Determination of 14 Organophosphorus Pesticide Residues in Mutton by Gel Permeation Chromatography-Gas Chromatography-Mass Spectrometry (GPC-GC-MS)

Junpeng ZHAO[1,2,3], **Richard Avoi**[4]*, **Azman Bin Atil @ Azmi**[4], **Jiao CHEN**[5], **Ling YUN**[6]

1. Tangshan Food and Drug Comprehensive Inspection and Testing Center, Tangshan 063000, China; 2. Hebei Agricultural Products Quality and Safety Testing Technology Innovation Center, Tangshan 063000, China; 3. Tangshan Institute of Industrial Technology for Functional Agricultural Products, Tangshan 063000, China; 4. Universiti Malaysia Sabah, Faculty of Medicine and Health Sciences, Malaysia; 5. Guizhou Forerunner College, Guiyang 550000, China; 6. Tangshan Center for Disease Prevention and Control, Tangshan 063000, China

Abstract [**Objectives**] This study was conducted to purify mutton samples by gel permeation chromatography (GPC). [**Methods**] Fourteen organophosphorus pesticide residues in samples were qualitatively and quantitatively analyzed by gas chromatography-mass spectrometry (GC-MS) in selective ion scanning mode (SIM). [**Results**] The organophosphorus pesticide standard solutions showed good linearity in the mass concentration range of 0.1 – 10.0 μg/ml with correlation coefficients (*r*) not lower than 0.999, and the detection limits (*S* = 3*N*) ranged from 0.01 to 0.05 mg/kg. The average recovery values were in the range of 80.2% – 99.7%, with relative standard deviations (*RSDs*, *n* = 3) in the range of 1.8% – 6.3%, at the addition levels of 0.5, 1.0 and 2.0 mg/kg. [**Conclusions**] The method is simple, sensitive and accurate, and can be used for the determination of organophosphorus pesticide residues in mutton.

Key words Mutton; Gas chromatography-mass spectrometry; Gel permeation chromatography; Organophosphorus; Pesticide residue

Organophosphorus pesticides (OPPs) are currently one of the most widely used three types of pesticides. They are widely used in agricultural production because of their high efficiency and broad spectrum, and their insecticidal mechanism is to inhibit the activity of insect cholinesterase. The extensive use of organophosphorus pesticides has caused great harm to human beings, animals and the environment. To this end, countries around the world have invested a lot of money, manpower and material resources to strengthen the research, development and application of pollution-free green pesticides, on the one hand. On the other hand, more and more strict pesticide residue limit standards are formulated to control the quality of food and improve the safety of food[1-6]. Therefore, it is of great significance to strengthen the research on the detection methods of organophosphorus pesticides, especially rapid, sensitive, accurate and simple detection methods.

Gel permeation chromatography-gas chromatography-mass spectrometry (GPC-GC-MS) is a synchronous analytical detection technique which combines GPC and GC-MS. It not only has the advantages of strong matrix interference resistance, high sensitivity and fast analysis speed, but also adopts the rapid determination mode of selective ion monitoring (SIM), achieving really fast GC-MS analysis. It has a wider quality detection range, simplifies the operation steps and saves reagents. The test results are accurate and reliable, and false positive test results are avoided[7-10]. In this paper, based on gel permeation chromatography (GPC) and gas chromatography-mass spectrometry (GC-MS), a method for the determination of organophosphorus pesticide residues in mutton was established, providing a basis for the safety assessment of pesticide residues.

Materials and Methods

Experimental materials

Mutton, provided by the experimental base of College of Animal Science, Hebei Agricultural University; mixed standard of 14 kinds of organophosphorus pesticides with a concentration of 1.0 mg/ml, Beijing Tanmo Quality Inspection Technology Co., Ltd.; n-hexane, acetonitrile, ethyl acetate, cyclohexane (all chromatographically pure), sodium chloride (guarantee reagent), TEDIA company; helium (purity > 99.99%).

Thermofisher ISQ gas chromatography-mass spectrometer, Finnigan Company, USA; TG-1701MS elastic quartz capillary column (30 m × 0.25 mm × 0.25 μm), Thermo Scientific Company, USA; analytical balance FA1104N, Beijing Beike Hengxin Scientific Equipment Co., Ltd.; vortex mixer HMS-350, Tianjin Hengao Technology Development Co., Ltd.; GPC gel chromatograph, J2 Science Company, USA.

Experimental methods

Preparation of standard solution Single standard stock solution: Fourteen kinds of single standard stock solutions of organophosphorus pesticides were prepared with a mass concentration of 100.0 μg/ml, respectively. The prepared solutions were stored in

a refrigerator at 4 ℃ for later use.

　　Preparation of mixed standard series solution: The standard stock solutions were diluted with n-hexane to standard series solutions of organophosphorus pesticides with concentrations of 0.1, 0.5, 1.0, 2.0, 4.0 and 10.0 μg/ml. The prepared solutions were stored in a refrigerator at 4℃ for later use.

Sample pretreatment　A 2 g of mutton sample was accurately weighed into a triangular flask with a stopper, which was added with 20 ml of petroleum ether, and the mixture was mixed with a vortex mixer for 2 min. After standing, the petroleum ether layer was extracted, and the residue in the triangular flask was washed with petroleum ether three times, 10 ml each time. The combined extract was filtered by anhydrous sodium sulfate (10 g), and the filtrate was concentrated to dryness under reduced pressure. The obtained concentrated was diluted with ethyl acetate: cyclohexane (volume ratio: 1 : 1) to a volume of 10.0 ml. After vortex-mixing for 2 min, the solution was filtered with 0.45 μm membrane, and purified with a gel permeation chromatography device to collect the effluent, which was concentrated to 2.0 ml under reduced pressure for analysis by GC-MS.

GC-MS analysis conditions　GC conditions: TG-1701MS chromatographic column (30 m × 0.25 mm × 0.25 μm); injection port 250 ℃; column temperature: the initial temperature 60 ℃ was kept for 1 min and then increased at 30 ℃/min to 160 ℃, which was kept for 1 min and then increased at 2 ℃/min to 220 ℃, which was kept for 2 min and then raised at 20 ℃/min to 280 ℃, which was kept for 4 min; carrier gas: helium (purity ≥ 99.999%) with a flow rate of 1.5 m/min; sample injection volume 1 μl; unsplit stream sampling.

　　MS condition: EI source temperature 230 ℃; electron energy 70 eV; transmission line temperature 280 ℃; selective ion scanning mode (SIM); solvent delay 5 min.

Results and Analysis

Qualitative analysis

　　According to the analysis conditions determined in this experiment, the mixed solution of 14 standards with a mass concentration of 1.0 μg/ml was injected to record the retention time. The total ion chromatogram of GC-MS is shown in Fig. 1. Table 1 shows specific qualitative and quantitative ions of OPPs.

　　As can be seen from Fig. 1, 14 compounds could be effectively separated within 44.0 min, with sharp peaks and good symmetry. There are no interfering peaks near the peaks, and the

intervals between peaks are appropriate, so the chromatographic detection conditions were ideal.
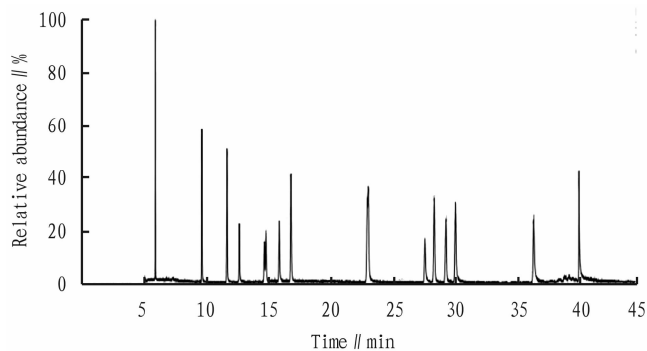

Fig. 1　GC-MS total ions of the mixture of 14 standards with a mass concentration of 1 μg/ml

Table 1　Retention time and characteristic ions of 14 OPPs in SIM mode

No.	Component name	Collision energy // eV	Retention time // min	Quantitative ion	Qualitative ion
1	Methamidophos	15	5.88	141	95
2	Dichlorvos	20	9.82	185	93
3	Acephate	10	11.83	136	94
4	Omethoate	14	12.82	156	110
5	Phorate	17	14.77	121	65
6	Dimethoate	25	15.83	125	79
7	Chlorpyrifos-methyl	12	18.78	286	93
8	Parathion-methyl	10	22.99	263	109
9	Fenitrothion	15	27.73	277	260
10	Malathion	15	28.28	127	99
11	Chlorpyrifos	20	29.10	197	169
12	Parathion	10	29.99	109	81
13	Isocarbophos	22	36.27	136	108
14	Triazophos	20	39.91	161	134

Linear relationship and detection limit

　　The mixed standard solutions of OPPs with mass concentrations of 0.1, 0.5, 1.0, 2.0, 4.0 and 10.0 μg/ml were determined in turn. Linear fitting was performed with mass concentration as the x-axis and peak area as the y-axis. The limit of detection (LOD) of the method was determined by the baseline noise ($S = 3N$), and the results are shown in Table 2. As shown in Table 2, there was a good linear relationship for various components in the mass concentration range of 0.1 – 10.0 μg/ml, with correlation coefficients (R) over 0.999, and the detection limits ranged from 0.01 to 0.05 mg/kg.

Table 2　Regression equations, correlation coefficients and detection limits of OPP compounds

Peak No.	Component name	Correlation coefficient	Correlation coefficient (r)	LOD // mg/kg
1	Methamidophos	$Y = 4.0483 \times 10^7 X - 4.5271 \times 10^6$	0.9995	0.02
2	Dichlorvos	$Y = 7.7613 \times 10^7 X - 5.6213 \times 10^6$	0.9992	0.02
3	Acephate	$Y = 6.8232 \times 10^7 X - 4.6231 \times 10^6$	0.9997	0.03
4	Omethoate	$Y = 6.8321 \times 10^7 X - 4.7238 \times 10^6$	0.9996	0.01
5	Phorate	$Y = 5.0173 \times 10^7 X - 6.8272 \times 10^6$	0.9990	0.04
6	Dimethoate	$Y = 4.2416 \times 10^7 X - 7.9235 \times 10^6$	0.9992	0.02

(Continued)

(Table 2)

Peak No.	Component	Correlation coefficient	Correlation coefficient (r)	LOD //mg/kg
7	Chlorpyrifos-methyl	$Y = 6.012\ 4 \times 10^7 X - 7.273\ 1 \times 10^6$	0.999 1	0.02
8	Parathion-methyl	$Y = 5.012\ 4 \times 10^7 X - 7.923\ 4 \times 10^6$	0.999 8	0.02
9	Fenitrothion	$Y = 7.281\ 4 \times 10^7 X - 6.923\ 4 \times 10^6$	0.999 3	0.01
10	Malathion	$Y = 4.612\ 3 \times 10^7 X - 3.712\ 4 \times 10^6$	0.999 5	0.02
11	Chlorpyrifos	$Y = 7.251\ 6 \times 10^6 X - 2.932\ 5 \times 10^5$	0.999 3	0.05
12	Parathion	$Y = 5.718\ 4 \times 10^7 X - 8.218\ 4 \times 10^6$	0.999 4	0.03
13	Isocarbophos	$Y = 4.916\ 5 \times 10^7 X - 5.812\ 5 \times 10^6$	0.999 1	0.02
14	Triazophos	$Y = 6.926\ 1 \times 10^6 X - 4.012\ 4 \times 10^5$	0.999 7	0.03

2.3 Recovery and precision Solutions prepared from 1.0 g of mutton was added with mixed standard solutions of OPP compounds, at addition levels of 0.5, 1.0 and 2.0 mg/kg, respectively, and each level was measured in parallel three times. The results are shown in Table 3. As shown in Table 3, the average recovery values were in the range of 80.9% − 98.4%, and the relative standard deviations (RSDs) were in the range of 2.0% − 6.7%, showing that the method is accurate and reliable.

Table 3 Recovery and RSDs of OPPs compounds (n = 3)   %

Component	0.5 mg/kg		1.0 mg/kg		2.0 mg/kg	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Methamidophos	81.5	3.3	92.3	3.7	96.6	4.8
Dichlorvos	85.6	2.7	89.9	2.7	93.0	4.6
Acephate	83.2	3.9	91.5	4.0	95.2	4.1
Omethoate	82.3	3.7	92.4	3.9	94.9	3.8
Phorate	80.2	4.1	86.2	3.4	89.7	1.8
Dimethoate	82.2	3.3	93.7	5.8	94.2	5.9
Chlorpyrifos-methyl	83.2	6.3	91.3	2.6	99.7	2.3
Parathion-methyl	82.4	4.3	89.5	3.8	94.2	4.9
Fenitrothion	80.9	3.7	86.5	4.5	97.4	4.2
Malathion	83.5	3.7	90.5	2.6	93.2	4.7
Chlorpyrifos	81.2	2.4	90.2	3.8	93.6	3.6
Parathion	81.5	4.1	88.0	4.5	94.4	4.1
Isocarbophos	85.7	4.9	91.7	3.6	93.8	2.7
Triazophos	82.5	5.3	89.2	4.0	92.7	4.6

Conclusions and Discussion

(1) A method for detection and analysis of 14 OPP compounds in mutton was established based on a gel permeation chromatography (GPC) purification system and gas chromatography-mass spectrometry (GC-MS) technique.

(2) The components of OPP mixed standard solution all showed a good linear relationship in the mass concentration range of 0.1 − 10.0 mg/L, with correlation coefficients (r) over 0.999. The detection limits (S = 3N) of the method ranged from 0.01 to 0.05 mg/kg.

(3) The average recovery values were in the range of 80.2% − 99.7% and the relative standard deviations (RSDs) were in the range of 1.8% − 6.3%, at the addition levels of 1.0, 2.0 and 4.0 mg/kg.

The method has the advantages of simple and rapid sample pretreatment, good impurity removal effect, high sensitivity and good reproducibility, and can be used for the detection of OPP compounds in mutton.

References

[1] Ministry of Health of the People's Republic of China, Standardization Administration of the People's Republic of China. GB/T5009. 218-2008 Determination of multi pesticide residues in fruits and vegetables[S]. Beijing: China Standards Press, 2008. (in Chinese).

[2] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of the People's Republic of China. GB/T 23204-2008 Determination of 519 pesticides and related chemicals residues in tea: GC-MS method[S]. Beijing: China Standards Press, 2009. (in Chinese).

[3] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. SN/T 0123-2010 Determination of organophosphorus multiresidues in foodstuffs of animal origin for import and export—GC-MS method[S]. Beijing: China Standards Press, 2010. (in Chinese).

[4] YAO CC, SHI ZH, CAO YZ, et al. Determination of 164 pesticide residues in animal fat by gel permeation chromatography-gas chromatography-tandem mass spectrometry[J]. Chinese Journal of Analysis Laboratory, 2010, 29(2): 84 − 92. (in Chinese).

[5] LU DS, XIONG LB, WEN YM, et al. Application of QuEChERS method coupled with online GPC-GC/MS for determination pesticide residues in vegetables and fruits[J]. Journal of Chinese Mass Spectrometry Society, 2011, 32(4): 229 − 235. (in Chinese).

Comparative analysis of models

In order to further illustrate the effect of CARE-BP neural network model in identifying the origin of millet, 130 characteristic wavelengths were used as input variables to construct full-spectrum BP neural network, support vector machine (SVM), partial least squares discriminant analysis (PLS) and K-nearest neighbor algorithm (KNN) models, respectively. Table 2 shows the identification results of millet from different producing areas using different models. As can be seen from the table, the identification effect of the CARE-BP model was better than other four models. CARE-BP model not only has strong generalization ability when the number of samples is small, but also can be applied to the analysis of complex nonlinear spectra, so it is an effective method to identification of millet from different places.

Table 2 Comparison of different modeling results

Model	Number of variables	Rate of identification∥%
Full spectrum BP	1 845	90.2
CARE-BP	130	98.1
SVM	130	97.5
PLS	130	93.1
KNN	130	95.9

Conclusions and Discussion

In this study, the BP algorithm based on NIRS was adopted to effectively distinguish millet from different producing areas. In order to simplify the model and eliminate redundant spectral variables, the CARS method was applied to extract characteristic wavelengths, and a CARS-BP neural network model was constructed. Compared with other three classification models (SVM, PLS and KNN), this model showed obvious advantages, and the discrimination accuracy was as high as 98.1%. The research results showed that the CARE-BP neural network model showed high accuracy and stability in feature extraction and classification tasks. Compared with the traditional methods of sensory evaluation and physical and chemical tests, NIRS combined with CARS-BP model can quickly and accurately identify the origin of millet, providing a new method for the authenticity identification and quality evaluation of millet.

References

[1] LI X, WANG HH, SHEN Q. Studies on the quality characteristics of different varieties of millet[J]. Journal of Chinese Institute of Food Science and Technology, 2017, 17(7): 248 –254. (in Chinese).

[2] TIAN X, CHE Q, YAN WM, *et al.* Discrimination of millet varieties and producing areas based on infrared spectroscopy[J]. Spectroscopy and Spectral Analysis, 2022, 42(6): 1841 –1847. (in Chinese).

[3] LIANG KH, ZHU DZ, SUN JM. Study on variety and regional factors related to nutrient quality in millet[J]. The Food Industry, 2017, 38(4): 192 –196. (in Chinese).

[4] FENG NH, HOU DH, YANG CY, *et al.* Discrimination of millet varieties and producing areas based on infrared spectroscopy[J]. Science and Technology of Food Industry, 2020, 41(8): 224 –229. (in Chinese).

[5] SI CJ. Application of system genetics method based on protein interaction in identification of plant functional genes[D]. Wuhan: Huazhong Agricultural University, 2021. (in Chinese).

[6] SHA M, LI LC, HUANG JL, *et al.* Effect of data processing method on identification of rice from different geographical origins by Raman spectroscopy[J]. Journal of Chinese Institute of Food Science and Technology, 2021, 21(5): 369 –376. (in Chinese).

[7] WANG Y, LI Y, YE HZ, *et al.* Geographical origin discrimination of *Campsis grandiflora* by near-infrared spectroscopy coupled with support vector machine[J]. Journal of Fuzhou University: Natural Science Edition, 2022, 50(4): 568 –573. (in Chinese).

[8] YANG J, MA X, GUAN H, *et al.* A recognition method of corn varieties based on spectral technology and deep learning model[J]. Infrared Physics & Technology, 2023(128): 104533.

Editor: Yingzhi GUANG   Proofreader: Xinxiu ZHU

◦◦◦

[6] HUANG C, GUO M. Optimization of purifying agent combination for detection of pyrethroid pesticide residues in cucumbers by orthogonal test[J]. Chinese Journal of Food Hygiene, 2012, 24(5): 438 –440. (in Chinese).

[7] KANG WJ. Application of gas chromatograph in detection of organophosphorus pesticide residues in vegetables[J]. Hunan Agricultural Sciences, 2010(1): 76 –78. (in Chinese).

[8] WANG DQ, HAN MH. Differences in stability of nine pesticides during the process of gas chromatography column[J]. Journal of Zhejiang Agricultural Sciences, 2010(1): 113 –115. (in Chinese).

[9] FRENICH AG, BOLANOS PP, VIDAL JLM. Multiresidue analysis of pesticides in animal liver by gas chromatography using triple quadrupole tandem mass spectrometry[J]. Journal of Chromatography, A, 2007, 1153: 194 –202.

[10] LIU GP, HUANG C, XUE RX, *et al.* Determination of 14 organophosphorus pesticides and 7 pyrethroids pesticides in five kinds of food by GPC and GC-MS/MS[J]. Chinese Journal of Food Hygiene, 2014, 26(4): 366 –372. (in Chinese).

Editor: Yingzhi GUANG   Proofreader: Xinxiu ZHU