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Study Title

PARAQUAT : Validation of a Residue Analytical Method for the Determination of Paraquat in Animal Products.

Data Requirement

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Author(s)

L Anderson

Study Completed On

11 February 1994

Performing Laboratory

ZENECA AGROCHEMICALS (ZENECA Limited)

JEALOTTS HILL RESEARCH STATION

BRACKNELL, BERKS, RG12 6EY

UK

Laboratory Project ID

93JH221

Study Number : 93JH221

Report Title : PARAQUAT : Validation of a Residue Analytical Method for the Determination of Paraquat in Animal Products.

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Sections §10(d)(1)(A), (B) or (C)

Company: ZENECA Inc.

Company Agent: M E. Rhodes Date: March 3, 1994

Regulatory Product Manager M.E. Rhodes
Title Signature

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Study Number : 93JH221

Report Title : PARAQUAT : Validation of a Residue Analytical Method for the Determination of Paraquat in Animal Products.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been conducted in compliance with the Principles of Good Laboratory Practice laid down in the United Kingdom Department of Health Compliance Programme (1989) which themselves are in accordance with Organisation of Economic Cooperation and Development Principles of Good Laboratory Practice ISBN 9264 12367 9.

Under the Memorandum of Understanding signed by both the United States of America and the United Kingdom this study is considered to satisfy the requirement that it be conducted in accordance with 40 CFR Part 160.

I believe that this study is valid for the purposes for which it was conducted and this report is a true reflection of the raw data generated

L. Anderson

Study Director

L Anderson
ZENECA Limited

R S Morrod

Sponsor

R S Morrod
Head of Department
ZENECA Limited

M. E. Rhodes

Submitter

Study No : 93JH221

Report Title : PARAQUAT : Validation of a Residue Analytical Method for the Determination of Paraquat in Animal Products.

QUALITY ASSURANCE STATEMENT

In accordance with Zeneca Agrochemicals policy and procedures for Good Laboratory Practice, the conduct of this study has been inspected/audited by the Quality Assurance Section at Jealotts Hill Research Station, Bracknell, Berks, RG12 6EY, UK.

Date of Inspection	Inspection/Audit	Date of Inspection Report
13 Oct 1993	Protocol	13 Oct 1993
02 Nov 1993	Study Conduct	10 Nov 1993
16 Dec 1993	Study Conduct	17 Dec 1993
26 Jan 1994	Report (RJ1600B)	04 Feb 1994
10 Feb 1994	Report (RJ1600B)	10 Feb 1994

In addition, the following facility and procedure inspections associated with this type of study have been carried out.

26 May 1993	Laboratory Facilities	30 Jun 1993
23 Jun 1993	Laboratory Procedures	12 Jul 1993
22 Oct 1993	Laboratory Procedures	26 Nov 1993

So far as can be reasonably established, the methods described and results incorporated in this report accurately reflect the raw data produced during the study.

Linda Elliott — J C Elliott
Quality Assurance Officer

14th February 1994
Date

Study Number : 93JH221

Report Title : PARAQUAT : Validation of a Residue Analytical Method for the Determination of Paraquat in Animal Products.

AUTHENTICATION

I, the undersigned, declare that this study was performed under my direction and that this report represents a true and accurate record of the results obtained.

L. Anderson
Study Director — L Anderson — 11/2/94 Date

Authorised for management by :

D W Bewick
Section Manager — D W Bewick — 11.02.94 Date

CONTENTS

Study Number : 93JH221

Report Title : PARAQUAT : Validation of a Residue Analytical Method for the Determination of Paraquat in Animal Products.

Author : L Anderson

SUMMARY

An analytical procedure has been validated for the determination of paraquat in animal products.

The method is intended to determine paraquat residues in animal products to a limit of 0.005 mg kg $^{-1}$.

The method was found to be accurate and precise down to the quoted limit of determination of 0.005 mg kg $^{-1}$ for the following matrices:

Muscle	: Mean Recovery = 89% CV = 7%
Skin and subcutaneoous fat	: Mean Recovery = 90% CV = 6%
Liver	: Mean Recovery = 85% CV = 9%
Fat	: Mean Recovery = 84% CV = 13%
Whole Egg	: Mean Recovery = 86% CV = 12%
Egg Yolk	: Mean Recovery = 81% CV = 13%
Egg White	: Mean Recovery = 92% CV = 4%

The above recovery and coefficient of variation (CV) values were calculated for a fortification range of 0.005 to 0.50 mg kg $^{-1}$.

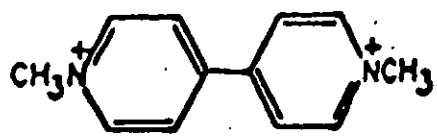
1 INTRODUCTION

The aim of this study was to produce validation data for a residue analytical method for the determination of paraquat in animal tissues using external standardisation.

This report describes the analytical procedures and presents validation data obtained when using the method described in Appendix 1 for the analysis of hen tissues and eggs. This study was carried out between October 1993 and February 1994 at Zeneca Agrochemicals, Residue Chemistry Section, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK.

The structure of paraquat is given below.

Figure 1.



Chemical Name (IUPAC) 1,1 dimethyl-4,4'-bipyridinium ion

2 MATERIALS AND METHODS

2.1 Test Material

Paraquat dichloride analytical standard ref: ASY20027-02S (purity 100%), was used for the method validation. It was obtained from Zeneca Agrochemicals, Quality Assurance, Safety and Environmental Dept (QSED), Yalding, Kent, ME18 6HN. Standard solutions in saturated ammonium chloride were prepared and 0.2, 1.0 or 10 $\mu g\ cm^{-3}$ concentrations used for fortification of the samples. Paraquat analytical standard is stable for at least 2 years at ambient temperatures. Paraquat standard solutions in saturated ammonium chloride have been shown to be stable at room temperature for 12 months.

2.2 Test System

The samples used for this study were obtained from a local supermarket. The various tissue types (muscle, skin and subcutaneous fat, liver and fat) were removed from the individual chickens and bulked to produce a sample for each tissue type. Eggs were similarly divided to produce separate samples of egg yolk and egg white. All samples were homogenised and stored at <-18°C until analysis.

2.3 **Analytical Procedure**

The analytical procedures were validated by fortifying each tissue type with paraquat from the limit of determination (0.005 mg kg^{-1}) to 0.50 mg kg^{-1}. Fortified and untreated samples were then subjected to the full analytical procedures (Appendix 1) and were analysed by high performance liquid chromatography with UV detection.

Summary of samples analysed :

Muscle, skin and fat, fat, whole egg, egg yolk, egg white

Control	- 4 replicate samples
Recovery 0.005 mg kg^{-1}	- 4 replicate samples
Recovery 0.05 mg kg^{-1}	- 4 replicate samples
Recovery 0.50 mg kg^{-1}	- 4 replicate samples

Liver

Control	- 4 replicate samples
Recovery 0.005 mg kg^{-1}	- 4 replicate samples
Recovery 0.10 mg kg^{-1}	- 4 replicate samples
Recovery 0.50 mg kg^{-1}	- 4 replicate samples

2.4 **Method**

In summary, samples (25g) were extracted three times by maceration in the presence of 10% trichloroacetic acid solution. The centrifuged homogenate was percolated through a column of cation exchange resin which retained the paraquat and some of the natural tissue constituents. The column was washed with 2.5% ammonium chloride solution and water to remove endogenous materials and the paraquat eluted with saturated ammonium chloride solution.

Final quantitative determination was by ion-pair reverse phase high performance liquid chromatography (HPLC) using UV detection.

Full details of the analytical method are included in Appendix 1.

3 RESULTS

3.1 Procedural Recoveries

TABLE 1 : Paraquat Method Validation Recovery Data.

Tissue Type	Fortification Level (mg kg^{-1})	% Recovery	Mean % Recovery	CV (%)
Muscle	0.005	80,84,84,77	81	4
	0.05	94,87,93,96	93	4
	0.50	94,93,95,92	94	1
Skin and subcutaneous fat	0.005	99,88,95,82	91	8
	0.05	85,83,93,89	88	5
	0.50	86,96,92,89	91	5
Liver	0.005	80,70,81,74	76	7
	0.10	85,85,92,88	88	4
	0.50	93,87,95,93	92	4
Fat	0.005	66,77,65,70	70	8
	0.05	95,101,90,86	93	7
	0.50	88,91,93,86	90	3
Whole Egg	0.005	75,84,81,72	78	7
	0.05	81,77,78,82	80	3
	0.50	97,99,101,100	99	2
Egg yolk	0.005	84,83,82,78	82	3
	0.05	72,60,67,78	69	11
	0.50	92,96,93,91	93	2
Egg white	0.005	90,95,96,87	92	5
	0.05	84,90,91,90	89	4
	0.50	94,96,95,95	95	1

3.2 **Residues in Untreated Samples**

Baseline noise/interferences at the retention time of paraquat in the control samples are quantified in terms of paraquat residue equivalents in Table 2.

TABLE 2 : Unfortified Control Sample Residues

Tissue Type	Paraquat Residue Equivalent (mg kg^{-1})
Muscle	0.0006, 0.0005, NP, NP
Skin and subcutaneous fat	NP, NP, NP, NP
Liver	NP, 0.0005, 0.0007, 0.0009
Fat	NP, NP, 0.0006, 0.0002
Whole Egg	0.0009, 0.0008, NP, NP
Egg yolk	NP, NP, NP, NP
Egg white	NP, NP, NP, NP

NP = No peak detected

No residues above the limit of determination (0.005 mg kg^{-1}) were measured in any of the control samples analysed in this study.

3.3 Linearity

HPLC detector linearity was confirmed by running a series of paraquat standards in the concentration range 0.0025 $\mu g\ ml^{-1}$ to 0.5 $\mu g\ ml^{-1}$.

A graph of HPLC detector response vs mass of paraquat was plotted and found to be linear in the region of interest (See Figure 2).

3.4 Limit of Determination

The limit of determination of this method was set at 0.005 mg kg^{-1} in this laboratory and is defined as the lowest level at which a residue can be precisely and accurately measured, i.e. where the resulting peak has a height or area of four times that of the baseline noise.

3.5 Effect of Diquat

Diquat does not interfere with the quantitation of paraquat. The separation of diquat and paraquat under the chromatographic conditions employed is shown in Appendix 1. A standard response curve was produced for both paraquat alone and paraquat mixed with a high concentration of diquat. No significant difference was noted between the two curves.

4 DISCUSSION

The filtration stage for analysis of egg whites was omitted due to difficulties in filtering the extracts resulting from homogenisation. This had no adverse effect upon the recovery of analyte through the method or the chromatographic separation of paraquat from coextractives.

5 CONCLUSIONS

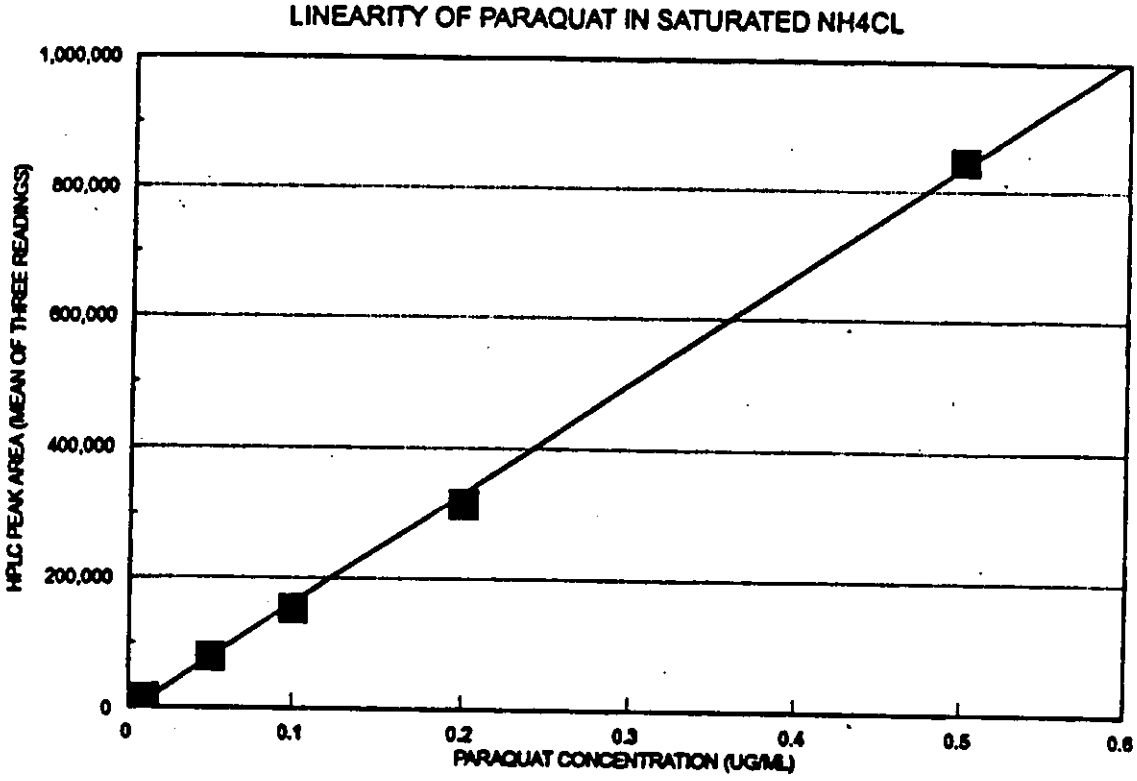
Paraquat can be determined accurately and precisely in animal products using the analytical procedure described in Appendix 1.

6 RAW DATA

All raw data relating to this study, and a copy of the final report will be stored in Zeneca Agrochemicals GLP Archives, Jealott's Hill Research Station, Bracknell, Berkshire. RG12 6EY; under Study Number 93JH221.

Document : RJ1600B
Disc : WP/94/CG/102
Reference : LA/CG
Date : 11 February 1994

FIGURE 2 : Graph of HPLC Detector Response and Paraquat Concentration



Injection Volume 350 μl
Correlation Coefficient 0.9991
Intercept 0.004

Appendix 1

STANDARD OPERATING PROCEDURE

RAM 004/04

THE DETERMINATION OF PARAQUAT IN ANIMAL PRODUCTS

A High Performance Liquid Chromatographic Method

Recipient : _____ Copy No : _____

STANDARD OPERATING PROCEDURE

RAM 004/04

THE DETERMINATION OF PARAQUAT IN ANIMAL PRODUCTS

A High Performance Liquid Chromatographic Method

Issue Date	:	Review Date	: Annually
Author	: L Anderson	Issuing Section	: Residue Chemistry

Authorised by : _____ D W Bewick
Section Manager

This SOP must not be photocopied. Further copies are available from the Environmental Sciences Department Administration Centre. (Contact C Gray or B Bylett extns 4322/3740).

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STANDARD OPERATING PROCEDURE

RAM 004/04

THE DETERMINATION OF PARAQUAT IN ANIMAL PRODUCTS

A High Performance Liquid Chromatographic Method

Issue Date	:	**Review Date**	: **Annually**
Author	: **L Anderson**	**Issuing Section**	: **Residue Chemistry**

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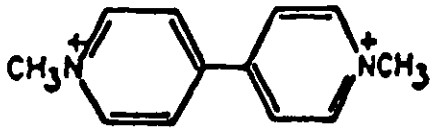
This SOP RAM supersedes RAM 004/03

If you received a copy of the 03 version of this SOP RAM it must be returned immediately to the Environmental Sciences Department Administration Centre.

CONTENTS

1. SCOPE

This method is suitable for the determination of residues of the herbicide paraquat in eggs and animal tissues and has a limit of determination of 0.005 mg kg^{-1}.



Paraquat
Chemical Name (IUPAC) 1,1 dimethyl-4,4'-bipyridinium ion

2. SUMMARY

Samples are extracted by maceration with 10% trichloroacetic acid solution.

The centrifuged homogenate is percolated through a column of cation exchange resin which retains the paraquat and some of the natural tissue constituents. The column is washed with 2.5% ammonium chloride solution and water to remove endogenous materials and the paraquat eluted with saturated ammonium chloride solution.

Final quantitative determination is by ion-pair reverse phase high performance liquid chromatography using UV detection.

3. PROCEDURE

3.1 Sample Preparation

Tissue samples should be removed from the freezer and allowed to stand at room temperature for approximately 30 minutes until it is possible for them to be sliced prior to mincing or homogenising. The mincing/chopping should be continued until a homogenous sample is obtained.

Samples which are removed from the freezer which have previously been homogenised, should only be allowed to thaw for the minimum time before breaking up and weighing out: this ensures that no partition of the endogenous water content can occur prior to analysis.

Egg samples should be thoroughly thawed and mixed before sub-sampling.

3.2 Extraction

3.2.1 Thoroughly mix the sample and weigh a representative aliquot (25 g) into a centrifuge bottle. Add 10% (w/v) trichloroacetic acid solution (TCA)(50 ml) and macerate for 5 minutes.

3.2.2 Centrifuge the homogenate at 3000 rpm for 10 minutes and transfer the supernatant to a 250 ml flask.

3.2.3 Re-extract by homogenising the tissue sample with two further portions of trichloroacetic acid solution (50 ml) and after each centrifugation combine the supernatant in the 250 ml flask.

3.2.4 Skin and subcutaneous fat and fat samples should be partitioned with hexane prior to ion-exchange resin extraction as follows;
The combined TCA extracts (150 ml) are shaken with hexane (100 ml) (1 min) and the layers allowed to separate until both layers clear. The lower aqueous layer is filtered and carried through the method as 3.2.5 below.

3.2.5 Filter the supernatant from centrifugation under vacuum using a glass fibre filter paper (Whatman GF/B) to remove fine particulates from extraction, rinse the residuum with further TCA.

3.2.6 Wash through filter paper and residuum with 300 ml deionised water.

3.2.7 Make up the combined TCA/water extract to 500 ml with deionised water.

3.3 **Cation exchange resin cleanup**

3.3.1 Prepare ion-exchange columns as follows:
Wash 3.5 g of resin with water into a burette (25 ml) containing a glass wool plug placed near the stopcock, or into a commercially prepared glass column with sintered glass plug.

3.3.2 Pass successively through the column at the rate of 5 ml/min saturated sodium chloride (20 ml) and water (50 ml).

Prepare a separate column for each sample.

3.3.3 Percolate the diluted TCA extracts through the prepared resin columns at a flow rate of 5-10 ml/min.

3.3.4 Wash the column with water (25 ml), 2.5% (w/v) ammonium chloride solution (100 ml), and water (50 ml) at a flow rate of 5-10 ml/min. (The columns can be left overnight at this stage provided the resin is covered with water).

3.3.5 Elute the paraquat from the resin with saturated ammonium chloride solution at a flow rate of about 1 ml/min as follows. Drain the water from the column ensuring that the resin only just remains covered with water. Top up the column with saturated ammonium chloride and collect the first 50 ml of the eluent into a 50 ml volumetric flask and mix.

NB. The recovery of paraquat from the resin is adversely effected if the flow rate of the eluent exceeds 1 ml/min.

3.3.6 Transfer an aliquot of the column eluent to HPLC vials for analysis.

4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The analytical conditions will depend on the equipment available. The following conditions have been found to be satisfactory in this laboratory.
Several method variables may be modified ie. mobile phases composition and flow rate to ensure resolution of the analyte from interferences in matrix or co-eluting peaks in the saturated ammonium chloride carrier if required.

Allow the mobile phase to equilibrate on the system until the baseline drift is negligible. Make 1 or 2 injections of saturated ammonium chloride to elute any coextractives before injecting standards and samples each day. Recycling the mobile phase overnight will reduce the time required for stabilisation of the system the following day.

4.1 Reverse Phase Ion Pair Chromatography

Equipment

Pump	:	Waters 501 pump
Injector	:	Waters Sample Processor model 712
Detector	:	Waters M-481 LC-UV detector
Chart Recorder	:	Kipp and Zonen BD40
Integration	:	Multichrom or similar

4.2 Analytical Conditions

Column	:	Hichrom Spherisorb S5P (25 cm x 4.6 mm i.d.)
Mobile Phase	:	Water:Methanol (90:10 v/v) + 0.1% sodium-1-octanesulphonate + 1.0% diethylamine + 0.8% orthophosphoric acid
Flow Rate	:	1.2 ml min^{-1}
Injection vol	:	350 μl
Detection	:	258 nm
Chart Speed	:	300 mm hr^{-1}

Column life is improved if the system is periodically flushed with water:methanol, and water to remove build up of mobile phase constituents.

Under these conditions the retention time of paraquat is about 9.0 minutes.

5 CONTROL AND RECOVERY EXPERIMENTS

5.1 At least one untreated control sample must be analysed with each set of samples to ensure that no unobserved contamination of the samples occurred prior to, or during the analysis, from matrix, solvents or materials.

5.2 At least two fortified control samples must be analysed with each set of samples to assess the analytical efficiency of the method. The level of fortification should reflect the magnitude of the paraquat residues anticipated. If no measured residue is expected, the recoveries should be fortified at 0.01 to 0.05 mg kg^{-1}.

In these laboratories using this method recoveries of between 70 and 110% of the added paraquat are expected.

6 TIME REQUIRED FOR ANALYSIS

Analysis time in this laboratory is estimated to be 8 hours for 8 samples inclusive of control and recovery samples.

7 CALCULATIONS

7.1 Make repeated injections (350 μl) of an analytical standard solution of paraquat into an HPLC operated under the conditions described in Section 4. When consistent responses are obtained measure the peak areas (or height) for the standard.

7.2 Inject 350 μl of the sample and similarly measure the response at the retention time of the paraquat peak in the standard.

7.3 Re-inject the standard after a maximum of four injections of sample solutions.

7.4 Calculate the paraquat residue value in the sample by a simple proportion calculation ie.

$$\text{Residue} = \frac{\text{sample response}}{\text{standard response}} \times \frac{\text{conc. in standard}}{\text{conc. in sample}}$$

$$\text{mg kg}^{-1} = \frac{\text{uV x sec}}{\text{uV x sec}} \times \frac{\mu\text{g ml}^{-1}}{\text{g ml}^{-1}}$$

7.5 Correct the measured residue for the mean percentage recovery of fortified control samples

ie. for a mean 80% recovery,

$$\text{corrected residue} = \text{measured residue} \times \frac{100}{80}$$

NOTE

In the case where laboratory data system/computing integrators are used the computer algorithm may adopt a slightly different method for calculation of results. For example, the VG-LS Multichrom laboratory data system uses the relative detector response factor calculated from an analytical standard solution as the basis for calculation of results. The final calculated result is, of course, the same as the above manual calculations.

8 LIMIT OF DETERMINATION

The limit of determination of this method in these laboratories has been set at 0.005 mg kg^{-1} and is defined as the lowest level at which a residue can accurately be measured; ie. where the resulting peak has a height or area of at least four times the baseline noise.

9 METHOD VALIDATION

In these laboratories to date the method has been applied to the analysis of eggs, muscle, skin and fat, kidney, fat and liver from poultry and bovine species.

No endogenous materials from these substrates have been observed to interfere with paraquat during the final chromatographic determination step.

9.1 Recovery Data for Validated Matrices

TABLE 1 : Paraquat Method Validation Recovery Data.

Tissue Type	Fortification Level (mg kg^{-1})	% Recovery	Mean % Recovery	CV (%)
Muscle	0.005	80,84,84,77	81	4
	0.05	94,87,93,96	93	4
	0.50	94,93,95,92	94	1
Skin and subcutaneous fat	0.005	99,88,95,82	91	8
	0.05	85,83,93,89	88	5
	0.50	86,96,92,89	91	5
Liver	0.005	80,70,81,74	76	7
	0.10	85,85,92,88	88	4
	0.50	93,87,95,93	92	4
Fat	0.005	66,77,66,70	70	8
	0.05	95,101,90,86	93	7
	0.50	88,91,93,86	90	3
Whole Egg	0.005	75,84,81,72	78	7
	0.05	81,77,78,82	80	3
	0.50	97,99,101,100	99	2
Egg yolk	0.005	84,83,82,78	82	3
	0.05	72,60,67,78	69	11
	0.50	92,96,93,91	93	2
Egg white	0.005	90,95,96,87	92	5
	0.05	84,90,91,90	89	4
	0.50	94,96,95,95	95	1

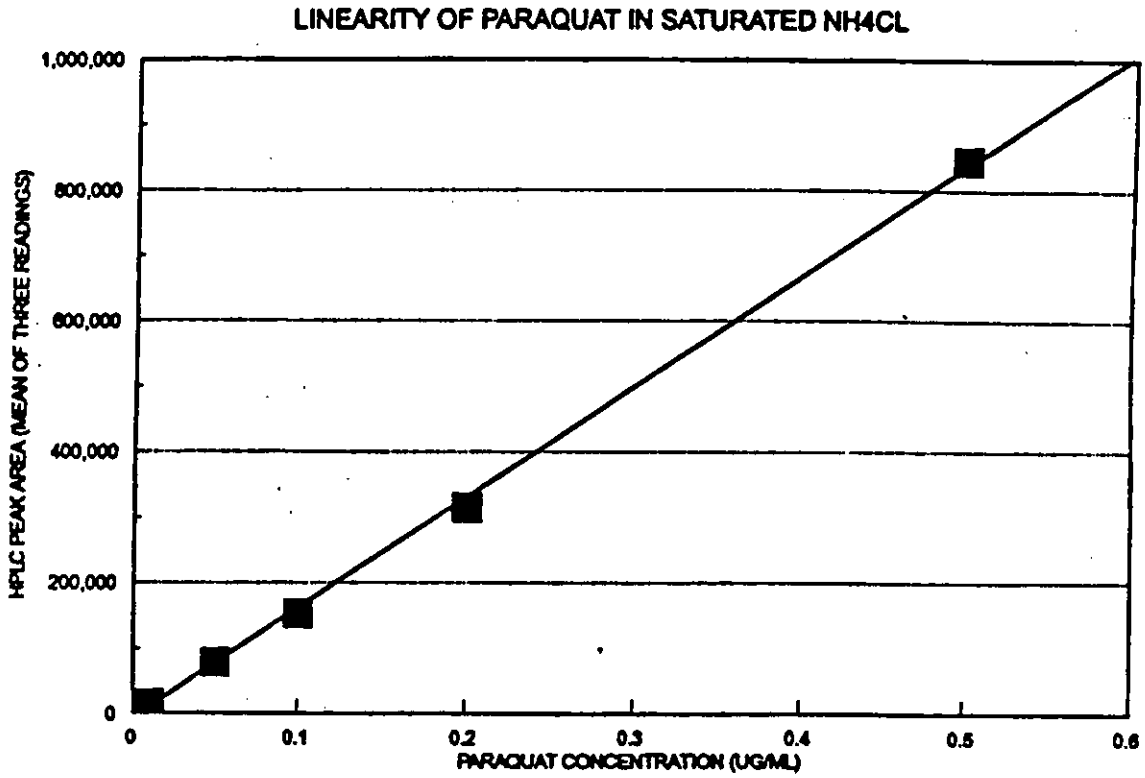
9.2 **Storage Stability**

Samples of egg and tissues for residue analysis should be stored deep frozen prior to analysis. This period should be kept to a minimum. Paraquat residues have been shown to be stable for at least 27 months in muscle (Reference 1), and at least 28 months in eggs (Reference 2)when stored at < -18° C.

9.3 **Linearity Data**

The linearity of the HPLC analysis of paraquat has been determined by running a series of paraquat standards in saturated ammonium chloride in the concentration range 0.0025 to 0.5 $\mu g\ ml^{-1}$.

FIGURE 1 : Graph of HPLC Detector Response and Paraquat Concentration



Injection Volume 350 μl
Correlation Coefficient 0.9991
Intercept 0.004

9.4 **Effect of Diquat**

Diquat does not interfere with the quantitation of paraquat. The separation of diquat and paraquat under the chromatographic conditions employed is shown in Appendix 5.4. A standard response curve has been produced for both paraquat alone and for paraquat mixed with a high concentration of diquat. No significant difference can be noted between the two curves.

10 **REFERENCES**

1. Anderson L., Boseley A.D. and Earl M.(1991). PARAQUAT: Storage Stability of Residues in Frozen Hen Muscle Tissue. ICI Agrochemicals Report RJ 0908B

2. Anderson L., Boseley A.D., Earl M.(1991). PARAQUAT: Storage Stability of Residues in Frozen Eggs. ICI Agrochemicals Report RJ 0912B

RAM No	:	RAM004/04
Disc No	:	WP/94/CG/83
Reference	:	ME/LA/CG
Date	:	11 February 1994

APPENDIX 1

Equipment

The following equipment may be used for this analytical method and is available from US suppliers. Similar equipment from UK suppliers was used in ZENECA Agrochemicals laboratories.

1. Equipment for initial sample preparation : Tecator Homogeniser, available from Thompson and Capper Ltd., 9-11 Hardwick Road, Astmoor Industrial Estate, Runcorn, Cheshire : or equivalent to the Waring Laboratory Micromizer (Cat No. FPC 60) Waring Products Division, Dynamic Corporation of America, New Hartford, CT 06057, USA.

2. Macerator : Polytron CH-6010, supplied by Northern Media, Hull, UK or Tekmar (3412-B25) available from Tekmar Co. PO Box 371856, Cincinnati, OH 45222-1856, USA.

3. Centrifuge : Wifug 2000E, supplied by Wifug, (Division of Eltex of Sweden), Lane Close Mills, Great Horton, Bradford, W Yorks : or equivalent available from Tekmar Co. Cincinnati, (as above).

4. Glass columns for chromatography of 1.0 cm i.d. (25 ml burettes are suitable).

5. High Performance Liquid Chromatograph e.g. Waters Model 501 pump, WISP 712 autosampler, Waters M481 LC-UV detector or equivalent instrument available from Waters, Millipore UK Ltd, Blackmoor Lane, Watford, Herts., WD1 8YW or from Waters Division of Millipore, 34 Maple Street, Milford, Mass., 01757, USA.

6. HPLC column S5P 25.0 cm x 4.6 mm i.d. available from Hichrom Ltd, 6 Chiltern Enterprise Centre, Station Road, Theale, Reading, Berks., RG7 4AA or Phase Separations Inc., 140 Water Street, Norwalk, CT06854, USA.,

APPENDIX 2

Reagents

1. Solvents : glass distilled hexane and methanol, from Rathburn Chemicals Ltd., Walkerburn, Scotland or from B & J Brand Solvents, Scientific Division, of Baxter Healthcare Corporation, USA. Tel 312-689-8410.

2. Analytical grade trichloroacetic acid, sodium chloride, orthophosphoric acid : from Fisons Scientific Equipment, Bishop Meadow Road, Loughborough, Leics. LE11 ORG or CMS, PO Box 1545, Houston, Texas 77251, USA.

3. Cation-exchange Resin particle size 0.15 - 0.30 mm. 52-100 mesh, sodium form and analytical grade ammonium chloride from BDH Laboratory Supplies, A Division of Merck Ltd., Broom Road, Poole Dorset. BH12 4NN or from Gallard Schlesinger, 584 Mineola Avenue, Carle Place, New York 11514-1731, USA.

4. Diethylamine and Sodium-1-octanesulphonate : Lancaster Synthesis Division, Eastgate, White Lund, Morecambe, Lancs. LA3 3DY or Lancaster Synthesis Inc., PO Box 1000, Windham, New Hampshire 03087, USA.

5. Ultra-pure water. (e.g. as produced by the Millipore Water Still).

APPENDIX 3

Hazards

Personnel untrained in the routine safe-handling of chemicals and good laboratory practices must not attempt to use this method. The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. For further information consult the Material Safety Sheets accompanying the chemicals or available from the suppliers.

In general, always wear safety glasses with side shields, work in a well ventilated area, avoid inhaling vapours and avoid contact of the chemicals with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

1. **TRICHLOROACETIC ACID**

   Serious risk of poisoning by inhalation, swallowing or skin contact.
   Causes severe burns.

2. **PARAQUAT**

   ICI Divisional Toxicity Classification is class 1 for solid reference material, class 3 for analytical solutions.
   Toxic by ingestion
   Harmful dust
   Avoid contact with eyes, skin and mouth. Avoid breathing dust.
   Wash hands and exposed skin before meals and after work.

   Ingestion of paraquat should be regarded as a dire emergency and action should be taken immediately. Details of remedial action/antidotes should be available in the laboratory.

3. **HEXANE**

   Highly flammable
   Avoid breathing vapour
   Avoid contact with eyes and skin
   (TLV 100ppm or 360 mg m^{-3})

4. **METHANOL**

   Highly flammable
   Serious risk of poisoning by inhalation or swallowing
   Avoid breathing vapour
   Avoid contact with eyes and skin
   (TLV 260 mg m^{-3})

5. **DIETHYLAMINE**

Harmful vapour
Harmful by skin absorbtion
Harmful if taken internally
Highly flammable
Avoid breathing vapour
(TLV 10 ppm or 30 mg m^{-3})

6. **ORTHOPHOSPHORIC ACID**

Causes burns
Avoid contact with eyes and skin
(TLV 1 mg m^{-3})

APPENDIX 4

Preparation of Analytical Standards

The following precautions must be taken when weighing out the analytical standard material :

1. Ensure good ventilation.
2. Wear appropriate protective clothing, safety glasses, gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash contaminated areas immediately.

Weigh out accurately, using a five figure balance, sufficient paraquat dichloride to allow dilution to give a 250 $\mu g\ cm^{-3}$ paraquat stock solution in a grade A volumetric flask. Make serial dilutions of this stock solution to give a range of working solutions from 0.0025 to 10.0 $\mu g\ cm^{-3}$ paraquat in saturated ammonium chloride solution. These standards should be used for the fortification of recovery samples and standards for HPLC analysis.

Solutions of paraquat dichloride in saturated ammonium chloride are stable at ambient temperatures under normal laboratory conditions for 12 months after preparation from the stock analytical standard.

APPENDIX 5

Typical HPLC traces for the determination of Paraquat
in Animal Tissues and Eggs

5.1 Paraquat Determination in Muscle, Skin and Fat, Liver and Fat (Hen)

Figure 1 : 0.025 $\mu g\ cm^{-3}$ Paraquat

Figure 2 : Untreated muscle sample at 0.5 $g\ cm^{-3}$

Figure 3 : Untreated muscle sample + 0.005 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 4 : Untreated liver sample at 0.5 $g\ cm^{-3}$

Figure 5 : Untreated liver sample + 0.50 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 6 : Untreated skin and fat sample at 0.5 $g\ cm^{-3}$

Figure 7 : Untreated skin and fat sample + 0.005 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 8 : Untreated fat sample at 0.5 $g\ cm^{-3}$

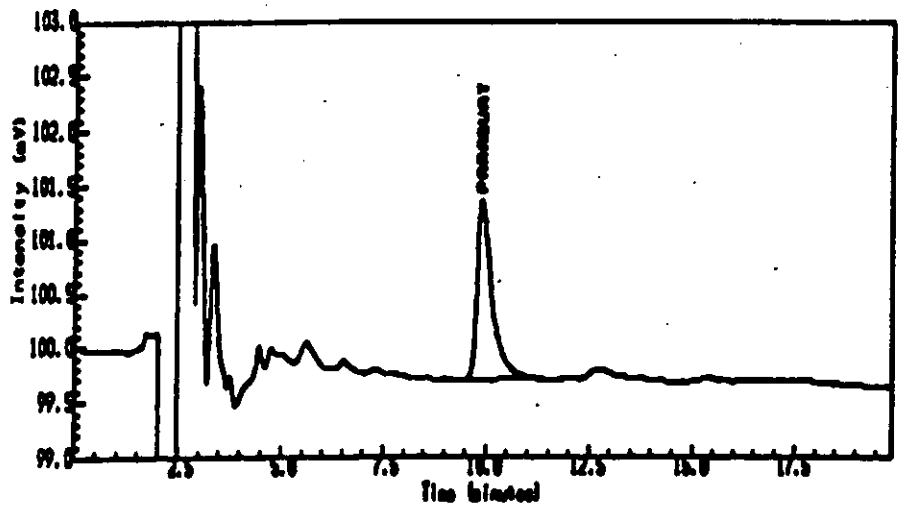
Figure 9 : Untreated fat sample at + 0.05 $mg\ kg^{-1}$ at 0.5 cm^{-3}

Figure 1

{RESIDUE} 23 ME4593A,7,1
Reported on 17-JAN-1994 at 14:34

Injection Report

Acquired on 16-JAN-1994 at 15:23



Sample Name : 0.025 STD
Sample Id : 4593/94/7
Sample Type : Standard Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak RT mins	Area uVs	ug/ml	Peak name	Width	RF slope	RF intercept
1 9.887	4307	0.025	PHENOL	21.1	157075.3750	38.3302

Totals		
Unknown	0	N/A
Quantified	4307	0.025
Grand Total	4307	0.025

ANALYSIS SUMMARY

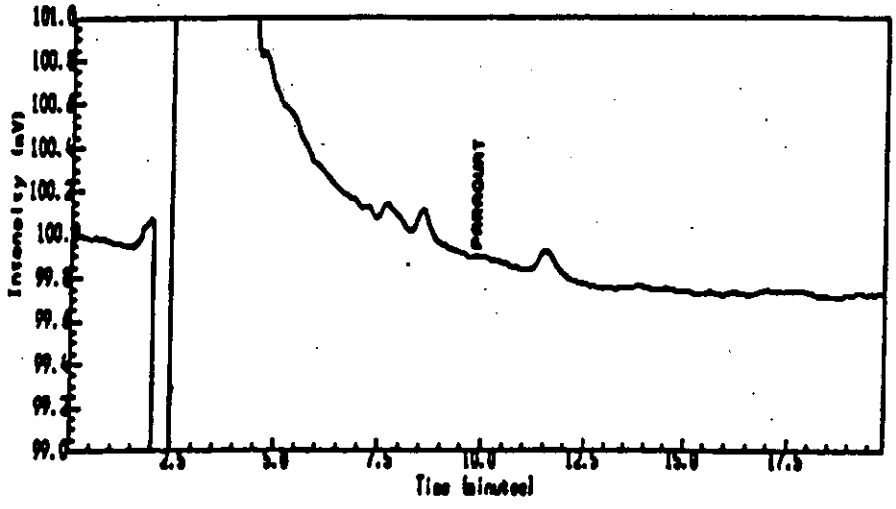
Method..... LAPQ
Run sequence..... LA4593
Calibration..... LAPQ

Figure 2

[RESIDUE] 23 ME4559A,2,1
Reported on 17-JAN-1994 at 14:53

Injection Report

Acquired on 8-JAN-1994 at 18:49



Sample Name : 17/1 94
Sample Id : 4559/94/2
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	ug/ml	Peak name	Width	RF slope	RF intercept
1	9.855	124	0.0002	PERMQUAT	8.3	1218447.7500	0.0000

Totals		
Unknown	0	N/A
Quantified	124	0.0002
Grand Total	124	0.0002

ANALYSIS SUMMARY

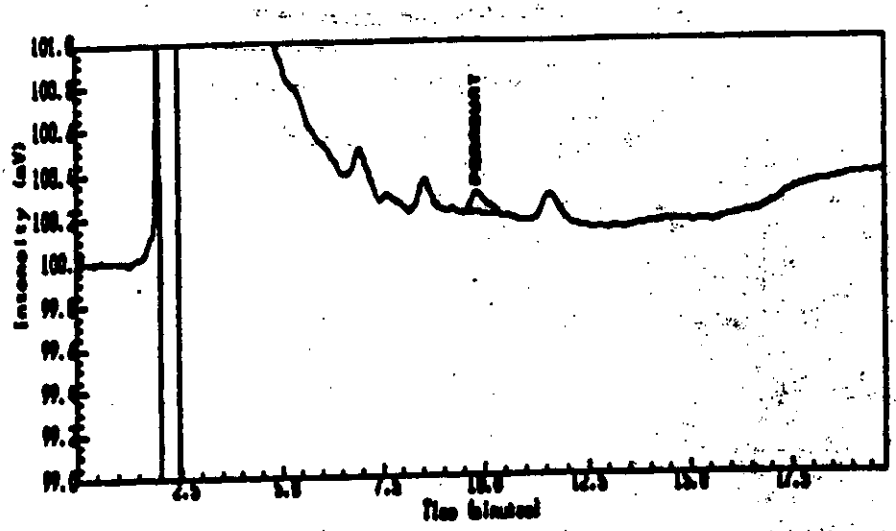
Method..... JAMPQT
Run sequence..... JAM4559
Calibration..... JAMPQT

Figure 3

[RESIDUE] 23 RE4559A,7,1
Reported on 17-JAN-1994 at 14:53

Injection Report

Acquired on 8-JAN-1994 at 20:55



Sample Name : R1 17/5 94
Sample Id : 4559/94/7
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak RT mins	Area uVs	ug/ml	Peak name	Width	RT Skew	RT Kurtosis
1 9.065	[illegible]	0.0045	ENDOSULF	25.6	[illegible]	0.0000

Totals		
Unknown	0	N/A
Certified	[illegible]	0.0045
Grand Total	[illegible]	0.0045

ANALYSIS SUMMARY

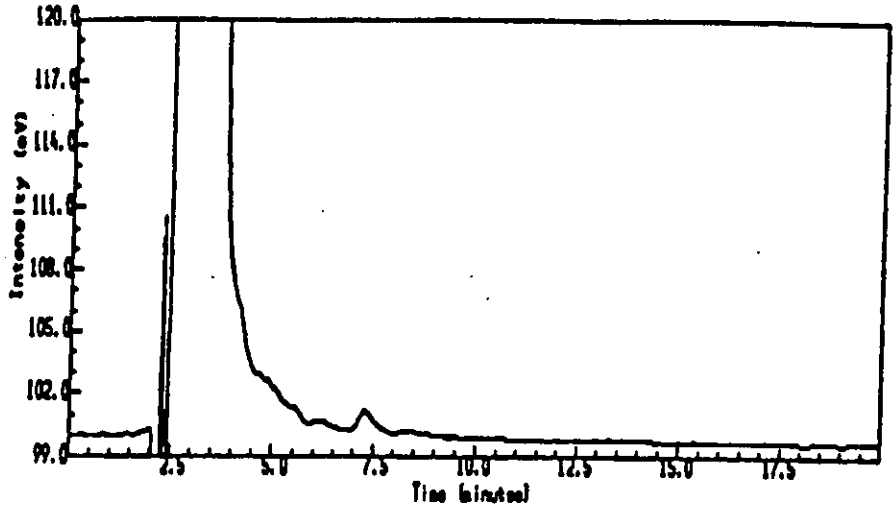
Method..... JANPQT
Run sequence..... JAN4559
Calibration..... JANPQT

Figure 4

[RESIDUE] 23 ME4542A,2,1
Reported on 17-JAN-1994 at 15:28

Injection Report

Acquired on 5-JAN-1994 at 22:49



Sample Name : 887/1 93
Sample Id : 4542/94/2
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	Width
1	9.450	103	I
2	9.514	823	28.8
3	10.037	230	7.0

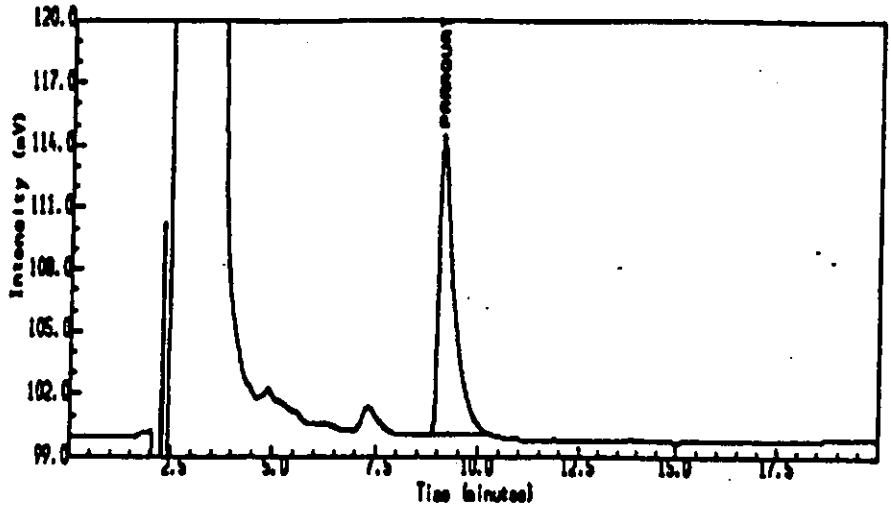
Totals	
Unknowns	0
Quantified	1157
Grand Total	1157

Figure 5

[RESIDUE] 23 ME4542A,3,1
Reported on 17-JAN-1994 at 15:29

Injection Report

Acquired on 5-JAN-1994 at 23:15



Sample Name : R1 887/2 93
Sample Id : 4542/94/3
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	ug/ml	Peak name	Width	RF slope	RF intercept
1	8.258	1033	0.0000		17.9	0.0000	0.0000
2	8.725	659	0.0000		11.9A	0.0000	0.0000
3	9.098	359867	0.4645	[illegible]	21.8	154865.2500	0.0000

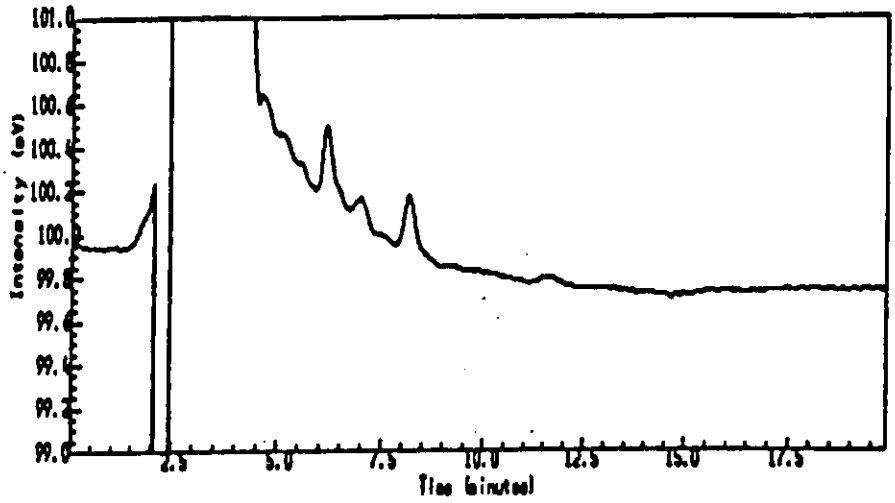
Totals		
Unknowns	0	N/A
Quantified	361359	0.4645
Grand Total	361359	0.4645

Figure 6

[RESIDUE] 23 ME4570A,2,1
Reported on 17-JAN-1994 at 14:57

Injection Report

Acquired on 11-JAN-1994 at 19:37



Sample Name : 18/1 94
Sample Id : 4570/94/2
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

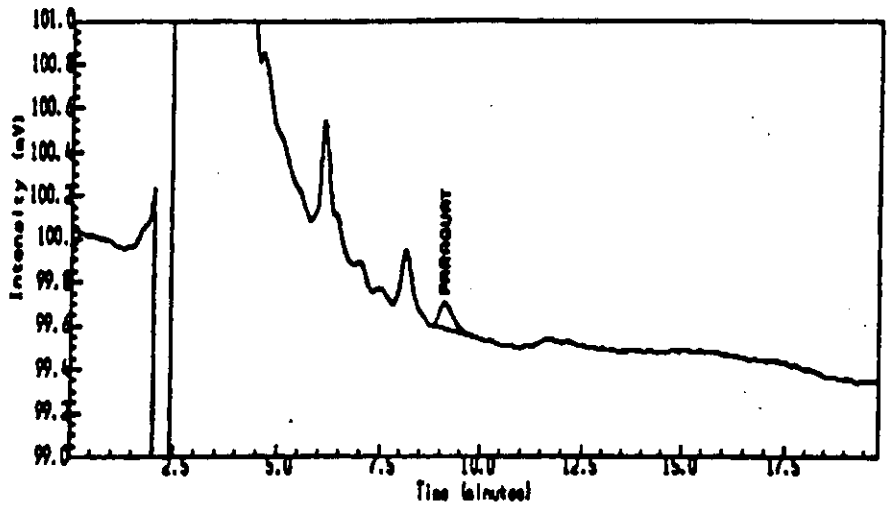
No peaks detected

Figure 7

[RESIDUE] 23 ME4570A,6,1
Reported on 17-JAN-1994 at 14:57

Injection Report

Acquired on 11-JAN-1994 at 21:18



Sample Name : R1 18/4 94
Sample Id : 4570/94/6
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	ug/ml	Peak name	Width	RF slope	RF intercept
1	9.130	2914	0.0044	PHENOXY	23.0	1322446.7500	0.0000

Totals

Unknowns	0	N/A
Quantified	2914	0.0044
Grand Total	2914	0.0044

ANALYSIS SUMMARY

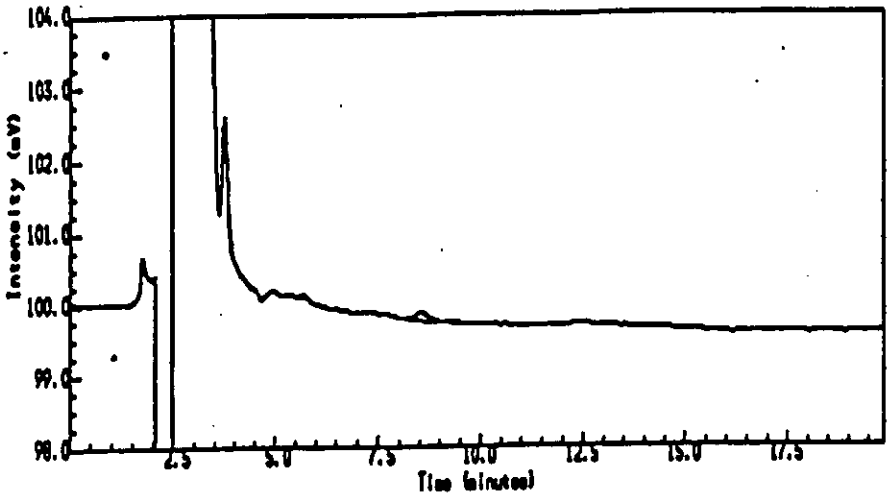
Method..... JANPQT
Run sequence..... JAN4570
Calibration..... JANPQT

Figure 8

[RESIDUE] 23 ME4608A,4,1
Reported on 19-JAN-1994 at 12:54
Modified on 19-JAN-1994 at 12:13

Injection Report

Acquired on 18-JAN-1994 at 19:26



Sample Name : 33/1 94
Sample Id : 4608/94/4
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	ug/ml	Peak name	Width	RF slope	RF intercept
1	8.554	2807	0.0000		19.8	0.0000	0.0000

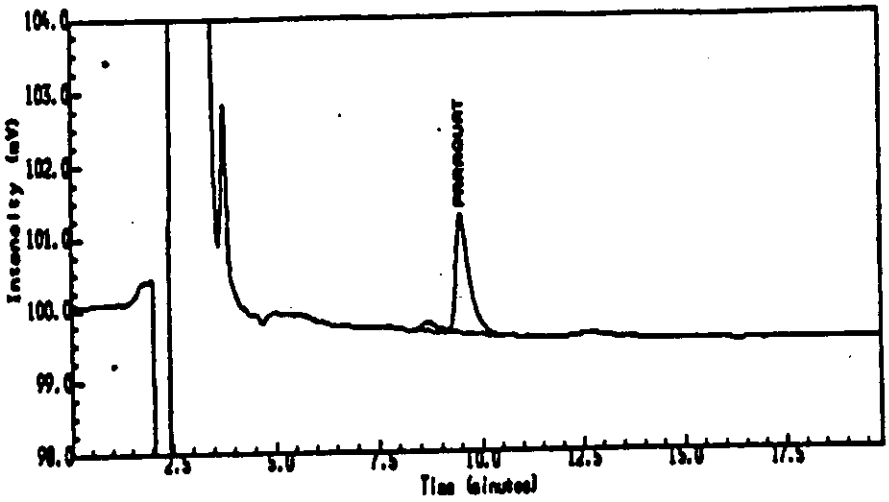
Totals		
Unknowns	0	N/A
Quantified	2807	0.0000
Grand Total	2807	0.0000

Figure 9

[RESIDUE] 23 ME4608A,13,1
Reported on 19-JAN-1994 at 12:54
Modified on 19-JAN-1994 at 12:13

Injection Report

Acquired on 18-JAN-1994 at 23:13



Sample Name : R2 33/7 94
Sample Id : 4608/94/13
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	uVs/dL	Peak name	Width	RF slope	RF intercept
1	8.693	2921	0.0000		21.8	0.0000	0.0000
2	9.453	35842	0.0477	PERMONT	19.8	1550887.0000	-1031.0000

Totals			
Unknown		0	N/A
Quantified		35842	0.0477
Grand Total		35842	0.0477

5.2 Paraquat Determination in Eggs (Hen)

Figure 1 : 0.025 $\mu g\ cm^{-3}$ Paraquat

Figure 2 : Untreated whole egg sample at 0.5 $g\ cm^{-3}$

Figure 3 : Untreated whole egg sample + 0.50 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 4 : Untreated egg yolk sample at 0.5 $g\ cm^{-3}$

Figure 5 : Untreated egg yolk sample at + 0.05 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 6 : Untreated egg white sample at 0.5 $g\ cm^{-3}$.

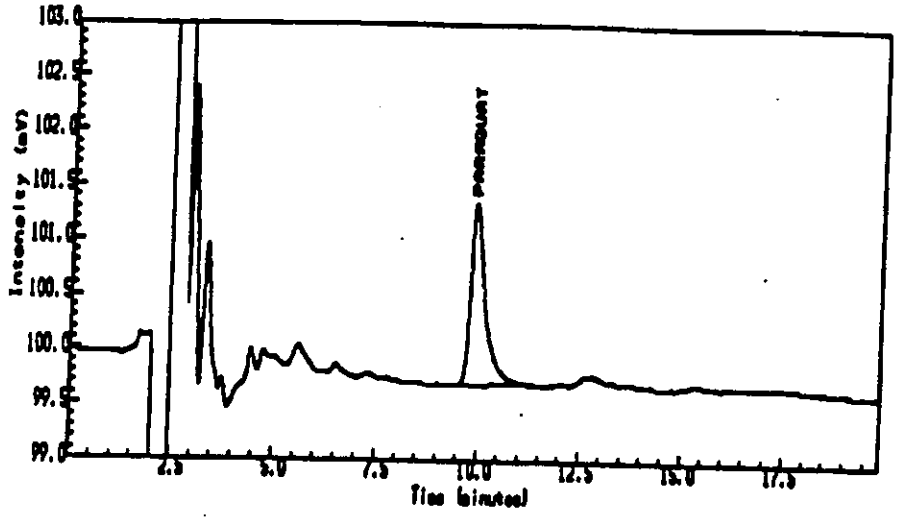
Figure 7 : Untreated egg white sample + 0.05 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 1

[RESIDUE] 23 ME4593A,7,1
Reported on 17-JAN-1994 at 14:34

Injection Report

Acquired on 16-JAN-1994 at 15:23



Sample Name : 0.025 STD
Sample Id : 4593/94/7
Sample Type : Standard Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	ug/ml	Peak name	Width	RF slope	RF intercept
1	9.857	40347	0.0250	[illegible]	21.1	1578075.3750	308.302

Totals		
Unknown	0	N/A
Quantified	40347	0.0250
Grand Total	40347	0.0250

ANALYSIS SUMMARY

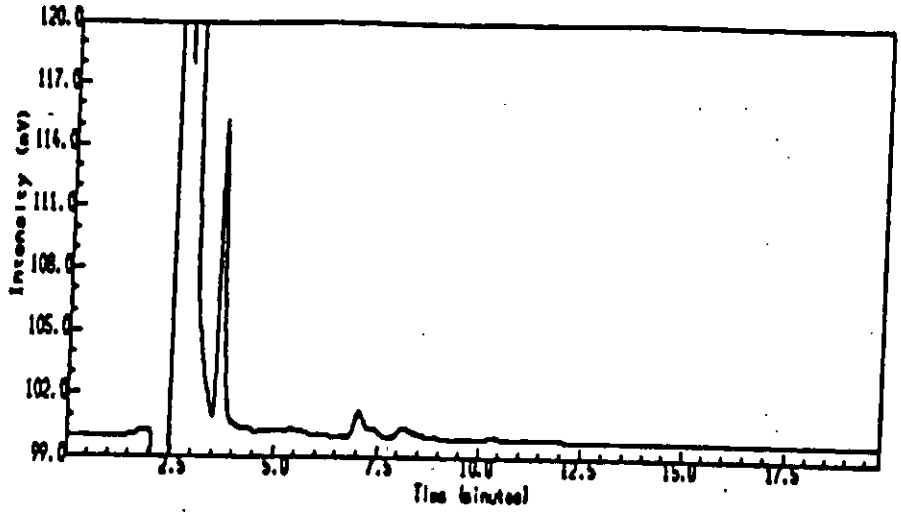
Method..... LAPQ
Run sequence..... LA4593
Calibration..... LAPQ

Figure 2

[RESIDUE] 23 RE4582A,4,1
Reported on 17-JAN-1994 at 15:14

Injection Report

Acquired on 13-JAN-1994 at 14:58



Sample Name : 24/1 94
Sample Id : 4582/94/4
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

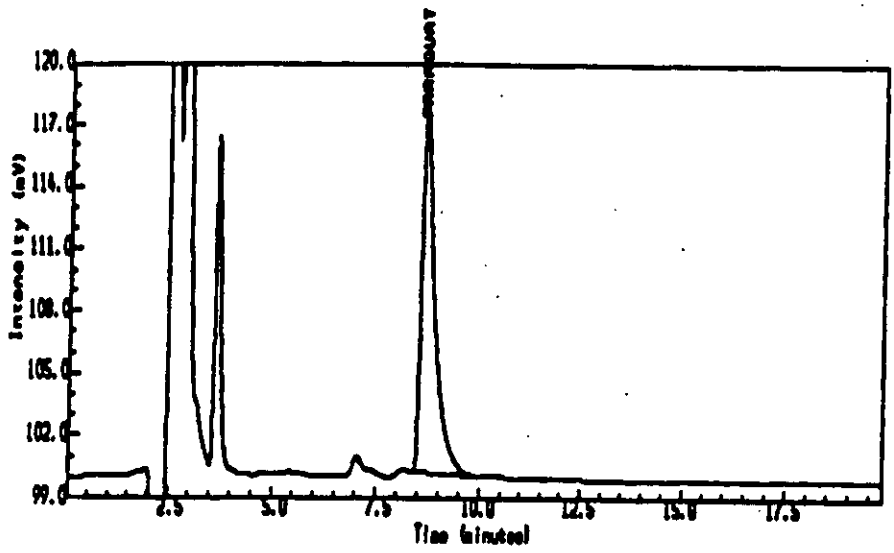
No peaks detected

Figure 3

[RESIDUE] 23 ME4582A,6,1
Reported on 17-JAN-1994 at 15:14

Injection Report

Acquired on 13-JAN-1994 at 15:49



Sample Name : R1 24/3 94
Sample Id : 4582/94/6
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	ug/ml	Peak name	Width	RF slope	RF intercept
1	8.639	394855	0.4834	ENDOSULF	18.6	193405.8750	0.0000

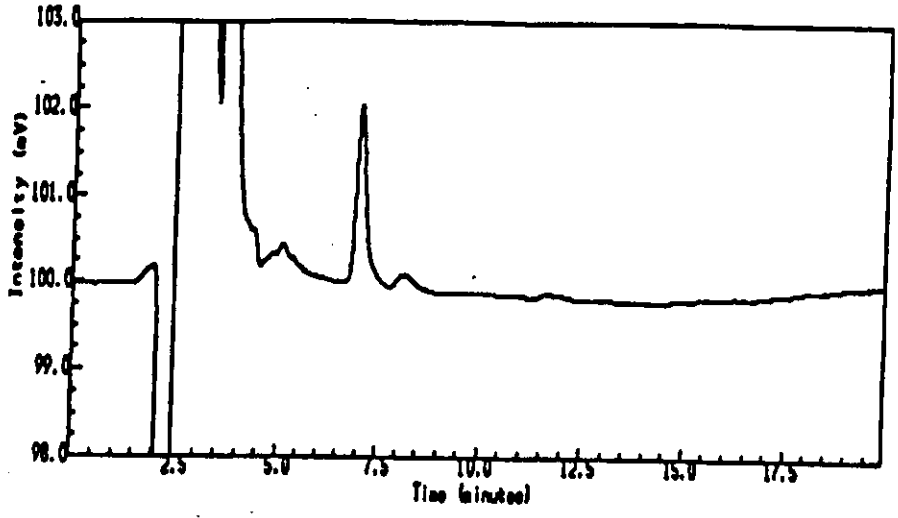
Totals		
Unknown	0	N/A
Quantified	394855	0.4834
Grand Total	394855	0.4834

Figure 4

(RESIDUE) 23 RE4574A,5,1
Reported on 17-JAN-1994 at 14:37

Injection Report

Acquired on 12-JAN-1994 at 15:54



Sample Name : 23/2 94
Sample Id : 4574/94/5
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

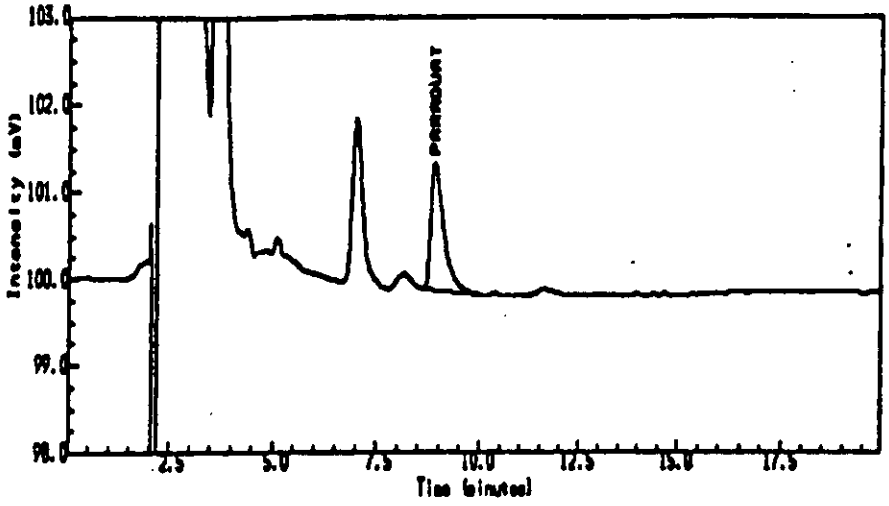
No peaks detected

Figure 5

[RESIDUE] 23 ME4574A,8,1
Reported on 17-JAN-1994 at 15:18

Injection Report

Acquired on 12-JAN-1994 at 17:09



Sample Name : R1 23/4 94
Sample Id : 4574/94/8
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uAs	ug/ml	Peak name	Width	RF slope	RF Intercept
1	8.885	31953	0.0351	PARAQUAT	19.8	1597193.7500	917.1275

Totals		
Unkrons	0	N/A
Quantified	31953	0.0351
Grand Total	31953	0.0351

ANALYSIS SUMMARY

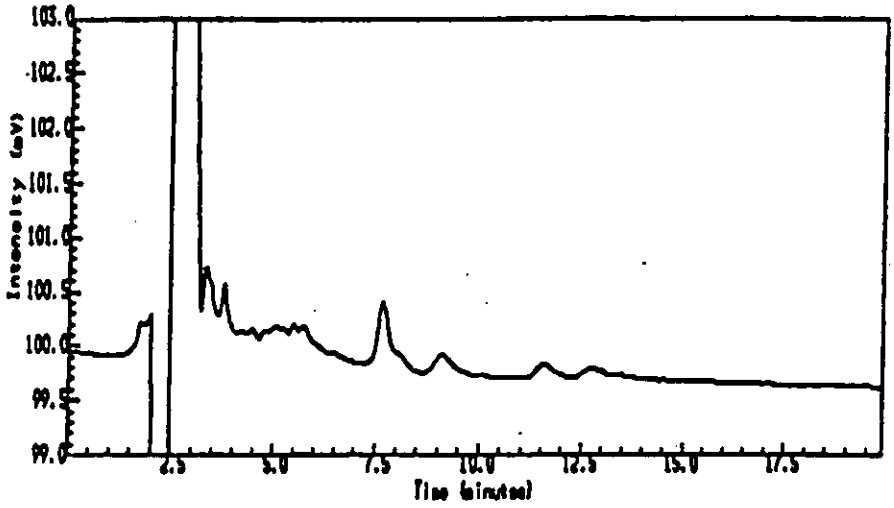
Method..... LAPQE
Run sequence..... LA4574
Calibration..... LAPQE

Figure 6

[RESIDUE] 23 ME4593A,5,1
Reported on 17-JAN-1994 at 14:34

Injection Report

Acquired on 16-JAN-1994 at 14:32



Sample Name : 38/2 94
Sample Id : 4593/94/5
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

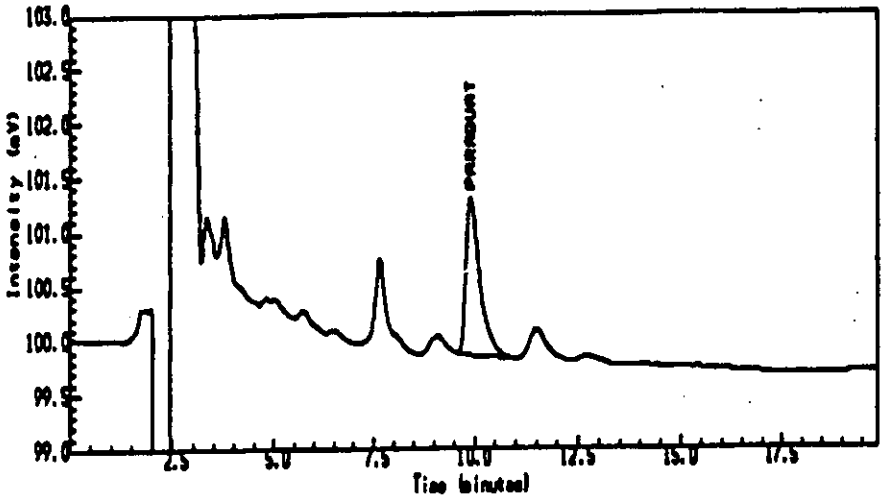
No peaks detected

Figure 7

[RESIDUE] 23 ME4593A,6,1
Reported on 17-JAN-1994 at 14:34

Injection Report

Acquired on 16-JAN-1994 at 14:58



Sample Name : R1 38/3 94
Sample Id : 4593/94/6
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area u&s	ug/ml	Peak name	Width	RF slope	RF intercept
1	9.887	3616	0.0021	ENFLURANE	20.5	1578075.3750	918.3022

Totals		
Unknowns	0	N/A
Quantified	3616	0.0021
Grand Total	3616	0.0021

ANALYSIS SUMMARY

Method..... LAPQ
Run sequence..... LA4593
Calibration..... LAPQ

5.3 Paraquat Residue Determination in Heart (Sheep), Liver (Bovine) and Kidney (Pig) (from RAM/0004/03).

Figure 1 : 0.1 μg cm^{-3} Paraquat

Figure 2 : Untreated heart sample at 0.5 g cm^{-3}

Figure 3 : Untreated heart sample + 0.10 mg kg^{-1} at 0.5 g cm^{-3}

Figure 4 : Untreated liver sample at 0.5 g cm^{-3}.

Figure 5 : Untreated liver sample + 0.10 mg kg^{-1} at 0.5 g cm^{-3}

Figure 6 : Untreated kidney sample at 0.5 g cm^{-3}

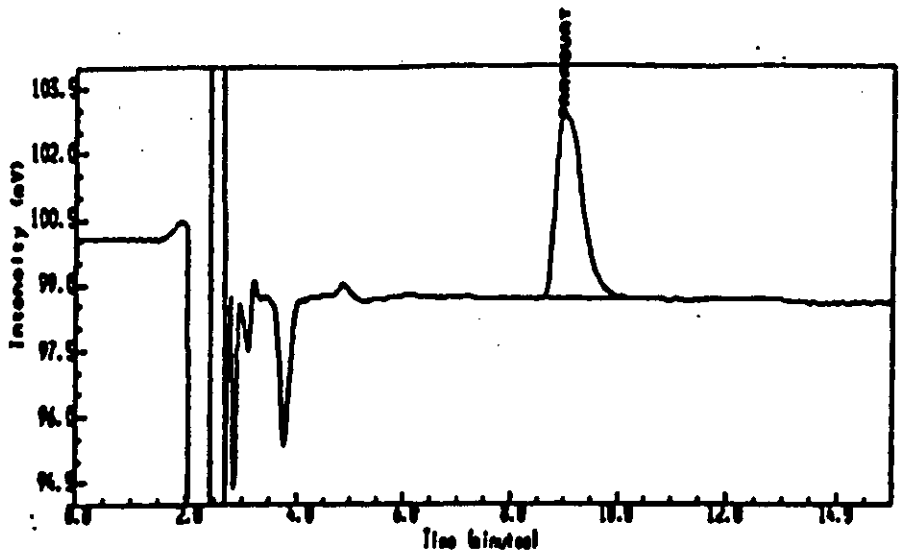
Figure 7 : Untreated kidney sample + 0.10 mg kg^{-1} at 0.5 g cm^{-3}

Figure 1

[RESIDUE] 23 DA424A,1,1
Reported on 14-NOV-1988 at 11:25

Injection Report

Acquired on 20-Oct-1988 at 10:29 by user TS



Sample Name : STD
Sample Id :
Sample Type : Standard Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.008	16557	0.008	RESIDUE
Residual		0	N/A	
Total		16557	0.008	

MISSING PEAKS

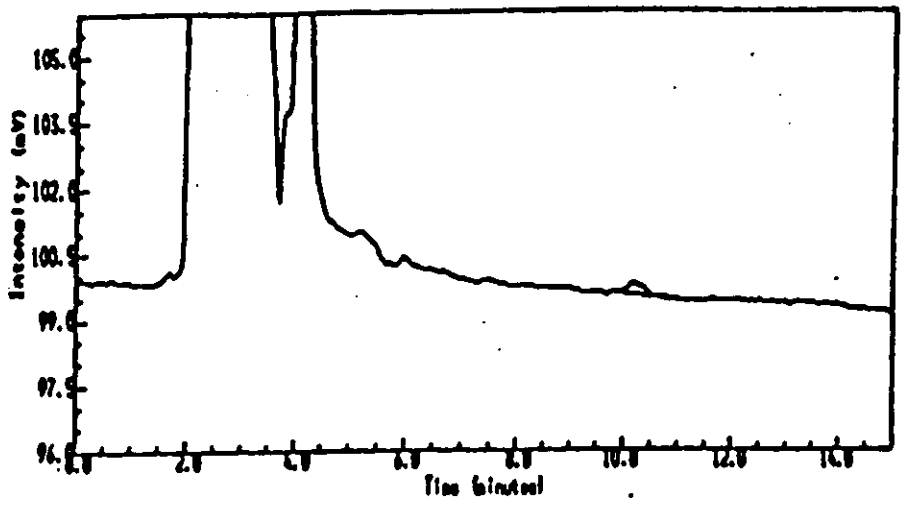
No missing peaks.

Figure 2

[RESIDUE] 22 DA454C,2,1
Reported on 14-NOV-1988 at 11:34

Injection Report

Acquired on 7-Nov-1988 at 15:07 by user T6



Sample Name : C7080/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 2

PEAK INFORMATION

Peak RT mins	Area uVs	ng/ng	Peak name
Residual	5738	N/A	
Total	0	0.000	

MISSING PEAKS

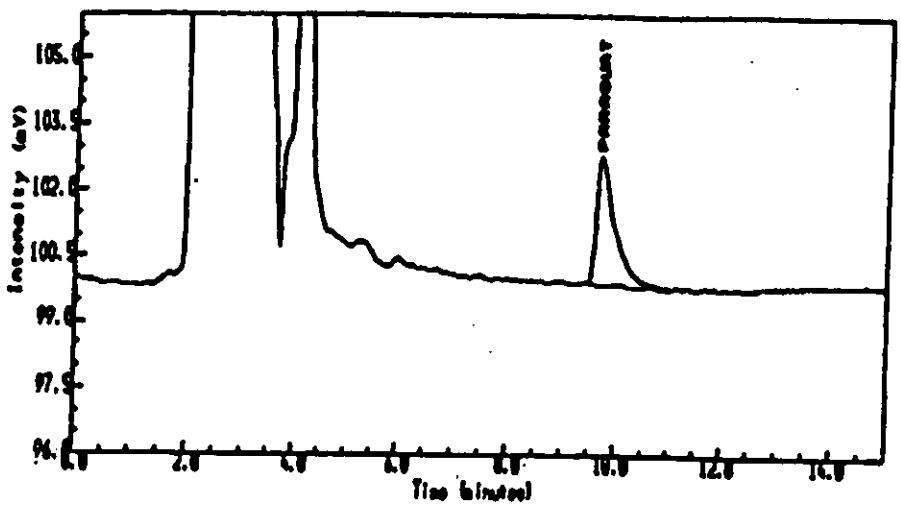
RT mins	Peak name
9.600	ENDRIN

Figure 3

[RESIDUE] 22 DA454C,3,1
Reported on 14-NOV-1988 at 11:32

Injection Report

Acquired on 7-Nov-1988 at 15:32 by user T6



Sample Name : R1 7080/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 3

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.728	6530	0.050	PERCENT
Residual		0	N/A	
Total		6530	0.050	

MISSING PEAKS

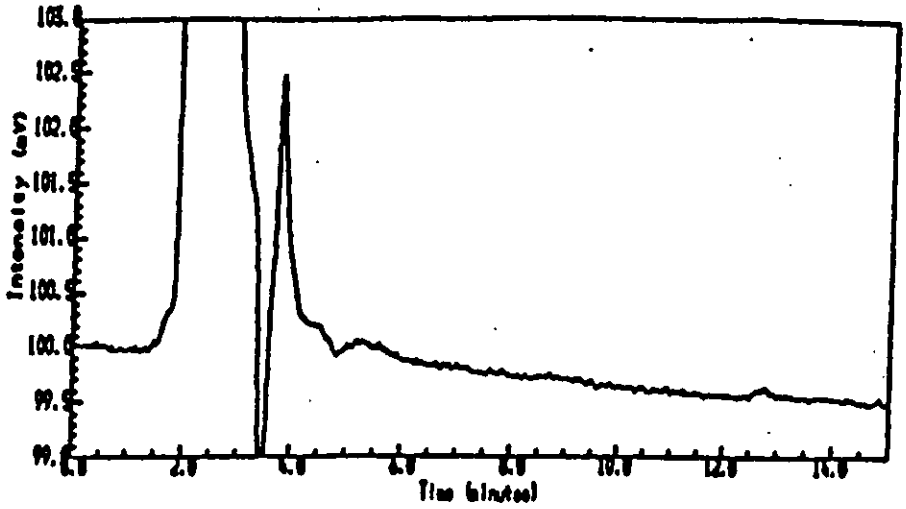
No missing peaks.

Figure 4

[RESIDUE] 22 DA459E,2,1
Reported on 21-NOV-1988 at 16:56

Injection Report

Acquired on 18-Nov-1988 at 17:33 by user T7



Sample Name : C7081/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 2

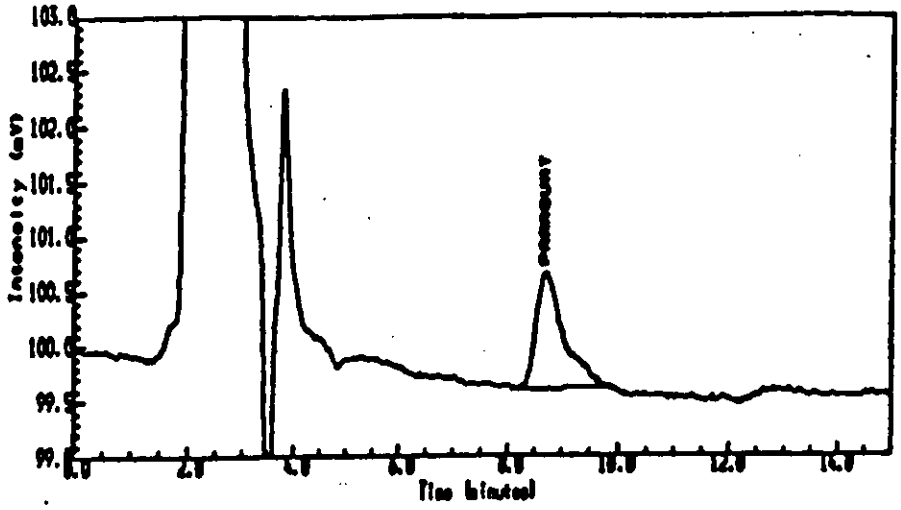
No peaks detected

Figure 5

[RESIDUE] 22 DA459E,3,1
Reported on 21-NOV-1988 at 16:35

Injection Report

Acquired on 18-Nov-1988 at 17:54 by user T7



Sample Name : R17081/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 3

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	8.704	37324	0.033	PARQUAT
Residual		0	N/A	
Total		37324	0.033	

MISSING PEAKS

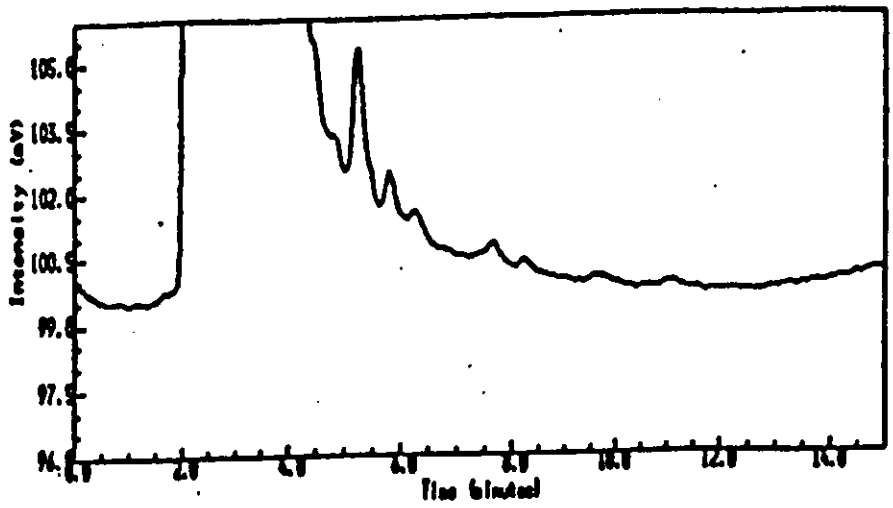
No missing peaks.

Figure 6

[RESIDUE] 22 DA454C,7,1
Reported on 14-NOV-1988 at 11:36

Injection Report

Acquired on 7-Nov-1988 at 17:13 by user T6



Sample Name : C7082/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 7

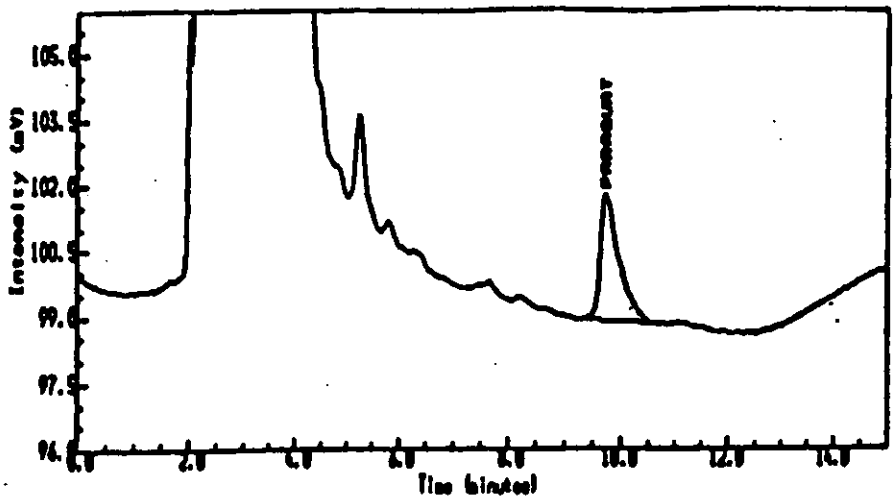
No peaks detected

Figure 7

[RESIDUE] 22 DA454C,8,1
Reported on 14-NOV-1988 at 11:37

Injection Report

Acquired on 7-Nov-1988 at 17:38 by user T6



Sample Name : RI 7082/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 8

PEAK INFORMATION

Peak	RT mins	Area Ub	mg/kg	Peak name
1	9.749	6704	0.087	PYRIDALYL
Residual		0	N/A	
Total		6704	0.087	

MISSING PEAKS

No missing peaks.

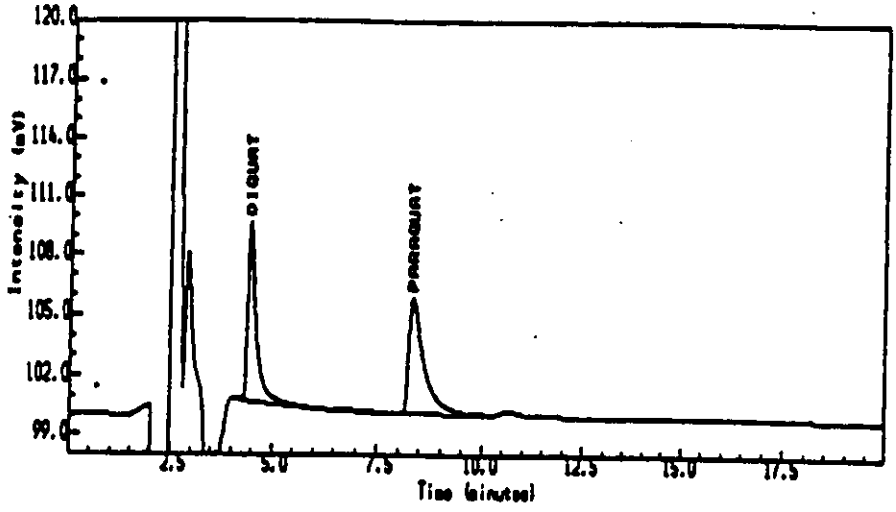
5.4 **Paraquat and Diquat Mixed Standard**

Figure 1 : 0.1 $\mu g\ cm^{-3}$ Paraquat + 0.5 $\mu g\ cm^{-3}$ Diquat

[DEVELOP] 23 D9267-25A,11,1
Reported on 17-JAN-1994 at 16:40

Injection Report

Acquired on 16-NOV-1993 at 18:26



Sample Name : D9267/25E
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 1