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DETERMINATION OF PARAQUAT IN BLOOD AND URINE BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY-MASS SPECTROMETRY.

Abstract: Paraquat (PQ) is a toxic quaternary ammonium compound widely used in agriculture. Numerous cases of paraquat intoxication have been reported either accidentally or intentionally as suicidal attempts.

A method for the determination of the herbicide PQ in blood and urine samples was developed using liquid chromatography-(electrospray ionization) mass spectrometry (LC-ESI-MS), following extraction with Oasis[®] WCX solid-phase cartridges. Chromatographic separation was achieved using an Atlantis[®] HILIC silica column, eluted isocratically with acetonitrile and ammonium formate (200mM) buffer, pH 3.8, at a 300 $\mu\text{L}/\text{min}$ flow rate. Quantitation was achieved by the addition of ethyl paraquat as internal standard (IS). The compounds were detected monitoring two ions for PQ (m/z 185 and m/z 171) and m/z 213 for the IS. The method was applied to determine PQ in two cases: a non fatal case, a 42-year-old female with 0.13 $\mu\text{g}/\text{mL}$ PQ concentration in blood and 6.29 $\mu\text{g}/\text{mL}$ in urine and a lethal case, a 51-year-old male with 0.27 $\mu\text{g}/\text{mL}$ PQ concentration in blood.

The authors developed a specific, sensitive and rapid assay for the identification and quantification of PQ, very important for monitoring suspected paraquat intoxications in hospitals and subsequently help in the treatment of these patients.

1. Introduction

The herbicide paraquat (1,1'-dimethyl-4,4'-dipyridyl cation, PQ), has been encountered in several cases of accidental and suicidal poisonings. The dichloride salt of paraquat or methyl viologen is well known under the trade name *Gramoxone*[®] (a 20% aqueous solution). Concentrated liquid formulations have been responsible for most (and more severe) poisonings than granular forms, which contain less PQ. Although normal use of the herbicide does not present a serious health risk, many successful suicide attempts are often the result of the ingestion of concentrated forms of PQ [1-2].

Paraquat has low but rapid gastrointestinal absorption (5-10%). Peak plasma concentrations appear in less than 2 h. Following ingestion, PQ is actively transported to all major organs, especially to the lung, where it is reduced to form highly reactive free radicals. It is slowly excreted unchanged in urine and feces [3-4].

A wide variety of analytical techniques have been reported to determine PQ in biological samples, including gas chromatography (GC) [5-6], GC/mass spectrometry (MS) [7], high-performance liquid chromatography (LC) [8-13], LC/MS [14-15] and LC/MS/MS [16-19]. However, most of them require a complicated and a time-consuming sample pretreatment.

Because this substance is ionic, solid-phase extraction (SPE) and HPLC usually have been accomplished with the aid of an ion-pairing reagent such as an alkyl sulphonic acid. LC-MS provides the analyst with a more sensitive and highly selective analytical method for paraquat. Strong cation exchange-based SPE methods that do not require ion-pairing reagents have been employed successfully, but the strong salts or strong acids used for elution are difficult to remove and are serious impediments to optimal LC-MS analysis. In order to overcome these problems, a new type of sorbents has been developed [20] for the retention of quaternary ammonium compounds and strongly basic organic compounds. The Oasis WCX sorbent incorporates a weak cation-exchanger bound to a polymeric reverse-phase particle. An Atlantis HILIC column was utilized for LC using no ion-pairing reagents.

This work presents two cases due to oral ingestion of *Gramaxone*[®], and describes a sensitive, specific, and rapid LC-ESI-MS method used to detect, confirm and quantify PQ in blood and urine samples.

2. Case reports

The first report describes a successful clinical case regarding the intoxication of a 42-year-old woman by a presumed lethal dose of paraquat. After hospital treatment this patient has shown a gradual return to normal spirometry values from the marked reduction that occurred at the time of paraquat intoxication. PQ was detected in blood and urine at levels of 0.13 µg/mL and 6.29 µg/mL, respectively. Blood and urine samples were sent again to our laboratory 7 days after intoxication. PQ was not detected in blood and PQ urine concentration found was 0.18 µg/mL. She survived.

The second case was a 51-year-old man admitted to the hospital with the information of a suicide attempt with intentional intake of *Gramaxone*[®]. Treatment with repeated activated charcoal hemoperfusion was attempted (total time 14h). The patient developed acute renal and respiratory failure. He died from multiple organ failure 8 days after intoxication.

At autopsy, the internal examination revealed that both lungs were solid with acute hemorrhagic edema and both kidneys were deeply congested. Histological findings showed marked pulmonary congestion with numerous hemosiderin-laden macrophages.

The paraquat blood concentration found was 0.27 µg/mL.

3. Materials and methods

3.1. Chemicals and reagents

Paraquat and ethyl paraquat (used as internal standard) were supplied by Sigma-Aldrich-Chemie GmbH (Steinheim, Germany). Each standard solution was prepared

in methanol (1 mg/mL) and stored in plastic bottles at +4°C. Acetonitrile and methanol were HPLC-grade and were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid was from Riedel-de Haën (Seelze, Germany). Deionised and purified water was obtained using a Milli-Q system (Millipore, Molsheim, France). Solid-phase cartridges Oasis® WCX, (60 mg, 3 cc) were purchased from Waters (Milford, MA). The phosphate buffer (pH 7.0) was prepared by dissolving 0.12 g of NaH₂PO₄ into a 100 mL volumetric flask and brought to volume with deionized water. Ammonium formate buffer 200 mM (13.0 g/L) was prepared with deionized water, and the pH adjusted to 3.6 with formic acid. The mobile phase was filtered through a 0.20 µm filter (Schleicher & Schuell) and degassed in an ultrasonic bath for 15 min just before use.

3.2. Instrumentation

The chromatographic system (LC) used was a Waters 2695 Alliance System and Atlantis® HILIC silica column (2.1x150 mm, 5 µm). The mobile phase consisted of acetonitrile and 200 mM ammonium formate buffer, pH 3.6, (30:70, v/v) at a 300 µL/min flow rate. The column temperature was maintained at 35°C. The injection volume was 10 µL.

A Waters 996 photodiode array detector operated on a 210-400 nm wavelength scan with a 1.2 nm resolution. The UV absorbance was measured at 258 nm.

Mass spectrometry detection (MS) was carried out on a Waters ZQ 2000 single quadrupole mass spectrometer with an electrospray ionization (ESI) performed in positive mode. Full-scan spectra were recorded from *m/z* 130-500, at a scan time of 0.5 s and an interscan delay of 0.1 s. The other main instrument settings were: capillary voltage 3.5 KV; cone voltage 40 V; extractor 4 V; ion energy 0.5; source temperature 120°C; desolvation temperature 400°C; cone gas (N₂) flow rate 0 L/h and desolvation gas (N₂) flow rate 600 L/h.

Instrument control, data acquisition and processing were achieved using Waters Empower software (Milford, MA).

3.3. Sample preparation

Controls and calibration samples were prepared by spiking drug-free whole blood and urine samples with standard solutions.

A 1 mL aliquot of whole blood or 1 mL of urine was spiked with 50 µL of internal standard (10 µg/mL) and diluted with 2 mL of acetonitrile. Then the samples were vortex mixed and centrifuged for at 2500 rpm for 10 min. Extraction cartridges (Oasis® WCX, 3cc) were conditioned with 1 mL of methanol followed by 1 mL of deionized water. Each sample was loaded through a cartridge. It was then washed with 1 mL of phosphate buffer (pH 7) followed by 1 mL of deionized water and 1 mL of methanol. After drying under vacuum for 10 min, elution was carried out with 1.5 mL of acetonitrile/water/TFA (84:14:2, v/v). The eluate was evaporated to dryness under a nitrogen gas flow at 40°C. The residue was dissolved in 100 µL of methanol and an aliquot (10 µL) was injected into the LC-ESI-MS system.

4. Results and discussion

Calibration curves for paraquat in blood and urine samples were linear from 0.010 to 2.0 µg/mL in blood ($y = 0.0142x + 0.1497$ with $r^2 = 0.9994$) and from 0.025 to 10.0 µg/mL in urine ($y = 0.0141x + 1.347$ with $r^2 = 0.9994$).

The detection limit of PQ in blood and urine samples was 0.004 µg/mL and 0.007 µg/mL respectively (LOD , $S/N=3$) and the lower limit of quantification (LOQ , $S/N=10$) was 0.012 µg/mL in blood and 0.024 µg/mL in urine. For intra-day and inter-day precision determinations, five replicate analyses were performed at each of the three studied concentrations. Relevant validation data for recovery and precision are presented in Table I. The method proved to be precise for paraquat, both in terms of intra-day and inter-day analysis, with coefficients of variation (CV) less than 20%. In selectivity study, an analysis of blank blood samples showed there were no interfering peaks at the elution time of paraquat or the internal standard (ethyl paraquat).

Quantitation employed the selected ion-recording mode (SIR) using the m/z corresponding to the most abundant product ion $[M+H]^+$ at m/z 185 for paraquat and m/z 213 for the internal standard. Paraquat fragment ions, m/z 171 and m/z 144, were due to the loss of the methyl group corresponding to $[M+H-CH_3]^+$ and due to the loss an HCN molecule $[M+H-CH_3-HCN]^+$, respectively. Both SIR and Scan acquisitions were performed in centroid mode. SIR mass chromatograms and mass spectrum in SCAN mode (m/z 185) of the paraquat detected in the blood sample (case 2) are shown in Fig. 1.

The proposed solid-phase extraction procedure and LC-ESI-MS method provided an accurate assay for the determination of paraquat in blood and urine.

The authors developed a specific, sensitive and a rapid assay for the identification and quantification of paraquat, very important for monitoring suspected paraquat intoxications in hospitals and consequently help in the treatment of these patients. The procedure has also been applied to a fatal death case involving paraquat poisoning.

5. References

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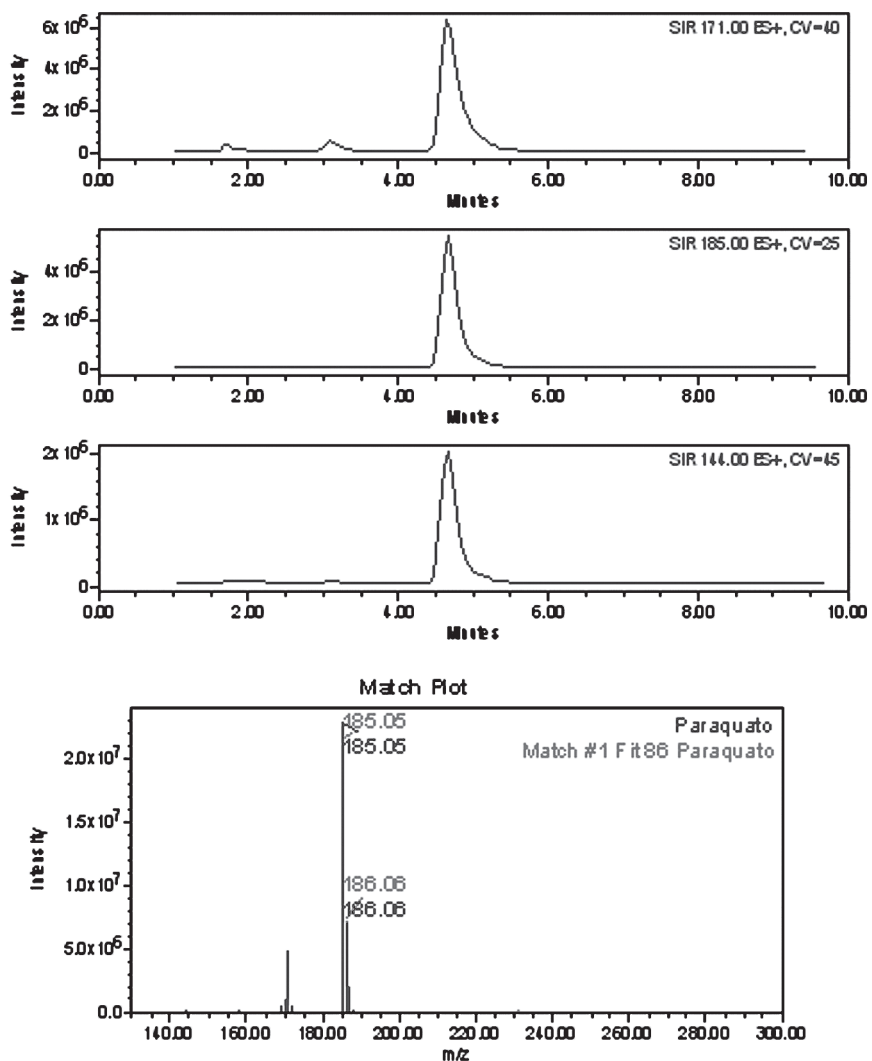


Figure 1 – SIR mass chromatograms and mass spectrum in SCAN mode (m/z 185), of paraquat in postmortem blood sample (case 2).

	Concentration level ($\mu\text{g/mL}$)	Recovery (%)	Intra-day CV (%)	Inter-day CV (%)	n
BLOOD	0.025	60 ± 8.7	10.4	4.2	5
	0.25	58 ± 7.7	6.0	6.7	5
	1	67 ± 1.8	10.2	17.3	5

Table I – Validation data of recovery and precision for paraquat.