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Optimized liquid chromatography tandem mass spectrometry approach for the determination of diquat and paraquat herbicides

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ABSTRACT

Liquid chromatography tandem mass spectrometry (LC–MS/MS) determination of quaternary ammonium herbicides diquat (DQ) and paraquat (PQ) can be very challenging due to their complicated chromatographic and mass spectrometric behaviors. Various multiple reaction monitoring (MRM) transitions from radical cations $M^{+\bullet}$ and singly charged cations $[M{-}H]^+$, have been reported for LC–MS/MS quantitation under different chromatographic and mass spectrometric conditions. However, interference peaks were observed for certain previously reported MRM transitions in our study. Using a Dionex Acclaim® reversed-phase and HILIC mixed-mode LC column, we evaluated the most sensitive MRM transitions from three types of quasi-molecular ions of DQ and PQ, elucidated the cross-interference phenomena, and demonstrated that the rarely mentioned MRM transitions from dications M^{2+} offered the best selectivity for LC–MS/MS analysis. Experimental parameters, such as IonSpray (IS) voltage, source temperature, declustering potential (DP), column oven temperature, collision energy (CE), acid and salt concentrations in the mobile phases were also optimized and an uncommon electrospray ionization (ESI) capillary voltage of 1000 V achieved the highest sensitivity. Employing the proposed dication transitions 92/84.5 for DQ and 93/171 for PQ, the direct aqueous injection LC–MS/MS method developed was able to provide a method detection limit (MDL) of 0.1 μg/L for the determination of these two herbicides in drinking water.

1. Introduction

Diquat (DQ, 1,1′-ethylene-2,2′-bipyridylium dication, CAS Registry Number [2764-72-9]) and paraquat (PQ, 1,1′-dimethyl-4,4′-bipyridylium dication, CAS Registry Number [4685-14-7]), are fast-acting and non-selective contact quaternary ammonium herbicides [1,2]. The corresponding commercial formulation ingredients, diquat dibromide (CAS Registry Number [85-00-7]), and paraquat dichloride (CAS Registry Number [1910-42-5]) are widely used to control weeds in agriculture (crop/fruit/vegetable) fields and aquatic environment due to their high water solubility and low vapor pressure, which facilitate solution preparation and spray application [3]. About 1.36 million kilograms of PQ and 68 thousand kilograms of DQ were used for agriculture in the United States in 2007 according to US Geological Survey [4].

Human exposure to PQ and DQ through ingestion, inhalation or dermal contact is believed to cause toxic effects on the lungs, heart, liver, kidney and central nervous system. An oral dose of 30 mg/kg of PQ is fatal and it has been reported to be a possible risk factor for Parkinson's disease [3,5,6]. PQ and DQ are also toxic to insects, algae, fish and other wildlife in the environment. Based on their toxicity, PQ and DQ are classified as moderately hazardous by the World Health Organization (WHO) [7], and considered as priority pollutants. The Ontario Ministry of the Environment has set the regulatory objective levels of 10 μg/L for PQ and 70 μg/L for DQ in drinking water, and 0.5 μg/L for DQ in surface water [8]. The US Environmental Protection Agency (EPA) also established a health advisory level of 20 μg/L for DQ, and a maximum contamination level of 3 μg/L for PQ in drinking water. In Europe, an individual pesticide including PQ and DQ cannot exceed 0.1 μg/L in drinking water, while total pesticides cannot exceed 0.5 μg/L [9].

Although gas chromatography (GC) [10] was historically employed for the measurement of DQ and PQ, capillary electrophoresis (CE) [11,12] and ion-pair or hydrophilic interaction liquid chromatography (LC) [13–16] are more commonly used in the analysis due to the cationic characteristic of these herbicides. Mass spectrometric detection is preferred over the non-selective detectors such as ultraviolet (UV) spectroscopy. Various ionization technologies such as fast atom bombardment (FAB) [17], particle beam (PB) [18], atmospheric pressure chemical ionization (APCI) [19], and electrospray ionization (ESI) [20], as well as various mass analyzers such as single quadrupole [21], triple quadrupole [22,23], sector [8], ion trap [24] and time-of-flight [25], have all been employed for the determination of DQ and PQ residues in

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different sample matrices. Among them, LC coupled with tandem mass spectrometry (LC–MS/MS) through ESI is the most popular quantitative approach because it offers superior sensitivity, selectivity, and simplicity.

After a careful review of the published LC–MS/MS studies, we found that the mass spectrometric behaviors of DQ and PQ were complex. Three types of quasi-molecular ions, radical cations $M^{+\bullet}$ (*m/z* 184 for DQ and *m/z* 186 for PQ), singly charged cations $[M-H]^+$ (*m/z* 183 for DQ and *m/z* 185 for PQ) and dications M^{2+} (*m/z* 92 for DQ and *m/z* 93 for PQ), were observed in the ESI mass spectra of DQ and PQ [26,27]. Neither the MRM transitions nor the ESI capillary voltages used in the literature for the quantitation of DQ and PQ were consistent. At least eight different MRM transitions each for DQ and PQ from their radical cations or singly charged cations were reported, while the published ESI capillary voltage ranged from 1500 to 5500 V. Meanwhile, few published studies suggested using dications M^{2+} as precursor ions for MRM analysis.

In 2011, we developed a direct aqueous injection LC–MS/MS method, using a Dionex Acclaim® reversed-phase and HILIC mixed-mode LC column, to measure six quaternary ammonium herbicides including DQ and PQ with a MDL of 1 μg/L for both of them in environmental water (unpublished data). Previously published MRM transitions 183/157 for DQ and 186/171 for PQ showed the highest responses in our initial experiment. Therefore, they were used for quantitation in that method. However, interference peaks were frequently observed for DQ and PQ in our LC–MS/MS chromatograms. In order to eliminate the interference peaks and establish a more accurate and reliable LC–MS/MS method for the determination of DQ and PQ, we evaluated typical MRM transitions resulting from three types of quasi-molecular ions of DQ and PQ, as well as isotopically-labeled analogs d4-DQ and d8-PQ, which were used as internal standards to carry out isotope dilution mass spectrometric (IDMS) analysis. Cross-interferences between DQ and PQ were identified for certain MRM transitions from radical cations and singly charged cations. The results in this study demonstrated that MRM transitions from the dications offered the best selectivity and accuracy for the LC–MS/MS analysis of DQ and PQ. Experimental parameters that could significantly influence MRM signals, such as IS voltage, source temperature, DP, column oven temperature, CE, acid and salt concentrations in the mobile phases were optimized to attain the desired method detection limit of 0.1 μg/L by direct aqueous injection. The more specific MRM transition from dications and the uncommon 1000 V ESI capillary voltage are recommended based on our experiment in order to obtain more accurate and sensitive quantitation results.

2. Experimental

2.1. Chemicals and reagents

Individual custom-made standard stock solutions of DQ and PQ were purchased from Ultra Scientific Analytical Solutions (Ottawa, ON, Canada). Stock solutions of d4-DQ and d8-PQ were purchased from Absolute Standards Inc. (Hamden, CT, USA). Certificates of analysis verified cation concentrations of 1000 μg/mL in each stock solution. Native and isotopically-labeled intermediate standard solutions were prepared by mixing the corresponding DQ and PQ stock solutions, and then further diluting with water to prepare working solutions. Because of the high capacity of the target compounds to form coordination complexes with metal ions, plastic labwares or silanized glasswares were used to avoid their adsorption onto the glass surfaces. Clean glassware was silanized first by rinsing/filling with Sylon CT silanizing solution (Supelco, Mississauga, ON, Canada) for one minute, then rinsed twice with toluene, three times with methanol and finally with NANOpure™ water. The silanized glassware was air-dried in the fumehood overnight.

ACS reagent grade ammonium formate (NH_4COOH) and formic acid (HCOOH) were purchased from Sigma Aldrich (Oakville, ON, Canada). HPLC grade acetonitrile (CH_3CN) was purchased from Fisher Scientific (Ottawa, ON, Canada). The high purity water used for aqueous mobile phases was produced by passing reverse osmosis water through a Barnstead NANOpure™ water purification system (Mississauga, ON, Canada). 10 mL 25% w/v sodium thiosulphate solution dropper bottles were purchased from ACP Chemicals Inc. (Montreal, QC, Canada).

2.2. Instrument analysis

All standards and samples were stored in polypropylene or polyethylene containers at 5 ± 3 °C. Prior to instrumental analysis, aqueous solutions were allowed to equilibrate to room temperature before 1 mL of each liquid was transferred to a 1.8 mL plastic HPLC vial. Then 10 μL of a 500 μg/L internal standard solution was added to each vial. The LC/MS-MS determination was achieved using a Shimadzu Prominence/20 series (Columbia, MD, USA) HPLC system coupled to an Applied Biosystems 4000 Q-trap (Foster City, CA, USA) mass spectrometer. Aqueous standard solutions or fortified samples containing both PQ, DQ and corresponding labeled compounds were injected into an Acclaim® Mix-mode HILIC-1 (reversed-phase/HILIC) 3 μm 150 mm × 3 mm LC column (Dionex, Sunnyvale, CA, USA). Mobile phases were ammonium formate (40 to 160 mM) and formic acid (0–0.5%, v/v) in water (A) and acetonitrile (B). The flow rate was 0.45 mL/min. The initial gradient was 0% B, held for 0.5 min then increased to 60% B at 6 min, 100% B at 7 min, and returned to 0% B at 7.5 min. The LC column was then conditioned for another 5.5 min resulting in a total run time of 13 min. Mass spectral data were acquired in positive ESI mode and processed with Analyst 1.5.2 software. Curtain gas, nebulizer gas and auxiliary gas of the MS were set at 25, 40 and 50 psi, respectively. Collision gas used in the MS/MS experiments was set at 6. High purity nitrogen was used for all gases. Entrance potential and collision cell exit potential were kept at 10 and 5 V, respectively.

3. Results and discussion

3.1. Mass spectrometry study of DQ and PQ

Compared to other small molecules with molecular weight less than 200, doubly-charged bipyridylium ions DQ and PQ have mass spectra complicated by the presence of even- and odd-electron species. As mentioned earlier, three types of quasi-molecular ions, M^{2+}, $M^{+\bullet}$ and $[M-H]^+$ have been reported in ESI mass spectra of DQ and PQ. The highly active dication structures of DQ and PQ could be the primary source of the multiple types of quasi-molecular ions observed. During the ESI process, the M^{2+} ions existing directly in the solution could also capture an electron, possibly via charge exchange, to form $M^{+\bullet}$ ($[M^{2+}+e^\bullet]$) ions or lose a proton to form $[M-H]^+$ ($[M^{2+}-H^+]^+$) ions, and the $M^{+\bullet}$ ions could further lose a radical $H^\bullet$ to form $[M-H]^+$ ($[M^{+\bullet}-H^\bullet]^+$) ions. The relative intensities of these ions can vary with different MS instruments [27]. In addition, adduct ions of DQ and PQ with a negative counterion in the solution such as chloride, bromide or with a negative counterion from ion-pairing agents in the mobile phases such as acetate, trifluoroacetate, pentafluoropropionate, heptafluorobutyrate, were also observed [8].

Evans et al. investigated detailed MS/MS fragmentation pathways for $[M-H]^+$ and M^{2+} of DQ and PQ using ion trap MS [24]. Product ion spectra of all three types of quasi molecular ions were studied in detail by MS/MS on a 4000 Q-trap instrument. As labeled in Figs. 1 and 2, most fragment ions of doubly and single charged cations are similar to those identified in the previous study. The only difference is that one major fragment ion of DQ^{2+} in Fig. 1A is