EPA Method 1699: High Selective Multi-residue HRGC/HRMS Pesticide Analysis Applied to Food Samples

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Introduction

Among recent official methods introduced by the United States Environmental Protection Agency (U.S. EPA) EPA Method 1699 can be found [1]. This method is used for the determination of organochlorine, organo-phosphorus, triazine and pyrethroid pesticides in environmental samples by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) using isotope dilution and internal standard quantitation techniques. This EPA method is generally applied to aqueous, solid, tissue and biosolids matrices.

Purpose:

The aim of this study was to extend the scope of applicable matrices for this method to include food samples. Furthermore the compatibility of this method with *QuEChERS* extracts has been investigated.

Experimental Conditions

Standard and sample preparation:
According to the EPA method 1699 standard mixtures were prepared either from neat material or commercially available solutions. ^{13}C-labeled standards were obtained from CIL (Cambridge Isotope Laboratories), native pesticides and deuterium-labeled standards from Dr. Ehrenstorfer GmbH Analytical Standards. Tea and rucola salad samples were prepared via the *QuEChERS* method.

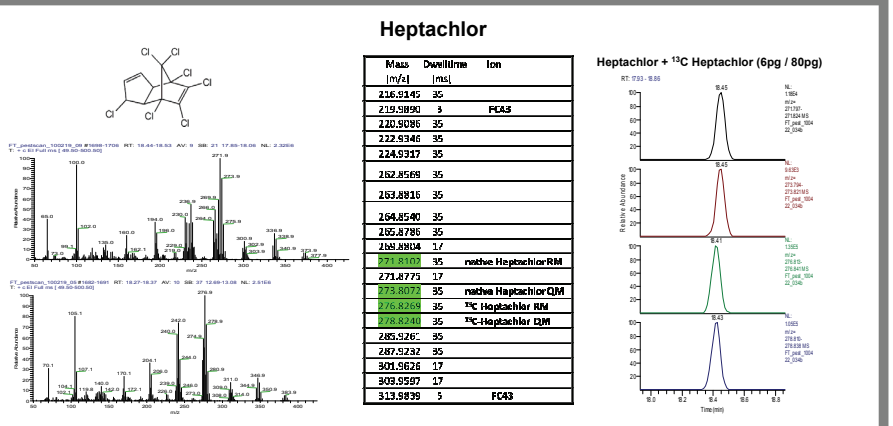
GC and MS Conditions:
Extracts and standards were analyzed on a Thermo Scientific DFS high resolution sector field mass spectrometer coupled to two Thermo Scientific TRACE GC Ultra gas chromatographs supported by an extra-wide Thermo Scientific TriPlus XT autosampler. The selected ionization mode was EI positive with an electron energy of 45eV.

FIGURE 1. Experimental Setup
Thermo Scientific DFS with two TRACE GC Ultra and TriPlus Autosampler

GC parameters	
Carrier Gas Flow Rate	1.2 ml/min
Oven Temp. Program	100°C (2min)
	9.0°C/min – 180°C
	1.5°C/min – 200°C (3min)
	6.0°C/min – 290°C (3min)
Transfer Line Temp.	250°C
SSL or PTV Injector	250 C or Temp. Program
MS Parameter	
Ionization Mode	EI positive Ion
Electron Energy	45eV
Ion Source Temperature	250 °C
Resolution (10% valley)	10000
AS Parameter	
Injection Volume	1 microliter

A high resolution MS multi-window selected ion monitoring (SIM, MID) method was set up including the usage of suitable reference masses (FC43). A mass resolution of 10,000 (10 % valley) was employed for ultimate selectivity. Extracts and standards were injected in splitless mode either via a temperature programmable PTV injector or split/splitless injector on a 30 m DB17ms column (0.25mm ID, 0.25 um film thickness).

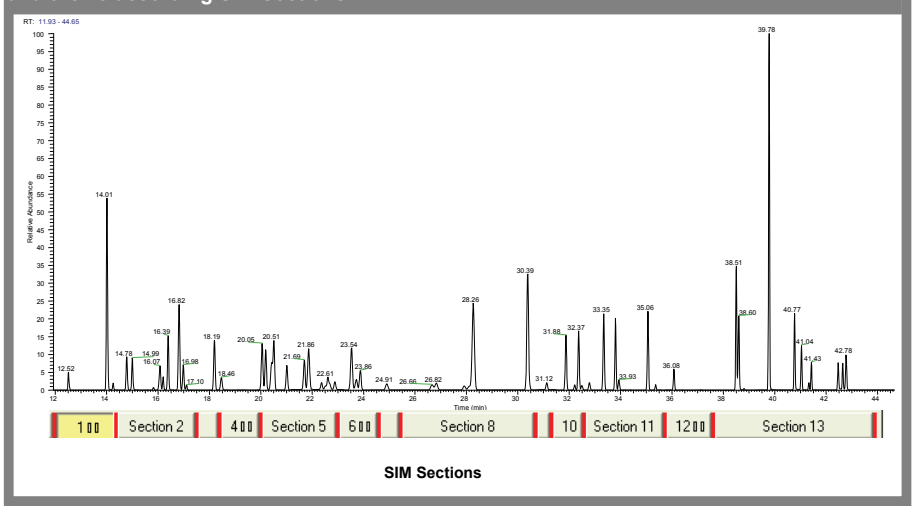
Figure 2: Example of setting up the SIM Method for Heptachlor and $^{13}C_{10}$ Heptachlor



Results and Discussion

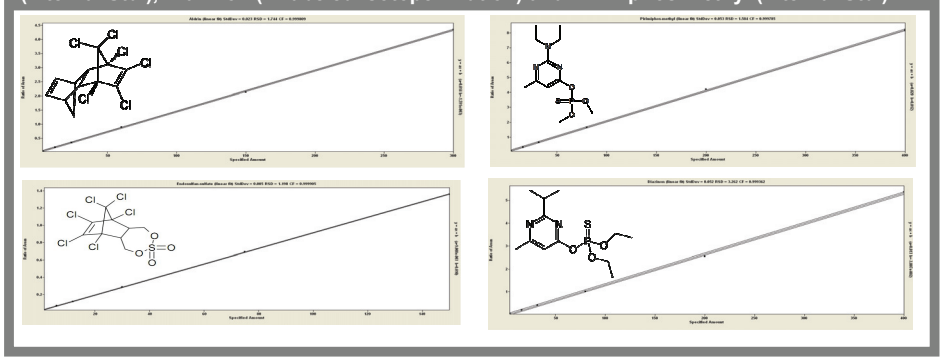
With the above described experimental conditions the combined selectivity of the GC column and high resolution of the DFS mass spectrometer ensured an unambiguous identification of all measured pesticides. Figure 3 shows the TIC chromatogram of 61 native pesticides and 32 ^{13}C- or D- labeled standards.

FIGURE 3: TIC chromatogram of 61 native pesticides and 32 ^{13}C- and D-labeled standards and the 13 according SIM Sections



Calibration of the system by isotope dilution technique was carried out by injection of 6 calibration solutions and calculation of the relative response factor for each pesticide. Figure 4 shows typical calibration curves for 4 selected pesticides.

Figure: 4 Calibrations curves for Aldrin (^{13}C-labeled Isotope Dilution), Endosulfansulfate (Internal Std.), Diazinon (D-labeled Isotope Dilution) and Pirimiphos-methyl (Internal Std.)



The instrument sensitivity using this EPA method was proven with standard measurements and resulted for most of the measured compounds in the fg/µl (ppt) range. Even for pesticides with strong tendency to fragment like Aldrin, Dieldrin, Endrin, Nonachlor and Endosulfan a sub ppb sensitivity could be achieved. The instrument detection limit for Endosulfan-sulfate is about 1pg/µl (Endosulfan-sulfate in Matrix, Figure 6). No significant difference between PTV and SSL injections have been found. It should be noted that in general detection and quantitation levels are limited by matrix interferences and noise rather than by the instrument performance. Exceptions are a few OP pesticides and captan, where the intense formation of small fragments limits the achieved sensitivity. Detection limits of some of the OP pesticides strongly depend on the activity of the injection port liner.

Figure 5: Native and internal standards of EPA Method 1699 with instrumental LODs

#	RT [min]	MID sec.	name	QM [m/z]	RM [m/z]	LOD [pg/µl]
1	8.01	1	Methamidophos	94.0058	95.0136	8.000
2	12.07	2	Tecnazene	260.8732	258.8761	0.008
3	13.85	2	13C6-HCB	289.8303	291.8273	0.003
4	13.86	2	HCB	283.8102	285.8072	0.003
5	14.1	2	Phorate	260.0128	262.0086	0.092
6	14.6	3	13C6-alpha-BHC	224.9317	222.9346	0.019
7	14.61	3	Alpha-BCH	218.9116	220.9086	0.031
8	14.83	3	Desethylatrazine	172.039	174.036	0.047
9	15.65	4	Diazinon-d10	314.1638	282.1074	0.290
10	15.86	4	Diazinon	276.0698	304.1011	0.070
11	15.89	4	Quintozene	236.8413	238.8384	0.047
12	16.01	4	Diazinon-oxon	273.1004	288.1239	0.063
13	16.2	4	13C3-Atrazine	218.1038	220.1009	0.103
14	16.21	4	Atrazine	215.0938	217.0908	0.048
15	16.62	4	Simazine	201.0781	203.0752	0.046
16	16.61	4	13C6-gamma-BHC	224.9317	222.9346	0.015
17	16.63	4	gamma-BCH	218.9116	220.9086	0.024
18	16.78	4	13C6-Fonofos	252.0503	253.0537	0.030
19	16.79	4	Fonofos	246.0302	247.0336	0.256
20	16.9	4	Disulfoton	274.0285	275.0318	0.434
21	17.97	5	13C6-beta-BHC	224.9317	222.9346	0.015
22	17.99	5	Beta-BHC	218.9116	220.9086	0.024
23	18.23	5	13C4-Heptachlor	276.8269	278.824	0.059
24	18.25	5	Heptachlor	271.8102	273.8072	0.036
25	19.8	5	13C6-delta-BHC	224.9317	222.9346	0.014
26	19.82	5	delta-BHC	218.9116	220.9086	0.024
27	19.95	5	Chlorothalonil.	265.8786	263.8816	0.019
28	20.15	5	13C12-Aldrin	269.8804	271.8775	0.026
29	20.19	5	Aldrin	262.8569	264.854	0.067
30	20.27	5	Chlorpyriphos-methyl	285.9261	287.9232	0.009
31	20.76	5	13C12-PCB-52	301.9626	303.9597	0.014
32	21.42	6	Parathion-methyl	263.0017	264.0051	0.168
33	21.59	6	Ametryn	227.1205	228.1238	0.047
34	21.63	6	Pirimiphos-methyl	290.0728	276.0572	0.050
35	22.1	6	Metribuzin	198.0701	199.0735	0.060
36	22.13	6	Dacthal	300.8807	298.8836	0.044
37	22.2	6	Octachlorostyrene	272.8413	270.8443	0.055
38	22.25	6	D10-Chlorpyriphos	324.0202	326.0172	0.036
39	22.62	6	Chlorpyriphos	313.9574	315.9545	0.226
40	23.25	7	Fenitrothion	277.0174	260.0146	0.045
41	23.28	7	Malathion	283.9942	285.002	0.575
42	23.4	7	13C10-Oxychlordane	269.8804	271.8775	0.073
43	23.45	7	Oxychlordane	262.8569	264.854	0.189
44	23.21	7	Parathio-ethyl-D10	301.0958	302.0992	0.000
45	23.59	7	Parathion-ethyl	291.033	292.0364	0.167
46	23.6	7	Chlorpyriphos-oxon	269.949	271.9462	0.115
47	24.58	8	13C12-Heptachlor-epox.	269.8804	271.8775	0.081
48	24.63	8	Heptachlor-epoxide	262.8569	264.854	0.208

#	RT [min]	MID sec.	name	QM [m/z]	RM [m/z]	LOD [pg/µl]
49	26.29	8	13C10-t-Chlordane	269.8804	271.8775	0.091
50	26.3	8	t-Chlordane	262.8569	264.854	0.227
51	26.45	8	13C10-t-Nonachlor	269.8804	271.8775	0.084
52	26.51	8	t-Nonachlor	262.8569	264.854	0.220
53	27.59	8	c-Chlordane	262.8569	264.854	0.224
54	27.75	8	13C9-alpha-Endosulfan	269.8804	271.8775	0.161
55	27.8	8	alpha-Endosulfan	262.8569	264.854	0.349
56	27.66	8	D5-Cyanazine	245.1204	247.1175	0.004
57	27.89	8	Cyanazine	240.089	242.0861	0.353
58	27.96	8	o,p-DDE	246.0003	247.9974	0.029
59	27.98	8	13C12-PCB-79	301.9626	303.9597	0.010
60	30.12	8	13C12-p,p-DDE	258.0406	260.0376	0.014
61	30.15	8	p,p-DDE	246.0003	247.9974	0.037
62	30.16	8	13C12-Dieldrin	269.8804	271.8775	0.043
63	30.19	8	Dieldrin	262.8569	264.854	0.124
64	30.89	9	Captan	263.9653	265.9623	1.048
65	31.67	10	o,p-DDD	235.0081	237.0052	0.012
66	32.01	10	Disulfoton-Sulfone.	213.0173	214.0251	0.073
67	32.17	10	Perthane	224.152	223.1487	0.087
68	32.25	10	13C12-Endrin	269.8804	271.8775	0.112
69	32.28	10	Endrin	262.8569	264.854	0.226
70	32.56	10	13C10-c-Nonachlor	269.8804	271.8775	0.068
71	32.57	10	c-Nonachlor	262.8569	264.854	0.173
72	33.14	11	13C12-o,p-DDT	247.0484	249.0454	0.013
73	33.16	11	o,p-DDT	235.0081	237.0052	0.017
74	33.62	11	p,p-DDD	235.0081	237.0052	0.010
75	33.72	11	13C9-beta-Endosulfan	269.8804	271.8775	0.185
76	33.74	11	beta-Endosulfan	262.8569	264.854	0.345
77	34.88	11	13C12-p,p-DDT	247.0484	249.0454	0.012
78	34.89	11	p,p-DDT	235.0081	237.0052	0.013
79	35.18	11	Endrin-aldehyd	249.8491	251.8456	0.062
80	35.9	11	Endosulfan-sulfate	262.8569	264.854	1.066
81	38.35	12	Hexazinone	171.0882	172.0916	0.104
82	38.42	12	13C8-Mirex	241.8581	243.8551	0.017
83	38.44	12	Mirex	236.8413	238.8384	0.023
84	38.44	12	13C12-Methoxychlor	239.1475	240.1508	0.012
85	38.45	12	Methoxychlor	227.1072	228.1106	0.022
86	38.64	12	Endrin-ketone	249.8491	247.8521	0.248
87	39.64	13	Phosmet	160.0399	161.0432	0.019
88	40.62	13	13C6-Permethrins-Pk1	189.1011	190.1045	0.020
89	40.63	13	Permethrins-Peak_1	183.081	184.0843	0.040
90	40.89	13	13C6-Permethrins-Pk2	189.1011	190.1045	0.029
91	40.9	13	Permethrins-Peak_2	183.081	184.0843	0.091
92	39.63	13	Azinphos-methyl-d6	160.0511	161.0544	0.154
93	39.63	13	Azinphos-methyl	160.0511	161.0544	0.125
94	42.33	13	Cypermethrins-Peak_1	163.0081	165.0052	0.485
95	42.51	13	Cypermethrins-Peak_2	163.0081	165.0052	0.515
96	42.64	13	Cypermethrins-Peak_3	163.0081	165.0052	0.354

The measurements of *QuEChERS* extracts from different food samples showed very good selectivity for most of the targeted pesticides with detection limits well below the range requested by food regulations. Figure 6 show 4 different pesticides in salad and tea matrix.

Figure 6: SIM Chromatogramm of four pesticides spiked at CS2 level into a salad matrix

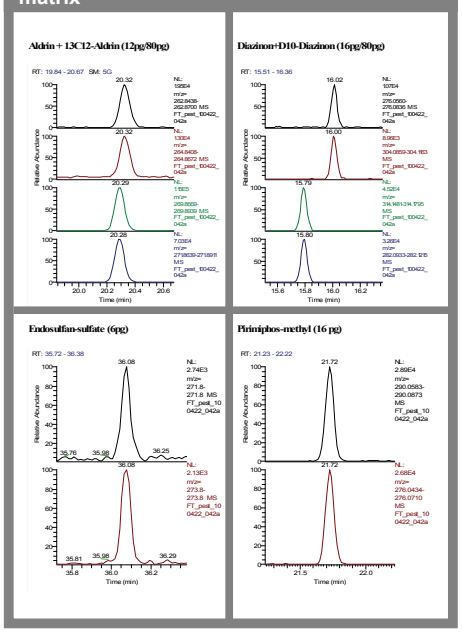
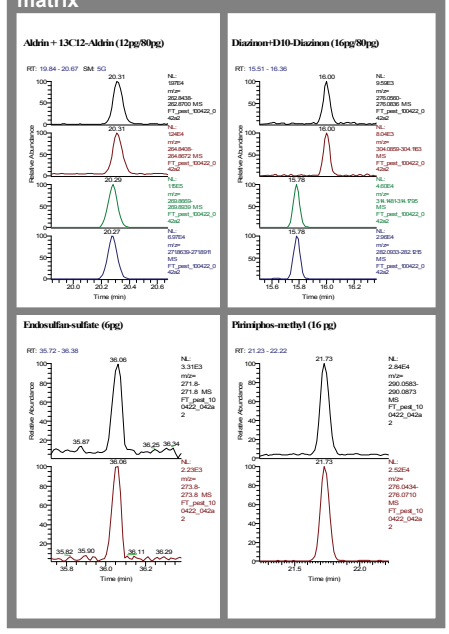


Figure 6: SIM Chromatogramm of four pesticides spiked at CS2 level into a tea matrix



Conclusions

It could be demonstrated that EPA method 1699 for measurement and determination of selected organochlorine, organo-phosphorus, triazine and pyrethroid pesticides in environmental matrices is also suitable for highly sensitive analysis of food samples using the QuEChERS sample preparation approach.

References

1. EPA Method 1699: Pesticides in Water, Soil, Sediment, Biosolids and Tissue by HRGC/HRMS, December 2007