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Comparison and optimisation of novel mass spectrometry ionisation sources

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Hiiele,
kes on mind alati vankumatult toetanud, kuid piisavalt arvustanud.

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II **Laaniste, A.**; Leito, I.; Rebane, R.; Lõhmus, R.; Lõhmus, A.; Punga, F.; Kruve, A. **Determination of neonicotinoids in Estonian honey by liquid chromatography-electrospray mass spectrometry**, *Journal of Environmental Science and Health Part B,* **2016**, 51(7), 455–464.

III **Laaniste, A.**; Leito, I.; Kruve, A. **Comparison of different ionization sources for the LC/MS analysis of pesticides**, submitted for publication in *Journal of American Society of Mass Spectrometry*.

IV **Laaniste, A.**; Kruve, A.; Leito, I. **Ensuring repeatability and robustness of poly(glycidyl methacrylate-co-ethylene dimethacrylate) HPLC monolithic columns of 3 mm id through covalent bonding to the column wall**, *Journal of Separation Science*, **2013**, 36, 2458–2463.

V **Laaniste, A.**; Marechal, A.; El-Debs, R.; Randon, J.; Dugas, V.; Demesmay, C. **"Thiol-ene" photoclick chemistry as a rapid and localizable functionalization pathway for silica capillary monolithic columns**, *Journal of Chromatography A*, **2014**, 1355, 296–300.

VI Marechal, A.; **Laaniste, A.**; El-Debs, R.; Dugas, V.; Demesmay, C. **Versatile ene-thiol photoclick reaction for preparation of multimodal monolithic silica capillary columns**, *Journal of Chromatography A*, **2014**, 1365, 140–147.

Author's contribution

Paper I: Participated in analysis of the results and writing of the text.

Paper II: Main person responsible for planning and writing the manuscript. Performed all the experimental work.

Paper III: Main person responsible for planning and writing the manuscript. Performed all the experimental work.

Paper IV: Main person responsible for planning and writing the manuscript. Performed all the experimental work.

Paper V: Performed all of the experimental work and participated in writing the manuscript.

Paper VI: Performed some of the experimental work.

ABBREVATIONS

APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionisation
APPI	Atmospheric pressure photoionisation
CRM	Charge residue model
DA-APPI	Dopant–assisted atmospheric pressure photoionisation
DESI	Desorption electrospray ionisation
EESI	Extractive electrospray ionisation
EERC	Estonian Environmental Research Centre
EI	Electron ionisation
ESI	Electrospray ionisation
GLM	General linear model
GM	Geometric mean
HESI	Heated electrospray ionisation
HPLC	High performance liquid chromatography
ID	Internal diameter
IEM	Ion evaporation model
LC	Liquid chromatography
LC/MS	Liquid chromatography/mass spectrometry
LoD	Limit of detection
LoQ	Limit of quantification
$\log P_{o/w}$	Logarithm of octanol-water partition coefficient
MALDI	Matrix-assisted laser desorption ionisation
ME	Matrix effects
MMI	Multimode ionisation
MMI–APCI	Multimode ionisation source with only APCI mode turned on
MMI–ESI	Multimode ionisation source with only ESI mode turned on
MRL	Maximum residue limit
MS	Mass spectrometry
MW	Molecular weight
OD	Outer diameter
PCA	Principal component analysis
PE	Process efficiency
pK_a	Negative logarithm of acid dissociation constant
PSA	Primary secondary amine
rpm	Revolutions per minute
RSD	Relative standard deviation
SRM	Selected reaction monitoring mode
s_g	Geometric standard deviation
S/N	Signal-to-noise ration
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
t_R	Retention time
UHPLC	Ultra high performance liquid chromatography
UV	Ultraviolet light

INTRODUCTION

Coupling of liquid chromatography (LC) and mass spectrometry (MS) has given a powerful and selective analytical tool for various applications ranging from routine monitoring of contaminants in environmental samples to the identification of novel synthesis products. This coupling became possible due to the invention of electrospray ionisation source. Liquid chromatography/mass spectrometry (LC/MS) has ever since developed rapidly, both in LC part and MS part. An important component from the sensitivity perspective is the ionisation source of MS, which is generating ions from the LC effluent. Ionisation is affected by many different factors, such as the properties of analytes, matrix components, source parameters, eluent composition etc. One way for obtaining the best results is having several different sources operating with different principles and choosing the optimal source for a specific analysis. Today there are many novel ion sources introduced in the literature and several of them are also available commercially. In order for analyst to be able to choose among them, a lot of work needs to be done to compare different sources.

Electrospray ionisation (ESI) source is most used source for generating ions in MS. Some other popular sources are atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photoionisation (APPI) sources. While they enable analysis of many different compounds, none of these sources is universal. Several novel sources are designed to give even better performance by lowering limits of detection and reducing matrix effects, such as heated ESI (HESI) sources, and to be able to analyse wider range of analytes, such as multimode (MMI) sources, which combine different ionisation modes in one source.

A novel nebuliser developed in our group for ESI has been characterised in this study as part of an effort to further enhance the ESI method (Paper I). Its novelty resides in the addition of nebuliser gas capillary inside the liquid capillary. This enhances the nebulisation process by generating finer droplets of effluent. However it needed optimisation and comparison with other sources.

During the thesis studies another possibility for enhancement of LC/MS method was researched: monolithic chromatographic columns (Papers IV–VI). Since monolithic columns are not as accessible as ionisation sources, we discuss the effect of ionisation sources in detail.

The aim of the thesis was two-fold: the comparison of different ionisation methods and secondly the optimisation of novel nebuliser. Also one of the aims was to compare the optimised novel ESI nebuliser with commercially available ESI nebuliser, in order to see which one has advantage in practical analysis (Paper II).

Different ionisation sources were compared to the performance of conventional ESI source under practical analysis conditions (Paper III). The comparison was performed on the basis of analysis of pesticides commonly analysed with LC/MS and having highly varying properties from the point of view of ionisation and compared with relevant statistical tests. It was also important to fulfil the aims of this work in the context of practical samples, such as garlic, honey, tomato etc., using relevant analytes (pesticides, drugs).

1. REVIEW OF LITERATURE

1.1. Liquid chromatography/mass spectrometry

LC/MS is widely used method for the determination of many different analytes, such as pesticides,[1–4] lipids,[5] amino acids,[6] pharmaceuticals,[7] polymers and their additives,[8,9] metabolites, etc.[10] in different matrices such as fruits and vegetables,[2] blood plasma,[1,5] bees,[11,12] human body fluids[10] etc. LC/MS is very diverse in its instrumentation, employing different stationary phases, pressures (HPLC vs UHPLC) and eluents in the LC part as well as a number of ionisation sources[13,14] (ESI, APCI, APPI, MALDI, EI etc.) and mass analysers (ion trap, triple quadrupole etc.) in the MS part. Since it has such a variety of instrumentation and its uses, there is a need to investigate the advantages and disadvantages of the different parts of LC/MS instrumentation and compare them with each other in order to find the best combinations for different applications. This study focuses on the MS ionisation sources part of LC/MS in order to choose between different sources on the basis of their advantages and disadvantages.

1.2. Ionisation sources in LC/MS

Since the introduction of electrospray ionisation (ESI) source by Dole et al.[15] and Fenn et al.[16] several new ionisation sources for generating gas phase ions from solution phase have been introduced and commercialised. The two main principles for atmospheric pressure ionisation (API) are based on liquid phase ionisation processes (ESI, HESI, DESI, EESI etc.) or gas phase ionisation processes (APCI, APPI).

The most popular sources in addition to ESI are the atmospheric pressure chemical ionisation (APCI)[13,17] and atmospheric pressure photoionisation (APPI) sources, which can be seen as adaption of the APCI concept.[18] Some of the new developments have offered additional capabilities to the 2 main ionisation processes. Heated electrospray ionisation (HESI) is a modification of the ESI source with additional sheath gas to further assist the nebulisation of effluent.[19,20] Among other new sources the multimode ionisation (MMI) source is of great interest.[13] In the MMI source the advantages of different ionisation techniques are combined such as ESI-APCI,[21–23] ESI-APPI[23] or APCI-APPI.[24] With MMI it is possible to analyse a wider range of analytes with different hydrophobicity, polarity, volatility, etc. than with the individual sources.[25]

With increasing number of ion sources available it is of growing interest for researchers and chromatography practitioners to find the optimal ionisation source for a given LC/MS analysis task – one that gives highest sensitivity and lowest limits of detection, is least prone to matrix effects, etc. ESI is commonly seen as the default LC/MS ionisation source for analysing many different compounds, but due to a number of recent developments in ionisation sources, it is

important to compare the performance of ESI source with the novel sources, to determine the most suitable ionisation mode for a given analytical task.

1.2.1. ESI and HESI

ESI is the most used ionisation source in coupling of LC with MS. ESI design is shown at Figure 1. The effluent coming from LC is sprayed into ionisation chamber by nebulising gas and generated ions are directed into MS entrance. ESI is usually considered more efficient for compounds that are ionised already in solution (i.e. have higher basicity) and, especially, if the formed ions have large hydrophobic moieties, that help the ionised compound to compete for the droplet surface, in order to escape to the gas phase.[26]

HESI is similar to ESI ionisation source differing only by the addition of sheath gas flow around the spray nozzle to further enhance the desolvation of the effluent droplets (Figure 1), one such example is Agilent "Jet Stream" source used in this study. The sheath gas is super-heated nitrogen surrounding the nebulising gas capillary, forcing the nebulised droplet spray to form a narrower plume. This creates a more focused zone in the ionisation chamber for the generation of ions, increasing sensitivity and the ionisation efficiency, while potentially reducing matrix effects.[20] HESI was introduced in 2009[20], therefore having far less published comparison material with other sources than ESI. HESI has been used, e.g., for the analysis of lipids in blood plasma,[5] pesticides in grapefruit, orange, pear and sweet pepper,[19] wide variety of pharmaceuticals,[27] steroidal lactones,[28] glucuronide in bile[29] and pteridines in insect pigments.[30]

The ionisation process in electrospray-like ionisation sources is described mainly by two models: ion evaporation model (IEM) and charge residue model (CRM). In the spray plume the droplets will divide into smaller droplets via Coulomb fission. IEM proposes that when the droplets have shrunk to sufficiently small size, the analyte ion can leave the droplet into the gas phase, thus replacing the Coulomb fission process. CRM, however, proposes that the droplet will divide into smaller droplets via Coulomb fission and the final droplet containing only one analyte molecule will decrease in size due to solvent evaporation, until there is only charged analyte ion remaining in gas phase.[14,31,32] It is likely that both mechanisms occur depending on the analyte molecule. For smaller molecules IEM is shown to be more likely and for macromolecules CRM is the main mechanism.[31]

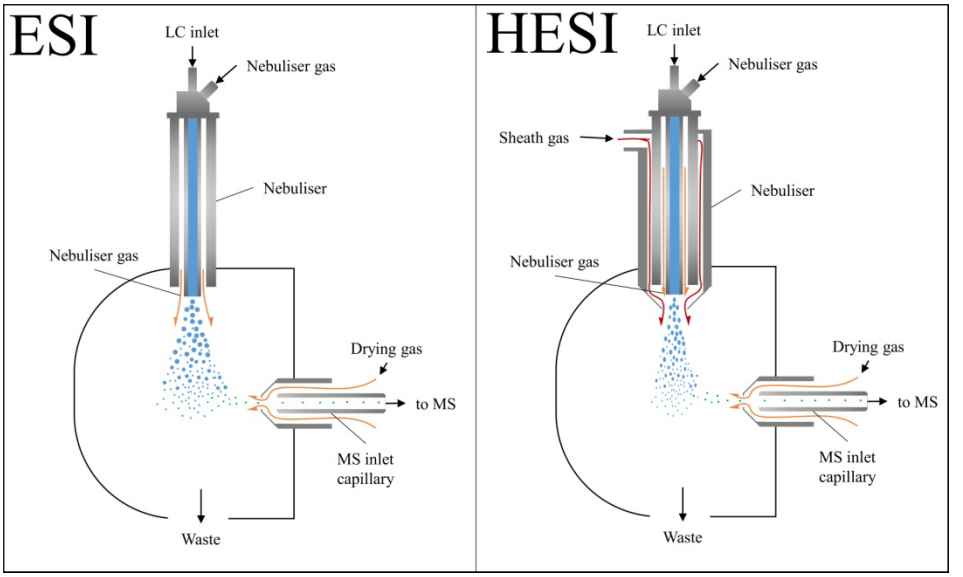

Figure 1. Instrumental principles of ESI and HESI.

1.2.2. APCI and APPI

In APCI and APPI the effluent is nebulised similarly as in ESI, but there is an additional heating to enhance the transition of analyte molecules to gas phase. The analyte is generally assumed to remain neutral in liquid phase and ionisation is assumed to occur largely in gas phase, indirectly via corona needle in APCI or by UV-lamp in APPI. However, recent results[33] cast some doubt on the assumption that APCI ionisation occurs only in the gas phase. It has been found that volatility is not required and that the parameters that govern ionisation in ESI and APCI sources are surprisingly similar. This implies that a combination of ionisation mechanisms might operate in the APCI source.[33] However, based on 40 compounds and contrasting the large number of studies, where compounds can be analysed with APCI but not with ESI, this similarity in ionisation parameters needs to be researched more closely in order to draw clearer conclusions. Also it would be interesting to see if APPI exhibits the same behaviour in regard of ionisation efficiencies. It is largely accepted, that APPI and APCI are usually used for the analysis of more volatile and less polar compounds.[13]

According to the traditional understanding in the case of APCI (Figure 2) the corona needle ionises first the nebulising gas and solvent molecules, that are present in large excess in the ionisation chamber. Subsequently the analyte molecules are ionised by the previously ionised gas and/or solvent molecules, thus the ionisation is determined by gas phase ion chemistry.[9,13,34]

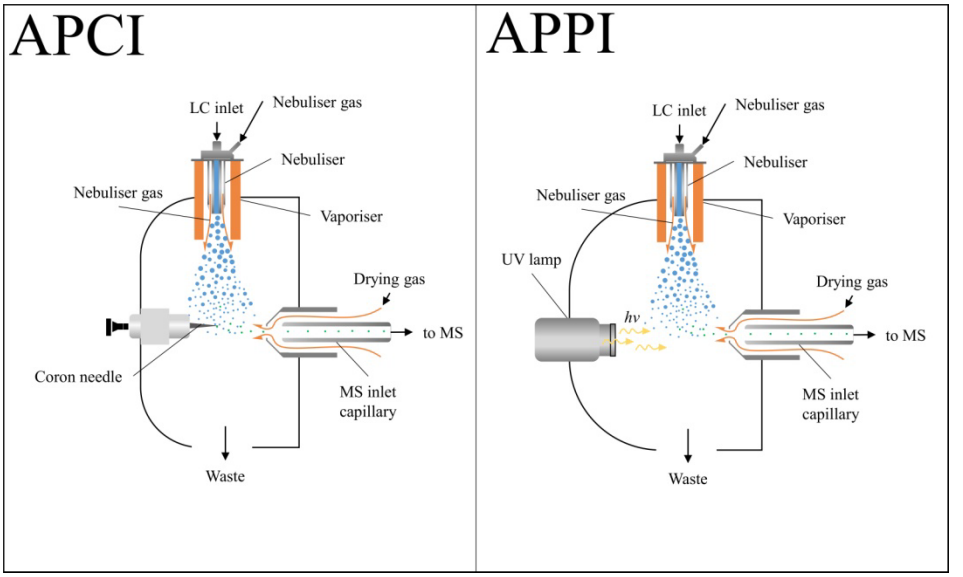

Figure 2. Instrumental principles of APCI and APPI.

APPI uses UV-lamp instead of corona needle for the ionisation (Figure 2). Since UV lamp does not necessarily need the molecule to reach it physically to ionise it, there is also a good probability that UV-lamp ionises the analyte directly. The ionisation mechanism of APPI follows similar mechanism as APCI, however the common gases (N_2, O_2) in the source are not ionised since they have higher ionisation energies (>12.6 eV) than the energy of the photons emitted from the commonly used krypton lamp (10.6 eV). Thus it is either solvent molecules, analyte molecules or molecules of added photoactive substances (dopants) that are ionised via ultraviolet light. The subsequent ionisation of analyte molecule depend on ionisation energies and proton affinities of analyte and other participating molecules. A dopant is usually used in order to enhance the ionisation efficiency of analyte in APPI. Dopant addition serves the purpose of generating more primary ions which can then ionise the analyte molecules.[25,35] Different compounds have been used as a dopant (e.g. acetone, anisole, tetrahydrofuran, benzene), but toluene is most commonly used, for it has proven to be the most robust dopant.[25,36]

1.2.3. Multimode sources

The combination of different ionisation sources may offer combined advantages over the use of the respective sources separately.[25] The principle of multimode ionisation source is shown in Figure 3 with the example of ESI–APCI source. Multimode source relies on the theoretical advantage of using two separate ionisation mechanisms such as ESI and APCI to complement each other and thus may lead to higher sensitivities, reduced matrix effects and robustness with

different matrices, without the need of switching between ionisation sources.[25] There are many different combinations of multimode sources such as APCI–APPI,[24] ESI–APCI,[21,22] etc. and each of these combinations needs to be studied separately, because the combined ionisation sources may complement each other, but may also inhibit the performance of each other. For example APPI and APCI are more similar in their operating principle than ESI and APCI and significant differences in the operation of respective multimode sources may arise. Interestingly, the ESI–APCI source has been shown to have higher sensitivity compared to the respective ionisation sources separately[21] while APCI–APPI source has been shown to have lower signal intensities than the individual sources.[24]

Several different multimode sources are in use, but the concept is still novel and a sufficient amount of data has not yet been collected for drawing general conclusions in comparison with other ionisation sources.

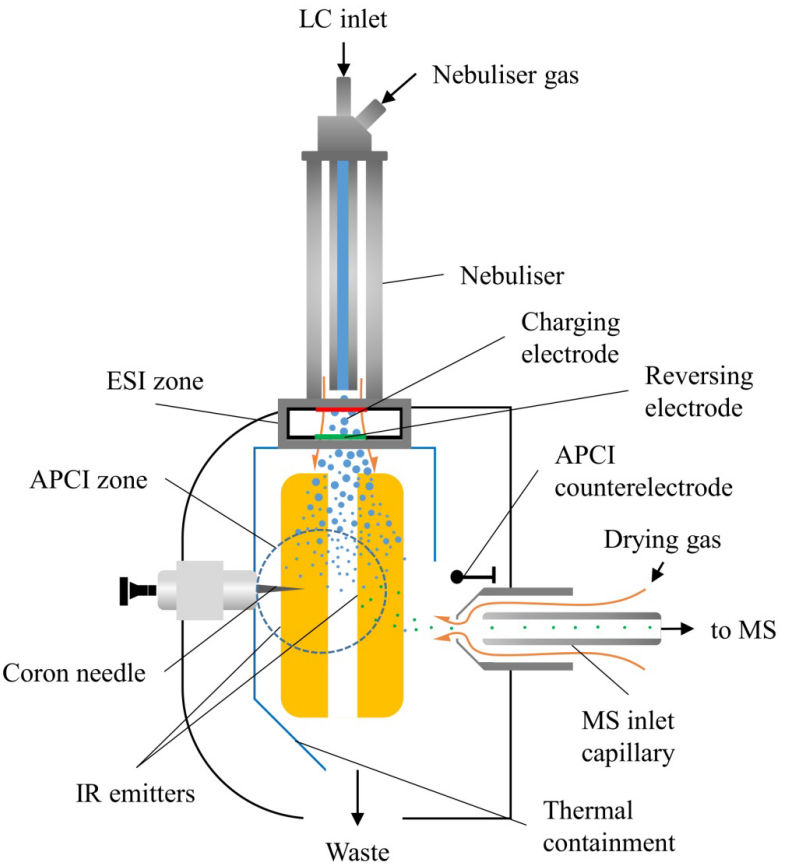

Figure 3. Instrumental principles of ESI-APCI multimode source.

1.2.4. Other sources

Many other ionisation sources exist, which differ from the previous ones, such as electron ionisation (EI), where gaseous neutral analyte molecules are ionised in collision with high-energy electrons in order to produce radical cations.[37] EI produces a lot of fragments and is not suitable when molecular ions are desired. Traditionally EI has been the standard ion source in gas chromatography-mass spectrometry, but lately has found uses in coupling with LC also.[38] A softer ionisation method compared to EI is the matrix-assisted laser desorption ionisation (MALDI), where the sample is mixed with organic matrix for assisting the desorption and is then irradiated with a short laser pulse to produce a plume of ionised analyte that is subsequently analysed by MS. MALDI has become an especially powerful imaging tool for tissues.[39] However, the coupling of online LC with MALDI is problematic.[40]

In desorption electrospray ionisation (DESI)[41] a spray from ESI source is directed at the surface of solid sample, thus desorbing and ionising the analyte molecules from the surface. MS inlet is positioned at an angle of the bouncing droplets from the surface containing ionised analyte. This allows for direct analysis of analytes from surfaces. Extractive electrospray ionisation (EESI)[42] is also a variant of ESI where the effluent containing neutral analyte is sprayed at an angle with another ESI nebuliser spraying a solvent solution. In collision of the two sprays the analyte molecules are ionised. EESI allows for direct analysis of liquid matrices such as water and urine. DESI and EESI, as variants of ESI, can also be used for the analysis of wide variety of analytes.

These are only a selection of the vast number of available ionisation sources. There is a lot more diversity and variations within each ionisation method and also with combinations of different sources.[37]

1.3. Advantages and limitations of different sources

Mostly the traditional ESI source has been compared with APCI and/or APPI sources. HESI and MMI ionisation sources have received much less attention, partly because they are novel sources. The most important comparison parameters have been the limit of detection (LoD),[7,8,43–57] signal-to-noise ratio (S/N)[7,48,50,53,56,58] and matrix effects (ME).[19,46–48,50,55,56,59–61] Linearity[47,48,53,56,57] and sensitivity (as calibration graph slope)[44,48,51,53] have also been considered. The results published in the literature agree only in very general terms. Significant differences are evident in more specific aspects. The results are affected by the analytes used, matrix, solvent composition, ionisation source parameters, chromatographic separation etc.[31]

1.3.1. Linearity

Linear range can be an important characteristic in comparison of different ionisation sources. For the analysis of estradiol ESI, APCI and APPI have been used, both in negative and positive mode. ESI in positive mode yielded narrower linear range than APCI or APPI. For negative mode the linear ranges for ESI, APCI and APPI were comparable.[48] Cai and Syage[53] compared ESI, APCI and APPI in positive mode and also found the narrowest linear range for the ESI source. Titato et al.[57] found comparable linear ranges for pesticides in comparison of ESI with APCI.

1.3.2. Matrix effects

One crucial parameter of an ionisation source is the matrix effect – ionisation suppression or enhancement caused by co-eluting matrix components. Matrix effect results usually in decreased (less often enhanced) analyte signal therefore causing underestimation (less often overestimation) of analyte quantity in the sample. Matrix effect can be influenced by the matrix type, chemical properties of the analyte, sample pretreatment, separation, instrumentation used etc.[62–64] Therefore matrix effect can be very troublesome to eliminate. It would be preferable to use an ionisation source that is less prone to matrix effect. Therefore this parameter has been often used for comparison of ionisation sources. APCI and APPI have been often compared to ESI in terms of matrix effect. In general more matrix effect has been observed for ESI,[46,48,56,59–61] however, in some cases ESI has performed better than APCI or APPI.[47,50,55] For example Hanold et al.[65] observed that APPI was much less susceptible to ion suppression than ESI and APCI. The differences in the extent of matrix effect have been related to the different ionisation mechanisms of ESI and APCI/APPI,[62] but as the factors contributing to matrix effect are diverse, the conflicting results in the literature are not surprising. Also variations between different varieties of electrospray sources have been observed. Stahnke et al.[19] have shown that ESI was less prone to matrix effects than HESI, although the opposite would be expected due to HESI's improved ion desolvation and confinement of the spray by thermal gradient.[20]

1.3.3. Limit of detection, signal-to-noise ratio and sensitivity

LoD is among the most often used comparison parameters for ionisation sources. A number of authors have observed comparable or lower LoD values for ESI compared to APCI or APPI when analysing pharmaceuticals,[50] pesticides,[44,47,56] anabolic steroids,[45] phytoestrogens,[51] triazines, phenylureas,[46] aflatoxin M1[56] and flavonoids.[43] But the opposite has been observed for lipids,[53] sulfonate esters,[49] polymer additives,[8] estradiol[48] and pyrene derivatives[52]. It can be concluded that ESI and APCI/APPI are compound-dependent, as Thurman

et al.[44] also concluded in case of pesticides. Comparing the compounds in the previous studies reveals that finding compound-property dependence patterns is complicated. It does seem, however, that compounds lacking ionic functional groups are performing better in APCI or APPI, in agreement with the classical ionisation models.

In several papers ionisation sources have been compared on the basis of S/N at a given concentration. S/N can be improved greatly if noise levels could be reduced, thus leading to potentially lower LoDs. Higher noise levels can originate from matrix components, solvent clusters and contaminants.[66,67] Thus different ionisation sources can have significantly different S/N ratios, as also observed in literature. It has usually been observed that APCI gives higher S/N values than ESI[48,53] and APPI comparable or higher S/N values than APCI.[7,48,53,58,65] However, Garcia-Ac et al.[50] have shown the opposite: higher S/N for ESI than for APCI and APPI.

Sensitivity can be measured as the calibration graph slope. Based on the data obtained by Keski-Rahkonen et al.[48] and Cai and Syage[53] it can be concluded that the best sensitivity in these studies was observed for APPI, followed by APCI. The lowest sensitivity was observed for ESI. A gain in analyte peak areas (up to 4 times) has been reported for HESI compared to ESI,[5] therefore it can be expected that HESI should have at least comparable if not better sensitivity compared to conventional ESI.

1.4. Novel developments in ESI sources

There have been a number of novel developments for ESI sources. Some of the examples include modifying the nebuliser capillary tip[68], implementing a wire into the liquid capillary[69] and also the previously mentioned HESI with adding a super-heated desolvation gas capillary[20], which has been commercialised by Agilent and is called “Jet Stream” ESI source.

Maxwell et al.[68] showed that an asymmetrically cut ESI emitter tip offers increased sensitivity (approximately two times) compared to the conventional emitter tip geometry. Additionally, Reschke et al.[69] compared emitters with different internal diameters (ID) in the range of 5 μm to 360 μm and found that larger ID results in higher signals even though the reverse is generally accepted from both theory and practice[70]. However, both of these studies were carried out for nano-ESI emitters and their conclusions cannot be automatically transferred to the pneumatically assisted nebulisers implemented in the conventional high flow rate ESI sources.

Bajic et al.[71] have described the addition of a wire (preferably from a conducting material) into the liquid capillary. According to the results presented in ref [71] this addition improves ESI sensitivity by up to 3 times (for Reserpine) depending on the flow rate of the liquid. The sensitivity improvement due to the additional wire may result from two factors. First, the additional wire reduces the effective cross-sectional area of the liquid capillary. Secondly, more surface

is available in the nebuliser tip where electrochemical reactions – producing charge excess for the droplets – can take place.

Among other novel nebuliser designs we have recently introduced (Paper I) a novel concept of nebuliser design. An additional capillary – carrying the nebuliser gas – was installed *inside* the liquid capillary. The advantages of the prototype – lowering of LoD values by up to 250 times – were shown for four analytes even without optimisation of the nebuliser design.[72] This nebuliser design is called 3R nebuliser.

It has also been described in the literature[64] that different analytes as well as standards and samples may have somewhat different optimal ionisation and mass-spectrometer parameters. This indicates that samples with different complexity may result in somewhat different optima and also nebuliser design suitable for one analyte may be less beneficial for another analyte. Therefore it is very important to test the newly developed ESI nebuliser under different conditions (e.g. standards vs samples, different analytes).

2. EXPERIMENTAL

2.1. Chemicals

Standards of carbendazim (99.0%), thiabendazole (98.5%), pymetrozine (99.0%), thiamethoxam (99.0%), vamidothion (99.0%), methiocarb sulfoxide (96.0%), chloridazon (98.0%), imidacloprid (99.5%), acetamiprid (98.5%), methiocarb sulfone (99.0%), thiacloprid (98.0%), imazalil (97.5%), thiophanate-methyl (97.5%), metribuzin (99.0%), pyrimethanil (99.0%), fenpropimorph (97.0%), spiroxamine (97.5%), propoxur (99.5%), triasulfuron (97.5%), bupirimate (98.0%), paclobutrazol (98.5%), methiocarb (98.5%), azoxystrobin (99.5%), epoxiconazole (98.5%), myclobutanil (97.5%), fenhexamid (99.0%), fluquinconazole (98.5%), flusilazole (99.5%), mepanipyrim (99.0%), bitertanol (98.0%), propiconazole (97.5%), triazophos (81.0%), methoxychlor (98.5%), ditalimfos (99.5%), tebufenozide (99.0%), benalaxyl (99.5%), pyrazophos (97.0%), buprofezin (99.0%), indoxacarb (99.5%), trifloxystrobin (99.5%), quinoxyfen (99.0%), pirimiphos-ethyl (98.5%) and hexythiazox (99.3%) were obtained from Dr. Erhenstorfer (Augsburg, Germany). Acetonitrile (HPLC grade) for sample pretreatment and chromatographic separation was acquired from Sigma-Aldrich (St. Louis, United States). Methanol (HPLC grade) for chromatographic separation was acquired from J. T. Baker (Deventer, Netherlands). Toluene (99.9%) was acquired from Sigma-Aldrich (St. Louis, United States). Ultra-pure water was obtained with a Millipore Milli-Q Advantage A10 setup (Millipore, USA). For sample pretreatment anhydrous $MgSO_4$ (99.2%) and glacial acetic acid, for acidification of acetonitrile, were acquired from Lach-Ner (Neratovice, Czech Republic), NaCl and sodium acetate from Reakhim (Leningrad, former Soviet Union) and primary-secondary amine (PSA) sorbent from Supelco (Bellefonte, USA). The aqueous mobile phase component (0.1% formic acid) for UHPLC were prepared from formic acid (98.0%, Riedel-de Haën, Switzerland) and dissolved in ultra-pure water. The buffer (pH = 2.8) for HPLC was prepared from formic acid,1 mM ammonium acetate (99.0%, Fluka Chemie AG, Buchs, Germany) dissolved in ultra-pure water.

2.2. Instrumentation

In Papers I and II measurements were performed on an Agilent Series 1100 LC/MSD Trap XCT (Agilent Technologies, Santa-Clara, USA). The instrument was equipped with a binary pump, an autosampler and a thermostatted column compartment. The injection volume was 5 or 10 μL, depending on analysis. For the separation, a 250 mm long Zorbax Eclipse XDB-C18 column with an Eclipse XDB-C18 12.5 mm pre-column (both with an internal diameter of 4.6 mm and particle size of 5 μm) was used. The mass spectrometer uses a quadrupole ion trap mass analyser. For instrument control, an Agilent ChemStation for LC Rev. A. 10.02 and MSD Trap Control version 5.2 were used. The ion transportation

parameters were optimised for each analyte at a chromatographic flow rate via MSD Trap Control software.[64] All of the analyses were carried out in positive mode. The mass spectrometer was operated in the selected reaction monitoring mode (SRM). Full MS^2 spectra were recorded.

In this study the dimensions and parameters of the novel nebuliser developed in our group were optimised. The optimised novel nebuliser was compared with a commercial nebuliser (with also optimised parameters according to procedure described by Kruve et al.[64]). Observed MS^2 were independent of the nebuliser used.

In Paper I for the analysis of carbendazim, thiabendazole, imazalil and methiocarb[72] gradient elution with methanol and buffer solution (pH = 2.8) was used. The linear gradient started at 20% methanol and was raised to 100% within 15 min, then the column was eluted for 7 min with methanol. After that the methanol content was lowered to 20% in 3 min. Stabilisation time of 7 min was used between injections. Eluent flow rate was 0.8 ml/min.

In Paper II for the analysis of honey samples gradient elution (flow rate 0.8 mL/min) was used with acetate buffer and methanol. The methanol percentage (v/v) was raised from 40 to 100% in 17 min, maintained at 100% for 5 min and lowered back to 40% in 3 min. The stabilisation time between runs was 7 min.

In Paper III, for the comparison of different ionisation modes, an Agilent 6495 Triple Quad LC/MS/MS instrument (Agilent Technologies, Santa-Clara, USA) was used. The UHPLC instrument was Agilent Infinity 1290, equipped with binary pump, an autosampler, a thermostatted column compartment. An Agilent Zorbax RRHD SB-C18 2.1×50 mm column with 1.8 μm particles was used for analyte separation. The injection volume was 1 μl. The mass spectrometer uses a triple quadrupole mass analyser and has exchangeable ion sources. 7 different ionisation modes were used: ESI source, HESI source, APPI source with and without dopant and MMI source with simultaneous ESI and APCI ionisation, as well as both ESI and APCI separately. In the context of this work the term ionisation mode means both different sources as well different ionisation approaches within the same source (APPI with and without dopant; MMI source with simultaneous ESI and APCI, as well as ESI and APCI separately). For instrument control Agilent MassHunter Workstation version B.07.00 was used. The fragmentation voltages were optimised using the MassHunter Optimizer software. Sequential injections were made while changing collision energy in steps to find the values where most intense fragments were formed. After automatic fragmentation optimisation it was confirmed and fine-tuned manually. Manufacturer's default source parameters were used for the analysis in the case of all sources.

In Paper III UHPLC analysis of 41 pesticides in the comparison of different ionisation modes gradient elution was used with formic acid aqueous solution and acetonitrile at 0.3 ml/min flow rate. Acetonitrile percentage (v/v) was raised from 10 to 100% in 6 min, maintained at 100% for 1 min and returned to 10% in 1 min. The stabilisation time between runs was 0.5 min.

For DA-APPI the dopant (toluene) was infused after column with infusion pump from KD Scientific (Holliston, United States). The flow rate of dopant was optimised within 0–1.75 ml/h range for all 41 pesticides. The lowest flow rate that gave the best peak areas for the largest number of compounds was chosen. 0.5 ml/h proved to be optimal for that.

For sample pretreatment, a centrifuge (Centrifuge 5430R) and stirrer (Mix-Mate from Eppendorf (Hamburg, Germany)) were used.

2.3. Selectivity

Fragmentation was used to ensure selectivity and 1–3 fragment ions were monitored, depending of the specific compound, instrument and analysis. The corresponding precursor ions and product ions with other parameters can be seen in Table 1 for Paper I and II, in Table A – 1 for 41 compounds used in Paper III and in Table A – 2 for optimisation experiments.

Table 1. Fragmentation paths for compounds used in Paper I and II with retention times.

Paper I				Paper II			
Compound	t_R (min)	Precur-sor ion	Prod. ion	Compound	t_R (min)	Precur-sor ion	Prod. ion
carbendazim	8.3	192	160	thiamethoxam	5.5	314	210; 180
thiodicarb	14.3	202	175	imidacloprid	6.7	256	209; 175
imazalil	14.0	297	201	acetamiprid	7.7	223	126; 187
methiocarb	16.4	226	169	thiacloprid	8.8	253	126; 186

In Paper II for the analysis of honey samples, selectivity was ensured by monitoring two ion transitions. After finding positive samples, an additional third ion transition for confirmation was used. Samples where all three ions could not be detected, that is at least one was not detected, were assigned as negative. Additional confirmation of positive samples was achieved by monitoring the abundance ratio of the signals of two most intense fragment ions. The acceptable boundaries for the abundance ratio were calculated from 64 calibration samples from eight days with a concentration range from LoD up to 0.3 mg/kg. The acceptable ratio was found as the mean ratio ± two standard deviations of the ratio found in the calibration samples. Analysis of the positive samples was repeated with the same criteria.

It is also interesting to note that in the case of thiamethoxam the observed fragment ions were different from those commonly reported in the literature (Figure 4). In the literature, the common fragments, corresponding to the thiamethoxam molecular ion ($[M+H]^+$), are 211 and 181[73–75]. In this work, thiamethoxam was primarily observed as a Na^+ adduct with fragments 210 and 180

and the $[M+H]^+$ ion was much less intense. This Na^+ adduct effect was studied with ion-trap and triple quadrupole (with MMI source) instruments. While on the triple quadrupole instrument, the $[M+H]^+$ ion for thiamethoxam (with observed fragments 211, 181, 210 and 180) was more intense than $[M+Na]^+$, the effect was the same as observed on the ion-trap instrument, that is with $[M+Na]^+$ fragments 211 and 181 were absent and 210 and 180 were most intense. Thus, the observed fragments were suitable for detection of thiamethoxam in case of neonicotinoid detection in honey samples.

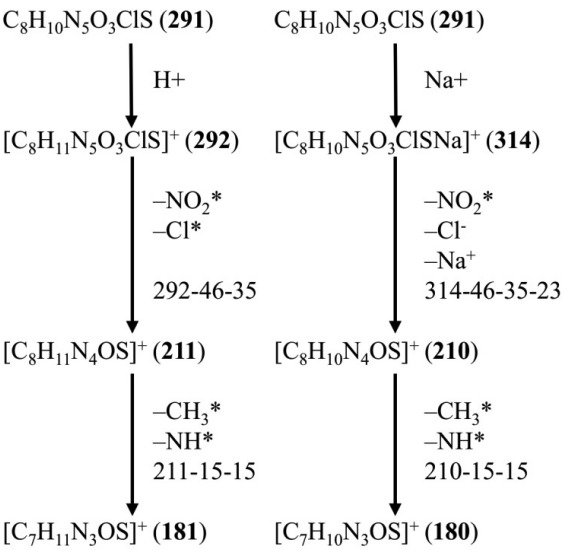

Figure 4. Proposed fragmentation scheme of thiamethoxam. The fragments marked with asterisk (*) are proposed to leave as radicals.

2.4. Samples for Paper II

The honey samples were collected between 2005 and 2013 and were provided from different sources, thus different storage conditions had been applied. The majority of honey samples, 141, were the same as used by Rebane and Herodes[76], 114 samples were obtained from the Estonian Environmental Research Centre (EERC) and 39 samples from Estonian beekeepers.

Samples used by Rebane and Herodes[76] were mostly stored at room temperature since their collection in 2005–2010, samples from the EERC (2010–2013) were kept in a dark room, designated only for honey samples with the temperature kept at 10–17 °C, honey sample collection was completed by one person from markets, stores and fairs in their original packages. After acquiring the samples directly from beekeepers in January 2014 (collected by beekeepers mostly in the summer of 2013, with some samples from 2011 and 2012), they were kept in a refrigerator at –20 °C.

2.5. Sample pretreatments

2.5.1. QuEChERS in the comparison of nebulisers in Paper I

15 ml of 1% acetic acid in acetonitrile, 6 g $MgSO_4$ and 1.5 g anhydrous sodium acetate was added to 15 g of homogenised sample. Shaken vigorously for 1 min and centrifuged at 5000 rpm for 1 min. The extract was transferred to tube containing 50 mg PSA + 150 mg anhydrous $MgSO_4$ per 1 ml of extract. It was shaken again and centrifuged at 5000 rpm for 1 min.[77]

2.5.2. QuEChERS for honey samples pretreatment in Paper II

For sample pretreatment, the modified QuEChERS method[77] was used. 1 g of honey was dissolved in 10 ml of purified water and 10 ml of acetonitrile. 4 g of $MgSO_4$ and 1 g of NaCl were added and shaken for 1 min, followed by centrifugation for 3 min at 4400 rpm. An acetonitrile fraction of 1 ml was pipetted into a 2 ml centrifuge tube with 150 mg of $MgSO_4$ and 25 mg of PSA for clean-up, followed by stirring for 1 min. Tubes were centrifuged for 1 min at 5000 rpm and the supernatant was taken for analysis. For every honey sample, sample pretreatment was performed, followed by subsequent analysis on the same day.

2.5.3. QuEChERS for garlic and tomato samples in comparison of ionisation modes in Paper III

For sample pretreatment modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used.[78] 15 ml of 1% acetic acid in acetonitrile was added to 15 g of tomato or 5 g of garlic homogenised sample. In the case of garlic, 10 ml of ultrapure water was also added, because the water content is much lower in garlic matrix. Subsequently 6 g of $MgSO_4$ and 1.5 g of sodium acetate were added. The mixture was stirred and centrifuged for 7 min at 5000 rpm. 3.33 ml of the acetonitrile fraction was pipetted into 15 ml centrifuge tube with 500 mg of $MgSO_4$ and 170 mg of primary secondary amine (PSA) for clean-up, followed by stirring and centrifugation for 7 min at 5000 rpm. The supernatant was taken for analysis. Samples were analysed in both extract and clean-up steps for the calculation of matrix effects with spiking of the blank sample, blank extract and blank extract clean-up steps.

2.6. Data analysis in experiments

2.6.1. Calculation of validation parameters

Upper limit of linear range was evaluated via visual inspection of residuals graph. The lower limit of linear range was determined by relative residuals. The limit of relative residuals was set to 20% as suggested in the SANCO guidelines (SANCO/12571/2013).[79]

LoD was determined either by S/N or by standard deviation of residuals. S/N was used for the preliminary characterisation of novel 3R nebuliser, because it is often used as one of the comparison parameters in case of novel developments in ion sources. In the S/N approach the lowest concentration that gave S/N value of at least 3 was assigned as LoD. In the residuals approach the LoD was calculated according to the ICH validation guidelines:[80,81]

$$\mathrm{LoD} = 3.3 \times \frac{standard\ deviation\ of\ residuals}{slope} \tag{1}$$

Both standard deviation of residuals and slope were determined in the LoD region, covering concentrations over approximately an order of magnitude. If it was not possible to confirm the calculated LoD with another fragment ion, then the lowest concentration where the peak of confirmatory ion was seen, was taken as LoD. The LoQ was determined by

$$\mathrm{LoQ} = 10 \times \frac{standard\ deviation\ of\ residuals}{slope}. \tag{2}$$

S/N values from triple quadrupole mass spectrometer were obtained with the MassHunter software with signal definition as area and noise definition as Auto-RMS (root-mean-square of the baseline over time window). S/N values from ion-trap mass spectrometer for the comparison of nebulisers were obtained with Data Analysis software version 5.2, which calculates noise over the whole chromatogram except the peaks.

Sensitivities of the ion sources were compared on the basis of calibration graph slopes in the linear range. As the slope values ranged over several orders of magnitude ratios of slopes were compared instead.

For the comparison of sensitivity, S/N and LoD values, geometric mean (GM) as well as geometric standard deviation (s_g) were used according to formulas,

$$\mathrm{GM} = \sqrt[\mathrm{n}]{a_1 \times a_2 \times \cdots a_\mathrm{n}} \tag{3}$$

where a_n is the compound-wise ratio value of sensitivity, S/N or LoD values between two ionisation modes and n is the sum of all product ions over the 41 compounds detected. Formula for geometric standard deviation is defined as follows,

$$s_g = \exp\left(\sqrt{\frac{\sum_{i=1}^{n}(\ln\frac{a_i}{\mu_g})}{n}}\right) \quad (4)$$

where n is the same as in GM formula, a_i is the same as a_n in GM formula, μ_g is the geometric mean of the ratio values of sensitivity, S/N or LoD values between two ionisation modes. The comparison of geometric means of the LoD, S/N and sensitivity ratios was done with HESI, because it gave the best results for these 3 parameters.

Matrix effect was calculated as follows:

$$\mathrm{ME} = \frac{c_{(\mathrm{found})}}{c_{(\mathrm{spiked})}} \times 100\% \quad (5)$$

where $c_{(\mathrm{found})}$ is the analyte concentration calculated from the analysis results and $c_{(\mathrm{spiked})}$ is the theoretical analyte concentration in the spiked sample. Matrix effect determinations were performed over a time period of 6 months in 4 different series. Within series the extraction step of the sample pretreatment was performed with blank samples and 3 replicates of spiked matrix samples. Part of the blank extract was spiked and samples for analysis were taken from each solution in this step. The sample clean-up step was then performed with the 3 spiked matrix samples, blank extract and 3 replicates of the spiked extract. After the clean-up the blank sample was spiked and again samples for analysis were taken from each solution. Analysis of the samples was done in duplicate.

In Paper III for repeatability determination 9 replicates in garlic matrix were used. Repeatabilities obtained with the different ionisation sources were compared using the *F*-test and relative standard deviation of the signals of spiked garlic extracts (see Table A – 5). At first the statistical differences in variances of signals were established with comparison of the best performing source (ESI) with others using the *F*-test. Then the relative standard deviation was used to estimate if the statistical difference is of practical significance. If the relative standard deviation was over 10% for the source with higher repeatability standard deviation, then the difference was considered significant in practice.

ANOVA, GLM and PCA analysis, as well as preparation of figures was performed with the R free software environment for statistical computing and graphics version 3.2.0 with packages pca3d and rgl (for PCA). Data were scaled and centred before analysis.

2.6.2. Proportions analysis

In Paper II neonicotinoids in honey samples were analysed for the comparison of novel nebuliser with commercial nebuliser. Honey samples were acquired from different years and for positive honey samples confidence intervals (the borders where in case of normal distribution the true value of the observed

parameter is with given condifence probability) were calculated. Proportions with 95% confidence intervals were calculated on a year-wise basis using the following formula:

$$p = \frac{n_{\text{pos}} + 2}{n + 4};\ W = 2 \times \sqrt{\frac{p(1-p)}{n+4}} \tag{6}$$

where p is proportion, n_{pos} is the number of positive samples, n is the overall number of samples and W is the error margin at a 95% confidence level.[82]

2.6.3. Principal component analysis (PCA)

In Paper III PCA was performed for analytes with molecular parameters: retention time (t_R), acidity of the conjugate acid (i.e. pK_a of protonated analyte), octanol-water partition coefficient (log$P_{o/w}$), molecular weight (*MW)* (values and additional information in Table A – 4) and separately for LoD and matrix effect data.

Three principal components were used for the analysis of molecular parameters, describing 97% of variance in data. Then the data points on the 3D plot of the 4 molecular parameters were analysed in the context of LoD and ME values. Separately for each ionisation mode the LoD and ME values were divided into 4 groups of equal size, where each group had 25% of the data points. Then the PCA plot was analysed in order to see if the LoD and ME values were in an observable correlation with the PCA.

Another PCA was done to compare ionisation modes using only LoD or ME data, in order to see the profile differences of the different ionisation modes. Three principal components were used for LoD and ME, describing 84% and 67% of variance, respectively.

If one point of data was missing for a compound the whole compound was omitted because of the requirement of PCA that the data matrix is complete. Thus, altogether 36 compounds were used for LoD and 40 compounds were used for ME profile analysis. In the analysis of LoD PCA plot myclobutanil was deliberately omitted from the dataset, because it had LoD results that were heavily influenced by existence and detection concentration levels of qualitative fragmentation ions.

2.6.4. Full factorial design for the optimisation of 3R nebuliser

Full factorial design[83] was used to plan the nebuliser optimisation experiments for both pesticides (standard solution and spiked garlic sample, both 1 mg/kg) and pharmaceuticals. For specifying most crucial parameters a two level design was used for 5 parameters (for instrumentation details see Figure 11):

1. Capillary B ID (B_ID): 0.25 mm and 0.50 mm;
2. Capillary C (C) presence: Yes (value 1 in GLM model) or No (value 0 in GLM model);
3. Capillary C ID (C_ID): 90 µm and 175 µm (corresponding OD were 230 and 360 µm, therefore both could be implemented only if Capillary B ID was 0.5 mm);
4. Capillary C pressure (Cp): if present: 8 bar and 14 bar;
5. Capillary A pressure (CA): 5 and 12 psi;
6. Capillary Voltage (CapV): 2500 V and 4000 V.

The parameter levels were chosen according to both previous experiences and to cover a wide range of possible parameter values (for gas pressures and capillary voltages). For example the approximate dimensions of commercial ESI nebuliser are 150 µm ID and 250 µm OD for liquid capillary, 575 µm ID and 1700 µm OD for gas capillary.

Due to the technical reasons – mainly the limited long-term stability of the MS and the analytes – it was impossible to include more parameter levels into the parameter effect study, though more information on the nebulisation mechanism could be gathered this way. In order to detect parameters significantly influencing the ESI/MS sensitivity a two level data analysis was performed. First, the parameters statistically significantly influencing the ESI/MS signal were detected with ANOVA. Thereafter, the parameters, previously found to be statistically significant, were implemented into a GLM. GLM was used to estimate the physical impact (how large signal increase/decrease occurs due to a change of a parameter value) of each parameter and all possible two-parameter interactions. This two-stage data treatment is necessary as some parameters being statistically significant may have considerably lower influence on the ESI/MS signal than other parameters also being statistically significant. Before data treatment both parameter levels and obtained peak areas were scaled in order to obtain comparable results. The GLM model was obtained in the form:

$$Analyte\ Signal = \sum_i^6 coef_{\mathrm{i}} \times parameter_{\mathrm{i}} + \frac{1}{2}\sum_k^6 \sum_l^6 coef_{\mathrm{kl}} \times interaction_{\mathrm{kl}} \qquad (7)$$

where only two parameter interactions were considered as follows:

$$interaction_{\mathrm{kl}} = parameter_{\mathrm{k}} \times parameter_{\mathrm{l}} \qquad (8)$$

The aim of the GLM model is not a full and accurate description of the electrospray ionisation process, but revealing nebuliser design elements and working parameters that have significant impact on ESI/MS signal. The impact of each parameter or parameter interaction can be estimated from the absolute value of the coefficients – the larger the absolute coefficient the larger is the impact of the parameter-parameter interaction.

2.7. Design of experiment for ionisation modes comparison

A high concentration was selected for matrix effect and repeatability determination in order to avoid the loss of signal due to ionisation suppression with sources that give higher LoD values. This concentration was mostly at the upper part or near the upper limit of linear ranges, corresponding to the concentration range where the maximum residue limits (MRLs) of most of the compounds are in garlic and tomato (0.01–0.5 mg/kg). The concentration in matrices was approximately 0.1 mg/kg in garlic and in tomato. The corresponding solvent concentrations of the pesticides in the analysed samples were approximately 0.05 mg/kg and 0.16 mg/kg.

Manufacturers' default source parameters were used for all ionisation sources. It was impractical to use individual source parameters for each compound in a study like this, as the gas flow rates and temperatures take a lot of time for stabilisation and therefore cannot be reasonably varied within a run. Additionally, different solvent compositions are expected to have somewhat different optimal source parameters. At the same time this study includes a large number of analytes, with very different retention times, and therefore eluting in very different solvent compositions. The average optimal parameter set is therefore expected not to deviate significantly from the default values.

SRM was used instead of full scan mode. For the absolute comparison of ionisation efficiencies in the sources full scan monitoring would be more proper. However, full scan would be impractical (especially keeping in mind selectivity), since SRM is mainly used in regular analysis of complex samples in order to ensure selectivity.

Besides the ionisation mode the results depend on compounds and elution conditions as well as on the MS system and ion source design and in order to obtain general conclusions these need to be cancelled out or accounted for. The compound dependence is accounted for by including compounds with varying properties. Dependence on the elution conditions and MS system is cancelled out by using all the MS sources on the same MS and with the same chromatographic method.

Dependence on the ion source design cannot be directly addressed in this experimental design, so that rigorously speaking, the results are applicable only to the sources used in this work. However, it has been demonstrated recently[84] that the relative order of the compounds by ionisation efficiency largely follow the same trend across different mass analysers, indicating that the main processes responsible for ionisation are the same in different instruments regardless of different source design. Also, since all manufacturers of ion sources adhere to the same general goals – trying to produce as robust and sensitive ion sources as possible – it is expected that the general conclusions are valid for the same source types from different manufacturers.

3. RESULTS AND DISCUSSION

3.1. Comparison of ionisation sources

Due to the large amount of different ionisation sources and the constant development of novel sources, it is important to compare different sources in order to find the optimal source for different applications. The aim of the comparison of different ionisation modes was to determine the ionisation source providing highest sensitivity and robustness for the analysis of pesticides. Thurman et al.[44] showed that for different pesticide classes different ionisation sources (ESI or APCI) were optimal. Therefore it is interesting to see if the novel MMI source offers useful properties of combined sources and therefore minimising the need to use different sources. 7 different ionisation modes – ESI, HESI, direct APPI, DA-APPI and MMI with ESI and APCI mode simultaneously and separately, were used for the analysis of pesticides in tomato and garlic.

Pesticides are widely used for crop protection and the large number of different pesticides demands strict control over the MRLs established by EU and other authorised organisations[3,85,86]. Pesticides differ widely by polarity, acid/base properties, hydrophobicity etc. thus several ionisation sources have been used for their analysis including ESI, APCI and HESI[19,44,47,86].

3.1.1. Comparison of chromatogram profiles

The peak profiles of different ionisation modes can give useful information about the general picture of ionisation efficiencies. As can be seen in Figure 5 relative peak areas of the compounds depend strongly on ionisation mode. For example, pirimiphos-ethyl gives large peaks in HESI and ESI modes (orange peak at 5.9 min in Figure 5), while for other ionisation modes the peak area of pirimiphos-ethyl is comparable to other compounds. Also much smaller (or absent) peaks of MMI-APCI are noticeable compared with other ionisation modes at the same concentrations. It is interesting that for both APPI modes the peak areas of different compounds are much closer to each other than for other ionisation modes (but at the same time, generally lower than with other modes). From data in Figure 5 both APPI modes seem to be less discriminating between compounds based on peak areas.

It has been shown that ionisation efficiency of compounds in ESI is affected by the ionisation in the solution phase and by competition for the surface of the droplets[14,87]. Recently it has been demonstrated that the relative order of compounds in ionisation efficiency scales is similar between ESI and APCI[33]. From Figure 5 also the similar profiles of chromatograms obtained with ESI and APCI can be observed. However, the chromatograms in Figure 5 seem to show similar profiles of MMI-APCI with ESI and HESI, but MMI-ESI leads to a

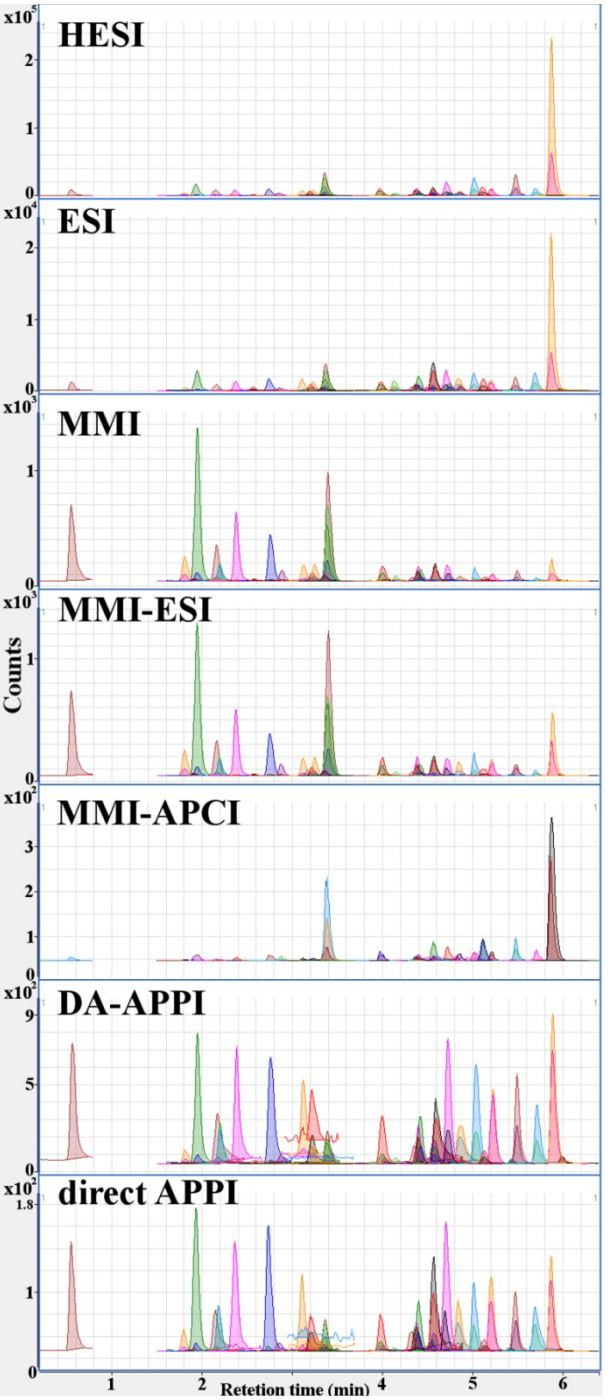

Figure 5. Chromatograms of 41 pesticides with different ionization modes. Analytes were injected in acetonitrile at 0.01 mg/kg concentration. Note the different spans of the signal axis.

surprisingly different peak profile. The peak profiles of the 3 MMI modes are inconsistent across different data series acquired on different time, giving sometimes the profile of MMI-APCI in Figure 5 and sometimes the profile of MMI-ESI in Figure 5 for all 3 modes. It can be concluded that MMI source itself is not very robust in its performance between series. For other ionisation sources the profiles matched throughout the experiments carried out over a period of one year.

A possible explanation for the less discriminating peak profiles of APPI is the direct ionisation mechanism. In the case of APPI the analyte molecule can be ionised both directly and indirectly.[14,18,88] This potential reason needs to be researched more closely, in order to draw clearer conclusions. To the best of our knowledge there have been no studies relating the APPI ionisation efficiency with molecular parameters.

3.1.2. Repeatability

ESI and HESI displayed the best repeatability standard deviations pooled over all studied compounds: 3.1% and 3.4% respectively (Table A – 5), which can be considered acceptable. For both direct APPI and DA-APPI the average relative repeatability standard deviations were higher: 11.6% and 12.2%, respectively. The MMI ionisation modes display still worse repeatability: the average relative standard deviations were 18.3%, 34.3% and 23.0% for MMI, MMI-APCI and MMI-ESI, respectively, over the 41 compounds. The worst individual repeatabilities for ESI and HESI were 8.2% and 9.0%, for fenhezamid and tebufenozide, respectively. For direct APPI, DA-APPI, MMI, MMI-APCI and MMI-ESI the worst repeatabilities were 43.4% (methiocarb sulfone), 52.9% (ditalimfos), 37.8% (benalaxyl), 267.7% (pyrimethanil) and 38.4% (quinoxyfen), respectively.

It needs to be considered that poor repeatability of an ionisation mode also influences determination of other parameters with the same ionisation mode (mainly with MMI ionisation modes), including matrix effect values. This can be seen in Figure 6, where matrix effect values are shown for 2 compounds. For mepanipyrim the repeatability with some ionisation modes is poor, thus leading to huge variability in signal enhancement and suppression. However, the goal was to get a general picture of the performances of different nebulisers, not the absolute values of ionisation suppression/enhancement, thus in spite of the high variability of matrix effects the obtained information is still useful for overall picture.

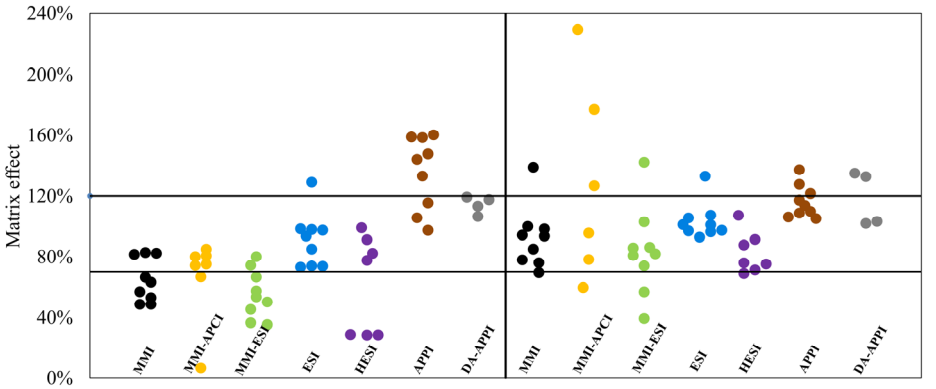

Figure 6. Example of matrix effect values over all series of data for chloridazon and mepanipyrim with all 7 ionization modes presented on a beeswarm plot. The high scatter of mepanipyrim matrix effect values in the case of MMI-APCI and MMI-ESI modes is caused by overall poor repeatability of mepanipyrim response with the MMI ion source.

3.1.3. Matrix effects

Matrix effect values were first analysed with *t*-test (95% confidence), comparing the values with 100%. If matrix effects were not present then no significant difference from 100% should be observed over the data of 4 different series. Analysis showed that out of the 41 compounds there were no significant differences from 100% for 9, 9, 18, 12, 7, 3 and 19 compounds in case of ESI, MMI, MMI-APCI, MMI-ESI, HESI, direct APPI and DA-APPI respectively in garlic and in tomato 36, 27, 16, 28, 36, 34 and 40 compounds in case of ESI, MMI, MMI-APCI, MMI-ESI, HESI, direct APPI and DA-APPI respectively. It can be seen that matrix effect values in case of MMI-APCI and DA-APPI were not significantly different for close to half of the compounds in garlic and for most of the compounds in case tomato (except MMI-APCI). The *t*-test results are influenced by both average matrix effect and the repeatability observed with the respective source. MMI-APCI showed high variability (see section 1.12.2) of the results, which therefore may mask some important deviations from 100%. Additionally statistical differences may be insignificant in practice. Thus matrix effect values were also compared with the limits (70–120%) established by the SANCO/12571/2013 guideline, which considers trueness (process efficiency) values between 70 and 120% as acceptable. Though process efficiency incorporates both matrix effect and recoveries from sample pretreatment, the matrix effect has to be at least in the same range to provide acceptable results.

The results for garlic and tomato are shown in Figure 7. The data has been pooled over four independent data series (over 8000 datapoints) showing all individual datapoints. The same general picture was observed in all 4 data series, recorded during a time period of 6 months in the case of all three MMI ionisation modes, HESI, ESI and direct APPI and in 2 data series during one

month in the case of DA-APPI. Figure 7 reveals that all three MMI ionisation modes and HESI suffer from ionisation suppression or enhancement for a significant number of compounds (although, as mentioned above in a number of cases the poor repeatability can be the cause of large difference from 100%). HESI performs better than the three MMI ionisation modes but in the case of HESI there are still only 65% and 70% of the compounds (in garlic and tomato matrix, respectively) within the acceptable matrix effect limits as suggested by SANCO. These results for HESI are in agreement with those observed by Stahnke et al.[19] Direct APPI has significant ionisation enhancement for garlic, with only 57% of the compounds within the limits, but shows better results for tomato, with 82% within limits. ESI and DA-APPI exhibit the least ionisation enhancement or suppression: 89% and 83% of the compounds, respectively, were within the acceptable limits in garlic and 93% and 91%, respectively, in tomato. It can be concluded that ESI and DA-APPI have least matrix effects in the case of tomato and garlic samples.

As would be expected, all ionisation modes have stronger ionisation enhancement or suppression in garlic samples than in tomato samples. Garlic is considered one of the worst matrices from the LC/MS matrix effect perspective, whereas tomato is a relatively simple matrix.[89] The three MMI ionisation modes and HESI still display significant ionisation suppression or enhancement in tomato matrix. Only direct APPI, DA-APPI and ESI have over 80% of the data points within the acceptable limits for tomato samples.

The results on matrix effect published in literature are conflicting. Some studies show ESI to be less prone to matrix effect compared to APCI or APPI,[46,48,56,59–61] but other studies show the opposite.[47,50,55] This is most probably due to the variability of compounds and matrices analysed and elution conditions used. Since APPI/APCI have different mechanisms of ionisation compared to ESI, it is not unexpected that depending on the analyte and the co-eluting matrix components the results may vary. Based on the results of this study it can be concluded that for a large variety of small neutral molecules (containing both nitrogen and oxygen bases with very different ionisation sites) ESI seems to have from the point of view of matrix effect advantage over APPI, 3 MMI modes and HESI.

Comparing the ionisation modes compound-wise gave interesting results. Compounds that had statistically significant difference from 100% and gave consistently out of limits matrix effect values in all series after complete sample pretreatment were identified. Ionisation of pymetrozine was suppressed in all 4 measurement series in all ionisation modes except for ESI (in garlic and tomato) and DA-APPI (in tomato). The most probable reason is that pymetrozine is the first eluting compound with retention time t_R = 0.57 min (dead time 0.50 min), while all other compounds have retention time over 1.7 minutes. With this retention time pymetrozine co-elutes with early-eluting matrix components as well as possible salt residues from the sample pretreatment. In spite of this the matrix effect of pymetrozine in the ESI source is within the acceptable limits even under such conditions.

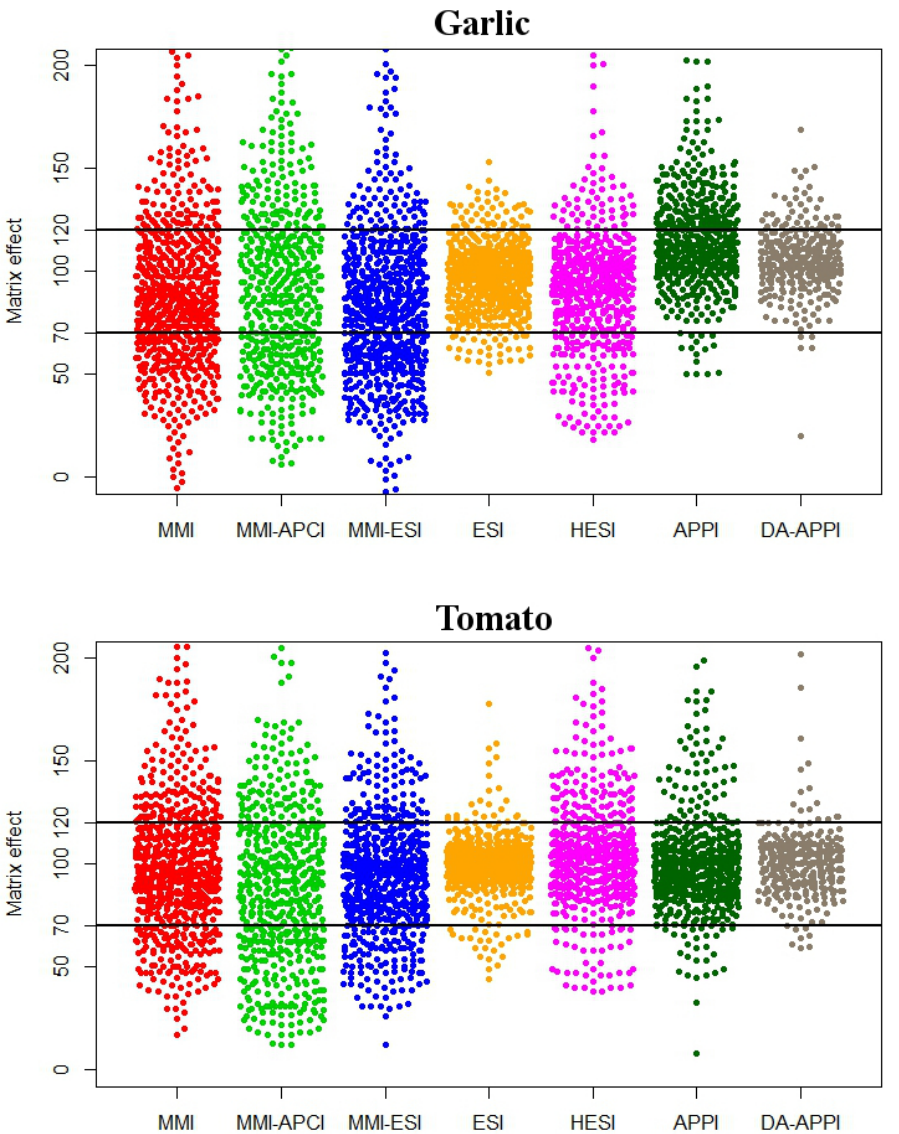

Figure 7. Matrix effects in garlic and tomato for different ionization sources. Lines at 120% and 70% refer to the recommended limits suggested by SANCO. The data have been pooled over four independent data series and each point on figure marks one data-point.

From the obtained data it is possible to assess the ability of MMI to combine advantages of ESI and APCI ionisation. For MMI strong ionisation suppression or enhancement was observed for 5 compounds. To 4 out of these compounds suppression was observed either with MMI-APCI or with MMI-ESI and for one compound (pymetrozine) with both of these modes. Therefore to these 4 compounds MMI fails to cope with matrix effect by having an alternative ionisation

mechanism. On the other hand for 7 compounds MMI-APCI produced strong ionisation suppression or enhancement and for 2 compounds strong ionisation suppression with MMI-ESI in garlic samples, but MMI did not show ionisation suppression or enhancement. Similar trends were also observed for tomato matrix. Therefore the advantages of MMI tend to be strongly compound-dependent. Data in Figure 7 indicates that MMI is by matrix effect comparable with MMI-ESI and inferior to ESI, thus offering no real advantage.

The general trend of the ionisation enhancement and suppression across the chromatogram reveals more ionisation suppression than enhancement in the first half of the chromatogram and more enhancement in the second half of the chromatogram for all three MMI modes. The observed ionisation suppression may be caused by polar or ionic compounds in the extract and eluting in the beginning of the chromatogram. No other significant trends were observed.

PCA analysis of matrix effects within a series and also in the context of molecular parameters showed no correlations. That is to be expected, since matrix effect is also dependent on the matrix components and concentrations of the components among other variables. Thus it was not expected that ME could be explained by analytes molecular parameters alone.

3.1.4. Linearity

The linear ranges for ESI (Figure 8 and Table 2) are in general wide and extend to low concentrations. ESI is closely followed by HESI, DA-APPI and MMI. All three have on average narrower linear ranges than ESI. The MMI-ESI source has slightly wider linear ranges that extend to lower concentrations compared to direct APPI and MMI-APCI ionisation modes. However, for MMI-ESI and especially for MMI-APCI the linear range for many compounds could not be determined, because for these compounds no linearity was observed (MMI-ESI and MMI-APCI) or the number of data points with significant signal was too small for linearity determination (MMI-APCI).

In the case of ESI the linear ranges could be determined for all compounds except for thiophanate-methyl (signals were obtained for concentrations range of less than 1 order of magnitude). Three compounds did not have a linear range with HESI, 2 compound with MMI, 24 with MMI-APCI, 10 with MMI-ESI, 7 with APPI and 6 with DA-APPI, because there were either not enough points for the given compound or linearity was not observed in the analysed range. In the case of 8 compounds (fenhexamid, mepanipyrim, bitertanol, methoxychlor, ditalimfos, tebufenozide, benalaxyl and quinoxyfen) the linear ranges could not be determined neither with MMI-ESI nor with MMI-APCI, but could be determined in with MMI. For trifloxystrobin linear range could not be determined with any arrangement of the MMI source.

When comparing the linear ranges compound-wise it was observed that ESI gave wider linear ranges for 68% of compounds compared to HESI, 72% compared with MMI, 94% compared with MMI-APCI, 84% compared with

MMI-ESI, 88% compared with direct APPI and for 60% of compounds with DA-APPI. Compounds that gave no linear range for one of the modes were not included in the percentage calculation. For ESI the geometric mean improvement of linear range width compared to other ionisation modes were 1.4, 1.5, 1.7, 1.5, 1.6 and 1.3 times for HESI, MMI, MMI-APCI, MMI-ESI, direct APPI and DA-APPI, respectively and the largest increase in linear range with ESI source compound-wise was 4.5, 5.0, 3.0, 5.0, 3.3 and 3.3 times, respectively. The largest decrease in linear range with ESI was 1.7, 1.3, 1.0, 1.1, 1.3 and 1.7 times narrower, respectively. Comparing the lower limits of linear ranges gives important information on the performance of ionisation modes in terms of quantitation at low analyte levels. Out of the 41 compounds 11 compounds in case of HESI had linear range extended to lower concentrations than ESI source. For MMI, MMI-APCI, MMI-ESI, direct APPI and DA-APPI these numbers were 12, 0, 5, 1 and 7, respectively.

It can be concluded that ESI has superior linear range, both in terms of width and lower limit of linear range, compared to HESI, MMI, MMI-APCI, MMI-ESI, direct APPI and DA-APPI. These results are not fully in line with those found in literature,[48,53] where ESI has generally not been excelling in terms of linearity. However it has to be kept in mind that the linear ranges are highly dependent on the compound analysed, as can be seen in Figure 8.

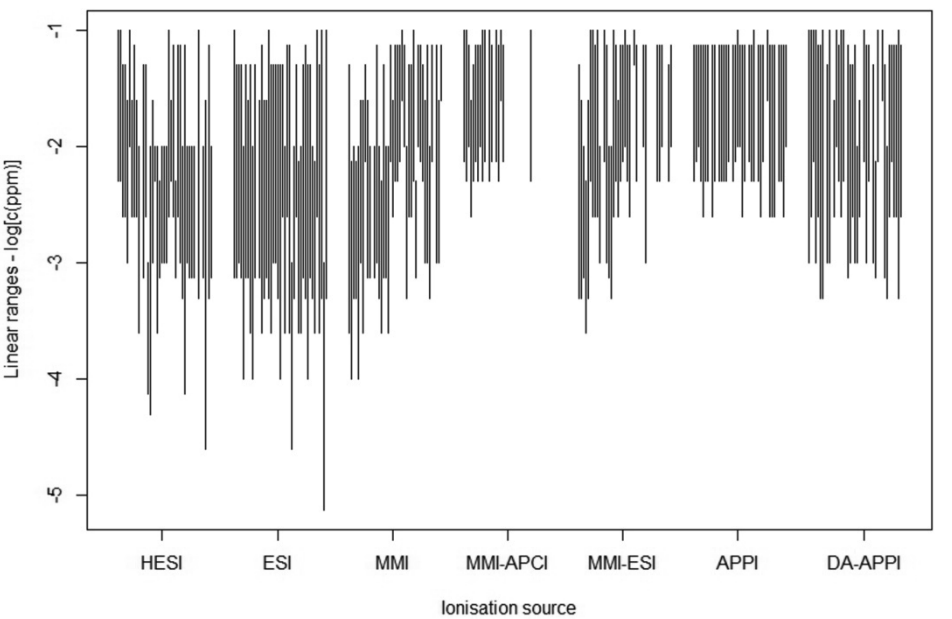

Figure 8. Linear ranges of different ionization modes. Each line marks a linear range for one compound. Absent lines denote compounds for which linear range could not be determined with the given ionization mode.

Table 2. Linear range upper limits (Uls, μg/kg) and lower limits (LLs, μg/kg) for 41 pesticides. Magnitude of each linear range (ML) is also shown. Compounds were chosen to cover different retention times.

	HESI			ESI			MMI			MMI-APCI			MMI-ESI			APPI			DA-APPI		
	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]
pymetrozine	100	5.0	1.50	100	0.75	2.25	50	0.25	2.25	100	7.5	1.25	50	0.50	2.00	75	5.0	1.25	100	1.0	2.00
thiamethoxam	100	5.0	1.50	50	0.75	1.75	7.5	0.10	1.75	100	5.0	1.50	25	0.50	1.75	75	7.5	1.00	100	2.5	1.75
vamidothion	50	2.5	1.25	50	1.0	1.50	10	0.50	1.50	75	10	0.75	10	0.75	1.25	75	10	0.75	100	7.5	1.25
methiocarb sulfoxide	50	2.5	1.25	50	0.75	1.75	7.5	0.50	1.25	25	2.5	1.00	5.0	0.25	1.25	75	5.0	1.25	100	1.0	2.00
chloridazon	25	1.0	1.25	10	0.10	2.00	10	0.10	2.00	50	5.0	1.00	25	0.50	1.75	75	2.5	1.50	75	2.5	1.50
imidacloprid	100	10	1.00	50	0.75	1.75	25	1.0	1.25	75	7.5	1.00	100	5.0	1.50	75	5.0	1.25	75	0.5	2.25
acetamiprid	25	2.5	1.00	25	0.75	1.50	25	0.25	2.00	75	5.0	1.25	100	2.5	1.75	75	5.0	1.25	100	0.5	2.50
methiocarb sulfone	75	2.5	1.50	50	0.25	2.25	50	7.5	0.75	75	10	0.75	75	2.5	1.50	[*1]			[*1]		
thiacloprid	25	2.5	1.00	10	0.10	2.00	25	0.75	1.50	100	5.0	1.50	100	2.5	1.75	75	2.5	1.50	50	1.0	1.50
imazalil	10	0.25	1.75	50	0.75	1.75	10	0.75	1.25	100	7.5	1.25	10	1.0	1.00	75	5.0	1.25	100	1.0	2.00
thiophanate-methyl	[*1]			[*1]			[*1]			[*1]			[*1]			[*1]			[*1]		
metribuzin	50	0.75	1.75	25	0.75	1.50	10	0.75	1.25	100	5.0	1.50	100	7.5	1.25	75	5.0	1.25	25	2.5	1.00
pyrimethanil	50	2.5	1.25	75	0.25	2.50	75	1.0	1.75	75	7.5	1.00	75	1.0	1.75	75	5.0	1.25	100	10	1.00
fenpropimorph	1.0	0.1	1.25	25	0.50	1.75	10	0.50	1.50	[*1]			10	0.75	1.25	75	5.0	1.25	75	5.0	1.25
spiroxamine	10	0.1	2.50	25	0.75	1.50	5.0	0.25	1.25	100	7.5	1.25	10	0.50	1.50	75	5.0	1.25	100	2.5	1.75
propoxur	25	1.0	1.25	100	0.50	2.50	50	0.75	1.75	75	5.0	1.25	100	2.5	1.75	75	7.5	1.00	100	5.0	1.50
triasulfuron	10	5.0	0.50	50	0.25	2.25	10	0.75	1.25	100	25	0.75	75	5.0	1.25	[*1]			[*1]		
bupirimate	10	0.25	1.75	50	1.0	1.50	10	0.25	1.75	75	7.5	1.00	25	2.5	1.00	75	5.0	1.25	75	0.75	2.00
paclobutrazol	5.0	0.75	0.75	50	1.0	1.50	75	7.5	1.00	[*1]			75	5.0	1.25	75	7.5	1.00	50	1.0	1.50
methiocarb	10	1.0	1.00	50	0.50	2.00	25	2.5	1.00	[*1]			75	7.5	1.00	100	10	1.00	50	2.5	1.25

Table 2. Continuation.

	HESI			ESI			MMI			MMI-APCI			MMI-ESI			APPI			DA-APPI		
	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]	UL	LL	ML[*2]
azoxystrobin	10	1.0	1.00	50	0.10	2.50	75	5.0	1.25	[*1]			100	10	1.00	75	10	0.75	75	1.0	1.75
epoxiconazole	10	1.0	1.00	50	2.5	1.25	75	5.0	1.25	[*1]			75	5.0	1.25	75	2.5	1.50	10	1.0	1.00
myclobutanil	100	2.5	1.75	10	0.25	1.75	75	7.5	1.00	[*1]			75	2.5	1.50	75	5.0	1.25	25	2.5	1.00
fenhexamid	25	5.0	0.75	75	2.5	1.50	100	25	0.75	[*1]			[*1]			[*1]			[*1]		
fluquinconazole	75	2.5	1.50	75	0.25	2.50	75	10	0.75	[*1]			100	50	0.50	75	7.5	1.00	100	7.5	1.25
flusilazole	5.0	0.75	0.75	1.0	0.025	1.75	10	0.50	1.50	[*1]			75	5.0	1.25	75	5.0	1.25	75	1.0	1.75
mepanipyrim	75	2.5	1.50	10	0.50	1.50	50	2.5	1.25	[*1]			[*1]			100	5.0	1.50	75	5.0	1.25
bitertanol	75	1.0	1.75	50	2.5	1.25	50	2.5	1.25	[*1]			[*1]			[*1]			[*1]		
propiconazole	10	0.5	1.50	7.5	0.25	1.50	100	5.0	1.50	[*1]			75	10	0.75	75	5.0	1.25	50	1.0	1.50
triazophos	75	0.1	3.00	10	0.25	1.75	5.0	0.75	0.75	100	5.0	1.50	75	1.0	1.75	75	2.5	1.50	7.5	0.75	1.00
methoxychlor	10	1.0	1.00	50	0.75	1.75	75	10	0.75	[*1]			[*1]			75	7.5	1.00	100	7.5	1.25
ditalimfos	10	0.75	1.25	75	0.50	2.25	75	7.5	1.00	[*1]			[*1]			[*1]			[*1]		
tebufenozide	10	0.75	1.25	50	0.10	2.50	50	5.0	1.00	[*1]			[*1]			100	25	0.75	100	25	0.75
benalaxyl	10	0.75	1.25	50	0.75	1.75	25	1.0	1.25	[*1]			[*1]			75	2.5	1.50	50	0.75	1.75
pyrazophos	[*1]			10	0.50	1.50	75	1.0	1.75	[*1]			75	5.0	1.25	75	2.5	1.50	10	0.5	1.50
buprofezin	100	0.5	2.50	7.5	0.25	1.50	10	0.50	1.50	[*1]			75	5.0	1.25	75	2.5	1.50	75	2.5	1.50
indoxacarb	[*1]			100	2.5	1.75	75	7.5	1.00	[*1]			75	10	0.75	[*1]			75	7.5	1.00
trifloxystrobin	10	0.75	1.25	50	0.25	2.25	[*1]			[*1]			[*1]			75	5.0	1.25	75	2.5	1.50
quinoxyfen	25	0.025	3.00	100	0.50	2.50	75	1.0	1.75	[*1]			[*1]			75	5.0	1.25	75	2.5	1.50
pirimiphos-ethyl	75	0.5	2.25	1.0	0.0075	2.25	25	1.0	1.25	[*1]			50	5.0	1.00	75	2.5	1.50	100	0.5	2.50
hexythiazox	10	0.75	1.25	100	0.50	2.50	75	25	0.50	[*1]			75	10	0.75	75	10	0.75	75	2.5	1.50

[*1] – linear range could not be determined either because of the concentration range or no linear range was observed

[*2] – magnitude of linear range: 1 represents 1 order of magnitude span of linear range

3.1.5. Limit of detection, signal-to-noise ratio and sensitivity

Sensitivity, S/N and LoD describe closely related performance abilities: to produce a large number of analyte ions (ionisation efficiency) and to produce as little noise as possible. These parameters were studied in acetonitrile as solvent, because the aim was to compare the performance of the different ionisation modes unaffected by matrix effects. From the results (Table 3 and raw data in Table A – 6 and Table A – 7) it can be seen that in terms of sensitivity, similarly to S/N, HESI source performs the best. HESI performance is closely followed by ESI. Also MMI and MMI-ESI show similar results, but have lower sensitivity than ESI. MMI-APCI and both APPI setups are the least sensitive and have lowest S/N. As expected, DA-APPI gave higher sensitivity (3 times) than direct APPI.

It is interesting to note that according to the LoD results (Table 3 and Figure 9) ESI is on the average comparable to HESI, although the latter has by an order of magnitude better sensitivity (slopes). A possible interpretation is that HESI is more effective than ESI in ionising both the analyte, and the matrix components.[67] This leads to the increase of the signal, but also to the increase of (chemical) noise. As a result, the standard deviation of residuals at low concentrations increases and causes higher LoDs (see Equation 1). In Figure 9 in comparison with ESI and HESI, MMI-ESI, MMI and DA-APPI ionisation modes give significantly higher LoD values. The highest LoD values were found with MMI-APCI and direct APPI and DA-APPI ionisation modes. In compound-wise comparison in Table 3 MMI-APCI and both APPI modes give the highest LoDs, which is in agreement with the general picture seen with S/N and sensitivity.

For DA-APPI increase in sensitivity has been reported in the literature previously when analysing pharmaceuticals[7] and flavonoids.[43] In another study improvement of LoD was not observed when adding toluene as dopant when analysing polymer additives.[8] In this study DA-APPI does not perform much better than direct APPI. This is somewhat surprising, because one would expect that dopant would enhance the ionisation of analyte and not affect the noise levels. This was not the case in this study. Although analyte ionisation was indeed enhanced, the noise levels were elevated also, cancelling out the gain of enhanced ionisation, resulting in comparable LoD values.

Although in the literature ESI has been found to have lower S/N than APCI or APPI,[48,53] this study and the one carried out by Garcia-Ac et al.[50] demonstrate that ESI has higher S/N.

Table 3. Comparison of S/N, sensitivity and LoD. APPI denotes direct APPI and DA-APPI denotes dopant assisted APPI in acetonitrile as solvent. Geometric means (GM) were calculated from respective parameters ratios of two ionisation sources with geometric standard deviation (s_g).

	S/N		**Sensitivity**			**LoD**	
	GM	s_g	GM	s_g		GM	s_g
HESI/ESI	3.2	2.3	11	1.7	ESI/HESI	1.1	2.2
HESI/MMI	4.2	3.1	83	3.8	MMI/HESI	4.5	6.1
HESI/MMI–APCI	11	4.5	378	2.8	MMI–APCI/HESI	40	7.9
HESI/MMI–ESI	4.7	3.8	57	3.4	MMI–ESI/HESI	6.1	7.3
HESI/APPI	16	5.2	362	3.1	APPI/HESI	33	4.5
HESI/DA–APPI	22	6.1	104	2.7	DA–APPI/HESI	32	4.6

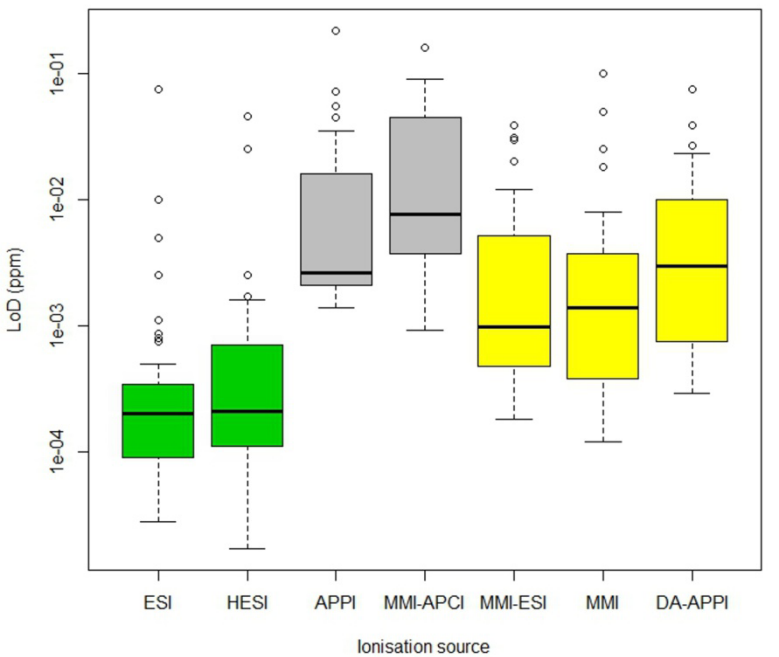

Figure 9. Boxplots of LoDs for different ionisation modes. Measurements were carried out in acetonitrile as solvent.

3.1.6. Principal Component Analysis of LoD results

PCA was used in order to gain better understanding on which properties facilitate low LoD values on one or another ionisation source. The LoD PCA plot for 3 principal components explaining 84% of data variance is presented in Figure 10. From the PCA plot the ionisation modes grouped into 3 distinct groups. First group shows that HESI and ESI have similar profiles, with the fluctuations in the LoD values follow similar logic (not the same values). The correlation of the LoD values of ESI and HESI was high (R^2=0.87). APPI and DA-APPI also follow similar logic in LoD values and the 3 MMI modes form a third group. The correlation of the LoD values were not as high as ESI and HESI had shown: APPI and DA-APPI had R^2=0.56 and others were even worse. These groups seem to be formed based on ionisation mechanisms. However, MMI-ESI would be expected to be more similar to ESI, but this is not the case. It seems that there is some other process affecting the 3 MMI modes that seems to be sufficiently different from the ESI ionisation mechanism. One of the reasons could be that MMI-ESI has distinctly different geometry than HESI and ESI.

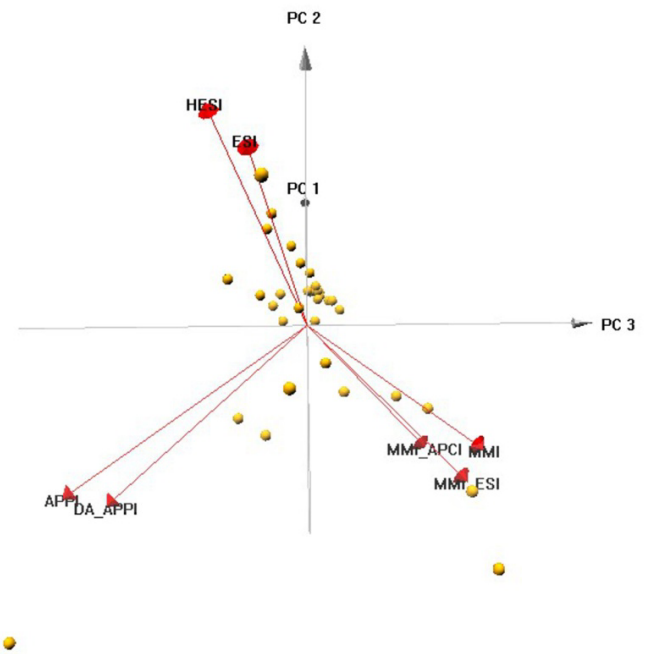

Figure 10. PCA plot for LoDs of different ionization modes. 84% of data variance is explained by first 3 principal components.

PCA was also carried out on the basis of the molecular parameters shown in Table A – 4 in Appendices. It was attempted to group the data points on the 3D plot according to LoD results, i.e. the LoD results did not affect the plot. However there were no clear correlations between the molecular parameters and LoD results. It can be because too few compounds were analysed for such analysis.

3.1.7. Conclusions of comparison

The ionisation mechanism in different ionisation modes is an active research field.[14,31,35,63] Nevertheless, some general trends are evident. Interestingly, the MMI source underperformed in comparison to the traditional ESI, although in theory it should have performed better,[21,25] because of the addition of APCI. It is the opinion of the authors that at least part of the reason is that the geometry of the MMI ionisation chamber is optimal neither for ESI nor for APCI. The nebuliser capillary tip of the MMI source is situated at a much larger distance from the MS inlet than in the conventional ESI source. Also there is a small separating wall perpendicular to MS inlet functioning as separator of the ESI and APCI regions in the source. Because of this separating wall some part of the nebulised effluent is directed further away from MS inlet, causing decreased sensitivities and increased LoDs. This design of the ionisation chamber can be the reason why MMI-ESI performs better than MMI-APCI when the respective traditional APCI has in some cases shown to perform better than traditional ESI.[43–45,48,49,51,57]

The unexpected underperformance of HESI may also be caused by the source design. HESI uses additional sheath gas to confine the spray plume for enhanced desolvation of droplets.[20] However, the addition of sheath gas seems to increase matrix effects both in this study and in the report by Stahnke et al.[19] HESI is significantly more prone to chemical noise, which is the generation of ions from matrix components other than the analyte.[67] Thus the advantage in sensitivity, achieved by more efficient ionisation of the analyte is partially offset by the increase in chemical noise.

The poor sensitivity and high LoD values of direct APPI can be explained by the compounds properties used for the comparison. Although the pesticides vary significantly in polarity (log*P* between –0.2 to 5), they are still small molecules having ionisable functional groups (all have at least one nitrogen or oxygen atom in molecule), thus the poor performance might be caused by the compounds that are not APPI specific. DA-APPI showed a significant improvement compared with direct APPI in the case of matrix effects and linear range. However, as the overall conclusion, the sensitivity and LoD values of DA-APPI are still much inferior to ESI.

3.2. Novel 3R nebuliser for ESI source

Novel 3R nebuliser offers a new concept of the ESI source introduced by Kruve et al.[72]. While the commercial nebuliser has spraying gas around the effluent only, the novel nebuliser introduces additional gas inside the effluent as is shown in Figure 11. It is hypothesised that the inner nebuliser gas gives additional nebulising power to generate smaller droplets and enhanced ionisation of analytes.

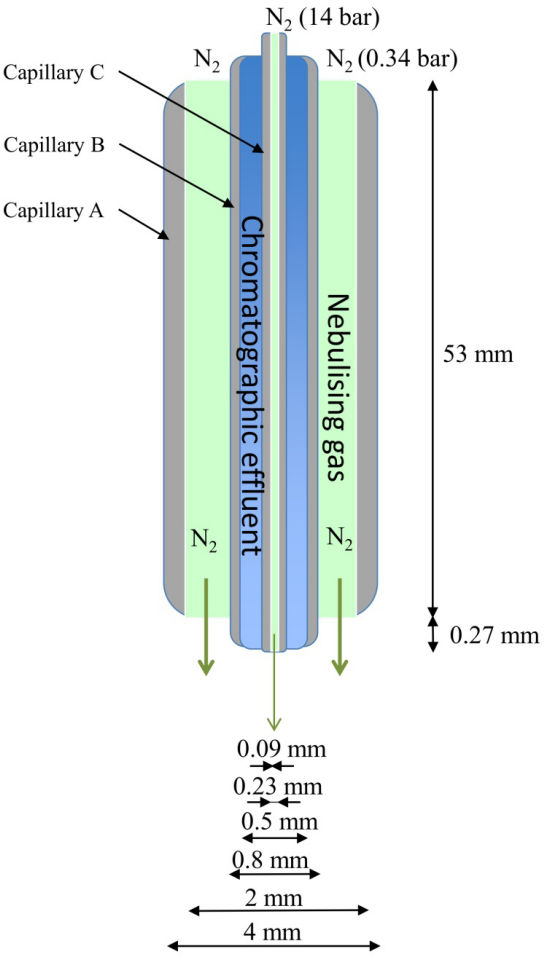

Figure 11. The dimensions of optimized 3R nebuliser with optimal gas pressures used.

3.3. 3R nebuliser optimisation

In order to determine the influence of the nebuliser parameters (as opposed to the parameters of the MS itself) on the analyte signals it is important to know if the MS parameters (the ones that influence ion transport inside the MS) need to be optimised simultaneously with the nebuliser parameters or may be fixed. For establishing this, 5 analytes (3 pesticides, namely thiamethoxam, paclobutrazol, etofenprox and 2 pharmaceuticals, namely meropenem and ertapenem) were chosen randomly and MS parameters were optimised (according to the procedure described by Kruve et al.[64]) for different nebuliser designs – two different ID-s of capillary B, with and without Capillary C for 3R nebuliser and a commercial nebuliser (altogether 5 different nebuliser designs).

The optimisation plots provided by the MS software (intensity versus optimised parameter value) were compared for different nebulisers and it was observed for all nebulisers and all analytes that only the optimal capillary voltage – the voltage applied between ESI nebuliser and MS entrance – changed significantly from nebuliser to nebuliser. Therefore all MS parameters excluding Capillary Voltage were individually optimised for analytes with the commercial nebuliser and fixed for nebuliser parameters optimisation. The used MS ion optics and fragmentation parameters are presented in the Table A – 2.

3.3.1. Testing different nebuliser parameters

The primary optimisation of nebuliser parameters was carried out using a two-level full factorial design model, where the parameter level values were chosen according to our previous experience (specified in chapter 1.10.3). The optimisation was performed using two types of samples: pesticides in solvent and pesticides in garlic extract. The results for pesticides (both solvent and garlic samples) and pharmaceuticals were analysed with ANOVA and a GLM model was used to estimate dependence of each analyte's signal on nebuliser parameters. From ANOVA results (data not shown) it was observed that for the majority of analytes most of the parameters and two-parameter combinations influenced statistically significantly the peak areas of the analytes. Therefore, all parameters and two parameter interactions were chosen for all analytes into the GLM model. Even though the actual relation between the analyte signal and parameter values may not be linear the GLM model is a good approximation (data shown in section 1.14.2) for describing the effects of different nebuliser parameters on the analyte signal.

The GLM coefficients for each analyte are given in Table A – 8. In Figure 12 the coefficients were averaged over all analytes and samples, because we aim to find nebuliser parameters that are suitable for a wide range of analytes in both simple and complex matrices. The error bars indicate the standard deviations of the averages. As the parameter values were scaled and centred, before fitting the GLM model, comparison of the coefficients can be used for comparing the physical significance of the parameters and parameter combinations. It can be seen from Figure 12 as well as from the non-averaged data in table A – 8, that for the analytes (both pesticides and pharmaceuticals) some of the nebuliser parameters tend to be significantly more important than others (in the parameter value ranges used in this study) and these effects are independent of the analyte and matrix. For example in our case the gas pressure in Capillary C tends to influence the MS response more significantly than the other studied parameters. Capillary B ID and Capillary C ID are somewhat less significant. All of these three parameter coefficients are also statistically significantly different from 0 according to the t-test, indicating their overall importance in nebulisation process for the variety of analytes and matrixes studied. The fact that optimisation results agree well for different analytes and different matrixes

(as indicated by the t-test) confirms that the 3R nebuliser with optimal parameters can be universally used for analytes of wide polarity range and matrixes of different complexity.

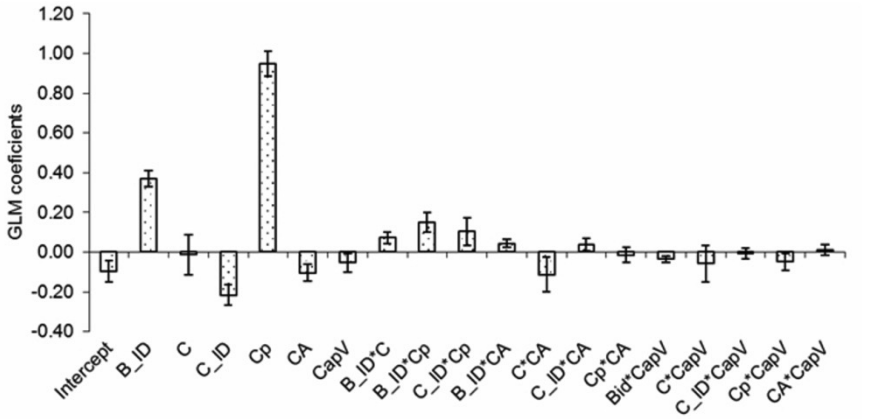

Figure 12. GLM coefficient values for different parameters and parameter combinations (abbreviations described in section 1.10.3). The values are given as averages over all pesticides and error bars represent standard deviations of the average coefficients. Similar results were also observed for pharmaceuticals (see Appendices Table A – 8).

3.3.2. Finding optimal nebuliser parameters and creating a model describing effects of different parameters

The coefficients averaged over all analytes and matrices for the 3 most significant parameters were used to predict the analytes signals:

$$S_{analyte} = (0.37 \pm 0.04) \times B_{ID_S} - (0.22 \pm 0.05) \times C_{ID_S} + (0.95 \pm 0.06) \times Cp_S \quad (9)$$

where $S_{analyte}$ is the scaled predicted analyte signal, B_ID_s, C_ID_s and Cp_s are the scaled values of Capillary B ID, Capillary C ID and gas pressure in Capillary C. Standard deviation of the obtained coefficients is presented as ±. Correlations between the predicted signals and measured signals (scaled within one analyte and sample type e.g. solvent or garlic extract) was studied for all analytes. For the majority of the compounds 60 to 80% of the signal variation (i.e. R^2 between 0.6 and 0.8) can be explained by the variation of only these three nebuliser parameters (Capillary B ID, Capillary C ID and gas pressure in Capillary C). The GLM model in our approximation is used as an indicative tool, therefore the descriptive properties of this model are sufficient according to the observed R^2 values. Importantly, the correlation analysis for standard solutions and samples was carried out separately as our model only aims to account for signal variation due to nebuliser and not for signal variation due to matrices.

It can be concluded from these data that the major changes in ESI/MS signal can be described by changes in capillary dimensions and nebuliser gas pressure (Capillary C). It is clear that in the dimension range that was used in this work a capillary B with a wider ID and capillary C with smaller ID should be preferred, but first of all, and independently of the capillary dimensions, the increase of gas pressure in Capillary C increases analyte signal. On the other hand, the "conventional" nebuliser gas pressure (CA in Figure 12) is significantly less influential. In this study we have used gas pressures to describe the gas flow rates in the capillaries, because pressures are better accessible experimentally. It is of interest if the actual effect arises from gas pressure or flow rate (either volumetric ml/min or linear velocity mm/s). In order to answer this question we used two different capillary C-s (different ID – 90 and 175 um) at 5 different pressures (6 to 14 bar) resulting in 5 different gas flow rates for each Capillary C. Also 8 different eluent flow rates were used for each capillary C gas pressure. Thereafter we correlated the results for both Capillary C-s. The correlation between the results obtained with both capillary C-s was carried out and it was observed that the best correlation was observed if gas pressures (not flow rates) were used (R^2 0.880 with gas pressure compared to R^2 of 0.022 with gas flow rate). The slope of the correlation line was 0.959. Therefore we conclude that using the gas pressures in the GLM is justified.

3.3.3. Influence of eluent flow rate on the ionisation

In order to study the effect of eluent flow rate on the ionisation efficiency a model compound imazalil was chosen and the peak areas of imazalil were studied at different eluent flow rates (0.05 to 0.4 ml/min) and different Capillary C gas pressures (Figure 13A). It was observed that independently of the Capillary C gas pressure imazalil signals decreased with increasing eluent flow rate (maximum decrease observed was 2 times, eluent flow rate changed from 0.05 to 0.4 ml/min, at Capillary C pressure 14 bar). This finding is very similar to that of Page et al.[90], who observed that the ion transportation efficiency decreases with increasing mobile phase flow rate. The decrease of ion transport efficiency may be the major cause of this effect also in our instrument. On the other hand, independently of the eluent flow rate higher Capillary C gas pressures yielded higher signal intensities, thereby indicating that higher capillary C gas pressure increases the number of desolvated ions reaching the mass analyser. The highest signal increase while using Capillary C pressure 14 bar instead of 6 bar was more than 4 times, occurring at flow rate 0.05 ml/min.

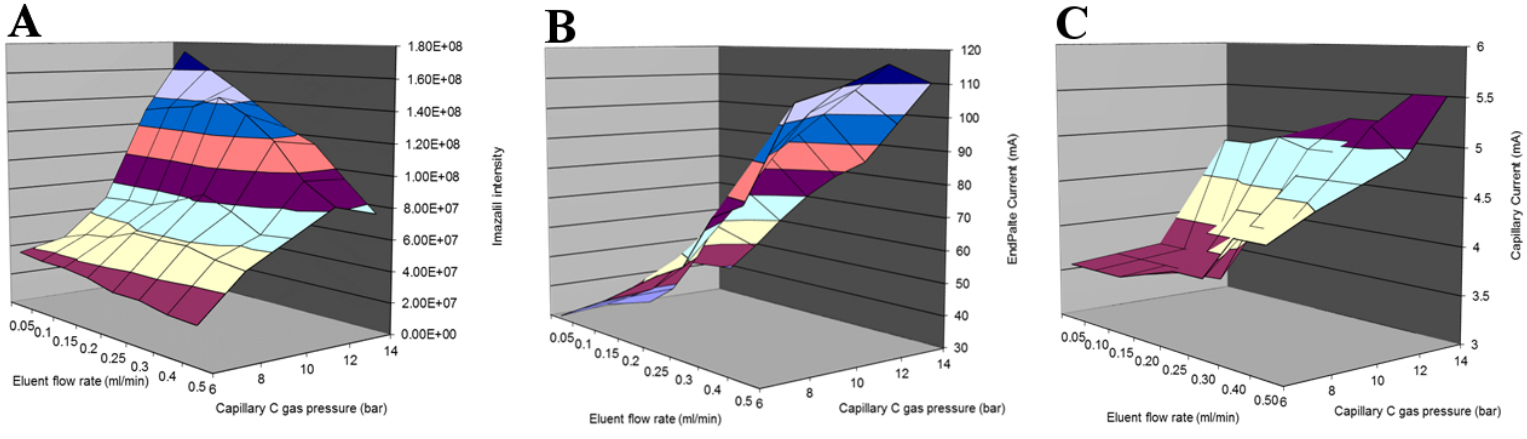

Figure 13. Relationships of different parameters to Capillary C pressure. A: Imazalil MS signal intensities depending on the eluent flow rate and Capillary C gas pressure. B: End plate current (mA) depending on the flow rate and Capillary C gas pressure. C: Capillary current (mA) depending on the flow rate and Capillary C gas pressure (Cp).

Additionally, the endplate current and capillary current – measuring the number of ions (including solvated ions) neutralised at the MS entrance and ion transportation capillary, respectively – were studied (Figure 13 B and C). It was observed that both higher eluent flow rates and higher gas pressures lead to the increase of the end plate and capillary current. The increase of endplate and capillary current with increasing gas pressure is most probably due to the fact that the spray plume widens if the inner nebuliser gas pressure increases. Therefore besides more ion entering the capillary, more ions also collide with the MS entrance. On the other hand increasing gas pressure increases MS signal. This means that even though more ions are lost in the MS ion optics, more ions also reach the ion trap. Therefore it can be concluded that increasing the inner capillary gas pressure increases the ionisation efficiency. It has been previously described by Page et al.[90], that the net ion current entering the mass analyser is not the most important parameter affecting sensitivity. Even more important is the number of desolvated ions reaching mass analyser, as only these ions actually contribute to the useful signal.

3.3.4. Universality of 3R nebuliser

The 3R nebuliser with optimised parameters (capillary B ID 0.5 mm, C ID 90 μm and capillary C pressure 14 bar) was compared with the commercial Agilent nebuliser originally implemented in the used MS system in order to evaluate the universality of the novel 3R ESI nebuliser. Comparison of sensitivity and LoD for 20 different analytes (Table 4) was done with both nebulisers. The analytes ranged from polar (oxamyl with log*P*= –0.5) to highly non-polar (etofenprox with log*P*=6.7). Four different sample matrices with varying complexity –ranging from solvent to the very complex garlic matrix – were used for pesticides (for 4 pharmaceuticals only solvent was used), resulting in 68 analytematrix combinations. Within the same matrix the comparison of nebulisers was done on the same day in order to minimise variations due to other factors.

Initial results[72] indicated that 3R nebuliser might be more sensitive than commercial ESI nebuliser (when comparing the results of the 3R nebuliser with and without the inner gas capillary). Subsequent experiments during optimisation showed the calibration graph slopes to be statistically insignificantly different for 3R nebuliser in comparison with commercial nebuliser.

On the other hand, the achievable LoD values were found to be different for two nebulisers (LoD values are presented in Table 4). In the case of two pesticides – imazalil and spiroxamine – the sensitivity was very high with both nebulisers. Therefore the LoD in solvent was indicated to be below <0.0001 mg/kg, which is significantly below the required working range for pesticide analyses. The data obtained are interpreted in the context of significant

Table 4. LoD (mg/kg) values for pesticides and pharmaceuticals with the 3R and commercial nebuliser and the geometric mean improvements with 3R nebuliser.

	Solvent			Garlic			Tomato			Onion		
	3R	commercial nebuliser	**Improve-ment**	3R	commercial nebuliser	**Improve-ment**	3R	commercial nebuliser	**Improve-ment**	3R	commercial nebuliser	**Improve-ment**
Carbendazim	0.001	0.01	**10.0**	0.005	0.01	**2**	0.001	0.005	**5**	0.0005	0.01	**20**
Thiabendazole	0.0005	0.0005	**1.0**	0.0001	0.0001	**1**	0.0001	0.0005	**5**	0.0001	0.0001	**1**
Acetamiprid	0.01	0.005	**0.5**	0.01	0.1	**10**	0.0005	0.005	**10**	0.005	0.005	**1**
Mepanipyrim	0.0005	0.0005	**1.0**	0.05	0.005	**0.1**	0.0005	0.001	**2**	0.0005	0.005	**10**
Methiocarb	0.005	0.005	**1.0**	0.5	0.5	**1**	0.001	0.005	**5**	0.005	0.01	**2**
Oxamyl	0.050	0.1	**2.0**	0.05	0.1	**2**	NA	NA	**NA**	0.1	0.05	**0.5**
Thiacloprid	0.0005	0.0005	**1.0**	0.001	0.001	**1**	0.0001	0.0005	**5**	0.0001	0.0005	**5**
Imidacloprid	0.050	0.050	**1.0**	0.005	0.05	**10**	0.005	0.1	**20**	0.005	0.05	**10**
Thiamethoxam	0.0005	0.1	**200.0**	0.001	0.01	**10**	0.0005	0.01	**20**	0.001	0.01	**10**
Imazalil	<0.0001	<0.0001	**NA**	0.005	0.01	**2**	0.0001	0.0001	**1**	0.005	0.01	**2**
Spiroxamine	<0.0001	<0.0001	**NA**	0.005	0.05	**10**	0.0001	0.001	**10**	0.005	0.005	**1**
Buprofezin	0.050	0.500	**10.0**	0.005	0.005	**1**	0.0005	0.001	**2**	0.005	0.001	**0.2**
Vamidothion	0.0005	0.010	**20.0**	0.005	0.005	**1**	0.005	0.01	**2**	0.01	0.01	**1**
Hexythiazox	0.005	0.005	**1.0**	0.1	0.05	**0.5**	0.005	0.01	**2**	0.05	0.05	**1**
Etofenprox	0.0005	0.001	**2.0**	0.001	0.005	**5**	0.0005	0.0005	**1**	0.001	0.005	**5**
Fluquinconazole	0.0005	0.0005	**1.0**	0.01	0.01	**1**	0.0005	0.001	**2**	0.05	0.01	**0.2**
Ofloxacin	0.0008	0.0008	**1.0**									
Norfloxacin	0.0008	0.0009	**1.2**									
Ciprofloxacin	0.0011	0.0009	**0.8**									
Sulfadimethoxine	0.0003	0.0034	**10.0**									
			2.4			**1.9**			**3.9**			**1.9**

improvement for the ESI/MS users. We consider that the lowering of LoD by more than 2 times might be important for practical users (although even smaller changes can be statistically important). Altogether approximately 30% of the analytes showed significantly lower LoDs (improvement more than 2 times) with 3R nebuliser (24 out of 68 analyte-matrix combinations). It is worth mentioning that improvement was achieved not only for an easy matrix – solvent – but also for very troublesome matrices such as garlic and onion. Only 3 samples out of 68 (4%) showed significantly higher (more than 2 times) LoD values with the new 3R nebuliser. The remaining analyte-matrix combinations did not show significant change of LoD. On the average (across all analytes and matrices) the 3R nebuliser gave LoD improvement of 2.5 times. That is higher than the average results reported in[72] in initial results, where the average was 1.7 times better for the 3R nebuliser in comparison with commercial nebuliser (according to the geometric mean).

The reason for the lower LoDs of 3R nebuliser compared to the commercial nebuliser, while sensitivity was not affected, is revealed by looking at the chromatograms. Figure 14 displays the chromatograms of carbendazim with the native commercial nebuliser and 3R nebuliser. It is observed that in the case of the native nebuliser the background noise is around 50 times higher than with the 3R nebuliser. Even though these data are recorded in MS/MS mode this shows that on the average the 3R nebuliser has clear advantages over the native commercial nebuliser.

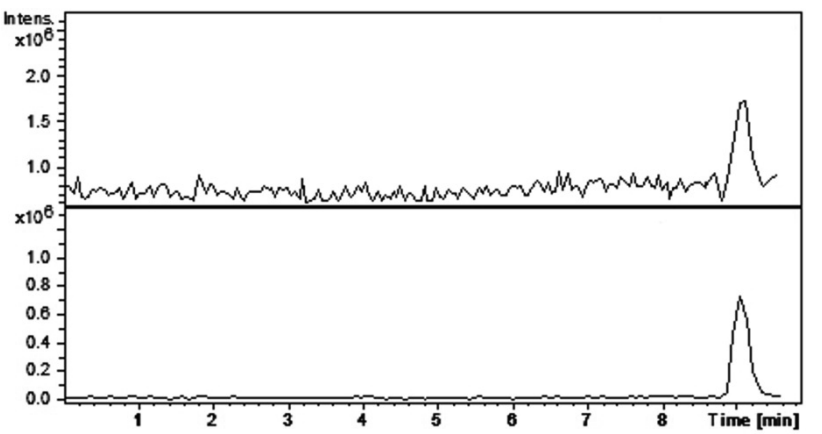

Figure 14. Comparison of carbendazim chromatograms at 0.01 mg/kg in solvent with commercial (above) and 3R nebuliser (below).

In addition to the universal applicability the 3R nebuliser also shows better robustness. The robustness of 3R nebuliser was compared with commercial nebuliser with 20 consecutive injections of garlic extract spiked with imazalil, carbendazim and thiabendazole (Figure 15).[72] Garlic was chosen because it is a complex matrix giving more matrix effects than many others.[89] For commercial nebuliser the signal decreased by 57% for imazalil with sudden decrease

starting from the 6th injection, while maximum decrease of 34% was observed for 3R nebuliser over a much smoother decline. 45% and 16% increase in signal was observed in case of carbendazim and thiabendazole, while for 3R nebuliser the corresponding numbers were 15% and 29%. While the results are worse for thiabendazole in case of 3R nebuliser, the sudden decline and the amplitude of the decline for the two other compounds are drastic for the commercial nebuliser. Also, during this work novel 3R nebuliser has been used for over four years without any need for replacement of details. On the other hand commercial nebuliser needs maintenance (capillary replacement more than once a year) and it is prone to clogging as well as nebuliser capillary tip contamination. Thus it can be concluded that 3R nebuliser is more robust compared to commercial nebuliser.

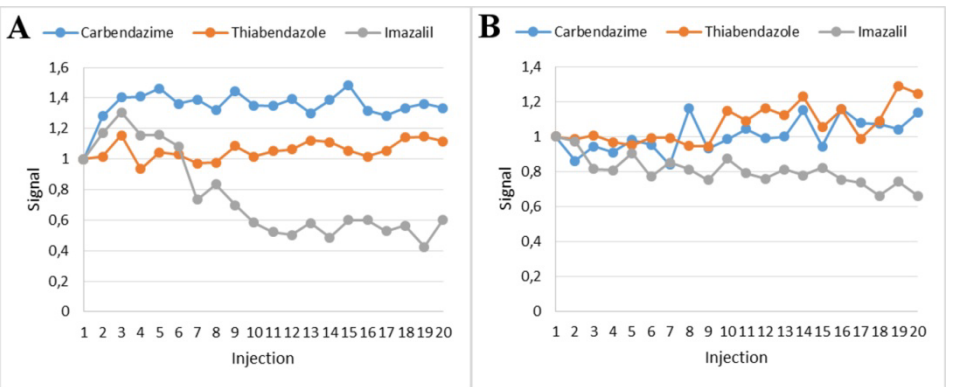

Figure 15. Comparison of robustness of the native commercial nebuliser (A) with the novel 3R nebuliser (B). Robustness was measured by change of the MS signal for either nebuliser during repetitive injections of garlic extract spiked with carbendazim, thiabendazole and imazalil.

3.4. Practical applications of the novel 3R ESI source: neonicotinoids in honey

The optimised nebuliser was compared with commercial nebuliser on a practical analysis example in order to determine how it performs under "field conditions". One important application is residue analysis, such as determination of neonicotinoids in honey samples.

Neonicotinoids, e.g. imidacloprid and acetamiprid, are a relatively new class of insecticides. Neonicotinoids affect the nervous system of the insects through nicotinic acetyl choline receptors. These pesticides are widely used because they act as strong agonists, activating the nicotinic acetyl choline receptors of insects. However, the effect is not as significant for vertebrates. They are therefore highly toxic for insects, but are generally considered only moderately harmful to vertebrates.[91–93]

Besides determination of the exposure of bees to neonicotinoids, it is increasingly important to determine neonicotinoids in products consumed by humans. This is illustrated by a study published in 2014 on the analysis of 573 fruit and 850 vegetable samples collected randomly from a market in the Aegean region in Turkey between 2010 and 2012. From 186 pesticide residues determined in the study, one of the three most frequently detected pesticides was acetamiprid (in over 20 fruit and over 120 vegetable samples). In addition, imidacloprid was frequently detected (in over 20 fruit and over 40 vegetable samples). Thiamethoxam was observed less frequently.[2]

3.4.1. Results of nebulisers comparison

For the analysis of honey samples the 3R nebuliser and the native ESI nebuliser were compared in terms of fitness for purpose. The MRLs of neonicotinoid pesticides are low. Thus, the analysis of neonicotinoids is a trace analysis and it is important to choose the nebuliser that gives the lowest LoDs and has linear ranges extending to low concentrations. Thus the two nebulisers were compared by LoD, linear range, process efficiency and repeatability (as RSD).

The LoD results for commercial nebuliser were 0.088 mg/kg, 0.030 mg/kg, 0.020 mg/kg and 0.0031 mg/kg for thiamethoxam, imidacloprid, acetamiprid and thiacloprid, respectively. For 3R the LoD values were 0.10 mg/kg, 0.018 mg/kg, 0.0056 mg/kg and 0.0014 mg/kg for thiamethoxam, imidacloprid, acetamiprid and thiacloprid, respectively. In the case of LoD values 3R presents advantages compared to the commercial nebuliser. Ratios of LoD values of 3R nebuliser to commercial nebuliser give 0.9 for thiamethoxam, 1.7 for imidacloprid, 3.7 for acetamiprid and 2.2 for thiacloprid with average of 2.1 times improved LoD in case of 3R nebuliser. Literature overview shows that the LoD for neonicotinoids in honey were in the range of 0.15–160 μg/kg for thiamethoxam, 0.03–33 μg/kg for imidacloprid, 0.04–0.5 μg/kg for acetamiprid and 0.02–0.5 μg/kg for thiacloprid[77,94–98]. While the values for thiamethoxam and imidacloprid obtained in this work are within the range found in literature, for acetamiprid and thiacloprid they are 3–11 times higher for 3R nebuliser and 6–40 times higher with the commercial nebuliser. However, LoD values are strongly dependent on the approach used for their determination.[80] Our previous knowledge shows that the approach used in this work gives very conservative values and thus results in significantly higher values than LoD estimates based on signal-noise ratio. Because the difference between LoQ and LoD is usually only multiplication of the latter with 3.3 or 3, the obtained LoQ values are also more conservative due to the method used for LoD determination.

In Table 5 and Figure 16 the linear ranges and LoQ values for both nebulisers and MRL values are shown. Comparing LoQ values with the MRL values shows that with the commercial nebuliser only LoQ for thiacloprid is below the MRL value, but with the 3R nebuliser both acetamiprid and thiacloprid LoQ values were below MRL and LoQ for imidacloprid is at MRL.

As can be seen in Table 5 the linear range is better or comparable for the 3R nebuliser (as compared to the commercial nebuliser). The linear range extends to lower concentrations for all neonicotinoids except thiamethoxam. For thiacloprid the linear range of the 3R nebuliser is stretching to both larger and smaller concentrations compared to commercial nebuliser, while for the latter linear range barely reaches the LoQ value. It can be assumed that concentrations of the 4 neonicotinoids in real honey samples are more likely at smaller concentrations below MRL values, thus it is preferred to use the 3R nebuliser for determination of neonicotinoids in honey samples.

Table 5. Comparison of novel 3 nebuliser with commercial nebuliser.

	m/z transitions		LoQ (mg/kg)		MRL (mg/kg)	Linear range (mg/kg)	
	Precursor ion	Product ions	Commercial	3R		Commercial	3R
thiamethoxam	314	210[b], 180	0.27	0.31	NA[a]	0.27–2.0	0.31–2.0
imidacloprid	256	175[b], 209	0.092	0.055	0.05	0.092–0.62	0.055–0.31
acetamiprid	223	126[b], 187	0.062	0.017	0.05	0.062–0.624	0.017–0.16
thiacloprid	253	126[b], 186, 226	0.0095	0.0043	0.2	0.012–0.078	0.0047–0.16

[a] MRL for thiamethoxam (0.01 mg/kg) is given as sum of thiamethoxam and clothianidin,
[b] quantitation ion.

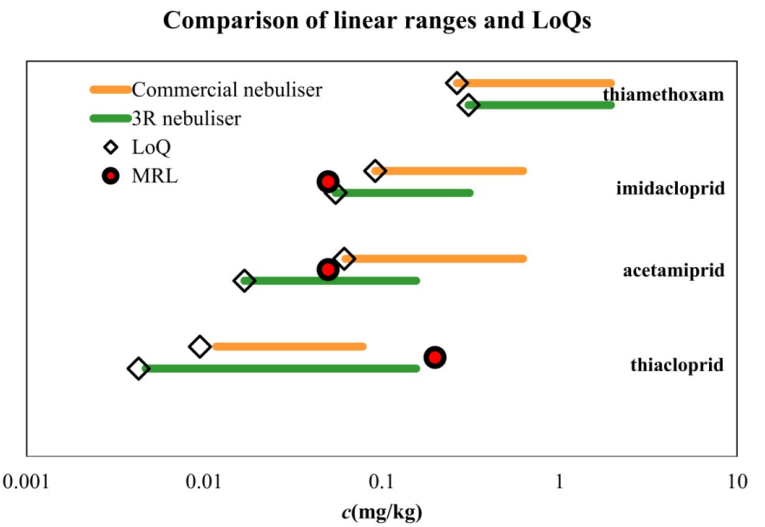

Figure 16. Comparison of linear ranges and LoQs.

According to SANCO/12571/2013 suggestions, repeatability values should be below 20%. As can be seen in Figure 17 the pooled RSD values of the 3R nebuliser were below the suggested 20%, with only upper confidence limit for thiacloprid at low end of linear range slightly over 20%. For commercial nebuliser, pooled RSD values were also below the limit, but upper confidence intervals for acetamiprid and thiamethoxam stretched significantly above the limit.

According to SANCO, the recovery values should be between 70–120%. In the context of LC/ESI/MS it is appropriate to compare these limits to process efficiency (PE) not to recovery, because PE also takes into account possible ionisation suppression. In Figure 18 the PE values with the SANCO limits and confidence intervals are shown. For 3 of the neonicotinoids – thiamethoxam, imidacloprid, thiacloprid – the PE values with confidence intervals were within recommended limits. For acetamiprid the averages were outside the limits at high end of the linear range and lower confidence intervals stretch outside of limits in case of low end of linear range. This however was not a problem in the current analysis since acetamiprid was expected at low concentrations (if at all) and PE values at low end of linear range were acceptable.

Considering the results from validation the 3R nebuliser is superior to commercial nebuliser for the residue analysis of the 4 neonicotinoids in honey and can be recommended for analysis of neonicotinoids in honey samples. Although honey samples can vary a lot in composition and only 3 different honey sorts were used for validation, it is safe enough to assume that the 3R nebuliser would prove also more efficient than commercial nebuliser for other honey types based on validation results.

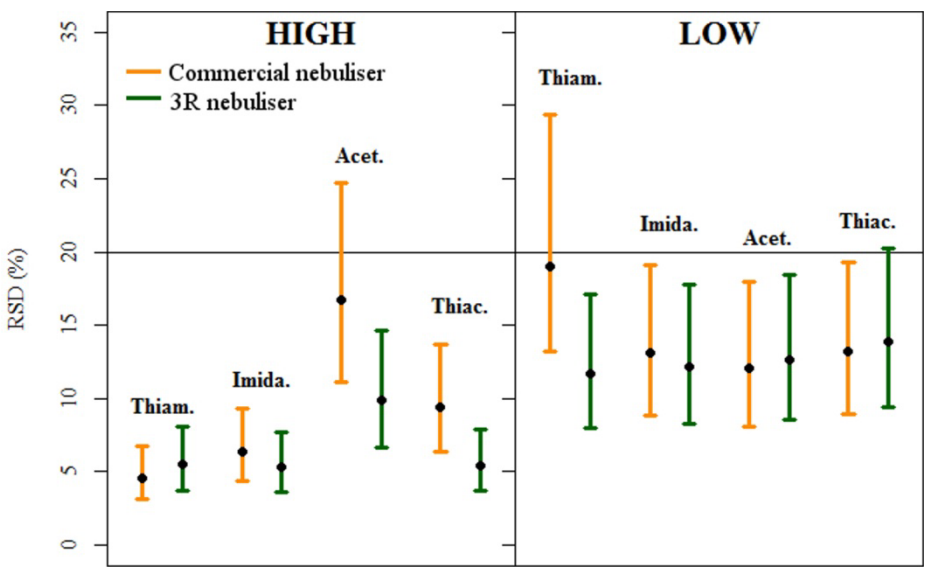

Figure 17. Comparison of RSD values for all analytes with both ESI nebulisers used. Error bars denote confidence interval at 95%. Suggested RSD values by SANCO are shown as lines at 20%.

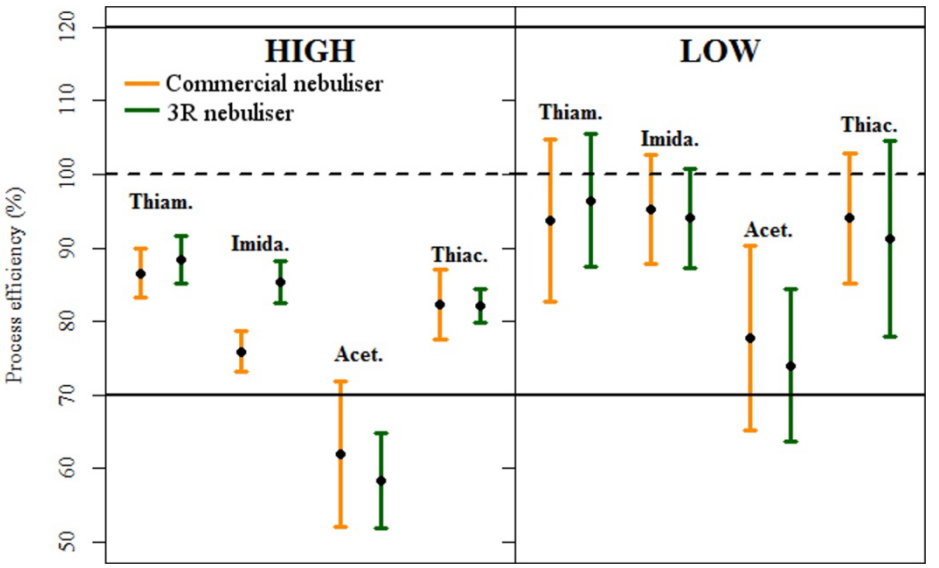

Figure 18. Comparison of process efficiency values for all analytes with both ESI nebulisers used. Error bars denote confidence interval at 95%. Suggested process efficiency values by SANCO should be between lines at 70% and 120%.

SUMMARY

There are numerous novel developments aiming to improve the interface between LC and MS. Novel commercial ion sources need to be compared with each other in order to evaluate their advantages. For some novel ion sources the comparison has been done in this study, occasionally with surprising results.

Comparison of the conventional ESI source with some of the newer developments in commercial ionisation sources, such as HESI, MMI (ESI-APCI) and APPI sources demonstrated that the ESI source is on an average the most robust and sensitive method for generating ions in the interface of LC and MS. While HESI proved to be superior in sensitivity and LoD over most other sources used in comparison and DA-APPI was robust when considering matrix effects, only ESI was among the leaders in all comparison parameters. The LoD values of ESI were comparable to HESI and the best matrix effects were observed with ESI source. ESI source also proved to be comparable or better in repeatability, linear ranges and sensitivity with respect to other sources.

The novel 3R nebuliser for ESI source recently developed in our group was compared to the conventional ESI source. Optimization of nebuliser parameters was successfully concluded during the study. Analysis of the main nebuliser parameters affecting the ionisation showed that the inner gas capillary pressure and internal diameter as well as the liquid capillary internal diameter had the strongest influence on the results. The importance of the outer gas capillary pressure, responsible of the nebulisation in the conventional ESI nebuliser, proved to be much less important than the gas pressure in the inner gas capillary, showing the advantage of the novel approach to ESI nebuliser.

The 3R nebuliser was demonstrated to give on the average 2.5 times (up to 200 times) lower limits of detection and less noise than the native commercial ESI source. After the optimisation of the novel nebuliser the advantage of lower LoDs became even more pronounced. It was also shown that the 3R nebuliser is more robust than the conventional one. The advantages of the 3R nebuliser were put to test with a practical analysis of neonicotinoids in honey. It is a trace analysis and thus is demanding of the ionisation source to give good results with as low LoD as possible. In the comparison with the native commercial nebuliser the 3R nebuliser proved to be comparable or better.

The aims of the thesis were fulfilled successfully, but many more novel ion source developments need to be evaluated and the work on improving the LC/MS method as such is far from done. This study is expected to add valuable information for the improvement of the LC/MS method and proposes a new direction for the improvement of ESI source.

SUMMARY IN ESTONIAN

"Uudsete massispektromeetria ionisatsiooniallikate võrdlemine ja optimeerimine"

Vedelikkromatograafia-massispektromeetria (LC/MS) on laialt kasutatav meetod väga mitmekesiste analüüside teostamiseks nii rutiin- kui teaduslaborites. Ionisatsiooniallikas on kahe meetodi (vedelikkromatograafia ja massispektromeetria) ühendamiseks oluline liides, mille abil tekitatakse kromatograafist tulevast vedelikust analüüdimolekulide ioonid, mida analüüsitakse massispektromeetriga. Erinevaid ionisatsiooniallikaid on mitmeid ning pidevalt täiendatakse olemasolevaid ja leiutatakse uusi. Seetõttu on vajalik võrrelda erinevaid uusi ionisatsiooniallikaid, et leida millised on spetsiifilise töö jaoks parimad.

Antud töö käigus võrreldi mitmeid uusi ionisatsiooniallikaid kommertsiaalselt levinud elektropihustus-ionisatsiooni allikaga (ESI). Võrdluses kasutati atmosfäärirõhulist fotoionisatsiooni allikat (APPI), kuumutatud elektropihustus-ionisatsiooni (HESI) ja mitmikmeetoditega (MMI) allikat. Võrdluseks kasutatavad parameetrid olid avastamispiir (LoD), tundlikkus, maatriksefektid (ME), lineaarne ala ja korduvus. Parimaid tulemusi saadi kommertsiaalse ESI allikaga. Samuti häid tulemusi näitas HESI allikas tundlikkuse osas, kuid jäi märkimisväärselt alla ME tulemuste poolest ESI ja dopandiga abistatud APPI allikatele. Kehvimad tulemused olid MMI allikatega.

Samuti oli töö eesmärgiks meie töögrupis eelnevalt väljatöötatud uudse ESI allika pihusti (3R) optimeerimine, mis viidi edukalt lõpule. Optimeerimise tulemused näitavad, et ionisatsiooni mõjutab oluliselt enam vedelikukapillaari sees asuv pihustusgaasi kapillaar võrreldes kommertsiaalsel allika välimise pihustusgaasi kapillaariga. Uudse 3R pihusti eelised seisnevad peamiselt madalamates avastamispiirides ja robustsuses võrreldes kommertsiaalse allikaga.

Samuti täideti edukalt uudse pihustiga seotud teine eesmärk: võrreldi kommertsiaalse pihustiga praktilise analüüsi näitel. Selleks oli jälgede määramine neonikotinoidide analüüsil meeproovidest. 3R pihustile leiti madalamale ulatuvad lineaarsed alad, madalamad või samaväärsed avastamispiirid ja parem korduvus võrreldes kommertsiaalse allikaga.

Antud töös leitud erinevate ionisatsiooniallikate võrdlusandmed ning uudse ESI pihusti iseloomustamisel leitud eelised lisavad olulist informatsiooni väga mitmekesisele MS ionisatsiooniallikatE temaatikale. Teadlased püüdlevad ühest küljest aina universaalsemate allikate poole, kuid samas otsitakse allikaid väga spetsiifiliste analüüside teostamiseks, mis annaksid parimaid tulemusi. Selleks on vajalik aina uuesti võrrelda praeguseid parimaid allikaid uudsete leiutistega, et leida effektiivseim liides LC/MS meetodile.

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APPENDICES

Table A – 1. Compound fragmentation data and acquisition time windows. For two compounds denoted with asterisk after the compound name (*) confirmatory fragment was not observed. Asterisk (*) at the product ion marks the quantitative ion. Retention time window was 0.6 min for all compounds. CE marks the collision energy of fragmentation.

Name	Pre-cursor ion	Pro-duct ion	CE (V)	Ret. time (min)
acetamiprid	223	187	20	2.373
		126*	20	2.373
azoxystrobin	404	372	24	4.379
		344	24	4.379
		329*	36	4.379
benalaxyl	326	208	20	5.057
		148*	20	5.057
bitertanol	338	269*	10	4.635
		251	10	4.635
bupirimate	317	166	30	4.004
		108*	30	4.004
buprofezin	306	201	20	5.27
		116*	20	5.27
chloridazon	222	193	24	2.165
		104*	24	2.165
ditalimfos	300	244	10	4.834
		148*	10	4.834
epoxiconazole	330	121*	20	4.389
		101	40	4.389
fenhexamid	302	178	35	4.463
		143*	35	4.463
fenpropimorph	304	147*	36	3.235
		130	24	3.235
		117	20	3.235
fluquinconazole	376	349	20	4.503
		307*	28	4.503
flusilazole	316	247	20	4.516
		165*	28	4.516
hexythiazox	353	228	20	6.102
		168*	20	6.102
imazalil	297	159*	30	2.818
		109	30	2.818
imidacloprid	256	209	20	2.218
		175*	20	2.218
indoxacarb	528	203*	35	5.502
		150	35	5.502
mepanipyrim	224	209	20	4.587
		183	20	4.587
methiocarb	226	169	10	4.16
		122	30	4.16
		121*	10	4.16
methiocarb-sulfone	258	201	10	2.578
		122*	10	2.578
methiocarb-sulfoxide	242	185*	15	1.927
		168	15	1.927
methoxychlor *	345	161*	30	4.786
metribuzin	215	187*	20	3.168
		145	20	3.168
myclobutanil	289	170	30	4.37
		125*	30	4.37
paclobutrazol	294	165	30	4.001
		139	30	4.001
		125*	30	4.001
pirimiphos-ethyl	334	198*	24	5.988
		182	20	5.988
propiconazole	342	342	20	4.783
		159*	20	4.783
propoxur	210	168*	10	3.258
		111	10	3.258
pymetrozine *	218	105*	20	0.507
pyrazophos	374	222	20	5.253
		194*	36	5.253
pyrimethanil	200	183	20	3.201
		107*	20	3.201
quinoxyfen	308	272	28	5.629
		197*	36	5.629
spiroxamine	298	144*	30	3.237
		100	30	3.237
tebufenozide	353	297*	10	4.9
		133	10	4.9
thiacloprid	253	186	20	2.707
		126*	20	2.707
thiamethoxam	292	211*	15	1.814
		181	15	1.814
thiophanate-methyl	343	192	20	3.2
		151*	20	3.2
triasulfuron	402	167*	20	3.383
		141	20	3.383
triazophos	314	162*	20	4.761
		119	40	4.761
trifloxystrobin	409	206	15	5.538
		186*	15	5.538
vamidothion	289	146*	20	1.913
		118	20	1.913
mepanipyrim		106*	20	4.587

Table A – 2. MS ion optics and fragmentation parameters for analytes used in the optimisation study of 3R nebulizer.

	Analyte	Retention time (min)	Skimmer (V)	Capillary Exit (V)	Octopole 1 DC (V)	Octopole 2 DC (V)	Trap drive	Octopole RF (Vpp)	Lens 1 (V)	Lens 2 (V)	Fragmentation Amplitude (V)	Fragmentation Cut Off (*m/z*)	*m/z* of precursor ion	*m/z* of product ion
Method 1 for pesticides	oxamyl	7.8	55	99	13	1.9	44	112	–4.7	–60	0.66	90	242	185
	thiamethoxam	9.1	47	50	11	2.2	44	95	–4.2	–56	3.00	113	292	211
	carbendazim	8.5									0.65	84	192	160
	imidacloprid	10.3	54	95	11	2.1	44	116	–4.4	–63	2	92	256	209
	thiabendazole	9.6									1	105	202	175
	acetamiprid	11.2	65	120	12	2.0	53	125	–3.5	–65	0.54	76	223	126
	vamidothion	10.9									0.62	103	310	146
	thiacloprid	12.0	44	112	13	1.7	50	120	–4.2	–66	2	92	253	126
	spiroxamine	15.2	61	124	15	2.4	53	107	–4.2	–66	0.71	95	298	144
	imazalil	13.9									2	125	297	201
	mepanipyrim	17.3	60	125	12	2.0	53	125	–4.0	–70	0.57	110	224	183
	fluquinconazole	17.1									0.84	182	376	349
	methiocarb	16.5									0.42	99	226	169
	buprofezin	18.8	45	100	12	2.5	44	125	–4.2	–65	0.57	61	306	201
	hexythiazox	19.3									0.54	74	353	228
	etofenprox	20.9	47	124	18	2.4	44	173	–4.4	–78	0.50	86	359	189
Method 2 for pharmaceuticals	norfloxacin		40	128	12	1.7	40	187	–5.0	–60	0.79	129	320	276
	ciprofloxacin										0.75	166	332	288
	ofloxacin										0.50	99	362	261
	sulfadimethoxine										0.50	110	311	245

Table A – 3. Default parameters of different ionisation modes used in Paper III.

Ionisation mode	Gas temp. (^{0}C)	Gas flow (14 l/min)	Nebuliser pressure (psi)	Capillary voltage (V)	Vaporiser temp. (^{0}C)	Corona current (μA)	Charging voltage (V)	Sheath gas temp. (^{0}C)	Sheath gas flow (l/min)	Nozzle Voltage (V)
HESI	200	14	20	3000				400	11	1500
ESI	200	14	15	4000						
MMI	200	14	60	2500	200	4	2000			
MMI-ESI	200	14	60	2500	200		2000			
MMI-APCI	200	14	60	2500	200	4				
APPI	200	14	40	2000	350					

Table A – 4. The molecular parameters values used in PCA of LoD and ME. The logP values were calculated as averages over several data sources, because there was no way to discriminate between different sources.

Compound	log$P$[a]	MW	pK_a	t_R (min)
pymetrozine	–0.02[99,100,101]	217	4.1[102]	0.58
thiamethoxam	0.57[99,100,103]	292	1[104]	1.83
vamidothion	–2.02[99,105]	287	–0.8[104]	1.93
methiocarb sulfoxide	1.07[100]	241	–1.6[104]	1.96
chloridazon	1.17[99,106]	222	0.7[104]	2.18
imidacloprid	0.76[99,100,107]	256	2.3[104,108]	2.21
acetamiprid	1.36[99,100,101]	223	0.7[109]	2.39
methiocarb sulfone	1.04[100]	257	–1.6[104]	2.60
thiacloprid	1.74[99,100,110]	253	0.8[104,108]	2.77
imazalil	3.44[99,107]	297	6.5[111]	2.84
thiophanate-methyl	1.92[99,112]	342	–0.5[104]	3.19
metribuzin	1.73[99,100,107]	214	7.1[113]	3.13
pyrimethanil	2.76[99,101]	199	3.5[101]	3.23
fenpropimorph	4.69[99,100,114]	303	7[115]	3.34
spiroxamine	3.86[99,100,116]	297	8.8[117]	3.34
propoxur	1.43[99,100,107]	209	–1.5[104]	3.26
triasulfuron	1.22[99,100]	402	2.6[104]	3.38
bupirimate	3.47[107]	316	4.4[118]	3.99
paclobutrazol	3.16[99,100,119]	294	2.4[104,108]	4.03
methiocarb	2.95[99,100,107]	225	–1.5[104]	4.16
azoxystrobin	3.46[99,100,120]	403	–0.2[104,108]	4.40
epoxiconazole	3.37[99,107]	330	2.5[104,108]	4.42
myclobutanil	3.07[99,100,107]	289	2.3[104]	4.36
fenhexamid	4.56[99,100,101]	302	–2.7[108]	4.42
fluquinconazole	3.44[99,121]	376	0.9[104]	4.53
flusilazole	3.61[99,100,122]	315	2.5[123]	4.60
mepanipyrim	5.19[99,121]	223	2.9[124]	4.59
bitertanol	4.09[99,125]	337	2.3[104]	4.64
propiconazole	3.84[99,101]	342	2.6[104,108]	4.87
triazophos	3.47[99, 107]	313	–0.2[104]	4.74
methoxychlor	5.24[99, 100,112]	346	–4.8[108]	4.87
ditalimfos	3.2[99]	299	–7.5[104,108]	4.78
tebufenozide	4.53[99,100,107]	352	–2.2[104]	4.89
benalaxyl	3.49[99,107]	325	–1.1[104,108]	5.04
pyrazophos	3.8[99,107]	373	–1.4[104]	5.23
buprofezin	4.2[99,100,101]	305	4.9[104,126]	5.13
indoxacarb	4.56[99,100,101]	528	–1.6[104,108]	5.44
trifloxystrobin	4.54[99,100,101]	408	2.4[108]	5.49
quinoxyfen	4.9[99,120]	308	3.6[120]	5.69
pirimiphos-ethyl	4.9[99,107]	333	5[104,108]	5.91
hexythiazox	4.06[99,100]	353	–7[108]	6.01

[a] calculated as averages over several sources

Table A – 5. Comparison of repeatabilities between ESI and different sources. APPI denotes direct APPI and DA-APPI denotes dopant assisted APPI.d

Compound	F-test (ESI/MMI)	F-test (ESI/MMI-APCI)	F-test (ESI/MMI-ESI)	F-test (ESI/AJS)	F-test (ESI/ direct APPI)	F-test (ESI/DA-APPI)	rstd (ESI)	rstd (MMI)	rstd (MMI-APCI)	rstd (MMI-ESI)	rstd (AJS)	rstd (APPI)	rstd (DA-APPI)
pymetrozine	0.0%	0.0%	0.0%	43.0%	0.1%	0.0%	1.3%	7.9%	7.0%	9.5%	1.7%	5.1%	6.0%
thiamethoxam	0.0%	0.0%	0.0%	21.4%	0.0%	1.4%	2.9%	13.8%	21.1%	12.7%	1.8%	23.4%	7.4%
vamidothion	0.0%	0.2%	0.0%	26.7%	0.3%	2.7%	5.1%	21.5%	17.7%	36.0%	3.3%	16.9%	11.6%
methiocarb sulfoxide	0.0%	0.0%	0.0%	12.0%	0.0%	0.0%	1.4%	15.5%	17.1%	24.6%	2.6%	8.1%	6.1%
chloridazon	0.0%	0.0%	0.0%	65.9%	0.0%	0.0%	1.6%	18.9%	40.6%	10.5%	1.9%	9.5%	9.2%
imidacloprid	3.8%	0.3%	0.2%	2.3%	4.0%	0.3%	5.6%	12.4%	18.3%	19.1%	2.2%	12.3%	17.7%
acetamiprid	0.0%	0.1%	0.0%	2.6%	23.0%	48.5%	4.3%	24.1%	16.8%	20.1%	1.7%	6.8%	5.6%
methiocarb sulfone	1.6%	9.2%	55.6%	49.7%	0.0%	0.0%	5.8%	14.9%	3.1%	7.2%	4.5%	43.4%	43.2%
thiacloprid	0.1%	0.1%	0.0%	9.7%	20.9%	2.7%	4.0%	15.6%	15.1%	19.2%	2.0%	6.3%	9.1%
imazalil	0.0%	0.0%	0.0%	48.6%	0.1%	10.3%	3.5%	15.5%	91.4%	26.2%	2.7%	13.9%	6.4%
thiophanate-methyl	0.0%	0.0%	0.0%	77.6%	0.0%	0.0%	1.6%	14.7%	19.3%	32.2%	1.4%	8.1%	20.9%
metribuzin	0.1%	0.0%	0.0%	69.1%	1.7%	5.5%	3.9%	14.1%	24.7%	29.9%	4.5%	9.7%	7.8%
pyrimethanil	1.0%	0.0%	0.0%	86.4%	9.4%	0.0%	3.7%	10.0%	267.7%	31.9%	3.4%	6.9%	25.8%
fenpropimorph	0.0%	0.0%	0.0%	7.7%	2.6%	3.7%	3.3%	27.3%	170.8%	19.4%	1.6%	7.7%	7.0%
spiroxamine	0.0%	0.0%	0.0%	29.8%	6.8%	72.3%	3.8%	26.2%	37.3%	34.7%	2.5%	7.5%	4.3%
propoxur	0.0%	0.0%	0.0%	43.5%	0.2%	0.0%	3.2%	19.4%	40.6%	18.8%	4.2%	10.7%	13.1%
triasulfuron	0.0%	0.0%	0.0%	25.7%		0.0%	2.4%	21.9%	31.3%	32.6%	3.7%		21.0%
bupirimate	0.0%	0.0%	0.0%	80.1%	0.2%	0.0%	1.7%	27.6%	16.7%	12.5%	1.8%	5.7%	6.8%
paclobutrazol	0.0%	0.0%	0.0%	35.6%	0.1%	0.0%	2.6%	26.6%	20.4%	22.7%	1.8%	10.4%	11.2%
methiocarb	0.0%	0.1%	0.0%	80.1%	0.0%	0.2%	2.4%	27.1%	9.3%	33.5%	2.6%	39.7%	8.1%
azoxystrobin	0.0%	0.0%	0.0%	5.4%	21.5%	0.4%	2.7%	17.1%	21.7%	23.3%	5.6%	4.2%	8.2%
epoxiconazole	0.0%	0.0%	0.0%	64.4%	12.0%	34.1%	3.6%	18.3%	26.9%	22.3%	4.3%	6.5%	5.1%
myclobutanil	0.0%	0.0%	0.0%	35.4%	0.2%	0.1%	3.2%	14.1%	24.7%	19.5%	4.5%	10.8%	12.5%

Table A – 5. Continuation.

Compound	F-test (ESI/MMI)	F-test (ESI/MMI-APCI)	F-test (ESI/MMI-ESI)	F-test (ESI/AJS)	F-test (ESI/ direct APPI)	F-test (ESI/DA-APPI)	rstd (ESI)	rstd (MMI)	rstd (MMI-APCI)	rstd (MMI-ESI)	rstd (AJS)	rstd (APPI)	rstd (DA-APPI)
fenhexamid	0.2%	0.0%	19.0%	61.6%		0.0%	8.2%	29.2%	41.3%	13.3%	6.7%		37.9%
fluquinconazole	0.0%	0.0%	0.0%	97.5%		0.1%	3.6%	16.2%	19.0%	31.3%	3.6%		14.8%
flusilazole	0.0%	0.0%	0.0%	81.5%	3.0%	1.2%	3.8%	33.3%	42.8%	31.0%	4.2%	8.8%	9.96%
mepanipyrim	0.0%	0.0%	0.0%	38.5%	2.8%	7.3%	2.6%	11.4%	38.1%	37.2%	3.6%	6.1%	5.1%
bitertanol	0.0%	0.0%	0.0%	33.5%	0.1%	0.0%	3.5%	21.0%	26.9%	22.0%	5.1%	13.4%	23.3%
propiconazole	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	1.3%	7.2%	24.9%	17.6%	4.2%	6.1%	5.5%
triazophos	0.0%	0.0%	0.0%	28.7%	7.7%	0.2%	2.6%	26.4%	25.8%	24.3%	3.9%	5.1%	8.7%
methoxychlor	0.6%	0.0%	0.0%	42.5%	0.0%	1.6%	3.5%	10.2%	26.2%	16.9%	4.7%	15.4%	8.6%
ditalimfos	0.4%	0.4%	0.0%	38.1%	0.0%	0.0%	5.0%	15.8%	15.6%	23.0%	6.9%	27.4%	52.9%
tebufenozide	0.0%	0.1%	0.0%	0.3%	0.0%	0.0%	2.7%	14.4%	9.7%	16.1%	9.0%	17.4%	14.6%
benalaxyl	0.0%	0.0%	0.0%	16.4%	2.0%	6.0%	2.1%	37.8%	34.6%	28.1%	3.5%	5.1%	4.1%
pyrazophos	0.0%	0.0%	0.0%	46.1%	0.0%	0.0%	1.5%	15.9%	27.5%	22.3%	2.0%	8.4%	6.5%
buprofezin	0.0%	0.0%	0.0%	6.4%	0.0%	0.0%	1.5%	18.8%	24.6%	22.7%	3.0%	7.9%	6.9%
indoxacarb	2.2%	2.9%	0.1%	71.6%		1.4%	4.5%	10.9%	10.4%	17.0%	3.9%		11.4%
trifloxystrobin	0.1%	0.0%	0.0%	10.1%	0.6%	52.3%	2.4%	9.9%	17.6%	26.1%	4.4%	7.0%	3.0%
quinoxyfen	0.0%	0.0%	0.0%	5.1%	0.0%	0.0%	1.0%	10.5%	35.7%	38.4%	2.2%	10.2%	4.7%
pirimiphos-ethyl	0.0%	0.0%	0.0%	36.0%	2.9%	45.4%	2.2%	15.3%	16.0%	19.5%	1.6%	5.1%	2.9%
hexythiazox	0.0%	0.5%	0.0%	70.0%		2.7%	2.8%	20.0%	8.6%	19.0%	2.4%		6.5%

Table A – 6. LoD values for 41 pesticides using 7 different ionisation modes, together with retention times. Grey background means that qualitative ion was absent. Orange background means that either qualitative or both ions were not detected. Yellow background means that qualitative ion was not detected at calculated LoD value and the lowest concentration where qualitative ion was observed was used as the LoD value.

		LoD (µg/kg)						
compound	t_R	**MMI**	**MMI-APCI**	**MMI-ESI**	**ESI**	**HESI**	**APPI**	**DA-APPI**
pymetrozine	0.58	1.0	1.2	0.45	0.87	1.4	2.1	2.0
thiamethoxam	1.83	0.24	0.93	0.27	0.20	1.7	2.5	3.2
vamidothion	1.93	1.2	5.9	0.74	0.80	1.0	35	9.6
methiocarb sulfoxide	1.96	0.25	2.5	0.18	0.27	0.61	1.7	0.75
chloridazon	2.18	0.34	1.4	0.26	0.12	0.42	25	2.5
imidacloprid	2.21	0.53	3.2	0.63	0.30	1.1	2.0	0.95
acetamiprid	2.39	7.5	50	7.5	2.5	2.5	25	5.0
methiocarb sulfone	2.60	5.0	53	4.8	0.29	0.71		75
thiacloprid	2.77	0.26	10	2.5	0.13	0.73	1.4	0.75
imazalil	2.84	0.47	1.5	0.92	0.33	0.26	10	10
thiophanate-methyl	3.19	50			75	46		
metribuzin	3.13	2.5	25	2.5	0.75	0.36	2.4	10
pyrimethanil	3.23	1.4	29	1.0	0.15	0.13	2.7	6.6
fenpropimorph	3.34	0.18	1.3	0.59	0.091	0.089	2.1	1.8
spiroxamine	3.34	0.13	1.5	0.19	0.063	0.086	2.6	1.1
propoxur	3.26	1.0	4.0	0.43	0.29	0.71	25	7.5
triasulfuron	3.38	1.4	16	0.45	0.21	0.28		
bupirimate	4.00	0.12	1.4	0.48	0.060	0.075	2.2	0.64
paclobutrazol	4.03	3.7	90	1.5	0.50	0.44	5.7	13
methiocarb	4.16	2.4	58	1.9	0.15	0.17	55	21
azoxystrobin	4.40	0.61	7.7	2.2	0.11	0.18	34	3.8
epoxiconazole	4.42	0.75	12	0.92	0.090	0.14	2.2	2.1
myclobutanil	4.36	100	49	1.6	10	25	3.6	3.0
fenhexamid	4.42	25		39	5.0	1.2	72	13
fluquinconazole	4.53	6.5	71	10	0.21	0.11	6.3	6.1
flusilazole	4.60	0.43	4.3	0.94	0.044	0.028	2.2	0.71
mepanipyrim	4.59	2.9	163	9.0	0.21	0.14	5.0	2.2
bitertanol	4.64	6.2	46	20	1.1	1.6	16	23
propiconazole	4.87	2.0	10	2.0	0.25	0.040	1.9	0.67
triazophos	4.74	0.38	3.4	0.48	0.047	0.11	1.6	0.73
methoxychlor	4.87	7.9	90	30.8	0.34	0.43	7.9	10
ditalimfos	4.78	3.3	43	12	0.13	0.14	217	39
tebufenozide	4.89	3.7	57	5.5	0.11	0.21	25	27
benalaxyl	5.04	0.55	5.9	0.45	0.057	0.15	2.2	0.39
pyrazophos	5.23	0.37	4.7	0.52	0.12	0.17	1.7	0.34
buprofezin	5.13	0.34	4.1	0.67	0.14	0.19	2.1	2.7
indoxacarb	5.44	7.3	26	9.8	0.39	0.21	9.2	14
trifloxystrobin	5.50	1.5	5.8	0.69	0.028	0.017	1.4	0.35
quinoxyfen	5.69	2.7	8.7	2.0	0.049	0.034	2.1	0.36
pirimiphos-ethyl	5.91	0.31	6.0	0.30	0.031	0.062	1.5	0.29
hexythiazox	6.01	18	33	30	0.090	0.021	45	8.3

Table A – 7. Sensitivity (slopes) values for 7 different ionisation modes for 41 pesticides.

	Slopes of calibration graphs						
compound	MMI	MMI-APCI	MMI-ESI	ESI	HESI	APPI	DA-APPI
pymetrozine	$2.5 \cdot 10^5$	$1.9 \cdot 10^4$	$2.5 \cdot 10^5$	$4.1 \cdot 10^5$	$4.1 \cdot 10^6$	$4.7 \cdot 10^4$	$2.2 \cdot 10^5$
thiamethoxam	$1.3 \cdot 10^5$	$1.2 \cdot 10^4$	$1.2 \cdot 10^5$	$2.3 \cdot 10^5$	$2.5 \cdot 10^6$	$1.3 \cdot 10^4$	$4.0 \cdot 10^4$
vamidothion	$1.6 \cdot 10^4$	$1.5 \cdot 10^3$	$1.8 \cdot 10^4$	$1.9 \cdot 10^4$	$2.4 \cdot 10^5$	$9.6 \cdot 10^2$	$3.2 \cdot 10^3$
methiocarb sulfoxide	$6.1 \cdot 10^5$	$6.1 \cdot 10^4$	$6.5 \cdot 10^5$	$9.5 \cdot 10^5$	$7.5 \cdot 10^6$	$8.4 \cdot 10^4$	$2.8 \cdot 10^5$
chloridazon	$2.6 \cdot 10^5$	$2.0 \cdot 10^4$	$2.5 \cdot 10^5$	$4.9 \cdot 10^5$	$6.1 \cdot 10^6$	$3.4 \cdot 10^4$	$9.2 \cdot 10^4$
imidacloprid	$6.9 \cdot 10^4$	$7.8 \cdot 10^3$	$6.1 \cdot 10^4$	$1.1 \cdot 10^5$	$1.1 \cdot 10^6$	$2.8 \cdot 10^4$	$7.9 \cdot 10^4$
acetamiprid	$2.9 \cdot 10^5$	$2.5 \cdot 10^4$	$2.5 \cdot 10^5$	$6.0 \cdot 10^5$	$5.6 \cdot 10^6$	$7.8 \cdot 10^4$	$2.4 \cdot 10^5$
methiocarb sulfone	$5.6 \cdot 10^3$	$1.8 \cdot 10^3$	$4.8 \cdot 10^3$	$1.8 \cdot 10^5$	$5.0 \cdot 10^5$	*	*
thiacloprid	$2.6 \cdot 10^5$	$2.7 \cdot 10^4$	$2.4 \cdot 10^5$	$8.4 \cdot 10^5$	$8.7 \cdot 10^6$	$7.7 \cdot 10^4$	$2.5 \cdot 10^5$
imazalil	$2.1 \cdot 10^5$	$1.7 \cdot 10^4$	$1.9 \cdot 10^5$	$1.6 \cdot 10^5$	$1.8 \cdot 10^6$	$4.6 \cdot 10^3$	$1.6 \cdot 10^4$
thiophanate-methyl	*	*	*	*	*	*	*
metribuzin	$5.9 \cdot 10^4$	$3.8 \cdot 10^3$	$7.1 \cdot 10^4$	$6.8 \cdot 10^5$	$4.2 \cdot 10^6$	$4.2 \cdot 10^4$	$1.4 \cdot 10^5$
pyrimethanil	$3.1 \cdot 10^4$	$2.1 \cdot 10^3$	$3.4 \cdot 10^4$	$3.9 \cdot 10^5$	$4.5 \cdot 10^6$	$2.8 \cdot 10^4$	$1.3 \cdot 10^5$
fenpropimorph	$7.2 \cdot 10^5$	*	$7.0 \cdot 10^5$	$8.0 \cdot 10^5$	$7.8 \cdot 10^6$	$1.1 \cdot 10^4$	$3.8 \cdot 10^4$
spiroxamine	$2.1 \cdot 10^6$	$8.7 \cdot 10^4$	$1.7 \cdot 10^6$	$2.0 \cdot 10^6$	$2.1 \cdot 10^7$	$2.4 \cdot 10^4$	$9.2 \cdot 10^4$
propoxur	$9.7 \cdot 10^4$	$8.1 \cdot 10^3$	$9.3 \cdot 10^4$	$5.6 \cdot 10^5$	$5.5 \cdot 10^6$	$7.3 \cdot 10^3$	$3.9 \cdot 10^4$
triasulfuron	$4.5 \cdot 10^4$	$6.5 \cdot 10^2$	$2.9 \cdot 10^4$	$1.7 \cdot 10^5$	$2.3 \cdot 10^6$	*	*
bupirimate	$2.0 \cdot 10^5$	$1.9 \cdot 10^4$	$1.7 \cdot 10^5$	$5.9 \cdot 10^5$	$6.4 \cdot 10^6$	$2.3 \cdot 10^4$	$8.5 \cdot 10^4$
paclobutrazol	$7.8 \cdot 10^3$	*	$7.5 \cdot 10^3$	$1.2 \cdot 10^5$	$1.0 \cdot 10^6$	$3.2 \cdot 10^3$	$9.0 \cdot 10^3$
methiocarb	$1.7 \cdot 10^4$	*	$1.3 \cdot 10^4$	$6.9 \cdot 10^5$	$3.2 \cdot 10^6$	$6.6 \cdot 10^2$	$1.3 \cdot 10^4$
azoxystrobin	$1.1 \cdot 10^5$	*	$1.0 \cdot 10^5$	$6.4 \cdot 10^5$	$1.2 \cdot 10^7$	$2.3 \cdot 10^4$	$4.6 \cdot 10^4$
epoxiconazole	$6.2 \cdot 10^4$	*	$6.7 \cdot 10^4$	$9.6 \cdot 10^5$	$7.6 \cdot 10^6$	$3.2 \cdot 10^4$	$7.4 \cdot 10^4$
myclobutanil	$1.5 \cdot 10^4$	*	$1.7 \cdot 10^4$	$2.0 \cdot 10^5$	$1.2 \cdot 10^6$	$1.2 \cdot 10^4$	$2.9 \cdot 10^4$
fenhexamid	$1.4 \cdot 10^3$	*	*	$1.8 \cdot 10^4$	$2.1 \cdot 10^5$	*	*
fluquinconazole	$8.2 \cdot 10^3$	*	$8.4 \cdot 10^4$	$2.2 \cdot 10^5$	$1.5 \cdot 10^6$	$4.0 \cdot 10^3$	$1.4 \cdot 10^4$
flusilazole	$1.0 \cdot 10^5$	*	$8.4 \cdot 10^4$	$1.6 \cdot 10^6$	$1.6 \cdot 10^7$	$5.9 \cdot 10^4$	$1.2 \cdot 10^5$
mepanipyrim	$1.7 \cdot 10^4$	*	*	$3.6 \cdot 10^5$	$3.5 \cdot 10^6$	$1.0 \cdot 10^4$	$5.1 \cdot 10^4$
bitertanol	$4.4 \cdot 10^3$	*	*	$9.1 \cdot 10^4$	$5.0 \cdot 10^5$	*	*
propiconazole	$3.7 \cdot 10^4$	*	$4.7 \cdot 10^4$	$8.0 \cdot 10^5$	$7.5 \cdot 10^6$	$2.9 \cdot 10^4$	$7.2 \cdot 10^4$
triazophos	$2.8 \cdot 10^5$	$1.7 \cdot 10^4$	$2.3 \cdot 10^5$	$2.0 \cdot 10^6$	$2.8 \cdot 10^7$	$6.7 \cdot 10^4$	$2.3 \cdot 10^5$
methoxychlor	$3.0 \cdot 10^3$	*	*	$6.8 \cdot 10^4$	$6.4 \cdot 10^5$	$2.4 \cdot 10^3$	$6.5 \cdot 10^3$
ditalimfos	$1.0 \cdot 10^4$	*	*	$5.6 \cdot 10^5$	$1.0 \cdot 10^7$	*	*
tebufenozide	$8.5 \cdot 10^3$	*	*	$2.9 \cdot 10^5$	$1.5 \cdot 10^6$	$1.2 \cdot 10^3$	$4.8 \cdot 10^3$
benalaxyl	$7.9 \cdot 10^4$	*	*	$1.3 \cdot 10^6$	$2.4 \cdot 10^7$	$3.4 \cdot 10^4$	$1.4 \cdot 10^5$
pyrazophos	$5.3 \cdot 10^4$	*	$7.1 \cdot 10^4$	$5.8 \cdot 10^5$	*	$3.3 \cdot 10^4$	$8.9 \cdot 10^4$
buprofezin	$6.1 \cdot 10^4$	*	$5.1 \cdot 10^4$	$8.2 \cdot 10^5$	$9.0 \cdot 10^6$	$8.5 \cdot 10^3$	$2.5 \cdot 10^4$
indoxacarb	$4.5 \cdot 10^3$	*	$6.5 \cdot 10^3$	$4.8 \cdot 10^4$	*	*	$6.8 \cdot 10^3$
trifloxystrobin	*	*	*	$1.0 \cdot 10^6$	$3.2 \cdot 10^7$	$2.9 \cdot 10^4$	$8.1 \cdot 10^4$
quinoxyfen	$1.9 \cdot 10^4$	*	*	$1.3 \cdot 10^6$	$1.9 \cdot 10^7$	$2.9 \cdot 10^4$	$9.3 \cdot 10^4$
pirimiphos-ethyl	$2.6 \cdot 10^5$	*	$4.6 \cdot 10^5$	$7.5 \cdot 10^6$	$1.4 \cdot 10^8$	$5.1 \cdot 10^4$	$1.8 \cdot 10^5$
hexythiazox	$3.5 \cdot 10^3$	*	$3.1 \cdot 10^3$	$8.7 \cdot 10^4$	$5.2 \cdot 10^6$	$1.3 \cdot 10^3$	$6.8 \cdot 10^3$

* Not enough points on the calibration graph

Table A – 8. Results from GLM, coefficients for each parameter (column) and for each analyte (row).

	Analyte	Intercept	B_ID	C	C_ID	Cp	CA	CapV	B_ID*C	B_ID*Cp	C_ID*Cp	B_ID*CA	C*CA	C_ID*CA	Cp*CA	Bid*CapV	C*CapV	C_ID*CapV	Cp*CapV	CA*CapV
Standard solution	carbendazim	0.24	0.59	–0.38	–0.42	1.21	–0.19	–0.06	–0.14	0.53	–0.30	–0.07	–0.31	0.18	0.04	–0.07	–0.20	0.04	–0.09	0.05
	thiabendazole	0.35	0.55	–0.72	–0.32	1.38	–0.31	–0.17	–0.14	0.51	–0.44	–0.05	–0.02	0.08	–0.08	–0.12	0.02	0.03	–0.21	0.07
	acetamiprid	0.24	0.56	–0.30	–0.33	1.08	–0.31	–0.07	0.13	0.23	–0.29	–0.01	0.08	0.05	–0.24	–0.07	–0.41	0.13	–0.01	0.04
	mepanipyrim	–0.20	0.22	0.03	–0.26	1.22	0.02	0.06	0.19	–0.05	0.24	0.22	–0.11	0.00	–0.09	–0.11	–0.41	0.06	0.10	–0.14
	methiocarb	–0.03	0.36	–0.12	–0.32	1.28	–0.25	–0.16	0.15	0.07	0.04	0.07	0.22	–0.10	–0.13	–0.06	0.03	–0.07	–0.01	0.11
	oxamyl	0.11	0.63	0.25	–0.59	0.84	–0.25	–0.08	–0.13	0.54	–0.12	–0.11	–0.52	0.33	0.10	–0.05	–0.11	0.02	–0.13	–0.07
	thiacloprid	0.10	0.50	–0.21	–0.46	1.28	–0.19	–0.21	0.08	0.24	–0.12	0.02	–0.21	0.12	0.00	–0.05	0.17	–0.07	–0.19	0.09
	imidacloprid	0.25	0.73	–0.28	–0.27	0.83	0.19	–0.04	0.05	0.41	–0.30	–0.10	–0.88	0.28	0.19	–0.11	–0.30	0.05	0.01	0.27
	thiamethoxam	0.15	0.54	–0.18	–0.40	1.12	–0.10	–0.20	–0.13	0.49	–0.20	–0.03	–0.51	0.17	0.24	–0.07	0.02	–0.04	–0.10	0.05
	imazalil	–0.02	0.42	–0.09	–0.45	1.30	0.01	–0.12	0.07	0.20	0.04	0.09	–0.47	0.10	0.12	–0.11	–0.16	0.07	–0.11	–0.12
	spiroxamine	–0.02	0.38	–0.10	–0.40	1.29	–0.04	–0.11	0.13	0.11	0.05	0.07	–0.33	0.15	–0.10	–0.08	–0.16	0.06	–0.13	–0.09
	buprofezin	0.12	–0.49	–0.83	1.15	0.31	0.47	–0.17	0.12	–0.45	–0.13	0.27	–0.87	–0.03	–0.16	0.13	0.32	–0.21	–0.16	0.12
	vamidothion	–0.10	0.54	0.47	–0.59	0.86	–0.14	–0.06	0.05	0.29	0.16	–0.08	–0.64	0.38	–0.06	–0.06	–0.14	0.08	–0.24	0.05
	hexythiazox	–0.84	0.19	1.43	–0.25	0.10	–0.11	–0.65	0.09	0.03	1.02	0.30	0.81	–0.49	0.00	–0.03	1.53	–0.50	–0.06	0.16
	etofenprox	–0.60	0.33	0.85	–0.25	0.62	–0.43	–0.53	0.11	0.10	0.74	0.19	1.26	–0.55	–0.43	–0.12	0.88	–0.10	–0.13	0.39
	fluquinconazole	–0.12	0.56	0.36	–0.54	0.88	0.05	0.18	0.07	0.29	0.14	0.08	–0.45	0.14	0.13	–0.01	–0.31	–0.12	0.43	–0.33

Table A – 8. Continuation.

	Analyte	Intercept	B_ID	C	C_ID	Cp	CA	CapV	B_ID*C	B_ID*Cp	C_ID*Cp	B_ID*CA	C*CA	C_ID*CA	Cp*CA	Bid*CapV	C*CapV	C_ID*CapV	Cp*CapV	CA*CapV
Garlic sample	carbendazim	0.23	0.52	–0.73	–0.25	1.21	–0.18	–0.25	–0.17	0.49	–0.41	–0.02	–0.11	0.10	–0.04	–0.09	0.23	0.00	–0.31	0.02
	thiabendazole	0.28	0.47	–0.82	–0.28	1.31	–0.20	–0.30	–0.05	0.33	–0.46	0.00	–0.19	0.18	–0.05	–0.08	0.27	–0.03	–0.27	0.08
	acetamiprid	–0.12	0.29	–0.29	–0.31	1.32	–0.17	–0.29	0.37	–0.22	0.11	0.21	0.14	0.05	–0.39	–0.09	0.29	0.04	–0.35	0.16
	mepanipyrim	–0.04	0.17	–0.49	–0.05	1.34	0.17	–0.46	0.33	–0.25	0.00	–0.02	–0.71	0.16	0.24	–0.05	1.12	–0.23	–0.30	-0.20
	methiocarb	0.07	-0.29	–0.69	–0.04	0.42	0.28	–0.03	0.19	–0.49	–0.01	–0.33	–0.26	–0.01	0.40	0.35	–0.01	0.02	–0.49	–0.24
	oxamyl	–0.04	0.16	–0.50	0.43	0.94	–0.49	–0.01	–0.33	0.45	0.07	0.02	0.13	0.05	–0.12	0.04	–0.25	–0.24	–0.07	0.12
	thiacloprid	0.01	0.38	–0.33	–0.29	1.30	–0.05	0.00	0.18	0.03	–0.07	0.07	–0.29	0.15	0.07	–0.03	–0.22	–0.03	0.00	–0.01
	imidacloprid	–0.42	0.59	0.58	–0.40	0.70	0.34	0.65	–0.02	0.36	0.47	0.12	–0.71	0.13	0.24	0.02	–1.57	0.24	0.53	–0.49
	thiamethoxam	0.21	0.49	–0.64	–0.30	1.25	0.02	–0.10	–0.07	0.36	–0.37	0.01	–0.39	0.12	0.12	–0.05	–0.06	0.02	–0.21	0.07
	imazalil	–0.06	0.23	–0.31	–0.25	1.43	–0.10	0.00	0.24	–0.13	0.06	0.08	–0.04	0.00	–0.10	–0.01	–0.16	–0.01	–0.04	0.17
	spiroxamine	–0.15	0.36	0.03	–0.34	1.14	–0.14	–0.30	0.12	0.08	0.15	0.06	–0.03	0.09	–0.20	–0.10	0.20	0.00	–0.22	–0.10
	buprofezin	–0.57	–0.02	1.11	0.01	0.40	–0.98	–0.21	0.60	–0.66	0.83	–0.07	1.51	–0.03	–0.77	–0.24	–0.45	0.40	0.23	0.12
	vamidothion	0.02	0.48	–0.26	–0.30	1.09	–0.21	–0.12	–0.11	0.41	–0.10	–0.03	–0.31	0.20	–0.08	–0.07	–0.18	0.07	–0.20	–0.04
	hexythiazox	–0.51	0.15	0.93	0.04	0.24	0.00	0.14	0.16	–0.08	0.70	0.20	0.59	–0.17	0.10	0.00	0.26	–0.13	0.56	–0.06
	etofenprox	–0.52	0.36	0.77	–0.22	0.72	0.28	–0.04	0.16	0.05	0.64	0.25	–0.28	–0.22	0.11	–0.01	0.25	–0.19	0.25	–0.01
	fluquinconazole	0.09	0.60	–0.05	–0.34	0.64	–0.08	0.62	0.20	0.15	–0.20	0.16	–0.18	0.02	–0.02	0.08	–0.40	0.03	0.28	0.06
Pharma–ceuticals	sulfadimethoxine	0.29	0.55	–0.82	0.05	1.16	–0.33	0.65	–0.06	0.41	–0.39	–0.05	0.21	–0.03	0.46	0.04	–1.33	0.21	0.27	–0.06
	norfloxacin	–0.72	0.37	0.75	–0.14	0.67	–0.08	0.04	–0.02	0.24	0.87	0.02	–0.07	0.02	–0.10	–0.01	0.06	–0.11	–0.19	0.05
	ciprofloxacin	–0.51	0.45	0.39	–0.11	0.84	–0.33	0.31	0.02	0.26	0.61	0.01	0.47	–0.17	–0.22	0.02	–0.62	0.10	0.04	0.01
	ofloxacin	–0.61	0.42	0.72	–0.03	0.44	0.08	0.14	0.22	0.04	0.68	0.03	–0.61	–0.10	0.30	–0.03	–0.27	0.18	–0.26	0.02
	average	–0.10	0.37	–0.01	–0.22	0.95	–0.10	–0.05	0.07	0.15	0.10	0.04	–0.11	0.04	–0.01	–0.04	–0.06	–0.01	–0.05	0.01
	standard deviation of the mean	0.05	0.04	0.10	0.05	0.06	0.04	0.05	0.03	0.05	0.07	0.02	0.09	0.03	0.04	0.02	0.09	0.03	0.04	0.03

PUBLICATIONS

Curriculum Vitae in English

I Personal information

1.	Name:	Asko Laaniste
2.	Date of birth:	21st of September, 1987
3.	Citizenship:	estonian
4.	Address:	Tiigi 57–10, Tartu
	Phone, e-mail:	58313727 asko.laaniste@gmail.com
5.	Current position:	University of Tartu, chemist
6.	Education:	2012–…, University of Tartu, doctoral studies
		2010–2012, University of Tartu, master's studies, MSc
		2007–2010, University of Tartu, bachelor's studies, BSc
7.	Language skills:	estonian mother tongue
		english fluent in speech and writing
		japanese basic understanding of speech
8.	Professional career:	01.2014 – … University of Tartu, chemist

II Research and development work

1. Main fields of research:

LC/MS, method validation, sample pretreatment, pesticides, HPLC, monolithic columns

2. Publications:

Anneli Kruve, Ivo Leito, Koit Herodes, Asko Laaniste, Rünno Lõhmus, *Enhanced Nebulization Efficiency of Electrospray Mass Spectrometry: Improved Sensitivity and Detection Limit*, J. Am. Soc. Mass Spectrom. 2012, 23, 2051–2054

Asko Laaniste, Anneli Kruve, Ivo Leito, *Ensuring repeatability and robustness of poly(glycidyl methacrylate-co-ethylene dimethacrylate) HPLC monolithic columns of 3 mm id through covalent bonding to the column wall*, J. Sep. Sci., 2013, 36, 2458–2463

Asko Laaniste, Audrey Marechal, Racha El-Debs, Jerome Randon, Vincent Dugas, Claire Demesmay, *"Thiol-ene" photoclick chemistry as a rapid and localizable functionalization pathway for silica capillary monolithic columns*, J. Chromatogr. A, 2014, 1355, 296–300

Audrey Marechal, Asko Laaniste, Racha El-Debs, Vincent Dugas, Claire Demesmay *Versatile ene-thiol photoclick reaction for preparation of multimodal monolithic silica capillary columns*, J. Chromatogr. A. 2014, 1365, 140–147

Jaanus Liigand, Anneli Kruve, Piia Liigand, Asko Laaniste, Marion Girod, Rodolphe Antoine, Ivo Leito, *Transferability of the electrospray ionization efficiency scale between different instruments*, J. Am. Soc. Mass Spectrom., 2015, 26, 1923–30.

Asko Laaniste, Anneli Kruve, Ivo Leito, Riin Rebane, Rünno Lõhmus, Ants Lõhmus, Fredrik Punga, *Determination of neonicotinoids in Estonian honey by LC/ESI/MS with novel nebulizer*, Journal of Environmental Science and Health, Part B, accepted for publication.

Asko Laaniste, Ivo Leito, Anneli Kruve, *Comparison of different ionization sources for the LC/MS analysis of pesticides*, submitted to Rapid Communications in Mass Spectrometry.

3. Research grants and scholarships:

2013 – Kristjan Jaak Scholarships for part-time studies in Lyon, France.

2014 – Doctoral school scholarship for participation in 38th ISEAC.

2015 – Estonian Students Fund in USA for financial support to worthy Estonian candidates, who demonstrate through academic excellence and community leadership their capacity for, and commitment to, making a contribution to Estonian society.

2015 – Kristjan Jaak Scholarships for participation in HPLC 2016 conference.

4. Other administrative and professional activities:

ValChrom project "Development of software for validation of chromatographic methods" (with registration number 3.2.1201.13-0020) under the submeasure "Supporting the development of R&D of info and communication technology" funded by the EU Regional Development Fund.

Roles in the project (10.2015 – end of project in 08.2015): one of the software testers and developers for correspondence with validation guidelines (ICH, AOAC, EuraChem, IUPAC, EMA, FDA, NordVal); programming of the ValChrom software on QureDesign platform, overview of the progress of testing, promoting the program at HPLC 2015.

III Teaching activities

Analüütilise keemia praktikum I

Since 2007 actively participated in popularization of science amongst elementary and high school students. Had a leading role in development and organization of chemistry workshop for "Teaduslaager" 2009–2014 (science camp for elementary school students on summers) and chemistry workshop for "Õpikojad" since 2010 (year-long practical workshops for elementary and high school students).

Also part of Estonian Olympiad committee since 2012, responsible for organization of the olympiad, generation of the exercises for the participants and training of Estonian students for International Chemistry Olympiad. Also a volunteer for European Science Olympiad in 2016.

IV Professional self-improvement and conferences

30.11.2013 – Training for teaching assistants, Tartu, Eesti.

02.–07.2013 – Visited the 1st University in Lyon for 5 months, studying functionalization of monolithic silica columns in prof. J. Randon's group.

11. October 2013 – Eesti XXXIII Keemiapäevad (Estonian XXXIII Chemistry Days).
04.–05. March 2014 – TÜ and TTÜ collaborative doctoral school's conference "FMTDK Teaduskonverents 2014".
16.–20. June 2014 – The 38th International Symposium on Environmental Analytical Chemistry.
Spring 2015. – Estimation of measurement uncertainty in chemical analysis, MOOC, Tartu, Eesti.
21.06.2015. – SFC – Principles, Instrumentation, Method Development, and Applications, Geneva, Switzerland.
21.–25. June 2015 – 42nd Symposium on high performance liquid phase separations and related techniques (HPLC 2015).
09.–10.06.2016. – 16th International Chromatography School, Zagreb.
19.–24.06.2016. – 44th International Symposium on high performance liquid phase separations and related techniques (HPLC 2016), San Francisco, USA.

Curriculum Vitae in Estonian

I Üldandmed

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2. Sünniaeg:	21.09.1987
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5. Praegune töökoht:	Tartu Ülikool, keemik
6. Haridus:	2012 …, Tartu Ülikool, doktoriõpe
	2010 2012, Tartu Ülikool, magistriõpe, MSc
	2007 2010, Tartu Ülikool, bakalaureuseõpe, BSc
7. Keelteoskus:	eesti keel emakeel
	inglise keel sujuv nii kõnes kui kirjas
	jaapani keel arusaamine lihtsamast kõnest
8. Teenistuskäik:	01.2014 – … aastaTartu Ülikool, keemik

II Teaduslik ja arendustegevus

1. Peamised uurimisvaldkonnad:
   LC/MS, metoodikate valideerimine, proovi eeltöötlus, pestitsiidid, HPLC, monoliitsed kolonnid

2. Publikatsioonide loetelu:

Anneli Kruve, Ivo Leito, Koit Herodes, Asko Laaniste, Rünno Lõhmus, *Enhanced Nebulization Efficiency of Electrospray Mass Spectrometry: Improved Sensitivity and Detection Limit*, J. Am. Soc. Mass Spectrom. 2012, 23, 2051–2054

Asko Laaniste, Anneli Kruve, Ivo Leito, *Ensuring repeatability and robustness of poly(glycidyl methacrylate-co-ethylene dimethacrylate) HPLC monolithic columns of 3 mm id through covalent bonding to the column wall*, J. Sep. Sci., 2013, 36, 2458–2463

Asko Laaniste, Audrey Marechal, Racha El-Debs, Jerome Randon, Vincent Dugas, Claire Demesmay, *"Thiol-ene" photoclick chemistry as a rapid and localizable functionalization pathway for silica capillary monolithic columns*, J. Chromatogr. A, 2014, 1355, 296–300

Audrey Marechal, Asko Laaniste, Racha El-Debs, Vincent Dugas, Claire Demesmay *Versatile ene-thiol photoclick reaction for preparation of multi-modal monolithic silica capillary columns*, J. Chromatogr. A. 2014, 1365, 140–147

Jaanus Liigand, Anneli Kruve, Piia Liigand, Asko Laaniste, Marion Girod, Rodolphe Antoine, Ivo Leito, *Transferability of the electrospray ionization efficiency scale between different instruments*, J. Am. Soc. Mass Spectrom., 2015, 26, 1923–30

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Asko Laaniste, Ivo Leito, Anneli Kruve, *Comparison of different ionization sources for the LC/MS analysis of pesticides*, submitted to Rapid Communications in Mass Spectrometry.

3. Saadud uurimistoetused ja stipendiumid:

2013 – Kristjan Jaagu osalise õppe stipendium teadustööks Lyonis, Prantsusmaal.

2014 – Doktorikooli stipendium osalemaks 38.. ISEAC konverentsil.

2015 – Eesti Üliõpilaste Toetusfond USAs stipendium toetamaks finantsiliselt silmapaistvaid üliõpilasi.

2015 – Kristjan Jaagu stipendium osalemiseks HPLC 2016 konverentsil.

4. Muu teaduslik organisatsiooniline ja erialane tegevus:

ValChrom projekt "Development of software for validation of chromatographic methods" (registreerimisnumbriga 3.2.1201.13-0020) alamüksuse "Supporting the development of R&D of info and communication technology" toetatud EU Regional Development Fund poolt.

Roll projektis (10.2015 – end of project in 08.2015): valideerimisalase tarkvara testimine ja arendamine vastavusse peamiste valideerimisjuhenditega (ICH, AOAC, EuraChem, IUPAC, EMA, FDA, NordVal); ValChrom tarkvara programmeerimine, ülevaade testimistest.

III Õppetöö

Analüütilise keemia praktikum I

Alates 2007. aastast olen olnud teaduse populariseerija põhikooli ja gümnaasiumiastme õpilaste seas. Olen omanud juhtivat rolli "Teaduslaager" 2009–2014 ja "Õpikojad" 2010–2016 väljatöötamisel ja läbiviimisel.

Samuti olen Eesti keemiaolümpiaadi organiseeriva komitee liige alates 2012. aastast, viies läbi treeninglaagreid, koostades ülesandeid ning valmistades Eesti õpilasi ette rahvusvaheliseks olümpiaadiks. Lisaks võtan vabatahtlikuna osa Euroopa Teadusolümpiaadi EUSO 2016 organiseerimisest.

IV Erialane enesetäiendus ja konverentsid

30.11.2013 – Õppeassistentide koolitus, Tartu, Eesti.

2013 – Tegin teadustööd Lyoni 1. Ülikoolis 5 kuud, prof. J. Randoni grupis, mis tegeleb monoliitsete kolonnide valmistamise ja funktrionaliseerimisega.

11.10.2013. – Eesti XXXIII Keemiapäevad

04.–05.03.2014. – TÜ ja TTÜ doktorikooli konverents "FMTDK Teaduskonverents 2014".

16.–20.06.2014. – The 38th International Symposium on Environmental Analytical Chemistry

Kevad 2015. – Mõõtemääramatuse hindamine keemilises analüüsis, Tartu, Eesti.
21.06.2015. – SFC – Principles, Instrumentation, Method Development, and Applications, Genf, Šveits.
21.–25.06.2015. – 42nd International Symposium on high performance liquid phase separations and related techniques (HPLC 2015).
09.–10.06.2016. – 16th International Chromatography School, Zagreb.
19.–24.06.2016. – 44th International Symposium on high performance liquid phase separations and related techniques (HPLC 2016), San Francisco, USA.

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