

Determination of organochlorine pesticide residues in milk by gel-permeation chromatography (GPC) cleanup and GC-ECD

LabTech, Inc.

Organochlorine pesticides are broad-spectrum, high efficient, inexpensive pesticides, which were widely used worldwide. However, the strong stability makes organochlorine pesticides accumulate largely through the food chain, which eventually influences human beings health. Because of its strong stability, long half-life, the organochlorine pesticides residue exceeded maximum residue limit (MRL) happens frequently. This study develops a method to determine organochlorine pesticides residue in dairy. This method shows great recovery and easy operation.

1. Experimental Section

1.1 Instruments and reagents

AutoClean automatic gel-permeation chromatography system (LabTech, Inc., Boston, USA)

EV331 rotary evaporator (Beijing LabTech Instrument Co., Ltd., Beijing)

GC-ECD

Shaker

Nitrogen blowing instrument

Ethyl acetate (redistilled for use)

Cyclohexane (redistilled for use)

Acetone (redistilled for use)

Sodium chloride (baked for 4h at 140 °C)

Anhydrous sodium sulfate (baked for 4h at 140 °C)

1.2 Sample Preparation

1.2.1 Sample

Take milk, homogenized.

1.2.2 Extraction

Accurately weigh 20.00g of homogenized milk sample to 200ml Erlenmeyer flask, 40ml of acetone, shaking 30min, adding 6g of sodium chloride, shake well, then add 30ml of petroleum ether, shaking 30min, after standing layer, transfer the organic phase to 100ml Erlenmeyer flask, dehydrate with anhydrous sodium sulfate, and take 35ml in a rotary evaporator and concentrated to 1ml, add 2ml of ethyl acetate - cyclohexane (1 + 1), and the solution was then concentrated, so repeated three times, final volume to 10ml with ethyl acetate - cyclohexane (1 + 1), to be cleanup.

1.2.3 Cleanup

GPC cleanup conditions:

Flow rate: 5ml/min

Cleanup column: 20 × 300mm SX-3

Mobile phase: ethyl acetate - cyclohexane (1 + 1)

Collection time: 12min-16min

The cleaned samples was collected in the nitrogen blowing instrument, evaporated to near dryness under nitrogen blowing at 50 °C, to volume 1ml with n-hexane, for further GC analysis.

1.3 Instrument analysis conditions

1.3.1 chromatographic conditions

Gas: GC-ECD

Column: TM-Pesticide 1 30m × 0.53mm × 1.0um

Column temperature program: heating at 170 °C for 1 minute, and then heat to 210 °C with the rate of 5 °C/min, and then heat to 230 °C with the rate of 2 °C/min, keeping on 10min.

Sample loading Inlet temperature: 280 °C

Detector temperature: 300 °C

Split ratio: splitless

Injection volume: 1 uL

1.3.2 GC-ECD spectra

Chromatogram result under the chromatographic conditions is as follows:

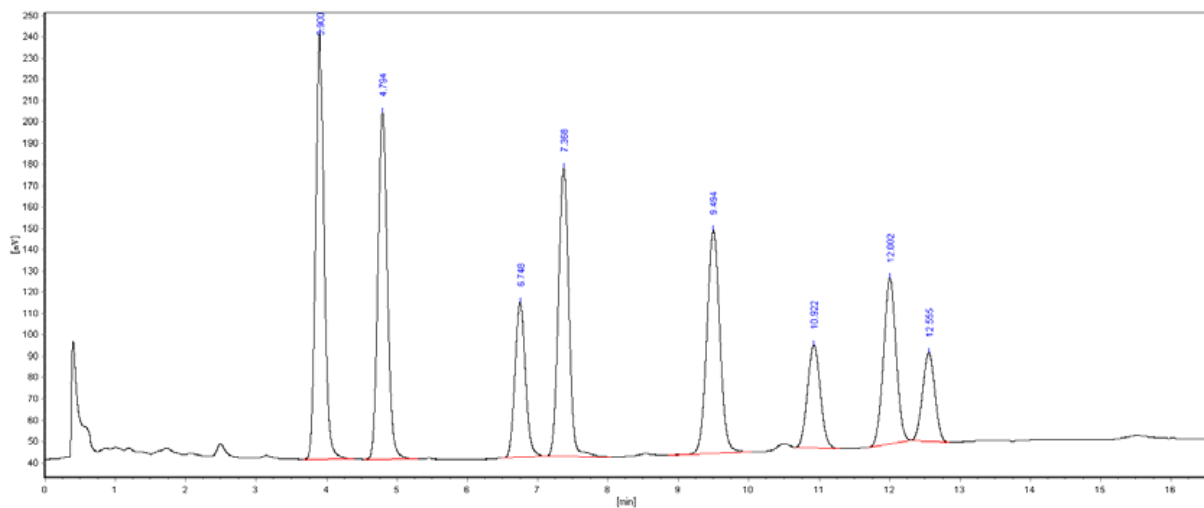


Figure 1, 8 GC-ECD chromatogram of organochlorine pesticides
(From left to right: α -666; β -666; γ -666; δ -666; P, P-DDT; O, P-DDT; P, P-DDE; P, P-DDD)

2. Results and discussion

2.1 Standard curve

2.2.1 Optimization of GPC cleanup conditions

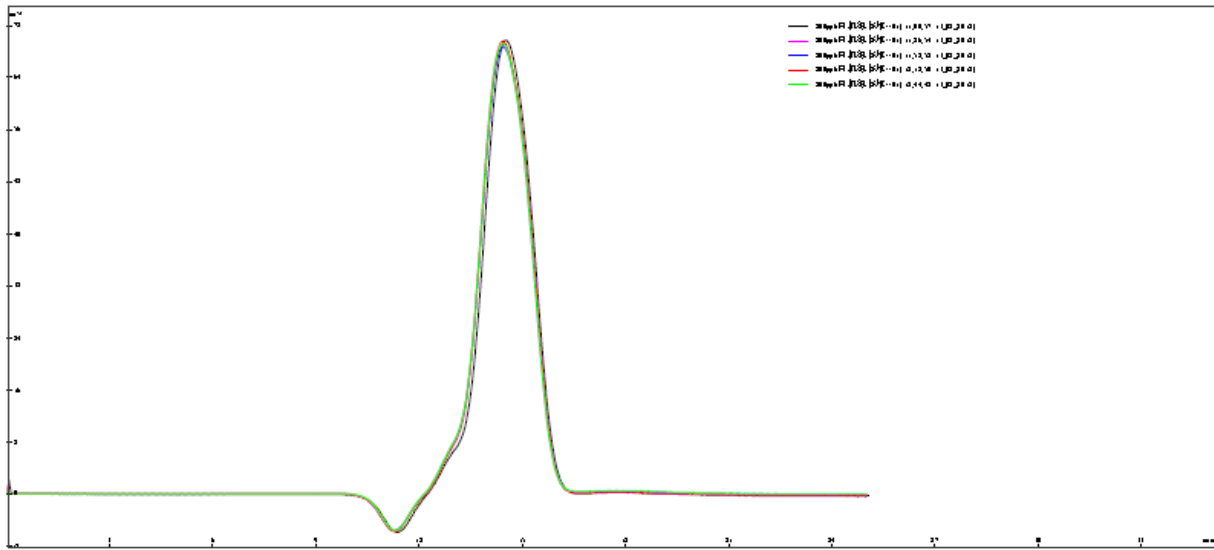


Figure 2. GPC cleanup reproducibility

Take standard mixture of 8 organochlorine pesticides, dilute to 200ppb with ethyl acetate: cyclohexane (1 + 1), as a GPC cleanup loading sample, using a UV detector at 254nm conditions to detect and get chromatogram shown in Figure 2; Loading sample 5 consecutive times, good reproducibility. Identify collection time of 12min-16min.

2.1.2 Optimization of the chromatographic conditions

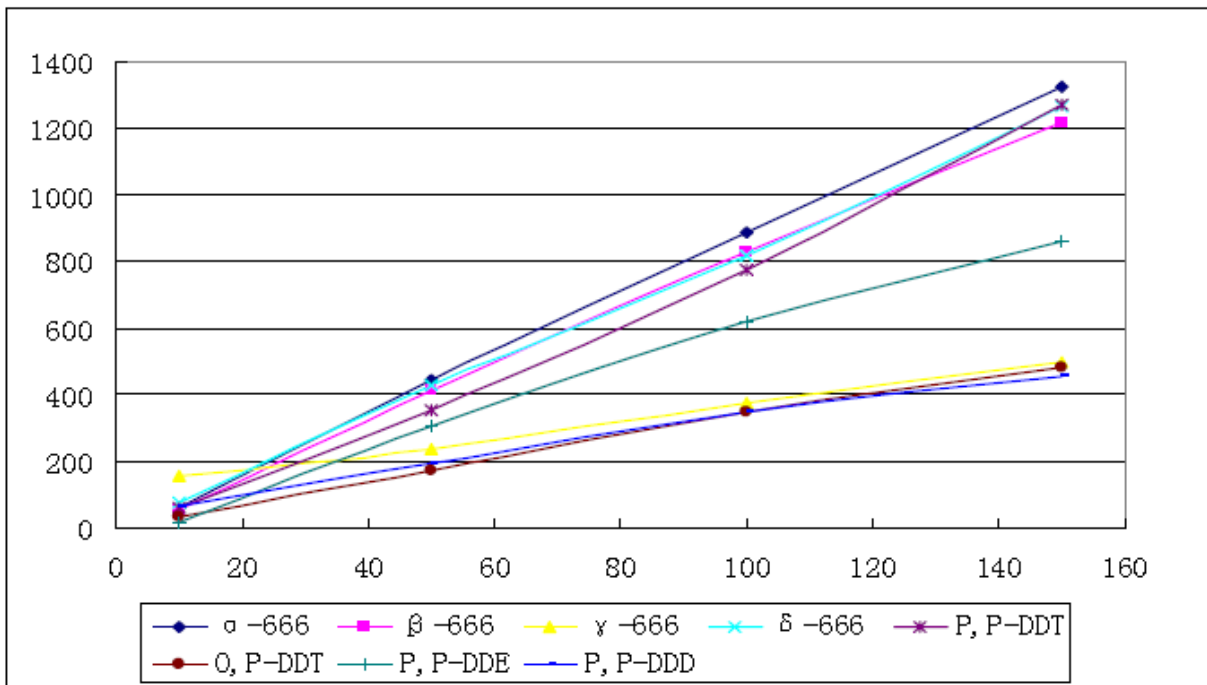


Figure 3. Regression curves of 8 organochlorine pesticides

To calculate the standard curve regression equation and linear range by setting sample concentration as abscissa (X) and the peak area of the vertical axis (Y). In the range of 10ppb-150ppb, linearity is good. The linear equation are:

| Pesticide | Regression equation | Regression coefficient |
|---------------|------------------------|------------------------|
| α -666 | $y = 9.0287x - 21.465$ | $R^2 = 0.9996$ |
| β -666 | $y = 8.3155x - 15.119$ | $R^2 = 0.9993$ |
| γ -666 | $y = 2.4953x + 120.71$ | $R^2 = 0.9965$ |
| δ -666 | $y = 8.4382x - 8.4691$ | $R^2 = 0.9994$ |
| P,P-DDT | $y = 8.6550x - 7.7220$ | $R^2 = 0.9958$ |
| O,P-DDT | $y = 3.2334x + 7.0878$ | $R^2 = 0.9960$ |
| P,P-DDE | $y = 6.0085x - 14.612$ | $R^2 = 0.9922$ |
| P,P-DDD | $y = 2.8035x + 46.326$ | $R^2 = 0.9903$ |

2.2 Recovery precision

In the optimization conditions, standards are added in a sample, and cleanup by LabTech AutoClean GPC system, GC-ECD analysis and the resulting spectrum as shown in Figure 4.

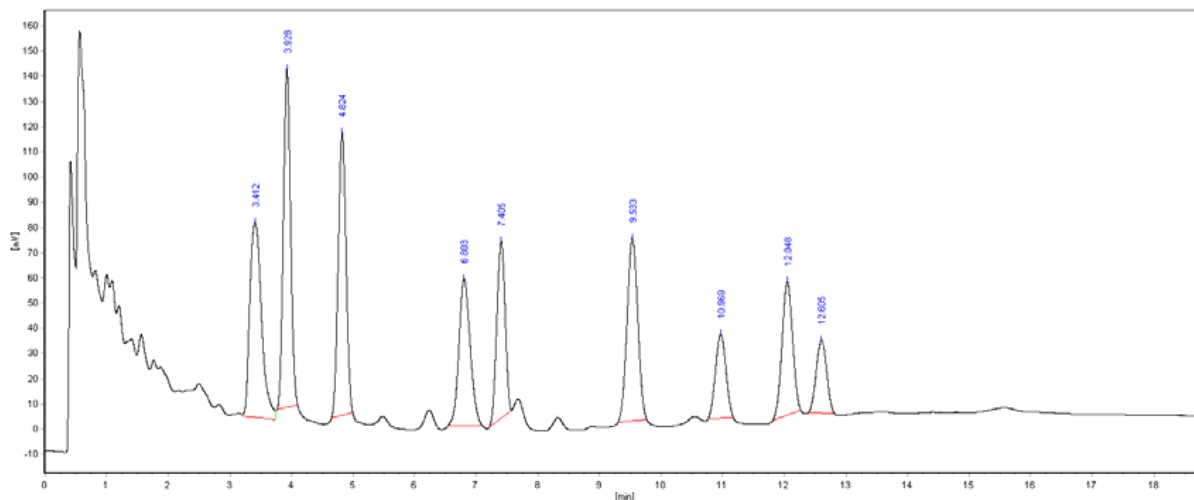


Figure 4. GC-ECD spectra of 8 organochlorine standards in milk by GPC cleanup

2.2.1 Recovery rate of standards

Recovery experiment is tested for the accuracy of determination of organochlorine pesticides in foods by spiking and recovery experiments, and results are shown in the below.

| Number | Pesticide | Addition amount (mg/kg) | Measured amount (mg/kg) | Recovery (%) | RSD (%) |
|--------|---------------|-------------------------|-------------------------|--------------|---------|
| 1 | α -666 | 0.1 | 0.122 | 122.1 | 3.03 |
| | | 0.1 | 0.129 | 129.7 | |
| | | 0.1 | 0.125 | 125.8 | |
| 2 | β -666 | 0.1 | 0.123 | 123.6 | 1.82 |
| | | 0.1 | 0.128 | 128.0 | |
| | | 0.1 | 0.127 | 127.0 | |
| 3 | γ -666 | 0.1 | 0.077 | 77.0 | 8.18 |
| | | 0.1 | 0.089 | 89.0 | |
| | | 0.1 | 0.078 | 78.0 | |
| 4 | δ -666 | 0.1 | 0.087 | 87.5 | 1.49 |
| | | 0.1 | 0.089 | 89.8 | |
| | | 0.1 | 0.089 | 89.8 | |
| 5 | P,P-DDT | 0.1 | 0.132 | 132.6 | 2.17 |
| | | 0.1 | 0.129 | 129.9 | |
| | | 0.1 | 0.127 | 127.0 | |
| 6 | O,P-DDT | 0.1 | 0.119 | 119.9 | 3.69 |
| | | 0.1 | 0.113 | 113.8 | |
| | | 0.1 | 0.122 | 122.3 | |
| 7 | P,P-DDE | 0.1 | 0.111 | 111.1 | 2.62 |
| | | 0.1 | 0.107 | 107.6 | |
| | | 0.1 | 0.105 | 105.5 | |
| 8 | P,P-DDD | 0.1 | 0.100 | 100.4 | 6.10 |
| | | 0.1 | 0.098 | 98.0 | |
| | | 0.1 | 0.109 | 109.8 | |

2.3.1 Results of real sample

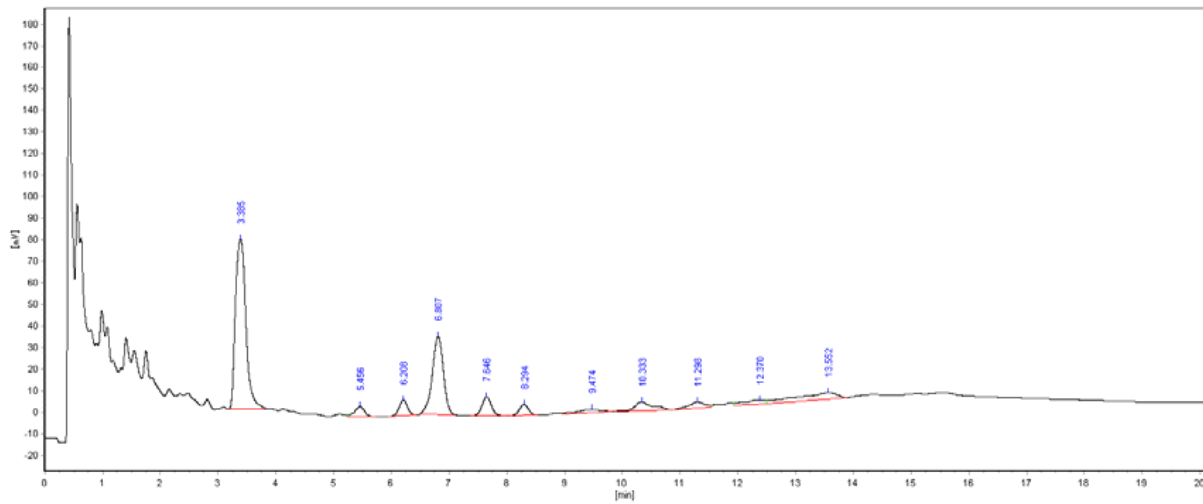


Figure 5. GC-ECD chromatogram of milk sample after cleanup

3. Conclusion

In this application note, a method is created to cleanup and analyze of 8 organochlorine pesticide residues in milk. The operation is convenient and the recovery rate is ideal.

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