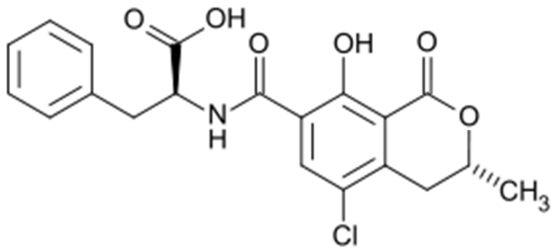
Determination of Ochratoxin A in Wine by LC-on-line-SPE-MS/MS by using Spark Holland Symbiosis™ system

Introduction:

Ochratoxin A(OTA), a toxin produced by Aspergillus ochraceus, Aspergillus arbonarius and Penicillium verrucosum, is one of the most abundant food-contaminating mycotoxins. It is also a frequent contaminant of water-damaged houses and of heating ducts. Human exposure can occur through consumption of contaminated food products, particularly contaminated grain and pork products, as well as coffee, wine grapes and dried grapes. The toxin has been found in the tissues and organs of animals, including human blood and breast milk. Ochratoxin A, like most toxic substances, has large species- and sex-specific toxicological differences.[1]



[1] text from Wikipedia[1]

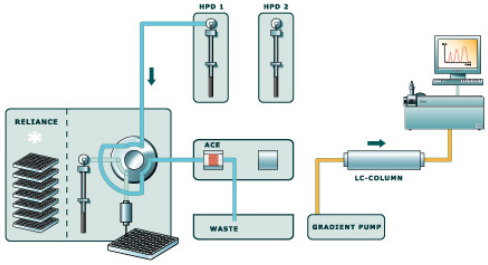
This application note describes the method development for the determination of OTA and the measurement of real wine samples.
Note that no internal standard is used for this application.

Method.

In the low pH range OTA becomes non-polar. Therefore the SPE method is developed under acidic conditions; all used SPE solvents contain 0.2% Formic acid (FA).
The method development started with the screening of the 6 reversed phase sorbents in the Method Development cartridge tray (Spark p/n:0822.660). After selecting the best cartridge sorbent the method was optimized by increasing the organic content in the wash solvent. After the method was developed three wine samples and one grape juice sample are acquired.

The final on-line SPE (XLC)method is:

Cartridge:	HySphere C18HD (p/n:0822.609)
Solvation	1 mL 0.2% FA in Methanol (5 mL/min)
Equilibration	1 mL 0.2% FA in water (5mL/min)
Sample application	1 mL 10% MeOH in 0.2% FA (5 mL/min)
Wash 1	1 mL 30% MeOH in 0.2% FA (5 mL/min)
Elution time	4.5 minutes LC gradient



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Autosampler method

Injection Mode:	Partial loopfill
Injection volume:	50 µL
Speed:	Normal
HeadSpace pressure:	Off
Air segment:	On
Cooling:	Off

Wash method:

Wash volume (ul)	Wash solvent
1000	40% ACN 0.2% FA

LC method

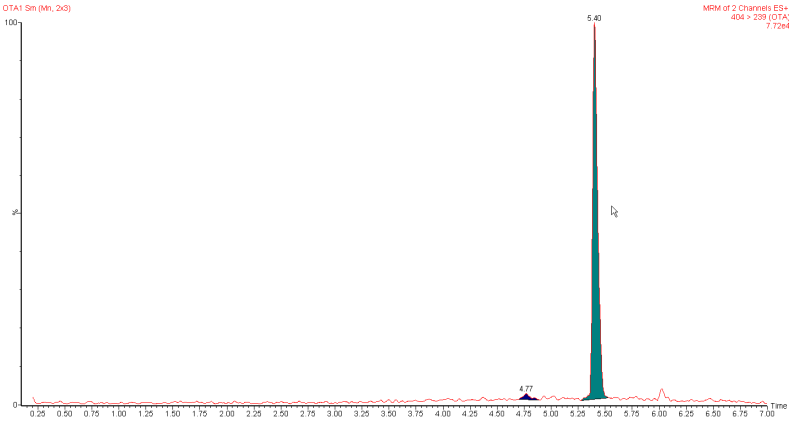
For the chromatography a Water Xterra -C18 (50*2.1 mm 3µ) column is selected. Mobile phase A is 0.2% formic acid in water and mobile phase B is 0.2% formic acid in Methanol.

Pump time	Pump flow (ml/min)	Pump Fraction A %	Pump Fraction B %
00:00:01	0.20	80	20
00:00:05	0.20	80	20
00:04:00	0.20	5	95
00:04:30	0.20	5	95
00:05:00	0.30	80	20
00:07:00	0.20	80	20

MS method

A Waters Quattro ™ Premier was used for the detection of OTA.
After tuning the MS two fragments are selected .
Q3 fragment 239 is selected for quantification.
Q3 fragment 241 is only used for confirmation purpose.

Chan	Reaction	Dwell(secs)	Cone Volt.	Col.Energy	Delay(secs)	Compound
1	: 404.00 > 239.00	0.100	25.0	22.0	0.005	OTA
2	: 406.00 > 241.00	0.100	25.0	22.0	0.005	OTA



50 µL 10 ng/mL OTA standard.

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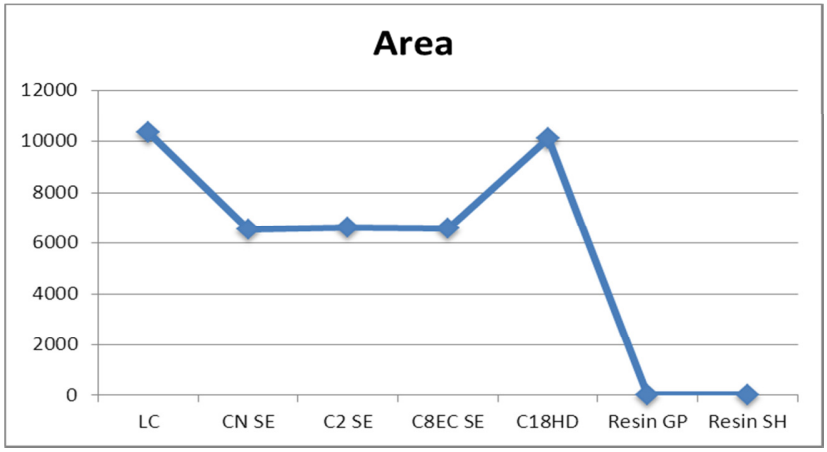
Results.

A 10 ng/ml OTA standard is spiked in 0.2% FA in water and injected using the LC modes of the Symbiosis™ Pro system. This gives an indication of the expected signal height and retention time.

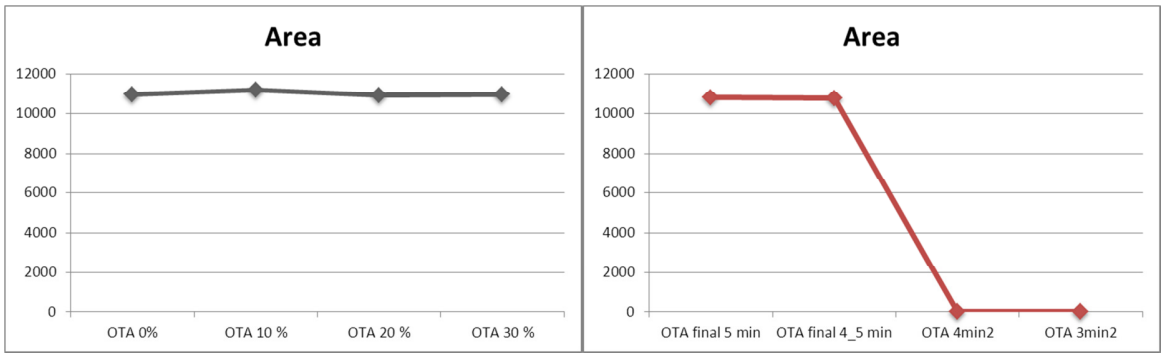
On-line SPE Method development.

After the standard LC injection the 6 Spark HySphere reversed phase sorbents are tested using a neat solution, the sorbent screening procedure. The cartridge wash is performed with 0.2% FA in water without any organic.



Area response of the neat solution with LC and of the 6 HySphere cartridge's SPE

The HySphere C18HD gives the best recovery (close to 100%) and is selected to be used for this application. After the selection of C18HD as the sorbent the application is further optimized.
First the highest percentage of organic in the wash determined. During the sorbent screening the cartridge was washed with water containing 0.2% Formic acid. The system is set up to automatically add 10%, 20% and 30% of methanol to the wash solution. After the wash optimization the shortest possible elution time is determined.



% MeOH in cartridge wash

Elution time (in Min) after 30% MeOH wash

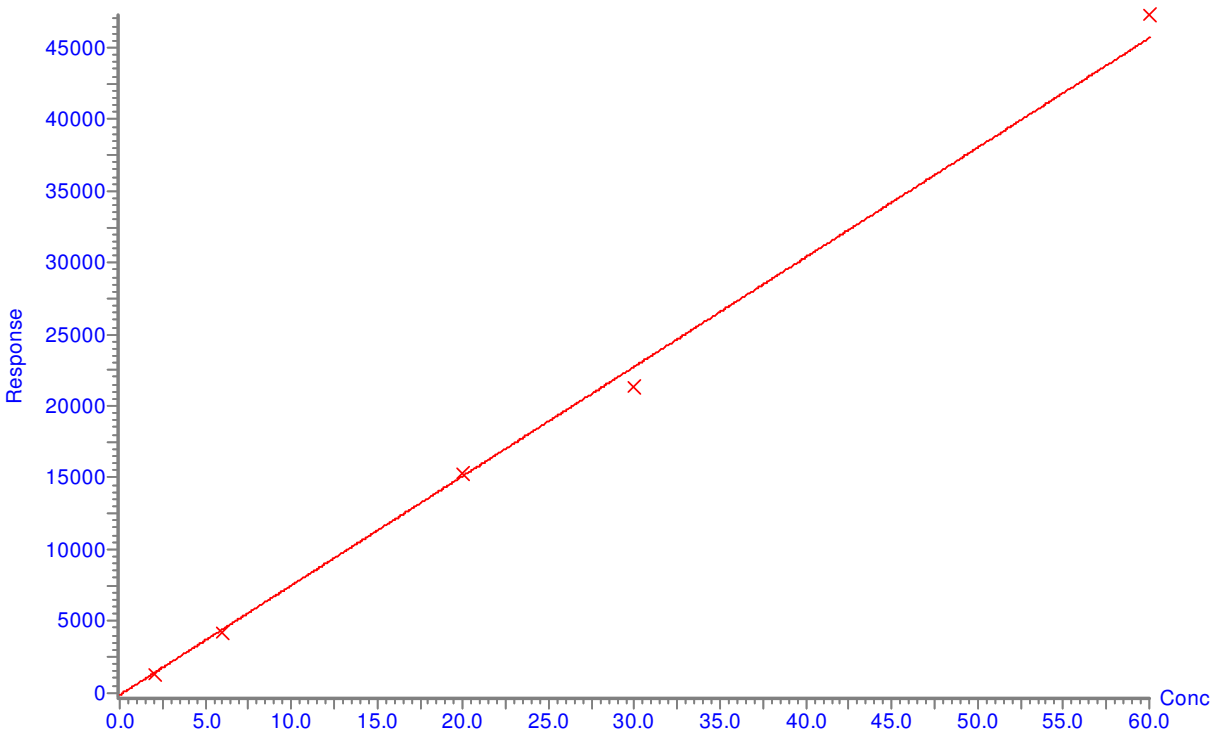
Calibration curve

After the method is optimized a calibration curve is created ranging from 2 till 60 ng/mL in 0.2% formic acid in water.

Name	Std. Conc (ng/mL)	Area	Calc. Conc.	%Dev
OTA 1	**2**	1343.188	2.0	-2.1
OTA 2	**6**	4163.079	5.7	-5.8
OTA 3	**20**	15262.19	20.2	0.9
OTA 4	**30**	21286.59	28.1	-6.4
OTA 5	**60**	47288.95	62.1	3.6

Calibration curve data (data generated by Quanlynx integration part of Masslynx)

Compound name: OTA
Correlation coefficient: r = 0.998773, r^2 = 0.997547
Calibration curve: 763.56 * x + -151.223
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None



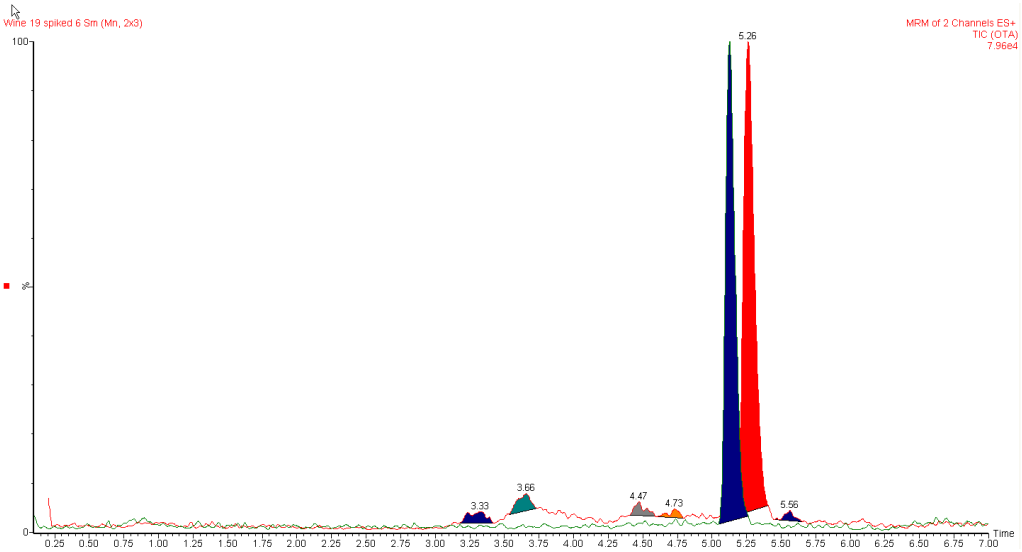
Calibration curve with a correlation coefficient of R= 09987 and a 1/X weighting.

Real wine samples.

After the creation of a calibration curve the three wine samples and one grape juice are analuzed.
Three wine samples are measured. Two of the wine samples are also spiked with 6 ng/mL OTA.
This to check for matrix effect issues. Also one filtered Grape juice sample is measured.

sample name	average ng/mL
Grape juice	0.9
Wine 10	0.6
Wine 17	0.3
Wine 17 spiked 6	6.3
Wine 19	0.3
Wine 19 spiked 6	6.3

Results of the real wine and grape juice samples



Red peak is OTA in Spiked wine 19 (6.3 ng/mL) and blue peak is the calibration point 2 (6 ng/mL)
(the spiked wine chromatogram has a 0.1 min time offset)

Conclusion:

From this study it can be concluded that within a time frame of one day it is possible to develop a XLC-MS method for the determination of OchraToxin A(OTA) with a recovery of almost 100%.
For OTA in neat solution the calibration curve correlation coefficient is more than 0.99% .
The % deviation is between -6.4 and +1 for the calibration curve and no internal standard is used for this application. The spiked wine samples show no matrix effect compared to the un-spiked sample.
The results acquired with on-line SPE are compatible with the currently used off-line SPE method.
Due to the limited time the wash of the cartridge is not further optimized and left to 30% Methanol in 0.2% formic acid.