Comparison of Extraction Techniques for Sediment and Biota Matrices

R&D Technical Report E1- 027/TR

James Deuchar and Gill Hardy

Dissemination Status
Internal: Released to Regions
External: Released to Public Domain

Statement of Use
This document provides information on the availability and use of extraction techniques for solid matrices.

CONTENTS

KEYWORDS

Extraction, Sediment, Biota, Matrices, Gas Chromatography, Solvent, Comparison, Mass Spectrometry

1 Executive Summary

The Environment Agency is required to monitor against the "standstill" requirement of European Committee (EC) Directives concerning the contamination of solid material (soil, sediment, plant, and animal tissue) by pesticides and other organic compounds.

The traditional Soxhlet extraction of these compounds from the solid is time consuming and uses significant solvent quantities with high energy and water resource. Newer techniques such as Microwave digestion (MASE), Accelerated Solvent Extraction (ASE) and Supercritical Fluid Extraction (SFE) are more efficient, using considerably less solvent, less energy and do not require water.
However the improved efficiency of these techniques could give higher recoveries of organic compounds absorbed in solid materials and produce a step-change in the environmental data.

By using an analytical protocol for extracting certified reference material (sediment and animal tissue) by each technique, analytical results are obtained which can be statistically evaluated. This enables comparison of the extraction techniques with respect to the uncertainty (experimental error) and the difference in numerical values of the final results.

The project data shows that the different extraction techniques do not produce significantly different data when compared to Soxhlet extraction.

2 Project Description

2.1 Origin

The project is concerned with the duty of the Environment Agency to monitor contamination of solid materials by organic pollutants such as pesticides using analytical instrumentation which can provide comparable long term information, enabling critical assessment of the compliance with EC Directives. Also there is a requirement to undertake this procedure with due regard to cost effectiveness and reduce any adverse environmental effects arising from this analysis.

An appreciation of the analytical methodology, in particular the extraction stage of the analysis, is required to enable an understanding of the results obtained from the monitoring exercise.

2.2 Background

Traditionally the extraction of organic compounds from solid such as sediments (river beds) and fish tissues (biota) preparation has involved the use of one technique on which all historical information is based. This technique known as 'Soxhlet' involves the continual heating and subsequent cooling (condensing) of a large volume of solvent 200-250 millilitres (ml) in a glass container (refluxing). A weighed portion of the solid sample is held in a porous container (paper or glass thimble) suspended above the boiling solvent and directly below the stream of condensing solvent. The condensing solvent therefore continually returns any extracted material from the solid to the solvent contained in the flask. This process is continued for a period of 24-48 hours, a time necessary to ensure consistent extraction of organic compounds contained in the solid material. As there is no absolute means of determining the true total quantity of organic material held within the solid, the final result will depend on the efficiency of the extraction. The latter depends on the nature of the solvent, its boiling point and the pressure under which the process is performed. Higher pressures and temperatures will force the solvent into the pores (holes) of the solid and enable more efficient extraction of organic material held within. Thus an increased amount of organic material may be extracted by these techniques in comparison with Soxhlet.

This is the basis of modern extraction equipment. See Section 2.2

However due to its long term use most of the monitoring information is based on the Soxhlet extraction technique. Thus it is still the reference method with which all other extraction methods are generally compared to judge their equivalence.

2.3 Improvements

This common sample processing procedure was labour intensive, time consuming and had high solvent and water usage. Simply automating this process was therefore not considered totally satisfactory. The increasing understanding of the importance of the sample matrix and its interaction with organic semivolatile compounds led to new developments in the form of the following techniques: -
(a) Sonication
(b) Accelerated Soxhlet Extraction (assumed equivalent to Soxhlet)
(c) Microwave–Assisted Solvent Extraction
(d) Accelerated Solvent Extraction
(e) Supercritical Fluid Extraction

An overview of these techniques as to their operating principles, solvent usage, robustness and environmental considerations is given in Section 3.

3 Extraction Techniques

3.1 Sonication

Both ultrasonic probe (Sporstoel *et al.* 1983, Marsden 1995) and ultrasonic bath (Fuoco *et al.* 1996) have been used to accomplish this technique.

Extraction efficiency has been related to pulsing which increases the solvent/sample contact. Similar recoveries to Soxhlet were only obtained if the sonicator was correctly tuned (Marsden 1995). Multiple extractions are necessary with low solvent volumes (20ml). Differences between the two techniques were encountered when analysing dioxins and dibenzofurans (chlorinated compounds) and when analysing Polynuclear Aromatic Hydrocarbons (PAH's), sonication being more efficient for the latter. Overall applicability to a range of compounds is therefore questionable and the absence of any automation limits this technique to laboratories having only a small number of samples. Reproducibility of technique between analysts is likely to be poor due to the high degree of manual operations.

3.2 Accelerated Soxhlet Extraction

This technique recognises the deficiency in extraction kinetics of the basic Soxhlet apparatus operating at low temperatures and extracts the sample in a thimble immersed in the boiling solvent. Subsequently the thimble is raised above the boiling solvent to enable rinsing with condensed solvent. Reduction of the solvent level by distillation then provides a means of collection for re-use or disposal.

Solvent re-use however is rarely practical for trace analysis work. The extraction is totally automated and reduces the extraction time by a factor of 4 to 10 e.g. 6 hours compared to the normal 24. This enables a reduction in water consumption (used for the condenser to cool the hot solvent) by the same factor.

Solvent usage is still high 150 – 250mls but no vapour escapes to the atmosphere.

In practice the equipment is not without problems such that a watching brief is required by the analyst especially to supplement any reduction in the solvent level and ensure no sample goes to dryness at the solvent reduction stage.

This technique is currently in operation at two of the National Laboratory Services (NLS) sites.

This represents an 'evolutionary' rather than a 'revolutionary' change and as such gives results consistent with the Soxhlet technique described in 2.1

It is therefore considered appropriate to use this technique as the 'reference' for comparison with the newer techniques.

3.3 Microwave–Assisted Solvent Extraction (MASE)

This technique is based on the fact that the absorption of microwave energy is approximately proportional to the relative permittivity of the sample or solvent (Barnabas *et al.* 1995).

The sample with a suitable solvent (volume usually 10 to 20mls) is placed in a non microwave-absorbing vessel and sealed. A computer programmable microwave oven provides accurate control of extraction time while monitoring pressure build-up and possible solvent vapour escape. Under typical operating conditions, the extraction temperature may be several times higher than the atmospheric boiling point with pressures in the range 10 to 20 atmospheres. This reduces the extraction time to minutes (15 to 20), requiring considerably less solvent compared to the Soxhlet technique (a factor of 10). Up to 15 samples can be extracted at once (c.f. automated Soxhlet 6 samples per unit).

A considerable reduction in electrical power is achievable and water consumption is negligible. Both instruments are similarly priced.

A major drawback is that the extract and sample have to be filtered manually and centrifugation may be necessary in some cases (e.g. fine sediment).

Although this technique has been used for a variety of analytes (e.g. organotin, petroleum hydrocarbons, and phthalate esters), surprisingly there are few references to those compounds being considered in this report.

In the extraction of chlorinated pesticides from soil (Fish *et al.* 1996), recoveries of eleven compound were studied producing recoveries which were equal or exceeded the current Environmental Protection Agency (EPA) method. In extracting PAH's by this technique (Chee *et al.* 1996), an optimum set of parameters was found by trial experiments which gave improved extraction efficiencies compared to the Soxhlet technique.

An alternative technique known as Focused Microwave-assisted extraction (FMW) uses open containers with water-cooled condensers. This purportedly eliminates compound degradation, as the temperature of extraction is limited to the boiling point of the solvent (Letellier *et al.* 1999). Solvent usage is 30mls per sample with a 10 minute extraction time. Results are comparable with those obtained by the standard Soxhlet technique. Addition of water up to 30% was shown to improve recovery of PAH's when analysing the SRM1941a reference sediment.

3.4 Accelerated Solvent Extraction (ASE)

This technique uses the same principles as MASE but uses conventional oven heating and high pressure under total computer control. The sample is placed in a stainless-steel container and pressurised up to 200 atmospheres by pumping in solvent. The vessel is heated (range 50 to 200°C) and the pressure relieved periodically by opening a vent valve. After static extraction for 5-10 minutes, the remaining extract is flushed from the sample container by fresh solvent followed by a nitrogen gas purge. Solvent usage is low (15ml for a 10gram sample) and the extraction time approximately 20 minutes. Automation enables unattended sequential extraction of sample batches.

The instrument although fairly recent has found wide support in the environmental field and the EPA method 3545 has been validated for all of the compounds studied in this project.

In particular, extraction of PAH's from HS-6 reference material gave recoveries (Heemken *et al.* 1997) comparable to Soxhlet, only if the sample was dried. This indicates that the solvent has to diffuse into the sample matrix for reliable extraction.

3.5 Supercritical Fluid Extraction SFE

Unlike the previous techniques that depend on liquid solvents, SFE uses a gas in its supercritical fluid state to perform sample extraction. Supercritical fluids have low viscosity, no surface tension and favourable diffusion properties compared to liquids, all of which combine to enhance extraction of compounds from porous solids (Poole and Poole 1996). The properties of these fluids can be altered by manipulating temperature, pressure and by addition of small volumes of solvent modifiers (e.g. methanol). Carbon dioxide (CO_2) is mainly used due to its low critical constants, low toxicity and its environmentally friendly aspect compared to the more toxic solvents.

Samples are weighed into small stainless steel containers and any void volume reduced by addition of inert material (e.g. celite, sodium sulphate). The extraction process is totally automatic with set temperatures, pressures, and modifier addition computer controlled.

The carbon dioxide is pumped into the vessel and the supercritical fluid state is obtained by application of high pressure and an appropriate extraction temperature. A short static extraction (e.g.15 minutes) is performed, followed by a dynamic one where the compounds are transferred to the adsorbent trap. As carbon dioxide is a gas at normal temperatures, there is no final solvent evaporation stage as required by the other techniques, the extracted compound being trapped on an appropriate adsorbent (e.g. florisil) which can be eluted with small volumes of solvent (1-2 mls). The adsorbent also acts, as a clean-up step again reducing the number of sample preparation stages required before chromatographic analysis. Time per sample extraction is approximately one hour but produces an extract not requiring solvent evaporation.

Similar to ASE, recoveries are affected by high water content, necessitating drying of the samples usually by mixing with sodium sulphate.

Extraction efficiences of PAH's from marine sediments (Heemken *et al.* 1997) by SFE have been shown comparable to ASE, while in-house experience has shown good precision and recovery for the remaining compounds studied in this project. It was therefore considered advantageous to include this technique for evaluation overriding its exclusion by the original project proposal.

As the extraction efficiency is dependent on a number of factors (diffusion of analyte out of pores, displacement from one adsorption site to another, replacement of analyte molecules on adsorbent sites by superfluid critical fluid) this technique is not considered as robust as the solvent based ones.

Instrumentation is fully automated but is more complicated than other techniques, requiring a highly experienced and technically competent analyst.

3.6 Environmental Considerations

All the techniques (except sonication) depend on the use of higher temperatures and/or pressures than the Soxhlet to shorten the extraction time, which consequently gives a reduction in electrical power consumption.

Another benefit derives from water-cooling being unnecessary and although solvent is still required, the necessary volume is considerably reduced.

The techniques are more easily automated providing improvements in labour costs and safety.

A summary of relevant information on these techniques is given in Table 1.1

Table 1.1 Efficiency and Environmental Considerations

	Soxtherm	Microwave	ASE	SFE	Sonication
Sample Handling					
Minimum Solvent (ml.)	150	15	15	5	100 to 250
Time of Extraction (min)	360	15	15	45	30 to 60
Maximum No. of Samples Per Run	6	15	24	8	6
No. of samples possible per day*	6	45 (three batches)	72 (three batches)	8 (overnight running)	12 (two batches)
Services					
Water	2 litres/min	No	No	No	No
Carbon Dioxide	N/A	N/A	N/A	1 litre/min	N/A
Compressed Air	Yes	No	Yes	No	No
Nitrogen	No	No	Yes	No	No
Helium	No	No	No	Yes	No
Electricity (VA)	1000	230	500	748	300
Features					
Extraction Selectivity	Average	Poor	Poor	Good	Poor
Automation**	Semi	Semi	Full	Full	Manual
Safety Features	Yes	Yes	Yes	Yes	No
Purchase cost	£10,000	£15,000	£40,000	£40,000	Under £10,000

* Estimated by taking account of total extraction time.
** Based on analyst's manual time during extraction

However before using more environmentally friendly instrumentation there is a need for comparison with the Soxhlet technique to understand their effect on future monitoring data. The sonication technique was considered unsuitable for further investigation due to the absence of any significant automation at present.

4   Experimental Procedure

4.1 Materials

In order to compare these techniques, solids containing known amounts of organic compounds are required.

By limiting the organic compounds to certain groups (i.e. chlorinated pesticides, polychlorinated biphenyls, polynuclear aromatic hydrocarbons) commercial materials (certified reference standards) are available whose constituents have been well characterised by several laboratories undertaking their analysis using set conditions and procedures.

The two chosen after review of those commercially available were
(1)Sediment SRM-1941a "Organics in Marine Sediment"
(2) Biota SRM-297 "Organics in Mussel Tissue"

A review of the literature (Wise *et al.* 1991, Schantz *et al.* 1993, and Wise *et al.* 1995) showed the main solvent used for certification of both reference materials was dichloromethane.
This solvent was therefore employed in the extraction process where possible.

4.2 Material preparation

The preparation of the solid material prior to analysis consists of the following stages:

4.3 Drying and sieving (sediments)

This stage is necessary when excess water is present or when certain particle sizes are important.

4.4 Extraction

The stage with which this project is predominately concerned.
As analytical determinations are subject to an uncertainty in the final result (experimental error), it is necessary to prepare the reference material extracts in a predetermined manner in order to gain meaningful information.
Two weighed portions in different containers are extracted at the same time and this procedure is then repeated over a set number of days (15).
The results obtained can then be used to calculate the uncertainty in the final reported value.

4.5 Clean-Up of Extract

This stage removes unwanted material coextracted with the organic compounds and which will interfere with the analytical stage.
It was found necessary for both high-pressure solvent techniques (ASE and MASE) when extracting sediment and was essential for all techniques when extracting biota.
Extracts, which required cleanup, were subjected to the same cleanup treatment.

4.6 Analysis of Material Extract

This stage identifies and measures the concentration of each separate organic compound.

The actual compounds measured are given in Table 4.1

Table 4.1 Compounds Analysed in Reference Materials

Compound	Concentration in Sediment μg/kg	Concentration in Biota μg/kg
PCB's		
PCB 52	6.89	115
PCB 101	11.0	128
PCB 118	10.0	130.8
PCB 138	13.38	134
PCB 153	17.6	145.2
PAH's		
Fluoranthrene	981	163.7
Benzo(b) Fluoranthrene	740	46.4
Benzo(k) Fluoranthrene	361	20.2
Benzo(a) Pyrene	628	15.63
Benzo(ghi) Perylene	525	22.0
Indeno(1,2,3 cd)pyrene	501	14.2
Chlorinated Pesticides		
Hexachlorobenzene	70	0
cis Chlordane	2.33	17.2
trans Nonachlor	1.26	18.0
4,4 (p,p) DDE	6.59	43.0
4,4 (p,p) DDD (TDE)	5.06	43.0
2,4 (o,p) DDT *	6.0	8.5
4,4 (p,p) DDT **	1.25	3.91

* Not certified values

** Values obtained by Gas Chromatography Electron Capture Detection only

Full experimental details of the above stages are given in Chapter 8, Appendix 1

5   Discussion

5.1 Introduction

All stages of the analytical methodology are considered in respect to their contribution to the accuracy of the final result. Statistical tests are then applied to determine whether the modern techniques give 'significantly' different results when compared to Soxhlet extraction.

5.2 Extraction

The addition of the monitoring standards, Alpha HCH and PCB 198 to assess the validity of the extraction step and the cleanup stage respectively, was found of limited use due to their variability even within batches. Overall the concentrations were sufficient to conclude that there were no procedural errors at these stages.
The extracts obtained for all the techniques and matrices (i.e. sediment and biota) were therefore considered valid.
The blanks summarised in Tables 9.1 and 9.2 of Appendix 2 and indicate no significant reagent contaminants.
It was considered inappropriate to blank correct the final results due to the necessary use of an artificial blank (i.e. diatomaceous earth).
The use of a common solvent (dichloromethane) was found to provide similar results across all solvent techniques except the ASE (Accelerated Solvent Extraction)
The ASE produced unexpected low recoveries for PAH's and indicates that solvent selection and pressure may be critical for this technique.
The SFE technique does not use common solvents and thus required initial experimentation to find the optimum conditions for extracting PAH's.
This indicates the difficulty in comparing these techniques purely on extraction efficiency.

5.3 Cleanup

A decision whether to 'clean-up' was taken based on the selectivity of each technique.
Neither the Soxtherm nor SFE required a cleanup step when considering the sediment matrix. Inspection of the RSD (Relative Standard Deviations) Tables 9.4, 9.5 respectively, indicate that the cleanup step for the Microwave and ASE extracts did not apparently affect the precision of the final result in comparison with the Soxtherm and SFE techniques.
All biota extracts were cleaned up and Tables 9.7 to 9.9 indicate an apparent improvement in the performance of the microwave and ASE techniques when compare to the Soxtherm technique.

5.4 Analysis

Inspection of the chromatograms showed difficulties in the analysis of the following compounds.
- NONACHLOR
Accurate integration of the chromatographic peak was found almost impossible due to baseline shifts and gave an over estimate of its true value.
- PCB52
Abnormal high recoveries for all techniques indicated background interference.
- pp DDT
Excessively high recoveries due to background interference from numerous peaks in vicinity of this peak leading to baseline integration errors.

• BENZO(a)PYRENE, INDENO(1,2,3-cd)PYRENE and BENZO(GHI)PERYLENE

It was found impossible to eliminate large cholesterol peaks derived from the biota even with the extensive cleanup employed. These severely interfered with the analysis of these compounds such that no meaningful results were possible.

5.5 Comparison of techniques

For this project the Soxtherm results in Table 9.3 Sediment Reference Material and Table 9.7 Biota Reference Material were used as the standard against which the results for the other techniques were compared (Sediment Tables 9.4 to 9.6 and Biota Tables 9.8 to 9.9)

5.5.1 Precision, The "F" Test

This statistical procedure compares the standard deviations of two sets of results and provides a means of assessing whether they are "significantly" different.

A result is "Significant" when the experimentally determined F value is greater than the theoretically derived value.

The Sediment results are given in Table 5.1 and the Biota results in Table 5.2

Table 5.1 Statistical F Test of Sediment Reference Material

Compound \ F Values	Microwave		ASE		SFE	
	Exp. F	Significant	Exp. F	Significant	Exp. F	Significant
HEXACHLOROBENZENE	1.47	No	8.51	*Yes	1.81	No
PCB 52	9.32	*Yes	47.91	*Yes	1.23	No
FLUORANTHENE	3.32	**Yes**	3.12	No	3.03	No
PCB 101	2.85	No	1.14	No	3.00	No
cis CHLORDANE	1.25	No	6.21	*Yes	1.10	No
trans NONACHLOR	1.17	No	18.72	*Yes	1.68	No
pp DDE	1.12	No	3.36	*Yes	1.36	No
PCB 118	1.05	No	1.36	No	4.68	*Yes
pp TDE	1.70	No	2.08	No	1.35	No
op DDT	1.05	No	2.51	No	4.90	**Yes**
PCB 153	2.01	No	2.26	No	1.08	No
pp DDT	1.49	No	2.44	No	10.62	**Yes**
PCB 138	3.49	*Yes	1.31	No	5.15	*Yes
BENZO(b)FLUORANTHENE	2.24	No	3.57	*Yes	3.04	No
BENZO(k)FLUORANTHENE	1.05	No	2.36	No	2.03	No
BENZO(a)PYRENE	1.92	No	1.63	No	1.02	No
INDENO(1,2,3-cd)PYRENE	1.19	No	1.10	No	1.51	No
BENZO(GHI)PERYLENE	2.14	No	1.02	No	1.50	No

* Indicates improvement in precision compared to Soxtherm.

Relative magnitudes of population standard deviation unknown so F (0.025) is used.

Theoretical F-Value with 14,14 Degrees of Freedom is 2.97

Theoretical F-Value with 14,13 Degrees of Freedom is 3.08

Theoretical F-Value with 13,12 Degrees of Freedom is 3.25

Consideration of the "significant results shows:

While the ASE technique gives an improved precision for certain chlorinated pesticides compared to the Soxtherm technique; the Microwave and SFE techniques provide better precision for PCB's.

Of the compounds indicating worse precision than the Soxtherm technique, the Microwave Fluoranthene result reflects the considerable difference in the experimental means obtained (Soxtherm 675, Microwave 1175) and may derive from exceedance of the calibration range in this instance. For the SFE technique, the DDT isomer results indicate a possible breakdown under the more vigorous extraction conditions required by this technique to extract the PAH compounds.

Table 5.2 Statistical F Test of Biota Reference Material

Compound \ F Value	Microwave		ASE	
	Exp. F	Significant	Exp. F	Significant
HEXACHLOROBENZENE	1.45	No	1.71	No
PCB 52	1.24	No	1.60	No
FLUORANTHENE	1.41	No	1.02	No
PCB 101	1.98	No	1.98	No
cis CHLORDANE	2.95	No	7.00	**Yes**
trans NONACHLOR	1.07	No	1.20	No
pp DDE	1.17	No	1.70	No
PCB 118	1.37	No	1.75	No
pp TDE	1.32	No	1.62	No
op DDT	1.66	No	12.41	*Yes
PCB 153	2.26	No	1.20	No
pp DDT	10.25	*Yes	7.37	*Yes
PCB 138	16.97	*Yes	1.18	No
BENZO(b)FLUORANTHENE	4.43	*Yes	5.45	*Yes
BENZO(k)FLUORANTHENE	1.99	No	2.54	No

* Indicates improvement in precision compared to Soxtherm.
Relative magnitudes of population standard deviation unknown so F 0.025 used
Theoretical F-Value with 14,14 Degrees of Freedom is 2.97
Theoretical F-Value with 14,13 Degrees of Freedom is 3.08
Theoretical F-Value with 14,9 Degrees of Freedom is 3.79
Theoretical F-Value with 13,9 Degrees of Freedom is 3.84

Consideration of the "significant results shows:

The results again show some evidence of improved precision compared to the Soxtherm technique with only the ASE cis chlordane result being worse. The latter cannot be attributed to any specific reason.

Overall both sets of results indicate that the techniques have at least comparable precision to Soxtherm when extracting Sediment and Biota reference material. The sediment results for the ASE are in agreement with an expected improvement in precision when full automation of the technique is possible.

5.5.2 Accuracy, The "T" Test

This statistical procedure compares the means of two sets of results and assesses whether they are "significantly" different.

As the mean is the criteria by which the results are judged, any visual difference in this value obtained by other techniques would affect the perceived accuracy of the final result.

As the project compares techniques rather than optimising methods, this does not allow a definite comparison of the mean obtained with the certified reference values. However inspection of tables in Appendix 2 show general agreement between the two values when the 95% confidence limits are taken into account.

The experimental mean can therefore be used to assess whether the apparent accuracy of a result is affected by the choice of extraction technique.

The Sediment results are given in Table 5.3 and the Biota results in Table 5.4

Table 5.3 Statistical T Test of Sediment Reference Material

Compound \ T Value	Microwave		ASE		SFE	
	Exp. T	Significant	Exp. T	Significant	Exp. T	Significant
HEXACHLOROBENZENE	0.52	No	2.02	No	0.34	No
PCB 52	1.88	No	2.64	**Yes**	0.55	No
FLUORANTHENE	1.98	No	2.41	**Yes**	0.86	No
PCB 101	0.97	No	0.96	No	0.87	No
cis CHLORDANE	2.52	**Yes**	2.79	**Yes**	3.30	**Yes**
trans NONACHLOR	1.34	No	1.81	No	0.73	No
pp DDE	0.34	No	1.60	No	0.15	No
PCB 118	0.42	No	1.20	No	1.90	No
pp TDE	0.47	No	1.16	No	1.42	No
op DDT	1.48	No	0.53	No	0.38	No
PCB 153	0.51	No	0.23	No	0.59	No
pp DDT	2.73	**Yes**	1.14	No	1.43	No
PCB 138	1.48	No	0.60	No	0.46	No
BENZO(b)FLUORANTHENE	1.18	No	1.43	No	0.74	No
BENZO(k)FLUORANTHENE	1.43	No	0.34	No	0.79	No
BENZO(a)PYRENE	0.17	No	0.48	No	0.17	No
INDENO(1,2,3-cd)PYRENE	0.85	No	1.53	No	1.20	No
BENZO(GHI)PERYLENE	1.27	No	1.61	No	1.37	No

Theoretical T-Value at 95% Confidence Interval is 2.00
Theoretical T-Value at 95% Confidence Interval is 2.01

Consideration of the "significant" results shows:
The Soxtherm gave a higher cis Chlordane result when compared to the other three techniques.
The Microwave pp DDT and PCB 52 result can be discounted due to the reasons given in Section 5.4
The ASE Fluoranthene result is indicative of the large difference between its mean (328) compared to that of the Soxtherm (675)

Table 5.4 Statistical T Test of Biota Reference Material

Compound \ T Value	Microwave		ASE	
	Exp. T	Significant	Exp. T	Significant
HEXACHLOROBENZENE	0.22	No	2.33	**Yes**
PCB 52	0.11	No	1.31	No
FLUORANTHENE	1.63	No	1.60	No
PCB 101	0.98	No	1.06	No
cis CHLORDANE	0.50	No	3.13	**Yes**
trans NONACHLOR	1.50	No	1.51	No
pp DDE	1.96	No	2.21	**Yes**
PCB 118	0.03	No	1.48	No
pp TDE	0.03	No	0.94	No
op DDT	2.66	**Yes**	0.53	No
PCB 153	0.39	No	0.23	No
pp DDT	0.05	No	0.32	No
PCB 138	1.08	No	1.81	No
BENZO(b)FLUORANTHENE	0.90	No	0.82	No
BENZO(k)FLUORANTHENE	1.86	No	0.68	No

Theoretical T-Value at 95% Confidence Interval is 2.00
Theoretical T-Value at 95% Confidence Interval is 2.01

Consideration of the "significant" results shows:
The Microwave op DDT result is closer to the certified reference value than the Soxtherm result.
The ASE Hexachlorobenzene result can be discarded, as there is no certified reference value for this compound.
The ASE pp DDE result arises from a low Soxtherm value when compared to the certified reference value. Both comparison techniques give a better correlation with the latter value.
The ASE cis chlordane result is appreciably greater than either the Soxhlet or Microwave techniques but the reason is unknown.

5.6 Validity of "T" Test

The relevance of this statistical test to this project can be shown by comparing two sets of results where an obvious difference in means is observed i.e. Microwave and ASE extraction of PAH's.

This comparison is given in Table 5.5

Table 5.5 Comparison of Microwave and ASE Extraction for Sediment Reference Material

Compound \ T Value	ASE Mean	Microwave Mean	Exp. T	Significant	% Mean Difference
HEXACHLOROBENZENE	49.4	102.9	2.40	**Yes**	**53.1**
PCB 52	14.5	20.6	1.23	No	24.5
FLUORANTHENE	327.7	1175.2	3.60	**Yes**	**92.6**
PCB 101	8.6	14.5	1.81	No	37.3
cis CHLORDANE	1.6	1.8	0.45	No	8.8
trans NONACHLOR	0.9	2.7	2.99	**Yes**	**79.9**
pp DDE	4.5	6.6	1.26	No	27.1
PCB 118	6.3	7.9	0.79	No	15.8
pp TDE	4.4	6.9	1.56	No	32.3
op DDT	7.1	9.1	0.89	No	17.8
PCB 153	12.2	13.2	0.26	No	5.1
pp DDT	6.9	4.0	1.83	No	32.4
PCB 138	10.7	13.7	0.85	No	16.8
BENZO(b)FLUORANTHENE	336.3	688.2	2.39	**Yes**	**51.7**
BENZO(k)FLUORANTHENE	309.1	504.3	1.73	No	34.8
BENZO(a)PYRENE	362.8	435.2	0.65	No	12.5
INDENO(1,2,3-cd)PYRENE	247.6	485.3	2.23	**Yes**	**48.5**
BENZO(GHI)PERYLENE	243.1	557.8	2.58	**Yes**	**60.3**

%Mean Difference = (Mean1-Mean2)/((Mean1+Mean2)/2)

Thus where the experimental means are obviously different between the two methods this is confirmed by the "T" test results.

In this instance, a variation of ± 25% or greater in the mean value indicates a "significant" difference.

6   Conclusion

The majority of the statistical results in Tables 5.3 and 5.4 are found to be not "significant". Those compounds, which are "significantly" different, often reflect analytical difficulties in their determination and thus cannot be considered representative of the true performance of the extraction technique.

For both reference materials, the majority of compounds show no meaningful differences between Soxhlet and the other extraction techniques. Thus none of the methods considered would produce an obvious stepwise increase in concentrations reported.

The experience gained in undertaking this project provides the following summary of instrument performance.

•Most Efficient Techniques (Based on sample capacity and extraction times)
ASE, Microwave

•Most Environmentally Friendly (Based on resources and solvent usage)
ASE, Microwave

•**Most Simplistic (Based on operation)**
Microwave

•Most Automated Techniques
ASE, SFE

•Most Selective Technique (Based on raw data)
SFE

The newer techniques are more environmentally acceptable in their use of solvents and energy resources and instrument automation enables faster sample preparation and should lead to an improved service to the Environment Agency.

7   References

Marsden P., Sample extraction Techniques (1995), Environmental Testing and Analysis, 4, No. 2 (i) 30; (ii) 32; (iii) 33; (iv) 34.

Sporstoel,S., Gjoes, N., Carlberg, C.E. (1983), Extraction Efficiencies for Organic Compounds found in Aquatic sediments, Analytica Chimica Acta, **151**, No. 1, 231-235

Fuoco, R., Colombini, M.R., Ceccarini, A. (1996) Analytical Quality Control for the Determination of Polychlorobiphenyls in Environmental Matrices, Mikrochimica Acta, 123, 175-183

Barnabas, I.J., Dean, J.R., Fowlis, I.A., Owen, S.P. (1995) Extraction of Polycyclic Aromatic Hydrocarbons from Soils using Microwave Energy, Analyst, 120, No. 7,1897-1904

Fish, J.R., Revesz, R. (1996), Microwave Solvent Extraction of Chlorinated Pesticides from Soil, LCGC International. 14 No. 3 (i) 231; (ii) 232; (iii) 234.

Chee, K.K., Wong, M.K., Lee, H.K. (1996) Optimisation of Microwave assisted Solvent Extraction of Polycyclic Aromatic Hydrocarbons in Marine Sediments using a Microwave Extraction System with High Performance Liquid Chromatography – Fluorescence Detection and Gas Chromatography – Mass Spectrometry, Journal of Chromatography *A*. 723 No. 2, 259-271

Letellier, M., Budzinski, H., Garriques, P. (1999) Focused Microwave Assisted Extraction of Polycyclic Aromatic Hydrocarbons, LCGC International, 12, No. 4, 222-225

Heemken, O.P., Theobald, N., Wenclawiak, B.W. (1997) Comparison of ASE and SFE with Soxhlet, Sonication, and Methanolic Saponification Extractions for the Determination of Organic Micropollutants in Marine Particulate Matter, Analytical Chemistry, 69, 2171-2180

Poole, C.F., Poole, S.K. (1996) Trends in Extraction of Semivolatile Compounds from Solids for Environmental Analysis, Analytical Communications, 33, 11H-14H

Wise, S.A., Benner, B.A., Christensen, R.G., Koster, B.J. Kurz, J., Schantz, M.M., Zeisler, R. (1991) Preparation and Analysis of Frozen Mussel Tissue Reference Material for the Determination of Trace Organic Constituents, Environmental Science Technology, 25 No. 10, 1695-1704

Schantz, M.M., Parris, R.M., Kurz, J., Ballschmiter, K., Wise, S.A. (1993) Comparison of Methods for the Gas Chromatographic Determination of PCB Congeners and Chlorinated Pesticides in Marine Reference Materials, Fresenius Journal of Analytical Chemistry, 346, 766-778

Wise, S.A., Shantz, M.M., Benner, B.A., Hays, M.J., Schiller, S.B. (1995) Certification of Polycyclic Aromatic Hydrocarbons in a Marine Sediment Standard Reference Material, Analytical Chemistry, 67, 1171-1178

Cheeseman, R.V., Wilson, A.L. (1989) A Manual on Analytical Quality Control for the Water Industry, Revised by Gardner, M.J. Water Research Centre report no NS 30.

8 Appendix1: Experimental Details

8.1 Sample Preparation

8.1.1 Reference Materials

Both Sediment SRM-1941a "Organics in Marine Sediment" and Biota SRM-297 "Organics in Mussel Tissue" were obtained from Promochem, Hertfordshire after competitive quotation. The sediment was transferred to a large container from the supplied 50 gram bottles and shaken for 30 minutes to remove any batch variation.
The water content was approximately 2.2%
The biota was used as supplied since mixing was considered inappropriate due to its stringy nature. Further processing was considered inadvisable.
The water content was approximately 12.5 %
Both materials were used without further drying to ensure no loss of compounds by volatilisation.

8.1.2 Chemicals

Analar Grade solvents comprising dichloromethane, hexane, diethyl ether, benzene, acetone.
For calibration and blank determinations, a modified form of diatomaceous earth (ISOLUTE HM-N) was used as an inert material.
Certified reference standards representing the chosen organochlorine pesticides, polychlorinated biphenyls and polynuclear aromatic hydrocarbons.

8.1.3 Standards

Three calibration standards representing 100, 50 and 10μg/kg solid were prepared.
Alpha HCH and PCB198 were used as the monitoring standards.
Aldrin and Hexabromobenzene were used as internal standards.
All standards were dissolved in acetone and made up in a volumetric flask
Addition of the internal standard to the extract is performed before analysis.

8.1.4 Extraction Equipment

The reference Soxhlet extraction was carried out using two Gerhardt Automatic Soxtherm units with water cooled condensers. Each unit is capable of extracting 6 samples each. Extraction time at atmospheric pressure was 6 hours with 150mls of solvent. (Boiling temperature 39.75°C).
The Microwave extractions were carried out on a CEM Corporation MARS5 system. Extraction time using 25ml of solvent was for 15 minutes at full power.
The ASE extractions were using a Dionex ASE 2000 Accelerated Solvent Extractor operated at a temperature of 100°C and 2000 psi pressure with 15ml solvent for 10 minutes.
The SFE extractions were carried out using an 8 sample chamber Hewlett Packard 7680T Superfluid Critical Extractor using a three step extraction procedure. The first step extracted the sample at 80°C and a carbon dioxide pressure of 121bar with a flow rate of 2ml/min (millilitres per minute) for 20minutes. The second step used carbon dioxide modified with 5%methanol at a pressure of 335bar and flow rate of 4ml/min to extract the sample at a temperature of 120°C for 40minutes.The final step used the same conditions without the methanol modifier and for only 15minutes. Total extraction time per sample was 2 hours.
Gel Permeation chromatography was performed on a Hewlett Packard 1100 High Pressure Liquid Chromatograph (HPLC) comprising an isocratic pump, autosampler (fitted with a 500

microlitre loop), ultraviolet detector and an Isco Foxy fraction collector. Envirogel Guard and Cleanup High Resolution columns were obtained from Waters Ltd. Calibration of the column was done using a mix of peanut oil, trifluralin, perylene and sulphur at a flow rate of 5ml/min dichloromethane. Separation time was 25minutes.

This cleanup removes the biota fish oil and the sediment sulphur.

Solid Phase Adsorption Chromatography was performed on an automated Zymark Benchmate system using 3ml Florisil cartridges eluted with a mixture of 50:50 hexane/diethyl ether. The cartridge traps any residual polar material not removed by the Gel Chromatography.

The exact procedure followed for each technique was a result of initial testing as to the steps required to produce a chromatographically suitable extract.

8.1.5 Sediment Procedure

A cleanup step was found unnecessary for Soxtherm and SFE, while for ASE and MASE this step was essential.

Weigh NIST 1941A Sediment reference material into separate method extraction vessels.

Use Isolute HM-N material if more weight required by the extraction technique.

Weigh Isolute HM-N material into an extraction vessel. (Blank)

 If Isolute has been added to the reference material, the same amount is added to the Blank

Spike each reference material and the Isolute blank with the extraction monitoring standard (alpha HCH).

For Soxtherm and ASE techniques, add copper powder to each container to remove sulphur.

Extract all 3 prepared samples using dichloromethane.

Prepare 1ml of each of the three calibration standard in separate vials.

If the technique is ASE or MASE, transfer the final extract to a labelled autosampler vial and add the cleanup monitoring standard (PCB198) to the vial, then carry out the Gel Permeation cleanup on the sample and calibration extracts.

If the technique is ASE or MASE, carry out the Solid Phase Chromatography cleanup on the Gel permeation eluates

For Soxtherm and SFE techniques,transfer to an appropriately labelled 10ml volumetric flask and make up to the mark with dichloromethane.

Remove 1ml and add internal standard.

For ASE and MASE, add internal standard to the final eluates and transfer to an insert contained in an autosampler vial. Cap and label vial.

Repeat the exercise over 15 days.

If extraction is considered unsatisfactory (i.e. extract lost, dryness etc), repeat with further material.

8.1.6 Biota Procedure

Weigh NIST 2974 biota reference material into separate method extraction vessels. Use Isolute HM-N material if extraction technique requires more material.
Weigh Isolute HM-N material into an extraction vessel. (Blank)
If Isolute has been added to reference material, add same amount to blank
Spike each reference material and Isolute blank with the extraction monitoring standard (alpha HCH).

Extract all 3 prepared samples using dichloromethane.
Transfer extracts to a labelled autosampler vial and add cleanup monitoring standard (PCB198) to the vial.

Prepare 1ml of each of the three Calibration Standards in separate vials.
Process these standards as samples.

Perform the Gel Permeation cleanup on the sample and calibration extracts.
Perform the Solid Phase Chromatography cleanup on the Gel permeation eluates
Add internal standard to all cleaned up eluates and then transfer final extract to an insert contained in an autosampler vial. Cap and label vial.

Repeat the exercise over 15 days.
If extraction is considered unsatisfactory (i.e. extract lost, dryness etc), repeat with further material.

8.1.7 Analysis

All extracts were analysed on a Hewlett Packard 5980 Gas Chromatograph fitted with a Gerstel cis-3 injection port and attached to a Hewlett Packard 5972 Mass Selective Detector.
The Gas Chromatograph was equipped with a 5% phenylmethyl silicone capillary column, 0.25mm i.d., 0.25 micron film thickness, 30 metre length (Hewlett Packard low bleed HPMS5).

The Gas Chromatograph oven temperature program profile was:
40°C for 0.5minute, 15°C per minute to 250°C, hold for 0.5minute, 2.0°C per minute to 300° for 8.9minute giving a total runtime of 45 minutes.

The Gas Chromatograph inlet was operated in constant flow mode with a pressure of 7psi (pounds per square inch) at 40°C.
Parameters for the cis injector port operated in the solvent vent mode were:
43°C for 0.3minute, 720°C per minute to 280°C for 5minutes, 720°C per minute for 10minutes; Splitless time 2.0minutes; injection volume 12.5microlitres.

The Mass Spectrometer was operated in the Selective Ion Mode, detecting the following ion masses for each compound as given in Table 8.1

Table 8.1 Masses used For Mass Spectrometric Selective Ion Monitoring

Compound	Ion Masses	Compound	Ion Masses	Compound	Ion Masses
Aldrin	263, 261, 265	PCB 52	220, 292	Fluoranthene	202, 200, 203
AlphaHCH	217, 181,183	PCB 101	326, 328	Benzo(b) Fluoranthene	252, 250, 253
HexaChloro benzene	284, 286	PCB 118	326, 328	Benzo(k) Fluoranthene	252, 250, 253
cis Chlordane	373, 375, 377	PCB 153	360, 362	Benzo(a) Pyrene	252, 126, 253
trans Nonachlor	409, 407, 411	PCB 138	360, 362	Indeno(1,2,3 cd) Pyrene	276, 138, 277
pp DDE	246, 248, 318	PCB 198	430, 432, 428	Benzo(ghi) Perylene	276, 138, 277
Dieldrin	263, 265, 261			Hexabromo benzene	552, 392
pp TDE	235, 237, 165				
op DDT	235, 237, 165				
pp DDT	235, 237, 165				

The extracts were analysed in small batches with a set of three calibration standards. Where the extract had been cleaned up, the calibration standards were also processed through the cleanup procedures.

8.1.8 Results Validation

Blank determinations were used to first assess any possible reagent or sample preparation contaminants.

All chromatograms were checked to ensure integration parameters were correct for the peaks measured and then reprocessed.

Matching the retention time of each peak with the retention time in the calibration standards identified the compounds in the samples.

Concentrations were determined by comparing peak heights of identified compounds in the sample with the corresponding peak heights in the calibration standards. Differences in final extract volume were corrected by applying the ratio of the internal standard peak heights in the sample and calibration standard.

8.1.9 Statistical Calculations

The final results were exported directly into a Microsoft Excel spreadsheet.

Calculations of within and between batch standard deviations are according to Cheeseman and Wilson (1989). Results outside four times the total standard deviation of the mean were rejected and the calculation repeated. Derivation of the total standard deviation provides estimates of the Relative Standard Deviation. Comparison of the experimental mean with the certified reference concentration provides estimates of the bias.

These statistical results are given in Tables 9.1 to 9.9, Appendix 2

9   Appendix 2: Experimental Results

Table 9.1 Sediment Reference Material
Blank Results

Micrograms compound per kilogram material

Compound	Soxtherm Mean	Conf. ±	Microwave Mean	Conf. ±	ASE Mean	Conf. ±	SFE Mean	Conf. ±
ALPHA HCH	31.96	6.75	26.75	1.87	22.67	1.94	22.99	3.00
HEXACHLOROBENZENE	3.08	2.04	0.30	0.13	0.19	0.06	0.46	0.24
PCB 52	3.93	1.18	2.27	1.22	2.81	1.23	4.03	1.12
FLUORANTHENE	25.14	2.59	4.14	1.19	7.67	1.40	4.65	1.17
PCB 101	0.62	0.08	0.67	0.55	0.55	0.28	0.22	0.12
cis CHLORDANE	0.82	0.30	0.00	N/a	0.00	N/a	0.00	N/a
trans NONACHLOR	0.49	0.27	0.02	0.03	0.00	N/a	0.29	0.39
pp DDE	1.37	0.54	0.09	0.04	0.38	0.21	0.47	0.33
PCB 118	0.67	0.23	0.05	0.03	0.57	0.24	0.31	0.27
pp TDE	1.34	0.28	0.13	0.06	0.39	0.22	0.08	0.15
op DDT	1.04	0.43	0.19	0.07	0.43	0.23	0.84	0.93
PCB 153	0.31	0.18	0.05	0.03	1.70	1.10	0.08	0.14
pp DDT	2.02	0.38	0.16	0.07	0.52	0.35	0.89	0.58
PCB 138	0.56	0.20	1.22	2.26	1.52	1.01	0.21	0.19
PCB 198	0.00	N/a	36.23	4.82	35.13	3.08	0.00	N/a
BENZO(b)FLUORANTHENE	4.05	1.59	0.84	0.53	4.53	1.03	1.30	1.03
BENZO(k)FLUORANTHENE	2.96	1.00	0.94	0.45	4.43	1.37	2.01	1.16
BENZO(a)PYRENE	3.18	1.32	1.18	1.07	5.49	1.68	1.12	0.82
INDENO(1,2,3-cd)PYRENE	2.96	0.99	0.40	0.46	6.41	1.76	0.60	0.61
BENZO(GHI)PERYLENE	3.03	0.81	0.66	0.70	6.93	1.95	0.81	0.74

Conf. = 95% Confidence Limit

Table 9.2 Biota Reference Material

Blank Results

	Micrograms compound per kilogram material					
Compound	Soxtherm Mean	Conf. ±	Microwave Mean	Conf. ±	ASE Mean	Conf. ±
ALPHA HCH	58.20	7.04	56.42	7.24	70.27	7.46
HEXACHLOROBENZENE	0.40	0.10	0.23	0.14	0.69	0.12
PCB 52	4.12	0.68	2.07	0.52	3.61	1.21
FLUORANTHENE	3.41	0.65	5.16	0.94	5.74	0.75
PCB 101	0.93	0.21	1.35	1.16	1.38	0.52
cis CHLORDANE	0.05	0.05	0.00	N/a	0.37	0.39
trans NONACHLOR	0.48	0.26	0.16	0.22	0.81	0.30
pp DDE	0.46	0.18	0.30	0.26	0.97	0.16
PCB 118	1.04	0.36	0.18	0.15	0.72	0.61
pp TDE	1.23	0.55	0.57	0.27	2.05	0.20
op DDT	2.39	2.16	0.66	0.28	1.39	0.42
PCB 153	0.92	0.25	0.16	0.17	0.67	0.21
pp DDT	1.03	0.41	0.23	0.12	0.93	0.45
PCB 138	1.27	0.45	0.15	0.15	0.65	0.26
PCB 198	79.50	6.50	75.77	8.36	84.33	6.04
BENZO(b)FLUORANTHENE	2.56	0.77	0.51	0.21	0.63	0.28
BENZO(k)FLUORANTHENE	2.23	0.70	0.59	0.19	1.17	0.25

Conf. = 95% Confidence Limit

Table 9.3 Sediment Reference Material

Extraction Technique: Soxtherm

Compound	Ref. ug/kg	ExpMean ug/kg	Conf. ±	Bias %	Rel.Std %	s_w	s_b	s_t	df
HEXACHLOROBENZENE	70.0	89.5	15.1	27.9	30.5	12.594	24.214	27.293	14
PCB 52	6.9	37.1	12.3	439.0	59.9	12.268	18.553	22.242	14
FLUORANTHENE	981.0	675.3	77.6	-31.2	20.7	100.589	97.442	140.047	14
PCB 101	11.0	11.2	1.2	1.5	19.6	1.390	1.691	2.189	14
cis CHLORDANE	2.3	3.8	0.7	67.5	29.0	0.684	0.901	1.132	14
trans NONACHLOR	1.3	1.8	0.9	40.9	88.8	1.422	0.678	1.576	14
pp DDE	6.6	7.3	1.9	11.1	47.0	1.251	3.201	3.436	14
PCB 118	10.0	8.9	1.7	-10.9	35.2	1.672	2.652	3.135	14
pp TDE	5.1	6.1	1.1	20.2	33.3	1.403	1.458	2.024	14
op DDT	6.0	6.0	1.5	0.4	43.6	1.535	2.134	2.628	14
PCB 153	17.6	11.5	3.2	-34.9	49.7	2.311	5.199	5.689	14
pp DDT	1.3	9.6	1.7	666.7	32.3	3.070	0.408	3.097	14
PCB 138	13.4	9.0	3.5	-32.5	67.0	1.416	5.879	6.047	13
BENZO(b)FLUORANTHENE	740.0	500.6	56.4	-32.4	20.4	72.510	71.597	101.901	14
BENZO(k)FLUORANTHENE	361.0	338.9	56.4	-6.1	28.8	37.248	90.395	97.769	13
BENZO(a)PYRENE	628.0	415.6	76.9	-33.82	33.4	81.222	112.6	138.809	14
INDENO(1,2,3-cd)PYRENE	501.0	386.2	64.9	-22.92	30.3	67.625	95.6	117.099	14
BENZO(GHI)PERYLENE	525.0	390.8	68.8	-25.56	31.8	63.607	106.7	124.180	14

ug/kg = Micrograms compound per kilogram solid
Ref. = Certified reference value
Conf.= 95%Confidence Limits on Experimental mean (ExpMean)

Rel.Std = Relative Standard Deviation
sw = Standard Deviation (Within batch)
sb = Standard Deviation (Between batch)
st = Total Standard Deviation
df = Degrees of Freedom

Table 9.4 Sediment Reference Material

Extraction Technique: Microwave

Compound	Ref. ug/kg	ExpMean ug/kg	Conf. ±	Bias %	Rel.Std %	s_w	s_b	s_t	df
HEXACHLOROBENZENE	70	102.9	18.3	47.0	32.1	15.637	29.138	33.069	14
PCB 52	6.89	20.6	4.0	198.6	35.4	3.003	6.640	7.288	14
FLUORANTHENE	981	1 175.2	141.3	19.8	21.7	101.327	234.168	255.151	14
PCB 101	11	14.5	2.0	31.7	25.5	2.380	2.831	3.698	14
cis CHLORDANE	2.33	1.7	0.6	-25.4	57.5	0.502	0.865	1.000	14
trans NONACHLOR	1.26	2.7	0.8	115.3	53.5	1.212	0.798	1.451	14
pp DDE	6.59	6.6	2.0	0.9	54.9	1.336	3.398	3.652	14
PCB 118	10	8.4	1.2	-15.7	26.1	1.527	1.586	2.201	14
pp TDE	5.06	6.9	1.5	36.2	38.2	1.000	2.438	2.635	14
op DDT	6	9.1	1.5	51.0	30.4	1.561	2.270	2.755	14
PCB 153	17.6	13.3	2.2	-24.5	30.2	3.454	2.037	4.010	14
pp DDT	1.25	4.0	1.4	221.6	63.6	1.121	2.295	2.555	14
PCB 138	13.38	13.6	1.8	1.8	23.7	2.432	2.124	3.229	14
BENZO(b)FLUORANTHENE	740	690.5	85.5	-6.7	22.4	103.315	114.681	154.355	14
BENZO(k)FLUORANTHENE	361	505.4	56.1	40.0	20.0	62.452	79.788	101.323	14
BENZO(a)PYRENE	628	431.3	57.6	-31.32	24.1	46.277	93.2	104.065	14
INDENO(1,2,3-cd)PYRENE	501	483.7	69.4	-3.45	25.9	44.514	117.2	125.342	14
BENZO(GHI)PERYLENE	525	562.0	103.6	7.05	33.3	75.461	171.1	187.034	14

ug/kg = Micrograms compound per kilogram solid
Ref. = Certified reference value
Conf.= 95%Confidence Limits on Experimental mean (ExpMean)

Rel.Std = Relative Standard Deviation
sw = Standard Deviation (Within batch)
sb = Standard Deviation (Between batch)
st = Total Standard Deviation
df = Degrees of Freedom

Table 9.5 Sediment Reference Material

Extraction Technique: ASE

Compound	Ref ug/kg	ExpMean ug/kg	Conf. ±	Bias %	Rel.Std %	s_w	s_b	s_t	df
HEXACHLOROBENZENE	70	49.4	5.4	-29.4	32.5	6.755	14.543	16.035	13
PCB 52	6.89	14.5	1.8	110.4	34.3	1.896	4.597	4.972	13
FLUORANTHENE	981	327.7	45.5	-66.6	35.5	27.285	113.100	116.345	13
PCB 101	11	8.6	1.3	-21.9	37.6	1.686	2.755	3.230	13
cis CHLORDANE	2.33	1.5	0.3	-37.4	42.1	0.274	0.550	0.614	14
trans NONACHLOR	1.26	0.8	0.2	-32.7	49.8	0.138	0.399	0.422	14
pp DDE	6.59	4.2	1.1	-36.1	51.1	0.938	1.936	2.151	14
PCB 118	10	5.9	1.5	-41.1	52.0	1.146	2.840	3.062	14
pp TDE	5.06	4.1	0.8	-19.2	43.1	0.958	1.482	1.765	14
op DDT	6	6.6	2.4	9.7	67.0	2.348	3.733	4.410	14
PCB 153	17.6	11.4	5.1	-35.4	79.2	4.135	7.993	8.999	13
pp DDT	1.25	6.5	1.1	418.4	40.5	0.921	2.454	2.621	14
PCB 138	13.38	10.0	4.1	-25.5	74.1	3.838	6.300	7.377	13
BENZO(b)FLUORANTHENE	740	336.3	30.9	-54.5	30.9	31.727	99.058	104.015	13
BENZO(k)FLUORANTHENE	361	309.1	36.5	-14.4	33.4	39.508	95.250	103.118	13
BENZO(a)PYRENE	628	338.7	101.6	-46.07	57.5	66.556	183.0	194.767	14
INDENO(1,2,3-cd)PYRENE	501	231.1	64.0	-53.87	54.2	36.354	119.8	125.148	14
BENZO(GHI)PERYLENE	525	226.9	70.6	-56.79	59.2	42.872	127.2	134.227	14

ug/kg = Micrograms compound per kilogram solid
Ref. = Certified reference value
Conf.= 95%Confidence Limits on Experimental mean (ExpMean)

Rel.Std = Relative Standard Deviation
sw = Standard Deviation (Within batch)
sb = Standard Deviation (Between batch)
st = Total Standard Deviation
df = Degrees of Freedom

Table 9.6 Sediment Reference Material
Extraction Technique: SFE

Compound	Mean ug/kg	ExpMean ug/kg	Conf. ±	Bias %	Rel.Std %	s_w	s_b	s_t	df
HEXACHLOROBENZENE	70	81.9	11.2	17.1	24.8	9.336	18.032	20.306	14
PCB 52	6.89	43.8	11.1	535.0	45.8	7.951	18.405	20.049	14
FLUORANTHENE	981	848.1	135.0	-13.5	28.7	72.325	232.729	243.708	14
PCB 101	11	12.7	2.1	15.4	29.9	1.630	3.423	3.791	14
cis CHLORDANE	2.33	1.2	0.6	-47.0	92.6	0.239	1.118	1.143	14
trans NONACHLOR	1.26	2.2	0.7	76.6	54.6	0.848	0.872	1.216	14
pp DDE	6.59	7.0	1.6	6.3	42.0	1.962	2.196	2.944	14
PCB 118	10	5.1	0.8	-48.5	28.1	0.658	1.291	1.449	14
pp TDE	5.06	4.1	1.0	-19.7	42.8	1.310	1.146	1.741	14
op DDT	6	5.4	3.4	-10.4	108.2	1.283	5.673	5.816	13
PCB 153	17.6	13.6	3.3	-22.5	43.4	3.521	4.761	5.921	14
pp DDT	1.25	6.2	5.8	398.5	162.0	2.708	9.725	10.095	13
PCB 138	13.38	10.3	1.5	-23.3	25.9	0.971	2.481	2.664	14
BENZO(b)FLUORANTHENE	740	608.8	98.3	-17.7	29.2	67.254	164.329	177.559	14
BENZO(k)FLUORANTHENE	361	422.3	77.2	17.0	33.0	69.833	120.556	139.321	14
BENZO(a)PYRENE	628	434.9	77.5	-30.75	32.2	56.548	127.9	139.864	14
INDENO(1,2,3-cd)PYRENE	501	275.1	79.6	-45.09	52.3	51.938	134.1	143.791	14
BENZO(GHI)PERYLENE	525	262.5	84.2	-50.00	57.9	57.221	140.8	151.996	14

ug/kg = Micrograms compound per kilogram solid
Ref. = Certified reference value
Conf.= 95%Confidence Limits on Experimental mean (ExpMean)

Rel.Std = Relative Standard Deviation
sw = Standard Deviation (Within batch)
sb = Standard Deviation (Between batch)
st = Total Standard Deviation
df = Degrees of Freedom

Table 9.7 Biota Reference Material

Extraction Technique: Soxtherm

Compound	Ref. ug/kg	ExpMean ug/kg	Conf. ±	Bias %	Rel.Std %	s_w	s_b	s_t	df
HEXACHLOROBENZENE	0.0	1.0	0.4	n/a	65.5	0.485	0.414	0.637	14
PCB 52	115.0	163.5	30.2	42.2	33.3	17.710	51.568	54.524	14
FLUORANTHENE	163.7	102.6	20.5	-37.3	36.1	11.719	35.114	37.018	14
PCB 101	128.0	115.3	21.1	-9.9	33.0	14.741	35.079	38.050	14
cis CHLORDANE	17.2	10.6	2.1	-38.2	36.1	3.237	2.055	3.835	14
trans NONACHLOR	16.6	13.4	4.1	-19.5	55.3	3.815	6.319	7.381	14
pp DDE	43.0	27.5	6.6	-36.1	43.6	5.014	10.859	11.961	14
PCB 118	130.8	113.2	23.6	-13.5	37.6	18.019	38.589	42.588	14
pp TDE	43.0	39.6	9.7	-7.9	44.2	6.654	16.200	17.514	14
op DDT	8.5	7.1	3.7	-15.9	92.6	3.867	5.373	6.620	14
PCB 153	145.2	98.9	28.9	-31.9	52.6	18.365	48.749	52.094	14
pp DDT	3.9	2.3	1.0	-40.9	72.0	0.433	1.609	1.666	13
PCB 138	134.0	83.1	26.3	-38.0	54.9	5.282	45.276	45.583	13
BENZO(b)FLUORANTHENE	46.4	20.2	5.1	-56.4	45.3	3.947	8.270	9.164	14
BENZO(k)FLUORANTHENE	20.2	15.2	5.0	-24.7	56.5	2.964	8.076	8.603	13

ug/kg = Micrograms compound per kilogram solid
Ref. = Certified reference value
Conf.= 95%Confidence Limits on Experimental mean (ExpMean)

Rel.Std = Relative Standard Deviation
sw = Standard Deviation (Within batch)
sb = Standard Deviation (Between batch)
st = Total Standard Deviation
df = Degrees of Freedom

Table 9.8 Biota Reference Material

Extraction Technique: Microwave

Compound	Ref. ug/kg	ExpMean ug/kg	Conf. ±	Bias %	Rel.Std %	s_w	s_b	s_t	df
HEXACHLOROBENZENE	0.0	1.0	0.3	n/a	50.9	0.182	0.497	0.529	14
PCB 52	115.0	168.4	27.1	46.4	29.1	30.845	38.006	48.947	14
FLUORANTHENE	163.7	161.1	17.3	-1.6	19.4	11.256	29.125	31.224	14
PCB 101	128.0	150.2	15.0	17.3	18.0	11.680	24.395	27.046	14
cis CHLORDANE	17.2	12.3	3.6	-28.7	53.7	2.180	6.211	6.583	14
trans NONACHLOR	16.6	20.7	4.2	24.6	36.9	1.467	7.497	7.639	14
pp DDE	43.0	48.2	7.2	12.1	26.8	4.516	12.110	12.925	14
PCB 118	130.8	112.1	20.2	-14.3	32.5	15.744	32.824	36.404	14
pp TDE	43.0	39.9	8.4	-7.2	38.2	3.763	14.756	15.228	14
op DDT	8.5	17.7	4.7	108.7	48.1	4.120	7.468	8.529	14
PCB 153	145.2	110.2	19.2	-24.1	31.4	14.187	31.628	34.664	14
pp DDT	3.9	2.3	0.3	-41.6	22.8	0.269	0.445	0.520	14
PCB 138	134.0	112.0	6.1	-16.4	9.9	5.992	9.304	11.067	14
BENZO(b)FLUORANTHENE	46.4	25.8	2.4	-44.4	16.9	1.990	3.872	4.353	14
BENZO(k)FLUORANTHENE	20.2	26.1	3.4	29.2	23.4	3.275	5.138	6.093	14

ug/kg = Micrograms compound per kilogram solid
Ref. = Certified reference value
Conf.= 95%Confidence Limits on Experimental mean (ExpMean)

Rel.Std = Relative Standard Deviation
sw = Standard Deviation (Within batch)
sb = Standard Deviation (Between batch)
st = Total Standard Deviation
df = Degrees of Freedom

Table 9.9 Biota Reference Material
Extraction Technique: ASE

Compound	Ref. ug/kg	Mean ug/kg	Conf. ±	Bias %	Rel.Std %	s_w	s_b	s_t	df
HEXACHLOROBENZENE	0.0	2.1	0.6	n/a	39.7	0.708	0.440	0.834	9
PCB 52	115.0	241.4	49.2	109.9	28.5	64.451	24.294	68.878	9
FLUORANTHENE	163.7	165.4	26.8	1.0	22.6	32.241	19.061	37.455	9
PCB 101	128.0	158.5	38.3	23.8	33.8	24.496	47.607	53.540	9
cis CHLORDANE	17.2	30.5	7.3	77.5	33.2	7.559	6.767	10.146	9
trans NONACHLOR	16.6	21.3	4.8	28.6	31.5	4.990	4.517	6.731	9
pp DDE	43.0	54.5	11.1	26.9	28.6	11.073	10.977	15.591	9
PCB 118	130.8	177.3	40.3	35.5	31.8	38.856	40.828	56.362	9
pp TDE	43.0	52.6	9.8	22.4	26.1	9.956	9.469	13.740	9
op DDT	8.5	5.9	1.3	-31.0	32.0	1.899	0.391	1.879	9
PCB 153	145.2	106.4	34.0	-26.8	44.7	24.421	40.798	47.549	9
pp DDT	3.9	2.5	0.4	-34.8	24.1	0.595	0.149	0.614	9
PCB 138	134.0	145.8	30.0	8.8	28.8	20.029	36.935	42.016	9
BENZO(b)FLUORANTHENE	46.4	15.5	2.8	-66.6	25.3	3.348	2.052	3.927	9
BENZO(k)FLUORANTHENE	20.2	18.7	3.9	-7.6	28.9	3.984	3.636	5.394	9

ug/kg = Micrograms compound per kilogram solid
Ref. = Certified reference value
Conf.= 95%Confidence Limits on Experimental mean (ExpMean)

Rel.Std = Relative Standard Deviation
sw = Standard Deviation (Within batch)
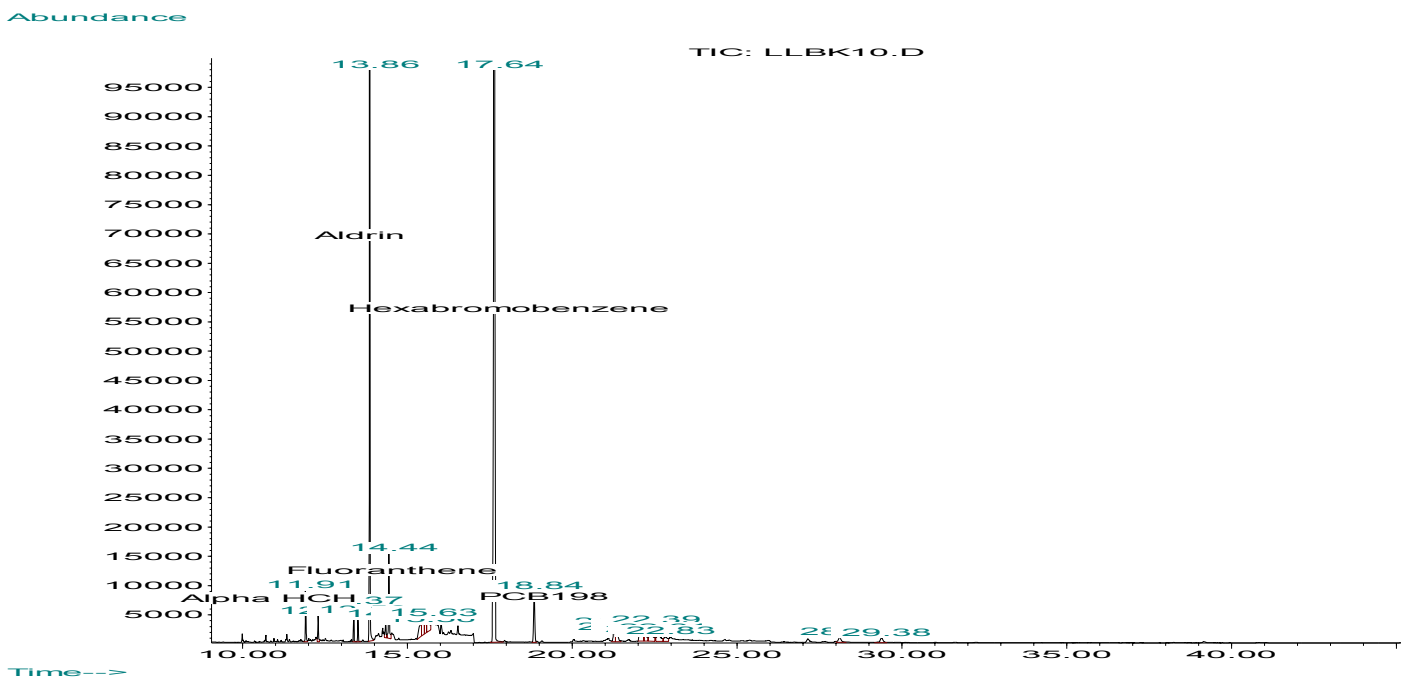sb = Standard Deviation (Between batch)
st = Total Standard Deviation
df = Degrees of Freedom

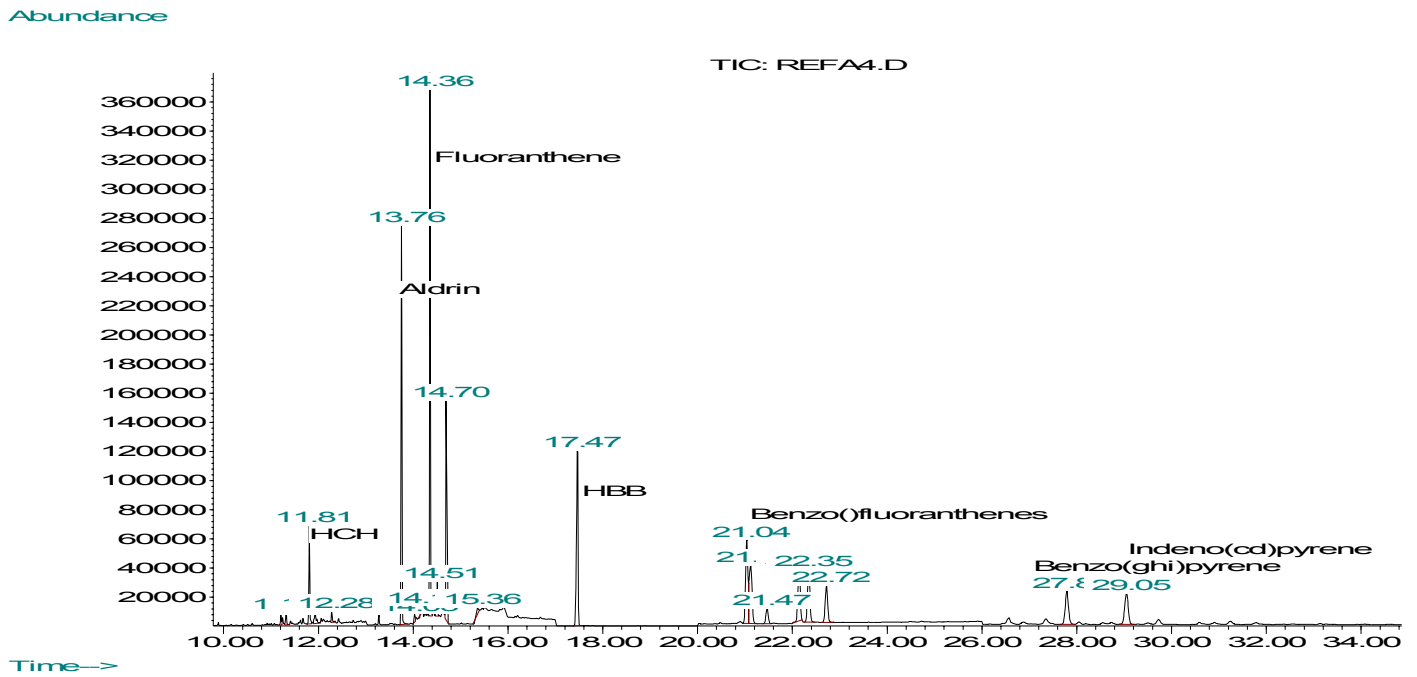# 10   Appendix3:Example Chromatograms (Total Ion Current TIC)

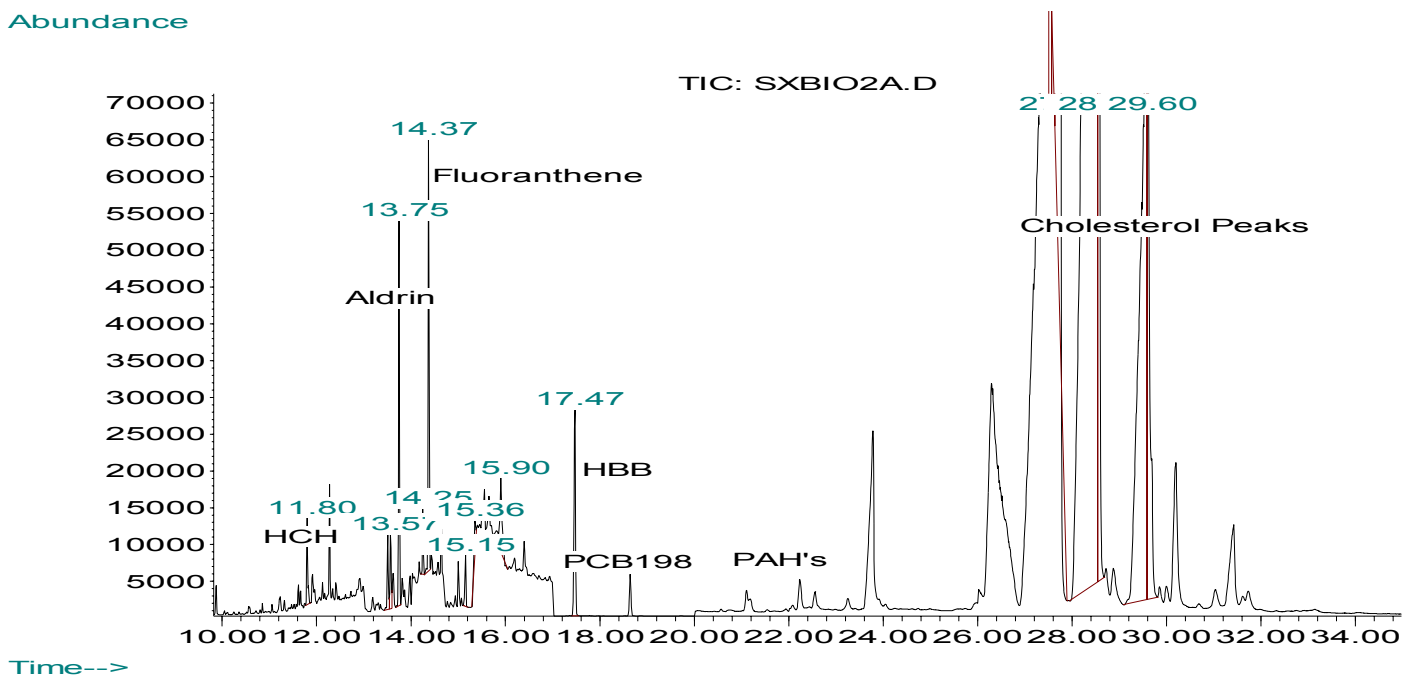## 10.1 Calibration Standard 50µg/kg dried weight
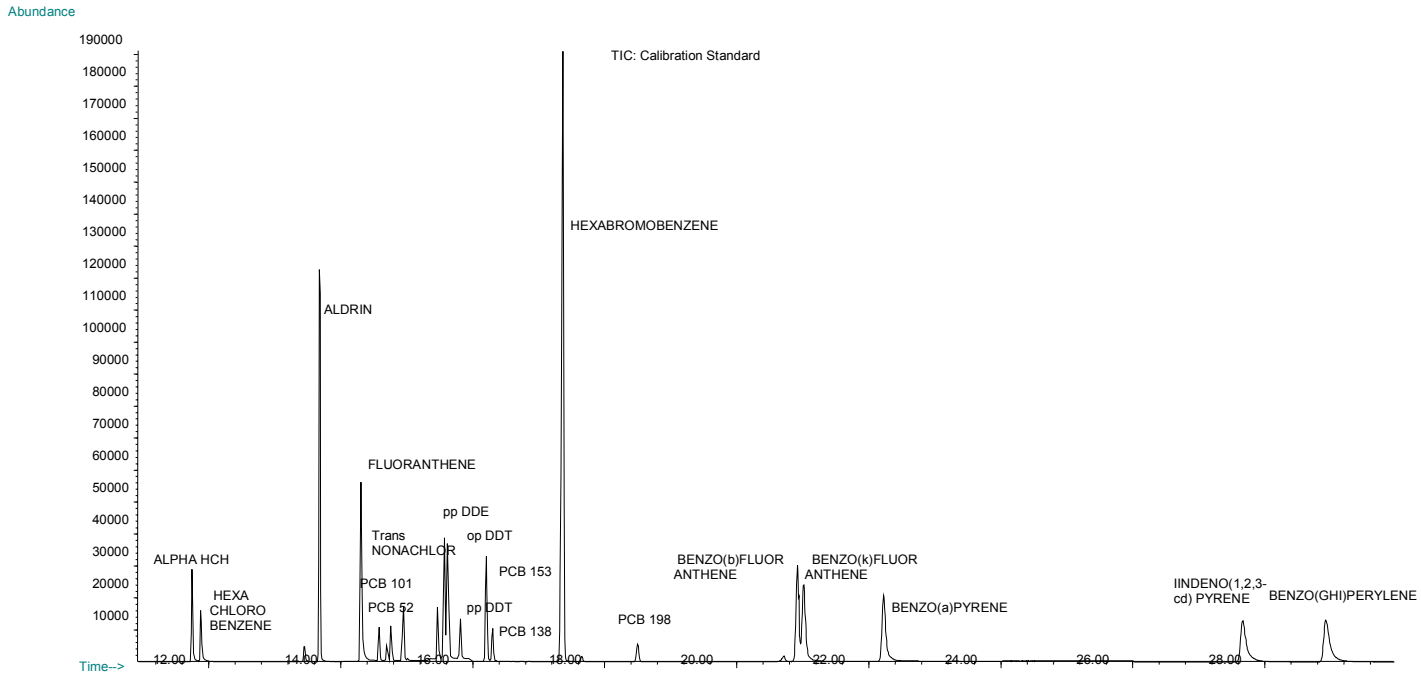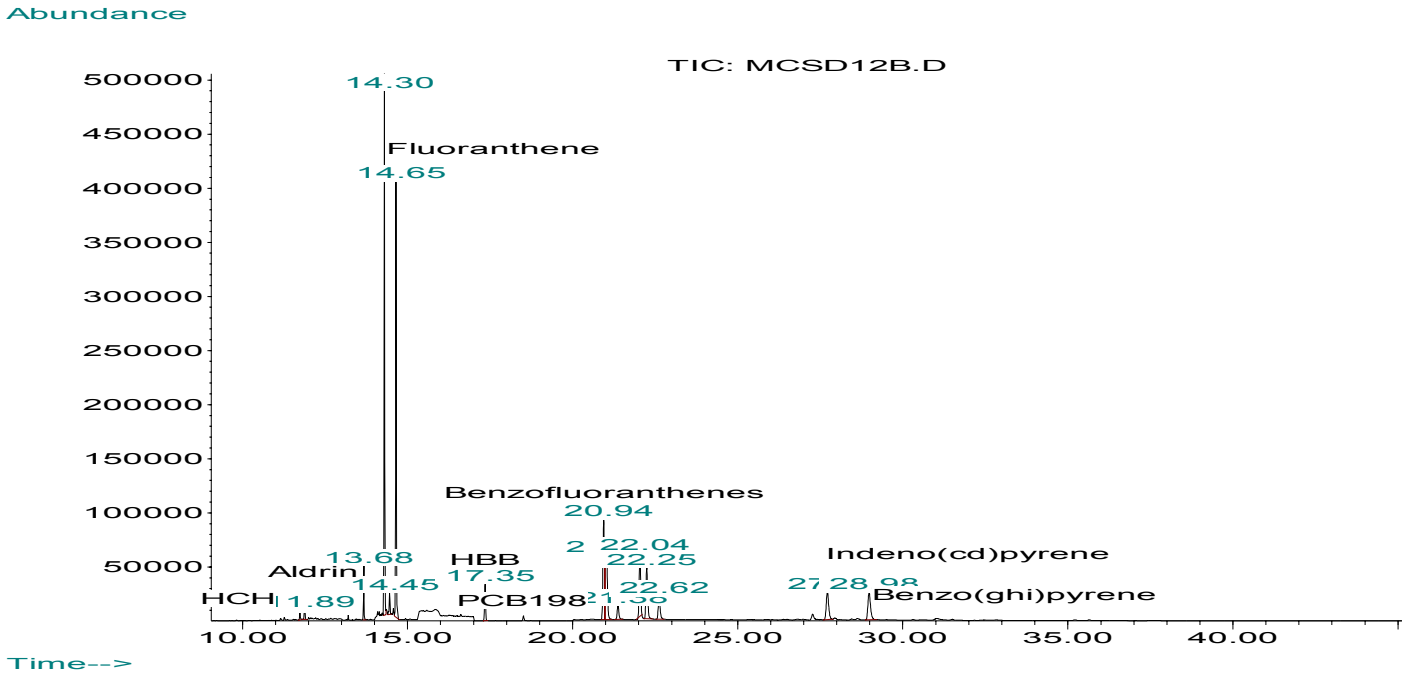

10.2 Blank

10.3 Soxtherm Extraction: Sediment

10.4 Soxtherm Extraction: Biota

10.5 Microwave Extraction: Sediment

Abundance

TIC: MCSD12B.D

14.30

Fluoranthene

14.65

Benzofluoranthenes
20.94

2 22.04
22.25

Aldrin
13.68

HBB
17.35

Indeno(cd)pyrene
27 28.98

14.45
HCH 1.89
PCB198 21.36
22.62
Benzo(ghi)pyrene

Time-->

10.6 Microwave Extraction: Biota

Abundance

TIC: MCBIO9A.D

13.67

Aldrin

28 29.17

Chlolesterol Peaks

13.22

Fluoranthene

14.31

14.65

Hexabromobenzene

11.89

29.79

11.74

15.81

PAH's
22.35

28.47

HCH

16.30

18.50

PCB198 22.07

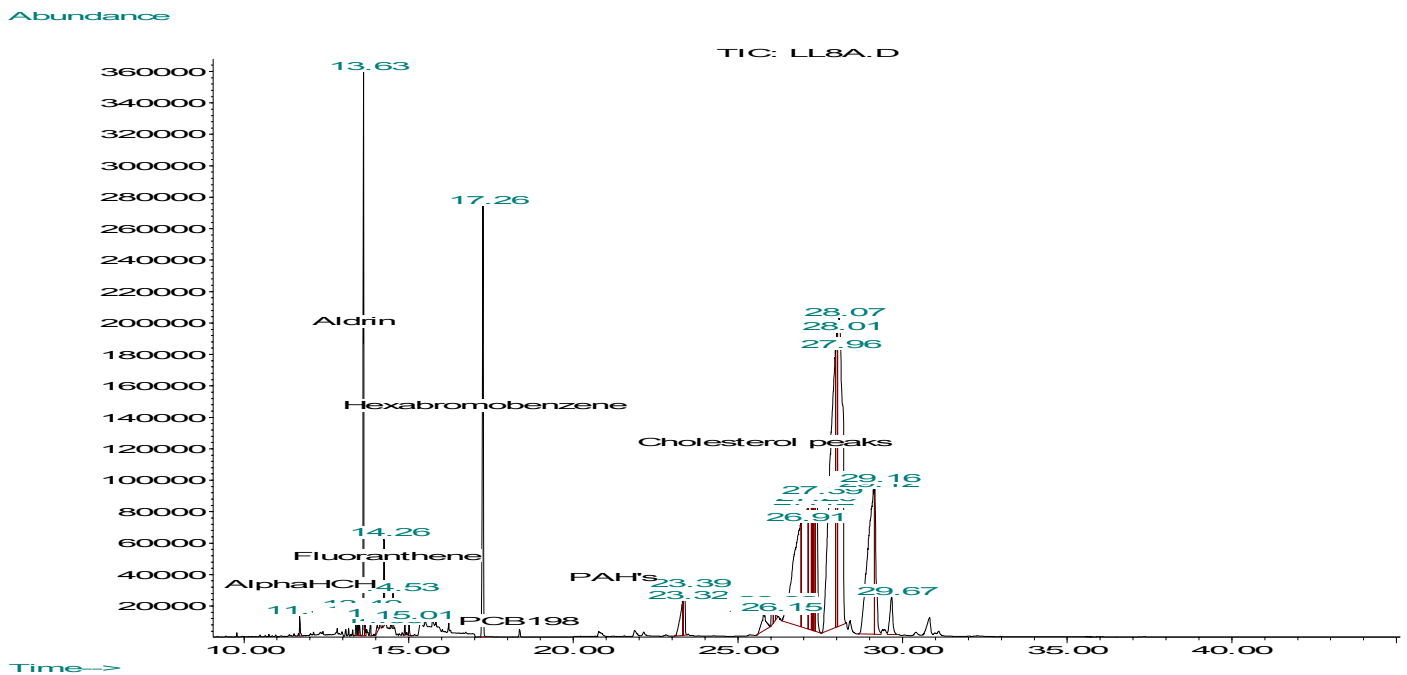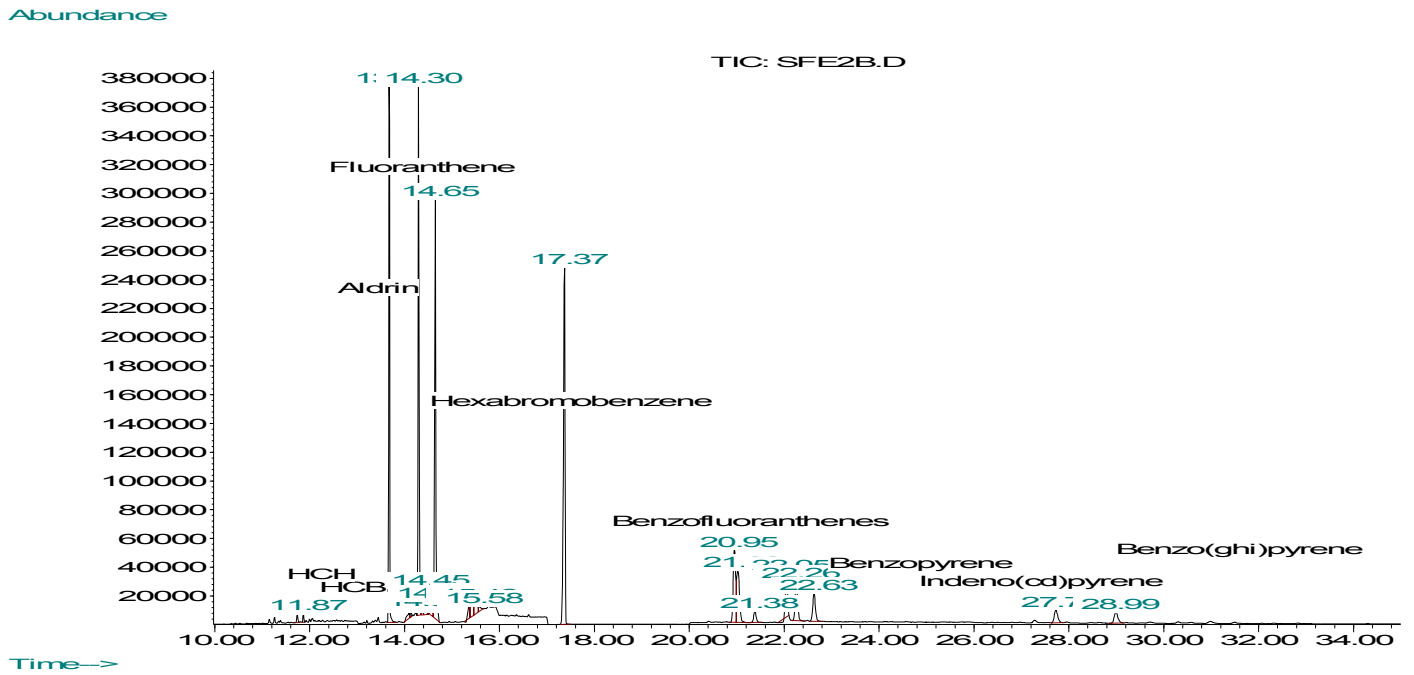Time—>
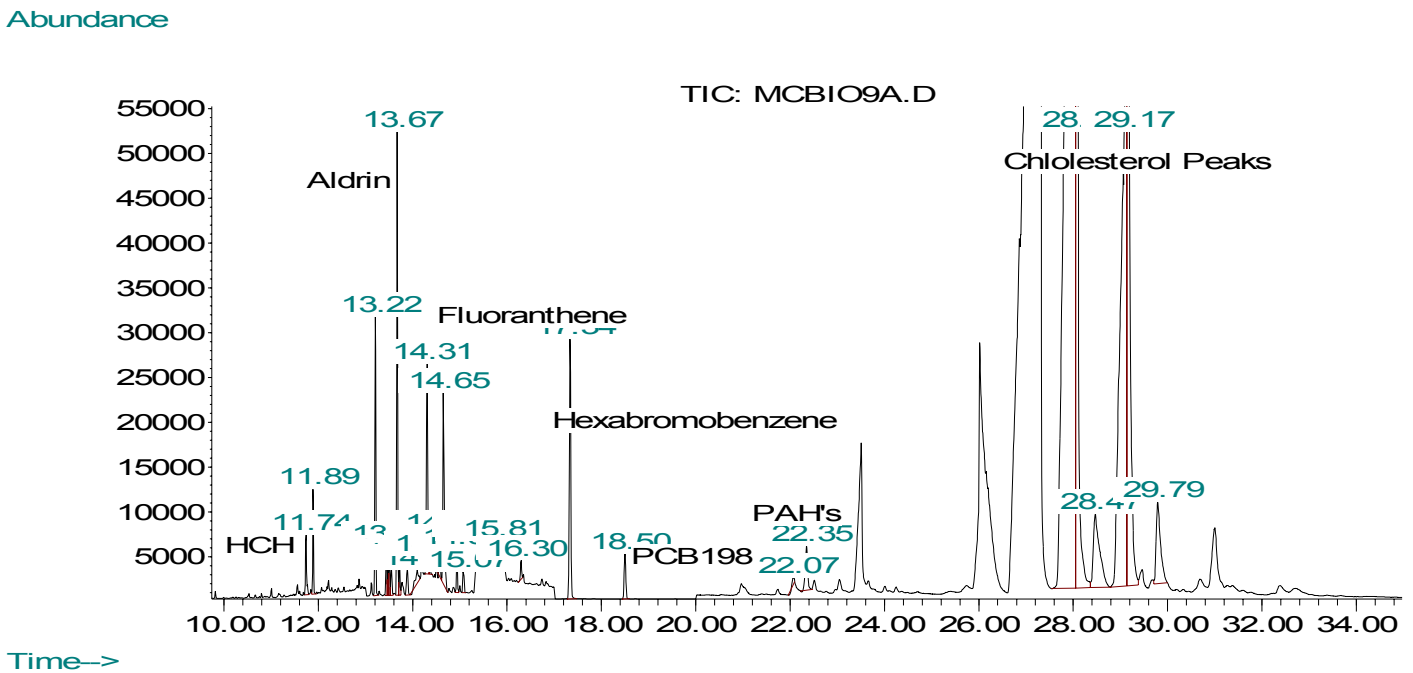
10.7 Accelerated Solvent Extraction (ASE): Sediment
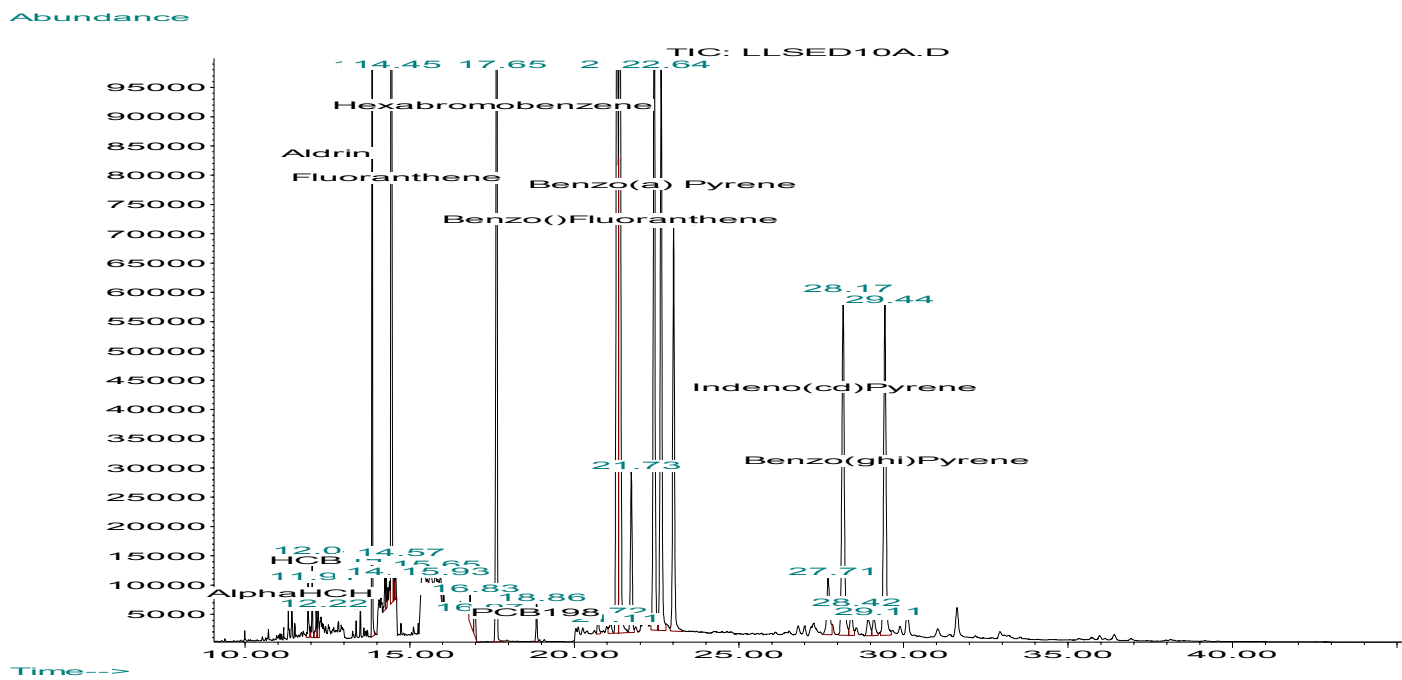
10.8 Accelerated Solvent Extraction (ASE): Biota

10.9 SuperFluid Critical Extraction (SFE): Sediment

Abundance

TIC: SFE2B.D

Fluoranthene

Aldrin

Hexabromobenzene

Benzofluoranthenes

Benzopyrene

Benzo(ghi)pyrene

Indeno(cd)pyrene

HCH

HCB

10.10 SuperFluid Critical Extraction (SFE): Blank

Abundance

TIC: SFEBK2.D

Aldrin

Hexabromobenzene

HCH