ELSEVIER

Journal of Chromatography A, 688 (1994) 67–73

JOURNAL OF CHROMATOGRAPHY A

Determination of diquat and paraquat in water using high-performance liquid chromatography with confirmation by liquid chromatography–particle beam mass spectrometry

Indira Kambhampati[a], Kurt S. Roinestad[a,*], Thomas G. Hartman[a], Joseph D. Rosen[b], Elaine K. Fukuda[a], R. Lee Lippincott[c], Robert T. Rosen[a]

[a]*Center for Advanced Food Technology, Cook College, Rutgers University, New Brunswick, NJ 08903, USA*
[b]*Department of Food Science, Cook College, Rutgers University, New Brunswick, NJ 08903, USA*
[c]*Department of Environmental Protection and Energy, CN 409, 401 E. State Street, Trenton, NJ 08625, USA*

First received 17 May 1994; revised manuscript received 7 September 1994

Abstract

A method was developed for the extraction of diquat and paraquat from environmental water samples by ion-exchange column chromatography. Determinations were performed using high-performance liquid chromatography with diode array detection. The HPLC method was developed to be compatible with LC–MS techniques. Results were confirmed with LC–particle beam (electron ionization) MS, where a unique ionization process was observed.

Recoveries in drinking water were over 75% for both diquat and paraquat spiked at concentrations of 5–10 μg/l. The method presented is uniquely specific since a diode array UV spectrum is obtained as well as an particle beam mass spectrum, insuring correct identification and quantitation of diquat and paraquat.

1. Introduction

Since their introduction approximately 35 years ago, the bipyridinium compounds paraquat (1,1′-dimethyl-4,4′-bipyridinium dihalide, PQ) and diquat (1,1′-ethylene-2,2′-bipyridinium dihalide, DQ) have been extensively employed as rapid-acting herbicides. They are effective for terrestrial and aquatic weed control and as an aid in crop desiccation, pasture renovation and seed bed preparation [1,2].

Both herbicides are extremely soluble in water and essentially insoluble in organic solvents. They are thermally stable, non-volatile, and retain their identity in both acidic and neutral conditions. However, DQ is known to degrade slowly at pH greater than 9 [3] and PQ hydrolyses at pH above 12 [1].

A variety of analytical techniques have been developed to determine residues of PQ and DQ in various matrices. Previously developed methods have included utilization of gel-filtration and ion-exchange liquid chromatography (LC) [3], gas chromatography with nitrogen–phosphorous-specific detection (GC–NPD) [4], enzyme-linked immunoassay (ELISA) [5,6] and high-performance liquid chromatography (HPLC). An inherent disadvantage of many published methods is

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00889-2

lack of analytical specificity which may result in identification and quantitation difficulties, especially in complex matrices. The United States Environmental Protection Agency (EPA) prefers methodology where confirmation of identity by mass spectrometry (MS) is possible. Yoshida et al. [7] and Barcelo et al. [8] both in 1993, published work on the determination of PQ and DQ by HPLC using thermospray MS to confirm their presence. The thermospray spectra of Yoshida et al. showed a single ion, at m/z 186 for PQ and m/z 183 for DQ, but Barcelo et al.'s work showed major ions present at m/z 187 and 184. This indicates an instrument-specific spectrum. Both papers used the same solvents and modifiers in the mobile phase. Brown et al. [9] published a particle beam (PB; 70 eV) mass spectrum of PQ, identical to the one presented in this paper, as well as a calibration curve. Few details were presented in this paper concerning HPLC conditions or recoveries, but the work confirms that the particle beam mass spectrum can be consistently obtained.

Behymer et al. [10] of the EPA concluded that thermospray HPLC–MS, because of the relatively soft ionization process, did not provide the variety of structurally significant fragment ions characteristic of conventional electron ionization (EI) mass spectrometry. Therefore, thermospray mass spectra are not considered absolute confirmation of identity. However, HPLC–PB-MS, since it provides 70 eV EI library-searchable spectra, is useful in identifying target as well as additional analytes. Previously published works have explored the use of HPLC–PB-MS methods for the determination of less volatile organic environmental pollutants [11–13].

HPLC techniques have been developed for the determination of DQ and PQ in water, but these methods rely upon non-volatile ion-pairing reagents to facilitate chromatography. These reagents contain insoluble sodium and potassium salts, phosphates and sulfonates, which are not amenable to MS confirmation of DQ and PQ, since these salts quickly precipitate and clog the LC–MS interface.

In our study, we focused on developing a simple yet specific and sensitive method for determining DQ and PQ in drinking water. The method utilizes HPLC with a diode array UV detection. The mobile phase is MS compatible so that confirmation can be obtained via PB-MS (EI mode).

2. Experimental

2.1. Chemicals and reagents

Analytically pure paraquat dichloride and diquat dibromide were obtained from Chem Service (West Chester, PA, USA). Ethyl viologen was purchased from Aldrich (St. Louis, MO, USA). Caffeine was purchased from Sigma (St. Louis, MO, USA). Ammonium hydroxide, ammonium acetate, HPLC-grade acetonitrile and HPLC water were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Standard solutions

PQ (100 mg) was dissolved in 100 ml HPLC-grade water to obtain a stock solution of 1 μg/μl and was further diluted to concentrations of 0.1 and 0.01 μg/μl. Standard solutions of DQ were prepared in the same manner. The concentration of both the DQ and the PQ solutions were recalculated to reflect the dissociation of the bromine and chlorine anions. The internal standard caffeine (50 mg) was dissolved in 100 ml of HPLC-grade water to obtain a concentration of 0.5 μg/μl.

2.3. Sample preparation

A 1–4-l sample of drinking water (pH adjusted to pH 8.5 with ammonium hydroxide) was decanted into a Millipore (Milford, MA, USA) 10-l stainless-steel pressure vessel, sealed and attached to a nitrogen tank. The vessel was then connected via PTFE tubing to a 30 cm × 1.5 cm glass column equipped with PTFE end fittings. The glass column was packed with 10 g of Dowex ion retardation resin (Sigma) which was previously conditioned by sequentially washing it with 100 ml of hexane, 100 ml of methanol and

100 ml of HPLC-grade water. The drinking water sample was then pumped through the ion-exchange column at a flow-rate of 25 ml/min. The DQ and PQ were eluted from the resin with 800 ml of 90% 0.2 *M* ammonium acetate and 10% acetonitrile. The eluate was collected in a 2000-ml round-bottom flask and rotary evaporated to 10 ml in a water bath at 70°C.

2.4. Instrumentation conditions

Analyses of DQ and PQ were performed by injecting a 200-μl aliquot of the sample into a Varian (Sunnyvale, CA, USA) Vista 5500 high-performance liquid chromatograph linked to a Varian Polychrome 9065 diode array detector and interfaced to a Techouse PC 386 DX data system. The column used for chromatographic separation was an Alltech (Deerfield, IL, USA) 250 mm × 4.6 mm, 100 Å mixed-phase C_{18}/cation-exchange column. The initial mobile phase consisted of 100% 0.05 *M* ammonium acetate (adjusted to pH 5.7 with glacial acetic acid). At 4 min, the mobile phase was switched to 90% 0.05 *M* ammonium acetate and 10% acetonitrile (adjusted to pH 4.5 with glacial acetic acid). The flow-rate of the system was 1 ml/min. The column temperature was held at 25°C. DQ and PQ were quantitated via diode array detection at 306 and 254 nm, respectively. Confirmation was obtained using the HPLC interfaced to a Vestec (Houston, TX, USA) Model 201 LC–MS system which was equipped with a PB (universal) interface. The mass spectrometer was operated in the EI mode. The filament voltage was 70 eV. The filament current was 200 μA. The source temperature was held at 220°C. The HPLC conditions were identical to those obtained off-line. A Teknivent (St. Louis, MO, USA) Vector/One MS data system was used for acquiring and processing data.

2.5. Internal standard

A 250-μg amount of caffeine (Sigma) was added to the final sample to obtain a 25 μg/ml concentration.

2.6. Quantitation

A calibration curve consisting of 14.5, 7.25, 1.45, 0.145 and 0.0725 μg standards (on-column, in triplicate) was constructed for PQ. A calibration curve consisting of 10.7, 5.35, 1.07, 0.107 and 0.0535 μg standards (on-column, in triplicate) was constructed for DQ. Caffeine was used as the internal standard (254 nm) and a response factor consisting of the area of the sample divided by the area of the internal standard was calculated for each level. The response factor for each standard was plotted against the on-column μg amount.

3. Results and discussion

3.1. Extraction procedure

Initial experiments to extract DQ and PQ from distilled water were conducted with various ion-exchange resins including a strong cation-exchange resin and a mixed-bed ion-exchange resin. Recoveries from these resins were below 30% for both DQ and PQ. However, extraction with Dowex ion retardation resin (50–100 mesh) resulted in acceptable recoveries (see Section 3.3) for both DQ and PQ in distilled water as well as drinking water, provided that the pH of the sample was adjusted to pH 8.5 with ammonium hydroxide before being pumped through the ion-exchange column.

3.2. HPLC conditions

An Alltech 250 mm × 4.6 mm, 100 Å mixed-phase C_{18}/cation-exchange column was used to separate DQ and PQ from other components of the drinking water matrix. A chromatogram of DQ and PQ displaying separation of these two components is presented in Fig. 1. Retention of analytes on the mixed-mode column that is used for our method is highly dependent on the pH of the mobile phase. Attempts to improve the resolution of DQ and PQ by increasing the pH of the mobile phase however, resulted in un-

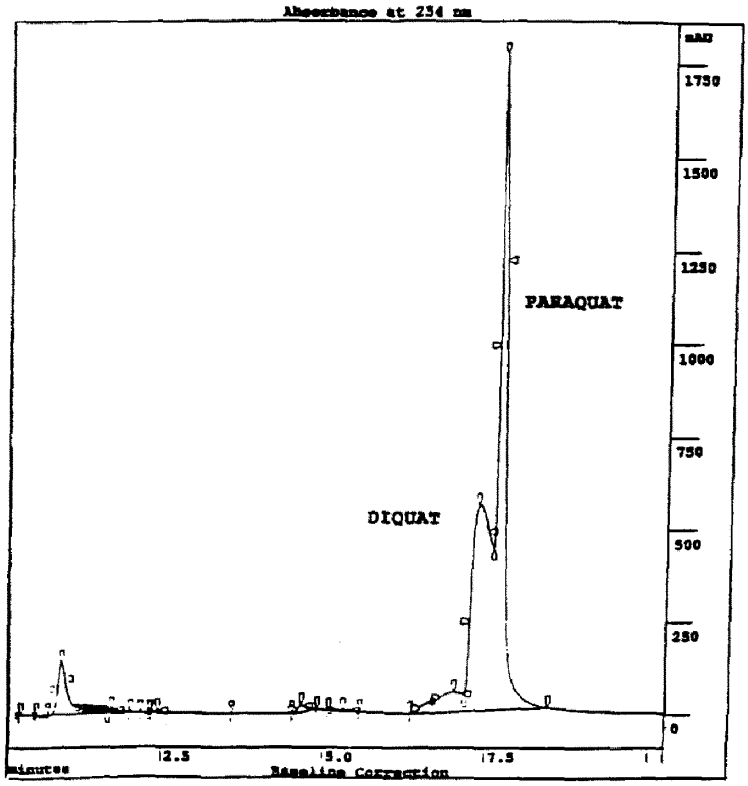

Fig. 1. Diquat and paraquat (20 μg each) chromatographed with LC–MS-compatible conditions.

acceptable chromatography. Lowering the pH of the mobile phase resulted in two very sharp peaks with identical retention times. In the case of coelution, however, it is still possible to quantitate each component as their diode array spectra are significantly different. DQ strongly absorbs at 306 nm while PQ exhibits virtually no absorbance at this wavelength. In systems where DQ and PQ coelute, diode array detection, as well as PB-MS, are useful in confirming the presence of PQ or DQ in the sample. The initial mobile phase to affect the separation of DQ from PQ consisted of 100% 0.05 *M* ammonium acetate (adjusted to pH 5.7 with glacial acetic acid). At 4 min, the mobile phase was switched to 90% 0.05 *M* ammonium acetate and 10% acetonitrile (adjusted to pH 4.5 with glacial acetic acid). This system contains only volatile buffers and is compatible with LC–MS, which is used to confirm the presence of DQ and PQ.

3.3. Internal standard, surrogate standard and recovery studies

Studies were conducted to choose an appropriate internal standard and surrogate standard for all subsequent analysis for DQ and PQ in drinking water. Caffeine was chosen as the internal standard whereas experiments conducted in our laboratory indicate that ethyl viologen (a homologue of PQ in which the methyl groups are replaced with ethyl groups) is a suitable surrogate standard since it is structurally very similar to DQ and PQ and is recovered from drinking water via our method for extracting DQ and PQ (recovery of surrogate

standard = 85.9% at the 1 μg/ml spiked level, average of duplicate analyses). A chromatogram of PQ and ethyl viologen is presented in Fig. 2. Recovery of DQ at 0.535 μg/ml in distilled water was conducted in triplicate and was determined to be 85.3% (R.S.D. = 2.8%). Recovery for PQ at 0.725 μg/ml was found to be 96.1% (R.S.D. = 0.9%). Recovery for DQ at the 5.3 μg/l level in distilled water was conducted in duplicate and was determined to be 86.3% (R.S.D. = 1.5%). That of PQ at the 7.3 μg/l level was 97.6% (R.S.D. = 4.0%). In drinking water, the same spiked levels were extracted and analyzed. Recoveries were determined to be 82.8% (R.S.D. = 2.4%) for DQ at the 0.535 μg/ml level, 84.2% (R.S.D. = 3.6%) for PQ at the 0.725 μg/ml level, 78.0% (R.S.D. = 2.0%) for DQ at the 5.3 μg/l level and 83.0% (R.S.D. = 4.8%) for PQ at the 7.3 μg/l level. All recovery studies were performed employing diode array as the detection system.

3.4. Mass spectrometry

EI (70 eV) particle beam mass spectra for DQ and PQ were generated and are presented in Figs. 3 and 4. The mechanism of ion formation of the compounds PQ and DQ is different than for non-ionic covalent molecules. With PQ and DQ, a singly charged mass spectrum results from a doubly charged species and must involve electron capture with subsequent charge elimination from one site, leaving an ion with a +1 charge state. With DQ, a $M^{2+} - H^{+}$ ion is also observed at m/z 183 and is larger than the M^{+} at m/z 184. We believe the mechanism of the formation of

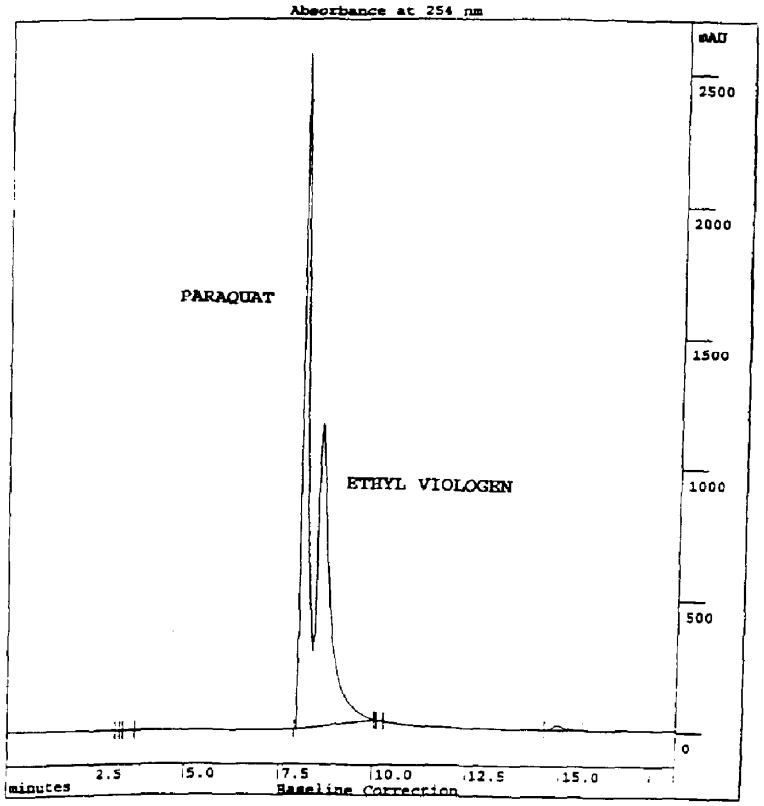

Fig. 2. Chromatogram of 25 μg of paraquat and 10 μg of ethyl viologen (surrogate standard).

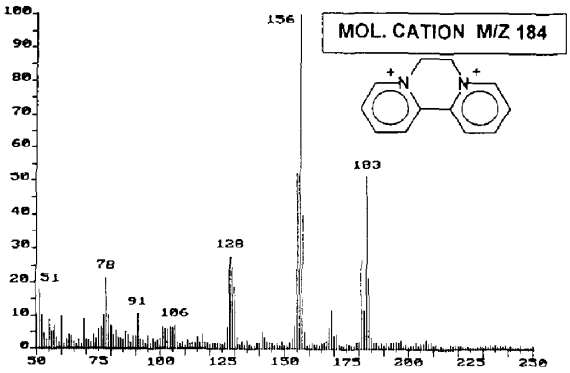

Fig. 3. Particle beam mass spectrum of diquat.

the ions for the above quats involves a disruption of the solvated particle (exiting from the PB interface) containing PQ and DQ with a subsequent release of the preformed molecular cations. This phenomenon is caused by the impact of electrons with the water particles containing the analyte. The observation supporting this hypothesis is that ions were not observed with the filament emission set to zero, no matter what changes were made in the focusing controls, and assuring that all other source voltages were indeed on. The observations described above support a secondary ionization process, i.e. desorption ionization. It was also observed that PQ does partially decompose with heat in the ion source of the mass spectrometer. While the mass chromatogram for the molecular ion shows a very sharp peak with no tailing eluting from the HPLC, the ions corresponding to the free base (m/z 156 and below) do show tailing.

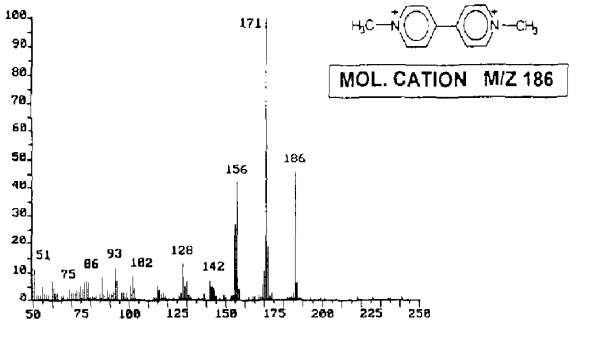

Fig. 4. Particle beam mass spectrum of paraquat.

This relates to material hitting the ion source walls.

In general, LC–PB-MS is not as sensitive as other HPLC methods of detection (this relates to electrostatic particle loss in the aerosol portion of the Vestec universal interface [13]) and absolute confirmation of these pesticides at low μg/l levels (<25 μg/l) may only be possible by selected ion monitoring (SIM). However, levels above 25 μg/l can be easily confirmed in the full scan mode.

The method presented allows the reliable identification of both DQ and PQ in drinking water samples. Work will continue and different water samples from other environmental sources will be tested to ascertain the ruggedness and uniformity of this method.

Acknowledgements

This research was supported by funds from the New Jersey Department of Environmental Protection and Energy. The Center for Advanced Food Technology is an initiative of the New Jersey Commission on Science and Technology. NJAES Publication No. D10570-1-94.

References

[1] E.V. Reed (Editor), *FDA Surveillance Index for Pesticides; PB82-913299*, National Technical Information Service, Springfield, VA, 1981.

[2] H.H. Funderburk, in P.C. Kearney and D.D. Kaufman (Editors), *Degradation of Herbicides*, Marcel Dekker, New York, 1969, pp. 283–298.

[3] I. Nakagiri, K. Suzuki, Y. Shiaku, Y. Kuroda, N. Takasu and A. Kohama, *J. Chromatogr.*, 481 (1989) 434–438.

[4] S.U. Khan, *Bull. Environ. Contam. Toxicol.*, 14 (1975) 745–749.

[5] J. Van Emon, J. Seiber and B. Hammock, *Anal. Chem.*, 58 (1986) 1866–1873.

[6] J. Van Emon, J. Seiber and B. Hammock, *Bull. Environ. Contam. Toxicol.*, 39 (1987) 490–497.

[7] M. Yoshida, T. Watabiki, T. Tokiyasu and N. Ishida, *J. Chromatogr.*, 628 (1993) 235–239.

[8] D. Barcelo and G. Durand, *J. Chromatogr.*, 647 (1993) 271–277.

[9] M.A. Brown, I.S. Kim, F.I. Sasinow, R.D. Stephens, in M.A. Brown (Editor), *Liquid Chromatography/Mass Spectrometry —Applications in Agricultural, Pharmaceutical, and Environmental Chemistry*, American Chemical Society, Washington, DC, 1990, pp. 198–214.
[10] T.D. Behymer, T.A. Bellar and W.L. Budde, *Anal. Chem.*, 62 (1990) 1686–1690.
[11] T.A. Bellar and W.L. Budde, *Anal. Chem.*, 60 (1988) 2076–2083.
[12] L.B. Clark, R.T. Rosen, T.J. Hartman, J.B. Louis and J.D. Rosen, *Int. J. Environ. Anal. Chem.*, 45 (1991) 169–178.
[13] L.B. Clark, R.T. Rosen, T.G. Hartman, J.B. Louis, I.H. Suffet, R.L. Lippincott and J.D. Rosen, *Int. J. Environ. Anal. Chem.*, 47 (1992) 167–179.