

Cod: 2.1020

AUTOMATED GPC CLEAN-UP IN ENVIRONMENTAL SAMPLES: METHOD PERFORMANCE DETERMINATION AND STUDY OF THE EFFECTS OF COLUMN CALIBRATION AND DURABILITY OVER POP'S REAL SAMPLES RECOVERIES

R. Bocciolini¹, G. Croce¹, M. Lolini¹, M. Baldassini¹, S. Bianchi¹, S. Ninci¹, E. Dell'unto¹, A. Di Baia¹, F. Mannelli², A. Manni³

¹ARPAT, Environmental Protection Agency of Tuscany - Central Area Laboratory, Florence, Italy

²ARPAT, Environmental Protection Agency of Tuscany - South Area, Arezzo, Italy

³Chemical Research 2000 s.r.l - Rome, Italy

Introduction

The EPA methods for some POPs' analysis (EPA 1613B for PCDD/DFs and EPA 8270D for PCBs and PAHs), allow for the use of Gel Permeation Chromatography (GPC) as one of the extract cleanup systems that laboratories can use prior to GC-MS analysis (method EPA 3640A "Gel Permeation Cleanup"). Gel-permeation chromatography is a size exclusion cleanup procedure: an organic solvent flows throughout a stationary phase composed of hydrophobic porous gels containing cross-linked divinylbenzene-styrene. This system is used to purify extracts of complex samples (wastes, soils and sediments) to remove high molecular weight co-extracted matrix constituents, such as polymeric materials, lipids, humic acids, etc., which could damage the GC or MS system. It can also remove sulphur contamination from sediment samples. However, it doesn't remove chromatographic interference peaks of analytes with similar chemical characteristics. In this case it is better to use, in sequence, different clean up methods based on chemicals characteristics such as polarity (i.e. SPEs by Basic silica gel, Alumina, Fluorisil).

Materials and methods

The automated GPC Cleanup system used daily in the Central Area Laboratory of the Environmental Protection Agency of Tuscany (ARPAT), is a module of the PrepLinc™ Platform Automated Sample Preparation System (J2 Scientific, MO, USA). It includes an in line UV detector ($\lambda=254$ nm). Sample is injected directly onto a glass Express™ packed column. The Express Column is a smaller repackable column than the one indicated in the official method, therefore a smaller amount of solvent can be used and the chromatographic run is faster (35 min. using the Express column vs. more than 1h in standard column). The Express Column is composed of a S-X3 Bio-beads® stationary phase with 100% Methylene Chloride as the mobile phase.

As reported in the 3640A EPA method, it is necessary to determine the elution times of target analytes in order to collect them. The Corn Oil GPC calibration working solution (Table 1) used in our laboratory is diluted 1:20 in methylene chloride from a certified reference standard (O2Si, Charleston, USA) containing UV absorptive substances. A UV chromatogram is used for establishing dump and collect time for target analytes.

During the purification step with GPC Cleanup, it is not necessary to determine where each analyte elutes, but rather to determine a window where all analytes of interest elute together off the column. It is also necessary to determine the smallest window possible to save solvent, run time and post-purification evaporation time.

In EPA Method 1613B for the determination of PCDD/DFs, the collect fraction is wide, starting with the recovery of more than 85% of phthalate and stopping after elution of perylene, just before sulphur elutes (about 13 minutes using the Express column). The same collection time is reported for semivolatiles compounds in the EPA Method 3640A. Differently for the PCBs the above calibration method prescribes a reduced collect window starting with 95% of methoxychlor and ending after perylene elution, but before sulphur elutes (about 10 minutes with an Express column).

In an effort to minimize the solvent volume that must be evaporated after GPC Cleanup, it was prepared a standard solution combining the "Corn Oil GPC calibration working solution" (Table 1) with a laboratory prepared standard CLWS "GPC calibration laboratory's working solution" (Table 2), from different ISOGUIDE 34 CRMs containing target analytes, in the ratio 14: 1 v / v.

Results and discussions

UV chromatograms from the Corn Oil Working Standard Solution and the Laboratory's Calibration Standard are reported in fig. 1 and 2.

As shown above, it's possible to precisely determine the GPC collection time, beginning from the methoxychlor tail and stopping 30 seconds after perylene-sulphur valley. The collect window is reduced to eight minutes (about 40 ml). This alternate GPC calibration method was subjected to national interlaboratory proficiency tests. It showed very good results in recovery and repeatability.

A GPC Cleanup system was used in a proficiency test of PCBs in soil, in order to determine the laboratory's quality performance. A sample aliquot was analyzed ten times to determine 28 PCBs congeners. The sample, added with labelled compound spiking solutions (P48-M-ES and P48-W-ES-STK Wellington Laboratories, Canada), was extracted by automated Soxhlet using methylene chloride, and then purified by GPC cleanup with an Express Column. The extract, spiked with a labelled internal standard solution (P48-RS-STK Wellington Laboratories, Canada), was concentrated to 100 μ L and injected on a GC/MS. The concentrations of the PCB congeners in the sample are reported in Table 3. They were calculated by isotopic dilution using high resolution mass spectrometry (AutoSpec Premier™ Waters).

The GPC Cleanup system was also used to purify furnace waste extracts in a PCDD/DF proficiency test. Six aliquots of sample, spiked with a labelled compound solution (EN 1948 ES Wellington Laboratories, Canada), were extracted by automated Soxhlet using toluene and purified by GPC Cleanup followed by manual elution through a acidic multilayer SPE column. The PCDD/DFs congener concentrations were calculated by isotopic dilution, using high resolution mass spectrometry. They are reported in Table 4.

Table 5 summarizes the results obtained in these national proficiency tests, measured by z-score parameter.

Working with the isotopic dilution method, makes it possible to monitor GPC purification efficiency, in all samples, by comparing labelled extraction standard recoveries with internal standard recoveries. If the internal standard recoveries don't fall in the expected method range, it will be necessary to repeat the analysis, and to look for the cause; which could be an incorrect GPC calibration.

As reported in Table 3, the average PCB internal standard recoveries of 72-104% is within the range requested by other EPA methods (EPA Method 1668B); as reported in Table 4, the average PCDD/DFs internal standards' recovery of 86-91% is well within the range reported in the EPA Method 1613B.

The GPC Cleanup column can be used for a number of samples due to the fact that everything injected on the column is eluted from the column over time. But after many samples there can be a general lowering in recoveries or abnormal variations in the internal standard recoveries within the same analytes; most commonly in the case of PAHs. When this happens, a careful analysis of the GPC collect window must be performed.

If after having verified the flow rate stability and performing a new GPC calibration, no time variations in the collect window are noticed, more accurate tests must be performed using a solution containing a mix of relative response factor solutions containing both native and labeled PAHs, PCBs, and PCDD / DFs. A special GPC collection program was used consisting of three collect windows: the central is the one usually used in the purification step, while the other ones are respectively set two minutes before and two after. Gas chromatographic analysis of the three fractions will confirm whether or not the collection of the analytes of interest is in the set window.

By mass chromatographic analysis, it was determined that for PCDD/DFs and PCBs all target congeners were eluted in the second fraction, but not all the PAHs were recovered. The smallest and lightest congeners (naphthalene, acenaphthene and so on) were found quantitatively in the 2nd fraction. However, others were found quantitatively in the 3rd fraction, as shown in figure 3 for Pyrene and figure 4 for Benzo(g,h,i)perylene. For Indeno(1,2,3-c,d)pyrene (figure4), Benz[a]anthracene and Chrysene (native 12C m/z 228 and labelled 12d m/z 240) and for Dibenzo[a,h]anthracene (12C m/z 278 and 12d m/z 292) it was possible to notice a partition, although less prominent than the previous, in the 3rd fraction with particular regard to the native compounds rather than labelled ones. This partition happened even within groups characterized by similar ratio m/z (native or deuterated), that are expected to have similar chemical behaviour (figure 5).

Conclusion

The new standard solution developed by our laboratory, allowed us to minimize the collection eluate volume from GPC Cleanup, with a consequent reduction in evaporation time. This is crucial to avoid losses of analytes, especially semivolatile compounds such as PAHs.

Moreover for PAHs it is necessary to pay attention, in routine analysis, to the labelled internal standards recoveries, especially within m/z 252 and 264 mass chromatograms, because they can suggest a column's deterioration and indicate that the column stationary phase needs replaced.

Reference

1. Method EPA 3640A-1 rev 1 September 1994 "Gel Permeation Cleanup";
2. Method EPA 1613, October 1994. "Tetra-through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS";
3. Method EPA 8270D rev 5 July 2014 "Semivolatile organic compounds by gas chromatography/mass spectrometry" - V Update SW-846 compendium;
4. Method EPA 1668B November 2008 "Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS"

Table 1 “Corn Oil GPC calibration working solution”

compound	approximately concentration mg/l
Corn oil	12500
Bis(2-ethylhexyl) phthalate	500
Pentachlorophenol	100
Perylene	10
Sulphur	40

Table 2 CLWS “GPC calibration laboratory’s working solution”

	approximately concentration pg/ul
16 IPA: Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pirene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pirene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene	57 each compound
PCB: 101-PCB	1.5
17 congeners Mixer ¹² C PCDD-PCDF	0.95 each congeners

Table 3: “PCBs proficiency test results” ug/kg	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Average values	CV%	z score
T ₃ CB-28+T ₃ CB-31	0,56	0,42	0,47	0,55	0,53	0,50	0,38	0,71	0,64	0,49	0,53	18,6	-1,55
T ₄ CB-52	3,32	3,32	4,04	3,74	4,03	3,35	3,30	3,76	4,74	4,56	3,82	13,8	-1,19
T ₄ CB-81	0,08	0,10	0,10	0,07	0,10	0,09	0,07	0,09	0,09	0,10	0,09	13,3	2,93
T ₄ CB-77	0,95	1,22	0,97	1,03	1,12	0,95	0,86	1,05	1,41	1,22	1,08	15,4	-0,63
P ₅ CB-95	8,03	9,71	9,25	9,32	8,80	8,78	7,80	9,51	10,45	11,67	9,33	12,1	-0,85
P ₅ CB-101	14,71	18,77	17,06	16,49	16,46	16,10	14,30	16,77	18,72	21,13	17,05	11,9	-0,58
P ₅ CB-99	5,61	7,48	6,45	6,50	6,31	6,21	5,38	6,55	7,12	8,14	6,58	12,6	-0,76
P ₅ CB-110	17,06	21,37	19,91	19,55	19,85	18,48	17,37	19,11	22,33	25,40	20,04	12,4	-1,02
P ₅ CB-118	16,50	21,96	19,47	19,56	18,56	18,29	16,22	18,68	21,61	22,09	19,30	10,9	-0,76
P ₅ CB-114	0,52	0,69	0,59	0,50	0,77	0,56	0,52	0,59	0,72	0,75	0,62	16,2	0,96
P ₅ CB-105	8,40	10,72	9,61	9,47	8,62	9,00	7,38	9,68	10,40	10,27	9,36	10,9	-0,59
P ₅ CB-126	0,16	0,22	0,19	0,22	0,21	0,17	0,14	0,19	0,19	0,21	0,19	12,9	-0,99
H ₆ CB-151	2,36	2,83	2,77	2,66	2,86	2,59	2,32	2,84	3,14	3,43	2,78	12,0	-0,90
H ₆ CB-149	10,13	12,48	11,71	11,18	11,23	10,85	9,36	11,33	12,55	13,78	11,46	11,0	-1,13
H ₆ CB-146	2,18	2,70	2,55	2,40	2,63	2,48	2,10	2,53	2,84	2,89	2,53	10,1	-0,80
H ₆ CB-153	16,04	20,52	18,80	17,81	17,75	17,50	15,21	18,80	20,11	21,75	18,43	10,9	-1,05
H ₆ CB-138	19,82	25,21	23,20	21,45	21,72	21,38	18,38	22,71	24,83	25,34	22,41	10,3	-0,87
H ₆ CB-128+ H ₆ CB-167	4,82	6,12	5,67	5,48	5,57	5,26	4,57	5,44	6,11	6,39	5,54	10,3	-1,03
H ₆ CB-156	2,74	3,57	3,19	3,22	3,17	3,04	2,61	3,13	3,51	3,46	3,17	9,8	-0,42
H ₆ CB-157	0,65	0,85	0,82	0,73	0,88	0,73	0,63	0,74	0,84	0,80	0,77	11,2	-0,74
H ₇ CB-187	3,22	4,00	3,15	3,58	4,21	3,52	3,82	4,29	4,60	4,99	3,94	15,2	-0,75
H ₇ CB-183	1,47	1,87	1,49	1,66	2,04	1,61	1,75	1,84	2,10	2,29	1,81	14,8	-0,78
H ₇ CB-177	1,76	2,13	1,99	1,88	2,05	1,88	1,84	2,08	2,22	2,37	2,02	9,4	-0,79
H ₇ CB-180	8,77	11,75	10,59	10,01	10,15	9,77	9,32	11,26	11,19	12,18	10,50	10,4	-0,47
H ₇ CB-170	4,38	5,51	5,14	4,82	5,05	4,83	4,60	5,20	5,61	5,64	5,08	8,4	-0,76
H ₇ CB-189	0,16	0,20	0,18	0,17	0,24	0,17	0,19	0,18	0,20	0,21	0,19	12,5	-0,91
Sample average internal standard (IS) recovery %	80	72	94	83	90	98	100	104	83	86			

Table 4: “PCDD/DF proficiency test results” ng/kg	S1	S2	S3	S4	S5	S6	Average values	CV%	z score
2,3,7,8-TCDD	38,0	38,0	41,0	41,6	40,6	40,4	40,0	3,8	-0,07
2,3,7,8-TCDF	1493,2	1506,2	1439,6	1482,8	1481,4	1460,8	1.477,4	1,6	-0,37
1,2,3,7,8-PeCDD	231,3	240,3	234,3	233,4	245,9	228,9	235,7	2,7	-0,23
1,2,3,7,8-PeCDF	1009,9	1062,9	1019,3	1034,6	1063,0	1079,4	1.044,9	2,7	0,01
2,3,4,7,8-PeCDF	1616,2	1653,7	1654,3	1604,1	1721,0	1634,2	1.647,4	2,5	-0,4
1,2,3,4,7,8-HxCDD	177,2	174,2	176,1	173,5	178,7	179,6	176,6	1,4	-0,15
1,2,3,6,7,8-HxCDD	417,9	402,4	402,5	416,4	410,9	424,8	412,5	2,2	-0,52
1,2,3,7,8,9-HxCDD	375,1	385,4	359,7	381,6	368,3	388,2	376,4	2,9	-0,53
1,2,3,4,7,8-HxCDF	741,3	723,6	746,1	760,1	768,6	764,3	750,7	2,3	-0,13
1,2,3,6,7,8-HxCDF	713,2	729,0	730,6	746,1	743,0	758,7	736,8	2,2	-0,06
2,3,4,6,7,8-HxCDF	679,1	686,6	689,2	638,5	683,6	695,6	678,8	3,0	-0,51
1,2,3,7,8,9-HxCDF	34,8	35,4	33,1	33,5	60,3	52,6	41,6	28,3	-1,71
1,2,3,4,6,7,8-HpCDD	1325,6	1357,9	1367,7	1327,2	1305,5	1336,7	1.336,9	1,7	-0,27
1,2,3,4,6,7,8-HpCDF	1052,8	1026,6	1026,8	995,5	1046,5	973,7	1.020,4	3,0	-0,36
1,2,3,4,7,8,9-HpCDF	241,0	239,3	241,7	229,5	234,4	239,9	237,7	2,0	0,1
OCDD	801,4	806,1	794,9	831,5	832,2	866,8	822,2	3,3	-0,27
OCDF	335,8	319,5	330,5	342,4	340,6	337,7	334,4	2,5	-0,48
Σ PCDD/DF ng WHO-TEQ/kg	1135,6	1153,3	1146,0	1136,8	1182,6	1154,9	1.151,6	1,5	-0,74
Sample average internal standard (IS) recovery %	87	86	86	87	91	87			

Table 5: “proficiency test rating”	Acceptable		Warning (W)	Action (A)	Definition: Z score = (x-X)/s x = laboratory value X = proficiency test value s = assigned standard deviation
	z-score ≤ 1	1 < z-score ≤ 2	2 < z-score ≤ 3	z-score > 3	
PCBs proficiency test Organohalogen Compounds	19	6	1	-	914
PCDD/PCDFs proficiency test	17	1	-	-	

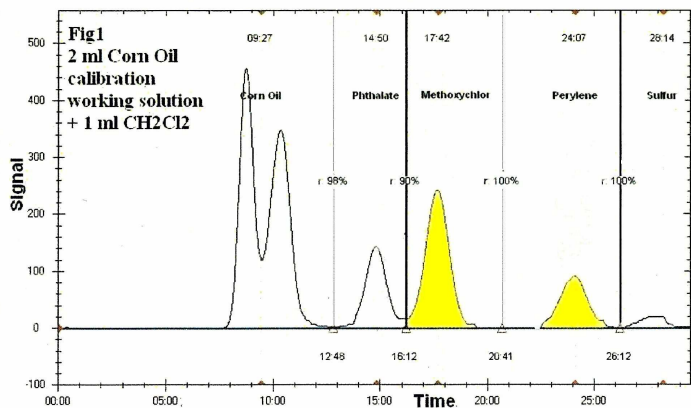


Figure 1
UV Chromatogram "Corn Oil GPC calibration working solution"

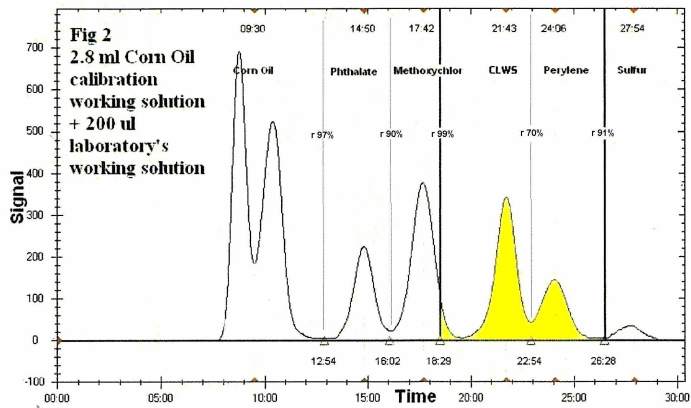


Figure 2
UV Chromatogram "laboratory's calibration standard solution"

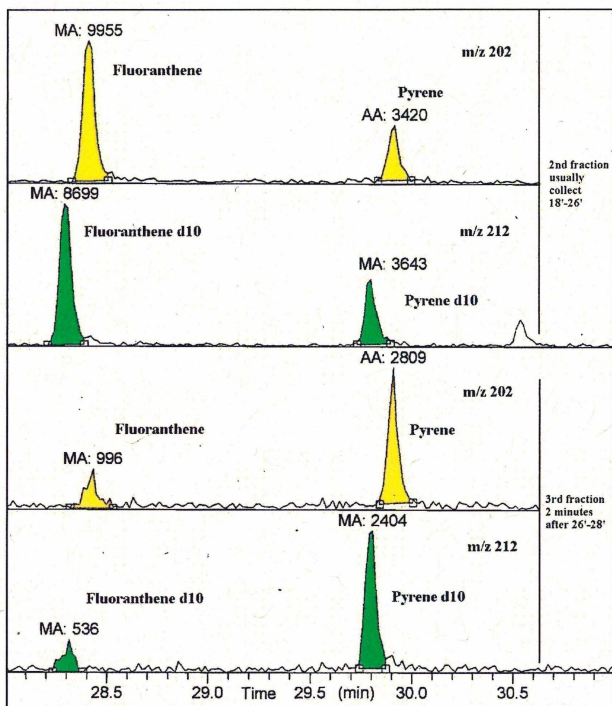


Figure 3
Comparison 2nd and 3rd fraction for 12C m/z 202 and 10d m/z 212

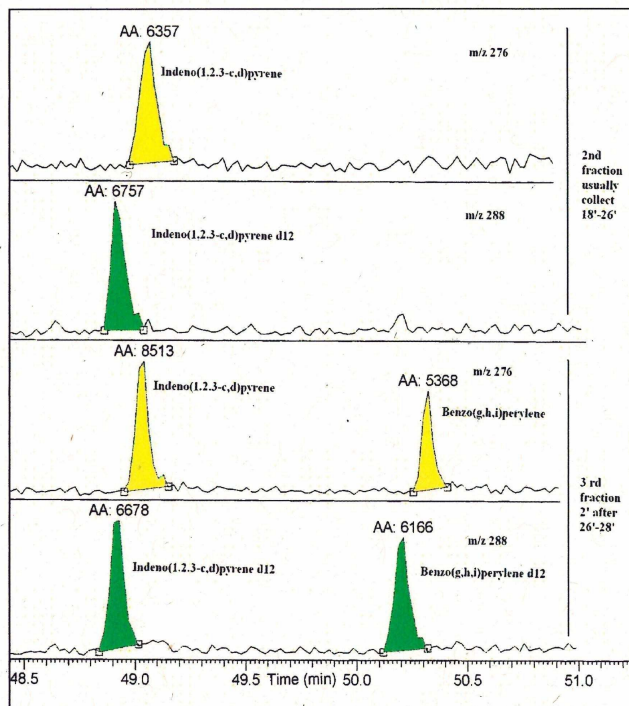


Figure 4
Comparison 2nd and 3rd fraction for 12C m/z 278 and 12d m/z 288

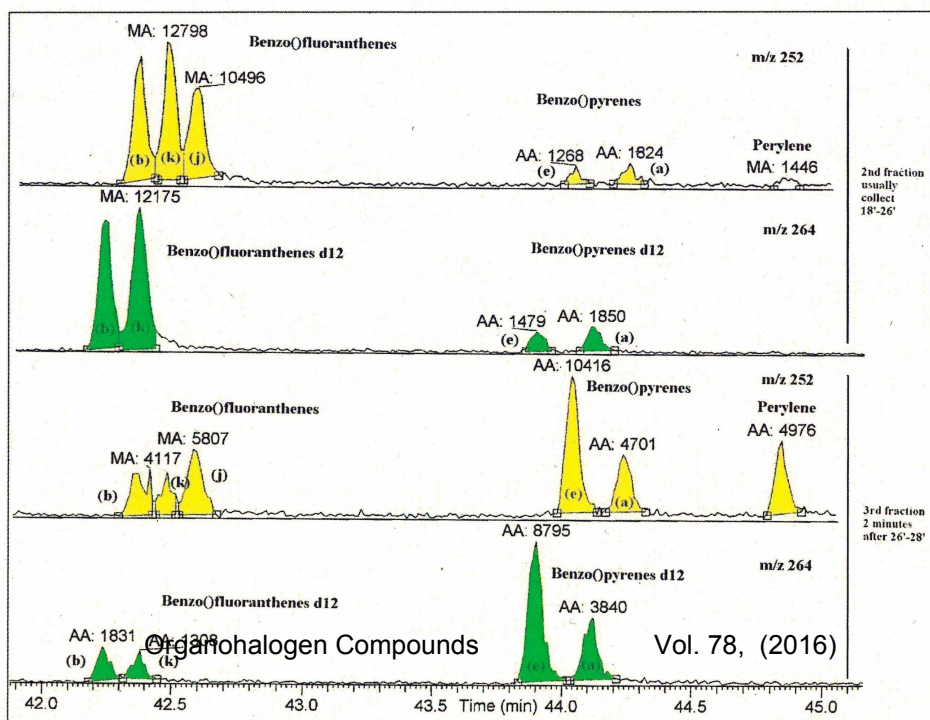


Figure 5
Comparison 2nd and 3rd fraction for 12C m/z 252 and 12d m/z 264