Note d'application

Advancing Endocrine Disrupting Compound Analysis Through Integrated Technology and Workflow Solutions

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Abstract

This application note describes the use of Oasis HLB sample preparation in combination with Xevo TQ MS for the analysis of endocrine disrupters to low ppt concentrations in groundwater, river water, and sewage effluent.

Benefits

- Using Solid Phase Extraction for isolation and enrichment of Endocrine Disruptors
- Use of LC-MS/MS with RADAR functionality to simultaneously acquire full scan data while maintaining the quality of MRM data
- Method development using IntelliStart Software
- Quantification of Endocrine Disruptors at low part-per-trillion concentrations

Introduction

Protecting the environment for present and future generations is an important responsibility undertaken by organizations all over the globe. It allows for higher quality of life, both directly and indirectly by reduced exposure to pollutants, maintenance of ecosystems, and better health through safer food and water supplies. For companies using water for consumed products or supplying water, the efficiency of treatment processes and quality of final products is also of high importance. For regulators, a key part of protecting both the environment and consumers is through the monitoring and discovery of substances of concern. This becomes increasingly important due to the high publicity related to newer emerging contaminants.

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] 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.

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.

This application note describes the use of Waters Oasis HLB sample preparation in combination with Xevo TQ MS for the analysis of endocrine disrupters to low ppt concentrations in groundwater, river water, and sewage effluent. It also describes the use of intelligent workflow tools for method development, as well as advanced LC-MS/MS analysis using Waters RADAR functionality to simultaneously acquire full scan data while maintaining the quality of MRM data.

Photo: Dave Gostisha/Ziptrivia llc

Experimental

UPLC conditions

System:	ACQUITY UPLC
Runtime:	5.30 min
Column:	ACQUITY BEH C_{18} 1.7 µm, 2.1 x 50 mm
Column temp.:	40 °C
Mobile phase A:	0.05% NH_4OH (aqueous)
Mobile phase B:	MeOH
Flow rate:	0.6 mL/min
Injection volume:	10 µL

Gradient

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

Time (min)	Flow rate	%A	%B
4. 4.30	0.60	65.0	35.0

MS conditions

MS system: Xevo TQ

Acquisition mode: RADAR Dual Scan-MRM

Ionization mode: ESI-

Capillary voltage: 2.0 kV

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

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 (p/n 720002543en) with final extract solvent composition mobile phase matched.

Xevo TQ MS setup (mass resolution, mass calibration, ion source optimization) was automated by IntelliStart Software. IntelliStart was also used to automatically develop fully optimized MRM acquisition methods for the endocrine disruptors targeted in this analysis. IntelliStart requires only the entry of basic compound information and automatically locates the precursor ion, optimizes cone voltage, locates product ions, and optimizes collision energy. Extracts from the IntelliStart method development report are shown in Figure 1.

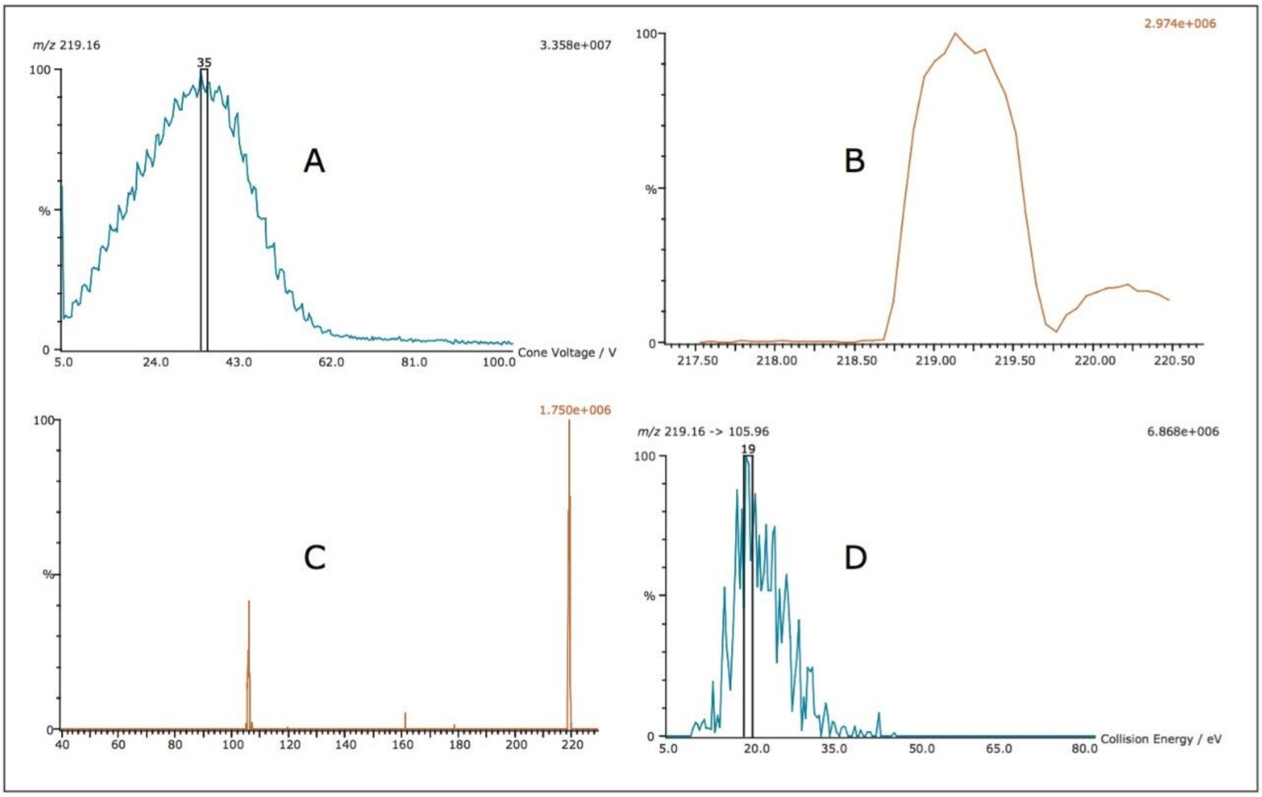


Figure 1. Extract from IntelliStart method development report showing optimization of cone voltage (A), optimized MS spectrum (B), location of product ions (C), and optimization of collision energy (D) for 4-nonylphenol.

The applied Xevo TQ MS method development workflow is shown in Figure 2. Multiple MRM transitions were produced by IntelliStart, and a selection of these were used based on selectivity and sensitivity in matrix. Table 1 provides a selection of the IntelliStart-produced MRM conditions for the endocrine disrupting compounds analyzed on Xevo TQ MS. Following IntelliStart method development, compound information along with key analytical parameters were exported to the QUANPEDIA compound database.

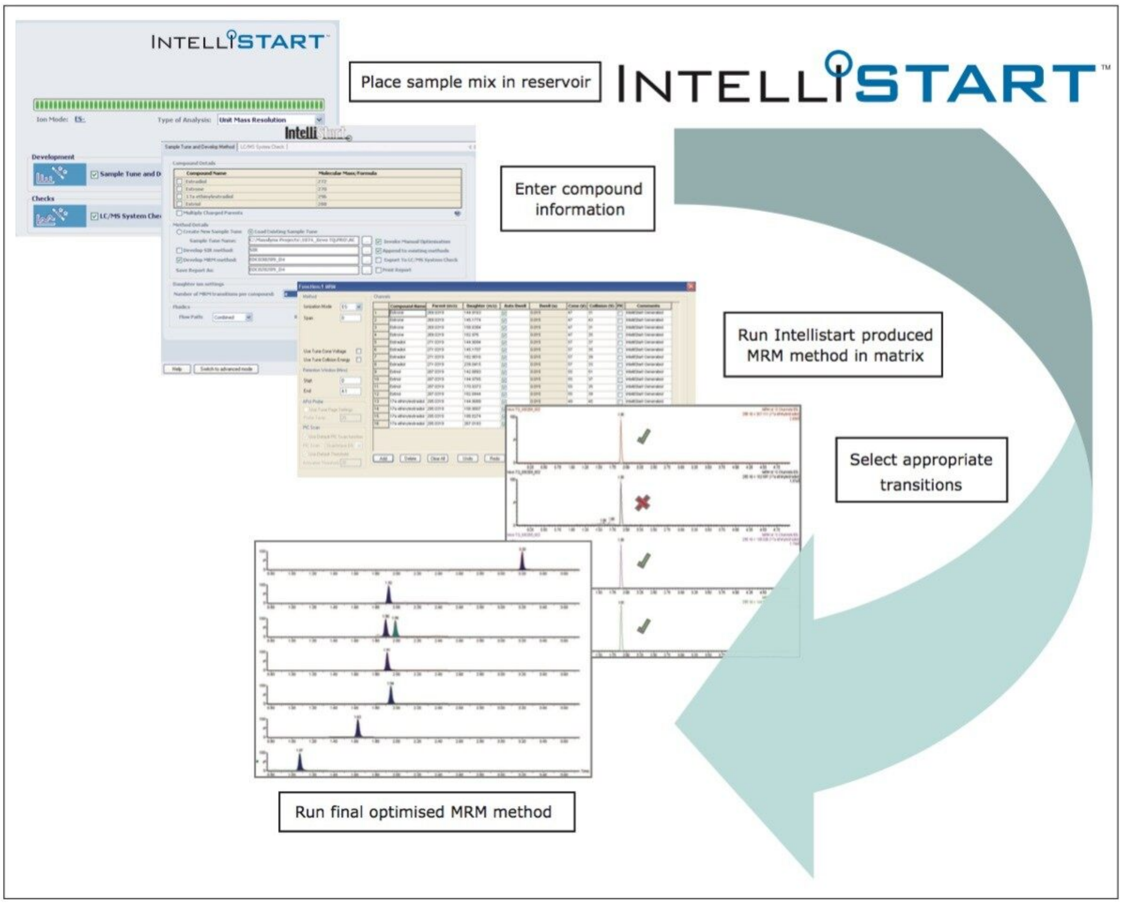
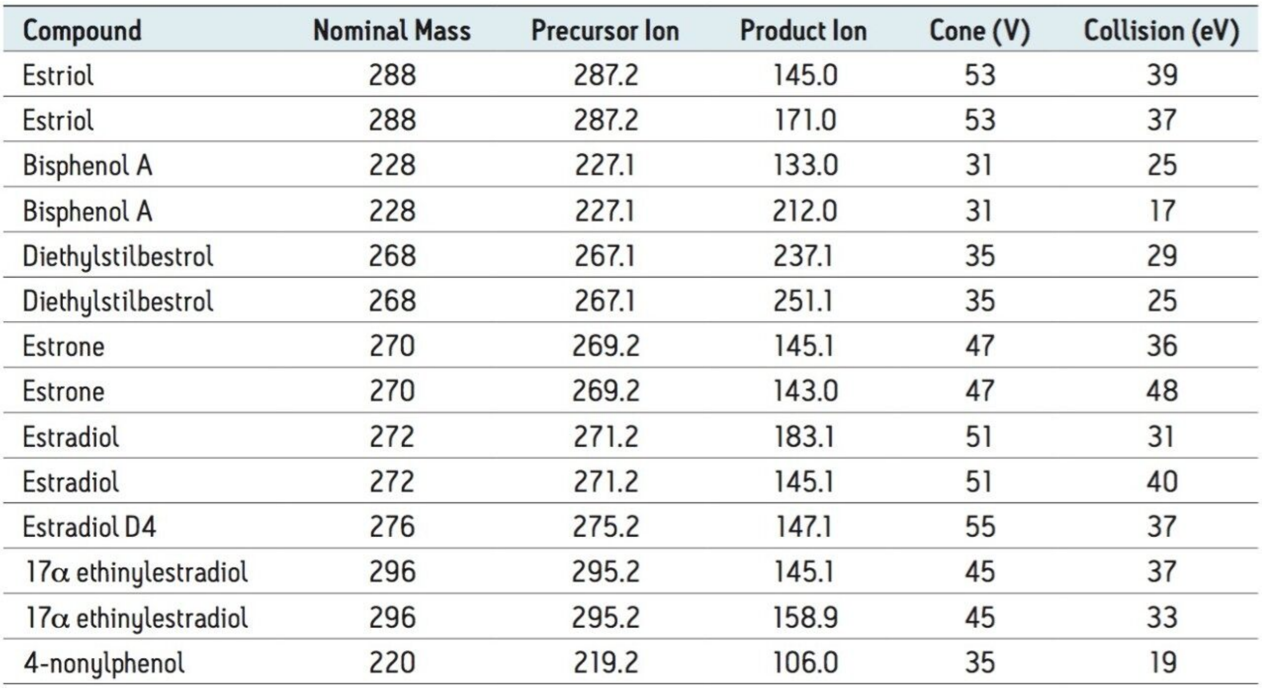


Figure 2. IntelliStart method development workflow on Xevo TQ MS.



Compound	Nominal Mass	Precursor Ion	Product Ion	Cone (V)	Collision (eV)
Estriol	288	287.2	145.0	53	39
Estriol	288	287.2	171.0	53	37
Bisphenol A	228	227.1	133.0	31	25
Bisphenol A	228	227.1	212.0	31	17
Diethylstilbestrol	268	267.1	237.1	35	29
Diethylstilbestrol	268	267.1	251.1	35	25
Estrone	270	269.2	145.1	47	36
Estrone	270	269.2	143.0	47	48
Estradiol	272	271.2	183.1	51	31
Estradiol	272	271.2	145.1	51	40
Estradiol D4	276	275.2	147.1	55	37
17α ethinylestradiol	296	295.2	145.1	45	37
17α ethinylestradiol	296	295.2	158.9	45	33
4-nonylphenol	220	219.2	106.0	35	19

Table 1. Selection of IntelliStart EDC MRM conditions.

In addition to MRM data, full scan data were acquired using the RADAR Dual Scan-MRM mode of the Xevo TQ MS. This functionality allows real time qualitative information about the nature of the sample matrix to be acquired at the same time as routine quantitative analyses.

Data acquisition and processing

MassLynx Software v.4.1 was used for data acquisition and spectral processing. TargetLynx Software was used to process MRM data, and TrendPlot was used for over-batch comparisons of selected parameters.

Results and Discussion

Spiked extracts of groundwater, river water, and sewage effluent showed high instrument selectivity and sensitivity to sub-ng/L levels with sample preconcentration 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. Figure 3 shows groundwater, river water, and sewage effluent extracts spiked at 1 ng/mL (equivalent to 5 ng/L with

Oasis HLB pre-concentration).

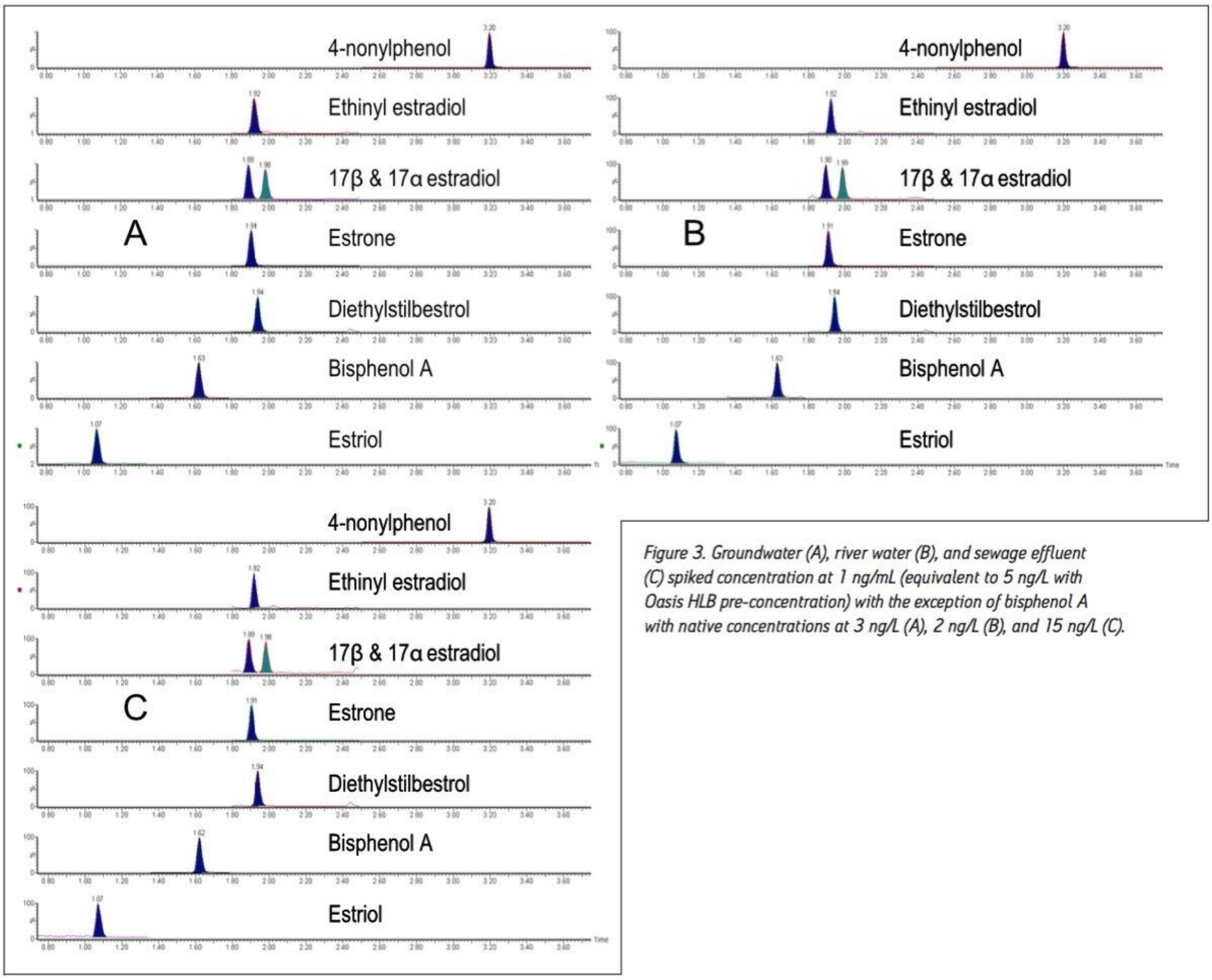


Figure 3. Groundwater (A), river water (B), and sewage effluent (C) spiked concentration at 1 ng/mL (equivalent to 5 ng/L with Oasis HLB pre-concentration) with the exception of bisphenol A with native concentrations at 3 ng/L (A), 2 ng/L (B), and 15 ng/L (C).

During data processing, the TrendPlot functionality of TargetLynx was used to trend some intra- batch parameters to monitor for any anomalies. Figure 4 shows internal standard D4 β-estradiol peak area plotted over one of the sample batches. This allows easy identification of possible spiking errors.

RADAR

Dual Scan-MRM matrix monitoring Full scan spectra were acquired alongside quantitative MRMs to monitor the background matrix in the 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, as well as provide information about non-targeted compounds.

Matrix monitoring for method development and QC

Figure 5 shows matrix monitoring of a spiked sewage effluent using RADAR Dual Scan-MRM mode. 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, suspected 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.

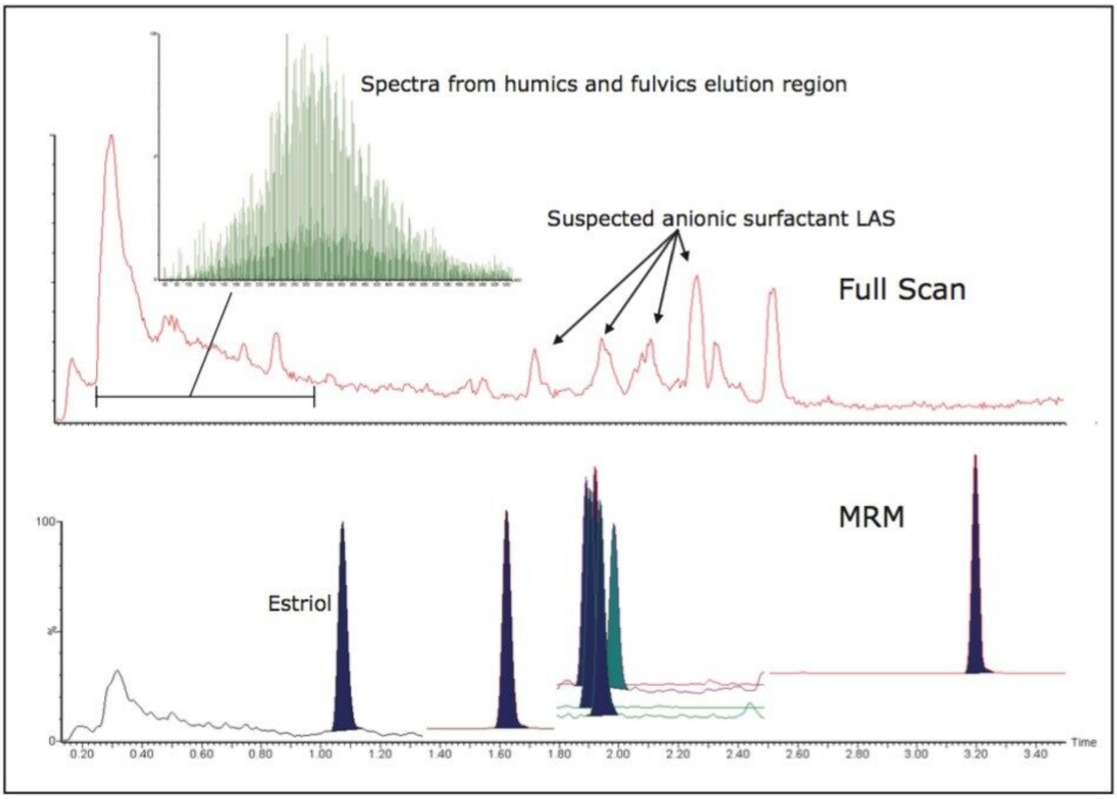


Figure 5. Matrix Monitoring of spiked sewage effluent using RADAR Dual Scan-MRM mode.

Matrix monitoring also allows observation of target ions that co-elute with matrix components. Figure 6 shows an expanded region of the same spiked sewage sample with co-elution of some matrix components including LAS. 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.

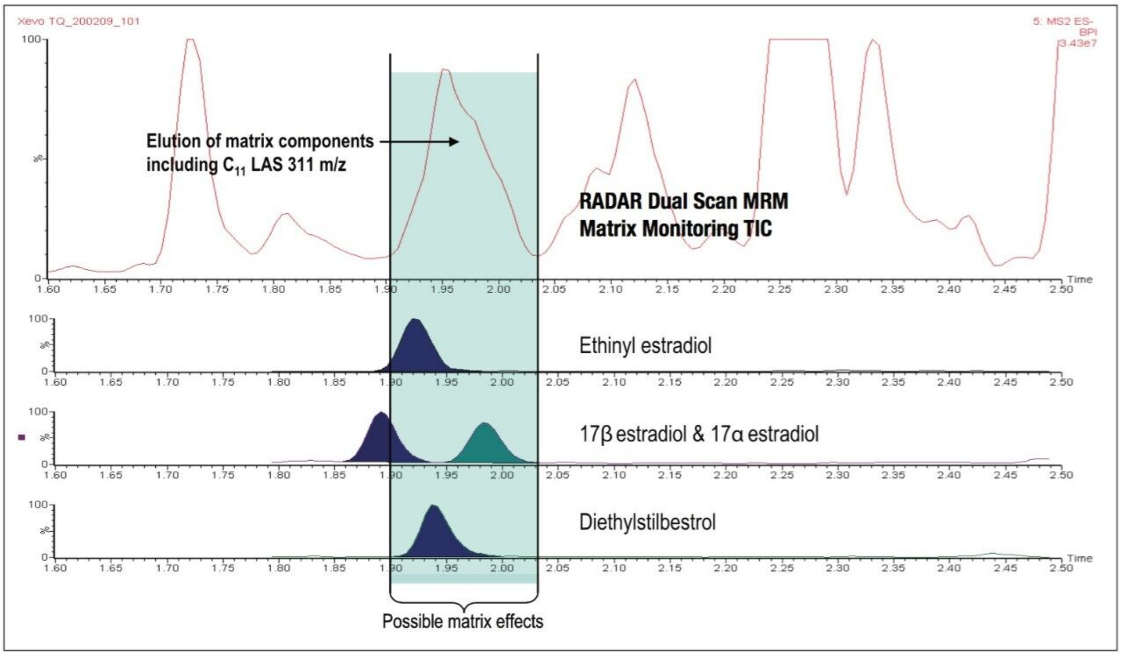


Figure 6. Expanded region of the same spiked sewage sample with some co eluting of some matrix components including LAS, observed using RADAR Dual Scan-MRM matrix monitoring.

Matrix monitoring for non-MRM targeted compounds

Using RADAR Dual Scan-MRM in a routine quantitation also allows for a retrospective look at non-targeted data acquired from a sample in the light of new information about potential contaminants.

RADAR Dual Scan-MRM can be used to 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 RADAR Dual Scan-MRM mode. Figure 7 shows overlaid TIC and extracted 264.8 *m/z* for the pentachlorophenol spiked sewage effluent, as well as a combined mass spectrum taken from the peak observed in the TIC.

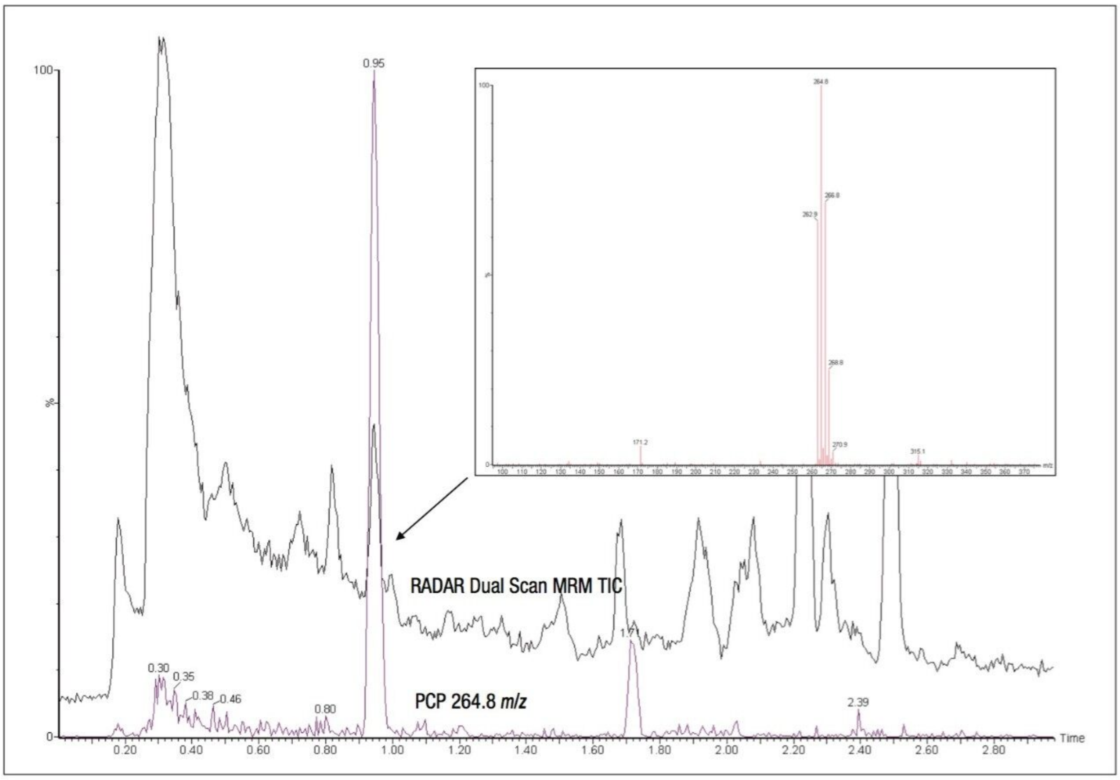


Figure 7. Overlay of TIC and extracted 264.8 m/z for the pentachlorophenol spiked sewage effluent, as well as a combined mass spectrum taken from the peak.

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.

The TrendPlot tool allowed for intra-batch anomalies in data to be quickly discovered and helps ensure quality results are produced.

RADAR 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. *Environmental Health Perspectives.* 1993 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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