# Automation of a Solid Phase Extraction Method for the Determination of Ochratoxin A in Wine Samples Prior to LC-MS/MS

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## Introduction

The emerging threats of mycotoxin contamination in our food, particularly ochratoxin A (OTA), have led to surveillance programs employing low-level screening methods in both industrial and government food safety laboratories. The persistant nature of this contaminant has led to its determination in a range of processed foods including cereals, beer and wine. Of particular interest to this investigation is the utility of automation to facilitate large scale quality control efforts in the preparation of samples prior to analysis by liquid chromatography-mass spectrometry-mass spectrometry (LC-MS/MS). A solid phase extraction method using application specific polymer column technology called the ISOLUTE Myco (60 mg/3 mL column) was developed from a multivariate statistical design of experiment (DOE). This method was employed to determine OTA concentrations in malbec wines from Argentina. The recovery for the extracted mycotoxin was 95-105% (<15% RSD) over a linear concentration range ($r^2$ = 0.997) of 0.4-4.0 µg/L. The optimized method was automated using a Biotage RapidTrace$^+$ workstation to ensure consistent method precision in high-throughput food safety laboratories. Feasibility of the direct transfer of the offline chemistry to the automated system was succesful.

## Objectives

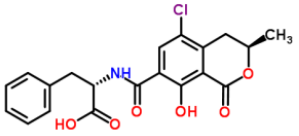
- Evaluation of application specific SPE for the ppb level targeted analysis of OTA in red wine.
- Automate the optimized manual procedure to support high-throughput quality control or food safety laboratories.

## References:

1) "Development of a QuEChERS-SPE strategy prior to UPLC-MS/MS for the determination of OTA in red wines" manuscript submitted to the Journal of Food and Agricultural Chemistry (Feb 2104)

2) "A novel extraction strategy prior to UPLC-MS/MS for the quantification of OTA in red wines" manuscript submitted to Food Chemistry (Nov 2013)

## Analyte of Interest




***Ochratoxin A***
***log P = 4.37 pKa=3.29***

***Figure 1:*** Chemical structure for ochratoxin A

## Experimental Procedure – Part I
## Method development at the Universidad Nacional de San Luis (manual prep)

## REAGENTS

Ochratoxin A, analytical standard, was obtained from Fluka (Steinheim, Germany). Acetonitrile, methanol, and water Optima® LC-MS grade were purchased from Fisher Scientific (Fair Lawn, New Jersey). Formic acid was obtained from Fisher Scientific (Loughborough, UK). Intermediate spiked samples were prepared using wine previously determined to be OTA-free. For the solid phase extraction step, 3cc and 60 mg ISOLUTE® Myco cartridges (Biotage, USA) were used.

## Mass Spectrometry

Mass spectrometry experiments were performed on a Quattro Premier™ XE Micromass MS Technologies triple quadrupole mass spectrometer with a ZSpray™ electrospray ionization source (Waters, Milford, USA). The source was operated in a positive (ESI+) mode at 350°C with $N_2$ as the nebulizer and the source temperature was kept at 150°C. The capillary voltage was maintained at 3.0 kV and the extractor voltage was set at 1.0 kV. Ultrapure nitrogen was used as desolvation gas with a flow of 800 L $h^{-1}$. Argon was used as collision gas at a flow of 0.18 mL $min^{-1}$. Detection was performed in multiple reaction monitoring (MRM) mode of selected ions at the first ($Q_1$) and third quadrupole ($Q_3$). The optimum MRM conditions are listed in Table 1. The data were acquired using MassLynx Mass Spectrometry Software (Waters, Milford, USA).

***Table 1****: Positive ion mode transitions for OTA*

| Transition (m/z) | Dwell (s) | Cone (V) | Collision (eV) | Type of Transition |
|---|---|---|---|---|
| 404.1 > 239.2 | 0.08 | 20 | 25 | Quantification |
| 404.1 > 341.1 | 0.08 | 20 | 25 | Confirmation |
| 404.1 > 358.2 | 0.08 | 20 | 20 | Confirmation |

## Chromatography

An Acquity™ Ultra High Performance LC system (Waters, Milford) equipped with autosampler injection and pump systems (Waters, Milford) was used. The autosampler vial tray was maintained at 15°C. The needle was washed with appropriate mixtures of acetonitrile and water. The separation was performed by injecting 25 µL sample onto an ACQUITY UPLC® BEH $C_{18}$ (Waters, Milford, USA) analytical column with 2.1 mm internal diameter × 50 mm length, and 1.7 µm particle size. The binary mobile phases consisted of water with 0.1% (v/v) of formic acid (A) and acetonitrile with 0.1% (v/v) of formic acid (B) delivered at 0.35 mL $min^{-1}$. The composition of the isocratic program was 70% A and 30% B. The total chromatographic run time was 2.0 min. The column was held at a temperature of 30ºC. A representative chromatogram was included in Figure 2.

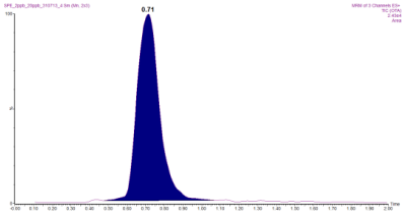



***Figure 2:*** Typical chromatogram obtained by this method

## Sample Preparation workflow

The optimized sample preparation workflow is detailed in Figure 3. Optimization experiments were detailed in previous work[1,2].

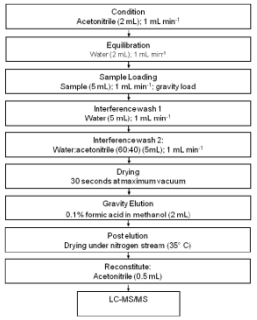



***Figure 3:*** Optimized solid phase extraction procedure

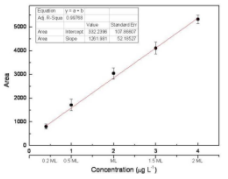



***Figure 4. Calibration curve***

## Method qualification

The qualification of the method was performed following the 2002/657/EC European Decision. Spiked wine samples (malbec variety) were used. Thus 5 blanks, 10 replicates at the ML (2.0 µg $L^{-1}$), and 5 replicates at 0.5ML (1.0 µg $L^{-1}$), 1.5ML (3.0 µg $L^{-1}$), 0.2ML (0.4 µg $L^{-1}$), and 2ML (4 µg $L^{-1}$) each were prepared. The obtained calibration curve is depicted in Figure 4.The analytical figures of merit, LoD, LoQ, RSD (%), and recovery values were calculated (Table 2).

***Table 2****: Figures of merit for SPE Myco-LC-MS/MS procedure*

| Concentration Level (µg $L^{-1}$) | RSD (%) | Recovery (%) | $r^2$ | LoD (µg $L^{-1}$) | LoQ (µg $L^{-1}$) |
|---|---|---|---|---|---|
| 0.4 (0.2ML) | 12.6 | 95.9 | 0.9977 | 0.14 | 0.41 |
| 1.0 (0.5 ML) | 14.3 | 107.2 | | | |
| 2 (ML) | 7.9 | 106.5 | | | |
| 3 (1.5ML) | 5.9 | 99.8 | | | |
| 4 (2ML) | 3.5 | 99.0 | | | |

## Experimental Procedure – Part II
## Method transfer to Biotage-US applications lab for feasibility of automation

Automation of the optimized SPE method was achieved with a Biotage RapidTrace$^+$ workstation (Figure 5). The automation parameters were detailed in Table 3.

***Figure 5:*** *RapidTrace+ automated SPE workstations*

***Table 3:*** *Automation parameters for the RapidTrace$^+$ workstation*

| Step | Source | Volume (mL) | Flow rate (mL $min^{-1}$) |
|---|---|---|---|
| Purge Cannula | MeCN | 5 | 30 |
| Purge Cannula | $H_2O$ | 5 | 30 |
| Condition | MeCN | 2 | 30 |
| Equilibration | $H_2O$ | 2 | 30 |
| Sample | Wine (neat) | 5 | 1 |
| Wash 1 | $H_2O$ | 5 | 1 |
| Wash 2 | 60/40 H2O/MeCN | 5 | 1 |
| Elute | 0.1% formic acid in MeOH | 2 | 1 |

A set of fortified cabernet samples were prepared over a gradient concentration range (5 levels x 2 replicates). The samples were processed using the parameters in Table 3. Post extraction, the recovered samples were evaporated using a Biotage TurboVap LV (45°C/20psi $N_2$). The samples were reconstituted with 0.5 mL 0.1% formic acid in acetonitrile. Linearity was verified over this concentration range of 0.4 to 1 ng $mL^{-1}$. ($r^2$ = 0.992). A second study was performed on 7 replicates of a fortified wine samples. The method repeatability (%RSD) was 7%.

Future work will include the feasibility in eliminating the evaporation step in this method. Feasibility of including beer matrices with these method parameters will also be evaluated.

## Conclusions

A UPLC-MS/MS method based on application specific ISOLUTE Myco SPE cartridges for sample clean-up was developed for the determination of OTA in red wine samples at the low levels required by international regulations. Technology transfer for automation of this method was demonstrated.

**Note:** The RapidTrace$^+$ workstation required an inexpensive bushing to accommodate the tabless cartridges.



Part Number: P63



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