QUANTITATIVE DETERMINATION OF PARAQUAT BY HPLC-DAD FOLLOWING CHEMICAL REDUCTION WITH SODIUM BOROHYDRIDE IN A FATAL INTOXICATION

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ABSTRACT: This report describes the analytical findings in a fatal case of massive paraquat ingestion. The objective of our work was to establish a reliable method for the analysis of the ionized paraquat using HPLC in combination with diode array detection following chemical reduction with sodium borohydride.

KEY WORDS: Paraquat intoxication; Sodium borohydride reduction; HPLC-DAD.

Problems of Forensic Sciences, vol. XLII, 2000, 36–40
Received 9 September 1999; accepted 16 May 2000

INTRODUCTION

The post-mortem samples (blood, urine, liver, kidney, and stomach contents) of a fifty-year old male, found dead at his home were delivered at the lab the day after the medicolegal autopsy, together with a bottle of Gramoxone® that was found next to the deceased. Gramoxone® is a 20% aqueous solution of the quaternary ammonium herbicide paraquat, the use of which is generally restricted to agriculturists. Although, the normal use of the herbicide does not represent a serious health risk, it has been encountered in several cases of suicidal poisonings, many of which unfortunately successful. These poisonings are often the result of the ingestion of concentrated forms of paraquat such as Gramoxone®. The quaternary ammonium herbicide paraquat is an ionized compound. Consequently, its determination is tedious and presents unique analytical challenges. The objective of our work was to establish a reliable method for the analysis of the ionized paraquat using high pressure liquid chromatography (HPLC) in combination with diode array detection (DAD).

General toxicological investigation

The routine screening by Emit® of the post-mortem blood and urine samples of the deceased disclosed the presence of tricyclic antidepressants (0.49 mg/l in urine only), benzodiazepines (0.9 mg/l blood; 0.3 mg/l urine), cotinine (3.4 mg/l blood; 0.3 mg/l urine), caffeine (0.5 mg/l blood; 0.1 mg/l urine). Further analysis with standard chromatographic methods of the blood, urine, and stomach contents confirmed the screening results, and simultaneously revealed the presence of diethyl parathion (0.1 mg/l urine; 187.2 mg/l stomach contents), mevinphos (61.0 mg/l blood; 19.0 g/l stomach contents), and paraquat. These quantitative results for paraquat were quite unreliable. Indeed, the extraction recovery of the ionized paraquat was low and variable due to the inappropriate analytical conditions. In an effort to provide sound quantitative data concerning paraquat in this intoxication case, our standard HPLC method [1] was adjusted.

EXPERIMENTAL

In a first effort to provide reliable quantitative data for paraquat in this intoxication case, the standard sample preparation was expanded. A chemical reduction of the fully ionized paraquat to a neutral tertiary amine was performed. Secondly, in order to compensate for losses during the entire sample preparation, an appropriate internal standard was needed. The commercially available analogue of paraquat, ethylparaquat, was selected.

HPLC-DAD system

The gradient HPLC system consisted of a model 126 pump and a type 210A manual injector fitted with a 50 µl sample loop from Beckman Instruments Inc. (San Ramon, CA). The Beckman model 168 photodiode array detector was operated in a 4-nm bandpass mode monitoring UV light from 220 to 300 nm. The display wavelength was 230 nm. Separation was achieved on an Aluspher® 100 RP-select B column (125 mm × 4.0 mm i.d., 5 µm particle size) from Merck (Darmstadt, Germany). The mobile phase was a mixture of 0.0125 M NaOH in water (solvent A) and 0.0125 M NaOH in methanol (solvent B). A linear mobile phase gradient was used from 90% A to 10% A during a 15 min time interval. Figure 1 shows a chromatogram of a real forensic blood sample from the presented case. As can be seen, the reduced form of paraquat and the internal standard show excellent chromatographic behavior under the above mentioned conditions.

Sample preparation

Liver and kidney samples were homogenized after dilution with an equal weight of water using an Ultra-Turax homogenizer from IKA (Staufen, Germany).

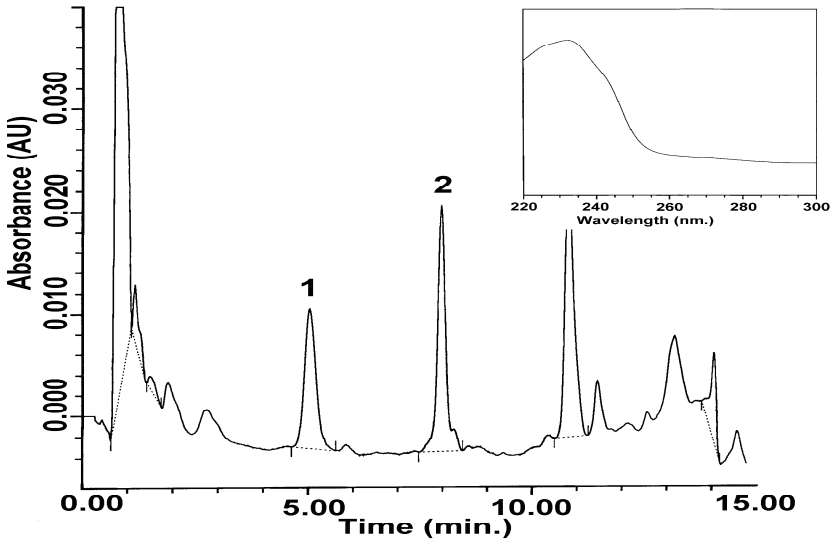

Fig. 1. HPLC-DAD chromatogram (monitored at 230 nm) of a forensic blood sample. The UV spectrum of the reduced form of paraquat is shown in the inset. Peak identification: 1. the reduced form of paraquat, and 2. the reduced form of ethylparaquat (IS).

Protein precipitation

Protein precipitation using 25% trichloroacetic acid (TCA) in water was applied as the initial clean-up step for blood, liver, and kidney homogenate. To that end, 1 ml of a suitable dilution of blood, liver and kidney homogenate was mixed with 1.5 ml of 25% aqueous TCA solution. After transferring the supernatant into a clean plastic test tube, the precipitate was washed with 2.5 ml of a 5% TCA solution in water. For urine and stomach contents, protein precipitation could be omitted.

Reduction procedure

The pH of 1 ml of a proper dilution of urine, stomach contents or supernatant was adjusted with sodium hydroxide (pH >10). Then, powdered sodium borohydride was added (250 mg) and the mixtures were heated in a thermoblock at 60°C for 25 minutes.

Extraction

After cooling down to room temperature, the obtained solutions were extracted with 8 ml of diethyl-ether. Next, the extracts were evaporated to dryness in an ice-bath under a gentle stream of nitrogen.

Quantitative analysis

Six point calibration curves were prepared in appropriate blank post-mortem matrices. The resulting calibration curves were calculated, using weighted linear regression (weighting factor 1/x). Forensic case samples were analyzed in duplicate.

RESULTS AND DISCUSSION

The reduction of bipyridinium compounds with sodium borohydride was first described by Van Dijk [3]. The direct application of this previously reported procedure to complex matrices such as whole blood and post-mortem tissue was unsatisfactory. For that reason, the reduction step was thoroughly optimized to our needs of a full post-mortem investigation. When transferring the supernatant after the protein precipitation, great care had to be taken that no sediment remained in the reaction medium, since remaining proteins extremely retard the reduction rate. During the entire process of sample preparation, paraquat and ethylparaquat were found to adsorb to glass surfaces, consequently only silanized glassware was used.

Table I summarizes some validation parameters. These figures clearly indicate that a reliable method was established to determine paraquat in post-mortem samples.

TABLE I. VALIDATION PARAMETERS

Statistical parameters		Blood	Urine	Stomach contents	Tissue
CV% (n = 6)		2.7	10.7	–	–
LOD [µg/l]		63	32	–	–
Linearity	Intercept	– 0.0042	0.0474	0.0395	– 0.0474
	Slope	0.1543	0.1608	0.0179	0.0250
	Correlation coefficient	0.9997	0.9999	0.9991	0.9983

Paraquat was detected in all available autopsy specimens. Concentrations greater than 5 mg/l were measured in both blood (5.05 mg/l) and urine (6.00 mg/l). A literature search learned that plasma levels exceeding 2 mg/l are likely to be fatal in most subjects [2]. Massive amounts were demonstrated in stomach contents (17 g/l), clearly indicating the oral intake of paraquat. A small amount of paraquat was detected in liver (4.86 mg/kg) in contrast to the high concentrations found in kidney (80.6 mg/kg). This may be explained by the high amount of Gramoxone® ingested by the victim in the presented case and a quick onset of death, resulting in an incomplete distribution. All of these data clearly confirm our initial findings of a massive, deadly Gramoxone® ingestion.

CONCLUSION

A straightforward and robust HPLC analysis following a optimized chemical reduction, was successfully used for the quantitative determination of paraquat in a forensic suicide case. We conclude that this victims main cause of death was paraquat ingestion although the presence of diethyl parathion, mevinphos, and some prescription drugs will definitively have contributed to the deadly outcome in this case.

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