

Direct Determination of Paraquat, Diquat, Mepiquat, Morpholine, and Chlormequat Pesticides Using Ion Chromatography and High Resolution Accurate Mass Spectrometry

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ABSTRACT

Purpose: Determine quaternary amine pesticides in homogenized vegetable and fruit samples using ion chromatography coupled with high resolution accurate mass spectrometry (IC-HRAMS).

Methods: Homogenized fruit and vegetable samples were extracted using European Research Laboratories (EURL) Quick Polar Pesticides (QuPPE) multiresidue extraction method.¹ Six cationic polar pesticides of interest in homogenized vegetable and fruit samples were separated by cation-exchange chromatography (CEX) on a Thermo Scientific™ Dionex™ IonPac™ CS17 cation-exchange column using an electrolytically-generated methanesulfonic acid (MSA) gradient (2 to 60 mM over 10 min) at 0.4 mL/min. The Dionex IonPac CS17 column was selected as the most suitable for separations of quaternary amines because it is optimized for hydrophobic ionic amine separations. Additionally, a Thermo Scientific™ Dionex™ IonPac™ Dionex prototype (JM006) cation exchange column was evaluated as a future product to separate diquat and paraquat. The quaternary amine ions were detected serially by suppressed conductivity and then by HRAM mass spectrometry in full scan and Parallel Reaction Monitoring (PRM) positive modes. The analysis was facilitated by the Thermo Scientific™ Dionex™ Integriion HPLC compact IC system coupled with Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer.

Results: Paraquat, diquat, mepiquat, and chlormequat exhibited good peak shape with peak asymmetries from 1.0 to 1.1 As(EP) and eluted from the column within 10 min. Mepiquat and chlormequat exhibited good chromatographic resolution with Rs >2. In contrast, diquat-paraquat with the carbon isotopic masses within 2 m/z fully coeluted but were easily resolved in MS/MS by HRAM MS. The four pesticides had good accurate mass, between <2 to 4 ppm m/z normalized to the true isotopic mass. The food samples did not contain any native paraquat, diquat, mepiquat, morpholine, and chlormequat. Good accuracy was found, with recoveries of spiked in reagents in the standards and the samples within 80 to 120%. Sensitivity (LODs), was single to double digit µg/L.

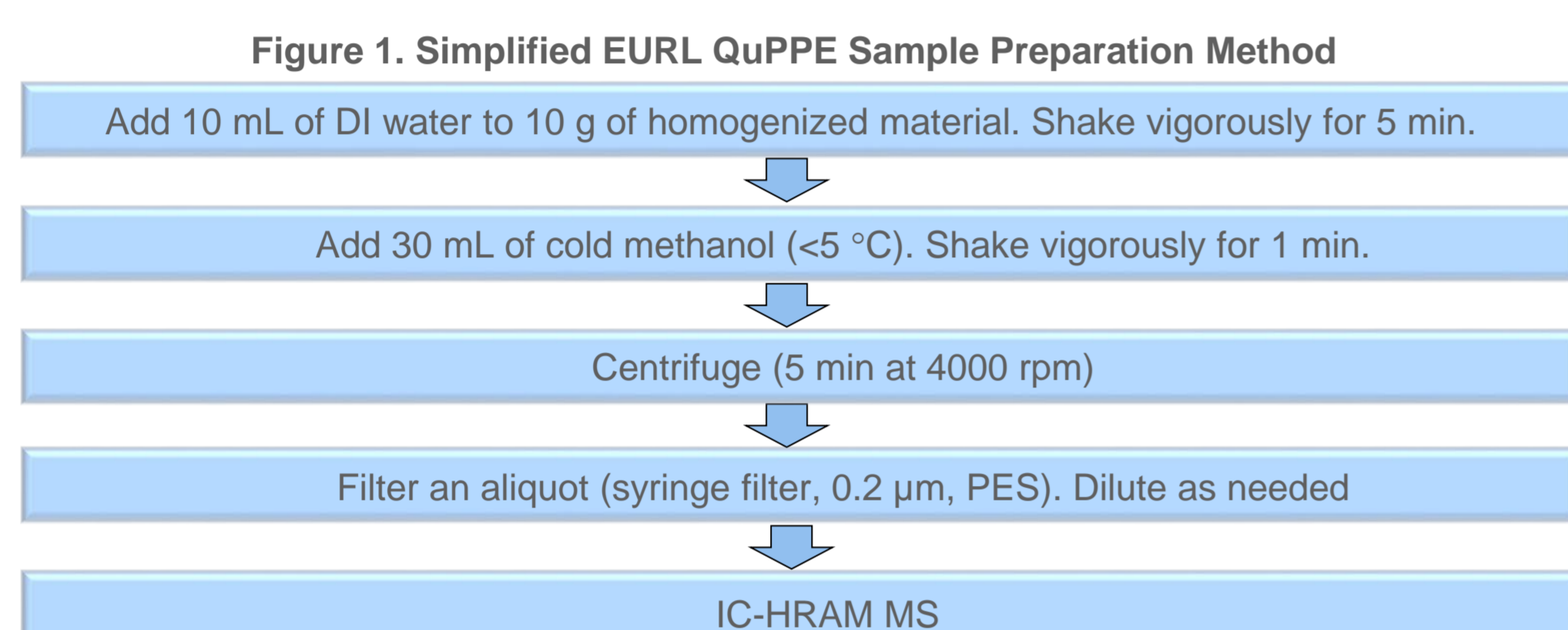
INTRODUCTION

Recently, anionic pesticides have been determined by IC-MS/MS using the Quick Polar Pesticides (QuPPE) method. However, a similar approach is lacking for cationic polar pesticides.²⁻⁵ Robust, sensitive analytical methods are needed to determine paraquat, diquat, and the related quaternary amine pesticides chlormequat, and mepiquat due to their acute and chronic toxicity. Paraquat and diquat are herbicides applied on crops and waterways, whereas chlormequat and mepiquat primarily function as growth regulators to improve fruiting and yields in grain crops. HPLC separations of these compounds often yield poor peak shapes and required ion-pairing reagents to achieve adequate peak shape. Quaternary amines are cationic and therefore best suited to CEX. Here we demonstrate direct determinations of quaternary amines in food products using CEX with serial detection by suppressed conductivity and high-resolution accurate mass spectrometry (HRAM MS) in full scan and MS/MS modes.

MATERIALS AND METHODS

Sample Preparation

Homogenized green bean, green pea, squash, prune, pear, and mixed apple-blue berry samples were prepared according to the EURL QuPPE extraction method and diluted 10-fold with deionized water (DI) prior to analysis (Figure 1).



Instruments

Dionex Integriion HPLC Compact system, Reagent-Free™ IC (RFIC™) model, with CD Conductivity Detector, and Thermo Scientific™ Dionex™ AS-AP autosampler

Thermo Scientific Q Exactive Focus Hybrid Quadrupole-Orbitrap mass spectrometer with HESI II probe

Figure 2 shows the IC-HRAM MS flow diagram.

Conditions

Ion Chromatography	
Column	Dionex IonPac CS17, 2 x 250 mm
MSA Gradient Separation	100 µL injection Equilibrate for 4 min at 2 mM MSA, 2 to 6.4 mM (0.1-2 min), 6.4 to 30 mM (2-5 min), 30 to 60 mM (5-7 min), 60 mM (7-9 min), 10 mM (9.0-10 min)
Flow Rate	0.40 mL/min, 10 min run
Eluent Source	Dionex EGC 500 MSA eluent generator cartridge, Dionex CR-CTC 600 trap column
Separation Temperatures	Column: 40 °C; CD Detector: 35 °C, Detector compartment: 20 °C
First Detection	Suppressed conductivity (CD), Dionex CERS 500e suppressor, 2 mm, 77 mA, external water mode at 0.5 mL/min delivered by an auxiliary pump
IC-MS interface	IC-MS Installation kit, acetonitrile desolvation solvent at 0.23 mL/min delivered by a second auxiliary pump
High Resolution Mass Spectrometry	
MS Detection	+ESI, 3.5V, HESI II, full scan, Parallel Reaction Monitoring MS/MS (PRM)
Gas	Sheath: 40, Aux: 5, Sweep: 1 Arb
Temperature	Capillary: 425 °C, Ion Transfer: 260 °C
Full scan	50-300 m/z, AGC 1e6, MIT: 100 mS, resolution: 30,000
PRM	AGC 2e5, MIT: 100 mS, 30,000 resolution, fixed first mass: 50.0 m/z, NCE 10-140V, inclusions list (Table 1)

Data Analysis

Thermo Scientific™ Xcalibur™, Thermo Scientific™ TraceFinder™

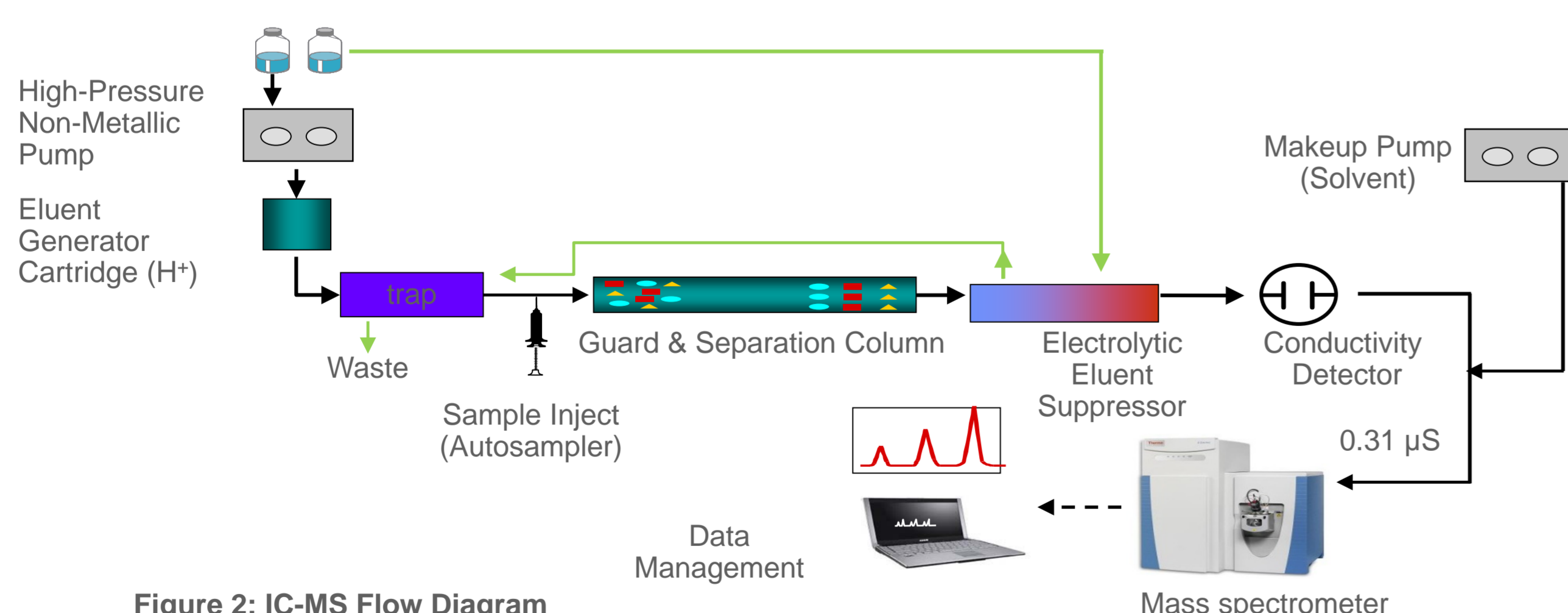


Table 1. Ions of interest and PRM conditions

Formula	Retention time (min)	Collision Energy (V)	PRM Window (min)	Formula
Trimethylsulfonium (TMS)	C3H9S+	5.18	50	3-6
Morpholine	C4H9NO	4.84	140	3-6
Mepiquat	C7H16N+	6.31	120	3-6
Chlormequat	C5H13CIN+	5.91	70	6-9
Diquat	C12H12N2+2	8.04	50	6-9
Paraquat	C12H14N2+2	8.05	30	6-9
ISTD d8-Paraquat	C12H6D8N2+2	8.05	50	6-9

RESULTS

Parallel Reaction Monitoring (PRM) Results

Figure 3 shows that trimethylsulfonium, morpholine, mepiquat, and chlormequat were chromatographically resolved and diquat and paraquat.

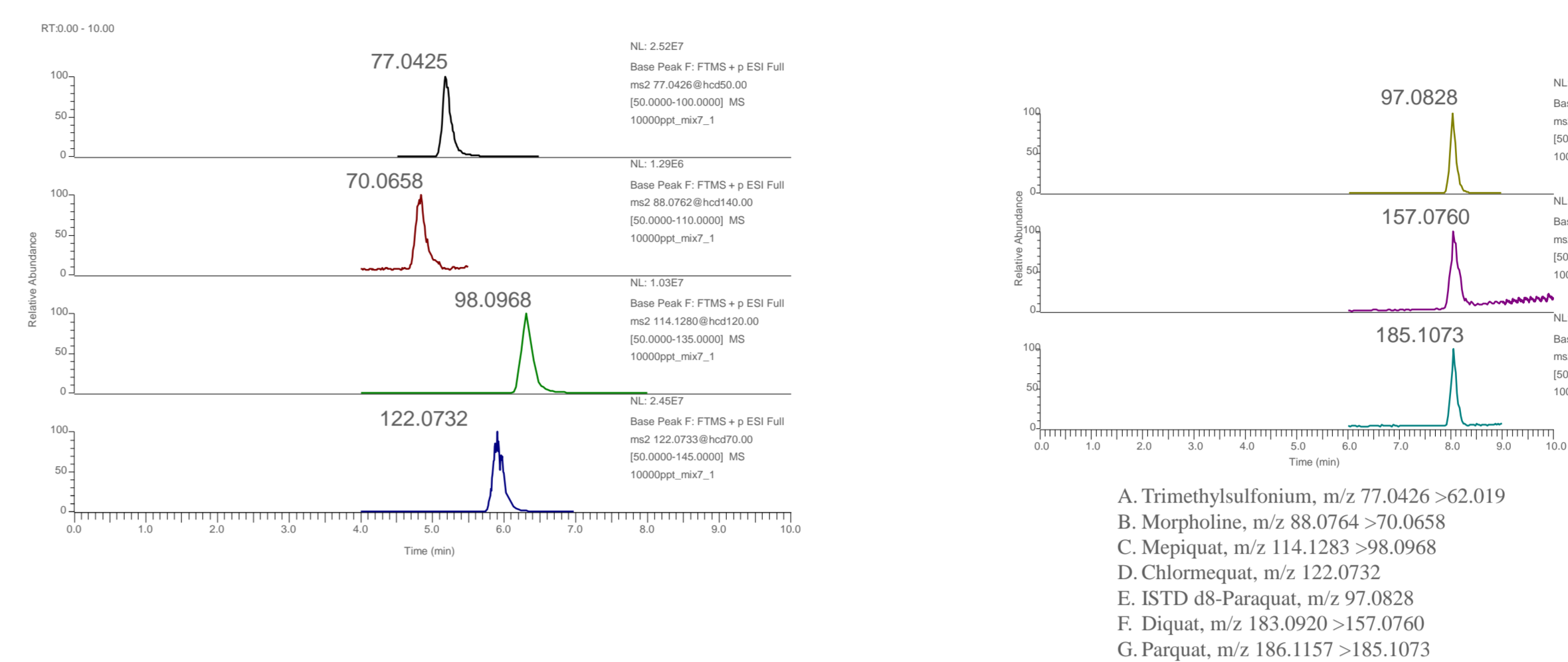


Figure 3. Chromatograms of ISTD and Six Cationic Pesticides Using PRM Mode

Table 2 shows that the accurate mass results are well within the EURL SANTE requirements.⁶ Figures 4 and 5 demonstrate the HRAM MS resolution of diquat and paraquat in 10x diluted (QePPE extracted) green beans and pear samples.

Table 2. Accurate Mass results of ISTD and six cationic pesticides using PRM mode

Precursor Ion	Confirming Ions ⁶	Accurate mass	Measured	SANTE ⁶ (< 5 ppm)
Chlormequat	124.070 58.066	122.0736	122.0736	--
Diquat	157.076 143.081	183.0920	183.0917	-1.6 ppm
Mepiquat	98.097 58.066	114.1283	114.1280	-2.6 ppm
Morpholine	70.066 68.050	88.0764	88.0763	-1.0 ppm
D8-Paraquat	97.5844 96.5797	97.0832	97.0828	-4.2 ppm
Paraquat	185.107 171.092	186.1146	186.1157	+0.5 ppm
TMS	62.019 61.011	77.0043	77.0043	--

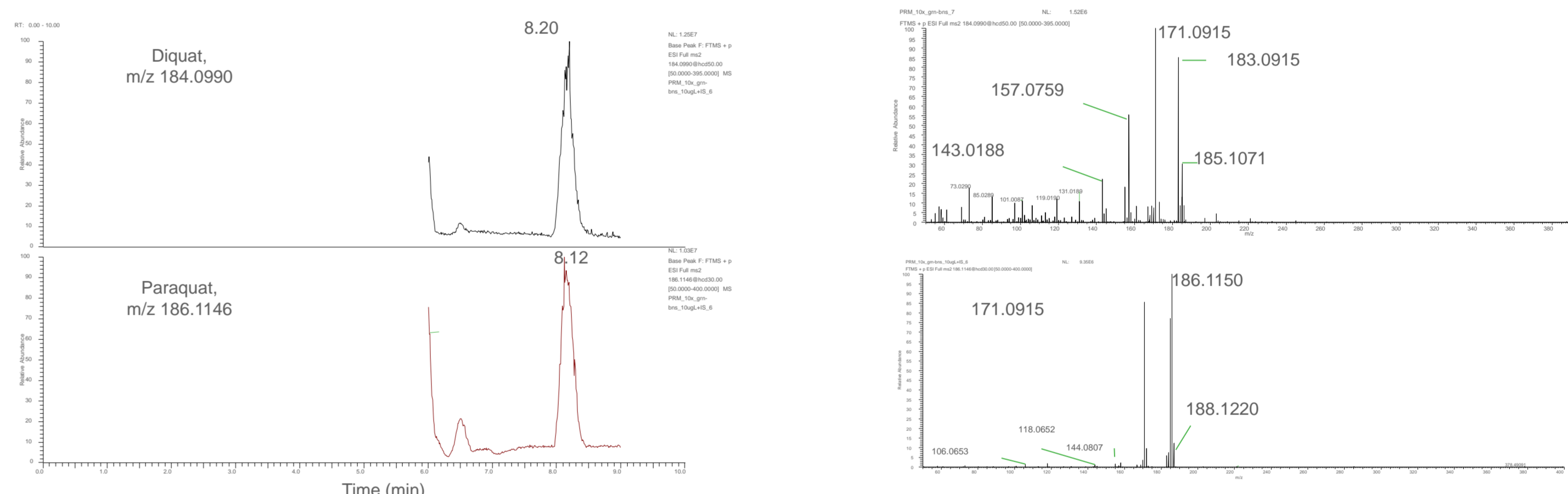


Figure 4. PRM chromatograms and spectra: Resolution of 20 µg/L diquat from 20 µg/L paraquat in QePPE extracted green bean sample

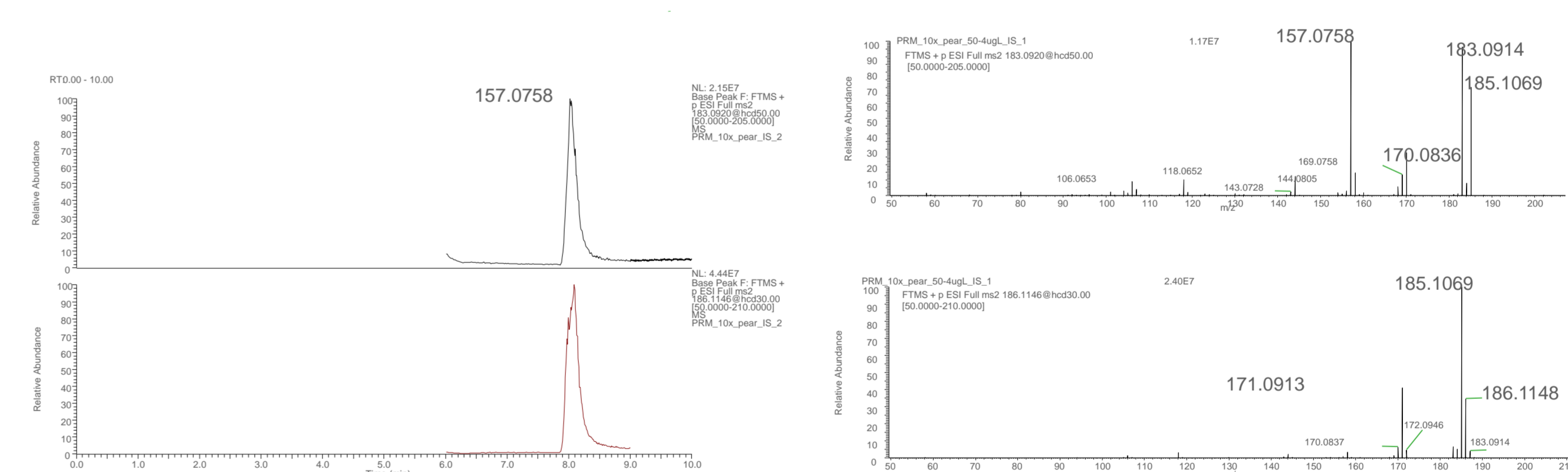


Figure 5. 10-fold diluted, QePPE extracted, pear sample

CONCLUSIONS

- Fast determinations of six quaternary amine pesticides (TMS, morpholine, mepiquat, chlormequat, diquat, and paraquat) in homogenized fruit and vegetable samples were demonstrated by IC-HRAM MS.
- TMS, morpholine, mepiquat, and chlormequat pesticides were chromatographically separated, whereas diquat and paraquat were resolved by accurate mass spectrometry.
- The JM0006 prototype column improved the chromatographic resolution of diquat and paraquat but more column development is needed.

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TRADEMARKS/LICENSING

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