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Analysis of multiple mycotoxins in cereals under ambient conditions using direct analysis in real time (DART) ionization coupled to high resolution mass spectrometry

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ABSTRACT

Direct analysis in real time (DART) ionization coupled to an (ultra)high resolution mass spectrometer based on orbitrap technology (orbitrapMS) was used for rapid quantitative analysis of multiple mycotoxins isolated from wheat and maize by modified QuEChERS procedure. After initial evaluation of ionization efficiencies for major groups of mycotoxins achievable with DART technology, sample preparation procedure and instrument parameter settings were optimized to obtain sensitive and accurate determination of most intensively ionizing toxins (deoxynivalenol, nivalenol, zearalenon, actyldeoxynivalenol, deepoxy-deoxynivalenol, fusarenon-X, altenuene, alternariol, alternariolmethylether, diacetoxyscirpenol, sterigmatocystin). The lowest calibration levels (LCLs) estimated for the respective analytes ranged from 50 to 150 $\mu g\,kg^{-1}$. Quantitative analysis was performed either with the use of matrix-matched standards or by employing commercially available ^{13}C-labeled internal standards (available for deoxynivalenol, nivalenol and zearalenon). Good recoveries (100–108%) and repeatabilities (RSD 5.4–6.9%) were obtained at spiking level 500 $\mu g\,kg^{-1}$ with isotope dilution technique. Based on matrix-matched calibration, recoveries and repeatabilities were in the range 84–118% and 7.9–12.0% (RSD), respectively. The trueness of data obtained for deoxynivalenol and zearalenon in wheat/maize by DART–orbitrapMS was demonstrated by analysis of certified reference materials (CRMs). Good agreement of these results with data generated by validated ultra-high pressure liquid chromatography–time-of-flight mass spectrometry method was documented.

1. Introduction

Mycotoxins represent a wide group of natural chemical contaminants originating from the secondary metabolism of pathogenic microscopic filamentary fungi, mainly *Fusarium*, *Alternaria*, *Aspergillus*, and *Penicillium* genera that may infest various food crops including cereals such as wheat, maize and barley. Depending on their nature and concentration levels, mycotoxins may induce toxic effects in human and/or farm animal, moreover, some of them, or their toxic transformation products, may be transferred into milk, eggs or occur in some animal's edible parts. Since mycotoxins are relatively stable compounds, they are not removed by heat treatment and can be found also in final processed foods. To protect consumer's health, the European Commission has set [Commission Regulation 1881/2006 (EC) amended by Commission Regulation 1126/2007 (EC)] [1] maximum levels for some mycotoxins, such as aflatoxins, ochratoxin A and major *Fusarium* toxins [deoxynivalenol (DON), zearalenon (ZEA) and fumonisins] in some unprocessed cereals and products thereof. Due to the dietary risk associated with representatives of the later group, trichothecene B, nivalenol (NIV), and trichothecenes A, T-2 and HT-2, have been evaluated by the European Food Safety Authority (EFSA) as candidates for future regulation. Recently, mycotoxins-related emerging risks have been discussed by scientists including, EFSA EMRISK panel [2]. Several studies have documented that the most common grain-contaminating genus of fungi, *Fusarium* spp., is also capable of producing other toxic secondary metabolites such as fusaproliferin, beauvericin, enniatins, and moniliformin. So far, only limited data is available on these metabolites. This is not only due to their late recognition but especially the late understanding of their role as mycotoxins. Another group of mycotoxins becoming under concern are ergot alkaloids occurrence of which in rye and other creaks is growing. The changing pattern of mycotoxins in European crops is currently often considered as the result of climatic changes.

Since occurrence of mycotoxins in cereals has been the issue of health concern for decades worldwide, enormous number of analytical methods/screening tests for control of their occurrence has been developed and characterized, in most cases they were val-

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idated for only one or closely related group of toxins. In the last decade, relatively large number of multi-mycotoxin methods (multiple analytes determined in single run) has been published [3–9]. In all of those applications, high or ultra-high pressure liquid chromatography (HPLC/U-HPLC) hyphenated with mass spectrometric detection (MS) employing triple quadrupole, ion trap or orbitrap mass analyzers has been used. To prevent the losses of targeted analytes and subsequent decrease of recoveries, most of methods use simple sample preparation procedures for isolation of target analytes without any specific purification steps.

In the few recent years, substantial developments have been done in the field of mass spectrometry enabling introduction of novel ambient desorption ionization techniques [10], such as direct analysis in real time (DART) [11], desorption electrospray ionization (DESI) [12], surface desorption atmospheric-pressure chemical ionization (DAPCI) [13] or atmospheric-pressure solids analysis probe (ASAP) [14]. These novel ion sources are characterized by remarkably high-throughput of analyses which can be carried out under ambient conditions without (chromatographic) separation of sample components prior to desorption/ionization or need of complicated and time demanding sample pre-treatment procedures. The DART technology, employed in this study, relies upon fundamental principles of atmospheric-pressure chemical ionization (APCI). Excited-state helium atoms produce reactive species for analyte ionization [11]. Numerous applications of DART ion source hyphenated with various types of mass spectrometers have been reported [15–25]. DART found its use in many areas of analytical chemistry as a tool for rapid qualitative analysis of diverse analytes. Due to relatively high signal fluctuation of ions intensities obtained by repeated DART measurements, internal standard has to be usually employed for compensation during quantitative analysis. However, implementation of Vapur™ gas ion separator and automatic sampling systems was reported to significantly improve the repeatability for some analytes [24].

In the current study, the possibility to use DART ion source coupled to an orbitrap-based (ultra)high resolution mass spectrometer for rapid control of a wide range of mycotoxins, potentially occurring in cereals, was investigated. To extract the target analytes, a simple procedure based on QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method [26] was employed. The data generated by DART–MS chromatographic separation-free approach were compared to those obtained by U-HPLC–TOFMS based method. To our best knowledge, this is the first study using a unique DART–orbitrapMS for detection/quantification of mycotoxins in plant matrices.

2. Experimental

2.1. Chemicals and standards

Standards of 3-acetyldeoxynivalenol (3-ADON), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-Glc), fusarenon-X (FUS-X), nivalenol (NIV), HT-2 toxin (HT-2), T-2 toxin (T-2), diacetoxyscirpenol (DAS), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), fumonisin B1 (FB1), fumonisin B2 (FB2), sterigmatocystin, zearalenone (ZEA), $^{13}C_{15}$-deoxynivalenol ($^{13}C_{15}$-DON), $^{13}C_{15}$-nivalenol ($^{13}C_{15}$-NIV) and $^{13}C_{18}$-zearalenone ($^{13}C_{18}$-ZEA) were supplied by Biopure (Tulln, Austria). Standards of deepoxy-deoxynivalenol (deepoxy-DON), altenuene, alternariol, alternariolmethylether (alternariol-met), ergocornine, ergocrystine and ergosine were obtained from Sigma–Aldrich (Steinheim, Germany).

To assess DART ionization of target mycotoxins, a composite standard acetonitrile solution containing each of analytes (isotope-labeled compounds not included) was prepared at concentration level of 5000 ng mL^{-1} and further diluted with acetonitrile to obtain solvent standards at 500 ng mL^{-1}. In later experiments, mixed acetonitrile standard (5000 ng mL^{-1}) containing 3-ADON, DON, FUS-X, NIV, DAS, sterigmatocystin, ZEA, deepoxy-DON, altenuene, alternariol and alternariol-met was used for preparation of matrix-matched calibration standards and spiking experiments. Individual solvent solutions of ^{13}C-labeled internal standards were prepared at 5000 μg mL^{-1} in acetonitrile. Matrix-matched standards in the concentration range from 10 to 1000 ng mL^{-1} (corresponding to 50–5000 μg kg^{-1} in matrix) were obtained by spiking of blank wheat and maize extracts (prepared by procedures described below), additionally, isotopically labeled compounds were added at level 100 ng mL^{-1} (500 μg kg^{-1} in matrix).

Acetonitrile and methanol, both of HPLC-grade, were supplied by Merck (Darmstadt, Germany). Pure water was obtained from Milli-Q purification system (Millipore, Bedford, MA, USA). Anhydrous magnesium sulphate, sodium chloride and ammonium formate (≥99% purity), were from Sigma–Aldrich (Steinheim, Germany). Primary secondary amine (PSA) sorbent was obtained from Varian (Harbor City, CA, USA); formic acid (≥98% purity) was from AppliChem GmbH (Darmstadt, Germany).

2.2. Samples and sample preparation

Modified QuEChERS procedure [26] was employed to extract target analytes from the examined matrices (wheat, maize and millet). 2 g of homogenized sample was weighed into a 50 mL polypropylene (PP) centrifuge tube, 7.5 mL of deionized water and 10 mL of acetonitrile were added. Vigorous shaking of the mixture (4 min) was followed by the addition of 4 g $MgSO_4$, 1 g NaCl, further shaking for 3 min and centrifugation (5 min, 10,000 rpm, 20 °C). 4 mL aliquot of the upper organic phase was transferred into a 15 mL PP tube containing 200 mg of PSA and 600 mg $MgSO_4$ and shaken for 3 min to perform solid phase extraction (SPE) clean-up of the extract. After centrifugation (3 min, 10,000 rpm, 20 °C), approx. 600 μL were taken for DART–orbitrapMS analysis. Prior to U-HPLC–TOFMS measurements, the extract was passed through a 0.2 μm filter and diluted with equal volume of water.

The validation of DART–MS method was performed with the use of blank wheat and maize samples which were pre-fortified with target mycotoxins (at levels 150 and 500 μg kg^{-1}, $n = 5$) and ^{13}C-labeled analogues available for DON, NIV and ZEA (500 μg kg^{-1}). After thorough mixing, the spiked matrix was incubated for 30 min prior to extraction. For additional quality control of obtained data, following certified reference materials (CRMs) were examined by DART–orbitrapMS and validated U-HPLC–TOFMS method: (i) CRM of maize flour with DON content (474 ± 30 μg kg^{-1}) from Biopure (Tulln, Austria); (ii) CRM of wheat flour containing DON (2800 ± 200 μg kg^{-1}) supplied by R-Biopharm (Darmstadt, Germany); (iii) CRM of ground millet containing ZEA (648 ± 140 μg kg^{-1}) provided by Institute of Reference Materials and Measurement (Geel, Belgium) and (iv) CRM of maize flour with ZEA (60 ± 9 μg kg^{-1}).

2.3. DART–orbitrapMS

DART–MS system used in this study consisted of a new commercial model of DART ion source (DART-SVP) with a 12 Dip-It™ tip scanner autosampler (IonSense, Saugus, MA, USA) coupled to the Exactive™ benchtop mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). 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 use of Vapur™ gas ion separator during DART ionization was essential

in order to maintain stable vacuum within the operating pressure limits of the Exactive™ instrument. Vapur™ interface also improved transport efficiency of ions from the sampling area to the atmospheric-pressure interface inlet of the mass spectrometer, thus enhancing both sensitivity and reproducibility of the measurement. The distance between the exit of the DART gun and the ceramic transfer tube of the Vapur™ was set to 10 mm, the gap between the ceramic tube and the inlet to the heated capillary of the Exactive™ was 2 mm.

DART–MS instrument was operated either in positive or negative ionization mode, optimized settings of the system parameters were as follows: (i) DART positive ionization: helium flow: 2.5 L min^{-1}; gas temperature: 350 °C; discharge needle voltage: −5000 V; grid electrode: +200 V; (ii) DART ionization negative ionization: helium flow: 2.5 L min^{-1}; gas temperature: 350 °C; discharge needle voltage: −5000 V; grid electrode: +350 V; (iii) mass spectrometric detection: capillary voltage: ±55 V; tube lens voltage: ±130 V; capillary temperature: 250 °C. Sheath, auxiliary and sweep gases were disabled during DART–MS analysis. The acquisition rate was set according to desired resolving power of the orbitrap mass analyzer, and was 10 spectra s^{-1} at 10,000 FWHM (full width at half maximum), 4 spectra s^{-1} at 25,000 FWHM and 2 spectra s^{-1} at 50,000 FWHM. In all cases, the mass resolving power was calculated for *m/z* 200.

Semi-automatic analysis of liquid samples was carried out with the use of 12 Dip-It™ tip scanner autosampler. Dip-It™ tips (IonSense, Saugus, MA, USA) were inserted into a holder and immersed in sample extracts placed in deepwell micro-plate (Life Systems Design, Merenschwand, Switzerland). The holder was mounted on the body of the autosampler. Subsequently, the Dip-It™ tips automatically moved at a constant speed of 0.5 mm s^{-1} through the helium gas beam in perpendicular direction to the axis leading from DART gun exit to the mass spectrometers inlet. Using the above moving speed, the time of desorption from the surface of each tip was 9 s; total run time of 12 analyses was approx. 4.2 min. To enable and/or enhance ionization of certain analytes, 2 mL autosampler vial containing dopant solution was placed in the distance of 20 mm from the DART gun exit. Aqueous solution of ammonia (25%, w/w, Penta, Chrudim, Czech Republic) and neat dichloromethane (Scharlau, Barcelona, Spain) were used in positive and negative ionization mode, respectively.

2.4. U-HPLC–TOFMS

For the critical assessment/comparison of new method performance characteristics, U-HPLC–TOFMS method employing Acquity™ Ultra Performance LC system equipped by Acquity™ UPLC HSS T3 column for sample separation and LCT Premier XE TOF (Waters, Bedford, MA, USA) mass spectrometer (resolving power of 12,000 FWHM) with electrospray ionization for analytes detection was used. More details are available in our recent paper [27].

3. Results and discussion

3.1. DART ionization of mycotoxins

In the initial phase of this study, the efficiency and practical applicability of DART technology for ionization of aflatoxins, *Fusarium* toxins, *Alternaria* toxins, ochratoxins, ergot alkaloids, and sterigmatocystin (analytes possessing relatively largely differing physico-chemical properties) was evaluated. For this purpose, solvent standards (acetonitrile) containing respective mycotoxin at level 500 ng mL^{-1} were analyzed (when considering sample preparation procedure described in Section 2, this corresponds to a relatively high concentration in matrix – 2500 μg kg^{-1}). In the first step, various settings of ionization gas temperature (100–400 °C), which represents one of the key parameters influencing the outcome of DART–MS analysis, were tested. The aim was to achieve as much as possible efficient thermo-desorption of analytes. As shown below, most mycotoxins could be transferred into gaseous phase at temperature 350 °C which was found as an optimal compromise between signal intensity and analytes thermal desorption speed (when this process is too fast, the number of points per desorption peak might be insufficient). Another optimized DART parameter was the grid electrode voltage. While the use of lower voltage (200 V) in positive ionization mode enabled approx. 50% intensity increase compared to 350 V setting, 350 V potential was optimal for analytes ionized in negative mode. In line with several studies concerned with ionization processes occurring in DART ion source [11,23], the benefit for ionization of some mycotoxins resulting from introducing dopant vapours (dichloromethane or ammonia) into the region between the ion source exit and Vapur™ interface ceramic tube inlet was demonstrated. Searching for target analyte ions in DART mass spectra was based on compliance of accurately measured masses and with their expected elemental composition; for confirmation, isotope patterns were taken into consideration. In following paragraphs, experimental data are presented in detail.

The list of ions generated by DART, when analyzing mycotoxin standard solutions, is provided in Table 1. As can be seen, most of the examined type B trichothecenes (3-ADON, DON, deepoxy-DON, FUS-X and NIV) could be effectively ionized in negative ion mode, forming anion adducts supposing dichloromethane vapours were present in the ionization region. No deprotonation of their molecules (process otherwise typical for DART ionization) took place, only signals corresponding to ions $[M+Cl]^-$ accompanied by the M+2 mass spectral peaks characteristic for chlorine-containing ions (see Fig. 1.), were originated. Unfortunately, the DON-3-Glc, a polar (poorly volatile) metabolite formed via glycosylation of DON did not provide detectable signal under any experimental conditions employed in this study. Contrary to type B trichothecenes, tested toxins of type A group were ionized in a positive polarity mode, yielding either single $[M+NH_4]^+$ adduct ion (HT-2, T-2) or both $[M+NH_4]^+$ and $[M+H]^+$ (DAS). Again, the presence of dopant vapours (aqueous ammonia in this case) was needed to assist the DART ionization. The intensities of HT-2 and T-2 toxin adduct ions were low thus making the detection of their trace levels in matrix samples rather difficult. On the other hand, DAS, thanks to intensive ionization yield, could be detected easily.

DART ionization of *Alternaria* toxins (altenuene, alternariol and alternariol-met) was enabled in negative mode, $[M-H]^-$ ions of analytes were formed. Additionally, $[M+Cl]^-$ adducts occurred in the presence of dichloromethane. While deprotonated molecules of alternariol and alternariol-met were approximately four times more intensive compared to corresponding chlorine adducts, altenuene yielded respective ions in an inverse intensity ratio. Analogous ions types, $[M-H]^-$ and $[M+Cl]^-$, were obtained also for ZEA.

Aflatoxins, another group of investigated compounds, were represented by AFB1, AFB2, AFG1 and AFG2. Unfortunately, poor ionization efficiencies were obtained under positive DART mode setting for all these very hazardous mycotoxins, no negative ions were formed. The intensity of $[M+H]^+$ ions declined in order AFB2 > AFB1 ≫ AFG1 ∼ AFG2. Poor efficiency of aflatoxins ionization by DART (APCI-like ionization technique) complies to results of several studies concerned with sensitivity of LC–MS analyses of these natural contaminants. Compared to APCI [10,11], electrospray ionization (ESI) [28,29] was documented to be the much better option for their control at ultra-trace levels which are of regulatory of interest (due to their high toxic potential maximum limits are in the range 0.0025–15.000 μg kg^{-1}, depending on matrix). On the other hand, sterigmatocystin, in spite of structural similarity with aflatoxins, is known to provide better sensitivity with an APCI interface

Table 1
Overview of most intensive mycotoxins ions detected under optimized DART–orbitrapMS conditions in solvent standard (500 ng mL^{-1}) and matrix effects observed during analysis of spiked wheat extract (500 ng mL^{-1}); expressed as % of analyte response in net solvent.

Compound	Elemental formula	Exact MW	Ionization mode	Detected ions			Average intensity in solvent standard (1×10^5 counts)	Matrix effect (%)[b]
				Ion	Elemental composition	Exact mass		
ADON	C17H22O7	338.1360	Negative	$[M+Cl]^-$	C17H22O7Cl	373.1049	47.9	19.0
DON	C15H20O6	296.1254	Negative	$[M+Cl]^-$	C15H20O6Cl	331.0943	31.0	39.0
Deepoxy-DON	C15H20O5	280.1305	Negative	$[M+Cl]^-$	C15H20O5Cl	315.0993	38.3	20.9
FUS-X	C17H22O8	354.1309	Negative	$[M+Cl]^-$	C17H22O8Cl	389.0998	14.6	11.6
NIV	C15H20O7	312.1204	Negative	$[M+Cl]^-$	C15H20O7Cl	347.0903	68.9	26.0
ZEA	C18H22O5	318.1462	Negative	$[M-H]^-$	C18H21O5	317.1394	27.3	17.9
				$[M+Cl]^-$	C18H22O5Cl	353.1150	10.4	20.2
DON-3-Glc	C21H30O11	458.1783	–	–	–	–	n.d.[a]	n.e.[c]
HT-2	C22H32O8	424.2092	Positive	$[M+NH_4]^+$	C22H36NO8	442.2435	6.9	n.e.[c]
T-2	C24H34O9	466.2197	Positive	$[M+NH_4]^+$	C24H38NO9	484.2541	11.6	n.e.[c]
DAS	C19H26O7	366.1673	Positive	$[M+H]^+$	C19H27O7	367.1750	4.9	26.5
				$[M+NH_4]^+$	C19H30NO7	384.2017	47.4	27.0
Altenuene	C15H16O6	292.0941	Negative	$[M-H]^-$	C15H15O6	291.0874	5.7	24.6
				$[M+Cl]^-$	C15H16O6Cl	327.0630	35.2	25.0
Alternariol	C14H10O5	258.0523	Negative	$[M-H]^-$	C14H9O5	257.0455	93.2	23.0
				$[M+Cl]^-$	C14H10O5Cl	293.0211	15.0	12.7
Alternariol-met	C15H12O5	272.0679	Negative	$[M-H]^-$	C15H1105	271.0612	48.1	26.0
				$[M+Cl]^-$	C15H12O5Cl	307.0368	4.1	24.4
AFB1	C17H12O6	312.0628	Positive	$[M+H]^+$	C17H13O6	313.0712	1.8	n.e.[c]
AFB2	C17H14O6	314.0785	Positive	$[M+H]^+$	C17H15O6	315.0868	2.4	n.e.[c]
AFG1	C17H12O7	328.0578	Positive	$[M+H]^+$	C17H13O7	329.0661	0.2	n.e.[c]
AFG2	C17H14O7	330.0734	Positive	$[M+H]^+$	C17H15O7	331.0712	0.3	n.e.[c]
Sterigmatocystin	C18H12O6	324.0628	Positive	$[M+H]^+$	C18H13O6	325.0707	64.2	23.1
OTA	C20H18ClNO6	403.0817	–	–	–	–	n.d.[a]	n.e.[c]
FB1	C34H59NO15	721.3879	–	–	–	–	n.d.[a]	n.e.[c]
FB2	C34H59NO14	705.3930	–	–	–	–	n.d.[a]	n.e.[c]
Ergocornine	C31H39N5O5	561.2946	–	–	–	–	n.d.[a]	n.e.[c]
Ergocristine	C39H39N5O5	657.2946	–	–	–	–	n.d.[a]	n.e.[c]
Ergosine	C30H37N5O5	547.2789	–	–	–	–	n.d.[a]	n.e.[c]

[a] Signal not detected (n.d.).
[b] Matrix effects in extract cleaned with PSA 50 mg mL^{-1}.
[c] Not estimated (n.e.)

[28]. In line with that fact, sterigmatocystin was effectively ionized and detected as $[M+H]^+$ by the DART–orbitrapMS.

No protonated/deprotonated molecules, their adduct or fragment ions were obtained under tested conditions for other mycotoxins (OTA, FB1, FB2, ergocornine, ergocrystine and ergosine) in net solvent. Similarly to DON-3-Glc, also these compounds are relatively polar, and, especially in case of fumonisins and ergot alkaloids, have relatively high molecular weight (MW), what may hamper a transfer of such analytes into gaseous phase. Of course, polarity and MW are not the only factors influencing the DART ionization of target analyte. For example, NIV and AFB1 are both polar and have similar MW; nevertheless, the latter one provides much lower signal (see Table 1). Unfortunately, fairly incomplete current knowledge on processes occurring during DART ionization does not allow us to explain these phenomena. In general, considering the analogy between DART and APCI techniques, our experimental results could be linked to behaviour of the above analytes under ESI/APCI conditions. While DART-amenable mycotoxins are well ionized in APCI, for the 'problematic' compounds, ESI was reported to be much more suitable ionization technique [29]. To facilitate and/or enhance DART ionization of troublesome mycotoxins, derivatization of polar functional groups, which enables avoiding hydrogen bonding, may represent a conceivable strategy. This approach (trimethylsilylation in particular case) has been successfully employed in the recent study by Zhou et al. [30] in order to enable DART ionization of some metabolites occurring in biological samples.

Taking into account the DART ionization feasibility (shown in Table 1), occurrence in food crops and maximum limits of mycotoxins established in Regulation No 1881/2006 (EC) and its amendments, further optimization and validation experiments were carried out only for 11 selected analytes (DON, NIV, ZEA, 3-ADON, deepoxy-DON, FUS-X, altenuene, alternariol, alternariol-met, DAS and sterigmatocystin).

3.2. Optimization of sample preparation procedure and mass spectrometer settings

To isolate rapidly multiple mycotoxins from tested cereals with satisfactory recoveries and to discriminate co-isolation of potentially interfering matrix as much as possible, modified QuEChERS-like procedure was employed. The target analytes present in a crude aqueous-acetonitrile extract were transferred during phases separation induced by anhydrous inorganic salts ($MgSO_4$ and NaCl added), into upper organic layer, while most of matrix components (mainly polar ones) were left in a bottom aqueous phase. However, some portion of (in crude extract abundant) matrix was, unavoidably, co-isolated into acetonitrile layer and interfered with DART ionization of target mycotoxins, signal suppression was very serious for most of them. To reduce, at least partly, such adverse phenomena, dispersive SPE clean-up utilizing PSA and $MgSO_4$ had to be employed. In this way, most of residual sugars, fatty and other organic acids could be removed. In Fig. 2, the improvement of DON signal (m/z 331.0943) in dependence on the amount of added PSA sorbent (0–100 mg/mL of extract) is illustrated. Considering sorbent cost/matrix effect decrease ratio, addition 50 mg PSA per 1 ml of acetonitrile extract (containing 200 mg matrix equivalent) was found as optimal. As shown in Table 1, under these conditions, the signal intensities of mycotoxin ions were in the range 11.6–39.0% intensity compared to

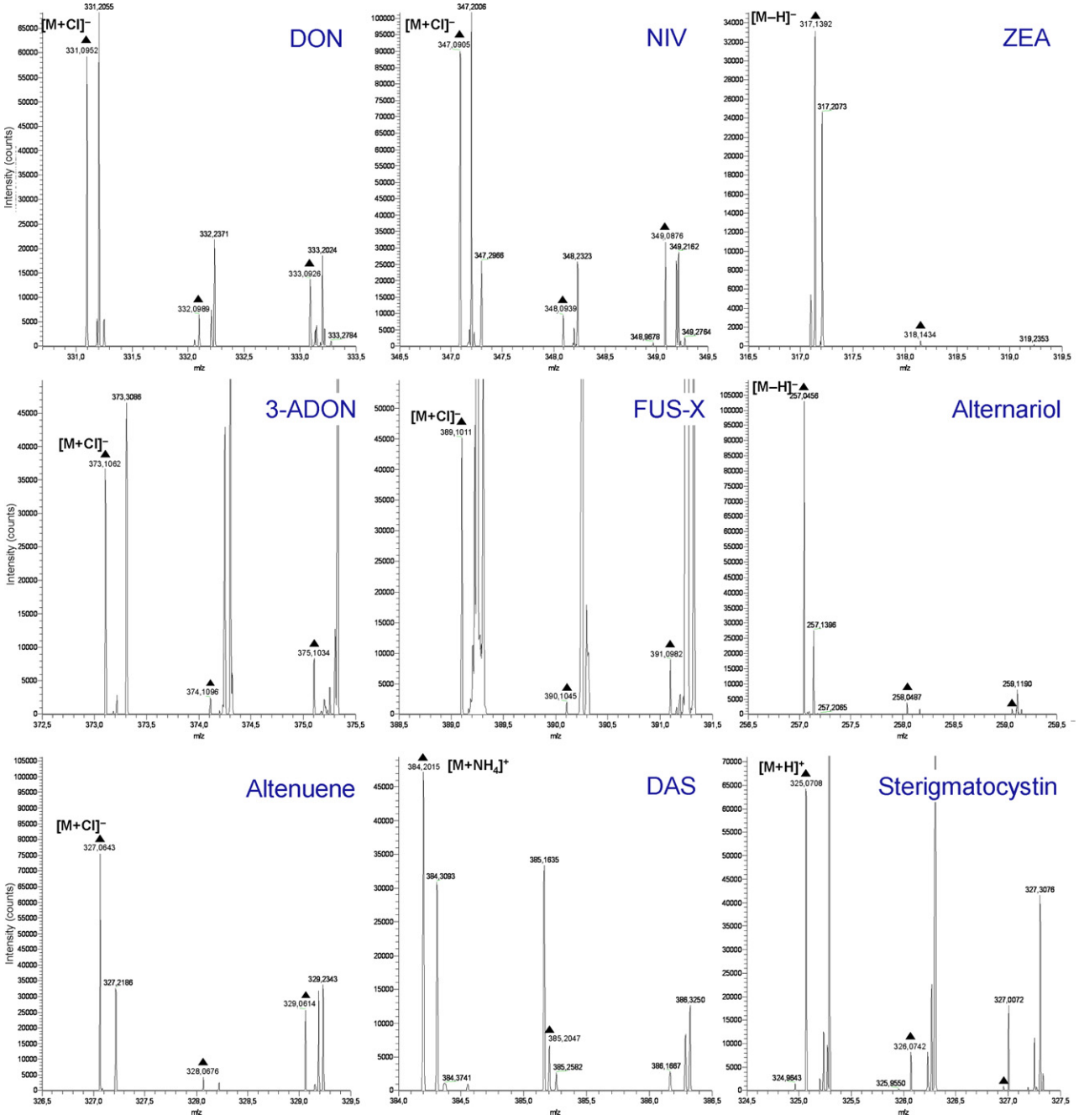

Fig. 1. Mass spectra of examined mycotoxins obtained by DART–orbitrapMS analyses of wheat extract (spike 500 μg kg^{-1}) at mass resolving power setting 50,000 FWHM. (▲) Ions yielded by target analytes.

those measured in net solvent. Worth to notice, matrix effects were more severe in DART–orbitrapMS compared to LC–MS methods which are commonly used for mycotoxins analysis. The extent of ion suppression in the latter approach was shown to be matrix-dependent [3,27], e.g. pronounced differences were documented between wheat and maize, obviously due to different nature of co-eluting matrix components. Contrary to that, in this study, the intensity of target analytes ionization was comparable for both tested matrices, wheat and maize. We assume this complies to almost identical total ion current, that was recorded for respective extracts, in spite of differences in co-extracts pattern observed in DART–orbitrapMS spectra (mainly pronounced in negative mode).

As documented in Fig. 1, numerous ions of matrix components with *m/z* values very close to both ions and isotope ions of analytes were present in the mass spectra of purified sample extracts. To achieve accurate mass measurement needed for unbiased identification and to ensure reliable quantitation, high resolving power of the instrument was utilized. The Exactive^{TM} mass spectrometer employed in our study enabled acquisition under four different mass resolving power settings, ranging from 10,000 to 100,000 FWHM. While the sensitivity of measurement was not influenced by the selected mass resolving power, this parameter defined the acquisition rate. Based on performed experiments, resolving power 50,000 FWHM was chosen optimal for the analyses, as it offered both satisfactory resolution of analyte/interference spectral peaks and reasonable acquisition rate (2 spectra s^{-1}). Since the typical width of the 'desorption' peak of target mycotoxins at lowest calibration levels (LCLs, discussed below) was no less than 6 s, min-

Table 2
Performance characteristics obtained by DART–orbitrapMS repeated analyses ($n = 5$) of wheat and maize spiked by selected *Fusarium* toxins, *Alternaria* toxins and sterigmatocystin at two concentration levels.

Compound	LCL[a] ($\mu g\,kg^{-1}$)	Repeatability and recovery experiments				
		Matrix	Spiking level ($\mu g\,kg^{-1}$)	Quantification based on: external calibration/isotope dilution		
				Determined value ($\mu g\,kg^{-1}$)	Repeatability (RSD, %)[b]	Recovery (%)
DON	80	Wheat	150	173/162	11.4/8.6	115/108
			500	561/532	8.2/6.1	112/106
		Maize	150	167/143	12.0/9.0	111/95
			500	583/479	9.9/5.6	116/96
NIV	70	Wheat	150	159/149	11.1/7.2	106/99
			500	519/476	8.5/5.4	118/104
		Maize	150	170/144	10.6/9.8	113/96
			500	521/501	7.9/6.0	104/100
ZEA	100	Wheat	150	139/143	14.3/7.6	93/95
			500	578/501	11.2/5.6	116/100
		Maize	150	166/154	12.1/8.1	110/102
			500	563/541	10.0/6.9	113/104
ADON	90	Wheat	150	142/–	9.9/–	92/–
			500	446/–	8.3/–	89/–
		Maize	150	168/–	11.5/–	112/–
			500	544/–	9.3/–	108/–
Deepoxy-DON	90	Wheat	150	169/–	8.5/–	113/–
			500	569/–	12.0/–	114/–
		Maize	150	136/–	11.3/–	91/–
			500	584/–	8.9/–	117/–
FUS-X	150	Wheat	150	172/–	16.5/–	115/–
			500	571/–	8.4/–	114/–
		Maize	150	180/–	15.9/–	120/–
			500	536/–	9.5/–	107/–
DAS	80	Wheat	150	129/–	13.2/–	86/–
			500	435/–	9.0/–	87/–
		Maize	150	123/–	12.3/–	82/–
			500	450/–	7.9/–	90/–
Altenuene	90	Wheat	150	165/–	12.1/–	110/–
			500	513/–	8.1/–	103/–
		Maize	150	139/–	13.6/–	93/–
			500	477/–	9.8/–	95/–
Alternariol	50	Wheat	150	133/–	11.0/–	89/–
			500	434/–	8.5/–	87/–
		Maize	150	129/–	9.7/–	86/–
			500	427/–	8.2/–	85/–
Alternariol-met	80	Wheat	150	125/–	12.0/–	83/–
			500	420/–	9.9/–	84/–
		Maize	150	133/–	9.2/–	89/–
			500	444/–	8.4/–	89/–
Sterigmatocystin	80	Wheat	150	148/–	11.8/–	99/–
			500	530/–	11.0/–	106/–
		Maize	150	139/–	13.0/–	92/–
			500	539/–	9.5/–	108/–

[a] Lowest calibration levels (LCLs).
[b] Relative standard deviation (RSD) calculated from 5 repeated analyses.

imum 12 points per peak were recorded. In case resolving power of 100,000 FWHM was set, the number of scans across the peak was lower than 9, thus not sufficient for quantitation. Employing 50,000 FWHM resolving power, good mass accuracy, with mass errors in the range from −3.6 to +3.8 ppm, was routinely obtained for tested compounds in examined samples, including extracts spiked at LCLs.

3.3. Quantitative analysis

Worth to notice, that most of published studies in the field of ambient mass spectrometry have employed DART–MS technique mainly for screening/profiling purpose, only few of them have documented its use for quantification of target analyte(s) considering general requirements for quality parameters of obtained results [31]. To fill in this gap, in the final phase of our study, we focused on demonstration of possible use of DART–orbitrapMS technique for reliable direct quantitative control of some mycotoxins in wheat and maize extracts prepared in a simple, quick way. For quantitative purposes, the most abundant ions yielded by respective mycotoxins (see Table 1) were used. With regard to achieved mass accuracy at resolving power setting 50,000 FWHM, narrow isolation window of 4 ppm could be employed to extract ion records (chronograms) of target analytes with high selectivity. For evaluation of repeatability (expressed as relative standard deviation, RSD, $n = 5$) of DART–orbitrapMS method, peak areas were preferred since they were shown to give better results compared to calculations based on peak heights. Typical RSDs for cereals spiked by mycotoxins at 500 $\mu g\,mL^{-1}$ level ranged from 8.1% to 14.3%, depending on respective compound, see Table 2. Further decrease of RSDs (4.7–8.7%) and improved linearity of calibration plots compared to external calibration, was obtained when

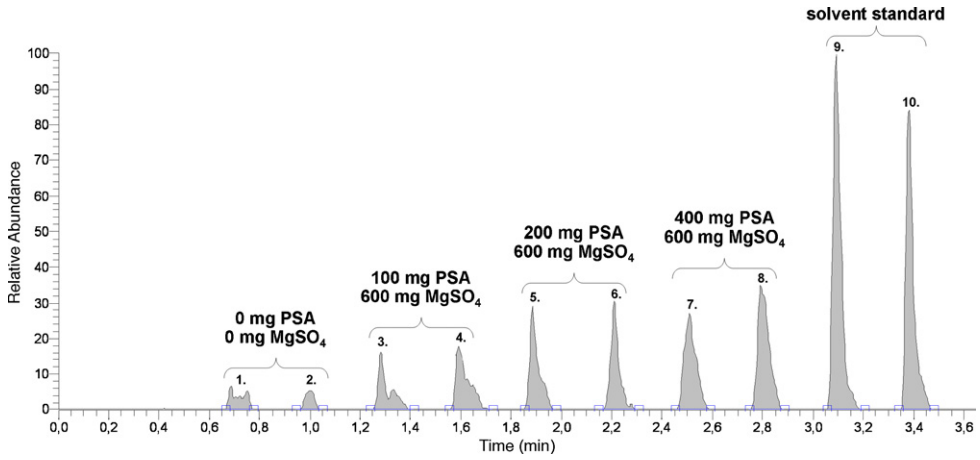

Fig. 2. The impact of dispersive SPE clean-up employing PSA and $MgSO_4$ on DON (m/z 331.0943 ± 4 ppm) signal intensity in wheat extract (spike 500 μg kg^{-1}). Given sorbent amounts were used for 4 ml of acetonitrile extract containing equivalent 800 mg of matrix; solvent standard concentration was 100 ng mL^{-1}.

isotopically labeled internal standards were employed for compensation of absolute signal fluctuation (Fig. 3). Regardless the use of isotope dilution technique for calibration, regression coefficients >0.99 were obtained for all 11 target analytes (measured in the range 50–5000 μg kg^{-1}). To characterize sensitivity of measurements, LCLs (concentrations which could be routinely detected and quantified within the long term period) were estimated, see Table 2. The common calculation of detection (LODs) and quantification (LOQs) limits based on signal-to-noise ratio could not be employed, as under narrow extraction window setting (4 ppm in particular case) no chemical noise was present in the record. Needed to emphasize that in case of regulated mycotoxins (DON, ZEA), DART–MS method LCLs allowed a reliable control of maximum limits established for tested matrices [1]. The recoveries of all target mycotoxins at both tested spiking levels 150 and 500 μg kg^{-1} were in the range 82–120% when external calibration based on matrix-matched standards was employed for quantification. Regarding the requirements for performance characteristics in analysis of regulated analytes [32], these were reliably met for both DON and ZEA.

The trueness of data obtained for DON and ZEA by DART–orbitrapMS based method was demonstrated by analysis of available certified reference materials (CRMs) containing incurred toxins, the results are presented in Table 3. As shown here, only slightly worse repeatability of measurement (expressed as RSD) was achieved when examining CRM extracts by earlier validated accurate U-HPLC–TOFMS method [27]. However, the benefit of rapid measurement enabled by ambient mass spectrometry was

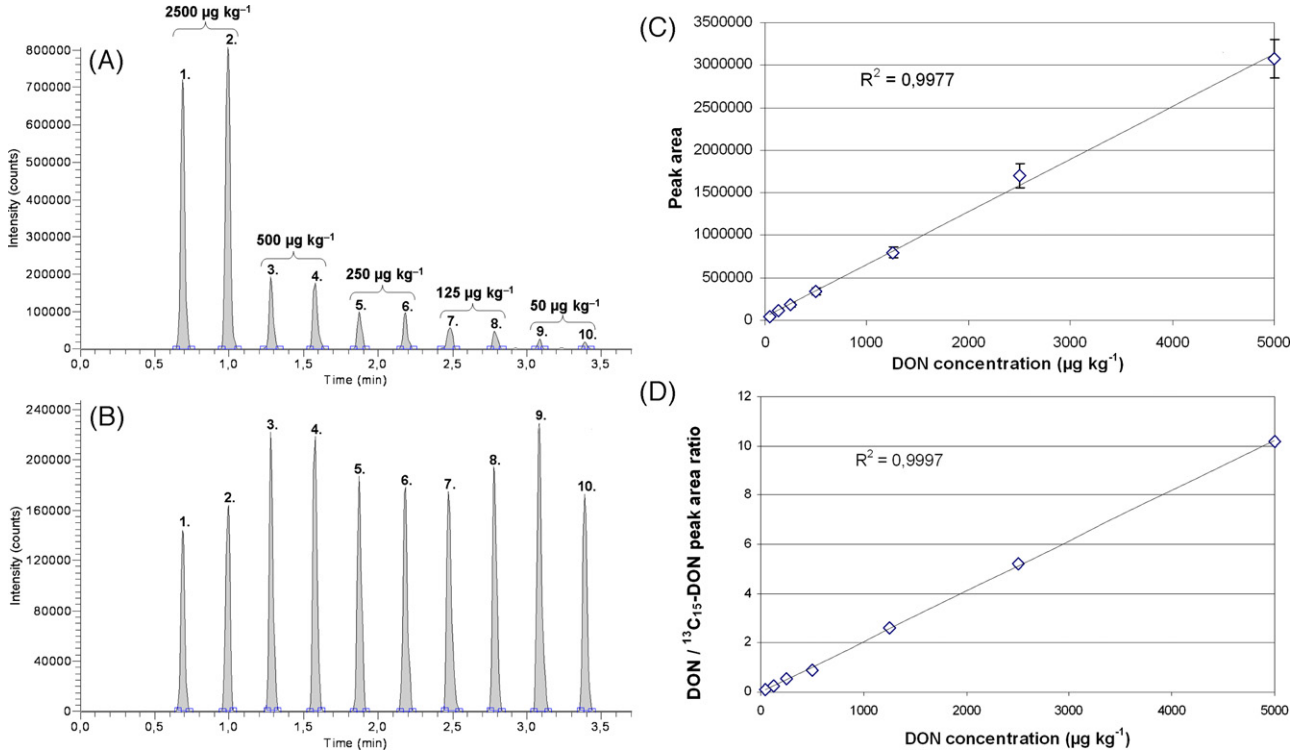

Fig. 3. Calibrations employing matrix-matched standards and isotope dilution. (A) Extracted target ion record: DON (m/z 331.0943 ± 4 ppm), concentration in the range 50–2500 μg kg^{-1}; (B) extracted target ion record: $^{13}C_{15}$-DON (m/z 346.1446 ± 4 ppm), concentration 500 μg kg^{-1}; (C) external calibration curve. (D) Isotope dilution calibration curve. Error bars are standard deviations calculated from three repeated injections.

Table 3
Comparison of trueness of data obtained by DART–orbitrapMS and U-HPLC–TOFMS analysis of certified reference materials.

Material description	Analyte (assigned/certified value)	DART–orbitrapMS External calibration/isotope dilution		U-HPLC–TOFMS[c]	
		Mean ($\mu g\,kg^{-1}$)	RSD (%)[a]	Mean ($\mu g\,kg^{-1}$)	RSD (%)[a]
CRM, maize flour	DON ($474 \pm 30\ \mu g\,kg^{-1}$)	459/486	9.0/5.9	500	4.1
CRM, wheat flour	DON ($2800 \pm 200\ \mu g\,kg^{-1}$)	2608/2819	6.7/5.4	2754	2.2
CRM, ground millet	ZEA ($648 \pm 140\ \mu g\,kg^{-1}$)	583[c]/613	7.5/6.0	637	3.4
CRM, maize flour	ZEA ($60 \pm 9\ \mu g\,kg^{-1}$)	<LCL[b]	–/–	55	5.5

[a] Relative standard deviation (RSD) calculated from 3 analyses.
[b] The concentration of analyte was below LCL of the method.
[c] Quantification was performed using wheat matrix-matched standards.

accompanied by the loss of sensitivity due to (unseparated) matrix interferences.

4. Conclusions

The results obtained within the comprehensive study concerned with critical assessment of DART–orbitrapMS potential to detect/quantify mycotoxins, that may occur in food matrices are summarized below:

(i) Of 24 tested mycotoxins, 11 target analytes could be efficiently ionized by DART technology. The ionization yield in QuEChERS-like wheat/maize extract was growing in order ZEA ~ FUS-X < 3-ADON ~ altenuene < DON ~ deepoxy-DON ~ alternariol-met ~ DAS < sterigmatocystin < NIV < alternariol. With an exception of ZEA, alternariol, alternariol-met and sterigmatocystin, adduct ions obtained by dopant introduction were the most intensive ($[M+Cl]^-$ for 3-ADON, DON, deepoxy-DON, FUS-X and altenuene; $[M+NH_4]^+$ for DAS). The matrix effects due to these ions suppression by co-isolated matrix were in the range 11.6–39.0% (the signal of standards in net solvent = 100%).

(ii) Only poor ionization of major trichothecenes A (T-2, HT-2) and some of tested aflatoxins (AFB1, AFB2) was achieved by DART. The ionization of OTA and other mycotoxins such as ergot alkaloids, fumonisins, and DON-3-Glc was not possible under employed experimental conditions. The derivatization procedure, which has to be rapid and reproducible, may represent a conceivable option to cope with, in this context, 'troublesome' mycotoxins.

(iii) In spite of narrower analyte scope and rather lower sensitivity compared to established LC–MS methods, DART–orbitrapMS represents a rapid, flexible, and easy to use approach. It was shown to be applicable for high-throughput control of maximum limits of ZEA and DON established in EU regulation for unprocessed wheat/maize. Regarding the latter major *Fusarium* toxin, this technique allows also control of its maximum limits in products prepared from these cereals. Comparable trueness of generated results were achieved by isotope dilution-based quantification and that employing matrix-matched calibration what documents good reproducibility of ions transfer from ionization region into MS detector (facilitated by automated sampler and gas ion separator).

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