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منظمة الأغذية والزراعة للأمم المتحدة | 联合国粮食及农业组织 | Food and Agriculture Organization of the United Nations | Organisation des Nations Unies pour l'alimentation et l'agriculture | Продовольственная и сельскохозяйственная организация Объединенных Наций | Organización de las Naciones Unidas para la Agricultura y la Alimentación

COMMITTEE ON COMMODITY PROBLEMS

INTERGOVERNMENTAL GROUP ON TEA

Nineteenth Session

New Delhi, 12 – 14 May 2010

REPORT OF THE WORKING GROUP ON MAXIMUM RESIDUE LEVELS BASED ON "TEA BREW"[1]

I. INTRODUCTION

At its Eighteenth Session held in Hangzhou, China (14 – 16 May, 2008), the Inter Governmental Group (IGG) on Tea, agreed to establish a Working Group on Maximum Residue Levels (MRLs) to have a look at the 'tea brew' in order to establish the feasibility of estimation of pesticide residue contents in tea brew which is consumed by human beings. In fact, the fixation of MRLs of pesticides should be based on tea brew.

II. BACKGROUND

The report of the existing Working Group on Maximum Residue Levels (MRLs) in tea constituted earlier by IGG on Tea, presented their report at the 18th Session of IGG at Hangzhou in May 2008, by the Coordinators (Dr. T. C. Chaudhuri and Dr. A. Scott). IGG thereafter debated the possibilities of fixation and achieving global harmonization of MRLs. Based on the deliberations at the said meeting, some decisions were taken on MRL issue, 'tea brew' in particular, under para 24 to 28 of the Report of the 18th Session of IGG on Tea, including constitution of a separate Working Group on Maximum Residue Levels (MRLs) on 'Tea Brew' (para 27) with the following objectives:

"Sampling of tea on the brew would be investigated by the WG under the leadership of Prof. Chen (China) with the assistance of Dr. Chaudhuri and Mr. Simrany (USA). This WG would:

- Combine the existing methodology of measuring residues in the tea brew, risk assessment and solubility of chemicals for establishing MRLs;
- Collect data on tea brew as well as leaf for the same sample;
- Investigate the legal considerations for establishing MRLs by this method;
- Consult appropriate international agencies, *e.g* JMPR, Codex Alimentarius and national authorities."

[1] Submitted by Dr T.C. Chaudhury (India).

III. ACTION PLANS

Individual efforts were made by the scientists in China, India and Japan to find out factual positions about the presence of pesticide residues in the brew of black and green tea, and then published the results that include methods for preparation of hot water brew for residue estimations. These factual data on the transfer of residue in tea brew from solid tea however confused the producers, traders and consumers of tea after finding the wide gap that exists of the residue contents between the solid tea and hot water brew as it is consumed. Experimental data on the transfer of residue for commonly used pesticides were also presented at the last IGG at Hangzhou which also showed a wide range of water solubility between the compounds.

This background information helped to formulate action plans for the WG on tea brew. This includes development of methodologies for estimations of residues in tea brew for both black and green tea; testing and validation of methods through international ring tests, collaboration of data with international bodies like, Codex JMPR. WG was also advised to meet during the intermittent period of IGG meetings to review the progress of the work of the group.

IV. MEETING OF THE WORKING GROUP ON MRLs IN TEA BREW

Based on the decisions at Hangzhou, the Working Group on tea brew met at the IGG headquarter at Rome on 20-22 May, 2009 and finalized the following action plans:

- Publish the methodology for tea brew, for the establishment of MRL in tea brew;
- Organize the collection of data on tea brew as well as leaf for the same sample;
- Investigate the legal considerations for establishing MRL by this method; and
- Consult appropriate international agencies, e.g. JMPR, Codex Alimentarius and national authorities.

A. ESTABLISHMENT OF METHODOLOGIES (DRY TEA INFUSTION/BREW)

Status: The methodology for preparation of tea infusion/brew for estimation of pesticide residue has been published. Thereafter, some specific actions were suggested in the WG meeting at Rome, in a time bound manner which are stated as under.

- Validation of the established methodology and organizing ring tests in different countries and laboratories as required by the regulators for three pesticides, Dimethoate, Bifenthrin, Imidachloprid (by 30th September 2009).
- Sampling: China for Green tea; India for Black tea; (medium particle), 7 day PHI (by 30th September 2009).
- Ring test labs, India (2), China (2), Sri Lanka (1), Indonesia (1), Kenya (1), US (1), Spcht (Germany 1), Bangladesh (1), total 10 labs.
- Methodologies for all three chemicals are to be specified along with dispatching of samples (by 30th September 2009) (for laboratories to submit results, 30th November 2009).
- To prepare the final document for submission to regulatory authorities on final data base and methodology. To be done by Prof. Chen and Dr. Chaudhuri (by 31st January 2010).

B. POLICY ISSUES

Status: For preparation of a document for submission to regulators.

Recommended action: The team leader designated for this action; Tea Board of India and Ministry of Agriculture, Department of Crop Protection, China. The Secretariat of IGG on tea will provide the necessary infrastructural support, as needed.

C. COUNTRY-SPECIFIC ACTIONS

Status: Methodology.

Action: Individual country specific actions may be supported through sharing of technical information gathered by the Working Group.

D. FUNDING

Status: Source to be identified.

Recommended action: Participating countries (producing / consuming) will bear the cost of country specific expenditure for analysis and sampling. In case of private laboratories, the issue of funding is open for consideration.

V. FOLLOW-UP ACTIONS

After the WG meeting at Rome on 20-22 May, 2009 and keeping in mind time targets, all actions were taken, brief of which is given as under:

1. Published methodology entitled 'Leaching of residues of certain pesticides from black tea to brew', Food Chemistry 2009,113:522-525 (Manikantan et. al, 2009) was considered to finalize SOP (Standard Operating Procedures) for analyzing residue content in 'solid tea' and in 'tea brew'. For black tea, SOP and data reporting sheets were written by India (UPASI-TRF in consultation with TRA) and for green tea same methods were refined and circulated by China.

2. Action Points on decisions of WG on Tea Brew, May 20-22, 2009, Rome was finalized by Dr. Chaudhuri in consultation with scientists - Dr. A. K. Barooah, Dr. N. Muraleedharan and Prof. Chen Zangmao in June 2009 and these were circulated to all concerned labs and nodal persons for taking further actions as assigned.

3. Field experiments were laid out in two places: India- TRA, Jorhat; and China – TRI, Hangzhou by July, 2009.

4. Black and green tea samples were dispatched along with SOP and reporting sheets to eight laboratories. For the rest two labs, samples could not be dispatched by Aug 2009.

5. Analysis of samples in respective labs were carried out and reported in between Sept09 – Jan10.

6. Compilation of data in Jan – Feb 2010 and report writing for submission to Codex through IGG.

For preparation of Policy Paper, Tea Board, India made an effort to identify a consultant but this could not be pursued further. However, a policy paper highlighting issues and giving justifications was compiled for presentation.

VI. RESULTS

Validation of established methodology (dry tea and tea infusion/brew) was done following international ring test organizing in six countries and 8 laboratories. Details of the results are given in Enclosure 1 and Annexure I, II and III.

VII. SUMMARY AND CONCLUSIONS

1. The performances of SOPs under the ring test for black and green tea and brew are satisfactory. Observations and summery of results are given under Conclusion in Enclosure 1. Constraints in few labs identified by the Working Group for conducting international ring test restricted them to analyze the samples, however, results are drawn based on analysis of available data, despite rare outliers.

Enclosure-I

Follow-up actions on the Decisions of the IGG Working Group on 'Tea Brew' on 20-22 May 2009, Rome.

I. Validation of established methodology (dry tea and tea infusion/brew)

Objective:

Validation of the established methodology for analysis of residues in 'dry tea' and 'tea infusion/brew' by organizing international ring test in eight countries involving 10 laboratories as required by the regulators.

Status

Dry Black tea tea samples were prepared from the leaves plucked on 5th day (PHI) after treatment with bifenthrin (@80g a.i./ha), imidacloprid (@22.25g a.i./ha) and dimethoate (@300g a.i./ha) in supervised field trials in India. Similarly Green tea samples were prepared from the leaves plucked on 7th day (PHI) after treatment with bifenthrin (@37.5g a.i./ha), imidacloprid (@45g a.i./ha) and dimethoate (@450g ai/ha) in supervised field trials in China. Both black tea and green tea samples were sent to 8 laboratories in 6 countries, viz, India (2), China (2), Sri Lanka (1), Indonesia (1), Kenya (1) and Bangladesh (1) for analysis under international Ring Test protocol.

Residues of three pesticides, viz., bifenthrin, imidacloprid and dimethoate, having different water solubility in both black tea and green tea as well as in the brew (liquor) were analysed in 6 laboratories. SOP for Black Tea and SOP for Green Tea for individual pesticides were followed and the results obtained are presented in Tables 1 to 12. The data were statistically analysed and evaluated using Horwitz ratio (HorRat) and Z scores.

Bifenthrin

SOP for determination of Bifenthrin residues in Black tea and brew: The performance of the method was satisfactory as indicated from acceptable HorRat and Z score values for both black tea and tea brew.

The SOP for determination of Bifenthrin residues in Green tea and brew: The performance of the SOP for determination of Bifenthrin residues in Green tea is satisfactory as indicated from acceptable HorRat and Z score values but the HorRat value was beyond the acceptable limit in case of tea brew.

Dimethoate

The SOP for determination of Dimethoate residues in Black tea and brew: The method performance was satisfactory as indicated from acceptable HorRat and Z score values for both black tea and tea brew, though the calculations were performed with very limited data.

The SOP for determination of Dimethoate residues in Green tea and brew: The method performance was satisfactory as indicated from acceptable HorRat and Z-score values for both green tea and tea brew.

Imidacloprid

The SOP for determination of Imidacloprid residues in Black tea and brew: The data showed large variation among the laboratories and the HorRat values were beyond the acceptable limits for both black tea and brew. However, the Z score for brew was satisfactory.

The SOP for determination of Imidacloprid residues in Green tea and brew: The method performance was satisfactory as indicated from acceptable Z score and HorRat values for both green tea and tea brew.

Summary and conclusion

The SOPs for determination of bifenthrin residues in dry black tea and its brew and dry green tea are satisfactory. However, the SOP for Green tea brew needs refinement. In case of dimethoate, both SOPs for black tea and green tea as well as their brew are satisfactory, though only limited data were available for black tea. The SOP for determination of imidacloprid residues in green tea and its brew is satisfactory. The method validation may be repeated for imidacloprid and dimethoate in Black tea.

The performances of SOPs for black tea, green tea and brew in general are good and can be considered as test methods, although refinement of SOPs is continuous process. Transfer of residue to tea brew/infusion depends on the solubility of the compounds which can be analysed precisely.

Annexure I. Compilation of Ring test results with statistical parameters (Annexure-I)

Annexure-I

(Compilation and Analysis of data by Dr Anup Barooah, TRA
Dr N. Muraleedharan, UPASI-TRF and
Dr T.C. Chaudhuri, NTRF, INDIA.)

FAO/IGG Ring Test Results

Validation of method of analysis of pesticide residues in tea and brew

Method: SOP for Black Tea

Table 1. Results for Bifenthrin residues in Black Tea

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)*	SD (±)*	RSD %	PRSD	Z score	HorRat value
1	Lab 1	1.910	1.717	0.430	25.08	14.65	0.45	1.71
2	Lab 2	1.010					-1.64	
3	Lab 3	2.148					1.00	
4	Lab 4	1.842					0.29	
5	Lab 5	1.670					-0.11	
6	Lab 6	0.043#						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1 to 5 only.
Data considered as outliers.
RNR: Result not reported. SD: Standard Deviation
RSD: Relative Standard Deviation PRSD: Predicted Relative Standard Deviation.

Table 2. Results for Bifenthrin residues in Black Tea Brew (liquor)

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)*	SD (±)*	RSD %	PRSD	Z score	HorRat value
1	Lab 1	0.022	0.031	0.015	47.26	26.75	-0.61	1.77
2	Lab 2	0.020					-0.75	
3	Lab 3	<0.1					-	
4	Lab 4	0.052					-2.06	
5	Lab 5	1.352#					-	
6	Lab 6	0.03					-0.07	
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1, 2, 4 and 6 only.
Data considered as outliers.
RNR: Result not reported. SD: Standard Deviation
RSD: Relative Standard Deviation PRSD: Predicted Relative Standard Deviation

Validation of method of analysis of pesticide residues in tea and brew

Method: SOP for Green Tea

Table 3. Results for Bifenthrin residues in Green Tea

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)	SD (±)	RSD %	PRSD	Z score	HorRat value
1	Lab 1	7.550	6.653*	0.341*	5.12	11.96	0.62	0.43
2	Lab 2	6.798					0.10	
3	Lab 3	7.486					0.58	
4	Lab 4	7.310					0.45	
5	Lab 5	4.120					-1.75	
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1 to 5 only.
RNR: Result not reported. SD: Standard Deviation
RSD: Relative Standard Deviation PRSD: Predicted Relative Standard Deviation.

Table 4. Results for Bifenthrin residues in Green Tea Brew (liquor)

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)*	SD (±)*	RSD %	PRSD	Zb score	HorRat value
1	Lab 1	0.026	0.052	0.055	106.90	24.78	-0.00	4.31
2	Lab 2	0.120					2.28	
3	Lab 3	<0.1					-	
4	Lab 4	<0.05					-	
5	Lab 5	0.009					-0.41	
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1, 2 and 5 only.
RNR: Result not reported. SD: Standard Deviation
RSD: Relative Standard Deviation PRSD: Predicted Relative Standard Deviation

Validation of method of analysis of pesticide residues in tea and brew

Method: SOP for Black Tea

Table 5. Results for Imidachloprid residues in Black Tea

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)	SD (±)	RSD %	PRSD	Zb score	HorRat value
1	Lab 1	0.026	0.240*	0.314*	131.04	19.68	-0.44	6.66
2	Lab 2	0.010					-0.50	
3	Lab 3	0.685					2.27	
4	Lab 4	0.239					0.44	
5	Lab 5	ND						
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1 to 4 only. ND: Not detected
RNR: Result not reported. SD: Standard Deviation
RSD: Relative Standard Deviation PRSD: Predicted Relative Standard Deviation

Table 6. Results for Imidachloprid residues in Black Tea Brew (liquor)

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)*	SD (±)*	RSD %	PRSD	Zb score	HorRat value
1	Lab 1	0.019	0.246	0.160	65.0	19.60	-0.68	3.32
2	Lab 2	0.122					0	
3	Lab 3	0.427					2.02	
4	Lab 4	<0.10						
5	Lab 5	ND						
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1 to 3 only. ND:Not detected.
RNR: Result not reported. SD: Standard Deviation
RSD: Relative Standard Deviation PRSD: Predicted Relative Standard Deviation

Validation of method of analysis of pesticide residues in tea and brew

Method: SOP for Green Tea

Table 7. Results for Imidachloprid residues in Green Tea

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)	SD (±)	RSD %	PRSD	Z score	HorRat value
1	Lab 1	4.690	4.989*	0.446*	8.95	12.48	-0.67	0.72
2	Lab 2	5.270					0.63	
3	Lab 3	5.462					1.06	
4	Lab 4	4.535					-1.02	
5	Lab 5	ND						
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1 to 4 only.
RNR: Result not reported.
RSD: Relative Standard Deviation
ND: Not detected
SD: Standard Deviation
PRSD: Predicted Relative Standard Deviation

Table 8. Results for Imidachloprid residues in Green Tea Brew (liquor)

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)*	SD (±)*	RSD %	PRSD	Z score	HorRat value
1	Lab 1	2.840	3.155	0.562	17.81	13.37	-0.56	1.33
2	Lab 2	3.768					1.09	
3	Lab 3	3.467					0.56	
4	Lab 4	2.543					-1.09	
5	Lab 5	ND						
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1 to 4 only.
RNR: Result not reported.
RSD: Relative Standard Deviation
ND: Not detected.
SD: Standard Deviation
PRSD: Predicted Relative Standard Deviation

Validation of method of analysis of pesticide residues in tea and brew

Method: SOP for Black Tea

Table 9. Results for Dimethoate residues in Black Tea

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)	SD (±)	RSD %	PRSD	Z score	HorRat value
1	Lab 1	0.004					0.71	
2	Lab 2	0.003					-0.71	
3	Lab 3	<0.05	0.004*	0.001*				
4	Lab 4	<0.05			20.20	37.10		0.54
5	Lab 5	RNR						
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for limited data from laboratories under SL No 1 and 2 only.
RNR: Result not reported. SD: Standard Deviation
RSD: Relative Standard Deviation PRSD: Predicted Relative Standard Deviation

Table 10. Results for Dimethoate residues in Black Tea Brew (liquor)

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)*	SD (±)*	RSD %	PRSD	Z score	HorRat value
1	Lab 1	0.024					-0.71	
2	Lab 2	0.028	0.026	0.003			0.71	
3	Lab 3	<0.05						
4	Lab 4	<0.05			10.88	27.475		0.40
5	Lab 5	ND						
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for limited data from laboratories under SL No 1 and 2 only.
ND: Not detected. RNR: Result not reported
SD: Standard Deviation. RSD: Relative Standard Deviation.
PRSD: Predicted Relative Standard Deviation

Validation of method of analysis of pesticide residues in tea and brew

Method: SOP for Green Tea

Table 11. Results for Dimethoate residues in Green Tea

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)	SD (±)	RSD %	PRSD	Z score	HorRat value
1	China Lab 1	0.650	0.602*	0.135*	22.45	17.15	0.36	1.31
2	China Lab 2	0.695					0.69	
3	India Lab 1	0.660					0.43	
4	India Lab 2	0.401					-1.49	
5	SriLanka Lab 1	2.700#						
6	Bangladesh Lab 1	RNR						
7	Kenya Lab 1	RNR						
8	Indonesia Lab 1	RNR						

*Calculated for data from laboratories under SL No 1 to 4 only.
#Considered as outlier.
ND: Not detected.
SD: Standard Deviation
PRSD: Predicted Relative Standard Deviation
RNR: Result not reported.
RSD: Relative Standard Deviation.

Table 12. Results for Dimethoate residues in Green Tea Brew (liquor)

Sl No	Laboratories	Residues (mg/kg)	Mean (mg/kg)*	SD (±)*	RSD %	PRSD	Z score	HorRat value
1	Lab 1	0.570	0.447	0.116	26.00	17.93	1.06	1.45
2	Lab 2	0.563					1.00	
3	Lab 3	0.424					-0.19	
4	Lab 4	0.361					-0.74	
5	Lab 5	0.315					-1.13	
6	Lab 6	RNR						
7	Lab 7	RNR						
8	Lab 8	RNR						

*Calculated for data from laboratories under SL No 1 to 5 only.
RNR: Result not reported.
RSD: Relative Standard Deviation
RNR: Result not reported.
SD: Standard Deviation
PRSD: Predicted Relative Standard Deviation

ANNEXURE II

SOP for determination of bifenthrin residues in black tea and tea infusion

1. Introduction

This method is applicable for the determination of bifenthrin residue in black tea and tea infusion.

2. Principle

2.1. Black tea

Bifenthrin is extracted with acetonitrile: water (2:1, v/v) mixture from the black tea samples. The extract is partitioned with sodium chloride and cleaned up through florisil column. Then it is eluted with ether/hexane mixture. The concentrated residue in the eluate is determined by GC with electron capture detector.

2.2. Tea brew

Two gram of made tea was infused in 100 mL of boiling water (ISO 3103 -1990). After 6 min of brewing, the water extract was filtered, cooled and partitioned with 100 mL of hexane (twice). The organic phase was passed through anhydrous sodium sulphate. The extract was concentrated by evaporating in a rotary vacuum evaporator and diluted with 10 mL hexane and analysed for the residues of bifenthrin. The spent leaves were dried between the folds of filter paper and residues were extracted following the method described above for black tea.

3. Extraction

Twenty gram of black tea sample is extracted with 150 mL of acetonitrile: water (2:1, v/v) by shaking it in a mechanical shaker for two hours. The contents are filtered and to the filtrate 200 mL of 4 percent NaCl and 60 mL of hexane are added. After partitioning, the hexane layer is passed through anhydrous sodium sulphate layer to a 500 mL round bottomed flask.

3.1. Clean up

The extract is evaporated to dryness on a rotary vacuum evaporator and the residue is dissolved in 10 mL hexane and again transferred to 125 mL separating funnel. The round bottomed flask is rinsed with 5 mL portions of hexane and the rinses are added to the separating funnel. About 30 mL acetonitrile–saturated with hexane is added to it and the acetonitrile layer is drained into a 250 mL round bottomed flask containing anhydrous sodium sulphate. The acetonitrile extract is evaporated to dryness at 60^0C. The concentrated residue is dissolved in 5 mL hexane and cleaned up by adsorption column chromatography using 10 g of 5 percent deactivated florisil and 100 mL of 6 percent diethyl ether in hexane as eluting solvent. Prior to elution the column is washed with 50 mL of hexane to remove the co-extractives. The eluate collected is concentrated at about 60^0C to dryness and diluted with 10 mL of hexane and injected into GLC, under the following conditions.

Instrument	:	Perkin Elmer Clarus 500 GC
Detector	:	Electron Capture Detector (ECD)
Column	:	Elite-5 (Cross bond 5 percent diphenyl – 95 percent dimethyl poly siloxane, 30 m length x 0.25 mm i.d. x 1 µm film thickness)
Gas flow rate		
Carrier (Nitrogen)	:	5 mL/min
Temperatures		
Injector	:	180°C
Detector	:	300°C
Oven	:	210°C
Sample volume injected	:	0.5µL

3.2. Preparation of acetonitrile saturated with hexane

Three portions of acetonitrile combined with one portion of hexane in a 125 ml separating funnel. Gently shaken and then collect the lower part of acetonitrile layer. This is called acetonitrile saturated with hexane.

4. Calculation:

$$\text{Concentration of residues (mg/kg)} = \frac{As \times C \times D}{A\,std \times W}$$

where,

As = "Sample" peak area corresponding to pesticide at its retention time
A std = "Standard" peak area corresponding to pesticide at its retention time
C = Concentration of pesticide in standard solution (ppm)
D = Sample dilution factor (mL)
W = Weight of tea sample (g) taken for analysis

In all the analysis, an equal volume of sample and standard solutions are injected into the GC.

SOP determination of imidacloprid residues in black tea and tea infusion

1. Introduction

This method is applicable for the determination of imidacloprid residue in black tea and tea infusion.

2. Principle

2.1. Black and green tea

Imidacloprid is extracted with acetonitrile from the black and green tea samples. The concentrated residue is determined by HPLC with DAD detector.

2.2. Tea brew

Two gram of made tea was infused in 100 mL of boiling water (ISO 3103 -1990). After 6 min of brewing, the water extract was filtered, cooled and partitioned with 100 mL of dichloromethane (twice). The organic phase was passed through anhydrous sodium sulphate. The extract was concentrated by evaporating in a rotary vacuum evaporator and diluted with 10 mL acetonitrile and analysed for the residues of imidacloprid. The spent leaves were dried between the folds of filter paper and residues were extracted following the method described above for black tea.

3. Extraction

Ten grams of tea sample is extracted with 100 mL of acetonitrile by shaking in a mechanical shaker for two hours. The contents are filtered, washed with 50 mL of acetonitrile and the extract is concentrated to dryness in a rotary vacuum evaporator with the water bath maintained at 85^{o}C.

3.1. Clean up

The concentrated residue is dissolved in acetonitrile and transferred to glass column packed with activated florisil (10 g) for clean up. Top and bottom of florisil, 1 cm layer of anhydrous sodium sulphate is packed. The compound is eluted with 100 mL acetonitrile. After evaporation, the samples are suitable diluted with acetonitrile and analysed in HPLC.

Instrument	:	HPLC (Agilent; Model 1100)
Detector	:	Diode array detector
Column	:	Zorbax Rx C18 (4.6 X 250mm)
Mobile phase	:	Acetonitrile: Water (35:65, v/v)
Flow rate	:	1.0 mL/min
Wavelength	:	270 nm
Injection volume	:	10 µL

4. Calculation:

$$\text{Concentration of residues (mg/kg)} = \frac{\text{As X C X D}}{\text{A std X W}}$$

where,

As = “Sample” peak area corresponding to pesticide at its retention time
A std = “Standard” peak area corresponding to pesticide at its retention time
C = Concentration of pesticide in standard solution (ppm)
D = Sample dilution factor (ml)
W = Weight of tea sample (g) taken for analysis

In all the analysis, an equal volume of sample and standard solutions are injected into the HPLC.

SOP for determination of dimethoate residues in black tea and tea infusion

1. Introduction

This method is applicable for the determination of dimethoate residue in black tea and tea infusion.

2. Principle

2.1. Black tea

Dimethoate is extracted with dichloromethane from the black tea samples. The extract is partitioned with hexane and cleaned up through silica gel column. Then it is eluted with dichloromethane. The concentrated residue in the eluate is determined by GC with nitrogen-phosphorus detector.

2.2. Tea brew

Two gram of made tea was infused in 100 mL of boiling water (ISO 3103 -1990). After 6 min of brewing, the water extract was filtered, cooled and partitioned with 100 mL of dichloromethane (twice). The organic phase was passed through anhydrous sodium sulphate. The extract was concentrated by evaporating in a rotary vacuum evaporator and diluted with 10 mL acetone and analysed for the residues of dimethoate. The spent leaves were dried between the folds of filter paper and residues were extracted following the method described above for black tea.

3. Extraction

Fifty grams of tea sample is extracted with 200 ml dichloromethane by shaking it in a mechanical shaker for two hours. The contents are filtered, to the filtrate add 1 g of activated charcoal, shake vigorously and the contents are filtered using Whatmann No. paper and washed with dichloromethane. The extract is passed through sodium sulphate funnel to a 500 ml round bottom flask. Add 3drops of propylene glycol and concentrate the extract to about 2 mL.

3.1. Clean up

To the concentrated residue in the round bottom flask, add about 50 mL of 15 percent aqueous methanol, shake vigorously. Transfer it to a 125 mL separatory funnel and add 40mL hexane and shake for 2 min. and allow the phases to separate. Drain the lower aqueous layer into a 500 mL round bottom flask. Repeat the process twice with an additional 40 mL of hexane; drain the aqueous extract into the flask. Combine the aqueous extraction re-partitioned with 20mL of dichloromethane (thrice). Combine the dichloromethane extract and evaporate to near dryness in a rotary vacuum evaporator. The concentrated residue is dissolved in hexane and transferred to a glass column packed with activated silica gel (10 g) using dichloromethane. In between the silica gel, 1 cm layer of anhydrous sodium sulphate is packed. Prior to elution, the column is washed with 50 ml of dichloromethane to remove the co-extractives and the washings are discarded. The compound is eluted with 200 mL of dichloromethane. After evaporation, the samples are suitably diluted with acetone and analysed in GC as per the conditions given below:

Instrument	:	Hewlett Packard 5890 series II Gas Chromatograph
Detector	:	Nitrogen Phosphorus Detector (NPD)
Column	:	HP-608 (PH ME Siloxane wide bore capillary 30 m x 0.53 mm x 0.5µ)
Gas flow rate	:	
Carrier (Nitrogen)	:	12.0 ml/min
Temperatures		
Oven	:	180^0C
Injector	:	225^oC
Detector	:	200^0C
Sample volume injected	:	0.5µL

4. Calculation:

$$\text{Concentration of residues (mg/kg)} = \frac{As \times C \times D}{A\,std \times W}$$

Where,

As = "Sample" peak area corresponding to pesticide at its retention time
A std = "Standard" peak area corresponding to pesticide at its retention time
C = Concentration of pesticide in standard solution (ppm)
D = Sample dilution factor (ml)
W = Weight of tea sample (g) taken for analysis

In all the analysis, an equal volume of sample and standard solutions are injected into the GC.

ANNEXURE III

SOP for determination of bifenthrin residues in green tea and tea infusion

1. Introduction

This method is applicable for the determination of bifenthrin residue in green tea and tea infusion.

2. Principle

2.1. Green tea

Bifenthrin is extracted with acetone from the green tea samples. The extract is cleaned up through florisil column. Then it is eluted with hexane/acetone/toluene mixture. The concentrated residue in the eluate is determined by GC with electron capture detector.

2.2. Tea brew

Two gram of made tea was infused in 100 mL of boiling water (ISO 3103 -1990). After 6 min of brewing, the water extract was filtered, cooled, and transferred to the 250mL graduated cylinder , by adding 30g NaCl and 150mL acetonitrile, shake vigorously 1min, balance 30min, take upper 100mL of acetonitrile in 500mL round bottomed flask, The extract was concentrated by evaporating in a rotary vacuum evaporator with the water bath maintained at 45℃, then N_2 drying. The spent leaves were dried between the folds of filter paper and residues were extracted following the method described above for green tea.

3. Extraction

Ten gram of green tea sample is extracted with 70mL of acetone by immersing overnight. The contents are filtered through anhydrous sodium sulphate layer to a 500 mL round bottomed flask. The extract is evaporated to dryness on a rotary vacuum evaporator and the residue is dissolved in 10 mL hexane.

3.1. Clean up

Prior to elution the column is washed with 10 mL of hexane to discard, then 1mL sample is transferred to 1g of 10% deactivated florisil column(1.0cm□id×15cm). The hexane/acetone/toluene(445:10:45,V/V/V) mixture eluate collected in 10 mL flask, and injected into GC, under the following conditions.

Instrument	:	Varian CP3800 or other type of GC
Detector	:	Electron Capture Detector (ECD)
Column	:	DB-1701(30 m length x 0.32 mm i.d x 0.25 µm film thickness)
Gas flow rate	:	
Carrier (Nitrogen)	:	2 mL/min
Temperatures		
Injector	:	260^0C
Detector	:	300^0C
Oven	:	Initial temperature 80 □, hold 1 min□ at the 10 □/ min rate of temperature, raise to 150 □, hold 1 min□then at the5 □/ min rate of temperature, raise to 260 □, hold 5 min□
Sample volume injected	:	1µL

4. Calculation:

Concentration residues (mg/kg)

$$C_x = \frac{Fa \times Cs \times Qs \times Vx \times Vex}{Fs \times Qx \times Vri \times M}$$

where,

Fa = "Sample" peak area corresponding to pesticide at its retention time
Fs = "Standard" peak area corresponding to pesticide at its retention time
Cs = Concentration of pesticide in standard solution (mg/L)
M = Weight of tea sample (g) taken for analysis
Qs = Standard Inject Volume, µL;
Vx = Sample Volume, mL;
Vex = Total Extraction Volume, mL;
Fs = Standard Area;
Qx = Sample Inject Volume, µL;
Vri = Part Extraction Volume, mL

In all the analysis, an equal volume of sample and standard solutions are injected into the GC.

SOP determination of imidacloprid residues in green tea and tea infusion

1. Introduction

This method is applicable for the determination of imidacloprid residue in green tea and tea infusion.

2. Principle

2.1. green tea

Imidacloprid is extracted with acetonitrile from the green tea samples by adding 20mL water. The extract is cleaned up through florisil column. Then it is eluted with hexane/acetone mixture. The concentrated residue in the eluate is determined by HPLC with DAD detector.

2.2. Tea brew

Two gram of made tea was infused in 100mL of boiling water (ISO 3103 -1990). After 6 min of brewing, the water extract was filtered, cooled and Transferred to the 250mL graduated cylinder, by adding 30g NaCl and 150mL acetonitrile, shake vigorously 1min, balance 30min, take upper 50mL of acetonitrile in 500mL round bottomed flask, The extract was concentrated by evaporating in a rotary vacuum evaporator with the water bath maintained at 40□, then N_2 drying. The spent leaves were dried between the folds of filter paper and residues were extracted following the method described above for green tea.

3. Extraction

Weigh 5g tea sample in 150mL Centrifuge bottle, add 25mL water for soaking one hour, then add 50mL acetonitrile, homogenous with IKA-18 homogenizer for one minute, centrifuge for 5 minutes with 5000 rpm, the organic solvent transferred to the 100mL graduated cylinder and repeat to add 50mL acetonitrile and homogenate and centrifuge steps, add 20g NaCl to graduated cylinder, shake vigorously 1min, balance 30min, take upper 40mL of acetonitrile in 250mL round bottomed flask, The extract was concentrated by evaporating in a rotary vacuum evaporator with the water bath maintained at 45℃, N_2 drying.

3.1. Clean up

The concentrated residue is dissolved in 3×2mL hexane/acetone (4:1,V/V) and transferred to glass column packed with 5g of 11% deactivated florisil column(1.6cm, id×25cm) for clean up. On the top and bottom of florisil, 2 cm layer of anhydrous sodium sulphate is packed. The compound is eluted with 20 mL hexane/acetone (4:1,V/V) and discard, the elute with 50mL hexane/acetone (1:1,V/V) was collected, and concentrated and evaporated with rotary vacuum evaporator with the water bath maintained at 40℃, the samples are diluted with 2mL acetonitrile and analysed with HPLC.

Instrument : HPLC (AGILENT 1100)
Detector : Diode array detector
Column : Aglient TC-C18 (4.6 X 250mm)
Flow rate : 1.0 mL/min
Wavelength : 270 nm
Mobile phase : Gradient Elution Procedure

Setp	Total time（min）	Flow rate (mL/min)	A: water (0.2% Phosphoric acid aqueous solution) %	B: acetonitrile(%)
0	0.00	1	90.0	10.0
1	30.00	1	40.0	60.0
2	35.00	1	90.0	10.0

Injection volume : 10 µL

4. Calculation:

Concentration residues (mg/kg)

$$C_x = \frac{Fa \times Cs \times Qs \times Vx \times Vex}{Fs \times Qx \times Vri \times M}$$

where,

Fa = "Sample" peak area corresponding to pesticide at its retention time
Fs = "Standard" peak area corresponding to pesticide at its retention time
Cs = Concentration of pesticide in standard solution (mg/L)
M = Weight of tea sample (g) taken for analysis
Qs = Standard Inject Volume, µL
Vx = Sample Volume, mL
Vex = Total Extraction Volume, mL
Fs = Standard Area
Qx = Sample Inject Volume, µL
Vri = Part Extraction Volume, mL

In all the analysis, an equal volume of sample and standard solutions are injected into the HPLC.

SOP for determination of dimethoate residues in green tea and tea infusion

1. Introduction

This method is applicable for the determination of dimethoate residue in green tea and tea infusion.

2. Principle

2.1. Green tea

Dimethoate is extracted with acetonitrile from the green tea samples by adding 20mL water. The extract is cleaned up through Envi-Carb SPE column. The concentrated residue in the eluate is determined by GC with flame phosphorus detector.

2.2. Tea brew

Two gram of made tea was infused in 100 mL of boiling water (ISO 3103 -1990). After 6 min of brewing, the water extract was filtered, cooled and Transferred to the 250mL graduated cylinder, by adding 30g NaCl and 150mL acetonitrile, shake vigorously 1min, balance 30min, take upper 50mL of acetonitrile in 500mL round bottomed flask, The extract was concentrated by evaporating in a rotary vacuum evaporator with the water bath maintained at 40□, then N_2 drying. The spent leaves were dried between the folds of filter paper and residues were extracted following the method described above for green tea.

3. Extraction

Weigh 5g tea sample in 150mL Centrifuge bottle, add 25mL water for soaking one hour, then add 50mL acetonitrile, homogenous with IKA-18 homogenizer for one minute, centrifuge for 5 minutes with 5000 rpm, the organic solvent transferred to the 100mL graduated cylinder and repeat to add 50mL acetonitrile and homogenate and centrifuge steps, by adding 20g NaCl, shake vigorously 1min, balance 30min, take upper 20mL of acetonitrile in 250mL round bottomed flask, The extract was concentrated by evaporating in a rotary vacuum evaporator with the water bath maintained at 45℃, N_2 drying.

3.1. Clean up

The concentrated residue is dissolved in 3×2mL acetonitrile /hexane (3:1,V/V) and transferred to Envi-Carb SPE column for clean up. The compound is eluted with 25mL acetonitrile /hexane (3:1,V/V), the elute was concentrated and evaporated with rotary vacuum evaporator with the water bath maintained at 45℃, the samples are diluted with acetonitrile and analysed in GC-FPD as per the conditions given below:

Instrument	:	Agilent 6890 N Gas chromatograph or other type of GC
Detector	:	Flame Phosphorus Detector (FPD)
Column	:	HP-17 (30 m x 0.32 mm x 0.25µ)
Gas flow rate	:	1.0 mL/min
Carrier (Nitrogen)	:	2.0 ml/min
Temperatures		
Injector	:	22^0C
Detector	:	250^0C
Oven	:	Initial temperature 100 □, hold 1 min□ at the 30 □/ min rate of temperature, raise to 220 □, hold 10 min□then at the 45 □/ min rate of temperature raising, raise to 250 □, hold 5 min□
Sample volume injected	:	1.0µL

4. Calculation:

Concentration residues (mg/kg)

$$C_x = \frac{Fa \times Cs \times Qs \times Vx \times Vex}{Fs \times Qx \times Vri \times M}$$

where,

Fa = "Sample" peak area corresponding to pesticide at its retention time
Fs = "Standard" peak area corresponding to pesticide at its retention time
Cs = Concentration of pesticide in standard solution (mg/L)
M = Weight of tea sample (g) taken for analysis
Qs = Standard Inject Volume, µL
Vx = Sample Volume, mL
Vex = Total Extraction Volume, mL
Fs = Standard Area
Qx = Sample Inject Volume, µL
Vri = Part Extraction Volume, mL

In all the analysis, an equal volume of sample and standard solutions are injected into the GC.

FORMAT FOR REPORTING THE RESULTS OF ANALYSIS OF PESTICIDE RESIDUES IN GREEN TEA/TEA INFUSION

Test Material Green tea/Tea infusion

Compound	LOD of instrument	Residues in mg/kg (3 decimal place)	% Recovery (if carried out)	Confimed by Mass Spec. Yes/No
Bifenthrin				
Dimethoate				
Imidacloprid				

Nodal person/Lab In-charge…….:

Name and address of Lab ……..:

Signature of analyst/Lab In-charge……..:

Return the completed result lasted by 30th NOV.2009

Green Tea sample(Treated)

Imidachloprid, 45 g.a.i./ha

Bifenthrin, 37.5 g.a.i./ha

Dimethoate, 450 g.a.i./ha

(7 day PHI)

19 July 2009, Hangzhou