POSSIBILITIES OF THE DETERMINATION OF MULTICOMPONENT PESTICIDE RESIDUES IN FOOD BY MASS SPECTROMETRIC TECHNIQUES

BÉLA KMELLÁR

PhD thesis summary and statement

Corvinus University of Budapest
Department of Applied Chemistry

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Name: Doctoral School of Food Sciences

Discipline: Food Sciences

Head of School: **Dr. Péter Fodor**
University professor
DSc
CORVINUS UNIVERSITY OF BUDAPEST
Faculty of Food Science
Department of Applied Chemistry

Supervisor: **Dr. Péter Fodor**
University professor
DSc
CORVINUS UNIVERSITY OF BUDAPEST
Faculty of Food Science
Department of Applied Chemistry

The approving signature of the Head of the Doctoral School and the Supervisor:

The candidate has met all the requirements determined in the Doctoral Code Book of the Corvinus University of Budapest. He took the observations and suggestions arising during preliminary examination when reworking this dissertation, thus the dissertation can be put to public defense.

....	....
Head of PhD School	Supervisor

The Local Doctoral Council for Life science of the Corvinus University of Budapest has been assigned in the resolution 8/03/2011 the following Thesis Committee for the public defense.

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1 INTRODUCTION

Almost 50 years, Rachel Carson published a book in 1962 called „Silent spring”. Although, it is not a scientific work, it opened the eyes of the scientists and intellectuals that the chemicals in the „new world” have a valuable risk and impact to the environment.

The excessive use of the wide scale of agrochemicals effect not just on the target plants, but may have a long term affect that can drive to ecological disaster. Although the ancient Sumerians used elemental sulfur as a plant protector, the usage of pesticides of current sense was started in the beginning of the 20th century. From the factors influencing the crop yield – although the weather is obviously not possible-, more chemicals were tried to eliminate the impact of negative factors.

The regulatory bodies tried to decrease their usage to the lowest level as possible because their negative impact on consumers.

The total exclusion is not realistic, which was reflected in the words of entomologist Ben Kantack, that:

„I prefer to find a salad in the market which contains a part per million (ppm) concentration of pesticides, than to find a cockroach in the salad with 40-50 infectious microorganisms between its legs.”

The food safety can be examined from physiological and regulatory point-of-view. It is safe, if the concentration of the questionable chemical is lower than the maximum residue level (MRL) settled by the legislative body.

These levels are not the same as the physiological limits in all cases (usually lower). The main reason of this is the conflict of interest between the legislators and the representatives of the agriculture sector.

The legislators intended to protect the consumers by decreasing the limits as possible, while the producers need to use chemicals to achieve the proper yield. The agricultural yield is an economic task that has to be taken into account by the legislative bodies, therefore the maximum residue levels are the results of such compromises. The compromises can only make sense if they complied with, so this can be verified.

The aim of the analysts is not to enforce or comply with the limits, but to develop such objective methods and measurements and serve results, whereby the above-described control and decision is feasible.

However, not only is this that has to be kept in mind. It is important that the analysis have to be fast and cost-effective. It means the speed of measurement, the reduction of solvent consumption of sample preparation and the maximalization of the number of compounds can be measured by one method, without loss of analytical information.

The analytical measurement technology and the joint development of new computer technology opened the till-then closed doors towards the multicomponent pesticide analysis, for which in my PhD thesis, I attempt to summarize the possibilities of today’s multicomponent pesticide analysis.

2 OBJECTIVES

The multicomponent pesticide methods showed/show a clear increasing tendency from the aspect of the number of compounds including in a method. This is limited by the capacity of the instruments, by the sample preparation technique – matrix - pesticide triangle, by the relationship between the detection technique and the co-eluting matrix components and also by the pesticides themselves. This indicates that the methods require a very high consistency.

In my PhD study, I targeted for the following partly related, partly separated topics by pushing the boundaries of the multicomponent pesticide methods. Here are as follows:

1. Developing a multicomponent method by LC-MS/MS technique that is suitable for the quantitative determination of large number (>150) of pesticides. My aims in this topic were the followings:

- Testing the „acetate buffered QuEChERS” method for the chosen 160 pesticides and for three representative matrices; researching the differences between the matrices
- Validation of the developed method
- Mapping of chromatographic and mass spectrometric interferences

2. Following this work, I focused the study on the effects of measurement parameters that I tested on 150 pesticides. My aims were the followings:

- Determination of ionization, chromatographic and mass spectrometric effects from the aspect of pesticide determination
- Comparison of UHPLC and HPLC performance and applicability

3. Including more than 150 pesticides in a single quantitative method would be justified, but it conflict to detection limits and is more laborious. Some LC-MS/MS instruments have a feature that allows component identification by spectrum libraries. My aim was to combine two methods to be cost-, and result-effective. One is feasible for the qualitative determination of 300 pesticides (the samples are screened), and after the occurrence of positive finding(s), we can determine the quantity of the detected pesticides with the second method. This second method is capable for the quantitative determination of less, but frequently detected pesticides. My aims in this topic were:

- Developing the qualitative and quantitative methods
- Develop the concept of the combination of the two methods
- Application of this method-combination on a large number of samples originating from the market those were prepared for analysis by the „acetate buffered QuEChERS” methodology
- Search for advantages and disadvantages of the two methods and their critical discussion

4. More than 90% of the analytical methods are focused on fruit and vegetables, crops and oil matrices. In contrast to this, analysis of matrices with low water content were marginalized and, partly related to this, is in its „infancy”. From the dry or dried samples, the spices and the tea can cause high background independently from the sample preparation method. For that reason, in this part, I focused the study not on the large number of pesticides, but on the elimination of matrix effect, with the following aims:

- Developing a sample preparation method for the determination of some chlorinated pesticides from spices and tea
- Developing a detection method by GC-MS/MS and GC×GC TOFMS coupled techniques
- Validating the two methods
- Critical comparison of the two methods from the aspect of matrix effects

I hope, with the realization of my goals I can focus the light on the limits and solutions of today’s multicomponent pesticide analysis, that can be a benefit for routine pesticide analysis requiring cost-effectiveness.

3 MATERIALS AND METHODS

The following chemicals were used for my investigations:
Analytical grade (mainly crystallized) pesticide standards were used for the method development. 1000-4000 µg/ml stock standard solutions were prepared from the analytical grade standards by dissolving them individually in organic (acetonitril, methanol, dimethyl formamid) solvents. After the preparation, the stock solutions and the analytical grade standards were kept in the freezer at -18 ºC. Standard mix solutions were prepared by dilution the stock solutions. For that and also for liquid chromatography eluent, deionized water was used. For the QuEChERS sample preparation, anhydrous $MgSO_4$, sodium acetate, PSA, sample vials with PTFE screw caps were used. The samples were evaporated under stream of nitrogen. Each sample was filtered through 0,45 µm (HPLC) or 0,22 µm (UHPLC) PVDF (polyvinylidene difluoride) filters. For HPLC separation, acetonitril and deionized water were applied. Small amount of formic acid, ammonium formiate and ammonium acetate were dissolved in the water phase in order to increase the ionization. Helium was solved as a carrier gas for gas chromatography analysis, the sample solutions were changed to ethyl acetate before GC analysis. Samples were stored in 2-ml sample vials.

The applied techniques:
LC-MS/MS, GC-MS, GC-MS/MS and GC×GC-TOFMS.

4 RESULTS

4.1 Development and application of a multicomponent method (>150 pesticide) by LC-MS/MS technique

From a wide list of pesticides I chose 160 relevant compounds those have a potential risk in Europe. I wanted to measure these with one method by HPLC-MS/MS technique. For that, I determined the optimal detection parameters of each in SRM mode and by merging these parameters, I constructed a method. Than, by applying a general HPLC column and a reversed phase gradient program, I did several runs in order to determine the retention time of each. I validated the analytical parameters of the developed method, like the linearity, limit of detection and robustness. I studied the „acetate buffered QuEChERS” methodology on the chosen orange, pear and tomato samples. I got sufficient limit of detection values for 150 pesticides from the 160 studied and more than 90% from the well detected ones showed appropriate recovery with this technique.

5.2 Study of the effects of operational parameters on the determination of 150 pesticides by LC-MS/MS technique

In this part I studied the effects of operational parameters on the determination of 150 pesticides by LC-MS/MS technique. From the ionization-assistant materials in ESI+ ionization, I studied the effects of 0,1% formic acid, 5 mM ammonium formiate and 5 mM ammonium acetate in water phase. Ammonium-content modifiers showed better ionization enhancement for some pesticides than formic acid. In contrast to this, the effect was suppression for many of them resulting higher detection limits. Taking into account that formic acid resulted low ppb level detection limits for each pesticide and these were under the current MRLs, there was no reason and it has no benefit to change to ammonium-content modifiers.

I investigated the effect of temperature for the compounds in HPLC application at 20-50°C. I experienced a negligible effect, although a constant temperature higher than room temperature (e.g. 30 °C) may be beneficial from the aspect of peak shapes and reproducibility.

I transferred the method to UHPLC column (1,8 µm particle size), that resulting in 20 minutes runtime instead of 45 minutes that was achieved in HPLC application. The disadvantage of the UHPLC column is that the ammonium-content modifiers so increased the backpressure and the conventional HPLC pumps could not serve it.

Co-elution of pesticides was frequent when injecting standard solutions containing large number of pesticides. Therefore I also studied whether they affect each-other in the ionization source. The investigation showed that the effect is strong suppression or enhancement for some pesticides and this effect is not primarily depends on the number of co-eluting compounds, but some pesticides are inclined, others are less prone for the co-elution. In any event, if the analyst measures an unknown sample that contains a pesticide at the concentration around the MRL, than, for the clarification, it is worth to re-measure the sample using the calibration without any affecting co-eluting pesticides.

5.3 Development and combination of two methods for the qualitative and quantitative determination of 300 pesticides by LC-MS/MS technique

I developed a multicomponent qualitative, so-called screening method for the identification of 300 pesticides and a quantitative method that is capable for the determination of less (~50), already identified and/or frequently detected pesticides, and their combination principle. The separation parameters were the same, consequently, the retention times can (also) constitute the basis of confirmation. Product ion spectrum served for basis of the identification from spectrum libraries. Two SRM transition of each pesticide served the chemical information. In this method I used calibration curves, but for the screening method, it was not necessary, because it was served just for identification. On the other hand, exactly this gives the potential of method combination, because there is no need to measure the samples quantitatively, if these are free of pesticides, which is cost-effective and the positive points are considered. I investigated critically the appearance of false positive and negative findings and the critical points, where the analyst may make a mistake and the advantages and disadvantages of the method combination. The combination was tested in 200 samples and it worked flawlessly.

5.4 Method development for the determination of some chlorinated pesticides from spices and tea by GC-MS/MS and GC×GC TOFMS techniques, a comparison study

My aim was to investigate the effects of matrix components on measurement those disturb the determination of some chlorinated pesticides from dry samples (spices and tea). For that, a simple sample preparation was applied based on a solid-liquid extraction with organic solvent. I developed GC-MS/MS and GC×GC TOFMS methods for measurement of some chlorinated pesticides. I defined the main analytical parameters, like the linearity, limit of detection and recovery. From the study I concluded that the comprehensive two dimensional gas chromatography in full scan mode allows not just the separation of target components from the matrix, but also the identification of non-targets, therefore it means an extraordinary analytical potential comparing to the GC-MS/MS technique. Besides, the limits of detection of the two methods are considered equivalent.

5 THESIS STATEMENTS

1. I developed a method for the determination of large number (>150) of pesticides by LC-MS/MS technique. I determined the analytical properties, limitations and I tested the applicability of the „acetate buffered QuEChERS” methodology.

This reveals that:

a) the developed method is adequate for 150 pesticides, but 10 compounds showed poor sensitivity.

b) the „acetate buffered QuEChERS” is capable for the extraction of large number of pesticides from high water content samples; the recovery values were between 70 and 120 % for more than 90 % of the investigated pesticides.

c) the validation parameters of the developed method meets the analytical requirements: the concentration-signal relationship is linear through three orders of magnitude; the limits of detection values were below 10 ng/kg and the measurements could be accomplished with standard deviation less than 20%.

2. I studied the chromatographic-mass spectrometric and analytical parameters of the elaborated multicomponent method.

In doing so, I proved that:

a) thermostation of the column at 30-35°C is ideal from the aspect of stability of chromatographic peaks. The application of higher temperature has no benefit; lower, uncontrolled temperature can cause retention errors (slips) if the laboratory temperature is unstable.

b) the ionization modifiers have affect on the retentions, but this effect is incidental and generally it is not significant. But it has to be taken into account if the analyst uses any kind of modifiers in the mobile phase.

c) the ammonium-content modifiers cause significant rise in the pressure on UHPLC column that can not executed by the normal HPLC pumps. The UHPLC application is solved using the formic acid modifier with similar limits of detection, linearity and robustness than it was achieved at HPLC application.

d) the ammonium-content modifiers are influencing the signal intensity in many cases positively, in some cases negatively comparing to formic acid. Since appropriate limits of detection concentration values could be achieved using formic acid, this is the optimal modifier from the three for multicomponent pesticide analysis using ESI+ ionization.

e) in standard mix solutions, the components can enhance or decrease the ionization of each-other that is not just co-elution, but also compound dependent. For that reason, the application of the less pesticides as possible in one standard mix solution is practical that confirm the justification of screening methods.

3. I elaborated the combination principle of a screening method that is capable for the identification of 300 pesticides and a quantitative method for the measurement of ~50 pesticides.

In doing so, I proved that:

a) the principle is right, logical and applicable in the practice. The EPI method is suitable for the identification of 300 pesticides from spectrum libraries, and with the MRM method we can measure the truly target pesticides.

b) the false positive finding can be reduced with sufficient analytical practice, the false negatives can occur frequently around limits of detection or as a consequence of co-elution, therefore the analyst have to take higher attention during manual evaluation of the screening chromatograms.

c) the database constructed from the calibration curves showed high standard deviation within matrices, the TPP-normalized database showed incidentally high differences within matrices. For this reason, samples with a pesticide detected around the MRL have to be re-measured and re-calibrated for its own matrix instead of a representative matrix.

d) as a result of routine application, half of the measured 200 samples were contaminated by minimum of one pesticide (from the 300), but I experienced that only 6% of the samples exceeded the current MRLs.

4. I applied and compared GC-MS/MS and GC×GC TOFMS techniques for the determination of some chlorinated pesticides from dried red pepper and tea samples.

In doing so, I proved that:

a) the simple sample preparation (10 times dilution) is suitable for the extraction of the selected chlorinated pesticides from spice samples, except chlorothalonil from red pepper and lindan from tea matrices, because these showed poor recovery.

b) the GC-MS/MS in full scan mode is not, but in MS/MS mode is moderately suitable for the measurement of investigated compounds. The reason of that is a large amount of co-eluting matrix components.

c) with the GC×GC TOFMS technique, complete detection could be achieved. Matrix effect also occurs, but it is less disturbing for the determinability.

d) because of the full scan mode and the very effective separation, the GC×GC TOFMS technique has a great potential for the identification of non-target compounds.

e) for simple matrices, where the one dimensional separation is sufficient, it is not worth to apply two dimensional separation. Whereas, for samples, resulting unknown and/or high background, the latter technique is beneficial.

6 PUBLICATIONS

In journals with impact factor:

2007 L. Abrankó, **B. Kmellár**, P. Fodor Comparison of extraction procedures for methylmercury determination by a SPME-GC-AFS system *Microchemical Journal*, **85** (2007), 122-126.

2008 **B. Kmellár**, P. Fodor, L. Pareja, C. Ferrer, M.A. Martínez-Uroz, A. Valverde, A.R. Fernandez-Alba Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography-tandem mass spectrometry *Journal of Chromatography A*, **1215** (2008) 37–50.

2010 **B. Kmellár**, L. Abrankó, P. Fodor and S.J. Lehotay Routine approach to qualitatively screening 300 pesticides and quantification of those frequently detected in fruit and vegetables using liquid chromatography tandem mass spectrometry (LC-MS/MS) *Food Additives and Contaminants*, **27**, No. 10, (2010), 1415–1430.

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2011 L. Polgár, **B. Kmellár**, P. Fodor Evaluation of the effect of pear stone cells ont he recovery of multi-class pesticides *Analytical Letters*, 2011 (in press)

Publications in conferences

International abstarct:

2007 **B. Kmellár**, R. Schäffer, P. Fodor Qualitative and quantitative determination of four pesticides in flowers by HPLC-ESI-MS/MS Winter Conference, Taormina, Italy, February, 2007. (on the date of lecture)

2008 **B. Kmellár**, C. Ferrer, M.A. Martínez-Uroz, L. Pareja, A Valverde, P. Fodor, A. Rodríguez Fernandez-Alba Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multiclass vegetables by liquid chromatography tandem mass spectrometry EPRW 2008 Conference, Berlin, Germany, June, 2008. (on the date of lecture)

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2009 **B. Kmellár**, L. Polgár, L. Abrankó, P. Fodor Combination of a screening and a quantitation method for the determination of 300 target pesticides by HPLC-MS/MS: achievements and pitfalls LAPRW 2009 Conference, Santa Fé, Argentina, June, 2009. (on the date of lecture)

2010 **B. Kmellár**, M. J. Gomez Ramos, M.A. Martínez Uroz, A. Rodríguez Fernández-Alba Determination of selected pesticides from dry spices by GC-MS/MS and GC×GC-TOFMS. A comparative study EPRW 2010 Conference, Strasbourg, France, June, 2010. (on the date of lecture)

L. Polgár, **B. Kmellár**, P. Fodor Comparison of three QuEChERS-based methods for the determination of multiclass pesticides from vegetable oils by HPLC-(ESI+)MS/MS EPRW 2010 Conference, Strasbourg, France, June, 2010. (on the date of lecture)