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COMMITTEE ON COMMODITY PROBLEMS

INTERGOVERNMENTAL GROUP ON TEA

NINETEENTH SESSION

New Delhi, India, 12 – 14 May 2010

REPORT OF THE WORKING GROUP ON THE DEVELOPMENT ON ESTABLISHING MRLs ON THE BREW[1]

I. INTRODUCTION

1. At the 18th Session of the IGG on Tea, which was held in Hangzhou, China in 2008, there was an agreement to establish a new Working Group (WG). On the para 27, Report of 18th Session of IGG on Tea, Hangzhou 14-16 May, 2008: "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 Working Group 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; and
- Consult with the related international agencies, e.g. JMPR, Codex Alimentarius and national authorities."

2. The Meeting of the Working Groups was conducted FAO in Rome from 20 to 22 May 2009 and instructed the WG to:

- Establish the methodology for measuring residues in the tea brew and dry tea; and
- Organizing the ring tests of green tea and black tea samples from tea garden sprayed with three pesticides (Bifenthrin, Imidacloprid and Dimethoate)in different countries and laboratories.

II. ACTIVITIES OF THE WORKING GROUP

3. Compilation of literature published on transfer rate of pesticide residue from made tea to tea brew showed a wide difference of pesticide residue in tea infusion which is depended largely by the water solubility of pesticide as early as reported in 1988 in China. The experimental data

[1] Submitted by Prof. Chen (China).

on the investigation from China, India and Japan are presented at the 18th IGG in Hangzhou, China.

4. According to the recommendation in the Rome Meeting, a validation of established methodology for determining the residues of dimethoate, bifenthrin and imidacloprid in the tea brew was planned to conduct through a ring test. The ring test are planned to conduct in 11 labs in eight countries. Due to the various reasons, the analysis was conducted in six labs of four countries: China (2), India (2), Sri Lanka (1) and Bangladesh(1).

5. As decided in the Rome Meeting, China recommended the method for green tea, and India recommended the method for black tea. A comparative determination of both methods was conducted with green tea and black tea samples fortified with three pesticides in different polarity. Investigation showed that the results of determination on the tea samples with low polarity pesticide (Bifenthrin) by two methods are similar; however, the results of determination on the tea samples with higher polarity pesticides (Imidacloprid and Dimethoate) showed a quite greater difference. Adding some water to the dry tea sample is necessary for improving the extractive rate of pesticides with high polarity from dry tea. If no water was added to the dry tea, the results of residue level of these pesticides with high polarity in dry tea samples will several hundred times lower than those in tea brew. So, the method is recommended to add 50% water (weight of dry tea) to the tea sample. The method is attached in this document. The results are listed in Table 1-4.

6. After the validation of the established methodology, three green tea samples (sprayed with three pesticides accordingly: Bifenthrin, Imidacloprid and Dimethoate) and three black tea samples (sprayed three pesticides as above) were sent to 8 labs in August of 2009. These tea samples were provided d by China (Green tea, TRI, Hangzhou, China,July 2009)) and India (Black tea, TRA, Jorhat, India, July 2009). The dosage was imidacloprid 22.25 g a.i. ha^{-1}(India) – 45 g.a.i. ha^{-1} (China), bifenthrin 37.5 g a.i. ha^{-1} (China) --80 g a.i. ha^{-1} (India), dimethoate 300 g a.i. ha^{-1} (India) – 450 g a.i. ha^{-1} (China), Spray volume 400 L/ha, Sprayer: Hand operated knapsack sprayer. The fresh tea shoots (one bud and two leaves) from treated tea plants was harvested at 5 days (India) or 7 days (China) after spraying.

7. The residue level of pesticide in the tea brew is closely related with the water solubility of pesticide. The higher water solubility of pesticide showed higher extractive rate of pesticide during the brewing process from made tea. The results of ring test is attached in Table 5and 6.

III. CONCLUSIONS

8. The Action Plan agreed at the 18th Session and the Working Group Meeting in Rome is being progressed. The methodology for determining the residue in tea brew was established and amended. The Working Group recommended investigating the following aspects in 2010-2011.

- Legal consideration for establishing MRLs of various pesticides in tea by considering the situation during brewing process;
- To establish one or two common methods for the determination of black tea and green tea after a further ring test;
- To conduct the experiment on the transfer rate of various pesticides used in tea production;
- To conduct the risk assessment of pesticide in made tea to drinker during the brewing process according to the above investigation on the transfer rate of various pesticides; and
- To put forward a suggestion on how to establish the MRL of pesticide in made tea in considering the transfer rate of pesticide from made to tea brew for discussion.

9. The funding problem should be considered and identified. It is recommended that IGG should contact with CFC or other organization for getting the supporting funds.

RING TEST FOR METHODOLOGY

Objective: According to the recommendation in the Rome Meeting, a validation of established methodology for determining the residues of dimethoate, bifenthrin and imidacloprid in the tea brew was planned to conduct through a ring test. The Black tea method is provided by India, the green tea method is provided by China. The comparative results of methodology are listed in Table 1-4.The results of tea samples are listed in Table 5-7.

Table1 Result of Green Tea (China Lab 1)

Pesticide Name	Made tea(mg/kg)		Tea infusion(mg/L)		Brewing rate%	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
Bifenthrin	7.55	5.89	0.026	0.019	0.34	0.32
Dimethoate	0.65	0.118	0.57	0.398	87.69	337.29
Imidacloprid	4.69	0.48	2.84	2.35	60.55	489.58

Table2 Result of Black Tea (China Lab 1)

Pesticide Name	Made tea(mg/kg)		Tea infusion(mg/L)		Brewing rate%	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
Bifenthrin	1.93	1.91	0.024	0.022	1.34	1.15
Dimethoate	0.028	0.004	0.023	0.024	82.14	600.00
Imidacloprid	0.294	0.026	0.156	0.190	53.15	730.77

Table3 Result of Green Tea (China Lab 2)

Pesticide Name	Made tea(mg/kg)		Tea infusion(mg/L)		Brewing rate%	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
Bifenthrin	6.798	4.11	0.120	0.110	1.77	2.68
Dimethoate	0.695	0.116	0.563	0.546	81.0	470.69
Imidacloprid	5.270	1.341	3.768	2.078	71.5	155.0

Table4 Result of Black Tea (China Lab 2)

Pesticide Name	Made tea(mg/kg)		Tea infusion(mg/L)		Brewing rate%	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
Bifenthrin	1.384	1.013	0.021	0.020	1.52	1.97
Dimethoate	0.021	0.003	0.022	0.028	104.8	933.3
Imidacloprid	0.257	0.010	0.135	0.122	52.5	1220.0

Method 1 : 50% water is added to the dry tea sample.
Method 2: No water is added to the dry tea sample.

Table 5 Results of analysis (Green tea sample)

	Bifenthrin			Imidacloprid			Dimethoate		
	Dry tea	Tea brew	Extractive rate %	Dry tea	Tea brew	Extractive rate%	Dry tea	Tea brew	Extractive rate %
China 1	7.55	0.026	0.34	4.69	2.84	60.55	0.65	0.57	87.69
China 2	6.79	0.12	1.77	5.27	3.77	71.50	0.69	0.56	81.0
India 1	7.49	<0.1	0.13	5.46	3.47	63.50	0.66	0.42	63.6
India 2	7.31	<0.05	0.068	4.53	2.54	56.1	0.40	0.36	90.0
Sri Lanka	4.12	0.01	0.242	ND	ND	---	2.70#	0.32	---

considered as outlier

Table 6 Results of analysis (Black tea sample)

	Bifenthrin			Imidacloprid			Dimethoate		
	Dry tea	Tea brew	Extractive rate %	Dry tea	Tea brew	Extractive rate%	Dry tea	Tea brew	Extractive rate %
China 1	1.93	0.024	1.24	0.294	0.156	53.15	0.028	0.023	82.14
China 2	1.38	0.021	1,52	0.257	0.135	52.50	0.021	0.022	104.8
India 1	2.15	<0.1	4.65	0.685	0.425	62.04	<0.05	<0.05	---
India 2	1.84	0.052	2.82	0.239	<0.1	41.84	<0.05	<0.05	---
SriLanka	1.670	1.352#	---	ND	ND	---	RNR	ND	---
Bangladesh	0.043#	0.03	---	RNR	RNR	RNR	RNR	RNR	RNR

RNR: result not reported
NR: not detected
considered as outlier

Table7 Relationship between the water solubility of tested pesticides and the extractive rate pesticide from made tea to tea brew

Pesticide	Number of lab*	Extractive rate of pesticide residue from made tea to tea infusion (%)		Water solubility (L/mg)
		Green tea	Black tea	
Bifenthrin	6	0.92 (5) **	0.26 (5)	0.1
Imidacloprid	6	63.26 (4)	55.32 (4)	610
Dimethoate	6	79.58 (4)	93.47 (2)	25000

*Information of Bifenthrin reported from two labs from China, two labs from India and one lab from SriLanka. Information of Imidacloprid and Dimethoate in green tea and Imidacloprid in black tea were reported from two labs from China, two labs from India, and that of Dimethoate in black tea was reported from two labs from China.
** Figure in the brackets is the numbers of lab whose information is used in the calculation.

SOP for the determination of Bifenthrin residues in Black tea and tea brew (provided by India)

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% 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% deactivated florisil and 100 mL of 6% 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% diphenyl - 95% dimethyl polysiloxane, 30 m length x 0.25 mm i.d x 1 µm film thickness)
Gas flow rate		
Carrier (Nitrogen)	:	5 mL/min
Temperatures		
Oven	:	210^0C
Injector	:	180^0C
Detector	:	300^0C
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:

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

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 GC.

SOP determination of imidacloprid residues in black tea and tea infusion
(Provided by India)

1. Introduction

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

2. Principle

2.1. Black tea

Imidacloprid is extracted with acetonitrile from the black 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	:	270nm
Injection volume	:	10 µL

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 HPLC.

SOP for determination of dimethoate residues in black tea and tea infusion (Provided by India)

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% 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)tor
Column	:	HP-608 (PH ME Siloxane wide bore capillary 30 m x 0.53 mm x 0.5µ)
Carrier (Nitrogen)	:	12.0 ml/min
Temperatures		
Oven	:	180^0C
Injector	:	225^0C
Detector	:	220^0C
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.

SOP for determination of bifenthrin residues in green tea and tea infusion (provided by China)

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		
Oven	:	Initial temperature 80 °C, hold 1 min, at the 10°C/min rate of temperature, raise to 150°C, hold 1 min, then at 5°C/min rate of temperature, raise to 260 °C, hold 5 min
Injector	:	260°C
Detector	:	300°C
Sample volume injected	:	1 µL

4. Calculation:

$$\text{Concentration of residues (mg/kg) } C_x = \frac{Fa \times Cs \times Qs \times Vx \times Vex}{Fs \times Qx \times Vri \times M}$$

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
(Provided by China)

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)
Carrier (Nitrogen) : 12.0 ml/min
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:

$$\text{Concentration of residues (mg/kg)} \quad C_x = \frac{Fa \times Cs \times Qs \times Vx \times Vex}{Fs \times Qx \times Vri \times M}$$

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
(Provided by China)

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		
Carrier (Nitrogen)	:	2.0 ml/min
Temperatures		
Injector	:	22^0C
Detector	:	250^0C
Oven	:	Initial temperature 100^0C, hold 1 min, at the 30^0C/min rate of temperature, raise to 220^0C, hold 10 min, then at the 45^0C/min rate of temperature raising, raise to 250^0C, hold 5 min
Sample volume injected	:	1.0µL

4. Calculation:

$$\text{Concentration of residues (mg/kg)} \quad C_x = \frac{Fa \times Cs \times Qs \times Vx \times Vex}{Fs \times Qx \times Vri \times M}$$

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.