



## Work Package 6: Pesticides

### Introduction

WP6 focuses on pesticides and, in particular, an important class of fungicides called strobilurins. Strobilurins were selected for study as they represent over one third of the fungicides used in cereals. There is potential for cumulative residues in the human diet, since residues have been reported in many different commodities (e.g. bananas, strawberries, and spinach). In 2008 the global sales of just one strobilurin (azoxystrobin) surpassed \$1 billion. It is therefore important that effective analytical methods are available for monitoring / control of residues from the use of these fungicides. Other current strobilurins include trifloxystrobin, pyraclostrobin, picoxystrobin, kresoxim-methyl and, dimoxystrobin.

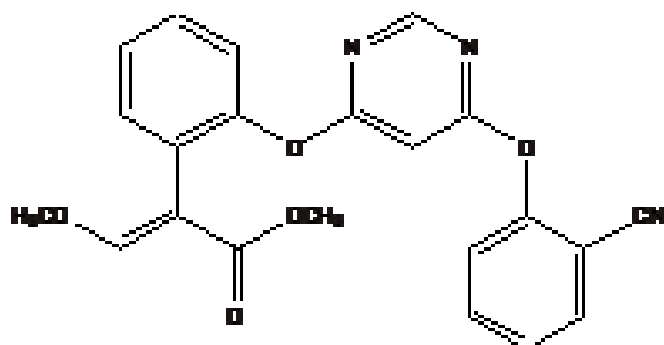


Figure 1. Chemical structure of a strobilurin (Azoxystrobin)

### Legislative limits in the EU

In the EU, Maximum Residue Level (MRL) for strobilurin fungicides vary according to the analyte / commodity combination. MRL data for wheat is listed in Table 1.

| Strobilurin     | MRL<br>(mg kg <sup>-1</sup> ) |
|-----------------|-------------------------------|
| Azoxystrobin    | 0.3                           |
| Trifloxystrobin | 0.05                          |
| Kresoxim-methyl | 0.05                          |
| Pyraclostrobin  | 0.1                           |
| Dimoxystrobin   | 0.1                           |
| Picoxystrobin   | 0.05                          |

Table 1. EU Maximum Residue Levels for strobilurins in wheat (EU Regulation (EC) No 396/2005)

### Project Aims

Strobilurins are normally analysed for in specialist laboratories using liquid chromatography (LC) coupled to mass spectrometry (MS), with sample extraction and clean-up via the QuEChERS<sup>1</sup> approach. This approach is suited to targeted, confirmatory residue analysis. In Biocop, alternative, screening methods using ambient ionization mass spectrometry have been assessed for the rapid detection of strobilurins.

### Work Package Progress and Results

Ambient ionization mass spectrometry (“ambient MS”) is a form of ionization in which ions are formed outside the mass spectrometer without the need for conventional sample preparation or separation. Recently several “ambient MS” techniques have been commercialized for use with a range of MS instruments. Since these different designs are likely to exhibit advantages and disadvantages in terms of cost, ease of use, safety, and sensitivity, BioCop partners<sup>2</sup> are currently evaluating three different techniques, namely:

- Desorption Electrospray Ionisation (DESI),
- Direct Analysis in Real Time (DART), and
- Atmospheric Pressure Solids Analysis Probe (ASAP).

To make this study as realistic as possible, spring wheat was sprayed with strobilurins to prepare samples with ‘incurred’ residues (i.e. added during normal farming practice rather than preparing ‘artificial’ samples by spiking grain in the laboratory). Two main approaches were used to introduce test samples into the MS. In the first approach a probe was dipped into whole grains or milled grains. In the second a suitable solvent (e.g. methanol) was used to prepare a crude sample extract.





## New Technologies to Screen Multiple Chemical Contaminants in Foods



**Figure 2.** Analyses of a grain sample by ambient ionization mass spectrometry (ASAP) – Note the minimal sample preparation required.

These experiments have proved that DART, DESI and ASAP based methods can provide rapid (< 1 minute per sample) qualitative or semi-quantitative screening for the presence of strobilurin residues in wheat grains at concentrations  $\leq$  MRLs. The main advantage of these techniques is that little, or no, sample preparation is required. Additional information, described by technique, is listed below:

### Dart

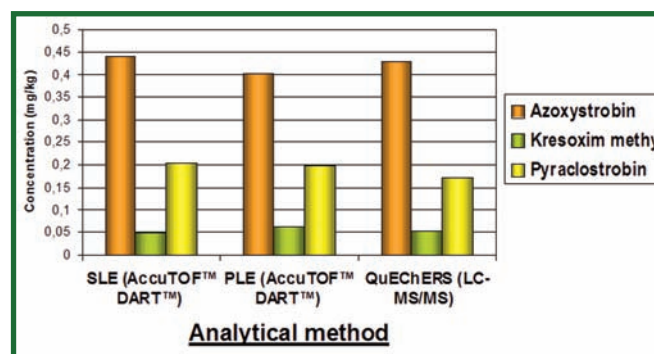
Quantification of strobilurins in solvent extracts were improved by the use of an internal standard. Analyte recoveries were 85%, with good measurement repeatability.

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).

DART provides simple and fast confirmation of strobilurins in wheat at MRL levels. This method was fully validated at a low spiking level (50 ug/kg) with the six strobilurins, (azoxystrobin, picoxystrobin, dimoxystrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin) all of which complied to requirements for official control of pesticide residues in food commodities.

Thanks to unique features of this novel technique, by passing time and labour consuming steps involved in conventional analytical procedures is possible. Since limited, or no sample preparation is required, additional convenience to MS-based analyses is delivered.

Direct Analysis in Real Time interfaced with Time-of-Flight Mass Spectrometry (DART-TOFMS): A New Challenge in Pesticide Residue Analysis was discussed in great detail at the EPRW 2008.



**Figure 3.** Incurred residue analysis - wheat grains

### DESI

DESI provided a sufficient number of product ions for confirmation of identity of both azoxystrobin and pyraclostrobin incurred in wheat grains.

### ASAP

Incurred residues of azoxystrobin were detected in whole grain without any sample preparation. Residues of azoxystrobin, kresoxim-methyl and pyraclostrobin incurred at 0.04- 0.4 mg/kg were detected in acetonitrile extracts. Further details of the work on DESI/DART has been published by Schurek et al<sup>3</sup>.





## Benefits of the BioCop Project

### Consumer

The ability of laboratories to use rapid methods for the screening of strobilurins (and other pesticide residues) should increase both the frequency and volume of testing. This, in turn, could lead to greater consumer protection.

### Food Industry

The food industry will benefit from the developed rapid screening technologies for the detection of residues of pesticides. For example the ability to check and screen raw materials for residues in “real time” could have a direct impact on production costs. The provision of high-throughput screening technology to the European food industry will therefore enhance their competitiveness.

### Scientist

From a scientific point of view this work package has produced a very rapid method for the detection of pesticide residues in grain. This method uses either none or very little solvent and therefore has the potential to reduce the amount of overall chemical waste in the environment.

## Training/Workshops

This work has been presented at a number of conferences including (i) The Latin American Pesticide Residue Workshop ([www.laprw2009.unl.edu.ar](http://www.laprw2009.unl.edu.ar)) and (ii) the Florida Pesticide Residue Workshop (<http://www.flworkshop.com/>). Further dissemination activities/events are planned for 2010 after the completion of an inter-laboratory study.

## Future Activity

During the remaining part of the project the performance characteristics of the three different ambient ionization mass spectrometry techniques will be critically assessed via an inter-laboratory study during winter 2009/10. It is envisaged that this work will

help define the Quality Assurance/Quality Control principles for this type of method and will facilitate the wider implementation of these technologies into routine control laboratories.

## Frequently Asked Questions

### Question:



The QuEChERS method for pesticide analysis is well established. Is there a need for another technique?

### Answer:



Whilst the QuEChERS sample clean-up method is the established technique in laboratories worldwide, these new ambient MS techniques have the potential to screen for many residues without any sample preparation/purification. These new methods are therefore more suited testing samples in “real-time”.

### Question:



Can ambient MS methods be automated?

### Answer:



Liquid samplers are available to assist with the preparation of samples prior to the ambient-MS measurement.

## References

- <sup>1</sup> <http://www.quechers.com/>
- <sup>2</sup> Fera, UK; VSCHT, Czech; and RIKILT, The Netherlands
- <sup>3</sup> Schurek, J, Vaclavik, H, Hooijerink, H.(Dick), Lacina, O., Poustka, J, Sharman, M., Caldow, M., Nielen, M. W. F., Hajslova, J: Journal Analytical Chemistry, **2008**, 80 (24), 9567-9575.

