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

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.

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 µm (p/n 883952-708)

Software:

- Agilent MassHunter Data Acquisition Software for triple quadrupole 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
- 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.

Experimental

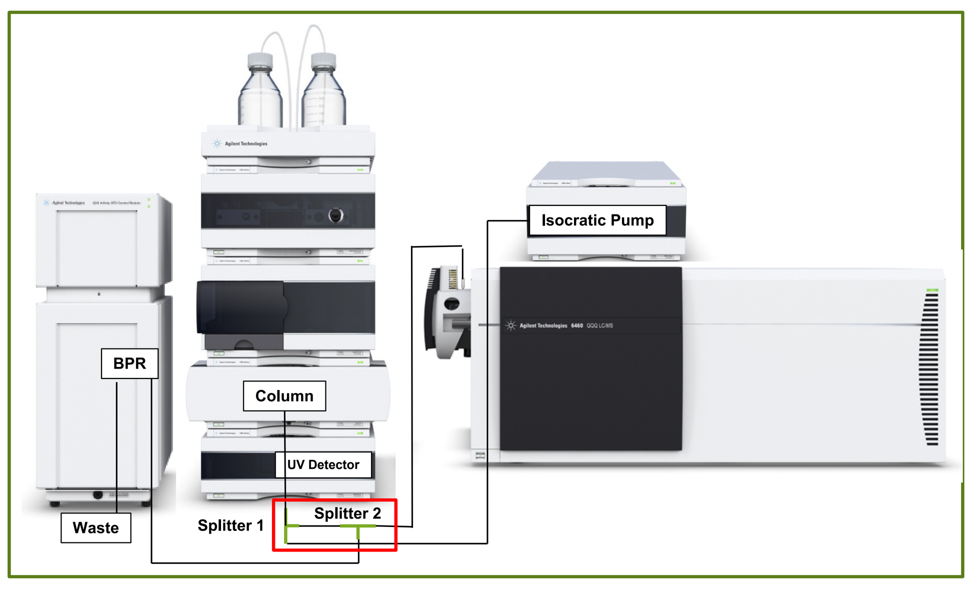

Figure 1) Configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6460 Triple Quadrupole LC/MS System. The column is directly connected to the splitter 1 in the splitter assembly (BPR = back pressure regulator, UV detector not used, Splitter Kit p/n: G4309-68715).

	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/µL 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).

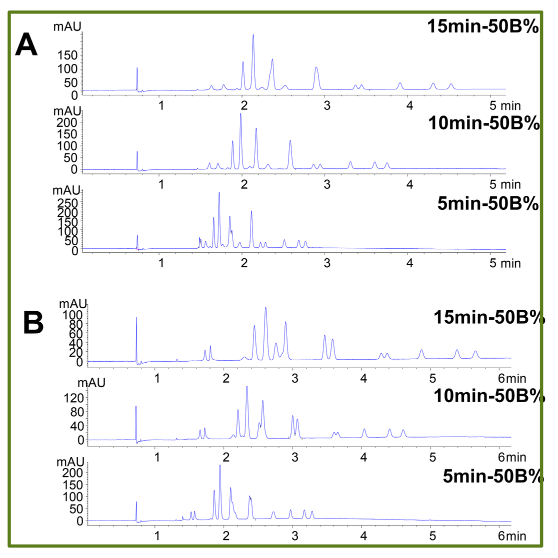

Figure 2) A) Separation of 17 pesticide compounds on a NH2 column with methanol as modifier by application of three gradients of different steepness.
B) Separation of 17 pesticide compounds on a NH2 column with ethanol as modifier by application of three gradients of different steepness.

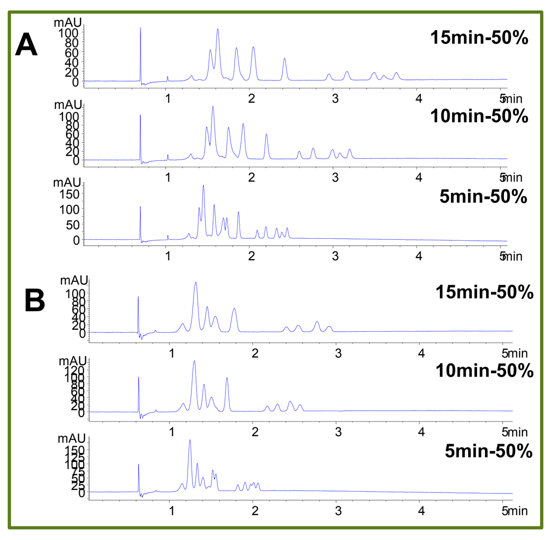

Figure 3) A) Separation of 17 pesticide compounds on a Rx-SIL column with methanol as modifier by application of three gradients of different steepness.
B) Separation of 17 pesticide compounds on a SB-CN column with methanol as modifier by application of three gradients of different steepness.

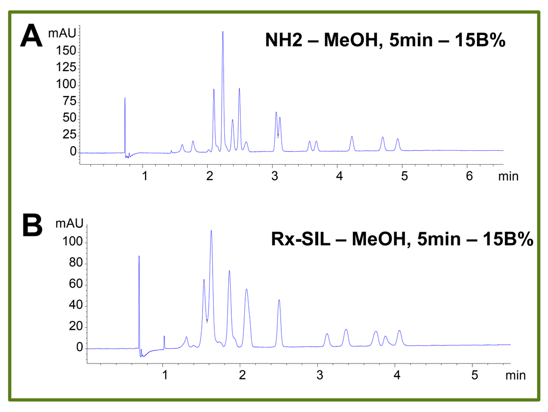

Figure 4) A) Separation of 17 pesticide compounds on a NH2 column with methanol as modifier and a 5 minutes gradient up to 15%B.
B) Separation of 17 pesticide compounds on a Rx-SIL column with methanol as modifier and a 5 minutes gradient up to 15%B.

Optimizing MS settings

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).

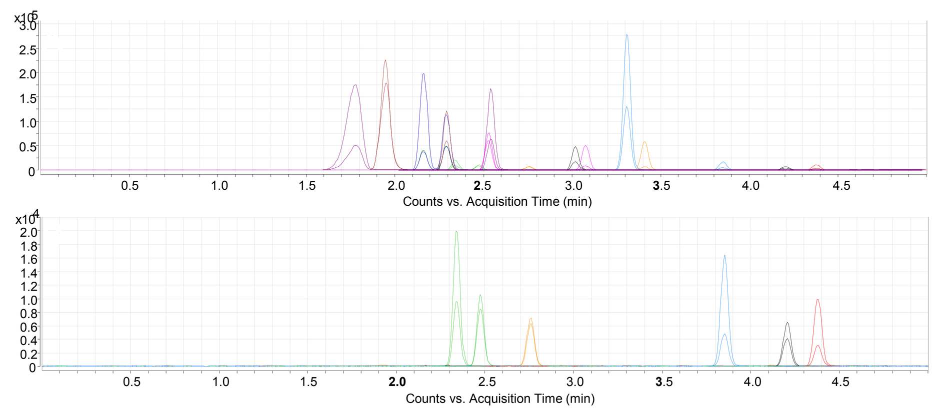

Figure 6) SFC separation of a mixture of 17 pesticides and detection by triple quadrupole MRM mass spectrometry at 100 ng/mL. A) MRM quantifier and qualifier of all 17 pesticide compounds. B) MRM quantifier and qualifier of the six lower abundant pesticide compounds.

Results and Discussion

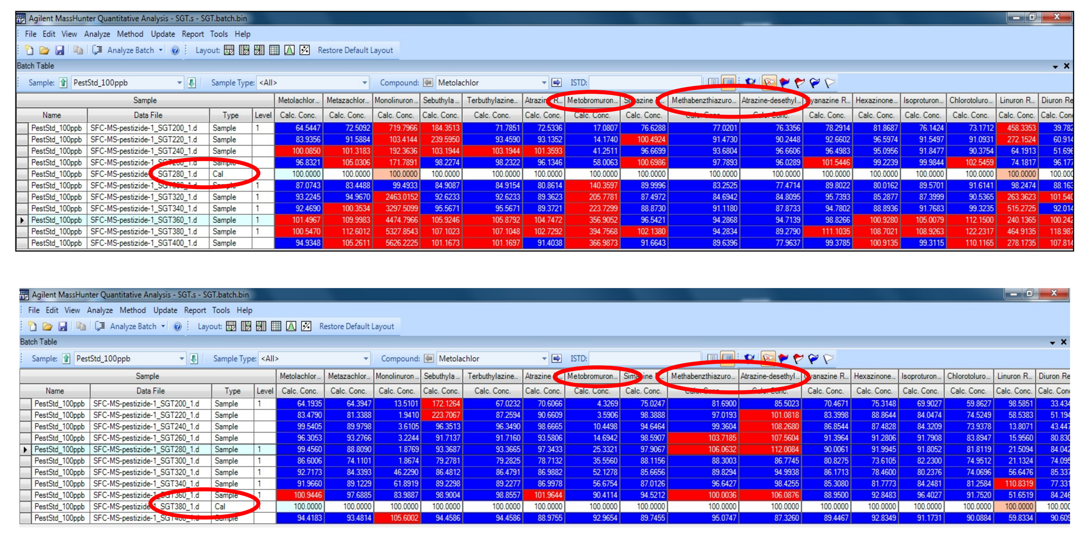

Figure 5) Data analysis of the data created by the methods obtained from MassHunter source optimizer for the optimization of the Agilent Jet Stream sheath gas temperature in the range of 200°C to 400°C, step size 20°C.

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.

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
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
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
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
Metobromuron	2.465	0.37	4.22	1.4	4.5	0.9997
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
Linuron	2.743	0.27	2.91	3.7	12.5	0.9998
Atrazine-desethyl	3.011	0.23	2.54	0.9	2.9	0.9997
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
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
Diuron	4.212	0.05	2.89	3.7	12.5	0.9997
Metoxuron	4.375	0.06	3.11	2.2	7.4	0.9990

Table 2) Performance results of the measurement of a sample comprising 17 pesticide compounds (Calibration from 1 to 1000 ng/mL, Limit-of-Detection, Limit-of-Quantification, linearity and statistical evaluation for retention time and area RSD [%] (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%.

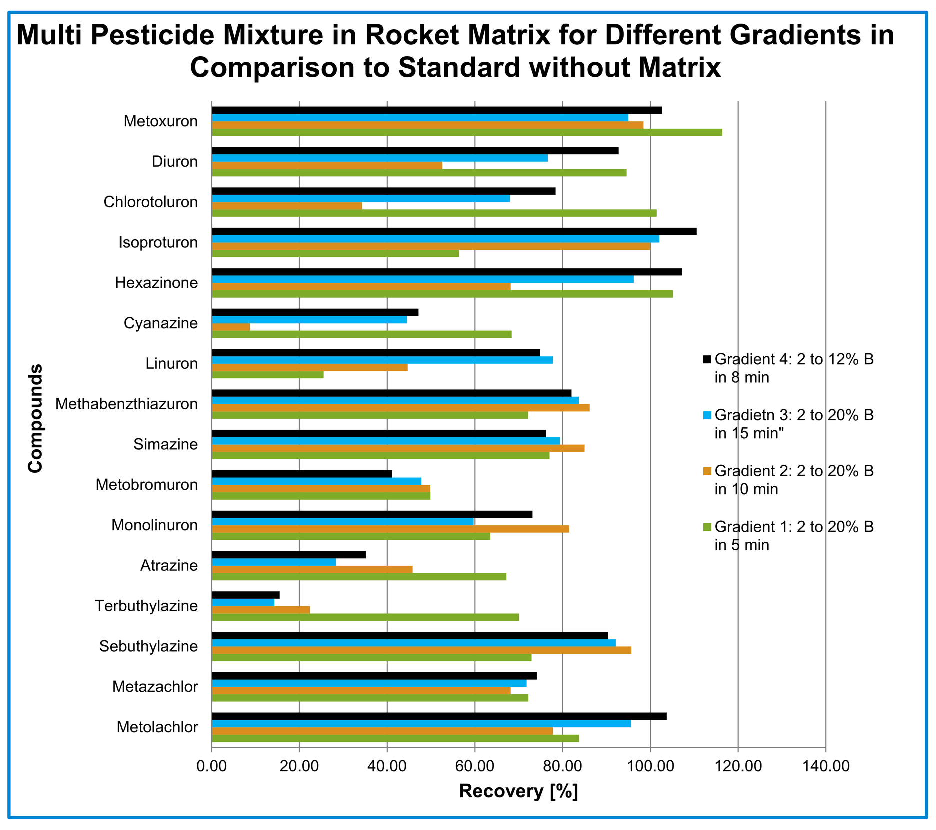

Figure 6) Comparison of matrix effects in different gradients of different steepness. The matrix effect is at its minimum for most of the compounds for gradient 4.

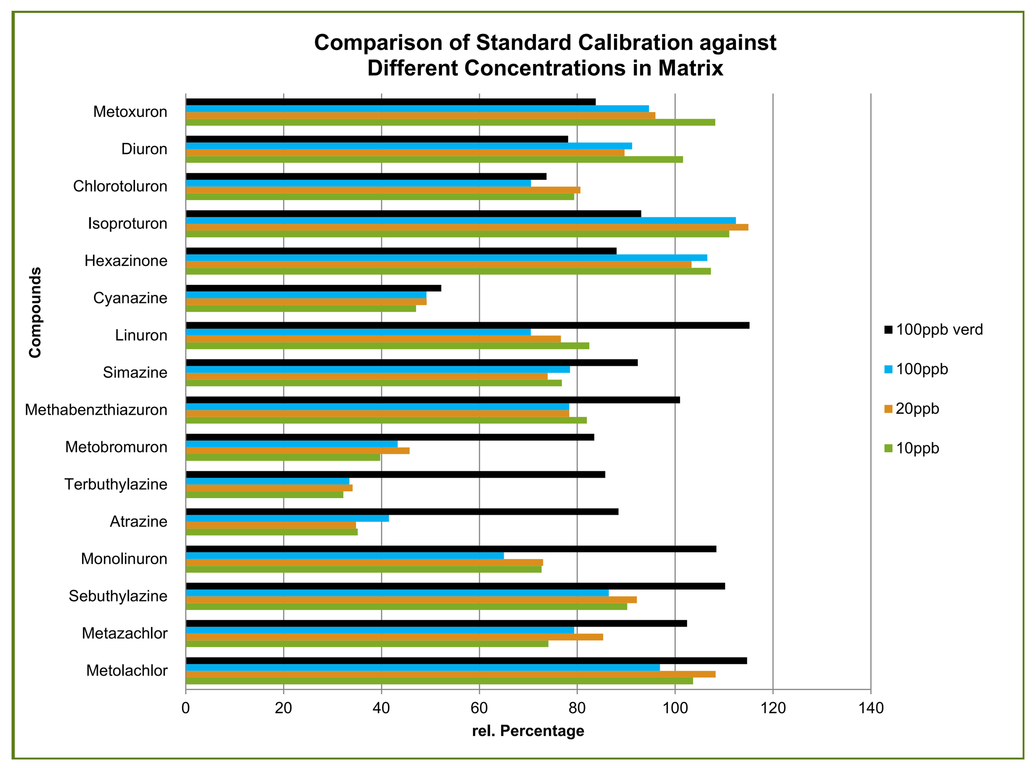

Figure 7) Comparison of spiked samples to a calibration in standard solution. Matrix effects are typically in a range of 70-120%. Matrix effects could be additionally minimized by sample dilution.

Conclusions

- This work demonstrates the workflow to optimize an Agilent 1260 Infinity Analytical SFC triple quadrupole 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.