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Matrix effects in liquid-chromatography electrospray mass-spectrometry

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ABBREVATIONS

$\%ME$	Relative signal intensity under the matrix effect conditions
A	Analyte peak area
b_1	Calibration graph slope
b_0	Calibration graph intercept
θ	Taylor cone's angle
C	Concentration of the analyte in the calibration sample
c_{calc}	Calculated concentration of the analyte in the original sample
c_{dil}	Calculated concentration of the analyte in the diluted sample
c_{spike}	analyte concentration in the spiked sample
d	Distance from capillary tip to the counter electrode
d_i	Dilution factor of the *i*th solution
ε_i	Relative residuals of the calibration graph
ε_0	Permittivity of the vacuum
E_0	Electric field
E_n	E_n number
ESI	Electrospray ionization
f	Fraction of the charge converted into gas-phase ions
HPLC	High performance liquid chromatography
K	Conductivity
k_A	Relative efficiency of formation of gas-phase ions A
MeCN	Acetonitrile
MS	Mass-spectrometry
MSPD	Matrix solid phase dispersion
P	Sampling efficiency of the mass spectrometer
Q	Charge of the droplet
QuEChERS	Quick Easy Cheap Effective Robust Safe
R	Radius of the droplet
r_c	Needle outer radius
V_{on}	ESI onset potential
V_{sample}	Volume of the sample extract
V_{sum}	Volume of the sample after dilution
V_t	volume flow rate
γ	Surface tension
U	Expanded uncertainty
u	Standard uncertainty
u_{RMS}	relative standard uncertainty of the sample peak area

INTRODUCTION

Liquid chromatography mass spectrometry (LC/MS) has been extensively used for identification and quantitation of different compounds. Different ionisation sources have been used to produce ions from the analyte in the chromatographic effluent and guide these ions into the mass spectrometer. Most commonly electrospray ionisation (ESI) is used as the interface between LC and MS. Despite the high popularity of ESI its response to an analyte is sensitive to compounds co-eluting with it in the effluent. These co-eluting compounds – normally originating from samples and being not present in standards – may either suppress (in most cases) or enhance (in rare cases) the ionisation of the analyte. Therefore under- or overestimated results can be obtained for samples. This phenomenon is called *matrix effect* and its occurrence is currently one of the main limitations of the otherwise successful LC/ESI/MS method.

In order to ensure the accuracy of the LC/ESI/MS results matrix effect should be either minimized (preferably eliminated) or taken into account. Different approaches, including improvement of chromatographic separation and sample preparation, have been suggested to minimize matrix effect. Traditional methods – such as isotope dilution, standard addition, internal standard – have been used as methods to take matrix effect into account.

The aim of this work was to give some insight into the possibilities to combat matrix effect by means of reducing the matrix effect influence or accounting for the matrix effect. A systematic overview of the matrix effect problem is presented, the previously used approaches for combating matrix effect are reviewed and two new approaches – extrapolative dilution and including matrix effect into uncertainty – have been proposed. Also sample preparation and ESI/MS parameter optimisation were studied to reduce the matrix effect.

Different sample preparation methods were evaluated from the point of view of matrix effects. Classical liquid-liquid extraction, liquid-liquid extraction with dispersive post-extraction clean-up (QuEChERS) and matrix solid-phase dispersion (MSPD) were tested.

Even though methods accounting for the ionisation suppression have been applied sensitivity of the LC/ESI/MS method is decreased and the detection limits become higher when matrix effect is not reduced. Therefore a combination of reduction and accounting for the matrix effect – an extrapolative dilution method – has been studied and validated within this work.

The matrix effect has been for a long time assigned only as a problem of co-eluting compounds and the possible reduction of matrix effect throughout ESI and MS parameters has not been applied frequently. Therefore different methods of optimization of ESI and MS parameters were tested to find a method and a parameter combination giving least matrix effect.

In the case of less critical applications the matrix effect does not need to be fully accounted for. Instead, its possible contribution can be included in the uncertainty budget of the result. An approach was developed in this study to evaluate the matrix effect uncertainty contribution across different commodity groups.

1. REVIEW OF LITERATURE

1.1. Liquid chromatography mass spectrometry

Liquid chromatography (LC) has been used to separate complex mixtures into components for a century. Since the first attempts to combine LC and mass spectrometry (MS) different interfaces to connect LC and MS have been tested. Due to the high solvent content in the LC effluent the ionisation of the sample has to be carried out at atmospheric pressure. Different atmospheric pressure ionization methods – electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI), atmospheric pressure photoionisation (APPI) – enable generating ions directly from the liquid phase and have been extensively used to interface LC and MS. Electrospray ionisation (ESI) has to date been the most frequently applied of them.

1.2. Electrospray ionisation

The solution to be ionised is sprayed through a stainless steel needle. This needle is maintained at a few kilovolts potential relative to the walls of the chamber and MS entrance.

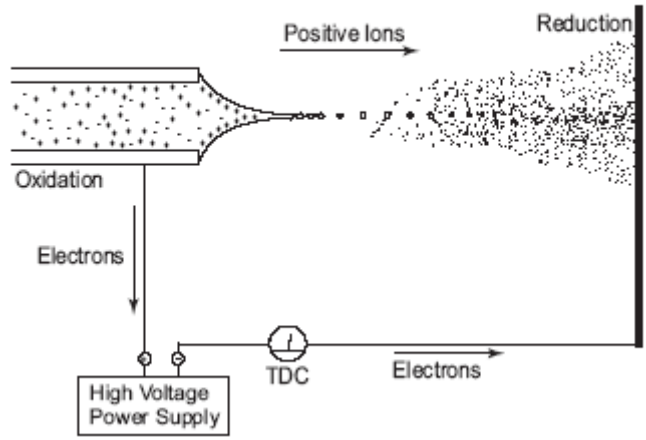

Figure 1. Electrospray ionisation scheme [Kebarle, 2009]

The applied electric field leads to the separation of positive and negative electrolyte ions at the tip of the needle. In the positive ESI mode the positive ions are concentrated on the surface of the liquid at the needle tip and negative ions are drawn inside the liquid. The repulsion of the positive ions on the sur-

face and the pull of the electric field overcome the surface tension and expand the liquid into the so-called Taylor cone. [Kebarle, 2000].

The electric field required for the electrospray process to occur is

$$E_0 = \left(\frac{2\gamma \cdot \cos\theta}{\varepsilon_0 \cdot r_c} \right)^{1/2} \tag{1}$$

where γ is the surface tension of the solution, θ is the Taylor cone's angle, ε_0 is the permittvity of the vacuum and r_c is the needle outer radius. [Kebarle 2000] For example, the combination V_c=2,000 V, $r_c=5\cdot10^{-4}$ m, d=0.02m leads to $E_0=1.6\cdot10^6$ V/m. [Kebarle , 2009]

And the potential V_{on} that needs to be applied between needle and MS entrance:

$$V_{on} \approx \left(\frac{r_c \gamma \cos\theta}{2\varepsilon_0} \right)^{1/2} \ln\left(\frac{4d}{r_c} \right) \tag{2}$$

where d is the distance from capillary tip to the counter electrode. Depending on the solvent and needle position the V_{on} values are from 2200 V (methanol) to about 4000 V (water). [Kebarle2009]

In ESI a continuous current is delivered to the needle and therefore an electrochemical oxidation – creating positive ions or removing negative – occurs in the needle. [Kebarle 2000]

It is assumed that the conversion of ions to electrons should occur at the metal-liquid interface. In case of Zn capillary Zn^{2+} ions can be detected in the spray solution. Ions have to convert into electrons because only electrons can flow through the metal wire supplying the electric current. The actual oxidation reaction depends on the electric potential present and on the chemical composition of the solution. [Blades 1991].

The occurrence of electrospray process is dependent on the presence of charges, which come from the partial separation of positive and negative ions in the Taylor cone. Therefore the ESI efficiency depends on the presence of electrolytes and a minimum 10^{-5} M of ionic substances is required in the solution. [Kebarle2000]

MS signal of the analyte A present in solution as ion A^+ depends on the total ion current as well as on the rate constant k_A

$$I\left(A^+, ms\right) = Pf \frac{k_A\left[\mathrm{A}^+\right]}{k_A\left[\mathrm{A}^+\right] + k_E\left[\mathrm{E}^+\right]} I \tag{3}$$

where $[E^+]$ denotes the net concentration of all ions present in the electrosprayed solution, P is the sampling efficiency of the mass spectrometer and f is the fraction of the charge on the droplet leaving the needle that is converted into gas-phase ions. k_A expresses the relative efficiency with which A is converted into gas-phase ions. [(Iribarne, 1976); (Kebarle, 2009); (de Hoffmann, 2004)]

It has been observed that while changing the total electrolyte concentration from 10^{-5} to 10^{-2} M the total ion current I changes very little. For a two-analyte mixture A and B the relative ionic currents registered by MS can be expressed as:

$$\frac{I_A}{I_B} = \frac{k_A C_A}{k_B C_B} \tag{4}$$

It has been observed that at constant electrolyte concentration 10^{-5} M, which is always present due to impurities from eg glassware, for $C_A=C_B < 10^{-5}$ M the k_A/k_B approaches 1. At very low initial concentrations all the analyte ions with high k_A and k_B are forced to the gas phase and deplete their concentration in the interior of the droplet and leads to apparent value $k_A/k_B=1$. It has also been found that k value increases for compounds with increasing surface activity. [Kebarle, 2000]

The compounds not present as ions in the solution can ionise via protonation or attracting a metal ion. For these compounds k_A depends also on the ionisation ratio $[AH^+]/[A]$.

The emerging liquid at Taylor cone disperses the liquid by Coulomb forces into a fine spray of charged droplets with diameter of about 1 μm. The radius of the formed droplet R and charge q can be calculated from

$$R \approx \left(\frac{V_f \cdot \varepsilon}{K} \right)^{1/3} \tag{5}$$

$$q \approx 0.7\left[8\pi\left(\varepsilon_0 \gamma R^3\right)^{1/2}\right] \tag{6}$$

where V_f is the volume flow rate, K is the conductivity of the solution and ε is the permittivity of the solution.

Due to the electric field the charged droplets migrate towards the MS entrance. Nowadays also an inert gas (so-called nebulizer gas) is used to assist the spraying.

A heated dry gas flowing from the MS side – the so-called drying gas – is used in the ESI chamber. This gas protects the mass spectrometer from influx of neutral molecules and facilitates the evaporation of the solvent from the drop-

lets. The diameter of the droplets is reduced and the charge density increases until the Rayleigh limit. At this point the Coulombic repulsion between ions becomes equal to the surface tension. “Coulombic explosion” tears the droplets apart producing a number of small daughter droplets. [Pramanik, 2002] At every Coulombic explosion the formed droplets carry about 2% of the parent droplets mass and about 15% of its charge [Kebarle 2009]. It has been also found that during the desolvation and formation of smaller droplets the composition of the solvent in the droplet can change considerably via preferential evaporation of the more volatile component(s) [Wang, 2010].

These daughter ions also undergo solvent evaporation and divide into smaller droplets through further “Coulombic explosions”. This sequence follows until the field due to surface charge density is strong enough to desorb ions from the droplet surface into the gas phase. This model of ion formation is called Iribarne-Thomson model or *ion evaporation model* and it has been found to describe formation of ions from small molecules. [(Fenn, 1989); (Pramanik, 2002)]

ESI is also able to produce ions, including multiply charged ions, from very large molecules, such as proteins. These ions are formed according to *charge residue model*. According to this model the evolution of droplets into smaller droplets occurs until all the solvent has evaporated and a charged residue is analysed with MS. The main source of these multiply charged ions are the parent droplets while the offspring droplets (daughter droplets) are the source of singly charged ions. [Kebarle, 2000]

1.3. Matrix effect

Matrix effect is the alteration of ionisation efficiency (MS response of the analyte) by the co-eluting compounds. This change of ionisation efficiency is usually assumed to occur in the ionisation source. Matrix effect may be present in LC/MS analysis even if MS^2 spectra are used for quantitation of the analytes because this phenomenon occurs before the ions reached mass spectrometer. Matrix effects causing signal enhancement or suppression have both been reported, resulting in over- and underestimated results respectively. [(Taylor, 2005), (Niessen, 2006)]

1.3.1. Evaluation of the matrix effect

Due to the possible over- or underestimation of the analyte concentration matrix effect needs to be studied during method development and/or validation. There are two main methods to study matrix effect. The first of these is by recording the *matrix effect profile.* For this the blank sample extract – free of the analyte – is injected into the LC column. At the same time a stream of the analyte solution is mixed with the chromatographic effluent and delivered into the ESI ioni-

sation source. MS monitors the analyte signal. The same is done while injecting a blank solvent into the LC column. If no matrix effect is present the MS intensities are equal for sample and solvent injection. For a region were ionisation suppression occurs the MS signal in the sample injection decreases and for ionisation enhancement increases. For a method, not affected by matrix effect, the analyte peak should elute away from the matrix effect region. The *matrix effect profile* method has been used in several papers for studying matrix effect[(Jain, 2006); (Klötzel, 2005)].

Two other methods for evaluating matrix effect have been proposed as well.

Secondly, quantitative estimation of matrix effect is possible. For this the standard in solvent with known concentration is prepared and analysed with LC/ESI/MS giving the peak area A_{standard}. Also a blank sample extract is prepared and spiked with the analyte at the same concentration level and thereafter analysed giving peak area A_{sample}. The matrix effect can be calculated:

$$\%ME = \frac{A_{\text{sample}}}{A_{\text{standard}}} \cdot 100\% \tag{7}$$

%ME value 100% indicates no matrix effect, less than 100% indicates ionisation suppression and *%ME* over 100% indicates ionisation enhancement due to coeluting sample compounds. [Taylor 2005, Niessen 2006]

In addition to the comparison of the peak areas also calibration graph slopes have been compared [Lehotay, 2010]. In this approach two calibration graphs are constructed, one in the solvent and the other in the post-extraction spiked samples. In this approach several aspects have to be kept in mind. First the intercepts of both calibration graphs have to be negligible so that the matrix effect would not depend on the concentration of the analyte. Also the linear range of the calibration graph has to be validated because differences while comparing standards and samples may occur.

A different approach to quantitation of matrix effect has been suggested by Lee and co-workers [Lee 2009]. LC/MS repose γ_{ij} of the analyte *i* in matrix *j* can be broken down into:

$$\gamma_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \tag{8}$$

where μ is the general mean, α_i is the effect of *i*-th analyte, β_j is the effect of *j*-th matrix compound, $(\alpha\beta)_{ij}$ is the interaction effect of *i*-th analyte and *j*-th matrix compound and ε_{ij} is the random error. According to developers of the method [Lee, 2009] it would be more accurate to use the interaction effect relative to the response in solvent z_{ij} as a measure of matrix effect:

$$\gamma_{ij} = \frac{(\alpha\beta)_{ij}}{z_{ij}} = \frac{x_{ij}}{z_{ij}} - 1 + \varepsilon_{ij} \quad (9)$$

1.3.2. Matrix effects properties

The extent of matrix effect has been shown to vary from sample to sample and from analyte to analyte.

In quantitative terms it has been observed that matrix effect varies on a very large scale from 10% (i.e. suppression of the signal 90%) [Niessen, 2006] up to few hundred per cent of ionisation enhancement. Lee et al [Lee, 2009] observed both strong ionisation suppression (*%ME* below 70%) for seven polar pesticides and strong ionisation enhancement (*%ME* 124 to 127%) for three pesticides via spiking of the blank sample extracts.

Stahnke et al [Stahnke, 2009] studied the *matrix effect profiles* for 150 pesticides. It was found that the *matrix effect profiles* for most pesticides are quite similar. On these *matrix effect profiles* distinct chromatographic peaks occurred, indicating presence of chromatographically well-resolved matrix compounds influencing the ionisation efficiency. Also it was found that neither short nor long retention times were free from matrix effect indicating the interferences from both polar and non-polar compounds.

On the other hand [Lehotay & Ae Son, 2010] showed that apple-blueberry sauce, peas and limes show significantly different matrix effects for a number of pesticides. Also *%ME* values tend to change from pesticide to pesticide (with different retention times).

It has also been observed that the matrix effect of the same analyte in the same mobile phase may strongly vary from sample to sample. In ref [Barnes, 1997] it was observed that for fenbutatin oxide ionisation suppression occurs in banana samples but for tomato and cucumber sample enhancement occurs. Also Matuszewski et al [Matuszewski, 2003] showed that the signal variability of spiked plasma samples is markedly higher between 5 different lots than within one lot indicating that plasma from different lots contain different (amount of) compounds responsible for matrix effects. Stahnke et al [Stahnke, 2009] showed that the *matrix effect profiles* for different batches of the same fruit or vegetable differ from each other. Karlsson et al [Karlsson, 2005] studied different toxins in mussel and liver samples and observed *%ME* variation from 16% to 134%. Dams et al [Dams, 2003] showed for urine samples that the matrix effect not only depends on the individual but also on the time the sample has been taken from the individual.

Therefore matrix effect should be carefully validated together with other validation parameters [Rogatsky, 2005]. In addition Marchi et al [Marchi, 2010] proposed a method to classify matrices according to the recoveries and matrix effects observed. In this sense it has to be kept in mind that matrix effect is very

variable and depends on the analyte, on the matrix and on the chromatographic separation of the analyte and matrix compounds.

Matrix effect has also been shown to depend on the nature of the analyte. Bonfiglio et al [Bonfiglio, 1999] it was found that in the case of more polar analytes the loss of sensitivity due to co-eluting compounds is higher compared to the less polar ones. Even stronger variation of response was observed from compound to compound than between different sample preparation methods.

LC/MS practitioners have noted that dilution of sample often helps to reduce matrix effects. No systematic study on the influence of dilution on matrix effects was available till current work [Paper II].

Also it has to be mentioned that sometimes *%ME* values over 100% may arise from some other matrix compound with the same *m/z* and give the fragments with the same *m/z* especially if MS with not very high *m/z* resolution is used [Rosen, 2010]

Matrix compounds, which elute together with analyte and produce ions (quasimolecular or adduct ions and their fragments if MS2 is used) with m/z similar to that of analyte, increase the analyte peak. This increase in signal must not be confused with ionisation enhancement by matrix compounds.

In addition to matrix effect also sample preparation and its recovery influence the accuracy of the whole method.

1.3.3. Compounds causing matrix effect

Co-eluting compounds causing matrix effect can be of different origin. First, ionic compounds in the solvent front near the dead time of the chromatogram may cause ionisation suppression if analyte is eluted too close to this region [(King, 2000); (Dams, 2003)]. Secondly the compounds causing analytes ionisation efficiency change may be present as normal chromatographic peaks [Stahnke, 2009]. While comparing suppression for Ringer solution and dialysates Lackmans et al observed differences only for low analyte concentrations [Lackmans, 2006]. Salts mainly caused ionisation suppression for medium and high concentrations but for low concentrations also endogenous compounds suppress ionization of the analytes.

Finally very hydrophobic and late eluting compounds from the previous runs may interfere with ionisation efficiency of the analyte. Often the compounds causing matrix effect are not ionised in the ESI source and therefore cannot be detected by MS.

Choi et al [Choi, 1999] observed 10 to 30% ionisation suppression (i.e. 90–70% *%ME* values) due to the late eluting components from the previous run. Similarly [Lagerwerf, 2000] observed considerable MS signal decrease after 9 injections of human plasma due to the saturation with the endogenous compounds, which are initially trapped on the column and elute after several injections.

Also, the decrease of MS response due to the contamination of the ion source was observed in the same work. Similarly in ref [Tan, 2009] a gradual decrease of the internal standard (escitalopram) response with increasing sequence number of the sample was observed, which could be explained by gradual contamination of the mass spectrometer during sequence analyses. Also ionisation enhancement for trichothecenes in cereal analyses via interface contamination has been observed [Klötzel, 2005].These effects should be avoided with adequate sample preparation. Also column switching techniques avoiding the matrix compounds from reaching the ESI/MS may be useful to avoid these problems [van Eeckhaut, 2009]. In [Karlsson, 2005] it was observed that after cleaning of the ionisation source stabilization for 24 h is needed before reproducible results can be achieved. This could also be related to the contamination of the ionisation source with time.

Also compounds with low volatility may cause matrix effect. It has been observed that ammonium sulphate – a non-volatile compound – causes ionisation suppression of phenacetin and caffeine compared to the solution of ammonium acetate. The amount of analyte precipitated on the surface of the MS entrance due to sulphate was increased twice for both analytes compared to acetate. [King, 2000]

It has been shown that some mobile phase additives eg formic acid and ammonium formate may cause ionisation suppression or enhancement depending on the concentration of the additive [Kowal, 2009]. In [Benijts, 2004] strong ionisation suppression for analytes in water samples in the presence of acid additives, which was not seen for the samples in the absence of additives, was observed. It was concluded that the matrix components were protonated in the presence of a strong acid and can move onto the surface of the droplet and outcompete the analyte molecules. Also the influence of different buffers was studied and found that above the critical concentration (5 mM for ammonium formate) severe ionisation suppression occurs.

Similarly Kebarle et al have reported that compounds with high proton affinity may also suppress the analyte response in ESI/MS [Kebarle & Tang, 1993].

Gonzalez-Marino et al found that presence of organic acids led to a stronger signal suppression compared to ammonium acetate for weak acids in negative ESI due to the protonation and loss of charge [Gonzalez-Marino, 2009].

Yamaguchi showed that 2-(2-Methoxyethoxy)ethanol could be used as a signal enhancing post-column mobile phase modifier for negative ESI mode [Yamaguchi, 1999].

For these reasons in order to obtain accurate quantitative analysis results the matrix effect should be minimal or should be compensated for.

1.3.4. Mechanism of matrix effect

In order to effectively fight against matrix effect it is important to know its origin and mechanism of action. It has been of question if matrix effect is a result of gas-phase or liquid-phase processes. Therefore King et al carried out a number of experiments to differentiate between gas-phase and liquid-phase processes [King, 2000].

One hypothesis taken was that the charged analytes in ESI may lose their charge due to the gas-phase reactions. Due to the fact that APCI did not show similar ionisation suppression this possibility was considered unlikely. [King, 2000]

In order to further rule this possibility out a dual ESI spray system was built. Into one of the needles chromatographic effluent was directed and into the other the analyte standard solution was infused to record the *matrix effect profile*. Even though the same sample was passed through the LC column no ionisation suppression was observed on the *matrix effect profile*. [King, 2000]

Another hypothesis was that the compounds reach gas phase as neutral compounds in ESI under matrix effect conditions. Therefore an APCI discharge needle was introduced into the system to enable charging of these neutral compounds. This system showed a very similar *matrix effect profile* to the original ESI conditions.

These three findings allow claiming that matrix effect is not a gas-phase phenomenon but originates from liquid-phase processes. [King, 2000]

Sample compounds may cause precipitation of the analyte. Therefore King measured the amount of analyte precipitated on the surface of the MS entrance from the standard and sample solutions. The amount of analyte on the MS entrance surface was considerably higher in the case of sample analyses and therefore it can be concluded that ionisation suppression is – as one reason – caused by analyte precipitation in droplets instead of emitting it to the gas phase as ions. [King, 2000]

Which compounds are affected by matrix effect? It is known that molecules with larger non-polar area – and consequently lower solvation energy in the polar solvents usually used in ESI – tend to have higher MS response and molecules with more polar surface and higher solvation energy tend to have lower MS response. It can be concluded that for compounds to be successfully analysed with ESI/MS both polar – necessary to enable ion formation – and nonpolar – increasing the fraction of analyte molecules on the surface of ESI droplet – fragments are needed in the molecule. Cech et al analysed 6 different peptides with different side groups of increasing hydrophobicity. It was observed that ESI/MS response follows the Gibbs free energy of transfer from water to octanol (for compounds not forming specific hydrogen bonds). It was also observed that at high concentrations – where the MS signal is saturated – compounds with high surface activity – such as surfactant octadecylamine – tend to suppress the ionisation of compounds with lower surface activity. [Cech,

2000]. Liang et al also found that compounds with higher hydrophobicity suffer less from ionisation suppression [Liang, 2003].

Even though ionisation enhancement is less common than ionisation suppression Zrostlikova et al found major ionisation enhancement for two pesticides – carbendazim and thiabendazole – in apple extracts [Zrostlikova, 2002]. It is discussed that this phenomenon may be present due to gas-phase proton transfer. Acidic matrix compounds may promote the formation of MH^+ for basic analytes.

1.4. Methods to reduce matrix effect

In order to achieve robust LC/ESI/MS method not significantly affected by matrix effect the number of coeluting compounds could be minimized via more effective sample clean-up or improving chromatographic resolution of the analyte peak from the peaks of interfering matrix compounds. Also more robust operational parameters such as a different ionisation source, ionisation mode or effluents flow rate could be used.

1.4.1. Operational parameters

Numerous authors have demonstrated that APCI shows less sensitivity to the co-eluting compounds on the ionisation efficiency than ESI does [Souverain, 2004]. King et al showed, based on the *matrix effect profile*, that APCI/MS signal is not influenced by protein precipitated dog plasma sample while ESI/MS signal showed strong suppression at the dead time of the chromatographic system [King, 2000]. Also Liang et al [Liang, 2003] found matrix effect differences for APCI and ESI. APCI showed ionisation efficiency enhancement for a target drug and ESI showed ionisation suppression for the same drug under otherwise identical conditions. The same effect was observed in [Alder, 2004] for a number of pesticides.

Bruins et al found for clenbuterol determination in urine that the respective matrix effects for APCI and ESI were 90% and 60% therefore significantly higher ionisation suppression was observed for ESI [Bruins, 1999].

Cappielo et al [(Cappiello, 2007); (Cappiello, 2008)] have shown that LC can be coupled with an electron-impact ionization source similar to the one used in GC-MS. This ionization mode was found to be influenced neither by the mobile phase nor by the matrix compounds thus being a matrix effect free ion source. This was demonstrated for atrazine, methomyl, aldicarb, propazine and terbutryn in river water and for ibuprofen and phenacetin in human plasma samples. Also it was shown that *matrix effect profiles* perfectly overlap for the standard and sample for human plasma samples with post-column infusion of phenacetin [Cappiello, 2008]. Unfortunately this interface is not commercially

available at present and also this ionisation source is compatible only with low flow rates.

Also positive and negative ionisation mode can show significantly different properties from matrix effects point of view [Thurman, 2001]. From data provided in [Kloepfer 2005] it can be seen that stronger ionisation suppression occurs for analytes analysed in the positive ionisation mode.

Also different ESI source geometries have been compared and higher ionisation suppression was found for the on-axis spray configuration. Lower ionisation suppression was observed with the orthogonal and especially the Z-spray. Small differences from source to source within the same source geometry were found [Holcapek, 2004]. From these data it can be concluded that part of the matrix effect is caused by the processes inside the MS – for example contamination of MS.

It has also been shown [Gonzalez-Marino, 2009] that different ESI sources from different manufacturers may show different matrix effect – for one system a strong ionisation suppression for parabens was observed while the other system showed signal enhancement for the same compounds – with the same sample preparation and chromatographic separation.

In addition decreasing the flow rate of the solution passing into the ESI interface may reduce the matrix effect. This may be so due to several reasons. First reduced flow rate significantly reduces the amount of organic material that needs to be ionised in unit time. Also the decreasing droplet size and increasing droplet surface area reduces the competition between the analyte and matrix compounds for desolvation and ionisation. In order to decrease the flow rate into ESI source a post-column flow splitting could be used. [(Van Eeckhaut, 2009), (Gosetti, 2010)]

In [Kloepfer, 2005] it was found that with decreasing flow rate it is possible to increase the sensitivity of the MS response. The optimal flow rate for each compound was found to be different. Also it was found that matrix effects – both suppression and enhancement – are significantly lower at 50 μl/min than at 200 μl/min. Kloepfer et al showed that as post column split reduces the flow rate matrix effect for some compounds are also reduced [Kloepfer, 2000].

Liang et al showed that the suppression of the internal standard caused by the target drug increases significantly with increasing flow rate, which was explained by the inverse relationship between the excess charge available on the droplet surface and flow rate according to the Enke's model [Liang, 2000].

Holcapek et al observed naphthalene-2-sulphonic acid signal decrease for matrix effect free system while increasing the flow rate and also the increase in suppression caused by diethylammonium acetate while increasing the flow rate [Holcapek, 2004]. Similarly Van De Steene et al have shown ionisation suppression decrease with decreasing the flow rate via post-column split [van de Steene, 2006]. Gangl et al [Gangl, 2001] used a nanosplitting device to reduce the flow rates down to 0.1 μl/min which resulted in three times higher signal intensity compared to flow rate of 200 μl/min in carvedilol analysis. According

to the *matrix effect profiles* obtained via post-column analyte addition it was observed that less ionisation suppression is present in the case of flow rate being 0.1 μl/min. As a drawback it was observed that conventional ESI needles may have non-optimal dimensions for some lower flow rates.

Andrews et al found for the nanosplitter signal improvement above 1000% (corresponding to the flow rate decrease from 200 μl/min to 0.1 μl/min) for indinavir and ritonavir according to the comparison of calibration graph slopes. However while comparing the signal improvements from concentration to concentration it was observed that for higher analyte concentrations the improvement decreased. Therefore it was concluded that even under microelectrospray conditions compounds at high concentrations may suppress the signal of low abundance mixture compounds. [Andrews, 2004]

Bahr et al compared the "micro" and "nano" ESI sources (1 μl/min and 30 nl/min respectively) and a considerably larger absolute signal for "nano" source was observed (depending on the compound 3 to 600 times). This phenomenon was explained via formation of considerably smaller initial droplets in "nano" source which also have higher charge-to-mass ratio. [Bahr, 1997]

1.4.2. Sample preparation

One possibility to eliminate matrix effect is to reduce the number and amount of co-eluting compounds via more efficient sample preparation. Bester et al showed a considerable improvement from matrix effects point of view according to the comparison of standard and matrix matched calibration slope by introduction of size exclusion chromatography as a sample preparation method [Bester, 2001]. At the same time in ref [Zrostlikova, 2002] no improvement of matrix effect was found while comparing gel permeation chromatography purified and untreated apple extracts for analyses of eight pesticides.

On the other hand Kloepfer et al found that for wastewater analyses the majority of the matrix effect is caused by the low molecular weight compounds and a more sophisticated clean up is needed to reduce the matrix effect [Kloepfer, 2005]. Similarly, Souverain et al found that in plasma samples most interfering compounds remaining after protein precipitation are polar compounds eluting in the beginning of the chromatogram and do not interfere with later eluting analytes [Souverain, 2004].

It has been observed that analytes influenced by the matrix effect depend on the sample preparation. For example in ref [Pizzuttu, 2009] it was found that for polar pesticides, eluting in the beginning of the chromatogram, the ionisation suppression is higher while using a more polar extraction solvent – acetonitrile – compared to the extraction with less polar acetone. This phenomenon was explained by the more effective extraction of polar compounds from the matrix by acetonitrile. The polar matrix compounds also elute in the beginning of the

chromatogram and cause suppression for co-eluting compounds. For very late eluting non-polar compounds a reverse tendency was observed due to higher amount of non-polar matrix extracted by acetone.

Also SPE has been studied from the matrix effect point of view. Karlsson et al found that SPE clean-up significantly improves the relative signal response for toxins [Karlsson 2005]. Benijts et al found that an additional washing step in SPE procedure may considerably influence the matrix effect and lead to significantly more accurate result (all *%ME* values exceeded 79%) [Benijts, 2004].

It should also be kept in mind that during sample preparation the sample may be contaminated with compounds causing matrix effect – eg polymers extracted from the plastic tubes used for sample preparation [(Jessome, 2006); (van Eeckhaut, 2009); (Mei, 2003)] or from SPE cartridge [Kloepfer, 2004]. Even different lab-waters with different purity grade have been shown to have different TIC-s indicating potentially different matrix effects when using these waters [Herath, 2010].

Even though sample preparation may decrease the matrix effect it also quite often increases the workload and time need for the analyses of one sample. Therefore often other means are sought for reduction of matrix effect.

1.4.3. Optimization of chromatographic resolution

Even though ionisation suppression regions tend to be wider than normal chromatographic peaks, the retention time ranges of these regions can be influenced in the same manner as normal peaks by changing chromatographic conditions. For doing this *matrix effect profiles* are used to monitor the shift of the ionisation suppression region with change of mobile phase and stationary phase. It has to be kept in mind that with changing chromatographic conditions also analyte's retention changes. [Nelsson, 2002]

Manini et al tested several approaches including using APCI instead of ESI as well as sample dilution but significant decrease of ionisation suppression was observed only after the chromatographic retention of the analytes was increased and a more efficient separation of analytes from the matrix components was achieved [Manini 2006].

Also Du et al introduced high-turbulence liquid chromatography as a measure to reduce the amount of lipids – identified as a main cause of matrix effect – from the plasma samples. The improvement of this method was validated via the RSD values of the calibration graph slopes over 5 different plasma lots. The highest RSD was 8.0 % for terfenadine. [Du, 2008]

Lackmans et al suggested several operational means to overcome matrix effect, among these guiding effluent into waste for first minutes of the chromatographic run, on-line desalting by using water as a mobile phase during the first minutes of the chromatographic run and column switching. [Lackmans, 2008]

One possible way for optimising chromatography is using UPLC, in ref [Van De Steene, 2008] it was found that while using UPLC the matrix effect for pharmaceuticals in surface water is considerably reduced. Also, the remaining influences in ionisation caused by matrix compounds were found to be eliminated (within precision of 30%) via correction with internal standard of high structural similarity. In case of UPLC analyses reduction of matrix effects may be attributed chromatographic separation of the analyte from matrix compounds and also to the reduction of the flow rate.

1.4.4. Sample dilution

Matrix effect – the influence of co-eluting compounds – can be reduced while reducing the amount of co-eluting compound via sample preparation or dilution of the samples. It has to be kept in mind that with reducing the amount of matrix compounds via dilution also the amount of analyte decreases. Dilution has been described as a method reducing matrix effect in several papers.

Sancho et al [Sancho 2002] found that for xenobiotics 10-fold dilution of the urine sample increased the accuracy from 63 to 86%.

For organophosphorous pesticides with intermediate polarity strong ionisation suppression (*%ME* values 17 to 35%) was observed. After two-fold dilution the *%ME* values increased to around 50%. In order not to lose sensitivity via additional dilution a matrix matched calibration in combination with two-fold dilution was used. For more polar pesticides the matrix effect was eliminated via two-fold dilution (*%ME* change from 71–93% to around 100%). [Garcia-Valcercelm, 2009]

A six- and three-fold intensity increase of the ofloxacin and labelled ciprofloxacin was observed in [Lee, 2007] while using a two-fold dilution of the original sample. Therefore in these conditions ionisation suppression was reduced six to twelve times.

Similalry to dilution injecting less sample could be used to reduce matrix effect.

Sagawa et al showed a change of matrix effect with sample dilution. From the dilution graphs it was observed that for some analytes (deoxynivalenol) the sample needs to be diluted 600 times to reach the matrix effect free situation [Sagawa, 2006].

It has been shown that sample preparation, if containing a pre-concentration step, may significantly increase the matrix effect (decreased *%ME* values) [Dams, 2003].

For benzoxazinoid derivatives in plant material (root and foliage) considerable decrease in matrix effect or even elimination of ionisation suppression was observed while using up to 16 fold dilution of the sample [Villagrasa, 2007]. Hernando et al observed the necessity to dilute the salmon extract 4 times to achieve coinciding response for the spiked sample and standard in solvent in avermectin residue analyses [Hernando, 2007].

Lackmans et al [Lackmans, 2006] found that the ionisation suppression caused by salts in the beginning of the chromatogram was eliminated by injecting 1 µl of sample solution instead of 5 µl. Similarly Gru et al showed that smaller injection volumes result in less ionisation suppression and higher intensities [Gru, 2010].

Hirabayashi et al [Hirabayashi, 2007] found that for peptide analyses the sample amount influences the signal intensity. For 0.5 µg sample compared to 0.005 µg sample about 90% ionisation suppression was observed and for 0.05 µg 20% to 40% ionisation suppression was observed. Also according to the matrix effect plots the increased sample size results in a wider range of ionisation suppression. Also a non-linear relationship between the peak area and sample amount was observed due to the change in matrix effect values depending on the sample size.

Similarly Heller et al varied the analyte/matrix ratio and found that injecting smaller volumes of the sample into the analytical column could potentially decrease matrix effect [Heller, 2007].

1.5. Methods to account for matrix effect

Reducing or eliminating matrix effect is often very laborious – several additional sample preparation steps – or decreases sensitivity – eg dilution. Therefore also accounting for the matrix effect is very important for ESI users.

Only rarely – eg in [Karlsson, 2005] – it has been found that matrix effect could be measured during method development and used later for correction of the sample analyses results or fully eliminated during method development. Therefore more sophisticated methods to account for matrix effect have been developed.

1.5.1. Internal standard usage

Internal standards (IS) have been tested to correct for matrix effect in several papers.

Villagrasa et al found that internal standard was effective in compensation of matrix effect only for the analyte eluting nearest to the internal standard in plant samples [Villagrasa, 2007].

Mei et al observed strong ionisation suppression in both APCI and ESI for analyte and internal standard (compounds not identified in the paper). Due to coelution of these compounds same phenomenon occurred for both compounds and the accuracy of this method remained within 85 to 122% [Mei, 2003].

For nine basic pharmaceuticals Van De Steene et al [Van De Steene, 2006] have shown remarkable improvement of accuracy (corrected *%ME* values close to 100%) while using four different structural analogue standards in combination with post-column flow splitting. For the analyses of the same phar-

maceuticals Van De Steene et al [Van De Steene, 2008] observed that for different samples the differences remain even after applying the structurally analogous standards.

Benijts et al used 15 isotopically labelled internal standards to compensate for matrix effect. For the analytes which did not have an isotope labelled standard available the closest eluting isotopically labelled IS of another analyte was used. It was found that this procedure brings accuracy close to 100% [Benijts, 2004 35].

As a drawback Klötzel et al [Klötzel, 2005] found that analyte and the IS can suppress each other's ionization when co-eluting. Therefore a non-co-eluting standard was used in further analyses.

Also different matrix effect values were found for analyte and IS in [Singh, 2008], respectively 8.1 to 10.2% (different concentrations) for analyte centchroman and 3.3% for IS tamoxifen. Unfortunately neither the calculation scheme of the matrix effect values nor the statistical comparison of these values is presented in the report.

Even on-line internal standard addition methodology has been introduced and validated. In the on-line system the internal standard is introduced into the sample injection loop on-line from a microreservoir containing the IS solution. Compared to the off-line method in the sense of accuracy and precision [Alnouti, 2006].

1.5.2. Isotope dilution

Isotope dilution has been suggested as the most accurate method to account for matrix effect due to the similar chemical and physical properties of the isotope labelled standard and analyte. Also analyte and its isotope labelled standard should co-elute and be affected by the same matrix compounds.

Chin et al [Chin, 2004] showed that for olanzapine the ionisation suppression effect could not be corrected with deuterated (D_3-olanzapine) in human plasma.

As a worst case study Lindegardh et al showed that the ionisation suppression of piperaquine and its D_6-IS were both 75%. The suppression was caused by the phosphate buffer in the sample eluting at the dead time of the chromatographic run [Lindegardh, 2008].

Sancho et al compared isotope labelled standards to structural analogues in the analyses of xenobiotics and found that in the case of similar matrix level structural analogues may compensate for the matrix effect even when not co-eluting with the analyte [Sancho, 2002].

It has been observed [(Stokvis, 2005); (Rychlik, 2008)] that for the deuterated standards the retention times may shift compared to the analyte. This may lead to similar problems as with structural analogues – the matrix compounds co-eluting with the sample are significantly different resulting in different ionisation suppression.

Jemal et al observed changes of the analyte (mevalonic acid) intensity to isotope labelled standard intensity ratio changes from urine batch to batch. This indicates differences in ionisation suppression for analyte and its isotope labelled standard. Therefore it was concluded that isotope dilution works well only if the method exhibits nearly zero matrix effect. Also it has been pointed out that the analyte and IS responded differently to the presence of ammonium hydroxide as an additive. [Jemal, 2003]

Liang et al showed with post-column infusion experiments that both analyte and isotope labelled standard suppress each other's ionisation in the ESI source. It was shown that this effect occurs for all analytes included in the study independent of whether deuterated or ^{13}C standard was used. The level of suppression for the ^{13}C standard caused by the analyte was slightly lower compared to the suppression for the deuterated standards caused by the analyte. The authors also found that in the presence of high IS concentration the range linear response of the analyte is significantly narrower than without the IS. Thus, not too high IS concentrations should be used. [Liang, 2003]

Wang et al showed that for the sample with heavy ionisation suppression the ratio of analyte carvedilol response to IS (D_5) response changes with dilution of the sample. This indicates different ionisation suppression of the analyte and the IS by the sample matrix. Also the analyte was found to elute slightly later compared to its IS. [Wang, 2007]

Saini et al showed that the matrix effect for mevalonic acid was 46% while for the internal standard (deuterated mevalonic acid) was 73% in plasma samples [Saini, 2006].

1.5.3. Post-column standard addition

Due to the fact that matrix effect strongly depends on the compound causing matrix effect as well as on the analyte retention time the physico-chemical properties of the internal standard are very important while selecting the internal standard. In order to overcome the problem of not matching retention times a post-column introduction of an internal standard has been tested. Post-column standard addition is a similar method to the internal standard. Only the standard is added to the chromatographic effluent. The intensity change of the internal standard response from the standard to the sample is used for correction of the analyte signal.

Both structural analogues as well as isotopically labelled compounds may be applied as efficient post-column internal standards. In [Choi, 1999] ^{13}C-tebufenozide was used as a post-column internal standard for both tebufenozide and hydroxybufenozide in a wheat hay extract and effective signal compensation was found for both analytes. The authors concluded that isotope-labelled standards are not necessarily required.

In addition to post-column standard addition segmented post-column standard addition has been used to visualize and account for matrix effect. Instead

of delivering a constant flow of analyte solution between the LC exit and ESI interface into the effluent alternate packets of analyte-containing solvent and analyte-free solvent were infused into a LC effluent. The periodic spikes of analyte-containing solvent were much narrower than the analyte peak eluting from the column and the height of these peaks is affected by the matrix effect in the same manner as the analyte eluting from the LC column. The variation of the spike heights was used to correct the analyte peak area affected by the matrix effect. It was found that applying the segmented analyte addition does not change the matrix effect of the chromatographic peak. But after correcting the peak area using the heights of the spike peaks the analyte content in a honey sample and the analyte content in a standard were in agreement within the confidence interval. [Kaufmann, 2005]

Stahnke et al [Stahnke, 2009] have shown the similarity of *matrix effect profiles* for a number of pesticides. They also found that the post-column standard addition remarkably improved the accuracy of the analyses result for a number of matrixes. For example for 14 pesticides in avocado the average *%ME* value was corrected from 40 to 94% with compensation via carbendazim signal. Similar improvements were seen for other matrixes as well. Still, for complicated matrixes such as avocado, cauliflower, tea, grapefruit, rocket and some others more than half of the pesticides included in the study (all together 150 pesticides) showed matrix effect value less than 60%.

1.5.4. Standard addition

Standard addition is one of the most common methods in analytical chemistry aiming to account for the interferences coming from the sample matrix.

Standard addition can be carried out if the matrix effect is constant over the whole calibration range and the intercept of the calibration line is zero. Ito et al showed that for constant signal suppression rate and independency of the analyte's concentrations the obtained quantitative results from the standard addition were in agreement with the theoretical values [Ito, 2001].

Kowal et al [Kowal, 2009] has shown that standard addition and isotope labelled (deuterated) standard produce comparable results for 20 real samples over a wide concentration range for pesticide analyses in different water samples. In a similar way Licea-Perez et al in [Licea-Perez, 2008] showed that the extrapolated results from the standard addition method agreed with the matrix-matched calibration for testosterone and 5α-dihydrotestosterone determination in serum. This may be due to the extensive three step sample preparation – liquid-liquid extraction followed by derivatisation and solid-phase extraction – resulting in very clean samples.

As a drawback it must be mentioned that standard addition needs at least two LC/MS runs per sample. Also it must be validated that the intercept of the calibration line is insignificantly different from zero and the matrix effect should not change with the change of the analyte concentration.

1.5.5. Matrix matched calibration

Matrix matched calibration is most often used in routine analyses laboratories to account for matrix effect. Also SANCO [SANCO/2007/3131] suggests matrix matched calibration for pesticide residue analyses as method to account for matrix effect.

Van de Steene et al have pointed out that due to variable matrix effect from sample to sample a representative matrix should be used for preparing the calibration solutions [van de Steene, 2006].

Unfortunately it has been shown that finding the representative matrix (see section Matrix effect properties) may be very complicated or even impossible.

1.5.6. *Echo*-peak technique

In order to make it possible to use the analyte as an internal standard the *echo*-peak technique has been introduced. In the *echo*-peak technique an sample and a standard solution containing analyte with known concentration are injected consecutively into the analytical column within a short time period. First the sample is injected directly into the separation column under isocratic conditions. After a short time (eg 1 min) the column switching valve position is changed to direct the mobile phase trough the pre-column into the analytical column. Thereafter the standard solution is injected and a gradient program is initiated. Due to this set-up the analyte from the sample peak elutes first and right after it elutes the analyte peak from standard solution. [Zrostlikova, 2002]

Also a set-up with reversed order of injections could be used to elute the standard peak before the peak from the sample. It has been shown by [Zrostlikova, 2002] that the results obtained with different set-ups are different. This could be explained by the differences in the elution profile of the matrix compounds. Alder et al found that injection of the standard before the sample gives better results [Alder, 2004].

Correction of the matrix effect can be achieved via *echo*-peak technique if the peaks from sample and standard are affected by the co-eluting sample compounds in the same manner. In other words the co-eluting compounds from the sample should be the same for both peaks. For calculation of the quantitative results the calibration plot is constructed from the peak area ratios.

According to ref [Zrostlikova, 2002] the *echo*-peak technique can only be implemented if the peaks are not tailing. In the case of tailing peaks the area of the second eluting peak is considerably increased at the expense of the first peak and over- or underestimated results could be obtained.

In the case of real sample analyses the concentration of the standard should not be very high because this may lead to overlooking of the sample peak and to a false negative result. [Alder, 2004]

1.5.7. Comparison of different methods to account for matrix effect

Only few reports are available about comparison of different methods aiming to account for the matrix effect.

Lehotay et al [Lehotay 2010] compared solvent calibration with matrix-matched calibration and the *echo*-peak technique. According to the authors interpretation the matrix-matched standard best compensated for the matrix effect (mean *%ME* value 89%). Difficulties were met in using the *echo*-peak technique when the number of pesticides used in the study was increased – the peaks from the sample and from the standard became chromatographically unresolved.

2. EXPERIMENTAL

2.1. Reagents

Solvents – acetonitrile (J.T.Baker, Deventer, The Netherlands), methanol (J.T.Baker), acetone (J.T.Baker and Rathburn Chemicals Ltd, Walkerburn, Scotland, UK), dichloromethane (EM Science, Gibbstown, USA), petroleum ether with boiling range of 40 – 60 ˚C (Riedel-de Haën, Seelze, Germany) – were of sufficient purity. Used water was purified with Millipore Simplicity 185 (MILLIPORE GmbH, Molsheim, France). Salts, magnesium sulfate, sodium sulfate, sodium chloride and sodium acetate were from Reakhim (Leningrad, Soviet Union). Before usage the magnesium sulfate was baked for 5 h at 500 ˚C in a muffle furnace to remove possible phthalate impurities. Sodium sulfate was freed from water and organic impurities by baking at above 400 ˚C for 6 h. Glacial acetic acid (Lach-Ner, Neratovice, Czech Republic) was used to improve stability of base-sensitive pesticide residues in the final extract of the QuEChERS method [Lehotay, 2005].

Pesticide standard substances were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions of approximately 1000 mg/kg in the appropriate solvent were prepared. Stock solution for carbendazim was 80 mg/kg because of its poor solubility. For spiking appropriate dilutions were made.

In the MSPD sample preparation method C_8 sorbent (Agilent) with average particle size 59 μm, average pore size 60 Å, surface area 546 m^2/g, carbon loading 12% was used. The sorbent was not endcapped. Primary Secondary Amine (PSA) (Supelco, Bellefonte, USA) was used in the QuEChERS method.

Formic acid (Riedel-de-Haën) and ammonium acetate (Fluka Chemie AG, Buchs, Germany) were used for preparing HPLC eluents.

2.2. Samples

Fruits and vegetables were obtained from a local trade center and market. All of the fruits and vegetables were tested for their pesticide content. Sample preparation was carried out for all of the matrixes and the obtained extracts were injected into the LC/MS system. A few of the fruits already contained some of the pesticide residues that were under study. The fruits that already contained pesticide residues were left out of the data treatment for these pesticide residues. Others were used as blank matrixes in this study.

2.3. Sample Preparation

The analyses were carried out as in a routine analysis laboratory. No special measures were taken to consider potential variability of physical properties, e.g. pH of fruits.

Three sample preparation methods: buffered QuEChERS method [Lehotay, 2005], Luke method [Cunniff, 1997] and matrix solid phase dispersion (MSPD) [Blasko, 2005] were used. In cases where sample preparation method is not mentioned the buffered QuEChERS method was used.

About 200 g portion of sample was weighed and thereafter chopped and homogenized for 1 min at 4500 rpm. All three sample preparation methods were carried out from the same homogenizate.

2.3.1. QuEChERS method

15.00 g of the homogenized sample was placed into a 50 ml polyethylene centrifuge tube. 15 ml of 1% acetic acid in acetonitrile (v/v), 6 g of anhydrous magnesium sulfate and 1.5 g of anhydrous sodium acetate were added and the tube was vigorously shaken by hand for 1 min in order to ensure that the solvent interacts well with the entire sample and that crystalline agglomerates are broken down sufficiently. The tube was centrifuged at 3000 rpm (900 g) for 1 min. The upper layer, the extract, was introduced into a glass centrifuge tube, which contained 50 mg of PSA and 150 mg of anhydrous magnesium sulfate per 1 ml of extract. The tube was sealed and shaken vigorously for 30 s. Tube was centrifuged at 3000 rpm for 1 min. The clear supernatant was used for the analysis. The pre-concentration factor was 1.

2.3.2. Luke method

Changes were made to the original AOAC 985.22 procedure [Cunniff, 1997]. The amount of homogenized sample was reduced from 100 g to 50 g. Solvent volumes were reduced accordingly. 50.00 g of homogenized fruits was weighed and 100 ml of acetone was added. The mixture was blended additionally at high speed for approximately 2 min. The mixture was filtered through Büchner funnel that was fitted with filter paper. Slight vacuum was applied to achieve optimal filtration speed. The extract was collected into a 500 ml suction flask. The volume of extract was measured with the measuring cylinder and 50 ml of the extract was placed into a 500 ml separatory funnel, 50 ml of petroleum ether and 50 ml of dichloromethane were added. The funnel was shaken vigorously for 1 min. Lower, aqueous layer was separated and upper organic phase was dried by passing through a sodium sulfate layer into a round bottom flask. The aqueous phase was returned into separatory funnel and ca 3 g of sodium chloride was added. Aqueous phase was twice extracted with 50 ml dichloromethane during 1 min. The lower, dichloromethane phase was dried by passing through a sodium sulfate layer and combined with the previous extracts. The volume of the extract was reduced to approximately 2 ml under slight vacuum in a rotary evaporator. The remaining solvent was removed under a stream of nitrogen. 20 ml of methanol was added to the almost dry residue. The extract

was filtered through a 0.45 μm syringe filter (Whatman, PTFE, 0.45 μm, diameter 13 mm). The pre-concentration factor was from 0.8 to 1 depending on fruit analysed.

2.3.3. Matrix solid-phase dispersion

Some modifications were made to the original MSPD procedure [Blasko, 2005]. Instead of 0.5 g of homogenized sample used in the original procedure we used 1.0 g of sample. Also the amounts of C_8 sorbent, elution solvent dichloromethane and added methanol were doubled. 1.00 g of the homogenized sample was placed into a mortar and was mixed with 1 g of C_8 sorbent for 5 min to obtain a homogeneous mixture. The mixture was transferred into a 5 ml polyethylene column and eluted with 20 ml of dichloromethane in 5 ml portions by applying a slight vacuum. The eluate was collected into two test tubes. 1 ml of methanol was added to both portions. Solutions were combined and concentrated under a stream of nitrogen to 1 ml by slightly warming. The pre-concentration factor of this method was 1.

2.4. LC/MS Parameters

For experiments the LC/MS method contained 3 to 14 pesticide residues. Chromatographic separation was carried out on a 250 mm long Zorbax Eclipse XDB-C18 column, with internal diameter 4.6 mm, particle size 5 μm. An Eclipse XDB-C18 12.5 mm long precolumn was used with internal diameter 4.6 mm and particle size 5 μm. An autosampler was used to inject 10 μl of sample solution. Gradient elution with methanol and buffer solution (pH = 2.8) was used. Buffer as well as methanol contained 1 mM ammonium acetate and 0.1% formic acid. The linear gradient started at 20% methanol and was raised to 100% within 15 min, then the column was eluted 17 min with methanol and the content of methanol was lowered to 20% in 3 min. Eluent flow rate was 0.8 ml/min.

The samples were analyzed with Agilent Series 1100 LC/MSD Trap XCT (Santa-Clara, USA) instrument using electrospray ionization in the positive ion mode and MS^2 for detection and quantification of the pesticide residues. The LC instrument was equipped with a binary pump, autosampler, thermostated column compartment and diode array detector. The scheme of the mass spectrometer is shown in Figure 2. The mass spectrometer uses quadrupole ion trap mass analyzer. For instrument control Agilent ChemStation for LC Rev. A. 10.02 and MSD Trap Control version 5.2 were used. Data analyses were performed using Data Analysis for LC/MSD Trap 3.2. The MS parameters are presented in Table 1 and Table 2, if not stated otherwise.

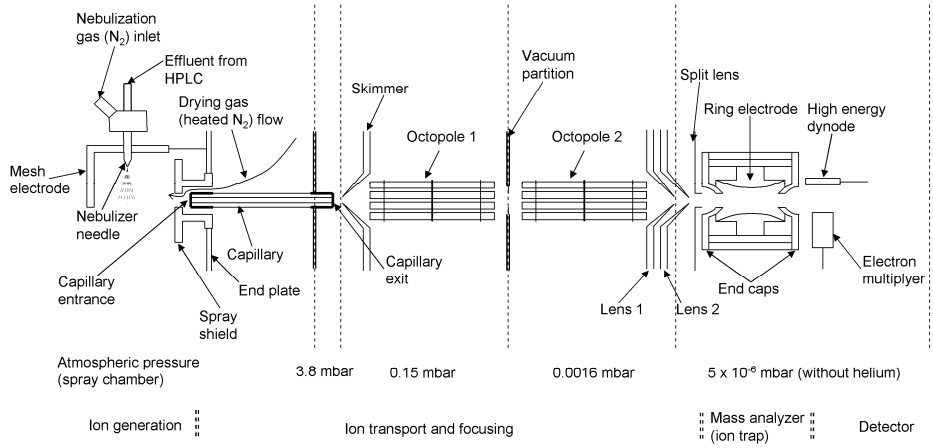

Figure 2. Design of the ion trap mass spectrometer with an ESI source

Table 1. Analyzed pesticide residues, CAS numbers, retention times and fragmentation parameters.

Pesticide residue	CAS[a]	t_R[a]	MS[a]	MS^2 [a]	Fragmentation potential (V)
Aldicarb sulphoxide	1646-87-3	6.7	207	132	0.31
Aldicarb sulphone	1646-88-4	7.4	240	223	0.43
Demeton-S-methyl sulphoxide	301-13-2	8.0	247	169	0.48
Carbendazim	10605-21-7	8.3	192	160	0.54
Methomyl	16752-77-5	8.4	163	122	0.39
Thiabendazole	148-79-8	9.5	202	175	0.46
Methiocarb sulphoxide	2635-10-1	10.2	242	185	0.48
Methiocarb sulphone	2179-25-1	11.1	258	201	0.44
Aldicarb	116-06-3	12.8	213[b]	116	0.41
Imazalil	35554-44-0	14.0	297	201	0.56
Thiodicarb	59669-26-0	14.3	355	163	0.44
Phorate sulphoxide	2588-03-6	14.8	277	199	0.49
Phorate sulphone	2588-04-7	15.1	293	247	0.38
Methiocarb	2032-65-7	16.4	226	169	0.41

[a] CAS – chemical abstracts service number, t_R – retention time, MS – *m/z* of parent ion $[M+H]^+$, MS^2 – *m/z* of the fragment used for quantitation.
[b] $[M+Na]^+$

Table 2 Parameters of the MS detector in separate time segments.

Time (min)	Capillary (V)	Skimmer (V)	Capillary Exit (V)	Octopole 1 DC (V)	Octopole 2 DC (V)	Trap drive	OctopoleRF (Vpp)	Lens 1 (V)	Lens 2 (V)
0.00–12.25	–1175.1	30	69.18	12.8	1.13	31.57	80	–3	–52.37
12.25–15.89	–1267.21	35.5	73	11	1.12	34.51	90	–3.9	–50
15.89–20.00	–1270	27.87	73	11.11	0.85	34.8	109.51	–4.02	–69.75

Due to the shift of the optimum ranges of the MS parameter occurring over time the parameters were reset for the analyses described in sections 3.3 and 3.4. Those parameters are presented in the text in Table 9. Also the chromatographic method was shortened to 32 min: gradient started at 20% methanol and was raised to 100% within 15 min, then the column was eluted 7 min with methanol and methanol's content was lowered to 20% in 2 min and between injections 5 min stabilization time was used.

During all experiments the calibration solutions and samples were run in the same batch in randomised order.

2.5. Statistical tests

T-test, F-test, ANOVA, PCA and PLS were carried out as statistical tests for data treatment. All statistical test were carried out at 95% confidence level and using the R soft ware [R Development Core Team, 2008].

The analysis results were compared with the spiked concentrations using the E_n values, defined as follows:

$$E_n = \frac{\left|c_{calculated} - c_{actual}\right|}{\sqrt{U_c^2(c_{calculated}) + U_c^2(c_{actual})}} \tag{10}$$

where c_{actual} is the concentration on which the sample was spiked, $c_{calculated}$ is the concentration calculated and $U_c(c_{added})$ and $U_c(c_{found})$ are the respective expanded uncertainties. The E_n values are interpreted as follows: $|E_n| \leq 1$ means agreement, $|E_n|$ 1 means disagreement. [ISO Guide 43-1]

3. RESULTS AND DISCUSSION

The main results obtained in this study are shortly described in the following sections. Further details on the experimental conditions, analytical methods and validation results can be found in the original publications.
Section 3.2.1 in based on the [Paper I, section 3.2.2 is based on [Paper II], section 3.3 is based on [Paper V], section 3.4 is based on [Paper III] and section 3.5 is based on [Paper IV]. Data presented and discussed in sections 3.1, 3.2.3, 3.2.4 and 3.2.5 have not been previously published.

3.1. Evaluation of matrix effect

Matrix effect can be quantitatively evaluated with two different methods. First, for quantitative evaluation, blank sample extracts are spiked with analyte and the corresponding peak areas are compared to the peak areas of the standard with the same analyte concentration.

Recently in addition to comparison of peak areas a comparison of calibration graph slopes has been introduced as a tool for evaluation of matrix effects [Lehotay & Son, 2010]. One calibration graph is prepared in the solvent and the other in blank matrix extract. This method has both advantages and disadvantages. As an advantage, the signals for both sample and standard are averaged over a wide concentration range and therefore a more accurate result should be obtained. As a drawback it must be mentioned that not always is the slope the only difference between the calibration lines made in sample matrix extract and in solvent. In several papers it has become obvious that the intercept of the calibration lines may be different from zero and also different between the calibration lines made in sample matrix extracts and in solvent. For example in [Villagrasa, 2007] it appeared that for benzoxazinoids in solvent, root samples and foliage extract solutions the intercepts were both different from zero as well as different between the different calibration curves. Similar phenomenon was observed in [Manini, 2006] for DL-phenylmercapturic acid with different matrix load.

In order to further study the possible tendencies occurring over different concentration ranges with matrix effect four calibration graphs were constructed – one in solvent and three in garlic extracts of different concentrations (10, 25 and 50%). The calibration lines were constructed for four pesticides: methomyl, thiabendazole, imazalil and methiocarb. Garlic extract was specifically selected as matrix because on an average it behaves as a worst case matrix from the point of view of matrix effects in LC/ESI/MS analyses of polar pesticides(see section 3.4.2 for details).

For each concentration the *%ME* was calculated according to eq (7). The obtained *%ME* values were averaged over all concentrations. Also the matrix effect was evaluated via comparison of the calibration graph slopes. The matrix

effect values estimated via these two approaches were compared according to the t-test.

For one of the pesticides – methomyl – the intercepts of the corresponding calibration graphs were statistically significantly different from each other. The calibration lines are presented in Figure 3.

The t-test results reveal that the matrix effect values calculated over a whole line and separately for each point are statistically significantly different for methomyl in 10% and 25% of garlic extract. Also the intercepts of the methomyl calibration lines were significantly different (t-test results) from each other. For example in 25% of extract the average *%ME* over all concentrations was 46% (±10%) while the *%ME* calculated according to the standard line slopes was 65%. For methomyl in 10% of the garlic extract the respective values were 58% (±7%) and 70%. Similar results were found for imazalil in 25% garlic extract (*%ME* values 83% (±16%) and 63% respectively). These results demonstrate that the *%ME* values are very sensitive even to small variations of experimental conditions and cannot be used for correction of the measurement results in a straightforward way.

For other pesticides both intercepts and differently calculated *%ME* values were not statistically significantly different.

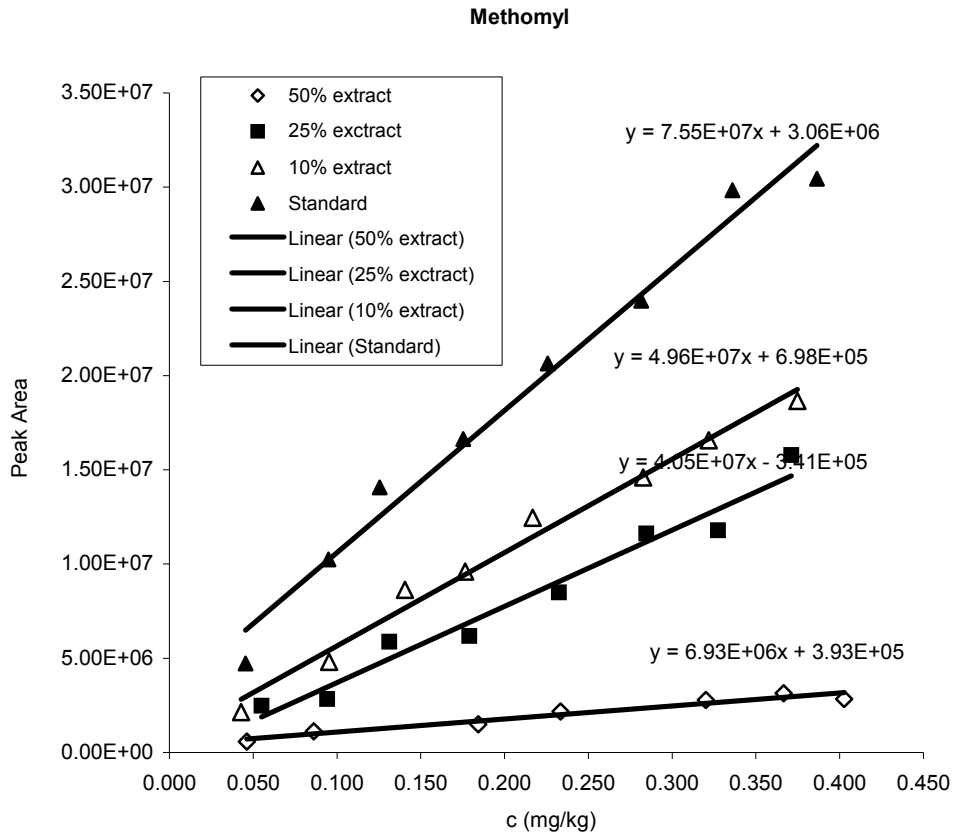

Figure 3 Calibration graphs with the different concentrations of the garlic extract

For visualizing the matrix effect over the chromatogram a standard solution is infused into the ionisation source while the sample is eluted through the column. The obtained *matrix effect profile* can be used to optimise the chromatography of the analyte – analyte elution should preferably occur in the matrix-effect-free zone. This kind of a profile can be seen in Figure 12.

In this study mainly the comparison of analyte peak areas in sample matrix and solvent were used for evaluation of matrix effect.

3.2. Reducing matrix effect

In this study different approaches were tested to reduce matrix effect. In principle either the amount of co-eluting compounds can be reduced or the instrumental parameters of the ionisation system can be modified so that co-eluting compounds have less influence on the analyte signal.

The amount of co-eluting compounds can be reduced in different ways. The amount of sample compounds co-extracted during sample preparation can be reduced by using a more efficient sample preparation procedure. The improvement of sample preparation often leads to very laborious and time consuming sample preparation procedures. Therefore it is often more practical to improve the chromatographic separation of analyte from the matrix components instead.

The aim of these procedures is to reduce the number of ons competing for the surface of the droplet in the ESI source. This competition can be reduced as well by injecting smaller sample amounts into the column or dilution of the sample.

3.2.1. Sample preparation

Three different sample preparation methods – Luke method [Cunniff, 1997], QuEChERS method [Lehotay, 2005] and MSPD method [Blasco, 2005] – were tested from the matrix effects point of view. Luke method is a classical liquid-liquid extraction method with a concentration step. QuEChERS is a novel approach based on a liquid-liquid extraction, which also includes sample cleanup with dispersive solid phase extraction in the second step. MSPD (matrix solid phase dispersion) as an example of solid phase extraction was used. For more details see Experimental section.

For evaluation of matrix effect dependence on sample preparation an apple sample was spiked 6 times with the standard mixture of the 14 pesticides with the concentration of 0.1 mg/kg. From Figure 4 it can be seen that the Luke method is generally less influenced by the coeluting matrix components (*%ME* closer to 100%) than the QuEChERS method. Statistically significant differences (t-test values less than 0.05) were found for demeton-S-methyl sulphoxide, carbendazim, methomyl, aldicarb, phorate sulphone and methio-

carb. Matrix effects for the MSPD method are also smaller than those of the QuEChERS method. Between these two methods differences are statistically significant for aldicarb sulphoxide, aldicarb sulphone, demeton-S-methyl sulphoxide, carbendazim, methomyl, thiabendazole, methiocarb sulphone and aldicarb. For three pesticides (methomyl, thiabendazole and aldicarb) matrix effect in the MSPD method was statistically smaller than in case of Luke method. The repeatability of these matrix effect values was very good for all pesticides. RSD values lower than 15% were observed.

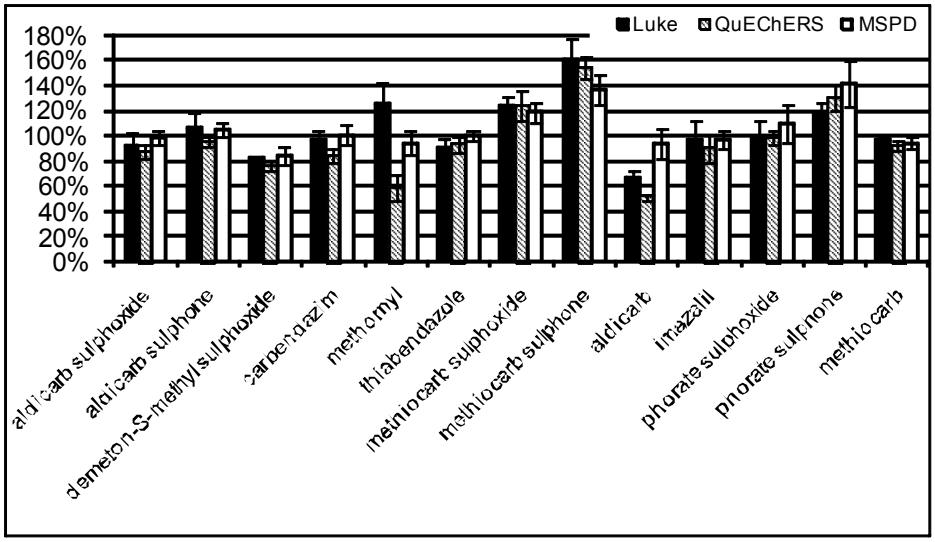

Figure 4 Matrix effects for Luke, QuEChERS and MSPD methods in apple sample spiked with standard mixture at the concentration level of 0.1 mg/kg. The error bars are the standard deviations over 5 parallel measurements.

The sample preparation methods were also compared in other samples – tomato, sweet pepper, orange, raspberries, banana, cucumber, lemon, blackcurrants, peach, grape, apple, grapefruit, pear, red currants and leek – at 1.0, 0.1 or 0.01 mg/kg concentration level. In each fruit/vegetable one sample preparation and one spiking concentration were used. The variability of matrix effects over different fruits tends to be higher than within one fruit (one variety of apple) for all pesticides irrespective of the sample preparation method. Based on the F-test the Luke method is more variable over different fruits than in one fruit for 5 pesticides (demeton-S-methyl sulphoxide, methiocarb sulphoxide, aldicarb, phorate sulphone and methiocarb), the QuEChERS method for 7 pesticides (demeton-S-methyl sulphoxide, carbendazim, methomyl, thiabendazole, methiocarb sulphone, aldicarb and phorate sulphone) and the MSPD method for 8 pesticides (aldicarb sulphoxide, aldicarb sulphone, demeton-S-methyl sulphoxide, carbendazim, thiabendazole, methiocarb sulphoxide, methiocarb sulphone and

methiocarb). The RSD values over different fruits were as high as 50% for some pesticides (methiocarb sulphone for MSPD). Lower variability is observed for pesticides eluting in the beginning of the chromatogram. The high variability shows that different fruits give quite different matrix effects as the variability of matrix effect (caused by system performance and sample preparation repeatability) in one variety of apple was much smaller. Therefore it can be concluded that matrix effect can not be evaluated in one sample and used for correction in another sample due to the possible overestimation/underestimation of the results if matrix used for correction leads to lower/higher *%ME* values.

3.2.1.1. Positive matrix effect in the case of thiodicarb

Positive matrix effect (signal enhancement) is significantly less commonly encountered in LC/ESI/MS than negative matrix effect (signal suppression). In addition it is more difficult to reliably identify: an apparent matrix effect can be caused by matrix compounds co-eluting with the analyte and accidentally giving ions with the same m/z under the used LC and MS conditions [Rosen, 2010]. Many authors reporting positive matrix effect do not scrutinize their data enough in order to prove that the signal enhancement is indeed due to matrix effect. An opposite example is given by Shah et al [Shah, 2009] who discovered positive matrix effect in determination of nitrosoamines in human urine. Matrix effects around 120% were consistently found using two different approaches (post-column infusion and comparison of mean areas of post-extraction spikes to standard solutions of the same concentration) at three different concentrations (60, 400 and 2000 ng/ml).

In this study thiodicarb was included in the standard mixture of the pesticides but is discussed separately below due to its uncommon behaviour – independent of sample preparation method a strong ionisation enhancement (*%ME* up to 264%) was observed for thiodicarb. For this reason thiodicarb was taken under closer study. 11 blank apple samples of different varieties were prepared with QuEChERS sample preparation method, 5 parallels each. Also blank solvent – MilliQ water – was taken for sample preparation and obtained results are presented in Figure 5. It can be seen that all samples independent of the apple variety cause strong ionisation enhancement for thiodicarb. Enhancement is also seen for the extract obtained with blank solvent extraction. It is therefore difficult to make conclusions on the actual origin of the matrix effect in the case of thiodicarb: besides matrix components also compounds originating from sample preparation – eg such as dissolved salt – can cause ionisation enhancement for thiodicarb.

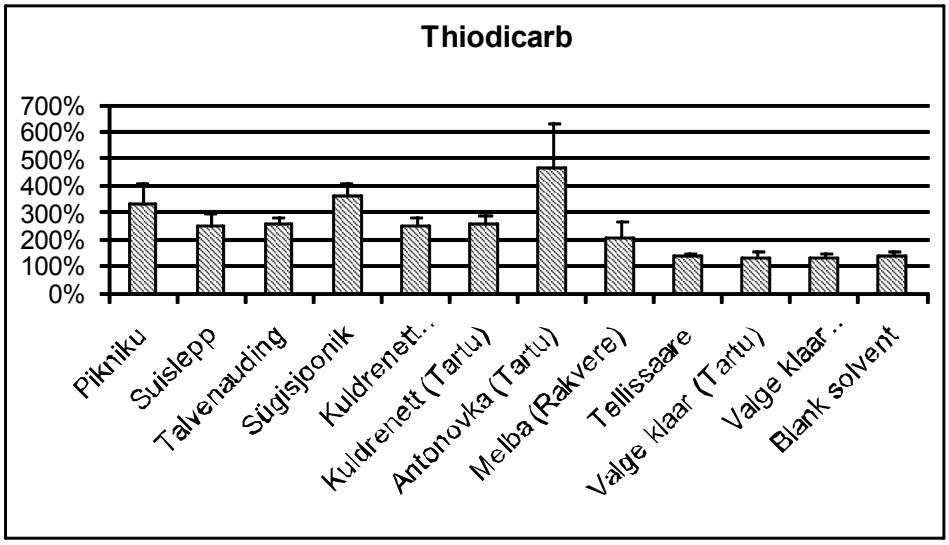

Figure 5 Matrix effects of thiodicarb in different apple samples using the QuEChERS sample preparation method. The error bars are presented as standard deviations over 5 parallel measurements.

As a comparison in [Banerjee, 2008] observed a 20% signal suppression for thiodicarb under different sample preparation and chromatography conditions. Though in present work ionisation enhancement instead of suppression was observed also different chromatographic conditions and instrumentation was used, which might strongly influence the results. Therefore ionisation enhancement of thiabendazole in our case is not related only to the properties of thiodicarb but also to the properties of the method.

There is at present no solid knowledge available about the possible mechanism of action of the positive matrix effect (differently from negative matrix effect). The most reasonable hypothesis is that the matrix compounds responsible for the positive matrix effect selectively bind to the analyte molecule or ion and such complex has higher affinity for the drop surface. Further investigations – centered around identification of the compounds causing matrix effects – are necessary in order to prove or reject this hypothesis.

3.2.1.2. Matrix effect at different concentrations

As the estimation of matrix effects over different fruits was carried out at three concentration levels (0.01 mg/kg, 0.1 mg/kg and 1.0 mg/kg) conclusions about matrix effect dependence on concentration can also be made. Though the average values of matrix effects did not show statistically significant differences between the used concentrations it can be seen that at 0.01 mg/kg level the matrix has stronger influence on ionization efficiency of the analyte. This

conclusion is based on statistically significant differences (F-test) between the standard deviations of *%ME* at different concentrations. The variability of results was statistically significant for 5 pesticides (aldicarb sulphoxide, aldicarb sulphone, aldicarb, phorate sulphoxide and methiocarb) for Luke as well as for the QuEChERS method. Differences in MSPD extracts of different concentrations occurred for 4 pesticides (aldicarb sulphone, thiabendazole, imazalil and methiocarb). This high variability of matrix effect at 0.01 mg/kg level compared to 1.00 mg/kg level was not caused by system instability, because reproducibility of calibration solutions of 0.01 mg/kg did not show higher variability than calibration solution of 1.00 mg/kg and 0.10 mg/kg did (no statistically significant differences based on F-test). In Figure 6 aldicarb's *%ME* values can be seen for different concentrations for QuEChERS sample preparation.

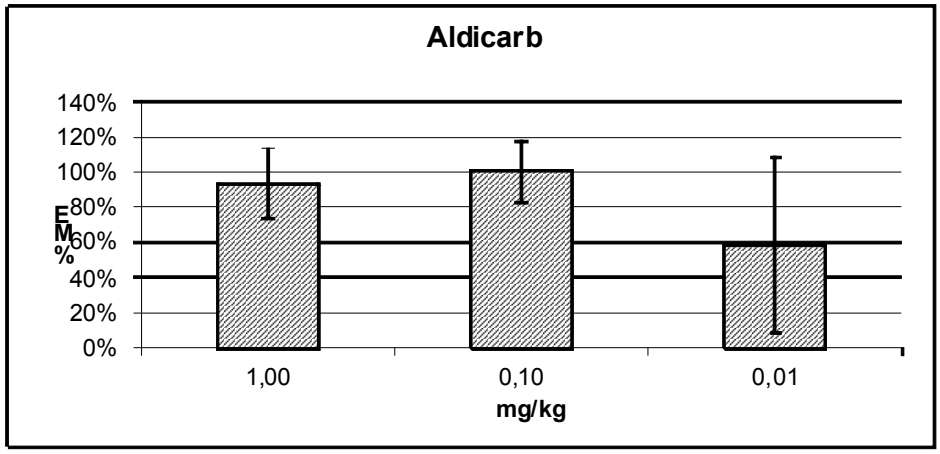

Figure 6 Aldicarb's matrix effect with QuEChERS sample preparation at different concentration levels (1.0 mg/kg corresponds to tomato, sweet pepper, orange, and raspberries, 0.1 mg/kg corresponds to banana, cucumber, lemon, blackcurrant and peach and 0.01 mg/kg to grape, apple, grapefruit, pear, red currant and leek). The error bars indicate the standard deviations over 5 spiked samples.

In order to get more information about the matrix effect dependence on the analyte concentration – a QuEChERS apple extract was spiked with pesticide standard mixture in the concentration range from 0.01 mg/kg to 4.0 mg/kg. In the same concentration range calibration solutions were prepared and matrix effect values at each concentration level were calculated. The results for methomyl, thiabendazole and aldicarb are presented in Figure 7. It can be seen that at lower concentrations independent of the pesticide analyte ionisation suppression is observed. The suppression decreases at higher concentrations and stabilises for aldicarb above 0.5 mg/kg at matrix effect value 80%. For

methomyl and thiabendazole at 1.0 and 4.0 mg/kg ionisation enhancement is observed.

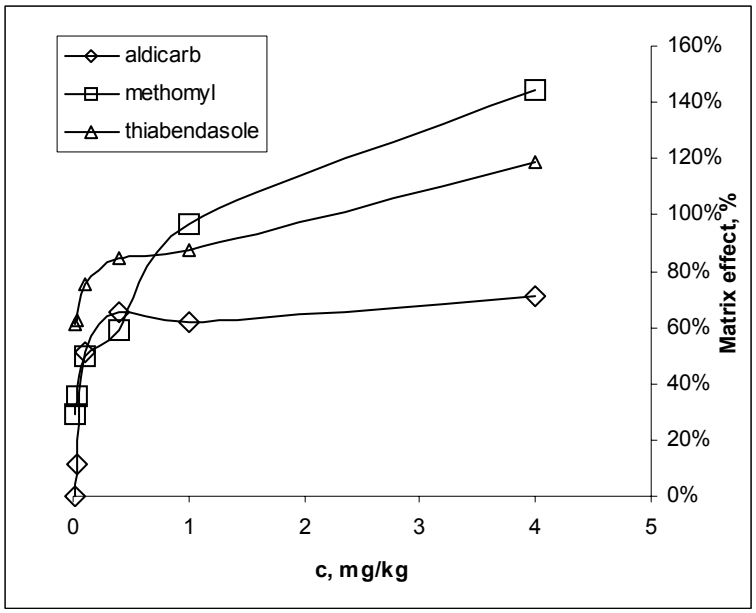

Figure 7 Matrix effects at different analyte concentrations in apple QuEChERS extract for aldicarb, methomyl and thiabendazole.

This kind of matrix effect change from 29 to 145% (methomyl) with concentration change is not always observed but is possible and has to be accounted for. Also it has to be kept in mind that ionisation suppression is more likely at lower analyte concentrations.

3.2.1.3. Detecting the co-eluting compounds causing matrix effect

It appeared that the UV chromatograms may provide information about the possible matrix effect in the case when the co-eluting compound causing the matrix effect has UV absorbance. It was observed for aldicarb peaks in apple extract (QuEChERS sample preparation) that the MS peak shape of analyte is strongly changed from variety to variety most probably due to the co-eluting compounds. At the same time the peak shape was consistently the same within single apple variety. These co-eluting compounds can be seen on the UV-chromatograms recorded in parallel to the MS^2 chromatogram (Figure 8).

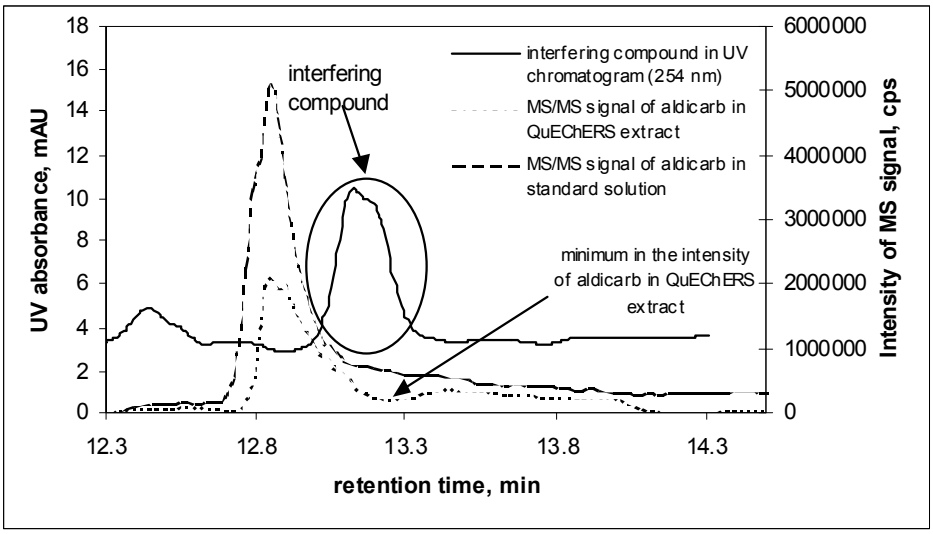

Figure 8 MS^2 chromatograms for aldicarb peak in standard and in sample overlaid with samples UV chromatogram (254 nm).

It can be seen from Figure 8 that next to aldicarb an interfering compound elutes and causes a change in the peak shape of the pesticide. In this case the compound causing ionisation suppression is present as a normal chromatographic peak.

From apple variety to variety the variability in matrix effect values for aldicarb was remarkable – from 92 to 36%. This variation is considerably higher than the one measured within one apple variety (RSD 2% for Aldicarb in QuEChERS apple extracts). Also the peak areas of the interfering peak varied a lot – from 87 to 7 mAU·s. Matrix effect and the interfering peak's peak area display some correlation (Table 3). This effect was found to be reproducible from day to day.

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Table 3 Matrix effect of aldicarb in different varieties of apple, areas and heights of the interfering compound peaks (QuEChERS method).

Apple variety	Matrix effect	Area (mAU·s)	Height (mAU)
Sügisjoonik (Estonia)	36%	36	2
Kuldrenet (Estonia)	46%	28	2
Talvenauding (Estonia)	52%	87	7
Sibulõun (Estonia)	82%	11	1
Jonagored (Poland)	92%	7	1

It can be concluded from the above described experiments that because of the high between-variety variability of matrix effect it can not be estimated once and used afterwards for matrix effect correction in different varieties of the same fruit. In addition, it becomes obvious from the analysis of different apple varieties that matrix matched calibration even within one fruit may lead to erroneous results. Therefore other possible methods accounting for or reducing matrix effect – eg dilution, *echo*-peaks technique, post column standard infusion, isotope dilution – were tested.

3.2.2. Dilution of the sample

It has been shown in the literature that dilution of the sample solution reduces the matrix effect due to the reduction of the amount of co-eluting compounds. At the same time, the exact behaviour of matrix effect in the case of dilution has not been systematically investigated. In order to study how matrix effect changes with dilution a garlic extract was spiked at 1.0 mg kg^{-1} with methomyl, thiabendazole, aldicarb, imazalil and methiocarb. Garlic extract was specifically picked for this study because according to the experience in our laboratory garlic is the worst case matrix with respect to matrix effects (see section 3.3.2 for details) in the case of determination of these pesticides. The standard solution in solvent with the 1.0 mg/kg concentrations was prepared as well. Both, the spiked sample extract and the standard solution were diluted with the solvent. For each dilution the matrix effect was determined. The dilution factor *d* was calculated for each dilution as follows:

$$d = \frac{V_{sample}}{V_{sum}}, \tag{11}$$

where V_{sample} is the volume of the initial sample extract taken for dilution and V_{sum} is the final volume after dilution. For each dilution the *%ME* was calculate according to eq 7. The matrix effect *%ME* was plotted against dilution factor as is exemplified in Figure 9.

From Figure 9 it can be seen that the more diluted (smaller dilution factor) sample the lower is the ionisation suppression (matrix effect values approach 100%). However, dilution graphs similar to Figure 9 are not always seen. Also for unknown samples the *%ME* can not be always calculated. Therefore the calculated concentration of the sample (eq 12) can be plotted against dilution factor.

The sample concentration in the original sample taken for dilution can be calculated:

$$c_{calc,i} = \frac{c_{dil,i}}{d_i} \tag{12}$$

where $c_{\mathrm{dil,\ i}}$ is the concentration of the diluted sample calculated from the calibration graph and *i* denotes the *i*-th dilution.

The experiments made in this work indicate that three different kinds of dilution plots may exist (Figure 10).

Firstly, when analyte ionization is not influenced by the matrix effect then the analyte concentrations in the sample found from all dilutions are similar and vary only due to random errors. This situation is shown in Figure 10a on the example of methomyl in rye extract. In this case the average analyte concentration should be presented as a result $c_{\mathrm{sample,\ calc}}$.

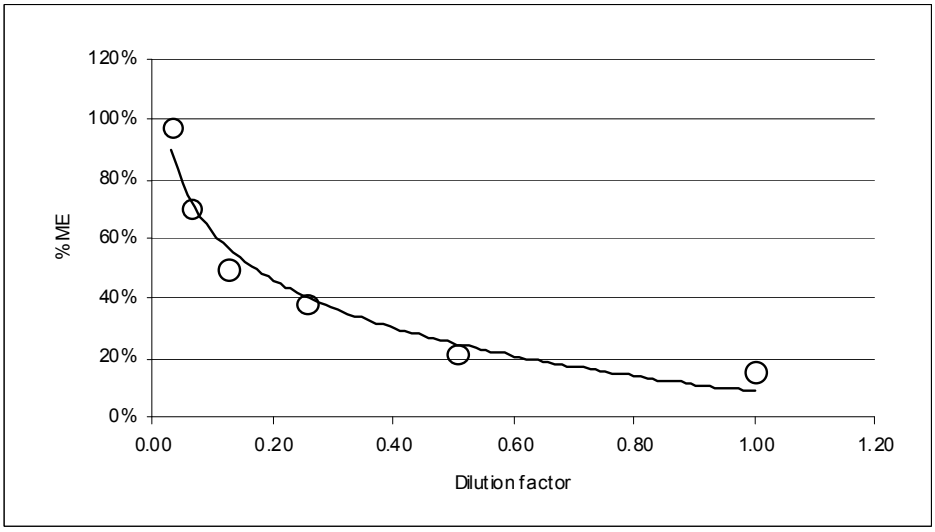

Figure 9 Matrix effect values for methiocarb in garlic sample at different dilutions (initial concentration 1.0 mg kg^{-1}).

Secondly, matrix effect can become eliminated when sample solution is sufficiently diluted. An example of this case is presented in Figure 10b. It can be seen that starting from a dilution factor of ca 0.1 to 0.15 the found analyte concentration in the sample does not change any more with the additional dilutions. Thus, the matrix effect can be considered eliminated. In this case the concentration calculated from the most diluted solution or the average of a few of the last dilutions should be used as the concentration of analyte in sample $c_{\text{sample,calc}}$.

As a third possibility the dilution may fail to fully eliminate the matrix effect because quantitation limit might be reached at higher dilutions. An example of this situation is shown in Figure 10c. In this case extrapolation to matrix-free solution has to be made. Least squares regression line was fitted through these points. The intercept of the line indicates the analyte concentration in a "matrix-free" solution and should be used as $c_{\text{sample, calc}}$. This approach has been named *extrapolative dilution approach*.

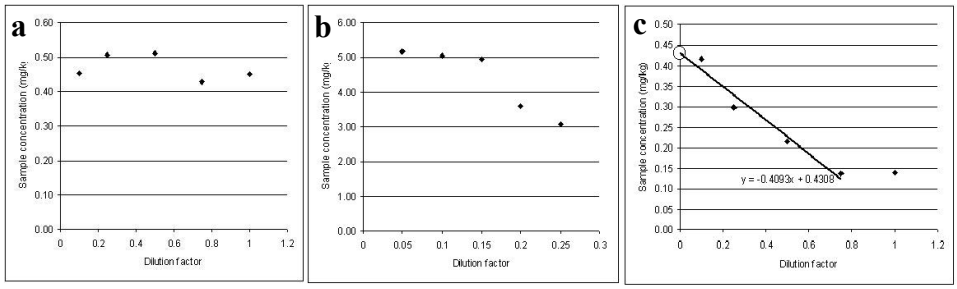

Figure 10 Examples of different dilution plots. On y-axis an analyte concentration calculated back into the original sample and on x-axis the dilution factor is plotted. a) methomyle in rye; b) methiocarb in tomato; c) aldicarb in apple.

The validation of the proposed calculation scheme was carried out with the spiked extracts of tomato, cucumber, apple, rye and garlic, obtained with QuEChERS sample preparation. 1 ml aliquots of the blank extract were spiked with five pesticides – methomyl, thiabendazole, aldicarb, imazalil and methiocarb – at two concentration levels (approximately 0.5 and 5.0 mg/kg, exact concentrations are given in Table 4 as c_{spike}). For 0.5 mg/kg samples 0.1, 0.25, 0.5 and 0.75 dilutions were prepared and analyzed. From the dilution profile it became obvious that dilutions with dilution factor of 0.05 and 0.02 were also needed for methomyl analysis in garlic. For 5.0 mg/kg samples the dilution factors d were 0.25, 0.2, 0.15, 0.1, 0.05 and 0.025.

For all samples $c_{\text{sample, calc}}$ were calculated according to the dilution plot obtained via the above described methods. The results are presented in Table 4. It can be seen that according to the E_{n} values (eq 10) all of the results are

acceptable. Therefore it can be concluded that in the case of analyte concentrations above the limit of quantitation the extrapolative dilution can be used as a method to both reduce and account for matrix effect. As drawbacks high workload, multiple injections per sample and time consumption can be outlined. Also samples with low analyte concentrations (near limit of quantitation) are not very convenient for extrapolative dilution analyses.

3.2.3. *Echo*-peak technique

Matrix effect has a number of troublesome properties. First of all matrix effect is both analyte and sample dependent, other compounds and different samples can only occasionally be used to fully account for matrix effect. Therefore the calibration should be carried out within the sample of interest. This in principle can be done by using the extrapolative dilution approach but in addition to that a method also applicable for lower concentrations should be found. In literature the *echo*-peak technique has been used to account for matrix effect [(Zrostlikova, 2002) (Alder, 2004)]. In this method an internal standard – containing the same target analytes – is injected into the column either a few seconds up to a minute before or after the sample injection. With this approach the analyte originating from the internal standard elutes very close to the analyte from the sample and can therefore account for matrix effect. This is only applicable if the elution zone of the coeluting compound is wide enough to influence both peaks in a similar way.

The *echo*-peak method was used also in this work (Figure 11). Unfortunately it became obvious in the course of the experiments that this method can only be used for samples that do not require gradient elution. It can be seen from Figure 10 that analytes eluting in the beginning of the chromatogram are about 2 min separated from the internal *echo*-peak. This time difference of retention times is too large to allow the peaks to elute in the same matrix effect zone especially when compound causing ionisation suppression is eluting as a normal chromatographic peak. For compounds more retained in the column the analyte peak from sample and its *echo*-peak from standard elute closer to each other. For compounds eluting after 12 min the peaks are eluting so close that they are not separated from each other at all and integration of these peaks separately is impossible.

Because gradient elution is used in the majority of LC methods the *echo*-peak approach was not studied further.

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Table 4 Spiked concentrations, calculated concentrations and observed E_n values for the test samples with extrapolative dilution

0.5 mg/kg	Cucumber				tomato				apple				rye				garlic			
	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n
methomyl	0.63	0.21	0.45	0.43	0.57	0.20	0.46	0.29	0.37	0.07	0.47	0.77	0.47	0.15	0.47	0.01	0.38	0.07	0.47	0.70
thiabendazole	0.59	0.09	0.42	0.91	0.56	0.07	0.43	0.88	0.52	0.06	0.44	0.64	0.51	0.05	0.45	0.66	0.42	0.05	0.45	0.28
aldicarb	0.39	0.06	0.48	0.72	0.39	0.06	0.48	0.84	0.43	0.06	0.49	0.55	0.45	0.06	0.50	0.40	0.57	0.06	0.49	0.56
imazalil	0.77	0.11	0.67	0.44	0.57	0.09	0.68	0.58	0.82	0.13	0.70	0.48	0.80	0.15	0.71	0.30	0.57	0.10	0.60	0.18
methiocarb	0.66	0.09	0.52	0.83	0.68	0.08	0.53	0.93	0.61	0.13	0.54	0.27	0.66	0.08	0.55	0.70	0.43	0.08	0.50	0.45
5 mg/kg	cucumber				tomato				apple				rye				garlic			
	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n	$c_{sample,calc}$	$u(c_{sample,calc})$	c_{spike}	E_n
methomyl	4.57	1.03	4.70	0.06	4.11	0.53	4.82	0.68	6.63	1.66	4.94	0.51	4.68	0.51	5.16	0.48	4.12	0.55	5.05	0.84
thiabendazole	5.40	0.90	4.43	0.54	5.94	0.78	4.55	0.89	5.39	2.04	4.66	0.18	5.59	1.06	4.87	0.34	5.42	0.40	4.75	0.84
aldicarb	4.50	0.45	4.97	0.53	4.35	1.95	5.11	0.19	4.44	0.47	5.23	0.83	5.02	0.43	5.47	0.52	5.77	0.66	5.25	0.40
imazalil	7.09	0.91	7.04	0.03	7.32	0.96	7.22	0.05	6.63	0.47	7.40	0.81	8.04	1.17	7.73	0.13	4.50	1.16	6.41	0.82
methiocarb	6.58	0.79	5.44	0.72	5.06	0.85	5.58	0.31	6.31	0.69	5.72	0.43	6.33	0.62	5.98	0.28	5.42	0.36	5.73	0.43

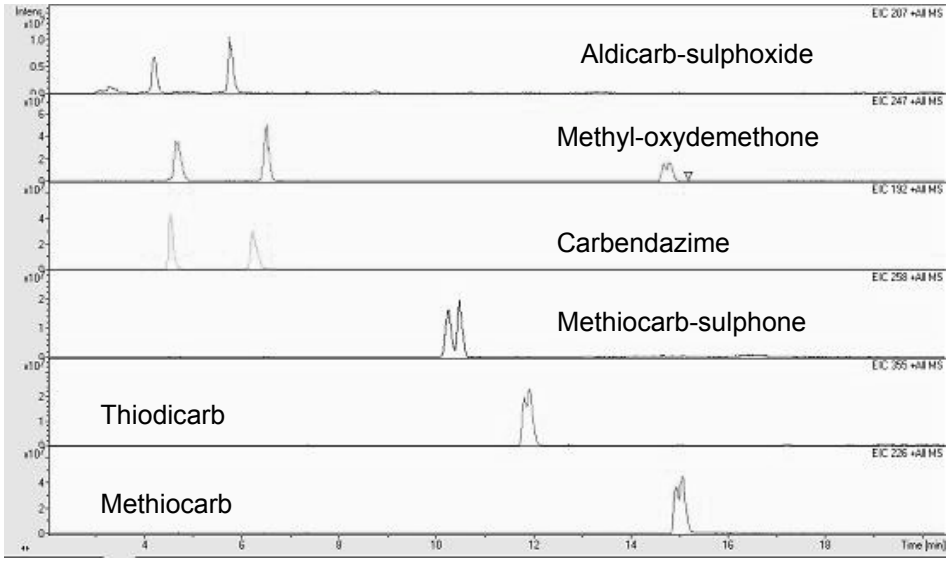

Figure 11 *Echo*-peak method for 6 pesticides with gradient elution. Time between sample and internal standard injection was 1 min plus approximately 30 sec taken for completing the injection.

3.2.4 Post column standard infusion

In order to study the applicability of post-column standard infusion *matrix effect profiles* for two analytes – aldicarb and thiodicarb – were compared. Aldicarb was chosen because of its sensitivity to ionisation suppression (Figure 8 and Table 3) and thiodicarb was chosen because of its tendency to give ionisation enhancement. Both analytes were infused into the effluent with the concentration corresponding to average concentration of 0.5 mg/kg analyte peak in the effluent. For both analytes the injected sample was a QuEChERS extract of apple sample. These profiles are shown on Figure 12.

Comparing the infusion results for aldicarb and thiodicarb for the same extract shown in Figure 12 leads to some principally important conclusions. It can be seen that aldicarb and thiodicarb undergo different ionisation efficiency changes in some regions of the chromatogram. For example at the dead time 2.8 min aldicarb shows strong ionisation enhancement but thiodicarb ionisation suppression. This may be so due to the fact that at the dead time a high concentration of salts is eluting from the column. Aldicarb forms an intense sodium adduct ion, which is used as a precursor in the MS^2 method and also for this *matrix effect profile*. At the same time thiodicarb is suppressed due to high electrolyte concentration and possible formation of sodium adducts instead of protonation, which might cause the precipitation of the droplets is ESI source.

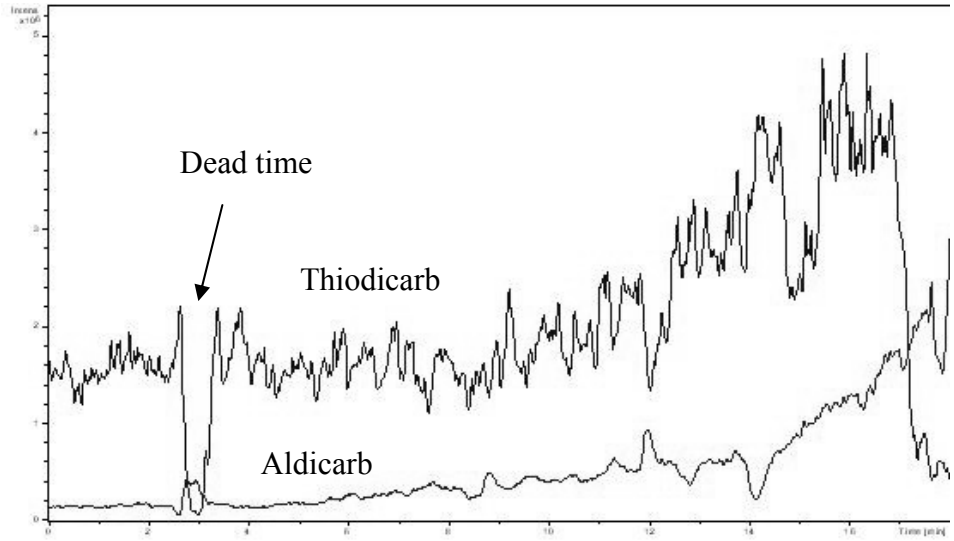

Figure 12 MS^2 intensities (matrix effect profiles) for aldicarb and thiodicarb for the infusion experiment when the same extract is injected to the analytical column.

Similar effect is seen at 12 minutes: aldicarb shows ionization enhancement, but thiodicarb suppression. The opposite is observed at 14.2 min where aldicarb shows ionisation efficiency suppression but thiodicarb shows enhancement. At 14.8 min thiodicarb shows ionisation suppression but for aldicarb no significant ionisation efficiency changes were observed. Similar differences between ionisation efficiency changes for the two pesticides were observed for other varieties as well. Also it was noted that these two pesticides undergo different ionisation efficiency changes at different mobile phase compositions, which is most probably caused by the different type of precursor ions (protonation and sodium adducts). For both pesticides ionisation efficiency increases as the methanol concentration in effluent increases, but when aldicarb shows higher ionisation efficiency for 100% methanol, then thiodicarb almost does not undergo ionisation at 100% methanol composition.

3.2.5 Isotope labelled standards as internal standards

Isotope labelled internal standards – carbendazim-D_4, methomyl-D_3 and thiabendazole-D_6 – were tested as means of correcting for matrix effect. The samples were spiked with 0.5 mg/kg standard solution and also isotope labelled standards were added 0.5 mg/kg. The accuracy was tested for the worst-case matrix garlic. The accuracy for the carbendazim analyses was 106% indicating a similar behaviour between carbendazim and its deuterated form. On the other hand for methomyl and thiabendazole the accuracies were 35 and 49%, respec-

tively. These data confirmed that isotope labelled internal standards do not always correct for the matrix effect. In addition it has to be mentioned that all of these analytes and their corresponding deuterated standards elute within 2 minutes. Therefore the coeluting compounds for these peaks should be relatively similar.

3.3 Accounting for matrix effect in pesticides LC/ESI/MS analyses via background spectra

Signals in MS^1 spectra recorded at the analyte retention time – the so-called background ions – may be divided into two types. First type ions can be seen in all spectra even if no analyte or sample is injected. These ions originate from the impurities of solvents, buffers, plastic- and glassware used [(Jassome, 2006), (van Eeckhaut, 2009)]. Even for pure water gradient elution presence of this kind of ions has been shown [Herath, 2010]. Second type ions, in the case of sample analysis ionisable matrix compounds may be present. In addition to giving ions, the matrix compounds may either cause ionisation suppression/enhancement of the analyte signal or have no influence on analyte ionisation.

3.3.1 Data treatment with PLS and PCA

Principal component analysis (PCA) was used in data treatment to choose the most influential background ions from the scan spectra (MS^1) for the PLS regression.
The intensities of the ions in the scan spectra were first scaled and centered:

$$S_i^s = \frac{I_i - mean(I)}{stdev(I)} \tag{13}$$

where I_i is the intensity of the ion with m/z *s* in scan spectra of the sample or standard *i*, *mean(I)* is the mean of the ion's intensity over all samples and standards, *stdev(I)* is the standard deviation of the ion's intensity over all samples and standards. The same was carried out with the MS^2 peak areas of the analytes.

For each analyte the scaled and centered MS^2 peak area and spectra were gathered into the spectra matrix *S*, which was broken down into score matrix *T* and loading matrix *P*.

14

$$S = T \cdot P = \begin{pmatrix} MS^2{}_1 & S_1^{100.0} & S_1^{100.2} & \dots & S_1^{1000.0} \\ MS^2{}_2 & S_2^{100.0} & S_2^{100.2} & \dots & S_2^{1000.0} \\ \dots & \dots & \dots & \dots & \dots \\ MS^2{}_{24} & S_{24}^{100.0} & S_{24}^{100.2} & \dots & S_{24}^{1000.0} \end{pmatrix} = \begin{pmatrix} t_1^1 & t_1^2 & \dots & t_1^a \\ t_2^1 & t_2^2 & \dots & t_2^a \\ \dots & \dots & \dots & \dots \\ t_{26}^1 & t_{26}^2 & \dots & t_{26}^a \end{pmatrix} \cdot \begin{pmatrix} p_1^{100.0} & p_1^{100.2} & \dots & p_1^{1000.0} \\ p_2^{100.0} & p_2^{100.2} & \dots & p_2^{1000.0} \\ \dots & \dots & \dots & \dots \\ p_a^{100.0} & p_a^{100.2} & \dots & p_a^{1000.0} \end{pmatrix} \quad (14)$$

where *a* denotes the number of principal components constructed. In this study only first two principal components were used for further analysis. The ions were chosen into the PLS according to the PCA plots of the first two principal components. The absolute loading value (matrix *P*) of the ion intensity kept in the reduced data set had to be at least 0.2 and in the case of highly correlated ion intensities the ones with the highest loading values were chosen into the data set. For a number of pesticides also the analyte precursor and fragment ions appeared significant according to the PCA plot, but were left out from PLS analyses due to the fact that MS^2 peak areas were regarded more accurate than the intensities of the scan spectra ions.

The aim of the PLS was to establish a calibration function for estimation of the analyte concentration (corrected for the matrix effect) from its MS^2 peak area and the intensities of background ions. For PLS regression the data was first centered:

$$X_i = I_i - mean(I) \quad (15)$$

The PLS regression model consists of two equations. First the spectral part is broken down according to eq 16:

$$X = T \cdot P + E = \begin{pmatrix} MS_1 & X_1^1 & \dots & X_1^j \\ MS_2 & X_2^1 & \dots & X_2^j \\ \dots & \dots & \dots & \dots \\ MS_i & X_i^1 & \dots & X_i^j \end{pmatrix} = \begin{pmatrix} t_1^1 & t_1^2 & \dots & t_1^a \\ t_2^1 & t_2^2 & \dots & t_2^a \\ \dots & \dots & \dots & \dots \\ t_i^1 & t_i^2 & \dots & t_i^a \end{pmatrix} \cdot \begin{pmatrix} p_1^0 & p_1^1 & \dots & p_1^j \\ p_2^0 & p_2^1 & \dots & p_2^j \\ \dots & \dots & \dots & \dots \\ p_a^0 & p_a^1 & \dots & p_a^j \end{pmatrix} + \begin{pmatrix} e_1^0 & e_1^1 & \dots & e_1^j \\ e_2^0 & e_2^1 & \dots & e_2^j \\ \dots & \dots & \dots & \dots \\ e_i^0 & e_i^1 & \dots & e_i^j \end{pmatrix} \quad (16)$$

where *X* is the matrix of ion intensities where the number of rows *i* is equal to the number of samples and standards. Columns in matrix *X* correspond to the ion intensities used in the PLS regression and where the first column corresponds to the MS^2 analyte peak areas (also centred). Matrix *T* is the matrix of scores and matrix *P* is the matrix of loadings and *E* is the matrix of residual errors indicating the unexplained variation in the *X* matrix. *a* denotes the number of linear combinations included in the PLS model. The number of background ion intensities *j* used in the PLS regression was chosen according to the PCA plots of the first two principal components and the number of ions varied from 4 (imazalil and thiabendazole) to 9 (aldicarb).

For each analyte the concentration *c* is related to the spectra matrix according to the matrix *T*:

$$c = T \cdot q + f = \begin{pmatrix} c_1 \\ c_2 \\ \dots \\ c_i \end{pmatrix} = \begin{pmatrix} t_1^1 & t_1^2 & \dots & t_1^a \\ t_2^1 & t_2^2 & \dots & t_2^a \\ \dots & \dots & \dots & \dots \\ t_i^1 & t_i^2 & \dots & t_i^a \end{pmatrix} \cdot \begin{pmatrix} q_q \\ q_2 \\ \dots \\ q_a \end{pmatrix} + \begin{pmatrix} f_q \\ f_2 \\ \dots \\ f_a \end{pmatrix} \quad (17)$$

where c is the matrix of analyte concentrations in the samples and standards. The number of columns in this matrix is one because for each analyte a separate PLS regression was constructed because for each pesticide the scan spectra of effluent were different. q is a loading vector and f is also vector of residual errors.

The validation function "Leave One Out" was used during the PLS analyses in R software.

The type of the ions (first or second type background ions) was recognized from the ion intensity versus matrix effect plots. The first type of ions show a correlation coefficient above 0.5 were assigned as background ions being influenced by matrix in a similar way as the analyte. Ions showing correlation coefficient below –0.5 were assigned as matrix compounds causing matrix effect, therefore second type of ions. Ions showing correlation coefficient between –0.5 and 0.5 were assigned as ions with mixed tendencies. The correlation coefficients were taken quite low due to a very complex nature of the scan spectra. The scan spectra were averaged over the retention time of the analyte peak. The minimum correlation coefficient found was –0.7932 for *m/z* of 111.0 for methiocarb and the maximum correlation coefficient was 0.2294 for *m/z* of 212.0 for methomyl.

3.3.2 Finding influential background ions

Throughout this paragraph the term "sample" indicates the post-extraction spiked blank matrix extract and "standard" indicates the analytes in solvent. Garlic and onion samples were purchased from local market. 14 different varieties of garlic and 4 varieties of onion were used. Each variety was used for preparation of one spiked sample. Pesticide concentrations in the spiked samples were approximately 1.5, 1.2, 0.9, 0.75, 0.5 and 0.2 mg/kg. In the calculations exact concentrations were used. Only for carbendazim the concentrations were about 10 times lower. Also 6 standard solutions with the same concentration range as well as 2 blank solvents were included in the analyses.

For every pesticide peak both the SRM signal and the MS^1 spectrum (referred to as "scan spectrum" throughout this work) at the retention time of the pesticide were recorded. Therefore two different MS methods were set up:

1. The mass spectrometer was operated in the selected reaction monitoring mode (SRM). Full MS^2 spectra were recorded (m/z from 100.0 to 350.0). Each fragmentation was observed independently in a separate time window. For quantitation the following transitions were used: 192.0 –> 160.0, 163.0 –> 122.0, 202.0 –> 175.0, 213.0 –> 116.0, 297.0 –> 201.0 and 226.0 –> 169.0 for

carbendazime, methomyl, thiodicarb, aldicarb, imazalil and methiocarb, respectively.

2. The mass spectra from m/z 100.0 to 1000.0 with the interval of 0.2 m/z units were recorded. The scan spectra were averaged over the analyte peak elution times: 7.7 – 9.1 min, 8.2 – 9.2 min, 8.9 – 9.9 min, 12.6 – 13.3 min, 13.4 – 14.2 min and 16.3 – 16.7 min for carbendazim, methomyl, thiabendazole, aldicarb, imazalil and methiocarb.

In order to find the most influential background ions principal component analysis (PCA) on scaled and centred variables was carried out. Four to nine most influential ions – based on their contributions into the first and second principal component – were chosen for each pesticide into the PLS calibration model in addition to the MS^2 peak area calibration. The absolute loading value (matrix *P*) of the ion intensity kept in the reduced data set had to be at least 0.2 and in the case of highly correlated ions the ones with the highest loading values were chosen into the data set.

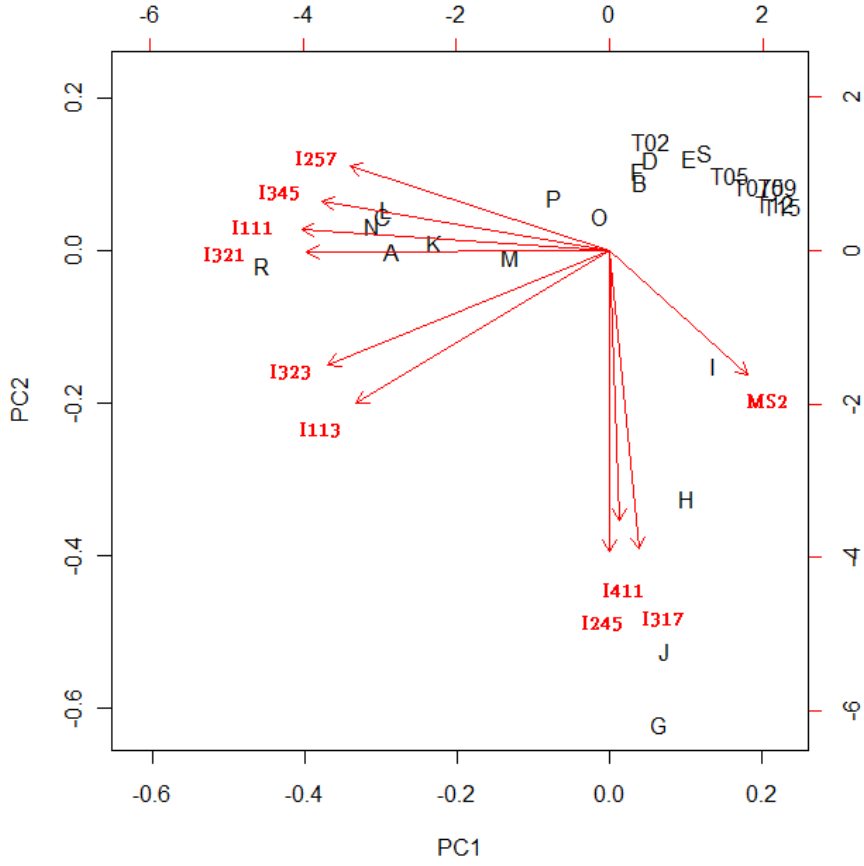

Figure 13 PCA plot for the first two principal components. Standards are labelled with "T", onion samples are "G", "H", "I" and "J" and all the other data points are garlic samples.

The obtained biplots of the first two principal components for aldicarb are presented in Figure 13. Three different regions can be seen from the biplots of aldicarb in Figure 13. On the top right the spot with least matrix effect – the standards and solvent blanks – is seen. The garlic samples make up a large area on the upper-left region of the biplot and all four onion samples are separated to the down-right. Therefore the three different regions are separated on biplot. Also these groups are in accordance with the observed matrix effect: the standards do not have matrix effect, onions have low ionisation suppression and garlic samples tend to have strong ionisation suppression. All these are separated on the first principal component axis. For all pesticides similar tendencies are seen on the biplots. Also from Figure 13 it can be seen that even though only 4 onions were included in the study, compared to the 14 garlic samples, the onion samples are very well separated from standards in solvent and garlic samples.

For all pesticides the most influential ions are presented in Table 5. The ions are classified according to their correlation with the matrix effect value (Section 3.3.1). For each pesticide in each sample and standard a matrix effect value *%ME* was calculated according to eq 7. Thereafter the intensities of the ions found by PCA were correlated with the *%ME*. For the first type of ions, affected by matrix effect similarly to the analyte, there is positive correlation between the *%ME* value and their concentration. In this study none of the ions showed this kind of behaviour. In the case of ions that cause ionisation suppression the analyte *%ME* value decreases with increase of the ion intensity. For each pesticide a few ions of this type were identified (Table 5). Also a number of ions with no clear tendencies were found. These ions were not eliminated from the further analyses but were kept in the partial least square analysis (PLS).

As an example in Figure 14 the absolute intensities of ion with m/z 345.0 from imazalil scan spectra vs. the respective matrix effect are presented. It can be seen that lower *%ME* values are found at higher intensities of the ion 345.0. Therefore it can be concluded that 345.0 may be an ion, which originates from the sample and causes ionisation suppression (or is in correlation with compounds causing ionisation suppression) and is therefore the second type of background ion.

From Figure 14 it can be seen that the correlation is a bit hazy. The probable reason is that in the real samples more than one compound coelutes with the analyte and each of them may suppress/enhance the analyte signal in a different way and no single compound is exclusively responsible for the matrix effect. Therefore all of the ions selected from the PCA biplots should be included in the model used for quantitative analysis.

Table 5 Background ions intensity of which decreases (R<–0.5) with increasing *%ME*. For ions indicated as "mixed" neither decreasing nor enhancing effect could be assigned (–0.5<R<0.5).

Pesticide	Decrease	Mixed
Carbendazim	157.0, 273.0	212.0, 217.0, 301.0, 303.8, 342.0, 344.0
Methomyl	157.0	212.0, 217.0, 303.8, 379.2
Thiabendazole	157.0	379.6, 426.0, 935.0
Aldicarb	111.0, 113.0, 245.0, 321.0, 325.0,	257.0, 317.0, 345.0, 411.0
Imazalil	103.0, 111.0, 231.0, 345.0	
Methiocarb	111.0, 113.0, 217.0, 303.0, 419.0	

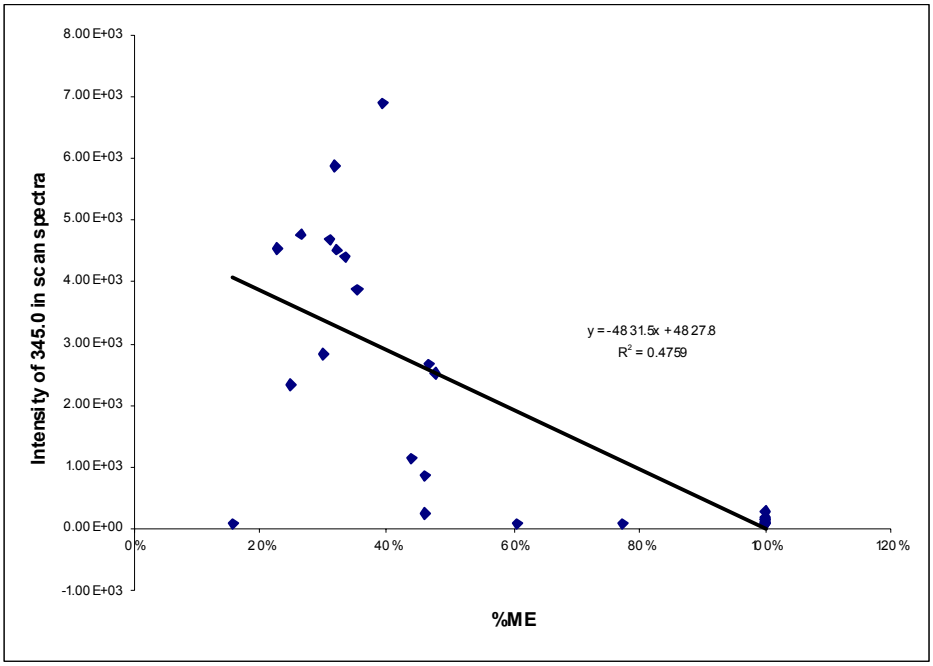

Figure 14 The correlation plot between the intensity of ion m/z = 345.0 co-eluting with the imazalil in the scan spectra and *%ME* values for imazalil in the same samples and standards.

3.3.3 Calculating the analyte concentration with PLS

The test set, containing 19 samples and standards, was used for creating the PLS models. The average errors of predictions are presented in the upper part of Figure 15. It can be seen that for the training set the errors generally decrease with the increasing number of components. Depending on the pesticide 1 to 6 components can be assigned as optimal according to these plots.

It was seen for all pesticides that the MS^2 peak area of the analyte is the first component with the highest contribution. In the second and third component the intensity of background ions in the scan spectra becomes important and they are used to correct for the matrix effect to the MS^2 peak area. It was observed that the intensities of the ions, which are shown in Table 5 as compounds causing matrix effect, are included in the second component of PLS regression with positive sign. This is in agreement with the proposed nature of these ions as being the second type of background ions. The higher is the intensity of these ions the more suppression they cause to the analyte ionisation. Therefore the apparent MS^2 peak area for the analyte is lower and the signal taken for calculation needs to be increased in order to achieve accurate results.

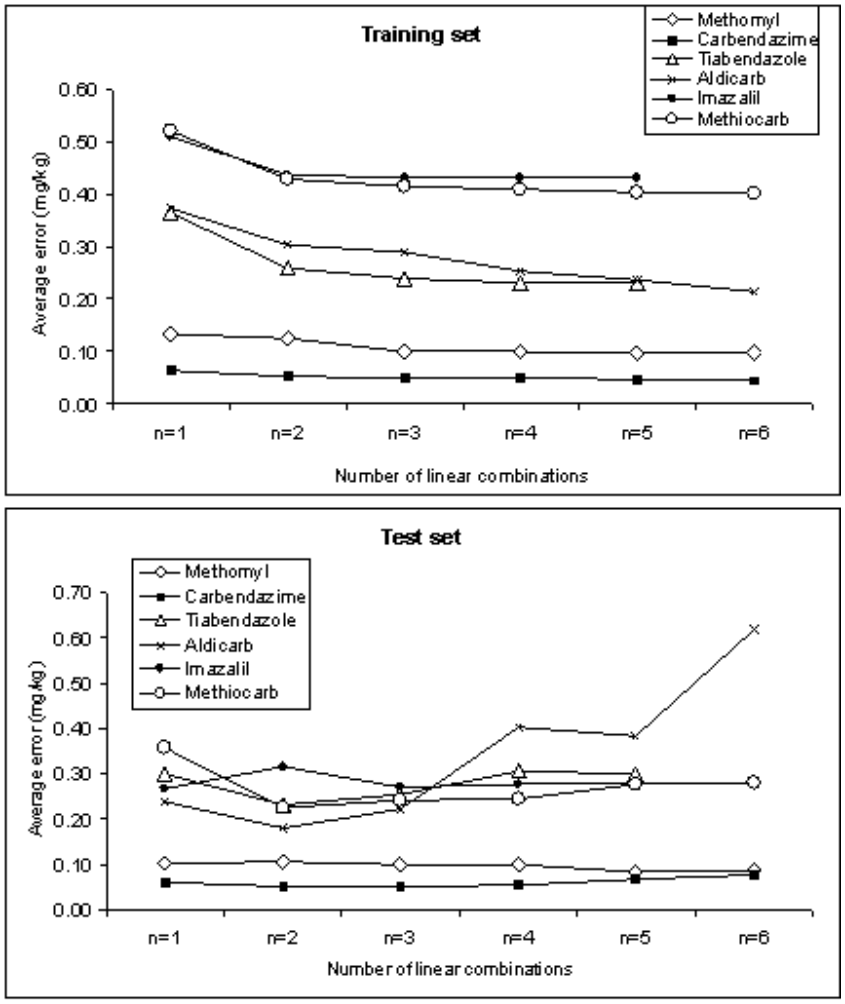

Figure 15 Comparison of average error of predication for training set (above) and test set (below) depending on the number of components used in the PLS regression model.

In order to validate the obtained PLS models, the models were used to estimate pesticide concentrations in a test set of seven samples and standards (3 garlic samples, 1 onion sample, 2 standards and one blank sample). The matrix effect values for the test set samples and standards are presented in Table 6. The average errors found for the test set for each pesticide are presented in the lower part of Figure 15. It can be seen that the tendencies of the errors are different from the ones shown for the training set. For the training set the errors generally decrease with the increasing number of components used in the PLS model. Contrary to that in the case of the test set for all pesticides a minimum in the average relative error occurs at 2 to 3 components. Obviously, if a larger number of components is used then the model is over-trained and starts generating noise always present in data sets. Therefore the final number of components used in the PLS models for all pesticides was chosen to be 3. The results obtained for each pesticide are presented in Table 7.

It has to be mentioned, that the average absolute errors for training and test set are not easily comparable due to the differences in the distribution of the concentrations of the samples and standards included in the training set and test set. Unfortunately as the solvent blanks are also included in both sets the relative errors cannot be used.

It is evident that the average errors are relatively small and for all of the results the calculated results agree with the spiked concentration within ±50% (as assigned uncertainty), which is usually considered a reasonable uncertainty for trace analysis.

Table 6 Matrix effect values for the samples in test set.

	carbendazim	methomyl	thiabendazole	aldicarb	imazalil	methiocarb
Garlic	51%	86%	58%	33%	39%	1%
Onion	76%	114%	78%	64%	61%	35%
Garlic	44%	63%	47%	23%	32%	1%
Garlic	46%	86%	37%	30%	44%	2%
Standard	100%	100%	100%	100%	100%	100%
Standard	100%	100%	100%	100%	100%	100%
Solvent	100%	100%	100%	100%	100%	100%

Table 7 Test set samples spiked concentrations (mg/kg) and estimated concentrations (mg/kg) with PLS with 3 components and the concentrations calculated via solvent calibration

		Garlic	Onion	Garlic	Garlic	Standard	Standard	Solvent	Average error (mg/kg)
Methomyl	Spiked	0.89	0.87	0.48	0.89	1.49	0.90	0.00	
	PLS	0.74	1.02	0.56	0.90	1.46	0.88	–0.13	0.10
	Solvent calibration	0.74	1.01	0.38	0.74	1.42	0.87	–0.07	0.11
Carbendazim	Spiked	0.25	0.25	0.14	0.25	0.42	0.25	0.00	
	PLS	0.17	0.23	0.15	0.20	0.44	0.34	–0.02	0.05
	Solvent calibration	0.14	0.21	0.07	0.12	0.38	0.28	–0.02	0.07
Tiabendazole	Spiked	1.14	1.11	0.61	1.14	1.91	1.14	0.00	
	PLS	0.84	1.07	0.92	0.78	2.14	1.41	–0.14	0.25
	Solvent calibration	0.65	0.90	0.30	0.38	1.74	1.18	–0.09	0.38
Aldicarb	Spiked	0.92	0.90	0.50	0.92	1.54	0.92	0.00	
	PLS	1.20	0.85	0.78	0.55	1.34	1.00	0.05	0.22
	Solvent calibration	0.25	0.58	0.06	0.22	1.37	0.97	–0.11	0.43
Imazalil	Spiked	1.13	1.11	0.61	1.13	1.89	1.13	0.00	
	PLS	1.25	0.83	1.19	0.91	1.89	1.29	0.09	0.27
	Solvent calibration	0.32	0.60	0.19	0.38	1.78	1.12	–0.20	0.49
Methiocarb	Spiked	0.99	0.97	0.54	0.99	1.66	1.00	0.00	
	PLS	0.88	1.09	0.86	0.60	1.31	0.95	–0.01	0.24
	Solvent calibration	–0.10	0.28	–0.11	–0.10	1.47	1.01	–0.12	0.69

In addition to the PLS model the conventional solvent calibration was used to estimate the concentrations of the samples and standards in the test set. The obtained calculated concentrations are also presented in Table 7. It can be seen that for all the pesticides the average errors are considerably higher when solvent calibration is used for calculations of the results. Only for methomyl, which does not undergo strong ionisation suppression (Table 6) PLS and solvent calibration have about the same accuracy. Thus it can be concluded that for samples undergoing ionisation suppression/enhancement the scan spectra could be used to recognize the presence of matrix effect and also to correct for it.

It can be seen from Table 7 that methomyl and carbendazim show considerably smaller average errors than other analytes. For carbendazim it has to be kept in mind that the concentrations of the samples and standards were more than 3 times lower, ranging from 0.43 to 0.00 mg/kg, compared to other analytes. Therefore still a significant improvement in the average error was found when using PLS regression. For methomyl it can be seen that the average errors are very similar for PLS regression and solvent calibration. This can be easily explained while comparing the matrix effect values. For methomyl the *%ME* values range from 63 to 114% for the test set. For other analytes the range of matrix effect present in the test set is much wider and therefore the influence of matrix compounds via scan spectra has to correct more for the result.

The PLS regression calibration method presented here may be considered as too labour-intensive for everyday usage at a routine analysis laboratory. Therefore a different approach can be recommended for these laboratories. During validation procedure quality control samples with known matrix effect should be run in MS^2 and scan mode and scan spectra should be used to find the ions causing matrix effect via PCA and PLS regression analyses. These ions assigned as causing matrix effect should be monitored also in routine analyses (using the MS^2 and scan mode alteration) and if found in the scan spectra PLS regression or other means should be applied to overcome/compensate for matrix effect.

3.4 Matrix effect as an uncertainty source

Due to the complex and variable nature of matrix effect described above – eg concentration dependence, sample variety dependence and retention time dependence – it is obvious that accounting for matrix effect is not always possible. On the other hand, very often the analysis of pesticides is done under circumstances where high accuracy (low measurement uncertainty) of the results is not required. Thus, a potentially useful way to handle matrix effect would be to estimate how much would the uncertainty of the result increase, if matrix effect cannot be avoided and remains uncorrected, and include this additional uncertainty contribution into the combined uncertainty of the result.

Therefore it became of interest if and how it would be possible to include matrix effect in the uncertainty calculations.

The estimation of uncertainty was broken down into two major steps. First, the estimation of the matrix effect variability by using different matrices and analyte concentrations was carried out. This is called the *matrix effect graph* approach in this work. For constructing this graph calibration solutions were prepared in different extracts and the measured peak areas were used for calibration graph construction. The averaged deviations from the regression line were used as a measure of uncertainty. In the second step the sample analyses were carried out via matrix matched calibration in similar matrices and the calculated result was presented with the uncertainty estimated from the matrix effect graph.

3.4.1 Calibration for uncertainty calculation

Calibration in matrix was used. Each calibration solution was prepared in a different fruit matrix, all matrixes were from the same commodity group as the samples in order to overcome possible systematic errors that may be present in single-matrix-calibration. Calibration solutions and samples were analysed within the same chromatographic batch in randomized order. This converts possible drift effects into scatter of calibration points around the calibration line. The calibration equation:

$$A = \beta + \alpha \cdot C \tag{18}$$

was obtained according to the unweighted least squares linear regression, where A is the (generic) peak area, C is the concentration, β is the intercept and α is the slope of the calibration graph.

The concentration of the pesticide residues in the sample c_{sample} was calculated as:

$$c_{\text{sample}} = \frac{A_{\text{sample}} - \beta}{\alpha} \tag{19}$$

where A_{sample} is the peak area of the pesticide in the sample. The combined standard uncertainty of the concentration $u_c(c_{\text{found}})$ is the function of uncertainty of the slope and intercept of the calibration graph and the sample peak area. The largest contribution to the sample peak area uncertainty is due to the matrix effect. For estimating this uncertainty we propose the method of *matrix effect graph*.

3.4.2 Matrix effect graph

The uncertainty of the concentration, $u_c(c_{found})$, is the function of uncertainty of the slope and intercept of the calibration graph and the sample peak area. The largest contribution to the sample peak area uncertainty is due to the matrix effect. For estimating this uncertainty, we propose the matrix effect graph method.

For constructing the matrix effect graph a calibration line using seven spiked extracts from different fruits is obtained (Figure 16). These fruits can be either from the same commodity group as the samples are or from different commodity groups. On the matrix effect graph the relative residuals of this calibration graph are plotted against the time of the measurement. The obtained matrix effect graphs are presented in Figure 17.

In our case matrix effect graphs for each pesticide were constructed by using data from measurements carried out at time intervals of approximately one month during half a year period. For every measurement seven samples were prepared as described in 2.3.1 with seven fruits from the fruiting vegetables commodity group or seven fruits from different commodity groups (as defined by SANCO [SANCO/2007/3131]). In this study two sets of samples were prepared: tomato, cucumber, melon, sweet corn, zucchini, sweet pepper, eggplant from fruiting vegetables group and lemon (citrus fruit), garlic (bulb vegetable), rye (cereals), eggplant (fruiting vegetable), beans (legume vegetables), apple (pomes fruit), gooseberries (berries) from different commodity groups.

To obtain the spiked extracts 1 ml of each supernatant is spiked with the standard mixture of thiabendazole, aldicarb, imazalil and methiocarb. The concentrations of the pesticides in the spiked extracts cover the range of 0.1 to 1.0 mg/kg. For every measurement of the calibration graph new samples were prepared and concentration levels randomized, so that a given fruit was used at a different concentration level each time. These extracts are analyzed with LC/ESI/MS and a calibration graph with regression line is constructed

$$A_i = b_0 + b_1 \cdot C_i + \varepsilon_i \tag{20}$$

A_i and C_i are the peak area and concentration corresponding to the i-th spiked extract, b_0 and b_1 are the intercept and slope of the regression line and ε_i is the residual of the i-th measurement. The residuals ε_i characterize the spread of points around the calibration line. This spread is caused by two main sources. First by a random error caused by system instability (e.g. spray instability, space charge effects) during each measurement. Secondly, and more importantly, by matrix effect, which influences each pesticide in the measurement of each extract differently.

As the response of MS detection can have substantial day-to-day variation the absolute values of residuals ε_i can be used only within day. At the same time the relative unsigned residuals ε_i^r defined according to eq 21

$$\varepsilon_{\mathrm{i}}^{\mathrm{r}} = \frac{\left|\varepsilon_{\mathrm{i}}\right|}{b_0 + b_1 \cdot C_{\mathrm{i}}} \tag{21}$$

are significantly less affected by the system parameters and can be pooled over a long time period (by using eq 21).

Thus the relative residuals resulting from the measurements performed over a long time period are calculated and plotted on a graph – a matrix effect graph. Pooling of data over a long time period enables efficient averaging. This is very important for getting reliable uncertainty estimates as matrix effect is a highly variable phenomenon. The matrix effect graph can be easily used to calculate relative standard uncertainties of the sample peak areas that originate from variations of the LC/ESI/MS system that occur over a several months time period and the matrix effect. Standard uncertainty is uncertainty at standard deviation level, i.e. encompassing roughly 68% of the possible values and is expressed by *u*. This standard uncertainty – the relative standard uncertainty of the sample peak area – is found as the root mean square of the relative residuals and is termed $u^{\mathrm{r}}_{\mathrm{RMS}}$:

$$u^{\mathrm{r}}_{\mathrm{RMS}} = \sqrt{\frac{\sum_{\mathrm{j}=1}^{n} \left(\varepsilon_{\mathrm{j}}^{\mathrm{r}}\right)^2}{n-2}} \tag{22}$$

n is the overall number of measurements (including all days and all matrixes).

Data should be added continuously to the matrix effect graph, for example once a month. In Figure 17 the matrix effect graph*s* obtained over half a year period for thiabendazole, aldicarb, imazalil and methiocarb are presented.

Both time effects and strong outliers can be tracked. Strongly outlying fruits and vegetables for which the matrix effect is significantly larger than the statistically derived $u^{\mathrm{r}}_{\mathrm{RMS}}$ can be identified and studied separately. In our case it was observed, that standards prepared in garlic strongly deviate from the regression line in the case of methiocarb and garlic was identified as the worst-case matrix for methiocarb.

17

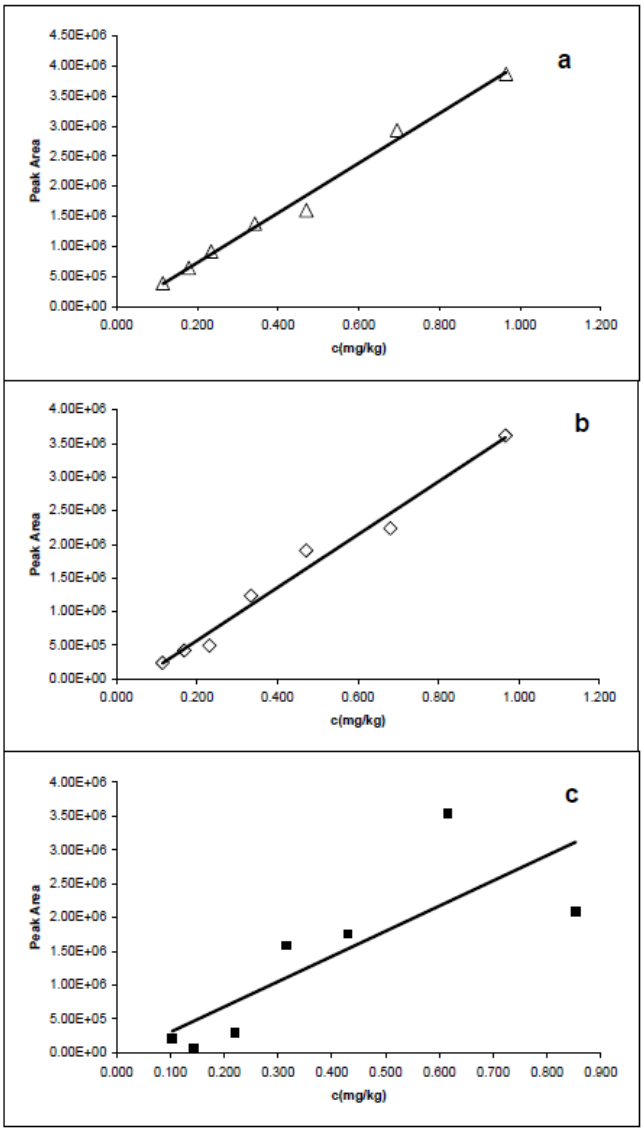

Figure 16 Calibration graphs for aldicarb determination: a) single-day single-matrix calibration (traditional "matrix-matched" calibration) (value of pooled relative residuals 0.07) b) single-day calibration with each concentration level spiked into the extract from a different fruit of the same commodity group (value of pooled relative residuals 0.16) and c) single-day calibration with each concentration level spiked into the extract from a different fruit of a different commodity group (value of pooled relative residuals 0.62).

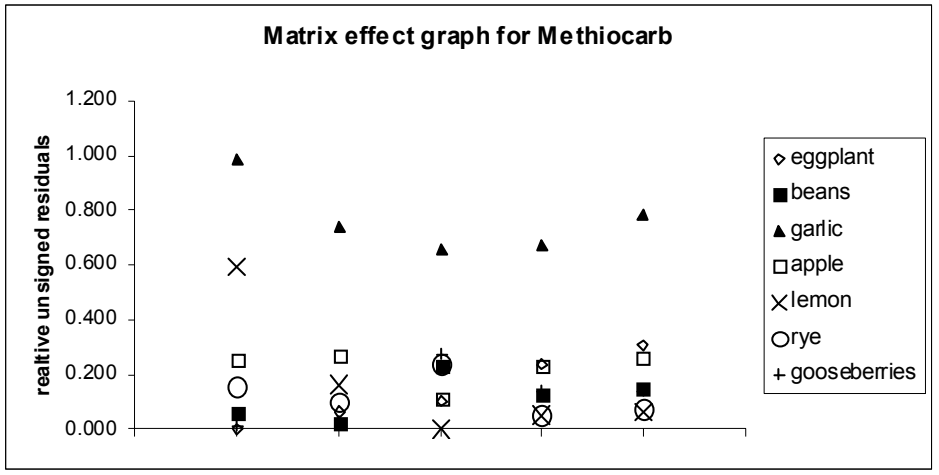

Figure 17 Matrix effect graphs for methiocarb over 5 measurements from data of the different commodity groups (the measurements have been carried out with approximately one month interval).

To obtain the matrix effect graphs measurements were carried out during half a year period using seven extracts. Three measurement cycles were used in the case of the fruiting vegetables group and five cycles for fruits from different commodity groups. The relative residuals were pooled and the calculated u^{r}_{RMS} values were 0.048, 0.281, 0.060 and 0.118 found from the matrix effect graph*s* obtained over fruiting vegetables for thiabendazole, aldicarb, imazalil and methiocarb respectively. The u^{r}_{RMS} values for the same pesticides calculated over different commodity groups were 0.162, 0.419, 0.253 and 0.354 respectively. It can be seen that u^{r}_{RMS} values calculated over different commodity groups are noticeably higher than the ones calculated within fruiting vegetables commodity group for all pesticides. This may be explained with the potentially higher variability of the matrix effect over different commodity groups.

Comparison of the u^{r}_{RMS} and pooled repeatability data reveals that, depending on the pesticide and on the way the u^{r}_{RMS} was calculated, the uncertainty due to the matrix effect makes up different share of u^{r}_{RMS}. For thiabendazole and imazalil the u^{r}_{RMS} value calculated over the fruiting vegetables group has the largest contribution form repeatability. On the other hand, the u^{r}_{RMS} calculated for aldicarb and methiocarb over fruiting vegetables and for all pesticides calculated over different commodity groups is made up mainly from uncertainty arising from the matrix effect (70 to 90% depending on pesticide).

The u^{r}_{RMS} was used as relative uncertainty of the sample peak area according to eq 23. Therefore the uncertainty originating from matrix effect as well as peak area repeatability is included in the uncertainty of sample peak areas.

$$u(A_{\text{Sample}}) = u^{r}_{\text{RMS}} \cdot A_{\text{Sample}} \tag{23}$$

For calculating the uncertainty of c_{sample} (see eq 19) in addition to uncertainty of A_{sample} also the uncertainty due to the calibration graph (i.e. the uncertainties of b_1 and b_0) has to be taken into account. The uncertainty contributions were combined to yield the combined (i.e. taking into account all relevant uncertainty sources) standard uncertainty $u_c(c_{\text{sample}})$ and the contribution percentages of the uncertainty sources were calculated by using the Kragten method [Kragten, 1994]. Recovery was not included as an uncertainty source as all the samples studied were spiked after sample preparation. The aim of this study was to evaluate the uncertainty arising from matrix effect and the uncertainty due to sample preparation is thus not included in the uncertainty budget.

$$u_C(C_{sample}) = \sqrt{\begin{aligned}&\left[\frac{\partial C_{sample}}{\partial A_{sample}} u(A_{sample})\right]^2 + \left[\frac{\partial C_{sample}}{\partial A_1} u(A_1)\right]^2 + \left[\frac{\partial C_{sample}}{\partial A_2} u(A_2)\right]^2 + \ldots + \left[\frac{\partial C_{sample}}{\partial A_n} u(A_n)\right]^2 \\ &+ \left[\frac{\partial C_{sample}}{\partial C_1} u(C_1)\right]^2 + \left[\frac{\partial C_{sample}}{\partial C_2} u(C_2)\right]^2 + \ldots + \left[\frac{\partial C_{sample}}{\partial C_n} u(C_n)\right]^2\end{aligned}} \quad (24)$$

The largest contribution to the uncertainty $u_c(c_{\text{sample}})$ originates from the sample peak area uncertainty. This finding did not depend on the way $u^{\text{r}}_{\text{RMS}}$ was calculated. For $u^{\text{r}}_{\text{RMS}}$ calculated within the fruiting vegetables commodity group sample peak area contribution to the final uncertainty was 79.9% for thiabendazole, 64.3% for aldicarb, 89.7% for imazalil and 65.6% for methiocarb. For $u^{\text{r}}_{\text{RMS}}$ calculated over different commodity groups the contributions of the sample peak uncertainty were higher: 98.1%, 88.1%, 99.4% and 94.5% respectively.

Uncertainties are presented in Table 8 as expanded uncertainties U found as follows:

$$U = k \cdot u_{\text{c}}(c_{\text{sample}}) \quad (25)$$

k is the coverage factor and is in our case equal to 2. This leads to approximately 95% coverage of the U, i.e. ca 95% of the obtained values are expected to lie within the expanded uncertainty interval.

Matrix effect graph approaches were validated by analysing 15 post-extraction samples spiked at 0.5 mg/kg level. The samples were prepared from tomato, cucumber and sweet corn extracts. The analysis results were compared with the spiked concentrations using the E_{n} values.

It can be seen from Table 8 that for all samples but one (out of 120) the spiked (c_{added}) and calculated (c_{sample}) concentrations agree according to E_{n} values, indicating that the uncertainty estimates are realistic or somewhat conservative (with 120 comparisons of uncertainties at roughly 95% coverage, as a statistical average, six could lead to E_{n} values above 1).

The relative standard uncertainties are 5.7% for thiabendazole, 19.4 – 22.4% for aldicarb, 6.8 – 6.9% for imazalil and 17.8 – 18.0% for methiocarb, for $u^{\text{r}}_{\text{RMS}}$ calculated over fruiting vegetables commodity group. The relative standard

uncertainties corresponding to u^{r}_{RMS} calculated over different commodity groups are 17.5 – 17.7%, 35.1 – 38.1%, 27.4 – 27.7% and 44.0 – 46.8%.

For methiocarb the uncertainty, calculated using u^{r}_{RMS} obtained over different commodity groups, is very high and it is questionable if this is applicable even in the case of routine analysis where also uncertainty arising from sample preparation has to be taken into account. As it was mentioned above and can be seen from Figure 17 garlic is the worst case matrix for methiocarb and strongly deviates from the regression line. Therefore the u^{r}_{RMS} values excluding the relative residuals from garlic extract were calculated for comparison. Without the garlic extracts the value of u^{r}_{RMS} for methiocarb would be 0.199 and the relative standard uncertainties would be 26.3 to 27.6%. According to the E_n values all results for methiocarb would still agree with spiked concentrations c_{sample}. In our interpretation this is a case where the second useful feature of the matrix effect graph – the possibility to identify problematic matrixes for possible in-depth study – becomes evident.

3.5 Optimisation of the ESI/MS parameters aiming at minimizing the matrix effect

Finally it became of interest if matrix effect is only controlled by the chemical properties of the sample, mobile phase and analyte or if optimising the ESI ionisation source and mass spectrometer parameters may reduce or even eliminate the matrix effect. Therefore an optimisation procedure for ESI and MS parameters focusing at reducing the matrix effect was developed and evaluated.

The initial MS parameters, used in our laboratory for a long time and established by optimization through standard infusion in solvent, are presented in Table 9. Fragmentation voltages were considered independent of the solvent composition and flow rate and were therefore fixed to 0.46, 0.41 and 0.58 V for thiabendazole, aldicarb and imazalil, respectively, throughout the study.

3.5.1 Optimization of ESI gases

The nebulizer gas pressure, dry gas flow rate and dry gas temperature cannot be optimized via software. The instrument manufacturer suggests choosing values for these parameters according to the eluent composition and flow rate. Therefore two-level full factorial design was used to optimize the gas flow rates. Capillary voltage was included in the optimization of the gas parameters but was afterwards further optimized together with other MS parameters. Previous experiments have shown that only the intensity of aldicarb changes when changing the dry gas temperature (maximum intensity at 350°C). Out of the pesticides under study aldicarb is the one with the lowest sensitivity and with the highest detection limit. Therefore temperature was fixed to 350°C.

Table 8 Concentrations and expanded uncertainties ($k = 2$) found in spiked samples. Results not agreeing according to E_n values are given in bold.

	Thiabendazole					Aldicarb					Imazalil					Methiocarb				
	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n
u_{RMS} within one commodity group																				
cucumber	0.463	0.008	0.514	0.059	0.854	0.516	0.012	0.478	0.208	0.182	0.651	0.012	0.656	0.090	0.058	0.573	0.009	0.681	0.243	0.442
cucumber	0.464	0.008	0.461	0.053	0.049	0.517	0.012	0.494	0.218	0.104	0.652	0.013	0.666	0.091	0.152	0.574	0.009	0.665	0.238	0.384
cucumber	0.456	0.008	0.458	0.052	0.043	0.508	0.012	0.495	0.218	0.063	0.641	0.012	0.662	0.091	0.236	0.564	0.009	0.743	0.265	0.671
cucumber	0.466	0.008	0.463	0.053	0.062	0.519	0.012	0.513	0.229	0.028	0.654	0.013	0.667	0.091	0.134	0.576	0.009	0.736	0.263	0.608
cucumber	0.463	0.008	0.468	0.054	0.101	0.516	0.012	0.488	0.214	0.132	0.650	0.012	0.692	0.095	0.431	0.573	0.009	0.550	0.198	0.113
sweet corn	0.468	0.008	0.491	0.056	0.395	0.522	0.012	0.386	0.154	0.877	0.658	0.013	0.682	0.094	0.262	0.579	0.009	0.595	0.213	0.074
sweet corn	0.470	0.008	0.448	0.051	0.432	**0.524**	**0.012**	**0.358**	**0.139**	**1.184**	0.661	0.013	0.716	0.098	0.562	0.582	0.009	0.567	0.204	0.074
sweet corn	0.463	0.008	0.455	0.052	0.152	0.516	0.012	0.390	0.156	0.808	0.651	0.013	0.644	0.088	0.073	0.573	0.009	0.704	0.251	0.521
sweet corn	0.477	0.008	0.473	0.054	0.068	0.532	0.012	0.443	0.187	0.471	0.670	0.013	0.738	0.101	0.668	0.590	0.009	0.803	0.287	0.740
sweet corn	0.477	0.008	0.491	0.056	0.242	0.532	0.012	0.433	0.181	0.545	0.670	0.013	0.711	0.097	0.410	0.590	0.009	0.700	0.250	0.437
tomato	0.463	0.008	0.433	0.049	0.600	0.516	0.012	0.381	0.151	0.893	0.651	0.013	0.668	0.092	0.184	0.573	0.009	0.637	0.228	0.278
tomato	0.469	0.008	0.477	0.055	0.134	0.523	0.012	0.482	0.210	0.197	0.659	0.013	0.626	0.086	0.383	0.581	0.009	0.817	0.292	0.807
tomato	0.470	0.008	0.463	0.053	0.133	0.524	0.012	0.508	0.226	0.067	0.660	0.013	0.726	0.099	0.657	0.581	0.009	0.745	0.266	0.614
tomato	0.470	0.008	0.466	0.053	0.070	0.524	0.012	0.488	0.214	0.167	0.661	0.013	0.696	0.095	0.366	0.582	0.009	0.806	0.289	0.777
tomato	0.469	0.008	0.476	0.054	0.129	0.523	0.012	0.462	0.198	0.308	0.659	0.013	0.748	0.102	0.861	0.580	0.009	0.737	0.263	0.593

	Thiabendazole					Aldicarb					Imazalil					Methiocarb				
	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n	c_{added}	$U(c_{added})$	C_{found}	$U_c(c_{found})$	E_n
u_{RMS} over different commodity groups																				
cucumber	0.463	0.004	0.514	0.180	0.283	0.516	0.006	0.478	0.358	0.106	0.651	0.006	0.656	0.363	0.015	0.573	0.004	0.681	0.616	0.175
cucumber	0.464	0.004	0.461	0.163	0.016	0.517	0.006	0.494	0.373	0.061	0.652	0.006	0.666	0.368	0.038	0.574	0.004	0.665	0.604	0.151
cucumber	0.456	0.004	0.458	0.162	0.014	0.508	0.006	0.495	0.373	0.037	0.641	0.006	0.662	0.366	0.059	0.564	0.004	0.743	0.662	0.269
cucumber	0.466	0.004	0.463	0.163	0.020	0.519	0.006	0.513	0.391	0.016	0.654	0.006	0.667	0.368	0.033	0.576	0.004	0.736	0.658	0.243
cucumber	0.463	0.004	0.468	0.165	0.033	0.516	0.006	0.488	0.367	0.077	0.650	0.006	0.692	0.381	0.108	0.573	0.004	0.550	0.518	0.043
sweet corn	0.468	0.004	0.491	0.172	0.130	0.522	0.006	0.386	0.272	0.498	0.658	0.006	0.682	0.376	0.066	0.579	0.004	0.595	0.551	0.029
sweet corn	0.470	0.004	0.448	0.158	0.141	0.524	0.006	0.358	0.247	0.670	0.661	0.006	0.716	0.393	0.141	0.582	0.004	0.567	0.530	0.029
sweet corn	0.463	0.004	0.455	0.161	0.050	0.516	0.006	0.390	0.275	0.459	0.651	0.006	0.644	0.357	0.018	0.573	0.004	0.704	0.633	0.207
sweet corn	0.477	0.004	0.473	0.167	0.022	0.532	0.006	0.443	0.325	0.272	0.670	0.006	0.738	0.404	0.168	0.590	0.005	0.803	0.708	0.300
sweet corn	0.477	0.004	0.491	0.172	0.080	0.532	0.006	0.433	0.315	0.313	0.670	0.006	0.711	0.390	0.103	0.590	0.005	0.700	0.630	0.173
tomato	0.463	0.004	0.433	0.153	0.195	0.516	0.006	0.381	0.267	0.507	0.651	0.006	0.668	0.369	0.046	0.573	0.004	0.637	0.583	0.109
tomato	0.469	0.004	0.477	0.168	0.044	0.523	0.006	0.482	0.361	0.115	0.659	0.006	0.626	0.347	0.096	0.581	0.004	0.817	0.719	0.329
tomato	0.470	0.004	0.463	0.163	0.044	0.524	0.006	0.508	0.386	0.039	0.660	0.006	0.726	0.398	0.165	0.581	0.004	0.745	0.664	0.246
tomato	0.470	0.004	0.466	0.164	0.023	0.524	0.006	0.488	0.367	0.097	0.661	0.006	0.696	0.383	0.092	0.582	0.004	0.806	0.711	0.316
tomato	0.469	0.004	0.476	0.168	0.042	0.523	0.006	0.462	0.342	0.179	0.659	0.006	0.748	0.409	0.217	0.580	0.004	0.737	0.658	0.237

Table 9. Optimization range, default values and starting conditions used in this work for the MS parameters. The optimisation order of parameters is as the order in table (See Figure 2 for detailed scheme of ESI/MS used in this study).

Parameter		Range		Starting conditions		
	Default values (Target mass m/z 300)	Min	max	Thiaben-dazole	Aldicarb	Imazalil
Capillary (V)	–3500	–5000	0	–2500	–2500	–2500
Skimmer (V)	40	0	150	44.4	51.6	41.8
Cap Exit (V)	200	0	360	122.9	103.3	122.9
Oct 1 DC (V)	12	2.5	100	15.33	13.85	14.34
Oct 2 DC (V)	2.5	0	12	1.18	2.1	1.7
Trap Drive	78	–60	60	33.7	33.9	42.4
Oct RF (Vpp)	200	0	300	63.9	78.7	93.4
Lens 1 (V)	–5	–25	5	–4.8	–4.3	–5.3
Lens 2 (V)	–60	–100	0	–73.4	–89.7	–63.1

The parameters taken for optimization were nebulizer gas pressure, dry gas flow rate and capillary voltage. The parameter levels were 40 and 50 psi (276 and 345 kPa), 8 and 10 l/min, 1500 and 2500 V, respectively. The optimization was carried out with both pesticide standard and spiked garlic sample with a concentration of 0.5 mg/kg. In order to effectively study the influence of the ESI/MS parameters on both sensitivity and matrix effect a sample with strong ionization suppression was used. A garlic extract – obtained by buffered QuEChERS method (Section 2.3.1) – was used as an example of a worst-case matrix. The same sample was used throughout the study.

The results of optimization of the parameters of the ESI gases are presented in Table 10. Analyses of variance (ANOVA) was carried out with these data (Table 10). It was observed that nebulizer gas flow rate, was statistically significantly influencing the sensitivity for aldicarb in both standard and sample. In case of imazalil statistically significant influence of nebulizer gas on the results was observed only in sample. Dry gas flow rate was statistically significantly influencing the sensitivity for thiabendazole and aldicarb in standard. Capillary voltage was statistically significantly influencing the signal intensity for thiabendazole in standard and imazalil in samples.

Table 10 The peak areas for standard and sample (0.5 mg/kg) and matrix effect values while optimizing of the gas flow rates. Other parameters were kept at default values. The temperature of the dry gas was fixed to 350°C.

			Standard Peak Areas			Sample Peak Areas			Matrix effect (%)		
Nebulizer (psi)	Dry Gas (l/min)	Capillary (V)	Thiaben-dazole	Aldicarb	Imazalil	Thiaben-dazole	Aldicarb	Imazalil	Thiaben-dazole	Aldicarb	Imazalil
40	8	1500	1.66E+08	7.14E+05	3.71E+07	9.63E+07	3.57E+05	1.42E+07	58%	50%	38%
40	8	2500	1.28E+08	8.45E+05	2.94E+07	7.61E+07	4.43E+05	1.56E+07	59%	52%	53%
40	10	1500	2.04E+08	1.05E+06	3.26E+07	9.87E+07	3.64E+05	1.30E+07	48%	35%	40%
40	10	2500	1.67E+08	1.04E+06	4.68E+07	9.28E+07	3.95E+05	2.08E+07	56%	38%	44%
50	8	1500	1.75E+08	8.99E+05	2.96E+07	9.11E+07	2.98E+05	1.13E+07	52%	33%	38%
50	8	2500	1.39E+08	8.73E+05	3.96E+07	8.06E+07	3.41E+05	1.50E+07	58%	39%	38%
50	10	1500	2.00E+08	1.17E+06	3.04E+07	9.63E+07	3.38E+05	1.33E+07	48%	29%	44%
50	10	2500	1.71E+08	1.15E+06	4.55E+07	9.87E+07	3.19E+05	1.50E+07	58%	28%	33%

For example for thiabendazole in standard the intensity is 56% higher for parameter set of 50 psi nebulizer gas, 10 l/min dry gas and capillary voltage 1500 V compared to 40 psi nebulizer gas, 8 l/min dry gas and capillary voltage 2500 V.

Parameter interactions were also estimated with ANOVA analyses. It was found that for thiabendazole and aldicarb in sample both nebulizer gas pressure and capillary voltage as well as dry gas flow rate and capillary voltage combinations statistically significantly influence the sensitivity. Therefore it can be concluded that the true performance optimum with respect to the ESI parameters can not be found according to the usual one-parameter-at-time optimization procedure, the combinations have to be studied and evaluated as well.

From the sensitivity – maximum signal – point of view the optimal conditions for the standard and the sample were somewhat similar. For thiabendazole and imazalil the optimal gas flow rates for analyzing sample were nebulizer gas 50 psi and dry gas 10 l/min. For aldicarb the optimal nebulizer gas pressure and dry gas flow rate were 40 psi and 10 l/min respectively for standard but 50 psi and 10 l/min for sample. For each parameter combination the matrix effect *%ME* (eq 7) was calculated. Optimal gas flow rates from the matrix effects point of view agreed well with the optima giving highest sensitivity for sample analyses.

The optimal capillary voltages were 1500 V for thiabendazole and 2500 V for imazalil for both standard and sample. These differences between analytes can be due to the changes in eluent composition corresponding to the elution time of different analytes.

3.5.2 Optimization of MS parameters

Optimization of MS parameters was carried out via four different procedures A, B, C and D.

Procedure A was a modification of the conventional manufacturer-recommended standard infusion in combination with parameter ramping provided by the software. For infusion a chromatographic effluent at the pesticide retention time (0.5 mg/kg standard injection) was used. The infusion rate of the effluent was 0.1 ml/h (0.00167 ml/min). In this way the solvent composition corresponded to the solvent in which the pesticide normally reaches the ionization source after chromatography.

Procedure B was the modification of the procedure A to additionally simulate the flow rate of the solvent reaching the ionization source. Therefore a tee-piece was used to mix the chromatographic solvent (0.8 ml/min), with the composition corresponding in the case of each pesticide to the effluent at the retention time of that pesticide, and the pesticide standard solution (3 mg/kg 0.1 ml/h). The analyte concentration in the mixed solvent was chosen to be approximately equal to the average analyte concentration in the effluent at the peak retention time (corresponding to 0.5 mg/kg standard injection). The flow

rate of the standard solution was negligible compared to the eluent flow rate and did not change the eluent composition markedly.

A modification of Procedure B – a **Procedure C** – was proposed in order to try to account for the matrix effect occurring in samples during chromatographic analyses. The standard solution in Procedure B was replaced with a sample (spiked garlic extract) solution to imitate matrix effect conditions. Even though in real chromatographic analysis only a fraction of the matrix compounds co-elute with any particular analyte the entire sample was used for two reasons. First it is technically complicated to separate the sufficient amount of the co-eluting compounds. Secondly, using a full sample increases the number of compounds that reach ionization source together with the analyte and therefore the ionization suppression for the full sample cannot be smaller and is also usable as a solution causing matrix effect.

Procedures A, B and C were repeated on 3 different days and the found optima agreed within 10% or better. The optima found are presented in Table 11.

All optima were saved in LC/ESI/MS methods and standards as well as samples (spiked garlic extracts) with pesticides at concentration 0.5 mg/kg were analyzed by using these methods. The efficiency of these optimization methods was evaluated by comparing pesticide peak areas for each LC/ESI/MS method. Results are presented in Table 11.

It was found that the parameter values obtained with procedure B – where analyte standard was pumped to the ESI source with the flow rate of the chromatographic effluent – resulted in significantly higher peak areas for all pesticides in both standards and samples than the optima found with procedures A and C. The optima found with procedure B gave almost 10, 2 and 2.5 times higher ionization efficiencies compared to optima found with procedure A for thiabendazole, aldicarb and imazalil, respectively. Comparing procedures B and C gave unexpected results. It was of interest if the presence of matrix – procedure C – helps to find the true optimum. It was found that under chromatographic conditions the parameters obtained by procedure B gave 2 (aldicarb in sample) to 1.2 (thiabendazole in sample and standard) higher sensitivity compared to procedure C. This tendency was found to be well repeatable from day to day. Therefore the proposed procedure C can not be used to find the parameter values giving the highest sensitivity in matrix effect conditions.

Table 11 The optimal values found with optimization procedures A, B and C (nebulizer pressure 50 psi, drying gas flow rate 10 l/min and temperature 350°C)

	Thiabendazole			Aldicarb			Imazalil		
Procedure	A	B	C	A	B	C	A	B	C
Capillary (V)	–3270.49	–1639.34	–1885.25	–4352.46	–1885.25	–3278.69	–3163.93	–2377.05	–2704.92
Skimmer (V)	41.80	56.56	76.34	41.80	56.56	39.34	51.64	46.72	49.18
Cap Exit (V)	157.38	91.48	104.75	98.36	44.26	0.00	157.38	89.02	0.00
Oct 1DC (V)	15.82	11.11	19.60	15.82	13.08	14.93	17.30	13.30	12.89
Oct 2DC (V)	1.11	1.20	0.00	3.54	3.87	3.42	1.64	1.57	1.90
Trap Drive	32.88	36.14	34.34	35.46	34.65	36.74	44.50	41.68	41.24
Oct RF (Vpp)	83.61	68.85	85.52	83.61	68.85	108.20	157.38	78.69	68.85
Lens 1 (V)	–4.84	–5.82	–6.31	–7.30	–6.80	–16.15	–6.31	–4.84	–7.30
Lens 2 (V)	–64.59	–75.41	–100.00	–82.30	–100.00	–81.97	–80.82	–98.36	–88.52
Standard peak area	1.13E+06	9.69E+06	7.78E+06	2.26E+06	4.44E+06	2.18E+06	6.60E+07	1.71E+08	1.07E+08
Sample peak area	5.76E+05	4.82E+06	3.88E+06	3.87E+05	5.08E+05	3.11E+05	9.86E+06	1.36E+07	7.95E+06
Matrix effect	50.3%	49.8%	49.8%	17.2%	11.4%	14.3%	14.9%	8.0%	7.4%

The parameter set providing highest sensitivity (procedure B) was taken for further validation with a **Procedure D** a two-level full factorial design with the center-point under chromatographic conditions. The parameter set obtained by procedure B was taken as the center point. The parameters taken into the design were chosen according to the signal versus parameter value graph provided by the software during software-based optimization via procedure B. The four most influential parameters, while changing the parameter value ±25% from the optima, were taken for further study with procedure D. These parameters were capillary voltage, direct current of the first octopole, trap drive and second lens voltage. The parameter levels were chosen to cause approximately ±25% intensity change on the signal versus parameter value graph provided by procedure B.

Results from factorial design – procedure D – confirmed that the optima found by procedure B were the true optima also for the chromatographic conditions for standards from the sensitivity point of view. A different situation was observed for samples. It can be seen on the example of aldicarb in Figure 18 that the values found with optimization procedure B, though giving better results than A or C, do not lead to the highest sensitivity. Similar situations were found for thiabendazole and imazalil as well. Therefore it can be concluded that the classical MS software based optimization procedures are unable to effectively account for the interactions between different MS parameters and multilevel experimental designs should be used to find the true optima for the sample analyses.

For each combination of factors it was also possible to calculate the matrix effects. Similarly to ESI parameters, described above, it was found that parameters giving higher intensity for the standard solution do not necessarily give lower ionization suppression (matrix effect). In fact reverse tendencies for all pesticides and almost all parameters were found. Working at conditions away from optimal sensitivity values can decrease ionization suppression by more than 3 times (*%ME* change from 7% to 23% found for imazalil).

Therefore it can be concluded that parameters giving the highest sensitivity do not match with the parameters providing the lowest ionization suppression. As the parameters can not be found with normal optimization procedures procedure D – a factorial design – should be used for this purpose.

As it can be seen from these results, the matrix effect value depends on the MS parameters. Therefore, contrary to the popular viewpoint, not only the ionization process determines the extent of matrix effect, but also by ion transport in the MS.

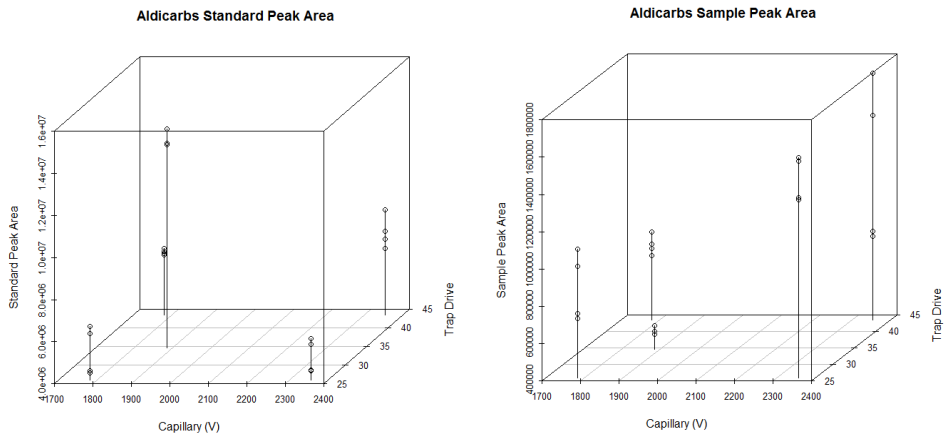

Figure 18. Aldicarb peak areas in standards and samples for different Capillary voltages and Tarp Drive values. In the center point other parameters were kept constant while at corner points Octopole 1 DC and Lens 2 V were changed as well.

SUMMARY

The aim of this work was to study the properties of the matrix effect in LC/ESI/MS analysis, to develop and evaluate approaches to combat the matrix effect as well as evaluate such approaches proposed in the literature. This work is carried out on the example determination of polar pesticides in fruits and vegetables.

More efficient sample preparation, dilution of the sample (especially if realized as extrapolative dilution), as well as optimisation of the ESI/MS parameters were found to be useful as means of matrix effect reduction. Post-column standard infusion, isotope dilution, using background spectra and the *echo*-peak technique were tested to account for the matrix effect. The results indicate that post-column standard infusion as well as internal standard method are not reliable methods for taking the matrix effect into account due to its complex nature and possible mismatch of analyte and standard properties. The *echo*-peak technique was found to be of limited use – only if gradient elution is not required. The approaches based on extrapolative dilution and usage of the background spectra were found useful in taking matrix effects into account.

From the matrix effects reduction point of view the focus was on evaluating different sample preparation methods. The classical liquid-liquid extraction, liquid-liquid extraction with dispersive post extraction clean-up (QuEChERS) and matrix solid-phase dispersion (MSPD) were tested.

Even though methods for accounting for the ionisation suppression have been applied before, sensitivity of the method decreases and the detection limits become higher in the presence of matrix effect. Therefore a combination of reduction and accounting for the matrix effect – the extrapolative dilution method – has been studied and validated within this work. This method allows following the reduction of the matrix effect by using the analyte concentration vs dilution factor plot. In cases where matrix effect cannot be eliminated with dilution analyte concentration can be calculated with extrapolation to the zero dilution factor.

In case of applications where low uncertainty is not critical the matrix effect does not need to be fully accounted for but can be incorporated into the uncertainty estimate. Therefore a within-commodity-group calibration together with the fruit-to-fruit matrix effect uncertainty calculation has been elaborated during this study.

Also, it was demonstrated that background ions from MS^1 spectra can be employed to correct for the matrix effects.

The matrix effect has for a long time been treated only as a problem of co-eluting compounds and the possible reduction of matrix effect via optimisation of ESI and MS parameters has usually not been considered. In this work different ESI and MS parameter optimisation methods were tested to find a combination of ESI and MS parameters giving the lowest matrix effect. It was found that different optimisation methods lead to different optima.

In this work some insight into the possibilities to combat matrix effect was given by means of reducing the matrix effect, accounting for the matrix effect as well as optimising the ESI/MS parameters according to the lowest ionisation suppression. As a result of this work it was shown that matrix effect can be reduced or accounted for to some extent.

SUMMARY IN ESTONIAN

"Maatriksefektid vedelikkromatograafilisel massispektromeetrilisel analüüsil elektropihustus-ionisatsiooniallikas"

Vedelikkromatograafilisel (LC) massispektromeetrilisel (MS) analüüsil kasutatavas elektropihustus-ionisatsiooniallikas (ESI) esinevaid analüütide ionisatsiooniefektiivsuse muutusi, mis on tingitud analüüdiga koos elueeruvatest ühenditest, nimetatakse maatriksefektideks. Käesoleva töö eesmärgiks oli tuua selgust maatriksefektide olemusse ja töötada välja ning hinnata meetodeid maatriksefekti vähendamiseks või arvesse võtmiseks.

Maatriksefekti vähendamise strateegiad võib suures plaanis jagada kolmeks: proovi-ettevalmistamise parendamine, massispektromeetri parameetrite sobiv optimeerimine ja maatriksefekte arvesse võttev andmetöötlus. Leiti, et erinevad proovi-ettevalmistusmeetodid annavad küllaltki erineva ulatusega maatriksefekte. Samuti on sama prooviettevalmistuse juures maatriksefektid erinevatele analüütidele erinevad. See on tingitud nii analüütide erinevatest füüsiko-keemilistest omadustest kui ka erinevatest analüütidega kaasaelueeruvatest ühenditest.

Kasutades erinevaid optimeerimismeetodeid ESI/MS parameetite optimeerimiseks leiti, et saadud parameetrite optimumid on küllaltki erienevad lähtuvalt optimeerimisel kasutatud eluendi koostisest ja voolukiirusest. Leiti, et kõrgeim tundlikkus saavutatakse, kui eluent vastab enim kromatografilisele efluendile. Samas selgus, et erinevate parameetrite (nii ESI kui ka MS) väärtuste juures saadakse ka erinevad maatriksefekti väärtused, kusjuures vähima maatriksefekti andsid parameetrite väärtused, mis ei vastanud kõrgeimale tundlikkusele. Seega õnnestus näidata, et osa maatriksefektist tekib paratamatult ka MS sees, mitte ainult ESI allikas.

Testiti erinevaid tuntud meetodeid maatriksefekti arvesse võtmiseks – lisamismeetodit, sisestandardimeetodit, kolonnijärgset standardi lisamist, isotooplahjendust, *echo*-piikide tehnikat. Erinevad meetodid osutusid andma erinevate analüütide jaoks erinevat täpsust. Seejuures ei olnud ükski meetod rakendatav kõigil juhtudel.

Uudse meetodina töötati välja ekstrapoleeriva lahjenduse meetod, mis samaaegselt nii vähendab maatriksefekti kui ka võtab seda arvesse. Valideerimise käigus leiti, et ekstrapolatiivne lahjendus töötab efektiivselt nii erinevate analüütide kui ka erinevate maatriksite jaoks. Samas, et ektrapolatiivse lahjenduse meetod tõstab märgatavalt analüüsi töömahukust ning ühe proovi analüüsimiseks kuluvat aega.

Seetõttu osutus huvipakkuvaks, kas ja kuidas saaks maatriksefekti (töömahuka) vähendamise asemel kaasata seda ühe komponendina analüüsitulemuse määramatuse arvutusse. Selleks töötati välja niinimetatud "maatriksefekti graafiku" lähenemine. Selle lähenemise järgi teostatakse kalibreerimine valmistades iga kalibreerimispunkti lahuse erineva maatriksi ekstraktis. Saadud kalibreerimisgraafiku hälbeid kasutatakse iseloomustamaks maatriksefekti poolt

põhjustatud varieeruvust ning nende keskmistamisel leitakse maatriksefekti arvesse võttev komponent määramatuse arvutuse jaoks. See lähenemine valideeriti 4 analüüdi jaoks 3 maatriksi viies sordis.

Samuti uuriti ning tõestati võimalus maatriksefekti arvesse võtta läbi MS taustaspektris esinevate ioonide intensiivsuste kasutades osalist vähimruutude meetodit.

Seega käesoleva töö tulemusena on uuritud maatriksefekti omadusi nii analüüdist, proovi ettevalmistusest kui ka maatriksist lähtuvalt ning välja töötatud kaks uut meetodit maatriksefekti käsitlemiseks LC-MS analüüsil: (1) uus meetod maatriksefekti arvestamiseks – "ekstrapoleeriv lahjendamine" – ning (2) "maatriksefekti graafiku" lähenemine maatriksefekti kaasamiseks määramatuse arvutusse.

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8. Work experience
   January 2007 – ... – University of Tartu
   October – December 2007, University of Helsinki
   July – August 2008, University of Helsinki
   Summer 2006 – MAAG Dairy Industry, chemist

II. Science

1. Main research areas
   LC-ESI-MS, LC-UV, pesticide analysis, sample preparation, ESI-MS matrix effects and ionization efficiency, statistical data analysis
2. Publications
   A. Kruve, M. Haapala, V. Saarela, S. Franssila, R. Kostiainen, T. Kotiaho "Feasibility of Capillary Liquid Chromatography – Microchip-Atmospheric Pressure Photoionization – Mass Spectrometry for Pesticide Analysis in Tomato" *Analytica Chimica Acta* In press

A. Kruve, K. Herodes, I. Leito "Partial least squares regression as a tool to account for matrix effect in pesticides LC/ESI/MS analyses via background spectra" *Rapid Commun. Mass Spectrom.* 25 (2011) 1159

I. Leito, A. Kruve ""Measurement Science in Chemistry" consortium – a new force in analytical chemistry higher education in Europe" *Anal. Bioanal Chem.* 397 (2010) 1635

A. Kruve, K. Herodes, I. Leito "Electrospray ionization matrix effect as an uncertainty source in LC/ESI/MS pesticide analysis" *J .AOAC*, 93 (2010) 306

A. Kruve, K. Herodes, I. Leito "Optimization of electrospray interface and quadrupole ion trap mass spectrometer parameters in pesticide liquid chromatography/ electrospray ionization mass spectrometry analysis" *Rapid Commun. Mass Spectrom.* 24 (2010) 919

A. Kruve, K. Herodes, I. Leito "Extrapolative dilution as an effective quantization method to account for matrix effects in pesticides LC/ESI/MS analyses" *Anal. Chim. Acta* 651 (2009) 75

A. Kruve, A. Künnapas, K. Herodes, I. Leito "Matrix effects in pesticide multi-residue analysis by liquid chromatography-mass spectrometry" *J. Chrom. A* 1187 (2008) 58

M. Oss, A. Kruve, K. Herodes, I. Leito "Electrospray Ionization Efficiency Scale of Organic Compounds" *Anal. Chem.* 82 (2010) 2865

I. Leito, K. Herodes, M. Huopolainen, K. Virro, A. Künnapas, A. Kruve, R. Tanner "Towards the electrospray ionization mass spectrometry ionization efficiency scale of organic compounds" *Rapid Commun. Mass Spectrom.* 22 (2008) 379

A. Kruve, A. Lamos, J. Kirillova, K. Herodes "Pesticide residues in commercially available oranges and evaluation of potential washing methods", *Proceedings of Estonian Academy of Sciences. Chemistry* 56 (2007) 134

A. Vaarmann, A. Kruve, A. Zharkovsky "Vesicular glutamate transporter and glutamic acid decarboxylase transcription levels are altered in the brain of cystatin B-deficient mice", *Journal of Neurochemistry* 101 (2007) 66

3. Scholarships and awards:

December 2006 – Estonian-Rotalia Academic Fund, Including. Scholarship

4. Other activities:

1th Baltic MS conference, Palanga, Latvia, 2007, oral presentation

International Conference on Metrology of Environmental, Food and Nutritional Measurements 2008, Budapest, poster presentation

Internship in Agilent facility in Waldbronn (2-day) in February, 2009

5th Conference by Nordic Separation Science Society, Tallinn, Estonia, 2009, oral presentation

International Mass Spectrometry Conference 2009, Bremen, Germany, oral presentation

Instructing students in a project in Viimsi high school, "School as a science laboratory"

14th Nordic MS Conference, 2010 Uppsala, poster presentation

Bayer Science and Eduation, 2010 Lindau Germany, poster presentation

5. Teaching activities:

Analytical Chemistry I
Analytical Chemistry Seminar I
Laboratory Course of Analytical Chemistry I
Laboratory Course of Analytical Chemistry II

Advanced Chromatography
Statistics in Analytical Chemistry
Statistics in Analytical Chemistry II

6. Supervising:

Ragne Auling (MSc, 2009) "Development of the LC-MS method for determination of glyphosate residues in wheat"

Asko Laaniste (BSc, 2010) "The preparation of glycidyl methacrylate and ethylene dimethacrylate-based monolithic columns for HPLC"

Hanno Evard (BSc, 2010) "Packing normal phase columns for liquid chromatography"

Gert Suurkuusk (MSc, 2010) "Validation of the gas chromatographic method for THC, CBD and CBN determination"

Kerli Lauk "(MSc, 2010) "Statistical methods for evaluation of data from interlaboratory comparison with small number of participants"

III. Specialized training

Introduction for Sample Preparation (for chromatography). Lectors: Harold M. McNair, Sandy Fuchs. Tartu, Estonia. 2007

Waters LC/MS training course "Mass Spectrometry", 2008, Helsinki, Finland

Measurement Science in Chemistry Summer School 2008, Celje, Slovenia

International Conference on Metrology of Environmental, Food and Nutritional Measurements 2008, Budapest, Hungary

6th Workshop on Proficiency Testing in Analytical Chemistry, Microbiology and Laboratory Medicine: Current Practice and Future Directions 2008, Rome, Italy

Internship in Agilent facility in Waldbronn (2-day) in February, 2009.

IV Other interests

Driver's license for category B, blood donor, sports (cross-country skiing, running), reading, travelling.

CURRICULUM VITAE

I Üldine

1. Ees- ja perekonnanimi: Anneli Kruve
2. Sünni aeg ja koht: 26.05.1985 Rakvere, Estonia
3. Kodakontsus: Estonian
4. Aadress, telefon, e-mail: Riia 11–10, Tartu 51010, Estonia
   +37253499807 anneli.kruve@ut.ee
5. Töökoht: Jaanuar 2007 – praeguseni, Tartu Ülikool, keemik
6. Haridus:
   - September 2007 – praeguseni – Tartu Ülikool, doktorantuur
   - September – December 2007 – Helsingi Ülikool (keemia), vahetusüliõpilane
   - 2006–2007 – Tartu Ülikool, MSc (analüütiline ja füüsikaline keemia), *cum laude*
   - 2003–2006 – Tartu Ülikool, BSc (keemia), *cum laude*

7. Keeled:
   Eesti keel – emakeel
   Inglise keel – väga hea
   Soome keel – kesktase
   Vene keel – kesktase
   Saksa keel – algtase
   Rootsi keel – algtase

8. Töökogemus
   Jaanuar 2007 – ... – Tartu Ülikool
   Oktoober – Detsember 2007, Helsingi Ülikool
   Juuli – August 2008, Helsingi Ülikool
   Suvi 2006 – MAAG Piimatööstus, keemik

II Teadustöö

1. Peamised uurimisvaldkonnad
   LC-ESI-MS, LC-UV, pestitsiidide analüüs, prooviettevalmistus, ESI-MS maatriksefektid ja ionisatsiooniefektiivsus, kemomeetria
2. Publikatsioonid
   A. Kruve, M. Haapala, V. Saarela, S. Franssila, R. Kostiainen, T. Kotiaho "Feasibility of Capillary Liquid Chromatography - Microchip-Atmospheric Pressure Photoionization – Mass Spectrometry for Pesticide Analysis in Tomato" *Analytica Chimica Acta* In press
   A. Kruve, K. Herodes, I. Leito "Partial least squares regression as a tool to account for matrix effect in pesticides LC/ESI/MS analyses via background spectra" Rapid Commun Mass 25 (2011) 1159

I. Leito, A. Kruve ""Measurement Science in Chemistry" consortium – a new force in analytical chemistry higher education in Europe" *Anal. Bioanal Chem.* 397 (2010) 1635

A. Kruve, K. Herodes, I. Leito "Electrospray ionization matrix effect as an uncertainty source in LC/ESI/MS pesticide analysis" *J AOAC*, 93 (2010) 306

A. Kruve, K. Herodes, I. Leito "Optimization of electrospray interface and quadrupole ion trap mass spectrometer parameters in pesticide liquid chromatography/electrospray ionization mass spectrometry analysis" *Rapid Commun Mass* 24 (2010) 919

A. Kruve, K. Herodes, I. Leito "Extrapolative dilution as an effective quantization method to account for matrix effects in pesticides LC/ESI/MS analyses" *Analytica Chimica Acta* 651 (2009) 75

A. Kruve, A. Künnapas, K. Herodes, I. Leito "Matrix effects in pesticide multiresidue analysis by liquid chromatography-mass spectrometry" *J. Chrom. A* 1187 (2008) 58

M. Oss, A. Kruve, K. Herodes, I. Leito "Electrospray Ionization Efficiency Scale of Organic Compounds" *Anal. Chem.* 82 (2010) 2865

I. Leito, K. Herodes, M. Huopolainen, K. Virro, A. Künnapas, A. Kruve, R. Tanner "Towards the electrospray ionization mass spectrometry ionization efficiency scale of organic compounds" *Rapid Commun. Mass Spectrom.* 22 (2008) 379

A. Kruve, A. Lamos, J. Kirillova, K. Herodes "Pesticide residues in commercially available oranges and evaluation of potential washing methods", *Proceedings of Estonian Academy of Sciences. Chemistry* 56 (2007) 134

A. Vaarmann, A. Kruve, A. Zharkovsky "Vesicular glutamate transporter and glutamic acid decarboxylase transcription levels are altered in the brain of cystatin B-deficient mice", *Journal of Neurochemistry* 101 (2007) 66

3. Stipediumid ja auhinnad:

Detsember 2006 – Estonian-Rotalia Academic Fund, koos stipendiumiga

4. Konverentsiettekanded:

1th Baltic MS conference, Palanga, Latvia, 2007, suuline ettekanne

International Conference on Metrology of Environmental, Food and Nutritional Measurements 2008, Budapest, poster ettekanne

Internship in Agilent facility in Waldbronn (2-day) in February, 2009

5th Conference by Nordic Separation Science Society, Tallinn, Estonia, 2009, suuline ettekanne

International Mass Spectrometry Conference 2009, Bremen, Germany, suuline ettekanne

Instructing students in a project in Viimsi high school, "School as a science laboratory"

14th Nordic MS Conference, 2010 Uppsala, poster ettekanne

Bayer Science and Eduation, 2010 Lindau Germany, poster etekanne

5. Õppetöö:

Analüütiline keemia I
Analüütilise keemia seminar I
Analüütilise keemia praktikum I
Analüütilise keemia praktikum II
Kromatorgraafia ja massispektromeetria

Statistika analüütilises keemias
Statistika analüütilises keemias II

6. Juhendamised:
   Ragne Auling (MSc, 2009) "Development of the LC-MS method for determination of glyphosate residues in wheat"
   Asko Laaniste (BSc, 2010) "Glütsidüülmetakrülaadi ja etüleendimetakrülaadi baasil monoliitsete vedelikkromatograafia kolonnide valmistamine"
   Hanno Evard "Normaalfaas vedelikkromatograafiliste täidiskolonnide valmistamine"
   Gert Suurkuusk (MSc, 2010) "Validation of the gas chromatographic method for THC, CBD and CBN determination"
   Kerli Lauk "(MSc, 2010) "Statistical methods for evaluation of data from inter-laboratory comparison with small number of participants"

III. Koolitused

"Introduction for Sample Preparation (for chromatography)" Harold M. McNair, Sandy Fuchs, Tartu, Eesti, 2007
Waters LC/MS treeningkursus "Mass Spectrometry", 2008, Helsingi, Soome
Measurement Science in Chemistry Suvekool 2008, Celje, Slovenia
International Conference on Metrology of Environmental, Food and Nutritional Measurements 2008, Budapest, Ungar
6th Workshop on Proficiency Testing in Analytical Chemistry, Microbiology and Laboratory Medicine: Current Practice and Future Directions 2008, Rooma, Itaalia
Agilendiga koostöö Waldbronnis Saksamaa (2-päeva) veebruar, 2009.

IV Muud huvid

Juhiload (B kategooria), doonor, sports (jooksmine, murdmaasuusatamine), lugemine, reisimine.

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS

1. **Toomas Tamm.** Quantum-chemical simulation of solvent effects. Tartu, 1993, 110 p.
2. **Peeter Burk.** Theoretical study of gas-phase acid-base equilibria. Tartu, 1994, 96 p.
3. **Victor Lobanov.** Quantitative structure-property relationships in large descriptor spaces. Tartu, 1995, 135 p.
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55. **Eve Koort.** Uncertainty estimation of potentiometrically measured ph and pK_a values. Tartu, 2006, 139 p.
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68. **Aime Lust.** Charge state of dopants and ordered clusters formation in CaF_2:Mn and CaF_2:Eu luminophors. Tartu, 2007, 100 p.
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70. **Mari Reinik.** Nitrates, nitrites, N-nitrosamines and polycyclic aromatic hydrocarbons in food: analytical methods, occurrence and dietary intake. Tartu, 2007, 172 p.
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72. **Erki Enkvist.** Synthesis of adenosine-peptide conjugates for biological applications. Tartu, 2007, 114 p.
73. **Svetoslav Hristov Slavov**. Biomedical applications of the QSAR approach. Tartu, 2007, 146 p.
74. **Eneli Härk.** Electroreduction of complex cations on electrochemically polished Bi(*hkl*) single crystal electrodes. Tartu, 2008, 158 p.
75. **Priit Möller.** Electrochemical characteristics of some cathodes for medium temperature solid oxide fuel cells, synthesized by solid state reaction technique. Tartu, 2008, 90 p.
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77. **Ave Sarapuu.** Electrochemical reduction of oxygen on quinone-modified carbon electrodes and on thin films of platinum and gold. Tartu, 2008, 134 p.
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100. **Darja Lavõgina.** Development of protein kinase inhibitors based on adenosine analogue-oligoarginine conjugates. Tartu 2010, 248 p.
101. **Laura Herm.** Biochemistry of dopamine D_2 receptors and its association with motivated behaviour. Tartu 2010, 156 p.
102. **Terje Raudsepp.** Influence of dopant anions on the electrochemical properties of polypyrrole films. Tartu 2010, 112 p.
103. **Margus Marandi.** Electroformation of Polypyrrole Films: *In-situ* AFM and STM Study. Tartu 2011, 116 p.
104. **Kairi Kivirand.** Diamine oxidase-based biosensors: construction and working principles. Tartu, 2011, 140 p.