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HPLC-MS/MS determination and the postmortem distribution or postmortem redistribution of paraquat and its metabolites in four fatal intoxication cases

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ABSTRACT

HPLC-MS/MS analysis and postmortem distribution or postmortem redistribution of paraquat and its two metabolites in poisoning death cases were reported. Paraquat, monoquat, and paraquat monopyridone were extracted from the sample with acetonitrile or methanol, respectively, detected by ZORBAX HILIC Plus (4.6 × 100 mm, 3.5 μm) chromatographic column, with 0.1 % formic acid aqueous solution - 0.1 % formic acid acetonitrile solution (v/v) as mobile phase. Paraquat, monoquat, and paraquat monopyridone had a good linear relationship within the range of 10–1000, 1–400, and 1–1000 ng/mL (or g), the correlation coefficient (r) were all ≥ 0.9996. Their detection limits were lower than 1 ng/mL (or g). The detection accuracy was 91.25~113.44 %. The intra-day and inter-day precision were 1.51–3.99 % and 1.92–4.93 %, respectively. This method was used to detect and analyze four rare paraquat poisoning cases. The distribution of paraquat, monoquat, and paraquat monopyridone is uneven, which is relatively high in the heart, blood, lung, and kidney. Heart blood/Peripheral blood ratio of paraquat, monoquat, paraquat monopyridone concentration in two poisoned cases were 1.4, 2.0, 1.5 and 1.9, 1.3, 1.2, which showed a location dependent postmortem redistribution. This is the first time that HPLC-MS/MS and the postmortem distribution or postmortem redistribution of paraquat metabolites in poisoned death cases have been reported. This research provides scientific basis for forensic identification of paraquat poisoning cases and extraction of biological specimen.

1. Introduction

Paraquat (PQ) is a moderately toxic organic heterocyclic contact defoliant and herbicide, most of which are in the form of sulfate or chloride. PQ was synthesized in the 19th century and used as a chemical indicator due to its low price, high efficacy and availability. It has been widely used as herbicide in more than 120 countries in the world since 1962 [1]; in recent years, cases of PQ poisoning and death due to accidental ingestion, suicide and homicide occurred frequently, so, it has been banned in several countries around the world.

PQ is absorbed into the human or animal body mainly through the gastrointestinal tract, respiratory tract, skin and mucous membranes, and then distributed in almost all tissues and organs. Literature reported [2] that the metabolic pathways of rats, sheep, pigs, cows, goats and laying hens after PQ poisoning were studied, and the research showed that the metabolic pathways of PQ in these animals were similar, the final metabolites in the body including monoquat, paraquat monopyridone (MP), 4-Carboxy-1-methyl pyridinium ion (MINA), and paraquat dipyridone (DP). The current research on PQ mainly focused on the detection of PQ in biological sample [3,4] and forensic toxicokinetics [5–9], however, the HPLC-MS/MS detection of the metabolites of PQ in biological samples have not been reported in China and other countries.

As far as our knowledge, in this study, a liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was the first time established for the detection of two major metabolites (monoquat and MP) of PQ in biological samples. Moreover, the

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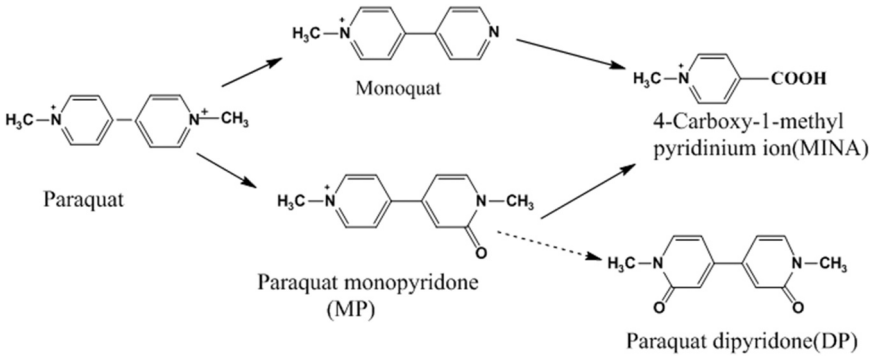

Fig. 1. Proposed metabolic pathway of PQ in animals.

metabolites of PQ in humans and animals were compared in order to determine whether metabolites are consistent among different species. Finally, the forensic toxicokinetics of PQ metabolites were studied by analyzing human poisoning cases, and this could help the identification work in PQ poisoning cases. Fig. 1.

2. Materials and methods

2.1. Standards and reagents

Standards of monoquat and MP were obtained from Shanghai Meidixi (Shanghai, China), standard solution of PQ dichloride (1 mg/mL) were purchased from Bailingwei (Beijing, China). Methanol, acetonitrile, and cyclohexane of analytical purity were obtained from Damao (Tianjin, China). Formic acid, acetonitrile, and methanol of chromatographic grade were all obtained from Sigma-Aldrich (Shanghai, China). Deionized high purity water was generated from Millipore Simplicity 185 Ultrapure Water System (USA).

The standard solution used in this experiment was prepared by using chromatographic grade methanol to a stock solution at a concentration of 1 mg/mL and stored in a refrigerator at 4 °C. Biological samples for this research were collected at the time of autopsy, and all samples were stored at − 80 °C before analysis.

2.2. Instruments

The detection of PQ and its two metabolites (monoquat and MP) was performed by HPLC-MS/MS with an Agilent 1260 infinity HPLC system (Agilent, USA), coupled with a 6460 Triple Quadrupole Mass Spectrometer (Agilent, USA). Mass hunter B.06.00 workstation (Agilent, USA) and analyst software were used for the data acquisition and quantification.

2.3. Sample collection

In this study, the human specimens examined were all signed an informed consent form before collected, and stored in a refrigerator at − 80 degrees before testing. The number of Approval Form for Ethical Review of Medical Research in Shanxi Medical University is 2020GLL022.

2.4. Sample pretreatment

Procedure for analyzing PQ and monoquat: tissue organ was ground with a tissue homogenizer. Body fluids (200 μL) and homogenized tissue (200 mg) was mixed with an equal volume of Ultrapure water, vortex for 30 s. Then, 1.2 mL analytically acetonitrile was added into the mixture after vortex mixing for 30 s followed by centrifugation at 4500 rpm/min for 8 minThe supernatant was transferred into another glass test tube, and blow to dry at 50 °C of nitrogen. The residues were reconstituted in 200 μL of methanol and vortex for 1 min. The complex solution was transferred to a 1.5 mL centrifuge tube, centrifuged at 12,000 rpm/min for 3 min, and the supernatant was passed through a 0.22 μm organic membrane and 5uL was injected into HPLC-MS/MS system for analysis. The graphic scheme of the sample preparation of PQ and monoquat are shown in Fig. 2.

Procedure for analyzing MP: Body fluids (200 μL) and homogenized tissue (200 mg) was mixed with 200 μL Ultra-purity water followed by vortex for 30 s. To the mixture was added 2 mL of methanol after vortex mixing followed by centrifugation at 4500 rpm for 8 minThe supernatant was transferred into another glass test tube and washed with 2mLof cyclohexane. The methanol layer was evaporated to dryness under a stream of nitrogen gas at 50 °C. The residues were dissolved in 200 μL of methanol, then vortex for 1 min, the complex solution was transferred into a 1.5 mL centrifuge tube, centrifuged at 12,000 rpm/min for 3 min, and the supernatant was passed through a 0.22 μm organic membrane and 5uL was injected into HPLC-MS/MS system for analysis. The graphic scheme of the sample preparations are shown in Fig. 3.

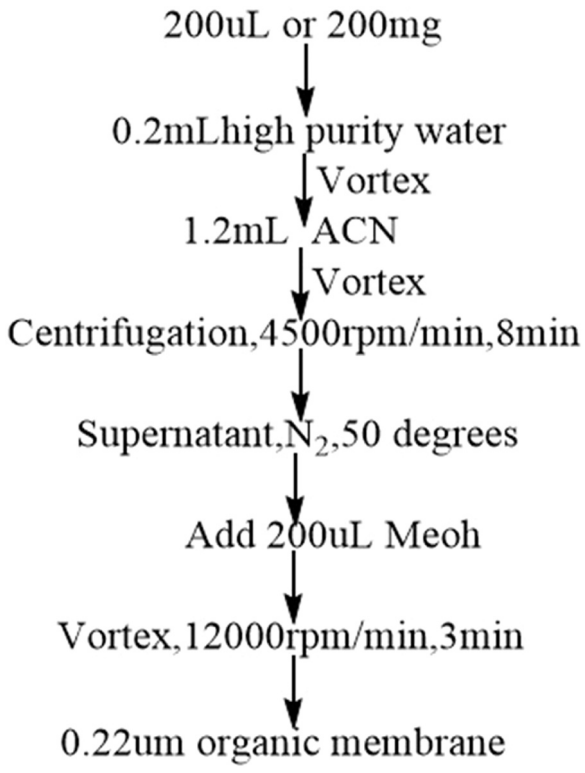

Fig. 2. The sample preparation of PQ and monoquat in biological samples.

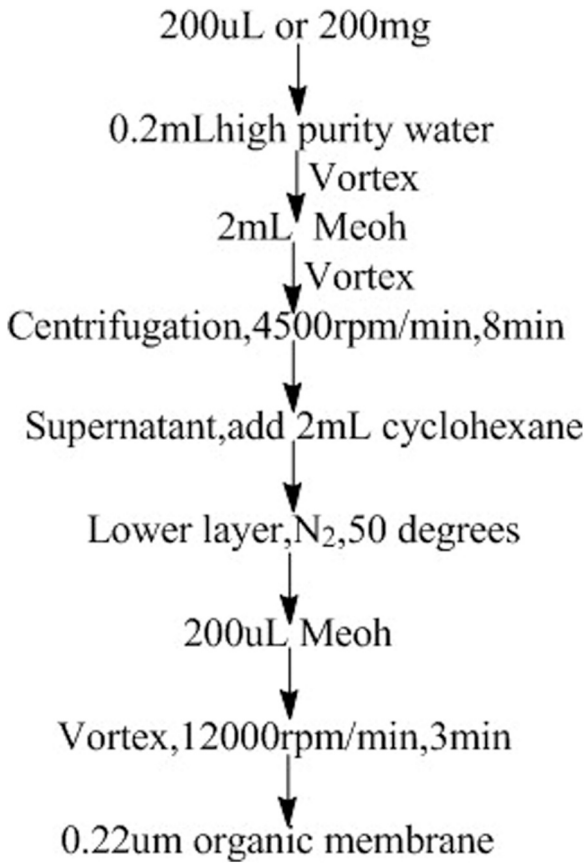

Fig. 3. The sample preparation of MP in biological samples.

2.5. HPLC-MS/MS conditions

For the analysis of PQ and monoquat: the HPLC system used was an Agilent 1260 series (Agilent, USA) with a ZORBAX HILIC Plus C_{18} column (4.6 × 100 mm, 3.5 μm), the column temperature was set at 25 °C, operated at a flow rate of 0.25 mL/min, the mobile phase consisted of 0.1 % formic acid/water (solvent A) and 0.1 % formic acid/acetonitrile (solvent B). The gradient parameters were consisted of a linear gradient from: the initial ratio of the organic phase is 70 %, 90 % at 9 min, then 70 % at 10 min, retention time to 15 min, the injection volume is 5 μL.

For the analysis of MP: the apparatus was Model 1260 high performance liquid chromatograph, the column was ZORBAX HILIC Plus C_{18} column (4.6 × 100 mm, 3.5 μm), the mobile phase was distilled water-acetonitrile (both contained 0.1 % formic acid, V/V = 50/50), the column temperature was maintained at 25 °C and the flow rate was set to 0.4 mL/min.

Ionization of analysis was carried out using the following setting of ESI in the positive mode. Gas flow: 11 L/min, ion spray voltage: 4000 V, atomized drying gas temperature: 300 °C; sprayer: 15 psi. Under the selected HPLC-MS/MS conditions, the chromatographic behaviors of PQ, monoquat, and MP were good, and the retention times were 11.95, 10.54 and 5.26 min, respectively. The MRM chromatograms of blank samples and spiking samples with the analyte were shown in Figs. 4 and 5.

2.6. Method validation

The HPLC-MS/MS data obtained by MRM mode were used for method validation. Blank blood, urine and liver samples were fortified, and the methods were validated by evaluating the corresponding linearity, sensitivity, accuracy, precision, LOD, and LOQ [10]. The specificity was tested by comparing the chromatograms of spiked samples with blank blood samples (Fig.4). Linearity was determined by calculating the area of the peak with blank blood, urine and liver spiked at different concentrations, which were shown in below: the linear concentration of PQ: blood: 10, 25, 50, 100, 250, 500, 1000 ng/mL; urine: 10, 50, 100, 200, 500, 1000, 2500, 5000 ng/mL; liver: 10, 25, 50, 100, 250, 500, 1000 ng/g. The linear concentration of monoquat: blood: 1, 5, 10, 20, 40, 80, 100, 200, 400 ng/mL; urine: 1, 5, 10, 25, 50, 100, 250, 500 ng/mL; liver: 5, 10, 25, 50, 100, 200, 400 ng/g. The linear concentration of MP: blood and urine: 1, 10, 25, 50, 100, 250, 500, 1000 ng/mL; liver: 5, 10, 25, 50, 100, 250, 500, 1000 ng/g. LOD and LOQ were determined as the target concentrations at which the signal-to-noise ratios are equal to 3 and 10, respectively.

Standard were added to blank matrix samples with three concentrations of low, medium, and high, and 6 parallel quality control samples were collected for each concentration point. The samples were analyzed according to the established sample pretreatment and detection methods. The intra-day and inter-day precision and accuracy of the methods were assessed by detecting two spiked samples of different concentrations in whole blood, urine, and liver to construct the calibration curve. The intra-day precision was determined separately by spiking samples for 3 times on one single day. Inter-day precision was assessed by spiking an extracted sample prepared daily for 3 consecutive days and analyzed in triples. The intra-day and inter-day precisions were evaluated by the relative standard deviation (RSD). The accuracy was determined by analyzing 3 replicates of blood samples at low, medium and high quality control (QC) concentrations on three different days. The detection limit was determined at a signal to noise ratio of 3.

3. Results

3.1. MS spectrometry conditions

In MRM analysis, the ionization efficiencies for the target by positive and negative modes were compared. For all the target compounds, the response of $[M+H]^+$ were significantly higher than those of $[M-H]^-$. Thus, mass spectrometry was performed in positive ionization mode. For quantitative determination with MRM, the frequently used Full Scan mode typically cannot deliver significantly higher sensitivity than the multiple reactions monitoring mode (MRM) of tandem mass spectrometry, therefore, the fragmentor and CE values were optimized to achieve the best ionization efficiency. MS/MS analysis was performed using nitrogen as the collision gas, collision-induced dissociation voltage of the analysis (Fragmentor), collision energy (CE) and other parameters were summarized as Table 1.

3.2. Method validation of PQ, monoquat, and MP

With blank blood samples, no interfering peak was present for the target compounds. The linearity was evaluated using blank

Table 1
MS parameters of PQ, monoquat, and MP.

Target	Precursor ion (*m/z*)	Product ion	Fragmentor	CE (v)	ESI(±)
PQ	186.1	171.1*	88	20	+
		77.1		57	
Monoquat	171.1	155.1	135	35	+
		77.1*		46	
MP	202	174.1*	100	25	+
		94.1		34	

*: Quantitative ion.

Table 2
Standard calibration curve, LOD, LOQ of PQ, monoquat, MP in blood, liver, and urine (ng/mL or ng/g).

Target	Sample	Calibration curve	Range	Correlation coefficient (r)	LOD	LOQ
PQ	Blood	y = 505.400274x+608.662061	10–1000	0.9998	0.9998	1.13
	Liver	y = 427.476401x+5956.518145	10–1000	0.9996	0.9996	1.77
	Urine	y = 61.538063x+7330.519286	10–5000	0.9997	0.9997	0.77
Monoquat	Blood	y = 401.3794x+1276.3972	1–400	0.9999	0.9999	1.93
	Liver	y = 25.0958x+617.4015	5–400	0.9996	0.9996	4.17
	Urine	y = 305.029281x+356.757636	1–500	0.9998	0.9998	3.13
MP	Blood	y = 13.5482x+45.9828	1–1000	0.9999	0.9999	4.40
	Liver	y = 4.3406x+43.6997	5–1000	0.9996	0.9996	20.00
	Urine	y = 1.101231x+14.767393	1–1000	0.9995	0.9995	2.00

blood, urine, and liver samples by calculating the area of the target peaks, good linearity of the standard calibration curve was achieved the selected concentration ranges for all targets. Low LODs 0.23 ng/mL and LOQs 0.77 ng/mL were obtained. Precision was evaluated by intra-day and inter-day analysis. Blank blood samples were spiked with the target at three levels. Intra-day precision was determined by analyzing three parallel samples of three concentrations on the same day, inter-day precision was assessed by spiking an extracted sample prepared daily for three days and analyzed in triples. The precision was expressed as relative standard deviation (RSD). Good precision was achieved for the spiked blood samples, with intra-day RSD ranging in 1.51–3.99 % and inter-day RSD ranging in 1.92–4.93 %. These results were shown in Tables 2–3, respectively.

3.3. PQ poisoning cases

The established HPLC-MS/MS methods were applied to the determination of PQ, monoquat, and MP in four PQ poisoning cases. The MRM chromatography of PQ and monoquat in the urine of case2 were shown in Fig. 4, the MRM chromatography of MP in the urine of case2 were shown in Fig.5.

3.3.1. Case 1

A man,27 years old, who drunk herbicide solution of PQ, at 17 : 00 on December 19, 2017, died on December 29, 2017. A legal autopsy was done on January 3, 2018 followed by a toxicological examination of blood, stomach content, lung, and liver. According to the method established in this study, PQ, monoquat, and MP were detected in blood, stomach content, lung, and liver specimens. The quantitative results were displayed as Table 4.

3.3.2. Case 2

A female, was admitted to the hospital at 14:14 on September 10, 2018 with "oral PQ for about six hours". The patient took oral PQ solution about 6 h before admission, followed by nausea, vomiting, abdominal pain, and other symptoms, unconscious disorder, and then rushed to the local hospital to wash the stomach. Then the patient was transferred a superior hospital. After blood perfusion, hormones, Baogan pills, anti-infection, and other treatments, first acute liver and renal failure, followed by pulmonary fibrosis, and gradually deteriorated, and eventually died on September 29, 2018. After that, pathological anatomy was performed, and liver, kidney, blood, urine, and stomach contents were taken for forensic toxic analysis. The quantitative results were shown in Table 5.

Table 3
Accuracy and precision of PQ, monoquat, and MP in blood sample (x ± s, n = 3).

Target	Conc (ng/mL)	Accuracy(x ± s, %)	Intra-day precision (%)	Inter-day precision (%)
PQ	20	91.25 ± 8.83	2.45	4.62
	500	98.23 ± 3.37	2.70	3.34
	800	98.54 ± 2.88	2.57	3.87
Monoquat	5	101.54 ± 2.69	3.10	4.81
	200	96.25 ± 5.30	2.03	4.57
	320	95.89 ± 4.98	2.58	4.93
MP	5	113.44 ± 3.9	3.99	4.34
	400	99.76 ± 0.86	1.51	2.86
	800	96.32 ± 2.28	2.91	1.92

Table 4
PQ, monoquat, and MP concentrations in tissue and body fluids.

Specimens	PQ (μg/g or μg/mL)	Monoquat (ng/g or ng/mL)	MP (ng/g or ng/mL)
Blood	3.0	20	38
Stomach content	1.7	5.9	n.d.
Liver	0.56	0.52	0.53
Lung	3.6	1.0	2.5

n.d.: Not detectable.

3.3.3. Case 3

A female, was given PQ solution on December 26, 2016, and died after being treated in the hospital; heart, muscle, blood, peripheral blood, bile, urine, vitreous humor, cerebrospinal fluid, were taken for forensic toxic analysis. The concentration of PQ, monoquat, and MP were shown in Table 6.

3.3.4. Case 4

A male, on the afternoon of July 6, 2017, he had symptom of sweating after taking a "cap" of PQ pesticides. Afterwards, he was sent to the hospital for rescue, and gastric lavage treatment was invalid, and on July 18, 2017, he was caused by pulmonary fibrosis

Table 5
PQ, monoquat, and MP concentrations in tissue and body fluids.

Specimens	PQ (μg/g or μg/mL)	Monoquat (ng/g or ng/mL)	MP (ng/g or ng/mL)
Liver	0.095	0.71	n.d.
Kidney	0.12	0.11	n.d.
Heart blood	0.31	0.12	n.d.
Urine	0.083	0.58	0.21
Stomach content	0.58	n.d.	n.d.

n.d.: Not detectable.

Table 6
PQ, monoquat, and MP concentrations in tissue and body fluids.

Specimens	PQ (μg/g or μg/mL)	Monoquat (ng/g or ng/mL)	MP (ng/g or ng/mL)
Heart	0.84	3.4	12
Muscle	2.5	1.2	0.98
Heart blood	2.2	4.0	6.9
Peripheral blood	1.6	2.0	4.7
Bile	0.22	2.0	5.4
Urine	3.5(ng/mL)	0.77	n.d.
Vitreous fluid	n.d.	n.d.	0.82
Cerebrospinal fluid	0.47	0.29	n.d.
Heart blood/ Peripheral blood	1.4	2.0	1.5

n.d.: Not detectable.

Table 7
PQ, monoquat, and MP concentrations in tissue and body fluids.

Specimens	PQ (μg/g or μg/mL)	Monoquat (ng/g or ng/mL)	MP (ng/g or ng/mL)
Heart	1.5	7.6	n.d.
Liver	1.5	12	1.8
Spleen	1.5	11	3.4
Lung	1.5	3. 6	1.2
Kidney	2.5	4.5	3.3
Brain	0.70	1.2	n.d.
Muscle	1.9	11	4.0
Stomach content	0.88	6.3	n.d.
Heart blood	1.8	2.9	2.2
Peripheral blood	0.97	2.3	1.8
Vitreous fluid	0.16	n.d.	n.d.
Bile	6.1	0.75	1.5
Urine	5.3	15	41
Pericardial effusion	2.1	0.45	1.6
Heart blood/ Peripheral blood	1.9	1.3	1.2

n.d.: Not detectable.

and finally died on July 20, 2017. A few days later, the autopsy was performed to collect tissue organs such as heart, liver, spleen, lung, kidney, brain, muscle, stomach content, blood, venous blood, vitreous humor, bile, urine, pericardial effusion and so on. The test results of PQ, monoquat, and MP were shown in Table 7.

4. Discussion

4.1. HPLC-MS/MS methods

According to domestic and global studies reported previously, the detection methods of PQ in biological samples including ultraviolet spectrophotometry [11,12], thin layer chromatography [13], HPLC [14,15], HPLC-MS/MS [3,16–20], GC [21], GC-MS [6,22–25], and many other methods. The GC method was suitable for the analysis of samples with easy gasification, good thermal stability, and relatively low boiling point while the HPLC method was suitable for ionic compounds with strong polarity, high boiling point, poor thermal stability, relatively large molecular weight and low volatility. Due to the limitation of volatility and thermal stability, most of the compounds can be analyzed by HPLC-MS/MS method.

Detected PQ by GC or GC-MS, there were some problems of strong residual and low sensitivity, moreover, derivatization was needed before instrumental analysis due to the strong polar characteristic of PQ. At the same time, the sensitivity of HPLC detection method were relatively low, it could not meet the needs for identification of poisoning cases and scientific research. However, the application of HPLC-MS/MS has high sensitivity, no need for derivatization, and the pretreatment process is simple. The chemical reagents used are less harmful to the experimenter and the environment, and have strong applicability in actual detection cases. The extraction methods of PQ in biological samples included liquid-liquid extraction (LLE) [26,27], solid phase extraction (SPE) [20], and direct precipitation of protein with acetonitrile or perchloric acid [17], however, there were some shortcomings of large demand for specimen, long processing time and high cost. In related research reports, PQ was mainly detected in blood and urine; however, there were other specimens such as organ fluids and tissues involved in daily poisoning cases, which also need to be accurately and quickly qualitative and quantitative. This requires a complete, simple, and efficient method in biological samples need to be established.

At present, the related research of metabolites of PQ has not been reported so far. Because PQ is metabolized very quickly in the body, about 90 % of PQ is excreted in the form of the original body within 48 h of taking PQ [1]. For PQ poisoning cases, the original body has often been undetectable due to the delay of rescue or death process. However, a clear forensic identification of the poison of PQ has not been able to carry out according to clinical symptoms and related pathological changes. Therefore, the detection of the metabolites of PQ could have great significance for solving the above problems.

As far as our knowledge, this study was the first time to establish a HPLC-MS/MS sensitive method for monoquat and MP detection in biological samples. The minimum detection limits for monoquat and MP were 0.58 and 0.60 ng/mL, respectively. Regarding to the pretreatment, PQ and its metabolites in tissue organs, body fluids and other biological samples were precipitated by acetonitrile or methanol to precipitate proteins and then directly analyzed by HPLC-MS/MS. Since the required sample amount is small (200 μL or 200 mg), time-saving, simple , fast sensitive and environmental friendly, the established approach is suitable for forensic examination and the actual inspection case.

4.2. Metabolic pathway of PQ

PQ can be absorbed into the body through a variety of pathways, including the gastrointestinal tract, respiratory tract, skin and mucous membranes. Due to the polarity of the molecule, which is a salt, around 10–30 % of it is absorbed into the body orally and very little by other routes. Because PQ produces a large amount of reactive oxygen radicals after entering the body, causing cell membrane lipid peroxidation and cell damage [28–30]. PQ's chemical structure is similar to endogenous polyamines, typeI, type II alveolar cells and tracheal Clara cells have an active uptake system for this substance. There is a competitive relationship between PQ and polyamines, thus a large amount of PQ accumulate in the lung which is the target organ after poisoning [31], PQ's concentration in the lung is 10–90 times higher than that in the plasma after poisoning. Because PQ is rapidly metabolized in the body, about 90 % of it is excreted in the form of the original body through the kidney within 48 h, and there has been no literature reported about the detection of PQ metabolites in vivo. According the literature [4], the main metabolites of PQ in animals are MP, DP, monoquat, and MINA. Chiaki Fuke [32] synthesized MP in vitro by liver microsome incubation.

In this study, through the detection of PQ poisoning cases, PQ metabolites were detected in heart, liver, spleen, lung, kidney, brain, muscle, testis, stomach, stomach content, vitreous humor, bile, urine, pericardial effusion, cerebrospinal fluid, heart blood, and peripheral blood. By comparing of the results of animal experiments with the realistic forensic cases, it was showed that the metabolic pathway of PQ in animals is consistent with that in humans.

PQ has two metabolic pathways in the body, through the two intermediate metabolites, monoquat and MP, and finally metabolized to MINA. The results of this experiment proved that after oral gavage of PQ solution, the content of MP in tissues and body fluids was significantly higher than that of monoquat, so the MP pathway should be the main metabolic pathway in human and animals.

4.3. Postmortem distribution

Concentration of PQ and its metabolites (monoquat and MP) in postmortem tissues and liquid fluids were shown in Table4–7. The concentration of PQ was generally high in bile, urine, spleen, and low in peripheral blood, brain and vitreous humor. This is because that PQ selectively accumulates in the lung tissue after entering the body and most of it is excreted in the form of protoplasm within 48 h after entered the body, and the kidney as the main metabolic organ could cause acute kidney injury [33], resulting in urinary retention. Other scholars have only tested PQ on the postmortem distribution of PQ poisoning [34], and its content distribution was different from this study that might due to the difference in dose or individual differences.

The concentration of MP was very high in urine, spleen, kidney, and low in brain and vitreous humor. This may be related to the accumulation of PQ in the lung tissue and excretion through the kidney.

Monoquat was more distributed in urine, kidney, and spleen, however, low in brain, vitreous humor and heart blood. The distribution trend of the two metabolites were roughly similar, both were very high in urine and kidney. This is because that PQ is mainly excreted by the kidney and is distributed higher in the kidney and urine and most of the metabolites are distributed in the body fluids. In contrast, PQ is distributed less in peripheral blood, thus the content of metabolites after metabolism is relatively low.

4.4. Postmortem redistribution

Postmortem redistribution (PMR) refers to the process of changing the concentration of drugs (poisons) in the body [35–37], especially the change of the concentration of the heart blood, which is position-dependent and time-dependent. The larger the ratio of heart blood to peripheral blood, the more likely this phenomenon occur [38]. In the Cases 2–3, the concentration of PQ and its metabolites in heart blood was much higher than in peripheral blood.

Bi Siyuan's study proved that time-dependent PMR occurs after oral gavage of PQ in mice. The Vd of PQ is 1.2–1.6 L/kg [5], but PQ is strong polar bipyridine compound and easy to pass through the biological barrier. In the acidic environment of gastric solution, PQ is relatively stable and its uneven distribution in different organs after death causes. PQ easily diffused from the reservoir to surrounding tissues or blood, which might also be one of the reasons for its redistribution after death.

In addition, the ratio of MP and monoquat in heart blood and peripheral blood was also very high, which indicated the PMR might also occur.

5. Conclusion

As far as our knowledge, this study was the first time a HPLC-MS/MS method and postmortem distribution or postmortem redistribution of the metabolites of PQ and its two metabolites in poisoned death cases have been reported. Heart blood and peripheral blood should be collected for analysis and PMR should be taken into consideration for the forensic identification of PQ related poisoning cases. Our study also proved the metabolic pathway of PQ in human was as the same as the reported in animal.

CRediT authorship contribution statement

Hongjuan Ma: Methodology, Writing, Formal analysis, Data curation. **Meng Hu:** Supervision. **Chao Zhang:** Supervision. **Juan Jia:** Supervision. **Shanlin Fu:** Supervision. **Zhiwen Wei:** Conceptualization. **Keming Yun:** Conceptualization.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2023.111606.

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