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Quantitation of Paraquat Residue in Milk Using the Agilent 6470 Triple Quadrupole LC/MS

Chromatographic separation of paraquat residue from milk matrix using HILIC-Z column chemistry

Figure 1. Agilent 1290 Infinity II LC coupled to an Agilent 6470 triple quadrupole LC/MS.

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Abstract

Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) is a quaternary amine extensively used as a non-selective contact herbicide. Having moderate toxicity, paraquat run-off from application areas and its presence in food products have been major concerns for human health. The presence of paraquat residue is reported in agricultural consumer products such as milk. Using mass spectrometry for the detection of this residue provides enhanced sensitivity and shortened sample preparation compared to other analytical methods. However, the linearity, accuracy, and reproducibility of the result in matrix is heavily dependent on the chromatographic separation of paraquat from the sample matrix components. This study describes a high-performance LC/MS method for sensitive and selective quantitation of paraquat in milk matrix. Chromatography was set in such a way that paraquat was well retained yet well resolved from endogenous sample matrix by using Agilent InfinityLab Poroshell 120 HILIC-Z column chemistry. This approach avoids the use of strong ion pair agents, drastically simplifying the analysis. The method is demonstrated to be highly sensitive, reproducible, and shows good and consistent recoveries over multiple batches.

Introduction

Milk is regarded as a complete food because of its rich nutrient density, containing a good mix of protein, fat, carbohydrates, vitamins, and essential minerals for maintaining good health. Due to the widespread consumption of milk, pesticides such as paraquat in agricultural consumer products are a concern because of their moderate toxicity levels (Figure 2).
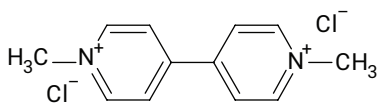

Figure 2. Chemical structure of paraquat.

A QuEChERS-based extraction of pesticide residue from milk matrix is found to be a very easy sample preparation to adopt. A highly selective multiple reaction monitoring (MRM)-based LC/MS/MS method was developed using an Agilent 6470 triple quadrupole LC/MS (LC/TQ).

Zwitterionic chemistry with a proprietary bonding technique in HILIC-Z columns offers powerful separation, stability across a wide pH range, and excellent peak shape compared to traditional bare silica phase HILIC columns.

Experimental

Chemicals and reagents

LC/MS pesticide standard was purchased from Sigma-Aldrich. This standard was used for method development and analysis of milk samples. Agilent Bond Elut QuEChERS extraction kit (part number 5982-5650) and dispersive kit (part number 5982-5156CH) were used for sample preparation. LC/MS-grade solvents such as acetonitrile and water were purchased from Honeywell (Charlotte, NC, USA). Ammonium formate was purchased from Sigma-Aldrich (now of Merck). Formic acid, MS grade was purchased from Fluka (now of Honeywell).

Instrument configuration

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 6470 triple quadrupole LC/MS (G6470B)

Table 1. Chromatography conditions.

Parameter	Value
Mobile Phase A	20 mM ammonium formate in water at pH = 3 (pH adjusted with formic acid)
Mobile Phase B	20 mM ammonium formate in water/acetonitrile (10/90)
Flow Rate	0.1 mL/min
Injection Volume	20 µL
Column Temperature	35 °C
Sample Diluent	Acetonitrile/water (60/40)
Needle Wash	MeOH/acetonitrile/water (25/50/25)
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (p/n 685775-924)

Table 2. Gradient.

Time (min)	%A	%B
0	20	80
1	20	80
5	27	73
6	20	80
8	20	80
Postrun	2 minutes	

Table 3. MS source parameters.

Parameter	Value
Ionization Source	AJS ESI
Ionization Mode	ESI Positive
Gas Temperature	250 °C
Gas Flow	7 L/min
Nebulizer	30 psi
Sheath Gas	390 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	3,500 V
Nozzle Voltage	0 V

Table 4. MRM parameters.

Compound ID	Precursor m/z	Product m/z	Fragmentor (V)	CE	CAV	Ionization
Paraquat	171	155	158	36	4	ESI Positive
	171	103	158	32	4	ESI Positive
	171	77.1	158	44	4	ESI Positive
	171	51.2	158	60	4	ESI Positive

Sample preparation

```
Add 10 mL of acetonitrile to 10 mL of milk.
            ↓
Vortex for 1 minute.
            ↓
Add QuEChERS kit (p/n 5982-5650).
            ↓
Shake vigorously for 1 minute.
            ↓
Centrifuge at 6,000 rpm for 6 minutes.
            ↓
Transfer the supernatant to a 15 mL dSPE tube
(p/n 5982-5156CH).
            ↓
Vortex for 1 minute.
            ↓
Centrifuge at 8,000 rpm for 8 minutes.
            ↓
Transfer the supernatant to a 2 mL vial.
```
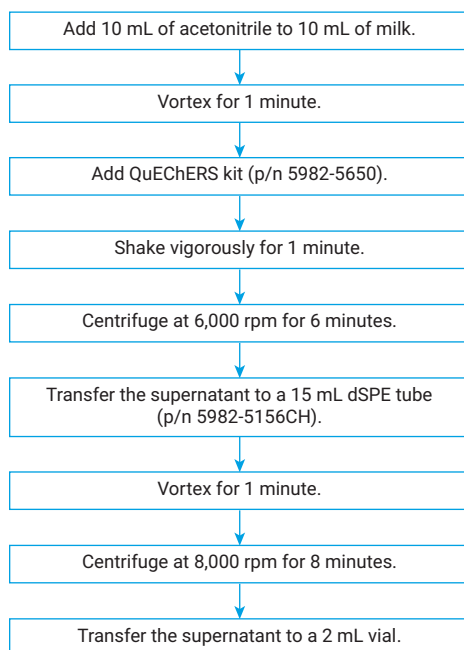
Figure 3. Flowchart for sample preparation.

Data acquisition and data analysis

All samples were acquired using the Agilent MassHunter data acquisition software version 10.1. Chromatograms were viewed through Agilent MassHunter qualitative analysis software version 10.0. Quantitation of each batch was carried out using Agilent MassHunter quantitative analysis software version 10.1.

Results and discussion

Individual stock solutions were made and mixed to appropriate volumes of stock solutions to make working standards. Working standards prepared were of concentrations 10 and 1 ppm. Individual volumes of working standards are spiked in 10 mL of milk samples and followed the sample preparation to generate procedural standard calibration (prespike) points.

Table 5. Dilution chart for prespike calibration curve.

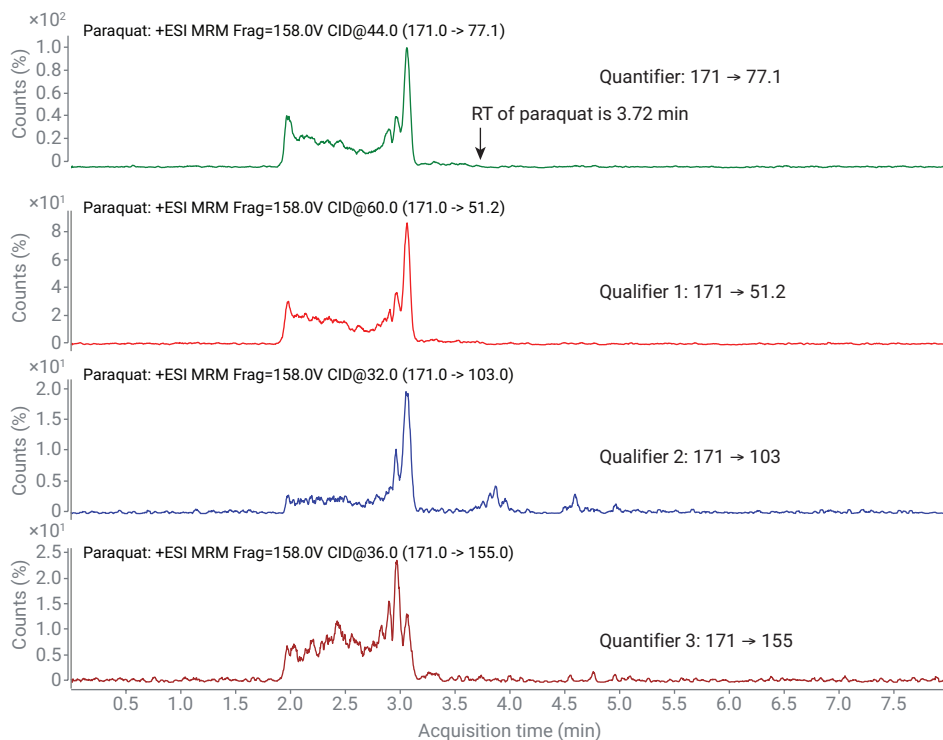| Working Standard Concentration (ppm)	Volume Taken (µL)	Volume of Milk (mL)	Obtained Concentration (ng/mL)
1	50	10	5
1	100	10	10
1	200	10	20
10	50	10	50
10	100	10	100
10	200	10	200

Figure 4. Extracted ion chromatogram (EIC) from blank milk matrix. Chromatograms showing matrix interference between 2 to 3 minutes.

Procedural standard calibration curves were made and found to be linear from 5 to 200 ng/mL with both linear regression and 1/x (Figure 6). Regression coefficient values were above 0.9950.

Spike levels were determined as per the maximum residue limits mentioned in BIS (Bureau of Indian Standards) regulation. Recovery% was calculated based on this experiment. Four spike samples were prepared at 10 ng/mL and injected in six replicates.

10 ppb = 10 ng/mL of milk sample pesticide residue absolute in 10 mL would be 100 ng. Therefore, 100 µL from 1 ppm needs to be spiked in 10 mL of milk sample.
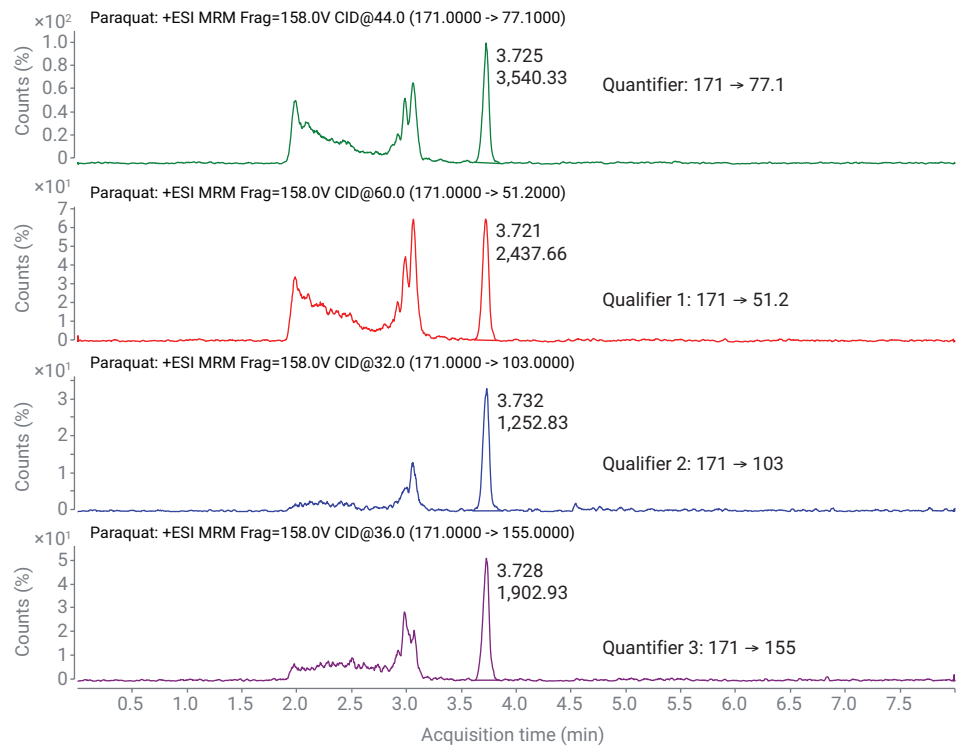

Figure 5. Extracted ion chromatogram (EIC) from procedural calibration standard at 10 ng/mL in milk matrix. Chromatograms showing a clear separation between matrix components and analyte.
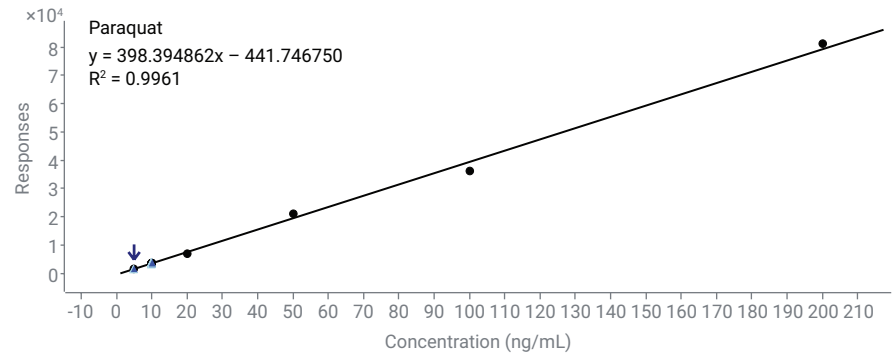

Figure 6. Procedural standard calibration curves from 5 to 200 ng/mL.

Batch Table

Sample: ⌃ MATRIX BLANK ▾ ⌄ Sample Type: <All> ▾ Compound: ⟨ PARAQUAT ▾ ⟩ ISTD:

		Sample			PARAQUAT...	PARAQUAT Results					Qualifier (171.0 -> 155...)		Qualifier (171.0 -> 103...)		Qualifier (171.0 -> 51.2...)	
⚠	▽	Name	Type	Level	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Accuracy	Ratio	MI	Ratio	MI	Ratio	MI
⚠		SOLVENT BLANK	Blank			3.576	8	☐	1.12811532		62.7	☐	57.6	☐		☐
⚠		MATRIX BLANK	MatrixBlank			3.714	24	☐	1.17023590			☐	60.8	☐		☐
⚠		MATRIX BLANK	MatrixBlank			3.694	32	☐	1.18887582			☐		☐	126.4	☐
		MB 5 PPB	Cal	1	5.00000000	3.728	1616	☐	5.16497360	103.3	51.1	☐	23.0	☐	70.7	☐
		MB 10 PPB	Cal	2	10.00000000	3.732	3532	☐	9.97397532	99.7	55.5	☐	34.9	☐	71.5	☐
		MB 10 PPB	Cal	2	10.00000000	3.725	3574	☐	10.07974127	100.8	51.5	☐	34.9	☐	68.1	☐
		MB 10 PPB	Cal	2	10.00000000	3.728	3552	☐	10.02425245	100.2	54.6	☐	32.9	☐	70.4	☐
		MB 10 PPB	Cal	2	10.00000000	3.725	3630	☐	10.21961238	102.2	52.3	☐	34.2	☐	66.9	☐
		MB 10 PPB	Cal	2	10.00000000	3.728	3567	☐	10.06315914	100.6	55.2	☐	33.7	☐	67.7	☐
		MB 10 PPB	Cal	2	10.00000000	3.728	3577	☐	10.08765126	100.9	52.2	☐	31.8	☐	64.4	☐
		MB 10 PPB	Cal	2	10.00000000	3.728	3491	☐	9.87230830	98.7	55.3	☐	33.2	☐	69.5	☐
		MB 10 PPB	Cal	2	10.00000000	3.728	3529	☐	9.96707273	99.7	53.9	☐	31.7	☐	68.7	☐
		MB 20 PPB	Cal	3	20.00000000	3.732	6843	☐	18.28420486	91.4	55.8	☐	33.4	☐	69.2	☐
		MB 50 PPB	Cal	4	50.00000000	3.725	20971	☐	53.74810549	107.5	52.8	☐	33.1	☐	68.0	☐
		MB 100 PPB	Cal	5	100.000000...	3.728	36329	☐	92.29618552	92.3	52.3	☐	32.7	☐	68.4	☐
		MB 200 PPB	Cal	6	200.000000...	3.728	81316	☐	205.218757...	102.6	52.0	☐	31.7	☐	67.8	☐
⚠		SOLVENT BLANK	Blank			3.770	24	☐	1.16948037			☐	12.9	☐	180.3	☐
⚠		SOLVENT BLANK	Blank			3.628	8	☐	1.12829932			☐	13.4	☐		☐
		MATRIX BLANK	MatrixBlank			3.721	20	☐	1.15959948		150.3	☐	41.4	☐	144.3	☐
⚠		MATRIX BLANK	MatrixBlank ⌄			3.718	15	☐	1.14695631		48.2	☐	297.6	☐		☐
		BRACKETING STD	QC	1	5.00000000	3.728	1543	☐	4.98309177	99.7	56.5	☐	35.7	☐	72.5	☐
		BRACKETING STD	QC	1	5.00000000	3.728	1505	☐	4.88575379	97.7	58.9	☐	34.4	☐	73.9	☐
		SPIKE 1-1	QC	2	10.00000000	3.725	3506	☐	9.90841182	99.1	51.0	☐	34.6	☐	68.9	☐
		SPIKE 1-1	QC	2	10.00000000	3.725	3744	☐	10.50758374	105.1	52.6	☐	33.9	☐	66.1	☐
		SPIKE 1-1	QC	2	10.00000000	3.725	3641	☐	10.24684913	102.5	52.7	☐	32.6	☐	67.5	☐
		SPIKE 1-1	QC	2	10.00000000	3.728	3542	☐	10.00009885	100.0	55.9	☐	32.4	☐	67.8	☐
		SPIKE 1-1	QC	2	10.00000000	3.728	3560	☐	10.04435924	100.4	51.2	☐	36.2	☐	72.2	☐
		SPIKE 1-1	QC	2	10.00000000	3.725	3617	☐	10.18743517	101.9	51.8	☐	34.4	☐	65.8	☐
		SPIKE 1-2	QC	2	10.00000000	3.728	3710	☐	10.42198936	104.2	53.5	☐	33.7	☐	68.9	☐
		SPIKE 1-2	QC	2	10.00000000	3.725	3577	☐	10.08806116	100.9	54.2	☐	37.6	☐	69.9	☐
		SPIKE 1-2	QC	2	10.00000000	3.728	3518	☐	9.94033772	99.4	57.8	☐	32.8	☐	70.5	☐
		SPIKE 1-2	QC	2	10.00000000	3.728	3660	☐	10.29570883	103.0	55.0	☐	34.5	☐	68.3	☐
		SPIKE 1-2	QC	2	10.00000000	3.728	3566	☐	10.06056524	100.6	54.3	☐	33.3	☐	68.7	☐
		SPIKE 1-2	QC	2	10.00000000	3.725	3551	☐	10.02289310	100.2	52.8	☐	33.6	☐	71.0	☐
		SPIKE 1-3	QC	2	10.00000000	3.718	4027	☐	11.21807930	112.2	50.9	☐	32.8	☐	70.3	☐
		SPIKE 1-3	QC	2	10.00000000	3.718	3817	☐	10.69052749	106.9	52.8	☐	32.2	☐	67.4	☐
		SPIKE 1-3	QC	2	10.00000000	3.718	3622	☐	10.19949171	102.0	55.4	☐	35.5	☐	70.4	☐
		SPIKE 1-3	QC	2	10.00000000	3.718	3784	☐	10.60788915	106.1	55.4	☐	34.6	☐	67.3	☐
		SPIKE 1-3	QC	2	10.00000000	3.721	3846	☐	10.76191930	107.6	52.5	☐	34.3	☐	69.7	☐
		SPIKE 1-3	QC	2	10.00000000	3.721	3600	☐	10.14402026	101.4	52.3	☐	35.1	☐	73.5	☐
		SPIKE 1-4	QC	2	10.00000000	3.721	3732	☐	10.47674266	104.8	50.9	☐	32.5	☐	70.1	☐
		SPIKE 1-4	QC	2	10.00000000	3.718	3724	☐	10.45714244	104.6	58.1	☐	36.7	☐	69.6	☐
		SPIKE 1-4	QC	2	10.00000000	3.714	3770	☐	10.57094762	105.7	55.6	☐	35.1	☐	67.9	☐
		SPIKE 1-4	QC	2	10.00000000	3.718	3667	☐	10.31215809	103.1	53.7	☐	35.9	☐	70.0	☐
		SPIKE 1-4	QC	2	10.00000000	3.714	3435	☐	9.73073688	97.3	59.7	☐	39.4	☐	73.1	☐
		SPIKE 1-4	QC	2	10.00000000	3.718	3700	☐	10.39485800	103.9	58.9	☐	35.2	☐	72.4	☐

Figure 7. Calibration table showing the accuracy, recovery, and MRM ion ratio of paraquat in procedural calibration standards, bracketing standards, and spike samples.

All four spike samples and their six replicate injections showed a recovery% that varies between 97% and 112% (Figure 8).
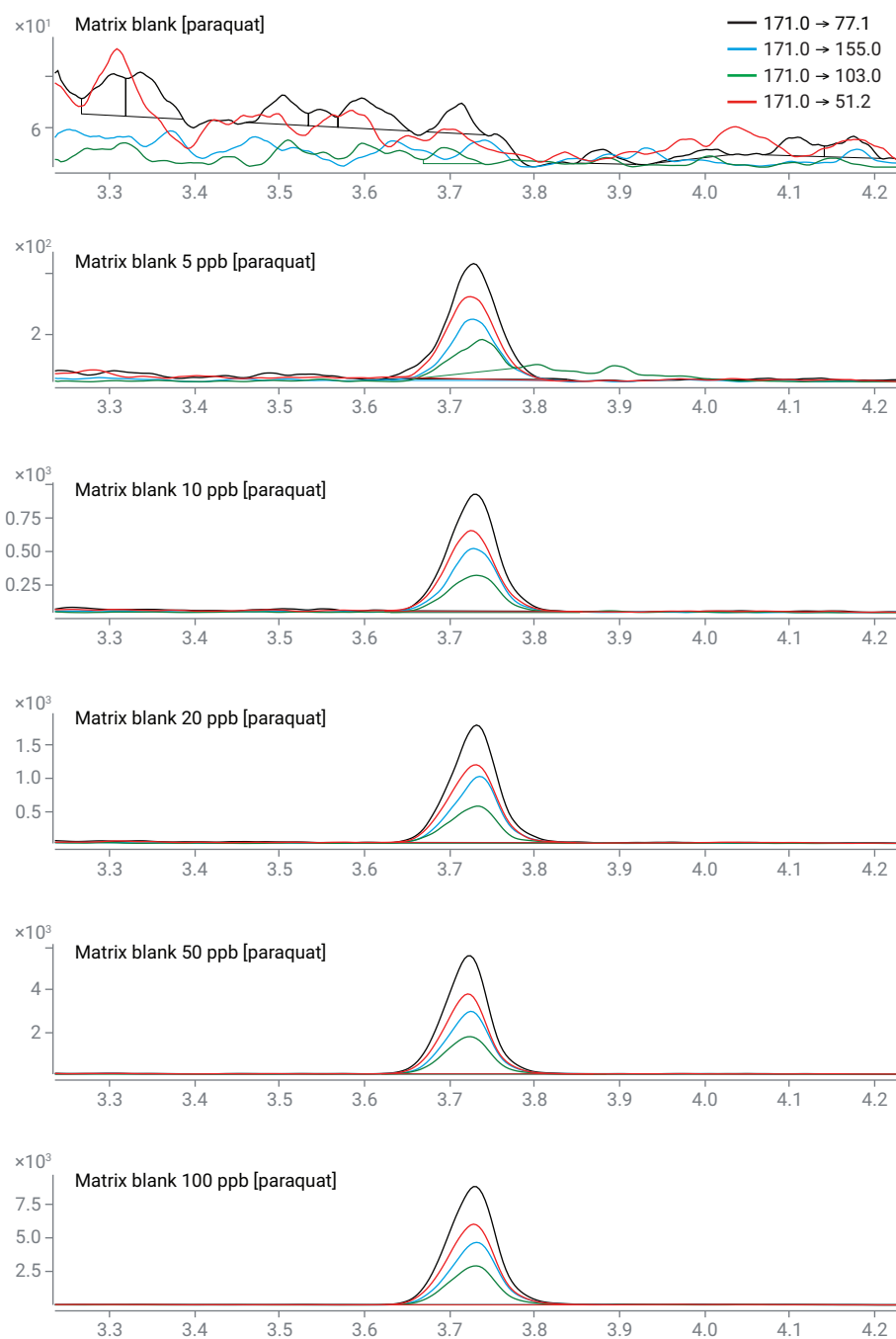

Figure 8. Compounds-at-a-glance feature showing chromatograms of quantifier and qualifiers of calibration standards used in matrix-based linearity.
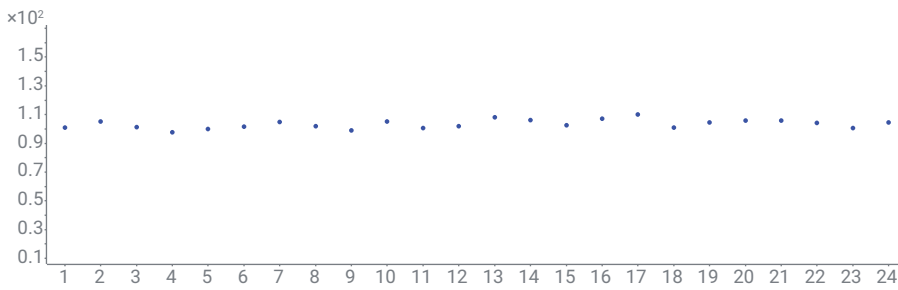
Figure 9. Matrix plot of recovery% of four spike samples prepared and injected in six replicates.
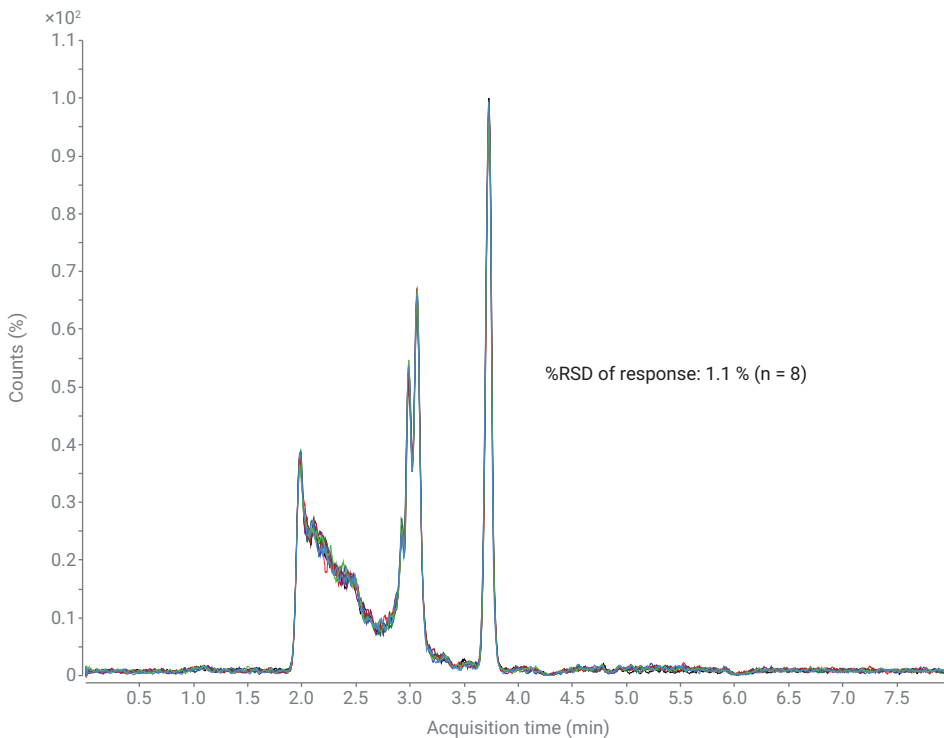

%RSD of response: 1.1 % (n = 8)

Figure 10. Overlaid total ion chromatogram of paraquat in procedural calibration standard at 10 ng/mL in milk sample showing %RSD of 1.1% (n = 8).

Conclusion

A highly sensitive, selective, and reproducible method based on MRM was developed to quantify paraquat residue in milk matrix. QuEChERS-based extraction and dSPE-based sample cleanup were adopted for sample preparation. Chromatography based on HILIC-Z chemistry gave good separation of matrix components from the analyte, which is critical to achieve the method performance. Acceptable recovery was obtained, with consistent results at the maximum residue limit. This method can be adopted for routine analysis of milk samples for the quantitation of paraquat residue.

References

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