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MULTIRESIDUE ANALYSIS OF PESTICIDES IN DRINKING WATER USING LC/MS/MS WITH ULTRA PERFORMANCE LC™

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OVERVIEW

- Purpose
  - UPLC™/MS/MS for 28 pesticides mixture in drinking water
- Methods
  - Comparison between UPLC™/MS/MS and HPLC/MS/MS
- Results
  - Increase Through-put, Sensitivity and Separation efficiency

INTRODUCTION

On April 1st, 2004, LC/MS was adopted as the analytical tool for the official methods of multiresidue pesticides analysis in drinking water in Japan. In past decade, mass spectrometers have been greatly improved. However, the conventional high-throughput methods result in poor chromatographic separation. Chromatographic separation is very important, especially for multiresidue analysis, in order to achieve reproducibility and accuracy of methods, since coelution of interfering matrix extracted from environmental samples cause ion suppression.

Ultra performance LC™ (UPLC™) is new technology that can achieve high-throughput separation of analytes without losing resolution. The purpose of this study was to develop the multiresidue method of pesticides in drinking water using LC/MS/MS and UPLC™, and to compare the new method with the traditional method.

Tab.1 List of Pesticide

Pesticide Name			
Thiram	Asulam	Diuron	Tricyclazole
Bentazone	Bensulide	Methomyl	Azoxystrobin
Carbofuran	Mecoprop	Benomyl	Halosulfuron-methyl
2,4-D	Carbaryl	Benfuracarb	Flazasulfuron
Triclopyr	Thiophanate-methyl	Probenazole	Thiodicarb
Iprodione	Carpropamid	Daimuron	Siduron
Oxine-copper	Dalapon	Bensulfuron-methyl	MBC*

*MBC:Benomyl Metabolite, Methyl benzimidazol-2-ylcarbamate

MS CONDITIONS

- Waters Micromass® Quattro *micro* triple quadrupole MS
- Ionization Mode ESI+ and ESI-, Simultaneous Polarity Switching
- Capillary Voltage 3.5kV
- Source Temperature 120℃
- Desolvation Temperature 500℃
- Desolvation Gas Flow 500L/Hr
- Cone Gas Flow 25L/Hr
- Inter-Scan Delay 100ms
- Inter-Channel Delay 20ms

HPLC CONDITIONS

- Waters Alliance 2695 solvent manager system
- Waters Atlantis dC_{18} Column
  - 2.1 x 150mm, 3.5μm, 40℃
  - Reverse phase C_{18} particles
- Injection Volume 10μL
- Flow Rate 0.2mL
- Mobile Phase
  - A: H_2O
  - B: MeOH
  - C: 0.1% Formic Acid
- Gradient

Time	%A	%B	%C	Curve	Flow
0.0	90	5	5	1	0.2mL/min
2.0	55	40	5	6	0.2mL/min
15.0	4	95	1	6	0.2mL/min
20.0	90	5	5	11	0.2mL/min

- Total Run Time 30min

UPLC CONDITIONS

- Waters ACQUITY Ultra Performance LC™ System (UPLC™)
- Waters ACQUITY Ultra Performance LC™ Column
  - 1.0 x 100mm, 1.7μm, 50℃
  - Bridged Ethane-Silicon C_{18} particles ($BEHC_{18}$)
- Injection Volume 10μL
- Flow Rate 0.15 mL
- Mobile Phase
  - A: 0.05% formic Acid
  - B: Acetonitrile
- Gradient

Time(min)	%A	%B	Curve	Flow
0.00	100	0	1	0.15mL/min
0.20	100	0	6	0.15mL/min
0.40	80	20	6	0.15mL/min
4.75	30	70	6	0.15mL/min
5.00	5	95	6	0.15mL/min
5.01	100	0	11	0.15mL/min

- Total Run Time 6min

Tab.2 MRM Parameters for UPLC™

Pesticide Name	Ionization Mode	Transition	Dwell Time (m sec)	Cone Voltage (V)	Collision Voltage (eV)	Pesticide Name	Ionization Mode	Transition	Dwell Time (m sec)	Cone Voltage (V)	Collision Voltage (eV)
Thiram	ESI+	241 > 88	40	10	20	Diuron	ESI+	233 > 72	20	20	20
Bentazone	ESI-	239 > 132	20	30	30	Methomyl	ESI+	163 > 88	20	10	10
Carbofuran	ESI+	222 > 165	20	20	10	Benomyl	ESI+	291 > 160	50	20	30
2,4-D	ESI-	219 > 161	20	30	10	Benfuracarb	ESI+	411 > 195	20	20	20
Triclopyr	ESI-	254 > 196	50	20	10	Probenazole	ESI+	224 > 40.7	40	20	10
Iprodione	ESI+	330 > 244.9	20	25	15	Daimuron	ESI+	269 > 151	20	20	10
Oxine-copper	ESI+	146 > 118	20	40	20	Bensulfuron-methyl	ESI+	411 > 149	20	30	20
Asulam	ESI+	231 > 156	20	20	10	Tricyclazole	ESI+	190 > 136	20	40	30
Bensulide	ESI+	398 > 158	50	15	25	Azoxystrobin	ESI+	404 > 371	50	20	20
Mecoprop	ESI-	213 > 141	20	20	10	Halosulfuron-methyl	ESI+	435 > 182	20	20	20
Carbaryl	ESI+	202 > 145	20	20	10	Flazasulfuron	ESI+	408 > 182	40	30	20
Thiophanate-methyl	ESI+	343 > 151	20	20	20	Thiodicarb	ESI+	355 > 88	20	20	20
Carpropamid	ESI+	334 > 139	20	20	20	Siduron	ESI+	233 > 94	20	20	30
Dalapon	ESI-	141 > 97	80	20	10	MBC	ESI+	192 > 160	20	20	20

- Transitions for HPLC/MS/MS were same as UPLC™ except Bensulide. (398>158 for UPLC, 398>356 for HPLC).
- The dwell time for HPLC/MS/MS was 100 ms to 200 ms.

RESULTS

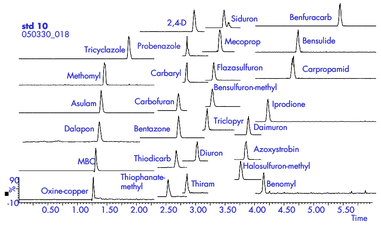


Fig.1 UPLC™ MRM Chromatograms

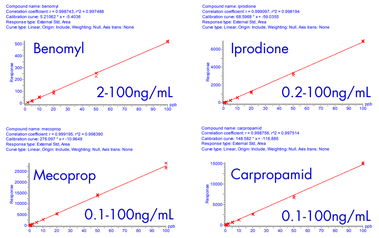
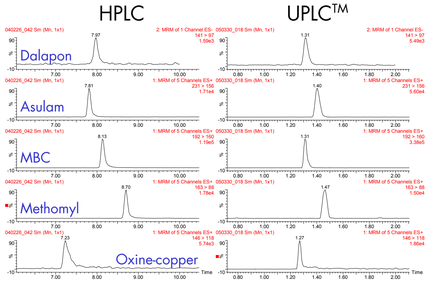


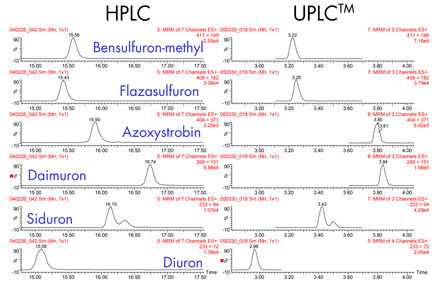
Fig.2 UPLC™ Calibration Curves

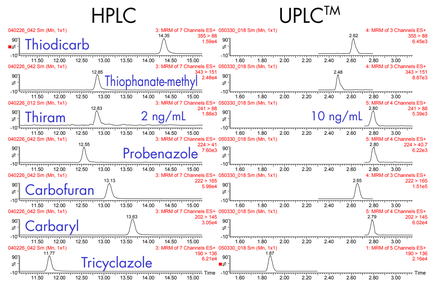
Tab.3 Correlation Coefficient of Calibration Standard in UPLC™

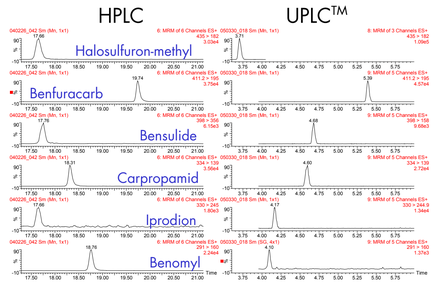
Pesticide Name	Correlation Coefficient (R)	Range of Calibration Curve (ppb)	Pesticide Name	Correlation Coefficient (R)	Range of Calibration Curve (ppb)
Thiram	0.998	2 - 100	Diuron	0.999	0.2 - 100
Bentazone	0.999	0.1 - 100	Methomyl	0.997	0.5 - 100
Carbofuran	0.998	0.1 - 100	Benomyl	0.999	2 - 100
2,4-D	0.999	0.5 - 100	Benfuracarb	0.999	0.2 - 100
Triclopyr	0.997	1 - 100	Probenazole	0.998	1 - 100
Iprodione	0.999	0.2 - 100	Daimuron	0.999	0.1 - 100
Oxine-copper	0.989	1 - 100	Bensulfuron-methyl	0.999	0.1 - 100
Asulam	0.994	0.1 - 50	Tricyclazole	0.998	0.2 - 100
Bensulide	1.000	0.2 - 100	Azoxystrobin	0.999	0.2 - 100
Mecoprop	0.999	0.1 - 100	Halosulfuron-methyl	0.999	0.1 - 100
Carbaryl	0.994	0.1 - 100	Flazasulfuron	0.999	0.1 - 100
Thiophanate-methyl	0.999	0.2 - 100	Thiodicarb	0.999	1 - 100
Carpropamid	0.999	0.1 - 100	Siduron	0.997	0.2 - 100
Dalapon	0.997	1 - 100	MBC	0.998	0.1 - 100

- Pesticides concentrations of standard solutions were prepared between 0.1 and 100 ng/mL (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL).
- Injections of these standards were run 3 times.
- Fig.2 shows calibration curves of 4 pesticides for instance.
- Tab.3 shows the correlation coefficients of calibration curves in UPLC™.
- The lowest points of calibration curves represent LOD with over 3 signal-noise ratio.
- The lowest maximum residue limit (MRL) of pesticides in this study is 25ng/mL as a sample solution. Thus LOD of this method is enough low. (In the official methods, the sample preparation consists of a solid-phase extraction (SPE). The pesticides extracted from 500mL drinking water sample are dissolved in 1mL.)









Tab.4 Comparison of Reproducibility from UPLC™ and HPLC

Pesticide Name	UPLC 10ppb	HPLC 25ppb	Pesticide Name	UPLC 10ppb	HPLC 25ppb
Thiram	15.4%	-	Diuron	8.6%	3.6%
Bentazone	1.7%	0.8%	Methomyl	5.5%	7.4%
Carbofuran	7.1%	0.6%	Benomyl	6.4%	4.5%
2,4-D	2.1%	1.6%	Benfuracarb	0.7%	-
Triclopyr	3.2%	9.5%	Probenazole	15.3%	8.4%
Iprodione	5.4%	3.0%	Daimuron	1.4%	1.2%
Oxine-copper	1.5%	-	Bensulfuron-methyl	3.7%	2.8%
Asulam	0.5%	2.4%	Tricyclazole	6.0%	2.6%
Bensulide	7.4%	2.4%	Azoxystrobin	4.3%	1.3%
Mecoprop	1.7%	1.8%	Halosulfuron-methyl	2.0%	1.9%
Carbaryl	2.7%	0.7%	Flazasulfuron	7.8%	2.7%
Thiophanate-methyl	14.1%	1.5%	Thiodicarb	7.1%	3.6%
Carpropamid	0.9%	2.2%	Siduron	7.5%	2.4%
Dalapon	5.1%	6.9%	MBC	3.9%	1.9%

- Tab.4 shows the reproducibility of UPLC™ and HPLC.
- UPLC™
  - This result was obtained from 3 times runs for calibration curves described above.
- HPLC
  - This result was obtained from 3 injections of the same concentration in a row.

Tab.5 Comparison of Peak Height from UPLC™ and HPLC

Pesticide Name	UPLC average peak height	HPLC average peak height	ratio of UPLC /HPLC	Pesticide Name	UPLC average peak height	HPLC average peak height	ratio of UPLC /HPLC
Thiram	5053	8653	0.58	Diuron	9792	12780	0.77
Bentazone	30165	23892	**1.26**	Methomyl	7973	18177	0.44
Carbofuran	136795	62083	**2.20**	Benomyl	905	1637	0.55
2,4-D	4940	3369	**1.47**	Benfuracarb	40081	35609	1.13
Triclopyr	4430	3045	**1.45**	Probenazole	4490	7312	0.61
Iprodione	10435	1826	**5.71**	Daimuron	189475	98461	**1.92**
Oxine-copper	5691	5561	1.02	Bensulfuron-methyl	58739	25178	**2.33**
Asulam	40785	17285	**2.36**	Tricyclazole	11867	60935	0.19
Bensulide	7415	6047	**1.23**	Azoxystrobin	5400	2947	**1.83**
Mecoprop	48998	11717	**4.18**	Halosulfuron-methyl	88757	28703	**3.09**
Carbaryl	72013	30860	**2.33**	Flazasulfuron	28275	29575	0.96
Thiophanate-methyl	4994	24650	0.20	Thiodicarb	3193	15278	0.21
Carpropamid	20652	34715	0.59	Siduron	37666	9352	**4.03**
Dalapon	2625	1409	**1.86**	MBC	219056	115560	**1.90**

- Tab.5 shows Comparison of Peak Height from UPLC™ and HPLC
- UPLC
  - This result was obtained from 3 times runs for calibration curves described above.
- HPLC
  - This result was obtained from 2 injections.

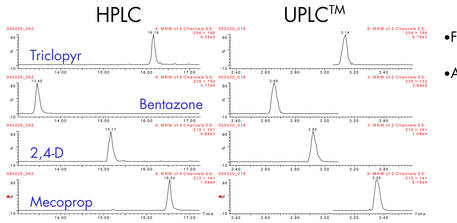


Fig.3 Comparison of LC Separation from UPLC™ and HPLC
10 ng/mL, 10μL

- Fig.3 shows the comparison of a peak separation from UPLC™ and HPLC.
- Although the total run time of UPLC™ method is 5 times shorter than HPLC, same LC separation was obtained.

CONCLUSIONS

- The high through-put quantification method for 28 pesticides was developed for UPLC™/MS/MS.
- The chromatographic run times were 30min for the HPLC method and 6min for the high through-put UPLC™ method, respectively.
- For over two third of 28 pesticides, the signal increased 1.2 to 5.9 times was observed in the UPLC™ compared with HPLC.
- Although the run times were reduced to one-fifth, the chromatographic resolution was kept at the same level.

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