

Comparison of different methods aiming to account for/overcome matrix effects in LC/ESI/MS on the example of pesticide analyses

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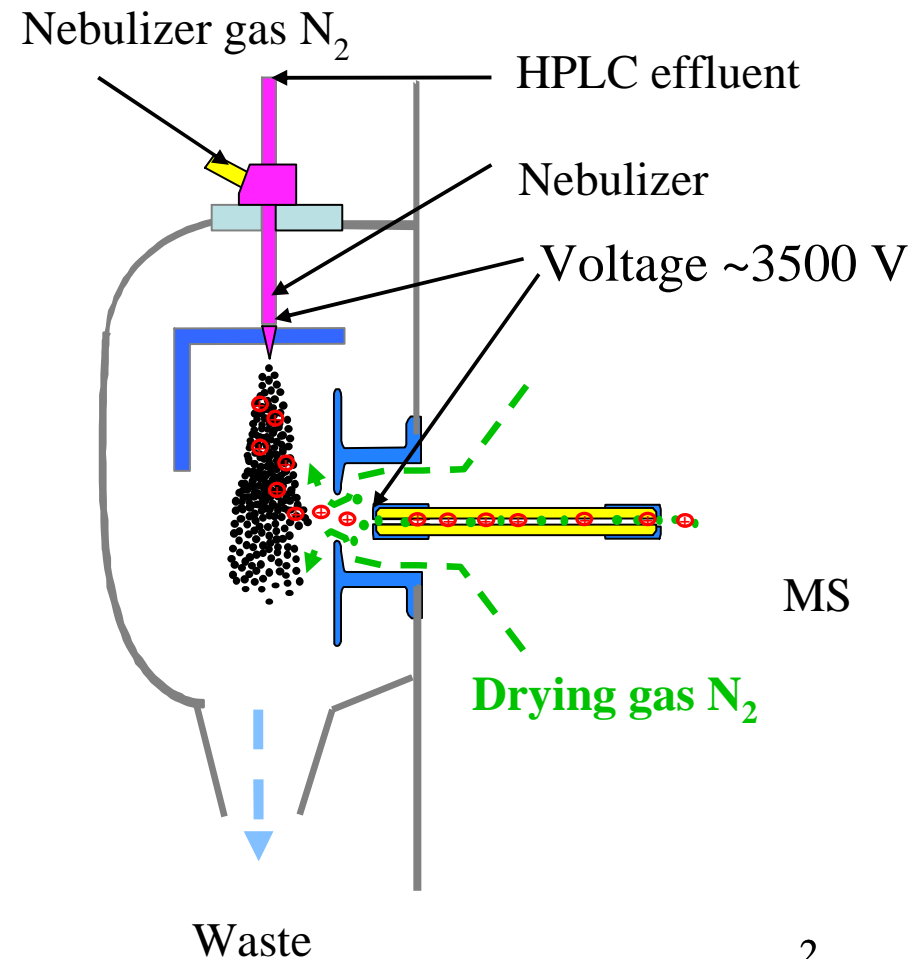
<http://dx.doi.org/10.1039/C3AY26551J>

*Matrix effects in liquid-
chromatography electrospray
mass-spectrometry*

Anneli Kruve

Electrospray ionization

- ESI is used to connect LC and MS
- LC effluent is sprayed into small droplets
- Droplets divide into smaller droplets
- From the surface of small droplets ions can reach gas phase



Contents

- Matrix effects in LC-ESI-MS, their presence and evaluation
- Approaches for combating matrix effects
 - Extrapolative dilution
 - Sample preparation
 - Accounting for matrix effects
 - ESI optimization to reduce matrix effects
- Conclusions

Matrix effect

- Ionization efficiency in ESI depends on:

- Solvent composition

- ESI parameters

- Compounds co-eluting with analyte

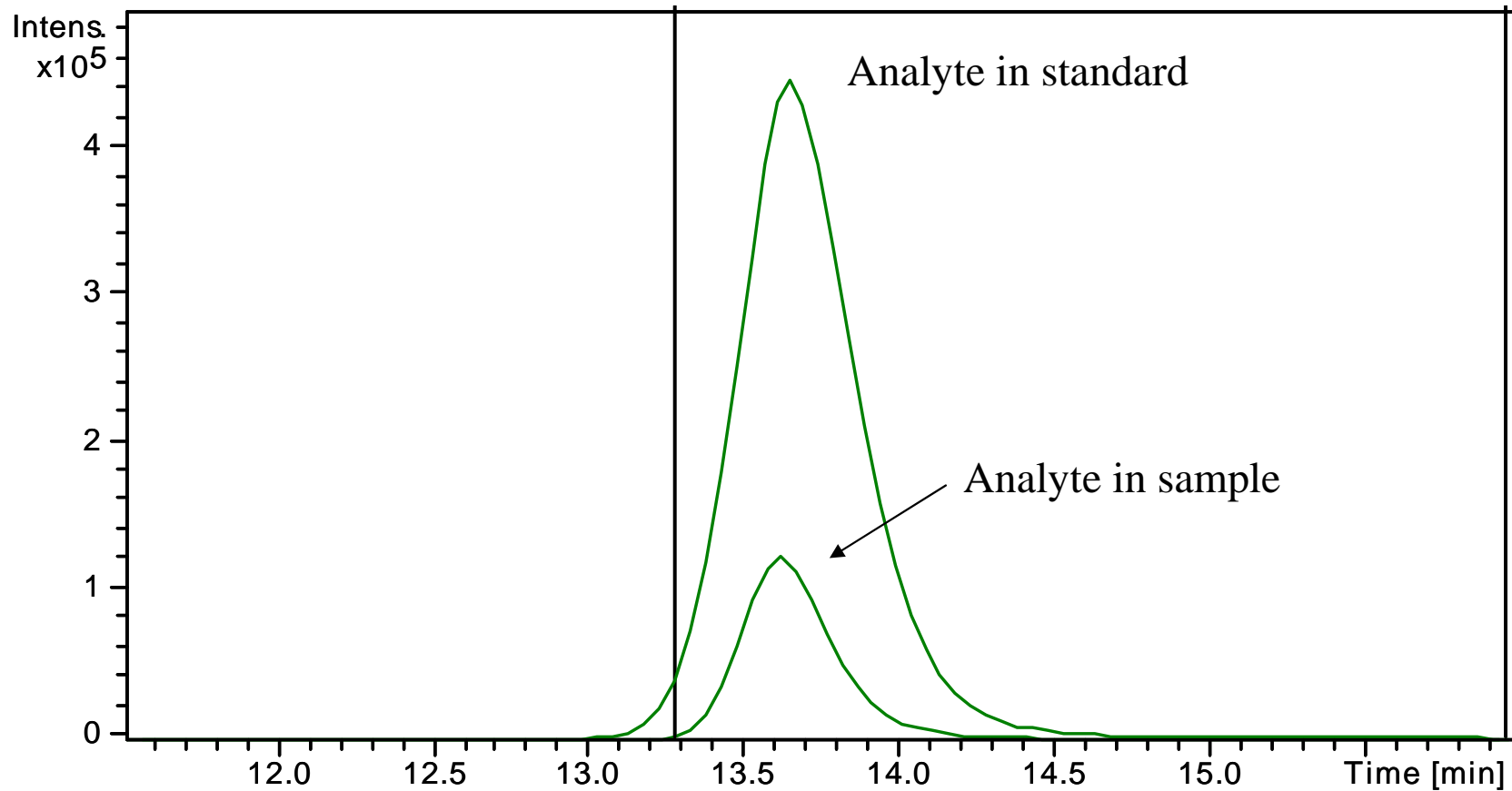
Are kept constant during analyses

Are not present in standards but are present in samples

Same amount of analyte gives different signal in sample and in standard

Matrix effect

How does matrix effect look like?



Combating matrix effect

- Reducing matrix effects
 - Sample preparation
 - Dilution of the sample
 - Instrumental parameters
- Taking matrix effect into account
 - Correcting results
 - Uncertainty

Evaluation of matrix effect

- Is expressed as a ratio of analyte signal in sample and in standard: $\%ME$

$$\%ME = \frac{PeakArea_{Sample}}{PeakArea_{Standard}} \cdot 100\%$$

$$\%ME = \frac{CalibrationGraphSlope_{Sample}}{CalibrationGraphSlope_{Standard}} \cdot 100\%$$

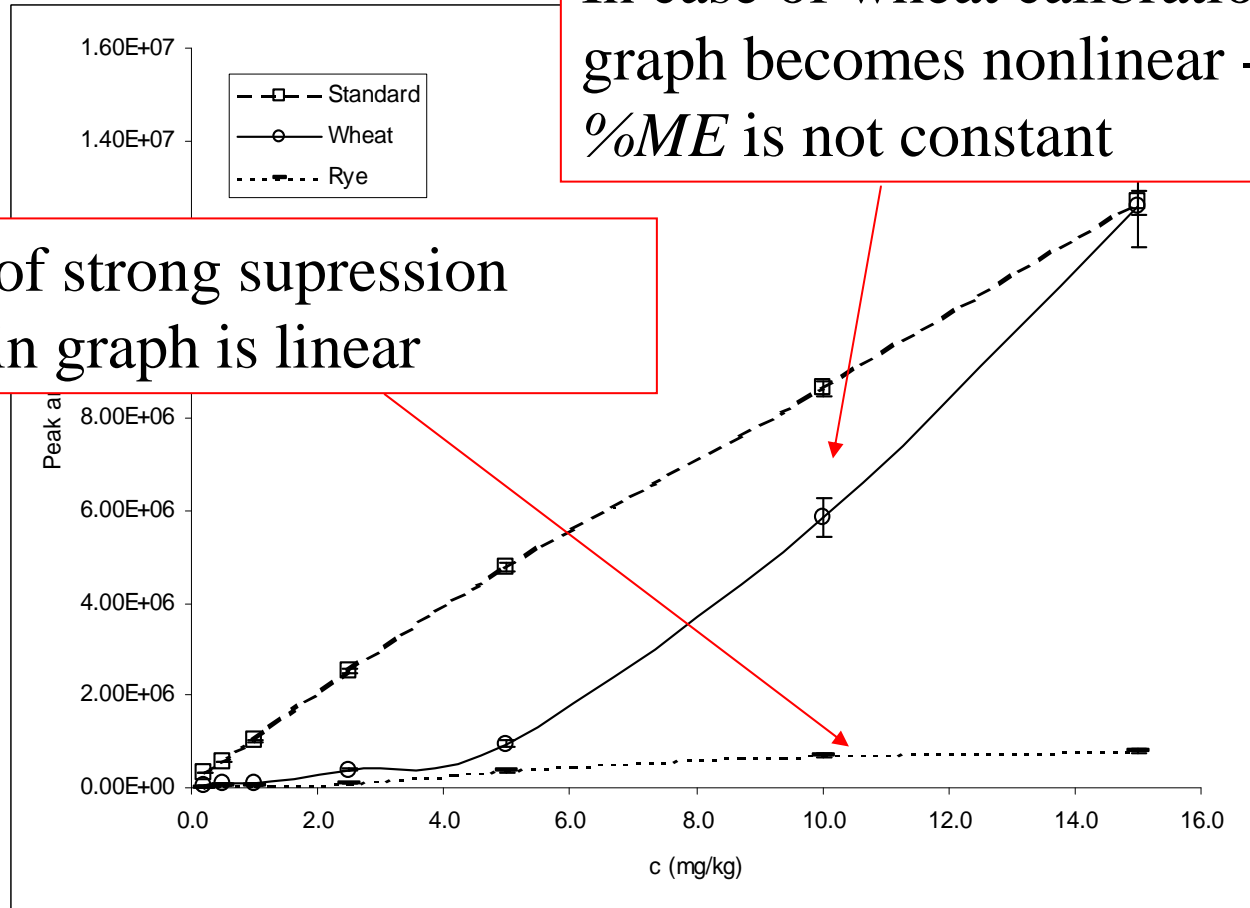
- $\%ME$ 100% - no matrix effect
- $\%ME < 100\%$ - ionization suppression
- $\%ME > 100\%$ - ionization enhancement

Glyphosate calibration graph in cereals

Slopes vs Peak Areas

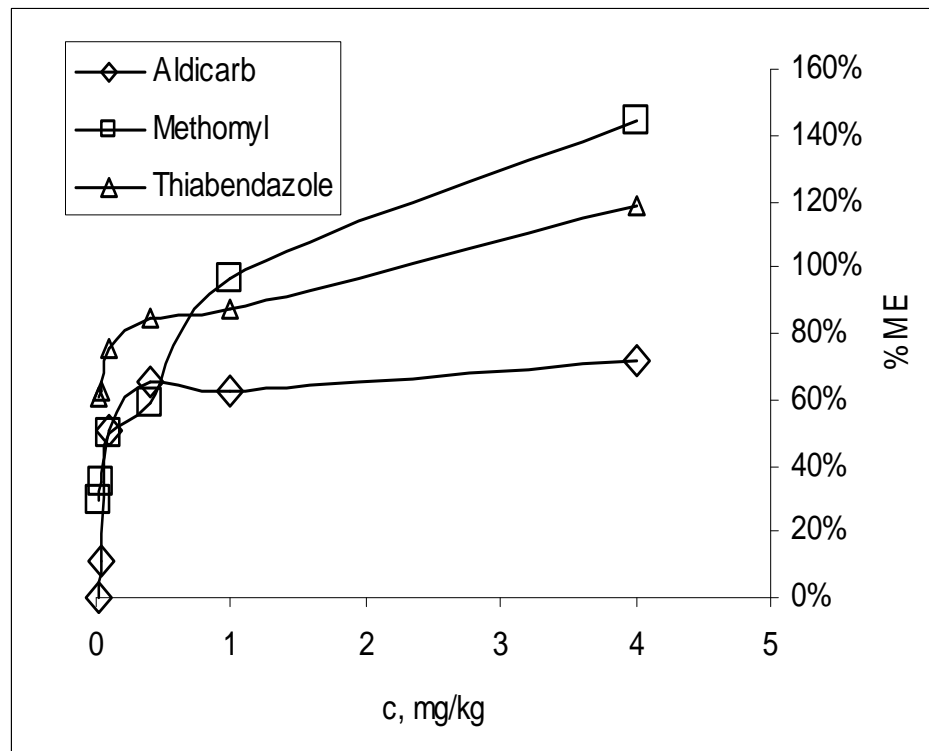
In case of wheat calibration graph becomes nonlinear - $\%ME$ is not constant

In case of strong suppression calibration graph is linear



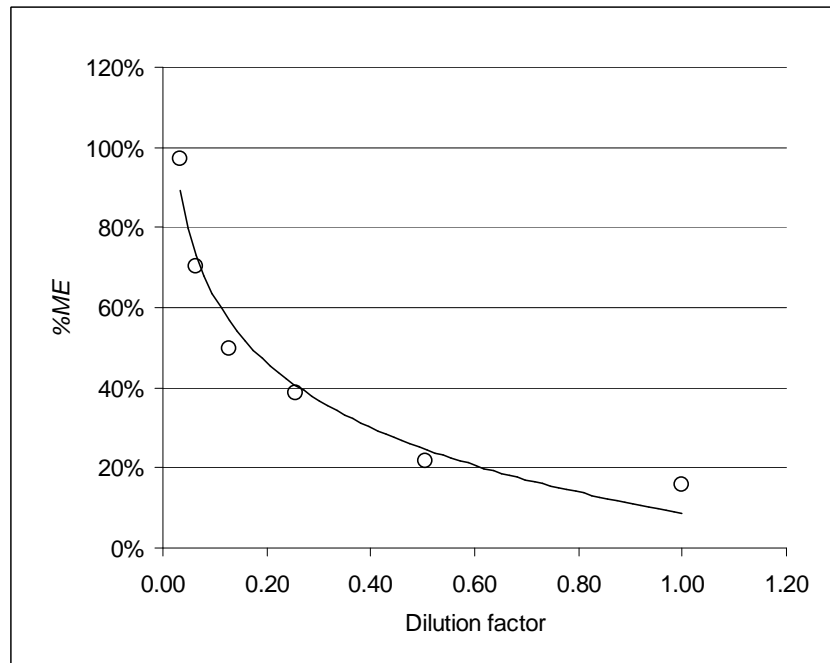
Matrix effect's dependence on analyte concentration

Garlic sample

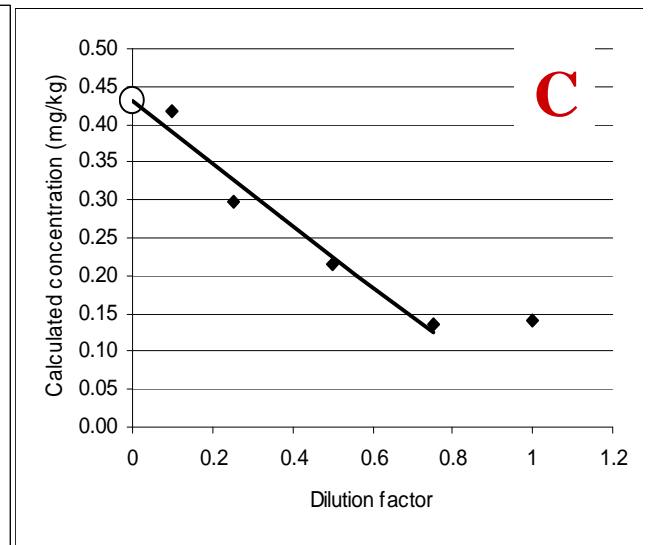
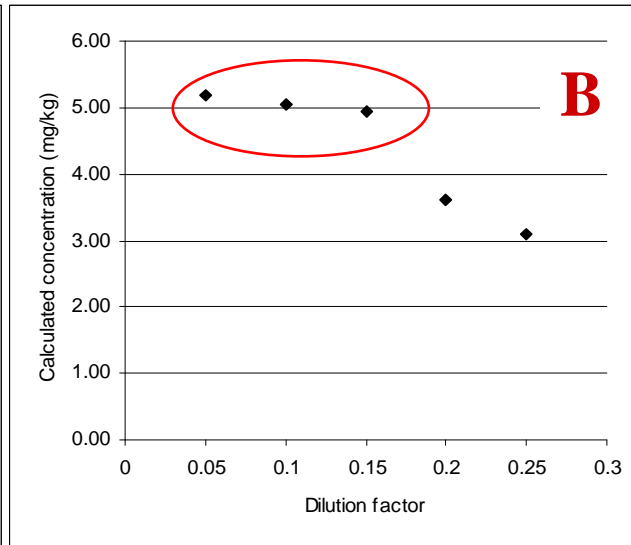
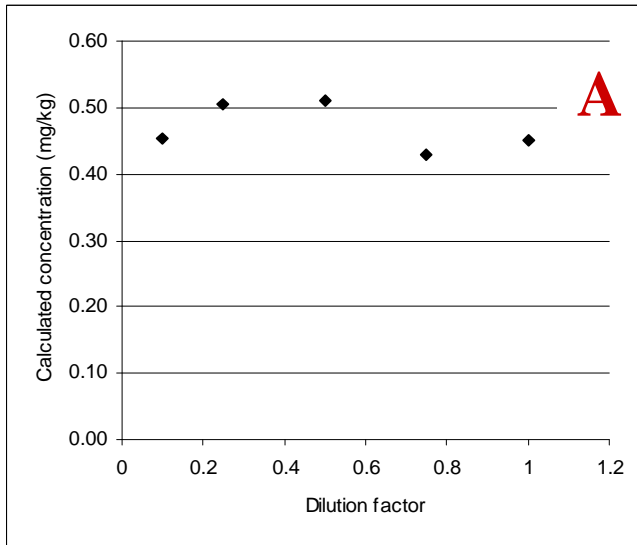


- $\%ME$ depends on the analyte concentration in the sample
- Risk of underestimated results at lower concentrations
- $\%ME$ **can not be used for correction of the analysis results**

Sample dilution



- The amount of co-eluting compounds is reduced
- Matrix effect is reduced
- Matrix effect may or may not be eliminated



No matrix effect

Dilution eliminates matrix effect

Dilution does not eliminate matrix effect

Analyte concentration is calculated as the average of all the measurements

Analyte concentration is the average of 3 most diluted samples

Analyte concentration is estimated as the intercept of the plot

Validation

- 5 fruits and vegetables, spiked with 5 pesticides at 2 concentration levels
 - 11 observations of situation A
 - 6 observations of situation B
 - 33 observations of situation C
- According to E_n scores all of the calculated concentrations agreed with the spiked concentrations



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Combating matrix effects in LC/ESI/MS: The extrapolative dilution approach

Anneli Kruve, Ivo Leito, Koit Herodes*

Institute of Chemistry, University of Tartu, Jakobli 2, 51014 Tartu, Estonia

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ABSTRACT

Liquid chromatography electrospray mass spectrometry – LC/ESI/MS—a primary tool for analysis of low volatility compounds in difficult matrices – suffers from the matrix effects in the ESI ionization. It is well known that matrix effects can be reduced by sample dilution. However, the efficiency of simple sample dilution is often limited, in particular by the limit of detection of the method, and can strongly vary from sample to sample.

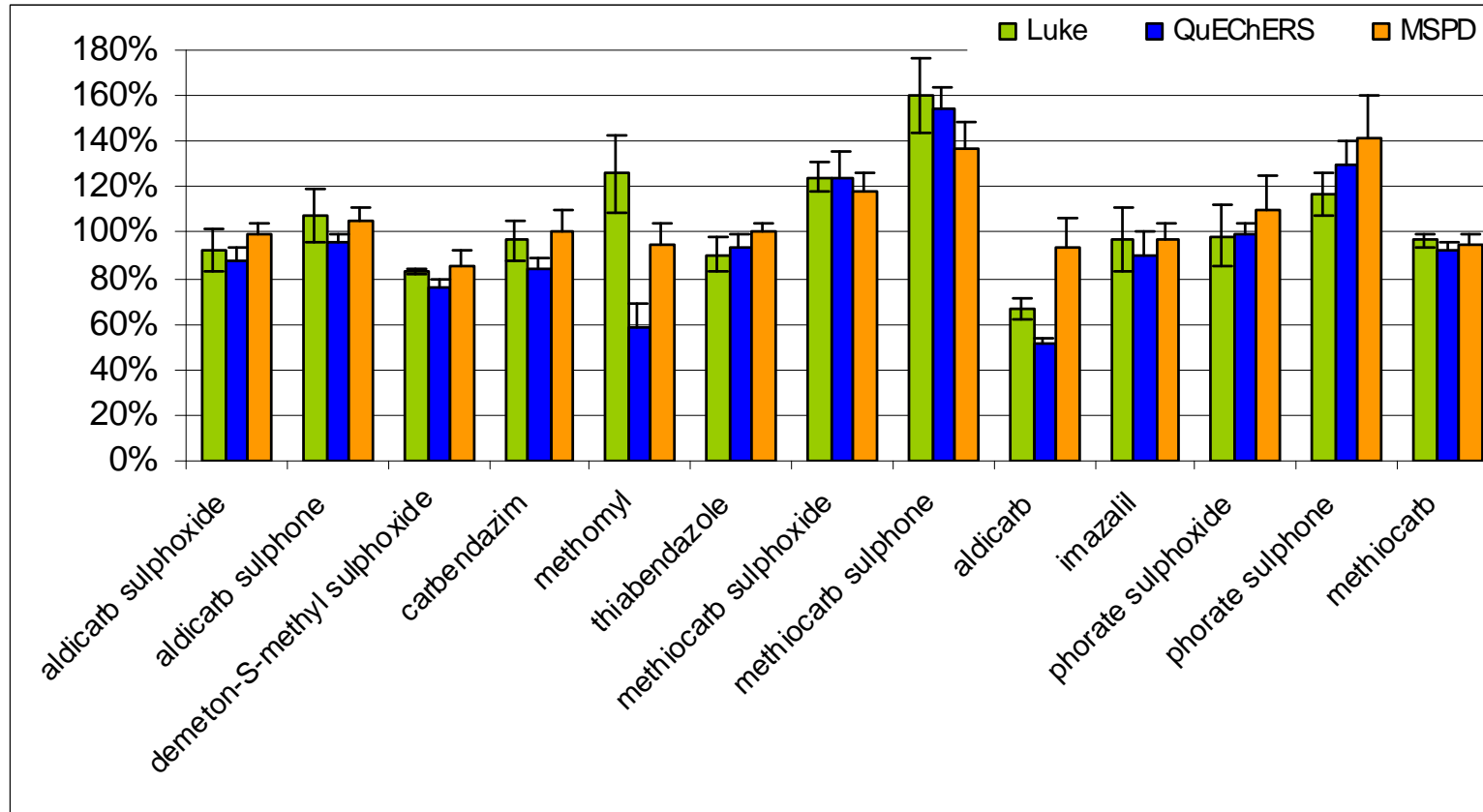
In this study matrix effect is investigated as the function of dilution. It is demonstrated that in some cases dilution can eliminate matrix effect, but often it is just reduced. Based on these findings we propose a new quantitation method based on consecutive dilutions of the sample and extrapolation of the analyte content to the infinite dilution, i.e. to matrix-free solution.

The method was validated for LC/ESI/MS analysis of five pesticides (methomyl, thiabendazole, aldicarb, imazalil, methiocarb) in five matrices (tomato, cucumber, apple, rye and garlic) at two concentration levels (0.5 and 5.0 mg kg⁻¹). Agreement between the analyzed and spiked concentrations was found for all samples. It was demonstrated that in terms of accuracy of the obtained results the proposed extrapolative dilution approach works distinctly better than simple sample dilution.

The main use of this approach is envisaged for (a) method development/validation to determine the extent of matrix effects and the ways of overcoming them and (b) as a second step of analysis in the case of samples having analyte contents near the maximum residue limits (MRL).

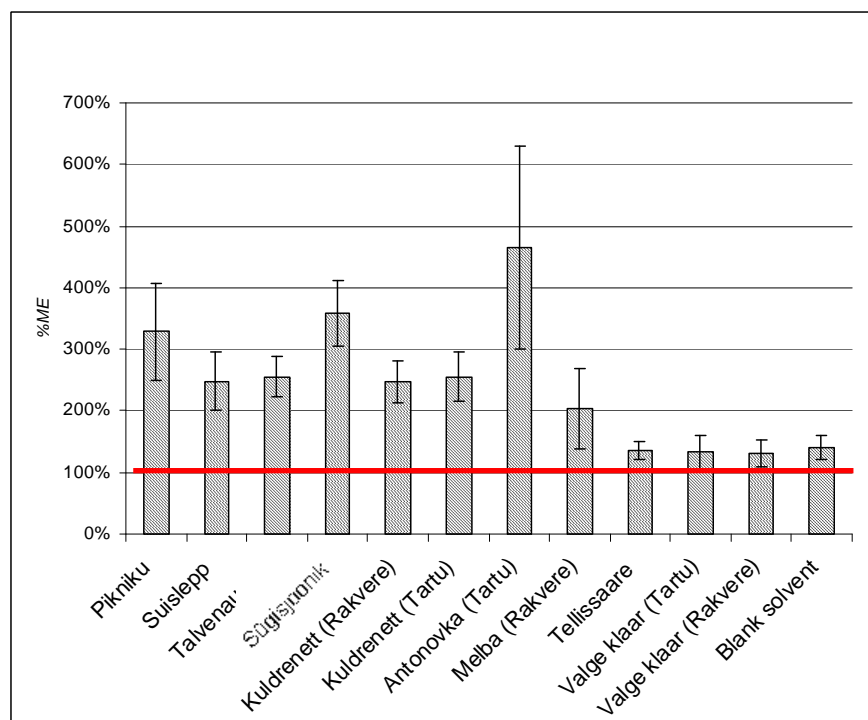
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Sample preparation



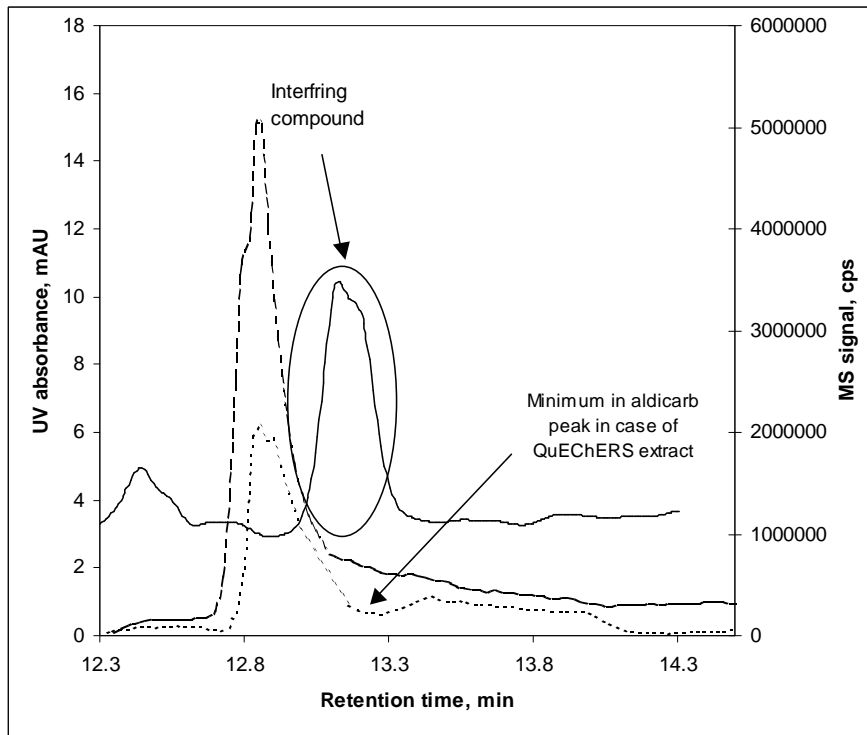
Luke and MSPD result in less matrix effect

Thiodicarb



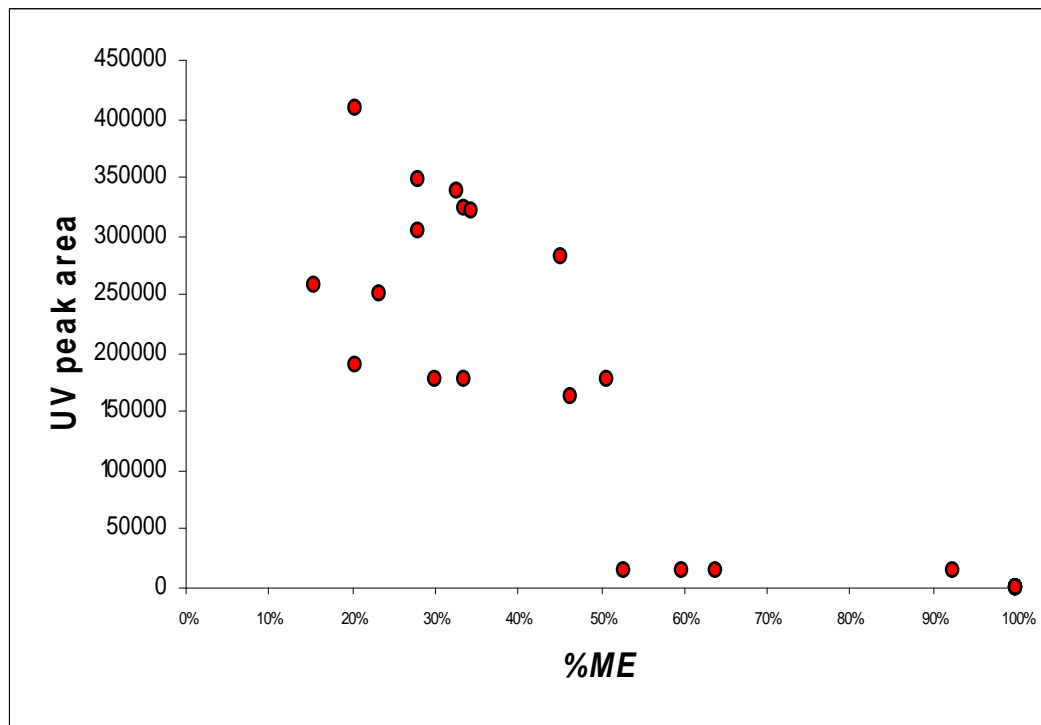
- In all samples ionization enhancement was observed
- Enhancement occurred with all sample preparation methods

“Seeing” matrix effect



- Next to aldicarb a peak elutes in the UV-chromatogram
- The shape of aldicarb peak is distorted

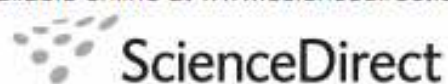
Correlation between the UV peak and matrix effect



- Measurements are carried out at different analyte concentrations!



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Journal of Chromatography A, 1187 (2008) 58–66

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Matrix effects in pesticide multi-residue analysis by liquid chromatography–mass spectrometry

Anneli Kruve, Allan Künnapas, Koit Herodes*, Ivo Leito

Institute of Chemistry, University of Tartu, Jakobi 2, 51014 Tartu, Estonia

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Abstract

Three sample preparation methods: Luke method (AOAC 985.22), QuEChERS (quick, easy, cheap, effective, rugged and safe) and matrix solid-phase dispersion (MSPD) were applied to different fruits and vegetables for analysis of 14 pesticide residues by high-performance liquid chromatography with electrospray ionization–mass spectrometry (HPLC/ESI/MS). Matrix effect, recovery and process efficiency of the sample preparation methods applied to different fruits and vegetables were compared. The Luke method was found to produce least matrix effect. On an average the best recoveries were obtained with the QuEChERS method. MSPD gave unsatisfactory recoveries for some basic pesticide residues. Comparison of matrix effects for different apple varieties showed high variability for some residues. It was demonstrated that the amount of co-extracting compounds that cause ionization suppression of aldicarb depends on the apple variety as well as on the sample preparation method employed.

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Keywords: Fruits and vegetables; Multi-residue pesticide analysis; Ionization suppression; Electrospray ionization; Matrix effect; LC/MS

Hypothesis

- If for aldicarb a compound causing matrix effect can be seen in UV, then for other analytes such compounds may exist also
 - Scanned mass spectra
 - Background ions

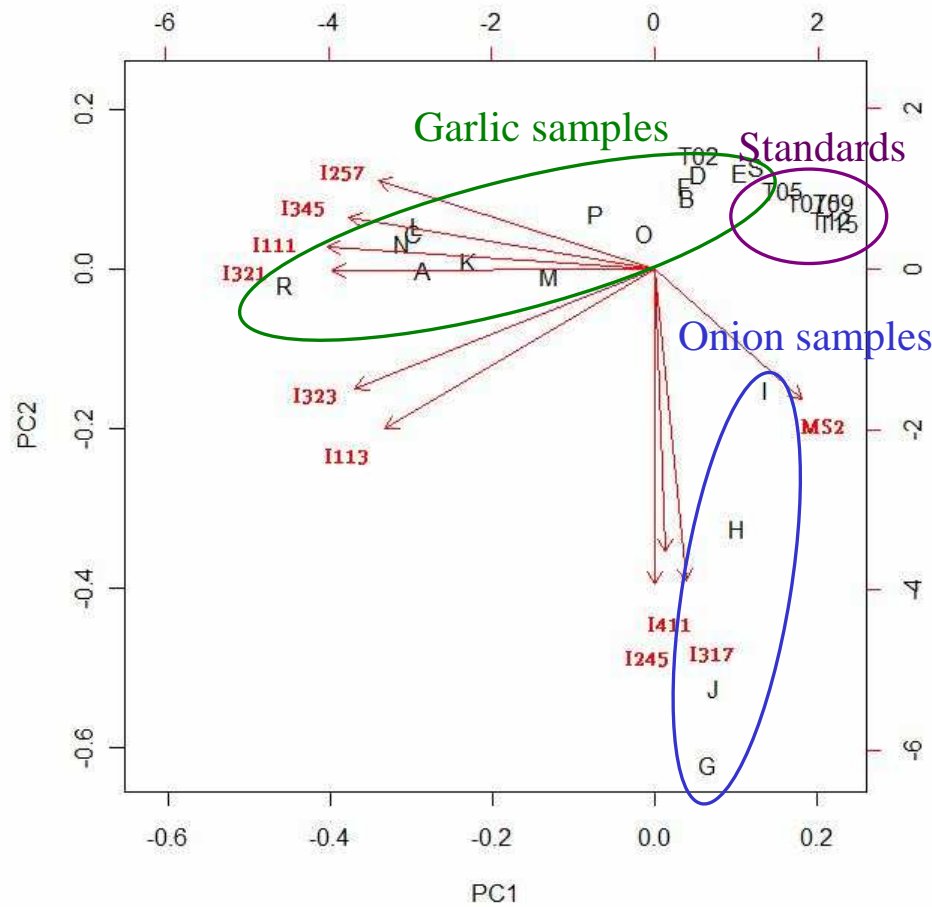
Background ions

- Are always there
 - Solvent impurities
 - Plasticizers
- Originate from the sample
 - Co-extracted compounds

Intensity changes
due to matrix effect

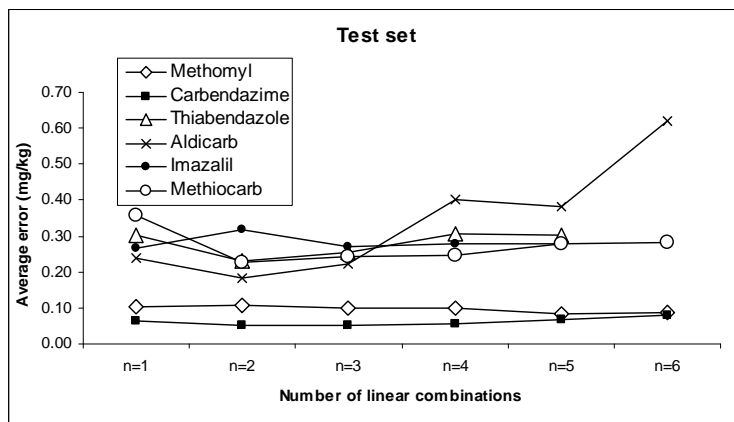
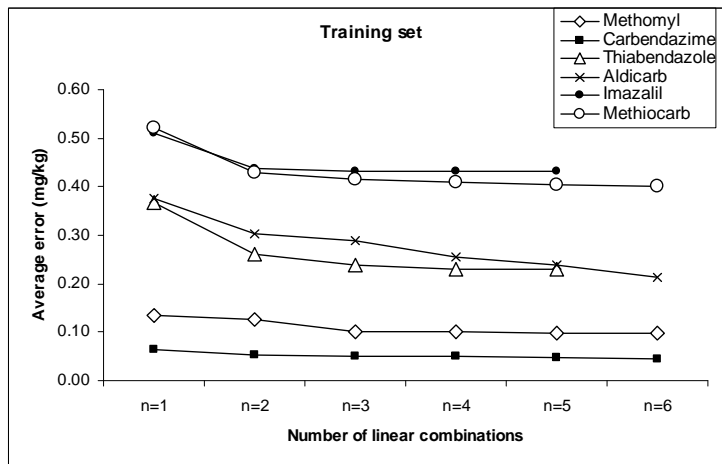
May cause matrix
effect

Scanned Spectra



- PCA was used to select background ions varying most from standards to samples

Correction of analysis results



- Background ions intensities together with analyte peak area were used in PLS regression to calculate the analyte concentration

Results

Methom	Aldicarb			
		PLS	0.22	
Carbenc		Solvent calibration	0.43	
Thiaben	Imazalil			
		PLS	0.27	
Aldicarb		Solvent calibration	0.49	
Imazalil	Methiocarb			
		PLS	0.24	
Methioc		Solvent calibration	0.69	

Rapid Commun. Mass Spectrom. 2011, 25, 1159–1168
(wileyonlinelibrary.com) DOI: 10.1002/rcm.4971

Accounting for matrix effects of pesticide residue liquid chromatography/electrospray ionisation mass spectrometric determination by treatment of background mass spectra with chemometric tools

Anneli Kruve*, Koit Herodes and Ivo Leito

Institute of Chemistry, University of Tartu, Ravila 14a, Tartu, Estonia 50411

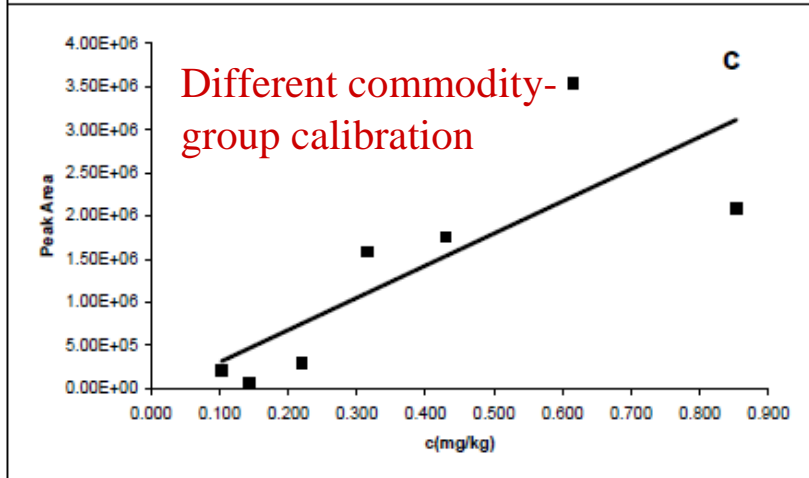
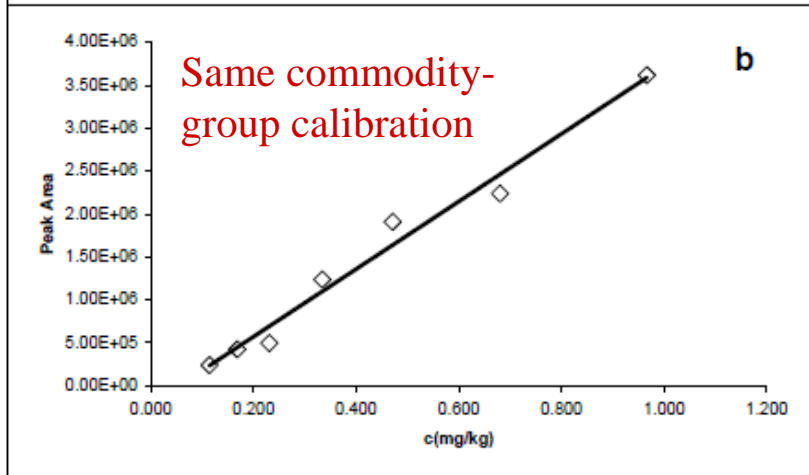
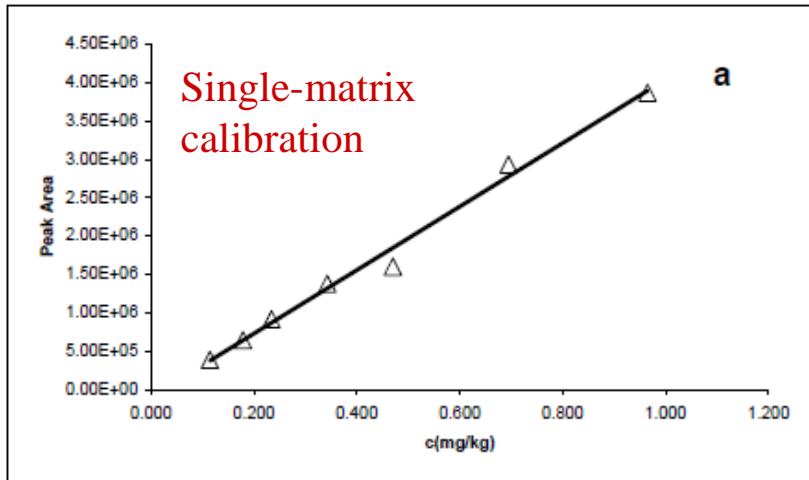
Matrix effect (ME) – ionisation suppression or enhancement – in liquid chromatography/electrospray ionisation mass spectrometry (LC/ESI-MS) is caused by matrix components co-eluting with the analytes. ME has a complex and not fully understood nature. ME is also highly variable from sample to sample making it difficult to compensate for. In this work it was studied whether the background ion signals in scanned mass spectra of the LC effluent at the retention time of the analyte offer some insight into the presence and extent of matrix effect. Matrix effects for six pesticides – thiabendazole, carbendazim, methomyl, aldicarb, imazalil and methiocarb – in garlic and onion samples used in the study varied from 1% (suppression 99%) to 127% (enhancement 27%) depending on the pesticide and sample. Also standards in solvent and solvent blanks were included in the study. The ions most strongly varying from sample to sample – and therefore best describing the changes in sample composition and ME – were selected for quantification according to principal component analysis (PCA) for all six pesticides under study. These ions were used to account for ME via partial least-squares (PLS) regression. The calibration set was constructed from 19 samples and standards and the obtained calibration function was validated with seven samples and standards. The average errors from the test set were from 0.05 to 0.27 mg/kg for carbendazim and imazalil, respectively (the respective average pesticide concentrations were 0.22 and 0.88 mg/kg). The PLS results were significantly more accurate compared to the conventional solvent calibration resulting in average errors from 0.07 to 0.69 mg/kg for carbendazim and methiocarb, respectively. Copyright © 2011 John Wiley & Sons, Ltd.

Matrix effect as an uncertainty source

- If low uncertainty is not needed in the analysis then matrix effect can be included as an uncertainty source
- *Matrix effect graph* approach
- Matrix-matched calibration

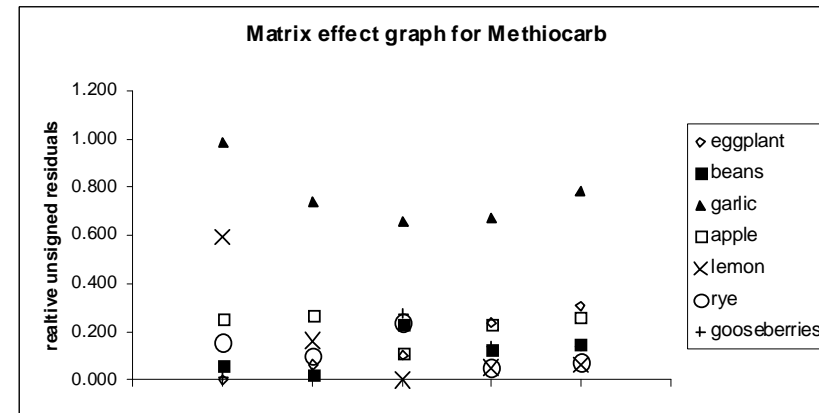
Matrix effect graph

- Each calibration solution is prepared in a different matrix
 - Same commodity group
 - Different commodity groups



$$A_i = b_0 + b_1 \cdot C_i + \varepsilon_i$$

$$\varepsilon_i^r = \frac{|\varepsilon_i|}{b_0 + b_1 \cdot C_i}$$



$$u_{\text{RMS}}^r = \sqrt{\frac{\sum_{j=1}^n (\varepsilon_j^r)^2}{n-2}}$$

$$u(A_{\text{Sample}}) = u_{\text{RMS}}^r \cdot A_{\text{Sample}}$$

Validation

- 15 samples were spiked with 4 pesticides and the results were calculated
- According to E_n scores all of the calculated concentrations but one agreed with the spiked concentrations while using u_{RMS}^r calculated in the same commodity group
- Using different commodity groups results in higher uncertainty – all results agreed with spiked concentrations

Electrospray Ionization Matrix Effect as an Uncertainty Source in HPLC/ESI-MS Pesticide Residue Analysis

ANNELI KRUVE, KOIT HERODES,¹ and IVO LEITO

University of Tartu, Institute of Chemistry, Jakobi 2, 51014 Tartu, Estonia

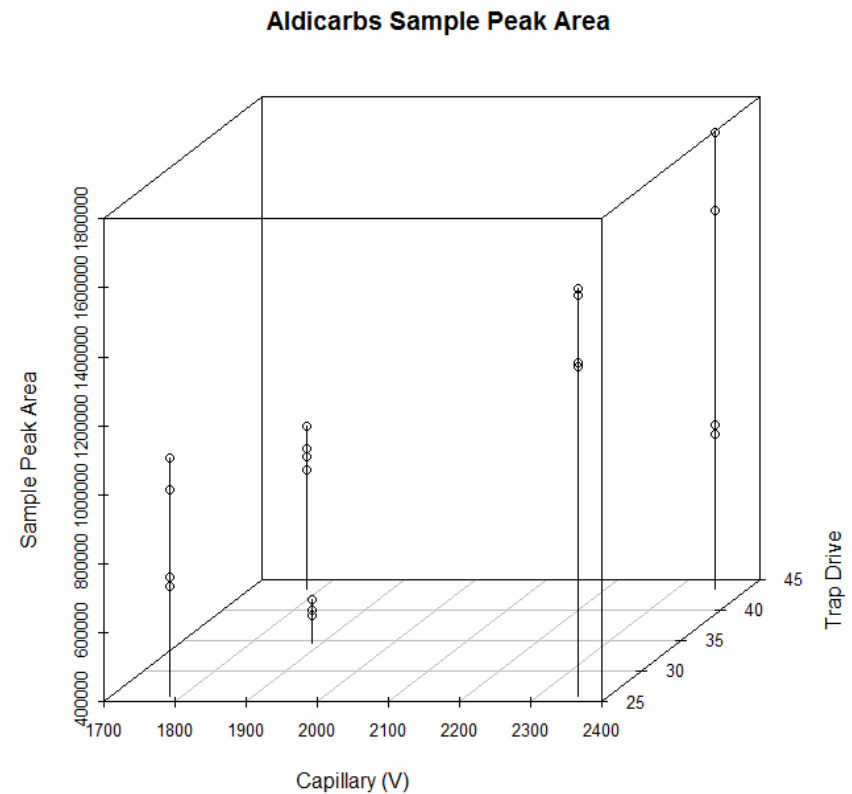
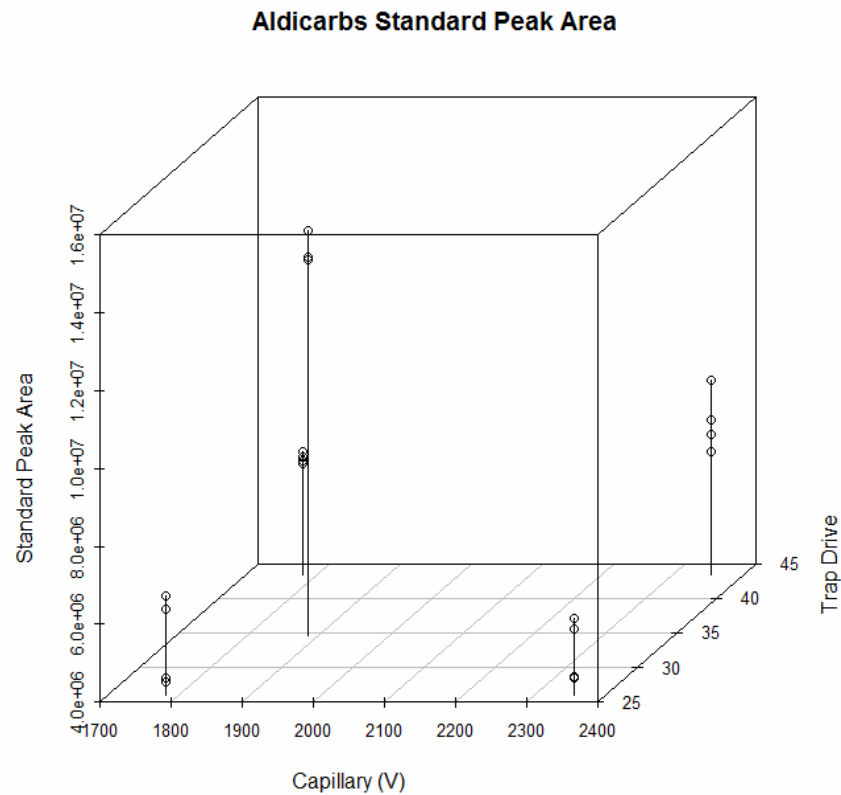
The matrix effects in HPLC/electrospray ionization (ESI)-MS analysis are difficult to compensate for because of their large variability. It is, therefore, often more practical to include uncertainty due to the matrix effect into the uncertainty budget rather than try to compensate. This work presents an empirical approach—the matrix effect graph approach—for estimating the uncertainty due to the matrix effect in HPLC/ESI-MS analysis of pesticide residues in fruits and vegetables. At certain time intervals (1 month), a calibration graph using extracts of different fruits/vegetables as calibration solutions is prepared, and a regression line is fitted through these data. These fruits/vegetables may be either from the commodity group of the samples or from different commodity groups. The relative residuals of the calibration point peak areas are calculated and plotted against the measurement time. We term the resulting graph the matrix effect graph. The root mean square of the relative residuals is calculated and used as the estimate of relative

During the last decade, HPLC/MS, especially combined with electrospray ionization (ESI), has been firmly established among the instrumental methods used in routine analytical laboratories (1). HPLC/ESI-MS is rightly considered to be one of the most reliable and universal methods of detection and identification of different organic analytes in complex matrixes at trace levels. The ability to produce multiple stage mass spectra (MS^n) decreases the possibility of misinterpretation (2, 3). For a long time, this ability was also thought to remove all possible analytical interferences. During the early years of HPLC/MS, concerns were expressed that the HPLC part of an HPLC/MS system would largely lose its importance as a separation tool (separation would be done by the mass spectrometer), and that its use would be often restricted to sample purification (4). Time has shown that, as a rule, these concerns have not materialized. As a general case, the ionization efficiency in an ESI source, and thus the analyte response (peak area), strongly depends on compounds coeluting with the analyte (5–7). The term “matrix effect” has been coined to denote this dependence. Matrix effect has been called the “Achilles heel” of quantitative HPLC/MS analysis (2), and it is now

Is matrix effect dependent on something else ... ?

- According to common understanding ... **NO**
- ESI parameters influence on matrix effect was studied
 - 3 different optimization strategies were used
 - Intensity optima and matrix effect optima do not coincide
 - Matrix effect can be reduced with appropriate ESI/MS parameters
 - ESI/MS parameters **DO influence** the %ME

Parameter optima for standards and samples



Optimization of electrospray interface and quadrupole ion trap mass spectrometer parameters in pesticide liquid chromatography/electrospray ionization mass spectrometry analysis

Anneli Kruve*, Koit Herodes and Ivo Leito

Institute of Chemistry, University of Tartu, Ravlia 14a, 50411 Tartu, Estonia

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Optimization of both the ionization process and ion transportation in the mass spectrometer is of crucial importance in order to achieve high sensitivity and low detection limits and acceptable accuracy in liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) analysis. In this paper four optimization procedures of electrospray interface and quadrupole ion-trap mass spectrometer parameters (ESI-MS) (nebulizer gas and drying gas flow rate, end plate voltage, capillary voltage, skimmer voltage, octopoles direct current and radio frequency, trap drive and lens voltages) were studied on three pesticides – thiabendazole, aldicarb and imazalil. The results demonstrate that the methodology of optimization strongly influences the effectiveness of finding true optima of the operating parameters. Both eluent flow rate and composition during optimization have to mimic the situation during real analysis as closely as possible in order to achieve parameters giving the highest sensitivity. Therefore, post-column addition of analyte to the mobile phase identical in composition to the one in which analyte elutes during real analysis combined with software-based optimization was found to be the most effective and fastest method for achieving intensity maxima. The parameters most strongly affecting ion formation and transportation, hence sensitivity, were capillary voltage, direct current of the first octopole, trap drive and the second lens for all pesticides under study. In addition to sensitivity and detection limit matrix effect was considered in the optimization process. It was found that the matrix effect can be reduced but

Summary

- Matrix effect depends on ...
 - ... analytes, matrices and concentrations
 - ... sample preparation
- Extrapolative dilution
- Result correction via background ions
- Uncertainty calculation
- ESI/MS parameter optimization



Ivo Koit Minu pere Riin Karin Karl Merit Triin Maris Anna Anna-Helena Olga Kaisa
 Lauri Geven Artur Rain Elin Jaan Allan Lauri Signe Ivar Eva Ingrid Erik Ester Maju
 Siret Kaarel Asko Hanno Gert Ragne Vahur Kristo Kerli Tapio Risto

Thank you!

