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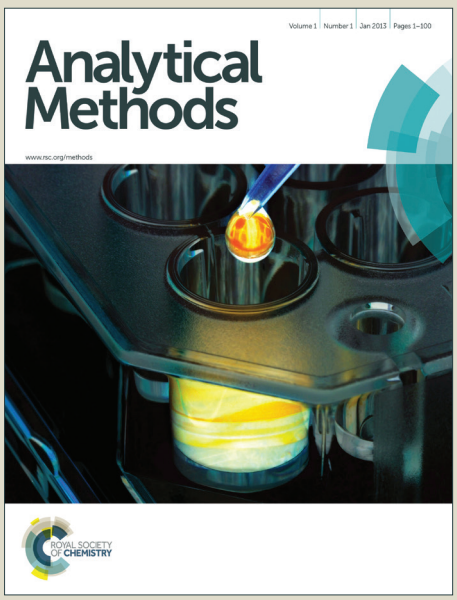

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Reversed-phase High Performance Liquid Chromatography Method for the Determination of Paraquat in the Whole Blood

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Abstract: In this paper, high performance liquid chromatography for quantification of paraquat in the blood is established. Samples were treated after joining hydrochloric acid for precipitation of protein, through acetonitrile extraction, and by ultrasound, centrifugation, filtration such pretreatment processes. In addition, Sodium heptane/acetonitrile/water (1.82 g/50 mL/450 mL) buffer solution was used as mobile phase and C_{18} column as the stationary phase and variable wavelength UV detector was carried out on samples of paraquat liquid chromatography separation and determination. The linear range was 0.3 ~30 μg mL^{-1} (R=0.9999) with the minimum detection limit of paraquat 0.026 μg mL^{-1} (S/N≧3). The limit of quantification for paraquat was 0.08 μg mL^{-1}. Moreover, the method proposed here is sensitive, accurate. Furthermore, the method proposed here will have a bright further in widely practical application.

Key words: High performance liquid chromatography; Paraquat; Blood analysis; Clinical analysis

1. Introduction

Paraquat (1,1-dimethyl-4,4-dipyridinium chloride) (PQ), a quaternary nitrogen herbicide, has been widely used in several crops in view of its great efficiency and low cost, and sold in about 130 countries for the use on farms, plantations and estates and in non-agricultural weed control[1]. However, undesirable characteristics include high toxicity for plants and aquatic organisms[2] and increasing cases of accidental or intentional intoxication of humans have been widely reported.[3] Due to the widely exists in rural, there are seventy percent of the people to commit suicide by it. The lung is one of the primary target organs of paraquat. It may cause lung congestion, pulmonary fibrosis, hemorrhage, edema, hyperplasia and degeneration, etc and pulmonary fibrosis is an important toxic effect of paraquat. It is also known to produce "Parkinsonism" in man.[4] In China, the intentional ingestion of paraquat has caused a large number of human fatalities.

Thus far, there are lots of reports of the analysis of paraquat in water, such as using GC-MS[5], flow system[6], capillary electrophoresis[7-9], high performance liquid chromatography (HPLC)[10-12], capillary electrophoresis mass spectrometry[13] and thin layer chromatography[14]. Compared with other reported methods, HPLC method has attracted increasing attention due to its excellent properties such as high separation efficiency, highly sensitive, wide range of applications and it has been

developed and extensively researched among the biological fluids and the practice. As far as we know, no reports have been published for a quantitative determination method for paraquat in whole blood.

In this paper, a selective, sensitive, accurate and reliable method was developed to determine the concentrations of paraquat by combining preconcentration procedures using the HPLC with UV and fluorescence detection and the design for pretreatment method has also been improved. Twice extracted with an organic solvent acetonitrile, the filtrate was blown with nitrogen stream, sodium heptane/acetonitrile/water (1.82 g/50 mL/450 mL) buffer solution as the mobile phase, C_{18} column as the stationary phase and a variable wavelength UV detector, paraquat concentration 0.3 ~ 30 μg mL^{-1} range and the peak area was a good linear relationship; On the concentration of 0.30 μg mL^{-1} of paraquat for 11 consecutive parallel determinations relative standard deviation was 4.7% and the detection limit was 0.026 μg mL^{-1} (calculated according to IUPAC standard method). The method is sensitive, accurate and satisfactory results.

2. Experimental

2.1 Apparatus and Reagents

Chromatographic experiments were performed using a Waters 600 pump (Milford, MA, USA) equipped with a Rheodyne 7725i injector (Cotati,

CA, USA) and a Waters 2487 dual λ absorbance detector. Chromatographic data were acquired and processed by Empower chromatography manager 2.0 software. The detection wavelengths were set at 290 nm. The flow rate was set at 1.0 mL min^{-1}, and the column temperature at 25 ^{o}C. Microsyringe (50 μL) was used to inject a certain volume of sample that the injection volume was 10 μL. The hold-up time was determined from the first perturbation of the base line. A XTerraRP18 Chromatography column (150 mm*4.6 mm, 5 μm, Waters Corporation) was connected as stationary phase. High-speed centrifuge (TG16G Xiamen Jingyi Industrial Technology Co.). PHS225 pH meter (Shanghai Precision & Scientific Instrument Company). Ultrasound equipment (ULTRASONIC CLEANER SB32000). Milli-Q Gradient pure water manufacturing system, Filter device (membrane pore size of about 4.5 μm).

Sodium heptane (98%, chromatographic pure, Shanghai Jingchun reagents Ltd.), Acetonitrile (99.9%, chromatographic pure, Shanghai Chemical Reagent Research Institute), Hydrochloric acid (36.0~38.0%), Phosphoric acid (≥ 85%), Triethylamine (≥ 99%, AR Shantou Xilong Chemical Co.), Water (freshly distilled twice-distilled water). Paraquat reference substance (99.5% U.S. Dima technology companies). Blood of the poisoned person was proved by Zhangzhou Hospital. All of the chemicals were used directly without further purification.

2.2 Preparation of standard solution

All working standard solutions were freshly prepared every week and stored at 4 °C. 0.0305 g paraquat which was dried at 100 °C for 2 h before use reference substance plus twice-distilled water was dissolved in 100 mL volumetric flask. Preparation of standard solution (3.0×10^{-4} μg mL^{-1}) was stored at 4 ^{o}C refrigerator. Standard solution was prepared by diluting the working fluid.

2.3 Sample pretreatment

Healthy human blood and patient's blood were provided from Zhangzhou Hospital. 1.0 mL healthy human blood (add a certain amount of known concentrations of paraquat reference) is mixed with 0.40 mL of hydrochloric acid (0.50 μg mL^{-1}), ultrasound 5 min, in a mixture of 2.0 mL of acetonitrile, ultrasound 15 min, then high-speed centrifuge to 8000 r min^{-1} for 15 min speed, remove the supernatant. Then mix 2.0 mL of acetonitrile and centrifugation 10 min at 8000 r min^{-1} speed. Merge two supernatants. The solution was filtered through 0.45 μm pore membrane filter, the filtrate evaporated to a stream of nitrogen 1.0 mL. The same manner to the paraquat of patient's blood sample. Adopted HPLC external standard method. Human procedures were in agreement with the guidelines of Nuremberg Code, Declaration of Helsinki, Belmont Report and the regional ethics committee for human experiments. The human experiments were approved by the Zhangzhou department of health.

2.4 Operating conditions

Mobile phase flow rate: 1.0 mL min^{-1}; column temperature: room temperature 25 ^{o}C; detection wavelength: 290 nm; Injection volume: 10 μL; paraquat retention time: about 3.7 ~ 4.2 min.

2.5 Analytical procedure

10 μL sample injection in XTerraRP18 column (150 mm × 4. 6 mm). Triethylamine-phosphate buffer solution (approximately pH 2.0) and sodium heptane acetonitrile-water as the mobile phase. To 1.0 mL min^{-1} flow rate, column temperature of 25 ^{o}C in conditions, detected at 290 nm. Set 1.0 mL min^{-1} of water flow rate, 25 ^{o}C under the conditions of the column and detected at 290 nm. This separation of work completed within six minutes. The method has been successfully the analysis of real samples, reproducibility and accuracy.

3. Result and discussion

3.1 Optimization of sample preparation

Sample pretreatment is a critical step in the analysis of drugs from whole blood. Three methods including protein precipitation, solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are often used for extraction of drugs. LLE is a traditional extraction method and useful for extraction of paraquat from whole blood. However, LLE usually cause emulsion formation and mutual solubility of the analytes in two phases, or it is difficult to extract the drug from the tissues.

In previous reports of HPLC analysis of paraquat in water, the retreatment procedure was conducted only by dilution and centrifugation of the samples. It would be difficult to apply that method to whole blood. Other method before the extraction, the hydrochloric acid was added, by ultrasound, the acetonitrile was added, by ultrasound, centrifugation to separate in our method. However, the sample was directly extracted by trichloroacetic acid solution (TCA).[15] The permeability of trichloroacetic acid solution was not good enough for the direct extraction, because that the blood samples are complicated (without the ultrasonic treatment，many drugs are difficult to extract from the other components of the blood). Therefore, the efficiency of the extraction in our methods is much better. Moreover, it was without subsequent complex processing.

3.2 Chromatogram of standard for mixtures of paraquat

The calibration standards for paraquat were analyzed by HPLC. Chromatogram of 15 μg mL^{-1} paraquat reference substancence solution of HPLC was shown in Fig. 1, and healthy human blood added with 15 μg mL^{-1} paraquat reference was shown in Fig. 2. The results of representative chromatogram indict that both of them have the same retention time.

Fig. 1

Fig. 2

3.2 Linearity

Linearity was checked by constructing the calibration curves using spiked drug which 1.0 mL blood from seven healthy human (whole blood), mixed with different concentrations of paraquat reference. Preparation of 0.30 μg mL^{-1}, 1.5 μg mL^{-1}, 3.0 μg mL^{-1}, 6.0 μg mL^{-1}, 12 μg mL^{-1}, 15 μg mL^{-1}, 30 μg $mL^{-1}$was used here. The relationship between the peak area and the amount added to the sample can be described by the following equations (Fig. 3): y = 6189.7x-786.51. The regression equations of the calibration curves were then used to calculate the analyte concentrations in patient's blood. The correlation coefficient was 0.9999 for paraquat.

Fig. 3

At a signal to noise ratio (SNR) of 3.3, the limit of detection (LOD) for paraquat was calculated to be 0.026 μg mL^{-1}. Taking SNR=10, the limit of quantification limit (LOQ) for paraquat was calculated to be 0.079μg mL^{-1}. The detection limit illustrates that the paraquat at 0.30~30 μg mL^{-1} range has good sensitivity and a wide linear range.

3.3 Precision and accuracy

The accuracy and precision data of the proposed method can be seen in Table 1. Linearity was checked by constructing the calibration curves using 1.0 mL healthy human blood (whole blood), sequentially added paraquat to 0.08 μg mL^{-1}, 0.5 μg mL^{-1}, 1.8 μg mL^{-1}, 3.0 μg mL^{-1}, 9.0 μg mL^{-1} were evaluated, the relative standard deviation (RSD) values were calculated which were 6.6%, 5.8%, 5.5%, 5.1% and 2.3%, respectively. The RSD value of paraquat was improved by increasing of the spiking level. In the same way, the RSD values for five spiking levels with paraquat at 0.3, 0.6, 1.2, 2.4 and 8.4 μg mL^{-1} were also evaluated, which were 5.9%, 3.3%, 3.6%, 9.4% and 7.6%, respectively. In addition, on the concentration of 0.30 μg mL^{-1} of paraquat for 11 consecutive parallel determinations RSD was 4.7%.

Average recovery values were calculated by the paraquat standard solution added to the whole blood samples. Experimental recoveries of analyte are shown in Table 1. The average recovery values were between 97.9~107.4% (n=5) for five spiking levels with paraquat.

Table 1

3.4 Application of the method to analysis of paraquat in clinical tests

This method was applied for toxicological examination in the case of suspected paraquat intoxication. Blood samples from four patients with paraquat poisoning were used as the HPLC analysis. The concentration of

paraquat of their blood samples was detected before and after perfusion. The analysis results to the two of cases in clinical tests are shown in table 2 and table 3, each chromatogram refers to each check in table 2 and table 3 was shown in Fig. 4 and Fig. 5, respectively. Fig. 4 showed that they did not interfere with absorption at 290 nm at range of 3.7 to 4.2. The results proved the rapid and robustness of the method which was successfully applied to the analysis of paraquat in clinical tests.

Table 2

Fig. 4

Table 3

Fig. 5

4. Conclusion

In this study, HPLC method was developed and successfully applied for the determination of paraquat in blood. The method of the pretreatment of blood analysis sample was simple, and the treated samples have no interference to the test results. In addition, the linear range was 0.30~30 μg mL^{-1} with the minimum detection limit of paraquat 0.026 μg mL^{-1} (S/N≧3). The method proposed here is sensitive and accurate, which will have a bright further in widely practical application.

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Figure Captions

Fig. 1 Chromatogram of paraquat reference substance

Fig. 2 Healthy human blood samples added with Paraquat reference substance (15 μg mL^{-1}) chromatogram

Fig. 3 Working curve of blank whole blood plus reference substance

Fig. 4 The relative chromatograms (a-f) of determination of the concentration of paraquat in blood of case 1, Mr Huang. Inset is the chromatograms of blank whole blood.

Fig. 5 The relative chromatograms (a-f) of determination of the concentration of paraquat in blood of case 2, Mr Chen.

Table captions

Table 1. Determination results and recoveries of paraquat in blood samples (n = 5)

Table 2. Determination of the concentration of paraquat in blood of case 1, Mr Huang

Table 3. Determination of the concentration of paraquat in blood of case 2, Mr Chen