# A Fast, Sensitive and Comprehensive Assay to Quantify Pesticide Residues in Botanical and Non-botanical Dietary Supplements using GC/MS/MS and LC/MS/MS coupled with QuEChERS Extraction Method


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## INTRODUCTION

Pesticides have been used over 4500 years, and pesticide residues in foods are often stipulated by regulatory bodies in many countries. Based on the types of pests, pesticides could be classified into herbicides, rodenticides, bactericides, fungicides, and larvicides. However, according to chemical structure, pesticides could be classified into organophosphate, carbamate, organochlorine, pyrethroid, triazines, triazoles, and neonicotinoids. This paper targeted to develop a fast, sensitive and comprehensive method to quantify 112 pesticide residues (see *Table 1*) in botanical and non-botanical dietary supplements with GC/MS/MS and LC/MS/MS coupling with "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) and dSPE (dispersive solid phase extraction) method.

| Pesticide # | Pesticide Name | Pesticide Type | Pesticide # | Pesticide Name | Pesticide Type | Pesticide # | Pesticide Name | Pesticide Type |
|---|---|---|---|---|---|---|---|---|
| 1 | Acephate | Organophosphate | 38 | Deltamethrin | Pyrethrin | 75 | Mecarbam | Organophosphate |
| 2 | Alachlor | Organochlorine | 39 | Diazinon | Organophosphate | 76 | Methacrifos | Organophosphate |
| 3 | Aldrin | Organochlorine | 40 | Dichlofluanid | organonitrogen | 77 | Methamidophos | Organophosphate |
| 4 | Dieldrin | Organochlorine | 41 | Dichlorvos | Organophosphate | 78 | Methidathion | Organophosphate |
| 5 | Azinphos-ethyl | Organophosphate | 42 | Dicofol (Dicofol p,p') | Organochlorine | 79 | Methoxychlor p,p' | Organochlorine |
| 6 | Azinphos-methyl | Organophosphate | 43 | Dimethoate | Organophosphate | 80 | Mirex | Organochlorine |
| 7 | BHC-alpha | Organochlorine | 44 | Omethoate | Organophosphate | 81 | Monocrotophos | Organophosphate |
| 8 | BHC-beta | Organochlorine | 45 | Endosulfan I | Organochlorine | 82 | S-421 | Organochlorine |
| 9 | BHC-delta | Organochlorine | 46 | Endosulfan II | Organochlorine | 83 | Paraoxon-ethyl | Organophosphate |
| 10 | BHC-epsilon | Organochlorine | 47 | Endosulfan sulphate | Organochlorine | 84 | Parathion-ethyl | Organophosphate |
| 11 | Lindane | Organochlorine | 48 | Endrin | Organochlorine | 85 | Paraoxon-methyl | Organophosphate |
| 12 | Bromophos-methyl | Organophosphate | 49 | Ethion | Organophosphate | 86 | Parathion-methyl | Organophosphate |
| 13 | Bromophos-ethyl | Organophosphate | 50 | Etrimphos (Etrimfos) | Organophosphate | 87 | Pendimethalin | organonitrogen |
| 14 | Bromopropylate | Other (diphenyl) | 51 | Fenchlorphos (Ronnel) | Organophosphate | 88 | Pentachloroaniline | Organochlorine |
| 15 | Chlordane-cis | Organochlorine | 52 | Fenchlorphos oxon | Organophosphate | 89 | Quintozene | Organochlorine |
| 16 | Chlordane-oxy | Organochlorine | 53 | Fenitrothion | Organophosphate | 90 | Pentachlorothioanisole | Organochlorine |
| 17 | Chlordane-trans | Organochlorine | 54 | Fenpropathrin | Pyrethrin | 91 | Pentachloroanisole | Organochlorine |
| 18 | Chlorfenvinophos | Organophosphate | 55 | Fensulfothion | Organophosphate | 92 | Permethrin cis | Pyrethrin |
| 19 | Chlorpyrifos-ethyl | Organophosphate | 56 | Fensulfothion-oxon | Organophosphate | 93 | Permethrin trans | Pyrethrin |
| 20 | Chlorpyrifos-methyl | Organophosphate | 57 | Fensulfothion-oxon sulfone | Organophosphate | 94 | Phosalone | Organophosphate |
| 21 | Cyfluthrin I | Pyrethrin | 58 | Fensulfothion sulfone | Organophosphate | 95 | Phosmet | Organophosphate |
| 22 | Cyfluthrin II | Pyrethrin | 59 | Fenthion | Organophosphate | 96 | Piperonyl butoxide | Organophosphate |
| 23 | Cyfluthrin III | Pyrethrin | 60 | Fenthion sulfone | Organophosphate | 97 | Pirimiphos-ethyl | Organophosphate |
| 24 | Cyhalothrin (lambda) | Pyrethrin | 61 | Fenthion sulfoxide | Organophosphate | 98 | Pirimiphos-methyl | Organophosphate |
| 25 | Cypermethrin I | Pyrethrin | 62 | Fenthion-oxon | Organophosphate | 99 | N-desethyl-pirimiphos-methyl | Organophosphate |
| 26 | Cypermethrin II | Pyrethrin | 63 | Fenthion-oxon sulfoxide | Organophosphate | 100 | Procymidone | Organochlorine |
| 27 | Cypermethrin III | Pyrethrin | 64 | Fenthion-oxon sulfone | Organophosphate | 101 | Profenofos | Organophosphate |
| 28 | Cypermethrin IV | Pyrethrin | 65 | Fenvalerate | Pyrethrin | 102 | Prothiofos | Organophosphate |
| 29 | Cypermethrin alpha | Pyrethrin | 66 | Flucythrinate | Pyrethrin | 103 | Pyrethrin I | Pyrethrin |
| 30 | Cypermethrin beta | Pyrethrin | 67 | Fluvalinate-tau I | Pyrethrin | 104 | Pyrethrin II | Pyrethrin |
| 31 | DCPA (Chlorthal-dimethyl) | Organochlorine | 68 | Fonofos | Organophosphate | 105 | Cinerin I | Pyrethrin |
| 32 | DDD-o,p' (o,p TDE) | Organochlorine | 69 | Heptachlor | Organochlorine | 106 | Cinerin II | Pyrethrin |
| 33 | DDD-p,p' (p,p' TDE) | Organochlorine | 70 | Heptachlor endoepoxide | Organochlorine | 107 | Jasmolin I | Pyrethrin |
| 34 | DDE-o,p' | Organochlorine | 71 | Heptachlor exooepoxide | Organochlorine | 108 | Jasmolin II | Pyrethrin |
| 35 | DDE-p,p' | Organochlorine | 72 | Hexachlorobenzene | Organochlorine | 109 | Quinnalphos | Organochlorine |
| 36 | DDT-o,p' | Organochlorine | 73 | Malaoxon | Organophosphate | 110 | Tecnazene | Organochlorine |
| 37 | DDT-p,p' | Organochlorine | 74 | Malathion | Organophosphate | 111 | Tetradifon | Organochlorine |
| | | | | | | 112 | Vinclozolin | Organochlorine |

*Table 1*: The information of 112 Pesticides

## METHODOLOGY

**Sample Preparation and Extraction:**

About 3.00 grams of sample and internal standards (IS) were extracted by "QuEChERS" extraction method using H2O, HOAc, MeCN, MgSO4, NaOAc and ceramic homogenizer followed by dSPE method.

**GC-MS/MS Conditions**

**GC system**: Agilent 7890B GC system including 7693 auto-sampler (see *Table 2* for parameters)
Column: HP-5MS (15m x 250 um x 0.25 um) (Agilent)
Oven Temperature Program: see *Table 3*.

**MS detector**: Agilent Triple Quadrupole 7000 D
MS Parameters: see *Table 4*.

| | |
|---|---|
| Inlet Heater (°C) | 280 |
| Front column Flow (mL/min) | 1.08 |
| Back column Flow (mL/min) | 1.28 |
| Quench Gas Flow (mL/min) | 2.25 |
| Collision Gas Flow (mL/min) | 1.50 |
| Aux Heater (°C) | 300 |

*Table 2*: GC Parameters

| | Rate (°C/min) | Temperature (°C) | Hold Time (min) | Run Time (min) |
|---|---|---|---|---|
| Initial | | 60 | 1 | 1 |
| Ramp 1 | 40 | 170 | 0 | 3.75 |
| Ramp 2 | 10 | 310 | 0.75 | 18.5 |

*Table 3*: GC Oven Temperature Gradient

| | |
|---|---|
| Source | EI |
| Scan Mode | dMRM |
| Source Temperature | 280 °C |
| RT-LOCK | Yes |

*Table 4*: MS Parameters

## RESULTS and DISCUSSIONS

### Sample Extraction

For sample extraction, we started from using USP extraction solvent, which is proper for botanical matrix, but not proper for non-botanical matrix. The AOAC extraction solvent was then tried, but not proper for dry powder sample like dietary supplements (*Table 5*). More extraction solvents were further investigated, and Dyad Labs' solvent can efficiently extract pesticides from both matrix (*Figure 1*). Different dSPEs were also compared for sample clean-up, and Agilent Fatty Sample dSPE (part#5982-5122) was selected for both matrix. During the sample extraction, some pesticides have hydrogen bonding with the active sites on container surface. In order to prevent this bonding issue, the analyte protectant solvent (sorbitol and gulonolactone in $H_2O$) was used (*Table 6*). The final optimized extraction procedure is shown in *Figure 2*.

| Analyte | Recovery (%) | |
|---|---|---|
| | AOAC Method | Dyad Method |
| Azinphos-ethyl | 25.84 | 95.00 |
| Fenchlorphos oxon | 27.35 | 84.75 |
| Fenitrothion | 60.65 | 98.25 |
| Heptachlor | 66.25 | 91.50 |
| Malaoxon | 23.85 | 115.25 |
| Methidathion | 28.40 | 96.75 |
| Paraoxon-methyl | 15.65 | 74.50 |

*Table 5*: Extraction Solvent Comparison

AOAC Extraction    Dyad Extraction

*Figure 1: The comparison of extraction solvents*

| Analyte | Nominal Conc. (ng/mL) | Result (ng/mL) | |
|---|---|---|---|
| | | Without AP* | With AP* |
| Malathion | 150 | 115 | 167 |
| Pendimethalin | 150 | 67.5 | 136 |

*Table 6: The comparison of with and Without AP (*AP: Analyte Protectant)*

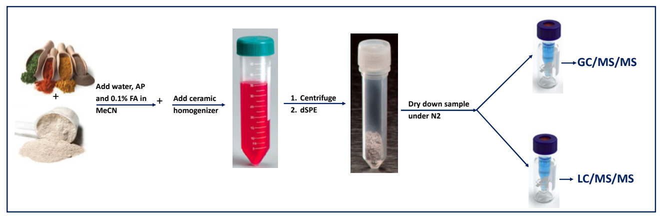


*Figure 2: The procedure of pesticides sample preparation with QuECHERS and dSPE method*

### Specificity

The specificity results indicated that there is no interference between analyte and IS. The blank diluent and matrix extracts have no no interference at analyte and IS expected retention time. Thus, the method is specific. The representative chromatograms are shown in *Figures 3-4*.

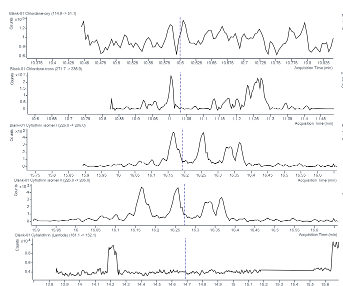

*Figure 3*: The representative chromatogram of diluent.

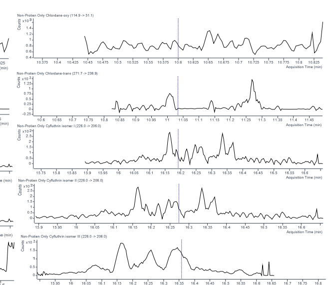

*Figure 4*: The representative chromatogram of blank non-botanical matrix extract.

### Linearity

The curve range of 2.00-1,000 ng/mL, covering nine points at 2.00, 5.00, 10.0, 20.0, 50.0, 100, 200, 500 and 1,000 ng/mL, was successfully validated. Each pesticide has its own specific range among 2.00-1,000 ng/mL based on USP 561. The regression is quadratic with 1/x as the weighing factor (*Figure 5*). The correlation coefficient $R^2$ is > 0.995 (*Figure 5*). The representative chromatograms for ULOQ were in *Figure 6*.

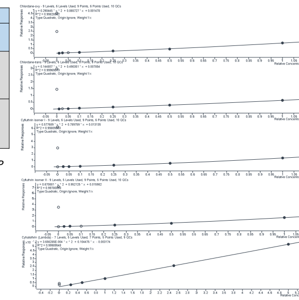

*Figure 5*: Representative Calibration Curves

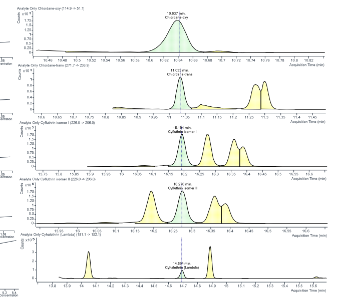

*Figure 6*: Representative Chromatograms of ULOQ

### Accuracy and Precision

The accuracy and precision were investigated with post-spiking pesticides in blank botanical and non-botanical matrix at lower, medium and high regions of the established range of the calibration curve (*Tables 7-8*).

| Analyte | | QC Levels (Accuracy %) | | |
|---|---|---|---|---|
| | | Low QC (50.0 ng/mL) | Medium QC (100 ng/mL) | High QC (150 ng/mL) |
| Chlordane-oxy | Rep. 1 | 102 | 82 | 81.1 |
| | Rep. 2 | 83.7 | 91.9 | 88.5 |
| | Rep. 3 | 80.2 | 108 | 91.2 |
| | Average | 88.6 | 94 | 86.9 |
| Chlordane-trans | Rep. 1 | 107 | 112 | 122 |
| | Rep. 2 | 115 | 112 | 123 |
| | Rep. 3 | 104 | 103 | 88.5 |
| | Average | 109 | 109 | 111 |
| Cyfluthrin I | Rep. 1 | 101 | 119 | 87.2 |
| | Rep. 2 | 92.4 | 101 | 113 |
| | Rep. 3 | 87.2 | 116 | 111 |
| | Average | 93.5 | 91 | 104 |
| Cyfluthrin II | Rep. 1 | 101 | 118 | 88.1 |
| | Rep. 2 | 94.8 | 99.3 | 109 |
| | Rep. 3 | 76.5 | 122 | 112 |
| | Average | 90.7 | 113 | 103 |
| Cyfluthrin III | Rep. 1 | 108 | 120 | 93.1 |
| | Rep. 2 | 102 | 104 | 126 |
| | Rep. 3 | 97.7 | 115 | 116 |
| | Average | 103 | 113 | 112 |

*Table 7*: The Representative Accuracy (%) Data

| Analyte | | %Accuracy (150 ng/mL) | Analyte | | %Accuracy (150 ng/mL) |
|---|---|---|---|---|---|
| Chlordane-cis | Accuracy (%) | 86.3 | Cyfluthrin I | Accuracy (%) | 108 |
| | | 83.5 | | | 98.7 |
| | | 80.2 | | | 98.7 |
| | | 82.6 | | | 92.9 |
| | | 83.3 | | | 96.4 |
| | | 87 | | | 99.2 |
| | Average | 83.8 | | Average | 89.899.1 |
| | SD | 2.49 | | SD | 2.8 |
| | RSD% | 2.97 | | RSD% | 3.2 |
| Chlordane-trans | Accuracy (%) | 99.9 | Cyfluthrin II | Accuracy (%) | 101 |
| | | 94.9 | | | 95.9 |
| | | 91.8 | | | 92.9 |
| | | 92.5 | | | 85.8 |
| | | 97.3 | | | 84.4 |
| | | 98.3 | | | 73.4 |
| | Average | 98.3 | | Average | 88.9 |
| | SD | 3.25 | | SD | 9.8 |
| | RSD% | 3.25 | | RSD% | 11 |
| | | | Cyfluthrin II | Accuracy (%) | 104 |
| | | | | | 103 |
| | | | | | 102 |
| | | | | | 95.6 |
| | | | | | 102 |
| | | | | | 100 |
| | | | | Average | 101 |
| | | | | SD | 2.83 |
| | | | | RSD% | 2.83 |

*Table 8*: The Representative Precision Data

## CONCLUSIONS

This method is a fast, specific, sensitive and comprehensive method, and firstly published to simultaneously quantify 112 pesticides in both botanical and non-botanical dietary supplements.