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## Fully automated liquid-liquid extraction method using a GERSTEL Dual Head Robotic with Robotic Pro of organochlorine pesticides and polychlorinated biphenyls in water

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### Introduction

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This application note describes the method development of an on-line automated solution of liquid-liquid extraction using a GERSTEL Robotic and Robotic Pro dual head MultiPurpose Sampler. This method uses an automated liquid-liquid extraction (LLE) using the GERSTEL QuickMix.

Pesticides are closely monitored compounds within the water industry. Over use of these on fields can affect ecosystems in close proximity and also widely affect food chains. A wide range of organochlorine pesticides are used for their pest-killing activity but can become toxic to other lifeforms when these pesticides leach from farmland into nearby water courses – this also has an effect on water sources destined to become part of the potable water system. Another class of compounds, polychlorinated biphenyls, are one such type of compound that heavily affect food chains and webs. This chemical pollutant which was once widely used in industry is very lipophilic and as a result builds up in animal fatty tissue if it is released into the environment. This can also affect the human population should they build up in livestock meant for human consumption. As a result of this, the Drinking Water Inspectorate imposes strict limits for the detection of these compounds in water. The following work demonstrates how these compounds can be extracted and analysed in this matrix.

The new GERSTEL Robotic and Robotic Pro is an evolution of the now well-established XT rail. One such evolution is the introduction of different tools which are situated in the heads of the Robotic system; the most common tools are that of headspace tool for headspace methods, Universal Syringe Module (USM) and the Preparation Syringe Module (PSM) for liquid handling. A big feature of this technology is that with the Robotic pro, automated exchange of different modules during a sample preparation sequence is possible. This enables a much greater range of syringes to be used in one prepsequence and further opens the possibilities that can be accomplished with automated sample preparation. With a traditional XT, only two syringe types could be used at any one time. The new technology enables the analyst to have three switchable modules. If a dual head is employed, four useable modules could be in use. Another noticeable difference between that of the XT and Robotic is that movement of the heads is now much more fluid and efficient, with the sampling heads moving directly to destination, taking the shortest and quickest route to the destination rather than sticking to an X, Y, Z route. These small savings in time per sample can be multiplied up over a full sequence, resulting in data being obtained faster.



*By clicking the above picture, you are able to watch a short video on the full extraction sequence using the Robotic/Robotic Pro*



**Figure 1. GERSTEL Dual Head Robotic MultiPurpose Sampler, mounted to an Agilent GC-MS**

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### Instrumentation

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Dual Head GERSTEL Robotic/Robotic Pro 2metre rail.  
GERSTEL QuickMix  
GERSTEL CIS4 Programmable Temperature Vaporiser inlet  
Maestro software integrated  
Agilent 7890B GC with a 5977B mass spectrometer with High Efficiency Source (HES)  
Agilent MassHunter software (Quant/Qual – B.07.04)

## Method

The method employed for this work utilises a Liquid-Liquid Extraction of a mixture of OCPs and PCBs from water. The System was operated in Selected Ion Monitoring mode. This work was on a 30M 0.25mm I.DDB-5MS UI column.

To a pre-prepared 20mL vial containing 18ml sample, 600µl solvent was added using the GERSTEL Robotic Pro Head, enabled with a PSM module with 2.5mL syringe. Calibration standards were automatically prepared at concentrations of 5, 25, 50, and 100 ng/L in purified water by automated addition of concentrated stock standard by the GERSTEL Robotic Pro equipped with a USM with 10µL syringe. This requires the sampling head to switch modules from PSM to USM within the sequence. Table 1 shows the volume of stock solutions used to spike the calibration standards.

Final Conc [ng/L]	Spike Conc [µg/L]	Vol (mL)	Vol spike (µL)
10	5.0	18	3.6
25	5.0	18	9
50	50.0	18	1.8
100	50.0	18	3.6
200	50.0	18	7.2

Table 1. Calibration spiking

Sample mixing was performed using the GERSTEL QuickMix at 1500rpm for 5 minutes. A small volume of a polar solvent was added post extraction to break up emulsions. 10 µL was then taken from the top solvent layer and injected directly into the Cooled Injector System (CIS) inlet.

## Results

Automation of the preparation of the calibration standards (auto spiking) was demonstrated to give excellent linearity. Figure 2 shows the typical linearity achieved when using automation to spike the calibration standards. Figure 3 shows the peak response for triallate at 5ng/L in tap water. Use of the automated technique improves linearity as accuracy, precision and reproducibility are improved. This is partly due to eliminating the human error in manual pipetting as these techniques are dependent on operator technique and ability.

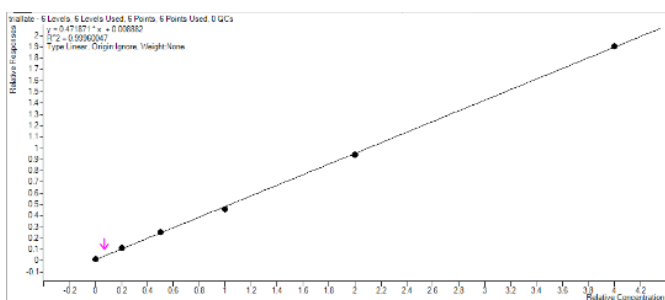


Figure 2 linearity of triallate.  $R^2 = 0.9996$ .

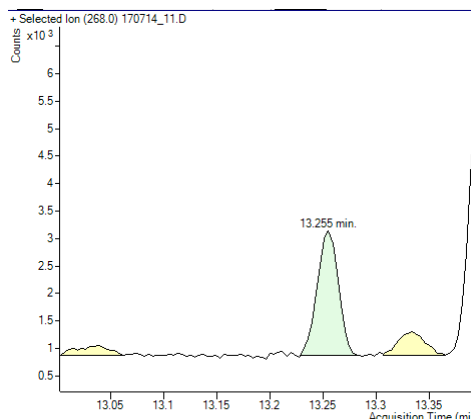


Figure 3a. triallate 5ng/l spike in tap water.

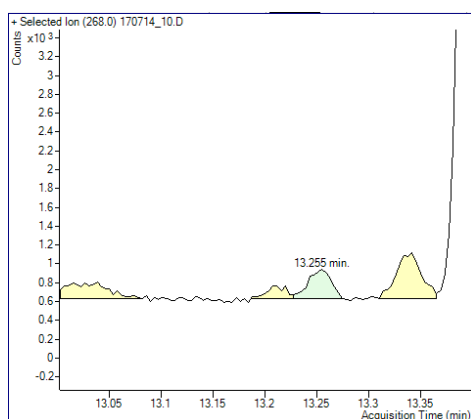


Figure 3b. triallate blank

The internal standard was added as part of the automated procedure and though full %RSD recovery test have yet to be carried out, the %RSD calculated for D<sub>6</sub> gamma-HCH for n=6 was 4.28%. Table 2 gives the data for this calculation.

10	170714_2_D	Cal	1	14/07/2017 17:06	0.0100	13.255	8630	0.0110	0.0110	12.601	76729
25	170714_3_D	Cal	2	14/07/2017 17:32	0.0250	13.255	19398	0.0262	0.0262	12.601	75734
50	170714_4_D	Cal	3	14/07/2017 17:59	0.0500	13.255	34903	0.0476	0.0476	12.601	76141
100	170714_5_D	Cal	4	14/07/2017 18:25	0.1000	13.255	73520	0.0986	0.0986	12.601	78261
200	170714_6_D	Cal	5	14/07/2017 18:52	0.2000	13.255	140723	0.2011	0.2011	12.601	73805
blank	170714_7_D	Cal	0	14/07/2017 19:19	0.0000	13.255	940	0.0005	0.0005	12.601	69110

Table 2. Calibration and internal standard data for triallate/D<sub>6</sub>-gamma HCH.

Figures 4 and 5 show the same data for PCB 28.

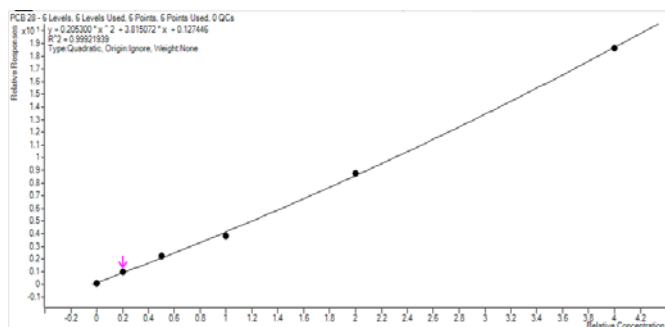


Figure 4.  $R^2 = 0.9992$  for PCB 28

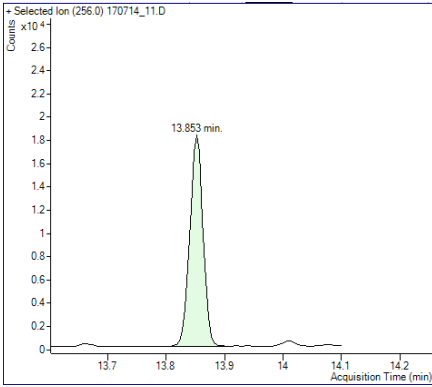


Figure 5a. PCB 28 5ng/l spike in tap water

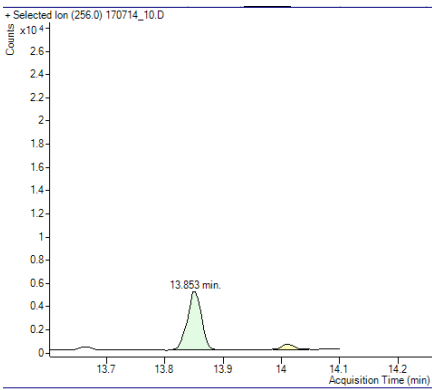


Figure 5b. PCB 28 blank

The work was also repeated on an Agilent 7010 triple quadrupole with high efficiency source (HES). Laboratories sometimes prefer the use of this type of instrument for increased selectivity, especially when dealing with more complex types of environmental matrices. This is also advantageous when injecting large volumes of sample due to increased sample background. The results for triallate and PCB 28 can be seen below as a comparison.

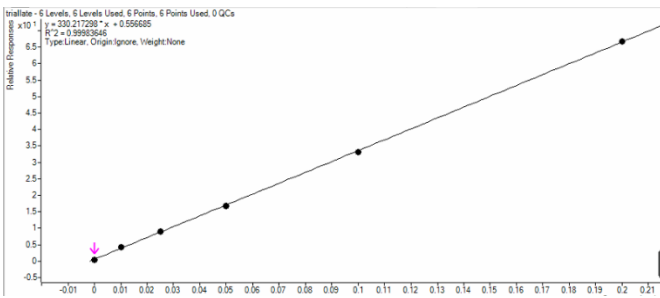


Figure 6. Linearity of triallate.  $R^2 = 0.9998$

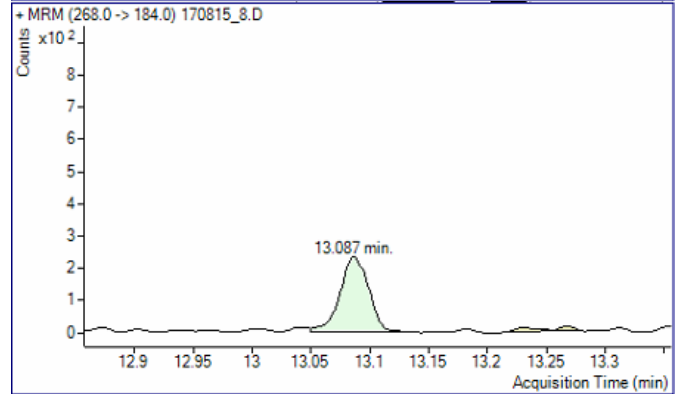
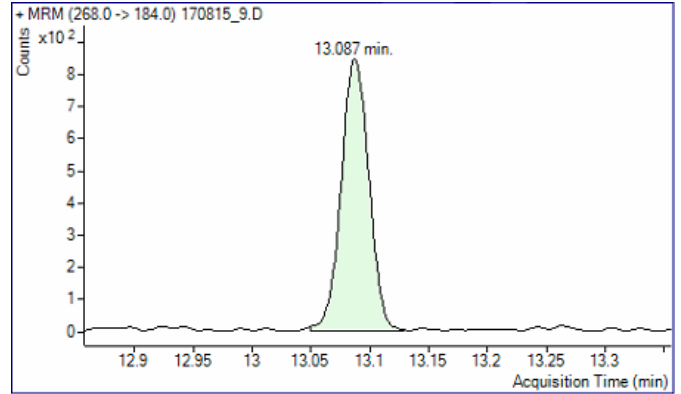


Figure 7. 5ng/L triallate (top) Blank (bottom)

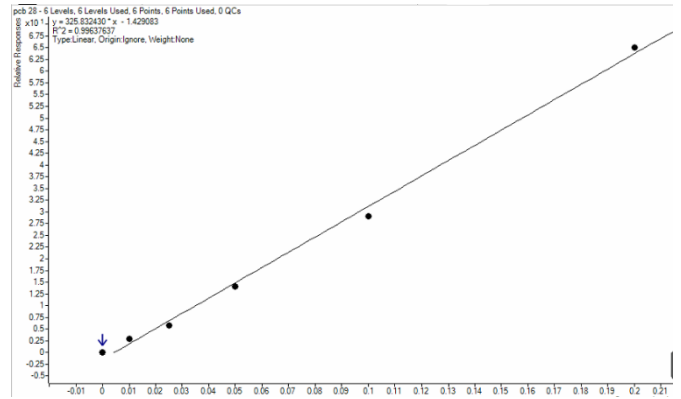


Figure 8. Linearity of PCB28.  $R^2 = 0.9964$

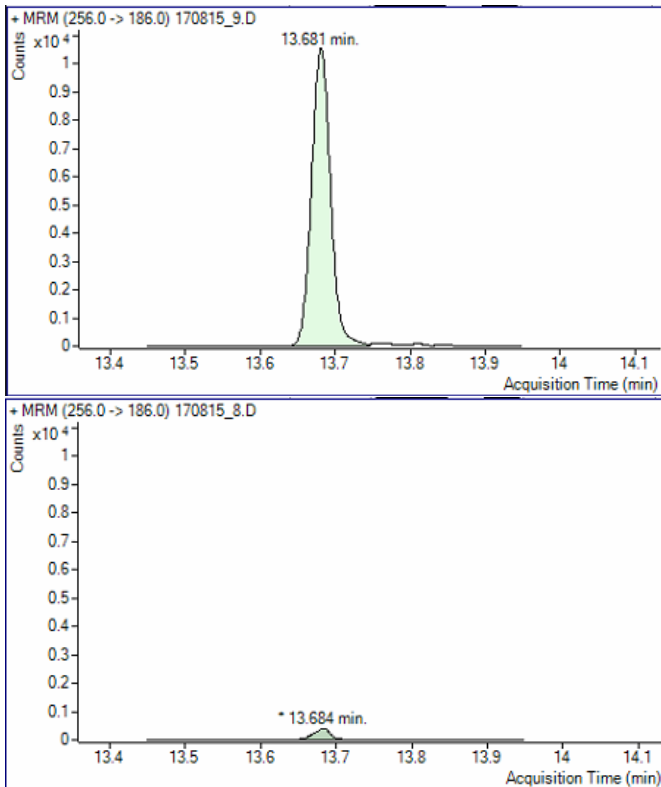


Figure 9. 5ng/L PCB28 (top) Blank (bottom)

The automated method removes the requirement for a laboratory analyst to perform manual LLE. For a batch of samples the manual method required two analysts, and three batches could be extracted in one day. If samples were extracted every day over the course of the year of the 260 working days 228 days of manual extraction for each analyst can be saved. When multiplied up by the two staff used for the manual method this equates to 3900 hours or 456 days of working time assume that it takes one hour to prepare samples for the automated method by one analyst.

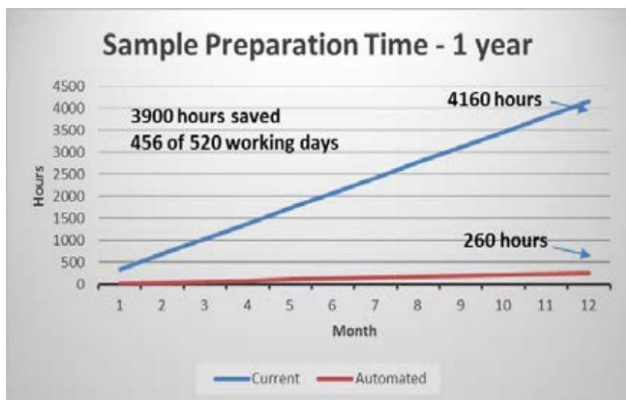


Figure 10. Sample preparation time

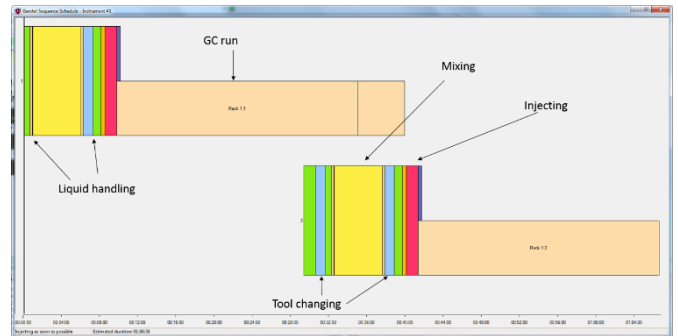


Figure 11. Maestro PrepSequence.

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. 55 samples can be prepared and ran in a 24 hour period. This would equate to an increase in throughput of over 100% compared to a manual method where 1 day is spent preparing and an equal amount of instrument time is required for acquisition. Figure 11 shows the automated PrepAhead function of Maestro. For comparison, 10 samples analysed sequentially would take 6 hours, but when the PrepAhead function is utilised, 10 samples takes 4 hours 40 minutes.

Figure 12 illustrates the potential savings for the main extraction solvent of extracting equal numbers of samples for the manual preparation and automated solution. The consumption saving does not take into consideration the amount of solvent that is currently used in the lab to wash the glass vessels used in the sample preparation. This washing solvent would be reduced to the amount of solvent taken to clean the syringes in between sampling steps.

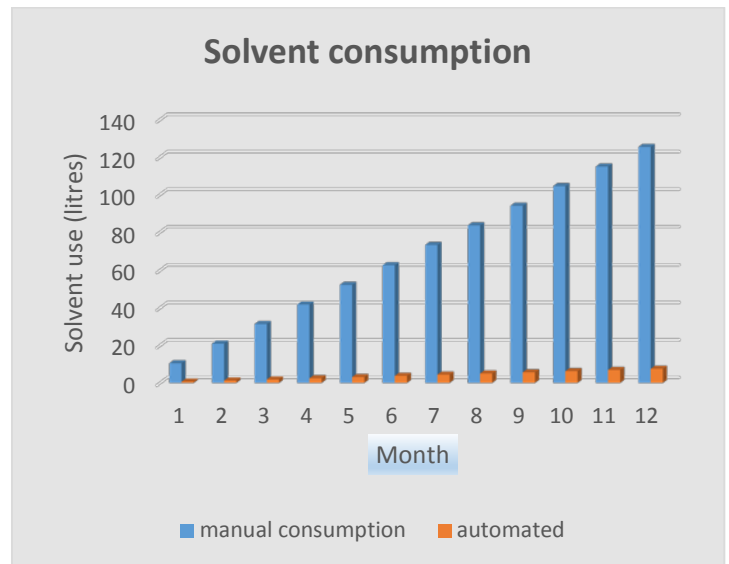


Figure 12. Solvent consumption over 12 months (based on extracting 30 sample per day for 260 days of the year).



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## Discussion

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The automation and miniaturisation of the customer OCP method has been demonstrated to;

- Improve sample throughput by 100%
- Save 3900 working hours in manual preparation time
- Reduce solvent consumption by a minimum of 120 litres
- Reduce solvent disposal cost
- Reduce solvent storage space requirements
- Reduce the need to collect large sample sizes – reduces storage space and solvent used to clean glassware
- Reduce the health and safety risks to analysts
- Give reliable accurate and reproducible data

Use of the Quick Mix does now enable the user to reduce GC run times to match extraction times which would again increase sample capacity.

This work demonstrates how the improved technology is able to be used for methods developed on previous GERSTEL MPS XTs. Further investigation is required to optimize the method for some of the compounds, a larger injection volume, optimization of the LLE or instrument settings may give adequate responses for quantitation. Emulsion formation can be a problem for dirty water samples but inclusion of the CF-200 Centrifuge to the automation would be possible if this was found to be an issue. Please [contact Anatune](#) if you need any further information on this technique.