

## Chromatography Technical Note No AS171

# Initial Work for Automation of Polycyclic Aromatic Hydrocarbons in Water

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### Introduction

This application note describes the method development of an on-line automated solution for Polycyclic Aromatic Hydrocarbons (PAHs). 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 PAHs are non-polar, hydrophobic compounds that do not ionise so are extracted well into organic solvents.

The original method is a LLE using 1000mL of sample extracting into 5mL cyclohexane. The automated method created was scaled down to use a 20mL vial, with 18mL of sample and 500µL solvent.

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 with Agilent 5977B (HES)

### Instrumentation

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

### Method

A suite of eighteen PAHs were prepared firstly at a concentration of 1µg/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 PAHs. This work was on a 30M 0.25mm I.D DB-5MS UI column.

Calibration standards were prepared at concentrations of 10, 25, 50, 100, and 200ng/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)
10	50	18	3.6
25	50	18	9
50	500	18	1.8
100	500	18	3.6
200	500	18	7.2

Table 1. Calibration Standards preparation (auto spiking)

These aqueous standards were extracted using a cyclohexane. 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. 10µL 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. 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 can be saved.

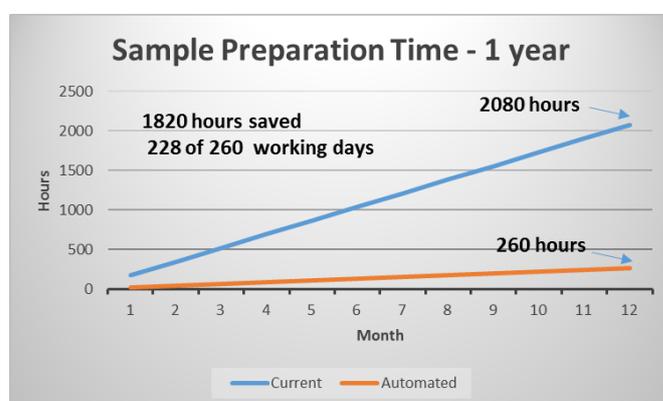


Figure 2. Sample Preparation time

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<sup>PLUS</sup>, which enables rapid cooling of the GC oven, 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 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 5mL cyclohexane for extraction, by automating and scaling down the sample preparation the amount of solvent used could be reduced by around 70 litres of cyclohexane per year. This is based on extracting into 500µL rather than 5mL 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.

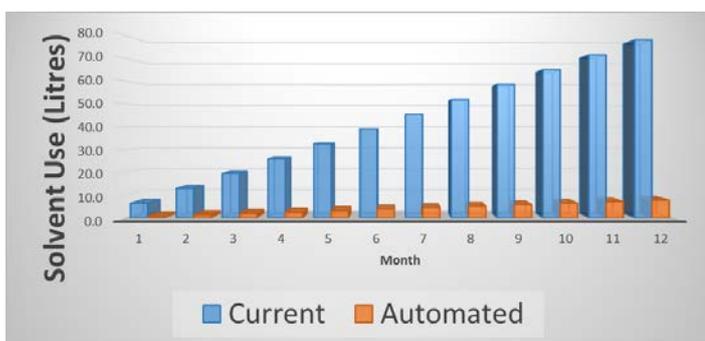


Figure 4 – Solvent consumption

Automated sample preparation also included the preparation of calibration standards. The linearity and peak responses were excellent for all of the PAHs. Linearity was  $\geq 0.995$  for all compounds with no weighting or 1/x weighting applied. Figure 5 shows the typical linearity achieved when using automation to spike the calibration standards. Figure 6 shows the peak response for benzo(a)anthracene 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.

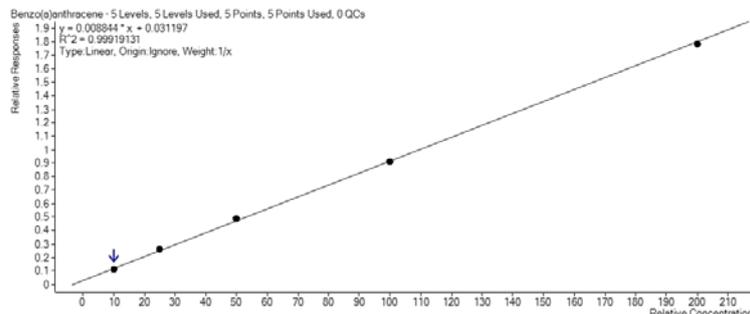


Figure 5. Linearity, benzo(a)anthracene

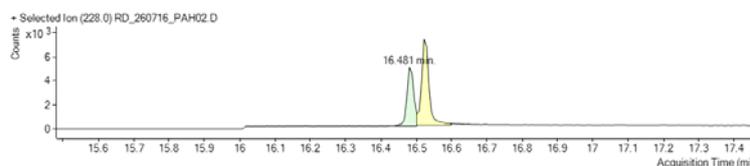


Figure 6. benzo(a)anthracene, 10 ng/L

Although precision experiments have not yet been performed on all of the eighteen PAHs, % Relative Standard Deviation (%RSD) has been calculated on the benzo(a)anthracene-d12 internal standard as an example. The internal standard was added as part of the automated procedure and the %RSD calculated for benzo(a)anthracene-d12 was 7.0%. Table 2 gives the data for this calculation.

Name	Data File	Type	Level	Acq. Date-Time	Vial	Benzo(a)anthracene Method		Benzo(a)anthracene Results				Benzo(a)Anthracene-d12 (ISTD) Results	
						Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	RT	Resp.
Cal 1 10ng/L	RD_260716_PAH02.D	Cal	1	26/07/2016 13:25	2	10	16.48	6546	9.3	9.3	92.6	16	5613
Cal 2 25 ng/L	RD_260716_PAH03.D	Cal	2	26/07/2016 13:58	3	25	16.48	15229	26.4	26.4	105.6	16	57513
Cal 3 50 ng/L	RD_260716_PAH04.D	Cal	3	26/07/2016 14:30	4	50	16.48	32573	51.5	51.5	103.0	16	66962
Cal 4 100 ng/L	RD_260716_PAH05.D	Cal	4	26/07/2016 15:02	5	100	16.48	51629	99.7	99.7	99.7	16	56535
Cal 5 200 ng/L	RD_260716_PAH06.D	Cal	5	26/07/2016 15:35	6	200	16.48	111117	198.1	198.1	99.1	16	62307
Blank had	RD_260716_PAH07.D	Sample		26/07/2016 16:07	7		16.48	953	0.0	0.0		16	57722

Table 2. Benzo(a)anthracene-d12 internal standard RSD data.

## Discussion

The automation and miniaturisation of the current SX O PAH method has been demonstrated to;

- Improve sample throughput
- Save 1820 working hours in manual preparation time
- Reduce solvent consumption by a minimum of 75 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

This initial investigation demonstrates how the improved sensitivity of the 5977B can offer the option for automation of LLE for PAHs in water. The automated technique uses 18mL of sample, 0.5mL solvent and an agitator at 750rpm to extract the PAHs. The agitation was sufficient for the extraction of the PAHs when spiked into a clean water, further investigation may be required to show the agitation is sufficient to extract the PAHs from real samples or if the extraction recovery could be improved by a more vigorous technique. Emulsion formation can be a problem for dirty water samples but inclusion of the CF200 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.