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Analysis of Highly Polar Pesticides in Food of Plant and Animal Origin by IC-MS/MS

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Introduction

Ion chromatography (IC) is increasingly being used in combination with unit- and high resolution mass spectrometry (MS) for the detection of highly polar pesticides [1]. Automatic generation of eluents and continuous automatic suppression (deionization and neutralization) ensure safe connection of IC to mass spectrometers. The method presented here employs IC-MS/MS for the analysis of highly polar anionic pesticides in food of plant and animal origin previously extracted by the QuPPe method.

Materials and Methods

Sample preparation was based on the Quick Polar Pesticides (QuPPe) method by the EURL-SRM [2]. Final extracts were diluted 5-fold for the analysis with IC-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 [2].

IC-MS/MS Instrumentation Details

IC Instrument	Thermo Scientific™ Dionex™ Integrion™ HPIC™ system
Column	Thermo Scientific™ Dionex™ IonPac™ AS19, 2x25mm and AS24, 2x25mm
Potassium hydroxide (KOH) Gradient Separation	15 mM (7 min), 15 to 36 mM (5 min), 36 mM (8 min), 36 to 70 mmol (0.5 min), 70 mmol (4.5 min), 70 to 15 mM (0.5 min), 15 mM (4.5 min)
IC Flow rate	0.3 mL/min
Eluent Source	Thermo Scientific™ Dionex™ EGC 500™ KOH eluent generator cartridge
Suppressor	Dionex ASRS® 300; 2mm
Separation Temperatures	Column: 32°C, Detector Compartment: 30°C, Suppressor: 15°C
Flow rate Make-up Solvent	0.15 ml/min acetonitrile (MS-Grade)
MS Instrument	Triple Quadrupole AB Sciex QTrap 5500
Ion Source	ESI Turbo Ion Spray, negative mode
Curtein gas (nitrogen)	30 psi
Ion Spray Voltage	-4500 V
Gas Flow	Gas 1: 60 psi, Gas 2: 60 psi
Temperature of Gas 2	600°C

Separation and Peak Shapes

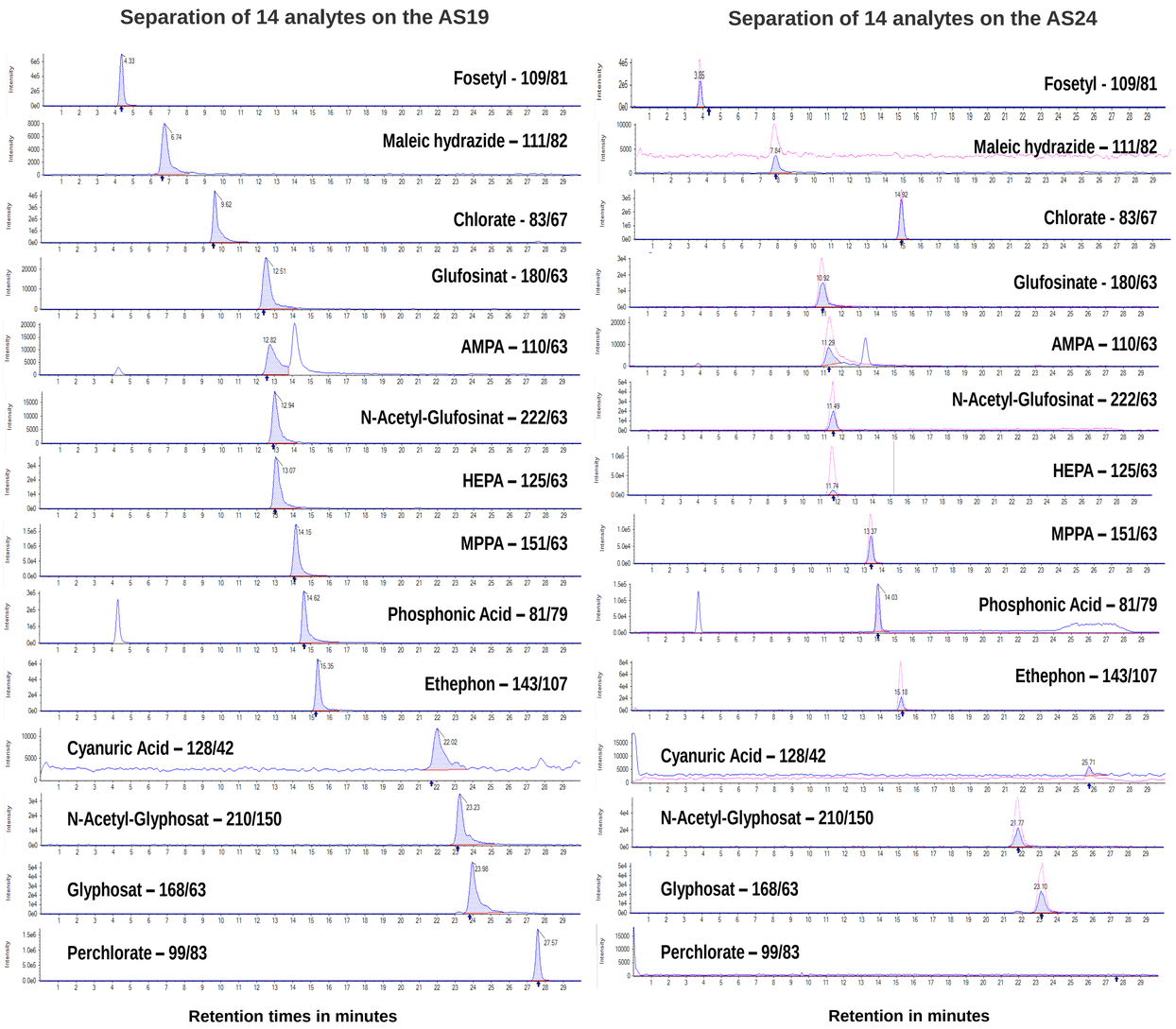

Figure 1: *Peak shapes of 14 anionic polar pesticides in double destilled water.*

14 anionic polar pesticides were separated on an AS19 column. The AS24 column, being more selective for polarizable substances, shows more retention for chlorate and perchlorate with perchlorate requiring >30 min to elute. Because of the higher capacity of AS24 some analytes show narrower, less tailing, peaks compared to AS19. Baseline separation was obtained between fosetyl (A), phosphonate (B), ethephon (C) and phosphate (D) (see Figure 2). These analytes are mass-spectrometrically interfered by phosphate, which is naturally present at high levels in many samples. This complicates quantifications near the LOQ, which is especially relevant in case of organically produced food and food for infants. Separation between fosetyl and phosphonate is also important.

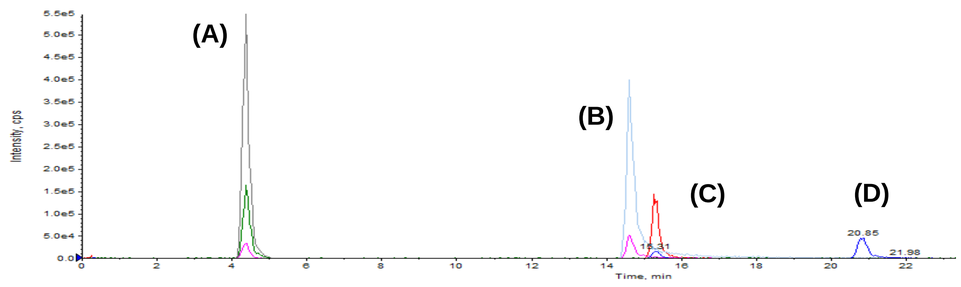

Figure 2: *Separation of Fosetyl (A), Phosphonic acid (B) and Ethephon (C) from Phosphate (D).*

Using Make-up Solvents to Increase Signal Intensities

The IC-eluent, which is neutralized by the suppressor, consists of nearly pure water. To facilitate evaporation and ionization of the analytes, organic solvents were tested as make-up solvents (additives to the eluent). These are admixed to the eluent, using a T-connector, just prior to entering the ion-source. High purity acetonitrile, methanol and isopropanol were admixed to the IC eluent (flow: 0.3 mL/min) at 0.08, 0.15, 0.23, 0.3, and 0.38 mL/min. Figure 3 exemplarily shows how the make-up solvent influx into the eluent flow affected the peak areas of glyphosate.

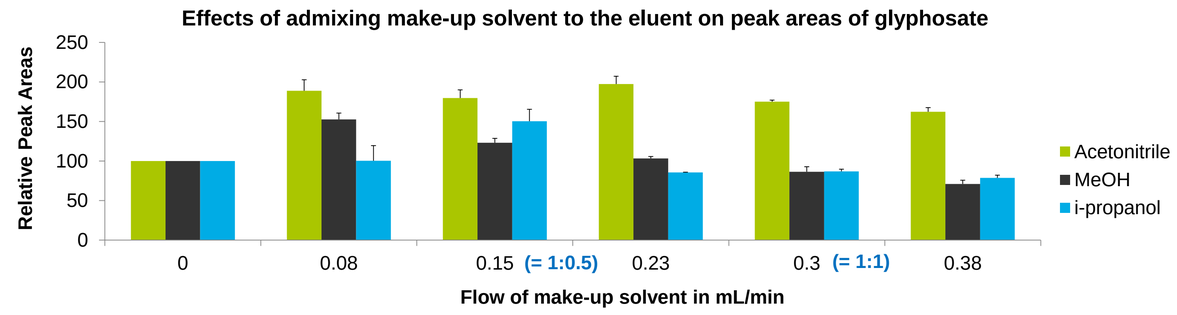

Figure 3: *Increase of peak areas of glyphosate by solvent addition using a make-up pump*

Impact of Dilution

Recovery experiments in soy (5 g sample weight), lemon (10 g) swiss chard (10 g), rhubarb (10 g) and cucumber (10 g), were conducted using QuPPe. The samples were spiked at 0.1 mg/kg (soy 0.2 mg/kg) and diluted at different rates in order to study the influence of dilution on matrix effects and retention time (RT). Recoveries were calculated using 1-point matrix matched calibration (n=3).

Matrix effects (MEs) were different from matrix to matrix but in general more moderate compared to LC-MS/MS. MEs could be reduced by diluting extracts and were largely eliminated at 10-fold dilution they. Heavy matrix load affected analyte retention, especially in the case of lemon and soy (see Figure 4). This effect was virtually eliminated by 5-fold dilution.

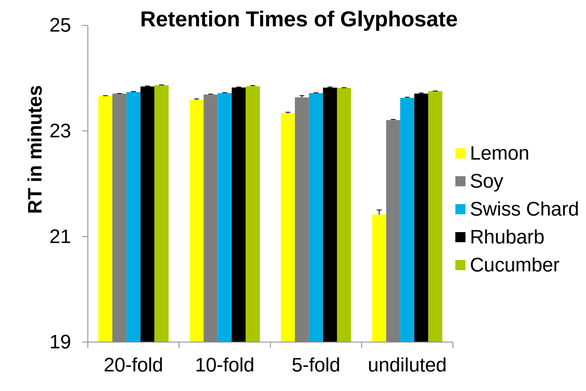

Figure 4*: Comparisson of retention times at different dilution factors (exemplary for glyphosate)*

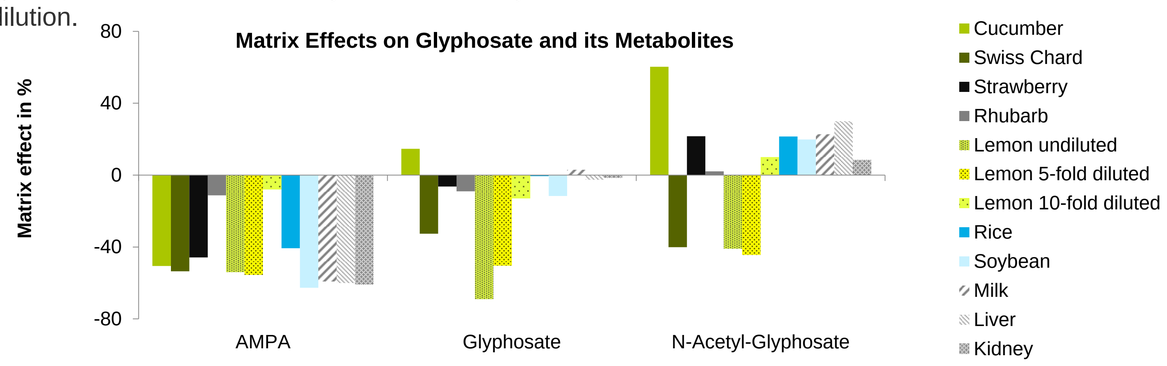

Figure 5: *Matrix effects on glyphosate, AMPA and N-acetyl-glyphosate in extracts of various matrices at 5-fold dilution*

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, milk, liver and kidney and 5 g for rice.

Analyte	Matrix	Spiking Level in mg/kg Matrix	Mean Recovery in %	Variation Coefficient in %	Analyte	Matrix	Spiking Level in mg/kg Matrix	Mean Recovery in %	Variation Coefficient in %
Glyphosate 168/63			96	2.3	Glyphosate 168/63			116	7.3
Glyphosate 168/150			100	3.7	Glyphosate 168/150			111	10.4
AMPA 110/63			97	5.9	AMPA 110/63	**Rice**	0.1	96	2.4
AMPA 110/79			106	3.7	AMPA 110/79			97	6.2
N-Acetyl-Glyphosate 210/63			104	2.7	N-Acetyl-Glyphosate 210/63			88	14.1
N-Acetyl-Glyphosate 210/150			107	3.4	N-Acetyl-Glyphosate 210/150			89	13.8
Glufosinate 180/63			99	4.6	AMPA 110/63	**Milk**	0.05	102	5.2
Glufosinate 180/95			99	3.4	AMPA 110/79			101	4.6
MPPA 151/63	**Cucumber**	0.02	103	4.0	AMPA 110/63	**Liver**	0.05	88	5.3
MPPA 151/133			108	4.3	AMPA 110/79			103	7.2
N-Acetyl-Glufosinate 222/63			103	3.0	AMPA 110/63	**Kidney**	0.05	106	9.0
N-Acetyl-Glufosinate 222/59			99	6.6	AMPA 110/79			115	7.0
Ethephon 143/107			90	5.2					
Ethephon 143/79			72	10.8					
Fosetyl 109/81			101	3.1					
Fosetyl 109/63			106	2.3					
HEPA 125/63			101	14.5					
HEPA 125/95			100	4.9					

Summary

Using IC-MS/MS in anion exchange mode, with automatic eluent generation and continuous automatic suppression 14 anionic polar pesticides were separated within 30 minutes. Because of a higher capacity on the AS24 peaks were more narrow than on the AS19, but perchlorate could not be eluted within 30 minutes. Using acetonitrile as a make-up solvent, peak areas could be increased almost 2-fold in case of glyphosate compared to non using make-up solvent. Performing 5-fold dilution matrix effects, especially retention time shifts, could be reduced. Recovery rates in validation experiments ranged between 70% and 120% with RSDs <15%.

Literature: [1] Rajski et al.: Journal of AOAC International Vol 101, No.2, 2018 ; [2] QuPPe Method: https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Baden-Württemberg