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Short communication

# Application of $^{13}\text{C}$ -labelled polychlorinated biphenyl congener 153 as internal standard in the gas chromatographic–mass spectrometric analysis of polychlorinated biphenyls

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## Abstract

We developed a method of qualitative and quantitative GC–MS analysis of polychlorinated biphenyls (PCBs) in soil samples, to be able to determine the biodegradation efficiency for individual PCBs.  $^{13}\text{C}_{12}$ -Substituted PCB 153 was used as internal standard. The analyses were performed on a DB-5 capillary column. The identification procedure was based on the correlation analysis of the measured and published relative retention  $r_{12}$  data using [ $^{13}\text{C}_{12}$ ]PCB 153 instead of octachloronaphthalene (OCN). [ $^{13}\text{C}$ ]PCB 153 was also used for quantification of individual PCBs in soil samples.

**Keywords:** Soil; Environmental analysis; Polychlorinated biphenyls

## 1. Introduction

Polychlorinated biphenyls (PCBs) are widespread toxic organopollutants [1]. Their analysis plays an important role in the monitoring of PCB contamination, persistence and removal studies. The development of an appropriate analytical method is fundamental for study of the processes of biodegradation, where changes in composition are followed. The method should enable us to obtain exact information on both quantitative and qualitative (congener-specific) composition of PCBs in samples. The use of labelled compounds is very helpful in such studies and there are two ways that they can be

applied. One of them is the use of PCBs labelled with radioactive carbon isotope  $^{14}\text{C}$  [2].

$^{14}\text{C}$ -labelled PCBs are used as tracers for investigations of their fate, biotransformations, pathways, recoveries, etc. The other employs [ $^{13}\text{C}$ ]PCB as an internal standard in gas chromatography [3].

Chlorinated biphenyls are usually analyzed by high-resolution capillary gas chromatography with electron capture or mass spectrometric detectors. The classical internal standard method of PCB analysis usually employs octachloronaphthalene (OCN) [4], DDT [5] or some of the characteristic PCBs as the internal standard [6]. A disadvantage of OCN and DDT is that they are compounds of different structure and thus their chemical behaviour differs from PCBs during the analytical procedure. Use of a PCB congener not present in the sample as the internal standard is another possibility. In this work we

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present our experiences and the advantages of using [ $^{13}\text{C}$ ]PCB 153 as the internal standard in both qualitative and quantitative GC–MS analysis.

## 2. Experimental

### 2.1. Chemicals

[ $^{13}\text{C}_{12}$ ]PCB 153 was obtained from Cambridge Isotope Laboratories, Andover, MA, USA (a concentration of  $41.7 \text{ ng } \mu\text{l}^{-1}$ ). PCB commercial mixtures Delor 103 and 106 (corresponding, in the degree of chlorination, to Aroclor 1242 and 1260, respectively) were obtained from Chemko Strážské, Slovak Republic (concentrations  $1.244 \text{ } \mu\text{g } \mu\text{l}^{-1}$  and  $1.117 \text{ } \mu\text{g } \mu\text{l}^{-1}$ , respectively); and soil samples (loess chernozem) were obtained from Institute of Radiochemistry, Research Center Rossendorf, Germany. The solvents used were *n*-heptane, acetone and hexane (all analytical-reagent grade), from Lachema, Brno, Czech Republic.

### 2.2. Preparation of soil samples

Soil samples were spiked with  $80 \text{ } \mu\text{l}$  of Delor 103 (concentration  $1.244 \text{ } \mu\text{g } \mu\text{l}^{-1}$ ) and  $250 \text{ } \mu\text{l}$  of [ $^{13}\text{C}_{12}$ ]PCB 153 (concentration  $41.7 \text{ ng } \mu\text{l}^{-1}$ ). After 1 day, homogenized soils samples ( $1 \text{ g}$ ) were extracted in a Soxhlet apparatus with a hexane–acetone (3:1) mixture for 4 h, and filtered through glass wool which was subsequently washed with acetone. The organic contaminants from soil in the organic solvent portion were removed with concentrated sulphuric acid (until decolorization). The material was then washed with distilled water (to neutral pH), and filtered through a layer of anhydrous sodium sulphate to remove water residues. Finally, the organic extract was evaporated on a rotary evaporator to preconcentrate PCBs and the residue dissolved in  $200 \text{ } \mu\text{l}$  *n*-heptane.

### 2.3. GC–MS

The analyses of PCBs were performed on a high-resolution gas chromatograph, model Varian 3400

(Zug, Switzerland) with a Finnigan MAT 800 ion-trap detector (San Jose, CA, USA).

A DB-5 capillary column (J&W Scientific, Folsom, CA, USA),  $30 \text{ m} \times 0.25 \text{ mm}$  I.D., with a film thickness of  $0.25 \text{ } \mu\text{m}$ , was used. The capillary column was tested with tetradecane at  $125^\circ\text{C}$ , obtaining its capacity factor of  $k' = 7.025$  and a number of theoretical plates of  $4100 \text{ m}^{-1}$ . Helium was employed as the carrier gas with a linear velocity of  $27.5 \text{ cm s}^{-1}$ , as measured at  $80^\circ\text{C}$ . All the measurements were carried out in full-scan mode, with a mass range  $m/z$  from 150 to 450. An injected sample volume was  $1 \text{ } \mu\text{l}$  in the splitless mode; the temperature program initiated at  $80^\circ\text{C}$  for 0.8 min, then with a fast gradient of  $25^\circ\text{C min}^{-1}$  to the start temperature  $T_s$  of  $140^\circ\text{C}$ , followed by a gradient  $\Delta T$  of  $3^\circ\text{C min}^{-1}$  to the final temperature of  $290^\circ\text{C}$ . Under these conditions, all the PCBs eluted during the temperature gradient (the temperature program was optimized in our previous work [7]). The detector and injector temperature was  $250^\circ\text{C}$  in all analyses.

## 3. Results and discussions

### 3.1. Qualitative PCB analysis

The temperature program applied to PCB analyses was previously [7] optimized to obtain linear regression between the relative retention data published by Mullin et al. [4]  $r_{12M}$  and our measured data  $r_{12}$  (related to OCN). On the basis of the linear regression obtained we were able to identify PCB congeners with commercial mixtures as the standard, without the need for every individual PCB standard. In this work [ $^{13}\text{C}_{12}$ ]PCB 153 was used as the internal standard instead of OCN. Linear regression for measured data related to [ $^{13}\text{C}_{12}$ ]PCB 153 and the cited data related to non-labelled PCB 153 was obtained cf. Fig. 1.

The labelled PCB 153 eluted 2 s before the unlabelled one and overlapping occurred, so it is possible to say that the chromatographic shift is negligible for our purpose (cf. Fig. 2). On the basis of the mass spectra the [ $^{13}\text{C}_{12}$ ]PCB 153 was easy to recognize. Non-labelled hexachlorinated PCBs have characteristic masses of  $m/z$  358–366, the corre-

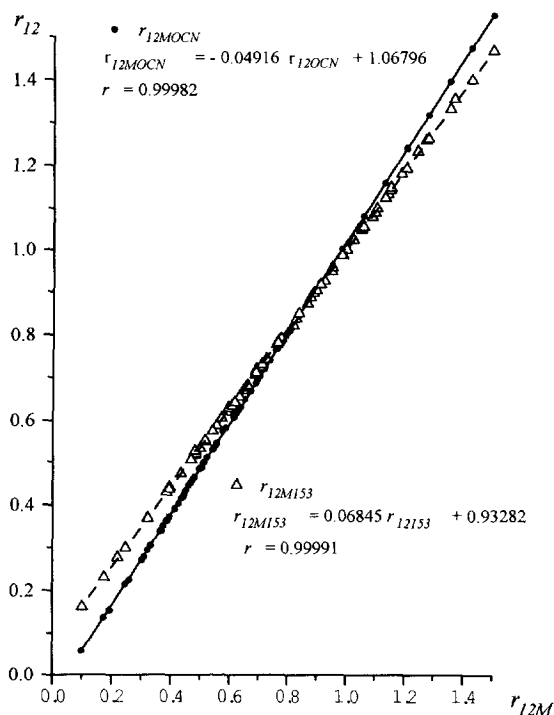


Fig. 1. Comparison of linear regressions between cited ( $r_{12M}$ ) and measured ( $r_{12}$ ) retention data which were related to OCN ( $r_{12MOCN}$ ,  $r_{12OCN}$ ) and to labelled ( $r_{12153}$ ) and non-labelled PCB 153 ( $r_{12M153}$ ). (See Section 3.1).

responding mass peaks of [ $^{13}\text{C}_{12}$ ]PCB 153 (where all twelve  $^{12}\text{C}$  in the molecule are replaced by  $^{13}\text{C}$ ) are 12 u higher than of those of non-labelled PCBs (cf. Fig. 3).

We analyzed Delor 103 with [ $^{13}\text{C}_{12}$ ]PCB 153 where no overlapping was found. In Delor 106 there are non-labelled PCB 153 and 132 which coelute with [ $^{13}\text{C}_{12}$ ]PCB 153, but using the most intensive peaks of [ $^{13}\text{C}_{12}$ ]PCB 153, the mass spectrum of individual congeners can be identified.

### 3.2. Quantitative PCB analysis

The mass spectrometric response of [ $^{13}\text{C}_{12}$ ]PCB 153 obtained was slightly lower than that of the corresponding mass peak of the non-labelled PCB153 as it is apparent from Fig. 2. This finding differs substantially from that of Sipolla and Himberg [8] who found a response more than three times lower for [ $^{13}\text{C}_{12}$ ]PCB 126. This they tried to explain as an error caused by heart cutting while working with a GC two dimensional system [9].

To be able to quantify the content of PCBs a calibration curve was measured for the non-labelled PCB 153. The calibration curve is described by the equation  $A^{12}\text{C}/A^{13}\text{C}=1.070 m^{12}\text{C}/m^{13}\text{C}$  with a cor-

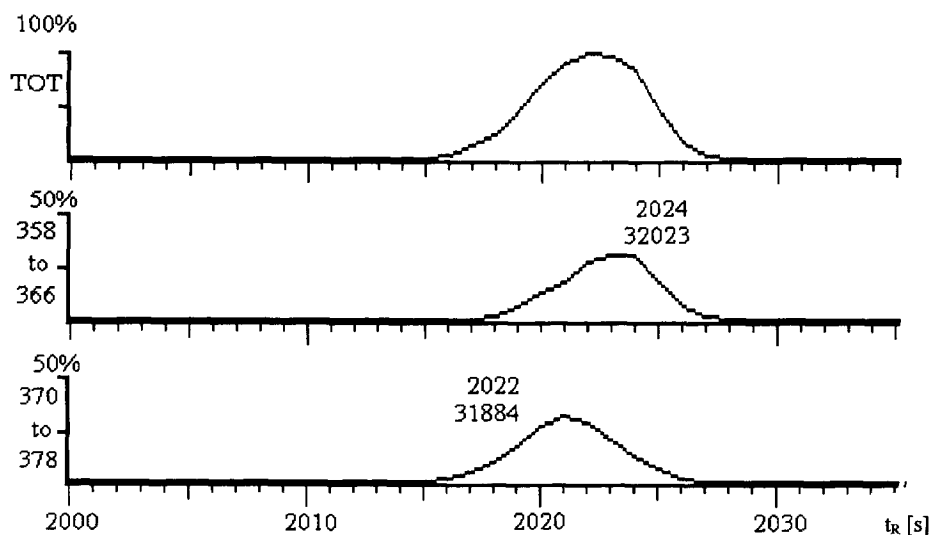
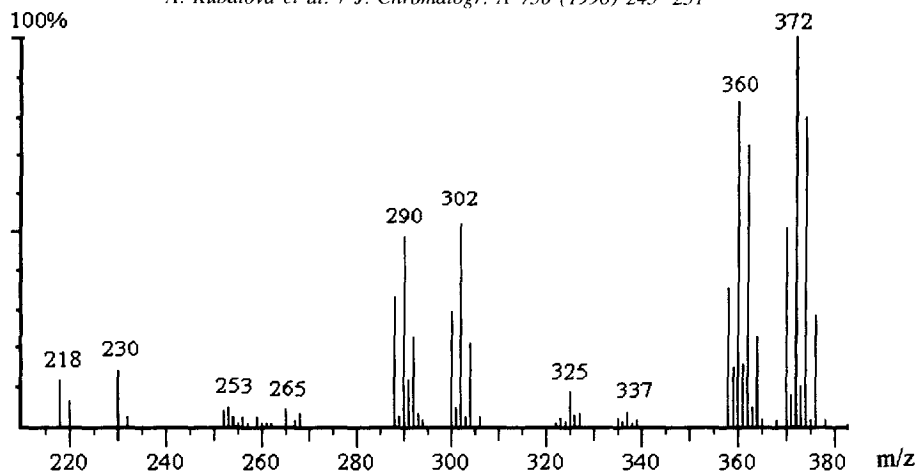
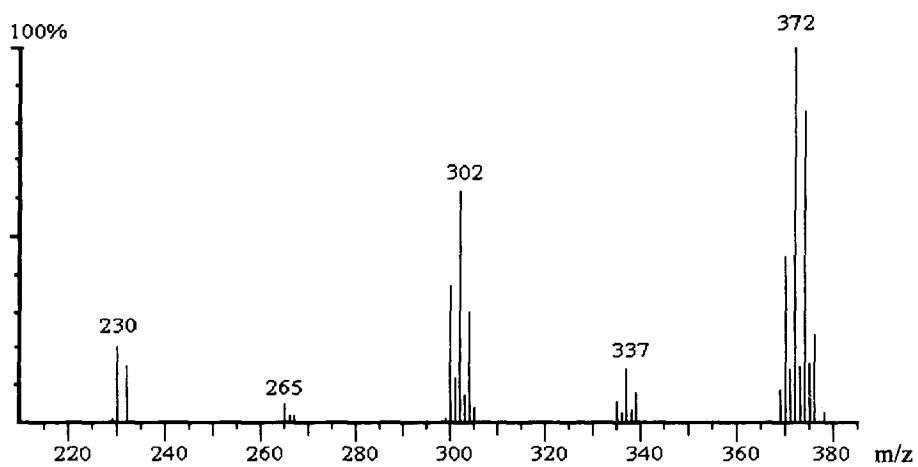


Fig. 2. TIC and mass chromatograms of a 1:1 mixture of [ $^{13}\text{C}_{12}$ ]PCB153 (concentration  $41.7 \text{ ng } \mu\text{l}^{-1}$ ) and [ $^{12}\text{C}$ ]PCB153 (concentration  $40 \text{ ng } \mu\text{l}^{-1}$ ) with retention times in seconds and integrated responses in counts.

a)

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b)



c)

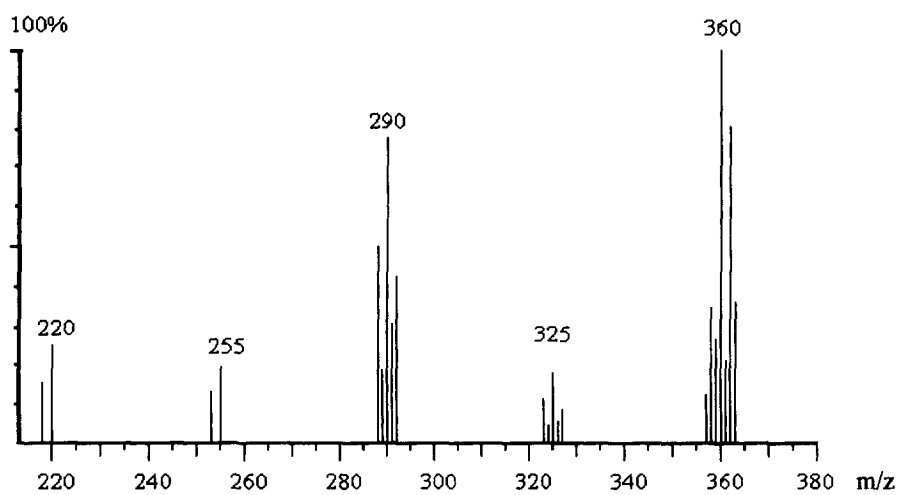


Fig. 3. (a) Mass spectrum of a 1:1 mixture of [ $^{13}\text{C}_{12}$ ]PCB153 and [ $^{12}\text{C}$ ]PCB153; (b) mass spectrum of [ $^{13}\text{C}_{12}$ ]PCB153; (c) mass spectrum of [ $^{12}\text{C}$ ]PCB153.

relation coefficient of  $r=0.998$ .  $A^{12}\text{C}/A^{13}\text{C}$  represents a ratio of the measured responses of the  $^{12}\text{C}$  and  $^{13}\text{C}$  congeners and  $m^{12}\text{C}/m^{13}\text{C}$  a ratio of the corresponding masses of the prepared standard samples. The slope of the calibration curve also confirms a lower response of the labelled PCB using our standards.

We have investigated the reproducibility of relative responses (related to [ $^{13}\text{C}_{12}$ ]PCB 153) for 25 peaks of Delor 103. For non-coeluting congeners, a good reproducibility with a S.D. of 7.4% was found (cf. Table 1). The coeluting congeners had worse reproducibility, probably due to inaccurate automatic integration.

A large standard deviation was found for congeners 26/25 and we assume that this was mainly

due to trace amounts of these congeners present in the Delor 103 samples. PCB 48/47 also had a poor reproducibility. We checked the mass spectra in this case and found siloxanes from the stationary phase coeluting with the PCBs. A high standard deviation of congeners 72/71 and 41/64 was probably caused by closely similar retention times [4]. As it is known from literature [5], the equipment should be recalibrated for the mass response every day, this was also confirmed by our work.

In our previous work [7] while studying biodegradation processes it was found that samples on which Delor 103 was spiked were biodegraded. We are able to find a change in the composition larger than 10% of the separated congeners present—where the coeluting congeners had to be taken as a sum.

Table 1  
Relative responses of 25 peaks representing PCB congeners in Delor 103

PCB Number <sup>a</sup>	Relative responses related to $^{13}\text{C}_{12}$ PCB 153						Average	S.D.	S.D.%
	1	2	3	4	5	6			
4	0.216	0.211	0.193	0.179	0.179	0.170	0.192	0.019	10
6	0.055	0.056	0.052	0.063	0.056	0.042	0.054	0.007	13
<sup>b</sup> 8/5	0.525	0.500	0.497	0.466	0.469	0.432	0.481	0.033	7
19	0.089	0.086	0.084	0.080	0.081	0.072	0.082	0.006	7
18	1.015	0.982	0.908	0.938	0.876	0.810	0.922	0.074	8
17	0.384	0.354	0.410	0.329	0.346	0.331	0.359	0.032	9
15	0.120	0.109	0.117	0.108	0.111	0.105	0.112	0.006	5
16	0.404	0.382	0.360	0.363	0.331	0.316	0.359	0.032	9
32	0.285	0.265	0.279	0.244	0.268	0.239	0.263	0.018	7
<sup>b</sup> 26/25	0.208	0.197	0.162	0.064	0.190	0.173	0.166	0.053	32
<sup>b</sup> 28/31	1.882	1.785	1.731	1.737	1.683	1.536	1.726	0.115	7
33	0.725	0.718	0.709	0.657	0.651	0.601	0.677	0.049	7
22	0.345	0.324	0.335	0.328	0.307	0.297	0.323	0.018	6
45	0.070	0.067	0.067	0.063	0.066	0.066	0.067	0.002	3
52	0.318	0.302	0.291	0.274	0.289	0.259	0.289	0.021	7
49	0.227	0.226	0.209	0.202	0.213	0.194	0.212	0.013	6
<sup>b</sup> 48/47	0.282	0.247	0.912	0.439	0.280	0.228	0.398	0.263	66
44	0.325	0.314	0.303	0.290	0.245	0.271	0.291	0.030	10
42	0.123	0.130	0.105	0.120	0.115	0.108	0.117	0.009	8
37	0.220	0.206	0.210	0.207	0.201	0.189	0.206	0.010	5
<sup>b</sup> 72/71	0.146	0.043	0.102	0.210	0.034	0.030	0.094	0.073	77
<sup>b</sup> 41/64	0.095	0.083	0.071	0.093	0.077	0.161	0.097	0.033	34
40	0.062	0.066	0.053	0.063	0.060	0.061	0.061	0.004	7
74	0.158	0.144	0.155	0.138	0.152	0.136	0.147	0.009	6
<sup>b</sup> 70/76	0.304	0.288	0.267	0.286	0.267	0.257	0.278	0.018	6
<sup>b</sup> 95/66	0.358	0.325	0.337	0.346	0.351	0.304	0.337	0.020	6
153	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0

Measured in full scan mode for  $m/z=100-400$ . Six parallel experiments were evaluated.

<sup>a</sup> Systematic numbering of PCBs according IUPAC nomenclature [10].

<sup>b</sup> Coeluting PCBs.

### 3.3. Analysis of soil samples

The goal of our work was to develop a method of quantitative determination of the biodegradation efficiency for individual PCBs in soil samples. The method was verified as follows: The soils were

contaminated with Delor 103 and before the extraction spiked with [ $^{13}\text{C}_{12}$ ]PCB 153 as the internal standard. The extracts of soil samples were analyzed under an optimized temperature program. No discrimination was observed for the major polychlorinated biphenyls in Delor 103; see Fig. 4 for a

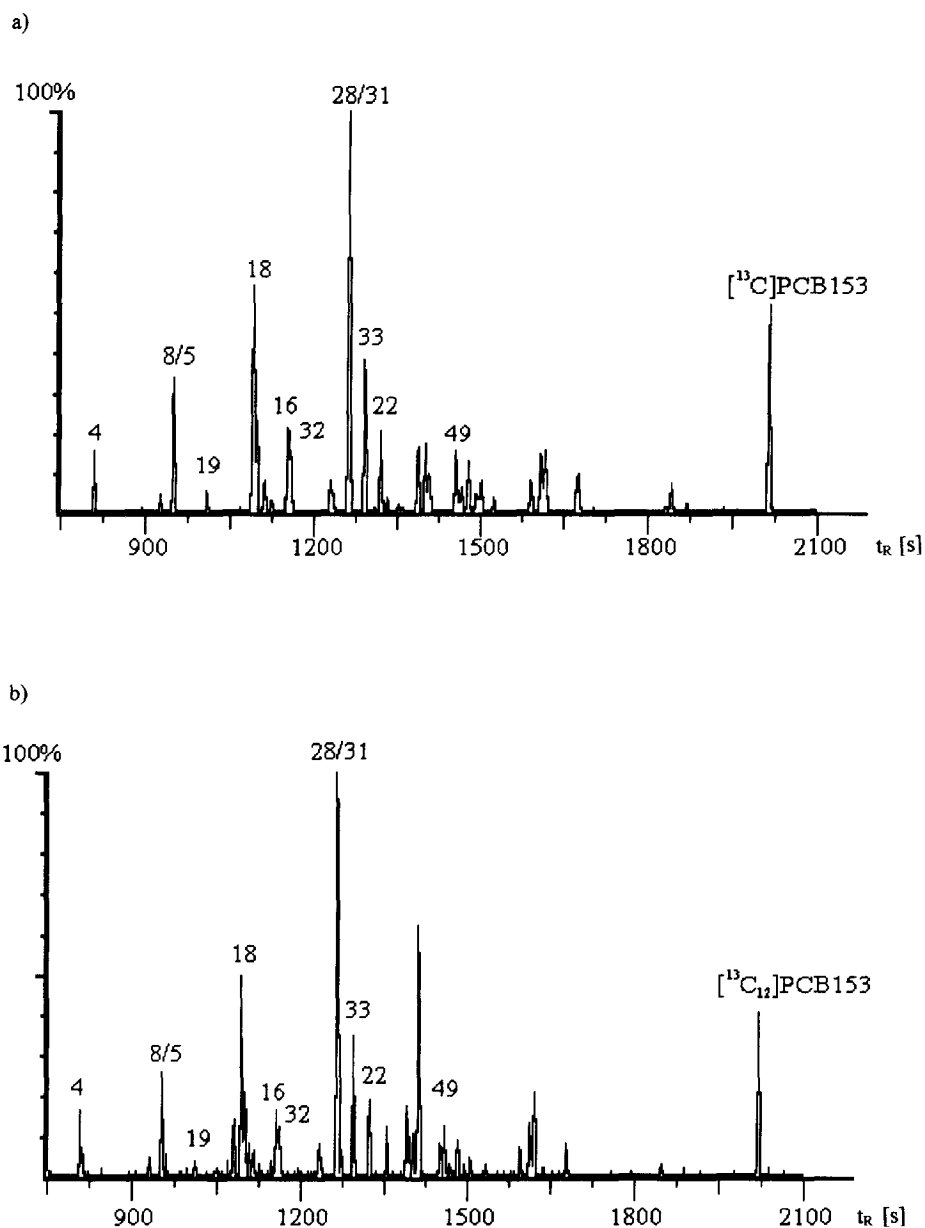


Fig. 4. Comparison of chromatograms of Delor 103 with the internal standard [ $^{13}\text{C}_{12}$ ]PCB 153. (a) Delor 103 with the internal standard [ $^{13}\text{C}_{12}$ ]PCB 153 used for spiking the soil; (b) after extraction from the soil.

comparison of the chromatogram of the standard with that of the extracted sample. The only difference between the two chromatograms probably were impurities present in the extracted samples (cf. Fig. 4b).

The extraction recovery of Delor 103, calculated as a sum of only five major PCB congeners and related to [ $^{13}\text{C}_{12}$ ]PCB 153, was 85%. When based on the response of 25 peaks of Delor 103 congeners, the value was 80%. This method could still be improved using multiple ion detection.

$^{14}\text{C}$ -labelled PCB 77 was used with success for the PCB recovery check from aqueous media used for the cultivation of degrading fungi. In this experiment large losses caused by evaporation were, surprisingly, found after one month of standing in the laboratory. Approximately 30% of the activity remained in the liquid sample, 10% was sorbed on the glass walls of the Erlenmeyer flasks and 30% was absorbed in sterile cellulose stoppers, while the remaining 30% probably escaped into the atmosphere [11].

#### 4. Conclusions

The isotopic internal standard [ $^{13}\text{C}_{12}$ ]PCB 153 used fulfils very well the requirement of a close chemical relatedness to the substance to be determined—PCB congeners. However the method is rather costly due to the price of the labelled PCB(s), the problem can be solved by their synthesis [2]. The results obtained by the GC–MS method demonstrate its applicability to both qualitative and quantitative analysis:

1. we have confirmed the suitability of [ $^{13}\text{C}_{12}$ ]PCB 153 for congener specific analysis of PCBs based on a linear relationship between the cited [4] and measured retention data.
2. the method was found to be reproducible (average S.D. 7.4%).
3. the method is convenient for the analysis of soil

samples and can be recommended for PCB biodegradation studies.

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