13th EPRW 2020

PD-04

Analysis of highly polar pesticides in food of plant and animal origin with CESI–MS/MS

Ann-Kathrin Wachtler, Cristin Wildgrube, Dorothea Mack, Anja Barth, Michelangelo Anastassiades, Ellen Scherbaum and Walter Vetter

E-Mail: Ann-Kathrin.Wachtler@cvuas.bwl.de

Introduction

Capillary Electrophoresis (CE) is a technique for the separation of charged molecules in solutions and is well established for the analysis of bio molecules. The principle of separation in electrophoresis is based on the movement of charged analytes in electric fields. By applying a strong electric potential on the capillary, charged analytes migrate along the capillary based on their tendency to move in the electric field (electrophoretic mobility) and the electro-osmotic flow of the electrolyte. This brings new characteristics to the separation of ionic analytes compared to IC and LC. Ionic or ionizable pesticides are thus potential candidates for CE-type separations. An interface was recently introduced, that combines CE outlet and electrospray needle in one device. This facilitates connection to mass spectrometric detectors. The ultra-low flow-rates of only few nL/min are reported to positively influence electrospray ionization leading to a reduced impact of co-eluting matrix components on analyte signals and thus to higher ionization yields.

Materials and Methods

Sample preparation was based on the Quick Polar Pesticides (QuPPe) method by the EURL-SRM. Final extracts were diluted 5-fold for the analysis with CESI-MS/MS. For matrices containing high amounts of proteins and fat (e.g. of animal origin), analysis entailed addition of EDTA during extraction and additional clean-up steps, such as protein precipitation by means of acid or acetonitrile, a dSPE C_{18} step (for the removal of fat) and finally an ultracentrifugation to remove peptides >10kDa [1]. Calibration standards and dilutions were prepared using the BGE as solvent.

CESI-MS/MS Instrumentation Details

CESI Instrument:	AB Sciex CESI 8000 Plus ESI-MS-System
Separation Capillary	Beckman Coulter OptiMS Silica sufrace cartridge 30 µm ID x 91 cm
Background electrolyte (BGE)	15/20/65 acetic acid/methanol/water
Conductive liquid	10/90 acetic acid/water
Focussing buffer	50 mM NH_4-acetate (pH 7)
Separation mode	30 kV; reversed polarity; 5 psi pressure

CESI Separation method

Step	Pressure (psi)	Time	Direction	Voltage	Solution	Description
Rinse 1	100	2 min	Forward flow		0.1 M HCl	Conditioning
Rinse 2	100	2 min	Forward flow		0.1 M NaOH	
Rinse 3	100	2 min	Forward flow		BGE	
Rinse 4	100	2 min	Reverse flow		Conductive liquid	
Inject 1	5	10 sec	Forward flow		Focussing buffer	
Inject 2	10	20 sec	Forward flow		Sample	
Inject 3	5	10 sec	Forward flow		Focussing buffer	
Inject 4	5	30 sec	Forward flow		BGE	
Separation	5	16 min	**Reverse polarity**	30 kV		
Ramp-down	5	5 min	**Reverse polarity**	1 kV		

MS Instrument	Triple Quadrupole AB Sciex QTrap 5500
Ion Source	Nanospray source, negative mode
Curtein gas (nitrogen)	5 psi
Ion Spray Voltage	~ -2200 V
Gas Flow	off
Temperature of Gas 2	50°C

Separation and Peak Shapes

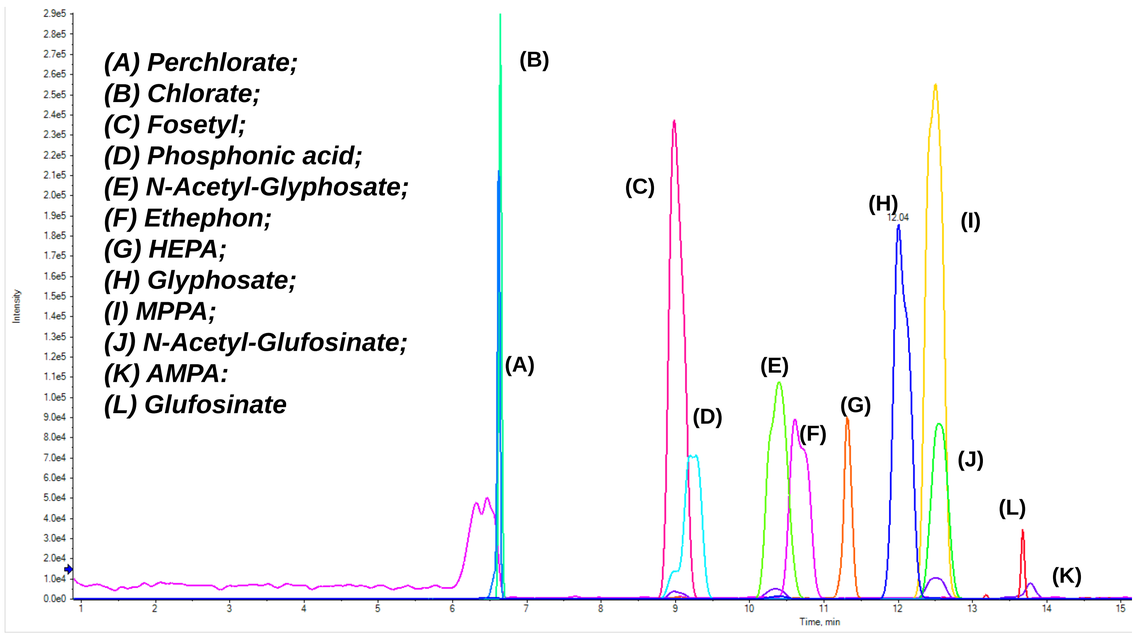

Figure 1: *Separation of 12 anionic polar pesticides in 5-fold diluted cucumber extract (dilution in BGE) at 0.2 µg/mL: (A) Perchlorate; (B) Chlorate; (C) Fosetyl; (D) Phosphonic acid; (E) N-Acetyl-Glyphosate; (F) Ethephon; (G) HEPA; (H) Glyphosate; (I) MPPA; (J) N-Acetyl-Glufosinate; (K) AMPA: (L) Glufosinate*

Influence of BGE Composition

The analytes are separated using a surrounding background electrolyte (BGE). To increase signal intensities of the analytes, different compositions of the BGE were tested, e.g. using organic solvents to improve evaporation in the ion source. Solutions of 0.2 µg/mL of a standard mix were repeatedly injected (n=10) in BGE as solvent, in undiluted as well as in 5- and 10-fold diluted QuPPe extracts of Swiss chard. The impact of varying the content of acetic acid, buffer, methanol and acetonitrile in the BGE was studied. Figure 2 and 3 show a comparison of exemplary average peak areas of glyphosate. Increasing the amount of acetic acid and methanol also increased peak intensities. Using formic acid (e.g. 0.1 or 1%) or alkaline conditions (10mmol NH_4 acetate +10% methanol pH 9) no signals could be detected by the MS.

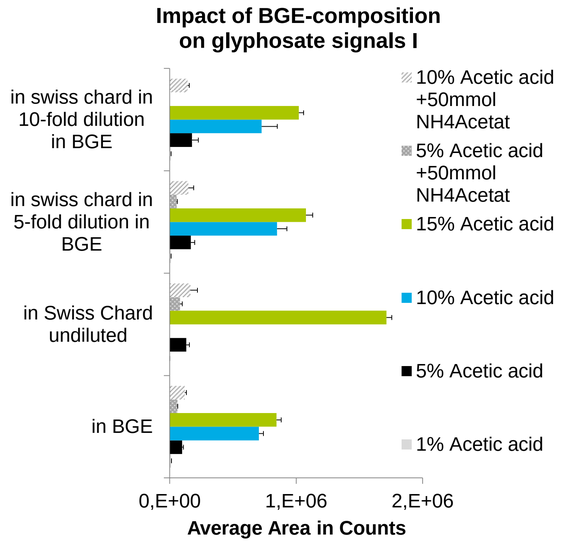

Figure 2: *Comparison of BGE solutions containing different amounts of acetic acid with varying amounts of NH_4Acetate*

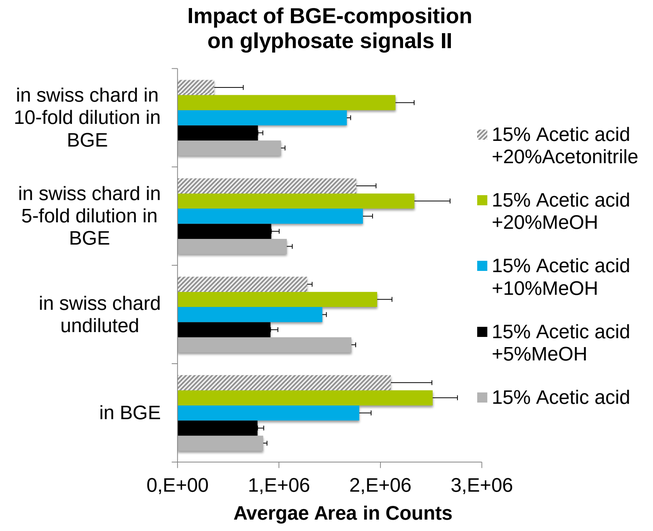

Figure 3: *Comparison of BGE solutions containing different amounts of methanol / acetonitrile*

Matrix Effects

Matrix effects were studied in extracts of plant and animal origin commodities. Most analytes showed moderate suppressions (up to -30%) or enhancements (up to +35%) (see figure 4). AMPA and glufosinate were heavily suppressed (between -50% and -98%). Figure 2 shows a signal enhancement of glyphosate using 15% acetic acid in BGE. Interestingly, this effect was observed when using additional methanol in the BGE. Dilution reduced signal suppressions, as shown exemplary for AMPA in figure 5.

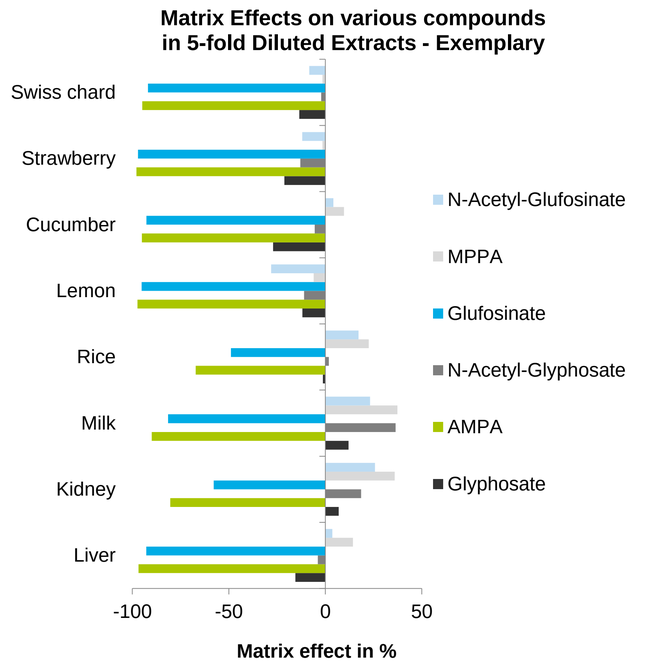

Figure 4: *Exemplary matrix effects in 5-fold diluted extracts of plant and animal origin samples.*

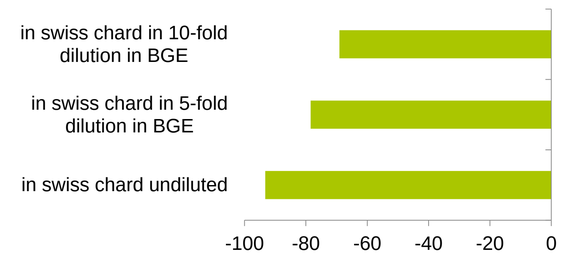

Figure 5: *Influence of dilution on signal suppressions (exemplarily for AMPA).*

Exemplary Validation Data

Validation was performed using isotopically labelled internal standards and 2-point matrix matched calibration (n=5). The sample weight was 10 g for cucumber and milk.

Analyte	Spiking Level in mg/kg Matrix	Mean Recovery in %	Variation Coefficient in %	Analyte	Spiking Level in mg/kg Matrix	Mean Recovery in %	Variation Coefficient in %
Cucumber				**Milk**			
Glyphosate 168/63	0.05	92	2.8	Glyphosate 168/63	0.1	113	7.6
Glyphosate 168/124		95	17.0	Glyphosate 168/124		118	11.0
AMPA 110/63		113	11.3	AMPA 110/63	0.2	78	36.4
AMPA 110/79		79	18.9	AMPA 110/79		86	26.7
N-Acetyl-Glyphosate 210/63		86	5.9	N-Acetyl-Glyphosate 210/63	0.1	99	5.9
N-Acetyl-Glyphosate 210/124		98	7.8	N-Acetyl-Glyphosate 210/124		96	12.1
Glufosinate 180/63		99	18.3	Glufosinate 180/63	0.2	*81**	13.5
Glufosinate 180/85		90	46.6	Glufosinate 180/95		*99**	25.0
MPPA 151/63		103	6.3	MPPA 151/63	0.05	114	6.8
MPPA 151/133		103	4.2	MPPA 151/133		112	10.4
N-Acetyl-Glufosinate 222/63		*111**	*32.3*	N-Acetyl-Glufosinate 222/63		*88**	16.5
N-Acetyl-Glufosinate 222/59		*116**	*34.6*	N-Acetyl-Glufosinate 222/59		*77**	15.7
Ethephon 143/107		116	15.0	Ethephon 143/107	0.1	111	9.4
Ethephon 143/79		112	3.9	Ethephon 143/79		116	9.9
Fosetyl 109/63		105	7.9	Fosetyl 109/63		101	4.3
Fosetyl 109/81		96	3.6	Fosetyl 109/81		106	7.5
HEPA 125/95		107	6.3	HEPA 125/95		111	6.9
TFA 113/69	0.01	115	19.2	TFA 113/69		97	7.1
TFA 113/133		112	19.0	TFA 113/133		100	22.3

**without ILIS*

Summary

Capillary electrophoresis has been connected to mass spectrometry to separate anionic polar pesticides within 15 minutes. Using 15% acetic acid and methanol in the background electrolyte, signal intensities of the analytes were increased. Acetonitrile as organic additive didn't improve sensitivity further. When using formic acid or alkaline conditions, the analytes were not satisfactorily ionized. Matrix effects between -30% and +35% were observed for most analytes, except for AMPA and glufosinate where suppressions ranged between -50% and -98%. Performing 5-fold dilution, matrix effects could be considerably reduced and peak shapes improved. Recovery rates in validation experiments were between 70% and 120%.

Literature: [1] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Baden-Württemberg