1 PURPOSE & PRINCIPLE

Emamectin (in use as a benzoate salt) is a member of the avermectin insecticides. Avermectins cause premature moulting in crustaceans.

Teflubenzuron and diflubenzuron are broad spectrum pesticides. They are chitin synthesis inhibitors and are effective in the moulting stage of insect and crustacea development/ growth.

Of the above in-feed chemicals, emamectin benzoate is the only one which currently has approval for use in marine fish farms as have the bath chemicals deltamethrin and azamethiphos.

At present this method will consider the quantification of emamectin benzoate alone.

For each marine cage fish farm the predictive model determines the expected area of impact around the farm; the extent of this area is the Allowable Zone of Effect (AZE).

Samples collected for analysis are referred to as 'near field' sediments and 'far-field' sediments. Near-field sediments are collected up to 25 metres from the cage edge; far-field sediments are collected between 25 metres and (typically) up to 100 metres from the cage edge. However, if the 100 metre measurement is *within* the AZE, then the far-field sediment will be taken at >100 metres, to take it outside the AZE. Both near and far-field sediments are covered by this method.

In practice, near-field sediment samples are usually taken at the cage edge and far-field sediment samples are taken outside the AZE. Near-field sediment samples may also be identified as Allowable Zone of Effect (AZE) samples; far-field sediment samples may also be identified as Outside AZE samples.

Appropriate quantities of wet sediment samples are taken to be equivalent to ~20 g dry-weight near-field sediment or ~2 g dry-weight far-field sediment. Note that for very high moisture content samples (>~60% moisture content) the target weight may not always be achievable due to capacity limitations of the methodology. Whilst this scenario is expected to occur infrequently, the reported method detection limit will be adjusted appropriately to account for any lesser dry-weight equivalent.

All sediment samples are extracted by shaking with acetonitrile in the presence of magnesium sulphate drying agent. The extracts are cleaned with cation exchange SPE cartridges. The extracts are finally re-constituted in 5ml of methanol / water.

The determinands are separated, identified and quantified by liquid chromatography with high resolution accurate mass spectrometric detection.

Quantification is performed by comparison of peak areas in the sample chromatogram with a range of standard concentrations constructed into a calibration curve. Internal standard correction is used in the calculation of results.

The criteria for choice of internal standards are based on the very low probability that they will be found in the environmental samples and their similar behaviour in the LC-MS system to the analytes. The components are identified by comparison with the standards using retention time, and accurate mass.

2 PERSONNEL

The work detailed in this procedure may be carried out by any member of staff with a relevant competency record. It may also be carried out by a trainee under supervision, on the authority of the Senior Chemist or Unit Manager.

3 SAFETY

A full CoSHH assessment related to this method can be found in ES-TORG-C-216. The principle safety hazards are listed below:

3.1 ACETONE is extremely flammable, and may form explosive vapour-air mixtures. Avoid inhaling vapour and excessive skin contact.

3.3 SOLID STANDARD MATERIALS should be assumed to be toxic and handled with care.

3.4 PREPARED STANDARDS have an excessive proportion of acetone. Treat these standards as if they were pure acetone (3.1).

3.6 ACETONITRILE is extremely flammable, and may form explosive vapour-air mixtures.

3.7 METHANOL is also toxic by ingestion and skin contact. Avoid inhaling vapour and excessive skin contact.

4 PERFORMANCE CHARACTERISTICS

4.1 **Range**: The analytical range is presented in the table below for a 2000µL injection. This range can be extended by extract dilution (see 9.12) or re-analysis using a smaller sample size.

Table 1: Instrument Range and Effective Sample Range

Determinand	Instrument Range (ng/L)	Effective Sample Range* (ng/Kg – dry-weight)
Emamectin Benzoate	10-300	3.4 -125

*Sediment equivalents are based on 60% extraction efficiency and 20g sediment

4.2 **Method Detection Limit (MDL) and Minimum Reporting Value (MRV):**

Table 2: MDL, EQS and MRV

Determinand	Proposed EQS ng/Kg dry-weight		Target MDL ng/Kg dry-weight		MDL Achieved ng/Kg dry-weight		MRV ng/Kg dry-weight	
	Far-field	Near-Field	Far-field	Near-Field	Far-field	Near-Field	Far-field	Near-Field
Emamectin Benzoate	12	120	0.2	2	3.4	33.7	3.4	33.7

4.3 **Bias, Precision and Accuracy**: The integrity of quantitative results will be ensured by the analysis of quality control standards throughout the batch. Also, system suitability will be assessed before the analysis of any samples.

Detailed validation and performance data can be accessed via this link (link not valid).

4.4 **Interferences:** The probability of occurrence of undetected interferences is considered to be highly unlikely for the following reasons:

- All components are analysed using high resolution mass spectrometry (HRMS) at a resolution of 70,000.
- Compounds with identical chemical formulae and retention time will interfere with the analysis. Care is taken with mobile phase and LC column selection to ensure interferences are separated from the true analytes of interest.
- Emamectin analysis utilises the cationic chemical properties of this compound during the sample preparation. Therefore any interfering compounds would be required to show the same characteristics and have a near identical mass.
- Small concentrations of systematic interference/ contribution are taken into account in the calibration curve. However interference can occur by sample matrix suppression of ionisation. Low peak area values (<70% of expected) for the Internal Standards may indicate the presence of a suppressant.

4.7 **Expanded Uncertainty:** See 'Uncertainty Data' file.

5 REAGENTS

5.1 **Water** - Use only Millipore Milli-Q_{plus} ultra-pure deionised water at a setting of 18.2M

5.2 **Methanol**- VWR HPLC Grade or equivalent

5.3 **Acetonitrile** - VWR HPLC Grade Grade or equivalent

5.4 **Acetone**- VWR HPLC Grade or equivalent

5.5 **Magnesium Sulphate** – $MgSO_4$

5.6 **Formic Acid** – Sigma Aldrich HPLC grade or equivalent

5.7 **Ammonium formate ≥99.0%** - Sigma Aldrich mass spectrometry grade or equivalent

5.8 **Acetic Acid**. Fishers, Analytical reagent Grade

5.9 **Ammonia solution** - S.G. 0.88 (35%). Analytical grade. Typically Fisher Scientific.

5.10 **Acetic Acid Solution** –

- Using a 10 ml measuring cylinder (6.10) add 8 ± 0.2 ml acetic acid (5.5) to 500 ml water (5.1)

5.11 **Elution solvent 1: 3.5% Ammonia solution – (in methanol)**

- In a 500 ml measuring cylinder (6.10) add 50 ± 1 ml ammonia solution (5.6) to 300 ml methanol (5.2)
- Make up to 500 ml with methanol (5.2)

5.12 **Elution solvent 2: 0.7% Ammonia solution – (in acetonitrile)**

- In a 500 ml measuring cylinder (6.10) add 100 ± 1 ml elution solvent 1 (5.11) to 400 ml acetonitrile (5.3)

5.13 **2-Propanol -** VWR HPLC Grade or equivalent

STOCK CALIBRATION STANDARDS

Table 3: Reagents 5.14, 5.15, 5.16

1ºStd Reagent No.	Determinand	1º Std Reagent Conc (mg/L)*	Vol 1º Std **req'd (µL) in 25ml	2º Std Reagent Conc (mg/L)	2ºStd Reagent No.	Vol 2º Std req'd (µL) in 25ml	3º Std Reagent Conc (µg/L)	3ºStd Reagent No.
5.14	Emamectin B.	250	100	1.0	5.15	250	10	5.16
	*Teflubenzuron****	*500*		*2.0*			*20*	
	*Diflubenzuron****	*1000*		*4.0*			*40*	
	*Ivermectin****	*500*		*2.0*			*20*	

nominal values **volume required is dependent on the actual 1º stock concentration * components are present in calibration mix but are not currently used in methodology*

5.14 **Mixed Fish farm Chemicals Primary Calibration Standard (250 mg/L)**

Current custom mix as AccuStandard supplied by Kinesis. Equivalent standards from other suppliers who meet ISO Guide 34 requirements may also be used.

NOTE: This custom mix also contains teflubezuron, diflubenzuron and ivermectin at the nominal concentrations given in Table 3 above. Equivalent standards are not required to contain these additional components.

- Mixed Fish Farm calibration standard supplied as custom mix containing emamectin benzoate at a nominal concentration of 250 mg/L.
- Standard supplied in 1ml glass ampule stored in fridge at 3 ± 2°C until use.
- Expiry as certified by supplier.
- On use, transfer the prepared standard to a clean 2ml amber vial (6.12).
- Clearly label as Fish Farm PRIMARY CALIBRATION STANDARD. Record the concentration, date and other necessary details in the relevant standards record form as detailed in ES-TORG-P-002.
- Store the transferred standard at 3 ± 2°C.

- Primary standards must be replaced after **12 months** or sooner if comparison with check standards indicates a problem or if the custom mix has a shorter expiry date.

5.15 **Secondary Stock Calibration Composite Standard (1.0 mg/L)**

- Using the Hamilton MicroLab Diluter (6.7), dispense 100 µl PRIMARY CALIBRATION STANDARD (5.14) with 800 µl acetonitrile in to a clean dry 25 ml volumetric flask (6.8).
- Make up to the 25 ml mark with acetonitrile (5.3) and mix thoroughly.
- Transfer to a clean 30 ml PTFE sealed screw cap amber vial (6.12).
- Clearly label as Fish Farm SECONDARY CALIBRATION STANDARD. Record the concentration of each component, the date and other necessary details in the relevant standards record form as detailed in ES-TORG-P-002.
- Store at 5°C ± 3°C.
- Secondary standards must be replaced after **8 months** or sooner if comparison with check standards indicates a problem or if any primary stock materials expire.

5.16 **Tertiary Stock Calibration Standard (10 µg/l)**

- Using the Hamilton MicroLab Diluter (6.7), dispense 250 µL Fish Farm SECONDARY CALIBRATION STANDARD (5.15) with 30 µL of acetonitrile (5.3) into a 25 ml volumetric flask (6.8).
- Make up to the 25 ml mark with acetonitrile (5.3) and mix thoroughly.
- Transfer to a clean 30 ml PTFE sealed screw cap amber vial (6.13).
- Clearly label as Fish Farm TERTIARY CALIBRATION STANDARD. Record the concentration of each component, the date and other necessary details in the relevant standards record form as detailed in ES-TORG-P-002.
- Store at 5°C ± 3°C.
- Tertiary standards must be replaced after **4 months** or sooner if comparison with check standards indicates a problem or if any primary stock materials expire.

5.17 **Quaternary Stock Calibration Standard (1 µg/l**)

- Using a gas-tight syringe (6.23), dispense 200 ± 5 µl Fish Farm TERITARY CALIBRATION STANDARD (5.16) into a 10 ml glass LC autosampler vial.
-
- Using a 5 ml autopipettor, add 1800 ± 100 µl acetonitrile (5.3) into the vial.
- Clearly label as Fish Farm QUARTERNARY CALIBRATION STANDARD.
- Quaternary standards must be used on day of preparation only.

INDEPENDENT STANDARDS FOR QUALITY CONTROL

Table 4: Reagents 5.18, 5.19, 5.20

1ºStd Reagent No.	Determinand	1º Std Reagent Conc (mg/L)*	Vol 1º Std **req'd (µL) in 25ml	2º Std Reagent Conc (mg/L)	2ºStd Reagent No.	Vol 2º Std req'd (µL) in 25ml	3º Std Reagent Conc (µg /L)	3ºStd Reagent No.
5.18	Emamectin B.	250	100	1.0	5.19	250	10	5.20
	*Teflubenzuron****	*500*		*2.0*			*20*	
	*Diflubenzuron****	*1000*		*4.0*			*40*	
	*Ivermectin****	*500*		*2.0*			*20*	

nominal values **volume required is dependent on the actual 1º stock concentration * components are present in independent mix but are not currently used in methodology*

Where possible primary independent mix (5.18) should be of a different source from the primary calibration mix (5.14). Preferably this will be a separate supplier/ manufacturer. However, this may not be possible, in which case a separate batch from the same supplier/ manufacturer would be suitable.

5.18 **Mixed Fish farm Chemicals Primary Independent Standard (250 mg/L)**

Current custom mix as AccuStandard supplied by Kinesis. Equivalent standards from other suppliers who meet ISO Guide 34 requirements may also be used.

NOTE: This custom mix also contains teflubezuron, diflubenzuron and ivermectin at the nominal concentrations given in Table 4 above. Equivalent standards are not required to contain these additional components.

- Mixed Fish Farm calibration standard supplied as custom mix containing emamectin benzoate at a nominal concentration of 250 mg/L.
- Standard supplied in 1ml glass ampule stored in fridge at 3 ± 2°C until use.
- Expiry as certified by supplier.
- On use, transfer the prepared standard to a clean 2 ml PTFE sealed screw cap amber vial (6.12).
- Clearly label as Fish Farm PRIMARY INDEPENDENT STANDARD. Record the concentration, date and other necessary details in the relevant standards record form as detailed in ES-TORG-P-002.
- Store the transferred standard at 3 ± 2°C.
- Primary standards must be replaced after **12 months** or sooner if comparison with check standards indicates a problem or if the custom mix has a shorter expiry date.

5.19 **Secondary Stock Independent Composite Standard (1.0 mg/L)**

- Using the Hamilton MicroLab Diluter (6.7), dispense 100 µl PRIMARY INDEPENDENT STANDARD (5.18) with 500 µl acetonitrile in to a clean dry 25 ml volumetric flask (6.8).
- Make up to the 25 ml mark with acetonitrile (5.3) and mix thoroughly.
- Transfer to a clean 30 ml PTFE sealed screw cap amber vial (6.12).
- Clearly label as Fish Farm SECONDARY INDEPENDENT STANDARD. Record the concentration of each component, the date and other necessary details in the relevant standards record form as detailed in ES-TORG-P-002.
- Store at 5°C ± 3°C.
- Secondary standards must be replaced after **8 months** or sooner if comparison with check standards indicates a problem or if any primary stock materials expire.

5.20 **Tertiary Stock Independent Standard (10 µg/l)**

- Using the Hamilton MicroLab Diluter (6.7), dispense 250 µL Fish Farm SECONDARY INDEPENDENT STANDARD (5.19) with 30 µL of acetonitrile (5.3) into a 25 ml volumetric flask (6.8).
- Make up to the 25 ml mark with acetonitrile (5.3) and mix thoroughly.
- Transfer to a clean 30 ml PTFE sealed screw cap amber vial (6.13).
- Clearly label as Fish Farm TERTIARY INDEPENDENT STANDARD. Record the concentration of each component, the date and other necessary details in the relevant standards record form as detailed in ES-TORG-P-002.
- Store at 5°C ± 3°C.
- Tertiary standards must be replaced after **4 months** or sooner if comparison with check standards indicates a problem or if any primary stock materials expire.

INTERNAL STANDARDS

The stock materials (neat materials) for these standards are to be sourced from a reputable supplier and be accompanied by a certificate of purity.

Table 5: Reagents 5.21, 5.22, 5.23, 5.24

Stock Reagent No.	Determinand	Weight req'd (mg)/ Volume (ml)	1° Std Reagent Conc (mg/L)*	1°Std Reagent No.	Vol 1° Std **req'd (µL) in 10ml	2° Std Reagent Conc (mg/L)	2°Std Reagent No.	Vol 2° Std req'd (µL) in 25ml	3° Std Reagent Conc (µg/L)	3°Std Reagent No.
5.21.1	6-C13-Diflubenzuron	25/50	500	5.22.1	200	10	5.23	1000	400	5.24
5.21.2	*Abamectin****	*50/50*	*1000*	*5.22.2*	*200*	*20*			*800*	

nominal values **volume required is dependent on the actual 1° stock concentration * components are present in internal standard primary mix but are not currently used in methodology*

NOTE: The volumes shown in the above table are nominal volumes in the case of 1º STD required and actual volumes in the case of 2º STD required. If the actual 1º Stock concentrations differ from those shown in the table then the nominal volumes required shall by corrected by multiplying by a factor F, where **F =** stock concentration expected / actual concentration.

5.21 **Pure standards.** As provided by Sigma Aldrich. Equivalent standards may also be used.

5.22 **Primary Stock Internal Standards (500 / 1000 mg/L)**

- Each of the solids (5.21.1-2) shall be weighed directly, but individually, into 25 ml volumetric flasks (6.8).
- Weigh to an accuracy of ± 0.0001g the nominal amounts of pure certified solid required, as detailed in the Table 5 above.
- Dissolve in acetone (5.1) and make up to the mark according to the instructions on how to prepare standards from solids detailed in ES-TORG-P-002.
- Transfer each prepared standard to a clean 30 ml PTFE sealed screw cap amber vial (6.13)
- Clearly label as Fish Farm INTERNAL STANDARDS. Record the concentration and weight of each component, date and other relevant details of the standards in the relevant standards record form as detailed in ES-TORG-P-002.
- Store these standards at 5ºC $\pm$ 3ºC.
- Primary internal standards must be replaced only if there are signs of deterioration or unaccountable weight change between uses.

5.23 **Secondary Stock composite Internal Standard (10/ 20 mg/l)**

Prepare a secondary internal standard using the Hamilton MicroLab Diluter.

- Using the Hamilton MicroLab Diluter (6.7), dispense the required volume of each PRIMARY INTERNAL STANDARD stock (5.22) required to give the concentrations in the Table 5 above with 30 µL acetonitrile (5.3) into a 10 ml volumetric flask (6.8) .
- Make up to the 10 ml mark with acetonitrile (5.3) and mix thoroughly.
- Transfer to a clean 10 ml PTFE sealed screw cap amber vial (6.12).
- Clearly label as Fish Farm SECONDARY INTERNAL STANDARD**.** Record the concentration of each component, the date and other relevant details of the standards in the relevant standards record form as detailed in ES-TORG-P-002.
- Store these standards at 5ºC $\pm$ 3ºC.
- Secondary internal standards must be replaced only if there are signs of deterioration or unaccountable weight change between uses.

5.24 **Tertiary Stock composite Internal Standard (400/ 800 µg/l)**

- Using the Hamilton MicroLab Diluter (6.7), dispense 2 x 500 µl aliquots of the SECONDARY INTERNAL STANDARD (5.23), each with 1000 µl acetonitrile (5.3) into a 25 ml volumetric flask (6.8).
- Make up to the 25 ml mark with acetonitrile (5.3) and mix thoroughly.
- Transfer to a clean 30 ml PTFE sealed screw cap amber vial (6.13).
- Clearly label as Fish Farm TERTIARY INTERNAL STANDARD**.** Record the concentration of each component, the date and other relevant details of the standards in the relevant standards record form as detailed in ES-TORG-P-002.
- Store at $5^{o}C \pm 3^{o}C$.
- Tertiary internal standards must be replaced only if there are signs of deterioration or unaccountable weight change between uses.

CONFIRMATION OF NEW STANDARDS

Once standards have been prepared it is essential to confirm that the declared concentration is correct. All standards have to be validated prior to being accepted as fit for purpose and a full explanation of the validation procedure is detailed in procedure ES-TORG-P-002 (14.2). However it is not necessary to compare new internal standards provided only one internal standard lot is used for a batch.

5.25 **WORKING CALIBRATION STANDARDS**

- Prior to working calibration standard preparation, a corresponding number of labelled CX (100 mg/6 ml) cartridges (6.15) must be conditioned on an SPE manifold by following steps 9.3.1 to 9.3.3.
- Using gas-tight syringes (6.23), dispense the appropriate volume of tertiary calibration standard (5.16) or quaternary calibration standard (5.17) as described in Table 6 below onto the corresponding labelled conditioned cartridge.
- Working calibration standards are then prepared as per samples from step 9.3.5 onwards.
- Working calibration standards are routinely freshly prepared on the day of analysis alongside a batch of samples. These can be used up to 48 hours after preparation and cartridge clean up step.

  NOTE: It is permissible to analyse a batch of samples using a previous set of calibration standards prepared using the same tertiary calibration standard (5.24) and the same lot number of SPE cartridges (6.15).

Table 6: Working Standards 5.25

Compound	WORKING CALIBRATION STANDARD CONCENTRATIONS (ng/l)							
	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Volume of 3^{O} Cal Std 5.16 (µl)/	-	-	-	-	50 ± 2	75 ± 2	100 ± 2	150 ± 5
Volume of 4^{O} Cal Std 5.17 (µl)	0	50 ± 2	100 ± 2	200 ± 5	-	-	-	-
Standard no.	5.25.1	5.25.2	5.25.3	5.25.4	5.25.5	5.25.6	5.25.7	5.25.8
Emamectin Benzoate conc	0	10	20	40	100	150	200	300
Teflubenzuron conc	0	20	40	80	200	300	400	600
Diflubenzuron conc	0	40	80	160	400	600	800	1200
Ivermectin conc	0	20	40	80	200	300	400	600

- Note: Standard 7 (5.25.7) is to be further prepared in triplicate to give three Instrument Performance Standards (IPS - 5.25.9). See 10.2 to explain purpose of IPS.

- Note: A further aliquot of standard 3 (5.25.3) is to be prepared to give a System Suitability Standard (SS – 5.25.10). See 10.1 to explain purpose of System Suitability Standard.

- Note: Standard 1 will also be used to assess as a Blank QC. There is no requirement for a further preparation since Blank assessment will be made using the Standard 1 calibration instrument injection. See 10.7 to explain the purpose of a Blank.

  NOTE: Ensure that there are a sufficient number of CX (100 mg/6 ml) cartridges (6.15) of the same lot number to prepare the complete analytical batch. Record lot used on ES-TORG-S-216.

5.26 WORKING INDEPENDENT CALIBRATION CHECK STANDARD (ICCS)

- Prepare an ICCS as for working calibration standard 7 but replacing tertiary calibration standard (5.16) with tertiary independent standard (5.20).

- See 10.3 to explain purpose of ICCS.

5.27 PROCESS BLANK (P BLK)

- An empty extraction vessel (6.20) is used as a Process Blank.

- Follow from step 9.2.5 onwards.

- See 10.4 to explain purpose of the Process Blank.

5.28 UNSPIKED PROCESS CHECK STANDARD (UPCS)

A supply of far-field sediment matrix is used to prepare an Unspiked Process Check Standard. The sediment is known to have never been exposed to the component(s) of interest and so would not be expected to return positive results due to matrix properties. A single UPCS is required per batch.

- Remove the sample from the freezer and allow to thoroughly defrost at ambient temperature. Use a clean spatula to mix thoroughly.

- Using a clean spatula, transfer 26 ± 0.2 g of sediment into to a clean, dry, labelled 125 ml glass screwcap extraction vessel (6.20).
- Follow from step 9.2.5 onwards.
- See 10.5 to explain purpose of the Process Check Standard: Spiked – Unspiked Matrix.

5.29 SPIKED PROCESS CHECK STANDARD (SPCS)

The same supply of far-field sediment matrix used for Process Blank above (5.27) is used to prepare a Spiked Process Check Standard. A single SPCS is required per batch.

- Remove the sample from the freezer and allow to thoroughly defrost at ambient temperature. Use a clean spatula to mix thoroughly.
- Using a clean spatula, transfer 26 ± 0.2 g of sediment into to a clean, dry, labelled 125 ml glass screwcap extraction vessel (6.20).
- Using a 250 µl gas-tight syringe (6.23), dispense the 120 µl of tertiary calibration standard (5.16) onto the PCS sediment in the extraction vessel.
- Ensure that the spike liquid soaks into the body of the sediment without running onto the glassware. Distribute evenly throughout the sediment.
- Leave to stand for a minimum of 30 minutes.
- Follow from step 9.2.5 onwards.
- See 10.5 to explain purpose of the Process Check Standard: Spiked – Unspiked Matrix.

5.30 Liquid Chromatography Mobile Phases

5.30.1 Mobile Phase A

- Measure 400 ml ± 50 ml of water (5.1) into a 500 ml measuring cylinder (6.10)
- Using a 1 ml autopipettor add 0.5 ml 5M Ammonium formate solution (5.30.7)
- Using a 1 ml autopipettor add 0.5 ml formic acid (5.6)
- Using a 25 ml measuring cylinder add 10 ±0.5 ml methanol (5.2)
- Make up to 500ml with water (5.1)
- Transfer to an HPLC solvent reservoir
- This solution is stable for one month. Clearly label HPLC reservoir with a preparation and expiry date and analyst initials.
- It is permissible to top-up the reservoir with additional fresh reagent whilst within the labelled expiry date. Topping-up cannot be carried out beyond the labelled reservoir expiry date.

5.30.2 **Mobile Phase B**

- Measure 400 ml ± 50 ml of methanol (5.2) into a 500 ml measuring cylinder (6.10)
- Using a 1 ml autopipettor add 0.5 ml 5M Ammonium formate solution (5.30.7)
- Using a 1 ml autopipettor add 0.5 ml formic acid (5.6)
- Make up to 500 ml with methanol (5.2)
- Transfer to an HPLC solvent reservoir
- This solution is stable for two months. Clearly label HPLC reservoir with a preparation and expiry date and analyst initials.
- It is permissible to top-up the reservoir with additional fresh reagent whilst within the labelled expiry date. Topping-up cannot be carried out beyond the labelled reservoir expiry date.

5.30.3 **Mobile Phase C**

- Measure 400 ml ± 50 ml of water (5.1) into a 500 ml measuring cylinder (6.10)
- Using a 1 ml autopipettor add 0.5 ± 0.01 ml formic acid (5.6)
- Make up to the 500 ml mark with water (5.1).
- Transfer to an HPLC solvent reservoir
- This solution is stable for one month. Clearly label HPLC reservoir with a preparation and expiry date and analyst initials.
- It is permissible to top-up the reservoir with additional fresh reagent whilst within the labelled expiry date. Topping-up cannot be carried out beyond the labelled reservoir expiry date.

5.30.4 **Mobile Phase D**

- Measure 400 ml ± 50 ml of methanol (5.2) into a 500 ml measuring cylinder (6.10)
- Using a 1 ml autopipettor add 0.5 ± 0.01 ml formic acid (5.6)
- Make up to the 500 ml mark with methanol (5.2)
- Transfer to an HPLC solvent reservoir
- This solution is stable for two months. Clearly label HPLC reservoir with a preparation and expiry date and analyst initials.
- It is permissible to top-up the reservoir with additional fresh reagent whilst within the labelled expiry date. Topping-up cannot be carried out beyond the labelled reservoir expiry date.

5.30.5 **Auto-sampler wash solution (Magic Mix)**

- Measure 350 ml acetonitrile (5.3) into a 500 ml measuring cylinder (6.10).
- Add 100 ml acetone (5.4).
- Make up to the 500 ml mark with 2-Propanol (5.13)
- Transfer to an HPLC solvent reservoir
- This solution is stable for 24 months. Clearly label HPLC reservoir with a preparation and expiry date and analyst initials

5.30.6 **Dilution solvent (60:40 water: methanol plus internal standard)**

- Measure 60ml of water (5.1) into a 100 ml volumetric flask(6.8)
- Using a 1ml autopipettor (6.11) add 1ml tertiary internal standard(6.24)
- Top up to the mark with methanol (5.2).
- Transfer to a 125 ml glass bottle.(6.20)
- This solution must be refrigerated at 5±3ºC and is stable for 24 Days

5.30.7 **5M Ammonium formate solution**

- Measure 35 ml ± 5 ml of water (5.1) into a 100 ml beaker (6.9)
- Weigh 15.75 ± 0.1 g ammonium formate (5.7) in a weighing boat and transfer to the beaker. Swirl to dissolve if necessary.
- Wash the weighing boat with minimal quantity of water and add to the beaker.
- Transfer to a 50 ml volumetric flask (6.8) and make up to the mark with water (5.1)
- Transfer to a 60 ml glass reagent vial. (6.13)
- This solution is stored at 5±3ºC and is stable for three months. Clearly label bottle with a preparation and expiry date and analyst initials.

6 EQUIPMENT

Glassware must be scrupulously cleaned before use as detailed in procedure ES-TORG-P-005. Volumetric glassware shall be grade B or better, unless stated otherwise.

6.1 Thermo Scientific Exactive Plus Orbitrap high resolution accurate mass analyser.

6.2 LC System: (Thermo EQUAN Max) Thermo Scientific ultimate 3000 LC system consisting of: LPG Pump, RS Pump, and column compartment

6.3 Laboratory Analytical Balance: Typically Mettler AT261, 5 figure decimal place.

6.4 Laboratory Top Pan Balance: Typically Mettler PB602, 2 figure decimal place.

6.5 Zymark Turbovap II concentrator. 50 ml cell rack.

6.6 Zymark Turbovap tubes. Nominal 50 ml with 1.0 ml endpoint.

6.7 Hamilton MicroLab Diluter (MicroLab 600 Series).

6.8 Volumetric flasks, glass, 10 ml, 25 ml, 50 ml, 100ml, 500 ml.

6.9 Beakers: Nominal volumes.

6.10 Measuring cylinders. Glass 10ml, 25ml, 50 ml, 500 ml.

6.11 Auto Pipettors: Variable volume, 1 ml and 5 ml capacity. Typically Gilson.

6.12 2 and 10 ml amber glass vials. Kburnside

6.13 30 ml and 60ml amber glass vials.

6.14 IST VacMaster sample processing station or equivalent (i.e. a 20 position vacuum manifold fitted with tube rack, individual on/ off PTFE stopcocks and vacuum control valves - or equivalent, Edwards 2-stage Rotary Vacuum Pump, or equivalent, manifold tube rack).

6.15 SPE cartridges: Strong Cation Exchange, 100 mg/ 6 ml (Retain CX or equivalent)

6.16 60ml (nominal volume) sample reservoirs

6.17 10 ml (nominal volume) PTFE clear screw top headspace vials with TFE/ Silicone liner, or equivalent.

6.18 Oven, capable of maintaining a temperature of 105^{o}C

6.19 Timers – for indication only (refer to ES-CAL-P-002 see 14.9).

6.20 125 ml and 250 ml glass screwcap extraction vessels

6.21 50 ml conical flasks

6.22 Reciprocating Shaker. Typically Gerhardt. Capable of 200 cycles per minute with timer

6.22 Gas-tight syringes. Glass 100 µl, 250 µl.

6.23 500 ml glass bottles for reagent storage.

7 ENVIRONMENTAL CONTROL

Manipulations using solvent and magnesium sulphate must be carried out over vented benching, in a fume cupboard or with LEV as appropriate. The preparation of standards and all necessary spiking activities (e.g. with internal standard) must be carried out in the Preparation Laboratory. Final analysis will be carried out in the Instrument Laboratory where the LC-HRAM instrument is housed.

8 SAMPLING & SAMPLE PRESERVATION

See 'Sample Stability Data', and 'Extract Stability Data' files.

Samples are collected in 250 ml aluminium cans which have been pre-rinsed with hexane as described in ES-MACH-PT-901. Samples are collected using a 0.025 m^2 van Veen grab and sub sampled through top opening flaps on the grab. The samples are frozen prior to transportation and are stored frozen in the dark below -18°C until taken for analysis.

Samples are returned to the laboratory as soon as possible. Once received by the laboratory the sample is registered and stored at -18°C or lower until required for preparation.

Maximum sample holding time for this method has been determined as 287 days.

Maximum extract holding time for this method has been determined as 24 days.

9 ANALYTICAL PROCEDURE

Calibration standards, Process Blank, Process Check Standard, Independent Calibration Check Standard and Instrument Performance Standards must be prepared at the same time as the samples are prepared for analysis. Ideally, calibration standards should also be prepared at the same time although it is permissible to use SPE treated calibration standards up to 48 hours old.

Prior to sample analysis, ensure that samples have been logged onto NEMS and that they have an appropriate sample number.

9.1 Determination of % Dry Solids

NOTE: Since determination of moisture content is also required for method ES-TORG-P-207, this may already have been assessed. Check before commencing.

Before sample preparation can commence, moisture content of each sample must be determined as follows.

9.1.1 Remove the sample from the freezer and allow to thoroughly defrost at ambient temperature

9.1.2 Weigh a clean dry 50 ml conical flask (6.21) to 2 decimal places. Note the weight (W1).

9.1.3 Using a clean spatula thoroughly mix the sediment and transfer 20 g ± 10 g to the pre-weighed conical flask. Return sample to the freezer when finished.

Note: If any water lies on top of the sediment it must be thoroughly mixed into the sediment matrix before sub sampling.

9.1.4 Re-weigh the flask, note the weight (W2) and place in an oven (6.18) at 105 ± 5°C for a minimum of 18 hours.

9.1.5 Remove the conical flask from the oven and cool in a desiccator to ambient temperature.

9.1.6 Re-weigh and take note of the weight (W3) of the flask plus dried sediment.

9.1.7 Place the conical flask back in the oven for a minimum of 2 hours

9.1.8 Remove the conical flask from the oven and cool in a desiccator to ambient temperature.

9.1.9 Re-weigh and take note of the weight (W4) of the flask plus dried sediment.

9.1.10 Repeat steps 9.1.6 to 9.1.9 until weights are consistently within 0.02 g and not continuing to drop.

9.1.11 Record weights W1, W2, W3 and W4 and enter on ES-TORG-S-216.

9.1.12 Use the following calculation to determine % dry solids:

$$\%\ Dry\ Solids = \frac{100\ x\ (weight(W4) - weight(W1))}{\big(weight(W2) - weight(W1)\big)}$$

NOTE: For samples which have already had % Dry Solids predetermined for another procedure - weight (W1) not required so leave cell blank, enter weight (W2) as 100 and enter weights (W3 and W4) each as % dry solids result. This is a manipulation of the calculation above.

9.2 **Sample Preparation for Extraction**

9.2.1 Use the % Dry Solids (9.1.12 above) to calculate the minimum target wet weight to take, accounting for the target minimum dry solids equivalent weight for the sediment location:

Near-field sediment: minimum of 2 g dry solids equivalent
Far-field sediment: minimum of 20 g dry solids equivalent

- Ensure the sediment type is known for each sample and identify the type on ES-TORG-S-216.
- Use the % Dry Solids (9.1.12 above) to calculate the minimum target wet weight to take:

$$Mimimum\ target\ wet\ weight\ (g) = \frac{minimum\ dry\ solids\ equivalent\ weight\ (g) x\ 100}{\%\ dry\ solids}$$

- The minimum target wet weight is calculated on ES-TORG-S-216.

9.2.2 Remove the sample from the freezer and allow to thoroughly defrost at ambient temperature. Use a clean spatula to mix thoroughly.

Note: If any water lies on top of the sediment it must be thoroughly mixed into the sediment matrix before sub sampling.

9.2.3 Using a clean spatula, transfer the minimum target wet-weight quantity of sediment ± 0.2 g

as determined in step 9.2.1 into to a clean, dry, labelled 125 ml glass screwcap extraction vessel (6.20). Record the actual weight of wet sample taken on worksheet ES-TORG-S-216. Return sample to the freezer after use.

Note: For many samples, especially those containing grit or stones, it may not be possible to ensure a sample weight within the above tolerance. It is therefore permissible to take a higher weight as long as the achieved weight is no more than 2g greater than the target.

Note: for heavily watered samples it may not be possible to transfer the full aliquot of target wet-weight into the 125 ml glass screwcap extraction vessel. It must be ensured that the addition of sediment must not exceed approximately 70 ml of the vessel in order to ensure there is sufficient remaining volume for addition of acetonitrile and to allow extraction by shaking.

9.2.3 Calculate the actual dry weight equivalent sample weight as follows:

$$Actual\ sample\ equivalent\ dry\ weight\ (g) = \frac{sample\ wet\ weight\ (g)\ x\ \%\ dry\ solids}{100}$$

- The actual sample equivalent dry weight taken is calculated on worksheet ES-TORG-S-216.

9.2.4 Add 50 ml acetonitrile to the bottle. Screw the cap down tightly and shake on a mechanical shaker (6.22) at 200 cycles per minute for 20 minutes.

9.2.5 Using a clean spatula add 4 g ± 0.5 g $MgSO_4$ (5.5) to each sample bottle. Cap the bottle and manually shake to mix the $MgSO_4$ and sediment thoroughly for 30 ± 10 seconds.

9.2.6 If moisture is seen to still be present, add additional $MgSO_4$ to ensure excess moisture is absorbed and manually shake to mix the $MgSO_4$ and sediment thoroughly for 30 ± 10 seconds.

9.2.7 Place the bottle back on the mechanical shaker and shake for a further 20 minutes at 200 cycles per minute.

9.2.8 If there are no visible signs of magnesium sulphate add a further 4 g ± 0.5 g $MgSO_4$ (5.5) and repeat from 9.2.7.

9.2.9 Allow the particulates to settle for 5 min and decant the supernatant liquid into a clean 50 ml measuring cylinder (6.10). Decant the maximum possible quantity of the supernatant acetonitrile (avoid decanting solids as best possible). If required centrifuge the extract.

9.2.10 Discard the original sample bottle.

9.2.11 Measure the volume of supernatant liquid and return to a new labelled 125ml or 250ml bottle (6.20). Record the volume and enter on ES-TORG-S-216.

9.2.12 Add an equivalent volume ± 1 ml of acidified water (5.10) to the supernatant liquid using the same measuring cylinder used for the sample.

9.3 Extract Clean Up

Note: The clean-up is designed to operate under gravity and at atmospheric pressure. However, on rare occasions the flow-rate through the cartridge(s) may be very slow in which case gentle vacuum may be applied to aid the flow-rate (typical flow-rates 2-5 ml/min).

9.3.1 Place appropriately labelled CX (100 mg/6 ml) cartridges (6.15) on the SPE manifold to accommodate all samples, QC's and calibration standards. Record the batch number of CX cartridge on ES-TORG-S-216 and ensure the same lot number is used for entire batch including calibration standards.

NOTE: Cartridges should not be reused.

9.3.2 Condition each cartridge as follows (Note that initial vacuum assistance may be required to break the air lock):

- Using a 5 ml autopipette, add 2 ± 0.1 ml acetonitrile (5.3) to each cartridge and allow to drain to the bed top under gravity.
- Using a 1 ml autopipette, add 1 ± 0.1 ml elution solvent 1 (5.11) to each cartridge and allow to drain to the bed top under gravity.
- Using a 5 ml autopipette, add 3 ± 0.1 ml elution solvent 2 (5.12) to each cartridge and allow to drain to the bed top under gravity.
- Using a 5 ml autopipette, add 2 ± 0.1 ml acetonitrile (5.3) to each cartridge and allow to drain to the bed top under gravity.
- Add acidified water (5.10) to the rim of each cartridge and allow to drain to the bed top under gravity.

9.3.3 Repeat all steps in 9.3.2 but retain the final portion of acidified water in the body of cartridge, allowing sufficient space for the reservoir adaptor.

9.3.4 Attach a 60ml reservoir (6.16) to the cartridge.

9.3.5 Transfer the extract in the sample bottle to the reservoir/ CX SPE stack and open the stopcock. Add extract as quickly as possible whilst ensuring no over-flowing of the reservoir.

9.3.6 Add 1 ml methanol (5.2) to the sample bottle, swirl to contact the glass surface and as soon as possible transfer to the reservoir/ CX SPE stack.

9.3.7 Allow to drain to the top of the adsorbent bed (vacuum assistance may be required). When the extract reaches the top of the SPE bed add a final 5 ± 0.1 ml of acidified water using a 5 ml autopipette.

9.3.8 Remove the reservoir.

Note: Unless specified, eluate from stages 9.3.1-9.3.8 is drained to waste.

9.3.9 Using a 5 ml autopipette, add 2.5 ± 0.1 ml acetonitrile to the cartridge and drain to waste.

9.3.10 Set up each cartridge to allow collection of eluate in a turbovap tube (6.6). Using a 1 ml autopipette, add 1 ml ± 0.1 ml elution solvent 1 (5.11) and allow to drain to the top of the bed.

9.3.11 Using a 1 ml autopipette, elute with a further 3 portions of 1ml of ± 0.1ml elution solvent 2 (5.12), allowing each to drain to top of bed before addition of the next.

9.3.12 Using a pipette bulb push the residual elution solvent from the cartridge.

9.3.13 Use the Zymark turbovap concentrator (6.5) set at 40ºC and sensor of 2 minutes to reduce extract volume.

9.3.14 Using a 1 ml autopipette, add 1 ml ± 0.1 ml of methanol to the extract, set the turbovap to sensor and concentrate the extract to 1 ml endpoint.

9.3.15 Using the Hamilton diluter (6.7), add 50 µl of tertiary internal standard (5.24) with 1000 µl of methanol (5.2) diluent to the reduced extract in the turbovap tube.

9.3.16 Using a 5 ml autopipette, add 3 ml ± 0.1 ml water (5.1) to the extract in the tube, agitate the sample to mix thoroughly and transfer to a clean, labelled 10 ml vial.

9.3.17 Store in a refrigerator at 5°C ± 3°C until required for analysis.

Note: Sample extract stability is 24 days.

9.4 LC-MS INSTRUMENT CONDITIONS

Parameters which control peak integration, quantification, calibration, component identification, report formats etc. are stored in the relevant master method definition files in Tracefinder 4.1 software. Component retention times and detector response factors may vary with LC column age and MS tune status. Tolerances are given in the System Suitability parameters.

The instrument shall be set up in accordance with the manufacturer's instructions using the conditions tabulated below.

Table 7: LC Conditions

Instrument	LC System: (Thermo EQUAN Max) Thermo Scientific Ultimate 3000 LC system
Trapping Column	Hypersil Gold aQ 20mm x 2.1mm x 12µm
Analytical Column	Accucore aQ 100mm x 2.1mm x 2.6µm
Guard Column	ZORBAX Eclipse Plus C18 4.6mm x 5 mm x 1.8 µm
Column Oven Temperature	25ºC
Mobile Phases	Mobile phase A: 98% H_2O, 2% Methanol, 5mM Ammonium formate, 0.1% formic Acid. Mobile phase B: 98% Methanol, 2% H_2O, 5mM Ammonium formate, 0.1% formic Acid. Mobile phase C: 0.1% formic Acid in H_2O Mobile phase D: 0.1% formic Acid in Methanol
LPG Pump (Loading)	Ramp 70%-0% Mobile phase C

	30%-100% Mobile phase D Flow 0.75 ml/min Equil:9.3 min Begin: 3min Duration: 0.1 min Constant: 6min Return: 0.1min
Pump (Analytical)	Multistep gradient, 0.3ml/min, 0-9min: 100% Mobile phase A 9min: 70% Mobile phase B 11min: 100% Mobile phase B 16.4min: 100% Mobile phase B 16.5min: 100% Mobile phase A 19 min: 100% Mobile phase A
Injection Volume	400 µl
Total Programme Time	19 minutes

Table 8: HESI Source Parameters

Sheath gas flow rate	40
Aux gas flow rate	10
Sweep gas flow rate	1
Spray voltage ([kV])	3.5kV
Spray current (µA)	-
Capillary temp	325 °C
S-lens RF level	50.0
Aux gas heater temp (˚C)	325 °C

Table 9: Orbitrap Detector Acquisition Settings

Scan range (Positive & Negative)	Full MS, 200-1000m/z
Resolution	70,000
Scan time negative	0 – 11.5 minutes
Scan time positive	11 – 19.5 minutes
AGC Target	1e6
Max IT	200ms

Table 10: Detector Quantitation Masses

Compound	**Mass**
Emamectin B.	886.5311
Diflubenzuron-C13-6	315.0448
*(Abamectin)**	*(890.5260)*

** Component is present in internal standard but are not currently used in determinand reporting methodology*

Table 11: Autosampler Settings

Syringe filling speed	200 µl/s

Filling speed –Wash 1	20 µl/s
Filling speed –Wash 2	20 µl/s
Inject to	LC Viv 1
Injection Speed	100 µl/s
Pre injection delay	500 ms
Post injection delay	500 ms
Post clean with solvent 1	1
Post clean with solvent 2	1
Valve clean with solvent 1	2
Valve clean with solvent 2	2
Clean Vol	100%
Transfer time	1600 s
Elution time	900 s

9.5 **Requirements for Good Performance**: These cover the maintenance and operation of the liquid chromatograph and mass spectrometer.

- **Liquid Chromatograph**: Ensure the correct columns have been fitted and there is sufficient mobile phase to complete the run sequence. The elution programme should be suitable for keeping the LC column and liquid lines clean. Performing conditioning injections before the sequence is started will ensure the system reaches equilibrium and the current mobile phase has reached the column.

- **Mass Spectrometer**: The ion source must be clean and at its optimal temperature and vacuum. The capillary must be in good condition and properly positioned (pay particular attention to this after syringe pump infusion work). The mass spectrometer must be correctly tuned according to the manufacturers' guidelines. Optimum temperatures, vacuum and gas settings are shown in the tables above.

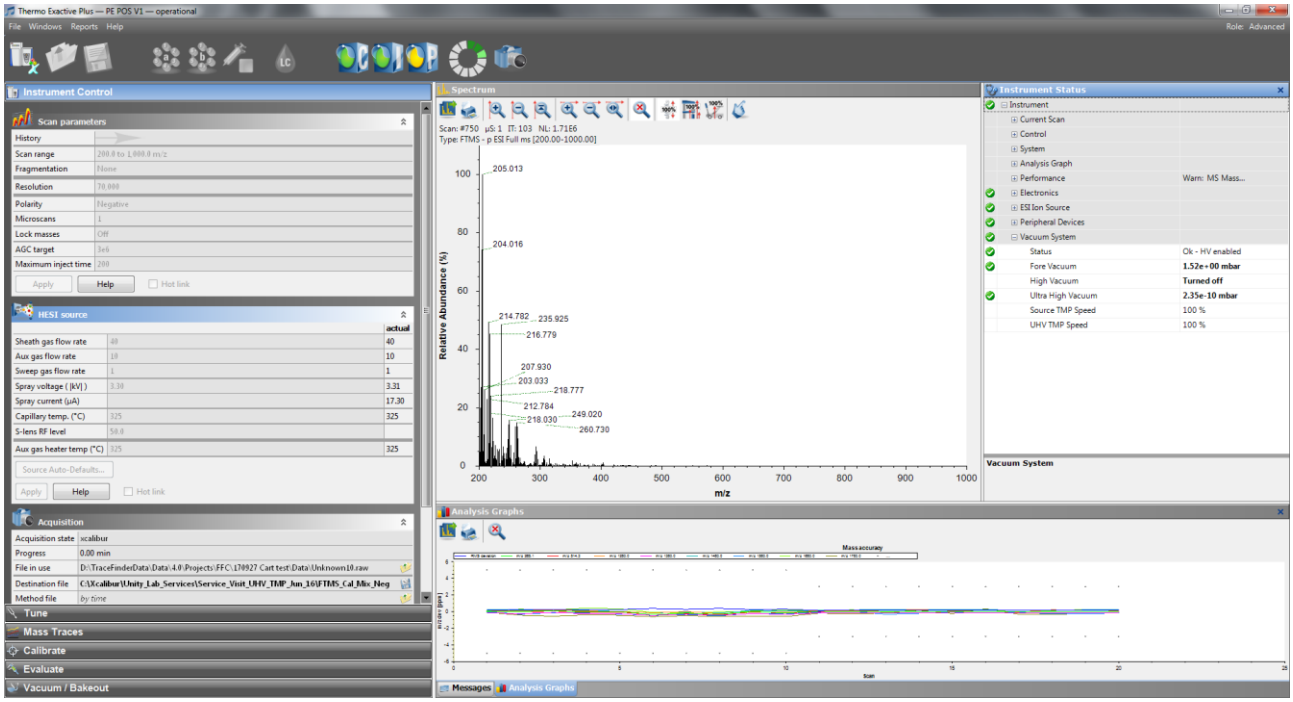
9.6 Evaluate the current instrument condition:

- On orbitrap tune page ensure that no red warnings appear for any of the criteria listed in the Instrument status panel. (Yellow/ Blue warning status are acceptable for MS mass calibration entries)

- Ultra-High Vacuum < $3.0e^{-10}$ mbar

- If necessary, (for example, following any major maintenance on the source or a significant reduction of instrument response) carry out instrument tuning according to manufacturer's guidelines.

- An example of a typical acceptable tune page output is given below:



9.7 A minimum of two conditioning injections should be run prior to the System Suitability check to ensure the current batch of mobile phases has been pumped through and to equilibrate the LC column. Conditioning injection solution may be made up of combined previous calibration standards – there is no prerequisite for known concentration levels.

Note: If there are time constraints, the 'purge' function on the LC pump can be used in addition. This also has the effect of stabilising the MS operating conditions.

9.8 System Suitability Standard must be run and assessed prior to final analysis. Standard 3 (reagent 5.25.10) is used for this purpose following instrument conditions listed in 9.4.

Check that the System Suitability checks pass the visual checks and the required parameters detailed below. Three critical areas are visually checked on all chromatograms.

- Peak resolution - Peak resolution is not critical since the individual quantitation masses produce discrete chromatograms for each component. The chromatogram in Appendix A shows the superimposed HRAM chromatograms for all components

- Peak Shape - Peak tailing is difficult to avoid with many compounds in LC. Some critical parameters are defined below.

- Baseline Integrity - Poor baselines are unlikely to be observed as HRAM analysis removes virtually all of the non–analyte contribution. Poor baselines are a possible symptom of gas flow problems.

- Peak Acquisition Windows – it is important that windows are sufficiently aligned to capture the entirety of the target peak including any tailing.

Table 12: System Suitability Criteria

Compound	Parameter	Minimum Criteria
Emamectin Benzoate	Peak Height	Greater than or equal to 120000 counts
C13 Diflubenzuron	Peak Area	Greater than or equal to 20 million counts
Emamectin Benzoate	Peak width at base	Less than 0.25 min
Emamectin Benzoate	Retention Time	± 0.15 min of previous system suitability

- Record details onto the appropriate control charts.

- If all system suitability criteria are satisfied then samples may be run. If any criteria are not satisfied then the system must be returned to a satisfactory state before analysis can recommence.

  If any one of these criteria is not met instrument is not suitable for use and may require tuning or maintenance. **Analysis should not commence until satisfactory system suitability results are obtained.**

Note: Poor column performance will be indicated by a significant shift in retention times and/ or split peaks. However shifts in retention time are expected immediately after pump replacement or invariably, after column replacement. In these circumstances after discussion with a senior chemist it may be possible to commence analysis with a retention time shift system suitability failure.

- See 10.1 to explain purpose of System Suitability Standard.

9.9 Load the autosampler: Up to 20 samples constitute a batch. Multiple batches may be incorporated into a single instrument run. An example order of an instrument run sequence is shown below:

System Suitability injection (5.25.10)
Calibration Standards (5.251-8)
Independent Calibration Check Standard (5.26)
Instrument Performance Standard (5.25.9)
Process blank (5.27)
Unspiked Process Check Standard (5.28)
Spiked Process Check Standard (5.29)
Samples (up to 10)
Instrument Performance Standard (5.25.9)
Samples (up to 10)
Instrument Performance Standard (5.25.9)

- Create an acquisition batch using the FFC_V2 master method in Tracefinder and enter the sequence. Save the batch using a unique identifying filename :FFC-DATE-INITIALS EXTRACTION ANALYST-INITIALS INSTRUMENT ANALYST

9.10 Run the sequence.

9.11 Once the acquisition of all the samples is completed perform the quantitative analysis using the system software.

9.12 Occasionally it may be necessary to dilute some samples to bring them within the calibration range. If possible the dilution should be targeted at producing a result which is 40 - 75% of

the calibration range. Precautions must be taken to eliminate the risk of cross contamination. The diluter should be purged at least 5 times with the tip of the dispenser wiped between each use.

When diluting samples it is important that the correct solvent ratios and amount of internal standard is maintained. To ensure this use Dilution solution (5.30.6) to prepare any required dilutions and use the volumes outlined in the table below

Table 13: Dilution Table

Dilution	Volume Extract (µl)	Volume 5.30.6 (µl)
2	2500	2500
5	1000	4000
10	500	4500
20	250	4750
40	125	4875

10 QUALITY CONTROL

The aim of quality control procedures is to provide and maintain confidence that the analytical method is satisfying the defined criteria with respect to accuracy and precision. A number of measurements are taken to ensure a sufficient quality of analysis. Three main areas have been identified as important; LC system performance, calibration, and control of extraction and clean-up.

See procedure SPC 002 for the details on the construction and maintenance of control charts.

10.1 **System Suitability Check (SSC)** – This is a minimum requirement for a Pre-treatment Recovery method type.

A single system suitability standard must be run prior to instrument analysis. Fixed limit charts are used to assess instrument performance prior to analysis. Plot results on the appropriate fixed limit control charts and assess suitability before commencing instrument analysis. Details of preparation and System suitability Criteria are given in Section 5.25.10.

10.2 **Instrument Performance Standard (IPS)** – This is a minimum requirement for Pre-Treatment Recovery method type.

Within each instrument run, an Instrument Performance Standard is analysed at intervals throughout the run to check the integrity of the instrument. It must be ensured that each sample has an Instrument Performance Standard run before and after it to ensure integrity of sample results. Plot results on the appropriate fixed limit control charts. The charts should be examined and assessed before reporting of sample results. Details of IPS preparation are given in Section 5.25.9.

10.3 **Independent Calibration Check Standard (ICCS)** – This is a minimum requirement for Pre-treatment Recovery method type.

Within each instrument run, a single Independent Calibration Check Standard is analysed immediately after the calibration standards. Standard is used to check the integrity of the calibration. Plot results on the appropriate fixed limit control charts. The charts should be

examined and assessed before reporting of sample results. Details of ICCS preparation are given in Section 5.26.

10.4 **Process Blank (P Blk)** – This is a minimum requirement for Pre-treatment Recovery method type.

Within each preparation batch, a single Process blank is analysed as a sample. A process blank is to check for process contamination. There is no requirement to plot process blank results. The results should be examined and assessed before reporting of sample results. Record the analyst and date extracted on ES-TORG-S-216. Details of preparation are given in section 5.27.

10.5 **Process Check Standard: Spiked-Unspiked matrix (SPCS-UPCS) -** This is a minimum requirement for a Pre-Treatment Recovery method type. Within each preparation batch, a single PCS-spiked – PCS-unspiked matrix is analysed as a sample. The PCS-spiked matrix and the PCS-unspiked matrix are prepared as follows:

- **PCS-spiked matrix**: A portion of low-level unspiked matrix is spiked with a known quantity of the analyte(s) of interest to bring the analyte(s) to a concentration between 50 and 90% of the method range. This is analysed.
- **PCS-unspiked matrix**: A portion of low-level unspiked matrix is analysed.

The method efficiency is calculated from the difference of the two QC standards and is used to measure performance in the same way as a PCS. The results are plotted on the appropriate individuals limit control charts within NWA. The charts should be examined and assessed before reporting of sample results. Details of preparation are given in Section 5.28 and 5.29.

Additional QC measures

10.6 **Calibration Check (CC) –** since this method makes use of a multi-point calibration curve, a Calibration Check QC measure is required. Within each run, a Calibration Check is assessed. This is a measure of the calibration curve in terms of correlation coefficient. The calibration check result should be assessed according to fixed limits; in this case the correlation coefficient (R^2) must be greater than 0.995. There is no requirement to plot calibration check results. The results should be examined and assessed before reporting of samples.

10.7 **Blank** – Since this method is used to analyse a challenging determinand at extremely low concentrations, a blank is required to ensure that there is no significant cross-contamination during calibration standard preparation. Emamectin benzoate is known to be particularly 'sticky' and so working at such low concentrations there is a risk to the integrity of low level calibration if any cross-contamination is not detected and immediately addressed.

Standard 1 is used as a Blank during injection of the calibration standards. The following criterion must be met to ensure satisfactory method performance:

Table 14: Blank Criterion

Compound	Parameter	Minimum Criteria
Emamectin Benzoate	Std 1 peak area	Not found or less than 10% of Std 2

11 CALCULATING AND REPORTING OF RESULTS

11.1 Use the Tracefinder chromatography software to process the raw data on the basis of peak area and utilising the internal standard correction facility of the program.

Note: Few components are expected to have a linear fit for their calibration therefore a quadratic fit must be used.

11.2 Check that all the standard peaks have been correctly identified. Check each chromatogram to ensure that peaks are properly shaped and that baselines have been drawn correctly. Change the integration parameters and re-integrate if necessary.

11.3 Review the calibration data to ensure a satisfactory calibration has been obtained and that the adjusted R^2 value is 0.995 or greater. If necessary, the analyst's discretion can be used to improve the calibration using the tools available in Tracefinder.

11.4 Compare the retention times of components in Instrument Performance Standard with those showing positive results in the samples. The retention time must be within 10 seconds of the nearest IPS. Discuss any results out with these tolerances with the Senior Chemist before taking further action.

11.5 Quantification is based upon a regression curve of concentration against response, where response is given by:

$$Response\ (analyte) = \frac{Peak\ area\ (analyte)}{Peak\ area\ (internal\ standard)}$$

11.6 Check that the Instrument Performance Standard, Independent Calibration Check Standard and Process Check Standard results fall within the required limits on the control charts and that the procedural blank is satisfactory. Provided these results are acceptable, sample results are calculated using the equation below:

$$X = \frac{[\,(Y * D) - B] * V * 100 * SV}{[R * VE * M\,]} \qquad ng/Kg$$

where; X = The final result in ng/kg dry-weight
Y = Raw result from the integrated chromatogram peak (ng/L)
D = Dilution factor (if applicable)
B = Process blank for the batch
R = within batch Process Recovery Factor (%)
M = Equivalent dry-weight mass of sediment (g)
V = Final extract volume (ml) = 5 ml
SV = Volume of acetonitrile solvent used for extraction = 50 ml
VE = Volume of decanted acetonitrile extract (ml)

MRVs to be used are quoted in Table 2. Deviations of these MRVs will occur for either or both of the following scenarios:

- Any samples for which the minimum target wet-weight was not achievable, any 'less than' results will reported with a sample specific adjusted MRV to reflect the reduced sample weight.

- Any samples for which the volume of supernatant measured in step 9.2.11 is less than 40 ml, any 'less than' results will reported with a sample specific adjusted MRV to reflect the reduced extract supernatant volume.

11.7 Instrument results are transferred on report template – Fishfarm Report ES-TORG-P-216.

11.8 Results are calculated on spreadsheet ES-TORG-S-216 which must be dated and the responsible analyst recorded. The results spreadsheet must have a unique identifying filename to include the date of analysis.

11.9 The results are entered onto the LIMS Database.

Note: Only components which require dilution shall be reported from diluted sample analysis. All other components results are reported from the undiluted sample analysis.

11.10 In addition to dry-weight results, the following parameters are also reported by NEMS:

- Moisture content
- Emamectin benzoate concentration as ng/Kg wet-weight

- Enter data for W1, W2, W3 and W4 for each sample onto the LIMS Database.

- NEMS uses the equation below to calculate the % moisture content.

$$\%\ Moisture\ Content\ (MC) = \frac{100\ x\ \big(weight(W2) - weight(W1)\big) - \big(weight(W4) - weight(W1)\big)}{\big(weight(W2) - weight(W1)\big)}$$

- NEMS converts the reported emamectin benzoate ng/Kg dry weight result to ng/Kg wet weight using the equation below:

$$ng/Kg \text{ wet weight} = \text{Emamectin ng/Kg dry weight x } \frac{(100 - \%MC)}{100}$$

12 CONFIRMATION

Confirmation of component identity is not necessary since the HRAM / Retention Time combination is suitable as an absolute identifier of the presence of the compound. Provided the quality control check standards within the run are within their limits then the analytical result shall be considered as valid.

13 REFERENCES

13.1 Thermo Dionex UltiMate 3000 HPLC Operators manuals.

14 RELATED DOCUMENTS

14.1 SEPA, National Procedure SPC 001: Guidance on Routine Analytical Quality Control Measures in the Laboratory.

14.2 SEPA, National Procedure ES-TORG-P-002: General Preparation, Handling & Storage of Trace Organics Standards and Maintenance of Standards Records.

14.3 SEPA National Procedure. ES-TORG-P-003: Maintenance, Calibration & Use of the Hamilton 500 Series Diluters. SEPA National Procedure.

14.4 COSHH ES-TORG-C-216: COSHH Assessment for ES-TORG-P-216 Determination of Fish Farm Medicines in Marine Sediments by LC-MS/MS.

14.5 SEPA, National Procedure ES-MACH-PT-901: Sampling of Marine and Estuarine Subtidal Sediments for Chemical Analysis.

14.6 SEPA, National Procedure SPC 002: Use of NWA quality analyst for set up and review of control charts

14.7 ES-TORG-S-216: Results Spreadsheet for Specific Fish Farm Medicines in Marine Sediments by LC-HRAM

APPENDIX A

Chromatogram of Diflubenzuron $^{13}C_6$ and Emamectin