ATMOSPHERIC PRESSURE GAS CHROMATOGRAPHY (APGC) COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY (MS/MS) FOR THE QUANTITATIVE ANALYSIS OF PESTICIDES AND PCBs REGULATED BY THE STOCKHOLM CONVENTION

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Introduction

The Stockholm Convention on persistent organic pollutants (POPs) entered into force to protect human health and the environment in 2004 [1]. For most organic compounds the most sensitive and selective analytical method has been gas chromatography coupled to mass spectrometry (GC-MS). High resolution GC/high resolution MS (HRGC/HRMS) has been used for the analysis of POPs and samples with low levels or limited material available for analysis [2]. Ionization of most POPs has been commonly undertaken by electron ionization (EI), and to lower extent by negative electron capture chemical ionization (CI) [3]. Several pesticides show extensive fragmentation, which complicates the analysis, often resulting in relatively high detection limits.

Atmospheric pressure chemical ionization (APCI) was initially developed in the seventies [4], and may be an alternative to both EI and CI ionization. Atmospheric pressure gas chromatography (APGC) is a soft (low-energy) ionization technique in the gas phase using a corona needle for ionization. APGC often generates only molecular or quasi-molecular ions. Recently, development in atmospheric pressure gas chromatography (APGC) has resulted in a very sensitive technique not only for the analysis of polycyclic aromatic hydrocarbon (PAHs), nitrogen-heterocyclic polyaromatic hydrocarbons (NPAHs) [5] and petroleum biomarkers [6], but also for brominated compounds [7] and pesticides [8].

The aim of this study was to develop a sensitive and accurate method for instrumental analysis of several POPs on the Stockholm Convention using APGC-MS/MS in small amounts (< 0.5 ml) of human plasma and to validate the developed APGC method. For comparison, HRGC/HRMS analysis of the same POPs was carried out on the same set of 89 samples from a human monitoring study and 8 quality assurance/quality control(QA/QC) samples.

Materials and methods

Samples

The study population included in this analysis was pooled from two cross-sectional cohort studies of non-diabetic overweight and obese postmenopausal women living in the Montreal (Quebec,-Canada) metropolitan area who were examined from 2003 to 2007. Reference blood plasma used as quality control (QC) was acquired from the Örebro University Hospital, Sweden. The participants were sampled in the morning after an overnight fast. The samples were stored in amber glass bottles at - 20°C before extraction and analysis. All details on the sample preparation are described by Salihovic et al [2].

APGC-MS/MS

An Agilent 7890A GC system (Palo Alto, USA) was coupled to a triple quadrupole MS, Xevo TQ-S (Waters Corporation, UK), equipped with an APGC source. GC separation was achieved using a fused silica DB-5MS capillary column, 30 m×0.25 mm i.d., film thickness 0.25 m (SGE Analytical Science, Victoria, AUS). The oven temperature was as follows: 180 °C (2 min); 3.5 °C/ min to 260 °C; 6.5 °C/min to 300 °C (4 min). Splitless injections of 1 µL using a single gooseneck deactivated liner from Restek, were carried out at 280 °C. Helium was used as carrier gas at a constant flow rate of 2.0 mL/min. The GC interface temperature was set to 310 °C

using N_2 as the make-up gas at 370 mL/min. The cone gas (N_2) was set at 170 L/hr, and the auxiliary gas (N_2) at 300 L/hr. The APCI corona needle was operated in current mode at 1.5 µA. To reduce protonation which competes with charge transfer ionization, the source was kept dry at 150 ℃. Two MRM transitions were measured for all compounds using argon as the reactant gas with collision energy of 20 to 40 eV depending on the compound.

HRGC/HRMS

HRGC/HRMS analyses were performed on a Micromass Autospec Ultima (Waters, Milford, MA, USA) operating at >10000 resolving power using EI ionization at 35 eV. Measurements were performed in the selective ion recording (SIR) mode, monitoring the two most abundant ions of the molecular bromine or chlorine cluster. The MS was coupled to a 6890N GC (Agilent Technologies, Atlanta, GA, USA). The GC separation was performed using the same column and oven program as for the APGC system. Splitless injections of 2 µL were carried out at 275 ℃.

Results and discussion

Linearity

Quantification of the native PCBs and pesticides was obtained by means of a 6 point calibration curve in the range from 0.04 to 300 pg/µL. The average relative response factor (RRF) was good and coefficients of determination (r^2) for all compounds over the calibration range are >0.995, indicating good linearity.

Table 1 RRF and r^2 of the calibration curve

	CS L 0.04 pg/uL	CS 0.1 0.4 pg/uL	CS 1 4 pg/uL	CS 2 40 pg/uL	CS 3 100 pg/uL	CS 4 300 pg/uL	average	STD	RSD(%)	r	r^2
PCB#52	1.25	1.39	1.33	1.40	1.49	1.44	1.38	0.08	6.1	0.9998	0.9996
PCB#101	0.96	1.12	0.99	1.04	1.04	1.07	1.04	0.06	5.3	0.9999	0.9998
PCB#138	1.05	1.02	1.03	0.93	0.94	1.05	1.00	0.05	5.3	0.9988	0.9975
PCB#153	1.24	0.98	0.99	1.02	0.95	1.01	1.03	0.11	10	0.9996	0.9992
PCB#180	1.84	1.63	1.56	1.48	1.69	1.69	1.65	0.12	7.6	0.9993	0.9987
HCB	*	0.65	0.73	0.76	0.85	0.72	0.81	0.17	9.7	0.9977	0.9948
o,p DDE	0.91	0.90	0.93	0.96	0.97	0.92	0.93	0.03	2.9	0.9998	0.9996
p,p DDE	1.64	1.31	1.12	1.42	1.34	1.28	1.35	0.17	8.4	0.9994	0.9988
Cischlordane	0.12	0.15	0.16	0.15	0.15	0.17	0.15	0.02	11	0.9985	0.9970

*CSL not included in average RRF calculation due to HCB contamination of solvents or below LOD.

Repeatability

To validate the performance of the APGC system, repeatability (area and RRF) was studied by 10 consecutive injections of two low level calibration standards, CS 0.1 (0.4 pg/µL) and CS 1 (4 pg/µL) (Table 2). For the area repeatability of all compounds, the 0.4 pg/uL standard showed better relative standard deviation (RSD) ranging from 8.0 to 21%. For the repeatability calculated on RRFs (relative to the ^{13}C internal standard), APGC showed good RSDs for injections of both the 4 pg/µL (3.6-5.5%) and the 0.4 pg/µL standard (3.1-16 %).

QA/QC

QA/QC samples were analysed following the same clean-up and extraction procedure as for the real samples. The limits of detection (LOD) of the analytical method were defined at signal-to-noise ratio (S/N) of 3:1. Comparison of the data from the two instruments was good. In Figure 1 the average levels accessed by high resolution MS and APGC are given for analysis of the QA/QC samples using a 95% confidence interval. As can be seen from the figure the results are in very good comparison.

Application to real samples

To demonstrate the applicability of APGC, more than 80 blood plasma samples were analyzed by APGC for the target compounds. A combined chromatogram of a representative human plasma sample (0.5 mL) is given in Figure 2. The MRM channel for PCB#118, 153, 105, 138, 180, 170 was magnified 4 times, while trans-nonachlor was magnified 50 times.

Table 2 Area repeatability and RRF repeatability for two low level calibration standards

	CS 0.1 (0.4 pg/µL)				CS 1 (4 pg/µL)			
	Area Repeatability		RRF repeatability		Area Repeatability		RRF repeatability	
	STD	RSD (%)	STD	RSD (%)	STD	RSD (%)	STD	RSD (%)
PCB#52	290	8.0	0.076	5.2	10114	28	0.053	3.8
PCB#101	481	11	0.049	4.4	12318	29	0.053	5.1
PCB#138	420	11	0.033	3.1	11083	28	0.052	3.8
PCB#153	392	11	0.057	5.7	10994	30	0.040	4.9
PCB#180	346	11	0.100	6.4	8682	29	0.050	3.6
HCB	276	15	0.068	9.1	5138	28	0.054	5.0
o,p DDE	348	9.7	0.067	7.3	10521	29	0.035	5.1
p,p DDE	574	11	0.099	7.3	14274	28	0.045	5.0
Cischlordane	78	12	0.012	7.6	1966	30	0.063	5.2
Transchlordane	57	15	0.011	11	1261	31	0.008	5.5
Trans-nonachlor	26	21	0.008	16	1261	31	0.005	3.8

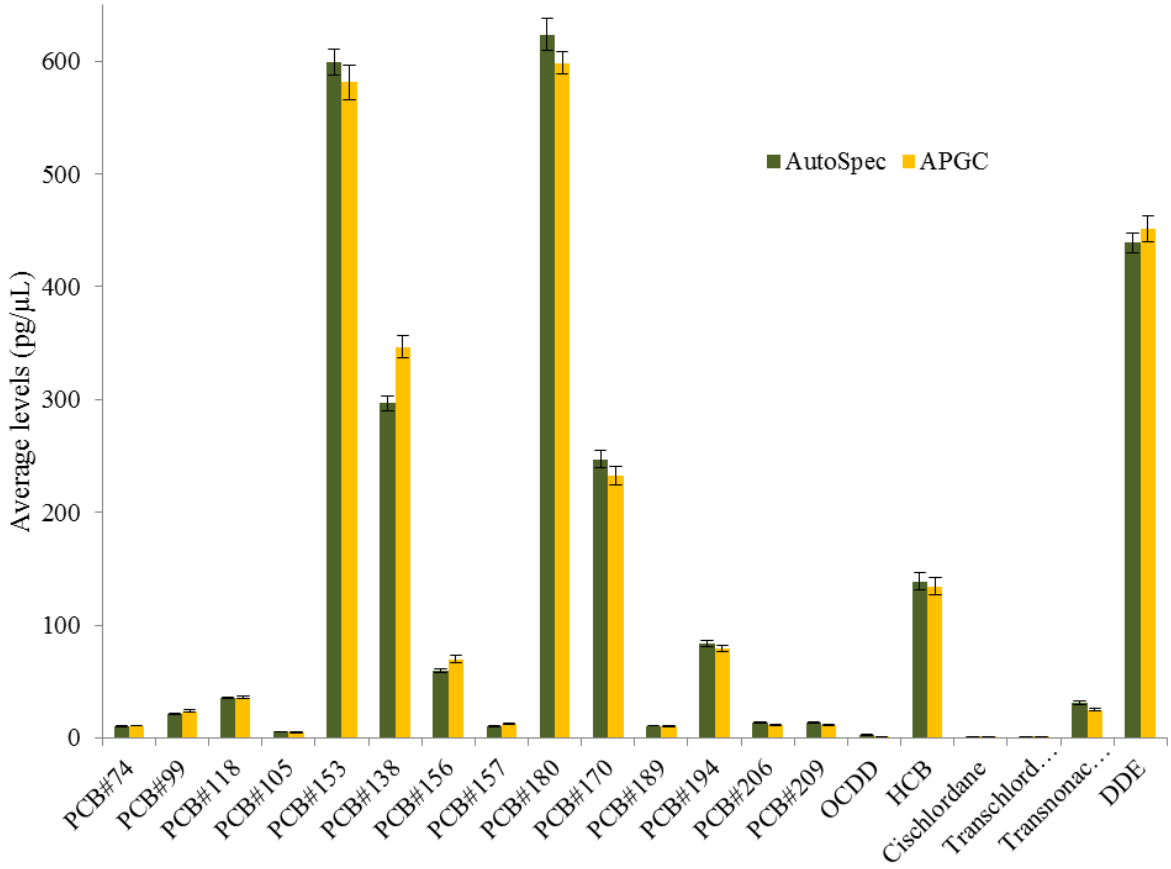

Figure 1 Comparison on POPs concentrations analysed by APGC and AutoSpec

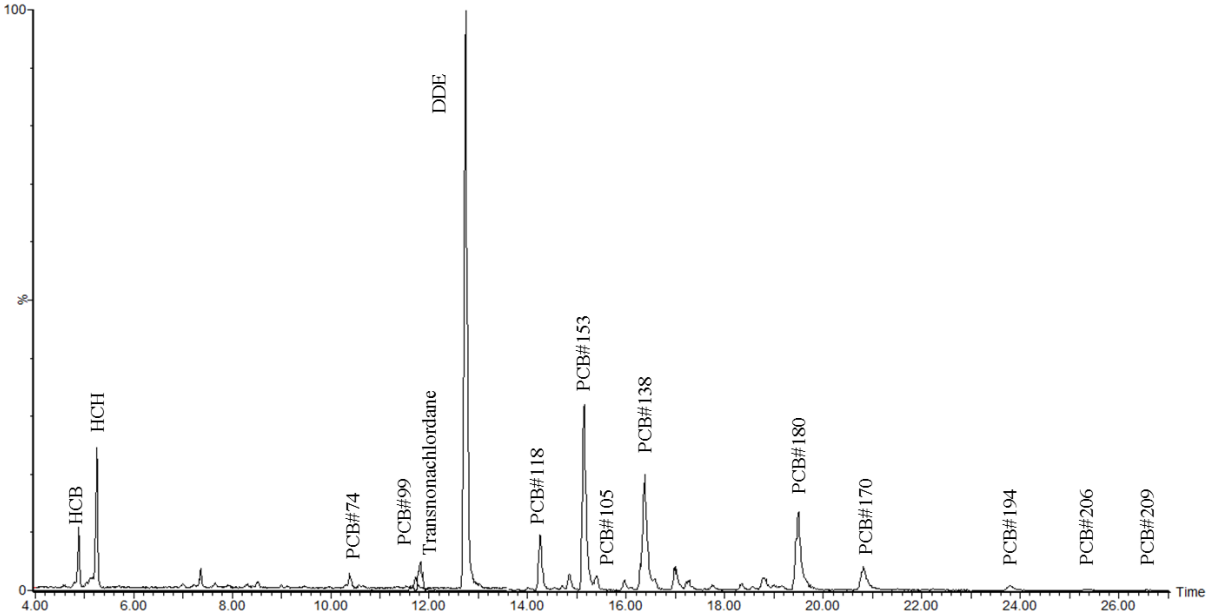

Figure 2 Combined chromatograms of the native compounds in real sample study on APGC

Conclusion

All POPs showed very good linearity over the range from 0.04 to 300 pg/µL with good r^2 values (>0.995). Area repeatability for the 0.4 pg/µL standard was good (8.0-21%) For the RRF repeatability, which is more relevant when using isotope dilution quantification, very good RSDs were seen for all compounds, 4 pg/µL (3.6-5.5%) and 0.4 pg/µL (3.1-16 %). The excellent sensitivity obtained using the APGC indicates that APGC is a powerful alternative that can easily meet the specification of high resolution GC/MS systems

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