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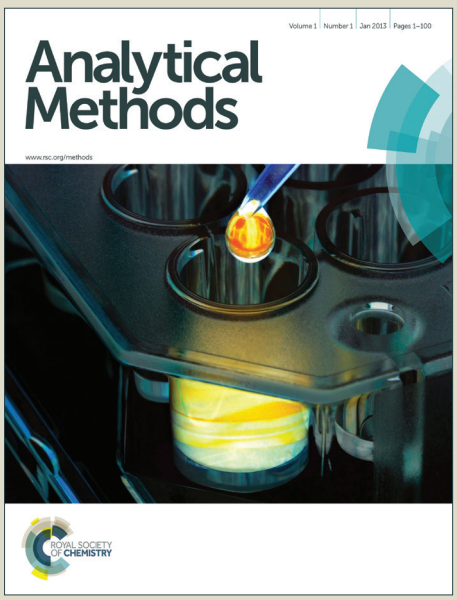

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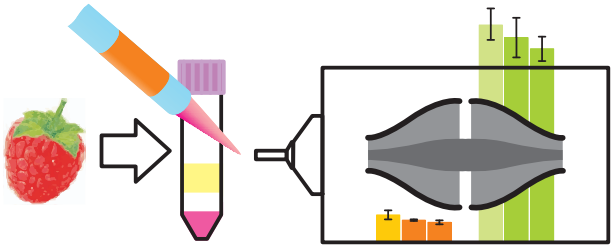

Rapid and Quantitative Analysis of Pesticides in Fruits by QuEChERS Pretreatment and Low-Temperature Plasma Desorption/Ionization Orbitrap Mass Spectrometry

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ABSTRACT

Ambient desorption/ionization high-resolution mass spectrometry (ADI-HR-MS) is a powerful method for the analysis of complex samples. Recently, direct analysis in real time (DART) MS and low-temperature plasma probe (LTP) MS demonstrated potential in direct qualitative pesticide residue screening and quantitative analysis of pesticides in liquid extracts. In the present study, a LTP-HR-MS method for quantitative pesticide residue analysis in fruit extracts was developed and evaluated with respect to the European Union (EU) legislation on pesticides. In particular, this study focused on pesticides in different fruit matrices that were reported to often exceed legal maximum residue levels (MRL) in Germany in the past (namely Acetamiprid, Cyprodinil, Fenhexamid, and Fludioxonil; see report on of the German Federal Office of Consumer Protection and Food safety in 2009). After method optimization, pesticides in spiked and unspiked fruit QuEChERS extracts were identified successfully by LTP-Orbitrap-MS via accurate mass measurements (<4 ppm). The method is considered useful for MRL verification.

Matrix-matched calibration was applied for quantification because it was found that the fruit matrix (still present during extract analysis) has a significant effect on analyte ion abundance. Linear working ranges greater than four orders of magnitude were achieved. Limits of quantification ranged from 0.001 mg/kg to 0.07 mg/kg (which is significantly below permitted MRLs). Measurement precision was below 15% and method precision typically close to 14% relative standard deviation. Finally, the validated LTP-HR-MS method was tested with unspiked fruit samples bought at a local grocery store. Pesticide residues of Cyprodinil and Fludioxonil (0.003 – 0.03 mg/kg) were readily detected. These results were directly compared to a standard liquid chromatography electrospray HR-MS method and found to be in good agreement.

INTRODUCTION

Modern agricultural industry uses pesticides to protect crops from pest infestation and diseases. In the European Union (EU) up to 440 approved pesticides are in use to ensure high crop yield and consistent food production.[1] Since most of the applied chemicals show adverse health effects, maximum residue levels (MRL) were set in force by the EU[2] and United States Environmental Protection Agency (US EPA).[3] Quantification of pesticide residues in agricultural products is routinely done to ensure consumer safety, but requires sensitive analytical techniques. Methods established for sub-MRL verification are typically based on either liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS).[4] These methods are very powerful and used routinely. However, time-consuming sample preparation and separation steps are necessary that currently limit applicability as fast screening tool for sub-MRL verification in large numbers of samples. In the last decade, promising methods for direct analysis were developed that reduced the need of sample preparation and demonstrated success in qualitative and semi-quantitative detection. Applications were recently reviewed by Monge *et al.*[5] and include, for example, explosives, illicit drugs, and biomarkers. Ambient desorption/ionization (ADI) MS was also successfully applied in qualitative and semi-quantitative pesticide residue analyses[6]. For example, DART-MS (direct analysis in real time, developed by R. B. Cody[7]) was used to detect agrochemical residues on fruits and vegetables. For qualitative screening purposes, swabs were used for fruit and vegetable skin sampling that were then probed directly by DART-MS.[8,9,10] Quantitative analysis of agrochemical residues in plant products required extraction with the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) procedure prior to DART-MS or desorption electrospray ionization MS (DESI-MS) analysis.[9,11] Cajka *et al.*[12] analyzed fungicides in fruit extracts and explored the recovery levels under different conditions during the extraction step. They concluded that the surface extraction method

would be best due to the high recovery rates for the selected fungicides despite the high solvent consumption. A quantitative approach towards direct monitoring of xenobiotics on fruit and vegetable peel with DART-MS was recently introduced by Farré *et al.*[13] Results for the content of selected pesticides on fruit peel obtained with direct analysis were found to be in good agreement with results from LC/ESI-MS after sample preparation. Direct analysis of pesticide standards on fruit peel was also demonstrated using atmospheric pressure glow discharge mass spectrometry (APGD-MS)[14]. Achieved LODs on apple skin were in the ng-range and estimated concentrations (μg/mg) below EU regulations.

Another plasma-based desorption/ionization source is the low-temperature plasma (LTP) that was introduced by Harper *et al.*[15]. In contrast to the direct-current powered DART source, LTP is based on a dielectric-barrier discharge, which is sustained in helium by applying a high-frequency alternating current voltage. Typically, LTP is operated at low power (below five W) and low temperature (slightly above room temperature) and, therefore, allows nondestructive analysis of temperature-sensitive samples. Performance characteristics of LTP-MS in terms of ionization capabilities were recently compared with ESI-MS and APCI-MS.[16,17] The Cooks group[18] first used low-temperature plasma mass spectrometry (LTP-MS) to analyze several agrochemicals in foodstuff and water samples. Fruits and vegetables were qualitatively screened for pesticides on the surface. For semi-quantitative analysis, QuEChERS extracts were spiked with standards of pesticides at different concentrations. In a different study, a handheld mass spectrometer was utilized with LTP and paper-spray ionization, respectively, for *in situ* analysis of agrochemical residues on fruit skin, but with a rather time-consuming standard addition method for semi-quantitative analysis.[19]

In the majority of published studies, direct analysis of agrochemical residues without any sample preparation was performed qualitatively. For quantitative information, an additional sample preparation step is required, which usually comprises extraction of the analytes from

the sample. While direct quantitative analysis of postharvest agrochemicals on the surface of fruits or vegetables is feasible, this procedure is complicated for pesticides that are applied during the growth phase because they tend to diffuse from peel to flesh of the plant.[20] In the latter case, sample preparation in form of an extraction step from the homogenized sample would provide more reliable results.

In moving this field of ADI-MS forward, a key would be the ability to also perform accurate quantification in addition to the benefits mentioned above. This study focuses on validation of LTP-MS for quantitative analysis of pesticide residues in fruit extracts. Exemplarily, important fungicides and insecticides were selected based on a 2009 publication of the German Federal Office of Consumer Protection and Food safety in which MRL violations for produce sold in Germany were reported e.g. for Acetamiprid, Cyprodinil, Fenhexamid, and Fludioxonil.[21] Acetamiprid is an insecticide and applied to the plant in form of a spray similar to Cyprodinil and Fenhexamid (both fungicides). Fludioxonil is another fungicide and typically used for seed or postharvest treatment. In this study, it was evaluated which time-consuming steps of standard methods could potentially be eliminated or have a significant influence on the analytical results in LTP-MS. Special attention was paid to the pesticide extraction from the fruit matrix, which was carried out with the QuEChERS approach that includes an analyte extraction step from the homogenized sample followed by a cleanup step. Samples obtained from the extraction step (extraction samples) and after an additional cleanup step (cleanup samples) were submitted to quantitative analysis with LTP-Orbitrap-MS. Several experiments were conducted to evaluate the performance of the method: First, calibration curves with standard solutions of each pesticide were determined. Second, potential matrix effects in grape and raspberry were evaluated with matrix-matched calibration and spiked samples. In further analyses, matrix-matched calibration was used to determine linear ranges and limits of quantification (LOQs). Precision and accuracy of LTP-

MS as well as precision of the entire method including sample preparation were determined at both low and high pesticide concentrations. Finally, real unspiked produce, grape and raspberry, were bought at a local grocery store and analyzed for pesticide residues. To test the stability of the method over time, real samples were analyzed twice in intervals of half a year and quantitative results were directly compared to quantification with an established LC/ESI-HR-MS method.

EXPERIMENTAL SECTION

Reagents

Acetamiprid, Cyprodinil, Fenhexamid, and Fludioxonil were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and used without further purification. Extraction reagents magnesium sulfate, sodium chloride, sodium citrate tribasic dehydrate, sodium citrate dibasic sesquihydrate, and cleanup tubes with PSA SPE (Supel QuE, Sigma-Aldrich/Supelco, Steinheim, Germany) for the QuEChERS procedure were purchased from Sigma-Aldrich Chemie GmbH. All solvents were ordered from Sigma-Aldrich Chemie GmbH and Fluka Chemie GmbH (Buchs, Switzerland) in the highest quality available. Dilutions were performed with double-distilled water (Aquatron A4000D, Barloworld Scientific, Nemours Cedex, France).

LTP probe

The LTP probe was home-built based on the source design first described by Harper *et al.*[15] Small modifications to the design were carried out during an optimization study. In short, the desorption/ionization source features a dielectric-barrier discharge in a quartz glass capillary (7.0 mm o.d., 1.0 mm i.d.), which is sustained between an inner stainless-steel pin electrode (1 mm diameter) and an outer copper ring electrode (20 mm wide, 27 mm distance to

capillary exit). A high-voltage alternating current waveform of 10 kV_{pp} at 30 kHz is applied to the inner pin electrode (power supply model Minimax3, Information Unlimited, Amherst, MA, USA) to generate the plasma. Teflon covers were used to shield both electrodes to guarantee safe operation. An in-house built ion-source housing was used to cover the apparatus during analysis for safety and to avoid disturbance of the plasma from laboratory air currents. Helium (high purity grade 4.6, Westfalen AG, Muenster, Germany) was used as discharge gas and fed into the capillary by use of a T-piece (Swagelok Company, Solon, OH, USA) at a flow rate of 300 mL/min. The mass spectrometer inlet capillary was extended in length (34 mm) compared to conventional ESI operation. LTP probe was positioned at an angle of 60° to the sample well plate in front of the MS inlet. Probe-to-MS-inlet distance was 2 mm and probe-to-sample-well-plate distance was 7 mm. Sample well plate was placed as close as possible to the inlet capillary (at approx. 1 mm distance). A detailed scheme of the experimental setup is included in the supporting information (*cf.* Figure S-1). The well plate temperature was held constant at approximately 423 K during analysis by use of a heating foil (Telemeter Electronic GmbH, Donauwörth, Germany). It is noteworthy that the pesticides studied here could not be observed without additional heating using the home-built source described above. In this regard, additional heating clearly aids thermal desorption of the analytes studied here.

LTP-MS analysis

Standard stock solutions of Acetamiprid, Cyprodinil, Fenhexamid and Fludioxonil were prepared in acetonitrile (ACN) with concentrations of $4.49x10^{-3}$, $4.44x10^{-3}$, $3.31x10^{-3}$, and $4.03x10^{-3}$ mol/L, respectively. For analysis, 10-µL-aliquots from the standard solutions were pipetted onto the heated glass sample well plate in front of the mass spectrometer inlet and sampled instantly with the LTP probe. An Exactive HCD (Thermo Scientific, Bremen, Germany) high-resolution mass spectrometer was used as the detection system. Operating

parameters were optimized for Cyprodinil (EIC, m/z 226.1340) and are listed in the supporting information (*cf.* Table S-1). Mass calibration was performed with an ESI source (model Ion Max, Thermo Scientific) with ESI positive ion calibration solution (20 µg/mL caffeine, 1 µg/mL MRFA and 0.001% Ultramark 1621) and ESI negative ion calibration solution (2.9 µg/mL sodium dodecyl sulfate, 5.4 µg/mL sodium taurocholate and 0.001% Ultramark 1621). All analyses were performed in triplicate. Ionized species produced transient mass spectrometric signals, which were recorded and processed with Thermo Excalibur software 2.1 (Thermo Scientific) and Origin Pro 8.0 (OriginLab, Northampton, MA, USA), respectively. A mass window of 1 ppm for all isotope signals of each compound was chosen for area determination of the detected transients.

Standard calibration curves with LTP-MS

For external calibration, standard stock solutions were diluted with ACN to concentrations of $5x10^{-5}$, $1x10^{-5}$, $5x10^{-6}$, $1x10^{-6}$, $5x10^{-7}$, $1x10^{-7}$, $5x10^{-8}$, $1x10^{-8}$, $5x10^{-9}$, and $1x10^{-9}$ mol/L for each pesticide. Analyses were performed using the methods and parameters described above.

Matrix-matched calibration with LTP-MS

For matrix-matched calibration experiments, grape and raspberry samples were purchased from a local supermarket in Muenster, Germany, in organic quality and were tested for pesticides with LC/ESI-MS. All fruits were free from pesticides studied here. Standard pesticide solutions in ACN were added to each 10 g of fruit homogenized by a stick blender. Final pesticide concentrations were $5x10^{-5}$, $1x10^{-5}$, $5x10^{-6}$, $1x10^{-6}$, $5x10^{-7}$, $1x10^{-7}$, $5x10^{-8}$, $2x10^{-8}$, $1x10^{-8}$, and $7x10^{-9}$ mol/L. Spiked samples were homogenized and subjected to QuEChERS extraction: First, 10 g of the homogenized fruit were mixed with 10 mL ACN. After addition of 4 g magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate tribasic dehydrate, and 0.5 g sodium citrate dibasic sesquihydrate the mixture was shaken for 1 min and centrifuged

for 5 min at 3000 rcf (Centrifuge 5416, Eppendorf AG, Hamburg, Germany). Second, the raw extract was subjected to an additional cleanup step, which consisted of pipetting a 6-mL extract aliquot in a primary secondary amine (PSA) cleanup tube followed by shaking for 30 sec, and centrifugation for 5 min at 3000 rcf. In conventional methodology, this extract is exposed to separation and detection with LC/ESI-HR-MS (described below). In LTP-MS, extract aliquots were investigated that were taken before (with heavy matrix load) and after the cleanup step (less matrix). Consequently, matrix-matched calibration was used to determine matrix effects in samples after the extraction step and after the cleanup step. To determine the performance characteristics of the method, linear range, and LOQs were derived and calculated from matrix-matched calibration curves after linear regression analysis.

Precision

Measurement precision and method precision were determined as follows. To determine the measurement precision of LTP-MS, one fruit sample of each fruit was spiked with all pesticides ($1x10^{-7}$ mol/L and $1x10^{-5}$ mol/L) and then analyzed in triplicate both after the extraction step and the cleanup step. To determine the precision of the entire method including sample preparation, three fruit samples were spiked with all pesticides ($1x10^{-7}$ mol/L and $1x10^{-5}$ mol/L). Each sample was extracted and subjected to the additional cleanup step prior to triplicate analysis after each step.

Recovery studies with LTP-MS

For recovery studies, pesticide standards were added to one fruit samples of each fruit at known concentrations of 5x10-7 and 1x10-5 mol/L and analyzed in triplicate after extraction and cleanup. Recovery values were calculated using matrix-matched calibration of each pesticide.

Analyses of real samples with LTP-MS

One package of conventional grapes and one package of conventional raspberries were purchased from a local supermarket. For analysis, all fruits were homogenized and subjected to QuEChERS extraction and cleanup procedures as described above. Samples were analyzed after the extraction step and after the cleanup step. Pesticide residue levels were calculated after matrix-matched calibration.

To evaluate the stability of the LTP-HR-MS method over a period of six month, fruit and calibration samples were stored in a freezer at 255 K and analyses were repeated after six month.

Validation of LTP-MS by LC/ESI-MS

LTP-MS results for pesticide residues in unspiked fruit samples were validated by comparing them directly to LC/ESI-MS experiments. For LC/ESI-MS measurements, QuEChERS extracts after the cleanup step were analyzed. Separation of pesticides was carried out on a Discovery C18 column (Supelco, Bellefonte, USA, column length 150 mm, inner diameter 3.0 mm, particle size of 5 µm) with an Accela LC system (Thermo Scientific, Bremen, Germany). Sample injection volume was 10 µL. A gradient program was developed with a mobile phase containing an ammonium acetate buffer (10 mM, pH 5.0) and Acetonitrile (ACN) at a flow rate of 300 µL/min as follows: from 0 min to 1 min 5 % ACN, from 1 min to 13 min increasing content of ACN towards 50 %, from 13 min to 14 min 50 % ACN, from 14 min to 20 min further increasing content of ACN towards 95%, from 20 min to 23 min 95% ACN, from 23 min to 24 min decreasing content of ACN towards 5% and an equilibration time of 6 min. Mass spectrometric detection was carried out on an Exactive HCD (Thermo Scientific) instrument equipped with an ESI source (model Ion Max, Thermo Scientific). Operating parameters were optimized for Cyprodinil (EIC, m/z 226.1340) and are listed in the supporting information (*cf.* Table S-2). All analyses were performed in duplicate.

Thermo Excalibur software 2.1 (Thermo Scientific) and Origin Pro 8.0 (OriginLab) were used for data analysis and processing, respectively.

RESULTS AND DISCUSSION

For the determination of the performance characteristics of an LTP-Orbitrap-MS method for quantitative pesticide residue analysis in fruits, four different pesticide species (*cf.* Table 1) were investigated in grape and raspberry fruit matrices.

Table 1

In LTP-MS, pesticides were readily detected as protonated (Acetamiprid at *m/z* 223.0744, *cf.* Figure S-2, Cyprodinil at *m/z* 226.1340, *cf.* Figure 1, and Fenhexamid at *m/z* 302.0708, *cf.* Figure S-2) or deprotonated (Fludioxonil at *m/z* 247.0323, *cf.* Figure 1) compounds with little fragmentation.

Figure 1

In case of Cyprodinil at *m/z* 242 and Fludioxonil at *m/z* 263, oxidized species (addition of one oxygen atom) were observed at relatively low abundance. Similar observations for aromatic compounds can be found in the literature, however, the oxidation process is not fully resolved.[16] Because only traces of those oxidized species were detected and their formation was not constant, they were not included in the analysis procedure.

Pesticides analyzed in grape and raspberry matrices could be clearly identified with their exact masses and distinguished from other species present in the sample. Mass accuracy was typically below 2 ppm and 4 ppm in positive and negative ionization mode, respectively.

Matrix effects in LTP-MS

As an ambient technique with direct desorption and ionization of the sample, LTP-MS can suffer from ion suppression through matrix effects. Accordingly, the potential presence of matrix effects in pesticide screening was studied here as well. Exemplarily, Figure 2 shows the transient mass spectrometric signal of Fludioxonil (EIC, *m/z* 247.0323) in spiked raspberry

matrix after extraction and after additional cleanup, respectively, and compared to Fludioxonil in a standard solution.

Figure 2

At the beginning of the experiment, a decrease of the initial transient peak height of Fludioxonil (*m/z* 247.0323) was observed in both extraction and cleanup sample (up to 80%) compared to a standard solution. However, total peak areas of the transient mass spectrometric signals were integrated and used for further calculations. In the extraction sample, signal reduction (peak area) to 64 ±1% relative to the peak area of the standard solution was observed. It is assumed that ion suppression due to matrix effects from the homogenized fruit were the cause for this observation. This assumption is supported by the fact that the cleanup sample did not exhibit suppression effects (104 ±4 % peak area). Consequently, the overall presence of matrix effects was studied for each pesticide in more detail and for both fruit matrices.

Figure 1 shows exemplarily mass spectra for Cyprodinil and Fludioxonil in standard solution at a concentration of $5x10^{-5}$ mol/L. Additional diagrams in Figure 1 show calibration curves obtained with pure standards and matrix-matched standards, respectively, in grape and raspberry after the cleanup step. Comparable mass spectra and calibration curves of Acetamiprid and Fenhexamid can be found in the SI (*cf.* Figure S-2).

Matrix effects were determined by quantification of pesticide spikes of known concentration (peak area) with a matrix-matched calibration. The calculated amount was then plotted versus the spiked concentration. Without any matrix effects, the ideal linear regression between spiked concentration and calculated concentration would yield a curve with a slope of 1 and an intercept of 0. If the calculated values of slope and intercept do not include these values in their confidence range then the matrix influences the ion signal of the analyte. It was found that most of the pesticides studied here were prone to matrix effects after extraction and

cleanup and in both fruit matrices. In raspberry and grape matrix, all pesticides suffered from considerable signal suppression after the extraction step (for example, up to 45% signal suppression in raspberry for Cyprodinil at $1x10^{5}$ mol/L). Signal suppression after extraction presumably resulted from the high matrix content in the sample. For example, after extraction, raspberry samples still contained a considerable amount of plant pigments. Ultimately, this can lead to competitive ionization in the LTP source and signal suppression of analyte species. After the cleanup step, Cyprodinil (*m/z* 226.1340) still showed slight ion signal suppression in grape and raspberry. In contrast, the Acetamiprid signal (*m/z* 223.0744) was enhanced in both matrices. Fenhexamid suffered from slight signal suppression in raspberry but not in grape whereas Fludioxonil did not exhibit matrix effects after the cleanup step neither in the raspberry nor in the grape sample. Ion signal enhancement could result from an altered desorption process in matrix or a more complicated ionization process. Based on these experiments on matrix effects, it is recommend to use matrix-matched calibration in LTP-MS pesticide analyses with QuEChERS pretreatment. This was done so for all experiments discussed below.

Calibration and limits of quantification

Matrix-matched calibration curves for all investigated pesticides were obtained over more than four orders of magnitude (approximately $1x10^{-8} – 5x10^{-5}$ mol/L) with regression coefficients ranging from $R^2 = 0.991$ to $R^2 = 0.999$. Linear ranges were comparable within pesticides and similar results were obtained for the extraction and cleanup samples.

An F-test[22] was performed on the data and showed a lack of homogeneity of variance over the calibration range. In this case, simple linear regression would assume homogeneous variance and, in turn, yield false results. Therefore, a weighted linear regression was performed for quantitative analysis.

LOQs were calculated using the weighted linear regression curve and its residual standard deviation. In Figure 3, LOQs for all pesticides in extraction and cleanup samples in both fruit matrices are depicted. In addition, the MRL for each pesticide is indicated for reference as a dashed gray line.

Figure 3

All LOQs were found to be significantly below the MRL: LOQs vary from 0.001 mg/kg (Cyprodinil in grape after cleanup) to 0.07 mg/kg (Fludioxonil in grape after extraction). Because these LOQs are well below the MRL for pesticides, this method is considered to be an attractive tool for MRL verification in fruits.

Because matrix effects exist (discussed above), they also affect LOQs in matrix-containing samples, i.e. after the first QuEChERS extraction. For Fludioxonil, LOQs in raspberry and grape extraction samples are increased by a factor of 2 and 20, respectively, compared to the corresponding cleanup samples (0.02 mg/kg and 0.07 mg/kg in extraction samples compared to 0.01 mg/kg and 0.003 mg/kg in cleanup samples). However, these LOQs are still significantly below the MRL. It is noteworthy, however, that a single QuEChERS extraction step followed by direct LTP analysis would still be sufficient to detect pesticide residues over a wide concentration range (approximately $5x10^{-8}$ – $5x10^{-5}$ mol/L) and with LOQs (0.001 – 0.07 mg/kg) below the MRL.

Precision and recovery

Measurement precision was determined as RSD of a triplicate analysis of one sample (measurement precision of LTP-MS) and as RSD of three equally treated sample preparations (precision of the entire method with sample preparation). Recovery rates were determined

from spiked fruit samples that were compared with the matrix-matched calibration. Obtained results are summarized in Table 2.

Table 2

Precision of the LTP-MS method varies for all pesticides over a range of 1% to 15%. Here, Cyprodinil shows lower RSDs <9% while Acetamiprid exhibits higher RSDs of up to 15%. In previous studies, LTP-MS showed RSDs of 10 – 30% in liquid samples with matrix.[18,23] Precision of the entire method including sample preparation remains at a comparable level (up to 14% RSD) for most cases. In three cases of the complete study, however, higher RSD values of up to 27% were found, i.e. in grape after extraction for Acetamiprid and Fenhexamid, and in raspberry after cleanup for Fenhexamid. It is assumed that these higher RSDs were a result of the sample preparation step. Errors could also result from the manual handling of standards. The observed variation for the LTP-MS method is relatively high compared to the conventional LC/ESI-MS method (typically RSDs of less than 2% for measurement precision and of up to 7% for the entire method with sample preparation were obtained in this study). In the future, precision of the LTP-MS method could be improved via utilization of an automated sample introduction system. This approach is likely to improve the precision of the entire method.

Regarding the obtained recovery rates, no clear trend was observed with changing matrix or pesticide species. Recovery rates range from 69% to 133% for all pesticides and matrices. Interestingly, analysis with LC/ESI-MS, which was used for validation, produced similar recovery rates for all species (75% - 132%, data not shown here). These recovery experiments demonstrate that this direct analysis approach with LTP-MS is capable of detecting pesticide residues in grape and raspberry matrices.

Real samples

After validation, LTP-MS was used to determine pesticide residues in grape and raspberry that were bought at a local grocery store. Residues were detected and subsequently quantified in both fruits for Cyprodinil and Fludioxonil. Extracts of the fresh fruits were analyzed directly after QuEChERS pretreatment and again after storage for several month (six month between measurement, e.g., to test for potential drift of the method). For validation purposes, pesticide residue levels in identical samples were also determined with an established LC/ESI-MS method. Quantitative results were derived from matrix-matched calibration curves with weighted linear regression and residual standard deviation. Data obtained with LTP-MS and LC/ESI-MS are displayed in Figure 4.

Figure 4

MRL for Cyprodinil defined by the EU are 5 mg/kg and 10 mg/kg in grape and raspberry, respectively, while the MRL for Fludioxonil are 5 mg/kg in both fruits. In the real samples, pesticide residues of Cyprodinil and Fludioxonil were found, but significantly below MRL in both fruits. Contents were close to the LOQs determined for both the LTP-MS and LC/ESI-MS methods, but still quantifiable. In grape, a concentration of 0.003 mg/kg was found for Cyprodinil. Here, similar results could be observed for either the extraction and cleanup samples and at different points in time (six month between measurements). In raspberry, Cyprodinil residues amounted to a concentration of 0.03 mg/kg, however, with a higher variation of residue levels between the two measurements. For Fludioxonil in grape, a concentration of 0.002 mg/kg could be obtained for the cleanup samples. Here, its content in the extraction samples was below the LOQ and could only be reported qualitatively. In raspberry samples, Fludioxonil residues accounted for a concentration of 0.02 mg/kg. In general, a variation between LTP-MS measurements of extraction samples (E1 at day 0, E2 after six month) and cleanup samples (C1 at day 0, C2 after six month) was observed. The

degree of the variation seems to be depending on the matrix and is more pronounced in the raspberry matrix. However, the exact cause should be evaluated in a future study with a larger dataset.

Calculated confidence intervals from the multiple measurements were found to be similar for samples analyzed with LTP-MS and for samples analyzed with LC/ESI-MS (*cf.* Figure 4). Analysis of real unspiked samples shows that LTP-MS is capable of quantifying pesticide residues in fruit extracts and detecting possible MRL exceeding. A comparison of determined residue levels by LTP-MS and LC/ESI-MS is shown in Figure 5 by contrasting the obtained concentrations.

Figure 5

Levels from the LTP-MS analysis were calculated as a mean of the results obtained from both extraction and cleanup samples determined on different days. Though the levels vary, there is still a good agreement of the average values between LTP-MS and LC/ESI-MS. The higher variation in LTP-MS is partly caused by the results obtained on two different analysis days. Analysis with LC/ESI-MS includes only results from one experimental day. To summarize the analysis of real samples it can be concluded that LTP-MS in combination with QuEChERS extraction is suitable for MRL verification.

CONCLUSIONS

In this study, LTP was coupled to Orbitrap MS, optimized, and validated for quantitative analysis of pesticide residues in fruit extracts. Relevant pesticides in the EU and Germany, which exceeded MRL in the past, were investigated (namely Acetamiprid, Cyprodinil, Fenhexamid, and Fludioxonil). Sample preparation was performed with the QuEChERS procedure. Fruit matrix still present in extracted samples and cleanup samples was found to

have an effect on the analytical response. Therefore, matrix-matched calibration was used throughout the study. For most pesticides, linear ranges cover more than four orders of magnitude with regression coefficients from $R^2 = 0.995 – 0.999$. Limits of quantification (0.001 – 0.07 mg/kg) after only the extraction step were found to be significantly below the defined MRL. Precision of the entire method was typically below 14% RSD (n = 3) with no difference between extraction and cleanup samples. Recovery rates were found to be comparable to results obtained from LC/ESI-MS analyses with QuEChERS pretreatment. After the validation, capabilities of LTP-MS were tested for quantification of pesticide residues in real unspiked samples. Detected pesticides were successfully quantified in extraction as well as in cleanup samples with one exception in which the concentration of the pesticide in the fruit was below the LOQ. Real unspiked samples were additionally analyzed by LC-ESI/MS for LTP-MS method validation. Pesticide residue levels and their confidence intervals were in the same range for both methods.

It can be concluded that, for the pesticides studied here, LTP-HR-MS is a) a fast screening method for pesticide residues in fruit extracts, b) suitable for flagging pesticides that exceed MRL, and c) a useful tool for sub-MRL verification in normal and organic fruits. Further, it was found that the additional QuEChERS cleanup step improved the LOQs, but was not necessary for achieving relevant MRL concentration ranges. Clearly, one limitation of LTP-MS is currently its limited precision compared to LC/ESI-MS. In the future, improvements could be achieved with e.g. automated sample application onto the sample plate, and internal standardization. Finally, LTP-MS analysis is not limited to liquid or dried liquid samples but can be extended towards quantitative analysis in, e.g., juice and water samples.

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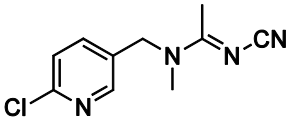
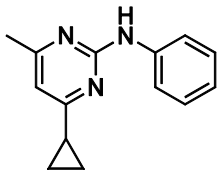
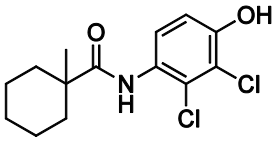
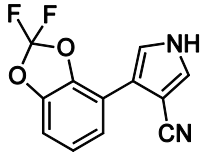
Table 1: List of studied compounds and corresponding species detected in LTP-HR-MS.

Pesticide	Structure	Detected species	Exact mass of detected species	Mass accuracy [ppm]
Acetamiprid		$(M+H)^+$	223.0744	0.9
Cyprodinil		$(M+H)^+$	226.1340	0.7
		$(M+O+H)^+$	242.1290	0.3
Fenhexamid		$(M+H)^+$	302.0708	-1.9
Fludioxonil		$(M-H)^-$	247.0323	4.1
		$(M+O-H)^-$	263.0273	4.0

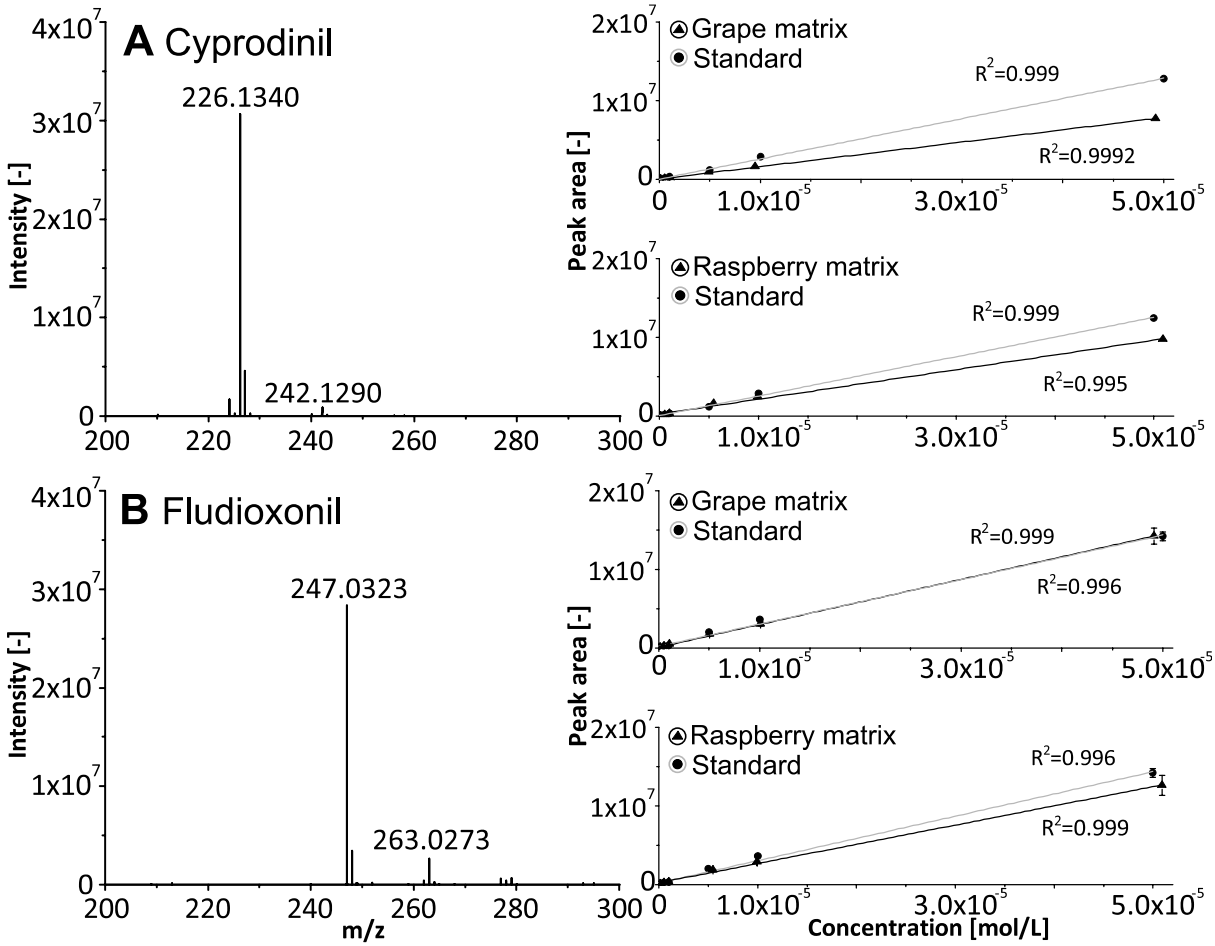


Figure 1: LTP-HR-MS mass spectra of A) Cyprodinil and B) Fludioxonil. Additional diagrams show the corresponding calibration curves for standards (black) and matrix-matched standards in grape and raspberry matrix after cleanup (gray). Most abundant signals correspond to the protonated/deprotonated species (*cf.* Table 1).

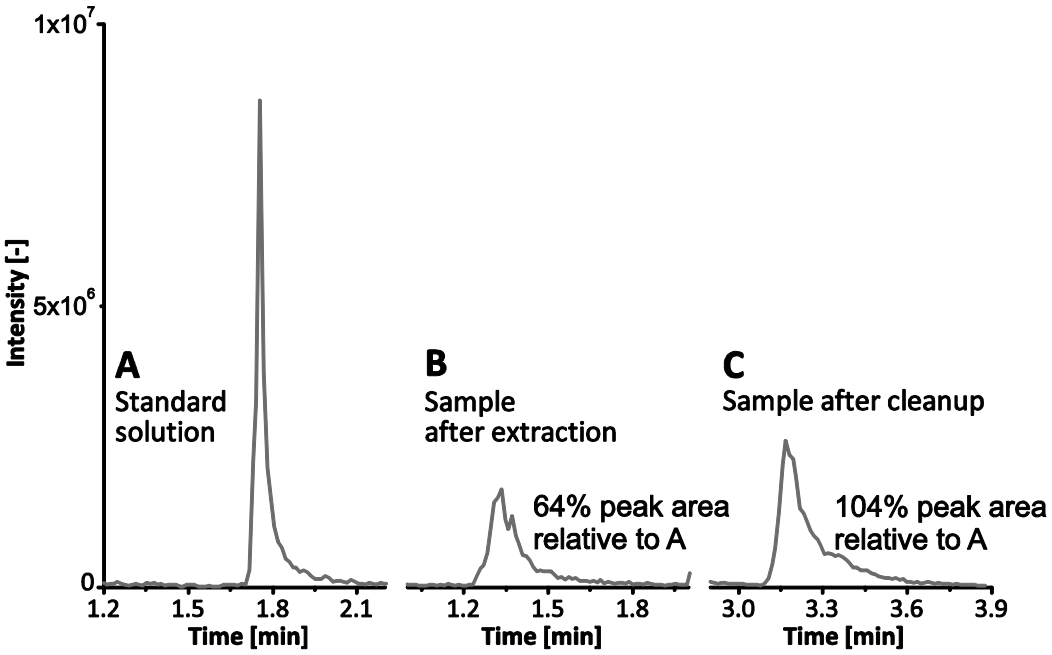


Figure 2: Transient mass spectrometric signal of Fludioxonil (EIC m/z 247.0323) obtained with LTP from A) standard solution, B) extraction sample from raspberry matrix, and C) cleanup sample from raspberry matrix.

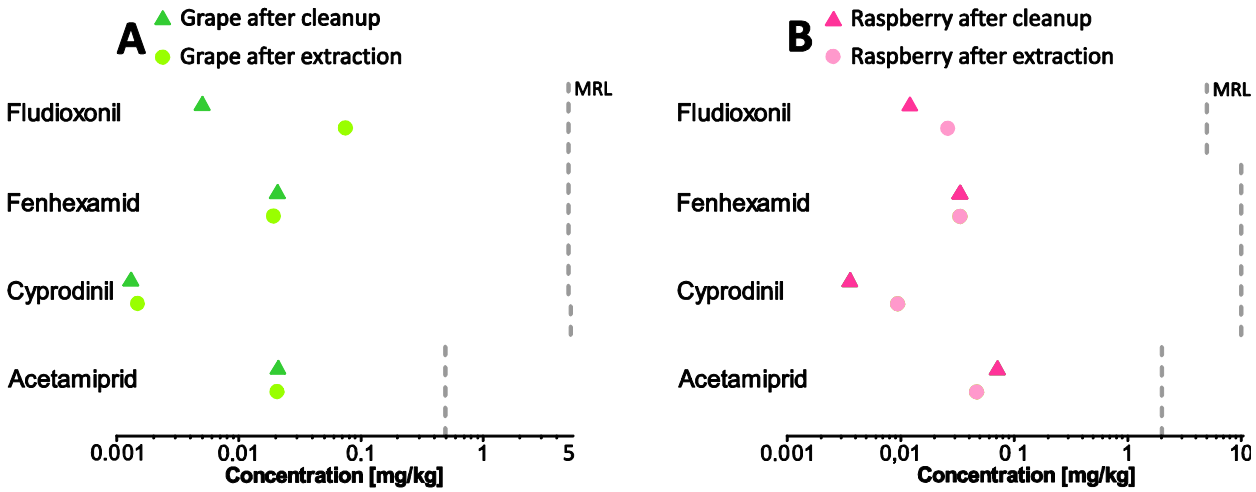


Figure 3: LOQs for pesticides obtained with direct LTP-HR-MS analysis after extraction (circles) and cleanup (triangles) in A) grape matrix (green) and B) raspberry matrix (pink). Pesticide maximum residue levels (MRL) for EU legislation are indicated as a dashed line.

Table 2: LTP-HR-MS measurement precision, overall method precision and recovery rates in extraction and cleanup samples for analyzed pesticides in raspberry and grape matrices.

Pesticide	Matrix	Analysis step	Measurement precision RSD [%]	Precision of entire method RSD [%]	Recovery [%]
Acetamiprid	Raspberry	Extraction	<14.6	<13.7	80 - 104
		Cleanup	<12.1	<12.5	77 - 89
	Grape	Extraction	<14.7	<26.6	72 - 132
		Cleanup	<15.2	<9.7	79 - 81
Cyprodinil	Raspberry	Extraction	<6.5	<14.1	84 - 104
		Cleanup	<8.5	<7.7	96 - 108
	Grape	Extraction	<7.3	<9.9	104 - 121
		Cleanup	<8.6	<10.5	111 - 133
Fenhexamid	Raspberry	Extraction	<10.3	<7.6	95 - 117
		Cleanup	<11.5	<20.7	71 - 103
	Grape	Extraction	<13.5	<25.2	77 - 123
		Cleanup	<10.7	<5.2	69 - 77
Fludioxonil	Raspberry	Extraction	<13.5	<11.3	88 - 92
		Cleanup	<10.0	<6.2	76 - 94
	Grape	Extraction	<9.6	<7.3	94 - 102
		Cleanup	<10.0	<7.3	80 - 94

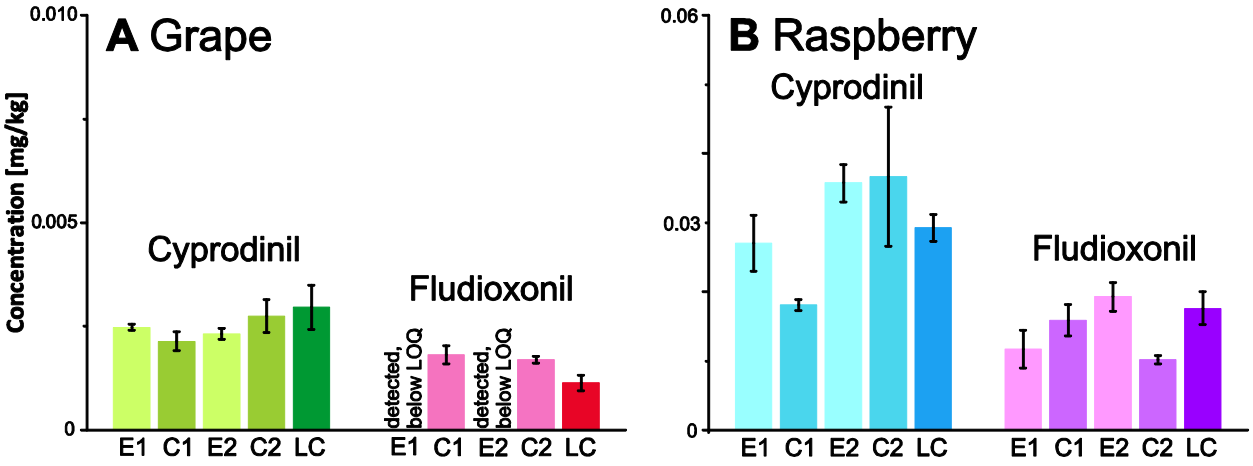


Figure 4: Fruits bought at a local supermarket tested for pesticide residues by LTP-Orbitrap-MS and LC/ESI-Orbitrap-MS: A) residue levels for Cyprodinil (shades of green) and Fludioxonil (shades of pink) in grape. B) Cyprodinil (shades of blue) and Fludioxonil (shades of violet) in raspberry matrix. Residue levels were obtained from LTP-MS analysis at different days after extraction (E1, E2) and after cleanup (C1, C2) and compared to LC/ESI-MS analysis (LC).

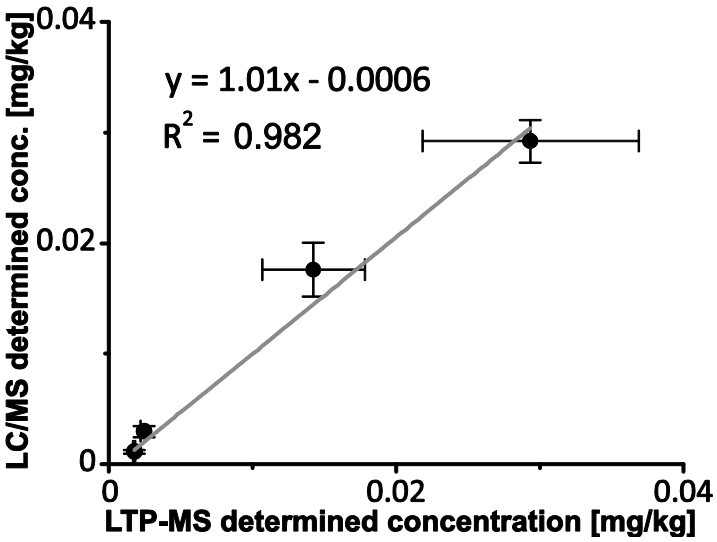


Figure 5: Comparison of residue levels of Cyprodinil and Fludioxonil determined with LTP-MS and LC/ESI-MS.

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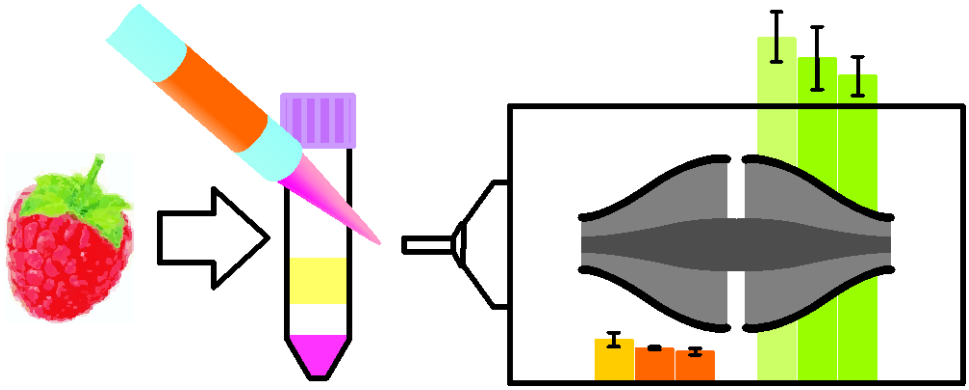
TABLE OF CONTENT



Low-temperature plasma ambient desorption/ionization high-resolution mass spectrometry is validated for quantitative analysis of pesticides in real samples.