USE OF AUTOMATED COLUMN CHROMATOGRAPHY CLEAN UP WITH REDUCED SOLVENT VOLUME IN POPS ANALYSIS

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Introduction

Analysis of various matrices for polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) has traditionally involved Soxhlet extraction (typically up to 24-36 h), followed by preparative multi column chromatography steps. This process can include acid-base-neutral silica, pure acidified silica, alumina, and carbon columns. For the acid-base combination mixtures have typically contained 22% or 44% sulfuric acid mixed with silica and 33% sodium hydroxide mixed with silica.

Attempts at automating sample extraction and clean up have been undertaken resulting in development of short time-frame Pressurized Liquid Extraction (PLE) – taking a total processing time of ~ 1 h as opposed to Soxhlet extraction – and the advancement of automated chromatography column sample clean up.[1] Because of rising solvent costs in recent years an attempt has been made to develop reduced volume programs for automated chromatography clean up. Results are presented here.

Materials and methods

The automated system contains a control module that pilots valve drive modules connected to a pump and pressure modules responsible for solvent flow in the valve module. The current model has a built in computer that does not need a stand-alone pc. Easy programming and software editing allows the creation of custom made sequences of events that drive the required solvent at the right place at the right moment. The system operates at low pressure (5-30 psi). Flow rates of up to 10-15 mL/min are used. The work described here consisted of using a high capacity acid base column (20 g of acidified silica and 20 g of alkaline silica), an alumina column (11 g) and a carbon column (0.34 g carbon). These columns are packed in disposable Teflon tubes individually sealed in Mylar packaging.

The new program used is shown in Table 1 on the left. The columns are conditioned with hexane at the beginning of the program during steps 1-3. Then the sample is loaded in hexane (step 4) and the HC-ABN column is eluted with hexane (step 5) to transfer the PCDD/Fs and PCBs onto the alumina column. The system is then flushed with 10% dichloromethane (DCM) in hexane (step 6), followed by elution of the alumina column with 70 mLs of 10% DCM/hexane (step 7). All PCBs elute off the alumina column in this step and are collected as Fraction 1. This includes the co-planary PCBs. Step 8 is flushing the system with pure dichloromethane which is collected to assure that all PCBs have been eluted. Step 9 elutes the remaining PCDD/Fs off the alumina and onto the carbon column. Step 10 flushes the system with toluene followed by elution of the carbon column with toluene (step 11) which is collected as Fraction 2 containing the PCDD/Fs. The final hexane purge in step 12 is also collected in this fraction. Note that the advantage of this program is that the PCBs and PCDD/Fs are completely separated into two different fractions. Therefore every sample needs only two GC/MS injections as opposed to three when the co-planary PCBs elute into the PCDD/F fraction. Steps 1-6 and 9-10 are going to waste and are not collected.

In addition to having separate PCB and PCDD/F fractions the new reduced volume program in Table 1 uses only 345 mLs of solvent compared to the 750 mLs for the original program which is shown on the right. Hence

New HC ABN program				Original HC-ABN program			
Step	**Flo**	**Volum**	**Description**	**Step**	**Flo**	**Volum**	**Description**
1	10	20	Condition ABN hexane	1	10	20	Wet silica hexane
2	10	10	Condition Alumina hexane	2	10	20	Flush bypass hexane
3	10	10	Condition Carbon hexane	3	10	20	Wet alumina hexane
4	5	30	Load Sample	4	10	20	Wet carbon hexane
5	10	120	Elute Hexane	5	10	90	Condition silica hexane
6	10	10	Change to 10% DCM in hexane	6	10	12	Toluene rinse
7	10	70	Elute 10% DCM in hexane	7	10	40	Pre-elute toluene
8	10	10	Change to DCM	8	10	12	EtAc/toluene 50/50 flush
9	10	45	Elute DCM	9	10	10	Elute EtAc/toluene
10	10	10	Change to Toluene	10	10	12	50/50 DCM/hexane flush
11	5	30	Elute Toluene	11	10	20	Pre-elute 50/50
12	10	10	Hexane Purge	12	10	12	Flush hexane
				13	10	30	Pre-elute hexane
Total Volume:		345		14	5	14	Sample load
Total Hexane:		242		15	10	90	Elute silica hexane
Total DCM:		63		16	10	12	Flush 2% DCM in hexane
				17	10	60	Elute 2%
				18	10	12	Flush 50/50 DCM - hexane
				19	10	120	Elute 50/50
				20	10	12	EtAc/toluene 50/50 flush
				21	10	4	EtAc/toluene elute
				22	10	12	Hexane flush
				23	10	10	Hexane elute
				24	10	12	Toluene flush
				25	5	75	Toluene Elute
				Total volume:		751	

Table 1 Original and reduced volumes in clean up program (all volumes in mLs, flows in mL/min).

the amount of solvent used in the new program is only about 45% of the original. The new program can be run in circa 40 min which results in faster turnaround time and less labor costs. Analysis was carried out with a Thermo Trace Ultra GC coupled with a DFS HRMS using a 60 m Dioxin-2 column with DB-5 like phase.

Results/Discussion We analyzed PCDD/Fs and PCBs in peanut butter, top soil and fish oil (Table 2). The recoveries of the ^{13}C labeled isotope dilution standards are shown. The peanut butter and top soil were first extracted with the PLE system and then processed on the automated column clean up system. Recoveries found

	peanut butter	top soil	fish oil
2378-T4CDF	80%	75%	78%
2378-T4CDD	89%	87%	86%
12378-P5CDF	92%	97%	101%
23478-P5CDF	78%	79%	86%
12378-P5CDD	83%	88%	93%
123478-H6CDF	84%	77%	78%
123678-H6CDF	75%	62%	64%
234678-H6CDF	69%	60%	62%
123789-H6CDF	86%	81%	82%
123478-H6CDD	88%	78%	80%
123678-H6CDD	72%	67%	67%
123789-H6CDD			
1234678-H7CDF	78%	71%	71%
1234789-H7CDF	96%	83%	84%
1234678-H7CDD	82%	78%	79%
OCDF			
OCDD	80%	81%	83%

		peanut butter	top soil	fish oil
33'44'-T4CB	77	72%	70%	77%
344'5-T4CB	81	73%	72%	75%
233'44'-P5CB	105	68%	71%	67%
2344'5-P5CB	114	71%	69%	67%
23'44'5-P5CB	118	67%	69%	68%
2'344'5-P5CB	123	67%	69%	65%
33'44'5-P5CB	126	76%	76%	71%
233'44'5-H6CB	156	65%	63%	60%
233'44'5'-H6CB	157	59%	58%	55%
23'44'55'-H6CB	167	65%	61%	56%
33'44'55'-H6CB	169	69%	67%	65%
233'44'55'-H7CB	170	59%	57%	54%
22'344'55'-H7CB	180	58%	56%	53%
233'44'55'-H7CB	189	64%	62%	58%

Table 2 ^{13}C recoveries for three different matrices processed with Pressurized Liquid Extraction (not for fish oil) and automated column chromatography using the reduced volume program.

were excellent. The work showed that all target PCBs (WHO-12 plus pcb-170 and -180) were collected efficiently in the separate fraction. Reducing the conditioning and wetting steps from the original program

proved to have no significant effect on the recoveries. The new reduced solvent method has also been used for the combination of classical (regular size) abn – alumina – carbon columns with a total volume of ~ 250 mLs. Further work with XL-ABN columns is in progress.

Our reduced solvent volume approach to automated sample clean up has been demonstrated to be very effective, both in terms of reduced labor costs (shorter sample prep time) and reduced solvent use, and is also giving excellent recoveries with difficult matrices. This demonstrates once again the importance of automation in today's laboratory work.

References:

1. Focant, JF, Shirkhan, H, Patterson Jr, DG, (2009) *Organohalogen.Cmpds*, 71, 2438-2443.