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Trace analysis of pesticide residues in water by high-speed narrow-bore capillary gas chromatography–mass spectrometry with programmable temperature vaporizer

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Abstract

A method for the rapid trace analysis of 17 residual pesticides in water by narrow-bore capillary (I.D. 100 μm) gas chromatography–mass spectrometry (GC–MS) using a programmable temperature vaporizer (PTV) was discussed. The method consisted of a large-volume injection (40 μl) by a PTV, high-speed analysis using a narrow-bore capillary column and MS detection. The PTV with solvent vent mode was very useful for large-volume injection into a narrow-bore capillary column because the injected solvent volume could be reduced to less than 2 μl. The analysis time was 8.5 min [less than 50% of the analysis time using conventional columns (I.D. 250 μm)]. A 10-ml volume of river water was extracted by dichloromethane (4 ml), and then the extract was condensed to 1 ml. This extract was analyzed. Mean recoveries for river water spiked at 100 pg/ml ranged from 83.4 to 96.7%. The limit of detections of the 17 pesticides ranged from 1 to 100 pg/ml.

Keywords: Large-volume injection; Programmable temperature vaporizer; Narrow-bore columns; Pesticides

1. Introduction

Theory of capillary gas chromatography has already demonstrated that the application of narrow-bore capillary columns (I.D.<0.1 mm) brings a number of advantages [1]. Reduction of the column diameter can increase the efficiency (and consequently, the resolution) and drastically reduce analysis times [2,3]. But one of major limitations for the use of narrow-bore columns is the lack of compatible instrumentation for trace level analysis. Broadened and distorted or even split peaks may result by band broadening in space if large sample volumes are injected into a narrow-bore column because the inner diameter of capillary column is very small (<100 μm) [4,5]. But the introduction of a large sample volume is a simple and efficient way to increase sensitivity. When conventional columns are used, well known injection techniques such as hot splitless, programmable temperature vaporizer (PTV) [6–8], cold on-column are widely used for trace level analysis and especially, the PTV has the potential to inject large sample volumes into a narrow-bore column to analyze trace level analytes as solvent can

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be eliminated from the inlet [9]. Further, the PTV is very useful for the trace analysis of pesticides because the open liner used for the PTV is very inert [10,11]. On the other hand, alternative on-column injection should be discouraged for the trace analysis of pesticides because the surface of the uncoated guard column used for this injection is active. A PTV method using a narrow-bore column has not yet been reported for the analysis of pesticide residues in environmental water. In this work, a PTV with solvent vent mode using a narrow-bore column was investigated for the trace analysis of 17 pesticides regulated in drinking water in Japan. The preliminary investigations discuss the optimization of several PTV parameters on peak areas and peak shapes of pesticides. Other important aspects of PTV injection combined with narrow-bore capillary column with respect to trace level analysis of pesticides – the reproducibility and linearity of peak areas and limits of detection (LODs) – are discussed.

2. Experimental

2.1. Materials and reagents

Dichlorvos, fenobcarb, simazine, diazinon, propyzamide, chlorothalonil, iprobenfos, fenitrothion, benthiocarb, isoprothiolane, isoxathion, chloronitrofen, EPN, diazinon-oxon, fenitrothion-oxon, isoxathion-oxon, EPN-oxon were purchased from Hayashi (Osaka, Japan). Dichloromethane was purchased from Wako (Osaka, Japan). Standard stock solutions of each analyte (10 ppm in dichloromethane) were made by dilution of 1000 ppm solutions prepared by dissolving 10 mg of pesticide standards in dichloromethane. Stock solutions were stored at −20°C and used every day to prepare fresh working standard solutions for optimization studies, as well as pesticide mixtures for fortification studies.

2.2. Apparatus and operation

A Hewlett-Packard (HP) 6890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with two inlet systems (PTV and split/splitless) and a HP 7683 automatic sampler was used. The capillary columns were a 10 m×0.1 mm I.D. for high-speed analysis and a 30 m×0.25 mm I.D. fused-silica capillary column coated with cross-linked methyl silicone with a film thickness of 0.25 μm (HP-1, Hewlett-Packard) for conventional analysis. A HP 5973 mass-selective detector was interfaced with the gas chromatograph, with the capillary column inserted directly into the ion source.

Large sample volumes (40 μl) were injected into the analytical columns with multiple injections (5 μl per one injection×8 injections). The delay time between injections was set to 2 s in order to remove the solvent from the injector. During multiple injections into a PTV inlet (1.6 min), the split vent valve was opened at a split vent flow-rate of 50 ml/min and column head pressure was set to 0 p.s.i. (and consequently, the column flow-rate is zero) (1 p.s.i.=6894.76 Pa). After multiple injection of sample, the split vent valve was closed (2.1 min) until the valve was opened again to eliminate the remaining solvent from the liner. The volume of the open baffle liner used in the PTV was 120 μl. The PTV temperature program was as follows: 0°C (1.7 min), 250°C/min to 250°C (2 min), 100°C/min to 300°C (6 min). The column oven temperature program was as follows: 80°C (3.7 min), 100°C/min to 150°C (0 min), 30°C/min to 250°C (3 min). The carrier gas was helium with an initial inlet pressure of 70.12 p.s.i. The time relationships of oven temperature, PTV temperature, column head pressure and split vent flow are shown in Fig. 1. The mass conditions were as follows: ionization energy, 70 eV: ion source temperature, 230°C; quadrupole temperature, 150°C. Full-scan gas chromatography–mass spectrometry (GC–MS) chromatograms were obtained by scanning the quadrupole from 40 to 400 m/z with a 0.2-s scan. For quantitative analysis of pesticides, selected-ion monitoring (SIM) was used.

2.3. Sample preparation

Samples of 10 ml each of river water and drinking water were spiked at 100 pg/ml with the dilute solution containing 100 ng/ml of each pesticide. These samples were extracted with 4 ml dichloromethane and then the extracts were concentrated to 1 ml. This extract was analyzed for validation purposes.

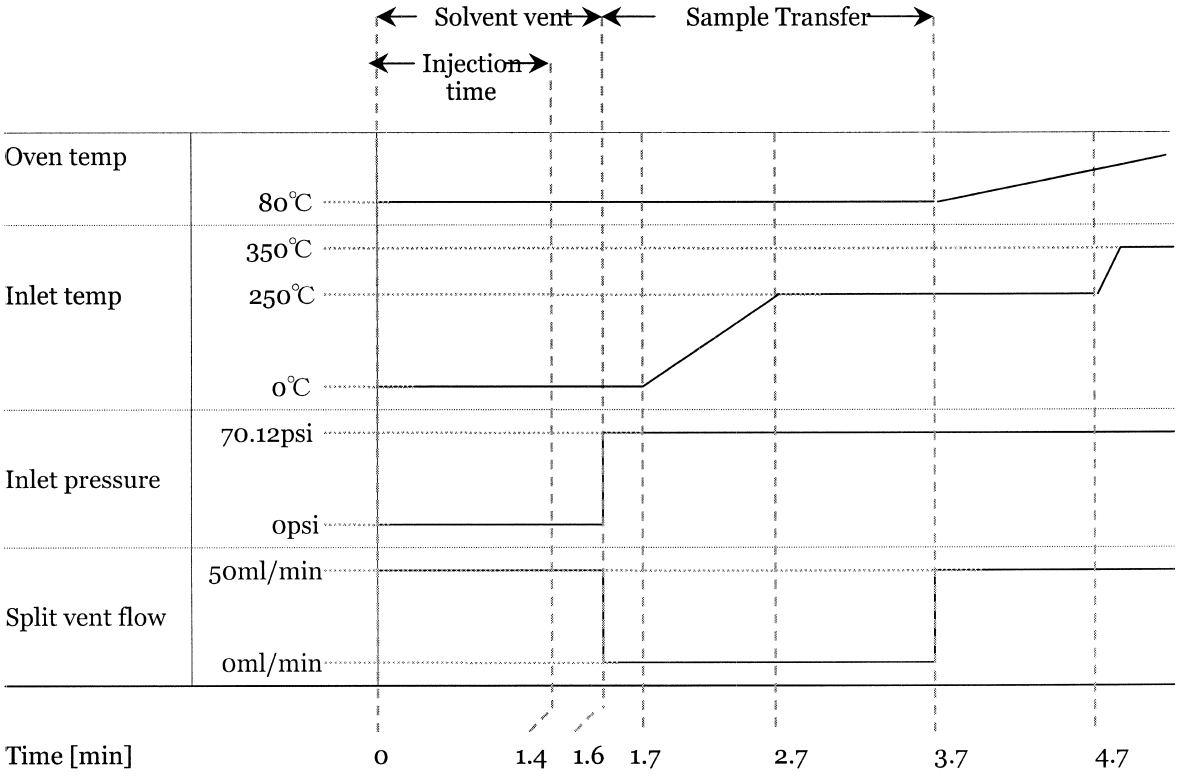

Fig. 1. Time relationships of oven temperature, PTV temperature, column head pressure and split vent flow.

3. Results and discussion

3.1. Optimization of PTV parameters for combination with a narrow-bore column

For a PTV with solvent vent mode, the boiling point (b.p.) of the solvent chosen should be lower than that of the most volatile analytes because the compounds of interest must be separated from the solvent without significant loss. Typically, the wider the b.p. difference of solvent and analyte is, the better the PTV technique works. In this work, dichloromethane was chosen for its low b.p. and other physical properties. Further, reactivity of pesticides is more of a concern with a PTV than with conventional split/splitless injection because the analytes stay in the inlet for a much longer time. Therefore, it is imperative that the open baffle liner without the packing materials is used. It is also important that larger sample volumes be introduced by multiple injections using an automatic sampler in order to prevent losses of sample via the split exit [11]. Here injection can be repeated several times to concentrate the analytes in the open baffle liner. With this injection technique, overflow of the liner does not occur because the volumes per injection are limited to 5 μl. But if large amounts condensed solvent remain in the liner, these solvents are transferred to the GC column and flooding occurs on the head of the GC column and chromatography suffers [12]. Consequently, the elimination of solvents prior to the next injection was very important. To eliminate the excessive solvent and concentrate the analytes in the liner, several PTV parameters were optimized.

3.1.1. Split vent flow

Fig. 2 shows the influence of split vent flow on the peak area for the pesticides investigated. The study was performed for 30-μl sample volume (6×5 μl) under the conditions described in Fig. 1. As can be seen, for all pesticides investigated, the largest peak

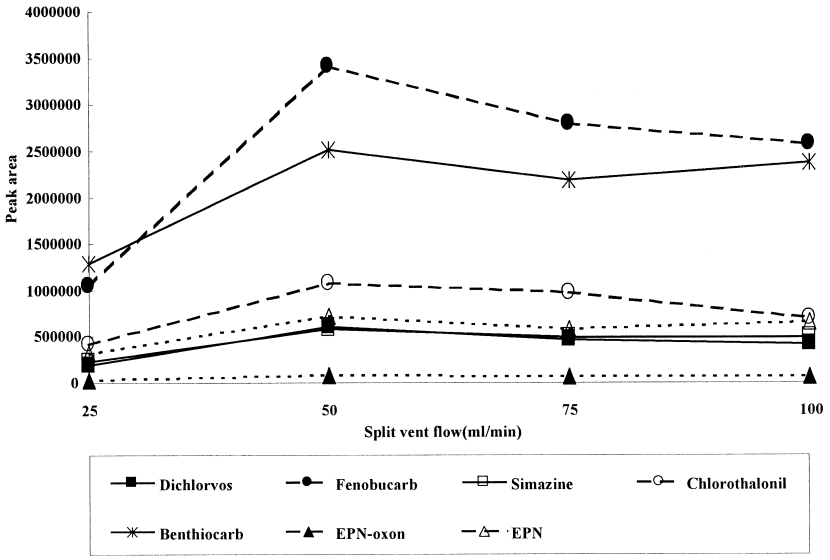

Fig. 2. Effect of split vent flow on peak areas. Initial PTV temperature: 0°C; purge time: 3.7 min; delay time between injections: 0 s; concentration of pesticides 20 ppb.

areas were obtained at the split vent flow of 50 ml/min. At 25 ml/min, the smallest peak area of all pesticides was observed because of liquid rinsing or flooding of the liner at low split flow-rates. Further, all of pesticides, except dichlorvos, produced a well-behaved peak in the 25–100 ml/min range. This indicates that the volume of remaining solvent in the liner was less than 3 μl because a splitless injection of greater than 3 μl (consequently more than 3 μl solvent was transferred to the column) with a narrow-bore column led to peak distortion of several pesticides due to flooding of the column. On the other hand, dichlorvos has the lowest boiling point of the pesticides investigated and it can therefore be presumed that this pesticide could not be completely trapped by the narrow band width in the liner at high split flow-rates (>75 ml/min). This initial broad band width led to peak distortion at 75 and 100 ml/min of split flow-rates. Therefore, the split vent flow was set to 50 ml/min.

3.1.2. Purge time

The purge time is the opening time of split vent valve after the transfer of the analytes to the column.

Too long a sample transfer time can give rise to extra column peak broadening. It is obvious that the transport process is influenced emphatically by the volume of the liner used. Thus, the open baffle liner used can be expected to decrease the sample transfer time because of its small inner volume. This is important because rapid transfer decreases the total analysis time and minimizes thermal degradation of labile pesticides in the hot liner. The effect of purge time on the peak area and peak shape and degradation was studied. In this work, the time in which the final temperature was reached (250°C) was 2.7 min. As a result, too short a purge time (2.7 min) led to the loss of all the pesticides but as far as the influence of the purge time on the peak area of pesticides investigated is concerned, no significant variations were observed when the purge time was varied from 3.2 min to 4.7 min, with a 20 ppb standard mixture as described in Section 2.2. For the peak shape and thermal degradation of pesticides, all the pesticides did not decompose and have well-behaved peaks. Therefore, a 3.7-min purge time was selected.

3.1.3. PTV temperature

The initial PTV temperature is very critical as long as the open liner is used because the analytes will be quantitatively retained in the liner by cold trapping only. It is found that in order to retain the analytes in the liner by cold trapping only, the initial PTV

temperature should be about 250°C below the boiling point of the analytes. The influence of initial PTV temperature on the peak area was studied. Further, the influence of the final PTV temperature on the thermal degradation of pesticides was studied. Fig. 3 shows the variation in peak area of the main pesticides investigated when the initial PTV temperature varied from −20°C to 15°C, with a 20 ppb standard mixture. As can be seen, in general, 0°C should be recommended to perform the analysis, as the retention power in the open liner by the cold trapping seemed to be more effective in preventing losses during both injection and solvent elimination steps. This temperature must be low enough to avoid excessive losses of the analytes of interest by coevaporation with the solvent and simultaneously adequate for efficiently performing solvent evaporation. On the other hand, the final temperature of 250°C gave the best results for the desorption of all trapped pesticides. Too high a final PTV temperature led to the decomposition of thermal liable pesticides (fenobcarb, chlorotalonil, oxons).

3.1.4. Maximum sample volume

For the multiple injection to inject large volumes into the narrow-bore column, sample volume per injection and the number of injections are important. In this work, the influence of injection volume per injection and the number of injections on the peak area were investigated

3.1.4.1. Injection volume per injection

The quantity to be injected per injection was evaluated at 1, 2, 3 and 5 μl. The number of injections was set to 6. Increasing the injection volume, peak abundance became higher but peak shape of dichlorvos was broadened at 1~4 μl and the well-behaved peak was observed at only 5 μl. This phenomenon indicates that dichlorvos cannot be trapped in the liner by cold trapping only and the solvent volume of 5 μl trapped this pesticide by solvent effect. Thus, the injection volume per injection was set to 5 μl.

3.1.4.2. The number of injections

At an injection volume of 5 μl, the number of injections was evaluated from 1 to 10. Fig. 4 shows the influence of the number of injections on the peak area for the pesticides investigated. As can be seen, at less than four-times (injection volume was 20 μl), the peak shape of dichlorvos was broadened and its peak area was drastically decreased at more than nine-times (45 μl). From five- to eight-times (25~40 μl) all peaks shape were satisfactory. Therefore, an eight-times injection (40 μl total volume) was selected as the optimum value.

After having performed these optimization pro-

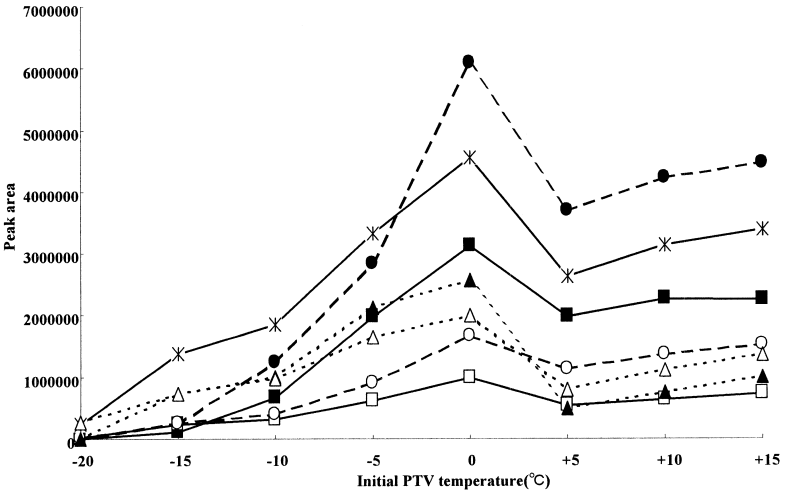

Fig. 3. Effect of initial PTV temperature on peak areas. Delay time between injections: 2 s; purge time: 3.7 min; split vent flow: 50 ml/min; concentration of pesticides 10 ppb. Symbols as in Fig. 2.

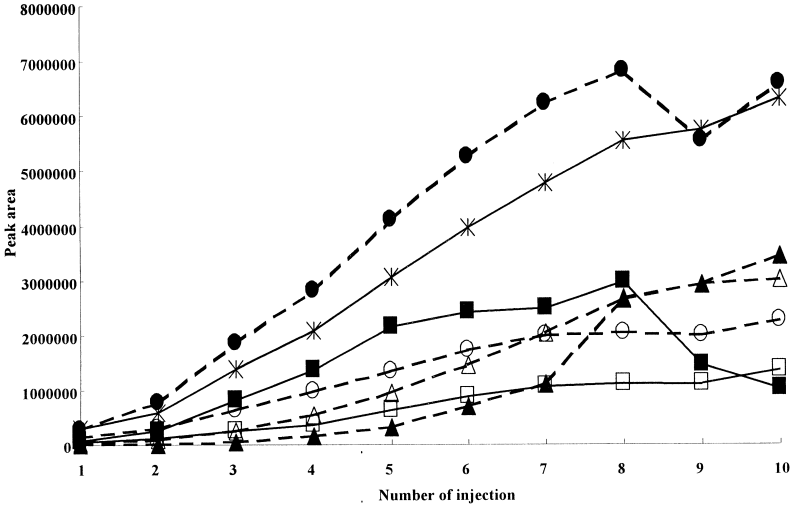

Fig. 4. Effect of the number of injection on peak areas. Initial PTV temperature: 0°C; delay time between injections: 2 s; purge time: 3.7 min; split vent flow: 50 ml/min; concentration of pesticides 10 ppb. Symbols as in Fig. 2.

cedures, the PTV introduced a large sample volume (40 μl) into a narrow-bore column under the analytical conditions described in the Experimental section.

3.2. Translating optimized methods for conventional columns to narrow-bore columns

Using narrow-bore columns, different operational

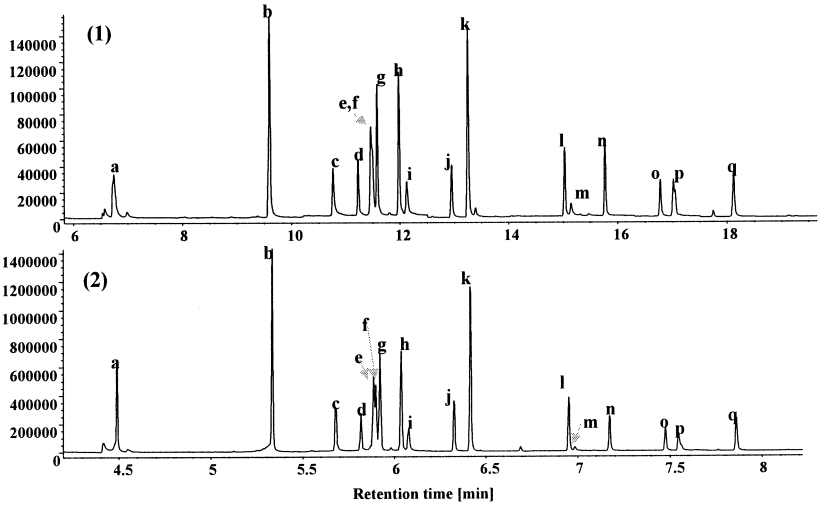

Fig. 5. SIM chromatograms of 17 pesticide standards on (1) a 30 m×0.25 mm I.D., 0.25um HP-1 column, (2) a 10 m×0.1 mm I.D., 0.1 μm HP-1 column (concentration: 10 ppb). Peaks: a=dichlorvos, b=fenobcarb, c=simazine, d=diazinon-oxon, e=propyzamide, f=chlorothalonil, g=diazinon, h=iprobenfos, i=fenitrothion-oxon, j=fenitrothion, k=benthiocarb, l=isoprothiolane, m=isoxathion-oxon, n=isoxathion, o=chloronitrofen, p=EPN, q=EPN-oxon.

Table 1
Analysis of standard solution: limits of detection, correlation coefficients and RSDs of 17 pesticides

Pesticide	Correlation coefficient		LOD, ppt		RSD, %	
	PTV[a] 10 ppt–10 ppb	Splitless[b] 100 ppt–100 ppb	PTV[a] (S/N=3)	Splitless[b] (S/N=3)	PTV[a] (n=5)[c]	Splitless[b] (n=5)[d]
Dichlorvos	0.999	0.992	10	300	5.3	4.5
Fenobcarb	0.999	0.999	5	250	2.4	2.7
Simazine	0.997	0.998	50	140	3.0	2.9
Diazinon-oxon	0.995	0.990	10	500	6.2	5.9
Propyzamide	0.994	0.997	40	180	4.0	3.7
Chlorothalonil	0.998	0.997	40	220	2.8	4.2
Diazinon	0.999	0.996	10	400	1.5	2.5
Iprobenfos	0.997	0.995	20	350	6.9	5.7
Fenitrothion-oxon	0.992	0.995	80	800	9.9	8.7
Fenitrothion	0.995	0.997	20	420	8.1	6.7
Benthiocarb	0.999	0.996	20	120	1.4	2.7
Isoprothiolane	0.999	0.992	20	350	2.5	4.5
Isoxathion-oxon	0.994	0.993	800	5000	9.7	11.2
Isoxathion	0.999	0.997	200	1500	8.5	10.2
Chloronitrofen	0.995	0.994	40	280	8.8	7.8
EPN-oxon	0.997	0.996	300	2200	9.8	8.7
EPN	0.995	0.994	30	860	5.3	5.7

[a] Injection volume: 40 μl (8×5 μl).
[b] Injection volume: 2 μl.
[c] Concentration: 1 ppb.
[d] Concentration: 10 ppb.

conditions have to be used. Since little information is currently available on the use of high-speed capillary GC, the transfer for standard validated operating procedures developed for conventional capillary columns into operating procedures for narrow-bore columns might be difficult and definitely hamper their use in a routine environment. In this respect, the development of method translation software is very helpful for translating standard operating procedures for a conventional column to an operating procedure for a narrow-bore column [2]. After performing the analyses on the standard column, the optimized conditions introduced in the methods for the new column are calculated in order to obtain the best chromatogram. In this work, the usefulness of the method translation software was illustrated with the analysis of 17 pesticides. The chromatograms obtained for the conventional column and narrow-bore column are compared in Fig. 5. From these chromatograms, it is obvious that resolution is very similar on both columns but the separation of propyzamide and chlorothalonil on the narrow-bore

Table 2
Recoveries and LODs for fortified river water at 0.1 ppb

Pesticide	Recovery (%)		
	River water		LOD (ppt) at S/N=3
	Mean	RSD	
Dichlorvos	89.2	5.4	1
Fenobcarb	94.5	6.2	1
Simazine	97.8	6.4	10
Diazinon-oxon	96.7	5.9	1
Propyzamide	85.7	6.7	5
Chlorothalonil	89.6	7.4	5
Diazinon	92.1	8.5	2
Iprobenfos	88.9	6.9	4
Fenitrothion-oxon	91.3	7.7	10
Fenitrothion	90.7	8.1	4
Benthiocarb	85.6	6.3	2
Isoprothiolane	89.2	4.8	2
Isoxathion-oxon	91.4	8.4	100
Isoxathion	88.9	9.1	20
Chloronitrofen	91.6	6.6	5
EPN-oxon	85.7	7.4	30
EPN	83.4	6.7	4

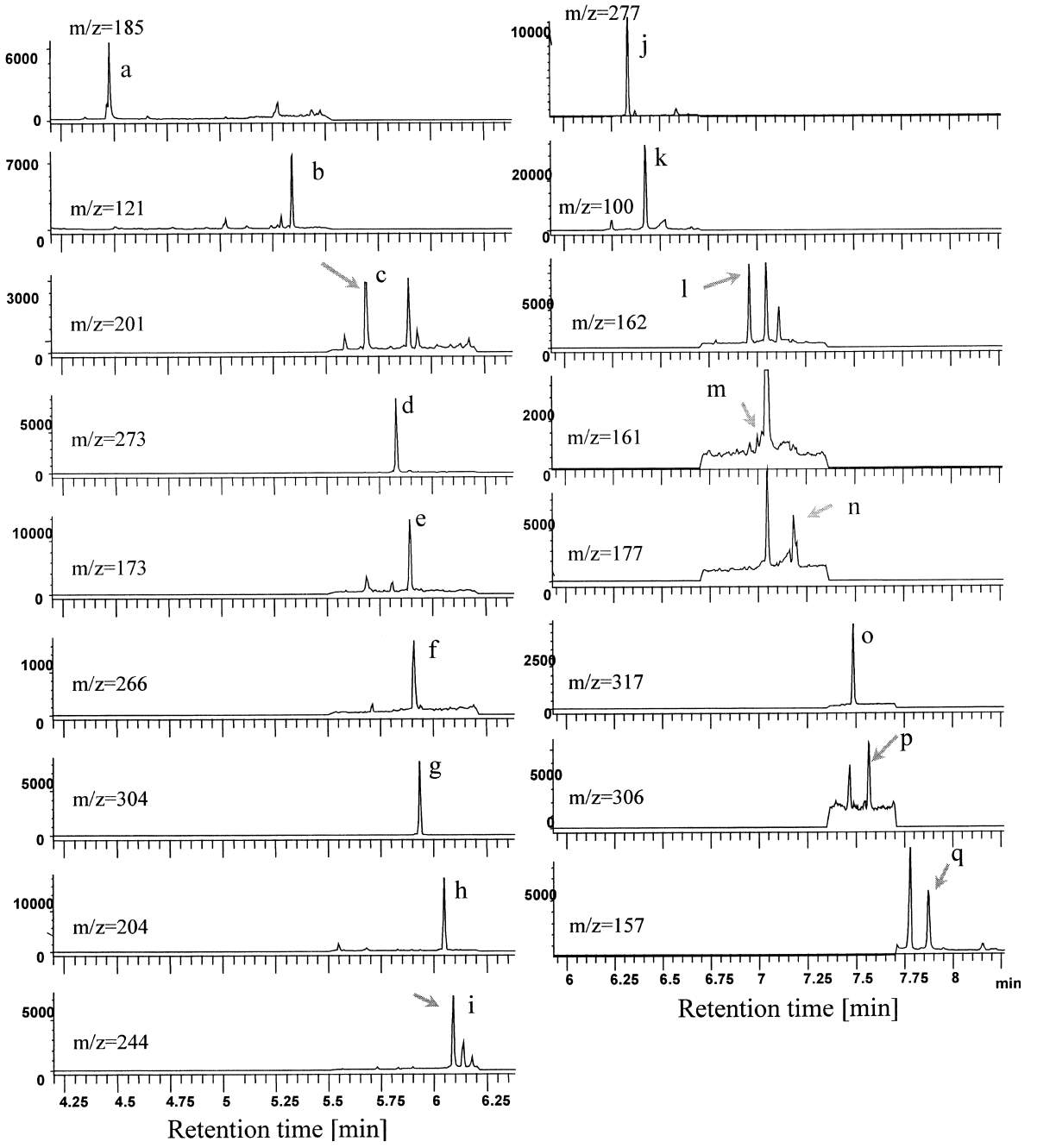

Fig. 6. SIM chromatograms of 17 pesticide standards in river water (concentration: 0.1 ppb). Peaks: a=dichlorvos, b=fenobcarb, c=simazine, d=diazinon-oxon, e=propyzamide, f=chlorothalonil, g=diazinon, h=iprobenfos, i=fenitrothion-oxon, j=fenitrothion, k=benthiocarb, l=isoprothiolane, m=isoxathion-oxon, n=isoxathion, o=chloronitrofen, p=EPN, q=EPN-oxon.

column was better than that on the conventional column. Moreover, the analyses on the narrow-bore column was much faster. From the last eluting peak (EPN), a speed gain factor of 2.3 was calculated.

3.3. Validation of the method

3.3.1. Repeatability

To evaluate the precision of the method developed in this work, analyses of standard mixtures of pesticides with concentrations of 1 ppb were performed. Results are presented in Table 1 with results of the splitless injection. Relative standard deviations (RSDs) of the 17 pesticides ranged from 1.4 to 9.9%. RSDs of oxons were too high because these peak abundances were very small. But the trace analysis of these pesticide metabolites have not been reported for a PTV because of these reactive and thermal liable properties. The RSD data obtained from the PTV were acceptable and similar to those obtained for splitless injection.

3.3.2. Linearity

Analyses of standard solutions with concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 ppb were used to test linearity. Results are presented in Table 1 with results of the splitless injection. Correlation coefficients of the 17 pesticides on the PTV ranged from 0.992 to 0.999. Further, the PTV led to higher correlation coefficients of 12 pesticides than those obtained for the splitless injection. These results illustrate that adsorption of pesticides on the surface of liner used in injector was depressed by large-volume solvents.

3.3.3. Limit of detection

Theoretical LODs were determined taking into account the usual definitions: for each pesticide, a signal equal to three-times the signal-to-noise ratio was considered the LOD. LODs of each pesticide are presented in Table 1. The injection volume for the PTV was 20-times larger than those for splitless injection. Nevertheless, the difference in LODs between the two injections ranged from 3 to 50 times. These results illustrate that there were losses due to liquid rinsing or flooding of the liner and depression of adsorption in the PTV.

3.4. Accuracy

Recovery tests were performed in order to study accuracy. These tests were based on the addition of known amounts of pesticides to samples. The areas of the peaks obtained when these samples were analyzed were compared with the areas of the peaks obtained when analyzing standard solutions with the same concentration by the sample procedure. Mean recoveries and the SIM chromatograms obtained in the analysis of fortified river water are shown in Table 2 and Fig. 6. For the fortified river water, the recoveries were all $>70\%$, which may be considered adequate for a routine analytical method. The SIM chromatograms obtained from fortified river water were reasonably clean and did not show the presence of significant peaks from the river water that prevent the quantitation. In addition, the LODs of all pesticides were much lower than 1/10 of maximum residue levels (MRLs) regulated in Japan.

4. Conclusions

The use of a PTV with solvent vent mode offered new possibilities for the implementation of narrow-bore column in high-speed, large-volume injection. Therefore, this combination appears to be suited for high-speed trace level analysis. Further, this proposed method was applied to the analysis of 17 pesticides residues in river water. In this way, analytical speed could be improved considerably with the same LOD, compared with the conventional column. It was necessary to select an open baffle liner because the pesticides investigated are very reactive and thermal liable compounds. Using this method, a 3–50-fold gain in concentration sensitivity could be achieved, compared with splitless injection. The present system can be used as a routine technique for high-speed and trace analysis.

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