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An HPLC Quantitative Analysis of Paraquat in Human Plasma: A Helpful Tool for Diagnosis and Evaluation of Treatment of Paraquat Poisoning in Vietnamese Hospitals

Phuong Vu Anh[a*], Ngan Nguyen Thi[b], Nguyen Thi Kim Thuong[c], Hung Ha Tran[d], Thao Ta Thi[e*]

[a,b]*Poison Control Center, Bach Mai Hospital, Hanoi, Vietnam*

[a,b,c,d,e]*Faculty of Chemistry, VNU University of Science, Hanoi, Vietnam*

[d]*Department of Intensive Care Medicine, Emergency and Toxicology, Hanoi Medical University, Hanoi, Vietnam*

[a]*Email: vuanhphuong86@gmail.com*

[b]*Email: hatranhungpcc@gmail.com*

[c]*Email: ngannt05101993@gmail.com*

[d]*Email: tathithao@hus.edu.vn*

[e]*Email: kimthuongbibi@gmail.com*

Abstract

In this paper, the paraquat (PQ) concentrations in plasma of poisoned patients were determined by high performance liquid chromatography (RP-HPLC) with a DAD detector followed simple extraction of PQ from plasma. The sample was simply pretreated with 15% trichloroacetic acid for deproteinization and directly injected to HPLC system. PQ in plasma was separated on a C8 column HPLC system using 2 channel mobile phase (A and B) with a volume ratio of 5:95, respectively. Channel A was 5% acetonitrile (ACN) and Channel B was a mixture of phosphate buffer (pH 2.5), sodium 1-heptanesulphonate (0.11% w / v), KCl (0.20% w / v), polyethylene glycol G400 (0.20% v / v) and methanol (20% v / v).

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* Corresponding author.

The flow rate of mobile phase was 0.5 mL/min. The method detection limit (MDL) is 0.013 μg/ mL and the quantitative limit is 0.040 μg/ mL. The recovery of PQ in plasma samples was 96.1% - 105.9 % at 5 different concentrations ranging from 0.040 μg / mL to 10.00 μg/ mL. The within- and between-day relative standard deviations were all less than 0.82% and 1.43% respectively. The method was successfully applied for determining paraquat concentrations in plasma samples of 31 acute paraquat poisoned patients at Poison Control Center, Bach Mai hospital, Vietnam. Quantitative results revealed that plasma PQ level was a key factor for prognosis and hemoperfusion using resin membrane had significant effect in removing PQ from the blood.

Keywords: Paraquat; Plasma sample; RP – HPLC; Vietnamese patients.

1. Introduction

Paraquat (1,l' -dimethyl4,4'-bipyridyl chloride, paraquaternary bipyridyl, PQ) is a highly effective contact herbicide but extremely toxic to humans. It is quick-acting and non-selective compound for weed control and inactive upon contact with soil [1]. There have been numerous patients with PQ poisoning due to accidental or suicidal ingestions worldwide [2-4]. The mortality rate of PQ poisoning is very high, usually around 70-85% in several studies [5-8]. The fatal outcome of paraquat poisoning is dose dependent and several indexes exist correlating plasma paraquat concentrations with the probability of death. In the treatment of paraquat poisoning, some promising therapies aimed to eliminate paraquat such as hemoperfusion using activated charcoal filter or resin filter were evaluated clinically.

In Vietnam, paraquat herbicide (there is not any of diquate) is widely used with many trade names. Annually, there are around 1000 paraquat poisoned patients (mainly by killing themselves). The current treatment of PQ poisoning mainly includes diminishing absorption of PQ, increasing elimination of PQ from the body, immunosuppressant therapy and administration of antioxidants. Hemoperfusion (HP) commonly performed is the most effective way to eliminate PQ from blood, which is an important factor related to prognosis, reducing mortality and significantly improve outcomes for PQ poisoned patients. Therefore, quantitative analysis of paraquat in human plasma is fundamental for emergency physicians indicate HP but still lack in hospital laboratories. At present, various methods have been applied to the analysis of PQ in plasma, which include spectrophotometry (UV-Vis) [9,10], GC/mass-spectrometry (MS) [11,12], high-performance-liquid chromatography (HPLC) [13-18], HPLC/MS [19], capillary electrophoresis (CE) [20]. However, some of these methods require special equipment or professional skills.

Therefore, in our study, a simple, rapid and inexpensive procedure utilizing HPLC with ultraviolet (UV) detection was established to determine PQ plasma level in 31 patients with acute PQ intoxication at Vietnam Poison Control Center, Bach Mai hospital, Hanoi, Vietnam from May to August, 2015. The results also revealed the effectiveness of HP in clearing plasma PQ.

2. Experimental

2.1. Chemicals and Reagents

Paraquat dichloride was supplied by Sigma – Aldrich (St. Louis, MO, USA). Methanol, acetonitrile, potassium

chloride, phosphoric acid, polyethylenglycol G400, trichloroacetic acid, sodium 1-heptanesulforic acid and trethylamine were obtained from Merck (Darmstadt, Germany).

Paraquat salts was dried in an oven at 110°C for 3 hr and then cooled in a desiccator. The process was repeated to a constant weight. Stock solutions of paraquat (1mg/mL) was prepared from dried paraquat in appropriate deionized water. The working standard solutions were daily prepared by diluting the stock solution in deionized water and were stored at 4^0C in a refrigerator.

2.2. Sampling and sample preparation

In order to get a representative plasma matrix, a pool of whole plasma was obtained from healthy man in Vietnam Institute of Hematology and Blood Transfusion (Vietnam) was used to make blank sample and standard samples (by adding known amount of paraquat in to blank plasma).

Whole blood sample were also obtained from 31 patients having PQ poisoning admitted to Poison Control Center of Bach Mai Hospital. The 3 ml of blood samples were drawn from the veins of the forearm and immediately transferred to the respective vessels containing heparin. Plasma was separated by centrifugation at 4000 rpm for 15 min and stored under -20^0C until analyzed.

An aliquot of 1.00 mL plasma was pipette into the sample vial of 5 mL, following by 1 mL TCA 5%. Samples were vortex mixed left 1 min at room temperature and centrifuged for 15 min at 4000 rpm in Universal 320 (Hettich, Germany). Supernatant was then filtered through a 0.45 mm pore membrane and ready for HPLC analysis.

2.3. HPLC instrument and operating conditions

An Agilent 1200 HPLC system consists of a model 1200 binary pump SL, a model 1200 series On-line vacuum degasser, a model 1200 thermo-statted column compartment SL, a model 1200 series autosampler and a model 1200 diode array detector (Agilent technology, USA). PQ was separated on a reversed phase column, a Zorbax C_8 (150 mm x 4.6 mm, 5 µm) and a C_8 saveguard column (20 mm x 4.0 mm, 5 µm) from Agilent technology (USA). A 30 µL injected PQ was eluted at 30^0C at flow rate of 0.5 mL/min and detected by UV absorption at 259 nm with an absorbance unit full scale set at 0.02. Mobile phase solution containing 1.10 g sodium heptanesulfonic acid and 2.00g KCl and 2.00 ml polyetylenglycol G400; 200 mL MeOH in 900 mL of deionized water was made. The pH was adjusted to 2.5 by H_3PO_4 before diluted 1000 mL Acetonitrile was then added to yield a 5% (v/v) proportion.

3. Results and discussion

3.1. Optimization of Chromatographic Conditions

On clinical demand, the described HPLC system was designed to rapidly identify and quantify the presence of paraquat in plasma. For that purpose, 30 μL of the extracts obtained from the blood samples were analyzed, as described in Section 2.2.

Paraquat is a hydrophilic compound and positive ion in water at pH 2.5. The separation of paraquat out of plasma matrix could be performed by C8 column in the present of 1.10 mg/mL sodium heptane sulfonate as an ion pairing reagent. At low ion concentration of negative ions, the PQ^{2+} ions was not generated ion pair complexes and completely made the possible elution method PQ of poor, low optical signal and unbalanced. However, as the negative ion concentration was too high, causing the negatively charged complexes efficient low elution. KCl was used to create the environment for the electrolyte buffer solution so as increased levels of this electrolyte charged substances soluble in solution than making the analytes were eluted faster. In this study, using polyethylene glycol (PEG) as MIXEL helped analytes in co-ion pair complex can easily move in the chromatography column. Another hand, PEG being as out-viscosity substances will help to reduce the polarization of matching quality mobile phase analysis and for good separation efficiency.

Several mixtures of acetonitrile and orthophosphoric acid/ dihydrophosphate buffer (pH 2.5) were evaluated as possible mobile phases. It is clear that a combination of orthophosphoric acid/ dihydrophosphate buffer (pH 2.5) and acetonitrile in a ratio of 95 : 5 (v/v) was the most suitable for separating PQ from potentially interfering compounds.

Under the conditions that was presented in section 2.2, the chromatograms of blank plasma sample (Fig. 1A) and spiked human plasma sample containing paraquat (Fig. 1B) were shown. The separation proved that there is not any interference between peak of paraquat and the peaks of other substances from plasma. The retention time of paraquat was 11.89 ± 0.02 min.

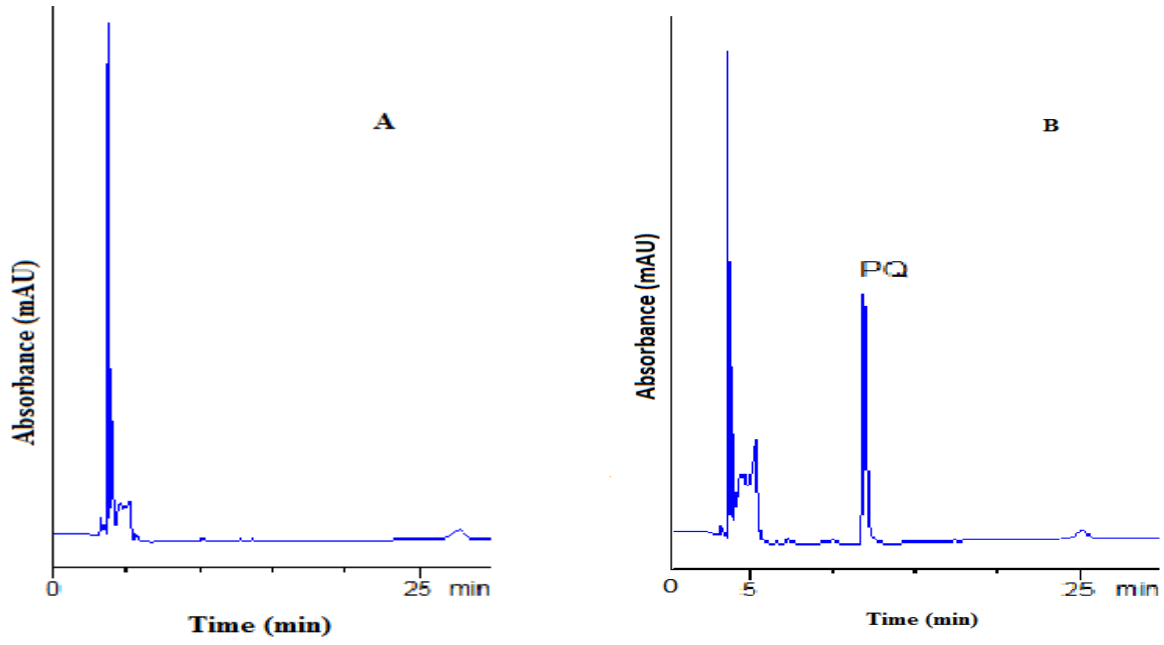

Figure 1: HPLC chromatograms of paraquat in plasma samples.

(A) Blank human plasma, (B) Blank human plasma containing 10 µg/mL PQ

3.2. Validation of analytical method

3.2.1. Linearity

Linearity was checked by generating calibration curves using 1.0 mL of healthy human plasma, spiked with different amount of paraquat to get final PQ concentrations in plasma 0.1, 0.5, 1.00, 5.00, 10.00 µg/mL. The relationship between peak area and amount of paraquat added to the plasma sample can be described by the equation y=0.84+205.06x with r^2= 0.9999. The regression equations for the calibration curves were then used to calculate the paraquat concentrations in plasma of patients.

The LOD and LOQ of the analytical method were calculated based on 3σ and 10 σ rule with blank plasma samples. At a signal-to-noise ratio of 3, the limit of detection of the method (MDL) was 0.013 µg/ml. Taking signal-to-noise ratio of 10, the limit of quantification (LOQ) for paraquat was calculated to be around 0.040 µg/ml PQ for plasma sample.

3.2.2. Precision and accuracy

The spiked samples were used and treated then analyzed as described procedure. The precision was determined by intra-day repeatability and inter-day reproducibility. Intra-day precision was determined by analyzing a spiked sample as same concentration on the same day (n = 5). The relative standard deviation (RSD) values were calculated and were below than 1% for different concentration of PQ in the linearity range. The same procedure was also repeated on different days (n = 4) to determine the inter-day precision. The RSD for intra-day precision was increased by reducing the spiking but almost less than 5%.

The trueness of the studied analytical method was evaluated in term of percentage recovery (%).This recovery was studied by analyzing three times for three paraquat concentrations. The data for the accuracy of the proposed method (Table 1) proved that the mean value of recovery was 96,11% - 105,89% whereas relative standard deviation (RSD) was less than 2.21%.

Table 1: Recovery for paraquat in human plasma sample (n=5)

Amount added (μg/mL)	Amount detected (μg/mL)	Relative recoveries (%)	RSD (%)
0.10	0.11 ± 0.003	105.9	2.83
0.50	0.48 ± 0.013	96.1	2.71
1.00	0.99 ± 0.03	98.2	2.21
5.00	4.99 ± 0.02	99.9	0.37
10.00	10.02 ± 0.03	100.2	0.27

3.3. Application of the proposed method to determine PQ in clinical setting

On admission, patients with acute PQ poisoning were diagnosed on the basis of a medical history of PQ ingestion, the clinical symptoms, and the urinary dithionite test which is a simple and quick method for confirming paraquat poisoning.

Paraquat concentrations in plasma samples from 31 PQ poisoned patients treated at the PCC were determined by HPLC proposed procedure. The results of plasma paraquat concentrations interpreted relative to the time since ingestion (Fig. 2) revealed almost patients hospitalized within 10 hours with plasma PQ levels higher than 10 μg/mL had fatal outcome. Thus, the plasma PQ concentration on admission may be provide important information for prognosis.

In order to assess the validity of HP treatment for blood clearance of PQ, the plasma PQ concentration was determined before and after each HP in the patients received HP treatment (Table 2). The results showed that HP had significant effect in removing PQ from the blood, especially the first HP.

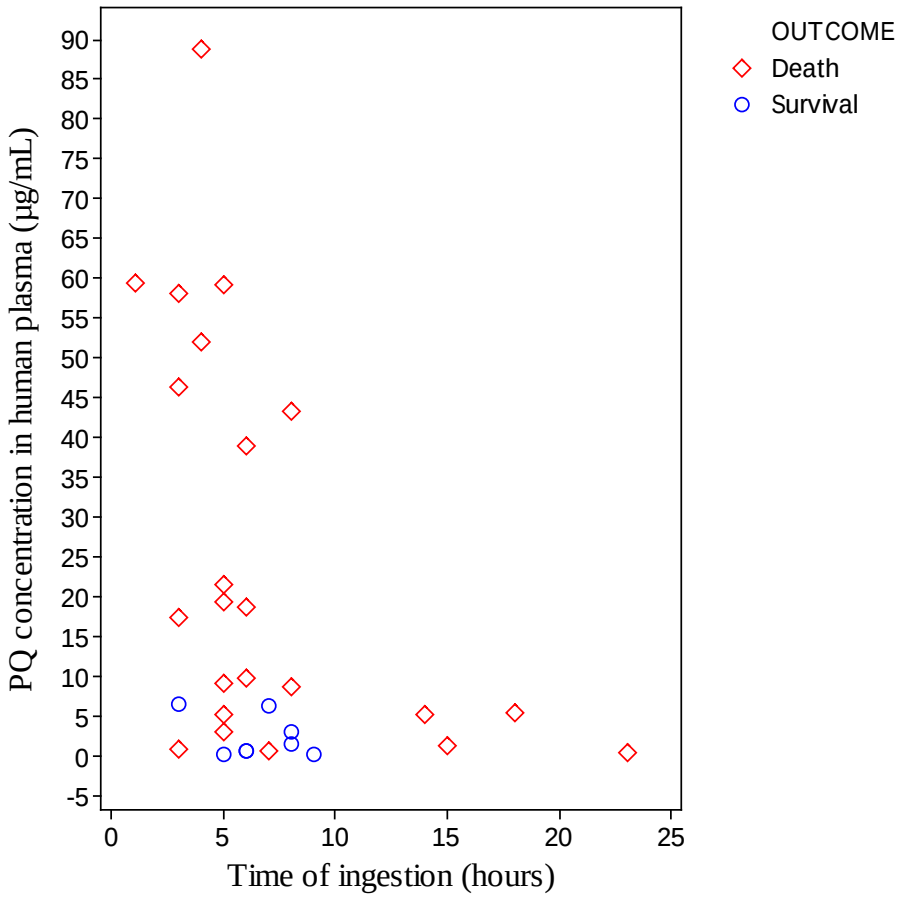

Figure 2: the paraquat concentration on admission in suvival and fatal patients

Table 2: The paraquat concentration in 31 patients with acute PQ poisoning

Number	Age	Gender	Time Ingestion (hour)	PQ concentration in human plasma (µg/mL)					
				First	After HP1	Before HP2	After HP2	Before HP3	After HP3
1	18	Female	6	18.68					
2	21	Male	5	21.48					
3	60	Male	6	38.98					
4	42	Female	8	8.6					
5	63	Female	6	9.77					
6	22	Female	8	3.09	0.26		0.05	0.12	<0.04
7	22	Female	5	19.3	4.40				
8	48	Male	14	5.10	2.32		1.90		
9	51	Female	6	0.63	0.03		<0.04		
10	21	Female	3	17.45					
11	26	Male	8	43.2					
12	54	Female	4	88.66					
13	31	Male	5	0.21					
14	31	Male	18	5.36	0.76		0.48	0.45	0.13
15	27	Female	23	0.51	0.24	0.05	<0.04		
16	55	Male	7	6.34	0.76		0.41		0.24
17	31	Male	4	52.0	3.60		1.70	2.47	1.44
18	18	Male	8	1.56					
19	34	Male	3	6.60	0.48	0.27	<0.04	<0.04	<0.04
20	21	Male	15	1.34	0.50	0.16	<0.04	<0.04	
21	23	Male	5	9.12					
22	55	Female	3	57.97					
23	38	Male	1	59.29					
24	48	Male	3	0.78					
25	24	Female	9	0.29					
26	16	Female	5	59.10	1.31				
27	14	Female	6	0.58					
28	14	Male	7	0.59	0.05				
29	15	Female	3	46.30	1.60		0.60		
30	37	Male	5	3.00	0.05				
31	16	Female	5	5.16	0.23		0.08	0.29	0.04

4. Conclusions and Recommendations

The simple and sensitive method for quantification of paraquat in human plasma was developed using HPLC with UV detection. The sample treatment in this study consisted of only a protein precipitation step by TCA 15% with high recovery and lower limit of detection. The obtaned results of PQ concentrations in Vietnamese human plasma reveiled the useful data for diagnosis and evaluation of treatment of paraquat poisoning in Vietnam. The analytical method was suitable and valuable for diagnosis and prognosis of PQ poisoning, and could be used as an effective tool for studies on modality of extracorporeal elimination of PQ in Vietnaeses laboratories in hospitals at central scale. Compared with very expensive instrument such as LC-MS/MS, HPLC can only be considered as moderat one but it is a very useful instrument that need to be immediately equipped in poison control center of Vietnamese hospitals.

Conflict of interest

The authors declare no conflict of interest associated with this manuscript.

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