Anal. Chem. **2008,** *80,* 9567–9575

Control of Strobilurin Fungicides in Wheat Using Direct Analysis in Real Time Accurate Time-of-Flight and Desorption Electrospray Ionization Linear Ion Trap Mass Spectrometry

Jakub Schurek,[†] Lukas Vaclavik,[†] H. (Dick) Hooijerink,[‡] Ondrej Lacina,[†] Jan Poustka,[†] Matthew Sharman,[§] Marianne Caldow,[§] Michel W. F. Nielen,[‡,||] and Jana Hajslova*[,†]

Department of Food Chemistry and Analysis, Institute of Chemical Technology Prague, Technická 5, 6 Prague 16628, Czech Republic, RIKILT Institute of Food Safety, P.O. Box 230, 6700 AE Wageningen, The Netherlands, Central Science Laboratory, Sand Hutton, York, U.K. YO41 1LZ, and Wageningen University, Laboratory of Organic Chemistry, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

Ambient mass spectrometry has been used for the analysis of strobilurin residues in wheat. The use of this novel, challenging technique, employing a direct analysis in a real time (DART) ion-source coupled with a time-of-flight mass spectrometer (TOF MS) and a desorption electrospray ionization (DESI) source coupled with a linear ion trap tandem MS (LIT MS^n), permitted a direct screen of the occurrence of target fungicides in treated grains in less than 1 min. For quantification purpose by DART-TOF MS, an ethyl acetate extract had to be prepared. With the use of a prochloraz as an internal standard, the performance characteristics obtained by repeated analyses of extract, spiked at 50 $\mu g\ kg^{-1}$ with six strobilurins (azoxystrobin, picoxystrobin, dimoxystrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin), were in the following range: recoveries 78−92%, repeatability (RSD) 8−15%, linearity (R^2) 0.9900−0.9978. The analysis of wheat with incurred strobilurin residues demonstrated good trueness of data generated by the DART-TOF MS method; the results were in a good agreement with those obtained by the conventional approach, i.e., by the QuEChERS sample handling procedure followed by identification/quantification employing high-performance liquid chromatography coupled with tandem mass spectrometry (LC−MS/MS). Tandem mass spectrometry using DESI-LIT MS^n provided a sufficient number of product ions for confirmation of the identity of azoxystrobin and pyraclostrobin in incurred wheat samples.

Among other pesticides, which originate from natural fungicidal derivatives,[1,2] the strobilurin class of compounds play an important role in an effective control of various types of plant pathogens, such as powdery mildews (ascomycetes) and rusts (basidiomycetes). The various strobilurins differ in their systemic properties, some of them are partially systemic and others redistribute themselves around the plant in the wax layer/epidermal cells by vapor action. In addition to strobilurins' fungicidal effect, these chemicals may induce physiological alteration (e.g., increase of endogenous cytokinin levels, stimulation of ethylene biosynthesis, increase in CO_2 assimilation), thus performing as bioregulators, particularly in cereals which become ripe in a shorter period of time as compared to nontreated ones. The resulting longer retention of green leaf tissue and significant yield enhancements are the benefits of the their use in agriculture.[3] With the exception of kresoxim-methyl, strobilurins are not classified as internationally accepted Pesticide Action Network Bad Actors,[4] meaning that they are not (i) highly acutely toxic, (ii) cholinesterase inhibitors, (iii) known/probable carcinogens, (iv) known groundwater pollutants, or (v) known reproductive or developmental toxicants. With regards to strobilurins, widespread use (for instance global consumption of azoxystrobin which is registered for over 400 crop/disease systems[2] was more than 5000 t in 2007[8]), development of analytical procedures enabling a high throughput control of their residues, particularly in cereals and a wide range of other commodities such as soft fruit, is needed.[5−7]

* To whom correspondence should be addressed. E-mail: jana.hajslova@vscht.cz.

[†] Institute of Chemical Technology Prague.

[‡] RIKILT Institute of Food Safety.

[§] Central Science Laboratory.

[||] Wageningen University.

(1) Bartlett, D. W.; Clough, J. M.; Godwin, J. R.; Hall, A. A.; Hamer, M.; Parr-Dobrzanski, B. *Pest Manage. Sci.* **2002**, *58*, 649–662.

(2) Sauter, H.; Steglich, W.; Anke, T. *Angew. Chem.* **1999**, *111*, 1416–1438.

(3) Grossmann, K.; Reztlaff, G. *Pestic. Sci.* **1997**, *50*, 11–20.

(4) Pesticide Action Network International: PAN Pesticide Database-Chemicals [Internet]. Available from http://pesticideinfo.org/List_Chemicals.jsp?ChemClassList=Strobin&dUseList=y&dDPR_Chem_Code=y&dEPA_PCCode=y&dCAS_No=y&dClassList=y&ChemRegList=&ChemUseList=&dS_BA=y&dEPA_Reg=yFAO/WHO. Codex Alimentarius: Pesticide Residues in Food-Maximum Residue Limits [Internet]. Available from http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp, accessed September 17, 2008.

(5) Informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC [Internet]. Available from http://ec.europa.eu/food/plant/protection/resources/mrl_pesticide.pdf, accessed March 25, 2008.

(6) Pesticides Safety Directorate (PSD), PDS Databases [Internet]. Available from https://secure.pesticides.gov.uk/MRLs/, accessed March 25, 2008.

(7) FAO/WHO Codex Alimentarius: Pesticide Residues in Food-Maximum Residue Limits [Internet]. Available from http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp, accessed March 25, 2008.

10.1021/ac8018137 CCC: $40.75 © 2008 American Chemical Society
Published on Web 11/13/2008

Several approaches have been described so far for analysis of strobilurin fungicides in food crops. High performance-liquid chromatography (HPLC) coupled with diode array detector (DAD),[9,10] or employing mass spectrometric (MS) detection[11−13] represent the most common methods of choice. It should be noted that the latter detection principle enabled introduction of various multiresidue methods capable to determine not only strobilurins but even hundreds of other pesticides in a single run.[14−16] However, time and labor consuming isolation/purification steps are involved in some of them; moreover, careful tuning of detection conditions setting is required.[17−20]

Only recently introduced ambient ionization techniques such as desorption electrospray ionization (DESI)[21] and/or direct analysis in real time (DART),[22] when interfaced with mass spectrometry, have a potential to examine samples in their native conditions. The DART ion source, investigated in this study, is based on a heat induced desorption (occurring typically at 150−350 °C) of sample components by metastable helium, generating ionized water clusters. The latter provides a soft ionization of sample molecules by proton transfer.[23] Thanks to unique features of this novel technique, additional convenience to MS-based analyses is delivered: bypassing of sample preparation or its reduction to a minimum is possible. The DART ion source coupled with a time-of-flight mass spectrometer (TOF MS) has already been reported as a useful tool for determination of pharmaceuticals,[24] fragrances,[25] soft drink additives,[26] or control of drug abuse,[22] offering faster alternative to LC-based approaches. Rather surprisingly, until now, none of the published applications has been concerned with analysis of pesticide residues in plant matrixes. DESI ion trap (IT) MS has been applied previously to the direct analysis of DEET, alachlor, and atrazine on leaves and vegetables and to the analysis of acetochlor in groundwater using solid-phase extraction (SPE) membranes.[27,28] The aim of the present study was to explore the potential of the DART-TOF MS technique to screen and, possibly, to quantify residues of the most common strobilurin fungicides employed for treatment of wheat. Complementary to that, the potential for confirmatory analysis of strobulirins in incurred wheat samples using DESI-LIT MS^n was investigated.

EXPERIMENTAL SECTION

Standards and Chemicals. All strobilurin standards (see Table 1 for list and structures of compounds) and prochloraz (internal standard) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock standard solutions (1000 mg L^{-1}) were prepared in ethyl acetate and stored at −20 °C. Working standard mixtures in ethyl acetate, containing 10 mg L^{-1} of each strobilurin, were diluted and used for spiking wheat of grain samples at 50 μg kg^{-1} (validation study). For calibration, matrix-matched standards in the range 6−1200 μg kg^{-1} (6, 12, 48, 120, 240, 600, and 1200 μg kg^{-1}) were prepared by dilution of the working standard in ethyl acetate crude extract (see Sample Preparation). Prochloraz (100 μg L^{-1} in ethyl acetate), an internal (syringe) standard, was added to matrix matched standards.

Ethyl acetate, acetonitrile, and methanol, HPLC grade, were provided by Merck (Darmstadt, Germany). Anhydrous sodium sulfate (Na_2SO_4) and magnesium sulfate ($MgSO_4$), obtained from Merck, were heated at 500 °C for 1 h to remove residual water and then stored in a desiccator. Poly(ethylene glycol), PEG 600, obtained from Sigma-Aldrich (St. Louis, MO) was used as a mass calibrant.

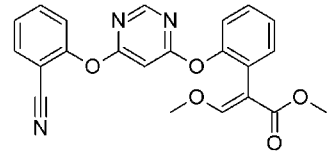
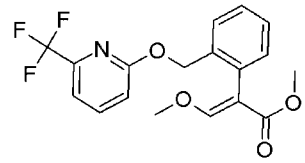
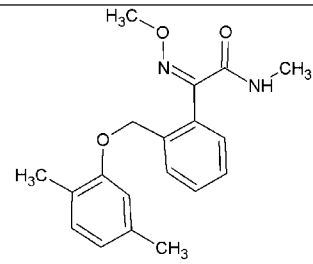
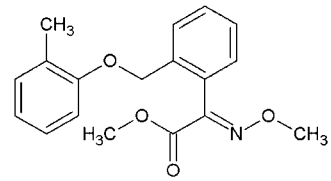
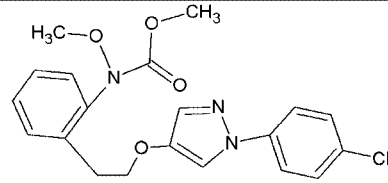
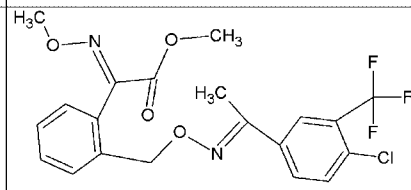
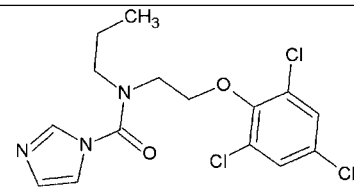
Test Samples. Four individual plots of approximately 400 m^2 of spring wheat (cultivar Paragon) were used to prepare incurred samples. Plot 1 was untreated. Plots 2, 3, and 4 were treated with azoxystrobin (as Amistar, Syngenta), kresoxim-methyl (+epoxiconazole as Landmark, BASF), and pyraclostrobin (as Vivid, BASF), respectively. Aliquots of the grain samples were milled using a Glenn Creston Ultra Centrifugal mill (model ZM100) fitted with a 1.0 mm cutter/grater.

Sample Preparation. While no sample preparation was needed for qualitative screening of strobilurins in test samples, matrix extracts, prepared in the way described below, had to be prepared for the quantitation of target analytes.

Sample Preparation for DART-TOF MS for Quantitative Analysis. Milled wheat grains (12.5 g) and 10 g of Na_2SO_4 were placed into a glass beaker and extracted for approximately 2 min with 50 mL of ethyl acetate using an Ultra-Turrax homogenizer (10 000 rpm). The suspension was filtered under vacuum through a layer of Na_2SO_4, and then the beaker and filter cake were rinsed with 25 mL of ethyl acetate. Combined filtrates were evaporated using a vacuum rotary evaporator (temperature maximum 40 °C, pressure 220 mbar) to an approximate volume of 20 mL. Concentrated crude extract was transferred into a volumetric flask, and the final volume was made up with ethyl acetate to 25 mL (0.5 g mL^{-1}) prior to DART-TOF MS analysis.

(8) Agranova, Crop Protection Actives-A Commercial and Technical Information Database [Internet]. Available from http://www.a2os.com/, accessed September 17, 2008.
(9) Abreu, S. M.; Caboni, P.; Cabras, P.; Garau, V. L.; Alves, A. *Anal. Chim. Acta* **2006**, *572*, 292–293.
(10) Boudina, A.; Emmelin, C.; Baaliouamer, A.; Paisse, O.; Chovelon, J. M. *Chemosphere* **2007**, *68*, 1280–1288.
(11) Polati, S.; Bottaro, M.; Frascarolo, P.; Gosetti, F.; Gianotti, V.; Gennaro, M. C. *Anal. Chim. Acta* **2006**, *579*, 146–151.
(12) Sannino, A.; Bolzoni, L.; Bandini, M. *J. Chromatogr., A* **2004**, *1036*, 161–169.
(13) Taylor, M. J.; Hunter, K.; Hunter, K. B.; Lindsay, D.; Le, S. *J. Chromatogr., A* **2002**, *982*, 225–230.
(14) Lambropoulou, D. A.; Albanis, T. A. *Anal. Bioanal. Chem.* **2007**, *389*, 1663–1683.
(15) Soler, C.; Pico, Y. *Trends Anal. Chem.* **2007**, *26*, 103–115.
(16) Pico, Y.; Font, G.; Ruiz, M. J.; Fernandez, M. *Mass Spectrom. Rev.* **2006**, *25*, 917–960.
(17) Medana, C.; Calza, P.; Baiocchi, C.; Pelizzeti, E. *Curr. Org. Chem.* **2005**, *9*, 859–873.
(18) Nunez, O.; Moyano, E.; Galceran, M. T. *Trends Anal. Chem.* **2005**, *24*, 683–703.
(19) Pico, Y.; Blasco, C.; Font, G. *Mass Spectrom. Rev.* **2004**, *23*, 45–85.
(20) Hajslova, J.; Zrostlikova, J. *J. Chromatogr., A* **2003**, *1000*, 181–197.
(21) Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306*, 471–479.
(22) Cody, R. B.; Laramee, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77*, 2297–2293.
(23) Venter, A.; Nefliu, M.; Cooks, R. G. *Trends Anal. Chem.* **2008**, *27*, 284–290.
(24) Williams, J. P.; Patel, V. J.; Holland, R.; Scrivens, J. H. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1447–1452.
(25) Haefliger, O. P.; Jeckelmann, N. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1361–1366.
(26) Cajka, T.; Vaclavik, L.; Riddellova, K.; Hajslova, J. *LC GC Eur.* **2008**, *21*, 250–256.
(27) Mulligan, C. C.; Talaty, N.; Cooks, R. G. *Chem. Commun.* **2006**, 1709–1711.
(28) Mulligan, C. C.; McMillan, D. K.; Noll, R. J.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3729–3734.

Table 1. Physico-Chemical Properties of Tested Strobilurins and Internal Standard

Compound	CAS number	Formula	Structure	log Kow	Water solubility *(mg L^{-1})*
Azoxystrobin	215934-32-0	$C_{22}H_{18}N_3O$		2.5	6.0
Picoxystrobin	117428-22-5	$C_{18}H_{17}F_3NO_4$		3.6	3.1
Dimoxystrobin	149961-52-4	$C_{19}H_{23}N_2O_3$		3.1	Not available
Kresoxim-methyl	143390-89-0	$C_{18}H_{20}NO_4$		3.4	2.0
Pyraclostrobin	175013-18-0	$C_{19}H_{19}ClN_3O_4$		4.0	4.6
Trifloxystrobin	141517-21-7	$C_{20}H_{20}F_3N_2O_4$		4.5	0.6
Prochloraz (i.s.)	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$		4.1	34.4

For direct screening, sample was introduced to the DART ion source manually using filter paper envelopes (80 g/m^2; Filtrak, Barenstein, Germany) containing approximately 1 g of milled wheat grain.

Sample Preparation for DESI-LIT MS^n Analysis. An OMIX (Varian, Palo Alto, CA) C18 microextraction pipet tip was filled with approximately 150 mg of milled wheat grains. Three volumes of 100 μL of methanol were added, and the percolate was collected. The methanol was evaporated to dryness under a gentle stream of nitrogen gas at 45 °C, and the residue was redissolved in 25 μL of methanol and centrifuged for a couple of minutes. The supernatant thus obtained was used for DESI analysis.

Sample Preparation for LC–MS/MS. The QuEChERS-based procedure was applied: 5 g of sample was mixed with 5 g of water

Table 2. Identification Characteristics of Strobilurins Using DART-TOF MS and LC−MS/MS Analytical Parameters

	analytical method						
	DART-TOF MS		LC−MS/MS				
compound	exact mass of $[M + H]^+$ ions[a] (m/z)	exact mass of fragment ions produced by CID[b] (m/z)/relative abundances of fragment ions (600 μg kg^{-1}, %)	retention time (min)	precursor ion (m/z)	cone (V)	collision (eV)	product ion (m/z)
azoxystrobin	404.1246	344.1031/5	5.19	404	20	25	344
		372.1037/2			20	15	372
picoxystrobin	368.1109	205.0880/5	7.19	368	10	19	145
		145.0670/1			10	9	205
dimoxystrobin	327.1708	205.0980/4	7.47	327	15	20	116
		116.0530/2			15	9	205
kresoxim-methyl	314.1392	206.0830/3	7.52	314	10	12	222
		131.0730/0.5			10	6	267
pyraclostrobin	388.1964	194.0830/5	7.79	388	20	15	164
		163.0620/4			20	12	194
trifloxystrobin	409.1375	206.0830/5	8.09	409	20	18	186
		186.0540/2			20	13	206

[a] Ions observed through standard DART-TOF MS analysis, orifice 1 voltage set to 20 V. [b] Ions observed through DART-TOF MS analysis utilizing CID, orifice 1 voltage set to 55 V.

and extracted using 10 mL of acetonitrile in 50 mL PTFE vessel. The mixture was then vortexed for 1 min. Subsequently, 4 g of $MgSO_4$ and 1 g of NaCl were added, and the mixture was shaken for another minute. To provide a well-defined phase separation, centrifugation at 11 000 rpm for 5 min was used. An aliquot (1 mL) of the extract was cleaned up with 50 mg of primary-secondary amine (PSA) and dried with 150 mg of $MgSO_4$. Following centrifugation, the supernatant was placed into a vial, so that the final concentration of matrix was 0.5 g mL^{-1}.

Samples Analysis. *DART-TOF MS.* For DART-TOF MS analyses, the system consisting of a DART ion source (IonSense, Danvers) and a JEOL AccuTOF LP high-resolution TOF mass spectrometer (JEOL Europe, SAS, Croissy sur Seine, France). Ethyl acetate extracts were introduced using an AutoDART HTC PAL autosampler (Leap Technologies, Carrboro) and Dip-it tips (IonSense, Danvers). The sampling tip was immersed into the crude extract and placed automatically in front the DART gun exit near the source, the mass spectrometer axis at a precise optimized position. In the case of direct examination of solid samples, a handmade envelope containing milled wheat grains was placed directly into the DART gas stream.

The DART ion source was operated in a positive ion mode with helium as the ionizing medium at a flow rate of 2.9 L min^{-1}. The gas beam was heated to 200 °C (direct analysis of wheat grain) or 300 °C (analysis of ethyl acetate extracts). The distance between the exit of the DART gun and inlet of the mass spectrometer was 12 mm. The discharge needle voltage of the DART source was set to a positive potential of 3000 V and was perforated, and the grid electrode voltages were +150 and +250 V, respectively. Accurate mass profiles were acquired within the range m/z 100−500, the spectra recording interval was 0.2 s, 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-TOF MS instrument. When cone-induced dissociation (CID) was used for analyte confirmation, the value of the orifice 1 voltage was increased to 55 V (from 20 V).

The trueness and precision of the method were assessed within the validation procedure; wheat grains were spiked at concentration 50 μg kg^{-1} ($n = 5$). The quantification was carried out using matrix-matched calibration and internal standard correction.

DESI-LIT MS^n. A dedicated DESI-MS setup was used consisting of a Prosolia (Indianapolis, IN) DESI ion source equipped with a rotational and an *x-y-z* positioner, a motorized sample stage with an *x-y-z* positioner, two CCD cameras with ×60 magnifying optics, and associated monitors and fitted onto a Thermo Fisher (San Jose, CA) model LXQ linear ion trap (IT) mass spectrometer. The desolvation gas was nitrogen (120 psi), the trap gas was helium, and the electrospray needle was at 5.5 kV. Full scan and data-dependent MS^n product ion spectra were acquired. The DESI spray consisted of methanol, water, and formic acid (50/50/0.1 v/v) and was delivered by the integrated syringe pump at 2.5 μL min^{-1}. The incident angle was 55°, and the MS inlet angle was at 10°. Methanol samples (1−5 μL) were deposited onto the wells of microscope slides (Marienfeld, Baden-Württemberg, Germany).

LC−MS/MS. Waters 2695 series liquid chromatograph (Waters, Manchester, U.K.), equipped with a quaternary pump, was interfaced via electrospray ionization (ESI) to a Quattro Premier XE mass spectrometer (Waters, Manchester, U.K.). For the LC separation, a reversed phase Discovery C18 analytical column (150 mm × 3 mm × 5 μm, Supelco, Bellefonte, PA) was employed. Sample injection volumes were 5 μL, the mobile phase was water containing 10 mM ammonium acetate/methanol, and the gradient was as follows: 0−3 min methanol, 70%; 3−14 min, methanol 70−100%; 14−20 min, column equilibration, methanol 70%. A constant flow rate of 0.3 mL min^{-1} was employed.

For operation in the MS/MS mode, argon 99.995% was a collision gas (flow rate of 0.2 mL min^{-1}). The capillary voltage was 3.5 kV, the desolvation temperature was set to 350 °C, and the source temperature was 120 °C. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode detecting two pairs of selected ions (a parent and a product ion, see Table 2), which were analyzed for each pesticide at a dwell

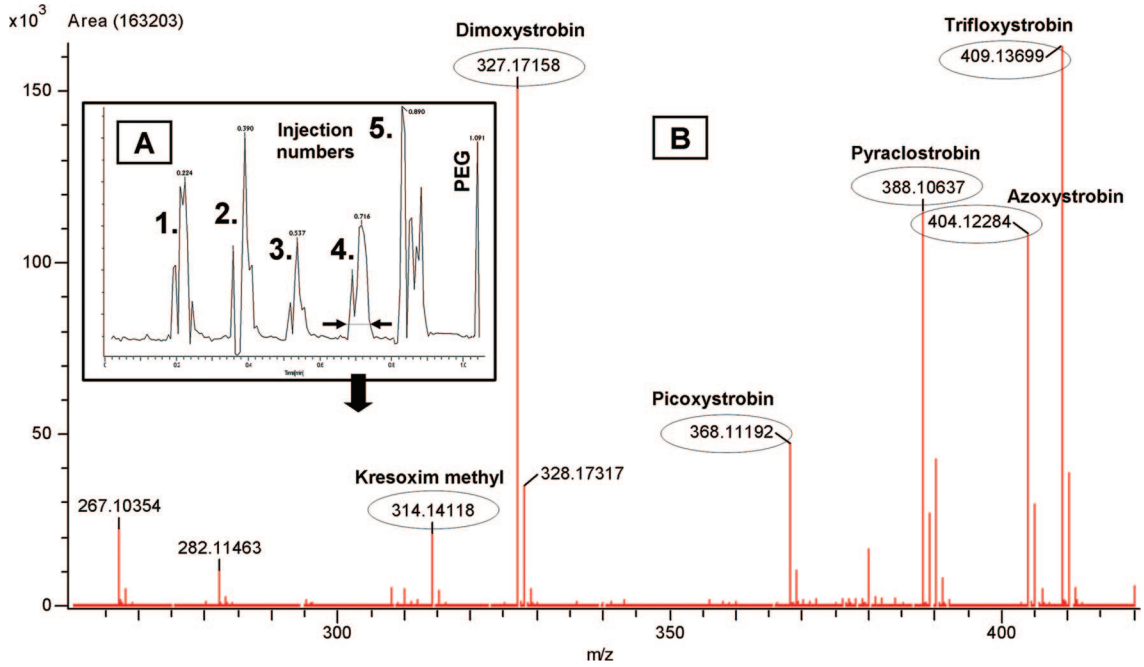


Figure 1. DART-TOF MS analysis of crude ethyl acetate extract of wheat grains spiked with strobilurins at 50 μg kg^{-1}. (A) Total ion current (five repeated injections plus PEG 600). (B) Zoomed part of $[M + H]^+$ mass profile in the *m/z* range 310−410 (example for injection four).

Table 3. Accuracy of Mass Measurement in DART-TOF MS Analysis of Spiked Crude Ethyl Acetate Extracts and Incurred Residues Samples ($n = 5$)

	mass drift uncorrected/mass drift corrected					
	spiked extracts[a]			incurred wheat samples[b]		
compound	average measured mass (m/z) $[M + H]^+$	average difference from exact mass (mDa)	relative standard deviation of average measured mass (10^{-3}, %, $n = 5$)	Average measured mass (m/z) $[M + H]^+$	average difference from exact mass (mDa)	relative standard deviation of average measured mass (10^{-3}, %, $n = 5$)
azoxystrobin	404.0912/404.1241	−33.40/−0.50	6/0.07	404.0871/404.1245	−37.50/−0.10	6/0.01
picoxystrobin	368.0874/368.1091	−23.49/−1.80	5/0.15			
dimoxystrobin	327.1002/327.1701	−70.59/−0.70	14/0.08			
kresoxim-methyl	314.0474/314.1389	−91.80/−0.30	18/0.03	314.0318/314.1388 8	−107.40/−0.32	24/0.03
pyraclostrobin	388.1203/388.1951	−76.10/−1.30	16/0.12	388.1101/388.1958	−86.30/−0.60	17/0.06
trifloxystrobin	409.1102/409.1371	−27.30/−0.40	5/0.04			

[a] Concentration of analytes was 50 μg kg^{-1}. [b] Concentration of incurred residues is shown in Table 5.

time of 5 ms and used for quantitative/qualitative purposes. The quantification was carried out using matrix-matched standards.

RESULTS AND DISCUSSION

Qualitative Analysis Using DART-TOF MS. Similar to atmospheric pressure ionization (API) that is routinely used for LC−MS/MS analysis of strobilurins, protonated molecules ($[M + H]^+$) were obtained under conditions of DART ionization. In the first phase of experiments, three wheat samples, each containing incurred residues of azoxystrobin, kresoxim-methyl, and pyraclostrobin, were directly analyzed by DART-TOF MS. Because of the limited thermal stability of filter paper in which milled wheat grains were placed into the DART source, the highest temperature for ionization was 200 °C. All target analytes were reliably detected as far as internal mass drift correction was carried out using PEG 600 in methanolic solution (200 μg L^{-1}) for this purpose. In addition to the direct examination of solid samples, the ethyl acetate extract obtained from blank wheat spiked with six strobilurins at 50 μg kg^{-1} each was analyzed. Although also the acetonitrile/water mixture (QuEChERS) and other solvents can be used for effective extraction of target compounds from grains, ethyl acetate was shown in our preliminary experiments as the optimal for obtaining a high intensity of MS signal. To their further enhancement, the temperature of the DART ion source was increased up to 300 °C. A typical record of five repeated injections of this sample (online mass calibrant included), together with a positive-ion DART mass profile in the *m/z* range corresponding to target strobilurins (exact masses are summarized in Table 2) as obtained in the fourth injection of a particular run, is presented in Figure 1. Large fluctuation of signal intensity is shown here, the relative standard deviation for the response of analytes was around 60% ($n = 5$). Table 3 summarizes the information on mass errors as obtained by DART-TOF MS analysis of both types of samples (incurred grains and spiked

extract) under experimental conditions. These data are divided into two parts, (i) mass drift uncorrected and (ii) mass drift corrected via an injection of a mass calibrant at the end of each run. The "average difference from the theoretical exact mass" was relatively low when mass corrected (ranging from −0.1 to −1.8 mDa). Actually correction is essential since the uncorrected data were biased significantly (ranging from −23.5 to −107.4 mDa).

Analyte ions were (tentatively) identified on the basis of elemental composition calculation. MassCenter JEOL software (version 1.3.8e) algorithm assigned it to the target compound according to the measured value of $[M + H]^+$ and the expected representation of elements in this protonized molecule (entered by operator). Similarly, isotopic ions could be processed to assess the whole isotopic pattern of the target compound. In Figure 2, incurred residue identification based on elemental composition is presented using kresoxim-methyl as an example. It should be noted, that according to Commission Decision 2002/657/EC[29] a minimum of three identification points is required for the confirmation of identity of a particular analyte. The mass resolution, expressed as full width at half-maximum (fwhm), obtained by the AccuTOF mass spectrometer, is in the range 5000−5200 for all target analytes. This value does not comply with the high-resolution MS category, for which at least 10 000 at 10% valley (approximately 20 000 fwhm) is required.[29] Under the DART-TOF MS conditions, only one identification point was earned. Note that the intensities of the in-source collision-induced dissociation (CID) fragment ions mentioned also in Table 2 were too low, and their detection in matrix-matched standards was only possible when strobilurins concentrations were ≥600 μg kg^{-1}. Of course additional identification points can be earned by LC−MS/MS, as stated by EU method validation guidelines.[29−31] As an alternative, we briefly studied the potential of DESI-LIT MS^n for confirmatory analysis of the incurred wheat samples.

Confirmatory Analysis Using DESI-LIT MS^n. Direct analysis of milled grains by DESI was feasible but in practice not to be recommended: grains were easily blown away from the surface by the desolvation gas. Also the use of double-sided tape was only partly successful in this respect. Therefore the grains were first put onto a bed of C18 material packed in a micropipet tip and simply percolated with methanol. The extract thus obtained was not expected to yield a quantitative recovery but to contain sufficient pesticides residues for confirmatory analysis by DESI-LIT MS^n. The $[M + H]^+$ ions of azoxystrobin and pyraclostrobin at m/z 404 and 388 could be coveniently detected in the incurred wheat grains, in accordance with the DART results shown in Figure 1 and Table 2. The $[M + H]^+$ ion of kresoxim-methyl and its MS/MS product ions upon CID were easily detected as an impurity in a mancozeb pesticide formulation but not in the incurred wheat grains, probably because of the relatively low level of approximately 50 μg kg^{-1} in the wheat and the incomplete recovery in the simplified sample preparation prior to DESI analysis. A typical DESI-LIT MS^2 product ion spectrum of wheat incurred with pyraclostrobin is given in Figure 3A. Major product ions can be seen at m/z 356, 296, and 194, probably caused by the loss of methanol, acetic acid, and the entire substituted methylaniline substructure. According to Commission Decision 2002/657/EC,[29] the parent ion and the three product ions can yield together 5.5 identification points, which is beyond the minimum requirement of 3. On the other hand, in ambient MS there is obviously no retention time criterium which can be complied with, so additional point requirements might be advocated for these techniques. Azoxystrobin yields one dominant product ion in DESI-LIT MS^2 at m/z 372 caused by the loss of methanol but still not enough identification points. Therefore MS^3 (m/z 404 → 372 →) was performed yielding the product ion spectrum of Figure 3B. Major product ions are obtained at m/z 344, 316, 303, 287, and 172, yielding all together 10 identification points for azoxystrobin in incurred wheat grains.

Quantification Using DART-TOF MS. As indicated above and illustrated again in Figure 4 (azoxystrobin used as an example) for quantitative analysis using the DART ion source, it is necessary to use an internal standard (IS) to compensate relatively high variation of analytes ion intensities in repeated (automated) analyses. In our study, prochloraz yielding $[M + H]^+$ at m/z 376.03984 was used as the IS, since this value was within the mass range of the strobilurins ($[M+H]^+$ from 314.1392−409.1375). Moreover, because of the three chlorine atoms contained in its molecule, this compound can be easily recognized by characteristic isotopic pattern. At a concentration of 100 μg L^{-1}, the difference from exact mass was 0.24 mDa (n = 5). In this particular case, the unique DART setup enabled measurement of the seven point calibration curve within 2 min. After internal mass drift correction, a calibration graph could be constructed by plotting analyte concentrations (X-axis) against an peak area ratio of the target strobilurin/prochloraz response (Y-axis). As an example, the calibration plot of azoxystrobin corrected by the internal standard showed a correlation coefficient R^2 of 0.9954 (Figure 4). For all strobilurins, matrix-matched standard calibration curves were linear within the tested range (6−1200 μg kg^{-1}). Regression coefficients (R^2) were not lower than 0.99.

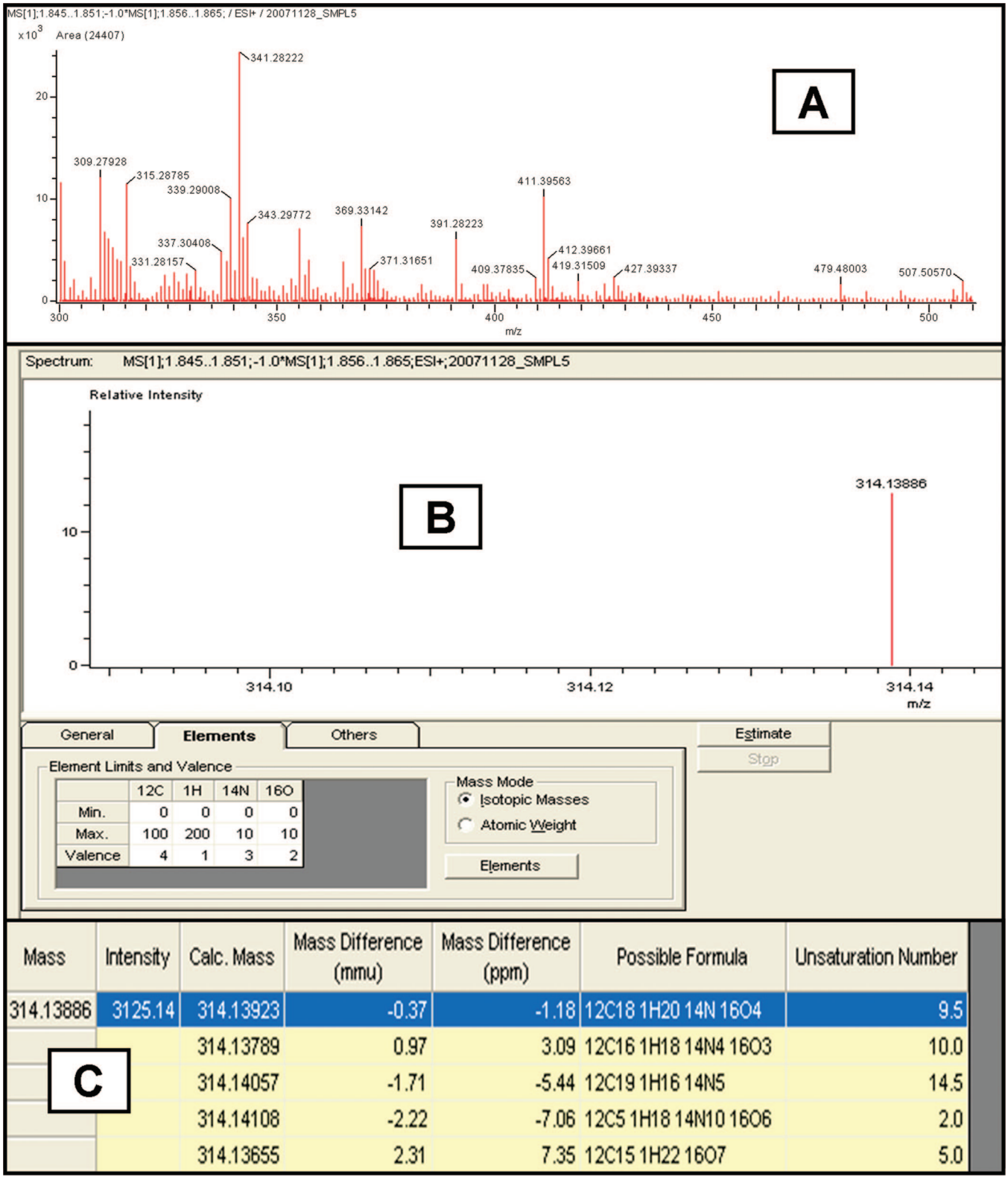
DART-TOF MS versus LC−MS/MS. The performance characteristics of DART-TOF MS and the "reference" LC−MS/MS method employing QuEChERS sample preparation are summarized in Table 4. Although the latter procedure enabled lower limits of quantification (LOQs) and better repeatability of measurements, the values obtained by DART-TOF MS were still satisfactory, meeting requirements (i.e., mean recoveries in the range 70−120%, with a RSD ≤ 20%) stated in Document No. SANCO/2007/3131 (Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed).[30]

The LOQs for all of target analytes were low enough (at least 30 μg kg^{-1}) to enable reliable control of EU and other international maximum residue levels (MRLs) established for strobilurins. The only exception was kresoxim-methyl: though it could be detected at this concentration, the requirement stated in the Commission Directive 97/57/EC[31] for LOQ > (0.5)(MRL) was not met. This compound had also the highest LOQ in LC−MS/MS, since, in

(29) Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results [Internet]. Available from http://ec.europa.eu/food/food/chemicalsafety/residues/lab_analysis_en.htm, accessed May 20, 2008.

(30) Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed. Document No. SANCO/2007/3131 from October 31, 2007 [Internet]. Available from http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf, accessed May 24, 2008.

(31) EC (European Communities), Council Directive 97/57/EC Establishing Annex VI to Directive 91/414/EEC Concerning the Placing of Plant Protection Products (PPPs) on the Market. *Off. J. Eur. Commun.* **1997**, *L265*, 87.



Mass	Intensity	Calc. Mass	Mass Difference (mmu)	Mass Difference (ppm)	Possible Formula	Unsaturation Number
314.13886	3125.14	314.13923	-0.37	-1.18	12C18 1H20 14N 16O4	9.5
		314.13789	0.97	3.09	12C16 1H18 14N4 16O3	10.0
		314.14057	-1.71	-5.44	12C19 1H16 14N5	14.5
		314.14108	-2.22	-7.06	12C5 1H18 14N10 16O6	2.0
		314.13655	2.31	7.35	12C15 1H22 16O7	5.0

C

Figure 2. DART-TOF MS direct analysis of wheat grains, element composition report. (A) Mass profile of the BIOCOP test sample with incurred kresoxim-methyl. (B) Zoomed part of mass profile showing in particular $[M + H]^+$ of kresoxim-methyl. (C) Estimating elemental composition from accurate mass measurement, the highlighted column stands for target analyte.

addition to $[M + H]^+$ ions, also competitive $[M + NH_4]^+$ adducts with the mobile phase component (ammonium acetate) are formed.

To demonstrate the potential of the novel DART-TOF MS approach to generate accurate data, wheat grains containing incurred residues of three strobilurins were analyzed. In Table 5, good agreement of results with data generated by LC−MS/MS is documented. Furthermore, the overall cost, the labor intensity as well as laboratory throughput is fairly better when employing the DART based-procedure. The total time required for analysis

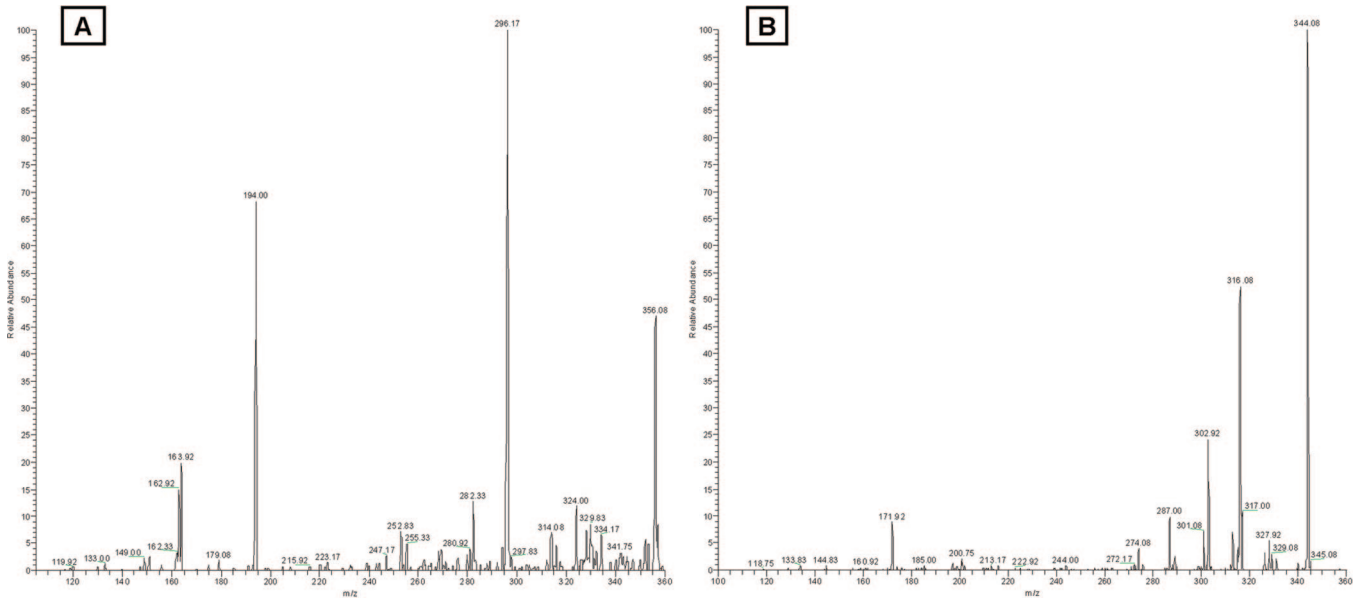


Figure 3. DESI-LIT MS^n confirmatory analysis of wheat samples incurred with strobilurin fungicides. (A) MS^2 full scan product ion spectrum of pyraclostrobin (*m*/*z* 388). (B) MS^3 full scan product ion spectrum of azoxystrobin (*m*/*z* 404 → 372). The concentrations as determined by LC−MS/MS are given in Table 5.

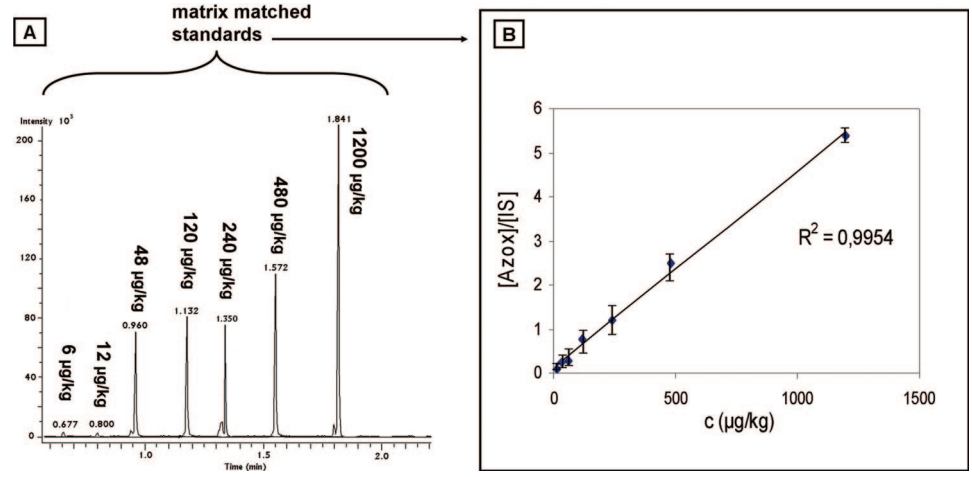


Figure 4. Quantification using DART-TOF MS. (A) Single ion current of *m*/*z* 404.1246 (azoxystrobin $[M + H]^+$ value) in matrix matched calibration standards (6−1200 μg kg^{-1}) measured within a single run. (B) Working curve of azoxystrobin (analyte to internal standard intensity ratio plotted versus analyte concentration).

Table 4. Selected Performance Characteristics of DART-TOF MS and LC−MS/MS Method Applied to Strobilurin Analysis in Cereals and Current Maximum Residues Limits (MRLs)[a]

	MRL (μg kg^{-1})			DART-TOF MS				LC−MS/MS			
compound	EU	Codex Allimentarius	U.K.	LOQs (μg kg^{-1})	repeatability (RSD, %)	recovery (%)	linearity (R^2)	LOQs (μg kg^{-1})	repeatability (RSD, %)	recovery (%)	linearity (R^2)
azoxystrobin	300		300	5	8	92	0.9954	1	3	90	0.9991
picoxystrobin	50[b]		50[c]	12	15	92	0.9933	2	4	93	0.9990
dimoxystrobin			50[c]	10	11	85	0.9860	2	4	88	0.9989
kresoxim-methyl	50	50	50	30	13	90	0.9900	4	6	89	0.9978
pyraclostrobin	100[b]	200	200	12	11	78	0.9912	2	5	96	0.9995
trifloxystrobin	50[b]	200	20[c]	6	12	83	0.9978	2	3	90	0.9980

[a] LOQ were estimated at S/N = 12. Recoveries and repeatability were measured at concentration level 50 μg kg^{-1} ($n = 5$). [b] 1EC Provisional MRLs. [c] U.K. Temporary MRLs.

of a sample batch consisting of six wheat grains and a seven point calibration curve (on matrix-matched standards) was approximately 1.5 h, while 5 h were needed when using the LC−MS/MS approach.

Table 5. Amount of Strobilurin Incurred Residues in Wheat Grain Measured By DART-TOF MS and LC-MS/MS ($n = 3$)

	DART-TOF MS		LC−MS/MS	
compound	mean (μg kg^{-1})	RSD ($n = 3$, %)	mean (μg kg^{-1})	RSD ($n = 3$, %)
azoxystrobin	445	6	429	2
kresoxim-methyl	45	17	52	4
pyraclostrobin	202	8	187	3

CONCLUSIONS

The results obtained within this pilot study employing ambient mass spectrometry can be summarized as follows: (1) DART-TOF MS and DESI-LIT MS allowed rapid, direct qualitative screening for the presence of strobilurin residues in wheat grains at concentration levels lower or close to MRLs. Tandem mass spectrometry is required to earn a sufficient number of identification points for confirmatory analysis, as shown by DESI-LIT MS^n. (2) Quantitative analysis of strobilurins in wheat by DART-TOF MS could be performed using a simple ethyl acetate extraction. Internal standard has to be added to overcome rather poor repeatability (absolute) of analyte responses. (3)The performance characteristics obtained within a DART-TOF MS validation study (spiked wheat sample) complied with EU regulation requirements for official control of pesticide residues in food commodities. (4) Analysis of samples containing incurred strobilurin residues documented a good trueness of DART-TOF MS based results, comparable with that obtained by the conventional analytical approach employing LC−MS/MS.

In the follow up studies, the research will be focused on exploring the possibility to expand the screening scope of ambient mass spectrometry by more pesticide/matrix combinations.

ACKNOWLEDGMENT

This work was realized as a part of the European Commission funded Integrated Project FOOD-CT-2004-06988 "BIOCOP (New Technologies to Screen Multiple Chemical Contaminants in Foods)" coordinated by Queen's University (Belfast, U.K.) and also within the scope of Research Projects MSM 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic. The authors wish to appreciate the support provided by JEOL (EUROPE) SAS (Croissy-sur-Seine, France). The DESI-LIT MS^n facility was granted to Wageningen University by the Dutch Science Foundation (NWO).

Received for review August 29, 2008. Accepted October 7, 2008.

AC8018137