Cape Peninsula
University of Technology

AN APPROACH TO THE IMPROVEMENT OF THE SELENIUM ANALYSIS PROCESS OF THE WESTERN CAPE PROVINCIAL VETERINARY LABORATORY

VOLUME II

A Research Dissertation submitted

by

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MAGISTER TECHNOLOGIAE: QUALITY

in the

Faculty of Industrial and Systems Engineering

CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

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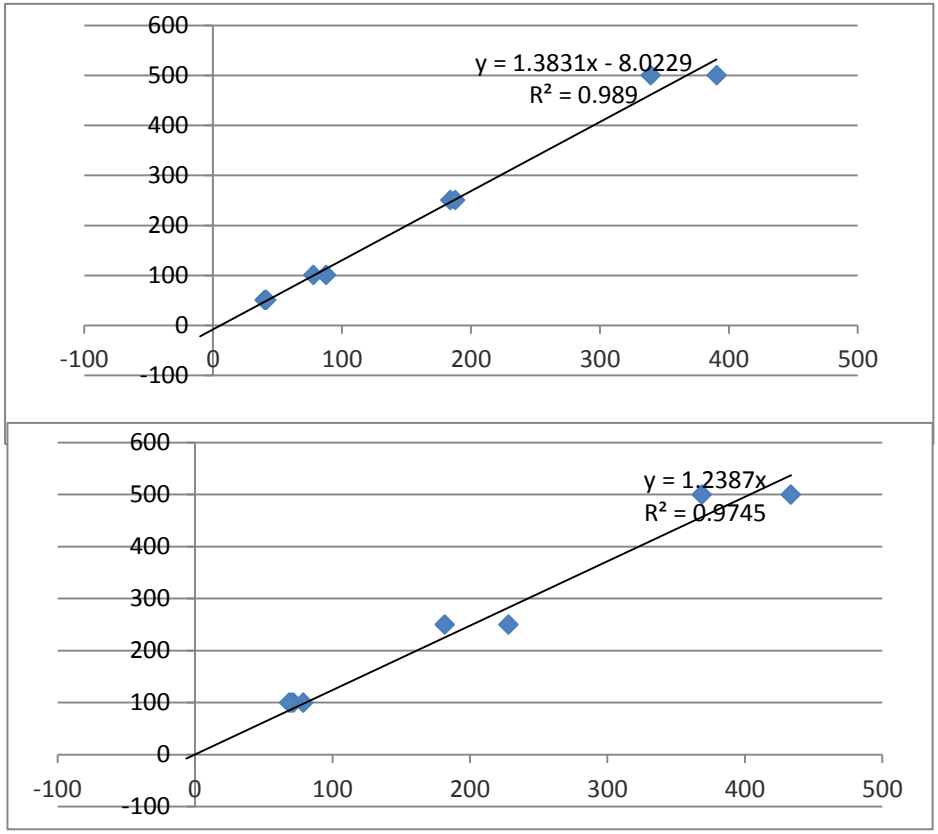
ANNEXURE 1: Council for Scientific and Industrial Research (CSIR) Laboratory Results. May 2010

Id	Se in ppb	% Recovery	Prep Conc
* blk a	<30		
* blk b	<30		
* 1ppb a	<30		
* 1ppb b	<30		
* 10ppb a	<30		
* 10ppb b	<30		
* 50ppb a	40	79.8	50
* 50ppb b	41	82.3	50
* 100ppb	88	87.5	100
* 100ppb b	78	77.7	100
* 250ppb a	184	73.6	250
* 250ppb b	188	75.0	250
* 500ppb a	390	78.1	500
* 500ppb b	339	67.9	500
Average		77.7	
blk a	<30		
blk b	<30		
1ppb a	<30		
1ppb b	<30		
10ppb a	<30		
10ppb b	<30		
100ppb a	72	71.7	100
100ppb b	79	78.8	100
100ppb a	70	70.0	100
100ppb b	69	68.6	100
250ppb a	228	91.3	250
250ppb b	182	72.6	250
500ppb a	369	73.8	500
500ppb b	433	86.7	500
Average		76.7	
LC1	1241		
LC2	1286		
LC3	1296		
LC4	1374		



Annexure 2: Research Standard Operating Procedure for Selenium Analysis Process

Cape Peninsula University of Technology	**Faculty of Engineering: Department Quality**
	Fluorometric method for Selenium analysis

1. Introduction

This method is used for the analysis of Selenium in biological liver samples, which have been overnight digested on an open heat block and thereafter prepared for fluorometric analysis. The basis for this method is the reaction of 2,3 diaminonapthalene (DAN) with Se(IV) to form a fluorescent Se-DAN complex. A filter fluorimeter is used to measure the fluorescence of this complex in order to calculate the concentration of Selenium in the given sample.

2. Personnel

The person performing the procedure should be fully trained to be able to conduct this analytical laboratory process.

3. Limitation and precision of method

3.1 The method is applied to biological liver samples.
3.2 Selenium concentration range that can be tested is 1 – 500 ppb.

4. Equipment and Materials

4.1 Digestion tubes (20ml x 150mm. Borosilicate glass or equivalent)
4.2 Digestion block (custom made), 135 bored holes with depth of ± 70mm.
4.3 Programmable temperature controller connected to digestion block
4.4 Separating funnels (250, 500 and 1000ml)
4.5 Volumetric flasks (A grade - approved for use)
4.6 100 – 1000µl Eppendorf pipette (BIO-040)
4.7 100µl Eppendorf pipette (BIO-058)
4.8 1000µl Eppendorf pipette (BIO-061)
4.9 100 and 1000µl new Eppendorf pipette tips
4.10 Glass beakers (250ml for general use)
4.11 Plastic Beaker or container for storage of tube lids
4.12 Spatula
4.13 Plastic weighing vessels and beakers for reagent preparation
4.14 Glass graduated and bulb pipettes for reagent preparation (approved for use)
4.15 1000ml volumetric flask (A grade, approved for use)
4.16 2000ml Measuring cylinder (approved for use)
4.17 Water-bath (BIO-030)
4.18 Thermometer (BIO-104)
4.19 Thermometer (BIO-091)
4.20 Multipurpose shaker (BIO-015)
4.21 Analytical balance (4 decimal place accuracy) (BIO-003 or BIO-004)
4.22 Repeat pipette (1-5ml) (BIO-080)
4.23 Pipette tips for repeat pipette (1-5ml)
4.24 Spectrofluorimeter, Perkin Elmer LS55

5 Reagents (All analytical reagent grade quality)

5.1 Acid Mixture: 1 (Perchloric Acid 60%): 5 (Nitric Acid 65%). Carefully pour 1600ml of Nitric Acid into a 2000ml measuring cylinder, fill up to the 2000ml mark with Perchloric Acid. Pour Acid mixture into brown glass storage bottle. Mixture is stable indefinitely.

5.2 1:1 Dilution of HCl: Measure equal volumes of Hydrochloric Acid (32%) and deionised water. Pour deionised water into storage vessel followed by Hydrochloric acid. Solution is stable for 12 months.

5.3 EDTA solution: Weigh out 0.81g NaH_2EDTA into a glass beaker. Transfer into 1000ml volumetric flask. Make up to the mark with deionised water. Solution is stable for 2 months.

5.4 0.1M HCl: Pour approximately 200ml deionised water into a 1000ml volumetric flask. Pipette 9.8ml Hydrochloric Acid (32%) into flask. Make up to the mark with deionised water.

5.5 1M HNO_3: Pour approximately 200ml deionised water into a 1000ml volumetric flask. Pipette 68.7ml Nitric Acid (65%) into the flask and make up to the mark with deionised water.

5.6 Cyclohexane

5.7 2,3 Diaminonapthalene (DAN) solution: (to be made up just before use): See point 7.5.2 for preparation details

NOTE: DAN is light sensitive and the activity of the reagent will decrease in the presence of light. Handle the reagent as far as practically possible in a dark room.

5.8 Standards:

5.8.1 Selenium stock standard: 1000ppm bought from Merck

5.8.2 Selenium standard solution: 10ppm – pipette 1ml of the 1000ppm stock standard into a 100ml volumetric flask. Make up to volume with 1M HNO_3 (stable for 6 months)

5.8.3 Working standards: dilute the 10ppm standard as follows with 1M HNO3. (stable for 6 months):

Blank: pour 1M HNO_3 into a 100ml volumetric flask up to mark.

100µl standard solution (5.8.2) in 100ml volumetric flask = 10ppb (ng/ml)

500µl standard solution (5.8.2) in 100ml volumetric flask = 50ppb (ng/ml)

1000µl standard solution (5.8.2) in 100ml volumetric flask = 100ppb (ng/ml)

2500µl standard solution (5.8.2) in 100ml volumetric flask = 250ppb (ng/ml)

5000µl standard solution (5.8.2) in 100ml volumetric flask = 500ppb (ng/ml)

6 Safety/ precautionary measures

6.1 A laboratory coat must be worn during the testing procedure.

6.2 Gloves should be worn when handling DAN solution, cyclohexane and acids.

6.3 Observe the safety instructing for the safe handling and disposal of chemicals as specified in Material Safety Data Sheets for chemicals used in Biochemistry.

7 Procedure

7.1 Weigh out ±0.1000g to one decimal point, of dried liver. Include an in-house liver control sample, LC2009 with each sample batch. Three blank tubes consisting of 1ml of 0.1M HNO_3 and three tubes for each standard containing 1ml of standard are also prepared with the samples.

7.2 Add 4ml of acid mixture. Place in digestion block in perchloric acid fume cupboard (BIO-035). Switch temperature controller ON to 'PTN1' programme.
(Please refer to P – BIO – E – 016 for details programming the temperature controller). The set programme should be as follows:

Step 1: Heat to 120°C (± 10°C)

Step 2: 1 hour @ 120°C (± 10°C)

Step 3: Heat to 160°C (± 10°C)

Step 4: 6 hours @ 160°C (± 10°C)

Step 5: Maintain @ 120°C (± 10°C) for 30 hours. (This programme is stored)

Digest overnight.

The block should be @ 120°C (± 10°C) the next morning. Record the temperature on form P – BIO – F – 072 with thermometer BIO-091.

7.3 Set the water bath at 60°C (± 5°C) and switch ON.

7.4 Remove the tubes from the digestion block and place into a rack inside the fume cabinet. Allow the tubes to cool for a few minutes. Add 1ml 1:1 HCl to the tubes. Place the tubes into the digestion block again and leave for 30 minutes at 120°C (± 10°C).

7.5 Remove tubes from digestion block after the 30 minutes is over and allow them to cool for a few minutes before adding the following reagents:

7.5.1 10 ml EDTA solution (Add this reagent in the general use fume cabinet (BIO-033)

7.5.2 1ml DAN solution

NOTE: The tubes can stand in the fume cabinet (BIO-033) covered with a paper towel until the DAN solution has been prepared. Once the DAN solution is ready for use, carry the tubes to the dark room (room 55) and add the DAN solution in the dark room. All procedures must take place in the dark room once the DAN has been added to the tubes!

2,3 Diaminonapthalene (DAN) solution preparation

- Every precaution should be taken that dark conditions be maintained throughout the preparation of DAN for analysis. This includes weighing out in a dark room, closing water bath with cover when DAN solution is in it, and purification of DAN steps performed in a dark room.
- Calculate the quantity of DAN required. 1ml of the DAN solution is used for each standard, blank and sample. 0.1000g DAN / 100ml 0.1M HCL.
- Weigh out the DAN in a darkened room into a plastic weighing vessel and transfer to a separating funnel. Ensure that the tap is closed tightly.
- Add the correct volume of 0.1M HCL to the separating funnel, i.e. 40ml 0.1M HCL with 0.0400g DAN.
- Carry the separating funnel to the water bath, which has heated to 60°C (± 5°C). Place into the water bath and secure by placing the string at the tap around the neck of the funnel, close the lid of the water bath and set the timer for 10 minutes. Record the temperature of the water bath on form P – BIO – F – 074.
- Remove the funnel from the water bath and carry the separating funnel to the dark room. Shake the content of the funnel manually until the DAN has dissolved completely. Be careful to hold the funnel lid firmly while shaking. Release the pressure build-up periodically. Place funnel into the stand.
- Pipette 40ml of cyclohexane into the funnel using the repeat pipette (BIO-080). Shake for 1 minute manually again. Place funnel into the stand and let the funnel stand in dark room for 60 minutes.
- Pour out the bottom DAN layer into the next separating funnel. Add 25ml cyclohexane and shake for 1 minute. Let funnel stand for 45 minutes. Pour the bottom layer into the next separating funnel. Add 25ml cyclohexane, shake for 1 minute and allow to stand for 45 minutes. The DAN is now ready for use.

7.5.3 5ml Cyclohexane

Note: These reagents must be added in this order.

7.6 After the addition of the cyclohexane. Put the lids on the tubes immediately and place in the sample rack. Shake the tubes in the rack on the multipurpose shaker for 1 minute.

7.7 Place the tubes into the 60°C water-bath and cover with lid for 40 minutes. Switch the water bath off and scoop out most of the hot water out and replace with tap water. Allow the tubes to stand for 5 minutes.

7.8 Return the tubes to the dark room shake again on the multipurpose shaker for 1 minute. Allow the tubes to stand for 30 minutes before reading.

8 Filter Fluorimeter Operation

8.1 Refer to the Perkin-Elmer model LS55 operation manual for detailed operation.

8.2 The fluorescence from the cyclohexane layer is read using the Spectrofluorimeter in a darkened room.

8.3 Switch on the instrument 60 minutes prior to reading samples to warm-up the lamp.

8.4 Instrument settings:

8.4.1 Emission wavelength: 520nm

8.4.2 Excitation wavelength: 375nm

8.5 Sipper unit accessory is used to aspirate the top layer of cyclohexane.

8.6 The instrument *concentration application* is used to calculate the concentration of selenium in each sample derived after known standards are each read in triplicate and calibration data is obtained.

8.7 Each known standard concentration is entered manually as parts per billion (ppb / ng/ml). The concentration reported for each sample is thus given in ppb.

8.8 To calculate the final concentration for the samples for reporting:

- Selenium results are reported as mg/kg (ppm)

ppb / ng/ml result x 1000 = mg/kg (ppm), but 0.1000g samples were weighed out initially therefore, mg/kg (ppm) x 10 = actual Selenium concentration in mg/kg (ppm).

9 Quality control

9.1 An internal liver control is run with every batch of samples. An International Certified Reference Material sample is run on six research trial runs.

10 Disposal

10.1 Once the samples have been read, the entire content of every tube is poured over into a separating funnel. The cyclohexane layer will settle on top.

10.2 Dispense each layer separately into glass bottles and mark each clearly. Waste is to be removed be waste removal company according to chemical waste regulations.

11 References

11.1 WC PVL Standard Operating Procedure. 1999. *P-BIO-M-001: Fluorometric Method for Selenium Analysis*. In-house Quality document.

11.2 Koh, T. & Benson, T. H. 1983. Critical Re-appraisal of Fluorometric Method for Determination of Selenium in Biological Materials. *Journal for the Association of Official Analytical Chemistry*, 66(4):918-926.

11.3 Perkin-Elmer. Model LS55 Spectrofluorimeter *Operator's Manual*.

Annexure 3: Standard Operating Procedure for Selenium Analysis Process of WC PVL Biochemistry Section

	DEPARTMENT OF AGRICULTURE **Directorate: Veterinary Services** Western Cape Provincial Veterinary Laboratory Quality System
	Fluorometric method for Selenium analysis

1 Introduction

This method is used for the analysis of Selenium in various sample types, e.g. liver, whole blood and feed. The basis for this method is the reaction of 2,3 diaminonapthalene (DAN) with Se(IV) to form a fluorescent Se-DAN complex. A filter fluorimeter is used to measure the fluorescence of this complex in order to calculate the concentration of Selenium in the given sample.

2 Personnel

The person performing the procedure should be fully trained or should perform the test under the supervision of a competent person.

Competent/ trained personnel:

2.1 Veterinary Technologist: Biochemistry and relief officer.

3 Limitation and precision of method

3.1 The method can be applied to a wide range of biological samples including organs such as liver and kidney, feed, leaves and other plant material as well as blood, urine and milk.

3.2 Selenium concentration range that can be tested is 1 – 500 ppb.

3.3 The precision and accuracy of the method has been monitored over a period of 15 months using an in-house laboratory liver control sample (BIO_LC2005) and a certified reference material from the National Institute of Standards (Bovine Liver 1577b). Refer to table below for details.

3.4 Precision of method over 15 months

	No. of detections	Se (ppm)	CV (%)
BIO_LC2005	56	1.35	8.81
Bovine Liver 1577b	44	0.72	10.69

Accuracy of method over 15 months

	Se detected (ppm) (n = 44)	Certified Se value (ppm) (NIST insert)
Bovine Liver 1577b	0.72 ± 0.08[a]	0.73 ± 0.06[a]

[a] Mean ± standard deviation

4 Equipment and Materials

4.2 Digestion tubes (20ml x 150mm. Borosilicate glass or equivalent)

4.3 Digestion block (custom made), 135 bored holes with depth of ± 70mm.

4.4 Programmable temperature controller connected to digestion block

4.5 Separating funnels (250, 500 and 1000ml)

Annexure 3: Standard Operating Procedure for Selenium Analysis Process of WC PVL Biochemistry Section

4.6 Volumetric flasks (A grade - approved for use)
4.7 100 – 1000µl Eppendorf pipette (BIO-040)
4.8 100µl Eppendorf pipette (BIO-058)
4.9 1000µl Eppendorf pipette (BIO-061)
4.10 100 and 1000µl new Eppendorf pipette tips
4.11 Glass beakers (250ml for general use)
4.12 Plastic Beaker or container for storage of tube lids
4.13 Spatula
4.14 Plastic weighing vessels and beakers for reagent preparation
4.15 Glass graduated and bulb pipettes for reagent preparation (approved for use)
4.16 1000ml volumetric flask (A grade, approved for use)
4.17 2000ml Measuring cylinder (approved for use)
4.18 Water-bath (BIO-030)
4.19 Thermometer (BIO-104)
4.20 Thermometer (BIO-091)
4.21 Multipurpose shaker (BIO-015)
4.22 Analytical balance (4 decimal place accuracy) (BIO-003 or BIO-004)
4.23 Repeat pipette (1-5ml) (BIO-080)
4.24 Pipette tips for repeat pipette (1-5ml)
4.25 Spectrofluorimeter, Perkin Elmer LS55

5 Reagents (All analytical reagent grade quality)

5.2 Acid Mixture: 1 (Perchloric Acid 60%): 5 (Nitric Acid 65%). Carefully pour 1600ml of Nitric Acid into a 2000ml measuring cylinder, fill up to the 2000ml mark with Perchloric Acid. Pour Acid mixture into brown glass storage bottle. Mixture is stable indefinitely.

5.3 1:1 Dilution of HCl: Measure equal volumes of Hydrochloric Acid (32%) and deionised water. Pour deionised water into storage vessel followed by Hydrochloric acid. Solution is stable for 12 months.

5.4 EDTA solution: Weigh out 0.81g NaH_2EDTA into a glass beaker. Transfer into 1000ml volumetric flask. Make up to the mark with deionised water. Solution is stable for 2 months.

5.5 0.1M HCl: Pour approximately 200ml deionised water into a 1000ml volumetric flask. Pipette 9.8ml Hydrochloric Acid (32%) into flask. Make up to the mark with deionised water.

5.6 1M HNO_3: Pour approximately 200ml deionised water into a 1000ml volumetric flask. Pipette 68.7ml Nitric Acid (65%) into the flask and make up to the mark with deionised water.

5.7 Cyclohexane

NOTE: DAN is light sensitive and the activity of the reagent will decrease in the presence of light. Handle the reagent as far as practically possible in a dark room.

Annexure 3: Standard Operating Procedure for Selenium Analysis Process of WC PVL Biochemistry Section

5.8 2,3 Diaminonapthalene (DAN) solution: (to be made up just before use): See point 7.5.2 for preparation details

5.9 Standards:

5.9.1 Selenium stock standard – 1000ppm (available from Merck or other ISO9002 accredited company)

5.9.2 10ppm – pipette 1ml of the 1000ppm stock standard into a volumetric flask. Make up to volume with 1M HNO_3 (stable for 6 months)

5.9.3 Working standards: dilute the 10ppm standard as follows with 1M HNO3. (stable for 6 months):
Blank: pour 1M HNO_3 into a 100ml volumetric flask up to mark.
100µl standard in 100ml volumetric flask = 10ppb (ng/ml)
500µl standard in 100ml volumetric flask = 50ppb (ng/ml)
1000µl standard in 100ml volumetric flask = 100ppb (ng/ml)
2500µl standard in 100ml volumetric flask = 250ppb (ng/ml)
5000µl standard in 100ml volumetric flask = 500ppb (ng/ml)

6 Safety/ precautionary measures

6.2 A laboratory coat must be worn during the testing procedure.

6.3 Gloves should be worn when handling DAN solution, cyclohexane and Acids.

6.4 Observe the safety instructing for the safe handling and disposal of chemicals as specified in Material Safety Data Sheets for chemicals used in Biochemistry.

7 Procedure

7.2 Weigh out 0.100g of dried liver or feed sample or 1ml of whole blood and place in digestion tubes. Four blank tubes consisting of 1ml of 0.1M HNO_3 and four tubes for each standard containing 1ml of standard are also prepared with the samples.

7.3 Add 4ml of acid mixture. Place in digestion block in perchloric acid fume cupboard (BIO-035). Switch temperature controller ON to 'PTN1' programme. (Please refer to P – BIO – E – 016 for details programming the temperature controller). The set programme should be as follows:

Step 1	Heat to 120°C (± 10°C)
Step 2	1 hour @ 120°C (± 10°C)
Step 3	Heat to 160°C (± 10°C)
Step 4	6 hours @ 160°C (± 10°C)

Step 5 Maintain @ 120°C (± 10°C) for 30 hours. (This programme is stored)
Digest overnight.

The block should be @ 120°C (± 10°C) the next morning. Record the temperature on form P – BIO – F – 072 with thermometer BIO-091.

7.4 Set the water bath at 60°C (± 5°C) and switch ON.

7.5 Remove the tubes from the digestion block and place into a rack inside the fume cabinet. Allow the tubes to cool for a few minutes. Add 1ml 1:1 HCl to the tubes. Place the tubes into the digestion block again and leave for 30 minutes at 120°C (± 10°C).

7.6 Remove tubes from digestion block after the 30 minutes is over and allow them to cool for a few minutes before adding the following reagents:

7.6.1 15 ml EDTA solution (Add this reagent in the general use fume cabinet (BIO-033)

7.6.2 1ml DAN solution

NOTE: The tubes can stand in the fume cabinet (BIO-033) covered with a paper towel until the DAN solution has been prepared. Once the DAN solution is ready for use, carry the tubes to the dark room (room 55) and add the DAN solution in the dark room. All procedures must take place in the dark room once the DAN has been added to the tubes!

<u>2,3 Diaminonapthalene (DAN) solution preparation</u>

- Calculate the quantity of DAN required. 1ml of the DAN solution is used for each standard, blank and sample. 0.100g DAN / 100ml 0.1M HCL.
- Weigh out the DAN in a darkened room into a plastic weighing vessel and transfer to a separating funnel. Ensure that the tap is closed tightly.
- Add the correct volume of 0.1M HNO_3 to the separating funnel.
- Carry the separating funnel to the water bath, which has heated to 60°C (± 5°C). Place into the water bath and secure by placing the string at the tap around the neck of the funnel, close the lid of the water bath and set the timer for 10 minutes. Record the temperature of the water bath on form P – BIO – F – 074.
- Remove the funnel from the water bath and carry the separating funnel to the dark room. Place funnel into the stand. Shake the content of the funnel manually until the DAN has dissolved completely. Be careful to hold the funnel lid firmly while shaking. Release the pressure build-up periodically.
- Pipette 40ml of cyclohexane into the funnel using the repeat pipette (BIO-080). Shake for 1 minute manually. Let the funnel stand for 60 minutes.

Annexure 3: Standard Operating Procedure for Selenium Analysis Process of WC PVL Biochemistry Section

- Pour out the bottom DAN layer into the next separating funnel. Add 25ml cyclohexane and shake for 1 minute. Let funnel stand for 45 minutes. Pour the bottom layer into the next separating funnel. Add 25ml cyclohexane, shake for 1 minute and allow to stand for 45 minutes. The DAN is now ready for use.

7.6.3 5ml Cyclohexane

Note: These reagents must be added in this order.

7.7 After the addition of the cyclohexane. Put the lids on the tubes immediately and place in the rack. Shake the tubes in the rack on the multipurpose shaker for 1 minute.

7.8 Place the tubes into the 60°C water-bath for 40 minutes. Switch the water bath off and scoop out most of the hot water out and replace with tap water. Allow the tubes to stand for 5 minutes.

7.9 Return the tubes to the dark room shake again on the multipurpose shaker for 1 minute. Allow the tubes to stand for 30 minutes before reading.

8 <u>Filter Fluorimeter Operation</u>

8.2 Refer to the Perkin-Elmer model LS55 operation manual for detailed operation.

8.3 The fluorescence from the cyclohexane layer is read using the Spectrofluorimeter in a darkened room.

8.4 Switch on the instrument 30 minutes prior to reading samples to warm-up the lamp.

8.5 Instrument settings:

8.5.1 Emission wavelength: 520nm

8.5.2 Excitation wavelength: 375nm

8.6 Sipper unit accessory is used to aspirate the top layer of cyclohexane.

8.7 The instrument *concentration application* is used to calculate the concentration of selenium in each sample derived after known standards are each read in triplicate and calibration data is obtained.

8.8 Each known standard concentration is entered manually as parts per billion (ppb / ng/ml). The concentration reported for each sample is thus given in ppb.

8.9 To calculate the final concentration for the samples for reporting:

- <u>Whole Blood</u> Selenium results are reported as ng/ml (ppb)
- <u>Liver or Feed</u> Selenium results are reported as mg/kg (ppm)

ppb / ng/ml result x 1000 = mg/kg (ppm), but 0.1000g samples were weighed out initially therefore, mg/kg (ppm) x 10 = actual Selenium concentration in mg/kg (ppm).

Annexure 3: Standard Operating Procedure for Selenium Analysis Process of WC PVL Biochemistry Section

8 Quality control

8.1 An internal liver control is run with every batch of samples. If a feed sample is analysed an internal feed control is also run with that batch of samples. The control values are checked against a standard reference material.

9 Disposal

9.1 Once the samples have been read, the entire content of every tube is poured over into a separating funnel. The cyclohexane layer will settle on top.

9.2 Dispense each layer separately into glass bottles and mark each clearly. Waste is to be removed be waste removal company according to chemical waste regulations.

10 References

10.1 Koh, T. and Benson, T. H. 1983. Critical Re-appraisal of Fluorometric Method for Determination of Selenium in Biological Materials. *Journal for the Association of Official Analytical Chemistry*, 66(4):918-926.

10.2 Perkin-Elmer. Model LS55 Spectrofluorimeter *Operator's Manual*.

Annexure 4: Research Standard Operating Procedure for Modified Selenium Analysis Process

Cape Peninsula University of Technology	**Faculty of Engineering: Department Quality**
	Method for determination of selenium by hydride generation atomic absorption spectrometry (HGAAS)

1. Introduction

This method is used for the analysis of selenium in biological liver samples, which have been microwave digested and thereafter prepared for hydride generation atomic absorption spectrometry. The principle for this method is the hydride vapour generation of selenium increases the sensitivity of the general atomic absorption technique for the detection of this analyte element.

2. Personnel

The person performing the procedure should be fully trained to be able to conduct this analytical laboratory process.

3. Limitation and precision of method

3.1 The method is applied to biological liver samples.
3.2 Selenium concentration range that can be tested is 1 – 500 ppb.

4. Equipment and Materials

4.1 Microwave pressure sample vessels (Polyfluoralkan (PFA) or equivalent)
4.2 Microwave-heated pressure digestion apparatus
4.3 Programmable temperature controller connected to digestion block
4.4 Volumetric flasks (A grade - approved for use), 25ml, 50ml, 500ml and 1000ml
4.5 100 – 1000µl Eppendorf pipette (BIO-040)
4.6 100µl Eppendorf pipette (BIO-058)
4.7 1000µl Eppendorf pipette (BIO-061)
4.8 100 and 1000µl new Eppendorf pipette tips
4.9 Spatula
4.10 Plastic weighing vessels and beakers for reagent preparation
4.11 Glass graduated and bulb pipettes for reagent preparation (approved for use)
4.12 1000ml volumetric flask (A grade, approved for use)
4.13 2000ml Measuring cylinder (approved for use)
4.14 Water-bath (BIO-030)
4.15 Thermometer (BIO-104)
4.16 Thermometer (BIO-091)
4.17 Analytical balance (4 decimal place accuracy) (BIO-003 or BIO-004)
4.18 Repeat pipette (1-5ml) (BIO-080)
4.19 Pipette tips for repeat pipette (1-5ml)
4.20 Flow injection hydride system, with sample loop, GBC HG 3000
4.21 Atomic absorption spectrophotometer (AAS), with measurement recording system, background correction and heated quartz cell, GBC Avanta

Cape Peninsula University of Technology	**Faculty of Engineering: Department Quality**
	Method for determination of selenium by hydride generation atomic absorption spectrometry (HGAAS)

4.22 Element-specific lamp for selenium

5 Reagents (All analytical reagent grade quality)

5.1 General: The concentration of the trace elements in the reagents and water used shall be low enough not to affect the results of the determination. A blank should be measured simultaneously with the test samples with every analysis run to control contamination and carry over with selenium in the reagents and apparatus used.

5.2 Nitric Acid: Not less than 65% (mass fraction), of approximately ρ (HNO_3) = 1,4 g/ml.

5.3 Diluted nitric acid: Mix 100ml nitric acid (4.2) with water to 1000ml.

5.4 Hydrogen peroxide: not less than 30% (mass fraction).

5.5 Hydrochloric acid 30%: Mass concentration of approximately ρ (HCL) = 1,15 g/ml

5.6 Standard solution hydrochloric acid: 2ml hydrochloric acid (5.5) made up to 100ml volumetric flask

5.7 Diluted hydrochloric acid: 3% (mass fraction) as carrier solution for the use of the flow-injection procedure. Dilute 90ml of hydrochloric acid (5.5) made up to the mark of 1000ml deionised water.

5.8 Sodium borohydride solution: 2g/l i.e. Dissolve 2g of sodium hydroxide pellets in deionised water, add 2g of sodium borohydride and make up to 1000ml with deionised water in a 1000ml volumetric flask. Prepare a fresh solution daily and, when necessary, filter before use. When analysis procedure is of longer time, it is recommended to cool the sodium borohydride solution, i.e. with ice around the flask, during its use in the HGAAS measurement.

WARNING: It is essential to observe the safety instructions for working with sodium borohydride. Sodium borohydride forms hydrogen with acids and this can result in an explosive air/hydrogen mixture. A permanent extraction system shall be provided at the point where measurements are carried out.

5.9 Standards:

5.9.1 Selenium stock solution: c(Se) = 1000ppm (1000mg/l). The stock solution is commercially available. It is advisable to use certified stock solutions such as bought from the supplier Merck

5.9.2 Selenium standard solution: 10ppm (10mg/l) – pipette 1000µl of the 1000ppm stock standard into a 100ml volumetric flask. Make up to volume with standard solution hydrochloric acid (5.6) (The solution is stable for at least 3 months)

5.9.3 Selenium calibration solutions: For the preparation of the five calibration solutions, take aliquots of 100µl, 500µl, 1000µl, 2500µl and 5000µl of the selenium standard solution (5.9.2) into 100ml flasks

and make up to mark with standard solution hydrochloric acid (stable for 3 months):
Blank: pour Standard Soln HCL (5.9.2) into a 100ml volumetric flask up to mark.
100µl standard in 100ml volumetric flask = 10ppb (ng/ml)
500µl standard in 100ml volumetric flask = 50ppb (ng/ml)
1000µl standard in 100ml volumetric flask = 100ppb (ng/ml)
2500µl standard in 100ml volumetric flask = 250ppb (ng/ml)
5000µl standard in 100ml volumetric flask = 500ppb (ng/ml)

6 Safety/ precautionary measures

6.1 A laboratory coat must be worn during the testing procedure.

6.2 Gloves should be worn when handling acids.

6.3 Observe the safety instructing for the safe handling and disposal of chemicals as specified in Material Safety Data Sheets for chemicals used in Biochemistry.

7 Digestion Procedure

7.1 Weigh out ±0.5000g to one decimal point, of dried homogenised liver into microwave pressure sample vessels. Include an in-house liver control sample, LC2009 with each sample batch. Three blank tubes consisting of 1ml of standard solution HCL (5.6), and three tubes for each standard containing 1ml of standard are also prepared with the samples.

7.2 Set the water bath at 80°C (± 5°C) and switch ON.

7.3 Add 5ml of nitric acid (5.2) and 2.5ml hydrogen peroxide (5.4) using reaction vessels of 100ml size. The reaction vessels are locked and fixed in the microwave digestion system.

7.4 Microwave Digestion procedure:
Ramp to temp 15 minutes
Hold at 200^{o}C 20 minutes

7.5 Remove the reaction vessels from the microwave system in an extractor hood and let them de-aerate carefully before opening.

7.6 Place open vessels into water bath with a temperature of 80^{o}C for 20 minutes to degas the extraction solution by allowing the brown (nitrose) gases to pass off.

7.7 Remove from water bath after 20 minutes is over and allow them to cool for a few minutes to room temperature, it is quantitatively transferred to a 25ml volumetric flask and filled up to the mark with deionised water.

7.8 If measuring is not done immediately after extraction the test solution must be stored in adequate vessels to prevent loss or carryover of selenium.

Annexure 4: Research Standard Operating Procedure for Modified Selenium Analysis Process

Cape Peninsula University of Technology	**Faculty of Engineering: Department Quality**
	Method for determination of selenium by hydride generation atomic absorption spectrometry (HGAAS)

8 **Selenium reduction procedure**

8.1 Dilute 10ml of the test solution (7.7) into a 50ml flask, add 5ml hydrochloric acid (5.5) and heat for 20 minutes in a water bath at 80^{o}C with opened flask.

8.2 After cooling down to room temperature the flask is filled up with water.

9 **Spectrometer settings of the flow-injection hydride generation atomic absorption spectrometer (HGAAS)**

9.1 Recommended instrument settings as specified in operating manual of the manufacturer:

9.1.1	Wavelength:	196.0 nm
9.1.2	Bandpass:	1.0 nm
9.1.3	Lamp Current	10.0 mA
9.1.4	Flame Conditions	lean flame
9.1.5	Read time	9 seconds
9.1.6	Replicates	3
9.1.7	Calibration	Conc least squares

9.2 The HG3000 automatic hydride generator is a continuous flow vapour generation system. It incorporates a peristaltic pump which continuously pumps sample (or rinse solution) and reagents into a mixing manifold.
The mixed solution flows through a reaction coil where the metal hydride is formed and then into a gas-liquid separator where the hydride is removed from the bulk liquid using an inert carrier gas. The hydride is then fed into a fused quartz absorption cell which is mounted over the burner of the atomic absorption spectrophotometer (AAS).

9.3 Switch the AAS on at least an hour before use to allow the instrument to warm up and stabilise.

9.4 Select the selenium application method and optimise the lamp.

9.5 Switch the HG3000 unit on and allow water to be pumped through the system in order to check that the peristaltic pump mechanism is operating properly.

9.6 Zero the instrument with a blank sample. At this stage it is important to make a note of whether the running concentration of the apparatus is not fluctuating erratically, as this is an indication of instrument stability and will effect results. Only once the running concentration stabilises should calibration samples and test samples be measured on HGAAS.

9.7 Ensure that inert gas flow is enabled.

9.8 Insert appropriate feeder pipes into their respective reagent bottles

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9.9 All measurements are pre-programmed to be taken in triplicate. Calibration samples are then read and the concentration of each is used to obtain calibration data in order to set up a calibration curve.

9.10 The calibration curve derived from five known calibration samples, (which were processed simultaneously with samples), is used calculate the concentration of selenium in each sample..

9.11 Each sample is then read and the concentration reported for each sample.

10 Calculation

10.1 To calculate the final concentration for the samples for reporting: The selenium mass fraction of the weighted test sample (W_s) is calculated according to the following formula

$$W_s = (c_t - c_b) \times V_1 \times V_3 \times D/(V_2 \times m \times 1000)\ mg/kg\ (ppm)$$

Where:

- c_t is the concentration of selenium in the test solution, µg/l;
- c_b is the concentration of selenium in the blank solution, µg/l;
- m is the mass of the test portion, g;
- V_1 is the volume of the test solution after microwave digestion procedure (i.e. 25ml);
- V_2 is the volume (aliquot) of the test solution after microwave digestion procedure (i.e. 10ml);
- V_3 is the volume for pre-reduction step (i.e. 50ml);
- D is the dilution factor (i.e. 1 when no further dilution is done);
- 1000 is the constant factor to calculate from selenium concentration expressed in ug/ml to ug/l.

8 Quality control

8.1 An internal liver control is run with every batch of samples. An International Certified Reference Material sample is run on five research trial runs.

9 Disposal

9.1 Dispense waste into glass bottles and mark each clearly. Waste is to be removed be waste removal company according to chemical waste regulations.

Cape Peninsula University of Technology	**Faculty of Engineering: Department Quality**
	Method for determination of selenium by hydride generation atomic absorption spectrometry (HGAAS)

10 References

10.1 BSi Group Headquarters. 2010. *Draft BS EN 16159 Animal feeding stuffs – Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (extraction with 65% nitric acid and 30% hydrogen peroxide*. BSI Group Headquarters:London

10.2 Koh, T. & Benson, T. H. 1983. Critical Re-appraisal of Fluorometric Method for Determination of Selenium in Biological Materials. *Journal for the Association of Official Analytical Chemistry*, 66(4):918-926.

10.3 GBC Scientific Equipment Pty Ltd. 1995. *AA Hydride System HG3000, EHG3000 & MC3000 Operation & Service Manual*. Published in-house: Australia

ANNEXURE 5

Draft for Public Comment

Annexure 5: International Standard Operating Procedure for Selenium Analysis Method.
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Form 36

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Supersession information: If you are aware of a current national standard which may be affected, please notify the secretary (contact details below).	

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Responsible Committee Secretary: **Committee Service Centre (BSI)**
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Introduction

This draft standard is based on European discussions in which the UK has taken an active part. Your comments on this draft are welcome and will assist in the preparation of the consequent British Standard. Comment is particularly welcome on national, legislative or similar deviations that may be necessary.

Even if this draft standard is not approved by the UK, if it receives the necessary support in Europe, the UK will be obliged to publish the official English Language text unchanged as a British Standard and to withdraw any conflicting standard.

UK Vote

Please indicate whether you consider the UK should submit a negative (with reasons) or positive vote on this draft.

Submission of Comments

- The guidance given below is intended to ensure that all comments receive efficient and appropriate attention by the responsible BSI committee. **Annotated drafts are not acceptable and will be rejected.**

- All comments must be submitted, preferably electronically, to the Responsible Committee Secretary at the address given on the front cover. Comments should be compatible with version 6.0 or version 97 of Microsoft Word for Windows, if possible; otherwise comments in ASCII text format are acceptable. **Any comments not submitted electronically should still adhere to these format requirements.**

- All comments submitted should be presented as given in the example below. Further information on submitting comments and how to obtain a blank electronic version of a comment form are available from the BSI website at: **http://www.bsigroup.com/en/Standards-and-Publications/Current-work/DPCs/**

Template for comments and secretariat observations

Date: xx/xx/20xx	Document: **ISO/DIS xxxx**

1	2	(3)	4	5	(6)	(7)
MB	**Clause No./ Subclause No./Annex (e.g. 3.1)**	**Paragraph/ Figure/ Table/Note**	**Type of comment**	**Comment (justification for change) by the MB**	**Proposed change by the MB**	**Secretariat observations on each comment submitted**
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 16159

September 2010

ICS 65.120

English Version

Animal feeding stuffs - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (extraction with 65% nitric acid and 30% hydrogen peroxide)

Aliments des animaux - Dosage du sélénium par spectrométrie d'absorption atomique par génération d'hydrures (SAAGH) après digestion par micro-ondes (extraction avec de l'acide nitrique à 65 % et du peroxyde d'hydrogène à 30 %)

Futtermittel - Bestimmung von Selen mit Atomabsorptionsspektrometrie-Hydridtechnik (HGAAS) nach Mikrowellen-Druckaufschluss (Extraktion mit 65% Salpetersäure und 30% Wasserstoffperoxid)

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. prEN 16159:2010: E

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Foreword

This document (prEN 16159:2010) has been prepared by Technical Committee CEN/TC 327 "Animal feeding stuffs", the secretariat of which is held by NEN.

This document is currently submitted to the CEN Enquiry.

1 Scope

This European Standard specifies a method for the determination of selenium in animal feeding stuffs by hydride generation atomic absorption spectrometry (HGAAS) after microwave pressure digestion.

The limit of quantification is 0,5 µg/l of the test solution. Using a test portion of 0,5 g and a volume of the test solution of 25 ml after pressure digestion the limit of quantification is calculated as 0,125 mg/kg in the sample.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies).

EN 13804:2002, *Foodstuffs – Determination of trace elements - Performance criteria, general considerations and sample preparation*

3 Principle

Selenium is determined in the test solution by hydride generation atomic absorption spectrometry (HGAAS) after microwave pressure digestion and a pre-reduction step.

The dried and homogenised feeding stuff test sample is digested by nitric acid and hydrogen peroxide under pressure and high temperatures in a microwave-heated pressure digestion system.

Selenium ions of the test solution are reduced with hydrochloric acid to selenium (IV) and converted to selenium hydride (SeH_2) by sodium borohydride. This selenium hydride is transferred by a gas stream into a heated measurement cell and decomposed. The absorption at the selenium line at 196,0 nm corresponds to the amount of selenium.

NOTE Selenium (VI) is not determined by the hydridisation as described here. It is therefore necessary to adjust the digestion conditions and to exercise a pre-reduction step with hydrochloric acid to yield only selenium (IV).

Other digestion procedures with the same extraction efficiency or other measurement systems like FI-HGAAS or HG-ICP-AES are possible (see Annex D).

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

4 Reagents

4.1 General

The concentration of the trace elements in the reagents and water used shall be low enough not to affect the results of the determination. A blank should be measured simultaneously with the test samples at every day of analysis to control contamination and carry over with selenium in the reagents and apparatus used.

4.2 Nitric acid, not less than 65% (mass fraction), of approximately ρ (HNO_3) = 1,4 g/ml

NOTE high purity is essential to avoid potential contamination. Therefore only use nitric acid available with high purity or perform an extraction by a sub-boiling distillation.

4.3 Diluted nitric acid, mix 100 ml nitric acid (4.2) with water to 1 l

4.4 Hydrogen peroxide, not less than 30% (mass fraction)

NOTE that high purity is essential to avoid potential contamination. Commercially available hydrogen peroxide for analysis should be tested for contamination of trace elements.

4.5 Hydrochloric acid, 30%, mass concentration of approximately ρ (HCl) = 1,15 g/ml

4.6 Diluted hydrochloric acid, e.g. about 3% (mass fraction), as carrier solution for the use in the flow-injection-procedure

EXAMPLE Dilute approximately 90 ml of hydrochloric acid (4.5) to 1 l with water.

4.7 Sodium borohydride solution, e.g. c = 2 g/l

Dissolve 2 g of sodium hydroxide pellets in water, add 2 g of sodium borohydride and dilute to 1 000 ml with water into 1000-ml-flask (5.4). Prepare a fresh solution daily and, when necessary, filter before use. When the analysis procedure is of longer time it is recommended to cool the sodium borohydride solution, i.e. with ice around the flask, during its use in the HGAAS measurement.

Sodium borohydride, stable aq. solution, 4,4 M in 14 M NaOH (e.g. from Alfa Aesar, Karlsruhe) is also commercially available.

NOTE The concentration by mass of the sodium borohydride solution may vary with the system and the instructions of the relevant shall therefore be observed.

WARNING – It is essential to observe the safety instructions for working with sodium borohydride. Sodium borohydride forms hydrogen with acids and this can result in an explosive air/hydrogen mixture. A permanent extraction system shall be provided at the point where measurements are carried out.

4.8 Selenium stock solution, c (Se) = 1 000 mg/l

The stock solution is commercially available. It is advisable to use certified stock solutions.

Otherwise dissolve 1,4053 g of selenium dioxide (SeO_2) and 2 g sodium hydroxide in approximately 50 ml water, and dilute to 1 000 ml with water.

4.9 Selenium standard solution, c (Se) = 1 mg/l

Dilute i.e. 100 µl the stock solution (4.8) in a 100-ml-flask (5.4) to a concentration of 1 mg/l. The selenium standard solution shall contain an adequate amount of hydrochloric acid, e.g. 2 ml of hydrochloric acid (4.5) per 100 ml.

NOTE The standard solution is stable for at least three months.

4.10 Selenium calibration solutions

For the preparation of five calibration solutions following procedure is recommended: Take aliquots of 0 µl, 250 µl, 500 µl, 1 000 µl and 1 500 µl of the selenium standard solution (4.9) into 100-ml-flasks (5.4). After

addition of 20 ml of nitric acid (4.3) and 10 ml of hydrochloric acid (4.5) the calibration solutions are heated for 20 min in a water bath at 80°C (see pre-reduction step 6.2.2). After cooling down to room temperature the flasks (5.4) are filled up with water and the calibration solutions are measured.

The selenium concentrations of the calibration solutions are: 0 µg/l; 2,5 µg/l; 5 µg/l; 10 µg/l and 15 µg/l (see Table 1).

Table 1 — Recommended calibration solutions (4.10) for the determination of selenium

Selenium (Se)	Concentration of calibration solution (4.10) after pre-reduction procedure	Aliquot of selenium standard solution (4.9) transferred in 100-ml-flasks (5.4) (pre-reduction step)
Calibration standard 1	**0 µg/l**	**0 µl**
Calibration standard 2	**2,5 µg/l**	**250 µl**
Calibration standard 3	**5 µg/l**	**500 µl**
Calibration standard 4	**10 µg/l**	**1 000 µl**
Calibration standard 5	**15 µg/l**	**1 500 µl**

Choose the concentrations of the calibration solutions so as not to exceed the linear range of the calibration function. It is recommended to use a minimum of 5 calibration solutions with different concentrations. The calibration solutions are measured from the lowest to the highest concentration. In general the calibration curve should be linear. Using a non-linear calibration function is possible if it is well-described.

NOTE Prepare fresh calibration solutions (inclusive pre-reduction step) at the day of analysis.

5 Apparatus and equipment

5.1 General

To minimise the contamination, all apparatus which come into direct contact with the sample and the solutions should be carefully pre-treated according to EN 13804.

5.2 Microwave-heated pressure digestion apparatus with inert reaction vessels, i.e. made of Polytetrafluorethen (PTFE), Polyfluoralkan (PFA), FEP or quartz, suitable for digestion temperatures of more than 200°C

NOTE 1 The microwave oven should be generally persistent against corrosion and especially his whole electronics should be protected against corrosion to ensure safe operation. The ventilation should transfer the acid vapours to an extractor hood.

NOTE 2 The reaction vessels should have a safety valve designed for a pressure of 1 000 kPa.

5.3 **Pipettes, volumetric and/or graduated,** 2 ml, 2,5 ml, and 10 ml

5.4 **Flasks,** 25 ml, 50 ml, 100 ml, 500 ml and 1 000 ml

5.5 **Flow-injection hydride system,** with sample loop, i.e. 500 µl

5.6 **Atomic absorption spectrometer (AAS),** with measurement recording system, background correction, heated quartz cell and accessories for the hydride procedure

5.7 **Element-specific lamp for selenium**

NOTE an electrodeless discharge lamp (EDL) is to prefer instead of a hollow-cathode lamp.

5.8 **Ultrasonic bath and/or water bath**

5.9 **Analytical balance,** accurate to 0,1 mg

6 Procedure

Sampling and preparation of a test sample is not part of the method. A recommended sampling method and method for sample preparation is given in ISO 6497 [1] and ISO 6498 [2].

NOTE The use of a stationary or especially for mineral feeds of a rotary riffler for mass reduction and the use of a sieve size of 0,5 mm or lower for particle size reduction is recommended because of low weights of ≤ 0,5 g of the test portions to ensure homogeneity.

6.1 Preparation of the test solution

NOTE The following extraction procedure leads in most cases to results for selenium and for other minerals and trace elements which correspond to the total contents of these elements. For some specific problems check whether modifications of the digestion program or other acid mixtures are necessary.

The weight of a test sample depends on the organic percentage of the sample material and from the size of the reaction vessels of the microwave digestion system.

Using reaction vessels of 20 to 100 ml sizes respectively a test portion of 0,2 to 0,5 g of the homogenised and to a particle size of ≤ 0,5 mm or lower grinded test sample are weighed to 1 mg exactly for digestion.

Add i.e. 5 ml nitric acid (4.2) and 2,5 ml hydrogen peroxide (4.4) using reaction vessels of 100 ml size, the reaction vessels are locked and fixed in the microwave digestion system (5.2).

NOTE For the pre-reaction let the reaction vessels bleed before the pressure digestion is started.

WARNING — For some samples heavy reactions may result after addition of nitric acid and hydrogen peroxide. Therefore let the reactions fade off at room temperature, i.e. over night.

To avoid potential contamination and/or carry over parallel to the test samples a blank is to digest for control. A steam stripping of the reaction vessels with nitric acid is therefore recommended.

The digestion with the microwave system is performed with a temperature program adapted to the matrices considering the operating manual of the manufacturer.

WARNING — For samples with unknown composition firstly a digestion procedure with low test portions are to perform. In particular cases heavy reactions with hydrogen peroxide could appear.

Also formation of highly explosive compounds is possible when organic matrices are digested. Too high weights could result in uncontrollable reactions.

In principle the pressure digestion is started with low power then continuously increased to the maximum permitted power supply for a distinct time to achieve a temperature of more than 200°C. The digestion requires about 15 minutes to 30 minutes. Afterwards the system is cooled down.

NOTE With a digestion temperature of 200°C a sufficient extraction of selenium (and other elements) is obtained. In general, it applies that the quality of the digestion will become better with increasing digestion temperature. Digestion temperatures greater than 280°C can lead to increased formation of volatile selenium (VI).

Remove the reaction vessels from the microwave system in an extractor hood and let them de-aerate carefully before opening. Let the vessels stand opened for about 20 minutes to pass off brown (nitrose) gases. The use of an ultrasonic bath or a water bath with a water temperature of about 80°C (5.8) is recommended to degas the extraction solution.

When loosing reaction gases within the microwave digestion the whole extraction procedure is to repeat with reduced test portion; this is very obvious when the volume of the extraction solution is reduced after the pressure digestion procedure.

Finally when the extraction solution has achieved room temperature it is quantitatively transferred to a 25- or 50-ml-flask (5.4) and filled up to the mark with water. For graduated reaction vessels the extraction solution could directly be filled up to the mark with water.

The extraction solution should be clear. When there are suspended particles in the extraction solution let them drop to the ground of the flask or either filtrate or centrifuge the solution before transferring it to a vessel of PP, PFA or FEP. If the measuring is not done immediately after the extraction the test solution must be stored in adequate vessels to prevent a loss or a carryover of selenium.

6.2 Measurement of the test solution

6.2.1 Pre-dilution of the test solution

It is very important that the acid concentration of the (diluted) test solution corresponds to that of the calibration solutions because the signal height for measuring selenium by HGAAS depends on the acid matrix.

Therefore when the measured selenium concentration of a sample exceeds the linear range of the calibration function, a dilution of the test solution (6.1) with nitric acid (4.3) instead with water is necessary.

EXAMPLE With a (linear) calibration function of calibration solutions of 2,5 µg/l to 15 µg/l feeding stuff samples with selenium concentrations of 0,625 mg/kg to 3,75 mg/kg are to measure without further dilution using 0,5 g test portion, a 25-ml-flask (5.4) after microwave pressure digestion, an aliquot of 10 ml of the digestion solution for pre-reduction using a 50-ml-flask (5.4). Thus samples with higher selenium concentrations are to dilute in that way that the selenium concentration of the pre-reduced test solution falls within the calibration function.

6.2.2 Pre-reduction of the (pre-diluted) test solution

Dilute 10 ml of the test solution (6.1) or the pre-diluted test solution (6.2.1) into a 50-ml-flask(5.4), add 5 ml hydrochloric acid (4.5) and heat for 20 min in a water bath at 80°C with opened flask. After cooling down to room temperature the flask is filled up with water.

6.2.3 Spectrometer settings of the flow-injection hydride generation atomic absorption spectrometer (HGAAS)

To devise a test schedule, first adjust the apparatus as specified in the operating manual of the manufacturer, then optimise the settings, paying particular attention to gas flow times and the amounts of sodium borohydride introduced. Typical settings are listed in Table 2.

Table 2 — Typical settings of HGAAS for measuring selenium

Temperature of the cell	900°C
Wave length	196,0 nm
Slit width	2,0 nm
Signal processing [a])	Peak height with background correction
Smoothing	0,5 s
Integration time	15 s
[a]) Nearest to the limit of quantification a signal processing by peak area is recommended.	

6.2.4 HGAAS determination

The pre-reduced test solutions (6.2.2), if necessary pre-diluted previously (6.2.1), and the selenium calibration solutions (4.10) are measured directly with an atomic absorption spectrometer with electrically heated quartz cell coupled to a flow-injection-hydride-system. Use of a 500 µl sample loop is recommended.

The apparatus should be programmed in such a way that first the sample loop is filled with the pre-reduced test or pre-reduced calibration solution. Then the test- or calibration solution is transferred to a mixing unit with diluted hydrochloric acid (4.6) and mixed with sodium borohydride solution (4.7). The resulting gas/liquid mixture is separated by an argon-flowed separator. The argon steam sorts out the metal hydrides to the quartz cell for atomisation reaction and measuring the atomic absorption of selenium.

Firstly the selenium calibration solutions (4.10) are measured, then the (pre-diluted) test solutions (6.2.2).

Check the linear range of the calibration function. If the concentration of the test sample is outside the linear range dilute with nitric acid (4.3) and not with pure water. When carrying out prolonged series of measurements, it is advisable to check the zero and the calibration at intervals.

Significant background signal appears in the case of the hydride generation technique, either by matrix effects or by using higher concentrations of nitric acid or hydrochloric acid. Add amido sulphuric acid after the pre-reduction step when disturbances from nitric acid appear.

Copper concentrations in the test solution of more than 750 µg/l could lead to a signal depression. Measuring copper and selenium concentrations simultaneously by ICP-AES could be useful: If the selenium concentration of the test solution by ICP-AES is higher to that of HGAAS and if the copper concentration is high a depression effect by copper seems to be possible. Then dilute the test solution with diluted nitric acid (4.3) or take 1 ml of a 0,5% solution of 1,10 - phenanthroline into 10 ml of the test solution (= 0,05% of 1,10 - phenanthroline within the test solution) for complexation of copper to measure selenium by HGAAS without a signal depression (see 6.2.2).

For unknown matrix effects use the standard addition procedure.

As an analytical control, reference samples having reliable known selenium contents shall be analysed parallel with all the series of samples analysed, the reference samples being subjected to all the steps in the method starting from digestion. Blank solutions prepared by subjecting them to all the steps in the method shall also be determined.

7 Calculation

In general the calibration curve and the element concentration of the test solution is calculated by the AAS system itself.

The selenium mass fraction of the weighed test sample (W_s) is calculated due to following formula:

$$W_S = (c_t - c_b) \times V_1 \times V_3 \times D / (V_2 \ x \ m \ x \ 1\ 000) \quad mg/kg \qquad (1)$$

where

c_t is the concentration of selenium in the test solution, µg/l;

c_b is the concentration of selenium in the blank solution, µg/l;

m is the mass of test portion, g;

V_1 is the volume of test solution after microwave digestion procedure (i.e. 25 or 50), ml;

V_2 is the volume (aliquot) of test solution after microwave digestion procedure (i.e. 10), ml;

V_3 is the volume for pre-reduction step (i.e. 50), ml;

D is the dilution factor (i.e. 1 when no further dilution is done);

1 000 is the constant factor to calculate from selenium concentration expressed in µg/ml to µg/l.

EXAMPLE:

Using m = 0,5 g as test portion, a 25-ml-flask (5.4) for microwave digestion (= V_1), an aliquot of 10 ml after digestion (= V_2) and a 50-ml-flask (5.4) for the pre-reduction step (= V_3) - when no further dilution (D = 1) is done the selenium mass of the sample (= W_s) is calculated as:

$$W_S = (c_t - c_b) \times (25 \times 50 \ \times \ 1) / (0{,}5 \times 10 \times 1\ 000) = (c_t - c_b) \times 1 \ \ 250 / 5 \ \ 000 = (c_t - c_b) / 4 \quad mg/kg$$

8 Precision

8.1 General

Details of an interlaboratory test done in 2009 on the precision of the method are summarised in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

8.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5% of the cases exceed the values of r given in Table 3.

8.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5% of the cases exceed the values of R given in Table 3.

Table 3 — Precision data

Matrix	mean, mg/kg	r, mg/kg	R, mg/kg
Cow feed	0,305	0,072	0,192
Turkey feed	0,313	0,054	0,134
Mineral piglet feed	6,87	1,94	4,23
Mineral cow feed	73,6	6,9	34,5

9 Test report

The test report shall specify:

a) information necessary for complete identification of the sample;

b) the test method used, with reference to this European Standard;

c) the test results obtained and the units in which they are specified;

d) data of sampling and sampling procedure (if known);

e) date when the analysis was finished;

f) operating details not specified in this European Standard, or regarded as optional, together with details of any incidents occurred when performing the method may have influenced the test result(s).

Annex A
(informative)

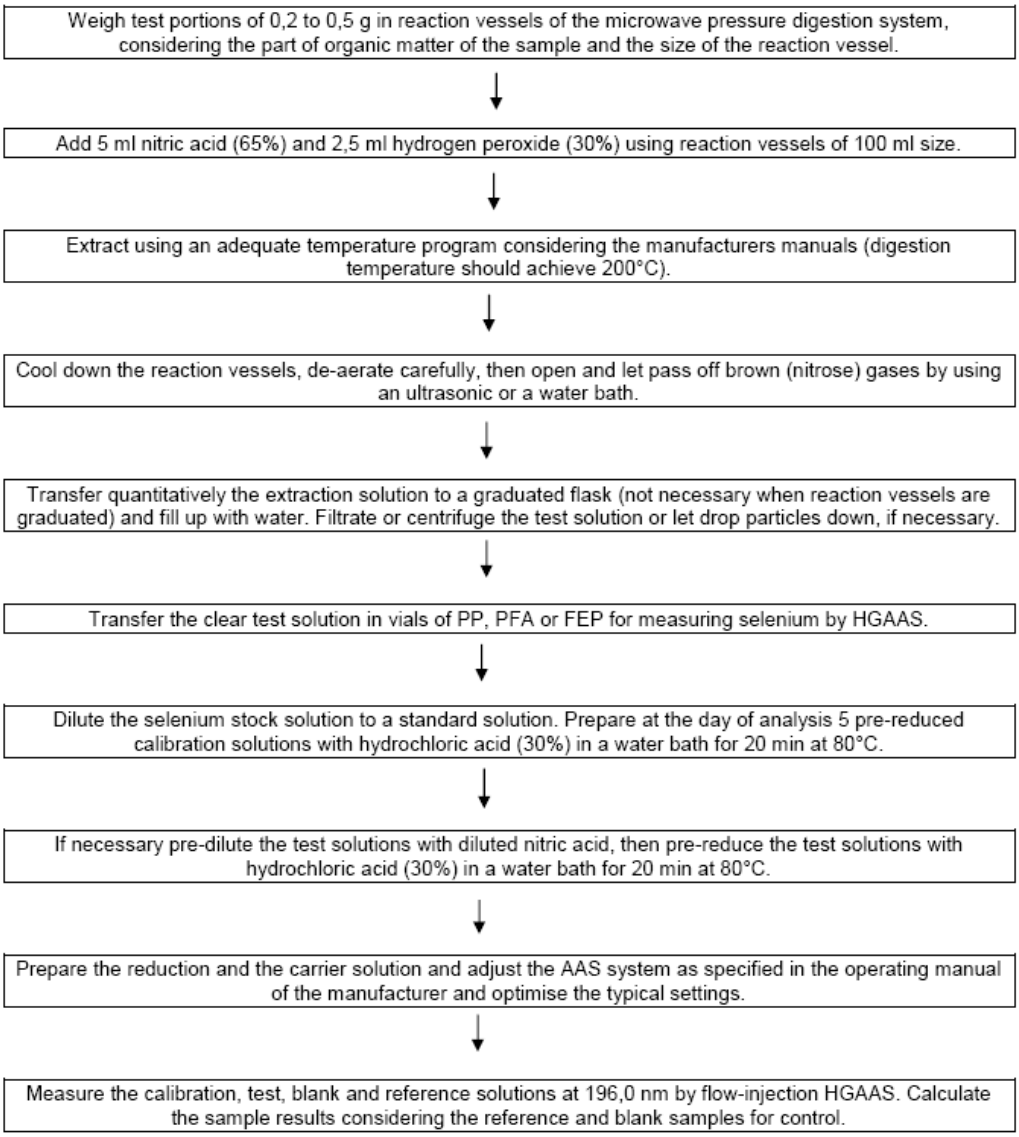
Results of the interlaboratory tests

Table A.1 — Precision data

Matrix tested	Cow feed	Turkey feed	Mineral piglet feed	Mineral cow feed	Turkey feed	Mineral piglet feed
Subsamples fully prepared for direct weighing	yes	yes	yes	yes	no	no
No. of labs	19	19	19	19	18	18
No. of outlier labs	1	4	0	4	4	1
No. of non-compliant labs	0	0	3	2	0	2
No. of valid labs	18	15	16	13	14	15
Mean value, mg/kg	0,305	0,313	6,87	73,6	0,246	6,76
s_r, mg/kg	0,026	0,019	0,69	2,5	0,016	0,58
r, mg/kg	0,072	0,054	1,94	6,9	0,045	1,62
RSD(r), %]	8,5	6,1	10,1	3,4	6,5	8,6
S_R, mg/kg	0,069	0,048	1,51	12,3	0,037	1,57
R, mg/kg	0,192	0,134	4,23	34,5	0,103	4,39
RSD(R), %	22,5	15,3	22,0	16,7	14,9	23,2
HORRAT(R)	1,2	0,8	1,8	2,0	0,8	1,9

Annex B
(informative)

Flowchart



Annex C
(informative)

Adjustment of a microwave oven

The adjustment of the microwave oven is an essential step for its proper use. By this procedure it should be established how a certain adjustment of the oven corresponds with the actual supplied (effective) power.

The microwave oven should be adjusted periodically. The way of adjusting a microwave oven depends on the electronic system that has been applied by the manufacturer. If for the oven a linear relationship holds between supplied power and the adjustment scale, it is calibrated at two positions (for example at 40% and 100% power). It is recommended, however, to calibrate at more positions in order to verify linearity.

If there is no linearity between adjustment scale and the supplied power, an adjustment at more positions is required. The instructions of the manufacturer should be observed.

Annex D
(informative)

Alternative digestion procedure with the same extraction efficiency: Acid digestion with a mixture of 65% nitric acid and 70% perchloric acid (7:3 by volume) at atmospheric pressure

NOTE Work with perchloric acid should only be undertaken if safety precautions are followed and care, caution, chemical knowledge and common sense are used. It should be pointed out that the safety depends not only on rules (see D.5), time- and temperature-controlled automated decomposition, special hood, exhaust system and sprinkler-washing system, but also on conscientious co-workers with a sense of responsibility.

D.1 Selenium calibration solutions

Add 0 ml, 0,2 ml, 0,5 ml, 1,0 ml, 1,5 ml and 2,0 ml of a 1 000 µg/l selenium standard solution into 100-ml-flasks. Add 3,6 M hydrochloric acid (not to mark).

Pre-reduction of Se (VI) to Se (IV) of standards

The calibration solutions are then heated for 30 min at 100°C and after cooling dilute the solutions to the mark of the flask. The final concentrations of the calibration solutions are:

0 µg/l, 2 µg/l, 5 µg/l, 10 µg/l, 15 µg/l and 20 µg/l.

D.2 Preparation of the test solution

Weigh 1 g dry or 5 g wet test portion in a sample tube (80 ml), i.e. a Kjeldahl tube. Add 15 ml of 65% nitric acid, 4 ml of 70% perchloric acid and 2 ml of concentrated sulphuric acid, the reagents should be of ultra pure quality. Immediately after addition of the acid mixture the digest is initiated. Automatic digestion of the test solution is performed using an electrically heated block of aluminium connected to a microprocessor for control of temperature and time according to a standard digestion program [11][12][13][14].

D.2.1 Digestion program

Step	Temperature, °C	Time, h
1	70	1
2	120	2
3	170	2
4	220	1

D.3 Measurement of the test solution

D.3.1 Pre-reduction of Se (VI) to Se (IV) of samples.

Add some deionised water and 15 ml concentrated hydrochloric acid to the sample test tube and transfer the test solution to a 50-ml-flask. Then heat the solution (40 ml of solution) for 30 min at 100°C [12]. After cooling dilute the solution using 3,6 M hydrochloric acid.

D.3.2 Atomic absorption spectrometer (HGAAS-procedure)

The test solution is measured using HGAAS as described in section 6.2.3.

The test solution can also be determined by using FI-HGAAS or HG-ICP-AES according to user's equipment and recommendations from the manufacturer.

D.4 Ten rules for automated wet ashing with perchloric acid

1. Elaborate always a new temperature program and a suitable mixture of the oxidizing acids for material with unknown chemical properties. The development has to be tested stepwise and by visual control.

2. Max 5 g material wet wt (1 g DM) and containing no more than 500 mg fat are allowed when using 15 ml oxidizing acid mixture (HNO_3/$HClO_4$: 7/3 (v/v)).

3. Add oxidizing acids to the samples to digest always in form of a mixture. Never separately!

4. Mark the meniscus on the tube for control of decreasing acid mixture during the ashing procedure.

5. Digest samples at ambient temperature for 3-5 hours before starting the ashing program.

6. Prevent bumping of acid solution at boiling. It is disastrous for the analysis!

7. Solubilise fat and fatty acids at 132°C until homogeneity of phases.

8. Dark colour during digestion indicates danger. Remove the tubes from the block and repeat the digestion after addition of HNO_3. Selenium can be lost by charring.

9. Stop digestion temperature at 180°C in the morning. Rise temperature to 225°C and digest according to the program, only tubes with light coloured solutions.

10. Hoods are made from polypropylene, exhaust tubes and fan of PVC. Wash regularly the whole system from hood to the fan with water.

Bibliography

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[4] EN 14627:2005, *Foodstuffs – Determination of trace elements – Determination of total arsenic and selenium by hydride generation atomic absorption spectrometry (HGAAS) after pressure digestion*

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[6] CEN/TS 15621:2007, *Animal feeding stuffs – Determination of minerals and trace elements by ICP-OES after microwave digestion*

[7] VDLUFA, chapter VII, 2.1.3 (2007) – *Microwave heated pressure digestion procedure*

[8] VDLUFA, chapter VII, 2.2.2.4 (2003) – *Determination of selenium in feeding stuffs after pressure digestion*

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[10] ISO 17379-2:2006, *Water quality – Determination of selenium – Part 2: Method using hydride generation atomic absorption spectrometry (HGAAS)*

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ANNEXURE 6

Annexure 6: Eurachem Quantifying Uncertainties. Page 1 of 2

4. The Process of Measurement Uncertainty Estimation

4.1. Uncertainty estimation is simple in principle. The following paragraphs summarise the tasks that need to be performed in order to obtain an estimate of the uncertainty associated with a measurement result. Subsequent chapters provide additional guidance applicable in different circumstances, particularly relating to the use of data from method validation studies and the use of formal uncertainty propagation principles. The steps involved are:

Step 1. Specify measurand

Write down a clear statement of what is being measured, including the relationship between the measurand and the input quantities (*e.g.* measured quantities, constants, calibration standard values *etc.*) upon which it depends. Where possible, include corrections for known systematic effects. The specification information should be given in the relevant Standard Operating Procedure (SOP) or other method description.

Step 2. Identify uncertainty sources

List the possible sources of uncertainty. This will include sources that contribute to the uncertainty on the parameters in the relationship specified in Step 1, but may include other sources and must include sources arising from chemical assumptions. A general procedure for forming a structured list is suggested at Appendix D.

Step 3. Quantify uncertainty components

Measure or estimate the size of the uncertainty component associated with each potential source of uncertainty identified. It is often possible to estimate or determine a single contribution to uncertainty associated with a number of separate sources. It is also important to consider whether available data accounts sufficiently for all sources of uncertainty, and plan additional experiments and studies carefully to ensure that all sources of uncertainty are adequately accounted for.

Step 4. Calculate combined uncertainty

The information obtained in step 3 will consist of a number of quantified contributions to overall uncertainty, whether associated with individual sources or with the combined effects of several sources. The contributions have to be expressed as standard deviations, and combined according to the appropriate rules, to give a combined standard uncertainty. The appropriate coverage factor should be applied to give an expanded uncertainty.

Figure 1 shows the process schematically.

4.2. The following chapters provide guidance on the execution of all the steps listed above and shows how the procedure may be simplified depending on the information that is available about the combined effect of a number of sources.

ANNEXURE 7

Annexure 7: UKAS Expression of Uncertainty. Page 1 of 4

7 STEP BY STEP PROCEDURE FOR EVALUATION OF MEASUREMENT UNCERTAINTY

The following is a guide to the use of this code of practice for the treatment of uncertainties. The left hand column gives the general case while the right hand column indicates how this relates to example K4 in Appendix K. Although this example relates to a calibration activity, the process for testing activities is unchanged.

	General case	Example K4: Calibration of a weight of nominal value 10 kg of OIML Class M1
7.1	If possible determine the mathematical relationship between values of the input quantities and that of the measurand: $y = f(x_1, x_2 \ldots x_N)$ See Appendix D for details.	It will be assumed that the unknown weight, W_X, can be obtained from the following relationship: $W_X = W_S + D_S + \delta l_d + \delta C + Ab$
7.2	Identify all corrections that have to be applied to the results of measurements of a quantity (measurand) for the stated conditions of measurement.	It is not normal practice to apply corrections for this class of weight and the comparator has no measurable linearity error, however, uncertainties for these contributions have been determined, therefore: Drift of standard mass since last calibration: 0 Correction for air buoyancy: 0 Linearity correction: 0 Effect of least significant digit resolution: 0

Annexure 7: UKAS Expression of Uncertainty. Page 2 of 4

	General case	Example K4: Calibration of a weight of nominal value 10 kg of OIML Class M1
7.3	List systematic components of uncertainty associated with corrections and uncorrected systematic errors treated as uncertainties. Seek prior experimental work or theory as a basis for assigning uncertainties and probability distributions to the systematic components of uncertainty. Calculate the standard uncertainty for each component of uncertainty, obtained from Type B evaluations, as in Table 1. For assumed rectangular distributions: $u(x_i) = \frac{a_i}{\sqrt{3}}$ For assumed triangular distributions: $u(x_i) = \frac{a_i}{\sqrt{6}}$ For assumed normal distributions: $u(x_i) = \frac{U}{k}$ or consult other documents if the assumed probability distribution is not covered in this publication.	Source of uncertainty / Limit (mg) / Distribution W_S Calibration of std. mass — 30 — Normal (k = 2) D_S Drift of standard mass — 30 — Rectangular δC Comparator linearity — 3 — Rectangular δAb Air buoyancy — 10 — Rectangular δI_d Resolution effects — 10 — Triangular Then: $u(x_1) = u(W_S) = \frac{30}{2} = 15$ mg $u(x_2) = u(D_S) = \frac{30}{\sqrt{3}} = 17.32$ mg $u(x_3) = u(\delta C) = \frac{3}{\sqrt{3}} = 1.73$ mg $u(x_4) = u(Ab) = \frac{10}{\sqrt{3}} = 5.77$ mg $u(x_5) = u(\delta I_d) = \frac{10}{\sqrt{6}} = 4.08$ mg
7.4	Use prior knowledge or make trial measurements and calculations to determine if there is going to be a random component of uncertainty that is significant compared with the effect of the listed systematic components of uncertainty. Random components of uncertainty also have to be considered as input quantities.	From previous knowledge of the measurement system it is known that there is a significant random component of uncertainty.
7.5	If a random component of uncertainty is significant make repeated measurements to obtain the mean from Equation (3): $\bar{q} = \frac{1}{n}\sum_{j=1}^{n} q_j$	Three measurements were made of the difference between the unknown weight and the standard weight, from which the mean difference was calculated: $\overline{W_S} = \frac{0.015 + 0.025 + 0.020}{3} = 0.020$ g

Annexure 7: UKAS Expression of Uncertainty. Page 3 of 4

	General case	Example K4: Calibration of a weight of nominal value 10 kg of OIML Class M1
7.6	Either calculate the standard deviation of the mean value from Equations (5) and (6): $s(q_j) = \sqrt{\frac{1}{n-1}\sum_{j=1}^{n}(q_j - \bar{q})^2}$ $s(\bar{q}) = \frac{s(q_j)}{\sqrt{n}}$ or refer to the results of previous repeatability evaluations for an estimate of $s(q_j)$ based on a larger number of readings, using Equation (7): $s(q_j) = \sqrt{\frac{1}{m-1}\sum_{j=1}^{m}(q_j - \bar{q})^2}$ $s(\bar{q}) = \frac{s(q_j)}{\sqrt{n}}$ where m is the number of readings used in the prior evaluation and n is the number of readings that contribute to the mean value.	A previous Type A evaluation had been made to determine the repeatability of the comparison using the same type of 10 kg weights. The standard deviation was determined from 10 measurements using the conventional bracketing technique and was calculated, using Equation (5), to be 8.7 mg. Since the number of determinations taken when calibrating the unknown weight was 3 this is the value of n that is used to calculate the standard deviation of the mean using Equation (6): $s(\overline{W}_X) = \frac{s(W)}{\sqrt{n}} = \frac{8.7}{\sqrt{3}} = 5.0$ mg
7.7	Even when a random component of uncertainty is not significant, where possible check the instrument indication at least once to minimise the possibility of unexpected errors.	
7.8	Derive the standard uncertainty for the above Type A evaluation from Equation (8): $u(x_i) = s(\bar{q})$	This is then the standard uncertainty for the Type A evaluation: $u(x_6) = u(\overline{W}_R) = s(\overline{W}_R) = 5.0$ mg

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Annexure 7: UKAS Expression of Uncertainty. Page 4 of 4

	General case	Example K4: Calibration of a weight of nominal value 10 kg of OIML Class M1
7.9	Calculate the combined standard uncertainty for uncorrelated input quantities using Equation (1) if absolute values are used: $u_c(y) = \sqrt{\sum_{i=1}^{N} c_i^2 u^2(x_i)} \equiv \sqrt{\sum_{i=1}^{N} u_i^2(y)}$, where c_i is the partial derivative $\partial f / \partial x_i$, or a known sensitivity coefficient. Alternatively use Equation (11) if the standard uncertainties are relative values: $\frac{u_c(y)}{\lvert y \rvert} = \sqrt{\sum_{i=1}^{N} \left[\frac{p_i u(x_i)}{\lvert x_i \rvert} \right]^2}$, where p_i are known positive or negative exponents in the functional relationship.	The units of all standard uncertainties are in terms of those of the measurand, i.e. milligrams, and the functional relationship between the input quantities and the measurand is a linear summation; therefore all the sensitivity coefficients are unity (c_i=1).
7.10	If correlation is suspected use the guidance in paragraph D3 or consult other referenced documents.	None of the input quantities is considered to be correlated to any significant extent; therefore Equation (1) can be used to calculate the combined standard uncertainty: $u(W_X) = \sqrt{15^2 + 17.32^2 + 4.08^2 + 1.73^2 + 5.77^2 + 5.0^2}$ = 24.55 mg.
7.11	Either calculate an expanded uncertainty from Equation (2): $U = k \cdot u_c(y)$ or, if there is a significant random contribution evaluated from a small number of readings, use Appendix B to calculate a value for k_p and use this value to calculate the expanded uncertainty.	U = 2 x 24.55 mg = 49.10 mg It was not necessary to use Appendix B to determine a value for k_p. In fact the effective degrees of freedom of $u(W_X)$ are greater than 5000 which gives a value for k_{95} = 2.00.
7.12	Report the result and the expanded uncertainty in accordance with Section 6.	The measured value of the 10 kg weight is 10 000.025 g ± 0.049 g. The reported expanded uncertainty is based on a standard uncertainty multiplied by a coverage factor k = 2, providing a coverage probability of approximately 95%. The uncertainty evaluation has been carried out in accordance with UKAS requirements.

ANNEXURE 8

Annexure 8: UKAS Uncertainties for Test Results. Page 1 of 4

APPENDIX N
UNCERTAINTIES FOR TEST RESULTS

N1 Introduction

N1.1 ISO/IEC 17025:2005 requires that "testing laboratories shall have and apply procedures for estimating uncertainty of measurement".

N1.2 It is recognised that the present state of development and application of uncertainties in testing activities is not as comprehensive as in the calibration fields, to which much of this document is addressed. It is therefore accepted that the implementation of ISO/IEC 17025:2005 criteria on this subject will take place at an appropriate pace, which may differ from one field to another. However laboratories should be able to satisfy requests from clients, or requirements of specifications, to provide statements of uncertainty.

N1.3 Testing laboratories should therefore have a defined policy covering the evaluation and reporting of the uncertainties associated with the tests performed. The laboratory should use documented procedures for the evaluation, treatment and reporting of the uncertainty.

N1.4 Some tests are qualitative in nature, i.e., they do not yield a numeric result. Therefore there can be no meaning in reporting uncertainties directly associated with the test result. Nevertheless, there will be uncertainties associated with the underlying test conditions and these should be subject to the same type of evaluation as is required for quantitative test results.

N1.5 The methodology for estimation of uncertainty in testing is no different from that in calibration and therefore the procedures described in this document apply equally to testing results.

N2 Objectives

N2.1 The objective of a measurement is to determine the value of the measurand, ie the specific quantity subject to measurement. When applied to testing, the general term measurand may cover many different quantities, for example:

the electrical breakdown characteristics of an insulating material;

the strength of a material;

the concentration of an analyte;

the level of emissions of electromagnetic radiation from an appliance;

the quantity of micro-organisms in a food sample;

the susceptibility of an appliance to electric or magnetic fields;

the quantity of asbestos particles in a sample of air.

N2.2 A measurement begins with an appropriate specification of the measurand, the generic method of measurement and the specific detailed measurement procedure. Knowledge of the influence quantities involved for a given procedure is important so that the sources of uncertainty can be identified.

Annexure 8: UKAS Uncertainties for Test Results. Page 2 of 4

N3 Sources of uncertainty

N3.1 There are many possible sources of uncertainty. As these will depend on the nature of the tests involved, it is not possible to give detailed guidance here. However the following general points will apply to many areas of testing:

(a) Incomplete definition of the test - the requirement may not be clearly described, e.g. the temperature of a test may be given as 'room temperature'.

(b) Imperfect realisation of the test procedure; even when the test conditions are clearly defined it may not be possible to produce the theoretical conditions in practice due to unavoidable imperfections in the materials or systems used.

(c) Sampling - the sample may not be fully representative. In some disciplines, such as microbiological testing, it can be very difficult to obtain a representative sample.

(d) Inadequate knowledge of the effects of environmental conditions on the measurement process, or imperfect measurement of environmental conditions.

(e) Personal bias and human factors; for example:

- Reading of scales on analogue indicating instruments.

- Judgement of colour.

- Reaction time, e.g. when using a stopwatch.

- Instrument resolution or discrimination threshold, or errors in graduation of a scale.

(f) Values assigned to measuring equipment and reference materials.

(g) Changes in the characteristics or performance of measuring equipment or reference materials since the last calibration.

(h) Values of constants and other parameters used in data evaluation.

(i) Approximations and assumptions incorporated in the measurement method and procedure.

(j) Variations in repeated observations made under similar but not identical conditions - such random effects may be caused by, for example, electrical noise in measuring instruments, short-term fluctuations in the local environment, e.g. temperature, humidity and air pressure, variability in the performance of the person carrying out the test and variability in the homogeneity of the sample itself.

N3.2 These sources are not necessarily independent and, in addition, unrecognised systematic effects may exist that cannot be taken into account but contribute to error. This is one reason that participation in inter-laboratory comparisons, participation in proficiency testing schemes and internal cross-checking of results by different means are encouraged.

N3.3 Information on some of the sources of these errors can be obtained from:

(a) Data in calibration certificates - this enables corrections to be made and uncertainties to be assigned.

(b) Previous measurement data - for example, history graphs can be constructed and can yield useful information about changes with time.

Annexure 8: UKAS Uncertainties for Test Results. Page 3 of 4

(c) Experience with or general knowledge about the behaviour and properties of similar materials and equipment.

(d) Accepted values of constants associated with materials and quantities.

(e) Manufacturers' specifications.

(f) All other relevant information.

These are all referred to as Type B evaluations because the values were not obtained by statistical means. However the influence of random effects is often evaluated by the use of statistics; if this is the case then the evaluation is designated Type A.

N3.4 Definitions are given in paragraph 3.10 for Type A evaluations and in paragraph 3.11 for Type B evaluations. Further detail on the means of evaluation is given in Sections 4 and 5.

N3.5 It is recognised that in certain areas of testing it may be known that a significant contribution to uncertainty exists but that the nature of the test precludes a rigorous evaluation of this contribution. In such cases, ISO/IEC 17025:2005 requires that a reasonable estimation be made and that the form of the reporting does not give an incorrect impression of the uncertainty.

N3.6 In some fields of testing it may be the case that the contribution of measuring instruments to the overall uncertainty can be demonstrated to be insignificant when compared with the repeatability of the process. Nevertheless, such instruments have to be shown to comply with the relevant specifications, normally by calibration.

N3.7 Some analysis processes appear at first sight to be quite complex, for example there may be various stages of weighing, dilutions and processing before results are obtained. However it will sometimes be the case that the procedure requires standard reference materials to be subject to the same process, the result being the difference between the readings for the analyte and the reference material. In such cases, much of the process can be considered to be negatively correlated and the uncertainty of measurement can be evaluated from the resolution and repeatability of the process; matrix effects may also have to be considered.

N4 Process

N4.1 The process of assigning a value of uncertainty to a measurement result is summarised below:

(a) Identify all sources of error that are likely to have a significant effect, and their relationship with the measurand.

(b) Assign values to these using information such as described in N3.3, or in the case of Type A evaluations, calculate the standard deviation using Equations (5) and (6).

(c) Consider each uncertainty component and decide whether any are interrelated and whether a dominant component exists (see D3 and Appendix C respectively).

(d) Add any interdependent components algebraically (i.e., account for whether they act together or cancel each other) and derive a net value.

(e) Express each uncertainty value as the equivalent of a standard deviation (see paragraph 3.24), taking into account any sensitivity coefficients (see paragraphs 3.28 to 3.35).

(f) Take the independent components and the values of any derived net components and, in the absence of a dominant component, combine them by taking the square root of the sum of the squares. This gives the combined standard uncertainty (Equation (1)).

Annexure 8: UKAS Uncertainties for Test Results. Page 4 of 4

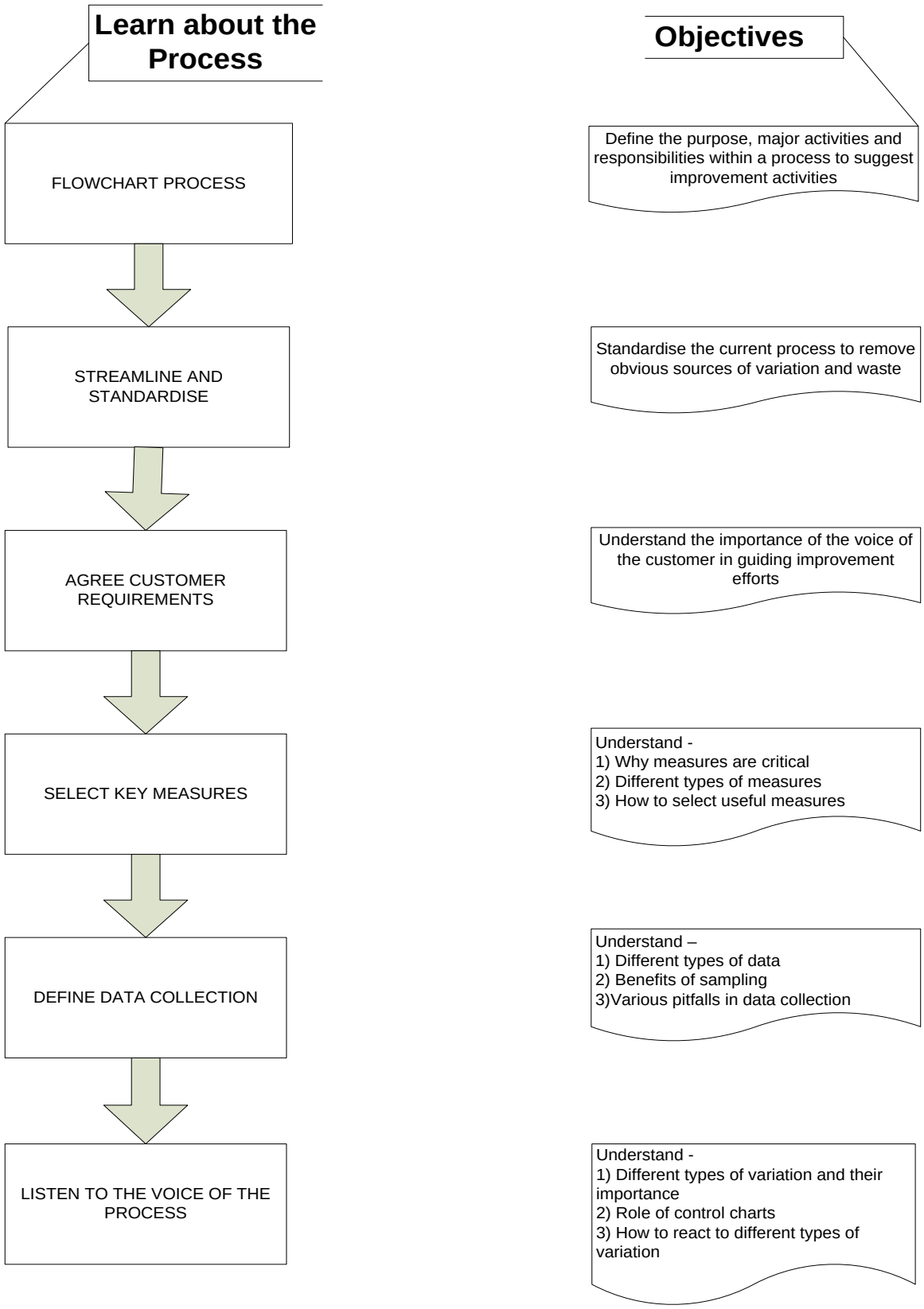
(g) Multiply the combined standard uncertainty by a coverage factor *k*, selected on the basis of the coverage probability required, to provide the expanded uncertainty *U* (see paragraphs 3.42 to 3.44).

(h) Report the result and, if required, the expanded uncertainty, coverage factor and coverage probability in accordance with Section 6.

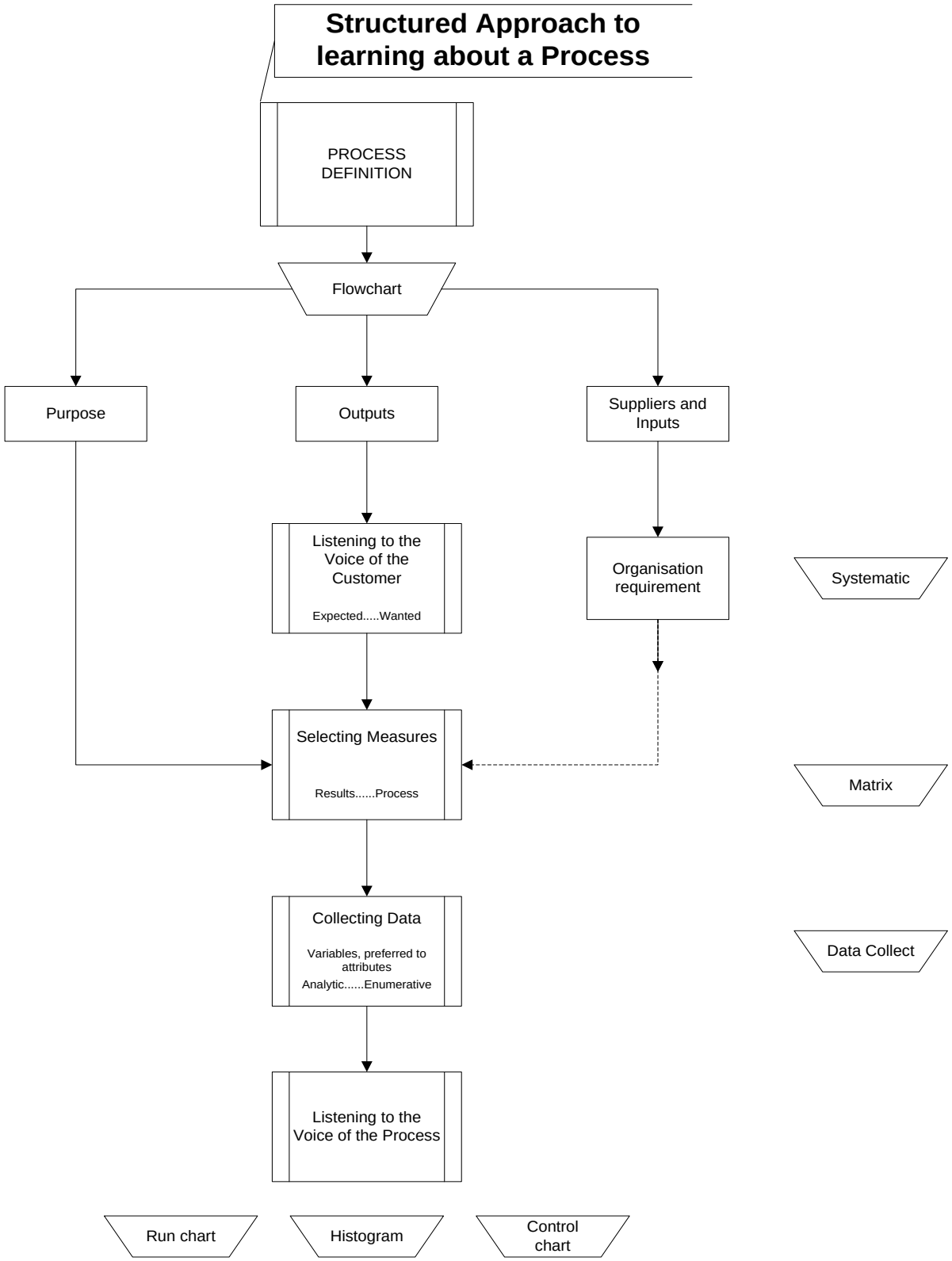
N4.2 If one uncertainty contribution is significantly larger than the others then modifications may be required to this procedure. In the case of a dominant component derived from Type B evaluation, see Appendix C. If the non-repeatability of the system is significant, and its effects are evaluated by using a Type A analysis, it may be necessary to use the procedure in Appendix B.

N4.3 Further information regarding uncertainty evaluation for testing activities can be obtained from specialist publications that address particular fields of testing, such as are described in References [7] and [8].

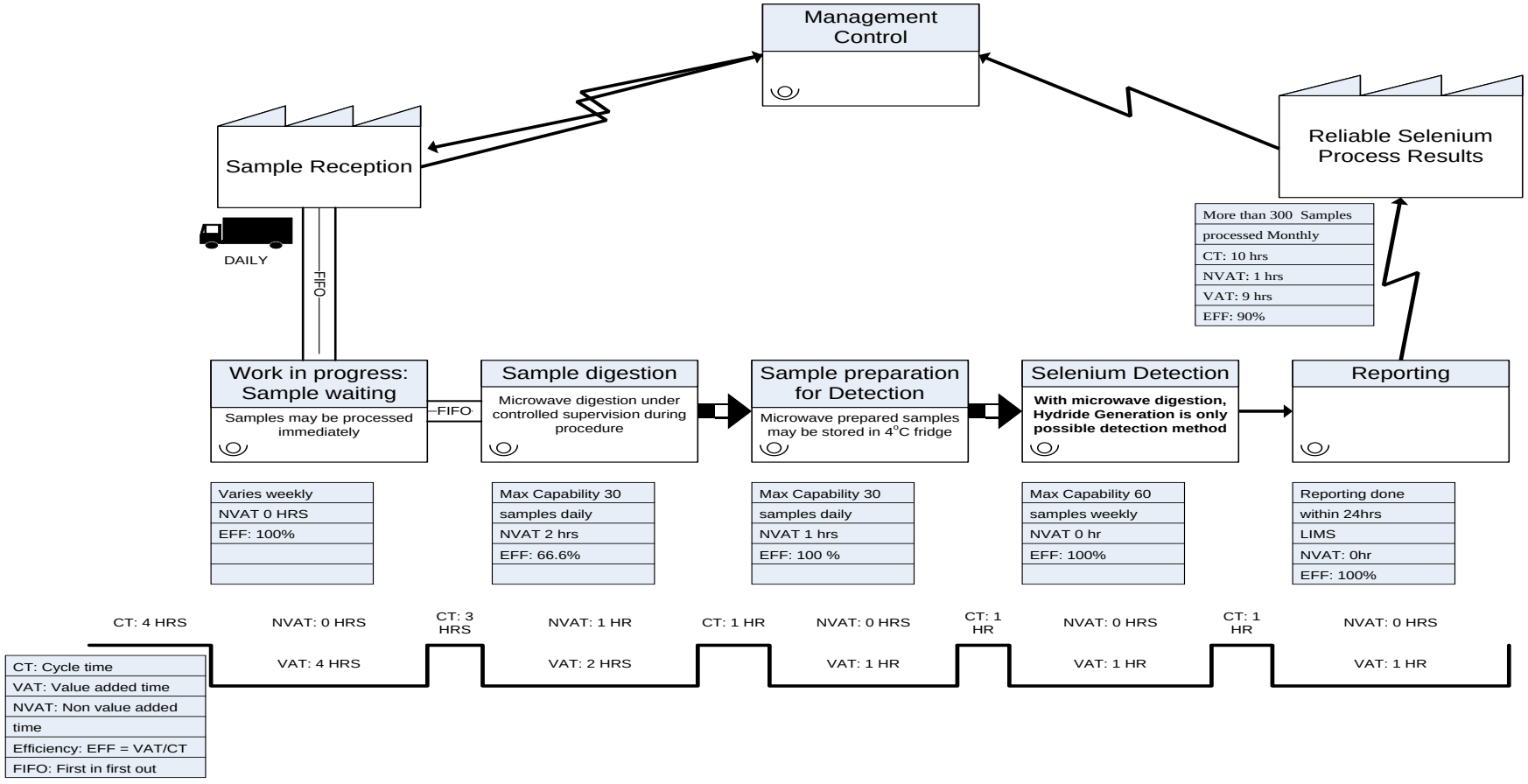
ANNEXURE 9A: Six Sigma: Learn about the Process (**Source**: Process Management International, 2009)



ANNEXURE 9B: Six Sigma: Learn about the Process (**Source:** Process Management International, 2009)



ANNEXURE 10: Value Stream Map of Future State Selenium Analysis Process (**Source**: Own Source)



ANNEXURE 11A: Current State Selenium Process Run, 8 June 2011

Fl Concentration results
Generated on 06-08-2011
at time 15:39:14

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.06.08.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	1.368	19.064	1.00	
10ppb	10.000	1.872	19.064	1.00	
10ppb	10.000	-1.191	19.064	1.00	
50ppb	50.000	5.089	19.064	1.00	
50ppb	50.000	5.805	19.064	1.00	
50ppb	50.000	5.990	19.064	1.00	
100ppb	100.000	14.410	19.064	1.00	
100ppb	100.000	14.075	19.064	1.00	
100ppb	100.000	14.297	19.064	1.00	
250ppb	250.000	43.163	19.064	1.00	
250ppb	250.000	40.814	19.064	1.00	
250ppb	250.000	38.765	19.064	1.00	
500ppb	500.000	88.765	19.064	1.00	
500ppb	500.000	83.676	19.064	1.00	
500ppb	500.000	83.391	19.064	1.00	

Fit equation:
Y = 0.175 x + -2.454
Correlation 0.9985
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC2009	145.632	22.997	16.479	1.00	liver	
1	55.417	7.231	16.479	1.00	liver	
2	124.775	19.352	16.479	1.00	liver	
3	136.133	21.337	16.479	1.00	liver	
4	151.309	23.989	16.479	1.00	liver	
5	1434.881	248.305	16.479	1.00	liver	
6	149.197	23.620	16.479	1.00	liver	
7	667.768	114.245	16.801	1.00	liver	
8	90.122	13.296	16.479	1.00	liver	
9	169.705	27.204	16.801	1.00	liver	
10	139.286	21.888	16.801	1.00	liver	
x	1417.445	245.258	16.801	1.00	liver	
xx	637.137	108.892	16.801	1.00	liver	
blank	32.448	3.217	16.801	1.00	standards	
10ppm	21.330	1.274	16.801	1.00	standards	
50ppm	62.169	8.411	16.801	1.00	standards	
100ppm	110.996	16.944	16.801	1.00	standards	
250ppm	248.551	40.983	16.801	1.00	standards	
5000ppm	555.018	94.541	16.801	1.00	standards	

ANNEXURE 11B: Current State Selenium Process Run, 14 June 2011

Fl Concentration results
Generated on 06-14-2011
at time 16:28:54

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.06.14.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	0.434	16.722	1.00	
10ppb	10.000	0.529	16.722	1.00	
10ppb	10.000	0.387	16.722	1.00	
50ppb	50.000	2.402	16.722	1.00	
50ppb	50.000	2.735	16.722	1.00	
50ppb	50.000	2.527	16.722	1.00	
100ppb	100.000	6.111	16.722	1.00	
100ppb	100.000	6.159	16.722	1.00	
100ppb	100.000	6.778	16.722	1.00	
250ppb	250.000	14.912	16.722	1.00	
250ppb	250.000	15.661	16.722	1.00	
250ppb	250.000	14.874	16.722	1.00	
500ppb	500.000	29.609	16.722	1.00	
500ppb	500.000	29.991	16.722	1.00	
500ppb	500.000	30.184	16.722	1.00	

Fit equation:
Y = 0.06 x + -0.083
Correlation 0.9994
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC2009	149.062	8.901	16.852	1.00	liver	
1 trial	45.844	2.680	16.852	1.00	liver	
2 trial	131.226	7.826	16.852	1.00	liver	
3 trial	147.021	8.778	16.852	1.00	liver	
4 trial	135.954	8.111	16.852	1.00	liver	
5 trial	1805.339	108.726	16.852	1.00	liver	
6 trial	161.356	9.642	16.852	1.00	liver	
7 trial	785.308	47.248	16.852	1.00	liver	
8 trial	49.412	2.895	16.852	1.00	liver	
9 trial	188.733	11.292	16.852	1.00	liver	
10 trial	293.693	17.618	16.852	1.00	liver	
BL	22.649	1.282	16.852	1.00	blood	
10	37.532	2.179	16.852	1.00	blood	
50	77.352	4.579	16.852	1.00	blood	
100	139.837	8.345	16.852	1.00	blood	
250	328.967	19.744	16.852	1.00	blood	
500	630.141	37.896	16.852	1.00	blood	

ANNEXURE 11C: Current State Selenium Process Run, 21 June 2011

Fl Concentration results
Generated on 06-21-2011
at time 15:50:52

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.06.21.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	2.006	16.121	1.00	
10ppb	10.000	2.045	16.121	1.00	
10ppb	10.000	1.884	16.121	1.00	
50ppb	50.000	8.899	16.121	1.00	
50ppb	50.000	8.937	16.121	1.00	
50ppb	50.000	8.704	16.121	1.00	
100ppb	100.000	17.563	16.121	1.00	
100ppb	100.000	17.472	16.121	1.00	
100ppb	100.000	17.852	16.121	1.00	
250ppb	250.000	43.611	16.121	1.00	
250ppb	250.000	44.962	16.121	1.00	
250ppb	250.000	45.031	16.121	1.00	
500ppb	500.000	89.496	16.121	1.00	
500ppb	500.000	88.963	16.121	1.00	
500ppb	500.000	90.119	16.121	1.00	

Fit equation:
Y = 0.179 x + -0.072
Correlation 0.9999
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC	134.694	24.036	16.768	1.00	liver	
1	45.350	8.045	16.768	1.00	liver	
2	122.235	21.806	16.768	1.00	liver	
3	136.303	24.324	16.768	1.00	liver	
4	130.811	23.341	16.768	1.00	liver	
5	1458.633	260.997	16.768	1.00	liver	
6	135.007	24.092	16.768	1.00	liver	
7	629.917	112.672	16.768	1.00	liver	
8	58.289	10.361	16.768	1.00	liver	
9	170.753	30.490	16.768	1.00	liver	
10	264.562	47.280	16.768	1.00	liver	
x	134.320	23.969	16.768	1.00	liver	
xx	132.448	23.634	16.768	1.00	liver	

ANNEXURE 11D: Current State Selenium Process Run, 29 June 2011

Fl Concentration results
Generated on 06-29-2011
at time 16:20:23

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.06.29.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	1.522	15.892	1.00	
10ppb	10.000	1.578	15.892	1.00	
10ppb	10.000	1.639	15.892	1.00	
50ppb	50.000	9.038	15.892	1.00	
50ppb	50.000	8.849	15.892	1.00	
50ppb	50.000	8.589	15.892	1.00	
100ppb	100.000	18.949	15.892	1.00	
100ppb	100.000	19.437	15.892	1.00	
100ppb	100.000	19.892	15.892	1.00	
250ppb	250.000	45.971	15.892	1.00	
250ppb	250.000	44.418	15.892	1.00	
250ppb	250.000	46.724	15.892	1.00	
500ppb	500.000	95.604	15.892	1.00	
500ppb	500.000	94.773	15.892	1.00	
500ppb	500.000	87.928	15.892	1.00	

Fit equation:
Y = 0.185 x + -0.095
Correlation 0.9987
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	118.535	21.890	15.592	1.00	liver	
1	51.182	9.398	15.592	1.00	liver	
2	126.887	23.439	15.592	1.00	liver	
3	138.070	25.513	15.592	1.00	liver	
4	128.219	23.686	15.592	1.00	liver	
5	1471.848	272.888	15.592	1.00	liver	
6	134.360	24.825	15.592	1.00	liver	
7	613.744	113.736	15.592	1.00	blood	
8	59.706	10.979	15.592	1.00	blood	
9	169.671	31.374	15.592	1.00	blood	
10	269.207	49.835	15.592	1.00	blood	
x	120.697	22.291	15.592	1.00	liver	
xx	119.436	22.057	15.592	1.00	liver	
LC Renee	119.743	22.114	15.592	1.00	liver	

ANNEXURE 11E: Current State Selenium Process Run, 5 July 2011

Fl Concentration results
Generated on 07-05-2011
at time 17:11:24

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.07.05.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	1.796	22.987	1.00	
10ppb	10.000	1.507	22.987	1.00	
10ppb	10.000	0.601	22.987	1.00	
50ppb	50.000	8.245	22.987	1.00	
50ppb	50.000	8.088	22.987	1.00	
50ppb	50.000	8.485	22.987	1.00	
100ppb	100.000	17.139	22.987	1.00	
100ppb	100.000	20.462	22.987	1.00	
100ppb	100.000	17.338	22.987	1.00	
250ppb	250.000	47.281	22.987	1.00	
250ppb	250.000	49.328	22.987	1.00	
250ppb	250.000	46.796	22.987	1.00	
500ppb	500.000	94.143	22.987	1.00	
500ppb	500.000	94.671	22.987	1.00	
500ppb	500.000	95.465	22.987	1.00	

Fit equation:
Y = 0.192 x + -0.793
Correlation 0.9996
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	120.477	22.298	21.815	1.00	liver	
1	55.630	9.869	21.815	1.00	liver	
2	121.364	22.468	21.815	1.00	liver	
3	147.113	27.403	21.815	1.00	liver	
4	132.003	24.507	21.815	1.00	liver	
5	1419.938	271.358	21.815	1.00	liver	
6	146.419	27.270	21.815	1.00	liver	
7	643.825	122.605	21.815	1.00	liver	
8	55.165	9.780	21.815	1.00	liver	
9	166.522	31.123	21.815	1.00	liver	
10	256.424	48.354	21.815	1.00	liver	
Std10	-13.429	-3.367	21.815	1.00	blood	
Std50	19.160	2.879	21.815	1.00	blood	
Std100	64.531	11.575	21.815	1.00	blood	
Std250	221.305	41.623	21.815	1.00	blood	
Std500	460.186	87.408	21.815	1.00	blood	
x	95.126	17.439	21.815	1.00	blood	
xx	93.268	17.083	21.815	1.00	blood	

ANNEXURE 11F: Current State Selenium Process Run, 12 July 2011

Fl Concentration results
Generated on 07-12-2011
at time 15:34:36

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.07.12.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	1.596	16.470	1.00	
10ppb	10.000	1.489	16.470	1.00	
10ppb	10.000	1.919	16.470	1.00	
50ppb	50.000	8.634	16.470	1.00	
50ppb	50.000	9.238	16.470	1.00	
50ppb	50.000	8.630	16.470	1.00	
100ppb	100.000	18.832	16.470	1.00	
100ppb	100.000	17.989	16.470	1.00	
100ppb	100.000	18.027	16.470	1.00	
250ppb	250.000	43.326	16.470	1.00	
250ppb	250.000	43.356	16.470	1.00	
250ppb	250.000	46.381	16.470	1.00	
500ppb	500.000	86.236	16.470	1.00	
500ppb	500.000	87.869	16.470	1.00	
500ppb	500.000	84.272	16.470	1.00	

Fit equation:
Y = 0.172 x + 0.525
Correlation 0.9994
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	131.522	23.164	16.266	1.00	liver	
1	45.496	8.356	16.266	1.00	liver	
2	126.480	22.296	16.266	1.00	liver	
3	121.048	21.361	16.266	1.00	liver	
4	135.972	23.930	16.266	1.00	liver	
5	1405.282	242.421	16.266	1.00	liver	
6	141.555	24.891	16.266	1.00	liver	
7	601.012	103.979	16.266	1.00	liver	
8	66.305	11.938	16.266	1.00	liver	
9	184.214	32.234	16.266	1.00	liver	
10	236.818	41.289	16.266	1.00	liver	
x	129.541	22.823	16.266	1.00	blood	
xx	128.548	22.652	16.266	1.00	blood	

ANNEXURE 11G: Current State Selenium Process Run, 21 July 2011

Fl Concentration results
Generated on 07-21-2011
at time 16:56:40

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.07.21.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	0.822	18.669	1.00	
10ppb	10.000	0.729	18.669	1.00	
10ppb	10.000	0.930	18.669	1.00	
50ppb	50.000	8.805	18.669	1.00	
50ppb	50.000	8.904	18.669	1.00	
50ppb	50.000	8.727	18.669	1.00	
100ppb	100.000	17.330	18.669	1.00	
100ppb	100.000	17.782	18.669	1.00	
100ppb	100.000	17.873	18.669	1.00	
250ppb	250.000	49.947	18.669	1.00	
250ppb	250.000	47.140	18.669	1.00	
250ppb	250.000	46.827	18.669	1.00	
500ppb	500.000	96.126	18.669	1.00	
500ppb	500.000	99.309	18.669	1.00	
500ppb	500.000	96.953	18.669	1.00	

Fit equation:
Y = 0.198 x + -1.403
Correlation 0.9996
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	124.864	23.261	18.156	1.00	liver	
LC2009 2	155.710	29.354	18.156	1.00	liver	
CRM	67.449	11.920	18.156	1.00	liver	
1	48.651	8.207	18.156	1.00	liver	
2	130.483	24.371	18.156	1.00	liver	
3	127.972	23.875	18.156	1.00	liver	
4	128.731	24.025	18.156	1.00	liver	
5	1388.027	272.769	18.156	1.00	liver	
6	134.427	25.150	18.156	1.00	liver	
7	591.596	115.453	18.156	1.00	liver	
8	56.584	9.774	18.156	1.00	liver	
9	164.403	31.071	18.156	1.00	liver	
10	252.082	48.390	18.156	1.00	liver	
x	123.887	23.068	18.156	1.00	blood	
xx	68.000	12.029	18.156	1.00	blood	

ANNEXURE 11H: Current State Selenium Process Run, 29 July 2011

Fl Concentration results
Generated on 07-29-2011
at time 14:51:08

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.07.29.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	1.913	17.621	1.00	
10ppb	10.000	1.918	17.621	1.00	
10ppb	10.000	1.483	17.621	1.00	
50ppb	50.000	10.478	17.621	1.00	
50ppb	50.000	9.583	17.621	1.00	
50ppb	50.000	10.371	17.621	1.00	
100ppb	100.000	21.315	17.621	1.00	
100ppb	100.000	18.764	17.621	1.00	
100ppb	100.000	21.139	17.621	1.00	
250ppb	250.000	51.916	17.621	1.00	
250ppb	250.000	50.878	17.621	1.00	
250ppb	250.000	47.563	17.621	1.00	
500ppb	500.000	96.018	17.621	1.00	
500ppb	500.000	94.267	17.621	1.00	
500ppb	500.000	95.390	17.621	1.00	

Fit equation:
Y = 0.19 x + 0.872
Correlation 0.9991
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	113.010	22.394	17.682	1.00	liver	
CRM	53.287	11.020	17.682	1.00	liver	
1	43.579	9.171	17.682	1.00	liver	
2	121.500	24.011	17.682	1.00	liver	
3	113.419	22.472	17.682	1.00	liver	
4	128.179	25.283	17.682	1.00	liver	
5	1390.454	265.681	17.682	1.00	liver	
6	141.710	27.860	17.682	1.00	liver	
7	657.011	125.998	17.682	1.00	liver	
8	47.559	9.929	17.682	1.00	liver	
9	131.104	25.840	17.682	1.00	liver	
10	257.753	49.960	17.682	1.00	liver	
x	106.567	21.167	17.959	1.00	blood	
xx	74.800	15.117	17.959	1.00	blood	

ANNEXURE 11I: Current State Selenium Process Run, 3 August 2011

Fl Concentration results
Generated on 08-03-2011
at time 14:41:33

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.08.03.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	1.901	16.575	1.00	
10ppb	10.000	1.638	16.575	1.00	
10ppb	10.000	2.064	16.575	1.00	
50ppb	50.000	9.134	16.575	1.00	
50ppb	50.000	10.648	16.575	1.00	
50ppb	50.000	8.962	16.575	1.00	
100ppb	100.000	17.525	16.575	1.00	
100ppb	100.000	17.897	16.575	1.00	
100ppb	100.000	18.837	16.575	1.00	
250ppb	250.000	46.513	16.575	1.00	
250ppb	250.000	44.945	16.575	1.00	
250ppb	250.000	46.681	16.575	1.00	
500ppb	500.000	92.688	16.575	1.00	
500ppb	500.000	96.532	16.575	1.00	
500ppb	500.000	84.928	16.575	1.00	

Fit equation:
Y = 0.183 x + 0.159
Correlation 0.9976
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	133.749	24.582	16.436	1.00	liver	
CRM	53.499	9.928	16.436	1.00	liver	
1	50.120	9.311	16.436	1.00	liver	
2	143.562	26.374	16.436	1.00	liver	
3	136.482	25.081	16.436	1.00	liver	
4	135.551	24.911	16.436	1.00	liver	
5	1382.858	252.675	16.436	1.00	liver	
6	136.920	25.161	16.436	1.00	liver	
7	604.422	110.529	16.436	1.00	liver	
8	84.583	15.604	16.436	1.00	liver	
9	174.164	31.962	16.436	1.00	liver	
10	250.827	45.961	16.436	1.00	liver	
x	129.466	23.800	16.897	1.00	liver	
xx	48.926	9.093	16.897	1.00	liver	

ANNEXURE 11J: Current State Selenium Process Run, 11 August 2011

Fl Concentration results
Generated on 08-11-2011
at time 16:54:17

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.08.11.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	2.183	16.726	1.00	
10ppb	10.000	1.969	16.726	1.00	
10ppb	10.000	2.142	16.726	1.00	
50ppb	50.000	9.762	16.348	1.00	
50ppb	50.000	10.757	16.348	1.00	
50ppb	50.000	9.434	16.726	1.00	
100ppb	100.000	18.737	16.348	1.00	
100ppb	100.000	19.033	16.348	1.00	
100ppb	100.000	22.128	16.348	1.00	
250ppb	250.000	46.657	16.348	1.00	
250ppb	250.000	48.551	16.348	1.00	
250ppb	250.000	47.821	16.348	1.00	
500ppb	500.000	92.113	16.348	1.00	
500ppb	500.000	89.510	16.348	1.00	
500ppb	500.000	96.767	16.348	1.00	

Fit equation:
Y = 0.185 x + 0.912
Correlation 0.9987
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	140.134	26.777	16.828	1.00	liver	
LC2009 2	126.974	24.348	17.267	1.00	liver	
Feed Contro		40.725	8.429	16.828	1.00	feed
Feed CRM		25.625	5.642	16.828	1.00	feed
CRM	50.515	10.236	16.828	1.00	liver	
1	43.054	8.859	16.828	1.00	liver	
2	124.812	23.949	16.828	1.00	liver	
3	130.452	24.990	16.828	1.00	liver	
4	132.944	25.450	16.828	1.00	liver	
5	1383.993	256.357	16.828	1.00	liver	
6	123.425	23.693	16.666	1.00	liver	
7	636.594	118.409	16.666	1.00	liver	
8	60.495	12.078	16.666	1.00	liver	
9	162.813	30.963	16.666	1.00	liver	
10	260.538	49.000	16.666	1.00	liver	
x	128.133	24.562	17.267	1.00	liver	
xx	110.210	21.254	17.267	1.00	liver	

ANNEXURE 11K: Current State Selenium Process Run, 16 August 2011

Fl Concentration results
Generated on 08-16-2011
at time 17:40:55

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.08.16.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor
10ppb	10.000	2.099	16.821	1.00	
10ppb	10.000	1.929	16.821	1.00	
10ppb	10.000	1.536	16.821	1.00	
50ppb	50.000	9.680	16.821	1.00	
50ppb	50.000	10.441	16.821	1.00	
50ppb	50.000	8.556	16.821	1.00	
100ppb	100.000	18.168	16.821	1.00	
100ppb	100.000	18.382	16.821	1.00	
100ppb	100.000	22.166	16.821	1.00	
250ppb	250.000	49.791	16.821	1.00	
250ppb	250.000	48.137	16.821	1.00	
250ppb	250.000	48.497	16.821	1.00	
500ppb	500.000	98.889	16.821	1.00	
500ppb	500.000	96.730	16.821	1.00	
500ppb	500.000	98.313	16.821	1.00	

Fit equation:
Y = 0.196 x + -0.154
Correlation 0.9996
****
Unknown sample results

Std#	Conc*Fact (ppb)		Intens.	BG	Factor	Info
LC 2009	145.480	28.389	16.368	1.00	liver	
1	74.761	14.514	16.368	1.00	liver	
2	139.206	27.158	16.368	1.00	liver	
3	145.439	28.381	16.368	1.00	liver	
4	159.109	31.063	16.368	1.00	liver	
5	1230.140	241.200	16.368	1.00	liver	
6	153.319	29.927	16.368	1.00	liver	
7	572.645	112.199	16.368	1.00	liver	
8	70.108	13.601	16.368	1.00	liver	
9	114.017	22.216	16.368	1.00	liver	
10	73.829	14.331	16.368	1.00	liver	
x	154.104	30.081	16.368	1.00	liver	
xx	152.728	29.811	16.368	1.00	liver	

ANNEXURE 11L: Current State Selenium Process Run, 24 August 2011

Fl Concentration results
Generated on 08-24-2011
at time 16:11:21

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.08.23.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)	Intens.	BG	Factor
10ppb	10.000	2.137	17.565	1.00
10ppb	10.000	3.481	17.565	1.00
10ppb	10.000	2.311	17.565	1.00
50ppb	50.000	11.332	17.565	1.00
50ppb	50.000	10.607	17.565	1.00
50ppb	50.000	10.621	17.565	1.00
100ppb	100.000	22.001	17.565	1.00
100ppb	100.000	21.225	17.565	1.00
100ppb	100.000	22.510	17.565	1.00
250ppb	250.000	55.261	17.565	1.00
250ppb	250.000	53.803	17.565	1.00
250ppb	250.000	54.323	17.565	1.00
500ppb	500.000	96.966	17.565	1.00
500ppb	500.000	97.600	17.565	1.00
500ppb	500.000	95.438	17.565	1.00

Fit equation:
Y = 0.193 x + 2.204
Correlation 0.9979
****
Unknown sample results

Std#	Conc*Fact (ppb)	Intens.	BG	Factor	Info
LC 2009	123.850	26.092	16.271	1.00	liver
CRM	44.234	10.736	16.271	1.00	liver
Sample 1	130.113	27.300	17.441	1.00	liver
Sample 2	125.996	26.506	16.271	1.00	liver
Sample 3	124.716	26.259	16.271	1.00	liver
Sample 4	130.460	27.367	16.271	1.00	liver
Sample 5	1456.350	283.099	16.271	1.00	liver
Sample 6	198.774	40.543	16.271	1.00	liver
Sample 7	684.309	134.191	16.271	1.00	liver
Sample 8	62.199	14.201	16.271	1.00	liver
Sample 9	125.359	26.383	16.271	1.00	liver
Sample 10	248.106	50.058	16.271	1.00	liver
x	129.035	27.092	16.271	1.00	
xx	49.636	11.778	16.271	1.00	

ANNEXURE 11M: Current State Selenium Process Run, 29 August 2011

Fl Concentration results
Generated on 08-29-2011
at time 15:18:11

****
Measurement conditions
Method: C:\FLWINLAB\METHODS\ExistingProcess11.08.29.mth
Analyst:
Comments: Default concentration method

Ex. wavelength (nm): 375
Em. wavelength (nm): 520
Ex. slit (nm): 10.0
Em. slit (nm): 10.0
Integration time (s): 1.00
Em. filter: open

Sipper parameters:
Pump time(s): 8.0
Delay time(s): 1.0
Purge time(s): 0.0
Purge direction forwards
****
Reference sample results

Std#	Conc*Fact (ppb)	Intens.	BG	Factor
10ppb	10.000	2.043	16.005	1.00
10ppb	10.000	1.896	16.005	1.00
10ppb	10.000	1.678	16.005	1.00
50ppb	50.000	10.056	16.005	1.00
50ppb	50.000	9.605	16.005	1.00
50ppb	50.000	8.987	16.005	1.00
100ppb	100.000	17.333	16.005	1.00
100ppb	100.000	18.163	16.005	1.00
100ppb	100.000	18.339	16.005	1.00
250ppb	250.000	44.008	16.005	1.00
250ppb	250.000	42.683	16.005	1.00
250ppb	250.000	42.434	16.005	1.00
500ppb	500.000	94.393	16.005	1.00
500ppb	500.000	93.685	16.005	1.00
500ppb	500.000	89.212	16.005	1.00

Fit equation:
Y = 0.184 x + -0.43
Correlation 0.9987
****
Unknown sample results

Std#	Conc*Fact (ppb)	Intens.	BG	Factor	Info
LC 2009	114.887	20.652	15.634	1.00	liver
CRM	45.123	7.850	15.634	1.00	liver
Sample 1	47.200	8.231	15.634	1.00	liver
Sample 2	129.251	23.288	15.634	1.00	liver
Sample 3	133.856	24.133	15.634	1.00	liver
Sample 4	135.840	24.497	15.634	1.00	liver
Sample 5	1331.610	243.928	15.634	1.00	liver
Sample 6	125.709	22.638	15.634	1.00	liver
Sample 7	619.824	113.311	15.634	1.00	liver
Sample 8	51.734	9.063	15.634	1.00	liver
Sample 9	173.681	31.441	15.634	1.00	liver
Sample 10	233.875	42.487	15.634	1.00	liver
x	115.563	20.776	15.801	1.00	
xx	44.399	7.717	15.801	1.00	

ANNEXURE 12: Data Analysis Calculations

Chapter 5.5.1 Total Regression Analysis Summary: Average of all standard calibration curves

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999978
R Square	0.999956
Adjusted R Square	0.999941
Standard Error	1.532624
Observations	5

4.597871

ANOVA

	df	SS	MS	F	Significance F
Regression	1	159473	159473	67891.59	1.25E-07
Residual	3	7.046805	2.348935		
Total	4	159480			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.362516	0.97762	0.370815	0.735412	-2.74871	3.473739	-2.74871	3.473739
X Variable 1	5.6741	0.021777	260.5602	1.25E-07	5.604797	5.743402	5.604797	5.743402

Table 5.4 Linearity

r^2 0.9999 and r 0.9999 indicates good positive linearity
F-ANOVA = 67891.59. Large number implies significant linearity.

Regression Equation: $y = bx + a$
a = 0.362516
b = 5.6741
Thus: $y = 5.6741x + 0.362516$

Total Regression Analysis: Average Calibration Curve Readings

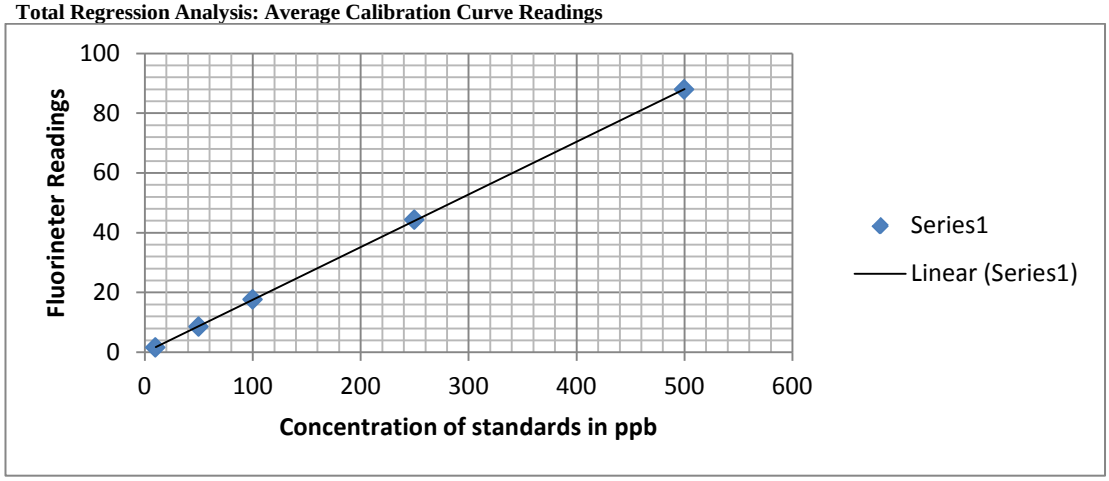


Table 5.4 Calibration uncertainties:

$S_{y/x}$ = random calibration uncertainties
$S_{y/x}$ = 1.532624

S_b = uncertainties in slope
S_b = 0.021777

S_a = uncertainties in intercept
S_a = 0.97762

Both S_b and S_a ‹ $S_{y/x}$. This indicates good general precision.
S_a/S_b = 44.89. This provides an indication that the range of standards requires more standards on the lower end of the calibration curve.
S_a‹ S_b provides an indication the extent of the range of standards is sufficient.

Table 5.4. Calibration uncertainties

Determination of the significance of standard error ($S_{y/x}$) of regression

f-test for variance in standard curve and variance of CRM

$f_{calc} = \frac{(S_{y/x})^2}{\sigma_{CRM}{}^2}$

$= \frac{8.53^2}{1.53^2} = 29.99 \sim 30 \rightarrow$

$f_{crit} = 7.146$

f_{calc} › f_{crit} thus there is significant evidence to reject the null hypothesis which states residuals are dispersed by random error.

Residuals are more widely dispersed than can be accounted for by random error. This could be evidence of non-linearity. Thus systematic error is present in the analytical process.

Uncertainty in slope: 0.021777 (Given by ANOVA)

Uncertainty in intercept: 0.97762 (Given by ANOVA)

Confidence limits in x intercept (b) at 95% confidence

CL of b: $b \pm t_{crit}S_b$
CL of b: 5.6741 ± (3.18 x 0.021777)
CL of b: 5.6741 ± 0.0693

Confidence limits in y intercept (a) at 95% confidence

CL of a: $a \pm t_{crit}S_a$
CL of a: 0.3625 ± (3.18 x 0.97762)
CL of a: 0.3625 ± 3.1089

Table 5.4 Limit of detection: Instrument Response (LOD Y intercept: Minimum Limit of detection according to ISO 11843 Method)

$Y_{lod} = a + 3S_{y/x}$
$Y_{lod} = 0.3625 + (3 \times 1.532624)$
$Y_{lod} = 0.3625 + (4.497872)$
$Y_{lod} = 4.960388 \sim 4.96 \rightarrow$

Table 5.4 Limit of detection: Analyte Concentration (LOD X intercept: Minimum Limit of detection according to ISO 11843 Method)

$X_{lod} = \frac{Ylod - a}{b}$
$X_{lod} = \frac{(a + 3Sy/x) - a}{b}$
$X_{lod} = \frac{4.9603 - 0.3625}{5.6741}$
$X_{lod} = \frac{4.5978}{5.6741}$
$X_{lod} = 0.810313529 \sim 0.81 \rightarrow$

Therefore this method could only detect selenium concentrations larger than 0.81ppb. All selenium concentrations detected were far above this value.

Table 5.4 Limit of Quantitation (LOQ Y intercept: Minimum Limit considered satisfactory for quantitative analysis)

$Y_{loq} = a + 10S_{y/x}$
$Y_{lod} = 0.3625 + (10 \times 1.532624)$
$Y_{lod} = 0.3625 + (15.32624)$
$Y_{lod} = 15.68874 \sim 15.69 \rightarrow$

Table 5.4 Limit of Quantitation (LOQ X intercept: Minimum Limit considered satisfactory for quantitative analysis)

$X_{loq} = \frac{Yloq - a}{b}$
$X_{loq} = \frac{(a + 10Sy/x) - a}{b}$
$X_{loq} = \frac{15.68874 - 0.3625}{5.6741}$
$X_{loq} = \frac{15.32624}{5.6741}$
$X_{lod} = 2.701087397 \sim 2.70 \rightarrow$

Therefore this method could only quantify selenium concentrations larger than 2.70ppb. All selenium concentrations observed were far above this value

Table 5.4 Calibration sensitivity

$b = 5.6741 \neq 0$
The method can thus be said to be calibration sensitive to selenium

Table 5.4 Calibration Sensitivity

Analytical sensitivity

$C_s = \frac{instrument\ response}{concentration\ of\ analyte}$

$C_s = \frac{5.6741\ response\ units}{1ppb}$

C_s = 5.6741 abs units/ppb

Inverse analytical sensitivity

$S_c = \frac{concentration\ of\ analyte}{instrument\ response}$

$S_c = \frac{1ppb}{5.6741\ response\ units}$

S_c = 0.176239401~ 0.18 ppb/abs units→

Current state selenium analysis method is thus said is responsive in the region 0.18ppb of analyte concentration, for each change in instrument response units given by the apparatus.

Chapter 5.5.2 CRM Calculations

Table 5.5 Accuracy and Uncertainty of CRM

<u>95% CL and CI</u>

For unknown population σ:

$\bar{x} \pm \frac{ts}{\sqrt{n}}$

Where t is obtained from t-table, df: n-1 at 95% confidence level.

T_{crit} = 2.57

$0.5235 \pm \frac{2.57\ x\ 0.083861}{\sqrt{6}}$

$0.5235 \pm \frac{0.21552277}{\sqrt{6}}$

0.5235 ± 0.087986802 ~0.0880

0.5235 ± 0.09→

The current state selenium analysis process is therefore said to provide average measurements for known CRM of 0.52 ± 0.09. The true value of the CRM is 0.56 ± 0.07.

Chapter 5.5.3 Precision, Bias and Horrat Analysis

Precision of CRM

%RSD (Relative Standard Deviation) = $\left[\frac{SD}{\bar{x}}\right] \bullet 100$

%RSD = $\left[\frac{0.083861}{0.5235}\right] \bullet 100$

%RSD = [0.160192932] $\bullet$ 100

%RSD = 16.01929322 ~ 16.02% →

The current state selenium analysis process is 16.02% imprecise

Analytical Bias

% Bias = $\left[\frac{\bar{x} - \mu}{\mu}\right] \bullet 100$

% Bias = $\left[\frac{0.5235 - 0.56}{0.56}\right] \bullet 100$

% Bias = -6.517857143 ~ -6.52% →

The current state selenium analysis process has a consistent -6.5% deviation from the true value.

Horwitz function based on CRM RSD

HORRAT = RSD_{obs} / RSD_{calc}

Conversion of CRM mean to percentage: 52.35pmm x 0.0001 = 0.005235%

Conversion to relative amount required by Horwitz function: $\frac{0.005235\%}{100}$

$RSD_{calc} = \pm 2^{(1-0.5\log \mathbf{C})}$

$RSD_{calc} = \pm 2^{(1-0.5\log \mathbf{0.00005235})}$

$RSD_{calc} = \pm 2^{3.14}$

$RSD_{calc} = \pm 8.818551213 \sim 8.82 \rightarrow$

HORRAT = RSD_{obs} / RSD_{calc}

= $\frac{16.02}{8.82}$ = 1.820454545 ~ 1.82→

The horrat ratio based purely on CRM measurements obtained for the selenium analysis process is calculated at 1.82%

Horwitz function based on overall process RSD

HORRAT = RSD_{obs} / RSD_{calc}

Analysis of data obtained from all samples processed provided an overall process RDS at 18,43472 ~ 18.43% (Annexure 14)

CRM used as relative amount required by Horwitz function as calculated above:

RSD_{calc} = ± 8.818551213 ~ 8.82→

HORRAT = RSD_{obs} / RSD_{calc}

$= \frac{18.43}{8.82}$ = 2.089569161 ~ 2.09→

The horrat ratio based on overall process performance from sample measurements for the selenium analysis process is calculated at 2.09%

Chapter 5.5.6 Total Analytical Uncertainty

Uncertainty ΔTE

$$s_{x_0} = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(y_0-\bar{y})^2}{b^2\sum(x_i-\bar{x})^2}}$$

$$s_{x_0} = \frac{1.532624}{5.671}\sqrt{\frac{1}{13}+\frac{1}{5}+\frac{(52.35-32.0116831)^2}{5.671^2(159480)}}$$

$$s_{x_0} = 0.270108739\sqrt{0.277004586}$$

$$s_{x_0} = 0.142161538 \sim 0.14 \rightarrow$$

Where:
b is the slope 5.6741 as given by regression statistics
$s_{y/x}$ is 1.532624 as given by regression statistics
y_0 is the response in the unknown
$\bar{y}$ is mean response obtained from standard calibration curve samples
$\sum(x_i - \bar{x})^2$ is 159480 obtained from:

Standard	Difference	$\bar{x}^2$
10	-172	29584
50	-132	17424
100	-82	6724
250	68	4624
500	318	101124
Ave:182	**Total**	**159480**

ANNEXURE 13: Quantitative Method Characteristics			
NAME OF METHOD CHARACTERISTIC		**DESCRIPTION**	**DERIVED FROM**
Calibration standard	Number of standards	Number of standards in calibration curve	n
	Number of replicates of standards	Number of replicate measurements of each standard in the curve	m
	Standard spread	Range of standards	List the lowest and highest concentrations of standards used in the standard calibration curve.
	Matrix of blank	Name of reagent	
Linearity	**Linearity Correlation**: Correlation coefficient	The "goodness of fit" of the line	$r = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\Sigma(x_i - \bar{x})^2 \Sigma(y_i - \bar{y})^2}}$
	Linearity Correlation: Coefficient of determination	Often the correlation coefficient r is expressed as r^2: the coefficient of determination or coefficient of variance	$r^2 = \frac{regression\ sum\ of\ squares}{total\ sum\ of\ squares} = \frac{SSR}{SST}$
	Linearity Correlation: The residual	The residual equals the difference between the observed value Y and the predicted value of Y.	$e_i = Y_i - \hat{\hat{Y}}_i$
	Significant Linearity: t_{stat} or t_{calc} of slope	t_{stat} or $t_{statistic}$ in linear regression is used to determine the existence of significant correlation. Also known as $t_{calc.}$ If $t_{calc} > t_{crit}$, there is significant linearity. This t statistic is provided in the column titled *t Stat* by Microsoft Excel. The value for t_{crit} is	$t_{stat} = \frac{b_1 - \beta_1}{s_{b_1}}$, where $s_{b_1} = \frac{s_{YX}}{\sqrt{SSX}}$, and SSX = $\sum_{i=1}^{n}(x_i - x)^2$ Or $T_{calc} = r\sqrt{\frac{n-2}{1-r^2}}$ The t_{crit} follows a t distribution with n -2 degrees of freedom.

		obtained from a t table.	
	Significant Linearity: f_{calc} ANOVA	f-test provides an alternate approach to testing whether the slope in simple linear regression is statistically significant. F test is calculated by the ratio of the variance due to regression divided by the error variance. It is given by the ANOVA table in Microsoft Excel.	F = $\frac{MSR}{MSE}$, where MSR = $\frac{SSR}{k}$, and MSE = $\frac{SSE}{n-k-1}$ f follows f distribution with *k* and *n-k-1* degrees of freedom.
	Significant Linearity: f_{crit}	Obtained from the f-table, if $f_{calc} > f_{crit}$, significant linearity is deemed to exist.	Calculated from f-table or given by Microsoft Excel as Significance F
	Significant Linearity: Regression SS > Residual SS	Regression SS also known as Regression sum of squares (SSR), is equal to the sum of the squared differences between predicted value of Y and the mean of Y. Residual SS also known as The error sum of squares (SSE), is equal to the sum of the squared differences between the observed value of Y and predicted value of Y. Significant linearity exists where SSR>SSE.	Relation between x and y SSR = SST – SSE > SSE = $\sum_{i=1}^{n}(Yi - \widehat{Y_i})^2$.
	Significant Linearity: Regression MS > Residual MS	Significant linearity exists where Regression MS or regression mean square (MSR) > Residual MS or Error mean square (MSE)	Regression MS = Regression mean square MSR = $\frac{SSR}{k}$ Residual MS = Error mean square MSE = $\frac{SSE}{n-k-1}$, where k = number of independent or explanatory variables in the

			regression model.
	Significant Linearity: Comment on significance of linearity	If $F_{calc} \rightarrow F_{crit}$, significant linearity is deemed to exist.	
	Significant Linearity: % Variation in detection of sample	When the coefficient of determination (coefficient of variance) is multiplied by 100, it indicates the percentage of variation in y associated with variation in x.	Variation % in absorbance due to variation to the variation in concentration in standards.
Regression parameters	Slope	b also known as b_1 = slope of population or gradient, represents the expected change in Y per unit change in X. It represents the average amount the Y changes (either positively or negatively) for a particular unit change in X.	Also indicated by 'x variable' coefficient on ANOVA table in Microsoft Excel
	Intercept	a also known as b_0 = the Y intercept for the population, represents the average value of Y when X equals 0.	Also indicated by 'intercept' coefficient on ANOVA table in Microsoft Excel
	Regression line equation	For Least-Squares method, the sample regression equation representing the straight line regression model can be represented as follows: $\hat{y}_i = b_1 x_i + b_0$ or y = bx + a	$\hat{y}_i = b_1 x_i + b_0$, where $\hat{y}_i$ is the predicted value of Y for observation *i*, and x_i is the value of x for observation *i*.
Calibration uncertainties	**Standard error of the regression:** Significance of standard error of regression	Standard error of regression, also known as Standard error of the estimate, or Residual standard deviation or Random calibration	$S_{y/x} = \sqrt{\frac{SSE}{n-2}} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2}}$ or $S_{y/x} = \left[\frac{\sum(y_i - \hat{y}_i)^2}{n-2}\right]^{\frac{1}{2}}$

		uncertainty, is a measure of the amount of error accrued in predicting a y value for each given x value. This statistic measures the variability of the actual y values from the predicted y values. It is a measure of variation around the fitted line of regression and is measured in units of the dependent variable y.	where y residuals = y_i - $\hat{y}_i$
	Significance of standard error of the regression: Comment on F test result	To determine calibration uncertainty in relation to standard error of regression, a f-test is conducted uses the variance of standard error of regression as the numerator and the variance obtained from replicate measurements of the CRM as denominator, to calculate F_{calc}. As this has direct bearing on sample analysis, a CRM sample is used in this calculation. However the F_{crit} for this f-test derived from standard calibration curve sample group with n-2 df.	If $F_{calc} > F_{crit}$ the null hypothesis is rejected. Significant evidence to say that the residuals are more widely dispersed than can be accounted for by random error. Therefore alternate hypothesis is accepted. It may be necessary to investigate further if Null hypothesis is rejected in order to ascertain the reason why residuals are significantly widespread, as this could be evidence of non-linearity. If $F_{calc} < F_{crit}$, the Null hypothesis is accepted and it is stated that there is significant evidence to conclude that residuals are dispersed due to random error.
	Uncertainty of slope	Standard error of slope or s_{b_1} is given by Microsoft Excel as Standard Error of x variable. The statistic determines the sensitivity of the calibration function or the	$s_{b_1} = \frac{s_{y/x}}{\sqrt{\sum(x_i - \bar{x})^2}}$

		rate at which the signal changes with concentration.	
	Uncertainty of intercept	Standard error of the intercept s_{a_1} is the error associated to the actual intercept point of the line. The intercept of the regression line has implications for the smallest detectable signal (measured response) and the corresponding lowest concentration.	$s_{a_1} = s_{y/x} \sqrt{\frac{\sum x_i^2}{n \sum (x_i - \bar{x})^2}}$
	Ratios of slope and intercept uncertainties: S_a and $S_b < S_{y/x}$	To test for precision of an analytical method determine if **:** S_a and $S_b < S_{y/x}$. If **:** S_a and $S_b < S_{y/x}$ then it points to good general precision of analytical method	
	Ratios of slope and intercept uncertainties: S_a/S_b	This ratio or test statistic tells the analyst if more standards are required above or below centroid. Ideal value is 6, which means standards are adequate and regression line is balanced. Ratio ‹6 means more standards required on higher end, Ratio ›6 means more standards required on the lower end of regression line.	
	Ratios of slope and intercept uncertainties: $S_b<S_a$	To determine if the working range of standards is wide enough or sufficient, S_b should be less than S_a.	

	Confidence limits at 95%: 95% CL of b: $b \pm tS_b$	The corresponding confidence level for b associated to S_b (interval of the slope), is calculated using the t-statistic for (n-2) degrees of freedom.	It is reported as two values (upper and lower) either mean with a ± sign or as two separate values
	Confidence limits at 95%: 95% CL of a: $a \pm tS_a$	The corresponding confidence level for a, associated to S_a (y intercept), is calculated in the same way as that of the slope.	It is reported as two values (upper and lower) either mean with a ± sign or as two separate values
Limit of detection (LOD)	Response representing the LOD	The LOD represents the level below which we cannot be confident whether or not the analyte is actually present, formally defined as "the concentration of analyte required to give signal equal to background (blank) plus three times standard deviation of the blank for 10 measurements"	$Y_{lod} = Y_{blank} + 3s_{blank}$
	Concentration representing LOD	Also known as the X_{lod}, refers to the associated concentration of the Y_{lod}	$X_{lod} = \frac{(Y_{blank} + 3sd_{blank}) - a}{b}$
	LOD from regression statistics	When a situation is presented where no blank was measured, regression statistics may be used to determine the LOD	$X_{lod} = \frac{Y_{lod} - a}{b}$ or $X_{lod} = \frac{(a + 3s_{y/x})}{b}$ or $X_{lod} = \frac{3s_{y/x}}{b}$
	LOD from propagation of errors method	Alternative method to determine LOD	$X_{lod} = \frac{3\left\{{s_{blank}}^2 + {s_a}^2 + \left[\frac{a}{b} \cdot {s_b}^2\right]\right\}^{1/2}}{b}$ or $X_{lod} = \frac{3\{{s_{blank}}^2 + {s_a}^2\}^{1/2}}{b}$
Limit of quantitation (LOQ)	Signal representing the LOQ	LOQ refers to the smallest concentration or mass which can be quantitatively analysed	$Y_{loq} = Y_{blank} + 10s_{blank}$

		with reasonable reliability by a given procedure	
	Concentration representing the LOQ	Also known as the X_{loq}, refers to the associated concentration of the Y_{loq}	$X_{loq} = \frac{(y_{blank} + 10sd_{blank}) - a}{b}$
	LOQ from regression statistics		$X_{loq} = \frac{10s_{y/x}}{b}$
Sensitivity	Calibration sensitivity	This parameter is only a reflection of whether the instrument is sensitive or not, and does not reflect the degree of sensitivity of an instrument.	A method is said to be calibration sensitive if $b \neq 0$, where b is the slope of a regression line.
	Analytical sensitivity	The analytical calibration sensitivity of the calibration curve, C_s, is the rate of change of the signal intensity with changes in the concentration of the analyte or the slope	$C_s = \frac{instrument\ response}{per\ concentration\ unit\ of\ analyte}$
	Inverse analytical sensitivity	To determine how responsive a method is to small changes in concentration, S_c is used. S_c is the inverse of the rate of change of the signal intensity (or instrument reading) with changes in the concentration of analyte	$S_c = \frac{concentration\ unit\ of\ analyte}{instrument\ response}$
Specificity	Evidence from reputable CRM that method is selective / specific for selenium.	A statement made from information extracted from the Certificate provided with the CRM is adequate for this purpose.	
Accuracy and Precision of CRM			

Analysis of CRM	**Mean measurement**	Average measurement of CRM	
Components of CRM measurement	T test to determine significant difference between the mean and true value	To test the accuracy of a method, replicate measurements of CRM are taken and a t-test conducted to determine if there is significant difference between the mean observed from replicate tests and the true mean measurement. A one-sample t test is then conducted. If $t_{calc} \rangle t_{crit}$ at a defined confidence level (e.g. 95%), then it may be said that there is a statistical difference between the mean values.	$$t_{calc} = \frac{\lvert \bar{x} - \mu_0 \rvert}{\frac{s}{\sqrt{n}}}$$ where: $\bar{x}$ = mean of test results of a sample μ_0= "true" or reference value s = standard deviation of test results n = number of replicate tests and t_{crit} is obtained from the student's t-table (df = n-1)
	95% CL and CI	When precision of a method is untested, the true population σ is unknown. The population standard deviation is estimated by computing s and a t-table statistic is used to compute CI Should the population σ be known, a z-table statistic is used to compute CI.	<u>For unknown population σ:</u> $$\bar{x} \pm \frac{ts}{\sqrt{n}}$$ Where t is obtained from t-table, df: n-1 at 95% confidence level. <u>For known population σ:</u> $$\bar{x} \pm \frac{z\sigma}{\sqrt{n}}$$ Where z is obtained from the z table, df: n-1, at 95% confidence level.
	Precision	Precision is the closeness with which results of replicate analysis of a sample agree. It is a measure of the dispersion or scattering	$$\%RSD = \left[\frac{SD}{\bar{x}}\right] \cdot 100$$

		around the mean value and usually expressed in terms of standard deviation. The larger the standard deviation, the worse the precision. % Relative standard deviation (RSD) is a measure of precision.	Where: SD = standard deviation $\bar{x}$ = mean
	Bias	Bias is the consistent deviation of analytical results from the "true" value, and is caused by systematic error in a procedure. Bias has a definite value, an assignable cause and is about the same magnitude for replicate measurements. Bias is determined using measurements obtained from the CRM.	% Bias = $\left[\frac{x-\mu}{\mu}\right]100$ Where: x = mean of a series of CRM measurements μ = the accepted CRM value
Analysis of Samples	**Analysis of samples (measurements)**	Measurements of samples (instrument readings)	
	Components of sample measurements: Precision	As for CRM Precision	As calculated for CRM Precision, given the mean value and standard deviation.
	Components of sample measurements: Uncertainty of inferred value	Sx_0 is the standard error for the sample (uncertainty in unknown) for x_0, and x_0 is the predicted value or unknown sample concentration.	$s_{x_0} = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(y_0-\bar{y})^2}{b^2\sum(x_i-\bar{x})^2}}$
	Components of sample measurements: % RSD of sample analysis	Precision is the closeness with which results of replicate analysis of a sample agree. It is a measure of the dispersion or scattering	%RSD = $\left(\frac{s_{x_0}}{x_0}\right)\cdot 100$ Where:

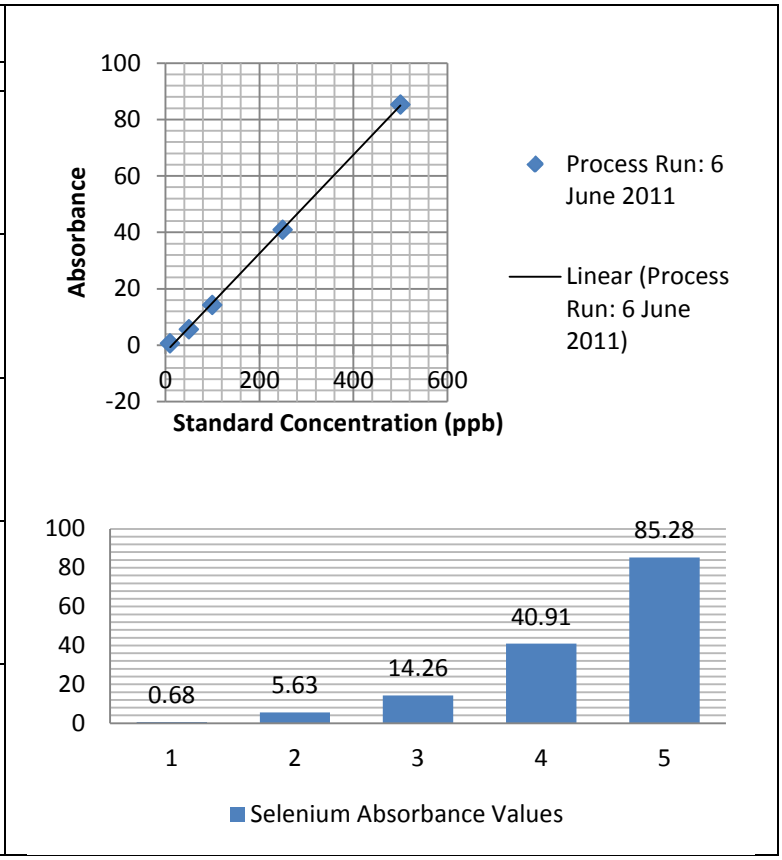
		around the mean value and usually expressed in terms of standard deviation. The larger the standard deviation, the worse the precision. % Relative standard deviation (RSD) is a measure of precision. Using regression uncertainty, Sx_0 is the standard error for the sample (uncertainty), and x_0 is the predicted value or unknown sample concentration.	$s_{x_0} = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(y_0-\bar{y})^2}{b^2\sum(x_i-\bar{x})^2}}$ and the unknown sample concentration may be calculated using the regression equation: $y_0 = bx_0 + a$ *thus:* $x_0 = \frac{y_0 - a}{b}$
	Components of sample measurements: CL of sample analysis	Error in concentration is minimal, if the signal from the unknown lies in the middle of the signals of all the standards, otherwise known as the centroid. The confidence limits of unknown concentration x_0 is calculated as CRM with unknown population σ, with student t-table value, and given as an interval.	Uncertainty at centroid: $s_{x_0} = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m}+\frac{1}{n}}$ $x_0 \pm tSx_0$ where: t-statistic obtained from t table and df n-2
	Components of sample measurements: Horwitz ratio	The Horwitz Function is a useful index of method performance with respect to precision. When the value of RSD and the mean value of analyte concentration have been determined, the Horwitz ratio, HORRAT, can be calculated.	HORRAT = RSD_{obs} / RSD_{calc} Where: RSD_{obs} = %RSD (precision) $RSD_{calc} = \pm 2^{(1-0.5\log \mathbf{C})}$ Where **C** = (mean of analyte in percentage) / Horwitz function conversion factor (100)

ANNEXURE 14: Overall Process Precision (%RSD)

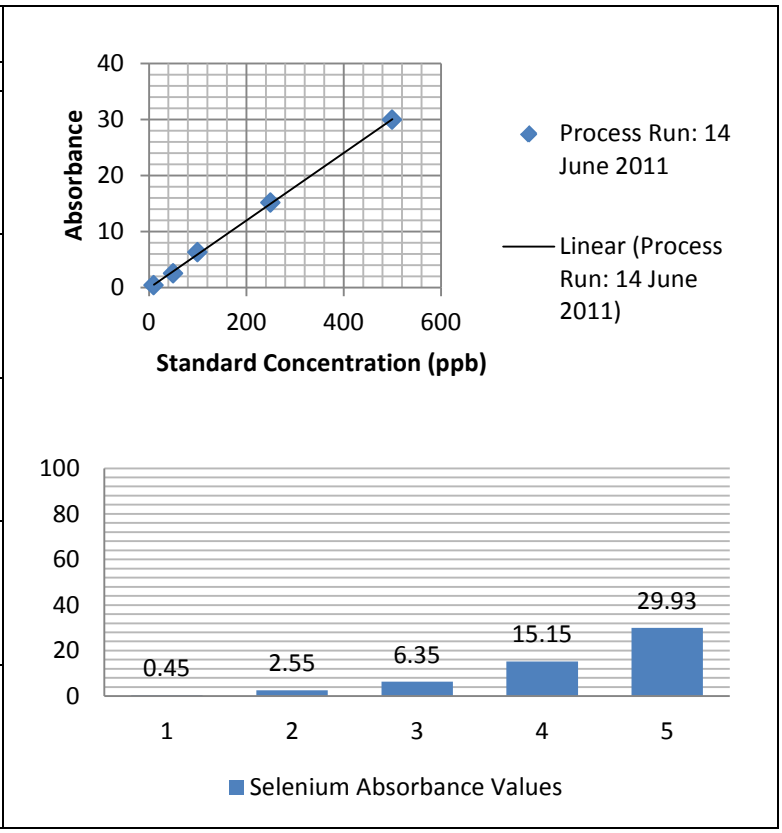
SAMPLE MEASUREMENTS	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**	**Sample 9**	**Sample 10**	**LC 2009**	**CRM**
Process Run 1	55.417	124.775	136.133	151.309	1434.881	149.197	667.768	90.122	169.705	139.286	145.632	
Process Run 2	45.844	131.226	147.021	135.954	1805.339	161.356	785.308	49.412	188.733	293.693	149.062	
Process Run 3	45.35	122.235	136.303	130.881	1458.633	135.007	629.917	58.289	170.753	264.562	134.694	
Process Run 4	51.182	126.887	138.07	128.219	1471.848	134.36	613.744	59.706	169.671	269.436	118.535	
Process Run 5	55.63	121.364	147.113	132.003	1419.938	146.419	643.825	55.165	166.522	256.424	120.477	
Process Run 6	45.496	126.48	121.048	135.972	1405.282	141.555	601.012	66.305	184.214	236.818	131.522	
Process Run 7	48.651	130.483	127.972	128.731	1388.027	134.427	591.596	56.584	164.403	252.082	124.864	
Process Run 8	43.579	121.5	113.419	128.179	1390.454	141.71	657.011	47.96	131.104	257.753	113.01	0.67449
Process Run 9	50.12	143.562	136.482	135.551	1382.858	136.92	604.422	84.583	174.164	250.827	133.749	0.5328
Process Run 10	43.054	124.812	130.452	132.944	1383.993	123.425	636.594	60.495	162.813	260.538	140.134	0.53499
Process Run 11	130.113	125.996	124.716	130.46	1456.35	198.774	248.106	62.199	125.359	684.309	123.85	0.50515
Process Run 12	74.761	139.206	145.439	159.109	1230.14	153.319	572.645	70.108	114.017	73.829	145.48	0.45123
Process Run 13	47.2	129.251	133.856	135.84	1331.61	125.709	619.824	51.734	233.875	173.681	114.887	0.44234
Average	**56.6459**	**128.291**	**133.694**	**135.781**	**1427.643**	**144.783**	**605.521**	**62.5125**	**165.795**	**262.557**	**130.454**	**0.5235**
Std dev	23.5899	6.66109	10.1250	9.24103	129.7748	19.3603	119.440	12.7150	30.4906	141.003	12.2106	0.08386
RSD%	**41.6444**	**5.19219**	**7.57324**	**6.80584**	**9.090143**	**13.3719**	**19.7252**	**20.3400**	**18.3905**	**53.7039**	**9.36009**	**16.0192**
Process Average RSD%		**18.43472**										

ANNEXURE 15: Analysis of Individual Standard Calibration Curves of Process Runs

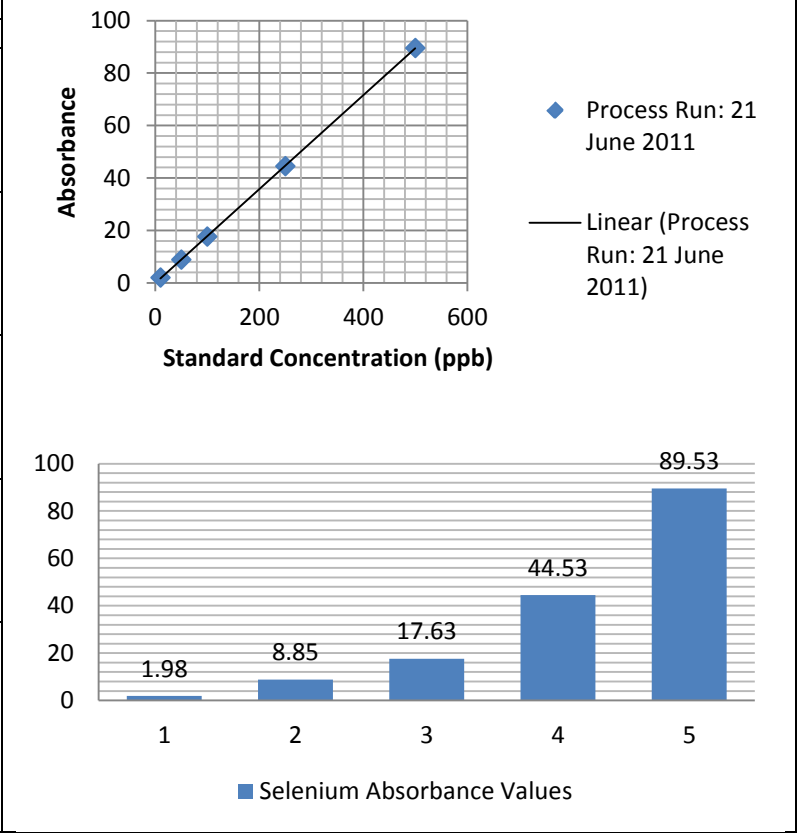
Process Run Date	**8 June 2011**	
Blank		19.064
10ppb	1.368	
10ppb	1.872	
10ppb	-1.191	
Calibration Std 10 Average		**0.68**
50ppb	5.089	
50ppb	5.805	
50ppb	5.99	
Calibration Std 50 Average		**5.63**
Std 100	14.41	
Std 100	14.075	
Std 100	14.297	
Calibration Std 100 Average		**14.26**
Std 250	43.163	
Std 250	38.765	
Std 250	40.814	
Calibration Std 250 Average		**40.91**
Std 500	83.391	
Std 500	88.765	
Std 500	83.676	
Calibration Std 500 Average		**85.28**



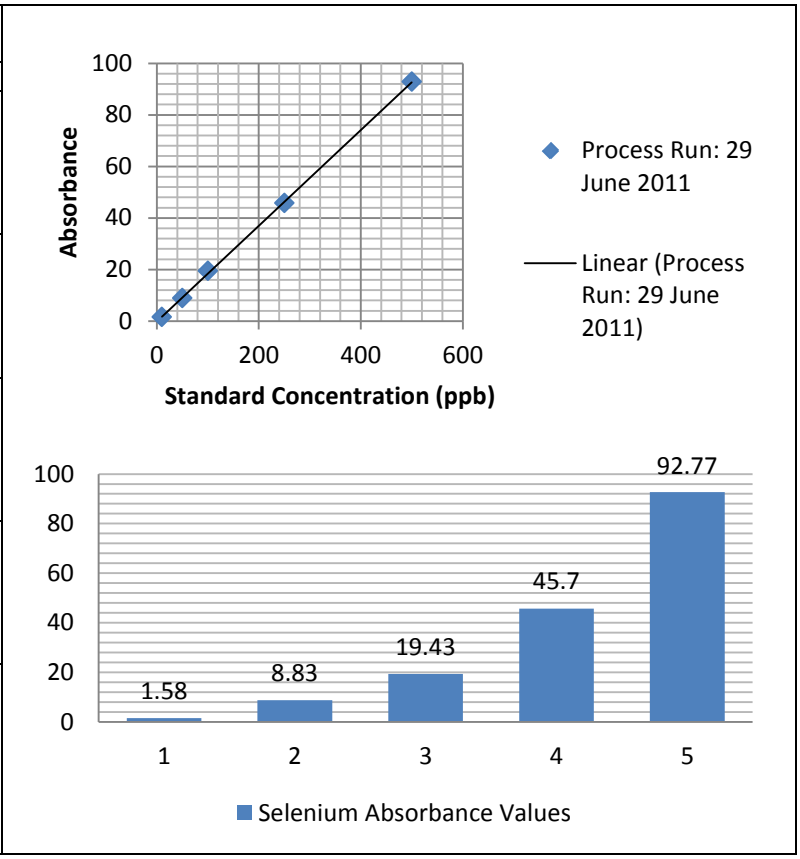
Process Run Date	**14 June 2011**	
Blank		16.722
10ppb	0.434	
10ppb	0.529	
10ppb	0.387	
Calibration Std 10 Average		**0.45**
50ppb	2.735	
50ppb	2.527	
50ppb	2.402	
Calibration Std 50 Average		**2.55**
Std 100	6.778	
Std 100	6.159	
Std 100	6.111	
Calibration Std 100 Average		**6.35**
Std 250	14.912	
Std 250	15.661	
Std 250	14.874	
Calibration Std 250 Average		**15.15**
Std 500	29.609	
Std 500	30.184	
Std 500	29.991	
Calibration Std 500 Average		**29.93**



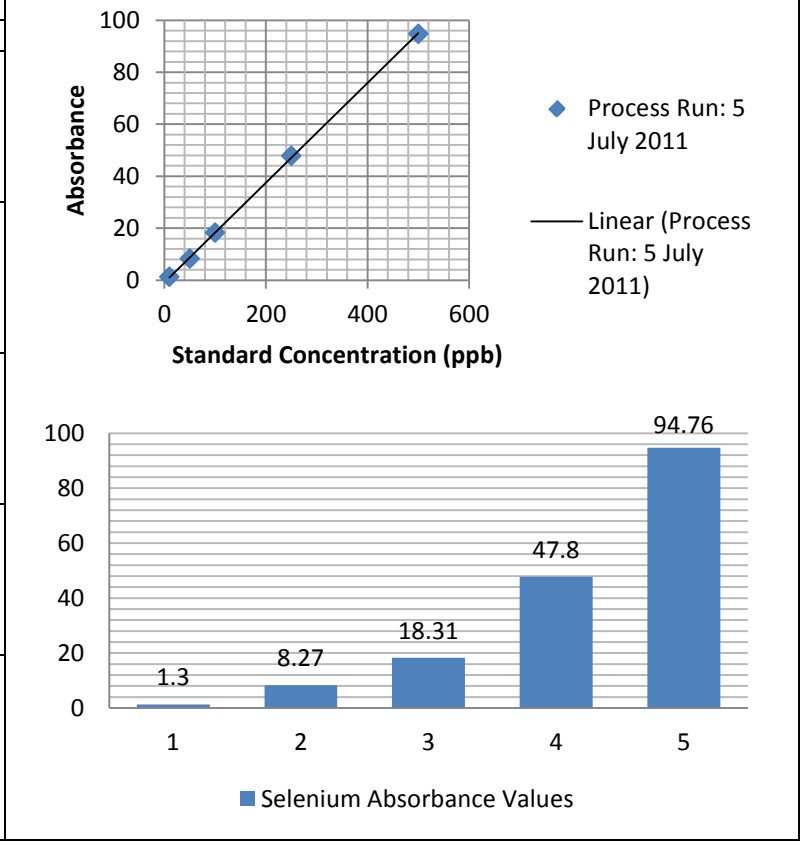
Process Run Date	**21 June 2011**	
Blank		16.121
10ppb	2.006	
10ppb	2.045	
10ppb	1.884	
Calibration Std 10 Average		**1.98**
50ppb	8.899	
50ppb	8.937	
50ppb	8.704	
Calibration Std 50 Average		**8.85**
Std 100	17.563	
Std 100	17.472	
Std 100	17.852	
Calibration Std 100 Average		**17.63**
Std 250	45.031	
Std 250	43.611	
Std 250	44.962	
Calibration Std 250 Average		**44.53**
Std 500	89.496	
Std 500	88.963	
Std 500	90.119	
Calibration Std 500 Average		**89.53**



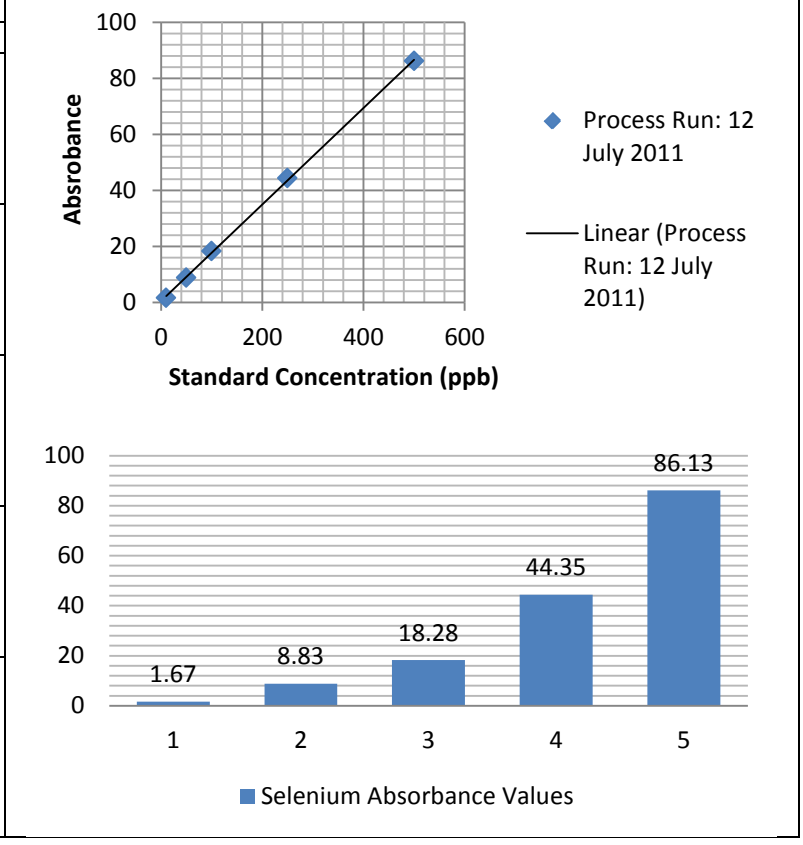
Process Run Date	**29 June 2011**	
Blank		15.892
10ppb	1.522	
10ppb	1.578	
10ppb	1.639	
Calibration Std 10 Average		**1.57**
50ppb	9.038	
50ppb	8.849	
50ppb	8.589	
Calibration Std 50 Average		**8.53**
Std 100	18.949	
Std 100	19.437	
Std 100	19.892	
Calibration Std 100 Average		**19.43**
Std 250	45.971	
Std 250	44.418	
Std 250	46.724	
Calibration Std 250 Average		**45.70**
Std 500	95.604	
Std 500	87.928	
Std 500	94.773	
Calibration Std 500 Average		**92.77**



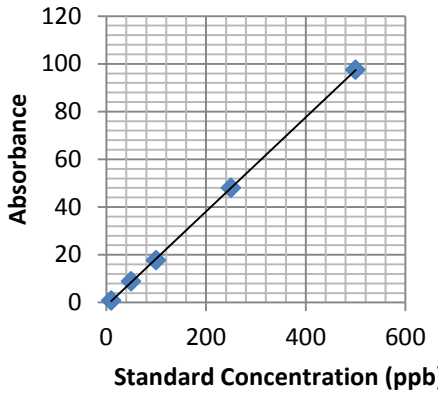
Process Run Date	**5 July 2011**	
Blank		22.987
10ppb	1.796	
10ppb	1.507	
10ppb	0.601	
Calibration Std 10 Average		**1.30**
50ppb	8.245	
50ppb	8.088	
50ppb	8.485	
Calibration Std 50 Average		**8.27**
Std 100	17.139	
Std 100	20.462	
Std 100	17.338	
Calibration Std 100 Average		**18.31**
Std 250	47.281	
Std 250	49.328	
Std 250	46.796	
Calibration Std 250 Average		**47.80**
Std 500	94.143	
Std 500	94.671	
Std 500	95.465	
Calibration Std 500 Average		**94.76**

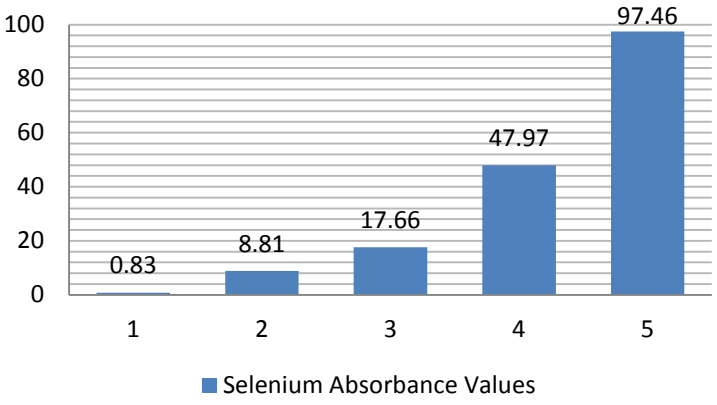


Process Run Date	**12 July 2011**	
Blank		16.470
10ppb	1.596	
10ppb	1.489	
10ppb	1.919	
Calibration Std 10 Average		**1.67**
50ppb	8.634	
50ppb	9.238	
50ppb	8.63	
Calibration Std 50 Average		**8.83**
Std 100	18.832	
Std 100	17.989	
Std 100	18.027	
Calibration Std 100 Average		**18.28**
Std 250	43.326	
Std 250	43.356	
Std 250	46.381	
Calibration Std 250 Average		**44.35**
Std 500	86.236	
Std 500	87.869	
Std 500	84.272	
Calibration Std 500 Average		**86.13**

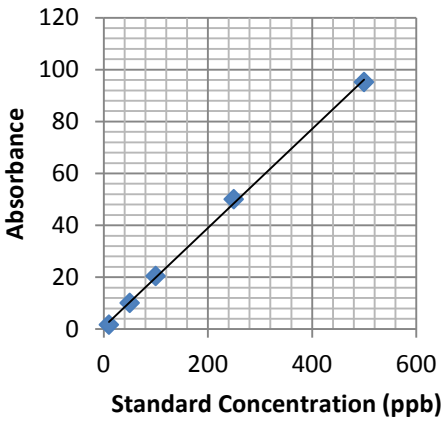


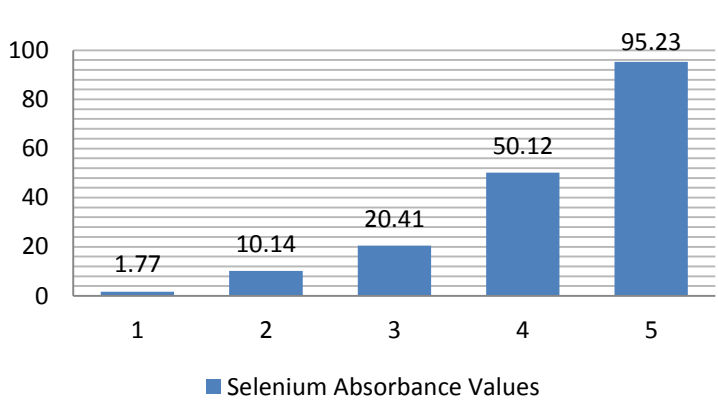
Process Run Date	21July 2011	
Blank		18.669
10ppb	0.822	
10ppb	0.729	
10ppb	0.93	
Calibration Std 10 Average		**0.83**
50ppb	8.805	
50ppb	8.904	
50ppb	8.727	
Calibration Std 50 Average		**8.81**
Std 100	17.33	
Std 100	17.782	
Std 100	17.873	
Calibration Std 100 Average		**17.66**
Std 250	49.947	
Std 250	47.14	
Std 250	46.827	
Calibration Std 250 Average		**47.97**
Std 500	96.126	
Std 500	99.309	
Std 500	96.953	
Calibration Std 500 Average		**97.46**



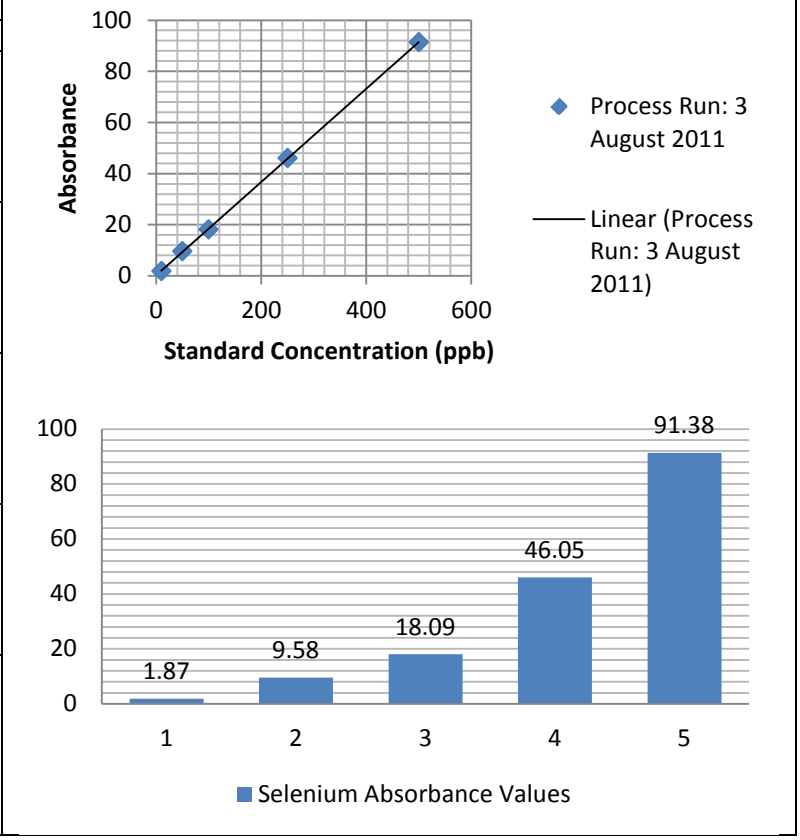


Process Run Date	29 July 2011	
Blank		17.621
10ppb	1.913	
10ppb	1.918	
10ppb	1.483	
Calibration Std 10 Average		**1.77**
50ppb	10.478	
50ppb	9.583	
50ppb	10.371	
Calibration Std 50 Average		**10.14**
Std 100	21.315	
Std 100	18.764	
Std 100	21.139	
Calibration Std 100 Average		**20.41**
Std 250	51.916	
Std 250	50.878	
Std 250	47.563	
Calibration Std 250 Average		**50.12**
Std 500	96.018	
Std 500	94.267	
Std 500	95.39	
Calibration Std 500 Average		**95.23**

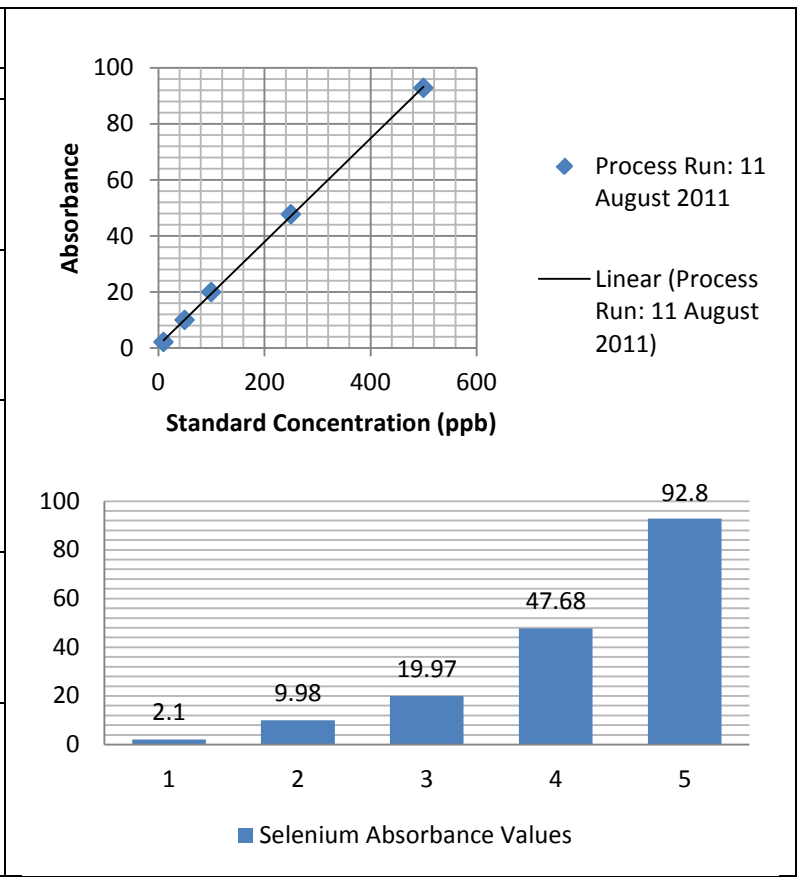




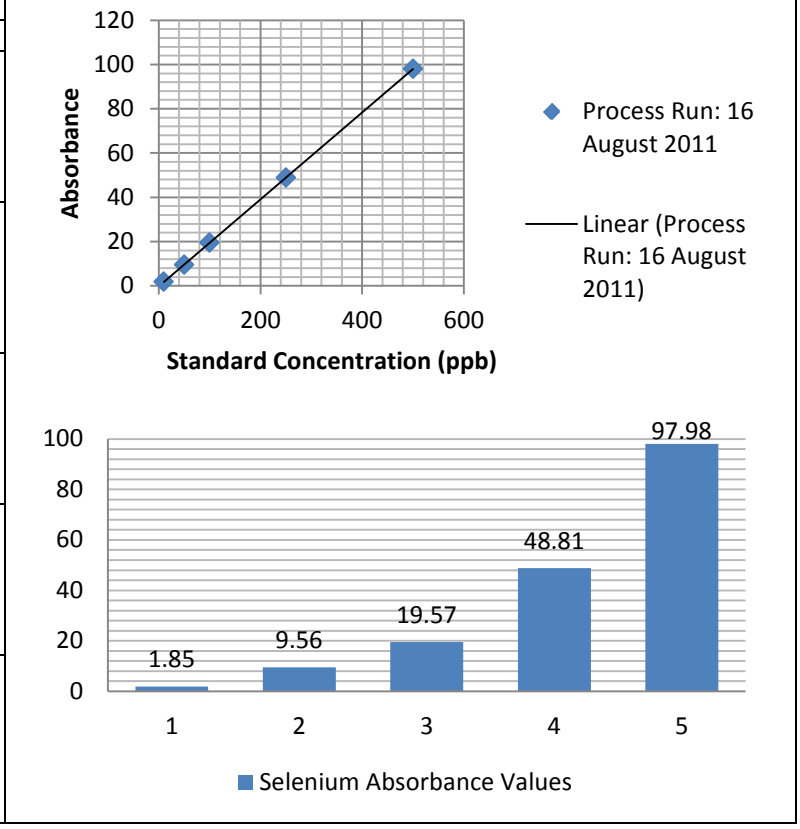
Process Run Date	**3 August 2011**	
Blank		16.575
10ppb	1.901	
10ppb	1.638	
10ppb	2.064	
Calibration Std 10 Average		**1.87**
50ppb	9.134	
50ppb	10.648	
50ppb	8.962	
Calibration Std 50 Average		**9.58**
Std 100	17.525	
Std 100	17.897	
Std 100	18.837	
Calibration Std 100 Average		**18.09**
Std 250	46.513	
Std 250	44.945	
Std 250	46.681	
Calibration Std 250 Average		**46.05**
Std 500	92.688	
Std 500	96.532	
Std 500	84.928	
Calibration Std 500 Average		**91.38**



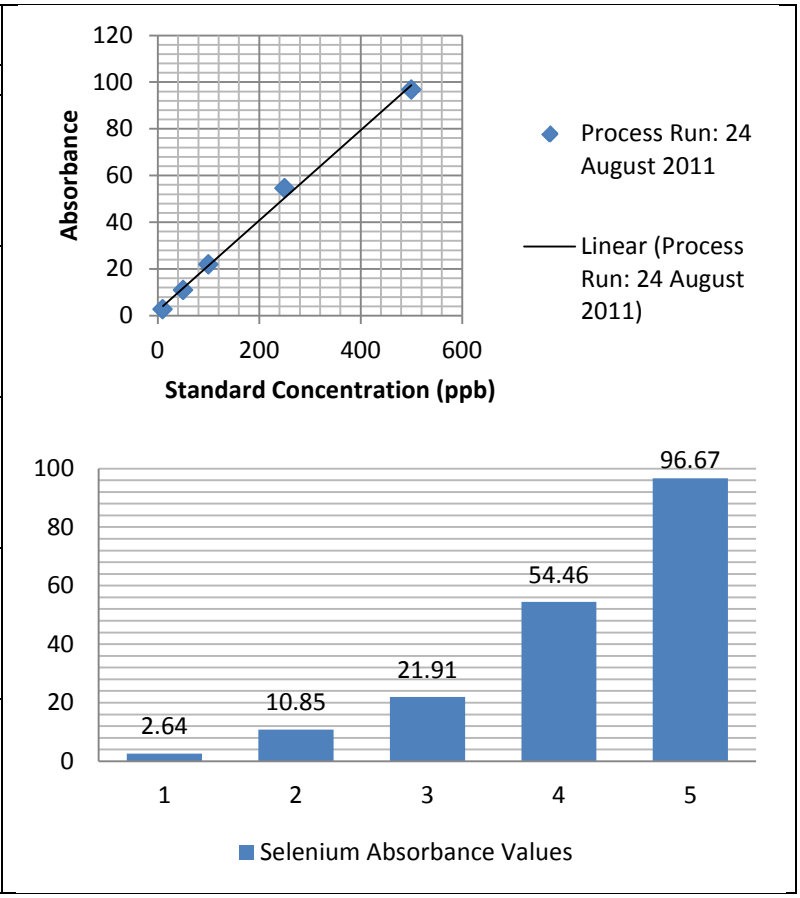
Process Run Date	**11 August 2011**	
Blank		16.726
10ppb	2.183	
10ppb	1.969	
10ppb	2.142	
Calibration Std 10 Average		**2.10**
50ppb	9.762	
50ppb	10.757	
50ppb	9.434	
Calibration Std 50 Average		**9.98**
Std 100	18.737	
Std 100	19.033	
Std 100	22.128	
Calibration Std 100 Average		**19.97**
Std 250	46.657	
Std 250	48.551	
Std 250	47.821	
Calibration Std 250 Average		**47.68**
Std 500	92.113	
Std 500	89.51	
Std 500	96.767	
Calibration Std 500 Average		**92.80**



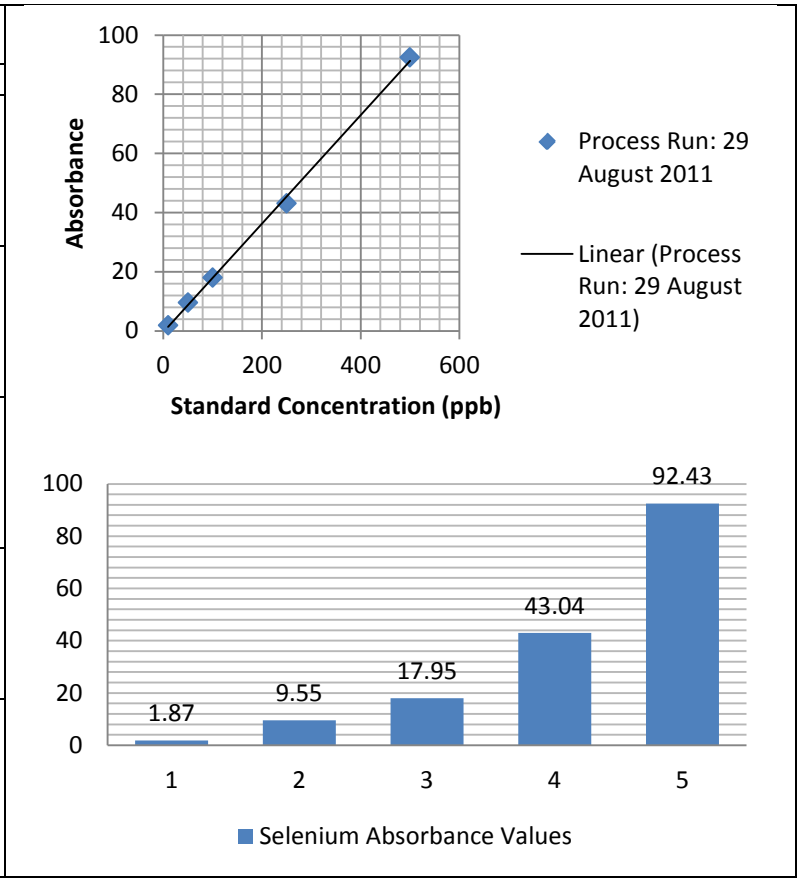
Process Run Date	**16 August 2011**	
Blank		16.821
10ppb	2.099	
10ppb	1.929	
10ppb	1.536	
Calibration Std 10 Average		**1.85**
50ppb	9.68	
50ppb	10.441	
50ppb	8.556	
Calibration Std 50 Average		**9.56**
Std 100	18.168	
Std 100	18.382	
Std 100	22.166	
Calibration Std 100 Average		**19.57**
Std 250	49.791	
Std 250	48.137	
Std 250	48.497	
Calibration Std 250 Average		**48.81**
Std 500	98.313	
Std 500	98.889	
Std 500	96.73	
Calibration Std 500 Average		**97.98**



Process Run Date	**24 August 2011**	
Blank		17.565
10ppb	2.137	
10ppb	3.481	
10ppb	2.311	
Calibration Std 10 Average		**2.64**
50ppb	11.332	
50ppb	10.607	
50ppb	10.621	
Calibration Std 50 Average		**10.85**
Std 100	22.001	
Std 100	21.225	
Std 100	22.51	
Calibration Std 100 Average		**21.91**
Std 250	55.261	
Std 250	53.803	
Std 250	54.323	
Calibration Std 250 Average		**54.46**
Std 500	96.966	
Std 500	97.6	
Std 500	95.438	
Calibration Std 500 Average		**96.67**



Process Run Date	**29 August 2011**	
Blank		16.005
10ppb	2.043	
10ppb	1.896	
10ppb	1.678	
Calibration Std 10 Average		**1.87**
50ppb	10.056	
50ppb	9.605	
50ppb	8.987	
Calibration Std 50 Average		**9.55**
Std 100	17.333	
Std 100	18.163	
Std 100	18.339	
Calibration Std 100 Average		**17.95**
Std 250	44.008	
Std 250	42.683	
Std 250	42.434	
Calibration Std 250 Average		**43.04**
Std 500	94.393	
Std 500	93.685	
Std 500	89.212	
Calibration Std 500 Average		**92.43**



Single Factor ANOVA: 10 ppb Calibration Curve Standard

	Process 1	Process 2	Process 3	Process 4	Process 5	Process 6	Process 7	Process 8	Process 9	Process 10	Process 11	Process 12	Process 13
	1.368	0.434	2.006	1.522	1.796	1.596	0.822	1.913	1.901	2.183	2.099	2.137	2.043
	1.872	0.529	2.045	1.578	1.507	1.489	0.729	1.918	1.638	1.969	1.929	3.481	1.896
	-1.191	0.387	1.884	1.639	0.601	1.919	0.93	1.483	2.064	2.142	1.536	2.311	1.678
Average	0.683	0.45	1.978333	1.579667	1.301333	1.668	0.827	1.771333	1.867667	2.098	1.854667	2.643	1.872333
Std dev	1.64238	0.072339	0.08399	0.058518	0.623482	0.223859	0.100593	0.249717	0.214947	0.113583	0.288767	0.730925	0.183647

10 ppb Calibration Standard

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Process 1	3	2.049	0.683	2.697411
Process 2	3	1.35	0.45	0.005233
Process 3	3	5.935	1.978333	0.007054
Process 4	3	4.739	1.579667	0.003424
Process 5	3	3.904	1.301333	0.38873
Process 6	3	5.004	1.668	0.050113
Process 7	3	2.481	0.827	0.010119
Process 8	3	5.314	1.771333	0.062358
Process 9	3	5.603	1.867667	0.046202
Process 10	3	6.294	2.098	0.012901
Process 11	3	5.564	1.854667	0.083386
Process 12	3	7.929	2.643	0.534252
Process 13	3	5.617	1.872333	0.033726

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13.71274	12	1.142728	3.7753	0.002216	2.147926
Within Groups	7.869823	26	0.302685			
Total	21.58256	38				

c = 13

n = 39

DF between groups c-1 12

DF within groups n-c 26

MSA 1.142728

MSW 0.302685

F calculated= MSA/MSW 3.7753

Null hypothesis H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = \mu_8 = \mu_9 = \mu_{10} = \mu_{11} = \mu_{12} = \mu_{13}$

Alternate hypothesis H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6 \neq \mu_7 \neq \mu_8 \neq \mu_9 \neq \mu_{10} \neq \mu_{11} \neq \mu_{12} \neq \mu_{13}$

Since the calculated f-test statistic is greater that the f crit test statistic obtained from the table, the null hypothesis is therefore rejected. There is not enough evidence to accept the null hypothesis stating that the mean concentration of selenium in the 10ppb calibration curve standard among the thirteen different process runs does not vary. The alternate hypothesis is therefore accepted stating that there is significant evidence to conclude that the mean selenium concentration among the thirteen different process runs varies.

Single Factor ANOVA: 50 ppb Calibration Curve Standard

	Process 1	Process 2	Process 3	Process 4	Process 5	Process 6	Process 7	Process 8	Process 9	Process 10	Process 11	Process 12	Process 13
	5.089	2.402	8.899	9.038	8.245	8.634	8.805	10.478	9.134	9.762	9.68	11.332	10.056
	5.805	2.735	8.937	8.849	8.088	9.238	8.904	9.583	10.648	10.757	10.441	10.607	9.605
	5.99	2.527	8.704	8.589	8.485	8.63	8.727	10.371	8.962	9.434	8.556	10.621	8.987
Average	5.628	2.554667	8.846667	8.825333	8.272667	8.834	8.812	10.144	9.581333	9.984333	9.559	10.85333	9.549333
Std dev	0.475864	0.168215	0.125005	0.225434	0.199941	0.34988	0.088707	0.488777	0.927755	0.688953	0.948307	0.414597	0.53667

50ppb Calibration Standard

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Process 1	3	16.884	5.628	0.226447
Process 2	3	7.664	2.554667	0.028296
Process 3	3	26.54	8.846667	0.015626
Process 4	3	26.476	8.825333	0.05082
Process 5	3	24.818	8.272667	0.039976
Process 6	3	26.502	8.834	0.122416
Process 7	3	26.436	8.812	0.007869
Process 8	3	30.432	10.144	0.238903
Process 9	3	28.744	9.581333	0.860729
Process 10	3	29.953	9.984333	0.474656
Process 11	3	28.677	9.559	0.899287
Process 12	3	32.56	10.85333	0.17189
Process 13	3	28.648	9.549333	0.288014

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	173.548	12	14.46234	54.89464	2.44E-15	2.147926
Within Groups	6.849863	26	0.263456			
Total	180.3979	38				

c = 13

n = 39

DF between groups c-1 12

DF within groups n-c 26

MSA 14.46234

MSW 0.263456

F calculated= MSA/MSW 54.89

Null hypothesis H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = \mu_8 = \mu_9 = \mu_{10} = \mu_{11} = \mu_{12} = \mu_{13}$

Alternate hypothesis H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6 \neq \mu_7 \neq \mu_8 \neq \mu_9 \neq \mu_{10} \neq \mu_{11} \neq \mu_{12} \neq \mu_{13}$

Since the calculated f-test statistic is greater that the f crit test statistic obtained from the table, the null hypothesis is therefore rejected. There is not enough evidence to accept the null hypothesis stating that the mean concentration of selenium in the 50ppb calibration curve standard among the thirteen different process runs does not vary. The alternate hypothesis is therefore accepted stating that there is significant evidence to conclude that the mean selenium concentration among the thirteen different process runs varies.

Single Factor ANOVA: 100 ppb Calibration Curve Standard

	Process 1	Process 2	Process 3	Process 4	Process 5	Process 6	Process 7	Process 8	Process 9	Process 10	Process 11	Process 12	Process 13
	14.41	6.111	17.563	18.949	17.139	18.832	17.33	21.315	17.525	18.737	18.168	22.001	17.333
	14.075	6.159	17.472	19.437	20.462	17.989	17.782	18.764	17.897	19.033	18.382	21.225	18.163
	14.297	6.778	17.852	19.892	17.338	18.027	17.873	21.139	18.837	22.128	22.166	22.51	18.339
Average	14.26067	6.349333	17.629	19.426	18.313	18.28267	17.66167	20.406	18.08633	19.966	19.572	21.912	17.945
Std dev	0.17043	0.372011	0.198411	0.471596	1.863746	0.476116	0.290813	1.424734	0.676181	1.878187	2.249017	0.647107	0.537263

100ppb Calibration Standard

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Process 1	3	42.782	14.26067	0.029046
Process 2	3	19.048	6.349333	0.138392
Process 3	3	52.887	17.629	0.039367
Process 4	3	58.278	19.426	0.222403
Process 5	3	54.939	18.313	3.473551
Process 6	3	54.848	18.28267	0.226686
Process 7	3	52.985	17.66167	0.084572
Process 8	3	61.218	20.406	2.029867
Process 9	3	54.259	18.08633	0.457221
Process 10	3	59.898	19.966	3.527587
Process 11	3	58.716	19.572	5.058076
Process 12	3	65.736	21.912	0.418747

Process 13	3	53.835	17.945	0.288652

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	534.8185	12	44.5682	36.22487	3.86E-13	2.147926
Within Groups	31.98834	26	1.230321			
Total	566.8068	38				

c = 13

n = 39

DF between groups c-1 12

DF within groups n-c 26

MSA 44.57

MSW 1.23

F calculated= MSA/MSW 36.22

Null hypothesis H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = \mu_8 = \mu_9 = \mu_{10} = \mu_{11} = \mu_{12} = \mu_{13}$

Alternate hypothesis H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6 \neq \mu_7 \neq \mu_8 \neq \mu_9 \neq \mu_{10} \neq \mu_{11} \neq \mu_{12} \neq \mu_{13}$

Since the calculated f-test statistic is greater that the f crit test statistic obtained from the table, the null hypothesis is therefore rejected. There is not enough evidence to accept the null hypothesis stating that the mean concentration of selenium in the 100ppb calibration curve standard among the thirteen different process runs does not vary. The alternate hypothesis is therefore accepted stating that there is significant evidence to conclude that the mean selenium concentration among the thirteen different process runs varies.

Single Factor ANOVA: 250 ppb Calibration Curve Standard

	Process 1	Process 2	Process 3	Process 4	Process 5	Process 6	Process 7	Process 8	Process 9	Process 10	Process 11	Process 12	Process 13
	43.163	14.912	43.611	45.971	47.281	43.326	49.947	51.916	46.513	46.657	49.791	55.261	44.008
	38.765	15.661	44.962	44.418	49.328	43.356	47.14	50.878	44.945	48.551	48.137	53.803	42.683
	40.814	14.874	45.031	46.724	46.796	46.381	46.827	47.563	46.681	47.821	48.497	54.323	42.434
Average	40.914	15.149	44.53467	45.70433	47.80167	44.35433	47.97133	50.119	46.04633	47.67633	48.80833	54.46233	43.04167
Std dev	2.200705	0.443812	0.800662	1.175901	1.343903	1.755209	1.71812	2.27359	0.957474	0.955251	0.869842	0.738919	0.846079

250ppb Calibration Standard

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Process 1	3	122.742	40.914	4.843101
Process 2	3	45.447	15.149	0.196969
Process 3	3	133.604	44.53467	0.64106
Process 4	3	137.113	45.70433	1.382742
Process 5	3	143.405	47.80167	1.806076
Process 6	3	133.063	44.35433	3.080758
Process 7	3	143.914	47.97133	2.951936
Process 8	3	150.357	50.119	5.169213
Process 9	3	138.139	46.04633	0.916757
Process 10	3	143.029	47.67633	0.912505
Process 11	3	146.425	48.80833	0.756625

Process 12	3	163.387	54.46233	0.546001
Process 13	3	129.125	43.04167	0.71585

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3187.416	12	265.618	144.36	1.29E-20	2.147926
Within Groups	47.83919	26	1.839969			
Total	3235.255	38				

c = 13

n = 39

DF between groups c-1 12

DF within groups n-c 26

MSA 265.62

MSW 1.84

F calculated= MSA/MSW 144.36

Null hypothesis H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = \mu_8 = \mu_9 = \mu_{10} = \mu_{11} = \mu_{12} = \mu_{13}$

Alternate hypothesis H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6 \neq \mu_7 \neq \mu_8 \neq \mu_9 \neq \mu_{10} \neq \mu_{11} \neq \mu_{12} \neq \mu_{13}$

Since the calculated f-test statistic is greater that the f crit test statistic obtained from the table, the null hypothesis is therefore rejected. There is not enough evidence to accept the null hypothesis stating that the mean concentration of selenium in the 250ppb calibration curve standard among the thirteen different process runs does not vary. The alternate hypothesis is therefore accepted stating that there is significant evidence to conclude that the mean selenium concentration among the thirteen different process runs varies.

Single Factor ANOVA: 500 ppb Calibration Curve Standard

	Process 1	Process 2	Process 3	Process 4	Process 5	Process 6	Process 7	Process 8	Process 9	Process 10	Process 11	Process 12	Process 13
	83.391	29.609	89.496	95.604	94.143	86.236	96.126	96.018	92.688	92.113	98.313	96.966	94.393
	88.765	30.184	88.963	87.928	94.671	87.869	99.309	94.267	96.532	89.51	98.889	97.6	93.685
	83.676	29.991	90.119	94.773	95.465	84.272	96.953	95.39	84.928	96.767	96.73	95.438	89.212
Average	85.27733	29.928	89.526	92.76833	94.75967	86.12567	97.46267	95.225	91.38267	92.79667	97.97733	96.668	92.43
Std dev	3.023768	0.292631	0.578584	4.212394	0.665445	1.801036	1.651573	0.887085	5.911102	3.676488	1.117955	1.111379	2.809263

500ppb Calibration Standard

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Process 1	3	255.832	85.27733	9.14317
Process 2	3	89.784	29.928	0.085633
Process 3	3	268.578	89.526	0.334759
Process 4	3	278.305	92.76833	17.74426
Process 5	3	284.279	94.75967	0.442817
Process 6	3	258.377	86.12567	3.243732
Process 7	3	292.388	97.46267	2.727692
Process 8	3	285.675	95.225	0.786919
Process 9	3	274.148	91.38267	34.94113
Process 10	3	278.39	92.79667	13.51656
Process 11	3	293.932	97.97733	1.249824

Process 12	3	290.004	96.668	1.235164
Process 13	3	277.29	92.43	7.891959

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11472.98	12	956.0816	133.1538	3.61E-20	2.147926
Within Groups	186.6872	26	7.180278			
Total	11659.67	38				

c = 13

n = 39

DF between groups c-1 12

DF within groups n-c 26

MSA 956.0816

MSW 7.1803

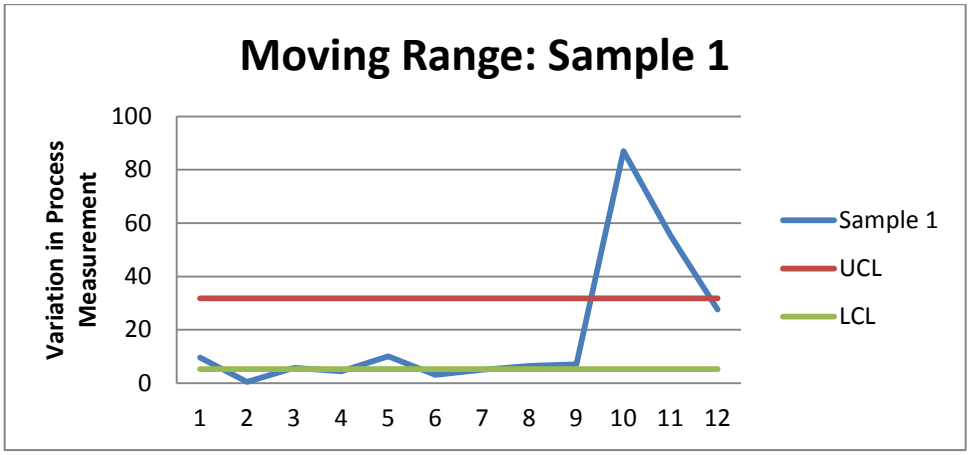
F calculated= MSA/MSW 133.15

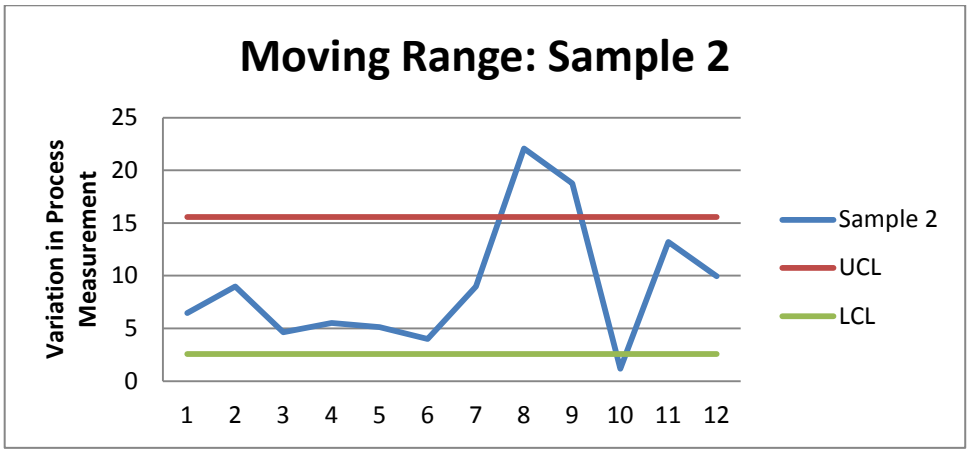
Null hypothesis H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = \mu_8 = \mu_9 = \mu_{10} = \mu_{11} = \mu_{12} = \mu_{13}$

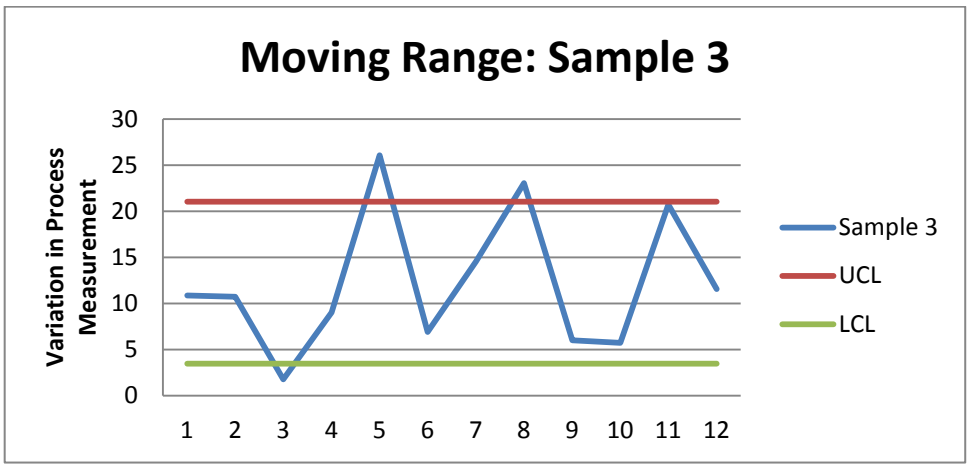
Alternate hypothesis H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6 \neq \mu_7 \neq \mu_8 \neq \mu_9 \neq \mu_{10} \neq \mu_{11} \neq \mu_{12} \neq \mu_{13}$

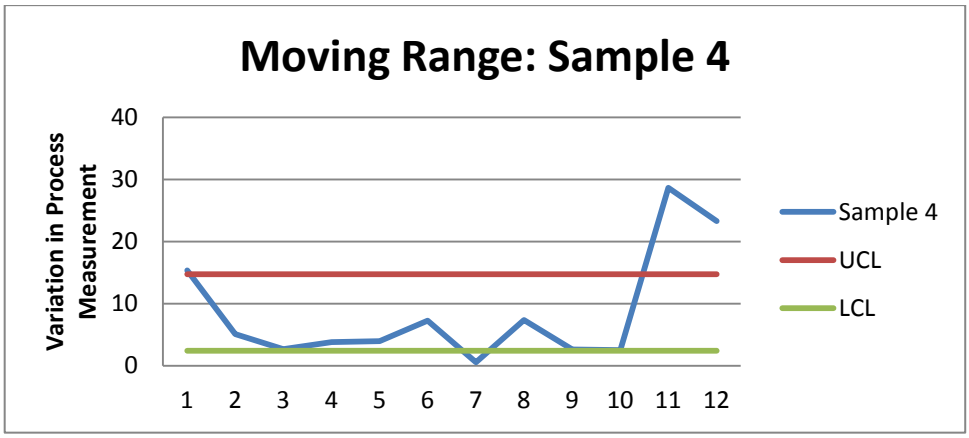
Since the calculated f-test statistic is greater that the f crit test statistic obtained from the table, the null hypothesis is therefore rejected. There is not enough evidence to accept the null hypothesis stating that the mean concentration of selenium in the 500ppb calibration curve standard among the thirteen different process runs does not vary. The alternate hypothesis is therefore accepted stating that there is significant evidence to conclude that the mean selenium concentration among the thirteen different process runs varies.

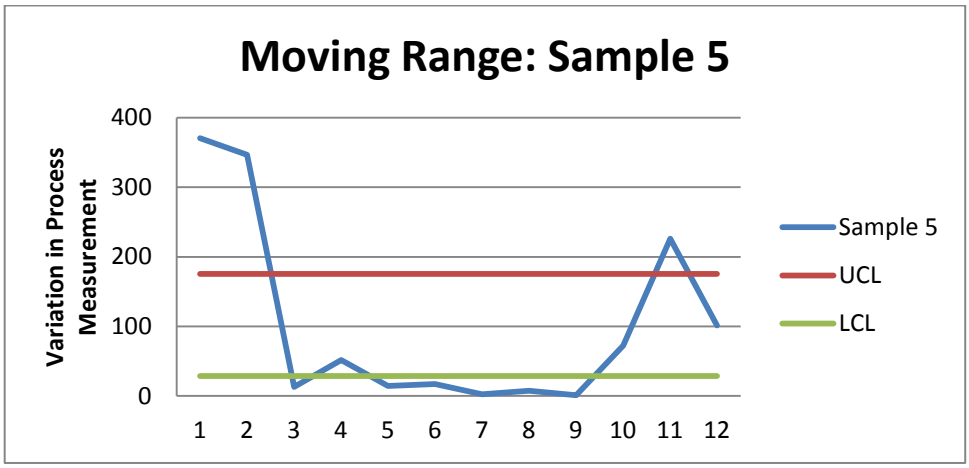
ANNEXURE 16: Individual Sample Moving Range Charts

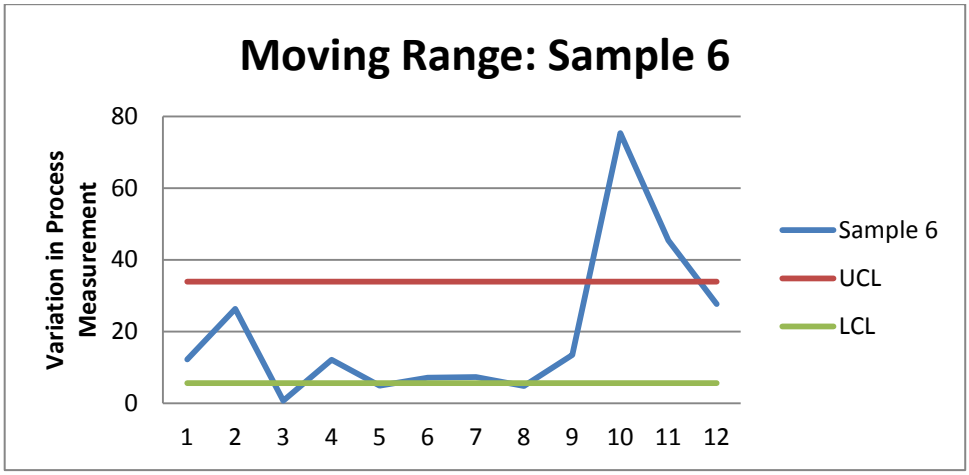


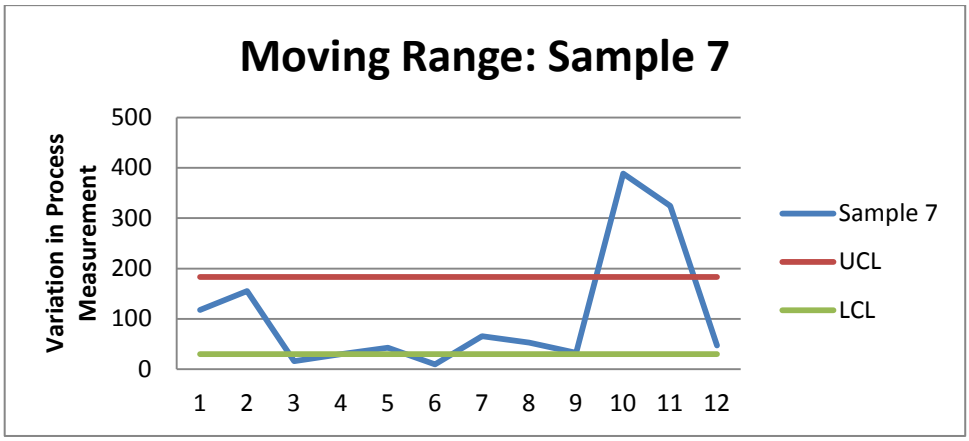


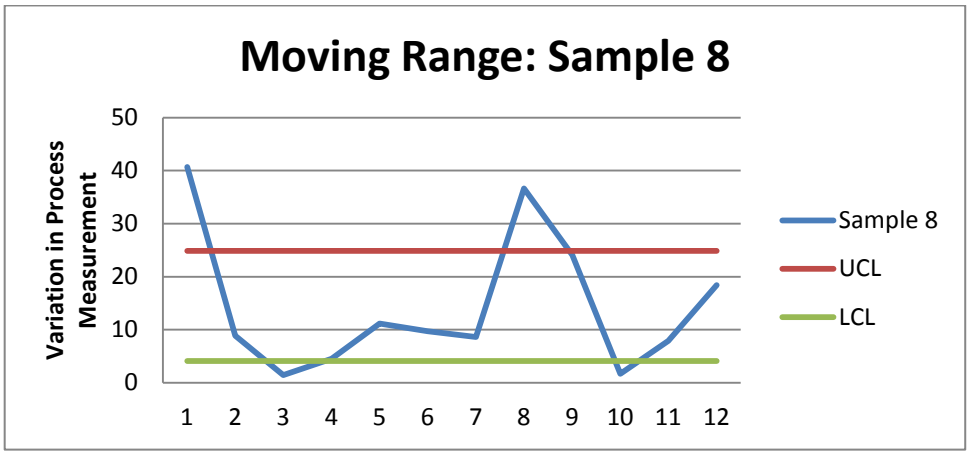


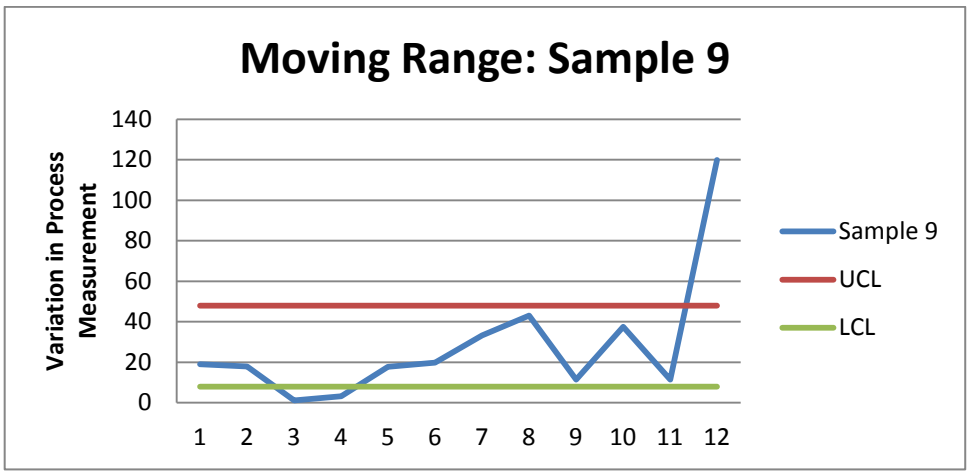

Moving Range: Sample 4
Variation in Process Measurement
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12
Sample 4
UCL
LCL

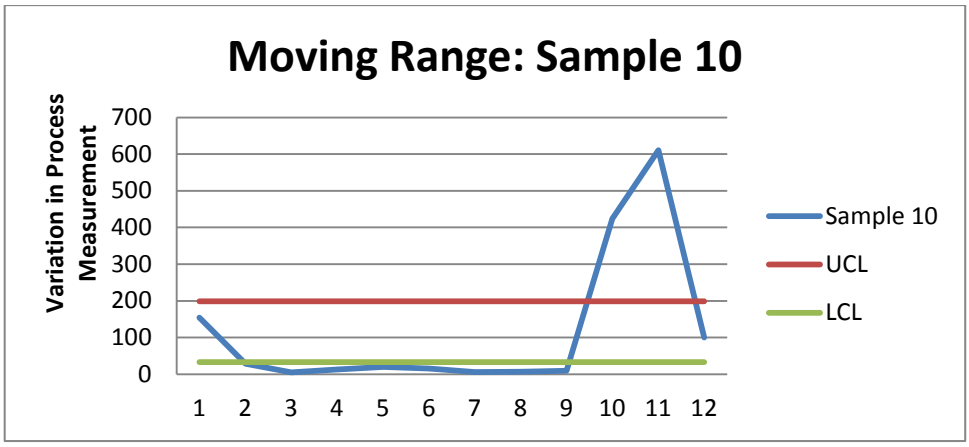

Moving Range: Sample 5
Variation in Process Measurement
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12
Sample 5
UCL
LCL

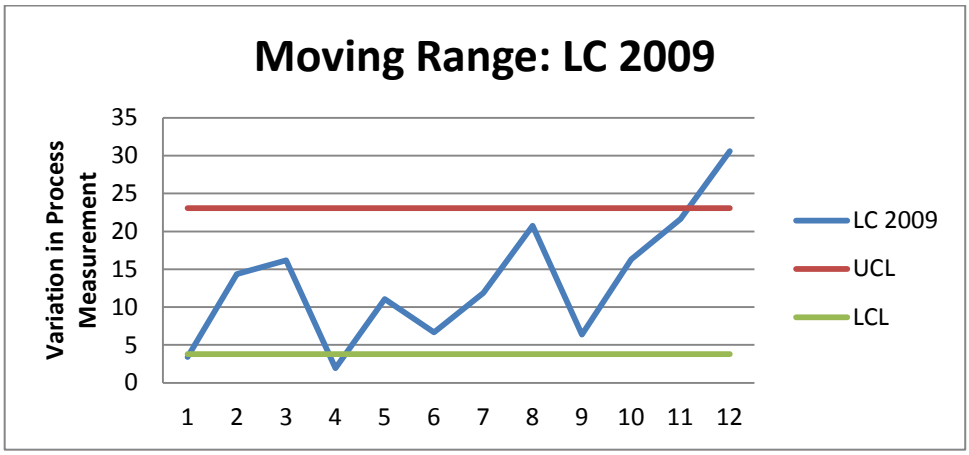

Moving Range: Sample 6
Variation in Process Measurement
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12
Sample 6
UCL
LCL


Moving Range: Sample 7
Variation in Process Measurement
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12
Sample 7
UCL
LCL


Moving Range: Sample 8
Variation in Process Measurement
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12
Sample 8
UCL
LCL


Moving Range: Sample 9
Variation in Process Measurement
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12
Sample 9
UCL
LCL


Moving Range: Sample 10
Variation in Process Measurement
700
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12
Sample 10
UCL
LCL


Moving Range: LC 2009
Variation in Process Measurement
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12
LC 2009
UCL
LCL

ANNEXURE 17: Modified Selenium Analysis Process FMEA Assessment

Item and Function	Potential failure mode	Potential effects of failure	Δ	SEV	Potential Causes of failure	OCC	Detection method & quality controls	DET	RPN	Recommended actions
Sample Registration	Mistaken switching of samples	Erroneous results		4	Carelessness/ Inadequate procedure	4	Internal Lab worksheet: Selenium analysis	5	80	Initiate use of internal control worksheet: Selenium analysis
Digestion failure	Microwave Apparatus failure	Process halted	Δ	8	Maintenance schedule not upheld	2	Uphold strict maintenance schedule	8	128	Ensure maintenance schedule upheld
					Unforeseen equipment failure	2	Unavoidable	10	160	None. Previous selenium method as backup.
	Microwave vessel failure	Select samples: Rework		8	'Wear and tear' of microwave vessels	3	Visual inspection of microwave vessels before use	8	192	Incorporate a 'Visual Inspection' step in Selenium Analysis SOP
Reduction phase failure	Water bath failure	Delay in process results		5	Unforeseen equipment failure	2	Unavoidable	10	100	Have back-up water bath on standby
	Thermometer failure	Delay in process results		5	Unforeseen equipment failure	3	Unavoidable	10	150	Calibrate thermometers before analysis
Incorrect measurement parameters (detection instrument)	Technician error	Delay in process results		8	Incompetence / carelessness	3	Adequate training / Human error	3	72	Ensure process operator competent to perform analysis
AA failure	AA apparatus failure	Process halted	Δ	8	Maintenance schedule not upheld	2	Uphold strict maintenance schedule	8	128	Ensure maintenance schedule upheld
					Unforeseen equipment failure	4	Unavoidable	10	320	None. Previous selenium method as backup.
	Lamp failure	Delay in process results		6	Routine 'wear and tear'	4	AA error message: Lamp fused	5	120	Ensure spare lamp in stock
HG failure	HG apparatus failure	Process halted	Δ	8	Maintenance schedule not upheld	2	Uphold strict maintenance schedule	8	128	Ensure maintenance schedule upheld.
					Unforeseen equipment failure	2	Unavoidable	10	160	None. Previous selenium method as backup.
	Breakage in HG piping system	Delay in process results		6	Routine 'wear and tear'	3	Visual check: No reagent being aspirated	5	90	Ensure spare piping in stock
Reporting: Electronic system	LIMS offline	Delay in result reporting		7	Unforeseen or unavoidable	3	None: General WCPVL failure mode	8	168	None: wait until system online again

Δ Delta = critical characteristic which may require special controls or affect safety, or compliance with government regulations

SEV = Severity rating (1 to 10)

OCC = Occurrence frequency (1 to 10)

DET = Detection rating (1 to 10)
RPN = Risk priority number (RPN) = (SEV) x (OCC) x (DET)

FMEA Evaluation Criteria: Severity, Occurrence and Detection

Severity Evaluation Criteria		
Effect	**Criteria: Severity of Effect**	**Rank**
Hazardous – without warning	Very high severity ranking when a potential failure mode affects safety of personnel and/or involves noncompliance with government regulation without warning	10
Hazardous – with warning	Very high severity ranking when potential failure mode affects safety of personnel and/or involves noncompliance with government regulation with warning	9
Very High	Process halted	8
High	Uncertainty in results – needs rework	7
Moderate	Results obtainable from process, but reduced level of performance. Controls out of spec	6
Low	Results obtainable from process, but reduced level of performance. Controls in spec	5
Very Low	Delayed process results. Controls in spec	4
Minor	Minor delay in process results. Controls in spec	3
Very Minor	Process results not available electronically	2
None	No effect	1*

*__Note:__ *Zero (0) rankings for severity, occurrence and detection are not allowed*

Occurrence Evaluation Criteria			
Rank	**CPK**	**Failure Rates**	**Probability of failure**
10	≥ 0.33	> 1 in 2	Very High: Failure almost inevitable
9	≥ 0.33	1 in 3	
8	≥ 0.51	1 in 8	High: Repeated failures
7	≥ 0.67	1 in 20	
6	≥ 0.83	1 in 80	Moderate: Occasional failures
5	≥ 1.00	1 in 400	
4	≥ 1.17	1 in 2000	Low: Relatively few failures
3	≥ 1. 33	1 in 15 000	
2	≥ 1.50	1 in 150 000	Remote: Failure is unlikely
1*	≥ 1.67	≤ 1 in 500 000	

*__Note:__ *Zero (0) rankings for severity, occurrence and detection are not allowed*

Detection Evaluation Criteria		
Detection	**Criteria**	**Rank**
Absolute uncertainty	Potential cause and subsequent failure mode cannot be detected	10
Very Remote	Very remote probability potential cause and failure mode will be detected by built-in control	9
Remote	Remote probability potential cause and failure mode will be detected by built-in control	8
Very Low	Very low probability potential cause and failure mode will be detected by built-in control	7
Low	Low probability potential cause and failure mode will be detected by built-in control	6
Moderate	Moderate probability potential cause and failure mode will be detected by built-in control	5
Moderately high	Moderately high probability potential cause and failure mode will be detected by built-in control	4
High	High probability potential cause and failure mode will be detected by built-in control	3
Very high	Very high probability potential cause and failure mode will be detected by built-in control	2
Almost certain	Certain probability potential cause and failure mode will be detected by built-in control	1*

*__Note:__ *Zero (0) rankings for severity, occurrence and detection are not allowed*

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