

The Application of Pesticides in the Production of Medicinal Plants in China

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Pesticide residues in Traditional Chinese Medicines (TCMs)

- In the early 1980s, starting the pesticide residue researches of TCMs
- Focusing on the researches of cumulative toxicity pesticides, such as: BHC, DDT, Pentachloronitrobenxene (PCNB) and drinox
- Investigating the half-life periods of pesticides in TCMs

The major projects of pesticide resides in TCM

- Dring 'the 9th Five-Year Plan' (1996-2000), the project of "Standardization of the Quality of Chinese Meteria Medica", including the researches of residual limits of the organochlorine (OCPs) in 71 kinds of TCM
- Improved the research level on pesticide resides in TCM
- Disclosed the polluted conditions of organic pesticides in commonly used TCMs.
- Providing a substantial data base for Good Agricultural Practice (GAP) on materials of TCM

The major projects of pesticide resides in TCM

- Dring 'the 10th Five-Year Plan' (2000-2005), the project of "Researches on the detection and limited standards of 50 kinds of TCMs", including the researches of residual limits of the pyrethroids and organicphosphorus in 50 kinds of TCM
- The achievements were officially recorded in the 2005 version of Chinese Pharmacopoeia



Analytical methods and residual limits of pesticides of TCM in Chinese Pharmacopoeia

- Chinese Pharmacopoeia (CP, 2000 version): establishing the methods and residual limits of nine organochlorines (OCPs)
- CP (2005 version): adding "Detection methods of three pyrethroid residues in TCM",
 "Detection methods of twelve organic phosphorus residues in TCM"
- CP (2010 Edition): recording the analytical methods for three pyrethroids, nine OCPs, and twelve organophosphorous pesticides (OPPs)



Standards of pesticide residues in TCM

- Green Standards of Medicinal Plants and Preparations for Foreign Trade and Economy of Ministry of Commerce of the People's Republic of China (GSMPP): ten Organochlorines (OCPs) (BHC, DDT, pentachloronitrobenxene (PCNB), Dieldrin)
- CP (2010 Edition): stipulated the maximum residue levels (MRLs) of nine OCPs, including BHC, DDT, and PCNB, only for *Radix et Rhizome Glycyrrhizae* and *Radix Astragali*

The maximum residue levels (MRLs) of pesticides in TCM

Organochlorines (OCPs)	Standards	TCMs	Limit Standards (mg/kg)
α -BHC, β -BHC,		Radix et Rhizome Glycyrrhizae and Radix Astragali	0.2
γ-BHC , δ- BHC	GSMPP	Medicinal Plants and Preparations	0.1
DDT: pp'-DDE, op'-DDT, pp- 'DDD, pp'-DDT	CP (2010 Edition)	Radix et Rhizome Glycyrrhizae and Radix Astragali	0.2
<i>սսս</i> , pp -սս 1	GSMPP	Medicinal Plants and Preparations	0.1
Pentachloronitrobenxene (PCNB)	CP (2010 Edition)	Radix et Rhizome Glycyrrhizae and Radix Astragali	0.1
	GSMPP	Medicinal Plants and Preparations	0.1
Dieldrin	GSMPP	Medicinal Plants and Preparations	0.02

Prohibited pesticides in China (23 kinds) (Ministry of Agriculture of the People's Republic of China)

- Announcement No. 199 (18): BHC, DDT, strobane, dibromochloropropane, chlordimeform, EDB, nitrofen, aldrin, dieldrin, mercury compounds, arsenide, plumbum compounds, N,N'-methylene bis -(2 -amino 1,3,4 -thiadiazole) (Bis-ADTA), fluoroacetamide, gliftor, tetramine, sodium fluoroacetate, silatrane
- Announcement No. 274 (5): methamidophos, parathion-methyl, parathion, monocrotophos and phosphamidon (Table 1)

Table 1 Prohibited pesticides in China

	П		

Classification	Pesticide
Organochlorine pesticide (7)	НСН
	DDT
	Camphechlor
	Dibromochloropane
	EDB
	Aldrin
	Dieldrin
Organophosphorus pesticide (5)	Methamidophos
	Parathion-methyl
	Parathion
	Monocrotophos
	Phosphamidon

Continued



Formamidine pesticide (1)	Chlordimeform
Organofluorine pesticide (1)	Fluoroacetamide
Fungicide (4)	Mercury compounds
	Bis-ADTA
	Arsenide
	Plumbum compounds
Herbicide (1)	Nitrofen
Rodenticide (4)	Gliftor
	Tetramine
	Sodium fluoroacetate
	Silatrane

Prohibited pesticides in the production of vegetable, fruit tree, tea and TCM

(Ministry of Agriculture of the People's Republic of China)

Announcement No. 199 (14): phorate, isofenphos-methyl, terbufos, phosfolan-methyl, sulfotep, demeton, carbofuran, aldicarb, ethoprophos, phosfolan, coumaphos, fonofos, isazofos, fenamiphos (Table 2)

Table 2 Prohibited pesticides in the production of vegetable, fruit tree, tea and TCM

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Classification	Pesticides
Organophosphorus pesticide (12)	Phorate
	Isofenphos-methyl
	Terbufos
	Phosfolan-methyl
	Sulfotep
	Demeton
	Ethoprophos
	Phosfolan
	Coumaphos
	Fonofos
	Isazofos
	Fenamiphos
Carbamate pesticide (2)	Carbofuran
	Aldicarb



The principle of pesticide application in the production of TCM in GAP

- Complying with Good Agricultural Practice (GAP) of State Food and Drug Administration (SFDA)
- Complying with the regulations of Ministry of Agriculture of the People's Republic of China
- Applying high effect, low toxicity and residue pesticides in the production of TCM



The Application of pesticides in the production of TCM in GAP

- Insecticides (21): including organochlorine
 (4), organophosphorus (9), pyrethroid (4),
 formamidine (1), others (3)
- Fungicides (31)
- Plant growth regulator (1)
- Total: **53**

Classification	Pesticides	Application
Organophosphorus pesticides	Trichlorfon	Gentiana scabra Sophora flavescens Platycodon grandiflorus Lonicera japonica Ggastrodia elata Angelica dahurica Houttuynia cordata Scrophularia ningpoensis Comus officinalis Alisma orientalis Erigeron breviscapus Andrographis paniculata Pogostemon cablin
	Dichlorvos	Cornus officinalis Ginkgo biloba Lonicera japonica Polygonum capitatum Pogostemon cablin

		Codonopsis pilosula Cornus officinalis
Organophosphorus pesticides	Dimethoate	Ggastrodia elata Gynostemma pentaphyllum Aconitum carmichaelii Ligusticum chuanxiong Tussilago farfara Alisma orientalis Pogostemon cablin
	Pphoxim	Fritillaria ussuriensis Schisandra chinensis Platycodon grandiflorus Lonicera japonica Rehmannia glutinosa Codonopsis pilosula Ggastrodia elata Coptis chinensis Artemisia annua Andrographis paniculata

		Panax ginseng Astragalus membranaceus Isatis indigotica Schizonepeta tenuifolia
Fungicides	Carbendazim	Angelica sinensis Platycodon grandiflorus Lonicera japonica Rehmannia glutinosa Dioscorea opposita Ginkgo biloba Codonopsis pilosula Salvia miltiorrhiza Gynostemma pentaphyllum Aconitum carmichaelii Ligusticum chuanxiong Angelica dahurica Houttuynia cordata Coptis chinensis Artemisia annua Scrophularia ningpoensis Comus officinalis Dendrobium candidum Pseudostellaria heterophylla Polygonum capitatum Erigeron breviscapus Pogostemon cablin

Fungicides	Chlorothalonil	Fritillaria ussuriensis Isatis indigotica Ligusticum chuanxiong Dendrobium candidum Pseudostellaria heterophylla
	Thiophanate-methyl	Fritillaria ussuriensis Gentiana scabra Astragalus membranaceus Angelica sinensis Platycodon grandiflorus Ligusticum chuanxiong Angelica dahurica Tussilago farfara Coptis chinensis Dendrobium candidum Andrographis paniculata

		Fritillaria ussuriensis Panax ginseng Gentiana scabra Schisandra chinensis Astragalus membranaceus
Fungicides	Mancozeb	Lonicera japonica Salvia miltiorrhiza Angelica dahurica Houttuynia cordata Artemisia annua Pseudostellaria heterophylla Erigeron breviscapus
	Bordeaux mixture	Isatis indigotica Panax ginseng Schisandra chinensis Schizonepeta tenuifolia Lonicera japonica Rehmannia glutinosa Aconitum carmichaelii Ophiopogon japonicus Angelica dahurica Tussilago farfara Coptis chinensis Comus officinalis Dendrobium candidum Alisma orientalis Pogostemon cablin



Fungicides	Zineb Thiophanate	Panax quinquefolius Platycodon grandiflorus Rehmannia glutinosa Ophiopogon japonicus Tussilago farfara Comus officinalis Dendrobium candidum		
	Thiophanate	Schizonepeta tenuifolia Lonicera japonica Gynostemma pentaphyllum Scrophularia ningpoensis Pseudostellaria heterophylla Alisma orientalis Pogostemon cablin		

Detection technology of pesticide residues in TCM

- Developing various analysis methods of pesticide residues in TCM rapidly
- Different extraction and clean-up methods: liquid-liquid extraction (LLE), solid phase extraction (SPE), solid-phase micro-extraction (SPME) and so on



Analytical methods

- The application of GC, LC, GC-MS, LC-MS and multiple-stage mass spectrometry techniques
- Remarkably increasing the qualitative capability, the detection sensitivity, detection limits and detection coverage of pesticide residues in TCM

Case studies

Degradation dynamics of carbendazim, daconil and deltamethrin in *Folium Isatidis*

Folium Isatidis (Daqingye), the leaf of the Isatis indigotica plant





Beijing Tong Ren Tang Hebei Yutian Good Agricultural Practice base



Folium Isatidis (Daqingye)

- Main active constituents: indirubin and indigotin
- Indirubin: exhibiting anticancer and antiinflammatory activities
- Treating fever, skin eruptions, jaundice, acute dysentery, mumps, inflammation of the throat, erysipelas, and carbuncle
- Inhibiting ConA-mediated mouse T lymphocyte activation and proliferation



Carbendazim

- Methyl 2benzimidazolecarbamate
- Systemic benzimidazole fungicide
- Treating leaf spots, blotches and blights, root rots, and other diseases in plants



Daconil

- 2,4,5,6-tetrachloro-1,3benzenedicarbonitrile
- A non-systemic fungicide
- Effective against a broad range of plant pathogens attacking many agronomicand vegetable crops



Deltamethrin

- (S)--cyano-3-phenoxybenzyl-(1R)cis-3-(2,2-dibromovinyl)-2,2dimethylcyclopropane carboxylate
- A synthetic type II pyrethroid insecticide
- Used in veterinary products to control lice, flies, and ticks on cattle, sheep, and pigs
- Controling numerous insect pests on fruits, vegetables, and field crops.



Formulated Pesticides

- 50% ultramicro-wettability of carbendazim (Shandong Sishui Fengtian Pesticide Co., Ltd., China. Batch No. 040608)
- 75% daconil wettable powder (Fine Chemicals Limited Company of Jiangsu, Batch No. 040601).
- 12.5% deltamethrin emulsifiable concentrates (Bayer Pharma Co., Batch No.050302)



Field trials

- Performed on Beijing Tong Ren Tang Hebei
 Yutian Good Agricultural Practice base
- Setting up three treatments (including a control), with 3 replicates each of 24 m²
- Using 27 field plots in total
- Separating each plot by a protective row.



The design of field trials

I -1	I -ck	I -2	II -1	II -ck	II -2	Ⅲ-1	III-ck	III-2
II -1	II -ck	II -2		III-ck	III-2	I -1	I -ck	I -2
			•					
III-1	III-ck	III-2	I -1	I -ck	I -2	II -1	II -ck	II -2

Processing: I - Carbendazim; II - Daconil; III - Deltamethrin

Level: I -ck; I -1; I -2; II -ck; II -1; II -2; III -ck; III -1; III -2

ck-control; 1-recommended normal dose; 2-double the maximal dose



Sample collection

- Representative samples (≈1 kg) were taken using a method of five-plot sampling 2 h after spraying and 1, 3, 7, 14, 21, 30, 45, and 60 d after spraying
- All samples were frozen at -18°C until pesticide residues were analyzed



Degradation dynamics of carbendazim

- **Extracted residues of carbendazim** from *Folium Isatidis* with acetone
- Purified by liquid/liquid partition
- Determined by HPLC



Statistical analysis

- The degradation kinetics of carbendazim in Folium Isatidis was determined by plotting residue concentration against time
- The rate equation was calculated from the first order rate equation: $C_t = C_0 e^{-kt}$ where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration, and k is the rate constant in days⁻¹.
- The half-lives $(t_{0.5})$ were determined from the k value for each experiment, $t_{0.5}=\ln 2/k$

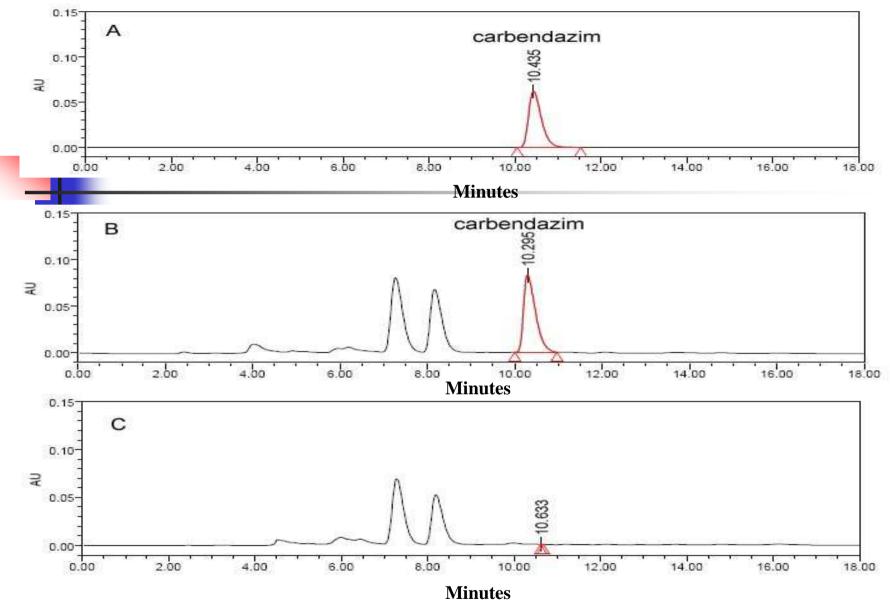


Figure 1. HPLC chromatograms for the analysis of carbendazim A: Carbendazim standard B: Sample with carbendazim C: Sample without carbendazim

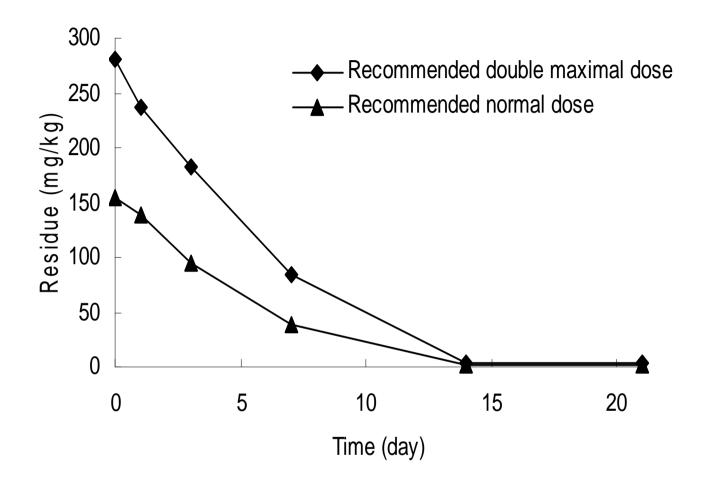


Figure 2. The degradation curves of carbendazim in Folium Isatidis

Carbendazim degradation dynamics

- Residues were undetectable by day 30 after application.
- The half-life of carbendazim at the recommended double maximal dose in *Folium Isatidis* was 2.92 days, and the dynamics could be described by the equation (first-class model degradation, C=306.65e^{-0.2371t}) with square of coefficient *R*²=0.9372.
- The half-life of carbendazim at the recommended normal dose in *Folium Isatidis* was 2.68 days, and the dynamics could be described by the equation (C=169.30e^{-0.2585t}) with square of coefficient *R*²=0.9411.

The harvest interval for Folium Isatidis

- From the results, 21 days after spraying, carbendazim residues (1.27 mg kg⁻¹ at the normal dose and 3.39 mg kg⁻¹ at the double maximal dose) were higher than the maximum residue limit allowed in China (GB 2763-2005, ≤0.5 mg kg⁻¹).
- The harvest interval for *Folium Isatidis* should be 23 days after spraying with the normal dose and 28 days after spraying with the double maximal dose of carbendazim.

Degradation dynamics of Daconil

- Extracted residues of Daconil from Folium Isatidis with acetidin
- Cleaned up using a home-made florisil cartridge previously activated with nhexane (the cartridge was filled with 1 g anhydrous sodium sulfate, 3 g florisil, 1 g anhydrous sodium sulfate)
- Separated directly by EQUITY-1701 capillary column (30 m × 0.25 mm × 0.25 mm)
- Determined using GC-ECD

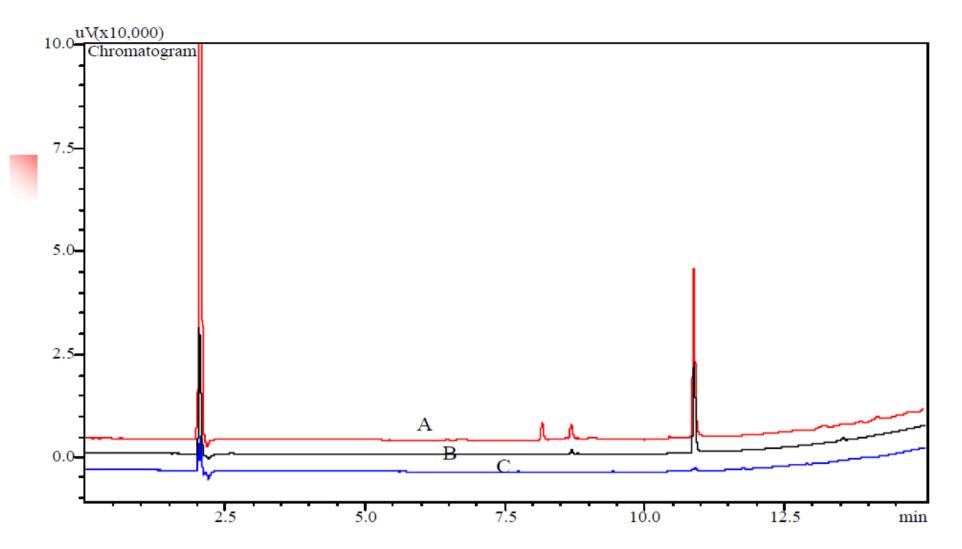


Figure 3. GC Chromatograms for the analysis of Daconil: Sample (A), Standard (B), and Blank (C)

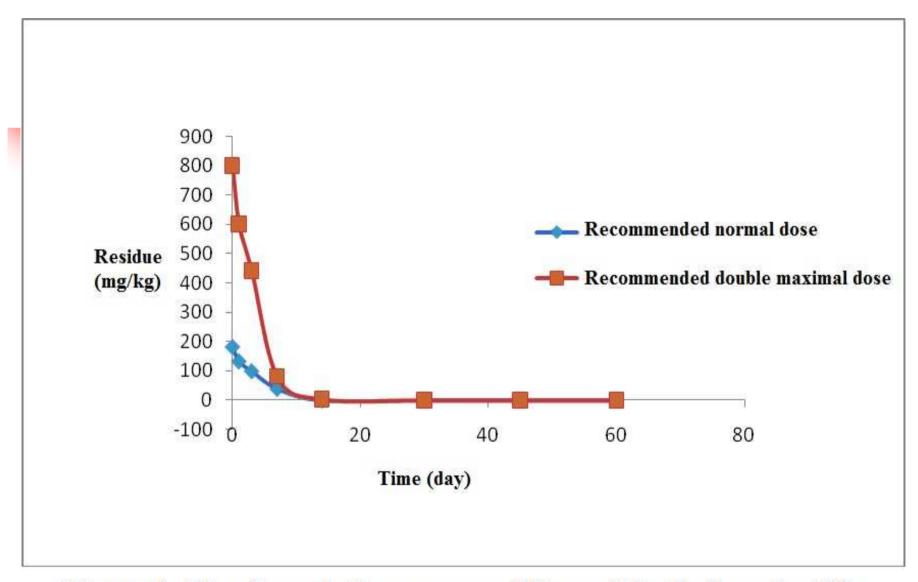


Figure 4. The degradation curves of Daconil in Folium Isatidis

Daconil degradation dynamics

- The dual-chamber models are used for the characteristic initial degradation.
- Choosing 15 days as the turning point, the degradation equation of daconil at the recommended double maximal dose in *Folium Isatidis* is $C = 885.7192e^{-0.3325t} + 6.3452 e^{-0.0709t}$; for recommended normal dose of daconil in Folium Isatidis is $C = 176.4787e^{-0.2127t} + 0.3324 e^{-0.0174t}$
- For high-concentration of daconil, early half-life $t_{0.5}=2.08$ days, the coefficient $r_{high1}=0.9825$ and the late half-life $t_{0.5}=9.77$ days and the coefficient $r_{high2}=0.9263$
- For low concentration of daconil, early half-life $t_{0.5}=3.26$ days and the coefficient $r_{\rm low1}=0.9960$, and the late half-life $t_{0.5}=39.83$ days and the coefficient $r_{\rm low2}=0.9652$

The harvest interval for Folium Isatidis

- China's national standard GB 2763-2005: maximum residual of daconil on vegetables ≤5 mg kg⁻¹.
- The safety interval of high and low concentrations of daconil in *Folium Isatidis* is 17 days.



Degradation dynamics of deltamethrin

- Extracted residues of deltamethrin from Folium Isatidis with acetidin
- Cleaned up using a home-made florisil cartridge previously activated with petroleun ether:Diethyl Ether (4:1, v/v) (the cartridge was filled with 2 g anhydrous sodium sulfate, 1 g neutral alumina, 2 g florisil)
- Separated directly by DB-5MS capillary column (0.25mm×30m,0.25mm)
- Determined using GC-ECD

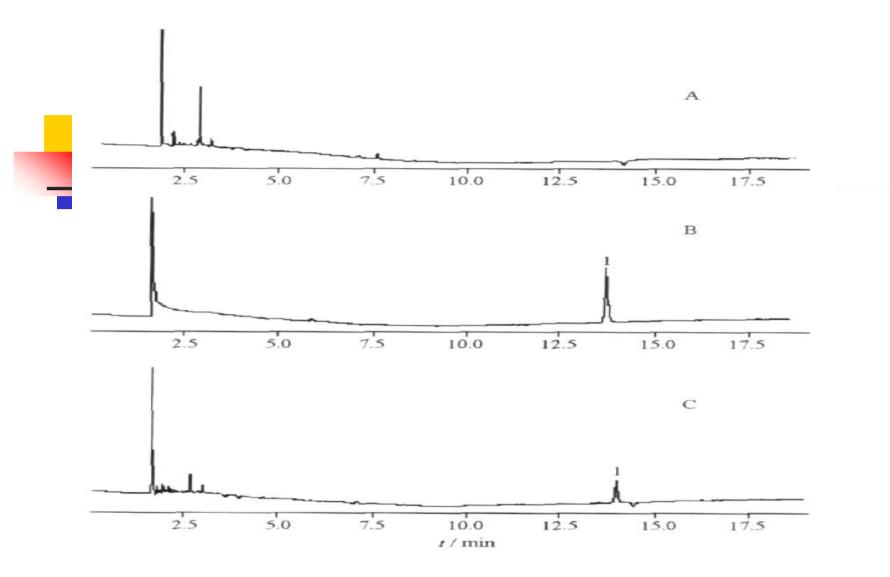


Figure 5. GC Chromatograms for the analysis of deltamethrin:
Blank (A), Standard (B), and Sample (C)
1- deltamethrin

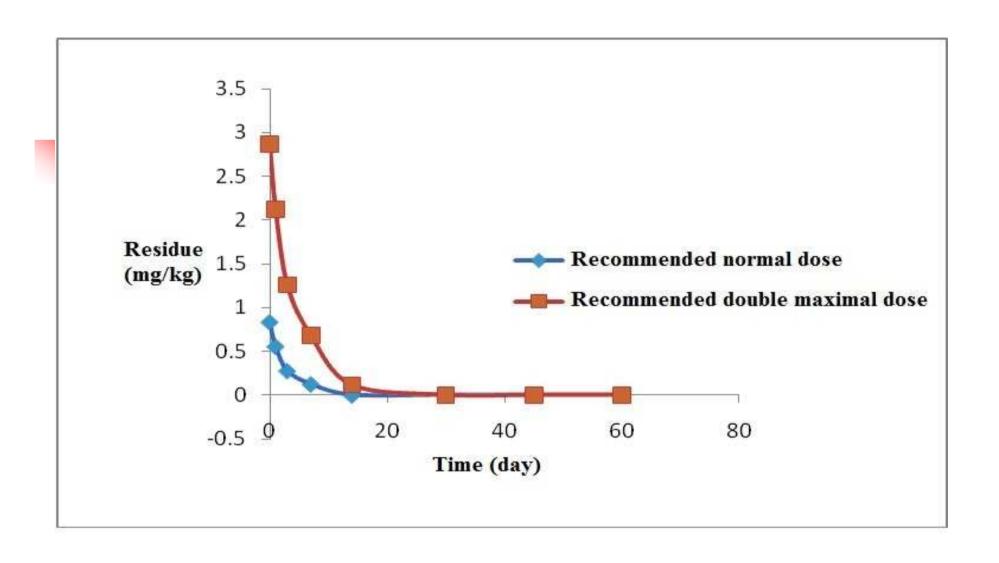


Figure 6. The degradation curves of deltamethrin in Folium Isatidis

Deltamethrin degradation dynamics

- The half-life of deltamethrin at the recommended double maximal dose in *Folium Isatidis* was 3.31 days, and the dynamics could be described by the equation (first-class model degradation, C=2.6714e^{-0.2091t}) with square of coefficient r=0.9976.
- The half-life of deltamethrin at the recommended normal dose in *Folium Isatidis* was 2.61 days, and the dynamics could be described by the equation (C=0.7323e^{-0.2652t}) with square of coefficient *r*=0.9866.

The safety interval for Folium Isatidis

- China's national standard GB 14928.4-94: maximum residual of deltamethrin on vegetables of leaves ≤0.5 mg/kg.
- The safety interval of high and low concentrations of deltamethrin in *Folium Isatidis* is 8.01 and 1.44 days, respectively.

Conclusions

- China's national standard GB 2763-2005 carbendazim residues ≤0.5 mg kg⁻¹; GB 2763-2005 daconil residues ≤5 mg kg⁻¹; GB 14928.4-94 deltamethrin residues ≤0.5 mg kg⁻¹
- The safety interval of high and low concentrations of carbendazim in *Folium Isatidis* is 28 and 23 days, respectively.
- The safety interval of high and low concentrations of daconil in *Folium Isatidis* is 17 days.
- The safety interval of high and low concentrations of deltamethrin in *Folium Isatidis* is 8.01 and 1.44 days, respectively.





Simultaneous determination of 18 organophosphorus pesticides in Chinese medicinal health wines by GC-FPD

- Consumed as liquor in many areas of Asia
- Playing an important role in Asian life, culture and diet since ancient times
- High nutritive value, special flavor and health function
- The quality and safety of wine attracting more interest for consumers in the world



Organophosphorus pesticides (OPPs)

- The most frequently applied pesticides worldwide
- Applied to the raw herbs to reduce disease and insect pests
- Resulting in the presence of OPPs residues in agricultural products and derivative food commodities
- Evaluating food safety and possible risks to human health



Determination of OPPs

- **Extracted and cleaned up by** acetone-dichloromethane (1: 1, *V/V*)
- Determined by GC-FPD
- Confirmed by GC-MS

18 OPPs

dichlorovos, phorate, dimethoate, diazinon, disulfoton, parathion-methyl, fenitrothion, malathion, fenthion, durshan, parathion, isocarbophos, quinalphos, methidathion, ethion, triazophos, phosmet, phosalone





Chinese medicine health wine samples

25 kinds, 80 samples: ningxiahong (20), Chinese jing wine (13), lianhua Chinese spirits (5), yedaolugui wine (3), fenglin wine (5), diyi wine (3), hawthorn wine (2), yishebian wine (2), yishewang wine (1), yisheshengbao wine (1), shiguogong wine (1), cordyceps sinensis wine (1), sanbian wine (2), red jingtian wine (1), ginseng wine (1), felicitous plant wine (1), herba saussureae involucratae wine (1), tall gastrodia tuber wine (1), chinese magnoliavine fruit wine (1), desertliving cstanche wine (1), ningxiner wine (2), zhuyeqing wine (2), jiafang wine (2), gucixiaotongye (4), guogong wine (3)

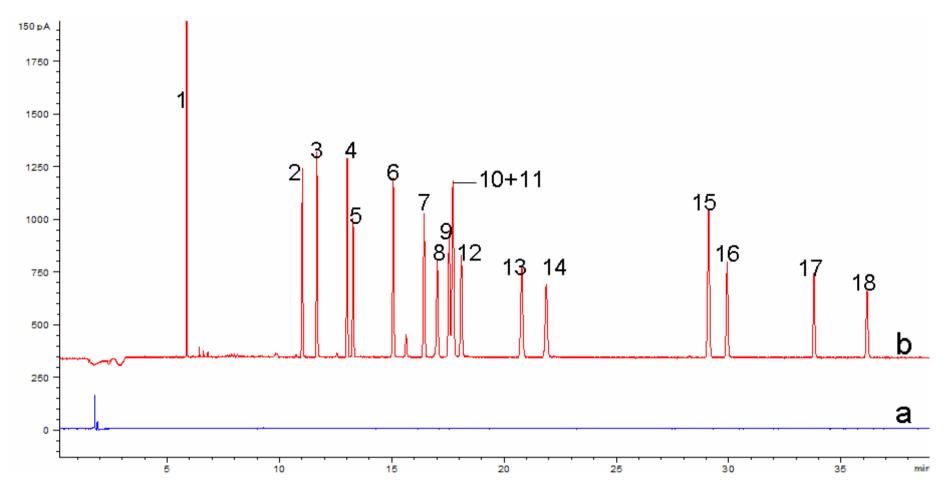


Figure 7. GC-ECD chromatograms of (a) control sample and (b) spiked sample of 18 OPPs. 1. dichlorovos 2. phorate 3. dimethoate 4. diazinon 5. disulfoton 6. darathion-methyl 7. fenitrothion 8. malathion 9. fenthion 10+11. durshan+ parathion 12. isocarbophos 13. quinalphos 14. methidathion 15. ethion 16. triazophos 17. phosmet 18. phosalone

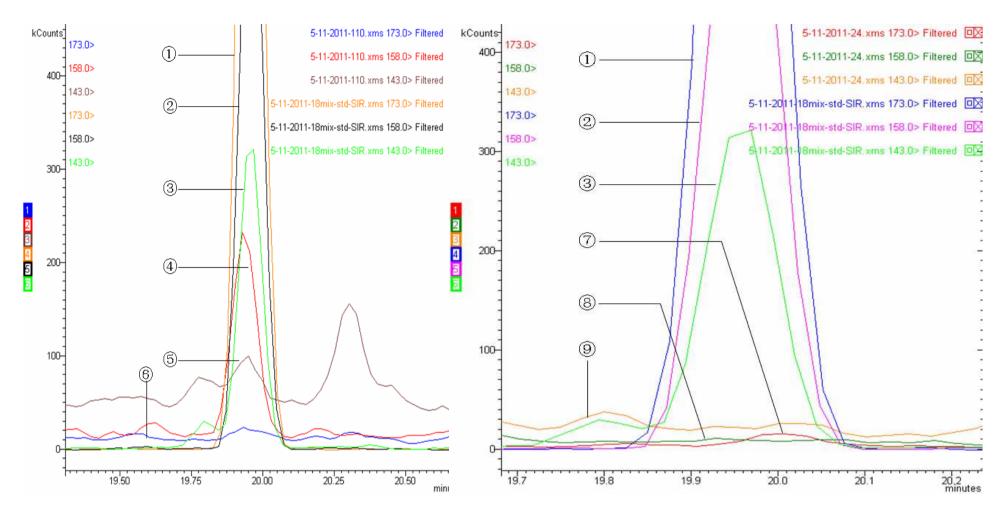


Fig. 3. Selected ion monitoring chromatogram of malathion from GC-MS confirmation: the comparison between standard substance and positive sample (No.37) (a) and the comparison between standard substance and negative sample (No.60) (b) ①: malathion quantitative ion (173) in standard solutions ②: malathion qualitative ion 1 (158) in standard solutions ③: malathion qualitative ion 2 (143) in positive sample (No.37) ⑤: malathion qualitative ion 2 (143) in positive sample (No.37) ⑦: malathion quantitative ion (173) in negative sample (No.60) ③: malathion qualitative ion 1 (158) in negative sample (No.60) ⑨: malathion qualitative ion 2 (143) in negative sample (No.60)

No.	Name	Batch Number	Chinese medicine raw material	Analytes and Content (ng/mL)
34	lianhua Chinese spirits	20090321	lotus plumule, cloves, cassia bark, taiwan angelica root, villous amonmum fruit, nutmeg	malathion (11.9); phosalone (10.4)
36	lianhua Chinese spirits	20090823	lotus plumule, cloves, cassia bark, taiwan angelica root, villous amonmum fruit, nutmeg	malathion (20.2); phosalone (9.7)
37	lianhua Chinese spirits	20100110	lotus plumule, cloves, cassia bark, taiwan angelica root, villous amonmum fruit, nutmeg	malathion (12.7); phosalone (10.6)
38	lianhua Chinese spirits	20090420	lotus plumule, cloves, cassia bark, taiwan angelica root, villous amonmum fruit, nutmeg	malathion (12.3); phosalone (10.2)
54	yisheshengbao wine	20091008	sharpleaf galangal fruit, siberian solomonseal rhizome, barbury wolfberry fruit, common yam rhizome, cassia bark, chinese date	phosmet (10.0)
55	yishebian wine	20090815	sharpleaf galangal fruit, siberian solomonseal rhizome, barbury wolfberry fruit, common yam rhizome, cassia bark, chinese date	phosmet (<loq)< td=""></loq)<>
61	red jingtian wine	home made	kirilow rhodiola root and rhizome	methidathion (<loq)< td=""></loq)<>
62	ginseng wine	home made	ginseng	malathion (8.2); phosmet (37.9)

Table 4. The contents of 18 OPPs in 8 positive samples (EU, 0.01-10 mg kg-1)



Conclusions

- A suitable method is proposed for extracting 18 OPPs in CM health wine.
- GC-FPD was applied for determining these pesticides in these wine samples from different origins.
- Total analysis time is 50 min (10 min for extraction plus 39 min for chromatography).



Summary

- The inappropriate use of pesticides in the production of medicinal plants has seriously affected the quality of TCM
- Implementation of GAP for Chinese medicinal plants is an important way to ensure the quality of TCM



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