# Micro Flow LC and its Application on Food Safety Analysis

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## Overview

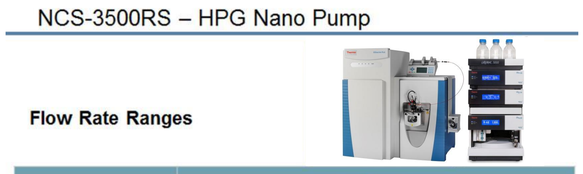
**Purpose:** To demonstrate a robust and high sensitivity quantitative method for pesticies in Food matrices utilizing microflow LC-MS/MS.

**Methods:** Nine samples matrices which diluted 1000 times and spiked with 381 pesticides with 5 concentrations from 20 ng/L (20ppt) to 1000 ng/L (1000 ppt or 1 ppb) were directly injected for microflow LC and attached to a Q Exactive High Resolution Mass Spectrometer for sample analysis.

## Introduction

Operating electrospray in the low nL/min flow rate was wildly used by proteomic research in the past two decades which can significantly improve the ionization efficiency on analytes, as well as reduce ion suppression and ionization bias during the electrospray ionization (ESI) process. However, the column of the nano flow LC suffered the inconsistency due to the column loading factor for food sample matrices. Also most liquid chromatography mass spectrometry (LC-MS) based analyses operate at much higher flow rate than required for nano-electrospray operation. This flow rate mismatching generally limits the utility of nano-ESI in most LC-MS applications.

We present an ESI source and MS interface that effectively combine micro-flow LC to achieve high sensitivity in LC-MS analysis. The only modification is changing all transfer tubing by using 50 µ ID of Viper connectors and also change the ESI capillary metal tubing too. The system is shown in Figure 1, which can be switching between three operation mode by changing the flow selector (Figure 2) and can convert from nano to capillary and also micro flow mode. The switching is taking less than 10 minutes.




| Flow Selector Type | Total Flow Rate (Sum of Channel A and B) | | |
|---|---|---|---|
| | Nominal | Minimum | Maximum |
| NAN | 500 nl/min | 50 nl/min | 1000 nl/min |
| CAP | 5 µl/min | 500 nl/min | 10 µl/min |
| MIC | 25 µl/min | 2.5 µl/min | 50 µl/min |

**Figure 1. Ultimate 3000 RSL – HPG Pump with Q Exactive**

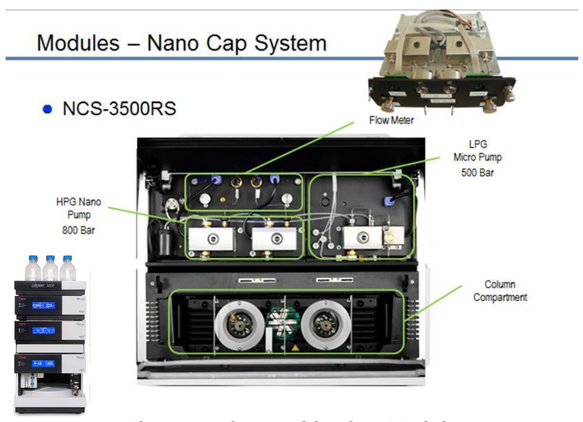



**Figure 2. Changeable Flow Module**

Micro Flow LC parameter is shown in Figure 3, the 1 mm x 100 mm column was using in this study. The 0.32 x 100 mm, 1.9 u, C-18 Hypercial aQ column was used at beginning of the experiment design for the best of the separation, however, due the high back pressure from 0.32 mm column which back pressure can be exceed the 800 Bar limits over 20 µl/min of flow rate. The system consists two set of pumps, one is HPG 800 Bar binary Nano pump and the other one is LPG 500 Bar ternary pump. Both pump can be operated independently, the original design is to use LPG pump as loading pump for in line SPE clean up which has building valves in the column compartment.

**Table 1. MFLC parameters**

| | |
|---|---|
| **MFLC** | Ultimate 3000 RSL |
| **Flow rate** | 30 µL/min |
| **Mobile phase** | A= Water with 0.1% FA and 5 mM ammonium formate<br>B= Methanol with 0.1% FA and 5 mM ammonium formate |
| **Gradient** | 0% B - 100% B (12 min), 100% B (14 min), 10% B (17 min) |
| **Analytical column** | Acclaim™ C18, 3 um, 1 x 150 mm |
| **Column temperature** | 40ºC |
| **Injection volume** | 5 µl |
| **MS Q Exactive Ionisation** | Electrospray, Positive mode |

## Results and Discussion

The results shown in Figure 4, is clearly demonstrated that the same 1 ppb sample will give the response of 2.21 E6 versus 2.27 E7 by inject the same amount of sample and kept the FWHM of chromatographic peak at the same with the flow rate of 300 µl/min versus 30 µl/min. The sensitivity increased about 10 times. The solvent usage reduced 90% and with the benefit which has much less solvent needs to disposal.

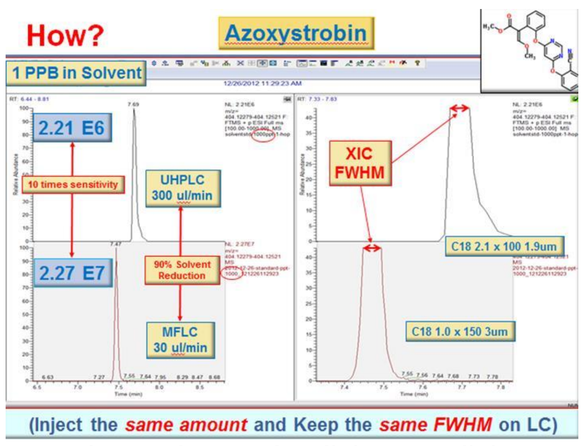



**Figure 4. Flow rate of 30 µl/min vs. 300 ul/min**

The reason for this sensitivity increase can be explained in the Figure 5. Peak concentration pC is defined as the mass of injection divided by peak volume which is the total solvent eluted under the chromatography peak. Since the injection volume is the same then the peak concentration is depending the solvent volume under peak. In this case the volume under the MFLC peak is 6 µl with a 12 seconds peak width and the peak volume under the UHPLC is 60 µl.

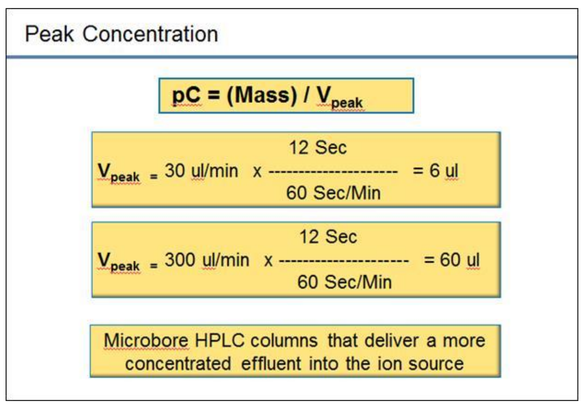



**Figure 5. Peak Concentration Calculation**

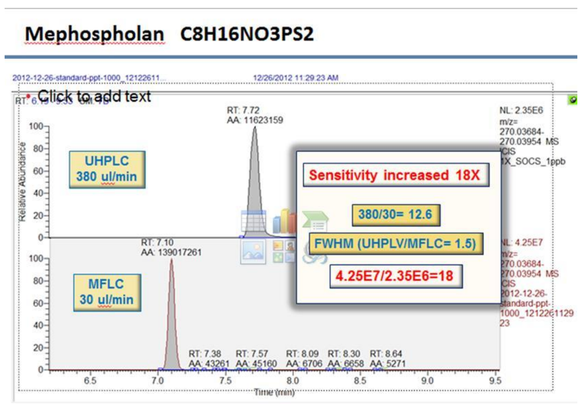



**Figure 6. Sensitivity increase, 30 µl/min *vs* 380 µl/min**

The additional calculation will be applied if the peak width is not equally. Figure 6 demonstrated that the sample run under the UHPLC had the peak width 50% more than the sample run under the MFLC. The calculation will involved with the factor that 380/30 equals to 12 then multiplied by a factor of 1.5 and this will result a 18 times better sensitivity. The calculation from 2.35E6/4.25E7 did show a 18 times increase on sensitivity. Figure 7 is a list of compounds on this study and the difference on the sensitivity gains represent in Red are the peak intensity between MFLC and UHPLC.

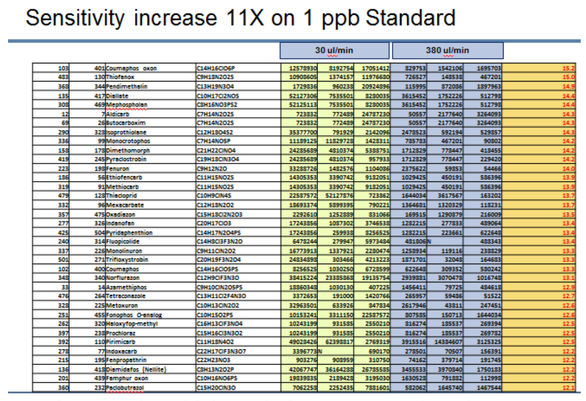



**Figure 7. Sensitivity gain between MFLC and UHPLV**

The calibration curve of 381 pesticides by using MFLC can be seen on Figure 8 on nine different matrices as Spinach, Honey, Wheat Flour, Raisin, Beet, Hazelnut, Orange, Clementine Orange and Avocado. There are 5 levels of calibration of 20, 100, 200, 400 and 1000 ng/L (ppt), and each level consists of 4 samples, 2 of them are solvent standards and the 2 are 1000 times dilution matrix standard. It is clearly there is no matrix interference in Azinphos methyl oxone among all matrices since it matches very well between solvent standard and matrix matched standard.

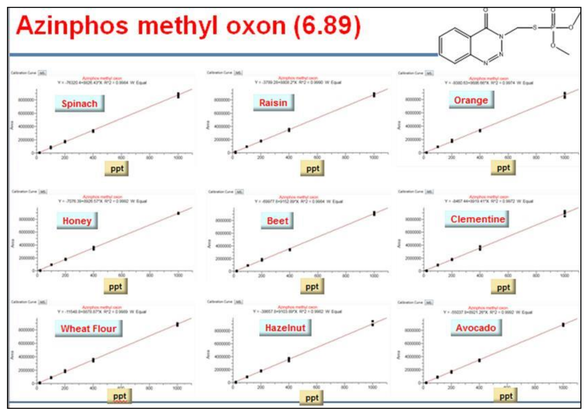



**Figure 8. Azinphos methyl oxon in 9 matrices**

## Conclusion

- A simple and highly sensitive microflow-based LC-MS/MS method was developed to quantify Pesticides in Food matrices with more than 10 times of sensitivity improvement.
- ESI is a concentration dependent source and less amount of solvent will be in favor of the detection for the mass spectrometer.
- MFLC will use significant less amount of solvent during sample analysis, typically a 90% less solvent will be using, this is a significant cost saving in laboratory operation.
- Additionally, it has much solvent to dispose and extremely Environmental friendly, since laboratory is one of the pollution source to the environment.
- The workflow described in this study can be adopted for quantitation of more classes of containments in food such as mycotoxins, vet drugs, marine toxins and antibiotics with a better detection limits.

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