Chromatography Technical Note No AS167

Investigation of the Agilent 5977B with High Efficiency Source (HES) to Enable Electron Impact (EI) Analysis of Phenolic Compounds in Water

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Introduction

Phenolic compounds are a group of chemicals which are detectable to the human palate as metallic tastes at low concentrations in drinking water. The reaction of hypochlorite and phenolic acids produces chlorinated phenols a by-product as to does degradation of phenoxy herbicides. 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP) are most likely of the chlorinated phenolic compounds to occur in drinking-water as they are by-products of the disinfection process.

Due to their polarity the phenolic compounds do not chromatograph well on commonly used non-polar column phases such as DB-5MS. Peak tailing is often a consequence of this and can affect quantitative reproducibility. Derivatives of phenols produces more volatile and less polar compounds whose chromatographic behavior is more suited to non-polar column phases.

The phenolic and chlorinated phenolic compounds are weak to moderately acidic and pKa values of the compounds in this study were in the range of 5-10 and completely unionised in highly acidic conditions. After derivatisation with Pentafluorobenzoyl Chloride (PFBCl) the derivatives do not ionise under acidic or basic conditions which facilitates the LLE.

Traditionally the PFBCl derivatives would be analysed using an electron capture detector or Negative Chemical Ion (NCI) source. The aims of this study were automation of the current exaction procedure and to see if the improved sensitivity from the latest Agilent 5977B single quadrupole mass spectrometer with High Efficiency Source (HES) would facilitate a direct conversion of a method with NCI source to Electron Impact (EI) source.

This application note describes the method development of an on-line automated solution for phenolic and chlorinated phenolic compounds with derivatisation by PFBCl. Development was in conjunction with Paul Leather, Environment Agency from July to August 2016. This method uses and automated liquid-liquid extraction and derivatisation with vigorous agitation using the *m*Vorx.

Figure 1 GERSTEL Dual Head with Agilent 5977B HES

Instrumentation

Dual Head GERSTEL MPS 2
GERSTL *m*Vorx
Anatune CoolR^{PLUS}
Maestro software integrated
Agilent 7890 GC with a 5977B mass spectrometer with High Efficiency Source (HES)

Method

A suite of twenty six phenolic compounds were prepared firstly at a concentration of 2ug/mL and analysed in fullscan mode after derivatisation with PFBCl to determine the most abundant ions. A method in Selected Ion Monitoring (SIM) mode was then used to compare the sensitivity of a 5975C Triple Axis Detector with inert source, a 5977A detector with Extractor Ion source and the latest 5977B detector with HES. This work was on a 30M 0.25mm I.D DB-5MS UI column.

After further development of the SIM method the automation of the method was optimized. Solutions were prepared at concentrations of 50, 100, 500, 1000 and 2000 ng/L in purified water by automated addition of concentrated stock standards by the MPS. These aqueous standards were firstly acidified by addition of sulfuric acid to 8mL of water. An aqueous solution of salt was added and the sample vortex mixed. A mixture of PFBCl in isooctane was added followed by excess sodium hydroxide which aided the removal of excess chlorine produced from the derivatization.

After vigorous mixing using the *m*Vorx a small volume of a polar solvent was added to break up emulsions. 2 µL was then taken from the top isooctane layer and injected.

Results

Using the PrepAhead function of the Gerstel Maestro software the sample preparation for each sample is done immediately preceding the GC injection whilst the previous sample is running. In combination with the Anatune CoolR^{PLUS}, which enables rapid cooling of the GC oven, 60 samples can be prepared and ran in a 24 hour period. This would equate to an increase in throughput of 100% compared to a manual method where 1 day is spent preparing and 1 day of instrument time is required. Figure 2 shows the automated PrepAhead function of Maestro. The multi-coloured bands represent the sample preparation and beige bands GC run-time.

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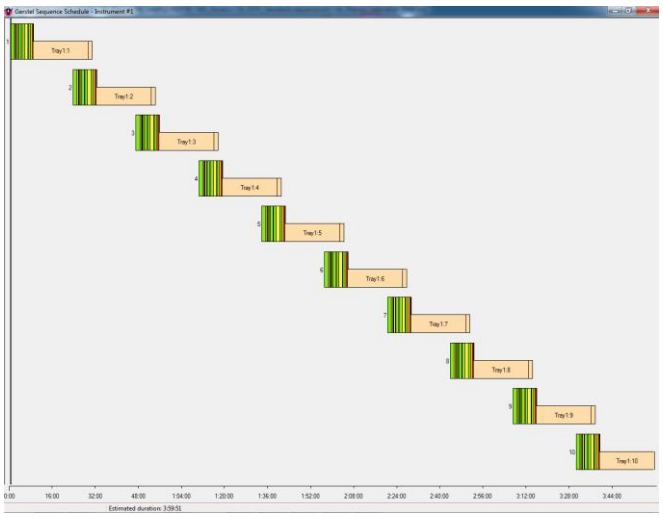

Figure 2 Maestro PrepAhead

Initially SIM ions including original molecular ions from the NCI method and the most abundant ions of 195,167 and 117 m/z were selected for comparison of the detector systems. Figure 3 shows the TIC chromatograms of the 5975C and 5977A and 5977A with 5977B respectively. Difference in retention time are due to column age. The difference in signal abundance between the detectors is clear. A comparison of signal to noise for selected peaks showed an increase of between 5-10 times between the 5977C and 5977B and 2-5 times between the 5977A and 5977B.

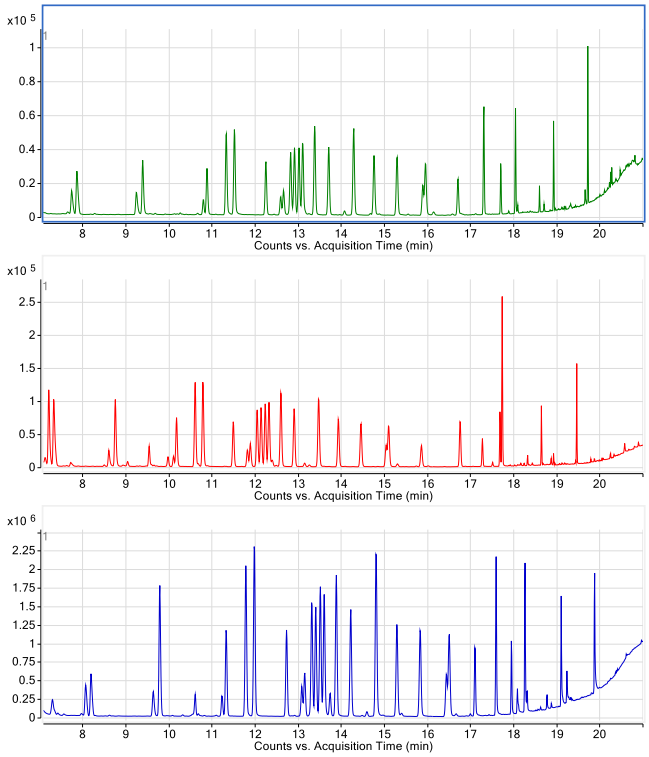

Figure 3 TIC chromatographs of 5975C (top), 5977A (middle) and 5977B (bottom) at 1ug/mL

After comparison of the mass spectrometers using the most abundant ions the method was developed to not include the 195,167 and 117 ions as these are products of the PFBCl and therefore not specific to the phenols. Figure 5 shows a comparison of 2,3-dichlorophenol and PFBCl which demonstrated this.

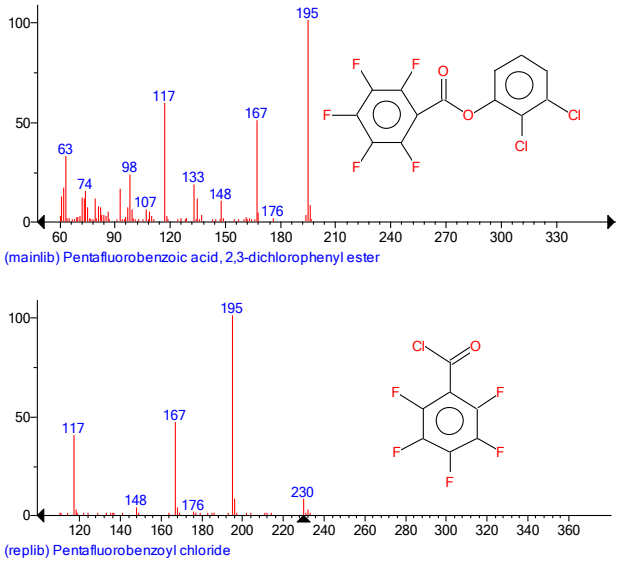

Figure 5 Pentafluorobenzoic acid 2,3-dichlorophenol ester (top) and PFBCl (bottom) fullscan mass spectra from NIST14

The removal of these abundant ions meant the molecular ions were selected for a more specific method. Table 1 shows the quantitation ions that were selected;

Compound	Quant Ion
Phenol-d6	293
Phenol	288
2-methyl phenol	302
3-methyl phenol	302
4-methyl phenol	302
2-Ethyl Phenol	316
2,6-dimethyl phenol	316
3-chlorophenol	322
4-chlorophenol	322
2,5-dimethyl phenol	316
2-Chlorophenol	322
2,4-dimethylphenol	316
2,3-dimethyl Phenol	316
3,5-dimethylphenol	316
2,3-dimethyl phenol	316
4-Chloro-2-methylphenol	336
3,4-dimethylphenol	316
4-Chloro-3-methylphenol	336
2,4-dichlorophenol-d3	361
2,5-dichlorophenol	356
2,4-dichlorophenol	356
2,6-dichlorophenol	356
2,3-dichlorophenol	356
4-chloro-3,5-dimethylphenol	350
2,4,6-trichlorophenol	390
2,4,5-trichlorophenol	390
2,3,5,6-tetrachlorophenol	424
Pentachlorophenol	458

Table 1 SIM ions for EI method

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The linearity and peak responses were excellent for the smaller phenolic compounds such as Phenol, methylphenols, ethylphenol, dimethylphenols, and monochlorophenols. The polychlorinated phenols had a lower peak responses so although the linearity plots gave acceptable results (>0.995) the peak responses at the lowest calibration level would not be acceptable based on the acquisition parameters with a 2µL injection.

Figure 6 shows an example of linearity for Phenol and peak response at the lowest calibration level of 50ng/L from the automated liquid-liquid extraction.

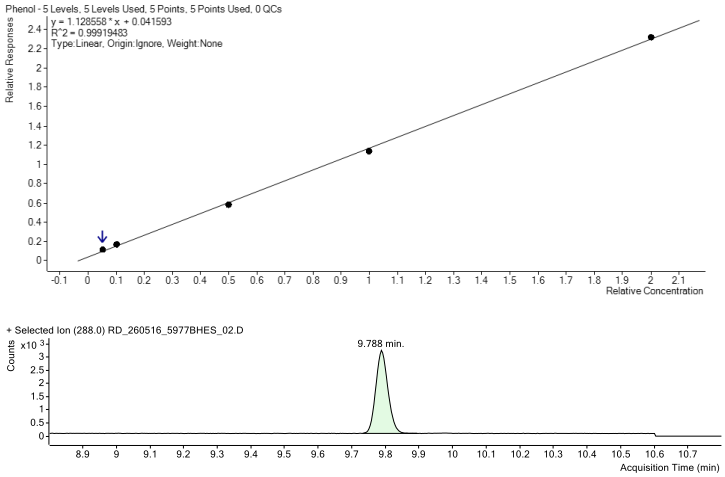

Figure 6 Linearity and peak response Phenol 50ng/L

Although precision experiments have not yet been performed on the 26 phenolic and chlorinated phenolic compounds, % Relative Standard Deviation (%RSD) was calculated on the phenol-d6 internal standard used in quantification of the analytes. %RSD 3.67%. 2,4-dichlorophenol-d3 was not assessed as the peak response was not very good for the polychlorinated phenols as mentioned previously.

Discussion

EI analysis may be preferential to laboratories due to savings of cost of NCI regent gases, or not having to switch sources between method, or having dedicated instruments for NCI analysis.

This initial investigation demonstrates how the improved sensitivity of the 5977B can offer the option of different approach to using NCI for phenols in water. Further investigation is required to optimize the method for polychlorinated phenols, a larger injection volume, optimization of the LLE or instrument settings may give adequate responses for quantitation.

One obvious drawback of this method would be having to use the 195,167 or 117 ions as qualifiers. For this reason further method development involved automation with an alternative derivatising agent that produces more specific ions in EI mode. This work is presented in application note AS168.

Please contact Anatune if you need any further information on this technique.

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