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Fast determination of paraquat in plasma and urine samples by solid-phase microextraction and gas chromatography–mass spectrometry

Lina Gao [a], Junting Liu [a,*], Chunyuan Wang [a], Guojie Liu [b], Xiaodong Niu [a], Cuixia Shu [a], Juan Zhu [a]

[a] School of Pharmacy, China Medical University, Shenyang 110001, PR China
[b] College of Basic Medical Science, China Medical University, Shenyang 110001, PR China

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ABSTRACT

A simple, sensitive and reliable gas chromatographic–mass spectrometric method (GC–MS) for quantifying paraquat concentration in biological samples has been developed, using ethyl paraquat as an internal standard. The method involved the procedures of sodium borohydride–nickel chloride ($NaBH_4$–$NiCl_2$) reduction and solid-phase microextraction (SPME) of the perhydrogenated products. GC–MS was used to identify and quantify the analytes in selected ion monitoring (SIM) mode. Under the optimal conditions, recoveries in plasma and urine samples were 94.00–99.85% and 95.00–100.34%, respectively. Excellent sample clean-up was observed and good linearities (r = 0.9982 for plasma sample and 0.9987 for urine sample) were obtained in the range of 0.1–50 μg/mL. The limits of detection (S/N = 3) were 0.01 μg/mL in plasma and urine samples. The intra-day precision was less than 8.43%, 4.19% (n = 3), and inter-day precision was less than 10.90%, 10.49% (n = 5) for plasma and urine samples, respectively. This method was successfully applied to the analysis of the biological samples collected from a victim who died as a result of ingestion of paraquat.

1. Introduction

Paraquat (1,1′-dimethyl-4,4′-bipyridinium chloride, PQ) is a highly effective contact herbicide that is marketed worldwide as a fast-acting, non-selective compound for weed control. PQ is classified as moderate toxic, but it is fatal to human. There have been numerous cases of PQ poisoning due to accidental or intentional swallowing of the commercial product [1–4]. The mechanism of PQ poisoning is associated with accumulation of reactive oxygen species (ROS) and toxic free radicals in various organs, especially in the lungs [5]. These result in oxidative injuries and eventually lead to irreversible pulmonary fibrosis and injuries of other vital organs [6–9]. Recent research showed that PQ exposure is correlated with Parkinson's syndrome [10,11].

Currently, no specific antidote for PQ is available. Since the severity and prognosis of PQ poisoning correlate well with their concentrations in plasma and urine, some treatment methods, e.g. dialysis, closely depend on the fast monitoring of the concentrations in plasma and urine less than 0.1 or 0.5 mg/L, respectively [12–14]. In cases, the victims with lower doses who eventually die within several weeks or a longer time are due to respiratory failure as a consequence of progressive and irreversible pulmonary fibrosis. Hence, the availability of a sensitive and rapid method capable of simultaneous determination of PQ in biological samples is essential in clinical and toxicological investigations.

Various methods have been applied to the analysis of PQ in biological samples, which include gas chromatography (GC) [15], gas chromatography–mass spectrometry (GC–MS) [16,17], high performance liquid chromatography (HPLC) [18–20], liquid chromatography coupled with mass spectrometry (LC–MS) [21–23], capillary electrophoresis (CE) [24,25] and derivative spectroscopy [26]. Some of them are time-consuming or extensive sample pretreatment procedures.

As well known, headspace solid-phase microextraction (HS-SPME) can adsorb analytes from gas phase of sample using a fiber [27–30]. It offers high throughput performance, abridges some procedures of the extended samples preparation, and saves time. Moreover it is reproducible, simple, and effective to eliminate interference compounds from the sample matrix with improvement in selectivity of the analysis. In our study, a simple, rapid and inexpensive procedure utilizing GC–MS with solid-phase microextraction (SPME) was established to determine PQ in biological samples.

* Corresponding author.
E-mail address: liujunting8@163.com (J. Liu).

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2. Experimental

2.1. Reagents and materials

PQ was purchased from Dikma Technologies Co., Ltd (USA), ethyl paraquat iodide (1,1′-diethyl-4,4′-bipyridinium iodide, EPQ) was manufactured by our laboratory [31] (purity ≥98%, used as an internal standard, I.S.), and Nickel chloride ($NiCl_2$, analytical grade) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shenyang, China). Sodium borohydride ($NaBH_4$), sodium hydrate (NaOH) and sodium chloride (NaCl) were analytical grade. Deionized water was produced by a Milli-Q Reagent Water System (Millipore China Co., Ltd, Shanghai, China). The drug-free plasma was obtained from the department of laboratory medicine of the First Affiliated Hospital of China Medical University. The drug-free urine was collected from a volunteer. The work was done in accordance with the guidelines by the Medical Ethics Committee of China Medical University.

Nickel chloride (10%, w/v) was prepared by dissolving 1 g of the crystals in 10 mL deionized water, which was prepared fresh before every analysis.

2.2. Preparation of standard solutions

PQ standard stock solution was made up at 1000 μg/mL in deionized water. Its working standard solution was then prepared through serial diluting of the stock standard solution with deionized water. The internal standard (I.S.) working solution was prepared by diluting the stock solution of EPQ at 100 μg/mL in deionized water. All standard solutions prepared were stored at 4 °C.

2.3. Sample preparation

An aliquot of 0.2 mL plasma or urine was pipetted into the sample vial of 12 mL, followed by 1 mL of 0.1 M NaOH and 40 μL of EPQ solution (100 μg/mL). Twenty milligrams of $NaBH_4$ and 200 μL of $NiCl_2$ solution (10%, w/v) were added to the vial. The reaction mixture was kept for 10 min at 60 °C.

2.4. Solid-phase microextraction

The sampling was performed by SPME technique with head space modality. At the end of the sample reaction time (10 min), 1 mL 20% (w/v) NaCl solution was added to the sample vial prior to tightly closed and heated (60 °C). The fiber of 100 μm polydimethylsiloxane coating (Supelco Bellefonate, PA) was introduced through the seal and exposed to the headspace of the sample vial for analyte extraction (20 min). SPME fiber was withdrawn into the needle, which was injected into the injection port of the GC–MS for 1 min, where the fiber was exposed again, and at the injection temperature the analyte was immediately desorbed into the column for separation and identification.

2.5. Instrumentation

GC–MS analyses both in scan and SIM mode were performed with a Shimadzu GC–MS QP 2010 (70 eV, electron impact mode). Chromatographic separation was achieved on a Rtx-5 (10 m × 0.18 mm I.D., 0.25 μm film thickness) cross-linked capillary column (Dikma, USA). The temperatures of injector, interface and ion source were 280, 280 and 200 °C, respectively. Helium was used as carrier gas at a flow rate of 1.01 mL/min with constant flow mode. Samples were introduced in the split-injection mode (10:1) and the oven temperature was initially at 70 °C for 1 min and programmed to 295 °C at a rate of 25 °C/min. The final temperature of 295 °C was held for 10 min.

Table 1
SIM parameters for GC–MS analysis of analytes in biological samples.

Analytes	Retention time (min)	Ions selected	MW
paraquat	6.75	96,181,<u>196</u>	196
EPQ (I.S.)	8.10	110,195,<u>224</u>	224

The significance of underlined values is molecular ion peak (as same as MW).

In the scanning mode, the mass range was 40–300 u at a rate of 0.5 s/scan. In the SIM mode, several characteristic ions for PQ and EPQ were used for peak-identification, while one ion underlined was selected for quantification (Table 1). All the GC–MS runs were performed in triplicate.

2.6. Methods for recoveries, quantification and linearity

Replicate ($n = 3$) plasma or urine samples (0.2 mL) were spiked with known amounts of PQ to yield final concentration of 1.0, 20, 50 μg/mL, the spiked samples were treated and analyzed as described above. Each run was evaluated in terms of percentage (%) recovery, defined as the measured concentration divided by the spiked concentration and multiplied by 100.

The regression equation of PQ spiked to plasma or urine depended on the peak area ratios with IS (4 μg). The concentration of standards ranged from 0.1 to 50 μg/mL (six concentrations are 0.10, 1.0, 5.0, 10, 20 and 50 μg/mL).

Precision, defined as the relative standard deviation, was determined by intra- and inter-day repetitions. PQ was added to blank plasma and urine. The concentration of PQ was determined as described above. Intra-day precision was determined by analyzing a spiked sample at three concentrations in triplicate on the same day ($n = 3$). The same procedure was repeated on different days ($n = 5$) to determine the inter-day precision.

2.7. Matrix effects

Two aqueous solution calibrations and two matrix calibrations samples were prepared at the same concentrations (including low (1.0 μg/mL) and high (50 μg/mL) concentrations) for evaluating the matrix effects. Both aqueous solution and matrix calibrations were performed by the same procedure. The matrix effect was estimated by comparing the peak areas from the samples of matrix calibrations to that from the corresponding samples of aqueous solution calibrations, and these were reported as percentage values.

2.8. Stability

The QC samples of medium (20 μg/mL) concentration were used to evaluate the freeze/thaw stability which were analyzed after three freeze/thaw cycles and stored at −20 °C for 14 days (stability samples, $n = 3$). For each freeze/thaw cycle, the stability samples were thawed and kept at room temperature for 4 h and refrozen at −20 °C for 24 h. Freshly prepared standard curves were used for quantification of the samples.

2.9. Quantification of PQ in blood and urine samples

Blood and urine samples were collected when the postmortem was performed in the Forensic Identification Service of China Medical University, which were pooled into polypropylene tubes and stored at −80 °C until analysis.

Urine sample of 0.2 mL was directly analyzed as described above. Blood sample of 1 mL was centrifuged for 10 min at 3500 rpm after adding 40 μL of EPQ solution (100 μg/mL). 0.2 mL aliquot of the supernatant was transferred into the sample vial, and then analyzed as described above.