread fungicides used for the post-harvest treatment of citrus species and it is therefore localized mostly on the surface of fruits. As citrus peel is often used as a spice for food processing and preparation, the carry-over of imazalil from citrus fruits to food products is an obvious and frequent event. Taking into account the relatively high stability of imazalil, the residual amount of this fungicide may not be negligible.

The goal of our comprehensive study was to quantitatively monitor the pathway of imazalil throughout a complete food preparation process. That is, imazalil-treated lemon batches were purchased from local markets, and the cleaning efficiency of household related washing procedures was determined first. After determining separately the imazalil contents of lemon peels and pulps, lemon peels were used to prepare a high-fat matrix cake that went through a usual baking /200 °C/ step, which models more closely the real household situation than spiking any food product with the standard solution of imazalil and avoiding any heat treatments to facilitate sample preparation issues.

In order to quantitatively extract imazalil from the baked fatty cake matrix, the sample preparation approach was adapted and validated on the basis of the EN 15662:2008 and the AOAC 2007.1 methods with three modifications, i.e., (i) hexane was added to the extraction solution, (ii) the cleaning step was applied after an overnight-long freeze-out step; (iii) the C₁₈ sorbent was added at the cleaning step of the sample preparation. Imazalil was quantified with the help of an HPLC-ESI-

MS/MS setup, while imazalil degradation was followed with an HPLC-ESI-QTOF-MS system.

As a conclusion, EN 15662:2008 method and its modifications generally resulted in better recovery values, mostly exceeding 90%, which meets the actual SANTE recommendations, provided the freezing step was included in the sample preparation process. The degradation of imazalil during the baking process was significantly higher when this analyte was spiked to the cake matrix in standard solution form than in the case of preparing the cake with imazalil-containing lemon peel (52% vs. 22%). This observation calls the attention to the careful evaluation of pesticide stability data that are based on solution spiking experiments.

Taking into consideration the washing procedures of the entire lemon samples as well, the overall carry-over rate of imazalil from the lemon until the baked cake products ranged between 55-76%. However, there is no regulation in the EU for pesticide residues in final food products except for some special commodities such as baby foods, this high level of

residual imazalil should trigger more real world scenario based experiments in this field.

HILIC, mixed-mode, and other aqueous normal-phase approaches for the liquid chromatography/mass spectrometry-based determination of challenging polar pesticides

The aim of the study was to evaluate the performance of different chromatographic approaches for the liquid chromatography/mass spectrometry (LC-MS(/MS)) determination of 24 selected highly polar pesticides and residues, namely, aminomethylphosphonic acid, amitrol, chlormequat, cyromazine, daminozide, diethanolamine, difenzoquat, diquat, ethephon, glufosinate-ammonium, glufosinate-N-acetyl, glyphosate, 2-imidazolidinethione, maleic hydrazide, mepiquat, 3-(methylphosphinico)propionic acid, morpholine, nereistoxin, paraquat, phosphorous acid, propylene thiourea, streptomycin, triethanolamine, and trimethylsulfonium iodide. The studied compounds that are unsuitable in most cases for conventional LC-

MS(/MS) multiresidue methods were tested under nine different chromatographic conditions, including two different hydrophilic interaction liquid chromatography (HILIC) columns, two mixed-mode columns (Sielc Technologies Obelisc N and Obelisc R), three normal phase columns operated in HILIC-mode (bare silica and two silica-based chemically bonded columns (Spherisorb cyano and amino)), and two standard reversed-phase C₁₈ columns. Different sets of chromatographic parameters in positive (for 17 analytes) and negative ionization modes (for nine analytes) were examined. In order to compare and contrast the different approaches, a semi-quantitative classification was proposed, which was calculated as the percentage of an empirical performance value consisting of three main features: (i) the capacity factor (k) to measure analyte separation from the void, (ii) the relative response factor (sensitivity) and (iii) peak shape based on analytes' peak width. While no single method was able to provide appropriate detection of all the 24 studied species in a single run, the best suited approach for the compounds ionized in positive mode was the one based on a UHPLC HILIC column with 1.8 μm particle size,

which provided appropriate results for 22 out of the 24 species tested. In contrast, the detection of glyphosate and aminomethylphosphonic acid could only be achieved with a zwitterionic-type mixed-mode column (Obelisc N). This column, however, proved to be suitable only for the pesticides detected in negative ion mode. Finally, the selected approach (UHPLC HILIC) was found to be useful for the determination of pesticides in oranges using HILIC-ESI-QTOF-MS with the limits of quantitation in the range from 0.003 to 0.56 mg kg⁻¹.

Determination of polar pesticides in olive oil and olives by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry and high resolution mass spectrometry

Quantifying polar pesticides is a real big challenge in itself, but the fatty food matrix also renders the analytical task often difficult. To solve this double challenge, two HPLC-MS methods were developed for the determination of polar pesticides in olive oil and olive samples. First, hydrophilic interaction liquid

chromatography (HILIC) separation followed by mass spectrometric detection with tandem mass spectrometry using a triple quadrupole instrument operated in multiple reaction monitoring mode (HILIC-ESI-MS/MS) was addressed. In the second method, the same chromatographic set-up was coupled to electrospray time-of-flight mass spectrometry (HILIC-ESI-TOF-MS). The selected polar pesticides included in the study were amitrol, cyromazine, diquat, paraquat, mepiquat, trimethylsulfonium (trimesium, glyphosate counterion) and fosetyl-aluminum. The sample preparation procedure was based on liquid partitioning with methanol. The performance of sample extraction was evaluated in terms of analyte recovery and matrix effects both in olive oil and in olive matrices. While the developed method for the determination of pesticides in olives and olive oil exhibited appropriate recoveries, the complexity of olive matrix restrains the performance and analytical parameters such as the limits of quantification, regardless of which instruments were used. The results obtained for olive oil were satisfactory, while, because of the high matrix complexity of olives, poor recovery rates were

obtained for diquat, paraquat and amitrol, although with a reasonable precision enabling its use in routine analysis. Similarly, matrix effects were minor in the case of olive oil (ca. 20% average signal), while significantly higher suppression was observed for olives (30-50%). The studied approaches were found to be useful for the determination of the selected pesticides in olive oil and olive matrices with the limits of quantitation below 5.0 $\mu\text{g kg}^{-1}$ in compliance with MRLs. However, the use of the tandem mass spectrometry (UHPLC(HILIC)-MS/MS) method still allowed the quantification of the target pesticides in compliance with the actual MRLs. Further progress should be conducted to minimize matrix effects and losses of these challenging analytes by means of additional tailored clean-up steps.

Related publications

Publications with impact factor

R. Nortes-Méndez, J. Robles-Molina, R. López-Blanco, **A. Vass**, A. Molina-Díaz, J. F. García-Reyes, Determination of polar pesticides in olive oil and olives by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry and high resolution mass spectrometry. *Talanta*, 2016; 158:222-228.

A. Vass, J. Robles-Molina, P. Pérez-Ortega, B. Gilbert-López, M. Dernovics, A. Molina-Díaz, J. F. García-Reyes, Study of different HILIC, mixed-mode, and other aqueous normal-phase approaches for the liquid chromatography/mass spectrometry-based determination of challenging polar pesticides. *Analytical and Bioanalytical Chemistry*, 2016; 408(18):4857-4869.

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Conferences

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A. Vass, E. Korpics, M. Dernovics, From the lemon surface until the baked food product: quantifile monitoring of the pathway of imazalil, 7. RAFA, Prgue, Czech Republic, 2015.11.03-06. ISBN 978-80-7080-934-1