Spinosyn A (m.w. 732.0), R=H
Spinosyn D (m.w. 746.0), R=CH3

Figure 1. Structures of spinosyn A and spinosyn D.

to obtain adequate sensitivity for the analytes. However, significant interfering peaks were still observed in the chromatograms of the purified extracts from green vegetables, cabbage, and especially green perrila. Moreover, because the presence of an excess amount of lipids and pigments like chlorophyll and carotenoid in the crude extracts decreased the affinity of the analytes for CH and silica, considerable quantities of spinosyns A and D were discharged during the cartridge-washing procedures.

Therefore, the study described in this paper focused on the development of a simple and reliable sample cleanup procedure for the determination of spinosad residues in vegetables and fruits by HPLC-UV with MS confirmation (Figure 2). Our improved routine method extends the current conventional multiresidue methods (6, 11–13) by incorporating spinosad into the regulatory monitoring program.

Experimental

Reagents and Materials

(a) *Solvents and chemicals.*—Acetonitrile, sodium chloride, ethyl acetate, anhydrous sodium sulfate, acetone, and cyclohexane were pesticide-analytical grade (Wako Pure Chemical, Osaka, Japan). Water was purified by using an Auto Pure WT100 system (Yamato Scientific, Tokyo, Japan). Acetonitrile and water only for HPLC were LC/MS grade (Wako Pure Chemical), and all other reagents were analytical grade (Wako Pure Chemical). Rapeseed oil, chlorophyll oil, and β-carotene were purchased from Wako Pure Chemical.

(b) *Analytical standards.*—The pesticide standards for spinosyns A and D were purchased from Hayashi Pure Chemical (Osaka, Japan). Each standard was dissolved in methanol to obtain a stock solution of 1 mg/mL. Equal volumes of the stock solutions were mixed and diluted with acetonitrile to obtain the mixed spiking solutions and working standard solutions. The standard solutions were stored in amber vials at 4°C.

(c) *Graphitized carbon-CH 2-layered column.*—Graphitized carbon (Supelclean ENVI-carb II) and CH (Bondesil-CH) were purchased from Supelco (Bellefonte, PA) and Varian (Harbor City, CA), respectively.

Apparatus

(a) *Dispersing unit.*—Ultra-Turrax T-25 with a stainless dispersion tool S25N-10G (IKA Labortechnik, Staufen, Germany).

(b) *Suction filter system (Figure 3).*—A Kiriyama funnel (Cat. No. SU-60; Nippon Rikagaku Kikai, Tokyo, Japan) fitted with a filter paper (Cat. No. 5A-60; Nippon Rikagaku Kikai) was connected by a vacuum SPC joint (Cat. No. 3047-19; Sibata Scientific Technology, Tokyo, Japan) to a 125 mL graduated cylindrical separatory funnel (Cat. No. 3027-19100; Sibata Scientific Technology). A maximum of 12 units was supported by a wooden stand (available by special order from Iwahana Kamidana, Takayama, Japan).

(c) *Rotary vacuum evaporator.*—Rotavapor with vacuum controller V-800 (Büchi Labortechnik, Flawil, Switzerland).

(d) *GPC-SPE cleanup system.*—An automated GPC cleanup system (Shimadzu, Kyoto, Japan) equipped with a CLNpak EV-2000 column (300 × 20 mm id), a CLNpak EV-G guard column (100 × 20 mm id), and an AO-50 column oven (Showa Denko, Tokyo, Japan) were used for sample cleanup. Acetone–cyclohexane (3 + 7) was the mobile phase, and the flow rate was 5 mL/min. Column temperature was maintained at 40°C, and the UV detector was set at 245 nm. Injection volume was 2 mL. As shown in Figure 4, a glass reservoir (16 mL; GL Sciences, Tokyo, Japan) connected to a flow-control tube (60 mm; Supelco) was used as the column. Glass wool (5–8 μm; Nippon Rikagaku Kidai) was placed at the bottom of the column as a filter, and 1 g CH, 0.5 g graphitized carbon, and 0.5 g anhydrous sodium sulfate were placed in series on the filter. This graphitized carbon-CH 2-layered column supported on the 50 mL tall beaker by a rack (available on special order from Iwahana Kamidana) was preconditioned with 10 mL cyclohexane followed by 10 mL acetone before use. A maximum of 16 columns was mounted in the GPC fraction collector.
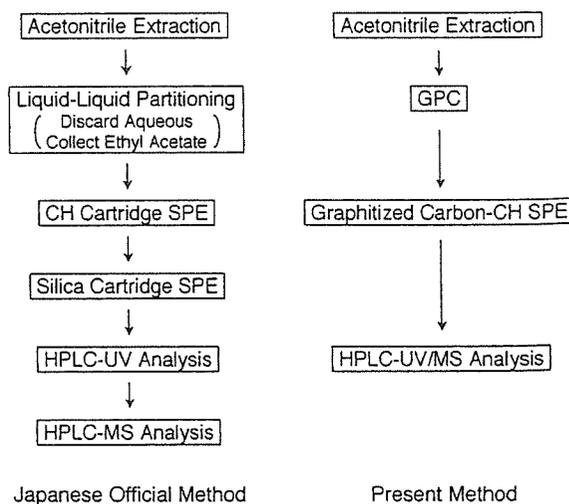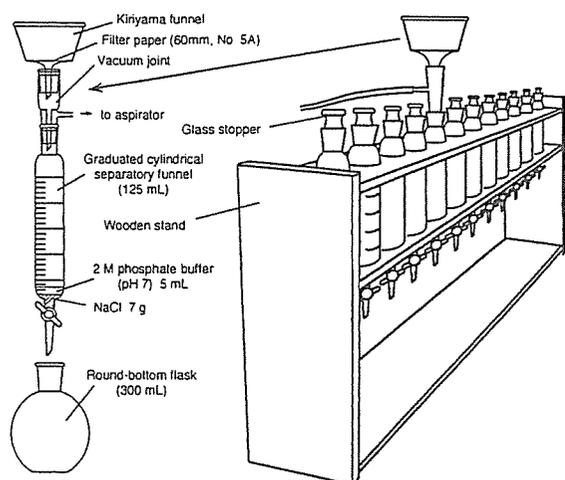

Japanese Official Method   Present Method

Figure 2. Flow charts of the Japanese official method and the present method for the determination of spinosad in vegetables and fruits.
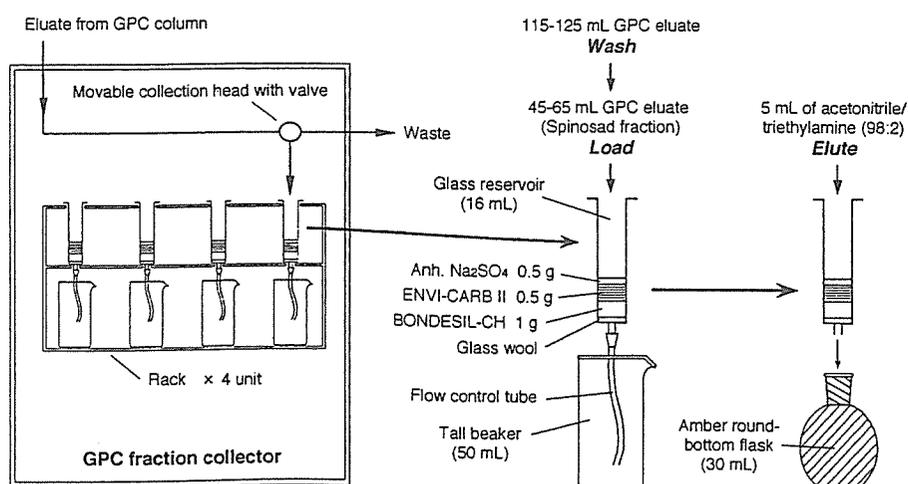
1.5 L/min; drying nitrogen gas flow rate, 10 L/min; heat block temperature, 200°C; curved desolvation line (CDL) temperature, 250°C; CDL voltage, 25 V; Q-array DC voltage, 55 V; Q-array RF voltage, 150 V. The chromatograms were recorded under full-scan (100–850 *m/z* at 0.75 scans/s) conditions. The data were processed with Shimadzu LCMS solution Ver.3.3 analysis software.

Sample Preparation

The vegetables and fruits were obtained from retail markets in Aichi Prefecture. Pesticide-free vegetables and fruits were used for the recovery test. About 500 g roughly chopped sample was chopped in a conventional food processor for 2 min to obtain a thoroughly mixed homogenate.

Extraction

A 20 g portion of the homogenate was weighed into a 250 mL centrifuge tube and extracted with 60 mL acetonitrile for 2 min by using a dispersing unit. The extract was centrifuged for 5 min at 3000 rpm, and the supernatant was filtered with a suction filter system (Figure 3) into a 125 mL graduated cylindrical separatory funnel containing 7 g NaCl and 5 mL 2 M phosphate buffer (pH 7). The contents of the centrifuge tube were again extracted with an additional 20 mL acetonitrile, and the extract was filtered into the same graduated cylindrical separatory funnel. The filtrate was shaken with a mechanical shaker for 5 min to salt out the water layer. The acetonitrile extract in a 300 mL round-bottom flask was evaporated to near dryness with a rotary vacuum evaporator. A 50 mL portion of ethyl acetate and 20 g anhydrous sodium sulfate were added to the residue, and the contents of the flask were sonicated for 1 min. The resultant mixture was filtered through glass wool placed above the narrow neck of a funnel into a 200 mL round-bottom flask. The 300 mL round-bottom flask was rinsed twice with 20 mL ethyl acetate, and the rinses were filtered through the glass wool. The filtrate was evaporated to near dryness with a rotary

Figure 3. Suction filter system using a graduated cylindrical separatory funnel.

(e) *HPLC-UV/MS system.*—A Shimadzu LCMS-2010A instrument equipped with a binary LC-10ADvp pump, an SIL-HTA autosampler, a CTO-10ACvp column oven, and an SPD-M10Avp photodiode-array detector was used for the HPLC-UV/MS analysis. The HPLC column was a CAPCELL PAK C18 MG II (100 × 2.0 mm id, 3 μm particle size) with a guard column (10 × 2.0 mm id, 3 μm particle size; Shiseido, Tokyo, Japan). The mobile phase was 10 mM ammonium acetate–acetonitrile (25 + 75), isocratic elution was used, and the flow rate was 0.25 mL/min. The column temperature was maintained at 40°C, and the detection wavelength was set at 245 nm. The single-quadrupole mass spectrometer was optimized by the instrument software in the electrospray positive ionization mode. Typical operating conditions were capillary voltage, 4.5 kV; nebulizer nitrogen gas flow rate,

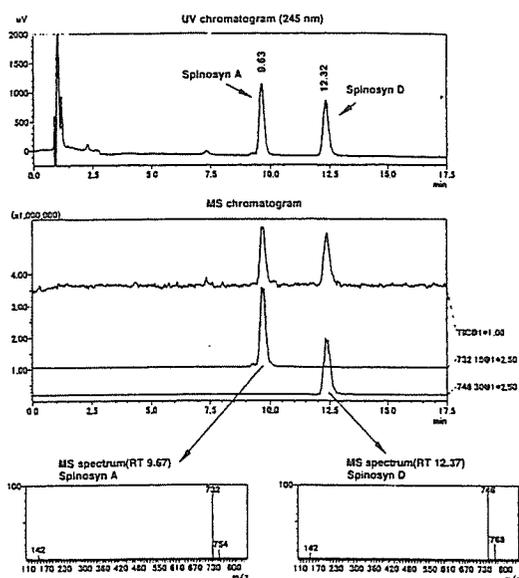**Figure 4. Graphitized carbon-CH 2-layered column cleanup after GPC.**

Figure 5. UV chromatogram, MS chromatograms, and MS spectra obtained for the standard mixture of spinosyn A and spinosyn D (each at 0.25 mg/mL).

vacuum evaporator. The residue was dissolved in acetone–cyclohexane (3 + 7), the volume was adjusted to 8 mL, and the mixture was centrifuged for 5 min at 3000 rpm. The supernatant was ready for cleanup.

Cleanup

A 2 mL aliquot of the extract (the supernatant from the previous step), equivalent to 5 g sample, was loaded into the GPC system. As shown in Figure 4, the first 45 mL eluate was discarded and the next 45–65 mL eluate (spinosad fraction) was directly loaded onto the 2-layered column. After an additional 65–115 mL eluate was discarded, the 2-layered column was automatically washed with the next 115–125 mL eluate. These automatic operations were conducted at 25 min intervals in the dark GPC fraction collector. After the 50 mL

tall beaker was exchanged for a 30 mL amber round-bottc flask and the flow-control tube was removed, the 2-layer column was eluted with 5 mL acetonitrile–triethylami (98 + 2). The eluate was evaporated to near dryness by usin₤ rotary vacuum evaporator, and evaporation was complet under a gentle stream of nitrogen. The residue was dissolv in acetonitrile–water (75 + 25), and the volume was adjust to 1 mL in an amber SPE concentration tube (GL Science The 1 mL test solution corresponded to 5 g sample.

HPLC-UV/MS Analysis

A 10 µL aliquot of the test solution was analyzed HPLC-UV/MS by using the parameters described in t Apparatus section. The UV chromatogram was used quantitation, which was based on the external standard meth₍ A minimum of 5 points was used for the calibration graph (p₍ area versus concentration) constructed for each analyte. I sum of both analyte concentrations was assumed to ₁ analytical value for spinosad. The MS chromatograms and ₦ spectra were used to confirm the identities of the analytes.

Recovery Test

The recovery test was conducted 5 times for each sample cabbage, green perilla, fig, and strawberry. Spinosad registered for cabbage, fig, and strawberry in Japan and ₁ been provisionally registered for green perilla in Ai Prefecture. A 0.5 mL aliquot of the standard solution of e₍ spinosyn at 2 or 10 µg/mL was added to 20 g chopped sam₱ and the sample was left for 60 min at room temperature bef extraction.

Extraction and Cleanup Studies

The rates of extraction of the analytes from water, elution profiles of the analytes, and representative matri (rapeseed oil, chlorophyll, and β-carotene) in the GPC and ₍ column cleanup procedures were investigated to finc suitable method for sample preparation. These tests w conducted 3 times through each procedure.

To determine the extraction rate from water, 0.5 spinosad solution containing spinosyns A and D, each

Table 1. Extraction of spinosyn A and spinosyn D from water, and elution of spinosyn A, spinosyn D, and representative matrixes from GPC and the CH column

| Compound | Extraction from water | GPC elution volume, mL | | | | | CH column elution | |
		30	35	45	55	65	Fraction 1	Fracti₍
Rapeseed oil		17	73	6			95	C
Chlorophyll			47	43	5	1	98	C
β-Carotene				88	7	2	99	(
Spinosyn A	94			65	32	4	0	9⁻
Spinosyn D	97			68	31	3	0	9₁

[a] Mean of 3 replicates.

Table 2. Elution of spinosyn A and spinosyn D in the extract of green perilla from a graphitized carbon-CH 2-layered column

Graphitized carbon, mg[a]	Compound	Recovery, %[b]		
		45–65 mL GPC eluate (load)	115–125 mL GPC eluate (wash)	5 mL acetonitrile–triethylamine (98 + 2; elute)
0	Spinosyn A	81	16	1
	Spinosyn D	85	14	1
	Chlorophyll	+++[c]	+	
	Carotenoid	+++	+	
100	Spinosyn A	49	33	16
	Spinosyn D	48	30	18
	Chlorophyll	+	++	+
	Carotenoid	++	++	
200	Spinosyn A	0	12	84
	Spinosyn D	0	11	86
	Chlorophyll			
	Carotenoid		++	+
300	Spinosyn A	0	0	101
	Spinosyn D	0	0	99
	Chlorophyll			
	Carotenoid		+	+
400	Spinosyn A	0	0	97
	Spinosyn D	0	0	95
	Chlorophyll			
	Carotenoid			
500	Spinosyn A	0	0	98
	Spinosyn D	0	0	102
	Chlorophyll			
	Carotenoid			

[a] Quantity of CH was 1000 mg in each case.

[b] Mean of 3 replicates.

[c] Recoveries shown as +++, ++, and + were evaluated by observation.

10 µg/mL, was added to 20 mL water in a separatory funnel. The water was thoroughly mixed with 80 mL acetonitrile, and the solution was transferred to a 125 mL graduated cylindrical separatory funnel containing 7 g NaCl and 5 mL 2 M phosphate buffer (pH 7). The mixture was shaken to salt out the water layer. The acetonitrile layer was evaporated to near dryness with a rotary vacuum evaporator, and evaporation was completed under a gentle stream of nitrogen. The residue was dissolved in ≤4 mL acetonitrile–water (75 + 25). Each compound was determined by HPLC-UV.

To optimize the GPC elution profile, 2 mL individual solution (spinosyns A and D, each at 50 µg/mL and rapeseed oil, chlorophyll, and β-carotene, each at 1000 µg/mL) was loaded into the GPC system. Each compound was determined by GPC with UV detection (GPC-UV).

To determine the elution profile in the CH column, 20 mL individual solution (spinosyns A and D, each at 10 µg/mL, and rapeseed oil, chlorophyll, and β-carotene, each at 200 µg/mL) was loaded onto the column packed with 1 g CH, which was then eluted with 10 mL acetone–cyclohexane (3 + 7); Fraction 1): The column was again eluted with 5 mL acetonitrile–triethylamine (98 + 2; Fraction 2). Each fraction was evaporated separately to near dryness with a rotary vacuum evaporator and dissolved in ≤4 mL acetone–cyclohexane (3 + 7). A 2 mL aliquot of the resulting solution was loaded into the GPC system. Each compound was determined by GPC-UV.

Results and Discussion

HPLC-UV/MS Conditions

Because spinosyns A and D are relatively poor polar compounds under neutral conditions (1), a reversed-phase

Table 3. Recovery of spinosyn A and spinosyn D from cabbage, green perilla, fig, and strawberry

Compound	Spiking level, µg/g	Cabbage		Green perilla		Fig		Strawberry	
		Avg. rec., %[a]	RSD, %	Avg. rec., %	RSD, %	Avg. rec., %	RSD, %	Avg. rec., %	RSD, %
Spinosyn A	0.05	88	7	92	8	86	8	92	8
	0.25	92	3	94	3	92	4	96	3
Spinosyn D	0.05	85	8	89	9	91	6	91	5
	0.25	93	4	93	5	92	3	95	4

[a] $n = 5$.

mobile phase, 10 mM ammonium acetate–acetonitrile (25 + 75), and a binary pumping system were used for routine HPLC analysis. Several mobile phase (isocratic and gradient elution of 10 mM ammonium acetate–acetonitrile from [10 + 90, v/v] to [95 + 5, v/v]) and stationary phase conditions were evaluated to obtain suitable conditions and analysis times for the separation of the analytes and other unidentified compounds in vegetables and fruits. Satisfactory results were obtained by using a CAPCELL PAK C18 MG II column with a mobile phase of 10 mM ammonium acetate–acetonitrile (25 + 75) with isocratic elution. By using the isocratic system, less fluctuation in the baseline of the UV chromatogram and a short analytical time were obtained. Spinosyns A and D were detected in their UV spectra at 245 nm, with retention times of 9.63 and 12.32 min, respectively (Figure 5). The calibration plots were linear in the concentration range of 0.05–50 µg/mL (correlation coefficient, 0.999). Furthermore, the peaks corresponding to spinosyns A and D were confirmed with high sensitivity and selectivity by their MS chromatograms and MS spectra in which characteristic molecular ions were observed at m/z 732.2 for spinosyn A and at m/z 746.2 for spinosyn D.

Extraction

Because spinosyns A and D are easily dissolved in acetonitrile under neutral conditions (1), we applied our simple and reliable multiresidue extraction method (6, 12, 13), which uses acetonitrile extraction and salting-out by the addition of NaCl in phosphate buffer (pH 7). As a result, spinosyns A and D were sufficiently partitioned (≥94%) into the acetonitrile layer (Table 1). A suction filter system (Figure 3) was devised to provide a more simple and rapid extraction. With the suction filter system, the extraction time for a large number of samples was shortened, compared with that of the traditional extraction system that uses an open top and side tabulation bell jar.

Cleanup

To develop a reliable autocleanup system before HPLC determination, GPC and a laboratory-made graphitized carbon-CH 2-layered SPE column were combined. GPC allows the compounds in the sample to be separated by a molecular size-exclusion mechanism (6, 12–15), CH is a medium-polarity sorbent that exhibits unique selectivities for specific
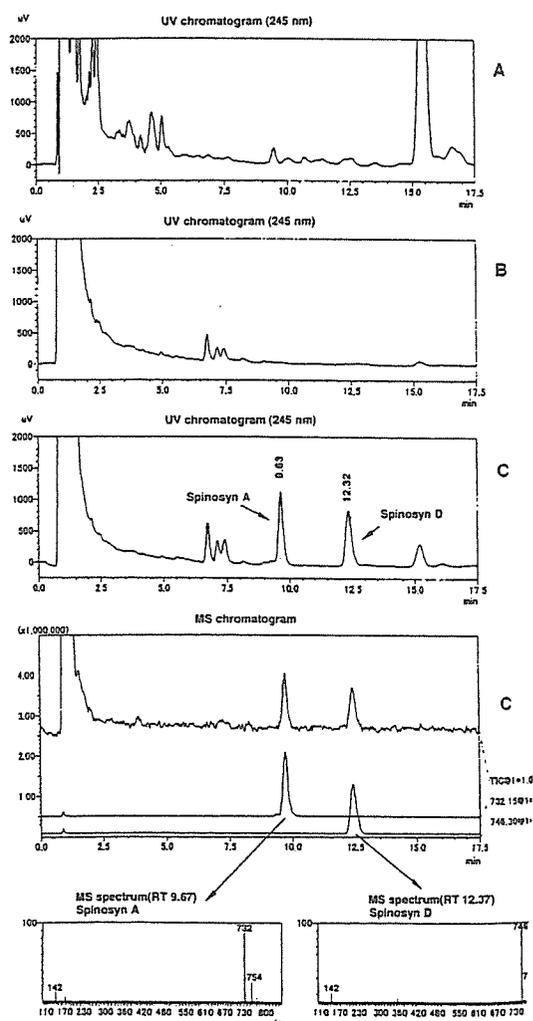

Figure 6. UV chromatograms, MS chromatograms and MS spectra of extracts of a green perilla: (A) bl; extract analyzed by the Japanese official method; (B) blank extract analyzed by the present method; (C) extract of sample spiked with spinosyn A and spinosyn D (each 0.05 µg/g) and analyzed by the present method.
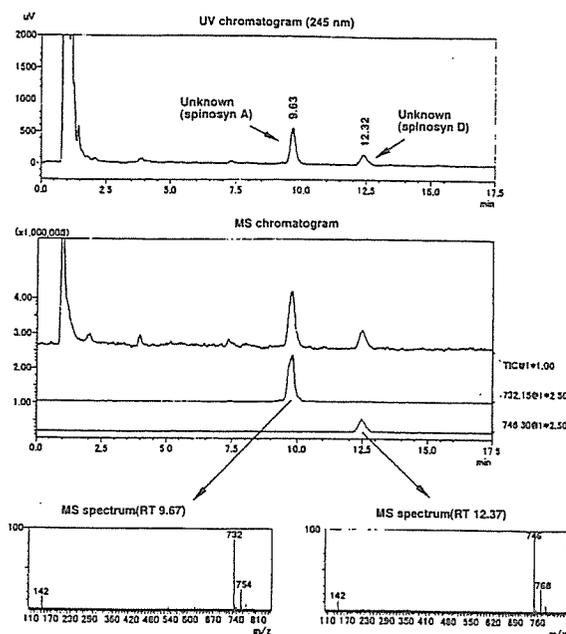
Figure 7. UV chromatogram, MS chromatograms, and MS spectra of a cabbage extract.

compounds (9, 10), and C18 for the HPLC separation is a poor polar sorbent. Therefore, we speculated that the GPC cleanup would be most selective when the analytes were compounds of a specific molecular size or a single compound such as spinosad; moreover, the selective GPC based on a separation mechanism different from that of the C18 separation would be effective for removing interferences from a sample before the HPLC determination. The elution profiles of spinosyns A and D from the GPC column are shown in Table 1. The mean elution times of spinosyns A and D were 10.6 ± 0.1 min (53 ± 0.5 mL) and 10.4 ± 0.1 min (52 ± 0.5 mL), respectively, and the relative standard deviation (RSD) was within 1%. A hard type gel CLNpak EV column restrains swelling during the change in solvents, and by using the special column oven, the column maintained at 40°C provides good reproducible elution times for the loaded compounds.

The spinosyns A and D eluted from the GPC column were directly retained on the CH sorbent when the GPC mobile phase was acetone–cyclohexane (3 + 7). Accordingly, spinosyns A and D loaded with 20 mL acetone–cyclohexane (3 + 7) were completely retained with an additional 10 mL acetone–cyclohexane (3 + 7; Fraction 1), and they were eluted with 5 mL acetonitrile–triethylamine (98 + 2; Fraction 2; Table 1). Rapeseed oil, chlorophyll, and β-carotene were eluted in Fraction 1 (Table 1); polarity matrixes and fatty acids such as stearic acid, palmitic acid, etc., were also eluted in Fraction 1 (data not shown). However, a significant part of the spinosad fraction (45–65 mL eluate) and the fraction containing chlorophyll and β-carotene still overlapped each other in the GPC elution (Table 1). Because the presence of an excess amount of pigments in the extracts of vegetables and

fruits, especially green vegetables, decreased the affinity of the analytes for the CH sorbent as mentioned above, a considerable amount of analytes may be discarded with the pigments during the SPE loading and washing procedure. Accordingly, because a 2-layered column with graphitized carbon placed on CH had played a significant role in retaining the pigments (6), we applied it to the determination of spinosad in this study. The elution profile of spinosyns A and D in the extract of green perilla, which contained 1.25 µg each of spinosyns A and D in 2 mL extract, equivalent to 5 g sample, is shown in Table 2. Although spinosyns A and D were discarded with the pigments in the SPE loading and washing procedures in the absence of graphitized carbon, they were completely retained when the quantity of the graphitized carbon used was >300 mg. From this result, we concluded that the amount of graphitized carbon needed to remove an excess amount of pigments from green vegetables was 500 mg. Thus, a 2-layered column (Figure 4) consisting 0.5 g graphitized carbon and 1 g CH was used for cleanup. A glass reservoir provided sufficient space for the addition of the GPC eluate. Anhydrous sodium sulfate prevented the graphitized carbon from floating in the added GPC eluate. Filtration through glass wool and the use of a flow-control tube resulted in an appropriate elution rate (approximately 2 mL/min).

Additional cleanup with silica-SPE (7, 9, 10) was not needed, and the cleaned sample extracts were not filtered through a 0.20–0.45 µm filter because the solutions were highly purified and certain types of filters were found to adsorb the analytes (data not shown).

Recovery Test

The recoveries from the representative vegetables and fruits, i.e., cabbage, green perilla, fig, and strawberry, and the corresponding RSD values are listed in Table 3. Spinosyns A and D spiked at 2 levels were sufficiently recovered in the range of 85–96% with a 3–9% RSD. Spinosyns A and D are relatively photodegradable compounds (1). Photodegradation increased with increasing purity during the sample cleanup procedures (data not shown). Sample cleanup in a dark GPC system, with the use of amber glass tools, might be effective for avoiding possible photodegradation of the purified analytes.

Interfering peaks were still observed in the chromatograms of the cleaned extracts of green perilla, which contains not only high levels of pigments but also various matrix components, prepared by the Japanese official method (Figure 6A). In contrast, with the use of the present method, these interfering components were effectively removed with minimal loss of analytes (Figure 6B and C).

The detection limits for spinosyns A and D in samples, which were estimated by Shimadzu LCMS solution analysis software as the lowest concentration of the analytes detectable with an RSD of 33% (= 3σ), based on the noise level in the chromatograms of the cleaned blank extract of green perilla and the respective standard peaks, were sufficient (0.005 µg/g by UV, 0.001 µg/g by MS) for monitoring spinosad residues in vegetables and fruits.

Table 4. Vegetables and fruits examined and the MRLs for spinosad

Sample	No. found/analyzed	Residue found, µg/g	MRL, µg/g[a] Before final draft	Final draft[b]
Apple	0/2		0.5	0.5
Asparagus	0/1		—	5
Broccoli	0/1		2	2
Cabbage	2/6	0.03, 0.02	2	2
Cabbage, Chinese	0/2		8	8
Iyo orange	0/1		0.3	0.3
Cucumber	0/2		0.5	0.5
Edible chrysanthemum	0/1		8	8
Eggplant	0/2		2	2
Fig	1/2	0.02	0.3	0.3
Japanese radish (leaf)	0/1		1	1
Japanese radish (root)	0/1		0.2	0.2
Lettuce	0/3		8	8
Melon	0/1		0.3	0.3
Qing gin cai	0/2		2	2
Sunny lettuce	0/4		8	8
Green perilla	0/3		—	10
Peach	0/1		0.2	0.2
Pimientos	0/1		2	2
Potherb mustard	0/2		5	5
Shallot	0/1		—	5
Spring onion	0/2		—	5
Strawberry	0/5		—	1
Tomato	0/3		0.5	0.5
Watermelon	0/1		0.3	0.3
Total	3/51			

[a] MRL = Maximum residue limit.
[b] Final draft of the Japanese positive list system.

Application to Commercial Samples

In 2004, the applicability of this method to routine analyses was tested with 51 commercial vegetable and fruit samples. All the commercial samples were successfully analyzed for spinosad without any seriously interfering peaks. Figure 7 shows the representative UV chromatogram, MS chromatograms, and MS spectra obtained from analysis of a cabbage sample in which spinosad was detected at a concentration of 0.02 µg/g. Details are listed in Table 4. Although spinosad was detected at concentrations of 0.02–0.03 µg/g in 2 cabbage samples and 1 fig sample, these residue levels were lower than the maximum residue limits (MRLs) of the final draft of the Japanese positive list system, which became effective on May 29, 2006.

Therefore, the newly developed method has an advant for such routine residue analyses. Additionally, ab 10 extracts prepared for the multiresidue analysis can processed within 1 working day (8 h).

Conclusions

An HPLC-UV/MS method was developed for determination and confirmation of spinosad in vegetables fruits. An autocleanup system combining GPC and graphitized carbon-CH 2-layered column SPE make possible to easily and effectively remove sample matri with minimal loss of analytes. The recovery and commer sample data show that this newly developed residue meth which uses a GPC-SPE cleanup system, is a simple, rapid, reliable tool for monitoring spinosad in vegetables and frui

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ノート

HPLC による食品中メトプレンの分析法

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Analytical Method of Methoprene in Foods Using HPLC

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We studied the determination of methoprene in foods by high-performance liquid chromatography (HPLC). The sample was extracted with acetonitrile and the extract was salted out by adding sodium chloride, allowing the acetonitrile layer to separate. The acetonitrile solution was washed with hexane saturated with acetonitrile, cleaned up on a Florisil column and determined by HPLC. The recovery of methoprene from spiked samples was 74.6–82.8%. In an evaluation of this method by 6 analytical laboratories, mean recoveries from spiked samples ranged from 79.4% to 84.6%. Repeatability relative standard deviation values were 2.3–8.8% and reproducibility relative standard deviation values were 8.8–23.6%. The detection limits were 0.001–0.02 μg/g and below the detection limit of the Notified Analytical Method.

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緒　　言

メトプレン Methoprene (Fig. 1) は，幼若ホルモン Juvenile Hormone (JH) の研究から開発された昆虫成長制御剤 (IGR) である．日本では農薬として登録されていないが，蚊，ハエの幼虫駆除を目的に衛生害虫駆除用として使用されている[1]．食品衛生法の残留基準は，玄米，小麦，大麦，ライ麦，とうもろこし，そばなどの穀類に 5.0 ppm，らっかせいに 2.0 ppm，マッシュルームに 0.2 ppm が設定されている．厚生労働省よりメトプレン試験法として，試料からアセトニトリル抽出後，塩化ナトリウムと水を加えて石油エーテルで再抽出し，5% 含水フロリジルカラム，次いで 6% 含水アルミナカラムで精製して GC-FID で定量する方法[2]が通知されている．しかし，この方法は他の農薬の試験法と比較して，フロリジル (25 g) とアルミナ (70 g) をそれぞれ用いたカラム精製を行うこと，定量方法に唯一 GC-FID を用いるなど繁雑な面がある．また，GC-FID を用いない分析法として，Chamberlain は，肥料中メトプレンをアセトニトリルで抽出し，ヘキサンに転溶後，フロリジルカラムで精製して HPLC で測定する方法[3]，田宮らは，米中メトプレンをヘキサンで抽出し，還流蒸留装置を用いて再抽出後，フロリジルカートリッジカラムで精製して HPLC で測定する方法[4]，遠藤らは，穀類中メトプレンをアセトンで抽出し，ヘキサンに転溶後，多孔性珪藻土カラムで脱脂，次いでフロリジルカートリッジカラムで精製して HPLC で測定する方法[*3]を報告している．また，Hill らは，ELISA を用いた分析法[5]を報告している．

今回著者らは，メトプレン試験法[2]の見直しを目的として，精製法の簡易化，GC-FID に比べて汎用され，操作も簡便な HPLC を用いる分析法の検討を行い，試料からア

$$OCH_3$$
$$(CH_3)_2-C-(CH_2)_3$$
$$CH-CH_2$$
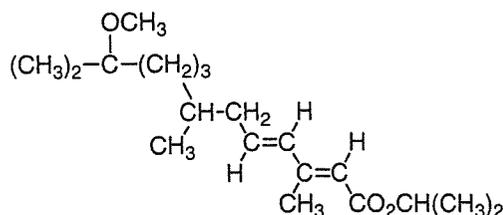$$CH_3 \quad C=C$$
$$H \quad C=C$$
$$CH_3 \quad CO_2CH(CH_3)_2$$

Fig. 1. Structure of methoprene

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*3 遠藤友紀子，今泉則子，渋谷　隆，前川吉明，日本食品衛生学会第 67 回学術講演会講演要旨集，p. 34 (1994).

セトニトリルで抽出し，塩析により水層を分離後，アセトニトリル層をヘキサンで洗浄，次いでフロリジル (5 g) カラムで精製して HPLC で定量する方法を開発し，数種類の農作物について添加回収試験を行ったところ，満足すべき結果が得られた．さらに，確立した分析法に従って，6 機関による共同評価試験を行ったところ，良好な評価結果が得られたので報告する．

実験方法

1. 試　　料

試料は市販の農作物 7 種（小麦，大麦，とうもろこし，そば，玄米，らっかせいおよびマッシュルーム）を用いた．

2. 試薬および標準品

試薬は和光純薬工業(株) の残留農薬試験用を用いた．

メトプレン標準品は林純薬工業(株) の残留農薬試験用を用い，20 mg をアセトン 20 mL に溶解して 1,000 μg/mL 標準原液を調製した．添加回収試験用の標準溶液は標準原液をアセトンで希釈した 2 μg/mL 溶液を，検量線作成用には標準原液をメタノールで適宜希釈した溶液を用いた．

ケイソウ土は和光純薬工業(株) のセライト 545 を用い，フロリジルは和光純薬工業(株) のフロリジル PR を 130℃ で 12 時間以上加熱後，デシケーター中で放冷して用いた．

3. 装置および測定条件

HPLC：(株)島津製作所製の LC-10 AD ポンプ，CTO-10A カラムオーブンおよび SPD-10AV 検出器，信和化工(株)製の STR ODS-Ⅱ カラム (4.6 mm i.d.×150 mm, 5 μm)，カラム温度 40℃，移動相メタノール-水 (90：10)，流速 0.8 mL/min，測定波長 265 nm．

GC/MS：(株)島津製作所製の GCMS-QP 5000, J & W Scientific 社製の DB-1 カラム (0.25 mm i.d.×30 m, 0.25 μm)，カラム温度プログラム 60℃ (1 min)→10℃/min→200℃→5℃/min→300℃ (5 min)，注入口温度 250℃，インターフェイス温度 300℃，キャリヤーガスヘリウム，キャリヤーガス流量 1 mL/min，注入量：1 μL，測定モード SIM (m/z 153, 111, 73)．

共同評価試験は，同等のカラムおよび条件を用いて行った．

4. 試 験 操 作

a. 抽出

細切した試料 10 g（マッシュルームは 20 g）を量り採り，穀類の場合は水 20 g を加えて 2 時間放置後，アセトニトリル 100 mL を加えてホモジナイザーでかくはん抽出した．ろ過助剤としてケイソウ土を加えて吸引ろ過後，残渣はさらにアセトニトリル 50 mL で抽出し，ろ過した．ろ液を合わせ，塩化ナトリウム 7 g を加えて振とう後，分離した水層を捨てた．アセトニトリル層をアセトニトリル飽和ヘキサン 20mL で洗浄し，ヘキサン層と一部

洗浄により生成する水層を捨てた後，アセトニトリル層を 40℃ 以下で減圧濃縮した．残渣を酢酸エチル 30 mL に溶解し，適量の無水硫酸ナトリウムを加えて脱水し，ろ過後，無水硫酸ナトリウムを酢酸エチル 10 mL で 2 回洗浄し，ろ過した．抽出液は 40℃ 以下で減圧濃縮後，残渣をヘキサン 5 mL に溶解した．

b. 精製

内径 15 mm，長さ 30 cm のクロマト管にフロリジル 5 g をエーテル-ヘキサン (1：19) で湿式充てんし，無水硫酸ナトリウム約 5 g を積層した．このカラムに a. の抽出で得られた試料溶液を負荷した後，エーテル-ヘキサン (1：19) 40 mL で洗浄し，エーテル-ヘキサン (3：17) 80 mL で溶出した．溶出液は 40℃ 以下で減圧濃縮し，残渣をメタノールで 2 mL に定容して HPLC 用の試験溶液とした．

c. 定量

3. の装置および測定条件に示した HPLC 条件で，メトプレンの標準溶液を用いて保持時間約 7 分のピークを測定してピーク面積法により検量線を作成した．試験溶液についても同様に操作し，標準溶液で作成した検量線を用いて，試料中のメトプレン濃度を求めた．

5. 添加回収試験

添加回収試験用の標準溶液 1 mL（マッシュルームは 2 mL）を添加（添加濃度 0.2 μg/g）し，1 時間後に抽出を開始した．

6. 共同実験による分析法の評価

共同実験は IUPAC のプロトコール[6]を参考にして，6 分析機関で，玄米，小麦，そば，らっかせいおよびマッシュルームの 5 農作物についてメトプレンの添加回収試験を行った．試料は各機関がそれぞれ購入したものを用い，5. の添加回収試験に示した方法でメトプレン標準品を添加して行った．各農作物について得られた 12 個（6 分析機関×2 個）の回収率を AOAC のコラボラティブスタディーガイドライン[7]に従って解析し，農作物ごとに回収率の平均値，併行再現性および室間再現性を求めた．再現性評価の基準として Horwitz の式（$RSD_R, \% = 2C^{-0.1505}$, C は質量分率）[8]を用いた．

結果および考察

1. 抽出および脱脂法

近年，食品中残留農薬の多成分分析では，アセトニトリルで抽出後，塩化ナトリウムを加えて塩析により水層を分離し，アセトニトリル層を減圧濃縮して次の精製操作を行うアセトニトリル抽出・水層分離法が一般化している[9],[10]．また，厚生労働省より通知される個別試験法などでは，アセトンによる抽出を原則としているが，メトプレンについてはアセトニトリル抽出法を採用している[2]．穀類，豆類からの抽出では，アセトニトリルはアセトンに比べて脂質成分の抽出が少なく脱脂操作を簡略化することが可能と考えられたことから，今回，アセトニトリル抽出・水層分離法を採用した．しかし，らっかせい，玄米などで

は脂質成分が少なからず抽出され，脂質成分の影響による
フロリジルカラムにおけるメトプレンや妨害成分の溶出挙
動のばらつきなども予想されたことから，少量のヘキサン
でアセトニトリル層を洗浄する操作を検討した．メトプレ
ンのアセトニトリル/ヘキサン分配率は 0.43 と高くないこ
とから，アセトニトリル層 150 mL に対して，アセトニ
トリル飽和ヘキサン 20 mL で 1 回洗浄したところ，メト
プレンのアセトニトリル層への残存率は 88.6±2.5% と良
好であったので，このアセトニトリル飽和ヘキサンによる
脱脂操作を加えた．

2．フロリジルカラムによる精製法

　従来の試験法では，フロリジル 25 g を充てんしたカラ
ム，次いでアルミナ 70 g を充てんしたカラムを用いて 2
段階の精製を行う．また，溶出溶媒としてエーテル/石油
エーテル混液を合計で 900 mL と大量に使用することか
ら，今回，フロリジル 5 g を充てんしたカラムを用いてメ
トプレンの精製効果を検討した．フロリジル (5 g) カラム
にメトプレン標準品を負荷し，エーテル/ヘキサン混液で
の溶出挙動を検討した．エーテル-ヘキサン (1:19)，エー
テル-ヘキサン (3:17)，次いでエーテル-ヘキサン (3:7)
の各 50 mL で順次溶出したところ，それぞれ 0%，78%，
22% のメトプレンが溶出したことからエーテル-ヘキサン
(3:17) での溶出が可能と考えられた．そこで，エーテル-
ヘキサン (1:19) 40mL で洗浄後，エーテル-ヘキサン (3:
17) の 20 mL ずつで順次溶出したところ，0〜20 mL の
画分に 0%，20〜40 mL の画分に 69%，40〜60 mL の画
分に 31% が溶出したことから，試験法としては溶出のば
らつきを考慮してエーテル-ヘキサン (3:17) 80 mL で溶
出することとした．

3．HPLC による定量

　これまでに報告されている HPLC 条件は，田宮ら[4]:
ODS カラム (4.6 mm i.d.×250 mm)，移動相 アセトニト
リル-水 (9:1)，流速 1 mL/min，測定波長 254 nm，溶出
時間約 15 分，遠藤ら[*1]: ODS カラム (4.6 mm i.d.×150
mm)，移動相メタノール-水 (9:1)，流速 1 mL/min，測

定波長 267 nm，溶出時間約 5 分である．ODS カラムは，
長さが 250 mm のものも使用されているが，より低い使
用カラム圧となり汎用されている 150 mm のものでも十
分に分離可能であったことから，実験方法に示した 150
mm のものを採用した．溶出溶媒はメタノール-水 (9:1)
を用い，検出波長は 265 nm とした．

4．添加回収試験

　添加回収試験の結果を Table 1 に示した．小麦など 7
種類の試料からの平均回収率は，74.6〜82.8% と良好で
あり，また，農作物由来の不飽和脂肪酸などによる妨害も
見られなかった．Fig. 2 にメトプレンの標準溶液，メトプ
レン標準品を添加した玄米の試験溶液，および玄米のブラ
ンク試験溶液のクロマトグラムを示した．

5．GC/MS による確認

　メトプレンの大気圧イオン化 LC/MS における測定感度
が低かったことから検出された場合の確認手段として，
HPLC 用の試験溶液をヘキサンに置換して GC/MS（SIM
モード）で測定する方法を検討した．実験方法に示した
GC/MS 条件で，m/z 153，111，73 のフラグメントイオン
をモニターすることにより，とうもろこしを除いて良好な
クロマトグラムが得られた．とうもろこしは，ヘキサンに
置換した試験溶液を中性アルミナカートリッジ（Waters
社，Sep-Pak Alumina N）に負荷した後，エーテル-ヘキ
サン (3:17) 20 mL で溶出する精製を追加することで良

Table 1.　Recoveries of Spiked Methoprene from Foods

Sample	Recovery, %[a]			
	1	2	3	Mean±RSD, %
Wheat	82.1	71.9	76.3	76.3±6.7
Barley	77.0	79.9	75.6	77.0±2.8
Corn	74.0	78.1	77.0	77.0±2.8
Buckwheat	73.6	79.4	84.5	79.4±6.9
Brown rice	82.8	90.3	82.6	82.8±5.3
Peanut	77.3	72.2	74.6	74.6±3.4
Mushroom	79.2	86.8	81.8	81.8±4.7

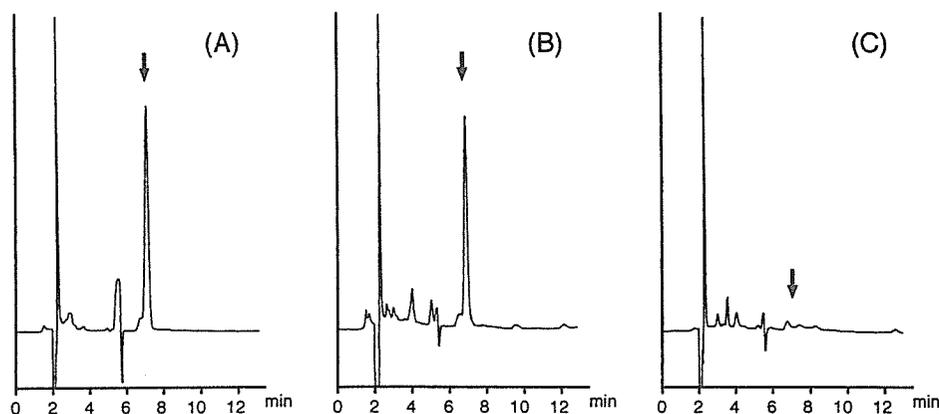[a] Samples were spiked with 0.2 μg/g of methoprene.

Fig. 2.　HPLC chromatograms of (A) methoprene standard (1 μg/mL), (B) brown rice fortified with methoprene (0.2 μg/g),
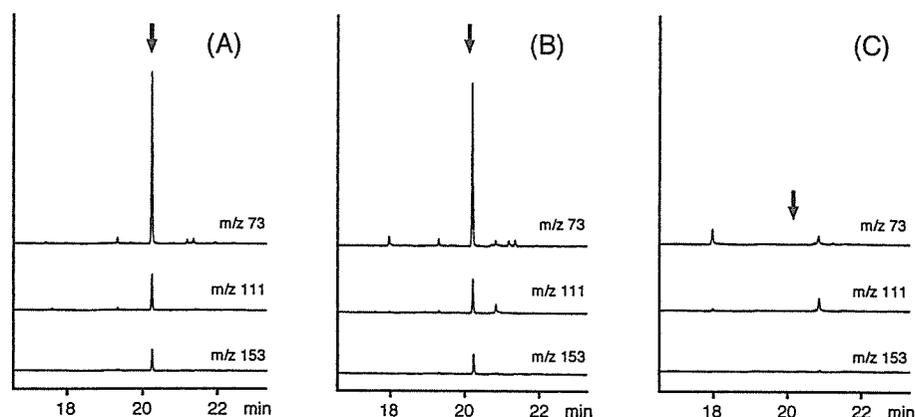(C) blank brown rice

Fig. 3. GC/MS (SIM) chromatograms of (A) methoprene standard (1 μg/mL), (B) brown rice fortified with methoprene (0.2 μg/g), (C) blank brown rice

Table 2. Interlaboratory Trial Results of Recovery Test Conducted by 6 Laboratories

Laboratory	Recovery, %[a]									
	Brown rice		Wheat		Buckwheat		Peanut		Mushroom	
	1	2	1	2	1	2	1	2	1	2
A	71.6	72.3	69.6	93.6	77.0	70.6	74.1	71.1	77.8	74.2
B	85.4	82.9	86.6	82.4	95.0	95.6	74.0	71.4	85.9	90.6
C	81.1	81.4	76.0	74.6	80.4	78.9	72.9	74.0	72.4	75.9
D	83.9	77.3	76.8	81.3	81.0	80.7	73.5	72.0	79.5	78.5
E	78.0	86.5	86.6	84.3	77.2	73.1	69.4	65.7	87.8	93.2
F	100.0	94.0	85.0	88.0	67.5	81.5	115.9	118.7	95.2	103.6
Mean	82.9		82.1		79.9		79.4		84.6	
Repeatability (RSD$_r$, %)	4.4		8.8		5.8		2.3		4.2	
Reproducibility (RSD$_R$, %)	10.3		8.8		10.9		23.6		12.0	
HORRAT value[b]	0.52		0.44		0.55		1.18		0.60	

[a] Samples were spiked with 0.2 μg/g of methoprene.

[b] HORRAT values are the observed RSD$_R$, %/RSD$_R$, % calculated from the Horwitz equation, RSD$_R$, % $= 2C^{-0.1505}$, $C =$ the estimated concentration.

好なクロマトグラムが得られた. Fig. 3 にメトプレンの標準溶液, メトプレン標準品を添加した玄米の試験溶液, および玄米のブランク試験溶液の SIM クロマトグラムを示した.

6. 本分析法の共同評価試験

本分析法の信頼性を確認する目的から, 6 か所の試験検査機関で添加回収試験を実施し, 回収率とそれらの平均値, 併行再現性の相対標準偏差 (RSD$_r$,%), 室間再現性の相対標準偏差 (RSD$_R$, %) および室間再現精度は食品試料の種類や分析法にかかわらず濃度の変数になっていることが報告されていることから[8], HORRAT 値 (Horwitz の式から予測した添加濃度 0.2 μg/g レベルの分析における標準的な RSD$_R$ (20%) と実測値から求めた RSD$_R$ の比)[7] を Table 2 に示した. 玄米など 5 種類の試料からの平均回収率は, 79.4% (らっかせい)～84.6% (マッシュルーム) と満足すべき結果であった. 標準溶液のクロマトグラムから S/N 比が 3 を示す農薬量を求めて得られた検出限界は, 0.001～0.02 μg/g であり, いずれの機関でも要求

される検出限界 0.02 μg/g[2] を満たしていた.

併行再現性 RSD$_r$ は, 2.3% (らっかせい)～8.8% (小麦) であった. 室間再現性 RSD$_R$ は, 8.8% (小麦)～23.6% (らっかせい) であった. らっかせいで 23.6% と高かったのは, 5 機関が 70% 前後の回収率であったのに対して 1 機関が 117% と高い回収率であったためである. HORRAT 値は, らっかせいを除いて 1.0 未満と室間再現性は良好であった. また, 1 機関で高い回収率となったらっかせいの併行再現性は良好であった. 以上から, 本分析法の妥当性が確認された.

ま と め

食品中に残留するメトプレンについて, 従来の試験法の見直しを検討した. アセトニトリルで抽出し, 塩析により水層を分離後, 少量のヘキサンで洗浄, 次いでフロリジルカラムで精製して HPLC で測定する分析法を検討したところ, 7 種類の農作物について良好な回収率が得られた. さらに, 共同実験による分析法評価の結果, その妥当性が

確認された.

文　　献

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Stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry for trace analysis of benzophenone and its derivatives in water sample

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Abstract

A simple and highly sensitive method called stir bar sorptive extraction (SBSE) and thermal desorption (TD)-gas chromatography-mass spectrometry (GC-MS), which is used for the determination of trace amounts of benzophenone (BP) and its derivatives, 2-hydroxy-4-methoxybenzophenone (BP-3) and 2-hydroxy-4-methoxy-4'-methylbenzophenone (BP-10), in river water samples, is described. A stir bar coated with polydimethylsiloxane (PDMS) is added to a 10 ml water sample and stirring is carried out for 120 min at room temperature (25 °C) in a vial. Then, the PDMS stir bar is subjected to TD-GC-MS. The detection limit is 0.5–1 pg ml^{-1} for BPs. The method shows good linearity and the correlation coefficients are higher than 0.997 for all the analytes. The average recoveries of the BPs are equal to or higher than 98.5% (R.S.D.: 1.5–5.1%). This simple, accurate, sensitive and selective analytical method may be used in the determination of trace amounts of BPs in river water samples.
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Keywords: Benzophenone; Water sample; Stir bar sorptive extraction (SBSE); Thermal desorption (TD); Gas chromatography-mass spectrometry (GC-MS)

1. Introduction

Benzophenone (BP) and its derivatives are the most commonly used sunscreen agents in cosmetics. However, various studies have revealed the estrogenic activity of BPs [1–3]. Therefore, BPs are considered to be endocrine disrupting chemicals (EDCs). To evaluate the potential risks of BPs, their determination requires highly sensitive and reliable methods. In the present study, we focused on the combined determination of BP, 2-hydroxy-4-methoxybenzophenone (oxybenzone, BP-3) and 2-hydroxy-4-methoxy-4'-methylbenzophenone (BP-10).

Several analytical methods for the determination of BPs in water and cosmetic samples have been reported, including liquid chromatography (LC) with UV detection, diode array detection (DAD) and mass spectrometry (MS) [4–8]. However, LC has low resolution and is frequently affected by the sample matrix. On the other hand, gas chromatography-mass spectrometry (GC-MS) was initially used for the determination of BPs [8–11].

Such sample preparation as online continuous liquid–liquid extraction (LLE) [9] and solid–phase extraction (SPE) [7,8] have been developed for the determination of BPs. However, LLE requires large volumes of organic solvents and additional concentration steps. On the other hand, although SPE requires small volumes of organic solvents, the manual version is tedious and time-consuming. Recently, solid–phase micro extraction

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(SPME) has been successfully used for the determination of BPs in water and urine samples [10,11]. However, the sensitivity of the above methods remains low. Because SPME with polydimethylsiloxane (PDMS) is by nature an equilibration technique that is based on the partitioning of an analyte between the stationary phase and the aqueous sample, the enrichment is dependent on the distribution coefficients of the analyte in the two phases. Therefore, the water/PDMS phase ratio is very important for sorptive extraction. The limited enrichment on the SPME fiber is mainly due to the volume of the PDMS phase (typically 0.5 µl or less), and increasing the volume of PDMS relative to the aqueous matrix is expected to markedly increase the enrichment of the analyte. Recently, a new sorptive extraction technique that uses a stir bar coated with PDMS was developed [12] and is known as stir bar sorptive extraction (SBSE). Its main advantages are high sensitivity and wide application range that includes volatile aromatics, halogenated solvents, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, preservatives, odor compounds and organotin compounds [12–18]. In addition, we have reported the determination of several EDCs in water samples and human biological samples [19–26].

The aim of this study was to determine trace amounts of BPs in water samples by the SBSE and TD-GC-MS method, which is simple, accurate and highly sensitive. The developed method was applied to river water samples.

2. Experimental

2.1. Materials and reagents

Benzophenone (BP) of environmental analytical grade and BP-d_{10} as internal standard were purchased from Kanto Chemical Inc. (Tokyo, Japan). 2-Hydroxy-4-methoxybenzophenone (oxybenzone, BP-3) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). 2-Hydroxy-4-methoxy-4'-methylbenzophenone (BP-10) was purchased from Lancaster Synthesis (Morecambe, England). The chemical structures are shown in Fig. 1. The other reagents were purchased from Wako Pure Chemical Inc. (Osaka, Japan). The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

Stock solutions (1.0 µg ml⁻¹) of BP, BP-3 and BP-10 standards were prepared by methanol. More than six-point calibrations (2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000 pg ml⁻¹) were prepared by the addition of purified water

and performed daily for all samples with the internal standards by using the SBSE method.

2.2. Instrumentation

TD was performed with a Gerstel TDS 2 thermodesorption system equipped with a Gerstel TDS A autosampler and a Gerstel Cooled Injection System (CIS) 4 programmable temperature vaporization (PTV) inlet. GC-MS was performed with an Agilent 6890N gas chromatograph equipped with a 5973N mass-selective detector with an ultra ion source (Agilent Technologies).

Stir bars coated with a 0.5 mm-thick PDMS layer (24 µl; Twister™: a magnetic stirring rod is placed inside a glass jacket and coated with PDMS) were obtained from Gerstel (Mülheim an der Ruhr, Germany). The stir bars were conditioned for 1 h at 300 °C in a flow of helium. Then, the stir bars were kept in new 2 ml vials until immediately prior to use. The stir bars could be used more than 50 times with appropriate re-conditioning (the stir bars were conditioned for 1 h at 300 °C in a flow of helium). For the extraction, a 20 ml headspace vial from Agilent Technologies (Palo Alto, CA, USA) was used.

2.3. TD-GC-MS conditions

The TDS 2 temperature was programmed to increase from 20 °C (held for 1 min) to 250 °C (held for 5 min) at 60 °C min⁻¹. The desorbed compounds were cryofocused in the CIS 4 at −150 °C. After the desorption, the CIS 4 temperature was programmed to increase from −150 to 300 °C (held for 10 min) at 12 °C s⁻¹ to inject the trapped compounds into the analytical column. Once an analyte is trapped by means of temperature control, the entire quantity is subjected to GC-MS. Injection was performed in the solvent vent mode. The separations were conducted on a DB-5ms fused silica column (30 m × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Agilent Technologies). The oven temperature was programmed to increase from 60 to 300 °C (held for 4 min) at 15 °C min⁻¹. Helium was used as the carrier gas at a flow rate of 1.2 ml min⁻¹. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode with electron ionization (ionization voltage: 70 eV).

2.4. Water samples

River water was sampled from two sites (points A and B) at Tama River, Tokyo, Japan. All samples were stored at 4 °C prior to use.
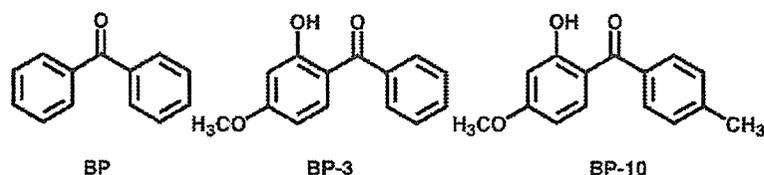

Fig. 1. Chemical structures of BPs.

2.5. Sample preparation

A 10 ml river water sample and an internal standard were added into a 20 ml headspace vial. A stir bar was added and the vial was crimped with a Teflon-coated silicone septum cap. SBSE was performed at room temperature for 0–180 min while stirring at 1000 rpm. After the extraction, the stir bar was easily removed, rinsed with purified water, dried with lint-free tissue and placed inside a glass TD tube. The TD tube was placed inside the TD system where the stir bar was thermally desorbed and subjected to GC-MS thereafter.

3. Results and discussion

3.1. Theoretical recovery

Table 1 shows $\log K_{o/w}$ and the theoretical recoveries of the compounds investigated in this work. The $K_{o/w}$ values were calculated with the log P predictor, which is available from Interactive Analysis Inc. (Bedford, MA, USA). Theoretical recovery was calculated with the following equations:

$$\text{Theoretical recovery} = \frac{K_{o/w}/\beta}{1 + K_{o/w}/\beta} = \frac{1}{\beta/K_{o/w} + 1}$$

Table 1
$\log K_{o/w}$ and theoretical recoveries of BPs by SBSE

Compound	$\log K_{o/w}$[a]	Theoretical recovery[b] (%)
BP	3.16	77.6
BP-3	3.79	93.7
BP-10	4.33	98.1

[a] $\log K_{o/w}$ values for all compounds as calculated with "Interactive Analysis log P predictor", as well as calculated recoveries.
[b] Sample volume 10 ml; phase ratio $\beta = 417$.

where $\beta = V_w/V_{PDMS}$, V_{PDMS} being the volume of PDMS and V_w the volume of water. The theoretical recoveries by SBSE were calculated on the basis of a 10 ml sample volume and a stir bar with a phase thickness of 500 μm (24 μl of PDMS). Bicchi et al. have reported on importance of. β [27]. An increase of β requires the decrease of recovery and long extraction time. The β calculated from the condition used by present method was 417. Moreover, because the theoretical recoveries of the BPs were equal to or higher than 77.6%, the extraction by SBSE was considered to have high recovery.

3.2. Optimization of GC-MS conditions

In the mass analysis of standard solutions using electron impact ionization (EI)-MS, m/z 105, 227 and 241 were observed as the main peaks of BP, BP-3 and BP-10, respectively. For the internal standard, BP-d$_{10}$, its main peak was detected at m/z 110 (Fig. 2). However, in the measurement of BP and BP-d$_{10}$ in the river water sample, the monitoring ions at m/z 105 and 110, respectively, could not be completely dissociated from the other peaks and therefore, the monitoring ions were set at m/z 182 and 192, respectively.

3.3. Optimization of TD conditions

The important parameters affecting TD from the PDMS stir bar were the TD temperature and the hold time. To optimize the TD temperature and the hold time, 5 ng ml^{-1} standard solutions of the BPs were used. The TD temperature profiles (150–275 °C) of the BPs in 10 ml standard solutions that were subjected to SBSE were determined by TD-GC-MS when the TD hold time was set at 5 min, and are shown in Fig. 3. The BPs were completely desorbed after the temperature reached approximately 250 °C. On the other hand, the TD hold time profiles (0–10 min)
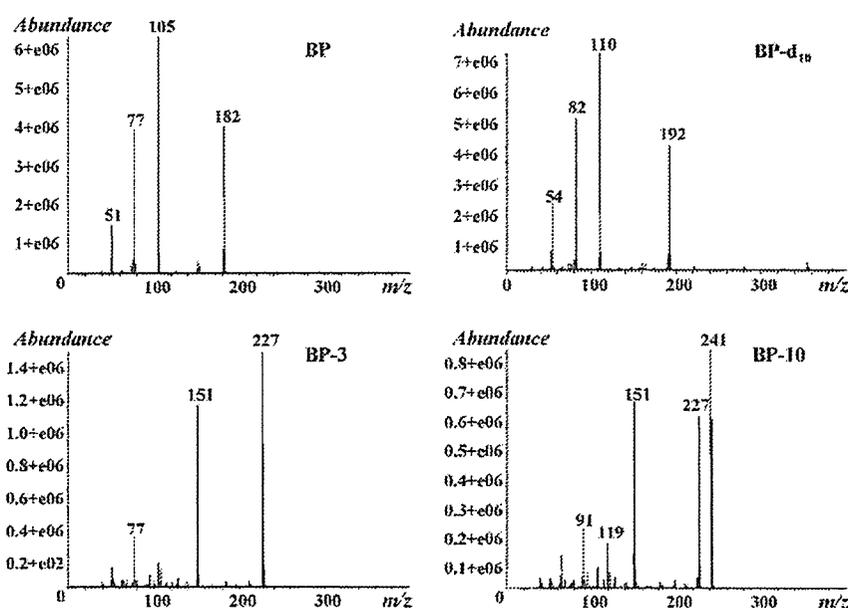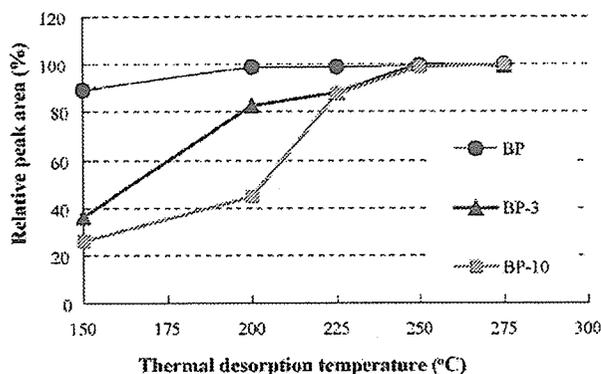

Fig. 2. Mass spectra of BPs.

Fig. 3. Optimum TD temperature for analysis of BPs by SBSE and TD-GC-MS method. The optimum TD temperature was examined in the range of 150–275 °C.

of the BPs in 10 ml standard solutions that were subjected to SBSE were determined by TD-GC-MS when the TD temperature was set at 250 °C, and are shown in Fig. 4. The BPs were completely desorbed after approximately 5 min. Therefore, the optimum TD temperature and hold time were set at 250 °C and 5 min, respectively.

3.4. Optimization of SBSE conditions

One important parameter affecting SBSE was the extraction time. Moreover, it has been reported that the impact of water/PDMS phase ratio, volume of PDMS, and sampling time on recovery were important [27]. To optimize the extraction time, 5 ng ml^{-1} standard solutions of the BPs were used. The extraction time profiles (0–180 min) of the BPs in 10 ml standard solutions that were subjected to SBSE were determined by TD-GC-MS, and are shown in Fig. 5. The BPs reached equilibrium after approximately 120 min. Therefore, this condition was used for the determination of BPs in water samples.

3.5. Figures of merit of SBSE and TD-GC-MS for determination of BPs

The calculated detection limits (LODs) of the BPs were 0.5–1 pg ml^{-1} for SBSE and TD-GC-MS, with the signal to
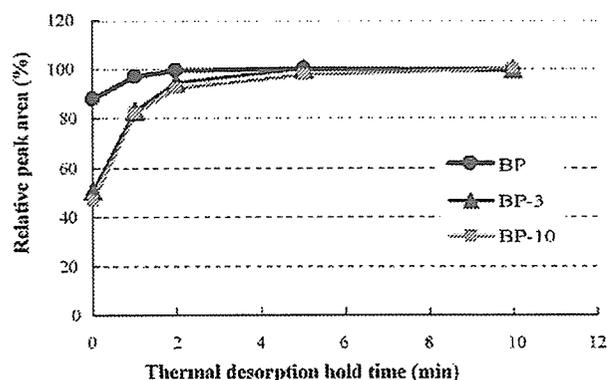

Fig. 4. Optimum TD hold time for analysis of BPs by SBSE and TD-GC-MS method. The optimum TD hold time was examined in the range of 0–10 min.
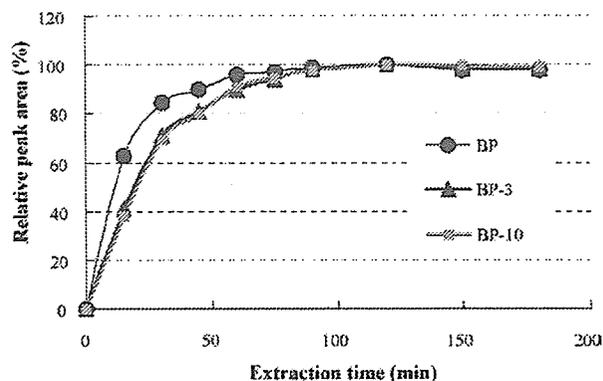

Fig. 5. Optimum extraction time for analysis of BPs by SBSE A PDMS-coated stir bar was added to 10 ml of standard solution (5.0 ng $^{-1}$) and stirring was commenced for 0–180 min at room temperature (25 °C) in a glass vial. The PDMS stir bar was then subjected to TD-GC-MS.

noise (S/N) ratio being 3. In addition, the limits of quantification (LOQs) of the BPs when S/N > 10 were 2–5 pg ml^{-1}. The method showed good linearity over the calibration range (2 or 5–5000 pg ml^{-1}) and the correlation coefficients (r) were higher than 0.997 for all the analytes. The figures of merit of the present method are summarized in Table 2. A comparison of the present SBSE method with the SPE [7,8] and SPME [10,11] methods used in previous studies was performed. The SBSE method was superior to the SPME method in terms of sensitivity. In addition, the SBSE method was applicable to a small volume of sample compared to the SPE method.

The recovery and precision of the method were assessed by replicate analysis (n = 6) of river water samples fortified at 100 and 1000 pg ml^{-1} levels. The non-spiked and spiked samples were subjected to SBSE and TD-GC-MS. The recovery was calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were obtained by using calibration curves obtained from standard solutions with the internal standard. The recovery and precision were 98.5–114.8% (R.S.D.: 1.5–5.1%) for the river water samples (Table 3). Therefore, the method enables the precise determination of standards and may be applicable to the determination of trace amounts of BPs in river water samples.

3.6. Determination of BPs in river water samples

A total of two river water samples were analyzed for BPs using the present method and the results are shown in Table 4.

Table 2
Figures of merit of SBSE and TD-GC-MS

Compound	LOD (pg ml^{-1})[a]	LOQ (pg ml^{-1})[b]	Range (pg ml^{-1})	Correlation coefficient (r)
BP	0.5	2	2–5000	0.999
BP-3	1	5	5–5000	0.998
BP-10	1	5	5–5000	0.997

[a] LOD: limit of detection (S/N = 3).
[b] LOQ: limit of quantification (S/N > 10).

Table 3
Recoveries of BPs in river water samples

Compound	Amount spiked			
	100 pg ml^{-1}		1000 pg ml^{-1}	
	Recovery (%)	R.S.D. (%)[a]	Recovery (%)	R.S.D. (%)
BP	98.5	1.6	99.3	2.2
BP-3	110.0	4.2	114.2	1.5
BP-10	114.8	5.1	113.5	2.4

[a] The recoveries and precision were also examined by replicate analysis (n = 6) of river water samples.

Table 4
Concentrations of BPs in river water samples

Compound	River water (pg ml)	
	A	B
BP	22.8	21.0
BP-3	12.9	8.9
BP-10	N.D.	N.D.

N.D. indicates not detected.

In the Tama River water samples, 21.0–22.8 pg ml^{-1} BP and 8.9–12.9 pg ml^{-1} BP-3 were detected by the present method. On the other hand, BP-10 was not detected in all river water samples. Typical SIM chromatograms of the river water sample (point A) are shown in Fig. 6. SBSE and TD-GC-MS enabled the successful determination of trace amounts of BPs in the river water sample. The BP levels in the river water samples were very low and could not be quantitfied by SPME-GC-MS [10,11]. However, the combination of SBSE and the TD-GC-MS method led to the successful determination of trace amounts of BPs in a small volume of water sample (10 ml).
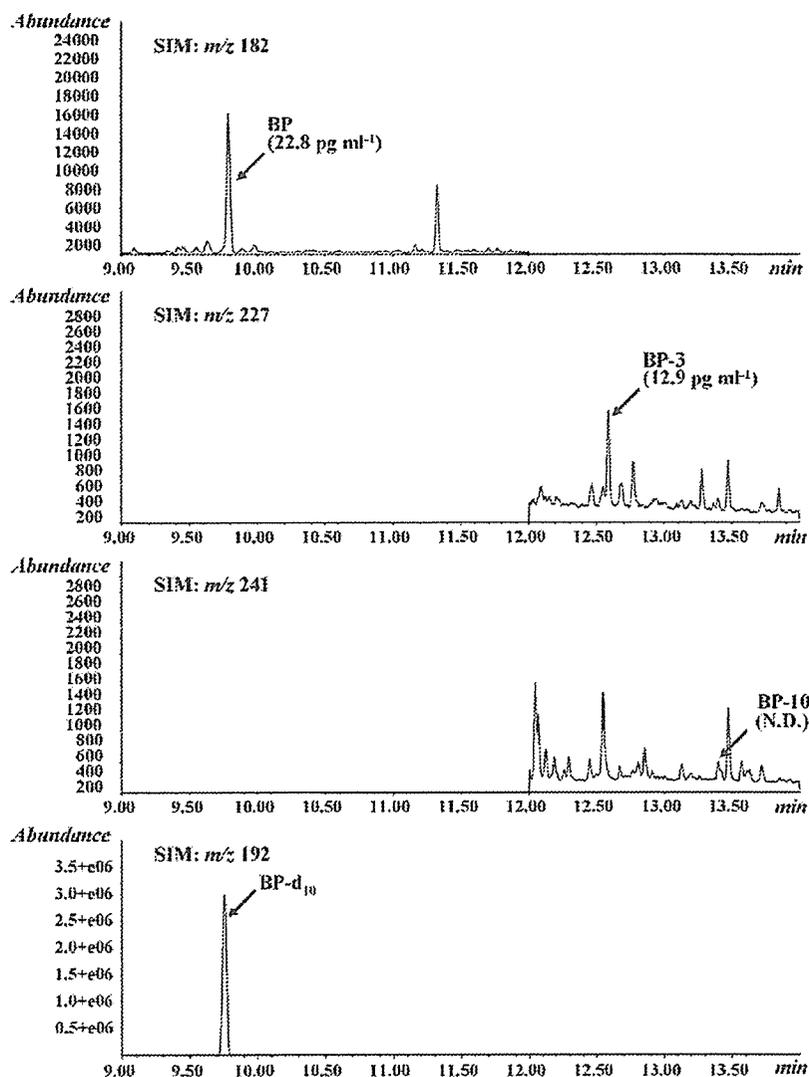

Fig. 6. SIM chromatograms of BPs and internal standard in river water sample (A).

4. Conclusions

The determination of trace amounts of BPs in river water samples using SBSE and TD-GC-MS was described. The proposed method has many practical advantages, including a small sample volume (10 ml) and simplicity of extraction; it is also solvent-free and has high sensitivity. The detection limits for BPs were of sub pg ml^{-1} level. In addition, the present method showed good linearity and high correlation coefficients using the internal standard. The recovery was high (98.5–114.8%) and the precision was good (R.S.D.: 1.5–5.1%) for the river water samples fortified at 100 and 1000 pg ml^{-1} levels. This simple, accurate and highly sensitive method is expected to have potential applications in various aqueous samples.

Acknowledgments

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報　文

遺伝子組換えトウモロコシ（Mon810系統）の定量PCR法を対象とした外部精度管理試験

（平成17年9月8日受理）

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Laboratory-performance Study of Quantitative PCR Methods to Analyze an Approved Genetically Modified Maize (Mon810 Line)

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　　A laboratory-performance study was carried out to investigate factors affecting the reliability of the quantitative PCR method to analyze an approved genetically modified (GM) maize (Mon810 line).
　　Test maize powdered samples were prepared as blind samples containing a high (assigned value; 5.45%) or low (assigned value; 0.35%) concentration of the Mon810 line. After confirmation of their homogeneity, they were provided to 27 laboratories participating in the collaborative study. The data were collected from all laboratories and statistically analyzed. Two laboratories, which used a Roche LightCycler (LC), reported significantly high test values. A further examination showed that the LC method is greatly affected by the equipment itself or PCR reagents, resulting in poor repeatability. On the other hand, some laboratories, which used ABI quantitative PCR equipment, reported erroneous test values. In these laboratories, the errors appeared to have been due to inadequate quality and/or yield of DNA. To identify factors affecting the test values, analysis of the measured values for the taxon-specific gene will be useful. Furthermore, the modified silica-gel membrane DNA extraction method made it possible to extract the required amounts of DNA more easily and in a shorter time than before.

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緒　　言

　近年のバイオテクノロジーの急速な進展に伴い，その基幹技術ともいえる遺伝子組換え技術が作物育種に応用され

るようになった．その結果開発された作物は遺伝子組換え（GM）作物と呼ばれ，1990年代後半に米国，カナダといった農業先進国において商業栽培が開始されて以降，東南アジアや南米などが栽培開始国に加わったことに伴い，

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