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A Simple Rapid Spectrophotometric Investigation of Diquat and its Applications

S. Pradhan[1], M. K. Rai[2], J. K Rai[3]

[1]School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, 492010, India

[2]Asso. Proffessor, So S in Chemistry, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, 492010

[3]Sr. Scientist,Chhattisgarh Council of Science and Technology, MIG25, Indravati colony, Raipur, Chhattisgarh, India

Abstract: ***A sensitive method for the determination of widely used bipyridium herbicide diquat using sodium borohydride in spectrophotometer has been described. Diquat on reduction with sodium borohydride in basic medium give a blue colored dye with absorbance maxima obtained at 620 nm. The method is calibrated over the concentration range of 2.6 to15 µg in a final solution of volume 10 ml (0.26 – 1.5 ppm). Various statistical parameters ie. molar absorptivity, Sandell's sensitivity, standard deviation and relative standard deviation have been calculated respectively. The method is simple, rapid, sensitive and satisfactorily applied for detection of diquat in samples of plant materials, soil, water and biological samples.***

Keywords: Spectrophotometer, Diquat, sodium borohydride, environmental, agricultural samples

1. Introduction

Diquat or diquat dibromide is a bipyridyl, water-soluble, nonselective, fast-acting herbicide widely used in indoor and outdoor, to control both land and aquatic weeds. In agriculture it is generally used for pre-harvest desiccation of variety of crops [1,2]. Upon application it has the tendency to reacts with molecular oxygen by free radical mechanism and generates super oxide anion, which may cause liquid peroxidation in cell membrane and ultimately cell death. Its intoxication leads to vomiting, diarrhea, and acute renal failure. The reported LD_{50} of diquat is 25 to 50 mg/kg [3-5]. Thus due to its potent toxicity and wide application several instrumental techniques have been reported from time to time like high-performance liquid chromatography combined with UV and fluorescence detectors(HPLC-UV-FD) [6]. liquid chromatography–electrospray ionization mass spectrometry[7], hydrophilic interaction chromatography (HILIC) [8], Capillary Electrophoresis[9], stopped-flow mixing technique[10], Gas chromatography–mass spectrometry (GC–MS) [11], LC-MS method[12], potentiometric determination[13], solid-phase extraction method[14], fluoro immunoassay combined with an optical transducer method[15] etc. Most of these instruments are very expensive and needs sophisticated handling and not suitable for routine analysis in small laboratory setup. In this paper an attempt is made for simple and rapid detection of diquat in various environmental and biological samples.

2. Methodology

2.1 Experimental

Instruments used in the experiment include A Systronics UV-VIS spectro photometric model 104, a Systronic pH meter model 335 and Remi C-854/4 clinical centrifuge of 1850 rpm. Analytical reagents and double distilled de mineralized water were used throughout the experiment.

A stock solution of 1000ppm in ethanol is prepared. Working standard solutions is prepared by appropriate dilution of the stock standard solution. Diquat(Sharda Worldwide Exports Pvt. Ltd, India), A 5 M aqueous solution of Sodium hydroxide, 5 %(w/v) aqueous solution of EDTA, 8M aqueous solution of Sulphuric acid, saturated ammonium chloride solution, 1%(w/v) sodiumborohydride and 100-200 mesh silicagel (BDH) for column chromatography are used throughout the experiment.

2.2 Preparation of calibration curve

To an aliquot of test solution containing 2.6 to 15 µg of diquat is taken in a 25 mL graduated test tube and made up to 10mL and to it, 1mL of 1 % sodiumborohydride is added and 2mL of 5M sodiumhydroxide is added and heated for ~ 15 mins on boiling water bath at a temperature range of 35-40^{0}C for full colour development. The blue coloured dye formed remains stable for ~12 hrs. The solution is then marked with water and absorbance is measured against reagent blank at 620 nm.

3. Applications

The utility of proposed method has been assess to detect diquat in various samples. Being ionic diquat is separated by using silica gel(5g) in a 25ml glass column with a glass wool above the stop cock. A fresh column is used in each experiment [16].

3.1 Detection of Diquat in plant materials

Different samples of plant materials like grains, foliage, (50g) are collected from areas where diquat had been sprayed. The samples were weighed, macerated and blended in a mixer. Then, 1mL of 5%EDTA is added to the blended sample and it is extracted using 25mL of 8M sulphuric acid. After extraction, the volume of the extract is made up to 250mL with water and allowed to pass through a silica gel column at a flow rate 4-5 mLmin^{-1}.The column is then

washed with water to remove over acidity left in the column. The absorbed diquat is then eluted by passing saturated ammonium chloride solution through the column. Then it is collected and diluted upto the mark with water in a calibrated flask. Finally, 1mL of aliquot is taken and diquat is determined by proposed method. (Table 1).

3.2 Detection of Diquat in soil samples

Soil samples are collected from different cultivated areas where diquat had been used. The samples (50g) is weighed and ground in a mixer. To the ground sample, 1mL of 5% EDTA is added, then filtered by adding 250ml of water using vacuum pump. The filtrate is then passed over a silica gel column at a fixed flow rate 5 mL^{-1}. The diquat present in the column is eluted by passing saturated ammonium chloride solution through the column at a flow rate of 2 mL^{1}. Then it is collected and diluted upto the mark in a calibrated flask. Finally, 1mL of aliquot is taken and diquat is determined by proposed method. (Table 1)

3.3 Detection of Diquat in water samples

A volume of 100mL runoff water is collected in PTTE bottles from different cultivated areas where diquat had been sprayed. The samples are filtered and 1mL of 5% EDTA is added to remove various metal ions. They are then allowed to pass through a silicagel column at a flow rate 5 $mLmin^{-1}$. The diquat present in the column is eluted by passing saturated ammonium chloride solution through the column at a flow rate of 2 mL^{-1}. Then, it is collected and diluted upto the mark in a calibrated flask. Finally, 1mL of aliquot is taken and diquat is derermined by proposed method. (Table 1).

3.4 Detection of Diquat in human samples

Blood and urine samples are collected from local pathology lab and synthetic samples are prepared by adding known amount of diquat to it. Prior to determination of diquat, 1 ml of 5% EDTA and 1 ml of 1% trichloro acetic acid is added to remove various metal ions and for deprotonisation respectively. The samples are then centrifuged at 1850 gm for 10 minutes and then 1 ml of above aliquot is then analysed by proposed method. (Table 2)

4. Results and Discussions

4.1 Spectral Characteristics

The absorption maxima of the blue coloured dye is measured at 620 nm against the reagent blank. The calibration curve is obtained at 0.26-1.5ppm.The molar absorptivity and Sandell's sensitivity were found to be $1.2x10^{6}$ L $mole^{-1}cm^{-1}$ and 0.002 µg cm^{-2} respectively. (Figure 1-2)

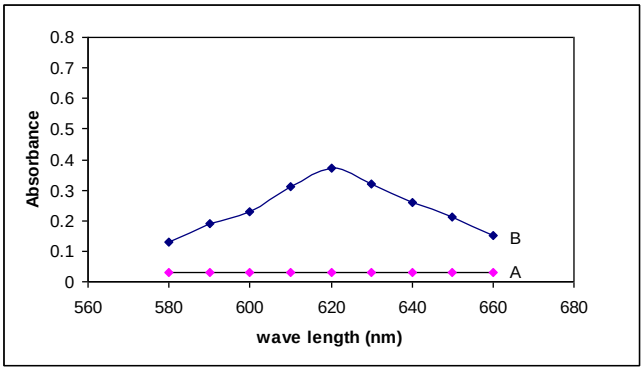

Figure 1: Absorption Spectra Of the dye and reagent blank.
A: Diquat = Reagent Blank
B: Concentration of 10µg/10ml.

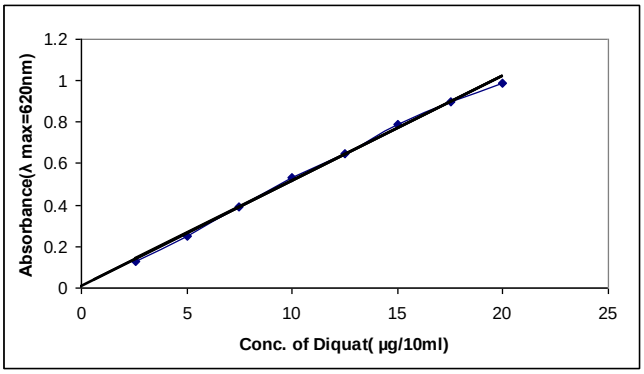

Figure 2: Calibration Curve for the determination of Diquat.

4.2 Optimization of Conditions:

Constant and maximum absorbance value were obtained when 1mL of 1% sodium borohydride is used for reduction. The absorbance value were found to be decreased when lesser amount of sodium borohydride is used. 2 mL of 5M NaOH is sufficient to form a coloured dye. Excess amount of sodium hydroxide decreases the intensity of the coloured dye. The optimum temperature for colour formation was found at around 35- 40^{o}C at pH 10-11. (Figure 3-7)

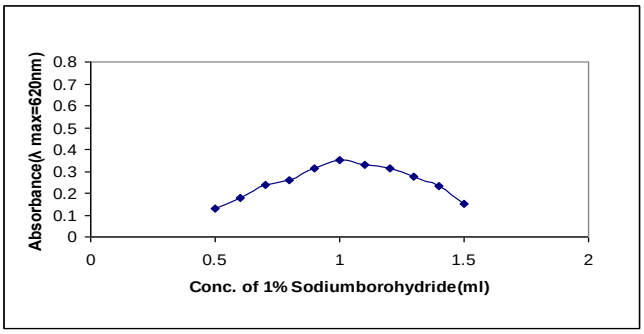

Figure 3: Effect of Sodiumborohydride on colour development.
Concentration of Diquat = 10µg/10ml.

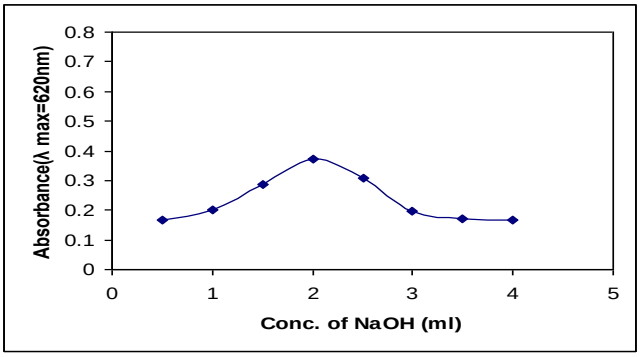

Figure 4: Effect of NaOH on colour development. Concentration of Diquat = 10µg/10ml.

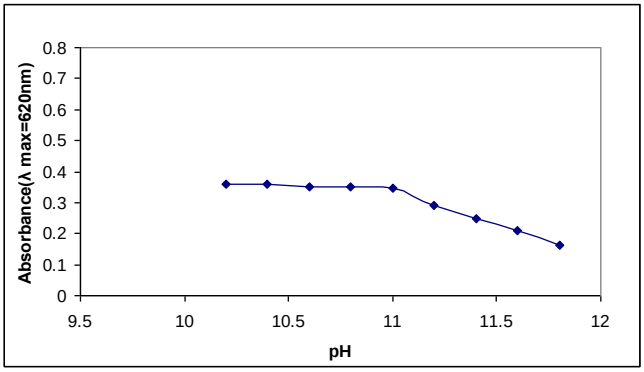

Figure 5: Effect of pH on colour development. Concentration of Diquat = 10µg/10ml.

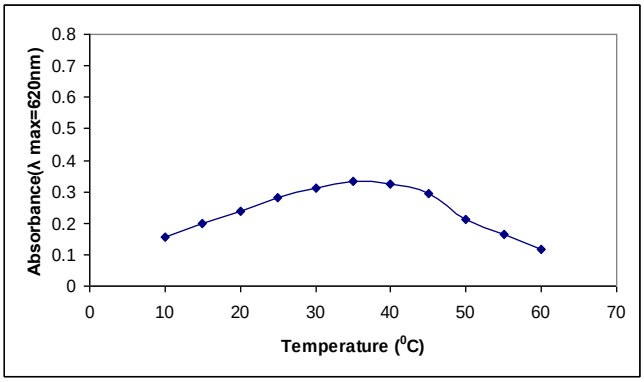

Figure 6: Effect of Temperature on colour development. Concentration of Diquat = 10µg/10ml.

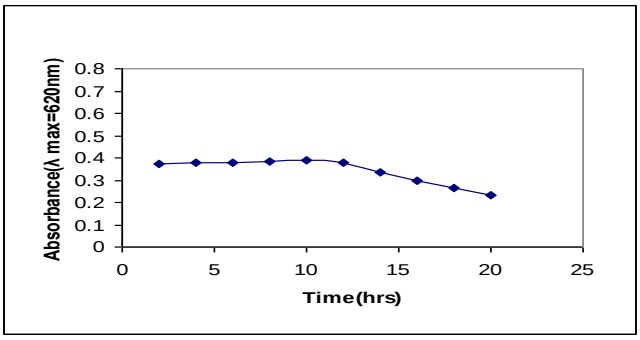

Figure 7: Effect of Time on stability of dye. Concentration of Diquat = 10µg/10ml**.**

4.3 Reproducibility

Precession of the experiment is checked by replicate analysis of solution containing 5 µg of diquat in 10 mL final solution over a period of 7 days. The standard deviation and relative standard deviation are found to be ± 0.0057 and 1.60 % respectively.

Table 1: Detection of Diquat in plant material & Environmental samples:(µg/10 ml)

Sample	Diquat Originally* found (a)	Diquat added (b)	Total Diquat found (c)	Difference (c-a)	Recovery c-a/bx100
Water[a]					
A	2.97	5	7.56	4.59	91.8
B	3.88	10	13.21	9.33	93.3
Soil[b]					
A	3.11	5	7.92	4.81	96.2
B	3.72	10	13.44	9.72	97.2
Grass[b]					
A	2.76	5	7.24	4.48	89.6
B	5.42	10	15.1	9.68	96.8
Sunflower					
Foliage[b]					
A	4.43	5	9.4	4.97	99.4
B	6.98	10	16.67	9.69	9.69

* : Mean of three (3) replicate analysis.
[a] : Size of sample 100 ml
[b] : Size of sample 50 g.

Table 2: Detection of Diquat in human samples

Sample[b]	Diquat added (µg/10 ml)	Diquat found[a] (µg/10 ml)	Recovery %
Urine			
A	5	4.47	89.4
B	10	9.01	90.1
Blood			
A	5	4.59	91.8
B	10	8.78	87.8

[a] : Mean of three (3) replicated analysis.
[b] : Size of sample 1 ml.

5. Conclusion

The advantage of the proposed method is its simplicity, accuracy, sensitivity of blue radical ion and easy availability of sodium borohydride. The proposed method is successfully applied to detect diquat in various samples of plant materials, urine, soil, blood etc.

6. Future Scope

- The practical utility of the proposed work is to make aware the common people, policy makers about the harmful effect of diquat in human life and necessary steps to be taken to replace it with life enhancing products.
- The proposed method being of low cost, accurate, reproducible might be utilised in small laboratories for controlling the excess use of diquat.

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References

[1] Richard P. Pohanish, in Sittig's Handbook of Pesticides and Agricultural Chemicals (Second Edition), 2015.
[2] Elisa M. Mazzaferro, Richard B. Ford, in Kirk & Bistner's Handbook of Veterinary Procedures and Emergency Treatment (Ninth Edition), 2012.
[3] Ramesh C. Gupta, James W. Crissman, in Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition), 2013.
[4] Keith R. Solomon, Glen Van Der Kraak, in Fish Physiology, 2013.
[5] Jones G M, Vale J A.Clinical Toxicology, **2000**, 38, 123.
[6] Fuke C, AraoT, MorinagaY, Takaesu H, Ameno K., Miyazaki T. Legal Medicine, **2000,** 4, 156.
[7] María A, Aramendía V, Lafont B F, Marinas A, Marinas J M, Moreno J M, Porras J M, Urbano F J, Food Chemistry **2006,** 97, 181.
[8] Alexander L.N, van NuijsIsabela Tarcomnicu, Adrian Covaci, Application of hydrophilic interaction chromatography (HILIC) for the analysis of polar contaminants in food and environmental samples, Journal of Chromatography A,Volume 1218, Issue 35, 2 September 2011, Pages 5964-5974.
[9] Po-Ling Chang , Ming-Mu Hsieh, and Tai-Chia Chiu, Recent Advances in the Determination of Pesticidesin Environmental Samples by Capillary Electrophoresis, Int. J. Environ. Res. Public Health 2016, 13, 409.
[10] Galeano Díaz,T, Durán Merás I, M F, Franco A. Water Research **2002,** 36,783.
[11] Menck de Almeida R, Yonamine M. Journal of Chromatography B, **2007,**853, 260.
[12] Ionara R. Pizzutti, Giovana M.E. Vela, André de Kok, Jos M. Scholten, Jonatan V. Dias,Carmem D. Cardoso, Germani Concenço, Rafael Vivian, Determination of paraquat and diquat:LC-MS method optimization and validation. Food Chemistry 209 (2016) 248–255.
[13] Hazem M.Abu Shawish, Nasser AbuGhalwa, MazenHamada, Abdel-HakemBasheer. Modified carbon paste electrode for potentiometric determination of diquat dibromide pesticide in water and urine samples, Materials Science and Engineering: C, Volume 32, Issue 2, 1 March 2012, Pages 140-145.
[14] Núñez O, Moyano E, Galceran M T. Journal of Chromatography A **2002,** 946, 275.
[15] Mallat E, Barzen C, Abuknesha R, Gauglitz G, Barceló D. Analytica Chimica Acta **2001**, 427, 165.
[16] Rai M K, Das J V, Gupta V K..; Talanta,**1997**,Dec 19;45(2):343-8.