National Standards of People's Republic of China

GB 5009.190-2014

National Food Safety Standard

Determination of Indicative PCB Content in Foods

Issued on: 2014-12-01 Implemented on: 2015-05-01

Issued by Ministry of Health of the People's Republic of China

Foreword

This standard has substituted for *GB/T 5009.190—2006 Determination of Indicative PCB Content in Foods and GB/T 22331—2008 Measurement of PCB Residue with Gas Chromatography in Aquatic Products.*

Compared with GB/T 5009.190—2006, this standard has the following main changes:

- Modified standard format

National Standard for Food Safety

Determination of Indicative PCB Content in Foods

1. Scope

Method I of this standard specifies the way to measure the content of PCBs (polychlorinated biphenyls) in food, including the indicative PCBs specified in the Global Environmental Monitoring System (GEMS)/food programme (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180), as well as PCB18, PCB33, PCB44, PCB70, PCB105, PCB128, PCB170, PCB187, PCB194, PCB195 and PCB199. Method II specifies the way to measure the content of PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180.

This standard is applicable to the measurement of indicative PCBs in samples of animal-derived food and oil or fat including fish, shellfish, eggs, meat, dairy and their products.

Method I: Gas chromatography-mass spectrography to dilute stable isotopes

2. Principles

The stable isotope dilution technique is applied in the standard to add 13C12-marked PCBs into the samples as the quantitative criterion. Sample solutions going through Soxhlet extraction shall be added to the internal standard of recovery after the chromatographic purification of column chromatography, separation and concentration. Then an analysis shall be made with gas chromatography-low resolution mass spectrometer, quadrupole mass selective ion monitoring (SIM)or ion trap tandem mass spectrometry multiple- reaction monitoring (MRM) and determination with the internal standard method shall be carried out.

3. Reagents and materials

3.1 Reagents

3.1.1. C_6H_{14}:Pesticide Analysis Grade

3.1.2. CH_2Cl_2:Pesticide Analysis Grade

3.1.3. C_3H6O:Pesticide Analysis Grade

3.1.4. CH_3OH:Pesticide Analysis Grade

3.1.5. C_8H_{18}:Pesticide Analysis Grade

3.1.6. Anhydrous Na_2SO_4: Top grade pure. We shall put the commercially available anhydrous Na_2SO_4 into the chromatographic column and rinse it twice with normal hexane and dichloromethane. The volume of solvent used every time shall be approximately twice that of anhydrous Na_2SO_4. After the rinse, we shall transfer the anhydrous Na2SO4 into the flask where it dries at 50 °Cbefore baking it at 225°C for 8 to 12 hours and storing it in the dryer.

3.1.7. H_2SO_4:95%~98%,top grade pure.

3.1.8. NaOH: top grade pure.

3.1.9. $AgNO_3$: top grade pure.

3.1.10. Silica gel for chromatography (75μm~250μm): We shall put the commercially available anhydrous Na_2SO_4 into the chromatographic column and rinse it twice with normal hexane and dichloromethane. The volume of solvent used every time shall be approximately twice that of silica gel. After the rinse, we shall transfer the silica gel into the flask which will be capped and placed in the oven to dry up at 50 ℃. Then we shall bake it at 180°C for 8 to 12 hours and place it in the reagent bottle with ground stopper after cooling, which will be stored in the dryer.

3.1.11. 44% acidated silica gel: Weigh 100 g of activated silica gel and drip 78.6 g of sulfuric acid in. Then shake it until lumps disappear before placing it into the reagent bottle with ground stopper, which will be stored in the dryer.

3.1.12. 33% alkaline silica gel: Weigh 100 g of activated silica gel and drip 49.2 g of 1 mol/L sodium hydroxide solution in. Then shake it until lumps disappear before placing it into the reagent bottle with ground stopper, which will be stored in the dryer.

3.1.13. 10% silver nitrate impregnated silica gel: Dissolve 5.6 g of silver nitrate in 21.5 mL of deionized water and drip it into 50 g of activated silica gel. Then shake it until lumps disappear before placing it into the reagent bottle with ground stopper, which will be stored in the dryer.

3.1.14. Alkaline aluminium oxide for chromatographic analysis: to bake it at 660 ℃ for 6 hours and place it into the reagent bottle with ground stopper, which will be stored in the dryer.

3.2 Standard solutions

3.2.1. Standard solutions for time window determination: comprising homologues when different PCBs appear at the first and last peaks on the DB-5ms chromatographic column. Please see A.1 at Appendix A.

3.2.2. Internal standard solutions for quantification: Please see A.2 at Appendix A.

3.2.3. Internal standard solutions for recovery: Please see A.3 at Appendix A.

3.2.4. Standard solutions for correction: Please see A.4 at Appendix A.

3.2.5. Standard solutions for accuracy and preciseness: Please see A.5 at Appendix A.

4. Equipment and facilities

4.1 Gas chromatography-quadrupole mass spectrometer (GC-MS) or gas chromatography-ion trap tandem mass spectrometer (GC-MS/MS).

4.2 Chromatographic column: DB-5ms column,30 m×0.25 mm×0.25 μm, or equivalent chromatographic column

4.3 Tissue homogenizer

4.4 Meat grinder

4.5 Rotary evaporators

4.6 Nitrogen concentrator

4.7 Supersonic cleaner

4.8 Oscillator

4.9 Analytical balance whose sensitivity is 0.1 g

4.10 Preparation for glassware instruments: all re-usable glassware shall be thoroughly rinsed after use in the following procedure.

a) To rinse the instrument with the last solvent;

b) To rinse it with normal hexane and acetone;

c) To rinse it with warm water that contains alkaline detergents;

d) To rinse it with hot water and deionized water

e) To rinse it with acetone, normal hexane and dichloromethane.

The combination of supersonic cleaners and warm water that contains alkaline detergents produces very good cleaning effects. Be careful not to damage the internal surface of the glassware instrument when cleansing it with a brush.

5. Analytical procedures

5.1 Specimen preparation

5.1.1. Pre-treatment

5.1.1.1 Samples collected on site shall be packaged with light-proof materials such as aluminum foil and brown glass bottles before being delivered to the lab in a small-sized refrigerator, where they are kept at -10℃ or lower.

5.1.1.2 Solid samples such as fish and meat can be freeze-dried or dried with anhydrous Na2SO4 and evenly mixed. Oil or fat can directly dissolve in the normal hexane for purification.

5.1.2. Extraction

5.1.2.1 Before extraction, we shall put an empty cellulose or fiberglass socket into the Soxhlet extractor and pre-extract it for 8 hours with normal hexane + dichloromethane (50+50) as the extraction solvent before drying.

5.1.2.2 We shall put 5.0 to 10.0 g of pre-treated samples into the above-mentioned extraction socket in 5.1.2.1 and add $^{13}C_{12}$-marked quantitative interior standard (3.2.2). Then we shall cover the samples with glass wool which will be put into the Soxhlet extractor after a 30-minute balance. We shall extract it for 18 to 24 hours with normal hexane + dichloromethane (50+50) as the extraction solvent while keeping the back-flow velocity at 3 to 4 times per hour

5.1.2.3 After the extraction, we shall transfer the extracting solution into the eggplant-shaped bottle while evaporating it in a rotatory way until it is almost dry. Where the analysis result must be calculated in terms of fat, fat content is needed to test the samples.

5.1.2.4 Determination of fat content: We shall determine the exact weight of the eggplant-shaped bottle before concentration and dry the solvent before weighing the bottle. The difference value of the two results shall be the fat content of samples. After the determination, we shall add a small amount of normal hexane to dissolve the residue in the bottle.

5.1.3. Purification

5.1.3.1 Purification with acid silica gel column

Decontaminating column filling: We shall fill in 4 g of activated silica gel, 10 g of acid silica gel, 2 g of activated silica gel and 4 g of anhydrous Na_2SO_4 in sequence from bottom to top after blocking the glass column end with glass wool (see the Fig D.1 in Appendix D). Then we shall pre-wash it with 100 ml of normal hexane.

Purification: We shall transfer all the condensed extracting solution to the column and rinse the eggplant-shaped bottle with about 5 mL of normal hexane for 3 or 4 times before transferring the washer liquid to the column.

When the liquid level lowers to the anhydrous Na_2SO_4 layer, we shall add 180 mL of normal hexane for elution and condense the eluent to about 1 mL. When the acidated silica gel layers are all discolored, it means that the fat content in the samples has gone beyond the column load limit. After the condensation of eluent, we shall produce a new acid silica gel purification column and repeat the above procedures until the sulfuric acid silica gel doesn't get discolored.

5.1.3.2 Purification with composite silica gel column

Decontaminating column filling: We shall fill in 1.5 g of silver nitrate impregnated silica gel, 1 g of activated silica gel, 2 g of alkaline silica gel, 1 g of activated silica gel, 4 g of acidated silica gel, 2 g of activated silica gel and 2 g of anhydrous Na_2SO_4 in sequence from bottom to top after blocking the glass column end with glass wool (see the Fig D.1 in Appendix D). Then we shall pre-wash it with 30 mL of normal hexane and dichloromethane (97＋3).

Purification: We shall transfer all condensed eluent that is purified in 5.1.3.1 to the column and rinse the eggplant-shaped bottle with about 5 mL of normal hexane for 3 or 4 times before transferring the washer liquid to the column. When the liquid level lowers to the anhydrous Na_2SO_4 layer, we shall add 50 ml of normal hexane and dichloromethane (97＋3) for elution and condense the eluent to about 1 mL.

5.1.3.3 Purification with alkaline aluminum oxide column

Decontaminating column filling: We shall fill in 2.5 g of baked alkaline aluminum oxide and 2 g of anhydrous Na_2SO_4 in sequence from bottom to top after blocking the glass column end with glass wool (see the Fig D.1 in Appendix D). Then we shall pre-wash it with 15 mL of normal hexane.

Purification: We shall transfer all condensed eluent that is purified in 5.1.3.2 to the column and rinse the eggplant-shaped bottle with about 5 mL of normal hexane for 3 or 4 times before transferring the washer liquid to the column. When the liquid level lowers to the anhydrous Na_2SO_4 layer, we shall add 30 mL of normal hexane (2×15 mL)for elution. Then when the liquid level lowers to the anhydrous Na_2SO_4 layer, we shall add 25 mL of dichloromethane and normal hexane(5＋95), and condense the eluent until it's nearly dry.

5.1.4. Treatment before analysis on the equipment

We shall transfer the purified sample solution into the sample introduction pipe and condense the nitrogen

when it flows down. Then we shall rinse the eggplant-shaped bottle with a small amount of normal hexane for three to four times and transfer the washing liquid into the sample introduction insert-pipe before condensing the nitrogen to about 50 µl. Then we shall add an appropriate amount of internal standard of recovery (3.2.3) and cap it before analysis on the equipment.

5.2 Reference conditions for equipment

5.2.1. Chromatographic condition

5.2.1.1 Chromatographic column: To carry out chromatographic separation with the 30-m DB-5ms (or other type which is equal to DB-5ms) quartz capillary column. The film thickness shall be 0.25 µm and the inner diameter 0.25 mm.

5.2.1.2 The temperature at the injection port shall be 300 ℃when using splitless injection.

5.2.1.3 The temperature of the chromatographic column rises in the following way: the initial temperature stays at 100 ℃for 2 min; then to 180℃at 15℃/min; then to 240℃at 3℃/min. Finally to 285 ℃at 10 ℃/min and stay for 10 minutes.

5.2.1.4 High-purity helium (purity>99.999%) shall be used as the carrier gas.

5.2.2. Mass spectrometric parameters

5.2.2.1 Quadrupole mass spectrometer

Ionization mode: Electron Impact Ion Source (EI); 70 eV. Ion detection mode: selected ion monitoring (SIM); The characteristic ion that is selected when detecting the PCBs is a molecular ion. See Table B.1 at Appendix B.

Ion source temperature shall be 250 ℃Transmission line temperature shall be 280 ℃Solvent delay will be 10 minutes.

5.2.2.2 Ion trap mass spectrometer

Ionization mode: Electron Impact Ion Source;70 eV. Ion detection mode: multiple- reaction monitoring(MRM);The parent ion that is selected when detecting the PCBs is a molecular ion (M+2 or M+4) and the daughter ion is a fragment ion(M-2Cl)that is formed when a molecular ion loses two helium atoms. See the Table B.2 at Appendix B. The ion trap temperature shall be 220℃the transmission line temperature shall be 280℃the manifold temperature shall be 40 ℃

5.3 Sensitivity examination

To inject 1 µL(20 pg)of CS1 solution and detect the GC-MS sensitivity. Detection ion SNR (Signal to Noise Ratio) of all 3-to-7-cl-substitution compounds shall be up to above 3, or the equipment shall be tuned again until it meets the requirement.

5.4 Qualification quantification of PCBs

5.4.1 Confirmation requirement for PCBs' chromatographic peak: The SNR of detected chromatographic peak shall be above 3 (See Fig C.1 or C.3 at Appendix C)

5.4.2 The abundance ratio of the two detected characteristic ion shall be within the theoretical range. Please see Table B.1 and B.2 at Appendix B.

5.4.3 To detect mass spectrum corresponding with the chromatographic peak (See Fig C.2 or C.4 at Appendix C). When the concentration is high enough, there shall be a fragment ion which loses two helium atoms (M-70). See Fig B.1 at Appendix B.

5.4.4 To detect the mass spectrum this corresponds with the chromatographic peak (See Fig C.2 or C.4 at Appendix C). There shall not be any fragment ion with molecular ions and two helium atoms (M+70)See Fig B.1 at Appendix B.

5.4.5 The retention time of determined PCBs shall be located at the time window which determines the standard solution via the analysis window. Time window determination standard solution comprises homologues when different PCBs appear at the first and last peaks on the DB-5ms chromatographic column. The time window shall be determined according to the retention period where different PCBs are by making analysis on window determination solution (1 μL) with the determined chromatographic condition and full-scan mass spectrum acquisition mode. Due to the overlap of retention periods of PCBs from three families in the DB-5ms chromatographic column, characteristic ions of these PCBs shall be detected in a single time window. To ensure the selectivity and sensitivity of the analysis, the detected characteristic ions at one window when determining the time window shall be as little as possible.

5.5 Statement of analysis result

5.5.1 During the standard, PCB28, PCB52, PCB118, PCB153, PCB180, PCB206 and PCB209 are quantified with the isotope dilution technique. Other target compounds shall be quantified with the internal standard method and the recovery calculation of internal standard for quantification shall be done with the internal standard method. The 20 target compounds determined in the standard include most PCBs' industrial products. There are 3 compounds in every family from PCB3 to PCB8. There is one in PCB9 or PCB10. See the Table A.4 in Appendix A. One 13C12-marked compound is used as internal standard for quantification in every family. See the Table A.2 in Appendix A. There are two internal standards of recovery to calculate the quantified internal standard recovery. See the Table A.3 in Appendix A.When calculating the quantified internal standard recovery, $^{13}C_{12}$-PCB101 shall be used as the internal standards of recovery for $^{13}C_{12}$-PCB28, $^{13}C_{12}$-PCB52, $^{13}C_{12}$-1PCB18 and $1^{13}C_{12}$-PCB153 and $^{13}C_{12}$-PCB194 shall be used as the internal standards of recovery for $^{13}C_{12}$-PCB180, $^{13}C_{12}$-PCB202, $^{13}C_{12}$-PCB206 and $^{13}C_{12}$-PCB209.

5.5.2 Relative Response Factor (RRF):In this standard, RRF is used for quantitative calculation. The RRF value is calculated with calibration standard solution. See the computational formulas at (1) and (2).

$$RRF_n = \frac{A_n \times c_s}{A_s \times c_n} \quad \cdots\cdots (1)$$

$$RRF_r = \frac{A_r \times c_s}{A_s \times c_r} \quad \cdots\cdots (2)$$

In the formula,

RRF_n— the relative response factor of target compounds to internal standard for quantification;

A_n—peak area of the target compounds;

C_s—concentration of internal standard for quantification (μg/L);

A_s—peak area of internal standard for quantification;

C_n—concentration of target compounds (μg/L);

RRF_r—the relative response factor of internal standard for quantification to internal standard for recovery;

A_r—peak area of the internal standard for recovery;

C_r—solutions of internal standard for recovery (μg/L);

The RRF values of the five thickness levels for different compounds shall have the relative standard deviation (RSD) of less than 20%. When the standard is met, we shall use average RRFn and average RRFr for quantitative calculation.

5.5.3 Content calculation: see (3) for calculation formula of PCBs content in samples

$$c_n = \frac{A_n \times m_s}{A_s \times RRF_n \times m} \quad (3)$$

In the formula,

C_n—PCBs content in the samples (μg/kg)

A_n—peak area of target compounds;

m_s—amount of internal standard for quantification into the samples(ng);

A_s—peak area of internal standard for quantification;

RRF_n—the relative response factor of target compounds to internal standard for quantification;

m—sampling weight(g)

5.5.4 Calculation of quantified internal standard of recovery: quantified internal standard of recovery(R) is calculated according to (4) and the quantitative value is symbolized with %.

$$R = \frac{A_r \times m_r}{A_r \times RRF_r \times m_s} \times 100\% \quad (4)$$

In the formula,

R—quantified internal standard for recovery, %;

A_s—peak area of quantified internal standard;

m_r—quantity of internal standard for recovery in the samples (ng)；

A_r—peak area of internal standard for recovery;

RRF_r—the relative response factor of quantified internal standard to internal standard for recovery;

m_s—quantity of internal standard for quantification in the samples,(ng).

Quantitative Results can have two digits after the decimal point.

5.5.5 Limit of detection: it is specified by this standard that the sample detection limit constitutes the sample solution concentration which corresponds with the response that meets the requirement of isotope abundance ratio when the SNR (Signal to Noise Ratio) is 3. See (5) for the computational formula of detection limit.

$$DL = \frac{3 \times N \times m_s}{H \times RRF_n \times m} \quad \text{(5)}$$

In the formula:

DL—limit of detection (μg/kg)

N— peak height of the noise

m_s—amount when quantified internal standard is added (ng)

H—peak height of quantified internal standard

RRF_n—the relative response factor of target compounds to internal standard for quantification;

m—sample volume,(g).

The sample base material, sampling volume, injected sample size, quantified internal standard of recovery, chromatographic separation, electrical noises and equipment sensitivity all may affect the sample detection limit, so the noise shall be acquired from the real sample spectrogram. The sample detection limit shall be reported when the outcome of target compounds hasn't come out yet.

6. Quality control and quality guarantee

6.1 Initial precision and accuracy test

The lab shall have the acceptable accuracy and preciseness before analyzing the real samples. Reliability of the analytical methods shall be verified by analyzing marked samples.

We shall use 3 or more blank samples whose base materials are similar to the real samples, in which we respectively add normal experiment solution with the right accuracy and preciseness. See Table A.5 for Appendix A. Then we shall respectively add standard solution of internal standard for quantification before analyzing the formulated marked samples in the same way as the real samples, and calculate the recovery of target compounds and the quantified internal standard of recovery. The estimated value of target PCBs for every sample shall range from 75% to 120% of the addition quantity. RSD<30%. The average of recovery for quantified internal standard shall range from 50% to 120%. And the quantified internal standard of recovery for single samples shall range from 30% to 130%.

Before being analyzed, real samples shall meet the above requirements. When the way to extract and purify the samples has been modified or the analyzer has been replaced, such experiments shall be repeated until the above requirements are met. The above experiments shall be done in the lab every 6 months to ensure that the above requirements are met.

If we can have standard reference materials which have base materials similar to samples, then we can apply normal reference materials to the accuracy and preciseness experiment instead of marked samples.

6.2 Quantified internal standard of recovery

Quantified internal standard shall be added to rectify the loss of target compounds during the extraction and purification of the samples. Quantified internal standard of recovery shall range from 30% to 130%. If the sample analysis outcome for quantified internal standard of recovery hasn't met the above requirement, samples shall be extracted, purified and analyzed on the equipment again.

6.3 Method blank

A method blank experiment shall be done on every group which has at most 15 samples.

6.4 Quality control sample

There are at most 15 samples for every group with a quality control sample. Quality control samples shall be standard reference material or marked sample with known concentration. The estimated value of target compounds shall range from 75% to 125% of the standard values.

6.5 Retention time window

Time window shall be carried out every week to determine the analysis of standard solution and the correctness of retention time window. When the chromatographic column has been replaced or cut, or the chromatographic parameter has been changed, time window shall be used to determine the standard solution and calibrate the retention time window.

6.6 Calibration of standard solution

Calibration standard solutions with 5 thickness levels shall be used for initial calibration. Calibration is regarded to be successful when the RSD of RRF is less than 20%. During the analysis, confirmatory tests

shall be done every 12 hours. CS3 in the calibration standard solutions shall be used for equipment analysis. The analysis outcome shall be within the scope of 20% of its constant value. Quantified internal standard of recovery shall be within

75% to 125%.

7. Others

Quantification limit of every target compounds shall be 0.5 μg/kg.

Method II Gas chromatographic method

8. Principle

With this method, we add PCB198, the quantified internal standard, into the samples and heat it in the water bath before vibrating extraction. After the sulfuric acid treatment and purification of chromatographic column, we shall make the measurement with the gas chromatographic- electron capture detector method so as to ensure the time qualification and internal standard quantification.

9. Reagent and material

9.1 Reagent

9.1.1 C_6H1_4:Pesticide Analysis Grade

9.1.2 CH_2C_{l2}:Pesticide Analysis Grade

9.1.3 C_3H6O:Pesticide Analysis Grade

9.1.4 Anhydrous Na_2SO_4: Top grade pure. We shall put the commercially available anhydrous Na_2SO_4 into the glass chromatographic column and rinse it twice with normal hexane and dichloromethane. The volume of solvent used every time shall be approximately twice that of anhydrous Na_2SO_4 . After the rinse, we shall transfer the anhydrous Na_2SO_4 into the flask where it dries at 50 ℃before baking it at 225°C overnight and storing it in the dryer when it cools down.

9.1.5 H_2SO_4:top grade pure.

9.1.6 Alkaline aluminium oxide for chromatographic analysis: to bake the commercially available chromatographic packing materials at 660 ℃for 6 hours and place it into the dryer for storage.

9.2 Standard solution

Indicative PCB standard solutions. See Table A.6 in Appendix A

10. Equipment and facilities

10.1.1 Gas chromatograph: with the electron capture detector(ECD)

10.1.2 Chromatographic column: DB-5ms column,30 m×0.25 mm×0.25 μm,or equivalent chromatographic column

10.1.3 Tissue homogenizer

10.1.4 Meat grinder

10.1.5 Rotary evaporator

10.1.6 Nitrogen concentrator

10.1.7 Supersonic cleaner

10.1.8 Vortex oscillator

10.1.9 Analytical balance

10.1.10 water bath oscillator

10.1.11 Centrifuge

10.1.12 Chromatographic column

11. Analytical procedure

11.1 Sample extraction

11.1.1 Solid sample:Weigh 5 g to 10 g of samples (accurate to 0.1 g) and place it into the conical flask with cover. Then add some quantified PCB198 and place it on the water bath oscillator for 2 hours with an appropriate amount of normal hexane + dichloromethane (50+50) as the extract solution. The water bath temperature shall be at 40 ℃and the vibrating rate shall be at 200 r/min.

11.1.2 Liquid sample (exclusive of oil and fat sample): Weigh 10 of samples (accurate to 0.1 g) and place it into the conical flask with cover. Then add quantified internal standard PCB198 and 0.5 g of sodium oxalate to be evenly mixed with 10 mL of methyl alcohol. Next, add 20 mL of aether and n-hexane (25+75) to be oscillated for 20 minutes and centrifuged at 3,000 r/ min for 5 minutes. Then transfer the supernatant liquor to the glass column which has 5 g of anhydrous Na2SO4. Add 20 mL of aether and n-hexane (25+75) in the residue and repeat the above procedure. Finally combine the extracting solution.

11.1.3 Transfer the extracting solution to the eggplant-shaped bottle and rotate to evaporate it until it is nearly dry. Fat content of the sample shall be determined if the analysis result is calculated by fat.

11.1.4 Determination of the sample fat: Get the exact weight of the empty eggplant-shaped bottle before concentration. Then dry the solvent and weigh the bottle and the residue again. The difference value of the two results shall be the fat content of samples.

11.2 Purification

11.2.1 Sulfuric acid purification

Transfer the condensed extracting solution into the 10-mL test tube and rinse the eggplant-shaped bottle for 3 to 4 times with approximately 5 mL of normal hexane before combining it with the concentrated solution. Meter the volume to the scale with normal hexane and add 0.5 mL of concentrated sulfuric acid. Then shake it for 1 minute and centrifuge it at 3,000 r/min, thus separating the sulfuric acid layer from the organic layer. If the upper solution is still colored, that means the fat still remains. So add an appropriate amount of

concentrated sulfuric acid and repeat the operation until the upper solution becomes colorless.

11.2.2 Purification with alkaline aluminum oxide column

Decontaminating column filling: We shall add a small amount of glass wool at the glass column end and fill in 2.5 g of baked alkaline aluminium oxide and 2 g of anhydrous Na2SO4 in sequence before rinsing it with 15 mL of normal hexane.

Purification: We shall transfer concentrated solution at 11.2.1 and rinse the eggplant-shaped bottle for 3 to 4 times with 5 mL of normal hexane before transferring it to the chromatographic column. When the liquid level lowers to the anhydrous Na2SO4 layer, we shall add 30 mL of normal hexane (2×15 mL) for elution. Then when the liquid level lowers to the anhydrous Na2SO4 layer,we shall add 25 mL of dichloromethane and normal hexane(5＋95) for elution, and condense the eluent until it's nearly dry.

11.3 Sample solution concentration

Transfer the sample solution in 11.2.2 to the sample introduction bottle and rinse the eggplant-shaped bottle with a small amount of normal hexane for 3 to 4 times. Then add the cleansing solution into the sample introduction bottle. Finally condense it to 1 mL under the nitrogen flow for GC analysis.

12. Determination

12.1 Chromatographic condition

12.1.1 Chromatographic column: DB-5ms column,30 m×0.25 mm×0.25 μm,or equivalent chromatographic column

12.1.2 Temperature at the injection port:290 ℃

12.1.3 Temperature raising procedure: starting temperature at 90 ℃,remaining for 0.5 minutes; to 200 ℃ at 15 ℃/min for 5 minutes; to 250°C at 2.5 ℃/min for 2 minutes; then to 265°C at 20℃/min for 5 minutes

12.1.4 Carrier gas: high-purity nitrogen (nitrogen>99.999%);column pressure is 67 kPa(10 psi)

12.1.5 Injection Volume: 1 μL of splitless sample introduction

12.1.6 Chromatographic analysis: to keep the qualitative time and compare the quantity with the peak height or peak area of samples and standard.

12.2 Qualitative analysis of PCBs

To make qualitative analysis with retention time or relative retention time. SNR(Signal to Noise Ratio) for the chromatographic peak of the detected PCBs shall be more than 3.

12.3 Quantitative determination of PCBs

12.3.1 Relative Response Factor(RRF)

To make quantitative calculation with RRF by using the internal standard method. To calculate RRF value according to (6)by using the calibrating standard solution sample introduction:

$$RRF = \frac{A_n \times c_s}{A_s \times c_n} \quad (6)$$

In the formula,

RRF—Relative response Factor of target compounds to internal standard of quantification;

A_n—Peak area of target compounds;

C_s—Concentration of internal standard of quantification(μg/L)；

A_s—Peak area of internal standard of quantification;

C_n—Concentration of target compounds (μg/L).

In the standard solutions, RSD (relative standard deviation) of the RRF value of all target compounds shall be less than 20%.

12.3.2 Content calculation

To calculate the content of PCBs in samples according to (7):

$$X_n = \frac{A_n \times m_s}{A_s \times RRF \times m} \quad (7)$$

In the formula,

X_n—content of target compounds(μg/kg)；

A_n— peak area of target compounds；

m_s—amount of quantified internal standard added to the sample(ng)；

A_s—peak area of quantified internal standard;

RRF—relative response Factor of target compounds to quantified internal standard;

m—sampling weight(g).

12.3.3 Limit of detection

It is specified by this standard that the sample detection limit constitutes the sample solution concentration which corresponds with the response that meets the requirement of relative retention time with triple SNR.See the computational formula at (8):

$$DL = \frac{3 \times N \times m_s}{H \times RRF \times m} \quad (8)$$

In the formula:

DL—limit of detection (μg/kg)

N— peak height of the noise

m_s—amount when quantified internal standard is added (ng)

H—peak height of quantified internal standard

RRF—the relative response factor of target compounds to internal standard for quantification;

m—sample volume,(g).

The sample base material, sampling volume, injected sample size, chromatographic separation, electrical noises and equipment sensitivity all may affect the sample detection limit, so the noise shall be acquired from the real sample spectrogram. The sample detection limit shall be reported when the outcome of target compounds hasn't come out yet.

13. Accuracy

Absolute differences of the two individual analysis results acquired under repetitive conditions shall be 20% of the arithmetic mean value or less.

14. Others

Quantification limit of every target compounds shall be 0.5 μg/kg.

Appendix A:

Standard solution for indicative PCB

Standard solutions used for time window determination, quantified internal standard, internal standard of recovery, standard series, accuracy and preciseness to determine the PCB content in food are specified from Table A.1 to A.6.

Table A.1 Standard solution to determine the time window of indicative PCBs tested with the GC-MS method

Compounds	Chlorine atom amount	Concentration mg/L
Biphenyl	0	2.5±0.25
PCB1	1	2.5±0.25
PCB3	1	2.5±0.25
PCB10	2	2.5±0.25
PCB15	2	2.5±0.25
PCB30	3	2.5±0.25
PCB37	3	2.5±0.25
PCB54	4	2.5±0.25
PCB77	4	2.5±0.25
PCB104	5	2.5±0.25
PCB126	5	2.5±0.25
PCB155	6	2.5±0.25
PCB169	6	2.5±0.25
PCB188	7	2.5±0.25
PCB189	7	2.5±0.25
PCB194	8	2.5±0.25
PCB202	8	2.5±0.25
PCB206	9	2.5±0.25
PCB208	9	2.5±0.25
PCB209	10	2.5±0.25

Table A.2 Standard solution for the quantified internal standard of indicative PCBs in the GC-MS method

Compounds	Chlorine atom amount	Concentration mg/L
$^{13}C_{12}$-PCB28	3	2.0
$^{13}C_{12}$-PCB52	4	2.0
$^{13}C_{12}$-PCB118	5	2.0
$^{13}C_{12}$-PCB153	6	2.0
$^{13}C_{12}$-PCB180	7	2.0
$^{13}C_{12}$-PCB202	8	2.0
$^{13}C_{12}$-PCB206	9	2.0
$^{13}C_{12}$-PCB209	10	2.0

Table A.3 Standard solution for the internal standard of recovery of indicative PCBs in the GC-MS method

Compounds	Chlorine atom amount	Concentration mg/L
$^{13}C_{12}$-PCB101	5	2.0
$^{13}C_{12}$-PCB194	8	2.0

Table A.4 Standard solution for indicative PCBs in the GC-MS method

Target compounds		Concentration μg/L				
		CS1	CS2	CS3	CS4	CS5
Natural compounds	PCB18	20	50	200	800	2000
	PCB28	20	50	200	800	2000
	PCB33	20	50	200	800	2000
	PCB52	20	50	200	800	2000
	PCB44	20	50	200	800	2000
	PCB70	20	50	200	800	2000
	PCB101	20	50	200	800	2000
	PCB118	20	50	200	800	2000
	PCB105	20	50	200	800	2000
	PCB153	20	50	200	800	2000
	PCB138	20	50	200	800	2000
	PCB128	20	50	200	800	2000
	PCB187	20	50	200	800	2000
	PCB180	20	50	200	800	2000
	PCB170	20	50	200	800	2000
	PCB199	20	50	200	800	2000
	PCB195	20	50	200	800	2000
	PCB194	20	50	200	800	2000
	PCB206	20	50	200	800	2000
	PCB209	20	50	200	800	2000
Quantified internal standard of isotope labeling	$^{13}C_{12}$-PCB180	400	400	400	400	400
	$^{13}C_{12}$-PCB202	400	400	400	400	400
	$^{13}C_{12}$-PCB206	400	400	400	400	400
	$^{13}C_{12}$-PCB209	400	400	400	400	400
	$^{13}C_{12}$-PCB28	400	400	400	400	400
	$^{13}C_{12}$-PCB52	400	400	400	400	400
	$^{13}C_{12}$-PCB118	400	400	400	400	400
	$^{13}C_{12}$-PCB153	400	400	400	400	400
Internal standard for recovery of isotope labeling	$^{13}C_{12}$-PCB101	400	400	400	400	400
	$^{13}C_{12}$-PCB194	400	400	400	400	400

Table A.5 Standard solution for indicative PCBs accuracy and preciseness experiment in the GC-MS method

Compounds	Concentration µg/L	Compounds	Concentration µg/L
PCB18	100	PCB138	100
PCB28	100	PCB128	100
PCB33	100	PCB187	100
PCB52	100	PCB180	100
PCB44	100	PCB170	100
PCB70	100	PCB199	100
PCB101	100	PCB195	100
PCB118	100	PCB194	100
PCB105	100	PCB206	100
PCB153	100	PCB209	100

Table A.6 Standard solution for indicative PCBs in the GC-ECD method

Compounds	Concentration µg/L				
	CS1	CS2	CS3	CS4	CS5
PCB28	5	20	50	200	800
PCB52	5	20	50	200	800
PCB101	5	20	50	200	800
PCB118	5	20	50	200	800
PCB138	5	20	50	200	800
PCB153	5	20	50	200	800
PCB180	5	20	50	200	800
PCB198(Quantified internal standard)	50	50	50	50	50

Appendix B

Characteristic ions and isotopic abundance ratio

Requirements to determine the characteristic ions and isotopic abundance ratio of indicative PCBs in food with the quadrupole mass spectrometer and ion trap mass spectrometer has been specified in Table B.1 and B.2.

Table B.1 Characteristic ions and isotopic abundance ratio of selected ion monitoring (SIM) of the quadrupole mass spectrometer

Homologues	Characteristic ions(m/z)	Ion type	Theoretical abundance	Determining ions
T_3CB	256/258	M/M+2	1.03	[a] [b]
T_4CB	290/292	M/M+2	0.78	[a] [b]
P_5CB	324/326	M/M+2	0.62	[a] [b]
H_6CB	358/360	M/M+2	0.52	[a] [b]
H_7CB	394/396	M+2/M+4	1.04	[a] [b]
O_8CB	428/430	M+2/M+4	0.89	[a] [a]
N_9CB	462/464	M+2/M+4	0.78	[a]
$D_{10}CB$	498/500	M+4/M+6	1.17	[a]
^{13}	270	M+2	—	—
^{13}	304	M+2	—	—
^{13}	338	M+2	—	—
^{13}	372	M+2	—	—
^{13}	406	M+2	—	—
^{13}	442	M+4	—	—
^{13}	476	M+4	—	—
^{13}	510	M+4	—	—

[a] Fragment ions that exist

[b] Fragment ions that can't exist

[c] These ions are fragment ions(M+35)with one molecular ion and a chlorine. The existence of these ions shows that disturbance of PCBs from adjacent families may occur.

Table B.2 Characteristic ions and isotopic abundance ratio of Multiple Reaction Monitoring (MRM) of the ion trap tandem mass spectrometer

Homologues	Parent ion (m/z)	Daughter ion	Theoretical abundance
T_3CB	258	186/188	2.00
T_4CB	292	220/222	1.00
P_5CB	326	254/256	0.67
H_6CB	360	288/290	0.50
H_7CB	396	324/326	1.00
O_8CB	430	358/360	0.80
N_9CB	464	392/394	0.67
$D_{10}CB$	498	426/428	0.55
$^{13}C_{12}$-T_3CB	270	198/200	2.00
$^{13}C_{12}$-T_4CB	304	232/234	1.00
$^{13}C_{12}$-P_5CB	338	266/268	0.67
$^{13}C_{12}$-H_6CB	372	300/302	0.50
$^{13}C_{12}$-H_7CB	408	336/338	1.00
$^{13}C_{12}$-O_8CB	442	370/372	0.80
$^{13}C_{12}$-N_9CB	476	404/406	0.67
$^{13}C_{12}$-$D_{10}CB$	510	438/440	0.55

Appendix C

Mass chromatogram and mass spectrum of PCBs determined with GC-MS

Fig C.1 and C.2 are the SIM chromatogram and mass spectrum to determine PCBs with the quadrupole mass spectrometer. Fig C.3 and C.4 are the MRM chromatogram and mass spectrum to determine the PCBs with the ion trap mass spectrometer.

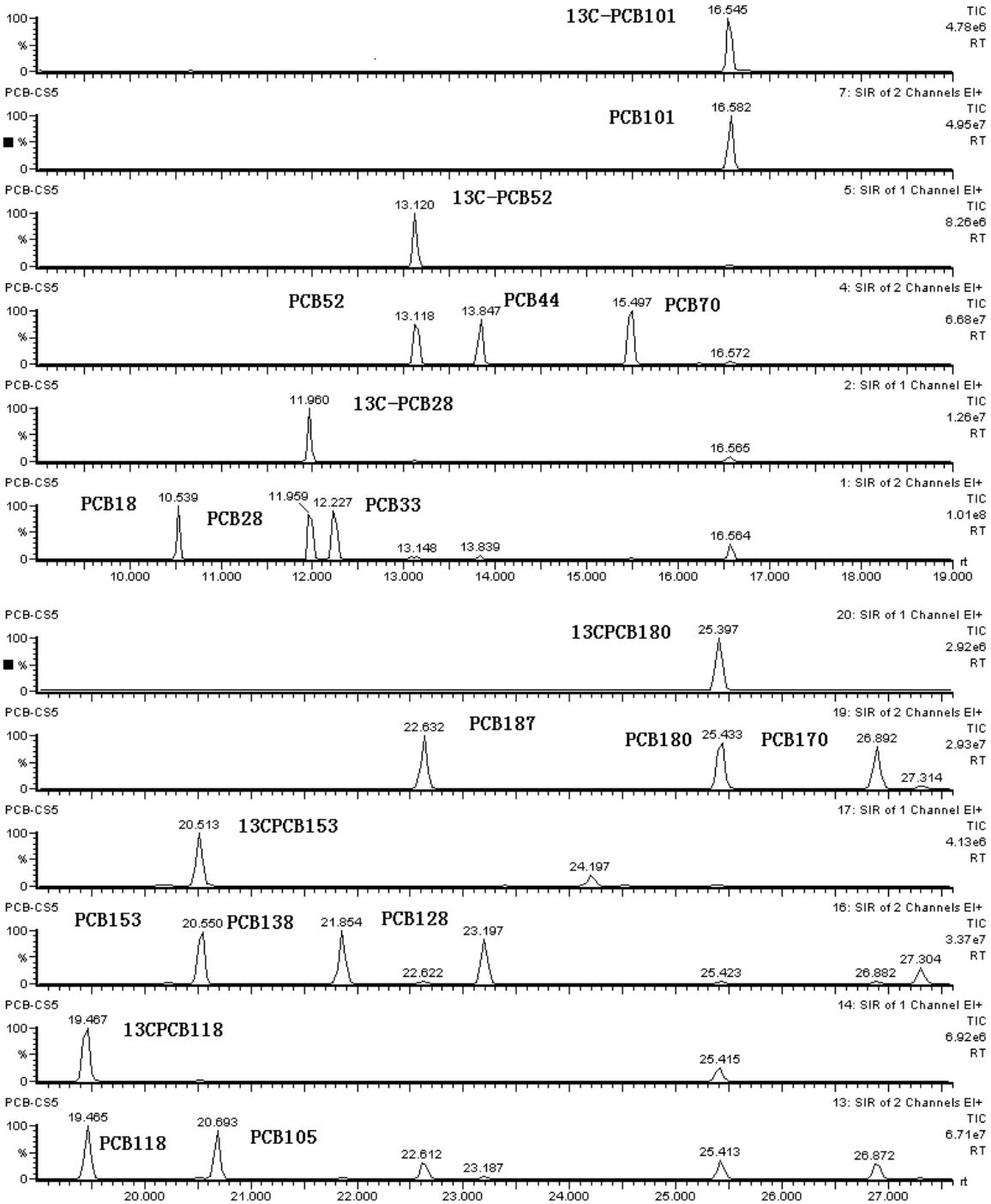
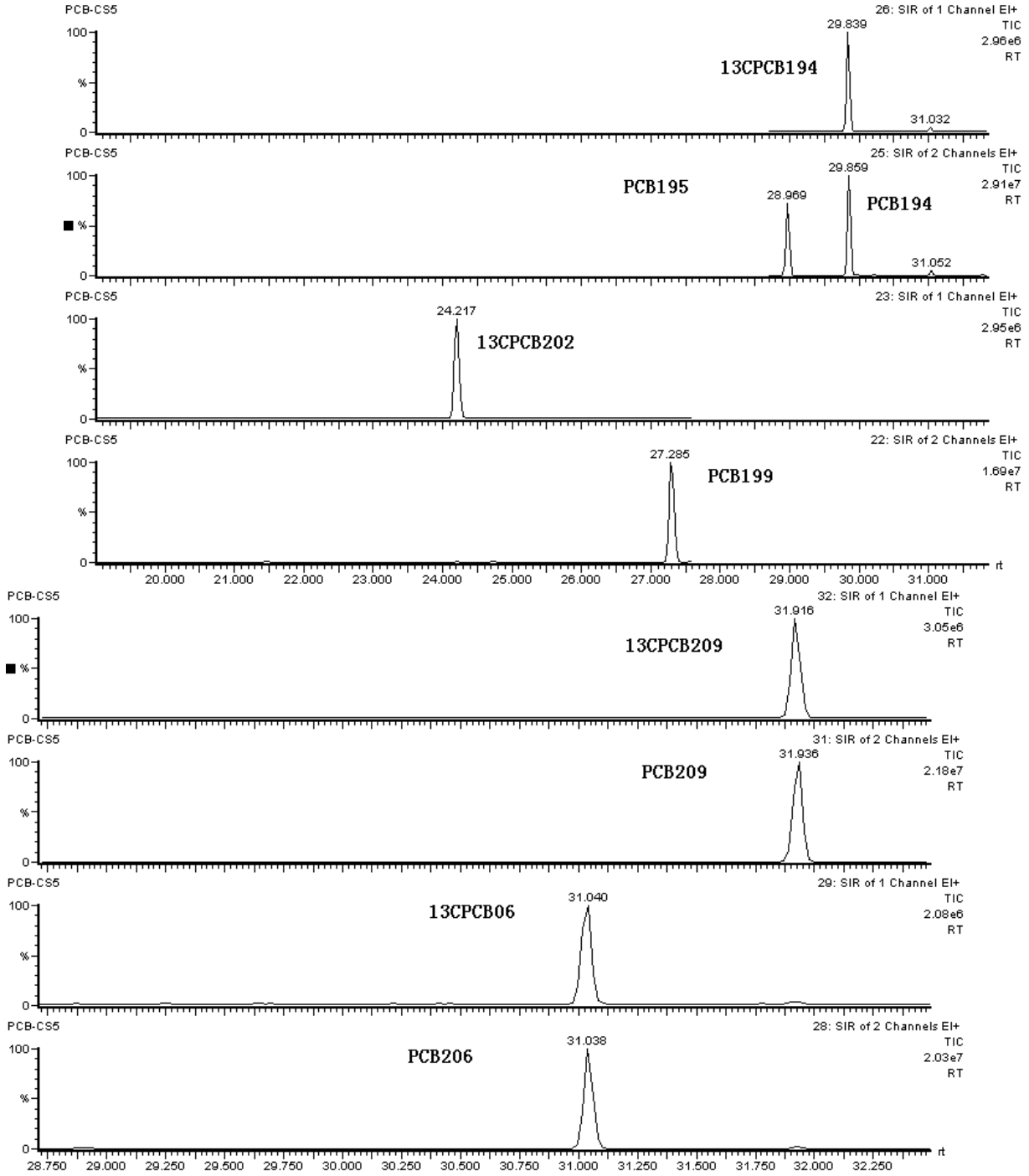


Fig C.1 Mass chromatogram of characteristic ions of target PCBs with SIM



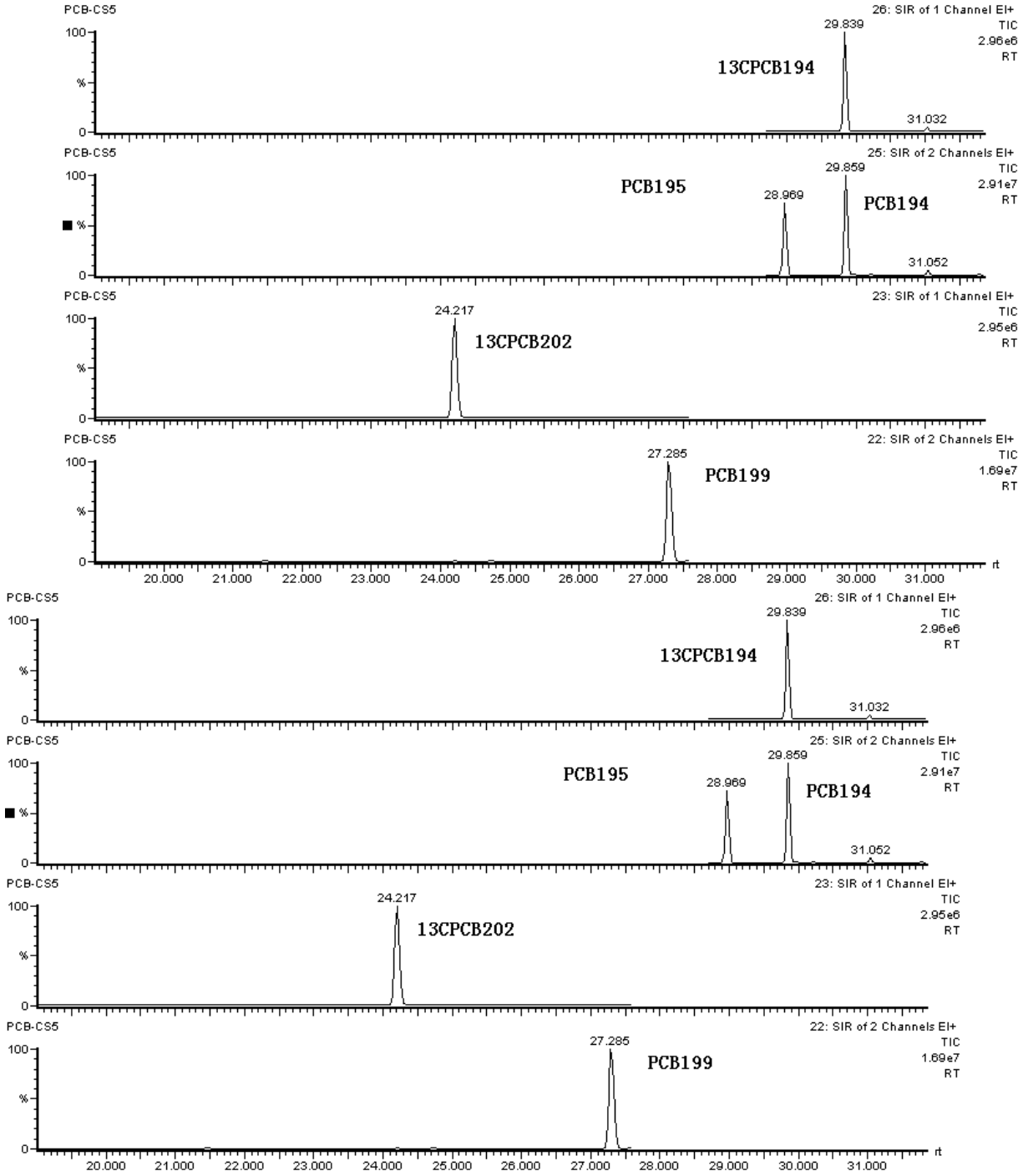


Fig C.1 (to be continued)

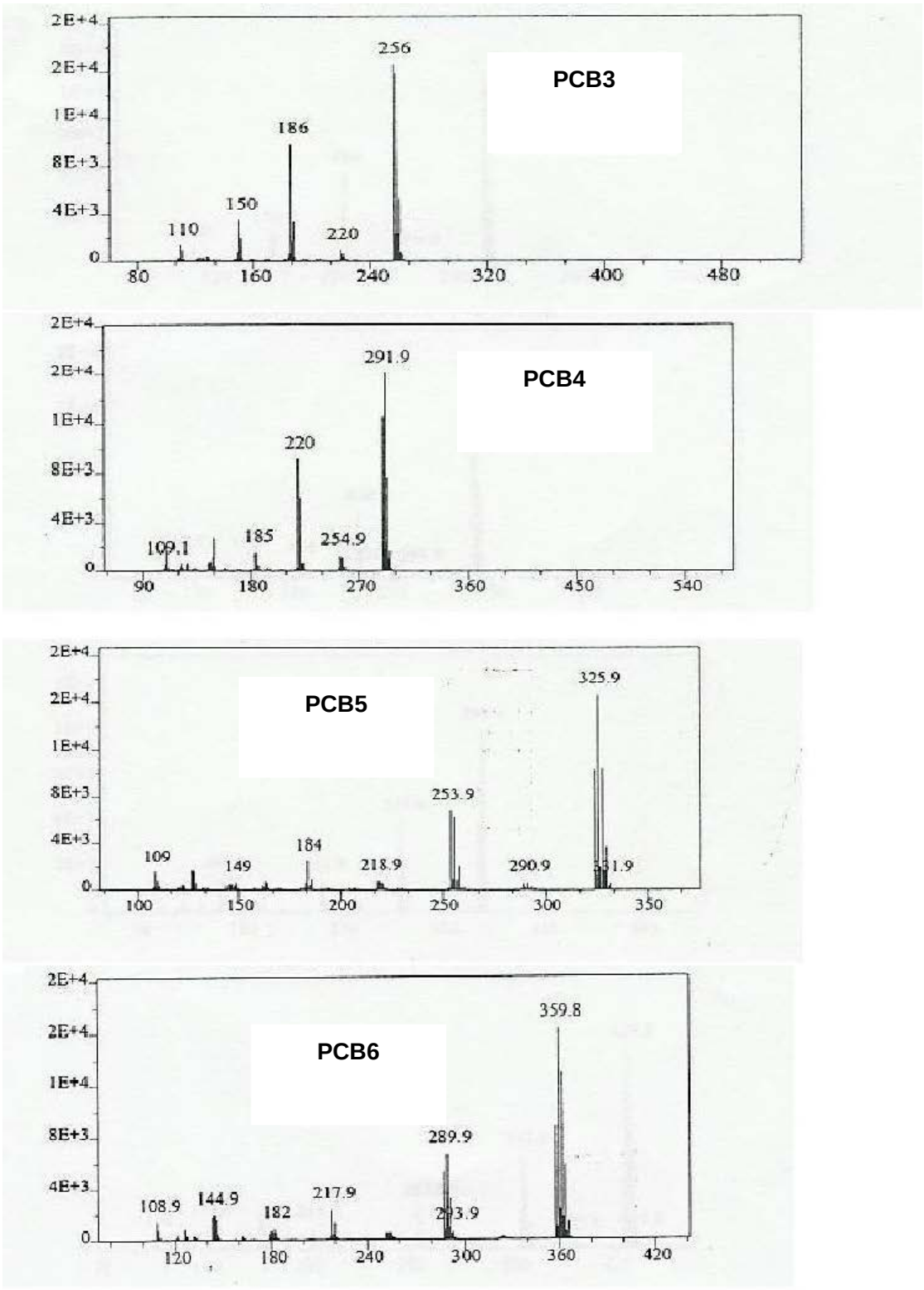


Fig C.2 Mass spectrum of characteristic ions of different PCBs with SIM

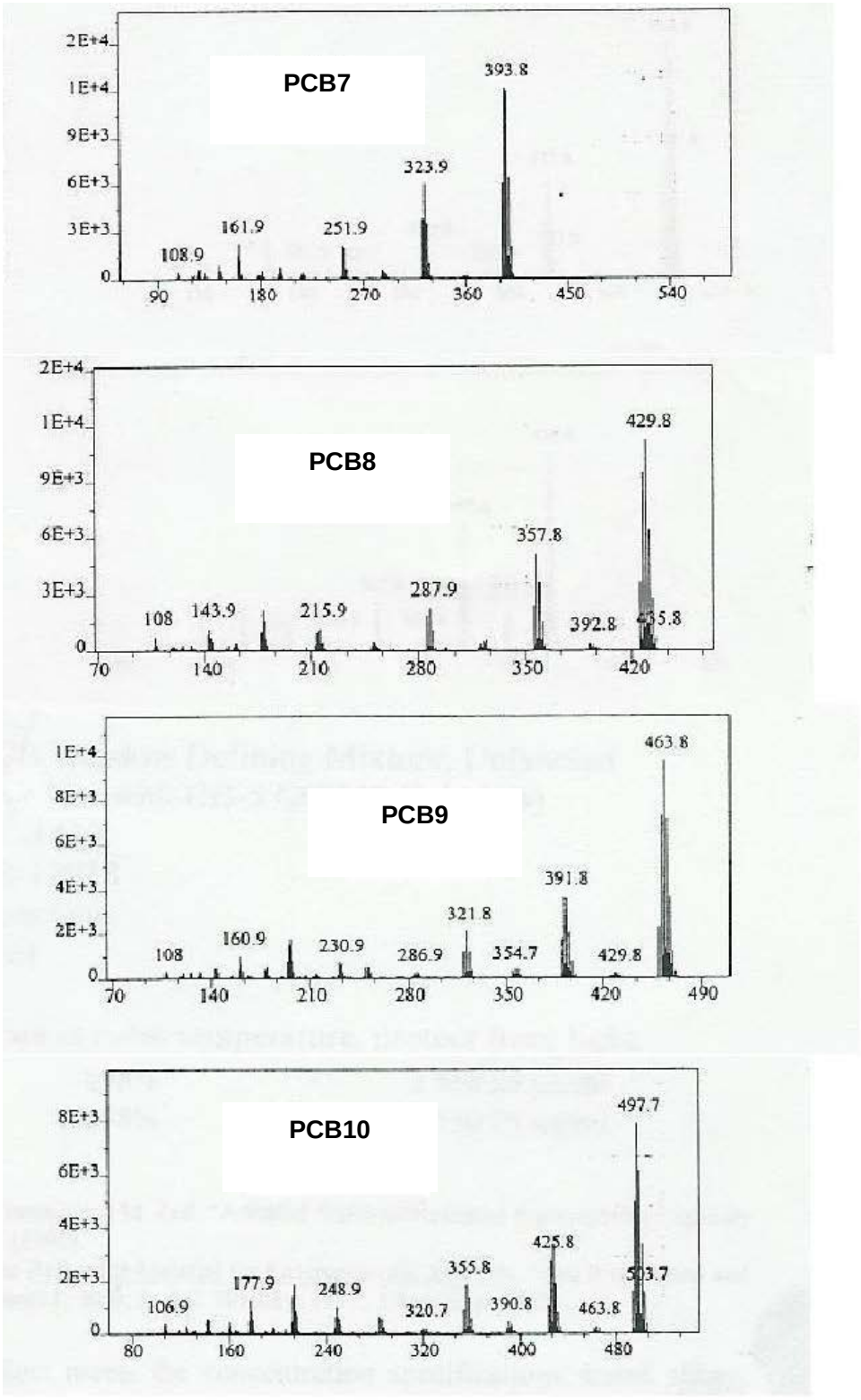


Fig C.2 (to be continued)

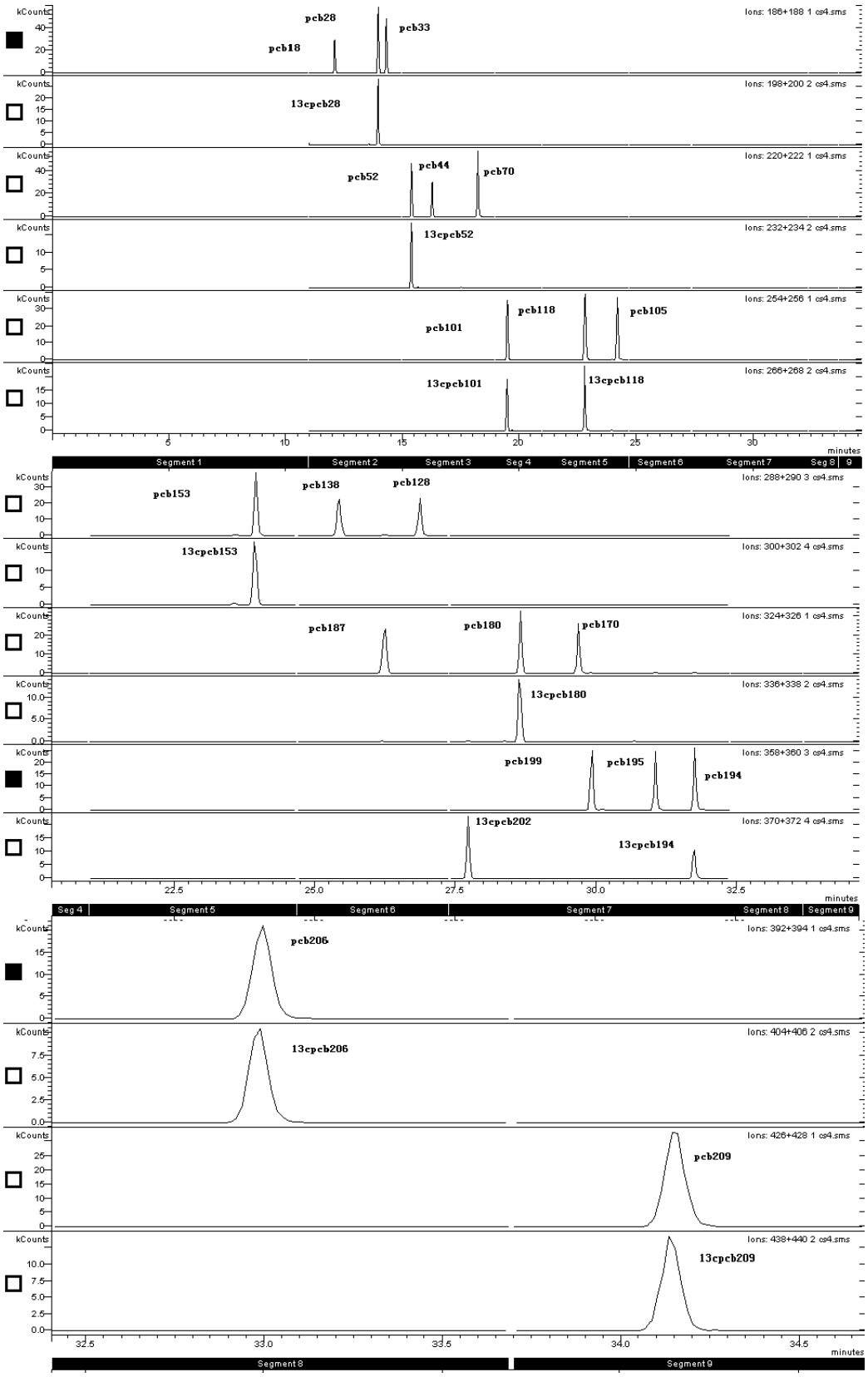


Fig C.3 Recombination ion chromatogram of different target PCBs daughter ions with MRM

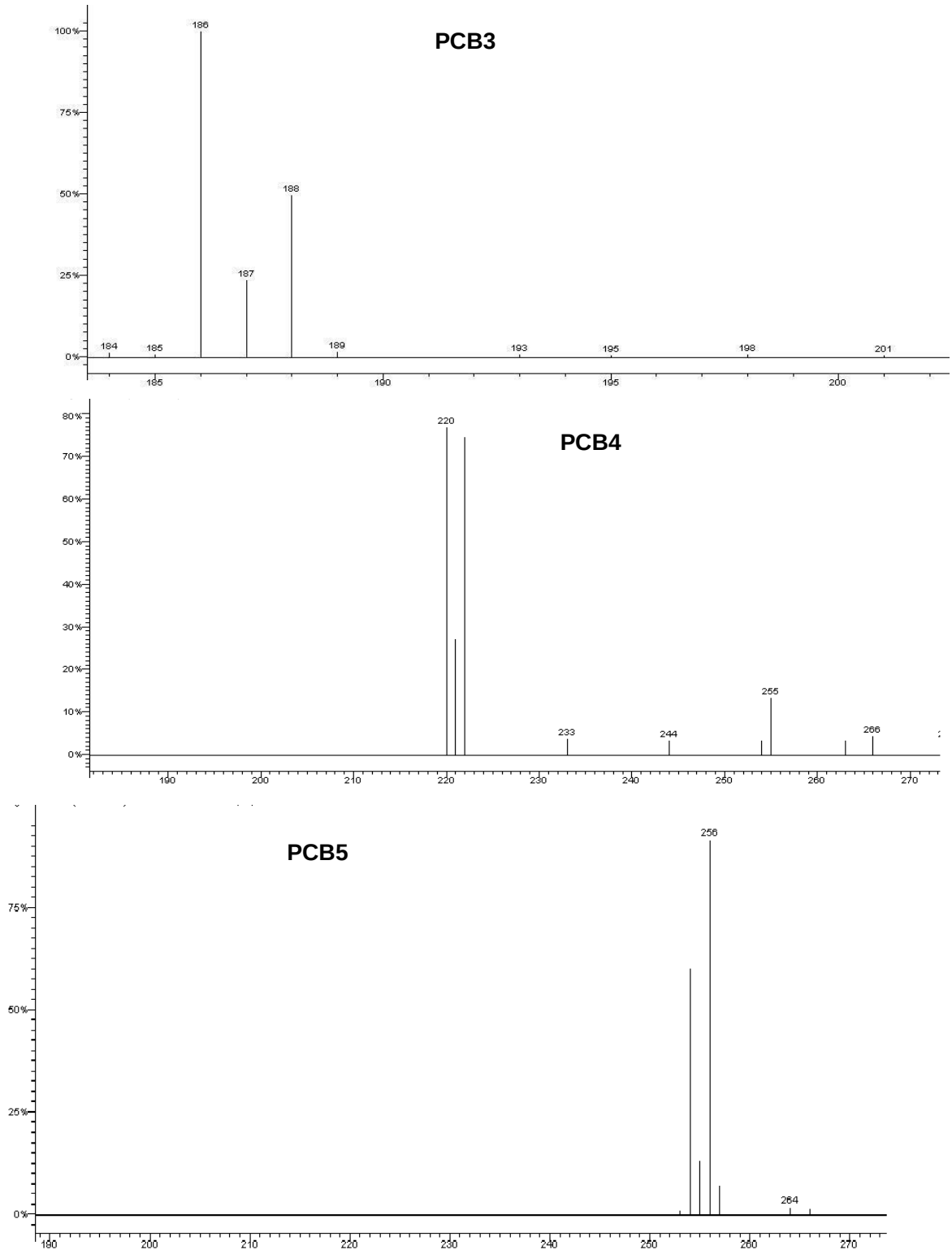


Fig C.4 Mass spectrum of different target PCBs daughter ions with MRM

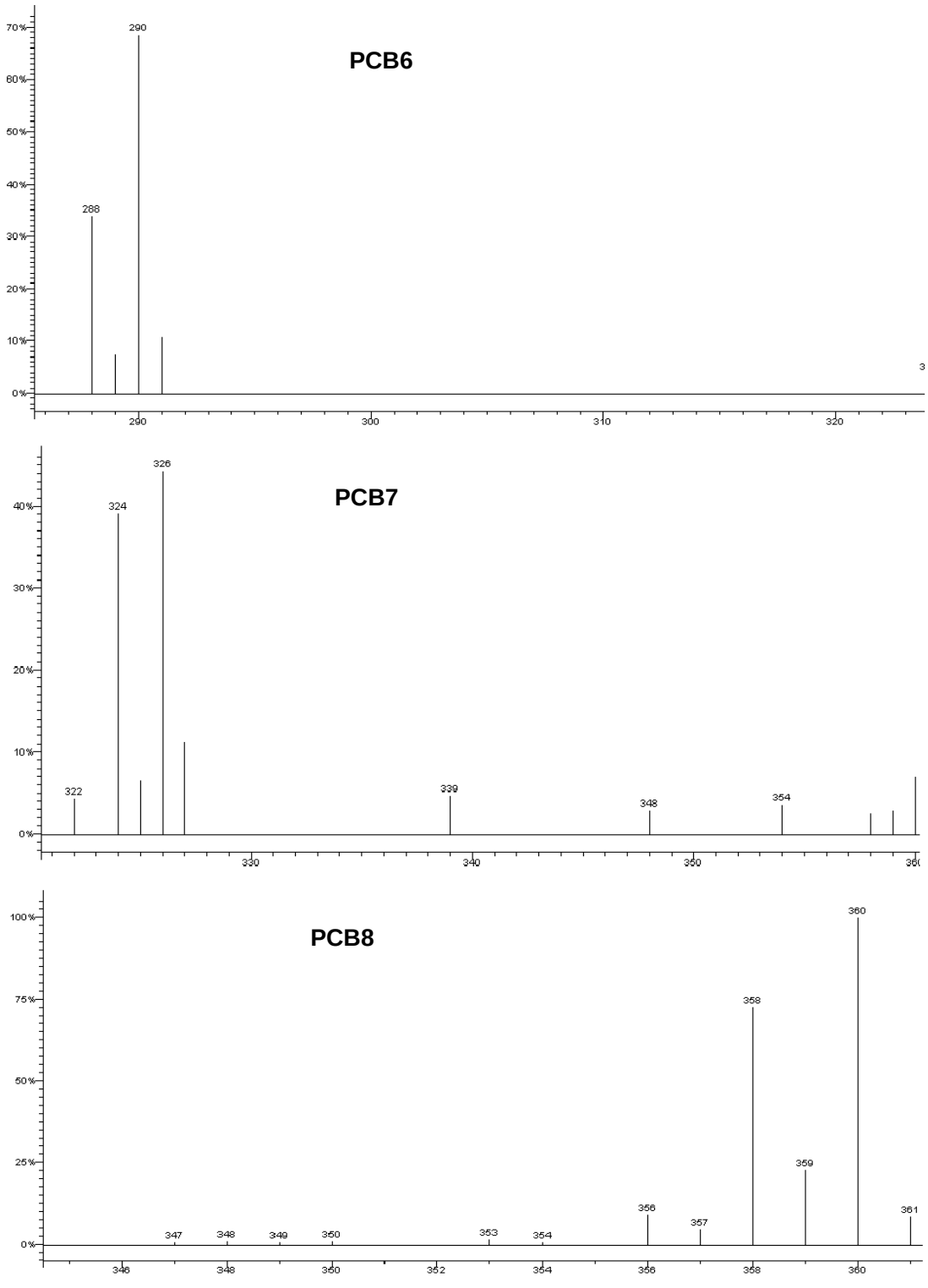


Fig C.4 (to be continued)

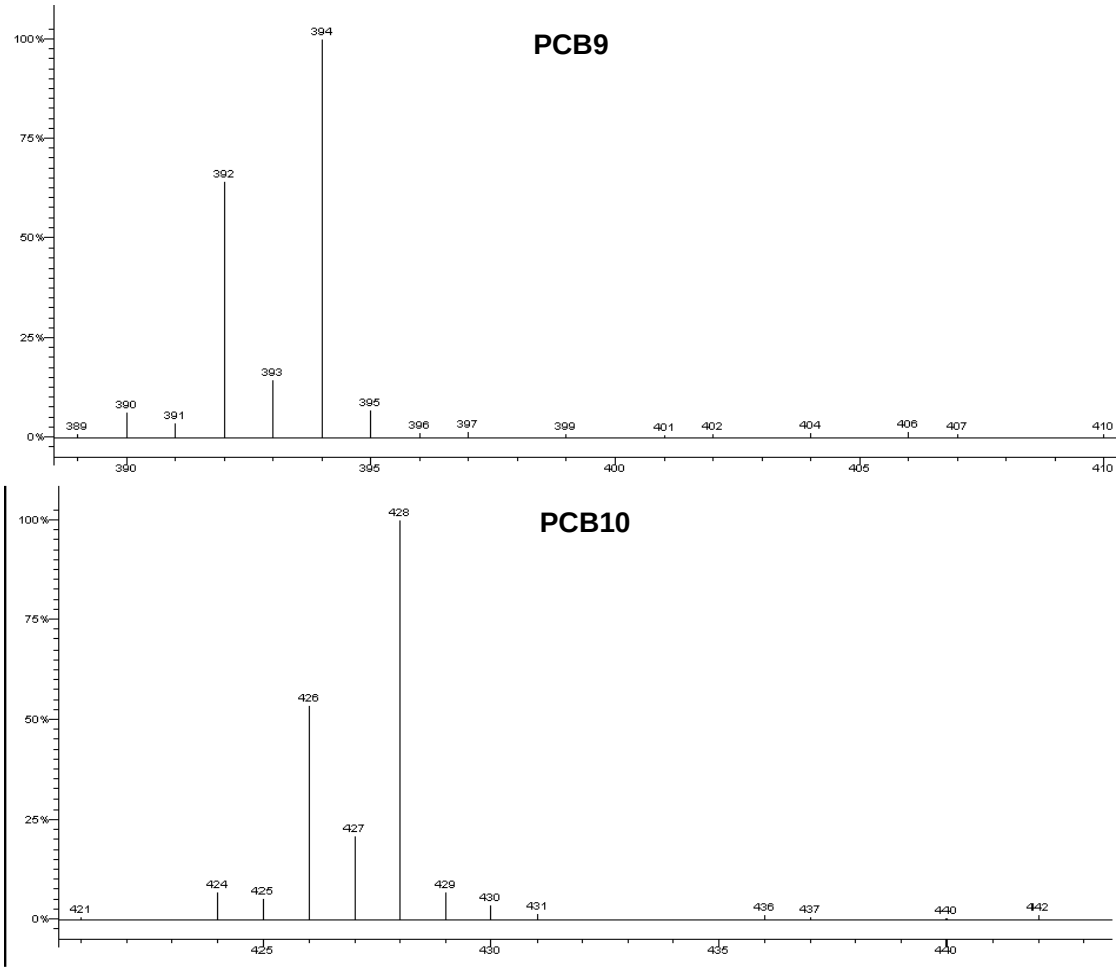


Fig C.4 (to be continued)

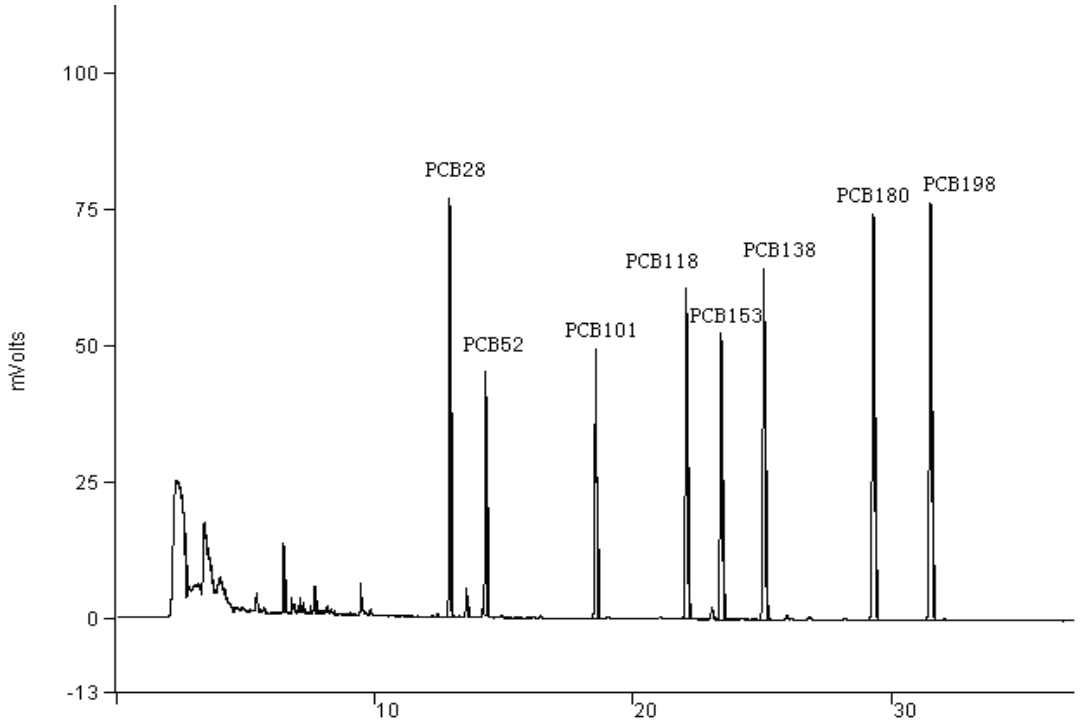


Fig C.5 Chromatogram of indicative PCB standard solution with GC-ECD

Appendix D

Purification flow chart

Fig D.1 is the decontaminating column schematic diagram;
Fig D.2 is GC-MS determination flow chart;
Fig D.3 is GC-ECD determination flow chart

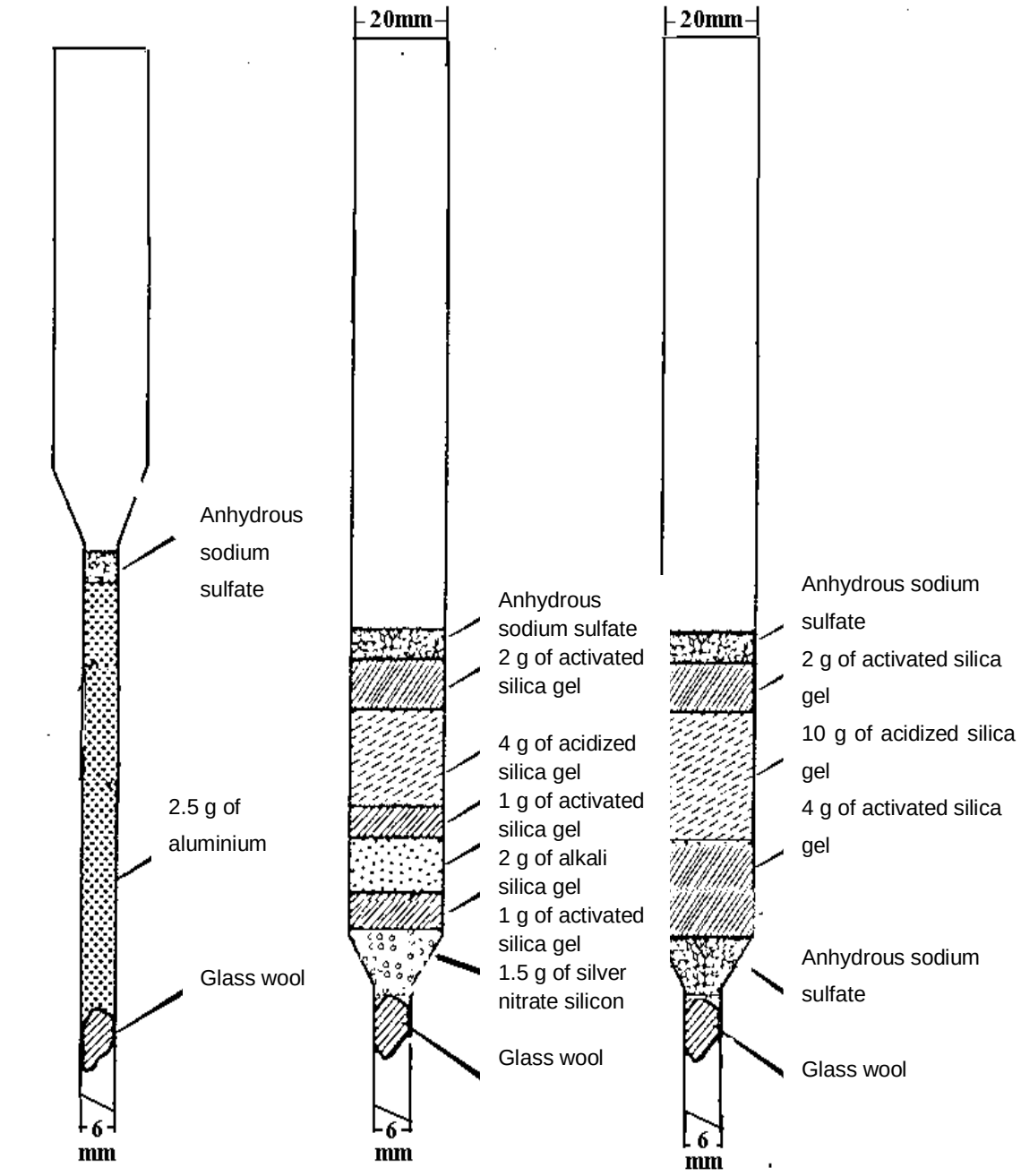


Fig D.1 Decontaminating column schematic diagram with GC-MS

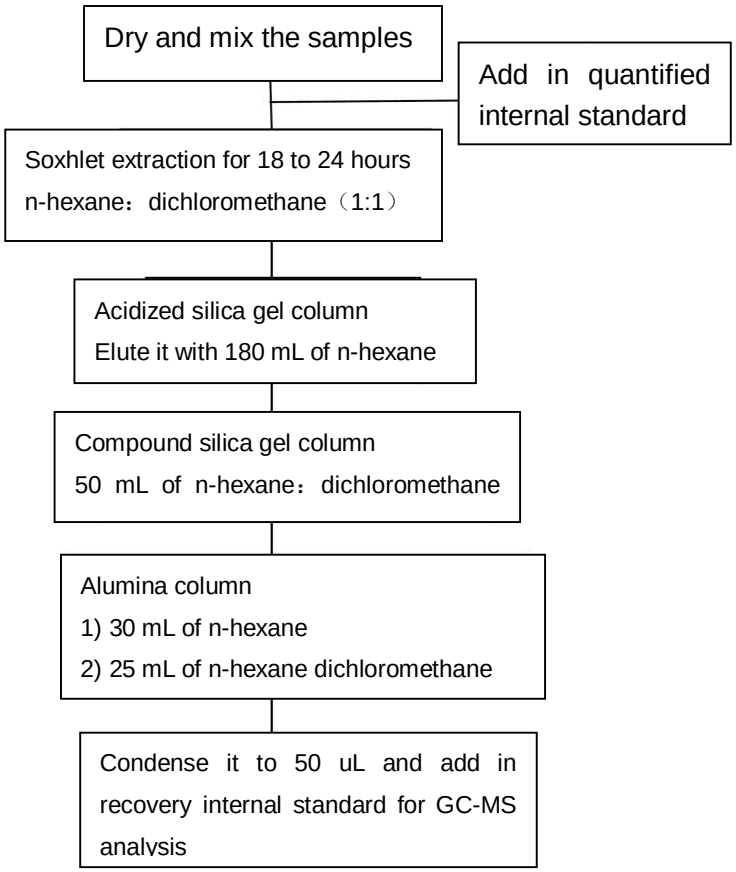


Fig D.2 Flow chart to determine the PCBs in food with isotope dilution gas chromatography-mass spectrometer

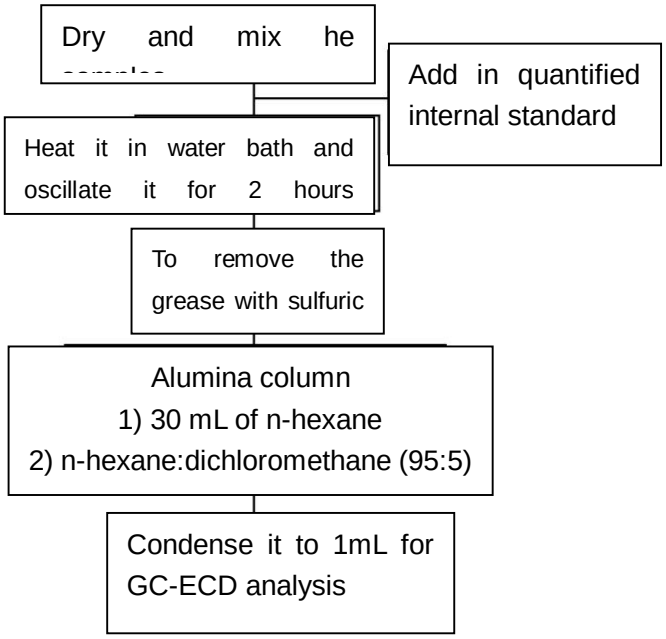


Fig D.3 Flow chart to determine the PCBs in food with the gas chromatography－electron capture detector