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High-performance liquid chromatography–atmospheric-pressure chemical ionization mass spectrometry as a new tool for the determination of the mycotoxin zearalenone in food and feed

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Abstract

A new method for the determination of the mycotoxin zearalenone (ZON) in food and feed, based on HPLC–MS with an atmospheric-pressure chemical ionization (APCI) interface after extraction from cereals and clean-up by either conventional solid-phase or immunoaffinity cartridges is presented. The APCI interface parameters are optimized to provide detection of ZON with maximum sensitivity after RP separation of ZON on a C_{18} column with acetonitrile–water (40:60, v/v) at 1 ml/min column flow without split. Using APCI-MS detection, the sensitivity of the method was improved by a factor of ca. 50 in comparison to HPLC with fluorescence detection, allowing determination of ZON down to 0.12 μg/kg maize which is well below present threshold values. Due to the selectivity of MS detection, it also was possible to quantitatively determine ZON both in raw extracts without clean-up using a normal-size (100 mm) chromatographic column or using only a short (20 mm) chromatographic column, when a clean-up was done to minimize possible interferences.

Keywords: Mycotoxins; Food analysis; Zearalenone

1. Introduction

Mycotoxins are generally considered to be toxic compounds that are produced by certain types of fungi which grow on plants of agricultural importance either before harvest or during storage [1]. Some of them (such as the aflatoxins) exhibit an extraordinary toxicity so that they are of special concern and consequently require regular monitoring in plant material destined for human consumption or as animal feed.

Zearalenone (ZON) [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic-acid-lactone] (Fig. 1a), is

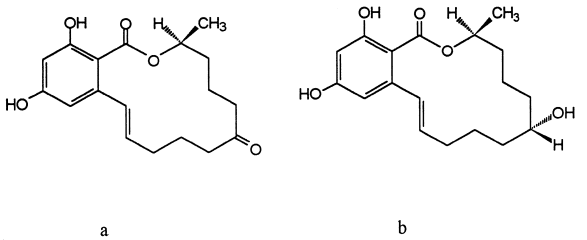

Fig. 1. Structures of zearalenone (a) and α-zearalenol (b).

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a secondary fungal metabolite that is produced by several *Fusarium* species, mainly *Fusarium graminearum* and *Fusarium culmorum*, which can infect corn, barely, oats, wheat and sorghum [2]. Of the different ZON derivatives that can be produced by *Fusarium* species, only *trans*-α-zearalenol [6-(10-hydroxy-6-*R*-hydroxy-*trans*-1-undecenyl) - β - resor - cyclic-acid-lactone] (α-ZOL), (Fig. 1b), has been observed to occur in naturally contaminated cereal grains [3]. Although its acute toxicity is considered to be much less than that of the aflatoxins [4], ZON is of concern due to its remarkable estrogenic activity and anabolic properties that have been discovered in rats, mice, poultry and swine. ZON and its derivatives can lead to hyperestrogenism and severe reproductive and infertility problems in animals, especially in swine [5]. Additionally, bioassays have indicated a carcinogenic activity for ZON. Further studies have to be carried out to decide whether ZON has to be considered a potential human carcinogen as well [6]. Based on its high biological activity and the frequency of its occurrence especially in wheat and maize, five countries (Austria, Brazil, France, Romania, Russia) have already established guideline or maximum tolerance values for ZON in food and in cereal products for human and animal consumption ranging from 30 to 1000 μg/kg [7].

A number of methods are presently available for the determination of ZON in different matrices such as various foods and feedstuffs, animal tissues, blood and urine [8,9]. Among these, TLC [10–12] and GC [12–14] methods have found wide application, but LC-based methods are usually preferred for the determination of ZON, since they allow a more straightforward determination than GC-based methods due to the simpler clean-up and they are more sensitive and specific than comparable TLC methods.

The most common methods of clean-up of the aqueous extracts are (a) liquid–liquid partitioning [15] and (b) solid-phase extraction on an aminopropyl column after an initial extraction step with basic acetonitrile and partitioning into dichloromethane [16]. Recently, a newly developed technique for the clean-up has been introduced which makes use of immunoaffinity columns (IACs) [17]. The use of the one-step clean-up with IACs employing an acetonitrile–phosphate buffer mixture represents a significant simplification compared to ''classical'' extraction methods that require tedious multiple extraction steps.

Furthermore, it provides extracts sufficiently clean to be amenable for HPLC with reversed-phase separation and direct fluorescence detection (FLD). Thus, HPLC–FLD has increasingly been used for the determination of ZON in different matrices [15,16,18]. Excitation is usually carried out at 275–280 nm and fluorescence emission is measured at 450–470 nm, allowing detection down to 2 ng ZON/g [18].

Electrochemical detection has also been used as a further approach for the detection of ZON and ZOL in corn [19], although not on a wider basis due to the detection method-inherent problems.

It is surprising to see that HPLC with MS detection has hardly been considered in this context despite its proven potential as a highly sensitive and specific detection technique in environmental [20] and food analysis [21]. Only few reports exist on the use of HPLC–MS for the determination of the various mycotoxins such as aflatoxins [22], trichothecenes [23–27] and other compounds [23,28]. In these reports, almost exclusive use of the thermospray interface for coupling HPLC and MS is made, since the thermospray interface can be considered the first interface that allowed coupling of HPLC–MS on a routine basis.

Since this initial work, HPLC–MS interfaces have undergone great development and significant improvement: after the interfaces of the first (direct liquid introduction, moving belt, particle beam) and second generation (thermospray, electrospray), a third generation of the so-called atmospheric-pressure ionization (API) interfaces is entering the market [29]. The API techniques which comprise both chemical ionization (atmospheric-pressure chemical ionization, APCI) and other ionization techniques (electrospray and ionspray) have opened a new window in HPLC–MS, allowing analysis of either low to medium polar analytes of low to medium molecular mass (APCI) or medium to highly polar analytes and ions preformed in solution of medium to high molecular mass (electrospray, ionspray). At the same time, the instruments have become smaller (usually of benchtop size), more rugged, compatible with the commonly used mobile phase flows and even with higher buffer concen-

trations [which allows direct transfer of a method from HPLC–UV, diode array detection (DAD) or FLD without adaptation] [30] without compromising the instruments' performance. Already these arguments would justify reconsidering HPLC–APCI-MS for the analysis of mycotoxins. In addition to this, the general applicability of HPLC–MS allows development of multimycotoxin methods which with the presently common detection methods (DAD, FLD) are either restricted due to their sensitivity or the lack of universal usability for the various mycotoxins.

Furthermore, the high selectivity of MS as compared to other detection techniques allows us to simplify and/or speed up the analysis of mycotoxins by (a) either simplifying the sample clean-up, leading eventually to the analysis of raw extracts without clean-up or (b) making use of a highly-specific extraction and clean-up step such as IAC and thus being able to reduce the degree of chromatographic separation required, leading finally to a flow-injection determination of the selectively isolated analytes.

In the following, HPLC–APCI-MS is applied for the first time to the determination of ZON in naturally contaminated and spiked corn samples. Instrumental parameters affecting the MS detection will be presented and the potential of HPLC–APCI-MS to increase sample throughput is discussed.

2. Experimental

2.1. Extraction and clean-up of ZON from corn samples

To obtain extracts from ZON-contaminated samples, 25.0 g of ground corn were homogenized and extracted with a mixture of 100 ml of acetonitrile (analytical grade, Baker, Gross-Gerau, Germany)–distilled water (75:25) with an Ultra Turrax T25 blender (IKA, Stauffen, Germany). After filtering through a Whatman GF/A filter, 80 ml of phosphate-buffered saline (PBS, pH=7.4) were added to 20 ml of the filtrate .

For the immunoaffinity clean-up, Easi-Extract IACs (Rhône Diagnostic Technologies, Glasgow, UK) were used. These were preconditioned with 15 ml of PBS and the diluted sample extract was applied to the IAC at a flow-rate of approximately 1.5 ml/min. After two washing steps each with 10 ml of distilled water, the column was dried under slight vacuum for ca. 5 min. ZON was eluted from the IAC with 5 ml of acetonitrile (HPLC grade, Donauchem, Vienna, Austria). After careful evaporation of the solution under a stream of nitrogen at 50°C, the residue was redissolved in 250 μl of acetonitrile–water (4:6, v/v) and filtered through a 0.22 μm membrane filter (Millex GV13, Millipore, Vienna) before HPLC analysis.

Alternatively, the extracts were purified by conventional solid-phase extraction using MycoSep multifunctional cleaning columns, type 224 (Romer, Union, MO, USA) for a simple ''one step'' clean-up. These columns consist of a mixture of different packing materials of both polar and apolar properties (charcoal, celite, alumina and ion-exchange resins) in a special format to provide an effective clean-up of the sample. For this type of clean-up, the corn extract is evaporated nearly to dryness. It is reconstituted in a mixture of acetonitrile–water (86:14) which is acidified by the addition of ca. 40 μl glacial acetic acid to 4 ml of the solution. Four ml of the acidified solution are transferred to a sample tube. Then the proprietarily designed clean-up column is inserted into the sample tube, forcing the raw extract to pass through the packing material. The purified extract (ca. 2 ml) can then be withdrawn from the layer on top of the packing material and directly injected into the HPLC system.

2.2. HPLC–MS analysis

Chromatographic separation and detection was carried out using a HP series 1100 HPLC–MS system (Hewlett–Packard, Waldbronn, Germany, and Palo Alto, CA, USA), consisting of a HP G1313A autosampler, a HP G1311A quaternary pump, a HP G1316A column thermostat set at 35°C and additionally equipped with a column-switching valve, a HP G1322A degasser unit, and a HP G1315A diode array UV–Vis detector coupled in series with the HP G1946A mass-selective detector, equipped with a HP G1947A APCI interface. DAD was performed at 192 nm (4 nm bandwidth) and at 220 nm (4 nm bandwidth) with a reference at 450 nm (80 nm

bandwidth), but yielded no signal that could be reasonably evaluated at the concentration levels investigated.

A Hypersil ODS analytical column of 100×2.1 mm and 5 μm particle diameter (Hewlett–Packard) was used for the chromatographic separation, preceded by a Hypersil ODS guard column (20×2.1 mm, 5 μm, Hewlett–Packard). Acetonitrile–water (40:60, v/v) was used as isocratic eluent at a column flow-rate of 1 ml/min. No split was applied when directing the eluent to the mass-selective detector. Different injection volumes in the range of 0.1 to 20 μl were used as indicated.

2.3. APCI interface design

The sensitivity of APCI-MS detection depends critically on the optimization of the detection parameters to a similar degree as is known for the thermospray interface [31]. They were thus optimized in a series of flow-injection analysis (FIA) sequences, both to find the most appropriate detection conditions and to characterize the interface's behaviour, the use of which has not been reported previously.

A scheme of the APCI interface is depicted in Fig. 2. A characteristic feature of this instrument is that the inlet coming from the HPLC system is neither directed on-axis nor off-axis (that is, inclined with a certain, small angle towards the ion optics axis) as this is the case for most other commercial APCI interface designs, but perpendicular to the ion optics axis. This prevents uncharged particles from entering the low-pressure region and finally the quadrupole and thus reduces significantly the background. The HPLC effluent enters the atmospheric-pressure chamber through a specially designed nebulizer needle which provides efficient nebulization under a wide range of column flows and solvent compositions. Evaporation of the solvent is further enhanced by heating the vaporizer to temperatures up to 400°C and by concurrently introducing a drying gas (up to 13 l/min) of nitrogen heated up to 350°C. The corona needle, situated perpendicular to the nebulizer needle, is kept at high potential resulting in a corona current typically in the low μA range, which supports ionization of the introduced molecules by the aid of the solvent molecules. The molecular and cluster ions are deflected and enter the first vacuum stage through an internally metal plated fused-silica capillary. The role of this capillary is first to create a molecular leak between the API chamber and the first vacuum stage and second to already initiate declustering of the solvent–analyte-cluster ions. Further declustering and partial fragmentation (the collision-induced dissociation, CID) can be achieved in the CID region, which is the region between the end of the transfer capillary and the first skimmer to which a voltage in the range of typically 10 to 100 V is applied. The ions that enter the high vacuum of the mass analyzer compartment through a second skimmer are further focused through an octapole mass filter. This octapole filter has the function of increasing the transmission of ions between the skimmer and the actual entrance of the quadrupole mass filter used for ion selection.

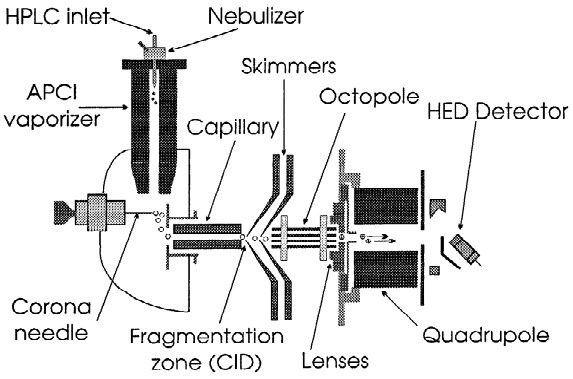

Fig. 2. Schematic diagram of the APCI interface used in this work.

2.4. Optimization of APCI parameters

Since the response in APCI-MS measurements is known to be strongly dependent on the interface parameters, these have to be optimized in order to obtain maximum sensitivity. This is done most conveniently in flow-injection mode by bypassing the column. In addition to reduced standard consumption, this has the advantage of introducing less contamination into the MS as compared to the rather common practice of interface parameter optimization in the continuous infusion mode.

Optimization was done by making successive injections (in a so-called FIA sequence) of 5 μl of a

1 ppm ZON standard while ramping the respective parameter. Recording the transient response in the scan mode (m/z 100–500) allows evaluation of both the sensitivity but also signal-to-noise ratio and fragmentation behaviour of the analyte under the chosen conditions.

The following parameters were evaluated: (a) ionization mode (positive or negative ion detection), (b) nebulizer gas flow, (c) vaporizer temperature, (d) fragmentor voltage, (e) corona current and (f) capillary voltage.

2.5. Calibration

Calibration of the method was done in selected ion monitoring (SIM) mode. As compared to scan mode, absolute signal intensity (sensitivity) decreased by a factor of ca. 2.75 (which already indicates that significant fragmentation of ZON occurs), but reproducibility and signal-to-noise ratio significantly improved due the increase in dwell time of the MS detection.

As an additional step towards making the analysis procedure more expedite, calibration was done by using different injection volumes (ranging from 0.1 to 20 μl) instead of solutions of different concentrations. Smaller volumes cannot be injected reproducibly with the autosampler configuration used, and when using larger injection volumes, peak shape was seriously affected due to the limited capacity of the chromatographic column.

2.6. Naturally contaminated and spiked samples

Both corn samples naturally infected with *F. graminearum*, untreated and spiked blank samples (where ZON could not be detected at concentrations higher than the limit of detection) were investigated.

2.7. Experiments with a single short column

In a further attempt to speed up the analysis procedure, analyses were performed using the ODS precolumn (of 20×2.1 mm dimensions) only, without an analytical column. The same chromatographic conditions were used as in the set-up using both a guard and an analytical column [acetonitrile–water (4:6) as isocratic eluent at 1 ml/min].

3. Results and discussion

3.1. Optimization of interface and detection parameters (Table 1)

3.1.1. Polarity of detected ions

Initial experiments were carried out to determine which ionization mode to use for the further optimization. Although mycotoxins are usually detected in the positive ion mode, where the $[M+H]^+$ quasimolecular ion is detected with highest abundance, we also obtained good sensitivity when operating the MS in the negative ionization mode. In this case, the $[M-H]^-$ ion was the base peak and no significant fragment ions were observed. Sensitivity was only lower by a factor of about 1.5 than in the positive ion mode under identical conditions. However, the stability of both the signal and the baseline was much worse than in positive ion mode, resulting both in an increased standard deviation (ca. 10% for

Table 1
Optimized parameter values of the APCI interface and the MS used for the determination of ZON

Parameter	Optimum value
Vaporizer temperature	400°C
Drying gas temperature	375°C
Drying gas flow	6 l/min
Drying gas pressure	50 p.s.i.g. (1 p.s.i.=6894.76 Pa)
Ionization mode	Positive
Fragmentor voltage	70 V
Capillary voltage	3000 V
Corona current	10 μA
MS operation mode	SIM, m/z=319, 0.2 min peak width, 1000 ms dwell time

five replicate 10 μl injections of a 1 ppm standard versus ca. 3% in the positive ion mode) and a significantly lower signal-to-noise ratio. Still, the possibility of detecting ZON also in the negative ion mode should be considered, first because of the higher selectivity of the negative versus the positive ionization mode and second because of the possibility to obtain (although in two separate chromatographic runs) two useful diagnostic ions of similar intensity which might be very useful, taking into account that LC–APCI-MS spectra generally exhibit very few if not only a single characteristic ion for one compound.

3.1.2. Vaporizer temperature

For thermospray MS, the vaporizer temperature is one of the parameters that have to be optimized the most carefully. For APCI, however, the vaporizer temperature is not a parameter as critical as for thermospray, provided the analyte is not too thermolabile and undergoes degradation when passing the short heated zone of the vaporizer. Thus, mainly the influence of the vaporizer temperature on the eluent evaporation and spray formation can be observed. Increasing the temperature in the range from 250 to 400°C improves the evaporation of the solvent and thus increases the response. Above 400°C, a thermal equilibrium seems to be reached where no further reduction in droplet diameter and size can be effected (due to the short residence time), thus the spray characteristics are not altered or improved further and the response shows no significant increase at higher temperatures. This observation is of course only valid for ZON and has to be reevaluated especially for thermolabile compounds where a decrease in sensitivity at higher temperatures is to be expected.

3.1.3. Drying gas flow and temperature

The drying gas flow was evaluated in the range of 4 to 12 l/min. An optimum response was found between 4 and 6 l/min. Above this range, the response decreases. At lower flow-rates, the spray becomes unstable and the signal-to-noise ratio deteriorates significantly. A value of 6 l/min drying gas flow was thus considered to be optimal (Fig. 3a).

From previous experiments, it was known that the temperature of the drying gas has only little influence

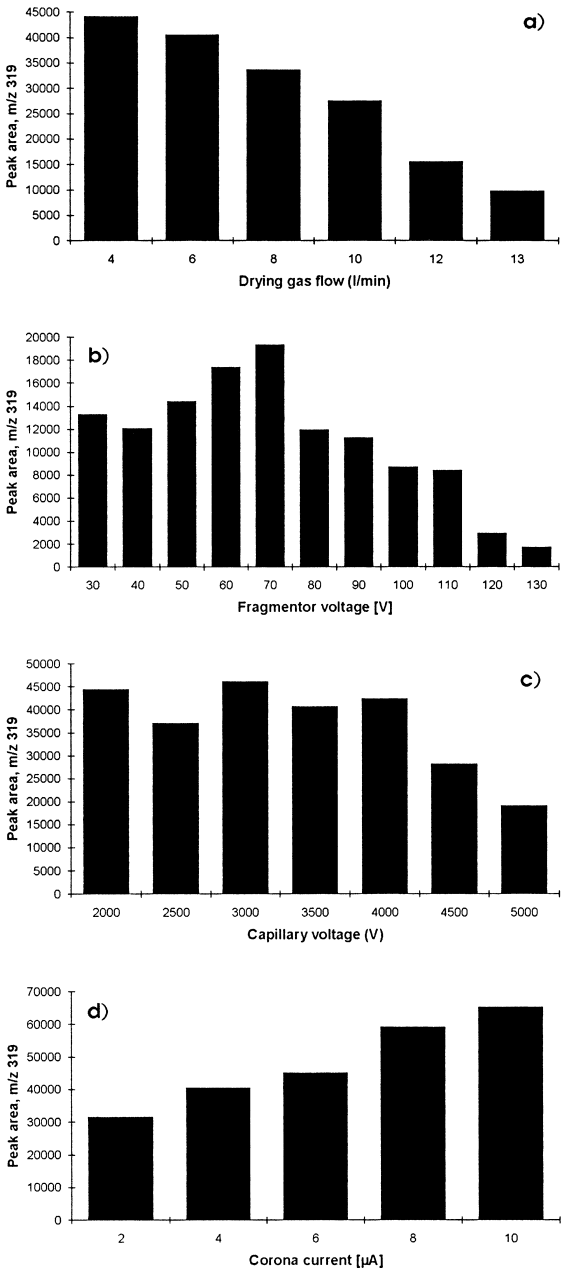

Fig. 3. Optimization of the APCI interface parameters for the detection of ZON in positive ionization mode: plots of the response of the quasimolecular peak of ZON at m/z 319 versus (a) drying gas flow, (b) fragmentor voltage, (c) capillary voltage, and (d) corona current.

on the response compared to the vaporizer temperature. It was thus set to a temperature slightly lower than the vaporizer (375°C).

3.1.4. Fragmentor voltage

A key parameter in tuning the instrument's sensitivity is the fragmentor voltage. At higher fragmentor voltage settings, CID can be initiated in the region between the end of the transfer capillary and the first skimmer cone. The fragmentor voltage was ramped between 30 and 130 V and a distinct optimum in the abundance of the quasimolecular ion peak was observed at 70 V (Fig. 3b). Particularly at low fragmentor voltages, a peak occurs at m/z 391 which is supposed to be an adduct of ZON but could not be identified. This peak disappears at higher fragmentor voltages. Already at low fragmentor voltages, significant fragmentation of the quasimolecular ion occurs, producing a $[M\text{-}OH]^+$ ion at m/z 301 which grows in intensity as the fragmentor voltage is increased. At high fragmentor voltage, this peak is more abundant than the $[M+H]^+$ peak. Another peak, occurring at m/z 149, is the most abundant fragment ion and becomes the base peak at fragmentor voltages above 80 V (Fig. 4a).

In negative ionization mode, no distinct trend could be observed when varying the fragmentor voltage, probably being obscured by the instability of the signal in general. The spectra show however very little fragmentation at fragmentor voltages up to 110 V, and only one adduct ion (at m/z 341) is observed at low fragmentor voltages as the single major signal apart from the quasimolecular ion peak (Fig. 4b).

3.1.5. Capillary voltage

Monitoring the intensity of the quasimolecular ion at m/z 319 allowed the capillary voltage to be optimized. In the range of 2000–4000 V, the response is only little dependent on the voltage, exhibiting a maximum at 3000 V (Fig. 3c). At capillary voltages above 4000 V, both the signal of the quasimolecular ion deteriorates and the signal-to-noise ratio decreases at the same time.

3.1.6. Corona current

The response of the analyte signal increases significantly with the corona current in the range of 2 to 10 μA. The (maximum usable) value of 10 μA was chosen, since not only the absolute signal, but also the signal-to-noise ratios were higher (Fig. 3d). In positive ion mode, the use of higher corona current settings is prevented in order to avoid arcing.

3.2. Quantitative analysis

The potential of the newly developed technique was investigated for quantitative analysis. Calibrations were carried out in different concentration ranges and under different conditions. A preliminary calibration was carried out in the 1–20 ppm range. As mentioned in Section 2, calibration was simplified by injecting different volumes of a 1 ppm (ng/μl) ZON standard into the HPLC–MS system instead of a fixed volume of standard solutions of different concentrations. The calibration graph was linear in the investigated range with a correlation coefficient of $r^2=0.997$ (see Table 2). To evaluate the limit of detection of the method, a second calibration was carried out in the concentration range 3.5–700 ppb. The detection limit was estimated to be 2.5 ng ZON/ml extract according to the calibration method as outlined in the German DIN 32645 standard [32]. This corresponds to a limit of detection of 0.12 μg ZON/kg maize which is sufficiently low to detect the infection by ZON much below the level of concern and by a factor of about 50 more sensitive than reported for the HPLC–FLD method [17].

3.2.1. Quantitative analysis using a single short column

Making use of a highly specific detector such as MS allows to sacrifice of some of the chromatographic or prechromatographic separation (=sample clean-up) while still being able to preserve a high degree of specificity and encountering only few interferences. To this aim, the chromatographic separation was carried out on the 20×2.1 mm precolumn only instead of making use of both the 20×2.1 mm precolumn and the 100×2.1 mm analytical column. Fig. 5 compares the separation on the system using (a) both an analytical and a precolumn and (b) using only a short precolumn. Compared to the system using the longer analytical column, some resolution is sacrificed, however, the ZON peak is

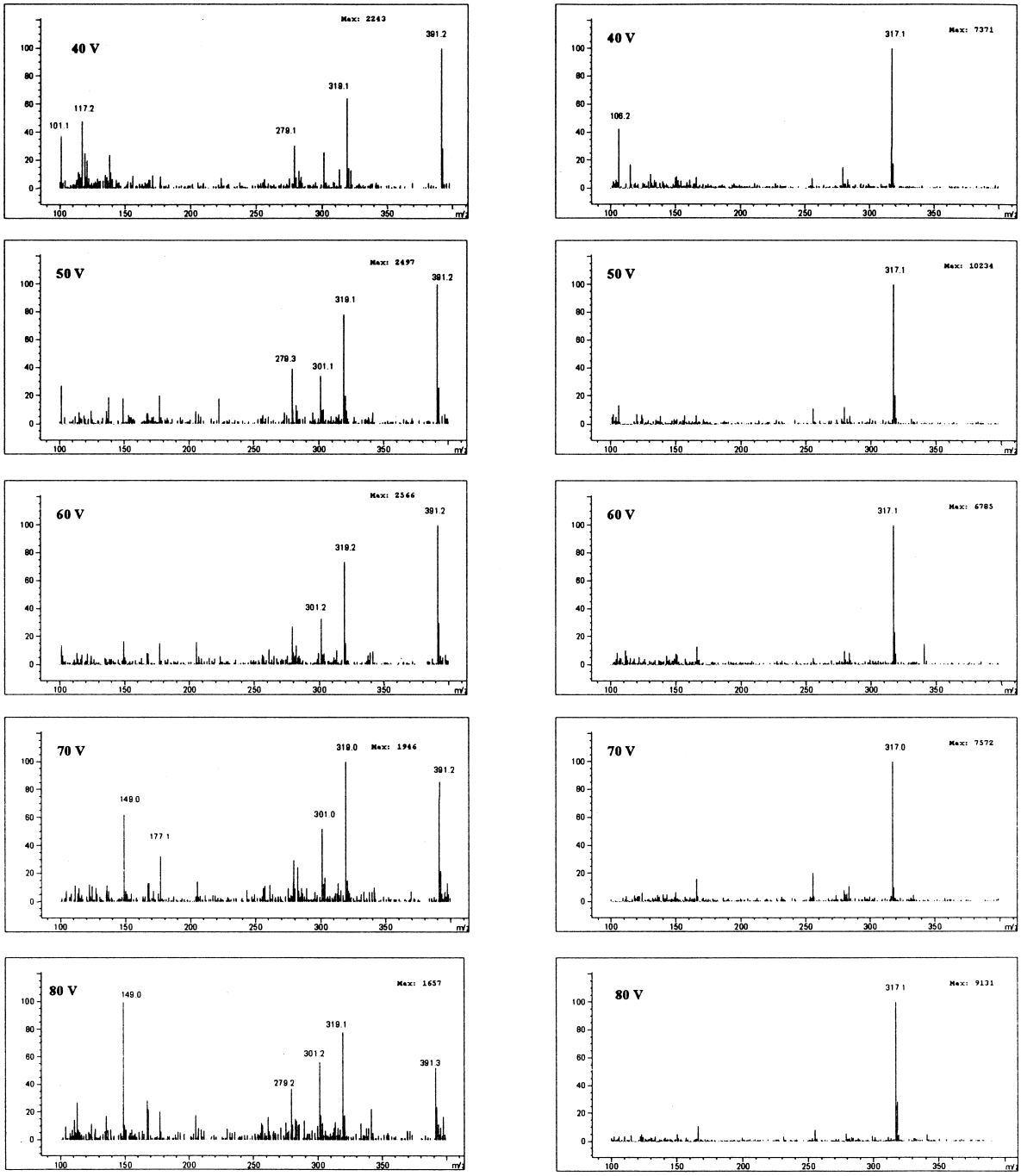

Fig. 4. Series of spectra of ZON acquired at different fragmentor voltage settings (values increasing from bottom to top in the range 40–130 V, as indicated in the spectra). (a) Positive ionization mode, and (b) negative ionization mode.

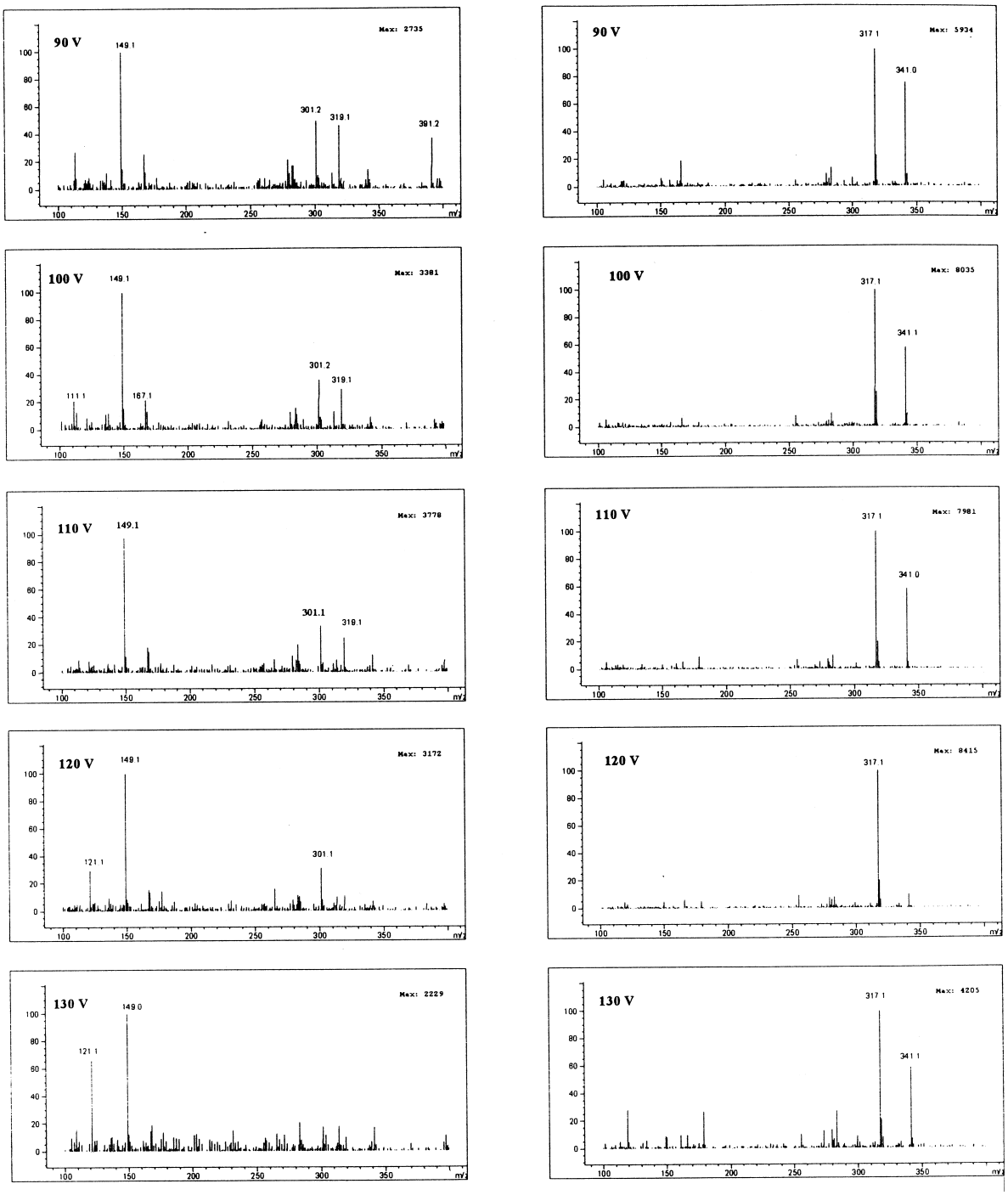

Fig. 4. (*continued*)

Table 2
Parameters of the calibration of the ZON determination by LC–MS in the SIM mode at $m/z=319$

Conditions	Concentration range (ng/μl)	Equation of regression line (for peak area)	Concentration levels (n)	Correlation coefficient (r^2)
Analytical column+precolumn	1–20	$y=14414x+3317$	5	0.9986
Precolumn only	1–10	$y=21750x-1052$	4	0.9992
Analytical column+precolumn	0.0035–0.7	$y=27121x+540$	6	0.9999

still well resolved from the interferences eluting directly in the void volume of the column. The advantage however is that the time needed for the complete separation is linearly reduced with the column length, that is by a factor of ca. 6 (from 4.8 min to 0.8 min). This allows a complete determination of ZON to be run in less than 2 min (as compared to 10 min when using a 100×2.1 mm column with precolumn) and thus to increase dramatically the throughput of the analytical method.

3.2.2. Analysis of spiked and naturally contaminated samples

The reproducibility and bias of the LC–APCI-MS determination of ZON was investigated by analyzing several naturally contaminated and spiked samples under different conditions. A sample spiked at 1 ppm was investigated under the following conditions: with and without immunoaffinity clean-up and using both pre- and analytical column and precolumn only. The quantitative results are presented in Table 3. The analysis using both pre- and analytical column have to be considered as very satisfying both in terms of reproducibility and bias, although already with this system a slight overestimation of the actual ZON concentration is observed. The results obtained using the precolumn only are still acceptable, although they show a higher overestimation and also a higher relative standard deviation. This behavior was to be expected and can most likely be attributed to inter-

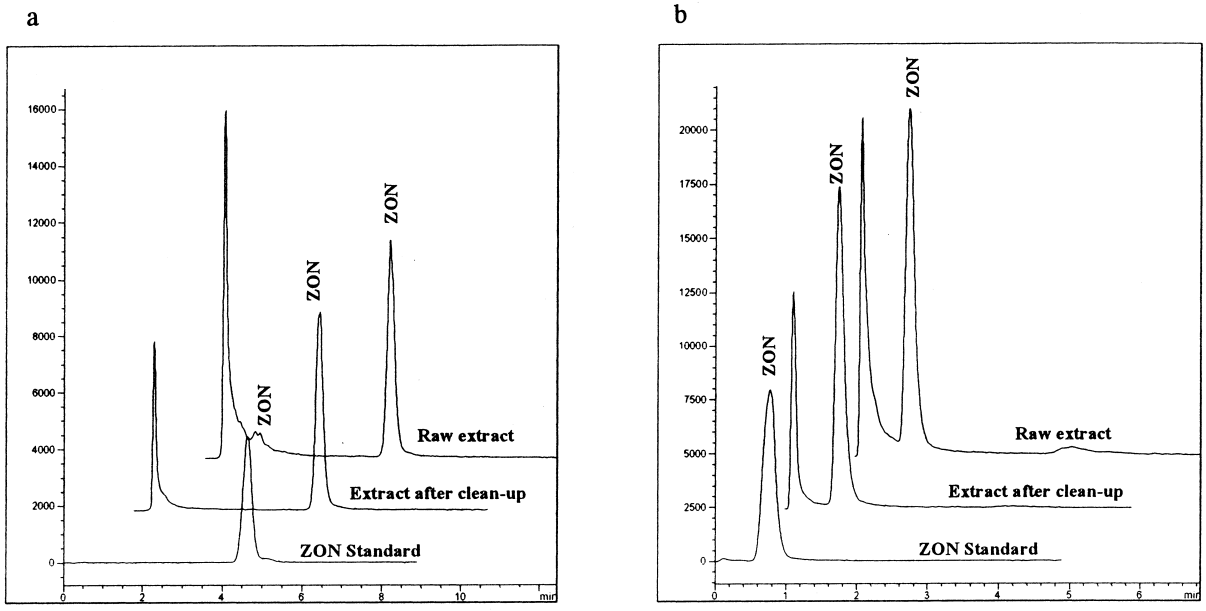

Fig. 5. Stackplot of the chromatograms of a 1 ppm ZON standard, a maize extract after clean-up spiked with 1 ppm and a raw extract (without clean-up), spiked at 1 ppm (from bottom to top), acquired using (a) both an analytical and a precolumn (time and signal offset: 10%), and (b) only a precolumn for the chromatographic separation (time offset: 20%, signal offset: 10%). Detailed conditions are given in Section 2.

Table 3
Quantitative results of the analysis of naturally and spiked corn samples

Conditions	Concentration level (ng/μl)	Found concentration (ng/μl)	Recovery (%)	Reproducibility, R.S.D. (%)
Analytical column+precolumn, with IAC	1.00 (spiked)	1.24	124	1.1 (n=3)
Analytical column+precolumn, without IAC	1.00 (spiked)	1.30	130	1.9 (n=3)
Precolumn only, with IAC	1.00 (spiked)	1.17	117	7.4 (n=3)
Precolumn only, without IAC	1.00 (spiked)	1.57	157	7.1 (n=3)
Analytical column+precolumn, without clean-up	0.0353 (spiked)	0.0276	78	1.6 (n=5)
Analytical column+precolumn, with MycoSep clean-up	0.0353 (spiked)	0.0351	99	6.9 (n=5)
Analytical column+precolumn, without clean-up	Naturally contaminated	0.0274	n.d.	5.6 (n=5)
Analytical column+precolumn, with MycoSep clean-up	Naturally contaminated	0.0130	n.d.	5.7 (n=5)

ferences which coelute with ZON and cannot completely be resolved on the short precolumn. The relative standard deviation (R.S.D.) values are generally satisfying, ranging from 1 to 2% at the ppm level to less than 8% at the ppb level.

In addition to the samples spiked at a high (ppm) level, further samples were investigated that were spiked at the ppb level to have a more realistic estimation of the method's characteristics. Analysing a spiked sample after a MycoSep column clean-up provided quantitative recovery in contrast to the analysis of a sample without clean-up, where a significant underestimation was observed (Table 3). In both cases, however, the reproducibility of the determination was good (R.S.D. $<$6%).

In general, one has to mention that the immunoaffinity clean-up provided cleaner extracts which could be analyzed with less interferences than the MycoSep multifunctional cleaning columns. But even using uncleaned, raw extracts the method works reliably. Injection of 30–50 raw samples was possible before the precolumn had to be replaced due to a loss in chromatographic resolution and deterioration of the peak shape.

4. Conclusion

The use of LC–APCI-MS provides a powerful technique for the determination of ZON in extracts of food and feed products. Under optimized conditions, down to 2.5 μg/l extract can be determined, corresponding to 0.12 μg ZON/kg corn. The high specificity of the MS allows the determination to be speeded up in two different ways: the first is to simplify and reduce sample clean-up to a minimum and to finally leave out sample clean-up completely. This strategy would reduce the time and effort for one analysis most significantly, since most of the (not automatable) workload lies in the sample preparation and clean-up. It compromises however the chromatographic performance with time, so that the use of a precolumn is mandatory which is to be replaced after ca. 50 injections of raw extracts.

The second approach is to reduce the analysis time by shortening the time of the chromatographic separation. Using a short (20 mm) instead of an analytical column of common length (100 mm) significantly reduces the analysis time. When the column diameter and/or the particle size is not reduced adequately, however, chromatographic resolution is reduced with the number of theoretical plates. Although ZON is resolved from the matrix, the analysis of spiked samples indicated a bias towards overestimation. This is however neither significant nor of great concern, since both of the two ways to speed up the analysis are basically intended to enable a fast and efficient screening of a large number of samples. In this case, it is more important to exclude all negative samples, leaving

only the few samples behind that are suspected to be ZON-contaminated. These samples can later on be analyzed using the standard chromatographic separation to actually confirm and accurately quantitate the ZON content.

As a perspective for the future, it can be expected that the use of short narrow-bore columns with smaller particle diameters will allow maintenance of chromatographic separation in a drastically reduced analysis time. This would allow both fast and accurate analysis at the same time. Furthermore, the use of APCI-MS detection for the LC analysis of mycotoxins will certainly give way to the development of multitoxin methods which are only scarcely reported in the literature up to now.

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