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Short communication

On-line sample preparation of paraquat in human serum samples using high-performance liquid chromatography with column switching

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Abstract

A new ion-pair high-performance liquid chromatographic method with column-switching has been developed for the determination of paraquat in human serum samples. The diluted serum sample was injected onto a precolumn packed with LiChroprep RP-8 (25-40 μm) and polar serum components were washed out by 3% acetonitrile in 0.05 *M* phosphate buffer (pH 2.0) containing 5 m*M* sodium octanesulfonate. After valve switching to inject position, concentrated compounds were eluted in the back-flush mode and separated on an Inertsil ODS-2 column with 17% acetonitrile in 0.05 *M* phosphate buffer (pH 2.0) containing 10 m*M* sodium octanesulfonate. The total analysis time per sample was about 30 min and mean recovery was 98.5±2.8% with a linear range of 0.1–100 μg/ml. This method has been successfully applied to serum samples from incidents by paraquat poisoning. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Paraquat is a bisquaternary ammonium compound used as a herbicide world-widely since 1962. The widespread uses of paraquat indicate the extensive availability and potential for accidental and intentional human exposure. Several hundred deaths have occurred after ingestion of paraquat and it is believed that an oral dose of only 1–2 g is fatal to most adults [1].

Several analytical methods have been reported to determine paraquat in blood, serum, urine and tissues following various clean-up procedures. These techniques include spectrophotometry [2–6], gas chromatography with nitrogen-specific detection (GC–NPD) [7,8], high-performance liquid chromatography (HPLC) [9–14], capillary electrophoresis [15,16] and immunoassay [17,18]. Spectrophotometric methods are based on the reduction of paraquat with sodium dithionate or glucose under alkaline conditions and GC methods are operating on

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boronhydride reduction to a tertiary amine or on injection-port pyrolysis.

The disadvantages of these techniques are laborious and time-consuming sample clean-up procedures such as cation-exchange chromatography [2], protein precipitation [3,14], ion-pair liquid–liquid extraction [5,13] and/or solid-phase extraction [4,9,11,12,16]. Therefore a fast and accurate analytical method for the estimation of paraquat exposure has been required.

In this study, a column-switching HPLC method [19–24] which involves on-line sample trace enrichment and direct injection of diluted serum samples was applied to the determination of paraquat in serum samples. This method was proved to be rapid, simple, specific and sensitive in the analysis of serum samples from five paraquat poisoning cases.

2. Experimental

2.1. Materials and reagents

Paraquat dichloride and sodium octanesulfonate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of HPLC grade. Polypropylene test tubes were used to avoid binding of paraquat to the surfaces of glassware.

2.2. Chromatographic system

The HPLC system consisted of a SpectraSystem P4000 pump (pump 1) (Thermo Separation Products, CA, USA), a Beckman 110B solvent delivery module (pump 2), a Rheodyne 7125 injector (Cotati, CA, USA), a Rheodyne 7000 switching valve and a SpectraSystem UV3000 detector. Data handling was performed by a PC1000 software program (Fig. 1).

The precolumn (20×3.9 mm I.D.) was tap-filled with LiChroprep RP-8 (25–40 μm, Merck, Darmstadt, Germany) and was changed after injection of 100 serum samples. The analytical column was an Inertsil ODS-2 column (150×4.6 mm I.D., 5 μm, GL Sciences, Tokyo, Japan) equipped with a Novapak C_{18} guard column (4×10 mm I.D., Waters Assoc., Milford, MA, USA).

The washing solvent and mobile phase were 3% acetonitrile in 0.05 *M* phosphate buffer (pH 2.0) containing 5 m*M* sodium octanesulfonate and 17% acetonitrile in 0.05 *M* phosphate buffer (pH 2.0) containing 10 m*M* sodium octanesulfonate, respectively. The flow-rates for both cases were 1.0 ml/min and the detection wavelength was 280 nm.

2.3. Analytical procedure

Prior to analysis, a 30 μl of the spiked serum or human serum samples was diluted with 30 μl of washing solvent.

Step 1 (0–4 min): After injecting 50 μl of diluted serum sample, the precolumn was washed for 4 min with washing solvent to remove the serum components. Meanwhile, the guard and analytical column were equilibrated with the mobile phase.

Step 2 (4–9 min): The valve was switched to inject position and the enriched compounds were eluted in the back-flush mode from the precolumn into the analytical column by mobile phase.

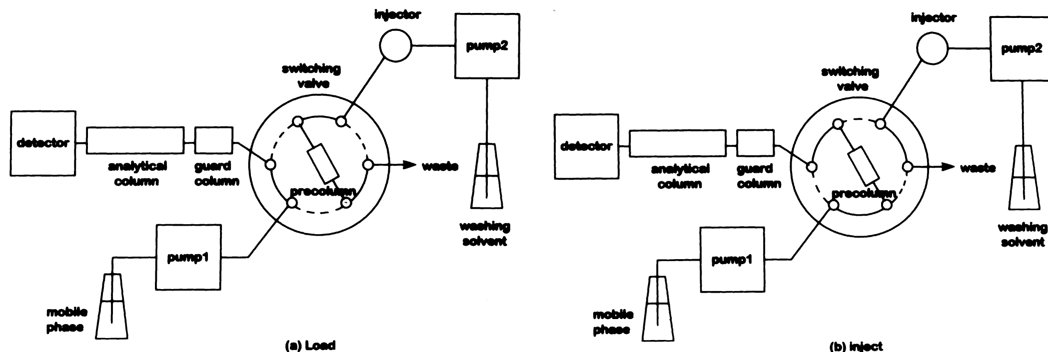

Fig. 1. Schematic diagrams of a six-port switching system.

Step 3 (9–30 min): The eluted compounds were separated on the analytical column. Meanwhile, the valve was moved to load position and the precolumn was re-equilibrated with the washing solvent for another injection.

Total analysis time per serum sample required about 30 min.

2.4. Quality parameters

The detection limit of paraquat was determined by a signal-to-noise ratio of paraquat peak being greater than 3:1.

Paraquat was spiked in serum in the range of 0.1–100 μg/ml and determined to obtain the mean absolute recovery. The precision [defined as the coefficient of variation (C.V.) of replicate analysis] and the accuracy (defined as the deviation between added and found concentration) of the assay were also evaluated.

3. Results and discussion

3.1. Column-switching procedure and chromatography

HPLC methods for quantification of paraquat in blood, urine, serum and tissues are conventionally based on ion-pair chromatography [9–14]. Off-line solid-phase extraction using Sep-Pak C_{18} cartridges [4,9,11,12,16] were popular for sample clean-up of paraquat from serum, urine and tissue samples.

However, on-line trace enrichment using column-switching is more simple, rapid and reproducible than those methods and in column-switching techniques [16–21], the proper choice of the precolumn packing material, washing solvent and washing time is critical in order to obtain complete recovery of paraquat from serum samples while removing serum components.

LiChroprep RP-8 (25–40 μm) was chosen as the precolumn packing because of the best recovery for paraquat among other packing materials including μBondapak Corasil/C_{18} (60%) and μBondapak phenyl (0%).

In using 3% acetonitrile in 0.05 *M* phosphate buffer (pH 2.0) containing 5 m*M* sodium octanesulfonate as washing solvent, the majority of serum components were relatively unretained while paraquat exhibited strong retention on LiChroprep RP-8 precolumn. In order to obtain the best recovery and clean chromatograms, the clean-up process was completed in 4 min by washing the precolumn at a flow-rate of 1.0 ml/min with washing solvent.

When an Inertsil ODS-2 column and 17% acetonitrile in 0.05 *M* phosphate buffer (pH 2.0) containing 10 m*M* sodium octanesulfonate for mobile phase were used, a clean and sharp chromatogram of paraquat was obtained from serum samples (Fig. 2). Retention time of paraquat was 14.6 min, and spectral analysis of paraquat peak in the chromatogram of serum samples together with authentic standard revealed no interfering peaks.

3.2. Quality parameters

The correlation of peak areas with the concentrations of paraquat in serum was linear in the range of 0.1–100 μg/ml with a correlation coefficient of 0.999. The detection limit of paraquat was 0.1 μg/ml with 25 μl of serum and mean recovery was 98.5±2.8%. The precision and the accuracy of the assay were evaluated over the same concentration range as above (Table 1) and C.V. varied from 1.1% to 3.3%, which indicates excellent results for biological sample analysis.

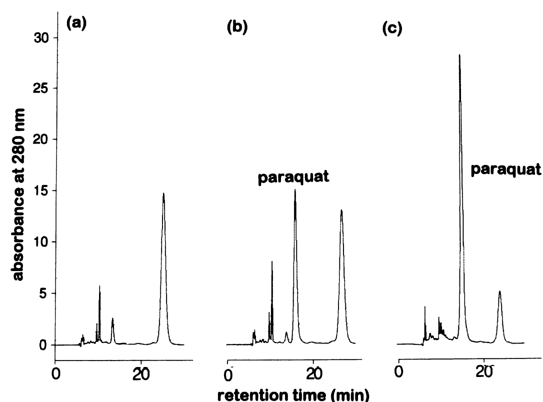

Fig. 2. Chromatograms of (a) blank serum, (b) blank serum spiked with paraquat (10 μg/ml), and (c) a serum sample from a paraquat incident.

Table 1
Reproducibility of paraquat in serum samples (n=5)

Concentration (μg/ml)		
Added	Found	C.V. (%)
Intra-day		
0.1	0.1	3.3
1.0	1.03	2.7
10.0	10.2	2.9
50.0	50.0	2.0
100.0	98.5	1.1
Inter-day		
0.1	0.1	3.2
1.0	1.02	2.8
10.0	10.4	2.9
50.0	49.8	1.8
100.0	99.3	1.2

3.3. Application of the method to human samples

The present method has been successfully applied to the analysis of serum samples from five paraquat poisoning incidents proving the advantage of the method developed in this study. The serum paraquat concentrations were found from 9.7 to 189.4 μg/ml.

4. Conclusion

For the determination of paraquat in serum, a simple column-switching HPLC method with direct injection of a small amount of diluted serum samples was developed. This method was successfully applied to the determination of paraquat from serum samples of paraquat incidents thanks to its excellent precision, sensitivity, specificity, and speed.

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