Simultaneous multi-determination and transfer of eight pesticide residues from green tea leaves to infusion using gas chromatography

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A method for determining eight pesticide (cyhalothrin, flufenoxuron, fenitrothion, EPN, bifenthrin, difenoconazole, triflumizole, and azoxystrobin) residues in made green tea as well as a tea infusion (under various brewing water temperatures; 60, 80, and 100ºC) using gas chromatography (GC) micro-electron capture detector (μECD) was developed and validated. The extraction method adopted the relatively commonly used approach of solid sample hydration, with the green tea hydrated before being extracted through salting out with acetonitrile followed by a cleanup procedure. The analytes were confirmed using GC-coupled to tandem mass spectrometry (GC/MS/MS) with a triple quadrupole. The linearity of the calibration curves yielded determination coefficients (R^2) > 0.995. Recoveries were carried out using blank samples spiked with all analytes at two levels. The results demonstrated that all pesticides were recovered within the range of 77–116% with a relative standard deviation (RSD) ≤ 14%. The quantification limits of 0.015 to 0.03 mg/kg were lower than the maximum residue limits (MRLs) set by the Korea Food and Drug Administration (KFDA) for all analytes (0.05–10 mg/kg). The infusion study indicated that cyhalothrin, flufenoxuron, and bifenthrin did not infuse into the tea brew from the made tea. Increases in brewing time resulted in increased transfer of azoxystrobin, fenitrothion, and difenoconazole from the made tea to the brew; however, this was not the case with triflumizole or EPN. We conclude that transfer of pesticides appeared to be dependent on their water solubilities and drinking a cup of tea is recommended to be at a water temperature of 60ºC.

Keywords: Pesticides; leaching; green tea; infusion; brewing; roasting; dried leaves

Introduction and objectives

- Tea is the most consumed drink in the world after water. Green tea is a 'non-fermented' tea that contains more catechins than black tea and could contribute to its strong antioxidant effects *in vitro* and *in vivo*. Additionally, green tea contents of certain minerals and vitamins increases the potential for antioxidant activity.
- Eight pesticides (cyhalothrin, flufenoxuron, fenitrothion, EPN, bifenthrin, difenoconazole, triflumizole, and azoxystrobin) with different physicochemical properties (volatility and polarity) were selected to evaluate the percent transfer of pesticide residue from dried (made) tea to infusion, as tea is subjected to an infusion process prior to human consumption.

MATERIALS and METHODS

- **Standards: flufenoxuron, triflumizole, fenitrothion, EPN, bifenthrin, cyhalothrin, difenoconazole, and azoxystrobin**
- ***n*-hexane, acetone: Merck HPLC grade (Germany)**
- **Nitrogen evaporator: Interface HyperVap HV-200**

Sample Preparation

Fresh green tea leaves

Caldron (350℃)

Roasting

Mixing

Drying after 3 times

GC analytical conditions (Agilent 6890 Series)

- **Detector: Electron capture detector (ECD)**
- **Temperature: Inlet (250℃), Det (320℃)**
- **Oven temperature**
  100℃ (2 min hold) → 20℃/min → 220℃ → 10℃/min → 300℃(6 min hold)
- **Flow rate: Carrier gas (N_2), column (1.0 mL/min)**
- **Column: DB-5 (30 m×0.25 mm, 0.25 um)**
- **Split mode Split (10:1)**
- **Injection volume: 1 uL**




Extraction

Sample

***n*-hexane**

Shaking (5 min)

Centrifugation (3000 rpm/min)

Evaporation

GC analysis

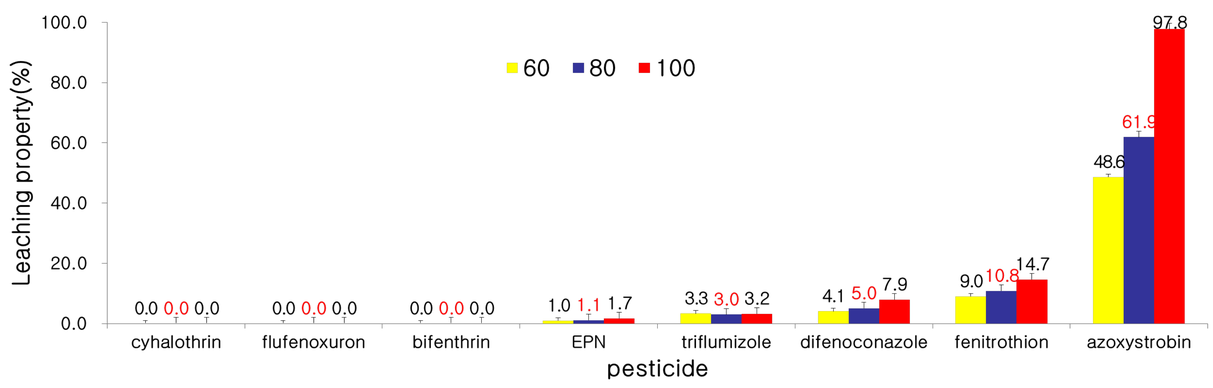
RESULTS and DISCUSSION

Recovery of 8 pesticides

Pesticide	Recovery (%)			
	Fortification (mg/kg)	Average ± C.V(%)	R^2	LOD (mg/kg)
Flufenoxuron	0.1	106±3.5	0.996	0.010
	0.5	89±8.5		
Triflumizole	0.1	103±5.6	0.998	0.005
	0.5	86±8.2		
Fenitrothion	0.1	116±3.1	0.996	0.005
	0.5	95±9.6		
EPN	0.1	98±6.1	0.998	0.0
	0.5	79±1.0		
Bifenthrin	0.1	89±3.1	0.995	0.005
	0.5	77±10.4		
Cyhalothrin	0.1	94±1.2	0.998	0.005
	0.5	81±8.1		
Difenoconazole	0.1	89±4.9	0.997	0.005
	0.5	87±10.8		
Azoxystrobin	0.1	91±4.7	0.997	0.005
	0.5	84±9.7		

C.V. (Coefficient of variation, %) = standard deviation(SD)/average × 100

Leaching efficiency by temperature

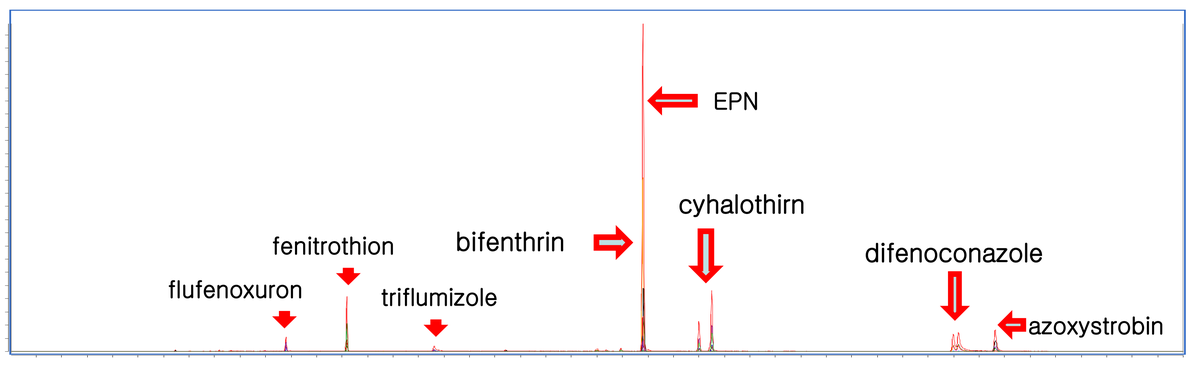


Analytical conditions of GC/MS/MS (Agilent 6890 Series)

Items	Conditions
Analytical column	5% phenyl methyl siloxane (30 m × 0.25 mm × 0.25㎛)
Transfer line temperature	280℃
Source temperature	320℃
Quadrupole temperature	Q1 and Q2 = 150℃

Compounds	M·W	Precursor	Product ion		Dwell	C·C	
Flufenoxuron	488.77	304.2	178.8	214	15	30	20
		125.6	98	51	15	10	40
Triflumizole	345.75	205.3	170.1	186	15	10	20
		277.2	179	205.9	15	30	20
Fenitrothion	277.23	277	109	79	15	20	40
		125	78.9	62	15	30	10
EPN	323.3	157	110.1	77	15	10	30
		169	141	51	15	10	40
Bifenthrin	422.88	180	165.1	179	15	20	20
		166	165.1	115.2	15	20	40
Cyhalothrin	449.85	181	127.1	115.1	15	30	30
		197	115.1	141.1	15	30	20
Difenoconazole	406.26	265	202.2	139.2	15	20	40
		323	173.1	265	15	40	20
Azoxystrobin	403.4	344.1	329.2	172.1	15	20	40
		388.1	360.1	299.9	15	10	30

Chromatogram of 8 pesticides



CONCLUSIONS

- **The recovery rates of pesticides in green tea extracts were in the range of 89 ~ 116% at fortification level of 0.1 mg/kg and 77 ~ 95 at fortification level of 0.5 mg/kg.**
- **Cyhalothrin, flufenoxuron, and bifenthrin were not extracted, and extraction efficiency decreased in the following order: EPN > triflumizole > difenoconazole > fenitrothion > azoxystrobin.**
- **EPN and triflumizole were extracted without the effect of brewing water temperature. Extraction efficiency of difenoconazole, fenitrothion, and azoxystrobin were increased when the temperature become high. Especially, 97.3% of azoxystrobin was extracted with the temperature of 100℃.**
- **The result shows that the brewing efficiency of pesticides from green tea was different according to solubility in water, and physicochemical properties.**

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