



# Analysis of alkylphenols using GC-MS/MS and automated SRM development

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## Keywords

Chromeleon Chromatography Data System, Automated build of quantitation method, Environment, Isomer integration, Linearity, Repeatability

## Goal

To describe the analysis of alkylphenol compounds in food and environmental matrices, illustrating the productivity and high-quality results from the GC-MS/MS system with automated selected reaction monitoring (SRM) development.

## Introduction

Alkylphenols and ethoxylated alkylphenols are used as surfactants in a broad range of applications, from processing wood and metals, to use as emulsifiers in polymerization, and even as solvents for flavorings.

Alkylphenols are endocrine disruptors with estrogen-like properties that have been shown to alter mammary gland development.<sup>1,2</sup> Additionally, both ethoxylated alkylphenols and alkylphenols are toxic to aquatic life. A subfamily of these chemicals, the nonylphenols, were once widely used in household laundry detergents. Although nonylphenol ethoxylate has been banned for use in household laundry detergents in both the E.U. and the U.S., it is still widely used in industrial laundry detergents and is particularly persistent in water.<sup>3</sup>

Due to their toxic qualities, production and use of nonylphenol and nonylphenol ethoxylates is prohibited in the E.U., and these chemicals are included in the list of hazardous compounds in the European Water Framework Directive.<sup>4,5</sup>

Current European regulations create high demand for analytical screening for these compounds at low levels in both water and food. Additionally, nonylphenol and nonylphenol ethoxylate have been part of a U.S. Environmental Protection Agency (EPA) action plan since 2010.<sup>3</sup>

## Experimental conditions

### Sample preparation

#### Water samples

To 1 L of sample; n-hexane was added and the mixture was shaken. After separation of the water and organic phases, the organic phase was removed and dried with anhydrous  $\text{Na}_2\text{SO}_4$ . An aliquot of the organic extract was evaporated to a volume of 3–4 mL and then evaporated under a gentle nitrogen stream to the final volume.

#### Solid samples

Into a glass jar, 10 g of the solid sample was weighed, then anhydrous  $\text{Na}_2\text{SO}_4$  and 40 mL of extraction solvent mixture (hexane and acetone) were added. The glass jar was sealed with a Teflon<sup>®</sup> seal and sonicated for 20 min. An aliquot of the sample extract was placed into a Kuderna-Danish apparatus. Another 40 mL of extraction solvent mixture was added to the sample and the extraction was repeated. An aliquot of the second extraction was added to the first extraction aliquot. The combined extracts were evaporated to a volume of 3–4 mL and then evaporated under a gentle nitrogen stream to the final volume.

### Method setup

Measurements were carried out using the Thermo Scientific<sup>™</sup> TRACE<sup>™</sup> 1310 gas chromatograph coupled to the Thermo Scientific<sup>™</sup> TSQ<sup>™</sup> Duo\* triple quadrupole mass spectrometer. The TRACE 1310 GC system was equipped with a programmed temperature vaporizing (PTV) multimode injection system to achieve optimum sample transfer into the analytical column for the critical alkylphenol compounds. The analytical conditions of the GC-MS/MS method are provided in Table 1.

The selected reaction monitoring (SRM) mode of the TSQ Duo\* GC-MS/MS system was chosen to achieve the lowest possible detection limits with high compound selectivity for the typical matrix-laden extracts from food or environmental samples.

The Thermo Scientific<sup>™</sup> Chromeleon<sup>™</sup> Chromatography Data System (CDS) software was used as the data system for sample analysis and quantitative data processing. The unique functionality of the Chromeleon CDS software is critical to the integration of the compound isomers into a single final result.

AutoSRM was used for the analytical method development. The automated SRM function is an important aid in optimizing the SRM transitions on all levels of method development. The precursor ion, product ion choice, and collision energy are optimized in a few logical, quick, and easy-to-follow steps. The result is a ready-to-use instrument and quantitation method.



**Figure 1. TSQ Duo GC-MS/MS system with Thermo Scientific<sup>™</sup> TriPlus RSH<sup>™</sup> Autosampler.**

\*Equivalent or better performance with the Thermo Scientific<sup>™</sup> TSQ<sup>™</sup> 9000 GC-MS/MS system

**Table 1. Recommended instrument conditions.**

<b>TRACE 1310 GC</b>	
Injection volume:	1 $\mu$ L
Inlet liner:	Siltec baffled liner (P/N 453T2120)
Carrier gas:	He, constant flow, 1.0 mL/min
Column type:	TG-5MS, 20 m length $\times$ 0.18 mm ID $\times$ 0.18 $\mu$ m film (P/N 26098-5780)
Oven program:	75 $^{\circ}$ C, 2.2 min 30.0 $^{\circ}$ C/min to 200 $^{\circ}$ C 20.0 $^{\circ}$ C/min to 300 $^{\circ}$ C 300 $^{\circ}$ C, 1.0 min
Transfer line Temp.:	320 $^{\circ}$ C
<b>PTV Injector</b>	
Injection mode:	Splitless with surge pressure
Surge pressure:	345 kPa
Surge time:	1 min
Split time, flow:	2 min, 30 mL/min
Inject temp.:	60 $^{\circ}$ C, 0.1 min
Transfer temp.:	600 $^{\circ}$ C/min to 280 $^{\circ}$ C, 5 min
Cleaning temp.:	870 $^{\circ}$ C/min to 325 $^{\circ}$ C, 15 min
Clean split flow:	25 mL/min
<b>TSQ Duo* Mass Spectrometer</b>	
Source temp.:	280 $^{\circ}$ C
Ionization:	EI, 70 eV
Emission current:	50 $\mu$ A
Mass resolution:	Q1, Q3 normal (0.7 Da at FWHM)
Collision gas:	Argon

### Compounds and transitions

The complete list of compounds and transitions as prepared by the AutoSRM tool is shown in Table 2.

All analytical information with retention times and SRM transitions is stored in the Chromeleon CDS method database. This information is also used for the quantitation processing method comprising a method including all compounds, their transitions, and retention times. This information storage strategy avoids potential human error in writing or copying from one file to another and simplifies the complete method setup.

## Results and discussion

The alkylphenol compound class includes many isomers, which do not elute as a single peak but as a group. These isomers require special attention for reliable signal integration and representation of the complete isomer peak area of the compound. The Chromeleon CDS software offers the ability to define a retention time range for the integration of these isomeric compounds, as provided in Table 3. Quantitation is performed using the elution time range for the complete isomer group.

In Figure 2, the complete MRM chromatogram is shown for a mixture of alkylphenols listed in Table 1.

### Automated SRM method development

Manual SRM method development can be labor intensive and time consuming. For every compound, one precursor ion, but preferably two or more precursor ions, need to be selected. Subsequently, the optimum collision energy and product ions for detection need to be defined for each precursor ion. When investigating dozens or hundreds of compounds, it is challenging for any analyst to perform this task.

In addition to all the necessary injections and data gathering, the primary challenge lies in handling the data and carefully monitoring which precursor yields the best product ion at which collision energy at a certain retention time, and performing this at the same time for dozens of compounds.

The automated SRM feature of the Chromeleon CDS software was designed to perform these tasks automatically using the following workflow:

1. A full-scan experiment identifies the target compounds.
2. The most intensive or selective ions are selected and put into a working list.
3. A product scan experiment is run in the next injection using the working list.
4. The method and the sequence for the product scan experiments is prepared automatically.
5. The product scan spectra are clearly shown by compound and by precursor ion.
6. The most intensive or selective product ions are selected and put into the working list.
7. Collision energy is optimized for sensitivity.

\*Equivalent or better performance with the TSQ 9000 GC-MS/MS system

8. The method and the sequence for the optimal collision energies is set up automatically.
9. The completed working list is stored as a compound database csv-file. This file can be imported or attached to the instrument method and is also used as the quantifying processing method.

The sequence of the AutoSRM steps is illustrated in Figures 4–5.

The Chromeleon CDS software can perform library searches for compound identification. Optionally, Chromeleon CDS software can import a list of compounds with retention times, etc. All of the data can be reviewed and edited manually.

## Quantitative calibration

The calibration curves (Figure 6) show good linearity over a range of 2 to 200 ppb. Even at the lowest level, the peaks are very clearly present, even for the isomer groups and the confirming ions.

## Repeatability in matrix

A spiked water sample was extracted and injected seven times. Table 4 shows repeatability of results at the relevant low concentration levels. The repeatability in a water matrix is excellent; all compounds show a repeatability below 5%.

## Trend reporting

The Chromeleon CDS software offers the ability to report trends and make flowcharts (Figure 7).

**Table 2. List of compounds in the method automatically prepared by AutoSRM.**

Parent Mass [m/z]	Product Mass [m/z]	RT [min]	Name
179	45	8.69	4-nonylphenol
179	73	8.69	4-nonylphenol, confirming ion 1
246	105	11.65	4-nonylphenol diethoxylate (SS2)
246	133	11.65	4-nonylphenol diethoxylate (SS2), confirming ion 1
339	73	11.67	4-octylphenol triethoxylate
339	117	11.67	4-octylphenol triethoxylate, confirming ion 1
339	161	11.67	4-octylphenol triethoxylate, confirming ion 2
357	73	10.25	BisphenolA
357	191	10.25	BisphenolA, confirming ion 1
185	45	8.66	D8-4-nonylphenol (SS1)
185	73	8.66	D8-4-nonylphenol (SS1), confirming ion 1
207	45	7.75	nonylphenol
207	73	7.75	nonylphenol, confirming ion 1
251	73	9.57	nonylphenol monoethoxylate
251	135	9.57	nonylphenol monoethoxylate, confirming ion 1
251	207	9.57	nonylphenol monoethoxylate, confirming ion 2
179	73	8.1	4-n-octylphenol, confirming ion 1
179	45	8.1	4-n-octylphenol
295	73	10.43	4-t-octylphenol diethoxylate
295	207	10.43	4-t-octylphenol diethoxylate, confirming ion 1
295	117	10.43	4-t-octylphenol diethoxylate, confirming ion 2
295	135	10.43	4-t-octylphenol diethoxylate, confirming ion 3
339	117	12.15	nonylphenol triethoxylate, confirming ion 1
339	73	12.15	nonylphenol triethoxylate
339	161	12.15	nonylphenol triethoxylate, confirming ion 2
207	73	7.04	4-t-octylphenol
207	45	7.04	4-t-octylphenol, confirming ion 1
295	207	11.4	nonylphenol diethoxylate, confirming ion 1
295	117	11.4	nonylphenol diethoxylate
295	135	11.4	nonylphenol diethoxylate, confirming ion 2
295	73	11.4	phenolnonylphenol diethoxylate, confirming ion 3



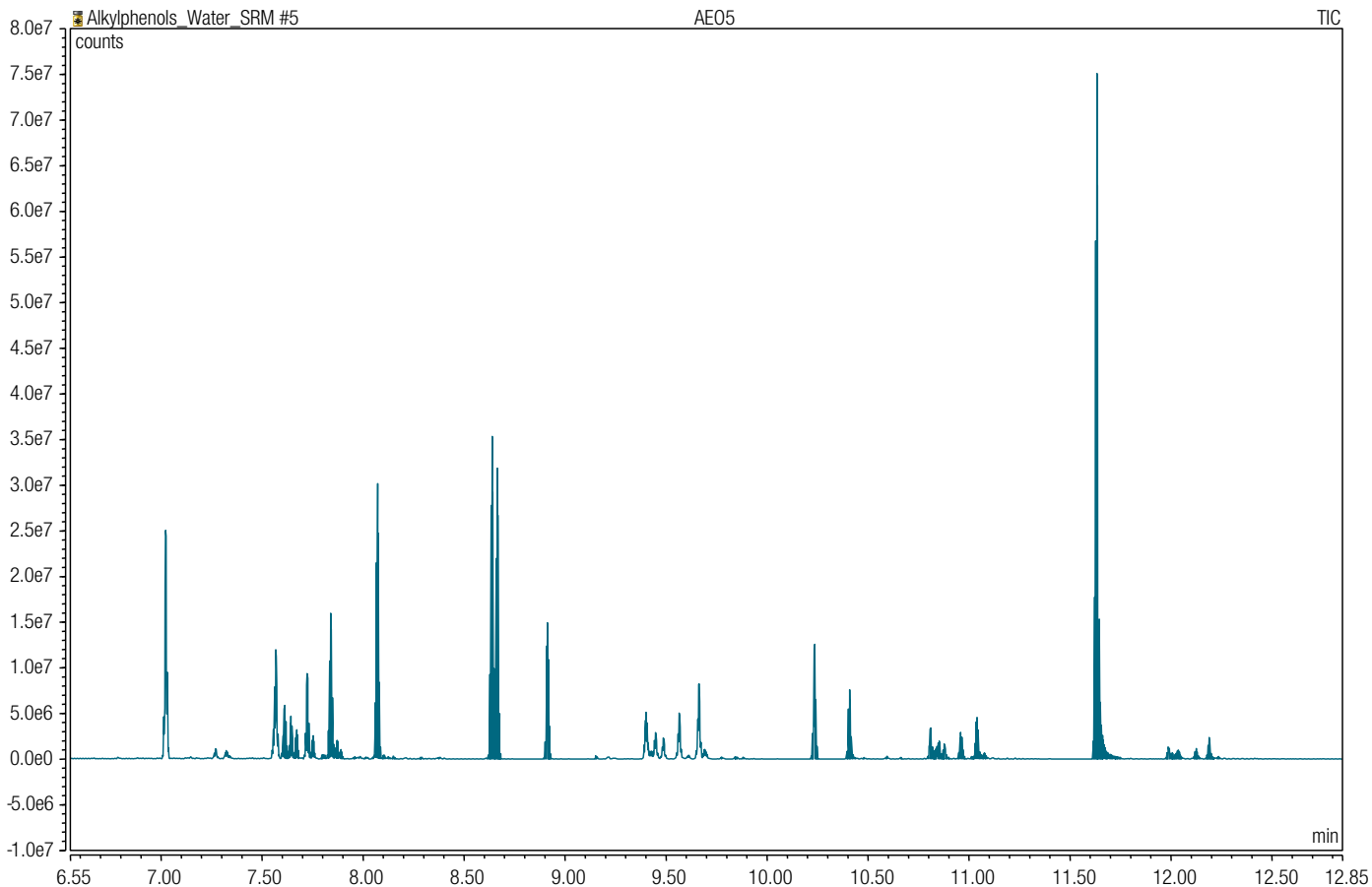


Figure 2. Chromatogram of alkylphenol standard at 100 ng/mL.

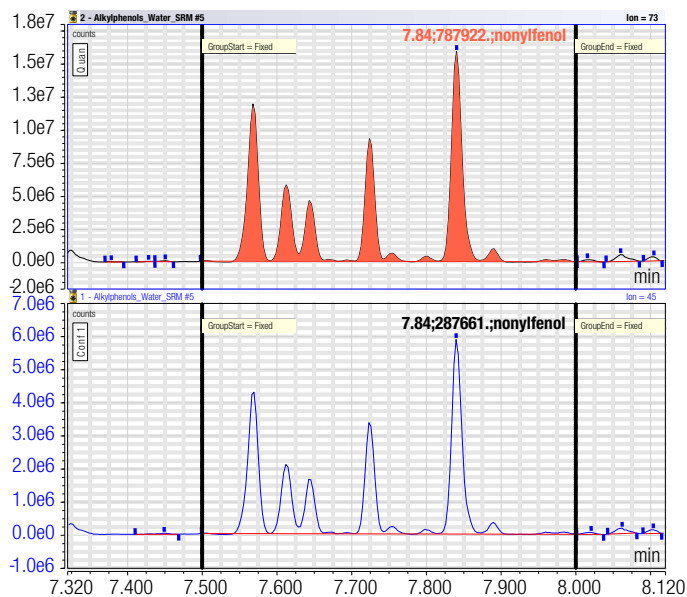


Figure 3. Chromatogram showing the integrated range of nonylphenol isomers.

Top: transition 207 > 73  
 Bottom: transition 207 > 45

Table 3. Isomer compound integration time ranges.

Target Compound	RT [min]
4-t-octylphenol	7.02
nonylphenol	7.5-8
4-n-octylphenol	8.07
D8-4-nonylphenol	8.64
4-nonylphenol	8.67
nonylphenol monoethoxylate	9.35-9.8
bisphenol A	10.23
4-t-octylphenol diethoxylate	10.41
nonylphenol diethoxylate	10.8-12
4-nonylphenol diethoxylate	11.63
4-octylphenol triethoxylate	11.65
nonylphenol triethoxylate	11.9-12.4

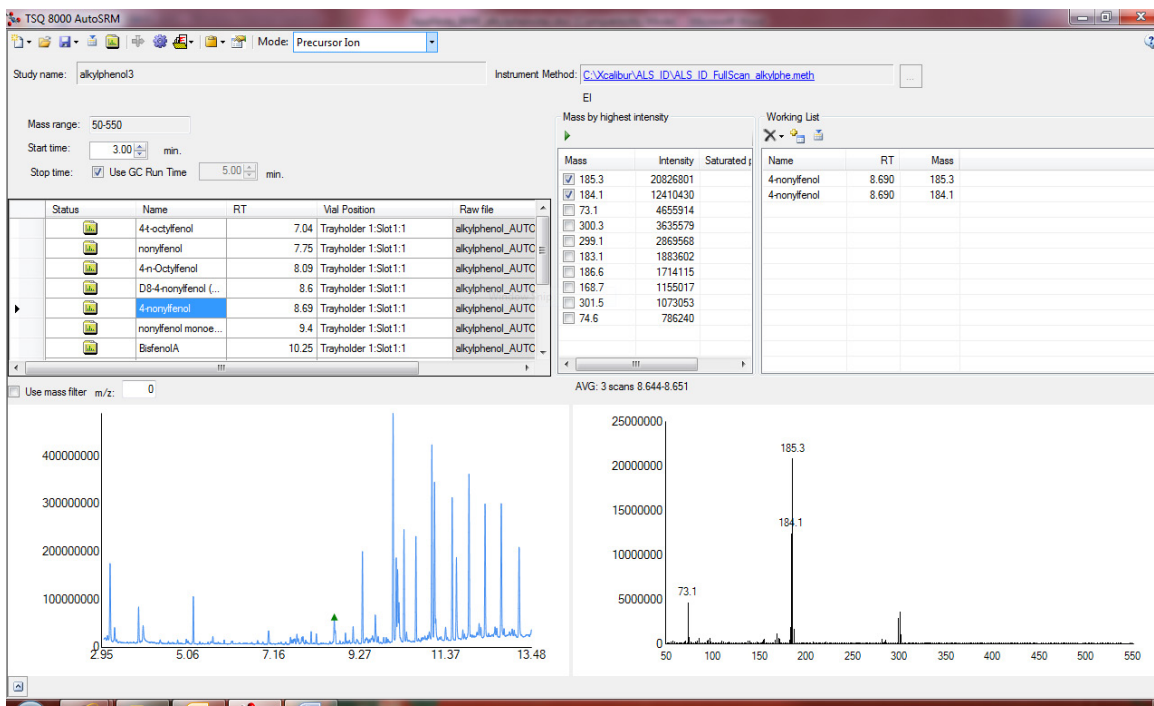


Figure 4. Chromeleon AutoSRM screen for automated GC-MS/MS method development.

- Upper left: The list of compounds and the retention times
- Upper right: The list of most intense ions in the working list
- Lower left: The full-scan chromatogram
- Lower right: The full-scan spectrum of a selected compound

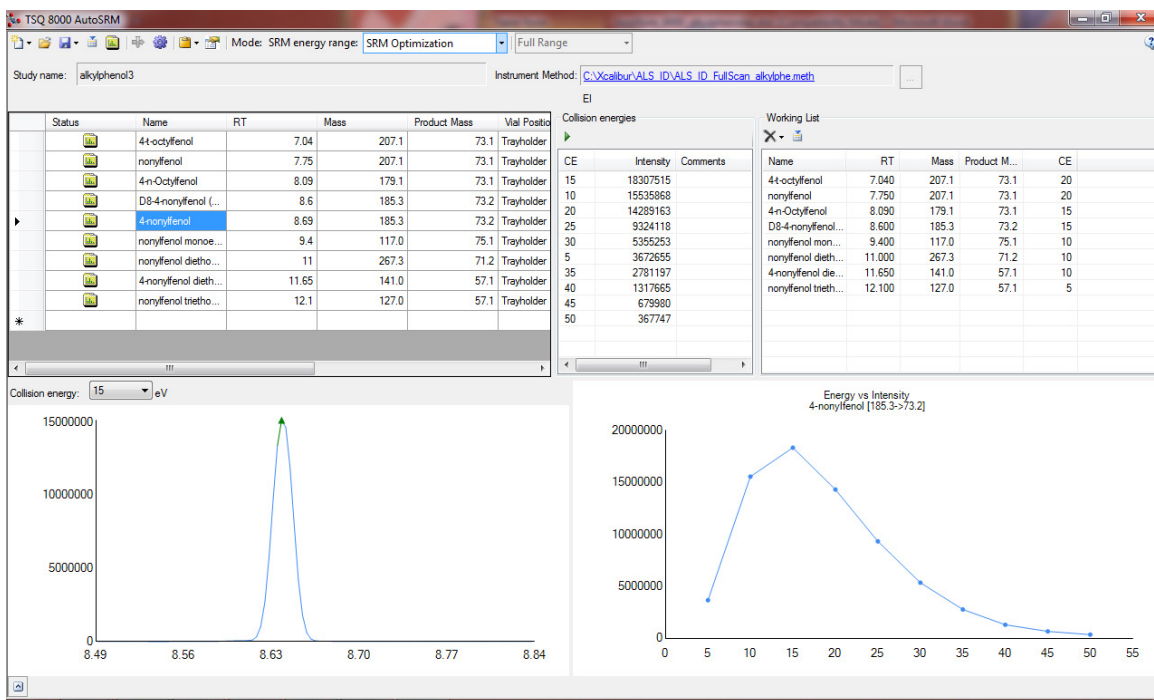


Figure 5. Optimization of the SRM collision energy by AutoSRM.

- Upper left: The list of compounds and the retention times
- Upper right: The list of applied collision energies and sorted by intensity of the product ion in the working list
- Lower left: The SRM peak, in this case  $m/z$  185 > 73
- Lower right: The graph of collision energy versus intensity

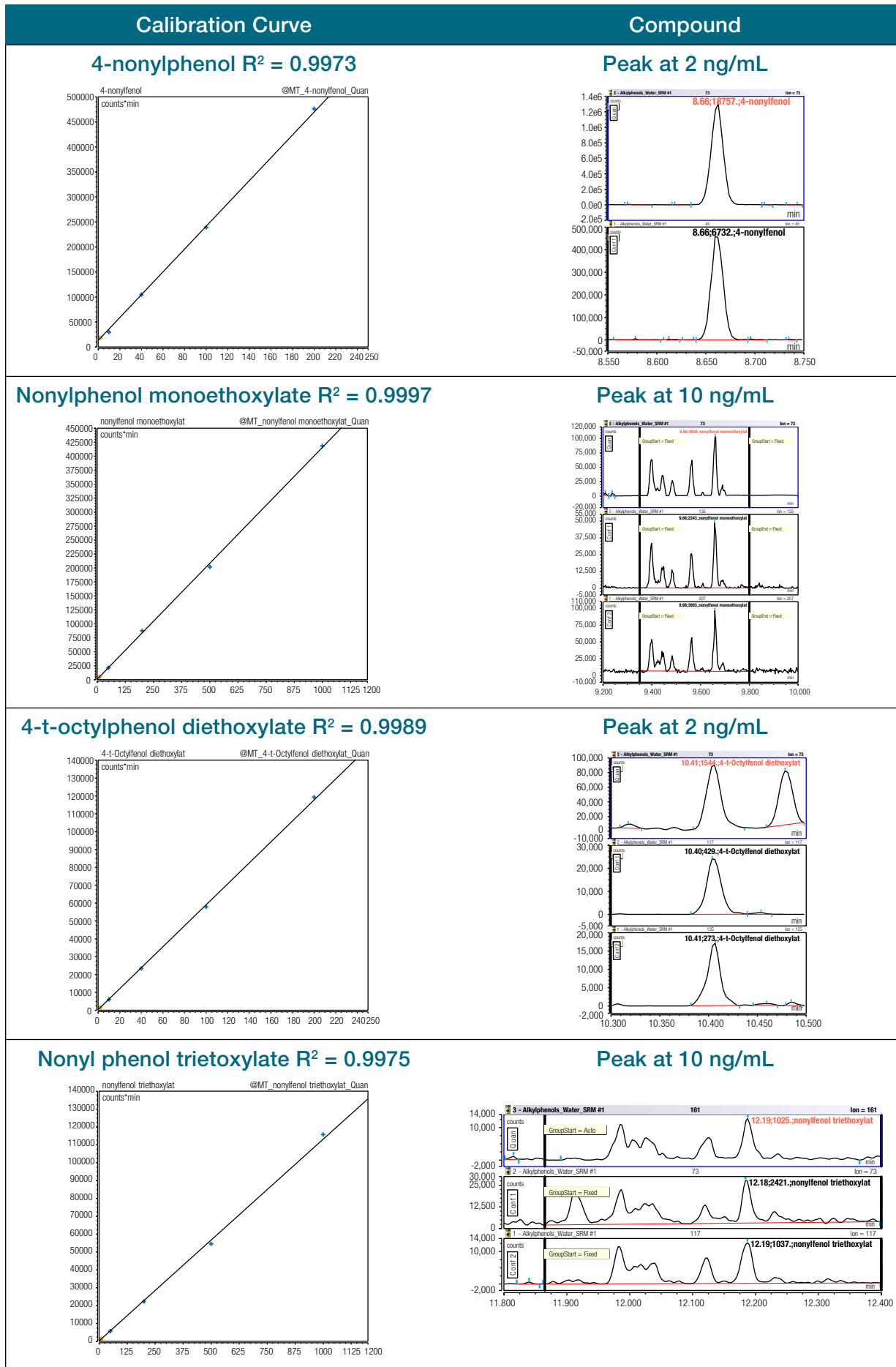


Figure 6. Calibration curves and peaks at lowest level.

Table 4. Method precision in % RSD measured, n = 7.

Compound	Avg. Conc. [ppb]	% RSD
4-n-octylphenol	81.9	1.49
4-nonylphenol	79.2	1.67
4-nonylphenol diethoxylate	731	4.25
4-octylphenol triethoxylate	86.4	3.59
4-t-octylphenol	95.7	1.23
4-t-octylphenol diethoxylate	97.9	2.1
bisphenol A	79.2	4.07
D8-4-nonylphenol	324.5	1.81
nonylphenol	370.3	1.55
nonylphenol diethoxylate	464.6	3.87
nonylphenol monoethoxylate	466	1.82
nonylphenol triethoxylate	513	4.23

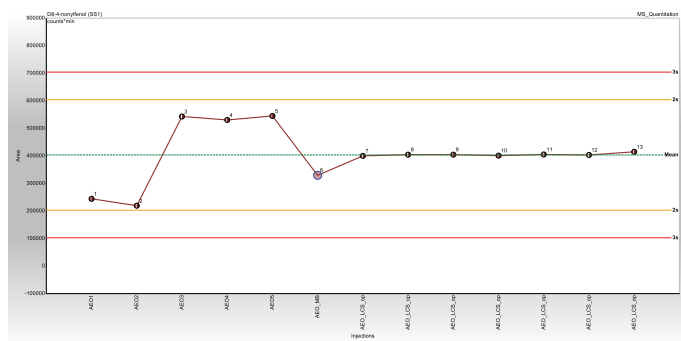


Figure 7. An example of the area of the internal standard in a short sequence, showing the average and the 2s and 3s values as horizontal bars. The analytical data of this sequence is well within the 2s width.

## Conclusions

- All alkylphenol compounds show good linear calibrations over the range of 2 to 200 ppb.
- The method has proven high precision and repeatability in a water matrix sample.
- AutoSRM is a useful tool for automated MRM method development and maintenance. It delivers an optimized method and avoids tedious and laborious method development.
- The Chromeleon CDS software offers excellent processing options for data reviewing, including ion ratio checks and reporting. All reporting can be optimized for each application requirement.

## References

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