CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Development and Independent Laboratory Validation of a Simplified Sample Preparation Method for the Determination of Paraquat and Diquat in Food Commodities

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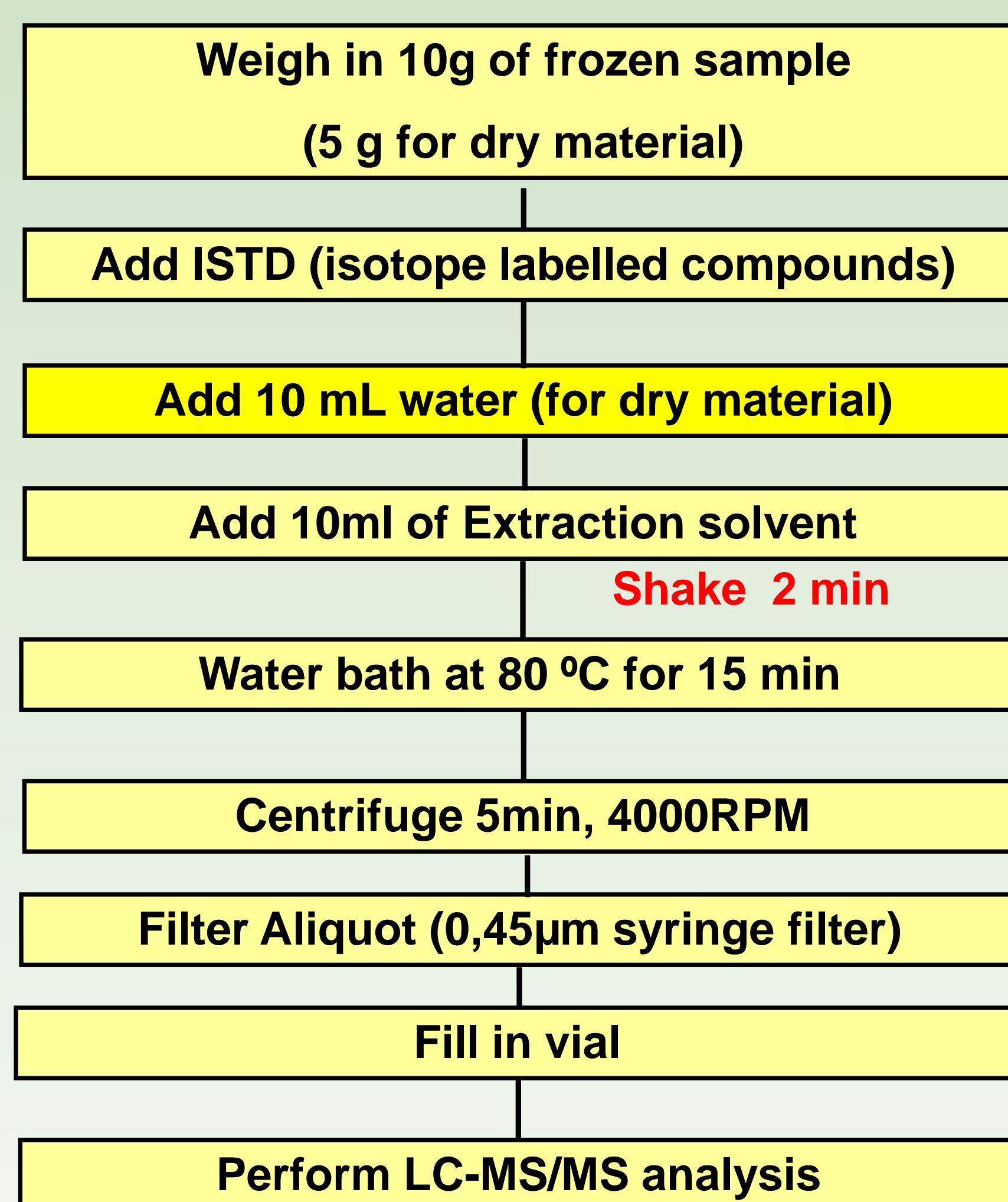1 Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstr. 3/2, 70736 Fellbach, Germany
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Introduction

Paraquat and diquat are non-selective contact herbicides used on a many crops worldwide. In the European Union diquat is used to defoliate potatoes to facilitate mechanical harvest and to dessicate cereals intended for animal feed (not human consumption). There are no approved agricultural uses of paraquat in the EU. By contrast, paraquat is used on more than 100 crops in 100 different countries so there is a need to monitor imported produce. The determination of paraquat and diquat is difficult, since due to their permanent ionic character and hydrophilic nature they are not amenable to multi-residue methods involving liquid-liquid-partitioning. Consequently they are generally excluded from pesticide residue monitoring programmes. Additionally, the high affinity of diquat and paraquat to bind to various surfaces requires special attention for the extraction of these compounds from food samples and the subsequent treatment and storage of extract solutions.

Experimental Procedure

Weigh in 10g of frozen sample (5 g for dry material)

Add ISTD (isotope labelled compounds)

Add 10 mL water (for dry material)

Add 10ml of Extraction solvent
Shake 2 min

Water bath at 80 ºC for 15 min

Centrifuge 5min, 4000RPM

Filter Aliquot (0,45µm syringe filter)

Fill in vial

Perform LC-MS/MS analysis

Extraction Solvents
45% MeOH+50% Water+5% FA
Water + 2% Formic Acid
MeOH + 5% Formic Acid
MeOH + HCl 0,1M (1:1)
MeOH + H_2SO_4 0,1M (1:1)

Figure 1: Procedure with different extraction solvents

Besides the different extraction solvents, the effect of shaking time (2 - 30 min), with and without heating, were also evaluated. Extraction with 0,6 N sulfuric acid (employed in the industry method) at 100 °C for times ranging between 30 min and 5h did not provide any improvement in the recovery of analytes compared to the method developed.

LC-MS/MS - Conditions

Column Obelisc R 2.1 x 150mm

Pre-column Obelisc R 2.1 x 10mm

Eluent A 20 mmol NH_4-formate in water pH 3 adjusted with formic acid

Eluent B Acetonitrile

Injection Volume 10 µL

Flow rate 0,4 mL min^{-1}

Results

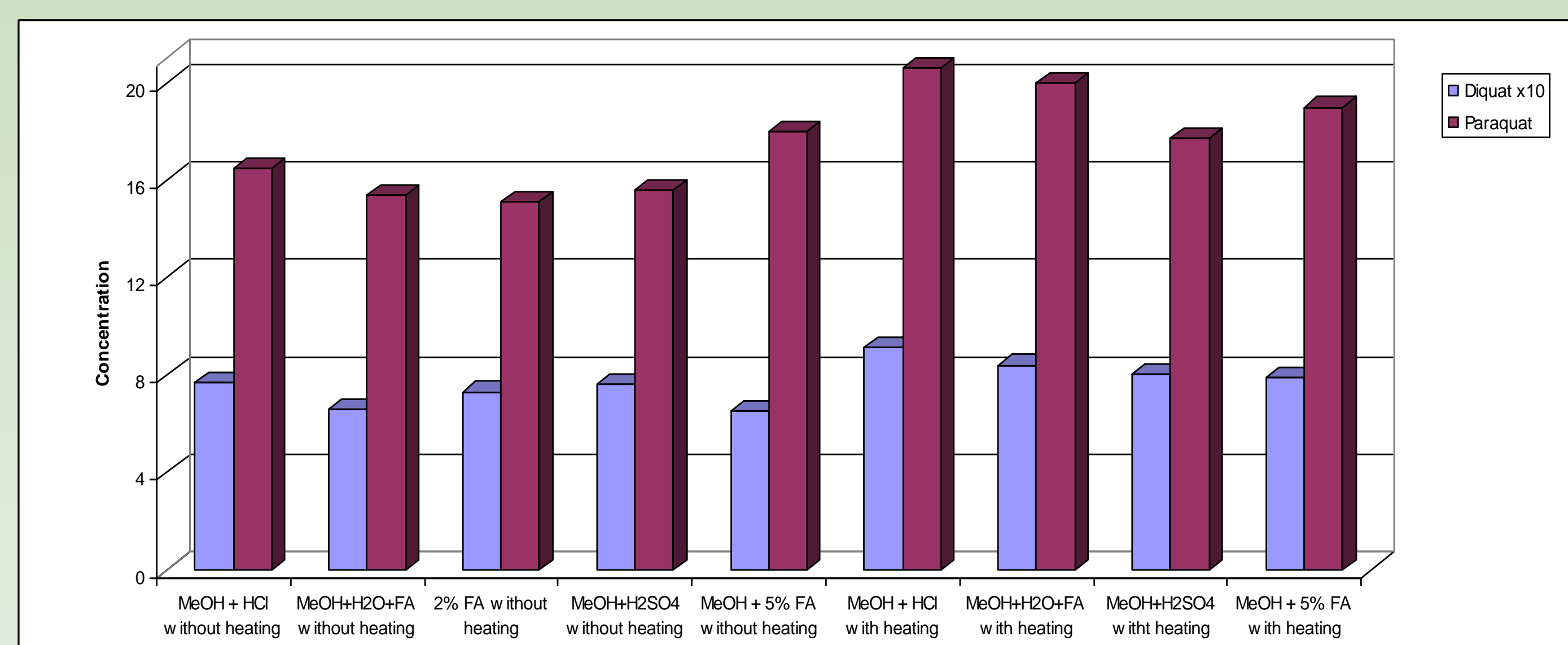*Figure 2*: Potatos treated with diquat and barley treated with diquat and paraquat

Figure 3: Comparison of the different extraction solvents on incurred wheat sample (n= 3)

* The extraction with water + 2% formic acid generated a viscous suspension upon heating and was discarded

An independent small-scale inter-laboratory validation of the developed method for diquat and paraquat in samples of potato and barley was performed in the three laboratories (CVUA, Fera and RIKILT). The precision and accuracy of the method were determined from recovery experiments (5 replicates) at spiking concentrations in the range 0.01 and 0.1 mg/kg.

	Potato				Barley			
	0,01 mg/kg		0,1 mg/kg		0,01 mg/kg		0,1 mg/kg	
	Rec	RSD	Rec	RSD	Rec	RSD	Rec	RSD
Diquat	94-103	2-5	100-120	4-9	100-116	1-9	99-117	3-10
Paraquat	95-116	6-7	105-114	1-7	92-119	5-7	101-114	2-7

Table 1: % Recoveries and % RSDs obtained for diquat and paraquat spiked on potato and barley performed in the three laboratories (CVUA, Fera, RIKILT)

Conclusion

The procedure established for diquat and paraquat involves extraction with MeOH + HCl 0,1M (1:1) at 80°C for 15 minutes. Analysis is performed by LC-MS/MS using the isotopically labelled compounds as ISTDs with the ISTDs helping to effectively eliminate errors caused by matrix-induced suppression in LC-MS/MS. Recoveries (n= 180) were in the range of 92 – 114% for all tested compound/ commodity/ spike concentration combinations. The good sensitivity of the method (LOQ 0,006 mg/kg), and the excellent recoveries and RSDs demonstrate that the method is suitable for routine analysis of these herbicides. The analysis of samples of barley and potato containing 'field incurred' residues of paraquat and diquat showed that the developed extraction procedure, although much simpler, yielded extraction efficiencies that are comparable to those of previously published more tedious methods. This work was conducted within the EU research project 'CONffIDENCE' (CONtaminants in Food and Feed: Inexpensive DetectioN for Control of Exposure) which is funded under the European 7th Framework programme. Further information on the project is available at *http://www.conffidence.eu*.