



*124th AOAC Annual Meeting  
Sep 26-29, 2010 in Orlando, USA*

**AOAC Inter-Collaborative Study Protocol  
for Determination of Several Hundreds  
of Pesticide Residues in Tea  
by GC-MS-SIM, GC-MS/MS & LC-MS/MS**

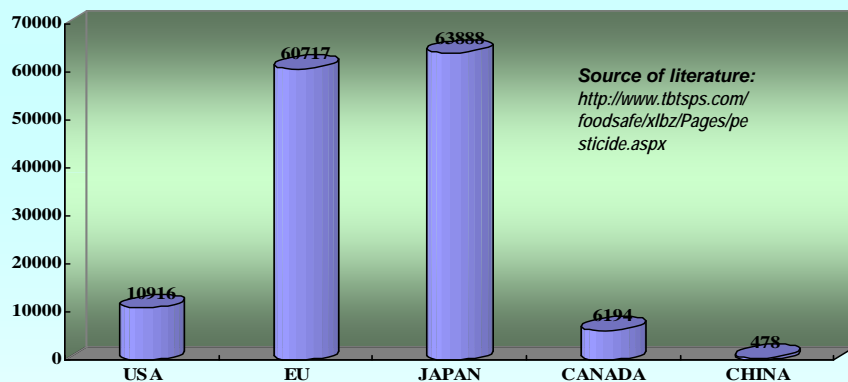
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***Sep 26-29, 2010***

# 1. Purpose and meaning

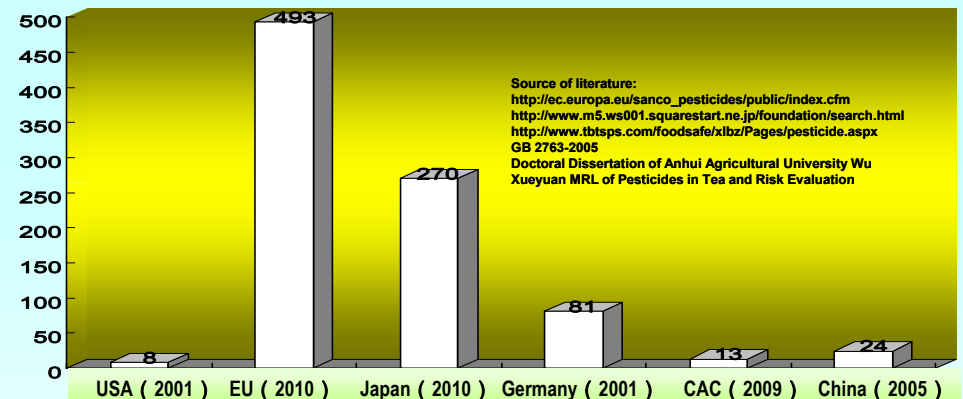
As everyone knows, the integration of global economy is stepping up, and the strategic positions of the world food safety and pesticide residue limit threshold for international trade are higher and higher, which call for high throughput residue analytical techniques.

- ① There are more and more and stricter and stricter limit standards for pesticide residues in edible agricultural products in countries all over the world.

Comparison of limit standards for pesticides residues in different countries ( Data from 2010)



Comparison of pesticide quantities with MRL for different countries



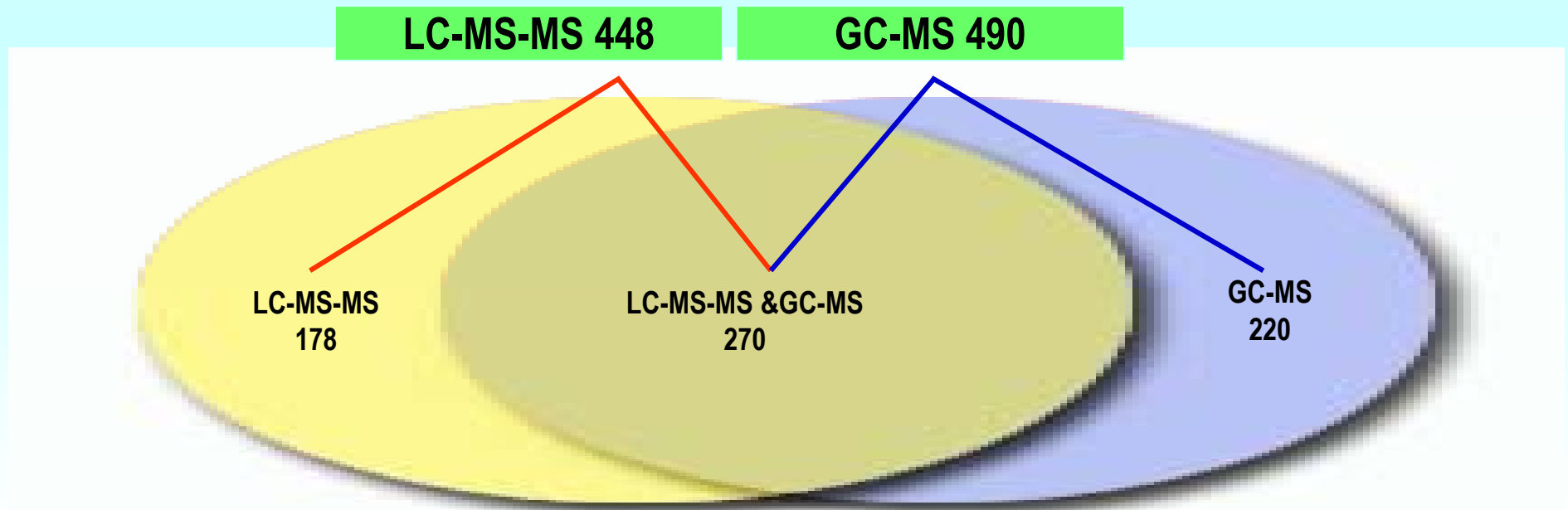
- ② The threshold of limit for pesticide residues in tea is high for international trade. At present, there are 17 countries and international organizations that have stipulated MRL levels for over 800 pesticide residues in tea.

EU, Germany, Holland, Switzerland, Hungary, Israel, CAC, China, Japan, Chinese Taiwan, Korea, USA, Australia, India, Kenya and South Africa (till 2006).

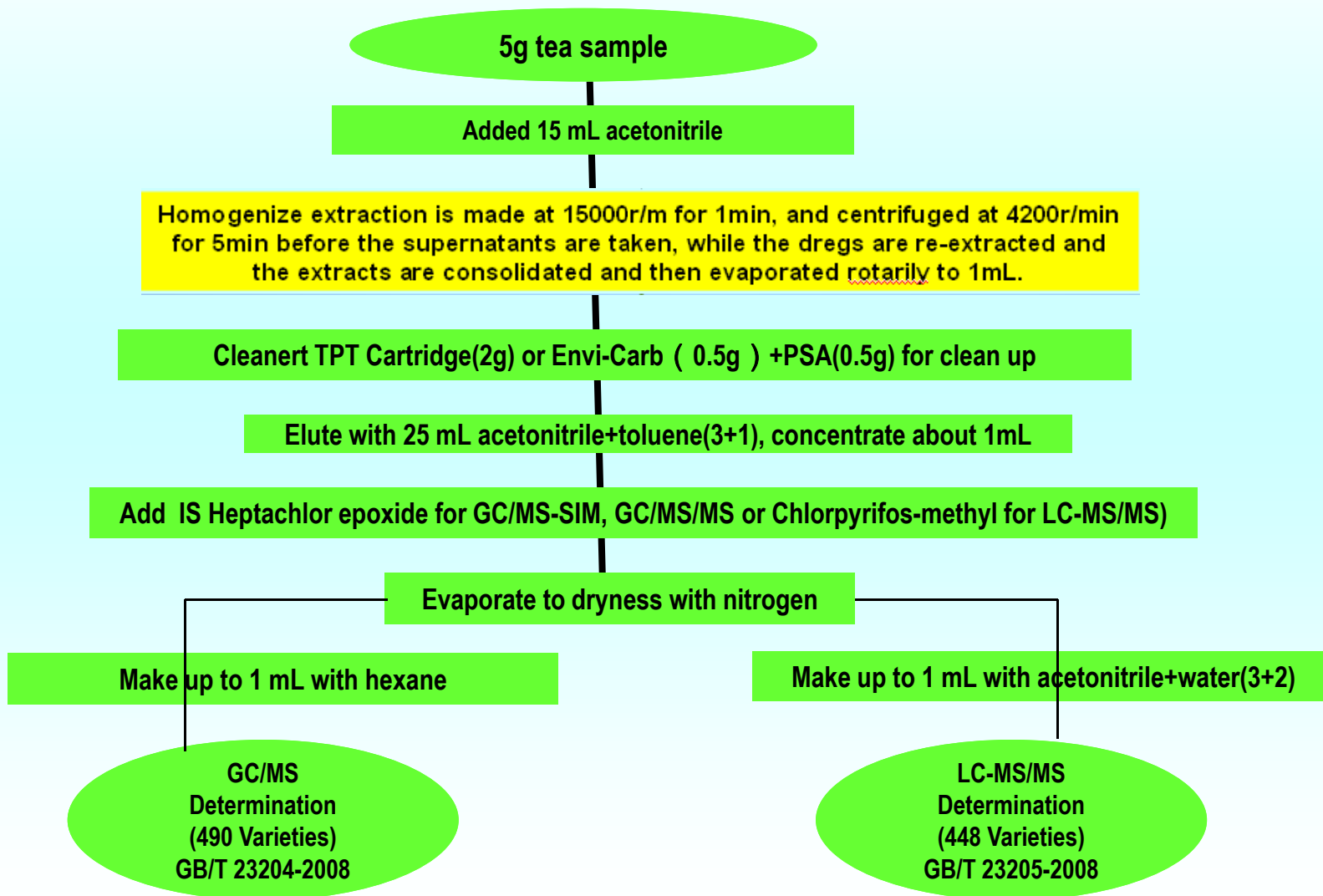
## *2. Application scope of the method*

The method is applicable to determination of 668 pesticide residues in Green tea, Red tea, Puer tea and Wulong tea by GC-MS-SIM, GC-MS/MS & LC-MS/MS.

Distribution of pesticide varieties determined by GC-MS & LC-MS-MS



### 3. GC-MS&LC-MS/MS Analysis program



## 4. Pesticide varieties for collaborative study: 30

*GC-MS-SIM, GC-MS/MS and LC-MS/MS Studies combined*

### ① GC-MS-SIM, GC-MS/MS: 30 varieties

classified per chemical compositions		classified per functions	
carbamate	1	acaricide	1
organophosphorus	5	herbicide	6
organohalogen	21	insecticide	11
pyrethroid	2	bactericide	12
other	1		

### ② LC-MS/MS: 30 varieties

classified per chemical compositions		classified per functions	
organosulfur	1	insect growth regulator	1
carbamate	2	plant growth regulator	1
pyrethroid	2	acaricide	2
organophosphorus	5	herbicide	5
organonitrogen	18	insecticide	9
other	2	bactericide	12

① GC-MS-SIM, GC-MS/MS :30 varieties

1	2,4'-DDE
2	4,4'-DDE
3	benalaxyl*
4	bifenthrin*
5	bromophos-ethyl
6	Chlorfenapyr
7	chlorthal-dimethyl
8	Diflufenican
9	Dimethachlor
10	Dimethenamid
11	Ethalfluralin
12	Ethoprophos*
13	Fenbuconazole
14	kresoxim-methyl*
15	Picoxystrobin*

16	pirimicarb
17	pirimiphos-methyl
18	Procymidone*
19	Propiconzole
20	Propyzamide
21	Pyrimethanil
22	Quinoxyfen
23	Tebufenpyrad*
24	Tefluthrin
25	Tetradifon
26	tolclofos-methyl
27	Triadimefon*
28	Trifluralin
29	Vinclozolin
30	Zoxamide

② LC-MS/MS : 30 varieties

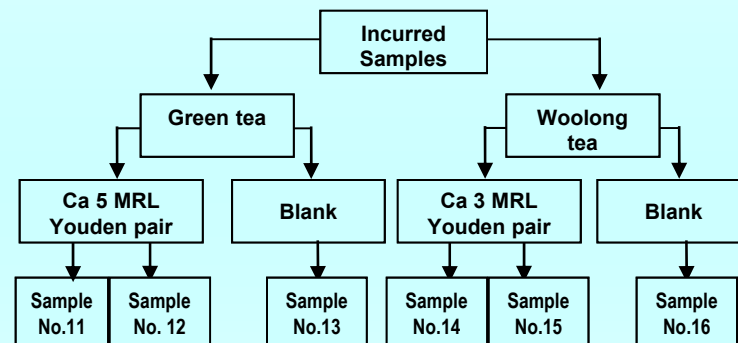
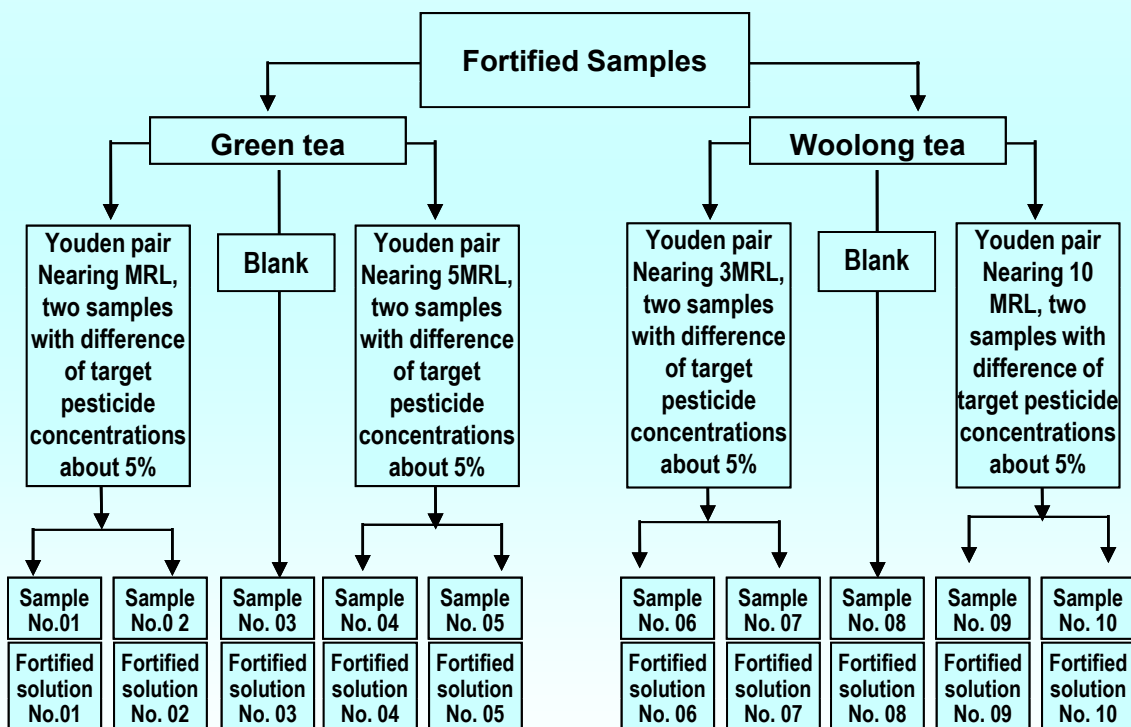
1	acetochlor
2	ametryn
3	azoxystrobin
4	benalaxyl*
5	bensulide
6	butralin
7	clomazone
8	diazinon
9	dibutyl succinate
10	Ethoprophos*
11	Etofenprox
12	Fenazaquin
13	Flutolanil
14	Furalaxyl
15	Indoxacarb

16	Iprobenfos
17	Isoprocarb
18	Isoprothiolane
19	kresoxim-methyl*
20	mefenoxam
21	phenothrin
22	Picoxystrobin*
23	pirimiphos-methyl
24	Procymidone*
25	prometon
26	propoxur
27	pyriproxyfen
28	Tebufenpyrad*
29	Triadimefon*
30	Trifloxystrobin

\* Indicates the pesticides to be determined by both GC-MS-SIM (GC-MS/MS) and LC-MS/MS

## 5. Sample quantizes for each laboratory to test:22

6 examination samples for pre-study and 16 official collaborative study samples make a total of 22.

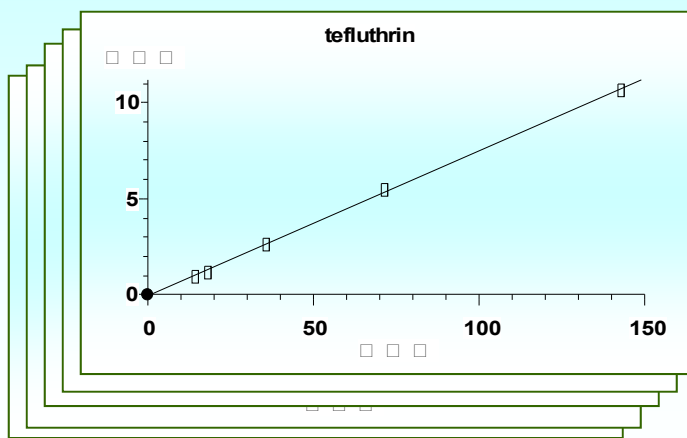


- 10ug/kg-2.0mg/kg. The concentration range of 30 target pesticides is 10ug/kg-2.0mg/kg.
- Fortified samples and incurred samples are designed in Youden Pair, with two samples for one pair and the difference of the concentration of the target pesticide is 5%.

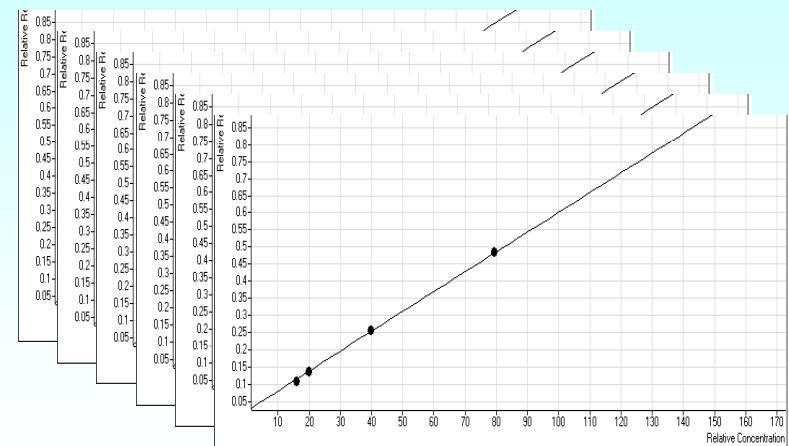
## 6. *Internal standard-Matrix-matched calibration curve quantification*

- ① GC-MS-SIM and GC-MS/MS uses Heptachlor epoxide as internal standard.
- ② LC-MS/MS uses chlorpyrifos-methyl as internal standard.
- ③ Draw a at least 5 point matrix matching calibration curve  $R^2 > 0.995$ .
- ④ See the parameter table for linearity range.

GC-MS



LC-MS/MS



# 7. Excel worksheet for data analysis for participants

- ① See Table 1 for method conditions and data sheet of qualitative and quantitative ions.
- ② See Table 2-1 and Table2-2 for GC-MS-SIM(GC-MS/MS) standard curve and analytical results.
- ③ See Table3-1 and 3-2 for LC-MS/MS standard curve and analytical results

This screenshot shows an Excel spreadsheet with multiple columns and rows. The columns are labeled with various parameters and data points. The spreadsheet is filled with numerical data, likely representing analytical results for GC-MS-SIM.

This screenshot shows a grid of data for GC-MS-SIM analysis. The grid is mostly empty, with the text '第 1 页' (Page 1) centered in the middle. The grid is outlined in green.

This screenshot shows a grid of data for GC-MS-SIM analysis, similar to the previous one. It features a grid with the text '第 1 页' (Page 1) in the center, indicating it is the first page of a multi-page document.

This screenshot shows a grid of data for GC-MS-SIM analysis, consistent with the other sheets. It contains a grid with the text '第 1 页' (Page 1) centered, representing the first page of the data sheet.

This screenshot shows a grid of data for GC-MS-SIM analysis, following the same format as the others. It displays a grid with the text '第 1 页' (Page 1) in the center, signifying the first page of the data.



## ***8. Statistical analysis and judgment criteria***

- ① See Table 4 for Comprehensive Statistical Sheet containing the test results of the collaborative study samples from participants summed up by Study Director
- ② Outlier values are judged per Dixon testing.
- ③ Statistical calculation is conducted per Statistical Manual of the AOAC (Youden, W.J., & Steiner, E.H., 1975, Arlington, VA)
- ④ Recoveries,  $RSD_r$ , and  $RSD_R$  are judged per the table below and “AOAC Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals”

<b>Concentration</b>	<b>Recovery limits</b>	<b>Repeatability (<math>RSD_r</math>)</b>	<b>Reproducibility (<math>RSD_R</math>)</b>
<b>10 <math>\mu\text{g/g}</math>(ppm)</b>	<b>80-115%</b>	<b>6%</b>	<b>11%</b>
<b>1 <math>\mu\text{g/g}</math></b>	<b>75-120%</b>	<b>8%</b>	<b>16%</b>
<b>10 <math>\mu\text{g/kg}</math>(ppb)</b>	<b>70-125%</b>	<b>15%</b>	<b>32%</b>

## *9. Identification of target pesticides*

Identification of target pesticides in this collaborative study tea adopts the standard from EU Document No. SANCO/10684/ 2009“Method Validation and Quality Central Procedures for Pesticide Residues Analysis in Food and Feed”.

Recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques

Relative intensity ( % of base peak )	GC-MS-SIM (relative)	GC-MS-MS,LC-MS/MS (relative)
> 50%	±10%	±20%
> 20%-50%	±15%	±25%
> 10%-20%	±20%	±30%
≤10%	±50%	±50%



## ***10. Equipment qualification***

**Make sure to check if the stability of the instrument is in agreement before and after testing each sample in order to prevent experimental errors incurred from sensibility drop and stability turbulence due to causes such instrument contaminations, etc.**

- ① Instrumental sensibility is calculated per internal standard matter noise ratios:  
GC-MS-SIM > 1000 ,  
GC-MS/MS > 2000 ,  
LC-MS/MS > 1000。**
- ② Instrumental stability (calculated per internal standard peaks):  
Retention time deviation < 3% (2 successive injections)  
Peak area deviation < 6% (2 successive injections)**

## 11. SPE equipment qualification

- ① Two cartridges are recommended for this collaborative study:
- 1) Cleanert TPT (10 mL, 2000 mg, Agela, Tianjin, China);  
(<http://www.agela.com/searchproduct.aspx?search=tpt>)
  - 2) Envi-Carb ( 0.5g/5mL, Supelco , USA+PSA(0.5g/3mL, Varian , USA )
- ② No matter which cartridge is selected, it shall be equipped with Visiprep 5-port flask vacuum manifold (RS-SUPELCO 57101-U, Sigma Aldrich Trading Co., Ltd), which is a solid-phase extraction equipment newly developed by Supelco.  
(<http://www.sigmaaldrich.com>)



Solid phase Extraction Equipment

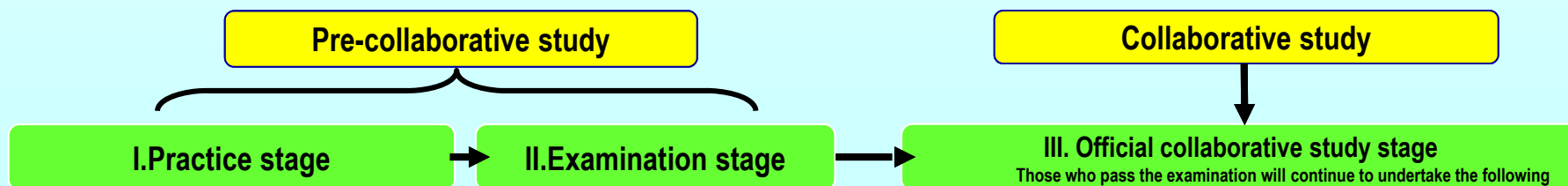


## ***12. Requirements for participating laboratories or personnel***

- ① As soon as Committee of Pesticides and Chemical Contaminants approves this Protocol, we will strive to recruit 20-30 laboratories from 15 countries and regions of the five continents worldwide.**
  
- ② Expertise necessary for participating laboratories and technical experts.**
  - 1) Skillful sample preparation techniques of pesticide residues:**
  - 2) Skillful analytical techniques with GC-MS-SIM, GC-MS/MS and LC-MS/MS and experience (operational experience, judging experience with trouble-shooting and maintenance and repair experience).**
  - 3) Precise mass spectrometric resolving technique for residual pesticides**

# 13. Pre-collaborative study

This collaborative study includes two programs of pre-collaborative study and official collaborative study and three stages as follows:



- ① This pre-study is added per proposal from the Committee of Pesticide and Chemical Contaminants, whose purpose is just what Dr. Krystyna explained explicitly: : “ pre-collaborative study by the participating laboratories to ensure the laboratories are qualified to run the method”
- ② This pre-study and official collaborative study will be required to finish within a certain time successively. When the examination samples are finished, the results from them will be immediately emailed to Study Director see the Table as left. Study Director will reply to the evaluation results of the examination samples, and it is up to laboratories themselves to decide if they will continue with the official collaborative study samples.

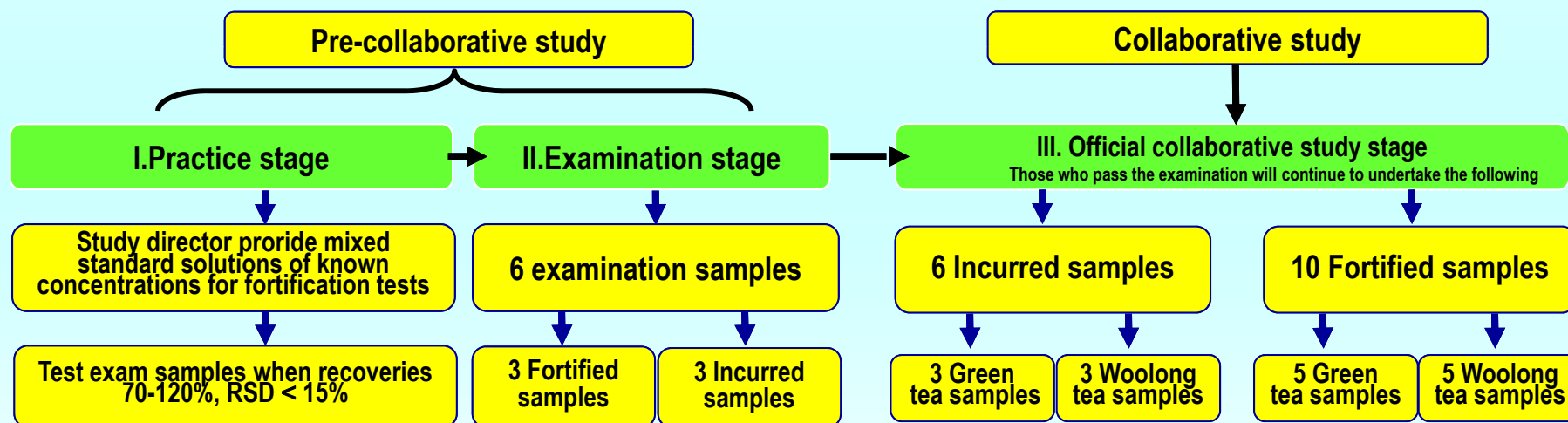
**Test Result Sheet of AOAC Pre-study Examination Samples**

Name of pesticides	Fortified samples			Pesticide names	Incurred samples		
	No.01	No.02	No.03		No.04	No.05	No.06
1				1			
2				2			
.....				.....			

Please take precautions that after completion of the pre-study the original chromatographs of the 6 samples and their relevant data shall be emailed to the Study Director together with this Test Result Sheet, and we will give a reply within 24 hours.

## 14. The detailed flow chart of AOAC collaborative study

We have designed the experiment protocols of pre-collaborative study followed by official collaborative study successively. We will mail the samples, mixed standard solutions, fortified solutions and other testing materials for pre-collaborative study and official collaborative study to the participants simultaneously at one time, and they are required to conclude the tests successively in three stages, and the flow chart is detailed as follows:



- ① The examination criteria for pre-collaborative study stage will follow recoveries and RSD as mentioned above. In principle, a laboratory that has 3 out of 6 examination samples failing to reach the evaluation criteria will no longer undertake the official collaborative study samples.
- ② Each laboratory, except for the practice stage in the pre-study stage, will undertake 6 samples in the pre-study examination stage and 16 samples in the official collaborative study stage, totaling 22 samples.



## ***15. Time frame for completion of collaborative study***

- ① As soon as Committee of Pesticides and Chemical Contaminants approves this Protocol, we will strive to recruit 20-30 laboratories from 15 countries and regions of the five continents worldwide**
- ② After the participating laboratories are decided, we will immediately prepare standard solutions, fortified solutions and relevant testing consumables, etc. for pre-collaborative and official collaborative study. Counted from the date of shipment of these testing materials in China, participants will be required to finish this collaborative study with 3 months.**

## ***16. Study Director's Promise***

**During the collaborative study period, Study Director will reply to any technical questions from participants within 24 hours; for questions in other aspects we will also do our utmost to help it out.**



## *17. Key control points*

- ① Rotary evaporation temperature: not exceeding 40°C.
- ② Instrumental stability: the difference of the response values of the adjacent two quality control standard solutions shall not exceed 6% for anyone.
- ③ Verify retention time/ ion abundance: ensure that each target pesticide is within the integration window and identifies correctly.
- ④ Make sure that integration lines of target pesticides are correct.
- ⑤ Prevent interferences from the reagents.
- ⑥ Avoid cross-interferences from the samples.



## ***18. Safety measures***

**Features of the method: Use only acetonitrile, toluene , hexanes, etc. instead of any organic reagents containing organic chlorine ; for one cartridge, one sample preparation may be used for determination of 668 pesticides, which conforms to the contemporary requirements for low-carbon environment protection, resources saving, environment-friendly and harmonious development.**

**However, acetonitrile, toluene and other reagents used in this experiment are of potential hazard to human health. Therefore, it is specially reminded that rubber gloves shall be worn in experiments and operation with care in the ventilated cabinet should be observed.**



# THANKS

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