Journal of Chromatography, 255 (1983) 483–490
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,148

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PARAQUAT AND DIQUAT IN URINE WITH RAPID SAMPLE PREPARATION INVOLVING ION-PAIR EXTRACTION ON DISPOSABLE CARTRIDGES OF OCTADECYL-SILICA

R. GILL*, S. C. QUA and A. C. MOFFAT
Home Office Central Research Establishment, Aldermaston, Reading, Berkshire RG7 4PN (Great Britain)

SUMMARY

A high-performance liquid chromatographic system is presented which separates the paraquat, diquat and 1,1′-diethyl-4,4′-bipyridyldiylium (internal standard) dications in under 7 min. An octadecyl-silica (ODS-silica) column is used with an eluent of 25% methanol (v/v) containing sodium heptanesulphonate (0.01 *M*) and a diethylamine–orthophosphoric acid buffer. The method is suitable for the analysis of paraquat and diquat in commercial weedkillers and human urine samples following poisoning.

A rapid procedure for the extraction of the herbicides from urine has been developed using disposable cartridges of ODS-silica pre-treated with sodium heptanesulphonate. The quaternary compounds are extracted as ion-pairs with the hydrophobic sulphonate anions.

INTRODUCTION

Paraquat (1,1′-dimethyl-4,4′-bipyridyldiylium) and diquat (1,1′-ethylene-2,2′-bipyridyldiylium) salts are non-selective weedkillers which have found extensive industrial and domestic application throughout the world. These herbicides are extremely toxic to man[1] and consequently they are encountered in cases of accidental, suicidal and homicidal poisonings[2,3]. Some commercial products are available which contain mixtures of both compounds[4] and hence rapid methods capable of analysing paraquat and diquat simultaneously in agricultural products and biological fluids are required for forensic and clinical purposes.

Various analytical techniques have been applied to the analysis of paraquat. A common method involves the reaction with sodium dithionite to give a stable blue radical-cation[5–9]. Similarly, diquat gives a green radical-cation and the overlapping absorption spectra present problems for simultaneous analysis[10]. A reduction of diquat interference in the analysis of paraquat has recently been demonstrated by the use of derivative spectroscopy[11]. A further problem for such colorimetric methods is that they cannot be adapted to include an internal standard for quantification.

0021-9673/83/0000–0000/$03.00

Sensitive radioimmunoassay (RIA) procedures are available for the determination of paraquat but the antisera do not cross-react with diquat[12–15]. Polarographic methods for paraquat in urine and serum have also been described but have not been applied to diquat[16].

Chromatographic methods have been widely used. The quaternary herbicides must undergo pyrolysis[17] or chemical reduction[18,19] before analysis by gas–liquid chromatography. Specificity can be further enhanced by coupling with a mass spectrometer[18]. These methods are very sensitive but chemical modification of the herbicides is clearly disadvantageous. Thin-layer chromatography has been used for underivatised paraquat and diquat[20–25] but quantification is less straightforward.

High-performance liquid chromatography (HPLC) would appear to offer the best approach for the analysis of quaternary ammonium compounds. HPLC has been used for the analysis of paraquat in formulations[26], urine[27], serum[28] and plant materials[29,30] but only one of these publications[27] has considered the simultaneous analysis of diquat. The HPLC packing materials used were a cation-exchanger[26,28], octadecyl-silica (ODS-silica)[29,30] and a material prepared by reacting γ-aminopropyltriethoxysilane with alumina[27]. In general, all chromatograms shown in these publications demonstrate poor peak shapes for the herbicides and this most probably arises from strong interactions with the silica used as the matrix for most of the packing materials.

The analysis of paraquat in biological fluids often requires the removal of interfering material and/or the concentration of the herbicide. Such extraction procedures have included the use of cation-exchange columns[5–7] or ion-pair extraction into organic solvents using bromothymol blue[31] or sodium dodecyl sulphate[9].

The present paper demonstrates a HPLC system using ODS-silica suitable for the analysis of both paraquat and diquat and its application to commercial weedkillers and urine. Furthermore, a new procedure for the rapid extraction of these quaternary herbicides from urine has been developed which involves ion-pair extraction on disposable cartridges of ODS-silica.

EXPERIMENTAL

Materials

Cetrimide (cetyltrimethylammonium bromide) and orthophosphoric acid (85%, AristaR) were obtained from BDH (Poole, Great Britain). Methanol (HPLC grade) was obtained from Rathburn Chemicals (Walkerburn, Great Britain). Diethylamine was puriss grade from Fluka (Fluorochem, Glossop, Great Britain). Sodium heptanesulphonate was obtained from Fisons (Loughborough, Great Britain). All other chemicals used were of analytical grade.

Paraquat dichloride (pure standard) was obtained from Applied Science Labs. (Pierce and Warriner, Chester, Great Britain). Diquat dibromide monohydrate (analytical standard) was obtained from ICI (Plant Protection Division, Yalding, Great Britain). 1,1′-Diethyl-4,4′-bipyridyldiylium diodide was obtained from ICI (Central Toxicology Laboratory, Macclesfield, Great Britain).

Disposable cartridges (*ca.* 10 × 9 mm I.D.) packed with ODS-silica (Sep-Pak C_{18}) were obtained from Waters Assoc. (Northwich, Great Britain).

Chromatography

Chromatography was performed with a Waters M6000 pump, a Rheodyne 7120 injection valve (fitted with a 20-μl loop) and a Perkin-Elmer LC-75 variable wavelength UV detector operated at 290 nm. The column (16 cm × 5 mm I.D., stainless steel) was packed with 3-μm ODS-Hypersil (Shandon Southern Products, Runcorn, Great Britain) using a slurry procedure, dispersing the packing material in isopropanol with hexane as the pressurising solvent.

The eluent was prepared by mixing orthophosphoric acid (13.5 ml; 0.20 moles), diethylamine (10.3 ml; 0.10 moles) and sodium heptanesulphonate (2.022 g; 0.01 moles) and diluting to 1000 ml with aqueous methanol (25%, v/v). A flow-rate of 1 ml/min was used with an operating pressure of 3400 p.s.i.

Reagent solutions

Alkaline cetrimide (0.05%). Cetrimide (500 mg) and concentrated ammonia (0.88 sp.gr., 5 ml) dissolved in water (1000 ml).

Alkaline sodium heptanesulphonate. Sodium heptanesulphonate (2 g) and concentrated ammonia (0.88 sp.gr., 2 ml) dissolved in water (100 ml).

Acidic methanol. Concentrated hydrochloric acid (10 ml) dissolved in methanol (1000 ml).

Internal standard. 1,1′-Diethyl-4,4′-bipyridyldiylium diiodide in water (100 μg/ml).

Preparation of Sep-Pak C_{18} cartridges for extraction

Samples and solutions were pushed through the Sep-Pak C_{18} cartridges with a gas-tight glass syringe (10 ml). The cartridges were prepared for use by the following scheme:

(i) Wash with water (5 ml), methanol (5 ml) then water (5 ml).
(ii) Pass alkaline cetrimide (5 ml).
(iii) Wash with water (5 ml), methanol (2 × 5 ml) then water (5 ml).
(iv) Pass alkaline sodium heptanesulphonate (10 ml).

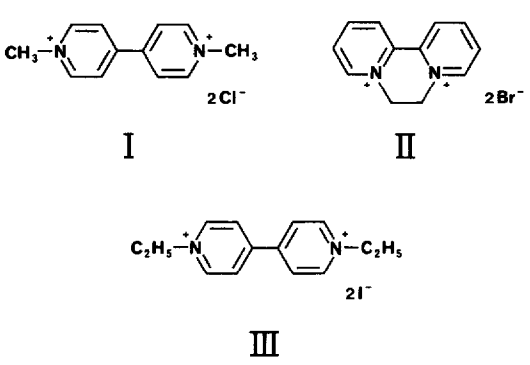
Urine extraction

Urine (1 ml) with internal standard solution added (100 μl, equivalent to 10 μg/ml in the urine) is made alkaline with concentrated ammonia (0.88 sp.gr., 200 μl) then passed through a pre-treated Sep-Pak cartridge (see above). The cartridge is washed with water (3 ml) and then methanol (3 ml), discarding the filtrates. The quaternary herbicides are then eluted with acidic methanol (5 ml) and the collected solution evaporated to dryness on a steam-bath. The residue is dissolved in the HPLC eluent (500 μl) and injected onto the HPLC column (typically 20 μl).

RESULTS AND DISCUSSION

The HPLC system was designed to separate paraquat, diquat and the chosen internal standard (1,1′-diethyl-4,4′-bipyridyldiylium ion), a diethyl homologue of paraquat (Fig. 1). The system uses an ODS-silica column with 3-μm packing material and an eluent of 25% methanol containing a diethylamine–orthophosphoric acid buffer (*ca.* pH 2.5) and sodium heptanesulphonate (0.01 *M*). Fig. 2 shows the separa-

tion of a standard mixture containing the three quaternary ammonium compounds where paraquat, diquat and the internal standard have capacity ratios, k', of 1.89, 2.23 and 2.67, respectively. The separation is complete within 7 min. It can be seen that the compounds show good peak shapes and approach baseline resolution.



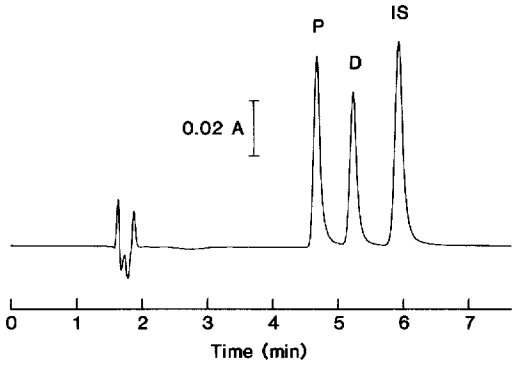


Fig. 1. Structures of quaternary herbicides: paraquat dichloride (I), diquat dibromide (II) and 1,1′-diethyl-4,4′-bipyridyldiylium diiodide (III, internal standard).

Fig. 2. Chromatogram of an aqueous standard mixture containing paraquat dichloride (P; 0.26 mg/ml, 1.00 mM), diquat dibromide monohydrate (D; 0.34 mg/ml, 0.94 mM) and 1,1′-diethyl-4,4′-bipyridyldiylium diiodide (IS; 0.47 mg/ml, 1.00 mM). Column: ODS-Hypersil, 3 μm (16 × 5 mm I.D.). Eluent: 25% methanol containing orthophosphoric acid (0.2 M), diethylamine (0.1 M) and sodium heptanesulphonate (0.01 M). Flow-rate: 1 ml/min. Detection: 290 nm (0.16 a.u.f.s.). Injection: 1.5 μl.

The mechanism of the chromatography most probably involves ion-pair formation between the polar quaternary ammonium ions and the heptanesulphonate anions. This was supported by an experiment using an eluent identical to that recommended except for the omission of sodium heptanesulphonate when the compounds showed no retention on the ODS-silica column. Other experiments showed that ion-pair chromatography with inorganic buffers in the eluent (*e.g.*, orthophosphoric acid–sodium hydroxide) gave badly tailing peaks. The addition of an amine or quaternary ammonium compound to an eluent has been widely used to reduce the peak tailing which is often observed for basic compounds on hydrocarbonaceous bonded packing materials[32,33]. These additives are believed to mask the residual silanol groups on the silica matrix. The inclusion of diethylamine in the present eluent led to a vast improvement in peak shape.

The quaternary ammonium ions (paraquat, diquat and the internal standard), show absorption maxima at 258, 309 and 260 nm, respectively, in the HPLC eluent. A detection wavelength of 290 nm provides a reasonable compromise for simultaneous analysis of paraquat and diquat giving comparable peak heights for similar concentrations of the three compounds (Fig. 2). Nevertheless, the absolute detection sensitivity for paraquat or diquat may be increased by choosing the appropriate detection wavelength.

The application of the HPLC system to the analysis of two commercial weed-killers is shown in Fig. 3. These products (Gramoxone and Weedol) contain paraquat and a mixture of paraquat and diquat, respectively. Rapid sample preparation involving dissolution of the products in water (1 mg/ml) followed by direct injection onto the HPLC column gave no interference from other components. A similar approach

can be applied to the analysis of urine containing high concentrations of the herbicides and Fig. 4 shows a post-mortem urine sample arising from a fatal dose of weedkiller. The urine (100 μl) was mixed with the internal standard solution (900 μl) before injection onto the column and the chromatogram shows a urine paraquat level of 707 μg/ml. The absence of diquat provides valuable information about the formulation of the weedkiller.

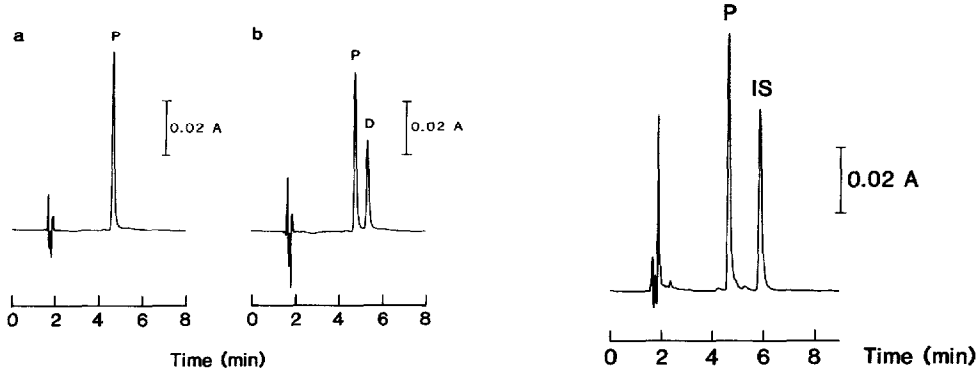


Fig. 3. Chromatograms of commercial weedkillers: (a) Gramoxone W solution; (b) Weedol granules. Samples prepared as aqueous solutions (1 mg/ml); injection volumes 1 and 8 μl, respectively. Chromatography as in Fig. 2. Peaks: P = paraquat; D = diquat.

Fig. 4. Chromatogram of post-mortem urine sample. Urine (100 μl) mixed with internal standard solution (900 μl) and 5 μl injected. Chromatography as in Fig. 2. Peaks: P = paraquat; IS = internal standard.

When the concentrations of the herbicides in urine are low it is no longer satisfactory to make direct injections onto the HPLC column. Fig. 5a shows the chromatogram arising from the direct injection of blank urine spiked with the internal standard (10 μg/ml) but this peak is obscured by endogenous compounds. In contrast, Fig. 5b shows the same urine following the sample preparation procedure described in this paper. Similarly, Fig. 5c shows the urine after spiking with paraquat dichloride (4.96 μg/ml) and diquat dibromide (4.83 μg/ml). The detection limits for the herbicides above the background of a blank urine were typically less than 1 μg/ml.

The extraction procedure uses disposable cartridges of ODS-silica (Sep-Pak C_{18}) which are pre-treated with a solution of sodium heptanesulphonate. It is supposed that the hydrophobic heptanesulphonate anions adsorb on the surface of the ODS-silica to give a material which acts as a cation exchanger. After addition of the internal standard, the urine is passed through a cartridge with retention of the quaternary herbicides as ion-pairs while the majority of the endogenous material is eluted. Before extraction the urine and cartridge are made alkaline with ammonia to ensure that endogenous amines are not protonated and hence are not retained as cations. Further clean-up is obtained by washing the cartridge with water and methanol before the herbicides are eluted with acidified methanol. Initial experiments showed that paraquat and diquat can show irreversible adsorption on the ODS-silica cartridges which probably represents interaction with the silica matrix of the material. However, this can be avoided by passing a dilute solution of cetrimide through the cartridges before treating with sodium heptanesulphonate. It appears that the cetrimide strongly adheres to the silica sites and is not displaced by the quaternary herbicides.

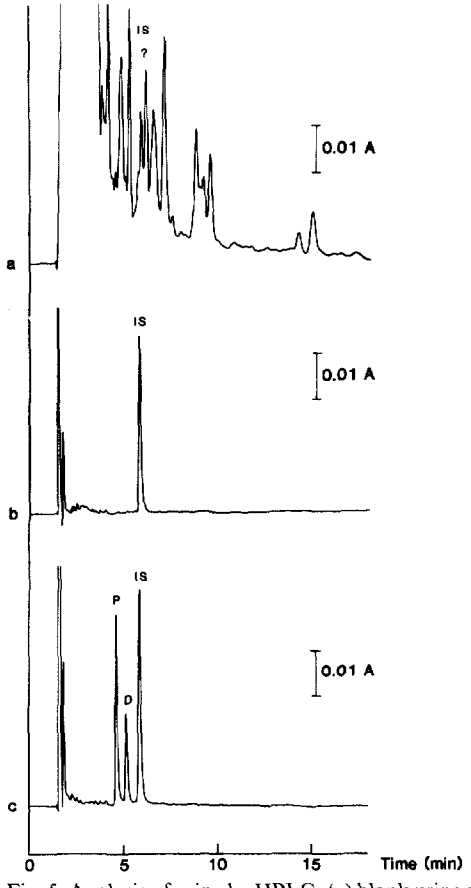


Fig. 5. Analysis of urine by HPLC: (a) blank urine spiked with internal standard (10 μg/ml), direct injection (20 μl); (b) blank urine spiked with internal standard (10 μg/ml) following extraction procedure on ODS-silica cartridges; (c) blank urine spiked with paraquat dichloride (4.96 μg/ml), diquat dibromide (4.83 μg/ml) and internal standard (10.0 μg/ml) following extraction procedure on ODS-silica cartridges. Chromatography as in Fig. 2. Peaks: P = paraquat; D = diquat; IS = internal standard.

The measured recoveries of paraquat, diquat and the internal standard at four different concentrations in urine (1, 10, 50 and 250 μg/ml) using the final extraction procedure are shown in Table I. It can be seen that all results exceed 90%.

Quantification was performed by peak height ratio measurements of paraquat (or diquat) relative to the internal standard. Extractions of either paraquat or diquat from urine (internal standard, 10 μg/ml) gave linear plots for peak height ratio against concentration up to 250 μg/ml. Further, the high precision of the method was demonstrated by repeating the analysis of urine samples, each sample being analysed six times. Two samples containing paraquat dichloride gave mean values of 4.32 and 98.8 μg/ml with coefficients of variation of 1.6% and 1.5%, respectively, while two samples containing diquat dibromide gave mean values of 4.28 and 93.7 μg/ml with coefficients of variation of 1.9% and 2.9% respectively.

The liquid–solid extraction procedure described in this paper is very rapid and

TABLE I

RECOVERIES OF PARAQUAT, DIQUAT AND INTERNAL STANDARD ADDED TO URINE USING SEP-PAK C_{18} EXTRACTION METHOD

Urine concentration (μg/ml)	*Recovery (%)*		
	Paraquat dichloride	*Diquat dibromide*	*Internal* standard*
1	98.0	94.7	100.0
10	92.6	90.2	96.7
50	95.6	95.5	101.7
250	96.7	97.5	100.8

* 1,1′-Diethyl-4,4′-bipyridyldiylium diiodide.

avoids the problem of emulsion formation often associated with ion-pair liquid–liquid extractions. Here, it is used in combination with HPLC but could equally be used with other analytical techniques (*e.g.*, colorimetric reaction). It may also be possible to apply the method, with suitable modifications, to the clean-up of other sample types (*e.g.*, blood, plant material, environmental samples). The method represents a new approach to the extraction and clean-up of quaternary herbicides but may prove to be valuable for other quaternary ammonium compounds (*e.g.*, anticholinesterase drugs). Furthermore, the method demonstrates how the selectivity of an extraction column can be controlled by coating the packing material with a suitable compound (*i.e.*, sodium heptanesulphonate) and this general approach could be useful for the design of other selective extraction columns.

In conclusion, the present HPLC system provides a suitable method for the simultaneous analysis of paraquat and diquat. The method has been applied to the analysis of the herbicides in urine following ingestion of weedkillers, with the development of a rapid sample preparation technique involving ion-pair extraction on cartridges of ODS-silica (Sep-Pak C_{18}). These methods should prove useful in forensic and clinical laboratories.

ACKNOWLEDGEMENT

The authors wish to thank ICI for providing the samples of diquat dibromide and 1,1′-diethyl-4,4′-bipyridyldiylium diiodide.

REFERENCES

1 T. J. Haley, *Clin. Toxicol.*, 14 (1979) 1.
2 K. Fletcher, in B. Ballantyne (Editor), *Forensic Toxicology*, John Wright, Bristol, 1974, p. 86.
3 R. D. Teare, *Med. Sci. Law*, 16 (1976) 9.
4 H. Martin and C. R. Worthing (Editors), *Pesticide Manual*, British Crop Protection Council, Nottingham, 5th ed., 1977.
5 S. L. Tompsett, *Acta Pharmacol. Toxicol.*, 28 (1970) 346.
6 D. J. Berry and J. Grove, *Clin. Chim. Acta*, 34 (1971) 5.
7 M. Bonini, M.-Cl. Saux, S. Larcebau and P. Castagnou, *J. Eur. Toxicol.*, (1973) 237.
8 J. Knepil, *Clin. Chim. Acta*, 79 (1977) 387.
9 D. R. Jarvie and M. J. Stewart, *Clin. Chim. Acta*, 94 (1979) 241.

10 S. H. Yuen, J. E. Bagness and D. Myles, *Analyst (London)*, 92 (1967) 375.
11 A. F. Fell, D. R. Jarvie and M. J. Stewart, *Clin. Chem.*, 27 (1981) 286.
12 T. Levitt, *Lancet*, ii (1977) 358.
13 T. Levitt, *Proc. Anal. Div. Chem. Soc.*, 16 (1979) 72.
14 M. J. Stewart, T. Levitt and D. R. Jarvie, *Clin. Chim. Acta*, 94 (1979) 253.
15 D. Fatori and W. M. Hunter, *Clin. Chim. Acta*, 100 (1980) 81.
16 G. Franke, W. Pietrulla and K. Preussner, *Z. Anal. Chem.*, 298 (1979) 38.
17 M. A. Martens and A. Heyndrickx, *J. Pharm. Belg.*, 29 (1974) 449.
18 G. H. Draffan, R. A. Clare, D. L. Davies, G. Hawksworth, S. Murray and D. S. Davies, *J. Chromatogr.*, 139 (1977) 311.
19 A. Van Dijk, R. Ebberinck, G. DeGroot, R. Maes, J. Douze and A. Van Heyst, *J. Anal. Toxicol.*, 1 (1977) 151.
20 G. S. Tadjer, *J. Forensic Sci.*, 12 (1967) 549.
21 R. A. de Zeeuw, P. E. W. van der Laan, J. E. Greving and F. J. W. van Mansvelt, *Anal. Lett.*, 9 (1976) 831.
22 H. D. Crone and E. M. Smith, *J. Chromatogr.*, 77 (1973) 234.
23 S. Tsunenari, *Forensic Sci.*, 5 (1975) 61.
24 M. B. Abou-Donia and A. A. Komeil, *J. Chromatogr.*, 152 (1978) 585.
25 M. Vandenheede, A. Heyndrickx and J. Timperman, *Med. Sci. Law*, 22 (1982) 57.
26 Y. Kawano, J. Audino and M. Edlund, *J. Chromatogr.*, 115 (1975) 289.
27 A. Pryde and F. J. Darby, *J. Chromatogr.*, 115 (1975) 107.
28 J. J. Miller, E. Sanders and D. Webb, *J. Anal. Toxicol.*, 3 (1979) 1.
29 L. Needham, D. Paschal, Z. J. Rollen, J. Liddle and D. Bayse, *J. Chromatogr. Sci.*, 17 (1979) 87.
30 D. C. Paschal, L. L. Needham, Z. J. Rollen and J. A. Liddle, *J. Chromatogr.*, 177 (1979) 85.
31 H. M. Stevens and A. C. Moffat, *J. Forensic Sci. Soc.*, 14 (1974) 141.
32 A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.*, 189 (1980) 299.
33 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.