REPORT

Rhodia Rattlechain lagoon duck samples: Determination of White Phosphorus Residues

Study Director: R E Butler

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Harlan Study Number: **41104675**

Study Completion Date:

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STUDY DIRECTOR AUTHENTICATION

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Harlan Study Number:	41104675
Study Title:	Rhodia Rattlechain lagoon duck samples: Determination of White Phosphorus Residues

This study was conducted in a facility operating to Good Laboratory Practice (GLP) within the national GLP monitoring programme, but the study report has not been audited by the Quality Assurance Unit. A formal claim of GLP compliance cannot therefore be made.

I the undersigned, hereby declare that this report accurately reflects the original data generated in the study.

Study Director: R E Butler

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Date: 22 MAR 2012

SUMMARY

The white phosphorus residues of Rhodia Rattlechain lagoon duck samples have been determined.

The amount of white phosphorus present (mg/kg) in the duck tissue samples and the limit of detection (mg/kg) for each sample is shown in the following table:

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)	Limit of Detection (LOD) (mg/kg of tissue)
Gizzard 1	5.09×10^{-2}	1.25×10^{-2}
Fat 1	1.51	2.39×10^{-2}
Kidney 1	<LOD	5.20×10^{-2}
Liver 1	<LOD	1.28×10^{-2}
Intestine 1	6.25	2.25×10^{-2}
Muscle 1	<LOD	1.28×10^{-2}
Gizzard 2	0.110	2.22×10^{-2}
Fat 2	0.831	4.25×10^{-2}
Kidney 2	<LOD	0.114
Liver 2	<LOD	2.23×10^{-2}
Intestine 2	<LOD	3.19×10^{-2}
Muscle 2	<LOD	2.22×10^{-2}

The amount of white phosphorus present (mg) in the duck gizzard contents and intestine contents and the limit of detection (mg) is shown in the following table:

Sample	Total White Phosphorus Content Present (mg)	Limit of Detection (LOD) (mg)
Gizzard 1 contents	0.530	1.29×10^{-4}
Intestine 1 contents	<LOD	1.29×10^{-4}
Gizzard 2 contents	9.80×10^{-2}	2.25×10^{-4}
Intestine 2 contents	<LOD	2.25×10^{-4}

GENERAL INFORMATION

Schedule

Experimental Starting Date:	10 January 2012
Experimental Completion Date:	26 January 2012

Archiving

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. Further retention or return of the data will be chargeable to the Sponsor.

1 INTRODUCTION AND PURPOSE

The purpose of this study is to determine the white phosphorus residues in Rhodia Rattlechain lagoon duck samples.

The procedure was be 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.

2 TEST ITEMS AND ANALYTICAL STANDARD SOLUTION

2.1 Test Items

The integrity of supplied data relating to the identity of the test items is the responsibility of the Sponsor.

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Gizzard 1
Description	:	Gizzard
Label	:	Gizzard 1
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Fat 1
Description	:	Fat
Label	:	Fat 1
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Kidney 1
Description	:	Kidney
Label	:	Kidney 1
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Liver 1
Description	:	Liver
Label	:	Liver 1
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Intestine 1
Description	:	Intestine
Label	:	Intestine 1
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Muscle 1
Description	:	Muscle
Label	:	Muscle 1
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Gizzard 2
Description	:	Gizzard
Label	:	Gizzard 2
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Fat 2
Description	:	Fat
Label	:	Fat 2
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark
Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Kidney 2
Description	:	Kidney
Label	:	Kidney 2
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark
Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Liver 2
Description	:	Liver
Label	:	Liver 2
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark
Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Intestine 2
Description	:	Intestine
Label	:	Intestine 2
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

Sponsor's identification	:	Rhodia Rattlechain lagoon duck samples Duck Muscle 2
Description	:	Muscle
Label	:	Muscle 2
Date received	:	10 January 2012
Expiry date	:	not available
Storage conditions	:	frozen at approximately minus 20°C, in the dark

2.2 Analytical Standard Solution

The integrity of supplied data relating to the identity, purity and stability of the phosphorus 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.

Sponsor's identification	:	Technical Yellow Phosphorus Certificate #538 Purity 99.83%
Phosphorus Standard Solution	:	704 mg/L in iso-octane
Suppliers reference	:	HFH100112
Harlan Laboratories Ltd. reference	:	ANCS/1012/001
Date received at Test Facility	:	10 January 2012
Expiry date	:	10 February 2012
Storage conditions	:	approximately minus 20 °C

3 DETERMINATION OF WHITE PHOSPHORUS RESIDUES

3.1 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.

3.2 Procedure

3.2.1 Standard Solution Preparation

A standard of white phosphorus was prepared (see section 2.1) as follows:

An aliquot (0.0704 g) of white phosphorus was diluted to a volume of 100 mL with iso-octane. The 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 using acetone and nitrogen 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.

The stock standard solution of white phosphorus was diluted using iso-octane to cover a nominal concentration range of approximately 0.005 to 0.25 mg/L.

3.2.2 Sample Preparation

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

Degassed glass distilled water was prepared by boiling glass distilled water vigorously and then purging with nitrogen as it cooled.

Gizzard Contents

The gizzard was dissected longitudinally between the crushing plates to expose the contents. The 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.

Intestine Contents

The contents of the intestine were quantitatively transferred to a conical flask using 60 ml of degassed glass distilled water.

Tissue Samples

Samples of gizzard, fat, kidney, liver, intestine and muscle were macerated and weighed into conical flasks for extraction (see following table).

Table 3.1

Tissue Sample	Mass of Tissue (g)
Gizzard 1	5.1670
Fat 1	2.7045
Kidney 1	1.2418
Liver 1	5.0314
Intestine 1	2.8672
Muscle 1	5.0623
Gizzard 2	5.0681
Fat 2	2.6537
Kidney 2	0.9860
Liver 2	5.0516
Intestine 2	3.5328
Muscle 2	5.0688

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 samples of gizzard and intestine 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, duplicate aliquots of the iso-octane extracts from the gizzard samples were diluted by factors of 100 (Gizzard 1) and 20 (Gizzard 2) and transferred to amber vials for analysis.

A sample blank was prepared by shaking a mixture of 30 mL of degassed glass distilled water with 10 mL of iso-octane as detailed for the samples.

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

3.2.3 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 0.005 to 0.25 mg/L.

Standard blank

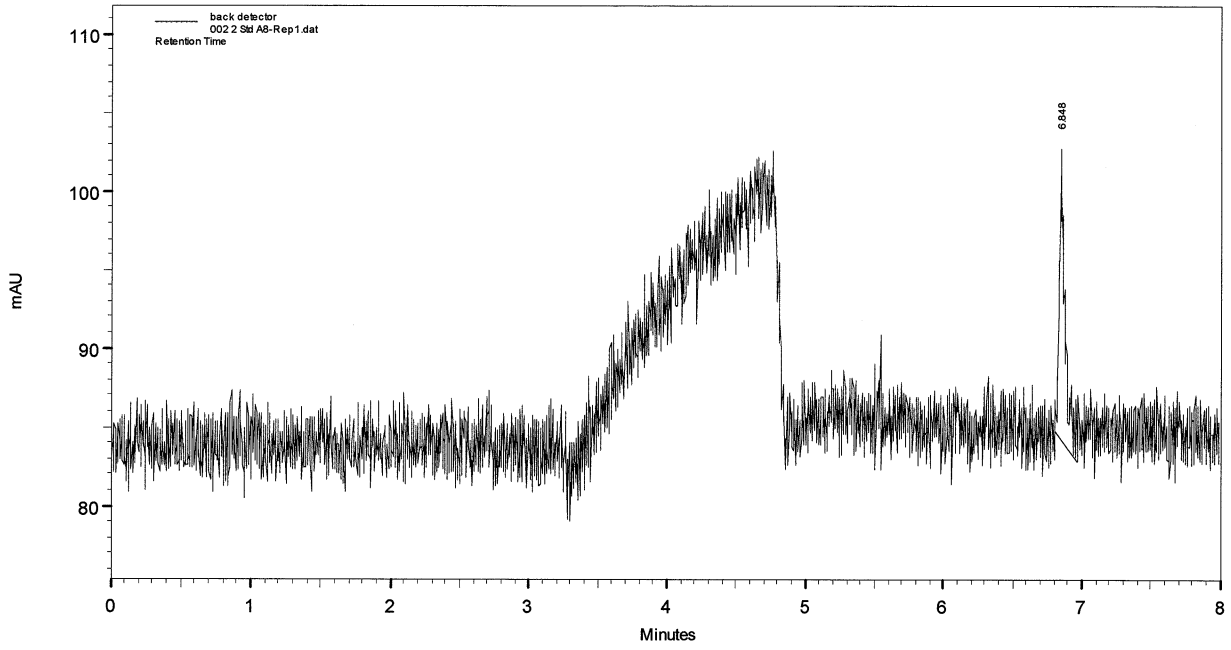
Iso-octane.

Analysis

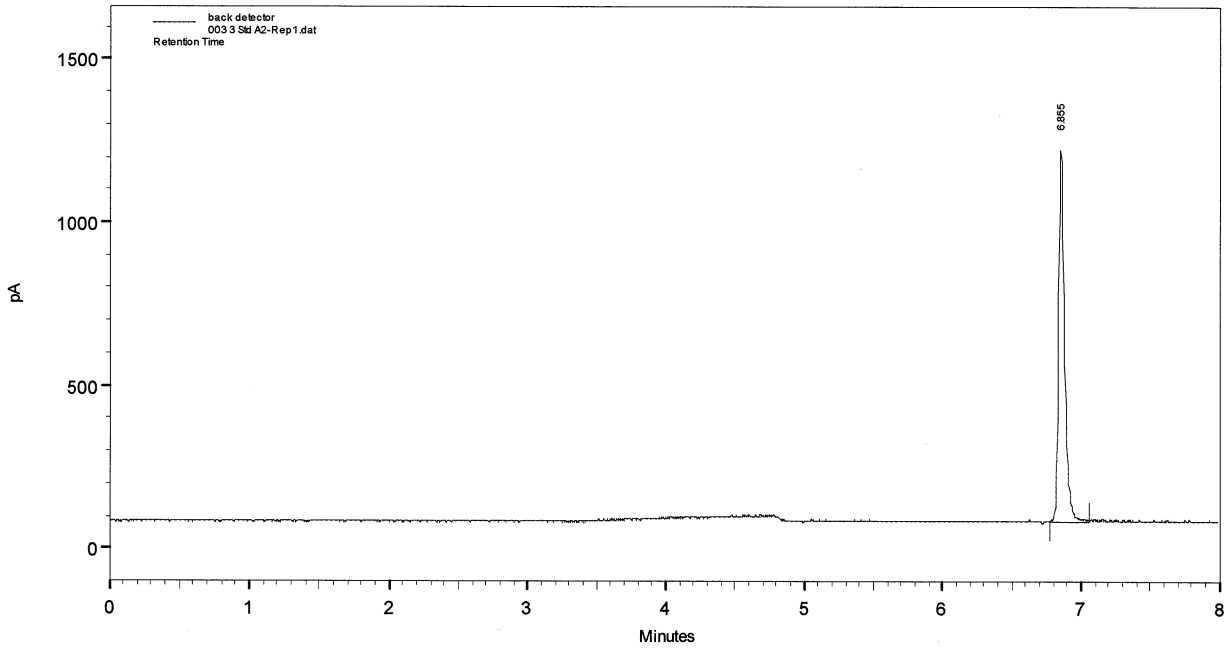
The standard and sample solutions were analysed by GC using the following conditions:

GC System	:	Agilent Technologies 6890, incorporating workstation
Column	:	TRB-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
Pressure	:	10.8 psi (constant pressure)
Retention time	:	~7 minutes

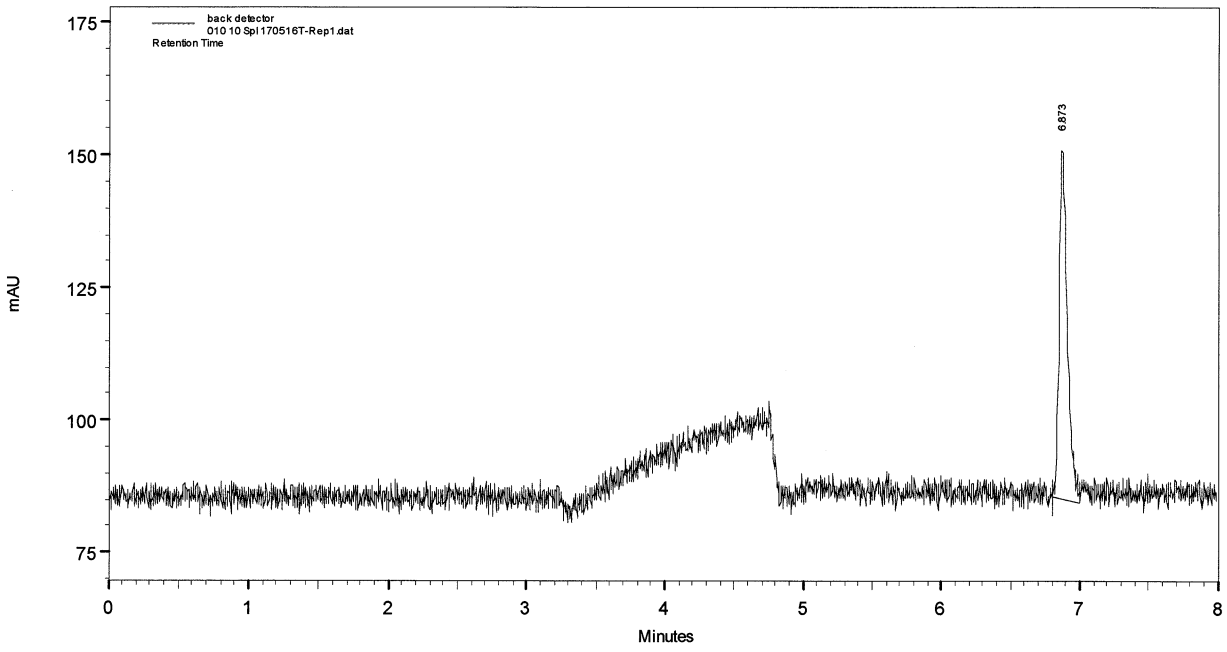
Typical Chromatography
Standard Solution 5.28 x 10^{-3} mg/L



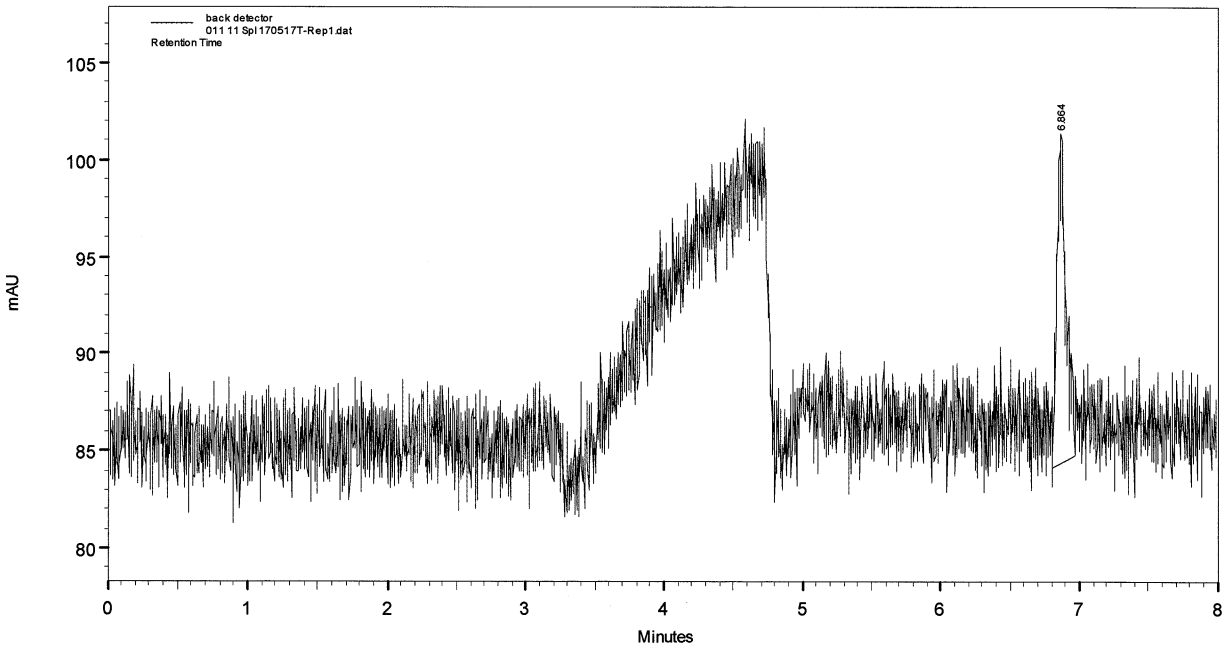
Standard Solution 0.264 mg/L



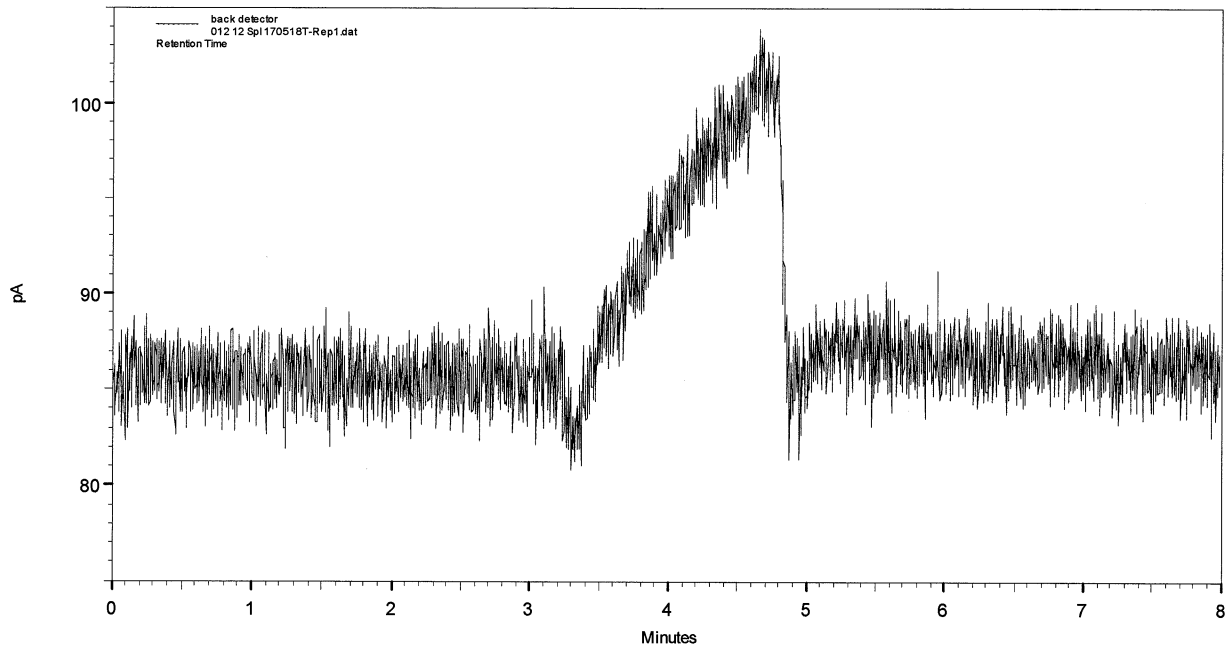
Typical Chromatography
Gizzard Tissue 1



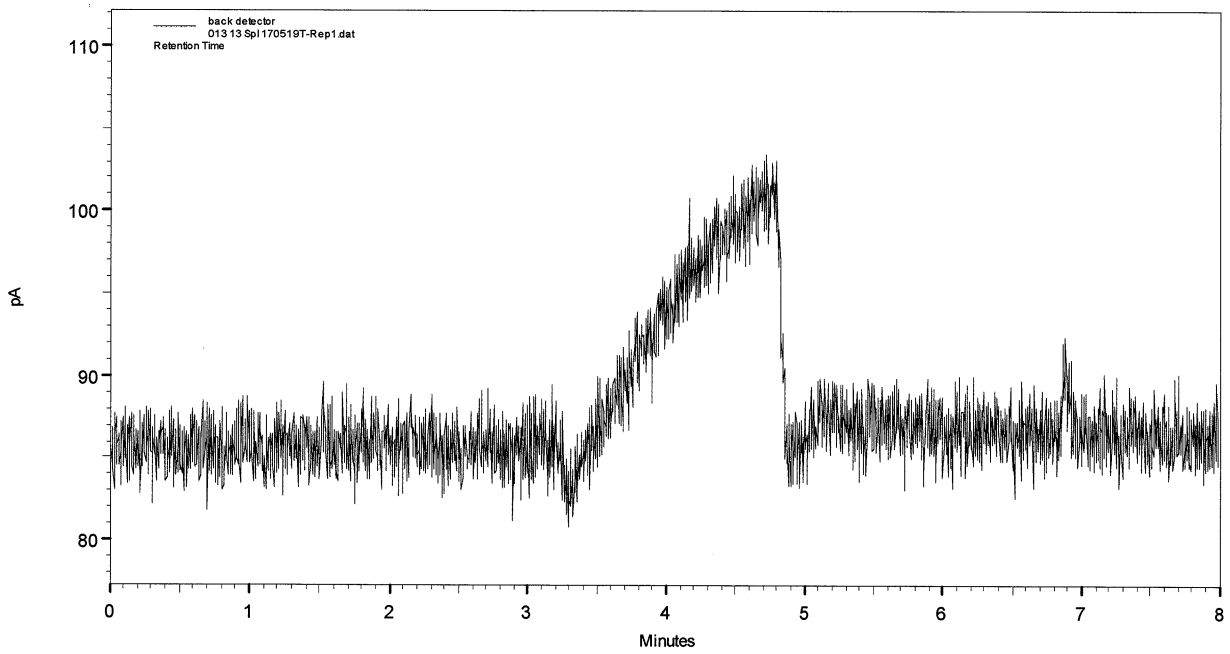
Fat 1



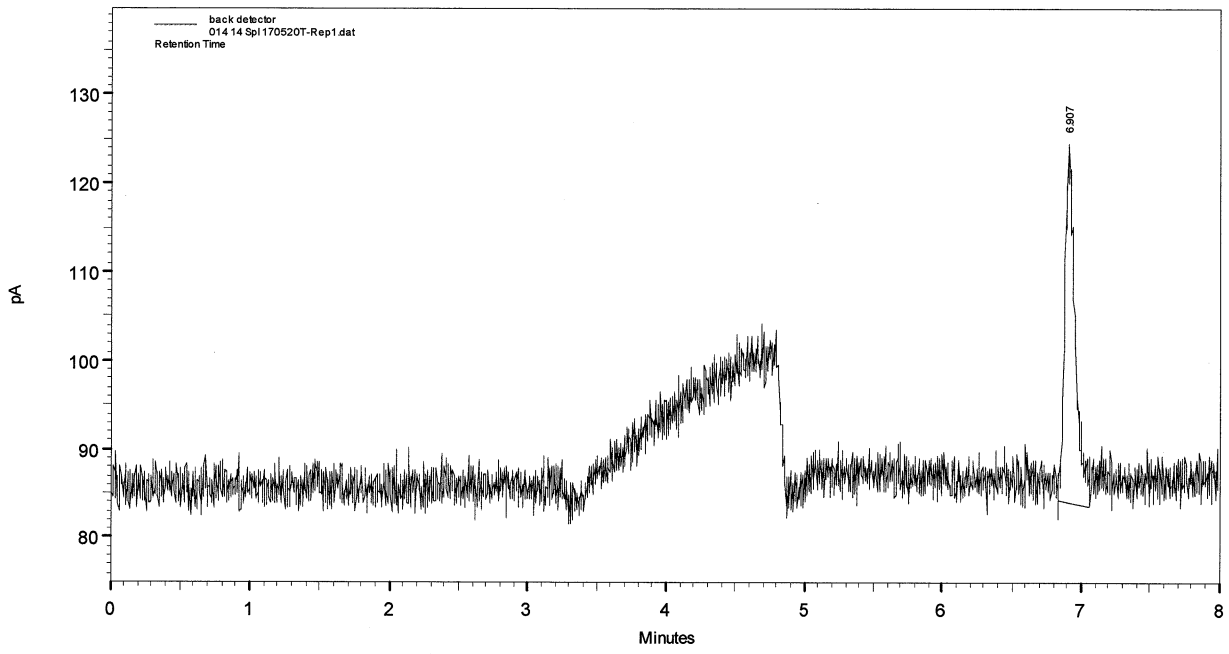
Typical Chromatography
Kidney 1



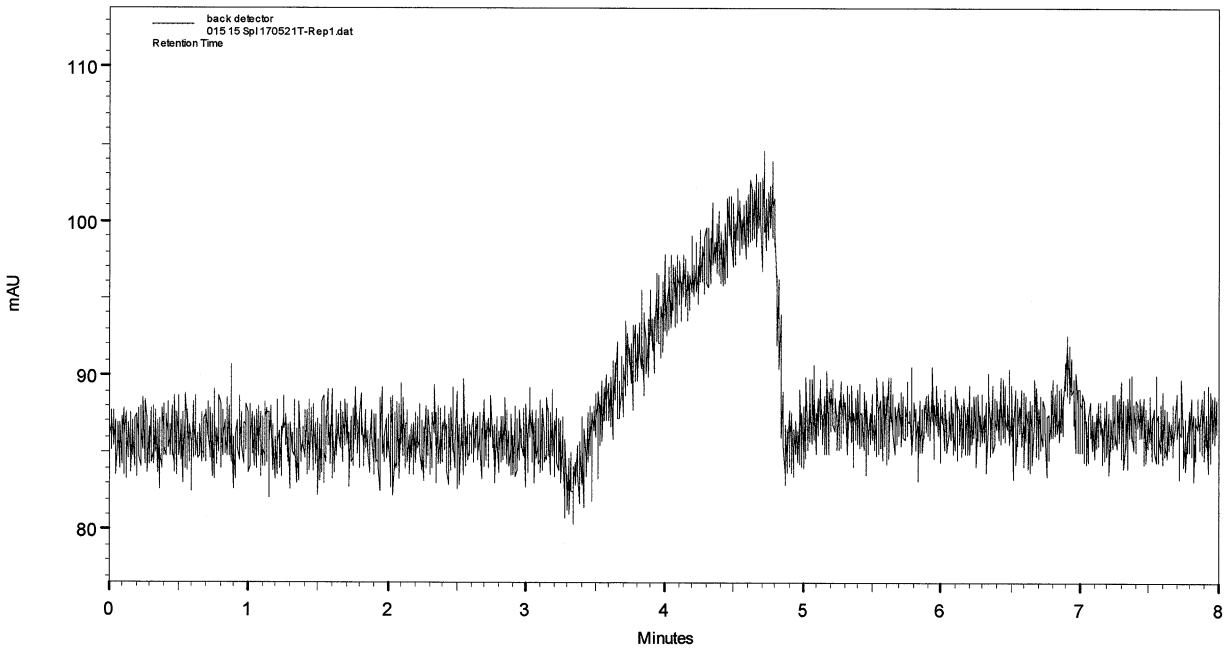
Liver 1



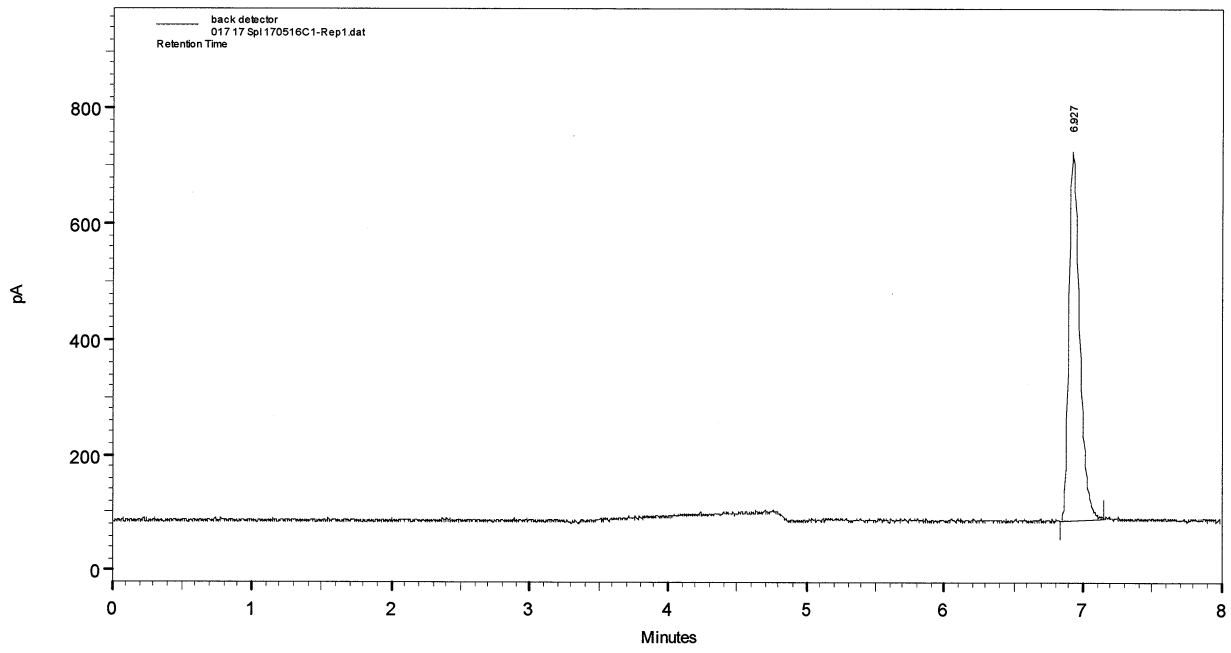
Typical Chromatography
Intestine Tissue 1



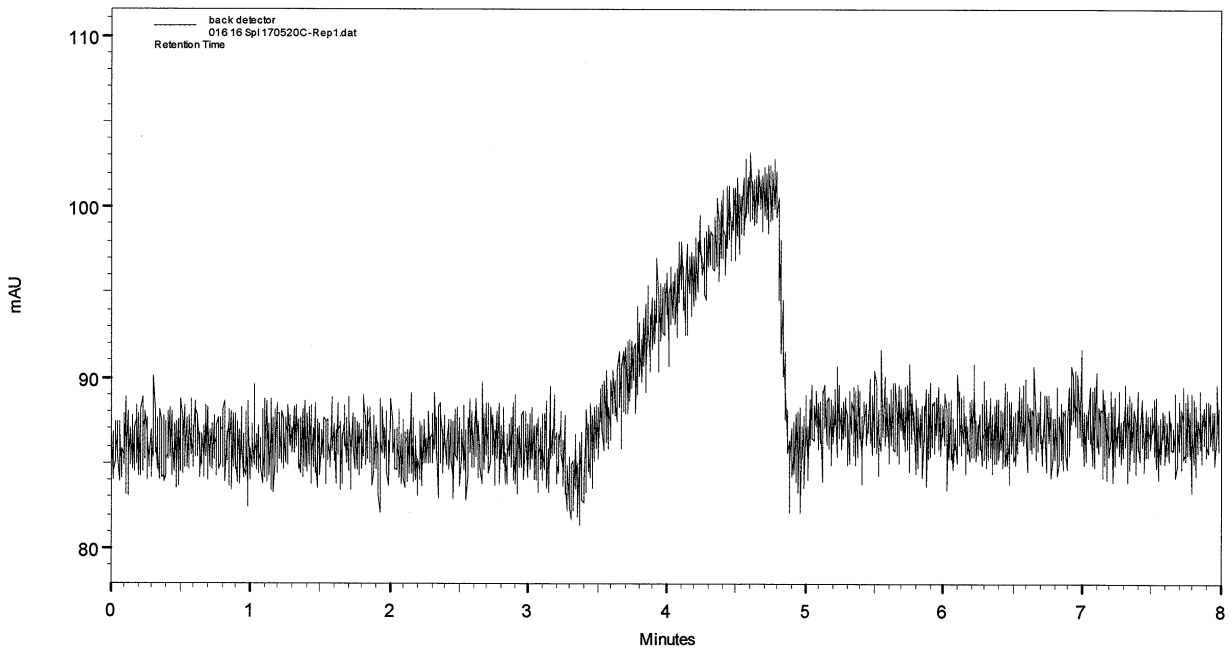
Muscle 1



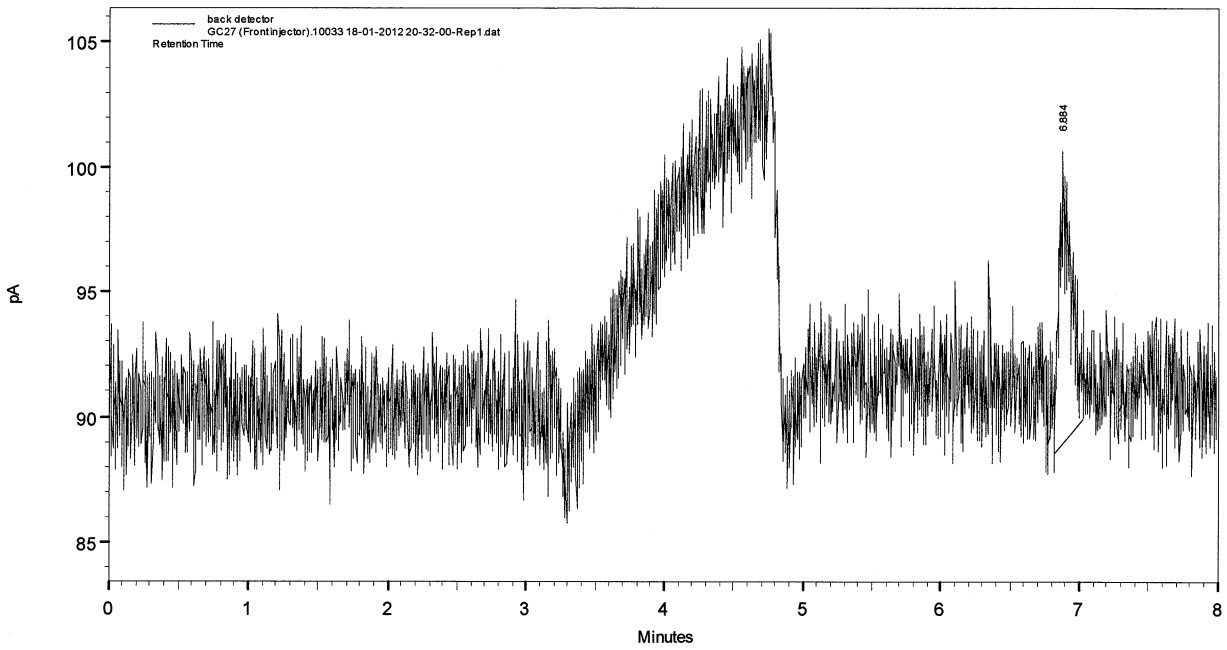
Typical Chromatography
Gizzard 1 Contents (x 100 dilution)



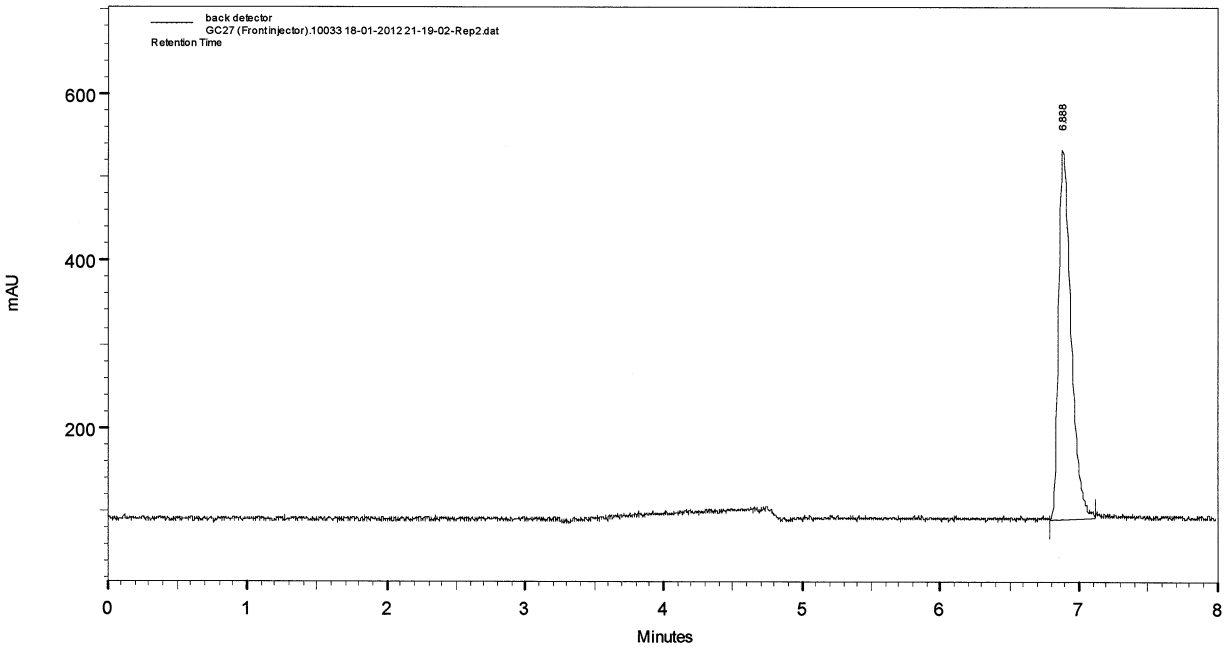
Intestine 1 Contents (no dilution)



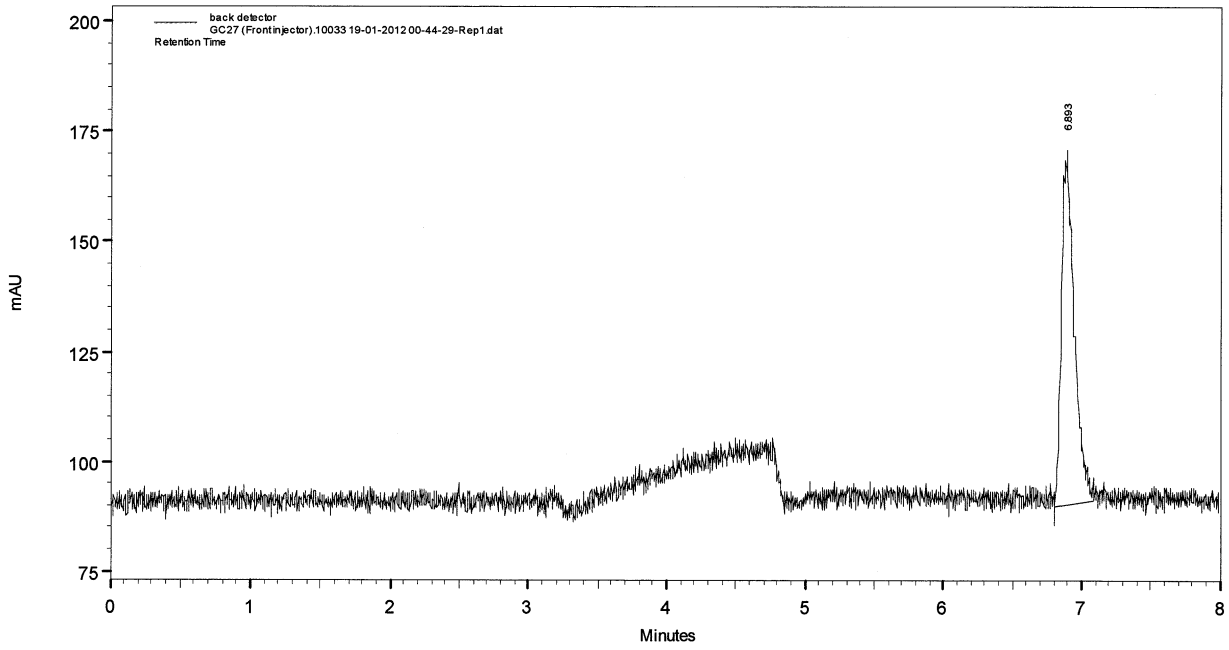
Typical Chromatography
Standard Solution 5.28 x 10^{-3} mg/L



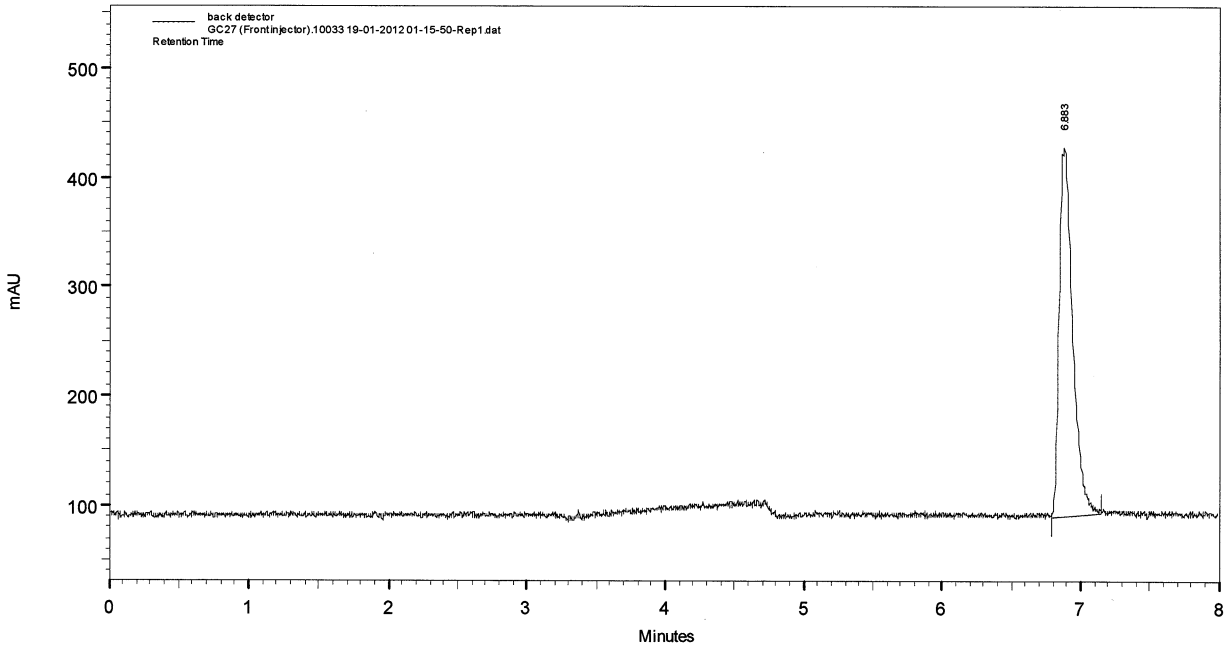
Standard Solution 0.264mg/L



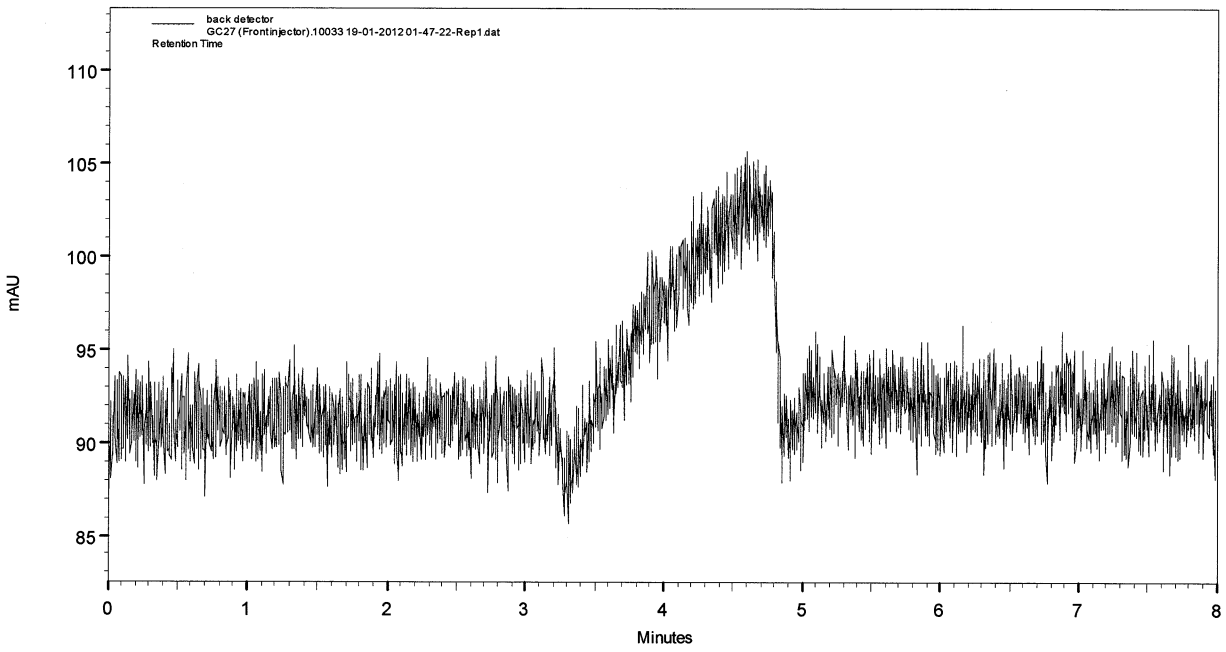
Typical Chromatography
Gizzard Tissue 2



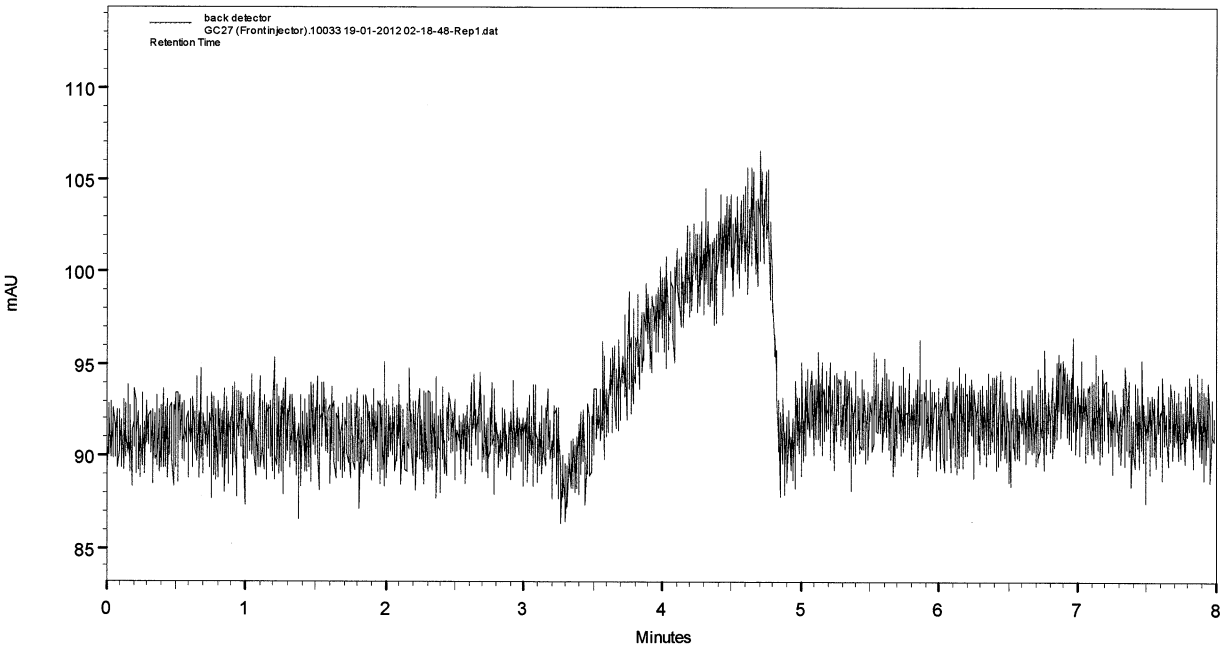
Fat 2



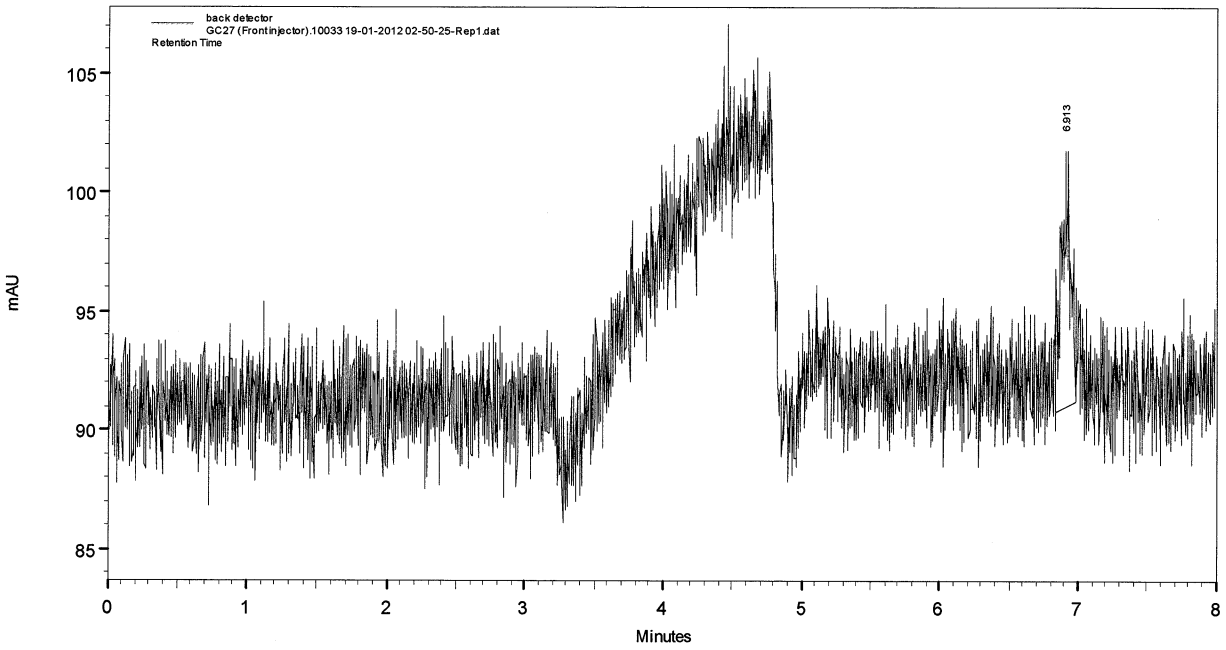
Typical Chromatography
Kidney 2



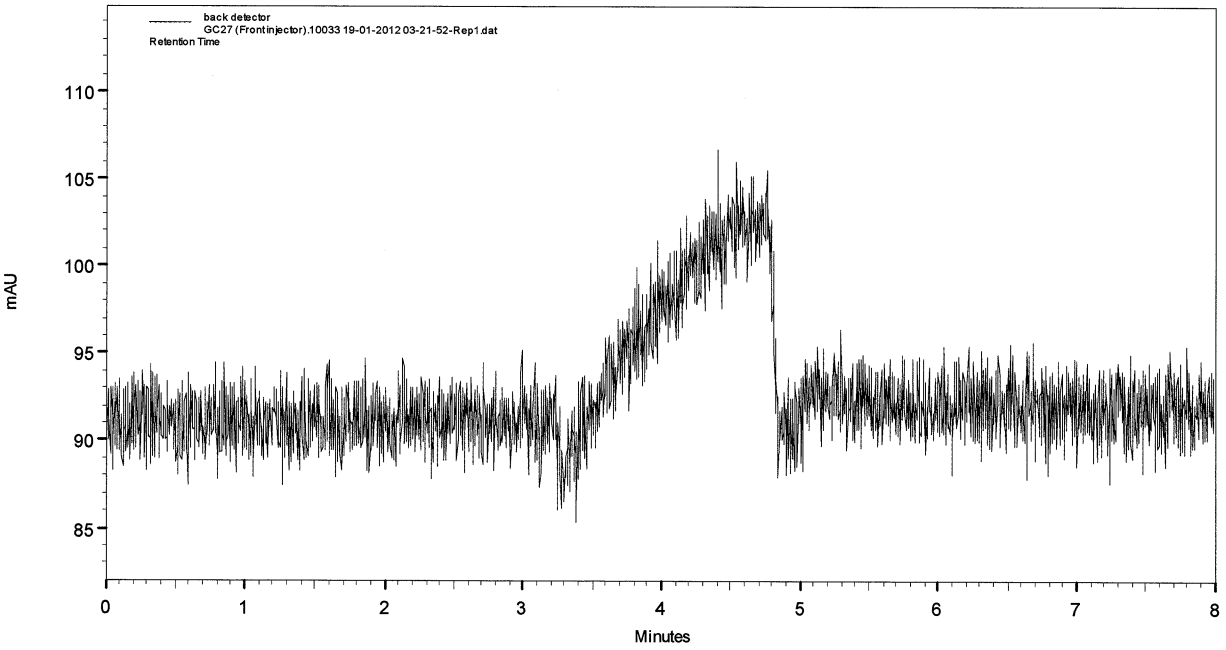
Liver 2



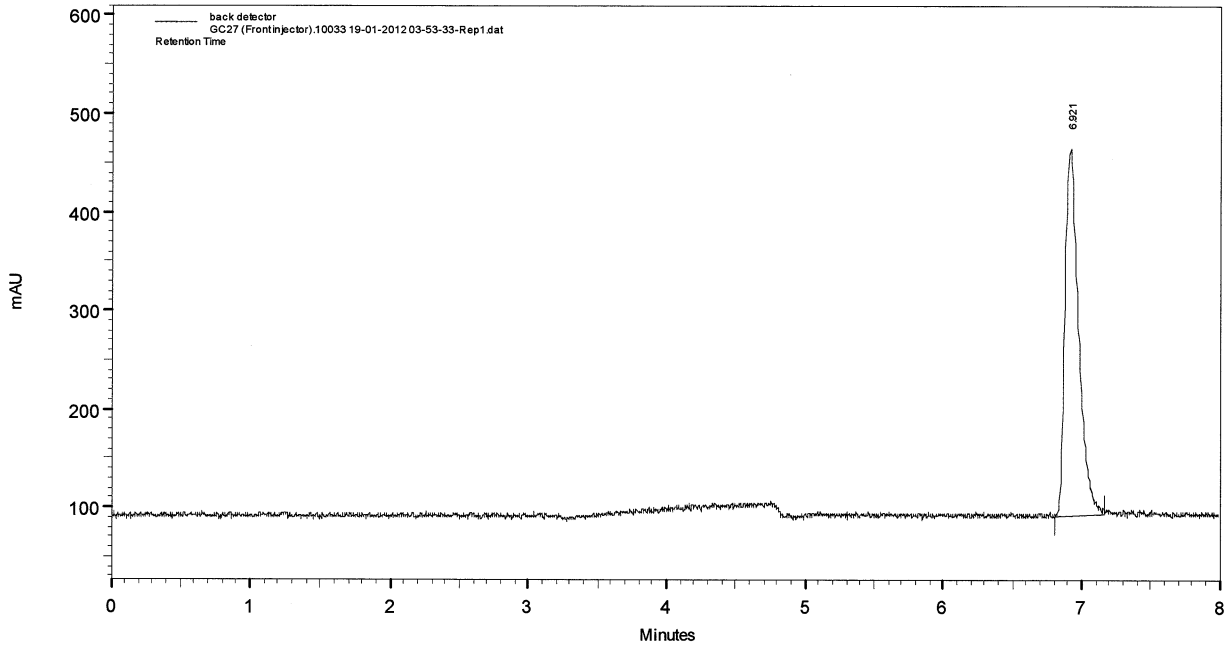
Typical Chromatography
Intestine Tissue 2



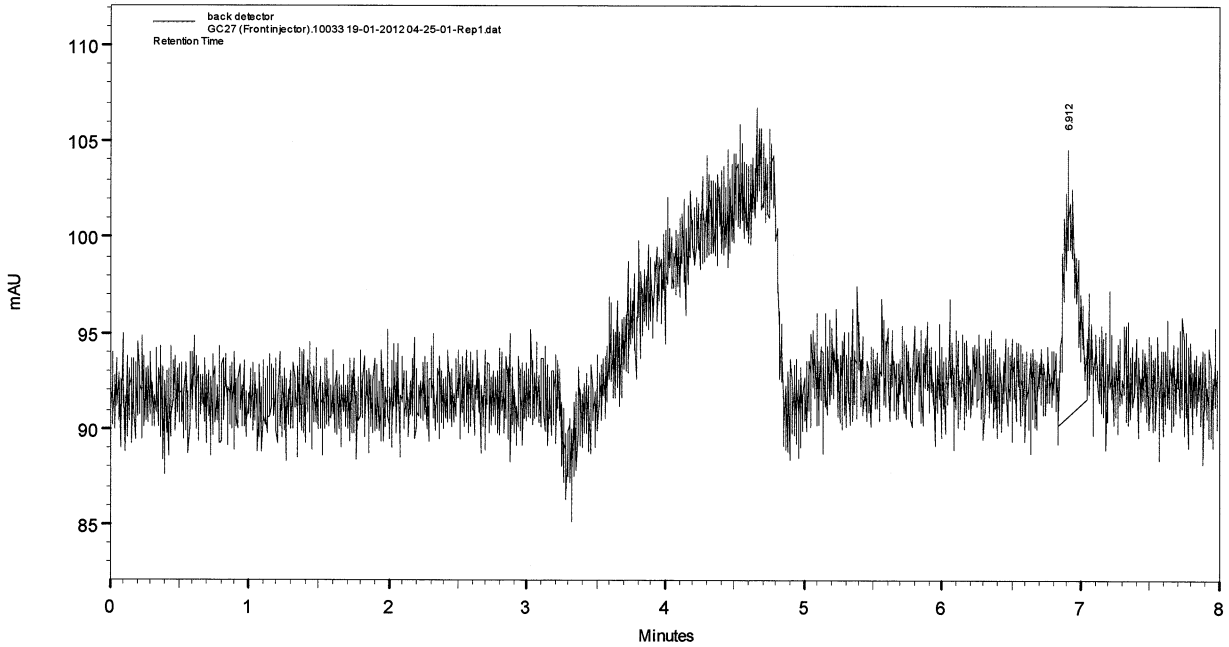
Muscle 2



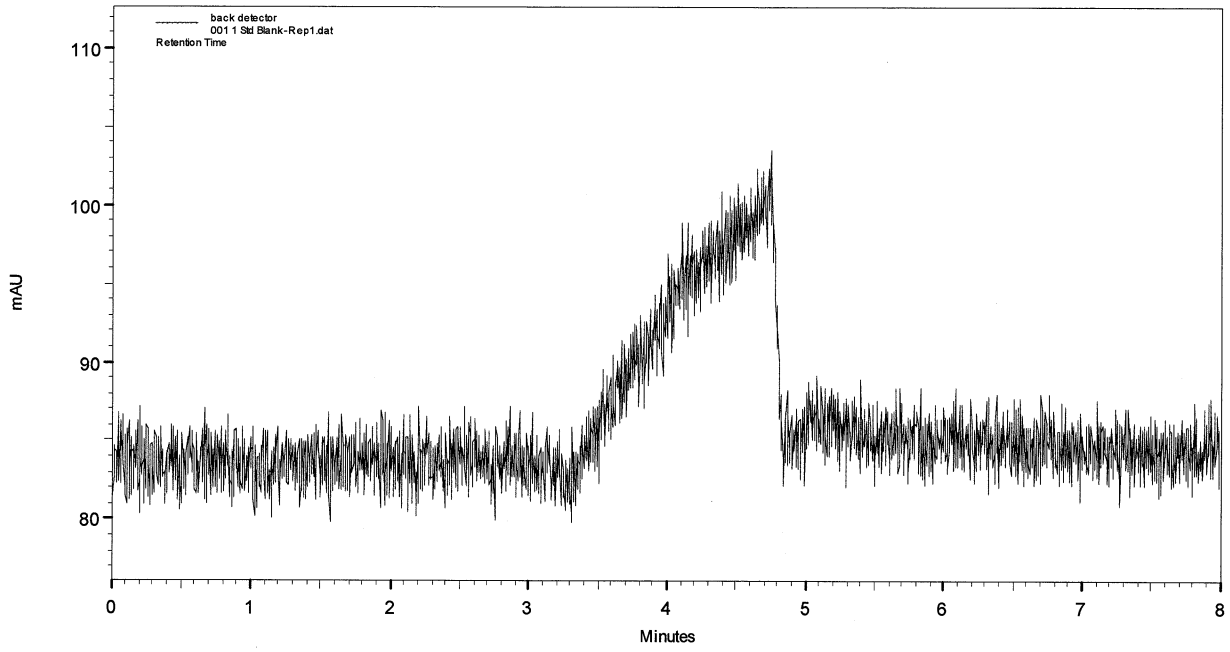
Typical Chromatography
Gizzard 2 Contents (x 20 dilution)



Intestine 2 Contents (no dilution)



Typical Chromatography
Solvent Blank



3.3 Calculations

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 and intestine contents was calculated using Equation 3.1.

Equation 3.1

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

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 (ml) of iso-octane used for extraction (20)
D = dilution factor (100 or 20 for gizzard contents)

The white phosphorus residue (mg/kg) in the tissue samples were calculated using Equation 3.2.

Equation 3.2

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

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 (mL) of iso-octane used for extraction (10)
M_{tis} = mass of tissue taken for extraction (g)

3.4 Results

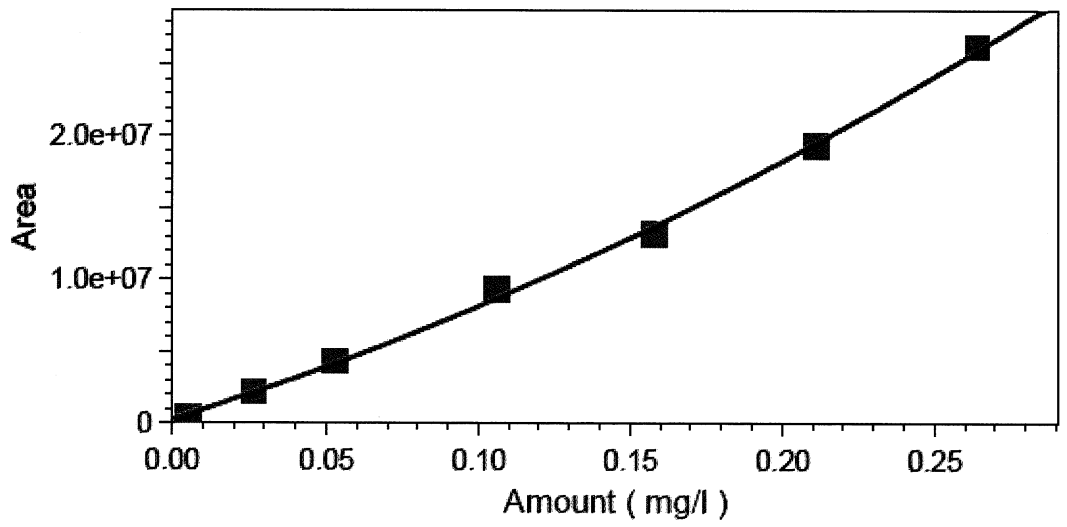
The mean peak areas relating to the standard and Duck 1 sample solutions are shown in the following table:

Table 3.2

Solution	Mean Peak Area
Standard blank	none detected
Standard 5.28 x 10^{-3} mg/L	4.2030 x 10^5
Standard 2.64 x10^{-2} mg/L	2.1610 x 10^6
Standard 5.23 x 10^{-2} mg/L	4.3232 x 10^6
Standard 0.106 mg/L	9.3164 x 10^6
Standard 0.158 mg/L	1.3220 x 10^7
Standard 0.211 mg/L	1.9323 x 10^7
Standard 0.264 mg/L	2.6195 x 10^7
Gizzard 1 tissue	2.1485 x 10^6
Fat 1	5.7340 x 10^5
Kidney 1	none detected
Liver 1	no measurable peak
Intestine 1 tissue	1.5426 x 10^6
Muscle 1	no measurable peak
Gizzard 1 contents	2.6152 x 10^7
Intestine 1 contents	no measurable peak

The calibration curve for the analysis from Table 3.2 is shown in Figure 3.1.

Figure 3.1



y = 1.12950e+008x^2 + 6.74000e+007x + 295651.
Goodness of fit (r^2): 0.998435

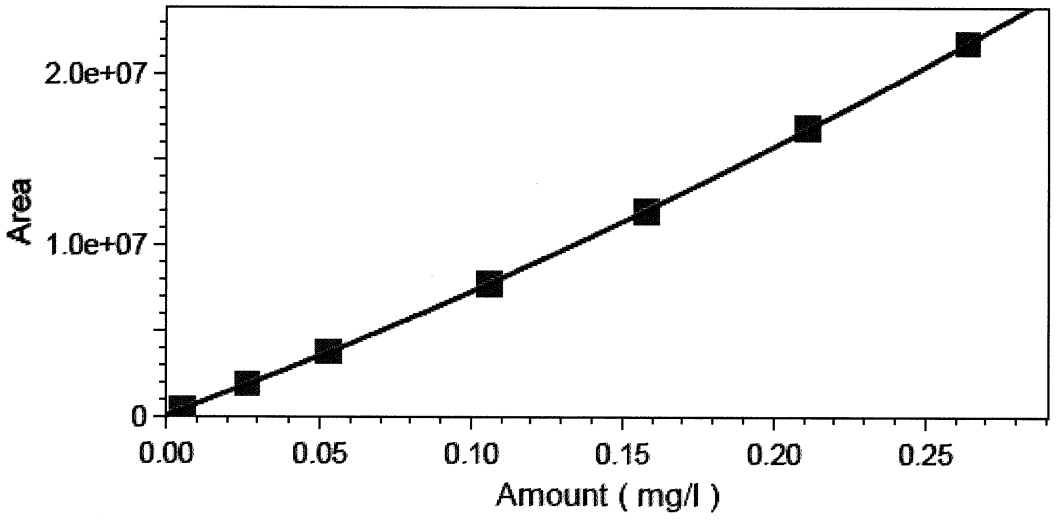
The mean peak areas relating to the standard and Duck 2 sample solutions are shown in the following table:

Table 3.3

Solution	Mean Peak Area
Standard blank	none detected
Standard 5.28 x 10^{-3} mg/L	4.5774 x 10^5
Standard 2.64 x10^{-2} mg/L	1.9346 x 10^6
Standard 5.23 x 10^{-2} mg/L	3.8142 x 10^6
Standard 0.106 mg/L	7.7650 x 10^6
Standard 0.158 mg/L	1.1976 x 10^7
Standard 0.211 mg/L	1.6871 x 10^7
Standard 0.264 mg/L	2.1746 x 10^7
Gizzard 2 tissue	3.9902 x 10^6
Fat 2	1.7668 x 10^7
Kidney 2	none detected
Liver 2	none detected
Intestine 2 tissue	3.9472 x 10^5
Muscle 2	none detected
Gizzard 2 contents	1.9994 x 10^7
Intestine 2 contents	5.8101 x 10^5

The calibration curve for the analysis from Table 3.3 is shown in Figure 3.2.

Figure 3.2



y = 6.18177e+007x^2 + 6.57107e+007x + 134036.
Goodness of fit (r^2): 0.999930

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 3.4

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)*
Gizzard 1 tissue	5.09×10^{-2}
Fat 1	1.51
Kidney 1	<LOD
Liver 1	<LOD
Intestine 1 tissue	6.25
Muscle 1	<LOD
Gizzard 2 tissue	0.110
Fat 2	0.831
Kidney 2	<LOD
Liver 2	<LOD
Intestine 2 tissue	<LOD
Muscle 2	<LOD

The white phosphorus concentration determined in the extracts of the gizzard contents and intestine contents and the resulting calculated total white phosphorus content (mg) is shown in the following table:

Table 3.5

Sample	Total White Phosphorus Content Present (mg)*
Gizzard 1 contents	0.530
Intestine 1 contents	<LOD
Gizzard 2 contents	9.80×10^{-2}
Intestine 2 contents	<LOD

* LOD: See Discussion Section 3.5.

3.5 Discussion

The limit of detection (LOD) for each analysis was based on three times baseline noise and calculated using the lowest calibration standard. This was determined to be 6.46×10^{-3} mg/L for the analysis of Duck 1 samples and 1.13×10^{-2} mg/L for the analysis of Duck 2 samples. The difference in LOD of the analyses was due to reduced sensitivity of the instrumentation for the second analysis. The LOD for each tissue sample has also been calculated in terms of mg/kg based on the mass of sample weighed.

3.6 Conclusion

The amount of white phosphorus present (mg/kg) in the duck tissue samples and the limit of detection (mg/kg) for each sample is shown in the following table:

Table 3.6

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)	Limit of Detection (LOD) (mg/kg of tissue)
Gizzard 1	5.09×10^{-2}	1.25×10^{-2}
Fat 1	1.51	2.39×10^{-2}
Kidney 1	<LOD	5.20×10^{-2}
Liver 1	<LOD	1.28×10^{-2}
Intestine 1	6.25	2.25×10^{-2}
Muscle 1	<LOD	1.28×10^{-2}
Gizzard 2	0.110	2.22×10^{-2}
Fat 2	0.831	4.25×10^{-2}
Kidney 2	<LOD	0.114
Liver 2	<LOD	2.23×10^{-2}
Intestine 2	<LOD	3.19×10^{-2}
Muscle 2	<LOD	2.22×10^{-2}

The amount of white phosphorus present (mg) in the duck gizzard contents and intestine contents and the limit of detection (mg) is shown in the following table:

Table 3.7

Sample	Total White Phosphorus Content Present (mg)	Limit of Detection (LOD) (mg)
Gizzard 1 contents	0.530	1.29×10^{-4}
Intestine 1 contents	<LOD	1.29×10^{-4}
Gizzard 2 contents	9.80×10^{-2}	2.25×10^{-4}
Intestine 2 contents	<LOD	2.25×10^{-4}