The Development and Validation of a Congener Specific Method for the Concurrent Determination of *Ortho*, Non-*ortho* Chlorobiphenyls, PCDDs and PCDFs

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Introduction

Certain non-*ortho* and mono-*ortho* congeners are recognized as the most toxic components of CB formulations. They are of particular interest because of their "dioxin-like" biological effects and it has been proposed that with suitable toxic equivalent factors (TEFs) these CBs should be regarded as additive with PCDDs and PCDFs in calculating toxic equivalent (TEQ) summations. There has been considerable interest in developing analytical schemes to obtain data on selected CBs and PCDD/PCDFs from a single sample. For biological samples the containment-enrichment procedure of Smith, Staling and Johnson[1] is often used for PCDD/PCDF analysis. This procedure has been automated[2,3] and equipment is commercially available. We therefore wished to retain the general scheme and apparatus of this method but to obtain a separate fraction containing non-*ortho* CBs and a further fraction containing all other CBs. This paper describes a solvent sequence that accomplishes this fractionation and presents some recovery and validation data.

Experimental

Method development: A standard mixture containing 10ng of *ortho* and non-*ortho* chlorobiphenyls was added to the top of a column containing 50mg of Amoco PX-21 carbon on 700mg glass fibres and was eluted with the appropriate solvent mixture. The eluate was collected in fractions according to Fig. 2. Following a part II clean-up (see below) the final extract was spiked with 100µl of a solution of hexabromobenzene (250pg/µl) and analysed by GC-ECD. Recoveries were calculated relative to hexabromobenzene.

Certified Reference Material BCR CRM 350 (chlorobiphenyls in mackerel oil): A similar clean-up system to that described by Smith Stalling and Johnson[1] was used. Fish oil (0.3± 0.05g) was applied to the top of a multicolumn system (see Fig. 1) and spiked with an internal standard solution containing $^{13}C_{12}$ *ortho* (CBs 28, 52, 101, 138, 180 and 194) and non-*ortho* (CBs 15, 77, 126 and 169) chlorobiphenyls at 10ng each. The column was then eluted with the appropriate solvent mixture and two fractions were collected (Fraction I and Fraction II, Fig. 1). Each fraction was subjected to a Part II clean-up as follows. The solvent was evaporated on a rotary evaporator to ca. 4ml and further reduced to a few µl in a 1.1ml vial after gentle blowing down under N_2. The residue was reconstituted in hexane. The extract was then applied to the top of a pasteur pipette packed with sulphuric acid/silica gel (40% acid on gel) and potassium silicate and was eluted with 20ml of hexane. Hexane was rotary evaporated to ca. 4ml transferred to a 1.1ml vial and gently blown

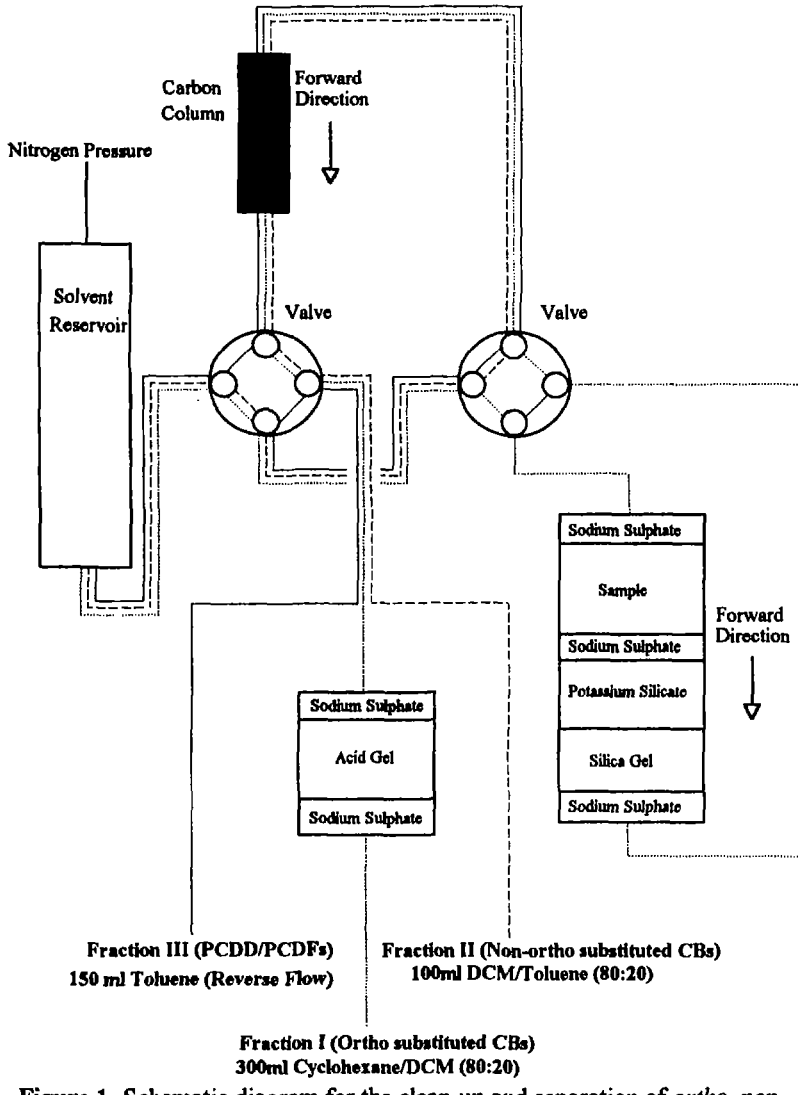

Figure 1. Schematic diagram for the clean-up and separation of *ortho*, non-*ortho* CBs and PCDD/PCDFs

down until just dry under a steady stream of N_2. 100µl of $^{13}C_{12}$ CB 202 was added (recovery standard) and the extracts were analysed by GC-MS.

GC-ECD: The extracts were analysed on a 5300 Mega Series Carlo Erba gas chromatograph equipped with a Ni-63 electron capture detector and a CTC-A200S autosampler using a DB-5 column (60m x 0.257mm, 0.1µm film thickness) in splitless mode. The oven temperature was programmed as follows : 100°C for 1min; ramp 12°C/min to 210°C, 4min; ramp 2°C/min to 250°C; ramp 5°C/min to 280°C, 20min. The carrier gas used was helium and the make-up gas was 5% argon-methane (40ml/min). The temperature of the injector port was 280°C and the ECD was maintained at 320°C.

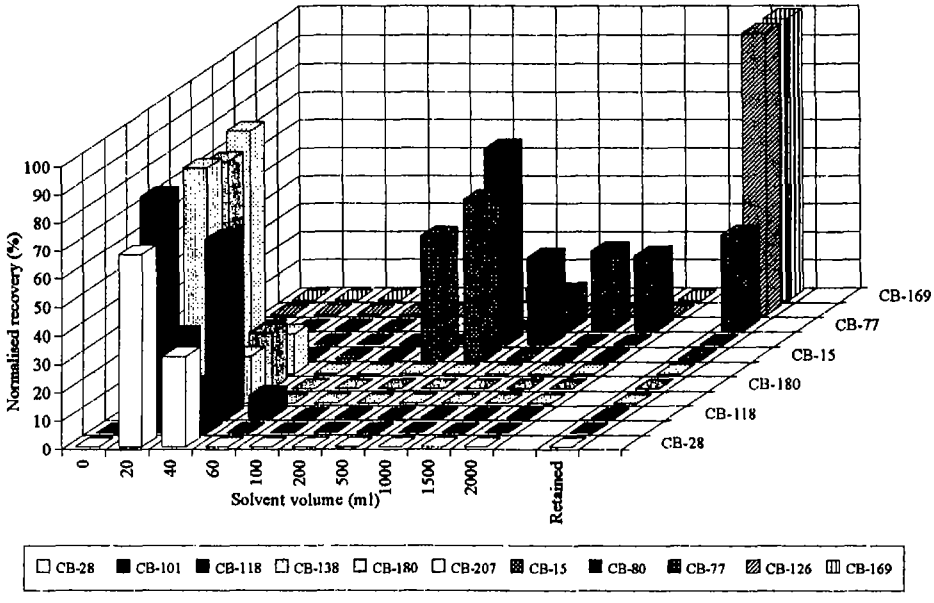

Figure 2. Fractionation of *ortho* and non-*ortho* CBs on the carbon column using a 50% mixture of cyclohexane and DCM

GC-MS: Analysis was accomplished using a VG 12-250 quadrupole mass spectrometer (*ortho* substituted fraction) or a VG Autospec (non-*ortho* fraction) coupled to a Carlo Erba HRGC. The column used was a Restek Rtx-5 (60m x 0.25mm x 0.1µm film thickness) in splitless mode. The oven temperature was programmed as follows: 100°C for 2min; ramp 20°C/min to 180°C, 7min; ramp 0.5°C/min to 190°C; ramp 5°C/min to 280°C, 10min.

Results and Discussion

The retention characteristics of CB congeners on a carbon column are controlled mainly by their substitution pattern. Non-*ortho* substituted congeners can more easily adopt a planar

configuration and are more strongly adsorbed on carbon than the corresponding non-planar (*ortho*-substituted) congeners. For example, when a 50% mixture of DCM and cyclohexane was used (Fig. 2), *ortho*-substituted CBs required up to 60ml of solvent to completely elute from the column whilst the first coplanar congener (CB-15) started eluting in the fraction between 100ml-200ml. As would be expected, CB-118 a mono-*ortho* substituted congener, showed a slightly stronger adsorption than the di and tetra *ortho* substituted CBs. This was more pronounced when cyclohexane (a weaker solvent) was used.

The nature of the solvent influences retention of solutes on activated carbon. For example, when 100% DCM was used, CB-118 quantitatively eluted within 40ml whilst, with cyclohexane CB-118 was retained even after 500ml of solvent have passed through the column. The cyclohexane/DCM solvent systems proved inadequate for the efficient elution of non-*ortho* CBs from the carbon column. For example, 96% of CB-169 was still retained on the column after elution with 2000ml of DCM. For this particular case, DCM/toluene solvent systems were further investigated.

Having established the retention characteristics of individual CB congeners on the carbon/glass fibre system for cyclohexane, DCM and toluene, a method for the separation of *ortho*, non-*ortho* substituted CBs and PCDD/PCDFs in three fractions was developed. The method is summarised in Fig. 1. The efficient separation of *ortho* and non-*ortho* congeners was evaluated by fractionating a standard mixture containing *ortho* substituted congeners at concentration 250 times higher than the corresponding non-*ortho*'s. Under these conditions only 0.1% of CB-118 was recovered in the second fraction. It was also found that an increase in the volume of the second fraction (Fraction II, Fig. 1) could result in low recoveries for 2,3,7,8 TCDF due to elution in this fraction. Thus 100ml DCM/toluene (80:20) was considered as the absolute maximum. The complete clean-up method, was validated for certain CB congeners by three replicate analyses (Table 1) of BCR Certified Reference Material CRM 350 (PCBs in Mackerel oil). Since CRMs are not available for non-*ortho* CBs and PCDD/PCDFs validation tests are more difficult to devise. Validation for PCDDs and

Table 1. Concentrations µg/Kg of selected CBs in BCR CRM 350

CBs	Certified	This Study		
		Concn.	% CV*	%Rec.
Ortho				
CB-28	22.5±4	20.1	8.6	64
CB-52	62 ±9	67	6.5	67
CB-101	164 ±9	163	1.0	72
CB-118	142 ±20	146	3.5	
CB-138	274 ±27**	306	3.0	78
CB-153	317 ±20	314	5.3	
CB-180	73 ±13	66	0.3	78
Non-*ortho*				
CB-15		1.08	29	85
CB-37		4.64	1.5	
CB-77		3.81	2.6	122
CB-126		0.75	8.2	87
CB-169		0.09	-***	93

*coefficient of variation; **not certified; *** not available (n=2)

PCDFs is in progress and is being approached by analysis of samples previously analysed in this laboratory by alternative methods. Preliminary and unvalidated results for non-*ortho* CBs in the BCR fish oil are also shown (Table 1).

Conclusion

The method described allows for the concurrent determination of *ortho* substituted CBs, a wide range of non-*ortho* substituted CBs (di-chloro to hexa-chloro) as well as PCDD and PCDFs. Critical separations such as mono-*ortho* CBs from early eluting di-chloro non-*ortho* CBs can be performed very efficiently. Furthermore, a refined separation of the non-*ortho* CBs from PCDD/PCDFs was achieved by eluting the carbon column with a 20% mixture of toluene in DCM. The PCDD/PCDF fraction was retained on the carbon column and quantitatively eluted by back flushing with 100% toluene. Some workers have used a similar scheme without separating non-*ortho* CBs from PCDD/PCDFs.[2-3] This has the disadvantage of requiring an increased number of channels in selected ion monitoring MS,[4] requiring multiple injections from a single fraction thus needing a greater volume and sacrificing sensitivity or requiring a further fractionation. The method will be applied to the determination of a selection of CB congeners, including non-*ortho* substituted CBs, in UK milk, eggs and meat. Results will be presented separately.

References

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