SHIMADZU

Application News

No.C87

Liquid Chromatography Mass Spectrometry

Analysis of Pesticides in Foods Using a Triple Quadrupole LC/MS/MS [LCMS-8030]

Pesticides are widely used in agricultural products to protect crops from insect infestation during cultivation and product transport. With the heightened concern for food safety, "Positive List System" was introduced in Japan on May 29, 2006. These limits ban the sale of food containing pesticides that exceed specified concentrations to ensure the safety of food circulating in the market. Currently, residue standards and analytical methods have been established for about 800 kinds of pesticides and veterinary pharmaceuticals, and this number is expected to increase even further. Therefore, there is increasing need for simpler analytical methods capable of simultaneous multi-residue analysis.
Here, using the LCMS-8030, we optimized methods for the simultaneous multi-residue analysis of 43 pesticides, and report the results of the analysis of actual vegetable extracts (paprika and leek) in solution.

Pesticides Screening Using a Method Package

In LC/MS/MS analysis, the measurement conditions, including the precursor and product ion *m/z* values, as well as collision energy voltage, etc., must all be set beforehand. As the number of analytes increases, so too does the amount of time and effort required to optimize each analysis condition.
Here, we conducted measurements using a simultaneous analysis method that screens for 167 substances that are included in the "residual pesticide LC/MS/MS method package" for pesticide screening in actual samples. Through the use of the residual pesticide method package, measurements can be conducted immediately without having to perform routine method such as optimization tasks.
A paprika solution, spiked with 10 µg/L each of imidacloprid, lufenuron,and 8 other pesticides, was used for the analysis. Fig. 1 shows an example of the pesticide screening results using the method package. The MRM results illustrated detection, with excellent sensitivity, of the positive list pesticides in the sample solution at the standard criterion concentration of 10 µg/L.

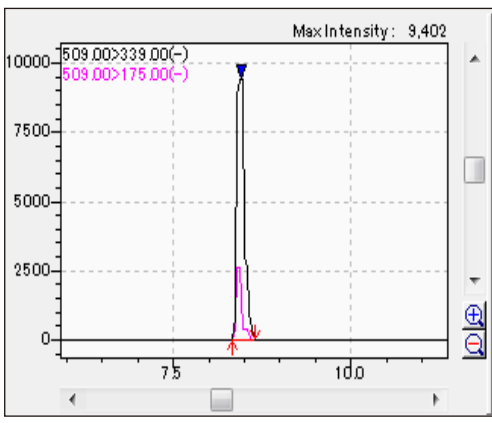
MRM measurement conditions screen
[Instrument Parameters View]

Type	Event#	+/−	Compound Name m/z	Time (0.000 min – 20.000 min)
MRM	143	+	Tricyclazole 190.00>163.00, 190.00>136.00	
MRM	144	+	Trifloxystrobin 409.10>186.10, 409.10>145.00	
MRM	145	+	Trifloxysulfuron 438.10>182.10, 438.10>257.00	
MRM	146	+	Triflumizole 346.10>73.10, 346.10>278.00	
MRM	147	+	TriflumizoleMetabolite 295.10>73.10, 295.10>215.00	
MRM	148	+	Triflumuron 359.10>156.00, 359.10>139.00	
MRM	149	+	Triflusulfuron-methyl 493.20>264.10, 493.20>238.00	
MRM	150	−	1-Naphthaleneacetic Acid 184.90>140.90	
MRM	151	−	2,4-D(2,4-PA) 218.90>160.95, 218.90>125.05	

MRM | Acq. Time: 0 - 20 min | Compound Name: Triticonazole

Ch	Precursor m/z	Product m/z	Pause Time (msec)	Dwell Time (msec)	Q1 Pre Bias(V)	CE	Q3
Ch1	318.20	70.10	1.0	5.0	−16.0	−21.0	−12.
Ch2	318.20	125.10	1.0	5.0	−17.0	−29.0	−23.
Ch3							
Ch4							

Analysis Window



ID#	Name	Ret. Time	m/z	Area	S/N Ratio	Mode
101	Ioxynil	No peak is detected.				
102	Iprovalicarb	No peak is detected.				
103	Isoxaflutole	No peak is detected.				
104	Linuron	No peak is detected.				
105	Lufenuron	8.454	509.00>33	72866	--	Auto
106	MCPA(MCP)	No peak is detected.				
107	MCPB	No peak is detected.				
108	Mecoprop (MCPP)	No peak is detected.				
109	Mefenpyr-diethyl	No peak is detected.				
110	Mepanipyrim	No peak is detected.				
111	Mesosulfuron-methyl	No peak is detected.				
112	Methabenzthiazuron	5.077	222.10>16	1946868	1779.72	Auto
113	Methiocarb	No peak is detected.				
114	Methomyl	No peak is detected.				

Fig. 1 Screening of Pesticides Using a Method Package

MRM Results of 43 Pesticides

We conducted MRM measurements of a standard solution of 43 pesticides using the MRM conditions incorporated in the method package. Fig. 2 shows the MRM chromatograms of the 43 pesticide substances in the mixed standard solution, and Table 1 shows the MRM transitions for each pesticide, in addition to the calibration curves.

Excellent linearity was obtained for each substance over the entire concentration range which varied from 0.5 or 1-100 ppb.

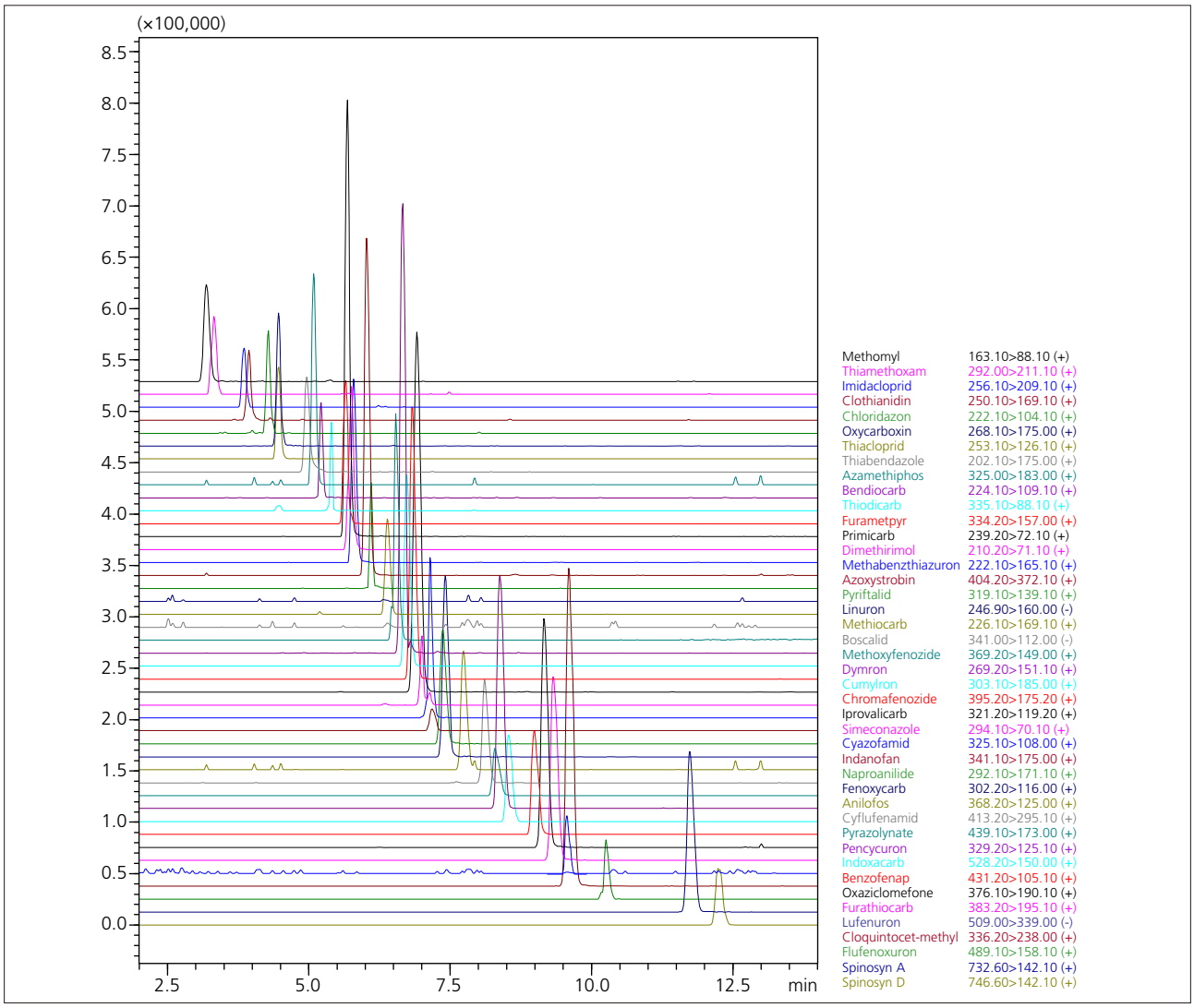


Fig. 2 MRM Chromatograms of 43 Pesticides in the Mixed Standard Solution

Table 1 MRM Transitions and Quantitative Results for 43 Pesticides

Compound	Polarity	Transition	Calibration curve(ppb)	r^2	Compound	Polarity	Transition	Calibration curve(ppb)	r^2
Methomyl	+	163.10>88.10	0.5-100	0.9995	Cumyluron	+	303.10>185.00	0.5-100	0.9999
Thiamethoxam	+	292.00>211.10	0.5-100	0.9998	Chromafenozide	+	395.20>175.20	0.5-100	0.9999
Imidacloprid	+	256.10>209.10	0.5-100	0.9992	Iprovalicarb	+	321.20>119.20	0.5-100	0.9997
Clothianidin	+	250.10>169.10	0.5-100	0.9992	Simeconazole	+	294.10>70.10	0.5-100	0.9991
Chloridazon	+	222.10>104.10	0.5-100	0.9994	Cyazofamid	+	325.10>108.00	0.5-100	0.9999
Oxycarboxin	+	268.10>175.00	0.5-100	0.9999	Indanofan	+	341.10>175.00	1-100	0.9999
Thiacloprid	+	253.10>126.10	0.5-100	0.9997	Naproanilide	+	292.10>171.10	0.5-100	0.9999
Thiabendazole	+	202.10>175.00	0.5-100	0.9990	Fenoxycarb	+	302.20>116.00	0.5-100	0.9999
Azamethiphos	+	325.00>183.00	0.5-100	0.9996	Anilofos	+	368.20>125.00	0.5-100	0.9999
Bendiocarb	+	224.10>109.10	0.5-100	0.9997	Cyflufenamid	+	413.20>295.10	0.5-100	0.9998
Thiodicarb	+	355.10>88.10	0.5-100	0.9999	Pyrazolynate	+	439.10>173.00	0.5-100	0.9999
Furametpyr	+	334.20>157.00	0.5-100	0.9997	Pencycuron	+	329.20>125.10	0.5-100	0.9998
Pirimicarb	+	239.20>72.10	0.5-100	0.9998	Indoxacarb	+	528.20>150.00	0.5-100	0.9995
Dimethirimol	+	210.20>71.10	0.5-100	0.9976	Benzofenap	+	431.20>105.10	0.5-100	0.9995
Methabenzthiazuron	+	222.10>165.10	0.5-100	0.9994	Oxaziclomefone	+	376.10>190.10	0.5-100	0.9999
Azoxystrobin	+	404.20>372.10	0.5-100	0.9997	Furathiocarb	+	383.20>195.10	0.5-100	0.9999
Pyriftalid	+	319.10>139.10	0.5-100	0.9998	Lufenuron	-	509.00>339.00	1-100	0.9997
Linuron	-	246.90>160.00	1-100	0.9998	Cloquintocet-mexyl	+	336.20>238.00	0.5-100	0.9998
Methiocarb	+	226.10>169.10	0.5-100	0.9999	Flufenoxuron	+	489.10>158.10	0.5-100	0.9992
Boscalid	-	341.00>112.00	0.5-100	0.9998	Spinosyn A	+	732.60>142.10	0.5-100	0.9997
Methoxyfenozide	+	369.20>149.00	0.5-100	0.9992	Spinosyn D	+	746.60>142.10	0.5-100	0.9999
Dymron (Daimuron)	+	269.20>151.10	0.5-100	0.9999					

Pesticide Analysis in Paprika and Leek Samples

MRM measurements were conducted for pesticides in actual sample solutions originating from paprika and leek. The LC/MS/MS analysis was conducted following preparation of each sample according to the method stipulated by Japan's Ministry of Health, Labour and Welfare Notification, "Multi-residue analysis of pesticides by LC/MS - Method Ⅰ."
Fig. 3 shows the MRM chromatograms obtained from analysis of the paprika and leak sample solutions.

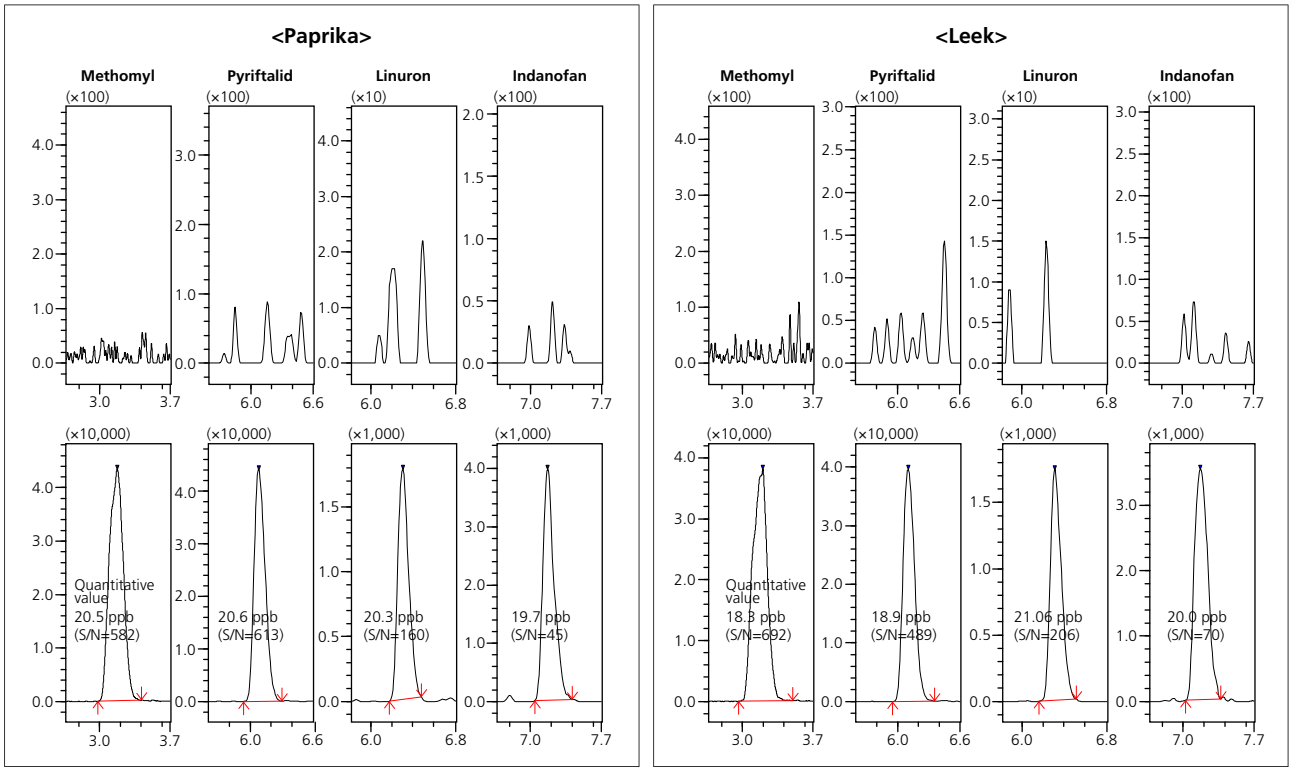


Fig. 3 MRM Results of Samples (Paprika/Leek) (upper: blank, lower: standards 20 ppb spiked)

The recoveries of the pesticides used to spike each sample are shown in Table 2. Good recovery results were obtained for the pesticides in the paprika, ranging from 96-111 %. The leek solution produced good recoveries, ranging from 70-110 % for nearly all of the pesticides. The lower recoveries might be attributable to an interfering component of the sample matrix and further examination of the method (sample pretreatment, etc.) is warranted with the aim of reducing matrix effects.

Table 2 Recovery of Pesticides (%)

Compound	Paprika	Leek	Compound	Paprika	Leek
Methomyl	100.7	89.5	Cumyluron	104.3	102.9
Thiamethoxam	107.0	75.2	Chromafenozide	100.3	99.9
Imidacloprid	(658.8)	94.7	Iprovalicarb	100.5	95.6
Clothianidin	102.2	107.0	Simeconazole	98.6	92.9
Chloridazon	110.7	93.8	Cyazofamid	103.7	95.7
Oxycarboxin	103.4	64.6	Indanofan	99.7	101.2
Thiacloprid	101.7	57.5	Naproanilide	100.8	99.1
Thiabendazole	96.3	53.4	Fenoxycarb	98.7	97.3
Azamethiphos	99.2	76.6	Anilofos	101.7	96.5
Bendiocarb	100.9	73.5	Cyflufenamid	99.5	98.8
Thiodicarb	102.0	84.4	Pyrazolynate	98.8	94.3
Furametpyr	101.7	75.7	Pencycuron	96.6	95.8
Pirimicarb	98.7	74.2	Indoxacarb	102.3	101.6
Dimethirimol	100.8	61.8	Benzofenap	98.5	98.4
Methabenzthiazuron	98.2	75.1	Oxaziclomefone	100.6	96.1
Azoxystrobin	(106.3)	(756.1)	Furathiocarb	102.6	95.7
Pyriftalid	100.5	92.2	Lufenuron	110.5	107.7
Linuron	99.2	102.7	Cloquintocet-mexyl	101.0	94.8
Methiocarb	106.3	89.0	Flufenoxuron	98.2	85.1
Boscalid	102.6	102.5	Spinosyn A	99.5	86.2
Methoxyfenozide	96.6	100.4	Spinosyn D	96.3	73.8
Dymron (Daimuron)	103.0	99.9			

Note: Recoveries for some items (values in parentheses) were difficult to calculate given that they were also detected in the blank.

Comparison of Sensitivity due to Differences in the Ammonium Acetate Concentration in Mobile Phase

Here, the LOQ's for all 43 pesticides measured was 10 µg/L or lower, indicating that they were all within the standard criterion. However, there were cases which required some sort of remedial measure, such as sample dilution to reduce the influence of the matrix and improve sensitivity.

To further increase detection sensitivity, we also evaluated the effect of reducing the ammonium acetate concentration of the aqueous and methanol mobile phases to 0.1 mmol/L.

Table 3 shows the LOQ of each pesticide measured using a salt concentration of 0.1 mmol/L, and the percent change in detection sensitivity with respect to the 5 mmol/L. It was confirmed that lowering the mobile phase salt concentration increased the S/N for almost all of the pesticides.

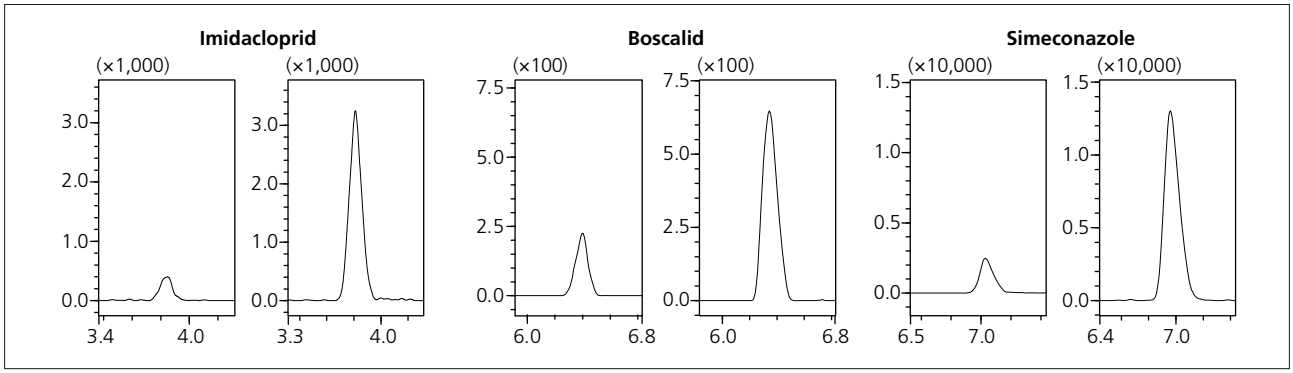


Fig. 4 Comparison of MRM Chromatograms (left: 5 mmol/L, right: 0.1 mmol/L)

Table 3 Comparison of Sensitivity at Different Salt Concentration

Compound	S/N (1 ppb)			Compound	S/N (1 ppb)		
	5 mmol/L	0.1 mmol/L	0.1 mmol/L / 5 mmol/L		5 mmol/L	0.1 mmol/L	0.1 mmol/L / 5 mmol/L
Methomyl	51	103	**2.0**	Cumyluron	175	533	**3.0**
Thiamethoxam	93	178	**1.9**	Chromafenozide	240	501	**2.1**
Imidacloprid	87	153	**1.8**	Iprovalicarb	186	222	**1.2**
Clothianidin	41	40	**1.0**	Simeconazole	177	288	**1.6**
Chloridazon	146	390	**2.7**	Cyazofamid	147	460	**3.1**
Oxycarboxin	80	369	**4.6**	Indanofan	33	34	**1.1**
Thiacloprid	261	546	**2.1**	Naproanilide	81	123	**1.5**
Thiabendazole	70	119	**1.7**	Fenoxycarb	262	340	**1.3**
Azamethiphos	297	470	**1.6**	Anilofos	299	304	**1.0**
Bendiocarb	75	231	**3.1**	Cyflufenamid	80	117	**1.5**
Thiodicarb	226	217	**1.0**	Pyrazolynate	236	237	**1.0**
Furametpyr	201	354	**1.8**	Pencycuron	160	226	**1.4**
Pirimicarb	204	250	**1.2**	Indoxacarb	266	281	**1.1**
Dimethirimol	154	171	**1.1**	Benzofenap	115	153	**1.3**
Methabenzthiazuron	71	171	**2.4**	Oxaziclomefone	257	479	**1.9**
Azoxystrobin	128	215	**1.7**	Furathiocarb	106	219	**2.1**
Pyriftalid	84	111	**1.3**	Lufenuron	28	115	**4.0**
Linuron	19	44	**2.3**	Cloquintocet-mexyl	357	417	**1.2**
Methiocarb	222	223	**1.0**	Flufenoxuron	219	502	**2.3**
Boscalid	56	229	**4.1**	Spinosyn A	549	619	**1.1**
Methoxyfenozide	53	77	**1.4**	Spinosyn D	137	288	**2.1**
Dymron (Daimuron)	99	177	**1.8**				

Table 4 Analytical Conditions

[Condition 1]
Mobile Phase A : 5 mmol/L Ammonium acetate - water
Mobile Phase B : 5 mmol/L Ammonium acetate - methanol
Gradient Program : 20 %B (0 min) – 70 %B (3 min) – 95 %B (10-15 min) – 20 %B (15.01-20 min)

[Condition 2]
Mobile Phase A : 0.1 mmol/L Ammonium acetate - water
Mobile Phase B : 0.1 mmol/L Ammonium acetate - methanol
Gradient Program : 20 %B (0 min) – 70 %B (3 min) – 95 %B (10-15 min) – 20 % B (15.01-20 min)
Column : Shim-pack XR-ODS Ⅱ (75 mmL. × 2.0 mmI.D., 2.2 µm)
Flow Rate : 0.2 mL/min
Injection Volume : 3 µL
Column Temperature : 40 °C

Probe Voltage : +4.5 kV (ESI-positive mode), -3.5 kV (ESI-negative mode)
Nebulizing Gas Flow : 3.0 L/min
Drying Gas Flow : 10 L/min
DL Temperature : 300 °C
BH Temperature : 500 °C
DL Voltage/Q-array Voltage : Using default values

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