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 anionic pesticides in food of plant and animal origin previously extracted by the Qu#Ppe method.

Materials and Methods

Sample preparation was based on the Quick Pesticides (Qu#Ppe) 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 eluates (such as protein precipitation by means of acid or acetone), a SPE C18 step (for the removal of fat) and finally an ultrafiltration to remove peptides >10 kDa [2].

IC-MS/MS Instrument Details

- **IC Instrument**: Thermo Scientific™ Dionex™ Integrity™ HPIC™ system
- **Column**: Dionex Chromolith® 250x4 mm, 10 µm, C18
- **Flow rate**: 1 mL/min
- **Injection volume**: 20 µL
- **Temperature**: 35°C
- **Detector**: UV 210 nm

Separation and Determination

**Separation**: Cucumber – 210/198

14 anionic pesticides were separated on an AS19 column. The AS24 column, being more selective for polarizable substances, shows more retention for chloride 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 for 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 quantification near the LOQ, which is especially relevant in case of organically produced foods and food for infants. Separation between fosetyl and phosphate is also important.

Using 14 anionic pesticides in anion exchange mode, with automatic eluent generation and continuous automatic suppression 14 anionic pesticides were separated within 30 minutes. Because of a higher capacity of the AS24 peaks were more narrow than on the AS19, but perchlorate could not be eluted within 30 minutes. Using acetylation 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%.

**Exemplary Validation Data**

Validation was performed using isotopically labelled internal standards and 2-point matrix matched calibration (m=3). The sample weight was 10 g for cucumber, milk, liver and kidney and 5 g for rice.

**Impact of Dilution**

Recovery experiments in soy (5 sample weight), lemon (10 g) sweet cherry (10 g), mushroom (10 g) and cucumber (10 g), were conducted using Qu#Ppe. 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 (m=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 using bigger eluents and were largely eliminated at 5-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.

**Exemplary Validation Data**

Validation was performed using isotopically labelled internal standards and 2-point matrix matched calibration (m=3). The sample weight was 10 g for cucumber, milk, liver and kidney and 5 g for rice.

**Impact of Dilution**

Recovery experiments in soy (5 sample weight), lemon (10 g) sweet cherry (10 g), mushroom (10 g) and cucumber (10 g), were conducted using Qu#Ppe. 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 (m=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 using bigger eluents and were largely eliminated at 5-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.