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Journal of Chromatography B, 775 (2002) 109–113

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

# Simple method for determination of paraquat in plasma and serum of human patients by high-performance liquid chromatography

P. Paixão[a,b], P. Costa[c], T. Bugalho[c], C. Fidalgo[c], L.M. Pereira[a,b,*]

[a] *Faculdade de Farmácia, Universidade de Lisboa, Av. Forças Armadas 1649-019 Lisboa, Portugal*

[b] *Unidade de Farmacoterapia, Nutrição e Estudos Biofarmacêuticos (UFNEB), Lisboa, Portugal*

[c] *General Intensive Care Unit, Hospital Distrital de Santarém, Av. Bernardo Santareno 2000-153 Santarém, Portugal*

Received 29 May 2001; received in revised form 17 April 2002; accepted 17 April 2002

**Abstract**

A simple and fast HPLC system is presented for quantifying paraquat in human plasma and serum using 1,1′-diethyl-4,4′-bipyridyldiylium (diethyl paraquat) as an internal standard. An octadecyl–silica column is used with an eluent of 10% acetonitrile (v/v) containing sodium 1-octanesulphonic acid (3.0 m*M*) and a diethylamine–orthophosphoric acid buffer (pH 3). Unlike with other techniques, sample treatment requires only the precipitation of protein contents by 6% perchloric acid (v/v) in methanol. The method has a limit of detection of 0.1 μg/ml and is linear up to 10 μg/ml. The serum of four patients and the plasma of one patient with paraquat intoxication's were analysed and positive identification and quantification was readily achieved. One of those patients survived, partially given the rapid disclosure of his levels of paraquat. Therefore, this method is suitable for quantification of paraquat in toxicological samples. It may be used as a prognostic tool in critical case detoxification and to quickly identify potentially salvageable patients for enrolment in new hemofiltration studies. © 2002 Published by Elsevier Science B.V.

*Keywords:* Paraquat

## 1. Introduction

Paraquat (1,1′-dimethyl-4,4′-bipyridinium chloride; Methyl Viologen; Gramoxone) is a desiccant and defoliant used in a vast variety of crops, originally prepared in 1882 but only introduced as a herbicide in 1959 [1]. Although its standard use does not present risks due to its inactivation by the natural components of the soil, the ingestion of paraquat can result in severe clinical situations [2]. Death usually occurs within 2 days of ingestion of 50 mg/kg by general organ failure or several weeks later for lower doses due to progressive and irreversible pulmonary fibrosis [3,4].

Treatment of paraquat poisoning remains ineffective, and current approaches rely on diminishing absorption of the remaining paraquat in the gastrointestinal tract [5], removal of blood paraquat by extracorporeal techniques and forced diuresis [6], and maintenance of vital functions. However, the fatal outcome is still dose dependent and several indexes exist correlating plasma-paraquat concentrations with the probability of death [7–9]. Briefly, if plasma-paraquat levels are above 2 μg/ml at 4 h, 0.6 μg/ml at 6 h, 0.3 μg/ml at 10 h or 0.1 μg/ml at 24 h post

*Corresponding author. Fax: +351-21-7938-715.
*E-mail address:* lpereira@ff.ul.pt (L.M. Pereira).

1570-0232/02/$ – see front matter © 2002 Published by Elsevier Science B.V.
PII: S1570-0232(02)00245-3

ingestion, chances are that the patient will not survive. The fast and accurate establishment of the intoxication level may help the physician to decide about the adequate treatment and may be an extra tool for the evaluation of the effects of some new therapeutic procedures [10].

Several methods are available to measure paraquat in plasma, including thin-layer chromatography [11,12], colorimetric methods [13,14], immunoassay [15], gas chromatography [16–18] and high-performance liquid chromatography [19–21].

Nakagiri et al. [19] presented an HPLC method with automated sample pre-treatment that quantified plasma paraquat and diquat in 8 min using special apparatus. Their limit of quantification was 0.1 μg/ml.

Croes et al. [20] described a method for paraquat quantification in plasma requiring an ion-pair extraction on disposable cartridges of end-capped octadecyl silica. Quantification was made using 1,1′-diethyl-4,4′-bipyridyldiylium as internal standard with a lower limit of quantification of 0.025 μg/ml.

Arys et al. [21] reported a method for quantification of paraquat in various human tissues. Their method requires a protein precipitation step, a chemical reduction with sodium borohydride step and an extraction step prior to analysis. Their blood limit of quantification was 0.063 μg/ml.

While some of these methods require special equipment, others are laborious and frequently demand complicated steps, or require extraction of paraquat using expensive disposable cartridges. These facts are important drawbacks in the use of such techniques as prognostic tools in paraquat poisoning, despite their excellent chromatographic properties, perhaps better suited for quality assurance.

This paper presents a simple, rapid and inexpensive HPLC method for quantification of paraquat in plasma and serum. This method has enough sensitivity for the fast evaluation of the intoxicated patient outcome using the normal clinical indexes following paraquat ingestion. In addition it may help to identify those patients that are potentially salvageable. It is being investigated their inclusion in clinical studies for testing of pharmacological therapies for paraquat poisoning, based on its early quantification.

## 2. Experimental

### *2.1. Reagents and standards*

Paraquat dichloride and 1-octanesulphonic acid were obtained from Sigma (St. Louis, MO, USA), perchloric acid (70%) and diethylamine from Merck (Darmstadt, Germany), methanol and acetonitrile (HPLC grade) from Riedel-de-Haën (Seelze, Germany), orthophosphoric acid from BDH Laboratory Supplies (Poole, UK), and diethyl Paraquat diiodide was kindly supplied by Dr. Bruce Woollen from Zeneca Pharmaceuticals (Macclesfield, UK).

### *2.2. HPLC analysis*

A high-performance liquid chromatographic system consisting of a model 1100 isocratic pump, a model 1100 thermostabiliser, and a model 1100 variable-wavelength UV–Vis detector (Hewlett-Packard, Waldbronn, Germany) was used. This system was linked to a PC running a control software HP ChemStation for LC which controls the apparatus and integrates the peaks. The samples were loaded manually via an Rheodyne valve (Cotati, USA) fitted with a 50-μl sample loop. Separation was achieved on a NovaPak $C_{18}$ column (150×4.6 mm, 5 μm particle size) from Waters Corporation (Milford, MA, EUA), and a $C_{18}$ pre-column (100×4.6 mm, 10-μm particle size) from HPLC Technologies (Macclesfield, Cheshire, UK). Paraquat and the internal standard were eluted at 25 °C at a flow-rate of 0.8 ml/min and monitored by UV absorption at 258 nm with an absorbance unit full scale set at 0.02. The mobile phase was an adaptation from Corasaniti et al. [22]. Briefly, a solution containing 3.0 m*M* of 1-octanesulphonic acid, 0.1 *M* orthophosphoric acid in 900 ml of deionized water was made and the pH was adjusted at 3.0 with the addition of diethylamine. Acetonitrile was then added to yield a 10% (v/v) proportion.

### *2.3. Sample preparation*

Stock solution of paraquat was 1 mg/ml in water and in order to make the calibration curve a further dilution was made to 50 μg/ml in water. The diethyl paraquat solution was freshly prepared with 210

μg/ml in water. Plasma, serum and water standards with known amounts of paraquat (0.0, 0.5, 1.0, 2.5, 5.0, 10.0 μg/ml) were prepared. To 200 μl of the different samples, 5 μl of I.S. (210 μg/ml) were added, followed by 45 μl of 6% perchloric acid (v/v) in methanol. Samples were vortex-mixed, left 10 min at −18 °C and centrifuged for 10 min at 2000 *g* in a Mini Centrifuge C-1200 (National Labnet, Woodbridge, USA). Supernatants were saved for HPLC analysis.

### *2.4. Patients and samples*

Patients were admitted at the General Intensive Care Unit, Hospital de Santarém, Santarém, Portugal, following Paraquat ingestion, resulting from either accidental or suicidal intakes, and after confirmation by qualitative urine tests. Blood samples were collected upon arrival on heparinized or non-heparinized tubes. After centrifugation, plasma or serum samples were tested and compared with the severity indexes whenever the blood was collected until 24 h after the ingestion of the herbicide. Often, either the time or/and the amount of the ingestion are difficult to establish, relying on patients' own recognisance or that of their relatives. Therefore an early and accurate assessment of the intoxication is crucial for appropriate medical action.

## 3. Results and discussion

On clinical demand, the described HPLC system was designed to rapidly identify and quantify the presence of paraquat in plasma and serum. For that purpose, 50 μl of the extracts obtained from the blood samples were analysed, as described in Section 2.3. Representative chromatograms are shown in Fig. 1. Blank plasma and serum samples were spiked with internal standard (Fig. 1A) and with paraquat and internal standard (Fig. 1B), without any interference between these substances and the peaks from plasma. The retention times of paraquat and internal standard are 6.5 and 9.5 min, respectively. Similar behaviour was observed with serum samples.

Calibration was performed by adding known amounts of paraquat to blank human plasma or serum in order to obtain final paraquat concentrations. These ranged from 0.5 μg/ml to 10 μg/ml with an internal standard concentration of 1.9 μg/ml. Results were plotted as the ratio of peak areas of paraquat to internal standard versus known con-

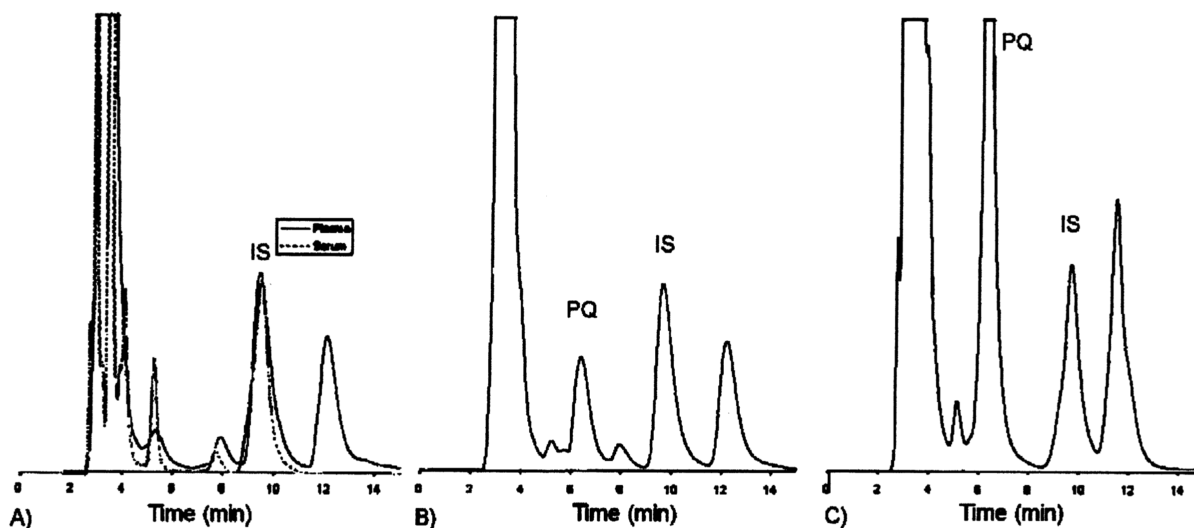


Fig. 1. HPLC chromatograms from plasma and serum samples. (A) Blank human plasma (—) and serum (- - -) with 1.9 μg/ml of internal standard added; (B) blank human plasma with 1 μg/ml of paraquat and 1.9 μg/ml of internal standard added; (C) plasma of a patient of a paraquat poisoning with 1.9 μg/ml of internal standard added. HPLC conditions used as described in text. PQ, paraquat; I.S., internal standard.

centration of paraquat. The calibration curves obtained were linear over the study concentrations range and could be extrapolated to at least 50 μg/ml (data not shown). The linear regression analysis for serum and plasma samples did not indicate a significant deviation from linearity as judged by the coefficient of determination ($r^2$=0.999 and $r^2$=0.998 for plasma and serum data, respectively) and by the pure error test for lack-of-fit which was not significant ($P>0.05$). The detection limit (LOD) was determined by the formula (3.3 SD)/$S$ where $S$ is the slope of the regression line and SD is the standard deviation of the $y$-intercept. The quantification limit (LOQ) was determined by the formula (10 SD)/$S$ with the same parameters as above. The LOD was around 0.1 μg/ml, and the LOQ was around 0.4 μg/ml for both plasma and serum. The plasma and serum paraquat concentrations of the unknown samples were interpolated on this calibration curve.

The intra-assay precision expressed as the coefficient of variation was studied by analysing four replicates of three paraquat concentrations (0.5, 2.5 and 10 μg/ml) and did not exceed 3.4% for plasma data, and 3.2% for serum data. The inter-day precision and accuracy is presented in Table 1. Five different concentrations were studied (as duplicates) over three different days. The mean inter-day coefficient of variation for plasma was 4.7%, with the highest value obtained for 0.5 μg/ml, and 3.1% with the highest value obtained for 0.5 μg/ml for serum. The recovery efficiency was studied by making a calibration curve in water following all the steps described in Section 2.3 and comparing with the results obtained in plasma. The mean plasma recovery value was 98.9% and the mean serum recovery value was 98.7% (Table 1).

Upon arrival at the ICU, paraquat serum levels of four patients were determined. Three of these patients had values much above the survival line (patient 1, 9.48±0.43 μg/ml at 17 h post-ingestion; patient 2, 5.76±0.16 μg/ml at 9 h; patient 3, 39.39±0.37 μg/ml at 5 h) and all had a fatal outcome. The other patient had a serum level below the survival line (0.13±0.02 μg/ml at 5 h post-ingestion) and survived the intoxication without any major complication, fully benefiting from the early clinical detoxification techniques. Another patient was also studied having a plasma paraquat level above the survivor line (patient 5, 20.50±0.07 μg/ml at 9.5 h post-ingestion) and had a fatal outcome.

## 4. Conclusions

An easy, fast, inexpensive and sensitive HPLC method is presented for the quantification of paraquat in human plasma and serum by UV detection. The sample treatment prior analysis consists only of a protein precipitation step by methanol:perchloric acid (6%). The method was used for the prognostic assessment of several human paraquat poisoning situations. All the outcomes were as predicted by the initial paraquat plasma or serum levels, although there were no 'near-borderline' levels that could result in more dubious results. Work is underway to adopt more aggressive critical care detoxification techniques whenever life threatening levels of

Table 1
Inter-day precision, recovery and accuracy of the determination of paraquat in plasma and serum

| Paraquat added (μg/ml) | Paraquat found (μg/ml) | | Recovery (%) | | C.V. (%) | |
|---|---|---|---|---|---|---|
| | Serum | Plasma | Serum | Plasma | Serum | Plasma |
| 0.50 | 0.59±0.03 | 0.52±0.04 | 100.06 | 98.81 | 4.75 | 7.25 |
| 1.00 | 1.10±0.05 | 1.02±0.06 | 100.70 | 103.03 | 4.36 | 5.98 |
| 2.50 | 2.40±0.06 | 2.46±0.12 | 91.69 | 98.87 | 2.50 | 4.68 |
| 5.00 | 4.80±0.15 | 4.98±0.18 | 93.10 | 98.48 | 3.08 | 3.56 |
| 10.00 | 10.09±0.18 | 10.07±0.31 | 98.70 | 100.74 | 1.77 | 3.05 |

Replicate assays were done as described in text. Values are mean±SD ($n$=6); recovery is the amount found in plasma compared with the same amount when done in water in percentage. The coefficient of variation is calculated by the formula (SD/mean)×100.

paraquat are early determined based on this rapid and easy quantification technique.

## Acknowledgements

We are grateful to Dr. Bruce Woollen from Zeneca Pharmaceuticals (Macclesfield, UK) for his kind help in supplying diethyl paraquat diiodide. This work was partially supported by the Portuguese Foundation for Science and Technology.

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