

Determination of 19 Organochlorine Pesticides Residues in Milk Powder by GPC-GC-MS

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Abstract: To establish the GPC - GC - MS detection method for determination of organochlorine pesticide residues in milk powder. After simple processing samples, concentration with multi-position concentrator, and online cleaning with GPC-GC-MS, injection into the mass spectrometer for analysis. Results showed that in the linear range of 5~100 ug/L, the correlation coefficient were >0.998, and the method detection limits (MDLs) were <0.5 ug/kg. The spiked recoveries at three levels of 10, 50, 100 ug/kg were in the range of 70 ~ 95%, the relative standard deviations (RSDs) (n=6) were <10%. This method had advantages of good accuracy and precision, simple operation, rapid, and was suitable for routine analysis.

Keywords: GPC - GC - MS, Organochlorine, Pesticide Residues, Milk Powder

1. Introduction

Organochlorine as broad-spectrum insecticides have been widely used, although now banned, but its easy to accumulate in the environment, difficult oxidation decomposition and high toxic, long-term residues in soil, through the food chain and biological enrichment of savings in various internal organs, and toxic. [1] Organic chlorine belongs to the nerve poison and viscera poison, [2] in the human body accumulates certain concentration, can interfere endocrine function, cause endocrine abnormality, even cause cancer. The maximum residue of organochlorine pesticides in milk and dairy products all around the world is very strict. The European Union stipulates that the residual of Endrin in milk and cheese is low to 0.8ug/kg. [3] Therefore, it is of great significance to establish an accurate, reliable and sensitive detection method for organochlorine pesticide residues in milk powder.

At present, the detection methods of organochlorine pesticide residues are mostly gas chromatography, gas chromatography-mass spectrometry, liquid chromatography-tandem mass spectrometry [4-6]. Due to the low content, and the complex matrix, pretreatment often need to use organic solvent assisted microwave, ultrasonic extraction, solid phase extraction, gel permeation chromatography, matrix dispersion methods such as solid

phase extraction [7-10] for purification and then concentrated on sample analysis. [11] dairy products contain fatty ingredients, and the sample pretreatment in the national standard is cumbersome, time-consuming and laborious, leading to large losses and low recovery rate. GPC (Gel Permeation Chromatography) is widely used to purify the sample, high efficiency to remove grease, pigment and high molecular weight protein distractors, processing complex samples. [12] In this study, GPC- GC-MS system was used to purify dairy products online, used eight-channel parallel concentrator for enrichment, Simplified pretreatment process. It provides a simple and reliable method for the detection of organochlorine pesticide residue in milk powder.

2. Method

2.1. Material and Reagent

19 organochlorine pesticide standards: O2si smart solutions; Sodium chloride, lead acetate: pure analysis; N-hexane: chromatographic purity; Acetone: chromatographic purity.

2.2. Instrument

GPC- GC-MS; Eight-channel parallel concentrator.

2.3. Method

2.3.1. Preparation of Mixed Standard Solution

Accurately weigh the standard product of pesticide of 10mg into 10mL volumetric flask, and the acetone/cyclohexane (3/7) is dissolved and dilute to the mark. This standard solution concentration is 1000mg/L. Diluted concentration was 1mg/L when use. Then use the 1mg/L standard solution to create concentrations ranging from 5ug/L to 100ug/L.

2.3.2. Analysis Conditions

(i) GPC Conditions

Chromatographic column: Shodex CLNpak ev-200 (2.1mm x 150mm); flow phase: acetone/cyclohexane (3/7, V/V); Flow rate: 0.1 mL/min; column temperature: 40°C sample quantity: 10 uL.

(ii) GC - MS Conditions

Chromatographic column: inert quartz tube: 5m x 0.53mm; Pre-column: rtx-5 MS, 5m x 0.25mm x 0.25mm; Analysis column: Rtx-5 MS, 25m x 0.25mm x 0.25m; Column temperature program: 82°C (5 min)_8°C/ min_300°C (7.75 min). No diversion time: 7 min; Solvent cutting time: 9.7min; Interface temperature: 300°C; Ion source temperature: 200°C. Collection mode: SIM.

(iii) Sample Pretreatment

Accurately weigh milk powder of 1g. Place the sample into 50mL plug centrifuge tube. Add 1g Sodium Chloride, 10mL acetone/n-hexane (1:1) solution. The vortex was mixed for 2min and the ultrasonic extraction was 15min. Add 500uL 2% lead acetate solution, statics after mixed 2min. centrifug 10min at a speed of 4000 r/m. Collect of supernatant liquid, the residue was ultrasonic extraction 15min with 10mL acetone/n-hexane, centrifugation, merge supernatant liquid. Concentration to 1mL with Eight-channel parallel concentrator. Measurement with GPC-GC-MS after filter.

(iv) GPC Positioning

The mixed standard solution was injected into GPC and separated by column, determine the peak time of organic chlorine. According to this time, confirm the injection time

program of switching valve and sample from GPC to GC-MS. This is the test method.

(v) GPC Purification Samples

Under the test method, Injection of processed sample 10uL. The components were collected during the peak time of organochlorine emission through switching of time programmed control valves. At other times, the column separates the fat and pigment from the sample and drained it into the waste liquid bucket.

(vi) GC - MS Analysis

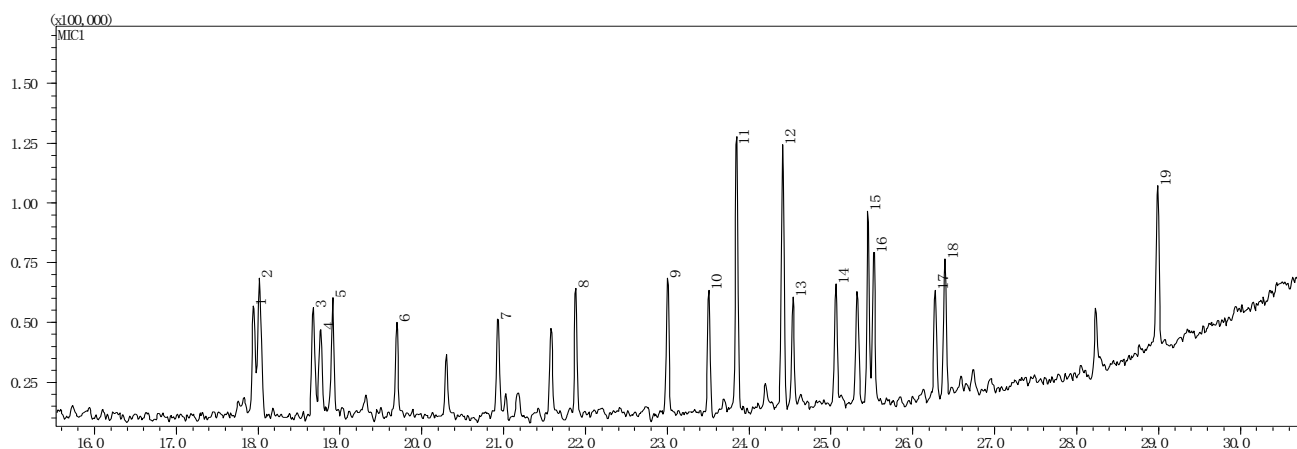
After GPC collects component, it gives a signal to GC-MS. GC-MS will start after receiving the trigger signal, The collected components will be injected into GC-MS through time program control valve. Then, the GPC purification process is finished and the samples are entered into GC-MS for analysis.

3. Result

3.1. Linear Equation, Correlation Coefficient and Method Detection Limit

In this experiment, the external standard method was used to make a mixture of 5, 10, 20, 50 and 100ug/L mixed standard solutions using acetone/cyclohexane (3/7, V/V). The standard equation of concentration and peak area was used to obtain the linear equation of 19 target compounds. 5ug/kg was added to the blank milk powder, and 12 samples were measured to calculate the standard deviation. The detection limits calculated according to the formula $C_L = K_i S_i \frac{C}{X}$, the Confidence factor $K_i=3$ [13]

19 kinds of organic chlorine mixed standard solution GC-MS MIC chromatogram, as shown in Figure 1; blank milk powder sample added low concentration (5ug/kg) organic chlorine mixed standard solution SIM collection chromatogram, as shown in Figure 2 (b). The linear equation, retention time (tR), correlation coefficient and method detection limit (MDL) as shown in table 1.



The pesticides represented by 1-19 digital annotations in the figure are shown in Table 1.

Figure 1. 19 kinds of organochlorine GC-MS MIC diagrams.

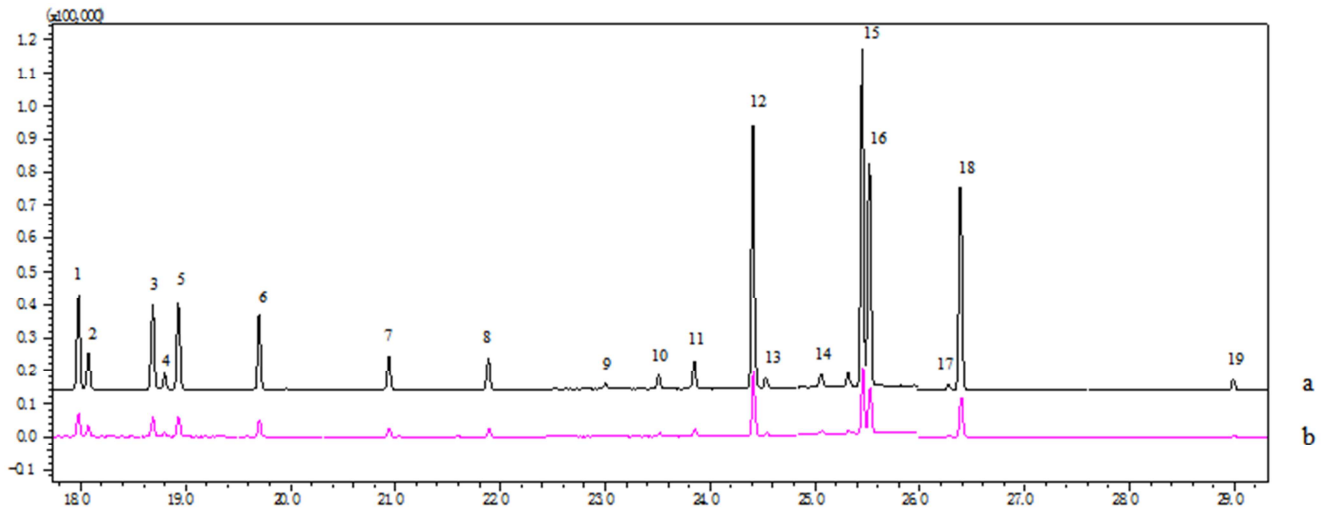


Figure 2. GC-MS/SIM chromatogram.

- (a) adding 19 kinds of organic chlorine mixed standard solution to milk powder samples.
 (b) adding 5ug/kg 19 kinds of organic chlorine mixed standard solution to milk powder samples.

Table 1. Linear equations, retention time (t_R), correlation coefficient (R) and MDL.

| Serial number | Compound | Linear equations | Retention time (t_R) | correlation coefficient (r) | MDL (ug/kg) |
|---------------|--------------------|-------------------|--------------------------|---------------------------------|-------------|
| 1 | α -HCH | $Y=134005X+370$ | 17.942 | 0.9986 | 0.10 |
| 2 | hexachlorobenzene | $Y=58850X+185$ | 18.017 | 0.9988 | 0.37 |
| 3 | β -HCH | $Y=105380X+92$ | 18.675 | 0.9990 | 0.18 |
| 4 | PCNB | $Y=10875X+8.5$ | 18.767 | 0.9985 | 0.45 |
| 5 | γ -HCH | $Y=200545X-121$ | 18.908 | 0.9994 | 0.10 |
| 6 | δ -HCH | $Y=96565X+269$ | 19.692 | 0.9992 | 0.15 |
| 7 | Heptachlor | $Y=10835X+66$ | 20.933 | 0.9997 | 0.12 |
| 8 | Aldrin | $Y=23340X+166$ | 21.883 | 0.9992 | 0.43 |
| 9 | Heptachlor epoxide | $Y=4810X+87$ | 23.000 | 0.9999 | 0.35 |
| 10 | trans-chlordane | $Y=24655X+555$ | 23.508 | 0.9993 | 0.24 |
| 11 | cis-chlordane | $Y=29140X+185$ | 23.808 | 0.9988 | 0.15 |
| 12 | P,P' -DDE | $Y=974425X-1076$ | 24.417 | 0.9989 | 0.10 |
| 13 | Dieldrin | $Y=11410X+82$ | 24.533 | 0.9990 | 0.08 |
| 14 | Endrin | $Y=10515X+57$ | 25.058 | 0.9999 | 0.48 |
| 15 | P,P' -DDD | $Y=1329750X-8320$ | 25.450 | 0.9989 | 0.15 |
| 16 | O,P' -DDT | $Y=902325X-4711$ | 25.517 | 0.9993 | 0.12 |
| 17 | Endosulfan sulfate | $Y=10985X-14$ | 26.275 | 0.9986 | 0.36 |
| 18 | P,P' -DDT | $Y=844300X-5858$ | 26.392 | 0.9989 | 0.22 |
| 19 | Mirex | $Y=38090X-46$ | 28.992 | 0.9990 | 0.20 |

As shown in Figure 1, the chromatographic peaks can basically be separated, and the chromatographic parameters are suitable. It can be seen from table 1 that, within the range of 5 to 100.0ug/L, 19 kinds of organic chlorine have a good linear relationship, $r > 0.998$, MDL < 0.5 ug/kg, which satisfies the detection requirements of trace pesticide residues.

3.2. Recovery and Precision

The samples of 1.0g blank milk powder were added to the standard solution of 10, 50 and 100ug/kg 19 organochlorine

mixtures, and 6 parallel samples were determined according to the 1.3 method, and the accuracy and precision of the method were investigated. [14] The SIM chromatogram of the 19 mixed standard solutions added to the powdered milk samples is shown in Figure 2 (a). The average recoveries and relative standard deviations (RSD) of the samples are shown in Table 2. From table 2, The average recovery rate of three concentration levels is 70 to 95%, RSD $< 10\%$, which meets the analysis requirements of various pesticide residues.

Table 2. The average recovery R (%) and the relative standard deviation RSD (%) ($n=6$).

| Serial number | Compound | Addition concentration/(ug/kg) | | | RSD (%) |
|---------------|-------------------|--------------------------------|------|------|---------|
| | | 10 | 50 | 100 | |
| 1 | α -HCH | 75.1 | 78.3 | 80.6 | 5.2~9.4 |
| 2 | hexachlorobenzene | 76.4 | 80.5 | 81.3 | 5.9~7.4 |
| 3 | β -HCH | 90.5 | 93.2 | 94.8 | 4.4~6.6 |
| 4 | PCNB | 85.6 | 88.2 | 84.3 | 2.2~3.3 |

| Serial number | Compound | Addition concentration/(ug/kg) | | | RSD (%) |
|---------------|--------------------|--------------------------------|------|------|---------|
| | | 10 | 50 | 100 | |
| 5 | γ -HCH | 77.8 | 75.2 | 79.7 | 4.8~5.3 |
| 6 | δ -HCH | 82.1 | 84.3 | 86.4 | 2.6~3.8 |
| 7 | Heptachlor | 74.3 | 78.9 | 75.8 | 3.2~4.5 |
| 8 | Aldrin | 72.8 | 76.3 | 74.4 | 2.8~3.6 |
| 9 | Heptachlor epoxide | 77.2 | 82.5 | 80.3 | 2.0~4.7 |
| 10 | trans-chlordane | 73.8 | 78.6 | 76.4 | 3.6~4.5 |
| 11 | cis-chlordane | 72.0 | 75.3 | 78.2 | 2.1~4.0 |
| 12 | P,P' -DDE | 77.6 | 83.3 | 79.2 | 2.8~3.9 |
| 13 | Dieldrin | 73.1 | 75.3 | 76.8 | 2.5~5.4 |
| 14 | Endrin | 81.6 | 83.4 | 85.3 | 2.1~4.8 |
| 15 | P,P' -DDD | 82.3 | 86.7 | 84.5 | 3.3~5.3 |
| 16 | O,P' -DDT | 75.2 | 79.8 | 82.1 | 3.5~6.1 |
| 17 | Endosulfan sulfate | 73.8 | 75.2 | 79.3 | 3.1~4.6 |
| 18 | P,P' -DDT | 70.8 | 72.4 | 76.5 | 2.4~3.9 |
| 19 | Mirex | 87.5 | 90.5 | 93.8 | 2.2~3.1 |

4. Discussion

4.1. Simplified Preprocessing

4.1.1. Use of On-line Gel Chromatographic Purification System

The national standard uses solid phase extraction to process samples, which takes a long time and is cumbersome in operation. Each step causes unpredictable loss, resulting in low results and low recovery rate. Therefore, it is time-consuming and energy consuming, and it is difficult to ensure the accuracy of the results.

Gel permeation chromatography is also known as dimensional exclusion chromatography, which is a chromatographic technique for separation by the size of solute molecules. The eluant was injected into the chromatographic column elution separation, and the samples of different molecular size and shape were eluted through the porous gel fixed phase. The large molecules were first eluted, and then the small molecules were eluted. It is a rapid method for the determination of molecular weight and molecular weight distribution. It has become an important means of separation and purification in the analysis of pesticide residues. It has obvious advantages to remove the large molecular substances such as fat and pigment in the sample. [15] GPC sample purification method is recommended by the EPA, the food and Drug Administration and the International Association of analytical chemists. In the FDA2905 a method and EPA 3640 method, the GPC method is used to separate the macromolecular impurities from the target. The advantages of GPC method, such as simple method, high degree of automation and high recovery rate, are gradually accepted by many laboratories, and more and more standards adopt GPC method for sample purification and separation. [16]

In this study, SHIMADZU online gel chromatography tandem three quadrupole GC-MS was used to locate and determine the time of separation of organochlorine. GPC was used to purify samples and remove fat impurities from macromolecules. Collect the components within the set time and automatically enter the GC-MS. Online separation and detection, process automation, simple steps, reducing the loss

of the experimental process. It not only guarantees the accuracy of the results, but also saves manpower and time.

4.1.2. Use of Parallel Concentrator

In this experiment, the 8 channel parallel concentrator is used to replace the traditional rotary evaporator. It has the functions of rotary evaporator and nitrogen blows instrument. Process automation does not require people to observe, and automatically fixed volume. When concentrated to 1mL, it will alarm and stop the nitrogen blow, which greatly liberates manpower. Because the concentrated liquid does not need to be transferred, the loss caused by operation is reduced. The operation is simpler and the result is more accurate.

4.2. The Results Are Accurate and Meet the Requirements of the Limit

In the range of 5.0 ~ 100.0ug/L, the linear relationship was good, and the method detection limit was < 0.5ug/kg. The recovery rate of pesticide was between 70 ~ 95% and RSD < 10%, and the results were accurate. The detection limit of the current national standard GB/T 23210 - 2008 milk powder is 4.2 ug/kg~2.0mg/kg. The limit of quantification for GB 23200.86-2016 [17] milk powder is 0.8 ug/kg. It can be seen that this method can meet the requirements of the national standard for detection limits and limit [18].

5. Conclusion

In this paper, GPC-GC-MS method for detecting organochlorine pesticide residues in milk powder was established. The process of pretreatment was optimized. The process was concentrated by multi-position concentrator to simplify the process of rotary steam and nitrogen blowing. The on-line GPC-GC-MS system was used to carry out on-line purification and automatic sampling analysis. In the concentration range of 5 to 100ug/L, the linear relationship, detection limit, precision and standard recovery were examined to confirm the index. The results are satisfactory: the linear relationship is good, the detection limit is low, the precision is good, and the recovery rate is high. Fully automated online GPC-GC-MS system, sample purification completely, the effect is good. The method is accurate, reliable

and easy to operate. It is suitable for the qualitative and quantitative determination of organochlorine pesticide residues in milk powder.

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