CHAPTER IV
RESULTS

4.1 Validation of the analytical method

4.1.1 Linearity range

The linearity range of fipronil and its derivatives were determined by GC-MS. The graphs were linear over the concentration range of 0.002-0.2 mg/L with the correlation coefficient of 0.999. Results are presented in Tables 4.1 - 4.4 and Figures 4.1 - 4.4

Table 4.1 Linearity range of fipronil

Parameter	**Conc.(mg/L)**	**Equation**	**r^2**	**Replication**	**Conc. level no.**
Linearity range	0.002-0.2	Y=497310x+357.7	0.9999	3	7

Level no.	**Conc.(mg/L)**	**Average %Recovery (3 rep.)**
1	0.002	101.1
2	0.005	96.5
3	0.01	97.7
4	0.02	96.9
5	0.05	94.2
6	0.1	93.8
7	0.2	82.0

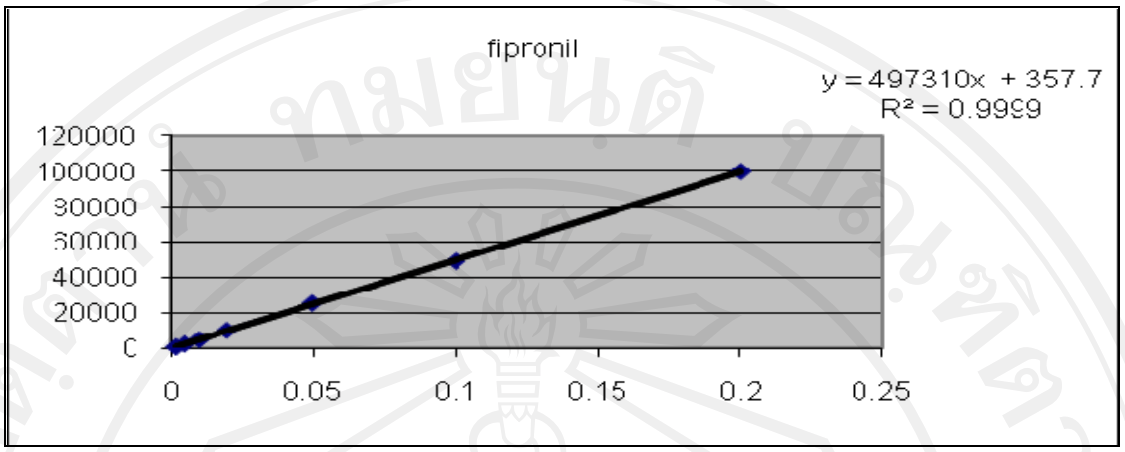

Figure 4.1 Linearity of fipronil

Table 4.2 Linearity range of fipronil-sulfide

Parameter	**Conc.(mg/L)**	**Equation**	**r^2**	**Replication**	**Conc. level no.**
Linearity range	0.002-0.2	Y=476251x+13.108	0.9995	3	7

Level no.	**Conc.(mg/L)**	**Average %Recovery (3 rep.)**
1	0.002	98.3
2	0.005	92.8
3	0.01	94.5
4	0.02	97.2
5	0.05	93.5
6	0.1	96.4
7	0.2	85.4

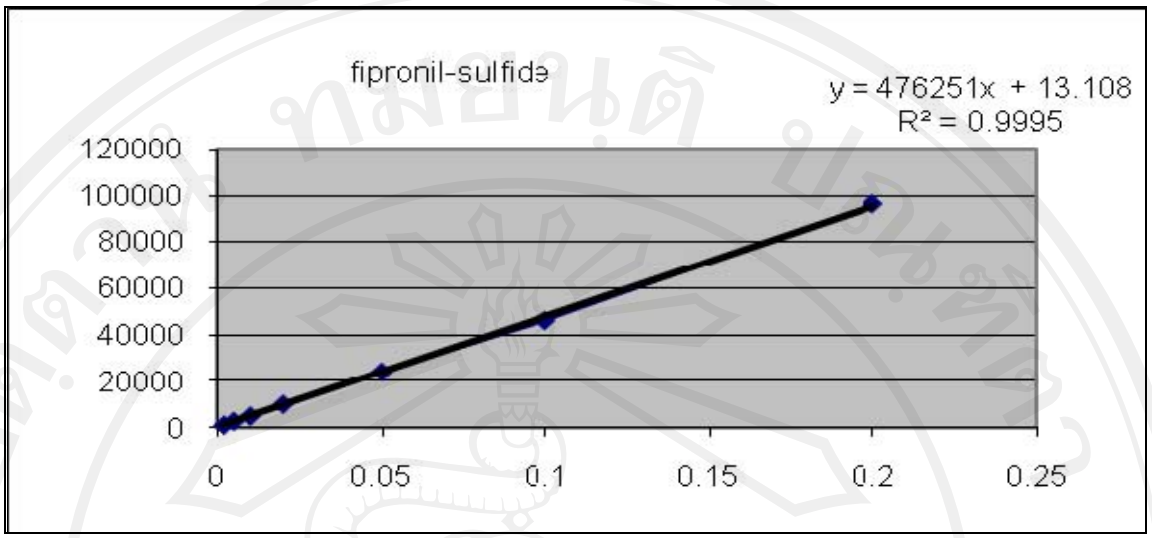

Figure 4.2 Linearity of fipronil-sulfide

Table 4.3 Linearity range of fipronil-sulfone

Parameter	**Conc. (mg/L)**	**Equation**	**r^2**	**Replication**	**Conc. level no.**
Linearity range	0.002-0.2	Y=263131x-164.69	0.9997	3	7

Level no.	**Conc.(mg/L)**	**Average %Recovery (3 rep.)**
1	0.002	92.0
2	0.005	90.9
3	0.01	92.8
4	0.02	95.0
5	0.05	91.4
6	0.1	98.6
7	0.2	89.9

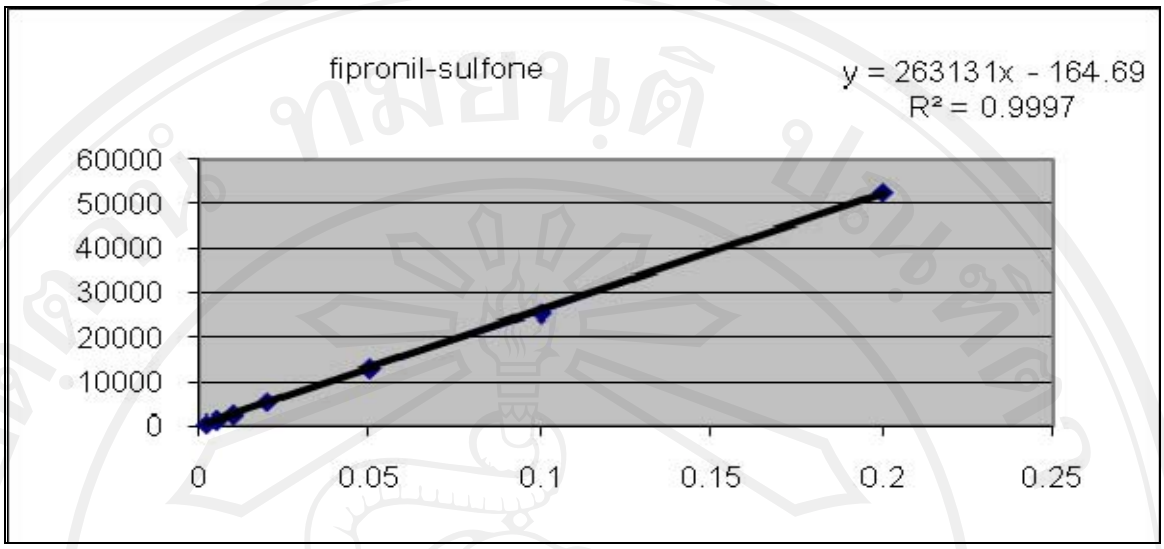

Figure 4.3 Linearity of fipronil- sulfone

Table 4.4 Linearity range of fipronil-desulfinyl

Parameter	**Conc. (mg/L)**	**Equation**	**r^2**	**Replication**	**Conc. level no.**
Linearity range	0.002-0.2	Y=249322x-303.68	0.9984	3	7

Level no.	**Conc.(mg/L)**	**Average %Recovery (3 rep.)**
1	0.002	96.7
2	0.005	95.6
3	0.01	98.4
4	0.02	95.9
5	0.05	89.3
6	0.1	97.2
7	0.2	91.2

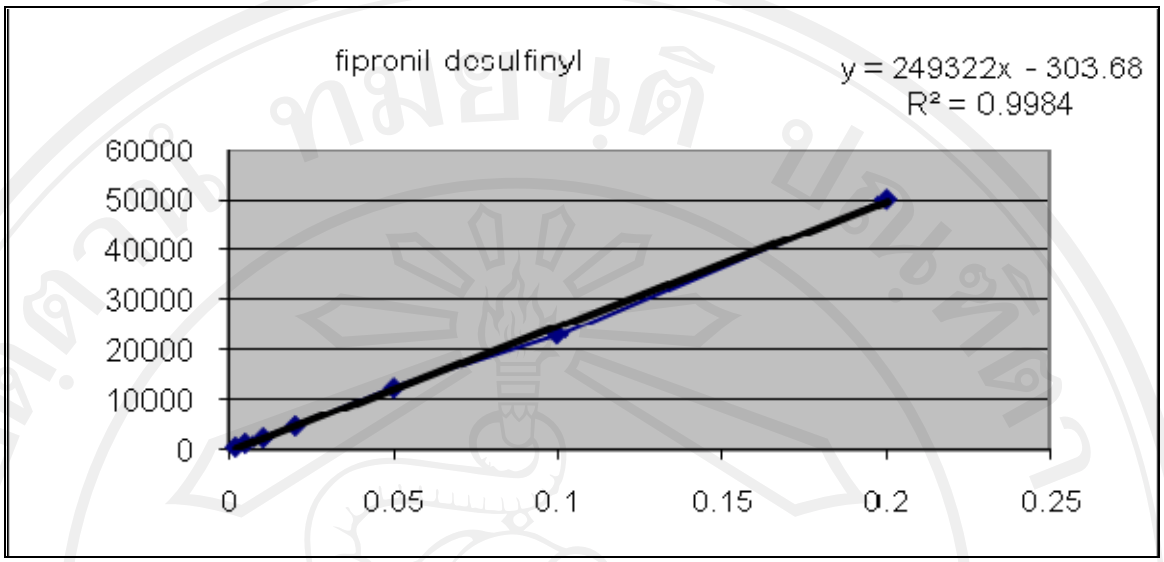

Figure 4.4 Linearity of fipronil - desulfinyl

4.1.2 The accuracy and the precision of the method

The accuracies and precisions for the determination of fipronil and its derivatives in sweet peppers were investigated by using standard addition method. Three levels concentration of standard fipronil or fipronil derivatives (fipronil-sulfide, fipronil-sulfone and fipronil-desulfinyl) of low, middle and high concentration levels at 0.002 mg/kg, 0.02 mg/kg and 0.2 mg/kg were added into the samples respectively and determined by GC-MS. The average percentage recoveries and the %CV were obtained. Results are shown in Tables 4.5- 4.28

Table 4.5 Percentage recovery and the precision of fipronil in sweet pepper samples blank added with 0.002 mg/kg of fipronil standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	812	0.0018	91.96
2	795	0.0018	90.06
3	797	0.0018	90.28
4	773	0.0018	87.60
5	842	0.0019	95.32
6	787	0.0018	89.17
7	784	0.0018	88.83
8	822	0.0019	93.08
9	871	0.0020	98.57
10	849	0.0019	96.11
	mean	0.0018	92.10
	SD	-	3.59
	%CV	-	3.90

Table 4.6 Percentage recovery and the precision of fipronil in sweet pepper samples blank added with 0.02 mg/kg of fipronil standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	9674	0.0185	92.63
2	10267	0.0197	98.43
3	9373	0.0179	89.69
4	9998	0.0192	95.80
5	8971	0.0172	85.76
6	8852	0.0169	84.60
7	9299	0.0178	88.97
8	9839	0.0188	94.25
9	8811	0.0168	84.20
10	9031	0.0173	86.35
	mean	0.0180	90.07
	SD	-	5.00
	%CV	-	5.55

Table 4.7 Percentage recovery and the precision of fipronil in sweet pepper samples blank added with 0.2 mg/kg of fipronil standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	101002	0.2034	101.69
2	93010	0.1872	93.61
3	100031	0.2014	100.71
4	95667	0.1926	96.30
5	97813	0.1969	98.47
6	95275	0.1918	95.90
7	94962	0.1912	95.59
8	95439	0.1921	96.07
9	95193	0.1916	95.82
10	95442	0.1921	96.07
	mean	0.1940	97.02
	SD	-	2.50
	%CV	-	2.58

Table 4.8 The precision of fipronil in sweet pepper samples blank added with 0.002 mg/kg of fipronil standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	812	0.0018	91.96
2	795	0.0018	90.06
3	797	0.0018	90.28
4	773	0.0018	87.60
5	842	0.0019	95.32
6	787	0.0018	89.17
7	784	0.0018	88.83
8	822	0.0019	93.08
9	871	0.0020	98.57
10	849	0.0019	96.11
11	1006	0.0018	89.76
12	1089	0.0019	97.32
13	970	0.0017	86.47
14	1031	0.0018	92.03
15	975	0.0017	86.93
16	1029	0.0018	91.85
17	1053	0.0019	94.04
18	993	0.0018	88.57
19	1050	0.0019	93.76
20	1012	0.0018	90.30
mean		0.0018	91.60
SD		-	3.44
%CV		-	3.73

Table 4.9 The precision of fipronil in sweet pepper samples blank added with 0.02 mg/kg of fipronil standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	9674	0.0185	92.63
2	10267	0.0197	98.43
3	9373	0.0179	89.69
4	9998	0.0192	95.80
5	8971	0.0172	85.76
6	8852	0.0169	84.60
7	9299	0.0178	88.97
8	9839	0.0188	94.25
9	8811	0.0168	84.20
10	9031	0.0173	86.35
11	8935	0.0197	98.46
12	8764	0.0193	96.63
13	8667	0.0191	95.60
14	8085	0.0179	89.40
15	8898	0.0196	98.06
16	8587	0.0189	94.75
17	8755	0.0193	96.54
18	8712	0.0192	96.08
19	8742	0.0193	96.40
20	8794	0.0194	96.95
mean		0.0186	93.0
SD		-	4.87
%CV		-	5.18

Table 4.10 The precision of fipronil in sweet pepper samples blank added with 0.2 mg/kg of fipronil standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	101002	0.2034	101.69
2	93010	0.1872	93.61
3	100031	0.2014	100.71
4	95667	0.1926	96.30
5	97813	0.1969	98.47
6	95275	0.1918	95.90
7	94962	0.1912	95.59
8	95439	0.1921	96.07
9	95193	0.1916	95.82
10	95442	0.1921	96.07
11	76003	0.1631	81.54
12	80078	0.1719	85.93
13	89665	0.1925	96.25
14	84322	0.1810	90.49
15	88990	0.1910	95.51
16	84266	0.1809	90.43
17	84409	0.1812	90.59
18	90354	0.1940	97.00
19	81123	0.1741	87.05
20	85540	0.1836	91.81
	mean	0.1877	93.84
	SD	-	4.99
	%CV	-	5.45

Table 4.11 Percentage recovery and the precision of fipronil-sulfide in sweet pepper samples blank added with 0.002 mg/kg of fipronil-sulfide standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	877	0.0018	91.26
2	903	0.0019	93.97
3	784	0.0016	81.58
4	853	0.0018	88.76
5	858	0.0018	89.28
6	847	0.0018	88.14
7	855	0.0018	88.97
8	787	0.0016	81.89
9	863	0.0018	89.80
10	881	0.0018	91.68
	mean	0.0018	88.53
	SD	-	3.59
	%CV	-	3.90

Table 4.12 Percentage recovery and the precision of fipronil-sulfide in sweet pepper samples blank added with 0.02 mg/kg of fipronil-sulfide standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	7219	0.0168	84.09
2	8280	0.0193	96.63
3	8089	0.0189	94.38
4	8157	0.0190	95.15
5	8251	0.0193	96.29
6	8252	0.0193	96.30
7	7530	0.0176	87.77
8	8202	0.0191	95.71
9	8285	0.0193	96.69
10	8334	0.0195	97.27
	mean	0.0188	94.03
	SD	-	4.43
	%CV	-	4.72

Table 4.13 Percentage recovery and the precision of fipronil-sulfide in sweet pepper samples blank added with 0.2 mg/kg of fipronil-sulfide standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	92432	0.1955	97.73
2	91123	0.1927	96.35
3	90089	0.1905	95.26
4	78157	0.1654	82.68
5	91654	0.1938	96.91
6	89252	0.1888	94.38
7	90830	0.1921	96.04
8	86202	0.1823	91.16
9	79828	0.1689	84.44
10	91334	0.1931	96.57
	mean	0.187	93.15
	SD	-	5.38
	%CV	-	5.78

Table 4.14 The precision of fipronil-sulfide in sweet pepper samples blank added with 0.002 mg/kg of fipronil-sulfide standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	877	0.0018	91.26
2	903	0.0019	93.97
3	784	0.0016	81.58
4	853	0.0018	88.76
5	858	0.0018	89.28
6	847	0.0018	88.14
7	855	0.0018	88.97
8	787	0.0016	81.89
9	863	0.0018	89.80
10	881	0.0018	91.68
11	975	0.0019	96.05
12	816	0.0016	80.22
13	917	0.0018	90.28
14	847	0.0017	83.30
15	898	0.0018	88.38
16	974	0.0019	95.95
17	940	0.0019	92.57
18	852	0.0017	83.80
19	867	0.0017	85.30
20	981	0.0019	96.65
mean		0.0018	88.89
SD		-	4.95
%CV		-	5.53

Table 4.15 The precision of fipronil-sulfide in sweet pepper samples blank added with 0.02 mg/kg of fipronil-sulfide standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	7219	0.0168	84.09
2	8280	0.0193	96.63
3	8089	0.0189	94.38
4	8157	0.0190	95.15
5	8251	0.0193	96.29
6	8252	0.0193	96.30
7	7530	0.0176	87.77
8	8202	0.0191	95.71
9	8285	0.0193	96.69
10	8334	0.0195	97.27
11	9821	0.0195	97.64
12	9162	0.0181	90.56
13	8565	0.0168	84.15
14	9670	0.0192	96.02
15	9377	0.0186	92.87
16	9261	0.0183	91.63
17	9974	0.0199	99.28
18	9351	0.0185	92.59
19	9435	0.0187	93.50
20	9928	0.0198	98.79
mean		0.0188	93.87
SD		-	4.37
%CV		-	4.64

Table 4.16 The precision of fipronil-sulfide in sweet pepper samples blank added with 0.2 mg/kg of fipronil-sulfide standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	92432	0.1955	97.73
2	91123	0.1927	96.35
3	90089	0.1905	95.26
4	78157	0.1654	82.68
5	91654	0.1938	96.91
6	89252	0.1888	94.38
7	90830	0.1921	96.04
8	86202	0.1823	91.16
9	79828	0.1689	84.44
10	91334	0.1931	96.57
11	85335	0.1966	98.28
12	79803	0.1837	91.86
13	77546	0.1785	89.25
14	79441	0.1829	91.44
15	81002	0.1865	93.25
16	83498	0.1923	96.15
17	78332	0.1803	90.16
18	79422	0.1828	91.42
19	88414	0.2037	101.85
20	83341	0.1919	95.96
mean		0.1871	93.56
SD		-	4.63
%CV		-	4.96

Table 4.17 Percentage recovery and the precision of fipronil-sulfone in sweet pepper samples blank added with 0.002 mg/kg of fipronil-sulfone standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	415	0.0019	95.45
2	378	0.0018	87.88
3	393	0.0018	90.95
4	375	0.0017	87.27
5	419	0.0019	96.27
6	396	0.0018	91.57
7	405	0.0019	93.41
8	341	0.0016	80.31
9	358	0.0017	83.79
10	349	0.0016	81.95
	mean	0.0018	88.89
	SD	-	5.59
	%CV	-	6.28

Table 4.18 Percentage recovery and the precision of fipronil-sulfone in sweet pepper samples blank added with 0.02 mg/kg of fipronil-sulfone standard using GC-MS for analysis

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	4864	0.0187	93.42
2	4574	0.0175	87.48
3	4815	0.0185	92.42
4	5038	0.0194	96.98
5	4923	0.0189	94.63
6	4606	0.0176	88.14
7	4934	0.0190	94.85
8	4847	0.0186	93.07
9	4656	0.0178	89.16
10	4522	0.0173	86.42
	mean	0.0183	91.66
	SD	-	3.60
	%CV	-	3.93

Table 4.19 Percentage recovery of fipronil-sulfone in sweet pepper samples blank added with 0.2 mg/kg of fipronil-sulfone standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	52776	0.2031	101.56
2	51387	0.1978	98.89
3	50996	0.1963	98.14
4	49806	0.1917	95.84
5	46788	0.1801	90.03
6	47825	0.1841	92.03
7	45144	0.1737	86.87
8	47847	0.1841	92.07
9	51656	0.1988	99.41
10	46079	0.1773	88.67
	mean	0.189	94.35
	SD	-	5.08
	%CV	-	5.38

Table 4.20 The precision of fipronil-sulfone in sweet pepper samples blank added with 0.002 mg/kg of fipronil-sulfone standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	415	0.0019	95.45
2	378	0.0018	87.88
3	393	0.0018	90.95
4	375	0.0017	87.27
5	419	0.0019	96.27
6	396	0.0018	91.57
7	405	0.0019	93.41
8	341	0.0016	80.31
9	358	0.0017	83.79
10	349	0.0016	81.95
11	457	0.0019	94.99
12	454	0.0019	94.49
13	430	0.0018	90.43
14	424	0.0018	89.42
15	413	0.0018	87.56
16	425	0.0018	89.59
17	441	0.0018	92.29
18	452	0.0019	94.15
19	451	0.0019	93.98
20	464	0.0019	96.18
mean		0.0018	90.60
SD		-	4.67
%CV		-	5.15

Table 4.21 The precision of fipronil-sulfone in sweet pepper samples blank added with 0.02 mg/kg of fipronil-sulfone standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	4864	0.0187	93.42
2	4574	0.0175	87.48
3	4815	0.0185	92.42
4	5038	0.0194	96.98
5	4923	0.0189	94.63
6	4606	0.0176	88.14
7	4934	0.0190	94.85
8	4847	0.0186	93.07
9	4656	0.0178	89.16
10	4522	0.0173	86.42
11	5007	0.0170	85.23
12	5898	0.0201	100.39
13	5832	0.0199	99.27
14	5754	0.0196	97.94
15	5823	0.0198	99.12
16	5631	0.0192	95.85
17	5343	0.0182	90.95
18	5114	0.0174	87.05
19	5599	0.0191	95.31
20	5283	0.0180	89.93
mean		0.0186	92.88
SD		-	4.66
%CV		-	5.01

Table 4.22 The precision of fipronil-sulfone in sweet pepper samples blank added with 0.2 mg/kg of fipronil-sulfone standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	52776	0.2031	101.56
2	51387	0.1978	98.89
3	50996	0.1963	98.14
4	49806	0.1917	95.84
5	46788	0.1801	90.03
6	47825	0.1841	92.03
7	45144	0.1737	86.87
8	47847	0.1841	92.07
9	51656	0.1988	99.41
10	46079	0.1773	88.67
11	45880	0.1942	97.10
12	47224	0.1999	99.94
13	42445	0.1796	89.82
14	43506	0.1841	92.10
15	42560	0.1801	90.07
16	41774	0.1768	88.40
17	42880	0.1815	90.75
18	43511	0.1842	92.08
19	43292	0.1832	91.62
20	42960	0.1780	89.01
mean		0.1864	93.22
SD		-	4.47
%CV		-	4.83

Table 4.23 Percentage recovery and the precision of fipronil-desulfinyl in sweet pepper samples blank added with 0.002 mg/kg of fipronil-desulfinyl standard using GC-MS for analysis

Replication	**Peak Area**	**Concentration (mg/kg)**	**%Recovery**
1	438	0.0018	90.53
2	410	0.0017	85.15
3	449	0.0019	92.64
4	414	0.0017	85.91
5	431	0.0018	89.18
6	428	0.0018	88.61
7	442	0.0018	91.30
8	464	0.0019	95.52
9	408	0.0017	84.76
10	468	0.0019	96.29
	mean	0.0018	89.90
	SD	-	4.07
	%CV	-	4.53

Table 4.24 Percentage recovery and the precision of fipronil-desulfinyl in sweet pepper samples blank added with 0.02 mg/kg of fipronil-desulfinyl standard using GC-MS for analysis

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	3688	0.0178	89.10
2	3322	0.0162	81.02
3	3309	0.0161	80.73
4	3593	0.0174	87.00
5	3930	0.0189	94.44
6	3731	0.0180	90.05
7	3882	0.0187	93.38
8	3953	0.0190	94.95
9	3417	0.0166	83.12
10	3895	0.0187	93.67
	mean	0.0177	88.75
	SD	-	5.55
	%CV	-	6.25

Table 4.25 Percentage recovery and the precision of fipronil-desulfinyl in sweet pepper samples blank added with 0.2 mg/kg of fipronil-desulfinyl standard using GC-MS for analysis

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	52114	0.2040	101.99
2	50198	0.1965	98.25
3	49894	0.1953	97.65
4	45632	0.1787	89.33
5	47791	0.1871	93.55
6	50032	0.1958	97.92
7	46092	0.1805	90.23
8	48013	0.1880	93.98
9	47976	0.1878	93.91
10	50054	0.1959	97.96
	mean	0.1910	95.48
	SD	-	3.95
	%CV	-	4.14

Table 4.26 The precision of fipronil-desulfinyl in sweet pepper samples blank added with 0.002 mg/kg of fipronil-desulfinyl standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	438	0.0018	90.53
2	410	0.0017	85.15
3	449	0.0019	92.64
4	414	0.0017	85.91
5	431	0.0018	89.18
6	428	0.0018	88.61
7	442	0.0018	91.30
8	464	0.0019	95.52
9	408	0.0017	84.76
10	468	0.0019	96.29
11	471	0.0018	89.77
12	439	0.0017	84.01
13	482	0.0018	91.75
14	492	0.0019	93.56
15	479	0.0018	91.21
16	426	0.0016	81.67
17	515	0.0020	97.70
18	497	0.0019	94.46
19	429	0.0016	82.21
20	425	0.0016	81.49
mean		0.0018	89.40
SD		-	5.01
%CV		-	5.62

Table 4.27 The precision of fipronil-desulfinyl in sweet pepper samples blank added with 0.02 mg/kg of fipronil-desulfinyl standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	3688	0.0178	89.10
2	3322	0.0162	81.02
3	3309	0.0161	80.73
4	3593	0.0174	87.00
5	3930	0.0189	94.44
6	3731	0.0180	90.05
7	3882	0.0187	93.38
8	3953	0.0190	94.95
9	3417	0.0166	83.12
10	3895	0.0187	93.67
11	4109	0.0163	81.46
12	5145	0.0205	102.34
13	4209	0.0167	83.48
14	4963	0.0197	98.67
15	4865	0.0193	96.70
16	4220	0.0167	83.70
17	5269	0.0210	104.84
18	4234	0.0168	83.98
19	4161	0.0165	82.51
20	5004	0.0199	99.50
mean		0.0180	90.23
SD		-	7.68
%CV		-	8.43

Table 4.28 The precision of fipronil-desulfinyl in sweet pepper samples blank added with 0.2 mg/kg of fipronil-desulfinyl standard using GC-MS for analysis

Replication	Peak Area	Concentration(mg/kg)	%Recovery
1	52114	0.2040	101.99
2	50198	0.1965	98.25
3	49894	0.1953	97.65
4	45632	0.1787	89.33
5	47791	0.1871	93.55
6	50032	0.1958	97.92
7	46092	0.1805	90.23
8	48013	0.1880	93.98
9	47976	0.1878	93.91
10	50054	0.1959	97.96
11	41674	0.1851	92.54
12	40409	0.1795	89.76
13	42134	0.1871	93.56
14	41357	0.1837	91.85
15	36213	0.1610	80.50
16	43647	0.1938	96.90
17	43012	0.1910	95.50
18	40578	0.1803	90.13
19	39884	0.1772	88.60
20	37213	0.1654	82.71
mean		0.1857	92.84
SD		-	5.26
%CV		-	5.76

4.1.3 Limit of detection (LOD) and Limit of quantification (LOQ)

The samples blank added with the mixed standard solutions of 0.005 mg/kg fipronil (fortified sample) and its derivatives were extracted and analyzed by GC-MS. Results of the LOD obtained by calculation of the SD value multiplied by 3 while results of the LOQ obtained by calculation of the SD value multiplied by 10 as shown in Tables 4.29- 4.40 and the peaks of mixed standard solutions are shown in Figures 4.5 – 4.6

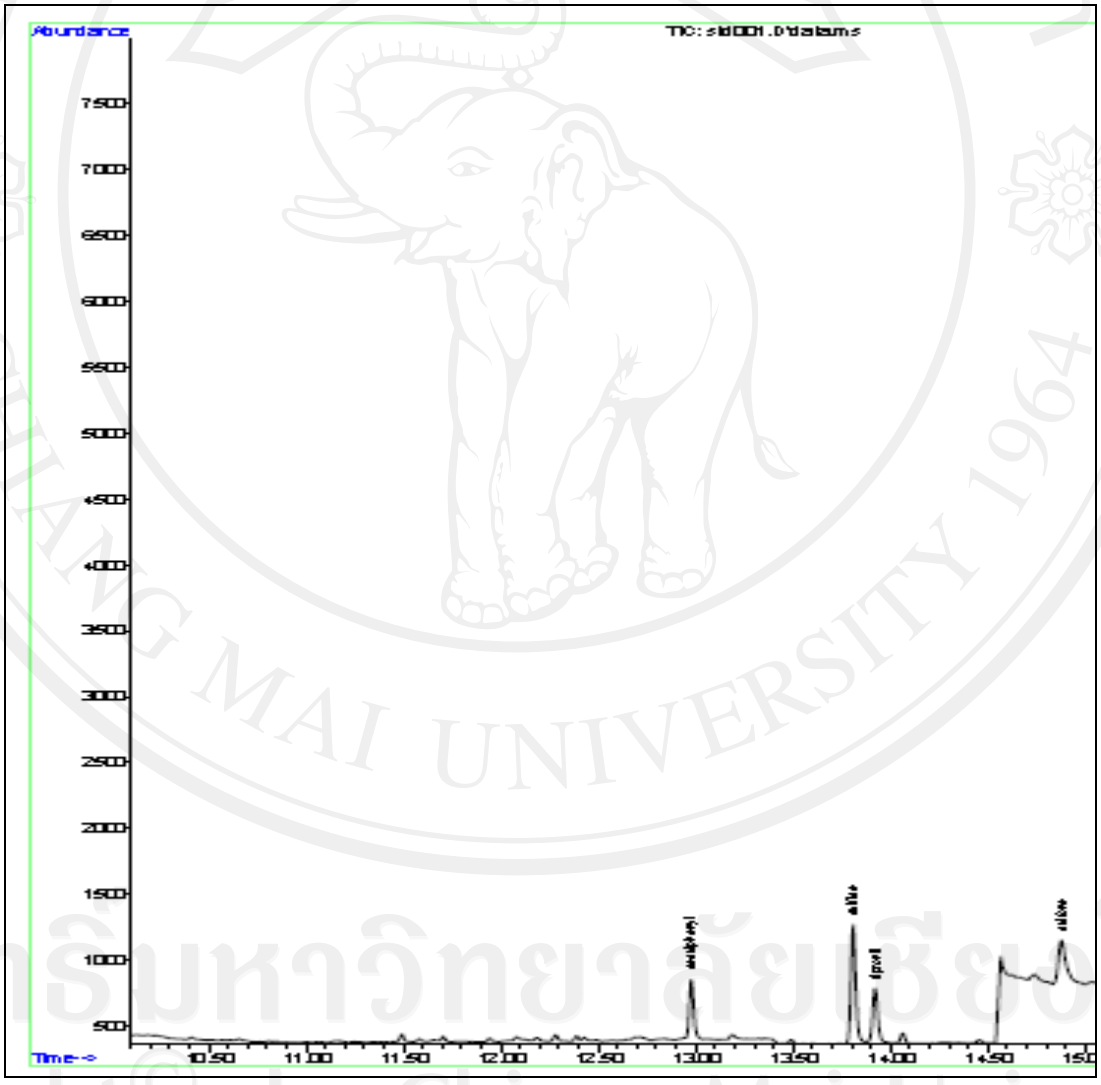

Figure 4.5 LOD of fipronil and its derivatives

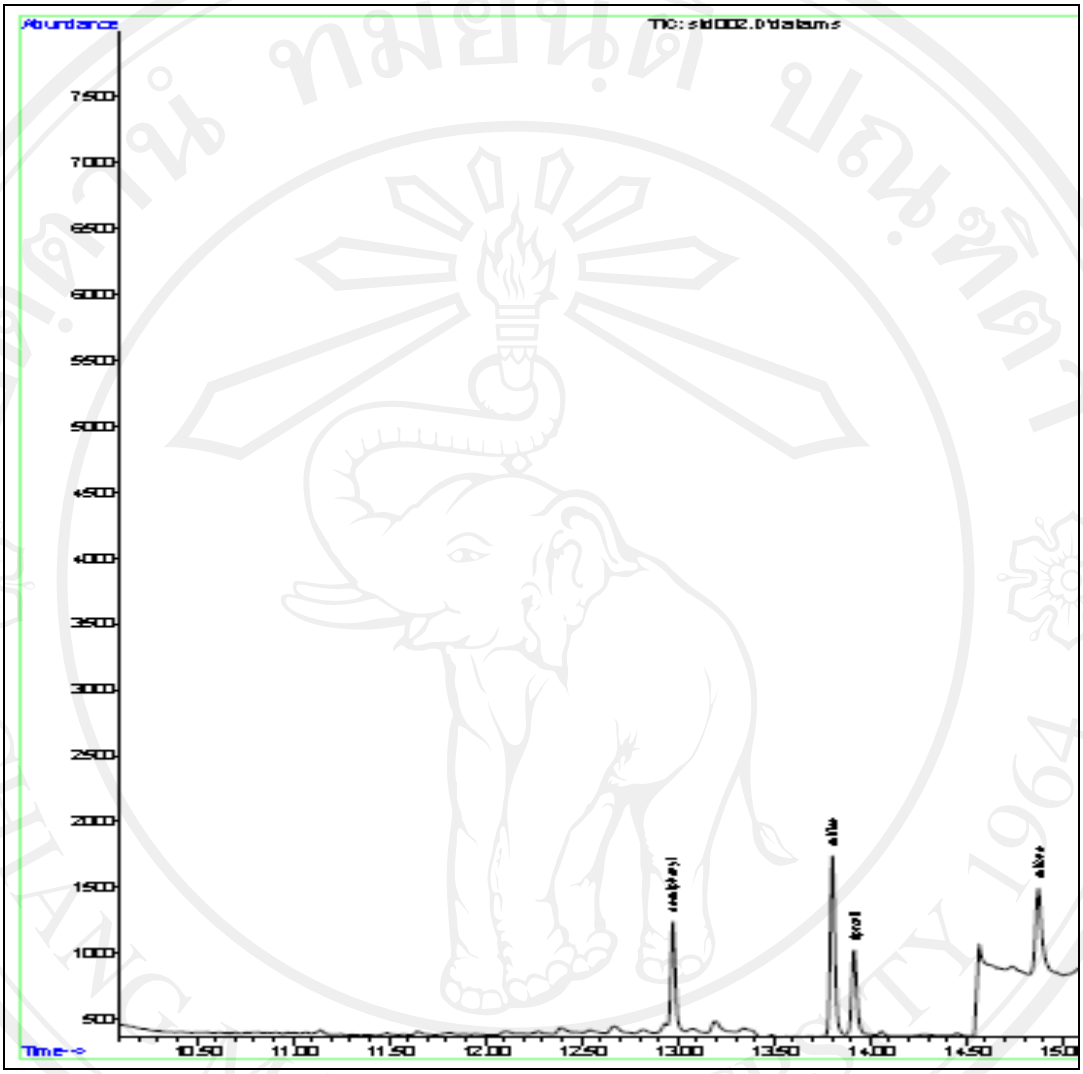

Figure 4.6 LOQ of fipronil and its derivatives

Table 4.29 The standard deviation value of fortified sample at 0.005 mg/kg of fipronil repeating analysis 10 times

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	2075	0.0041	82.16
2	2046	0.0040	80.96
3	2035	0.0040	80.50
4	2104	0.0042	83.36
5	2224	0.0044	88.32
6	2275	0.0045	90.43
7	2362	0.0047	94.03
8	2239	0.0044	88.94
9	2193	0.0044	87.04
10	2242	0.0045	89.07
Mean		0.0043	86.48
SD		0.0002*	-
%CV		5.22	-

*$LOD_{approx} = 3 \times 0.0002 = 0.0006$ approximately equals to 0.001 mg/kg

From the LOD $_{approx}$ value obtained (Table 4.29) the concentration of fortified sample at 0.001 mg/kg fipronil were extracted and analyzed by GC-MS for confirmation of LOD value that showed 3 times of the SD are indicated in Table 4.30

Table 4.30 The peak areas of fortified sample of fipronil at 0.001 mg/kg that shown 3 times of the noise (LOD)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	479	0.00091	90.82
2	447	0.00084	84.08
3	483	0.00092	91.66
4	464	0.00088	87.66
5	492	0.00094	93.55
6	473	0.00090	89.55
7	481	0.00091	91.24
8	495	0.00094	94.19
9	470	0.00089	93.13
10	428	0.00080	94.82
Mean		0.00090	89.18
SD		0.00004	-
%CV		4.85	-

$LOQ_{approx} = 10 \times 0.0002 = 0.002$ approximately equals to 0.002 mg/kg

From the LOQ $_{approx}$ (10 times of SD) obtained from Table 4.29 the concentration of fortified sample at 0.002 mg/kg fipronil were extracted and analysed by GC-MS for confirmation. Results showed that percentages of recovery are higher than 80% and the LOQ $_{approx}$ is 10 times of the noise as indicated in table 4.31

Table 4.31 The peak areas of fortified sample of fipronil at 0.002 mg/kg that shown 10 times of the noise (LOQ)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	812	0.0018	91.96
2	795	0.0018	90.06
3	797	0.0018	90.28
4	773	0.0018	87.60
5	842	0.0019	95.32
6	897	0.0020	101.48
7	868	0.0020	98.23
8	822	0.0019	93.08
9	871	0.0020	98.57
10	849	0.0019	96.11
	Mean	0.0019	94.06
	SD	0.0001	-
	%CV	4.68	-

The samples blank added with the mixed standard solution of 0.005 mg/kg fipronil-sulfide (fortified sample) were extracted and analyzed by GC-MS. Results of the standard deviation value shown in Table 4.32

Table 4.32 The standard deviation value of fortified sample at 0.005 mg/kg of fipronil-sulfide repeating analysis 10 times

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	2191	0.0045	81.59
2	2050	0.0042	84.08
3	2090	0.0042	83.21
4	2117	0.0043	82.71
5	2132	0.0044	83.34
6	2012	0.0041	82.51
7	2030	0.0042	83.25
8	1969	0.0040	80.72
9	2153	0.0044	81.39
10	2155	0.0044	80.14
Mean		0.0043	85.48
SD		0.0002*	-
%CV		3.62	-

*$LOD_{approx} = 3 \times 0.0002 = 0.0006$ approximately equals to 0.001 mg/kg

From the LOD $_{approx}$ value obtained (Table 4.32) the concentration of fortified sample at 0.001 mg/kg fipronil were extracted and analysed by GC-MS for confirmation of LOD value and the peak areas that showed 3 times of the noise are indicated in Table 4.33

Table 4.33 The peak areas of fortified sample of fipronil-sulfide at 0.001 mg/kg that shown 3 times of the noise (LOD)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	539	0.00086	86.46
2	552	0.00089	89.45
3	534	0.00085	85.31
4	532	0.00085	84.85
5	549	0.00089	88.76
6	541	0.00087	86.92
7	524	0.00083	83.01
8	518	0.00082	81.63
9	515	0.00081	80.94
10	544	0.00088	87.61
Mean		0.00090	85.50
SD		0.00003	-
%CV		3.40	-

LOQ $_{approx}$ = $10 \times 0.0002 = 0.002$ approximately equals to 0.002 mg/kg

From the LOQ $_{approx}$ (10 times of SD) obtained from Table 4.32 the concentration of fortified sample at 0.002 mg/kg fipronil-sulfide were extracted and analysed by GC-MS for confirmation. Results showed that percentages of recovery are higher than 80% and the LOQ $_{approx}$ is 10 times of the noise as indicated in table 4.34

Table 4.34 The peak areas of fortified sample of fipronil-sulfide at 0.002 mg/kg that shown 10 times of the noise (LOQ)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	797	0.0017	82.93
2	923	0.0019	96.05
3	884	0.0018	91.99
4	913	0.0019	95.01
5	858	0.0018	89.28
6	877	0.0018	91.26
7	865	0.0018	90.01
8	887	0.0018	92.30
9	893	0.0019	92.93
10	881	0.0018	91.68
Mean		0.0018	91.31
SD		0.0001	-
%CV		3.94	-

The samples blank added with the mixed standard solution of 0.005 mg/kg fipronil-sulfone (fortified sample) were extracted and analyzed by GC-MS. Results of the standard deviation value shown in Table 4.35

Table 4.35 The standard deviation value of fortified sample at 0.005 mg/kg of fipronil-sulfone repeating analysis 10 times

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	1162	0.0046	92.46
2	1044	0.0042	83.59
3	1017	0.0041	81.56
4	1038	0.0042	83.14
5	1023	0.0041	82.01
6	1055	0.0042	84.41
7	1040	0.0042	83.29
8	1047	0.0042	83.81
9	1056	0.0042	84.49
10	1079	0.0043	86.22
Mean		0.0042	84.50
SD		0.0002*	-
%CV		3.65	-

*$LOD_{approx} = 3 \times 0.0002 = 0.0006$ approximately equals to 0.001 mg/kg

From the LOD $_{approx}$ value obtained (Table 4.35) the concentration of fortified sample at 0.001 mg/kg fipronil were extracted and analysed by GC-MS for confirmation of LOD value and the peak areas that showed 3 times of the noise are indicated in Table 4.36

Table 4.36 The peak areas of fortified sample of fipronil-sulfone at 0.001 mg/kg that shown 3 times of the noise (LOD)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	203	0.00079	79.29
2	208	0.00081	81.50
3	228	0.00090	90.33
4	210	0.00082	82.38
5	224	0.00089	88.57
6	219	0.00086	86.36
7	235	0.00093	93.42
8	217	0.00085	85.47
9	221	0.00087	87.24
10	226	0.00089	89.45
Mean		0.00090	86.40
SD		0.00004	-
%CV		5.04	-

$LOQ_{approx} = 10 \times 0.0002 = 0.002$ approximately equals to 0.002 mg/kg

From the LOQ $_{approx}$ (10 times of SD) obtained from Table 4.35 the concentration of fortified sample at 0.002 mg/kg fipronil-sulfone were extracted and analysed by GC-MS for confirmation. Results showed that percentages of recovery are higher than 80% and the LOQ $_{approx}$ is 10 times of the noise as indicated in table 4.37

Table 4.37 The peak areas of fortified sample of fipronil-sulfone at 0.002 mg/kg that shown 10 times of the noise (LOQ)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	389	0.0018	90.13
2	408	0.0019	94.02
3	403	0.0019	93.00
4	375	0.0017	87.27
5	409	0.0019	94.23
6	386	0.0018	89.52
7	425	0.0020	97.50
8	427	0.0020	97.91
9	398	0.0018	91.98
10	379	0.0018	88.09
	Mean	0.0019	92.84
	SD	0.0001	-
	%CV	3.97	-

The samples blank added with the mixed standard solution of 0.005 mg/kg fipronil-desulfinyl (fortified sample) were extracted and analyzed by GC-MS. Results of the standard deviation value shown in Table 4.38

Table 4.38 The standard deviation value of fortified sample at 0.005 mg/kg of fipronil-desulfinyl repeating analysis 10 times

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	1189	0.0045	90.48
2	1122	0.0043	85.61
3	1106	0.0042	84.44
4	1126	0.0043	85.90
5	1130	0.0043	86.19
6	1077	0.0041	82.33
7	1022	0.0039	78.33
8	1133	0.0043	86.41
9	1059	0.0041	81.02
10	1046	0.0040	80.08
Mean		0.0042	84.08
SD		0.0002*	-
%CV		4.30	-

*$LOD_{approx} = 3 \times 0.0002 = 0.0006$ approximately equals to 0.001 mg/kg

From the LOD $_{approx}$ value obtained (Table 4.38) the concentration of fortified sample at 0.001 mg/kg fipronil-desulfinyl were extracted and analysed by GC-MS for confirmation of LOD value and the peak areas that showed 3 times of the noise are indicated in Table 4.39

Table 4.39 The peak areas of fortified sample of fipronil-desulfinyl at 0.001 mg/kg that shown 3 times of the noise (LOD)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	147	0.00088	88.06
2	155	0.00091	91.33
3	144	0.00087	86.83
4	134	0.00083	82.74
5	143	0.00086	86.42
6	145	0.00087	87.24
7	153	0.00091	90.51
8	129	0.00081	80.70
9	148	0.00088	88.47
10	159	0.00093	92.96
Mean		0.00090	87.53
SD		0.00004	-
%CV		4.26	-

$LOQ_{approx} = 10 \times 0.0002 = 0.002$ approximately equals to 0.002 mg/kg

From the LOQ $_{approx}$ (10 times of SD) obtained from Table 4.38 the concentration of fortified sample at 0.002 mg/kg fipronil-desulfinyl were extracted and analysed by GC-MS for confirmation. Results showed that percentages of recovery are higher than 80% and the LOQ $_{approx}$ is 10 times of the noise as indicated in table 4.40

Table 4.40 The peak areas of fortified sample of fipronil-desulfinyl at 0.002 mg/kg that shown 10 times of the noise (LOQ)

Sample No.	Peak Area	Concentration (mg/kg)	%Recovery
1	448	0.0018	92.45
2	408	0.0017	84.76
3	459	0.0019	94.56
4	424	0.0018	87.84
5	451	0.0019	93.03
6	438	0.0018	90.53
7	422	0.0017	87.45
8	474	0.0019	97.45
9	428	0.0018	88.61
10	441	0.0018	91.10
	Mean	0.0018	90.74
	SD	0.0001	-
	%CV	4.15	-

4.2 Optimum conditions for analyzing fipronil and its derivatives by GC-MS

After analyses of the fortified samples to validate the analytical method by testing linearity range, precision, accuracy, LOD and LOQ, the optimum conditions obtained for analysis of fipronil and its derivatives are indicated below:

Column : HP-5ms; 30 m. length x 250 um(i.d.) x 0.25 um.film Thickness

Temperature : injector 250° C, detector, 300 °C

Oven temperature program : 50 °C (1 min) 10 °C/min 100 °C (1 min) 20 °C/min
240 °C (2 min) 25 °C /min 280 °C (0 min)

Inject mode : splitless (purge on time 1 ml/min)

Carrier gas : helium, flow rate 1 ml/min

Injecttion volume : 2 ul

MSD Transfer Line : 280 °C

MS Source : 230 °C maximum250 °C

MS Quad : 150 °C maximum200 °C

Tune File : atune.u

Acquistion Mode : SIM

Ion selected for SIM acquisition: (in retention time order)

Fipronil desulfinyl **388,** 333, 369, 390

Fipronil **367**, 213, 369

Fipronil sulfide **351**, 255, 353, 420

Fipronil sulfone **383**, 215, 365,

(Quantitation ions were in bold)

4.3 Comparison of two types of SPE for cleaning up the samples extracts

4.3.1 Using alumina SPE for cleaning up the extracts

The sweet pepper samples added with the mix standard solution of fipronil at concentration of 0.002 mg/kg were extracted and cleaned up with 2 types of SPE (alumina and PSA) and analysed by GC-MS. Good percentages of recovery of the insecticides residues are shown in Figure 4.7 and Tables 4.42-4.45

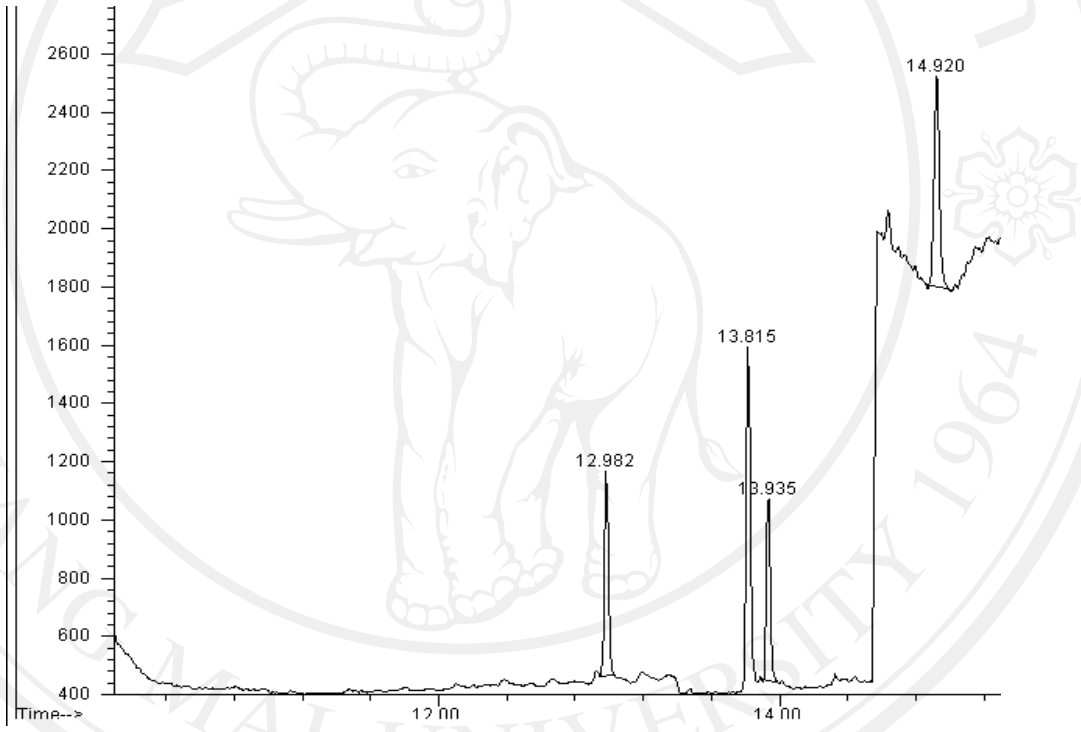

Figure 4.7 Chromatogram of fipronil residues in the sweet pepper samples added with 0.002 mg/kg of fipronil and cleaned up with alumina SPE

Table 4.41 Percentage recovery of fipronil in sweet pepper samples added with mixed standard of fipronil at 0.002 mg/kg and cleaned up with alumina SPE

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	545	0.0019	95.90
2	535	0.0019	94.73
3	547	0.0019	96.13
4	573	0.0020	99.17
5	542	0.0019	95.55
6	517	0.0019	92.63
7	565	0.0020	98.23
8	559	0.0020	97.53
9	471	0.0017	87.27
10	449	0.0017	84.70
	Mean		95.24
	%CV		5.02

Table 4.42 Percentage recovery of fipronil-sulfide in sweet pepper samples added with mixed standard of fipronil at 0.002 mg/kg and cleaned up with alumina SPE

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	1331	0.0019	94.87
2	1289	0.0018	89.58
3	1310	0.0018	92.23
4	1293	0.0018	90.09
5	1328	0.0019	94.49
6	1287	0.0018	89.33
7	1320	0.0019	93.48
8	1313	0.0019	92.60
9	1219	0.0016	80.77
10	1325	0.0019	94.11
	Mean		90.82
	%CV		4.58

Table 4.43 Percentage recovery of fipronil-sulfone in sweet pepper samples added with mixed standard of fipronil at 0.002 mg/kg and cleaned up with alumina SPE

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	489	0.0018	88.31
2	508	0.0019	92.65
3	503	0.0018	91.51
4	475	0.0017	85.12
5	509	0.0019	92.88
6	485	0.0017	87.40
7	525	0.0019	96.53
8	527	0.0019	96.98
9	497	0.0018	90.14
10	476	0.0017	85.35
	Mean		91.28
	%CV		4.64

Table 4.44 Percentage recovery of fipronil-desulfinyl in sweet pepper samples added with mixed standard of fipronil at 0.002 mg/kg and cleaned up with alumina SPE

Replication	Peak Area	Concentration (mg/kg)	%Recovery
1	698	0.0019	94.61
2	708	0.0019	96.88
3	679	0.0018	90.30
4	634	0.0016	80.08
5	681	0.0018	90.75
6	655	0.0017	84.85
7	672	0.0018	88.71
8	701	0.0019	95.29
9	688	0.0018	92.30
10	705	0.0019	96.20
	Mean		90.42
	%CV		5.88

4.3.2 Using PSA (primary-secondary-amine) SPE for cleaning up the extracts

The fortified samples at 0.002 mg/kg of fipronil were extracted (same as 4.3.1) and cleaned up with PSA-SPE. Results in quantity are shown in Figure 4.8 but percentages of recovery were very low, no study further for this SPE type.

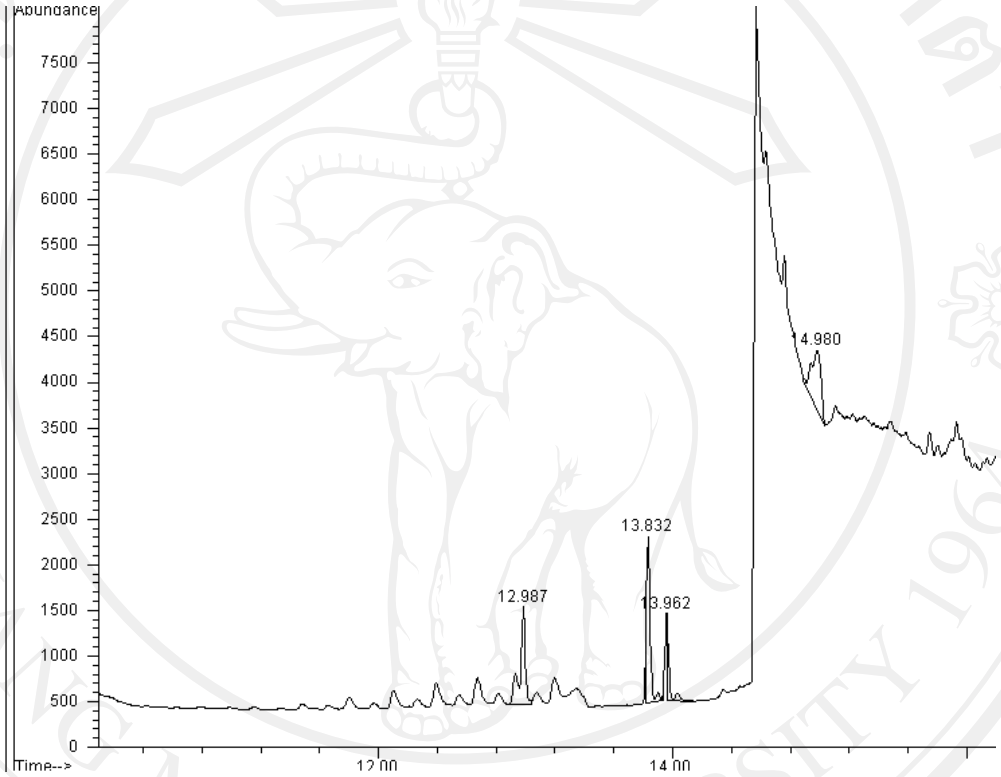

Figure 4.8 Chromatogram of fipronil residues in sweet pepper samples added 0.002 mg/kg of fipronil and cleaned up with PSA-SPE

4.4 Analysis of residues of fipronil and its derivatives in sweet peppers at the recommended rate

The sweet pepper plants were sprayed with fipronil at recommended rate (20 ml/20 L) 1, 2, and 3 times. The fruits were harvested to be analysed at 0, 1, 3, 5, 7, 10 and 14 days after sprayed. Results are shown in Tables 4.46-4.48 and disintegration of fipronil and its derivatives are shown in Figures 4.9-4.11

Table 4.45 The amount of fipronil and its derivatives residues in sweet peppers harvested 7 times for analysis by GC-MS after spraying **once** with fipronil at recommended rate

Time of spraying Day(s)	Amount of fipronil (mg/kg)	Amount of fipronil-sulfide (mg/kg)	Amount of fipronil-sulfone (mg/kg)	Amount of Fipronil-desulfinyl (mg/kg)
0	0.037	0.003*	0.013*	0.019*
1	0.033	0.002	0.009	0.015
3	0.019*	0.002	0.008	0.013
5	0.008	N.D.	0.002	0.003
7	0.008	N.D.	N.D.	0.002
10	0.005	N.D.	N.D.	0.001
14	0.004	N.D.	N.D.	N.D.

* Lower than MRL value (FAO/Codex MRL = 0.02 mg/kg)

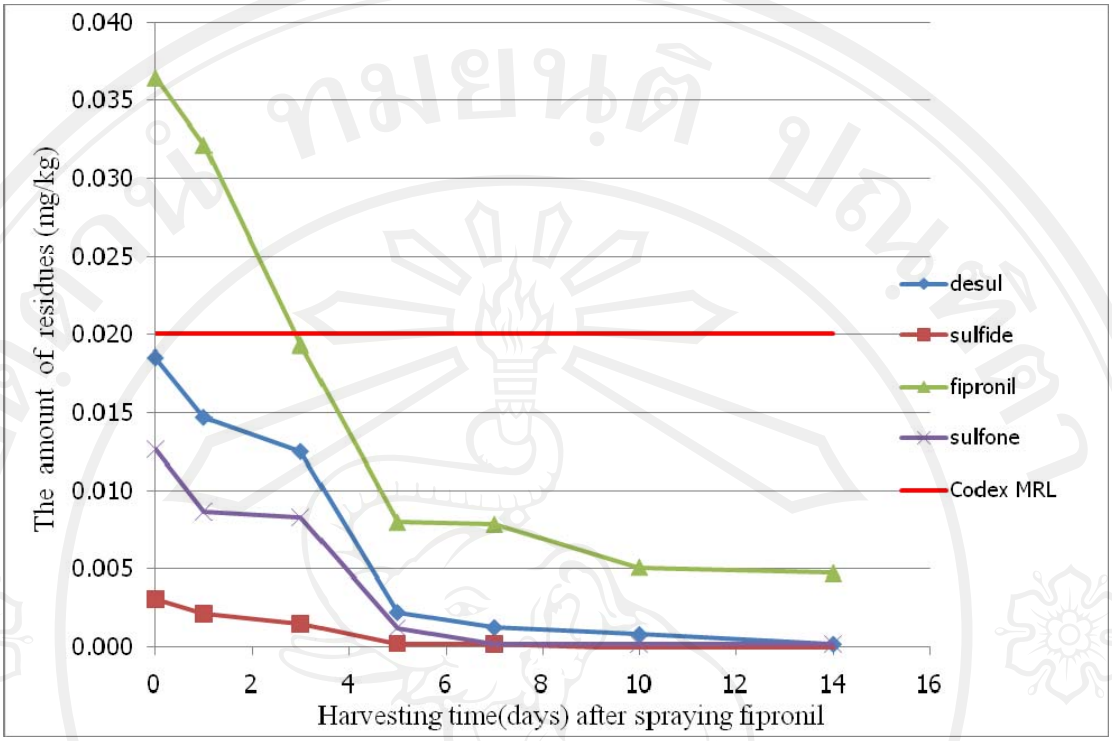

Figure 4.9 Disintegration of fipronil and its derivatives in sweet peppers after 1 spray at the recommended rate harvesting 7 times for analysis

Table 4.46 The amount of fipronil and its derivatives residues in sweet peppers harvested 7 times for analysis by GC-MS after spraying **twice** with fipronil at recommended rate

Time of spraying Day(s)	Amount of fipronil (mg/kg)	Amount of fipronil-sulfide (mg/kg)	Amount of fipronil-sulfone (mg/kg)	Amount of Fipronil-desulfinyl (mg/kg)
0	0.039	0.005*	0.018*	0.032
1	0.032	0.004	0.016	0.027
3	0.023	0.002	0.010	0.015*
5	0.013*	0.001	0.002	0.004
7	0.007	0.001	0.002	0.003
10	0.005	N.D.	0.001	0.003
14	0.004	N.D.	0.001	0.002

* Lower than MRL value (FAO/Codex MRL = 0.02 mg/kg)

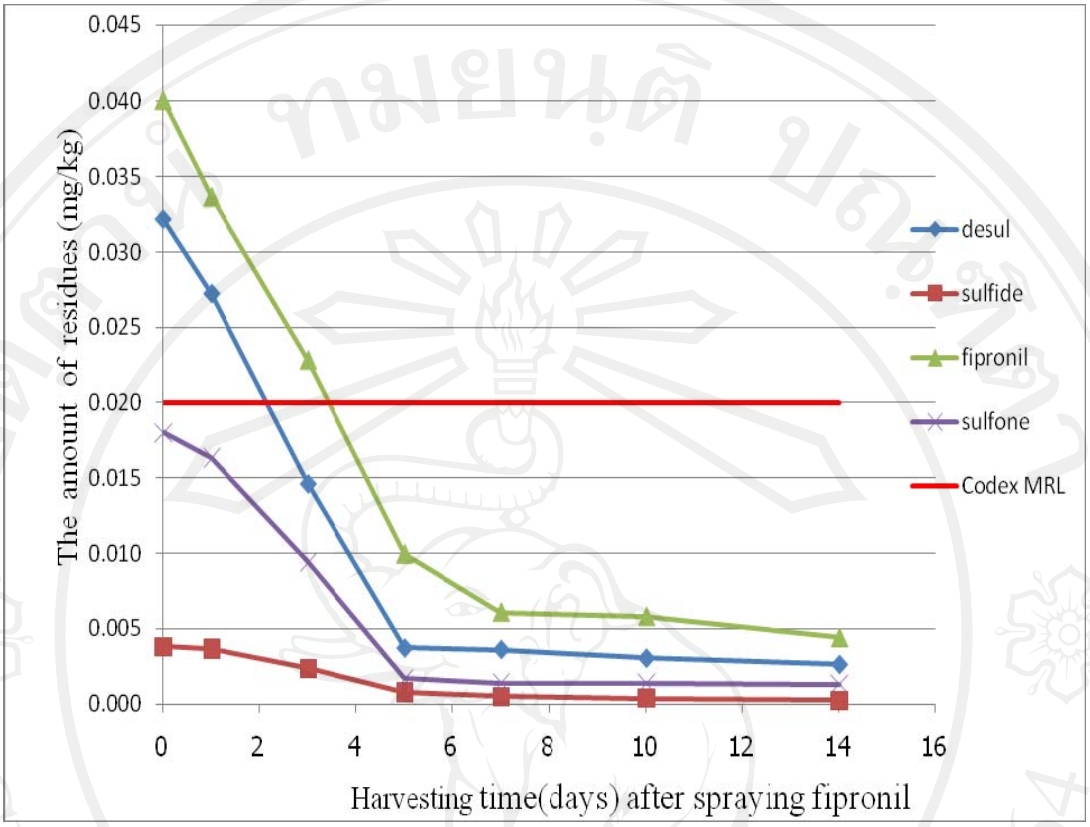

Figure 4.10 Disintegration of fipronil and its derivatives in sweet peppers after 2 sprays at the recommended rate harvesting 7 times for analysis

Table 4.47 The amount of fipronil and its derivatives residues in sweet peppers harvested 7 times for analysis by GC-MS after spraying **3 times** with fipronil at recommended rate

Time of spraying Day(s)	Amount of fipronil (mg/kg)	Amount of fipronil-sulfide (mg/kg)	Amount of fipronil-sulfone (mg/kg)	Amount of Fipronil-desulfinyl (mg/kg)
0	0.153	0.017*	0.073	0.116
1	0.083	0.012	0.043	0.084
3	0.073	0.009	0.037	0.045
5	0.021	0.002	0.004*	0.009*
7	0.016*	0.001	0.003	0.007
10	0.015	0.001	0.002	0.006
14	0.010	0.001	0.001	0.005

* Lower than MRL value (FAO/Codex MRL = 0.02 mg/kg)

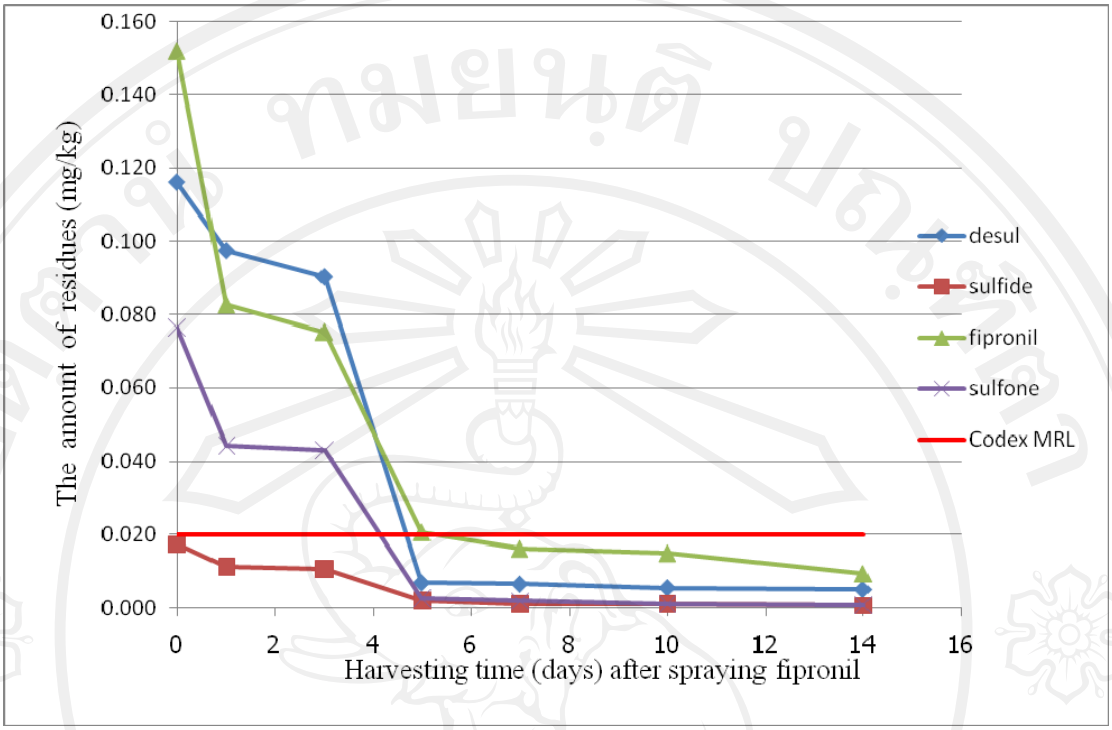

Figure 4.11 Disintegration of fipronil and its derivatives in sweet peppers after 3 sprays at the recommended rate harvesting 7 times for analysis

4.5 Analysis of residues of fipronil and its derivatives in sweet peppers at double concentration rate of recommendation

The sweet pepper plants were sprayed with fipronil at double concentration rate of recommendation (40 ml/20 L) 1, 2, and 3 times. The fruit were harvested to be analysed at 0, 1, 3, 5, 7, 10 and 14 days after sprayed. Results are shown in Tables 4.49-4.51 and disintegration of fipronil and its derivatives are shown in Figures 4.12-4.14

Table 4.48 The amount of fipronil and its derivatives residues in sweet peppers harvested 7 times for analysis by GC-MS after spraying with **once** fipronil at double concentration rate of recommendation

Time of spraying Day(s)	Amount of fipronil (mg/kg)	Amount of fipronil-sulfide (mg/kg)	Amount of fipronil-sulfone (mg/kg)	Amount of Fipronil-desulfinyl (mg/kg)
0	0.100	0.008*	0.019*	0.034
1	0.084	0.007	0.018	0.029
3	0.039	0.003	0.014	0.019*
5	0.024	0.002	0.003	0.003
7	0.020*	0.001	0.002	0.003
10	0.013	0.001	0.001	0.001
14	0.013	0.001	N.D.	0.001

* Lower than MRL value (FAO/Codex MRL = 0.02 mg/kg)

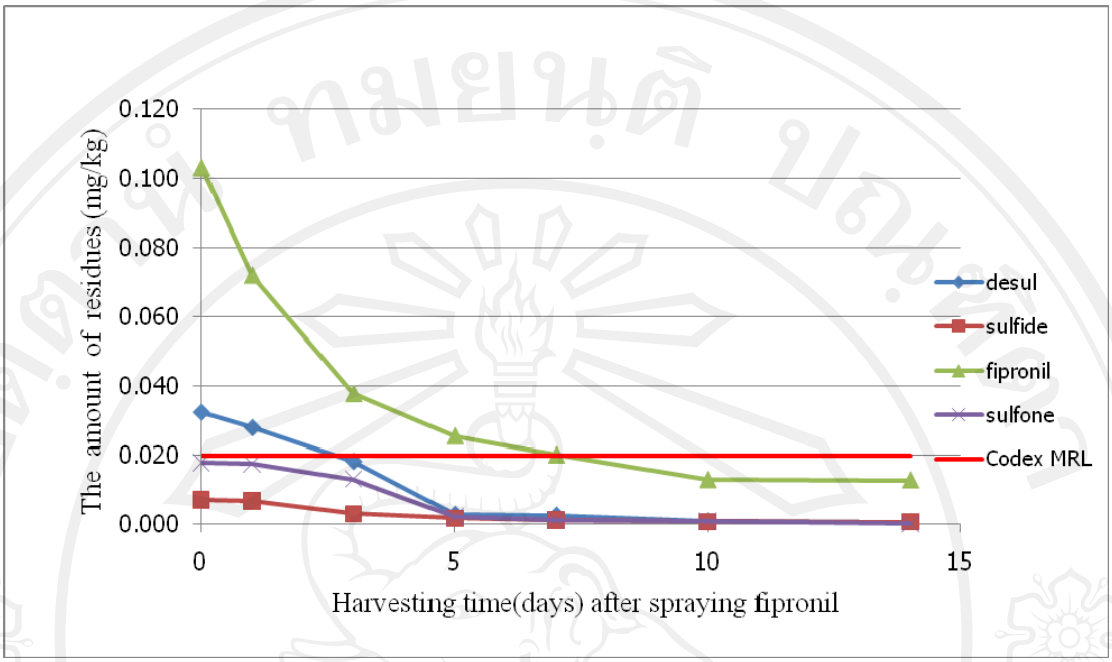

Figure 4.12 Disintegration of fipronil and its derivatives in sweet peppers after 1 spray at double concentration rate of recommendation harvesting 7 times for analysis

Table 4.49 The amount of fipronil and its derivatives residues in sweet peppers harvested 7 times for analysis by GC-MS after spraying with **twice** fipronil at double concentration rate of recommendation

Time of spraying Day(s)	Amount of fipronil (mg/kg)	Amount of fipronil-sulfide (mg/kg)	Amount of fipronil-sulfone (mg/kg)	Amount of Fipronil-desulfinyl (mg/kg)
0	0.093	0.010*	0.043	0.068
1	0.080	0.008	0.027	0.049
3	0.028	0.003	0.010*	0.019*
5	0.023	0.002	0.005	0.009
7	0.021	0.002	0.004	0.007
10	0.018*	0.001	0.002	0.003
14	0.015	0.001	0.002	0.003

* Lower than MRL value (FAO/Codex MRL = 0.02 mg/kg)

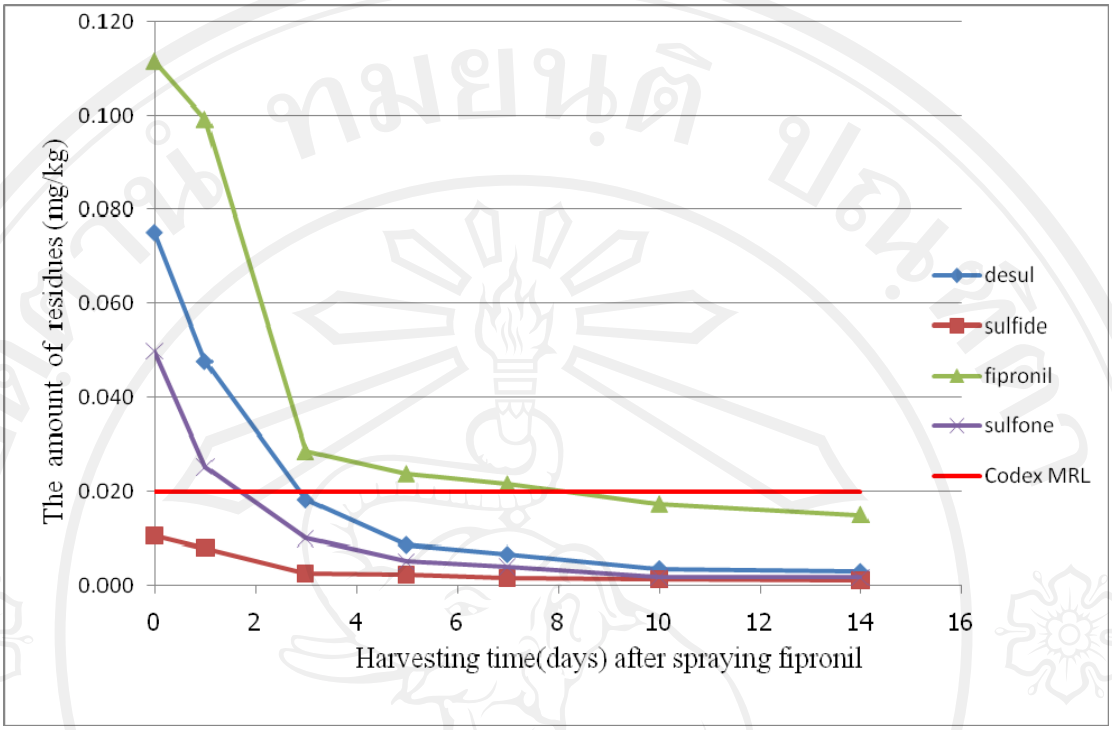

Figure 4.13 Disintegration of fipronil and its derivatives in sweet peppers after 2 sprays at double concentration rate of recommendation harvesting 7 times for analysis

Table 4.50 The amount of fipronil and its derivatives residues in sweet peppers harvested 7 times for analysis by GC-MS after spraying **3 times** fipronil at double concentration rate of recommendation

Time of spraying Day(s)	Amount of fipronil (mg/kg)	Amount of fipronil-sulfide (mg/kg)	Amount of fipronil-sulfone (mg/kg)	Amount of Fipronil-desulfinyl (mg/kg)
0	0.202	0.029	0.105	0.182
1	0.131	0.018*	0.066	0.097
3	0.041	0.004	0.013*	0.027
5	0.039	0.003	0.007	0.015*
7	0.031	0.003	0.006	0.013
10	0.024	0.002	0.004	0.011
14	0.001*	N.D.	0.002	0.002

* Lower than MRL value (FAO/Codex MRL = 0.02 mg/kg)

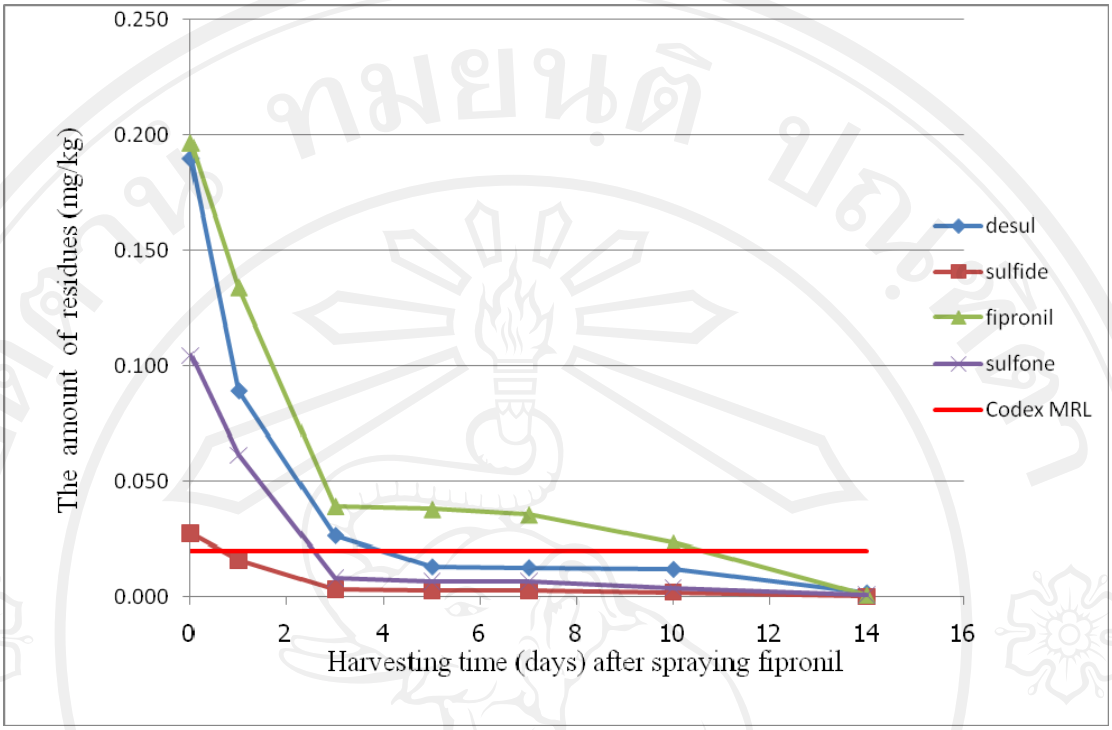

Figure 4.14 Disintegration of fipronil and its derivatives in sweet peppers after 3 sprays at double concentration rate of recommendation harvesting 7 times for analysis