Fast and Sensitive Analysis of Paraquat and Diquat in Drinking Water

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Overview

This application note describes a fast and sensitive LC-MS/MS method for the determination of Paraquat and Diquat in drinking water. Using the Ultra Quat HPLC column and the AB SCIEX API 3200™ LC/MS/MS System equipped with a Turbo V™ source, the limits of quantitation (LOQ) for this method in drinking water are 0.1 µg/L and 5 µg/L respectively for Diquat and Paraquat using a 10 µL injection volume without sample preparation prior to analysis.

Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride, $C_{12}H_{14}N_2Cl_2$), and Diquat (1,1'-ethylene-2,2'-bipyridilium dibromide, $C_{12}H_{12}N_2Br_2$), are non-selective and nonsystematic contact herbicides widely used in agriculture to control broadleaf and grassy weeds. The use of these herbicides is very important because weeds compete vigorously with crops for water, light and other nutrients. As a result, if they are not suppressed they reduce crop yields by up to 80%. However both Parquat and Diaquat are toxic and ingestion of either compound can have serious effects as they can alter reduction-oxidation activities in biological systems. The analysis of these highly charged dual quaternary amines is complicated because of their ionic nature and therefore Paraquat and Diquat are difficult to retain by standard reversed phase HPLC. For drinking water the United States Environmental Protection Agency (EPA) has established a maximum contaminant level of 20 µg/L for Diquat.[1] Paraquat is currently not regulated in drinking water to our knowledge.

The EPA 549.2 method for the analysis of Paraquat and Diquat uses reversed phase/ion-pair extraction utilizing C8 SPE cartridges followed by ion-pair LC with ultraviolet (UV) and/or photodiode array (PDA) detection.[2] This method is time-consuming and requires large sample volume, and suffers from stability and reproducibility problems associated with ion-exchange chromatography. Recently, various mass spectrometry (MS) methods have been developed for the analysis of these herbicides. Although these methods have lower limit of detection, an extensive cleanup is generally required.[3-4]

Experimental

Chemicals

De-ionized (DI) water (Type I reagentgrade, 18 MΩ-cm resistance) was produced in house. HPLC grade acetonitrile was purchased from J. T. Baker. Standards of Paraquat dichloride tetrahydrate and Diquat dibromide monohydrate were purchased from Supelco. The purity of these compounds was ≥99%. Internal standards (ISTD) D_8-Paraquat and D_4-Diquat (both 99.6% isotope enriched) were obtained from CDN Isotopes. Heptafluorobutyric acid (HFBA), 99%, was obtained from Sigma-Aldrich.

LC

An Agilent 1100 series equipped with degasser, quaternary pump, and autosampler was used. HPLC separation was performed on a Restek Ultra Quat 3µm (50x2.1mm) with guard column Ultra Quat 3 µm (20x2.1 mm) and an isocratic mobile phase of 95% water + 5% acetonitrile + 10mM of HFBA at a flow rate of 500 µL/min. The injection volume was set to 10 µL. Low concentrations of HFBA effectively shield the positive charges of Paraquat and Diquat, increasing interaction with the Ultra Quat stationary phase, resulting in more retention required to separate analytes from matrix components.

MS/MS

An AB SCIEX API 3200™ LC/MS/MS system equipped with a Turbo V™ source operating in Electrospray Ionization (ESI) mode and positive polarity was used. The following source and gas parameters were applied: TEM=700°C; CUR=15 psi; GS1=70 psi; GS2=60 psi; IS=5500 V; and CAD=7.

Compound dependent parameters, such as Declustering Potentials (DP), Collision Energies (CE), and Collision Cell Exit Potential (CXP) for each detected MRM transition are listed in Table 1. Two transitions a quantifier and a qualifier were monitored. A dwell time of 200 ms were used per transition.

Results and Discussion

At the analytical conditions used, Paraquat and Diquat preferentially form the singly charged [M_2^+-H^+] ions as the MS base peaks. However, the doubly charged [M_2^+] ions at m/z 92 (Diquat) and m/z 93 (Paraquat) and the radical [M+.] cations at m/z 184 (Diquat) and m/z 186 (Paraquat) have also been formed at much lower relative intensities. The MS/MS spectrum of singly charged Diquat (m/z 183) is quiet simple compared to that of the singly charged Paraquat (m/z 185) as illustrated in Figure 1.

Table 1. Detected MRM transitions for the analysis of Paraquat and Diquat

Analyte Name	MRM transition	DP (V)	CE (V)	CXP (V)
Paraquat [M_2^+-H^+]	185/170	40	30	3
	185/169	40	35	3
D_8-Paraquat [M_2^+-H^+]	193/178	40	29	3
Diquat [M_2^+-H^+]	183/157	35	32	3
	183/168	35	35	3
D_4-Diquat [M_2^+-D^+]	186/158	35	32	3

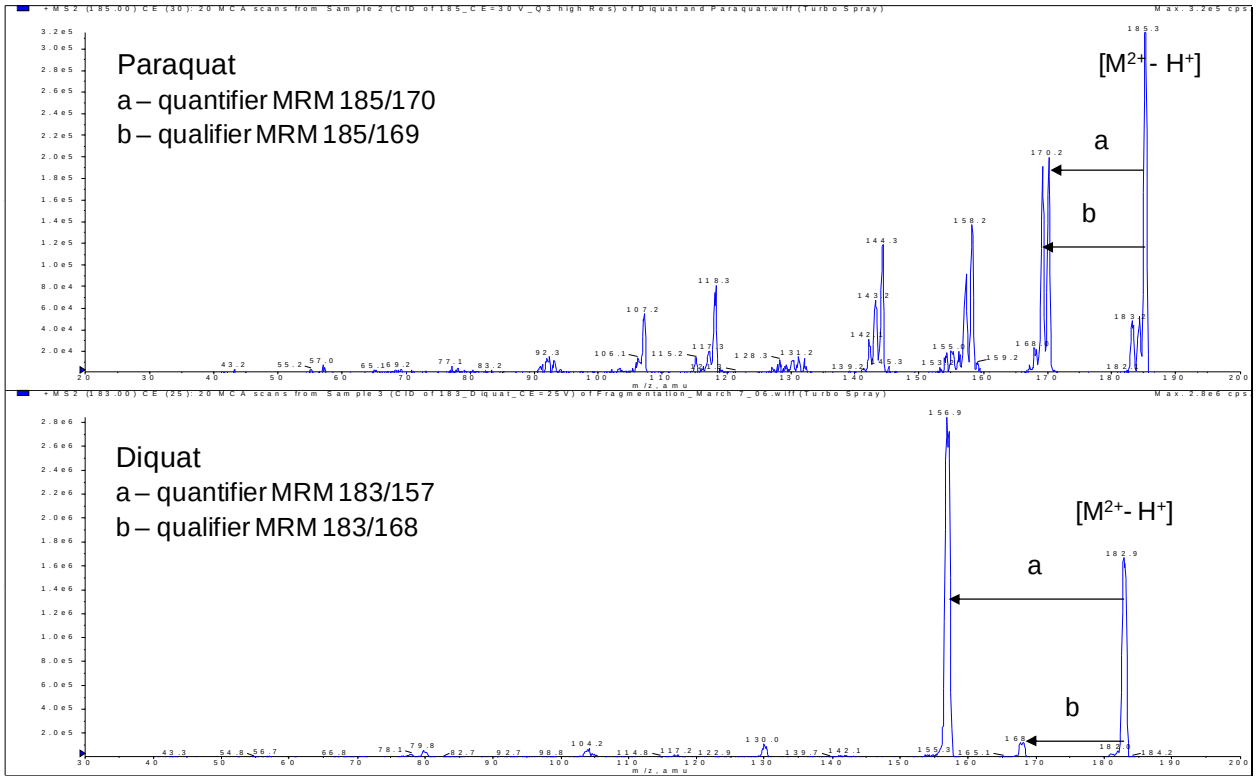

Figure 1. MS/MS spectra of Paraquat and Diquat

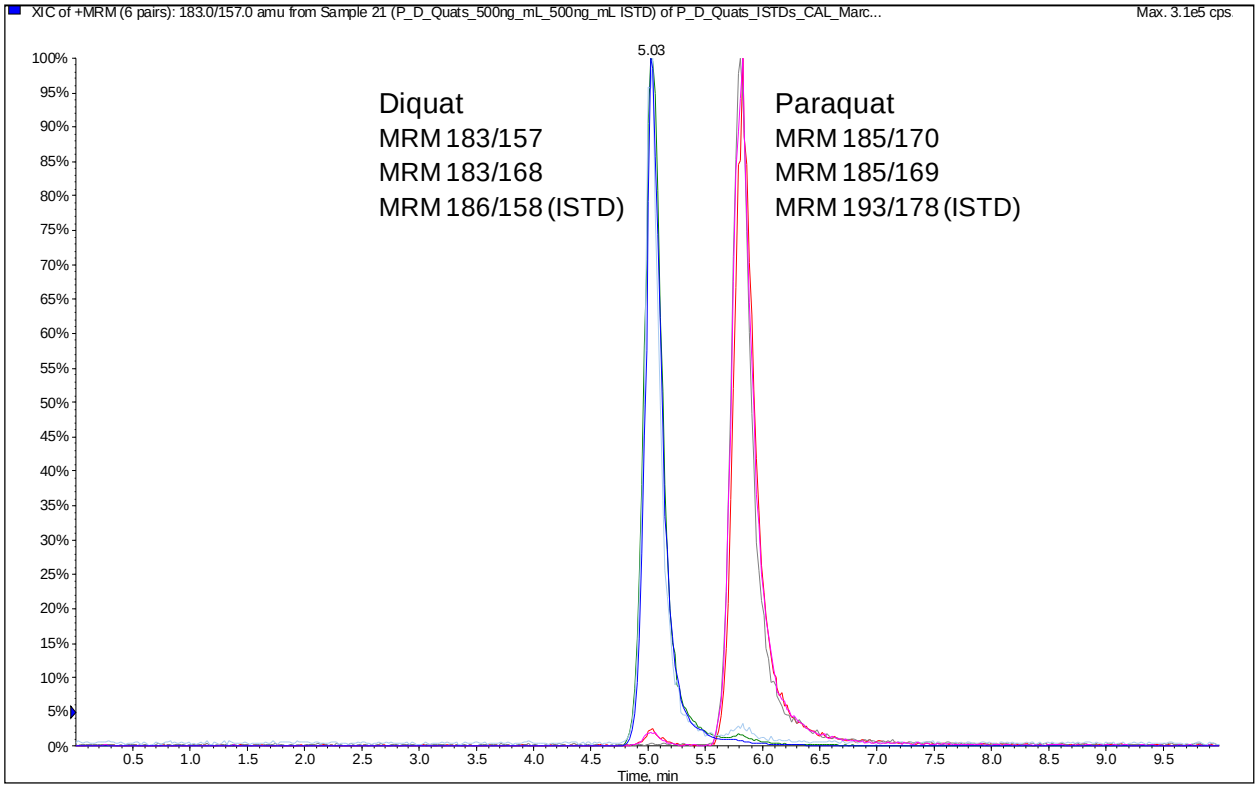

Figure 2. Separation and detection Diquat and Paraquat in drinking water (500 µg/L) by LC-MS/MS

For the highest sensitivity and selectivity used Multiple Reaction Monitoring (MRM) was used to quantify Paraquat and Diquat in DI water and drinking water.

Figure 2 shows the analysis of drinking water spiked with 500 µg/L of Paraquat, D_8-Paraquat, Diquat, and D_4-Diquat. Two MRM transitions were selected for each analyte.

Internal standards were used to improve the accuracy of quantitation, to compensate for matrix effects, and to correct for random and systematic errors in separation and detection. Triplicate injections of 9 concentrations of analytes in DI water and in drinking water, from 0.1 to 500 µg/L for Diquat and from 0.5 to 500 µg/L for Paraquat were used to investigate the performance of the developed method.

Correlation coefficients for calibration curves were >0.999, using a linear fit and 1/x weighting factor. These results indicate that quantification can be performed with good linearity and sensitivity. Figure 3 and 4 show the calibration curve for Diquat (MRM 183/157) and Paraquat (185/170) in the case of drinking water.

Table 2 summarizes the statistical parameters for the analysis of Diquat and Paraquat in drinking water. The limits of quantitation (LOQ) of the analytes were calculated from the chromatograms, at a signal-to-noise-ratio of >10.

Table 2. Summary of statistical parameters

Analyte Name	MRM transition	LOQ (µg/L)	Linear range (µg/L)	R^2
Diquat	183/157	0.1	0.1 – 5000	0.9996
Paraquat	185/170	0.5	0.5 – 5000	0.9999

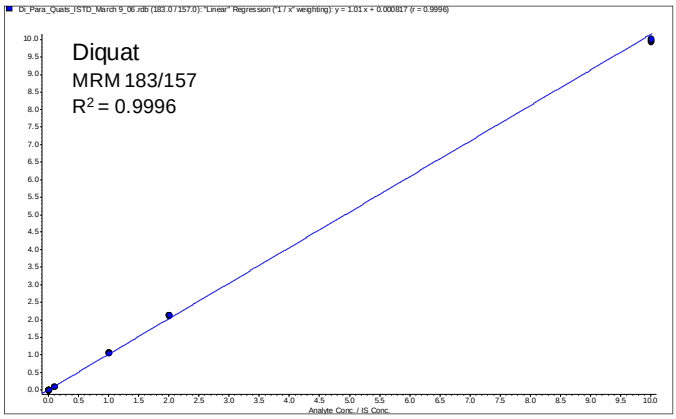

Figure 3. Calibration curve for Diquat (183/157) in drinking water from 0.1 µg/L to 5000 µg/L

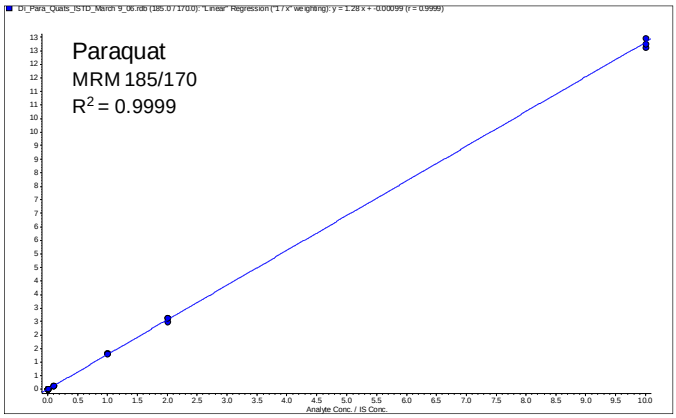

Figure 4. Calibration curve for Paraquat (185/170) in drinking water from 0.5 µg/L to 5000 µg/L

Summary

The use of the Restek Ultra Quat 3 µm HPLC column with an eluent containing Heptafluorobutyric acid allows sufficient separation of Paraquat and Diquat. Coupled to an API 3200™ LC/MS/MS systems enough sensitivity of detection is provided to inject water samples directly without any time-consuming sample preparation prior to analysis. The method was found to be robust, selective and sensitive.

References

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