

# Comparison of micro-flow and high-flow UHPLC/MSMS (incorporating polarity-switching) for the quantitative determination of multiple pesticide residues present in strawberry crude extract.

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## Introduction

SASA participates in the annual UK and coordinated EU surveillance programmes that monitor various UK and imported food & drink for the presence of pesticide residues and their metabolites on behalf of the Scottish Government.

Matrix effects in pesticide residue analysis are a constant concern for laboratories engaged in monitoring schemes. Various sample preparation and clean-up strategies can help minimise or ideally eliminate the effects of matrix. However, laboratories that prefer to analyse crude extracts directly by LC/MSMS are attracted to complementary instrument technologies that could improve this analysis of 'dirty' samples.

In our evaluation, we decided to compare micro-flow (25 µl/min) and high-flow (400 µl/min) LC/MSMS experiments via the analysis of crude extracts collected from strawberry samples received as part of the 2013 UK Pesticide Residues in Food annual surveillance program. A retail strawberry sample with incurred residues was selected to show the results from micro-flow LC and high-flow LC were comparable. In total, 192 pesticides and their metabolites were sought using a single Multiple Reaction Monitoring (MRM) experiment incorporating pos/neg ESI switching and scheduled MRM i.e. 364 MRMs in positive mode and 20 MRMs in negative mode.

## Experimental

### Sample preparation

10g of cryomilled strawberry samples were homogenised with ethyl acetate (matrix concentration ≅ 1g ml<sup>-1</sup>). Sample extracts were then solvent exchanged into methanol and filtered (0.45µm PTFE). No clean-up was employed.

Calibration standards were prepared in (organic) strawberry matrix.

Set-up for the ABSCIEX 5500QTRAP mass spectrometer and Shimadzu Nexera UHPLC

- Run time: 17 min
- Flow rate: 0.4 mL/min
- Mobile phase A: Water+5mM ammonium acetate
- Mobile phase B: Methanol+5mM ammonium acetate
- Column: Phenomenex Kinetex 2.6 µm, C18, 50x4.6mm with Phenomenex KrudKatcher (a disposable in-line filter)
- Injection volume: 3 µl
- Matrix-matched standards and real samples with incurred residues (extracted from strawberries (1g/mL matrix) in methanol)

Table 1.

Time (min)	%A	%B
0.1	75	25
0.7	40	60
11.8	2	98
13.8	2	98
14	75	25
17	75	25

Set-up for the ABSCIEX 5500QTRAP mass Spectrometer and Eksigent ekspert™ micro-LC200 system

- Run time 11 minutes
- Flow rate = 25 µl/min
- Mobile phase A: Water+5mM ammonium acetate
- Mobile phase B: Methanol+5mM ammonium acetate
- Column Triart C18 0.5 µm x 100mm
- Injection volume = 12 µl
- Matrix-matched standards and real samples with incurred residues (extracted from strawberries (1g/mL matrix) in methanol).

Table 2.

Time (min)	%A	%B
0	98	2
1	98	2
1.2	40	60
8	2	98
8.5	2	98
8.6	98	2
10	98	2

Note: Samples diluted 1:4 in eluent to reduce matrix effects and decrease system contamination.

## Results

This comparison shows that the micro-flow LC produced peaks of similar chromatographic integrity when compared with normal flow rates. Sensitivity i.e. peak areas were increased when micro-flow was used. The study also highlights that quantitative measurements between micro-flow and high-flow systems were comparable (table 3).

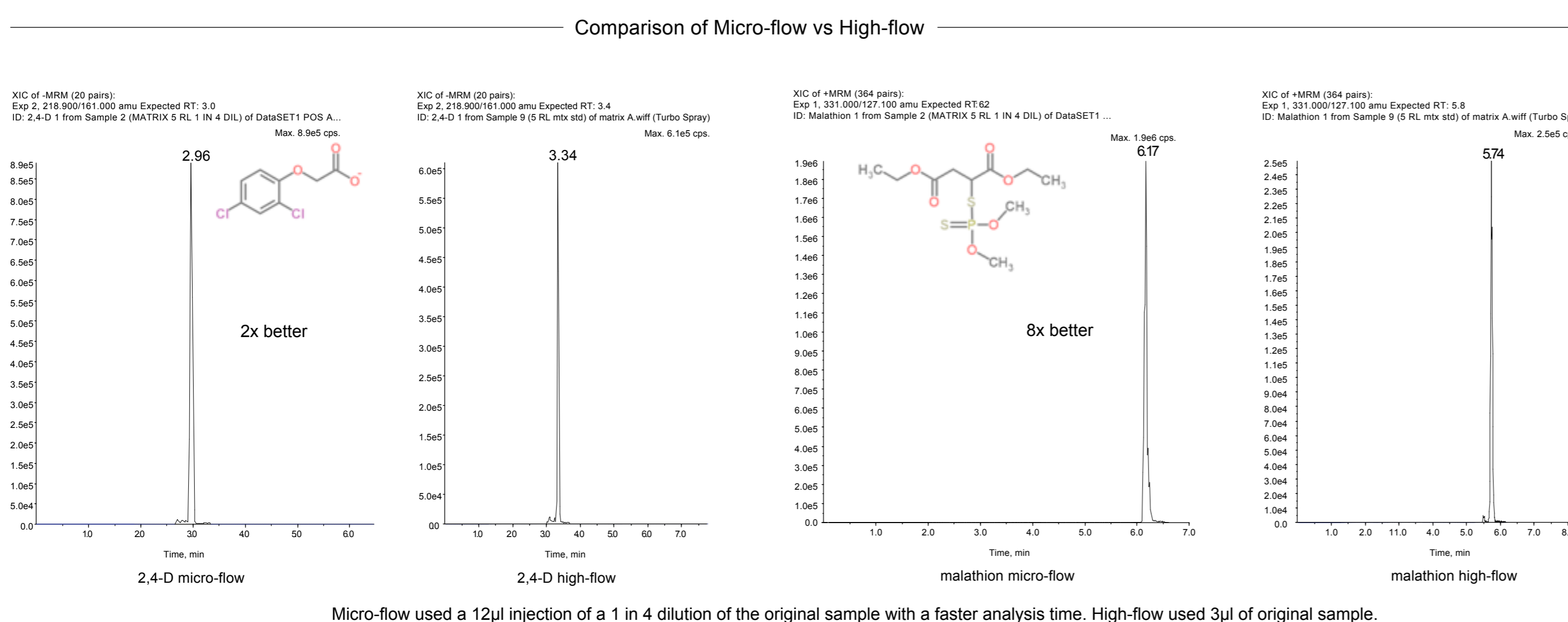
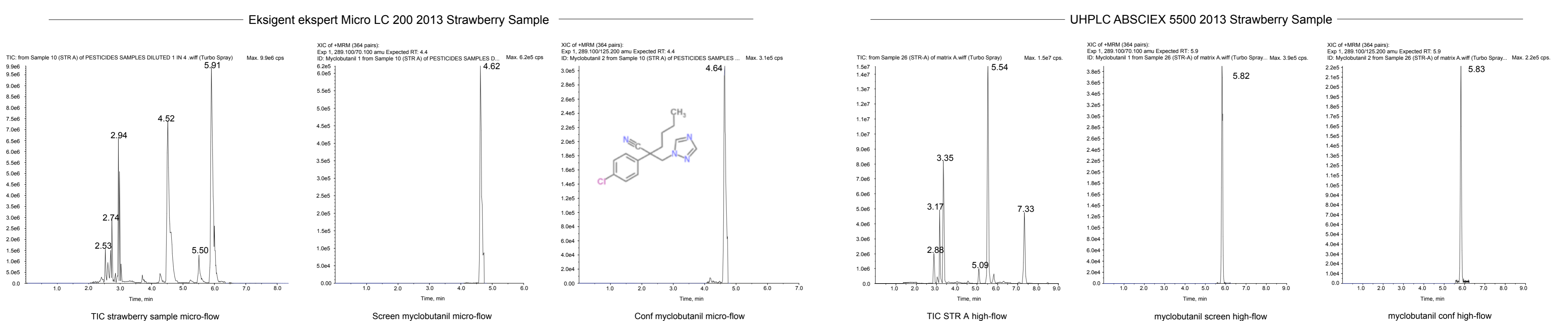


Table 3. Comparison of Incurred Residues in a 2013 Strawberry Sample

Residue	*UPLC Waters Premier XE (mg/kg)	UHPLC ABSCIEX 5500 (mg/kg)	Eksigent ekspert Micro LC 200 (mg/kg)
myclobutanol (+ve)	0.035	0.034	0.031
fludioxonil (-ve)	0.26	0.23	0.25



\*Original SASA quantitation required separate runs for screen and confirmation.



## Conclusions

This initial comparison study of micro-flow LCMS/MS versus high-flow LCMS/MS has demonstrated that micro-flow LCMS/MS can provide superior sensitivity for the analysis of multiple pesticide residues present in 'dirty' samples. Dilution of sample extract can reduce matrix effects thereby increasing the robustness of the analysis. Micro-flow can also offer significant cost savings due to high throughput of samples and less solvent consumption. In total 25x less solvent used per injection plus faster overall cycle time using this particular micro-flow method.