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Note

Anomalies in the high-performance liquid chromatographic determination of diquat in water samples

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Diquat (1,1′-ethylene-2,2′-bipyridyldiylium dication) is a non-selective herbicide suitable for both terrestrial and aquatic use. It is the only herbicide registered for use in waterways in New Zealand. The common method of analysis for diquat involves colorimetry either directly or after reaction with sodium dithionite[1]. The sensitivity of the method is about 0.1 mg/l and the dilute samples encountered in aquatic systems generally require an ion-exchange concentration step. A rapid and simple method for the analysis of diquat using high-performance liquid chromatography (HPLC) has recently been published[2]. The method was developed for use with formulations and forensic samples when concentrations of several hundred mg/l were being measured, but results were given which showed that the method was still accurate at about 1 mg/l.

The original authors used either aqueous solutions of over 100 mg/l, or more dilute solutions in the HPLC eluent. We have extended the use of the method to the analysis of dilute aqueous solutions by direct injection. By using a 1000-μl sample loop, concentrations of less than 0.01 mg/l may be analysed directly. This makes the method suitable for environmental samples and for analyses supporting herbicide efficacy and placement trials. The C_{18} cartridge concentration step recommended for bipyridylium herbicide residues in water[3] is also avoided.

Extension of the analytical method to these dilute samples was not without problems, however, and early experiments were confounded by highly variable responses from samples of around 1 mg/l and below. It was not uncommon for the response for diquat to vary by a factor of two between injections. Factors such as volume flushed through the loop and flow-rate through the loop were important. An added complication was that the diquat response was highly dependent on not only the type of injection solvent but also on the quality. For example, when using an identical sample loading technique, a 1 mg/l standard in tap water gave a peak half the size of that of a 1 mg/l standard in pure water. With the Sep-Pak recovery method of Gill *et al.*[2], similar levels of diquat could be extracted from both pure water and tap water standards, indicating that no breakdown of the chemical had occurred.

The present report investigates the cause of these anomalous results and outlines ways of avoiding problems.

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EXPERIMENTAL

Materials and reagents

Diquat solutions in different water samples were made from a single container of a 20% commercial aqueous concentrate formulation (Reglone, ICI Tasman, Upper Hutt, New Zealand). Orthophosphoric acid (85%) (Ajax Chemicals) and methanol (BDH) were analytical grade. Diethylamine (BDH) was reagent grade and 1-heptanesulphonic acid sodium salt was obtained from Sigma.

Apparatus

HPLC with isocratic elution was performed with a Spectra-Physics 740B pump, a Rheodyne 7120 injection valve fitted with a 20-μl loop and a Shimadzu SPD-2A variable-wavelength detector set at 309 nm. The column was a 15 cm × 4.6 mm I.D. stainless-steel column slurry-packed with Zorbax C_8 (DuPont, Wilmington, DE, U.S.A.) and was preceded by a 2-μm in-line filter (Rheodyne, Berkeley, CA, U.S.A.). The column and pre-filter were held in a Micromeritics 731 column oven set at 30°C. Integration of peaks was with a Spectra-Physics Minigrator.

HPLC conditions

The analytical conditions were based on the method of Gill *et al.*[2]. The eluent was water–methanol containing orthophosphoric acid (13.5 ml/l), diethylamine (10.3 ml/l) and 1-heptanesulphonic acid sodium salt (2 g/l). For samples likely to contain interfering peaks, water–methanol (75:25) at 2 ml/min was used, which gave a retention time for diquat of 150 sec. For cleaner samples, water–methanol (60:40) at 1.6 ml/min was used, giving a retention time for diquat of 100 sec. When larger sample loops and sample sizes of over 100 μl were used for samples below 0.1 mg/l, water–methanol (90:10) at 1.6 ml/min gave a retention time beyond the major injection baseline disturbance.

Water samples and injection solvents

Pure grade water was glass distilled and then passed through a Millipore Milli-Q water purifier. Tap water was from the Hamilton City water supply. River water was taken from the Waikato River in Hamilton City, and lake water was taken from Hamilton Lake, a turbid, mesotrophic lake. Both the river and lake water samples were allowed to stand overnight, but were not filtered, before sampling by pipette.

Stock solutions of 100 mg/l were made in both pure and tap water, and further diluted with the appropriate injection solvent. Stock solutions were kept no longer than four days, and dilute injection solutions were made fresh each day. A list of injection solvents used is given in Table I.

Method of filling the sampling loop

All injections were done with Hamilton gas-tight syringes. Volumes from 25 to 500 μl were injected using a 500-μl capacity syringe, and volumes from 1 to 5 ml with a 1- or 5-ml syringe. The samples were injected manually at approximately 2 ml/min, and a range of flushing volumes were used to load the 20-μl sampling loop. The sampling valve port was not flushed between injections unless a low-concentration sample followed a high-concentration sample in which case the port was flushed

with the low-concentration sample while in the inject position. After loading and injection, the valve was left in the inject position until the sample was eluted and the next sample was to be loaded.

RESULTS AND DISCUSSION

A series of tests using different flush volumes to fill the 20-μl sample loop with diquat standards of 1 mg/l made in solutions of different ionic strength suggested that an ion-exchange type adsorption of diquat on the stainless-steel loop was occurring. (While the experimental results presented are for a 20-μl sample loop, the same effect has been observed with larger loops of up to 1000 μl). The results of the tests are given in Table I. In all cases the relative peak heights were in good agreement with integrated areas for the diquat peak.

TABLE I

EFFECT OF SAMPLE LOOP FLUSH VOLUME ON PEAK HEIGHT OF DIQUAT USING INJECTION SOLUTIONS OF 1 mg/l IN SOLVENTS OF DIFFERENT IONIC STRENGTH

Zorbax C_8 column with water–methanol (60:40) containing 85% orthophosphoric acid (13.5 ml/l), diethylamine (10.3 ml/l) and 1-heptanesulphonic acid sodium salt (2 g/l). Flow-rate 1.6 ml/min. Detector wavelength was 309 nm at 0.04 a.u.f.s. sensitivity. Injection loop was 20 μl stainless steel with 0.51-mm bore. Flushing rate was approximately 2 ml/min. Solution identification: P = pure water; T = tap water; PM = pure water–methanol (80:20); TM = tap water–methanol (80:20); EP = HPLC eluent made with pure water; ET = HPLC eluent made with tap water; PNa = pure water plus $NaNO_3$ (10 mg/l); R = river water; L = lake water.

Flush volume (μl)	*Peak height (% of full scale) for different solutions*								
	P	*T*	*PM*	*TM*	*EP*	*ET*	*PNa*	*R*	*L*
25	13	14	14	14	12	12	11	13	13
50	16	16	15	15	14	14	13	15	15
100	17	17	16	15	15	15	15	16	16
200	23	20	27	17	16	15	17	20	18
500	36	20	41	20	16	16	18	21	20
1000	43	21	51	20	*	*	*	*	*

* Not determined.

Injections made with the two solutions of low ionic strength, P and PM, showed a steadily increasing peak height as the flush volume increased. With a 1000-μl flush, the peak height was over three times that achieved when the loop was flushed with 25 μl. The peak heights obtained with all other solutions were less affected by flush volume. As sample PNa shows, only 10 mg/l of an ionic compound needed to be added to pure water to bring the behaviour in line with solutions in tap (T), river (R) and lake (L) water. Injections made in the strongly ionic HPLC eluent solutions, EP and ET, showed the least effect from flush volume. All solutions showed an increase in peak height as the flush volume increased from 25 to 100 μl, but this is to be expected due to the parabolic flow profile of samples injected into narrow bore tubing[4,5]. At least 2–3 loop volumes are commonly recommended to completely fill a sample loop[5], but it could be as high as 10 (ref. 6).

Varying the sample flow-rate through the loop also affected the size of the diquat peak. For example, passing 1000 μl of a 1 mg/l diquat solution in pure water through the 20-μl sample loop at 6 ml/min gave a peak about 60% the size obtained if the flushing rate was 2 ml/min. The importance of low flow-rates for efficient ion-exchange adsorption is a well established principal.

When more concentrated solutions (10 and 100 mg/l) were used, the effect of changing flush volumes was less severe, but still significant. Differences between pure water and tap water were still evident for solutions of 10 mg/l. With these stronger solutions it is likely that the adsorption capacity of the sample loop is more readily exceeded.

Final proof of the adsorption of diquat onto the sample loop was obtained by flushing the loop with 1 ml of a diquat solution (1 or 10 mg/l in pure water), expelling the sample with air, flushing the loop with 100 μl of either pure water or the HPLC eluent, and then injecting the resultant solution. When pure water was used to flush the loop, the average peak height of the resulting diquat peak was 15% deflection at 0.04 a.u.f.s., the equivalent of 20 ng. When the HPLC eluent was used to flush the loop, the average peak height was less than 2% deflection at 0.04 a.u.f.s. Both 1 and 10 mg/l solutions gave similar sized peaks.

The existence of this adsorption phenomenon raises the possibility of adsorption onto other stainless-steel parts of the HPLC equipment. However, in view of the strongly ionic nature of the eluent, adsorption within the flowing part of the system (column walls, connecting tubing, frits) should be minimal, although it may increase peak tailing. The major problems arise from sample transfer and injection.

While the most obvious effect caused by diquat adsorption onto the sample loop was non-reproducibility, two less obvious effects were non-linearity and carryover. Although the detector response for diquat is linear for absolute levels injected, response curves resulting from samples, particularly those of low ionic strength, are non-linear below about 10 mg/l. Also, carryover can occur through several injections following high-concentration samples. To reduce this, the injector should be kept in the inject position through the analysis and flushed with the HPLC eluent between samples. The needles of syringes used for manual injections should be similarly cleaned. Many analysts and commercial autosampling devices employ a similar principle to load fixed sample loops, namely, a large initial flush of 10 or more loop volumes to clean the sampling valve lines of any previous sample or rinse solution prior to the first injection, followed by smaller flushes of 3–4 loop volumes for subsequent injections. This method is not suitable for diquat analysis.

The HPLC method for diquat analysis is satisfactory for the direct determination of low levels in environmental samples providing certain precautions are observed. All samples should be injected in an identical manner. The flush volume and flow-rate through the loop should be kept constant, and carryover eliminated by adequate rinsing between samples. The need to cleanse needle-to-valve connecting lines in autosamplers should not be overlooked. In some models it may be possible to replace these with PTFE tubing. Since the exact comparability of field samples in regard to ionic strength is unlikely, all samples should be spiked with salts or an aliquot of the HPLC eluent. Ideally, standards should be made by spiking blank samples, and a range of standard concentrations should always be used to confirm the response curve for the system.

This method for diquat should be suitable for paraquat. With attention to the details outlined in this note direct determination of both herbicides to low levels in water should be possible.

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