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Duck and Coot Tissues:

DETERMINATION OF WHITE PHOSPHORUS IN DUCK AND COOT TISSUES

PROJECT NUMBER: 0071/0786

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AUTHENTICATION

This report fully and accurately reflects the procedures used and data generated.

[redacted] ..... DATE: 29 JUN 2010

CONTENTS

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SUMMARY

The quantity of white phosphorus present in the gizzard contents of the supplied duck and coot tissues has been determined. The results are shown in the following table:

Sample	Total White Phosphorus Content Present (mg)	Total White Phosphorus Content Present (µg)
Duck Gizzard Contents	2.72×10^{-3}	2.72
Coot Gizzard Contents	0.186	186

The white phosphorus residue (mg/kg) in the supplied duck and coot tissue samples has also been determined. These results are shown in the following table:

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)
Duck Gizzard	0.281
Duck Skin and Fat	<0.02
Duck Liver	<0.02
Coot Gizzard	1.13

The procedures used were based on Johnston, JJ, Goldade, DA, Kohler DJ, Cummings, JL (2000). Determination of white phosphorus residues in ducks: an atomic emission detection/compound-independent calibration-based method of generating residue data for risk assessment and environmental monitoring. Environ Sci Technol 34(9):1856-1861.

Duck and Coot Tissues:

DETERMINATION OF WHITE PHOSPHORUS IN DUCK AND COOT TISSUES

1. INTRODUCTION

The objective of this study was to determine the white phosphorus residue in duck and coot tissue samples supplied by the Sponsor. The procedure was based on Johnston, JJ, Goldade, DA, Kohler DJ, Cummings, JL (2000). Determination of white phosphorus residues in ducks: an atomic emission detection/compound-independent calibration-based method of generating residue data for risk assessment and environmental monitoring. Environ Sci Technol 34(9):1856-1861.

Testing was conducted at Harlan Laboratories Ltd between 17 May 2010 and 20 May 2010.

2. ANALYTICAL STANDARD SOLUTION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	white phosphorus standard solution
Description	:	pale yellow solution
Concentration	:	922 mg/l prepared in iso-octane
Date received at Test Facility	:	24 November 2009
Storage conditions	:	room temperature, in the dark

The integrity of supplied data relating to the identity, purity and stability of the analytical standard solution is the responsibility of the Sponsor. Preparation of the standard solution was performed at the Sponsor's facilities at Oldbury, UK, and this procedure was witnessed and documented by a member of Harlan Laboratories Ltd staff with management responsibilities to maintain study integrity.

3. TISSUE SAMPLES

3.1 Identification and Storage Conditions

Sponsor's identification	:	25/03/10, Centre ID26, B648, Duck
Tissue Samples Supplied	:	Gizzard Liver Skin and Fat
Date received at Test Facility	:	10 May 2010
Storage conditions	:	stored frozen at approximately -20°C, in the dark

Sponsor's identification	:	20/04/10, Centre ID26, B393, Coot
Tissue Samples Supplied	:	Gizzard
Date received at Test Facility	:	10 May 2010
Storage conditions	:	stored frozen at approximately -20°C, in the dark

The integrity of supplied data relating to the identity and stability of the tissue samples is the responsibility of the Sponsor.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. DETERMINATION OF WHITE PHOSPHORUS RESIDUES

5.1 Method

The determination was carried out using a procedure based on Johnston, JJ, Goldade, DA, Kohler DJ, Cummings, JL (2000). Determination of white phosphorus residues in ducks: an atomic emission detection/compound-independent calibration-based method of generating residue data for risk assessment and environmental monitoring. Environ Sci Technol 34(9):1856-1861.

5.2 Procedure

5.2.1 Standard Solution Preparation

An aliquot (0.0922 g) of white phosphorus was diluted to a volume of 100 ml with iso-octane. Preparation of the standard solution was performed at the Sponsor's facilities at Oldbury, UK, due to legislative restrictions in handling the potentially reactive phosphorus. The white phosphorus was dried in acetone and then dried further using a tissue prior to weighing and diluting with iso-octane. This procedure was witnessed and documented by a member of staff at Harlan Laboratories Ltd with management responsibilities to maintain study integrity.

5.2.2 Sample Preparation

Each tissue sample was defrosted by placing the containers in a 20°C nominal temperature waterbath for a minimum of 1 hour.

Gizzard contents

The gizzard, once defrosted, was dissected longitudinal between the crushing plates to expose the contents. These contents were then transferred to a conical flask and the remaining tissue quantitatively rinsed using 60 ml of degassed glass distilled water* and added to the conical flask.

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* Degassed by boiling vigorously and then purging with nitrogen as it cooled.

Remaining tissues

Each tissue sample, once defrosted, was macerated and an aliquot of each macerated tissue sample (see following table) was then transferred to a conical flask for extraction.

Table 5.1

Tissue Sample	Mass of Tissue Taken (g)
Duck Gizzard	5.0785
Duck Liver	5.0122
Duck Skin	4.2471
Coot Gizzard	5.0906

Extraction of Samples for Analysis

Each tissue sample was suspended in 30 ml of degassed glass distilled water* and 10 ml of iso-octane added to each flask. To the two samples of gizzard contents containing 60 ml of degassed glass distilled water*, 20 ml of iso-octane was added. All flasks were purged with nitrogen to fill the headspace and shaken at approximately 150 rpm on a horizontal flat bed shaker for 18 hours, at ambient temperature, in the dark.

After the shaking period, the samples were allowed to stand for approximately 1 hour at room temperature prior to decanting into centrifuge tubes and centrifuging at 2500 rpm for 15 minutes. The iso-octane extract was then removed to a clean glass vessel and an aliquot taken for analysis in an amber vial. In addition, aliquots of the gizzard contents extract and gizzards tissue extract were diluted in duplicate by a factor of 10 and a factor of 100 with iso-octane and transferred to amber vials for analysis.

The remaining volumes of iso-octane extract were placed into storage at approximately -20°C, in the dark.

A sample blank was prepared by treating a mixture of 30 ml of degassed glass distilled water* and 10 ml of iso-octane as detailed for the samples.

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* Degassed by boiling vigorously and then purging with nitrogen as it cooled.

5.2.3 Analysis

The concentration of white phosphorus in the sample solutions was determined by gas chromatography (GC) with flame photometric detection (FPD).

Standards

Standard solutions of white phosphorus were prepared in iso-octane to cover a nominal concentration range of 0.01 to 0.25 mg/l.

Standard blank

Iso-octane

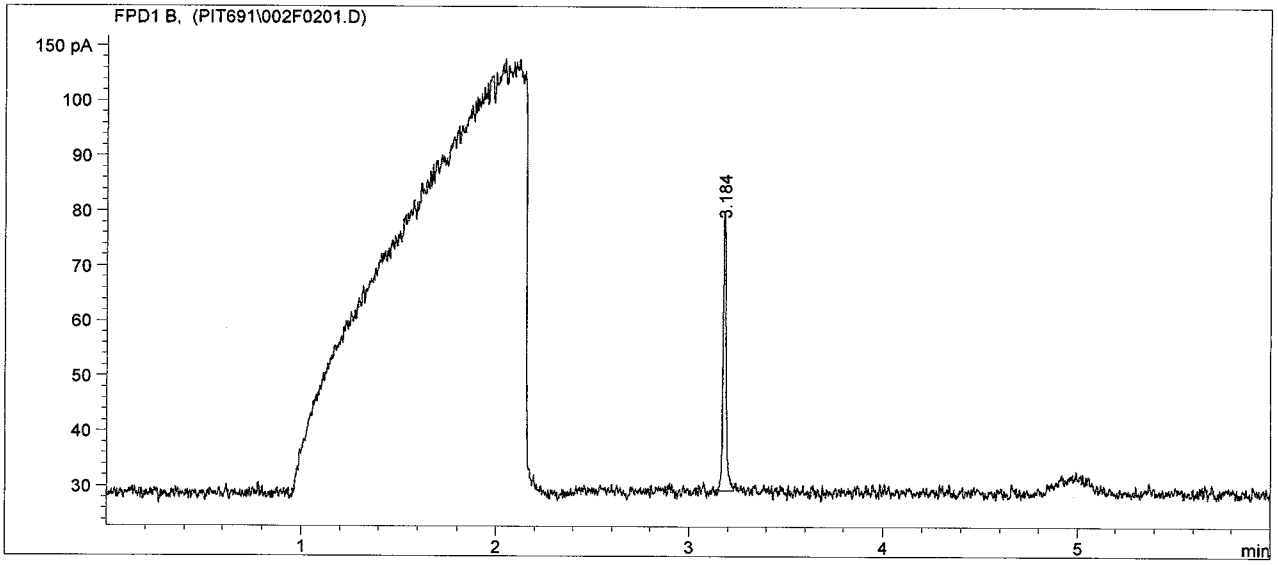
Analysis

The standard and sample solutions were analysed by GC using the following conditions:

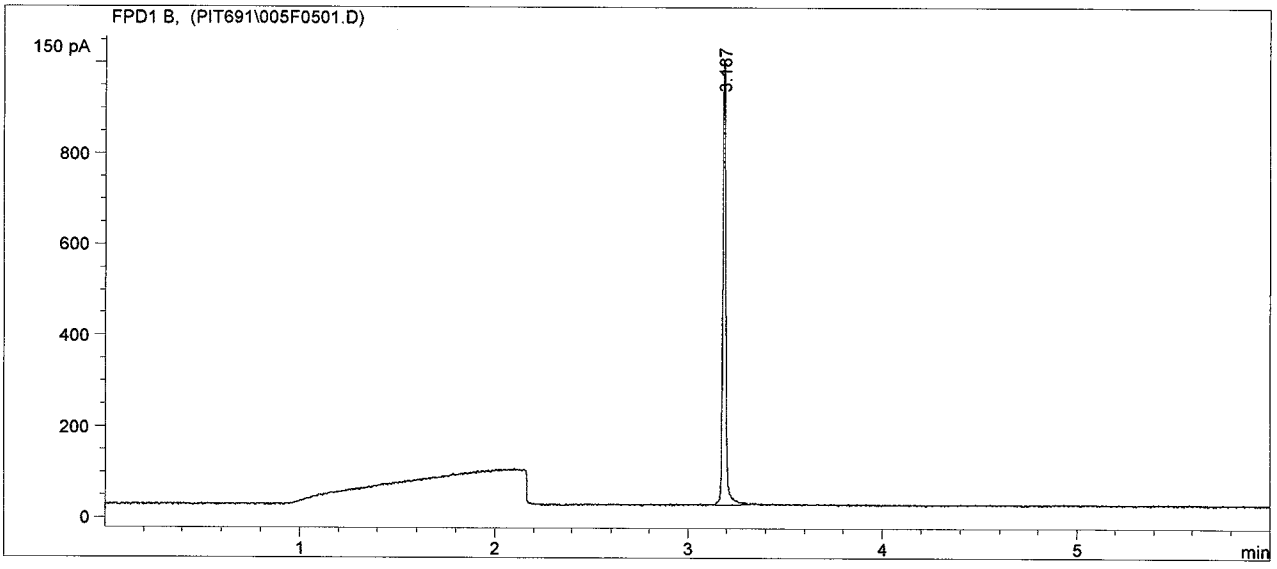
GC System	:	Agilent Technologies 6890, incorporating autosampler and workstation
Column	:	DB-1 (30 m x 0.25 mm id x 0.25 µm film)
Oven temperature program	:	initial 40°C for 0.5 minutes rate 20°C/minute final 150°C for 2 minutes
Injection temperature	:	250°C
FPD mode	:	phosphorus
FPD temperature	:	250°C
Injection volume	:	2 µl
Injection mode	:	splitless (purge on at 0.5 minute)
Carrier gas	:	nitrogen
Flow rate	:	0.7 ml/minute (constant pressure)
Retention time	:	~ 3.2 minutes

Typical Chromatography

Standard Solution 1.38 x 10^{-2} mg/l

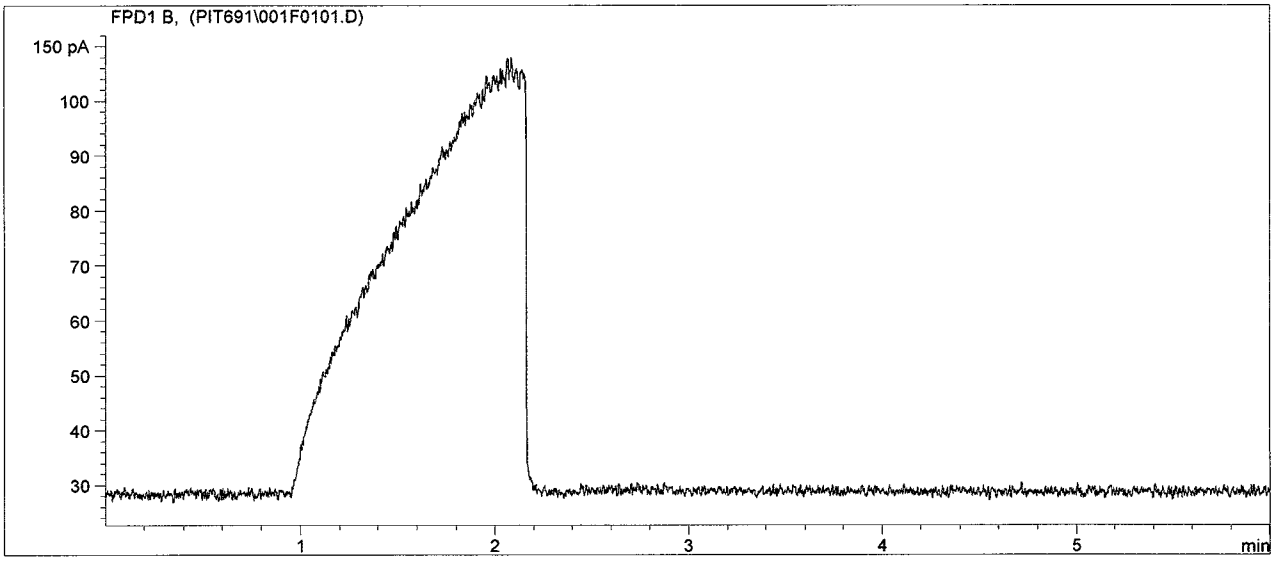


Standard Solution 0.138 mg/l

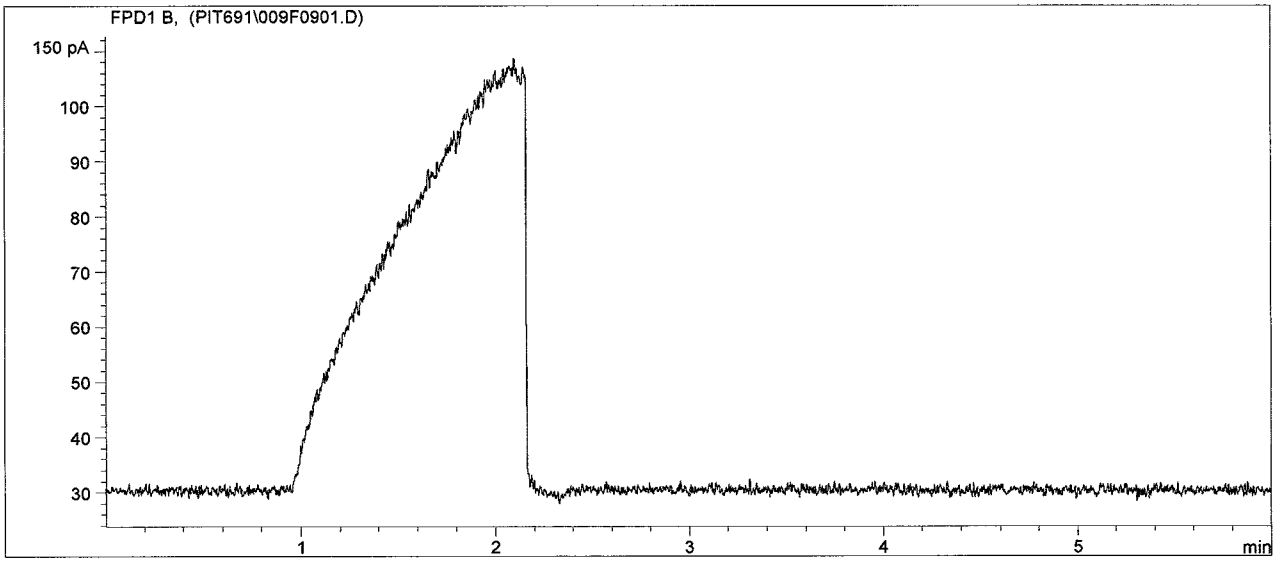


Typical Chromatography

Standard Blank

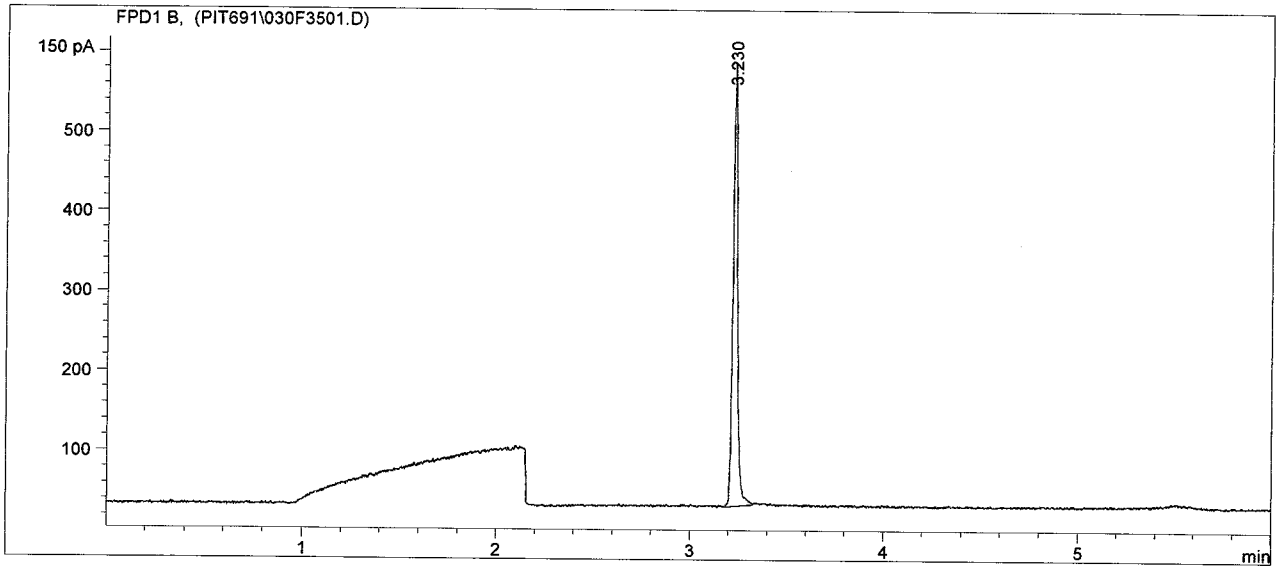


Sample Blank

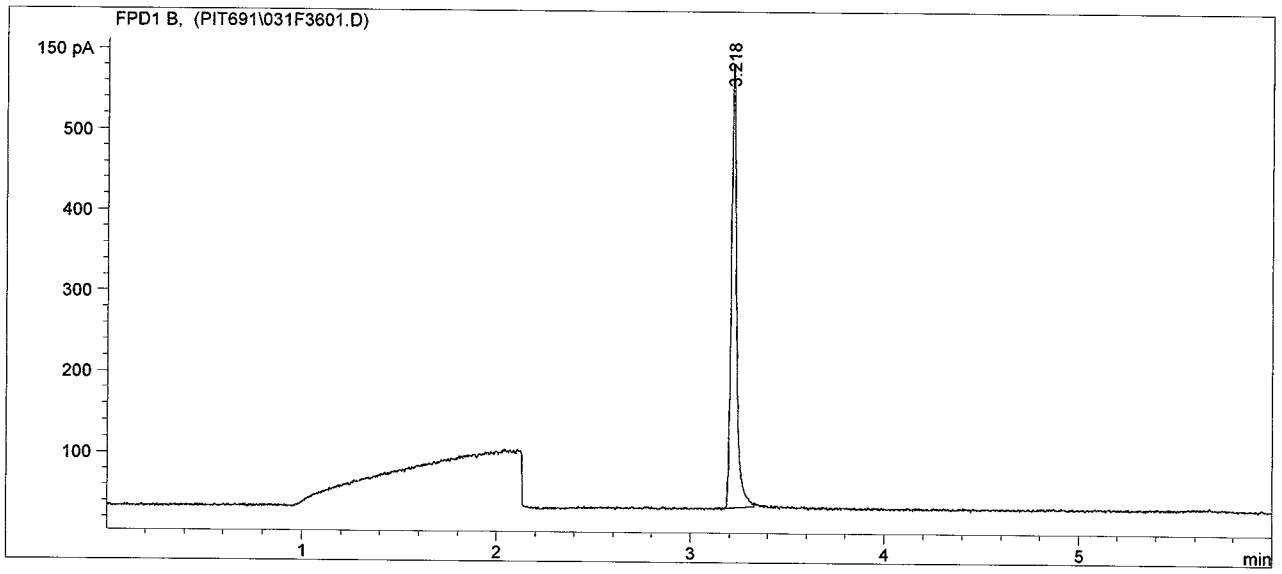


Typical Chromatography

Duck Gizzard Contents Solution (no dilution)

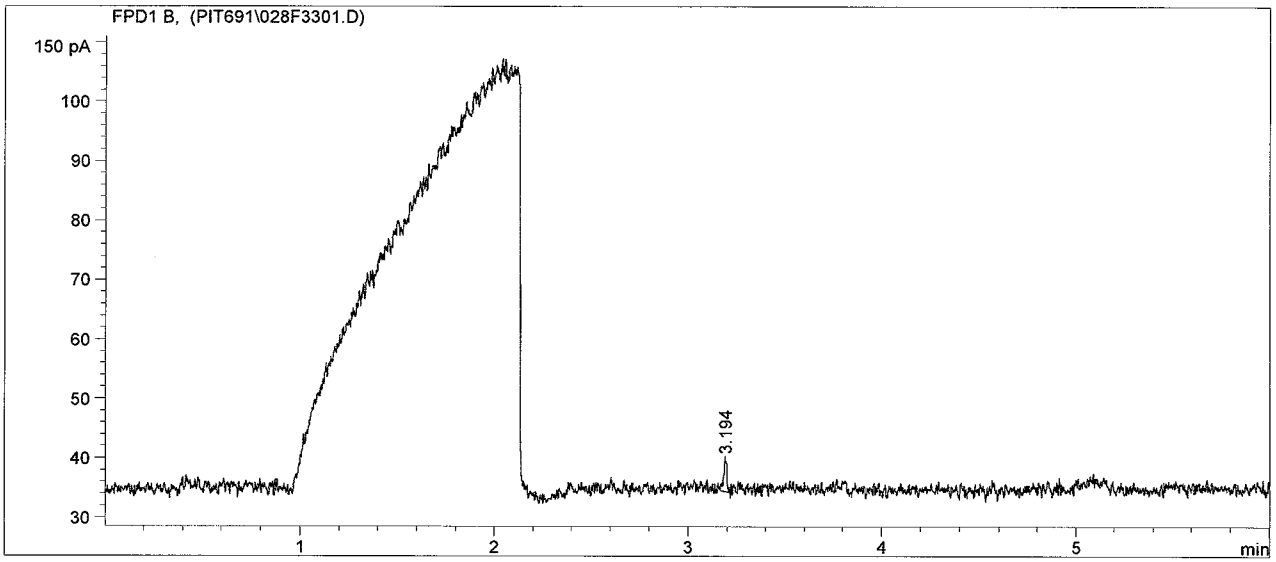


Duck Gizzard Tissue Sample Solution (no dilution)

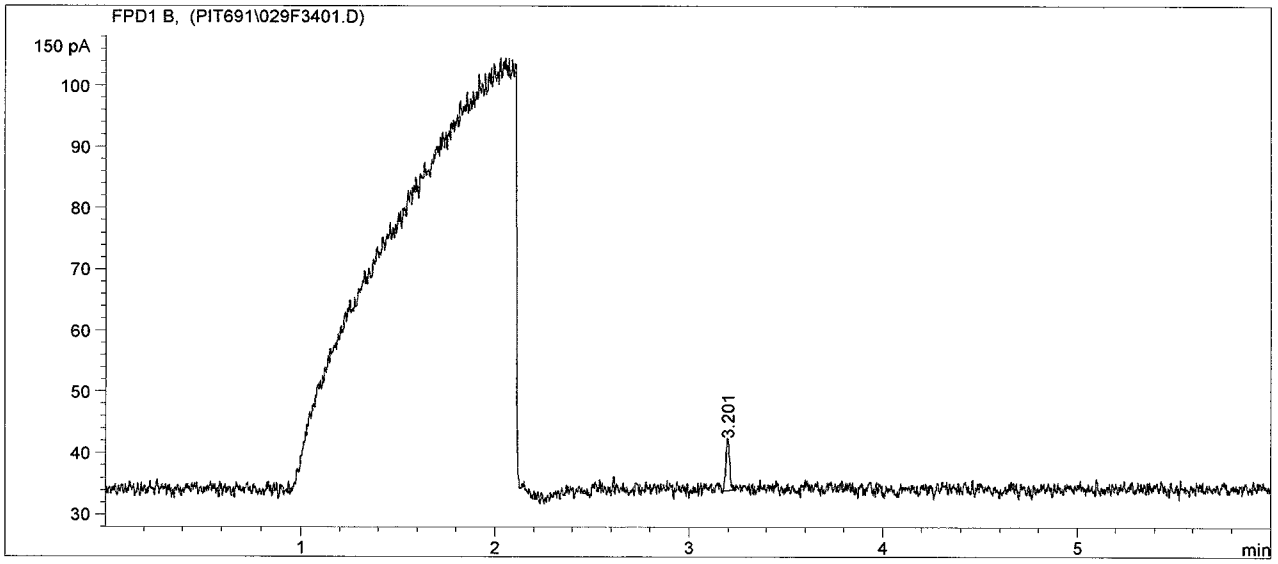


Typical Chromatography

Duck Liver Solution

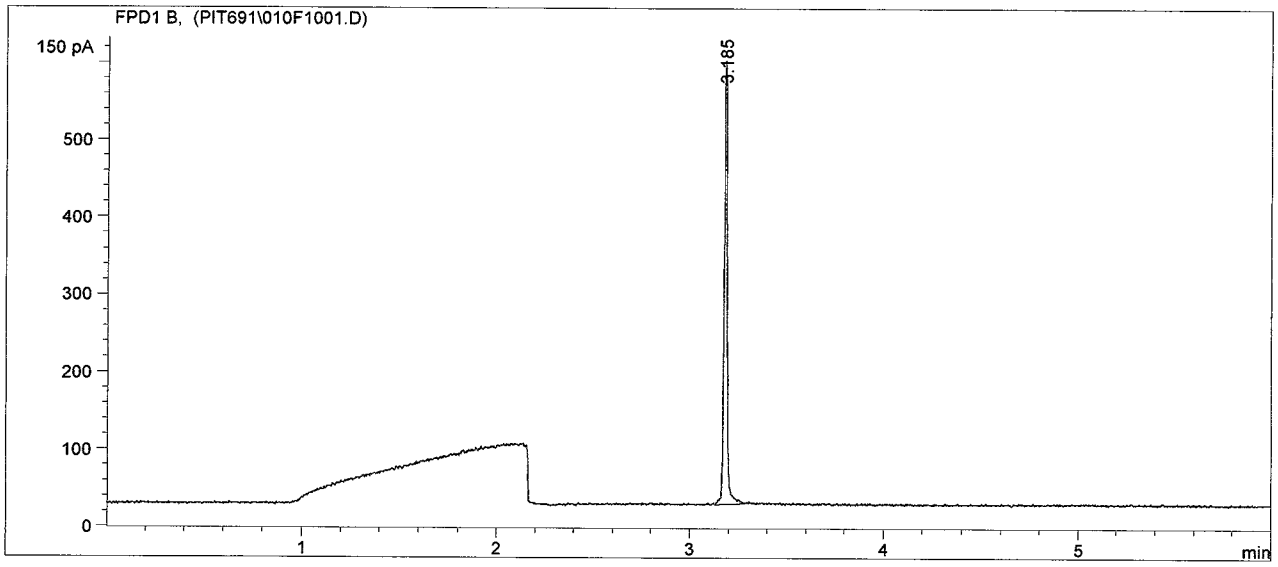


Duck Skin and Fat Sample Solution

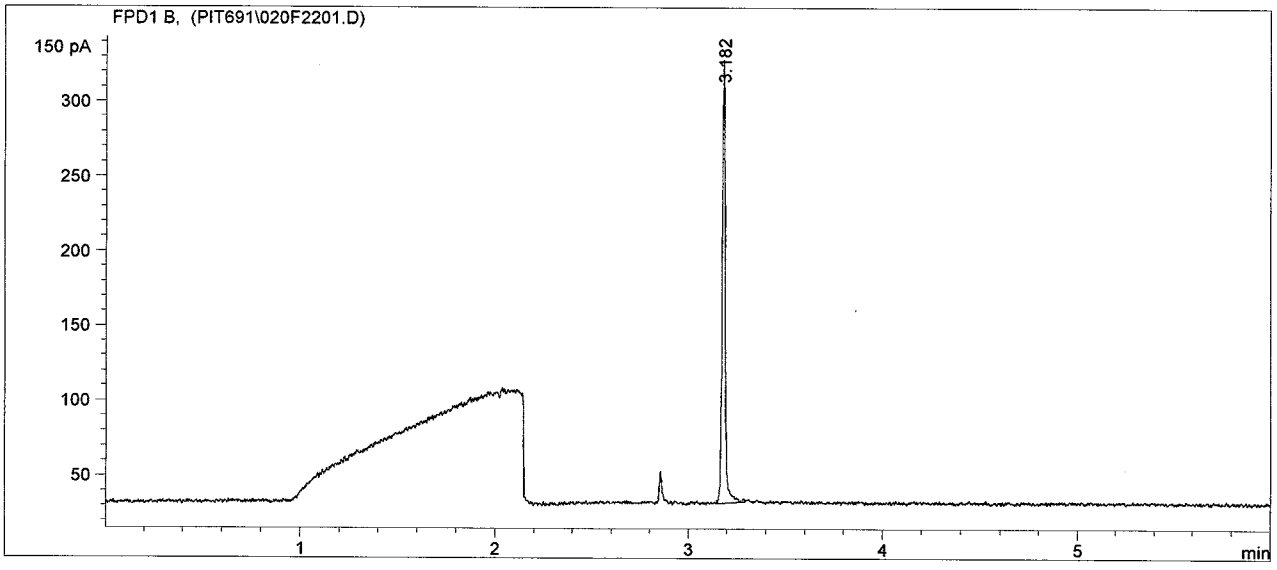


Typical Chromatography

Coot Gizzard Contents Solution (x100 dilution)



Coot Gizzard Tissue Solution (x10 dilution)



5.3 Calculation

The mean peak area and concentration of each standard were plotted on a calibration curve and the sample concentration (mg/l) interpolated from the curve. The concentration was then corrected for dilution factor if relevant.

The white phosphorus content (mg) present in the gizzard contents was calculated using Equation 5.1. These results have also been multiplied by 1×10^{-3} to present the values in µg within the report.

Equation 5.1

$$M_{wp} = C_{wp} \times \frac{V_{iso}}{1000}$$

where:

M_{wp} = mass of white phosphorus present in contents (mg)
C_{wp} = concentration of white phosphorus determined in the sample solution (mg/l)
V_{iso} = volume of iso-octane used for extraction (ml)

The white phosphorus residue (mg/kg) in the original tissue sample was calculated using Equation 5.2.

Equation 5.2

$$R_{wp} = C_{wp} \times \frac{V_{iso}}{1000} \times \frac{1}{M_{tis}} \times 1000$$

where:

R_{wp} = residue of white phosphorus (mg/kg)
C_{wp} = concentration of white phosphorus determined in the sample solution (mg/l)
V_{iso} = volume of iso-octane used for extraction (ml)
M_{tis} = mass of tissue taken for extraction (g)

5.4 Results

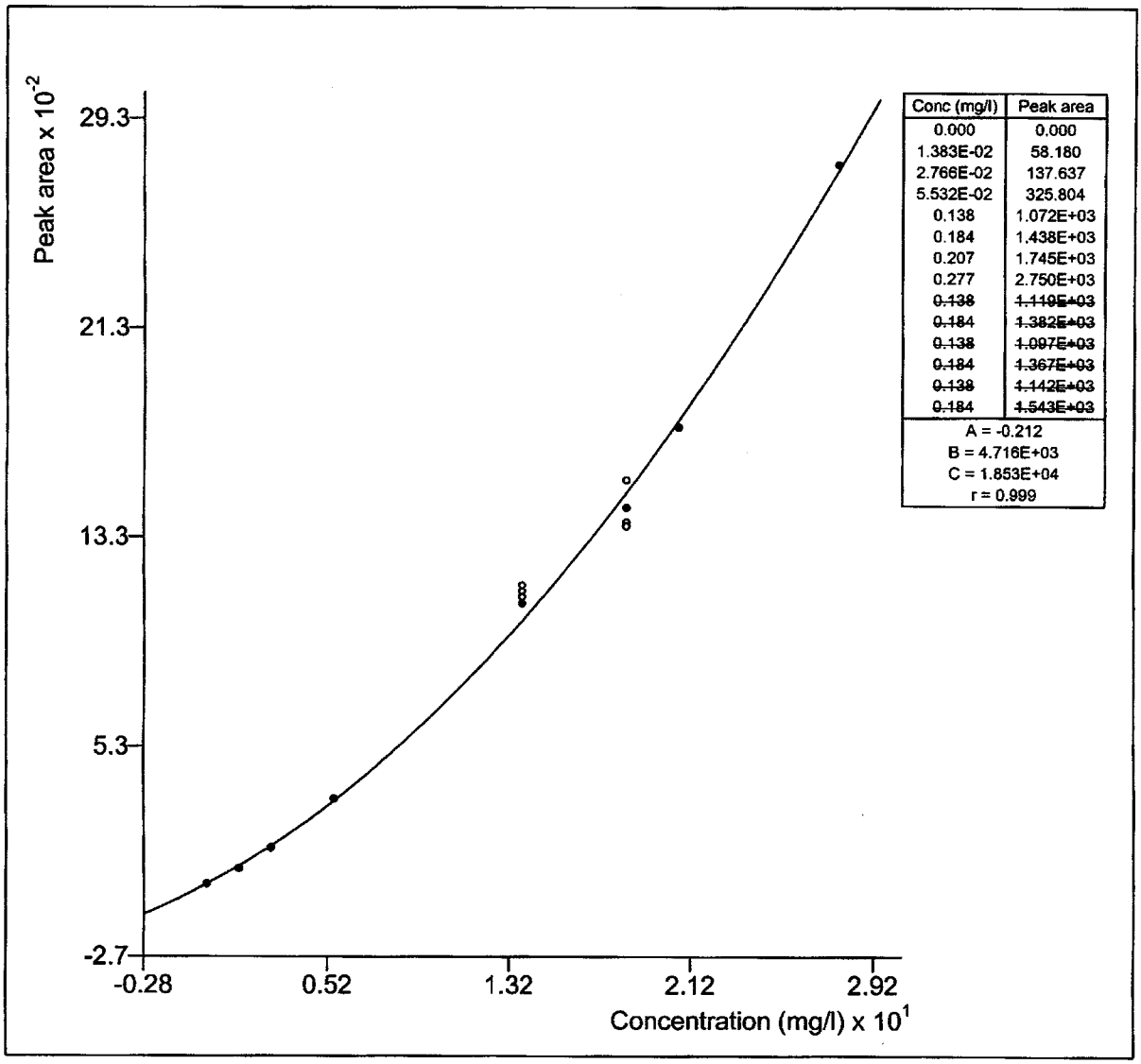
The mean peak areas relating to the standard and sample solutions are shown in the following table:

Table 5.2

Solution	Mean Peak Area
Standard blank	none detected
Standard 1.38 x 10^{-2} mg/l	51.180
Standard 2.77 x 10^{-2} mg/l	137.637
Standard 5.53 x 10^{-2} mg/l	325.804
Standard 0.138 mg/l	1.072 x 10^{3}
Standard 0.184 mg/l	1.438 x 10^{3}
Standard 0.207 mg/l	1.745 x 10^{3}
Standard 0.277 mg/l	2.750 x 10^{3}
Sample blank	none detected
Duck gizzard contents (no dilution)	979.170
Duck gizzard tissue (no dilution)	1.046 x 10^{3}
Duck liver	5.448
Duck skin and fat	11.609
Coot gizzard contents (x100 dilution, A)	620.178
Coot gizzard contents (x100 dilution, B)	573.939
Coot gizzard tissue (x10 dilution, A)	313.214
Coot gizzard tissue (x10 dilution, B)	348.273

The calibration curve from the sample analysis is shown in Figure 5.1.

Figure 5.1



Standards 0.138 mg/l and 0.184 mg/l were used throughout the analysis as check standards only.

The white phosphorus concentration determined in the duck gizzard contents extract and the coot gizzard contents extract and the resulting calculated white phosphorus content (mg and µg) present is shown in the following table:

Table 5.3

Sample	Concentration of White Phosphorus (mg/l)	Total White Phosphorus Content Present (mg)	Total White Phosphorus Content Present (µg)
Duck Gizzard Contents	0.136	2.72×10^{-3}	2.72
Coot Gizzard Contents	9.28	0.186	186

The white phosphorus concentration determined in each of the analysed tissue extracts and the resulting calculated white phosphorus residue (mg/kg) in the original tissue samples is shown in the following table:

Table 5.4

Tissue Sample	Analysed Concentration of White Phosphorus (mg/l) *	White Phosphorus Residue (mg/kg of tissue) *
Duck Gizzard Tissue	0.143	0.281
Duck Liver	<0.01	<0.02
Duck Skin and Fat	<0.01	<0.02
Coot Gizzard Tissue	0.573	1.13

5.5 Discussion

Trace levels of white phosphorus residue were found to be present in the duck skin and duck liver extracts. The limit of quantification based on 10 times signal/noise was calculated to be approximately 0.01 mg/l, equivalent to 0.02 mg/kg based on a nominal sample mass of 5 g.

For the duck and coot gizzard content and tissue extracts, the sample responses most appropriate with respect to the calibration standards were used for calculation of the definitive reported values.

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* See discussion, Section 5.5.

5.6 Conclusion

The quantity of white phosphorus present in the gizzard contents of the supplied duck and coot tissues has been determined. The results are shown in the following table:

Table 5.5

Sample	Total White Phosphorus Content Present (mg)	Total White Phosphorus Content Present (µg)
Duck Gizzard Contents	2.72×10^{-3}	2.72
Coot Gizzard Contents	0.186	186

The white phosphorus residue (mg/kg) in the supplied duck and coot tissue samples has also been determined. These results are shown in the following table:

Table 5.6

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)
Duck Gizzard	0.281
Duck Liver	<0.02
Duck Skin and Fat	<0.02
Coot Gizzard	1.13