

Trace determination of Octyl&Nonyl-phenols, related Ethoxylates and Bisphenol A using on-line SPE and Orbitrap LCMSMS

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ABSTRACT

Purpose: To demonstrate the feasibility of using a Thermo Scientific Focus Hybrid Quadrupole-Orbitrap MS with a Thermo Scientific™ EQuan MAX Plus™ system to meet method detection limits for compliance with EU Water Framework Environmental Quality Standards (EQS) for bisphenol A and key Alkylphenol and related Ethoxylates.

Methods: EQuan on-line SPE High Resolution Accurate Mass using Selected Ion Monitoring (SIM) and Parallel Reaction Monitoring (PRM).

Results:

Component	EQS Surface/ Marine Waters (ng/L)	Required LoD(ng/L)	Achieved LoD* Surface/ Marine/ Ground waters	Achieved LoD* WWTW effluent	(1) Predicted No Effect Concentration	(2) Proposed value
NP-1-EO	4800 (1)	480	50	50		
NP-2-EO	4800 (1)	480	7	7		
NP-3-EO	4800 (1)	480	7	7		
OP-1-EO	N/A	N/A	90	140		
OP-2-EO	N/A	N/A	4	15		
OP-3-EO	N/A	N/A	5	5		
4-Nonylphenol (branched)	300 (total NP)	30	15	25		
4-n-Nonylphenol			10	20		
Bisphenol-A	1400 (2)	140	40	60		
4-t-Octylphenol	100/10	10/1	4	4		

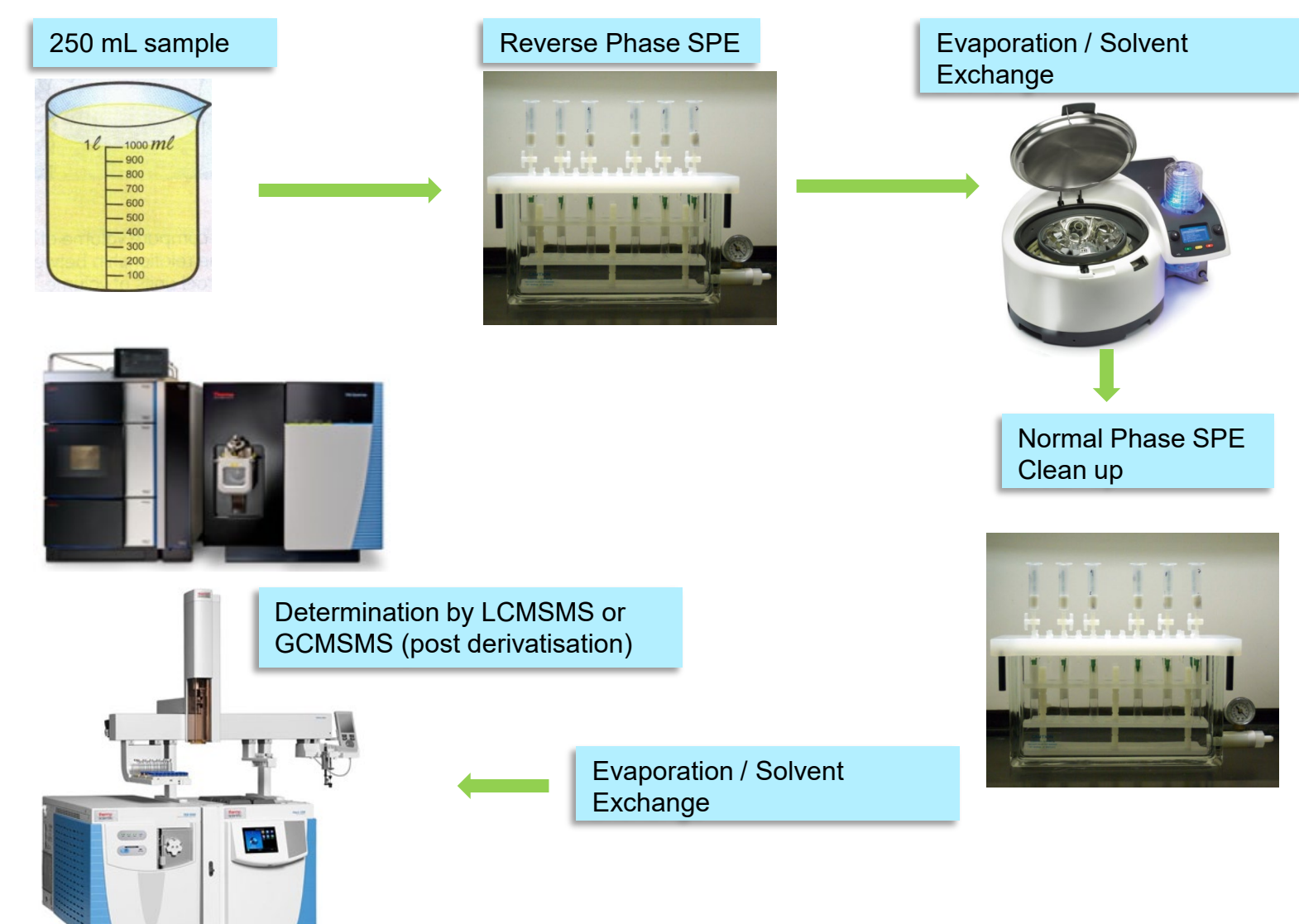
INTRODUCTION

The occurrence and effects of endocrine disrupting compounds (EDCs), particularly mixtures, in aquatic environments is a significant concern¹. Of the many EDCs, bisphenol A, Alkylphenols and related Ethoxylates are recognised as possessing significant estrogenic potency and risk to freshwater ecosystems and drinking water resources². Due to their environmental significance, the compounds were incorporated into the EU Water Framework Directive³, with low EQS limits of detection, which present a significant analytical challenge, particularly for routine monitoring methods requiring high throughput.

Current methods generally involve large-volume SPE; normal phase SPE clean up and size exclusion fractionation, derivatisation and GCMS or LCMS determination, which take considerable time, expense, and sampling logistics (Figure 1).

The aim of this work is to assess the feasibility and performance of using a 2.5 mL sample on-line solid phase extraction and a Q Exactive Focus Orbitrap MS, for the determination of the targets at the required EQS MDLs.

Figure 1. Typical workflow for Bisphenol A, Alkylphenols and related Ethoxylates analysis.



MATERIALS AND METHODS

Liquid Chromatography

Liquid Chromatography separations were carried out on EQuan MAX Plus™ system including binary analytical pump, CTC autosampler, quaternary loading pump and column compartment; see Figure 2. Alkylphenols and Bisphenol A were determined with negative ionisation using PRM; and the ethoxylates determined with positive ionisation using SIM. Two separate runs were carried out.

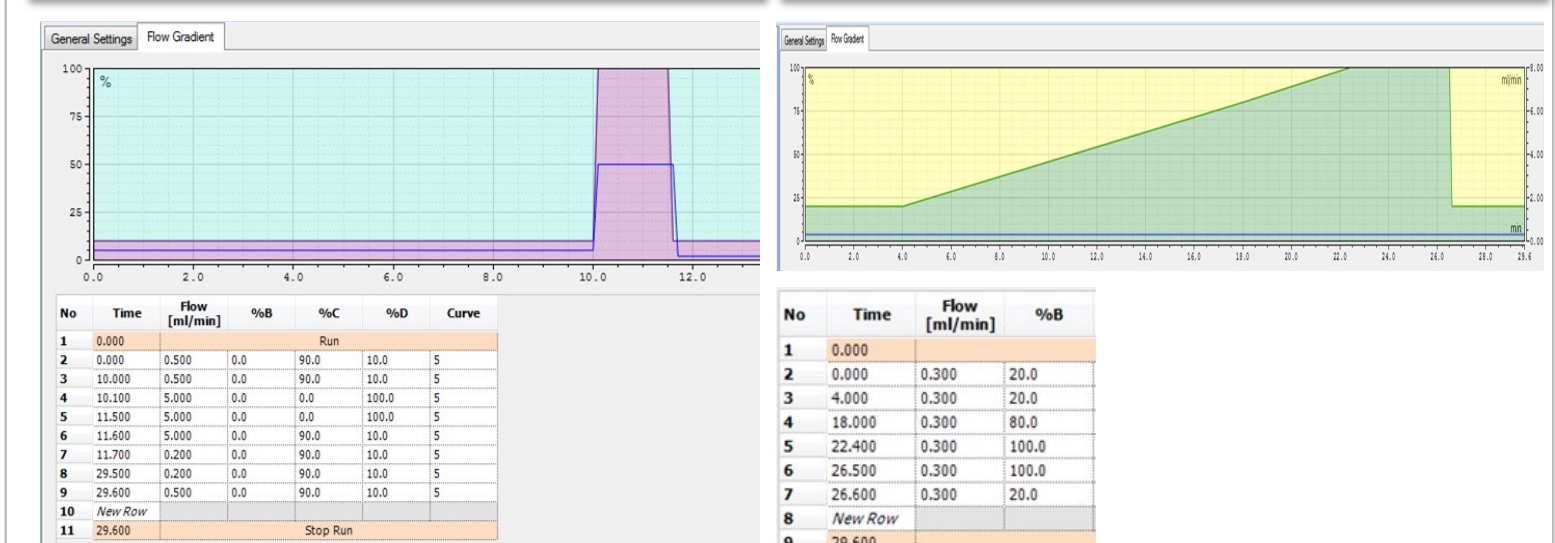
Loading Conditions:
Column: Thermo Scientific™ Hypersil GOLD™ aQ 12 µm 20 x 2.1, 2.6 µm
Injection volume: 2.5 mL
Loading rate: 0.5 mL/min
Loading solvent:
C - H₂O (0.1% Formic acid)
D - Methanol (0.1% Formic acid)

Analytical Conditions:
Column: Thermo Scientific™ Accucore™ aQ 100 x 2.1, 2.6 µm
Injection volume: 2.5 mL
Flow rate: 0.3 mL/min
Needle Wash Solvent: 50% MeOH & 50% ACN

Mobile phase:
A - H₂O (no buffer)
B - MeOH (no buffer)

Syringe and valve Cleaning Solvents:
1 - Acetonitrile: Acetone: Propan-2-ol:- 70:20:10
2 - Acetonitrile.

Post column infusion: Ammonia solution (1.5% w/v in methanol) 11-21 mins, 20µL/min.



Mass Spectrometry

The MS analysis was performed on a Q Exactive Focus bench-top high resolution mass spectrometer using heated electrospray ionization (HESI). The Alkylphenols and Bisphenol A were determined using Parallel Reaction Monitoring (PRM) where MS/MS data were collected at a resolving power of 17,500 (FWHM m/z 200) in negative polarity; see Figure 3. The Alkylphenol ethoxylates were determined using Single Ion Monitoring (SIM) where MS1 data were collected at a resolving power of 70,000 (FWHM m/z 200) in positive polarity.

Figure 2. Q Exactive Focus Mass Spectrometer, showing EQuan On-Line SPE System.



LCMS Analysis

Method Calibration

All calibration standards were prepared in LCMS grade water with 10% LCMS grade methanol; 2.5 mL volumes were used for analysis and the calibration was carried out using internal standardisation for the APs and Bisphenol A and external standardisation for the APEOs. Calibrants were prepared at between 10 and 1200 ng/L, as appropriate for each target.

Acquisition, Processing and Confirmation

The data were acquired, processed and confirmed using Thermo Scientific™ TraceFinder™ 4.1 software. Data confirmation using the accurate mass of the MS1 and MS2 Target and Confirming ions respectively; as well as the isotopic pattern of MS1 ions for APEOs determined by SIM.

UKAS Method Validation

The methods were validated in accordance with the United Kingdom Accreditation Service (UKAS) guidelines, using the following water types:

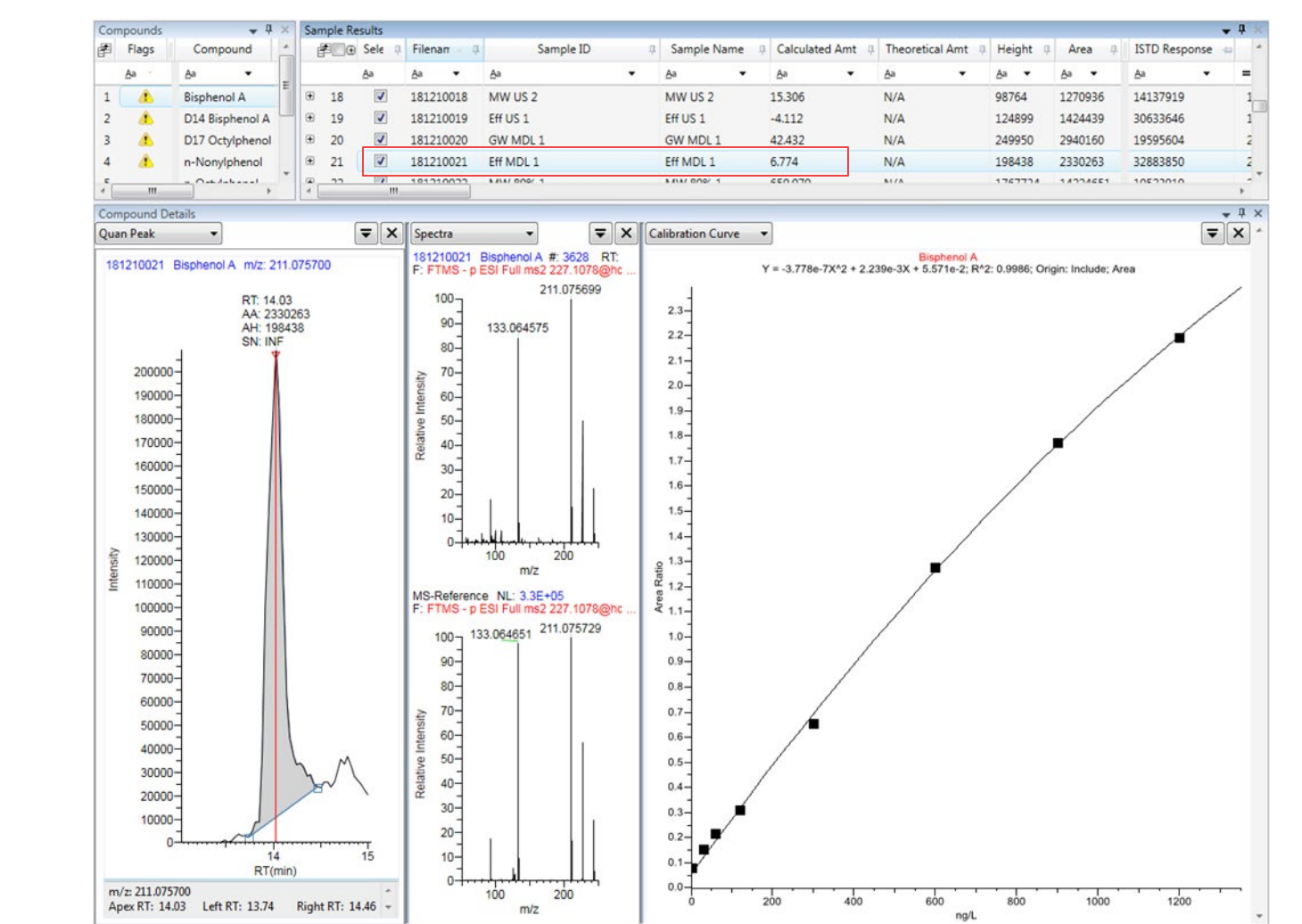
- Waste Water Treatment Works (WWTW) effluent: A works based in Glasgow with a population equivalent of > 200,000.
- Ground Water was supplied by AQUACHECK (UKAS).
- Marine (saline) water was from the Scottish Ayrshire coast.
- River water was sampled from the River Clyde, Lanarkshire, Scotland.

The method detection limits (MDL) were derived according to NS30⁴ documentation and procedures.

To derive the required statistics, 11 Validation batches comprising of duplicate samples were analysed. Matrix samples were spiked at a concentration which would give the minimum guaranteed response at the detector; appropriate blanks were also ran. To calculate the method detection limits, the within batch standard deviation was multiplied by a factor of 4.65. Method performance with respect to bias and precision was assessed using representative clean and dirty matrix types; in this case ground water and WWTW effluent. To monitor routine operational performance, Quality Control matrix spikes at 10% and 90% of calibration range where employed along with unspiked matrix samples for blank correction purposes; lastly limits for precision and bias were set at 12.5% and 25 % respectively.

Results

Figure 3. Bisphenol A Internal Calibration, showing chromatogram of spike near MDL in waste effluent. Negative mode PRM.



Figures 3 to 6 show typical calibration curves for bisphenol A, nonylphenol, nonylphenol mono-ethoxylate and t-octylphenol.

Also shown are chromatograms of procedural target spikes at concentrations close to the method detection limits, in the various matrices described in the UKAS Method Validation section.

Due to space limitations, the other targets are not shown but relevant data are tabulated in the Abstract Section.

Figure 4. Nonylphenol Internal Calibration, showing chromatogram of spike near MDL in waste effluent. Negative mode PRM.

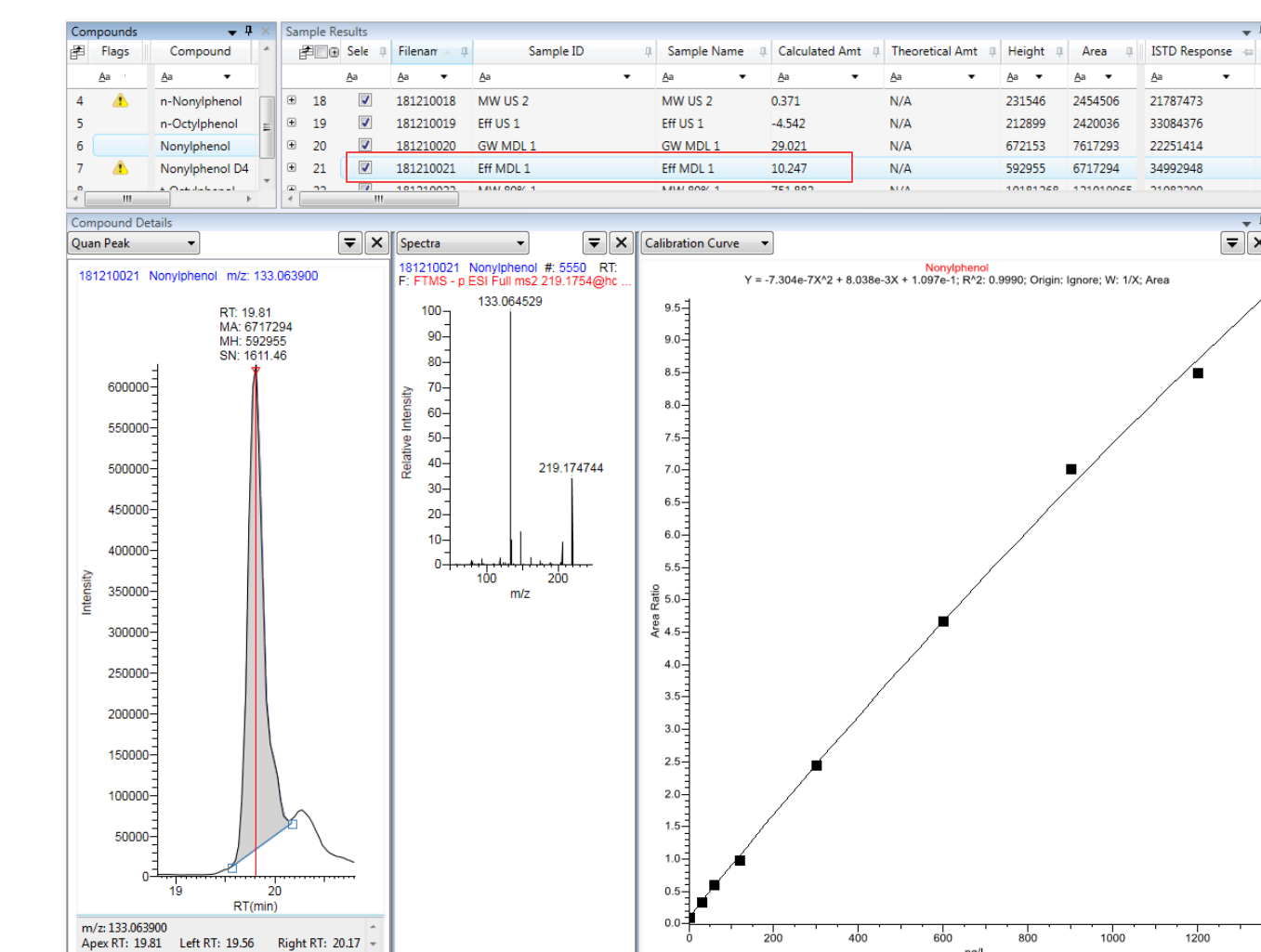


Figure 5. Nonylphenol Monoethoxylate Calibration, showing chromatogram of spike near MDL in Marine Water. Positive mode SIM.

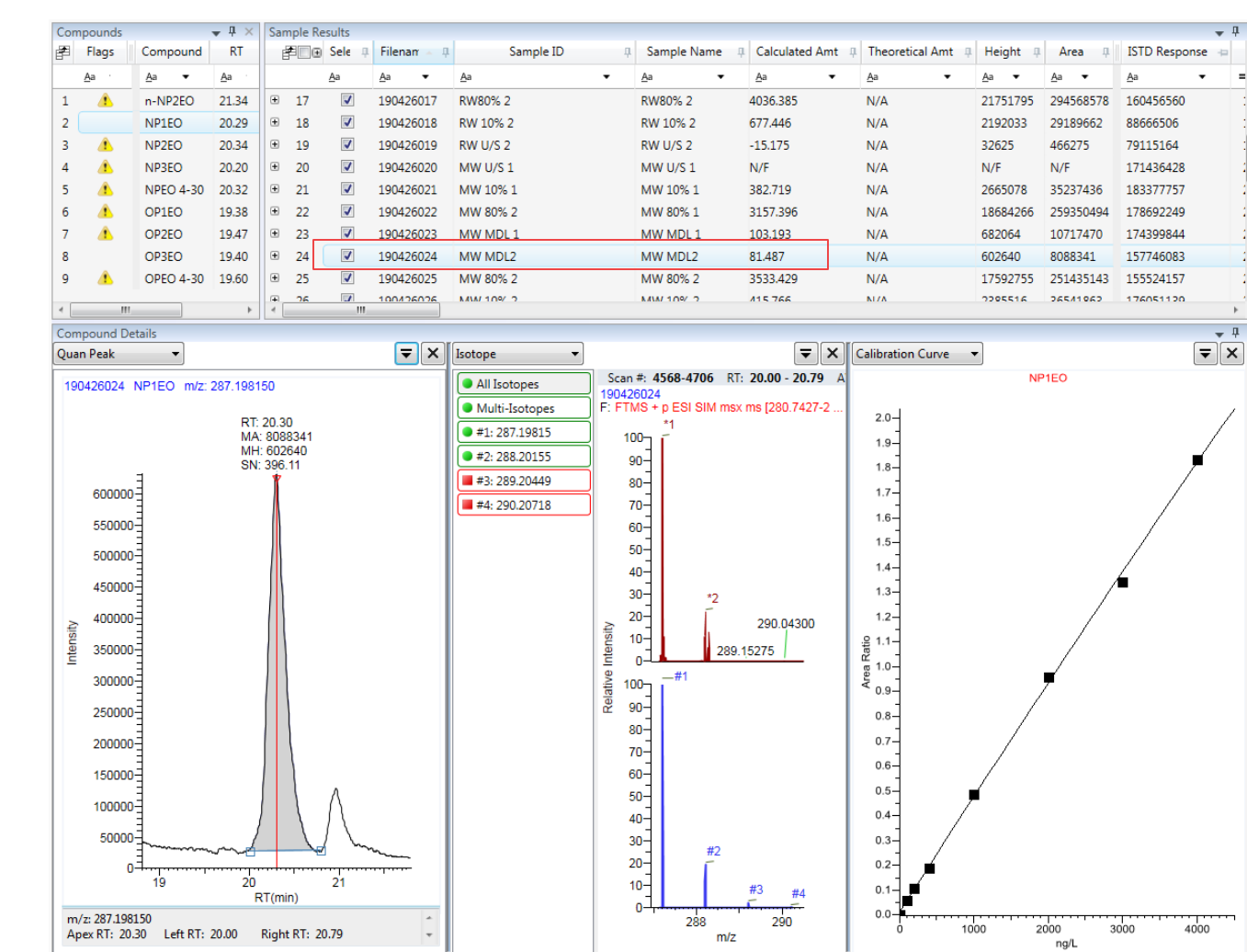
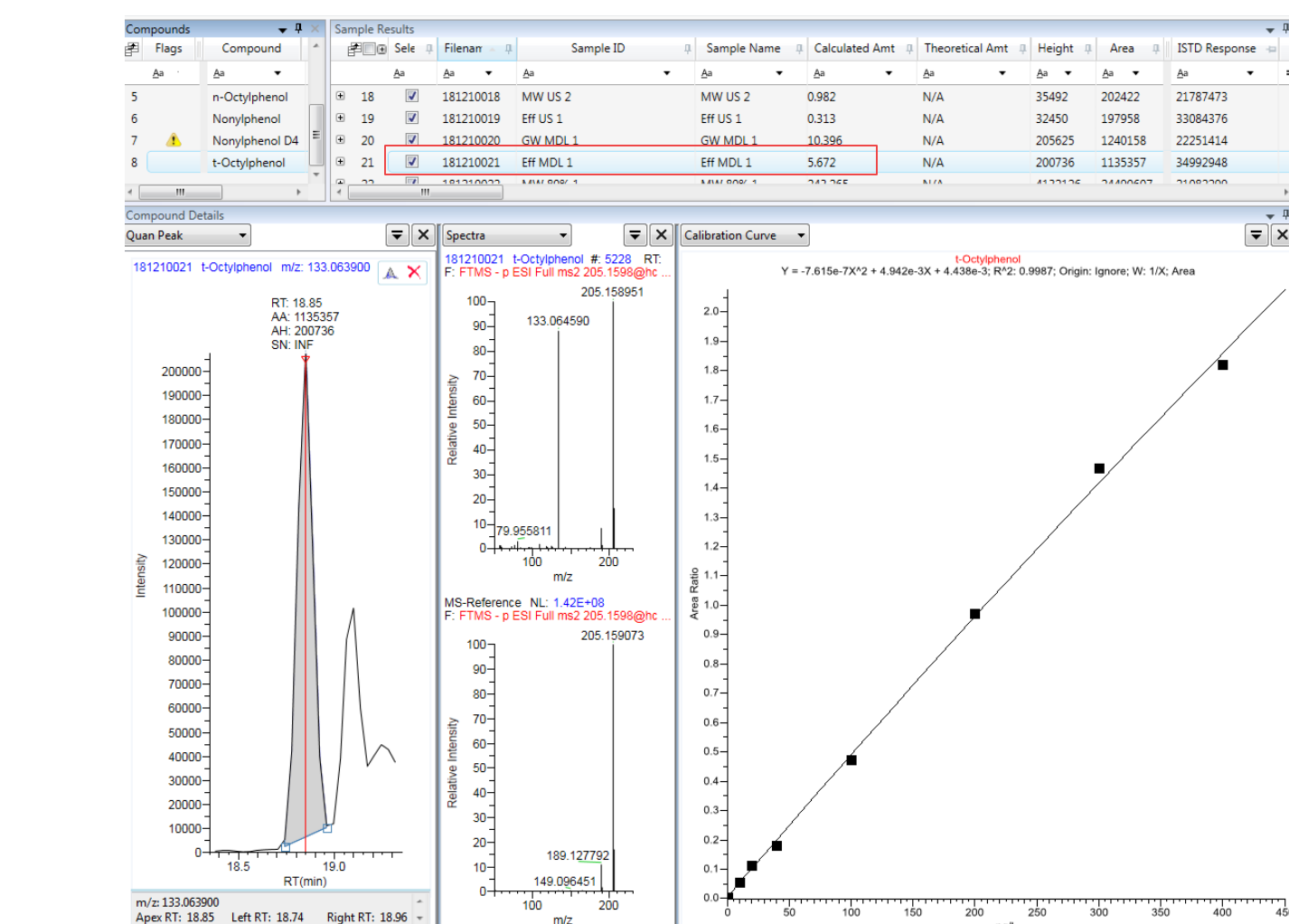


Figure 6. t-Octylphenol Internal Calibration, showing chromatogram of spike near MDL in waste effluent. Negative mode PRM.



DISCUSSION/CONCLUSIONS

- Using on-line SPE, with PRM (negative) and SIM (positive) acquisition for bisphenol A, the alkylphenols and related ethoxylates, the results have shown excellent method performance both in terms of quantitation and confirmation, and in speed of analysis compared to traditional approaches.
- Through rigorous validation using a wide range of representative sample types (waste water, ground water, river water and marine water), the methods have been shown to meet the stringent method detection limits required to comply with the EU Water Framework Directive and related Environmental Quality Standards.
- If the duration of current methods (see Figure 1) is assumed to be in the order of 10 hours, then the method described is approximately 30 times faster, which has implications in terms of sampling logistics, capital expense and maintenance, as well as expense of consumables.

REFERENCES

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