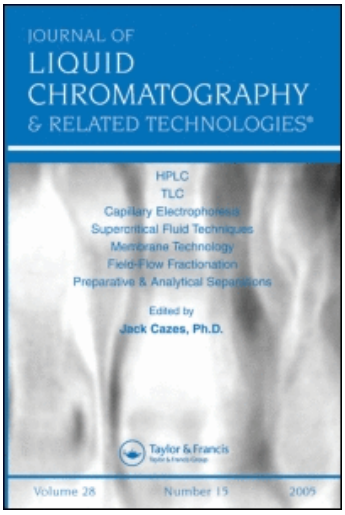

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SIMULTANEOUS QUANTITATION OF DIQUAT AND ITS TWO METABOLITES IN SERUM AND URINE BY ION-PAIRED HPLC

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ABSTRACT

We describe here the simultaneous quantitation of diquat (DQ) and its two metabolites (DQ-monopyridone and -dipyridone) in serum and urine. Serum or urine was mixed vigorously with ten times volume of trichloroacetic acid and centrifuged at 12,000 rpm for 5 min. A 20 μl of the upper layer was injected into the HPLC. The major operating conditions of the HPLC were as follows: Column; Zorbax ^{R} C8 (24cm x 4.6mm i.d.), Mobile phase; 5 % acetonitrile(v/v) containing ortho-phosphoric acid (0.2 M), diethylamine(0.1 M), and sodium octansulfonate(7.5 mM) and flow rate; 1.0 ml/min and UV detection by combined two UV detectors: 310 nm for DQ and 365 nm for DQ-monopyridone and DQ-dipyridone. Linear calibration curves for DQ, DQ-monopyridone and DQ-dipyridone were in the range of 0.1 - 10 μg/ml. In the serum and urine dosed intravenously DQ and its two metabolites were also detected.

INTRODUCTION

Herbicides containing diquat (DQ) only and combined with DQ and praquat (PQ) are widely used in Japan. An average of 200-400 deaths per year in Japan were registered during the past five years due to these herbicide poisoning(1).

Pharmacokinetic of DQ is summarized as a slight absorption after oral ingestion and rapid elimination into the urine with unchanges(2). *In vitro* studies using rats liver homoginate, however, we have identified two metabolites of DQ, DQ-monopyridone and DQ-dipyridone, as shown in Figure 1(3).

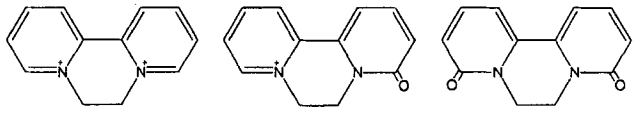
There has been reported DQ and PQ determination in urine and serum using HPLC(4-7), but not reported these metabolites.

We describe here simultaneous determination of DQ and DQ-monopyridone and DQ-dipyridone in serum and urine by HPLC after deprotenization by trichloroacetic acid.

MATERIALS AND METHODS

Reagents

We purchased methyl viologen as PQ(Sigma), $DQBr_2$ (Sigma), organic solvents (HPLC grade, Wako). DQ-monopyridone and DQ-dipyridone were isolated and purified after incubation with rat liver homoginates with $DQBr_2$ according to Fuke et al(3).



Diquat (DQ) **DQ-monopyridone** **DQ-dipyridone**

FIGURE 1. Chemical Structures of Diquat(DQ), DQ-monopyridone and DQ-dipyridone.

Sample preparation for HPLC analysis

Serum or urine samples (0.2-1.0 ml) was deprotenized vigorously with ten times volume of 10 % trichloroacetic acid solution and centrifuged at 12,000 rpm for five minutes. A 20 μl of the upper clean layer was injected into the HPLC.

Operating conditions of HPLC

The major operating conditions of the HPLC were as follows: Column; Zorbax ^{R} C8 (24 cm x 4.6 mm i.d.), Mobile phase; 5 % acetonitrile(v/v) containing ortho-phosphoric acid (0.2 M), diethylamine(0.1 M), and sodium octansulfonate(7.5 mM) and flow rate; 1.0 ml/min and UV detection by combined two UV detectors: 310 nm for DQ and 365 nm for DQ-monopyridone and DQ -dipyridone.

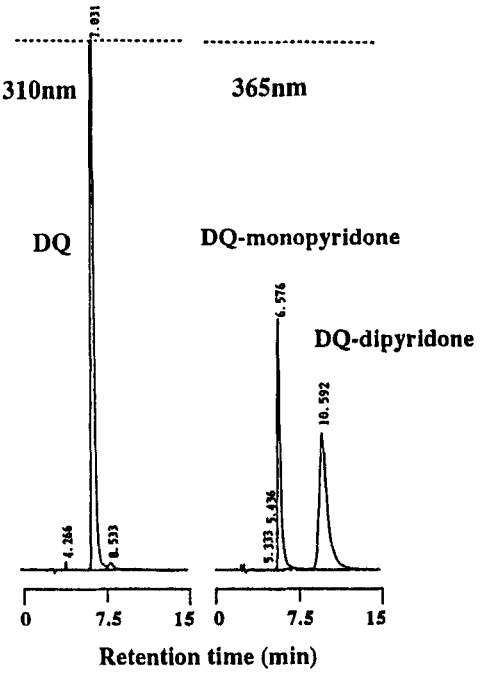


FIGURE 2. HPLC Chromatograms of Diquat(DQ), DQ-monopyridone and DQ-dipyridone in Rat Serum after Deprotenization by Trichloroacetic Acid Solution.

RESULTS AND DISCUSSION

Figure 2 shows typical HPLC chromatograms of blank serum spiked with DQ (10 μg/ml), DQ-monopyridone (5 μg/ml) and DQ-dipyridone (5 μg/ml). The retention time of DQ peak was 6.8 min at 310 nm, but not detected at 365 nm. The retention times of DQ-monopyridone and DQ-dipyridone peaks were 3.3 and

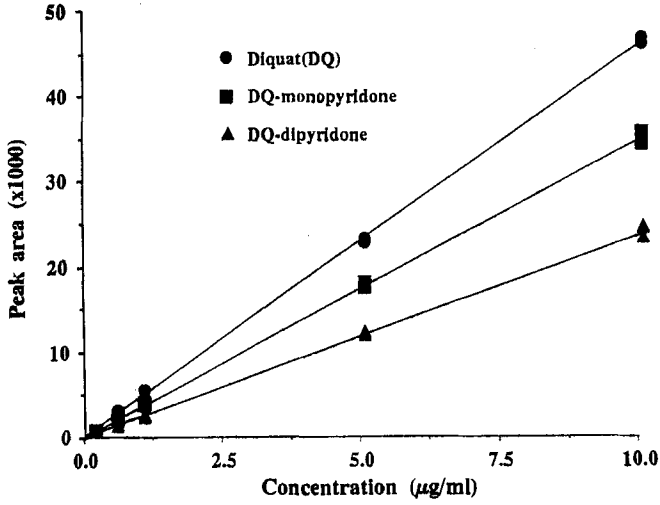


FIGURE 3. Calibration Curves of Diquat(DQ), DQ-monopyridone and DQ-dipyridone in Spiked with Rat Serum after Deprotenization by Trichloroacetic Acid Solution.

These peaks were clearly separated. The retention time of PQ peak at 10 μg/ml was 9.0 min at 310 nm (chromatogram not shown), but its peak was not detected at 365 nm in the analysis of 10μg/ml. PQ solution. No interference by PQ was observed for the quantitation of DQ itself and its two metabolites in this method. Similar chromatograms were also obtained in the analysis of urinary samples.

Calibration curves were produced using absolute peak area of DQ, DQ-monopyridone and DQ-dipyridone in the HPLC chromatograms versus concentrations in serum or urine samples. Figure 3 shows the calibration curves of DQ, DQ-monopyridone

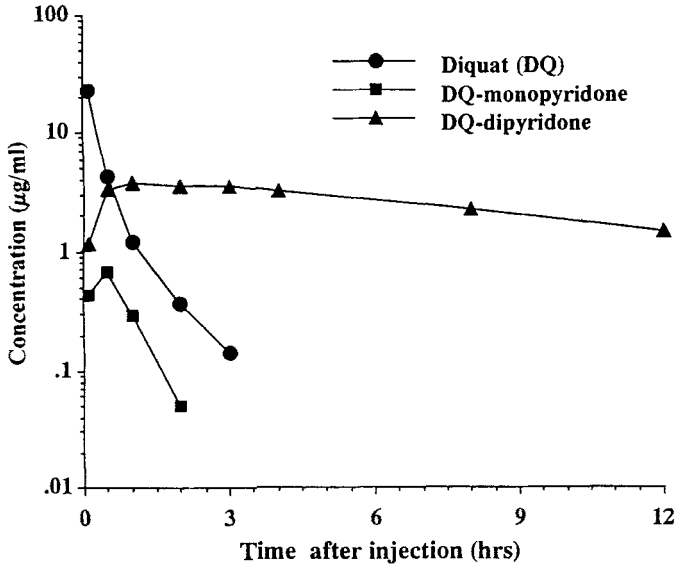


FIGURE 4. Time Course Changes of Diquat(DQ), DQ-monopyridone and DQ-dipyridone in Serum of a Rat Dosed Intravenously(10 mg/kg).

and DQ-dipyridone. Each linear calibration curve of DQ, DQ-monopyridone and DQ-dipyridone was in the range of 0.1 - 10 μg/ml, respectively. Each detection limit of Pq-monopyridone, DQ-monopyridone and DQ-dipyridone was 0.05 μg/ml, respectively. The precision of the method was examined using six replicate analyses of spiked serum and urine at 1.0 μg/ml. The value of the within-day coefficient of variation of the method and the day-to-day were less than 6%.

We applied this method to a rat intravenously injected DQ (10 mg/kg). Figure 4 shows time course changes of DQ, DQ-

monopyridone and DQ-dipyridone concentrations in serum. In urine collected for 24 hours, DQ, DQ-monopyridone and DQ-dipyridone were also detected. These results suggest that DQ is metabolized *in vivo* to DQ-monopyridone and DQ-dipyridone as our previous *in vitro* report(3).

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