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An unusual case of non-fatal poisoning due to herbicide 4-chloro-2-methyl phenoxyacetic acid (MCPA)☆

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ABSTRACT

MCPA (4-chloro-2-methyl phenoxyacetic acid) is a systemic hormone-type selective herbicide readily absorbed by leaves and roots. Use of MCPA for murder or attempted murder is very rare in Sri Lanka. However, a reported case of attempted murder by adding MCPA to water will be discussed in this paper.

Three extraction methods were carried out with urine samples spiked with MCPA, namely liquid–liquid extraction with chloroform, solid phase extraction using C18 cartridges and vortex mixing with methanolic hydrochloric acid. Based on the recovery results, solid phase extraction was selected as the most suitable method and applied in the analysis of urine and water samples. Identification of MCPA in urine, water and the suspected poison bottle was carried out by HPLC and was confirmed by GC–MS. 4-chloro -2- methyl phenol metabolite was also identified and confirmed in the urine sample of the patient by GC–MS. Quantitative analysis of MCPA was carried out by HPLC using a validated method where Zorbax XDB-C18 column was used with photo diode array detector.

In this case, presence of MCPA in one patient's urine sample collected four days after the incident was confirmed by GC–MS and found at a concentration of 0.83 μg/ml. MCPA was not identified in the urine samples collected after 13 days in other three patients. The water sample taken from the suspected water storage tank found to contain 101 μg/ml of MCPA. The results showed that HPLC combined with GC–MS is suitable for forensic analysis of MCPA in urine.

1. Introduction

MCPA (4-chloro-2-methyl phenoxyacetic acid) is a systemic hormone-type selective herbicide readily absorbed by leaves and roots [1]. Its uses include the control of annual and perennial weeds in cereals and grassland [1]. According to the WHO recommended classification of pesticides by hazard it is classified as a class III, slightly hazardous pesticide.

Sri Lanka has a major problem with intentional self-poisoning, with high total and youth suicide rates [2]. Being an agricultural country, the people in Sri Lanka has ready access to potent pesticides; hence suicide and homicide due to pesticides are very common. Most poisoning deaths in Sri Lanka are due to organophosphorus pesticides, but deaths from intentional self-poisoning with other pesticides, such as chlorophenoxy herbicides including MCPA are also reported [3,4]. However, use of MCPA for murder or attempted murder is very rare in Sri Lanka.

Different extraction procedures such as liquid–liquid extraction [5,6], solid phase extraction [7–9] and extraction with organic solvents after vortex mixing and centrifuging [10] have been reported in the literature for the extraction of MCPA from water, blood, urine and other tissues. Further, numerous methods for the identification and quantification of MCPA have been reported in the literature including UV [5], HPLC [6–8,10], GC [9], GC–MS [11] and LC–MS–MS [12]. In this study we present a case of attempted murder by MCPA poisoning confirmed by HPLC and GC–MS analysis of water and body fluids.

2. Materials and methods

2.1. Case

A family comprising four members including two children and a pregnant woman were suspected to be poisoned by a poison introduced to their water storage tank. The suspicion was aroused due to the abnormal behaviour seen in a labourer working in their house. The owner of the house had seen this man checking on the smell of water that was coming from the tap one day. Further, the

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Table 1
Validation results.

Selectivity	Verified against endogenous compounds due to matrices
RSD	
(0.5 ppm)	4.18%
(80 ppm)	3.85%
Percentage recovery at 100 μg/ml (solid phase extraction)	88.7%
Linearity	
10–100 μg/ml	0.999
0.5–2.0 μg/ml	0.999
LOD	0.38 μg/ml
LOQ	0.63 μg/ml

family members frequently fell ill during the previous few weeks. When questioning, it was revealed that the suspected person had added some unknown poison given by another to the water storage tank and he did so repeatedly about three to four times.

Water samples were taken from the suspected water storage tank and another water storage tank nearby which received water from the same source through the same pipe line. These water samples along with the suspected poison bottle were sent for analysis to the toxicology laboratory of the Government Analyst's Department.

After realizing what had happened, all the members of the family were admitted to the hospital for treatment. Urine samples were collected from these patients for analysis, but they were collected at varying time intervals and sent for further analysis (Table 2).

3. Experimental

Three extraction methods were carried out with urine samples spiked with MCPA, namely liquid–liquid extraction with chloroform, solid phase extraction using Bond Elut C18 (200 mg) cartridges and vortex mixing with methanolic hydrochloric acid. Based on the recovery results, solid phase extraction procedure using C18 cartridges was selected as the most suitable method of extraction and applied in the analysis of urine samples in this particular case. Water samples were analysed directly as well as after solid phase extraction.

The qualitative analysis in water was carried out using UV and GC–MS, respectively, while the quantitative analysis of MCPA in water was carried out using a validated HPLC method. The qualitative and quantitative analysis of MCPA in urine was carried out by GC–MS.

3.1. Materials

The standard MCPA was obtained from Sigma–Aldrich Laborchemikalien GMBH, Germany.

3.2. Standard and working solutions

Standard MCPA was dissolved in methanol at the concentration of 1 mg/ml (1000 ppm). The stock solution was diluted appropriately with methanol to obtain 100, 80, 60, 40, 20 and 10 ppm MCPA working standards. 10 ppm MCPA working standard was diluted appropriately with methanol to get working standards of 0.5, 1.0, 1.5, and 2.0 ppm, respectively. All the standards were fortified in methanol.

Calibration curves were constructed in the ranges 10–100 ppm and 0.5–2.0 ppm. The 0.5 ppm standard and 80 ppm standard were injected 10 times and relative standard deviation was calculated. Limit of detection (3 × noise) and limit of quantitation (10 × noise) was calculated using 0.5 ppm standard.

3.3. Liquid–liquid extraction [6]

5 M sulphuric acid (1 ml) was added to urine (10 ml) and kept at 90–95°C for 01 h in a stoppered glass tube and then allowed to cool to room temperature. The hydrolysed sample was extracted three times with chloroform (1 ml × 3). The aqueous and chloroform layers were separated by centrifugation for 5 min. The combined chloroform extract was shaken three times with 0.25 M phosphate buffer, pH 6.2 (1 ml × 3). The buffer layer was separated and made acidic with diluted sulphuric acid to pH 2. The resulted acidic solution was then extracted three times with chloroform (1 ml × 3). The chloroform extract was made dry with small amount of anhydrous sodium sulphate, filtered and evaporated (250 μl). The chloroform extract was injected to HPLC and GC–MS.

3.4. Solid phase extraction [7]

The C18 cartridges (200 mg) were conditioned with methanol (3 ml × 2) followed by 0.01 M phosphate buffer: methanol 80:20 (v/v), pH 2 (3 ml × 2). The urine sample (5 ml) was then passed through the column at a flow rate of 6–8 ml/min. The cartridges were then washed with the same buffer solution (6 ml). Finally, MCPA was eluted from the column with methanol (2 ml) and analysed by HPLC and GC–MS.

3.5. Extraction after vortex mixing and centrifugation [10]

Urine (1 ml) was vortex mixed with 2 ml of methanolic hydrochloric acid (2 ml of concentrated HCl in 1 L of methanol) for 30 s. The mixture was centrifuged for 5 min and the supernatant was taken for analysis by HPLC and GC–MS.

3.6. Recovery studies

A urine sample (100 ml) was spiked with MCPA standard to obtain 100 ppm MCPA in urine. Five aliquots of 5 ml each were

Table 2
Analytical results.

	Samples sent	Number of days passed after the incident	Results
Patient I	Blood	04	Not sufficient for analysis
	Urine		0.83 μg/ml of MCPA was identified
Patient II	Urine	13	MCPA was not identified
Patient III	Urine	13	MCPA was not identified
Patient IV	Urine	13	MCPA was not identified
Samples obtained from the crime scene investigation	Water sample taken from the suspected water storage tank	On the same day	101 μg/ml of MCPA was identified
	Water sample taken from the other storage tank nearby	On the same day	MCPA was not identified
	Suspected poison bottle	On the same day	MCPA was Identified