ELSEVIER

Journal of Chromatography B, 674 (1995) 193–196

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Simple method for analysis of diquat in biological fluids and tissues by high-performance liquid chromatography[1]

Cherukury Madhu[2], Zoltan Gregus[3], Curtis D. Klaassen*

Center for Environmental and Occupational Health, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160-7417, USA

First received 7 February 1995; revised manuscript received 11 July 1995; accepted 13 July 1995

Abstract

A simple HPLC method has been described to quantify diquat in biological fluids and tissues. This method permits separation and quantification of diquat from blood, bile, urine, liver and kidney. It does not require special pretreatment of the samples prior to analysis, nor a specially prepared analytical column. Various concentrations of diquat were added (10–300 nmol/ml or g) to fluids or tissues. Analysis of blank samples revealed no substances that interfere with diquat elution. Excellent recovery (95–105%) was obtained. Diquat (120 μmol/kg, i.v.) was injected to rats and quantified in bile, blood and liver. Concentration of diquat was higher in blood and bile than liver. Therefore, this method is applicable for quantification of diquat in toxicological samples, and may be used to determine structurally similar compounds such as paraquat.

1. Introduction

Diquat is a widely used non-selective herbicide. Because of its extreme toxicity to humans, it has been encountered in accidental, suicidal and homicidal poisonings [1–4]. Acute poisoning with large oral doses of diquat results in generalized necrosis, mainly of the liver, kidney and heart, and may be associated with mortality several hours to a few days after exposure [5]. The diquat-induced hepatic necrosis is thought to be due to production of superoxide anion radical, with the resultant formation of hydrogen peroxide and hydroxyl radical, leading to tissue destruction [6]. However, Smith [7] reported strain differences in diquat-induced hepatic necrosis, in that Sprague–Dawley rats are resistant whereas Fisher rats are sensitive. The reason for this strain difference is not known. Recently, we observed a marked inter-animal difference in Sprague–Dawley rats to diquat-induced oxidative stress and thought that the variation might be due to difference in diquat disposition. Therefore, quantification of diquat in biological samples was necessary to test this hypothesis.

Several methods are available to measure diquat in biological fluids [8–12] and in food crops [13], but not for tissues. These methods include thin-layer chromatography [14–19], colorimetry with an alkaline dithionite reaction [20] or bromthymol blue [8], gas chromatography

* Corresponding author.

[1] This work was supported by US Public Health Service Grant ES-03192.

[2] Present address: Allergan Company, Irvine, CA, USA.

[3] Present address: University Medical Center, Pécs, Hungary.

0378-4347/95/$09.50
SSDI 0378-4347(95)00306-1

[10], and high-performance liquid chromatography [9,11,13]. These methods are laborious because they require extraction of diquat from samples, using disposable cartridges, and require specially prepared analytical columns. Therefore, the purpose of the present study was to develop a simple, rapid and less expensive HPLC method for quantification of diquat in biological fluids that also could be used for tissues.

2. Experimental

2.1. Standards

Diethylamine, octane sulphonic acid and tetrahydrofuran were obtained from Sigma (St. Louis, MO, USA). Orthophosphoric acid and HPLC grade methanol were purchased from Fisher Scientific (St. Louis, MO, USA) and ultrapure perchloric acid (70%) from Alfa Products (Danvers, MA, USA). Diquat was a generous gift by Dr. I.F.H. Purchase of ICI Toxicological Laboratories (Macclesfield, Cheshire, UK).

2.2. Sample preparation

Sprague–Dawley rats (200–225 g) were obtained from Sasco (Omaha, NE, USA). They were provided tap water and lab chow (Ralston Purina, St. Louis, MO, USA) ad libitum and were housed at 22–23°C on a 12-h light–dark cycle.

2.3. HPLC analysis

HPLC was performed using a Waters Model 501 pump and a manual injector equipped with a 20-μl loop (Alltech Associates, Deerfield, IL, USA). The separation was carried out on a μ-Bondapack C_{18} column (30 cm × 3.8 mm; Waters Associates, Boston, MA, USA) with a guard column (25 mm × 3.8 mm) packed with pellicular C_{18} (Alltech). Diquat was eluted at a flow-rate of 1.0 ml/min and monitored by UV detection at 315 nm with full scale absorbance set at 0.02 AUFS. Quantification of diquat was based on integrated peak areas. Integration was performed using a Waters 840 chromatography data station system. The mobile phase was selected from Gill et al. [19] and was prepared by mixing deionized water (900 ml), octane sulfonic acid (650 mg), acetonitrile (75 ml), orthophosphoric acid (13.5 ml), diethylamine (10.3 ml), and tetrahydrofuran (40 ml) and filtered through a 0.45-μm filter (Millipore, MA, USA).

2.4. Extraction method

To 100 μl of blood, bile or urine, a known amount of diquat (0, 10, 30, 100 or 300 nmol) was added, followed by 400 μl of water. Then 500 μl of 70% methanol, containing 21 m*M* perchloric acid, was added. Samples were mixed by vortex-mixing and centrifuged for 4 min in a microfuge (Beckman). The resultant supernatants were saved for HPLC analysis.

To 1 g of liver or kidney, a known amount of diquat (0, 10, 30, 100 or 300 nmol) was added, followed by 4 ml of 70% methanol containing 21 m*M* perchloric acid, and homogenized with a Polytron (Brinkman) approximately 45 s, and centrifuged for 15 min at 1000 *g*. The resultant supernatants were saved for diquat analysis.

Standards were prepared by adding diquat to 70% methanol containing 21 m*M* perchloric acid and processed as above. In order to avoid the loss of diquat during sample processing and analysis, precautions were taken as described in Lauren and Agnew [21].

2.5. Animal experiments

Rats were anesthetized with urethane (1.2 g/kg, i.p.). After median laparotomy, the bile duct was cannulated with a 25-gauge needle attached to PE-50 tubing. Diquat (120 μmoles/kg, i.v.) was administered and bile collected for 30 min into preweighed Eppendorf tubes (1.5 ml) containing 500 μl of 0.25 *M* perchloric acid. Thereafter, blood and liver samples were taken and diquat was extracted as described above. Bile samples were centrifuged and the resultant supernatants were used for diquat analysis.

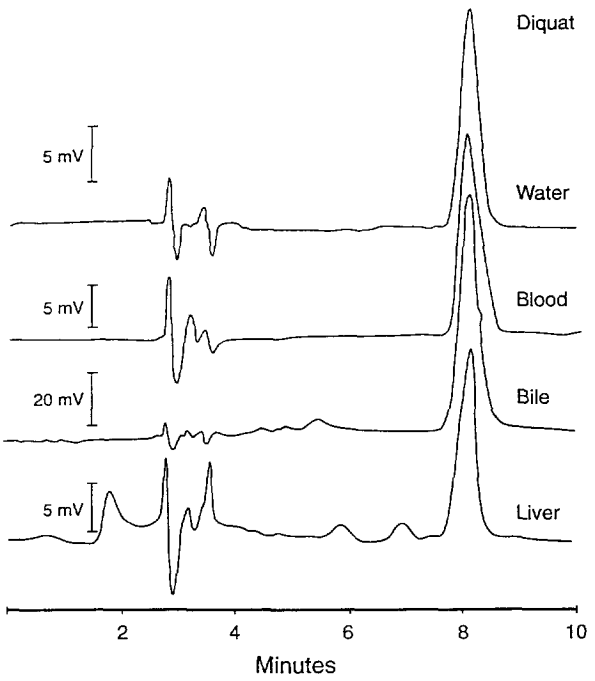

Fig. 1. Chromatograms of the diquat standard and biological samples from rats injected with diquat (120 μmol/kg, i.v.). Conditions of HPLC analysis are described in the text.

3. Results and discussion

Diquat (0.3125 nmol) was dissolved in water, and the biological samples (bile, blood and liver) collected from control rats were extracted as described in Section 2.4. Subsequently, 20 μl of the extracts was analyzed by HPLC, and diquat was monitored (315 nm) with a variable UV detector. Representative chromatograms are shown in Fig. 1. Blank samples contained no substances that interfere with diquat elution (chromatograms are not shown). With this method, diquat was eluted at approximately 8 min. Spectral analysis by photodiode-array detector of the diquat peaks in the chromatogram of biological samples and the diquat standard revealed no interfering peaks.

The peak areas of diquat are linearly related to quantity of diquat (0.0195–5 nmol) injected. Linear regression analysis of the standard curve indicated no significant deviation from linearity ($r^2 = 0.999$).

The interday assay precision and accuracy for the analysis of diquat (10–300 nmol/ml or g)

Table 1
Interday assay precision and accuracy of diquat in biological fluids and tissues

Sample	Diquat added (nmol/ml or g)	Found (nmol/ml or g)	Recovery (%)	C.V. (%)
Blood	10	9.49 ± 0.27	95	2.70
	30	28.4 ± 0.70	95	2.33
	100	96.8 ± 3.13	97	3.23
	300	289 ± 13.0	96	4.49
Bile	10	9.18 ± 0.367	92	3.99
	30	29.6 ± 0.723	98	2.44
	100	99.4 ± 2.89	99	2.90
	300	301 ± 2.00	100	0.66
Urine	10	10.5 ± 0.351	105	3.34
	30	28.8 ± 1.02	96	3.54
	100	102 ± 1.53	102	1.50
	300	345 ± 3.00	115	0.869
Liver	10	10.9 ± 0.173	109	1.59
	30	31.8 ± 0.379	106	1.19
	100	102 ± 3.86	102	3.78
	300	309 ± 7.57	115	2.45
Kidney	10	9.97 ± 2.32	100	23.2
	30	30.4 ± 0.64	101	2.10
	100	100 ± 2.47	100	2.47
	300	295 ± 4.51	98	1.53

Values are mean ± S.D., $n = 3$; recovery = [(diquat found in sample)/(diquat added)] × 100.

from the biological samples are presented in Table 1. The interday coefficient of variation for all the samples analyzed was <4.5% except for the kidney at 10 nmol/g. The recovery of diquat exceeded 95% for all the samples (Table 1). Therefore, this method is valid to quantify diquat from biological fluids and tissues.

Previously diquat was quantitated from biological fluids [9,22], tissues [23] and agricultural products [11,13]. These methods require either specially prepared analytical columns (which are not commercially available) or samples using disposable cartridges. Similarly, other methods require either silica solid-phase extraction cartridges [13] or column chromatography [11]. These methods are labor-intensive and time-consuming. The present method does not require either disposable cartridges for extraction of diquat or specially prepared analytical columns. Therefore, this method is simple and easy to use.

Diquat was measured in blood, bile and liver samples following administration of diquat (120 μmol/kg, i.v.) to rats. At 30 min after administration of diquat, the concentration of diquat (nmol/ml or g) in blood, bile and liver was 139 ± 17, 195 ± 22 and 15.3 ± 0.9 (mean ± S.E., $n = 6$), respectively (Table 2). Quantification of diquat disposition using this method revealed that the observed interanimal variation in diquat-induced oxidative stress [24] is due to differences in hepatic accumulation of diquat.

In conclusion, this method is simple, rapid and sensitive for determination of diquat in biological samples including tissues. This method is applicable for quantification of diquat, and possibly also of paraquat, from toxicological samples.

Table 2
Quantitation of diquat in bile, blood and liver samples of rats by HPLC

Sample	Diquat concentration (nmol/ml or g)
Bile	195 ± 22
Blood	139 ± 17
Live	15.3 ± 0.9

Diquat was measured in the samples 30 min after administration of diquat to rats (120 μmol/kg, i.v.). Values represent means ± S.E. of 6 animals.

References

[1] K. Fletcher, in B. Ballantyne (Editor), Forsenic Toxicology, John Wright, Bristol, 1974, p. 86.
[2] T.J. Haley, Clin. Toxicol., 14 (1979) 1.
[3] R. Vanholder, F. Colardyn, J.D. Reuck, M. Praet, N. Lameire and S. Ringoir, Am. J. Med., 70 (1981) 1267.
[4] P. Mahieu, Y. Bonduelle, A. DeCabooter, M. Gala, A. Hassoun, J. Koening and R. Lauwerys, Clin. Toxicol., 22 (1984) 363.
[5] S. Okonek, Med. Welt, 27 (1976) 1401.
[6] R.D. Fairshter and A.F. Wilson, Am. J. Med., 59 (1975) 751.
[7] C.V. Smith, Biochem. Biophys. Res. Comm., 144 (1987) 415.
[8] M. Akerblom, Bull. Environ. Contam. Toxicol., 45 (1990) 157.
[9] R. Gill, S.C. Qua and A.C. Moffat, J. Chromatogr., 255 (1983) 483.
[10] S. Kawase, S. Kanno and S. Ukai, J. Chromatogr., 283 (1984) 231.
[11] T. Nagayama, T. Maki, K. Kau, M. Iida and T. Nishima, J. Assoc. Off. Anal. Chem., 70 (1987) 1008.
[12] A. Pryde and F.J. Darby, J. Chromatogr., 115 (1975) 107.
[13] T.M. Chichila and S.M. Walters, J. Assoc. Off. Anal. Chem., 74 (1991) 961.
[14] M.B. Abou-Donia and A.A. Komeil, J. Chromatogr., 152 (1978) 585.
[15] R.A. de Zeeuw, P.E.W. van der Laan, J.E. Greving and F.J. van Mansvelt, Anal. Lett., 9 (1976) 831.
[16] H.G. Draffan, R.A. Clare, D.L. Davies, G. Hanksworth, S. Murray and D.S. Davies, J. Chromatogr., 139 (1977) 311.
[17] G.S. Tadjer, J. Forensic Sci., 12 (1967) 549.
[18] S. Tsunenari, Forensic Sci., 5 (1975) 61.
[19] M. Van den Heede, A. Heyndrickx and J. Timperman, Med. Sci. Law, 22 (1982) 57.
[20] S.H. Yuen, J.E. Bagness and D. Myles, Analyst (London), 92 (1967) 375.
[21] D.R. Lauren and M.P. Agnew, J. Chromatogr., 303 (1984) 207.
[22] I. Nakagiri, K. Suzuki, Y. Shiaku, Y. Kuroda, N. Takasu and A. Kohama, J. Chromatogr. 481 (1989) 434.
[23] S. Ito, T. Nagata, K. Kudo, K. Kimura and I. Imamura, J. Chromatogr., 617 (1993) 119.
[24] Ch. Madhu, Z. Gregus and C.D. Klaassen, J. Pharm. Exp. Ther., 263 (1992) 1008.