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Off-line supercritical fluid extraction/gas chromatography-mass spectrometry analysis of pesticides in fish

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Introduction

Pesticide residues in fish tissue are caused by environmental contamination and present a potential hazard to human health. Many states and the EPA issue consumption advisories based on the observed levels of found pesticides, so it is important to monitor them accurately. Monitoring these pesticides levels in fish require analytical methods with good recoveries and low limits of quantification. Other sample preparation techniques such as accelerated solvent extraction (ASE) may cause complicated matrix interferences and use more solvent than SFE. In this study, we evaluated supercritical fluid extraction as a sample preparation technique for the extraction of pesticides from fish tissue, coupled with gas chromatography tandem mass spectrometry (GCMS/MS) to quantify 18 prevalent pesticides. For this poster, walleye fish with a low lipid content (%L=0.9) was studied.

Sample and standards

Samples: Fish tissue is prepared by grinding frozen fillets, and freeze drying a portion of the homogenized tissue.
Standards and Reagents: Restek (32291) Organochlorine Pesticide Mix AB#1 (X1), AccuStandard S-8890 (A), AccuStandard CLP-032-R (SS), Fisher Alumina, basic (A941-500)

Sample preparation

0.5 grams of the dried fish tissue is placed into a 5 mL extraction vessel and spiked with 20 uL each of the X1A and SS spiking solutions (2.5 ug/mL) and allowed to dry. One gram of activated alumina is added on top of the spiked fish tissue. The alumina acts as a column to allow extraction and sample clean up in one step. The vessel is sealed and placed onto the SFE system. The extract was collected and brought to a 1.0 mL volume for injection into the GCMS.

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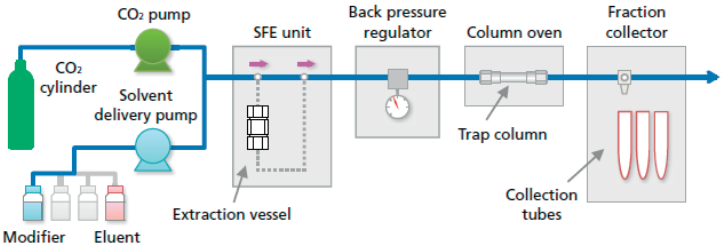
SFE extraction procedure

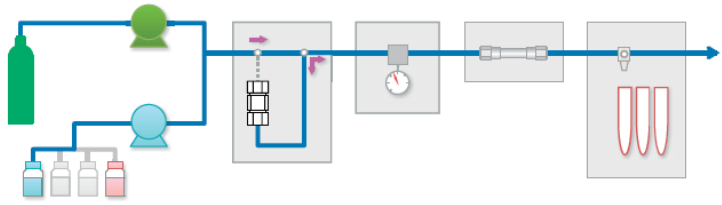
(1) Extraction vessel delivery and temperature control

A specified extraction vessel is transferred to the SFE unit and heated to the set temperature.



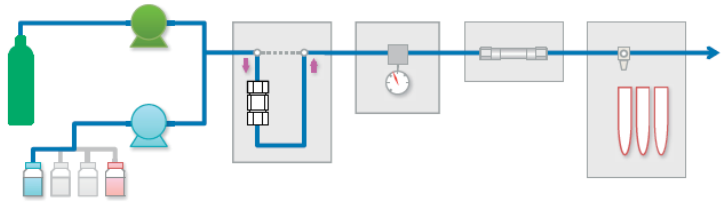
(2) Static extraction

When the temperature of the extraction vessel has reached the set temperature, the supercritical fluid is introduced and static extraction (i.e., in the absence of fluid flow) is allowed.



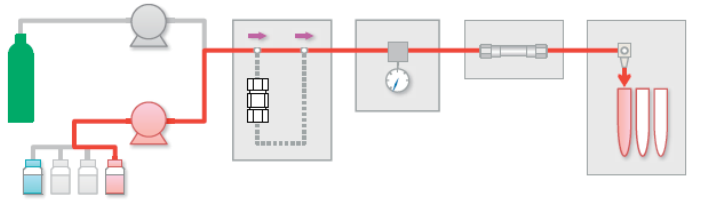
(3) Dynamic extraction

The extraction is dynamically performed by passing the supercritical fluid through the extraction vessel. The extraction material is taken from the extraction vessel and collected at atmospheric pressure after evaporation of CO_2 in the trap column downstream the back pressure regulator.



(4) Elution from the trap column and recovery of the extraction material

The delivery pump is used to deliver the eluent through the trap column, thereby eluting the extraction material, which is then recovered using a fraction collector.



Analytical Conditions

Extraction Conditions

Vessel Temp	:	50 °C
System Pressure	:	30.0 MPa
Flow rate	:	1.0 mL/min 100% CO2
Static Extraction time	:	25 min
Dynamic Extraction Time	:	30 min
Trap Column	:	Shimadzu C18, 4.6 x 50 mm, 5um
Column Temp	:	trap 20 °C, elute 50 °C
Column Rinse	:	Hexane, 2 mL/min

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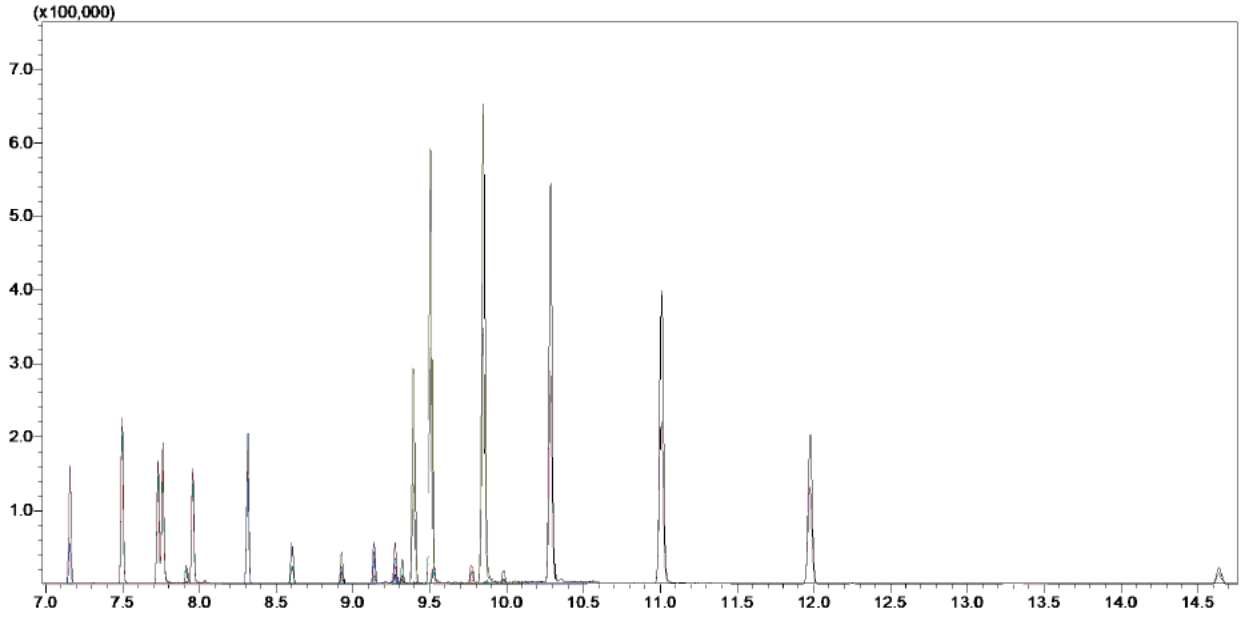
Analytical Conditions

GC-MS	: GCMS-TQ8040 (Shimadzu)
Sampler	: AOC-20i/s (Shimadzu)
Column	: SH-Rxi-5MS (30m x 0.25mmI.D., df=0.25µm, Shimadzu)
IF Temp.	: 290 °C IS Temp.: 230 °C Event Time: 0.3sec
Inj. Temp.	: 275 °C
Flow Control	: Linear Velocity, 43.5cm/sec
Inj. Mode	: Splitless (High Press. Inj., 250kPa, 1.5min)
Oven Temp.	: 50 °C (0.5min), 28 °C /min to 265 °C, 3 °C /min to 285 °C, 25 °C /min to 330 °C (1min)
Inj. Volume	: 1 µL

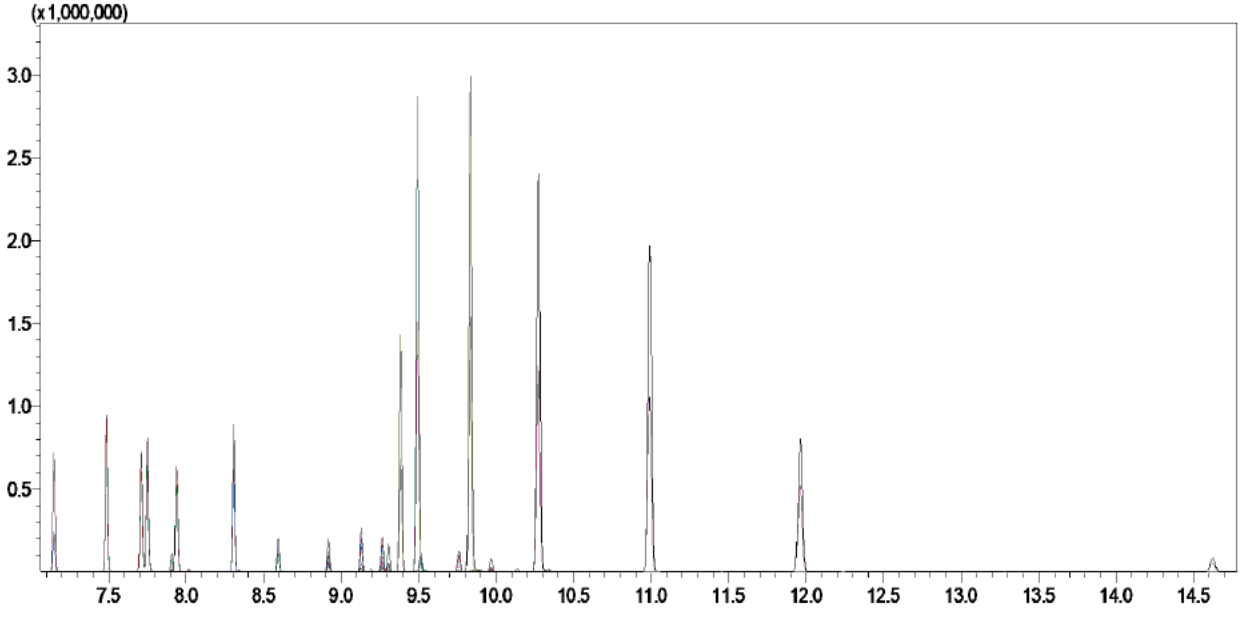
Sample Table

Compound Name	R.T.	Quant	Qual	Linearity 10-100 ppb R^2
2,4,5,6-tetrachloro-m-Xylene (surrogate)	7.137	244.0>209.1	171.0>136.2	0.998
alpha-BHC	7.465	180.9>144.9	218.9>182.9	0.999
gamma-BHC	7.667	180.9>144.9	218.9>182.9	0.998
Chlordene	7.909	302.9>231.9	304.9>231.9	0.984
Heptachlor	8.291	271.8>236.9	273.8>238.9	0.998
Aldrin	8.575	262.9>191.0	262.9>193.0	0.993
Heptachlor epoxide	8.894	352.8>262.9	354.8>264.9	0.996
trans-Chlordane	9.103	372.8>263.9	374.8>265.9	0.997
cis-Chlordane	9.242	372.8>263.9	374.8>265.9	0.999
trans-Nonachlor	9.301	406.8>299.9	406.8>334.9	0.998
4,4'-DDE	9.374	246.0>176.0	317.9>248.0	0.998
2,4'-DDD	9.483	235.0>165.0	237.0>165.0	0.999
Dieldrin	9.492	276.9>241.0	262.9>193.0	0.998
Endrin	9.733	262.9>191.0	262.9>193.0	0.999
4,4'-DDD	9.805	235.0>165.0	237.0>165.0	0.999
cis-Nonachlor	9.96	406.8>299.9	406.8>334.9	0.999
4,4'-DDT	10.248	235.0>165.0	237.0>165.0	0.999
Methoxychlor	10.967	227.1>169.1	227.1>212.1	0.999
Mirex	11.963	271.8>236.8	273.8>238.8	0.999
Decachlorobiphenyl (surrogate)	14.591	497.7>427.8	499.7>429.8	0.999

GCMS Chromatograms



10 ppb neat standard



50 ppb spiked walleye extract

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Recovery

A walleye sample known to contain very low levels of pesticides was used for this study. The walleye sample was extracted by SFE and spiked post extraction. Additional walleye sample were then spiked and then extracted by SFE to determine recoveries. Low recoveries were initially found and it was determined to be caused by a high trap column temperature. The trap column temperature was lowered to 20°C during the extraction process, and increased to 50°C for the elution with hexane.

Recovery Results

Pesticide	Walleye "post-spiked" extract (ppb found)	Walleye "pre-spiked" extract (ppb found)	Recovery %
2,4,5,6-tetrachloro-m-Xylene	48.39	45.27	93.6
alpha-BHC	49.83	26.47	53.1
gamma-BHC	51.46	44.10	85.7
Chlordene	52.20	52.23	100.1
Heptachlor	47.63	23.45	49.2
Aldrin	53.21	43.97	82.6
Heptachlor epoxide	54.12	48.44	89.5
trans-Chlordane	49.83	44.73	89.8
cis-Chlordane	52.50	48.13	91.7
trans-Nonachlor	52.47	46.29	88.2
4,4'-DDE	51.25	53.58	104.5
o,p'-DDD	52.44	47.87	91.3
Dieldrin	53.53	47.41	88.6
Endrin	53.36	44.46	83.3
4,4'-DDD	61.15	64.46	105.4
cis-Nonachlor	53.65	47.31	88.2
4,4'-DDT	44.41	17.90	40.3
Methoxychlor	46.10	20.01	43.4
Mirex	50.74	40.50	79.8
Decachlorobiphenyl	41.74	42.64	102.2

Improving Recovery

Good recovery for most pesticides was found, however several compounds still showed low recoveries. Past research by PA DEP BOL has found that adding some oil to the fish tissue may help improve recovery of pesticides in fish that have a low lipid content. A small amount of corn oil was added to the fish tissue which was then spiked before supercritical fluid extraction.

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Pesticide	Walleye "post-spiked" extract (ppb found)	Walleye "pre-spiked" extract with corn oil (ppb found)	Recovery %
2,4,5,6-tetrachloro-m-Xylene	48.39	45.28	93.6
alpha-BHC	49.83	40.07	80.4
gamma-BHC	51.46	40.46	78.6
Chlordene	52.20	44.35	85.0
Heptachlor	47.63	39.07	82.0
Aldrin	53.21	41.77	78.5
Heptachlor epoxide	54.12	44.14	81.6
trans-Chlordane	49.83	43.21	86.7
cis-Chlordane	52.50	44.99	85.7
trans-Nonachlor	52.47	43.71	83.3
4,4'-DDE	51.25	45.98	89.7
o,p'-DDD	52.44	42.71	81.5
Dieldrin	53.53	44.09	82.4
Endrin	53.36	44.36	83.1
4,4'-DDD	61.15	41.64	68.1
cis-Nonachlor	53.65	41.34	77.0
4,4'-DDT	44.41	40.88	92.1
Methoxychlor	46.10	45.14	97.9
Mirex	50.74	38.79	76.4
Decachlorobiphenyl	41.74	41.09	98.5

Summary

1. Good recovery for all pesticides was achieved by adjusting certain SFE parameters.
2. Adding oil to low lipid fish tissue may improve the recovery of certain pesticides.
3. Trap column temperature can affect recovery results.
4. A minimum amount of matrix interference was observed with this technique, reducing or eliminating the need for additional clean up steps before GC analysis.
5. Automated extraction of 48 samples can now be performed.

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