衞生署
中醫藥規管辦公室

Chinese Medicine
Regulatory Office
Department of Health

Technical Meeting on Testing Methods for the Amendments to the Limits of Pesticide Residues in Chinese Herbal Medicines

29th May 2024

政府中藥檢測中心
Government Chinese Medicines Testing Institute

衞生署
Department of Health

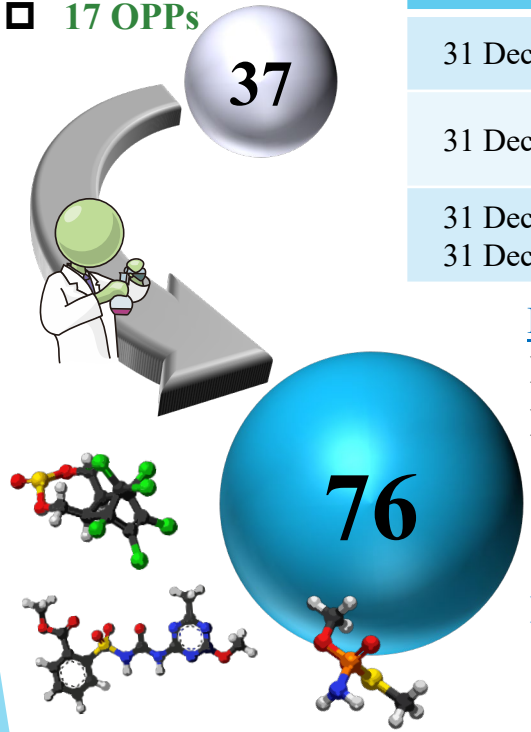
Content

衞生署
Department of Health

An Overview of the Amendments

Current Scope

- 20 OCPs
- 17 OPPs



Revise regulatory limits by phases

Date	Amendment
31 Dec 2021	1. Repeal the pesticide residue limits for Chm from animal & mineral origin
31 Dec 2024	2. Amendment of pesticide residue limits for **542** Chms from herbal origin (except DDT residues in Radix Panacis Quinquefolii (西洋參))
31 Dec 2026 31 Dec 2028	3. Amendment of DDT residue limit in Radix Panacis Quinquefolii (西洋參) in two phases

Revised Scope

- Adopt general limits set in Chinese Pharmacopoeia (ChP) 2020 Edition
- **55 pesticides in 33 categories** should not be detected
  - **22 OCPs**
  - **26 OPPs**
  - **7 Carbamate pesticides** and **others**
- **21 pesticides** not included in ChP2020, continue to adopt the current limits
  - **10 OCPs**
  - **11 OPPs**

衛生署
Department of Health

4 Revised Regulations

➢ 76 pesticide residues

Organophosphorus Pesticides

- **37 OPPs** in **30** categories

1. Methamidophos
2. Parathion-methyl
3. Parathion
4. Monocrotophos
5. Phosphamidon
6. Fenamiphos
7. Fonofos
8. Cadusafos
9. Coumaphos
10. Sulfotep
11. Terbufos
12. Phorate
13. Isofenphos-methyl
14. Demeton
15. Ethoprophos
16. Isazofos
17. Isocarbophos
18. Phosfolan
19. Phosfolan-methyl
20. Dichlorvos
21. Trichlorphon
22. Omethoate
23. Diazinon
24. Dimethoate
25. Malathion
26. Triazophos
27. Chlorpyriphos
28. Acephate
29. Ethion
30. Methidathion

Organochlorine Pesticides

- **32 OCPs** in **15** categories

1. HCH
2. DDT
3. Chlordimeform
4. Nitrofen
5. Aldrin
6. Dieldrin
7. Chlorsulfuron
8. Endosulfan
9. Fipronils
10. Dicofols
11. Chlordane
12. Endrin
13. Heptachlor
14. HCB
15. Quintozene

Carbamate and Others

- **7 pesticides** in **4** categories (carbamates & others)

1. Ethametsulfuron-methyl
2. Metsulfuron-methyl
3. Carbofuran
4. Aldicarb

- pesticides **"SHOULD NOT BE DETECTED"**
- pesticides continue to adopt the current limits

衞生署
Department of Health

Revised Regulations

Point to note

Current Regulations (OCPs)

No.	Name	Test Parameter(s)	MRL* (mg/kg)
1.	Aldrin and Dieldrin	Sum of Aldrin and Dieldrin	0.05
2.	DDT	Sum of *p,p*'-DDT, *o,p*'-DDT, *p,p*'-DDE and *p,p*'-TDE	1.0
3.	HCH	Sum of α-, β- and δ-isomers	0.3
4.	Lindane	Lindane (γ-isomer)	0.6

*** Denote "Maximum Residue Limit"**

 # Refer to Chinese Pharmacopoeia 2020 Edition for the limit of quantification for pesticide residue in Chinese herbal medicines

Revised Regulations (OCPs)

No.	Name	Test Parameter(s)	Regulatory Limit
1.	Aldrin	Aldrin	Should not be detected#
2.	Dieldrin	Dieldrin	
3.	DDT	Sum of *p,p*'-DDT, *o,p*'-DDT, *p,p*'-DDE and *p,p*'-TDE, expressed as **DDT**	
4.	HCH	Sum of α-, β-, δ- and γ-isomers, expressed as **HCH**	
5.	Endosulfan (***New***)	Sum of α-endosulfan, β-endosulfan and endosulfan sulphate, expressed as **endosulfan**	Should not be detected#
6.	Fipronil (***New***)	Sum of fipronil, fipronil desulfinyl, fipronil sulfone and fipronil sulfoxide, expressed as **fipronil**	

Revised Regulations

Point to note

Revised Regulations (OPPs)

No.	Name	Test Parameter(s)	Regulatory Limit
1.	Fenamiphos (*New*)	Sum of fenamiphos, fenamiphos sulphoxide and fenamiphos sulphone, expressed as **fenamiphos**	Should not be detected#
2.	Phorate (*New*)	Sum of phorate, phorate sulphoxide and phorate sulphone, expressed as **phorate**	
3.	Terbufos (*New*)	Sum of terbufos, terbufos sulfone and terbufos sulfoxide, expressed as **terbufos**	

Revised Regulations (Carbamate)

No.	Name	Test Parameter(s)	Regulatory Limit
1.	Carbofuran (*New*)	Sum of carbofuran and carbofuran-3-hydroxy, expressed as **carbofuran**	Should not be detected#
2.	Aldicarb (*New*)	Sum of aldicarb, aldicarb sulphoxide and aldicarb sulphone, expressed as **aldicarb**	

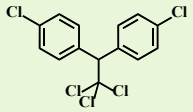
Refer to Chinese Pharmacopoeia 2020 Edition for the limit of quantification for pesticide residue in Chinese herbal medicines

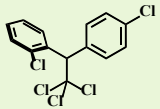
Residue Definition(s)

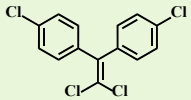
- Example

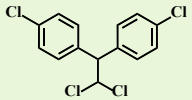
Current Regulations

Residual Level (mg/kg) = Sum of

(i) *p,p'*-DDT  +

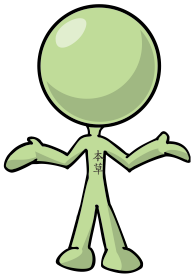
(ii) *o,p'*-DDT  +

(iii) *p,p'*-DDE  +

(iv) *p,p'*-TDE 

DDT

Maximum Residue Limit (1.0 mg/kg)

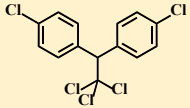


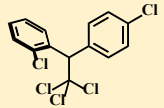
DDT

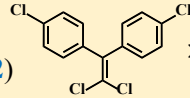
Should not be detected (except 西洋參)

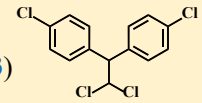
Revised Regulations

Residual Level (mg/kg) = Sum of

(i) *p,p'*-DDT (M.W. **354.48**)  +

(ii) *o,p'*-DDT (M.W. **354.48**)  +

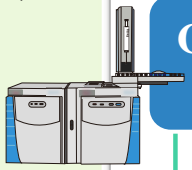
(iii) *p,p'*-DDE (M.W. **318.02**)  × $\frac{354.48}{318.02}$ +

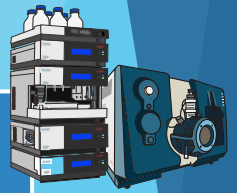
(iv) *p,p'*-TDE (M.W. **320.03**)  × $\frac{354.48}{320.03}$

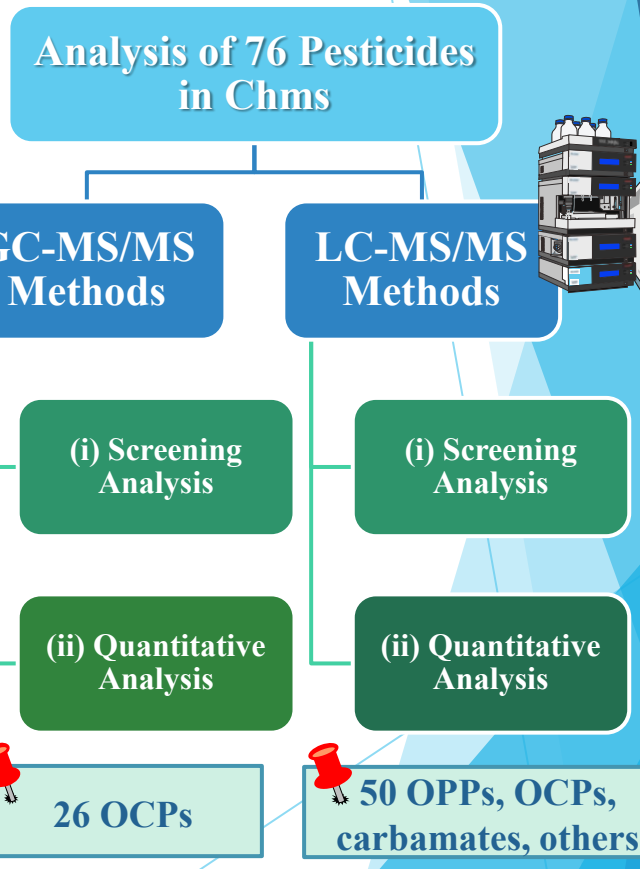
(expressed as DDT)

Method Summary

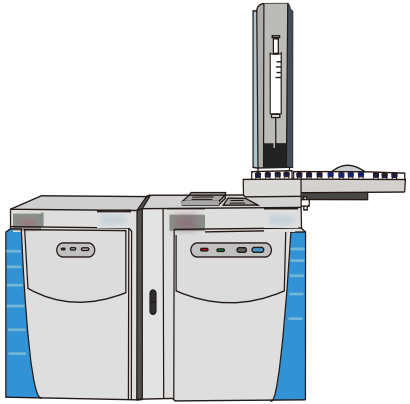
- Two techniques
  - GC-MS/MS (**26 OCPs**)
  - LC-MS/MS (**50 OPPs, OCPs, carbamates, others**)
- Two-tier approach
  - Screening Analysis
  - Quantitative Analysis
- Screening Analysis
  - Use isotopically labeled internal standards or internal standards
  - Set screening detection limit (SDL)
  - Conc. of analyte ≥ SDL → "**Suspected Positive**"
  - Subject to confirmation by quantitative method
- Quantitative Analysis
  - Use isotopically labeled internal standards
  - Refer to ChP2020 for the limits of quantification
- Matrix-matched calibration (All methods)
- Various clean-up kits
  - dSPE
  - SPE
  - Alumina

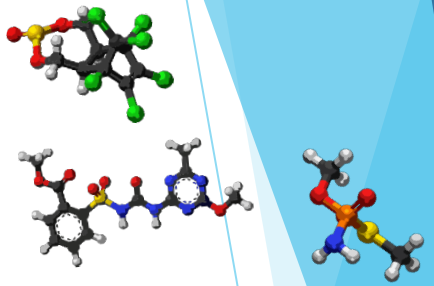






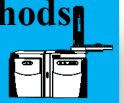
GC-MS/MS Methods





Targeted Pesticides

GC-MS/MS Methods



- 26 targeted OCPs

1.	α-BHC	**15.**	*o,p'*-dicofol
2.	β-BHC	**16.**	*p,p'*-dicofol
3.	δ-BHC	**17.**	*cis*-chlordane
4.	γ-BHC	**18.**	*trans*-chlordane
5.	*p,p'*-DDT	**19.**	oxychlordane
6.	*o,p'*-DDT	**20.**	Endrin
7.	*p,p'*-DDE	**21.**	Heptachlor
8.	*p,p'*-TDE	**22.**	*cis*-heptachlor epoxide
9.	Nitrofen	**23.**	HCB
10.	Aldrin	**24.**	PCNB
11.	Dieldrin	**25.**	PCA
12.	α-endosulfan	**26.**	MPCPS
13.	β-endosulfan		
14.	Endosulfan sulphate		

Suggested I.S. for Screening Analysis

1.	PCB 138-$^{13}C_{12}$
2.	PCB 52-$^{13}C_{12}$
3.	Dicofol-d^8

- **Solvent: *iso*-octane**

Suggested Labelled I.S. in Quantitative Analysis

1.	α-BHC-$^{13}C_6$	**10.**	Aldrin-$^{13}C_{12}$	**19.**	Endrin-$^{13}C_{12}$
2.	β-BHC-$^{13}C_6$	**11.**	Dieldrin-$^{13}C_{12}$	**20.**	Heptachlor-$^{13}C_{10}$
3.	δ-BHC-$^{13}C_6$	**12.**	α-endosulfan-$^{13}C_9$	**21.**	*cis*-heptachlor epoxide-$^{13}C_{10}$
4.	γ- BHC-$^{13}C_6$	**13.**	β-endosulfan-$^{13}C_9$		
5.	*p,p'*-DDT-$^{13}C_{12}$	**14.**	Endosulfan sulphate-$^{13}C_9$	**22.**	HCB-$^{13}C_6$
6.	*o,p'*-DDT-$^{13}C_{12}$	**15/16.**	Dicofol-d^8	**23.**	PCNB-$^{13}C_6$
7.	*p,p'*-DDE-$^{13}C_{12}$	**16.**	*cis*-chlordane-$^{13}C_{10}$	**24.**	PCA-$^{13}C_6$
8.	*p,p'*-TDE-$^{13}C_{12}$	**17.**	*trans*-chlordane-$^{13}C_{10}$	**26.**	MPCPS-$^{13}C_6$
9.	Nitrofen-d_4	**18.**	oxychlordane-$^{13}C_{10}$		

Matrix-matched Calibration

GC-MS/MS Methods

- Three types of matrix blank

Matrix Blank

Leaf, Flower and Cortex

Examples

款冬花(**花**)

淡竹葉(**葉**)

牡丹皮(**皮**)

Root and Stem

Examples

太子參(**根**)

天麻(**根莖**)

Seed and Fruit

Examples

胡蘆巴 (**果實及種子**)

菟絲子 (**果實及種子**)

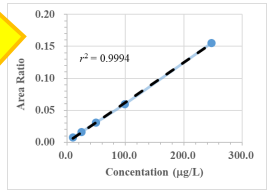
- Chms pre-determined to be free from the targeted pesticides

Undergo the experimental procedures to obtain **matrix extract**

e.g. 500 µL of extract + 500 µL corresponding mixed standard solution

- Calibration standards with matrix

GC-MS/MS analysis

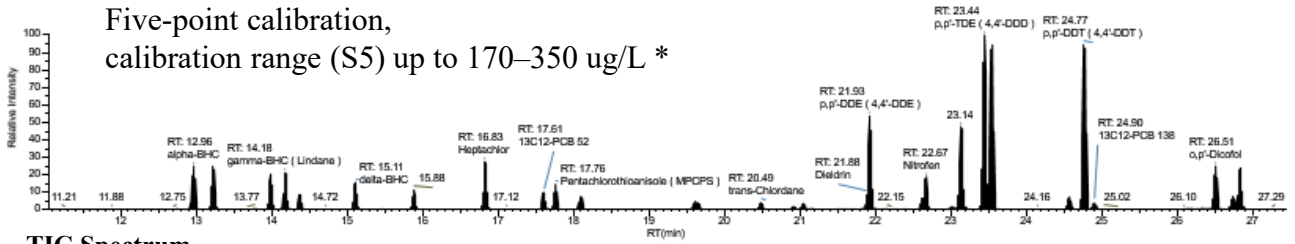


- Matrix-matched calibration curve

Linearity Range

GC-MS/MS Methods

- **Recommended Calibration Ranges:**
  Five-point calibration,
  calibration range (S5) up to 170–350 ug/L *



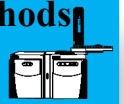
TIC Spectrum

Analyte	Retention Time* (min)	Analyte	Retention Time* (min)	Analyte	Retention Time* (min)
α-BHC	12.9	Aldrin	18.1	Nitrofen	22.6
HCB	13.2	Heptachlor epoxide	19.6	β-Endosulfan	23.0
β-BHC	13.9	Oxychlordane	19.6	*p,p'*-TDE	23.4
γ-BHC	14.1	*trans*-Chlordane	20.4	*o,p'*-DDT	23.5
PCNB	14.3	α-Endosulfan	20.9	Endosulfan sulfate	24.5
δ-BHC	15.1	*cis*-Chlordane	21.0	*p,p'*-DDT	24.7
PCA	15.8	Dieldrin	21.8	*o,p'*-Dicofol	26.5
Heptachlor	16.8	*p,p'*-DDE	21.9	*p,p'*-Dicofol	26.8
MPCPS	17.7	Endrin	22.6		

*** For reference only**

Experimental Procedures

GC-MS/MS Methods



- Weigh ~1 g of sample in a 50-mL tube
- Spike I.S.
- Soak the sample with ~5 to 10 mL of water for 15 min
- Shake with 15 mL of chilled 1 % acetic acid in ACN for 5 min
- Add $MgSO_4$ (6 g) and $C_2H_3NaO_2$ (1.5 g) and shake for 5 min
- Shake with **dSPE (5 in 1/Verde/Z-Sep+)** for 5 min
- Draw ~6 to 7 mL of supernatant and evaporate to near dryness
- Reconstitute with 4.5 mL of hexane
- Clean up with **Florisil SPE** by eluting with 15 mL of 15 % diethyl ether in hexane
- Evaporate the eluent
- Reconstitute with 0.3 mL of *iso*-octane

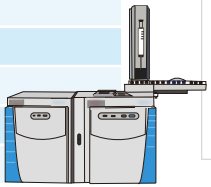
	dSPE Cleanup		
	5 in 1 (General)	**Verde (Intense Pigment)**	**Z-Sep+ (High Fat Content)**
Composition	$MgSO_4$ **(900 mg)** PSA **(150 mg)** GCB **(45 mg)** C18 **(150 mg)** SiO_2 **(150 mg)**	MgSO4 **(1200 mg)** PSA **(400 mg)** GCB **(80 mg)** Z-Sep+ **(480 mg)**	Z-Sep+ **(500 mg)**
Medicinal Parts	Root, Rhizome	Cortex, Leaf, Flower, Herba	Fruit, Seed
Examples	三七、人參、黨參、當歸等等	淡竹葉、合歡花、合歡皮等等	王不留行、地膚子、浮小麥等等

Instrument Set-up

GC-MS/MS Methods

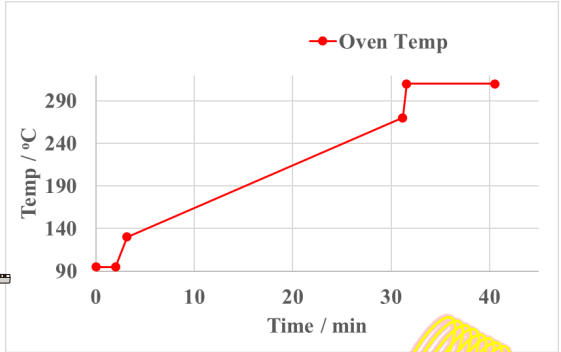
- Suggested GC-MS/MS conditions

GC-MS/MS System	Thermo Quantum GC-MS/MS or equivalent
Column	DB-5MS Ultra Inert, HP-5MS Ultra Inert (30 m × 0.25 mm, 0.25 µm), or equivalent
Injection Temp.	250 °C
Injector Mode	Splitless with surge (pulse)
Scan Mode	Product ion scan
Surge Pressure	150 kPa
Surge Duration	1 min
Injection Volume	1 µL
Carrier Gas	1.5 mL/min Helium
Ionization Mode	Electron impact
Ion Source Temp.	300 °C
Transfer Line Temp.	300 °C

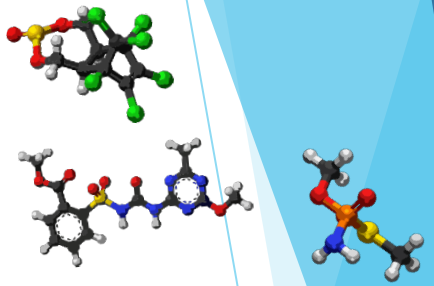


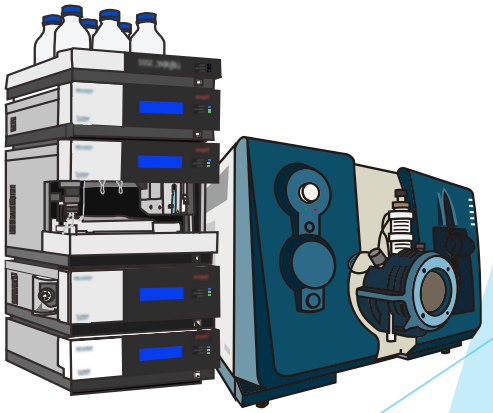
- Oven Program

Oven Temp.	95 °C (2 min) → 30 °C/min ramp → 130 °C (0 min) → 5 °C/min ramp → 270 °C (0 min) → 100 °C/min ramp → 310 °C (9 min)



LC-MS/MS Methods





Targeted Pesticides

LC-MS/MS Methods

- 50 targeted OPPs, OCPs, carbamate pesticides and others

1.	Methamidophos (**OP**)
2.	Parathion-methyl (**OP**)
3.	Parathion (**OP**)
4.	Monocrotophos (**OP**)
5.	Phosphamidon (**OP**)
6.	Chlordimeform (**OC**)
7.	Fenamiphos (**OP**)
8.	Fenamiphos sulphoxide (**OP**)
9.	Fenamiphos sulphone (**OP**)
10.	Fonofos (**OP**)
11.	Cadusafos (**OP**)
12.	Coumaphos (**OP**)
13.	Sulfotep (**OP**)
14.	Terbufos (**OP**)
15.	Terbufos sulphoxide (**OP**)
16.	Terbufos sulphone (**OP**)
17.	Chlorsulfuron (**OC**)
18.	Ethametsulfuron-methyl (**Others**)
19.	Metsulfuron-methyl (**Others**)
20.	Phorate (**OP**)
21.	Phorate sulphoxide (**OP**)
22.	Phorate sulphone (**OP**)
23.	Isofenphos-methyl (**OP**)
24.	*O*-demeton (**OP**)
25.	*S*-demeton (**OP**)
26.	Carbofuran (**Carbamate**)
27.	Carbofuran-3-hydroxy (**Carbamate**)
28.	Aldicarb (**Carbamate**)
29.	Aldicarb sulphoxide (**Carbamate**)
30.	Aldicarb sulphone (**Carbamate**)
31.	Ethoprophos (**OP**)
32.	Isazofos (**OP**)
33.	Isocarbophos (**OP**)
34.	Fipronil (**OC**)
35.	Fipronil desulfinyl (**OC**)
36.	Fipronil sulphone (**OC**)
37.	Fipronil sulphoxide (**OC**)
38.	Phosfolan (**OP**)
39.	Phosfolan-methyl (**OP**)
40.	Dichlorvos (**OP**)
41.	Trichlorphon (**OP**)
42.	Omethoate (**OP**)
43.	Diazinon (**OP**)
44.	Dimethoate (**OP**)
45.	Malathion (**OP**)
46.	Triazophos (**OP**)
47.	Chlorpyriphos (**OP**)
48.	Acephate (**OP**)
49.	Ethion (**OP**)
50.	Methidathion (**OP**)

Suggested I.S. and Labelled I.S. for Screening and Quantitative Analysis

1.	BDMC
2.	Acephate-d_3
3.	Dichlorvos-d_6
4.	Coumaphos-d_{10}
5.	Chlorpyriphos-d_{10}

- **Solvent: ethanol**
- Using **+ve mode** of ESI
- Using **−ve mode** of ESI

Matrix-matched Calibration

LC-MS/MS Methods

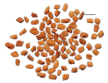
- Matrix blank

Examples


太子參(**根**)


天麻(**根莖**)


牡丹皮(**皮**)


胡蘆巴
(**果實及種子**)


菟絲子
(**果實及種子**)


通草
(**藤木**)


款冬花(**花**)


淡竹葉(**葉**)

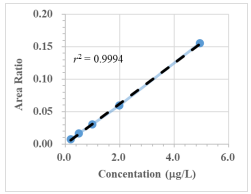
- Chms pre-determined to be free from the targeted pesticides

Undergo the experimental procedures to obtain **matrix extract**

E.g. 500 µL of extract + 500 µL corresponding mixed standard solution

- Calibration standards with matrix

LC-MS/MS analysis

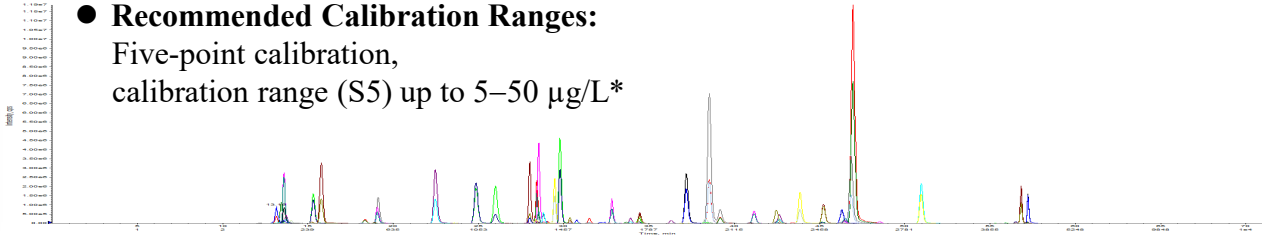


- Matrix-matched calibration curve

Linearity Range

LC-MS/MS Methods

- **Recommended Calibration Ranges:**
  Five-point calibration,
  calibration range (S5) up to 5–50 µg/L*



TIC Spectrum

Analyte	Retention Time* (min)
Methamidophos	13.1
Acephate	13.4
Omethoate	13.5
Chlordimeform	13.6
Aldicarb sulfoxide	13.7
Aldicarb sulfone	14.0
Monocrotophos	15.3
Phospholan methyl	15.8
3-hydroxycarbofuran	18.4
Trichlorfon	19.2
Dimethoate	19.1
Phosfolan	22.5
Aldicarb	23.7
Phosphamidon	25.5
Dichlorvos	28.0
Carbofuran	28.4
Fenamiphos sulfoxide	28.6

Analyte	Retention Time* (min)
Metsulfuron methyl	28.6
Fenamiphos sulfone	28.9
Chlorsulfuron	29.6
Ethametsulfuron methyl	29.9
Phorate sulfoxide	30.5
Phorate sulfone	30.9
Isocarbophos	31.6
Methidathion	33.0
Demeton	34.1
Terbufos sulfone	34.5
Terbufos sulfoxide	34.6
Parathion methyl	34.7
Malathion	37.2
Triazophos	38.6
Isazophos	39.2
Ethoprophos	40.9
Fenamiphos	42.3

Analyte	Retention Time* (min)
Parathion	43.6
Isofenphos methyl	44.9
Sulfotep	45.0
Fonofos	46.2
Coumaphos	46.7
Diazinon	46.8
Phorate	48.4
Cadusafos	50.9
Terbufos	55.9
Ethion	56.8
Chlorpyrifos	57.2

*** For reference only**

Experimental Procedures

LC-MS/MS Methods

- Weigh ~1 g of sample in a 50-mL tube
- Spike I.S.
- Soak the sample with ~5 to 10 mL of water for 15 min
- Shake with 15 mL of chilled 1 % acetic acid in ACN for 5 min
- Add $MgSO_4$ (6 g) and $C_2H_3NaO_2$ (1.5 g) and shake for 5 min
- Shake with **dSPE** for 5 min
- Draw 1.5 mL of supernatant and evaporate to near dryness under a **gentle steam of N_2**
- Reconstitute to 1 mL with solvent as below:
  - For **+ve mode**:
    49 % ACN, 21 % MeOH, 30 % H_2O with **0.1 % formic acid**
  - For **–ve mode**:
    49 % ACN, 21 % MeOH, 30 % H_2O
- Draw 0.7 mL of solution to a 2-mL tube with 300 mg of **Alumina (Neutral)**
- Vortex for 30 s
- Centrifuge at 14000 rpm for 1 min
- Transfer the supernatant to GC vial

	dSPE Cleanup
Composition	$MgSO_4$ (1400 mg), GCB (40 mg), C18 (150 mg)
Medicinal parts	Suitable for different medicinal parts (root, cortex, leaf, seed and etc.) of Chms

Instrument Set-up

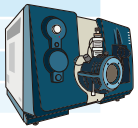
LC-MS/MS Methods

- Suggested UPLC conditions

UPLC System	Dionex/Ultimate 3000 Binary Analytical UPLC System or equivalent
Column	Phenomenex Synergi Fusion-RP 80 Å (2 mm × 50 mm, 4 µm), coupled with (ii) Acquity UPLC HSS T3 (2.1 mm × 150 mm, 1.8 µm) or equivalent
Flow Rate	100 µL/min
Auto-sampler Temp.	10 °C
Injection Volume	5 µL

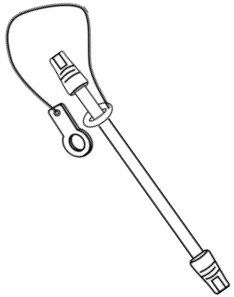
- Suggested MS/MS conditions

MS/MS System	Sciex 6500+ Triple Quad System or equivalent
Ionization Mode	Electrospray ionization
Polarity	+ve / –ve
Ion Spray Voltage	+ 5500 V; – 4500 V
Curtain Gas	20 psi
Gas 1	50 psi
Gas 2	60 psi
TEM	300 °C



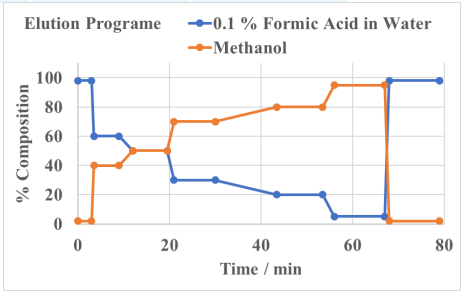
- Elution Program

Time (min)	Solvent A (%)	Solvent B (%)
0.0	98	2
3.0	98	2
3.5	60	40
9.0	60	40
12.0	50	50
19.5	50	50
21.0	30	70
30.0	30	70
43.5	20	80
53.5	20	80
56.0	5	95
67.0	5	95
68.0	98	2
79.0	98	2



Solvent A: 0.1 % formic acid in water;

Solvent B: Methanol



Quality Control Plan

Screening Analysis

(Suggested Quality Control Parameters)

- ✓ Precision of triplicate injections for single level calibration standard: **± 20 %** (RSD)
- ✓ Detectability check standard: **Detected**
- ✓ Method and sample blanks: **Not Detected**
- ✓ Standard check solution: **70–130 %**
- ✓ Relative retention time (RRT):
  - **± 0.5 %** (GC-MS/MS);
  - **± 2.5 %** (LC-MS/MS)

Quantitative Analysis

(Suggested Quality Control Parameters)

- ✓ r^2 of calibration curve: **> 0.990**
- ✓ Spike recovery: **70–120 %** or **60–130 %** based on method validation results
- ✓ Precision of injections: **± 20 %** (RPD or RSD)
- ✓ Standard check solution: **80–120 %**
- ✓ Relative retention time (RRT):
  - **± 0.5 %** (GC-MS/MS)
  - **± 2.5 %** (LC-MS/MS)
- ✓ At least two MRM transitions:

Relative Intensity (%)	Acceptance Criteria
> 50 %	± 20 %
> 20 to 50 %	± 25 %
> 10 to 20 %	± 30 %
≤ 10 %	± 50 %

References:
1. Chinese Pharmacopoeia 2020 Edition
2. SANTE/11312/2021

Conclusion

- ✓ Expression of residue level(s)
- ✓ Matrix blank for different medicinal parts of Chms
- ✓ Matrix-matched calibration standard solutions
- ✓ Internal standards and/or isotopically labeled internal standards
- ✓ Choice of suitable clean-up kits

Thank you

- **Acknowledgment**
  Government Laboratory 政府化驗所

- **Q&A Session**

Online Survey

衛生署
Department of Health