MEASUREMENT OF TRACE LEVEL DECHLORANE FLAME RETARDANTS IN FOOD AND FEED BY GC-MS/MS

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Introduction

Dechloranes are a class of chlorinated compounds that share bicyclo [2,2,1] heptene structure, resulting from a Diels-Alder condensation between one or two hexachlorocyclopentadiene molecules and various cyclic dienophiles. As a result of their structure and composition, dechloranes have both flame retardant and pesticide properties[1]. Dechlorane 602 (Dec 602), Dechlorane 603 (Dec 603), Chlordene Plus (CP) and Dechlorane Plus (syn and anti isomers, DP syn and DP anti) have been replacing Mirex after its ban in the 70's following its toxicity, persistence and high potential for bioaccumulation. At present, there are no restrictions and regulations for these compounds although they have been detected in environment[2], in biota[3] and in human serum samples[4].

With the aim of investigating possible routes of exposure, a GC-MS/MS method has been developed to measure dechlorane levels in food and feed. To the best of our knowledge this is the first time that GC-LRMS (GC-MS/MS) is used for dechlorane detection that is usually performed using GC-HRMS sector instruments. The introduction of such a technique is favourable as the triple quadrupole GC-MS/MS instrumentation is more widespread in routine analysis laboratories, requires lower starting investment, and is easier to operate than HRMS, although its performances are comparable to HRMS.
To ensure method sensitivity, PTV inlet injection parameters, that have been estimated as crucial, have been settled by means of Experimental Design (DoE), which allowed to reach high peak area with a suitable peak symmetry (no fronting or tailing). MRM transitions have been optimized for all the dechloranes, RRF calibration curve and LOQs have been calculated before the method was applied on real food matrices to have a preliminary idea of dechlorane levels in foodstuffs. When compared to food consumption habits, these first data on levels of selected dechloranes in food will allow preliminary estimation of dechlorane daily intake by food consumption.

Materials and methods

- Chemicals

DP syn, DP anti, DP syn $^{13}C_{10}$ labeled (99%), Dec 602 $^{13}C_{10}$ labeled (99%) standards were supplied by Cambridge Isotope Laboratories (CIL, Andover, MS, USA). Dec 602 (95%), Dec 603 (98%) were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada) and CP was from Wellington Laboratories (Guelph, ON, Canada). Mirex was purchased from Cluzeau Info Labo (France). The EC-1414solution of PCB-80 $^{13}C_{12}$ (99%), from CIL, was used as recovery labeled standards. Nonane (analytical standard grade, Fluka) was purchased from Sigma Aldrich (St. Louis, MO, USA).

- Samples preparation

Different food and feed sample matrices such as pork fat, beef fat, pork, milk, egg, fish, vegetable oils, corn and aluminum oxide additives for feed were analyzed. Sample preparation followed an ISO 17025 procedure, validated and currently used for PCDDs, PCDFs and cPBDs routine detection[5]. The main steps were: accelerated solvent extraction with ASE™ Dionex 350 and PowerPrep™ clean-up with mixed bed silica/alumina/carbon columns. Final extracts were stabilized in ultrapure nonane. Procedural blank samples consisting of 10 mL of

Milli-Q® water (Millipore, Brussels, Belgium) followed the same procedure and were analyzed as the other samples to assess method LOQs.

- GC-MS/MS system

All analysis were performed using an Agilent (Palo Alto, CA, USA) GC-MS/MS Triple Quad 7000C, connected to a GC 7890B. Analyses were carried out using He pure GC grade 99.9999% (Air Liquid) as the carrier gas at constant flow rate of 1.1 mL/min. The capillary column, an Agilent DB-5ms ultra inert (60m x 0.25mm x 0.25µm film thickness) typical for dioxin trace analysis, was directly connected to a PTV inlet, where 5µL of the final sample extract in nonane were injected by means of an autosampler 7693 from Agilent. Inlet initial temperature was 45°C for 1.3 min, then ramped up at 720°C/min to 320°C until the end of run. During inlet vent, vent flow was 120 mL/min and vent pressure was 10.5 psi. These parameters have been settled by means of Experimental Design (Face Centered Design). GC oven program temperature began at 140°C for 2.6 min, ramped at 100°C/min to 320°C and hold for 21.1 min, with a total run time of 25.5 min. The mass spectrometer GC-QQQ was connected to the GC system by means of a transfer line heated at 320°C. The ion source, hold at 280°C, was operating in Electron Ionization (EI) at 70eV; MS1 and MS2 quadrupoles where at 150°C and operated in Multiple Reaction Monitoring (MRM) mode, in order to increase method sensitivity and selectivity. Ultrapure Nitrogen and Helium were respectively the collision and the quench gas.

- Experimental Design for PTV inlet parameters

To ensure method sensitivity, a Face Centered Design (postulating a second order model) was performed to assess the best values for Initial Injector Temperature (T), Vent Flow (VF) and Vent Pressure (VP), since they affect sample transfer into the column. Optimum values, in fact, allow efficient solvent removal (nonane in this case) without removing analytes and thus enhancing signals, that is peak area. In this work also peak symmetry was taken into account and its acceptability interval was close to 1 (very symmetric peak with no fronting and no tailing), that is 0.9 – 1.1, since no peak smoothing was carried out. Table 1 outlines the boundaries of the experimental domain and the three levels chosen for each variable. The central levels were selected by means of a Full Factorial Design performed on a larger experimental domain (data not reported).

- **Table 1**: Experimental domain and factor levels.

Levels of the variables in the Experimental Design		-1	0	+1
initial injector temperature (°C)	T	30	45	60
vent flow (mL/min)	VF	20	60	100
vent pressure (psi)	P	1	10.5	20

A total of 17 experiments (three replicates of the center point) were performed. All experiments were performed in random order using a 10 pg/µL mixture of all the dechlorane standards in nonane. Peak area and peak symmetry were calculated by means of MassHunter Workstation software (Agilent); for all the computations and plots relative to Experimental Design, routines written in Matlab (Mathworks Inc., Natrick, USA) by the authors have been used.

- Identification and quantification by MS/MS Triple Quad

Quantification was by isotopic dilution technique applied to product ions. $^{13}C_{10}$ DP syn and $^{13}C_{10}$ Dec 602 were used as labeled internal standard since they are the only commercially available (in addition to Mirex). $^{13}C_{10}$ DP syn was used to quantitate DP syn and DP anti, while $^{13}C_{10}$ Dec 602 was used for all the others, since they have similar chemical structure. Recoveries were calculated using PCB-80 $^{13}C_{12}$ (99%), as already described elsewhere for dioxin and dioxin like compounds[6].
Calibration was carried out by injecting five points calibration solutions (mix of all the unlabeled standards, the two labeled standard and the recovery standard) from 0.5 to 100 pg/µL; the relative response factor (RRF) associated to each compound was established.

Results and discussion

A GC-QQQ MS/MS method was developed for Dechloranes trace level analysis in food and feed. The sample preparation, the chromatographic column were the same as a routine dioxin ISO 17025 procedure and, even if this layout could be improved and optimized for specific dechloranes analysis, it allows easy switch between dioxin and flame retardant analysis in routine laboratories. Dechloranes, in fact, are eluted out of the carbon column with the "mono-ortho PCB" fraction and recoveries are in the range 50-80% depending on the compound, which allows proper use of isotope dilution for quantification.

- Experimental Design and best PTV inlet parameters

A Face Centered Design with 17 experiments was performed, studying peak area and peak symmetry for each compound. Figure 1 shows the bar plot of the coefficients for one of the compounds (Mirex) and summarizes the results obtained. The trend is the same for all the other compounds (data not shown).

Factor	Effect on peak area	Effect on peak symmetry
T (x_1)	Negative quadratic effect → T at intermediate level = 45°C	Linear positive effect →T at high level = 60°C
VF (x_2)	Positive linear effect →VF at high level = 100 mL/min	Not significant
P (x_3)	(Almost significant) negative linear effect →P at low level = 1 psi	(Almost significant) positive linear effect →P at high level = 20 psi

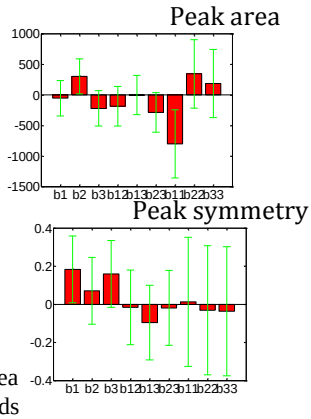

Figure 1: Bar plots (right) of the coefficients of the models for the two responses, peak area and peak symmetry, relative to Mirex. The coefficients estimated for all the other compounds follow the same trend. The brackets correspond to the confidence intervals at p=0.05. Summary (left) of the effects of the three factors on responses for all the compounds.

Final operative conditions represent the best compromise between increasing peak area and maintaining a suitable peak symmetry. Based on its strong negative quadratic effect on peak area, temperature was maintained at 45°C; vent pressure was fixed at intermediate level (e.g. 10.5 psi), because it had a positive effect on peak area, but a negative effect on peak shape; vent flow was set at high level (100mL/min) as it had a positive linear effect on peak area. When looking at the response surface relative to peak area (Figure 2) it is clear that the response can increase with vent flow in the outlined direction, outwards the experimental domain, still giving symmetric peaks.

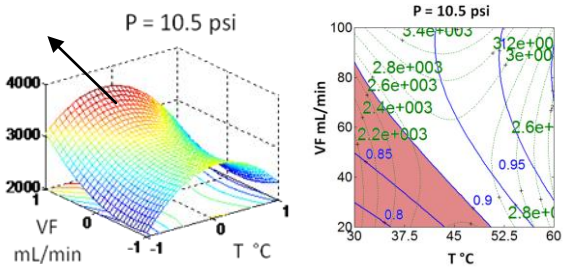

Figure 2: Response surface (left) for peak area of Mirex, as an example, in the plane temperature-vent flow at vent pressure 10.5 psi. Overlapped isoresponse plot (right) of peak area (green dotted lines) and peak symmetry (blu lines) at vent pressure 10.5 psi. The area in red highlights the experimental conditions that lead to non-acceptable values for peak symmetry

To assess if peak area increased significantly outside the experimental domain, in the direction suggested by the model, three replicates at vent flow 120 mL/min (VF = 1.2), temperature at 45°C (T = 0) and pressure at 10.5 psi (P = 0), were performed. By means of a t test it was assessed that actually peak area in this point was statistically higher than peak area with vent flow at 100 mL/min. In conclusion, the injection conditions that fulfilled the

aims of this work, were set as follows: initial inlet temperature = 45°C; vent flow = 120 mL/min, vent pressure = 10.5 psi.

- Method performances and results on real samples

Optimization of the MRM transitions was fulfilled and Table 2 shows the quantitative transitions for each compound. The major precursor ion for almost all the Dechloranes was the ion 272 m/z, the hexachlorocyclopenta-1,3-diene cation. It is the retro Diels-Alder product, whose formation is promoted from the inductive effects of six chlorine substituents, and because in some cases its development is combined with an aromatic compound formation (for example, furane from Dec 602). This is not the case of Dec 603, whose main fragment is not a retro Diels-Alder product.

Table 2: Quantitative MRM transition for Dechloranes.

t_R (min)	Compound	MRM transition	t_R (min)	Compound	MRM transition
8.03	$^{13}C_{12}$ PCB80	303.9 > 233.9	15.93	Dec 603	262.8 > 227.9
10.39	Mirex	272 > 237	22.17	$^{13}C_{10}$ DP syn	277 > 242
11.23	$^{13}C_{10}$ Dec 602	277 > 242	22.19	DP syn	272 > 237
11.25	Dc 602	272 > 237	24.16	DP anti	272 > 237
12.36	CP	272 > 237			

Method performances and levels in selected food are summarized in Table 3.

Table 3: Method validation and levels on real samples.

Compound	LOQ (pg/g fat)	Present in blank (n=4)	Pork fat (n=5)	Beef fat (n=1)	Fish (n=1)	Milk (n=6)	Pork meat (n=3)
Mirex	0.46	¼	1-2	60	40	2-6*	<LOQ*
Dec 602	0.41	2/4	1-2	70	10	1-2	1-2
CP	0.03†	No	<1	50	<LOQ	<1-3*	<LOQ*
Dec 603	0.07†	No	1-3	3	<LOQ*	<1*	<LOQ*
DP syn	0.74	¾	1-3	15	2	<1	5-6
DP anti	1.09	4/4	1-2	5	1	1-2	5-6

†iLOQ, since no detection in blanks; * interferences or matrix effect preventing good quantitation.

Calibration curve gaves good R^2 for each compound. Limits of quantitation (LOQ) were determined using blanks background levels or instrumental limits of quantitaton (iLOQ, determined statistically by replicate injections of the lowest calibration point) according to whether it was possible to measure a level in blanks or not. Real samples from the routine dioxin laboratory were injected to assess levels in different food matrices. Low levels, typically around 1-2 pg/g fat and below 10 pg/g fat were observed in all matrices except for beef fat. These levels must be further confirmed and cross compared to food habits to assess daily intake and predominance or not of this route of exposure for human.

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