# DETERMINATION OF DIQUAT IN HUMAN PLASMA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTOR

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## TÓM TẮT

# XÁC ĐỊNH DIQUAT TRONG HUYẾT TƯƠNG NGƯỜI BẰNG PHƯƠNG PHÁP SẮC KÍ LỎNG HIỆU NĂNG CAO VỚI DETECTOR MẢNG DIODE

*Phương pháp sắc kí lỏng hiệu năng cao với detector mảng diode được phát triển để xác định hàm lượng diquat trong mẫu huyết tương người. Các điều kiện sắc ký thích hợp: pha tĩnh là cột C8, pha động là hỗn hợp dung dịch đệm axetonitril và axit orthophosphoric / dihydroclorua (pH 2,5), tốc độ dòng 0,5 mL/phút, bước sóng phát hiện là 309 nm và thời gian lưu của diquat là 10,03 phút. Khoảng tuyến tính của phương pháp từ 0,5 đến 10 µg/mL, giới hạn phát hiện 0,1 µg/mL, giới hạn định lượng 0,35 µg/mL. Phương pháp đã được phát triển và áp dụng để xác định mẫu huyết tương bệnh nhân ngộ độc diquat tại Trung tâm Chống Độc, Bệnh viện Bạch Mai.*

***Từ khóa:*** *Diquat, high performance liquid chromatography, human plasma.*

## 1. INTRODUCTION

Diquat (DQ) (1,1'-ethylene-2,2'-bipyridylium dibromide) is widely use as non-selective contact herbicide with a rapid desiccant action similar to paraquat (PQ) (1,10-dimethyl-4,40-bipyridinium dichloride). [1] As a herbicide/algicide it is used to control broadleaf and grassy weed in non-crop and aquatic areas. In severe cases, multiple-organ failure can lead to mortality. Giselle's study indicated that the fatality rate of acute Diquat poisoning was 43%.

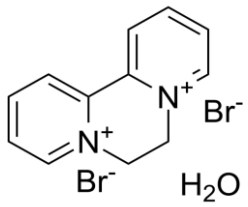


*Figure 1. Chemical structure of diquat*

No specific data have been published, but DQ is believed to have a volume of distribution equivalent to PQ due to similarities in molecular structure and physical properties, this value of PQ is 1.2 – 1.6 l $kg^{-1}$. The drug concentration in the blood peaks after 2 hours, followed by a rapid decrease and is eliminated in the urine. DQ is most concentrated in the kidney, followed by the liver. In the blood, DQ is not bound to plasma proteins. [2]

In recent times, acute DQ poisoning cases began to appear with an increasing number of patients, many serious poisoning cases occurred, causing many patients to die. At the Poison Control Center of Bach Mai Hospital, in the second quarter of 2021, 10 patients with DQ poisoning were hospitalized, of which 7 (70%) patients died. The DQ concentration in plasma has been used as the most common marker to evaluate the

possibility and severity of DQ poisoning. The Therefore, the development of a method for determining DQ in human plasma sample is very necessary.

Several methods have been reported for the determination of DQ such as second derivative UV-Vis absorption spectrometry (UV-Vis) [3][4], gas chromatography – flame ionization detector (GC-FID) [5], gas chromatography-mass spectrometry (GC-MS) [6][7], potentiometric sensor [8], high-performance liquid chromatography [9], high-performance liquid chromatography – UV detector (HPLC-UV) [10], liquid chromatography-mass spectrometry (LC-MS) [11], high-performance liquid chromatography – diode array detector (HPLC-DAD) [12], high-performance liquid chromatography – tandem mass spectrometry (HPLC-MS-MS) [13], liquid chromatography – tandem mass spectrometry (LC-MS-MS) [14], ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS-MS) [15].

In this research, we proposed a new, low cost, high sensitivity and selectivity analytical method for the determination of diquat in the plasma samples applying the HPLC-UV method. The method developed was applied at Posion Control Centre, Bachmai Hospital for diagnosis of diquat acute-poisoning from DQ-intoxicated patients. The collected data were used by the doctors for mortality prognosis and prescription of therapeutic treatments to diquat-intoxicated patients.

## 2. EXPERIMENTAL

### 2.1. Apparatus

An Agilent 1200 HPLC system includes of a model 1200 binary pump SL, a model 1200 series On-line vacuum degasser, a model 1200 thermostatted column compartment SL, a model 1200 series autosampler and a model 1200 diode array detector (Agilent technology, USA). A Zorbax Eclipse XDB-C8 (30 mm x 4.6 mm, 2.5 µm) and a C8 saveguard column (20 mm x 4.0 mm, 5 µm) from Agilent technology (USA). pH values of solutions were controlled with an S220 SevenCompact pH meter (Mettler Toledo, Switzerland).

### 2.2. Chemicals and reagents

All chemicals were of analytical grade or higher quality. Diquat in the dibromide salt form purchased from Sigma Aldrich (Hamburg, Germany) was used to prepare stock solutions of diquat at 100 ppm. Trichloroacetic acid (TCA), ammonia solution ($NH_3$ 25%), methanol, acetic acid (Ace), phosphoric acid ($H_3PO_4$) 85%, sodium 1-heptanesulfonate, potassium chloride (KCl) and polyethylenglycol 400 (PEG 400) were purchased either from Merck or Fluka (Germany).

### 2.3. Sampling and sample preparation

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.

Whole blood samples were also taken from DQ addicts admitted to the Bachmai Hospital PCC. A 3 ml blood sample was taken from a vein in the forearm and immediately transferred to the appropriate blood vessel with heparin. Plasma was separated by centrifugation at 8000 rpm for 7.5 minutes and stored at -20 °C until analysis.

1000 µL plasma aliquots were pipetted into a 5 mL sample vial, followed by pipette 100 µL TCA 50%. The sample was vortexed, left at room temperature for 1 minute, and centrifuged at Universal 320 (Hettich, Germany) at 8000 rpm for 7.0 minutes. The supernatant was then filtered through a 0.45 mm PTFE membrane and ready for HPLC analysis.

### 2.4. Analytical Procedure

The injected 30 µL DQ was eluted at a flow rate of 0.5 ml $min^{-1}$ and detected by UV absorbance at 309 nm in absorbance units set to 0.01 on a full scale. A mobile phase solution containing 1.20 g sodium heptane sulfonate and 2.00 g KCl and 2.00 ml polyethylene glycol G400. Prepared 200 mL of MeOH in 1000 mL of deionized water. The pH was adjusted to 2.5 with $H_3PO_4$ before diluting 1000 mL of acetonitrile to obtain the mobile phase.

### 2.5. Method Evaluation

After optimizing experimental conditions and instrumental parameters, the suitable conditions were selected to determine diquat. The developed method was evaluated linearity calibration curve was built in standard solutions from 0.5 to 10 ppm.

## 3. RESULTS AND DISCUSSION

### 3.1. UV-Vis Spectroscopy

The maximum wavelength of diquat was determined by UV-Vis spectroscopy. 10 mL of diquat was prepared by diluting 100 µg $mL^{-1}$ of diquat with deionized water. A typical UV spectrum is recorded at 400 - 190 nm and is shown in Figure 2. From this we can conclude that 309 is the maximum wavelength of diquat.

### 3.2. HPLC conditions

At pH 2.5, diquat are hydrophilic chemicals, cations in the water. Sodium heptane sulfonate is used as an ion pair reagent. $DQ^{2+}$ ions did not form an ion pair complex at low concentrations of negative ions, resulting in poor, weak, and imbalanced DQ elution techniques. However, if the negative ion concentration is too high, efficient elution of the negatively charged complex will not work. The electrolyte buffer was prepared using KCl and increased the amount of electrolyte charged compound soluble in the solution to allow the analyte to elute faster. In this work, polyethylene glycol (PEG), which is used as a mixel, allowed the analysis target of the coion pair complex to freely flow through the chromatography column. On the other hand, the high viscosity of PEG helps to reduce polarization in mobile phase analysis with reasonable quality and improve separation efficiency.

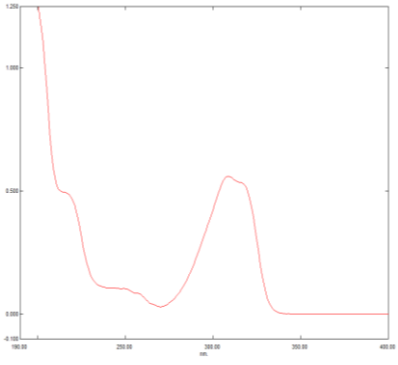

*Figure 2. UV-Vis Spectrophotogram of Diquat*

Many combinations of acetonitrile and orthophosphoric acid/dihydrochloride buffer (pH 2.5) have been tested as suitable mobile phases. It was clear that a gradient mixture of orthophosphoric acid/dihydrochloride buffer (pH 2.5) and acetonitrile was the most suitable for extracting DQ from potentially interfering chemicals.

Chromatograms of a blank plasma sample (Figure 3A) and a spiked human plasma sample with diquats (Figure 3B) were displayed under the conditions described in Section 2.3. Separation showed that the diquats peak did not interact with the peaks of other compounds in plasma. The retention time for diquats was 9.896 ± 0.02 minutes.

### 3.3. Validation of method

#### *3.3.1. Linearity*

Calibration curves were created using 1.0 mL of healthy human plasma spiked with different amounts of diquats and final plasma DQ concentrations of 0.5, 1.00, 2.00, 5.00, 10.00 to give 15.00 µg$ml^{-1}$. The formula y = -11.635 + 159.49x, $r^2$ = 0.9999 can be used to show the relationship between the peak area and the amount of diquats injected into the plasma sample. The patient's plasma diquat concentration was then calculated using the standard curve regression equation. The LOD and LOQ of the analytical methods were calculated using the rules 3 and 10 using blank plasma samples. The diquat detection limit (LOD) and quantification limit (LOQ) was determined to be approximately 0.10 and 0.35 µg$ml^{-1}$ DQ, respectively.

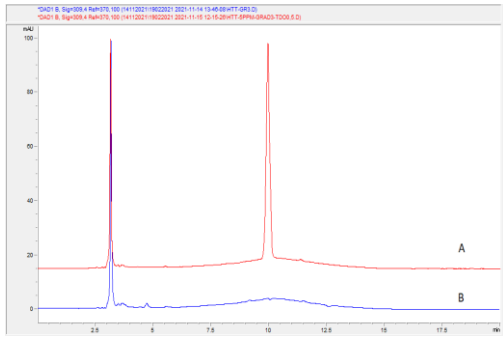


*Figure 3. HPLC Chromatogram of Diquat at human plasma sample: A: Diquat 5.0 µg$ml^{-1}$, B: blank sample*

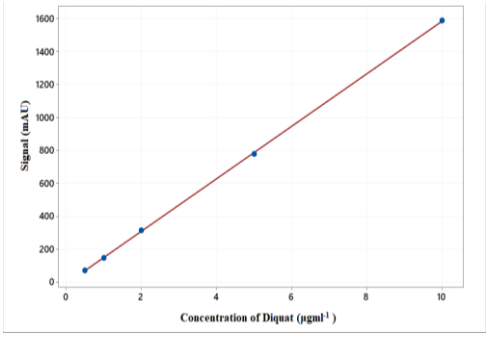


*Figure 4. Calibration curve of Diquat for increased concentration of diquat spiked in plasma samples from 0.5 to 10 µgml$^{-1}$*

### *3.3.2. Precision and accuracy*

The spiked samples were used, processed and analyzed according to the techniques shown. The accuracy was estimated using daytime reproducibility. On the same day (n = 5), spike samples of the same concentration were analyzed to determine intraday accuracy. Relative standard deviation (RSD) values were determined for various concentrations of DQ within the linearity range and were found to be less than 1%. Lowering the spikes improved the daily accuracy RSD, but only about 5%. The validity of the analyzed method was evaluated as a percentage. This recovery was examined three times for three different diquat doses. The average recovery was 95.83% to 98.36% and the coefficient of variation (RSD) was less than 2.30%.

*Table 1. Intraday accuracy of method*

| Concentration (µg ml$^{-1}$) | Coeffficient variance (n=5) |
|---|---|
| 1 | 2.28 |
| 5 | 0.67 |
| 10 | 0.24 |

## 4. APPICATION

Patients with acute DQ poisoning were identified at admission based on a urinary dithionite test, which is a rapid and easy way to confirm the history of DQ intake, clinical symptoms, and diquat poisoning. HPLC was used to measure diquat levels in plasma samples of 13 DQ addicts treated with Poison Control Center, Bach Mai Hospital. Because it is a new toxin in clinical practice, many properties and related problems in the treatment of acute DQ poisoning have not been clarified. For example, plasma DQ concentration at the time of admission can be used to predict mortality in acute poisoning patients as studied with PQ. The test can also be used to evaluate the effectiveness and compare the effectiveness of dialysis measures by determining the DQ concentration in the patient's blood and the DQ concentration in the blood after passing through the dialyzer. Or other similar studies to find out the most effective treatment regimen, helping to improve mortality and sequelae in acute poisoning with DQ herbicide.

## 5. CONCLUSION

Using HPLC with UV detection, we have established a simple and sensitive technique for quantifying diquat in human plasma. In this operation, the only sample treatment was the TCA 50% protein precipitation step, which showed good yields and reduced detection limits. The DQ concentration obtained from human plasma in Vietnam provided important information for the diagnosis and evaluation of diquat addiction therapy in Vietnam. The analytical approach is accepted and beneficial in the diagnosis and prognosis of DQ addiction and can be widely used as an efficient tool for the study of in vitro DQ removal modality in Vietnamese laboratories and hospitals. Compared to more expensive equipment like LC-MS/MS, HPLC is only a budget option, but it is a very valuable tool that needs to be installed in a toxicology control center in a Vietnamese hospital as soon as possible.

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