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Rapid Determination of Paraquat in Urine with Ion-Pair Extraction and Spectrophotometry

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Paraquat and diquat are herbicides that are widely used throughout the world. They are mainly used for preemergence and interrow treatment of weeds, and as desiccants.

Many occupational, accidental and suicidal intoxications with paraquat have been reported. Determination of paraquat in urine is a valuable tool for diagnosis of such intoxications and as a basis for treatment. Although most of the paraquat is excreted in the faeces when orally administered, up to 1000 mg/L has been found in urine after ingestion (Hayes 1982). The prognosis for recovery is good if the urinary concentration of paraquat does not exceed 200 mg/L during the first three hours after ingestion and if the level has dropped to 1 mg/L within three days.

Occupational exposure to herbicides is mainly dermal, as has been shown in many studies (cf. Kolmodin-Hedman and Åkerblom 1987). Minor amounts of paraquat are taken up through the intact skin (Wester et al. 1984), but the uptake through scratches and sores can be substantial, even fatal. When given subcutaneously to rats, most of the paraquat and diquat (73-98%) was excreted in an unaltered form in the urine (Hayes 1982). The determination of the dipyridylium ions in urine is regarded relevant as biological monitoring in occupational exposure studies with mainly cutaneous uptake.

A number of methods have been used for the determination of paraquat in urine. Calderbank and Yuen (1966) reported extraction of diquat from human excreta with a cation exchanger followed by spectrophotometric determination of the herbicides in the eluate after reduction with sodium dithionite. The RIA-technique was used by Levitt (1977). Draffan et al. (1977) and Kawase et al. (1984) converted paraquat and diquat to their perhydrogenated products, which were analysed by gas chromatography. Pyrolysis gas chromatography was used by Martens and Heyndrickx (1974).

Paraquat and diquat are good candidates for ion-pairing techniques. Gill et al. (1983) extracted these quaternary ions as heptanesulphonate ion-pairs on an octadecyl-silica disposable cartridge and determined them by HPLC. Solvent extraction of paraquat as an ion-pair with dodecylsulphonate from plasma was performed by Jarvie and Stewart (1979). Paraquat was then determined spectrophotometrically according to Calderbank (1961). Jarvie et al. (1981) improved the method by extracting with sulphosalicylic acid and determining paraquat colorimetrically as above directly in the organic phase.

Ion-pair extraction techniques have been used extensively in the analysis of drugs (e.g., Schill et al. 1983). Bromthymol blue (BTB) has been frequently used for the extraction of cations. In an early search for selective extraction of diquat in analysis of rapeseeds, BTB was found to be a good ion-pairing reagent (Åkerblom 1974). In the present study, which has been shortly presented earlier (Åkerblom 1978 a, b), the conditions for ion-pair formation between BTB and paraquat or diquat were investigated. The technique was applied in a quick method for urine analysis where basic equipment and reagents were used.

MATERIALS AND METHODS

A Varian 634 scanning spectrophotometer was used. A second derivative electronic device consisting of two integrated circuits (made at the electronic workshop, Varian, Sweden) was serially connected between the spectrophotometer and the recorder. Cuvettes with 3-mL volume and 4-cm pathlength were used.

All chemicals were reagent grade.

Dithionite solution was prepared by dissolving sodium dithionite (Merck, 0.2 g) in sodium hydroxide (0.3 M, 100 mL). The solution was used within 3 hr. Stannate solution was prepared by continuously shaking tin-(II)-chloride·$2H_2O$ (Merck, 1 g) gently in sodium hydroxide (3 M, 20 mL) until dissolved, and diluting to 30 mL with water. Fresh solution was prepared every day.

Diquat and paraquat reference substances were kindly provided by ICI Plant Protection Ltd.

An outline of the final method for determination of paraquat in urine is given in Figure 1.

Colorimetric determination of paraquat and diquat was used throughout the study. If not otherwise stated, the herbicides were reduced to free radicals prior to measurement. Paraquat was reduced with dithionite accor-

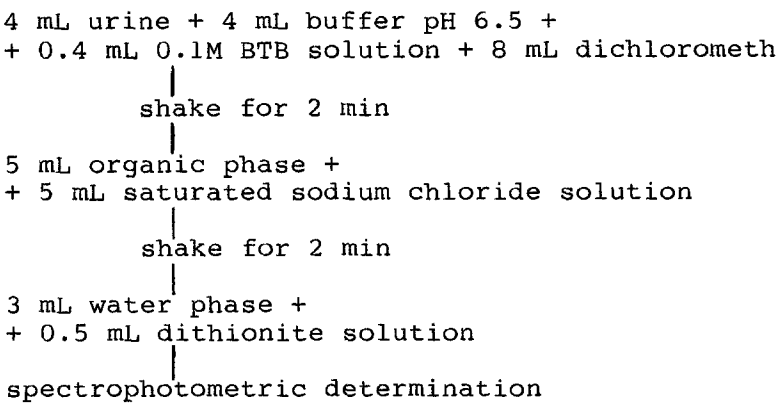

Figure 1. Flow chart for the determination of paraquat in urine

ding to Calderbank (1961). Final extract (3 mL) was fortified with dithionite solution (0.5 mL). Directly after gentle mixing, the normal and second derivative spectra were scanned between 420 and 370 nm. Diquat was reduced with stannate according to Kirsten (1964). Final extract (3 mL) was fortified with stannate solution (0.5 mL), gently mixed and, if needed, centrifuged. Exactly ten minutes after fortification normal and second derivative spectra were scanned between 400 and 350 nm.

The most suitable pH for extraction of the ion-pair was investigated. Sodium phosphate buffers (0.05 M in phosphate ion) of pH values of 4 to 9 were saturated with dichloromethane. Paraquat and bromthymol blue (BTB) were added to give concentrations 1 uM and 5 mM, respectively . Aliquots (8 mL) of these solutions were shaken with equal amounts of dichloromethane (previously saturated with the relevant buffer) for 20 min. The samples were centrifuged. The paraquat in the organic phase (aliquot of 5 mL) was extracted with saturated sodium chloride solution (5 mL), and the concentration was determined colorimetrically as described above.

For determination of extraction constants for the pairs diquat/BTB and paraquat/BTB, solutions of diquat or paraquat (0.01 mM) and BTB (0.03 to 1 mM) were made in sodium phosphate buffer (pH 6.5, 0.1 M in phosphate ion, saturated with dichloromethane). Aliquots were shaken with equal amounts of dichloromethane (saturated with buffer) for 20 min at 25°C. After centrifugation the concentration in the organic phase was determined colorimetrically as described above. The concentration in the aqueous phase (aliquot of 2 mL) was

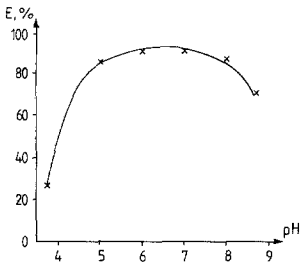

Figure 2. Influence of pH on the extraction (E) of paraquat as an ion-pair with bromthymol blue

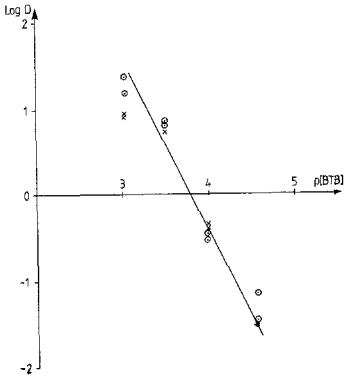

Figure 3. Distribution (D) between dichloromethane and water of paraquat (x) and diquat (o) as ion-pairs with bromthymol blue (BTB) at pH 6.5

determined colorimetrically after the addition of an equal amount of saturated sodium chloride solution and extraction of excess BTB with dichloromethane (1 mL). All studies were performed in duplicate.

The recovery of paraquat and diquat from urine was investigated with urine samples from eleven different persons of 4 - 57 years of age. The persons had no prior contact with paraquat or diquat. Urine (4 mL) was fortified with paraquat or diquat (final cation concentrations 0.2 ug/mL). One series of urine samples was fortified with paraquat to give final concentrations of 0.05 to 24 ug/mL. Sodium phosphate buffer (pH 6.5, 0.5 M in phosphate ion, 4 mL) and BTB (0.1 M in ethanol, 400 uL) was added. The solution was shaken vigorously on a wrist-action shaker with dichloromethane (8 mL) in a test tube for 2 min. After separation of phases 5 mL of the dichloromethane phase was extracted with an equal amount of saturated sodium chloride solution for 2 min. The herbicide concentration in the sodium chloride solution was determined colorimetrically as above.

RESULTS AND DISCUSSION

The most favourable pH for extraction was pH 6 - 7, see Figure 2. Bromthymol blue (BTB) is a dibasic acid with

pK_a values of 1.5 and 7.1 (Schill 1964). Diquat and paraquat are both divalent cations, and it was assumed that they could be extracted as pairs with the divalent BTB anion. However, as can be seen in Figure 2, the extraction optimum for paraquat/BTB is below pK_a 7.1, i.e., where the monovalent anion is predominant. The same was found to be true for diquat.

Thus, two molecules of BTB are paired with one molecule of the herbicide: $Q^{2+}_{aq} + 2X^{-}_{aq} \rightleftharpoons QX_{2,org}$ (where Q is paraquat or diquat and X is BTB).

The distribution D of the herbicide between the two phases is $[QX_2]_{org}/[Q^{2+}]_{aq}$ and is shown as a function of the BTB concentration in Figure 3. The distribution is governed by the extraction constant K_{ex} and the counter ion concentration ($K_{ex} = [QX_2]_{org}/[Q^{2+}]_{aq}\,[X^-]^2_{aq}$, $D = K_{ex}\,[X^-]^2_{aq}$). K_{ex} was calculated from the diagram in Figure 3 and was found to be $10^{-7.6}$ for both paraquat/BTB and diquat/BTB. The inclination of the line in the diagram satisfies the equation $\log D = \log K_{ex} + 2 \log [X^-]_{aq}$, thus proving the 1:2 pairing.

It was found that the BTB concentration had to be increased about 10 times for urine compared to pure solutions to give the same degree of extraction. The same influence of the matrix has been observed earlier (e.g., Åkerblom & Alex 1984). In order to ensure buffering of urine, which has pH values between 4 and 8, a 0.5 M buffer solution was used.

The influence of time on the degree of extraction of the ion-pair from urine was investigated; extraction times were 1 to 60 min. Maximum extraction of the ion-pair paraquat/BTB was achieved within 1 min; recoveries were 68-75%. The recovery of the pair diquat/BTB when extracted from urine fluctuated between 42 and 73% regardless of extraction time.

The ion strength of the aqueous solution influences the ion-pair formation negatively. This fact was used for extraction of the herbicides with saturated sodium chloride solution. Extraction was found to be complete within 0.5 min.

Recoveries of paraquat from fortified urine samples

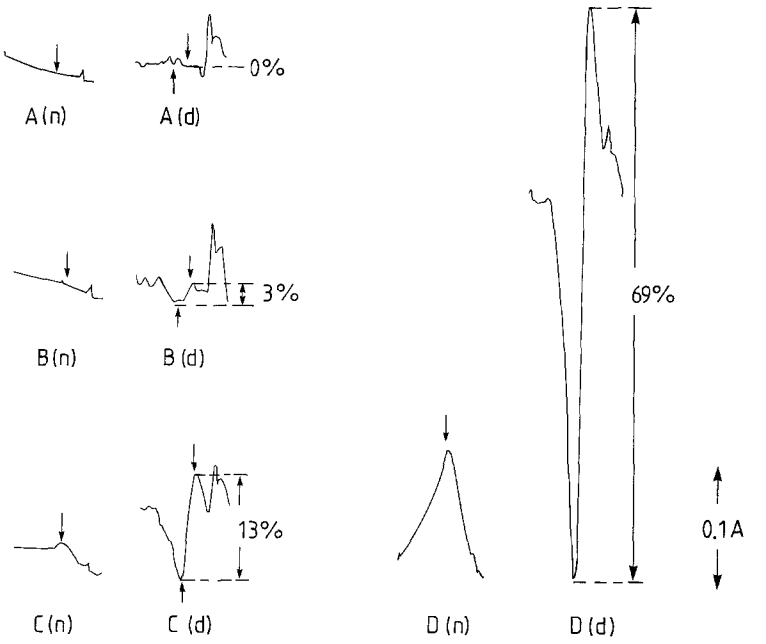

Figure 4. Normal (n) and 2nd derivative (d) spectra of reduced paraquat in urine. A no paraquat, B 0.02 mg/L, C 0.12 mg/L, D 0.56 mg/L paraquat in urine. Peak height is given in % of f.s.d. The maximum absorption was at 394 nm.

were not complete, but they were consistent at all concentrations tested; recoveries were 70 $\pm$ 3.4 % s.d. (n=22). The recoveries of diquat varied between 42 and 73% (55 $\pm$ 7.0 % s.d., n=18). This might be due to the fact that diquat is a rigid molecule with the two nitrogen atoms relatively close to each other. In paraquat the two aromatic rings can rotate, and the nitrogen atoms are apart. This facilitates the approach of two BTB molecules, one to each nitrogen atom. The method is thus suitable for quantitative work with paraquat but not with diquat. It can, howver, be used to give information of the levels of diquat in urine. The detection limit was 0.03 mg/L of paraquat in urine.

The method as described in the recovery studies uses reduction of the herbicides and a 4-cm pathlength cuvette. The use of second derivative scanning enhances the sensitivity appreciably as shown in Figure 4. This technique was discussed thoroughly by Fell et al. (1981). The calibration graph was linear up to a concentration of 2 mg/L urine for 4-cm cuvettes.

In emergency cases at places remote from well equipped laboratories the method presented here can be used without the final reduction of the quaternary ions. BTB is a common indicator in laboratories and schools. Chloroform or carbon tetrachloride can substitute for the dichloromethane if necessary. The spectrum of non-reduced paraquat or diquat can be measured manually (absorbance maxima 256 and 310 nm, respectively). In such cases it is suggested that the urine is diluted 10-fold before extraction with BTB. With 1-cm cuvettes, values above 10-50 mg/L can then be determined.

The method presented here is rapid and simple. Part of it can be used for the determination of higher levels of paraquat and diquat in urine in small laboratories remote from well equipped laboratories. When used in detail as described here a detection limit of 0.03 ug/mL is obtained in a 4 mL sample of urine. It is then suitable for biological monitoring of paraquat in exposure studies.

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