# METHOD FOR THE MEASUREMENT OF DECHLORANE 602 IN HUMAN SERUM

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## Introduction

Quite recently, the presence of significant amounts of several Dechlorane flame retardants were reported in environmental samples from Canada[1]. Several molecules were identified, e.g. Dec 602, 603, 604, Dechlorane Plus (DP), and Chlordene Plus (CP)[2]. So far, to our best knowledge, no data are available on the possible presence of such analytes in human specimen, particularly in Europe where very few data on any matrices are available. Therefore, the aim of this preliminary project was to develop a GC-MS method for the measurement of 602, 603, 604 and CP in human serum. The particularity of the 600 family molecules is to exhibit a bicycle [2,2,1]-heptene halogenared structure. During mass spectrometric analysis, they can undergo retro Diels-Alder reactions and typically form hexchlorocyclopentadiene (HCCPD) fragments (m/z = 272). The quantification of those analytes is most of the time carried out on the most abundant ions of this fragment cluster (e.g m/z 271.8102/273.8072 for 602). We decided to investigate the use of comprehensive two-dimensional gas chromatography (GCxGC) coupled to negative chemical ionisation (NCI) high resolution mass spectrometry (HRMS) to ensure sensitive, specific, and accurate quantification.

## Materials and Methods

### *Chemicals*

All parameters concerning the quality and potential pre-treatment of the entire chemical used for those analyses are the ones used in routine. The test matrix consisted in a QC pool made of French serum that was not artificially fortified in any toxicants.

### *Analytical procedure*

All samples were processed in an ISO17025 BELAC accredited laboratory. Sample sizes were 5 mL. Samples were extracted using solid-phase extraction (SPE) on non-endcapped C18 cartridges (1g/6 mL). The C18 cartridges were eluted with 3 x 5 mL of hexane. The 15 mL were loaded on multi-layer column made of 1g sodium sulfate / 1g activated silica / 2g of 22% sulphuric acid silicagel. Further elution with 15 mL of hexane was the performed. The evaporation is carried out in in a PowerVap 6 system (Fluid Management Systems Inc., Waltham, MA, USA) to 500 µL using GC-vial connected evaporation tubes. The final volume after gentle room temperature evaporation was 5 µL. Measurements were carried out on a JEOL AccuTOF GC system (JEOL Ltd., Tokyo, Japan). The GC oven (Agilent 6890) was equipped with a ZX1 - $LN_2$ Cooled Loop Modulation GC x GC System (Zoex Corp., Houston, TX, USA). The $^1$D GC column was an HT-8 (60 m x 0.25 mm ID x 0.25 µm df) (SGE, Villebon, France). The 2D GC column was an Rxi-17 (1.5 m x 0.25 mm ID x 0.25 µm df) (Restek, , Belletonte, PA, USA). The $P_M$ was 4 s, 400 ms of hot pulse duration. The temperature program was 140°C for 2 min, 15°C/min to 220°C for 7,5 min, 6°C/min to 250°C, 2°C/min to 265°C, 30°C/min to 310°C for 20 min. 1 µL of the final extract in nonane (5 µL) were injected into a split/splitless injector held at 275°C in splitless mode. Helium was used at 0.8 mL/min. The major MS parameters were an ion source temperature of 140°C, an ionisation voltage of 200 V, methane at 1 mL/min as reagent gas, an acquisition range from 30.00 to 700.00 m/z, a recording interval of 0.04 s (25 Hz), an accumulation time of 0.037 s, a data sampling interval of 0.5 ns, and a detector voltage of 2300 V. The mass accuracy of the instrument was ensured by frequent single point calibration checks. The EI spectra were recorded on a Peg4D from LECO ( Monchengladbach, Germany).

## Results and Discussion

### *Method development*

Before optimization, we did quick testing of human serum we had and only identified Dec 602 to be present in the samples. This was quite consistent with some calculations of Biota Sediment Accumulation Factors (BSAF) performed by Eric Reiner's group and that reported higher BSAF for Dec 602, compared to other dechloranes. From that stage, we focused our efforts on Dec 602 only. GCxGC with cryogenic modulation was used because it reduces the risk of chromatographic co-elution and enhances the instrumental detection limits (iDLs) of the MS instrument. The classical shape of the modulated GC peak for Dec 602 is illustrated in Figure 1. We decided to use HR time-of-flight (TOF) MS because the instrument was equipped with a specific ion source filament that allowed performing in NCI mode at temperatures as low as 140°C with a low thermal emission filament expressing good stability. The use of such reduced temperature was ideal to minimize dissociative electron capture (DEC) and enhance resonance electron capture (REC) to favor high MS signal for the parent ion cluster. This is important for isotope dilution (ID) (once $^{13}C$ labels will be available) accurate quantification, but also for enhanced specificity in the identification of the target molecule. Also, the cluster of the $Cl_{12}$-containing molecule is very specific and ion ratios can be calculated (Figure 1). Additionally, the use of full-scan HRMS allows performing elemental composition calculations on the top of classical library searching. An example of formula calculation based on the M+6 (m/z 613.64) ion of the parent cluster from an NCI mass spectra obtained from the more intense slice of the GCxGC peak cluster in Figure 2. The good mass accuracy (typically 5 ppm) permits the univocal identification of the good formula (0.21 mmu difference). The good formula is the third in the list but the first hit is not possible as it is for a molecule containing 10 $^{37}Cl$ (although we work on an M+6 ion), and the second hit is for a molecule containing 10 $^{16}O$ atoms, a very unlikely situation. As illustrated in Figure 3, additional differences appear between NCI and EI mass spectra. Interestingly, the HCCPD m/z 271.8102/273.8072 cluster that is used for quantification in EI is barely present in NCI, although its dechlorinated product (pentachlorocyclopentadiene – PCCPD – $C_5Cl_5$) is present in high abundance. Additionally, the cluster around m/z 340 represents an hexachloro-norbornene species ($C_9H_4OCl_6$). Those additional clusters present in the NCI spectra are extra pieces of evidences that reinforce the identification process and the global specificity of the method. This is especially valuable in situations where several of the 600 family analytes are presents and might closely elute or co-elute in some GC approaches.

### *Calibration study*

As of today, no $^{13}C$-labelled Dec 602 is available. We tested IS calibration of Dec 602 versus PCB153, PCB-180, and PCB-209. We wanted to test which of those would be the best candidate in terms of relative response factor (RRF), retention time, and similarity in recovery rates. Figure 4 (left) illustrates the calibration curve using different PCBs for Dec 602 quantification. PCB-209 showed the better RRF and linearity. It also had the closest retention time. Figure 4 (right) shows that the preliminary testing of the procedure on a QC pool made of Central European human serum highlighted the presence of Dec 602 at the ng/g lipid level.

## Conclusions

Once a $^{13}C$ Dec standard will be available, or after optimization of the use of one PCB for quantification, the method will be used to measure Dec 602 at background level in European population. An extended human biomonitoring study will be considered in the near future to confirm the presence of Dec 602 at measurable levels in human.

## Acknowledgements

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**References**

1. Sverko et al., Environ. Sci. Technol. (2010) 44, 574.
2. Shen et al., Environ. Sci. Technol. (2011) 45, 693.

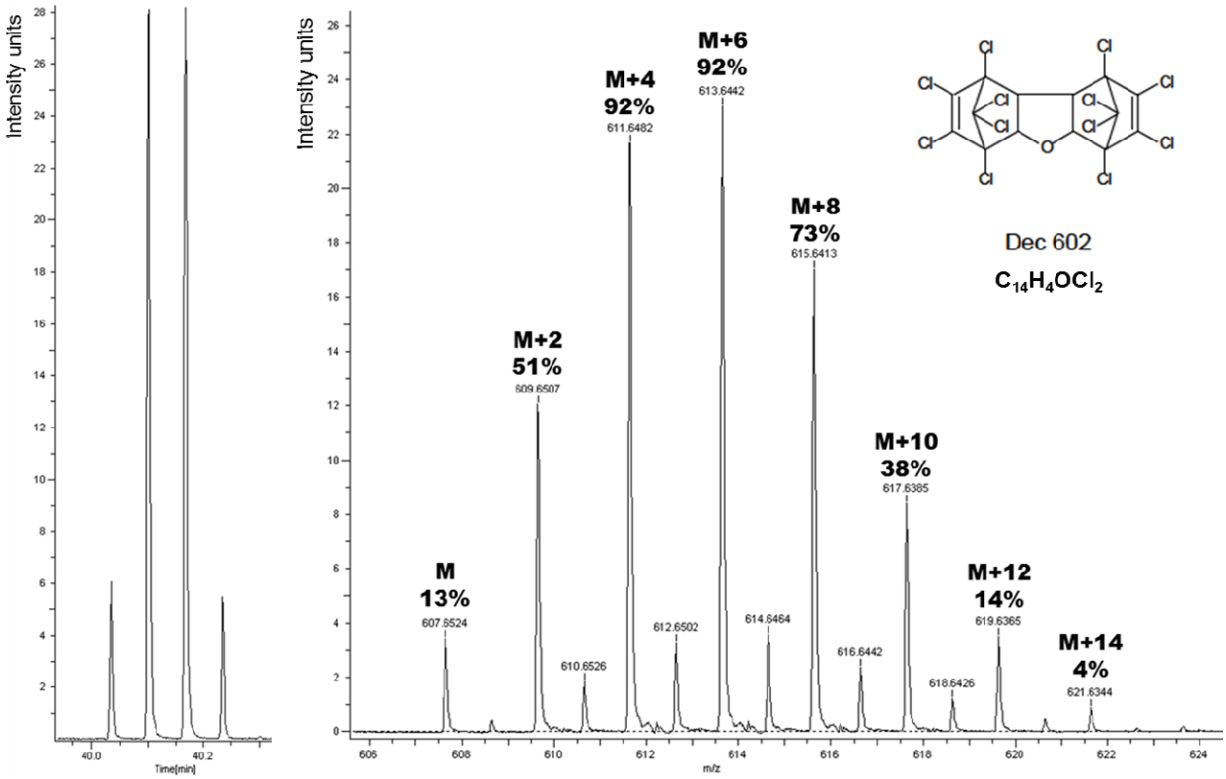
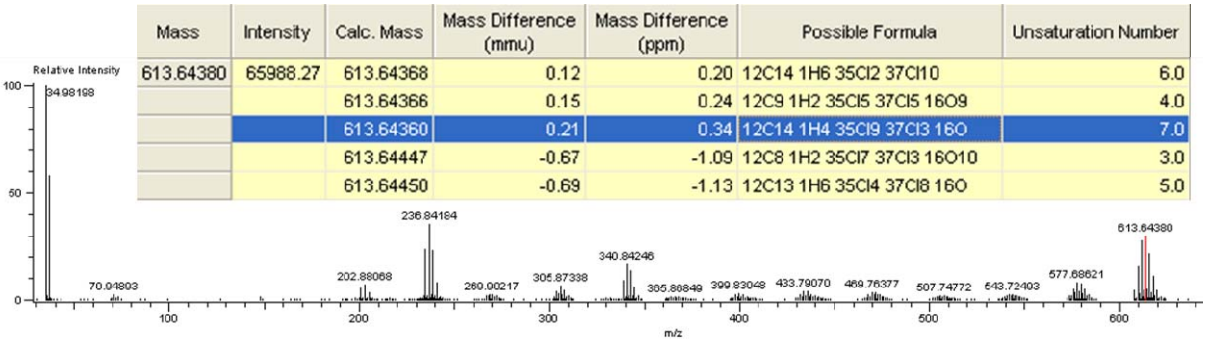



**Figure 1:** GCxGC modulated peaks for Dec 602 (400pg injected) (left) and MS parent ion cluster (right).




| Mass | Intensity | Calc. Mass | Mass Difference (mmu) | Mass Difference (ppm) | Possible Formula | Unsaturation Number |
|---|---|---|---|---|---|---|
| 613.64380 | 65988.27 | 613.64368 | 0.12 | 0.20 | 12C14 1H6 35Cl2 37Cl10 | 6.0 |
| | | 613.64366 | 0.15 | 0.24 | 12C9 1H2 35Cl5 37Cl5 16O9 | 4.0 |
| | | 613.64360 | 0.21 | 0.34 | 12C14 1H4 35Cl9 37Cl3 16O | 7.0 |
| | | 613.64447 | -0.67 | -1.09 | 12C8 1H2 35Cl7 37Cl3 16O10 | 3.0 |
| | | 613.64450 | -0.69 | -1.13 | 12C13 1H6 35Cl4 37Cl8 16O | 5.0 |

**Figure 2:** Elemental composition calculation for the M+6 ion of the Dec 602 MS parent cluster.

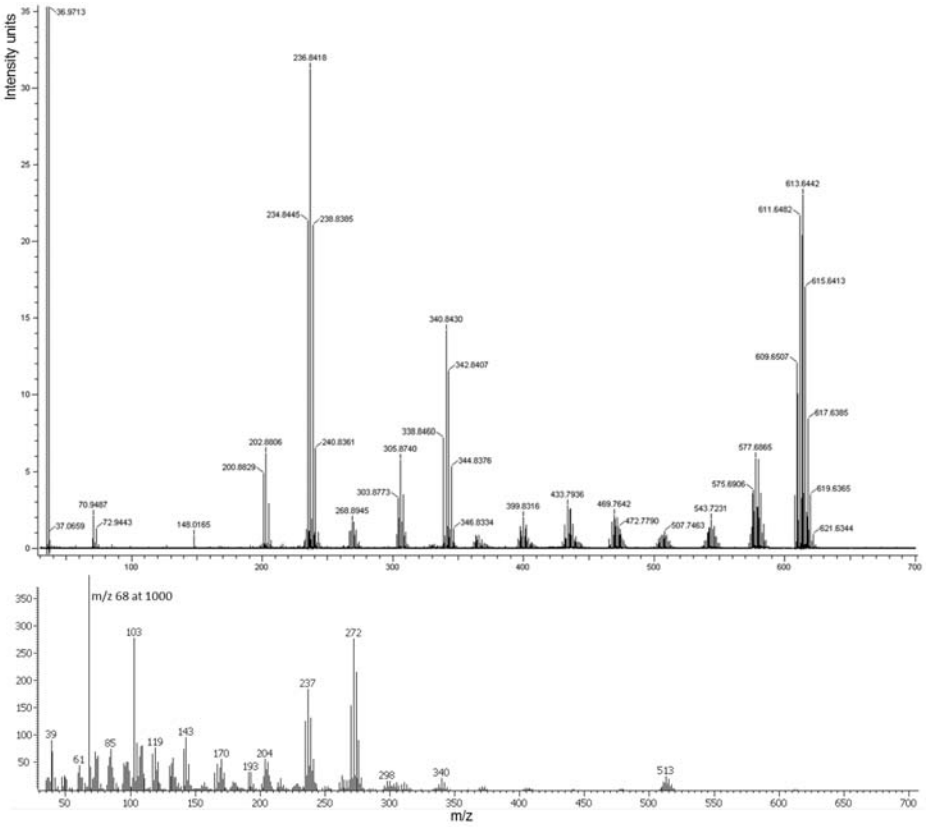



**Figure 3:** NCI (top) versus EI (bottom) MS signal for Dec 602 (400pg injected).

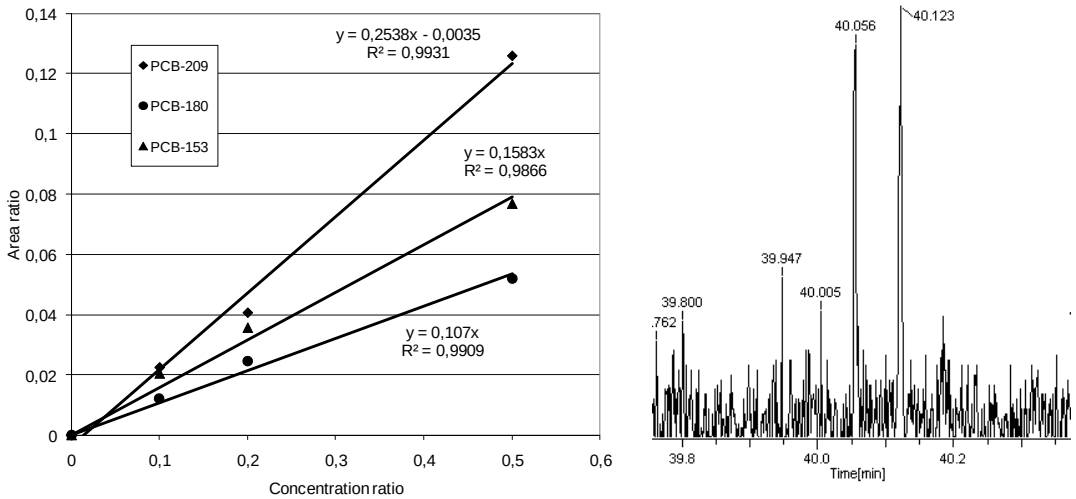



**Figure 4:** Calibration curve study using three different PCBs for quantification of Dec 602 (left) and Dec 602 signal in human serum (right).