TDL.ORG TEXAS DIGITAL LIBRARY

Journal of Regulatory Science

http:\\journalofregulatoryscience.org

Regulatory Science

Journal of Regulatory Science 05 (2017) 1–8

Determination of Paraquat and Diquat in Potato by Liquid Chromatography/Tandem Mass Spectrometer

Narong Chamkasem[a,*], Cynthia Morris[a], Tiffany Harmon[a]

[a]*Southeast Regional Laboratory, U.S. Food and Drug Administration, 60 Eighth Street NE, Atlanta, GA, 30309*

Abstract

A high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of paraquat and diquat in potatoes using a reversed-phase with weak anion-exchange and cation-exchange mixed-mode Acclaim™ Trinity Q1 column. Five grams of sample was shaken with fifteen milliliters of 1:1 MeOH: 0.1 N HCl for 10 min at room temperature and heated for 15 min at 80°C. After centrifugation, the supernatant was passed through an Oasis HLB SPE to retain suspended particulates and non-polar interferences. The sample was directly injected and analyzed for 10 min by LC-MS/MS with no sample concentration. Two multiple reaction monitoring (MRM) channels were monitored in the method for each target compound to achieve true positive identification. Two internal standards corresponding to each analyte were used to counter matrix suppression effect. Linearity of the detector response with a minimum coefficient of determination (r^2) of more than 0.997 was demonstrated in the range from 2 to 250 ng/mL for each analyte using matrix-matched standard. The average recovery for all analytes at 10, 25, 100, and 500 ng/g (n=5) are between 87-106% with a relative standard deviation of less than 12%.

Keywords: paraquat, diquat, potatoes, LC-MS/MS, determination

1. Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride), and diquat (1,1'-ethylene-2,2'-bipyridilium dibromide), are non-selective and nonsystematic contact herbicides widely used in agriculture to control broadleaf and grassy weeds. Usually, no significant residues are found in the crops when they are used as pre-emergence herbicides. However, when they are used as pre-harvest desiccants, residues can occur in the plant through translocation from the treated leaves to the roots such as in potatoes. Paraquat is considered one of the most toxic herbicides in the world [21, 16]. Paraquat can cause damage to the liver, lungs, and kidneys [5, 11]. It is a banned substance in the European Union; however, it is still applied in at least 90 countries, including Brazil and China [25]. Diquat is a non-selective herbicide that acts quickly to damage only those parts of the plant to which it is applied. It has been used in pre-harvest crop desiccation to facilitate mechanical harvest of many crops including potatoes, cereal, canola, soya, and alfalfa [15]. The tolerance of paraquat and diquat for potatoes in the U.S. are 0.5 and 0.1 μg/g, respectively [23].

Several methods and techniques including gas chromatography [2], capillary electrophoresis [24], and enzyme-linked immunoassay [19] have been used for paraquat and diquat in food and environmental samples. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) was used to determine both analytes in liquid samples to simplify the methodology and improve selectivity/sensitivity [6, 8]. The high polarity of paraquat rendered poor retention on conventional C18 columns. Ion-pairing reagent can be used to increase the retention, but it leads to signal suppression and low sensitivity when coupled with mass spectrometric detectors [4]. Hydrophilic Interaction Liquid Chromatography (HILIC) columns showed promising retention of polar compounds like quaternary ammonium [1, 18], but a buffer with high salt concentration (150-250 mM ammonium formate) was required to maintain optimal performance of the column. This high salt concentration affects the LC/MS method sensitivity due to ion-suppression.

Obelisc R columns were used to improve sensitivity of these analytes in food [14, 17]. These columns had poor efficiency and produced a broad peak shape of paraquat and diquat. A Syncronis HILIC column was used to determine paraquat in fruit and vegetables [26]. This method requires an optimal pH of the sample solution (pH 4-5) to obtain good peak shape and a very small injection volume (less than 2 uL) must be used to maintain a sharp peak shape. A mixed-mode Acclaim™ Trinity Q1 (Thermo Scientific) has reversed-phase, cation and anion exchange mechanisms that can retain ionic compounds as well

*Corresponding author: Narong Chamkasem, Phone: 404-253-2302. Fax: 404-253-1209. Email: narong.chamkasem@fda.hhs.gov

as neutral ones. It was used to analyze glyphosate, glufosinate, and 2,4-dichlorophenoxy acetic (2,4-D) in soybean and corn with good peak shape and sensitivity [9, 10]. It was developed by Thermo Scientific specifically for paraquat/diquat to replace the Acclaim™ mixed-mode HILIC-1 column [22, 12]. This column is very versatile and ideal for the analysis of very polar compounds having either negative or positive charge molecules.

Aqueous extraction with acid at high temperature was used successfully to extract free and bound paraquat/diquat in food matrices [14, 26, 20]. Pizzutti *et al.* (2016) thoroughly evaluated different extraction parameters, including solvent composition, temperature, sample filtration, and matrix effects to extract paraquat and diquat in cowpea beans [17]. They reported higher relative standard deviation (> 20%) for paraquat using methanol/HCl 0.5 mol/L even with the use of isotopically labelled internal standard. This problem may be caused by a wide peak shape of the paraquat peak separated by the Obelisc R column. The purpose of this study was to develop a quick, sensitive, and selective method to determine paraquat/diquat in potatoes by combining a short extraction procedure of acidic aqueous extraction at high temperature to improve extractability and a quick sample cleanup to improve method ruggedness and reproducibility. Another important purpose was also to expand the capability of the Acclaim™ Trinity Q1 mixed-mode column as the only column for the determination of cationic compounds of paraquat and diquat as well as anionic compounds such as glyphosate, glufosinate, and 2,4-D as part of the multi residue screening methods [9, 10].

2. Material and Methods

2.1. Chemicals and Materials

Pesticide standard mixes, all ≥ 99% purity, were purchased from LGC Standards (Manchester, NH) consisting of paraquat, diquat, paraquat dichloride d6, and diquat dibromide d4 monohydrate. Methanol, acetonitrile, and water were of HPLC grade, obtained from Fisher Scientific (Pittsburgh, PA), and used for HPLC mobile phase and extracting solvent. Acetic acid was obtained as 99.8% solution for mass spectrometry from Fluka (Buchs, Switzerland.). Ammonium acetate and hydrochloric acid (HCl) were ACS grade and purchased from Fisher Scientific (Pittsburgh, PA). Extracting solvent (1:1 methanol: 0.1% hydrochloric acid) was prepared by mixing 500 mL of methanol with 500 mL of 0.1 N HCl. Oasis HLB (60 mg) solid phase extraction cartridge was obtained from Waters (Milford, MA). EDP 3 electronic pipettes at different capacity (0-10 μL, 10-100 μL, and 100-1000 μL) were purchased from Rainin Instrument LLC (Oakland, CA) and were used for standard fortification and preparation.

A solution 0.5 M ammonium acetate/formic acid (pH 4.7) was prepared as follows: 19.25 g of ammonium acetate were dissolved in approximately 300 mL of water and adjust the pH with acetic acid (approx. 13.4 mL) until the pH was 4.7 (using pH meter), and the solution was adjusted to 500 mL with water. The HPLC mobile phase was prepared by mixing 350 mL of acetonitrile with 100 mL of water and 50 mL of 0.5 M acetate buffer.

2.2. Standard Preparation

The standard solutions of paraquat and diquat at 100, 10, and 1 μg/mL were prepared by dissolving the stock standard in 1:1 water:methanol solution. The solutions were maintained at 4°C in stocked polypropylene tubes to avoid glass adsorption. The internal standard (IS) solution of paraquat dichloride d6, and diquat dibromide d4 monohydrate at 1 and 10 μg/mL were prepared by dissolving the stock standard in 1:1 water: methanol solution. The calibration standards (at 2 to 250 ng/mL) were prepared in the extracting solvent and blank matrix extract (after the SPE cleanup) with IS solutions at 50 ng/mL for the calibration curves. These two calibration curves were used to quantify the analyte concentrations in the samples.

2.3. Sample Preparation and Extraction Procedure

Russet and yellow potatoes were obtained from a local market. The samples were ground using a food processor until a smooth texture was achieved. Five-gram samples were weighed out and placed into 50-mL centrifuge tubes (Fisher Scientific, Pittsburgh, PA), which were all fortified with native standard solutions at 10, 25, 100, and 500 ng/g (5 replicates). The samples were allowed to stand at room temperature for 1 hour and then stored in a freezer for two days to let the analytes be absorbed by the sample. A set of five non-fortified samples were also prepared and used for matrix matched standard. On the extraction day, the spiked samples were allowed to thaw to room temperature. The IS solution (95 μL) at the final concentration of 10 μg/mL was added into the sample so the concentration was 50 ng/mL for all samples. The extracting solvent (15 mL) was added to each tube using an automatic pipette. The tubes were capped tightly and shaken for 10 min on a SPEX 2000 Geno grinder (SPEX Sample Prep LLC, Metuchen, NJ) at 2,000 stroke/min. The sample tubes were heated in a water bath at 80°C for 15 min and immediately shaken for another 2 min on the Geno grinder. The sample tubes were cooled to room temperature and centrifuged at 3,000 x g for 5 min using a Q-Sep 3000 centrifuge (Restek, Bellefonte, PA). Three milliliters of the supernatant were passed through an Oasis HLB cartridge (no conditioning is required) into an autosampler vial. Ten microliters of sample was injected into the LC-MS/MS system. The calibration curves made of solvent and matrix blank were used to quantify the analyte concentration.

2.4. LC-MS/MS Analysis

LC-MS/MS analysis was performed by using a Shimadzu HPLC system. The instrument was equipped with two LC-20AD Pumps, a Sil-20AC autosampler, and a CTO-20AC column oven (Shimadzu, Kyoto, Japan), coupled with a 5500 Q-TRAP mass spectrometer from AB SCIEX (Foster City, CA). The Analyst software (version 1.6) was used for instrument control and data acquisition. Nitrogen and air from TriGas Generator (Parker Hannifin Co., Haverhill, MA) were used for nebulizer and collision gas in LC-MS/MS. An Acclaim™ Trinity Q1 (3 μm, 100 x 3 mm) from Thermo Scientific (Sunnyvale, CA) and a C18 SecurityGuard guard column (4 x 3 mm) from Phenomenex (Torrance, PA) were used for HPLC separation at

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	DP [a]	CE [b]	EP [c]	CXP [d]	Retention Time (min)
Paraquat. 1	186	171	30	28	10	28	4.5
Paraquat. 2	185	170	30	28	10	28	4.5
Paraquat Dichloride d6 (IS)	192	174	30	28	10	28	4.5
Diquat. 1	183	157.2	160	30	10	30	5.5
Diquat. 2	183	168.2	160	30	10	30	5.5
Diquat Dibromide d4 (IS)	186.2	158.2	160	32	10	10	5.5

[a]DP = declustering potential
[b]CE = collision energy
[c]EP = entrance potential
[d]CXP = collision exit potential

Figure 1: Retention time and MRM conditions for LC/MS analysis.

35°C with sample injection volume of 10 μL. The mobile phase is acetonitrile:water 7:3 containing 50 mM ammonium acetate (pH 4.7) at a flow rate of 0.5 mL/min for a total run time of 10 min. A diverter valve was activated to direct the HPLC mobile phase from the column to waste from 0 to 3 min then switched to the ion source for 4 minutes before being switched to waste for the rest of the run. The MS determination was performed in a positive electrospray mode with monitoring of the two most abundant MS/MS (precursor/product) ion transitions using a scheduled MRM program for 90 seconds for each analyte. Analyte-specific MS/MS conditions and LC retention time for the analytes are shown in Figure 1. The MS source conditions were as follows: curtain gas (CUR) of 30 psi, ion spray voltage (ISV) of 5500 volts, collisionally activated dissociation gas (CAD) was high, nebulizer gas (GS1) of 60 psi, heater gas (GS2) of 60 psi, source temperature (TEM) of 600°C.

3. Results and Discussion

3.1. Chromotography Optimization

Paraquat and diquat both possess positive charges in an aqueous solution, which makes it difficult to be retained by a reversed-phase column. The Acclaim^{TM} Q1 is a mixed-mode that contains weak cation and weak anion exchange active sites that make it suitable for the analysis of ionic compounds. It was used successfully to analyze glyphosate, a strong anionic compound, in soybean and corn [9]. Paraquat and diquat are strong cationic compounds and are moderately retained and separated on this column. Thermo Scientific developed this column specifically for these analytes [22]. This column is very versatile and very useful for the analysis of anionic or cationic compounds.

During the method development stage, different mobile phase parameters were evaluated, which included pH (2.8 to 5), acetonitrile concentration (0-100%), and salt concentration (0-100 mM). The most important parameter was the pH of the mobile phase. At a pH less than 3, both paraquat and diquat are strongly retained on the column because the weak cation exchange sites are fully ionized. At a higher pH (between 4 and 5), the column is less retentive and allows paraquat and diquat to be eluted with a moderate salt concentration solution. High salt concentration shortened the retention time of the analytes (at pH between 4 and 5) and decreased analyte response due to ion-suppression. Acetonitrile enhanced the retention of the analyte similar to the HILIC mode (Figure 2). It also increases the analyte response by reducing the sample droplets surface tension and increasing evaporation efficiency. All three of these parameters must be chosen appropriately to achieve the optimum separation and peak sensitivity for the target analytes. It was found that the mobile phase containing 7:3 acetonitrile water with 50 mM ammonium acetate at pH 4.7 produced the optimum condition for peak shape, retention time, and sensitivity of paraquat and diquat.

3.2. Evaluation of Matrix Effects

Sample matrix may reduce the detector response due to ion suppression at the ion source, thereby causing a quantification error [7]. Matrix effect (%ME) in the sample extract was calculated as the slope of the calibration curve of the analyte in the sample matrix divided by the slope of the calibration curve of the analyte in solvent and multiplied by 100 (Figure 3). Therefore, a value of 100% means that no matrix effect is present; a value less than 100% suggests matrix suppression, and a value more than 100% suggests there is matrix enhancement. Paraquat has matrix enhancement (112%) in yellow potato and matrix suppression (35%) in the Russet potato (Figure 4). On the other hand, diquat has matrix suppression for both matrices (35-40%). Since the matrix effect is dependent on the type of potato and not predictable, an internal standard is needed for accurate quantification. It is a good practice to use IS to only correct for matrix effect at the MS ion source and add the IS after the extraction just before analysis. However, it was found that, the recovery of paraquat spiked in potato samples at the low levels (25-100 ng/g) can be below 70% when the IS was

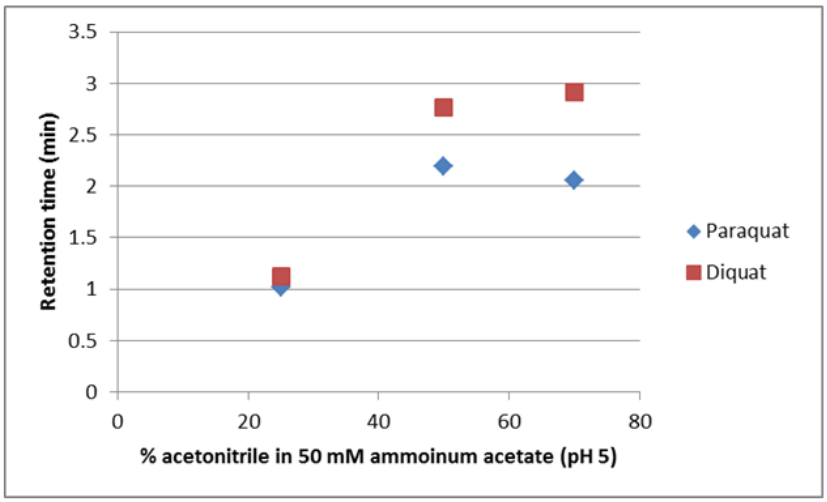


Figure 2: Retention time of Paraquat and Diquat vs. % of acetronitrile in the mobile phase containing ammonium acetate (pH = 5).

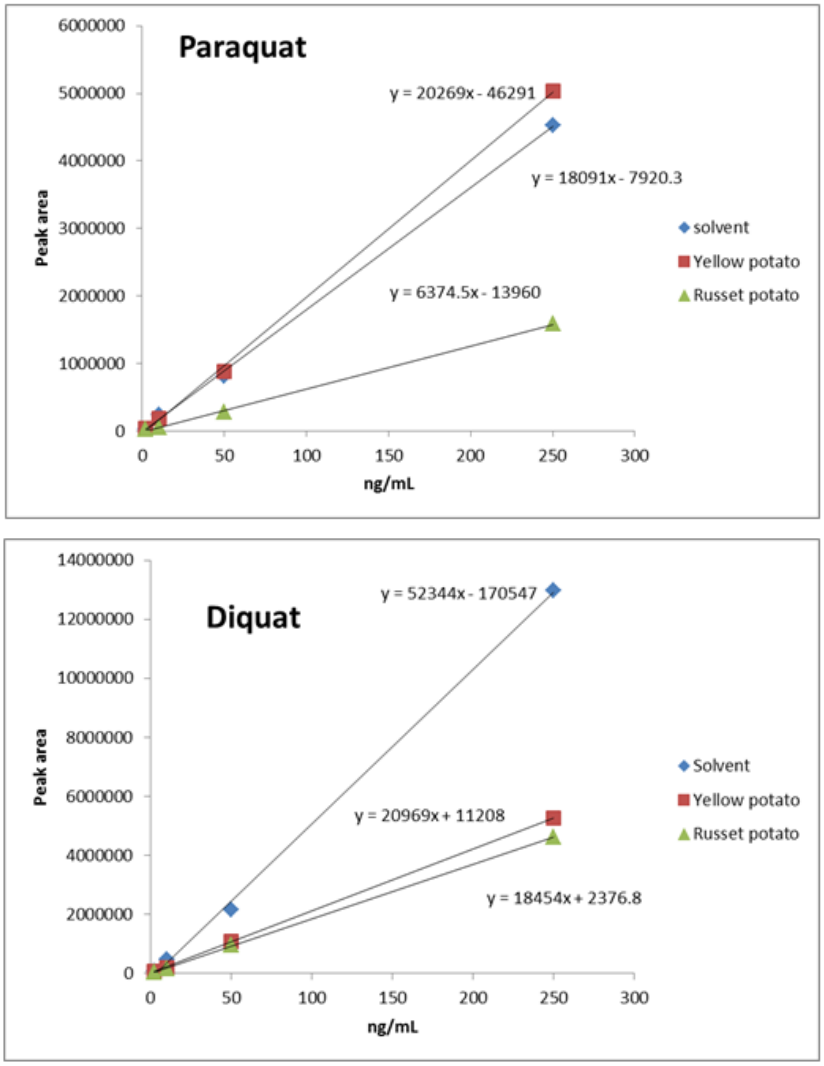


Figure 3: Callibration curves of analytes in solvent and in blank potato matrix

Yellow Potato

	Slope of cal. curve in solvent	Slope of cal. curve in matrix	Matrix effect (%ME)
Paraquat	18091	20269	112
Diquat	52344	20969	40

Russet Potato

	Slope of cal. curve in solvent	Slope of cal. curve in matrix	Matrix effect (%ME)
Paraquat	18091	6374	35
Diquat	52344	18454	35

Figure 4: Matrix effect evaluation in potato samples (using calibration curve with linear fit).

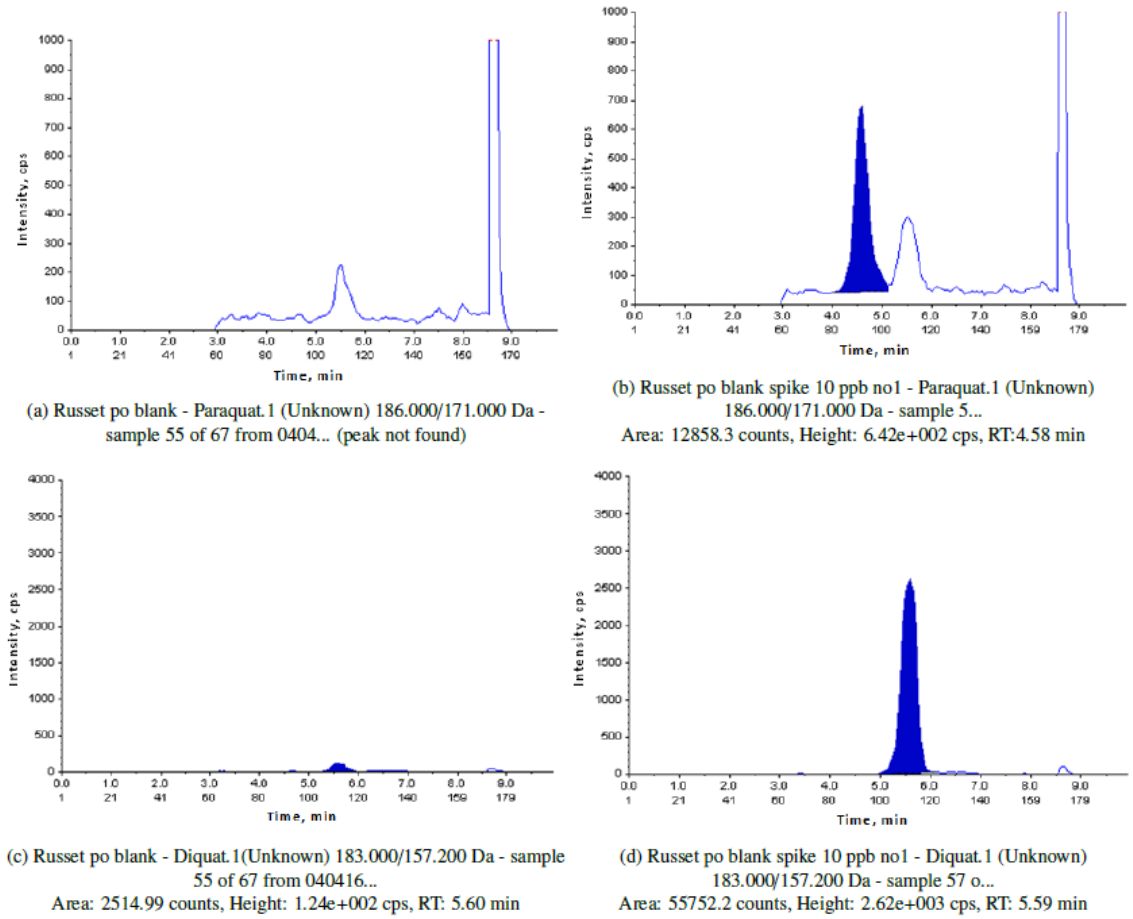
	Paraquat Recovery (%)	
Spike Level (ng/g)	IS after extraction	IS before extraction
25	70	104
50	63	100
100	65	110
500	80	96
2000	93	92

Figure 5: Recovery of paraquat spiked in Russet potato analyzed by two extraction methods (adding IS before and after extraction).

Matrix	Spike Level (ng/g)	Paraquat				Diquat			
		Std. in Matrix		Std. in Solvent		Std. in Matrix		Std. in Solvent	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Russet Potato	10	101	3.9	81	5.5	98	5.7	92	6.5
	25	89	9.2	86	10.4	93	4.1	93	4.3
	100	87	6.2	92	6.3	98	5.4	102	5.2
	500	91	8.3	98	8.0	94	3.1	98	3.1
Yellow Potato [a]	10	97	11.9	96	11.7	106	7.5	121	6.4
	25	97	10.6	96	10.6	98	6.7	102	6.3
	100	103	7.2	102	7.1	100	4.0	99	3.8
	500	101	7.1	100	7.0	100	4.3	98	4.0

[a]Note: The yellow potato has incurred residue diquat at 2 ng/g.

Figure 6: Recovery and precision data obtained in the validation experiments (n=5).



(a) Russet po blank - Paraquat.1 (Unknown) 186.000/171.000 Da - sample 55 of 67 from 0404... (peak not found)

(b) Russet po blank spike 10 ppb no1 - Paraquat.1 (Unknown) 186.000/171.000 Da - sample 5...
Area: 12858.3 counts, Height: 6.42e+002 cps, RT:4.58 min

(c) Russet po blank - Diquat.1(Unknown) 183.000/157.200 Da - sample 55 of 67 from 040416...
Area: 2514.99 counts, Height: 1.24e+002 cps, RT: 5.60 min

(d) Russet po blank spike 10 ppb no1 - Diquat.1 (Unknown) 183.000/157.200 Da - sample 57 o...
Area: 55752.2 counts, Height: 2.62e+003 cps, RT: 5.59 min

Figure 7: Chromatogram of Russet potato blank and Russet potato blank fortified at 10 ng/g of paraquat and diquat.

added after extraction versus just before extraction (Figure 5). A small finite amount of paraquat (both native and isotopically labelled) may be absorbed by the sample matrix. The similar issue was not observed in the diquat analysis. By adding the IS at the beginning of the extraction, the errors caused by a) matrix absorption, b) extraction solvent volume change (due to moisture from the sample or pipette error), and c) matrix suppression at the ion source, can be corrected. This practice was previously used by many researchers for paraquat/diquat analysis in food [18, 14, 17, 3].

3.3. Method Validation

The calibration standard solutions at the concentration from 2 to 250 ng/mL were prepared in sample matrices and extracting solvent with IS at the concentration of 50 ng/mL. These standard solutions were injected along with the fortified samples and sample blank. The calibration curves were linear fit with 1/x weighing (Figure 3). The linearity was evaluated and proved satisfactory, with coefficients of determination r^2 of more the 0.998. The specificity of the method was evaluated by analyzing reagent blank, blank sample, and blank sample spiked at the lowest concentration (10 ng/g). No relevant signal (above 30%) was observed at any of the transitions selected in the blank sample (except trace of incurred residue of diquat in yellow potato). A reagent blank was injected immediately after the 250 ng/mL standard and no analyte signals were detected above 10% of the 2 ng/mL standard.

The method detection limit (MDL) for each compound was calculated from 7 replicates of the lowest calibration standard in the Russet potato (8 ng/g). The MDL was calculated multiplying standard deviation of 7 replicates with t value at degree of freedom of 6 (t = 3.14). The MDL for paraquat and diquat were 0.4 and 0.3 ng/g, respectively. The method quantification limit (MQL) was three times the MDL, which were 1.2 and 0.9 ng/g, respectively.

Accuracy (recovery %) and precision (relative standard deviation or RSD %) were evaluated at the fortification levels of 10, 25, 100, and 500 ng/g in five replicates (Figure 6) using both calibration methods (solvent and matrix-matched standard curves with IS).

The average recovery of paraquat for both matrices at four spiking levels range from 87% to 103% using matrix-matched standard curve and from 81% to 102% using solvent standard curve with the RSD of less than 12% (for both methods). The average recovery of diquat for both matrices at four spiking levels ranged from 93% to 106% with the RSD of less than 8%

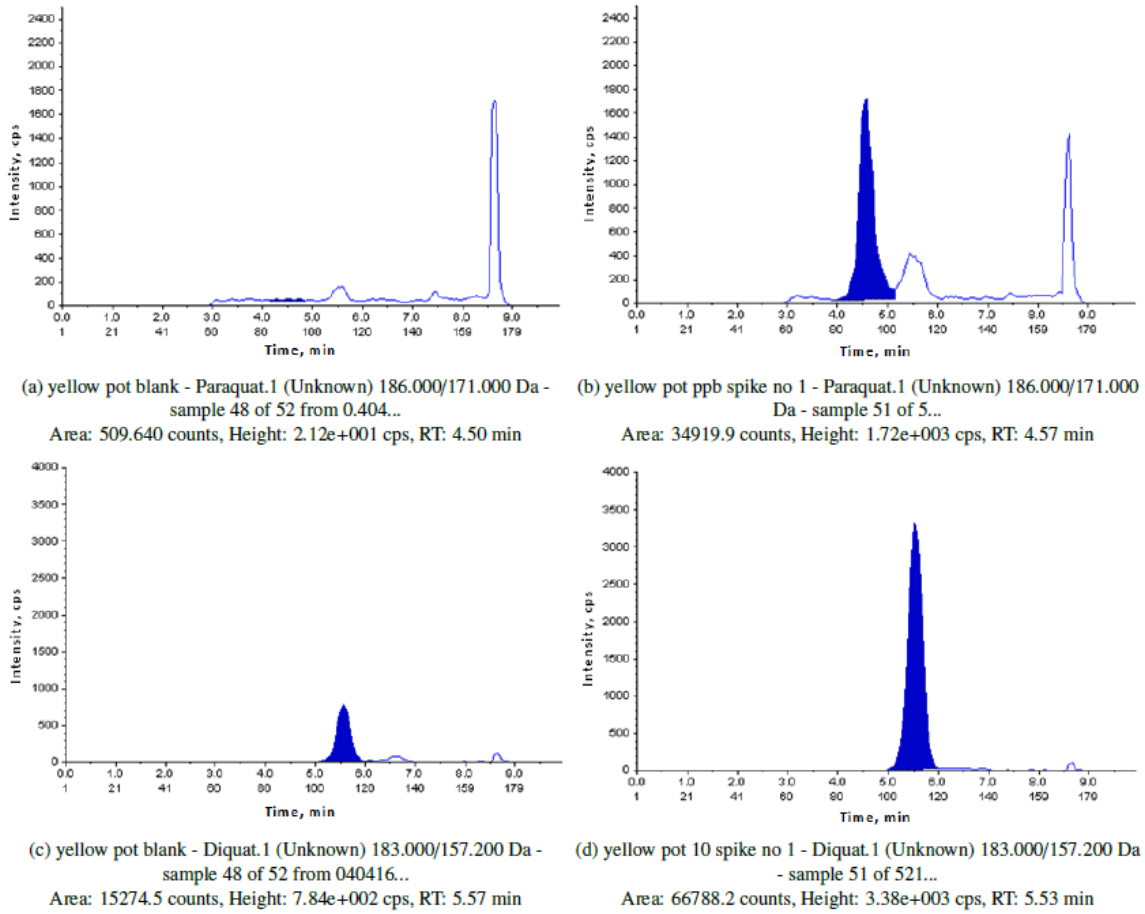


Figure 8: Chromatogram of yellow potato blank and yellow potato blank fortified at 10 ng/g of paraquat and diquat.

using matrix-matched standard curve and from 92% to 121% with the RSD of less than 7% using solvent standard curve (Figure 6). To simplify the procedure and for screening purposes, the calibration standard made from the extracting solvent (with IS) may be used to quantify paraquat and diquat in the potato samples. The standard curve gave the overall acceptable accuracy (within 70-120%) and precision (≤ 20%) as per the performance criteria of the SANCO guidelines [13]. Chromatograms of the Russet potato blank and the yellow potato blank was compared with chromatogram of potato blank fortified at 10 ng/g (Figure 7). Chromatograms of the yellow potato blank and the yellow potato blank fortified at 10 ng/g are shown in Figure 8. There was no significant difference in peak shape and retention time in both matrices. The yellow potato blank has incurred residue of diquat at approximately 2 ng/g.

4. Conclusion

This paper describes a quick, easy, and reliable 10-min LC-MS/MS method for the measurement of paraquat and diquat in potato samples with the lowest fortification level of 10 ng/g. The validation studies demonstrated that the high temperature extraction and the use of the mixed-phase mode Acclaim^{TM} Q1 column coupled with LC-MS/MS are robust and suitable for routine analysis of paraquat and diquat. Good recoveries and precisions were obtained within the acceptable range (70-120% recovery with ≤ 20% RSD). The Acclaim^{TM} Trinity Q1 column is very versatile for the analysis of polar pesticides, which normally are not retained on the reversed-phase column. This mixed-mode column provides a sharper peak shape and better resolution than the HILIC columns. The same column can be used to determine paraquat and diquat (cationic), glyphosate (anionic) and 2,4-D (relatively non-ionic), in food by just changing the appropriate mobile phase and acquisition methods [9, 10]. This approach will enable a chemist to perform a multiresidue pesticide testing on the same LC-MS/MS instrument without changing the column [3].

5. Declaration of Conflicting Interest

The authors declare no conflicts of interest.

6. Article Information

This article was received August 4th, 2016, in revised form November 25, 2016, and made available online March 31, 2017.

7. References

[1] Alexander, L. N., Nuijs, V., Tarcomnicu, I., & Covaci, A. (2011). Application of hydrophilic interaction chromatography for the analysis of polar contaminants in food and environmental samples. *Journal of Chromatography: A, 1218*, 5964-5974.

[2] Almeida, R. M., & Yonamine, M. (2007). Gas chromatographic-mass spectrometric Method for the determination of the Herbicides paraquat and diquat in plasma and urine samples. *Journal of Chromatography: B, 853*, 260-264.

[3] Anastassiades, M., Kolberg, D. I., Mack, D., Sigalova, I., Roux, D., & Fügel, D. (2013). *Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPe–Method)*. Retrieved July1, 2016, from *http : //www.eurl – pesticides.eu.*

[4] Aramendia, M. A., Borau, V., Lafont, F., Marinas, A., Marinas, J. M., Moreno, J. M., Porras, J. M., & Urbano, F. J. (2006). Determination of diquat and paraquat in olive oil by ion-pair liquid chromatography-electrospray ionization mass spectrometer (MRM). *Food Chemistry 97*, 181-188.

[5] Awadalla, E. A. (2012). Efficacy of vitamin C against liver and kidney damage induced by paraquat toxicity. *Experimental and Toxicologic Pathology, 64*, 431-434.

[6] Bassarab, P., Williams, D., Dean, J. R., Ludkin, E., & Perry, J. J. (2011). Determination of quaternary ammonium compounds in seawatersamples by solid-phase extraction and liquid chromatography-mass spectrometry. *Journal of Chromatography: A, 1218*, 673-677.

[7] Cassiano, N. M., Barreiro, J. C., Martins, L. R. R., Oliveira, R. V., & Cass, Q. B. (2009). Chromatographic methods validation for analysis of small molecules in biological matrices. *Qumica Nova, 32*,1021-1030.

[8] Castro, R., Moyano, E., & Galceran, M. T. (2001). Ion-trap versus quadrupole for analysis of quaternary ammonium herbicides by LC-MS. *Chromatographia, 53*, 273-278.

[9] Chamkasem, N., & Harmon, T. (2016). Direct determination of glyphosate, glufosinate, and AMPA in soybean and corn by liquid chromatography/tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 408*(18), 4995-5004.

[10] Chamkasem, N., & Morris, C. (2016). Direct Determination of 2,4-dichlorophenoxyacetic acid in Soybean and Corn by Liquid chromatography/tandem mass spectrometry. *Journal of Regulatory Science, 2*, 9-18.

[11] Chen, Y. W., Yang, Y. T., Hung, D. Z., Su, C. C., & Chen, K. L. (2012). Paraquat induces lung alveolar epithelial cell apoptosis via Nrf-2-regulated mitochondrial dysfunction and ER stress. *Archives of Toxicology, 86*, 1547-1558.

[12] De Bora, B. & Rohrer, J. (n.d.). *Improved Separation of Diquat and Paraquat Using the Acclaim Mixed-Mode HILIC-1 Column.* Retrieved from *http : //www.dionex.com/en – us/webdocs/*87647 – *AB* – 114 – *LC – Diquat – Paraquat – Acclaim – HILIC* – 1 – *AB*70230_*E.pdf*

[13] European Commission Directorate-General for Health and Food Safety. (2013). *Guidance document on analytical quality control and validation procedures for pesticide resuides analysis in food and feed.* Retrieved from *https : //ec.europa.eu/food/sites/food/files/plant /docs/pesticides_mrl_guidelines_wrkdoc_*11945.*pdf*

[14] Kolberg, D. I. S., Mack, D., Anastassiades, M., Hetmanski, M. T., Fussell, R. L., Meijer, T., & Mol, H. G. J. (2012). Development and independent laboratory validation of a simple method for the determination of paraquat and diquat in potato, cereals and pulses. *Analytical and Bioanalytical Chemistry, 404*, 2465-2474.

[15] Monsanto International sàrl & Monsanto Europe SA. (2010). The agronomic benefits of glyphosate in Europe. Retrieved from *http : // www.monsanto.com/products/documents/glyphosate – background – materials/agronomic%20benefits%20of%20glyphosate%20in %20europe.pdf*.

[16] Philbey, A. W., & Morton, A. G. (2001). Paraquat poisoning in sheep from contaminated water. *Australian Veterinary Journal, 79*(12), 842-843.

[17] Pizzutti, I. R., Vela, G. M. E., de Kok, A., Scholten, J. M., Dias, J. V., Cardoso, C. D., Concenço, G., & Vivian, R. (2016). Determination of paraquat and diquat: LC-MS method optimization and validation. *Food Chemistry, 209*, 248-255.

[18] Schaner, A., & Hickes, H. (2015). Quantitative analysis of paraquat in vegetation by stable isotope dilution and liquid chromatography/electrospray ionization-mass spectrometry. *Journal of AOAC International, 98*, 512-516.

[19] Selisker, M. Y., Herzog, D. P., Erber, R. D., Fleeker, J. R., & Itak, J. A. (1995). Determination of paraquat in fruits and vegetables by a magnetic particle based enzyme-linked-immunosorbent assay. *Journal of Agricultural and Food Chemistry, 43*, 544-547.

[20] Tadeo, J. L., Sanchez-Brunete, C., Perez, R. A., & Fernandez, M. D. (2000). Analysis of herbicide residues in cereals, fruits and vegetables. *Journal of Chromatogrophy: A, 882*, 175-191.

[21] Taylor, P. J., Salm, P., & Pillans, P. I. (2001). A detection scheme for paraquat poisoning: validation and a five-year experience in Australia. *Journal of Analytical Toxicology, 25*(6), 456-460.

[22] Thermo Scientific. (n.d.). *Acclaim Trinity Q1 Columns for trace analysis of diquat and paraquat.* Retrieved from *http : //www.dionex.com/en – us/webdocs /*113757 – *PS* – 20567 – *Acclaim – Trinity – Q*1 – *PS* 20567 – *EN.pdf*.

[23] Tolerances and Exemptions for Pesticide Chemical Residues in Food, 40 CFR 180 (2016). Retrieved July 1, 2016.

[24] Wigfield, Y .Y., McCormack, K. A., & Grant, R. (1993). Simultaneous determination of residues of paraquat and diquat in potatoes using high-performance capillary electrophoresis with ultraviolet detection. *Journal of Agricultural and Food Chemistry, 41*, 2315-2318.

[25] Yao, F., Liu, H., Wang, G., Du, L., Yin, X., & Fu, Y. (2013). Determination of paraquat inwater samples using a sensitive fluorescent probe titration method. *Journal of Environmental Sciences, 25*,1245-1251.

[26] Zou, T., He, P., Cao, J., & Li, Z. (2015). Determination of Paraquat in vegetables using HPLC-MS-MS. *Journal of Chromatographic Science, 53*, 204-209.