



Sep 13-16,2009 in Philadelphia,USA
123nd AOAC INTERNATIONAL Annual Meeting

The study of the stability of the 460 pesticides in the standard solutions and their metabolic process in tea by GC-MS and GC-MS/MS

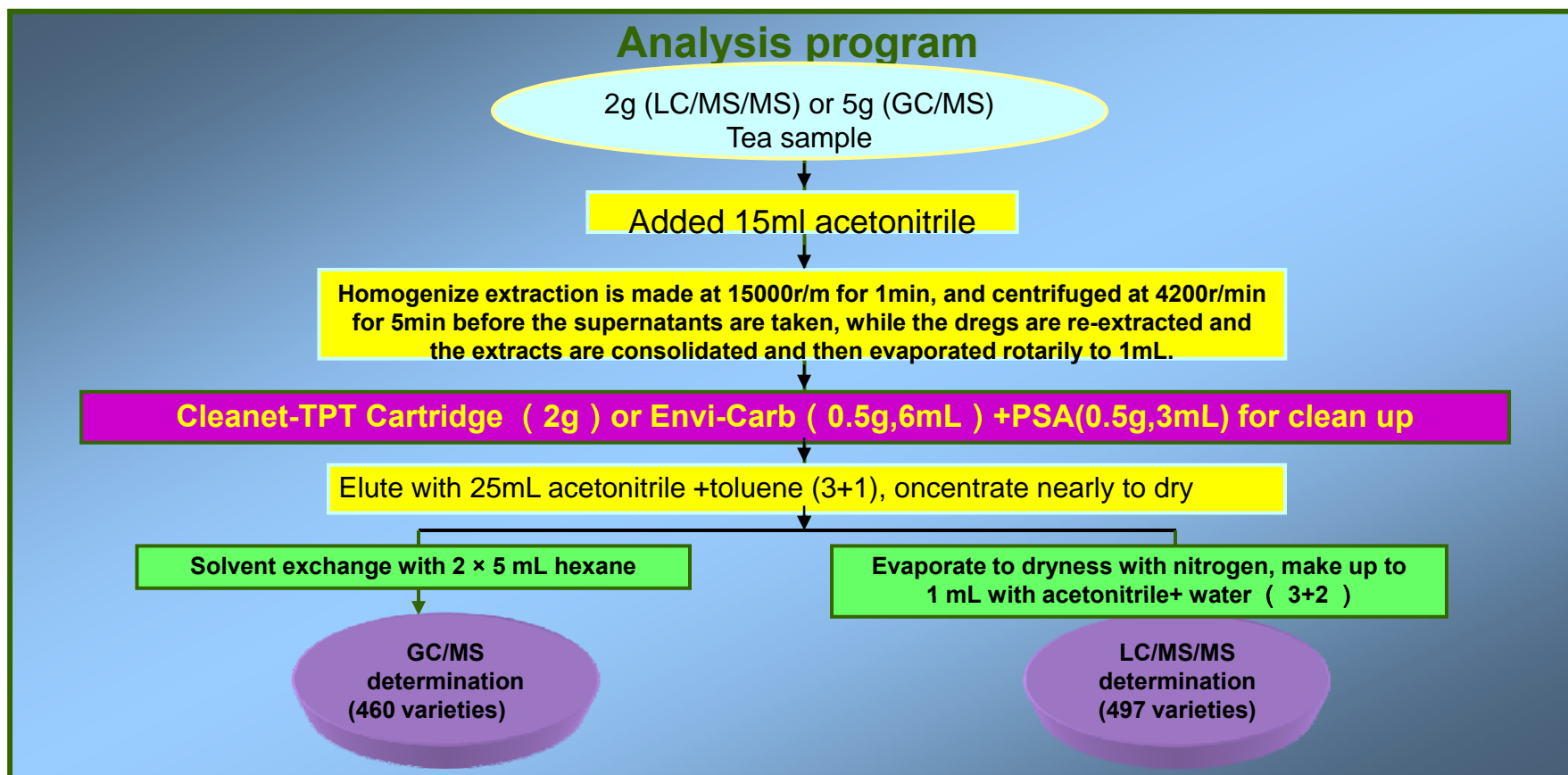
—The Ruggedness of the analytical methods of multiresidue pesticides in tea

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1. The multiresidue analytical method submitted to AOAC International for inter- collaborative study

The Chinese national standard GB/T23204-2008---the analytical method of 460 pesticide multiresidues in tea, which was introduced at the last 122nd AOAC Annual Meeting.



The organization of the AOAC inter-collaborative study breaks into two stages--- GC-MS/MS is chosen before LC-MS/MS.

2. For the purpose of organizing this AOAC inter-collaborative study, three stages of study work were undertaken after the 122nd AOAC Annual Meeting.

Stage I: The study of the stability of the mixed standard solutions of 460 pesticides (Nov. 26, 2008 - Feb. 26, 2009).

Stage II: The stability study of 385 pesticides in tea (Mar. 31, 2009 - Jun. 31, 2009).

Stage III: Preparing the youden pair samples for the inter-collaborative study and studying the ruggedness of the method (Jul. 9, 2009 to Sep. 10, 2009).

3. Why were these three stages of study work undertaken?

- ① To select the pesticides stable for a succession of three months out of the mixed standard solutions of six groups totaling 460 pesticides.**
- ② To study the metabolic patterns of 345 pesticides in tea and sort out the pesticides stable in tea for 2-3 months.**
- ③ To study the change patterns of the pesticides ratios in tea youden pair samples over three months, and investigate the scientificness of the youden pair sample preparation scheme.**

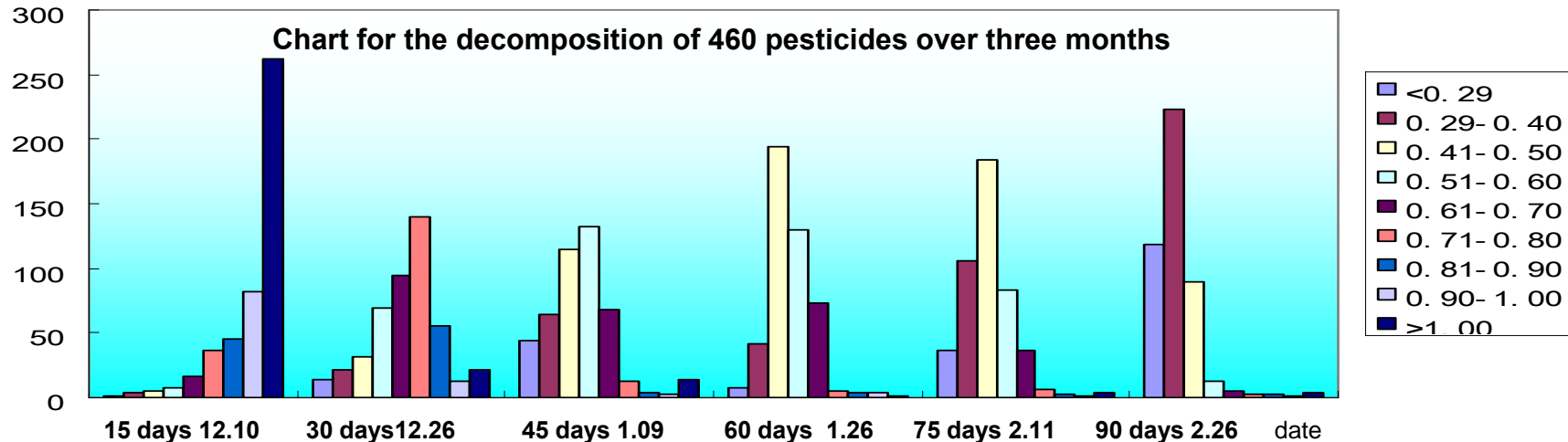
Stage I: The study of the stability of the mixed standard solutions of 460 pesticides

- ◆ **Experimental time: lasting three months from Nov. 26, 2008 to Feb.26, 2009.**
- ◆ **Pesticide varieties: 460 pesticides grouped into 6 of A, B, C, D, E and F, and each group in turn contains respectively 85 varieties, 95, 76, 83, 68 and 53.**
- ◆ **Detection means: GC-MS.**
- ◆ **Analytical results: determination is made every two days, A, B, C, D, E and F are all parallel experiments with 31 determinations conducted in 3 months and 28520 analytical data obtained. See Table 1 attached.**

1. The statistical analysis of the test data for stability of 460 pesticides over three months: ratio change

When the response values obtained from different pesticides on the first day are set as 1, those obtained later are compared with the former and the ratios are thus calculated. The change of this ratio reflects the rate of decomposition of the pesticides.

Nos. of pesticides



Ratio range*	15 days 12.10	30 days 12.26	45 days 1.09	60 days 1.26	75 days 2.11	90 days 2.26
<0.29	1	14	44	8	37	119
0.29-0.40	4	22	64	41	106	223
0.41-0.50	5	31	115	194	184	89
0.51-0.60	8	69	132	130	83	13
0.61-0.70	17	94	68	73	36	5

Ratio range*	15 days 12.10	30 days 12.26	45 days 1.09	60 days 1.26	75 days 2.11	90 days 2.26
0.71-0.80	36	140	13	5	6	3
0.81-0.90	45	55	4	4	3	2
0.90-1.00	82	12	3	4	1	1
>1.00	262	21	14	1	4	4

Conclusion: Statistical calculation is conducted at 80% confidence level for the ratio distribution of 460 pesticides (eliminate 10% max values and 10% min values), and the decomposition range falls within 0.73-1.31 by the time of 15 days, 0.45-0.86 by 30 days, 0.29-0.68 by 45 days, 0.40-0.64 by 60 days, 0.30-0.61 by 75 days and 0.20-0.47 by 90 days. Tests have found that most of the pesticides decompose rapidly between the first 30-45 days, with decomposition rate reaching 30%, and decompose slowly thereafter.

2. The statistical of analysis of the test results of Groups A, B, C, D, E and F on the 90th day of the study of the stability of the 460 pesticides

The statistical table of decomposition analyzed on the 90th day for 460 pesticides

Ratio range*	A	B	C	D	E	F	Total
<0.29	1	8	2	9	3	4	27
0.29-0.40	11	63	44	53	16	18	205
0.41-0.50	43	7	3	0	34	20	107
0.51-0.60	12	2	1	0	3	5	23
0.61-0.70	1	0	1	0	1	1	4
0.71-0.80	0	0	0	1	0	0	1
0.81-0.90	1	0	1	0	1	0	3
0.91-1.00	0	0	0	0	0	1	1
>1.00	1	0	0	0	1	0	2

Ratio range refers to the distribution range of the response ratios for the stability analyzed on Feb.26, 2009

It can be seen from the above-mentioned table that there are still 346 pesticides with ratio range above 0.29 analyzed on the 90th day while the standard solutions are placed at room temperature.

3. The test conclusions of the stability of the standard solutions of 460 pesticides

After the stability test for 3 months, there are 345 pesticides with the ratio of response value bigger than 0.29, belonging to those of relative stable pesticides, which can be adopted as the target for stability study in the next stage of tea samples.

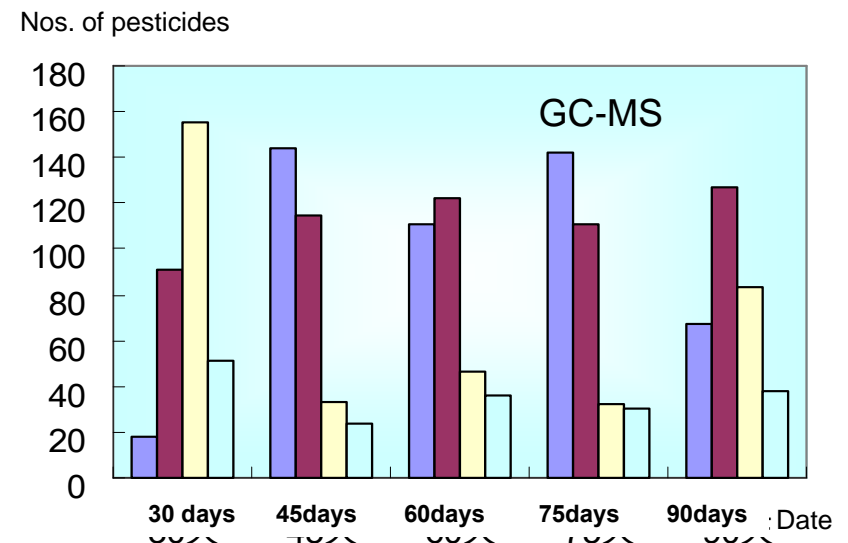
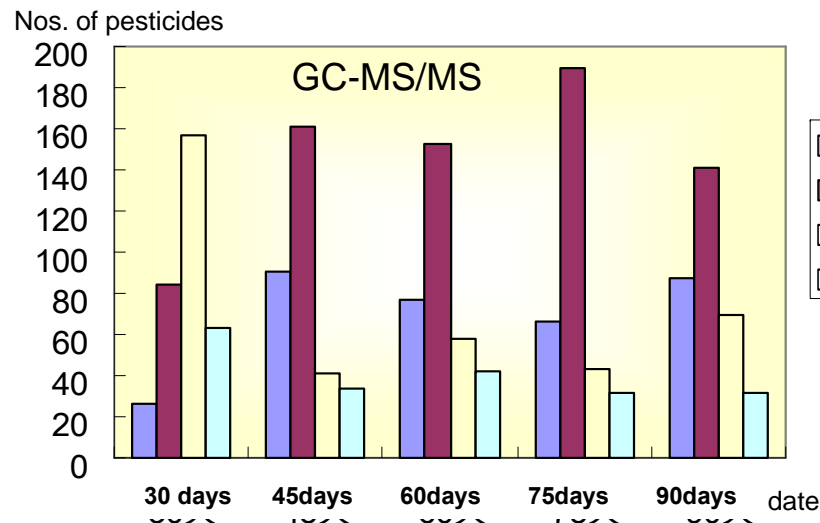
Stage II: The stability study of 345 pesticides in tea

- ◆ **Experimental time: three months from Mar.31,2009 to Jun.31,2009.**
- ◆ **Pesticide varieties: 367 pesticides breaking into four groups of A, B, C and D.**
- ◆ **Tea varieties: Wu Long and Green Tea are each analyzed by 2 persons.**
- ◆ **Detection means: GC-MS and GC-MS-MS**
- ◆ **Analytical results: the analysis is carried out every 5 days, with parallel testing of each tea and 26904 analytical test data obtained from 19 times of determination over 3 months, [See Table 2 attached.](#)**

1. Preparing the incurred tea samples and deciding the fixed values

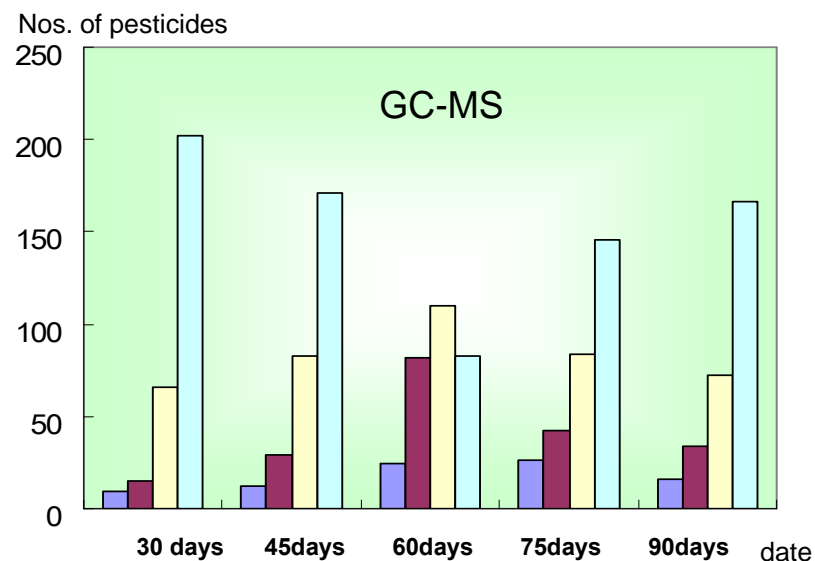
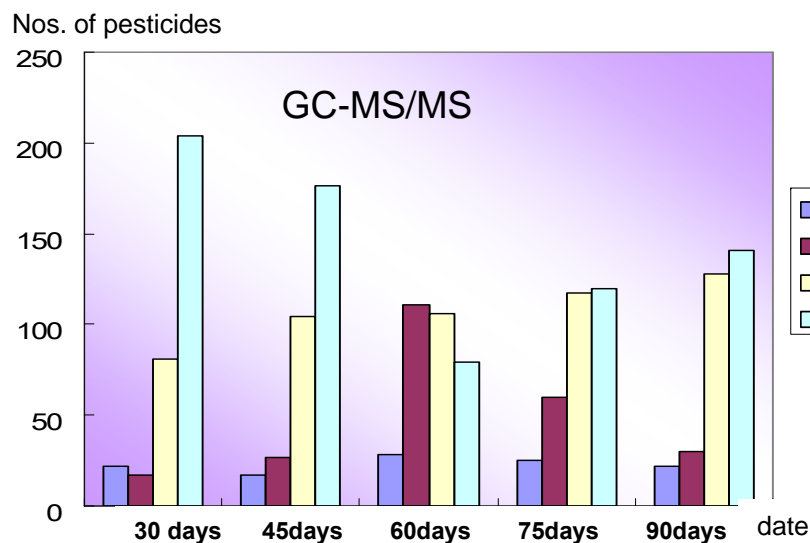
- ① Break 345 pesticides into 4 groups, and prepare them into mixed standard solutions.**
- ② Take a certain quantity of tea, grind and pass them through 10-15m sieve and make a uniform spray with the pesticides.**
- ③ Weigh a certain quantity of representative pesticide incurred samples and fix the values for 367 pesticide residual levels with GC-MS and GC-MS/MS.**

2. The statistical analysis of the test results of stability of 345 pesticides in Green Tea



- ① When the Green Tea incurred samples are stored for 30 days, approximately 35% pesticides have decomposed 30% (ratio less than 0.7); yet by 45 days, about 80% pesticides have decomposed 30%; obviously in the stage of 30-45 days, most of the pesticides have decomposed very quickly; in the stage of 45-90 days, the rate of decomposition of the pesticides tend to slow down with not very big differences.
- ② In general, about 80% of the pesticides decompose to the fixed value 70% below, and nearly 30% of the pesticides decompose to the fixed value 50% below within 90 days.
- ③ There are slight differences between the values of GC-MS/MS and GC-MS, which is mainly manifested in that the number of the pesticides with the pesticide content analyzed by GC-MS/MS greater than the fixed value 50% (ratio ≥ 0.5) is bigger than that of the pesticides by GC-MS.

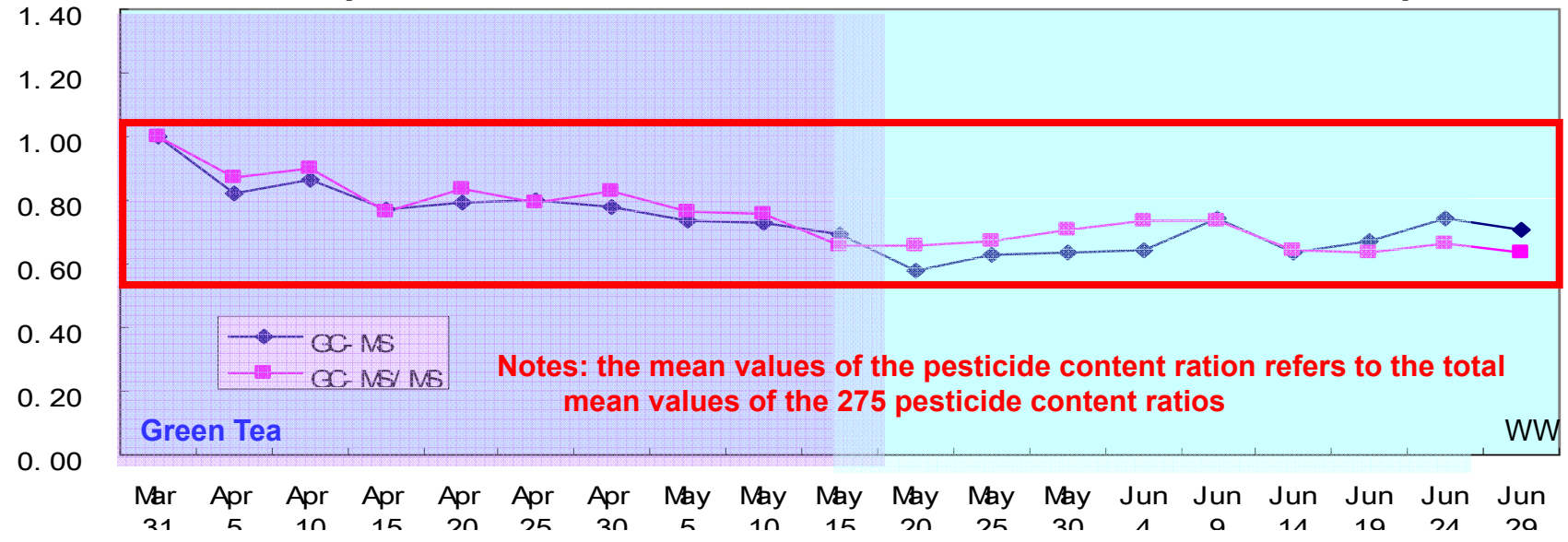
3. The statistical analysis of the test results of stability of 345 pesticides in Wu Long Tea



- ① Regarding Wu Long Tea, The determined values of approximately 90% of the pesticide content is greater than the fixed value content 70% (ratio>0.7) by the time of 30 days, yet this ratio drops to around 85% and 60% by 45 days and 60 days. There is no apparent decrease after 60 days, which proves that the pesticides in Wu Long Tea decompose rapidly after 45 days.
- ② In general, above 55% of pesticides in Wu Long Tea still can reach the fixed value content above 70% after the 90 days' decomposition, whose ratio is evidently higher than that of Green Tea. This also shows that pesticides in different tea matrices have different decomposition rate; they decompose rapidly in Green Tea but slowly in Wu Long Tea.
- ③ Unlike Green Tea, the analytical results of Wu Long Tea by both GC-MS/MS and GC-MS do not have marked differences. For Green Tea, However, the differences in the results by these two instruments are remarkable.

4. In the analytical results of the 345 pesticides, a total of 70 pesticides with no peaks, rapid decomposition, ratio less than 0.5 and RSD greater than 40% have been eliminated in the matrices of tea, and the mean values are calculated for the ratio of pesticide content of the reserved 275 pesticides, while the mean values of this ratio is being observed for its change with the time.

The mean values of the ration of pesticide content ① The pesticide metabolic curve in the Green Tea incurred samples

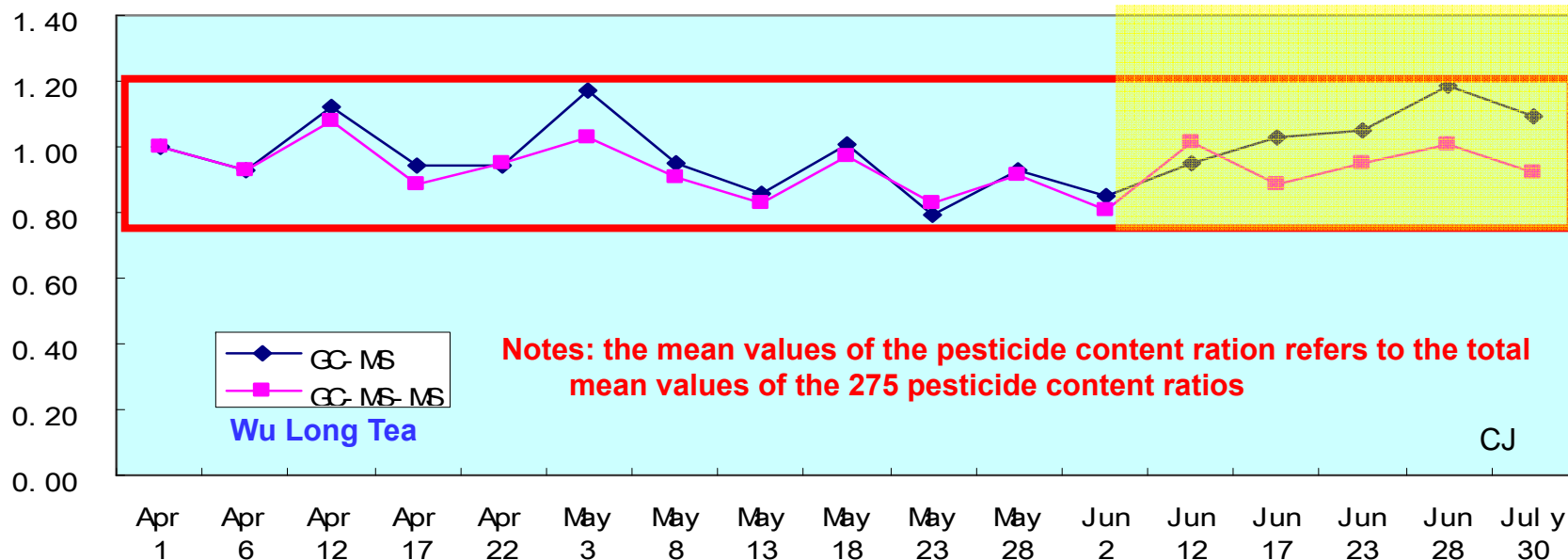


Method	Mar 31	Apr 5	Apr 10	Apr 15	Apr 20	Apr 25	Apr 30	May 5	May 10	May 15	May 20	May 25	May 30	Jun 4	Jun 9	Jun 14	Jun 19	Jun 24	Jun 29
GC-MS	1.00	0.82	0.86	0.77	0.79	0.80	0.78	0.73	0.73	0.69	0.58	0.63	0.63	0.65	0.75	0.64	0.67	0.74	0.71
GC-MS/MS	1.00	0.87	0.90	0.77	0.83	0.79	0.83	0.76	0.76	0.66	0.66	0.67	0.71	0.74	0.73	0.64	0.64	0.67	0.64

It can be seen from the metabolic curve: (1) the first 50 days is the period of pesticide rapid decomposition, while the last 40 days witnesses no apparent decomposition; (2) the analytical results by GC-MS/MS and GC-MS are basically identical.

② The pesticide metabolic curve in the Wu Long Tea incurred samples

The mean values of the ration of pesticide content



Method	Apr 1	Apr 6	Apr 12	Apr 17	Apr 22	May 3	May 8	May 13	May 18	May 23	May 28	Jun 2	Jun 12	Jun 17	Jun 23	Jun 28	July 30
GC-MS	1.00	0.93	1.12	0.94	0.94	1.17	0.95	0.86	1.01	0.79	0.93	0.85	0.95	1.03	1.05	1.19	1.09
GC-MS-MS	1.00	0.93	1.08	0.89	0.95	1.03	0.91	0.83	0.97	0.83	0.91	0.81	1.01	0.89	0.95	1.01	0.92

It can be seen from the metabolic curve that the mean values of the ratios have no apparent decomposition over the period of 3 months, and moreover the before 60 days, instrumental results of GC-MS/MS and GC-MS are basically the same, while after 60 days mistakes occur with the two analytical results, which fail to reflect the true metabolic process. In such a case, it will get confirmed until after 90 days when the test of youden pair samples have been finished.

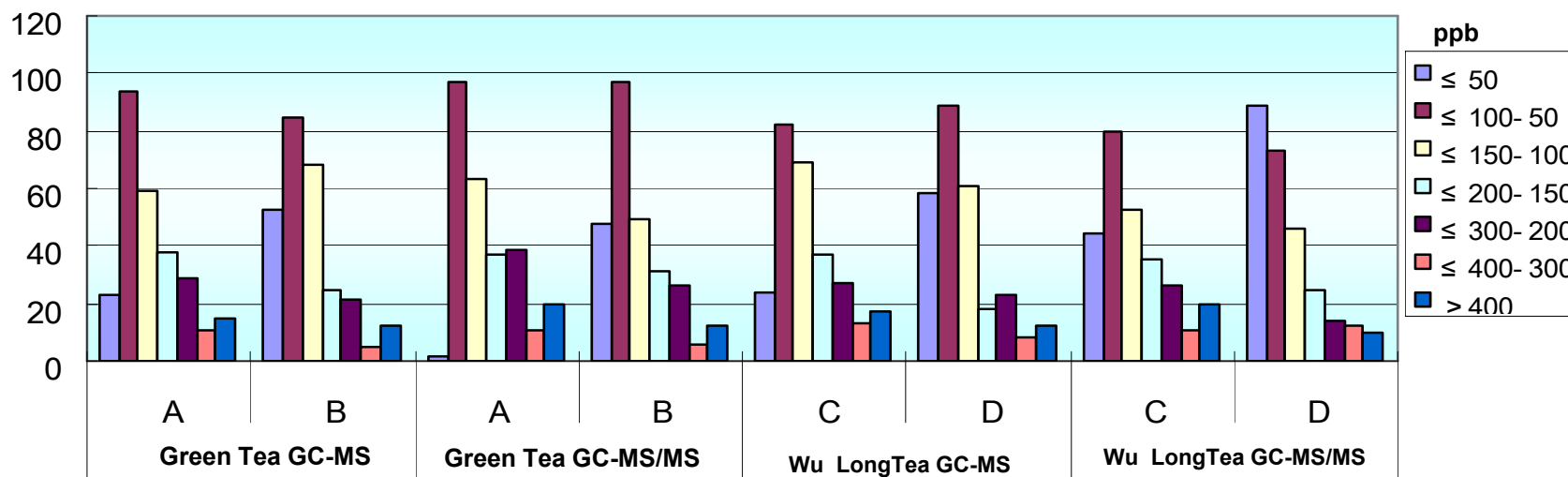
Conclusions reached in Stage II: 275 pesticides have been chosen as the target pesticides for the next stage study.

Stage III: Preparing the youden pair samples for the inter-collaborative study and studying the ruggedness of the method

- ◆ **Time: two months from Jul.9,2009 to Sep.10,2009.**
- ◆ **Pesticide varieties: 275.**
- ◆ **Tea varieties: Wu Long Tea and Green Tea.**
- ◆ **Analytical means: GC-MS/MS and GC-MS.**
- ◆ **Analytical results: determination is made every 5 days, with parallel tests for 3 samples and continuous analysis for 2 months as well as 39024 analytical data obtained. The analytical results are shown in [Table 3 attached](#).**

1. Deciding the concentrations of 275 pesticide residues in youden pair samples: weigh at random the tea taken from 5 different areas for content determination and get the mean values and RSD of the content of 5 parallel samples, in which most of RSD values fall within 15% and it can be assumed that the tea samples are relatively homogenous, while the mean content of the 5 parallel samples at this moment is the fixed value content. See Table 4 attached.

Nos of pesticides Distribution of the fixed value content for 275 pesticides in youden pair samples

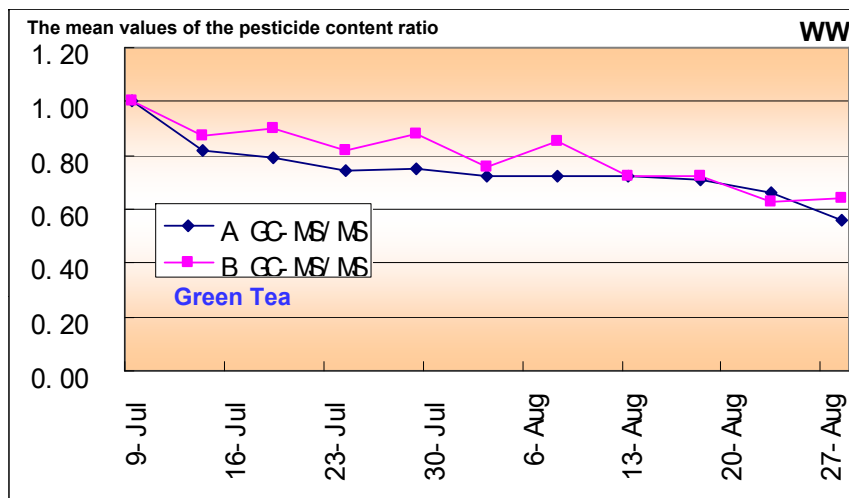
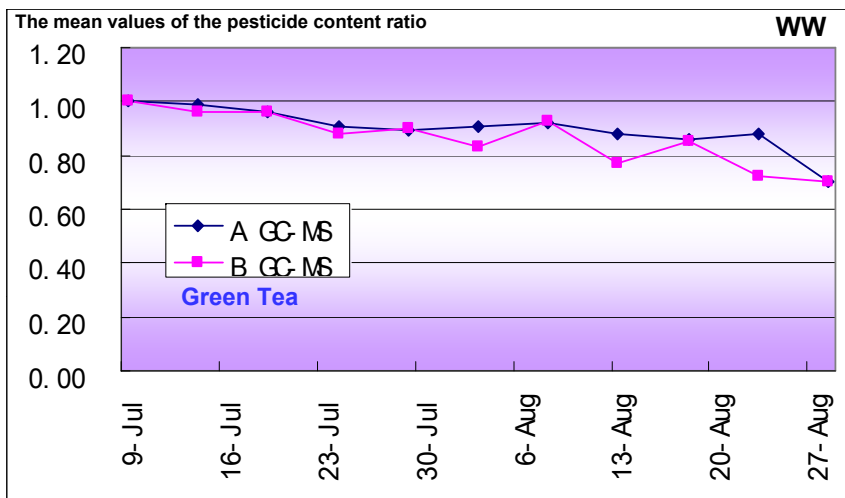


Distribution range of fixed value content /ppb	Green Tea GC-MS		Green Tea GC-MS/MS		Wu Long Tea GC-MS		Wu Long Tea GC-MS/MS		Distribution range of fixed value content /ppb	Green Tea GC-MS		Green Tea GC-MS/MS		Wu Long Tea GC-MS		Wu Long Tea GC-MS/MS	
	A	B	A	B	C	D	C	D		A	B	A	B	C	D	C	D
≤50	23	53	2	48	24	58	44	89	≤300-200	29	21	39	26	27	23	26	14
≤100-50	94	85	97	97	82	89	80	73	≤400-300	11	5	11	6	13	8	11	12
≤150-100	59	68	63	49	69	61	53	46	> 400	15	12	20	12	17	12	20	10
≤200-150	38	25	37	31	37	18	35	25									

Conclusions: through the fixed value determination of the 275 pesticides in youden pair samples, the residue levels all fall within 17 ppb-2.0 ppm range, which thorough complies with the requirements of international trade.

2. The statistical analysis of the decomposition rate of pesticide content of Youden pair samples (A/B)

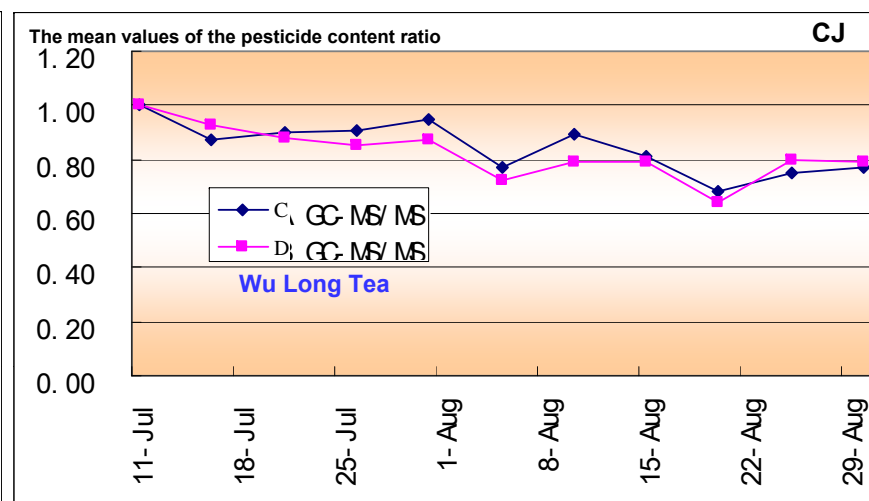
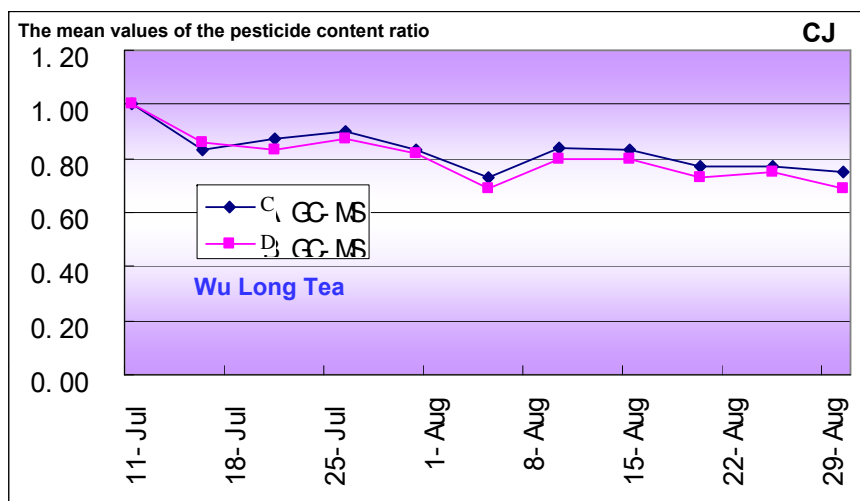
(1) The pesticide metabolic curve of Green Tea youden pair samples (A/B)



Method	Youden pair	9-Jul	14-Jul	19-Jul	24-Jul	29-Jul	3-Aug	8-Aug	13-Aug	18-Aug	23-Aug	28-Aug
GC-MS	A	1.00	0.99	0.96	0.91	0.89	0.91	0.92	0.88	0.86	0.88	0.70
GC-MS	B	1.00	0.96	0.96	0.88	0.9	0.83	0.93	0.77	0.85	0.72	0.70
GC-MS/MS	A	1.00	0.82	0.79	0.74	0.75	0.72	0.72	0.72	0.71	0.66	0.56
GC-MS/MS	B	1.00	0.87	0.9	0.82	0.88	0.76	0.85	0.72	0.72	0.63	0.64

- ① The pesticides in Green Tea youden pair samples decompose within 40% in nearly 2 months, which are basically identical with test results in Stage II.
- ② The analytical results of GC-MS/MS and GC-MS are slightly different.

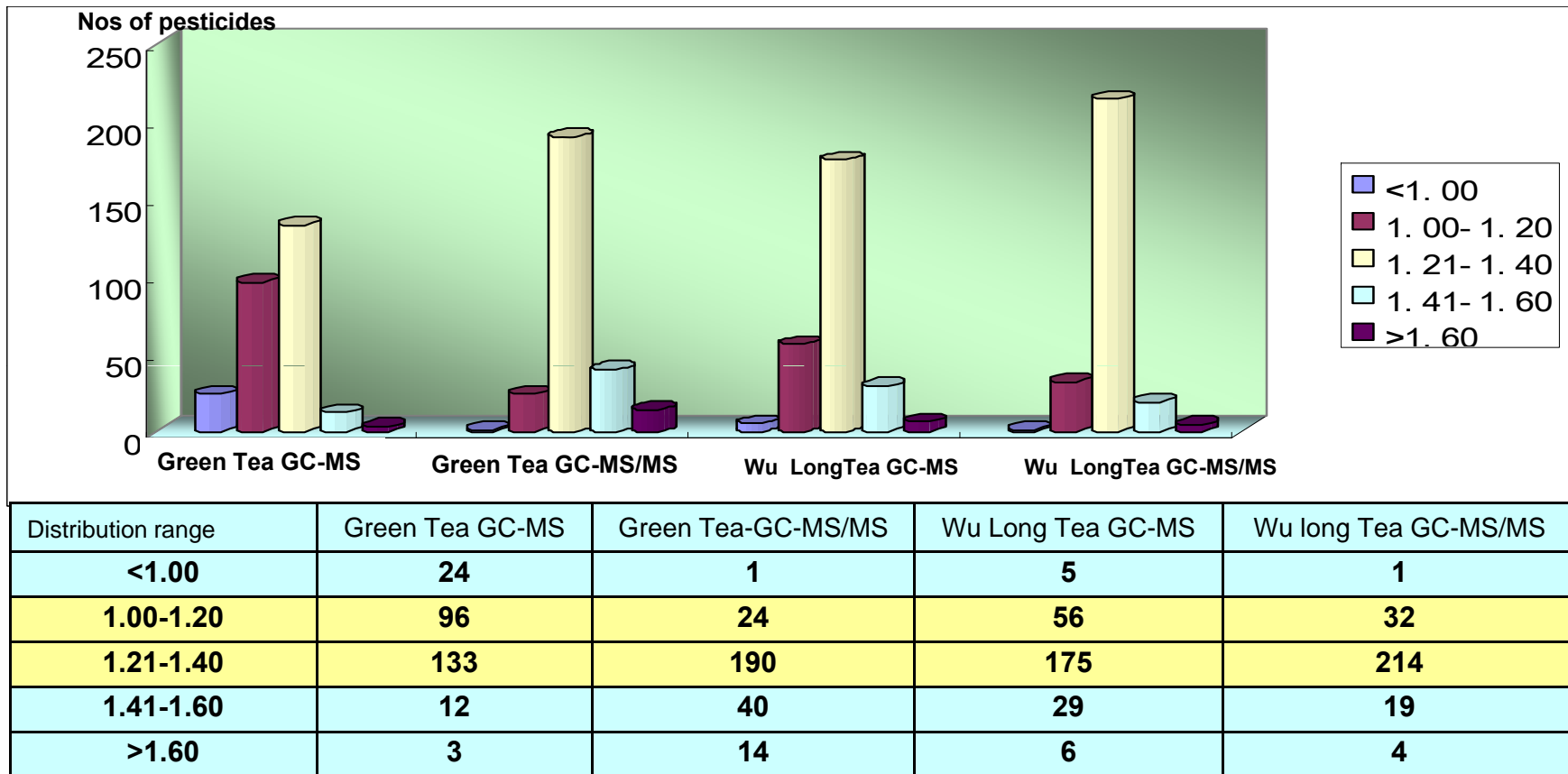
(2) Pesticide metabolic curve in Wu Long Tea youden pair samples (C/D)



Method	Youden pair	11-Jul	16-Jul	21-Jul	26-Jul	31-Jul	5-Aug	10-Aug	15-Aug	20-Aug	25-Aug	30-Aug
GC-MS	C	1.00	0.83	0.87	0.9	0.83	0.73	0.84	0.83	0.77	0.77	0.75
GC-MS	D	1.00	0.86	0.83	0.87	0.82	0.69	0.8	0.8	0.73	0.75	0.69
GC-MS/MS	C	1.00	0.87	0.9	0.91	0.95	0.77	0.89	0.81	0.68	0.75	0.77
GC-MS/MS	D	1.00	0.93	0.88	0.85	0.87	0.72	0.79	0.79	0.64	0.8	0.79

- ① Pesticides decompose slowly in Wu Long Tea youden pair samples, within 25% nearly 2 months, which has a slower decomposition rate than that of Green Tea.
- ② GC-MS/MS is very close to GC-MS in analytical results, without any system bias.

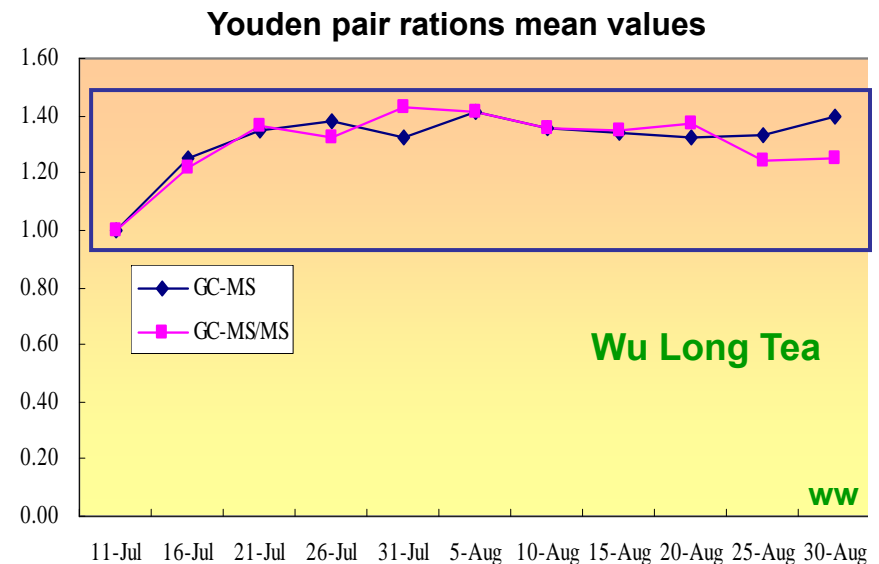
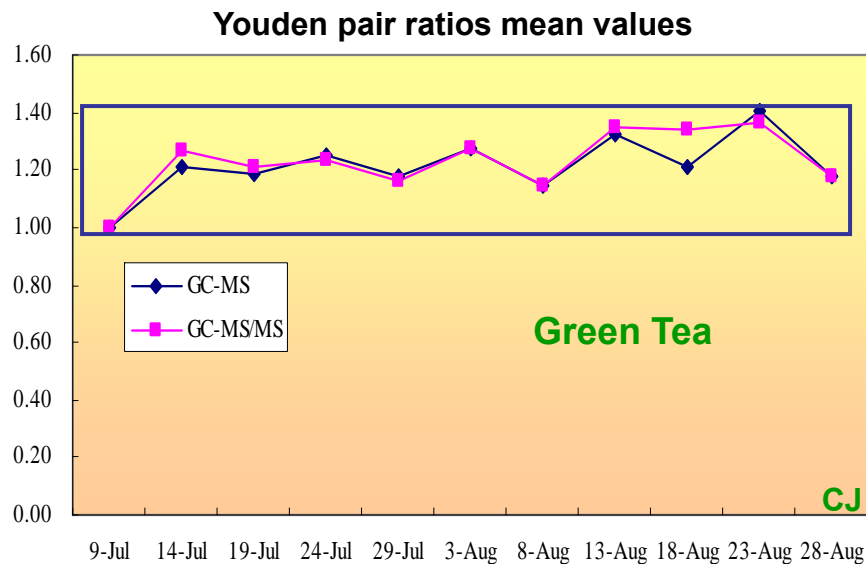
3. The statistical analysis of the distribution range of the ratios of fixed values for 275 pesticides in Youden pair samples



It can be seen from the above-mentioned data that the ratios of the youden pairs of above 85% pesticides fall within a range of 1.00-1.40; the ratios less than 1.0 and greater than 1.6 merely account for 5%, which can be said that most pesticides in youden pairs all get their reasonable content ratios.

The fixed value ratios of Youden pairs refer to the ratios of fixed values content A(C) with that of Sample B(D).

4. The statistical analysis of Youden pair ratios' change with time in Youden pair samples



It can be noticed from the change curve of the youden pair ratios over 2 months:

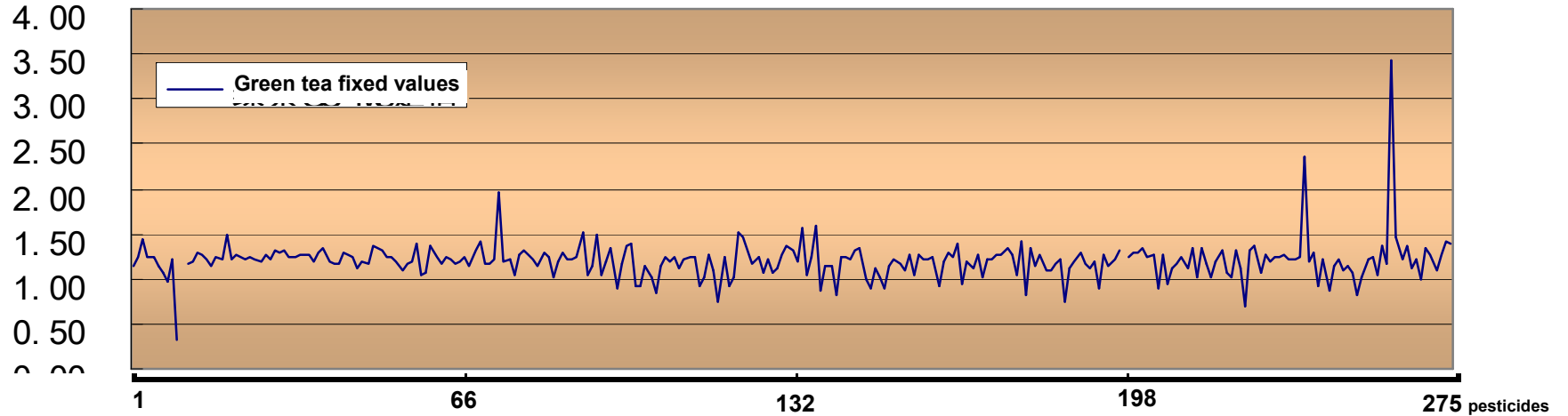
- (1) Be it Green Tea or Wu Long Tea and be it GC-MS/MS or GC-MS analysis, the absolute majority of pesticide youden pair ratios fall within the range of 1.0-1.4.
- (2) With the lengthening of time, pesticide content in tea becomes small, but youden pair ratios still remain the original value range, which demonstrates that in the process of sample determination in-lab accidental bias is very small and the analytical results are accurate and reliable, proving the method is of very good ruggedness.

Notes: Youden pair ratios' mean values refer to the total mean values of youden pair ratios for 275 pesticides

5. Comparison of youden pair ratio range with actual values for 275 pesticides in Green Tea by GC-MS

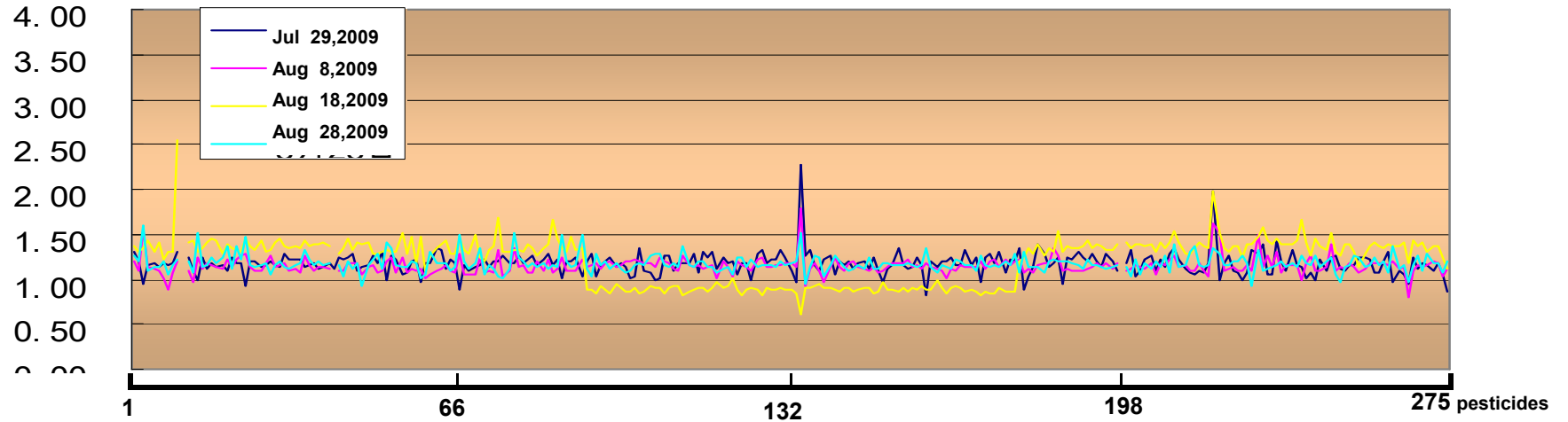
(1) Youden pair ratio fixed values for 275 pesticides in Green Tea

Mean values of pesticide ratio content



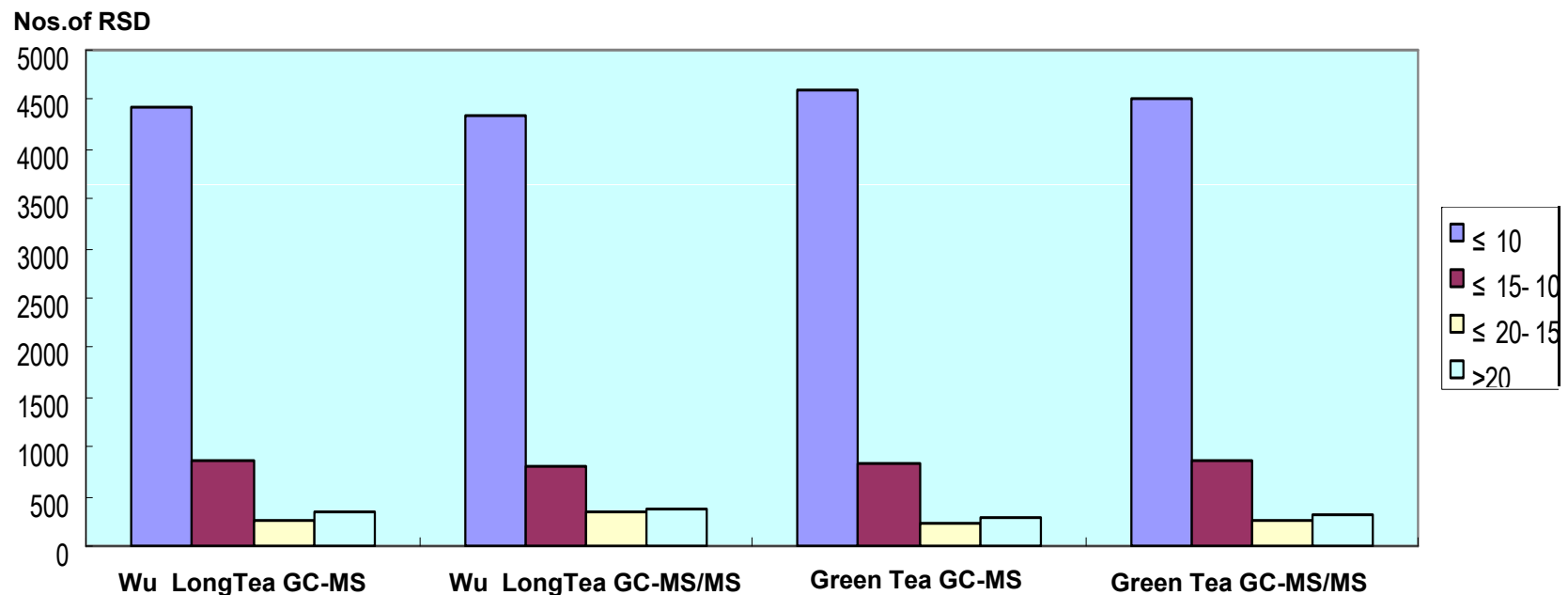
(2) Overlapping diagram of analytical values over 4 times for 275 pesticides in Green Tea

Mean values of pesticide ratio content



6. Statistical analysis of RSD for 275 pesticides in youden pair samples

Using both GC-MS/MS and GC-MS, 12 determinations are conducted on the 275 pesticides in both Green Tea and Wu Long tea youden pairs samples within 2 months(3 samples for every parallel testing), with 26000 RSD values. See [Table 5 attached](#).

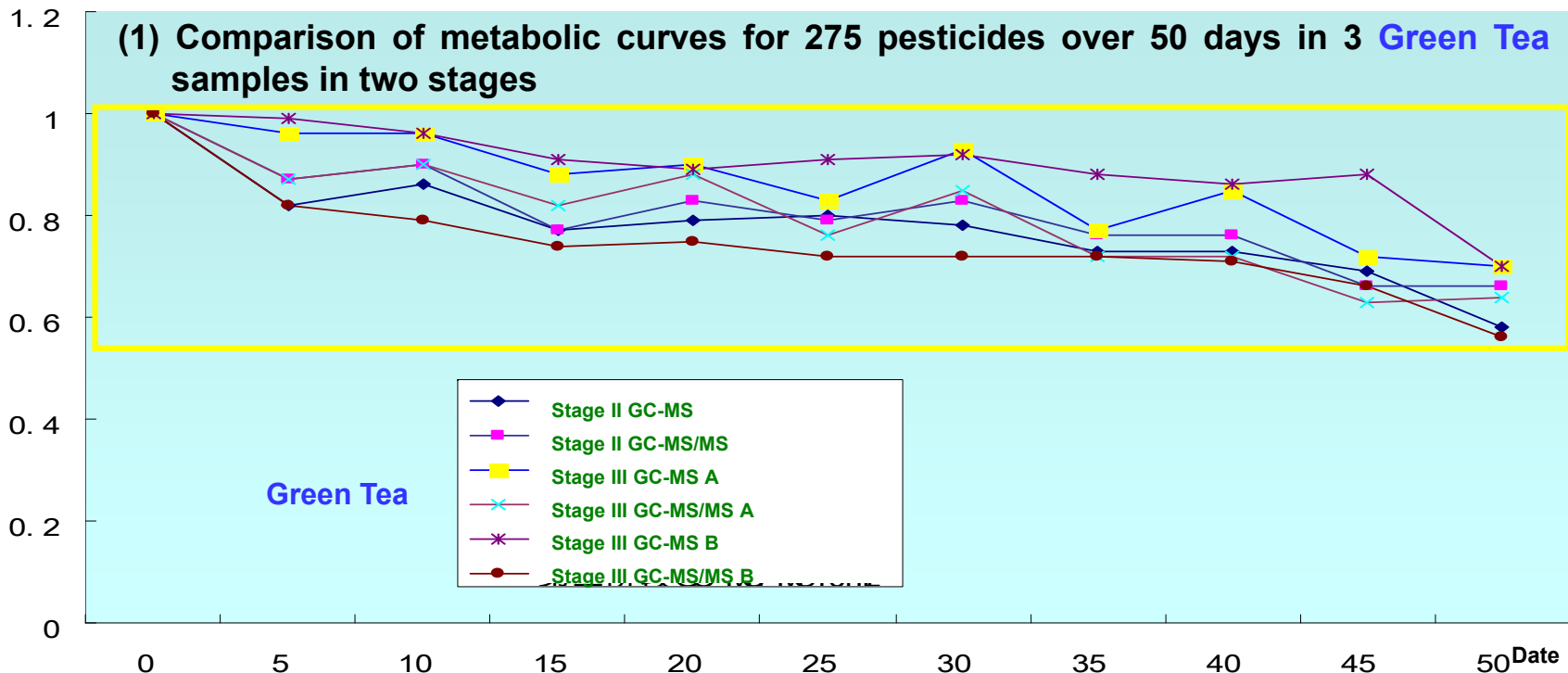


It can be viewed from the above figure: Above 75% pesticides have RSD $\leq 10\%$ in different tea matrices and under different instrumental conditions; above 90 pesticides have RSD $\leq 15\%$; Pesticides with RSD $\leq 20\%$ account for 94.5%, which demonstrates that the prepared tea youden pair samples are uniform, representative, and the method is of very good ruggedness.

7. Comparison of the analytical results of the pesticide stability in Green Tea and Wu Long Tea in Stage II and Stage III

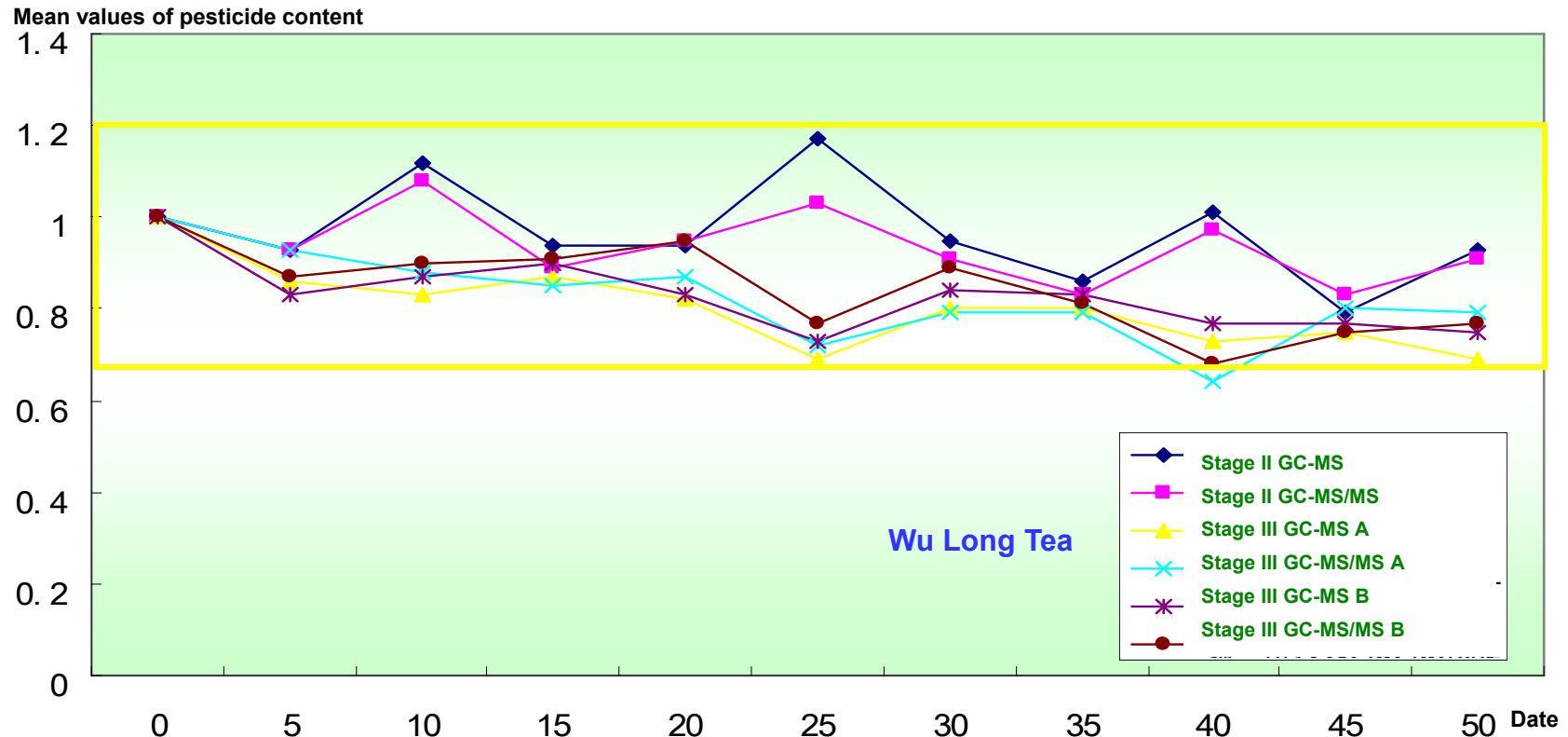
Below are the above-mentioned two stages, where different personnel use two different instruments for the study of 275 pesticides contained in 6 samples of two teas with their metabolic curves over 50 days placed together for a comparative analysis.

Mean values of pesticide ratio content



Metabolic curves for 3 Green Tea incurred samples over 50 days in two stages indicate: In the first 50 days pesticide contents rapidly decompose to 56%-70%, and the analytical results in the two stages are identical. Test results bias fall within 20% in different stages using different instruments by different lab personnel, without apparent system bias.

(2) Comparison of metabolic curves for 275 pesticides over 50 days regarding 3 Wu Long Tea samples in two stages



Metabolic curves for 3 Wu Long Tea incurred samples over 50 days in two stages indicate: In the first 50 days pesticide contents rapidly decompose to 69%-93%, suggesting a non-uniform decomposition, with a relatively big difference for the test results of the two stages, which reflects the values on the high side in Stage I, exceeding the original value 1.0 at many points. It is tentatively judged that the cause is traced to operations by different lab personnel.

8. Summary of the three stages of study

(1) Study of the stability for 460 pesticides in mixed standard solutions over a succession of 3 months

75 pesticides have been eliminated because of their instability in the mixed standard solutions

(2) study of metabolic pattern for 345 pesticides at ppb level in tea over a succession of 3 months

70 pesticides have been eliminated because of the quick metabolic process in tea

(3) Study of the metabolic pattern for 275 pesticides in tea youden pair samples over a succession of 3 months

It will need another month to finish the test, but no abnormalities have been found yet.

Pesticide varieties have been tentatively selected for organizing the inter-collaborative study on tea, and the ruggedness of the method has been verified, which has laid the fundamental conditions for organizing the AOAC inter-collaborative study.

Work to be done in Stage IV

1. Design the AOAC inter-collaborative study protocol and prepare Green Tea and Wu Long Tea youden pair samples containing 100-200 multiresidue pesticides. Choose a part of laboratories to carry out rehearsals in order to accumulate experience for officially organizing the inter-collaborative study.
2. Prepare the practice samples for testing the capability of inter-collaborative study participating laboratories in order to supply to the participants for practice before officially undertaking the inter-collaborative study samples.
3. Submit the inter-collaborative study protocol to AOAC International, and prepare the organization of inter-collaborative study for the world volunteering laboratories.
4. I would like to invite you experts, who are interested in this study, to participate, and right now you may register with us. We will be very happy to cooperate with you, and make this study an AOAC official method.

- ◆ The development of AOAC proves that very piece of her work is dedicated to excellence and perfection, therefore she has been able to create and maintain a glorious history for 125 years. In such a spirit, we are also constantly pursuing excellence and perfection. Therefore, we have encompassed our past year's work for AOAC in a circle and present it to everyone here. We hope that it will achieve a happy ending under the direction of every expert present today.
- ◆ Wish all of you have a happy ending to whatever business you undertake.
- ◆ Thank you very much!



Sep. 15, 2009

◆ A THOUSAND THANKS
◆ FOR THE FRIENDS OF THE WORLD !

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