Analysis of Diquat and Paraquat by QuPPe and LC-MS/MS – Critical Points

D. Mack, L. Moser, A. Wachtler E. Eichhorn and M. Anastassiades
E-Mail: eurl-srm@cvuas.bwl.de

EURL-SRM

CVUA STUTTGART

**Diquat (DQ)**

Paraquat (PQ)

Introduction

Diquat (DQ) and paraquat (PQ) are non-selective herbicides that are also widely used as crop-desiccants to facilitate harvesting e.g. in oily seeds, pulses, cereals and potatoes. Both are banned in the EU and their EU-MRLs are therefore set at the LOQs for most products, with some exceptions, such as PQ in rice and DQ in oats, various pulses, oil seeds, tree nuts and tree fruits. Analysis of DQ and PQ proves challenging due to matrix dependent extraction yields, and extraction conditions thus need to deviate from those of the original QuPPe method.

Critical Points to consider in PQ/DQ analysis

1. Variability of MRM-Ion-Ratios

Being double charged and showing redox reactivity DQ and PQ generate various precursor ions within the ESI-ion-source, with the most prominent being the following: a) **Dications** $[M]^{2+}$; b) **Radical cations** $[M]^{+\bullet}$; and c) **Deprotonated cations** $[M-H^+]^+$. Each of these forms has its own MS/MS-transitions. The relative generation rate of these precursor ions depends on the composition of the mobile phase during elution (including co-eluting matrix components) as well as on the design and conditions in the LC-MS/MS interface. Different matrices influence the pattern of generated parent ions differently (see below).
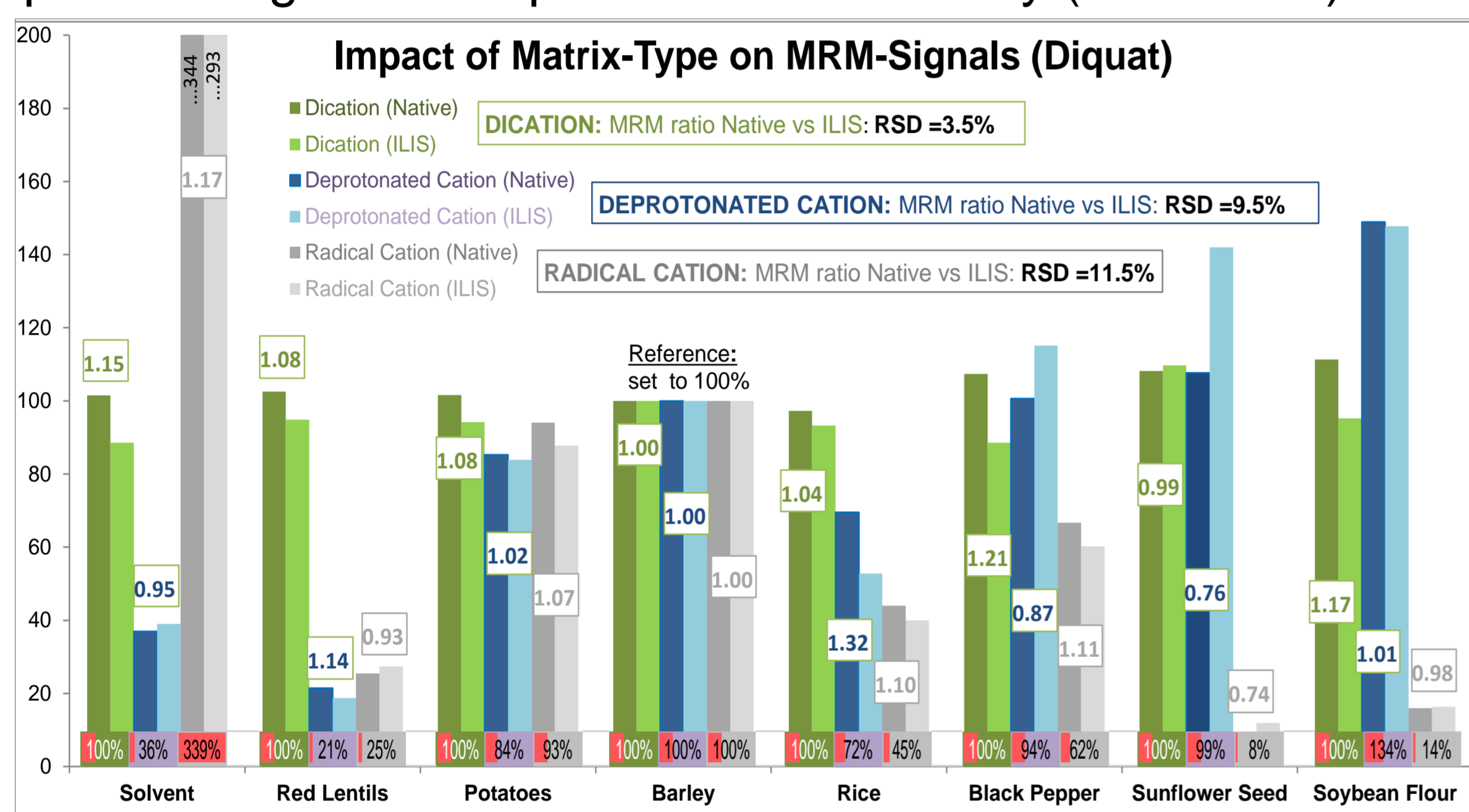

With the MRM-signal ratios being heavily matrix dependent, calculations should be made using ILIS-MRMs matching to those of the native analyte. In the example above, the ratios between the matching MRMs of ILIS and native compound were largely comparable for all precursor ions irrespective of the matrix. This is pre-requisite for ILIS-based calibrations that are not matrix-matched. In other experiments, however, only the dication-based MRMs showed a largely stable signal ratio to their matching ILIS-MRMs, whereas the respective ratios of the radical and the deprotonated precursors were fluctuating or even shifting depending on the matrix. Overall, MRMs based on dication-precursors were found to provide the least fluctuating signals and signal ratios and are to be preferred.

2. Standard Stability

PQ and DQ and their ILISs are sensitive to degradation in various solvents, especially when exposed to sunlight. The Figure exemplarily shows the DQ-stability in MeOH or MeOH/1% FA.

Water containing 10% ACN proved suitable for stock and working solutions, providing good stability for DQ even when exposed to light (Δ<10% after 29 months). DQ-D4 (ethylene labelled) appears to be more prone to degradation than native DQ.
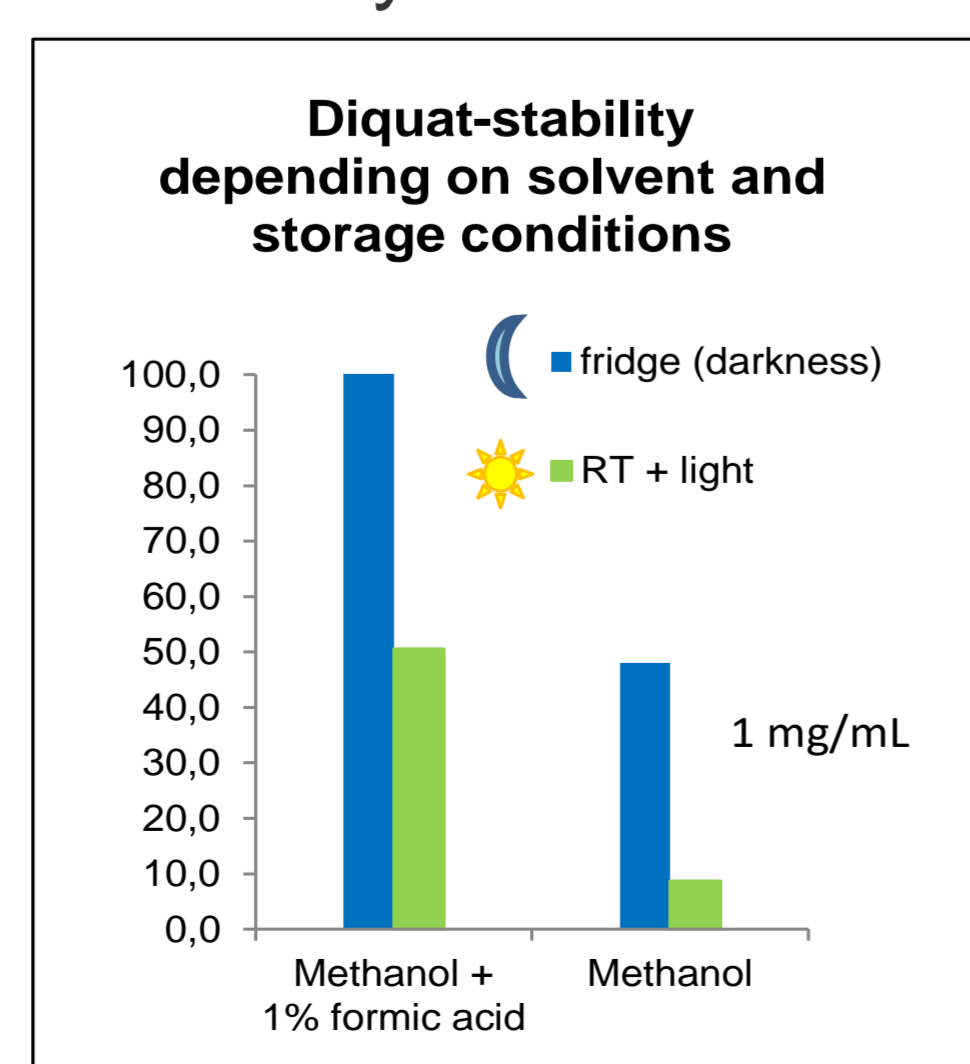
Ring-labelled DQ-D8 was synthesized by the EURL-SRM and proved to be much more stable than DQ-D4. DQ-D8 is now also commercially available.

3. Chromatography

The tendency of PQ and DQ to interact with surfaces often leads to tailing peaks and carry-over effects in LC-MS/MS. Clean and well primed chromatographic systems are needed to reduce these effects. The figure below (left) shows how different parts within the LC-system contribute to peak broadening. The figure in the middle shows how priming with DQ and PQ helps to block active sites and sharpen peaks. Standards in matrix are to be preferred as DQ/PQ may experience losses and carry-over to the next run if injected in pure solvent. The figure to the right shows typical peaks using different columns. A peak separation was achieved on a sulfobetain coated column.
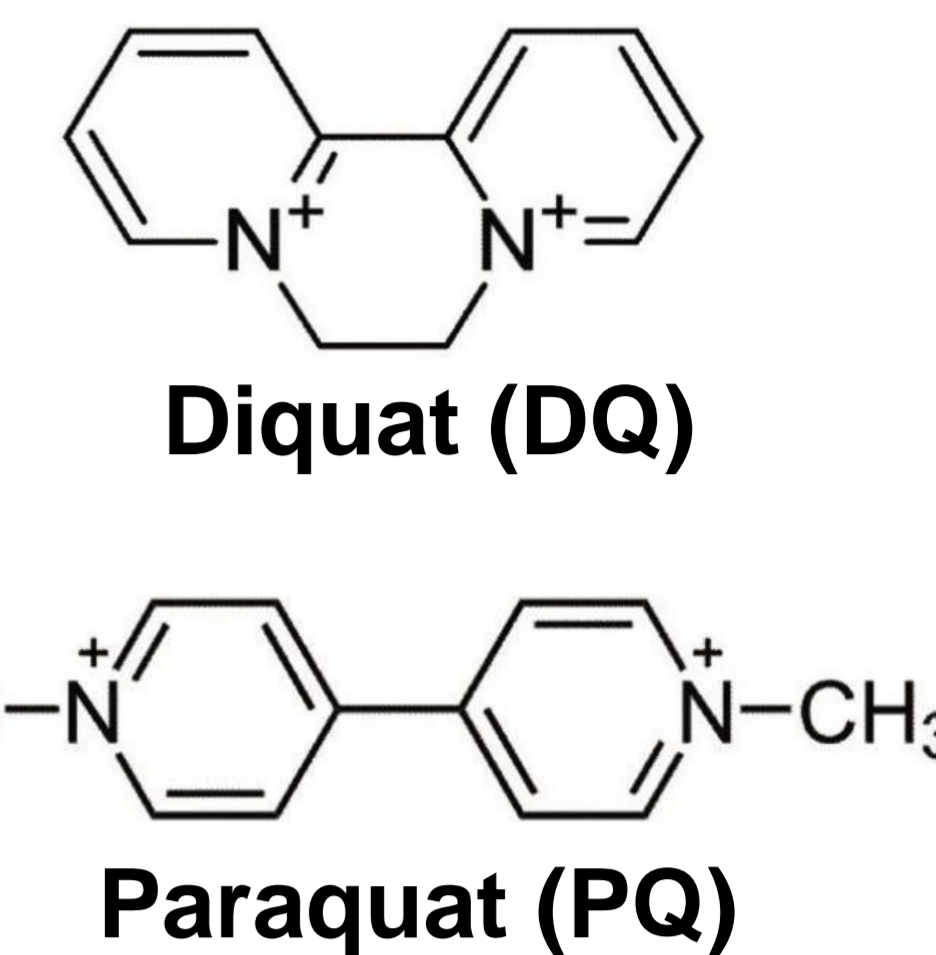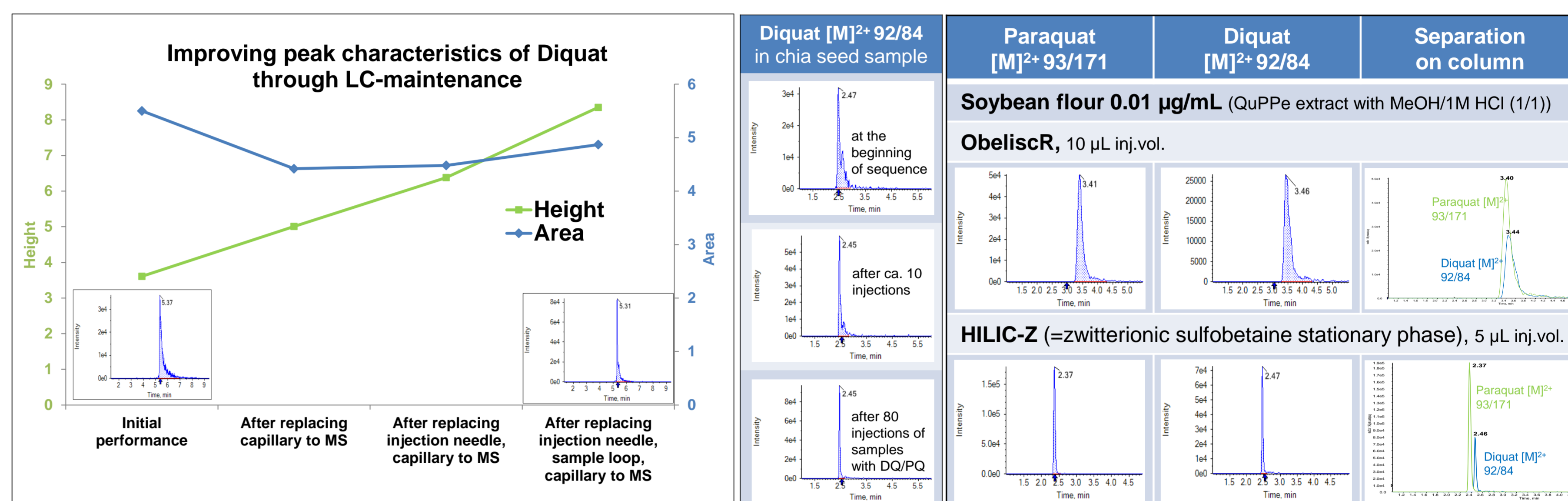
4. Extractability

PQ and DQ tend to interact with matrix during extraction and an equilibrium seems to be formed, that depends on matrix-type and extraction conditions. Very low recoveries were observed in oily seeds (e.g. flax, chia and rape seeds) and pulses, whereas fruits, vegetables and cereals seem less affected. In cases of strong interactions acidity helps to reduce interactions, and increase recovery rates, but a higher acidity than in the current QuPPe protocol is needed. Interestingly, the yields of incurred residues correlate well with the recoveries of spiked DQ/PQ, suggesting that incurred residues are subjected to the same equilibria (at least their readily extractable parts, see figure). Heating seems to slightly improve both extraction yields and absolute recovery rates, but acid-strength seems to play a bigger role in improving extraction yields (and recoveries). ILIS-based correction for recovery works well even for very poor absolute recoveries (see table below).
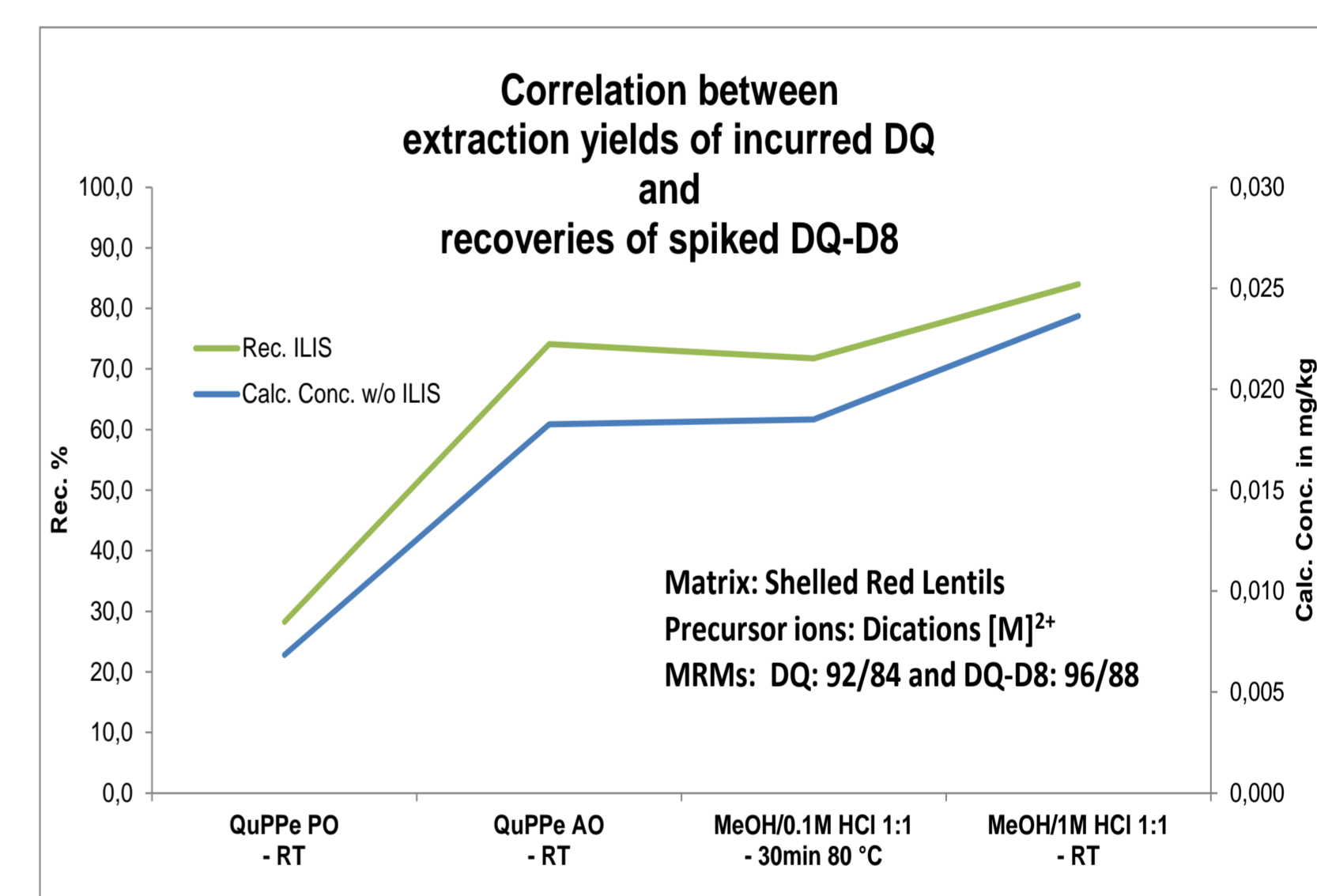
Absolute and ILIS-corrected recovery rates of spiked DQ and PQ (level 0.2 mg/kg)		QuPPe AO (EDTA/1% FA / RT)	QuPPE PQ/DQ 1M HCl / RT	QuPPe PQ/DQ 0,1M HCl / 80 °C	QuPPe PQ/DQ 1M HCl / 80 °C	QuPPe AO (EDTA/1% FA / RT)	QuPPe PQ/DQ 1M HCl / RT	QuPPe PQ/DQ 0,1M HCl / 80 °C	QuPPe PQ/DQ 1M HCl / 80 °C
		no ILIS				**with ILIS**			
Rice	DQ	95	87	81	95	109	105	108	99
	PQ	89	88	85	91	105	97	109	102
Chia	DQ	15	74	3	99	108	102	81	99
	PQ	12	60	9	88	107	102	102	96
Lentils (brown, unshelled)	DQ	12	50	28	74	110	104	113	100
	PQ	10	15	15	41	114	99	109	97

With QuPPe-AO (w. EDTA) the recoveries of PQ/DQ are higher than with QuPPe-PO, but in difficult matrices these remain low, bearing risks of FNs or severe underestimations if these approaches are used for screening. Such samples are recommended to be analyzed using PQ/DQ optimized conditions rather with the normal QuPPe conditions.

Analysis of market samples

Various market samples were analyzed for PQ and DQ. Exemplary data concerning linseeds and chia are shown below. Interestingly, organic samples also contained residues, in some cases even exceeding the MRLs.

Analyzed		PQ		DQ	
		> LOQ	> MRL	> LOQ	> MRL
		# samples (thereof organic)			
Linseeds (flax)	11 (9)	-	-	2 (1)	-
Chia seeds	13 (8)	5 (1)	3 (1)	-	-

**EPRW 2022
PD - 45**

Baden-Württemberg

Reference: www.quppe.eu