# Initial Work for Automation of Organo Chlorine Pesticides and Poly Chlorinated Biphenyls in Water

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## Introduction

This application note describes the method development of an on-line automated solution for OCPs and PCBs. Development was in conjunction with Paul Leather, Environment Agency in July-Aug 2016. This method uses an automated liquid-liquid extraction (LLE) using the GERSTEL *Agitator*.

The OCPs are non-polar, hydrophobic compounds that do not ionise so are extracted well into organic solvents.

One of the key challenges of automating the method for OCPs and PCBs is with the scaling factor that extraction from a large sample volume allows. The manual method utilises a LLE with a concentration factor of 100 times, this is followed with an evaporation step that further concentrates the sample by a factor of 10. Overall the manual sample preparation gives a concentration factor of around 1,000 times.

The method was scaled down to use a 20mL vial, with 18mL of sample and 500µL solvent. This gave a concentration factor of 36.

Direct injection from solvent layer requires the depth of the solvent to facilitate reproducible sampling. Tolerances on the glassware and volumes added contribute to the position of the solvent layer. The band of solvent is required to be of sufficient depth to ensure correct sampling. The GERSTEL MPS was carefully programmed to sample reproducibly.

Using the Agilent 5977B with High Efficiency Source (HES), the concentration factor losses from miniaturising the sample preparation were accounted for. The HES source has been shown to give around 20-50 times sensitivity gains in comparison with the 5973C that is currently employed for the method.

A large volume injection (LVI) can then be used to achieve a similar concentration factor to the original method.



**Figure 1. GERSTEL Dual Head MPS XT with Agilent 5977B**

## Instrumentation

Dual Head GERSTEL MPS XT
GERSTEL *Agitator*
Anatune CoolR[PLUS]
GERSTEL *CIS*
Maestro software integrated
Agilent 7890 GC with a 5977B mass spectrometer with High Efficiency Source (HES)

## Method

A suite of thirty nine OCPs and nineteen PCBs were prepared firstly at a concentration of 1ug/mL and analysed in fullscan mode to confirm the retention time. A method in Selected Ion Monitoring (SIM) mode was then used to quantitate the OCPs and PCBs. This work was on a 30M 0.25mm I.D DB-5MS UI column.

Calibration standards were prepared at concentrations of 5, 25, 50, and 100 ng/L in purified water by automated addition of concentrated stock standards by the MPS. Table 1 shows the volume of stock solutions used to spike the calibration standards.

| Final Conc [ng/L] | Conc Stock [µg/L] | Vol water (mL) | Vol spiked (µL) |
|---|---|---|---|
| 5 | 50 | 18 | 1.8 |
| 25 | 50 | 18 | 9 |
| 50 | 500 | 18 | 1.8 |
| 100 | 500 | 18 | 3.6 |

Table 1. Calibration Standards preparation (auto spiking)

These aqueous standards were extracted using a mixture of isohexane and acetone. Sample mixing was performed on the GERSTEL Agitator at 750rpm. 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

The automated method removes the requirement for a laboratory analyst to perform manual LLE. For each batch of samples the manual method required two analysts, and three batches could be extracted in one day. The automated method only requires a preparation step of aliquoting the 18mL of sample into the 20mL vial, all further steps were automated.

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.
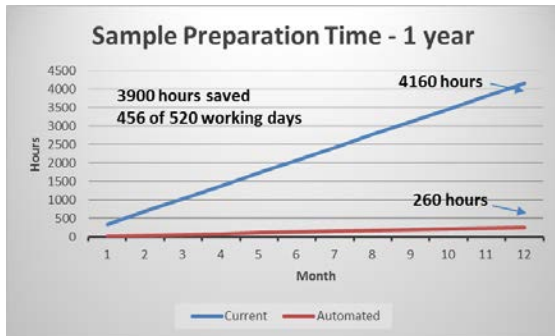
Figure 2. Sample Preparation time

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, 44 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 3 shows the automated PrepAhead function of Maestro. The multi-coloured bands represent the sample preparation and beige bands GC run-time.



Figure 3. Maestro PrepAhead

Solvent Consumption currently is 10mL Iso-hexane:Acetone (95:5) for extraction, by automating and scaling down the sample preparation the amount of solvent used could be reduced by around 155 litres of Iso-hexane and 8 litres of acetone per year. This is based on extracting into 500µL rather than 2mL and this could potentially be scaled further to 300µL. Figure 4 illustrates the potential savings for the main extraction solvent.

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.
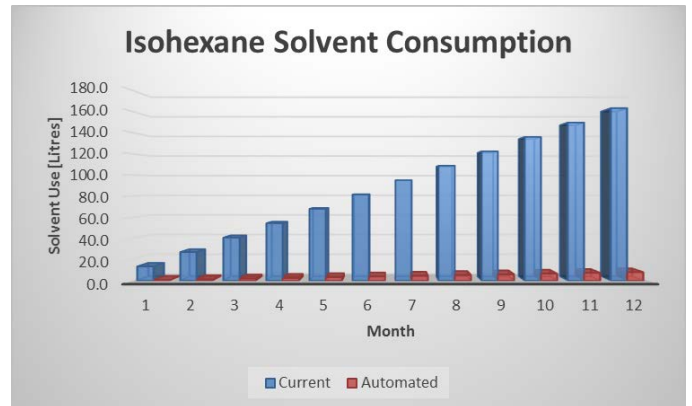


Figure 4. Solvent consumption

Automation of the preparation of the calibration standards (auto spiking) was demonstrated to give excellent linearity. Figure 5 shows the typical linearity achieved when using automation to spike the calibration standards. Figure 6 shows the peak response for trifluralin at the lowest calibration level. Using the automated technique improves linearity as accuracy, precision and reproducibility are improved. This is partly due to eliminating the human error in manual pipetting, these techniques are dependent on operator technique and ability. Automation of this pipetting also reduces risks of RSI.
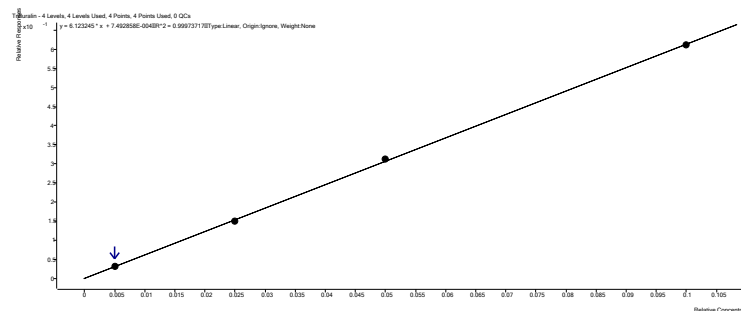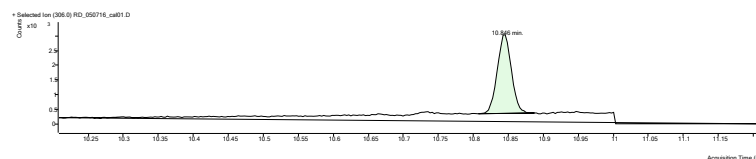


Figure 5. Linearity, Trifluralin



Figure 6. Trifluralin, 5ng/L

Although precision experiments have not yet been performed on all of the thirty nine OCPs and nineteen PCBs, % Relative Standard Deviation (%RSD) has been calculated on the trifluralin-d14 internal standard as an example. The internal standard was added as part of the automated procedure and the %RSD calculated for trifluralin-d14 was 5.5%. Table 2 gives the data for this calculation.

| Name | Data File | Type | Level | Acq. Date-Time | Vial | Exp. Conc. | RT | Resp. | Calc. Conc. | Final Conc. | Accuracy | RT | Resp. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 ng/L | RD_050716_cal01.D | Cal | 1 | 05/07/2016 12:28 | 1 | 0.005 | 10.85 | 3787 | 0.0051 | 0.0051 | 102.2 | 10.74 | 118135 |
| 25ng/L | RD_050716_cal02.D | Cal | 2 | 05/07/2016 13:06 | 2 | 0.025 | 10.85 | 16294 | 0.0243 | 0.0243 | 97.1 | 10.74 | 109024 |
| 50ng/L | RD_050716_cal03.D | Cal | 3 | 05/07/2016 13:44 | 3 | 0.05 | 10.85 | 35703 | 0.0509 | 0.0509 | 101.7 | 10.74 | 114369 |
| 100ng/L | RD_050716_cal04.D | Cal | 4 | 05/07/2016 14:22 | 4 | 0.1 | 10.85 | 70219 | 0.0997 | 0.0997 | 99.7 | 10.74 | 114830 |
| Blank Istd | RD_050716_cal05.D | Sample | | 05/07/2016 14:59 | 5 | | 10.85 | 453 | 0.0006 | 0.0006 | | 10.74 | 102474 |

Table 2. Trifluralin-d14 internal standard RSD data.

# *Discussion*

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 160 litres
- Reduce solvent disposal cost
- Reduce solvent storage space requirements
- Reduce the need to collect large sample sizes – reduces storage space, solvent used to clean glassware
- Reduce the health and safety risks to analysts
- Give reliable accurate and reproducible data
- Reduce instrument maintenance costs

To demonstrate sensitivity is sufficient for all the thirty nine OCPs and nineteen PCBs, further optimisation of the extraction and LVI methods should be carried out.

Not all compounds were quantifiable to the lowest concentration, some compounds demonstrated noisy blank responses. This was attributed partly to the system cleanliness and column age at time of testing as well as using solvent purchased from a different supplier to the customer method.

Certain compounds may also benefit from further sample enrichment on the extraction or optimisation of the LVI method. The original customer method uses a 30µL injection and only 10µL was injected for this initial work. The example given below (figure 8) is trans-chlordane at 5 ng/L and subsequent linearity (figure 7). The peak at 18.93 minutes is trans-chlordane, 19.30 is cis-chlordane and the peak at 18.75 is a matrix peak.
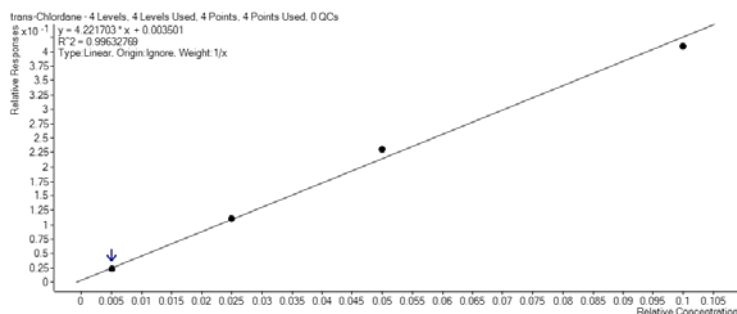


trans-Chlordane - 4 Levels, 4 Levels Used, 4 Points, 4 Points Used, 0 QCs
y = 4.221703 * x + 0.003501
R^2 = 0.99632769
Type:Linear, Origin:Ignore, Weight:1/x
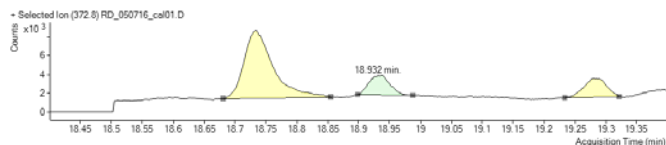
**Figure 7. Linearity Trans-Chlordane**



**Figure 8. Trans-chlordane 5ng/L**

This initial investigation demonstrates how the improved sensitivity of the 5977B can offer the option for automation of LLE for OCPs and PCBs in water. 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.

The automated technique uses 18mL of sample, 0.5mL solvent and an agitator at 750 rpm to extract the OCPs. The agitation was sufficient for the extraction of the OCPs when spiked into a clean water, further investigation may be required to show the agitation is sufficient to extract the OCPs from real samples or if the extraction recovery could be improved by a more vigourous technique.

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.