Australian Government

National Measurement Institute

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BP Data Validation Project Report

CONTENTS

1. INTRODUCTION

The NMI is Australia's principal metrological authority which carries with it the responsibility for maintaining and improving the nation's units and standards of measurement. The NMI achieves this through innovative development of new standards, new techniques and new reference materials underpinned by vigorous participation and collaboration in the international sphere of metrology. The international traceability of Australia's measurement system is guaranteed through it links with the International Bureau of Weights and Measures and other national and international measurement organisations.

The NMI Laboratory at The ARRC Building in Kensington, Western Australia is accredited by the National Association of Testing Authorities (NATA) to the International Laboratory Standard ISO/IEC 17025:2005 and its management systems are certified to AS/NZS ISO 9001:2008. Through the development of oil spill identification capabilities in the early 1990's and the establishment of a section dedicated to the analysis of organic compounds in environmental matrices, NMI has achieved a solid reputation in the field of environmental petroleum hydrocarbon-related analyses. This expertise has been called upon by many government agencies and environmental consultants during the past 20 years.

In January 2011 the NMI was requested by CSIRO Earth Science and Resource Engineering, as a qualified, independent and impartial laboratory, to collaborate on a project to assess and validate the methodology used to acquire analytical data from samples taken from the Gulf of Mexico.

2. OBJECTIVES

The purpose of this collaborative project was to provide CSIRO with a method to quantify the sample data to an acceptable industry standard. The objectives of the work to be performed at NMI Kensington were discussed and agreed between NMI and CSIRO prior to commencement of any analytical activity. The agreed objectives were as follows:

- To replicate the Gas Chromatography/Mass Spectrometer (GC/MS) instrumental conditions used in the field on the same GC/MS instrument set up in the CSIRO ARRC laboratory and validate the performance of the instrumental parameters.

- To set up GC/MS processing methods for the quantification of data collected in the Gulf in both full scan and Selected Ion Monitoring (SIM) modes.

- To validate the extraction method used by CSIRO in the field (listed in Appendix B) and assess the extraction efficiency and recovery of a predetermined list of analytes.

- To estimate the associated uncertainties of the analytical results.

3. ACTIONS

In order to address these project objectives, a number of actions were proposed by NMI Officers. They were:

(a) Preparation of artificial sea water

- Prepare artificial sea water in accordance with the procedure in Appendix A for validation. Measure 930 mL of sea water into 1 L amber glass containers for the recovery trials. Analyse the prepared seawater for contamination.

(b) Calibration

- Inject Benzene, Toluene, Ethylbenzene, Xylene/Total petroleum hydrocarbon (BTEX/TPH) calibration standards using the existing CSIRO acquisition methods to establish the linearity of the instrument and produce calibration curves.
- Include the deuterated surrogates and internal standard in the calibration standard mix to determine the relative response ratios for these compounds.
- Responses ratios for analytes not included in the calibration mix will be assigned response ratios corresponding to analytes of similar nature.
- Use appropriate factors to re-calculate the results.

(c) Analysis

- Spike three sets of seven prepared seawater samples with different concentrations of the CSIRO surrogate mix and the NMI recovery standard mix. The samples will be extracted according to the CSIRO procedure.

- The spike levels will be chosen to be at concentrations close to the practical quantification limit, a mid range and a high level typical of those found in the samples.

- The surrogate mix will be maintained at a concentration close to that of the undiluted surrogate mix used by the CSIRO scientists.

- Measure and record the volume of dichloromethane remaining after the extraction procedure for each sample.

- Add internal standard to an aliquot of the extract and inject onto the CSIRO GC/MS using their original full scan acquisition method.

(d) Verification

- Estimate the associated uncertainties in concentrations.

4. EXPERIMENTAL DESIGN AND TIMELINE

4.1 Method

The above actions (a)-(c) and partial performance of Action (d) were carried out in the period between February and April 2011. Data analysis continued until the production of the final report in July 2011.

Forty litres of artificial seawater water were prepared using the method in Appendix A in February 2011. A 930 mL aliquot was analysed before any validation work was performed to ensure that the water was free of contamination and suitable for the purpose. The prepared blank sea water was spiked in replicates of seven at each of the three concentration levels 5-11 ug/L, 11-22 ug/L and 43-110 ug/L for the various compounds detailed in Appendix C. These were then extracted in the 1 L amber glass containers with 15 mL of dichloromethane (vigorously shaken for 30 seconds, rested and shaken again for 1 min) and transferred to a 1L separating funnel for ease of separating the dichloromethane phase. The dichloromethane phase was removed and dried over anhydrous sodium sulphate into a 20 mL measuring cylinder. Measurement of the volume of each of the dichloromethane extracts was taken (see Appendix D).

A 1 mL aliquot of the dichloromethane extract was transferred to a GC vial and 2 µL of internal standard mix was added before injection on the GC/MS.

4.2 GC/MS Instrument Method

A series of standards of various concentrations (Appendix C) was injected on the same GC/MS instrument (Agilent 7890A GC and 5975C inert XL MSD) that was used in the field, in order to establish the instrument linearity and produce a calibration curve for quantification. The injector liner was changed as the responses for some of the calibration compounds were considered to be too low to produce satisfactory calibration curves. No additional optimisation of the instrument was performed.

The validation was performed in both full scan and selected ion monitoring (SIM) modes. Details of the instrument acquisition conditions are presented in Table 1. The instrument conditions are the same as those used in the field. The SIM acquisition method was altered to include the m/z 252, 276 and 278 ions so that all of the PAH compounds in the calibration standard were acquired.

Table 1 NMI GC/MS instrument acquisition parameters for both full scan and selected ion monitoring modes

GC Column:	J&W DB-5MS (60 m x 0.25 mm x 0.25 μm)			
GC Carrier gas:	Helium			
GC Conditions:	Column constant pressure:	25 psi		
	Injection mode:	Splitless		
	Injector temp:	310 °C		
	Injection volume:	1 μL		
	Initial oven temp:	40 °C		
	Initial oven hold time:	2 minutes		
	Oven program rate:	8 °C/minute		
	Oven final temp:	310 °C		
	Oven hold time:	30 minutes		
	Total run time	65.75 minutes		
MSD Full scan mode:	Interface Temperature:	300 °C		
	Mass range:	30 to 300 m/z		
	Acquisition time:	65.75 minutes		
	Filament/multiplier delay:	6.0 minutes		
MSD SIM mode:	Interface Temperature:	300 °C		
	Acquisition time:	65.75 minutes		
	Filament/multiplier delay:	6.0 minutes		
	Ion Group	Start Time (min)	Mass	Dwell Time (μs)
	Group 1	6.00	57	100
			92	100
			100	100
			106	100
			120	100
	Group 2	15.00	128	80
			136	80
			142	80
			152	80
			154	80
			156	80
			166	80
			170	80
			184	80
	Group 3	27.70	57	75
			66	75
			178	75
			184	75
			188	75
			192	75
			198	75
			202	75
			206	75
			212	75
			228	75
			230	75
			252	75
			276	75
			278	75

4.3 Quantification Method

The Agilent Chemstation software was used for quantification of all results. Quantification was based on internal standard corrected target ion area counts and retention times of reference compounds. Analytes were confirmed by retention time and qualifier ion data. p-Terphenyl was used as the internal standard and Toluene-D8, Naphthalene-D8 and Phenanthrene-D10 as surrogates. For those target analytes where a reference material was not available, the retention time, quantifier ion and qualifier ions supplied by CSIRO were used.

Table 2 lists the analytes in the full scan quantification method and their associated parameters. The SIM processing method used the same retention times and target ions as the full scan method. The compound identification by the SIM quantification method did not make use of qualifier ions.

Reference standards were not available for all compounds of interest. In these cases, reference standards of similar type were used for quantification. These are listed in Table 3.

The analyte identifications and associated parameters for those compounds listed in Tables 2 and 3 for which no calibration standard was available were not verified by NMI.

Table 2 Full scan processing method parameters.

Analyte	Retention Time (min)	Target ion m/z (quantification)	Qualifier ion(s) m/z (confirmation)
p-Terphenyl (INTERNAL STANDARD)	33.969	230	-
nC7	6.331	57	71
Toluene-D8	7.777	98	100, 70
Toluene	7.873	91	92, 65
nC8	8.54	57	85
Ethylbenzene	10.104	91	106
m + p-Xylene	10.16	91	106, 105, 77
o-Xylene	10.891	91	106, 105, 77
nC9	10.97	57	85
iPB	10.66	120	105
nPB	12.4	120	105
1-Methyl-3-ethylbenzene	12.58	120	105
1-Methyl-4-ethylbenzene	12.66	105	120
1,3,5-Trimethylbenzene	12.77	120	105
1-Methyl-2-ethylbenzene	13.01	120	105
1,2,4-Trimethylbenzene	13.4	120	105
nC10	13.4	57	85
1,2,3-Trimethylbenzene	14.07	120	105
nC11	15.72	57	85
nC12	17.829	57	43, 71, 85
Naphthalene-D8	17.877	136	137
Naphthalene	17.946	128	127, 129
iC13	18.11	57	85
iC14	19.29	57	85
nC13	19.87	57	85
2 + 1-Ethylnaphthalene	20.09	156	141
2-Methlynaphthalene	20.21	142	115
iC15	21.3	57	85
1-Methylnaphthalene	20.536	142	115
nC14	21.75	57	85
Biphenyl	21.767	154	153
2,6-Dimethylnaphthalene	22.3	156	141
2,7-Dimethylnaphthalene	22.34	156	141
1,3 + 2,7-Dimethylnaphthalene	22.57	156	141
1,6-Dimethylnaphthalene	22.66	156	141
iC16	22.82	57	85
1,4 + 2,3-Dimethylnaphthalene	22.96	156	141
1,5-Dimethylnaphthalene	23.06	156	141
nC15	23.53	57	85
1,2-Dimethylnaphthalene	23.23	156	141
Acenaphthylene	23.222	152	151, 150, 153
Acenaphthene	23.797	153	154, 152, 76
1,3,7-Trimethlynaphthalene	24.38	170	155
1,3,6-Trimethylnaphthalene	24.52	170	155
1,3,5 + 1,4,6-Trimethylnaphthalene	24.76	170	155

Table 2 Full scan processing method parameters (Cont.).

Analyte	Retention Time (min)	Target ion m/z (quantification)	Qualifier ion(s) m/z (confirmation)
2,3,6-Trimethylnaphthalene	24.86	170	155
1,2,7 + 1,6,7-Trimethylnaphthalene	25.07	170	155
1,2,6-Trimethylnaphthalene	25.12	170	155
nC16	25.19	57	85
1,2,4-Trimethylnaphthalene	25.37	170	155
1,2,5-Trimethylnaphthalene	25.51	170	155
Fluorene	25.514	166	165, 163
iC18	25.92	57	85
nC17	26.76	57	85
Pristane	26.81	57	183
1,3,6,7-Tetramethylnaphthalene	26.76	184	169
1,2,4,6 + 1,2,4,7 + 1,4,6,7-TeMN	27.01	184	169
1,2,5,7-Tetramethylnaphthalene	27.08	184	169
2,3,6,7-Tetramethylnaphthalene	27.22	184	169
1,2,6,7-Tetramethylnaphthalene	27.36	184	169
1,2,3,7-Tetramethylnaphthalene	27.42	184	169
1,2,3,6-Tetramethylnaphthalene	27.54	184	169
1,2,5,6 + 1,2,3,5-TeMN	27.81	184	169
nC18	28.25	57	85
Phytane	28.35	57	183
Dibenzothiophene	28.29	184	139, 152
Phenanthrene-D10	28.648	188	187, 189, 184
Phenanthrene	28.724	178	176, 179, 177
Anthracene	28.895	178	176, 179, 89
nC19	29.66	57	85
4-Methyldibenzothiophene	29.77	198	197
2 + 3-Methyldibenzothiophene	30.04	198	197
1-Methyldibenzothiophene	30.36	198	197
3-Methylphenanthrene	30.33	192	191
2-Methylphenanthrene	30.43	192	191
9-Methylphenanthrene	30.69	192	191
1-Methylphenanthrene	30.77	192	191
nC20	30.968	57	71, 43, 85
4-Ethyldibenzothiophene	31	212	165
4,6-Dimethyldibenzothiophene	31.11	212	165
2,4-Dimethyldibenzothiophene	31.3	212	165
2,6 + 3,6-Dimethyldibenzothiophene	31.41	212	165
3,7 + 1,4-Dimethyldibenzothiophene	31.65	212	165
1,6 + 1,8-Dimethyldibenzothiophene	31.75	212	165
1,3-Dimethyldibenzothiophene	31.9	212	165
1,9 + 1,2-Dimethyldibenzothiophene	31.96	212	165
3-Ethylphenanthrene	31.56	206	191
9 + 2 + 1-Ethylphenanthrene + 3,6-DMP	31.8	206	191
3,5 + 2,6-Dimethylphenanthrene	31.91	206	191

Table 2 Full scan processing method parameters (Cont.).

Analyte	Retention Time (min)	Target ion m/z (quantification)	Qualifier ion(s) m/z (confirmation)
2,7-Dimethylphenanthrene	31.98	206	191
1,3 + 3,9 + 3,10 + 2,10-DMP	32.15	206	191
1,6 + 2,9 + 2,5-DMP	32.26	206	191
nC21	32.28	57	85
1,7-Dimethylphenanthrene	32.34	206	191
2,3 + 1,9 + 4,9 + 4,10-DMP	32.46	206	191
1,8-Dimethylphenanthrene	32.69	206	191
1,2-Dimethylphenanthrene	32.89	206	191
Fluoranthene	32.665	202	200, 203, 101
Pyrene	33.425	202	200, 203, 201
nC22	33.5	57	85
nC23	34.67	57	85
nC24	35.79	57	85
nC25	36.9	57	85
Benz(a)anthracene	37.407	228	226, 229, 114
Chrysene	37.537	228	226, 229, 113
nC26	38.06	57	85
nC27	39.27	57	85
nC28	40.62	57	85
Benzo(b)fluoranthene	41.657	252	250, 253, 126
Benzo(k)fluoranthene	41.787	252	250, 253, 126
nC29	42.12	57	85
nC30	43.85	57	85
nC31	45.88	57	85
nC32	48.112	57	85
Indeno(1,2,3,c,d)pyrene	49.862	276	277, 138, 274
Dibenz(a,h)anthracene	49.992	278	279, 139
nC33	51.14	57	85
Benzo(g,h,i)perylene	51.805	276	138, 277, 274

Table 3 Analytes and associated reference standards used for quantification.

Analyte	Retention Time (min)	Reference standard used for quantification
p-Terphenyl	33.969	p-Terphenyl
nC7	6.331	nC7
Toluene-D8	7.777	Toluene-D8
Toluene	7.873	Toluene
nC8	8.54	nC7
Ethylbenzene	10.104	Ethylbenzene
m + p-Xylene	10.16	m + p-Xylene
o-Xylene	10.891	o-Xylene
nC9	10.97	nC7
iPB	10.66	nC7
nPB	12.4	nC7
1-Methyl-3-ethylbenzene	12.58	o-Xylene
1-Methyl-4-ethylbenzene	12.66	o-Xylene
1,3,5-Trimethylbenzene	12.77	o-Xylene
1-Methyl-2-ethylbenzene	13.01	o-Xylene
1,2,4-Trimethylbenzene	13.4	o-Xylene
nC10	13.4	nC7
1,2,3-Trimethylbenzene	14.07	o-Xylene
nC11	15.72	nC7
nC12	17.829	nC12
Naphthalene-D8	17.877	Naphthalene-D8
Naphthalene	17.946	Naphthalene
iC13	18.11	nC12
iC14	19.29	nC12
nC13	19.87	nC12
2 + 1-Ethylnaphthalene	20.09	2-Methlynaphthalene
2-Methlynaphthalene	20.21	2-Methlynaphthalene
iC15	21.3	nC12
1-Methylnaphthalene	20.536	1-Methylnaphthalene
nC14	21.75	nC12
Biphenyl	21.767	Biphenyl
2,6-Dimethylnaphthalene	22.3	1-Methylnaphthalene
2,7-Dimethylnaphthalene	22.34	1-Methylnaphthalene
1,3 + 2,7-Dimethylnaphthalene	22.57	1-Methylnaphthalene
1,6-Dimethylnaphthalene	22.66	1-Methylnaphthalene
iC16	22.82	nC12
1,4 + 2,3-Dimethylnaphthalene	22.96	1-Methylnaphthalene
1,5-Dimethylnaphthalene	23.06	1-Methylnaphthalene
nC15	23.53	nC12
1,2-Dimethylnaphthalene	23.23	1-Methylnaphthalene
Acenaphthylene	23.222	Acenaphthylene
Acenaphthene	23.797	Acenaphthene
1,3,7-Trimethlynaphthalene	24.38	1-Methylnaphthalene
1,3,6-Trimethylnaphthalene	24.52	1-Methylnaphthalene

Table 3 Analytes and associated reference standards used for quantification (Cont.).

Analyte	Retention Time (min)	Reference standard used for quantification
1,3,5 + 1,4,6-Trimethylnaphthalene	24.76	1-Methylnaphthalene
2,3,6-Trimethylnaphthalene	24.86	1-Methylnaphthalene
1,2,7 + 1,6,7-Trimethylnaphthalene	25.07	1-Methylnaphthalene
1,2,6-Trimethylnaphthalene	25.12	1-Methylnaphthalene
nC16	25.19	nC20
1,2,4-Trimethylnaphthalene	25.37	1-Methylnaphthalene
1,2,5-Trimethylnaphthalene	25.51	1-Methylnaphthalene
Fluorene	25.514	Fluorene
iC18	25.92	nC20
nC17	26.76	nC20
Pristane	26.81	nC20
1,3,6,7-Tetramethylnaphthalene	26.76	1-Methylnaphthalene
1,2,4,6 + 1,2,4,7 + 1,4,6,7-TeMN	27.01	1-Methylnaphthalene
1,2,5,7-Tetramethylnaphthalene	27.08	1-Methylnaphthalene
2,3,6,7-Tetramethylnaphthalene	27.22	1-Methylnaphthalene
1,2,6,7-Tetramethylnaphthalene	27.36	1-Methylnaphthalene
1,2,3,7-Tetramethylnaphthalene	27.42	1-Methylnaphthalene
1,2,3,6-Tetramethylnaphthalene	27.54	1-Methylnaphthalene
1,2,5,6 + 1,2,3,5-TeMN	27.81	1-Methylnaphthalene
nC18	28.25	nC20
Phytane	28.35	nC20
Dibenzothiophene	28.29	Dibenzothiophene
Phenanthrene-D10	28.648	Phenanthrene-D10
Phenanthrene	28.724	Phenanthrene
Anthracene	28.895	Anthracene
nC19	29.66	nC20
4-Methyldibenzothiophene	29.77	Dibenzothiophene
2 + 3-Methyldibenzothiophene	30.04	Dibenzothiophene
1-Methyldibenzothiophene	30.36	Dibenzothiophene
3-Methylphenanthrene	30.33	Phenanthrene
2-Methylphenanthrene	30.43	Phenanthrene
9-Methylphenanthrene	30.69	Phenanthrene
1-Methylphenanthrene	30.77	Phenanthrene
nC20	30.968	nC20
4-Ethyldibenzothiophene	31	Dibenzothiophene
4,6-Dimethyldibenzothiophene	31.11	Dibenzothiophene
2,4-Dimethyldibenzothiophene	31.3	Dibenzothiophene
2,6 + 3,6-Dimethyldibenzothiophene	31.41	Dibenzothiophene
3,7 + 1,4-Dimethyldibenzothiophene	31.65	Dibenzothiophene
1,6 + 1,8-Dimethyldibenzothiophene	31.75	Dibenzothiophene
1,3-Dimethyldibenzothiophene	31.9	Dibenzothiophene
1,9 + 1,2-Dimethyldibenzothiophene	31.96	Dibenzothiophene
3-Ethylphenanthrene	31.56	Phenanthrene
9 + 2 + 1-Ethylphenanthrene + 3,6-DMP	31.8	Phenanthrene
3,5 + 2,6-Dimethylphenanthrene	31.91	Phenanthrene
2,7-Dimethylphenanthrene	31.98	Phenanthrene

Table 3 Analytes and associated reference standards used for quantification (Cont.).

Analyte	Retention Time (min)	Reference standard used for quantification
1,3 + 3,9 + 3,10 + 2,10-DMP	32.15	Phenanthrene
1,6 + 2,9 + 2,5-DMP	32.26	Phenanthrene
nC21	32.28	nC20
1,7-Dimethylphenanthrene	32.34	Phenanthrene
2,3 + 1,9 + 4,9 + 4,10-DMP	32.46	Phenanthrene
1,8-Dimethylphenanthrene	32.69	Phenanthrene
1,2-Dimethylphenanthrene	32.89	Phenanthrene
Fluoranthene	32.665	Fluoranthene
Pyrene	33.425	Pyrene
nC22	33.5	nC20
nC23	34.67	nC20
nC24	35.79	nC20
nC25	36.9	nC20
Benz(a)anthracene	37.407	Benz(a)anthracene
Chrysene	37.537	Chrysene
nC26	38.06	nC20
nC27	39.27	nC20
nC28	40.62	nC20
Benzo(b)fluoranthene	41.657	Benzo(b)fluoranthene
Benzo(k)fluoranthene	41.787	Benzo(k)fluoranthene
nC29	42.12	nC32
nC30	43.85	nC32
nC31	45.88	nC32
nC32	48.112	nC32
Indeno(1,2,3,c,d)pyrene	49.862	Indeno(1,2,3,c,d)pyrene
Dibenz(a,h)anthracene	49.992	Dibenz(a,h)anthracene
nC33	51.14	nC32
Benzo(g,h,i)perylene	51.805	Benzo(g,h,i)perylene

5. TREATMENT OF RESULTS

No quantification of the acquired data was performed in the field by CSIRO. NMI produced internal standard quantification methods with associated calibration curves to quantify this data. In the absence of volumetric measurements a strategy was developed to enable quantification of the samples.

In an internal standard calibration method, the concentrations of the p-Terphenyl internal standard in both the calibration standards and the samples are critical to the determination of accurate results because they are used to normalise the respective responses. The CSIRO p-Terphenyl internal standard was topped up to the mark each day. Therefore a correction factor needs to be applied to each sample to account for the varying concentrations of p-Terphenyl.

5.1 Calculations and correction factor for the analyte concentrations in the sample

For each sample, the Chemstation software calculates the results in ug/mL using the NMI calibration curve and the concentration of the internal standard entered into the method.

This result (concentration in ug/mL) must be multiplied by the CSIRO internal standard concentration (p-terphenyl) and divided by the NMI internal standard concentration of 1.035ug/mL, to give the corrected concentration in ug/mL for the dichloromethane extract.

The correction factor is: CSIRO internal standard concentration ÷ 1.035

The formula for the corrected concentration in the extract is:

$$\text{Corrected Conc (ug/mL)} = \frac{\text{Calc Conc} \times \text{IS Conc}}{1.035}$$

Calc Conc = Concentration calculated from NMI calibration curve in ug/mL.
IS Conc = Concentration of CSIRO internal standard.

The CSIRO surrogate mix composite standard was topped up to the mark each day. This must be taken into account when calculating the surrogate recoveries in the samples. In the absence of volumetric data for sample and extract volumes, the sample surrogate recoveries should be used to correct analyte concentrations.

The formula to calculate the concentration of the analyte in the sample is:

$$\text{Conc in sample (ug/L)} = \frac{(\text{Corrected Conc} \times \text{Spiked Surr Conc}) \div \text{Corrected Surr Conc}}{0.930}$$

Corrected Conc = Concentration of analyte corrected for internal standard in ug/mL.
Spiked Surr Conc = Concentration of surrogate spiking solution in ug/mL.
Corrected Surr Conc = Amount of surrogate recovered in the sample, corrected for internal standard, in ug/mL.

The calculations are based on the following assumptions:

The amount of sample taken for extraction is 930 mL.
The recoveries of the surrogates in the sample and the associated analytes are equivalent. The responses of the analytes for which no reference material was available was assumed to be identical to that of a similar concentration of the reference compound selected for its quantification.

5.2 Calculation of LOR (Limit of Reporting)

The calculation of the LOR is based on the USEPA document "The Method Detection Limit Procedure of the US Environmental Protection Agency" (ref. 7.1). The results of replicate spikes were used to calculate the method detection level, which was then used to derive the method limit of reporting (LOR). The 95% confidence level was used. The lowest spiking level was based on the responses of the lowest calibration standard that could be detected on the GC/MS.

The method detection level (MDL) is calculated by:
$MDL = s \times t_{(n-1,\ 1-\alpha=0.95)}$
Where
n = number of replicate spikes
s = standard deviation of measured concentrations of n spike determinations
t = Student's t value at n-1 degrees of freedom and 1-α (95%) confidence level
(t = 1.943, when n=7 and $\alpha = 0.05$)
α = level of significance

Method LOR = 10 x MDL

A summary of the calculated limits of reporting (LORs) can be found in Appendix E.

5.3 Calculation of Uncertainties

The measurement uncertainties of each analyte are calculated from the following sources:

- Calibration – the calibration curve
- Precision – determined from recovery trials and includes volumetric, gravimetric and most other sources of uncertainty associated with the extraction process.

The following describes how the expanded measurement uncertainty for this test method is estimated. The approach taken is that described in the ISO GUM (ref. 7.2) and the Eurochem Guide (ref. 7.3).

The measurement process for each component can be described by the following equation:

$$C_c = \frac{r_c}{r_i} . \left(\frac{\partial c}{\partial r} \right) . \frac{V_{ext}}{V_{samp}} . d . \int prec$$

where:

C_c = concentration of analyte in test sample

r_c = measured instrument response to the analyte in the test sample

r_i = measured instrument response to the internal standard in the test sample

$\left(\frac{\partial c}{\partial r} \right)$ = change in concentration as a function of response

V_{ext} = volume of extract

V_{samp} = volume of sample

d = dilution factor

$\int prec$ = the effect of measurement precision

In practice, the function $\left(\frac{\partial c}{\partial r} \right)$ is determined experimentally as a calibration curve.

The dilution factor is to account for dilution of the analyte to maintain the response within detector linearity. Other factors that influence the uncertainty include the precision (taken from replicate recovery data). Extract and sample volume errors are incorporated in the precision data and therefore do not require a separate calculation. Only the uncertainty in the amount of internal standard added to the samples needs to be quantified, as the internal standard response uncertainty is incorporated in the calibration curve.

Appendix I presents an example of the process for the calculation of uncertainty values for Naphthalene.

6. RESULTS OF VALIDATION

6.1. Calibration

The coefficients of determination for the calibrations for all reference compounds are presented in Appendix F and G. In the full scan mode they range from 0.9910 to 0.9999. In the SIM mode they range from 0.9922-0.9999.

6.2. Practical limit of reporting

Using the method described in 5.2 the practical limits of reporting are presented in Appendix E.

6.3. Recovery Trials

The NMI conducted a number of recovery experiments to determine the variation in the extraction efficiencies of the method. A calibration curve using mainly Polycyclic Aromatic Hydrocarbons (PAH) calibration standards listed in Appendix C along with the Toluene-D8, Naphthalene-D8 and Phenanthrene-D10 that were used as surrogates by CSIRO, was run to determine the linearity range of the instrument and to quantify the data from the acquired samples. Data for the recovery reference components spiked into artificial seawater are contained in Appendix D.

6.4. Measurement Uncertainty

The sources of the uncertainty in the method are presented in Appendix H. The calculated uncertainties for the compounds for which standards were available are listed in Appendix J.

7. DISCUSSION

The method presented to the NMI for validation was reviewed and a number of practices were found to be of concern.

- The first of these was that 20 mL of sample was poured out from the one litre sample bottle for analysis by some other technique. The risk of losing undissolved oil floating on the surface of the water sample by following this procedure is significant.
- Neither the sample volumes nor the recovered solvent volumes were recorded at the time of analysis. Both of these values as well as the calibration standards would normally be required to quantify the sample components.
- The p-Terphenyl added as an internal standard was the only compound available for quantification. The use of p-Terphenyl as an internal standard in a complex matrix like oil has some disadvantages associated with it. Firstly, there was no way to determine whether the 230m/z ion used to quantify it, came solely from the ionization of the p-terphenyl or whether some component of the signal was due to other background sources from the oil. Secondly, the response of p-Terphenyl differs from those of the surrogates and analytes.

Addition of the internal standard to the same amount of extract prior to injection allowed retrospective calibration and comparison of results from injection to injection.

Squalene, added as a surrogate, could not be used as the molecular ion was not acquired in the initial data acquisition methods.

The solvent delay time was set too long in both the full scan and SIM acquisition methods, thus excluding the collection of data for some early eluting peaks such as Benzene.

The narrow range of ions selected in the SIM acquisition mode precluded the detection of a number of analytes. The only ions detected were those specified in the SIM mode acquisition method, for example, Benzene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Indeno(1,2,3,c,d)perylene, Dibenz(a,h)anthracene and Benzo(g,h,i)perylene were not detected as the ions 78, 228, 252, 276 and 278 were not included in the list.

Calibration standards were not available for all compounds of interest. In such cases the compound was assigned a standard of similar type as listed in Table 3. The different responses of the assigned standard and the compound to the MSD could affect the quantified concentration values.

The response of each surrogate compound in the calibration curve was used to calculate the recovery of the corresponding surrogate in each sample. The recovery values of the surrogates were then applied to all of the compounds in the sample to correct the concentrations. The presence of surrogates in the samples allows for the correction for the various volumes of dichloromethane recovered in the extraction.

The spiking method used for the surrogates in the field was shown to produce varying results when tested in the NMI laboratory. Adding the surrogates and the extracting solvent in quick succession before any agitation of the sample resulted in higher and more

consistent recoveries. The results obtained by the NMI's recovery experiments are therefore considered to be a best case scenario.

A single shakeout with one aliquot of solvent in a bottle with little headspace is not likely to result in the best extraction efficiency. The extraction solvent would be partially dissolved in the sample and adequate partitioning would not occur. The salinity of the matrix affects the solubility of the extraction solvent (dichloromethane) and analytes.

The cleanliness of the GC/MS system affects the response of individual components. This has significant impact on the limit of detection, especially for the later eluting compounds such as Indeno(1,2,3-cd)pyrene and Dibenz(a,h)anthracene. Standards injected at some regular intervals could have been used to monitor the performance of the system.

The calculation for the limits of reporting (LOR) for the various analytes makes use of the replicate recoveries. This can give a distorted view unless the uncertainty at this level is also taken into account. For example, the LOR calculated for Benzo(g,h,i)perylene is 1.5 ug/L, but the uncertainty at 5 ug/L is $\pm$ 10 ug/L (that is, $\pm$ 206 %). The uncertainty value at less than 5 ug/L is not calculated as it is meaningless. The later eluting peaks, that is, those peaks with retention times close to this have low responses and generally do not chromatograph well, especially after injections of several highly contaminated extracts. Thus, the method LOR for Benzo(g,h,i)perylene should be set to about 30-40 ug/L, in line with the later eluting PAHs.

In conclusion, there were a number of shortcomings in the method originally employed in the field. The limitations of the method would contribute significantly to the measurement uncertainty associated with the results. The analytical results can therefore only be considered to be semi-quantitative.

8. REFERENCES

8.1 USEPA 1984, 1997, "The Method Detection Limit Procedure of the US Environmental Protection Agency".

8.2 Guide to the Expression of Uncertainty in Measurement, ISO, Geneva, Switzerland.

8.3 Quantifying Uncertainty in Analytical Measurement, Eurachem, 2nd Edition, www.bam.de/eurochem/publications.htm, 2000.

8.4 USEPA Method 8270: Semi-Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS).

APPENDICES

APPENDIX A: Method for the Preparation of Artificial Seawater.

The method outlined below is based on that described in Kester *et al*., (1967). This method is well known as a standard approach for the preparation of artificial seawater, and is advantageous over other preparations since it is free from organic matter.

The method of Kester *et al*., (1967), uses two types of salts; those that are weighed in anhydrous form (Gravimetric Salts, Table 1) and those that contain water of hydration (Volumetric Salts, Table 2). In order to avoid precipitation of $CaCO_3$, $CaSO_4$, $SrCO_3$ or $SrSO_4$, solutions of gravimetric salts and volumetric salts must be made separately, and only combined after dissolution of all components. The procedure for preparing each solution is detailed below.

Equipment Required

1 x 1 litre Class A volumetric flask with glass stopper
1 x 500 ml Class A measuring cylinder
1 x 100 ml Class A measuring cylinder
1 x 500 ml conical flask or beaker
3 x 15 ml glass vials
1 x 10 ml glass vial
6 x 4 ml glass vials
1 x double ended spatula

Prior to use, all equipment must be thoroughly cleaned to avoid problems of contamination. To do this, each item is rinsed three times with a small quantity of analytical reagent-grade DCM (99.5 % pure). The glass vials, conical flask (or beaker) can also be fired overnight in a furnace at 500 ^{o}C to ensure complete removal of any trace organic impurities. Once cleaned, all items should be covered with aluminium foil until they are required.

Preparation of Solution of Gravimetric Salts

1. Fill a pre-cleaned 1 litre Class A volumetric flask with 500 mL MilliQ water.
2. Weigh the required number of grams of each salt as listed in Table 1 into an appropriately sized pre-cleaned vial (15 mL for NaCl and Na_2SO_4, 4 mL for all other salts).
3. Add each salt to the water in the volumetric flask in turn. Rinse each vial three times with a small quantity of MilliQ water to ensure complete transfer of the entire weight of salt. If any salt adheres to the neck of the volumetric flask when being transferred, wash with a small volume of MilliQ water.
4. Agitate the solution to achieve complete dissolution of each salt before the next is added.
5. Once all the salts have been added, stopper the volumetric flask and set aside.

Table 1. Gravimetric Salts.

Salt	Molecular Weight	Grams required per litre of solution
Sodium chloride, NaCl	58.44	23.926
Sodium sulphate, Na_2SO_4	142.04	4.008
Potassium chloride, KCl	74.56	0.677
Sodium bicarbonate, $NaHCO_3$	84.00	0.196
Potassium bromide, KBr	119.01	0.098
Boric acid, H_3BO_3	61.83	0.026
Sodium fluoride, NaF	41.99	0.003

Preparation of Solution of Volumetric Salts

1. Measure 250 mL MilliQ water into a beaker or conical flask.
2. Weigh the required number of grams of each salt as listed in Table 2 * into an appropriately sized pre-cleaned vial (15 mL for $MgCl_2.6H_2O$, 10 mL for $CaCl_2.2H_2O$ and 4 mL for $SrCl_2.6H_2O$).
3. Add each salt to the water in the conical flask (or beaker) in turn. Rinse each vial three times with a small quantity of MilliQ water to ensure complete transfer of the entire weight of salt.
4. Agitate the solution to achieve complete dissolution of each salt before the next is added.
5. Once all three salts have been mixed together, add this to the solution in the volumetric flask.
6. Rinse the conical flask three times with a small volume of MilliQ water, and add this to the solution in the volumetric flask, to ensure complete transfer of the volumetric salts.
7. Rinse the neck of the volumetric flask to wash any residue into the mixture in the bulb of the flask.
8. Agitate the contents of the flask to mix the two solutions.
9. Add MilliQ water to the volumetric flask so that the total volume reaches 1 litre. Stopper the flask and wrap the seal with either aluminium foil or Teflon tape. Refrigerate until required.

Table 2. Volumetric Salts.

Salt	Molecular Weight	Moles/L of solution	Conc.	G required per litre of solution
Magnesium chloride, $MgCl_2.6H_2O$	203.33	0.05327	1.0 M	10.8314
Calcium chloride, $CaCl_2.2H_2O$	147.03	0.01033	1.0 M	1.5188
Strontium chloride, $SrCl_2.6H_2O$	266.64	0.00009	0.1 M	0.0240

Method for calculation of weight of volumetric salts.

The original method gives the quantities in Moles per kilogram of solution. In this case, 1 kg of pure water is taken to be equivalent to 1 litre. The molecular weight of each salt is taken to represent one Mole, for example, 1 Mole of $MgCl_2.6H_2O$ is assumed to be equal to 203.33 g. The number of Moles per litre of solution can then be multiplied by the mass of one Mole (or, the molecular weight) to give the number of grams required for one litre of solution.

References:

Kester, D.R., Duedall, I.W., Connors, D.N., Pytkowicz, R.M., (1967) Preparation of Artificial Seawater. *Limnology and Oceanography*, 12(1), 176-179.

APPENDIX B: CSIRO Method of Analysis to be Validated.

Liquid/liquid extraction method for sea water samples collected on board the Ryan Chouest following the Deepwater Horizon oil spill May - September 2010.

Standards

D_8 Toluene, d_8 napththalene, d_{10} phenenathrene and deuterated 2,6,10,15,19,23-hexamethyltetracosane were combined in a mixed composite solution and used as surrogates for different groups of compounds with a known amount added to each sample before extraction.

Standards 1,1-binapthyl and p-terphenyl were combined in a second mixed composite solution and used as internal standards. A known amount was added to each sample at the end of the extraction just before injecting into the GC-MS.

The stock solutions were prepared in dichloromethane (DCM) and diluted to 100 mL in a volumetric flask and kept in a fridge.

Standard	Weight (mg)	Vol (mL)	Final Conc (µg/mL)
Toluene d_8	44.25	100	442.5
Naphthalene d_8	50.93	100	509.3
Phenanthrene d_{10}	51.46	100	514.6
2,6,10,15,19,23-Hexamethyltetracosane - d_{62}	53.39	100	533.9
1,1-binaphthyl	51.01	100	510.1
p-terphenyl	53.19	100	531.9

Because there were no facilities on board to weigh standards, only one stock solution was used throughout the whole voyage. Each day, the standards were allowed to come to room temperature and topped up to the volumetric mark. The remaining concentration in the mixed standard was calculated by subtracting the amount added to each sample from the total concentration, the results were kept on a spreadsheet.

Bottle Preparation

Amber glass sample bottles were rinsed three times with tap water, followed by three times with DI water to remove sea salts. The bottles were further rinsed with methanol, (2 x 20 mL) and DCM (2 x 20 mL). Once the DCM was evaporated, the bottles were put in the oven (150 °C) for a few hours, cooled and covered with aluminium foil and capped. The lids were rinsed with the same procedure but were air dried.

Sample Collection and Liquid/Liquid Extraction

1 L samples were collected at different intervals and their details recorded (UTC time, GPS position and observations). Samples were collected either by sub-sampling the outflow stream from the sensor array box or directly from the sea surface by lowering the bottle taped to a pole. A 20 mL aliquot was sub sampled into a scintillation vial. Aluminium foil

was placed on the mouth of the bottles and vials before capping and the scintillation vials were wrapped with Parafilm ® and stored in a fridge.

Once all samples were sub sampled, 20 µl of the deuterated surrogate standard mix was added to each bottle followed by 15 mL of DCM with a dispenser. Aluminium foil was placed on the mouth of the bottle and capped. This was then vigorously shaken for 30 s. The samples were rested and shaken again for 1 min, before allowing the sample to rest for at least 30 min or longer until the DCM separated out to the bottom of the bottle. The DCM phase was removed with a long Pasteur pipette and dried over a minimum amount of anhydrous sodium sulphate in a scintillation vial.

A 1 mL aliquot was transferred to a GC vial and 2 µL of internal standard mix was added.

GC-MS Conditions

The Samples were analysed on an Agilent 7890A GC connected to a 5975C MSD. Samples were injected (0.5 µL) onto a DB-5MS GC column (J&W, 60 m, 0.25 mm I.D., 0.25 µm film thickness) via a split/splitless injector at a constant carrier gas pressure of 25 psi and a temperature of 310 °C. The temperature program started at 40 °C (hold for 2 min.) with an 8 °C min^{-1} ramp to 310 °C (hold for 20 min.).

The MS was run on SIM mode with three different groups for selected compounds as shown in the table below with their approximate retention times.

Group 1			Group 2			Group 3		
m/z	**target**	RT (min.)	**m/z**	**target**	RT (min.)	**m/z**	**target**	RT (min.)
57	aliphatics		57	aliphatics		57	aliphatics	
92	Toluene	7.7	128	naphthalene	17.7	178	phenanthrene	28.5
100	d8-toluene (std)	7.6	136	d8-naphthalene (std)	17.6	184	dibenzothiophene	28.1
106	et-benzene	9.9	142	methylnaphthalenes	19.9-20.4	188	d10-anthracene (std)	28.4
	m/p-xylene	10.2	156	C2-naphthalenes	21.8-23	192	methylphenanthrenes	30-30.6
	o-xylene	10.7	170	C3-naphthalenes	23.2-25.3	198	methyldibenzothiophenes	29.4-30.2
120	C3-benzenes	12.2-14	184	C4-naphthalenes	24.1-27.6	206	C2-phenanthrenes	31.4-32.2
						212	C2-dibenzothiophenes	30.7-31.7
						230	p-terphenyl (std)	33.7

Quantification was carried out by comparison of the areas of individual components with the appropriate internal standard.

APPENDIX C: Concentrations of standards used for the calibration curve

				Linearity standard 6	**Linearity standard 5**	**Linearity standard 4**	**Linearity standard 3**	**Linearity standard 2**	**Linearity standard 1**	**Linearity standard 0**
	mL	Stock concentration	Mix concentration	5 ug/mL	2 ug/mL	1 ug/mL	0.5 ug/mL	0.2 ug/mL	0.1 ug/mL	Blank dichloromethane
Analyte		ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL
Benzene	2	615.8	12.316	6.158	2.463	1.232	0.616	0.246	0.123	0
Toluene	2	567.4	11.348	5.674	2.270	1.135	0.567	0.227	0.113	0
Ethylbenzene	2	531.8	10.636	5.318	2.127	1.064	0.532	0.213	0.106	0
M,p-Xylene	2	528.9	10.578	5.289	2.116	1.058	0.529	0.212	0.106	0
o-Xylene	2	587.1	11.742	5.871	2.348	1.174	0.587	0.235	0.117	0
C7	2	504.1	10.082	5.041	2.016	1.008	0.504	0.202	0.101	0
C12	2	506.8	10.136	5.068	2.027	1.014	0.507	0.203	0.101	0
C20	2	502.8	10.056	5.028	2.011	1.006	0.503	0.201	0.101	0
C32	2	509.7	10.194	5.097	2.039	1.019	0.510	0.204	0.102	0
Naphthalene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
1-Methylnaphthalene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
2-Methylnaphthalene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Acenaphthylene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Acenaphthene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Fluorene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Phenanthrene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Anthracene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Fluoranthene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Pyrene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Benz(a)anthracene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Chrysene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Benzo(b,k)fluoranthene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Indeno(1,2,3-cd)pyrene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Dibenz(a,h)anthracene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Benzo(g,h,i)perylene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Dibenzothiophene	10	100.0	10.00	5.000	2.000	1.000	0.500	0.200	0.100	0
Biphenyl	10	101.7	10.17	5.085	2.034	1.017	0.509	0.203	0.102	0
CSIRO surrogate mix										
d8-Toluene	2	534.0	10.68	5.340	2.136	1.068	0.534	0.214	0.107	0

				Linearity standard 6	**Linearity standard 5**	**Linearity standard 4**	**Linearity standard 3**	**Linearity standard 2**	**Linearity standard 1**	**Linearity standard 0**
(Appendix C continued)										
	mL	Stock concentration	Mix concentration	5 ug/mL	2 ug/mL	1 ug/mL	0.5 ug/mL	0.2 ug/mL	0.1 ug/mL	Blank dichloromethane
d8-Naphthalene	2	544.0	10.88	5.440	2.176	1.088	0.544	0.218	0.109	0
D10-Phenanthrene	2	507.5	10.15	5.075	2.030	1.015	0.508	0.203	0.102	0
Internal standards										
1,1binaphthyl		515.5								
p-terphenyl		517.8								

APPENDIX D: Summary of Results of Recovery Trials

(i) Summary of concentrations spiked into 930 mL of prepared blank artificial seawater, average recoveries, standard deviations and relative standard deviations:

Compound Name	expected recovery in 15mL DCM ug/mL (5ug spike) in 930mL sample (ug/L)	5ug spike				expected recovery in 15mL DCM ug/mL (10ug spike) in 930mL sample (ug/L)	10ug spike				expected recovery in 15mL DCM ug/mL (50ug spike) in 930mL sample (ug/L)	50ug spike		
		Average (%)	Std Dev	RSD (%)			Average (%)	Std Dev	RSD (%)			Average (%)	Std Dev	RSD (%)
nC7	10.84	81.73	18.93	23%		21.68	124.33	9.46	8%		108.41	86.60	15.71	18%
Toluene-D8	5.74	57.65	11.45	20%		11.48	81.02	12.70	16%		57.42	38.03	25.86	68%
Toluene	6.10	67.59	11.52	17%		12.20	90.85	10.14	11%		61.01	85.91	12.90	15%
Ethylbenzene	5.72	59.92	10.47	17%		11.44	79.77	8.08	10%		57.18	77.50	11.32	15%
m + p-Xylene	5.69	64.82	11.84	18%		11.37	83.65	8.01	10%		56.87	80.24	11.52	14%
o-Xylene	6.31	57.07	9.94	17%		12.63	76.82	7.81	10%		63.13	75.76	10.95	14%
nC12	10.90	50.31	7.56	15%		21.80	64.64	5.15	8%		108.98	68.23	7.77	11%
Naphthalene-D8	5.85	40.18	8.27	21%		11.70	50.24	8.45	17%		58.49	30.23	17.46	58%
Naphthalene	5.38	51.61	9.99	19%		10.75	57.54	6.42	11%		43.01	55.46	7.29	13%
2 Methyl Naphthalene														
1 Methyl Naphthalene														
Biphenyl														
Acenaphthylene	5.38	55.88	10.38	19%		10.75	50.14	5.57	11%		43.01	57.14	7.42	
Acenaphthene	5.38	55.70	9.81	18%		10.75	52.90	4.69	9%		43.01	58.25	7.25	12%
Fluorene	5.38	56.85	9.86	17%		10.75	49.43	4.47	9%		43.01	58.33	6.98	12%
Dibenzothiophene														
Phenanthrene-D10	5.46	54.12	7.44	14%		10.91	51.39	7.31	14%		54.57	68.59	8.55	12%
Phenanthrene	5.38	58.32	9.01	15%		10.75	57.29	4.63	8%		43.01	59.60	6.64	11%
Anthracene	5.38	62.55	10.38	17%		10.75	54.23	5.15	10%		43.01	60.51	6.47	11%
nC20	10.81	58.21	6.62	11%		21.63	61.78	5.35	9%		108.13	75.57	7.60	10%
Fluoranthene	5.38	62.65	10.02	16%		10.75	56.37	4.85	9%		43.01	60.78	6.16	10%
Pyrene	5.38	62.52	10.05	16%		10.75	55.20	4.76	9%		43.01	59.03	6.01	10%
Benz[a]anthracene	5.38	74.63	20.35	27%		10.75	59.16	5.53	9%		43.01	49.74	5.78	12%
Chrysene	5.38	61.49	10.14	16%		10.75	46.23	3.95	9%		43.01	58.27	5.34	9%
Benzo(b)fluoranthene	5.38	90.40	28.92	32%		10.75	64.77	6.76	10%		43.01	44.93	6.12	14%
Benzo(k)fluoranthene	5.38	98.11	21.94	22%		10.75	64.62	6.03	9%		43.01	89.81	8.30	9%
nC32	10.96	49.49	9.12	18%		21.93	9.95	10.44	105%		109.65	62.64	5.85	9%
Indeno(1,2,3,c,d)pyrene	5.38	192.13	64.51	34%		10.75	84.27	13.01	15%		43.01	93.85	12.36	13%
Dibenz[a,h]anthracene	5.38	148.69	49.59	33%		10.75	61.97	10.67	17%		43.01	70.63	10.00	14%
Benzo[ghi]perylene	5.38	28.11	5.10	18%		10.75	37.79	7.49	20%		43.01	80.28	13.56	17%

(ii) Summary of volumes of dichloromethane extract recovered in the liquid-liquid partitioning process for the analysis of spiked artificial seawater:

Volume of dichloromethane recovered in the extraction (mL)											
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Replicate 7	Replicate 8	Replicate 9	Average volume recovered	RSD (%)
5ug spike	4.2	3.8	7.2	8.3	10.8	6.6	4.3	-	-	6.5	40%
10ug spike	2.7	3.2	4	6	4.9	3.4	5.6	6.9	11.4	5.3	50%
50ug spike	3.7	5.4	7.2	7.8	9.8	7.8	4	-	-	6.5	34%

Replicates 8 and 9 were carried out to check the concentrations recovered when the spiking solution was added and mixed before the addition of dichloromethane for extraction.

(iii) Acceptable recovery range specified in Table 6 from US EPA Method 8270B, revision 2, Nov 1990:

Analyte	Acceptable % Spike Recovery
Naphthalene	21 - 133
Acenaphthylene	47 - 145
Acenaphthene	33 - 145
Fluorene	59 - 121
Phenanthrene	54 - 120
Anthracene	27 - 133
Fluoranthene	26 - 137
Pyrene	52 - 115
Benz(a)anthracene	33 - 143
Chrysene	17 - 168
Benzo(b,k)fluoranthene	11 - 162
Benzo(a)pyrene	17 - 163
Indeno(1,2,3-c,d)pyrene	1 - 171
Dibenz(a,h)anthracene	1 - 227
Benzo(g,h,i)perylene	1 - 219

APPENDIX E: Method Limit of Reporting (LOR) Calculation

Method LOR Calculation

(based on USEPA "The Method Detection Limit Procedure Of The US Environmental Protection Agency")

Compound Name	Average recovery (ug/L)	Std deviation	MDL = s*t(6, 95%) (ug/L)	LOR = 10*MDL (ug/L)
nC7	8.86	2.05	3.99	39.87
Toluene-D8	3.31	0.66	1.28	12.78
Toluene	4.12	0.70	1.37	13.65
Ethylbenzene	3.43	0.60	1.16	11.63
m + p-Xylene	3.69	0.67	1.31	13.08
o-Xylene	3.60	0.63	1.22	12.20
nC12	5.48	0.82	1.60	16.00
Naphthalene-D8	2.35	0.48	0.94	9.40
Naphthalene	2.77	0.54	1.04	10.44
Acenaphthylene	3.00	0.56	1.08	10.84
Acenaphthene	2.99	0.53	1.02	10.24
Fluorene	3.06	0.53	1.03	10.30
Phenanthrene-D10	2.95	0.41	0.79	7.89
Phenanthrene	3.14	0.48	0.94	9.41
Anthracene	3.36	0.56	1.08	10.84
nC20	6.29	0.72	1.39	13.91
Fluoranthene	3.37	0.54	1.05	10.47
Pyrene	3.36	0.54	1.05	10.50
Benz(a)anthracene	4.01	1.09	2.13	21.26
Chrysene	3.31	0.55	1.06	10.59
Benzo(b)fluoranthene	4.86	1.56	3.02	30.21
Benzo(k)fluoranthene	5.27	1.18	2.29	22.92
nC32	5.43	1.00	1.94	19.43
Indeno(1,2,3,c,d)pyrene	10.33	3.47	6.74	67.39
Dibenz(a,h)anthracene	7.99	2.67	5.18	51.80
Benzo(ghi)perylene	1.51	0.27	0.53	5.33

MDL = Method Detection Limit – based on 7 replicate recoveries and a 95% confidence level
LOR = Limit of Reporting

APPENDIX F: Coefficients of Determination of the Calibration Curves in Full Scan Mode.

Full scan calibration curve	
Analyte	Coefficient of determination R^2
C7	0.999894
Toluene-d8	0.999542
Toluene	0.99961
Ethylbenzene	0.9998
m + p-Xylene	0.999839
o-Xylene	0.999752
nC12	0.999693
Naphthalene-D8	0.999854
Naphthalene	0.999859
2 Methyl Naphthalene	0.999912
1 Methyl Naphthalene	0.999889
Biphenyl	0.99992
Acenaphthylene	0.999892
Acenaphthene	0.999914
Fluorene	0.999936
nC16	0.99969
Dibenzothiophene	0.999921
Phenanthrene-D10	0.999918
Phenanthrene	0.999941
Anthracene	0.999852
Fluoranthene	0.999867
Pyrene	0.999855
Benz(a)anthracene	0.996428
Chrysene	0.999876
nC28	0.99969
Benzo(b)fluoranthene	0.990916
Benzo(k)fluoranthene	0.995863
nC29	0.999999
Indeno(1,2,3,c,d)pyrene	0.993632
Dibenz(a,h)anthracene	0.978012
nC32	0.999999
Benzo(ghi)perylene	0.996098

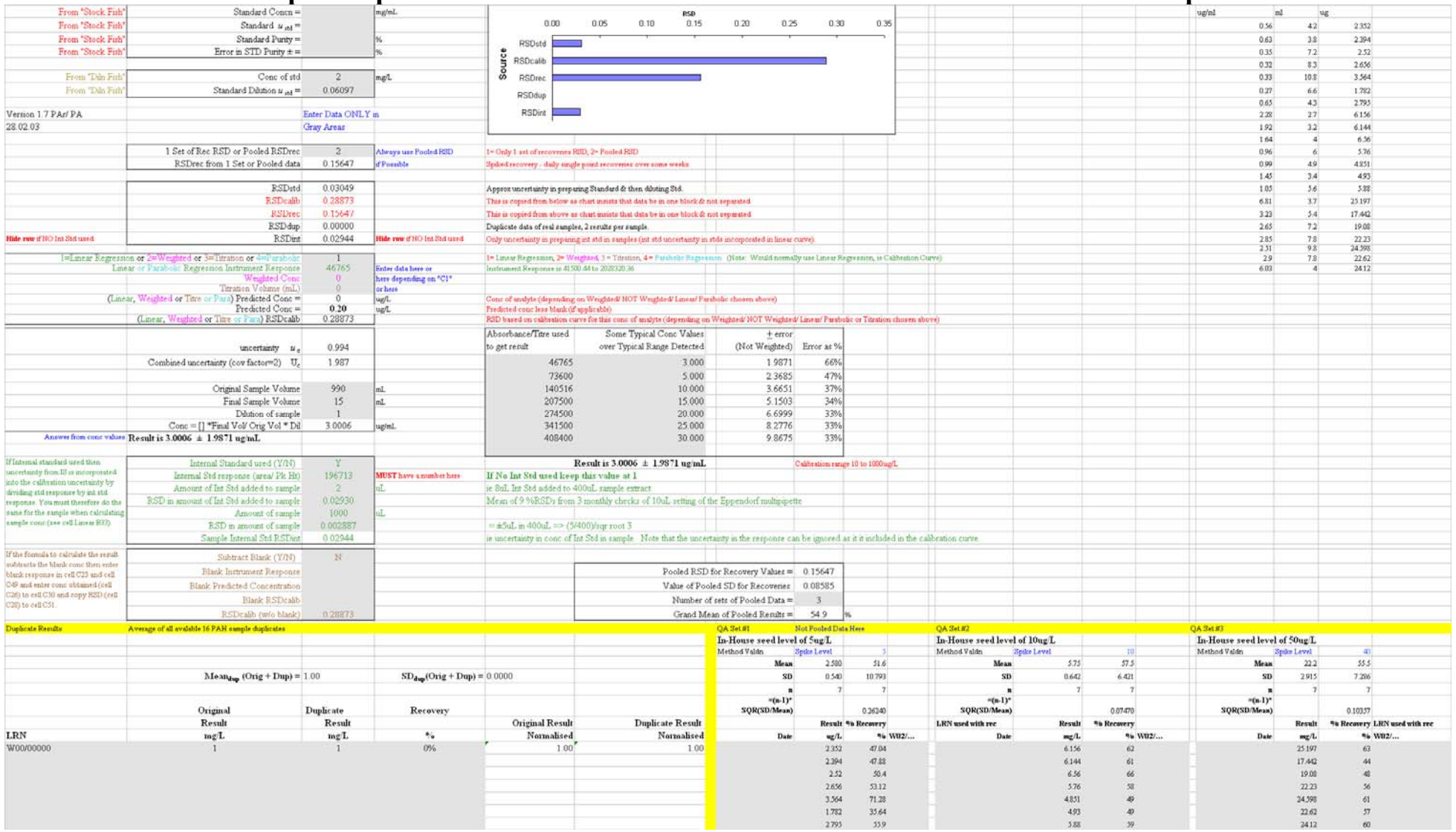
APPENDIX G: Coefficients of Determination of the Calibration Curves in SIM Mode:

SIM calibration curve	
	Coefficient of determination R^2
C7	0.996784
Toluene-d8	0.997148
Toluene	0.997021
Ethylbenzene	0.997493
m + p-Xylene	0.997337
o-Xylene	0.997487
nC12	0.996268
Naphthalene-D8	0.998033
Naphthalene	0.997942
2 Methyl Naphthalene	0.998583
1 Methyl Naphthalene	0.998235
Biphenyl	0.998218
Acenaphthylene	0.998945
Acenaphthene	0.999104
Fluorene	0.999824
nC16	0.999248
Dibenzothiophene	0.998905
Phenanthrene-D10	0.998932
Phenanthrene	0.998498
Anthracene	0.998812
Fluoranthene	0.998955
Pyrene	0.99906
Benz(a)anthracene	0.997577
Chrysene	0.997586
nC28	0.999251
Benzo(b)fluoranthene	0.994349
Benzo(k)fluoranthene	0.99217
nC29	0.996738
Indeno(1,2,3,c,d)pyrene	0.994811
Dibenz(a,h)anthracene	0.994316
nC32	0.996738
Benzo(ghi)perylene	0.995076

APPENDIX H: Sources of Uncertainties

Action	Uncertainty
Removal of 20 mL of sample from sample bottle	There is no way to quantitate loss of undissolved oil that may have been present on the surface of the water in the bottle
Sample volume not accurately determined	Variation in sample volume precludes calculation of accurate analyte concentrations
Recovered volume of Dichloromethane not measured	Variation in recovered dichloromethane precludes calculation of accurate analyte concentrations
Use of p-Terphenyl as Internal Standard	Presence of contaminants in the samples which produce ions at 230m/z can affect concentration calculations.
Dispensing of 2 uL of p-Terphenyl	Error in pipette/syringe volume
Dispensing of 1 mL of sample	Error in pipette/syringe volume
Shakeout	Vigour of shaking changes extraction efficiency
Dispensing of 20 uL of surrogates	Error in pipette/syringe volume
Preparation of standards	Volumetric variations. Purity of standards
Topping up of working standard	Adding solvent with a Pasteur pipette which has a variable drop size. Temperature of standard solution could be different.
Cleanliness of GC system	Response of individual compounds changes as the column and injector liner become dirty. Absence of regular calibration checks means system status was unknown.
p-Terphenyl as internal standard	The detector response for p-terphenyl is not the same as that for the surrogates and compounds of interest. Compound concentration calculation errors
Mass range too low, for example, squalene Solvent delay too long for benzene to be acquired TPH only use 57 – common ion A sixty minute run time is too long causing lower sensitivity for higher end components Order of adding the surrogates pre or post DCM Composition of sample – salinity of matrix affecting DCM solubility	The mass range in the acquisition was set too low to collect squalene (mass). Need inject a benzene standard to confirm the retention and then set the appropriate solvent delay.

APPENDIX I: Example of Spreadsheet for the Calculation of Measurement Uncertainties for Naphthalene



From "Stock Fish"	Standard Concn =		mg/mL	
From "Stock Fish"	Standard u_{std} =			
From "Stock Fish"	Standard Purity =		%	
From "Stock Fish"	Error in STD Purity ± =		%	
From "Dilu Fish"	Conc of std	2	mg/L	
From "Dilu Fish"	Standard Dilution u_{std} =	0.06097		
Version 1.7 PAst PA		Enter Data ONLY in		
28.02.03		Gray Areas		
	1 Set of Rec RSD or Pooled RSDrec	2	Always use Pooled RSD	1= Only 1 set of recoveries RSD, 2= Pooled RSD
	RSDrec from 1 Set or Pooled data	0.15647	if Possible	Spiked recovery - daily single point recoveries over some weeks
	RSDstd	0.03049		Approx uncertainty in preparing Standard & then diluting Std.
	RSDcalib	0.28873		This is copied from below as chart insists that data be in one block & not separated
	RSDrec	0.15647		This is copied from above as chart insists that data be in one block & not separated
	RSDdup	0.00000		Duplicate data of real samples, 2 results per sample.
Hide row if NO Int Std used	RSDint	0.02944	Hide row if NO Int Std used	Only uncertainty in preparing int std in samples (int std uncertainty in stds incorporated in linear curve).
	1=Linear Regression or 2=Weighted or 3=Titration or 4=Parabolic	1		1= Linear Regression, 2= Weighted, 3 = Titration, 4 = Parabolic Regression (Note: Would normally use Linear Regression, ie Calibration Curve)
	Linear or Parabolic Regression Instrument Response	46765	Enter data here or	Instrument Response is 41500.44 to 2028320.36
	Weighted Conc	0	here depending on "C1"	
	Titration Volume (mL)	0	or here	
	(Linear, Weighted or Titre or Para) Predicted Conc =	0	ug/L	Conc of analyte (depending on Weighted/ NOT Weighted/ Linear/ Parabolic chosen above)
	Predicted Conc =	**0.20**	ug/L	Predicted conc less blank (if applicable)
	(Linear, Weighted or Titre or Para) RSDcalib	0.28873		RSD based on calibration curve for this conc of analyte (depending on Weighted/ NOT Weighted/ Linear/ Parabolic or Titration chosen above)
	uncertainty u_c	0.994		
	Combined uncertainty (cov factor=2) U_c	1.987		
	Original Sample Volume	990	mL	
	Final Sample Volume	15	mL	
	Dilution of sample	1		
	Conc = [] *Final Vol/ Orig Vol * Dil	3.0006	ug/mL	
Answer from conc values	**Result is 3.0006 ± 1.9871 ug/mL**			

Absorbance/Titre used to get result	Some Typical Conc Values over Typical Range Detected	± error (Not Weighted)	Error as %
46765	3.000	1.9871	66%
73600	5.000	2.3685	47%
140516	10.000	3.6651	37%
207500	15.000	5.1503	34%
274500	20.000	6.6999	33%
341500	25.000	8.2776	33%
408400	30.000	9.8675	33%

Result is 3.0006 ± 1.9871 ug/mL Calibration range 10 to 1000ug/L

If Internal standard used then uncertainty from IS is incorporated into the calibration uncertainty by dividing std response by int std response. You must therefore do the same for the sample when calculating sample conc (see cell Linear R83).	Internal Standard used (Y/N)	Y	
	Internal Std response (area/ Pk Ht)	196713	MUST have a number here
	Amount of Int Std added to sample	2	uL
	RSD in amount of Int Std added to sample	0.02930	
	Amount of sample	1000	uL
	RSD in amount of sample	0.002887	
	Sample Internal Std RSDint	0.02944	
If the formula to calculate the result subtracts the blank conc then enter blank response in cell C23 and cell C49 and enter conc obtained (cell C26) to cell C30 and copy RSD (cell C28) to cell C51.	Subtract Blank (Y/N)	N	
	Blank Instrument Response		
	Blank Predicted Concentration		
	Blank RSDcalib		
	RSDcalib (w/o blank)	0.28873	

If No Int Std used keep this value at 1
ie 8uL Int Std added to 400uL sample extract
Mean of 9 %RSDs from 3 monthly checks of 10uL setting of the Eppendorf multipipette
= ±5uL in 400uL => (5/400)/sqr root 3
ie uncertainty in conc of Int Std in sample. Note that the uncertainty in the response can be ignored as it it included in the calibration curve.

Pooled RSD for Recovery Values =	0.15647
Value of Pooled SD for Recoveries	0.08585
Number of sets of Pooled Data =	3
Grand Mean of Pooled Results =	54.9 %

Duplicate Results — Average of all available 16 PAH sample duplicates

$Mean_{dup}$ (Orig + Dup) = 1.00 SD_{dup}(Orig + Dup) = 0.0000

LRN	Original Result ng/L	Duplicate Result ng/L	Recovery %	Original Result Normalised	Duplicate Result Normalised
W00/00000	1	1	0%	1.00	1.00

QA Set #1 — Not Pooled Data Here — In-House seed level of 5ug/L — Method Valdn — Spike Level 3

	Result	% Recovery	Date	ug/L	% WU2/...
Mean	2.580	51.6			
SD	0.540	10.793			
n	7	7			
=(n-1)* SQR(SD/Mean)		0.26240			
				2.352	47.04
				2.394	47.88
				2.52	50.4
				2.656	53.12
				3.564	71.28
				1.782	35.64
				2.793	55.9

QA Set #2 — In-House seed level of 10ug/L — Method Valdn — Spike Level 10

	Result	% Recovery	LRN used with rec	Date	mg/L	% WU2/...
Mean	5.75	57.5				
SD	0.642	6.421				
n	7	7				
=(n-1)* SQR(SD/Mean)		0.07470				
					6.156	62
					6.144	61
					6.56	66
					5.76	58
					4.851	49
					4.93	49
					5.88	59

QA Set #3 — In-House seed level of 50ug/L — Method Valdn — Spike Level 40

	Result	% Recovery	LRN used with rec	Date	mg/L	% WU2/...
Mean	22.2	55.5				
SD	2.915	7.286				
n	7	7				
=(n-1)* SQR(SD/Mean)		0.10357				
					25.197	63
					17.442	44
					19.08	48
					22.23	56
					24.598	61
					22.62	57
					24.12	60

Calculated RSD worksheet

Regress-O-Matic (Version 1.7 PAr/ PA 28.02.03) **See StatHelp.doc for instructions and discussion.**

Help

Put your calibration data into these cells. Add or delete rows as required. Use autofill to extend the formulas to new

Concentration (x)	Response (y)	x*y	x^2	y^2	Predicted y	Residual	Residual^2	Plotted Residual
0.2	0.21097	0.042	0.04	0.044508	0.2398	-0.0288	0.00083	-0.0288
0.5	0.5408	0.27	0.25	0.29247	0.5493	-0.0085	0.00007	-0.0085
1.0	1.0789	1.1	1	1.16413	1.0651	0.0139	0.00019	0.0139
2.0	2.0720	4.1	4	4.2931	2.0968	-0.0248	0.00061	-0.0248
5.0	5.2790	26	25	27.867	5.1918	0.0872	0.00760	0.0872
10.0	10.3111	103	100	106.319	10.3501	-0.0390	0.00152	-0.0390
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
		0	0	0.0000			0.00000	9999.0000
sum= 18.7	19.493	135	130.29	139.980			0.01083	
average= 3	3.249							
n= 6								

		rsd=	0.05204	Select the check box for a regression plot.	☑ Plot?
slope (m)=	1.0316696	SD_m	0.006132619	$+/-_m$= 0.01702688	plot range (relative to the stds)
intercept (c)=	0.03342	SD_c	0.022084	$+/-_c$= 0.06132	low= 0.1
r=	0.999929	r^2	0.9999		high= 1.4
t=	168.23	SD_x	0.1		

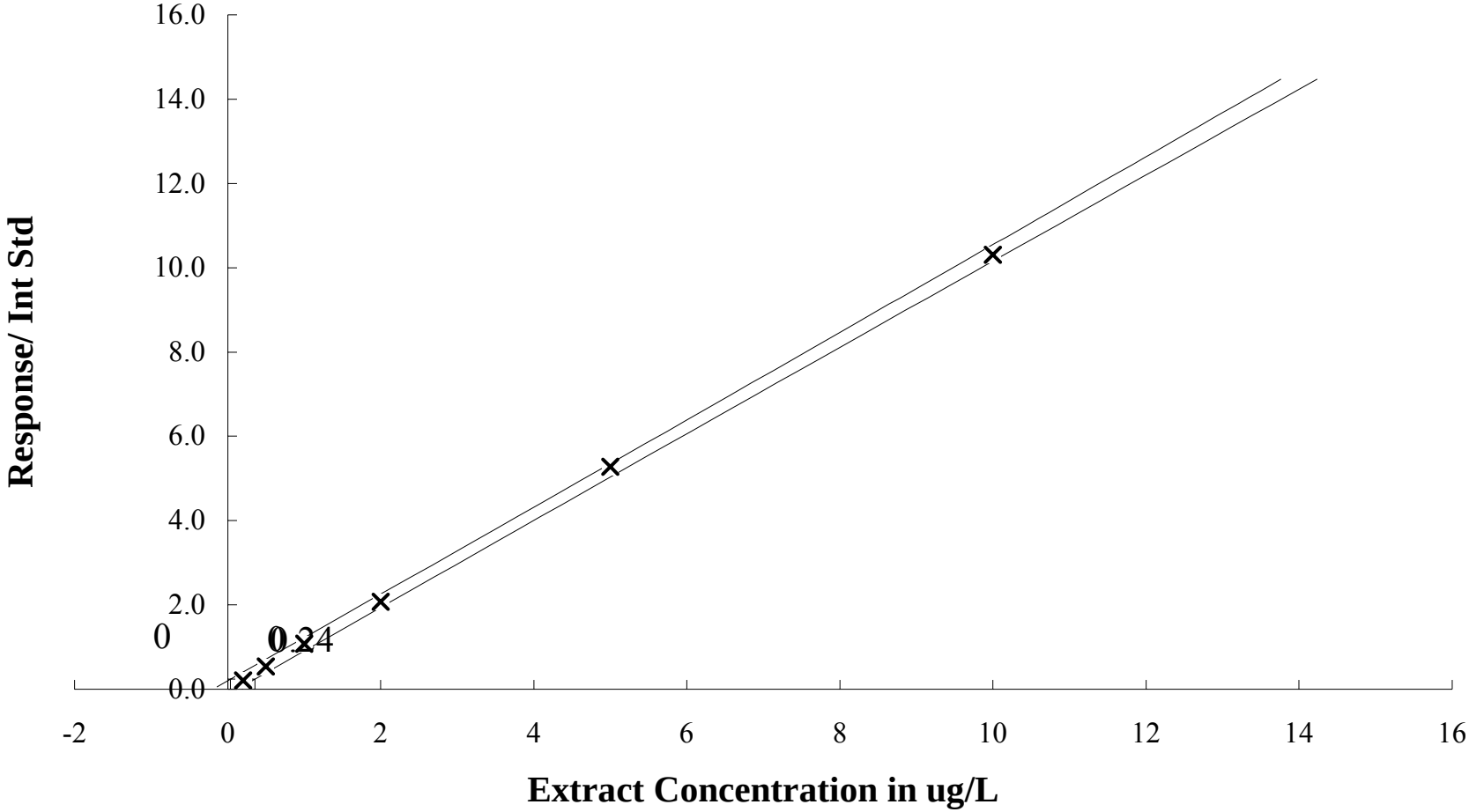
measured y =	0.238	replicates of y=	1
predicted x =	0.2		
+/-	0.2	RSD=	0.2887

ANOVA

Source	*Sum Sqr*	*dof*	*mean sqr*	*F-ratio*
Regression	76.6415	1	76.64150908	28300
Residuals	0.01083	4	0.002708162	
Total	76.65234173	5		

If Internal Stds used enter in response field the response divided by internal std response

Regression analysis of the naphthalene calibration curve



Plot of naphthalene concentration versus response

APPENDIX J: Uncertainty Values of Analytes at the Various Concentrations, Expressed in ug/L and in Percentage.

(i) Uncertainty values of analytes at the various concentration levels in ug/L:

Typical Uncertainty values of the analytes at the various concentration levels ± (ug/L)									
Concentration (ug/L)	3	5	10	15	20	25	30	50	100
Analyte									
Toluene	3	4	5	6	7	9	10	-	-
Ethylbenzene	2	3	4	5	7	8	10	-	-
m,p-Xylene	2	3	4	5	7	8	10	-	-
o-Xylene	3	3	4	5	7	8	10	-	-
C7	3	3	5	6	8	10	12	-	-
C12	3	3	4	5	6	7	8	-	-
C20	3	3	3	4	5	6	7	-	-
C32	-	33	35	37	40	44	48	69	128
Naphthalene	2	2	4	5	7	8	10	-	-
1-Methylnaphthalene	2	2	2	2	2	3	3	-	-
2-Methylnaphthalene	1	1	2	2	2	2	3	-	-
Acenaphthylene	2	2	3	5	6	8	10	-	-
Acenaphthene	2	2	3	5	6	7	9	-	-
Fluorene	1	2	3	4	6	7	9	-	-
Phenanthrene	1	2	3	4	5	7	8	-	-
Anthracene	3	3	4	5	6	7	9	-	-
Fluoranthene	2	2	3	4	6	7	8	-	-
Pyrene	2	2	3	4	6	7	8	-	-
Benz[a]anthracene	9	9	9	10	12	13	14	21	-
Chrysene	2	2	3	4	6	7	8	-	-
Benzo[b]fluoranthene	2	2	3	4	6	7	8	-	-
Benzo[,k]fluoranthene	9	9	10	10	11	12	13	19	-
Benzo[a]pyrene									
Indeno[1,2,3-cd]pyrene	-	19	19	19	20	21	23	29	52
Dibenz[a,h]anthracene	-	24	24	25	24	26	27	34	55
Benzo[g,h,i]perylene	-	10	11	11	12	14	15	21	40
D8 toluene	4	5	9	13	17	21	26	-	-
Naphthalene d8	3	4	8	11	15	19	22	-	-
Phenanthrene d10	2	2	3	5	6	7	9	-	-

(ii) Uncertainty values of analytes at the various concentration levels expressed in percentages:

Typical Uncertainty values of the analytes at the various concentration levels ± %									
Concentration (ug/L)	3	5	10	15	20	25	30	50	100
Analyte									
Toluene	116%	74%	46%	38%	35%	34%	33%	-	-
Ethylbenzene	80%	54%	38%	34%	33%	32%	32%	-	-
m,p-Xylene	72%	50%	37%	34%	33%	32%	32%	-	-
o-Xylene	95%	62%	41%	34%	34%	33%	32%	-	-
C7	95%	64%	46%	41%	40%	39%	39%	-	-
C12	90%	58%	36%	31%	29%	28%	27%	-	-
C20	89%	56%	34%	28%	26%	25%	24%	-	-
C32	-	668%	347%	246%	200%	175%	161%	137%	128%
Naphthalene	66%	47%	37%	34%	33%	33%	33%	-	-
1-Methylnaphthalene	52%	32%	17%	13%	11%	10%	10%	-	-
2-Methylnaphthalene	46%	28%	16%	12%	11%	10%	10%	-	-
Acenaphthylene	59%	44%	35%	33%	32%	32%	32%	-	-
Acenaphthene	54%	40%	32%	31%	30%	30%	30%	-	-
Fluorene	49%	37%	31%	30%	30%	29%	29%	-	-
Phenanthrene	46%	35%	29%	27%	27%	27%	27%	-	-
Anthracene	90%	58%	38%	32%	30%	30%	29%	-	-
Fluoranthene	62%	43%	31%	29%	28%	27%	27%	-	-
Pyrene	64%	44%	32%	29%	28%	28%	27%	-	-
Benz[a]anthracene	293%	178%	95%	69%	58%	52%	48%	42%	-
Chrysene	60%	42%	31%	29%	28%	27%	27%	-	-
Benzo[b]fluoranthene	60%	42%	31%	29%	28%	27%	27%	-	-
Benzo[,k]fluoranthene	315%	190%	98%	70%	56%	49%	45%	37%	-
Indeno[1,2,3-cd]pyrene	-	373%	188%	129%	101%	85%	75%	59%	52%
Dibenz[a,h]anthracene	-	488%	245%	166%	122%	105%	91%	67%	55%
Benzo[g,h,i]perylene	-	206%	107%	76%	62%	55%	50%	43%	40%
D8 toluene	140%	108%	91%	87%	86%	86%	85%	-	-
Naphthalene d8	95%	82%	77%	75%	75%	75%	75%	-	-
Phenanthrene d10	53%	39%	32%	30%	30%	29%	29%	-	-