Comparative study of four HILIC type columns for the analysis of anionic polar pesticides

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Introduction

The analysis of highly polar pesticides, such as glyphosate, is generally considered challenging. Recently, several hydrophilic liquid interaction chromatography (HILIC) columns became available for the analysis of these compounds. Here, we present the results of a comparative study involving four different HILIC columns and six different matrices of plant and animal origin (PO/AO). The results of a low-level validation as well as the matrix effects (ion suppression) obtained for each matrix-analyte combination with each of the four tested columns are compared.

Analytical methods

Extraction was conducted based on the QuPPe procedure: www.quppe.eu. For details on the LC conditions including gradients, injection volume, exemplary chromatograms etc. see also in the latest version of the QuPPe method. The following columns were tested: Anionic Polar Pesticides Column (‚APPC', Waters), Luna Polar Pesticides (‚LunaPP', Phenomenex), Raptor Polar X (‚PolarX', Restek), ObeliscN (Sielc). All extracts were measured with the same LC instrument (Waters I-Class) using the same column position and the same MS instrument (Sciex 5500 QTrap). For validation experiments, the homogenate was spiked prior to extraction (n=5) at three levels. For the matrices and low level concentration see Table 1. The presented results were measured using a 2-point matrix-matched calibration and isotopically labelled internal standards (IL-IS). Matrix effects were measured by comparing signals in solvent against signals in QuPPe extracts undiluted (DL1) as well as 5-fold (DL5), 10-fold (DL10) and 20-fold (DL20) diluted with acidified methanol/water, both spiked at 0.1 µg/mL (n=3).

Results

Overall, the highest number of successfully validated matrix-analyte combinations was counted with LunaPP (32 of 48 combinations), followed by APPC (31), PolarX (30) and ObeliscN (26). However, PolarX performed best in dry matrices (12/18) and both APPC and LunaPP performed best in AO matrices (12/18). Validation of fosetyl at low level, was successful on each column and matrix, except for ObeliscN (see below). Investigations on matrix effects (ME) exposed further differences between the columns (Fig. 1 and 2), with the PolarX showing overall more analytes with negligible ME (<±20%) (Fig.1c). AMPA was the compound most heavily affected by ME on each column, with ObeliscN performing best. The impact of further dilution on reducing matrix effects was also studied and is exemplarily shown for AMPA in Fig. 2. This measure was more effective on LunaPP (Fig. 2b) than on APPC (Fig. 2a).

Further remarks on the results and used columns

Evaluation of HEPA in bovine liver was not possible due to background levels [1]. Elution/retention of *N*-acetyl-glyphosate on the PolarX was generally not achieved in our laboratory. During the experiments, a background and high baseline of fosetyl with the ObeliscN was observed.

Summary and outlook

Despite the more moderate suppression on PolarX, LunaPP and APPC showed the higher number of successfully validated analyte-matrix combinations. However, each column had its advantages and drawbacks. We therefore believe that the final decision for a specific column should depend on the matrix and analyte scope of a lab. The project will be continued with further analytes, i.e. chlorate, perchlorate, phosphonic acid and TFA.

LITERATURE:

[1] EURL-SRM. Residue Findings of QuPPe-Compounds in Samples of Plant Origin from the German Market in 2023. 2024.

Table 1: Results of validation experiments at the respective lowest tested level.
‚✓' = Compound was successfully validated in the respective matrix at the given spiking level.
*No. of successfully validated compounds at low level / out of

Matrix	Column	Glyphosate	AMPA	NAGly	Glufosinate	MPPA	NAGlu	Fosetyl	Ethephon	HEPA	x/ total*
Lemon – 0.01 mg/kg	APPC	✓	✓	✓				✓	✓	✓	6/9
	LunaPP	✓	✓	✓	✓	✓	✓	✓	✓	✓	9/9
	PolarX	✓						✓	✓		3/9
	ObeliscN	✓		✓	✓		✓		✓	✓	6/9
Pumpkin – 0.01 mg/kg	APPC	✓		✓		✓	✓	✓		✓	6/9
	LunaPP	✓			✓		✓	✓	✓		5/9
	PolarX				✓	✓	✓	✓	✓	✓	6/9
	ObeliscN	✓				✓	✓				3/9
Bovine Liver – 0.01 mg/kg	APPC		✓			✓	✓	✓	✓	-	5/8
	LunaPP	✓		✓		✓	✓	✓	✓	-	6/8
	PolarX		✓		✓			✓	✓	-	4/8
	ObeliscN					✓	✓			-	2/8
Cow‘s Milk – 0.01 mg/kg	APPC	✓	✓			✓	✓	✓	✓	✓	7/9
	LunaPP		✓	✓		✓		✓	✓	✓	6/9
	PolarX		✓		✓		✓	✓	✓	✓	6/9
	ObeliscN	✓		✓		✓	✓		✓		5/9
Whole Grain Wheat – 0.02 mg/kg	APPC					✓	✓	✓		✓	4/9
	LunaPP	✓				✓	✓	✓			4/9
	PolarX				✓	✓	✓	✓	✓	✓	6/9
	ObeliscN		✓	✓		✓	✓		✓	✓	6/9
Soybean – 0.02 mg/kg	APPC					✓		✓		✓	3/9
	LunaPP						✓	✓			2/9
	PolarX		✓		✓	✓		✓	✓	✓	6/9
	ObeliscN	✓					✓		✓	✓	4/9

NAGly = *N*-acetyl-glyphosate; NAGlu = *N*-acetyl-glufosinate

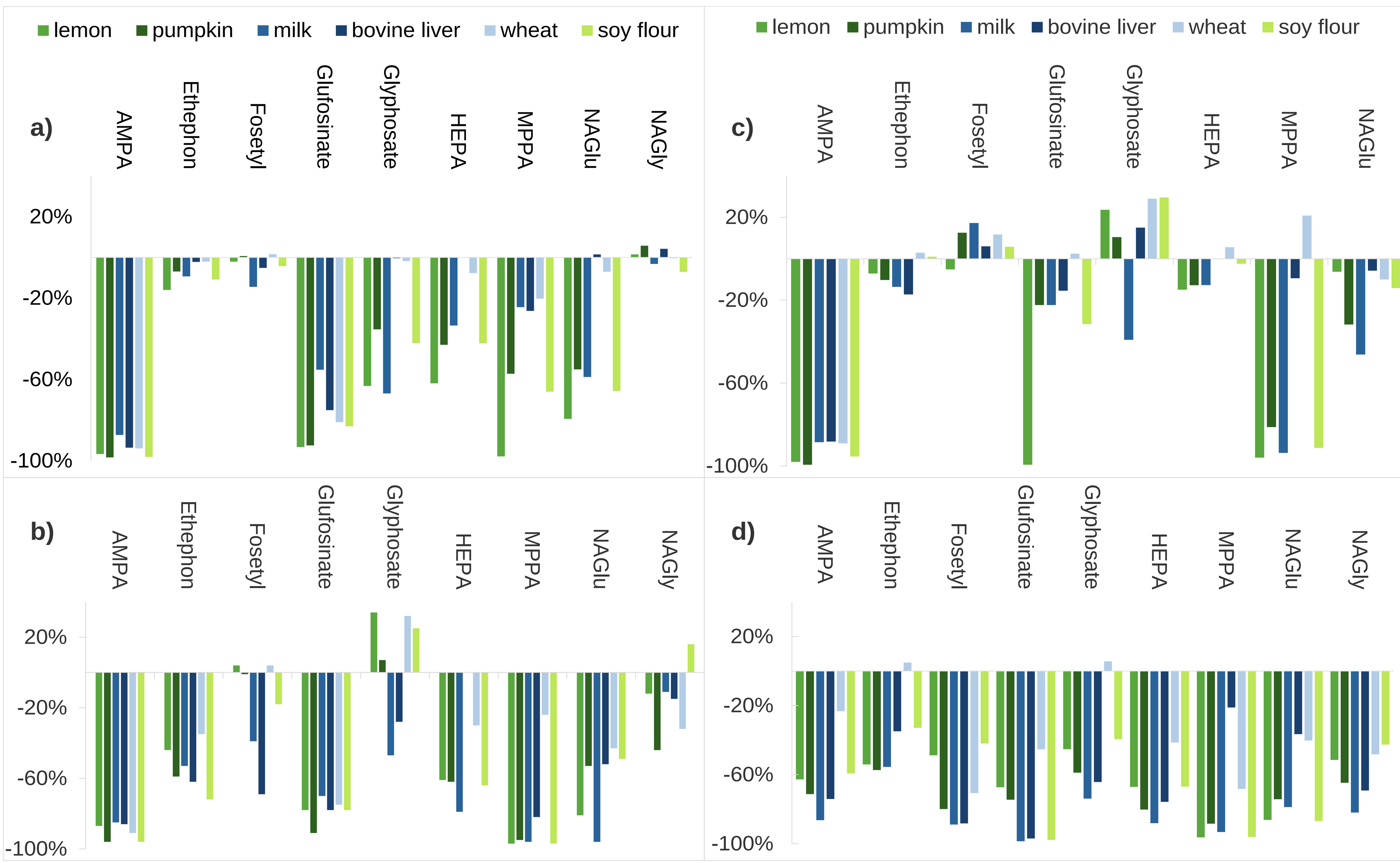

Fig. 1: Matrix effects in undiluted extracts on the APPC (a), LunaPP (b), PolarX (c), ObeliscN (d).

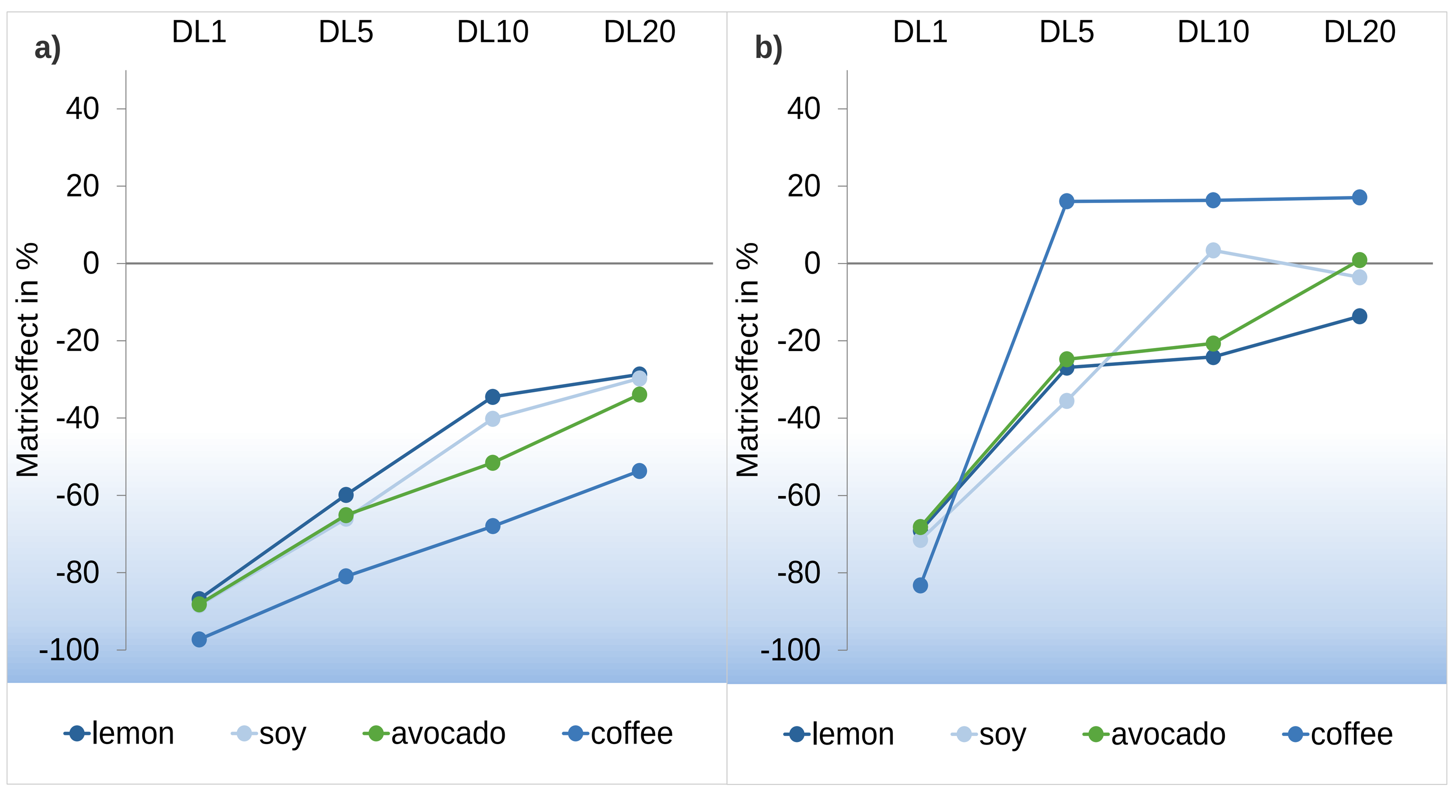

Fig. 2: Matrix effects of AMPA in different dilution steps (DL1 to DL20) exemplarily on the APPC (a) and LunaPP (b). DL = dilution factor, e.g. DL1 = undilted, DL5 = 5-fold diluted, and so on.

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