L12 DIRECT ANALYSIS IN REAL TIME – TIME-OF-FLIGHT MASS SPECTROMETRY: ANALYSIS OF PESTICIDE RESIDUES AND ENVIRONMENTAL CONTAMINANTS

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Introduction

Ambient desorption ionization mass spectrometry (MS) is a rapidly growing area representing an attractive alternative to conventional analytic approaches. Recently introduced ionization techniques, such as direct analysis in real time (DART)[1], desorption electrospray ionization (DESI)[2] or atmospheric pressure solids analysis probe (ASAP)[3], allow direct examination of various types of samples in the open atmosphere and at ground potential. Little or no sample treatment prior to analysis is required. Additionally, time-consuming separation of sample components, which is usually employed by chromatographic methods, can be omitted with ambient MS.[4]

The ionization process with DART is based on interactions of metastable atoms of gas with atmosphere (H_2O, O_2) and sample components. The gas (usually helium) flows through a tube divided into several compartments. In a discharge chamber, ions, electrons and metastables are formed. In the next step, charged species are removed from the gas stream and heated gas promotes the desorption process. Ionization of the sample occurs in the area between the ion source and a mass spectrometer inlet (sampling gap). DART provides relatively simple mass spectra characterized mainly by $[M + H]^+$ and $[M]^+$ in positive-ion mode or $[M-H]^-$ and $[M]^-$ in negative-ion mode.[1] It is worth to notice, that DART technique has common features with atmospheric pressure chemical ionization (APCI) as the formation of metastables take place in an electrical discharge.[1,4]

DART ion source can be hyphenated to any type of mass spectrometer. However, when coupled to a high-resolution time-of-flight mass spectrometer (TOFMS), accurate mass measurement is enabled, allowing the confirmation of target analyte identity and calculations of elemental compositions of "unknowns". For correct identification of "unknowns", it is essential to gain knowledge about the examined matrix to allow discrimination of potential compounds suggested by the software.

Until now, very few papers dealing with applications of DART have been published.[5-9] In following examples, the potential of DART–TOFMS technique for qualitative and quantitative analysis of *(i)* pesticide residues, in particular case, strobilurins in wheat grains, *(ii)* thiabendazole on cut-flower leaves, and *(iii)* rapid screening of brominated flame retardants (BFRs) in in-door dust extract, will be demonstrated.

Experimental

Chemicals

Pesticide standards (≥99 %) were obtained from Dr. Ehrenstorfer (Germany), decabromodiphenyl ether (BDE-209) standard (≥98 %) was provided by Cambridge Isotope Laboratories (USA). Solvents used for sample extractions and preparations of standard solutions were HPLC-grade. Poly(ethylene glycol) 600 was from Sigma-Aldrich (Germany), anhydrous Na_2SO_4 was supplied by Merck (Germany).

Sample Preparation

(i) An amount of 12.5 g of milled wheat grains was spiked with an internal standard (prochloraz) at a concentration of 250 ng g^{-1} and extracted by shaking with 50 ml of ethyl acetate and 10 g of anhydrous Na_2SO_4. The suspension was filtered and the volume was reduced by evaporation to 25 ml. Similarly, wheat grain extracts spiked with strobilurins (azoxystrobin, kresoxim methyl, pyraclostrobin, trifloxystrobin, dimoxystrobin and picoxystrobin) in the range from 12 to 1200 ng g^{-1} were prepared. Wheat grains with incurred residues of azoxystrobin, kresoxim methyl and pyraclostrobin (reference material) were processed as described above.

(ii) Flowers (roses) were purchased from local florists shop. The leaf was separated from the rest of flower and its surface was directly analyzed.

(iii) In-door dust containing BFRs (mainly BDE-209) was extracted using ASE 300 pressurized liquid extraction system (Dionex, USA): a hexane–acetone (1 : 1, v/v) mixture was used for extraction. The residues of extract were dissolved in isooctane.

DART–TOFMS Analysis

For DART–TOFMS analyses, the system consisting of a DART ion source (IonSense, USA), a JEOL AccuTOF LP high-resolution mass spectrometer [JEOL (Europe) SAS, France], and an AutoDART HTC PAL autosampler (Leap Technologies, USA) was used. Helium gas was flowed at 2.9 dm^3 min^{-1}, discharge needle voltage was ± 3000 V, while perforated and grid electrode voltages were set to ± 150 V and ± 250 V, for positive and negative-ion mode, respectively. Other system parameter settings were changed depending on examined analytes, as summarized in Table I. To monitor bromine fragment ions originated from BDE-209, the cone voltage of the mass spectrometer was adjusted as described in results section.

Automated introduction of liquid samples was carried out with the use of Dip-it™ tips (IonSense, USA). Solid samples (flower leaves) were introduced manually by placing them in front of DART source. Poly(ethylene glycol) 600 solution (200 μg ml^{-1}) was introduced at the end of each sample analysis to perform internal mass calibration (mass drift compensation). The mass resolution of the instrument

Table I
DART–TOFMS parameter settings

Analytes	Polarity	Beam temp. [°C]	Ion guide voltage [V]
Strobilurins	Positive	300	1000
Thiabendazole	Positive	150	800
BDE-209	Negative	300	600
Bromine ions	Negative	350	400

during the measurements was typically 6,000 full width at half maximum (fwhm).

Results

Analysis of Strobilurins

The strobilurins and prochloraz (internal standard) were detected as $[M + H]^+$ ions (see Fig. 1.). A high mass resolving power of TOFMS instrument enabled the identity confirmation of target analytes on the basis of elemental composition calculations; the differences between measured (accurate) and calculated (exact) masses ranged from –2.27 to 5.10 ppm.

Fig. 2. shows the total ion current (TIC) record of six injections of wheat grain extract spiked with strobilurins (240 ng g^{-1}) and prochloraz (250 ng g^{-1}). Unfortunately, the

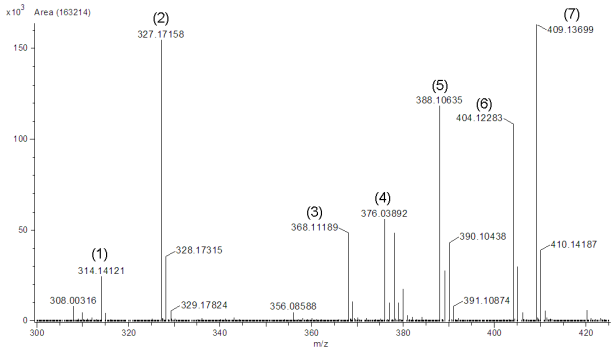

Fig. 1. **Dart positive mass spectrum of wheat grain extract spiked with strobilurins (240 ng g^{-1}) and prochloraz (250 ng g^{-1}); (1) Kresoxim methyl, (2) Dimoxystrobin, (3) Picoxystrobin, (4) Prochloraz, (5) Pyraclostrobin, (6) Azoxystrobin, (7) Trifloxystrobin**

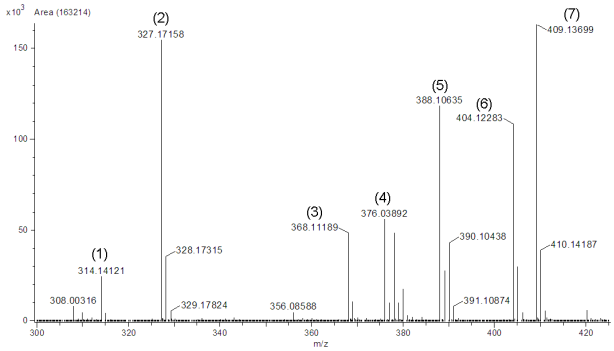

Fig. 2 **TIC chromatogram of six repeated wheat grain extract introductions [(1)–(6)] followed by PEG 600 solution (7)**

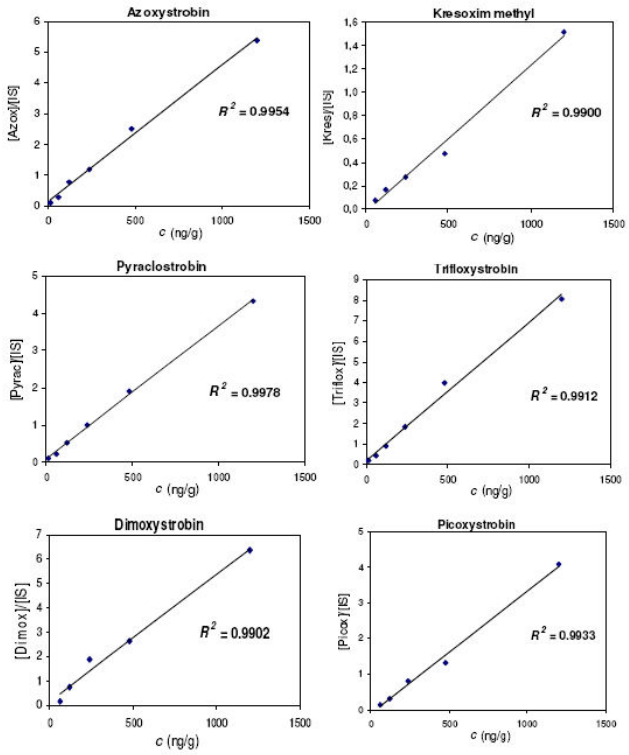

Fig. 3. **Examples of calibration curves of matrix-matched strobilurin standards**

absolute response of the detector, even when employing autosampler, was poorly repeatable because of the dependence of position of the sampling tip and the sampling gap. Therefore, an internal standard had to be used for quantification of strobilurin residues. Calibration plots obtained by analyses of matrix-matched standards (see Fig. 3.) were constructed by plotting the ratio of analyte/internal standard ion intensity *vs.* concentration of particular analyte. Acceptable linearity was obtained for tested concentration range, regression coefficients of calibration curves were higher than 0.99.

In the next step, basic performance characteristics of the method were estimated using spiked samples. The repeatability was in the range from 8 to 15 % (n = 6, 60 ng g^{-1}), LOQs (limits of quantification) ranged from 12 to 30 ng g^{-1}. Considering the European regulation requirements, this method can be useful for rapid control of strobilurin residues in wheat grains[10]. For comparative purposes, wheat grain sample containing incurred residues of strobilurins was analyzed using

Table II
DART–TOFMS and LC–MS/MS methods: Analysis of incurred residues in wheat grains

Analyte	Concentration[ng g^{-1}]	
	DART–TOFMS	LC–MS/MS
Azoxystrobin	445	429
Kresoxim methyl	45	52
Pyraclostrobin	202	190

in-house validated method employing liquid chromatography–tandem mass spectrometry (LC–MS/MS). A good agreement of the results generated by two alternative approaches is documented in Table II.

Direct Detection of Thiabendazole in Plant Leaf

In this experiment, the possibility to monitor pesticide residues directly from the surface of the flower leaf was examined. For this purpose, the temperature of gas beam was decreased to 150 °C. Fig. 4.(A) shows positive mass spectrum of the leaf surface obtained by DART–TOFMS. In zoomed mass spectrum (Fig. 4.(B)), ion m/z 202.04410 corresponding to protonated thiabendazole molecule $[C_{10}H_8N_3S]^+$ (theoretical mass m/z 202.04389) was observed. No other pesticide compounds were detected in examined sample.

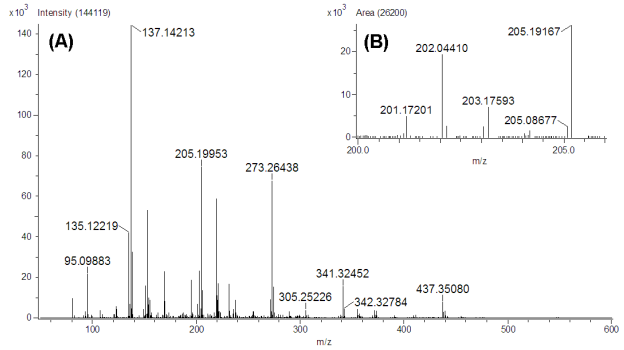

Fig. 4. **DART positive mass spectrum of flower leaf; (A) m/z 50–600, (B) m/z 200–206. The ion m/z 202.04410 corresponds to thiabendazole**

Screening of BFRs in In-Door Dust

The most common methods used in analysis of BFRs employ gas chromatography coupled to mass spectrometry (GC–MS) operated in negative chemical ionization mode (NCI)[11]. The ions $[^{79}Br]^-$ and $[^{81}Br]^-$ are typically the base peaks in NCI mass spectra of these compounds and due to their selectivity they are frequently used for quantification purposes[12]. Supposing some similarity of BFRs fragmentation under NCI conditions in GC–MS and negative APCI, DART–TOFMS was proposed as a suitable approach for rapid screening of BFRs.

In the first phase of this experiment, the ionization of BDE-209 by DART was investigated. As documented in Fig. 5.(A), phenolate anions resulting from the cleavage of the ether bridge and anions resulting from bromine abstraction were observed after introduction of BDE-209 standard solution. To induce fragmentation, the cone voltage was decreased from -20 V to -140 V. Under these conditions, intensive $[^{79}Br]^-$ and $[^{81}Br]^-$ ions were the only ions in recorded mass spectrum (Fig. 5.(B)).

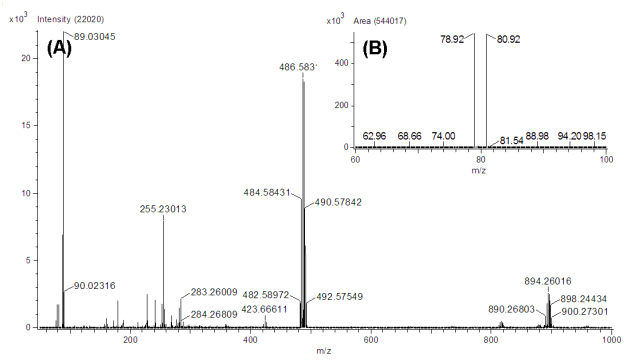

Fig. 5. **DART negative mass spectrum of BDE-209 standard solution (50 µg ml^{-1}); (A) beam temp.: 300 °C, cone volt.: –20 V, (B) beam temp.: 350 °C, cone volt.: –140 V**

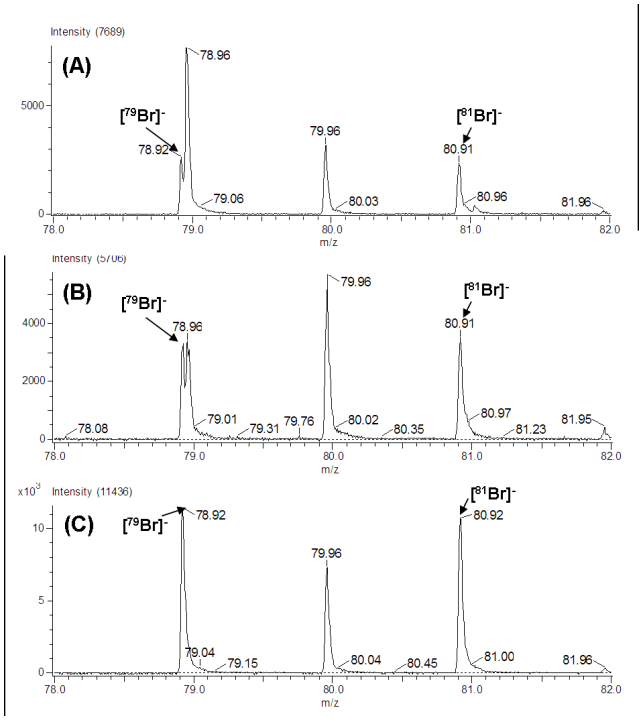

Fig. 6. **DART negative mass spectrum of in-door dust extract; (A) cone volt.: –140 V, (B) cone volt.: –200 V, (C) cone volt.:–240 V**

While it was not possible to detect BFRs in dust extract due to high chemical noise, both bromine ions were distinctly recognized when fragmentation was induced (Fig. 6.(A)). To remove interfering ion with a mass close to $[^{79}Br]^-$, an attempt to induce its fragmentation was undertaken. As shown in Fig. 6.(B) and Fig. 6.(C) this was achieved by further decrease of cone voltage value.

Conclusions

DART–TOFMS technique can be used for determination of strobilurin fungicides in milled wheat grain extracts obtained by simple extraction procedure without time-consuming chromatographic separation. This method withstands the regulation demands of the European Union for the control of pesticide residues; moreover, simplified workflow enables examination of many samples within a short time period.

Qualitative analysis of solid samples without any sample preparation is a challenging application of this novel technique. DART–TOFMS was shown to be a useful tool enabling rapid examination of plant surface and detection of pesticide used for flower treatment.

Preliminary results indicate the potential to introduce new concepts into rapid screening of BFRs by employing DART–TOFMS. In addition, the information provided by both negative and positive mass spectra should be exploited with the aim to detect the presence of other contaminants. Of course, more follow-up research is needed, with a special focus on quantification of target compounds and identification of unknowns.

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