Call for Voluntary Collaborators
Priority Project of AOAC INTERNATIONAL (2010)
Nov. 27, 2010)

High-Throughput Analytical Method for Determination of multi-Classes and multi-Kinds of 668 Residue Pesticides and Chemical Pollutants in Tea by GC-MS, GC-MS/MS and LC-MS/MS
— AOAC Collaborative Study Protocol (Draft)

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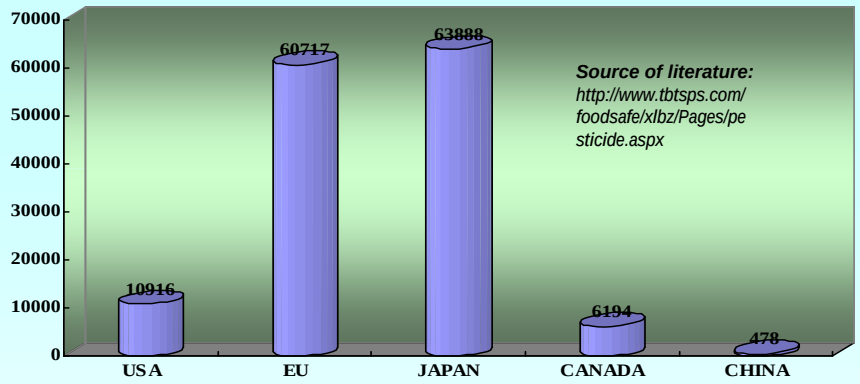
1. Purpose of AOAC collaborative study

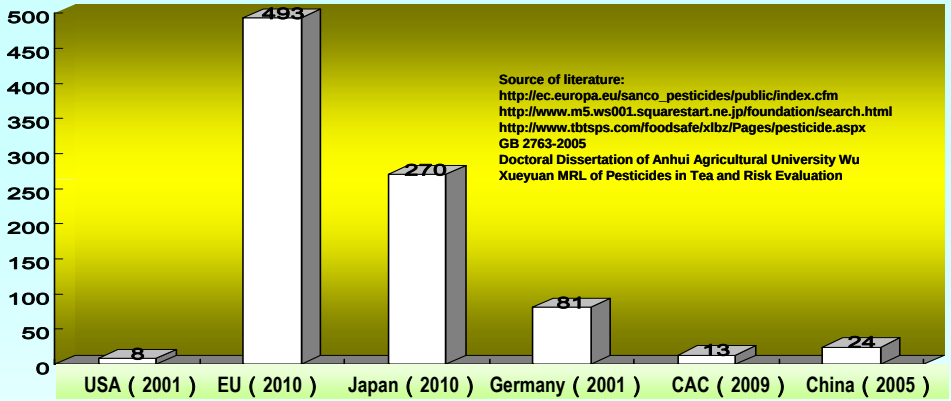
As everyone knows, the integration of global economy is stepping up, and the strategic positions of the world food safety and pesticide residue limit threshold for international trade are getting higher and higher, which calls for high throughput residue analytical techniques.

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



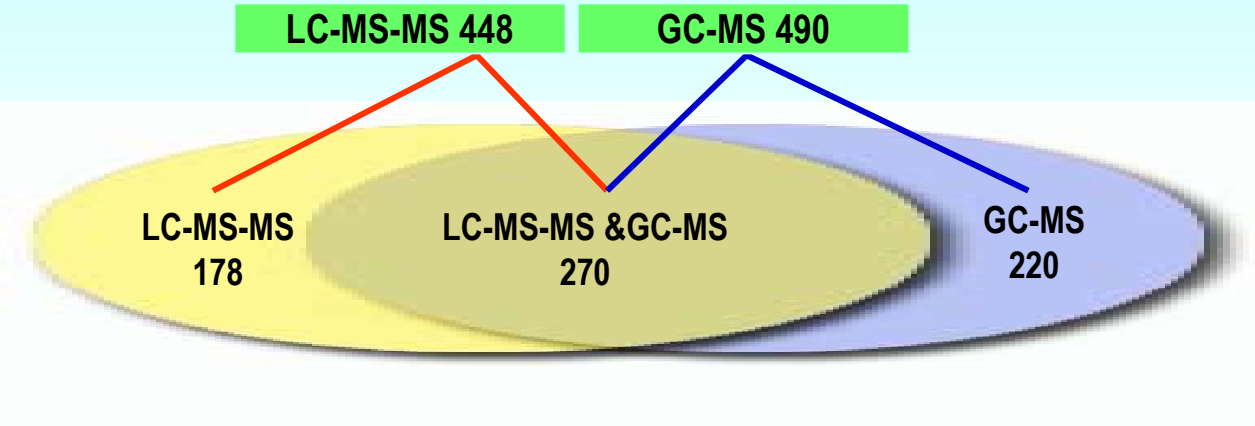
② The threshold of limit for pesticide residues in tea is high for international trade. At present, there are 17 countries and international organizations that have stipulated MRL levels for over 800 pesticide residues in tea.

EU, Germany, Holland, Switzerland, Hungry, 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 the determination of multi-classes and multi-kinds of 668 residue pesticides and chemical pollutants in Green tea, Red tea, Puer tea and Woolong tea by GC-MS, GC-MS/MS & LC-MS/MS. The limits of detection (LOD) for 490 pesticides determined by GC-MS were between 1.0-500 μg/kg, and for 448 pesticides determined by LC-MS/MS were between 0.03-4820 μg/kg. There are 482 pesticides with LOD≤100μg/kg for GC-MS method, accounting for 98% of the pesticides tested and there are 417 pesticides for LC-MS/MS, accounting for 93% of the pesticides analyzed; there are 264 pesticides with LOD≤10μg/kg for GC-MS method, accounting for 54% of the pesticides tested, 325 pesticides for LC-MS/MS method, making up 73% of the pesticides analyzed. There are 270 pesticides that can be analyzed by both GC-MS and LC-MS/MS. There are 264 pesticides with LOD≤100μg/kg for GC-MS method, accounting for 98% of the pesticides tested, 247 for LC-MS/MS, making up 91% of the pesticides analyzed; there are, however, 133 pesticides with LOD≤10μg/kg for GC-MS method, accounting for 49% of the pesticides tested, 200 pesticides for LC-MS/MS method, making up 74% of the pesticides analyzed.

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



3. Analytical procedures

②Centrifugalize

①Homogenize

③Concentration

The key control points

①Homogenize at 13500r/min for 1min;
②Centrifuge at 4200r/min for 5min;
③Concentrate to ca 1mL at 40℃;
④Elute by gravity for clean-up;
⑤Concentrate to ca 0.5mL at 40 ℃;
⑥Evaporate to dryness with nitrogen gas at 35 ℃
⑦Filter through 0.2μm membrane

④Clean-up

⑦Sampler vial

⑧Determine

⑥Evaporate with nitrogen

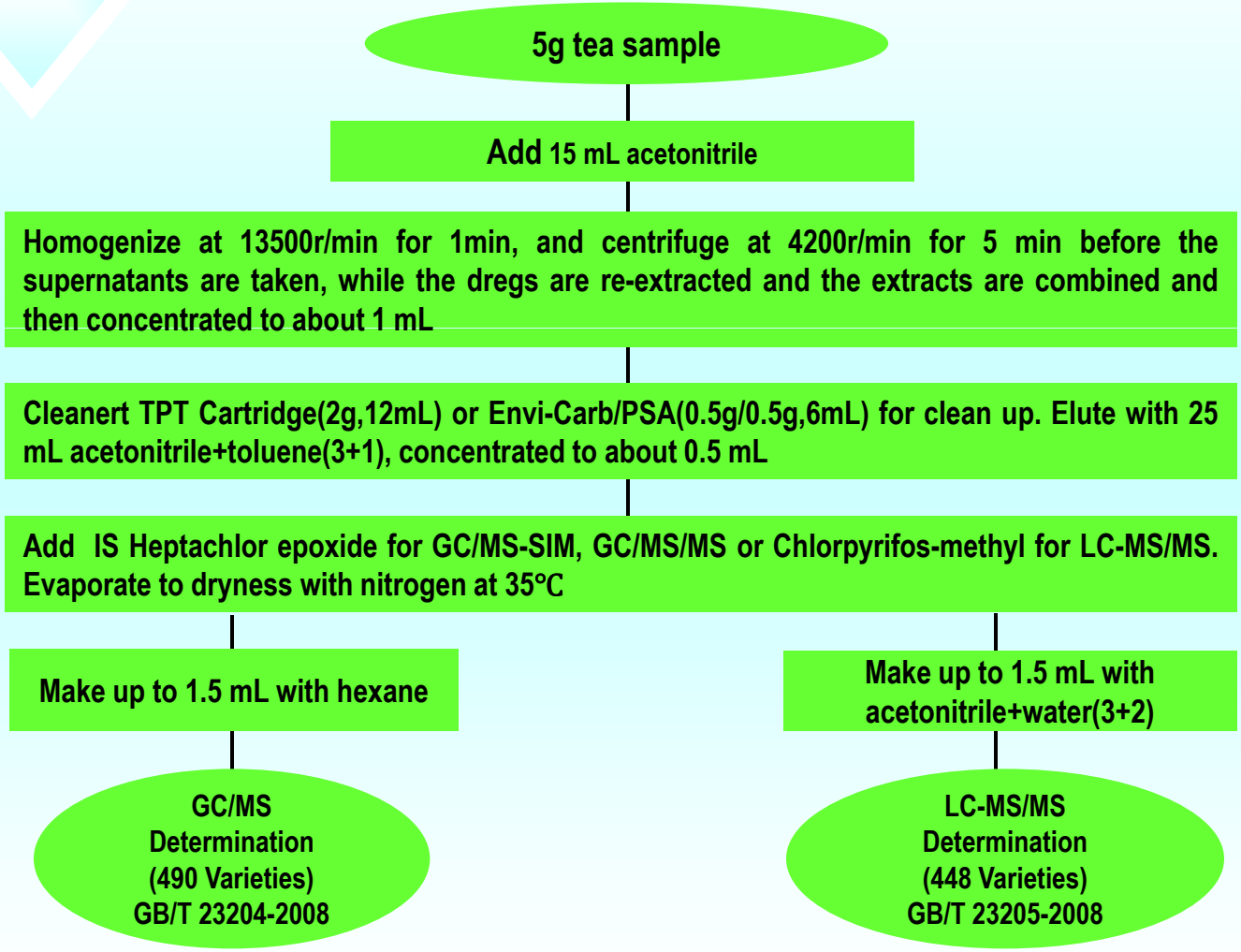
⑤Concentration Concentration

GC-MS-SIM

GC-MS-MS

LC-MS-MS

3. GC-MS(GC-MS/MS)&LC-MS/MS Analysis program



Study Director would like to remind all the participants, as a binding rule for voluntary participation: this inter-collaborative study is a unified and standardized action for all the collaborators from different countries all over the world, and each step involved in the experiment including reagents and testing materials shall not be altered or replaced without the prior consent of Study Director.

4. “Shrunken” AOAC collaborative study protocol

4.1 20 pesticides for GC-MS(GC-MS/MS) and LC-MS/MS determinations

No	GC-MS		LC-MS/MS	
	Pesticides	MRL/ (μg/kg)	Pesticides	MRL/ (μg/kg)
1	2,4'-DDE	200	acetochlor	10
2	benalaxyl	100	benalaxyl	100
3	bromophos-ethyl	100	bensulide	30
4	bromopropylate	100	butralin	20
5	chlorfenapyr	50000	chlorpyrifos	100
6	diflufenican	50	clomazone	20
7	dimethenamid	20	diazinon	20
8	fenchlorphos	100	ethoprophos	20
9	picoxystrobin	100	fenazaquin	10
10	pirimicarb	50	flutolanil	50
11	pirimiphos-methyl	50	imidacloprid	50
12	bifenthrin	5000	indoxacarb	50
13	propyzamide	50	kresoxim-methyl	100
14	pyrimethanil	100	phenothrin	50
15	Quinoxyfen	50	picoxystrobin	100
16	tebufenpyrad	100	pirimiphos methyl	50
17	tefluthrin	50	propoxur	100
18	tolclofos-methyl	100	tebufenpyrad	100
19	trifluralin	100	triadimefon	200
20	vinclozolin	100	trifloxystrobin	50

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

It will be extremely difficult in terms of resources, time and personnel for each participant to organize a collaborative study on hundreds of pesticides, so AOAC International has proposed an alternative “shrunken” protocol, which is to select two teas and two methods of GC-MS (GC-MS/MS) and LC-MS/MS for a respective determination of 20 pesticides totaling 40 pesticides to be involved in the collaborative study.

20 pesticide classes for GC-MS (GC-MS/MS) detemination

classified per chemical compositions		classified per functions	
Carbamate	1	Acaricide	2
Organophosphorus	4	Herbicide	4
Organohalogen	2	Insecticide	8
Pyrethroid	2	fungicide	6
Organonitrogen	9		
Other	2		

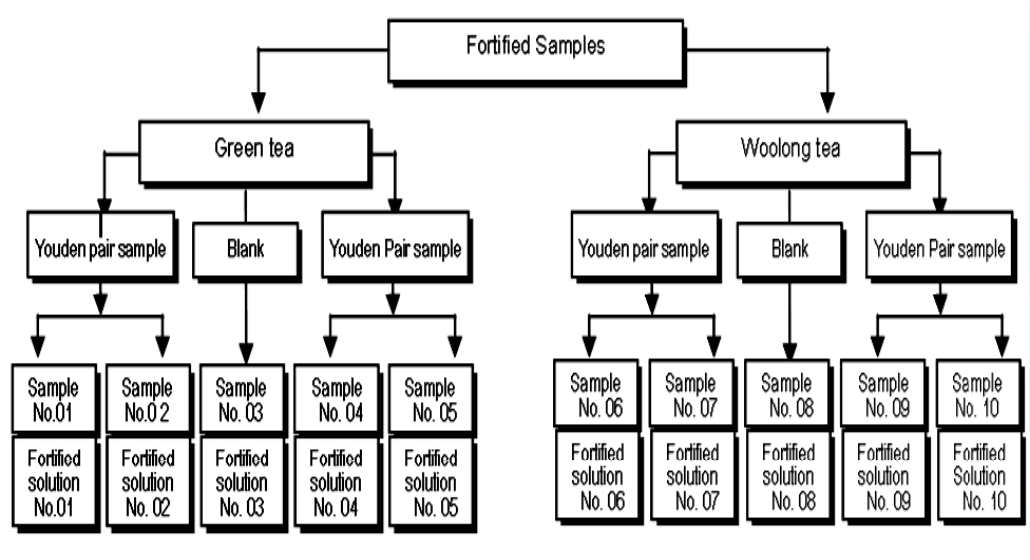
20 pesticide classes for LC-MS/MS detemination

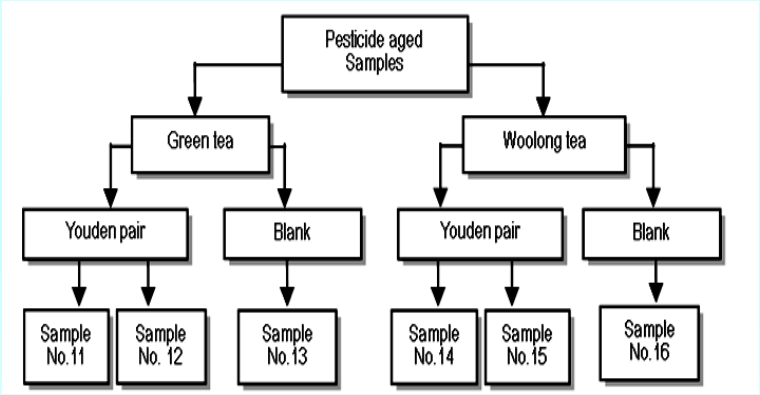
classified per functions		classified per chemical compositions	
Carbamate	2	Plant growth regulator	1
Organophosphorus	5	Acaricide	2
Organonitrogen	12	Herbicide	3
Other	1	Insecticide	8
		fungicide	6

4. “Shrunken” AOAC collaborative study protocol

4.2 Each participating laboratory makes a determination of a total of 16 collaborative study samples.

Each sample is only sufficient for one test, in other words, each participant will have only one testing opportunity for each sample, so he/she will have to make every effort to ensure an utmost success with one shot. Therefore, Study Director will emphasize again that this collaborative study must be dealt with full focus and attention. The compositions of these 16 collaborative study samples are as follows:

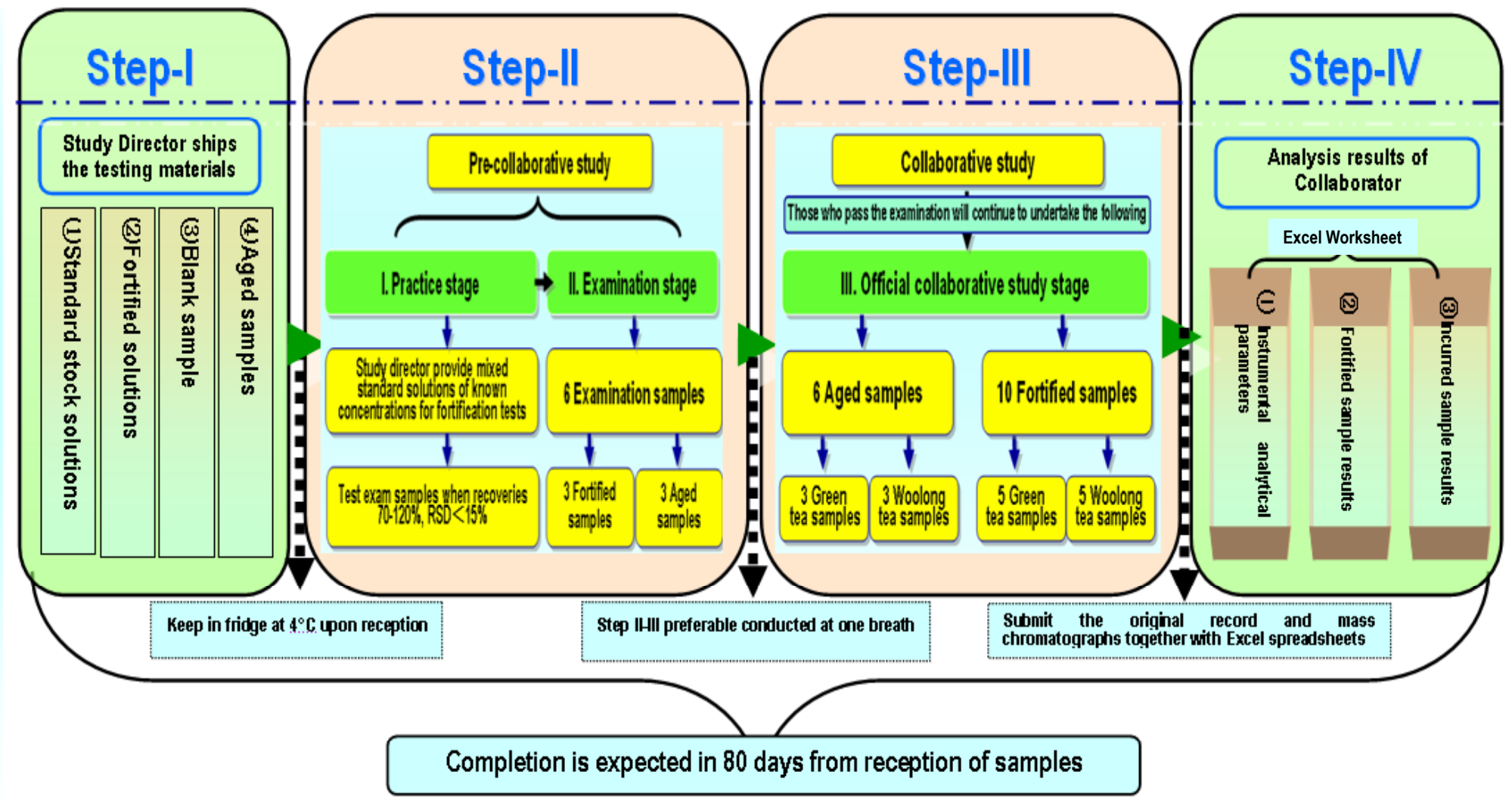




- **The concentration range of 20 target pesticides is 10ug/kg-2.0mg/kg equivalent to 1MRL-5MRL.**
- **Fortified samples and pesticide aged samples are designed in Youden Pair, with two samples for one pair and the difference of the concentration of the target pesticide is about 5%.**

***The serial number here represents the design concept of the collaborative study protocol only, the actual serial number of each sample shipped will be determined later.**

5. The flow chart of the AOAC collaborative study



Step-I Testing materials shipped by Study Director

(1)For Pre-collaborative study

- ◆ 20 pesticide mixed standard stock solutions: 2 ampoules (1.0mL X 2, 1 spare aged).
- ◆ Internal standard stock solutions: 2 ampoules (1.0 mL X 2, 1 spare aged).
- ◆ Green tea and woolong tea blank samples: 1 bottle each (200g x 2).
- ◆ Examination sample materials:

① 3 ampoules of fortified solutions (0.5mL x 3) for preparation of examination samples.

② 3 bags of green tea blank samples (5.0g x 3) for preparation of examination samples.

③ 3 bags of woolong tea aged samples (5.0g x3).

(2)For Collaborative study

- ◆ 2 ampoules (1.0mL x 2, 1 spare aged) of 20 pesticide mixed standard stock solutions for establishing at least 5 point matrix-matched internal standard calibration curve.
- ◆ 2 ampoule (1.0mL x 2, 1 spare aged) of internal standard stock solutions.
- ◆ 10 ampoules (0.5mL x 10) of fortified solutions for preparation of fortified samples.
- ◆ 10 bags of tea blank samples(5.0 g x 10): No. 01-No.05 is green tea; No.06-No.10 is woolong tea for preparation of fortified samples.
- ◆ 6 bags of aged tea samples (5.0 g x 6): No.11-No.13 is green tea; No.14-N0.16 is woolong tea.
- ◆ 1 bottle of green tea and woolong tea blank samples each (200g x 2) for preparation of matrix-matched internal standard calibration mixed solutions.

(3)For Quality Control

Quality control standard stock solutions: 2 ampoules (1.0mL X 2, 1 spare aged), used for checking the instrumental sensitivity and stability.

Step-II Pre-collaborative study

Step-II ① The significance

Because of the complexity of tea matrices, the magnitude of content of the research and the nature of technology involved, this collaborative study is by no means easy to be undertaken, and the objective of this Pre-collaborative study is well explained in the remarks of AOAC Sr. Director, Stakeholder Communications Krystyna McIve "pre-collaborative study by the collaborators to ensure the laboratories are qualified to run the method".

Study Director reminds everyone here once again: For each of these 16 collaborative study samples, each participant will have only one testing opportunity, which means success or failure is determined by just one shot. However, the practice samples that Study Director provides shall be sufficient for full practice or rehearsal in order to meet the objective of dexterous mastery of the key control points. We hope that you will exercise with full attention on the practice occasions that this pre-collaborative offers.

Step-II Pre-collaborative study

Step-II② Practice stage

①This collaborative study adopts internal standard-matrix-matched calibration curve quantification

- Prepare 5 portions of Green Tea and Woolong Tea blank sample solutions each for calibration points, add respectively 20,50,100,200 and 300 uL pesticides mixed standard working solutions and 40uL heptachlor epoxide internal standard working solution for GC-MS or GC-MS-MS (40μL chlorpyrifos methyl internal standardworking solution for LC-MS-MS) before mixing uniformly.
- Use nitrogen blow to dryness, dissolve residua by 1.5 mL hexane (GC-MS or GC-MS-MS) or 1.5 mL acetonitrile: water (3:2) (LC-MS/MS), mix thoroughly, and prepare to be 5 portions matrix-matched internal standard calibration mixed solutions. Establish 5 point matrix-matched internal standard calibration curve.

②practice spiked recovery test

- Each lab use blank tea samples and 20 pesticides mixed standard working solutions and practice spiked recovery test at 1MRL and 5MRL levels on one's own. If one reaches the acceptance criteria for pre-collaborative study, he may undertake examination samples.
- At least 5 point matrix matched internal standard calibration curve $R2 \geq 0.995$ for GC-MS (GC-MS/MS) or LC-MS/MS
- Recoveries fall within 70-120% with RSD<15% (n=5)

Study Director repeatedly reminds every participant: the acceptance criteria shall be met through practice. Repeated tests shall be reproducible on a consistent basis instead quality control criteria are met at one/two time(s) or occasionally. If one could meet the criteria one or two times, it only demonstrates that he or she hasn't mastered the core and key control points of this technique and it is recommended that more practice be undertaken. These cautions have a lot to do with whether he or she is able to pass the exam when testing the exam samples and more to do with whether analytical results from testing the 16 collaborative study samples are legitimate. Therefore, it is of utmost importance that the acceptance criteria be met through practice.

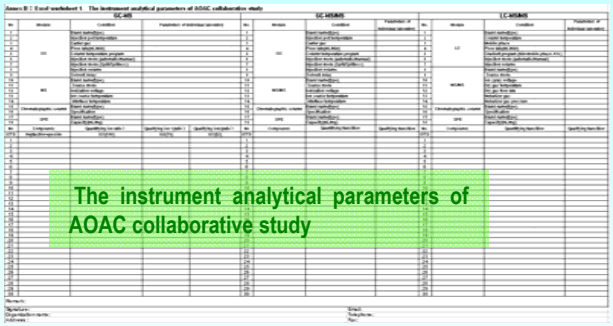
Step-II Pre-collaborative study

Step-II③ examination stage

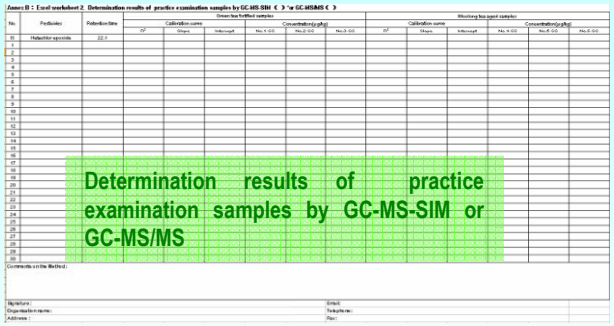
The Corresponding Serial Numbers of Centrifugal Tubes, Blank Samples and Fortified Solution Ampoule Bottles

Serial Nos.	No.01	No.02	No.03
Centrifugal tubes	No.01	No.02	No.03
Blanks samples	No.01	No.02	No.03
Fortified solutions	No.01	No.02	No.03

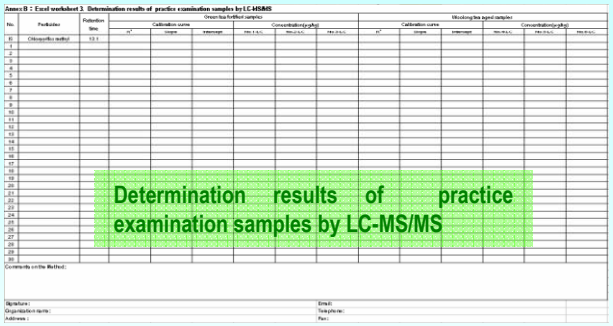
- Preparation and determination of fortified samples for Green Tea: add three bags of Green Tea blank samples No.1, No.2 and No.3 shipped by Study Director into three 80mL centrifugal tubes with the same serial numbers, and place three ampoule bottles contained fortified solutions No.1, No.2 and No.3 upright stand in front of the centrifugal tubes with the same serial numbers. Wait till solutions at the top of the ampoule bottle flow down to the bottom before opening the bottle and add all the fortified solutions into the three centrifugal tubes with the same numbers. Rinse the ampoule bottle three times separately and add the washings into the centrifugal tube. Wait for 20min until the solutions are fully absorbed by the sample before undertaking sample preparation per 6.1 Extraction and 6.2 Cleanup in the AOAC collaborative study. In the meanwhile, prepare 5 portions Green Tea matrix blank solutions and establish internal standard-matrix-matched calibration curve used for quantification of Green Tea fortified samples.
- Woolong Tea aged samples No.4-No.6 are prepared per the method as mentioned above. In the meanwhile, prepare 5 portions Woolong Tea matrix blank solutions and establish internal standard-matrix-matched calibration curve for quantification of Woolong Tea incurred samples.
- Verifying the test results against the following three methods:1) whether the retention time is within the time window; 2) whether integration line is correct; 3) mass spectrum resolution is checked if ion abundance complies with requirements of EU standard or AOAC standard.



The instrument analytical parameters of AOAC collaborative study



Determination results of practice examination samples by GC-MS-SIM or GC-MS/MS



Determination results of practice examination samples by LC-MS/MS

Study Director will provide ample feedback of the study results to each collaborator. Upon reception of Excel worksheet 1-3, mass spectrum chromatographs and raw data, Study Director will promptly reply to each collaborator regarding examination results within 48 hours for collaborators to decide whether to continue with the collaborative samples.

Step-II Pre-collaborative study

Step-II④ Acceptance criteria for pre-collaborative study

① At least 5 point matrix-matching internal standard calibration curve $R^2 \geq 0.995$ for GC-MS (GC-MS/MS) or LC-MS/MS.

② Recoveries fall within 70-120% with RSD<15% (n=5).

③ Ion abundance of the targeted pesticides should be in accordance with recommended maximum permitted tolerances of the AOAC regulation or EU regulation[2].

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%

Study Director specially reminds each participant here: the success of this collaborative study primarily depends on whether one is able to meet all the above-mentioned acceptance criteria. If any of index or combination(s) for recoveries, RSD ion abundance or R^2 exceeds 30%, the participating laboratory shall, in principle, be rendered incapable of continuing to undertake the official collaborative study samples.

Step-III Collaborative study

Step-III① Drawing at least 5 point matrix-matched internal standard calibration curve

- This collaborative study adopts matrix-matched internal standard calibration curve for quantification. GC-MS-SIM and GC-MS/MS uses Heptachlor epoxide as internal standard. LC-MS/MS uses chlorpyrifos-methyl as internal standard.

- Preparation procedures of 5 point matrix-matched internal standard calibration curve are as follows:
  Prepare 5 portions of Green Tea and Woolong Tea blank sample solutions each, add respectively 20,50,100,200 and 300 uL pesticides mixed standard working solutions and 40uL internal standard working solutions before mixing uniformly.
  Use nitrogen to blow to dryness, dissolve the residue by 1 mL hexane (GC-MS or GC-MS-MS) or 1mL acetonitrile: water (3:2) (LC-MS/MS), mix thoroughly, and prepare to be 5 point matrix-matched internal standard calibration mixed solutions. After determination, establish 5 point matrix-matched internal standard calibration curve.

From a practical view, the matrix used in calibration working curve shall have to be the same as that of the target samples, i.e. Green Tea samples shall be quantified with Green Tea matrix-mached internal standard calibration working curve while Woolong Tea samples shall be quantified with Woolong Tea matrix-mached standard calibratin working curve, which cannot be confused with. Matrix matched standard calibration working solutions should be prepared for immediate use.

Step-III Collaborative study

Step-III② Preparing collaborative fortified samples (taking 5 Green Tea fortified samples for instance)

Add 5 portions of Green Tea blank samples supplied by Study Director Nos1-5 to a 80mL centrifugal tube with the same serial numbers respectively. Place sequentially ampoule bottles containing the fortified solution with the same serial numbers upright in front of the corresponding centrifugal tubes. Wait till solutions at the top of the ampoule bottle flow down to the bottom before opening the bottle and add all the fortified solutions into the centrifugal tubes with the same numbers. rinse each ampoule bottle three times with 0.5 mL toluene and add the washings into the centrifugal tubes. Wait for 20min until the solutions are fully absorbed by the sample to form collaborative fortified samples before undertaking sample preparation per section 6.1 Extraction and 6.2 Cleanup in the AOAC collaborative study.

Serial Nos.	No.01	No.02	No.03	No.04	No.05
Centrifugal tubes	No.01	No.02	No.03	No.04	No.05
Blanks samples	No.01	No.02	No.03	No.04	No.05
Fortified solutions	No.01	No.02	No.03	No.04	No.05

Study Director specially reminds each participant: 1) a portion of fortified solutions corresponding to a portion of blank samples is sufficient for only one analysis. The concentrations of fortified solutions in each ampoule bottle are unknown to each participant, requiring him or her to take special precautions to transfer them thoroughly. 2) To avoid cross-interference of samples (especially high concentrations vs. low concentrations or contaminations of blank samples), samples preparations and determinations shall be conducted in accordance with the serial numbers, and the utensils for sample preparations used shall be thoroughly rinsed or cleansed one time after one use so as to prevent contamination of next sample preparation.

Step-III③ Aged samples are prepared per section 6-1 extraction and 6.2 cleanup in the collaborative study method.

Step-III Collaborative study

Step-III④ GC-MS（GC-MS/MS）or LC-MS/MS analytical requirements

① Before making determinations of collaborative samples, use quality control standard working solutions to check if the instrumental sensitivity and stability have met the requirements.

② After completion of testing a consignment of samples, re-run the quality control standard working solutions to check if the instrumental sensitivity and stability are in agreement before and after.

③ Abide strictly by qualitative and quantitative requirements of the method and check the retention time and ion abundance of each peak to ensure that each target pesticide peak is within the integration window and identified correctly.

④ Check to see if the integration line of each pesticide peak is selected correctly, and for pesticides with integration line problems adopt uniformly the manual integration mode from one peak valley to another peak valley.

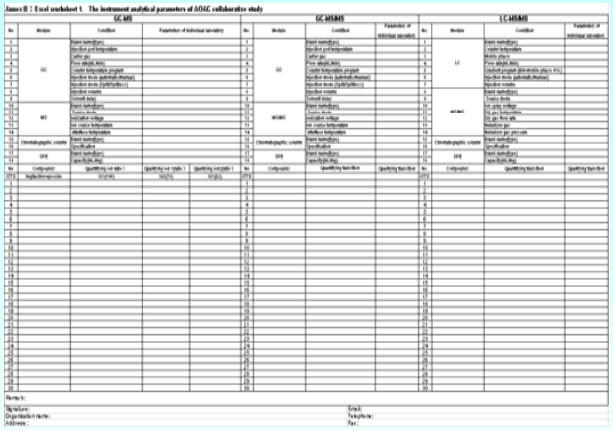
⑤ Collaborators shall submit to Study Director the official collaborative study results by Excel worksheets.

Step-IV Collaborators shall submit to Study Director the official collaborative study results by Excel worksheets.

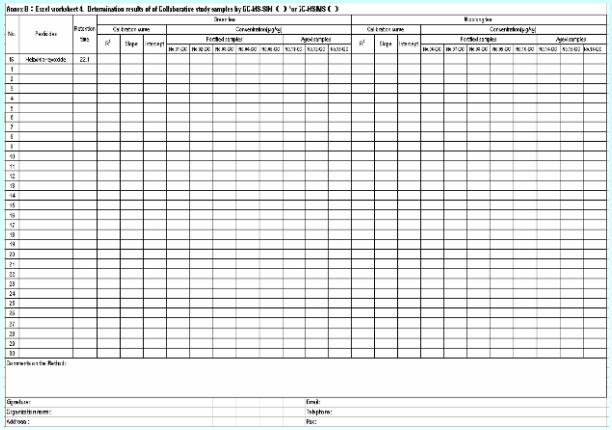
① The instrumental analytical parameters (qualitative and quantitative ions aged), see Excel worksheet 1 of Annex B of the collaborative study method.

② Determination results of Collaborative study samples by GC-MS or GC-MS/MS, see Excel worksheet 4 of Annex B of the collaborative study method.

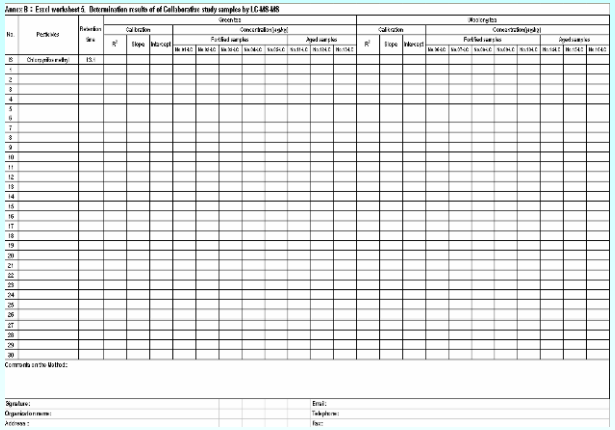
③ Determination results of Collaborative study samples by LC-MS/MS, see Excel worksheet 5 of Annex B of the collaborative study method.



The instrument analytical parameters of AOAC collaborative study



Determination results of Collaborative study samples by GC-MS or GC-MS/MS



Determination results of Collaborative study samples by LC-MS-MS

6. Judging criteria for statistical analysis of collaborative test results

Study Director enters the analytical results for collaborators into the following Table, per the statistical manual of the AOAC to statistical analysis of the test results[4].

Statistical analyss of collabcrative study results (for example)

Festicide: Pyrnetrozine

Lab	Youden pair (Green Tea)		Difference	SUM	Lab	Youden pair (Green Tea)		Difference	SUM
Sampe No.	S01-GC	S02-3C			Sample No.	S04-GC	S05-GC		
1					1				
2					2				
3					3				
...					...				
P					P				
Average,µg/kg					Average,µg/kg				
Add content µg/kg					Add content, µg/kg				
The average recovery,%					The average recovery,%				
SR					SR				
RSDR,%					RSDR,%				
R					R				
Sr					Sr				
RSDr,%					RSDr,%				
r					r				

- **Matrix-matched internal standard calibration curve R^2 > 0.995.**
- **Identification of target pesticides in this collaborative tea samples adopts EU Standard [2]**
- **Recoveries, RSDr and RSDR are judged per the "AOAC Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals"[3].**

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%

Concentration	Recovery limits	Repeatability (RSD_r)	Reproducibility (RSD_R)
10 µg/g(ppm)	80-115%	6%	11%
1µg/g	75-120%	8%	16%
10 µg/kg(ppb)	70-125%	15%	32%

7. Equipment qualification

① Instrumental sensitivity is calculated per internal standard reference noise ratios (S/N):

GC-MS-SIM > 1000

GC-MS/MS > 2000

LC-MS/MS > 500

② Instrumental stability (calculated per internal standard peaks):

Retention time deviation<3% (2 successive injections)

Peak area deviation<6% (2 successive injections)

③ GC-MS,GC-MS/MS and LC-MS/MS are all equipped with data processing software system.

Study Director cautions: 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 sensitivity drop and stability fluctuation due to instrument contaminations, etc.

8. SPE equipment qualification

8.1 Recoveries and RSD requirement

Based on the suggestions from experts at the community meeting of the past 124th AOAC Annual Meeting regarding multiresidues in tea and the two cartridges, Cleanert-TPT and Envi-Carb+PSA, originally recommended, a comparative experiment was conducted at 2MRL level on the cleanup efficiencies of cartridges of the same class having Envi-CARb+PSA (500mg/500mg, 6mL) from seven manufacturers such as UCT, SUPELCO, Agilent, Waters, Varian, CNW and Bestown with Cleanert-TPT for 340 pesticides (LC-MS/MS) and 227 pesticides (GC-MS, GC-MS/MS) in Green Tea and Woolong Tea, with the results as follows:

Evaluation of cleanup efficiencies of SPE cartridges against 340 pesticides (LC-MS/MS) and 227 pesticides (GC-MS/MS) in Green Tea and Woolong Tea

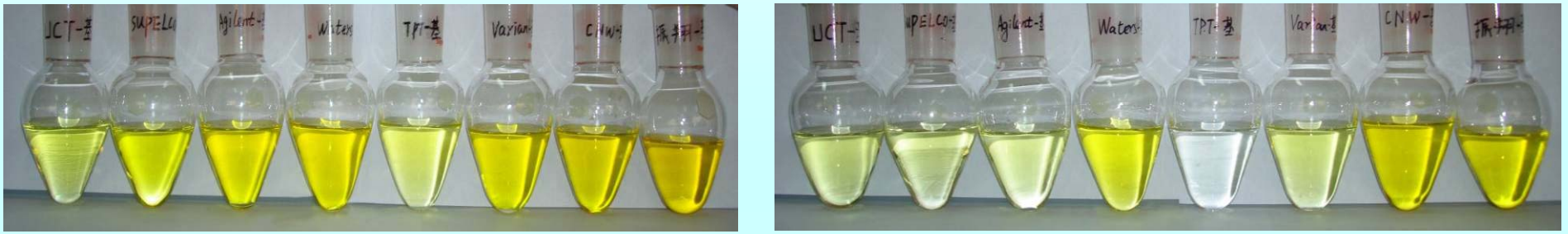
SPECartridge	Brands	UCT	SUPELCO	Agilent	Waters	Agela	Varian	CNW	Bestown
	fillers	GCB/ PSA	ENVI-CARB-II /PSA	Carbon/PSA	Carbon/PSA	Cleanert TPT	Carbon/PSA	CNWBOND GCB/PSA	PSA/Carb
elution time (min)		14 ~ 29	14 ~ 26	14 ~ 28	19 ~ 33	13 ~ 22	19 ~ 29	16 ~ 31	13 ~ 26
Elution flow rate (drop/min)	before Connecting the reservoir	40 ~ 55	62 ~ 90	40 ~ 110	43 ~ 77	70 ~ 115	60 ~ 95	55 ~ 106	50 ~ 110
	After Connecting the reservoir	83 ~ 115	80 ~ 120	87 ~ 147	68 ~ 120	108 ~ 156	80 ~ 123	95 ~ 145	100 ~ 162
Percentage of pesticides with recoveries between 70-120%		96.5	97.1	96.6	96.1	96.8	96.7	95.6	96.2
Percentage of pesticides with RSD<15%		98.1	98.2	98.7	98	98.4	97.2	97.8	98.2
Grading of depigmentation results		Ⅱ	Ⅱ	Ⅱ	Ⅲ	Ⅰ	Ⅱ	Ⅲ	Ⅳ

Remarks: 1) Except for Cleanert-TPT cartridge filler volume being 2000mg and cartridge volume 12mL, all other SPE cartridge filler volumes are 500mg. and the cartridge volume is 6mL. 2) Except for UCT cartridge's elution at 1.0 Kpa negative pressure, other cartridges are all by gravity elution. 3) Percentages of recoveries and RSD are the average values determined by three instruments of GC-MS, GC-MS/MS and LC-MS/MS. 4) Test results: Grade I is the good results from de-pigmentation of both teas; Grade II is the relatively good results from de-pigmentatioon of Woolong tea; Grade III is the relatively poor results from de-pigmentation of both Green Tea and Woolong Tea; IV is the poor results from de-pigmentation of both teas.

8. SPE equipment qualification

8.2 De-pigmentation levels

① Viewed from de-pigmentation results, Cleanert-TPT from Agela does the best, belonging to Grade I; GCB/PSA from UCT, ENVI-CARB-II/PSA from Supelco, Carbon/PSA from Agilent, and Carbon/PSA from Varian belong to Grade II; Carbon/PSA from Waters, GCB/PSA from CNW belong to Grade III; PSA/CARB from Bestown belong Grade IV.



(a) (b)

Fig.1 Comparison of depigmentation results from Green Tea (a) and Woolong Tea (b) by 8 cartridges (From left to right is UCT，SUPELCO，Agilent，Waters，Agela，Varian，CNW and Bestown）

② In terms of percentage of the number of pesticides with recoveries between 70-120% and RSD<15% (n=16), the absolute majority of pesticides are in conformity with the acceptance criteria of AOAC method.

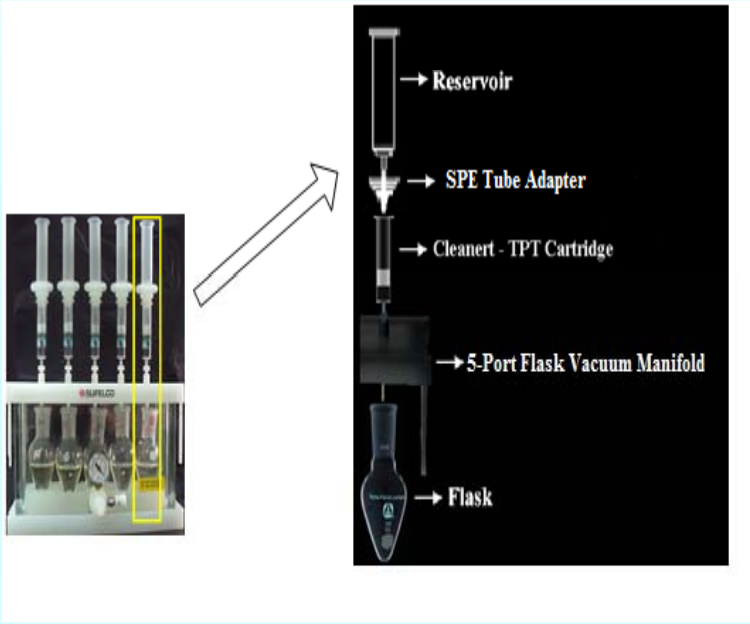
③ The conclusion was shown as follow:

- firstly, the Cleanert-TPT and Envi-Carb+PSA recommended as the first and second choice for AOAC cleanup cartridges based on 183312 data from our previous 912 experiments have been re-confirmed and test results once again prove that the efficiencies of both cartridges are of Ruggedness.
- secondly, this comparative experiment of cleanup efficiencies of 8 SPE cartridges for tea discovered that the remaining 6 SPE Carbon/PSA (500mg/500mg, 6mL), in terms of recoveries and RSD (n=16), have also met the acceptance criteria of AOAC method. If collaborators who use these new cartridges in the pre-collaborative study have also met the acceptance criteria of AOAC pre-collaborative study, they may use these cartridges newly recommended in the official collaborative study as well.

8. SPE equipment qualification

① Two cartridges are recommended for this collaborative study:
1) Cleanert TPT (12 mL, 2000 mg, Agela, Tianjin, China);
(http://www.agela.com/searchproduct.aspx?search=tpt)
2) Envi-Carb +PSA (0.5g/0.5g,6mL, Supelco,USA)

② No matter which cartridge is selected, it is recommended to use Visiprep 5-port flask vacuum manifold or equivalent (RS-SUPELCO 57101-U, Sigma Aldrich Trading Co., Ltd), which is a solid-phase extraction equipment newly developed by Supelco. (http:www.sigmaaldrtch.com)



Solid phase Extraction Equipment

The Supelco Visiprep 5-Port flask vacuum manifold recommended here is that it is relatively convenient to receive big volumes of cleansing liquid (32mL). If collaborators are able to solve the reception of big volume (32mL) in Cartridge cleanup process, they may use other SPE devices as well. For those labs that are willing to try Cleanert TPT but Cleaner TPT cartridges can not be easily accessed in the region, Study Director may provide necessary assistance.

9. Checking the interference and influences from the reagents

To prevent the interferences from reagents, blank tests shall be conducted on the reagents in the entire process of the method to confirm there are no interfering peaks, and each laboratory is requested to supply black test TIC chromatographs to Study Director.

10. Avoiding cross-interferences among the samples and influence from the reagents

To prevent cross-interferences among the samples, the blade of the homogenizer, after completion of extracting the previous sample and before extracting the next sample, shall be thoroughly cleansed 30 seconds homogenously with 2x50 mL acetonitrile in the same way as sample extraction so as to avoid the interferences on the samples of low or non pesticide content from the samples of high pesticide content, which is a key point in this collaborative study.

11. Sample testing sequence requirements

For collaborative sample testing, Study Director suggests that the following order shall be observed sequentially: before testing samples, conduct 2-3 repeated injections of quality control standard working solutions (to check instrumental sensitivity and stability), make successive injections of matrix-matched internal standard calibration mixed solutions with concentrations from low to high 1, 2, 3, 4 and 5 points (to draw an matrix-matched internal standard calibration curve), use sample testing solutions to test No.01, No.02…(from small number to big per the order of serial numbers) and finally repeat testing quality control standard working solutions 2-3 times (to check again instrumental sensitivity and stability).

12.Requirements for collaborators or personnel

Expertise necessary for collaborators 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. Period of completion of collaborative study and requirements

- After AOAC Pesticide and Chemical Contaminants Committee approves this protocol, we will spend two months recruiting collaborators worldwide, aiming at 20-30 laboratories from 15 countries and regions.
- As soon as the collaborators are finalized, we will immediately set about preparing the standard solutions, fortified solutions and relevant testing consumables, etc. used for pre-collaborative study and official collaborative study and ship them out in one lump by courier service. Counted from the date of shipping these testing materials from China, collaborators are kindly requested to finish this AOAC collaborative study within 3 months.
- Upon reception of these collaborative materials, collaborators shall immediately check if the testing materials received conform to those in the packing list and if the materials are sound and safe and fill in the Receipt Form (see Annex 1), which should be emailed or faxed to Study Director. Counted from the date of reception of these testing materials, collaborators are required to finish analytical tasks of all samples within 2 months at the latest.

14. Safety measures

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.

15. Study Director's hope, requests and promises

① Study Director is hoping to have 15 countries and regions and over 20-30 laboratories for each method (GC-MS, GC-MS/MS or LC-MS/MS) participating in this collaborative study.

② Study Director requests each participating laboratory to conclude this collaborative study within 80 days from reception of testing materials and to email or fax the analytical result data sheets, chromatographs, raw data, etc. to Study Director himself.

③ Study Director promises , during the collaborative study period, Study Director will reply to any technical questions from participants within 48 hours; For questions in other aspects we will also do our best to help out.

16. References

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2. Document No. SANCO/10684/ 2009“Method Validation and Quality Central Procedures for Pesticide Residues Analysis in Food and Feed
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5. Standardization Administration of P.R.China (2008) GBT 23204-2008 , Determination of 519 pesticides and related chemicals residues in tea—GC-MS method
6. Standardization Administration of P.R.China (2008) GB/T 23205-2008, Determination of 448 pesticides and related chemicals residues in tea—LC-MS-MS method
7. High-Throughput Analytical Techniques for determination of 653 multi-Classes and multi-Kinds of Residue Pesticides and Chemical Pollutants in Tea. Part I: The Tentative Probe into the Analytical Techniques of Multiresidues in Tea (J.AOAC Int. Manuscript ID-10-0008R.1)
8. High-Throughput Analytical Techniques for determination of 653 multi-Classes and multi-Kinds of Residue Pesticides and Chemical Pollutants in Tea. Part II: Comparative Study of Extraction Efficiencies of the Three Sample Preparation Techniques (J.AOAC Int. Manuscript ID-10-0215R.1)
9. High-Throughput Analytical Techniques for Determination of 653 multi-Classes and multi-Kinds Residue Pesticides and Chemical Pollutants in Tea. Part III: The Evaluation of the Cleanup Efficiency of SPE Cartridge Newly Developed for Multiresidues in Tea (J.AOAC Int. Manuscript ID-10-0251R.1)
10. High-Throughput Analytical Techniques for Determination of 653 multi-Classes and multi-Kinds Residue Pesticides and Chemical Pollutants in Tea. Part IV: Key Control Points and Error Analysis of AOAC collaborative Study Method (has formed the draft)
11. High-Throughput Analytical Techniques for Determination of 653 multi-Classes and multi-Kinds Residue Pesticides and Chemical Pollutants in Tea. Part V: Establishment of AOAC Collaborative Study Pesticide Degradation Kinetic Equations and Prediction for Detected Values of Multiresidues of Collaborative Study Samples (being written)

THANKS

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