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Multi-Residue Method for Determination of 85 Pesticides in Vegetables, Fruits and Green Tea by Stir Bar Sorptive Extraction and Thermal Desorption GC-MS

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Keywords

Multi-residue method, pesticides, vegetables, fruits, green tea, Stir bar sorptive extraction (SBSE), GC-MS.

Abstract

A multi-residue method to determine five groups of 85 pesticides - chlorinated, carbamate, phosphorous, pyrethroid and others - in vegetables, fruits and green tea has been developed using stir bar sorptive extraction (SBSE) coupled to thermal desorption and retention time locked (RTL) GC-MS. Pre-extraction with methanol and dilution with water prior to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds

with high log $K_{o/w}$ values (e.g. pyrethroid). The methanol extracts were diluted twofold and fivefold, and were simultaneously SBSE-enriched. The two stir bars were placed in a single glass thermal desorption liner and were simultaneously desorbed. The versatility of the method was exhibited by its good linearity (4-100 µg/kg, r^2 >0.9900) for 66 pesticides and limit of detection (LOD: < 5 µg/kg) for most of the analytes. The method enables to determine pesticides at low µg/kg in tomato, cucumber, green soybeans, spinach, grape and green tea.

INTRODUCTION

The determination of pesticide residues in agricultural products, plant and environmental samples has been major subject for many years because of their toxic potential risk for human health, persistence and tendency to bioaccumulate. Pesticide residues analysis is carried out by means of several steps, e.g. extraction with organic solvent followed by liquid-liquid partitioning (LLE), clean up by column chromatography or gel permeation chromatography (GPC), and a final chromatographic separation and determination. These processes usually contribute qualitative and quantitative to the analytical results. However, when using traditional sample preparation techniques, e.g. LLE, column chromatography and evaporation, most steps are tedious time-consuming, labor-intensive and complex. Moreover, usually an aliquot of extract is injected into chromatographic system (e.g. typical injection volume for GC is 1 µl). It may result in lack of sensitivity because only a fraction of the sample is used.

In contrast to conventional sample preparation techniques, solid phase microextraction (SPME), which is a simple, solvent-free technique allowing the extraction and concentration steps to be focused into a single step, has been successfully applied to the determination of pesticide residues in water, soil and food samples. Also, SPME provides high sensitivity because the whole extract can be introduced into the GC or HPLC by thermal desorption or liquid desorption. Although aqueous samples, e.g. water and beverage, could be analyzed without any further sample preparation, analysis of solid samples, e.g. soil, vegetables and fruits, are generally based on a headspace SPME (HS-SPME) or a solvent extraction of the analytes before direct immersion SPME (DI-SPME). Beltran et al. reported pyrethroid residues analysis in strawberry and tomato by use of DI-SPME. SPME fiber was directly immersed into slurry of samples with water and hexane/acetone (1:1) without any previous solvent extraction [1].

In 1999, a new extraction technique known as stir bar sorptive extraction (SBSE) using stir bars coated with 50-300 µl of polydimethylsiloxane (PDMS) was developed by Baltussen et al. [2]. The extraction mechanism and advantages are similar to those of SPME, and therefore the sensitivity of the technique, but the enrichment factor is ~100 times higher. Sandra et al. developed a multi-residue screening method of pesticides in vegetables, fruits and baby food by SBSE in combination with thermal desorption (TD)-retention-time-locked (RTL)-GC-MS [3]. Although an aliquot of methanol extract is tenfold diluted with water and SBSE is performed, the presence of pesticide residues is elucidated with RTL-GC-MS analysis in the scan mode. The authors indicated that SBSE-TD-RTL-GC-MS is promising for multi-residue analysis of GC amenable pesticides.

The aim of this paper was to apply SBSE-TD-RTL-GC-MS to determine five groups of 85 pesticides - chlorinated, carbamate, phosphorous, pyrethroid and others – at µg/kg levels, in vegetables (tomato, cucumber, green soybean and spinach), fruits (grape) and green tea.

EXPERIMENTAL

Instrumentation. The stir bars (Twister; the magnetic stirring rod is incorporated in a glass jacket and coated with PDMS) coated with 24 µl of PDMS were used. For the SBSE, 20 ml headspace vial with PTFE-coated silicone septa from Agilent technologies (CA, USA) were used. SBSE was performed by use of a multiple position magnetic stirrer (20 positions) from Global change (Tokyo, Japan). The thermal desorption (TD)-GC-MS analysis was performed with a GERSTEL TDU thermo-desorption unit equipped with a GERSTEL MPS 2 auto-sampler and a GERSTEL CIS 4 programmable temperature vaporization (PTV) inlet and an Agilent 6890N gas chromatograph with a 5973N mass-selective detector equipped with an ultra-ion source (Agilent Technologies).

Sample preparation. Vegetables, fruits and green tea samples were initially homogenized by use of an Ace Homogenizer (Nihon Seiki Seisakusho, Tokyo, Japan) or a Knife mill Grindomix GM 200 (Retsch, Haan, Germany), and 100 ml of methanol was added to 25 g of the homogenized sample in flask. The flask was then placed in an ultrasonic bath for 20 min. Four fractions of the blend were placed in a closed 40 ml vials and

centrifuged for 5 min at 3000 rpm. One to ten milliliter of the supernatant methanol phase was placed in a 20 ml headspace vial and 10 to 19 ml of Milli-Q purified water (Millipore, MA, USA) was added. A stir bar was added and then vial was crimped with PTFE-coated silicon septa. SBSE was simultaneously performed at room temperature (24 °C) for 60 min while stirring at 1000 rpm. After extraction, the stir bar was removed with forceps, dipped briefly in Milli-Q water, dried with a lint-free tissue, and placed in a glass liner of a thermal desorption system. The glass liner was then placed in the thermal desorption unit. No further sample preparation was necessary.

TD-RTL-GC-MS. The stir bar was thermally desorbed by programming the TDU from 20 °C (held for 1 min) to 280 °C (held for 5 min) at 60 °C/min. The desorbed compounds were cryo-focused in the PTV at –150 °C for subsequent GC-MS analysis. An empty baffled liner was used in the PTV injector. After desorption, the PTV was programmed from –150 °C to 280 °C (held for 5 min) at 600 °C/min to inject the trapped compounds on to the analytical column. Injection was performed in the splitless mode and the split valve was closed for 3 min. The separations were performed on a HP-5ms fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies). The oven temperature was programmed from 70 °C (held for 2 min) at 25 °C/min to 150 °C, at 3 °C/min to 200 °C and finally at 8 °C/min to 300 °C. This is the temperature program for the RTL screener option (Agilent Technologies). Helium was used as carrier gas. The head pressure was calculated using the RTL software so that chlorpyrifos methyl at a constant retention time of 16.59 min. The mass spectrometer was operated in the scan mode using electron-impact ionization (electron-accelerating voltage: 70V). The scan range was set from m/z 40 to 500 every 0.31 s. The main qualifier ion was used for determination.

Results and Discussion

Dilution of methanol extract. Since solid samples, e.g. vegetables, fruits and green tea, cannot be extracted directly by use of SBSE, methanol extraction of the analytes before SBSE is performed. Methanol extract was then diluted with Milli-Q water. Percent level of methanol may cause negative and positive effects for the recovery of solutes in aqueous sample. For the compounds with low log $K_{o/w}$ (< 2.5), the methanol may reduce recovery. For the compounds with high log $K_{o/w}$ (> 5.0), the methanol can prevent adsorption of the compounds to the glass wall of extraction vessel and the sample matrix. It results in high recovery. In addition, dilution process can change the amount of solutes in sample. This may also change extraction amount in SBSE. To evaluate the effect of dilution factor on the SBSE, fortified methanol extract of spinach sample (50 µg/L for all compounds, corresponding to approximate levels of 200 µg/kg of sample) was prepared. The dilution factor was varied over the range 1.7-20 (corresponding to 60-5% methanol). A 60-min extraction was performed. Figure 1 shows the results of representative pesticides with various log $K_{o/w}$ values.

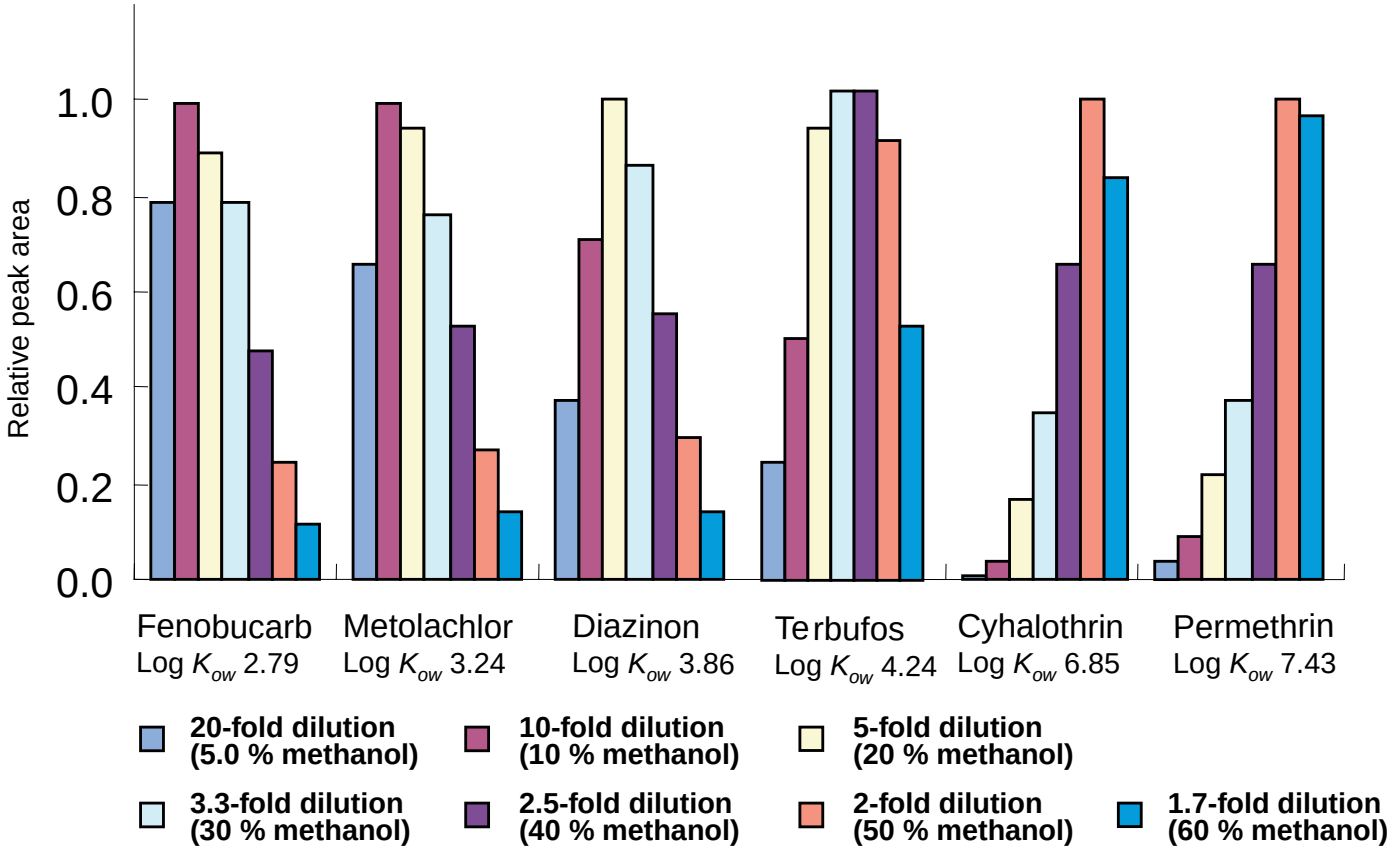

Figure 1. The effect of dilution factor of methanol extract on the SBSE. Methanol extract of blank spinach sample was fortified at 50 µg/L (corresponding to approximate level of 200 µg/kg of sample). The dilution factor was varied over 1.7-20 (corresponding to 60-5.0 % methanol). Relative peak area was normalized by the maximum peak area of each compound.

Log $K_{o/w}$ values were calculated with a SRC-KOWWIN software package (Syracuse Research, Syracuse, NY, USA) according to a fragment constant estimation methodology [4] for all analytes. For fenobucarb (log $K_{o/w}$ 2.79) and metolachlor (log $K_{o/w}$ 3.24), the response decreased when dilution factor decreased 10 to 1.7. This is due to decrease of partitioning coefficients by increase of methanol amount. For diazinon (log $K_{o/w}$ 3.86) and terbufos (log $K_{o/w}$ 4.24), the response increased when the factor decreased 20 to 5.0 or 20 to 2.5, and the response decreased when the factor decreased 3.3 to 1.7 or 2 to 1.7. The increased responses were because of major effect of sample amount and minor effect of methanol at the factor higher than 5.0 or 2.5. For cyhalothrin (log $K_{o/w}$ 6.85) and permethrin (log $K_{o/w}$ 7.43), although poor extractive behavior was observed at the factor higher than 3.3, the response increased significantly at the factor lower than 2.5. This is due to major effect of adsorption to glass wall and to sample matrix at the factor lower than 3.3. According to these results, several dilution factors can be selected for the SBSE of methanol extract of pesticides with various log $K_{o/w}$. Although SBSE can be performed in parallel, multiple analyses are necessary for several dilution factors for one sample. Since the TD system employed in this study can simultaneously perform thermal desorption of two stir bars in a single glass liner, two dilution factors can be selected for practical use. Twofold and fivefold dilution were selected because of high sensitivity of the pesticides with various log $K_{o/w}$ values. Figure 2 shows typical chromatograms.

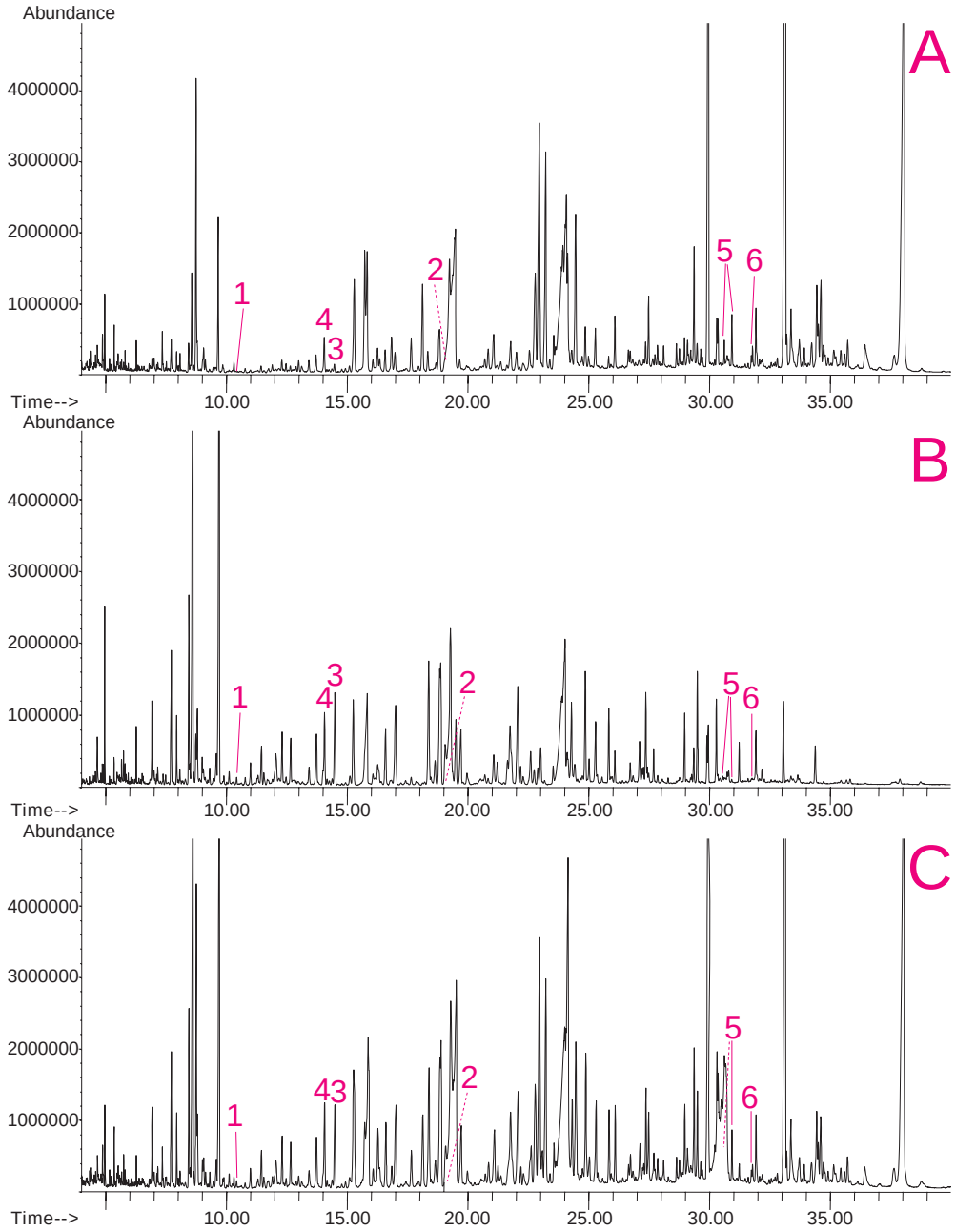

Figure 2. Typical chromatograms obtained by SBSE-TD-RTL-GC-MS of the fortified methanol extract of spinach sample (A) twofold (one stir bar), (B) fivefold (one stir bar), and (C) twofold and fivefold dilution (two stir bars). 1. Fenobucarb (log $K_{o/w}$ 2.79), 2. Metolachlor (log $K_{o/w}$ 3.24), 3. Diazinon (log $K_{o/w}$ 3.86), 4. Terbufos (log $K_{o/w}$ 4.24), 5. Cyhalothrin (log $K_{o/w}$ 6.85), 6. Permethrin (log $K_{o/w}$ 7.43). Methanol extract of blank spinach sample was fortified at 50 µg/L (corresponding to approximate level of 200 µg/kg of sample).

Method Validation and determination of pesticides in real samples. To validate the method, the linearity was firstly examined by analyzing fortified methanol extract of blank spinach samples. The extract was diluted twofold and fivefold, and were simultaneously SBSE-enriched (60 min). The two stir bars were simultaneously analyzed by TD-RTL-GC-MS in scan mode. For 66 compounds, the seven-points of matrix matched calibration curves were linear over the range 0.80 to 25 µg/L (corresponding to approximate levels of 4.0 to 100 µg/kg sample) with correlation coefficient (r^2) better than 0.9900. For 19 compounds, the r^2 were in the range of 0.9574-0.9885. The limit of detection (LOD) was estimated by six replicate analyses of the lowest-level calibration standard and calculating 3.36 times the standard deviation of the determination results. The LOD was calculated to be 0.12- 5.2 µg/L (corresponding to approximate levels of 0.62-26 µg/kg sample). Linearity and the LOD are summarized in Table 1.

Table 1. Linearity and LOD of SBSE-TD-RTL-GC-MS analysis of pesticides in fortified spinach sample.

Chlorinated pesticides	log $K_{o/w}$	r^2 [4-100 µg/kg][a]	LOD[b] [µg/kg]
Procymidone	2.59	0.9959	3.1
β-BHC	3.68	0.9991	3.9
δ-BHC	3.68	0.9937	2.0
Chlorobenzilate	3.99	0.9978	0.83
α-BHC	4.26	0.9997	1.6
γ-BHC(Lindane)	4.26	0.9996	1.5
p,p-DDD	5.87	0.9999	1.0
p,p-DDE	6.00	0.9999	1.0
Carbamate pesticides	**log $K_{o/w}$**	**r^2 [4-100 µg/kg][a]**	**LOD[b] [µg/kg]**
Pirimicarb	1.70	0.9751	4.2
Bendiocarb	1.72	0.9965[c]	24
Ethiofencarb	2.04	0.9574[c]	26
Isoprocarb	2.30	0.9798	3.5
Fenobucarb	2.79	0.9921	3.8
Methiocarb	2.87	0.9843	3.4
Diethofencarb	3.29	0.9885	1.7
Chlorpropham	3.51	0.9972	2.3
Thiobencarb	3.90	0.9984	1.1
Esprocarb	4.58	0.9996	1.0
Phosphorous pesticides	**log $K_{o/w}$**	**r^2 [4-100 µg/kg][a]**	**LOD[b] [µg/kg]**
Dichlorvos	1.90	0.9753	3.3
Fensulfothion	2.35	0.9981	2.9
Parathion-methyl	2.75	0.9920	2.2
Malathion	2.75	0.9938	2.3
Thiometon	2.88	0.9993	1.9
Isofenphos oxon	2.89	0.9936	3.0
Etrimfos	2.94	0.9985	1.3
Quinalphos	3.04	0.9974	1.0
Dimethylvinphos	3.16	0.9878	3.1
Fenitrothion	3.30	0.9959	1.5
Pyraclofos	3.37	0.9975	1.3
Phenthoate	3.47	0.9978	0.63
Ethoprophos	3.59	0.9957	4.1
Edifenphos	3.61	0.9958	1.8
Parathion	3.73	0.9995	1.2
Diazinon	3.86	0.9983	1.3
Fenthion	4.08	0.9986	1.0
E,Z-Chlorofenvinphos	4.15	0.9939	2.4
Pirimiphos-methyl	4.20	0.9994	0.92
Terbufos	4.24	0.9999	1.1
Phosalone	4.29	0.9980	0.80
EPN	4.47	0.9987	0.73
Tolclofos-methyl	4.56	0.9998	0.93
Isofenphos	4.65	0.9980	1.1
Chlorpyrifos	4.66	0.9999	1.0
Cadusafos	5.48	0.9992	2.4
Prothiofos	5.69	0.9997	1.0
Pyrethroid pesticides	**log $K_{o/w}$**	**r^2 [4-100 µg/kg][a]**	**LOD[b] [µg/kg]**
Fenpropathrin	5.62	0.9949	0.76
Cyfluthrin 1,2,3,4	5.74	0.9980	1.8
Deltamethrin	6.18	0.9957	2.6
Cypermethrin 1,2,3,4	6.38	0.9994	1.4
Flucythrinate 1,2	6.56	0.9992	1.6
Acrinathrin	6.73	0.9966	2.0
Fenvalerate 1,2	6.76	0.9986	1.8
Fluvalinate 1,2	6.81	0.9988	2.1
Cyhalothrin 1,2	6.85	0.9993	2.0
Tefluthrin	7.19	0.9999	1.4
Permethrin 1,2	7.43	0.9992	2.6
Halfenprox	8.35	0.9990	1.6
Other pesticides	**log $K_{o/w}$**	**r^2 [4-100 µg/kg][a]**	**LOD[b] [µg/kg]**
Benfuresate	2.80	0.9878	2.9
Mefenacet	2.80	0.9766	3.0
Cyproconazole	2.91	0.9934[c]	12
EPTC	3.02	0.9993	2.1
Metolachlor	3.24	0.9913	2.3
Chinomethionate	3.37	0.9953	1.6
Mycrobutanil	3.50	0.9647	3.2
Thenylchlor	3.53	0.9879	1.9
Fenarimol	3.62	0.9762	2.1
Butylate	3.85	0.9957	1.5
Tebconazole	3.89	0.9771	1.1
Bitertanol 1,2	4.07	0.9773	2.3
Propiconazole 1,2	4.13	0.9941	1.4
E-Pyrifenox	4.20	0.9750	1.0
Z-Pyrifenox	4.20	0.9720	1.3

[a] Linear range of the matrix matched calibration curve.

[b] The LOD was calculated as 3.36 times the standard deviation of replicate analyses (n=6) of blank spinach samples spiked at the lowest concentration of the calibration curve.

[c] Linear range was 24-100 µg/kg

red values show less than 0.9900

Table 1. Linearity and LOD of SBSE-TD-RTL-GC-MS analysis of pesticides in fortified spinach sample (cont.).

Mepronil	4.24	0.9789	3.0
Pretilachlor	4.29	0.9939	1.2
Buprofezin	4.30	0.9997	0.82
Pyrimidifen	4.59	0.9934	0.82
Tebufenpyrad	4.61	0.9986	0.63
Flutolanil	4.65	0.9784	2.8
Flusilazole	4.89	0.9865	1.2

Pendimethalin	5.18	0.9998	1.0
Difenoconazole 1,2	5.20	0.9924	1.1
Pyridaben	5.47	0.9988	0.85
Pyriproxyfen	5.55	0.9996	1.0
Imibenconazole	5.64	0.9991	0.62
Silafluofen	8.20	0.9990	0.76

[a] Linear range of the matrix matched calibration curve.
[b] The LOD was calculated as 3.36 times the standard deviation of replicate analyses (n=6) of blank spinach samples spiked at the lowest concentration of the calibration curve.
[c] Linear range was 24-100 µg/kg
red values show less than 0.9900

The proposed method was applied to several tomato, cucumber, green soybean, spinach, grape and green tea samples obtained from different markets. Determination of the pesticides in samples was carried out by a seven-points level matrix matched calibration or a five-points level standard addition calibration using fortified methanol extracts. Figure 3 shows typical chromatograms of a green tea sample. Table 2 shows the frequency of residue findings and concentration range of contaminated samples. Of the 25 samples analyzed, pesticide residues were detected in 12, of which 1 slightly below the maximum residue levels (MRLs) (Japanese Ministry of Health, Labor and Welfare) involving permethrin in spinach (2.0 mg/kg).

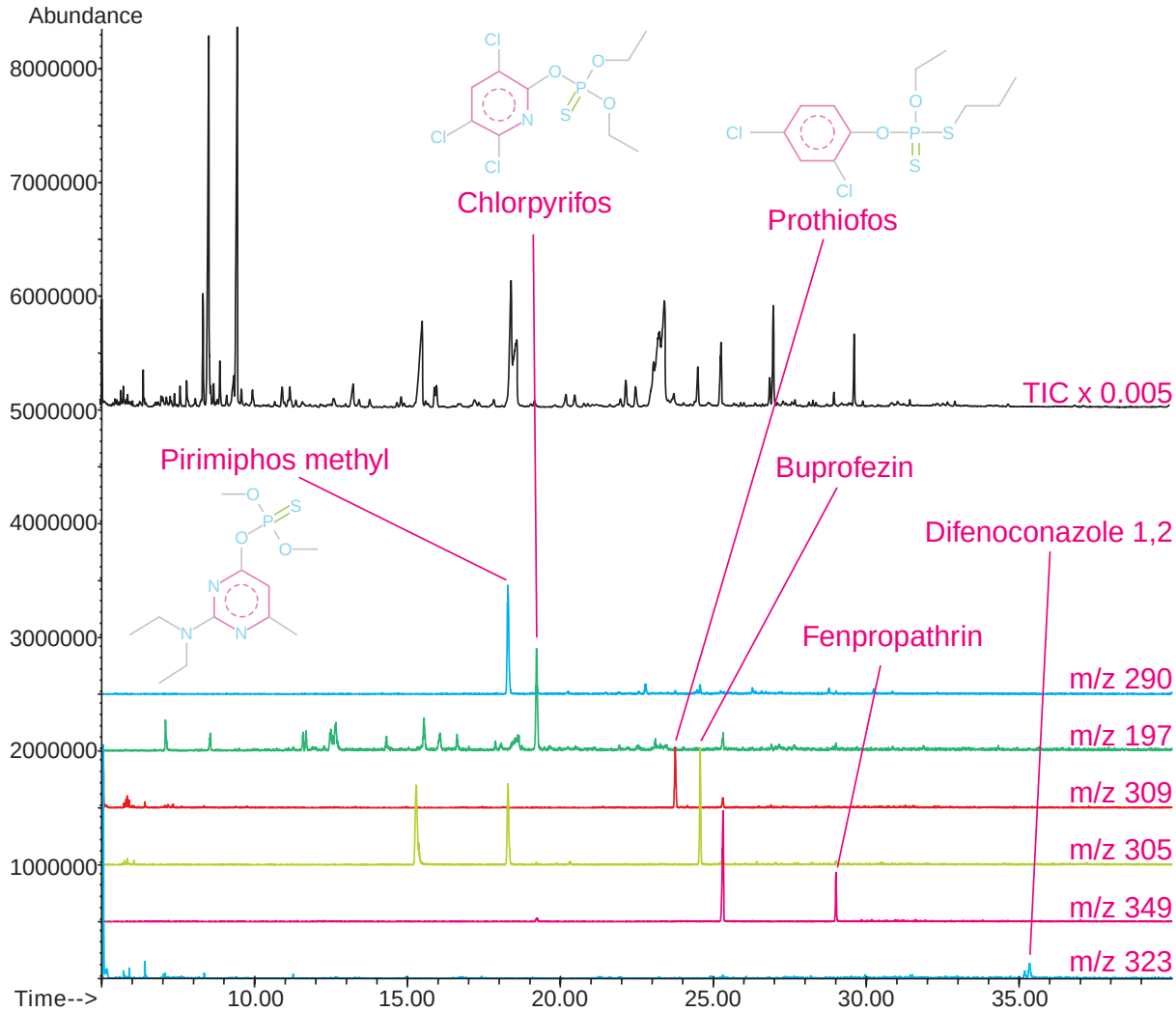

Figure 3. Typical chromatograms obtained by the SBSE-TD-RTL-GC-MS of a green tea sample.

Table 2. Pesticide concentrations in vegetables, fruits and green tea samples by SBSE-TD-RTL-GC-MS.

Matrix	Pesticide	No. of Samples	Concentration [mg/kg]	MRLs[a] [mg/kg]
Grape	Fenpropathrin	1	0.020	0.20
Tomato	Buprofezine	1	0.0060	-
	Chlorobenzilate	1	0.0017	-
	Procymidone	1	0.066	-
Cucumber	Chinomethionate	1	0.0044	0.50
	Procymidone	1	0.0031	5.0
Green soybeans	Chlorpyrifos	1	0.0066	0.050
	Cypermethrin	1	0.081	0.050
Spinach	Cypermethrin	2	0.0039-0.012	2.0
	p,p-DDD	1	0.0015	-
	Permethrin	1	1.8	2.0
Green tea	Buprofezine	3	0.025-0.032	-
	Chlorpyrifos	3	0.0029-0.017	3.0
	Difenoconazole	1	0.13	10
	Fenpropathrin	1	0.21	25
	EPN	1	0.015	0.10
	Pirimiphos methyl	3	0.0024-0.0077	10
	Prothiofos	2	0.017-0.044	5.0

[a] Maximum residue levels (Japanese Ministry of Health, Labor and Welfare)

Conclusion

The multi-residue method for determining 85 pesticides in vegetables, fruits and green tea using the SBSE followed by TD-RTL-GC-MS in scan mode was described. Combination of twofold and fivefold dilution of methanol extract for the SBSE analysis showed high sensitivity for the pesticides with various log $K_{o/w}$ values (LOD: 0.62-26 µg/kg). The method allowed determination of µg/kg levels of pesticide residues in vegetables, fruit and green tea.

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