ADVANCING ENDOCRINE DISRUPTING COMPOUND ANALYSIS THROUGH INTEGRATED TECHNOLOGY AND WORKFLOW SOLUTIONS

Paul Silcock[1], Keith Worrall[1], Cecilia Mazza[1], Joe Romano[1], Alan Wainwright[2], and Chris Hunter[2]
[1]Waters Corporation, [2]Environment Agency - National Laboratory Service, UK

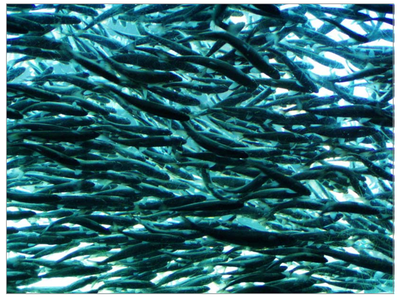
INTRODUCTION

Endocrine disrupting compounds (EDCs) have caused increased concern for organizations that monitor their occurrence in environmental and potable waters. These compounds often have physiological effects to humans and wildlife at very low concentrations.[1]

One class of EDCs are the estrogenically active substances. These, of course include natural and synthetic estrogens as well as alkylphenol compounds that mimic at the estrogenic receptor.[2] There is a need to monitor these compounds reliably to low parts per trillion (ppt) concentrations in often complex samples such as environmental surface waters and treated sewage. To achieve this, sophisticated sample preparation chemistries and powerful analytical systems are required in combination. Different approaches have been compared to analyze estrogenic substances and LC/MS/MS is highly applicable.[3]

Development and setup of reliable, highly sensitive, multi-analyte methods using LC/MS/MS often requires a significant time and resource investment from organizations, in addition to their current responsibilities. This means that the speed at which a quality result can be produced is a key parameter. With this in mind the ability to quickly setup and run new high performance methodologies quickly is clearly desirable.

LC/MS/MS traditionally offers selective and sensitive analysis in a targeted fashion. While this is still the priority for this type of instrumentation, there are advantages in acquiring information simultaneously that are non-targeted and can offer intra-sample quality control or discovery of non-targeted components.



METHODS

Sample preparation

Spiked groundwater, river water, and sewage effluent extracts were prepared using Waters Oasis HLB glass 5-cc/200-mg SPE cartridges. The protocol employed is based on a method described in the Waters' Environmental Chromatography Methods Guide (no.720002543en) with final extract solvent composition mobile phase matched.

UPLC conditions

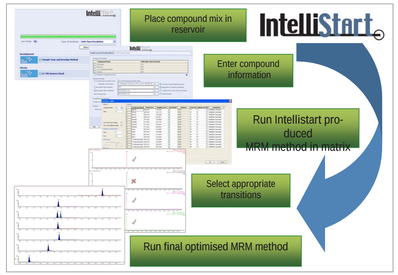
LC System:	**ACQUITY UPLC®**
Runtime:	5.30 min
Column:	ACQUITY® BEH C18 1.7 µm, 2.1 x 50 mm
Column Temp:	40°C
Mobile Phase:	A: 0.05% NH4OH (aqueous) B: MeOH
Flow Rate:	0.6 mL/min
Inj Volume:	10 µL

Time (min)	Flow rate	%A	%B
1. Initial	0.60	65.0	35.0
2. 3.00	0.60	5.0	95.0
3. 4.20	0.60	5.0	95.0
4. 4.30	0.60	65.0	35.0

MS conditions

MS System:	**Xevo TQ MS**
Acquisition Mode:	**Dual Scan-MRM**
Ionization Mode:	ESI Negative
Source Temp:	150 °C
Desolvation Temp:	650 °C
Desolvation Gas:	1100 L/hr
Cone Gas Flow:	20 mL/min
Collision Gas Flow:	0.18 mL/min

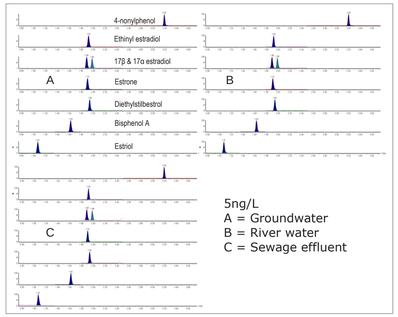
EDC Multiple Reaction Monitoring (MRM) conditions were automatically determined using Intellistart on XEVO TQ MS and this was incorporated into a simple method development workflow.



RESULTS & DISCUSSION

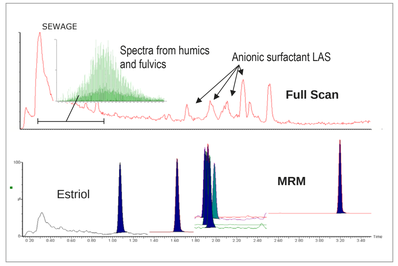
MRM selectivity and sensitivity

Spiked extracts of groundwater, river water, and sewage effluent showed high instrument selectivity and sensitivity to sub-ng/L levels with sample pre-concentration using Oasis HLB solid phase extraction. ACQUITY UPLC maintained good resolution between the critical pair 17α and 17β estradiol while eluting the last component at 3.2 min. This allowed a high sample throughput through the analytical system.

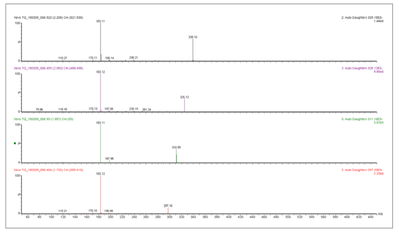


Dual Scan-MRM matrix monitoring for method development and QC

Full scan spectra were acquired alongside quantitative MRMs to monitor the background matrix in the each sample. This allows acquisition of data that is often missed during routine quantitative analysis and can help to highlight areas where methodology can be improved, offer intra-sample QC as well as provide information about non-targeted compounds.



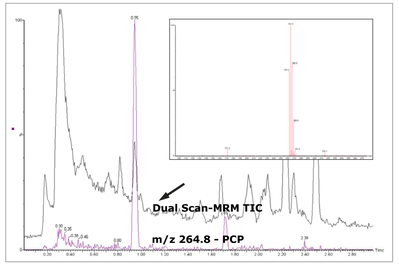
Using this acquisition mode, it was possible to discover background matrix components that originated from the sample and/or laboratory processes. Humic and fulvic substances, which could potentially cause undesirable matrix effects, can be seen eluting prior to the first analyte peak (estriol) giving higher confidence in the quantitative performance of that targeted component. In addition, anionic surfactant LAS (Linear alkylbenzene sulphonate) at high concentration can be observed in the chromatogram with spectra giving ions at 297, 311, 325, and 339 *m/z*. This was further confirmed using ScanWave™ product ion scanning revealing an intense characteristic 183 *m/z* product ion from each.



The specificity of the MRM acquisition allows quantitation of target analytes in the presence of matrix peaks, but the ability to investigate potential matrix effects for every sample can allow additional QC checks to be made and the continuous improvement of methodologies.

Dual Scan-MRM matrix monitoring for non-MRM targeted compounds

Dual Scan-MRM can be also used to retrospectively observe compounds in a sample that are not targeted in the original MRM experiment. To demonstrate this, another sewage extract was spiked to 20 ng/L equivalent with pentachlorophenol and analyzed alongside target MRMs for EDCs in Dual Scan-MRM mode.



CONCLUSION

- **IntelliStart Technology on Xevo TQ MS can streamline the workflow process of developing highly sensitive MRM acquisition methods for endocrine disrupter analysis. This means that less time is taken to set up methods and results can be generated on real samples faster.**
- **Xevo TQ MS gives high sensitivity and selectivity when applied to measurement of low levels of EDCs in groundwater, river water, and sewage effluent.**
- **ACQUITY UPLC allows high sample throughput while maintaining resolution of the critical isomers 17α and 17β estradiol.**
- **Dual Scan-MRM mode allows full scan data to be acquired simultaneously with MRM. This in turn allows matrix monitoring for method development, QC purposes, as well as discovery of non-targeted components.**

References

1.Colborn T, Soto A, vom Saal F. 1993 Environmental Health Perspectives 101(5):378–384.
2.A Pillon et al., Environ Health Perspect. 2005 113(3): 278–284.
3.M Silvia Díaz-Cruz et al. Journal of Mass Spectrometry. 2003 38(9): 917–923.

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