



Paraquat Analysis of Brain Samples from Paraquat-Exposed Squirrel Monkeys for Residues of Paraquat Final Report

DATA REQUIREMENT(S): EPA Guidelines OPPTS 860.1480 (1996)

AUTHOR(S): William J. Ray, Ph.D

STUDY COMPLETION DATE: January 21, 2011

PERFORMING LABORATORY: Syngenta Crop Protection, LLC
410 Swing Road
Greensboro, NC 27409 USA

LABORATORY PROJECT ID: Report Number: TK0012660
Task Number: TK0012660

SPONSOR: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

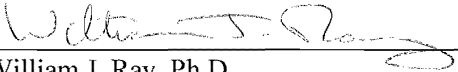
This page intentionally left blank.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

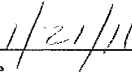
This study was conducted in accordance with the Environmental Protection Agency Good Laboratory Practice Standards (40 CFR Part 160), except for the following:

An in-phase audit was not conducted due to the safety factor in dealing with squirrel monkey brain tissues and therefore did not want to expose untrained QAU personnel to potential hazards.

A SOP was written on the proper handling of animal tissues, before the experimental start date, but it was not signed and issued before this study was finalized.



William J. Ray, Ph.D
Study Director



Date

Performing Laboratory: Syngenta Crop Protection, LLC
410 Swing Road
Greensboro, NC 27409 USA

QUALITY ASSURANCE STATEMENT

Study Title: Analysis of Brain Samples from Paraquat-Exposed Squirrel Monkeys for Residues of Paraquat

Study Director: Bill Ray

Study Number: TK0012660

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

<u>Inspection/Audit Type</u>	<u>Inspection/Audit Dates</u>	<u>Reporting Dates</u>
Audit Protocol	09-SEP-2010	09-SEP-2010
Audit Study Data	18-NOV-2010	18-NOV-2010
Audit Final Report (1 st)	20-DEC-2010	20-DEC-2010
Audit Final Report (2 nd)	10-JAN-2011	10-JAN-2011

Prepared By: Barbara S. North Date: 1/10/11
North, Barbara S.
Quality Assurance Consultant
Quality Assurance Unit
Syngenta Crop Protection LLC

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
William J. Ray, Ph.D	Study Director
Louis Mayer, B.S.	Study Coordinator

Study Dates

Study initiation date: September 13, 2010
Experimental start date: October 25, 2010
Experimental termination date: October 29, 2010

Deviations from the Guidelines

None

Others

The protocol, raw data, amendments and deviations or certified copies thereof, associated with this study and other documentation are archived in the Syngenta Crop Protection Archives at Syngenta Crop Protection, Greensboro, NC (USA).

TABLE OF CONTENTS

STATEMENT OF DATA CONFIDENTIALITY CLAIMS	2
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	3
QUALITY ASSURANCE STATEMENT	4
GENERAL INFORMATION	5
TABLE OF CONTENTS	6
1.0 EXECUTIVE SUMMARY	7
2.0 INTRODUCTION	7
3.0 MATERIALS AND METHODS	7
3.1 Test Substances	7
3.2 Test Matrix	8
3.3 Preparation and Storage of Analytical Standard Solutions.....	8
3.4 Sample Analysis.....	8
3.5 Instrumental Conditions	9
4.0 RESULTS AND DISCUSSION	9
4.1 Method Recovery	9
4.2 Residue Analysis.....	9
4.3 Circumstances Affecting the Data	10
4.4 Method Modifications	10
5.0 CONCLUSIONS	10
6.0 REFERENCES	10
TABLES SECTION	11
TABLE 1 Summary of Results	12
FIGURES SECTION	13
FIGURE 1 Representative Chromatogram of Paraquat in Reagent Blank.....	14
FIGURE 2 Representative Chromatogram of Paraquat in Control Sample (Primate Brain Tissue).....	15
FIGURE 3 Representative Chromatogram of Paraquat in Primate Brain Tissue (LOQ Recovery Sample 0.005 µg/g)	16
FIGURE 4 Representative Chromatogram of Paraquat in Primate Brain Tissue (Sample 682).....	17
FIGURE 5 Representative Chromatograms of Paraquat Calibration Standards	18
FIGURE 6 Representative Paraquat Calibration Curve	19

1.0 EXECUTIVE SUMMARY

The study objective was to analyze paraquat residues in brain tissues from Squirrel Monkeys exposed to paraquat in a laboratory setting. A total of 15 treated tissue samples and 1 control tissue sample were received from SRI international (Menlo Park, CA, USA), under the direction of Dr. DiMonte, on 10/13/2010 in frozen condition. All samples were analyzed for residues of paraquat according to Syngenta Method GRM012.02A (with minor modifications). The Limit of Quantitation (LOQ) for paraquat in the brain tissues was 0.005 µg/g (wet weight). The concurrent method recovery for paraquat from the brain tissue matrix was 96.86%, which verified the performance of this method on brain tissue.

The monkey brain tissue samples exhibited paraquat residues which ranged from 0.007 µg/g to 0.256 µg/g, except samples 664, 666 and 732 which were < LOQ.

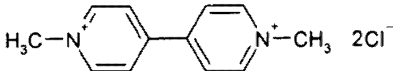
2.0 INTRODUCTION

The purpose of this study was to analyze paraquat residues in brain tissues from Squirrel Monkeys exposed to paraquat in a laboratory setting.

This study was conducted in compliance with EPA FIFRA GLP Standards, 40 CFR Part 160 [1].

3.0 MATERIALS AND METHODS

3.1 Test Substances

Common Name:	Paraquat Dichloride
CAS Name:	1,1'-dimethyl-4,4'-bipyridinium dichloride (9Cl)
CAS Number:	1910-42-5
IUPAC Name:	1,1'-dimethyl-4,4'-bipyridinium dichloride
Structure:	
Reference No.:	ASJ10083-03
Purity:	99.9%
Re-assay Date:	3/2012
Storage Condition:	Refrigerate
Source:	Syngenta Analytical Development & Product Chemistry

3.2 Test Matrix

The test system consists of Squirrel Monkey brain tissues from the cortical areas. The brain tissues were supplied by SRI International (Menlo Park, CA, USA), under the direction of Dr. DiMonte. These brains were collected from Squirrel Monkeys that were treated with paraquat in an investigational study assessing the potential effects of treatment on nigrostriatal function/integrity. These samples have been in frozen storage for approximately 3-4 years. They were prepared and shipped by SRI International and received by Bill Helke in frozen condition on 10/13/2010. All sample information is listed in Table 1.

3.3 Preparation and Storage of Analytical Standard Solutions

Individual primary stock solution of Paraquat Dichloride at the 100 ppb concentration in water was prepared according to the procedures described in Analytical Method GRM012.02A. The working calibration standards were prepared daily by serial dilutions from the 100 ppb stock standard resulting in final concentrations of 10 ppb, 5 ppb, 2.5 ppb, 1.0 ppb, 0.5 ppb, 0.25 ppb, 0.10 ppb, 0.05 ppb and 0.025 ppb (the final three standards were diluted from a 10 ppb stock standard). All final standards were diluted with 3 ml of acetonitrile and the appropriate amount of the 250mM ammonium formate (pH 3.7) buffer to make a final volume of 10 ml. All standards were stored in the refrigerator.

3.4 Sample Analysis

Method GRM012.02A, "Paraquat and Diquat: Residue Method for the Determination of Paraquat Dichloride (PP148) as Paraquat Cation and Diquat Dibromide (PP901) as Diquat Cation in Animal Matrices. Final Determination by LC-MS/MS" was used with minor modifications to determine the residue levels of paraquat. Retention times and response factors were similar, in relation to the minor modifications made in the method. The LOQ for Paraquat was 0.005 µg/g (ppm) for the brain tissues (wet weight), measured as the cation.

To summarize, brain tissues were extracted with 5ml of 10% w/v aqueous trichloroacetic acid, via polytron homogenization. A 75µl aliquot of each extract was transferred to a polypropylene tube in which 275µl of 250mM ammonium formate (pH 3.7) and 150µl of acetonitrile were added. Sample vials were hand shaken to thoroughly mix and then submitted for LC-MS/MS analysis.

Calculations for chromatographic analysis were conducted using a validated software application (Analyst 1.5, Applied Bio-systems and SAW version 6).

3.5 Instrumental Conditions

Shimadzu UFLC XR Autosampler/Binary Pump

Column: Waters Atlantis HILIC 3 μ m; 3 x 100 mm
Guard Column: Waters Atlantis HILIC 3 μ m
Column Heater Temp: 40°C
Mobile Phase A: 250 mM Ammonium Formate, pH=3.7
Mobile Phase B: Acetonitrile
Flow Rate: 1 mL/min
Injection Volume: 10 μ L

HPLC Isocratic Conditions:

%A	%B
95	5

AB Sciex Qtrap 5500 Mass Spectrometer:

Scan Type: MRM
Collision Energy: 25V
Dwell Time: 100ms
Electrospray Polarity: Positive
Capillary Potential: 3000V
Heater Temperature: 500°C
Gas (GS1 & GS2): 50L/hr
Declustering Potential: 35 V
Monitoring Ion Transition: Paraquat 186.1 (Q1) $\rightarrow$ 171.2 (Q3)

Total Runtime: 3.0 min

Approximate Paraquat RT: 2.45 min

4.0 RESULTS AND DISCUSSION

4.1 Method Recovery

The performance of the method for determination of total paraquat residues was demonstrated by fortifying one non-treated control sample (due to limited quantities of control sample) with Paraquat Dichloride prior to the extraction. This fortified sample was analyzed concurrently with the analytical sample set. The individual recovery for paraquat cation was 96.86% and is presented in Table 1.

4.2 Residue Analysis

The monkey brain tissue samples exhibited paraquat residues (wet weight) which ranged from 0.007 μ g/g to 0.256 μ g/g, except samples 664, 666 and 732 which were < LOQ. All of

Report Number: TK0012660

Page 9 of 19

Case ID: 220300429

SYNG-PQ-00044973

the individual results are presented in Table 1. Representative chromatograms of the control, recovery and treated samples for paraquat are presented in Figures 1 and 5. The calibration curve is shown in Figure 6.

4.3 Circumstances Affecting the Data

No circumstances occurred that may have affected the quality or integrity of the data generated during this study.

4.4 Method Modifications

The tissue sample weights were much lower (~30X), therefore final extraction solvents were varied to a lower volume to keep approximately the same weight to solvent ratio. Isocratic HPLC conditions were used, instead of the gradient conditions, to help reduce sample carryover between injections. A different mobile phase solvent ratio was used to improve peak shape and resolution of the peaks. All of these minor modifications are recorded in the raw data in the LNB.

5.0 CONCLUSIONS

The Squirrel Monkey brain tissues were successfully analyzed for paraquat residues as determined by a concurrent method recovery sample.

6.0 REFERENCES

1. Gardinal, P and Crook, S.J., Syngenta Analytical Method GRM012.02A. "Paraquat and Diquat: Residue Method for the Determination of Paraquat Dichloride (PP148) as Paraquat Cation and Diquat Dibromide (PP901) as Diquat Cation in Animal Matrices. Final Determination by LC-MS/MS", Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK

TABLES SECTION

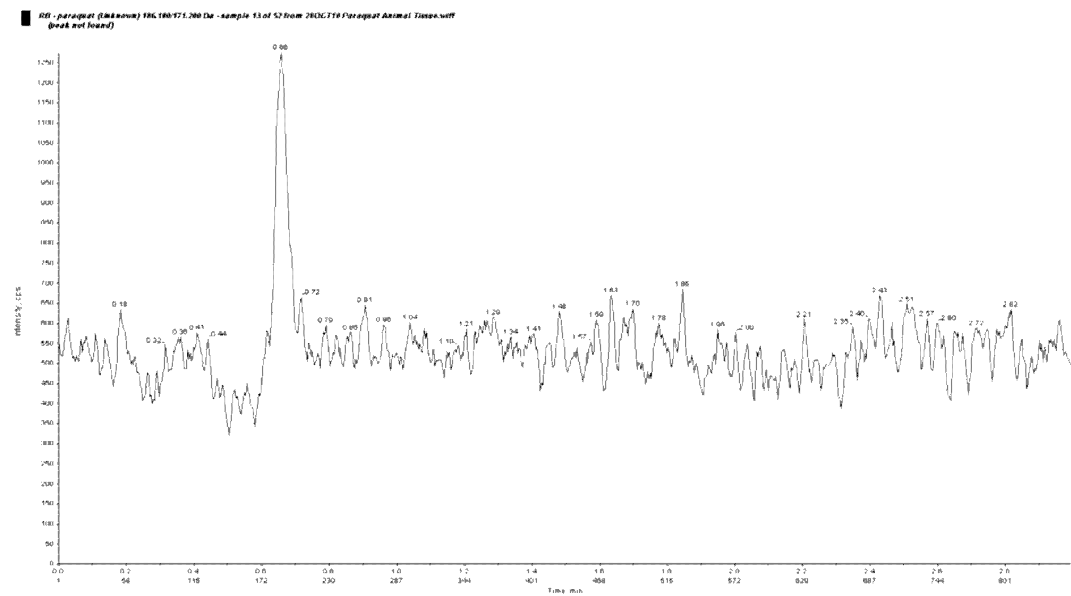
TABLE 1 Summary of Results

Sample ID	Sample Description	Date Analyzed	Paraquat $\mu\text{g/g}$ (ppm) ¹
RISP02110-0002	Brain Tissue-664	10/28/10	<0.005
RISP02110-0003	Brain Tissue-666	10/28/10	<0.005
RISP02110-0004	Brain Tissue-670	10/28/10	0.179
RISP02110-0005	Brain Tissue-672	10/28/10	0.221
RISP02110-0006	Brain Tissue-676	10/28/10	0.213
RISP02110-0007	Brain Tissue-678	10/28/10	0.007
RISP02110-0008	Brain Tissue-681	10/28/10	0.222
RISP02110-0009	Brain Tissue-682	10/28/10	0.256
RISP02110-0010	Brain Tissue-711	10/28/10	0.193
RISP02110-0011	Brain Tissue-716	10/28/10	0.179
RISP02110-0012	Brain Tissue-717	10/28/10	0.254
RISP02110-0013	Brain Tissue-718	10/28/10	0.214
RISP02110-0014	Brain Tissue-732	10/28/10	<0.005
RISP02110-0015	Brain Tissue-752	10/28/10	0.226
RISP02110-0016	Brain Tissue-753	10/28/10	0.241

1- $\mu\text{g/g}$ (ppm) based on wet weight, LOQ 0.005 $\mu\text{g/g}$

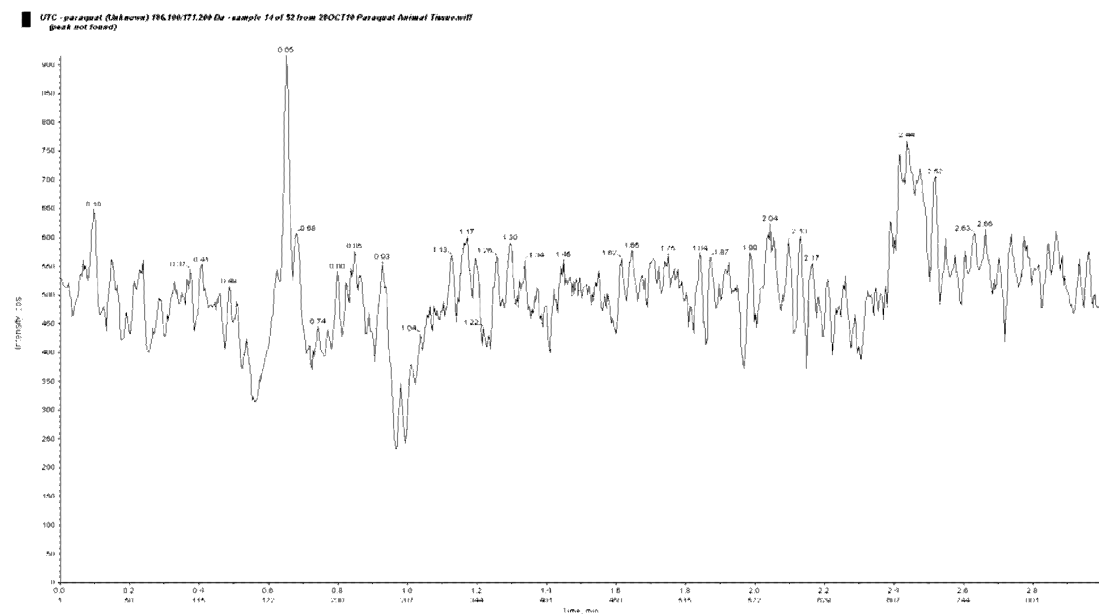
FIGURES SECTION

FIGURE 1 **Representative Chromatogram of Paraquat in Reagent Blank**



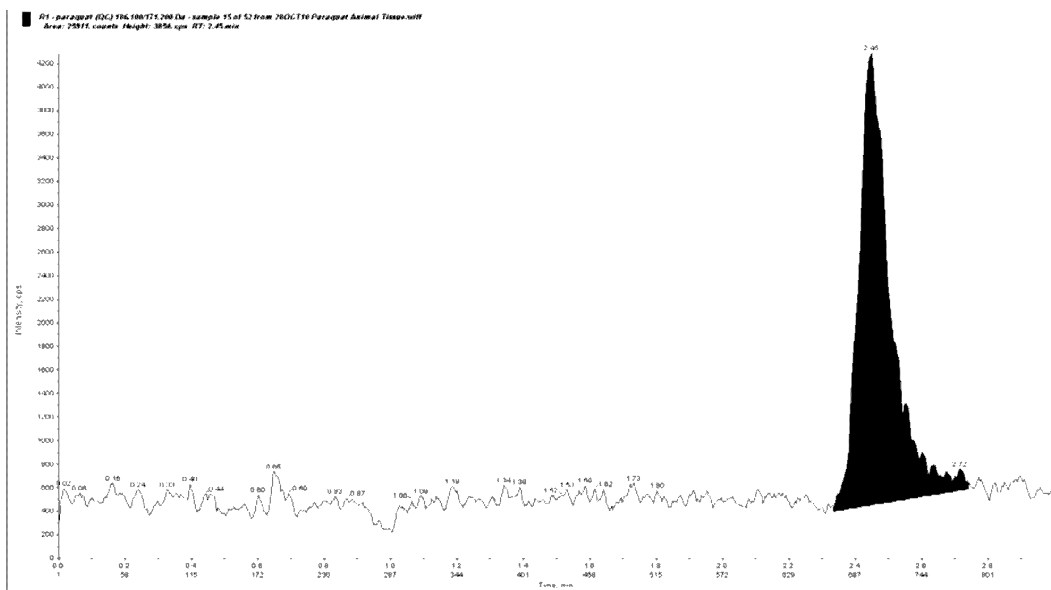
Sample: RB; 10 μ L injection volume; Quantification m/z: 186.1 $\rightarrow$ 171.2
 RT: 2.45 min; Expected: 0.00 pg, Found: 0.00 pg (< LOD)

**FIGURE 2 Representative Chromatogram of Paraquat in Control Sample
(Primate Brain Tissue)**



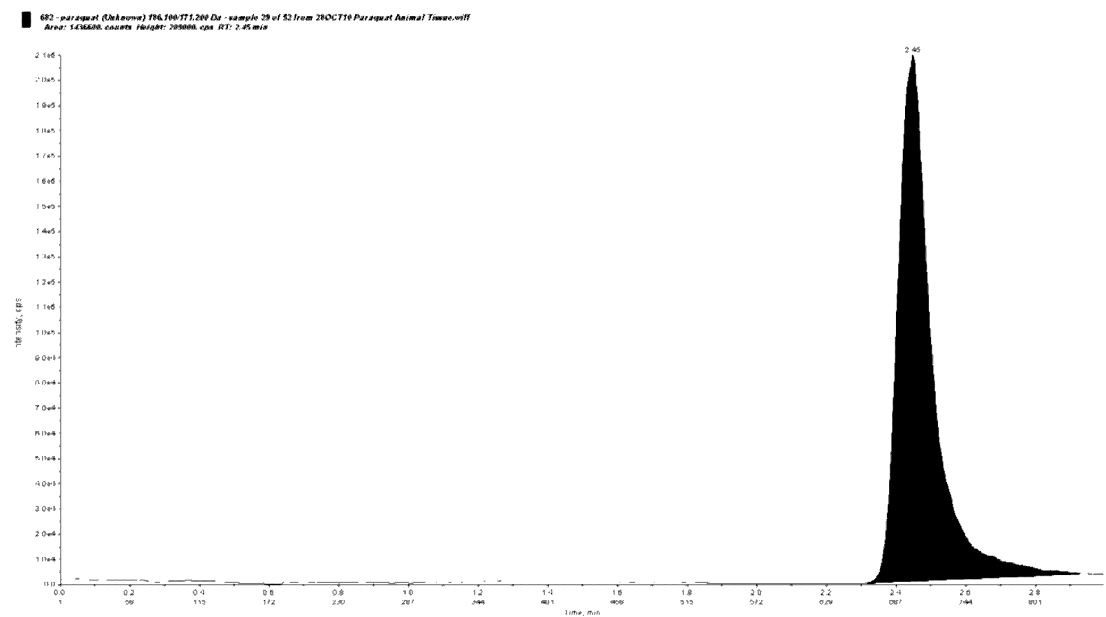
Sample: UTC; 10 μ L injection volume; Quantification m/z: 186.1 $\rightarrow$ 171.2
RT: 2.45 min; Expected: 0.00 pg, Found: 0.00 pg (< LOD)

FIGURE 3 Representative Chromatogram of Paraquat in Primate Brain Tissue (LOQ Recovery Sample 0.005 µg/g)



Sample: R1; 10 µL injection volume; Quantification m/z: 186.1 → 171.2
 RT: 2.45 min; Expected: 0.500 pg, Found: 0.442 pg (96.86%)

FIGURE 4 Representative Chromatogram of Paraquat in Primate Brain Tissue (Sample 682)



Sample: 682; 10 μ L injection volume; Quantification m/z: 186.1 $\rightarrow$ 171.2
RT: 2.45 min; Found: 19.710 pg (0.256 μ g/g)

FIGURE 5 **Representative Chromatograms of Paraquat Calibration Standards**

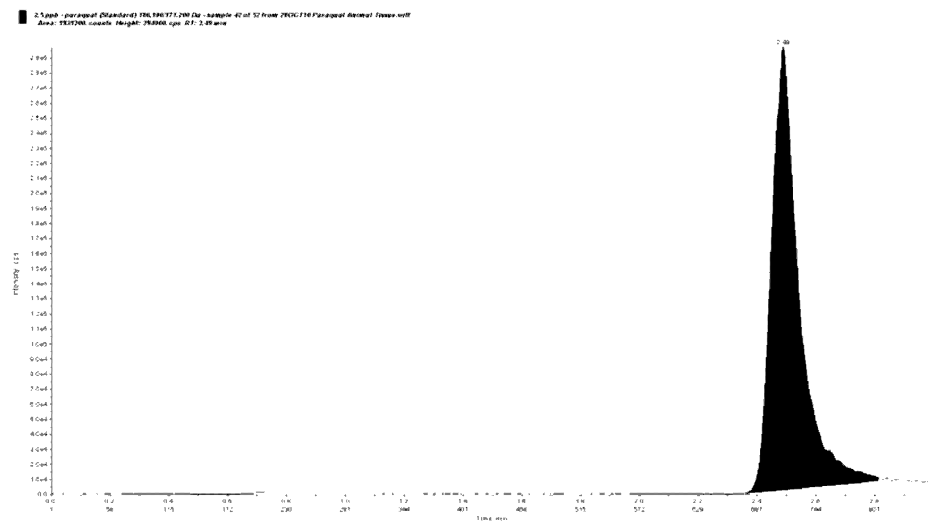
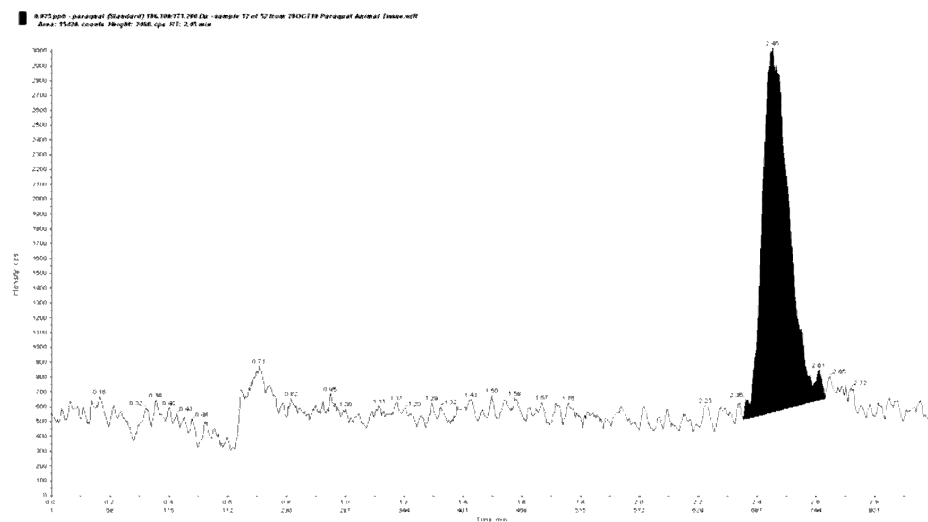
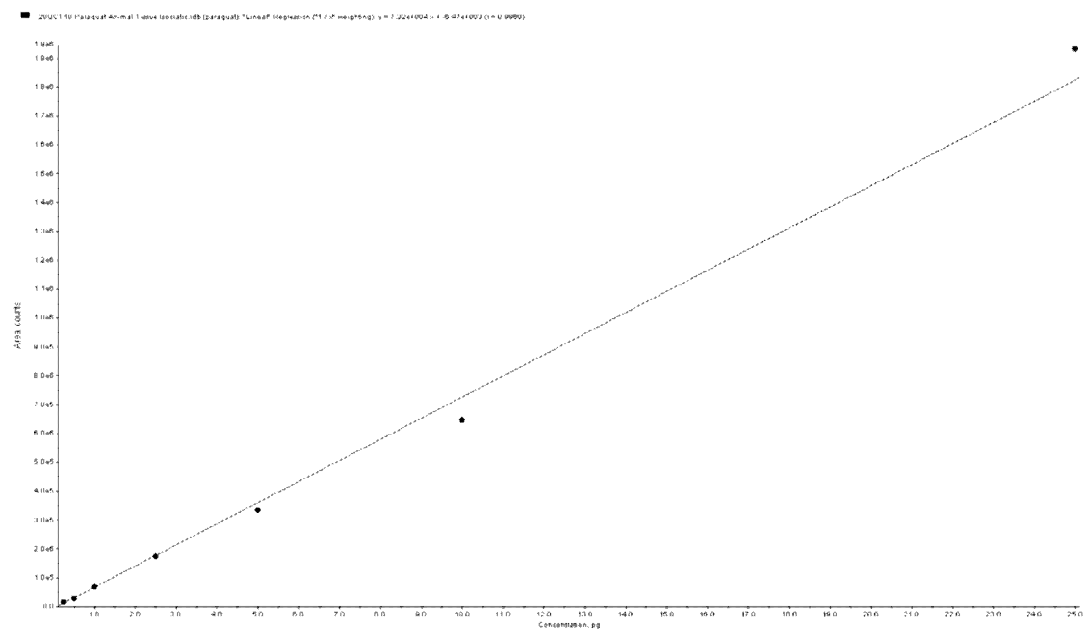


FIGURE 6 Representative Paraquat Calibration Curve



Paraquat Calibration Curve: 0.025 pg/μL – 2.5 pg/μL; 10μL injection volume;
0.25 pg – 25 pg on column; $r = 0.9968$