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	or Teflon (DESI) could be directly detected. With DART this was also possible for ziram. Before the instrumental analysis of fruit matrix, an extract had to be prepared following a straightforward procedure. The raw extracts were deposited on a slide (DESI), or rods were dipped into the extracts (DART), after which thiram and ziram could be rapidly detected (typically 10 samples in few minutes). In the case of thiram, the lowest calibration levels were 1 mg kg–1 (DART–TOFMS, DESI– MS2) and 0.1 mg kg–1 (DART–orbitrapMS). For ziram, the achieved lowest calibration levels were 0.5 mg kg–1 (DART–TOFMS) and 1 mg kg–1 (DART–orbitrapMS). In all cases, this was sufficiently low to test samples against EU-MRLs for a number of fruit crops. By using an internal standard (semi)quantitative results could be obtained.

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Abstract

Dithiocarbamates (DTCs) are fungicides, which require a specific single residue method for detection and verification of compliance with maximum residue limits (MRLs) as established for fruit and vegetables in the EU. In this study, the use of ambient mass spectrometry was investigated for specific determination of individual DTCs (thiram, ziram) in fruit. Two complementary approaches have been investigated for their rapid analysis: (*i*) direct analysis in real time (DART) combined with medium-high resolution/accurate mass time-of-flight mass spectrometry (TOFMS) and high-resolution/accurate mass Orbitrap MS, and (*ii*) desorption electrospray ionization (DESI) combined with tandem-in-time mass spectrometry (MS^2). With both techniques, thiram deposited on a glass surface (DART) or Teflon (DESI) could be directly detected. With DART this was also possible for ziram. Before the instrumental analysis of fruit matrix, an extract had to be prepared following a straightforward procedure. The raw extracts were deposited on a slide (DESI), or rods were dipped into the extracts (DART), after which thiram and ziram could be rapidly detected (typically 10 samples in few min). In the case of thiram, the lowest calibration levels were 1 mg kg^{-1} (DART–TOFMS, DESI–MS^2) and 0.1 mg kg^{-1} (DART–Orbitrap MS). For ziram, the achieved lowest calibration levels were 0.5 mg kg^{-1} (DART–TOFMS) and 1 mg kg^{-1} (DART–Orbitrap MS). In all cases, this was sufficiently low to test samples against EU-MRLs for a number of fruit crops. By using an internal standard (semi)quantitative results could be obtained.

Keywords: Ambient mass spectrometry; Desorption electrospray ionization; Dithiocarbamate fungicides; Direct analysis in real time; Fruits; Mass spectrometry

Introduction

Introduced 40–70 years ago, dithiocarbamate fungicides (DTCs) still represent an important class of plant protection product widely used in agriculture. They are characterized by a broad spectrum of activity against various plant pathogens, by low acute mammalian toxicity, and low production costs. In combination with modern systemic fungicides, they are also used to manage resistances and to broaden the spectrum of activity. It is therefore not surprising that the so-called "maneb group" (zineb, maneb, mancozeb, propineb, metiram) was not only one of the most frequently detected pesticides reported in the document on Monitoring of Pesticide Residues in Products of Plant Origin in the European Union, Norway, Iceland, and Liechtenstein, but this group also had the highest frequency in exceeding maximum residue limits (MRLs) (European Commission (EC), SEC(2007) 1411).

DTCs are non-systemic fungicides, the residues of which mostly remain on the surface of the sprayed crop. DTCs can be categorized into three sub-classes depending upon their carbon skeleton—dimethyldithiocarbamates (DMDs), ethylene bis(dithiocarbamtes) (EBDs), and propylene bis(dithiocarbamates) (PBDs). Without the presence of sodium salts, EBDs and PBDs forming polymeric chelates are almost insoluble in both, water and organic solvents. However, DMDs (*e.g.*, thiram, ziram, ferbam) are slightly soluble in water and some polar organic solvents. Due to the insolubility in common extraction solvents and also poor stability, they are not amenable to multi-residue methods. On this account, a single residue method which involves their conversion into carbon disulfide (CS_2) is commonly used. CS_2 is then detected spectrophotometrically or by gas chromatography with various detection options (Crnogorac and Schwack 2009). Inherent to this approach is that no information is obtained on the origin or the source of CS_2, *i.e.*, which DTC had been applied in the field. Although current EU legislation for routine enforcement is still based on the determination of DTCs as CS_2, specific maximum residue limits (MRLs) have been

established for thiram, ziram, and propineb, these therefore should be determined as such when required (European Community, Commission Directive 2007/57/EC).

Over the few recent years, a large number of novel ambient desorption ionization techniques, such as desorption electrospray ionization (DESI), atmospheric-pressure solids analysis probe (ASAP), direct analysis in real time (DART), and many others, have become available. Their main advantages compared to conventional techniques, involve the possibility of direct sample examination in the open atmosphere, minimal, or no sample preparation requirements, and, remarkably high sample throughput (Cody et al. 2005; Takats et al. 2004; McEwen et al. 2005).

DART represents one of APCI-related techniques employing a glow discharge for the ionization. Metastable helium atoms, originated in the plasma, react with ambient water, oxygen, or other atmospheric components to produce the reactive ionizing species. DART ion source was shown to be efficient for soft ionization of a wide range of both polar and non-polar compounds. DART produces relatively simple mass spectra characterized in most cases by $[M+H]^+$ in positive-ion mode, and $[M-H]^-$ in negative-ion mode. Coupling the DART ion source with a mass spectrometer (MS) with a high mass resolving power (*e.g.*, time-of-flight MS, Orbitrap MS) enabling accurate mass measurements allows confirmation of the target analyte identity and the calculation of elemental composition of "unknowns" (Cody et al. 2005; Hajslova et al. 2011).

In the case of DESI, an electrospray (ESI) needle is kept at high voltage and the spray is pneumatically assisted with nitrogen gas. DESI solvents are usually aqueous mixtures of methanol or acetonitrile with formic acid or acetic acid, and are sprayed at a few µl/min. The solvent spray is directed onto the surface to be analyzed. In the case of liquids, sample aliquots can be pipetted onto a surface, usually a glass or polytetrafluoroethylene (PTFE) slide. The pneumatically-assisted electrospray is used to produce charged solvent droplets

and gas phase solvent ions that collide with analytes on the sample surface. The ionization process closely resembles conventional ESI-MS; however, the sample is not present in the solvent or ionized during the electrospray process, and might be less vulnerable to ionization suppression caused by the presence of salts and other interfering matrix components. Similarly to ESI, the resulting mass spectra show mainly singly or multiply charged molecular ion of the analyte (Takats et al. 2004; Nielen et al. 2011).

In this study, we have addressed the challenge to develop a rapid method for the specific determination of individual DTCs based on the use of ambient mass spectrometry. Two complementary approaches have been investigated for rapid analysis of DTC fungicides in fruits by: (*i*) direct analysis in real time (DART) combined with medium-high resolution/accurate mass time-of-flight mass spectrometry (TOFMS) and high-resolution/accurate mass Orbitrap MS, and (*ii*) desorption electrospray ionization (DESI) combined with tandem-in-time mass spectrometry (MS^2).

Materials and methods

Chemicals and reagents

Standards of target DTCs thiram and ziram together with triphenyl phosphate (TPP) used as an internal standard (purity 99.0, 97.0, 99.0%, respectively) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich (Taufkirchen, Germany). Working standard solutions were prepared in acetonitrile (0.5 mg ml^{-1}). In the case of ziram, the dissolving process was supported by ultrasonication for 5 s. Acetonitrile (HPLC grade) was provided by Merck (Darmstadt, Germany). Magnesium sulfate and sodium chloride were delivered from Sigma-Aldrich and Lach-ner (Neratovice, Czech Republic), respectively. Poly(ethylene glycol), PEG 600, obtained from Sigma-Aldrich was used as a mass calibrant.

Ultra-pure water was produced by Milli-Q system (Millipore Corporation, Bedford, MA, USA).

For the DESI experiments acetonitrile and methanol from Biosolve (Valkenswaard, The Netherlands) were used. Formic acid, ammonium formate, sodium acetate, sodium hydrogen carbonate, DL-penicillamine, and a sodium hydroxide solution were from Sigma-Aldrich, magnesium sulfate, acetic acid, and ammonia from Merck. Primary secondary amine from Agilent Technologies (Santa Clara, CA, USA) was used to test the effect of clean up of the extract by dispersive solid phase extraction (dSPE). TMTD, a commercial plant protection product containing thiram, was purchased from a local agrochemicals supplier. Standards of ziram and propineb were purchased from Dr. Ehrenstorfer.

Instrumentation

DART–TOFMS

For DART–TOFMS analyses, the system consisted of a DART ion source (model DART-100, IonSense, Danvers, MA, USA), an AccuTOF LP medium-high resolution TOF mass spectrometer (JEOL (Europe) SAS, Croissy sur Seine, France), and an HTC PAL autosampler AutoDART-96 (Leap Technologies, Carrboro, NC, USA).

The DART ion source was operated in a positive ion mode with helium as the ionizing medium at a flow rate of 3.5 l min^{-1}. The gas beam was heated to 300°C. The discharge needle voltage of the DART source was set to –3000 V and the discharge/grid electrode voltages were +150/+250 V, respectively. The desorption time was 5 s. Accurate mass profiles were acquired within the range *m/z* 100–500, the spectra recording interval was 1 s (1 spectrum s^{-1}), and the peak voltage value was 1000 V. A solution of PEG 600 in methanol (200 µg l^{-1}) was used for mass axis calibration of the DART–TOFMS instrument.

MassCenter (JEOL) software (v. 1.3.0) was used for instrument control, data acquisition, and data processing. Mass spectral data were obtained by averaging of the mass spectra

recorded during the exposure of the sample to the DART gas beam; background ions were subtracted and a mass drift was corrected.

DART–Orbitrap MS

The DART–Orbitrap MS system consisted of a DART ion source (model DART-SVP) with a 12 Dip-It tip scanner autosampler (IonSense, Saugus, MA, USA) coupled to an Exactive high-resolution mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). A Vapur interface (IonSense, Saugus, MA, USA) was employed to hyphenate the ion source and the mass spectrometer. Low vacuum in the interface chamber was maintained by a membrane pump (Vacuubrand, Wertheim, Germany).

The DART ion source was operated in a positive ion mode with helium as the ionizing medium at a pressure of 4 bar. The gas beam was heated to 400°C. The discharge needle voltage of the DART source was set to –5 kV and the grid electrode voltage was +350 V, respectively. The parameters of the mass spectrometer were as follows: capillary voltage: +30V; tube lens voltage: +110V; capillary temperature: 250°C. The acquisition rate was set to 4 spectra s^{-1} corresponding to a mass resolving power of 25,000 FWHM (*m/z* 200). A constant speed of 0.5 mm s^{-1} was used for the Dip-It tip scanner autosampler.

XCALIBUR software (v. 2.1) was used for instrument control, data acquisition, and data processing. Mass spectral data were obtained by averaging of the mass spectra recorded during the exposure of the sample to the DART gas beam followed by the subtraction of background ions.

DESI–MS^2

An Omnispray DESI source from Prosolia Inc. (Indianapolis, IN, USA) was coupled to a LXQ linear ion trap from Thermo Fisher Scientific. A glass microscope slide with PTFE spots (Prosolia) was used as a sample substrate. The final spray solution was MeOH:H_2O (1:1 *v/v*) with 0.1% formic acid and 10 m*M* ammonium formate at a flow of 5 µl min^{-1}. The

spray voltage was 5 kV, the ion transfer tube was held at 50°C. The distance between spray tip and sample was 3 mm, and the distance between sample and the MS inlet was 0.5 mm. The nitrogen pressure was 120 psi. A modified ion transfer tube (Thermo Fisher Scientific) was used to minimize the distance between MS inlet and the sample. The angle between spray tip and sample was 55°. The sample slide moved at a speed of 250 µm s^{-1}.

Sample extraction

Procedure I for DART–MS analyses

Fruit samples (pear) were weight as whole (100–250 g) in 1-L polyethylene bags. After addition of acetonitrile (1 ml per 1 g of the sample) and internal standard solution (2 mg kg^{-1}), the bag was clipped shut, and the extraction was conducted for 15 min on a shaker (HS 260 basic, IKA, Staufen, Germany) operating at 180 min^{-1}. A volume of 0.7 ml of sample extract was put into the sampling hole of a deep well micro-plate for direct analysis. The final concentration of matrix was 1 g ml^{-1}.

For the validation experiments, appropriate volumes of thiram, ziram, and TPP from the stock solutions (all at 0.5 mg ml^{-1} in acetonitrile) were added in a polyethylene bag containing the whole fruit and the extraction solvent (1:1 ratio, *w/v*) to achieve concentrations of 5, 1, and 2 mg kg^{-1}, respectively.

Procedure II for DART–MS analyses

The QuEChERS-based procedure (Anastassiades et al. 2003) was applied: 5 g of roughly homogenized sample was mixed with 5 ml of water and extracted using 10 ml of acetonitrile in a 50 ml PTFE vessel. The mixture was vortexed for 1 min. Subsequently, 4 g of $MgSO_4$ and 1 g NaCl were added, and the mixture was shaken for another minute. The mixture was then fortified by internal standard solution (2 mg kg^{-1}). To provide a well-defined phase separation, centrifugation at 11,000 rpm for 5 min was used. A volume of 0.7 ml of sample extract was put into the sampling hole of a deep well micro-plate for direct analysis. The final

concentration of matrix was 0.5 g ml^{-1}. (*Note:* Roughly, medium, and finely homogenized samples were obtained by homogenization of approx. 400 g of pears for 15, 30, and 60 s using a homogenizer (2094 Homogenizer, Foss Tecator, Höganäs, Sweden).)

For the optimization experiments, appropriate volumes of thiram and ziram from the stock solutions (both at 0.5 mg ml^{-1} in acetonitrile) were added to either homogenized sample or the whole fruits to achieve the concentrations of 10 and 5 mg kg^{-1}, respectively. In the case of spikes added to homogenized fruit, the standard solutions were added to (*i*) the homogenized sample in a PTFE vessel, followed by an immediate extraction step; or (*ii*) the homogenized sample in a PTFE vessel, followed by an extraction step after 10 min. For spikes carried out before sample homogenization, the standard solutions were spread on the whole fruits (400 g) placed in a homogenizer. After sample homogenization, the QuEChERS extraction procedure followed. The internal standard (TPP) was added (2 mg kg^{-1}) before the centrifugation step.

For the validation experiments, appropriate volumes of thiram and ziram from the stock solutions (both at 0.5 mg ml^{-1} in acetonitrile) were spread on the whole fruits (400 g) present in a homogenizer to achieve the concentrations of 5 and 1 mg kg^{-1}, respectively (MRLs). After sample homogenization, the QuEChERS extraction procedure followed. The internal standard (TPP) was added (2 mg kg^{-1}) before the centrifugation step.

Note: Matrix-matched standards of thiram and ziram containing also an internal standard (TPP) were used for the quantification purposes (determination of recoveries). Calibration graphs were constructed by plotting the analyte concentrations (*x*-axis) against the ratio of the target DTCs and internal standard TPP response (*y*-axis).

Procedure I for DESI–MS^2 analyses

Two types of QuEChERS extraction were tested, a non-buffered version, the same as described in the section "*Procedure II for DART–MS analyses*" and an acetate-buffered

version (Lehotay et al. 2005). In short: 10 g of homogenized sample was extracted with 10 ml acetonitrile. A phase partitioning was induced by addition of salts. After vigorous shaking and centrifugation, the acetonitrile phase was measured. An optional clean up by dispersive SPE was done by transferring 0.5 ml to an Eppendorf cup containing 25 mg PSA and 150 mg magnesium sulfate. The cup was vortexed and centrifuged. For DESI–MS^2 analysis, 1.5 µl aliquots of the extracts were pipetted onto 1.5 mm ID PTFE spots printed on a microscope slide. After evaporation of the solvent under ambient conditions, the slide was put on a moving stage and the spots could automatically be positioned in the spray beam of the DESI source for detection of the analyte.

Procedure II for DESI–MS^2 analyses

As an alternative to the procedure involving extraction of a homogenized product, the rinsing procedure as described in the section "*Procedure I for DART–MS analyses*" was also performed. Again, 1.5 µl of extract were pipetted onto PTFE spots on the microscope slide for DESI–MS^2 measurement.

For both extraction procedures, azoxystrobin (arbitrarily chosen for method development purposes) was used as an internal standard, and was added to the extracts just before the DESI measurements.

Results and discussion

DART–MS experiments

Optimization of DART parameters

In the first part of our experiments, the relationship between the setting of various DART operating parameters and features of mass spectra generated under particular conditions was investigated. Two models of DART ion source (*i.e.*, DART-100, DART-SVP) were tested

within our experiments, each requiring optimization of particular settings. In general, helium beam temperature, flow rate, and desorption time were the major parameters affecting DART ion formation and effectiveness of their transmission into MS.

Protonated molecules $[M+H]^+$ were obtained under conditions of positive DART ionization when analyzing standards of thiram and ziram dissolved in acetonitrile. In the case of thiram, the $[M+H]^+$ ion corresponded to an elemental composition of $[C_6H_{12}N_2S_4+H]^+$, while that of ziram to $[C_6H_{12}N_2S_4Zn+H]^+$, each characterized by the characteristic isotope pattern.

The impact of gas beam temperature was monitored for temperatures 250, 300, 350, 400, and 450°C. For the model DART-100 the temperature of 300°C provided the highest responses for both analytes tested, while a temperature of 400°C was optimal for the model DART-SVP. Helium flow rates were also observed to have an influence on the DART–TOFMS responses of target analytes. This parameter was tested for 3.0; 3.5; 4.0; and 4.5 l min^{-1} (DART-100). A helium flow of 3.5 l min^{-1} gave the highest responses for both analytes. In the case of DART-SVP, a constant pressure of 4 bar is recommended, thus this value was kept during the all experiments.

Another important factor optimized was (thermo)desorption time. It is worth to mention that two different commercial autosampler devices were used to improve the quantitative DART measurements and also the throughput of analyses. The AutoDART autosampler (HTC PAL autosampler AutoDART-96) uses the robotic arm to deliver the sample from a deep-well reservoir into the sampling region where desorption at fixed position occurs. On the other hand, a 12 Dip-It tip scanner autosampler scans the glass-rod surfaces with samples through the DART gas stream at a constant speed. In the case of an AutoDART autosampler, the tested values of (thermo)desorption time included 1, 2, 5, 10 and 30 s. It was observed that 5 s provided sufficient intensity of ions. Longer desorption time led only to increased detection of various matrix co-extracts present in the sample extracts. For a 12 Dip-It tip

scanner autosampler, a constant speed of 0.5 mm s^{-1} was optimal providing the MS peak width of 5 s.

The last parameter considered was the use of a Vapur interface (DART-100), which is designed to improve collection of ions desorbed from the sample by collimating them for transfer into the MS. However, using this "ion concentrator", only a relatively slight increase of signal intensity was observed (approx. 20%) for thiram and ziram. On the other hand, a much higher signal intensity of the chemical background was observed. Therefore, a Vapur interface was not used in subsequent experiments (DART–TOFMS). However, the use of a Vapur gas ion separator during DART ionization was essential in order to maintain stable vacuum within the operating pressure limits of the Exactive instrument (Orbitrap MS).

Optimization of sample preparation for DART–MS

Once the DART parameters were optimized, the detection of thiram and ziram in real-world samples (fruits) was investigated. The recovery studies were performed with pears at a level of 10 mg kg^{-1} for thiram and 5 mg kg^{-1} for ziram. After that, the optimized method was tested at the levels of 5 mg kg^{-1} for thiram and 1 mg kg^{-1} for ziram, which represent the MRLs for this type of matrix.

In the initial phase of the experiment, we used a method developed by Crnogorac and Schwack (2007), which was modified in terms of using acetonitrile as an extraction solvent instead of water containing sodium hydrogen carbonate and DL-penicillamine (Procedure I). Employing this sample preparation method, recoveries of 94% and 102% and RSDs of 8% and 17%, were achieved for thiram and ziram, respectively. However, the drawback of this method was a high consumption of the solvent (1 ml per 1 g of sample) since the whole fruit (approx. 100–250 g) needed to be analyzed.

To overcome this limitation, we decided to develop an alternative strategy for sample preparation. Choosing QuEChERS-based procedure as a conceivable option, we were

concerned about recoveries, which were reported low when using this protocol for thiram analysis in matrices such as oranges and lettuce (Lehotay et al. 2005). This problem seems to be related to poor stability of DTCs in acidic media as well as to the action of various enzymes released during the extensive sample homogenization of biotic matrices (Roberts and Hutson 1999).

To learn more about DTCs breakdown, in the first part of experiments dealing with the QuEChERS-based procedure (Procedure II), we analyzed spikes of thiram and ziram added to differently homogenized pears (roughly, medium, finely). The best recoveries were obtained for finely homogenized samples (≈100%) supposing immediate extraction followed. However, as far as it started 10 min after addition of the spike, the degradation of both analytes was observed (≈40% decrease), thus, demonstrating their lower stability in the presence of pear matrix (**Figure 1-A**).

Figure 1

The lower recoveries of roughly and medium homogenized samples can be explained by low amount of water released from the sample. Therefore, we also investigated the addition of water to the sample to increase the "active" water content before the extraction (10 g of sample *vs.* 5 g of sample plus 5 ml of water). As a consequence, the recoveries of target analytes improved also for the roughly and medium homogenized samples as far as water was added to the sample before the extraction.

In the follow-up part of the experiments, we investigated the recoveries of thiram and ziram when both analytes were added to the sample before homogenization (≈400 g of pears were used for each experiment). Again, roughly, medium, and finely homogenized samples were analyzed. Under these conditions, the best recoveries were obtained for roughly homogenized

samples, probably due to less intensive interaction of target analytes with the matrix components (**Figure 1-B**). Further improvement of recoveries was observed as far as the pear samples were cryogenically homogenized (the samples were put into the freezer (–18°C) over night and were comminuted in frozen condition). Using this approach, the enzymatic activity reduces, thus, an improvement of recoveries of both target analytes was achieved.

To conclude, we recommend cryogenic homogenization with subsequent extraction of 5 g sample plus 5 ml of water per 10 ml of acetonitrile. Under these conditions, the recoveries 68% and 75%, and RSDs of 11% and 10%, were achieved for thiram and ziram, respectively, spiked at 10 mg/kg and 5 mg kg^{-1}, respectively. The recoveries of thiram and ziram spiked at their MRLs of 5 mg kg^{-1} and 1 mg kg^{-1}, respectively, were slightly lower: 65% and 73% with RSDs of 12% and 13%, respectively. It should be noted, that the use of isotopically labeled internal standards (currently available as d_{12}-thiram and d_{12}-ziram) can be an effective way to compensate for the losses of both analytes during the homogenization/extraction step. Unfortunately, the cost of isotopically labeled *vs.* native DTCs is currently prohibitively high (25 € *vs.* 0.1 €, respectively, per 1 mg); moreover, isotopic exchange (D–H) may occur, thus biasing the quantitative data. For the quantification purposes, the matrix-matched standards containing an internal standard (TPP) should be prepared shortly before the analysis of the extracts prepared by the QuEChERS method. When checking the stability of the matrix-matched standards, a decrease of responses of DTCs as high as 20% was observed after 2 hours.

Comparison of detection: TOFMS vs. Orbitrap MS

A limitation of the TOFMS used in this work was the relatively low mass resolving power (typically around 5,000 FWHM). Consequently, a high risk of interference of the target ion with matrix components with similar masses can be expected, especially since in ambient MS there is no LC separation. Therefore, an alternative high resolution MS system equipped with

an orbitrap mass analyzer offering enhanced mass resolving power was also used. The DART–Orbitrap MS system showed a high ability to eliminate matrix interferences. **Figure 2** clearly illustrates the benefit of high mass resolving power of this system—using the 25,000 FWHM resolving power allowed complete spectral separation of thiram from matrix interferences even if their intensities were higher compared to this analyte.

Figure 2

As illustrated in **Figure 3**, for quantitative analysis using a DART ion source, it is necessary to use an internal standard to compensate relatively high variation of the ion intensities of analytes. Triphenyl phosphate (TPP), yielding $[M+H]^+$ at *m/z* 327.079, was used for this purpose (spiked at a level of 2 mg kg^{-1}). Calibration curves obtained by analyses of matrix-matched standards were constructed by plotting the ratio of analyte/internal standard ion intensity *vs.* concentration of the particular analyte. Acceptable linearity was obtained for the tested concentration range; regression coefficients of calibration curves were >0.97.

Figure 3

Using DART–TOFMS, the limits of quantification (expressed as the lowest calibration levels, LCLs) were 1 mg kg^{-1} and 0.5 mg kg^{-1} for thiram and ziram, respectively, and 0.1 mg kg^{-1} and 1 mg kg^{-1} for thiram and ziram, respectively, when employing DART–Orbitrap MS. The high value of LCL of thiram in the case of TOFMS was attributed to the low mass resolving power of the TOFMS, resulting in insufficient resolution of thiram from matrix coextractants. However, even with this difference of sensitivity/selectivity, the achieved LCL for thiram by both instruments are acceptable for the control of the MRLs of

fruit commodities such as pears, apples, plums, and strawberries (EU-MRLs of 5, 5, 2, and 10 mg kg^{-1}, respectively). In the case of ziram, the obtained LCLs would be suitable to control the MRL of fruit commodities such as pears and plums (1 and 2 mg kg^{-1}, respectively). **Figure 4** shows MS records of both analytes at their MRLs in pear extract using DART–TOFMS and DART–Orbitrap MS. The records of blank samples are also presented to illustrate the selectivity of detection.

Figure 4

DESI–MS^2 experiments

Optimization of DESI–MS^2 parameters

As the first step, the MS response of different DTCs was tested by dilution of standard solutions into the spray solvent of the DESI source. Thiram was dissolved in acetonitrile. Ziram and probineb were dissolved in a mixture of DL-penicillamine and sodium hydrogen carbonate in water (at pH 12) as described by Crnogorac and Schwack (2007), which resulted in the disintegration of the polymer and formation of class-specific anions. Several spray solvent compositions were tested: aqueous methanol or acetonitrile, with and without addition of acids (acetic acid, formic acid, ammonia, and/or ammonium formate). In addition, the ion transfer tube temperature was varied. The ziram (transition *m/z* 120 → *m/z* 76 with a collision energy of 10 eV) and propineb (transition *m/z* 225 → *m/z* 191 with a collision energy of 20 eV) anions could be detected but only with relatively low sensitivity. Due to limitations of the linear ion trap in the difference between precursor and product ions that can be detected, some of the precursor/product ion combinations described by Crnogorac and Schwack (2007) could not be measured. Thiram was sensitively detected when using a water–methanol

mixture (1:1, *v/v*) with 0.1% formic acid as spray solvent. An $[M+Na]^+$ adduct (*m/z* 263) was obtained but fragmentation of this ion did not give sensitive product ions. After adding 10 m*M* of ammonium formate to the spray solution, the protonated molecules $[M+H]^+$ of thiram (*m/z* 241) were the most intense signal. This ion could be fragmented to *m/z* 196 and *m/z* 88 by applying a collision energy of 25 eV.

Next, the standards were deposited on PTFE spots printed on a glass slide and the different geometric parameters of the DESI source were optimized, measuring the most intense DTC transition. Unfortunately, only thiram could be detected by DESI–MS^2 (optimum conditions provided in the experimental section). Therefore further work with DESI–MS^2 was limited to this DTC.

Attempts to directly detect thiram from crop surfaces

One of the attractive features of DESI–MS is direct detection of analytes from a sample surface. The direct detection of a pesticide from a crop surface has been demonstrated by Garcia-Reyes et al. (2009). Although detection of thiram from a (small) surface area of a crop sample is not easily related to an MRL set in mg kg^{-1} whole crop, such direct detection might be very useful for fast qualitative screening purposes. Therefore, attempts were made to directly detect thiram deposited on pear leaves taped onto the microscope slide, but unfortunately without success. The signal was much lower compared to that of solvent standard deposits, and, in addition, the signal was interfered by matrix. Possible explanations for these phenomena include interference of the matrix on the ionization process and the fact that the leaves are a different material than the slides, which can influence wetting of the surface and the formation of secondary droplets which are thought to play an important role in the ion formation during DESI (Gao et al. 2010).

Indirect analysis through an intermediate extraction step

As an alternative to the direct surface analysis, different extraction methods, which are widely used in pesticide residue analysis, were studied. Since thiram dissolves well in acetonitrile, three variations of the QuEChERS extraction procedure were examined, a non-buffered version and an acetate buffered version with and without dSPE clean up. Here extraction was done using a spiked homogenized pear sample. Another approach involving a surface extraction of the intact product with acetonitrile was also carried out. Aliquots of the different extracts were pipetted onto the PTFE spots of the slide and then measured by DESI–MS^2.

Figure 5

Figure 5 shows a comparison between the different extraction methods (note that the signals for samples from the QuEChERS extraction are enhanced 15 times). As is clear from **Figure 5** the surface extraction provided the least suppression and highest response. Therefore this method was chosen for further work. The method was additionally tested on apple, pear, strawberries, and lettuce leaves, spiked with thiram at the respective MRLs of the different matrices (2–10 mg kg^{-1}). Immediately after surface extraction of the samples, 1.5 µl aliquots were deposited on the PTFE spots of the glass slide. The solvent evaporates quickly under ambient conditions leaving a stable thiram deposit. Thiram could be successfully detected from all crops tested.

Semi-quantitative analysis of samples

In solvent standards, thiram could be detected down to concentrations of 0.1 mg l^{-1} (corresponding to 0.15 ng absolute or ~0.1 ng mm^{-2}). No spot-to-spot carry-over occurred. The response increased with the amount of thiram deposited but the absolute response was of

limited reproducibility. By addition of an internal standard (in this case, rather arbitrary, azoxystrobin was used) for normalization of the response, semi-quantitative data could be obtained. Using solvent standards (0–1 mg l^{-1}) and averaging three analyses of the same spots, a five-point calibration curve was constructed. The linearity was considered sufficient (R^2 = 0.98) to allow at least semi-quantitative analysis. The applicability of the method to real-world samples was tested by spiking 6 pear samples at 10 mg kg^{-1} and measuring them against a calibration solution in matrix at the same level. The average recovery was 85% with an RSD of 26%.

Detection of thiram applied as TMTD

In the field, thiram is not applied as the pure substance. Instead the formulated plant protection products such as TMTD, which contains 80% of thiram, is suspended in water at 2 g l^{-1} and applied onto the crops. To test if the DESI–MS^2 method was also able to detect thiram when applied as TMTD, strawberries were spiked at the MRL of 10 mg kg^{-1} using both the TMTD suspension and a solution of thiram in acetonitrile. After spraying, the fruits were left for 30 min to allow drying and interaction with the surface.

Figure 6 shows that thiram could be easily detected both when deposited as TMTD and neat thiram. During MS measurement, product ion spectra are obtained which facilitate identification.

Figure 6

Figure 7 shows the product ion spectra of *m/z* 241 for a solvent standard and a strawberry spiked at 10 mg kg^{-1}. Both the quantifier at *m/z* 196 and the qualifier at *m/z* 88 can be seen in the correct ion ratio according to the applicable EU guideline (SANCO/10684/2009).

Figure 7

The results obtained demonstrate that the DESI–MS^2 method is suited for detection of thiram in real-world samples, and to provide at least a semi-quantitative concentration.

Conclusions

Ambient MS was investigated for rapid detection of DTCs in fruits. Two complementary ionization techniques were investigated: DART and DESI. With both approaches, an extraction step was found to be necessary to facilitate detection.

With DESI–MS^2 the best results were obtained using a surface extraction of the intact product. For extracts of homogenized product, severe suppression effects were observed. Thiram could be rapidly detected (typically 10 samples in few minutes) down to 0.1 mg l^{-1} in standard solutions and at MRL level in extracts from various fruits. Using an internal standard, semi-quantitative results could be obtained. With the current instrumentation and interface design, it was not possible to detect the other DTCs tested.

In the case of DART, also here an extraction step was employed. For detection, a glass rod was dipped into the raw extract and simply positioned between the DART source and MS detector. Using this strategy, thiram was easily detected. In addition, ziram could also be detected as an intact molecule after dissolution in acetonitrile. The detectability of both DTCs was demonstrated at MRL level in pears. Using an internal standard, quantitative analysis was possible.

Overall, despite a high solvent consumption, the surface extraction method combined with DART analysis would be the preferred method because of higher recovery and the applicability to two out of the three DTCs for which the individual MRLs have been established.

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Figure Captions

Figure 1. Dependence of thiram and ziram recovery on sample preparation conditions. (A) The pear samples were spiked by thiram and ziram at 10 mg kg^{-1} and 5 mg kg^{-1}, respectively, after completing homogenization of the matrix. For the extraction QuEChERS-based procedure with 10 g of samples per 10 ml of acetonitrile was used with subsequent immediate analysis by DART–TOFMS.

(B) The pear samples were spiked by thiram and ziram at 10 mg kg^{-1} and 5 mg kg^{-1}, respectively, before homogenization of the matrix. For the extraction QuEChERS-based procedure with 5 g of samples plus 5 ml of water per 10 ml of acetonitrile was used with subsequent immediate analysis by DART–TOFMS.

For the quantification (A) + (B) matrix-matched standards were used with TPP as an internal standard. The error bars represent standard deviations (n=3).

Figure 2. Thiram (m/z 240.996) in pear extract analyzed by DART–Orbitrap MS (0.1 mg kg^{-1}) and DART–TOFMS (1 mg kg^{-1}). The mass resolving power (R) is also indicated.

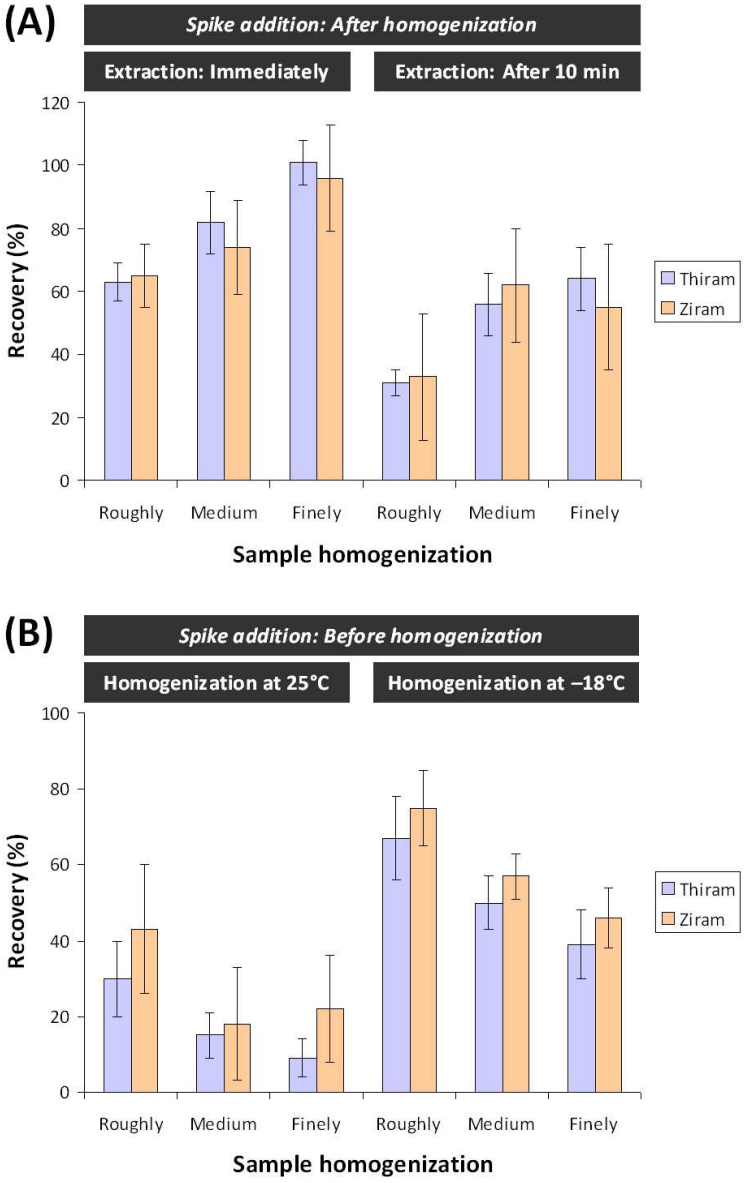
Figure 3. DART–Orbitrap MS and DART–TOFMS analysis of matrix-matched standards. Concentration of thiram (m/z 240.996) was in the range of 0.1–10 mg kg^{-1}. To illustrate the fluctuation of signal intensity, the internal standard TPP (m/z 327.079) is also shown (concentration 2 mg kg^{-1}).

Figure 4. DART–Orbitrap MS and DART–TOFMS analysis of spikes of thiram (*m/z* 240.996) and ziram (*m/z* 304.925) in the pear extracts at the MRLs of 5 and 1 mg kg^{-1}, respectively.

Figure 5. DESI–MS^2 analysis of thiram and azoxystrobin (internal standard) in various pear extracts deposited on PTFE spots.

Figure 6. DESI–MS^2 analysis of (*i*) blank strawberry extract, (*ii*) extract of strawberry spiked with a solvent standard solution of thiram (equivalent to 10 mg kg^{-1}), (*iii*) extract of strawberry sprayed with commercial crop protection product TMTD (equivalent to 10 mg thiram kg^{-1}). Each deposit in duplicate. To illustrate the fluctuation of signal intensity, internal standard (azoxystrobin) is also shown.

Figure 7. Scan of product ions of *m/z* 241 at a collision energy of 25 eV. (A) Solvent standard of thiram at a concentration of 10 µg ml^{-1}; (B) spiked strawberry sample by thiram at 10 mg kg^{-1}.



Dependence of thiram and ziram recovery on sample preparation conditions.
(A) The pear samples were spiked by thiram and ziram at 10 mg kg–1 and 5 mg kg–1, respectively, after completing homogenization of the matrix. For the extraction QuEChERS-based procedure with 10 g of samples per 10 ml of acetonitrile was used with subsequent immediate analysis by DART–TOFMS.
(B) The pear samples were spiked by thiram and ziram at 10 mg kg–1 and 5 mg kg–1, respectively, before homogenization of the matrix. For the extraction QuEChERS-based procedure with 5 g of samples plus 5 ml of water per 10 ml of acetonitrile was used with subsequent immediate analysis by DART–TOFMS.
For the quantification (A) + (B) matrix-matched standards were used with TPP as an internal standard. The error bars represent standard deviations (n=3).

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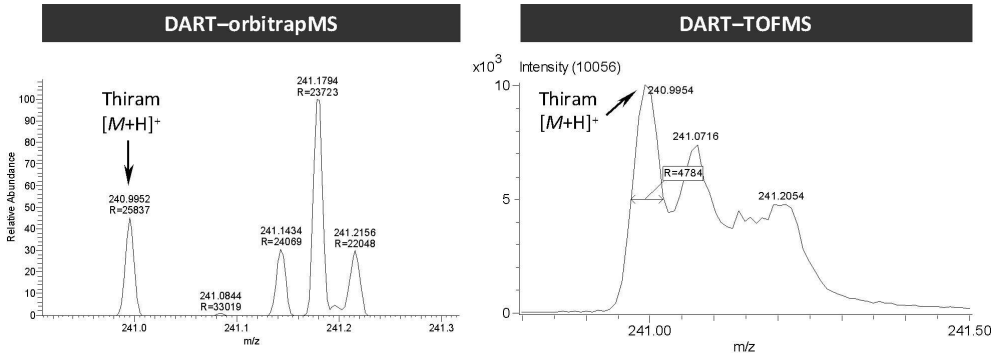
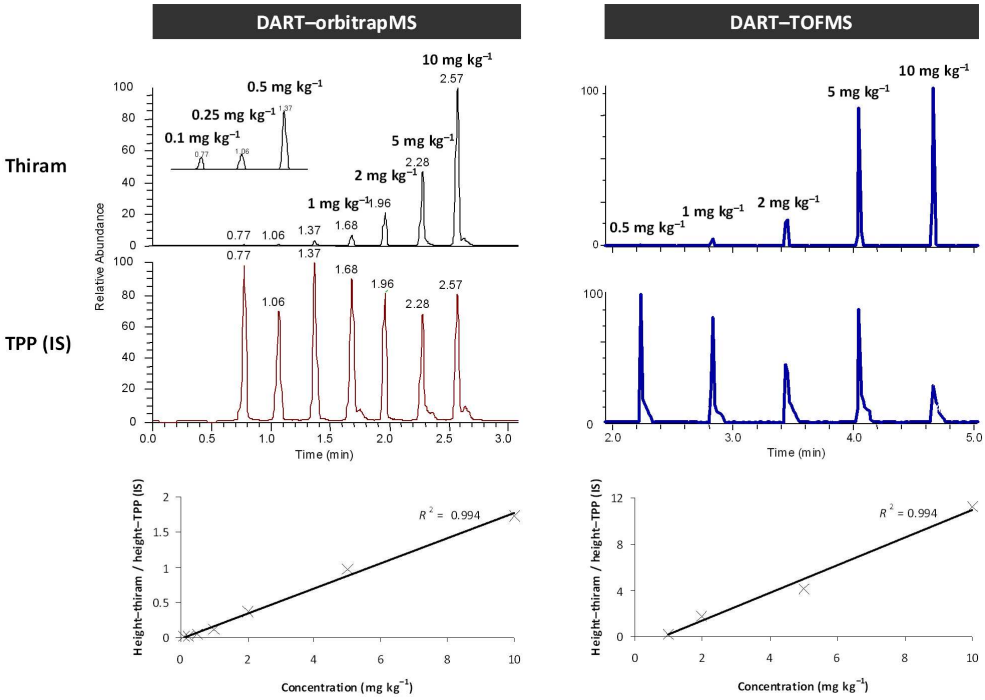


Figure 2. Thiram (m/z 240.996) in pear extract analyzed by DART–orbitrapMS (0.1 mg kg–1) and DART–TOFMS (1 mg kg–1). The mass resolving power (R) is also indicated.
529x190mm (96 x 96 DPI)



DART–orbitrapMS and DART–TOFMS analysis of matrix-matched standards. Concentration of thiram (m/z 240.996) was in the range of 0.1–10 mg kg–1. To illustrate the fluctuation of signal intensity, the internal standard TPP (m/z 327.079) is also shown (concentration 2 mg kg–1).
529x381mm (96 x 96 DPI)

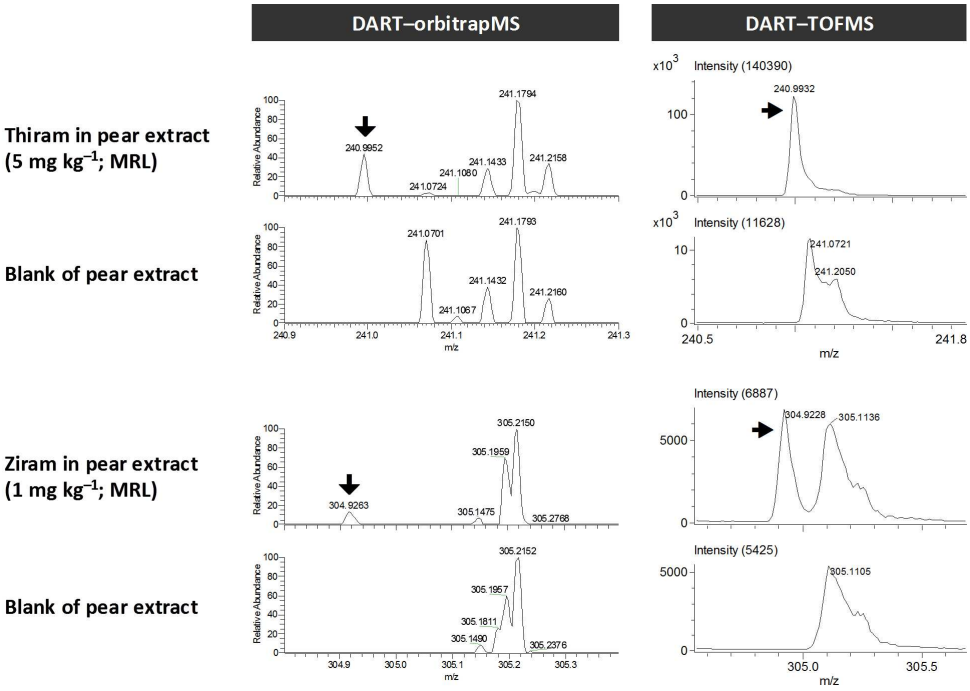
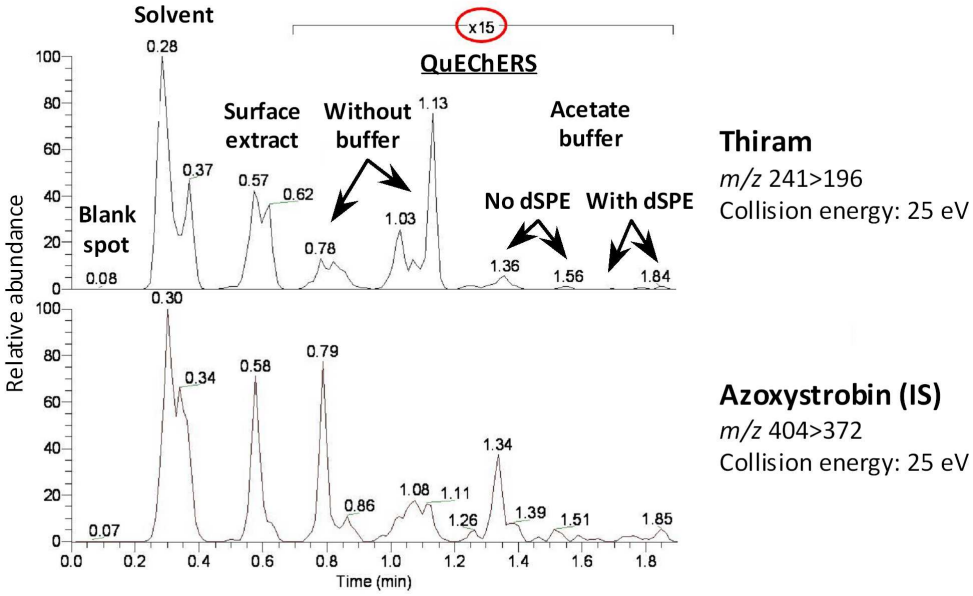
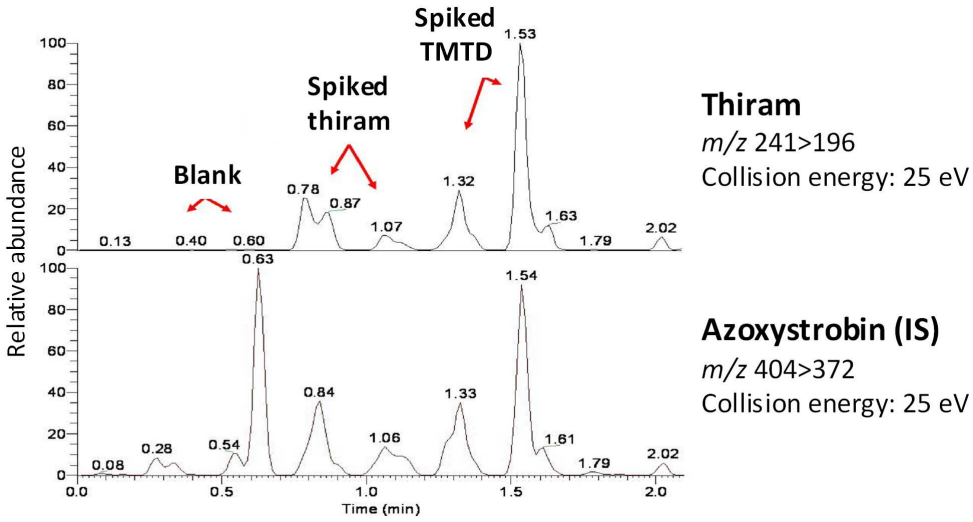


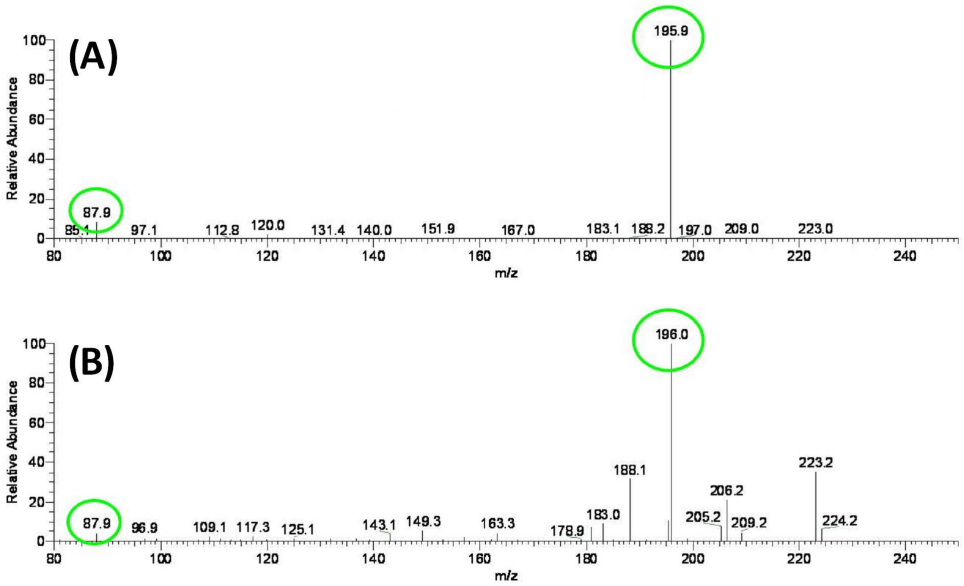
Figure 4. DART–orbitrapMS and DART–TOFMS analysis of spikes of thiram (m/z 240.996) and ziram (m/z 304.925) in the pear extracts at the MRLs of 5 and 1 mg kg–1, respectively.
529x370mm (96 x 96 DPI)



DESI–MS2 analysis of thiram and azoxystrobin (internal standard) in various pear extracts deposited on PTFE spots.
529x328mm (96 x 96 DPI)



DESI–MS2 analysis of (i) blank strawberry extract, (ii) extract of strawberry spiked with a solvent standard solution of thiram (equivalent to 10 mg kg–1), (iii) extract of strawberry sprayed with commercial crop protection product TMTD (equivalent to 10 mg thiram kg–1). Each deposit in duplicate. To illustrate the fluctuation of signal intensity, internal standard (azoxystrobin) is also shown.
529x285mm (96 x 96 DPI)



Scan of product ions of m/z 241 at a collision energy of 25 eV. (A) Solvent standard of thiram at a concentration of 10 µg ml−1; (B) spiked strawberry sample by thiram at 10 mg kg−1.
529x317mm (96 x 96 DPI)