Determination of Pesticide Residues in Tea: An AOAC Collaborative Study

UCT Part Numbers:
RFV0050CT - 50 mL centrifuge tubes
ECPSACB506 - 6 mL SPE cartridge with 500 mg GCB and 500 mg PSA
ECSS25K - Sodium sulfate, anhydrous, ACS grade, granular 60 mesh
AD0000AS - Cartridge adaptors
RFV0025P - 25 mL empty reservoirs

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Summary:

Tea is one of the most widely consumed beverages in the world [1]. The application of pesticides in tea cultivation is a common practice in order to increase production yields. Therefore it is important to test the teas for pesticide residues to ensure they are safe for human consumption. However, tea is one of the most complex matrices, which makes the extraction and cleanup of pesticides in tea very challenging. Dr. Guo-Fang Pang and his colleagues at the Chinese Academy of Inspection and Quarantine have developed an efficient and sensitive method to quantitatively determine multiclass pesticide residues in tea [2]. The method employs a solvent extraction using acetonitrile (MeCN), followed by a solvent reduction and a cleanup using solid phase extraction (SPE) cartridge packed with 500 mg each of graphitized carbon black (GCB) and primary secondary amine (PSA), the pesticides are then eluted with MeCN:toluene (3:1, v/v), concentrated down and analyzed by GC/MS, GC/MS/MS or LC/MS/MS.

Matrix matched calibration curves were constructed using organic green and Oolong teas, the responses for 20 representative pesticides were linear with R^2 ranging from 0.9960 to 1.0000. Excellent recoveries (89.5-116% for green tea & 79.3-107% for Oolong tea), and relative standard deviations (RSD% < 10%) were obtained using this simple yet effective method.

Procedure:

a) Weigh 5 ± 0.01 g of homogenized tea sample into a 50-mL centrifuge tube (UCT part#: **RFV0050CT**), add 15 mL of MeCN, and homogenize at 13500 rpm/min for 1 min using an IKA T-25 homogenizer.

b) Centrifuge at 5000 rpm/min for 5 min. Transfer the supernatant to a large test tube (20 x 150 mm).

c) Repeat the extraction with 15 mL of MeCN, and combine the supernatants.

d) Concentrate the extract to about 1 mL using a TurboVap evaporator at 40 ºC under a gentle stream of nitrogen.

e) Add about 2 cm of anhydrous sodium sulfate (Na_2SO_4, UCT part#: **ECSS25K**) to the 6 mL, dual layer SPE cartridge (UCT part#: **ECPSACB506**).

f) Connect a 25 mL empty reservoir (UCT part#: **RFV0025P**) to the top of the dual layer SPE cartridge using cartridge adaptor (UCT part#: **AD0000AS**).

g) Condition the cartridge with 10 mL of MeCN: toluene (3:1, v/v). Do not let the cartridge go dry from this point on.

h) Insert a 50-mL glass vial into the vacuum manifold. Apply the concentrated extract (from Step d) to the cartridge. Wash the test tube with 2 x 3 mL of MeCN: toluene (3:1 v/v) and transfer the rinses to the cartridge, apply a low vacuum to pass the rinse through the SPE cartridge and collect.

i) Continue to elute the extracts from the SPE cartridge with 25 mL of 3:1 MeCN: toluene.

j) Remove the 50-mL vial from the manifold, and concentrate the eluate to about 0.5 mL using TurboVap at 40 ºC under a gentle stream of nitrogen.

k) Add 40 µL of the internal standard solution, and appropriate amounts of pesticide working solution for matrix matched standards and evaporate to dryness under a gentle stream of nitrogen at 35 ºC.

l) Reconstitute with 1.5 mL of n-hexane (or initial mobile phase for LC/MS/MS analysis), vortex for 30 sec. and filter with a 0.2 µm syringe filter. The extract is now ready for instrumental analysis.

GS/MS method:

GC/MS: Agilent 6890N GC coupled to a 5975C MSD
Injector: 1 µL splitless injection at 280 ºC, 40 mL/min purge flow at 1.5 min
Liner: 4 mm splitless gooseneck (UCT part#: **GCLGN4MM**), packed with deactivated glass wool
GC capillary column: Restek Rtx®-1701, 30m x 0.25mm x 0.25µm
Oven temperature: Initial temperature at 40 ºC, hold for 1 min; ramp at 30 ºC/ min to 130 ºC; ramp at 5 ºC/ min to 250 ºC, ramp at 10 ºC/ min to 290 ºC, and hold for 5 min.
Solvent delay: 15.5 min
Carrier gas: Ultra-high pure Helium at a constant flow of 1.2 mL/min
MSD: Transfer line: 280 ºC; MS Source (ESI): 250 ºC; MS Quad: 150 ºC
Tune file: atune

Retention times, quantifying and qualifying ions with ion ratios

Peak No.	Pesticide	Retention time (min)	Quantify ion (ion ratio)	Qualify ion 1 (ion ratio)	Qualify ion 2 (ion ratio)
IS	Heptachlor epoxide	22.44	353(100)	355(81)	351(52)
1	Trifluralin	15.71	306(100)	264(85)	335(7)
2	Tefluthrin	17.67	177(100)	197(28)	161(4)
3	Pyrimethanil	17.73	198(100)	199(51)	200(6)
4	Propyzamide	19.39	173(100)	255(22)	240(10)
5	Pirimicarb	19.44	166(100)	238(20)	138(7)
6	Fenchlorphos	20.22	285(100)	287(69)	270(6)
7	Dimethenamid	20.21	154(100)	230(49)	203(25)
8	Tolclofos-methyl	20.35	265(100)	267(37)	250(11)
9	Pirimiphos-methyl	20.78	290(100)	276(87)	305(64)
10	2,4'-DDE	23.10	246(100)	318(35)	176(25)
11	Bromophos-ethyl	23.52	359(100)	303(83)	357(75)
12	4,4'-DDE	24.34	318(100)	316(78)	246(128)
13	Procymidone	25.22	283(100)	285(65)	255(13)
14	Picoxystrobin	25.37	335(100)	303(44)	367(7)
15	Quinoxyfen	27.63	237(100)	272(41)	307(32)
16	Chlorfenapyr	28.12	247(100)	328(57)	408(46)
17	Benalaxyl	28.23	148(100)	206(28)	325(5)
18	Bifenthrin	29.02	181(100)	182(15)	141(4)
19	Diflufenican	29.26	266(100)	394(21)	267(15)
20	Bromopropylate	29.90	341(100)	183(54)	339(51)

Results:

Linearity parameters of Green and Oolong tea

Pesticide	Linearity range (µg/kg)	Green tea R^2	Oolong tea R^2
Trifluralin	80-1200	0.9998	0.9963
Tefluthrin	40-600	0.9998	0.9995
Pyrimethanil	40-600	0.9999	0.9996
Propyzamide	40-600	0.9992	0.9999
Pirimicarb	40-600	0.9960	0.9999
Fenchlorphos	80-1200	0.9998	0.9991
Dimethenamid	16-240	0.9999	0.9996
Tolclofos-methyl	40-600	0.9998	0.9990
Pirimiphos-methyl	40-600	0.9988	1.0000
2,4'-DDE	160-2400	0.9996	0.9987
Bromophos-ethyl	40-600	0.9999	0.9988
4,4'-DDE	160-2400	0.9998	0.9985
Procymidone	40-600	0.9999	0.9991
Picoxystrobin	80-1200	0.9998	0.9985
Quinoxyfen	40-600	1.0000	0.9990
Chlorfenapyr	320-4800	1.0000	0.9997
Benalaxyl	40-600	0.9999	0.9991
Bifenthrin	40-600	0.9999	0.9971
Diflufenican	40-600	0.9999	0.9990
Bromopropylate	80-1200	0.9999	0.9968

Recovery and RSDs obtained from the spiked Green tea

Pesticide	Spiked (µg/kg)	Rec% 1	Rec% 2	Rec% 3	Rec% 4	Rec% 5	Ave	RSD% (n=5)
Trifluralin	200	91.5	91.5	88.5	91.5	88.5	**90.3**	1.8
Tefluthrin	100	93.1	93.1	93.1	93.1	93.1	**93.1**	0.0
Pyrimethanil	100	90.1	90.1	87.1	90.1	90.1	**89.5**	1.5
Propyzamide	100	99.1	99.1	99.1	99.1	99.1	**99.1**	0.0
Pirimicarb	100	114.1	105.1	120.1	120.1	120.1	**116**	5.7
Fenchlorphos	200	93.0	94.5	91.5	93.0	91.5	**92.7**	1.4
Dimethenamid	40	97.7	97.7	90.2	97.7	90.2	**94.7**	4.3
Tolclofos-methyl	100	93.1	93.1	90.1	93.1	93.1	**92.5**	1.5

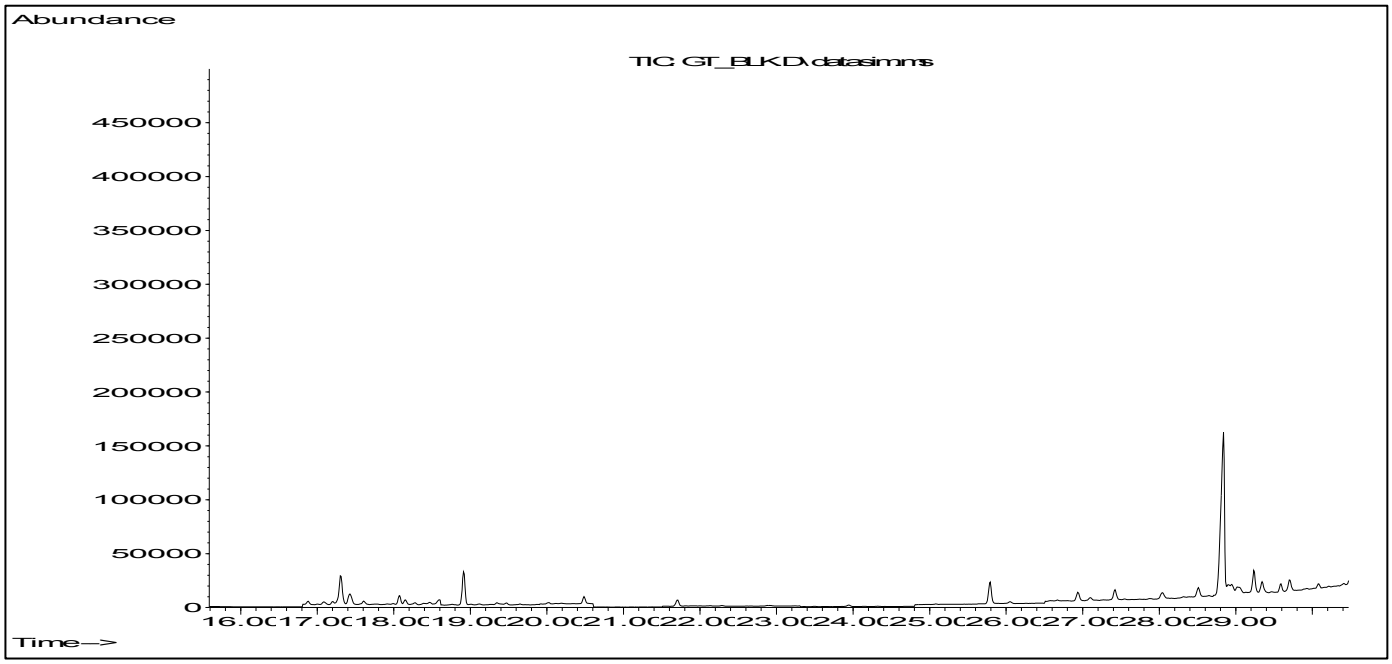
Pirimiphos-methyl	100	93.1	96.1	90.1	93.1	93.1	**93.1**	2.3
2,4'-DDE	400	93.0	94.5	90.8	93.0	91.5	**92.6**	1.6
Bromophos-ethyl	100	93.1	96.1	93.1	93.1	93.1	**93.7**	1.4
4,4'-DDE	400	93.0	94.5	92.3	93.0	92.3	**93.0**	1.0
Procymidone	100	96.1	96.1	93.1	96.1	93.1	**94.9**	1.7
Picoxystrobin	200	94.5	96.0	93.0	94.5	94.5	**94.5**	1.1
Quinoxyfen	100	90.1	90.1	90.1	93.1	90.1	**90.7**	1.5
Chlorfenapyr	800	94.5	96.7	92.6	94.1	94.1	**94.4**	1.6
Benalaxyl	100	96.1	96.1	96.1	96.1	96.1	**96.1**	0.0
Bifenthrin	100	93.1	96.1	93.1	93.1	93.1	**93.7**	1.4
Diflufenican	100	93.1	96.1	93.1	93.1	90.1	**93.1**	2.3
Bromopropylate	200	94.5	96.0	93.0	94.5	94.5	**94.5**	1.1

Recovery and RSDs obtained from the spiked Oolong tea

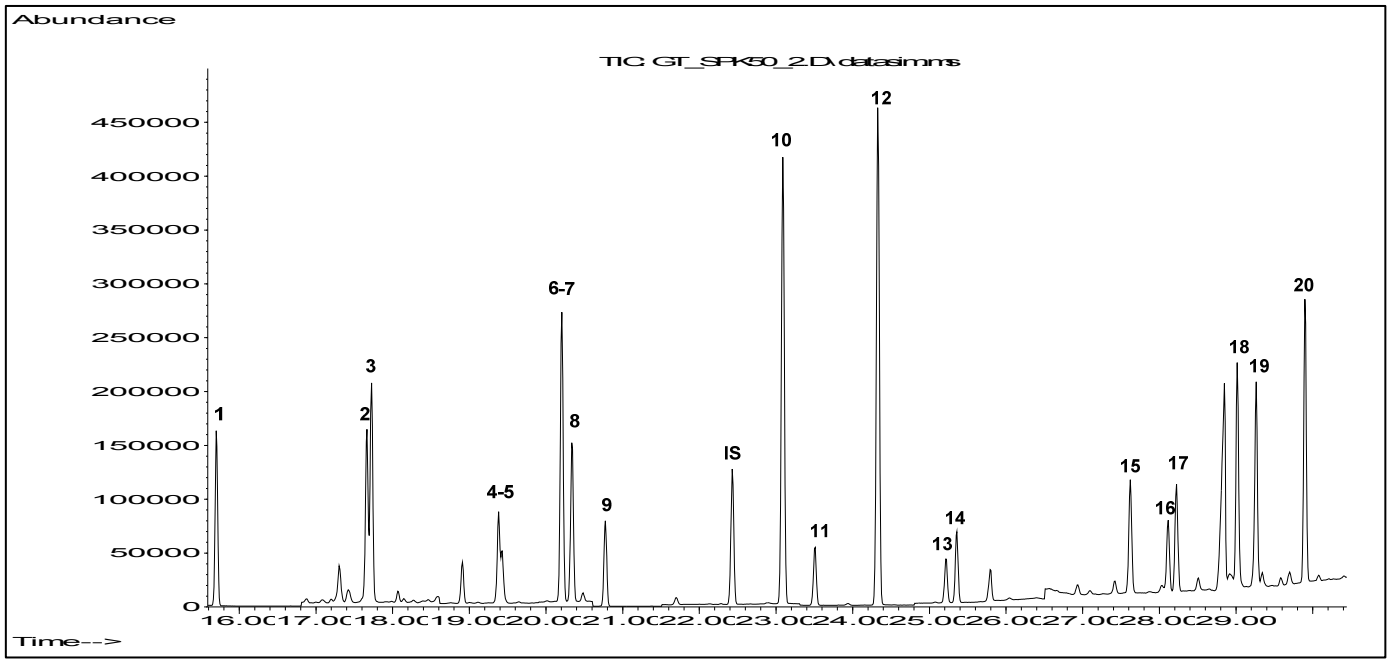
Pesticide	**Spiked** (µg/kg)	**Rec% 1**	**Rec% 2**	**Rec% 3**	**Rec% 4**	**Rec% 5**	**Ave**	**RSD%** (n=5)
Trifluralin	200	84.0	91.5	87.0	85.5	87.0	**87.0**	3.2
Tefluthrin	100	81.1	87.1	87.1	84.1	81.1	**84.1**	3.6
Pyrimethanil	100	78.1	81.1	81.1	78.1	78.1	**79.3**	2.1
Propyzamide	100	81.1	87.1	84.1	84.1	81.1	**83.5**	3.0
Pirimicarb	100	99.1	114.1	102.1	111.1	108.1	**107**	5.8
Fenchlorphos	200	81.0	87.0	84.0	85.5	82.5	**84.0**	2.8
Dimethenamid	40	82.7	90.2	82.7	82.7	82.7	**84.2**	4.0
Tolclofos-methyl	100	81.1	87.1	84.1	87.1	84.1	**84.7**	3.0
Pirimiphos-methyl	100	84.1	90.1	87.1	90.1	87.1	**87.7**	2.9
2,4'-DDE	400	85.5	87.0	89.3	87.8	84.0	**86.7**	2.3
Bromophos-ethyl	100	90.1	90.1	90.1	90.1	90.1	**90.1**	0.0
4,4'-DDE	400	85.5	87.8	84.8	86.3	84.0	**85.7**	1.7
Procymidone	100	87.1	87.1	87.1	87.1	78.1	**85.3**	4.7
Picoxystrobin	200	87.0	87.0	88.5	90.0	79.5	**86.4**	4.7
Quinoxyfen	100	93.1	102.1	96.1	99.1	87.1	**95.5**	6.0
Chlorfenapyr	800	87.0	91.5	91.9	94.9	84.7	**90.0**	4.5
Benalaxyl	100	93.1	96.1	96.1	96.1	90.1	**94.3**	2.8
Bifenthrin	100	90.1	93.1	96.1	93.1	87.1	**91.9**	3.7
Diflufenican	100	87.1	87.1	90.1	87.1	81.1	**86.5**	3.8
Bromopropylate	200	84.0	91.5	91.5	90.0	82.5	**87.9**	4.9

Chromatograms:

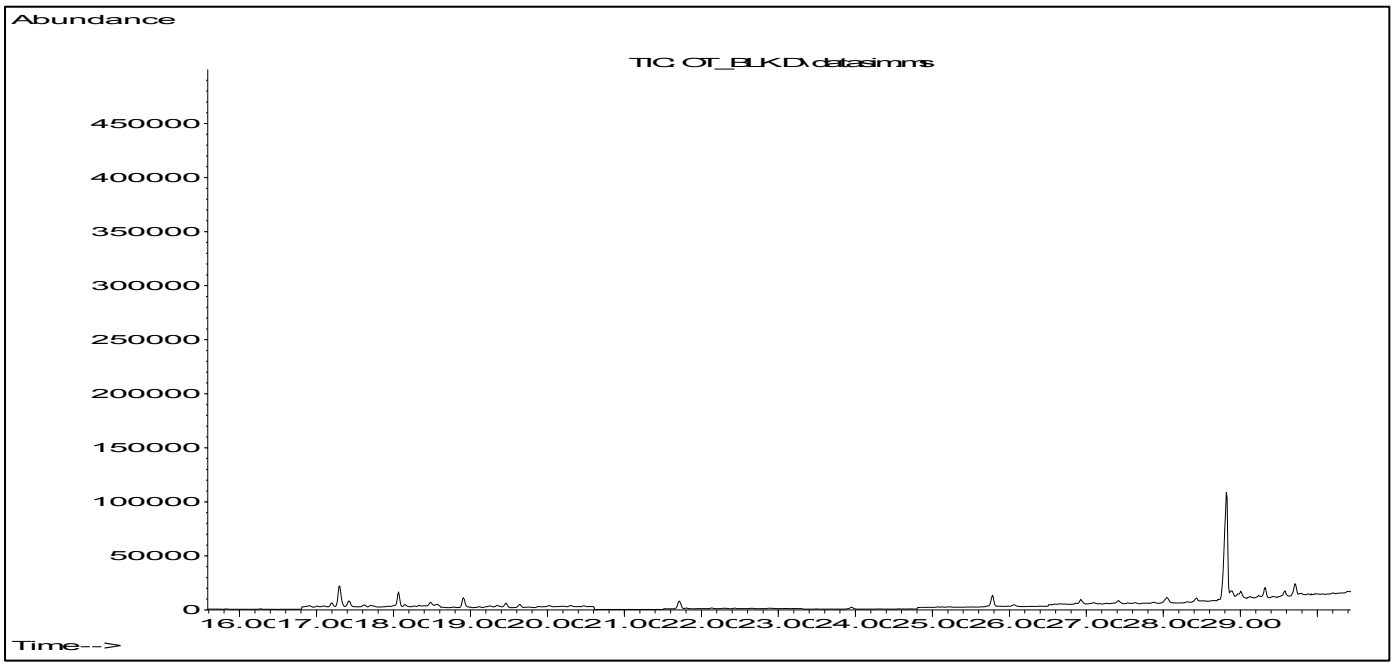
(a) Chromatogram of blank Green tea



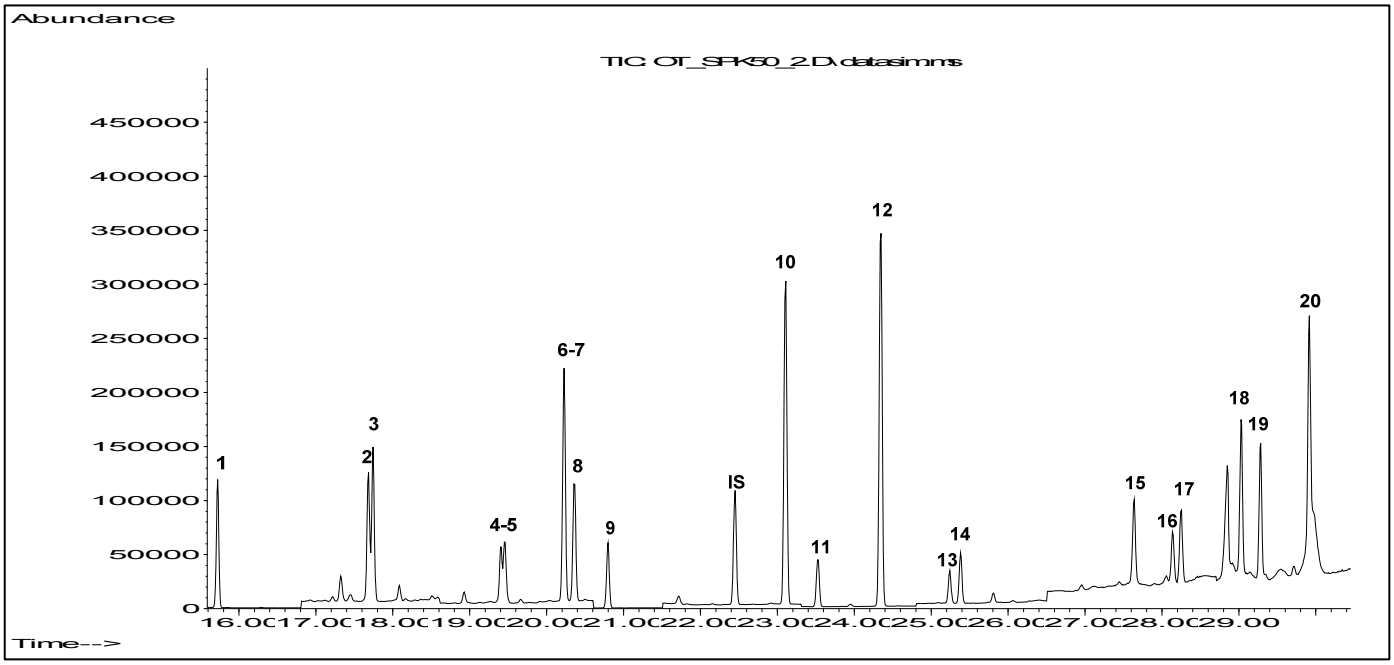
(b) Chromatogram of spiked Green tea



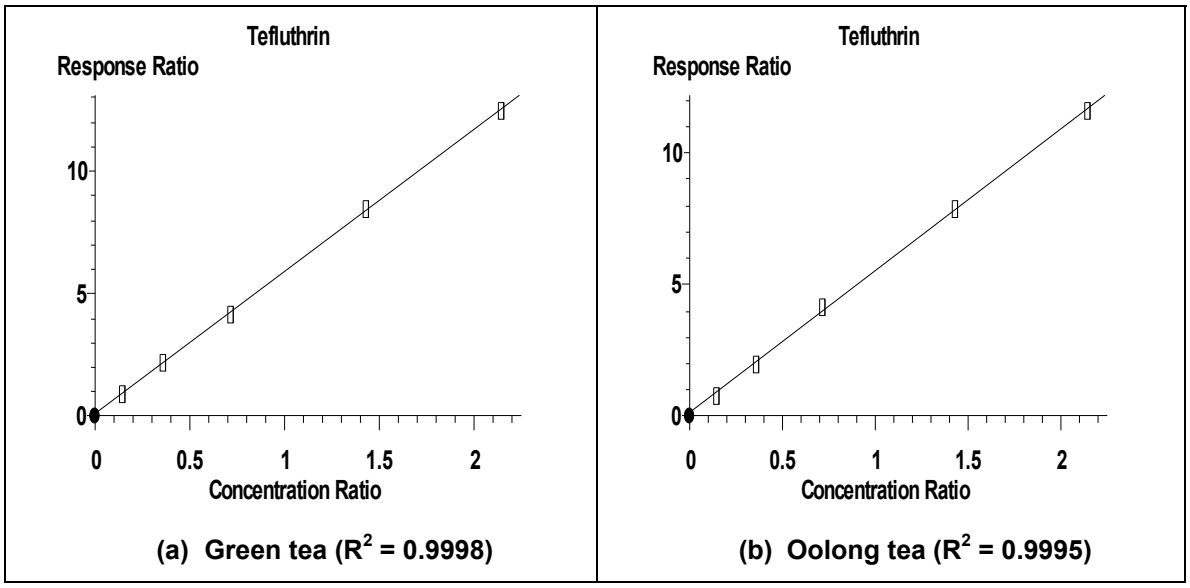
(c) Chromatogram of blank Oolong tea



(d) Chromatogram of spiked Oolong tea



Peak list in chromatograms (b) and (d): 1. Trifluralin; 2. Tefluthrin; 3. Pyrimethanil; 4. Propyzamide; 5. Pirimicarb; 6. Fenchlorphos; 7. Dimethenamid; 8. Tolclofos-methyl; 9. Pirimiphos-methyl; 10. 2,4'-DDE; 11. Bromophos-ethyl; 12. 4,4'-DDE; 13. Procymidone; 14. Picoxystrobin; 15. Quinoxyfen; 16. Chlorfenapyr; 17. Benalaxyl; 18. Bifenthrin; 19. Diflufenican; and 20. Bromopropylate.



Example calibration curves of Tefluthrin in Green and Oolong teas

References:

[1] http://en.wikipedia.org/wiki/Tea

[2] Pang GF, Fan CL, Zhang F, Li Y, Chang QY, Cao YZ, Liu YM, Li ZY, Wang QJ, Hu XY, and Liang P. High-throughput GC/MS and HPLC/MS/MS techniques for the multiclass, multiresidue determination of 653 pesticides and chemical pollutants in tea. J AOAC Int. 2011, 94(4), 1253-1296.

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • www.unitedchem.com • Email: methods@unitedchem.com