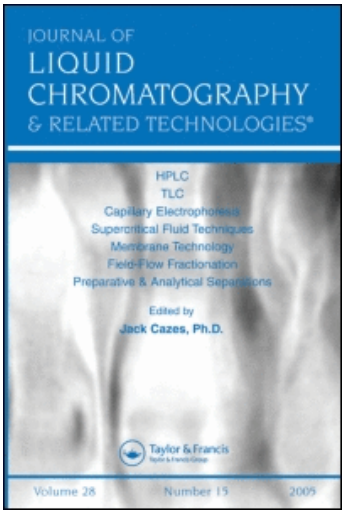

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OPTIMIZATION OF ATMOSPHERIC PRESSURE CHEMICAL IONIZATION INTERFACE PARAMETERS FOR THE SIMULTANEOUS DETERMINATION OF DEOXYNIVALENOL AND ZEARALENONE USING HPLC/MS

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ABSTRACT

In this paper, the influence of several atmospheric pressure chemical ionization (APCI) parameters were investigated in flow-injection for the simultaneous determination of deoxynivalenol (DON) and zearalenone (ZON) using liquid chromatography (LC) coupled to a mass spectrometer (MS). During the optimization procedure of the APCI interface, it was revealed that vaporiser temperature and capillary temperature had a strong influence on the MS signal of DON and ZON in the positive and in the negative ionization mode. In the positive mode a higher absolute signal of both compounds was obtained,

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while in the negative mode a higher selectivity was achieved. Thereafter, tube lens offset, corona discharged current, and capillary voltage were investigated in the negative mode; it was shown that only the tube lens offset had a large influence on the MS signal of the analytes. Finally, the optimized conditions were confirmed injecting and separating a standard mixture on a LC column prior to MS detection.

INTRODUCTION

The presence of mycotoxins in agricultural commodities can present a major health concern for animal and humans due to their biological activity (1). Deoxynivalenol (DON) and zearalenone (ZON) are two commonly analyzed mycotoxins, which often co-occur in cereals (2–5). The most common approach to determining DON is gas chromatography (GC) (6–8), providing good sensitivity. However, the main disadvantage is the requirement of derivatization of the sample. An alternative approach to avoid the derivatisation step has been developed by Tiebach (9) using LC-MS, applying direct liquid introduction (DLI). Since then, several additional methods have been published using different interfaces, such as fast atom bombardment (FBA) (10–11), thermospray (11–13), and plasmaspray (11), coupled to MS detector. The most recent approaches are based on HPLC coupled to MS using APCI interface (14,15). The generally applied method for ZON is based on HPLC with fluorescence detection since ZON has good fluorescence properties (16–18). However, the recent introduction of APCI has enabled LC-MS to become a more diverse tool and, as a result, new LC-MS methods have been published, including the analysis of ZON (19–21).

Since DON and ZON normally are analyzed with two completely different approaches (GC versus HPLC), two different analyses have to be carried out if both analytes are to be determined. Only recently, a simultaneous method for determination of trichothecenes and zearalenone has been developed (22). However, this method is based on GC-MS and requires a time-consuming derivatisation step and also has the disadvantage of a long GC run.

In this paper, the basis for a simultaneous determination of DON and ZON using LC coupled to an MS ion trap equipped with an APCI interface is presented. It is well known that interface parameters play a main role in enhancing the transmission response of the MS and, hence, these parameters were carefully optimized, since no data presently are available for a simultaneous analysis of the two mycotoxins. The absolute responses and spectra were studied in the positive and negative mode by ramping vaporizer and capillary temperatures in different combinations. Further investigations on tube lens

offset, corona discharged current, and capillary voltage were carried out only in the negative ionization mode due to its better selectivity. The optimized APCI conditions were tested by separating a standard mixture of DON and ZON using liquid chromatography prior to MS detection.

EXPERIMENTAL

Chemical and Reagents

DON and ZON standards were purchased from Sigma (Milano, Italy). Acetonitrile, ethanol, and methanol were of HPLC grade (Aldrich, Milano, Italy). Water was purified in a Super-Q Plus, Millipore, Waters System (Millipore, Milano, Italy).

Preparation of Standards Solutions

Standard solution of DON was prepared in ethanol, while ZON was dissolved in methanol obtaining two stock solutions at a concentration of 100 μg/mL and 1000 μg/mL, respectively. The stock solutions were tightly sealed and stored at $+4^{\circ}$C. The concentrations were checked regularly using a spectrophotometer (Shimadzu, Duisburg, Germany) according to the UV-max and the extinction coefficient ($\in$) reported in The Merck Index (23). The DON absorbance was measured in ethanol at 218 nm ($\in$ 4500), while ZON was determined in methanol at 274 nm ($\in$ 13,909) and at 316 nm ($\in$ 6020). Working standard solutions were prepared by taking an exact volume of the stock solution and evaporating it under a gentle stream of nitrogen and re-dissolving the residue in acetonitrile/water (1 : 1, v/v).

HPLC-MS Equipment

Chromatographic separation was performed using a SpectraSystem (Finnigan Mat, San Jose, CA, USA) consisting of a SCM degasser, a P4000 (low flow) quaternary pump, and an AS3000 autosampler. The HPLC system was coupled to an MS ion trap, LCQ-Deca (Finnigan Mat, San Jose, CA, USA) equipped with an APCI interface. The system was controlled with Xcalibur software, version 1.2 (Finnigan Mat, San Jose, CA, USA).

Optimized APCI Parameters

During the optimization of the APCI the following parameters were investigated: ionization mode (positive and negative); vaporizer temperature (ramped from 150°C to 350°C in steps of 50°C), and capillary temperature (increased from 150°C to 250°C in steps of 25°C). In order to determine the interactions of the vaporizer and the capillary temperatures, all capillary temperatures were tested at all vaporizer temperatures in both ionization modes. The other parameters were kept as follows: nitrogen carrier gas and helium sheath gas flows were set at 70 and 9 arbitrary units, respectively. The capillary voltage was held at 3 V in the positive mode and −3 V in the negative mode. All other settings were pre-optimized for DON by the autotune program in the continuous infusion mode, to achieve maximum transmission for DON protonated molecular ions $[M + H]^+$ and deprotonated molecular ions $[M - H]$.

The investigation was performed at a flow rate of 0.2 μL/min in flow-injection mode by bypassing the column. Optimization was carried out in acetonitrile : water 15 : 85 for DON and acetonitrile : water 50 : 50 for ZON, as estimated from the retention time of the chromatographic method. A short experiment was done shooting DON and ZON in acetonitrile : water 50 : 50 and 90 : 10, respectively, and no major changes were realized. Injections of 5 μL of 5 μg/mL standard solutions were done in triplicate for each mycotoxin, both in positive and negative mode, while ramping the respective parameters. The MS response was acquired in full scan mode (m/z 150–350) allowing evaluation of the peak area, the signal to noise ratio, and the spectrum. The evaluation of the parameters was based on the average of the peak area, calculated on the protonated molecular ions $[M + H]^+$ and deprotonated molecular ions $[M - H]^-$, in positive and negative ionization mode, respectively.

Once the vaporizer and the capillary temperature were set for the negative ionization mode, the interactions between the following parameters were investigated: tube lens offset (increased from −100 V to 100 V in steps of 50 V), corona discharged current (from 3 μA to 9 μA in steps of 2 μA), and capillary voltage (from −120 V to 0 V in steps of 20 V).

LC-MS Analysis at Optimized Conditions

For the chromatographic separation of the two analytes a liquid chromatography column XTerra RP-18 (150 mm × 2.1 mm, 3.5 μm, C18 end-capped, pore size 120 Å, Waters, Milano, Italy) was used. A linear binary gradient, at a flow rate of 200 μL/min was applied, starting with 1 min 100% water, increasing to 90% acetonitrile in 4 min, remaining at 90% acetonitrile for 7 min, then lowering to 0% acetonitrile in 1 min, followed by 100% water

for 2 min. After the run, the column was re-equilibrated in 100% water for 2 min. The APCI was used in the negative ionization mode and mass spectra were registered in full scan mode (m/z 150–350).

RESULTS AND DISCUSSION

Optimization of APCI Parameters

The LC-MS interface parameters were optimized in order to improve sensitivity and selectivity. This was done in both the positive and in the negative ionization mode by taking into account the interaction of the vaporizer and the capillary temperature. The influence of the studied parameters on the response of the target analytes and the interaction of these parameters were evaluated based on the protonated molecular ions $[M+H]^+$ m/z 297.3 for DON and $[M+H]^+$ m/z 319.4 for ZON in the positive mode.

The results (Figure 1) show that the DON and the ZON signals are enhanced at higher capillary temperatures and the absolute signal is higher when a vaporizer temperature below 300°C is applied. The best results for DON were obtained with a low vaporiser temperature. However, a vaporizer temperature that is too low does not allow for a satisfactory evaporation of the liquid solvent flowing from the LC system. In the positive ionization mode, a high capillary temperature (250°C) and a medium vaporizer temperature (250°C) have to be applied to work at optimized conditions using a flow rate of 0.2 μL min. A careful evaluation of the signal to noise (S/N) ratio demonstrated a low selectivity in the positive ionization mode. Indeed, despite the high absolute signals (based on peak area), the S/N ratio for DON never exceeded 60 when injecting as much as 25 ng of the compounds. For this reason the positive ionization mode was not further considered for DON analysis in this study.

The deprotonated molecular ions for DON ($[M-H]^-$ m/z 295.3) and for ZON ($[M-H]^-$ m/z 317.4) were evaluated for the optimization of the APCI parameters in the negative ionization mode and the results can be seen in Figure 2.

In the negative ionization mode, a higher signal is achieved when applying a lower capillary temperature, while the vaporiser temperature shows an optimum between 200°C and 300°C. In Figure 2 it can also be seen that DON has a 10 fold lower signal than ZON and, consequently, optimised parameters must mainly be based on DON detection. From Figure 2 it can be concluded that a vaporiser temperature of 200°C and a capillary temperature of 150°C would be preferable. However, the chosen parameters for APCI were 250°C for the vaporizer and 150°C for the capillary, due to the fact that 200°C in some cases might be insufficient for a complete evaporation of the solvent. The optimum of the

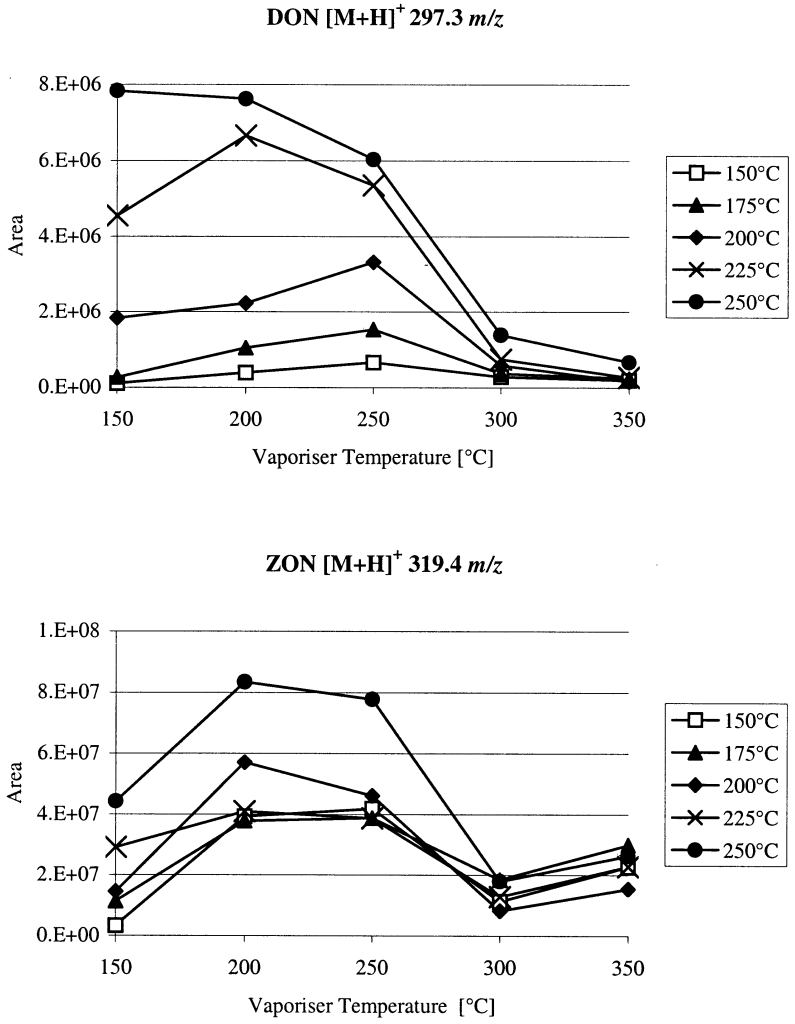


Figure 1. Influence of the vaporizer temperature and the capillary temperature (see legend) on DON and ZON responses in the positive ionization mode.

vaporizer temperature is linked to the LC flow rate and higher vaporizer temperatures are required when higher flow rates are utilized (data not shown).

After evaporating the solvent in the ionization chamber the analytes reach the first vacuum stage via the capillary. While the analytes never reach the set vaporizer temperature (due to the evaporation process), the analytes are fully

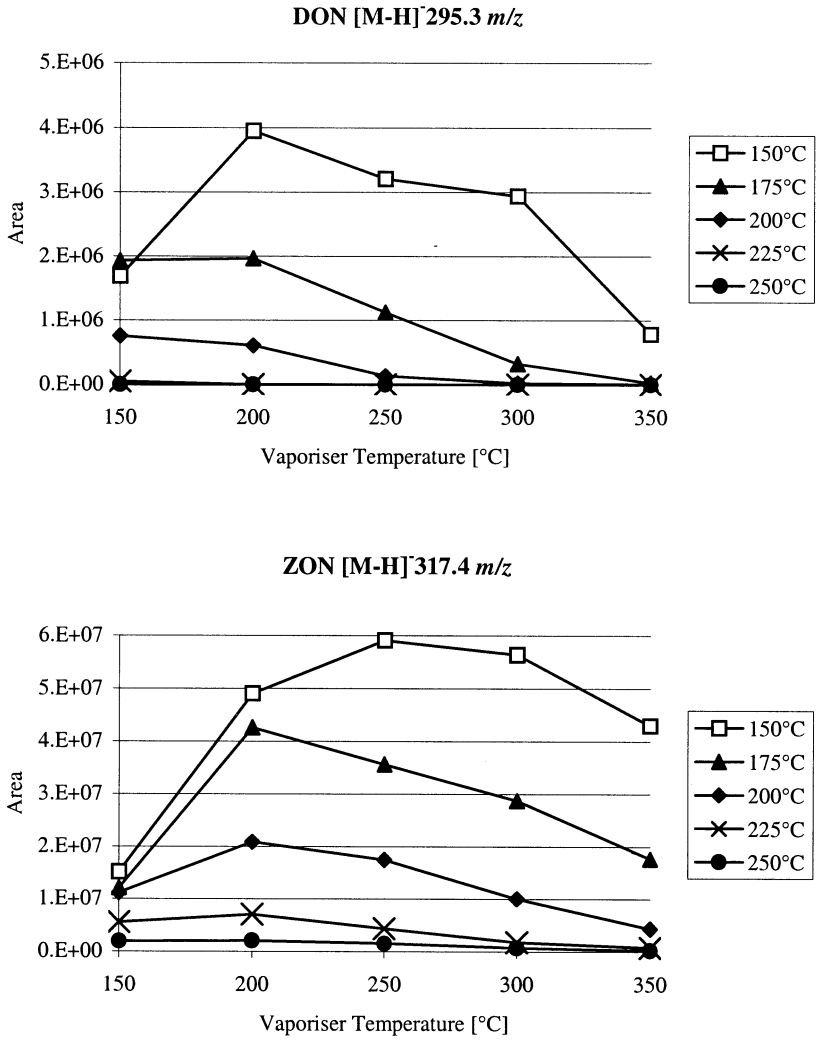
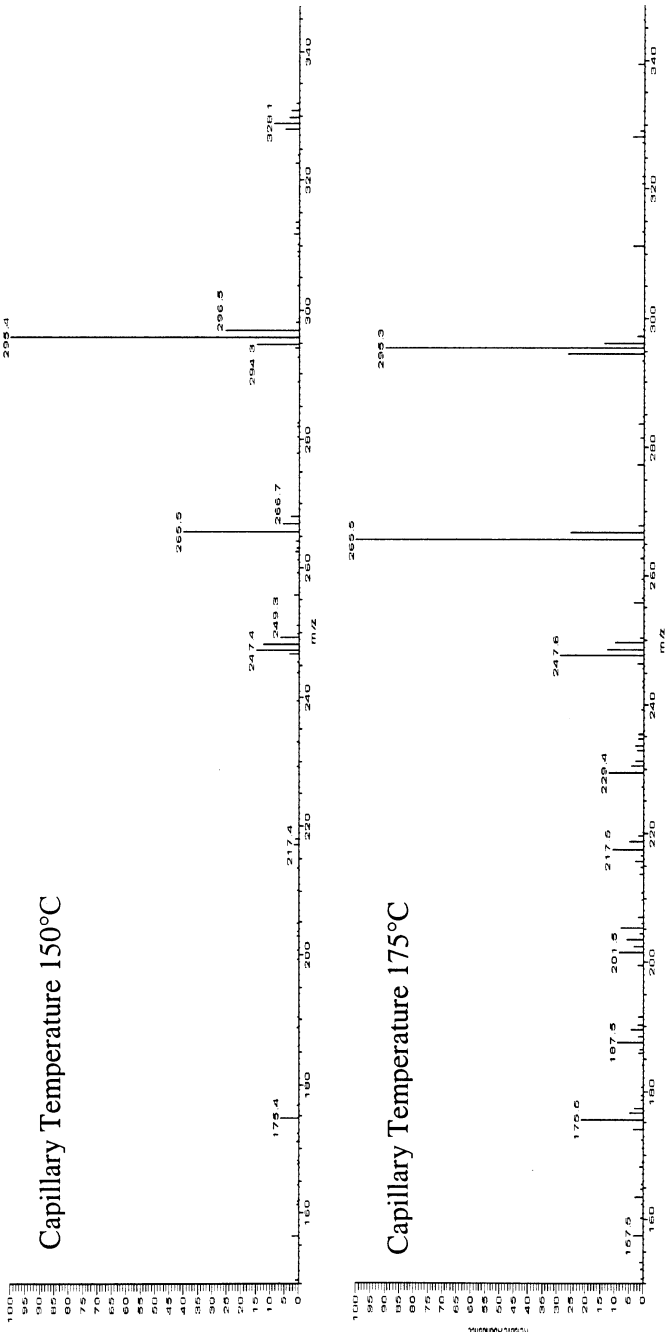


Figure 2. Influence of the vaporizer temperature and the capillary temperature (see legend) on DON and ZON responses in the negative ionization mode.

exposed to the capillary temperature. Consequently, thermally labile species will be more affected by the capillary temperature than by the vaporiser temperature. In Figure 3 the fragmentation pattern of DON in the negative ionization mode applying different capillary temperatures (150°C to 250°C) at a constant vaporiser temperature of 250°C, is shown.



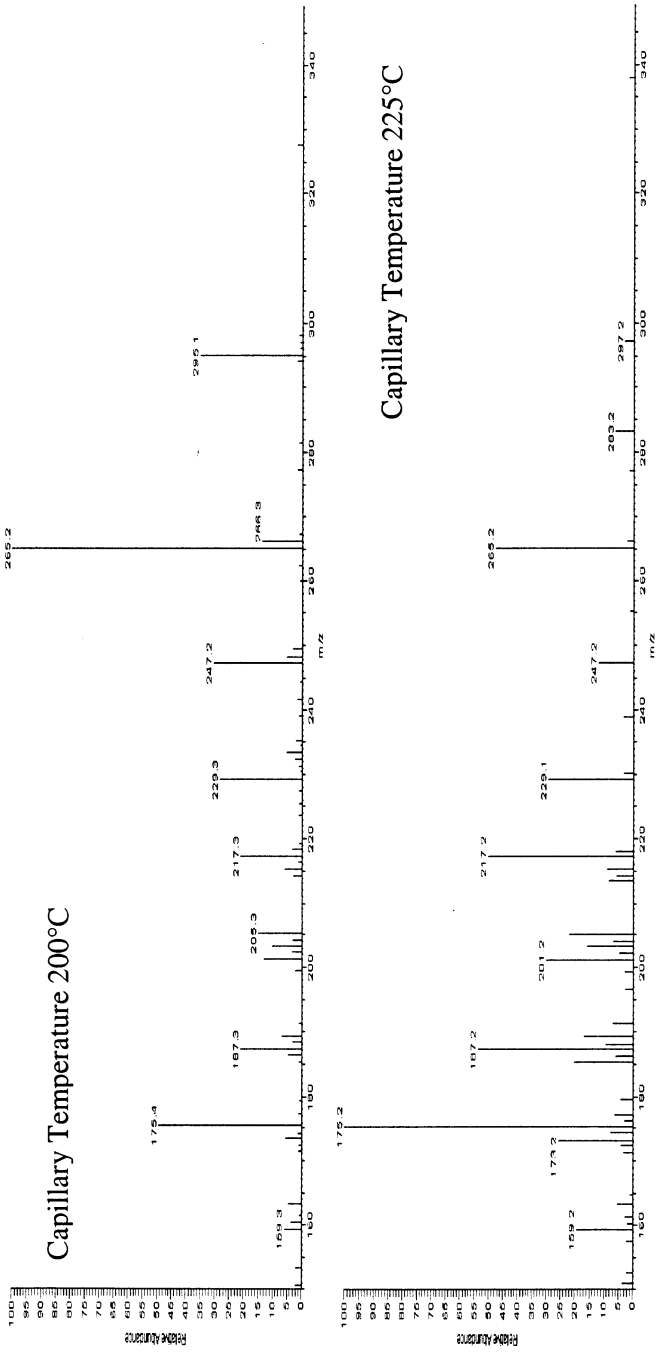
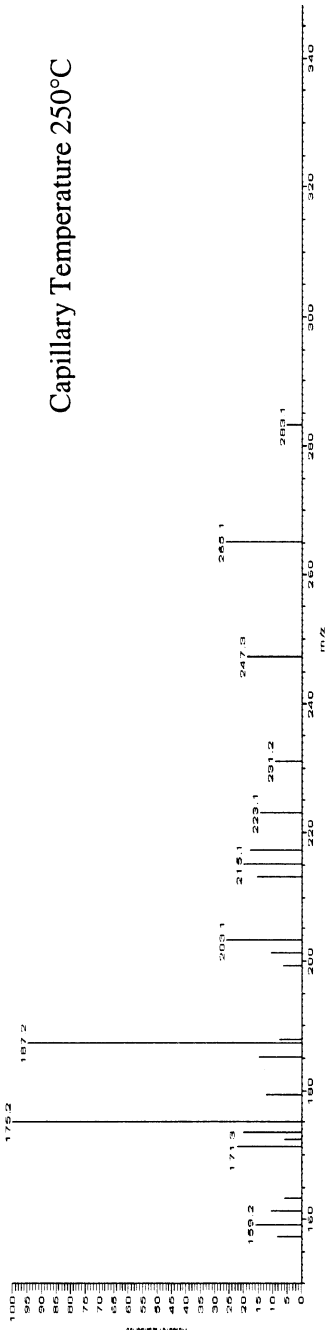


Figure 3. Spectra of DON acquired in the negative ionization mode setting the vaporizer temperature at 250°C, ramping the capillary temperature from 150°C to 250°C.

(*continued*)



At high capillary temperatures (225°C–250°C) the deprotonated molecular ion ($[M - H]^-$ m/z 295.3) has disappeared. At the chosen optimized conditions (vaporizer temperature 250°C, capillary temperature 150°C), m/z 295.3 ion gives the highest absolute signal in comparison to all other investigated combinations (except for 200°C vaporizer temperature, as discussed above). Despite the fact that a thermal degradation of DON lowers the absolute signal, it has the advantage of providing an identification fingerprint by the formation of two additional ions (m/z 265, 247), which help in excluding matrix interferences when not running in the MS/MS mode. The fact that these ions are derived from the degradation of the DON ion was confirmed by acquiring the same spectrum as the DON fragmentation pattern when applying direct infusion in MS/MS scan mode.

In order to further improve DON signal in negative ionization mode, the interaction between the tube lens offset and the corona discharged current was evaluated.

From Figure 4, it is evident that the corona discharged current is responsible only for minor changes, therefore, it was set at 5 μA, since values between 3 μA and 5 μA are generally recommended in order to avoid arching in the APCI due to too high value. Figure 4 clearly shows that setting of the tube lens offset has a strong influence on DON signal, hence, it was more extensively investigated between −100 V to 0 V confirming the value of −50 V as the one providing the highest signal (data not shown). Experiments carried out ramping

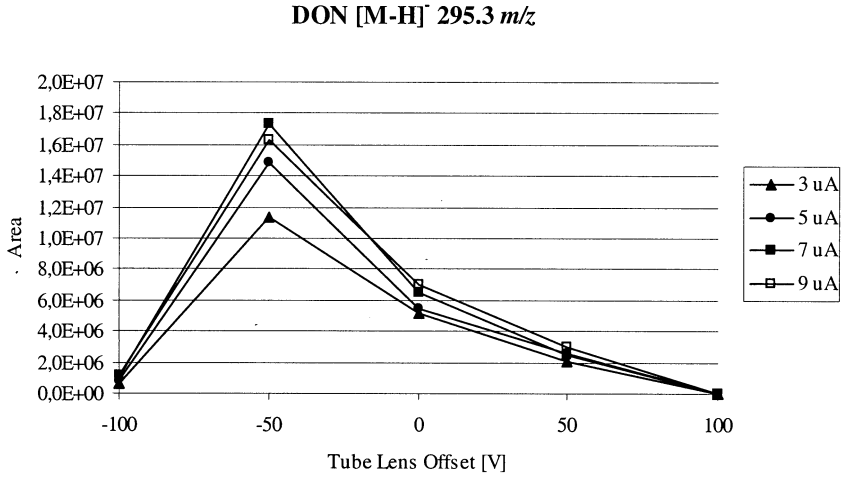


Figure 3. Continued.

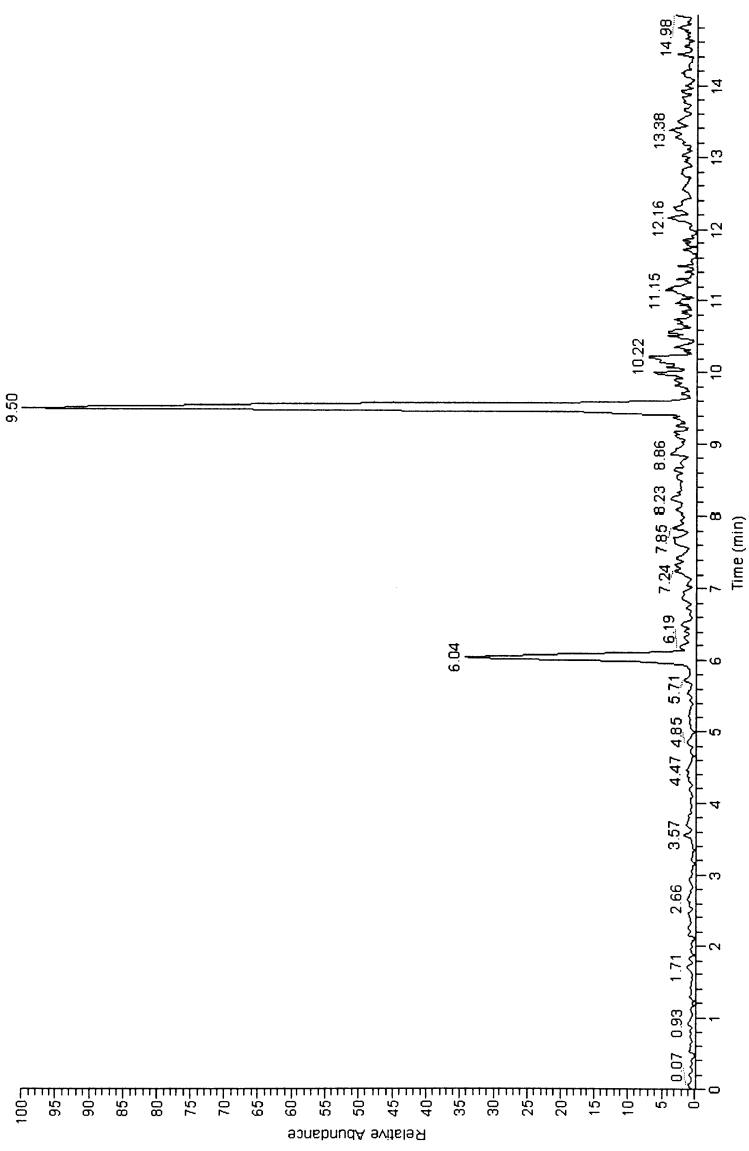


Figure 4. Influence of the tube lens offset and the corona discharged current (see legend) on DON response in the negative ionization mode.

the capillary voltage at the optimized conditions only showed slight changes in the DON signal, reaching an optimum response between –40 V and –20 V.

The achieved optimised instrument APCI parameters were: vaporizer temperature 250°C; capillary temperature 150°C; tube lens offset –50 V; corona discharged current 5 μA, and capillary voltage –20 V.

HPLC-MS Analysis at Optimized Conditions

The above optimised parameters, obtained by flow injection by-passing the column in the negative ionization mode, were further confirmed by analyzing a 5 μL mixed standard solution (2.5 μg/mL of each mycotoxin) through the LC-column. In this case, using a capillary temperature of 150°C the optimal vaporizer temperature was observed at 250°C for both DON and ZON, ramping from 150°C to 250°C

Applying the optimized APCI parameters, a standard chromatogram acquired in full scan mode, injecting 5 μL of a standard solution containing 500 ng/mL and 60 ng/mL of DON and ZON, respectively, was easily obtained as seen in Figure 5. Such concentrations are presently under discussion within the European Union as legal limit for food.

CONCLUSION

During the optimization of APCI parameters it has been shown that the ionization mode combined with the vaporizer temperature and the capillary temperature had a strong influence on the signal acquired in MS.

The optimized APCI parameters allows for a simultaneous detection of DON and ZON using LC-MS. Future investigation will be devoted to quantitative analysis, as well as the development and evaluation of a simultaneous extraction method for DON and ZON.

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