

QUANTIFICATION OF DIOXIN-LIKE PCBs: PRACTICAL OPTIMALISATION OF THE EN 1948-4 PROTOCOL

Idczak FG, Duchateau PJ, Nadin CJ, Petitjean SM, Bergmans BL

Institut Scientifique de Service Public (ISSeP), rue du Chéra 200, 4000 Liège, Belgium – b.bergmans@issep.be

INTRODUCTION

Some polychlorinated biphenyls (PCBs) with similar structure and lipophilic properties as the dioxins have been identified as having similar toxic health effects and are often referred to as dioxin-like PCBs. The World Health Organisation (WHO) has published a list of 12 PCBs with their associate relative toxicity equivalencies¹ and recommends the quantification of these compounds.

A new European standard “EN 1948-4: Stationary source emissions - Determination of the mass concentration of PCDDs/PCDFs and dioxin-like PCBs - Part 4: Sampling and analysis of dioxin-like PCBs” will be published at the end of the year. This standard specifies a framework of quality control requirements which should be fulfilled by any PCB emission sampling, extraction, clean-up, identification and quantification methods to be applied. The protocol presented in the standard gives basic information, but requires practical implementation and several optimisations to measure PCB concentrations of about 0.01 ng TEQ/Nm³.

MATERIAL AND METHOD

PCBs are sampled together with PCDDs/PCDFs on XAD-2 cartridges in accordance with EN 1948-1 requirements. Sampling uncertainty has been estimated and stays within performances of other reference laboratories². Purification steps have been optimised to separate PCBs and PCDDs/PCDFs into two independent fractions. Samples are spiked with ¹³C PCBs extract standards and extracted in toluene. The concentrated extract is subjected to a full automatic (Power Prep^(c)) multistep clean-up (Silica-Alumina-Carbon). The final extract (150-200 µl, in n-nonane) is analysed by HRGC-HRMS, using a MICROMASS Autospec ULTIMA (SIR Mode, RP 8500, 10% Valley) equipped with a HP-Agilent (GC 6890 Series) chromatograph. The different congeners are separated by a 60m x 0.25mm x 0.25µm CP-Sil 8 CB Low Bleed/MS CHROMPACK-VARIAN column (5% Phenyl - 95% Dimethylpolysiloxane). The injected volume is 1.5µl (Splitless, EPC Flow Ramp Mode), using a HP-Agilent 7683 Series autosampler.

RESULTS AND DISCUSSION

Selection of the Electronic Impact ionisation (EI) and trap current

The well-established standard ionisation energy of 70eV used in most of commercial instruments is aimed at making mass spectra fragments comparable for all type of instruments and is used for qualitative molecular determination through the mass spectra analysis. The energy necessary for the first ionisation of organic molecules is much lower and an energy excess leads to fragment ions and uncharged species which are not relevant in this application where only unimolecular fragments are examined. The response of each dioxin-like PCB, native and labelled ¹³C has been investigated in a large range of primary electron energy (from 28eV to 80eV). The mean areas of each peak of congeners obtained for different ionisation energies are showed in figure 1. The value of 35eV suggested in the EN 1948-4 gives a very bad result and an EI of 31eV was showed as being optimal for PCBs quantification.

The effect of the trap current on the sensitivity has been investigated in the range 200 to 650 µA. If the sensitivity of the PCBs signals grows when increasing trap current, the sensitivity of the reference gas signal follows the same rule and the lock mass can create interference with the peaks of the products of interest. User should pay attention to avoid any saturation of the signal by the tracer and the response of the reference gas has thus been studied in the same range of current. A trap current between 400 and 450 µA is optimal as showed in figure 2.

The effect of this parameter on the sensitivity of the method is quite important and should not be neglected. This value is instrument specific and each laboratory should optimise this parameter before quantifying PCBs.

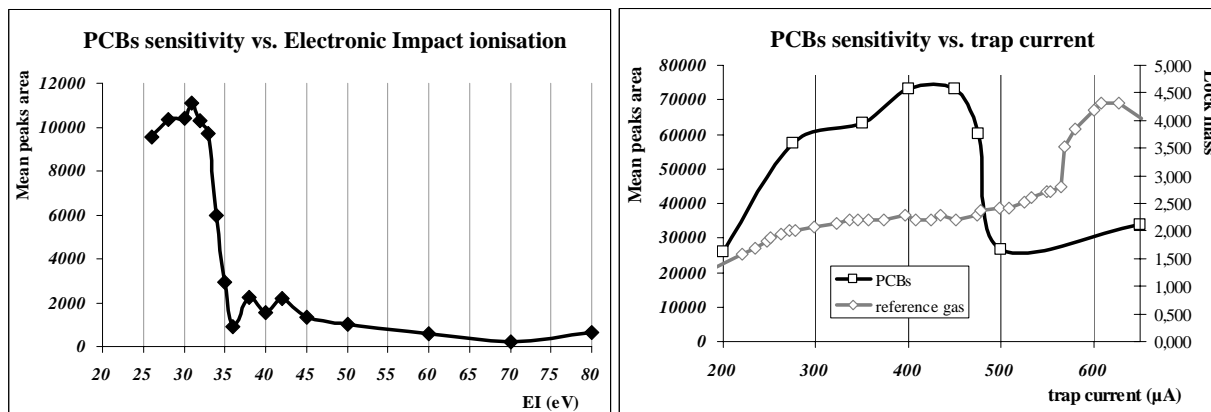


Figure 1: Effect of Electronic Impact ionisation on sensitivity

Figure 2: Effect of trap current on sensitivity

Choice of the mass reference gas and resolution power

The addition of compounds with known mass spectra is required to correct derive of the equipment in real time. Perfluorokerosene (PFK) is recommended by the EN 1948-4 for this application. However the large number of mass fragments produced by this substance interferes with the observation and interpretation of the products of interest. The use of Perfluorotributylamine (PFTBA) which gives a lower number of fragments is recommended. Furthermore, this gas consumes less energy for its ionisation, leaving more ions free for the compounds of interest.

Increasing the resolution power (RP) has a positive impact in term of selectivity and allowed a better separation of two adjacent mass peaks, but has a negative impact on the intensity of the signal. When the signal of the analyte decreased, the effects of minor interferences on the ability to detect this specific analyte become increasingly significant. There is a value of RP which optimised the “Signal-to-Noise Ratio” or S/N. As the mass reference gas influences the noise and thus the S/N, this parameter has also been taken into account in the optimisation of the RP. Figure 3 shows the relative sensitivity of mean PCBs response *versus* PFK and PFTBA as “lock mass” references for a mass resolving power in the range 10000 ± 3000. The optimal value for RP is around 8500 and not 10000 as specified in the EN 1948-4 protocol. As expected the use of PFTBA gives a better S/N ratio then PFK and is thus recommended.

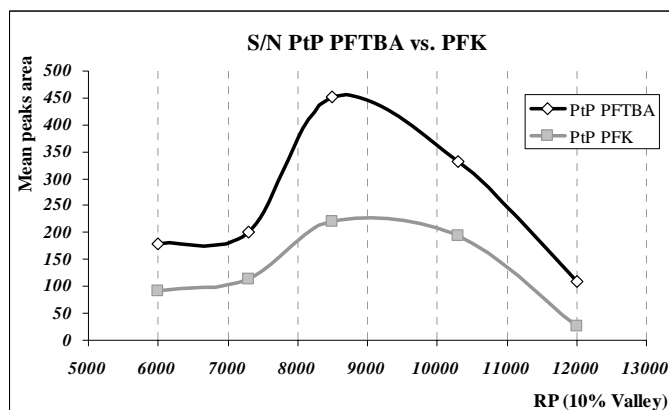


Figure 3: Effect of mass resolving power on S/N with PFK & PFTBA

GC Flow rate optimisation

The signal from a mass spectrometer is a series of mass scans recorded at regular intervals over time. The equipment reconstructed then a peak for a specified mass over time from a series of discrete measurements. It is generally accepted that a minimum of ten to thirteen points across a chromatographic peak are required to obtain accurate measurements. By adjusting the flow rate in the column, the operator can adapt the number of points keeping the scan rate of the equipment constant. Due to diffusion of the compounds in the column, a too low flow rate introduces noise and should thus also be avoided.

The Trennzahl (Tz) represents the number of baseline separated peaks between one pair of system homologues and could be used as measurement of the quality of the separation power of the GC. Tz for PCBs with 4 chlorine atoms has been studied for a flow rate between 0.7 and 1.7 l/min. Flow adjustment has easily been achieved by changing the pressure of the carrier gas at the head of the column. A flow rate of 0.9 ml/min with a column of diameter 0.25 mm gives the better Tz and this value of flow is used in the actual optimised protocol. This value is not in step with the EN 1948-4 recommendation (constant flow rate of 1 to 1.2 ml/min for all PCBs) and confirms the advantage of adapting the flow rate to each degree of chlorination.

For PCBs with higher numbers of chlorine atoms, the exact values are still under study, but first results showed that values of 1 to 1.2 l/min are realistic. We recommend thus to increase the flow rate from 0.9 to 1.2 ml/min depending on the degree of chlorination. This increase has also a positive impact on the purge at the end of the analysis and on acquisition time. This consideration is quite important in our configuration where a column of 60 m is used.

Target mass and dwell Time

En 1948-4 recommends quantifying every native dioxin-like PCB against its corresponding labelled ¹³C congener by using the monoisotopic ratio with statistically the higher relative intensities (ex: M/M+2 for TeCB).

Once against the intensity of the peak is only one parameter of the limit of quantification of the method and the real factor which should be optimised is the S/N ratio. Other monoisotopic ratio (M / M+2 / M+4 / M+6) for both native and labelled ¹³C PCBs have thus been studied and are presented in table 1. Some monoisotopic ratios, depending on the number of chlorine atoms and isotope, have been determined as being a better choice in term of S/N than the recommended ratio based on relative intensity. When the results are quite similar, the monoisotopic ratio with the higher relative intensity has been kept. We suggest to focus only on these monoisotopic masses when setting the target masses of the equipment and to adapt the dwell time regarding their theoretical relative intensity.

	12C	Monoisotopic mass	relative intensity	S/N PtP	13C	Monoisotopic mass	relative intensity	S/N PtP
Cl-4	M	289,9224	78	166	M	301,9626	78	29
	M+2	291,9194	100	330	M+2	303,9597	100	210
	M+4	293,9165	48	87	M+4	305,9567	48	87
Cl-5	M	323,8834	63	124	M	335,9236	63	169
	M+2	325,8804	100	22	M+2	337,9207	100	24
	M+4	327,8775	64	40	M+4	339,9177	64	118
Cl-6	M	357,8444	52	59	M	369,8847	52	60
	M+2	359,8415	100	431	M+2	371,8817	100	612
	M+4	361,8385	80	204	M+4	373,8788	80	260
	M+6	363,8356	36	24	M+6	375,8758	36	3
Cl-7	M	391,8054	44	19	M	403,8457	44	175
	M+2	393,8025	100	125	M+2	405,8428	100	124
	M+4	395,7995	96	100	M+4	407,8398	96	170
	M+6	397,7966	51	157	M+6	409,8369	51	33

Table 1: Relative intensity of best monoisotopic ratios depending on the degree of chlorination and isotope of PCBs.

External validation of the method

An external validation of the optimised protocol has been performed to ensure the quality of the work done within this study. Figure 4 shows a typical result obtained during the ringtest “12th Round of the International Intercalibration Study (IICS) organised by Intercal Sweden”. ISSeP has achieved a z-score = -0.20 for PCB #126 standard (congener with the higher toxicity equivalencies) which confirms the analytical quality of the method.

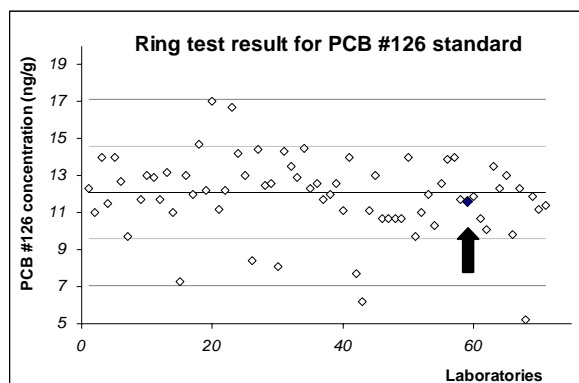


Figure 4: Ringtest for quantification of PCB #126 standard.

CONCLUSIONS

The protocol of the EN 1948-4 for dioxin-like PCBs quantification has been optimised during this study. For some analytical aspects the EN 1948-4 protocol is not optimal and other settings are proposed and justified in this paper. An external validation of the method has been performed to ensure the quality of the work done within this study.

The main modifications could be summarised as follow:

- The typical ionisation energy of 35 eV recommended by the EN 1948-4 and commonly used for PCDDs/PCDFs quantification is not optimal at all for PCBs. An EI of 31 eV is recommended for PCBs quantification.
- The trap current has a strong influence on the sensitivity and should be optimised for all instruments taking into account the gain in sensitivity for the PCBs and the risk of saturation by the “Lock mass” of the reference gas.
- The reference gas specified in the EN 1948-4 is not optimal and a better signal-to-noise ratio (S/N) is achieved using Perfluorotributylamine (PFTBA) as reference gas at a mass resolving power around 8500.
- Variation in flow rate can be easily obtained by a ramped pressure of the carrier gas at the head of the column. Increasing the carrier gas flow rate as the degree of chlorination is growing leads to a better T_z and has a positive impact on the acquisition time allowing the use of a longer column in routine analysis and thus a better sensitivity.
- Target masses should be set only on the monoisotopic masses which give the best S/N and the dwell time adapted regarding their theoretical relative intensity. This selection depends on the degree of chlorination and isotope.

ACKNOWLEDGMENTS

The authors wish to thank the team of ISSeP analytical laboratory and especially G. Zaffuto, A. Counasse, N. Jennes, V. Xhoris for help in sample preparation. They also express gratitude to A Galloy for technical help. ISSeP and the authors are also indebted to the Walloon Environment Directorate which has funded this study.

REFERENCES

- 1 van den Berg, M., et al.: “The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds” – Toxicological Sciences 93 (2006) pp. 223-241
- 2 Van Bavel, B. et al. : “Validation of method EN 1948 Part4: Determination of DL-PCB from stationary sources” – Organohalogen Compounds Vol. 71 (2009) pp. 2273-2277