VALIDATION OF A HIGH THROUGHPUT SEMI-AUTOMATED SAMPLE PREPARATION METHOD FOR PERSISTENT PESTICIDES AND POLYCHLORINATED BIPHENYLS IN HUMAN SERUM, BASED ON SOLID PHASE EXTRACTION

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Introduction

A serum extraction and cleanup method has been developed with the aim of increasing current sample through-put as well as improving overall performance, i.e., decrease sample to sample variability and optimization of overall recovery. To reach these goals, solid phase extraction employing automation on the Rapid Trace by Zymark Corporation has been implemented, using a Gilson 215-liquid handler to automate necessary sample pretreatment steps including among others, addition of internal standards. Cleanup of the serum extract is performed by application of the extract on an activated silica gel column, using the Zymark Rapid trace after necessary evaporation and reconstitution of the sample. Final cleanup is performed using gel permeation chromatography (GPC), on a Gilson HPLC system and a Gilson 215-liquid handler as an auto-injector and fraction collector.

Solid phase extraction is a sorbent extraction methodology in which the sample, in this case serum, after necessary pretreatment including denaturation and acidification is applied to a column containing a sorbent, where the target analytes are retained. Biogenic materials less retained on the sorbent are partially eluted from the column; traditionally C18 derivitized silica [1,2] has been used. Although, in more resent years, several manufacturers have released sorbents based on either styrene-divenyl benzene (SDB) polymers or SDB-polymers with functional groups. Some examples of SDB-polymers include OASIS-HLB (Waters), ENV and PPL (Varian) and ENV+ (Jones Chromatography). SDB polymers have previously been evaluated and applied in environmental research [3].

In the current method, automation of sample pretreatment using the Gilson 215-liquid handler is addressed, including reproducibility and accuracy measurements of internal standard additions. Quality Assurance/Quality Control (QA/QC) charts for selected analytes as well as relative standard deviations for the measurement of 11 PCBs and 11 persistent pesticides in 27 samples are reported.

Methods and Materials

Instrumentation

A 215-liquid handler by Gilson (Middleton, WI) equipped with a 204-syringe pump (Gilson) were used for automation of internal standard additions and denaturation of the serum samples prior to SPE extraction. SPE extraction was performed on the RapidTrace by Zymark corporation (Hopkinton, MA), which is a modular automated SPE system.

Gel permeation chromatography (GPC) was performed on an HPLC manufactured by Gilson equipped with a 215-liquid handler as an autoinjector and fraction collector, a dual pump head 302-

pump (Gilson), a UV/VIS-156 detector (Gilson), a 402-syringe pump and a HPLC column heater/ chiller model 7956 (Jones Chromatography) was used. The chromatographic separations were performed on a high resolution GPC column (10 µm, 50 Å, 7.5 I.D., 300 mm length, Polymer Laboratories, Amherst, MA).

Sample preparation method – general overview

Serum samples (1 g) are added to standard 16 x 100 mm test tubes, fitted with persable septum caps; the sample weight was recorded to an accuracy of 1 mg. The test tubes containing the serum are placed in an Automix on the Gilson 215 liquid handler; followed by unattended addition of internal standard (100 µl in methanol), concentrated formic acid (1 ml) and water (1 ml) with mixing by rotation (5 min) between each addition.

Samples are transferred to a Rapid Trace (Zymark Corporation) for unattended extraction of the samples. The procedure includes (*i*) conditioning of the sorbent, (*ii*) application of the sample, (*iii*) rinsing of biogenic material less retained on the sorbent to waste by elution with 0.1M HCl and 5% methanol solution, (*iv*) drying of the sorbent by means of high pressure (45 psi) nitrogen flow through the extraction cartridge and (*v*) elution of compounds of interest. The extract is then evaporated to dryness. The sample is reconstituted in hexane 500 µl and eluted through an activated silica gel column (0.9 g, activated at 180 °C). The sample is brought to a volume of 100 µl. Final cleanup of the extract prior to analysis is performed on a high pressure liquid chromatography (HPLC) system fitted with an high resolution GPC column, Polymer Laboratories, (Amherst, MA).

The samples are transferred to GC vials and fortified with a recovery standard solution using the 215-liquid handler (Gilson) prior to evaporation to a final volume of 10 ml for gas chromatography high resolution analysis [4].

Spiking reproducibility

Estimation of spiking reproducibility and accuracy on the Gilson 215-liquid handler was performed at four different spike levels, i.e., 25, 50, 100 and 200 µl. The instrumental parameters for the 215-liquid handler, used in the spike reproducibility experiment are given in Table 1. The reproducibility of the internal standard spike was estimated by spiking a known amount of a standard containing CB-153 (200 pg/µl) to a known amount of CB-138 standard (10 pg/µl), using 10 replicates for each spike level. For the two lowest spike volumes, 250 ml of the CB-138 standard were used, while 500 µl were used for the larger spike volumes, the amount of the standard in each sample tube was measured by means of analytical balance. In addition, five replicates for each spike level were constructed by

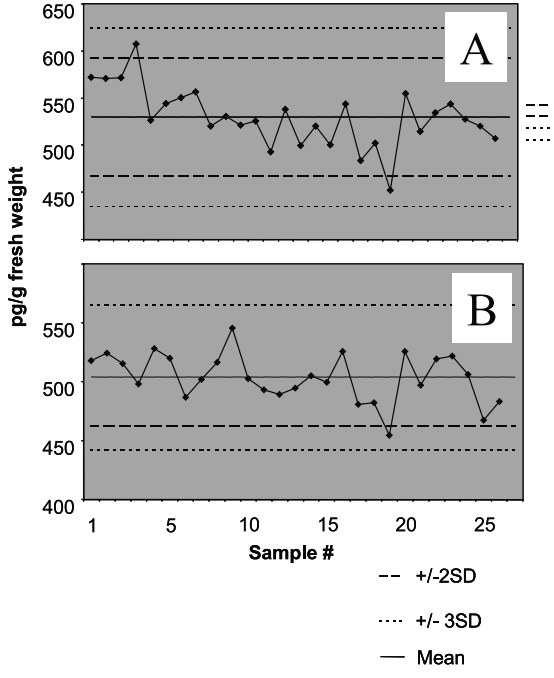
Table 1. Instrumental settings used for spiking reproducibility evaluation of Gilson 215-liquid handler. Solvent plug and air gap refers to volume drawn before standard and air gap refers to air volume in-between standard and solvent plug, respectively. Reproducibility as percent relative standard deviation (RSD) (n=10) and precision as percent deviation from mean standard made using analytical balance (n=5), are given.

Spike	Instrumental settings					Accuracy	
volume	Syringe size	Air gap	Solvent plug	Take-up speed	Dispense speed	Reproducibility	Precision
(ul)	(mL)	(ul)	(ul)	(mL/min)	(mL/min)	RSD	%-deviation
200	1	40	40	1	3	3.2	-0.8
100	1	40	40	1	3	1.9	0.5
50	1	40	40	1	3	1.5	4.5
25	0.1	20	20	1	3	3.2	9.3

Table 2. **Reproducibility of developed methodology expressed as relative standard deviation (RSD) from the analysis of 27 QA/QC samples comprised of unspiked human serum.**

Compound	Conc. average	RSD	Compound	Conc. average	RSD
	pg/g fresh weight			pg/g fresh weight	
PCB28	388	4	HCB	191	5
PCB52	68	8	β-HCH	386	12
PCB118	81	5	γ-HCH	52	6
PCB105	33	7	Heptachlorepoxide	132	8
PCB153	530	6	Oxychlor	308	9
PCB138	491	4	t-Nonachlor	479	4
PCB156	73	9	pp-DDE	431	8
PCB180	437	4	Dieldrin	173	7
PCB189	6	33	o,p'-DDT	29	13
PCB194	91	5	p,p'-DDT	704	5
PCB209	65	5	Mirex	504	4

Figure 1. QA/QC chart showing levels of CB-153 and Mirex in 27 replicate samples of human serum.



spiking CB-153 (25 to 200 µl) by use of Rainin Instrument Company Inc (Woburn, MA) SE3 automatic volumetric pipettes and recording the accurate amount added by means of analytical balance. Accuracy of the 215-liquid handler was estimated in relation to these five replicates with known amounts of CB-138 and CB-153. All samples were analyzed by GC/MSD Agilent Technologies (Atlanta, GA) in negative chemical ionization mode, monitoring for ions *m/z* 372 and 374. Reproducibility of the standard spike for each spike volume was calculated as relative standard deviation (RSD) of the area ratio CB-153 over CB-138, c.f. Table 2. Accuracy, of the standard spike at each spike volume were calculated as a percentage of the difference in mean value of the same area ratio, in-between the 10 samples spiked on the 215-liquid handler and the five references constructed on the analytical balance after necessary corrections of solvent density. The spiking standard (CB-153) was prepared in methanol, since this is the solvent used when serum samples are spiked. Hence the final sample was solvent transferred to trimethylpentane prior to GC/MS analysis.

Results and Discussion

The current automated methodology has significantly reduced the number of man-hours required for processing samples for PCB and persistent pesticide analysis. The manual steps in the method are currently comprised of evaporations and loading samples into racks for the automated systems and transferring of the final sample to GC vials followed by evaporation. The method has good reproducibility as illustrated in Table 2, showing RSDs in the range of 4 – 13 % for 10 PCBs (concentration over 33 pg/g fresh weight) and 11 persistent pesticides for 27 samples analyzed in two different batches, in two different weeks. The low sample to sample variability is most likely due to the high degree of automation; performing each task the same way for each sample and high purity of the final sample extract. There are no apparent trends observed in the QA/QC charts, as shown in Figure 1, for CB-153 and Mirex, respectively.

The 215-liquid handler has been shown to have a reproducibility of 3 % RSD or less when spike volumes in the range of 25 to 200 µl are used. In the current method, a spike volume of 100 µl has been implemented. The variability of the Ranin autopipetters for manual addition of internal standards to samples has a much lower variability than the 215-liquid handler for each of the spike volumes studied, i.e., 0.3 – 1.5 % RSD. The variability of the Ranin pipettes were estimated from the balance readings when constructing the 5 replicates used for the accuracy calculation, c.f., Methods and Materials, above. However, the variability of the 215-liquid handler is still in the range of what can be expected as normal variability of a GC/MS system and signal integration errors. The accuracy of the 215-liquid handler in comparison to the five replicate samples constructed on the analytical balance are also in an acceptable range as estimated to be 0.5 % in deviation from the mean value of the samples constructed on the analytical balance, for a spike volume of 100 µl. Automation and unattended processing of samples has improved the throughput in comparison with existing methods in our lab, although, the actual number of samples possible to process per given time has to be empirically found when the current method is implemented.

References

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