# USING MICRO-LC AND LC-MS/MS TO ENHANCE MYCOTOXIN ANALYSIS

**Stephen Lock and Ashley Sage**
**AB SCIEX, Warrington, UK**

## INTRODUCTION

Traditionally in mycotoxin screening of food samples, samples are extracted and analyzed by LC/MS/MS usually at LC flow rates which are in excess of 400 µl/min and in combination with high pressures with smaller particle size HPLC columns to maintain sharp peaks and fast chromatography. These flow rates produce fast speeds and excellent peak shapes and results, but have a draw back in that they require higher volumes of organic solvent. The consumption of HPLC organic solvents, such as acetonitrile and methanol, is a growing cost of analysis and its disposal has an environmental impact. Therefore, ways to reduce solvent consumption in pesticide residue testing will be beneficial to the environment and reduce running costs of a testing lab.

Here we present new data using micro flow LC, running at 20 µL/min, in combination with a LC-MS/MS method developed on an AB SCIEX QTRAP® 4500 system utilizing the *Scheduled* MRM™ algorithm. Initially this approach has been tested on a mixture of 6 mycotoxins to show its applicability in food analysis and data presented will compare Micro LC with traditional LC flow rates.

## MATERIALS AND METHODS

**Figure 1.** QTRAP® 4500 LC/MS/MS system

### Sample Preparation

For linearity and sensitivity tests calibration standards were prepared in 10% acetonitrile in water from concentrations 0.01 – 2 ppb. Samples was extracted using a simple solvent extraction. Flour (2 g) was mixed with a 80:20 mixture of acetonitrile: water (10 mL) in a 50 mL PTFE tube. The tubes were shaken (1 min) roller mixed (20 minutes) and centrifuged (10 min, 2500 rpm). The top layer (6 mL) was filtered using a Phenomenex PHENEX filter (15 mm RC Membrane 0.45 µm) and the supernatant (200 µl) was diluted with water (600 µl) containing 0.5% acetic acid and 5 mM ammonium acetate and then injected (10 µl).

### MS Conditions

In this work the QTRAP® 4500 LC/MS/MS system was used (Figure 1) in positive and negative mode using an ion spray voltage of 5500 V (positive mode) or -4500 V (negative mode) for the high flow injections the standard electrospray probe was used for the microLC work the source was fitted with an ESI probe designed for Micro LC[1]. The method was set up to detect 17 mycotoxins although in this initial study the samples just contained afflatoxins, ochratoxin A and zeranolen (Table 1). The source settings were adjusted for low flows and the MRM conditions were simply transferred from the high flow method system without any further modifications.

**Figure 2.** Eksigent ekspert™ microLC 200

**Table 1. MRM transitions with their micro LC retention times (RT) used to detect target mycotoxins using a single method with negative and positive polarity switching**

| Mycotoxin | RT (min) | Polarity | Ion | MRM (quantifier) | MRM (qualifier) |
|---|---|---|---|---|---|
| 15-Acetyldeoxynivalenol (15-AcDON) | 5.2 | positive | $[M+H]^+$ | 339/321 | 339/137 |
| 3-Acetyldeoxynivalenol (3-AcDON) | 3.9 | negative | $[M+CH3COO]^-$ | 397/307 | 397/59 |
| | | negative | $[M-H]^-$ | | 337/307 |
| Aflatoxin B1 (AFB1) | 5.8 | positive | $[M+H]^+$ | 313/285 | 313/128 |
| Aflatoxin B2 (AFB2) | 5.6 | positive | $[M+H]^+$ | 315/287 | 315/259 |
| Aflatoxin G1 (AFG1) | 5.6 | positive | $[M+H]^+$ | 329/243 | 329/200 |
| Aflatoxin G2 (AFG2) | 5.4 | positive | $[M+H]^+$ | 331/313 | 331/245 |
| Deoxynivalenol (DON) | 3.2 | negative | $[M+CH_3COO]^-$ | 355/295 | 355/59 |
| Diacetoxyscirpenol (DAS) | 5.4 | positive | $[M+H]^+$ | 384/307 | 384/247 |
| Fumonisin B1 (FB1) | 6.4 | positive | $[M+H]^+$ | 722/334 | 722/352 |
| Fumonisin B2 (FB2) | 7.0 | positive | $[M+H]^+$ | 706/336 | 706/318 |
| Fusarenon X (FUS X) | 3.5 | negative | $[M+CH_3COO]^-$ | 413/353 | 413/59 |
| HT-2 toxin | 6.1 | positive | $[M+NH_4]^+$ | 442/263 | 442/105 |
| Monoacetoxyscirpenol (MAS) | 5.3 | positive | $[M+H]^+$ | 342/265 | 342/307 |
| Nivalenol (NIV) | 2.8 | negative | $[M+CH_3COO]^-$ | 371/281 | 371/59 |
| Ochratoxin A (OTA) | 6.2 | positive | $[M+H]^+$ | 404/239 | 404/102 |
| T-2 toxin | 6.3 | positive | $[M+NH_4]^+$ | 484/215 | 484/185 |
| Zearalenon (ZON) | 6.2 | negative | $[M-H]^-$ | 317/131 | 317/175 |

### LC Conditions

The LC system used for the Micro LC tests was the Eksigent ekspert™ microLC 200 for the high flow injections a Shimadzu XR HPLC system was used. The Micro LC system was run at 20 µl/min which was over 20 times lower than used for the conventional 3.0 mm columns of the Shimadzu XR system which was run at 450 µl/min and an injection volume of 10 µl was used for both systems. The Micro LC separation used a 2.7 µm 0.5 x 100 mm Halo™ C18 column and the high flow separation used a Phenomenex ® Kinetix ® 2.6 µm C18 3 x 100 mm both held at 40 ºC and separations were carried out using the same gradient profile shown in table 2 from water to methanol both phases containing 5 mM ammonium acetate and 0.5% acetic acid.

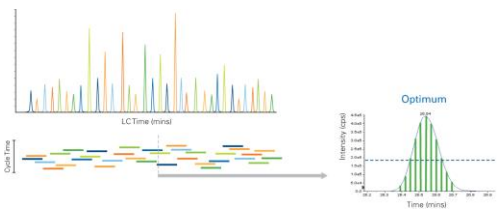


**Figure 3.** *scheduled* MRM™ **: -** When we know the elution time of the toxin, we use this to intelligently schedule the acquisition of the MRM for that pesticide so that, each MRM is monitored only across its expected elution time. This decreases the # of concurrent MRMs maintain both cycle and dwell time.

| Time (min) | Flow (µL/min) | A (%) | B (%) |
|---|---|---|---|
| -2 | 20 | 98 | 2 |
| 2 | 20 | 98 | 2 |
| 5 | 20 | 40 | 80 |
| 5.2 | 20 | 2 | 98 |
| 7 | 20 | 2 | 98 |
| 7.2 | 20 | 98 | 2 |
| 8 | 20 | 98 | 2 |

Table 2. LC gradient used for both Micro flow and high flow separation. For both separations used a 2 minute equilibration time.

## RESULTS

For this initial study 6 different mycotoxin standards which were available at the time were combined and diluted and their sensitivity and linear ranges assessed. Due to the dilution in the sample preparation an upper level of 2 ppb was all that was needed but detection at 0.05 ppb or below was also needed to meet current EU guidelines for cereal testing [2]. Table 3 and Figure 4 show that the micro LC method was linear and sensitive enough to meet current legislation.

| Compound | Calibration range | Linearity for MRM1 | Concentration of standard | Signal to noise (S/N) * |
|---|---|---|---|---|
| | | ('r' value) | ng/ml | |
| ZON | 0.02 - 2 ng/ml | 0.998 | 0.02 | 11 |
| AFB1 | 0.005 - 2 ng/ml | 0.998 | 0.005 | 12 |
| AFB2 | 0.01 - 2 ng/ml | 0.998 | 0.01 | 17 |
| AFG1 | 0.01 - 2 ng/ml | 0.996 | 0.01 | 10 |
| AFG2 | 0.01 - 2 ng/ml | 0.997 | 0.01 | 9 |
| OTA | 0.02 - 2 ng/ml | 0.995 | 0.02 | 11 |
| * Data calculated in MultiQuant™ software at close to LOD limit | | | | |

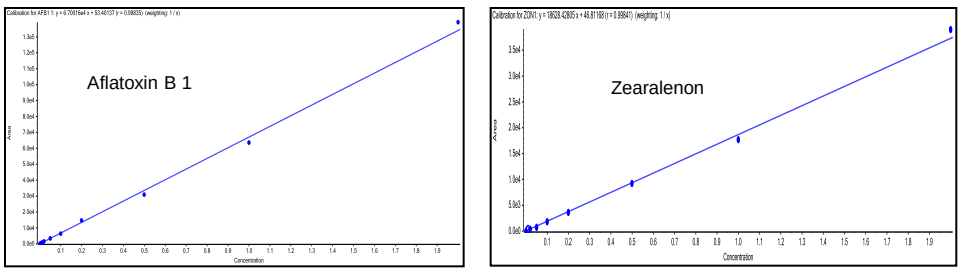


Figure 4. Calibration lines for mycotoxins Aflatoxin B 1 (0.005 – 2ppb) and Zearalenon (0.02 – 2 ppb).

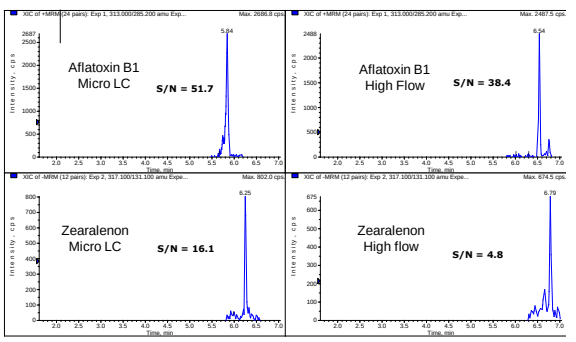


Figure 4. Comparison of a flow extract analyzed by the high flow and micro flow methods

Figure 5 shows a comparison of the analysis of an extract of flour spiked at a 2 µg/kg (2 ppb) with mycotoxins run on both the high flow method as well as the micro flow method. When comparing the two methods both were run using the same gradient and mobile phase as to not influence the sensitivity. The column chemistries were closely matched and it is evident that the micro flow method seems to be a factor of 2 – 3 times more sensitive in signal to noise compared to the high flow method Evidence of the low dead volume of the micro flow system is clearly demonstrated by the shorter retention times observed even at 20 times lower flow rate

| Component Name | Num. Values | Mean | Standard Deviation | Percent CV |
|---|---|---|---|---|
| AFB1 1 | 6 of 6 | 0.0632 | 0.0049 | 7.68 |
| OTA1 | 6 of 6 | 0.049 | 0.0056 | 11.44 |
| AFB2 1 | 6 of 6 | 0.0722 | 0.0078 | 10.87 |
| ZON1 | 6 of 6 | 0.0322 | 0.0044 | 13.59 |
| AFG1 1 | 6 of 6 | 0.0703 | 0.0092 | 13.02 |
| AFG2 1 | 6 of 6 | 0.0665 | 0.0091 | 13.67 |

Table 4. CV data from the repeat analysis of a 2 µg/kg spiked flour extract, no internal standard used.

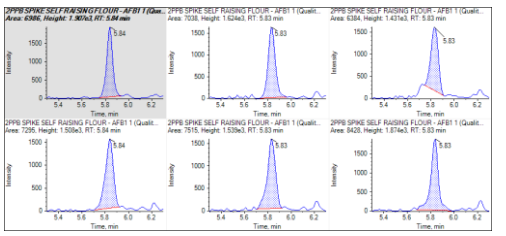

Figure 5. Chromatograms from the repeat analysis of a 2 µg/kg spiked flour extract for AFB1 .

The data in Table 4 and Figure 5 confirmed that the current Micro LC method has good reproducibility at the current EU legislation limits even with no internal standards and the chromatography currently still under development.

## CONCLUSIONS

Although the Micro LC method is still currently under development this study has clearly demonstrated that using Micro LC is a valid approach in mycotoxin analysis. The same MRM conditions were used for both methods so that method transfer is quick between an high flow method to the Micro LC method. The only MS optimization needed is the adjustment of the source settings for the lower flow rates. The method used was quick, sensitive, robust and reproducible but also provides a huge cost saving to labs. With LC grade acetonitrile running at a cost of £100/L this 3 day study could have cost about £ 100 with convention chromatography (0.6 ml/min running for 24hrs a day) and < £10 with Micro LC. Over a year this amounts savings of over £4000 (£90 x 50 weeks) in solvent consumption alone.

## REFERENCES

1. K. Mriziq et al.: 'Higher Sensitivity and Improved Resolution Micro flow UHPLC with Small Diameter Turbo V™ Source Electrodes and Hardware for use with the ExpressHT™-Ultra System' Technical Note Eksigent (2011) # 4590211-01
2. European Mycotoxins Awareness Network: 'Mycotoxins Legislation Worldwide (last updated February 2012)', http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=79

## TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures.