APPNOTE

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Multi-Residue Method for the Determination of Five Groups of Pesticides in Non-Fatty Food Samples by Dual Stir Bar Sorptive Extraction (Dual SBSE) and Thermal Desorption GC-MS

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Keywords

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

Abstract

A multi-residue method for determination of five groups of 85 pesticides - organochlorine, carbamate, organophosphorous, pyrethroid and others - in non-fatty food, e.g. vegetables, fruits and green tea is described. The method is based on stir bar sorptive extraction (SBSE) coupled to thermal desorption (TD) and retention time locked (RTL) GC-MS in the scan mode. Samples are extracted with methanol and diluted with water prior to SBSE. Dilution of the methanol extract before SBSE was optimized to obtain high sensitivity, and to minimize sample matrix effects (particularly for the pesticides with high log $K_{o/w}$ values). The optimized method consists of a dual SBSE extraction performed simultaneously on respectively a twofold and a fivefold diluted methanol extract. After extraction, the two stir bars are placed in a single glass thermal desorption liner and are simultaneously desorbed. The method showed good linearity (r^2 >0.9900) for 66 pesticides and high sensitivity (limit of detection: < 5 µg/kg) for most of the target pesticides. The method was applied to the determination of pesticides at low µg/kg levels in tomato, cucumber, green soybeans, spinach, grapes and green tea.

Introduction

In recent years much effort has been dedicated to the determination of pesticide residues in agricultural products, plant and environmental samples because of their potential risk of toxicity for human health, persistence and tendency to bio-accumulate. Pesticide residues analysis is generally carried out following several steps, e.g. extraction with organic solvent followed by liquid-liquid partitioning (LLE), clean up by column chromatography and/or gel permeation chromatography (GPC), concentration, and a final chromatographic separation and determination. In these traditional sample preparation techniques, most steps are tedious, time-consuming, labor-intensive, rather complex and they consume large amount of solvents. Solid phase extraction (SPE) and matrix solid-phase dispersion (MSPD) were recently introduced as alternative sam-ple preparation methods in pesticide residues analysis. These miniaturized methods can largely reduce solvent consumption. The major drawback is, however, the fact that the enrichment factor (original sample amount versus final extract volume) obtained with these techniques is rather limited and either concentration to small volume (< 1 mL) or large volume injection should be applied to compensate for lower overall sensitivity. For this reason, solid phase microextraction (SPME), which is a simple, solvent-less technique allowing the extraction and concentration in a single step, was introduced. SPME has been successfully ap-

plied to the determination of pesticide residues in various sample matrices, e.g. water, soil and food. Also, SPME provides enhanced sensitivity because the extracted fraction (on the fiber) can be introduced quantitatively into the GC by thermal desorption. Alternatively, the SPME fiber can be desorbed by liquid extraction, and the extract analyzed by HPLC. Although aqueous samples, e.g. water and beverages, can be analyzed without any further sample preparation by SPME, analysis of solid samples, e.g. vegetables and fruits, is either based on a headspace SPME (HS-SPME) or a solvent extraction of the analytes is performed before direct immersion SPME (DI-SPME).

In 1999, a new extraction technique using stir bars coated with 20-300 µL of polydimethylsiloxane (PDMS) was developed by Baltussen et al. [1]. This extraction technique is known as stir bar sorptive extraction (SBSE). The extraction mechanism and advantages are similar to those of SPME, but the enrichment factor, which is determined by the amount of extraction phase (PDMS), is up to 100 times higher. Several authors indicated that the SBSE method allows limit of detection (LOD) at the sub-ng/L level, particularly for compounds having more hydrophobic characteristics [2-5]. SBSE has been successfully applied to various types of samples in many fields, e.g. environmental, food and biological samples, as reported in recent reviews published by Baltussen et al. and David et al. [6, 7]. 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 [8]. As well as miniaturization of sample preparation, the SBSE-TD process made it possible to replace several steps in the traditional method, e.g. solvent exchange, concentration and clean-up. Moreover, although an aliquot of the initial extract is diluted with water prior to SBSE, detection of the presence of pesticide residues at µg/kg levels is possible using RTL-GC-MS analysis in scan mode. The authors indicated that SBSE-TD-RTL-GC-MS was promising for multi-residue analysis of GC amenable pesticides.

The aim of this paper was to optimize and validate the dual SBSE-TD-RTL-GC-MS method for the determination of five groups of 85 pesticides, including organochlorine, carbamate, organophosphorous, pyrethroid and other pesticides at µg/kg levels in vegetables (tomato, cucumber, green soybean and spinach), fruits (grape) and green tea.

Experimental

Materials

Two standard solutions of 47 and 50 pes-ticides mixtures at 10 µg/mL each in acetone were purchased from Kanto Kagaku (Tokyo, Japan). Some pesticides in these stock solutions are composed of several isomers: bitertanol 1, 2; E, Z-chlorofenvinphos; cyfluthrin 1, 2, 3, 4; cyhalothrin 1, 2; cypermethrin 1, 2, 3, 4; difenoconazole 1, 2; fenvalerate 1, 2; flucythrinate 1, 2; fluvalinate 1, 2; fosthiazate 1, 2; permethrin 1, 2; propiconazole 1, 2; and triadimenol 1, 2. For these compounds, the concentration (10 µg/mL) is the sum of the concentration of the individual isomers. Buprofezin, Fenpropathrin and Procymidone were also purchased from Kanto Kagaku, as individual solutions at 10 µg/mL in acetone. The 10 µg/mL stock standard solutions were then mixed and diluted with acetone to prepare a test mixture containing 100 solutes (85 and 15 isomeric analogues). The list of solutes is given in Table 1. The stock standard solutions were kept at -20 °C. Methanol, pesticide residues grade, was purchased from Wako (Osaka, Japan). Vegetables, fruits and green tea samples were obtained from different local stores in Tokyo Japan.

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 obtained from GERSTEL GmbH & Co. KG (Mülheim an der Ruhr, Germany). For the SBSE, 20 mL headspace vials 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 TD-RTL-GC-MS analysis was performed with a GERSTEL TDU thermal desorption unit equipped with a GERSTEL MPS 2 autosampler 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 then 100 mL of methanol was added to 25 g of the homogenized sample in the flask. The flask was then placed in an ultrasonic bath for 20 min. Four fractions of the extract were placed in closed 40 mL vials and centrifuged for 5 min at 3000 rpm. Various volumes of the supernatant methanol phase were

transferred to a 20 mL headspace vial and Milli-Q purified water (Millipore, MA, USA) was added to a volume of 20 mL. As final solutions, a twofold dilution (10 mL methanol extract + 10 mL water) and fivefold dilution (4 mL methanol extract + 16 mL water) were obtained. To the diluted samples, a stir bar was added and the vial was crimped with PTFE-coated silicone 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 prep-aration was necessary. Figure 1 shows a dual SBSE procedure for non-fatty food samples, e.g. vegetables, fruits and green tea. Reconditioning of the stir bas was done after use by soaking in Milli-Q purified water and a mixture of methylene chloride-methanol (1:1) for 24 h each ; the stir bars were then removed from the solvent and dried on a clean surface at room temperature for 1 h. Finally, the stir bars were thermally conditioned for 30 min at 300 °C in a flow of helium. Typically, 30 extractions could be performed with the same stir bar.

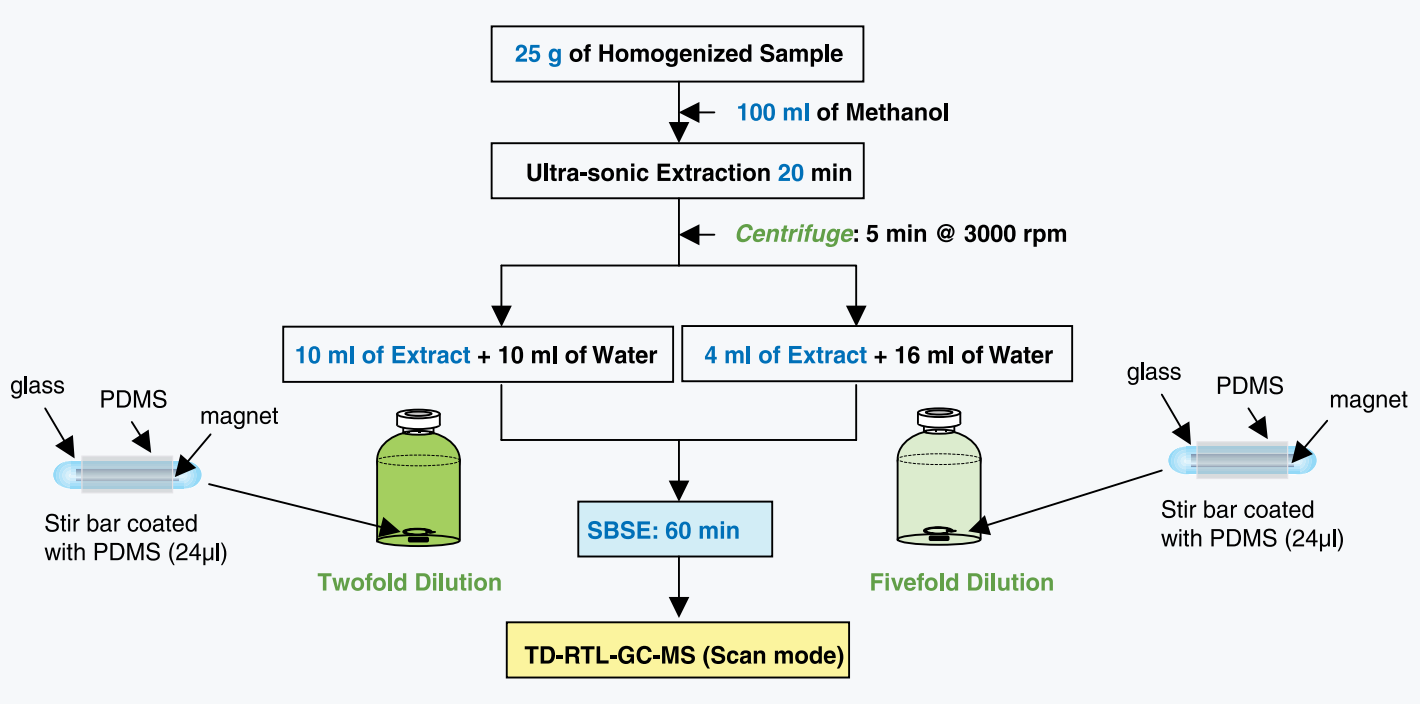

Figure 1: Dual SBSE procedure for non-fatty food samples.

TD-RTL-GC-MS. The stir bar was thermally desorbed by programming the TDU from 40 °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 tem-perature 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 eluted 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 selected ions for determination are shown in Table 1.

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Table 1: Pesticides studied and corresponding octanol-water partitioning coefficients (log $K_{o/w}$), selected ions for determination, linearity and limit of detection (LOD) obtained when fortified methanol extract of spinach sample was twofold and fivefold diluted, and simultaneously analyzed by Dual SBSE-TD-RTL-GC-MS in the scan mode.

No.	Compounds	Log $K_{o/w}$ [a]	m/z [b]	r^2 [4.0-100 µg/kg] [c]	LOD [d] [µg/kg]
Organochlorine Pesticides (OCPs)					
1	Procymidone	2.59	283	0.9959	3.1
2	β-BHC	3.68	183	0.9991	3.9
3	δ-BHC	3.68	183	0.9937	2.0
4	Chlorobenzilate	3.99	251	0.9978	0.83
5	α-BHC	4.26	183	0.9997	1.6
6	γ-BHC(Lindane)	4.26	183	0.9996	1.5
7	p,p-DDD	5.87	235	0.9999	1.0
8	p,p-DDE	6.00	246	0.9999	1.0
Carbamate pesticides					
9	Pirimicarb	1.70	166	0.9751 [e]	13
10	Bendiocarb	1.72	151	0.9965 [e]	24
11	Ethiofencarb	2.04	107	0.9574 [e]	26
12	Isoprocarb	2.30	121	0.9798 [f]	11
13	Fenobucarb	2.79	121	0.9921	3.8
14	Methiocarb	2.87	168	0.9843 [e]	20
15	Diethofencarb	3.29	267	0.9885 [e]	10
16	Chlorpropham	3.51	127	0.9972	2.3
17	Thiobencarb	3.90	100	0.9984	1.1
18	Esprocarb	4.58	222	0.9996	1.0
Organophosphorous Pesticides (OPPs)					
19	Dichlorvos	1.90	109	0.9753 [e]	20
20	Fensulfothion	2.35	293	0.9981 [e]	17
21	Parathion-methyl	2.75	263	0.9920	2.2
22	Malathion	2.75	173	0.9938	2.3
23	Thiometon	2.88	246	0.9993 [f]	5.7
24	Isofenphos oxon	2.89	229	0.9936 [f]	12
25	Etrimfos	2.94	292	0.9985	1.3
26	Quinalphos	3.04	156	0.9974	1.0
27	Dimethylvinphos	3.16	295	0.9878	3.1
28	Fenitrothion	3.30	277	0.9959	1.5
29	Pyraclofos	3.37	360	0.9975	1.3
30	Phenthoate	3.47	274	0.9978	0.63
31	Ethoprophos	3.59	158	0.9957	4.1
32	Edifenphos	3.61	310	0.9958	1.8
33	Parathion	3.73	291	0.9995	1.2
34	Diazinon	3.86	179	0.9983	1.3
35	Fenthion	4.08	278	0.9986	1.0

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Table 1 (cont.): Pesticides studied and corresponding octanol-water partitioning coefficients (log $K_{o/w}$), selected ions for determination, linearity and limit of detection (LOD) obtained when fortified methanol extract of spinach sample was twofold and fivefold diluted, and simultaneously analyzed by Dual SBSE-TD-RTL-GC-MS in the scan mode.

No.	Compounds	Log $K_{O/W}$ [a]	m/z [b]	r^2 [4.0-100 µg/kg] [c]	LOD [d] [µg/kg]
Organophosphorous Pesticides (OPPs) (cont.)					
36	E,Z-Chlorofenvinphos	4.15	267	0.9939 [e]	14
37	Pirimihos-methyl	4.20	290	0.9994	0.92
38	Terbufos	4.24	231	0.9999	1.1
39	Phosalone	4.29	182	0.9980	0.80
40	EPN	4.47	157	0.9987	0.73
41	Tolclofos-methyl	4.56	265	0.9998	0.93
42	Isofenphos	4.65	255	0.9980	1.1
43	Chlorpyrifos	4.66	314	0.9999	1.0
44	Cadusafos	5.48	159	0.9992	2.4
45	Prothiofos	5.69	309	0.9997	1.0
Pyrethroid Pesticides					
46	Fenpropathrin	5.62	349	0.9949	0.76
47	Cyfluthrin 1,2,3,4	5.74	226	0.9980 [g]	5.4
48	Deltamethrin	6.18	253	0.9957 [g]	7.8
49	Cypermethrin 1,2,3,4	6.38	163	0.9994 [g]	4.2
50	Flucythrinate 1,2	6.56	199	0.9992	1.6
51	Acrinathrin	6.73	181	0.9966	2.0
52	Fenvalerate 1,2	6.76	167	0.9986	1.8
53	Fluvalinate 1,2	6.81	250	0.9988	2.1
54	Cyhalothrin 1,2	6.85	181	0.9993	2.0
55	Tefluthrin	7.19	197	0.9999	1.4
56	Permethrin 1,2	7.43	183	0.9992	2.6
57	Halfenprox	8.35	263	0.9990	1.6
Other Pesticides					
58	Benfuresate	2.80	163	0.9878	2.9
59	Mefenacet	2.80	192	0.9766	3.0
60	Cyproconazole	2.91	222	0.9934 [e]	24
61	EPTC	3.02	128	0.9993	2.1
62	Metolachlor	3.24	238	0.9913	2.3
63	Chinomethionate	3.37	234	0.9953	1.6
64	Mycrobutanil	3.50	179	0.9647 [e]	3.2
65	Thenylchlor	3.53	127	0.9879	1.9
66	Fenarimol	3.62	251	0.9762 [e]	13
67	Butylate	3.85	217	0.9957	1.5
68	Tebconazole	3.89	250	0.9771 [f]	11
69	Bitertanol 1,2	4.07	170	0.9773	2.3
70	Propiconazole 1,2	4.13	173	0.9941	1.4

Table 1 (cont.): Pesticides studied and corresponding octanol-water partitioning coefficients (log $K_{o/w}$), selected ions for determination, linearity and limit of detection (LOD) obtained when fortified methanol extract of spinach sample was twofold and fivefold diluted, and simultaneously analyzed by Dual SBSE-TD-RTL-GC-MS in the scan mode.

No.	Compounds	Log $K_{o/w}$ [a]	m/z [b]	r^2 [4.0-100 µg/kg] [c]	LOD [d] [µg/kg]
Other Pesticides (cont.)					
71	E-Pyrifenox	4.20	262	0.9750	1.0
72	Z-Pyrifenox	4.20	262	0.9720	1.3
73	Mepronil	4.24	119	0.9789	3.0
74	Pretilachlor	4.29	238	0.9939	1.2
75	Buprofezin	4.30	305	0.9997	0.82
76	Pyrimidifen	4.59	184	0.9934	0.82
77	Tebufenpyrad	4.61	318	0.9986	0.63
78	Flutolanil	4.65	323	0.9784	2.8
79	Flusilazole	4.89	233	0.9865	1.2
80	Pendimethalin	5.18	252	0.9998	1.0
81	Difenoconazole 1,2	5.20	323	0.9924 [f]	11
82	Pyridaben	5.47	364	0.9988	0.85
83	Pyriproxyfen	5.55	136	0.9996	1.0
84	Imibenconazole	5.64	125	0.9991 [f]	6.2
85	Silafluofen	8.20	179	0.9990	0.76

[a] Log $K_{o/w}$ values are calculated with a SRC-KOWWIN software according to reference [31]
[b] Selected ion for determination
[c] Linear range of the matrix matched calibration curve (approximate level)
[d] The LOD (approximate level) 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
[e] Linear range was 24-150 µg/kg (approximate level)
[f] Linear range was 12-150 µg/kg (approximate level)
[g] Linear range was 12-100 µg/kg (approximate level)
red values show less than 0.9900 (r^2)

Results and Discussion

SBSE-TD-RTL-GC-MS analysis of pesticides

When SBSE is applied to solid samples, e.g. vegetables, fruits and green tea, there are two approaches as is the case of SPME. One is pre-extraction of the analytes before SBSE, and another is DI-SBSE for aqueous slurry of the samples. In this study, methanol extraction with ultrasonic bath was performed before SBSE because the former includes a dilution process that can reduce the matrix effect for SBSE process. The methanol extract was then diluted with Milli-Q water.

Since SBSE is by nature an equilibrium technique, the extraction of solutes from the aqueous phase into the PDMS phase is controlled by the partitioning coefficients. Recent studies have correlated this partitioning coefficient with the octanol-water distribution constant ($k_{o/w}$) [9-11]. Hydrophobic compounds with a high $k_{o/w}$ can be high recovery; by contrast, hydrophilic compounds with a low $k_{o/w}$, e.g. polar pesticides, can be low recovery [1].

For the present work, one hundred pesticides were first selected as model compounds across many chemical classes including a wide range of polarity, e.g. organochlorine pesticides (OCPs), carbamate pesticides, organophosphorus pesticides (OPPs), pyrethroid pes-ticides and other pesticides. SBSE-TD-RTL-GC-MS analysis of fortified methanol-water samples (1:9) (5 ng/mL for all compounds) was performed. Twenty-milliliter samples were SBSE-enriched for 60 min. Experimental recovery was calculated

by comparing the peak areas with those of a direct analysis of a standard solution for calibration curves, which was spiked on quartz wool placed in an empty thermal desorption liner. 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 [12] for all analytes. Figure 2 shows the total ion chromatogram (TIC) of the fortified methanol sample obtained by SBSE-TD-RTL-GC-MS.

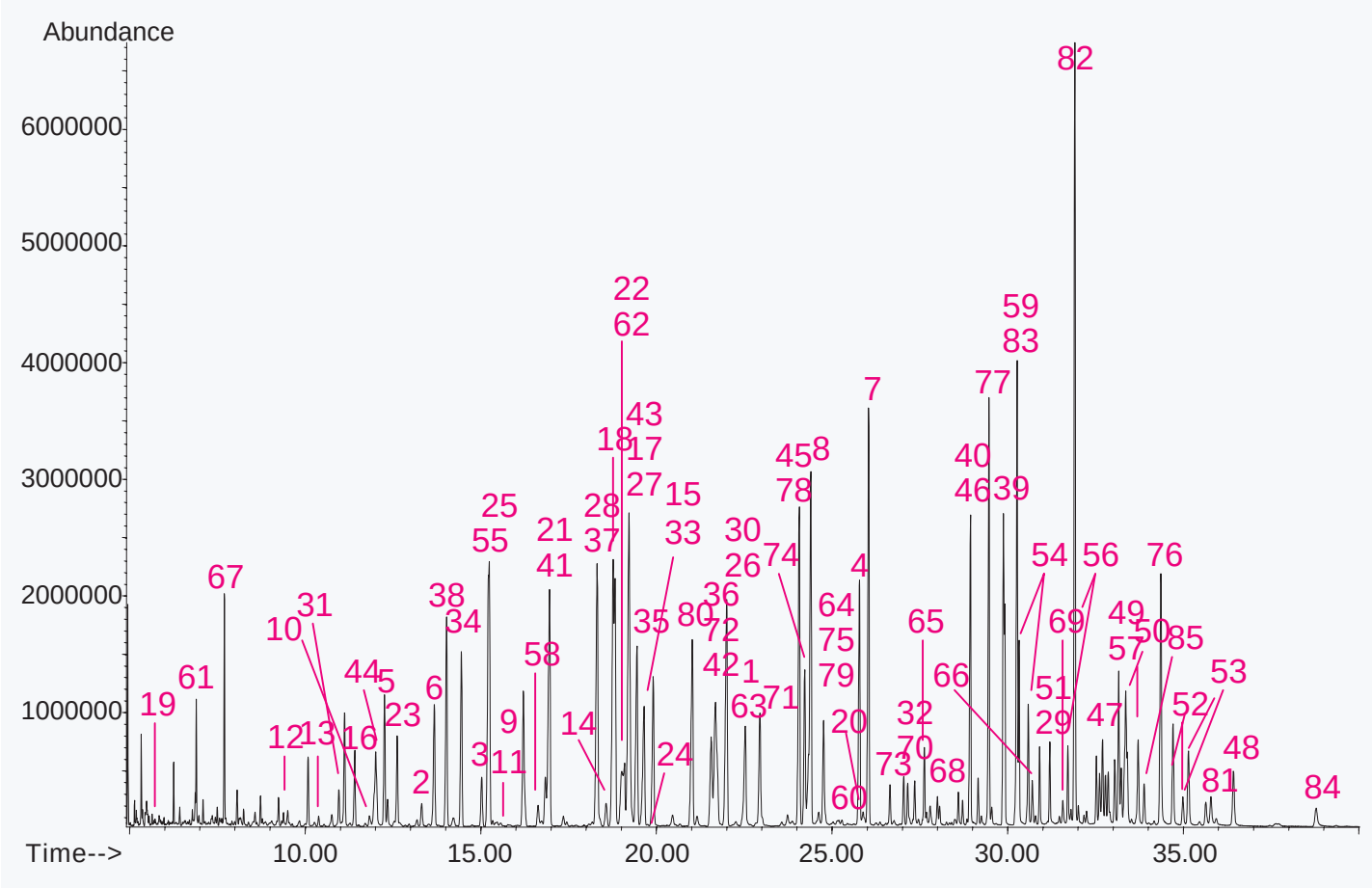

Figure 2: Total ion chromatogram obtained by SBSE-TD-RTL-GC-MS of a methanol-water sample (1:9) spiked with 85 pesticides at the 5.0 ng/mL level. Identification: see Table 1.

Eighty-five pesticides could be detected with a relative standard deviation (RSD) below 12 % (n = 6). The recovery was in the range of 0.74 % (pirimicarb; log $K_{o/w}$ = 1.70) to 75 % (flusilazole; log $K_{o/w}$ = 4.89). For fifteen pesticides, however, very high standard deviations (RSD > 20 %) were obtained or they could not be detected in the extract or in the direct analysis at all. These pesticides were: methamidophos (log $K_{o/w}$ = -0.92), acephate (log $K_{o/w}$ = -0.90), dimethipin (log $K_{o/w}$ = 0.66), tricyclazole (log $K_{o/w}$ = 1.40) and fosthiazate (log $K_{o/w}$ = 1.75), carbaryl (log $K_{o/w}$ = 2.35), acetamiprid (log $K_{o/w}$ = 2.55), dichlofluanid (log $K_{o/w}$ = 2.72), captan (log $K_{o/w}$ = 2.74), iprodion (log $K_{o/w}$ = 2.85), triadimenol (log $K_{o/w}$ = 2.95), lenacil (log $K_{o/w}$ = 3.09), pacrobutrazol (log $K_{o/w}$ = 3.36), captafol (log $K_{o/w}$ = 3.42) and dicofol (log $K_{o/w}$ = 4.28). These pesticides are either too polar (log $K_{o/w}$ < 1) or too thermolabile to be analyzed by SBSE-TD-GC-MS. The degradation of some pesticides during SBSE-enrichment and/or in the TD-PTV-GC system was already described before [8]. For these compounds SBSE followed by liquid desorption and LC-MS is recommended, as was illustrated with the analysis of iprodion in wine [22]. From the 100 test solutes, 85 compounds could thus be extracted and analyzed. Figure 3 shows a plot of the recovery obtained from the 85 pesticides as a function of their log $K_{o/w}$ Additionally, the equilibrium theoretical

line for the SBSE [1] of a 20-mL sample with a stir bar coated with 24 µL of PDMS is also drawn.

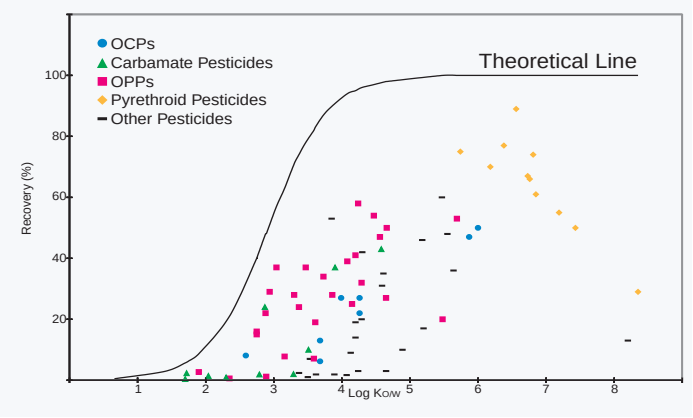

Figure 3: Theoretical and experimental recovery as function of log $K_{o/w}$ for 85 pesticides (see Table 1), obtained by SBSE-TD-RTL-GC-MS of a methanol-water sample (1:9) spiked at 5.0 ng/mL.

In general it is observed that the obtained recovery is lower than the theoretical value. For some solutes, such as the organophosphorous pesticides (OPPs), with log $K_{o/w}$ value between 3 and 4, the correspondence is quite good. For others, large deviations are observed. The difference between theoretical octanol-water distribution coefficients and practical PDMS-water distribution has already been mentioned in an earlier paper [1]. Moreover, the extraction time of 60 min is not long enough to reach full equilibrium, but a one hour extraction time was maintained for practical reasons. One important factor is however also the presence of 10 % methanol in the sample that influence the distribution between the aqueous phase and PDMS. Finally, it is also observed that the deviation is rather important for solutes with high log $K_{o/w}$ values (> 6), such as pyrethroid pesticides. Probably adsorption on the glass wall and matrix effects are most important for those solutes.

Nevertheless, the fact that 85 pesticides out of 100 can be extracted the methanol-water mixture, opens interesting possibilities. Even at relatively low recoveries, accurate quantification is possible using sorptive extraction techniques as was indicated by several authors [1, 13-15].

Importance of methanol-water dilution factor

The percent level of organic solvent, e.g. methanol, in aqueous sample used for SBSE enrichment can both have a negative and a positive effect on the recovery of solutes. For the compounds with low log $K_{o/w}$ (< 3.0), methanol can dramatically reduce partitioning coefficients between PDMS phase and aqueous sample [16]. For the compounds with high log $K_{o/w}$ (> 6.0), the methanol can minimize adsorption of the compounds to the glass wall of the extraction vessel [13] and also to the sample matrix [17], resulting in increased re-covery. In addition, polarity of the solvent mixture (in this case water:methanol) can also change the absolute and relative amount of sample matrix compounds that are co-extracted by SBSE.

To evaluate the effect of the dilution factor, a fortified methanol extract of spinach sample (50 ng/mL 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 (14 mL methanol + 6 mL water) to 20 (1 mL methanol + 19 mL water). A 60-min extraction was performed. Figure 4 shows the results of representative pesticides with various log $K_{o/w}$ values (2.35-7.43). Relative peak areas for each compound were normalized to the maximum peak area. For the pesticides with low log $K_{o/w}$, e.g. fensulfothion (log $K_{o/w}$ = 2.35), fenobucarb (log $K_{o/w}$ = 2.79) and metolachlor (log $K_{o/w}$ = 3.24), the highest response was obtained at dilution factor 10 (corresponding to 10 % methanol). The response decreased when the factor decreased from 10 to 1.7. Obviously, this is due to the decrease of the partitioning coefficients with increasing amounts of methanol.

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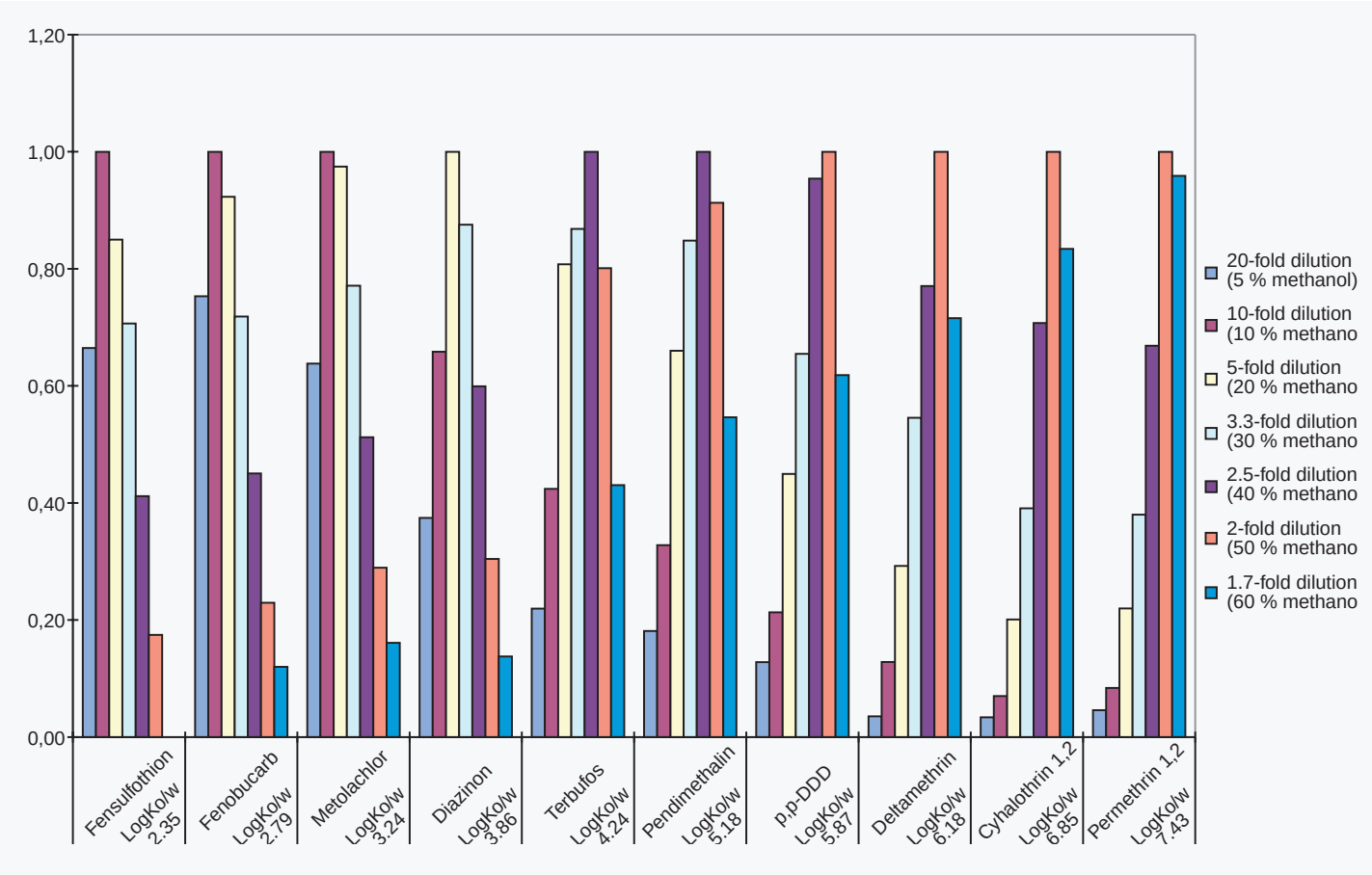

Figure 4: Comparison of the total ion chromatogram (TIC) obtained for extraction of a methanol extract of a spinach sample fortified at 50 ng/mL level (corresponding to 200 µg/kg of sample). The dilution factor was varied from 1.7 (60 % methanol) to 20 (5 % methanol). Relative peak area was normalized to the maximum peak obtained for each compound.

For the pesticides with medium log $K_{o/w}$, e.g. diazinon (log $K_{o/w}$ = 3.86), terbufos (log $K_{o/w}$ = 4.24) and pendimethalin (log $K_{o/w}$ = 5.18), the response increased when the dilution factor decreased from 20 to 5.0 (optimum for diazinon) or 2.5 (optimum for terbufos and pendimethalin). A further decrease in dilution factor (higher relative methanol concentration), leads again to reduced recovery. For the pesticides with high log $K_{o/w}$, e.g. p,p-DDD (log $K_{o/w}$ = 5.87), deltamethrin (log $K_{o/w}$ = 6.18), cyhalothrin (log $K_{o/w}$ = 6.85) and permethrin (log $K_{o/w}$ = 7.43), poor extractive behavior was observed at a dilution factor higher than 5.0. This is mainly due to adsorption of the solutes to the glass wall of the extraction vessel as well as the sample matrix. The highest response was obtained at the factor 2.0 (corresponding to 50 % methanol). According to these results, the dilution factor should be matched to the log $K_{o/w}$ of the analytes. This is however not possible in multi-residue analysis. For the multi-residue analysis of the 85 pesticides, a dual extraction was therefore selected as the optimum method. One extraction was performed on a twofold dilution extract (mainly targeting solutes with high log $K_{o/w}$) and one extraction was performed on a fivefold dilution extract (targeting solutes with low and medium log $_{Ko/w}$). The extraction can be performed simultaneously without increasing overall analysis time. Moreover, the thermal desorp-tion system employed in this study can simultaneously perform thermal desorption of two stir bars in a single glass insert.

Figure 5 shows a comparison of the total ion chromatograms (TIC) obtained for the fortified methanol extract of the spinach sample at 50 ng/mL (corresponding to approximate level of 200 µg/kg of sample) after respectively twofold (A) and fivefold (B) dilution. The chromatograms are compared to the combined desorption and analysis of two stir bars used in respectively twofold and fivefold diluted sample (C).

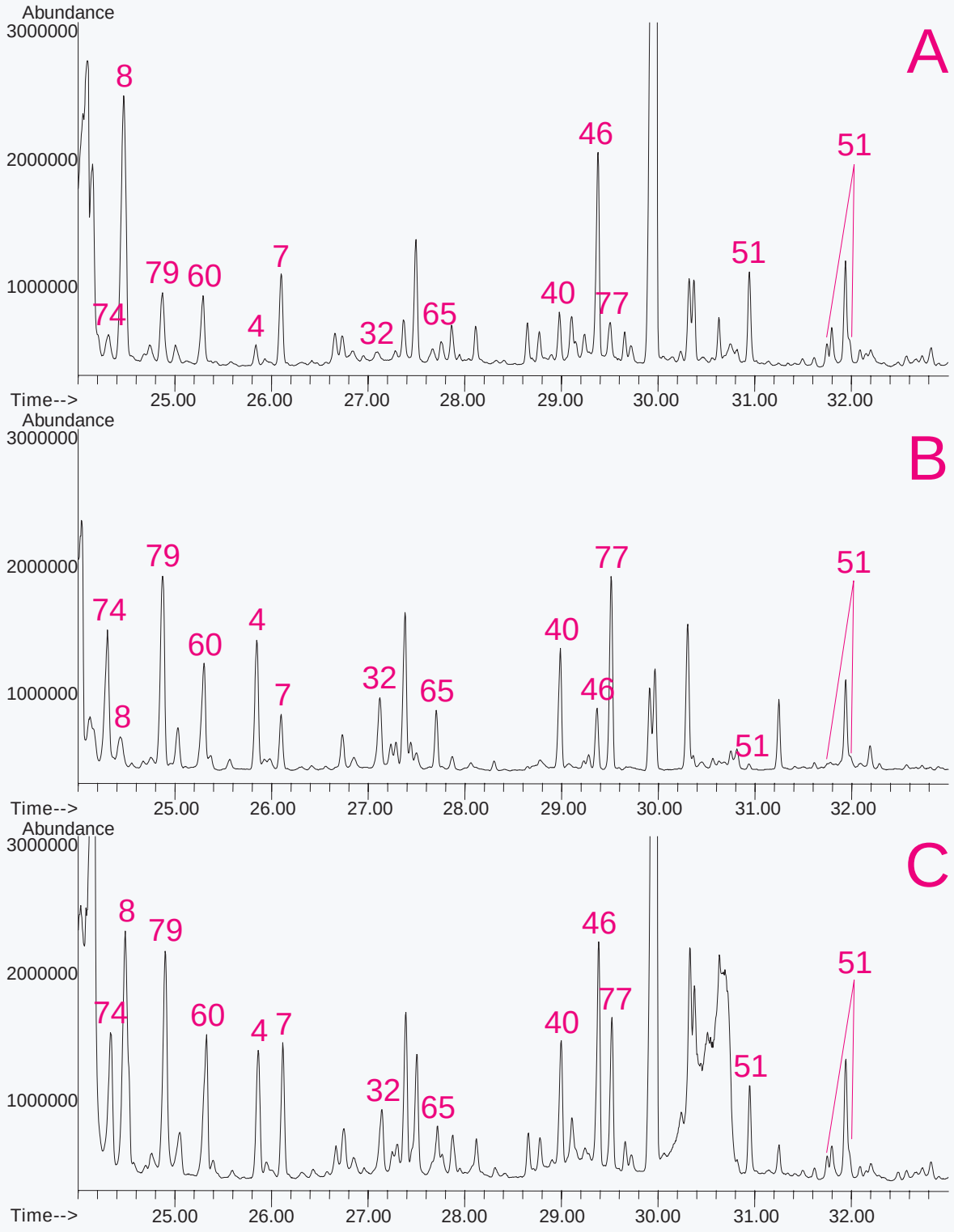

Figure 5: Comparison of the total ion chromatogram (TIC) obtained for extraction of a methanol extract of a spinach sample spiked at 50 ng/mL (corresponding to 200 µg/kg) using: A: twofold dilution (single stir bar); B: fivefold dilution (single stir bar); C: combined twofold and fivefold dilution (simultaneous analysis of two stir bars).

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Method Validation and determination of pesticides in real samples

As previous studies indicated, the effect of the sample matrix in SBSE could be compensated by use of a standard addition calibration method, a matrix matched calibration method or (isotope labeled) internal standard method [8, 18-20]. In this study, the standard addition method and the matrix matched cal-ibration method were used. To validate the method, a fortified methanol extract of blank spinach samples having seven concentration levels approximately 0.80 to 25 ng/mL, corresponding to concentration between 4.0 to 100 µg/kg. For each level, a dual SBSE enrichment was performed after respectively twofold and five fold dilutions. The two stir bars corresponding to the same sample (spiked level) were simultaneously analyzed by TD-RTL-GC-MS in the scan mode. For 66 compounds, good linearity of the seven-points of matrix matched calibration curves was achieved with correlation coefficient (r^2) above 0.9900. For 19 compounds, the r^2 were in the range of 0.9574-0.9885. The limit of detection (LOD) was calculated as 3.36 times the standard deviation obtained for six replicate analyses of the lowest-level sample. The LOD was calculated to be 0.63-26 µg/kg for the different pesticides. Linearity data and LOD values for the individual target compounds are listed in Table 1.

Finally, the method was applied to several tomato, cucumber, green soybean, spinach, grape and green tea samples obtained from different markets. Determination of the pesticides in the samples was carried out by a seven-point level matrix matched calibration or a five-point level standard addition calibration using fortified methanol extracts. Figure 6 shows typical chromatograms of a green tea samples. Figure 7 shows a comparison of the mass chromatograms (m/z 163) obtained for extraction of a methanol extract of spinach sample using A: fivefold dilution (single SBSE); B: combined twofold and fivefold dilution (dual SBSE); C: mass spectrum of cypermethrin 3 obtained for B. Cypermethrin 1,2,3,4 was determined at 3.9 µg/kg. Table 2 shows the frequency of residue detection and concentration range of contaminated samples. Out of 25 samples analyzed, pesticide residues were detected in 12 (48 %), of which 1 (permethrin in spinach) was close to the maximum residue levels (MRLs) allowed in Japan [21] (2.0 mg/kg).

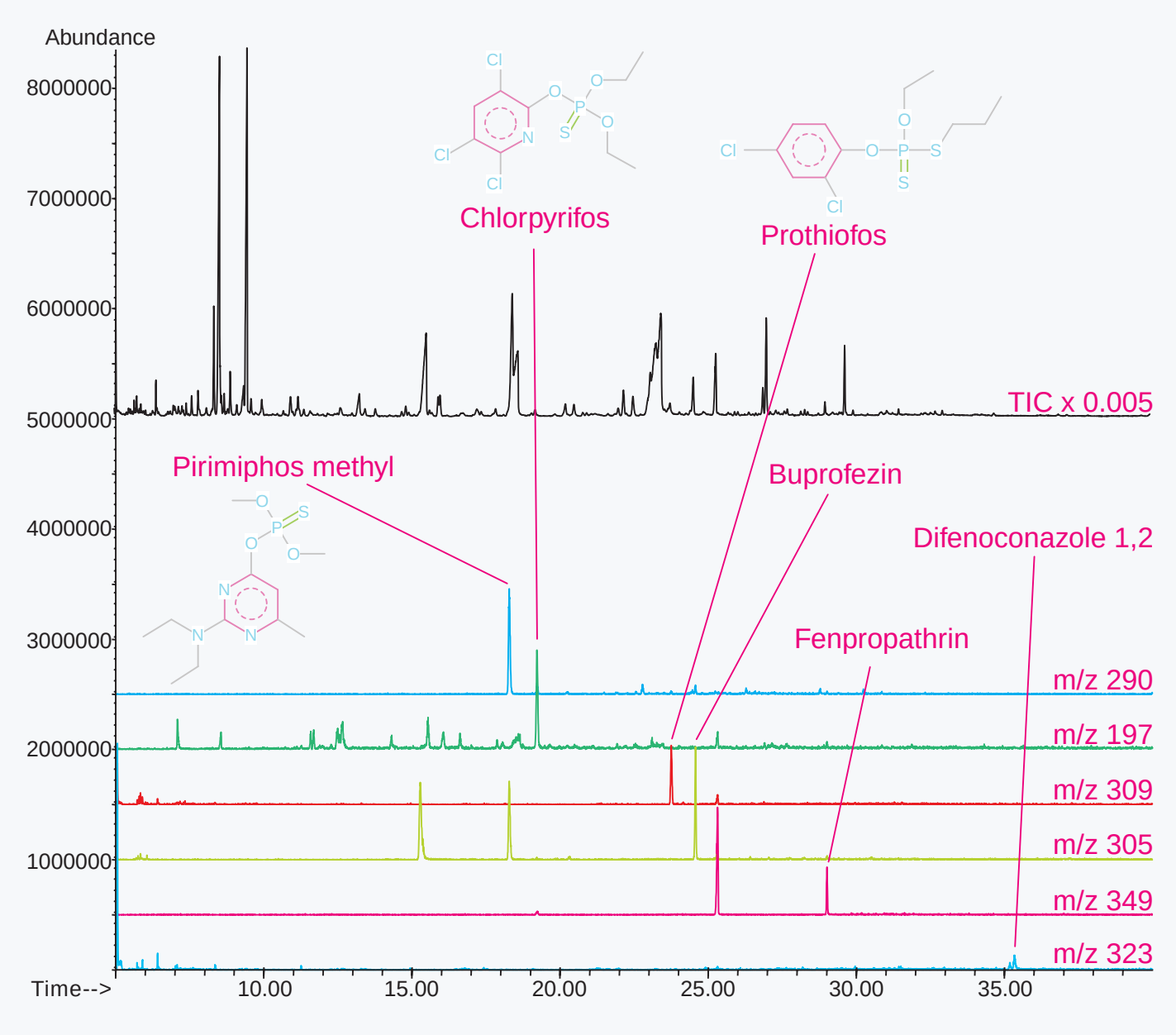

Figure 6: Total ion chromatogram and mass chromatogram obtained by Dual SBSE-TD-RTL-GC-MS of green tea sample.

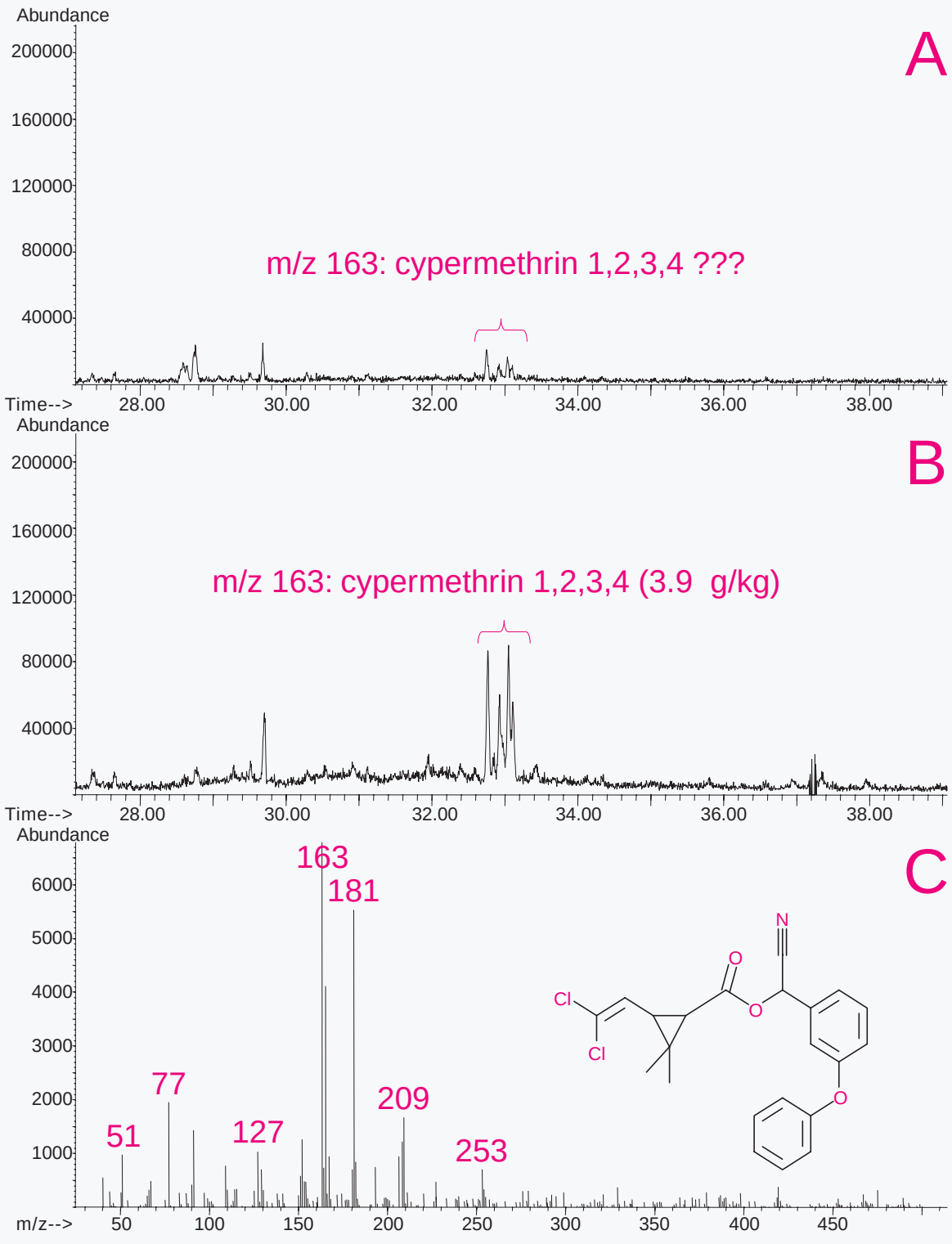

Figure 7: Comparison of mass chromatogram (m/z 163) obtained for extraction of a methanol extract of a spinach sample using: A: twofold dilution (single stir bar); B: combined twofold and fivefold dilution (simultaneous analysis of two stir bars); C: mass spectrum of cypermethrin 3 obtained for B; cypermethrin 1,2,3,4 in B was determined at 3.9 µg/kg (sum of the individual isomers) by use of matrix matched calibration method.

Conclusion

A multi-residue method for determination of 85 commonly used pesticides in vegetables, fruits and green tea is described. A dual stir bar sorptive extraction is performed on respectively a twofold and fivefold aqueous dilution of the methanol extract. Subsequently, the stir bars are simultaneously thermally desorbed and the enriched compounds are analyzed by retention time locked GC-MS in the scan mode. By using the dual extraction of respectively a twofold and fivefold aqueous dilution, a wide range of solutes with different octanol-water partitioning coefficients can be extracted and enriched, while matrix effects and adsorption on the glass wall of the extraction vessel are minimized. The method allows determination of µg/kg levels of pesticide residues in vegetables, fruit and green tea.

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