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APPLICATION OF CHROMA 2001 FOR IDENTIFICATION AND QUANTIFICATION OF SELECTED OCPs AND PCBs IN MULTICOMPONENT FOOD EXTRACTS

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The possibility of applying inexpensive CHROMA 2001 system for identification and quantification of selected organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in multicomponent food extracts was studied (fish, human milk). Chromatographic separation (GC-ECD) of the examined analytes was carried out on a ZB-5 capillary column (60 m; 0.25 mm i.d.; film thickness 0.25 μm). Two methods of the data acquisition and processing were created. Each analyte was identified by a comparison of the retention times (RTs) and relative retention times (RRT_{53} or RRT_{30+209}) of the peaks from calibration standards with peaks from cleaned-up extracts. In quantification, a one-point calibration curve method was used.

The results obtained indicate that the use of automatic data processing of multicomponent chromatograms by Chroma 2001 is limited. During identification of more than 14 analytes, manual data processing is required. Nevertheless, the application of CHROMA 2001 reduces considerably the time necessary for qualitative and quantitative GC analysis of multicomponent food extracts.

INTRODUCTION

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are abundant environmental contaminants included in Stockholm Convention list [Struciński *et al.,* 2000]. A combination of stability and propensity of OCPs and PCBs to form gas under appropriate environmental conditions means that they are subject to long-range atmospheric transportation, and a combination of their resistance to metabolism and lipophilicity means that they will accumulate in food chains [Jones & de Voogt, 1999].

Although the compounds mentioned represent two different types of pollution problems, they are often linked together in discussion on analytical methods [Hovander *et al.,* 2000; Schade *et al.,* 1998].

The determination of trace levels of OCPs and PCBs in environmental and food samples is a complicated procedure, consisting of many steps – sample homogenization, extraction from the matrix, clean-up and concentration, in the end gas chromatography (GC) separation and electron capture (ECD) or mass spectrometry detection (MS) [Thomas *et al.,* 1998; Juan *et al.,* 1999*;* Smedes & de Boer, 1997] .

A problem at the last step of GC analysis of cleaned-up extracts is the resulting information overloaded. A solution to this problem is utilization of computer-based pattern recognition techniques. Automated data quality assessment is becoming increasingly popular and old chart recorders and integrators are replaced by the advanced chromatographic data systems and computer software [Erickson, 1997].

The aim of our work was to apply Chroma 2001 system including integrator and computer software Chromax to identify and quantify selected OCPs and PCB congeners in some multicomponent food extracts.

MATERIALS AND METHODS

Standard solutions. Two kinds of standard solutions were used. First, there is a mixture of specific PCB congeners (IUPAC Nos: 28, 52, 101, 105, 114, 118, 128, 149, 138, 153, 156, 170, 180 and 187). Individual chlorobiphenyls mentioned, as well as PCB 30 and 209 (internal standards) were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). The different working standard solutions were prepared by adding the appropriate weight amounts of primary standards to isooctane (Baker ® ultra-resi analyzed quality). The second standard solution came from the Institute of Applied Environmental Research at the Stockholm University. It included selected OCPs (α-HCH, HCB, β-HCH, γ-HCH, p,p'-DDE, p,p'-DDD, p,p'-DDT), specific PCB congeners (IUPAC Nos: 29, 28, 52, 101, 118, 153, 105, 138, 156, 180, 189, 198), and PCB 53 as an internal standard.

Cleaned-up extracts. The cleaned-up extracts of human milk [Lulek *et al.,* 2002] and herring tissue [Eriksson *et al.,* 1997] were used in our study.

GC-ECD analysis. The identification and quantification of OCPs and PCBs were performed by GC/ECD (gas chromatography with electron capture detector). A Shimadzu GC-14 gas chromatograph equipped with a ^{63}Ni electron capture detector and split/splitless injector was used. Chromatographic separation of the examined compounds was carried out on a 60 m ZB-5, Phenomenex® (0.25 mm i.d.; film thickness 0.25 μm) fused silica capillary column (5% diphenyl polysiloxane, 95% dimethyl polysiloxane). The detector temperature was 320°C with nitrogen as make-up gas at a flow rate of 48.0 mL/min.

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The temperature program of the column was 0.5 min at 85°C; 25°C/min until 140°C and 3°C/min until 300°C, holding for 10 min.

Chromatographic data acquisition, integration and quantification were performed on a personnal computer Authentic MD using commercial Chroma 2001 system (Pol-Lab®).

RESULTS AND DISCUSSION

The application of Chroma 2001 in the analysis of human milk and fish extracts was preceded by creation of two methods for automatic data processing. Method I (milk) was designed for determination of specific PCB congeners (IUPAC Nos: 28, 52, 101, 105, 114, 118, 128, 149, 138, 153, 156, 170, 180, and 187), while method II (herring) enabled simultaneous analysis of selected OCPs (α-HCH, HCB, β-HCH, γ-HCH, p,p'-DDE, p,p'-DDD, p,p'-DDT) and PCBs (29, 28, 52, 101, 118, 153, 105, 138, 156, 180, 189, 198).

Prior to sample analysis, the elution order of analytes of interest was determined from data provided by Chroma 2001 system and by other laboratories using similar GC conditions [Frame, 1997; Szyrwińska, 2003].

Analytes were identified by the data system by setting allowable retention time windows for reference peak and analyte peak retention time. Two (method I - PCB 30 and PCB 209) or one (method II - PCB 53) internal standard reference peaks were designated an RRT (relative retention time) reference peaks/peak to further assist in proper peak identification through the use of relative retention times. With regard to the possibility of Chroma 2001 system, only 10 reference and analyte peak windows were set to automating data processing. The inter-day repeatability of the RTs and RRTs of all analytes recorded by Chroma 2001 system was calculated from fourteen replicate analyses of a standard mixture of the PCB congeners (method I) as well as OCPs and PCBs (method II). It was below 0.06% for RTs and below 0.01% for RRTs, except for PCB 180 and PCB 187. Peaks with a signal-to-noise ratio of three or less were regarded as not detected.

As it results from data presented in Figure 1, twelve PCB congeners were identified in human milk extract by Chroma 2001 using method I. In turn, in extract of herring tissue, the presence of all examined OCPs (7 compounds) and PCBs (12 compounds) was established by method II (Figure 2).

Quantitative measurements of PCBs and OCPs in the analyzed extracts of human milk and herring tissue were carried out on the basis of peak heights. The calibrations were performed with bracketing standards calculated to be ±10% of the concentration of each analytes as determined by a preliminary analysis. The linearity of the ECD response for each compound was determined by plotting calibration graphs of peak height/mass injected *ver.* mass injected [Wells *et al.,* 1992].

Between various options offered by Chroma 2001, the one-point calibration curve method was used for quantification in our study. The concentration of a specific analyte in a sample was calculated by Chromax. All transfers of data to forms and data reductions (*e.g.* concentration calculations, weight of milk and herring extracts and samples *etc.*) were checked by the analyst and introduced to calibration table before GC analysis. The software used the concentration amounts for the individual analytes entered into the peak table and the results from the calibration standard to generate the coefficients of a polynomial curve fit that was, in turn, used to calculate the analytical concentrations. The results of qualitative and quantitative analysis of PCBs and OCPs in the analyzed multicomponent food samples, obtained using method I and II, are presented in Table 1 and Table 2, respectively.

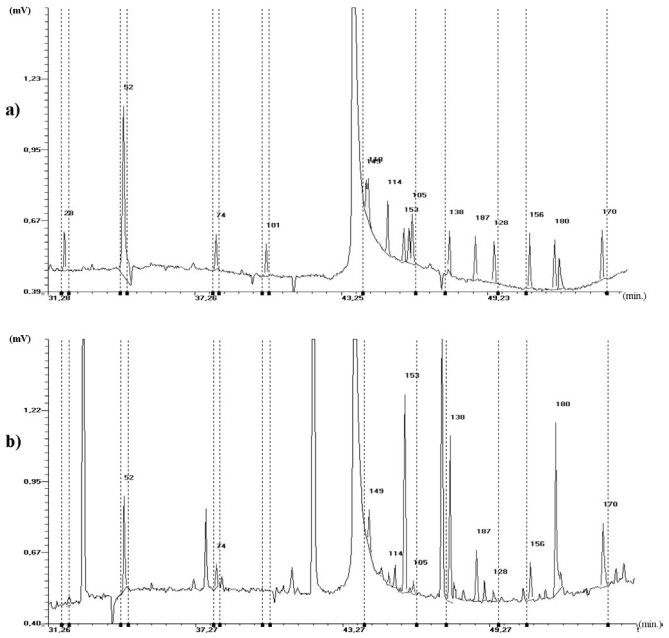

FIGURE 1. GC-ECD chromatograms recorded by Chroma 2001system (method I): a) standard solution of specified PCB congeners; b) cleaned-up extract of human milk.

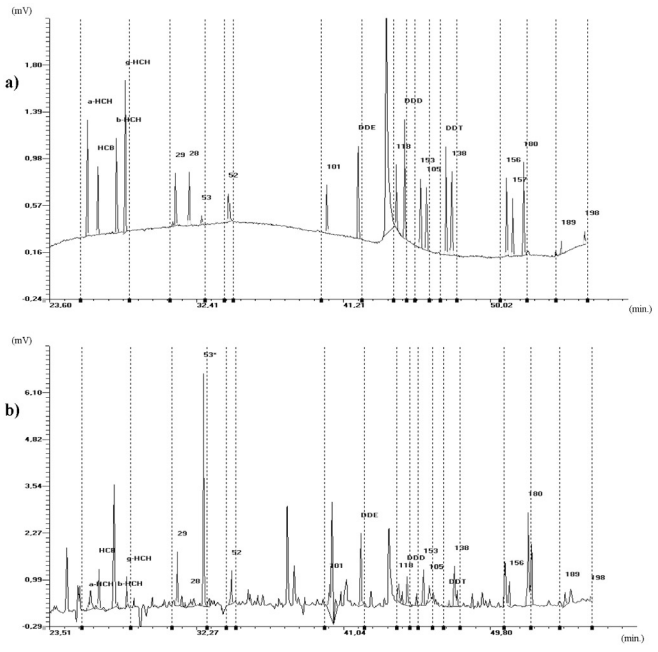

FIGURE 2. GC-ECD chromatograms recorded by Chroma 2001system (method II): a) standard solution of specified OCPs and PCB congeners; b) cleaned-up extract of herring tissue.

TABLE 1. Results of the determination of selected PCBs in human milk using Chroma 2001 system (method I).

Peak number	RT (min)	$RRT_{PCB\ 30+209}$	Analyte identified	Height (μV)	Determined content	
					(ng/g milk)	(ng/g fat)
1	34.342	0.379	PCB 52	358	0.2158	3.8877
2	38.107	0.420	PCB 74	81	0.2420	4.3598
3	44.358	0.489	PCB 149	151	0.3709	6.6823
4	45.147	0.498	PCB 114	42	0.0811	1.4614
5	45.797	0.505	PCB 153	763	1.5656	28.2084
6	46.150	0.509	PCB 105	43	0.0885	1.5951
7	47.652	0.526	PCB 138	637	1.6054	28.9251
8	48.728	0.538	PCB 187	192	0.4564	8.2240
9	49.412	0.545	PCB 128	37	0.0910	1.6390
10	50.932	0.562	PCB 156	139	0.2608	4.6987
11	51.967	0.573	PCB 180	660	1.3664	24.6193
12	53.897	0.595	PCB 170	243	0.5043	9.0871

TABLE 2. Results of the determination of selected OCPs and PCBs in herring tissue using Chroma 2001 system (method II).

Peak number	RT (min)	$RRT_{PCB\ 53}$	Analyte identified	Height (μV)	Determined content (ng/g fresh weight)
1	25.828	0.79	α-HCH	152	0.60
2	26.455	0.809	HCB	1040	3.34
3	27.542	0.843	β-HCH	86	0.64
4	28.093	0.859	γ-HCH	750	3.06
5	31.117	0.952	PCB 29	1427	2.89
6	31.922	0.977	PCB 28	151	1.47
7	34.348	1.051	PCB 52	852	12.24
8	40.188	1.229	PCB 101	721	10.13
9	42.065	1.287	DDE p,p’	1913	8.46
10	44.353	1.357	PCB 118	445	4.75
11	44.84	1.372	DDD p,p’	742	6.54
12	45.798	1.401	PCB 153	928	9.62
13	46.16	1.412	PCB 105	394	3.22
14	47.333	1.448	DDT p,p’	133	1.46
15	47.657	1.458	PCB 138	1055	11.00
16	50.932	1.558	PCB 156	627	4.14
17	52.067	1.593	PCB 180	2496	18.99
18	54.252	1.66	PCB 189	307	0.21
19	55.782	1.706	PCB 198	77	0.49

The quality of the results obtained using Chroma 2001 system was validated by its comparison with the results of quantitative analysis of selected PCB congeners (74, 105, 114, 128, 138, 153, 149, 156, 170, 180, and 187) in the same human milk sample, calculated on the basis of data from Chrompack integrator [Szyrwińska, 2003].

Figure 3 presents a comparison of mean concentrations of selected PCB congeners determined using both integrators. It is worth noting that the relative standard deviations (RSDs) of the means varied between 10.26% for PCB 138 to 16.85% for PCB 156, when Chrompack integrator was employed. In that case a better repeatability of the results of Chroma 2001 was obtained. For all the examined congeners, the RSD values were close to or less than 7.08% (range - 4.39% PCB 153 - 7.08% PCB 180).

Additionally the results presented in Figure 3 were compared on the basis of statistical criteria. Student’s paired t-test was employed to check whether the concentrations of the congeners examined determined at both acquisition and integration systems are significantly different. The calculated t values were in each case smaller than the critical value t_{crit} read from tables for a specified degree of freedom (f=n-1) and 95% confidence level.

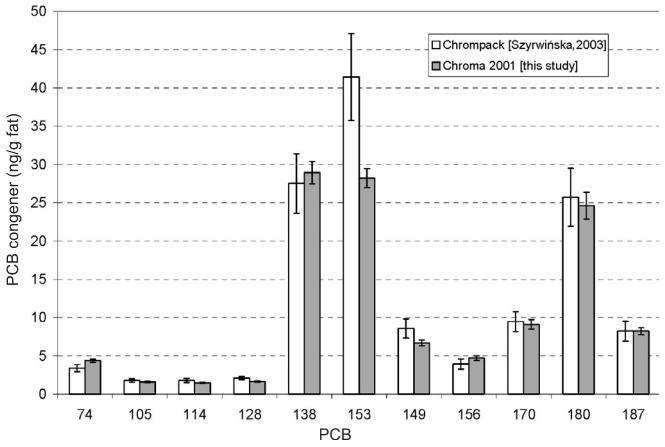

FIGURE 3. Comparison of the results of specified PCBs quantification obtained using two integrators of chromatographic data (n=3, mean ± SD).

CONCLUSIONS

The application of CHROMA 2001 reduces considerably the time necessary for qualitative and quantitative GC analysis of multicomponent food extracts. Nevertheless, the use of automatic data processing by Chroma 2001 and Chromax software is limited because during identification of more than 14 analytes manual data processing is required.

However, taking into consideration the low cost of Chroma 2001 system, it should be useful for the automating data processing in routine determination of indicator PCB congeners (PCB 28, 52, 101, 118, 138, 153 and 180) in food products.

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