N-Methyl carbamate pesticides analysis system

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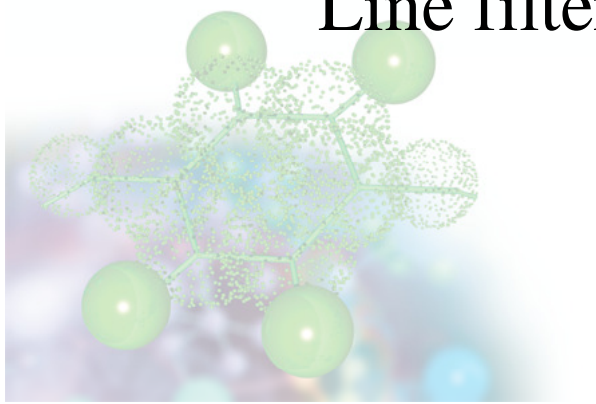
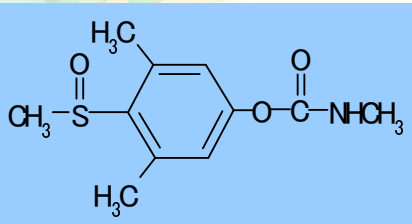
1. Three(3) types of measurement conditions
    1. Official methods
    2. 11 kinds of components simultaneous analysis
    3. 18 kinds of components simultaneous analysis
2. High sensitivity fluorescence detection
3. Tools for stable and high sensitivity analysis
   Line filter, Clean up column

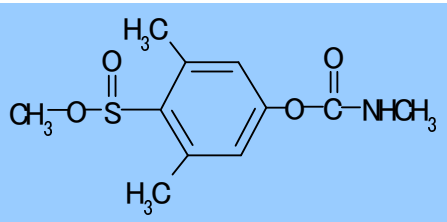


Structural formula of carbamate pesticides

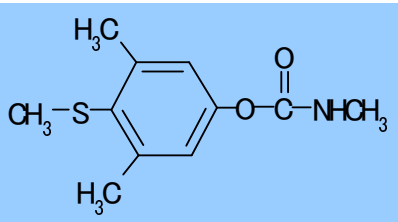
Oxamyl



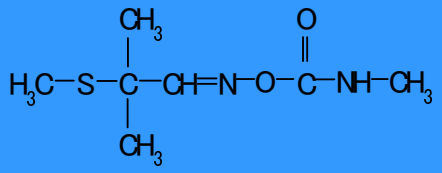
Sulfoxide



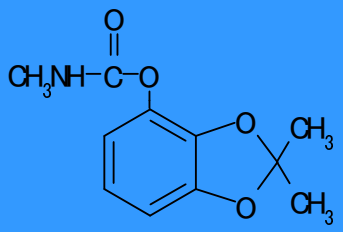
Methiocarb Sulfone



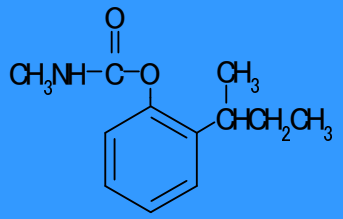
Methiocarb



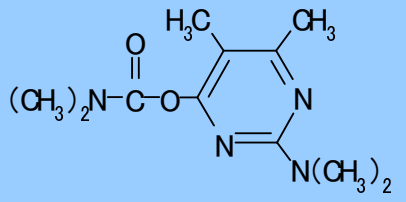
Aldicarb



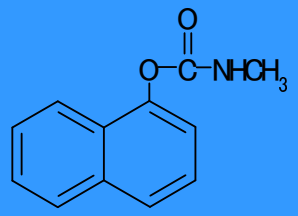
Bendiocarb



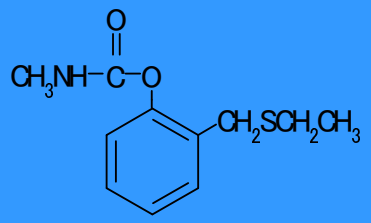
Fenobucarb



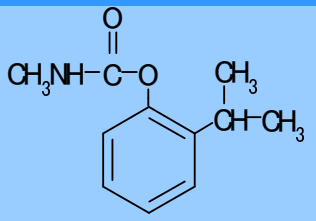
Pirimicarb



Carbaryl(NAC)



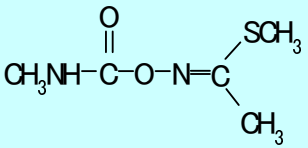
Ethiofencarb



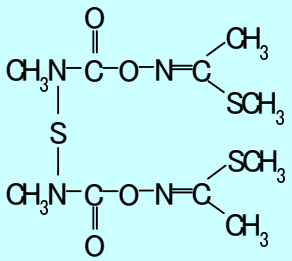
Isoprocarb

Metholcarb(MTMC)

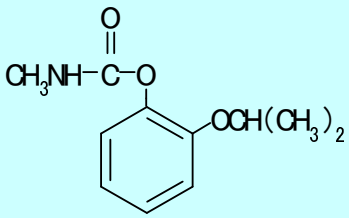
Xylylcarb(MPMC)



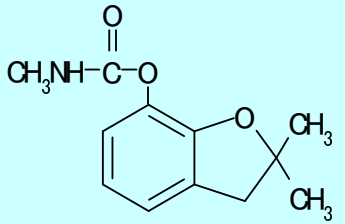
Methomyl



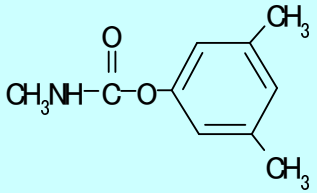
Thiodicarb



Propoxur(PHC)



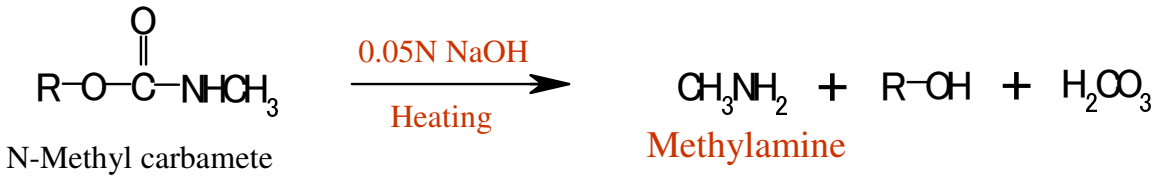
Carbofuran



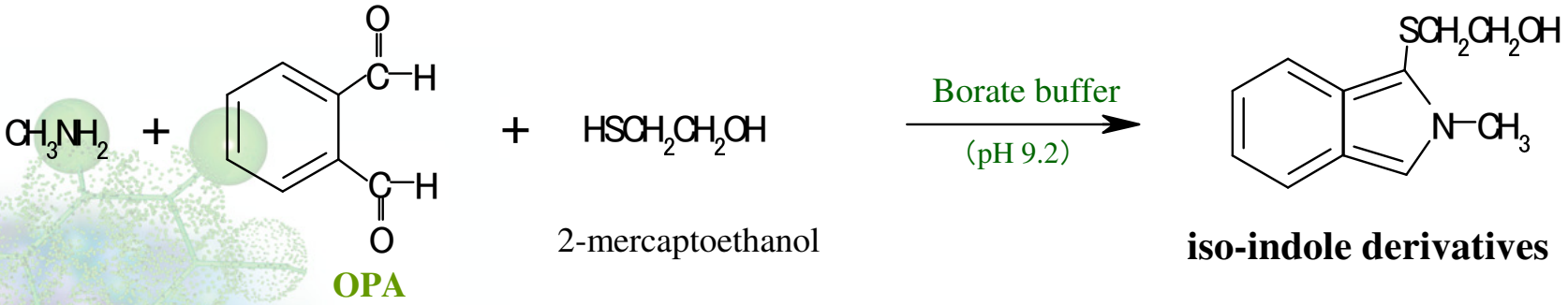
Macbal(XMC)

Principle of carbamate pesticides analysis

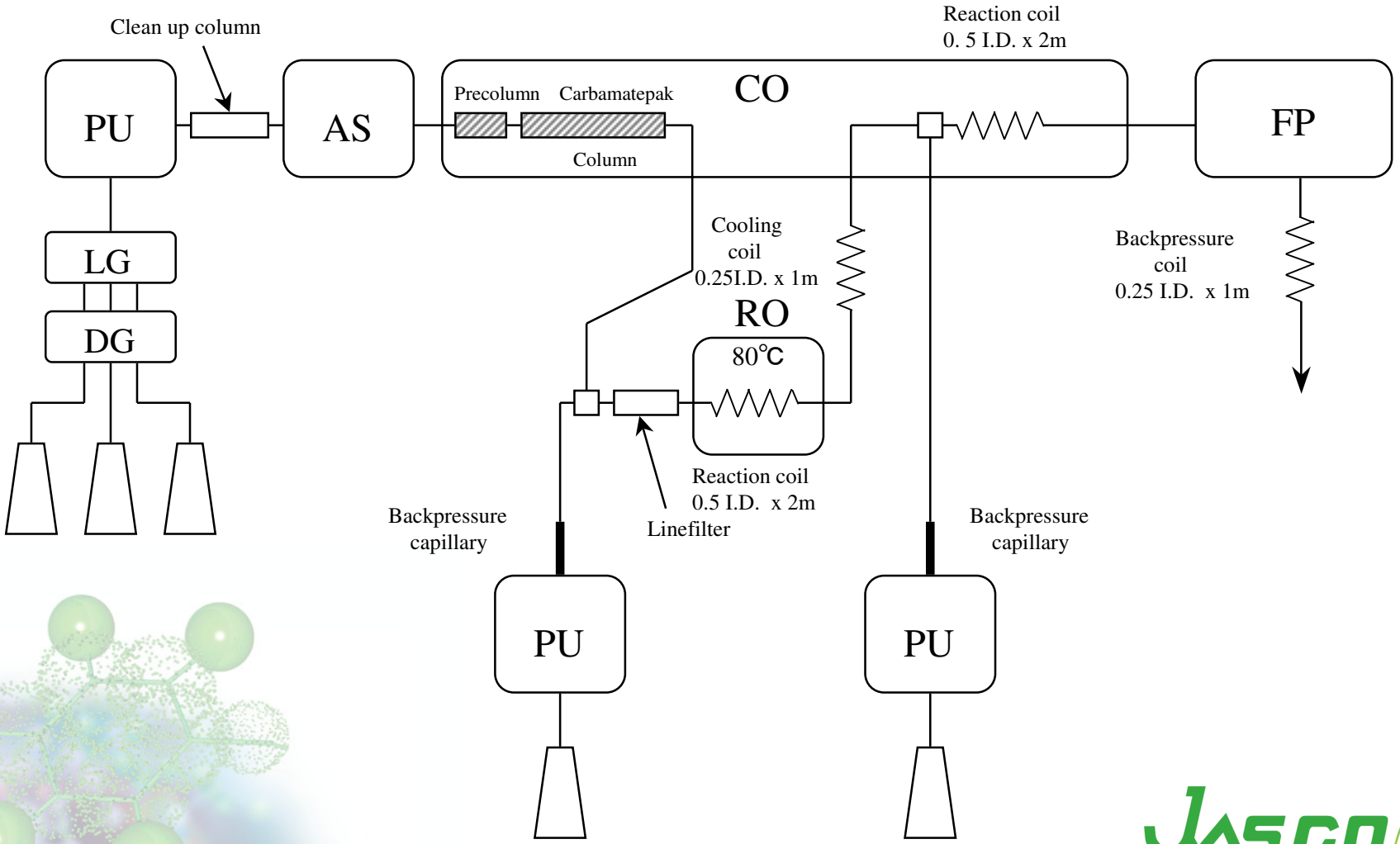
Step 1: Alkali hydrolysis



Step 2: Luminescence reaction with o-phtahalaldehyde (OPA)



Flow diagram of N-Methyl carbamate analysis system



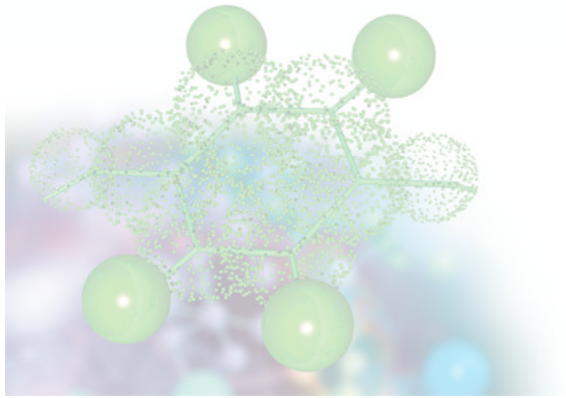
Measurement conditions specified in official method

Column:	Octadecylsilyl silica gel (3.9 mm I.D. x 150 mmL)
Column temperature:	40 degree C
Mobile phase:	A: H_2O, B: CH_3OH, C: THF
Flow rate:	0.6 ml/min
Reagents 1:	50 mMNaOH aq.
Reagent flow rate 1:	0.5 ml/min
Reagents 2:	50 mM $Na_2B_4O_7 \cdot 10H_2O$ / 0.4 mg OPA in 5 ml CH_3OH /2-mercaptoethanol (1000/5/1)
Reagent flow rate 2:	0.5 ml/min
Reaction temperature:	80 to 100 ˚C
Detection :	Ex; 340 nm, Em; 455 nm, Gain; x100

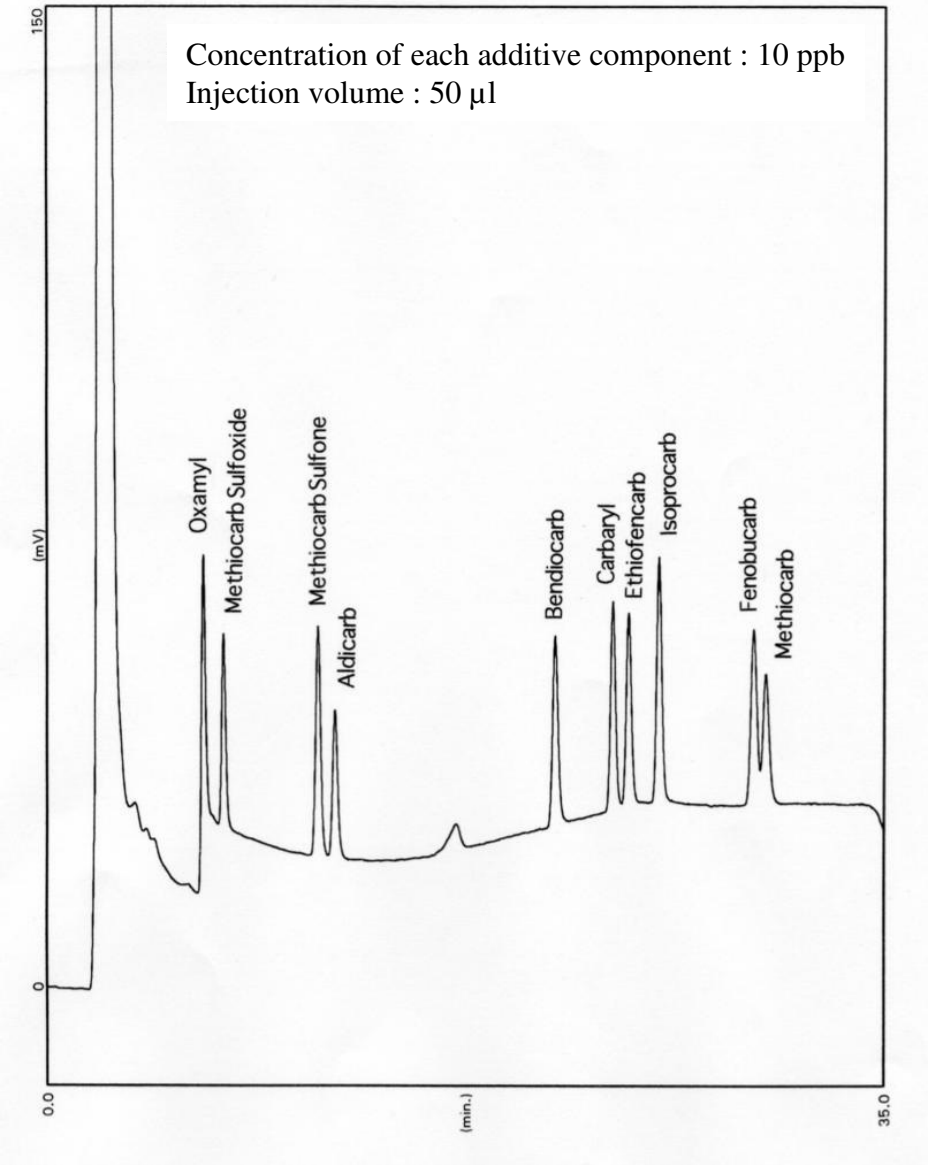
Gradient conditions

	A	B	C
0min	88%	12%	0%
0.1min	90%	0%	10%
20.0min	70%	0%	30%
29.9min	70%	0%	30%
30.0min	88%	12%	0%

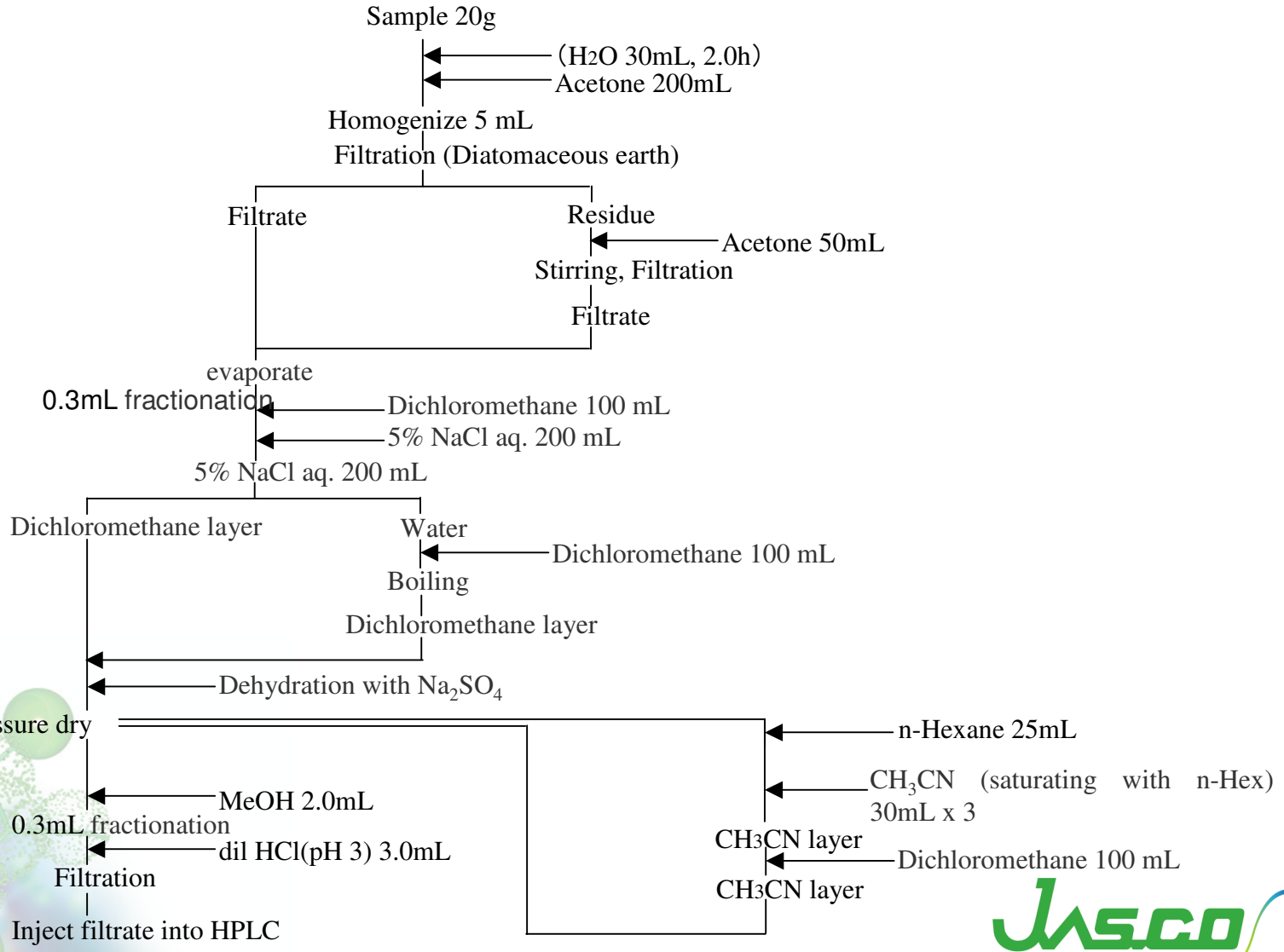
1cycle 40min



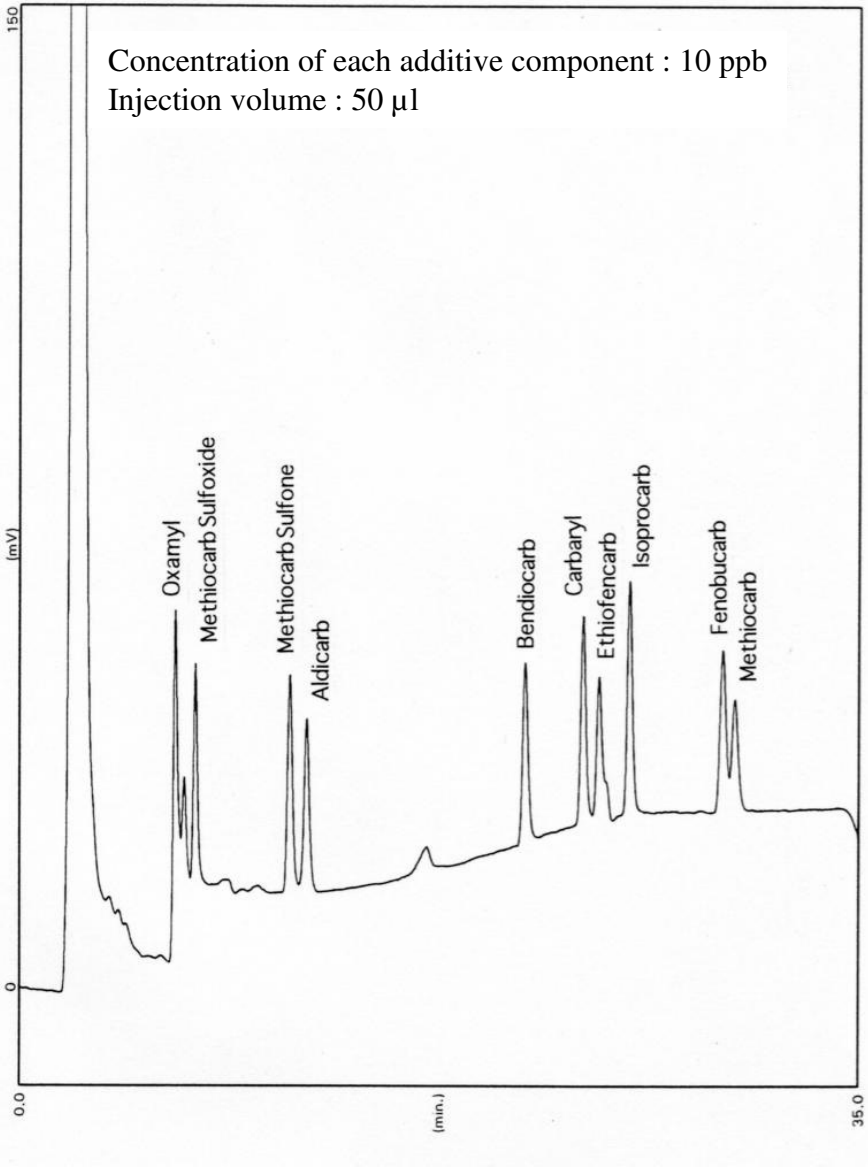
Chromatogram of carbamete pesticides (official method)



Extraction pre-treatment of carbamate pesticides in food (official method)



Chromatogram of carbamate pesticides added to extractions of rice (official method)



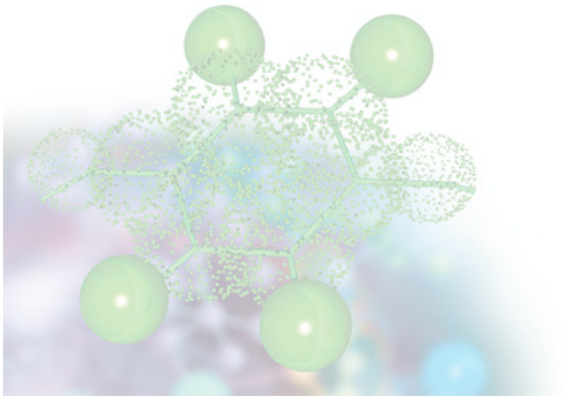
JASCO Measurement conditions for the separation of 11 kinds of components

Column:	CarbamatePak (4.0 mm I.D. x 150 mmL)
Column temperature:	40 degree C
Mobile phase:	A: H_2O, B: CH_3OH
Flow rate:	1.0 ml/min
Reagents 1:	50 mMNaOH aq.
Reagent flow rate 1:	0.5 ml/min
Reagents 2:	50 mM $Na_2B_4O_7 \cdot 10H_2O$ / 0.4 mg OPA in 5 ml CH_3OH /2-mercaptoethanol (1000/5/0.5)
Reagent flow rate 2:	0.5 ml/min
Reaction temperature:	80 ℃
Detection:	Ex; 339 nm, Em; 455 nm, Gain; x100

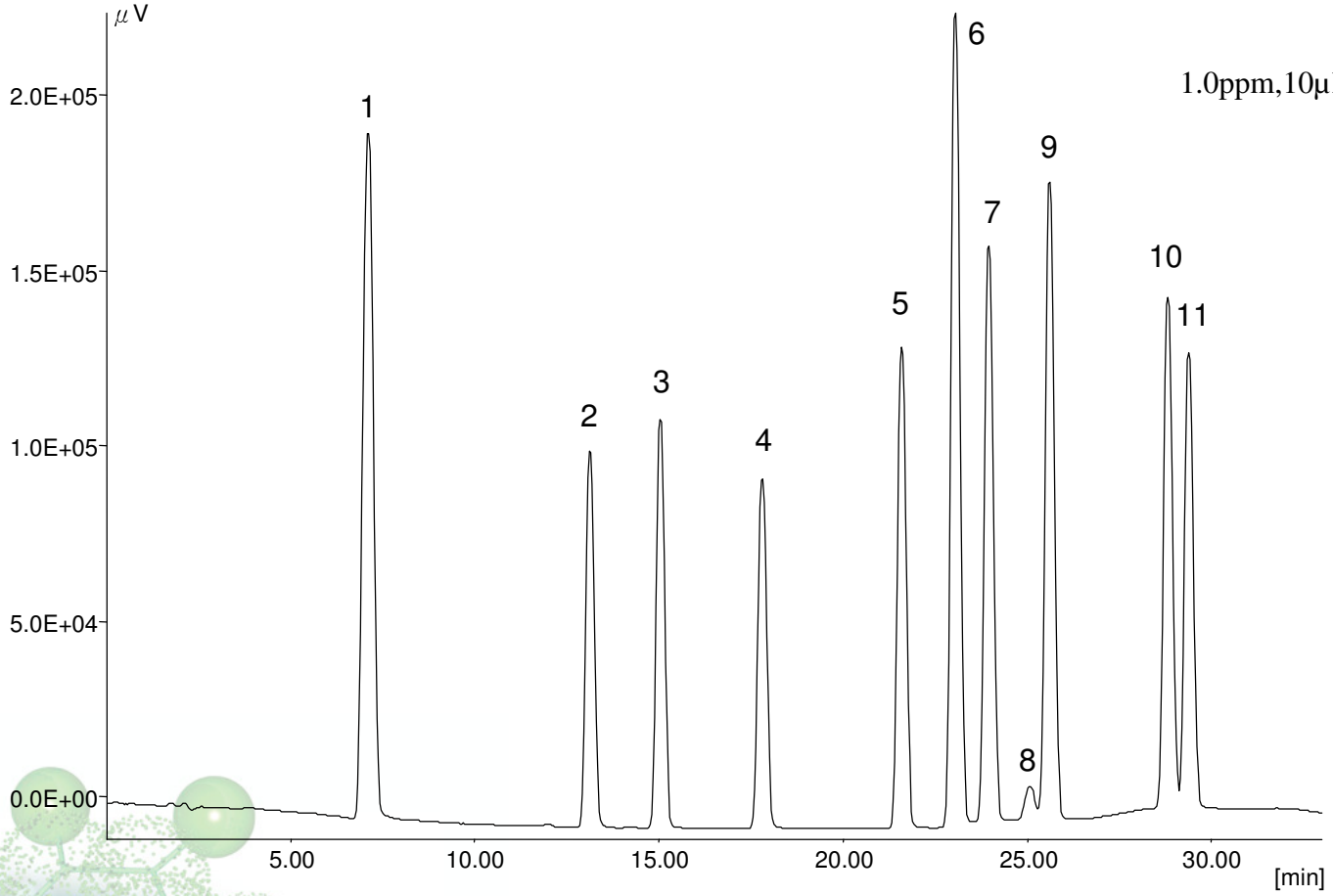
Gradient conditions

	A	B
0min	85%	15%
20.0min	35%	65%
25.0min	35%	65%
25.1min	0%	100%
30.0min	0%	100%
30.1min	85%	15%

1cycle 50min



Chromatogram of 11 components of carbamate pesticide (standard samples)



1. Oxamyl
2. Methiocarb sulfoxide
3. Methiocarb sulfone
4. Aldicarb
5. Bendiocarb
6. Carbaryl
7. Ethiofencar
8. Pirimicarb
9. Isoprocarb(MIPC)
10. Fenobcarb(BPMC)
11. Methiocarb

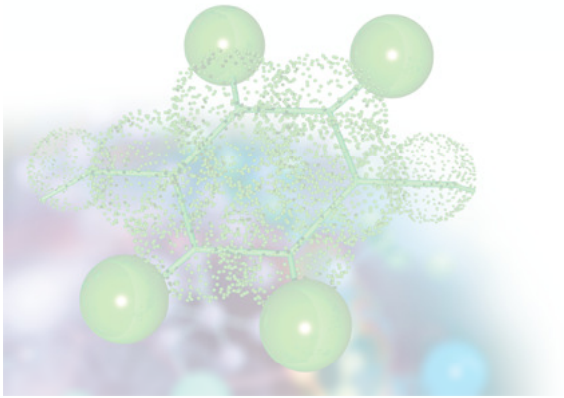
JASCO Measurement conditions for the separation of 18 kinds of components

Column:	CarbamatePak (4.0 mm I.D. x 150 mmL)
Column temperature:	40 ˚C
Mobile phase:	A: H_2O, B: CH_3OH, C: THF
Flow rate:	1.0 ml/min
Reagents 1:	50 mMNaOH aq.
Reagent flow rate 1:	0.5 ml/min
Reagents 2:	50 mM $Na_2B_4O_7 \cdot 10H_2O$ / 0.4 mg OPA in 5 ml CH_3OH /2-mercaptoethanol (1000/5/0.5)
Reagent flow rate 2:	0.5 ml/min
Reaction temperature:	80 ˚C
Detection:	Ex; 339 nm, Em; 455 nm, Gain; x100

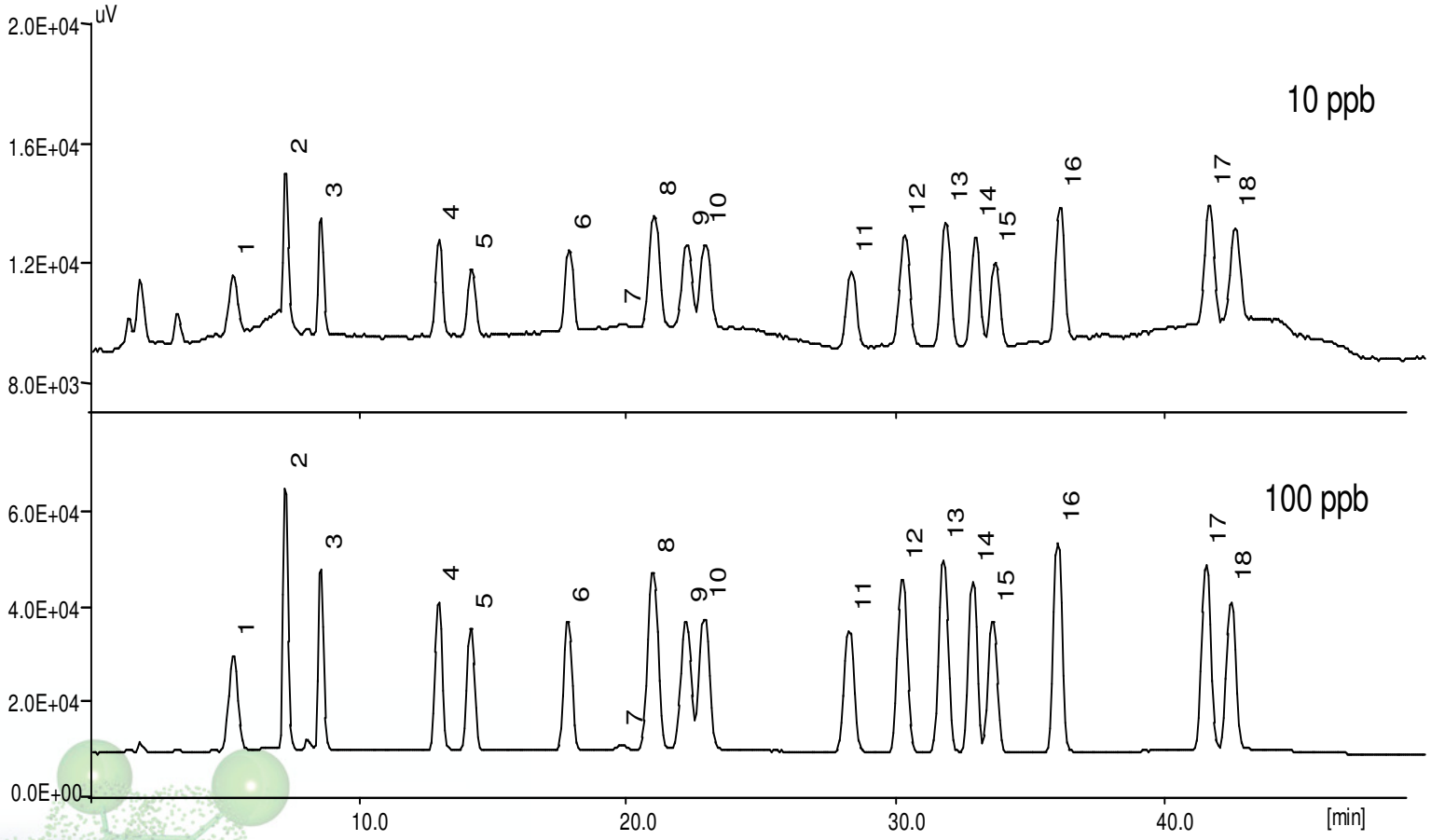
Gradient conditions

	A	B	C
0min	88%	12%	0%
0.2min	88%	12%	0%
3.0min	90%	0%	10%
20.0min	85%	0%	15%
35.0min	70%	0%	30%
40.0min	70%	0%	30%
40.1min	88%	12%	0%

1cycle 50min



Chromatogram of 18 kinds of components of carbamate pesticide (standard samples)



1. Oxamyl	7. Pirimicarb	13.Macbal(XMC)
2. Methomyl	8. Metholcarb(MTMC)	14.Carbaryl
3. Methiocarb sulfoxide	9. Propoxur(PHC)	15.Ethiofencarb
4. Aldicarb	10.Carbofuran	16.Isoprocarb(MIPC)
5. Methiocarb sulfone	11.Bendiocarb	17.Fenobcarb(BPMC)
6. Thiodicarb	12.Xylylcarb(MPMC)	18.Methiocarb

Detection limit of carbamate pesticides

Compornents	(A) Detection limit*	(B) Minimum standard detection concentration**	Ratio between (A) and (B)
Oxamyl	0.54	20	**37**
Methiocarb sulfoxide	0.36	50	**137**
Methiocarb sulfone	0.32	50	**152**
Aldicarb	0.56	20	**35**
Bendiocarb	0.36	20	**56**
Carbaryl	0.26	100	**396**
Ethiofencarb	0.26	100	**374**
Isoprocarb	0.22	500	**2190**
Fenobucarb	0.36	300	**836**
Methiocarb	0.42	50	**118**

* Injection volume

** Notice of Ministry of Health and Welfare No. 166

Reproducibility

（Concentration: 20ppb, Injection volume: 50µl, n=5）

Compoments	Retention time (min)			Peak height(µV)			Peak area (mV sec)		
	AV.	S.D.	R.S.D.(%)	AV.	S.D.	R.S.D.(%)	AV.	S.D.	R.S.D.(%)
Oxamyl	7.053	0.0049	0.069	30266	209	0.69	334369	2703	0.78
Methiocarb sulfoxide	12.030	0.0078	0.065	13909	162	1.17	167168	1797	1.08
Methiocarb sulfone	13.481	0.0103	0.076	6063	272	4.49	70661	2618	3.71
Aldicar	15.691	0.0124	0.079	20345	134	0.66	259049	4301	1.66
Bendiocarb	18.667	0.0122	0.065	15682	172	1.10	204956	4937	2.41
Carbaryl	19.892	0.0121	0.061	18425	131	0.71	226471	5722	2.53
Ethiofencarb	20.524	0.0120	0.059	14743	212	1.44	182936	6307	3.45
Isoprocarb	21.795	0.0161	0.074	18081	317	1.76	226641	6834	3.02
Fenobucarb	24.356	0.0192	0.079	13120	215	1.64	172654	2573	1.49
Methiocarb	25.003	0.0237	0.095	6712	154	2.30	90581	1729	1.91

AV.: Average　　S.D.: Standard deviation　R.S.D. : Relative standard deviation

GPC Pre-treatment system

Comparing with conventional pre-treatment method based on solvent extraction, using GPC for pre-treatment of residual pesticides makes possible to save a lot of time.

It is used for removing fat (Triglyceride etc.) and pigments (chlorophyl etc.)

Procedure

Sample

↓

Solvent extraction

↓

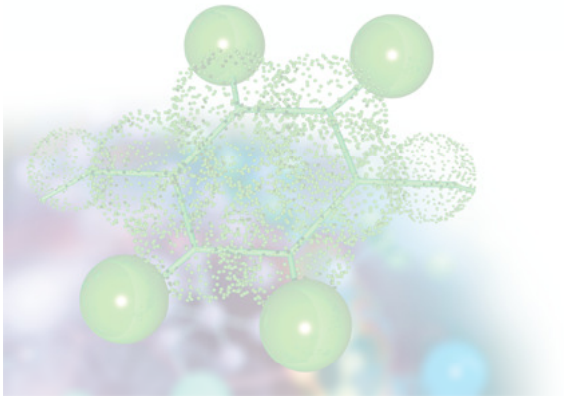
GPC Clean-up

↓

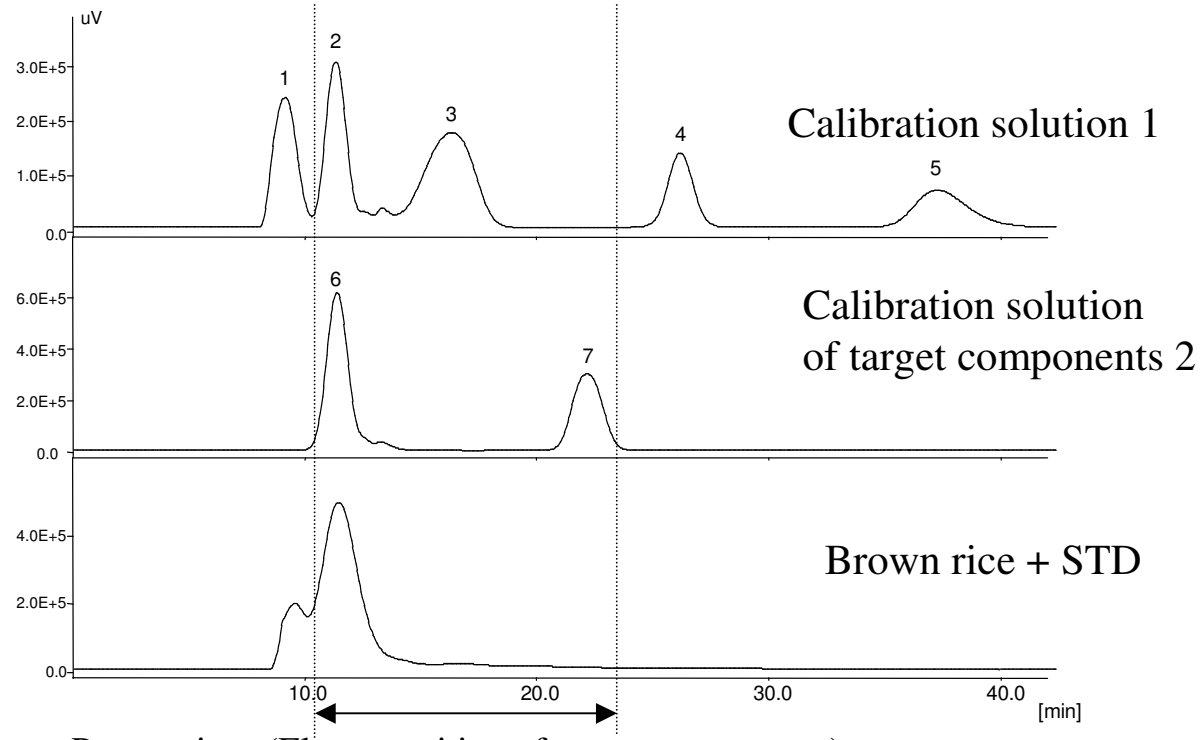
Preparative

↓

HPLC, GC



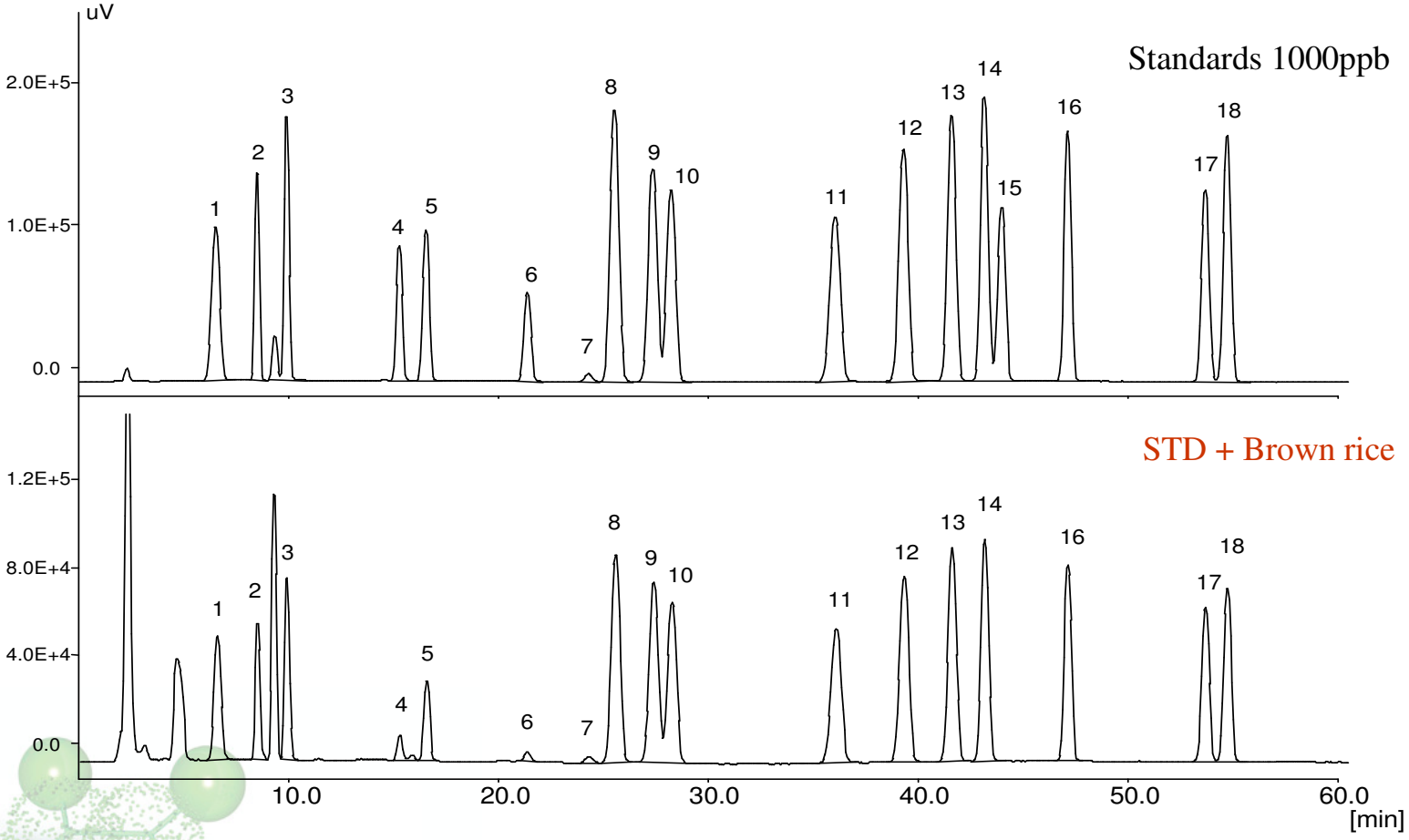
GPC Chromatogram of standard samples and brown rice



Preparatives (Eluent position of target components)

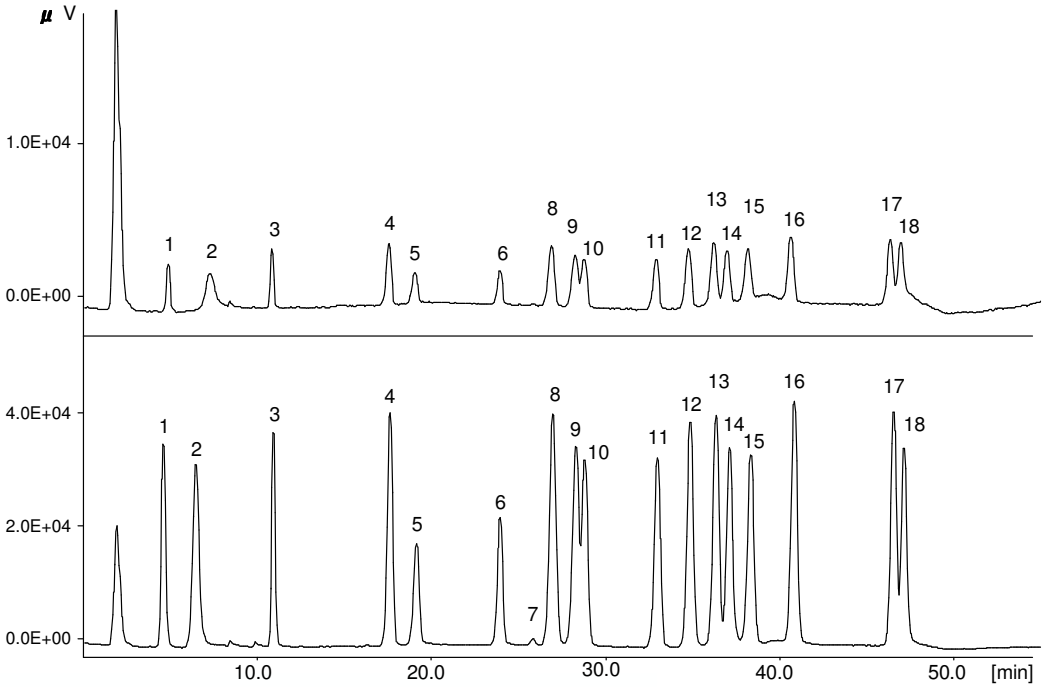
Preparative column:	Shodex CLNpak EV-2000 (20mm I.D. x 300mm L), Shodex CLNpak EV-G (20mm I.D. x 100mm L)
Column temperature:	room temperature
Solvent:	Cyclohexane/Ethyl Acetate(70/30)
Flow rate:	5.0 mL/min
Injection volume:	5.0 mL
Detection wavelength:	254 nm
Standard samples:	1. Cone oil , 2. Bis(2-ethyl hexyl) phthalete, 3. Methoxychlor, 4. Perylene, 5. Sulfer, 6. Quinomethionate, 7. Fulvalinate

Chromatogram of brown rice added carbamate pesticides



18 components of standards ware added to be 1 ppm

N-methyl carbamate analysis with semi-micro HPLC



[Conditions]

Column:	CarbamatePak (2.1mm I.D. x 150mmL)
Column temp.:	40 ° C
Eluent:	A; H2O / B; CH3OH / C; THF Gradient eluent 1cycle 55min
Flow rate:	0.2ml/min
Reaction Eluent-1:	50mM NaOH 0.1ml/min 80° C
Reaction Eluent-2:	OPA* 0.1ml/min 40° C
Wave length:	W.L.Ex 339nm, W.L.Em 455nm
Injection volume:	5μL

*) Sodium Tetraborate buffer/OPA/Mercaptoethanol (1000/5/0.05)

1. Oxamyl	7. Pirimicarb	13.Macbal(XMC)
2. Methomyl	8. Metholcarb(MTMC)	14.Carbaryl
3. Methiocarb sulfoxide	9. Propoxur(PHC)	15.Ethiofencarb
4. Aldicarb	10.Carbofuran	16.Isoprocarb(MIPC)
5. Methiocarb sulfone	11.Bendiocarb	17.Fenobcarb(BPMC)
6. Mthiodicarb	12.Xylylcarb(MPMC)	18.Methiocarb

Summary

- ✓ **The best method can be selected from 3 types of methods for the target components of pesticides**
- ✓ **The system offers a good reproducibility with high sensitivity over 35 times of standard**
- ✓ **Stable and high sensitivity analysis**