METHOD DEVELOPMENT FOR THE CONGENER SPECIFIC DETERMINATION OF *ORTHO* AND NON-*ORTHO* SUBSTITUTED POLYCHLORINATED BIPHENYLS IN FRUIT AND VEGETABLE SAMPLES

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Introduction

In recent years special concern has been focused on certain *ortho* and non-*ortho* polychlorinated biphenyls (PCBs) which exhibit potentially harmful effects. A limited number of these PCB congeners elicit dioxin-like toxic responses such as body weight loss, reproductive dysfunction, endocrine and teratogenesis[1,2,3]. Many of these PCBs, especially the non-*ortho* PCBs, are present in very low concentrations in fruit and vegetables and it is necessary to establish analytical methods for the extraction, fractionation, and quantification of these PCBs. We report the development of a method for the congener specific determination of selected mono-*ortho*, di-*ortho* and non-*ortho* PCBs in fruit and vegetable samples using an on-line clean-up procedure and gas chromatography-mass spectrometry (GC-MS) analysis.

Experimental

Method development: The procedure developed for the clean-up and fractionation of *ortho* and non-*ortho* PCBs in fruit and vegetable samples, using silica and carbon columns chromatography, is summarised in Figure 1. The fractionation of PCBs on carbon was assessed initially using a standard mixture containing 40ng each of *ortho* (28, 52, 101, 118, 138, 153, and 180) and non-*ortho* (PCBs 77, 126, and 169) PCBs, which was added to the top of a carbon column (glass, 10cm x 10mm i.d) containing 50mg of Amoco PX-21 carbon on 700mg glass fibres[4,5]. The column was eluted with hexane:dichloromethane (DCM) followed by DCM:toluene and the eluent was collected in fractions according to Figure 1 and analysed by GC-MS.

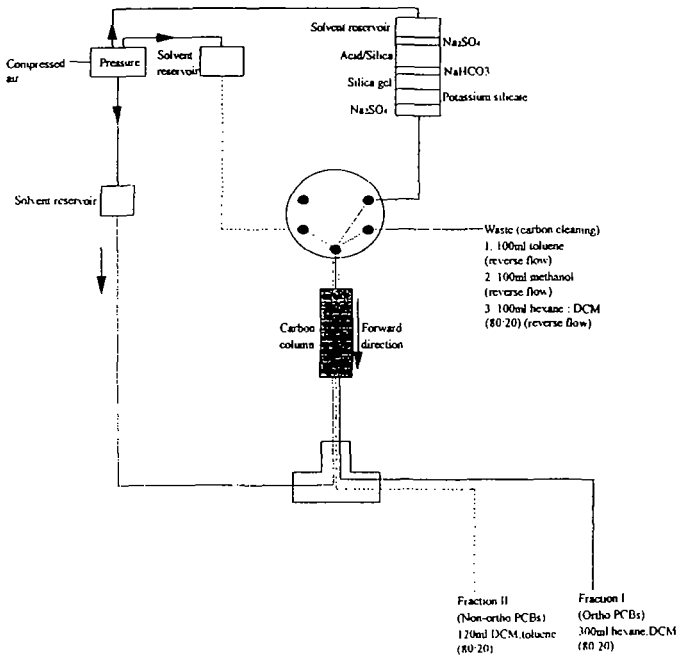

Figure 1. Schematic diagram for clean-up and separation of ortho and non-ortho PCBs.

Method validation: Four English apples (161g) were cut into small pieces and homogenised with 200ml of Analar water[6]. The resulting slurry was subdivided into four equal portions and stored in a freezer. A portion of the slurry was spiked with an internal standard solution containing $^{13}C_{12}$ *ortho* (28, 52, 101, and 138) and non-*ortho* (77, 126 and 169) polychlorinated biphenyls. Anhydrous sodium sulphate (100g) was added and the slurry was extracted with 3 x 40ml hexane. The extract was then applied to the top of a multicolumn (glass column, 25cm x 25mm i.d, packed from the bottom with glass wool, anhydrous sodium sulphate (1cm), potassium silicate (3cm), silica gel (5cm), sodium bicarbonate (1cm), sulphuric acid/silica gel mixture (5cm, 40:60) and anhydrous sodium sulphate (1cm)) which was connected to the carbon column. The extract was eluted with hexane:DCM (300ml, 80:20) and the carbon column alone was eluted with DCM:toluene (120ml, 80:20) to collect two fractions (Fraction I, mono-*ortho* and di-*ortho* PCBs and Fraction II, non-*ortho* PCBs). Each fraction was treated as follows: the solvent was evaporated on rotary evaporator to *ca* 4ml, transferred to a 1.1ml vial and gently blown down under a steady stream of compressed air until just dry. 50ul of $^{13}C_{12}$ PCB-180 (400pgul^{-1}) recovery standard was added and the fractions were analysed by GC-MS with selected ion monitoring.

Table 1. Normalised %Recoveries of standard mixture on the PX-21 carbon/glass fibre using different solvent systems

	Hexane:DCM (80:20)ml				DCM:Toluene (80:20)ml		
Congener	150	200	250	300	50	100	150
PCB-28	100	0	0	0	0	0	0
PCB-52	100	0	0	0	0	0	0
PCB-77[a]	0	0	0	0	99.3	0.7	0
PCB-101	100	0	0	0	0	0	0
PCB-118	93.6	3.9	2.5	0	0	0	0
PCB-126[a]	0	0	0	0	100	0	0
PCB-138	100	0	0	0	0	0	0
PCB-153	100	0	0	0	0	0	0
PCB-169[a]	0	0	0	0	100	0	0
PCB-180	100	0	0	0	0	0	0

[a]Non-*ortho* substituted PCBs

Apple, cabbage and potato samples were collected from South Wales and representative sub-samples sent to two other laboratories for PCB congener analysis. Samples were analysed by each laboratory using standard methods. The following PCBs were determined, mono-*ortho* (PCBs 105,114, 118, 123, 153, 157, 167 and 189), di-*ortho* (PCBs 28, 52, 101, 138, and 180) and non- *ortho* (PCBs 77, 81, 126 and 169).

Results and discussion

The retention characteristics of PCB congeners on the carbon column are controlled mainly by their substitution pattern and by nature of the solvent used in the elution[7]. Non-*ortho* substituted PCB congeners can more easily adopt a planar configuration and are more strongly adsorbed on carbon than the corresponding non-planar (*ortho*-substituted) congeners. The elution characteristics of the individual congeners (Table 1) show the clear separation of the *ortho*/mono-*ortho* and non-*ortho* fractions. Having established the retention behaviour of the individual PCB congeners on the carbon/glass fibre system for hexane, DCM and toluene (Table 1), a method for the separation of mono-*ortho*/di-*ortho* and non-*ortho* substituted PCBs in two fractions was developed. This method, summarised in Figure 1, employed hexane:DCM (300ml, 80:20) to elute all mono- and di-*ortho* PCBs and DCM:toluene (120ml, 80:20) to elute non-*ortho* PCBs as a separate fraction.

Table 2. Recoveries and Precision for PCBs in apple extracts

Recovery	PCBs 28	52	77	101	126	138	169
Sub-sample							
1	56	58	116	63	103	a	90
2	55	57	71	59	80	70	81
3	53	56	88	57	100	65	89
4	57	50	103	53	78	68	92
Av.	55	54	94	58	90	65	88
Precision							
%COV	3.1	6.5	21	6.7	14	9.5	5.6

[a]Not determined

Table 3. Interlaboratory comparison of the concentrations of PCB congeners in fruit and vegetable samples

Sample	Laboratory/PCB concentration ($ugKg^{-1}$)					
	Laboratory A		Laboratory B[a]		Laboratory C	
	$\Sigma 7^b$	$\Sigma 18^c$	$\Sigma 7^b$	$\Sigma 18^c$	$\Sigma 7^b$	$\Sigma 18^c$
Cabbage	0.3	0.5	0.3	0.4	_	_
Apple	1.7	2.8	2.0	2.2	2.6	3.0
Apple	1.4	2.5	0.5	0.6	0.9	1.2
Potato	1.0	2.1	0.4	0.9	1.2	1.7
Potato	0.7	1.7	0.6	1.1	0.4	0.7

[a]Samples analysed in Nottingham

$\Sigma 7$-sum of PCBs 28, 52, 101, 118, 138, 153 and 180

$\Sigma 18$-sum of PCBs 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189

The intralaboratory performance of the method for the efficient separation of mono-*ortho*/di-*ortho* and non-*ortho* PCB congeners was evaluated by analysis of homogenised apple samples spiked with mono-*ortho*/di-*ortho* and non-*ortho* PCBs. Recoveries for the PCB congeners investigated(Table 2) which ranged from 55-94% and precision (coefficient of variation, %COV) of 3.1-21% for the complete method were considered acceptable.

The total PCB concentrations in apple, cabbage and potato samples analysed by the method described and by two independent laboratories are shown in Table 3. In each case, the observed levels reflected the differences in limits of detection, which ranged from 0.1ug Kg^{-1} for laboratory A to <0.01 ug Kg^{-1} for laboratory B, since congeners not detected were considered to contribute a concentration equivalent to the detection limit for the purpose of calculating the total PCB concentration. Nevertheless, examination of the data indicates a good agreement between the laboratories for the sum of seven and eighteen congeners.

Conclusion

The method described allows for the concurrent determination of *ortho* and non-*ortho* substituted PCBs (di-chloro to hepta-chloro) in fruit and vegetable samples with acceptable recovery and precision.

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