Rokus A. de Zeeuw,[1] Ph.D.; Jaap Wijsbeek;[1]
Jan Piet Franke,[1] Ph.D.; Maciej Bogusz,[2] M.D.; and
Malgorzata Kłys,[2] Ph.D.

Multicenter Evaluation of Ultrafiltration, Dialysis, and Thermal Coagulation as Sample Pretreatment Methods for the Colorimetric Determination of Paraquat in Blood and Tissues

REFERENCE: de Zeeuw, R. A., Wijsbeek, J., Franke, J. P., Bogusz, M., and Kłys, M., **"Multicenter Evaluation of Ultrafiltration, Dialysis, and Thermal Coagulation as Sample Pretreatment Methods for the Colorimetric Determination of Parquat in Blood and Tissues,"** *Journal of Forensic Sciences*, JFSCA, Vol. 31, No. 2, April 1986, pp. 504-510.

ABSTRACT: Three methods of sample pretreatment for the rapid colorimetric determination of paraquat were compared: ultrafiltration, dialysis, and thermal coagulation. Spiked autopsy blood and tissue samples were examined in parallel in Groningen and Krakow and some samples were interchanged. All three methods gave recoveries between 87 to 102%; accuracy at the 20-mg/L level was within 10% of the target value and coefficients of variation in the 10 to 60-mg/L range were between 3 to 15%. Determinations in blood and liver in a fatal case of Gramoxone® poisoning showed excellent agreement. Because of its reliability, speed, and simplicity, ultrafiltration is the method of choice.

KEYWORDS: toxicology, paraquat, ultrafiltration, dialysis, thermal coagulation, comparative analysis

Paraquat poisoning remains a constant hazard in modern society. The prompt correct assessment of a paraquat intoxication is of decisive value in introducing lifesaving treatment and, on the other hand, may have important forensic science implications. There is, therefore, a strong need for rapid and reliable methods for paraquat determinations in biological samples. Most frequently, paraquat is determined colorimetrically after reaction with sodium dithionite in alkaline medium, which produces a blue-colored free radical [*1*]. A variety of modifications of this method has been published which differ mainly in the sample pretreatment step: Paraquat was isolated from blood and tissues by cation-exchange chromatography [*1,2*], ion pair extraction [*3*], dialysis [*4*], or thermal coagulation [*5*].

Besides colorimetry, other methods of paraquat determination include radioimmunoassay

Received for publication 19 Feb. 1985; revised manuscript received 27 June 1985; accepted for publication 3 July 1985.

[1]Professor of toxicology, research associate, and senior research associate, respectively, Department of Toxicology, State University, Groningen, The Netherlands.

[2]Chief toxicologist and toxicologist, respectively, Institute of Forensic Medicine, Copernicus Medical Academy, Krakow, Poland.

[6-8], gas chromatography [9,10], gas chromatography/mass spectrometry [11], and high-pressure liquid chromatography [12]. The latter methods, although claimed to be more selective than colorimetry, suffer from the fact that they are more complicated or time-consuming or both, and, therefore, not so useful for rapid determinations in emergency cases.

The purpose of the present study was to obtain an evaluation of sample pretreatment methods for the colorimetric determination of paraquat in blood and tissues. In addition to dialysis and thermal coagulation, we included ultrafiltration, a relatively new method to remove macromolecules such as proteins from biological samples. In view of the growing interest in multicenter evaluations, the investigations were carried out in parallel in Groningen and Krakow and some samples from a fatal case of paraquat poisoning were interchanged to estimate the interlaboratory variability.

Materials and Methods

Materials

Autopsy blood was used as received. Liver and kidney were minced with a Waring blender and homogenized in five parts of saline solution. Dialysis was performed using Visking dialysis tubing, diameter 21 mm, nominal cutoff 10 to 15 kilodaltons, supplied by Serva GmbH, Heidelberg (FRG). Ultrafiltration was done in MPS-1 reusable ultrafiltrations units with YMT membranes (cutoff 25 to 30 kilodaltons), obtained from Amicon Corp., Danvers, MA, USA. Paraquat standard solutions were prepared by mixing a standard (1 mg/mL) of pure paraquat dichloride (ICI Plant Protection Division, Experimental Dept., Yalding, Gt. Britain) with water, autopsy blood, or tissue homogenate. The biological samples were left for 2 to 4 h after spiking for equilibration. Specimens of blood and liver containing 10 to 60 mg/L of paraquat were used for recovery determinations and as precision standards. Accuracy was tested with spiked blood samples containing 20 mg/L of paraquat. Blank samples of tissue homogenate (containing no paraquat) were prepared and run in parallel with the spiked samples. Samples of blood and liver from a case of fatal paraquat (Gramoxone®) poisoning were also analyzed.

Methods of Sample Pretreatment

Dialysis—The method of Smirnakis et al [4] was used with minor modifications: 2 g of material were dialysed against 10 mL of water overnight. The clear dialysate was taken for colorimetry.

Ultrafiltration—From blood or tissue homogenate, 1 mL was diluted with 4 mL of water and centrifuged 3 min at 12 000 *g* (Krakow) or 10 min at 6000 g at 0°C (Groningen). Of clear supernatant, 1 mL was poured into the sample reservoir of the MPS-1 unit. The unit was centrifuged 15 min at 1000 *g* under an angle of 35°. Usually 300 μL of ultrafiltrate could be obtained in 15 min.

Thermal Coagulation According to Rejent et al [5]—From blood or tissue homogenate, 1 mL was diluted with 4 mL of water and heated in a boiling water bath for 15 min. The sample was then centrifuged 3 min at 12 000 *g* (Krakow) or 10 min at 6000 g at 0°C (Groningen) and the supernatant was filtered through a standard paper filter. The filtrate was taken for colorimetry.

Colorimetry

The samples obtained from the above-mentioned procedures were mixed with 2% sodium dithionite in 1*N* sodium hydroxide (NaOH) in proportions of 8:1. The absorbances were read within 1 min at 600 nm against a reference in microcuvettes of 100-μL capacity (Krakow) or semimicrocuvettes of 500-μL capacity (Groningen). Measurements were carried out by means of a SP8-100 spectrophotometer (Pye Unicam, Cambridge, England) in Groningen or a Specord

colorimeter (Carl Zeiss Jena, GDR) in Krakow. The reference was prepared by mixing a sample obtained from the appropriate procedure with 1*N* NaOH in proportion of 8:1. The blank samples were obtained by running an appropriate biological sample, containing no paraquat, through the total procedure. Besides the fixed wavelength determinations, the blank samples as well as aqueous paraquat solutions were scanned against the reagent blank (8 parts water + 1 part sodium dithionite) in the visible range of the spectrum (350 to 800 nm) by means of a Specord colorimeter.

Results and Discussion

In most colorimetric procedures paraquat is determined at a wavelength of about 390 nm [*1-3,5*]. However, readings taken at this wavelength may be strongly influenced by background absorbance from the matrix (for example, hemoglobins). This was checked by scanning the absorbance spectrum of blank blood, liver, and kidney samples and treated with sodium dithionite. For thermal coagulation, a very strong, broad background was observed for liver, peaking around 390 nm, while for blood and kidney a distinct background around 420 nm was observed (Fig. 1). Moreover, these backgrounds were found to vary in repetitive experiments, so that a correction method, using measurements at four different wavelengths [*1*], proved to be of little use. The background absorbance at 600 nm (that is, at the wavelength of the second maximum of the paraquat derivative) was much less than around 390 nm, comparable for all matrices and virtually constant during repetitive experiments. Thus, 600 nm would seem preferable for measuring samples heated by thermal coagulation. The samples prepared by dialysis and ultrafiltration showed less background absorbance, which remained almost the same, throughout the whole visible range of spectrum (Fig. 2). All further measurements were then made at 600 nm. Although this will lead to some loss in sensitivity for dialysis and ultrafiltration samples, chances for interference of other components will be less than around 400 nm.

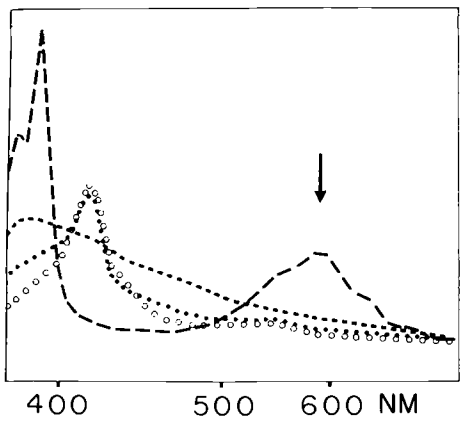

FIG. 1—*Paraquat absorption spectrum after treatment with dithionite and absorption spectra of blank blood, liver, and kidney after sample pretreatment by thermal coagulation.*

— — — — — *paraquat standard in water*
..... *blank blood*
---------- *blank liver homogenate, dilution 1:25*
00000000000 *blank kidney*

The arrow indicates the preferred wavelength for paraquat determinations. Note that the liver sample had to be diluted an extra five-fold to get the reading on the same scale with the curves for blood and kidney. Recorded on the Specord spectrometer.

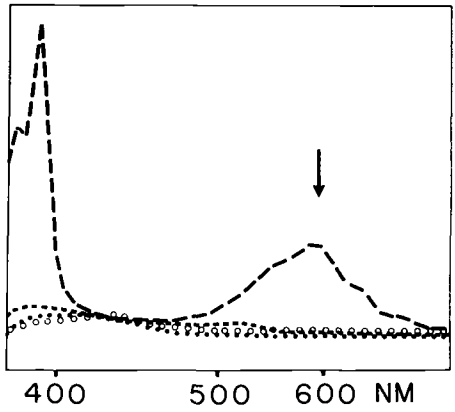

FIG. 2—*Paraquat absorption spectrum after treatment with dithionite and absorption spectra of blank blood, liver, and kidney after sample pretreatment by ultrafiltration.*

— — — — — *paraquat standard in water*
..... *blank blood*
---------- *blank liver homogenate, dilution 1:5*
00000000000 *blank kidney*

The arrow indicates the preferred wavelength for paraquat determinations. Recorded on the Specord spectrometer.

TABLE 1—*Percent recovery of paraquat in water standards and in biological material treated with different methods. Mean ± standard deviation (day-to-day variation) for determinations in the range of 10 to 60 mg/L.* K = *Krakow.* G = *Groningen.*

Method of Pretreatment	H_2O Standards	Blood	Liver
None	K 100 ± 7 (N = 40)		
	G 100 ± 2 (N = 12)		
Dialysis	K	102 ± 13 (N = 17)	95 ± 10 (N = 12)
	G	87 ± 11 (N = 10)	98 ± 11 (N = 9)
Ultrafiltration	K	89 ± 16 (N = 8)	93 ± 15 (N = 6)
	G	101 ± 5 (N = 9)	87 ± 3 (N = 9)
Coagulation	K	100 ± 11 (N = 16)	95 ± 11 (N = 5)
	G	nd	97 ± 4 (N = 9)

TABLE 2—*Accuracy of paraquat determinations in two blind tests. Samples: blood spiked with paraquat. Target: 20 mg/L. Mean values of three determinations.*[a]

Method of Pretreatment	1st Examination	2nd Examination
Dialysis	22.2	20.6
Ultrafiltration	19.6	19.9
Coagulation	18.8	20.7

[a]Not corrected for recovery losses.

TABLE 3—*Comparison of the applied methods of sample pretreatment.*

Method of Pretreatment	Sample Quality	Simplicity	Speed	Economy	Main Disadvantages
Dialysis	clean sample, low background absorbance	simple but troublesome for large series of samples	requires one day	cheap	time factor, not for large series
Ultrafiltration	clean sample, low background absorbance	very simple, convenient for large series	results within 60 min	filters about $1, each	check quality of filters visually
Thermal coagulation	samples sometimes opalescent, varying background absorbance	very simple, convenient for large series	results within 60 min	very cheap	needs careful standardization because of background absorption

TABLE 4—*Paraquat determinations in a case of Gramoxone poisoning. Parallel determinations in Krakow* (K) *and Groningen* (G). *Mean values of three to six determinations.*[a]

Method of Pretreatment	Blood, mg/L		Liver, mg/kg	
	K	G	K	G
Dialysis	35	41	127	123
Ultrafiltration	41	37	118	129
Coagulation	34	nd	125	136

[a]Not corrected for recovery losses.

The percent recovery figures of paraquat added to biological material, as well as day-to-day variations, are shown in Table 1. The values were calculated against the absorbances of standard solutions of paraquat in water treated with sodium dithionite without any pretreatment and run in parallel with biological samples. The values were obtained in Groningen and Krakow in a period of one year.

In general, the results in Groningen tended to be more precise than in Krakow, probably because of differences in instrumentation. The recovery figures for the two laboratories did not differ significantly. Furthermore, they were virtually constant over the concentration range studied. Some blood samples treated with thermal coagulation in Groningen yielded higher background absorptions at 600 nm as a result of turbidity, so that no precision figures could be given. This may be due to inadequate centrifugation at 6000 g as this phenomenon was never observed at 12 000 *g* in Krakow.

The accuracy of each method was checked in Krakow with blood standards containing 20 mg/L of paraquat in two series of independent determinations, as a blind test performed by different analysts. The results are presented in Table 2.

Because of the instability of the blue-colored free radical [*1*], absorbance measurements must be taken at constant times after adding the dithionite reagent. After dialysis and thermal coagulation, the color was stable for about 5 min, whereas after ultrafiltration fading sometimes started after 2 min. The reason for this may be traces of active compounds in the YMT membranes as this phenomenon appeared to be batch-dependent. Some earlier batches of membranes were found to be unacceptable in that they did not have a uniform thickness upon visual inspection to light transmission. The more recent batches were all of acceptable quality, but a check on light transmission before use may be recommended.

It can be concluded that all three methods of sample pretreatment provided adequate accuracy and precision when absorbance was measured at 600 nm. Ultrafiltration and dialysis provided the cleanest samples and the lowest background. Thermal coagulation appeared to be more prone to background interferences but it should be noted that this method was originally proposed for serum analysis [*5*]. Some other properties of the methods are compared in Table 3. In our opinion, ultrafiltration is the method of choice in view of reliability, simplicity, and speed. Also, the ultrafiltrate can be used directly for other analytical applications such as HPCL.

Samples of autopsy blood and liver, taken from a fatal case of Gramoxone poisoning in Krakow, were split and examined in parallel in the two laboratories. The results are shown in Table 4. Excellent agreement was obtained for all three methods.

References

[*1*] Calderwood, A. and Yuen, S. H., "An Ion Exchange Method for Determining Paraquat Residues in Food Crops," *Analyst*, Vol. 90, No. 1067, Feb. 1965, pp. 99-106.

[*2*] Berry, D. J. and Grove, J., "The Determination of Paraquat (1,1′-Dimethyl-4,4′-Bipyridylium Cation) in Urine," *Clinica Chimica Acta*, Vol. 34, No. 7, 1971, pp. 5-11.

[3] Jarvie, D. R. and Stewart, M. J., "The Rapid Extraction of Paraquat from Plasma Using an Ion-Pairing Technique," *Clinica Chimica Acta*, Vol. 94, No. 3, 1979, pp. 241-251.
[4] Smirnakis, Z., Coutselinis, A., Alevisopoulos, G., Pagou, M., and Adjutantis, G., "A Simple Method for the Estimation of Paraquat in Tissue," *Journal of Analytical Toxicology*, Vol. 6, No. 5, Sept./Oct. 1982, p. 266.
[5] Rejent, T. A., Balkon, J., and Donnelly, B., "A Paraquat Fatality—the Dilemma of Multiple Analyses," *Journal of Forensic Sciences*, Vol. 28, No. 2, April 1983, pp. 510-515.
[6] Levitt, T., "Radioimmunoassay for Paraquat," *The Lancet*, Vol. II, No. 8033, 13 Aug. 1977, p. 358.
[7] Stewart, M. J., Levitt, T., and Jarvie, D. R., "Emergency Estimations of Paraquat in Plasma. A Comparison of the RIA and ION Pair/Colorimetric Methods," *Clinica Chimica Acta*, Vol. 94, No. 3, 1979, pp. 253-257.
[8] Fatori, D. and Hunter, W. M., "Radioimmunoassay for Serum Paraquat," *Clinica Chimica Acta*, Vol. 100, No. 2, 1980, pp. 81-90.
[9] Martens, M. A. and Heyndrickx, A., "Determination of Paraquat in Urine by Pyrolysis Gas Chromatography," *Journal de Pharmacie de Belgique*, Vol. 29, No. 5, 1974, pp. 449-454.
[10] Van Dijk, A., Ebberinck, R., De Groot, G., Maes, R., Douze, J., and Van Heyst, A., "Rapid and Sensitive Assay for the Determination of Paraquat in Plasma by Gas-Liquid Chromatography," *Journal of Analytical Toxicology*, Vol. 1, No. 4, July/Aug. 1977, pp. 151-154.
[11] Draffan, G. H., Clare, R. A., Davies, D. L., Hawksworth, G., Murray, S., and Davies, D. S., "Quantitative Determination of the Herbicide Paraquat in Human Plasma by Gas Chromatographic and Mass Spectrometric Methods," *Journal of Chromatography*, Vol. 139, No. 2, 1977, pp. 311-320.
[12] Gill, R., Qua, S. C., and Moffat, A. C., "High-Performance Liquid Chromatography of Paraquat and Diquat in Urine with Rapid Sample Preparation Involving Ion-Pair Extraction on Disposable Cartridges of Octadecylsilica," *Journal of Chromatography*, Vol. 255, 1983, pp. 483-490.

Address requests for reprints or additional information to
Dr. Rokus A. de Zeeuw
Professor of Toxicology
Department of Toxicology
State University
Deusinglaan 2
9713 AW Groningen, The Netherlands