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Review

Matrix solid-phase dispersion extraction and the analysis of drugs and environmental pollutants in aquatic species

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ABSTRACT

The classical methods of analyte isolation using homogenization and liquid–liquid partitioning have served for several decades as the standard for the analysis of drugs and environmental pollutants in aquatic species. However, these methods often are costly in terms of analyst time and solvent use and often cannot be accomplished before the materials in question have gone to market. While there have been improvements in screening and determinative techniques, these classical extraction methods are now a limiting factor in residue monitoring. We present here an overview of tissue residue methods for the analysis of drugs and chlorinated pesticides in aquatic resources and offer a comparison to newer extraction technologies, such as solid-phase extraction, supercritical fluid extraction, and matrix solid-phase dispersion (MSPD), as alternatives. MSPD, in particular, shows a great potential to reduce labor and solvent costs and improve sample throughput for residue monitoring programs directed toward aquatic species.

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1. INTRODUCTION

Aquatic resources are monitored for the presence of tissue residues of chemical agents for two main reasons: (1) for food safety —to identify and remove from commercial markets any edible tissues that contain potentially hazardous levels of drug or other chemical residues and (2) for environmental monitoring —to help identify geographical areas where environmental quality may have been significantly compromised.

With increasing reliance on aquatic species as a

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source of dietary protein there is a strong public interest in the safety of edible aquatic resources. This interest is based on concerns about potential unacceptable health risks associated with eating fish containing residues of drugs and environmental pollutants [1]. Such residues may exist in both fish bought by consumers in commercial markets and in fish caught for recreational purposes from rivers, lakes, and oceans. Further, seafood sold in the markets of one country may often have been imported from another with different regulatory policies concerning drug and pesticide use in aquatic environments. For example, imports accounted for over 60% of the fish and shellfish consumed by the United States in 1990 [2]. Therefore, methods are needed for compounds that may be present in either domestic or international products. There is also a need for an international consensus regarding residue levels and concerns.

In this regard, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) serves as a scientific advisory body to FAO, WHO, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), and the Codex Committee on Food Additives and Contaminants, concerning the safety of residues of food additives, contaminants, and veterinary drugs. Recommended acceptable daily intake and maximum residue level (MRL) for these substances have been proposed by JECFA and are used by many countries to formulate regulations regarding chemical residues in foods —including aquatic food resources. The analytical needs of an effective residue monitoring program are in part determined by the MRLs as set by a nation's regulatory authorities. Appropriate analytical methods for these programs are recommended by the CCRVDF. A listing of reports and other documents published by the JECFA is available [3].

Although aquatic species are sporadically monitored for various environmental contaminants, existing environmental monitoring efforts are not designed to be of direct use in evaluating many aspects of seafood safety concerns. This is, in part, due to the fact that many environmental programs lack sufficient geographical scope and sufficient focus on the edible portions of many aquatic species. Because many studies are conducted by various university researchers and by state and federal agencies, the programs also tend to lack a common methodological approach to analysis.

As a result of public concern, the failure of environmental and drug monitoring programs to contribute valuable residue data for human food analysis, and the fact that present seafood monitoring and inspection programs lack both the frequency and direction sufficient to ensure effective implementation of current regulatory limits for seafood safety, several governmental bodies, including the US government, have declared their intention to develop a new seafood inspection system [4]. There is early recognition that the key to the success of this new system will be development and application of more efficient and cost-effective analytical methods.

Two of the major classes of chemicals that will need to be included in any aquatic food safety program and in existing and future environmental monitoring programs are drugs and chlorinated pesticides. This review offers a summary of existing methods for the analysis of many of the drugs and for chlorinated pesticides in aquatic species. Several major drawbacks of the methods are discussed and three relatively new methods that offer solutions to these problems are described.

2. METHODS FOR RESIDUE ANALYSIS OF DRUGS AND POLLUTANTS IN AQUATIC SPECIES

Analytical methods are needed for screening, quantitation, and confirmation of chemical residues in aquatic species for research and regulatory purposes. A review of the literature for methods used to extract, isolate, and quantify chemical (drugs and chlorinated pesticides) residues in aquatic species (Tables 1 [5–42] and 2 [43–77]) reflects the confusion currently felt in the field concerning which protocols are most efficient, accurate, reliable, and cost-effective. For example, most methods currently being used by monitoring agencies for pesticide analysis are based on five "classical" multi-residue methods, some developed over thirty years ago. These methods are commonly called the non-fatty (MOG), fatty (Mills), Luke, Storherr, and Krause methods. Together they detect approximately 321 pesticides or pesticide-related compounds [78]. Most of these methods have undergone rigorous multi-laboratory calibration studies, such as those

TABLE 1

REPRESENTATIVE EXTRACTION AND LIQUID CHROMATOGRAPHIC METHODS FOR DRUGS USED IN AQUACULTURE[a]

Compound(s)	Matrix	Sample preparation	Organic solvent (ml)	Analytical column	Mobile phase	Detection method and analysis time	Limit of detection	Reference
Tetracycline, oxytetracycline, chlortetracycline	Fish	Homogenization, filtration, SPE using Amberlite XAD2 resin	100	(A) Wako Gel (dimethylsilica) 10 μm or (B) Shimadzu Gel (polystyrene gel) 10 μm	(A) 0.05 *M* phosphate buffer–ACN (9:1) (B) 0.04 *M* KH_2PO_4–0.08 *M* EDTA–MeOH (1:1:8)	UV at 370 nm 15 min	20 ng TC, OTC 60 ng CTC on column	5
Tetracycline, oxytetracycline, chlortetracycline	Salmon muscle	Homogenization, SPE using Bond Elut C_{18}	29	Merck Hibar LiChroCART RP-18 7 μm	0.01 *M* oxalic acid–ACN–MeOH (73:17:10)	Photodiode array at 355 nm 15 min	90 ng/g TC 80 ng/g OTC 500 ng/g CTC	6
Oxytetracycline	RBT muscle	Homogenization, SPE using Amberlite XAD2 resin	825	Hypersil SAS 5 μm	0.1 *M* citric acid–0.1 *M* trisodium citrate–0.1 *M* Na_2EDTA–ACN (340:5:5:150)	UV at 370 nm 8 min	5 ng/g	7
Oxytetracycline	RBT muscle and liver	Homogenization, SPE using Sep-Pak C_{18}	10	Shandon ODS Hypersil 5 μm	[(5 g DAHP + 5 ml DEA)/810 ml water]–ACN–DMF (81:19:6)	UV at 365 nm 8 min	5 ng/g	8
Oxytetracycline	RBT serum, liver, muscle	Homogenization, liq–liq, SPE using Bond Elut C_{18}	5	Spheri ODS 5 μm	ACN–DMF–0.01 oxalic acid (27:6:67)	UV at 355 nm 8 min	50 ng/ml serum 50 ng/g muscle 100 ng/g liver	9
Oxytetracycline	Fish liver, muscle, slime, hide, vertebrae	Homogenization, SPE using Bondesil C_8 or C_{18}	11	Supelcosil LC-18 DB 5 μm	0.005 *M* phosphate buffer–ACN–THF (81:10:9)	UV at 357 nm 10 min	5 ng/g muscle 10 ng/g liver	10
Oxytetracycline	RBT plasma	Protein precipitation using trifluoracetic acid	0	Cyano Spheri-5 MPLC 5 μm	0.02 *M* oxalic acid–MeOH–DMF (95:5:5)	UV at 350 nm 6 min	4 ng on column	11
Oxytetracycline	Channel catfish muscle	MSPD	16	MicroPak C_{18} MCH-10	0.02 *M* oxalic acid–ACN–MeOH (70:27.5:2.5)	Photodiode array at 365 nm 6 min	50 ng/g (1.25 ng on column)	12
Sulfadimethoxine, ormetoprim	Catfish muscle, liver, kidney	Homogenization liq–liq	10	μ-Porasil	$CHCl_3$–MeOH–H_2O–conc. NH_4OH (1000:28:2:0.6)	UV at 288 nm 20 min	50 ng/g each	13
Sulfadimethoxine, ormetoprim	Chinook salmon muscle	Homogenization, SPE using Sep-Pak C_{18}	25	Ultrasphere ion-pair 5 μm	ACN–MeOH–0.1 *M* H_3PO_4 (17:10:73)	UV at 280 nm 30 min	200 ng/g each	14
Sulfadimethoxine	Channel catfish muscle	MSPD	16	MicroPak C_{18} MCH-10	0.017 *M* H_3PO_4–ACN (65:35)	Photodiode array at 270 nm 10 min	50 ng/g (1.25 ng on column)	15

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TABLE 1 *(continued)*

Compound(s)	Matrix	Sample preparation	Organic solvent (ml)	Analytical column	Mobile phase	Detection method and analysis time	Limit of detection	Reference
Sulfadiazine, sulfamerazine, sulfamethazine, sulfadimethoxine, sulfapyridine	Coho salmon muscle	MSPD	16	Supelcosil LC 18-DB	ACN–0.01 *M* ammonium acetate gradient composition	Photodiode array at 270 nm 57 min	*ca.* 100 ng/g SDZ 66 ng/g SMR 228 ng/g SMT 150 ng/g SDM 48 ng/g SP	16
Furazolidone	Atlantic salmon muscle and liver	Homogenization, liq–liq, SPE using Bond Elut NH_2	57	ODS Hypersil 3 μm	ACN–water (16:84) containing 0.001 *M* Na_2EDTA and 0.1 *M* KNO_3	UV at 400 nm 8 min	5 ng/g	17
OA, NA, PA, FZD, DFZ, NPN, FMZ, SDM, SMM, SSZ, SMR	Eel, yellowtail, RBT meat	Homogenization, liq–liq, SPE using alumina	367	Nucleosil C_{18}	THF–ACN–H_3PO_4–water (29:1:0.06:69.94)	UV at 260 nm 40 min	20 ng/g OA 40 ng/g SMR, FZD, NA 60 ng/g SMM, SSZ, SDM, DFZ 80 ng/g PA, NPN, FMZ	18
Oxolinic acid, nalidixic acid, piromidic acid	Eel, RBT, sweetfish, red sea bream, yellowtail tissue	Homogenization, SPE using Baker 10 Amino Cartridge	65	Nucleosil 3 C_{18} 3 μm	ACN–MeOH–0.01 *M* oxalic acid (3:1:6)	UV at 295 nm 8 min	50 ng/g each	19
Flumequine, oxolinic acid	Salmon plasma	SPE using Bond Elut C_2 or on-line polystyrene–divinylbenzene	10	Polystyrene–divinylbenzene PLRP-S 5 μm	ACN–THF–0.02 *M* orthophosphoric acid (20:15:65)	Fluorescence 262 nm excitation 380 nm emission 13 min	5 ng/ml OA 10 ng/ml FEQ	20
Flumequine, oxolinic acid	Atlantic salmon liver	Homogenization, liq–liq, on-line dialysis, on-line SPE using polystyrene–divinylbenzene	5	Polystyrene–divinylbenzene PLRP-S 5 μm	ACN–THF–0.02 *M* orthophosphoric acid (20:14:66)	Fluorescence 325 nm excitation 365 nm emission 15 min	4 ng/g OA 7 ng/g FEQ	21
Flumequine, oxolinic acid	Atlantic salmon muscle	Homogenization, liq–liq, on line dialysis, on-line SPE using polystyrene–divinylbenzene	10	Polystyrene–divinylbenzene 5 μm	ACN–THF–0.02 *M* orthophosphoric acid (20:15:65)	UV at 260 nm or fluorescence 325 nm excitation 365 nm emission 13 min	2 ng/g OA 3 ng/g FEQ using fluorescence	22
Flumequine, oxolinic acid	Salmon and RBT muscle and liver	Homogenization, liq–liq	10	PLRP-S polymer 5 μm	0.002 *M* H_3PO_4–ACN–THF (64:21:15)	Fluorescence 260 nm excitation 380 nm emission 12 min	5 ng/g OA 10 ng/g FEQ	23
Flumequine	Atlantic salmon muscle	Homogenization, liq–liq	43	ODS Hypersil 3 μm	0.1 *M* citric acid–MeOH–ACN–THF (60:30:5:5)	Fluorescence 324 nm excitation 363 nm emission 8 min	5 ng/g	24

Flumequine	Eel plasma, aquaria water	Liquid–liquid	4	Nucleosil	ACN–DMF–[(3 g H_3PO_4 + 1 g TMAC)/675 ml water] (125:200:675)	Fluorescence 245 nm excitation 350 nm emission	2 ng/ml	25
Oxolinic acid	RBT serum	SPE using Sep-Pak Accell or liq–liq	4	Nova-Pak C_{18} 4 μm	MeOH–(7.5 g/l $KH_2PO_4 \cdot 2H_2O$ + 2.5 g/l $Na_2HPO_4 \cdot H_2O$ (4:6)	UV at 258 nm 15 min	1 ng/ml	26
Oxolinic acid	RBT serum, muscle, liver	Serum - direct inj. Muscle, liver, homogenization, SPE using Bond Elut C_{18}	0 10	Regis Pinkerton GFF ISRP 5 μm	ACN–0.1 *M* KH_2PO_4 (1:9)	UV at 254 nm 12 min	10 ng/ml serum (direct inj.) 10 ng/g liver, muscle	27
Oxolinic acid	Salmon muscle	Homogenization, liq–liq	26	Partisil ODS-3 5 μm	ACN–MeOH–0.01 *M* oxalic acid (3:1:6)	Fluorescence 327 nm excitation 369 nm emission 4 min	1–2 ng/g	28
Oxolinic acid	Channel catfish muscle and bile	MSPD	16	Versapak C_{18} 10 μm	MeOH–0.05 *M* GAA gradient composition	UV at 260 nm 65 min	50 ng/g	29
Nalidixic acid	Channel catfish muscle and liver	MSPD	16	Verspak C_{18} 10 μm	MeOH–0.05 *M* GAA gradient composition	UV at 257 nm radio-label monitoring 65 min	20 ng/g muscle 60 ng/g liver using radioactivity	30
Nalidixic acid	RBT and amago salmon serum, muscle, liver, kidney, bile	Homogenization, liq–liq	260	ToyoGel DEAE-2SW	0.015 *M* phosphate buffer–ACN (65:35)	UV at 278 nm	Muscle 50 ng/g serum 50 ng/ml liver, kidney 100 ng/g bile 100 ng/ml	31
Miloxacin, M-1 metabolite	Eel, yellowtail, red sea bream and RBT muscle	Homogenization, SPE using Bond Elut C_{18}	110	L-column ODS	0.05 *M* NaH_2PO_4–ACN (65:35)	UV at 260 nm or fluorescence 325 nm excitation 365 nm emission 10 min	10 ng/g each	32
Enrofloxacin	Atlantic salmon and RBT serum, muscle, liver	Serum - SPE using C_2 muscle and liver - homogenization, liq–liq. SPE using C_{18}	2 61	PLRP-S polymer 5 μm	0.002 *M* H_3PO_4–ACN (8:2)	UV at 289 nm or fluorescence 278 nm excitation 440 nm emission 12 min	1 ng/ml serum 1 ng/g tissue using fluorescence	33
Sarafloxacin	Fish serum	SPE using Bond Elut C_2	2	PLRP-S polymer 5 μm	0.002 *M* H_3PO_4–ACN–MeOH (72:20:8)	Fluorescence 278 nm excitation 440 emission 7 min	5 ng/g	34
Enrofloxacin, sarafloxacin	Atlantic salmon muscle, liver	Homogenization, liq–liq	11	RLRP-S polymer 5 μm	0.002 *M* H_3PO_4–ACN–MeOH (73:19:8)	Fluorescence 278 nm excitation 440 nm emission 8 min	5 ng/g enrofloxacin 10 ng/g sarafloxacin	35

(Continued on p. 230)

TABLE 1 *(continued)*

Compound(s)	Matrix	Sample preparation	Organic solvent (ml)	Analytical column	Mobile phase	Detection method and analysis time	Limit of detection	Reference
Ciprofloxacin	RBT and african catfish plasma	liq–liq	3	Spherisorb-5 ODS	ACN–DMF–[(1.13 g H_3PO_4 + 0.38 g TMAC)/700 ml water] (1.5:1.5:7)	UV at 278 nm	–	36
Ampicillin	Yellowtail tissue	Homogenization, SPE using Sep-Pak Florisil	134	Nucleosil C_{18}	MeOH–0.02 *M* Na_2PO_4–0.01 *M* citric acid (15:42.5:42.5)	UV at 222 nm 40 min	30 ng/g (3 ng on column)	37
Thiamphenicol	Yellowtail tissue	Homogenization, liq–liq SPE using Florisil	–	TSK gel ODS-120T	MeOH–water (15:18)	UV at 225 nm 20 min	1.25 ng	38
Thiamphenicol, florfenicol, chloramphenicol	Yellowtail muscle	Homogenization, liq–liq, SPE using Sep-Pak Florisil	245	Chromatorex ODS 5 μm	MeOH–water (15:85)	UV at 225 and 270 nm 30 min	10 ng/g each	39
Febendazole	Channel catfish plasma, kidney, fat, muscle, bowel contents, urine	Homogenization, SPE using diatomaceous earth, liq–liq	49	MicroPak C_{18} MCH-10	Water–0.05 *N* H_3PO_4–ACN (6:6:88) gradient flow-rate	UV at 290 nm 30 min	low ng/g range	40
Malachite green	RBT muscle and liver	Homogenization, liq–liq	16	PLRP-S polymer 5 μm	0.02 *M* H_3PO_4–ACN–THF (49:40:1)	UV at 615 nm 6 min	1 ng/g muscle 10 ng/g liver	41
Malachite green	Pond and tap water	SPE using Baker 10 diol	2	μBondapak C_{18} 10 μm and PbO_2 postcolumn reactor	MeOH–(0.05 *M* Na acetate + 0.1 *M* GAA) (85:15)	UV at 618 nm 17 min	2.83 ng/l chromatic form 2.01 ng/l leuco form	42

[a] Abbreviations: ACN = acetonitrile; CTC = chlortetracycline; DAHP = diammoniumhydrophosphate; DEA = diethanolamine; DFZ = difurazone; DMF = dimethylformamide; FEQ = flumequine; FMZ = furamizole; FZD = furazolidone; GAA = glacial acetic acid; MSPD = matrix solid-phase dispersion; NA = nalidixic acid; NPN = nifurpirinol; OA = oxolinic acid; OTC = oxytetracycline; PA = piromidic acid; RBT = rainbow trout; SDZ = sulfadiazine; SDM = sulfadimethoxine; SMR = sulfamerazine; SMT = sulfamethazine; SMM = sulfamonomethoxine; SP = sulfapyridine; SPE = solid-phase extraction; SSZ = sulfisozole; TC = tetracycline; THF = tetrahydrofuran; TMAC = tetraethylammonium-chloride.

TABLE 2

METHODS FOR DETERMINATION OF CHLORINATED PESTICIDE RESIDUES IN AQUATIC RESOURCES[a]

Compound(s)	Matrix	Sample preparation	Detection	Reference
EPA "16" except for endosulfan I and II	Whole oyster homogenate	MSPD [8 ml acetonitrile–methanol (9:1)]	GLC–ECD	43
EPA "16" except for endosulfan I and II	Crayfish and lobster hepatopancreas	MSPD (8 ml acetonitrile)	GLC–ECD	44
DDT, DDE and DDD (*para* isomers); lindane; heptachlor; hept. epox.; aldrin; dieldrin; endrin	Fish muscle	MSPD (8 ml acetonitrile)	GLC–ECD	45
ΣDDT (also PCBs, PAHs)	Mussel, oyster	NOAA methods	GLC–ECD	46
ΣDDT, lindane, heptachlor, hept. epox., aldrin, dieldrin, α-chlordane, *trans*-nonachlor, mirex	Fish liver, whole mollusk	NOAA methods	GLC–ECD	47
ΣDDT, lindane, heptachlor, hept. epox., aldrin, dieldrin, α-chlordane, *trans*-nonachlor, mirex (also HCB, PCB, PAHs)	Oyster	NOAA methods	GLC–ECD	48
Endosulfan I and II, endosulfan sulfate	Whole crayfish	Modified EPA methods	GLC–ECD (another column for confirmation)	49
ΣDDT, lindane, heptachlor, aldrin, dieldrin, chlordane, mirex, methoxychlor, endosulfan, toxaphene (also PCBs, trifluralin)	Whole fish	Modified EPA methods	GLC–ECD (3 different columns for confirmation)	50
ΣDDT, lindane, heptachlor, aldrin, dieldrin, chlordane, mirex, methoxychlor, endosulfan, toxaphene (also PCBs, trifluralin)	Clam, oyster, mussel, and quahog	Modified EPA methods	GLC–ECD	51
DDT, DDE and DDD (*para* isomers); α-HCH; dieldrin; *trans*- and *cis*-chlordane; *trans*-nonchlor; octachlor epoxide (also HCB, PCBs)	Fish muscle (with and without skin)	FDA	GLC–ECD	52
DDE and DDD (*ortho* and *para* isomers)	Crayfish abdominal muscle and hepatopancreas	FDA (modified Florisil procedures)	GLC–ECD (another column for confirmation)	53
DDT, DDE and DDD (*para* isomers); *o,p'*-DDT, α- and β-HCH; lindane; heptachlor; hept. epox.; aldrin; dieldrin; oxychlordane; *trans*-nonachlor (also HCB, Aroclors 1254 and 1260)	Fish "edible portions"–all flesh and skin	FDA for high moisture, non-fatty food	GLC–ECD (another column and TLC for confirmation)	54

(Continued on p. 232)

TABLE 2 *(continued)*

Compound(s)	Matrix	Sample preparation	Detection	Reference
ΣDDT, ΣHCH, dieldrin, Σchlordane, *trans*-nonachlor, mirex, toxaphene (also Σchlorobenzenes, PCBs)	Fish liver	Solvent extraction, GPC for cleanup, Florisil column chromatography for fractionation	GLC–ECD (GLC–MS for confirmation)	55
EPA "16" except for endrin aldehyde	Oyster and clam	Solvent extraction, reversed GPC for cleanup, Florisil column chromatography for further cleanup	GLC–MS	56
ΣDDT, α-HCH, lindane, heptachlor, hept. epox., aldrin, dieldrin, endrin, *trans*- and *cis*-chlordane, oxychlordane, *trans*- and *cis*-nonachlor, mirex, methoxychlor, toxaphene (also HCB; Aroclors 1242, 1248, 1254 and 1260; dacthal, pentachloroanisole)	Whole fish composites	Solvent extraction, automated GPC for cleanup, Florisil column chromatography for cleanup and initial fractionation, silica gel chromatography for further fractionation	GLC–ECD (GLC–MS for confirmation)	57
Organochlorine pesticides	Fish	SFE, silica gel or alumina chromatography for cleanup	GLC	58
α- and β-Endosulfan; endosulfan sulfate, diol, ether and lactone	Fish	Semipreparative liquid chromatography–homogenization of tissue with trisodium citrate, disodium hydrogen orthophosphate, and Na_2SO_4; silicic acid/alumina chromatography for cleanup	GLC–ECD	59
DDT, DDE and DDD (*para* isomers); DDT and DDD (*ortho* isomers); dieldrin	Whole fish	Semipreparative liquid chromatography–homogenization of tissue with trisodium citrate, disodium hydrogen orthophosphate, and Na_2SO_4; silicic acid/alumina chromatography for cleanup	GLC–ECD	60
DDT, DDE and DDD (*para* isomers); lindane; hept. epox.; dieldrin; endrin; *trans*-chlordane; *cis*-nonachlor	Fish, crabmeat, shrimp, scallop	Solvent extraction, SPE columns (C_{18} and Florisil) for cleanup	GLC–ECD	61
p,p'-DDT-d8	Clam without gut contents	Solvent extraction, SPE columns for cleanup, Florisil column chromatography for further cleanup	GLC–MS	62
Endosulfan	Fish, oyster, and clam	Soxhlet extraction, AC for cleanup	GLC–ECD	63
DDT, DDE and DDD (*para* isomers); α-BHC; lindane; γ-chlordane; mirex (also chlorobenzenes, PCBs)	Whole mussel	Soxhlet extraction, AC for cleanup	Dual capillary column GLC	64

DDT, DDE, DDD, α-HCH, lindane, heptachlor, hept. epox., aldrin, dieldrin (also PCBs)	Fish muscle	Soxhlet extraction, AC for cleanup and fractionation	GLC–ECD	65
EPA "16" except for dieldrin, endrin aldehyde, and endosulfan sulfate	Fish muscle	Soxhlet extraction, AC for cleanup and fractionation	GLC–ECD	66
ΣDDT, HCH isomers, heptachlor, hept. epox., aldrin, dieldrin (also PCBs)	Whole fish and shellfish	Soxhlet extraction, AC for cleanup, saponification (alcoholic KOH) or one fraction	GLC–ECD	67
DDT, DDE and DDD (*pra* isomers); lindane; heptachlor; hept. epox.; aldrin; dieldrin; endrin; mirex; methoxychlor; toxaphene	Oyster, mussel, clam	Soxhlet extraction, liquid–liquid partitioning, AC for cleanup	GLC–ECD (3 different columns and TLC used for confirmation)	68
p,p'-DDT, toxaphene, parathion	Oyster	Soxhlet extraction, liquid–liquid partitioning, AC for cleanup	GLC–ECD	69
DDT, DDE and DDD (*para* isomers); *o,p'*-DDT (also PCBs)	Mussel	Soxhlet extraction, H_2SO_4 cleanup	GLC–ECD	70
DDT, DDE, β-HCH, dieldrin, hexachloroepoxide	Fish fat	Soxhlet extraction, on-line SEC for cleanup	GLC–ECD	71
DDT, DDE and DDD (*para* isomers); dieldrin	Fish	Solvent extraction, AC for cleanup	GLC–ECD	72
DDT, DDE and DDD; α- and β-HCH; heptachlor; dieldrin; endrin; α- and γ-chlordane; *trans*- and *cis*-nonachlor; toxaphene; compound E (also PCBs)	Fish	Solvent extraction, AC for cleanup	GLC–ECD	73
ΣDDT (also Aroclors 1242 and 1254, B[α]P)	Fish muscle	Solvent extraction, AC for cleanup	GLC–ECD (GLC–MS for confirmation)	74
Heptachlor, photodieldrin, *cis*-chlordane; photo-*cis*-chlordane (all [^{14}C]-labeled)	Whole fish	Solvent extraction, AC for cleanup	GLC–ECD and TLC Radioactivity also measured	75
ΣDDT (also PCBs)	Fish liver, whole mollusk	Solvent extraction, AC for cleanup and fractionation	GLC–ECD (GLC–MS for confirmation)	76
Lindane	Whole fish and fish liver, kidney and spleen	Solvent extraction, H_2SO_4 for cleanup	GLC–ECD	77

[a] Abbreviations: AC = adsorption chromatography; B[α]P = benzo[α]pyrene; BHC = benzene hexachloride; ECD = electron capture detector; EPA = United States Environmental Protection Agency; FDA = United States Food and Drug Administration; GLC = gas–liquid chromatography; GPC = gel permeation chromatography; HCB = hexachlorobenzene; HCH = hexachlorohexane; hept. epox. = heptachlor epoxide; MS = mass spectrometry; MSPD = matrix solid-phase dispersion; NOAA = United States National Oceanic and Atmospheric Administration; PAH = polyaromatic hydrocarbons; PCB = polychlorinated biphenyl; SEC = size-exclusion chromatography; SFE = supercritical fluid extraction; SPE = solid-phase extraction; TLC = thin layer chromatography.

needed to obtain official acceptance by the Association of Official Analytical Chemists (AOAC). These methods are the backbone of residue analysis protocols for governmental agencies such as the US Food and Drug Administration (FDA) [79], US Environmental Protection Agency (EPA) [80], and the US National Oceanic and Atmospheric Administration (NOAA) [81] (Table 2). These methods work well under certain conditions and for certain purposes. However, perhaps the greatest drawback to their continued use is their inefficiency as screening methods. These methods are sufficiently complex as to not allow the generation of relevant data in time to prevent contaminated foods from entering the marketplace. For example, the FDA is responsible for prohibiting interstate marketing of food containing illegal pesticide residues. In many cases, food is sold before the FDA completes the analyses needed to confirm the presence of the illegal residues [82]. Results are obtained too late to prevent enforceable removal of the contaminated product. A similar problem exists in screening for drugs that are commonly used in aquaculture.

A variety of methods for the analysis of drugs in aquatic species have been reported. Historically, microbial inhibition tests have been used to detect and quantitate antibiotic residues in fish tissues [83–89] but these tests often lack the specificity and sensitivity required for residue detection at MRLs, may be affected by non-specific inhibitors, do not detect microbiologically inactive metabolites [90], and often have a 20–24 h incubation time.

Colorimetric methods published for drug residue testing in fish generally lack the sensitivity and specificity required for residue analysis, but may be of use in experimental studies [91–93]. Experimental methods using radiolabelled drug and liquid scintillation counting [90,94–97] or whole body autoradiographic studies [98,99] have been used to provide information on pharmacokinetic behavior, metabolite formation, disposition, depletion rate, and extraction efficiencies for compounds used in aquaculture. These methods are, however, unsuited for use in routine drug residue analysis.

The development of rapid screening tests that are practical and rugged would allow for routine monitoring of larger numbers of samples in a shorter time period with greater sensitivity and selectivity than is often currently available using conventional methods. The development of these tests may soon be required by many governments as a part of the methods package needed for drug approval of new animal drugs [100]. Tests that show promise as screening methods for aquatic species include thin-layer chromatography (TLC) [101], high-performance liquid chromatography (LC) (Table 1), and ligand receptor techniques such as immunoassay [102–104], bacterial cell receptor assay [105], and radioimmunoassay [106,107]. These techniques have the greatest potential applicability to regulatory monitoring programs, but must be combined with newer extraction methods or used with a reference biological fluid, not requiring extraction, to indicate the residue level in a target tissue. However, the ability of a screening assay to accurately detect positive and negative samples must be evaluated based on performance parameters such as sensitivity, specificity, cross-reactivity, predictive values (positive and negative) and efficiency before the test can be included in a residue monitoring program.

Suspects identified with screening tests require quantitation and confirmation of the presence of residues exceeding the MRL in the target tissue; hence rapid tissue extraction, quantitation, and confirmatory methods must be available for regulatory purposes. For many such analyses, chromatographic methods provide the necessary specificity and sensitivity required for both qualitative and quantitative drug analyses. Liquid chromatography is the most commonly reported method for quantitation of drugs in aquatic species, but extraction procedures and/or sample pretreatment are often needed before injection on conventional reversed-phase analytical columns. Newer analytical columns employing internal surface reversed-phase or immunoaffinity packing permit direct injection of plasma and other liquid matrices that cannot ordinarily be used with conventional LC columns [27]. With these improvements LC procedures may approach screening tests in speed and simplicity [108]. As illustrated in Table 1, a variety of analytical columns and mobile phases are reported for a number of drugs. Reversed-phase C_{18} packing is most commonly used but other packings reported are dimethyl silica, polystyrene gel, ion-pair, polystyrene divinylbenzene, and Regis Pinkerton internal surface reversed-phase. Detection by UV using fixed- or variable-wavelength detectors or diode ar-

ray is most frequently reported, but fluorescence detection gives greater sensitivity for the quinolone antibiotics. Reported limits of detection for some of the LC methods listed in Table 1 are above the current MRLs of many countries and few of the methods have undergone interlaboratory validation studies, however. Therefore, methods validated for detection of drugs and pollutants about the MRL are needed for many compounds used in aquaculture.

Several multi-residue gas–liquid chromatographic methods (GLC) have also been reported for aquaculture drugs [109,110] but GLC is not as frequently utilized for analysis as LC (Table 1). Nevertheless, absolute confirmation of the presence of a compound required for regulatory action may often be secured using gas–liquid chromatography–mass spectrometric detection and confirmation (GLC–MS). LC–MS is becoming more available and will someday exceed GLC for the purpose of confirming many of the polar drugs used in aquaculture. However, even these technologies require the use of tissue isolation methods that are simple, fast, and efficient.

3. PROBLEMS DUE TO REQUIREMENTS FOR SAMPLE PREPARATION, ISOLATION, AND CLEANUP

Sample preparation, isolation, and cleanup are becoming the major rate-limiting factors in sample analysis as improvements in analytical methods proceed [111]. This fact is especially important in light of efforts to introduce rapid screening tests such as immunoassays. Several approaches have been used over the years for the preparation, isolation, and cleanup of drug and environmental residues from aquatic matrices. The classical approach to isolation of the drugs is homogenization followed by liquid–liquid partitioning. Liquid–liquid partitioning may also be used with biological fluids. Homogenization and liquid–liquid partitioning methods provide adequate separation of the drug from matrix but are often expensive in terms of time, labor, material use, and organic solvent disposal. Such approaches also tend to be highly nonspecific in their isolation of the target drug(s).

Furthermore, these methods may be generating more pollution than they are satisfactorily resolving. Table 1 gives an indication of the problem by showing a list of the required volumes of solvent for extraction of various drugs from aquatic tissues. Many of these solvents are of greater environmental concern than the compounds they are used to isolate. During extraction and isolation procedures much of this solvent volume is evaporated into the atmosphere, and solvents are often not disposed of properly. This leads to further contamination of the atmosphere, aquifers, aquatic habits, and resources.

As previously mentioned, a further problem with excessive use of solvents is that they make these methods very expensive to perform. The purchase price and subsequent disposal costs of organic solvents and wastes can be limiting factors in analyses performed by government agencies operating on a restricted budget. Therefore, costs for materials can limit the number of samples which can be consistently analyzed to provide a statistically sound evaluation of the magnitude of contamination.

Employee costs can also be a limiting factor in residue analysis. Official methods generally require extensive training of laboratory technicians in order to guarantee consistent, reliable results and most such methods are not amenable to automation.

All of the above problems indicate that the use of classical methods for screening purposes should be severely curtailed and phased out as new, more appropriate methods are developed and validated for sample screening.

4. TISSUE RESIDUE PROBLEMS CAUSED BY DRUGS

Diseases are the single most important cause of economic loss in intensive aquaculture [2] and necessitate the use of antibacterial and other therapeutic compounds to maintain the health and production of cultured species. Although there is a degree of variability there are numerous therapeutants which are consistently used worldwide in aquaculture [112–114]. These agents belong to a wide range of chemical and therapeutic classes such as antibacterials (*e.g.*, sulfonamides and potentiated sulfonamides, aminoglycocides, β-lactams, tetracyclines, quinolones, macrolides, etc.), parasiticides (*e.g.*, mebendazole and dichlorvos), disinfectants, piscicides, herbicides, algacides, anesthetics, water treatments, and dyes. This large range of chemical classes presents a problem for residue monitoring, and requires the use of screening tests to adequately detect the presence of illegal residues.

A residue can be defined as "any compound present in edible tissues of the target animal that results from the use of the sponsored compound, including the sponsored compound, its metabolites and any other substances formed in or on food because of the sponsored compounds use" [100]. Metabolites are considered to be as toxic as the parent compound unless shown otherwise [100]. Clearly, extraction and analytical methods used in monitoring programs must be capable of extracting and analyzing both parent drug and metabolites present in the tissues of interest at or less than MRLs. However, MRLs are not always static. Toxicological data are always being updated and the JECFA periodically issues recommendations for MRLs based on such available toxicology information for selected veterinary drugs including those used in aquaculture. Many countries have established MRLs based on JECFA recommendations for a number of these compounds. A listing of WHO publications containing JECFA recommended MRLs is available [3].

The use of therapeutants in aquaculture not only may result in unacceptable residues in edible tissues but also in the environment. Drugs used in aquaculture may be directly introduced into the environment, as with ectoparasiticides, or indirectly introduced in medicated feeds (via non-consumption of the feed, poor bioavailability, and limited biotransformation). The environmental degradation, accumulation, and persistence of these agents is affected by water temperature, sediment microenvironment, and factors affecting dispersion [115]. It has been estimated that 70 to 80% of orally administered oxytetracycline remains in the environment [116]. Furthermore, there are large variations in the persistence of antibiotics in sediments from fish farms. Furazolidone exhibits a very short half-life (18 h) [117] and oxytetracycline a half-life of 32 to 64 days depending on sediment conditions [116]. The environmental fate [115–122] and effects [117,122–127] of several compounds commonly used in aquaculture has been the subject of recent studies. However, the environmental metabolism, fate, and effects of most drugs introduced into the aquatic environment is poorly understood and relatively few methods are available for the multitude of compounds, environmental matrices, and environmental conditions that are of import in assessing the environmental impact of these compounds. Environmental impact assessment is now required for aquaculture drug approval in the United States [128]. Additionally, periodic monitoring of fish farm effluents for drug residues may be required. Therefore, methods of analysis of therapeutic agents in environmental samples are now part of the drug approval process and should be part of our continuing environmental concern.

Development of new methods of analysis of therapeutants was identified at a recent joint FDA-US Department of Agriculture (USDA) sponsored Interregional Research Project Number 4 (IR-4) meeting as a priority need in aquaculture [129]. In general, new methods for drug residue analysis in aquatic species must be suitable for screening, quantitating, and confirming tissue residues of drugs used domestically and drugs present in imported aquacultural products. The methods must also be suitable for analyzing environmental samples for drug and contaminant residues.

5. TISSUE RESIDUE PROBLEMS CAUSED BY PESTICIDES

Pesticides have been and continue to be applied extensively in the United States for agricultural purposes on animals, farmland, and forests and for mosquito control in urban areas. Many of these chemicals ultimately find their way into aquifers, rivers, lakes, and oceans mostly through transfer in the water itself, through adsorption to sediments and other organic layers in the water, or through air. In addition, because many aquaculture ponds are built on land that was formerly used for agriculture, the sediments and organic materials of these ponds could also contain high levels of pesticides.

Some of the pesticides that may be found in the environment are the chlorinated hydrocarbon, organophosphorus, carbamate, and pyrethrin/pyrethroid pesticides; chlorophenoxy acid, triazine, trichlorobenzoic acid, chlorophenol, and glyphosate herbicides; viticulture fungicides; and grain fumigants. The EPA has established tolerance levels for over 300 pesticides in various foods or food groups [130], and the FDA has determined action levels for many pesticides and their metabolites and degradation products in seafood [131]. Some of these pesticides are included in current seafood monitoring

programs conducted by groups such as the FDA and US National Marine Fisheries Service and also included in environmental monitoring programs conducted by groups such as the NOAA.

Most of the pesticides are readily degraded in the environment and therefore, are normally not a problem as tissue residues. However, some of the pesticides, especially the chlorinated hydrocarbons, such as DDT, are persistent in the environment and can be commonly found as residues in mammalian and aquatic species. Most of the chlorinated pesticides have been banned from wide-ranging use in this country for over twenty years, but they continue to be of concern to regulatory agencies because of their occurrence in food and their unknown health effects. Many are classified as suspected carcinogens [132].

In general, the persistence of the chlorinated pesticides and potential to undergo biomagnification, their continued use in some countries, and the concerns for their known and unknown toxicity make them a very important class for regulatory agency attention. State, federal, and international monitoring programs will need to continue to include this class of compounds in their testing for decades to come. Because efficient, cost-effective, universal methods for the extraction, detection, quantitation, and confirmation of these residues in aquatic matrices do not exist, a need for a better approach to analysis has recently been acknowledged. The research and development plans of regulatory agencies, such as the FDA [78,133] and the USDA Food Safety Inspection Service [134], currently include commitments to increase and improve capabilities for testing for pesticide residues.

6. FUTURE METHODS OF RESIDUE ANALYSIS

There have been recent advances in the field that offer several promising techniques as possible solutions to the problems caused by outmoded and complex analytical methods. Three techniques, supercritical fluid extraction (SFE), solid-phase extraction (SPE), and matrix solid-phase dispersion (MSPD), are receiving attention because they have the potential to greatly reduce analysis costs and reduce analyst-generated waste and pollution.

6.1. Solid-phase extraction

In the SPE process, a compound is isolated from a liquid sample based on its relative solubility in the liquid mobile phase compared to its solubility in a solid support-bound liquid stationary phase of differing polarity or to a solid support stationary phase of differing polarity. Isolation is accomplished by passing the analyte dissolved in solvent (organic or aqueous) through a column containing the stationary phase with subsequent elution using an appropriate solvent. Several solid-phase extraction methods have been developed to facilitate the extraction and cleanup of biological liquid and tissue samples.

For liquid matrices, acceptable residue recovery may be obtained using protein precipitation and direct injection of plasma without cleanup with SPE [11], but the many impurities present can affect the chromatogram and accumulate on the analytic column, thus resulting in increasing back pressure. SPE cleanup helps avoid these problems and works well with biological fluids such as plasma, urine, and cerebral spinal fluid. In addition, SPE extraction and analysis can be automated and done on-line [20–22] and/or with on-line dialysis and column switching.

Before SPE can be used with solid tissue (*e.g.*, muscle and liver), a separate homogenization step and often multiple filtration, sonication, centrifugation, and liquid–liquid cleanup steps are required. While SPE may improve cleanup of these solid tissue samples, the additional labor and materials costs make SPE less suitable, in some cases.

Solid-phase extraction methods published for fish tissues are often combinations of SPE with other methods such as homogenization, liquid–liquid partition, filtration, sonication, and centrifugation (Table 1). Because choice of SPE column depends on the matrix and on the particular compound of interest, a wide range of solid-phase columns of differing polarities have been used for drug extraction in fish and include C_2, C_8, C_{18}, NH_2, amberlite resins, and PLRP(polystyrene–divinylbenzene) polymers (Table 1).

6.2. Supercritical fluid extraction

With the SFE process, supercritical fluids [usu-

ally supercritical carbon dioxide (SC-CO_2)] are used in place of organic solvents to extract residues [135]. Carbon dioxide becomes a supercritical fluid if handled above its critical temperature and pressure. Because various chemicals and associated tissue lipids are soluble in the SC-CO_2, they are extracted and then collected once the pressurized CO_2 is brought back to atmospheric pressure. No large volumes of organic solvents are needed. One drawback to the procedure is that because the extracts contain contaminating lipids, a cleanup step is usually needed before samples can be injected onto instruments such as gas chromatography apparatuses. Cleanup is usually performed with gel permeation chromatography or adsorption chromatography with Florisil. In-line cleanup could be conducted by using disposable or reusable SPE cartridges or newer disc SPE technologies and changing the pressures of the supercritical fluid. Coupling this system directly to an LC–MS type interface or a GLC–MS interface could provide a complete analytical process for the desired analysis.

More work will be necessary to further develop this process. Its application to fish tissues [58] is quite limited. However, the process has the potential to provide a near solventless, in-line, automated process for the rapid analysis of the lipophilic chemical species from edible aquatic resources.

6.3. *Matrix solid-phase dispersion*

Of the three techniques being considered, matrix solid-phase dispersion, in particular, has the strongest potential to meet the demands of future residue monitoring of aquatic resources for drugs and pollutants.

In general terms, the process involves blending a tissue sample (0.1–1.0 g) with lipophilic polymer-derivatized silica particles [*e.g.*, octadecylsilyl (ODS)-derivatized silica (C_{18})], which simultaneously disrupts and disperses the sample. This mixture of C_{18} and tissue becomes part of a potentially multiphasic column that possesses unique chromatographic character. Elution of the MSPD column with a solvent or solvent sequence can provide a high-resolution fractionation of target analytes that can be further purified by simultaneous use of co-columns of Florisil, silica, or alumina. The final eluate can, in most cases, be directly analyzed or further concentrated or manipulated to meet the demands of the individual analysis. The extracts obtained from these methods are most often detected by LC (in the case with drugs) or GLC with electron capture detection or mass spectrometry (in the case with pesticides). However, they can also be used in immuno- [103] or receptor assays.

Additionally, the MSPD process is generic and can be modified for a particular application by (1) a change in the eluting solvent or solvent sequence, (2) use of a different polarity polymer or solid support, and (3) blending of the C_{18}/tissue in the presence of modifiers such as chelators, acids, bases, etc.

MSPD could also be used in conjunction with SFE. The water in biological matrices often interferes with the SFE process [135] and analysts have used samples blended with diatomaceous earth to remove water from the sample. However, blending samples first with polymer-coated silicas, as is done in the MSPD process, would remove water and provide an initial stage of fractionation at the point of elution of the analytes with supercritical fluid and modifiers.

In general, the three main advantages of MSPD are (1) it allows for rapid turnover of samples and hence, access to timely data on residue levels present in samples, (2) because of its required small sample size, it considerably decreases solvent use compared to the classical methods, which in turn decreases environmental contamination and increases worker safety, and (3) it is suitable to robotics automation. Therefore, MSPD has the potential to meet the future demands for conducting drug and pesticide analysis for large numbers and varieties of samples.

6.4. *MSPD applied to aquatic resources*

As seen in both Tables 1 and 2, MSPD has been used to provide for single or multi-residue analysis of various drugs and environmental contaminants in several aquatic matrices. Drugs isolated from aquatic animal tissues by this method include oxytetracycline [12] and sulfadimethoxine [15,103] from fortified channel catfish muscle and oxolinic acid as an incurred residue from channel catfish muscle and bile [29]. Reimer and Suarez [16] reported a multi-residue method for MSPD isolation and LC analysis of five sulfonamides in fortified salmon

muscle. Jarboe [30] has demonstrated its applicability to the isolation of incurred residues of nalidixic acid from channel catfish muscle and liver. Walker and Barker [103] evaluated the performance of several enzyme immunoassays for the detection of sulfadimethoxine residues using MSPD extracts of fortified channel catfish muscle as well. Other compounds used in aquaculture or related compounds have been extracted from various non-aquatic matrices using MSPD methods [136] and these methods could potentially be applied to aquatic matrices.

Pesticides extracted and isolated by this method include 14 chlorinated hydrocarbon pesticides from fortified whole oyster homogenate and crayfish hepatopancreas [43,44] and 9 chlorinated pesticides from fortified catfish muscle [45]. These methods are a significant advance in the ability to screen more samples due to their simplicity and efficiency.

7. DISCUSSION

Methods development for residue determination should focus on rapid screening tests, multi-residue capabilities, metabolite detection, and improved sensitivity [137]. Further, the use of determinative methods generally requires a method of isolating the compound(s) of interest from edible or marker tissues that is rapid, inexpensive, and does not generate large volumes of solvents for disposal. Classical isolation methods using homogenization and/or liquid–liquid partitioning of biological tissues and fluids may be sufficient for some applications but are poor for screening purposes because they are often lengthy, involving multiple steps and use large volumes of solvents (Table 1). Solvent disposal is becoming increasingly expensive and environmentally unsound. Therefore, methods using low solvent volumes are desirable. A main purpose of this review was to present a case for phasing out existing official methods in favor of newer technologies that require less sample, less solvent, less employee time, and less cost per sample. Newer techniques such as supercritical fluid extraction [135], solid-phase extraction (Tables 1 and 2), and MSPD (Tables 1 and 2) [136,138] offer alternative isolation strategies. When compared to the classical methods, these new methods greatly reduce labor and solvents costs and improve throughput. There are a few drawbacks to the new methods and more work is needed to further develop SPE, SFE, and MSPD for use with the many different types of matrices that may contain residues of chemical contaminants. However, of the three new methods, MSPD shows tremendous potential.

MSPD methods have been published for the isolation of a wide range of compounds in a variety of matrices indicating this approach may provide a generic technique for single and multi-residue extraction of drugs, environmental pollutants, and their metabolites. In particular, MSPD has already been used to provide a two-step process for the single- or multi-residue analysis of various drugs [12,15,16,29, 30,103] and chlorinated pesticides [43–45] in several aquatic matrices. This process, when compared to classical methods, has been estimated to reduce solvent use by approximately 98% and analysis time by 97%. Furthermore, once the MSPD column is prepared, the process of solvent elution, collection, and analysis can be automated by the use of robotics. Cost of analysis is decreased because less solvent is needed and fewer laboratory technicians need to undergo training. Safety and environmental protection are increased because less solvent is needed. Finally, data is generated more quickly because of the ease of the process and its potential to be automated. These features of MSPD make it a general and perhaps significantly useful method in designing future residue analysis screening programs for aquatic as well as other food animal resources.

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