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Application Note

A Method for the Rapid and Sensitive Determination of Ochratoxin A in Red Wine

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Abstract

The method described in this application note allows for the rapid and sensitive analysis of this compound in red wine, without the need for extraction and cleanup procedures.

Introduction

Under poor storage conditions, agricultural produce is often subject to fungal contamination. Penicillium and Aspergillus molds, which produce the natural product ochratoxin A, may be found on stored coffee beans, grapes and grain.[1] Ochratoxin A is carcinogenic in rats and may be associated with human kidney disease. Because of this a Maximum Residue Level (MRL) in grain of 5 µg/Kg has been proposed in the European Union.[2] The structure of ochratoxin A is shown in Figure 1.

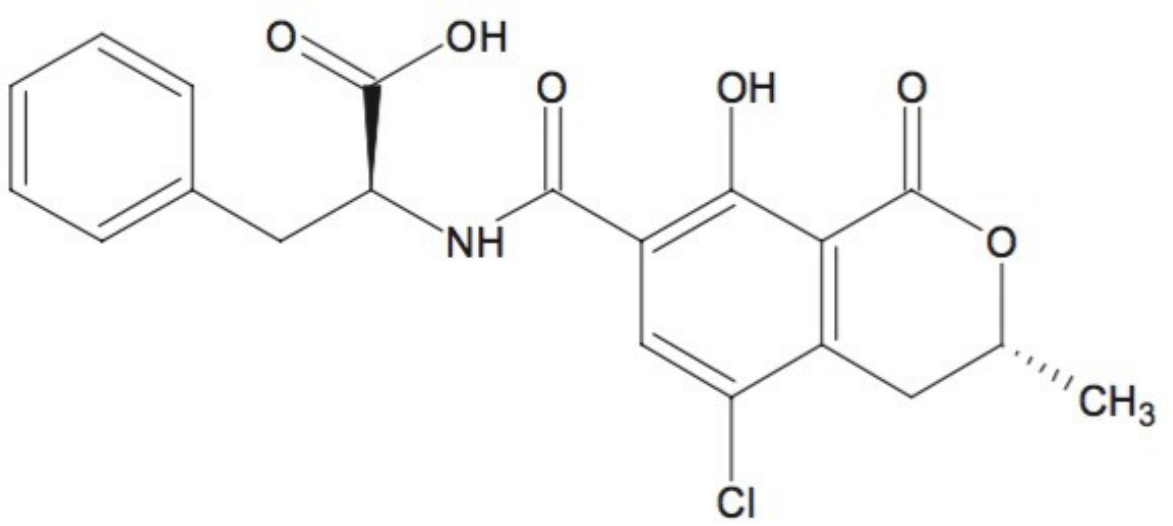

Figure 1. Ochratoxin A.

Many other foodstuffs, including beer and wine, may also contain unacceptably high levels of ochratoxin A. For this reason, a rapid and simple method was developed for its determination in red wine. No extraction or cleanup was performed. An aliquot of wine was analysed directly and a fast chromatographic gradient allowed a sample to be analysed every 10 minutes.

Experimental

All experiments were conducted using a Waters Alliance HPLC system, an XTerra 2.1 mm by 30 mm HPLC column and a Waters Micromass Quattro micro API triple quadrupole mass spectrometer. Mobile phase A was water with 1% formic acid. Mobile phase B was acetonitrile with 1% formic acid. 50 µL of red wine was loaded onto the HPLC column and the following solvent gradient was run at a flow rate of 0.4 mL/min.

Time	0 min	5% B

Time	1 min	5% B
Time	3 min	100% B
Time	6 min	100% B

The eluent was interfaced to the electrospray source of the mass spectrometer in positive ion mode. The MS/MS transitions selected for quantification and confirmation of the compound are shown in Table 1.

Precursor m/z	Product m/z	Dwell Time (s)	Cone Voltage (V)	Collision Energy (eV)
403.9	238.9	0.1	21	25
403.9	357.9	0.1	21	15
405.9	240.9	0.1	21	25

Table 1. MS/MS conditions for ochratoxin A.

In order to avoid contamination of the ion source, the LC eluent was diverted to waste for the first three minutes of each run.

Waters Micromass Quattro micro API triple quadrupole mass spectrometer.

Results and Discussion

Figure 2 shows the full scan MS spectrum of ochratoxin A under positive electrospray conditions.

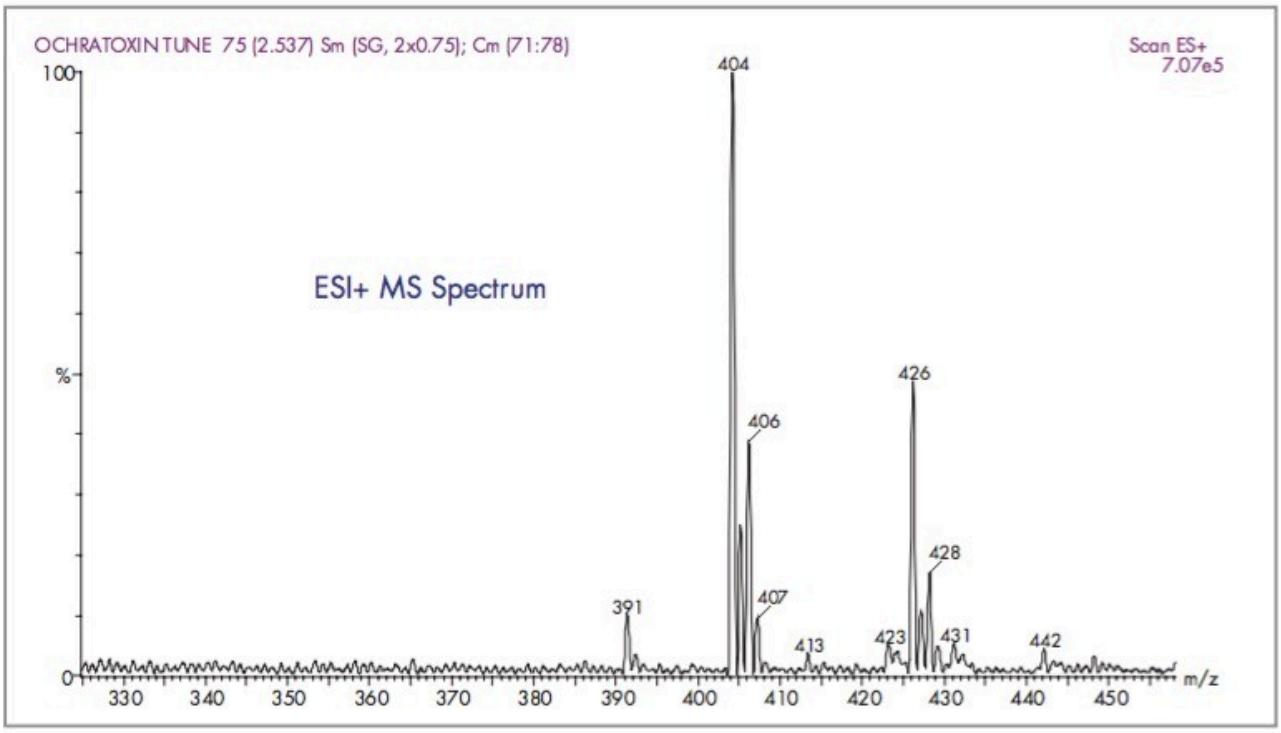

Figure 2. MS spectrum of ochratoxin A.

Figure 3 shows the chromatograms obtained from the analysis of red wine spiked at 0.25 µg/L.

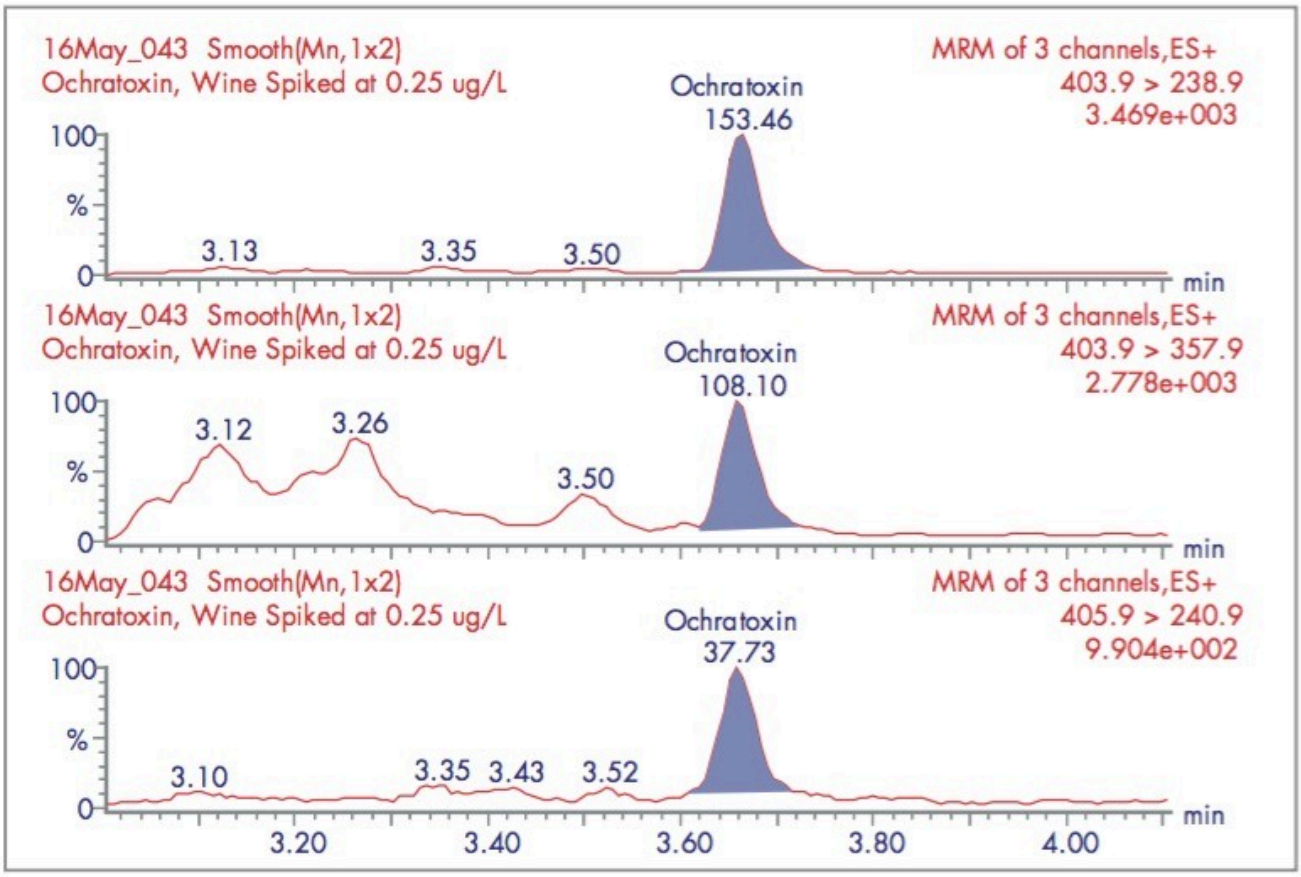

Figure 3. Chromatograms for 0.25 µg/L ochratoxin A in red wine.

A calibration graph was generated between 0.024 and 2.39 µg/L and is shown in Figure 4.

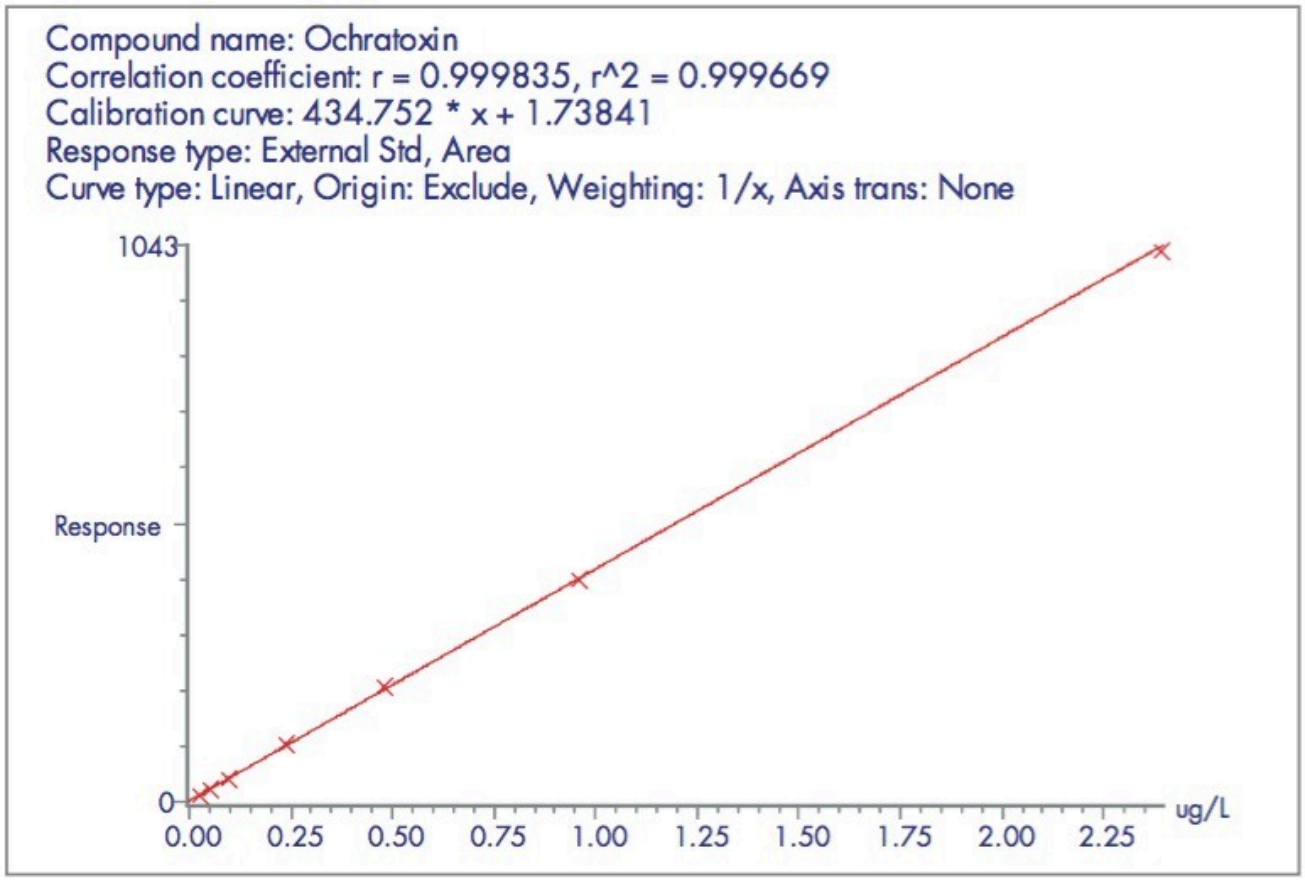

Figure 4. Calibration graph for ochratoxin A.

Conclusion

Given the concerns over the potential toxic effects of ochratoxin A in the human diet, it is necessary to study its occurrence in various foods. The method described above allows for the rapid and sensitive analysis of this compound in red wine, without the need for extraction and cleanup procedures.

References

1. van Egmond HP, Speijers GJA. *J. Nat. Toxins.* 1994, 3: 125.

2. Krivobok S, Seigle-Murandi F, Steiman R, Creppy EE. System. *Appl. Microbiol.* 1995; 18: 455.

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