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Optimized ultra performance liquid chromatography tandem high resolution mass spectrometry method for the quantification of paraquat in plasma and urine

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ABSTRACT

A simple, sensitive and specific ultra performance liquid chromatography coupled to electrospray tandem high resolution mass spectrometry (UPLC-ESI-HRMS/MS) method has been developed and validated for quantification of paraquat in plasma and urine. The sample preparation was carried out by one-step protein precipitation with acetonitrile. The paraquat was separated with a HILIC column in 10 min. Detection was performed using Q Exactive Orbitrap mass spectrometer by Targeted-MS/MS scan mode. Methodological parameters, such as ammonium formate concentration, formic acid concentration, spray voltage, capillary temperature, heater temperature and normalized collision energy were optimized to achieve the highest sensitivity. The calibration curve was linear over the concentration range of LOQ-1000 ng/mL. LOD was 0.1 and 0.3 ng/mL, LOQ was 0.3 and 0.8 ng/mL for urine and plasma, respectively. The intra- and inter-day precisions were <7.97% and 4.78% for plasma and urine. The accuracies were within the range 93.51-100.90%. The plasma and urine matrices had negligible relative matrix effect in this study. This method was successfully applied to determine paraquat concentration in plasma samples with hemoperfusion from 5 suspected paraquat poisoning patients.

1. Introduction

Paraquat (PQ) is a widely used non-selective and fast-acting contact quaternary ammonium herbicide. It is suitable for many agricultural uses due to its high solubility in water, low vapor pressure and its ability to bind to soil [1]. However, over the last few decades, more and more incidents about accidental and intentional poisoning of PQ have been reported, and the mortality rate is high especially in Asia [2–4]. The primary route of exposure to PQ is ingestion, as well as inhalation and dermal contact. Unlike many other poisons, there are no clinically effective detoxification agents for PQ poisoning and the treatments are often unsatisfactory or even useless. Once PQ absorption, the toxic effects can occur in multiple organs. It primarily accumulates in the lungs, resulting in acute pulmonary distress, and it also has drastic effects on the gastrointestinal tract, the kidneys, the liver, and the heart [5,6]. Additionally, PQ is believed to be associated with Parkinson's disease, because of its deleterious effects on the dopaminergic neurons [7,8].

The rescue for PQ poisoning consists of removing PQ from the stomach with bentonite and from the blood via hemoperfusion [5]. Recently, PQ levels in plasma and urine have been successfully used to predict survival or death using the nomogram method [9]. The concentration of PQ in plasma has a higher predictive value with respect to prognosis, and the urine PQ level contributes to a more rapid evaluation [10]. Therefore, monitoring plasma and urine concentrations of PQ can help to provide valuable information for diagnosis and prognosis.

Several methods have been reported for the determination of PQ in different matrices, including water [11–13], agricultural products [14,15] and biological matrices [3,16,17] with gas chro-

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matography (GC) [18], GC-mass spectrometry (MS) [19], high performance liquid chromatography (HPLC) [20] and HPLC–MS [17]. Capillary electrophoresis (CE) [21], CE-MS [22], enzyme-linked immunosorbent assays (ELISA) [23] have also been reported for the quantification of PQ. Among these methods, HPLC–MS is the most popular analytical approach for qualitative and quantitative determination of PQ, due to its doubly charged cationic characteristic in solution, which is beneficial to ionization and MS responses.

Due to the hydrophilicity of PQ, ion-pairing reagents such as heptafluorobutyric acids (HFBA) [24] and trifluoroacetic acids (TFA) [16], which can retain PQ on LC separation columns, were used in some HPLC–MS methods. Nevertheless, the sensitivity of MS detection was greatly decreased by the ion-pairing reagents in the mobile phase due to ion suppression, and ion-pairing reagents added extra contaminants to the MS system.

The Hydrophilic Interaction Liquid Chromatography (HILIC) is an alternative approach for separating polar compounds, followed by MS analysis [25]. It has been reported that HILIC is a variant of normal phase liquid chromatography (NP-LC), but the separation mechanism is more complicated than NP-LC. HILIC employs traditional polar stationary phases such as silica, amino or cyano like NP-LC, but the mobile phase used is similar to those employed in the reversed phase liquid chromatography (RP-LC) mode [26,27], that makes it has many specific advantages over conventional NP-LC and RP-LC. Currently, HILIC has been investigated as a novel tendency for the separation of polar compounds and charged substances in complex matrices that always elute near the void in RP-LC.

In the present study, a sensitive and simple analytical method for measuring PQ in plasma and urine samples by UPLC-ESI-HRMS/MS was described. The sample preparation was simple and fast, employing only one-step acetonitrile protein-precipitation. The use of HILIC column for separation, combined with tandem mass spectrometry for detection offered lower LOQ and better selectivity. In summary, it was sufficient to qualitative and quantitative determine PQ in clinical samples for diagnosis and prognosis in patients with PQ poisoning.

2. Materials and methods

2.1. Chemicals and reagents

Paraquat dichloride hydrate (PQ) and ethyl viologen dibromide (EV) were both purchased from Sigma-Aldrich (St. Louis, MO, USA). EV was chosen as the internal standard. Acetonitrile (MS grade) was obtained from Merck (Darmstadt, Germany). Formic acid and ammonium formate were both MS grade and acquired from Sigma-Aldrich (St. Louis, MO, USA). Water used in the experiment was deionized and purified by a Milli-Q system (Bedford, MA, USA).

2.2. Liquid chromatography

Chromatographic separation was achieved on an ACQUITY UPLC bridged ethyl hybrid (BEH) HILIC column (100 × 2.1 mm id, 1.7 μm particle size) from Waters Corp, with the column temperature set at 35 °C on the Thermo Scientific Dionex Ultimate 3000 RSLC system. Elution was performed with mobile phase A (0.5% formic acid in 40 mM ammonium formate) and B (acetonitrile) at 0.3 mL/min flow rate, following the gradient as follows: 75% B (0 min) → 75% B (hold on 1 min) → 25% B (linear decrease in 4 min) → 25% B (hold on 1 min) → 75% B (linear increase in 0.1 min) → 75% B (hold on 3.9 min), with a total run time of 10 min. The eluents from the first 2.5 min and the last 5.5 min were diverted to waste via the divert valve.

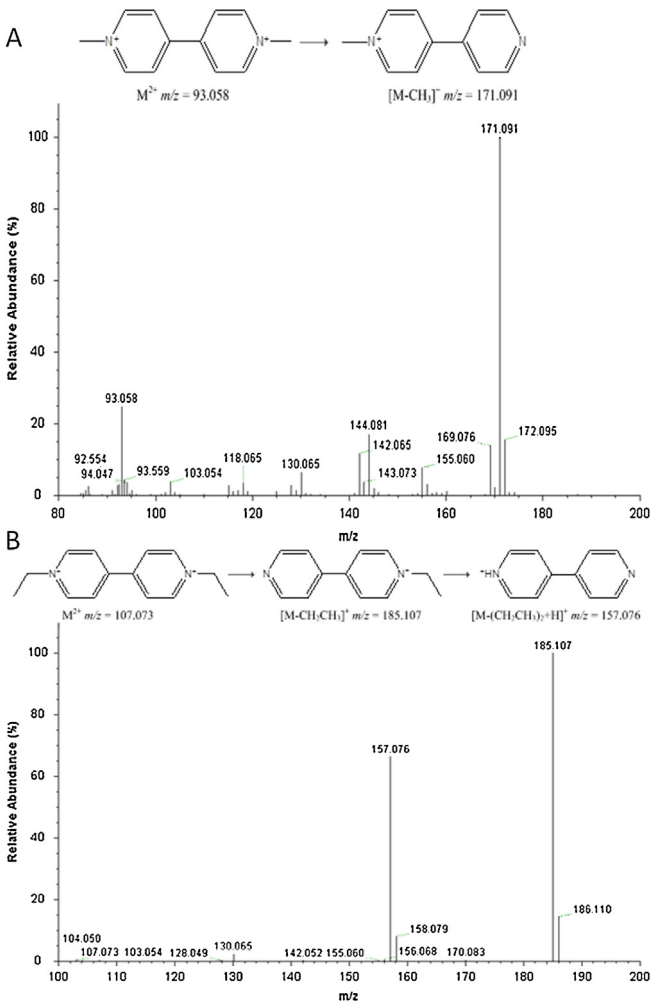

Fig. 1. Full product ion mass spectra of M^{2+} ion (*m/z* 93.058) of paraquat (A) and M^{2+} ion (*m/z* 107.073) of internal standard ethyl viologen (B) obtained in positive ion mode.

2.3. Mass spectrometry

The Q Exactive Orbitrap mass spectrometer equipped with heated electrospray ionization source (HESI-II) was operated in the positive ionization mode. Source conditions were optimized as follows: The spray voltage was 2.5 kV, capillary temperature 350 °C, heater temperature 550 °C, S-lens RF level 50, sheath gas flow rate 48, auxiliary gas flow rate 11 and sweep gas 3 (arbitrary units). Nitrogen was used for the collision gas in the HCD cell and damping gas in the C-trap. The mass spectrometer acquired the Targeted-MS/MS scan data at the resolution of 17500 (full width at half maximum at *m/z* 200). Precursor ion was selected in the quadrupole with 1 *m/z* isolation window, subsequently enriched in the C-trap and fragmented in the HCD cell. After fragmentation, all its product ions were acquired in the Orbitrap™. Under the optimized conditions, the full product ion mass spectra of PQ and EV obtained in positive ion mode was illustrated in Fig. 1. Instrument control, data acquisition and analysis were performed with Xcalibur 2.2 software.

2.4. Preparation of standard solutions and quality control (QC) samples

The stock solutions of PQ and EV were prepared individually with deionised water to get the cation concentration of