Derivative Spectroscopic Determination of Paraquat in Serum
Tsung-Li Kuo

A reliable and sensitive method is described for the quantitative determination of the herbicide paraquat in serum. It involves the formation of dodecyl sulfate–paraquat ion pairs, adsorption of these ion-pairs on XAD-2 resin, and determination of the sensitive derivative spectrum of reduced paraquat.

Analytical recovery of paraquat added to serum was about 98% at concentrations of 0.02–1.0 mg/L. Assay sensitivity is 0.005 mg/L, but this could be increased two- to fivefold by using greater volumes (4–10 mL) of serum/plasma without encountering problems of turbidity. The proposed method also avoids interferences from severe jaundice, which generally occurs in patients with paraquat poisoning.

Additional Keyphrases: toxicology · pesticides · chromatography, ion-pair

Paraquat, a notorious herbicide (N,N′-dimethyl-γ,γ'-dipyridylium dichloride), has been used extensively, commercially and domestically, and paraquat poisoning from accidental and intentional ingestions has been increasing (1–3). The measurement of paraquat in blood is important in assessing the severity and prognosis of paraquat intoxication (4,5). These applications necessitate analytical precision adequate to determine paraquat at submicrograms per milliliter of blood.

Paraquat in serum has been determined by sulfosalicylate deproteinization (6) and ion-pair extraction (7) followed by derivative spectroscopy, a sensitive and less-expensive way of generating derivative spectra by electronic processing (8). However, although the method of Jarvis et al. (6) is rapid, it is unsatisfactory for determining low concentrations of paraquat in poisoned patients with severe jaundice. On the other hand, analytical recovery of paraquat from plasma by the method of Fell et al. (7) is only 73.8% and is limited by the requirement of 2-mL sample sizes, because larger sample volumes will cause emulsion.

Recently, I demonstrated (9) that XAD-2 resin pretreated with an ion-pairing agent, sodium dodecyl sulfate (SDS), gave excellent adsorption of paraquat, the recovery of paraquat from serum being 86.6% in the range of 0.1–20 mg/L. However, to establish a more sensitive method, I developed a method combining derivative spectroscopy with modified XAD-2 column chromatography; I also describe sample pretreatment.

Materials and Methods

Instrumentation: I used a Model 240 (Shimazu, Kyoto, Japan) double-beam ultraviolet–visible recording spectrophotometer equipped with a Model OP 1-2 option program for derivative measurement. Scans were obtained with a 2-nm bandwidth, a scan speed of 180 nm/min, and an absorbance scale of 0.02 A full scale for paraquat concentrations of 0.01–0.1 mg/L or 0.2 A full-scale for 0.1–1.0 mg/L.

Reagents: Amberlite XAD-2 resin, 20–50 mesh (Sigma Chemical Co., St. Louis, MO), was purified according to the method of Stajic et al. (10) and stored refrigerated. Solvent mixtures were prepared by mixing equal volumes of methyl isobutyl ketone and isobutanol (Merck, Darmstadt, F.R.G.), then adding 2 g of SDS (Sigma Chemical Co.) per liter and saturating the organic phase with water. Alkaline dithionite solution was freshly prepared by dissolving 0.1 g of sodium dithionite (Merck) in 10 mL of 1 mol/L NaOH (Merck).

Standard: Standard solutions were prepared by dissolving weighed amounts of paraquat (methyl viologen; Sigma Chemical Co.), dried to constant weight before use, in distilled water or in fetal bovine serum (Bischo Laboratories, Grand Island, NY). These were kept in a plastic bottle at 4°C.

Procedure: To determine paraquat in serum, I slightly modified my previous method (9). Mix 2.0 mL of serum with 8.0 mL of 2.5 g/L SDS (optimal final concentration, 1 g/L) in a test tube and pass this solution through a syringe packed with 2 mL of purified resin. Elute, extract, and reduce paraquat with solvent mixtures, NaCl solution, and dithionite reagent, respectively, as in the previous method (9). Record the second-derivative spectrum of reduced paraquat from 410 to 380 nm in a 1-cm (1 mL) quartz semimicro cuvette vs a freshly prepared reagent blank. Run each sample four times to ensure precision of measurement. Measure the peak amplitude at 392 nm with respect to the adjacent satellite peak (at a longer wavelength). Prepare a standard curve by treating standards the same as test samples and calculate the sample concentrations by reference to this curve.

Pretreat serum samples with visibly severe jaundice with trichloroacetic acid (TCA). Mix 2 mL of serum with 8 mL of TCA (62.5 g/L) in a test tube. Centrifuge, and wash the precipitate twice with 2 mL of 50 g/L TCA solution. Combine the supernates (about 13 mL), then add 1.2 mL of 25 g/L SDS solution. Apply this mixture to the XAD-2 column and wash thoroughly with distilled water until the filtrate is neutralized (test with Hydrion pH paper). Elute and determine paraquat as described previously.

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Results

Standard curves and linearity. Standard curves were plotted as second-derivative peak amplitudes (arbitrary units) vs paraquat concentrations. The respective least-squares slopes, intercepts, and correlation coefficients of the results were: 31.97 (SD 0.11), −0.19 (SD 0.06), and 0.9999 for paraquat concentrations of 0.1–1.0 mg/L, and 302.0 (SD 4.6), −0.05 (SD 0.30), and 0.9990 for 0.01–0.1 mg/L concentrations. The detection limit is 0.005 mg/L.

Precision. The coefficients of variation (CV) for 0.1 and 1.0 mg of paraquat per liter (in bovine serum) were 3.6% and 2.6% (n = 12 each) within a run and 4.4% (n = 10) and 3.1% (n = 18) day to day.

Analytical recovery. The mean recovery of paraquat added to bovine serum in the range of 0.02 to 1.0 mg/L was 86.0% (Table 1). The recoveries of paraquat from serum with and without TCA treatment were not significantly different.

Interference. Figure 1A, a typical second-derivative spectrum of serum from a paraquat-poisoned patient with severe jaundice, determined by the method of Jarvie et al. (6), shows no absorption peak of paraquat at 392 nm. Figure 1B shows results for the same serum sample by the proposed method with TCA pretreatment; the absorption peak at 392 nm was clearly observed and the paraquat concentration in serum was estimated as 0.09 mg/L. In general, interference from hemolyzed blood (hemoglobin content as great as 20 g/L) is not significant by the proposed method.

Method comparison. The concentrations of paraquat in serum from 22 poisoned patients were determined by the proposed method and by that of Jarvie et al. (6) concurrently. The correlation between the two methods (Figure 2) was good (r = 0.9993), with a slope of 1.026 (SD 0.006) and intercept of 0.010 (SD 0.028) mg/L. The standard error of estimate was 0.078 mg/L.

Discussion

The major modification of the previous method (9) is that XAD-2 resin was used without SDS pretreatment. Alternatively, I mixed the serum samples with SDS before passing them through the XAD-2 column. This method is convenient for clinical chemists because the purified resin is already widely used in laboratories to extract drugs or poisons from biological materials.

The liver damage by paraquat makes jaundice not uncommon in patients with acute poisoning. Jarvie et al. (6) reported no significant interference of bilirubin (181 μmol/L) in the derivative spectrum of paraquat. However, when I used their method to assay a severely jaundiced blood sample, the resulting yellow background interference completely masked the paraquat absorption spectrum. Furthermore, the method of Jarvie et al. (6) has lower sensitivity because of the dilution of serum sample through the additions of protein-precipitating and dithionite reagents.

Neither this proposed method nor the method of Fell et al. (7) is subject to interference by high bilirubin concentrations in serum/plasma. The sensitivity of this method is equal to that of their method for 2-mL serum samples. Moreover, the sensitivity of the proposed method could be increased two- to fivefold by using 4–10 mL of serum samples, while avoiding the disadvantage of turbidity resulting from the method of Fell et al. (7).

This technique for the assay of paraquat by the combination of derivative spectroscopy and ion-pair liquid–solid extraction is more efficient and convenient than the traditional liquid–liquid extraction protocols (7).

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Table 1. Effect of TCA Treatment on the Recovery of Paraquat from Serum

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<tr>
<th>Paraquat concn., mg/L</th>
<th>Analytical recovery, %</th>
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<tr>
<td></td>
<td>With TCA</td>
<td>Without TCA</td>
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<tr>
<td>0.02</td>
<td>85.3 ± 2.1</td>
<td>86.3 ± 2.0</td>
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<td>0.05</td>
<td>85.0 ± 2.0</td>
<td>85.9 ± 2.0</td>
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<td>0.40</td>
<td>85.8 ± 1.5</td>
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<tr>
<td>1.00</td>
<td>85.3 ± 1.4</td>
<td>85.6 ± 1.6</td>
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<tr>
<td>Total mean</td>
<td>85.4 ± 1.8</td>
<td>86.0 ± 1.8</td>
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*Mean ± SD (n = 6).

Fig. 1. Second-derivative spectra of paraquat in serum from a poisoned patient with severe jaundice: (A) sulfosalicylic acid deproteinization method; (B) the proposed XAD-2 method.

Fig. 2. Comparison of serum paraquat by second-derivative spectroscopy after XAD-2 isolation, and by the sulfosalicylic acid deproteinization method (6).
Protein Markers of Nutrition Status as Related to Sex and Age
Edward Sechs and Larry H. Bernstein

We assessed age- and sex-related variation in those proteins commonly measured to evaluate protein catabolic losses in patients in acute care. We determined concentrations of iron and iron-binding capacity, transferrin, albumin, prealbumin, and retinol-binding protein in sera from 158 healthy blood donors and 48 hospitalized patients, then grouped the data according to age (by decade) and sex. There was no significant variation in either category. For the total nondiseased population the concentrations of three proteins (means SD, and range) were: transferrin, 2760, 510, 1740-3780 mg/L (population CV 18.5%); prealbumin, 296, 49, 200-390 mg/L (CV 16.6%); retinol-binding protein, 60, 127, 35-85 mg/L (CV 21.2%). Prealbumin and retinol-binding protein concentrations were highly correlated (r = 0.603). Because prealbumin can be easily monitored and its assay is subject to few interferences, we conclude that determination of prealbumin is suitable for assessing the existence and severity of nutritional metabolic deficits.

Additional Keyphrases: variation, source of, prealbumin (transthyretin) - retinol-binding protein; catabolic losses during acute illness - transferrin - iron and iron-binding capacity - albumin - reference interval

Because clinical assessment is unreliable for measuring nutritional deficits, evaluations of albumin and iron and the iron-binding capacity of serum are used in assessing the clinical status of patients with moderate to severe protein-calorie malnutrition. The laboratory also measures nitrogen balance and somatic protein losses, but changes in visceral proteins best reflect severe disturbances in energy metabolism.

In the present study our purposes were to: (a) evaluate the relation between age and sex of the study population and variation in these protein markers, (b) investigate correlations between the various markers, and (c) establish which of them are most responsive to clinical changes in patient status, given the known insensitivity of serum albumin and transferrin as markers in the presence of pre-existing diseases of liver, kidney, or gastrointestinal tract unrelated to nutrition status, and given the relationship of albumin to hydration state and of transferrin to iron metabolism and the long biological half-lives of these proteins in blood. This prompted our evaluation of prealbumin (transthyretin) and retinol-binding protein (RBP) (1) as specific tests for protein-calorie malnutrition and for their usefulness in monitoring repletion of protein stores.

Materials and Methods
We measured albumin, iron, and iron-binding capacity in serum with a BMC 8700 discrete automated analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Albumin was determined by the brom cresol green dye-binding method at 590 nm (2), iron by addition of Ferrozine, measured at 570 nm (3), and iron-binding capacity (TIBC) after saturating the available binding sites on transferrin. Transferrin was routinely calculated from TIBC to provide the data used for decisions as to clinical management.

Prealbumin and RBP were quantified by radial immunodiffusion with "M-Partigen" and "LC-Partigen" plates and standards, respectively (Behring Diagnostics, La Jolla, CA). For comparison with the values calculated from TIBC we also determined transferrin by radial immunodiffusion, using Quiplates and standards from Helena Laboratories, Beaumont, TX.

As controls we used blood obtained from 80 men and 78 women who were volunteer blood-bank donors, ages 20 to 69 years, with a mean of 16 volunteers in each age decade. To establish the correlation of protein markers in the hospital population, we also assayed blood samples from 48 Bridgeport Hospital patients, matched for sex with the control population but randomly selected for age. All but one of the patients had serum albumin concentrations <32 g/L. We also wanted to ascertain the changes in patients who had moderate to severe protein-calorie malnutrition who were being fed intravenously or by oral tube.

References