FENITROTHION (No.35)

CIPAC Peer Validation

CIPAC Peer Validation on the Analytical Method of
Tetramethyl Pyrophosphorothioate (TMPP)
in Fenitrothion Technical and Formulations
by Gas Chromatography

by
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1. INTRODUCTION

1.1 Scope

The results of peer validation on the analytical method for the determination of tetramethyl pyrophosphorothioate (TMPP) in fenitrothion technical material, fenitrothion wettable powder, fenitrothion emulsifiable concentrate and fenitrothion ultra-low volume liquid are reported.

The peer validation was performed under draft CIPAC guideline for analytical methods for the determination of relevant impurities referred to in FAO and/or WHO specifications for pesticide technical grade active ingredients and formulations (CIPAC/4622).

1.2 Samples

1) Fenitrothion technical (TC)
2) Fenitrothion wettable powder (WP)
3) Fenitrothion emulsifiable concentrate (EC-1)
4) Fenitrothion emulsifiable concentrate (EC-2)
5) Fenitrothion ultra-low volume liquid (UL-1)
6) Fenitrothion ultra-low volume liquid (UL-2)

1.3 Participants

Hirofumi Ushino	Hayashi Agro Science, Ltd. (JAPAN)
Hiroki Tokunaga	Hodogaya Contract Laboratory, Co., Ltd. (JAPAN)
Minako Yamamoto	Koei Techno Co., Ltd. (JAPAN)
Yasushi Asada	Sumitomo Chemical Co., Ltd., Organic Synthesis Research Laboratory (JAPAN)

2. ANALYTICAL METHOD

2.1 Outline of Method

TMPP in the test samples is determined by capillary gas chromatography using flame ionisation detection and internal standardisation (CIPAC/4660/m).

2.2 Method Development Prior to Peer Validation

The analytical method was developed by Osaka Laboratory, Sumika Chemical Analysis Service, Ltd., and accordingly the following data were obtained by this laboratory.

1) Confirmation of Analyte Identification

GC/MS spectrum of TMPP was obtained for standard and sample solutions under the operating conditions described in CIPAC/4660/m. TMPP was spiked to the sample solutions because TMPP was not contained in the technical material and formulations used for this study.

2) Specificity

Retention times of the ingredients in fenitrothion technical material and formulants in each formulation were checked with the solutions of TMPP standard, internal standard and fenitrothion technical material and formulations to check non-analyte interference.

3) Linearity

Calibration solutions whose concentrations were about 0.3, 0.6 and 0.9 mg/20ml were prepared respectively in duplicate using TMPP standard under the presence of fenitrothion technical material. The solutions were analyzed and the peak area ratios of TMPP to internal standard were plotted against the concentration of TMPP to make a calibration line.

4) Accuracy

The stock solution at an appropriate concentration of TMPP was fortified to fenitrothion technical material and formulations so that the fortified concentration of TMPP was at the level of specification. These solutions were analyzed, and the recoveries were calculated by the following equation:

$$R = \frac{C - C_0}{C_S} \times 100$$

where, R : recovery (%)
C : observed concentration (g/kg) of TMPP
C_0 : initial concentration (g/kg) of TMPP in fenitrothion technical material and formulations
C_S : fortified concentration (g/kg) of TMPP

5) Repeatability

Five separate sub-samples from a sample of fenitrothion technical material and formulations were analysed. Appropriate amount of TMPP was spiked to the samples because the contents of TMPP in the technical material and formulations were not enough to evaluate repeatability. Mean and relative standard deviation (RSD) were calculated from the analytical values.

6) Limit of Quantitation (LOQ)

The stock solution at an appropriate concentration of TMPP was fortified to fenitrothion technical material and formulations so that the signal to noise ratio of TMPP was about 10 on the chromatogram. These solutions were analyzed and signal to noise ratios were evaluated.

2.3 Peer Validation

The peer validation was conducted with four independent laboratories through the network of JAPAC. The participants are shown in **1.3.**

We requested the collaborators to conduct peer validation according to the prescribed protocol, describe operating conditions in detail, and attach the calibration curve and all chromatograms for each sample.

The investigated items are specificity, linearity, repeatability and LOQ. The details of each procedure are the same as those described in **2.2.**

3. REMARKS OF PARTICIPANTS

3.1 Analytical Conditions

Lab	Gas chromato-graph Integrator	Column	Column temp	Flow rate	Split flow (ml/min)
Proposed Method		DB-1 (0.25mm i.d.×30m, 1 μm)	100°C (0 min), ramp at 10°C/min to 300°C, then hold at 300°C for 20 min	He, 35 cm/min	20
1	Shimadzu GC-17A Shimadzu GCsolution	DB-1 (0.25mm i.d.×30m, 1 μm)	100°C (0 min), ramp at 10°C/min to 300°C, then hold at 300°C for 20 min	He, 1.2 ml/min	20
2	Shimadzu GC-2010 Shimadzu GCso-lution	DB-1 (0.25mm i.d.×30m, 1 μm)	100°C (0 min), ramp at 10°C/min to 300°C	He, 1.0 ml/min	20
3	Agilent 6890N Agilent Chem-Station	DB-1 (0.25mm i.d.×30m, 1 μm)	100°C (0 min), ramp at 10°C/min to 300°C, then hold at 300°C for 20 min	He, 1.0 ml/min	20
4	Shimadzu GC-2010 Shimadzu GCsolution	DB-1 (0.25mm i.d.×30m, 1 μm)	100°C (0 min), ramp at 10°C/min to 300°C, then hold at 300°C for 20 min	He, 1.0 ml/min	20

4. RESULTS AND DISCUSSION

4.1 Method Development

1) Confirmation of Analyte Identification

The total ion chromatograms and relevant MS spectra of the TMPP standard solution and sample solutions are shown in Figure 1. The MS spectra were consistent with the structure of TMPP.

2) Specificity

The peak of TMPP was separated from the peaks of fenitrothion and its related compounds in fenitrothion technical material. The peak was also separated from the peaks from formulants and peak of internal standard (Figure 2).

3) Linearity

The calibration line is shown in Figure 3. The equation of the calibration line and the correlation factor (*r*) are shown in the figure. The correlation factor was satisfactory, and the response of TMPP was linear over the concentration range of 0.3 to 0.9 mg/20 ml.

4) Accuracy

The recoveries were satisfactory as shown in Table 1.

5) Repeatability

The repeatability of this method was satisfactory with RSD values of 1.0 – 7.8 % as shown in Table 4.

6) LOQ

The quantitation limits of TMPP in TC and formulations were estimated to be 0.1 – 0.3 g/kg as shown in Table 3.

4.2 Peer Validation

1) Specificity

The peak of TMPP was separated from the peaks of fenitrothion and its related compounds in fenitrothion technical material. The peak was also separated from the peaks from formulants and peak of internal standard (Figure 4).

2) Linearity

The calibration line is shown in Figure 5. The equation of the calibration line and the correlation factor are shown in the figure. The correlation factor was satisfactory, and the response of TMPP was linear over the concentration range of 0.3 to 0.9 mg/20 ml.

3) Repeatability

The repeatability of this method was satisfactory with RSD values of 0.5 – 7.0 % as shown in Table 2. All RSD values were found to be smaller than 20%.

4) LOQ

The quantitation limits of TMPP in TC and formulations were estimated to be 0.2 – 1.6 g/kg as shown in Table 3.

5. CONCLUSION

For all samples, the analytical method was peer-validated in terms of specificity, linearity, accuracy, quantitation limit and repeatability. The RSDs of repeatability for technical material and all formulations were found to be smaller than 20% for all laboratories participated in this peer validation.

In conclusion, the proposed method was successfully peer-validated and was considered appropriate for the determination of TMPP in technical material, wettable powder, emulsifiable concentrate and ultra-low volume liquid.

Table 1 Recovery of TMPP

No.	Recovery (%)					
	TC	WP	EC-1	EC-2	UL-1	UL-2
1	85.6	83.1	93.0	78.3	95.6	84.5
2	86.1	81.8	108.6	77.9	96.7	84.4
Mean	85.9	82.5	100.8	78.1	96.2	84.5

Table 2 Repeatability for the Determination of TMPP

No.	Recovery (%)					
	TC	WP	EC-1	EC-2	UL-1	UL-2
1	2.80	1.09	1.53	1.88	1.56	2.21
2	2.85	1.07	1.78	1.88	1.58	2.21
3	2.85	1.11	1.51	1.88	1.54	2.18
4	2.87	1.09	1.54	1.87	1.51	2.21
5	2.90	1.09	1.73	1.83	1.52	2.24
Mean	2.85	1.09	1.62	1.87	1.54	2.21
RSD	1.3	1.3	7.8	1.2	1.9	1.0

Table 3 LOQ of TMPP

Item	TC	WP	EC-1	EC-2	UL-1	UL-2
LOQ (g/kg)	0.3	0.1	0.2	0.2	0.2	0.2
S/N at LOQ	9.1	6.9	6.0	6.4	12.4	6.3

Table 4 Repeatability for the Determination of TMPP

Lab	No.	Recovery (%)					
		TC	WP	EC-1	EC-2	UL-1	UL-2
1	1	2.93	1.22	1.24	2.01	1.39	2.37
	2	2.84	1.12	1.27	2.27	1.33	2.36
	3	2.96	1.12	1.17	1.97	1.43	2.41
	4	2.70	1.12	1.19	2.11	1.52	2.40
	5	2.71	1.10	1.30	2.10	1.44	2.38
	Mean	2.83	1.14	1.23	2.09	1.42	2.38
	RSD	4.3	4.2	4.4	5.5	4.9	0.9
2	1	2.97	1.25	1.30	1.77	1.63	2.48
	2	2.95	1.23	1.12	1.63	1.58	2.53
	3	2.97	1.24	1.12	1.70	1.61	2.48
	4	3.01	1.22	1.15	1.93	1.60	2.50
	5	3.01	1.25	1.10	1.66	1.63	2.52
	Mean	2.98	1.24	1.16	1.74	1.61	2.50
	RSD	0.9	1.1	7.0	6.9	1.3	0.9
3	1	3.33	1.39	1.57	1.92	1.70	2.64
	2	3.26	1.37	1.42	1.73	1.66	2.64
	3	3.31	1.38	1.48	1.93	1.67	2.64
	4	3.41	1.35	1.43	2.01	1.66	2.57
	5	3.10	1.36	1.41	1.95	1.65	2.42
	Mean	3.28	1.37	1.46	1.91	1.67	2.58
	RSD	3.5	1.2	4.5	5.5	1.2	3.7
4	1	2.92	1.16	1.23	1.81	1.57	2.53
	2	2.93	1.17	1.16	1.80	1.58	2.52
	3	2.93	1.20	1.17	1.92	1.59	2.52
	4	2.88	1.16	1.17	1.85	1.60	2.55
	5	3.00	1.19	1.16	1.87	1.57	2.52
	Mean	2.93	1.18	1.18	1.85	1.58	2.53
	RSD	1.5	1.5	2.5	2.6	0.8	0.5

Table 5 LOQ of TMPP

Lab	Item	TC	WP	EC-1	EC-2	UL-1	UL-2
1	LOQ (g/kg)	0.2	0.6	1.6	1.2	1.6	1.3
	S/N at LOQ	11.7	8.0	11.5	8.1	11.0	7.2
2	LOQ (g/kg)	0.6	0.3	0.3	0.5	0.3	0.5
	S/N at LOQ	12.5	7.0	12.3	11.8	8.0	10.6
3	LOQ (g/kg)	0.6	0.2	0.4	0.5	0.3	0.4
	S/N at LOQ	9.7	9.7	10.2	9.5	9.7	9.9
4	LOQ (g/kg)	0.3	0.1	0.2	0.2	0.2	0.2
	S/N at LOQ	10.8	8.2	8.7	8.9	10.2	11.2

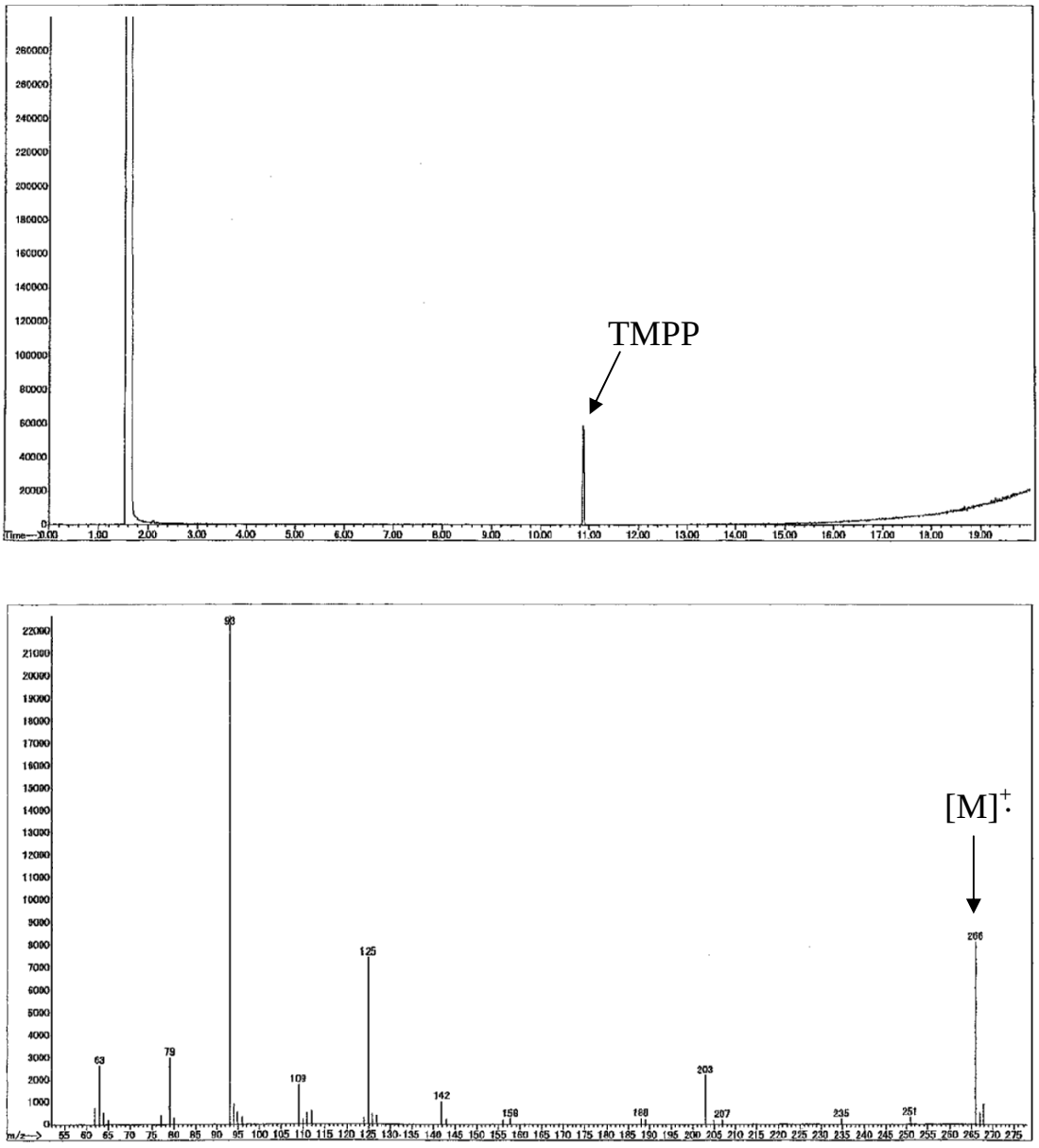

Figure 1-1 GC/MS Spectra of TMPP standard Solution
(Upper, Total Ion Chromatogram; Lower, MS Spectrum of TMPP)

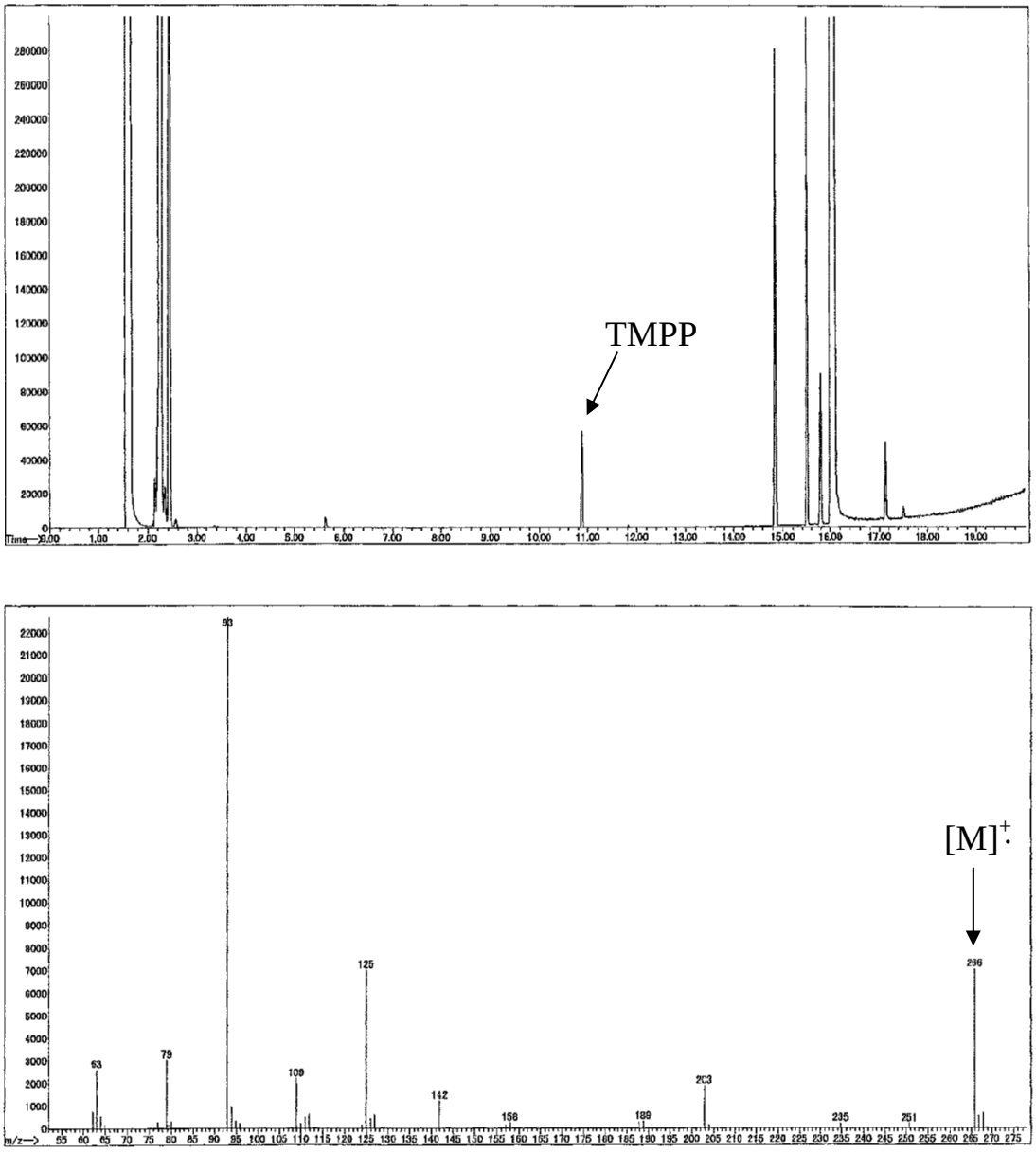

Figure 1-2 GC/MS Spectra of Sample Solution (Fenitrothion TC)
(Upper, Total Ion Chromatogram; Lower, MS Spectrum of TMPP)

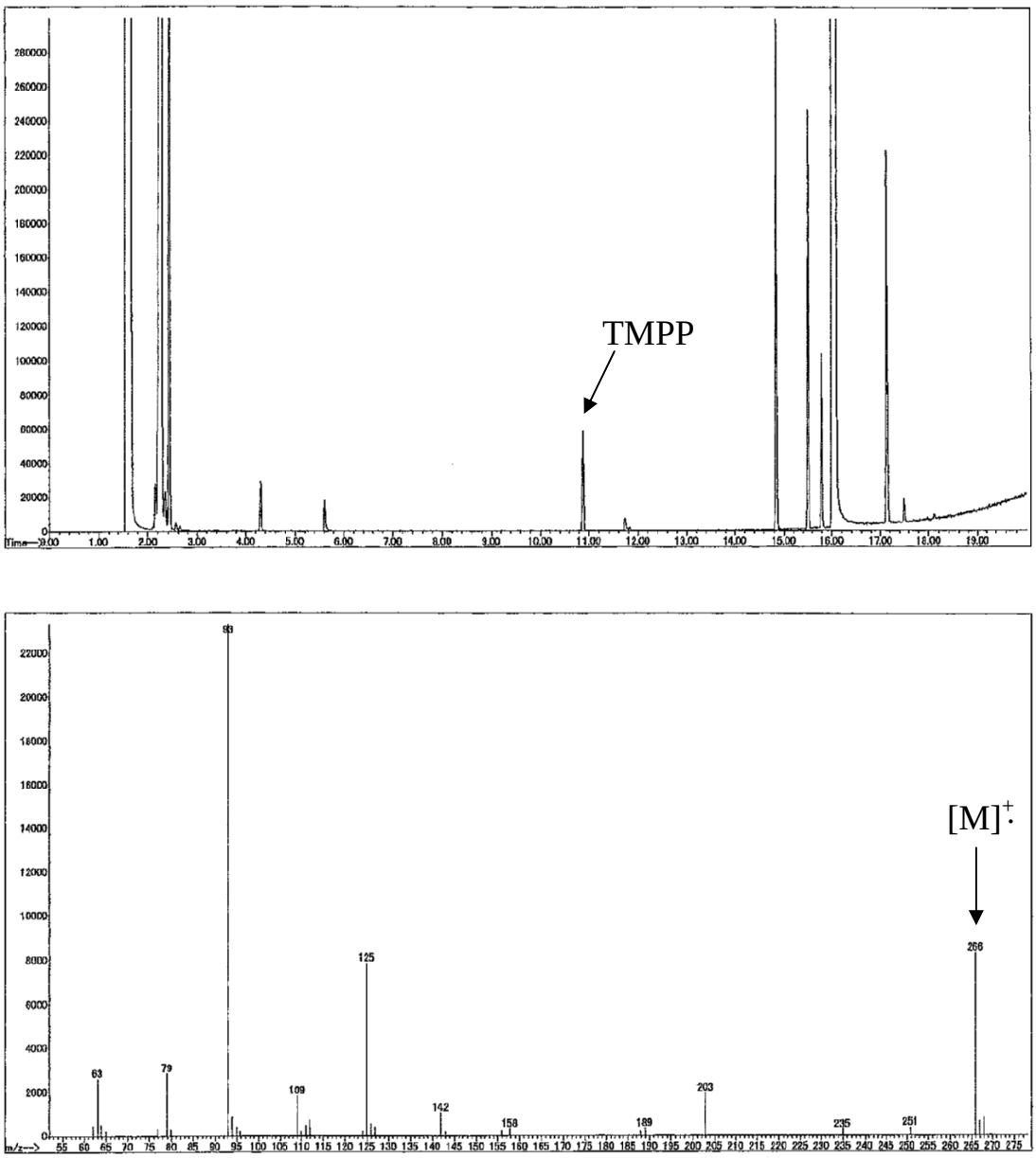

Figure 1-3 GC/MS Spectra of Sample Solution (Fenitrothion WP)
(Upper, Total Ion Chromatogram; Lower, MS Spectrum of TMPP)

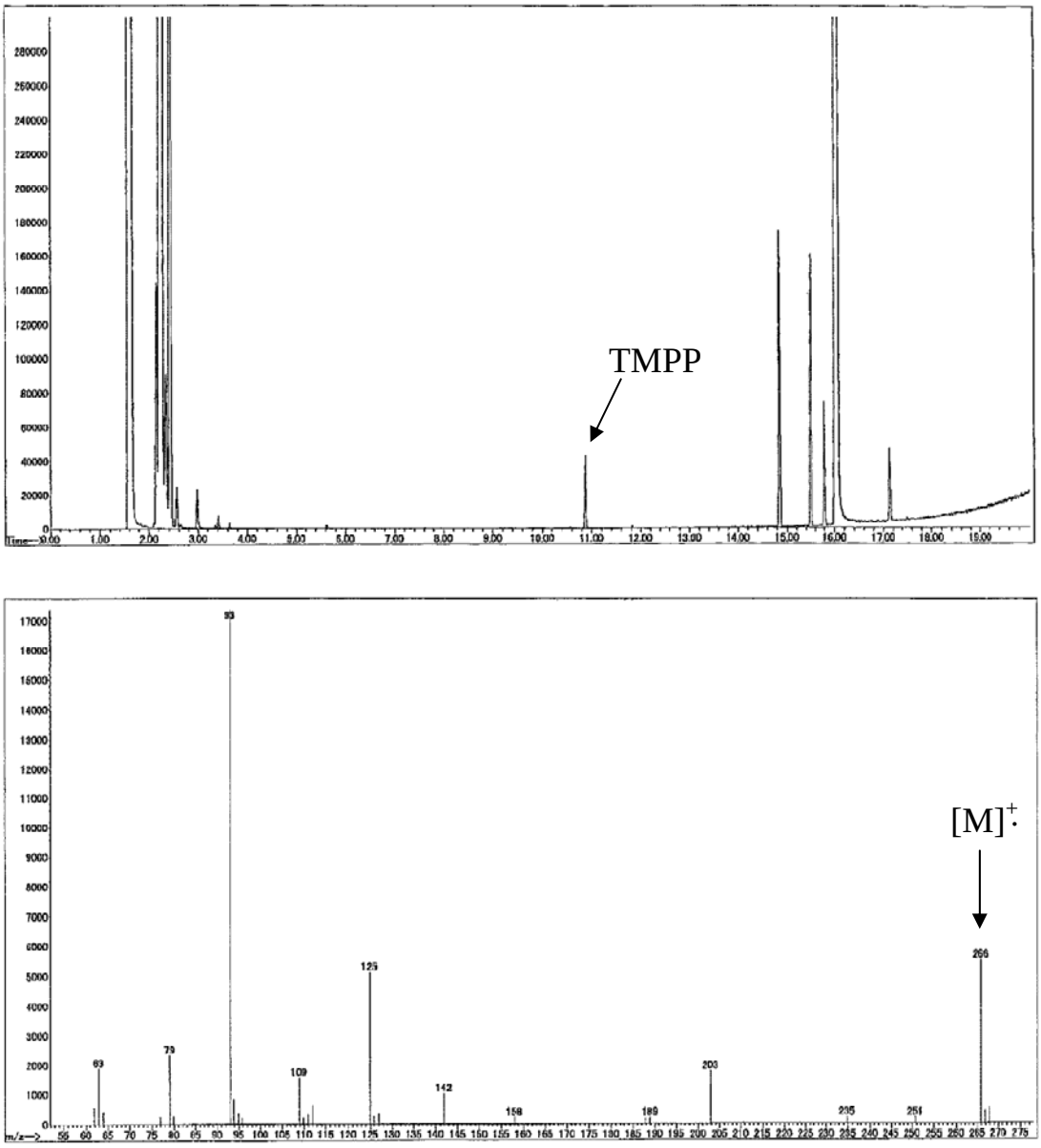

Figure 1-4 GC/MS Spectra of Sample Solution (Fenitrothion EC-1)
(Upper, Total Ion Chromatogram; Lower, MS Spectrum of TMPP)

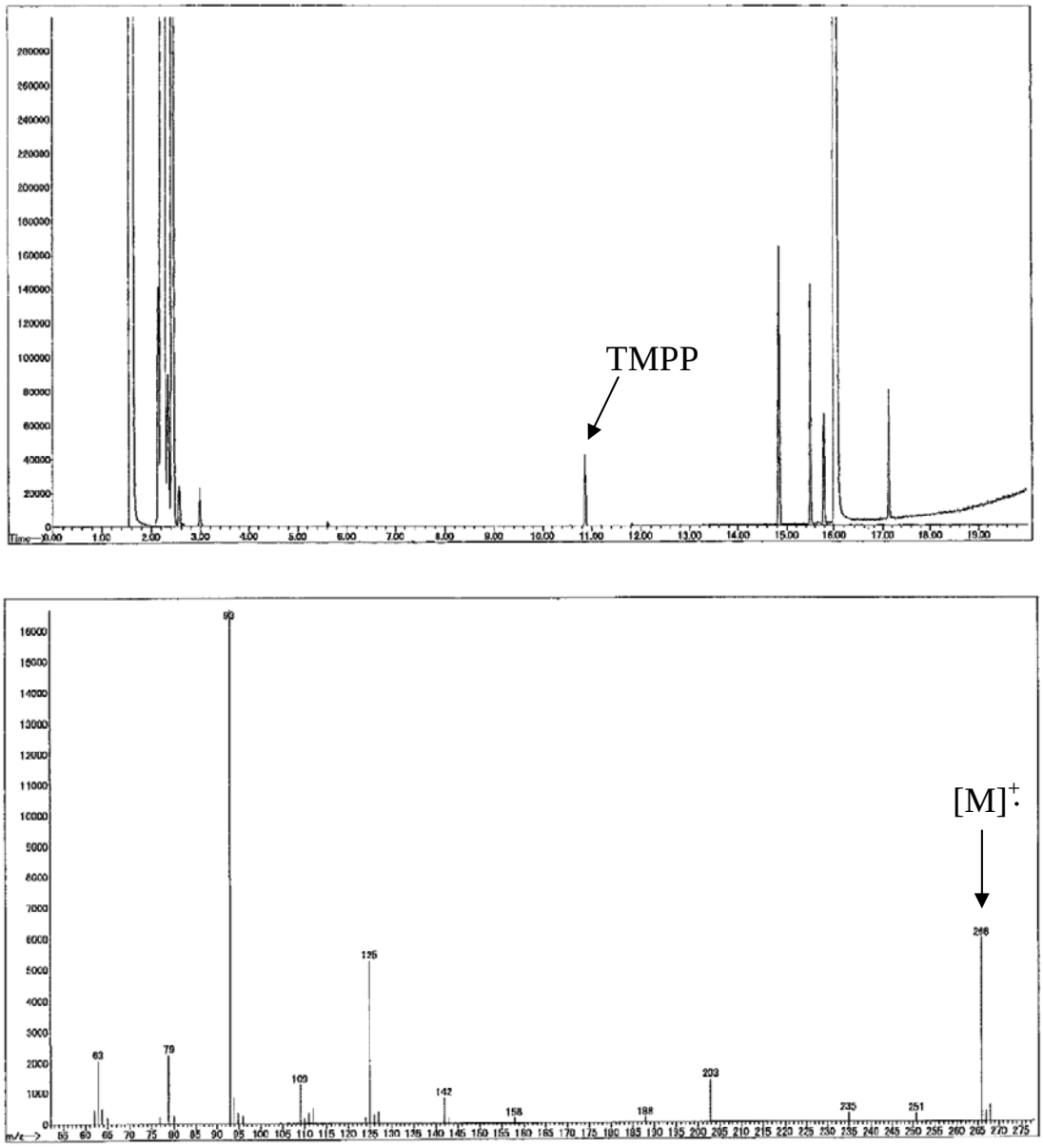

Figure 1-5 GC/MS Spectra of Sample Solution (Fenitrothion EC-2)
(Upper, Total Ion Chromatogram; Lower, MS Spectrum of TMPP)

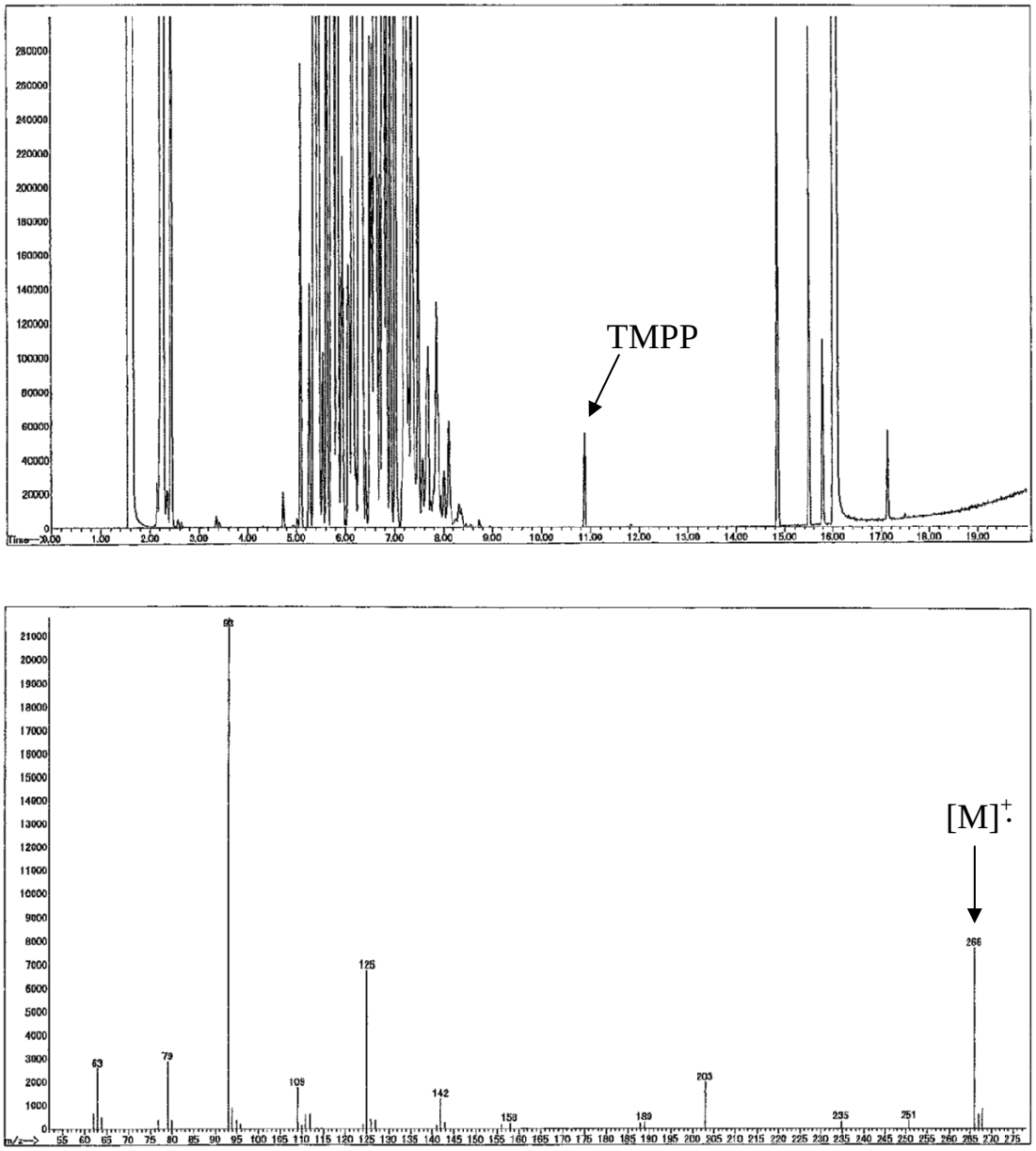

Figure 1-6 GC/MS Spectra of Sample Solution (Fenitrothion UL-1)
(Upper, Total Ion Chromatogram; Lower, MS Spectrum of TMPP)

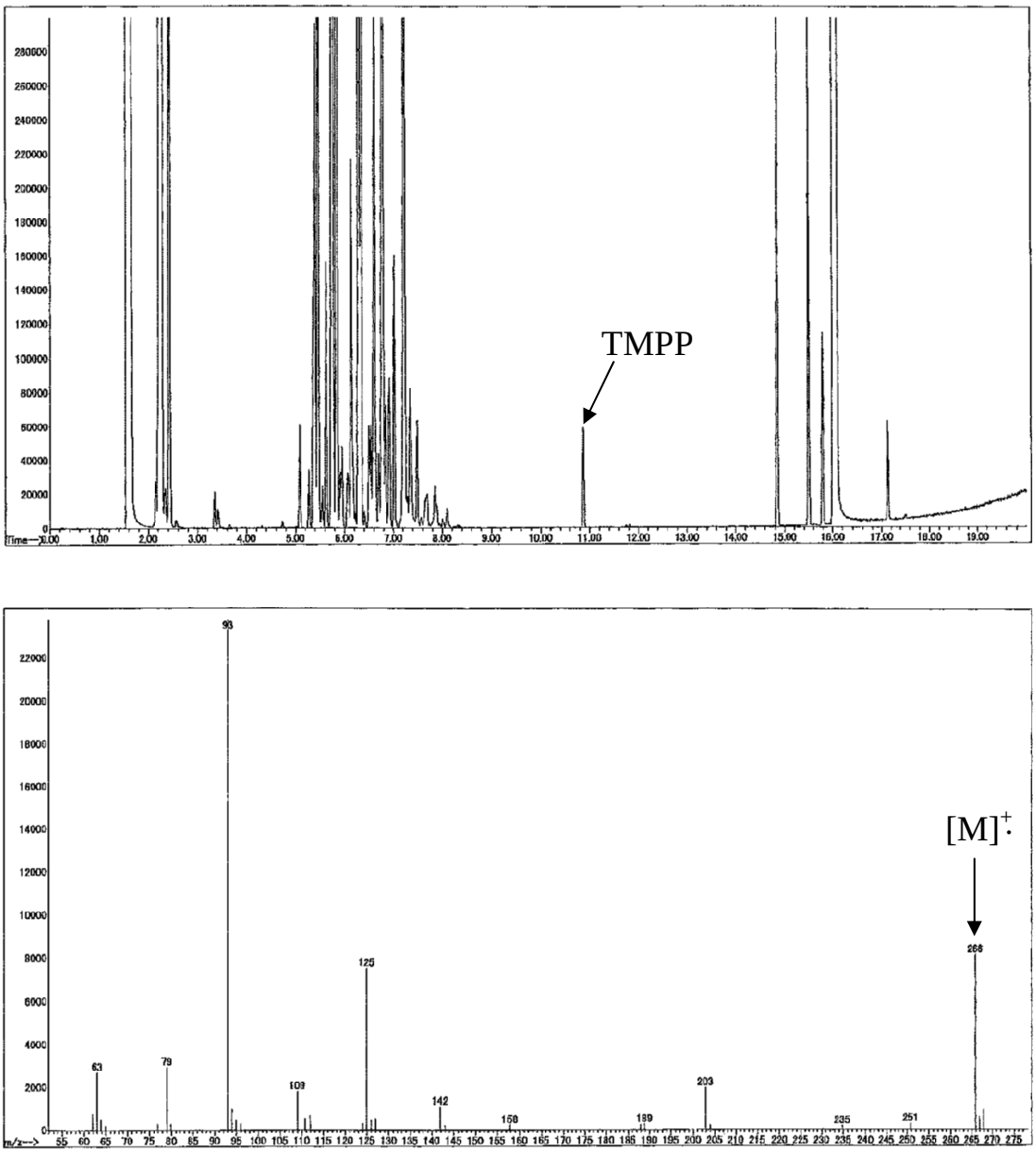

Figure 1-7 GC/MS Spectra of Sample Solution (Fenitrothion UL-2)
(Upper, Total Ion Chromatogram; Lower, MS Spectrum of TMPP)

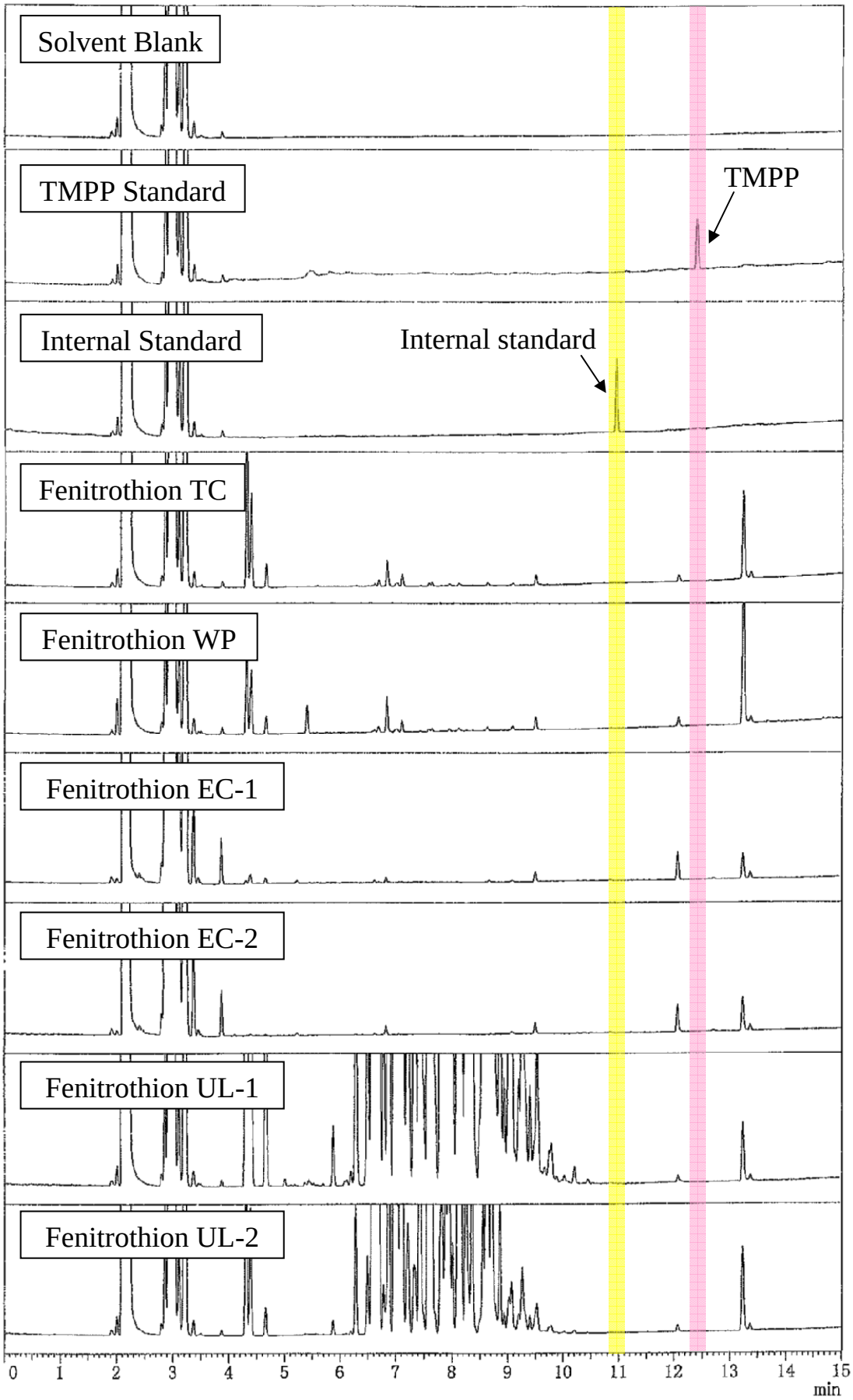

Figure 2 Specificity of Analytical Method

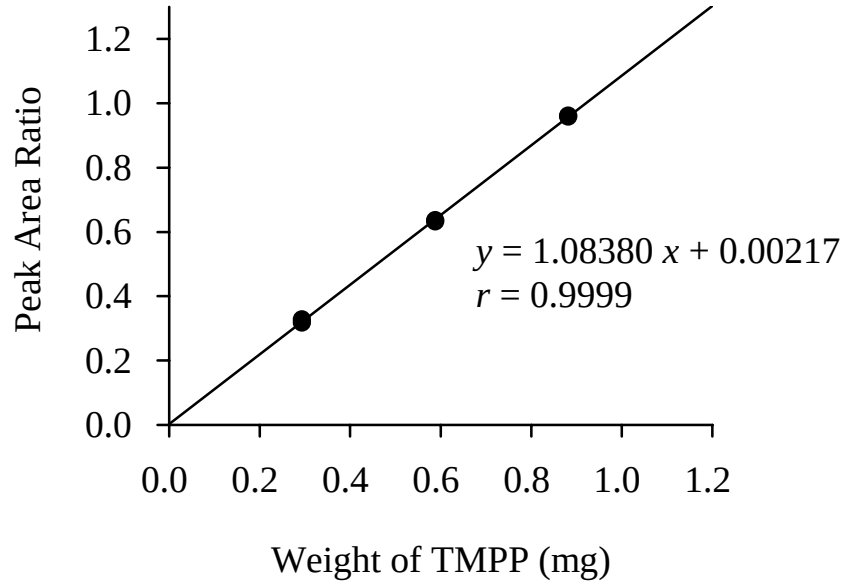

Figure 3 Calibration curve for the determination of TMPP

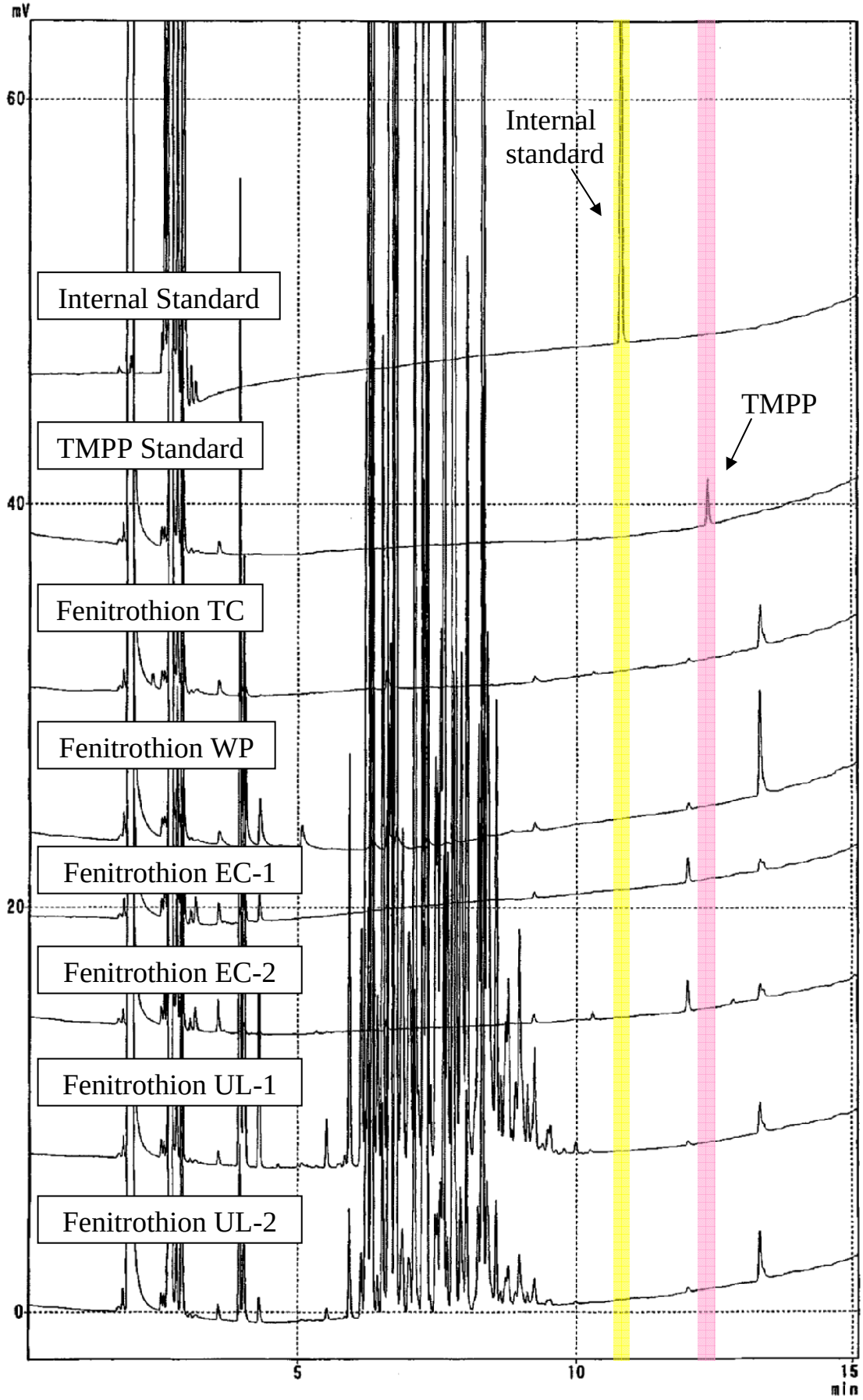

Figure 4-1 Specificity of TMPP (Lab 1)

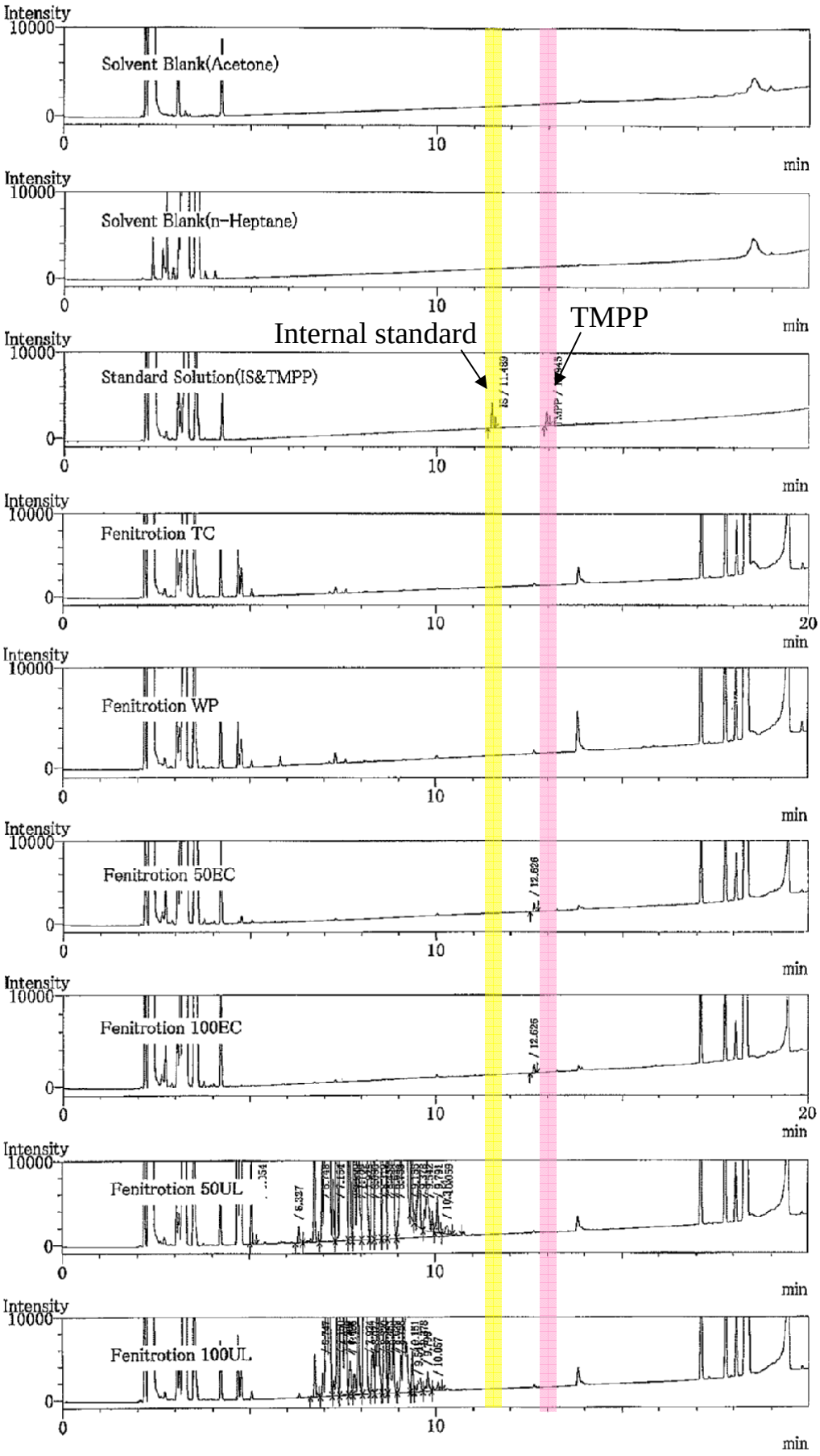

Figure 4-2 Specificity of TMPP (Lab 2)

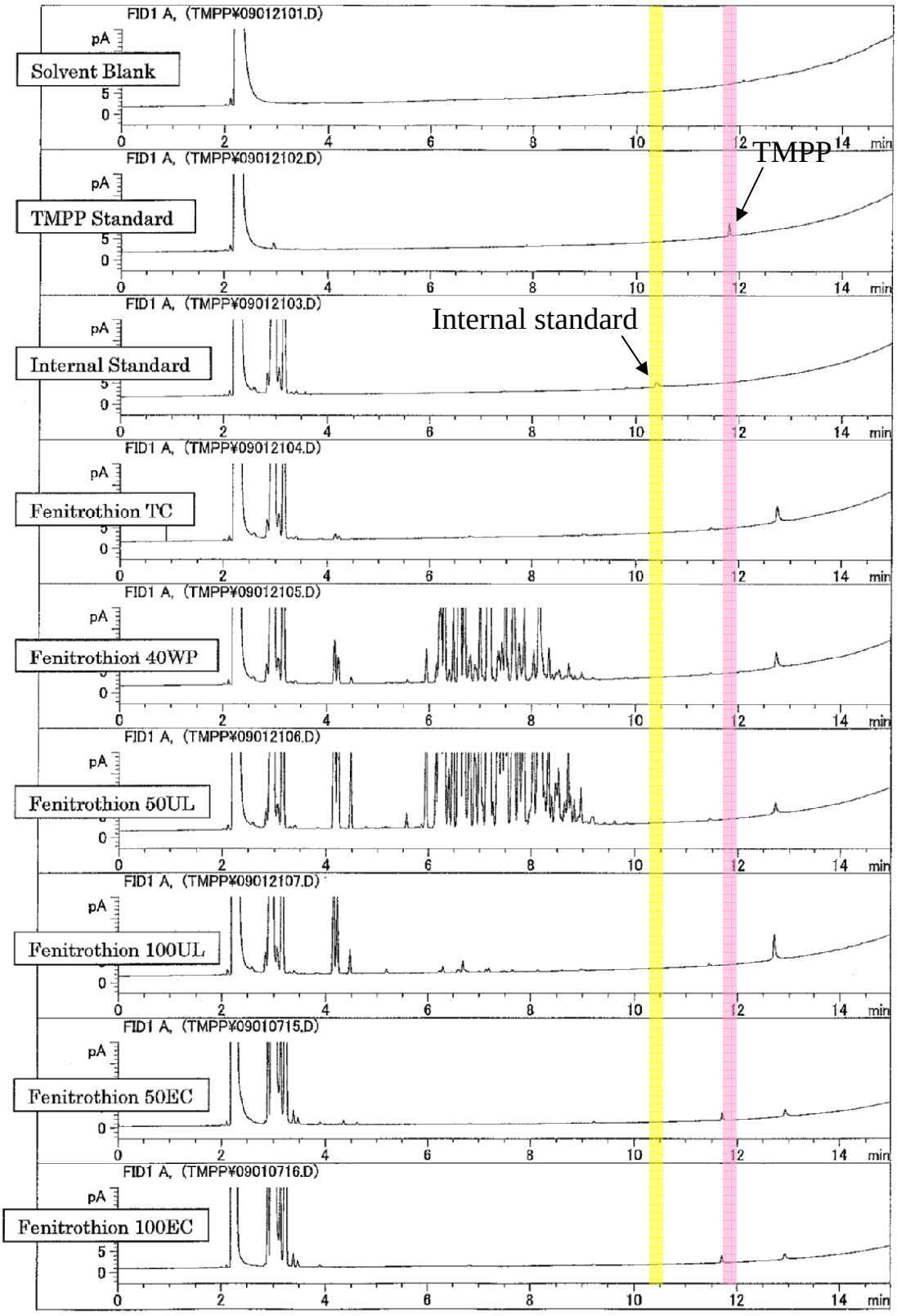

Figure 4-3 Specificity of TMPP (Lab 3)

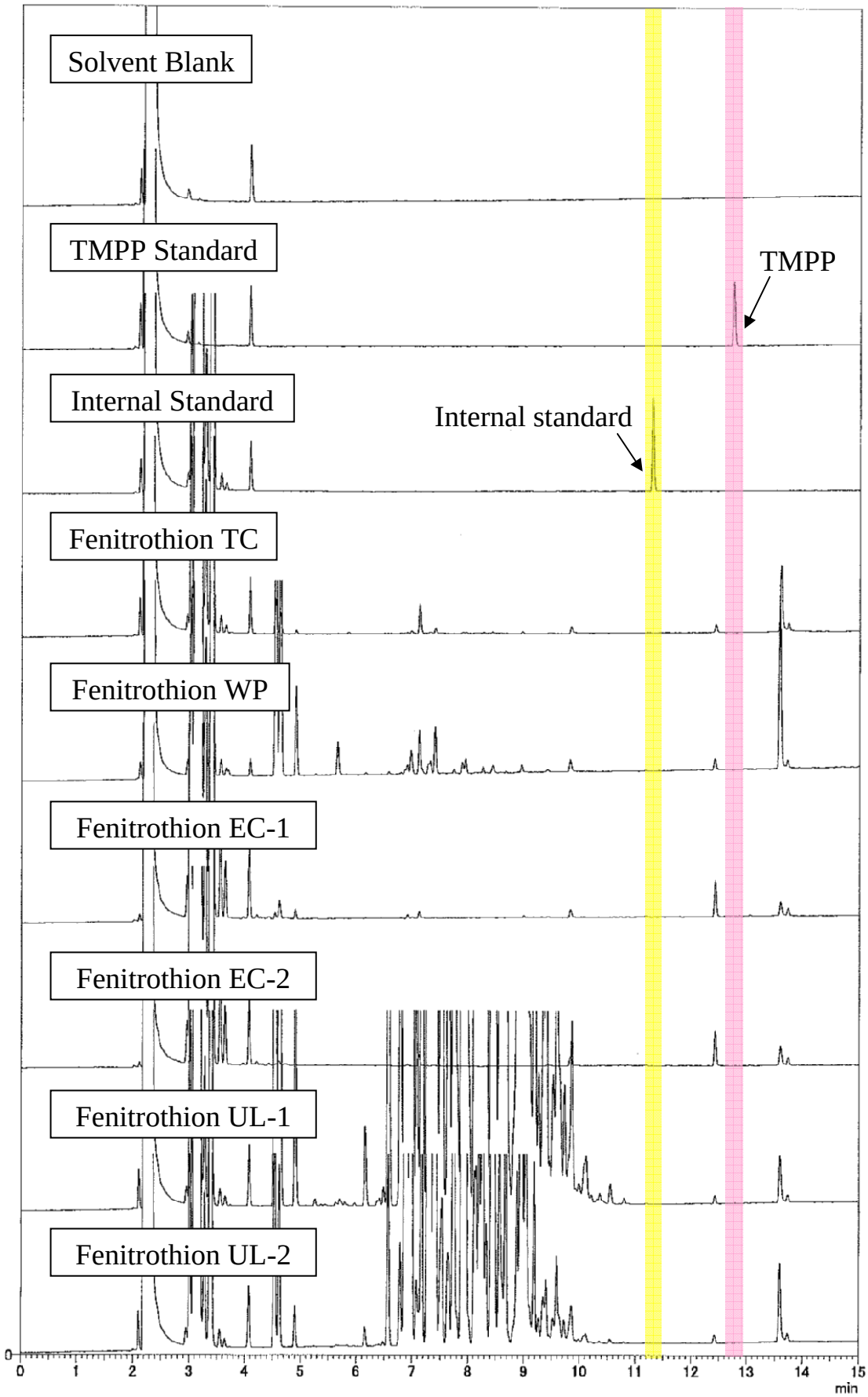

Figure 4-4 Specificity of TMPP (Lab 4)

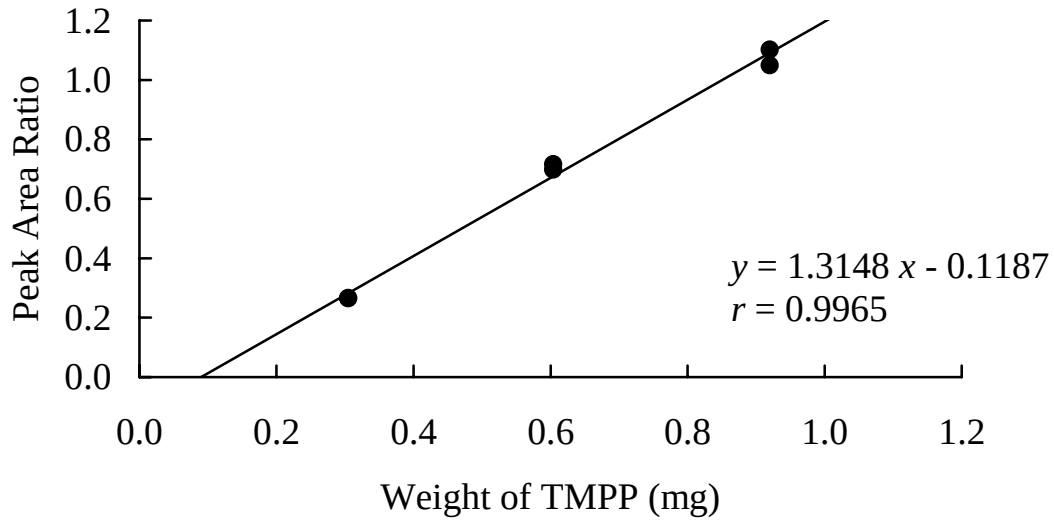

Figure 5-1 Linearity of TMPP (Lab 1)

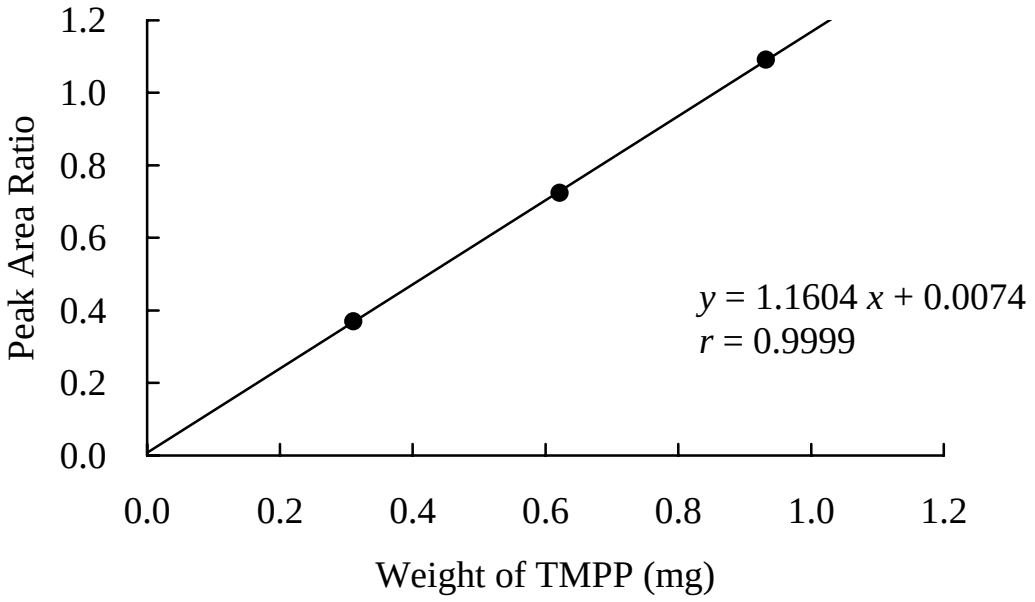

Figure 5-2 Linearity of TMPP (Lab 2)

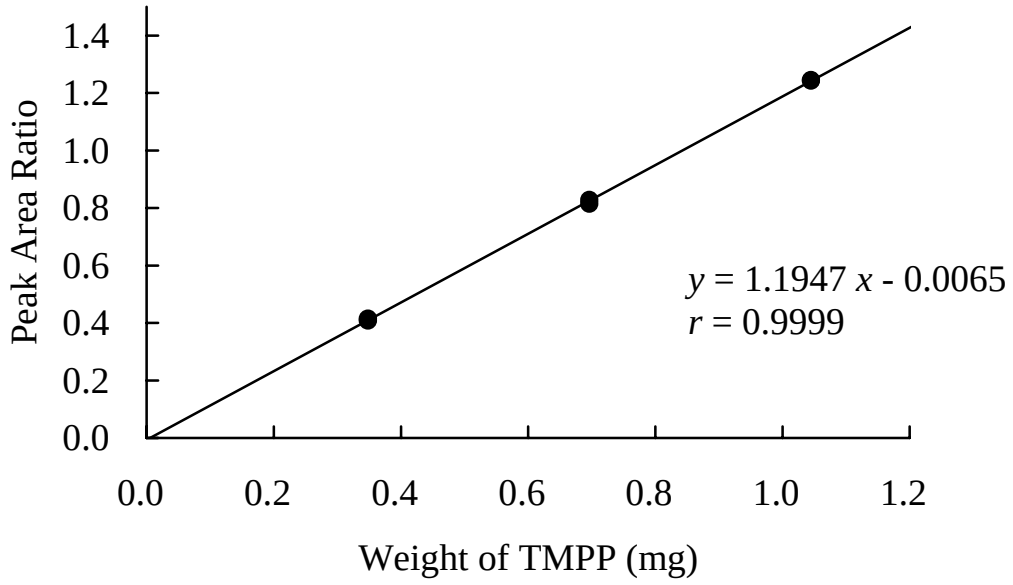

Figure 5-3 Linearity of TMPP (Lab 3)

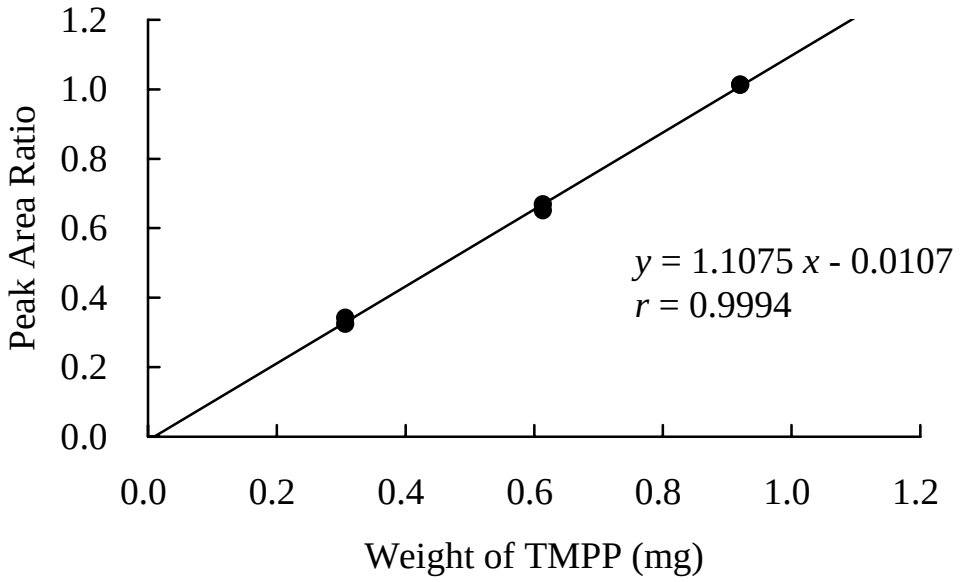

Figure 5-4 Linearity of TMPP (Lab 4)