

Review Article

A simple and rapid method for detection of paraquat in human plasma by high-performance liquid chromatography

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Abstract: We have developed an effective analytical method to determine the concentration of paraquat in human plasma by high-performance liquid chromatography (HPLC), which can provide methodological support for diagnosis, therapy, and prognostic evaluation of acute poisoning related to paraquat. The plasma samples were deproteinized with 35% perchloric acid. Then the paraquat was extracted from the samples and separated on Ultimate XB-C18 column with a mobile phase consisting of 0.1 M phosphate buffer (containing 75 mM sodium heptane sulfonate) and methyl cyanides (PH 3.0), flow rate of 1 mL/min, column temperature of 28 °C and a detection wave length on 258 nm. A good linearity was obtained within the range of 0.2 to 500 g/ml in human plasma. The average rate of absolute and relative recovery were about 100.6% and 101.31%, accompanied by the variations less than 3% and 6% respectively. The within- and between-day relative standard deviations were all less than 6%. After repeated freeze and thaw of plasma samples, no significant difference was observed for the concentration of paraquat ($P>0.05$). The method was validated by successfully applying in one patient with acute paraquat poisoning. Due to the celerity, accuracy and no-interference by other components of blood sample, this method was suitable for determination the concentration of paraquat in human plasma.

Keywords: Paraquat, HPLC, plasma concentration

Introduction

Paraquat is a quaternary ammonium compound used as herbicide throughout the world. This herbicide possesses severe toxicity for humans and live stocks with main poisoning route being oral administration [1]. There is a big variation of death rate of paraquat ingestion as reported by different studies and no effective therapeutic method existed in clinical practice. Paraquat can spread to lung, liver, kidney, thyroid gland and muscle through blood circulation, leading to multiorgan failure which is responsible for the death of paraquat poisoning. As the concentration of paraquat in blood was closely associated with survival rate and prognosis, the establishment of plasma paraquat concentration is helpful for the clinicians to evaluate the survival rate and predict the prognosis of the patients [2]. Multiple methods have been applied for detection of paraquat in blood, including ultraviolet spectrophotometry,

chromatography, and capillary electrophoresis [3-5]. The most important step for analysis is extraction of paraquat from the samples. Routine methods used for paraquat extraction are complex and time-consuming, including liquid-liquid extraction, solid phase extraction [6]. Therefore, they are not applicable in clinical practice. In our study, we established a sensitive, rapid and simple method to determine the concentration of paraquat in plasma samples by processing the samples with precipitation of protein and detecting with high-performance liquid chromatography (HPLC). This method possessed the suitability to evaluate of intoxicated patient, predict prognosis, and provide the help for clinic treatment.

Materials and methods

Reagents and apparatus

Chromatographic separation was performed by application of a HPLC system (Fu Li Co.,

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Zhejiang, China). The high-speed centrifuge was purchased from eppendorf Co. (Germany), the vortex mixer from IKA Co. (Germany), and Model AL-204 analytical balance from Mettler-Toledo (Switzerland), respectively. Standard Paraquat samples were acquired from Shang Li Fang Co. (Beijing, China). Sodium heptane-1-sulphonate, Methyl Cyanides, methanol, and triethylamine, of HPLC grade, were purchased from Sinopharm Chemical Reagent Co. (Beijing, China).

Methods

HPLC operating conditions: The HPLC column was an Ultimate XB-C18 analytical column (4.6 mm×250 mm i.d., 5 m) maintained at the temperature of 28°C. The mobile phase consisted of 0.1 mol/L phosphate buffer (containing 75 mM sodium heptanesulfonate) and methyl cyanides (88:12, v/v). The pH was adjusted to 3.0 with triethylamine. The sample was eluted at a flow rate of 1 ml/min and the column eluent was monitored by UV absorption at 258 nm.

Standard preparation: The stock solutions of paraquat were prepared by dissolving 2 mg standard substance with 1 ml ultrapure water in order to obtain a concentration of 2000 µg/ml. The stock solutions were stored at -40°C. Standard solutions of paraquat were prepared by serial dilution of the stock solutions to yield a final concentration of 2, 20, and 200 g/ml, respectively.

Sample preparation: 0.5 ml of plasma sample and 100 l of 35% perchloric acid were vortex-mixed for 1 min in the eppendorf tube. The resulting mixture was centrifuged for 5 min at 13,000 g. Finally, 20 l supernatant was injected to the chromatograph for analysis.

Specificity testing: Chromatograms were obtained after the analysis of the following samples, including blank plasma, 200 g/ml standard solutions, blank plasma + standard solutions, and samples to be measured. From the chromatogram, we can determine the appearance time of paraquat and exclude possible endogenous interferences.

Building a calibration curve: Paraquat was added to the blank plasma to yield final concentrations of 0.2, 0.5, 2, 5, 20, 50, 200, and 500 g/ml. A calibration curve was generated to determine the linear relationship between different plasma concentration of paraquat and

correspondent peak area. Regression equation and the lower limit of detection were acquired from the calibration curve.

Absolute and Relative recovery rate: Paraquat was added to the blank plasma to yield final concentrations of 2, 20, and 200 g/ml. The absolute recovery rate of paraquat was calculated by comparing peak areas of samples at above-mentioned three concentration levels with corresponding concentration standard solutions. Relative recovery rate, sometimes called accuracy, was calculated by evaluating peak areas of five replicate biological samples (same as the absolute recovery study).

Intra-day and inter-day precision: Paraquat was added to the blank plasma to yield final concentrations of 2, 20, and 200 g/ml. The intra-day precision was calculated by analyzing peak areas of the samples at three different concentrations in quintuplicate on the same day. The intra-day precision was also determined by repeating the same procedure for three consecutive days. The intra-day and inter-day precision were depicted as the relative standard deviations (RSDs).

Stability testing: Paraquat was added to the blank plasma to yield final concentrations of 2, 20, and 200 µg/mL. The stability of the test was assessed by monitoring the concentrations of paraquat in following conditions: fresh solutions, stored at -20°C for 1 day and 7 day, after one or two cycles of freeze (-20°C)/thaw (room temperature). Five paralleled paraquat samples were analyzed in different concentrations and time points.

Statistical analysis

All measurement data were shown as Means ± SEM. The result of precision was described as relative standard deviation (RSD). Statistical significances between two and three groups were assessed by the method of least significant difference (LSD) and ANOVA, respectively. Values of $P < 0.05$ were considered statistically significant.

Results

The chromatographic conditions used in our research resulted in a retention time of 7 minutes for paraquat. For plasma samples, no significant interference was found for the retention time of paraquat. A representative chro-

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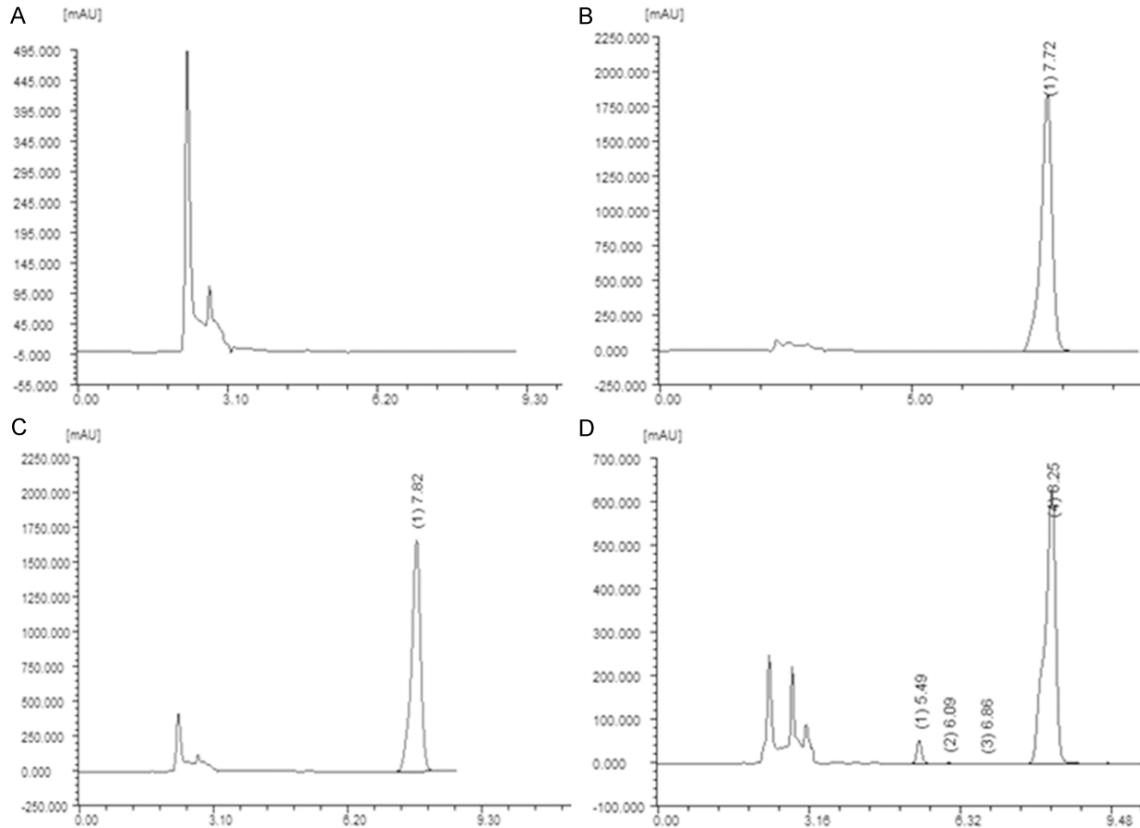


Figure 1. Representative chromatograms of blank plasma sample. (A) Standard solution (200 µg/mL); (B) Blank plasma sample supplemented with standard solution (200 µg/mL); (C) And the patient plasma sample (D). The retention time of paraquat is 7 min.

Table 1. Recovery of paraquat at different concentrations (n=5)

Paraquat added (µg/ml)	Absolute recovery (%)	RSD (%)	Relative recovery (%)	RSD (%)
2	99.75±2.70	2.70	99.08±5.67	5.72
20	100.65±2.56	2.55	103.93±5.38	5.17
200	101.40±2.94	2.90	100.90±4.41	4.37

Table 2. Intra-day and inter-day precision of paraquat (n=5)

Paraquat added (µg/mL)	Intra-day		Inter-day	
	Mean detected (µg/mL)	RSD (%)	Mean detected (µg/mL)	RSD (%)
2	2.03±0.11	5.56	2.03±0.10	5.11
20	21.09±0.80	3.40	20.82±0.93	4.45
200	204.38±6.37	3.12	203.17±7.26	3.57

matogram of different samples was shown in **Figure 1**. The results indicated a high specificity for this method.

The calibration curve generated from different plasma levels of paraquat was linear over the range from 0.2 to 300 g/ml. The minimum detectability of paraquat for the method was 0.1 g/ml. The regression equation was $Y=0.000011X-0.9956$ ($r=0.998$), in which X was the peak area and Y was the concentration of paraquat in plasma (g/ml).

The mean of absolute recovery and relative recovery rate of paraquat, determined at three paraquat plasma concentrations, were 100.6% (RSD <3%) and 101.31% (RSD <6%), respectively (**Table 1**). The intra-day and inter-day precision were all less than 6% (**Table 2**).

The stability of paraquat in human plasma was also evaluated. After one or two cycles of freeze/thaw, no statistical significance was found within different periods (**Table 3**). Moreover, no obvious alteration was found for

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Table 3. The stability of paraquat after freezing/thawing (n=5)

Sample	2 µg/mL		20 µg/mL		200 µg/mL	
	Test results (µg/mL)	RSD (%)	Test results (µg/mL)	RSD (%)	Test results (µg/mL)	RSD (%)
Fresh solutions	2.01±0.12	6.16	21.11±0.80	3.78	204.13±6.06	2.97
1 cycle	2.05±0.08*	4.00	20.75±1.18*	5.70	203.96±7.07*	3.58
2 cycles	1.99±0.12*.#	5.87	20.59±0.70*.#	4.33	201.42±9.41*.#	3.63

Note: *P>0.05, compared with fresh solutions; #P>0.05, compared with 1 cycle.

Table 4. The stability of paraquat after storing (n=5)

Sample	2 µg/mL		20 µg/mL		200 µg/mL	
	Test results (µg/mL)	RSD (%)	Test results (µg/mL)	RSD (%)	Test results (µg/mL)	RSD (%)
Fresh solutions	2.01±0.12	6.16	21.11±0.80	3.78	204.13±6.06	2.97
Storing 1 day	2.01±0.10*	5.17	20.45±1.34*	6.56	208.71±11.99*	5.75
Storing 7 days	2.02±0.15*.#	7.22	20.24±1.40*.#	6.91	204.68±12.15*.#	5.94

Note: *P>0.05, compared with fresh solutions; #P>0.05, compared with storing for 7 days.

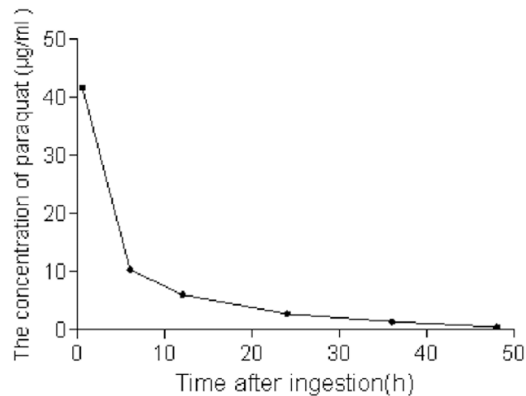


Figure 2. The plasma concentration-time curve of the patient with paraquat poisoning.

the results of detection after storing for 1 or 7 days (Table 4). Therefore, in human plasma, paraquat possessed relative stability for at least 7 days at -20°C and for two freeze/thaw cycles.

Clinical application

To determine the reliability of this method, we applied it to a 32-year-old man admitted to our department after intentionally ingesting 50 ml 20% paraquat solution for 30 minutes. Plasma samples were collected and tested for the levels of paraquat on admission, and at 6, 12, 24, 36, and 48 h after ingestion, respectively. The plasma concentrations of paraquat, detected by this method, were reduced gradually and the patient was survived (Figure 2). Therefore,

emergency medical treatments, including gastric lavage, catharsis, hemoperfusion and anti-oxygen free radical therapy are required in clinic in order to save patients' life.

Discussion

Paraquat is a quaternary ammonium herbicide which has been widely used throughout the world, especially in developing countries. Because of strong polarity and high boiling point, liquid chromatography, especially high-performance liquid chromatography (HPLC), was the most common method for detecting the concentration of paraquat in plasma. Different chromatographs, chromatographic columns, and mobile phases may alter the results of the detection significantly. In practice, a better detection result can be obtained when mobile phase is equipped with ion-pair reagent and chromatographic column with high enrichment property [7, 8]. Due to ionic molecular form of paraquat, the most common extraction method from specimens is solid phase extraction (SPE) [6]. Considering high costs and complicated steps of SPE, we selected a rapid, simple and convenient extraction approach of protein precipitation with perchloric acid [9]. In accordance with the Hara's results, the appearance times of endogenous substances in plasma were all less than 6 min and no significant interference was found for the detection of paraquat.

This method was successfully performed to determine the concentration of paraquat in

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plasma within 30 minutes, which possessed satisfactory peak shape and appropriate retention time. Moreover, the recovery and precision rate were within acceptable performance criteria. Finally, the present method has also been successfully applied in one case involving acute paraquat poisoning. In our study, we found the retention time of paraquat was affected by the with storage time of mobile phase. This may be associated with the changed composition of mobile phase ascribed to evaporation of methyl cyanides. Moreover, when the proportion of sodium heptanesulfonate was decreased or methyl cyanide was elevated, the retention time of paraquat can be shortened obviously (data not shown). In our research, we found a favorable experimental condition, including column temperature of 28°C, a mobile phase containing of 75 mM sodium heptanesulfonate and 12% methyl cyanides in volume ratio. In this condition, potential interference can be excluded and a satisfactory chromatogram can be obtained.

In conclusion, the proposed method provided a fast and simple procedure for the determination of paraquat in human plasma. Evaluating the paraquat poisoning timely and correctly should be useful in avoiding over-treatment and relieving patient's burden in clinical practice.

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Disclosure of conflict of interest

None.

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