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Chemical extraction techniques for the determination of drugs, pesticides and antibiotics used by the aquaculture industry

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

The finfish aquaculture industry utilises a wide spectrum of chemotherapeutants ranging from anti-sea lice treatments to antibiotics in the production of their marketed products. The impact of these compounds to non-target organisms coupled to their potential for environmental persistence is of major concern and the minimisation of their usage is the target for a regulatory regime under development by Fisheries and Oceans Canada (DFO). Accurate quantification of contaminant levels is essential to determine if Environmental Quality Standards (EQS) have been exceeded. Typically, sea lice treatments and antibiotics used in aquaculture have been adopted from terrestrial farming practices. This is reflected in the number of analytical methods available for the analysis of their active ingredients (a.i.) in soil and freshwater media compared to the marine environment. Traditional extraction methods either use large volumes of solvents harmful to human health and the environment, are labour intensive and time consuming, or are expensive due to instrument costs. Recently, a new technique has been developed called QuEChERS, an acronym for quick, easy, cheap, effective, rugged and safe which is gaining popularity for multi-class and multi-residue extractions from a variety of matrices due to its simplistic methodology. No matter what analytical method is developed or selected for routine analysis of samples, it should undergo a validation procedure to demonstrate its robustness and suitability for the intended purpose. For regulatory decision-making and enforcement where analytical results could be presented in court as part of a prosecution case it is critical that the analytical method is also accredited to ISO/IEC 17025 which would give confidence that procedures employed by the analytical laboratory adhered to a set of strict regulatory guidelines. If the analytical method is to be used solely for the purpose of monitoring, validation is still considered essential but accreditation is not.

1. INTRODUCTION

The Department of Fisheries and Oceans (DFO) is in the process of developing a regulatory regime for the minimisation of impacts from drug and pesticide usage in marine net pen aquaculture on non-target organisms, as committed to in the 2015 Aquaculture Activities Regulations (AAR) Regulatory Impact Analysis Statement (Canada Gazette 2014). Similar to the AAR regime already in place (to be integrated in the future General Aquaculture Regulation (GAR)), the regulatory framework for assessing pesticide and drug deposits will be on a site-by-site basis (not a bay or a group of farms) and will be risk-based, dependent on the active ingredient (a.i) used. The review papers prepared and science advice generated at the CSAS meeting will inform the design of a monitoring program for drugs and pesticides which will include pre-impact evaluation (predictive modelling) and post-deposit sampling design and assessment for compliance. This document provides an overview of some established analytical techniques available for the extraction of pesticides and therapeutants currently or previously used by the aquaculture industry from sediment samples collected from around cage sites and will help to guide Aquaculture Management in future requirements for chemical analyses.

The increasing worldwide demand for seafood (finfish, shellfish, etc.) has resulted in aquaculture production overtaking the wild caught fisheries in terms of supply (FAO 2018). In Canada, approximately 45 species of finfish and shellfish are farmed with the main species being Atlantic salmon, Rainbow trout, Artic charr, clams, mussels, and oysters (Fisheries and Oceans Canada 2014). Out of all these, the most financially important is Atlantic salmon (*Salmo salar*) with annual production exceeding 120,000 tonnes in 2017 (Fisheries and Oceans Canada 2019). Globally, Canada is the fourth largest producer of Atlantic salmon behind Norway, Chile and Scotland with production mainly in the Atlantic region (New Brunswick, Nova Scotia and Newfoundland and Labrador) and on the west coast (British Columbia). To meet the high demand for Atlantic salmon in the domestic and export markets, production relies on intensive rearing in both freshwater hatcheries and marine net cage facilities. Due to the high biomass of fish and stresses involved with such farming practises, pesticides and antibiotics are routinely used to treat fish for the prevalence of pests and diseases during the production cycle. All the chemotherapeutants used by the aquaculture industry have been adopted from terrestrial agriculture, veterinary and human health treatments. As a result of this, extensive research has been conducted regarding their toxicity, environmental effects (terrestrial soil and freshwater) and persistence in foods (produce and farmed meat). Limited research, however, has been conducted to investigate these parameters in the marine environment.

1.1. CHEMICAL TREATMENTS FOR SEA LICE

The main marine pests affecting salmon aquaculture are the external parasitic copepods (sea lice) *Lepeophtheirus salmonis* and *Caligus elongatus*. Within Canada, at the time of writing there are five chemical products approved by Health Canada which can be used to control these parasites, Salmosan®, Paramove® 50, Aquaparox 50, Slice® and Calicide®. Other chemical products are available, AlphaMax® was granted emergency registration in the autumn of 2009 to summer 2010 in southwest New Brunswick, however, this and Excis® are currently not approved for use in Canada. Imvixa® can be requested for emergency use through the Emergency Drug Release program (Fisheries and Oceans Canada 2018) or for investigational purposes if an investigator is granted an Experimental Studies Certificate (ACFFA 2019).

A laboratory (Appendix) was contracted by Fisheries and Oceans Canada (DFO) to develop and validate an analytical method to quantify active ingredient concentrations of anti-sea lice formulations plus selected antibiotics (Table 1) in collected marine sediment samples.

Table 1. Identified compounds of interest for method development and validation (Appendix), and post deposit monitoring purposes.

Sea lice treatments and their modes of application		Metabolite	Antibiotics
Bath	In-feed		
Interox® Paramove® 50 and Aquaparox 50 (hydrogen peroxide+)	Slice® (emamectin benzoate* a.i.)	Desmethyl emamectin benzoate	Erythromycin#
Salmosan® (azamethiphos a.i.)	Calicide® (teflubenzuron a.i.)		Florfenicol
AlphaMax® (deltamethrin a.i.)	Imvixa® (lufenuron a.i.)		Sulfadiazine
Excis® (cypermethrin a.i.)	Abamectin		Sulfadimethoxine
	Ivermectin		Trimethoprim
			Amoxicillin
			Oxytetraxycline

+ = analytical determination not performed – see Section 1.1.2.

* = detected and quantified by the contracted laboratory (Appendix) as emamectin (free base) not the benzoate salt

= detected and quantified by the contracted laboratory (Appendix) as erythromycin-H_2O (anhydroerythromycin metabolite)

1.1.1. Salmosan® 50 WP (azamethiphos a.i.)

Salmosan® 50 WP (Fish Vet Group, PMRA registration #32506) is a wettable powder formulation containing a 50% (w/w) concentration of the active ingredient azamethiphos (*S*-6-chloro-2,3-dihydro-2-oxo-1,3-oxazolo[4,5-*b*]pyridine-3-ylmethyl *O*, *O*-dimethylphosphorothioate, CAS No. 35575-96-3, log K_{ow} = 1.05, Figure 1) (BCPC 2018). Azamethiphos is an organophosphorous compound which acts as a cholinesterase inhibitor. It was initially developed as an insecticide to control flies, mosquitos and tsetse flies and other pests (BCPC 2018) but has since been adopted for use in aquaculture. Salmosan® is applied as a bath treatment in either tarped net pens or well boats at a concentration of 0.1 ppm azamethiphos for 30 min or up to 60 min (water temperature > 10°C or < 10°C, respectively) (Burridge 2013). Due to its low log K_{ow}, azamethiphos is highly water soluble and is therefore more likely to remain in the water column rather than binding to organic matter, e.g., in sediment on the sea floor. Salmosan Vet® 50% w/w, powder for suspension is a new formulation (Fish Vet Group 2018) has been introduced by the manufacturer which allows for a longer treatment period at higher water temperatures (1 h up to 15°C) at the same treatment concentration (0.1 ppm azamethiphos). This new formulation also has an increased product shelf life of up to 24 months but has not been registered by the Pesticide Management Regulatory Authority (PMRA) for use in Canada at the time of writing.

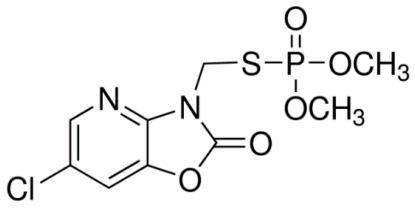


Figure 1. Chemical structure of azamethiphos.

1.1.2. Interox® Paramove® 50 and Aquaparox 50 (hydrogen peroxide a.i.)

Interox® Paramove® 50 (Solvay, PMRA registration #31393) and Aquaparox 50 (Alpha Chemical Ltd., PMRA registration #32401) are formulations containing 50% hydrogen peroxide (CAS No. 7722-84-1, log K_{ow} = -1.57) (Pesticide Properties DataBase 2018) which are applied as bath treatments within well boats. Hydrogen peroxide is a strong oxidising agent used mainly in the pulp and paper industry as a bleaching agent, in detergent manufacturing as a disinfectant and also as rocket propellant. Within aquaculture, it is used as a fungal treatment in freshwater hatcheries and as an anti-sea lice treatment in sea cages. Treatments for sea lice are performed in well boats, for Interox® Paramove® 50 the recommended treatment regime is at a concentration of 1200 to 1800 mg a.i./L for up to 40 minutes at water temperatures > 10°C to < 14°C. A similar regime is recommended for Aquaparox 50, 1200 to 1800 mg a.i./L for up to 30 minutes depending on the water temperature. Reports suggest that the mode of action is the formation of oxygen bubbles in the gut and haemolymph of the sea lice resulting in paralysis and subsequent dislodgement from the salmon (Bruno and Raynard 1994).

Hydrogen peroxide is highly reactive and readily decomposes to form water and oxygen.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Even though hydrogen peroxide is currently used as a treatment for sea lice, determination of this compound in collected sediment samples by the DFO contracted laboratory (Appendix) was deemed not practical due to the following factors:

1. Short half-life in seawater of 28 days at 10°C (Lyons et al. 2014).
2. Low log K_{ow} indicating that it will remain in the aquaeous phase therefore will not be environmentally persistent in sediment.
3. Rapid dilution and dispersal of discharged plume due to ocean currents.
4. Naturally present at low concentrations in the environment due to photocehmical reactions with organic matter and biological formation (Cooper et al. 1988).

1.1.3. Slice® (emamectin benzoate a.i.)

Slice® (Intervet International B.V.) is a pre-mix formulation containing 0.2% emamectin benzoate ((4''R)-4''-(methylamino)avermectin B1 benzoate, CAS No. 155569-91-8, log K_{ow} = 5.0 (pH 7), Figure 2), which is incorporated into fish feed. Application is via in-feed treatment at a recommended dose of 50 µg emamectin benzoate/kg/day for seven consecutive days (MSD Animal Health 2012). Emamectin affects glutamate-gated chloride channel modulation (BCPC 2018) and hence nerve impulses which results in paralysis of the sea lice and detachment from the host fish followed by death. Emamectin consists of two homologues:

a. (4''*R*)-4''-(methylamino)avermectin B1a, ≥ 90% abundance (Figure 3a).

b. (4''*R*)-4''-(methylamino)avermectin B1b, ≤ 10% abundance (Figure 3b).

Due to the mode of application, any uneaten medicated feed deposited onto the seafloor has the potential for the active ingredient to impact benthic faunal communities. Faeces containing any non-metabolised parent compound when settled onto the sea floor could also be a potential route of environmental impact. The octanol/water partition coefficient of emamectin benzoate is high (log K_{ow} = 5.0, pH 7) (BCPC 2018) which indicates that it has a tendency to partition onto organics such as sediment. It is therefore more persistent in the environment than both azamethiphos and hydrogen peroxide with a half-life in marine sediment of 164 to 175 days (Bright and Dionne 2005).

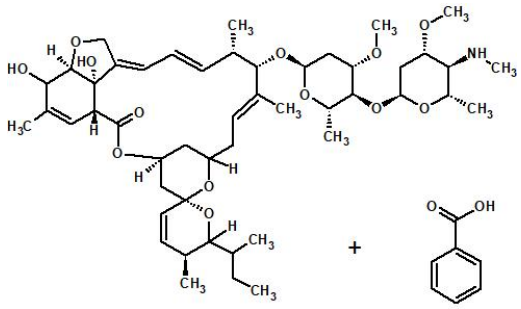


Figure 2. Chemical structure of emamectin benzoate.

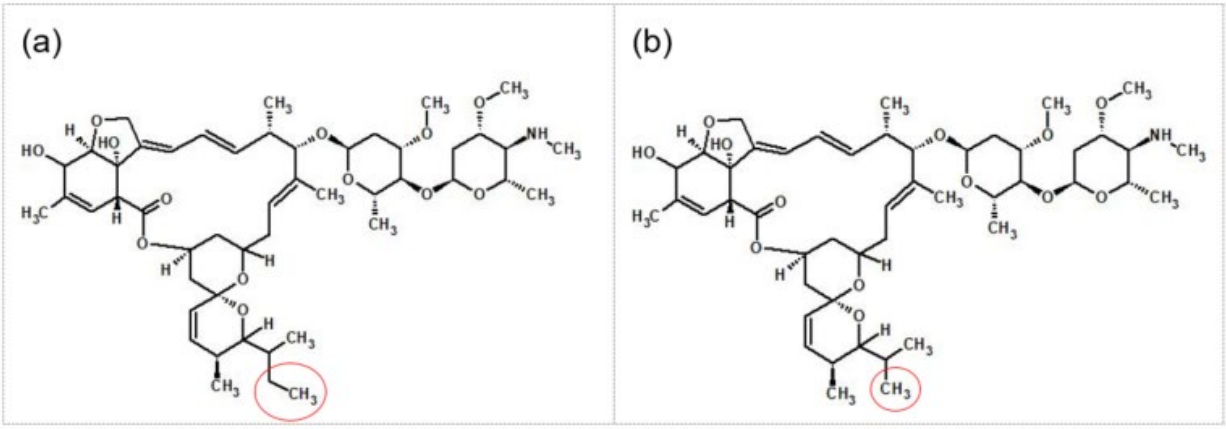


Figure 3. Chemical structures of emamectin homologues (a) emamectin B1a and (b) emamectin B1b.

1.1.4. Calicide® (teflubenzuron a.i.)

Teflubenzuron (CAS No. 83121-18-0, 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-fifluorobenzoyl)urea, log K_{ow} = 4.98 (pH 5), Figure 4 is a systemic growth inhibitor which targets chitin synthesis and affects moulting of the exoskeleton of target organisms. Terrestrially it is used to control a whole spectrum of crop pests such as, but not limited to, moths/butterflies, beetles, flies and aphids (BCPC 2018). In the UK it is approved for salmon aquaculture and marketed as Calicide® at a dose level of 10 mg/kg for seven consecutive days after incorporation into pelted fish feed (Committee for Veterinary Medicinal Products 1999). As with emamectin benzoate (Slice®), there is a high possibility that teflubenzuron will bind to sediment and persist in the environment due to its mode of application and its high log K_{ow} value. This treatment targets only the early life stages of the sea louse and thus must be used in combination with bath treatments to fully deal with all the life stages of the parasite. As a result

of limited use by industry, the manufacturer no longer supplies this product to Atlantic Canada (ACFFA 2019) resulting in its drug identification number (DIN) becoming dormant.

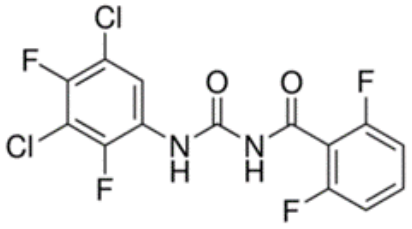


Figure 4. Chemical structure of teflubenzuron.

1.2. NON-APPROVED TREATMENT OPTIONS FOR SEA LICE

Two additional chemical products used to control sea lice are AlphaMax® and Excis® although these are not approved for use in Canada at the time of writing. AlphaMax® was, however, issued an emergency registration (ER) between autumn 2009 to summer 2010 (Burridge and Van Geest 2014) in southwest New Brunswick. These compounds have been included in this report for background information since regulators in Canada are still interested in them.

1.2.1. AlphaMax® (deltamethrin a.i.)

Deltamethrin (Figure 5) is a synthetic pyrethroid, (CAS No. 52918-63-5, (S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate), log K_{ow} = 4.6). It is a non-systemic pesticide with contact and stomach action which disrupts sodium channels and thus prevents nerve signals from being transmitted (BCPC 2018). The formulation is a 10 mg deltamethrin/mL concentrate which is used as a bath treatment at a target concentration of 2 µg deltamethrin/L seawater in tarped cages for 30 minutes. The high log K_{ow} value of deltamethrin indicates that it has an affinity towards organic material, therefore there is the risk of it binding to and persisting in the sediment environment when the treatment plume is dispersed and diluted after release.

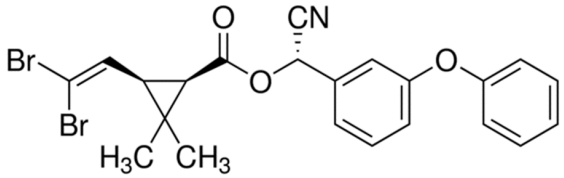


Figure 5. Chemical structure of deltamethrin.

1.2.2. Excis® (cypermethrin a.i.)

Cypermethrin (Figure 6) is a synthetic pyrethroid (CAS No. 52315-07-8, (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, log K_{ow} = 5.55). It is sold under the brand name Excis® (Norvartis Animal Health, Norway) and used as a bath treatment for the control of sea lice. Excis® has never been approved for use in the Canadian aquaculture industry. Like deltamethrin, it is a non-systemic insecticide with contact and stomach action but also exhibits anti-feeding action (BCPC 2018). The log K_{ow} value for cypermethrin is higher than that of deltamethrin which indicates that it will have a higher affinity towards organic material. Therefore, there is the risk of environmental persistence in sediments if it was ever approved for use in Canada.

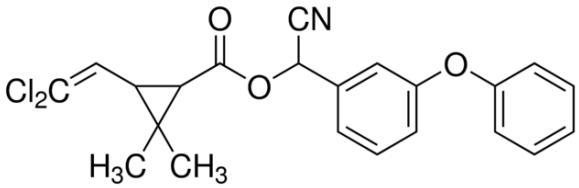


Figure 6. Chemical structure of cypermethrin.

1.3. ADDITIONAL TREATMENT OPTION FOR SEA LICE

1.3.1. Imvixa® (lufenuron a.i.)

Lufenuron (CAS No. 103055-07-8, (RS)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea, log K_{ow} = 5.12, Figure 7) is a benzoylurea compound which acts as a systemic pesticide used to control fleas in pets and mites and other pests. Recently it has been developed as a treatment to prevent and control sea lice infestations in salmon and is marketed as Imvixa® by Elanco Animal Health (Basel, Switzerland). It is registered for commercial use in Chile. In Canada, it can be requested for emergency use under an EDR (Fisheries and Oceans Canada 2018) and has also been used for investigational purposes in 2017 to 2018 (ACFFA 2019). Imvixa® is a 10% oral powder formulation which is incorporated into commercial feed at a dose of 5 mg lufenuron/kg/day for seven days for a total dose of 35 mg/kg. Smolts are treated in freshwater facilities then depurated for approximately seven days to excrete any unabsorbed material prior to moving to sea cages. The mode of action is similar to teflubenzuron in that it inhibits chitin synthesis and prevents moulting of the exoskeleton of target organisms; it also causes these organisms to stop feeding (BCPC 2018). The high log K_{ow} value indicates the ability of lufenuron to bioaccumulate and partition into organic matter (McHenry 2016). This study also showed that lufenuron and its metabolites were excreted by the fish while in the sea cages. A study using post-smolt salmon, body weight range of 107-185 g, housed in tanks with seawater and treated with [^{14}C]-lufenuron showed that fillet and pooled faecal samples contained lufenuron after 178 days post treatment (FAO and WHO, 2018). Due to its high log K_{ow}, lufenuron should stay bound to the faecal matter. Therefore any risk to the marine environment would primarily be as a result of the deposited excreta. Any lufenuron which does partition into sediment has the potential to be also persistent. A toxicity study conducted by (Brock et al. 2016) determined lufenuron was persistent in spiked sediment samples for at least 66 weeks (118% of initial values).

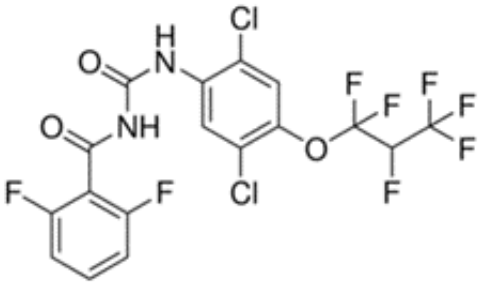


Figure 7. Chemical structure of lufenuron.

1.4. ANTIBIOTICS USED BY THE AQUACULTURE INDUSTRY

Other than the aforementioned sea lice treatments, the most abundantly used chemicals employed by the aquaculture industry are antibiotics administered in-feed for the control of

bacterial pathogens. Tables 1 and 2 and Figure 8 present the antibacterial compounds currently of interest for analytical determination which fall into the main classes of β-lactams[1], tetracyclines[2], macrolides[3], sulfonamides[4], phenicols[5].

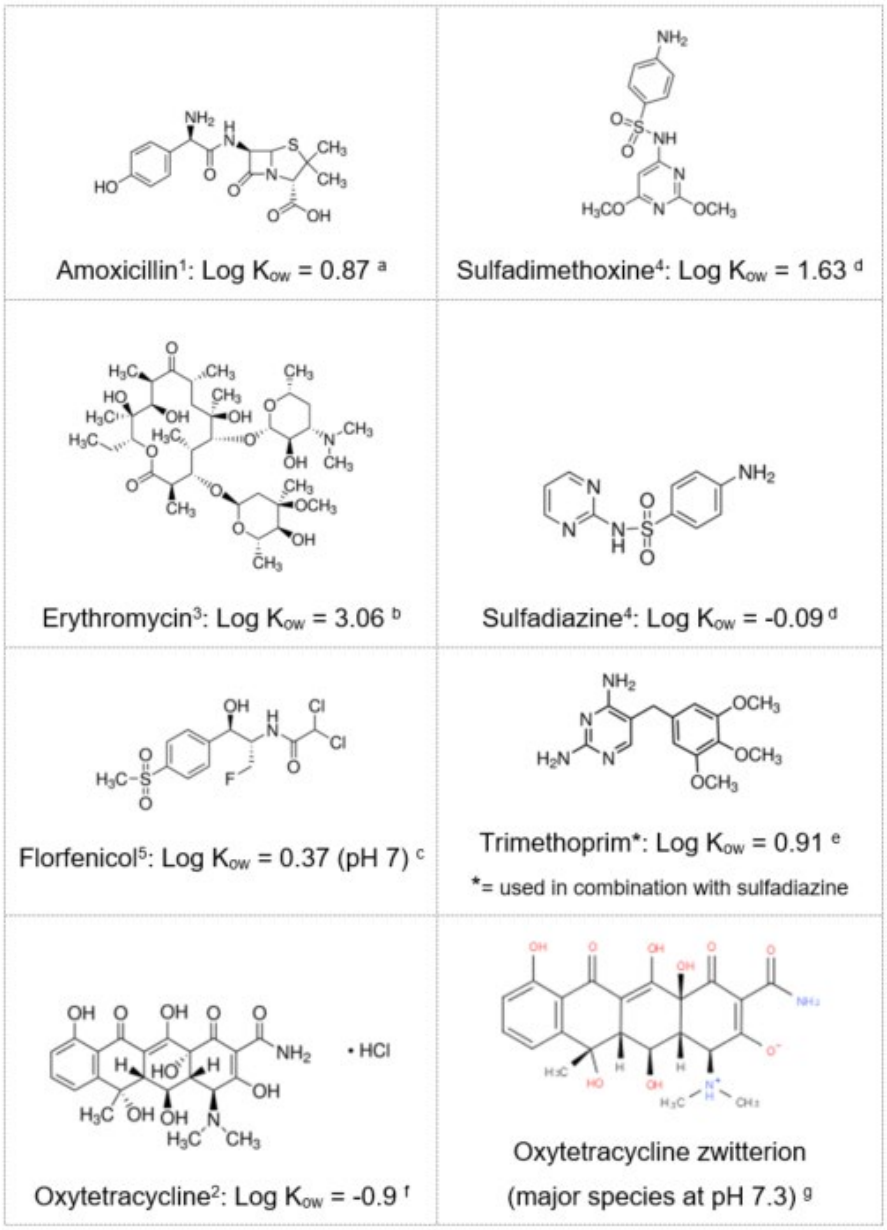
Table 2. Antibiotics of analytical interest currently used by the Canadian aquaculture industry.

Trade name	Active ingredient(s)	Compound class	Mode of administration	Treatment (but not limited to)	Mode of action
Amoxicillin	Amoxicilln	β-lactam	In-feed	Could be used in combination with other antibiotics to treat winter sores, strep infections, etc.	Inhibition of cell wall synthesis
Terramycin Aqua®	Oxytetracycline	Tetracycline	In-feed	BKD, Ricketsiae, mycoplasma and protozoa	Disruption to protein synthesis
Aquaflor®	Florfenicol	Phenicol	In-feed	Furunculosis (including atypical), fungal infections and *Moritella*/*Vibrio* winter sores	Protein synthesis inhibitor, based on peptydil transferases
Erythromycin	Erythromycin	Macrolide	In-feed	Strep, BKD and some protozoans	Inhibition of protein synthesis, primarily through transpeptidation/translocation modalities
Romet® 30	Ormetroprim*/ sulfadimethoxine	Sulfonamide	In-feed	Non-resistant furunculosis and fungal infections	Inhibitition of enzyme dihydrofolate synthetase
Tribrissen®	Trimethoprim/ sulfadiazine	Sulfonamide	In-feed	Non-resistant furunculosis and fungal infections	Inhibitition of enzyme dihydrofolate synthetase

* = Not identified for analytical determination.

Each of the listed antibiotics have low log K_{ow} coefficients, similar to azamethiphos (log K_{ow} = 1.05), with the exception of erythromycin which has a log K_{ow} = 3.06. Therefore it can be assumed that they are highly water soluble and thus would have low adsorption to organic matter and low persistence in the sediment environment. This, however, is not the case for tetracyclines, marcolides and fluoroquinolones (none listed in this report) which bind strongly to clay and organic matter since they are zwitterionic[1]. The mechanism for their adsorption is via cation exchange and the degree to which this occurs is pH dependant. Therefore, these compounds have the potential to be highly persistent in sediment, e.g., oxytetracycline having a half-life of up to 150 days in marine sediment (Brooks et al. 2008).

[1] Zwitterionic: a molecule with two or more functional groups, of which at least one has a positive and one has a negative electrical charge and the net charge of the entire molecule is zero.



a = PubChem. URL: https://pubchem.ncbi.nlm.nih.gov/compound/33613

b = PubMed Abstract. McFarland JW et al; J Med Chem 40: 1340-6 (1997).

c = Environmental Assessment for AquaFlor® for Freshwater-Reared Salmonids. Schering-Plough Animal Health Corp., 1/31/2007.

d = S. Thiele-Bruhn and M.O. Aust. Effects of Pig Slurry on the Sorption of Sulfonamide Antibiotics in Soil. Arch. Environ. Contam. Toxicol. 47, 31-39 (2004).

e = PubChem. URL: https://pubchem.ncbi.nlm.nih.gov/compound/5578#section=Octanol-Water-Partition-Coefficient

f = PubChem. URL: https://pubchem.ncbi.nlm.nih.gov/compound/54675779#section=Octanol-Water-Partition-Coefficient

g = CHEBI:133011 - oxytetracycline zwitterion. URL: https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A133011

Figure 8. Chemical structures of antibiotics currently or historically used in Canadian aquaculture.

2. SAMPLING CONSIDERATIONS

2.1. STANDARD OPERATING PROCEDURES

To obtain accurate data from collected samples, it is imperative that not only a suitable chemical analysis method be developed and validated (Section 4.2), but samples of consistent quality must be collected for analysis. It does not matter how robust the analytical method is, if the samples being tested have been collected inconsistently the generated results will be inaccurate and highly variable, and will not be representative of the sampled locations. To ensure reliable samples are collected, standard operating procedures (SOPs) should be developed to meet study objectives and in conjunction with laboratory requirements prior to field sampling. Factors to consider include, but are not limited to:

1. the types of matrix to be collected (e.g., water/sediment).
2. types of sample storage containers required.
3. types of samplers to be used (e.g., grab, corer, Niskin bottle, etc.).
4. depth and volume of the sediment sample to be collected from the grab/corer.
5. volume of water sample required and preservation method (if applicable).
6. storage conditions for the collected samples (on the boat and when onshore).
7. shipping procedure (if samples are to be analysed by an off-site third party laboratory).

A more detailed discussion relating to planning and design for post deposit monitoring is presented in Page et al. (2021).

2.2. SEDIMENT SAMPLING

In terms of physical samples to be collected for analysis, in-feed drug formulations used to treat sea lice contain lipophilic (high log K_{ow}) active ingredients which tend to bind to organic matter and thus remain persistent in the environment after treatment. Sediment therefore is the matrix of most interest when quantification of these chemical compounds is required. Sampling is typically performed using either grabs or corers with the analytical aliquot taken from the bulk sample. A significant issue with using grabs is washout of the collected sample which occurs when the flaps and/or jaws of the grab do not close properly upon retrieval thus allowing loss of sediment and overlying water thus compromising the integrity of the sample. Employing corers reduces this phenomena although corers do have their own issues as well. The depth at which to sub-aliquot the collected bulk sample for analysis should be based on the study or program objectives. Ideally for determination of most recent chemical deposition, the upper sediment layer (down to 2 cm) should be sampled (US EPA, Office of Water 2001). If a deeper profile is aliquoted (e.g., down to 5 cm, or deeper), there is a possible risk that depths >2 cm may not contain appreciable levels of the target analyte(s). This would result in dilution of the analyte(s) contained within the top 2 cm when the sample is analysed resulting in a lower concentration being determined than is actually present in-situ.

2.3. WATER SAMPLING

Bath treatments in either well boats or tarped netpens involves the use of formulations containing hydrophilic (low log K_{ow}) active ingredients. If determination of these compounds is required to confirm treatment or post discharge concentrations then water samples need to be collected for analysis. These can be collected using a variety of techniques, but the most favoured are Nyskin bottles for vertical profile samples or peristaltic pumping for set depths.

Dispersion and transport of treatment plumes after release or discharge is highly variable and depends on many factors (Page et al. 2015). Therefore the main challenge associated with water sampling is that without the aid of a visual indicator such as fluorescein dye, it is impossible to track the plume accurately and thus unable to sample with true confidence (Ernst et al. 2014). Another consideration is that unlike in-feed treatments which fall to the sea bed and remain persistent, dilution and dispersion of bath treatment plumes occurs rapidly due to ocean currents. Therefore water samples need to be collected at the time of treatment and within a few hours post discharge unlike sediment samples which can be collected up to a few months after application depending on the half-life of the active ingredient.

2.4. SAMPLE HANDLING

Along with consistent accurate sample collection, good sample handling is imperative to minimise positive or negative bias effects during analysis. Positive bias produces artificially high results usually as a result of cross contamination during field collection or laboratory extraction and analysis. Rinsing grabs or corers after use at each location would limit contamination at the sampling stage. Sample containers can also contribute to contamination if they have not been cleaned properly before each use. To eliminate this, certified pre-cleaned disposable containers can be purchased from chemical supply vendors which eliminates the possibility of dirty containers being used. Thorough cleaning of laboratory apparatus and equipment is also essential to mitigate contamination at the analytical level. Negative bias on the other hand results in decreased analyte concentrations. This is primarily as a result of analyte degradation due to improper storage and/or preservation prior to analysis. This can be limited by ensuring appropriate container materials and storage conditions are employed for the collected samples (US EPA, SESD 2017).

3. CHEMICAL EXTRACTION TECHNIQUES

3.1. OVERVIEW

Accurate quantification of chemical concentrations is essential for the evaluation of risk or impact. Over the past few decades, analytical methodologies and techniques available for the determination of pesticides, antibiotics and other chemical compounds in environmental, agricultural and biological matrices (blood, urine, faeces, tissue), to name a few, have grown and evolved at a rapid rate. These advancements have resulted in the reduction of sample preparation complexity and processing times and also lowered detection limits along with improving accuracy and precision of analysis.

The main steps involved with any analytical method are:

1. preparation of the sample prior to extraction.
2. extraction of the compound(s) of interest from the sample matrix.
3. clean up of the extract to remove any endogenous compounds that would interfere with the detection of the compound(s) of interest.
4. instrumental detection of the compound(s) of interest.

Points 2 and 3 are typically the most time consuming in terms of labour and constitutes about two-thirds (⅔) of the total analytical procedure (Points 1 to 3). In terms of total time, however, sample analysis (Point 4) normally takes the longest due to instrument run-time which can be overnight or longer depending on the number of samples and the chromatographic run time per

sample. Data processing of collected chromatograms and spectra is also time consuming for the analyst.

Development and validation of an analytical method will establish a set of data specific to that method, i.e., linear range, selectivity/specificity, LOD/LOQ, accuracy and precision, recovery, etc. (Section 4.2). This is a result of the extraction solvent(s) used, the chosen extraction technique, and type of analytical instrument employed for detection and quantification. If a subsequent method targeting the same analyte(s) and matrix type were to be developed using different conditions and instruments, it would produce different validation data since it is uncommon that two very different methods produce the same results.

To greatly improve accuracy and precision for an assay it is common practice to employ the use of internal standards which are added at a constant amount to the calibration standards, quality controls and test samples. Internal standards compensate for any loss, variability or signal enhancement/suppression of the target analyte(s) during analysis. For non-mass spectroscopy applications, internal standards are typically compounds of similar chemical properties (and thus similar behaviour) to the target analyte(s) when extracted and analysed. For mass spectrometry applications, internal standards are typically isotopically labelled versions of the target analyte(s). These are usually deuterium labelled, i.e., containing deuterium (^{2}H) in place of hydrogen (^{1}H) or ^{13}C labelled-containing ^{13}C atoms instead of ^{12}C atoms. Isotopically labelled internal standards behave exactly the same, and chromatograph extremely similar to their unlabelled analogue(s). The main drawback of using isotopically labelled internal standards is that they are not available for every compound and may need to be custom synthesised.

Many chemical extraction techniques are available depending on the compounds of interest and the type of matrices involved. State of the art techniques and those not commonly used for routine processing and analysis of samples by commercial laboratories include, but are not limited to, supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and matrix solid phase dispersion (MSPD). These techniques use less solvent volumes, are less time consuming and also have the advantage of higher extraction recoveries compared to more traditional techniques resulting in increased detection limits. However, the instrumentation is expensive plus a clean up step is usually required by, e.g., solid phase extraction (SPE) or gel permeation chromatography (GPC) prior to instrumental analysis. The instrumentation also require well-trained analysts to operate and maintain them. In contrast, the techniques which are employed on a more routine basis, because of lower equipment costs and complexity, include liquid-liquid extraction (LLE), Soxhlet extraction, pressurised liquid extraction (PLE) also known as accelerated solvent extraction (ASE) and SPE. LLE and Soxhlet extraction in comparison to the state of the art techniques use higher solvent volumes, are more time consuming and have slightly lower extraction efficiencies. They also have the drawback of using larger volumes of hazardous solvents such as dichloromethane, hexane, ethyl acetate and acetone which are extremely harmful to both the environment and human health. A relatively recent method initially developed for the determination of pesticides in fruits and vegetables is QuEChERS (pronounced ‘catchers’), an acronym for **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe. This method is now gaining popularity for the extraction of different classes of chemicals from a wide range of matrix types (e.g., environmental and biological).

Once samples have been processed, the extracts are analysed using chromatographic separation (either GC or HPLC/uHPLC) of the constituents based on their chemical properties followed by detection using an appropriate detector. The most commonly used detectors for GC systems are flame ionisation detector (FID), nitrogen-phosphorus detector (NPD) or electron capture detector (ECD). For HPLC/uHPLC systems, detection is typically by ultraviolet-diode array (UV-DAD), fluorescence (FLD) or photodiode array (PDA). These types of detectors afford high sensitivity and are relatively inexpensive due to their ‘simplistic’ nature but they do have

their limitations when trace or ultra trace detection and multi-class identification of compounds is required. For these situations the most suitable detector is the more expensive mass spectrometer (MS) which is an extremely sensitive, accurate and specific instrument which measures the mass-to-charge [m/z] ratio of ions. Several types of mass spectrometers are available ranging from tandem mass spectrometers to the more sophisticated, sensitive and accurate quadrupole time of flight (QToF) and ion trap mass analysers.

3.2. LIQUID-LIQUID EXTRACTION (LLE)

This method is based on the partitioning of compounds based on their relative solubility between two immiscible solvents, generally water and a non-polar organic solvent. The analytes in the water phase are partitioned into the non-polar solvent by shaking in separatory funnels (Figure 9) and the phases allowed to separate. The organic layer is collected and usually the extraction is repeated with the addition of fresh solvent to enhance the recovery efficiency of the analytes. The organic phases are combined, then evaporated and reconstituted to concentrate the analytes of interest which increases sensitivity during instrumental analysis. The most common problem when using this method is the formation of an emulsion during the shaking procedure. This is usually caused by the aqueous sample containing high levels of protein, free fatty acids, triglycerides, etc. which is more of a problem in biological samples but less so for environmental samples. This technique (based on USEPA 3510) was used by Ernst et al. (2014) to extract azamethiphos and deltamethrin from water samples collected after release from a tarped cage and well boat after sea lice treatments. Solid samples can also be extracted in this manner by first grinding or milling the sample down sufficiently then extracting it with a suitable organic solvent followed by centrifugation, purification and/or concentration prior to analysis. The advantages and disadvantages of this technique are:

Advantages	Disadvantages
Inorganic salts easily removed.	Labour intensive.
Short method development time.	Large volumes of organic solvents used.
Low cost.	Difficult to automate.
	Emulsion formation.

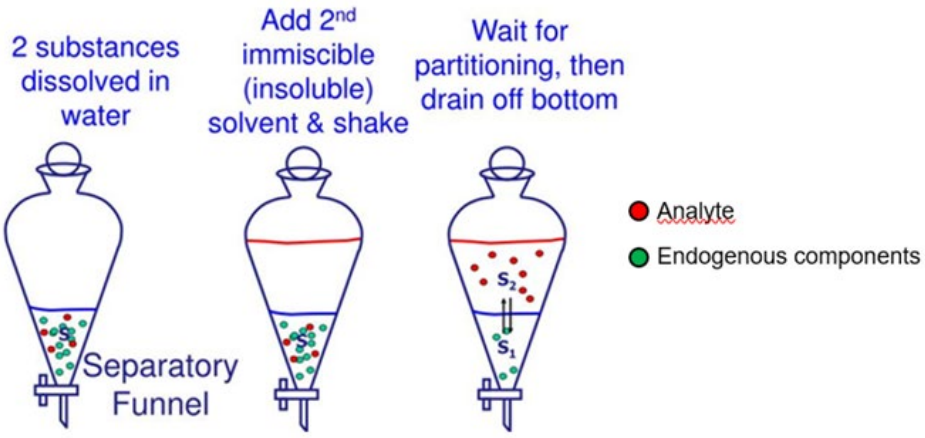


Figure 9. Typical liquid-liquid extraction set-up.

3.3. SOLID PHASE EXTRACTION (SPE)

This sample preparation technique is the one most widely used, it involves the separation of components within a sample by their chemical interaction with a chromatographic sorbent material (usually held in a syringe cartridge). There is a large selection of SPE sorbent materials available to extract countless compounds from different samples, e.g., environmental, biological and foods. The four main phase types available for SPE are reversed phase, normal phase, ion exchange (anion and cation) and adsorption. These phases include, but are not limited to, C18, C8, strong anion exchange (SAX), strong cation exchange (SCX), cyano (CN), anion exchange (AX), cation exchange (CX). The selection of the phase type will depend on the sample type, polarity and chemical characteristics of the compounds to be extracted. SPE overcomes many problems related to LLE such as incomplete phase separation, emulsion formation and low recoveries. It is much faster than LLE with a higher sample throughput and uses smaller solvent volumes. Non-volatile or semi-volatile compounds can be extracted from liquid samples or purified from solid samples which have been pre-extracted into solvent. The basic steps for SPE are presented in Figure 10.

Step 1) Pre-conditioning of the sorbent with organic solvent and/or water (or aqueous buffer) which wets the sorbent.

Step 2) Sample loading – any non-retained components will pass through the sorbent.

Step 3) Washing the sorbent to remove retained impurities with a suitable solvent. The impurities are removed by washing with a solvent that is strong enough to remove them but weak enough to leave the compound(s) of interest behind.

Step 4) Drying of the sorbent – usually under vacuum.

Step 5) Elution of the retained analytes with a suitable solvent.

Step 6) Evaporation, and reconstitution of the dried sample with a suitable solvent for analyse.

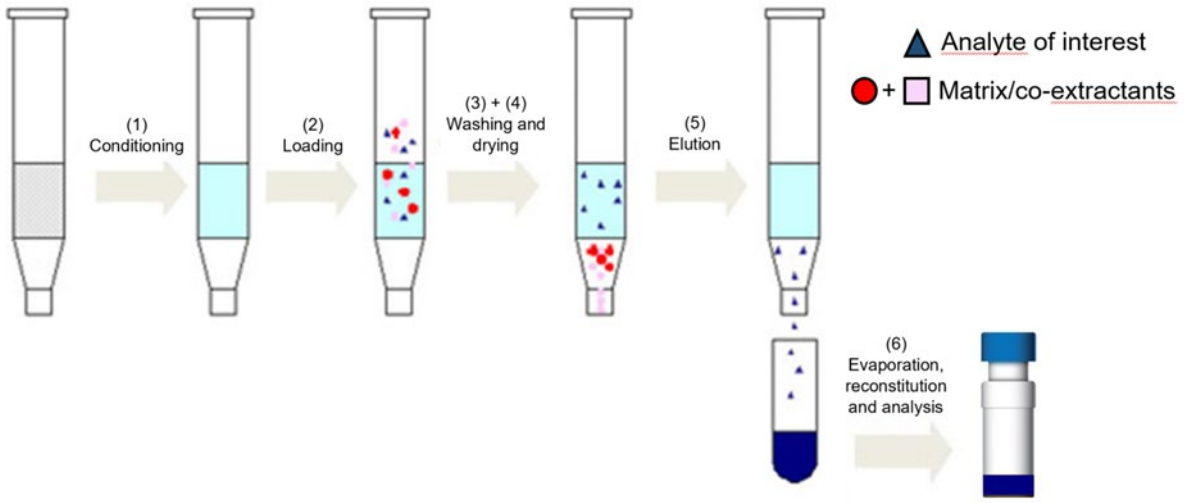


Figure 10. Representative solid phase extraction procedure.

Lyytikäïnen et al. (2003) used this technique to extract various classes of pesticides from reconstituted reference water samples followed by GC-ECD/NPD analysis which yielded typical recoveries in the range 66% to 124%. Stevens and Jones (2010) listed the advantages and disadvantages of this technique as:

Advantages	Disadvantages
Very selective.	Greater complexity/difficult to master.
Effective with a variety of matrices.	Lengthy method development.
Concentration effect.	Costly.
High recoveries.	Many choices of sorbents.
High reproducibility.	

3.4. SOXHLET AND PRESSURISED LIQUID EXTRACTION (PLE)

Soxhlet extraction (Figure 11a) was originally invented in 1879 by Franz von Soxhlet to extract fats from milk solids (Jensen 2007) but has since been improved and adapted for a wide range of solid matrix types. In practise the prepared solid sample is placed in a thimble holder and extracted by a suitable organic solvent which is continuously boiled and condensed filling the holder which is then siphoned back into the bulk solvent in the distillation flask when it reaches a certain level. This process is repeated until the analyte(s) of interest have been extracted from the sample. Due to the stepwise extraction method, i.e., filling/emptying of the sample chamber this extraction technique is very time consuming. Once the extraction is completed, the solvent is concentrated to a certain volume, usually by rotary evaporation then analysed using an appropriate chromatographic system. Akoto et al. (2016) used this method to determine organochlorine pesticide (OCP) residues in river sediment followed by GC-ECD analysis.

Pressurised liquid extraction (PLE), also known as accelerated solvent extraction (ASE, Figure 11b) is the modern automated version of the Soxhlet extractor that requires less solvent to extract samples. PLE houses the samples in steel cells then extracts them with solvent at

elevated temperatures and pressures which increases the solubility of the analytes and also the viscosity of the extracting solvent. This increases diffusion of analytes into the solvent and increases the extraction efficiency and also reduces the processing time. Depending on the instrument, up to 24 samples can be extracted sequentially with a smaller footprint compared to the traditional Soxhlet system for the same number of samples. Evaluation of this technique was performed for the extraction of pesticides in soil and it was found to perform better than the traditional Soxhlet method (Gan et al. 1999).

a) b)

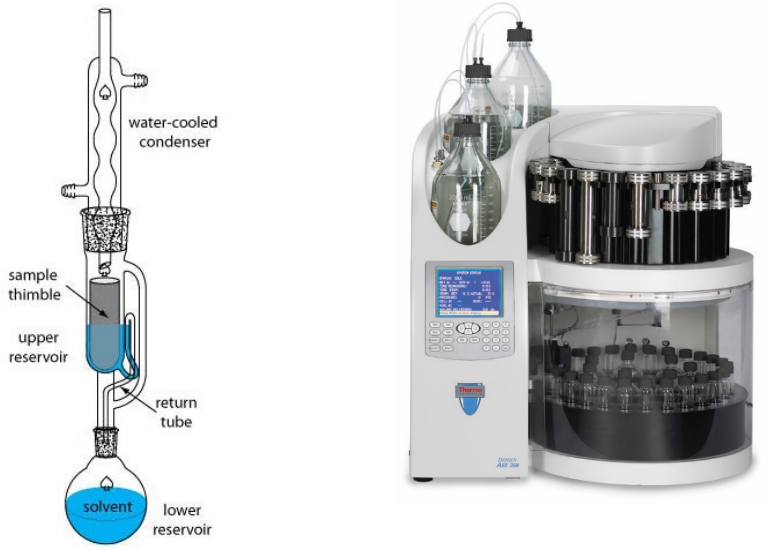


Figure 11. Representative diagram of a) a Soxhlet extractor and b) an accelerated solvent extractor.

3.5. QUICK, EASY, CHEAP, EFFECTIVE, RUGGED AND SAFE (QUECHERS)

The traditional extraction techniques described above have their advantages but also disadvantages, most notably the use of large volumes of hazardous solvents, complexity of method development and the cost of automated systems. In an attempt to improve the efficiency, cost effectiveness and safety, a new extraction technique was developed by Anastassiades et al. (2003) for the determination of pesticide residues in fruits and vegetables (Table 3). This technique was given the acronym QuEChERS (pronounced ‘catchers’) which stands for Quick, Easy, Cheap, Effective, Rugged and Safe.

They based the method on liquid extraction of homogenised samples using acetonitrile, a water miscible organic solvent extensively used for multi-residue extraction of pesticides from foods. This solvent is one of the most widely used for QuEChERS extractions since it can extract a wide range of polar and non-polar pesticides with the least number of co-extractants (Zhang et al. 2012). Slight modification such as acidification of the acetonitrile is sometimes required to enhance extraction efficiency depending on the chemical properties of compound(s) being extracted. Since acetonitrile is water miscible, it can be separated from the water phase by the addition of salt in a process called ‘salting out’ which also increases recovery of polar compounds – magnesium sulfate and sodium chloride were selected for water absorption and liquid-liquid partitioning. The cleanup step was achieved by dispersive-solid phase extraction (d-SPE) whereby a sub-sample of the acetonitrile phase was added to primary and secondary

amine (PSA) SPE sorbent materials, along with magnesium sulfate. The role of the PSA is to remove interferences such as fatty acids, ionic lipids and sugars and the magnesium sulfate absorbs any water still present in the organic phase. PSA is the most commonly used d-SPE sorbent material but other materials such as C18 and graphitised carbon black (GCB) can be added to the magnesium sulfate/PSA mix to further improve the clean-up step if required. After d-SPE cleanup, the extract can be analysed without further preparation, although some methods do perform an additional clean-up or concentration step prior to this. The developed method was fully established but not validated to determine the robustness of the procedures. The only validation parameter investigated was recovery experiments for a wide variety of pesticides fortified into lettuce and strawberry samples. Results demonstrated that the method did give good recovery, 86% (RSD = 2.7) to 102% (RSD = 2.8) and 87% (RSD = 3.0) to 102% (RSD = 3.4), respectively. Deltamethrin had recoveries of 97% (RSD = 2.9) for lettuce and 94% (RSD = 3.1%) for strawberry.

The Association of Official Agricultural Chemists (AOAC International) issued a validated method (Table 3) after conducting an inter-laboratory collaborative study (Lehotay 2007) which used 13 laboratories to investigate 20 insecticides, fungicides and herbicides in grapes, lettuce and oranges which gave LOQ < 10 ng/g. A second validated QuEChERS method is the European EN 15662 Method which again is for the determination of various pesticides found in fruits and vegetables (Table 3).

Table 3. Comparison of various QuEChERS methods.

Originator	Anastassiades et al. (2003)	AOAC (2007)	European EN 15662	Estil et al. (2016)	Brondi et al. (2011)	Berlioz-Barbier et al. (2014)	DFO contracted laboratory	SEPA (2018)
Matrix	Fruits and vegetables	Fruits and vegetables	Foods of plant origin	Marine sediment	Freshwater & sediment	Freshwater sediment	Marine sediment	Marine sediment
Class of chemicals	Pesticides	Pesticides	Pesticides	Pesticides - Pyrethroids	Pesticides	Pharmaceutical, hormones, pesticides, plasticiser	Pesticides, drugs and antibiotics	Pesticide (Emamectin benzoate)
Sample size	10 g Homogenised	15 g Homogenised	5 to 10 g Homogenised Add water to get 10 g weight	2 g – dry Spike with IS	10 g – dry	2 g – dry	5 g – wet Spiked with surrogates and standards mix	Wet sediment weighed to give: Near field - 2 g dry equiv. Far field – 20 g dry equiv.
Extraction solvent	Acetonitrile (10 mL)	1% Acetic acid in acetonitrile (15 mL)	• Acetonitrile (10 ml) • Spike with IS	• Add deionised H_2O (10 mL) • Add 1% acetic acid in acetonitrile (10 mL)	Acetonitrile (10 mL)	• Add water (10 mL) • Add acetonitrile (10 mL)	• Add deionised H_2O (5 mL) • Add acetonitrile (10 mL)	• Acetonitrile (50 mL)
Shake	Vortex, 1 min	No	Shake, 1 min (or longer)	Vortex after each above step	No	No	Shake 1 min, let stand 5 min	Shake – 200 cycles/min for 20 min
Partitioning	4 g Anh. $MgSO_4$ + 1 g NaCl and spike IS	1.5 g Anh. NaAce + 6 g anh. $MgSO_4$ and spike IS	4 g $MgSO_4$ + 1 g NaCl + 1 g $Na_3C_6H_5O_7$ + 0.5 g $C_{12}H_{18}Na_4O_{17}$	1.5 g NaAc + 2 g $MgSO_4$	4 g anh. $MgSO_4$ + 1 g NaCl	1.5 g Anh. NaAc + 6 g anh. $MgSO_4$ (pH = 4.8)	1.5 g Anh. NaAc + 6 g anh. $MgSO_4$	4 g ± 0.5 g $MgSO_4$
Shake and centrifuge	• Vortex mix,1 min • Add IS • Vortex mix, 30 sec • 5000 rpm, 5 min	• Shake vigorously 1 min • 1500 rcf, 1 min	• Shake, 1 min • Centrifuge 3000 g, 5 min	• Vortex, 1 min • 4000 rpm ,1 min • Freeze sample	• No shaking mentioned • 3000 rpm, 1 min	• Manual shake then vortex (30 sec) • 5000 rpm, 5 min	• Vortex mix 1.5 min • 4100 rpm, 5 min	• Manually shake 30 ± 10 sec. • Shake – 200 cycles/min for 20 min • Allow supernatant to settle
Supernatant sub-aliquot vol.	1 mL	1 to 8 mL	• 8 mL, freeze overnight then, • 6 mL for d-SPE	8 to 9 mL	5 mL	6 mL	1 mL	As much as possible and add equivalent vol. of acidified water
d-SPE Clean up	150 mg Anh. $MgSO_4$ + 25 mg PSA per mL extract	150 mg Anh. $MgSO_4$ + 25 mg PSA per mL extract	• 900 mg $MgSO_4$ + 150 mg PSA or • 900 mg GCB:$MgSO_4$ (1:59) + 150 mg PSA or • 900 mg GCB:$MgSO_4$ (1:19) + 150 mg PSA	Use roQ QuEChERS d-SPE clean up tube (part no. KS0-8926)	SPE cartridge containing 330 mg PSA, 330 mg C18 and 1 cm layer of $MgSO_4$ (conditioned with 3 mL acetonitrile	PSA/GCB (900 mg$MgSO_4$, 150 mg PSA, 15 mg GCB)	• 150 mg $MgSO_4$ + 50 mg C18. • Spike with IS	• SPE clean-up CX (100 mg/6 mL). • Gravity/atmospheric pressure operation, but gentle vacuum can be applied if required.
Shake and centrifuge	• Shake by hand or vortex mix, 30 sec. • 6000 rpm, 1 min	Centrifuge 1500 g rcf, 1 min	• Shake, 30 sec • Centrifuge 3000 g, 5 min	• Vortex, 1 min • Centrifuge 3000 rpm, 1 min	None since SPE cartridge used for cleanup	• Manual shake then vortex (30 sec) • Centrifuge 5000 rpm, 5 min	• Vortex 1 min • Centrifuge 4100 rpm, 5 min	N/A

Originator	Anastassiades et al. (2003)	AOAC (2007)	European EN 15662	Estil et al. (2016)	Brondi et al. (2011)	Berlioz-Barbier et al. (2014)	DFO contracted laboratory	SEPA (2018)
Sample concentrated before analysis?	No	No • But options available for different scenarios • Prior to analysis, sample centrifuged (1500 rcf, 1 min) prior to transferring to autosampler vial	No 5 mL + 50 µL formic acid (5%)	Yes • Filter (0.2 µM syringe filter) 5 mL aliquot and dry • Reconstituted with 50 µL acetone + 950 µL H_2O:methanol (1:1)	No	Yes • 55 mL Aliquot dried under N_2 at 40°C • Reconstituted with acetonitrile (500 µL) • Diluted 100 µL aliquot 10x with water:acetonitrile (89:11)	No Supernatant filtered (0.2 µM nylon filter) prior to analysis	• Concentrated at SPE extraction stage. • Internal standard spiked into extract. • Stable for 24 days at 5°C ± 3°C.
Detection	GC-MS	GC-MS and LC-MS/MS	GC-MS and/or LC-MS/MS	LC-MS/MS	GC-MS	LC-MS/MS	LC-MS/MS	LC-MS (HRMS – high resolution mass spectrometry)
Validated	No	Yes	Yes	No	Yes	Yes	Yes (no complete)	Yes

Since its inception QuEChERS has primarily been used for the determination of pesticides in fruits and vegetables. However of late, more research has been conducted for the determination of other classes of compounds such as veterinary drugs, antibiotics, pharmaceuticals, drugs, environmental contaminants, etc., found in a wide variety of matrix types. A literature review was conducted regarding extraction of various chemical compounds from many terrestrial soil types using QuEChERS and variations of it (Vera et al. 2013). However, few studies have been performed using marine sediment as the test matrix. A QuEChERS method followed by GC-MS analysis was evaluated for the determination of atrazine, fipronil and α- and β-endosulfan pesticides in both freshwater and sediment (Brondi et al. 2011 and Table 3). Validation of their method demonstrated determination of coefficient (R^2) of 0.9964 to 0.9972 and 0.9835 to 0.9993 for each of the analytes in water and sediment, respectively, with water samples showing the better coefficient of determination. The limit of detection (LOD) for each matrix were similar ca. 0.003 mg/L (mg/kg for sediment) although LOD for atrazine in sediment was a factor of 10 higher at 0.02 mg/kg. The limit of quantification was two times higher for atrazine in water (0.01 mg/L) compared to α- and β-endosulfan (0.005 mg/L) whereas in sediment the LOQ for atrazine (0.05 mg/kg) was five times higher than fipronil and α- and β-endosulfan (0.01 mg/kg). The extraction method they used was similar to that of (Anastassiades et al. 2003) except that a 5 mL acetonitrile extract was purified using a commercial SPE cartridge containing PSA, C18 and anhydrous magnesium sulfate.

The Scottish Environmental Protection Agency (SEPA) has published a detailed method for the determination of emamectin benzoate in marine sediment (SEPA 2018). Their method is a modified QuEChERS method with extraction achieved using acetonitrile and partitioning with magnesium sulfate (Table 3). The cleanup step is achieved using SPE cartridges containing cation exchange sorbent followed by concentration of the eluent prior to analysis. The method utilises different sample weights (2 g – near field, up to 25 m from cage and 20 g far field, 25 to 100 m (or > 100 m if 100 m measurement is within the Allowable Zone of Effect (AZE) and gives achieved method detection limits (MDLs) of 33.7 ng/kg and 3.4 ng/kg (dry weight), respectively.

Chromatography supply companies are a good source of application notes for sample preparation methods. Estil et al. (2016) published a technical note for the extraction and analysis of several pyrethroids from marine sediment (Table 3) based on a modified QuEChERS method. It is unclear whether this method has been validated to test its performance but it does show high recovery and precision for the tested analytes (range 87% to 108% recovery, 2% to 8% RSD).

Berlioz-Barbier et al. (2014) spiked blank freshwater sediment with pharmaceuticals, pesticides, a pesticide metabolite and a plasticiser then extracted the samples using a modified QuEChERS method (Table 3) followed by LC-MS/MS analysis. They did not use the original QuEChERS extraction media but compared those detailed in the AOAC (acetate buffer) and also the European test method guidelines (citrate buffer). They concluded that the acetate buffer provided better recovery of the tested compounds. They also evaluated the original d-SPE clean-up material against other combinations and found that a mix of PSA/GCB gave the better overall results. Their method was also validated for linearity, LOQs, linearity, recovery, and intra- and inter-day precision.

Based on the amount of research available, QuEChERS has been demonstrated to be an excellent technique for the extraction of many classes of compounds from foods and other matrices. However, since QuEChERS is considered a multi-residue method, i.e., it extracts numerous components of varying chemical classes and properties; it does compromise extraction efficiency and hence detection sensitivity for each tested analyte. Tailored extraction methods which target a specific class of compound, e.g., organophosphates, pyrethroids,

macrolides, etc. or a single analyte would deliver lower detection limits and hence lower limits of quantification for ultra-trace detection. Figure 12 shows representative steps for sediment extraction using QuEChERS. The main advantages and disadvantages are:

Advantages	Disadvantages
Minimum use of hazardous organic solvents.	Multi-residue extraction method, therefore reaching suitable recoveries for all the targeted analytes can be challenging.
Non-labour intensive.	Extraction methods tailored to specific classes of compounds could achieve lower detection limits.
Inexpensive compared to other methods.	Dirtier sample extracts resulting in increased analytical instrument maintenance.
Multi-residue extractions.	
Relatively high sample throughput.	
State of the art or specialised equipment not required for the extraction phase.	

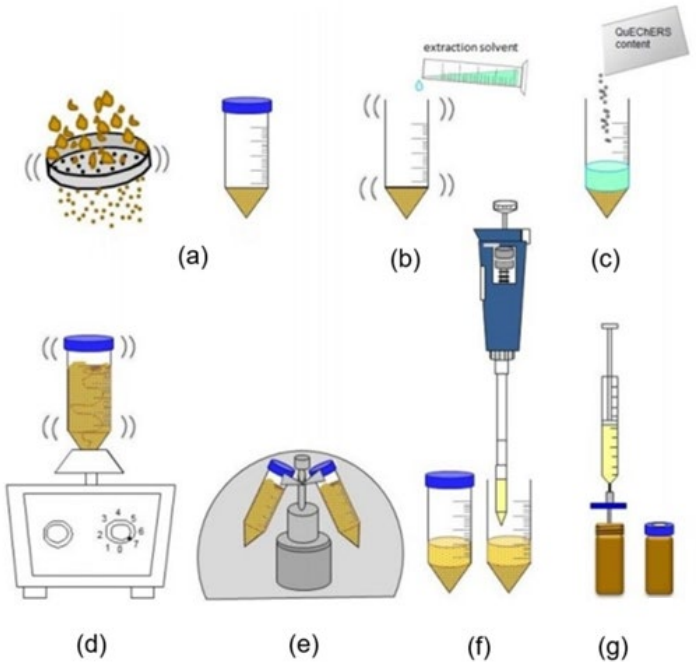


Figure 12. Representative processes for analysis of sediment by QuEChERS, (a) sample pretreatment and weighing; (b) extraction solvent addition and shaking; (c) addition of QuEChERS salts; (d) vortexing; (e) centrifugation; (f) sub-aliquoting of supernatant; and (g) filtering and analysis.

4. IMPORTANCE OF ACCREDITATION AND METHOD VALIDATION

4.1. ISO/IEC 17025 ACCREDITATION

Accreditation demonstrates that a test facility's quality management system and technical competence has met or exceeded stringent evaluation guidelines as defined by the relevant body. This assures the client that the testing facility is competent in performing specific types of testing and measurement which in turn gives them a high degree of confidence in the generated

data. Based on the client's requirements, whether compliance and enforcement, regulatory submission or monitoring depends if accreditation is required. The primary standard for testing and calibration laboratories is ISO/IEC 17025 which is an internationally recognised standard published jointly by the International Organisation for Standardisation (ISO) and the International Electrotechnical Commission (IEC). Within Canada, accreditation of testing facilities to ISO/IEC 17025 can be performed by either the Canadian Association for Laboratory Accreditation (CALA) or the Standards Council of Canada (SCC). To become accredited, a laboratory must submit an application portfolio to an accreditation body which includes information about its operations as well as some background and technical documentation:

1. A copy of ISO/IEC 17025:2017.
2. Quality Management System documentation (policies, procedures, documented processes).
3. Test methods and supporting standard operating procedures (SOPs).
4. Method validation data.
5. Internal audit records.
6. Management Review Records.
7. Demonstration of satisfactory participation in proficiency testing (a minimum of one successful testing round is required prior to gaining accreditation).

The application must also include its scope of accreditation, i.e., the method(s) for which the laboratory is seeking accreditation.

During the facility inspection by the accreditation body, the following documentation must be available for inspection, but not limited to:

1. Competency requirement for each position.
2. List of proficiency testing participation and reports from PT providers.
3. Internal quality control and document control.
4. Sample management.
5. Staff training.
6. Laboratory glassware cleaning.
7. Method verification and validation and validation records.
8. Confidentiality.
9. Equipment maintenance and equipment maintenance records.
10. Reagent preparation records.
11. Calibration certificates.
12. Test reports.
13. Complaints.

Any deficiencies noted during the facility inspection by the inspectors will be recorded in their report and the laboratory given an opportunity to rectify them within a specified time period. When the all the deficiencies have been resolved to the satisfaction of the accreditation body they make the final decision as to whether the laboratory can be accredited. Accredited laboratories are inspected on a regular schedule to maintain their status.

4.2. METHOD VALIDATION

Once a preliminary method has been developed which fulfills the analyst's or client's requirements, its performance should be fully tested to ensure that it is suitable for its intended purpose and that it yields valid scientific data which can be trusted at a given confidence level. The purpose of validation is to verify the suitability of the developed method along with the capacity of the analysts and laboratory (Rambla-Alegre et al. 2012). Table 2 in the Appendix presents data from selected studies where the respective analytical methods have been validated. Figure 13 shows a typical progression of the validation process.

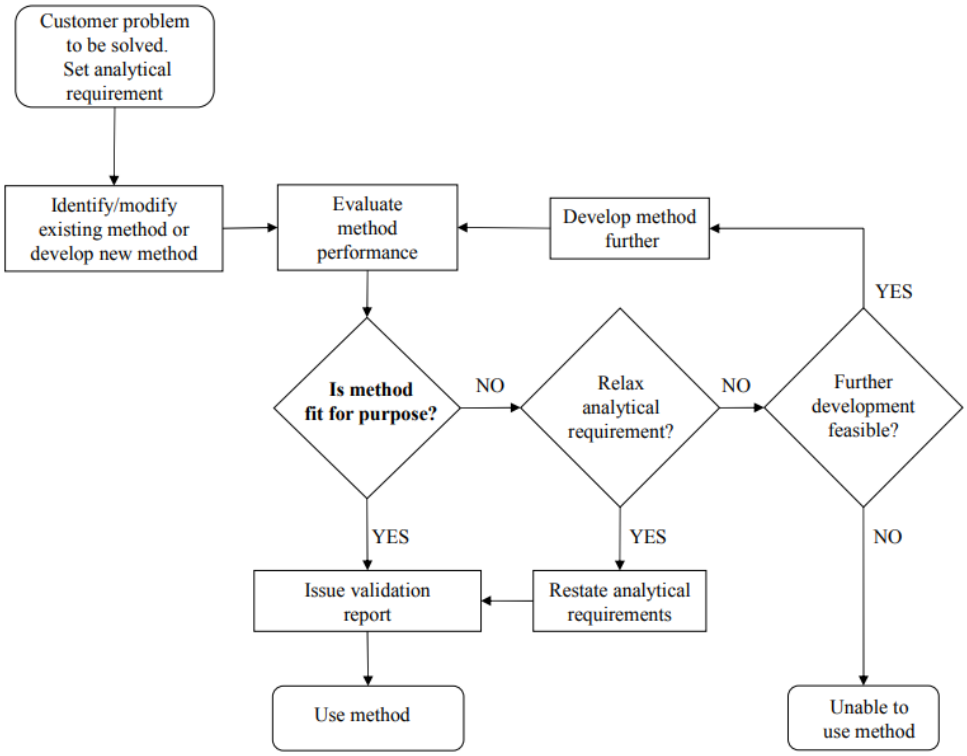


Figure 13. Progression of the method validation process, "The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and related topics" (Eurachem 2014).

These tests take the form of a method validation study whereby the developed method is assessed to determine whether it provides reproducible and precise results within a given set of criteria. Guidelines issued by the Food and Drug Administration (FDA), International Council of Harmonisation (ICH), European medicines Agency (EMA), Association of Analytical Chemists (AOAC) and International Organisation for Standardisation (ISO) specify parameters which should be considered as part of the validation process. It is up to the client and testing laboratory to decide the extent of validation required for the proposed method. Test parameters, with definitions, which should be considered, based on the (ICH 2005) document, are:

1. **System suitability:** Tests are based on the concept that the equipment, electronics, analytical procedures and samples to be analysed constitute an integral system that can be evaluated as such.
2. **Specificity:** The ability to assess unequivocally the analyte in the presence of components which may be expected to be present – impurities, degradants, matrix, etc.

3. **Limit of detection (LOD):** The lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value.
4. **Limit of quantification (LOQ):** The lowest amount of analyte in a sample that can be quantitatively determined with suitable accuracy and precision.
5. **Linearity:** The ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample.
6. **Range:** The interval between the lower and upper concentration of analyte in the sample which has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
7. **Accuracy:** Expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.
8. **Precision:** Expresses the closeness of agreement (degree of scatter) among a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions.
    a. **Repeatability:** Expresses the precision under the same operating conditions over a short time interval of time – also termed intra-assay precision.
    b. **Intermediate precision:** Expresses within-laboratories variations: different days, different analysts, different equipment, etc.
    c. **Reproducibility:** Expresses the precision between laboratories (collaborative studies).
9. **Robustness:** A measure of the analytical procedure's capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The validation study does not need to encompass all the aforementioned parameters but it should be as extensive as is required to show that the method is suitable for its intended purpose. For example, if the method is intended as a qualitative screening method there would be no requirement for LOQ or linearity validation testing. However, if the stability of the analyte in the matrix is in question then further tests such as storage stability should be incorporated into the validation protocol. Figure 14 presents a list of validation parameters recommended by various organisations.

Parameter	Organization
Specificity	ICH, USP
Selectivity	FDA, ISO 17025, IUPAC
Accuracy	FDA, ICH, ISO 17025, USP
Precision	USP, ICH, FDA, IUPAC
Repeatability	ICH, ISO 17025
Intermediate precision	ICH
Reproducibility	ICH, defined as ruggedness in USP, ISO 17025, FDA
Trueness	IUPAC
Linearity	ICH, ISO 17025, IUPAC, USP
Range	ICH, USP
Limit of detection	FDA, ICH, ISO 17025, IUPAC, USP
Limit of quantitation	ICH, ISO 17025, IUPAC, USP
Robustness	FDA, included in ICH as method development activity, ISO, USP
Ruggedness	IUPAC, USP, defined as reproducibility in ICH
Sensitivity	FDA
Recovery	FDA, IUPAC
Applicability	IUPAC
Measurement uncertainty	IUPAC
Stability	FDA

Figure 14. Summary of validation parameters from various organisations, Ramabla-Alegre et al. (2012).

If any significant changes are made to the validated method it should be re-validated, or at least verified using selected parameters to demonstrate that it is still suitable for its intended purpose. Examples of significant changes are, but not limited to:

1. extraction of different sample matrix type.
2. determination of additional analyte(s).
3. amendment(s) to the extraction procedure.
4. substitution of instrumentation (or parts of).
5. amendment to quantification range.
6. amendment(s) to instrumental conditions.

If the validated method is intended for single laboratory use then inter-laboratory testing may not be required. Also, if a testing laboratory develops a method which they see as their intellectual property they may refuse to conduct inter-laboratory testing to prevent competitors possessing the method. This type of test is, however, a valuable tool to demonstrate how robust the developed analytical method is and how competent personnel are at reconstructing the method. Testing can be as informal as a round-robin test where samples of known concentration are prepared by (e.g., the client) then sent to various independent laboratories (including the developmental lab) where they would be processed using the same methodology but possibly different equipment. The generated results from each facility would then be analysed for accuracy and precision to assess its robustness.

The Scottish Environmental Protection Agency (SEPA), accredited to ISO/IEC 17025:2005 by the United Kingdom Accreditation Service (UKAS) have published a comprehensive internal document (SEPA 2017) which documents the procedures required for validation of various chemical tests which they undertake.

4.2.1. Wet sediment weight versus dry sediment weight

Analysis of sediments for determination of pesticides, drugs and antibiotics concentrations can be performed using either wet sediment or dried sediment as detailed in numerous research

studies and analytical methodologies. It should be noted that if concentrations are determined based on “wet” or “as is” values, the data will be biased lower than if they were calculated using “dry” weight (Environmental Chemistry Consulting Services 2011). The contracted laboratory used sediment wet weights to validate and report concentrations based on the developed QuEChERS method. Therefore all their calculations were thus biased lower since the % moisture of the blank matrices used for fortification of calibration and quality control samples was not taken into account. For example the determined LOQ of emamectin was calculated to be 0.21 ng/g wet, therefore if the moisture content of the sample was, e.g., 20% then the LOQ based on dry weight is actually 0.2625 ng/g. Therefore if the validation and field sampled data are to be standardised then the % moisture should be determined and the values recalculated accordingly. The contracted laboratory, at the time of writing has confirmed that this would be accomplished for all previous and new samples.

Also, environmental quality standards (EQS) as part of post deposit monitoring will be calculated based on sediment dry weight concentrations, therefore reporting of standard units is crucial for this to be accomplished accurately.

Another reason to use dry weight would be to have consistency in reporting since the % moisture of collected samples would differ from location to location.

4.2.2. Bound pesticides

The pesticides and antibiotics utilised in the aquaculture industry are different chemically and will have different behaviours and fate once released in the environment. Some compounds may readily dissolve in water, remain bioavailable and be metabolised in time, while others may quickly adsorb to suspended particles and sediments, and bioaccumulate. Sediments are the ultimate pool of any treatment compounds released in the aquatic environment, therefore an understanding of the bioavailability and bioaccumulation of these compounds are essential to describe their fate and predict their toxicity potential.

A range of factors influence the distribution of a compound between suspended particles and sediments and water, but by far, the most important is their lipophilicity, expressed through the octanol-water partitioning coefficient (log K_{ow}). Compounds with a low log K_{ow} are water soluble, such as azamethiphos, with log K_{ow} = 1.05 (BCPC, 2018) and hydrogen peroxide, with log K_{ow} = -1.57 (Pesticide Properties DataBase, 2018) remain bioavailable and are unlikely to accumulate in tissue or sediments. When released into the aqueous environment azamethiphos disperses very quickly and is distributed through oceanic dispersion. With a half-life of 8.9 days, azamethiphos is degraded through photolysis and hydrolysis with the main product being 6-chloro-oxazolo(4,5-b) pyridin-2(3H)-on which is not expected to bind to sediment (PMRA 2016). Similarly, hydrogen peroxide decomposes quickly into oxygen and water, with a half-life estimated at about seven days. The rate of this reaction increases in the presence of organic matter and metals (Burridge et al. 2010).

Other pesticides and antibiotics utilised in aquaculture with log K_{ow} > 3, have a reduced water solubility and tend to adsorb onto organic matter, animal tissue or suspended particles and sediments. The latter is the ultimate bioaccumulation pool, such that there is an interest in understanding the interactions of these compounds to sediments and the factors that influence it. Deltamethrin (log K_{ow} = 4.6) and emamectin benzoate (log K_{ow} = 5.0) (BCPC, 2018) are two chemotherapeutants used in the aquaculture industry that display a high octanol-water partitioning coefficient and will most likely bioaccumulate through adsorption into the organisms’ lipids layers and the organic matter and sediment particles present in the water.

The type of interactions established within the sediments vary from weak van der Waals forces and hydrophobic partitioning to covalent bonds and sequestration and are not limited to one single type of interaction. The full range of possible interactions between pesticides and soil or sediments is well documented by Gevao et al. (2000). These interactions influence, to an extent if a compound remains bioavailable or it bioaccumulates and to what degree. Kümmerer (2009) found that the composition of the sediments or that of the soil determine the character and strength of the adsorption interactions. Li et al. (2017) found that the content, type and characteristics of the organic carbon present guides the strength of the adsorption in addition to the structure and hydrophobicity of the compounds themselves. To support this, Palmquist et al. (2012) offers the example of pyrethroid pesticides, which due to their hydrophobic character would be expected to bioaccumulate in aquatic species. Instead more than 97 % of the total pyrethroid pesticides introduced into the aquatic environment interacts with dissolved organic carbon and is adsorbed to suspended organic matter, soil, sediments and clay, decreasing its bioavailability.

Thus, a range of other factors dependent on the matrix influences the interactions between pesticides and sediments. These factors range from the total organic carbon concentration and composition, the particle size distribution within the sediments (that enables the active sorption area), the sorption and desorption processes from particles, and in particular the dissolved organic matter content. Soil pH is also of importance; adsorption increases with decreasing soil pH for ionisable pesticides, e.g., 2,4-D, 2,4,5-T, picloram, and atrazine (Hatzinger and Alexander 1995). Abiotic processes are predominant under reducing conditions when the level of enrichment is important (Tribovillard et al. 2006); these processes may lead to the depletion or formation of complexes. These complexes could create differences in extraction efficiencies. Formation of complexes could also be influenced by aging in soils (i.e. timing of application versus when chemical analyses are completed) with degradation potential declining with time (Hatzinger and Alexander 1995) and potentially influencing efficiencies of extraction methodologies. These points are particularly relevant to aquaculture considering that highly enriched sediments will likely be sampled around sites (Widenfalk 2002; Li et al. 2017; Rain-Franco et al. 2018; Vryzas 2018).

As discussed previously, there is a wide range of interactions that govern the distribution of a particular pesticide or chemotherapeutant between water and sediments or other particulates. When a compound is strongly retained onto the sediment surface, its bioavailability is significantly decreased, and so is its toxicity to the live organisms (Widenfalk 2002; Palmquistet al. 2012). Toxicity also varies from one living organism to another; for example the epibenthic species *Hyalella azteca* that lives at the water/sediment interface is affected differently than a sediment dwelling invertebrate, such as *Chironomus dilutus* that actively feeds on sediment particles because of the different exposure routes (Li et al. 2017). Some researchers argue that bioavailability is a better indicator than the total chemically extractable amount when assessing the exposure of an organism to a compound in the aquatic environment (Kümmerer 2009; Palmquist et al. 2012). The range of interactions varies from weak electrostatic van der Waals forces to covalent bonds, such that, if conditions in the aqueous environment were to change some pesticides adsorbed into the surface of the sediments through weaker interactions could be easily released and made bioavailable. Exhaustive techniques that obtain the total extractable compound are used to assess the toxicity potential, although the release and accessibility of these pesticides in the long run are still hard to predict (Burridge et al. 2010).Another factor to consider is the interaction of compounds, most notably pesticides, to sample containers during storage. Sharom and Solomon (1981) assessed the adsorption and desorption of the pesticide permethrin in solution to various container materials (polyethylene, polyvinylchloride, glass and Teflon). They found that agitation of the containers reduced the time for adsorption to reach a plateau compared to static samples. Temperature was also a factor

with lowest rates of adsorption seen at -17°C and the highest at 34°C for each material. Desorption of permethrin from each container was assessed by agitation of containers with double distilled water. Results showed that permethrin was easily desorbed from glass followed by Teflon polyethylene and polyvinylchloride. To minimise adsorption, sample containers and any associated glassware can be silanised to cover the surface with organofunctional alkoxysilane molecules. In a study conducted by Harrison et al. (2013) a silanisation procedure using dimethyldichlorosilane was performed on all glassware associated with the extraction procedure to reduce any adsorption effects. Results showed recovery of all tested compounds as 81% ± 22% using the silylated glassware compared to 45% ± 3% for the non-treated glassware.

Therefore, when selecting the most appropriate method for measuring compound concentrations in sediments, one has to consider many aspects from the storage container to the chemistry of the pesticides and the matrix complexity. These latest factors are especially important for the selection of the appropriate extraction technique. For bioavailable compounds, a liquid-liquid extraction (LLE) might be enough while for pesticides that interact more strongly with particulates in the aquatic environment, more exhaustive extraction techniques may be required, such as Soxhlet extraction, accelerated solvent extraction (ASE), supercritical fluid extraction, microwave assisted extraction or high temperature distillation. Di et al. (2015) evaluated the extraction efficiency of ten organochlorine pesticides from sediments prepared using four exhaustive extraction techniques: accelerated solvent extraction (ASE), microwave assisted extraction (MAE), QuEChERS and Ultrasonic Solvent Extraction (USE). The highest recovery was obtained when treating the samples using MAE and QuEChERS, while MAE showed a better reproducibility with low RSD values (Đurović-Pejčev et al. 2018) evaluated QuEChERS, solid-liquid extraction (SLE) and Soxhlet for the analysis of 12 pesticides belonging to different chemical classes in soils. The highest recovery was obtained when using QuEChERS, followed by SLE and Soxhlet, while the lowest detectable limits were obtained when using Soxhlet, QuEChERS and SLE. All three methods proved to be reproducible with RSD values < 19%.

5. SUMMARY AND CONCLUSIONS

There are many techniques available either manual or automated for the extraction of chemicals from sample matrices, although all techniques do involve some manual component for sample preparation prior to extraction. Instrumental methods tend to be more expensive due to the initial cost of the equipment but is less labour intensive, resulting in higher sample throughput which is essential for routine analysis. The converse is true for manual techniques. Extraction of a single analyte or several of the same chemical class within a sample can yield very high recoveries since the extraction method can be tailored to those specific analytes. Multi-residue extraction of analytes from different chemical classes from a single sample, however, requires a compromise on recovery since each analyte will react differently with the chosen extraction solvent(s) resulting in lowered detection limits. On the other hand, improved extraction methodologies and instrumental sensitivities are continuously lowering the limits of detection for chemical compounds.

Validation of developed analytical methods is critical to demonstrate its capabilities and robustness prior to its routine use especially for regulatory decision making and enforcement. If method development, validation and sample analysis are contracted out to a third party laboratory, it is recommended that they should be able to accredit the method to ISO/IEC 17025. This will give confidence to the client that the work has conducted to a specific set of guidelines to ensure the quality and integrity of the reported data especially if the generated data could be used in a prosecution case for enforcement.

QuEChERS is a new and developing technique which has been shown to be suitable for multi-residue and multi-class extraction of compounds within differing matrix types. It offers cost effective and rapid analysis of samples compared to the traditional methods and techniques. This has been demonstrated by the DFO contracted laboratory (not accredited) whose developed QuEChERS method along with a liquid/liquid extraction method for the quantification of various anti-sea lice treatments and antibiotics has shown acceptable accuracy and precision for each analyte investigated. The determined LOQs and LODs were also comparable, if not better than other published methods. However, additional work does need to be conducted for it to fulfill the needs of a fully validated method suitable for routine use. Stability experiments need to be performed to assess the degree of degradation (if any) of each of the tested analytes in sediment samples after collection. The method robustness also needs to be tested using different substrate types (other than muddy) likely to be encountered during field sampling for example in monitoring projects covering a wide geographic range. A comprehensive methodology document should also be provided for review. A potential knowledge gap is the bioavailability of bound compounds to benthic populations. Some research has shown that when pyrethroids are bound to sediment their bioavailability is reduced, whether this is the case for other compounds is unknown.

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7. APPENDIX

7.1. METHOD DEVELOPMENT AND VALIDATION BY A DFO CONTRACTED LABORATORY

INTRODUCTION

There has been extensive research conducted to investigate the effects and fate of pesticides, drugs and antibiotic residues in agricultural soils and freshwater sediments, yet only limited work for marine sediment. Fisheries and Oceans Canada contracted a third party laboratory to develop and validate an analytical method for the quantification of these chemical classes in marine sediment collected from spatial sampling around salmon aquaculture sites as part of a proposed national monitoring program. The compounds of interest identified prior to study initiation are listed in Table 1. This is not an exhaustive list since additional chemicals may be added in the future if they are considered relevant. In the event of this happening, the contracted laboratory would have to re-validate or verify their developed method and/or adapt their protocol to include other compounds while ensuring the LOQs are relevant for the application of regulatory thresholds.

The contracted laboratory initially trialled the QuEChERS method based on (Ikonomou and Surridge 2013) and (Benskin et al. 2016), AOAC 2007.01 and EN 15662 to simultaneously extract all the listed compounds from a single sediment sample with uHPLC-MS/MS detection. However, based on preliminary data they concluded that the antibiotics, amoxicillin and oxytetracycline hydrochloride could not be extracted efficiently by this single method and thus proceeded to utilise a McIlvaine buffer/EDTA solution extraction for these two compounds (Table 3). Robustness of the two extraction methods was demonstrated by validating for linearity, accuracy and precision, matrix effects, method detection limit (MDL), LOQ and recovery for each of the listed compounds. The control matrix used for preparation of fortified samples were sediment samples collected from reference stations located away from salmon cage sites in southwestern New Brunswick. These blank samples were screened for the presence of residues as part of the validation study. Results provided by the contracted laboratory did show that both extraction methods met the acceptance criteria for each of the validation parameters (Table A1) as defined by the study plan.

Table A1. Acceptance criteria for validation parameters as defined by the contracted laboratory.

Parameter	Measured as	Acceptance criteria
Accuracy	Mean % recovery	80 - 120%
Precision	RSD	< 20%
Selectivity	MRM* ratio	Within 30%
Linear range	R^2	> 0.995
Matrix effect/bias	% Deviation	< 50%

*=Multiple reaction monitoring

Coefficients of determination (R^2) for each of the analytes were > 0.995 with LOQs in the range 0.14 ng/g to 12.16 ng/g and method detection limits (MDLs) in the range 0.02 ng/g to 3.82 ng/g.

Accuracy of the method also met the acceptance criterion (actual range 81.4% to 118.3%). Therefore the developed method has been shown to be viable and robust for the extraction and quantification of the listed chemicals from marine sediment. The proficiency of an analytical method can be measured by its detection and quantification limits which is essential when attempting to determine trace or ultra-trace residue levels of analytes in samples which could be vital in applying environmental quality standards (EQS). Table A2 presents limit of detection (LOD) and limit of quantification (LOQ) data for various pesticides and antibiotics extracted from soil or sediment using various extraction techniques with detection by either GC-MS/MS or LC-MS/MS. All techniques gave different values of LOD and LOQ when compared against each other as is to be expected, but within each method the LOQs were typically about three times higher than the respective LODs. Therefore it is evident that different sample extraction techniques (and potentially different analysts/facilities/equipment) and instrumental conditions give different extraction efficiencies.

The method described by the contracted laboratory indicates that it is promising for the quantification of the listed pesticides, drugs and antibiotics based on the validation data. The method was subsequently trialled for the analysis of sediment samples collected as part of a Fisheries and Oceans Canada science project to inform the design of a post-deposit monitoring program and the current national Aquaculture Monitoring and Modelling Program (AMMP).

Table A2. Determined LODs and LOQs from representative studies.

Originator	DFO contracted laboratory	Salvia et al. (2012)	Leseur et al. (2008)	Yang et al. (2010)	Dong et al. (2015)	Radović et al. (2015)	Shi et al. (2014)	Moreno-González et al. (2015)	Syngenta (2014)
Matrix tested	Marine sediment (wet)	Terrestrial soil	Terrestrial soil (dried)	Agricultural soil (freeze dried)	Agricultural soil	River sediment (dried)	Surface sediment (wet)	Marine sediment (freeze dried)	Agricultural soil
Chemical classes	Pesticides, drugs and antibiotics	Steroids, veterinary and human drugs	Pesticides	Pesticides	Pesticides	Pharmaceuticals and pesticides	Antibiotics	Pharmaceuticals	Pesticide
Extraction method(s)	QuEChERS [1] and McIlvaine/EDTA [2]	QuEChERS	USE [3]; PLE [4] European DIN 12393 [5] QuEChERS [6]	QuEChERS	QuEChERS	Ultrasonic Solvent Extraction	Ultrasonic Solvent Extraction	Pressurised Liquid Extraction	Liquid extraction with SPE clean up
Detection	UPLC-MS/MS	LC-MS/MS	GC-MS	GC-MS	UHPLC-MS/MS	LC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS	LC-MS/MS
Validated	Yes (but incomplete)	Yes	Yes	Yes	Yes	Yes	Partially (Rec, LOD, LOQ only)	Partially (Rec, LOQ, LOD only)	Yes

Originator	DFO contracted laboratory	Salvia et al. (2012)	Leseur et al. (2008)	Yang et al. (2010)	Dong et al. (2015)	Radović et al. (2015)	Shi et al. (2014)	Moreno-González et al. (2015)	Syngenta (2014)
Investigated compound(s) of interest to aquaculture (LOD[a]; LOQ)[^] [^] Conc. units are ng/g unless otherwise stated	[1] Abamectin (B1a) (0.437; 1.397) [1] Azamethiphos (0.050; 0.163) [1] Cypermethrin (1.763; 5.607) [1] Deltamethrin (1.003; 3.197) [1] Desmethyl EB (0.054; 0.170) [1] Emamectin (0.063; 0.203) [1] Erythromycin-H_2O (0.253; 0.803) [1] Florfenicol (0.117; 0.367) [1] Ivermectin (1.233; 3.927) [1] Lufenuron (0.067; 0.217) [1] Sulfadiazine (0.060; 0.177) [1] Sulfadimethoxine (0.060; 0.187) [1] Teflubenzuron (1.220; 3.880) [1] Trimethoprim (0.067; 0.207) [2] Amoxicillin	Erythromycin (0.700, 2.140) Florfenicol (0.004, 0.013) Sulfadiazine (0.020, 0.055) Sulfadimethoxine (0.009, 0.026) Trimethoprim (0.006, 0.019)	Deltamethrin [3] (8; 25) [4] (3.8; 13) [5] (6; 20) [6] (14; 47)	Cypermethrin (6.2; 20.7) Deltamethrin (9.5; 31.8)	Emamectin benzoate (4.65 x 10^{-5} mg/L; 0.01 mg/kg) Lufenuron (2.4 x 10^{-4} mg/L; 0.05 mg/kg)	Amoxicillin (3; 10) Erythromycin (1; 3) Trimethoprim (1; 3)	Erythromycin (0.08; 0.03) Florfenicol (0.02; 0.25) Oxytetracycline (0.05; 0.19) Sulfadiazine (0.21; 0.41)	Erythromycin (0.45; 1.49) Trimethoprim (1.15; 3.83)	Abamectin comprising: Avermectin B1a (0.1; 0.5) Avermectin B1b (0.2; 0.5)

Originator	DFO contracted laboratory	Salvia et al. (2012)	Leseur et al. (2008)	Yang et al. (2010)	Dong et al. (2015)	Radović et al. (2015)	Shi et al. (2014)	Moreno-González et al. (2015)	Syngenta (2014)
	(1.597; 5.093) [2] Oxytetracycline (0.217; 0.677)								
MDL[+], LOD, LOQ calculations	MDL = $t_{(n-1,1-\alpha=0.99)}$ (S) where t=3.143 LOQ = 10 x SD of baseline noise	LOD = analyte conc. giving 3x background noise LOQ = analyte conc. giving 10x background noise	Concentrations corresponding to S/N of 3 (LOD) and 10 (LOQ)	LOD = 3x SD of baseline noise of a spiked soil LOQ = 10x SD of baseline noise of a spiked soil sample	LOD = 3 x SD of baseline noise LOQ = lowest spiked concentration	Concentrations corresponding to S/N of 3 (LOD) and 10 (LOQ)	Concentrations corresponding to S/N of 3 (LOD) and 10 (LOQ)	Concentrations corresponding to S/N of 3 (LOD) and 10 (LOQ)	LOD = 4x baseline noise of a control sample LOQ = lowest fortification sample (recovery 70 – 120%, RSD ≤ 20%)

+ = MDL (method detection limit) reported by the DFO contracted laboratory instead of LOD

KNOWLEDGE GAPS

Even though the method developed by the DFO contracted laboratory has shown potential for the analysis of the listed compounds, some points need to be addressed and further work needs to be conducted to demonstrate its robustness.

Analytical Method Document

A comprehensive methodology document detailing the analytical and extraction methodologies to determine pesticides, drugs and antibiotics in marine sediment has yet to be prepared by the contracted laboratory for review. At present the provided method is a brief stepwise description of the extraction procedure, it does not contain sufficient information to enable a third party laboratory to fully reconstruct the method. Such a document should detail, but not be limited to:

1. document control (issue date, version number, approval signature).
2. chemicals used (suppliers and grades).
3. equipment list.
4. reagents preparation (extraction solvents, mobile phases, etc.).
5. preparation of calibration standards (stock solution preparation, dilution scheme, etc.).
6. preparation of internal standards (stock solution preparation, dilution scheme, etc.).
7. preparation of surrogate standards (stock solution preparation, dilution scheme, etc.).
8. procedures for fortification of samples.
9. sample preparation prior to analysis.
10. detailed extraction procedures.
11. instrumental conditions (including software and version number used for data processing).
12. equations used to quantify data.
13. representative chromatograms for determined analytes.

Procedure ES-TOEG-P-216 (SEPA 2018) is an example of a comprehensive analytical method document which contains sufficient information to fully reconstruct the method for emamectin benzoate in marine sediment samples.

Accreditation

As detailed in Section 3.1, accreditation provides the client a degree of confidence in the generated results and that the work is conducted to a certain standard as dictated by the issuing body. The validation and analysis described here has been conducted by a laboratory that's is accredited by the BC Ministry of Environment's EDQA Program, BC Ministry of Health, Public Works Canada and CDC – Elite Legionella. However, they have not achieved ISO/IEC 17025 (the primary standard for testing and calibration laboratories) – at the time of writing, their accreditation is pending according to their website.

Storage stability of pesticides, drugs and antibiotics in collected samples

Sediment samples processed by the contracted laboratory were collected by DFO by either grab or diver cores and the top 1 cm or 2 cm is sub-sampled into I-Chem Certified 200 amber

bottles. Samples were then stored frozen at ca. -20°C until shipped to the contracted laboratory in insulated shipping boxes containing gel ice packs. When received, the samples were kept frozen until thawed for sub-aliquoting prior to analysis and the remainder of the samples returned to frozen storage. The chemical stability of the compounds from sampling to analysis is unclear therefore evaluation of storage stability, along with freeze/thaw stability would provide invaluable information. This stability has not been assessed as of this writing. Further work is therefore recommended to determine if degradation occurs and if so, to what extent.

Examination of available literature has shown that very limited research has been conducted to investigate the stability of theses classes of chemicals in marine sediment. Research conducted by (Lyytikäïnen et al. 2003) did address this question in relation to some triazine, organophosphate, organochlorine, pyrethroid and carbamate pesticides – although in terrestrial sediment and using none of the pesticides listed in this paper. Sediments were fortified with a pesticide mixture in the range 0.03 to 1.2 µg/g, one sample was sub-aliquoted (n=3) and analysed immediately for baseline determination. The remainder were stored at either -17°C or +3°C for up to 28 days and analysed at defined time-points. Results showed that all but one of the pesticides was stable when stored at -17°C whereas degradation was more evident in samples stored at +3°C which implies that temperature was a factor when storing samples. A study conducted by the U.S. Geological Survey, the California Department of Fish and Game's Water Pollution Control Laboratory and California Department of Pesticide Regulation (Hladik 2007) did not validate storage stability as part of their research. However, they did conduct analyses that showed sediment samples spiked with a mixture of pyrethroids were stable for one month. Also, collected samples containing pyrethroids stored over a one year period were found to have less that a 10% difference in quantified concentrations.

Storage of five classes of pesticides (triazines, organophosphates, organochlorines, pyrethroids and carbamates) spiked into freshwater sediment and soil then stored at -17°C and +3°C for up to 28 days did show stability for all tested compounds except for lindane (organochlorine) at -17°C. Samples stored at +3°C degraded by up to 10% by 8 to 13 days of storage whereas malathion (organophosphate) took one day to degrade to this level (Lyytikäïnen et al. 2003).

Limited research can be found in the literature pertaining to long-term storage stability or freeze/thaw effects of pesticides and antibiotics in sediment.

Quantification of anhydroerythromycin (erythromycin-H2O)

The macrolide antibiotic erythromycin was identified for investigation in the original list of compounds for which the method was developed. Rather than validating for erythromycin, its metabolite anhydroerythromycin (erythromycin-H2O), which is formed under acidic conditions and results in the removal of water (-H2O) from the parent molecule was assessed instead. The metabolite also possesses little antibacterial activity compared to the parent compound (Fiese and Steffen 1990, McArdell et al. 2003). Since this drug is administered as in-feed and has a log K_{ow} = 3.06 it is expected to adsorb onto organic solids and could be environmentally persistent in sediments. Therefore, validation for the parent drug should potentially be conducted as well, a good example being the validation of emamectin benzoate and its metabolite desmethyl emamectin benzoate.

Effect of differing sediment types

Validation of the extraction method by the contracted laboratory employed reference sediments collected from southwest New Brunswick which can be classed as a muddy substrate. Similarly, the contaminated samples collected for analysis originated from the same vicinities, i.e., the

same substrate type as the reference sediments. Therefore the extraction procedure has been shown to be suitable for muddy (high organic content) sediment types but its performance is unknown for other substrate types (e.g., sandy). The LOQs and MDLs determined by the contracted laboratory were established using muddy substrate (not clay, sand or gravel), therefore details need to be provided to ensure standard operating procedures are detailed enough to form the basis of a monitoring program. As a result, the analytical procedure should be re-checked using the other available substrate types. The method validation need not be fully repeated, at a minimum re-testing for the following parameters using the new substrate type would be recommended (Thompson et al. 2002):

1. Specificity/selectivity and LOD if the sample matrix differs from that used in the method development.
2. Accuracy (bias) – under repeatability or reproducibility conditions.
3. Precision – under repeatability or reproducibility conditions.