Quantitative Determination of multi-Pesticide Residues in Vegetables by Supercritical Fluid Chromatography Coupled to Triple Quadrupole Mass Spectrometry



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## Introduction

This work describes the development of a supercritical fluid chromatography (SFC) method for the separation of a multi-pesticide sample. Connecting the SFC to a Triple Quadrupole MS and optimization of the necessary MS parameters is discussed. Important performance parameters of the final method such as limits of detection (LOD), limits of quantification (LOQ), linearity, retention time, and area RSD are determined.

The obtained SFC/QQQ MS method is further optimized for the determination of pesticides in a complex food matrix. Several gradients of different steepness are applied to the analysis of a vegetable matrix spiked with different concentrations of a multi-pesticide standard. The optimum separation conditions are determined by software-aided batch comparison to identify the gradient with the lowest matrix impact.

## Experimental



Figure 1) Configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6460 Triple Quadrupole LC/MS System. The column is directly connected to the splitter 1 in the splitter assembly (BPR = back pressure regulator, UV detector not used, Splitter Kit p/n: G4309-68715).

# **Results and Discussion**

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		Sample			Metolachlor	Metazachlor	Monolinuron	Sebuthyla	Terbuthylazine	Atrazin R	Metobromuron	So azine 🦲	Methabenzthiazuro	Atrazine-desethyl	yanazine R	Hexazinone	Isoproturon	Chlorotoluro	Linuron R	Diuron Re
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	PestStd_100ppb	SFC-MS-pestizide-1_SGT200_1.d	Sample	1	64.5447	72.5092	719.7966	184.3513	71.7851	72.5336	17.0807	76.6288	77.0201	76.3356	78.2914	81.8687	76.1424	73.1712	458.3353	39.782
	PestStd_100ppb	SFC-MS-pestizide-1_SGT220_1.d	Sample		83.9356	91.5884	103.4144	239.5950	93.4590	93.1352	14.1740	100.4924	91.4730	90.2448	92.6602	96.5974	91.5497	91.0931	272.1524	60.914
	PestStd_100ppb	SFC-MS-pestizide-1_SGT240_1.d	Sample		100.0850	101.3183	192.3636	103.1944	103.1944	101.3593	41.2511	96.6699	93.6804	96.6606	96.4983	95.0956	91.8477	90.3754	64.1913	51.690
	PestStd_100ppb	SFC-MS-pestizide-1_SCT200_1.0	Sample		96.8321	105.0306	171.7891	98.2274	98.2322	96.1346	58.0063	100.6986	97.7893	96.0289	101.5446	99.2239	99.9844	102.5459	74.1817	96.17
	PestStd_100ppb	SFC-MS-pestizide SGT280_1.d	Cal	D	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.000
	PestStd_100ppb	SFC-MS-pestizide-1_SG1990_1	Carpie	1	87.0743	83.4488	99.4933	84.9087	84.9154	80.8614	140.3597	89.9996	83.2525	77.4714	89.8022	80.0162	89.5701	91.6141	98.2474	88.163
	PestStd_100ppb	SFC-MS-pestizide-1_SGT320_1.d	Sample	1	93.2245	94.9670	2463.0152	92.6233	92.6233	89.3623	205.7781	87.4972	84.6942	84.8095	95.7393	85.2877	87.3999	90.5365	263.3623	101.540
	PestStd_100ppb	SFC-MS-pestizide-1_SGT340_1.d	Sample	1	92.4690	100.3534	3297.5099	95.5671	95.5671	89.3721	223.7299	88.8730	91.1180	87.8733	94.7802	88.8936	91.7683	99.3235	515.2725	92.014
•	PestStd_100ppb	SFC-MS-pestizide-1_SGT360_1.d	Sample	1	101.4967	109.9983	4474.7966	105.9246	105.8792	104.7472	356.9052	96.5421	94.2868	94.7139	98.8266	100.9280	105.0079	112.1500	240.1365	100.242
	PestStd_100ppb	SFC-MS-pestizide-1_SGT380_1.d	Sample	1	100.5470	112.6012	5327.8543	107.1023	107.1048	102.7292	394.7568	102.1380	94.2834	89.2790	111.1035	108.7021	108.9263	122.2317	464.9135	118.987
	PestStd_100ppb	SFC-MS-pestizide-1_SGT400_1.d	Sample		94.9348	105.2611	5626.2225	101.1673	101.1697	91.4038	366.9873	91.6643	89.6396	77.9637	99.3785	100.9135	99.3115	110.1165	278.1735	107.814

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	Sample			Metolachlor	Metazachlor	Monolinuron	Sebuthyla	Terbuthylazine	Atrazine	Metobromuron	Sime ine	Methabenzthiazuro	Atrazine-desethyl	vanazine R	Hexazinone	Isoproturon	Chlorotoluro	Linuron R	Diuron R
Name	Data File	Туре	Level	Calc. Conc.	Calc. Conc.	Calc. Conc.	Calc. Conc.	Calo, Core	Cultur Corric.	Calc. Conc.	Calc. Conc.	Calc. Conc.	Calc. Conc.	Calc. Conc.	Calc. Co				
PestStd_100pp	b SFC-MS-pestizide-1_SGT200_1.d	Sample	1	64.1935	64.3947	13.5101	172.1264	67.0232	70.6066	4.3269	75.0247	81.6900	85.5023	70.4671	75.3148	69.9027	59.8627	98.5851	33.4
PestStd_100pp	b SFC-MS-pestizide-1_SGT220_1.d	Sample		83.4790	81.3388	1.9410	223.7067	87.2594	90.6609	3.5906	98.3888	97.0193	101.0818	83.3998	88.8644	84.0474	74.5249	58.5383	51.19
PestStd_100pp	b SFC-MS-pestizide-1_SGT240_1.d	Sample		99.5405	89.9798	3.6105	96.3513	96.3490	98.6665	10.4498	94.6464	99.3604	108.2680	86.8544	87.4828	84.3209	73.9378	13.8071	43.44
PestStd_100pp	b SFC-MS-pestizide-1_SGT260_1.d	Sample		96.3053	93.2766	3.2244	91.7137	91.7160	93.5806	14.6942	98.5907	103.7185	107.5604	91.3964	91.2806	91.7908	83.8947	15.9560	80.83
PestStd_100pp	b SFC-MS-pestizide-1_SGT280_1.d	Sample	1	99.4560	88.8090	1.8769	93.3687	93.3665	97.3433	25.3321	97.9067	106.0632	112.0084	90.0061	91.9945	91.8052	81.8119	21.5094	84.04
PestStd_100pp	b SFC-MS-pestizide-1_SGT300_1.d	Sample	1	86.6006	74.1101	1.8674	79.2781	79.2825	78.7132	35.5560	88.1156	88.3003	86.7745	80.8275	73.6105	82.2300	74.9512	21.1324	74.09
PestStd_100pp	b SFC-MS-pestizide-1_SGT320_1.d	Sample	1	92.7173	84.3393	46.2290	86.4812	86.4791	86.9882	52.1278	85.6656	89.8294	94.9938	86.1713	78.4600	80.2376	74.0696	56.6476	85.33
PestStd_100pp	b SFC-MS-pestizide-1_SGT340_1.d	Sample	1	91.9660	89.1229	61.8919	89.2298	89.2277	86.9978	56.6754	87.0126	96.6427	98.4255	85.3080	81.7773	84.2481	81.2584	110.8319	77.33
PestStd_100pp	b SFC-MS-pestizide-1_0-a1360_1.d	Sample	1	100.9446	97.6885	83.9887	98.9004	98.8557	101.9644	90.4114	94.5212	100.0036	106.0876	88.9500	92.8483	96.4027	91.7520	51.6519	84.24
PestStd_100pp	b SFC-MS-pestizide SGT380_1.d	Cal		100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.00
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Figure 5) Data analysis of the data created by the methods obtained from MassHunter source optimizer for the optimization of the Agilent Jet
Stream sheath gas temperature in the range of 200°C to 400°C, step size 20°C.
A)Sheath gas temperature of 280°C was taken as the reference (100%) for a one point calibration and compared to the values obtained for other temperatures.

## Experimental

Instruments:

- Agilent 1260 Infinity Analytical SFC Solution (G4309A):
- Agilent 6460 Triple Quadrupole LC/MS System (G6460A)
- Agilent 1260 Infinity isocratic Pump (G1310B)
- Splitter kit G4309-68715
- In addition, the following parts are required to run the SFC system for automated method development:
- Agilent 1290 Infinity Thermostatted Column Compartments (G1316C) with valve drive
- Two 1200 Infinity Series Quick-Change 8-position/9-port valves (G4230A) Agilent 1290 Infinity Valve Drive (G1170) with 1200 Infinity Series Quick-Change12-position/13-port valve (G4235A) Capillary kit for method development (p/n 5067-1595)

### Instrumental set-up

The recommended configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6460 Triple Quadrupole LC/MS System is shown in figure 1. The column is directly connected to a splitter assembly, which contains two combined splitters (and an additional check valve to prevent backflush of CO2 into the make-up pump and a solvent filter). At the first splitter the make-up flow coming from an isocratic pump is introduced into the flow path. This splitter is connected to the second one by a short 0.12 mm i.d. capillary. Here, the flow is split into the part going to the MS and the other part going to the back pressure regulator (BPR) of the SFC module. The connection to the MS is made by a 50 µm i.d. stainless steel capillary of 1 meter length. The split ratio is depending on the back pressure generated by this restriction capillary and the pressure set by the BPR. As a rule of thumb, a SFC back pressure of 120 bar diverts about 0.45 mL/min of the SFC flow to the ion source and 200 bar back pressure would divert about 0.6 mL/min to the ion source. Since electrospray MS is a concentration dependent detector this has no influence on signal intensity.

	Precursor Ion [m/z]	Fragentor (V)	Product Ion 1 [m/z]	Collsion Energy (eV)	Product Ion 2 [m/z]	Collsion Energy (eV)
Metolachlor	284.1	90	252.1	12	176.1	24
Metazachlor	278.1	70	210.1	4	134.1	20
Metobromuron	259.0	85	170.0	16	148.1	12
Hexazinone	253.1	85	171.1	12	71.1	32
Linuron	249.0	85	181.1	12	159.9	16
Cyanazine	241.1	100	214.1	12	104.1	32
Diuron	233.1/235.1	95	72.1	20	72.1	20
Metoxuron	229.1/231.1	135	72.1	16	72.1	16
Terbuthylazine	230.1	55	174.1	12	104.1	32
Sebuthylazine	230.1	85	174.1	12	104.1	36
Methabenzthiazuron	222.1	65	165.1	12	150.0	36
Atrazine	216.1	85	174.0	16	104	28
Monolinuron	215.1	95	148.0	16	125.9	12
Chlorotoluron	213.1/215.1	65	72.1	20	72.1	20
Isoproturon	207.1	95	165.0	12	72.1	20
Simazine	202.1	105	132.1	16	124.1	16
Atrazine-desethyl	188.1	90	146.0	16	104.0	24

Table 1) MRM conditions for pesticide compounds inherent in the used mixture obtained from MRM Optimizer (Dwell time: 10 ms, Cell acceleration voltage: 5V).

# **Results and Discussion**

### **Optimizing SFC separation**

In the first step of the optimization of the SFC/triple quadrupole MS method, the SFC component was optimized by DAD detection using higher concentration pesticide samples (10 ng/ $\mu$ L of each compound in the mixture). The setup of different gradients for the automated screening was done using the Agilent ChemStation Method Scouting Wizard. For the scouting experiments, three different types of column (amino, silica, and cyano) and three solvents of increasing polarity (isopropanol, ethanol, and methanol) were used (Figures 2 – 4).





B)Same data displayed with a sheath gas temperature of 380°C as reference value.

Lower values are color coded in blue and higher values are color coded in red.

	RT	RT RSD	Area RSD	LOD	LOQ	R2	Table 2) Performance results
Metolachlor	1.784	0.54	3.75	2.7	9.1	0.9996	
Metazachlor	1.947	0.53	2.46	1.6	5.2	0.9992	of the measurement of a
Sebuthylazine	2.158	0.42	0.42	0.2	0.7	0.9998	
Terbuthylazine	2.282	0.45	0.45	0.3	1.0	0.9992	sample comprising 1/
Atrazine	2.283	0.38	4.56	0.1	0.4	0.9998	
Monolinuron	2.338	0.38	3.07	0.3	1.0	0.9990	pesticide compounds
Metobromuron	2.465	0.37	4.22	1.4	4.5	0.9997	(Calibration from 1 to 1000
Simazine	2.525	0.31	2.73	0.2	0.7	0.9990	
Methabenzthiazuron	2.538	0.34	2.02	0.2	0.7	0.9995	ng/ml_Limit_of_Detection
Linuron	2.743	0.27	2.91	3.7	12.5	0.9998	ng/ me, ennit of Detection,
Atrazine-desethyl	3.011	0.23	2.54	0.9	2.9	0.9997	Limit-of-Quantification
Cyanazine	3.068	0.23	2.39	0.4	1.2	0.9999	
Hexazinone	3.307	0.12	2.38	0.1	0.3	0.9994	linearity and statistical
Isoproturon	3.404	0.11	3.31	0.4	1.2	0.9999	
Chlorotoluron	3.852	0.11	2.69	3.7	12.5	0.9998	evaluation for retention time
Diuron	4.212	0.05	2.89	3.7	12.5	0.9997	and area $DCD [0/1/n-10)$
Metoxuron	4.375	0.06	3.11	2.2	7.4	0.9990	anu area nod [%] (n=10).

### **Evaluation of matrix effects in real samples**

A QuEChERS extract of a rocket sample was spiked with 10, 20 and 100 ppb of the pesticide mix. These samples were compared to the same concentrations in standard solution and for different gradients (Figures 6 and 7). Matrix effects typically decrease the response but the effect is less prominent for shallower gradients with better separation. The typical recovery range is between 70 and 120%.



### Column:

- Agilent ZORBAX Rx-SIL, 4.6 x 150 mm, 5 μm (p/n 883975-901)
- Agilent ZORBAX SB-CN, 4.6 x 150 mm, 5 µm (p/n 883975-905)
- Agilent ZORBAX NH2, 4.6 x 150 mm, 5  $\mu$ m (p/n 883952-708)

#### Software:

- Agilent MassHunter Data Acquisition Software for triple quadruple mass spectrometer, Version 06.00.
- Agilent MassHunter MRM and Source Optimizer Software, Version 06.00
- Agilent MassHunter Qualitative Software, Version 06.00
- Agilent MassHunter Quantitative Software, Version 07.00
- Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.05 with Agilent ChemStation Method Scouting Wizard, Version A.02.03, (G2196AA).

SFC methods (final conditions in bold):

- SFC flow: 3 mL/min
- SFC Gradient 1: 0 min 2% B to 15, 10, 5 min 50% B. Stop time 15, 10, 5 minutes. Post time: 2 minute.
- SFC Gradient 2: 0 min 2% B to 5 min 15 % B to 6 min 15% B. Stop time 6 minutes. Post time: 2 minutes.
- **SFC Gradient 3:** 0 min 2% B to 5 min 20% B. Stop time: 5 minutes. Post time: 2 minutes.
- Modifier: **Methanol**, Ethanol, Isopropanol.
- BPR temperature: 60° C, BPR pressure: 120 bar
- Column temperature: 40°C

Figure 2) A) Separation of 17 pesticide compounds on a NH2 column with methanol as modifier by application of three gradients of different steepness.
B) Separation of 17 pesticide compounds on a NH2 column with ethanol as modifier by application of three gradients of different steepness.



### **Optimizing MS settings**



Figure 3) A) Separation of 17 pesticide compounds on a Rx-SIL column with methanol as modifier by application of three gradients of different steepness.

B) Separation of 17 pesticide compounds on a SB-CN column with methanol as modifier by application of three gradients of different steepness.

Figure 4) A) Separation of 17
pesticide compounds on a NH2
column with methanol as modifier
and a 5 minutes gradient up to
15%B.
B) Separation of 17 pesticide

compounds on a Rx-SIL column with methanol as modifier and a 5 minutes gradient up to 15%B.



- Injection volume: 5 µL, 3 times loop overfill.
- UV detection: 220 nm, band width 8 nm, ref. 360 nm, band width 100 nm, Data rate: 10 Hz (not used in the final SFC/MS method).

# Connection of the SFC to the MS by splitting and make up flow (final conditions in bold):

- Make up composition: Acetonitrile + 0.2% formic acid
- Make-up flow: 0.1 1.0 mL/min, Step: 0.1 mL/min; **0.5 mL/min**.
- Flow gradient: 0 min 0.5 mL/min to 5 min 0.3 mL/min.

#### MS method (final conditions in bold):

- Ionization mode: positive
- Capillary voltage: 2000 4500 V, Step 500 V, **2500V**.
- Nozzle voltage: 0 2000 V, Step 200 V, **2000V**.
- Gas flow: 5 13 L/min, Step 1 L/min, 8L/min.
- Gas temp.: 160 340 °C, Step 20°C, 220°C,
- Sheath gas flow: 8 12 L/min, Step 1L/min, **12 L/min**.
- Sheath gas temp.: 200 400°C, Step 20°C, **380** °C.
- Nebulizer pressure: 20 60 psi, Step 5psi, **25 psi**.
- MRM conditions: see table 1.

For the identification and optimization of all MRM transitions the MassHunter optimizer software was used. The makeup flow was optimized by testing different solvents and additives at different flow rates and manual inspection of the intensity of the resulting MRM transitions. All parameters of the Agilent JetStream source were optimized by means of the MassHunter source optimizer and data batch analysis by MassHunter Quantitative software (Figure 5).



Figure 6) SFC separation of a mixture of 17 pesticides and detection by triple quadrupole MRM mass spectrometry at 100 ng/mL. A) MRM quantifier and qualifier of all 17 pesticide compounds. B) MRM quantifier and qualifier of the six lower abundant pesticide compounds.

Figure 7) Comparison of spiked samples to a calibration in standard solution. Matrix effects are typically in a range of 70-120%. Matrix effects could be additionally minimized by sample dilution.

# Conclusions

- This work demonstrates the workflow to optimize an Agilent 1260 Infinity Analytical SFC triple quadruple mass spectrometer method for the analysis of multiple pesticides
- LOQs are typically below 2.9 ppb, retention time RSDs are below 0.4% and area RSDs below 4%.
- The advantage compared to HPLC methods is the good separation of a larger number of compounds in faster run time at comparable performance.
- The importance of optimization of the separation on matrix effects in real samples is demonstrated.