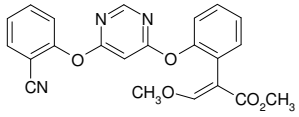
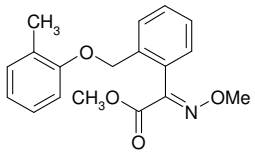
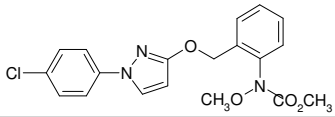
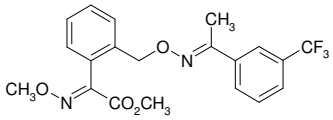
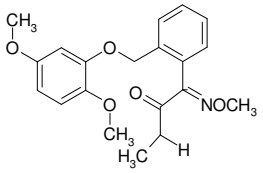
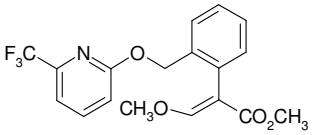
Analysis of stobilurins in wheat grains using DART-TOFMS

Strobilurins, systemic pesticides originated from natural fungicidal derivatives, play an important role in control of various plant pathogens.[1,2,3] Because of their unique protective properties, significant yield enhancements and longer retention of green leaf tissue, strobilurins have been widely used in agriculture since their introduction on the market in 1992.[3] As other pesticides, these compounds are involved in control and monitoring surveys undertaken by regulation authorities.[4] Some characteristics of strobilurins are shown in Table 1.

Table 1 Strobilurins: structure and MRLs in wheat.

Compound	CAS number	Structure	MRL (mg/kg) in wheat		
			UK	Codex	EU
Azoxystrobin	**131860-33-8**		**0.3**	**none**	**0.3**
Kresoxim methyl	**143390-89-0**		**0.05**	**0.05**	**0.05**
Pyraclostrobin	**175013-18-0**		**0.2**	**none**	**none**
Trifloxystrobin	**141517-21-7**		**0.02[a]**	**none**	**none**
Dimoxystrobin	**149961-52-4**		**0.05[b]**	**none**	**none**
Picoxystrobin	**117428-22-5**		**0.05[b]**	**none**	**0.05[a]**

[a] proposed MRL, [b] temporary MRL

The AccuTOF-DART system equipped with an AutoDart HTC PAL autosampler was used for the analysis of strobilurin residues (listed in Table 1) in wheat grain extracts. Crude extracts were prepared by shaking 12.5 g of sample with 50 mL of ethyl acetate and 5 mL of Na_2SO_4, the suspension was then filtered and the volume was made up to 25 mL by rota vapour. Within the validation, extracts spiked with strobilurins in the range from 12 to 1200 ng/g were analyzed. For quantitative analysis, prochloraz was used as an internal standard (samples were spiked with this internal standard at a level of 250 ng/g).

The DART ion source was operated in positive ion mode with helium as the ionizing medium at a flow rate of 2.7 L/min. The gas beam was heated to 300°C, and the optimal distance between the exit of the DART gun and inlet of the mass spectrometer was 12 mm. The discharge needle voltage set to positive potential of 3000 V, perforated and grid electrode voltages were +150 V and +250 V, respectively. Accurate mass spectra were acquired in a range of *m/z* 100–500 employing 0.2 s recording interval; the peaks voltage value was set to 1000 V. A solution containing a mixture of poly(ethylene glycol) PEG 600 and 200 was used for mass calibration. The same calibrant was introduced at the end of each sample analysis to compensate any mass drift. The mass resolution of the mass spectrometer was typically 6000 ± 500 (FWHM).

The examined samples were introduced automatically with the use of an AutoDart sampler and Dip-it™ tips. A sampling tip was immersed into the sample and then placed in front of the DART gun exit close to the source – mass spectrometer axis. Each sample was examined in six repeated runs. The TIC chromatogram of spiked wheat sample is shown in Figure 1. Due to the variability of absolute responses, the use of internal standard is obviously essential for quantitative measurements.

Figure 1 TIC chromatogram: "on-line" 6 repeated injections of spiked wheat extract followed by PEG mixture.

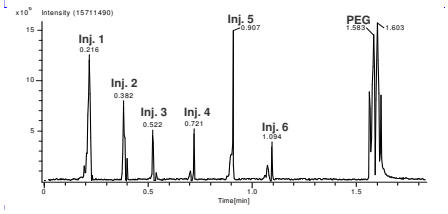


Figure 2 shows a positive-ion DART mass spectrum of crude ethyl acetate wheat extract spiked with strobilurins. Under these experimental conditions, both strobilurins and internal standard were detected as $[M+H]^+$. In Table 2, measured and exact masses are compared, the differences ranged from -1.95 to 2.51 mmu.

Figure 2 Strobilurins (240 ng/g) and prochloraz (250 ng/g) in wheat extract.

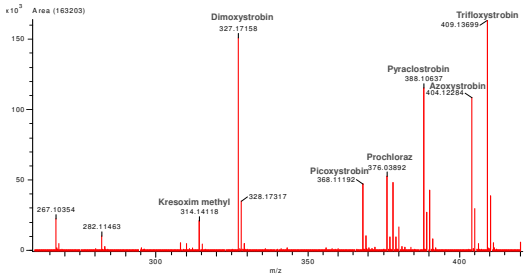


Table 2 Strobilurins identified in wheat extract by exact mass

Compound	Exact mass (mu) [M+H]⁺	Measured mass (mu) [M+H]⁺	Diference (mmu)	Elemental composition [M+H]⁺
Azoxystrobin	404.12465	404.12284	1.81	$C_{22}H_{18}N_3O_5$
Kresoxim methyl	314.13923	314.14118	-1.95	$C_{18}H_{20}NO_4$
Pyraclostrobin	388.19641	388.10637	0.04	$C_{19}H_{19}ClN_3O_4$
Trifloxystrobin	409.13752	409.13699	2.51	$C_{20}H_{20}F_3N_2O_4$
Dimoxystrobin	327.17087	327.17158	-0.71	$C_{19}H_{23}N_2O_3$
Picoxystrobin	368.11097	368.11192	-0.95	$C_{18}H_{17}F_3NO_4$
Prochloraz	376.03864	376.03892	-0.28	$C_{15}H_{16}Cl_3N_3O_2$

As Figure 3 shows, acceptable linearity was obtained for the analytes in the range from 12 to 1200 ppb.

The repeatability of measurements at a spiking level of 60 ng/g was in the range 8–15% ($n = 6$), limits of quantification (LOQs) ranged from 12 to 30 ng/g depending on the particular analyte. To prove the trueness of generated data, wheat grains with incurred strobilurin residues (reference material) were employed. Table 3 documents good agreement between the data obtained by DART-TOFMS and accredited LC–MS/MS method.

Figure 3 Calibration plots of strobilurins (analyte to internal std. intensity ratio plotted versus analyte concentration).

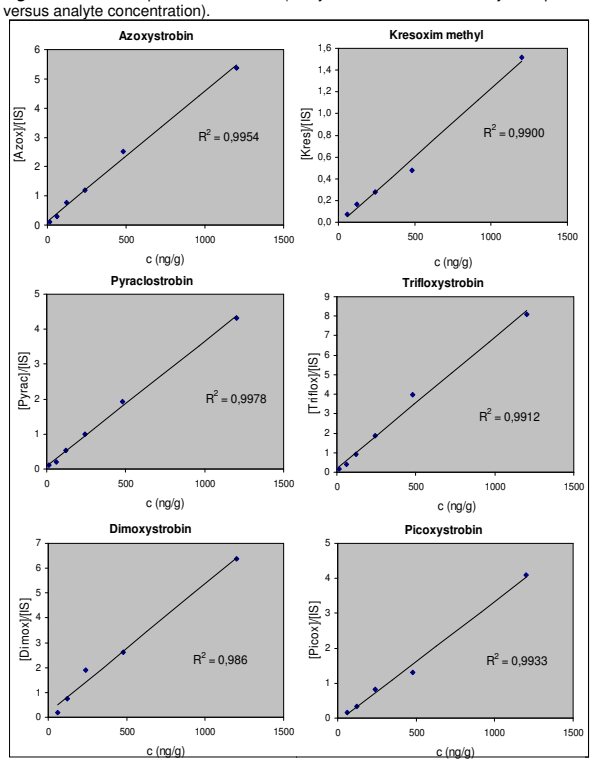


Table 3 Comparison of DART-TOFMS and LC–MS/MS method: analysis of wheat grain reference material.

Analyte	Concentration (ppb)	
	DART-TOFMS	LC–MS/MS
Azoxystrobin	445	429
Kresoxim methyl	45	52
Pyraclostrobin	202	170

Compared to conventional LC–MS/MS method, DART-TOFMS allowed significant decrease of analysis time, thus, enabled increase of sample throughput.

Although the detection limits are somewhat higher employing this new strategy as compared to the LC–MS/MS method, the DART-TOFMS enables convenient control of MRLs set for strobilurins residues in wheat grains which are in the range from 0.05 to 0.3 mg/kg.

References

1. Oerke, E.C., Dehne, H.W., Schonbeck, F., Weber, A., 1994. Crop Protection and Crop Production. Elsevier, Amsterdam, 808p
2. Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., Parr-Dobrzanski, B., 2002. Review: the strobilurin fungicides. *Pest Manage. Sci.* 58, 649–662.
3. Sauter, H., Steglich, W., Anke, T., 1999. Strobilurins: evolution of a newclass of active substances. Angew. Chem., Int. Ed. 111, 1416–1438.
4. http://ec.europa.eu/food/plant/protection/resources/mrl_pesticide.pdf