Loughborough University

APPLICATION OF LIQUID-LIQUID EXTRACTION FOR PRECONCENTRATION AND SUBSEQUENT DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS BY INDUCTIVELY COUPLED PLASMA-ISOTOPIC DILUTION MASS SPECTROMETRY IN ENVIRONMENTAL WATER SAMPLES

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ABSTRACT

The Semi –exact matching double isotope dilution mass spectrometry [SEMDIDMS] using Gas Chromatography hyphenated with inductively coupled mass spectrometry [GC-ICP – MS] was able to detect PBDE up to about 38.082 ng/L in 0.033g (50 µl) of final extract of 2L environmental synthetic model water containing 15 mg/L humic acid. Ultrasound Assisted Liquid-liquid extraction and H_2SO_4/KOH/$NaSO_4$ partitioning phases alongside with non-destructive lipid removal by activated silica gel column chromatography clean up technique produced cleaner extracts. This demonstrated that the method is sufficiently selective, fit for purpose and specific enough for the priority BDE congeners. The peaks were baseline resolved. The external calibration approach for PBDE analysis in environmental water samples was critically examined using the same sample preparation procedure. All BDE congeners and solvent, n- hexane [6.903] peak appeared at their characteristic retention time of 6.73 min [BDE-28], 7.46 min [BDE-47], 8.04 min [BDE-100], 8.29 min [BDE-99], 8.97 min [BDE-154] and 9.45 min [BDE-153]. The technique was applied for the analysis of PBDE in River Mole water samples with previous pollution history. The sample preparation methodology employed was Ultrasound Assisted Liquid-liquid extraction and H_2SO_4/KOH/$NaSO_4$ and activated silica gel chromatography clean up technique. The final residues values after preconcentration under nitrogen stream at 50°C range from -0.00846 to 0.000137g demonstrating the efficiency of clean up and preconcentration technique. The preconcentration factor of 40,000.SEMDIDMS result achieved quantitative mean spike recoveries with comparably lower and variable-defined uncertainties that lies within the QC control limit of 90-110 % according to LGC in –house standard. No PBDE congener was detected in calibration blanks, procedural blanks and River Mole water samples by the two methods. River mole water is as clean of priority PBDE congeners as the blank. External calibration passed all data quality control tests as stated in 40 CFR 136, EPA Quality standard. However, it was necessary to effect modifications in extraction processes and injection system to achieve quantitative recoveries. CCV and ICV check standard data revealed that their mean recovery and standard deviation values generally lies within the quality control limit. The calibratrion standards also generally lies within 80 - 120% according to 40 CFR 136, EPA Quality standard. The interval of about 24 hours interval between extraction process and partitioning yielded improved recoveries but with higher uncertainties than SEMDIDMS. Paired T test confirmed no statistically significance difference between SEMDIDMS external calibration except for BDE- 28 and BDE- 47.Hence further improvement on extraction and partitioning were recommended to achieve more quantitative recoveries with lower uncertainties. The use of 1,1 dibromocyclohexane as an

internal injection standard for PBDE analysis was validated by Paired T test that there is no statistically significance differences between its result and that of the SEMDIDMS. The instrumental Limit of detections [LODs] for priority congeners were in the range of 3.03 ng/g – 11.07 ng/g for m/z 79 –Br and 4.31 ng/g - 11.93 ng/g for m/z 81-Br. The method LOQs for external calibration were in the range of 0.31 ng/kg - 1.13 ng/kg and 0.44 ng/kg - 1.27 ng/kg for m/z 79 –Br and m/z 81-Br respectively. The method LOQs for internal standard approach were in the range of 0.16 ng/kg - 0.58 ng/kg and 0.23 ng/kg – 0.65 ng/kg for .m/z 79 –Br and m/z 81 Br -BDE respectiv The values of instrumental LODs, Method LODs and LOQs obtained for each congeners are generally higher than values obtained by SEMDIDMS [developed by the inorganic team] which states that Instrumental LOD ranged from 1 to 5 ng g^{-1} and the LOQ ranged from 0.10 to 0.14 ng kg^{-1} for the individual congeners. This comparative study further confirmed the use of IDMS as an absolute method for reference material production. The application of ID-ICPMS for the determination of total bromine achieved quantitative recovery.

Keywords: Semi exact matching double isotope dilution mass spectrometry, Ultrasonic – assisted Liquid Liquid extraction, External calibration, Limit of detection and Limits of quantititation

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ABBREVIATIONS

BDE - Brominated diphenyls ethers

BFRs - Brominated flame retardants

EU - European Union

GC– Gas Chromatography

HA - Humic acid

ICP - Inductively Coupled Plasma

IUPAC - International Union of Pure and Applied Chemistry

ID-ICPMS - Isotope dilution mass spectrometry

IDA - Isotope Dilution Analysis

LLE - Liquid Liquid Extraction

LODs - Limit of Detections

LOQs - Limit of quantifications

MAE - Microwave assisted extraction

MS – Mass spectrometry

NCI - Negative chemical ionization

NMIs - National Measurement Institutes

PBDEs - Polybrominated diphenyls ethers

PBBs - Polybrominated biphenyls

PCBs - Polychlorinated biphenyls

POPs - Persistent organic pollutant

PF - Particulate fraction

QMS - Quadrupole mass spectrometer

QA - Quality Assurance

QC - Quality Control

RF – Radio frequency

SEDIDMS- Semi-exact double isotope dilution mass spectrometry

SPM - Suspended particulate matter

UNEP- United Nations Environment Programme

UALLE-Ultrasonic –assisted liquid liquid extraction

WFD - Water Framework Directive

Mw. Molecular weight

1.0 INTRODUCTION

Polybrominated diphenyls ethers (PBDEs) are industrial aromatic organobromine chemicals grouped as brominated flame retardants (BFRs)[1]. They are used in a wide range of products such as electronics, polyurethane, polymers and textiles. These compounds attracted global concern due to concentration increase in the environment [2] and their link with adverse health related effects such as neurobehavioral toxicity, reproductive and feeding disorder in fish.[1] Potential health hazards of PBDEs in pregnant women and developing foetuses include induce neural defects and cardiac arrhythmia, impairment of motor skills, learning and memory, induce immuno-toxicity, disrupt endocrine functioning and impair reproductive development.[1,2,3,4,5]

PBDEs are highly hydrophobic and lipophilic compounds with low solubility in water [5,6] They undergo thermal and photolytic degradation.[7] They possess very low vapour pressure at room temperature.[7,8] The vapour pressure increases with increasing molecular weight and degree of bromination.[7,8] As a persistent organic pollutant (POP), they have been detected in biotic and abiotic complexes such as air[1,2], water, sediments, human body fluids and tissues (blood).[2] .Several legislation and environmental actions have been enacted to detect and further control its spread in the environment.[9,10]. Its usage, production, marketing, and importation have attracted ban by several developed countries but it is still in use in several Asia countries.[3,4]

The basic structure of a PBDE consists of two phenyl rings joined by an ether linkage, surrounded by various number of bromines.[1,2,3] There are 209 BDE congeners known at present, however, the focus of this study is on the six most abundance priority PBDE congeners . They are identified with their chemical structure as shown in figure 1. The following are lists of priority PBDEs studied with their basic properties which include IUPAC name, chemical abstract numbers, CAS and their molecular weights.

(a) 2,4,4'-Tribromodiphenyl ether (BDE-28, CAS 41318-75-6, $C_{12}H_7Br_3O$, Mol. Wt :406.89538 [g/mol][11]

(b) 2,2′,4,4′,5,5′-Hexa-bromodiphenyl ether (BDE-153, CAS 68631-49-2, $C_{12}H_4Br_6O$, Mol. wt : 643.58356 [g/mol]),[12]

(c) 2,2′,4,4′,5,6′-Hexa-bromodiphenyl ether (BDE -154, CAS 207122-15-4, $C_{12}H_4Br_6O$, 643.58356 [g/mol]), [13]

(d) 2,2',4,4',5-Pentabromodiphenyl ether (BDE-99 , CAS 60348-60-9, $C_{12}H_5Br_5O$, 564.6875 Mol. Wt [g/mol]) ,[14]

(e) 2,2',4,4'-Tetrabromodiphenyl ether, (BDE 47 CAS :5436-43-1, Mol wt 485.79144 [g/mol], $C_{12}H_6Br_4O$) [15]

(f) 2,2',4,4',6-Pentabromodiphenyl ether [BDE 100, CAS: 189084-64-8, Mol. Wt 564.6875 [g/mol], $C_{12}H_5Br_5O$] [16]

The basic chemical structure of priority BDE Congeners are as shown in figure 1.

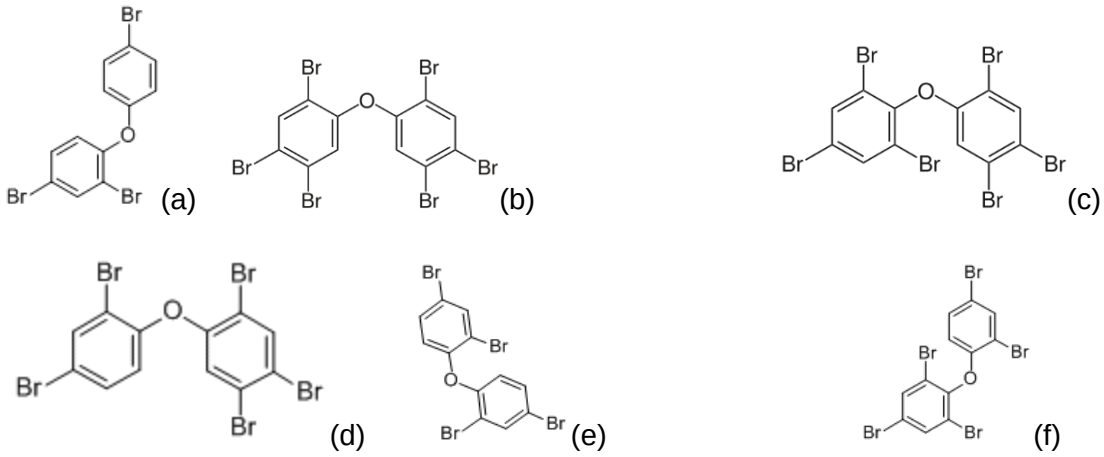

Figure 1: Structures of studied priority BDE Congeners

LGC and other members of European Association of National Metrology Instititues (EMRP) such as LNE, SYKE, TUBITAK UME, UBA, REG (HZG) participated in a project code named ENV08 WFD aimed at developing metrologically validated procedure of measurement for the BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154. The goal also includes developing appropriate methods for extraction and pre-concentration of these PBDE. The EUWFD 2008/105 EC specified that the LOQ should be smaller than 30 % of the Environmental Quality Standard (EQS) of 0.5 ng/L for the sum of PBDEs in whole water samples by 2014. However, EU WFD 2013 spanning 2015 -2021 recently released stipulates an EQS level of 140 ng/kg.[9,10]

The Inorganic analysis team of LGC, Queen's road, Teddington, Uk successfully developed Ultrasonic Assisted - Liquid-Liquid Extraction method for PBDEs preconcentration and Gas Chromatography Inductively Coupled plasma Isotope Dilution Mass spectrometry (GC-ICP-IDMS) for the quantification of PBDE's in both environmental model waters and real environmental water samples. The method achieved highly efficient clean up, and a pre-concentration factor of 40,000. Instrumental LOD ranged from 1 to 5 ng g^{-1} and the LOQ ranged from 0.10 to 0.14 ng kg^{-1} for the individual congeners. The method have been applied for the analysis of PBDEs in River Thames water samples and environmental model water containing 15 mg/L humic acid with recovery within the acceptable quality control (QC) limit. This project compares the result of determination of PBDE in River Mole using both SEMDIDMS and external calibration

Real water samples were collected on 28th August, 2014 from River Mole a tributary of the River Thames in southern England (near Hampton Court Palace). Its pollution history revealed that in 2003, Gatwick Airport Ltd pleaded guilty to charges of allowing chemical pollution to enter the river after a detergent, used to clean rubber and oil from the runway, was washed into Crawters Brook by airport workers. The Environment Agency estimated that about 5200 fish of 14 different species died as a result of the pollution. The airport was fined £30,000 by Lewes Crown Court. [17]

Additionally, this project also reports developed methodology for total bromine determination in PBDEs using ID-ICPMS. At the time of this project there was no single published publication for total bromine determination in PBDEs using ICP-IDMS. However S. Hill [19] and M Ohata et al [20] in different studies accurately determined bromine in plastics using ICP-IDMS. Common methods reported successfully for bromine determination in different matrices include ion chromatography (IC) with combustion method [21,24,25] , a thermal ionization mass spectrometry (TIMS) with combustion method [26,27], an inductively coupled plasma optical emission spectrometry (ICPOES) or inductively couple plasma mass spectrometry (ICPMS) with combustion method [28,29,30], a X-ray fluorescence (XRF) spectrometry[31,32,33] in indifferent matrices using different digestion methods. The project highlight developed ID-ICPMS procedure for total bromine determination PBDEs and verification of ICP-IDMS accuracy as a quantification strategy for PBDEs using Unlabelled CRM 2,4,4' –TriBDE (BDE-28).

2.0 AIMS

The main aim of this project is to compare and contrast the results of analysis of Polybrominated diphenyl ethers in River Mole water sample by Semi-exact double isotope dilution mass spectrometry, SEDIDMS (a primary method) with external calibration using GC-ICP-MS. The project also describes the analysis of PBDEs in environmental model water containing 15 mg/L humic acid by SEDIDMS. It further present the report of a preliminary investigation into the application of ID-ICPMS for determination of total bromine in PBDEs.

3.0 EXPERIMENTAL

3.1 Instrumentation

3.1.0 Gas chromatography hyphenated with Inductively Coupled Plasma-Mass Spectrometer (GC-ICP-MS)

The analysis of PBDE was carried out on Agilent 6890 gas chromatography (GC), equipped with an Agilent technologies 7683 series autosampler injector all controlled through

windows QXP professional (HP), Agilent Technologies Waldbrown Analytical Division B4, SYSTEM ₦ : G1030AX, System Serial ₦ : DE 0739183, Germany. The GC was coupled to an Agilent 7500ce (ICP MS2), inductively coupled plasma spectrometer, Octopole Reaction system via a heated transfer line and fitted with a DB-5MS capillary column 15 m x 0.25 mm, film thickness 0.25 µm. Control and operation of the coupled system was performed using Agilent Mass Hunter Software version installed on Microsoft windows XP professional DELL , Central processing unit (DELL Precision T3500, Other auxiliary equipment include: Agilent technologies G3292A recirculating chiller, Main Pump (Agilent part No: G1833 – 81004, Model : EDWARDS E2M18, S/N 37807071), Auxilliary pump: (Agilent Part No: G1833 -81004, Model : E2M18, EDWARDS PART No: A36324930, Mat No . 36324930XS, S/N 76284919). Gas cylinders: 10 ppm Xenon/oxygen/Helium, 200 bar, V: 150 bar hydrogen Cp grade Helium compressed. Carrier gas was Helium operated at splitless injection mode and injection volume of 2-2.5 µl.

a. **Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**: The Element 2 Sector Field –ICP-MS obtained from Thermo-Fisher Scientific Bremen, Germany) was used for quantitative determination of Bromine.
b. **Digestion Equipment**: CEM Discover Microwave unit (Asset No: A10166), 10 mL Quarts microwave vessels.
c. **Filteration Apparatus**: Whatman ® filter, FP 30/5.0 CN-S, 5.0 µm, 7 bar max, Lot No. G5444150 supplied by GE Health care UK Limited, Glass Syringe filter1 Lt sample bottle.
d. **Balance used**: Denver TC -2012 (S/N : T0122196,last calibrated on 23/10/13), five-figure analytical balance model XP205 from Mettler Toledo GmbH, (Greifensee, Switzerland, S/N 1129160109, location 6/14 –balance room, last calibrated 6/12/14), Oertling MD 31, Birmingham, UK S/N 892727, Calibrated 16/10/13), Sartorius Analytic (Sartorius A 2005 RM 4/20, SD 76, GC 8192 SN 37040253, UKAS calibration No. 0438 , Calibration date : 23-10-13).
e. **Shaker**: B100/ TW, ROTATEST SHAKER (obtained from LUCKHAM LTD, England; Asset No: 2054369)
f. **Separation apparatus**: Retort Stand, O-Clamp, Fume Chamber ID No 6/07A (Model :FUME C), Fisher brand® FB11004 Ultrasonic bath, Asset No:A10354. All electrical and electronics in this laboratory passed Hawkesworth Appliance testing Nov 2013, passed SCL Group -0800, 195 7254 (09-13), passed Electrical safety test.
g. **Glassware**: 2L capacity separating funnels (supplied by MBL England, 23/32, ISO 4800 certified), Four 2.5 L capacity amber bottles, I-CHEM Certified® 60 mL vial obtained from Thermo Fisher Scientific (Loughborough, Leicestershire, UK), glass

jug, 2 L amber glass bottles, 5 ml amber vials, 200ul amber injection vials and 25 ml measuring cylinder (supplied by Thermo Fisher Scientific, UK).

h. **Pipettes**: Eppendorf Multipette Xstream, balance room pipette – 6/18 (100 -1000µl, pipette by made by GILSON), Lab 6/1 pipette (50 -250 µl, pipette by made by GILSON) 250 µl – CP 250 Capillaries and piston, 250 ,mm long glass pasteur pippete unpluggeg, pippete tips.

i. **Other equipment include as follows**: All experiments were carried out in the Fume Chambers – ID No 6/07A (Model:FUME C), Model F/C ID N016/0813, F/C 6/0813. Sample concentrator supplied by TECHNE). PFTE evaporating dish used during quantitatation of humic acid for the preparation of model water. Thermo Scientific HERAUES oven, used in during evaporation of water from humic acid sample and drying of glassware.

j. B.D PLASTIPAK Injection syringe with metal needle was mostly used instead of plastic made pipettes as spiking tool to avoid contaminating enriched spikes with polybrominated diphenyl ethers that may be adsorbed to plastic, and Falcon tubes used as containers for ICP-MS microwave extracts and tuning solutions during bromine determination.

k. Ultrasonic bath: Fisherbrand model FB11004 from FisherScientific (Loughborough, Leicestershire, UK)

3.2 SAMPLES AND REAGENTS

3.2.1 Samples and reagents for Total Bromine determination by Semi exact – matching double Isotopic Dilution Mass Spectrometry

The acid used for sample digestion was ultra-high purity concentrated nitric acid (16M) (Certificate number T650423, CAS (7697-32-2), 67-69% supplied by Romil Ltd,Cambridge. For sample dilution, concentrated ammonia solution 32% (18M) (Hi Per Solv CHROMANORM) for HPLC obtained from VWR Chemicals France and ultra-high purity Milli-Q water were used. The standard solution (ca. >1000 mg kg^{-1}) of Br used was a primary standard solution prepared using high purity Sodium bromide, (Alfa Aesar, Putratronic,+99.9955% ,Br2 Red, CAS~~N~~7647-15-6 supplied by Johnson Matthey , UK opened on 06/10/08) by dissolving with water. Enriched ^{81}Br spike solution for SEMD-ICP-IDMS were prepared from 81Br enriched sodium bromide (NaBr, powder form), Bromine (Br-81, as NaBr 99%, CKT-81-Na, Element weight-50mg, Compound weight -64.4mg, Lot Number:139201 produced by Ck Gas Product Ltd for preparation of ^{81}Br Spike Ck gas, Hool UK), by dissolving with water. The concentration of ^{81}Br enriched spike solution was determined accurately by ID-ICPMS using the blend of spike and bromide standard solutions. The isotopic abundance of bromine is listed in IUPAC [44] for bromide standard

solution as well as sample, because the value could be accepted generally. The isotopic abundance from CRM certificates was used for 81Br enriched spike solution. The method was validated by analysis of Unlabelled Certified Material CRM: 2,4,4' – TriBDE (BDE 28) 50 µl / mL in Nonane obtained from Cambridge Isotope Laboratory, CIL UK.

3.2.2 Samples and reagents for the determination Polybrominated diphenyl ethers by Semi exact–matching double Isotopic Dilution Mass Spectrometry [SEMDIDMS] and external calibration

The sample blend resulted from addition of enriched PBDE standard mix (spike) to the sample, while calibration blend was produced from mixture of natural PBDE standards mix and enriched PBDE standard mix with the information below. The Natural PBDE standards mix of known concentration was prepared from six (6) individual priority congeners unlabelled certified standards which include 2,4,4'- triBDE (BDE -28) 50 µg/ml in nonane, unlabelled certified standard , (BDE -28 –CS , LOT #: SDBD – 010, PSO #:11F – 515); 2,2',4,4'- tetraBDE (BDE -47) 50 µg/ml in nonane, BDE -47–CS , LOT #: SDBD – 011, PSO #:11F – 516); 2,2',4,4',5- pentaBDE (BDE -99) 50 µg/ml in nonane, BDE -99–CS , LOT : SDAC – 029, PSO:10C – 695); 2,2',4,4',6 - pentaBDE (BDE -100) 50 µg/ml in nonane, BDE -100–CS , LOT: SDAD – 011, PSO:10G – 428); 2,2',4,4',5,5'- hexaBDE (BDE -153) 50 µg/ml in nonane, BDE -153–CS , LOT #: SDBJ– 010, PSO # :11J – 288); 2,2',4,4',5,6'- hexaBDE (BDE -154) 50 µg/ml in nonane, BDE -154–CS , LOT #: SCJF– 001, PSO :9G -332, obtained from Cambridge Isotope Laboratory, Uk. Isotopically Labelled Enriched Standard used included BDE-28 , Lot 01 04; BDE -47, Lot 01 06; BDE -99, Lot 01 13; BDE – 100, Lot 01 06; BDE – 153, Lot 01 13; BDE – 154 ,Lot 01 06 (50 µg/ml in Isooctane) supplied by ISC Science, Oviedo, Spain.

For external calibration standards, the natural PBDE standard mix (50 µg/ml in nonane) were diluted in hexane to give five calibration points (20, 40, 60,80,100 ng/g), plus a blank which covered the expected range for the samples. The Continuing calibration verification standard (CCV) was prepared at the mid-calibration point (60 ng/g) from same source as calibration standards while the Initial Calibration Verification check standard (CCV) was prepared directly from the main. The study also tested the ability 73.08256 ng/g 1,1 dibromocyclohexane diluted in n-hexane as an injection internal standard following following results of first external calibration run. Detail information about Calibration and check standards preparation is given in appendix A**.**

Other reagents used include Conc. H_2SO_4, 5% nitric acid, n-hexane and acetone (Optigrade for HPLC, supplied by PromoChem, LGC standards GmBH. n-hexane (for HPLC grade) supplied by PromoChem, LGC Standard, GmBH, Ultra high purity water (UHP water) (18

MΩ cm, <5ppb Total Organic Carbon) was prepared using an ELGA purelabflex system ELGA, Veolia Water (Marlow, UK).Potassium hydroxide, Sodium hydroxide, humic acid, Sodium Sulphate (99.0 % anhydrous granular) and methanol CHROMASOLV® were supplied by SIGMA ALDRICH, Poole, Dorset, UK while Sodium Chloride, analyte grade was supplied by Fisher Scientific, Uk.

3.2.2.1. Sampling preparation, pre-concentration and preservation

3.2.2.1.1 Cleaning of sample containers

This is very important procedure that ensures accurate PBDEs determination. All glassware and sample bottles were soaked in 1% (v/v) Mucasol ® (Brand GmbH, Main, Germany) detergent solution for 24 hours, 1% nitric acid for another 24 hours (to prevent the adsorption of PBDEs to the sample bottle), rinsed in hot tap water severally. They were thereafter rinsed three-times with UHP-water and allowed to dry. Prior usage, all glass wares were rinsed and ultrasonicatedly cleansed using acetone (HPLC grade) and n-hexane (HPLC grade) sequentially. The ultrasound waves help to disrupt proteins and cell membrane holding PBDEs and other contaminants [32,33,34,35,36] Also, sample bottle plastic caps and PTFE lining were soaked in 1% Mucasol detergent solution for twenty–four hours, washed in dish washer and subjected to same cleansing procedure like glassware to prevent contamination.

3.2.2.1.2 Sample Collection and Storage

Two types of samples examined included real water samples from River Mole and model environmental water (15 mg/L Humic acid solution) using SEMDIDMS and external calibration approaches for quantification. Water samples were collected with a 2 Lt capacity glass jug (supplied by Fisher Scientific Loughborough, Uk) into thoroughly cleaned 2.5 L amber glass bottles (to inhibit thermal and light degradation of PBDEs) with lids lined with PTFE obtained from Sigma Aldrich (Poole, Dorset, UK). At no point in sampling process did the sample come into contact with plastic. The samples were kept cool en-route the laboratory in an ice pack. They were immediately refrigerated on arrival at the laboratory at 4°C until analysis. Filtration was not performed based on European union directive because the suspended particulate matter must be included in the analysis.[9]

Samples were appropriately labelled as shown in appendix B. External calibration experiment was performed twice to enable investigations into the recoveries of PBDE in spiked water samples. The first experiment was labelled as ‘Run 1 ext’ and ‘Run 2 ext/int’ [use of internal standard]. For Run 1 external calibration, 2 Kg of water samples A4, E3 and A4 were spiked with 0.14599 g of 13 ng/g natural PBDE standard mix (supplied by

Cambridge Isotope Laboratory, Uk) while the other three samples A9, E4 and E3 were unspiked and analysed as raw water samples. Run 2 external /internal standard method utilised spiked samples A10, A11 and A12 for recovery studies. Samples C and B were procedural blanks analysed alongside each run to assess contamination from laboratory environment, useful for blank correction if necessary or re-preparation of samples as well as reanalysis if amount of analyte is greater than or equal to 10% or 2.2 times the PBDE method detection limit.[37].

3.2.2.1.3 Preparation of Environmental 15 mg/l Humic acid model water samples

1g of humic acid (1000 mg] was weighed into 1 litre of UHP water in a pre-cleaned 1 L capacity bottle of known weight (604.69 g) using Denver TC-2012 balance. The bottle was placed in Fisherbrand ultrasonic bath for 30 min to ensure uniform dissolution and equilibration. The solution was filtered using all glass100 ml capacity syringe filter instead of plastic to prevent contamination by organics. About 435.12 g filtrate was obtained from syringe filtrations using Whatman filter, FP 30/5.0 CN-S, 5.0 µm, 7 bar max, Lot No. G5444150 supplied by GE Health care UK. The sample bottle plus filtrate weighed 1039.81 g. To determine the mass of humic acid stock solution required to produce 15 mg/L model water, the actual concentration of humic acid in the filtrate must be known. Therefore, 32.67782 g, 31.0792 g and 48.6435 g of the filtrate and water (blank) were measured into pre-weighed PFTE evaporating dish and gently evaporated to dryness in the oven. The concentration of the stock humic acid was calculated as 964.75 mg/L from residue mass with blank correction. Therefore, 29.5 mg of stock humic acid was required by 2 Litre water (of recorded weight) to produce 15 mg/L model water. The remaining filtrate was refrigerated for future use. Detail of model water preparation is contained in Appendix C.

3.2.2.1.4 Preparation of Natural PBDE standard mixture

0.10767 g (156 µl) of each priority congener was weighed into a pre-weighed agilent amber vial to produce a standard PBDE mix. Density corrections were applied to the certified values to convert µg mL^{-1} to µg g^{-1} and produce a new concentration of about 11.69393 µg g^{-1} of each PBDE congener in this mix standard in n-hexane. For the preparation of sample blend, 0.11 g of mix PBDE standard in nonane was evaporated to dryness under nitrogen to enable dilution with 2.44 g methanol substituting nonane which is immiscible with water sample. This allows PBDEs to equilibrate in water. All standards were refrigerated (4 ˚C) but returned to ambient temperature before use. The concentration of PBDEs in the calibration blend is expected to be equivalent to that of the sample.

3.2.2.1.5 Preparation of Enriched Spike mixture (81Br Isotopically Labelled PBDE Standard)

The mixture of 0.2 g of BDE-28, 0.14 g of BDE-47, 0.18 g of BDE-99, 0.3 g of BDE-100, 0.2 g of BDE-153 0.31 g of BDE-47 ^{81}Br Isotopically Labelled Standard in isooctane (density of 0.690 g/ml at 20°C) was prepared in a pre-weighed 1.5 ml extremely high recovery (agilent technologies) vial. The final weight was recorded in triplicate. Isooctane is immiscible with water and was evaporated to dryness under nitrogen streams using sample concentrator (TECHNE) to enable dilution with 2.5 g methanol.

3.2.2.1.6 Preparation of sample blend

Each model water sample labelled R7,R8, R9 was spiked with 0.1877 g (0.15-0.156ul) of natural PBDE (x 25) standard mix (in methanol) with injection syringe the new total mass was recorded. The injection syringe was rinsed severally with methanol into the sample bulk to ensure quantitative transfer of the natural standard mix into the model water mixture. The sample blends were refrigerated to equilibrate for twenty –four hours before addition of 81-Br enriched isotopically labelled standard congener. In each case, the tip of the syringe needle was positioned below the surface of the water in the sample bottle before the addition. The final weight of vial was measured using Oertling MD31 balance. After the last step, the mixture was placed on B100/ TW, ROTATEST SHAKER (obtained from LUCKHAM LTD, England) for uniform equilibration for another twenty – four hours at room temperature before extraction.

3.2.2.1.7 Preparation of calibration blend

The mass of an empty 1.5 ml extremely high recovery (agilent technologies) injection vial was recorded in triplcates using Mettler Toledo balance. The spiking ratio of natural standard reference isotope $^{79-}$Br-BDE to enriched spike $^{81-}$Br-BDE adopted was 1:5 earlier tested on GC-ICP-MS. The peak intensity of isotope $^{79-}$Br was one fifth of $^{81-}$Br peak intensity. 0.09757 g of natural PBDE standard mix was added to 0.48266 g of $^{81-}$Br –BDE enriched Spike. The blend was evaporated to dryness at 50 °C under nitrogen and then reconstituted in 50 µl (0.0324 g) n-hexane for GC-ICP-MS analysis at optimised conditions in table 2.

3.2.2.2.0 Extraction process: ultrasonic assisted liquid - liquid extraction process

All sample types, for all calibration strategies passed through the same UALLE extraction procedure. The separating funnels, glass stoppers and taps were rinsed with acetone twice, dried in purified air and lastly with n-hexane twice again to remove any contamination due to trapped water and BFRs that might have occurred after the previous cleaning stages. Measuring cylinders were also cleaned in the same order.

Five pre-cleaned separating funnels were labelled with same sample bottle labels. For each sample**,** 20 mL n-hexane and 20 mL 3% m/v nitric acid were added to each sample in labelled amber bottle and was vigorously shaken for about 60 seconds. The bottle was placed on ultrasonic water bath for 30 min to enable hexane penetrate particles and membranes of any matrix trapping PBDEs, breaks droplets emulsions and extract PBDEs quantitatively. The mixture was transferred into labelled 2.5 L capacity glass separating funnel fitted with PTFE taps clamped to retort stands. It was assumed that the single partitioning has quantitatively transferred about 99.9% of PBDEs. Another 20 ml of n-hexane was added to the bottle to quantitatively transfer the water–solvent mixture and to extract any remaining PBDEs within the wall of the bottle. The bottle was placed in ultrasonic bath for two minutes and its content transferred into the separating funnel accordingly. This last step was repeated with additional 20 ml n-hexane for quantitative transfer and the separating funnel was corked. Solid water ice carbondioxide was added to keep the temperature of the bath to less than or equal to 20 $^{\circ}$C. Extraction was carried out with vigorous shaken for about 40 seconds with periodic venting. Phase separation of the resulting organic (on top) and aqueous layers (lower) was allowed for another 24 - hours. The aqueous layer was carefully collected in a beaker and discarded. For 'Run 1 ext' the phase separation was allowed for about three hours but for 'Run 2 ext' and 'Run 2 ext/int', it was allowed for 24 hours.

The above steps were repeated for samples using different sets of separating funnels labelled for individual samples to enable easy identification in case of contamination investigations.

3.2.2.2.1 Partitioning phases

The organic extract was subjected to three phases of clean-up procedure.

a. Partitioning with conc. H_2SO_4

The first partitioning was done by careful addition of about 2 ml of concentrated sulphuric acid (previously cleaned or partitioned in n-hexane) to the brownish jelly emulsion of PBDE hexane-extract [for model water]. The mixture was vigorously shaken (vertically and horizontally) for 60 seconds with periodic venting. It was allowed to settle down for about 10 min. Three separate layers was formed this include the top organic n-hexane-PBDE layer, middle layer brownish emulsion and lower aqueous layer. The aqueous layer was discarded appropriately. The second stage of sulphuric acid partitioning was repeated as above, the emulsion layer decreased in dimension there was increase in aqueous layer establishing that sulphuric acid had imparted polarity to more coextractants. The set up was allowed to settle for about 10 min. The aqueous layer was discarded.

b. Partitioning with 5% Sodium Chloride solution

The resulting extract was shaken with 5 ml of 5 % sodium chloride to remove traces of acid.

c. Partitioning with 20 % (m/v) potassium hydroxide

The extract was further shaken with 2 ml of 20 % (m/v) potassium hydroxide, shaking and allowed to settle for shorter period when clear layers had formed to prevent the alkaline degradation of PBDEs in the extract. The aqueous phase was discarded appropriately.

d. Partitioning with 5% Sodium Chloride solution

The resulting extract was shaken with 5 ml of 5 % sodium chloride, allowed to settle and the aqueous phase discarded. This step was repeated with another 5 ml of 5 % sodium chloride to remove traces of alkali. To dehydrate the trapped PBDEs extract, 5 g of anhydrous sodium sulphate was added to the organic layer was thereafter quantitatively transferred into I-CHEM Certified® 60 mL vial with a small volume of n-hexane. This procedure was repeated for all samples.

3.2.2.2.1 Clean-Up Stage

a. Preparation of solid phase chromatography column

These column chromatographic techniques involved the use of silica gel packed in Pasteur pipette. The Pasteur pipette packing was done by first clogging its tip with a clean glass wool. This was followed by the addition of 0.3 g of anhydrous sodium sulphate; Na_2SO_4. 0.2 g activated silica gel impregnated with silver nitrate; $AgNO_3$. 0.3 g of anhydrous sodium sulphate in ascending order. The anhydrous sodium sulphate traps any water-contaminant within the sample extract before reaching the impregnated activated silica gel and the glass wool. The packed column was preconditioned with about 5 ml of n-hexane to remove trapped air and background contaminants within the column. The n-hexane-extract was quantitatively passed through each silica gel column and the eluent collected into the 60 mL I-CHEM Certified® vial. The cleaned extract was pre-concentrated to about 0.5 mL under nitrogen stream at 50˚C This was quantitatively transferred into a 1.5 mL extreme high recovery vial (Agilent Technologies) for subsequent evaporation to dryness under nitrogen with gentle heating (<50˚C). The vial was crimp sealed after adding 50 µL of n-hexane and injected into GC-ICP-MS 2). About 16.67 % of n-hexane was lost during extraction and clean up stages. Basically, this clean-up procedure was done in accordance with the USEPA Draft Method 1614 for the analysis of PBDEs in wastewater and bio-solids, which among others, describes solid phase chromatography procedures and a destructive clean-up technique using an acid / alkaline, liquid / liquid partitioning for aqueous samples [38].

3.3. Procedures for accurate determination of total bromine by microwave digestion and isotopic dilution inductively coupled mass spectrometry

3.3.0 Sample preparation

Microwave digestion was employed (CEM Discover system) to ensure complete dissolution of samples. The microwave program presented in table 1 was selected based on LGC microwave standard operating procedure provided in Appendix D.

3.3.1 Preparation of natural standard stock solution

0.1086g of 77% Bromine, high purity sodium bromide was dissolved in 100ml of UHP water to produce natural stock standard solution of concentration 547.9 µg/g. 800 µl (7.9703 g) of the stock standard was further diluted with 111.1543 g UHP water to produce 39.287 µg/g working standard labelled as 'Dilution 1' in a clean falcon tube.

Table 1: Microwave Digestion program (CEM Discover system) conditions

	Standard mode		**CEM Program**				
Step	**Power (Watts)**	**Temperature (^oC)**	**Pressure (Psi)**	**Run time (min)**	**Hold time (min)**	**Stirrer speed**	**Cooling**
1	080	100	200	02.30	00.30	Time, off	off
2	100	130	230	02.00	01.00	Off	-
3	300	180	260	03.00	20.00	Off	off

3.3.2. Preparation and digested procedure blank [BLK 1 and BLK 2]

0.7198 g of Ultra high purity Nitric acid was weighed into 10 ml septum capped microwave vessel. The vessel was sealed and thereafter subjected to phases of microwave digestion protocol (table 2). At completion of the protocol, the digest vessel was placed in solid carbon dioxide and allowed to freeze. 0.5 ml (500 µl) of 32 % (v/v) concentrated ammonia solution was injected through the septum cap with injection syringe to avoid loss of volatile bromine analyte (if any). The vessel was gently shaken, returned into solid carbon dioxide (ice pack) and allowed to cool while still sealed. The alkalinized digest was removed from ice pack after no other visible reaction was observed within the vessel. The blank digest was quantitatively transferred with UHP water into a 50 mL falcon tube, capped and labelled as BLK 1 and BLK

2 appropriately. The sample was refrigerated prior to ICP-MS analysis using Element 2, Thermo Scientific, sector field ICP-MS.

3.3.3 Preparation of Mass Bias Correction Blends.

3.3.3.1 Preparation of Digested Calibration Blend (labelled as CB Digest)

The mixture of 0.1070g of NaBr dilution1(natural standard) and 0.1116 g of 81 Br Spike (supplied by Ck gas, SH2/107, 25 µg/g 81 Br, prepared) was accurately weighed into a pre-weighed 10 mL microwave vessel. The mixture was capped and allowed to freeze in the solid carbon dioxide. 500ul of ultra-high purity Nitric acid was added and then sealed. The blend was subjected to microwave digestion protocol. On completion of microwave digestion programme, the digest was alkalinized by addition of 500 µl of 32 % Concentrated Ammonia solution, injected through the septum cap using injection syringe to prevent any loss. The mixture was gently shaken for about 1 min and later kept in ice pack to cool to room temperature till no further visible reaction observed. The content was quantitatively transferred to a falcon tube and made up to 100 g with ultra-high purity water, capped and label appropriately prior ICP-MS analysis using Element 2, Thermo Scientific, sector field ICP-MS.

3.3.3.2 Preparation of 28 - PBDE Certified Reference Material (CRM) sample blend

0.1210g of CIL' 2,4,4' BDE 28 was accurately weighed into a pre-weighed 10 ml capacity microwave vessel labelled as '**28'** and the new total weight recorded. The content was evaporated to dryness at 50 ^{o}C under nitrogen using sample concentrator (TECHNE) to expel nonane. 0.1013 g of ^{81}Br Spike was added to the vessel to give a sample blend. The vessel was placed in the solid carbon dioxide (ice pack) to freeze. 500ul of ultra-high purity Nitric acid was added to the frozen blend and sealed. The mixture was subjected to microwave digestion programme. The resulting digest was cooled in ice pack followed by addition of 500 µl of concentrated ammonia solution through the septum cap using injection syringe and the mixture shaken gently. The digest was quantitatively transferred to a falcon tube with ultrapure water, capped and labelled appropriately before ICP-MS analysis**.**

3.3.3.3 Preparation of Undigested Calibration blend (CB Undigest MB)

The blend of 0.0991 g of dilution 1(natural standard, 39.287 µg/g) and 0.1017 g of 81 Br Spike prepared in a microwave vessel. The vessel was kept in ice pack to freeze. 500 µl of Concentration ammonia solution was added using injection syringe followed by addition of 500ul of ultra-high purity concentrated nitric acid. The calibration blend was not subjected to microwave digestion protocol to enable comparison between digested calibration blend with undigested calibration blend. The content was quantitatively transferred to a falcon tube and

made up to 100 g with ultra-high purity water, capped and label appropriately prior ICP-MS analysis using Element 2, Thermo Scientific, sector field ICP-MS.

4.0 ANALYSIS

4.1 GC-ICP-MS analysis of PBDEs by SEMDIDMS and External Calibration

For SEMIDMS, three replicate injections of each sample blend were made and bracketed on either sides with the measurement made on the calibration blend to correct for mass bias. The sequence was run orderly as calibration blank (hexane), test Calibration blend, calibration blank (hexane) twice, procedural blank (twice), sample blank (twice), calibration blend, sample blend, calibration blend, calibration blank(hexane) (as shown in Appendix E). This sequence was adopted to mitigate instrumental drift on the observed ratios. The Agilent Technologies Masshunter workstation ICP Data Analysis software (version B.01.01) was used for manual integration of the chromatographic signal. The software's ability to present chromatograms as a log function was found to assist with the integration of the peaks. Table 1 below describes the optimised instrumental condition for the analysis.

The same optimised conditions for SEMDIDMS [shown in table 2] were employed for external calibration ['Run 1 ext' and 'Run 2 ext'] and internal injection standard approach [Run 2 ext/int]. For Run 2 ext/int, the five calibration standards, CCV and ICV standards were prepared with n-hexane/internal standard mixture (73.0825 ng/g 1,1 dibromocyclohexane –n-hexane mixture). This internal standard gave peak area of greater than 800 cps same as top calibration standard (97.635 ng/g) for PBDE 28 with retention time 2.793 min as shown in figure 5**.** The analysis sequence involved measuring of three (3) calibration blanks in the beginning, then five (5) calibration standards (PBDE Mix standards from Cambridge Isotope laboratory, UK), followed by two (2) calibration blanks. Every three triplicate sample measurement was bracketed before and after by CCV standard, calibration blank and ICV standard to monitor signal drift. In addition the calibration was repeated at the end of the analysis as shown in appendix F.

Table 2: Instrumental operating conditions for GC-ICP -MS

Q-ICP-MS Parameters	
Forward power	680 W
Plasma gas flow rate	15.5 L min^{-1}
Carrier gas flow rate	0.6 L min^{-1}
Short program dwell time	0.05 s per isotope
Extended program dwell time	0.19 s per isotope
Monitored isotopes	^{79}Br, ^{81}Br (tuning ^{128}Xe)
Points per spectral peak	1
Optional gas	10 ppm Xe with 20% oxygen in a balance of He
% of optional gas	5.0%
Collision gas	Helium
Collision gas flow rate	2.5 mL min^{-1}
Rotary pumps	2
GC parameters	
Column stationary phase	DB-5MS UI (30 m x 0.25 mm id x 0.25 µm film thickness)
Carrier gas	Helium
Short flow program	3.0 mL min^{-1} initial, held for 9 min then ramped at 0.3 mL min^{-2} to 3.6 mL min^{-1} and held for 10 min
Short temperature program	110 °C initial, held for 1 min ramped at 28 °C min^{-1} to 300 °C and held for 11 min
Extended flow program	1.5 mL min^{-1} constant flow
Extended temperature program	90 °C initial held for 3 min ramped at 1 0°C min^{-1} to 140 °C and then ramped at 2°C min^{-1} to 300 °C and held for 35 min
Temperature of the transfer line metal tubing	300 °C
Temperature of the ICP-MS torch injector	300 °C
Injector technique	Splitless
Injector temperature	300 °C
Injection volume	2.5 µL

4.2 ICP – MS analysis of total bromine

ICP - MS Instrument -Thermo Scientific, Finnigan Element 2 (by Thermo Electron Corporation) run by Element Software Suite version 3.0 possess high mass resolution , outstanding sensitivity and signal to noise ratio possessing very low detection limit. The operating condition is as shown in table 3 and appendix G. Daily routine check test was carried out. Thermo Fisher ICP – MS – Element 2 was tuned specifically for bromine using 97.97 ng/g Br tuning standard in 1% ammonia to remove any acid trace, obtain maximum instrument stability, good and by tuning torch position, focus lens and sample gas at low resolution. Each sample blend was bracketed before and after with a calibration blend as: reagent blanks, calibration blend, sample blend, calibration blend, sample blend and so forth. The acronyms are HR, High resolution, MR medium resolution, LR Low resolution. Appendix G listed full tune parameters of for total bromine analysis. The transmission at high resolution and medium resolution were 1.93% and 8.288% respectively.

Table 3: Operating conditions of ICPMS for isotope dilution mass spectrometry

Tune parameter	**LR**	**MR**	**HR**
Plasma Power [Watt]	1320	1320	1320
Cool gas [L/min]	15	15	15
Sample gas [L/min]	0.980	0.980	0.980
Nebulizer	Glass Concentric		
Spray Chamber	Quartz, Double pass cooled to $2^{o}C$		
Cones	Nickel		
Isotope Monitored	^{79} Br and ^{81} Br		

5.0 RESULTS AND DISCUSSION

5.1 Application of SEMDIDMS quantification of PBDE in humic acid model water.

The result of recovery studies of PBDE in humic acid model water by SEMDIDMS is as summarised in table 4 below.

Table 4: SEMDIDMS result for PBDE analysis in 15 mg/L Humic acid model water

Concentration (ng/L)	37.260	36.595	37.340	38.082	37.399	36.953
Recovery range (%)	93 -95	94 -99	96 -101	91 -106	96 -99	91 -105
Maximum U (%)	2	6	5	3	5	5

Despite the presence of 15 mg/L humic acid in model water, the method was able to detect PBDE up to about 38.082 ng/L in 0.033g (50 µl) of extract. This demonstrated that the method is sufficiently selective, fit for purpose and specific enough for the priority congeners. The peaks and baseline were well resolved as shown in Figure 2 m/z 79 Br PBDE chromatogram. All BDE congeners and solvent, n- hexane [6.903] peak appeared at their characteristic retention time of 6.73 min [BDE-28], 7.46 min [BDE-47], 8.04 min [BDE-100], 8.29 min [BDE-99], 8.97 min [BDE-154] and 9.45 min [BDE-153]. The method still achieved the quality control limit 90 – 110 % with very low uncertainty.

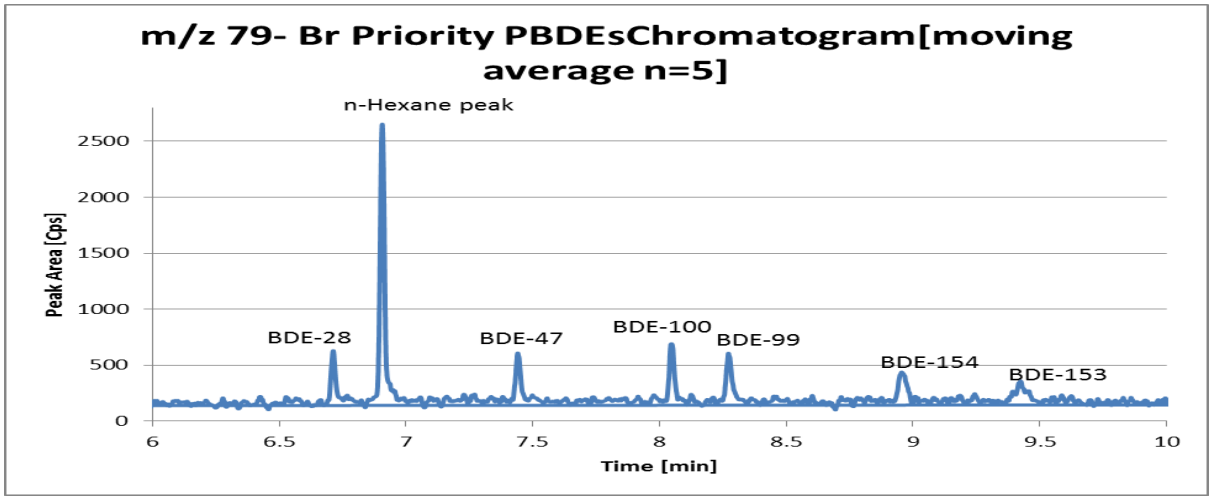

Figure 2: Chromatogram of PBDEs analysis in 15 mg/L Humic acid model water.

5.2 Comparative study of Polybrominated diphenyl ethers quantification using Semi Exact matching double IDMS and External calibration method

Following the previous success of LLE-GC-ICP-IDMS in the analysis of real particulate water sample (River Thames) and synthetic model water by LGC. The efficacy of the method was further certified by its application to River Mole water samples. The analysis of River Mole water samples by SEMDIDMS was performed by another analyst at LGC. This project compares results of quantification strategies by SEMDIDMS, external calibration [Run 1 ext, Run 2 ext], and later by internal injection standard correction [Run 2 ext/int] due to experience from external calibration approach. For data quality control, continuing calibration verification (CCV), initial calibration verification check standards were used for drift

monitoring as described in section 4.1. As a result of experience from external calibration approach, the analytical behaviour of 1,1 dibromocyclohexane [figure 3] diluted in n-hexane mixture [73.08256 ng/g] was tested in this experiment as a potential internal injection standard.

The efficiency of sample preparation process by Ultrasonic assisted Liquid –Liquid extraction, H_2SO_4/KOH/Na_2SO_4/NaCl partitioning and clean–up was monitored. The final residues values after preconcentration under nitrogen stream at 50°C range from 0.00005 to 0.00008g for 'Run 1 ext' and -0.00846 to 0.000137g for 'Run 2 ext/int' where mass of injection extracts were 0.06308g (about 100 µl) and 0.032063 g (50 µl) respectively shown in **appendix H.**

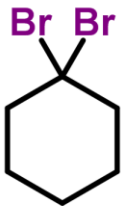

Figure 3: Structure and properties of 1,1-dibromocyclohexane

[ChemSpider ID:161452, Molecular Formula: C6H10Br2 ,Average mass: 241.951599 Da, Monoisotopic mass: 239.914917 Da, boiling point : 207.7±13.0 °C at 760 mmHg, density of 1.8 ± 0.1 g/cm3, vapour pressure of 0.3 ± 0.4 mmHg at 25°C [reproduced from reference 39)

5.2.1 Evaluation of the Instrumental limit of detections [LODs], Method Limit of

Detection and Limit of Quantification of Priority Congeners by external calibrationTable 5 is the List of Limit of detection, Method Detection Limits and Limit of Quantification of each priority PBDE Congener. The instrumental Limits of detections [LODs] were calculated from values of standard deviation values as shown in appendix J.[36] The calibration curves (R^2 = 0.995-0.999) were used for '**Run 1 ext' and 'Run 2 ext/int'.** LOQ was determined by multiplication of one-third LOD by 10. The LODs were in the range of 3.03 ng/g – 11.07 ng/g for m/z 79 –Br and 4.33 ng/g - 11.93 ng/g for m/z 81-Br as shown in table 5. The method LODs for 'Run1 ext.' were in the range of 0.09 ng/kg - 0.36 ng/kg for m/z 79-Br and 0.13 ng/kg - 0.38 ng/kg for m/z 79–Br and m/z 81 Br- BDE respectively. For 'Run 2 ext/int' , method LODs 0.05 ng/kg - 0.17 ng/kg and 0.07 ng/kg - 0.20 ng/kg for m/z 79 –Br and m/z 81 Br- BDE respectively.

Table 5: List of Instrument Limit of detections [LODs], Method Detection Limits and Limits of Quantification of each Congener applying external calibration approach

Run 1 Ext	m/z 79 Br			m/z 81 Br		
Congeners	**LOD ng/g**	**MTD LOD, [ng/kg]**	**LOQ, [ng/kg]**	**LOD, [ng/g]**	**MTD LOD, [ng/g]**	**LOQ, [ng/kg]**
BDE-28	3.03	0.09	0.31	7.63	0.23	0.78
BDE-47	8.90	0.27	0.91	6.46	0.20	0.67
BDE-99	4.88	0.15	0.50	4.33	0.13	0.44
BDE-100	3.74	0.11	0.38	4.33	0.38	1.27
BDE-153	11.07	0.36	1.13	11.93	0.36	1.21
BDE 154	8.35	0.26	0.85	6.96	0.21	0.71
Mean	6.66	0.21	0.68	6.94	0.25	0.85
Max	11.07	0.36	1.13	11.93	0.38	1.27
Min	3.03	0.09	0.31	4.33	0.13	0.44

The method LOQs for ‘Run 1 ext’ were in the range of 0.31 ng/kg - 1.13 ng/kg and 0.44 ng/kg - 1.27 ng/kg for m/z 79 –Br and m/z 81-Br respectively. The method LOQs for ‘Run 2 ext/int.’ were in the range of 0.16 ng/kg - 0.58 ng/kg and 0.23 ng/kg – 0.65 ng/kg for. for m/z 79–Br and m/z 81 Br- BDE respectively.

From the analysis above, the use of internal standard correction enhanced reduction in method LODs and LOQ. For ‘Run 1 ext’. and ‘Run 2 ext/int.’ the values of instrumental LODs, Method LODs and LOQs obtained for each congeners are obviously higher than values obtained by SEMDIDMS which states that Instrumental LOD ranged from 1 to 5 ng g^{-1} and the LOQ ranged from 0.10 to 0.14 ng kg^{-1} for the individual congeners. This confirms the ability of SEMDIDMS for quantitation of priority PBDEs at ultratrace levels.

Table 5 (a): List of Instrument Limit of detections [LODs], Method Detection Limits and Limits of Quantification of each Congener applying internal standard correction

RUN 2 EXT/INT	m/z 79 Br			m/z 81 Br		
Congeners	**LODs [ng/g]**	**MTD LOD [ng/kg]**	**LOQs [ng/kg]**	**LODs [ng/g]**	**MTD LODs, [ng/kg]**	**LOQ [ng/kg]**
BDE-28	3.03	0.05	0.16	7.63	0.12	0.40
BDE-47	8.90	0.14	0.46	6.46	0.14	0.46
BDE-99	4.88	0.08	0.26	4.33	0.07	0.23
BDE-100	3.74	0.06	0.20	12.46	0.20	0.65
BDE-153	11.07	0.17	0.58	11.93	0.19	0.63
BDE 154	8.35	0.13	0.44	6.96	0.11	0.37
Mean	6.66	0.10	0.35	8.29	0.14	0.46
Max	11.07	0.17	0.58	12.46	0.20	0.65
Min	3.03	0.05	0.16	4.33	0.07	0.23

5.2.2 Recovery Studies

The external calibration method [Run 1 ext] performance for priority PBDEs was evaluated by comparing concentrations and recoveries obtained with SEMDIDMS method [Run 3 IDMS]. The 2 kg water samples were spiked with 2 ng of each PBDE congener. No PBDE congener was detected in calibration blanks, procedural blanks and River Mole water samples. Therefore, blank correction was not performed for quantifying any congener. From this result, River Mole was as clean of priority PBDE congeners as the procedural blanks in all the runs. Tables 6 and 7 and figure 4 summarises the results of SEMDIDMS [RUN 3 IDMS] and 'Run 1 ext.'

Table: 6 Recovery studies of analysis of PBDE congeners in River Mole water by SEMDIDMS [Run 3 IDMS]

	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154
Concentration ng L^{-1} [n=3]	**1.002**	**0.998**	**1.001**	**1.018**	**0.996**	**0.982**
Recovery range [%]	**99 – 101**	**95 – 106**	**99 - 106**	**98 – 102**	**94 – 107**	**99 - 101**
Maximum U [%]	**13**	**6**	**15**	**6**	**21**	**7**

Table: 7 Recovery studies of analysis of PBDE congeners in River Mole water by External calibration [Run 1 ext]

	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154
Concentration ng L^{-1}	**0.696**	**0.624**	**0.558**	**0.676**	**0.502**	**0.591**
Recovery range [%]	**65 – 74**	**53 – 75**	**46 - 75**	**58 – 79**	**36-65**	**52 -70**
% RSD	6	17	27	16	29	15

From the tables 6 and 7 and figure 4, ‘Run 1 ext.’ achieved a low recovery compared to ‘Run 3 IDMS’ recovery values. ‘Run 1 ext.’ also recorded higher uncertainties [RSD%] compared to ‘Run 3 IDMS’. In addition, ‘Run 1 ext.’ recovery values failed the quality control recovery limit of 90 - 110 % according to LGC in-house quality standard. However, the quality control test performed on ‘Run 1 ext.’ [external calibration] check standards, CCV and ICV revealed that their mean recovery and standard deviation values generally lies within the quality control limit. The calibration standards also generally lies within 80 – 120 % according to 40 CFR 136, EPA Quality standard.[37] The recovery studies results from all the methods are presented in Appendix K labelled quality control data for recovery and their accuracy range. The accuracy assessment recovery range [P-2sp to P+ 2sp], 40 CFR 136, EPA Quality standard, where P is the mean recovery and sp is the standard deviation] also passed the quality control criteria. It was inferred that, the external calibration approach passed all data control tests. However, it was necessary to effect modifications in extraction processes and injection system to achieve quantitative recoveries.

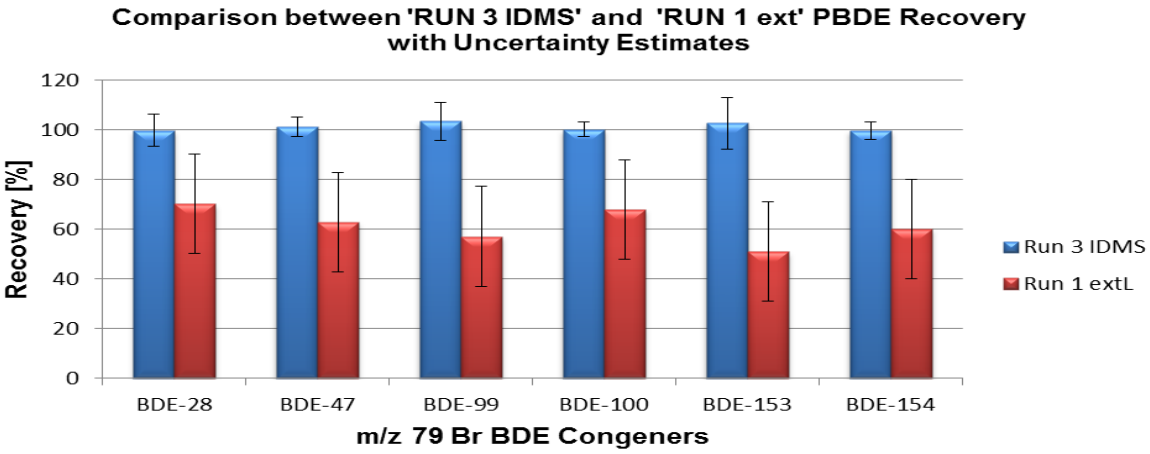

Figure 4: Comparison between SEMDIDMS and External calibration PBDE Recoveries and uncertainty estimates

SEMDIDMS as a primary method has clearly displayed unequal capacity to produce very accurate results than all classical calibration strategies.[40,41,42]

5.2.3. Possible causes of low spike recoveries by external calibration

In this project the causes of low recovery, higher uncertainties were investigated within the time limit and availability of some materials. Sources of error suspected include:

a. Organic and aqueous phase settlement time range

b. Injection / instrument drift with time exemplified in figures 8 and 9

c. Variations in instrumental sensitivity during a run [40,41]

d. Possible loss of PBDE to aqueous phase during extraction and partitioning processes [43,43,44,]

e. Evaporation of hexane extract 0.033 – 0. 062 g due to laboratory temperature.

f. The other effect of the instability of the GC run is that PBDE on injector or column break down differs due to matrix components in the extract. These may block active sites and reduce the amount of break down leading to less break down occurring for the extract compared to the standards.[45]

5.2.4 Use of 1,1 dibromocyclohexane as an Injection Internal Standard

The experiment was repeated with internal standard in [Run 2 ext/int]. After UALLE the phase separation was allowed enough time to settle. Although, the method development experience observed that PBDEs are congener-based. The evidence of fluctuations and low recovery necessitated a repeat of the recovery studies by the inclusion of a suitable internal

injection standard aimed at compensating for losses during sample preparation. However, the analytical properties of 1,1 dibromocyclohexane was tested even though there was no literature evidence of previous use. This internal standard gave peak area of greater than 800 cps same as top calibration standard (97.635 ng/g) for BDE- 28 with retention time 2.793 min as shown in figures 5 and 6.

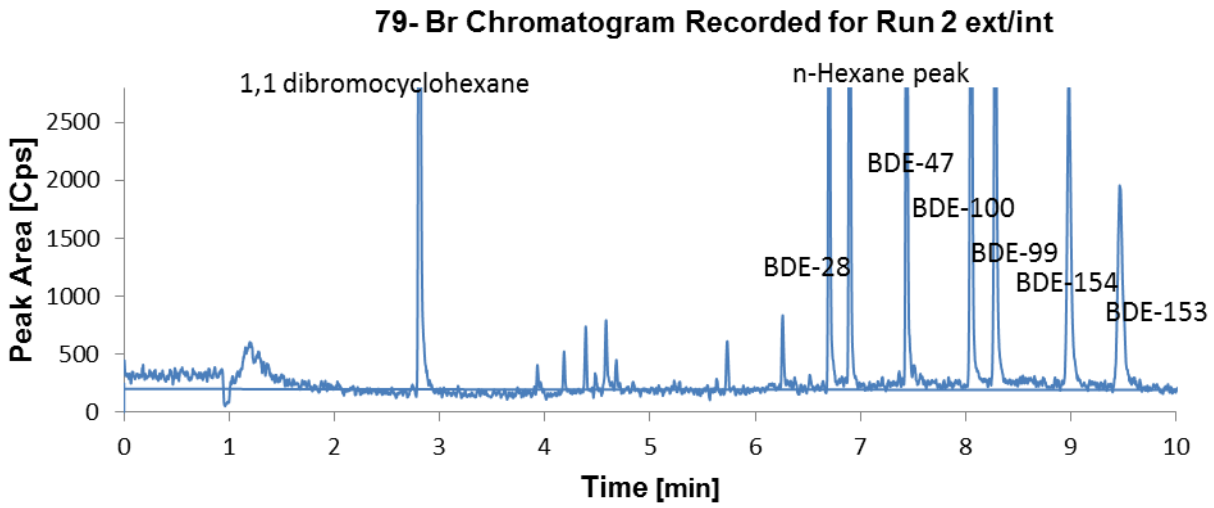

Figure 5: m/z 79 Br PBDE Congeners Chromatogram showing analytical behaviour of 1,1 dibromocyclohexane used as injection internal standard

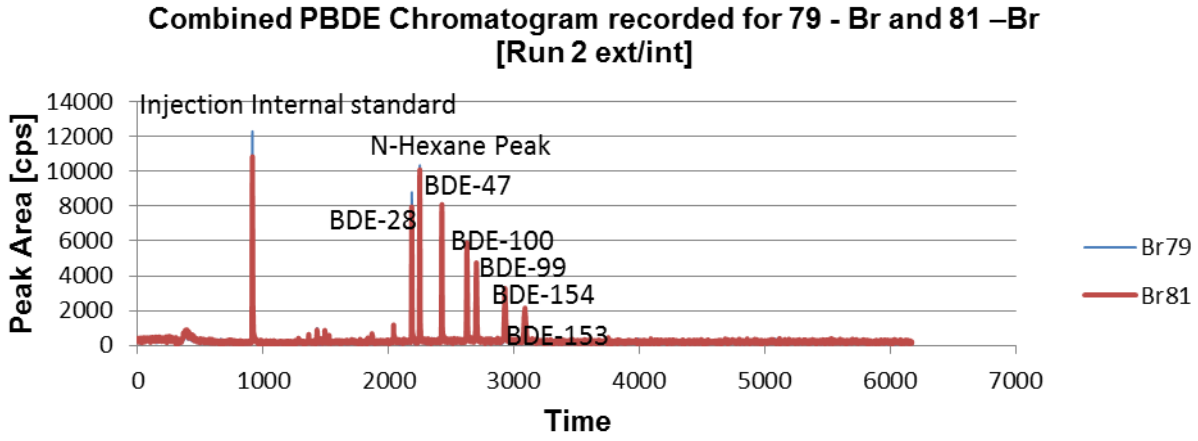

Figure 6: Combined PBDE Chromatogram recorded for m/z 79 Br and m/z 81 Br PBDE congeners

Figures 7, 8 and 9 serves as evidence of variation in recoveries for BDE-47 m/z 79-Br [for all classical methods tested] due to fluctuation in injections, instrument sensitivity [GC-ICP-MS] with time in between runs.[40,41,42] The plots were obtained for all m/z 79 BDE recoveries obtained from 'Run 1 ext,' 'Run 2 ext' and 'Run 2 ext/int.' as shown in appendix L to this project.

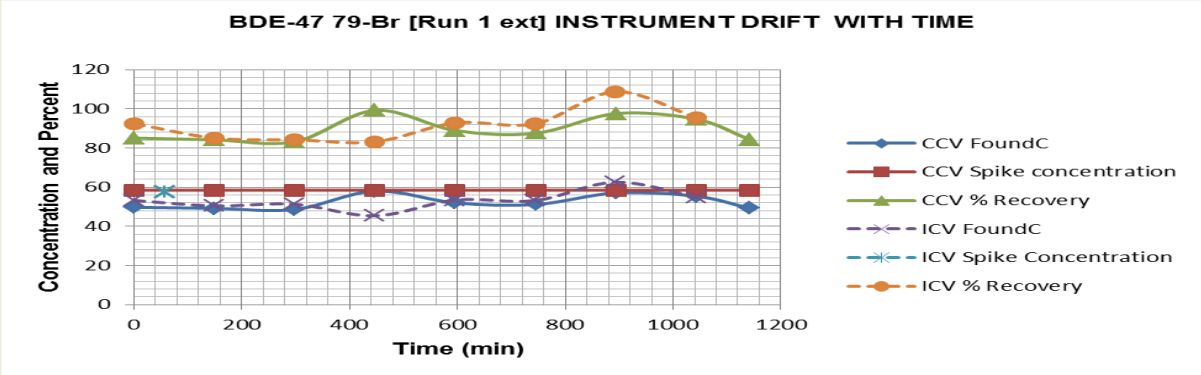

Figure 7: Plot of BDE-47 m/z 79 –Br Instrument Drift with time [Run 1 ext]

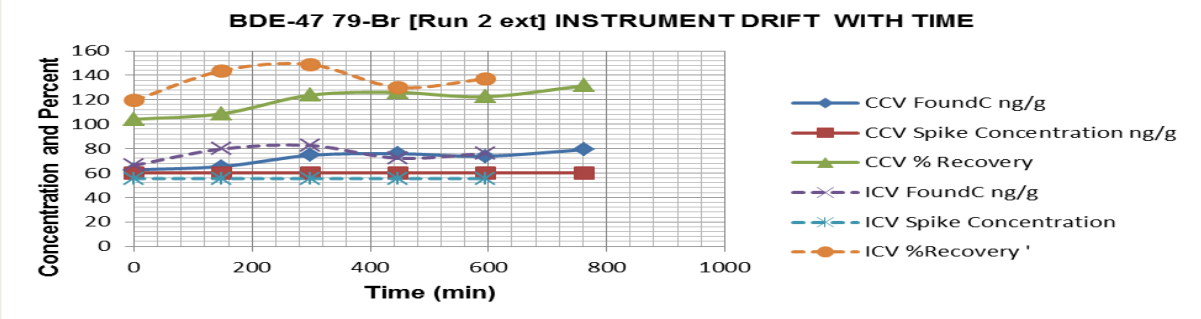

Figure 8: Plot of BDE-47 m/z 79 –Br Instrument Drift with time [Run 2 ext]

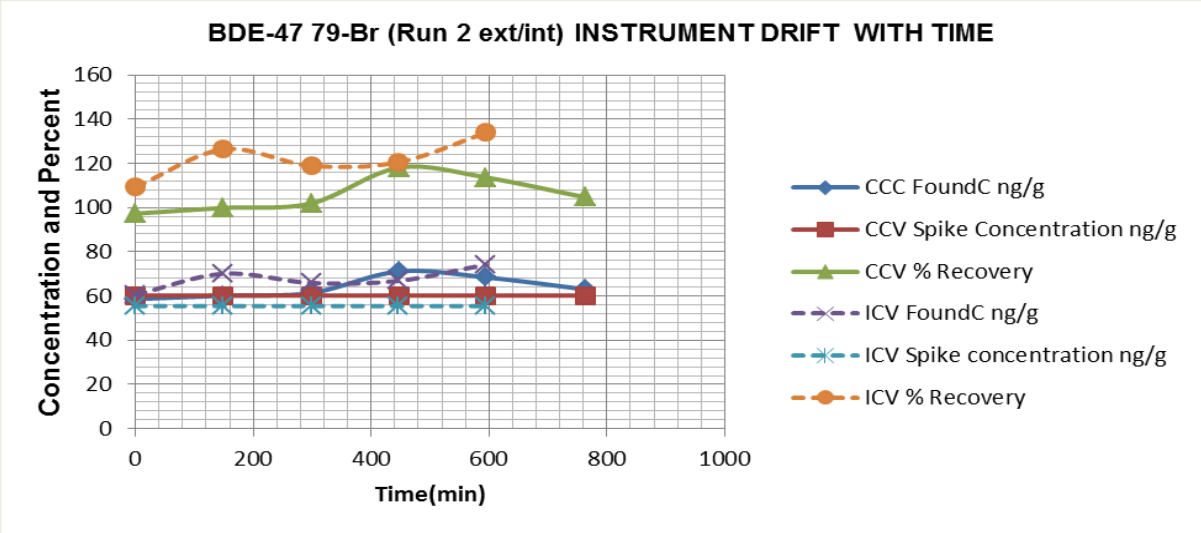

Figure 9: Plot of BDE-47 m/z 79 –Br Instrument Drift with time [Run 2 ext/int]

5.2.5 Result of repeat analysis

Reference appendix K**,** quality control data for recovery and their accuracy range the values of mean recoveries and standard deviations of calibration standards recoveries, CCV and ICV check standards [recoveries] established that 'Run 1 ext' and 'Run 2 ext/int' passed all

quality control test. The values lie within the control limit 90 – 100**%.** The summary of results presented in tables 8, 9 and figures 10 and 11 below shows that the mean percentage recovery of BDE-28, BDE-47 exceeded the control limit 90 -110% but with lower uncertainty estimate for 'Run 2 ext.' bias suspected. The mean recoveries of other congeners lie within the limit but with much higher uncertainty relative standard deviation RSD[%]. The results of internal standard corrected, 'Run 2 ext/int.' Shows that the mean recovery and uncertainty estimates lies within the control limit for BDE- 99 and BDE-100. Lower mean recoveries and highest uncertainties recorded for BDE-153 and BDE-154. There is evidently reduced recoveries for BDE-153 and BDE-154 in 'Run 2 ext/int' and higher uncertainties.

However, data bar and sparking tools in Figures 11 established that 'Run 3 IDMS' is more accurate than all the classical approach. Also, that 'Run 1 ext' [external calibration] will be able to give higher spike recoveries with some other modifications in the established LLE and partitioning processes that will be suggested later in this paper.

Table 8: Summary of spike recovery result for 'Run 2 Ext.' [external calibration]

SUMMARY	**79 Br**					
	BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Concentration ng L^{-1}	1.402	1.458	1.239	1.210	1.023	0.980
Recovery range [%]	127 - 153	129 -153	109 -144	106 – 139	67 - 125	67 – 121
Mean Recover [%][n=3]	141	143	126	121	103	100
RSD [%]	9	9	14	14	30	29

Table 9: Summary of spike recovery result for 'Run 2 ext/int' [with internal standard]

SUMMARY	**79 Br**					
	BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Concentration ng L^{-1}	1.132	1.176	1.029	0.961	0.820	0.796
Recovery range [%]	105 -120	107- 120	92 – 116	85 – 107	54 - 98	55 – 96
Mean recovery [%] [n=3]	114	116	105	96	83	81
RSD [%]	7	7	12	11	30	28

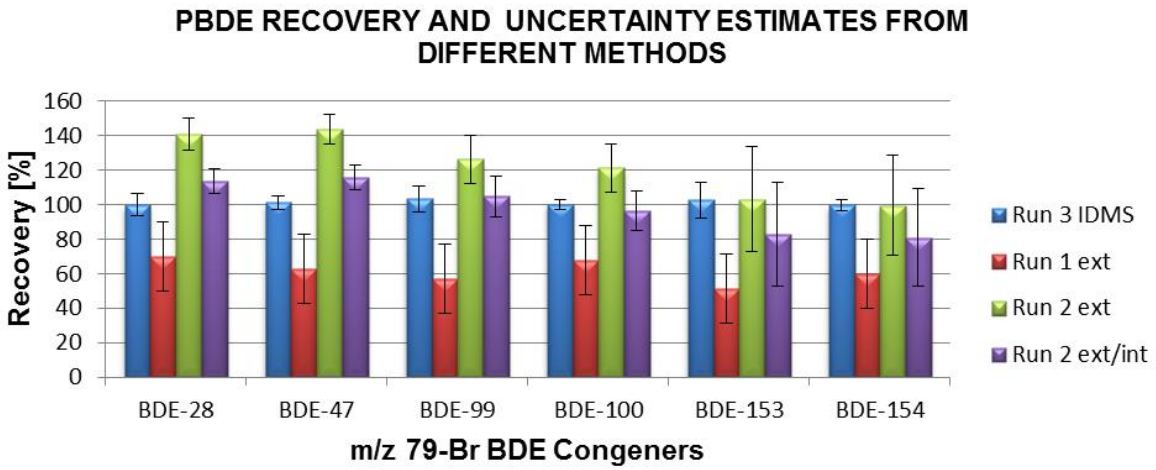

Figure 10: Summary of mean recoveries and uncertainty estimates from the four strategies

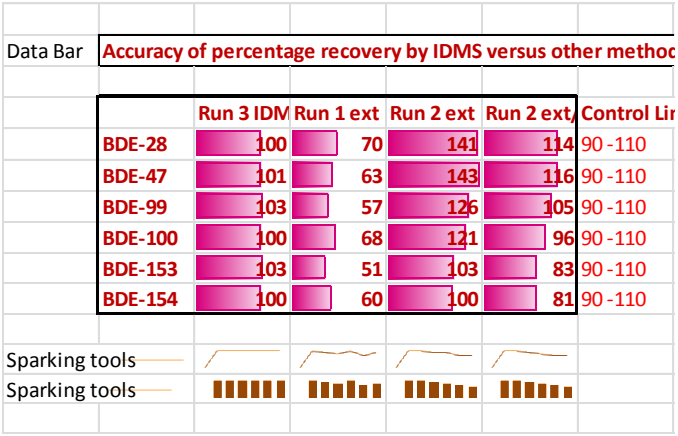

Figure 11: Data Bar and sparking tools describing the accuracy of IDMS over all other methods.

5.2.6 Statistical Comparison of all classical calibration strategies with SEMDIDMS

The results of different experimental strategies for quantification demands that a further statistical tool be engaged to obtain concrete statistical evidences of significance differences between method results. Consequently, table 10 below describes the results of a Paired Sample T test conducted to compare mean concentrations and recoveries obtained between 'Run 3 IDMS' versus 'Run 1 ext.'' 'Run 3 IDMS' versus 'Run 2 ext/int' [with internal standard correction], 'Run 3 IDMS' versus 'Run 2 ext. and 'Run 2 ext/int' versus 'Run 2 ext.'

Table 10: Sample Paired Test [two-tail] test table for comparison bêtween two methods

	Run 3 IDMS Versus Run 1 ext.		**Run 3 IDMS versus Run 2 ext/int.**		**Run 3 IDMS versus Run 2 ext.**		**Run 2 ext/int Versus Run 2 ext**	
Congener	**/t/**	**p-value**	**/t/**	**p-value**	**/t/**	**p-value**	**/t/**	**p-value**
BDE-28	7.514	0.017	-2.301	0.148	-4.845	0.040	-9.144	0.012
BDE-47	5.103	0.036	-3.044	0.093	-5.572	0.031	-9.556	0.011
BDE-99	4.868	0.040	-0.459	0.691	-2.656	0.117	-6.732	0.021
BDE-100	5.567	0.031	1.078	0.394	-2.214	0.157	-7.310	0.018
BDE-153	6.104	0.026	1.361	0.307	-0.161	0.887	-7.310	0.035
BDE-154	7.561	0.017	1.586	0.254	0.015	0.989	-4.921	0.039

Null Hypothesis, Ho = There is no significant difference between the results of the two techniques

Alternative Hypothesis, H1= There is significant difference between the results of the two

techniques.

Sample Paired –T- test assumes that the different measures are normally distributed or at least reasonably symmetric.The variables, mean concentrations and mean recoveries were plugged into an excel Microsoft worksheet designed by the author to give corresponding values of /t/ statistic and p-values. Figure 12 is the plot of /t/ statistic versus P-values obtained from Paired T test data derived from mean concentrations recovered by each method.

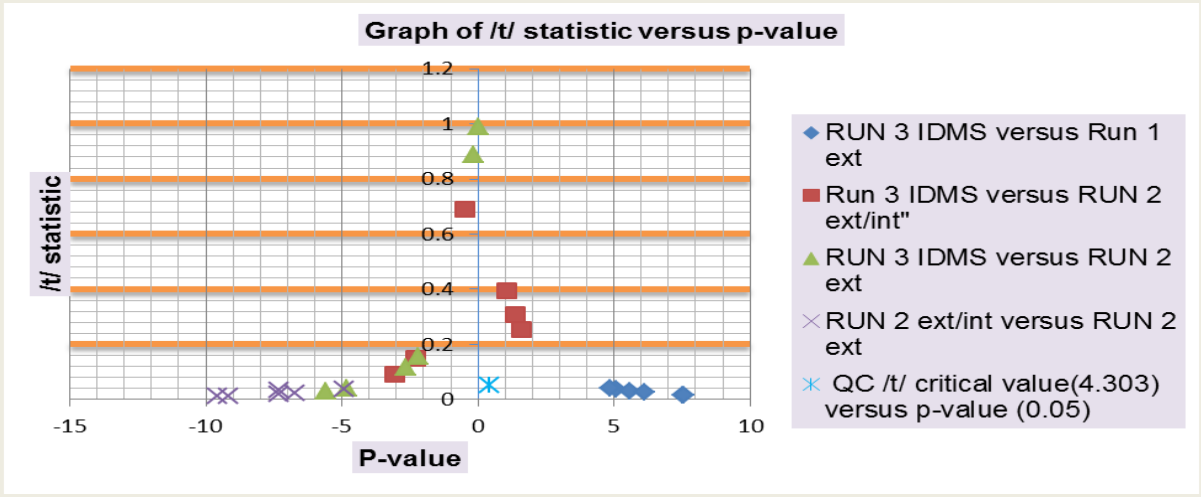

Figure 12 : Graph of /t/ statistic versus P-values obtained from Paired T test data derived from mean concentrations recovered by each method.

5.2.6.1 Quality Control limit for Sample Paired-T test

Each point on the graph represents a PBDE congener analysed by the paired method. Following the quality control conditions plotted as light blue point on the curve. Any congener and method pair value that lies above the QC/t/ critical value (4.303) versus p-value (0.05) point on the graph gave same result. Hence no statistical difference . Any method pair that lies at a point below the QC/t/ critical value (4.303) versus p-value (0.05) gave statistically significant results from each other.

The critical value is t_2 = 4.303 (P=0.05) at 95 % confidence limit obtained from t-table where tn-1= 3-1 = 2. Since three samples were analysed for each method. If t_2 = 4.303 (P=0.05) is less than t-calculated [/t/ statistics], then the null hypothesis is retained and there is no statistically significant differences between the two results. If t_2 = 4.303 (P=0.05) greater than t-calculated /t/ statistics, then the null hypothesis is rejected. The two methods gave statistically significantly different results. However, final inference was based on significance (2-tailed) referred to as p-value. P-value can be obtained on excel spread sheet by selecting the data region as used in this example. P-value = TTEST(W34:W38,X34:X38,2,1).

If the Sig (2-tailed) value is greater than > 0.05, there is no statistically significant difference between two methods. The differences between methods are likely due to chance and not likely due to any modification. If the sig (2-tailed) values is less than or equal to ≤ 0.05. Then, there is statistically significance difference between the two methods. The probability that the differences are due to sampling error and processing is less than 0.05.

From table 10 and figure 12 above, Sample paired T test reports as follows:

a. That the /t/ statistics and p-values decisions for the mean concentrations and recoveries of m/z 79 PBDE congener for each method are generally the same except for BDE-99 and BDE-153 in 'Run 3 IDMS' versus 'Run 1 ext.' as well as BDE – 47 and 'Run 3 IDMS' versus 'Run 2 ext/int' where /t/ statistics and p – values inference for mean recoveries contradicts that of mean concentration.
b. Obviously, there is no statistically significant difference between Run 3 IDMS result and 'Run 2 ext/int' [with internal standard]. The results are statistically the same.
c. There is statistically significant difference between results obtained using injection standard correction [Run 2 ext/int] and the one obtained without applying internal standard correction [Run 2 ext]..
d. There is evidently statistically significant difference between the results of Run 3 IDMS and Run 1 ext [external calibration].
e. 'Run 2 ext' compared with 'Run 3 IDMS' shows no statistically significance difference between IDMS [Run 3 IDMS] result and 'Run 2 ext' except for BDE- 28 and BDE- 47.Where there is statistical significance difference between the results of two congeners.

5.2.7 Advantages of IDMS techniques over external calibration strategy

IDMS is able to produce most accurate result than external calibration because it measures isotopic ratios and both the labelled enriched spike [which acts as a perfect internal standard] and the analyte are affected in the same magnitude by same variations in instrument sensitivity. Also, IDMS is able to correct for any transformation during sample preparation and instrumental analysis. IDMS is not affected by matrix effect because both the enriched spike and the analyte are the same element. No calibration graph required in IDMS.

Once sample and spikes have equilibrated, any possible loss of analyte from the isotope-diluted sample will not affect the final result. This is because any aliquot of the isotope-diluted will contain the same isotope amount ratio X R, and therefore, it is unnecessary to know the pre-concentration or dilution factor of the sample or to take into account any non-quantitative separation or evaporation step.[40,41] In addition, the uncertainty in the mass fraction measurement procedure are dependent on measurable and defined variables. The uncertainty are typically lower when compares with external calibration. The uncertainty contributing to the mass fraction of the calibration standard comes from the gravimetric dilution of a stock to give a working solution. The uncertainty in the measurement of the isotope ratios has to be experimentally determined or captured in a factor such as the overall

precision of results. IDMS only requires that the isotope ratios be measured with with high accuracy and high precision using suitable instruments.[40, 41, 42, 43]

IDMS is usually laborious and time consuming compared to external calibration. The cost of mass spectrometry and availability of suitable isotopic materials need to be considered. IDMS demands special training of analyst to acquire the skill less accurate results are often achieved.

5.3. Total bromine determination result discussion

The samples were dispersed in alkaline solution such as ultrapure ammonia solution to get rid of matrix effects, bromine loss associated with sample digestion and sample preparation M. Ohata et al [20] and S. Hill et al [19] recommended that "the solvent should be kept at alkaline condition to remove memory effect (memory effect which was steamed out from the drain of the spray chamber of ICPMS) , acid traces, maximum sensitivity and good stability. M. Ohata et al [20] discovered that the constant results were obtained by the dilution using NH_3 solution. This alkaline conditioning allowed reproducibility of measurement.

The optimum spiking ratio was derived from S. Hill et al [19] work when the optimum spiking ratio of 0.5 gave a percentage recovery of 101 %. An ICPMS is a powerful tool for quantitative multielement analysis as well as isotope ratio determination due to its high sensitivity, multielement capability, and wide linear dynamic range. An isotope dilution ID-ICPMS is known to be a primary method of measurements, which is one of the reliable analytical methods; therefore, it is recommended to be applied for the CRM development. To achieve maximum instrument, good stability, The Spray chamber (Quartz, Double Pass) and all ICPMS glassware ultrasonically cleansed (using Ultrasonicator Kerry) with Ammonia , 5% Sulphamic and thorough rinse in Ultra high purity water (Elga water) and air dried. The Nickel cone was ultrasonically cleansed in 1 % ammonia, rinsed in UHP water and air dried.

Loss of minimum bromine due to microwave acid digestion may be inevitable, however ID-ICPMS a primary method of measurement was able to compensate for Bromine loss because isotopic equilibrium between Br in sample and enriched isotope was ensured during the digestion procedure. The total bromine was calculated by substituting variables into Semi-exact matching double Isotope dilution mass spectrometry equation described reference [40, 41, 42.]

Table 11: Result for total bromine determination in Digested calibration blend.

Samples	Found Bromine Concentration	Standard Uncertainty	Expanded Uncertainty	Expected Bromine concentration	Recovery
	Br [µg/g]	U [µg/g]	U [%]	[µg/g]	(%)
Digest calibration blend_1	41.04	0.41	2.00	39.287	104.462
Digest calibration blend_2	40.80	0.42	2.07	39.287	103.8511
Digest calibration blend_3	40.79	0.36	1.78	39.287	103.8257
Mean values	40.88	0.40	1.95		**104.0463**

From **table 11** above, the expected total bromine concentration in mass bias correction blend was 39.287 ug/g. The observed concentration of Br In sample was 40.88 ± 0.40 ug /g with the standard uncertainty of 0.97 %.The mean recovery for digest calibration blend was 104.1 % (k=2). Therefore, the use of acid digestion gave a good recovery.

Table 12: Summary of result of total bromine determination in unlabelled 2,4,4'TriBDE (BDE-28) Certified Reference Material (CRM)

Sample	Found Concentration	Standard Uncertainty	Expanded Uncertainty	Expected Bromine Concentration	Expected standard uncertainty	% recovery
	Br µg/g	U µg/g	U % (k=2)	[µg/g]	Ui [µg/g]	
CIL PBDE 28_1	42.38	0.46	2.19	40.93	0.12	103.6
CIL PBDE 28_2	42.66	0.55	2.59	40.93	0.12	104.2
CIL PBDE 28_3	41.96	0.53	2.54	40.93	0.12	102.5
CIL PBDE 28_4	42.13	0.60	2.85	40.93	0.12	102.9
Mean values	42.28	0.54	2.54			103.3

The expected concentration of Br in 2,4,4' TriBDE (BDE-28) Certified Reference Material (CRM) analysed is 40.93 mg/kg with Standard uncertainty of 0.121563 mg/kg. The mean recovery for CIL PBDE 28 was 103.3%.The mean observed concentration of total bromine in CIL PBDE 28 was 42.28 ± 0.54ug/g with expanded uncertainty of 2.5 %.

Table 13: Percentage contributions to standard uncertainties for the determination of total Bromine in Unlabelled Certified Material CRM: 2,4,4'–TriBDE (BDE 28) by ID-ICPMS

Variables	c_z	m_x	m_y	m_{YC}	m_{zc}	R_Y	R_z	R_{BC}	R'_B	R'_{BC}
Budget (%)(n=4)	0.002	3.527	5.03	4.99	5.2585	0.001	0.050	19.308	24.441	37.387

Table 14: Percentage contributions to standard uncertainties for the determination of total Bromine in Undigested CB estimated by ID-ICPMS by ID-ICPMS

Variables	c_z	m_x	m_y	m_{YC}	m_{zc}	R_Y	R_z	R_{BC}	R'_B	R'_{BC}
Budget (%) (n=3)	0.003	7.54	6.42	8.35	8.63	0.0	0.0	0.08	17.28	51.54

R'_{BC} is the observed isotope amount ratio in the calibration blend; R'_B, Observed isotope amount ratio in the sample blend; R_{BC}, is the true isotope amount ratio in the calibration blend; R_Z, is the isotope amount ratio in the standard solution used to prepare the calibration blend; R_Y is the isotope amount ratio in the spike solution. m_{ZC} refers to the mass of natural standard solution added to the calibration blend. m_{YC}, is the mass of spike solution added to the calibration blend. m_Y, mass of spike solution added to the sample blend. m_X, mass of sample, C_Z refers to the concentration of Bromine in the sample which equals the ratio of mass fraction ,W_Z to atomic weight of m/z 79 Br obtained from IUPAC Data table.

From table 16 and 17 above the main contributory sources of uncertainty in this study include R_{BC}, Observed isotope amount ratio in the calibration blend (19.31 %), R'_B, Observed isotope amount ratio in the sample blend (24.44 %) and R'_{BC}, Observed isotope amount ratio in the calibration blend (37.38 %).S. Hill [19] and M.Ohatia [20] identified three different contributing sources to measurement uncertainties. These include variability of the technique due to transient signals, inhomogeneity in sampling and mixing with enriched spikes and abundances of the two naturally occurring isotopes 79 Br (50.69 %) and 81 Br (49.31%).

6.0 CONCLUSION

The external calibration technique provides results in agreement with IDMS however lower recovery and high uncertainties. The sample preparation methodology employed was Ultrasound-assisted Liquid-liquid extraction and H_2SO_4/KOH/$NaSO_4$ and activated silica gel chromatography clean up technique. Both 'Run 1 ext' and 'Run 2 ext' check standards and calibration standards fulfilled the Quality control requirements by LGC and 40 CFR PART 136.[37] No PBDE congener was detected in calibration blanks, procedural blanks and River Mole water samples by the two methods. River mole water is as clean of priority PBDE congeners as the blank. The agreement between Run 3 IDMS and Run 2 ext further confirmed that more quantitative better quantitative recoveries will be achieved by modification in Sample extraction and partitioning processes. Sample Paired T test also confirms this deduction. For Repeat experiment, 'Run 2 ext' after application of ultrasonic assisted Liquid-Liquid extraction, the water sample and n-hexane mixture was allowed more interaction overnight to allow enough time for n-hexane to extract more PBDE congeners into its phase. Partitioning processes was executed the next day. The instrumental Limit of detections [LODs] for priority congeners were in the range of 3.03 ng/g – 11.07 ng/g for m/z 79 –Br and 4.33 ng/g - 11.93 ng/g for m/z 81-Br. Method LODs and LOQs obtained for each congeners are obviously higher than values obtained by SEMDIDMS. 1,1 dibromocyclohexane performed expected function as an internal injection standard. Applicable to monitoring of low levels of PBDE in the environment

For total bromine determination, good recovery was obtained for the digested calibration blend when compared to the undigested calibration blend indicating that no significant losses of Br occurred during the addition of the spike. However there appears to be a positive bias of about 4%. Good recovery was obtained for PBDE 28 but also there appears to be a bias of 3%. This might be explained as a loss of solvent for the PBDE congener as it was opened in 2012 but it is more difficult to explain for the digested calibration blend. Although a five figure balance was not used on this occasion and triplicate weighing were not performed.. Therefore the reported uncertainty is a good estimate of what can be achieved. An expanded uncertainty of 2% was achieved.

6.1 Suggestions for future development:

a. External calibration should provide efficient recovery. Ensure overnight settlement n-hexane-water sample interaction [to allow all PBDEs move into the organic phase] before partitioning processes. Better extraction by performing two hexane extractions. After the first partition the aqueous phase could be returned to its original sample bottle. The hexane can be put in a smaller second separating funnel to

enable us re-extract the sample in the bottle a second time with hexane and transfer to the 2L separator and. Instrument stability must be ensured.

b. For determination of total bromine, determine the isotopic ratio of the bromine in the sample and compare this to the standard to determine if there is a measurable difference. Use of five figure balance with triplicate weighings to establish uncertainty of masses.Check traceability to other SI by analysing NIST CRM 1000 mg kg standard. Analyse matrix CRM certified for total bromine such as SRM 2258-BDE in 2,2,4- Trimethylpentane and SRM 3184 – Bromide Anion Standard solution. Always transfer the mixture into the microwave while frozen. To obtain better ratio, the use of Neptune high resolution Multi-collector ICP- MS is highly recommended for the entire analysis.

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APPENDICES

Appendix A: Preparation of Calibration standards for External Calibration experiment

Appendix A1- Preparation of calibration standards for run 1 external calibration (Run 1 ext)

	PREPARATION OF 11 PPM NATURAL PBDE MIX STANDARD							
	0.6548	**DENSITY CORRECTION**		**1.527184**				
CONGENER	**CONC µg/ml**	**DENSITY (g/ml)**	**6.62918**	**STD(g)**	**µg /g**	**Mass of PBDE (µg)**	**NONANE (g)**	**Conc. Of PBDE [µg/g]**
BDE-28	50	0.718	6.73649	0.10731	69.63788	7.472841	0.65342	11.43651
BDE-47	50	0.718	6.84523	0.10874	69.63788	7.572423	0.65342	11.58891
BDE-99	50	0.718	6.95631	0.11108	69.63788	7.735376	0.65342	11.83829
BDE-100	50	0.718	7.06546	0.10915	69.63788	7.600975	0.65342	11.6326
BDE-153	50	0.718	7.17384	0.10838	69.63788	7.547354	0.65342	11.55054
BDE-154	50	0.718	7.2826	0.10876	69.63788	7.573816	0.65342	11.59104

Weight of PBDEs plus vial [g] for preparation of 11 ppm Natural PBDE mix

	W1 (g)	W2 (g)	W3 (g)	MEAN(g)	STDEV (g)	SEM (g)	RSD %	LINEARITY	STD UNCERT
Dry Mass of Vial [g]	6.62918	6.62917	6.62919	6.62918	1E-05	5.77E-06	0.000151	0.000144	0.000144
BDE 28	6.73651	6.73648	6.73648	6.73649	1.73E-05	1E-05	0.000257	0.000144	0.000144
BDE 47	6.84523	6.84523	6.84523	6.84523	0	0	0	0.000144	0.000144
BDE 99	6.95633	6.95632	6.95631	6.95632	1E-05	5.77E-06	0.000144	0.000144	0.000144
BDE 100	7.06545	7.06546	7.06546	7.06546	5.77E-06	3.33E-06	8.17E-05	0.000144	0.000144
BDE 153	7.17384	7.17384	7.17385	7.17384	5.77E-06	3.33E-06	8.05E-05	0.000144	0.000144
BDE 154	7.2826	7.28259	7.2826	7.28260	5.77E-06	3.33E-06	7.93E-05	0.000144	0.000144

PREPARATION OF 1 PPM [intermediate standard]									
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN(g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
Dry mass of vial [g]	14.84439	14.84441	14.84442	14.84441	1.53E-05	8.82E-06	0.000103	0.000144	0.000144
PLUS HX	17.06145	17.05656	17.05633	17.05811	0.002892	0.00167	0.016953	0.000144	0.001676
PLUS PBDE	17.27126	17.27119	17.27111	17.27119	7.51E-05	4.33E-05	0.000435	0.000144	0.00015
HX (g)				2.21371					
Mass of 11 ppm PBDE mix standard (g)				0.21307					
PREPARATION OF 20 PPM									
VIAL (g)	28.22094	28.22091	28.22082	28.22089	6.24E-05	3.61E-05	0.000221	0.000144	0.000148
PLUS HX (g)	34.52366	34.52361	34.52358	34.52362	4.04E-05	2.33E-05	0.000117	0.000144	0.000146
PLUS PBDE (g)	34.63194	34.63198	34.63191	34.63194	3.51E-05	2.03E-05	0.000101	0.000144	0.000145
HX (g)				6.30273					
Mass of 1 ppm PBDE standard mix(g)				0.10833					

	Preparation of 1 ppm PBDE		Calibration Std PBDE Concentration	
CONGENERS	**MASS OF 11PPM PBDE[g]**	**HEXANE (g)**	**[µg/g]**	**[ng/g]**
BDE 28	0.21307	2.21371	1.118613	1118.613
BDE 47	0.21307	2.21371	1.140735	1140.735
BDE 99	0.21307	2.21371	1.10057	1100.57
BDE 100	0.21307	2.21371	1.12181	1121.81
BDE 153	0.21307	2.21371	1.114599	1114.599
BDE 154	0.21307	2.21371	1.106447	1106.447

	Preparation of 20 ng/g		**Calibration std PBDE Concentration**	
CONGENERS	**MASS OF 11PPM PBDE**	**HEXANE (g)**	**[µg/g]**	**[ng/g]**
BDE 28	0.10833	6.30273	0.01923	19.2259
BDE 47	0.10833	6.30273	0.01961	19.60611
BDE 99	0.10833	6.30273	0.01892	18.9158
BDE 100	0.10833	6.30273	0.01928	19.28085
BDE 153	0.10833	6.30273	0.01916	19.15691
BDE 154	0.10833	6.30273	0.01902	19.01681

	Preparation of 40 ng/g		**Calibration std PBDE Concentration**	
CONGENERS	**MASS OF 1PPM PBDE [g]**	**HEXANE (g)**	**[ng/g]**	**[µg/g]**
BDE 28	0.20536	6.10585	37.62206	0.037622
BDE 47	0.20536	6.10585	38.36607	0.038366
BDE 99	0.20536	6.10585	37.01523	0.037015
BDE 100	0.20536	6.10585	37.72959	0.03773
BDE 153	0.20536	6.10585	37.48705	0.037487
BDE 154	0.20536	6.10585	37.21289	0.037213

PREPARATION OF 60 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN(g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
Dry mass of vial (g)	27.88087	27.88086	27.88086	27.88086	5.77E-06	3.33E-06	2.07E-05	0.000144	0.000144
PLUS HX (g)	34.24927	34.24928	34.24927	34.24927	5.77E-06	3.33E-06	1.69E-05	0.000144	0.000144
PLUS PBDE (g)	34.57309	34.57308	34.57306	34.57308	1.53E-05	8.82E-06	4.42E-05	0.000144	0.000144
HX (g)				6.36841					
Mass of 1 ppm PBDE (g)				0.323803					

	Preparation of 60 ng/g PBDE		**Calibration std PBDE Concentration**	
CONGENERS	**MASS OF 1PPM PBDE [g]**	**HEXANE (g)**	**[ng/g]**	**[µg/g]**
BDE 28	0.323803	6.36841	56.87615	0.056876
BDE 47	0.323803	6.36841	58.00092	0.058001
BDE 99	0.323803	6.36841	55.95876	0.055959
BDE 100	0.323803	6.36841	57.03871	0.057039
BDE 153	0.323803	6.36841	56.67205	0.056672
BDE 154	0.323803	6.36841	56.25758	0.056258

Preparation of 80 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN(g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
Dry Mass of vial (g)	28.19372	28.19368	28.19366	28.19369	3.06E-05	1.76E-05	0.000108	0.000144	0.000145
PLUS HX (g)	34.42133	34.42134	34.42131	34.42133	1.53E-05	8.82E-06	4.44E-05	0.000144	0.000144
PLUS PBDE (g)	34.84471	34.84469	34.84471	34.84470	1.15E-05	6.67E-06	3.31E-05	0.000144	0.000144
HX (g)				6.22764					
Mass of 1 ppm PBDE (g)				0.423377					

	Preparation of 80 ng/g		**Calibration std PBDE Concentration**	
CONGENERS	**MASS OF 1PPM PBDE [g]**	**HEXANE (g)**	**[ng/g]**	**[µg/g]**
BDE 28	0.423377	6.22764	76.04721	0.076047
BDE 47	0.423377	6.22764	77.55111	0.077551
BDE 99	0.423377	6.22764	74.8206	0.074821
BDE 100	0.423377	6.22764	76.26456	0.076265
BDE 153	0.423377	6.22764	75.77432	0.075774
BDE 154	0.423377	6.22764	75.22014	0.07522

Preparation of 100 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN(g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
Dry mass of vial (g)	28.33115	28.33111	28.33109	28.33112	3.06E-05	1.76E-05	0.000108	0.000144	0.000145
PLUS HX (g)	34.58063	34.58062	34.58057	34.58061	3.21E-05	1.86E-05	9.3E-05	0.000144	0.000145
PLUS PBDE (g)	35.11378	35.11381	35.11383	35.11381	2.52E-05	1.45E-05	7.17E-05	0.000144	0.000145
HX (g)				6.24949					
Mass of 1 ppm PBDE (g)				0.5332					

	Preparation of 100 ng/g		**Calibration std PBDE Concentration**	
CONGENERS	**MASS OF 1PPM PBDE [g]**	**HEXANE (g)**	**[ng/g]**	**[µg/g]**
BDE 28	0.5332	6.24949	95.4389	0.095439
BDE 47	0.5332	6.24949	97.32629	0.097326
BDE 99	0.5332	6.24949	93.89951	0.0939
BDE 100	0.5332	6.24949	95.71168	0.095712
BDE 153	0.5332	6.24949	95.09642	0.095096
BDE 154	0.5332	6.24949	94.40094	0.094401

Preparation of CCV standard [60 ng/g]		**60 ng/g**							
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN(g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
Dry mass of vial (g)	28.30932	28.30928	28.30922	28.30927	5.03E-05	2.91E-05	0.000178	0.000144	0.000147
PLUS HX (g)	34.67158	34.67161	34.67156	34.67158	2.52E-05	1.45E-05	7.26E-05	0.000144	0.000145
PLUS PBDE (g)	34.99748	34.99746	34.99746	34.99747	1.15E-05	6.67E-06	3.3E-05	0.000144	0.000144
HX (g)				6.36231					
Mass of 1 ppm PBDE (g)				0.325883					

Preparation of 60 ng/g CCV standard		**60 ng/g**	**Calibration std PBDE Concentration**	
CONGENERS	**MASS OF 1PPM PBDE [g]**	**HEXANE (g)**	**[ng/g]**	**[µg/g]**
BDE 28	0.325883	6.36231	57.29638	0.057296
BDE 47	0.325883	6.36231	58.42947	0.058429
BDE 99	0.325883	6.36231	56.37221	0.056372
BDE 100	0.325883	6.36231	57.46014	0.05746
BDE 153	0.325883	6.36231	57.09078	0.057091
BDE 154	0.325883	6.36231	56.67324	0.056673

Preparation of new 11 ppm Natural PBDE standard mix for ICV preparation from other source

	Preparation of 11 ppm Natural PBDE Mix Standard for ICV standard preparation							
	Density of nonane, 0.6548 g/ml	**Density correction**		**1.527184**				
Congeners	**Conc. [µg/ml]**	**Density [g/ml]**	**6.62918 [vial mass plus]**	**Mass of 69.64 µg/g ppm stock Std [g]**	**[µg/g]**	**[µg PBDE]**	**Nonane, [g]**	**[µg/g]**
BDE 28	50	0.718	6.73649	0.10731	69.63788	7.472841	0.65342	11.43651
BDE 47	50	0.718	6.84523	0.10874	69.63788	7.572423	0.65342	11.58891
BDE 99	50	0.718	6.95631	0.11108	69.63788	7.735376	0.65342	11.83829
BDE 100	50	0.718	7.06546	0.10915	69.63788	7.600975	0.65342	11.6326
BDE 153	50	0.718	7.17384	0.10838	69.63788	7.547354	0.65342	11.55054
BDE 154	50	0.718	7.2826	0.10876	69.63788	7.573816	0.65342	11.59104

PREPARATION OF ICV [60 ng/g]		**60 ng/g**							
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN(g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
Dry mass of vial (g)	39.17203	39.17217	39.17217	39.17212	8.08E-05	4.67E-05	0.000206	0.000144	0.000151
PLUS HX (g)	71.32023	71.3202	71.32019	71.32021	2.08E-05	1.2E-05	2.92E-05	0.000144	0.000145
PLUS PBDE (g)	71.47983	71.47976	71.47975	71.47978	4.36E-05	2.52E-05	6.1E-05	0.000144	0.000146
HX (g)				32.14808					
Mass of 11 ppm stock PBDE (g)				0.159573					

	Preparation of ICV standard	**60 ng/g**	**Calibration std PBDE Concentration**	
CONGENERS	**MASS OF 11PPM PBDE [g]**	**HEXANE (g)**	**[ng/g]**	**[µg/g]**
BDE 28	0.159573	32.14808	56.76734	0.056767
BDE 47	0.159573	32.14808	57.52382	0.057524
BDE 99	0.159573	32.14808	58.76168	0.058762
BDE 100	0.159573	32.14808	57.74071	0.057741
BDE 153	0.159573	32.14808	57.33338	0.057333
BDE 154	0.159573	32.14808	57.5344	0.057534

Appendix A2:

TOPIC : REANALYSIS OF RIVER MOLE WATER SAMPLES EXTERNAL CALIBRATION AND INTERNAL STANDARD (RUN 2 EXT an d RUN 2 EXT/INT)

AIM : To investigate the sources low recovery of PBDEs of spiked water samples from previous experiment.

Stock					**Conc .**	**Conc.**
Vial [g]	**vial + 1,1, Dibromocyclohexane**	**plus hex**	**Mass of 1,1, dibromohexane**	**Mass of hexane(g)**	**mg/g**	**µg/g**
40.8623	40.9206	79.3443	0.0583	38.482	1.514994	1514.994

Internal standard solvent preparation			**Stock**	**Int.std/Hx stock**		**Mass of hexane**	**Int. Std/Hx**	
Mass of solvent Bottle (g)	**Bottle + hx(g)**		**Conc in [µg/g]**	**Mass of 50ul of 1514.994 µg/g int.std stock[g]**	**Mass of PBDE [µg]**	**Hexane(g)**	**Conc. [µg/g]**	**Conc. [ng/g]**
1384.97	2088.75		1514.994023	0.03395	51.43405	703.78	0.073083	73.08256
Evaluation by GC _ICP_MS								
Std give area of approx 800 cps same as top 100 ng/g PBDE 28 so is correct conc for use as internal standard								

	Preparation of 11 ppm Natural PBDE Mix Standard in Internal standard / n-hexane solvent							
	Density of nonane,0.6548 g/ml	**Density correction**		**1.527184**	**Conc. Of Stock**			
Congeners	**Conc [ug/ml]**	**Density [g/ml]**	**6.62918**	**Natural std [g]**	**[µg/g]**	**[µg]**	**nonane [g]**	**[ug/g]**
BDE 28	50	0.718	6.73649	0.10731	69.63788	7.472841	0.65342	11.43651
BDE 47	50	0.718	6.84523	0.10874	69.63788	7.572423	0.65342	11.58891
BDE 99	50	0.718	6.95631	0.11108	69.63788	7.735376	0.65342	11.83829
BDE 100	50	0.718	7.06546	0.10915	69.63788	7.600975	0.65342	11.6326
BDE 153	50	0.718	7.17384	0.10838	69.63788	7.547354	0.65342	11.55054
BDE 154	50	0.718	7.2826	0.10876	69.63788	7.573816	0.65342	11.59104

Date	**14-Aug-14**
Topic	**Preparation of New calibration natural standard PBDE mix with 1,1 Dibromocyclohexane (injection Internal standard)/hexane solvent [Hx/int]**

Preparation of 1 ppm PBDE									
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial [g]	14.67921	14.67925	14.6793	14.67925	4.51E-05	2.6E-05	0.000307	0.000144	0.000146
plus Hx/int	16.88029	16.88031	16.88037	16.88032	4.16E-05	2.4E-05	0.000247	0.000144	0.000146
PLUS pbde	17.09439	17.0944	17.09438	17.09439	1E-05	5.77E-06	5.85E-05	0.000144	0.000144
Hx/int(g)				2.20107					
Mass of of 11 ppm PBDE stock (g)				0.214067					

	Preparation of 1ppm PBDE		Calibration std PBDE Concentration	
Congeners	**Mass of 11ppm PBDE**	**Mass hx/int solvent [g]**	**[µg/g]**	**[ng/g]**
BDE 28	0.21407	2.20107	1.13028	1130.28
BDE 47	0.21407	2.20107	1.152632	1152.632
BDE 99	0.21407	2.20107	1.112049	1112.049
BDE 100	0.21407	2.20107	1.13351	1133.51
BDE 153	0.21407	2.20107	1.126224	1126.224
BDE 154	0.21407	2.20107	1.117987	1117.987

Preparation of 20 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial (g)	28.54762	28.54775	28.54785	28.54774	0.000115	6.66E-05	0.000404	0.000144	0.000159
Plus Hx/int (g)	34.84813	34.84812	34.84815	34.84813	1.53E-05	8.82E-06	4.38E-05	0.000144	0.000144
Plus PBDE (g)	34.95483	34.95483	34.95489	34.95485	3.46E-05	2E-05	9.91E-05	0.000144	0.000145
Hx/int solvent(g)				6.300393					
Mass of 1 ppm PBDE (g)				0.106717					

	20 ng/g		**Calibration std PBDE Concentration**	
Congeners	**Mass of 1PPM PBDE [g]**	**Mass hx/int solvent [g]**	**[µg/g]**	**[ng/g]**
BDE 28	0.10672	6.30039	0.01914	19.14479
BDE 47	0.10672	6.30039	0.01952	19.5234
BDE 99	0.10672	6.30039	0.01884	18.83599
BDE 100	0.10672	6.30039	0.01920	19.19951
BDE 153	0.10672	6.30039	0.01908	19.07609
BDE 154	0.10672	6.30039	0.01894	18.93658

Preparation of 40 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial (g)	28.3616	28.36159	28.3616	28.3616	5.77E-06	3.33E-06	2.04E-05	0.000144	0.000144
Plus Hx/int (g)	34.46227	34.46226	34.46222	34.46225	2.65E-05	1.53E-05	7.68E-05	0.000144	0.000145
Plus PBDE (g)	34.66695	34.66696	34.66694	34.66695	1E-05	5.77E-06	2.88E-05	0.000144	0.000144
Hx/int solvent (g)				6.100653					
Mass of 1 ppm PBDE (g)				0.2047					

	Preparation of 40 ng/g		**Calibration std PBDE Concentration**	
Congeners	**Mass of 1PPM PBDE [g]**	**Mass hx/int solvent[g]**	**[ng/g]**	**[µg/g]**
BDE 28	0.20470	6.10065	37.92517	0.037925
BDE 47	0.20470	6.10065	38.67517	0.038675
BDE 99	0.20470	6.10065	37.31345	0.037313
BDE 100	0.20470	6.10065	38.03356	0.038034
BDE 153	0.20470	6.10065	37.78908	0.037789
BDE 154	0.20470	6.10065	37.51271	0.037513

Preparation of 60 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial (g)	29.02687	29.02684	29.02683	29.02685	2.08E-05	1.2E-05	7.17E-05	0.000144	0.000145
Plus Hx/int (g)	35.3924	35.39238	35.39236	35.39238	2E-05	1.15E-05	5.65E-05	0.000144	0.000144
Plus PBDE (g)	35.71776	35.71778	35.71781	35.71778	2.52E-05	1.45E-05	7.05E-05	0.000144	0.000145
Hx/int solvent (g)				6.365533					
Mass of 1 ppm PBDE std mix(g)				0.325403					

	Preparation of 60 ng/g		Calibration std PBDE Concentration	
Congeners	**Mass of 1PPM PBDE [g]**	**Mass hx/int solvent[g]**	**[ng/g]**	**[µg/g]**
BDE 28	0.325403	6.365533	57.77943	0.057779
BDE 47	0.325403	6.365533	58.92207	0.058922
BDE 99	0.325403	6.365533	56.84747	0.056847
BDE 100	0.325403	6.365533	57.94457	0.057945
BDE 153	0.325403	6.365533	57.57209	0.057572
BDE 154	0.325403	6.365533	57.15104	0.057151

Preparation 0f 80 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial (g)	28.54899	28.54912	28.54918	28.5491	9.71E-05	5.61E-05	0.00034	0.000144	0.000155
Plus Hx/int (g)	34.76866	34.76864	34.76864	34.76865	1.15E-05	6.67E-06	3.32E-05	0.000144	0.000144
Plus PBDE (g)	35.19932	35.19935	35.19933	35.19933	1.53E-05	8.82E-06	4.34E-05	0.000144	0.000144
Hx/int solvent(g)				6.21955					
Mass of 1 ppm PBDE mix (g)				0.430687					

	80 ng/g		**Calibration std PBDE Concentration**	
Congeners	**Mass of 1PPM PBDE [g]**	**Mass of hex/int solvent [g]**	**[ng/g]**	**[µg/g]**
BDE 28	0.430687	6.21955	78.26877	0.078269
BDE 47	0.430687	6.21955	79.8166	0.079817
BDE 99	0.430687	6.21955	77.00632	0.077006
BDE 100	0.430687	6.21955	78.49247	0.078492
BDE 153	0.430687	6.21955	77.9879	0.077988
BDE 154	0.430687	6.21955	77.41754	0.077418

Preparation of 100 ng/g									
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial (g)	28.23051	28.23047	28.23046	28.23048	2.65E-05	1.53E-05	9.37E-05	0.000144	0.000145
Plus Hx/int (g)	34.48045	34.48044	34.48043	34.48044	1E-05	5.77E-06	2.9E-05	0.000144	0.000144
Plus PBDE (g)	35.0203	35.02034	35.02032	35.02032	2E-05	1.15E-05	5.71E-05	0.000144	0.000144
Hx/int solvent (g)				6.24996					
Mass of 1 ppm PBDE mix (g)				0.53988					

	Preparation of 100 ng/g Calibration standard		**Calibration standard PBDE Concentration**	
Congeners	**Mass of 1PPM PBDE [g]**	**Mass of hx/int solvent[g]**	**[ng/g]**	**[µg/g]**
BDE 28	0.53988	6.24996	97.63511	0.097635
BDE 47	0.53988	6.24996	99.56593	0.099566
BDE 99	0.53988	6.24996	96.06029	0.09606
BDE 100	0.53988	6.24996	97.91417	0.097914
BDE 153	0.53988	6.24996	97.28475	0.097285
BDE 154	0.53988	6.24996	96.57326	0.096573

Preparation of Continuing Calibration Verification [CCV] standard and Initial calibration verification check standard [ICV]

Preparation of ccv		**60 ng/g**							
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial (g)	28.84299	28.84304	28.84305	28.84303	3.21E-05	1.86E-05	0.000111	0.000144	0.000145
Plus Hx/int (g)	35.20286	35.20287	35.20287	35.20287	5.77E-06	3.33E-06	1.64E-05	0.000144	0.000144
Plus PBDE (g)	35.53528	35.53531	35.5353	35.5353	1.53E-05	8.82E-06	4.3E-05	0.000144	0.000144
Hx/int solvent (g)				6.35984					
Mass of 1 ppm PBDE mix (g)				0.33243					

	Preparation of CCV standard [60 ng/g]	**60 ng/g**	**Calibration std PBDE Concentration**	
Congeners	**Mass of 1PPM PBDE [g]**	**Mass hx/int solvent [g]**	**[ng/g]**	**[µg/g]**
BDE 28	0.33243	6.35984	59.07994	0.05908
BDE 47	0.33243	6.35984	60.2483	0.060248
BDE 99	0.33243	6.35984	58.127	0.058127
BDE 100	0.33243	6.35984	59.2488	0.059249
BDE 153	0.33243	6.35984	58.86794	0.058868
BDE 154	0.33243	6.35984	58.43741	0.058437

Preparation of ICV standard		**60 ng/g**							
	W1 (g)	**W2 (g)**	**W3 (g)**	**Mean(g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std uncert**
Dry mass of vial (g)	40.12606	40.12609	40.12604	40.12606	2.52E-05	1.45E-05	6.27E-05	0.000144	0.000145
Plus Hx/int (g)	72.27111	72.27118	72.27115	72.27115	3.51E-05	2.03E-05	4.86E-05	0.000144	0.000145
Plus PBDE (g)	72.42512	72.42497	72.42497	72.42502	8.66E-05	5E-05	0.00012	0.000144	0.000152
Hx/int solvent (g)				32.14508					
Mass of PBDE (g)				0.153873					

	Preparation of ICV Standard	60 ng/g	Calibration std PBDE Concentration	
Congeners	**Mass of 11 PPM PBDE [g]**	**Mass Hex/int solvent (g)**	**[ng/g]**	**[µg/g]**
BDE 28	**0.153873**	**32.14508**	**54.74471**	**0.054745**
BDE 47	**0.153873**	**32.14508**	**55.47423**	**0.055474**
BDE 99	**0.153873**	**32.14508**	**56.66799**	**0.056668**
BDE 100	**0.153873**	**32.14508**	**55.68339**	**0.055683**
BDE 153	**0.153873**	**32.14508**	**55.29057**	**0.055291**
BDE 154	**0.153873**	**32.14508**	**55.48443**	**0.055484**

APPENDIX B: SAMPLES AND SPIKED SAMPLES PREPARATION

APPENDIX B1: River mole sample water samples preparation uncertainty of oertling MD31 balance linearity ± 0.51

SAMPLE BOTTLE	DRY MASS OF BOTTLE (g)	MASS OF BOTTLE + WATER SAMPLE (g)	SAMPLE MASS (g)	LINEARITY	STANDARD UNCERTAINTY BOTTLE(UC),g	WATER(UC).g
A1	1371.9	3409.9				
	1371.8	3409.9				
	1371.7	3409.8				
MEAN	1371.8	3409.9	2038.1			
STDEV	0.1	0.057735				
SEM	0.057735027	0.033333		0.51	0.513258	0.511088
RSD%	0.007289692	0.001693				
A2	1366.6	3410				
	1366.6	3410				
	1366.6	3410				
MEAN	1366.6	3410	2043.4			
STDEV	2.78475E-13	0				
SEM	1.60777E-13	0		0.51	0.51	0.51
RSD%	2.03772E-14	0				

A3	1365.1	3374.2				
	1365.2	3374.1				
	1365.2	3374.3				
MEAN	1365.166667	3374.2	2009.0			
STDEV	0.057735027	0.1				
SEM	0.033333333	0.057735		0.51	0.511088	0.513258
RSD%	0.004229156	0.002964				
A4	1373.3	3409.9				
	1373.2	3409.9				
	1373.2	3409.9				
MEAN	1373.233333	3409.9	2036.7			
STDEV	0.057735027	0				
SEM	0.033333333	0		0.51	0.511088	0.51
RSD%	0.004204313	0				
A6	1365.5	3378.3				
	1365.7	3378.4				
	1365.6	3378.5				
MEAN	1365.6	3378.4	2012.8			
STDEV	0.1	0.1				
SEM	0.057735027	0.057735		0.51	0.513258	0.513258

RSD%	0.007322789	0.00296				
A7	1371.7	3403.1				
	1371.8	3403				
	1371.7	3402.9				
MEAN	1371.733333	3403	2031.3			
STDEV	0.057735027	0.1				
SEM	0.033333333	0.057735		0.51	0.511088	0.513258
RSD%	0.00420891	0.002939				
A8	1371.7	3402.5				
	1371.7	3402.6				
	1371.6	3402.5				
MEAN	1371.666667	3402.533	2030.9			
STDEV	0.057735027	0.057735				
SEM	0.033333333	0.033333		0.51	0.511088	0.511088
RSD%	0.004209115	0.001697				
A9	1366.9	3413.2				
	1366.9	3413.3				
	1366.9	3413.5				
MEAN	1366.9	3413.333	2046.4			

STDEV	2.78475E-13	0.152753				
SEM	1.60777E-13	0.088192		0.51	0.51	0.517569
RSD%	2.03727E-14	0.004475				
A10	1373	3359.6				
	1373.1	3359.7				
	1373.1	3359.6				
MEAN	1373.066667	3359.633	1986.6			
STDEV	0.057735027	0.057735				
SEM	0.033333333	0.033333		0.51	0.511088	0.511088
RSD%	0.004204823	0.001718				
A11	1375.2	3396.1				
	1375.1	3396				
	1375.3	3396				
MEAN	1375.2	3396.033	2020.8			
STDEV	0.1	0.057735				
SEM	0.057735027	0.033333		0.51	0.513258	0.511088
RSD%	0.00727167	0.0017				
A12	1373.7	3384.3				
	1373.6	3384.3				

	1373.7	3384.4				
MEAN	1373.666667	3384.333	2010.7			
STDEV	0.057735027	0.057735				
SEM	0.033333333	0.033333		0.51	0.511088	0.511088
RSD%	0.004202987	0.001706				
E1	1371.7	3402				
	1371.6	3401.9				
	1371.7	3401.9				
MEAN	1371.666667	3401.933	2030.3			
STDEV	0.057735027	0.057735				
SEM	0.033333333	0.033333		0.51	0.511088	0.511088
RSD%	0.004209115	0.001697				
E2	1371.3	3416.6				
	1371.3	3416.7				
	1371.3	3416.7				
MEAN	1371.3	3416.667	2045.4			
STDEV	0	0.057735				
SEM	0	0.033333		0.51	0.51	0.511088
RSD%	0	0.00169				

E3	1373.9	3384.8				
	1373.8	3384.8				
	1373.8	3384.8				
MEAN	1373.833333	3384.8	2011.0			
STDEV	0.057735027	5.57E-13				
SEM	0.033333333	3.22E-13		0.51	0.511088	0.51
RSD%	0.004202477	1.65E-14				
E4	1370.5	3337				
	1370.4	3337				
	1370.5	3337.1				
MEAN	1370.466667	3337.033	1966.6			
STDEV	0.057735027	0.057735				
SEM	0.033333333	0.033333		0.51	0.511088	0.511088
RSD%	0.004212801	0.00173				
C1	1374	3401.9				
	1374	3401.8				
	1373.9	3401.8				
MEAN	1373.966667	3401.833	2027.9			
STDEV	0.057735027	0.057735				

SEM	0.033333333	0.033333		0.51	0.511088	0.511088
RSD%	0.004202069	0.001697				
C2	1374.7	3405.4				
	1374.8	3405.3				
	1374.7	3405.4				
MEAN	1374.733333	3405.367	2030.6			
STDEV	0.057735027	0.057735				
SEM	0.033333333	0.033333		0.51	0.511088	0.511088
RSD%	0.004199726	0.001695				

APPENDIX B2: PREPARATION OF 11 PPM STOCK NATURAL PBDE STANDARD MIXTURE

CONGENERS	6.40601g	Weight of stock standard g	CONC, µg/ml	DENSITY, g/ml	MASS FRACTION (µg /g)	Mass of PBDE (µg)	Total mass of Nonane (g)	PBDE, (µg /g)
28 BDE	6.596343	0.190333	50	0.718	69.63788301	13.25438719	1.140483	11.62173148
47 BDE	6.79044	0.194097	50	0.718	69.63788301	13.51650418	1.140483	11.85156129
99 BDE	6.977703	0.187263	50	0.718	69.63788301	13.04059889	1.140483	11.43427731
100 BDE	7.16858	0.190877	50	0.718	69.63788301	13.29227019	1.140483	11.65494812
153 BDE	7.35823	0.18965	50	0.718	69.63788301	13.20682451	1.140483	11.58002751
154 BDE	7.546493	0.188263	50	0.718	69.63788301	13.11023677	1.140483	11.4953373

APPENDIX B3: PREPARATION OF 13 ng/g NATURAL PBDE MIX DILUTED IN METHANOL ADDED TO EACH SPIKED SAMPLES

	MASSES IN TRIPLICATES					REPEATABILITY			
	W1	**W2**	**W3**	**MEAN**	**STANDARD DEVIATION (g)**	**SEM(g)**	**RSV%**	**LINEARITY**	**STD UNCERT**
DRY MASS OF VIAL (g)	92.63678	92.63677	92.63679	92.63678	1E-05	5.7735E-06	1.07948E-05	0.000144	5.7735E-06
MASS OF VIAL + STOCK STD (g)	92.7446	92.74449	92.7444	92.74449	0.000100167	5.78312E-05	0.000108003	0.000144	5.7831E-05
MASS + VIAL+ STOCK STD+MEOH (g)	184.1873	184.18733	184.18725	184.18728	4.16333E-05	2.4037E-05	2.26038E-05	0.000144	2.4037E-05
BALANCE LINEARITY STANDARD UNCERTAINTY						0.000144			

W1,W2 AND W3 = WEIGHT IN TRIPLICATES

SEM= STANDARD ERROR OF THE MEAN

RSV =RELATIVE STANDARD DEVIATION

STD UNCERT = STANDARD UNCERTAINTY

APPENDIX B3: RIVER MOLE WATER SAMPLE SPIKING BY NATURAL STANDARD MIX, 13ng/g

CONGENERS	MASS OF 11 PPM, STOCK NATURAL STANDARD, M1 (g)	M2,MEOH (g)	[ng/g] PBDE
28 BDE	0.10782	91.44267	13.70318
47 BDE	0.10782	91.44267	13.97417
99 BDE	0.10782	91.44267	13.48215
100 BDE	0.10782	91.44267	13.74234
153 BDE	0.10782	91.44267	13.654
154 BDE	0.10782	91.44267	13.55415

APPENDIX B4:SPIKING OF RIVER MOLE WATER SAMPLES WITH 13.6 NG/G PBDE MIX IN METHANOL

						repeatability				MPm (g)
Samples	W1 (g)	W2 (g)	W3 (g)	mean	stdev	SEM, g	RSV%	linearity	Std Uncert	
A1	57.53772	57.53772	57.5377	57.53771	1.15E-05	6.67E-06	2.01E-05	0.000144	6.67E-06	
	57.39071	57.3908	57.39074	57.39075	4.58E-05	2.65E-05	7.98E-05	0.000144	0.000146	0.146963
A2	57.39073	57.39075	57.39077	57.39075	2E-05	1.15E-05	3.48E-05	0.000144	1.15E-05	
	57.24045	57.24045	57.24043	57.24044	1.15E-05	6.67E-06	2.02E-05	0.000144	0.000144	0.150307
A3	57.24045	57.24043	57.24045	57.24044	1.15E-05	6.67E-06	2.02E-05	0.000144	6.67E-06	
	57.08981	57.08982	57.0898	57.08981	1E-05	5.77E-06	1.75E-05	0.000144	0.000144	0.150633
A4	57.08983	57.08972	57.08979	57.08978	5.57E-05	3.21E-05	9.75E-05	0.000144	3.21E-05	
	56.94194	56.94197	56.94196	56.94196	1.53E-05	8.82E-06	2.68E-05	0.000144	0.000144	0.147823
E1	56.94195	56.94192	56.94191	56.94193	2.08E-05	1.2E-05	3.66E-05	0.000144	1.2E-05	
	56.79338	56.79333	56.79334	56.79335	2.65E-05	1.53E-05	4.66E-05	0.000144	0.000145	0.148577
E2	56.79333	56.79337	56.79336	56.79335	2.08E-05	1.2E-05	3.67E-05	0.000144	1.2E-05	
	56.64738	56.64734	56.64737	56.64736	2.08E-05	1.2E-05	3.67E-05	0.000144	1.2E-05	0.14599

W1,W2 AND W3 = WEIGHT IN TRIPLICATES (g)

SEM= STANDARD ERROR OF THE MEAN

RSV =RELATIVE STANDARD DEVIATION

STD UNCERT = STANDARD UNCERTAINTY

MPM = MASS OF 13.6 ng/g PBDE MIX IN METHANOL

APPENDIX B4: CONCENTRATIONS OF POLYBROMINATED DIPHENYL ETHERS [PBDE] SPIKE IN THE SAMPLES

	A1								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final extract (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.146963	2.013865	2038.067	2.038067	0.98812497	0.03274	61.51083	2	1.019033
BDE-47	0.146963	2.05369	2038.067	2.038067	1.007665997	0.03274	62.72726	2	1.019033
BDE-99	0.146963	1.981382	2038.067	2.038067	0.972186884	0.03274	60.51868	2	1.019033
BDE-100	0.146963	2.01962	2038.067	2.038067	0.990949178	0.03274	61.68664	2	1.019033
BDE-153	0.146963	2.006638	2038.067	2.038067	0.984579135	0.03274	61.2901	2	1.019033
BDE-154	0.146963	1.991962	2038.067	2.038067	0.977378443	0.03274	60.84186	2	1.019033

	A2								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final extract (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.150307	2.059679	2043.4	2.0434	1.007967	0.03274	62.91017	2	1.029839
BDE-47	0.150307	2.100411	2043.4	2.0434	1.0279	0.03274	64.15427	2	1.050205
BDE-99	0.150307	2.026457	2043.4	2.0434	0.991708	0.03274	61.89545	2	1.013229
BDE-100	0.150307	2.065566	2043.4	2.0434	1.010847	0.03274	63.08997	2	1.032783
BDE-153	0.150307	2.052288	2043.4	2.0434	1.00435	0.03274	62.68442	2	1.026144
BDE-154	0.150307	2.037278	2043.4	2.0434	0.997004	0.03274	62.22598	2	1.018639

	A3								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final extract (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.150633	2.064155	2009.033	2.009033	1.027437	0.03274	63.04689	2	1.032078
BDE-47	0.150633	2.104976	2009.033	2.009033	1.047755	0.03274	64.2937	3	0.701659
BDE-99	0.150633	2.030861	2009.033	2.009033	1.010865	0.03274	62.02997	4	0.507715
BDE-100	0.150633	2.070055	2009.033	2.009033	1.030374	0.03274	63.22709	5	0.414011
BDE-153	0.150633	2.056748	2009.033	2.009033	1.02375	0.03274	62.82065	6	0.342791
BDE-154	0.150633	2.041706	2009.033	2.009033	1.016263	0.03274	62.36121	7	0.291672

	A4								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final extract (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.147823	2.025649	2036.667	2.036667	0.99459	0.03274	0.99459	2	1.012825
BDE-47	0.147823	2.065708	2036.667	2.036667	1.014259	0.03274	1.014259	2	1.032854
BDE-99	0.147823	1.992976	2036.667	2.036667	0.978548	0.03274	0.978548	2	0.996488
BDE-100	0.147823	2.031439	2036.667	2.036667	0.997433	0.03274	0.997433	2	1.015719
BDE-153	0.147823	2.01838	2036.667	2.036667	0.991021	0.03274	0.991021	2	1.00919
BDE-154	0.147823	2.003619	2036.667	2.036667	0.983774	0.03274	0.983774	2	1.001809

	E1								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final extract (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.148577	2.035972	2030.267	2.030267	1.00281	0.03274	62.18608	2	1.017986
BDE-47	0.148577	2.076235	2030.267	2.030267	1.022642	0.03274	63.41587	2	1.038118
BDE-99	0.148577	2.003133	2030.267	2.030267	0.986635	0.03274	61.18304	2	1.001566
BDE-100	0.148577	2.041791	2030.267	2.030267	1.005676	0.03274	62.36382	2	1.020896
BDE-153	0.148577	2.028666	2030.267	2.030267	0.999212	0.03274	61.96293	2	1.014333
BDE-154	0.148577	2.01383	2030.267	2.030267	0.991904	0.03274	61.50977	2	1.006915

	E2								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final extract (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.14599	2.000527	2045.367	2.045367	0.978077	0.03274	61.10345	2	1.000263
BDE-47	0.14599	2.040089	2045.367	2.045367	0.99742	0.03274	62.31182	2	1.020044
BDE-99	0.14599	1.968259	2045.367	2.045367	0.962301	0.03274	60.11787	2	0.98413
BDE-100	0.14599	2.006245	2045.367	2.045367	0.980873	0.03274	61.27809	2	1.003122
BDE-153	0.14599	1.993348	2045.367	2.045367	0.974568	0.03274	60.88418	2	0.996674
BDE-154	0.14599	1.97877	2045.367	2.045367	0.96744	0.03274	60.4389	2	0.989385

	A10								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final injection (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.14599	2.000527	1986.567	1.986567	1.007027	0.031383	63.74488	2	1.000263
BDE-47	0.14599	2.040089	1986.567	1.986567	1.026942	0.031383	65.00549	2	1.020044
BDE-99	0.14599	1.968259	1986.567	1.986567	0.990784	0.031383	62.7167	2	0.98413
BDE-100	0.14599	2.006245	1986.567	1.986567	1.009906	0.031383	63.92707	2	1.003122
BDE-153	0.14599	1.993348	1986.567	1.986567	1.003414	0.031383	63.51613	2	0.996674
BDE-154	0.14599	1.97877	1986.567	1.986567	0.996075	0.031383	63.05161	2	0.989385

	A11								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final injection (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.14599	2.000527	2020.833	2.020833	0.989951	0.032063	62.39298	2	1.000263
BDE-47	0.14599	2.040089	2020.833	2.020833	1.009529	0.032063	63.62685	2	1.020044
BDE-99	0.14599	1.968259	2020.833	2.020833	0.973984	0.032063	61.3866	2	0.98413
BDE-100	0.14599	2.006245	2020.833	2.020833	0.992781	0.032063	62.5713	2	1.003122
BDE-153	0.14599	1.993348	2020.833	2.020833	0.986399	0.032063	62.16908	2	0.996674
BDE-154	0.14599	1.97877	2020.833	2.020833	0.979185	0.032063	61.71441	2	0.989385

	A12								
Congeners	**Mass of 13 ng/g natural standard PBDEs added (g)**	**Mass of PBDE [ng]**	**Mass of Water (g)**	**Mass of water [Kg]**	**Expected Conc. PBDE [ng/Kg]**	**Mass of Hexane final extract (g)**	**Expected Conc. PBDE [ng/g]**	**Vol. of water [L]**	**Expected Conc. PBDE [ng/L]**
BDE-28	0.14599	2.000527	2010.667	2.010667	0.994957	0.03157	63.36797	2	1.000263
BDE-47	0.14599	2.040089	2010.667	2.010667	1.014633	0.03157	64.62113	2	1.020044
BDE-99	0.14599	1.968259	2010.667	2.010667	0.978909	0.03157	62.34587	2	0.98413
BDE-100	0.14599	2.006245	2010.667	2.010667	0.997801	0.03157	63.54908	2	1.003122
BDE-153	0.14599	1.993348	2010.667	2.010667	0.991387	0.03157	63.14058	2	0.996674
BDE-154	0.14599	1.97877	2010.667	2.010667	0.984136	0.03157	62.6788	2	0.989385

APPENDIX B5: DILUTION OF 81Br ENRICHED PBDE MIX WITH METHANOL

	MASSES IN TRIPLICATES				**BALANCE LINEARITY 0.000144 g**				
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN (g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
DRY MASS OF VIAL	14.56198	14.56199	14.56198	14.56198	5.7735E-06	3.33333E-06	3.96478E-05	0.000144	0.000144039
VIAL + 81 Br ENRICHED SPIKE	15.54999	15.55	15.55002	15.55	1.5275E-05	8.81917E-06	9.82331E-05	0.000144	0.00014427
MASS OF SPIKE ADDED				**0.98802**					
VIAL + 81 Br ENRICHED SPIKE+METHANOL	20.71886	20.71889	20.71888	20.71888	1.5275E-05	8.81917E-06	7.37263E-05	0.000144	0.00014427
MASS OF METHANOL ADDED				**5.168873**					
	STOCK		ng/g	SPIKE (g)	PBDE (ng)	MEOH (g)	ng/g IN MEOH		
CONCENTRATION	104.8	µg/g	104800	0.98802	103.544496	5.168873333	**20.03231446**		

APPENDIX B6: ADDITION OF 20ng/g 81 Br ENRICHED SPIKE TO SPIKED SAMPLE TO FORM SAMPLE BLEND FOR SEMI – EXACT DOUBLE IDMS EXPERIMENT

			ADDITION OF 20ng/g 81 Br ENRICHED SPIKE TO SPIKED SAMPLE TO FORM SAMPLE BLEND						
SAMPLE			**SAMPLE SPIKED WITH 81 Br ENRICHED PBDE STANDARD**						
	W1 (g)	**W2 (g)**	**W3 (g)**	**MEAN (g)**	**STDEV (g)**	**SEM (g)**	**RSD %**	**LINEARITY**	**STD UNCERT**
A1	20.41481	20.41479	20.4148	20.4148	1E-05	5.7735E-06	4.89841E-05	0.000144	0.000144116
	20.27986	20.2798	20.27974	20.2798	6E-05	3.4641E-05	0.000295861	0.000144	0.000148108
	MASS OF ENRICHED SPIKE			0.135					
A2	20.27946	20.2794	20.27936	20.27941	5.0332E-05	2.90593E-05	0.000248194	0.000144	0.000146903
	20.14665	20.14664	20.14657	20.14662	4.3589E-05	2.51661E-05	0.000216359	0.000144	0.000146183
	MASS OF ENRICHED SPIKE (g)			0.132787					

A3	20.14671	20.14657	20.14657	20.14662	8.0829E-05	4.66667E-05	0.000401204	0.000144	0.000151373
	20.01506	20.01508	20.0151	20.01508	2E-05	1.1547E-05	9.99247E-05	0.000144	0.000144462
	MASS OF ENRICHED SPIKE [g]			0.131537					

Appendix C: Preparation of Humic acid [15 mg/L] stock solution.

Finding the concentration of Humic acid in Humic acid stock solution prepared by weighing 1 g of humic acid [supplied by Sigma – Aldrich, Uk] into 1 L of ultra - high purity (UHP) water. About 1000 mg is expected.

S/N	Mass of PTFE Evaporating dish[g]	Plus Humic acid solution [g]	PTFE Mass after ev aporation to dryness [g]	Mass of Humic acid Solution [g]	Mass of residue [g]	Residue correction [g]	Blank correction
1	57.58318	90.261	57.6125	32.67782	0.02932	0.02962	906.43
2	102.8177	133.8962	102.848	31.0785	0.0303	0.0306	984.60
3	71.7102	96.929	71.7352	25.2188	0.025	0.0253	1003.22
Blank	50.2855		50.2852		-0.0003	0	0
						Mean	**964.75 mg/L**
						Stdev	**51.4**
						CV%	**5.3**

Therefore, To produce 15 mg/L Humic acid, 1 Litre of water sample will require: $\frac{15 \times 1000ml}{964.75} = 15.54807mg$ **of the humic acid stock solution. Hence, 2L water sample will require =** $15.54807mg \times 2 = 31.09614mg$ **of Humic acid stock solution was added to each 2L water samples.**

Appendix D:Specification for the CEM Discover system and vessel type

- **Vessel type – Pyrex**
- **Maximum number of vessels – 1**
- **Maximum operating temperature 250 ^{o}C**
- **Maximum operating pressure 21bar**
- **Vessel capacity 10 ml**
- **Minimum volume of liquid for digestion 0.1 ml**
- **Maximum volume of liquid for digestion 5.0 m**

Appendix E : GC-ICP –MS Sequence of analysis of PBDE in Environmental model water [15 mg/L humic acid]

CB= Calibration blend

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C:\ICPMH\1\SEQUENCE\140714a.S - SMPL 7/14/2014 10:32 AM

	Method	Type	Vial	Data File	Sample	Comment	Dil/Lvl	ISTD Conc	Action on Failure	Skip	LC/GC Vial	Result
1	C:\ICPMH\1\METHODS\GC250214.m	Sample	1		hx		1.000					
2	C:\ICPMH\1\METHODS\GC250214.m	Sample	2		test CB		1.000					
3	C:\ICPMH\1\METHODS\GC250214.m	Sample	1		hx		1.000					
4	C:\ICPMH\1\METHODS\GC250214.m	Sample	1		hx		1.000					
5	C:\ICPMH\1\METHODS\GC250214.m	Sample	6		B 9		1.000					
6	C:\ICPMH\1\METHODS\GC250214.m	Sample	6		B 9		1.000					
7	C:\ICPMH\1\METHODS\GC250214.m	Sample	6		B 9		1.000					
8	C:\ICPMH\1\METHODS\GC250214.m	Sample	7		B 10		1.000					
9	C:\ICPMH\1\METHODS\GC250214.m	Sample	7		B10		1.000					
10	C:\ICPMH\1\METHODS\GC250214.m	Sample	7		B10		1.000					
11	C:\ICPMH\1\METHODS\GC250214.m	Sample	2		test CB		1.000					
12	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
13	C:\ICPMH\1\METHODS\GC250214.m	Sample	3		R 7		1.000					
14	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
15	C:\ICPMH\1\METHODS\GC250214.m	Sample	3		R 7		1.000					
16	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
17	C:\ICPMH\1\METHODS\GC250214.m	Sample	3		R 7		1.000					
18	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
19	C:\ICPMH\1\METHODS\GC250214.m	Sample	4		R 8		1.000					
20	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
21	C:\ICPMH\1\METHODS\GC250214.m	Sample	4		R 8		1.000					
22	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
23	C:\ICPMH\1\METHODS\GC250214.m	Sample	4		R 8		1.000					
24	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
25	C:\ICPMH\1\METHODS\GC250214.m	Sample	5		R 9		1.000					
26	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
27	C:\ICPMH\1\METHODS\GC250214.m	Sample	5		R 9		1.000					
28	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
29	C:\ICPMH\1\METHODS\GC250214.m	Sample	5		R 9		1.000					
30	C:\ICPMH\1\METHODS\GC250214.m	Sample	8		CB		1.000					
31	C:\ICPMH\1\METHODS\GC250214.m	Sample	1		hx		1.000					

S/N	Vial No	Sample
1	1	Calibration blank ,Hx
2	2	Test Calibration blend
3	1	Calibration blank , Hx
4	1	Calibration blank , Hx
5	6	Reagent blank , B9
6	6	Reagent blank , B9
7	6	Reagent blank , B9
8	7	Water blank (without humic acid & PBDE MIX) B10
9	7	Water blank (without humic acid & PBDE MIX) B10
10	7	Water blank (without humic acid & PBDE MIX) B10
11	**2**	Test CB

Appendix F:Sequence of analysis of PBDE in River Mole water samples by External calibration

Appendix F1: 'Run 1 ext' [External Calibration]

	Rjct	Data File	Acq. Date-Time	Type	Level	Sample Name
	####	001SMPL.D	14/08/2014 17:20	Sample		hx
	####	002SMPL.D	14/08/2014 17:44	Sample		hx
	####	003SMPL.D	14/08/2014 18:09	Sample		hx
	####	004SMPL.D	14/08/2014 18:34	Sample		std 4
	####	005SMPL.D	14/08/2014 18:59	Sample		std 2
	####	006SMPL.D	14/08/2014 19:24	Sample		std 5
	####	007SMPL.D	14/08/2014 19:49	Sample		std 1
	####	008SMPL.D	14/08/2014 20:13	Sample		std 3
	####	009SMPL.D	14/08/2014 20:38	Sample		hx cal blk
	####	010SMPL.D	14/08/2014 21:03	Sample		hx cal blk
	####	011SMPL.D	14/08/2014 21:28	Sample		
	####	012SMPL.D	14/08/2014 21:53	Sample		
	####	013SMPL.D	14/08/2014 22:18	Sample		
	####	014SMPL.D	14/08/2014 22:43	Sample		
	####	015SMPL.D	14/08/2014 23:07	Sample		
	####	016SMPL.D	14/08/2014 23:32	Sample		
	####	017SMPL.D	14/08/2014 23:57	Sample		
	####	018SMPL.D	05/08/2014 23:05	Sample		hx cal blk

Appendix F1: 'Run 1 ext' [External Calibration]

	Rjct	Data File	Acq. Date-Time	Type	Level	Sample Name
	####	019SMPL.D	05/08/2014 23:30	Sample		std icv
	####	020SMPL.D	05/08/2014 23:55	Sample		Sample A9
	####	021SMPL.D	06/08/2014 00:19	Sample		Sample A9
	####	022SMPL.D	06/08/2014 00:44	Sample		Sample A9
	####	023SMPL.D	06/08/2014 01:09	Sample		std ccv
	####	024SMPL.D	06/08/2014 01:34	Sample		hx cal blk
	####	025SMPL.D	06/08/2014 01:59	Sample		std icv
	####	026SMPL.D	06/08/2014 02:24	Sample		Sample E4
		027SMPL.D	06/08/2014 02:48	Sample		Sample E4
		028SMPL.D	06/08/2014 03:13	Sample		Sample E4
		029SMPL.D	06/08/2014 03:38	Sample		std ccv
		030SMPL.D	06/08/2014 04:03	Sample		hx cal blk
		031SMPL.D	06/08/2014 04:28	Sample		std icv
		032SMPL.D	06/08/2014 04:53	Sample		Sample E3
		033SMPL.D	06/08/2014 05:17	Sample		Sample E3
		034SMPL.D	06/08/2014 05:42	Sample		Sample E3

Appendix F1: 'Run 1 ext [External Calibration] [Continued]

	Rjct	Data File	Acq. Date-Time	Type	Level
	035SMPL.D	06/08/2014 06:07	Sample		std ccv
	036SMPL.D	06/08/2014 06:32	Sample		hx cal blk
	037SMPL.D	06/08/2014 06:57	Sample		std icv
	038SMPL.D	06/08/2014 07:21	Sample		Sample A4
	039SMPL.D	06/08/2014 07:46	Sample		Sample A4
	040SMPL.D	06/08/2014 08:11	Sample		Sample A4
	041SMPL.D	06/08/2014 08:36	Sample		std ccv
	042SMPL.D	06/08/2014 09:01	Sample		hx cal std
	043SMPL.D	06/08/2014 09:25	Sample		std icv
	044SMPL.D	06/08/2014 09:50	Sample		Sample E1
	045SMPL.D	06/08/2014 10:15	Sample		Sample E1
	046SMPL.D	06/08/2014 10:40	Sample		Sample E1
	047SMPL.D	06/08/2014 11:05	Sample		std ccv
	048SMPL.D	06/08/2014 11:30	Sample		hx cal blk
	049SMPL.D	06/08/2014 11:54	Sample		std icv
	050SMPL.D	06/08/2014 12:19	Sample		Sample E2
	051SMPL.D	06/08/2014 12:44	Sample		Sample E2
	052SMPL.D	06/08/2014 13:09	Sample		Sample E2

Appendix F1: 'Run 1 ext [External Calibration] [Continued]

Rjct	Data File	Acq. Date-Time	Type	Level	Rjct	
053SMPL.D	06/08/2014 13:34	Sample		std ccv		
054SMPL.D	06/08/2014 13:59	Sample		hx cal blk		
055SMPL.D	06/08/2014 14:24	Sample		std icv		
056SMPL.D	06/08/2014 14:49	Sample		hx		
057SMPL.D	06/08/2014 15:13	Sample		std ccv		
058SMPL.D	06/08/2014 15:38	Sample		std 4		
059SMPL.D	06/08/2014 16:03	Sample		std 2		
060SMPL.D	06/08/2014 16:28	Sample		std 5		
061SMPL.D	06/08/2014 16:53	Sample		std 1		
062SMPL.D	06/08/2014 17:18	Sample		std 3		
		Sample		Hx Cal blk		

Appendix F1: 'Run 1 ext [External Calibration] [Continued]

	Rjct	Data File	Acq. Date-Time	Type	Level	Sample Name
####	001SMPL.D	14/08/2014 17:20	Sample		hx	####
####	002SMPL.D	14/08/2014 17:44	Sample		hx	####
####	003SMPL.D	14/08/2014 18:09	Sample		hx	####
####	004SMPL.D	14/08/2014 18:34	Sample		std 4	####
####	005SMPL.D	14/08/2014 18:59	Sample		std 2	####
####	006SMPL.D	14/08/2014 19:24	Sample		std 5	####
####	007SMPL.D	14/08/2014 19:49	Sample		std 1	####
####	008SMPL.D	14/08/2014 20:13	Sample		std 3	####
####	009SMPL.D	14/08/2014 20:38	Sample		hx cal blk	####
####	010SMPL.D	14/08/2014 21:03	Sample		hx cal blk	####
####	011SMPL.D	14/08/2014 21:28	Sample			####
####	012SMPL.D	14/08/2014 21:53	Sample			####
	013SMPL.D	05/08/2014 21:01	Sample		std icv	013SMPL.D
	014SMPL.D	05/08/2014 21:25	Sample		proce blk	014SMPL.D
	015SMPL.D	05/08/2014 21:50	Sample		proce blk	015SMPL.D
	016SMPL.D	05/08/2014 22:15	Sample		proce blk	016SMPL.D
	017SMPL.D	05/08/2014 22:40	Sample		std ccv	017SMPL.D

Appendix F2: 'Run 2 ext/int' [Continued]

	Rjct	Data File	Acq. Date-Time	Type	Level	Sample Name
018SMPL.D	05/08/2014 23:05	Sample		hx cal blk	018SMPL.D	05/08/2014 23:05
	####	019SMPL.D	15/08/2014 00:47	Sample		std icv
	####	020SMPL.D	15/08/2014 01:12	Sample		Sample A10
	####	021SMPL.D	15/08/2014 01:36	Sample		Sample A10
	####	022SMPL.D	15/08/2014 02:01	Sample		Sample A10
	####	023SMPL.D	15/08/2014 02:26	Sample		std ccv
	####	024SMPL.D	15/08/2014 02:51	Sample		hx cal blk
	####	025SMPL.D	15/08/2014 03:16	Sample		std icv
	####	026SMPL.D	15/08/2014 03:40	Sample		Sample A11
	####	027SMPL.D	15/08/2014 04:05	Sample		Sample A11
	####	028SMPL.D	15/08/2014 04:30	Sample		Sample A11
	####	029SMPL.D	15/08/2014 04:55	Sample		std ccv
	####	030SMPL.D	15/08/2014 05:20	Sample		hx cal blk
	####	031SMPL.D	15/08/2014 05:44	Sample		std icv
	####	032SMPL.D	15/08/2014 06:09	Sample		Sample A12
	####	033SMPL.D	15/08/2014 06:34	Sample		Sample A12
	####	034SMPL.D	15/08/2014 06:59	Sample		Sample A12
	####	035SMPL.D	15/08/2014 07:23	Sample		std ccv
	####	036SMPL.D	15/08/2014 07:48	Sample		hx cal blk

Appendix G: Operating conditions of ICPMS for isotope dilution mass spectrometry

Tune Parameters	LR	MR	HR
Touch X – Pos. [mm]	3600	3600	3600
Touch Y – Pos. [mm]	2100	2100	`2100
Touch Z – Pos. [mm]	-3700	-3700	-3700
Plasma Power [Watt]	1320	1320	1320
Peri. Pump Speed [rpm]	8.00	8.00	8.00
Cool gas [L/min]	15	15	15
Aux gas [L/min]	1.05	1.05	1.05
Sample gas [L/min]	0.980	0.980	0.980
Additional 1[L/min]	0.000	0.0000.0	0.000
Add. 2/ GD gas Flow [L/min]	0.000	0.000	0.000
Guard Electrode:	Yes	yYes	Yes
Extraction [V]	-2000	-2000	-2000
Focus [V]	-1286	-1286	-1286

Appendix G: Operating conditions of ICPMS for isotope dilution mass spectrometry [continued]

Tune Parameters	LR	MR	HR
X- Deflection [V]	0.25	0.25	0.25
Y- Deflection [V]	0.75	0.75	0.75
Shape [V]	115.00	115.00	115.00
Rotation quadrupol 2 [V]	1.02	-1.90	-1.76
Rotation quadrupol 2 [V]	-0.50	-3.91	-0.48
Focus quadrupol 1 [V]	-2.62	5.51	4.65
Focus quadrupol 2 [V]	0.00	0.00	0.00
UaUb %	-0.340	-0.340	-0.340
Focus offset [%]	50.70	50.70	50.70
MATSUDA – Plate [V]	60.00	60.00	60.00
SEM-Deflection [V]	-75.00	-75.00	-75.00
SEM [V]	2700	2700	2700

Appendix G2 : Operating conditions of ICPMS for isotope dilution mass spectrometry

Isotope	Intensity AVG (cps)	Ratio AVG	Resolution AVG
Li 7 (LR)	216591.0		300
Ba 137 ++ (LR)	4701.3		313
In115 (LR)	1722376.0		317
Ba137(LR)	194879.2		313
Ba137016(LR)	260.0		420
U238(LR)	2731955.2		317
U238O16(LR)	136153.8		319
Ba137O16(LR)		0.0013	0
Ba137++/Ba137(LR)		0.0241	0
U238O16/U238(LR)		0.0498	0
Fe56(Mr)	90922.0		4263
In115(MR)	142763.0		4086
Ar38(HR)	6498772.5		8315

Appendix G2 : Operating conditions of ICPMS for isotope dilution mass spectrometry [Continued]

Ar40Ar40 (HR)	5169282.7		7712
In115(HR)	33353.9		7020

Note: LR – Low resolution, MR – medium resolution, HR – High Resolution

From the table above, transmission resolution was determined to enable us determine the quantity of actual bromine ion reaching the ICP – MS.

Therefore, For transmission at high resolution,

$$= \frac{HR}{LR} \times 100 = \frac{33353}{1722376} \times 100 = 1.93\%$$

Therefore, For transmission at Medium resolution,

$$= \frac{MR}{LR} \times 100 = \frac{142763}{1722376} \times 100 = 8.288\%$$

Appendix H: Masses of final injection extracts and residues from sample preparation

	05/08/2014											
	Analyst	**Olukayode Babarinde**										
	Activity	**Reconstitution of extraction in 50 ul hexane**										
				W1 (g)	**W2 (g)**	**W3 (g)**	**Mean (g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std Uncert**
E1	**Dry Mass of vial**			3.03873	3.03875	3.03876	3.03875	1.53E-05	8.82E-06	0.000503	0.000144	0.000144
	Vial + residue			3.03885	3.03885	3.03885	3.03885	0	0	0	0.000144	0.000144
	Mass of residue	**due + 50 ul hx**		3.10093	3.10094	3.10094	3.10094	5.77E-06	3.33E-06	0.000186	0.000144	0.000144
	Mass of residue						0.00010					
	Mass of 50 µl hx						0.06209					

				W1 (g)	**W2 (g)**	**W3 (g)**	**Mean (g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std Uncert**
E2	**Dry Mass of vial**			3.03265	3.03267	3.0327	3.03267	2.52E-05	1.45E-05	0.00083	0.000144	0.000145
	Vial + residue			3.03279	3.03278	3.03276	3.03278	1.53E-05	8.82E-06	0.000504	0.000144	0.000144

	Mass of residue	**due + 50 ul hx**	3.0941	3.09413	3.09413	3.09412	1.73E-05	1E-05	0.00056	0.000144	0.000144
	Mass of residue					0.00010					
	Mass of 50 µl hx					0.06134					

				W1 (g)	**W2 (g)**	**W3 (g)**	**Mean (g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std Uncert**
E3	**Dry Mass of vial**			3.0373	3.03732	3.03733	3.03732	1.53E-05	8.82E-06	0.000503	0.000144	0.000144
	Vial + residue			3.0374	3.0374	3.0374	3.03740	0	0	0	0.000144	0.000144
	Mass of residue	**due + 50 ul hx**		3.10018	3.010021	3.10022	3.07014	0.052065	0.03006	1.695847	0.000144	0.03006
	Mass of residue						0.00008					
	Mass of 50 µl hx						0.03274					

				W1 (g)	**W2 (g)**	**W3 (g)**	**Mean (g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std Uncert**
E4	**Dry Mass of vial**			3.00986	3.00985	3.00985	3.00985	5.77E-06	3.33E-06	0.000192	0.000144	0.000144

	Vial + residue		3.00991	3.00991	3.0099	3.00991	5.77E-06	3.33E-06	0.000192	0.000144	0.000144
	Mass of residue	**due + 50 ul hx**	3.07319	3.07319	3.07318	3.07319	5.77E-06	3.33E-06	0.000188	0.000144	0.000144
	Mass of residue					0.00005					
	Mass of 50 µl hx					0.06328					

				W1 (g)	**W2 (g)**	**W3 (g)**	**Mean (g)**	**Stdev (g)**	**SEM (g)**	**RSD %**	**Linearity**	**Std Uncert**
A9	**Dry Mass of vial**			3.04023	3.04023	3.04023	3.04023	5.44E-16	3.14E-16	1.79E-14	0.000144	0.000144
	Vial + residue			3.0403	3.04031	3.0403	3.04030	5.77E-06	3.33E-06	0.00019	0.000144	0.000144
	Mass of residue	**due + 50 ul hx**		3.10289	3.1029	3.1029	3.10290	5.77E-06	3.33E-06	0.000186	0.000144	0.000144
	Mass of residue						0.00007					
	Mass of 50 µl hx						0.06259					

				W1 (g)	W2 (g)	W3 (g)	Mean (g)	Stdev (g)	SEM (g)	RSD %	Linearity	Std Uncert
A4	**Dry Mass of vial**			2.98248	2.9825	2.98248	2.98249	1.15E-05	6.67E-06	0.000387	0.000144	0.000144
	Vial + residue			2.98254	2.98255	2.98256	2.98255	1E-05	5.77E-06	0.000335	0.000144	0.000144
	Mass of residue	**due + 50 ul hx**		3.04563	3.04563	3.04563	3.04563	5.44E-16	3.14E-16	1.79E-14	0.000144	0.000144
	Mass of residue						0.00006					
	Mass of 50 µl hx						0.06308					

				W1 (g)	W2 (g)	W3 (g)	Mean (g)	Stdev (g)	SEM (g)	RSD %	Linearity	Std Uncert
C1	**Dry Mass of vial**			3.02954	3.02955	3.02956	3.02955	1E-05	5.77E-06	0.00033	0.000144	0.000144
	Vial + residue			3.02961	3.0296	3.02961	3.02961	5.77E-06	3.33E-06	0.000191	0.000144	0.000144
	Mass of residue	**due + 50 ul hx**		3.09075	3.09075	3.09073	3.09074	1.15E-05	6.67E-06	0.000374	0.000144	0.000144
	Mass of residue						0.00006					
	Mass of 50 µl hx						0.06114					

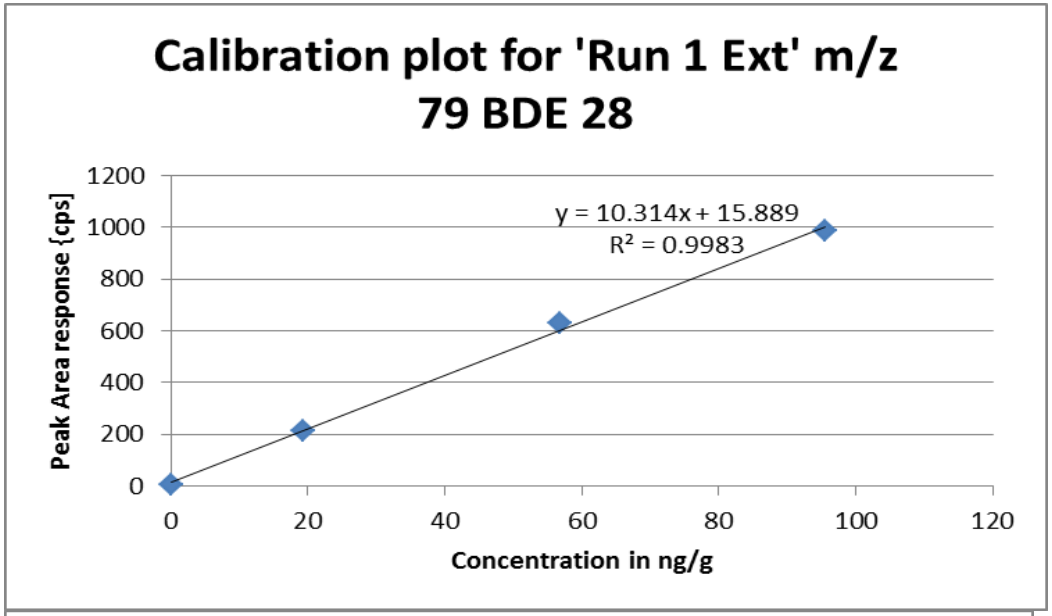
	RUN 2 EXT and RUN 2 EXT /INT										
				W1 (g)	W2 (g)	W3 (g)	Mean (g)	Stdev (g)	SEM (g)	RSD %	Linearity	Std Uncert
B	Dry Mass of vial			3.01728	3.01729	3.01728	3.017283	5.77E-06	3.33E-06	0.000191	0.000144	0.000144
	Vial + residue			3.01729	3.01733	3.01733	3.017317	2.31E-05	1.33E-05	0.000765	0.000144	0.000145
	Mass of residue	due + 50 ul hx/int		3.04974	3.04974	3.04974	3.04974	0	0	0	0.000144	0.000144
	Mass of residue						3.33E-05					
	Mass of 50 µl int /hx						0.032423					

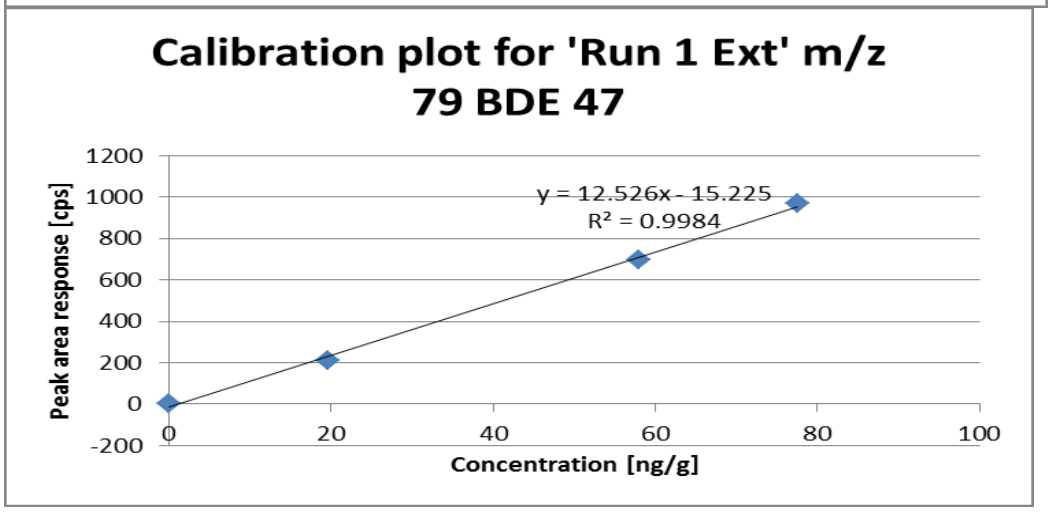
				W1 (g)	W2 (g)	W3 (g)	Mean (g)	Stdev (g)	SEM (g)	RSD %	Linearity	Std Uncert
A10	Dry Mass of vial			3.00553	3.00553	3.00552	3.005527	5.77E-06	3.33E-06	0.000192	0.000144	0.000144
	Vial + residue			3.00225	3.00226	3.00226	3.002257	5.77E-06	3.33E-06	0.000192	0.000144	0.000144
	Mass of residue	due + 50 ul hx/int		3.03363	3.03364	3.03365	3.03364	1E-05	5.77E-06	0.00033	0.000144	0.000144
	Mass of residue						-0.00327					
	Mass of 50 µl hx/int						0.031383					

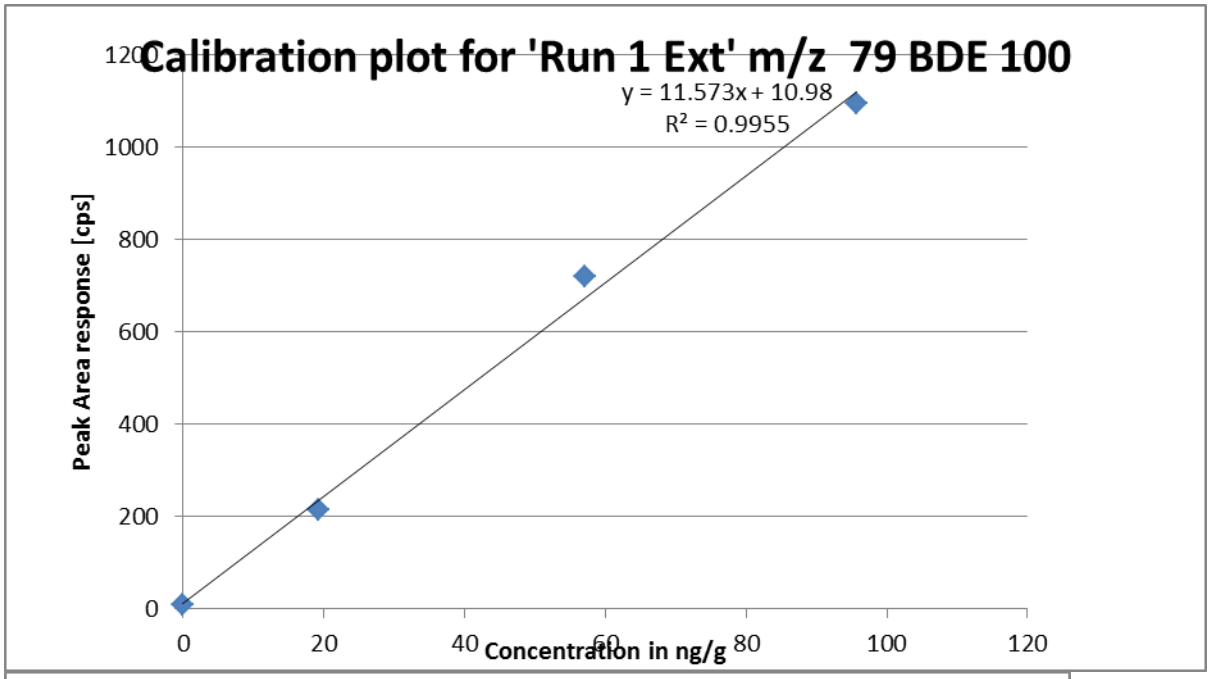
				W1 (g)	W2 (g)	W3 (g)	Mean (g)	Stdev (g)	SEM (g)	RSD %	Linearity	Std Uncert
A11	**Dry Mass of vial**			3.02301	3.023	3.02301	3.023007	5.77E-06	3.33E-06	0.000191	0.000144	0.000144
	Vial + residue			3.01456	3.01456	3.01453	3.01455	1.73E-05	1E-05	0.000575	0.000144	0.000144
	Mass of residue	**due + 50 ul hx/int**		3.04662	3.04661	3.04661	3.046613	5.77E-06	3.33E-06	0.00019	0.000144	0.000144
	Mass of residue						-0.008457					
	Mass of 50 µl hx /int						0.032063					

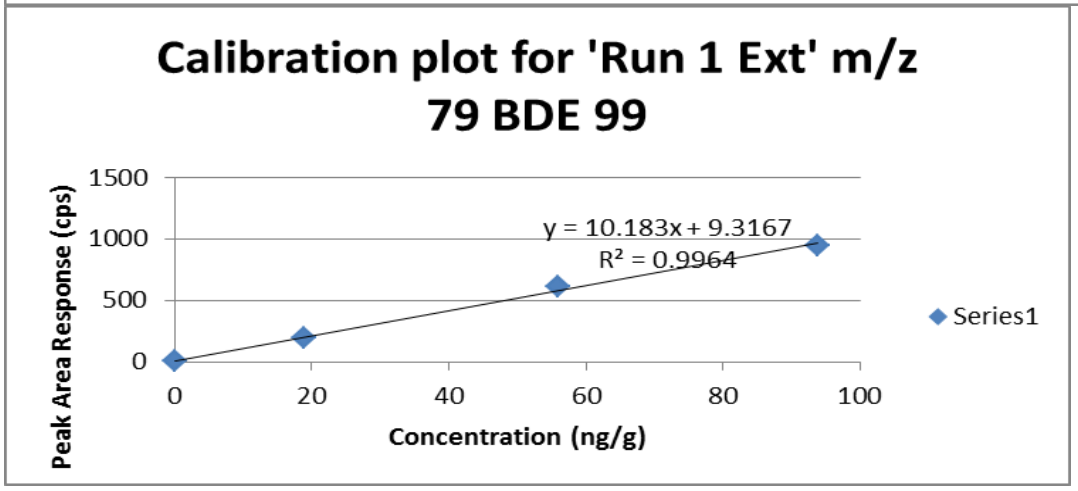
				W1 (g)	W2 (g)	W3 (g)	Mean (g)	Stdev (g)	SEM (g)	RSD %	Linearity	Std Uncert
A12	**Dry Mass of vial**			3.00858	3.00857	3.00859	3.00858	1E-05	5.77E-06	0.000332	0.000144	0.000144
	Vial + residue			3.00871	3.00872	3.00872	3.008717	5.77E-06	3.33E-06	0.000192	0.000144	0.000144
	Mass of residue	**due + 50 ul hx/int**		3.04028	3.04029	3.04029	3.040287	5.77E-06	3.33E-06	0.00019	0.000144	0.000144
	Mass of residue						**0.000137**					
	Mass of 50 µl hx/int						**0.03157**					

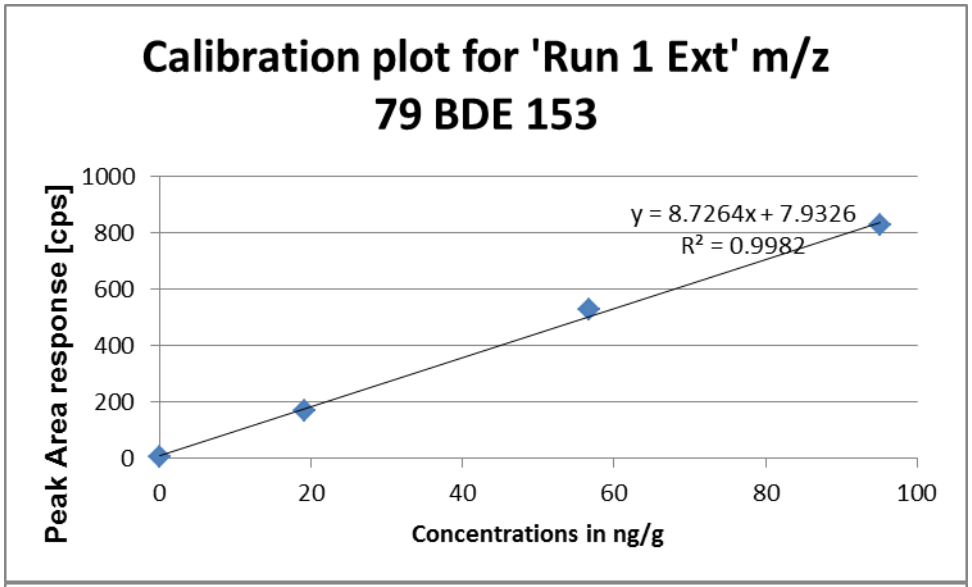
Appendix I : Calibration Plots For 'run 1 ext' and 'Run 2 ext '

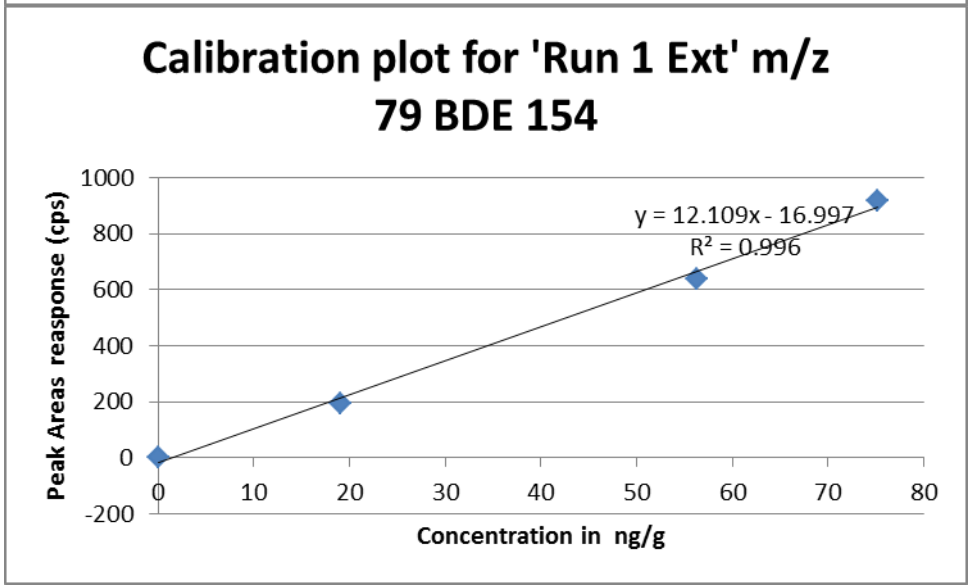




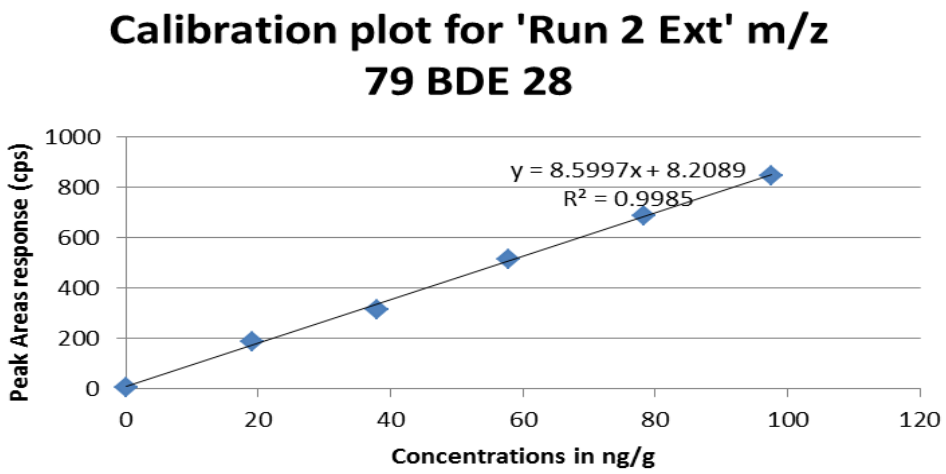

Calibration plot for 'Run 1 Ext' m/z 79 BDE 100
y = 11.573x + 10.98
R² = 0.9955
Peak Area response [cps]
Concentration in ng/g
0
200
400
600
800
1000
1200
0
20
40
60
80
100
120

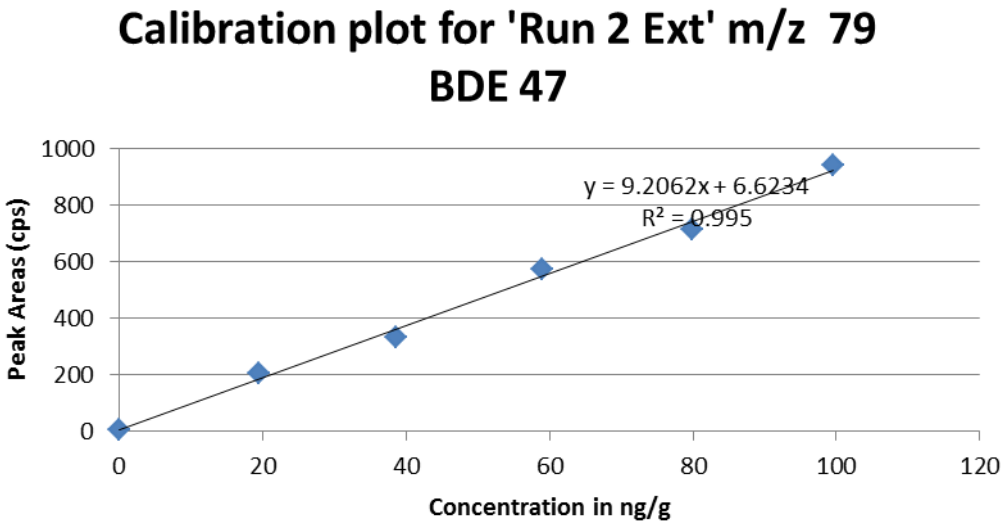

Calibration plot for 'Run 1 Ext' m/z 79 BDE 99
y = 10.183x + 9.3167
R² = 0.9964
Peak Area Response (cps)
Concentration (ng/g)
Series1
0
500
1000
1500
0
20
40
60
80
100

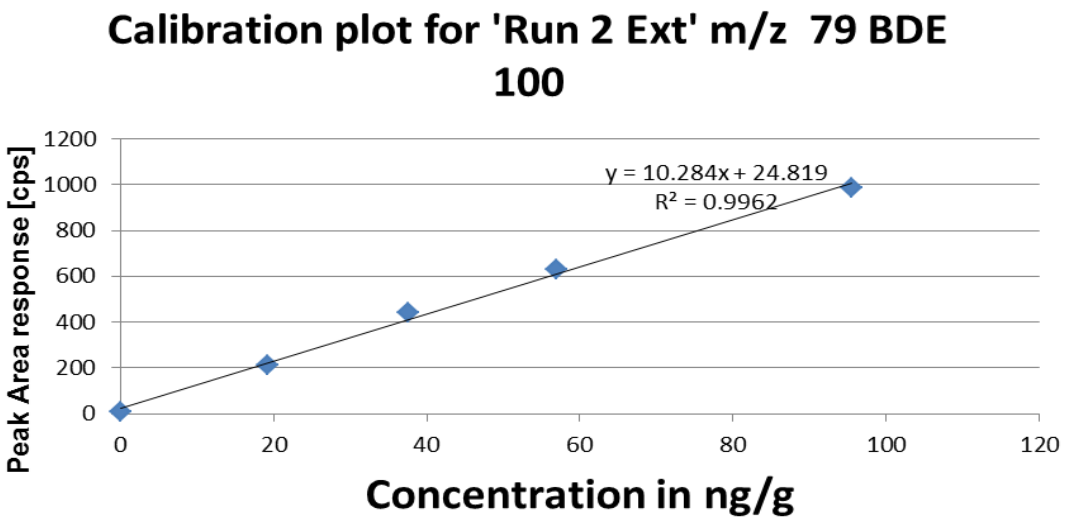

Calibration plot for 'Run 1 Ext' m/z
79 BDE 153
Peak Area response [cps]
1000
800
600
400
200
0
y = 8.7264x + 7.9326
R² = 0.9982
0
20
40
60
80
100
Concentrations in ng/g

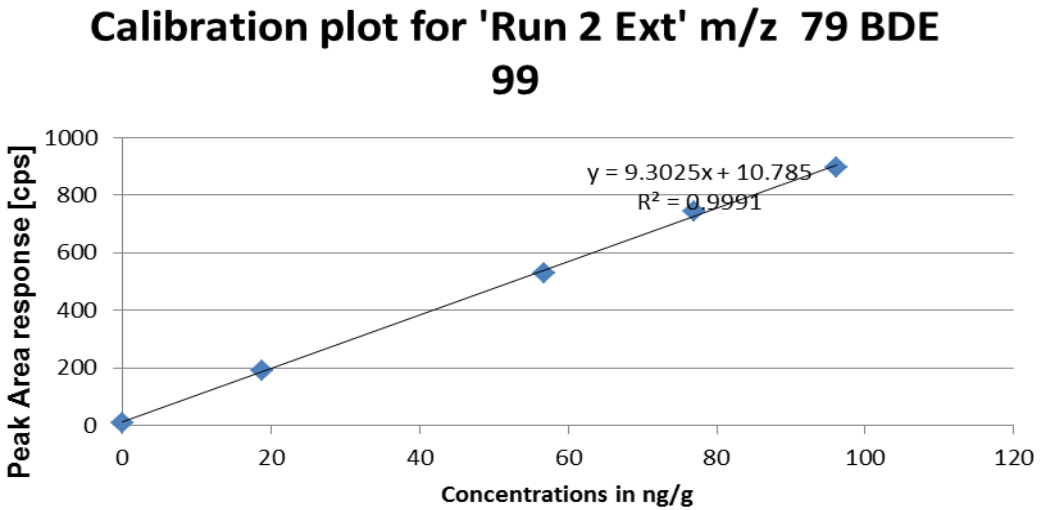

Calibration plot for 'Run 1 Ext' m/z
79 BDE 154
Peak Areas reasponse (cps)
1000
800
600
400
200
0
-200
y = 12.109x - 16.997
R² = 0.996
0
10
20
30
40
50
60
70
80
Concentration in ng/g

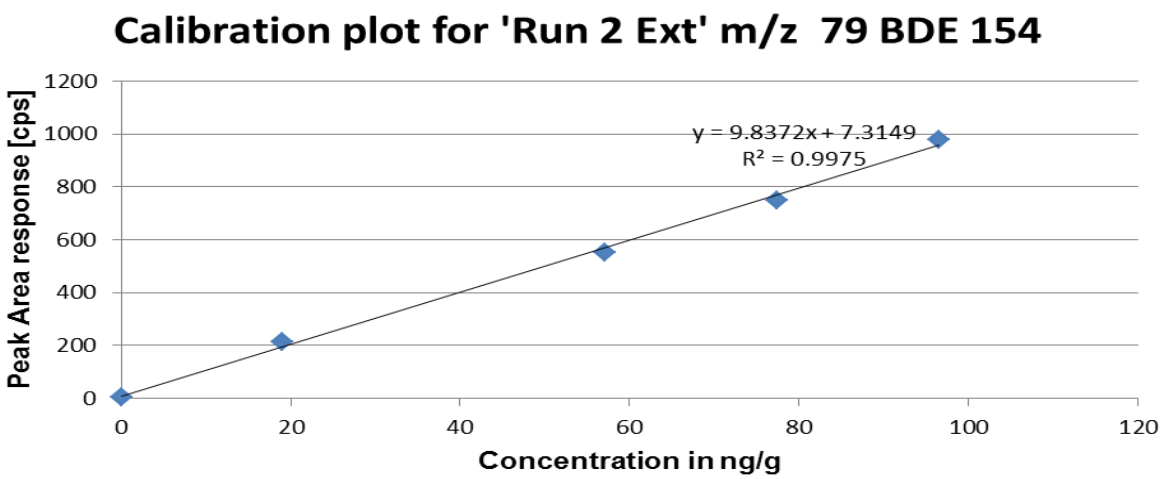
Appendix I2: Calibration plot for Run 2 ext.[External Calibration]

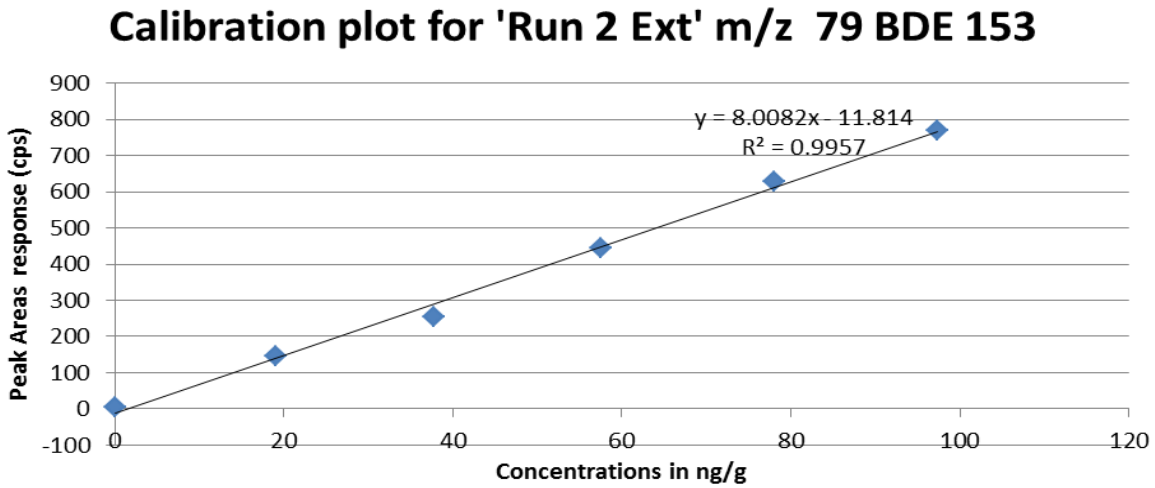





Calibration plot for 'Run 2 Ext' m/z 79 BDE 100
Peak Area response [cps]
y = 10.284x + 24.819
R² = 0.9962
Concentration in ng/g


Calibration plot for 'Run 2 Ext' m/z 79 BDE 99
Peak Area response [cps]
y = 9.3025x + 10.785
R² = 0.9991
Concentrations in ng/g


Calibration plot for 'Run 2 Ext' m/z 79 BDE 154
Peak Area response [cps]
1200
1000
800
600
400
200
0
y = 9.8372x + 7.3149
R² = 0.9975
0
20
40
60
80
100
120
Concentration in ng/g


Calibration plot for 'Run 2 Ext' m/z 79 BDE 153
Peak Areas response (cps)
900
800
700
600
500
400
300
200
100
0
-100
y = 8.0082x - 11.814
R² = 0.9957
0
20
40
60
80
100
120
Concentrations in ng/g

Appendix J: Estimation Of The Limit Of Detection from standard deviation values of slope and intercept of Calibration curve.

The measurement were recorded from separate analysis run of calibration standards prepared. The product moment correlation coefficient of all calibration curve for the measurement of instrument Limit of detection ranged between 0.995 - 0.9991. [as shown in the graph. All the formula shown below were plugged into an excel sheet prepared by the author to calculate the LOD. Let letter x represent the concentration of each congeners calibration standard in ng/g while letter 'y ' represents the corresponding peak areas measured in count per seconds [cps].

$$r = \frac{\sum_i \left\{ \left(x_i - \bar{x} \right) \left(y_i - \bar{y} \right) \right\}}{\left\{ \left[\sum_i \left(x_i - \bar{x} \right)^2 \right] \left[\sum_i \left(y_i - \bar{y} \right)^2 \right] \right\}^{1/2}}$$

Correlation coefficient is also reffered to as product moment correlation coefficient represented by 'r'. It is also called linear correlation coefficient. It measures the strenght and direction between our two variables , concentration of PBDE congeners (x) and intrument signal response (Peak area response). The term R-squared (R^2) is called the coefficient of Determination or square of the correlation coefficient. It denotes the magnitude of linear association between the concentration of PBDE congeners (x) and Peak area response, (y)

Table of values for determination of product moment correlation coefficient, gradient and Intercept of the linear calibration curve

x_i	y_i	$\left(x_i - \bar{x}\right)$	$\left(x_i - \bar{x}\right)^2$	$\left(y_i - \bar{y}\right)$	$\left(y_i - \bar{y}\right)^2$	$\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)$
Sums :						

Manual Determination of the intercept and Gradient of the calibration curve

Assumption: That all errors are in y-axis. The intercept and gradient of the calibration curve determine the line of regression of y on x. We maintained that instrumental signals, peak area response (cps) and concentrations in ng/g are plotted on y-axis and x-axis respectively. $\bar{x}$ represents the mean of concentrations in ng/g (x) values and $\bar{y}$ is the mean of the peak area response (cps) (y) values. 'b' represents the slope of least square line derived from application of in Equation (3) while 'a' represents the intercept calculated by application of equation (4) described below:

$$b = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sum_i (x_i - \bar{x})^2}$$ Equation (3)

$a = \bar{y} - b\bar{x}$ Equation (4)

Table of values for the Determination of the confidence limits for the slope and intercept

x_i	x_i^2	y_i	$\hat{y}_i$	$\lvert y_i - \hat{y}_i \rvert$	$(y_i - \hat{y}_i)^2$
$\sum_i x_i =$	$\sum_i x_i^2 =$				$\sum_i (y_i - \hat{y})^2 =$

Estimation of Random errors term statistics $s_{y/x}$ -The random errors in the values for the slope and intercept are estimated using equation (4).Note that term statistics $s_{y/x}$, estimates the random errors in the y –direction, utilizing y-residuals where $\hat{y}_i$ - values are the points on the calculated regression line corresponding to the individual x-values and was calculated using regression equation (5) above, $y_i - \hat{y}_i$ - values are termed y-residuals.

$$s_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y})^2}{n-2}}$$ Equation (4)

DETERMINATION OF THE CONFIDENCE LIMITS FOR THE SLOPE

6.2.1 Determination of the standard deviation of the slope, S_b

Equation (7) is utilized to calculate the standard deviation of the slope $S_{b,}$ given that the statistics

$$s_b = \frac{s_{x/y}}{\sqrt{\sum_i (x_i - \bar{x})^2}}$$ Equation (5)

Confidence limits for the slope, b

The confidence limits for the slope, b is expressed in equation (8), given that **b**= , **S_b =, n=.** The t- value for (n-2) = degrees of freedom and 95% confidence level is (t_{n-2}=). The 95% confidence limits for the slope b are:

$$= b \pm t_{(n-2)} s_b$$ Equation (6)

DETERMINATION OF THE CONFIDENCE LIMITS FOR THE INTERCEPT

6.3. 1 Determination of the standard deviation of the intercept, S_a.

Equation (8) below is utilized to calculate the standard deviation of the intercept, S_a .

Given that: n=6, the statistics, $s_{y/x} =$, $\sum_i x^2_i =$ and $\sum (x_i - \bar{x})^2 =$. These values were substituted in equation (7) as shown below:

$$s_a = s_{x/y}\sqrt{\frac{\sum_i x_i^2}{n\sum_i (x_i - \bar{x})^2}}$$ Equation (7)

Confidence limits for the intercept, a

The confidence limits for the intercept, a is expressed in equation (10), given that **a**= (calculated from equation (4), **S_a =?, n=?.** The t- value for (n-2) =? degrees of freedom and 95% confidence level is ? (t_{n-2}=?). The 95% confidence limits for the intercept, a are:

$$= a \pm t_{(n-2)} s_a$$ Equation (8)

Estimation Of The Limit Of Detection

The limit of detection of an analyte may be described as that concentration which gives an instrument signal (y) significantly different from the blank or background signal. Assuming that each point on the plot has a normally distributed variation. Limit of detection is expressed as the analyte concentration giving a signal (y) equal to the blank signal, y_B plus three (3) standard deviations of the blank, S_B. The, estimate of standard error of the gradient and the intercept, $S_{x/y}$ can be used in place of S_B while the calculated intercept (a) can be used as an estimate of

y_B,[1] the blank absorbance for the determination of the detection limit because the intercept should be a more accurate measurement of y_B than the singly measured y_1=? in table 1 above.

Therefore, the value of 'y' at detection limit = $y_B + 3S_B$. Equation (9)

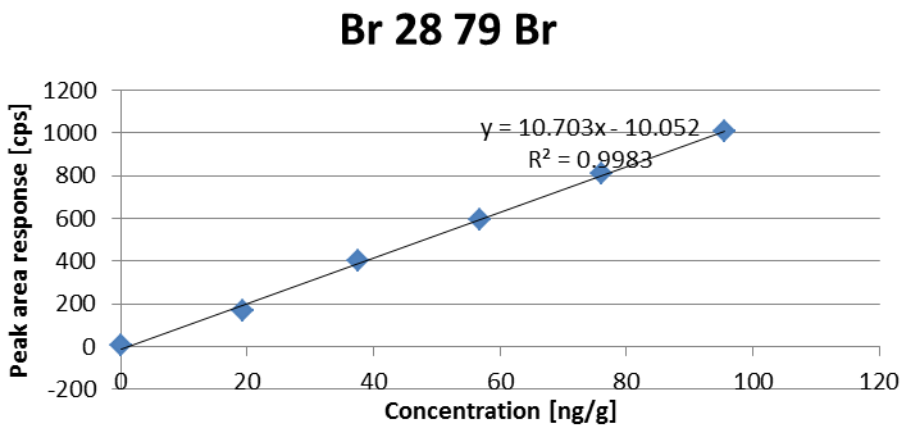
Then, calculate the limit of detection using regression line equation substituting the value of 'y' at detection limit obtained using equation [9].

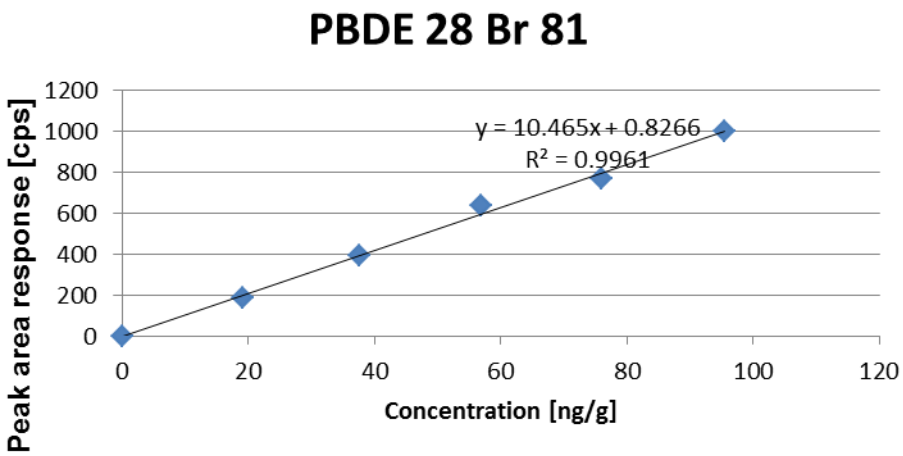
Appendix J1: Sequence of analysis of calibration standards by GC-ICP-MS used for LOD Calculation

S/N	Rjct	Data File	Acq. Date-Time		Level	Sample Concentration
1	001SMPL.D	14/08/2014 17:20	Sample	1	hx	
2	002SMPL.D	14/08/2014 17:44	Sample	2	Std 1	20 ng/g
3	003SMPL.D	14/08/2014 18:09	Sample	3	Std 2	40 ng/g
4	004SMPL.D	14/08/2014 18:34	Sample	4	std 3	60 ng/g
5	005SMPL.D	14/08/2014 18:59	Sample	5	std 4	80 ng/g
6	006SMPL.D	14/08/2014 19:24	Sample	6	std 5	100 ng/g
7	007SMPL.D	14/08/2014 19: 49	Sample	7	Std CCV	
8	008SMPL.D	14/08/2014 20:16	Sample	8	Std ICV	
9	009SMPL.D	14/08/2014 20:41	Sample	1	hx	
10	0010SMPL.D		Keyword	Standby		

Appendix J1: Tables of values and Calibration plots variables for the calculation of instrument limit of detection Using m/z 79 Br PBDE congeners

	BDE 28 79 Br			**PBDE 28 79 Br**	
S/N	**Conc ng/g**	**Peak area (cps)**		Conc ng/g	Peak area (cps)
1	0	4		0	1
2	19.2259	167.6336		19.2259	188.5458
3	37.62206	404.8044		37.62206	392.9643
4	56.87615	597.1153		56.87615	638.3791
5	76.04721	812.0802		76.04721	769.4068
6	95.4388	1006.781		95.4388	999.4818





BDE 28 79 Br

r= 0.9992

$s_{y/x}$ **= 17.51**

yB =a -10.05

Slope, b = 10.703 ± 0.61

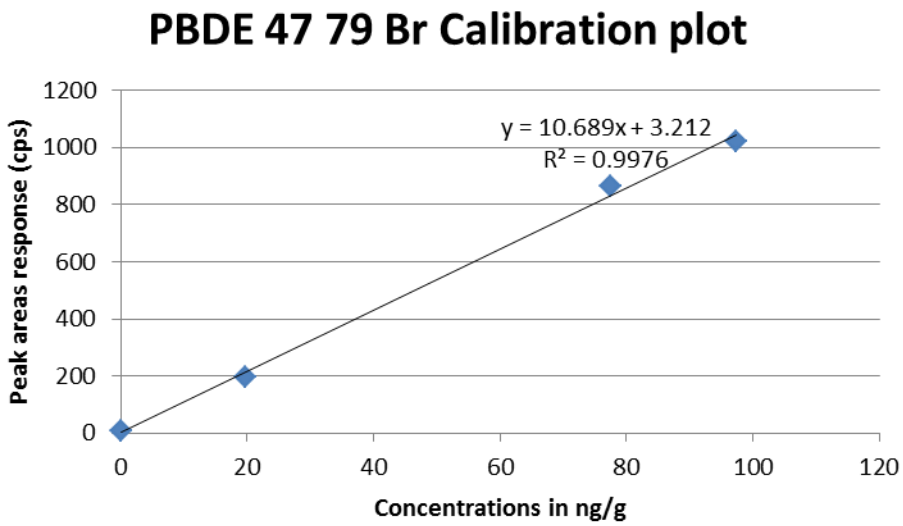
Intercept, a =-10.05 ± 25.18

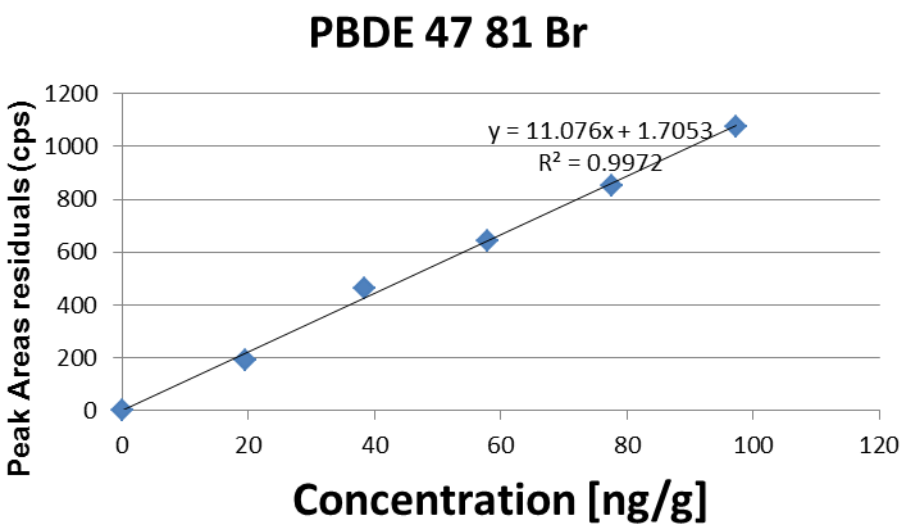
LOD= 3.03 ng/g

Peak area at LOD = 42.47 cps

S_{xo} = 1.97

					BDE 47 81 Br	
	PBDE 47 79 Br				**Conc ng/g**	**Peak area (cps)**
S/N	**Conc ng/g**	**Peak area (cps)**			0	3
1	0	9			19.60611	190.6612
2	19.60611	197.5125			38.36607	463.7922
3	97.32629	1021.506			58.00092	644.7697
4	77.55111	863.742			77.55111	853.0047
5					97.32629	1076.396
6						





BDE 47 79 Br.

r= 0.999

$s_{y/x} = 29.58$

y_B = a =3.21

Slope, b = 10.69 ± 1.59

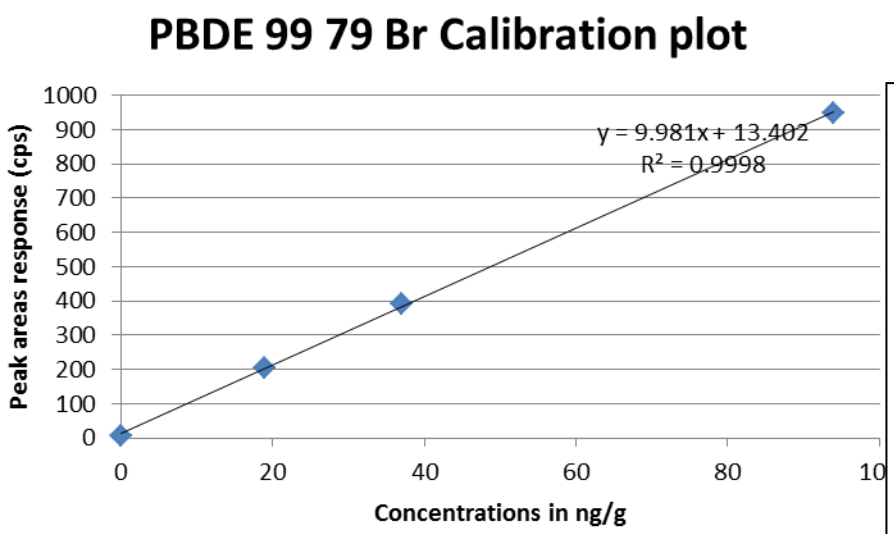
Intercept, a = 3.21 ± 100.09

LOD= 8.90 ng/g

Peak area at LOD = 91.94 cps

S_{xo} = 3.39 ng/g

	BDE 99 79 Br			BDE 99 81 Br		
S/N	**Conc ng/g**	**Peak area (cps)**		**Conc ng/g**	**Peak area (cps)**	
1	0	7		0	10	
2	18.9158	205.0408		18.9158	178.2671	
3	37.01523	389.6735		37.01523	384.2709	
4	93.89951	947.3492		93.89951	953.874	



BDE 99 79 Br.

r= 0.9999

$S_{x/y}$ = 7.28

y_B = a=13.4

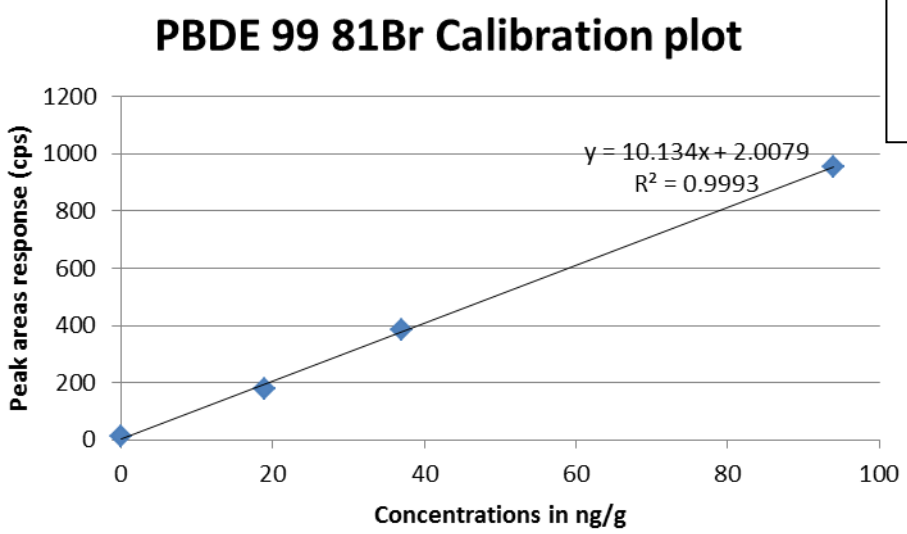
Slope ,b = 9.98 ± 0.45

Intercept, a = 13.40 ± 22.93

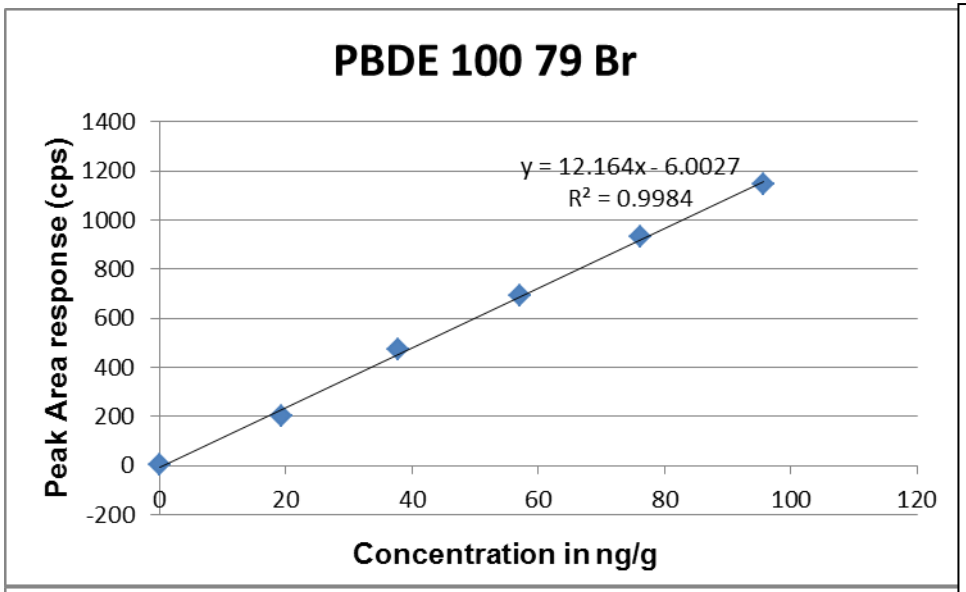
LOD= 4.88 ng/g

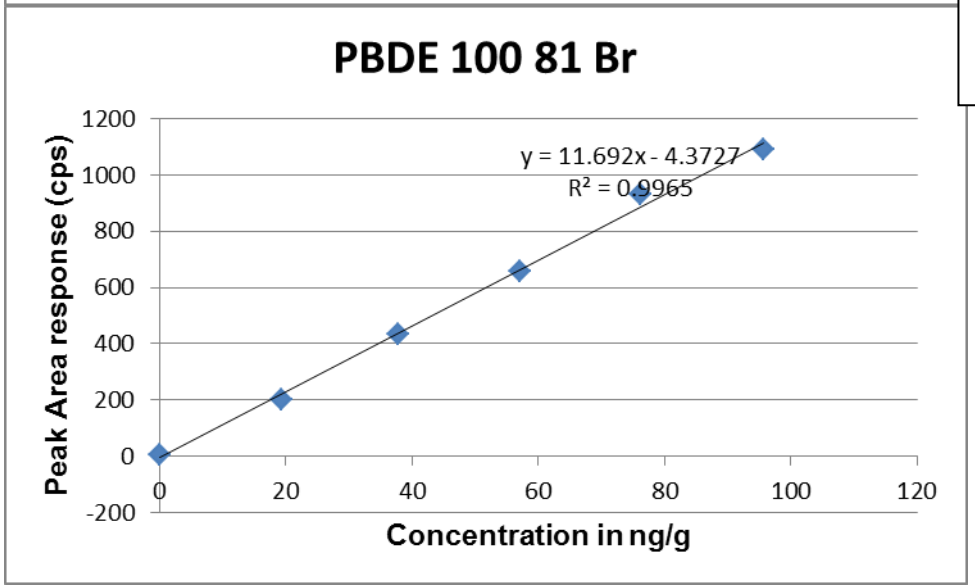
Peak area at LOD = 35.27 cps

S_{xo} = 0.895 ng/g



BDE 100 79 Br	Conc ng/g	Peak area (cps)	BDE 100 81 Br	Conc ng/g	Peak area (cps)	
S/N	0	2		0	7	
1	19.28085	199.652		19.28085	201.93	
2	37.72959	470.7211		37.72959	432.9113	
3	57.03871	693.9995		57.03871	656.3157	
4	76.26456	930.3446		76.26456	930.236	
5	95.71168	1146.38		95.71168	1089.496	





BDE 100 79 Br.

r= 0.999

$s_{y/x} = 19.16$

y_B=a=-6.00

Slope, b = 12.16 ± 0.66

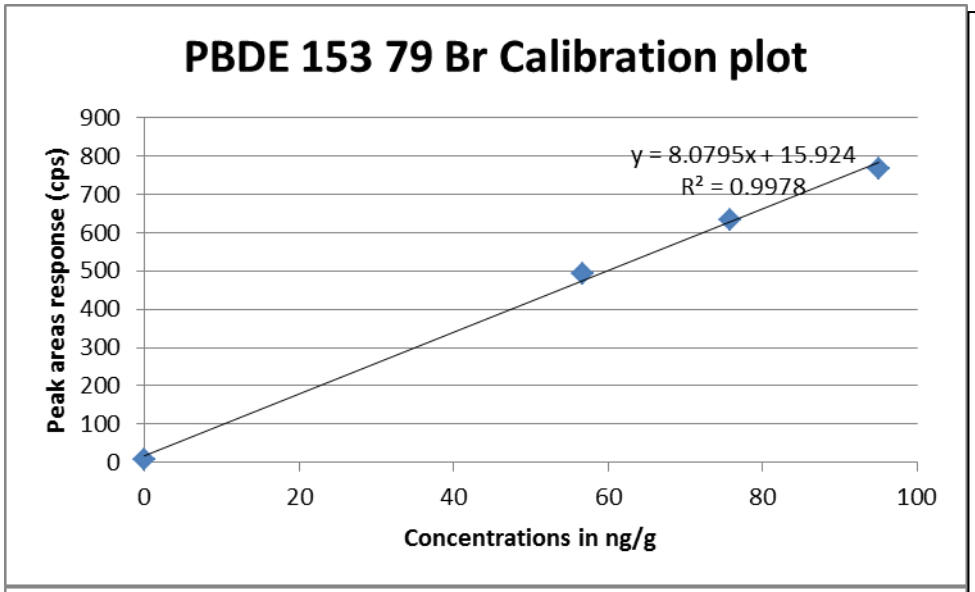
Intercept, a = -6.00 ± 38.49

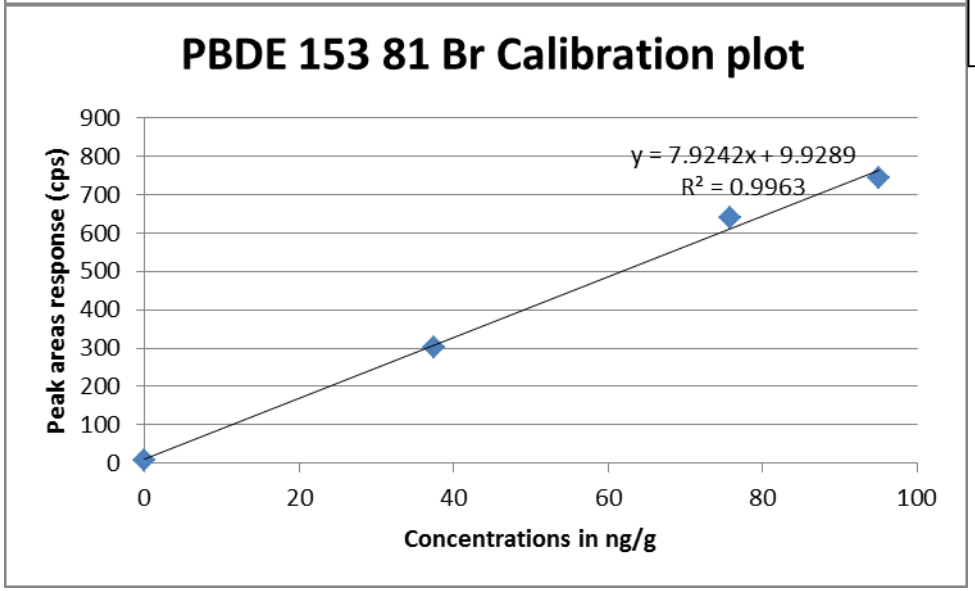
LOD= 3.74 ng/g

Peak area at LOD =51.46 cps

S_{xo} = 1.90 ng/g

	BDE 153 79 Br			BDE 153 81 Br		
S/N	Conc ng/g	Peak area (cps)		Conc ng/g	Peak area (cps)	
1	0	7		0	7	
2	95.09642	768.2856		95.09642	742.949	
3	56.67205	493.0494		75.77432	638.4254	
4	75.77432	633.7992		37.48705	302.4129	
5						





BDE 153 79 Br.

r= 0.999

$s_{y/x} = 19.20$

$y_{B=}$ a =15.92

Slope, b= 8.08 ± 1.16

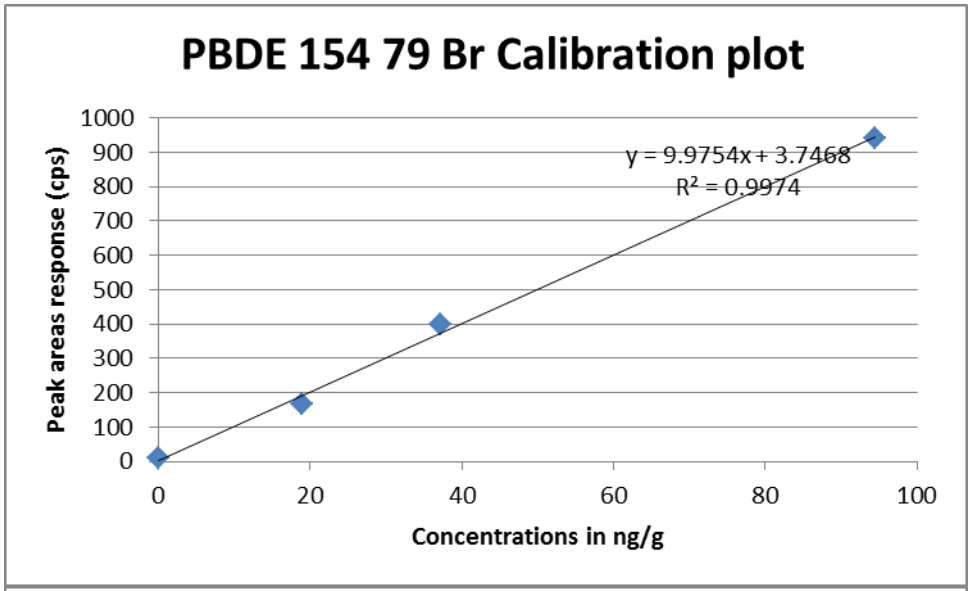
Intercept, a= 15.92 ± 77.94

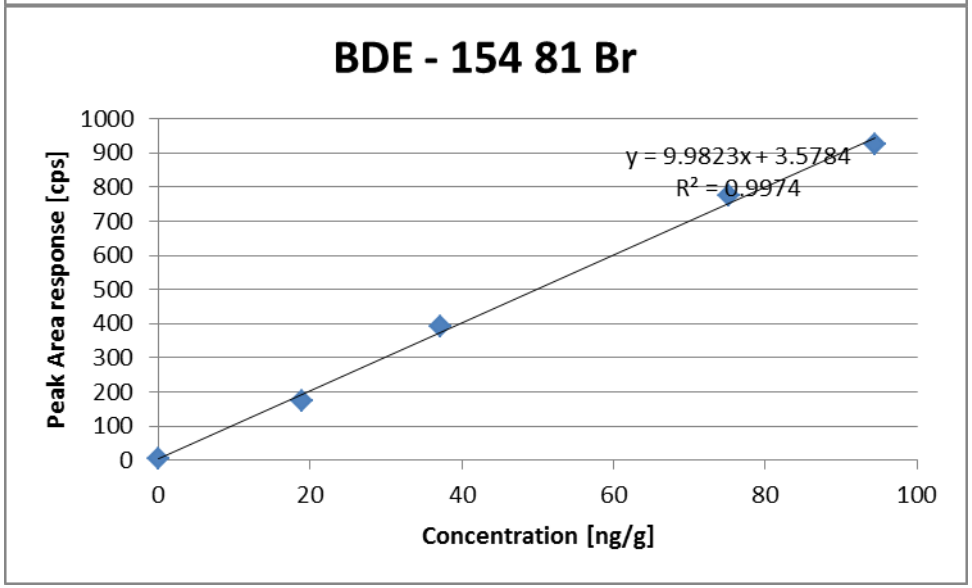
LOD= 11.07 ng/g

Peak area at LOD = 73.51 cps

S_{xo} = 3.13 ng/g

	BDE 154 79 Br			**BDE 154 81 Br**	
S/N	**Conc ng/g**	**Peak area (cps)**		**Conc ng/g**	**Peak area (cps)**
1	0	10		0	5
2	19.01681	167.7003		19.01681	172.7248
3	37.21289	398.5799		37.21289	392.697
4	94.40094	941.3151		75.22014	776.1228
5				94.40094	925.8522





BDE 154 79 Br.

r= 0.998

$s_{y/x} = S_B = 24.69$

yB= a = 3.75

Slope, b = 9.98 ± 1.53

Intercept= 3.74 ± 79.48

LOD= 8.35 ng/g

Peak area at LOD =71.55 cps

S_{xo} = 3.03 ng/g

Appendix K: Recovery Studies and Quality control data for CCV and ICV check calibration standard recovery and their accuracy range

APPENDIX K1 :SUMMARY OF PBDE RECOVERY STUDIES ON RIVER MOLE WATER SAMPLES By RUN 3 IDMS [SEMDIDMS]

	ng/kg PBDE					
	BDE-28	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**
Bottle A 1	0.982	0.979	0.982	0.998	0.977	0.963
Bottle A 2	1.002	0.998	1.001	1.018	0.996	0.982
Bottle A 3	1.021	1.018	1.021	1.038	1.016	1.001
0	0.000	0.000	0.000	0.000	0.000	0.000
0	0.000	0.000	0.000	0.000	0.000	0.000
0	0.000	0.000	0.000	0.000	0.000	0.000
	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154
mean	1.002	0.998	1.001	1.018	0.996	0.982
Max	1.021	1.018	1.021	1.038	1.016	1.001
min	0.982	0.979	0.982	0.998	0.977	0.963

Recovery Studies

Sample 1	101	106	106	102	107	101
Sample 2	99	103	99	100	94	99
Sample 3	99	95	105	98	106	99
Sample 4						
Sample 5						
Sample 6						
	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154
mean	100	101	103	100	103	100
Max	101	106	106	102	107	101
Min	99	95	99	98	94	99

U% : Uncertainty measurement

	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154
Sample 1	11	8	14	5	13	5
Sample 2	10	6	15	4	21	7
Sample 3	13	6	14	6	17	3
Sample 4						
Sample 5						
Sample 6						
	13	8	15	6	21	7
	7	4	8	3	11	4

BROMINE CONCENTRATION IN ng/Kg

	ng/kg Br					
	BDE-28	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**
Bottle 1	0.579	0.644	0.695	0.706	0.728	0.717
Bottle 2	0.590	0.657	0.709	0.720	0.742	0.731
Bottle 3	0.602	0.670	0.722	0.734	0.757	0.746
mean	0.590	0.657	0.709	0.720	0.742	0.732
Max	0.602	0.670	0.722	0.734	0.757	0.746
min	0.579	0.644	0.695	0.706	0.728	0.717
%	3.895	3.895	3.895	3.895	3.895	3.895

Summary

	BDE-28	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**
Concentration ng L^{-1}	1.002	0.998	1.001	1.018	0.996	0.982
Recovery range [%]	99 - 101	95 - 106	99 - 106	98 - 102	94 - 107	99 - 101
Maximum U [%]	13	6	15	6	21	7

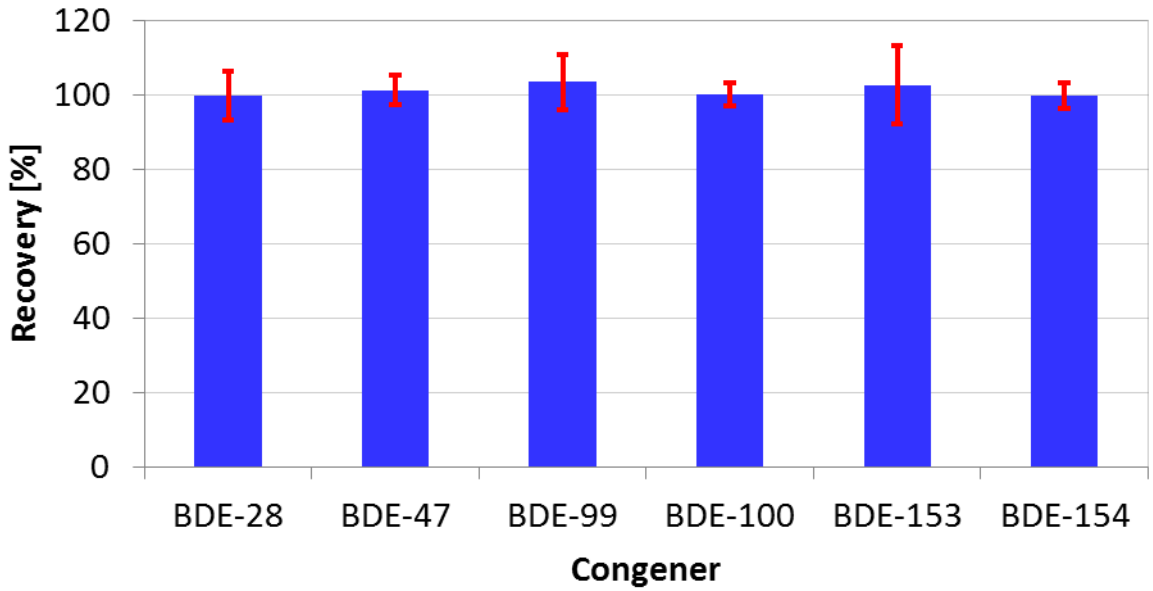


FIGURE 1: PBDE RECOVERY STUDIES IN RIVER MOLE WATER SAMPLES

B: SUMMARY OF PBDE RECOVERY STUDIES ON RIVER MOLE WATER SAMPLES By RUN 1 ext [External calibration]

Concentration of PBDE in [ng/kg]				**m/z 79 Br**		
	BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Bottle 1	0.648	0.500	0.446	0.577	**0.357**	0.507
Bottle 2	0.742	0.765	0.740	0.797	**0.653**	0.693
Bottle 3	0.699	0.606	0.489	0.654	**0.495**	0.572
Mean	0.696	0.624	0.558	0.676	0.502	0.591
Stdev	0.047	0.133	0.159	0.111	0.148	0.095
SEM	0.027	0.077	0.092	0.064	0.086	0.055
RSD%	6.739	21.375	28.410	16.476	29.519	15.998
Mean	0.696	0.624	0.558	0.676	0.502	0.591
Max	0.742	0.765	0.740	0.797	0.653	0.693
Min	0.648	0.500	0.446	0.577	0.357	0.507

Recovery Studies [%] of m/z 79 PBDE in Spiked River Mole Water sample by external calibration [Run 1 ext]

m/z 79 Br PBDEs						
	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154
Bottle 1	65	53	46	58	36	52
Bottle 2	74	75	75	79	65	70
Bottle 3	71	61	51	67	51	59
Mean	70	63	57	68	51	60
Stdev	5	11	16	11	15	9
SEM	3	6	9	6	8	5
RSD%	6	17	27	16	29	15
Mean	70	63	57	68	51	60
Max	74	75	75	79	65	70
Min	65	53	46	58	36	52

Concentration of m/z 81 Br PBDE in [ng/kg]

	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154	BDE 28
Bottle 1	0.599	0.589	0.448	0.603	0.346	0.500	0.599
Bottle 2	0.718	0.797	0.687	0.816	0.658	0.709	0.718
Bottle 3	0.687	0.679	0.527	0.697	0.476	0.582	0.687
Mean	0.668	0.688	0.554	0.705	0.494	0.597	0.668
Stdev	0.062	0.104	0.122	0.107	0.157	0.106	0.062
SEM	0.036	0.060	0.070	0.062	0.090	0.061	0.036
RSD%	9.245	15.145	22.017	15.150	31.739	17.684	9.245
Mean	0.668	0.688	0.554	0.705	0.494	0.597	0.668
Max	0.718	0.797	0.687	0.816	0.658	0.709	0.718
Min	0.599	0.589	0.448	0.603	0.346	0.500	0.599

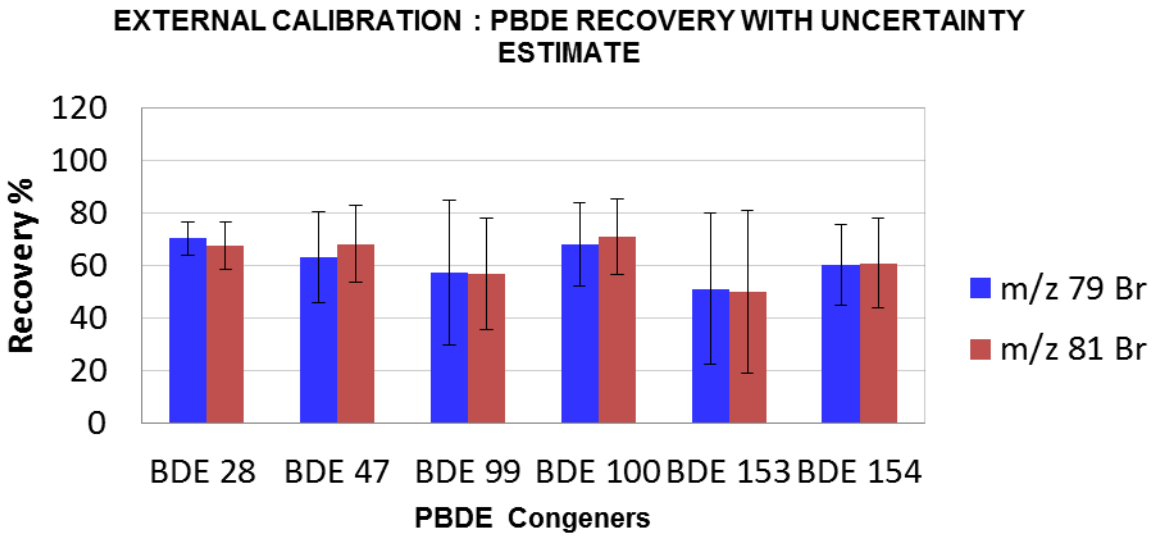
Recovery Studies [%] of m/z 81 PBDE in Spiked River Mole Water sample by external calibration [Run 1 ext]

m/z 81 Br	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154
Bottle 1	**60**	**58**	**46**	**60**	**35**	**51**
Bottle 2	72	78	70	81	66	72
Bottle 3	70	68	55	71	49	60
Mean	67	68	57	71	50	61
Stdev	6	10	12	10	16	10
SEM	4	6	7	6	9	6
RSD%	9	15	21	15	31	17
Mean	67	68	57	71	50	61

Max	72	78	70	81	66	72
Min	60	58	46	60	35	51

SUMMARY

m/z 79 Br	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Concentration ng L^{-1}[n=3]	**0.696**	**0.624**	**0.558**	**0.676**	**0.502**	**0.591**
Recovery range [%]	**65 – 74**	**53 – 75**	**46 - 75**	**58 – 79**	**36-65**	**52 -70**
% RSD	6	17	27	16	29	15



Recoveries and uncertainties of m/z 79 and m/z 81 Br in PBDE congeners in PBDE spiked river mole water samples by Run 1 ext.

C: SUMMARY OF PBDE RECOVERY STUDIES ON RIVER MOLE WATER SAMPLES By RUN 2 ext [External calibration]

Concentration of PBDE in [ng/kg] m/z 79 Br	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Bottle 1	1.432	1.512	1.082	1.066	0.676	0.665
Bottle 2	1.514	1.549	1.406	1.377	1.231	1.187
Bottle 3	1.26	1.313	1.228	1.187	1.162	1.089
Mean	1.402	1.458	1.239	1.210	1.023	0.980
Stdev	0.130	0.127	0.162	0.157	0.302	0.277
SEM	0.075	0.073	0.094	0.091	0.175	0.160
RSD%	9.246	8.706	13.100	12.956	29.568	28.301
Mean	1.402	1.458	1.239	1.210	1.023	0.980
Max	1.514	1.549	1.406	1.377	1.231	1.187
Min	1.260	1.313	1.082	1.066	0.676	0.665

Recovery Studies [%] of m/z 79 PBDE in Spiked [Run River Mole Water sample by external calibration [Run 2 ext]

Percentage recovery	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Bottle 1	142	147	109	106	67	67
Bottle 2	153	153	144	139	125	121
Bottle 3	127	129	125	119	117	111
Mean	141	143	126	121	103	100
Stdev	13	12	18	17	31	29
SEM	8	7	10	10	18	17
RSD%	9	9	14	14	30	29
Mean	141	143	126	121	103	100
Max	153	153	144	139	125	121
Min	127	129	109	106	67	67

m/z 81 Br PBDE Concentration in [ng/kg]

Concentration of PBDE in [ng/kg] m/z 81 Br						
	BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Bottle 1	1.432	1.499	1.161	0.792	0.74	0.66
Bottle 2	1.498	1.525	1.49	1.025	1.434	1.199
Bottle 3	1.2	1.307	1.29	0.859	1.32	1.096
Mean	1.377	1.444	1.314	0.892	1.165	0.985
Stdev	0.157	0.119	0.166	0.120	0.372	0.286
SEM	0.090	0.069	0.096	0.069	0.215	0.165
RSD%	11.369	8.248	12.619	13.448	31.954	29.049
Mean	1.377	1.444	1.314	0.892	1.165	0.985
Max	1.498	1.525	1.490	1.025	1.434	1.199
Min	1.200	1.307	1.161	0.792	0.740	0.660

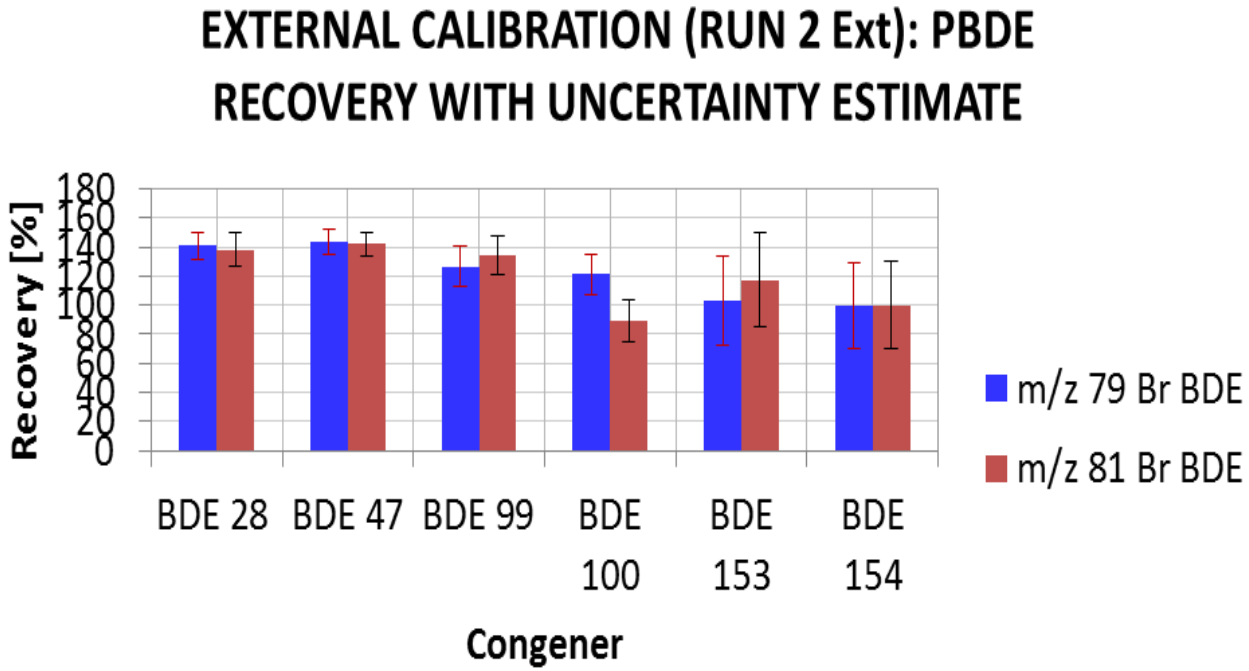
Recovery Studies [%] of m/z 81 PBDE in Spiked [Run River Mole Water sample by external calibration [Run 2 ext]

Percentage recovery	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Bottle 1	142	146	117	78	74	66
Bottle 2	151	151	153	103	145	122
Bottle 3	121	129	132	86	133	111
Mean	138	142	134	89	117	100
Stdev	16	12	18	13	38	30
SEM	9	7	10	7	22	17
RSD%	11	8	13	14	33	30
Mean	138	142	134	89	117	100
Max	151	151	153	103	145	122
Min	121	129	117	78	74	66

SUMMARY	79 Br					
Congeners	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Concentration ng L^{-1}	1.402	1.458	1.239	1.210	1.023	0.980
Recovery range [%]	127 - 153	129 -153	109 -144	106 - 139	67 - 125	67 – 121

SUMMARY	81 Br					
Congeners	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Concentration ng L^{-1}	1.377	1.444	1.314	0.892	1.165	0.985
Recovery range [%]	121 - 151	129 - 151	117 - 153	78 - 103	74 - 145	66 - 122

RSD %	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
79 Br	9	9	14	14	30	29
81 Br	11	8	13	14	33	30



Recoveries and uncertainties of m/z 79 and m/z 81 Br in PBDE congeners in PBDE spiked river mole water samples by Run 2 ext. {external calibration}

D: SUMMARY OF PBDE RECOVERY STUDIES ON RIVER MOLE WATER SAMPLES By RUN 2 ext/int [using 1,1 dibromocyclohexane as internal standard]

Concentration of PBDE in [ng/kg] m/z 79 Br

m/z 79 Br [ng/kg]	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154
Bottle 1	**1.16711**	**1.233**	0.909	**0.858**	**0.542**	**0.545**
Bottle 2	**1.18989**	**1.215**	1.134	**1.06**	**0.963**	**0.938**
Bottle 3	**1.037782**	**1.081**	1.043	**0.964**	**0.955**	**0.906**
Mean	1.132	1.176	1.029	0.961	0.820	0.796
Stdev	0.082	0.083	0.113	0.101	0.241	0.218
SEM	0.047	0.048	0.065	0.058	0.139	0.126
RSD%	7.250	7.060	11.003	10.518	29.364	27.407
Mean	1.132	1.176	1.029	0.961	0.820	0.796
Max	1.190	1.233	1.134	1.060	0.963	0.938
Min	1.038	1.081	0.909	0.858	0.542	0.545

Recovery Studies [%] of m/z 79 PBDE in Spiked [Run River Mole Water sample by internal standard [Run 2 ext/int]

Percentage recovery	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Bottle 1	**116**	**120**	**92**	**85**	**54**	**55**
Bottle 2	**120**	**120**	**116**	**107**	**98**	**96**
Bottle 3	**105**	**107**	**107**	**97**	**96**	**92**
Mean	114	116	105	96	83	81
Stdev	8	8	12	11	25	23
SEM	5	5	7	6	14	13
RSD%	7	7	12	11	30	28
Mean	114	116	105	96	83	81
Max	120	120	116	107	98	96
Min	105	107	92	85	54	55

m/z 81 Br PBDE Concentration in [ng/kg]

Concentration of PBDE in [ng/kg] m/z 81 Br	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154
Bottle 1	**1.163**	**1.242**	**0.961**	**0.852**	**0.609**	**0.548**
Bottle 2	**1.187**	**1.235**	**1.207**	**1.088**	**1.168**	**0.979**
Bottle 3	**1.003**	**1.117**	**1.104**	**0.96**	**1.138**	**0.95**
Mean	1.118	1.198	1.091	0.967	0.972	0.826
Stdev	0.100	0.070	0.124	0.118	0.314	0.241
SEM	0.058	0.041	0.071	0.068	0.182	0.139
RSD%	8.950	5.863	11.327	12.221	32.361	29.177
Mean	1.118	1.198	1.091	0.967	0.972	0.826
Max	1.187	1.242	1.207	1.088	1.168	0.979
Min	1.003	1.117	0.961	0.852	0.609	0.548

Recovery Studies [%] of m/z 81 PBDE in Spiked [Run River Mole Water sample by internal standard [Run 2 ext/int]

Percentage recovery	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Bottle 1	**115**	**121**	**97**	**84**	**61**	**55**
Bottle 2	**120**	**122**	**124**	**110**	**118**	**100**
Bottle 3	**101**	**110**	**113**	**96**	**115**	**97**
Mean	112	118	111	97	98	84
Stdev	10	7	14	13	32	25
SEM	6	4	8	7	19	14
RSD%	9	6	12	13	33	30
Mean	112	118	111	97	98	84
Max	120	122	124	110	118	100
Min	101	110	97	84	61	55

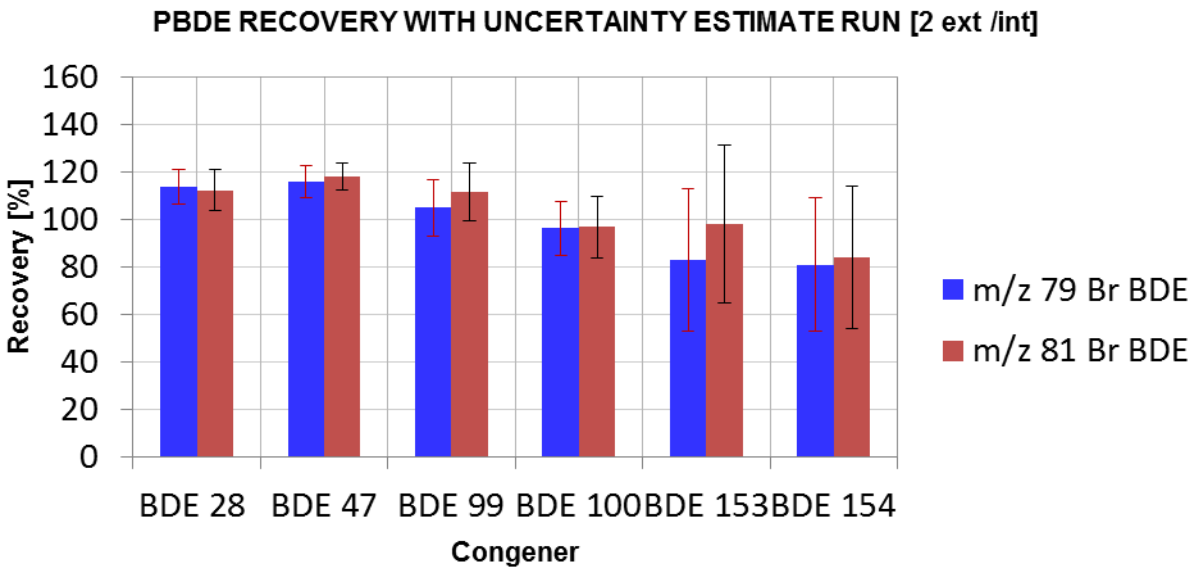
Summary of result

SUMMARY	79 Br					
	BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Concentration ng L^{-1}	1.132	1.176	1.029	0.961	0.820	0.796
Recovery range [%]	105 - 120	107- 120	92 - 116	85 - 107	54 - 98	55 - 96

	81 Br					
	BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
Concentration ng L^{-1}	1.118	1.198	1.091	0.967	0.972	0.826
Recovery range [%]	101 - 120	110 - 122	97 -124	84 - 110	61 - 118	55 – 100

%	**Percentage Recovery**					
	BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
79 Br	114	116	105	96	83	81
81 Br	112	118	111	97	98	84

RSD%	RSD%					
79 Br	7	7	12	11	30	28
81 Br	9	6	12	13	33	30



Recoveries and uncertainties of m/z 79 and m/z 81 Br in PBDE congeners in PBDE spiked river mole water samples

by Run 2 ext. {external calibration}

Appendix K2: RUN 1 Exterenal Calibration QC Data for m/z 79-Br BDE Congeners

m/z 79 Br BDE-28	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
mean, P	85.7	96.24	105.6
Max	104.1	114.98	119.2
Min	84.5	82.80	98.1
Stdev, Sp	6.026	10.41	
P -2sp	73.66	75.43	**Accuracy assessment recovery range**
P+2sp	97.77	117.05	

m/z 79 Br BDE -47	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	80.5	91.8	102.1
Max	99.2	108.7	115.9
Min	83.2	83.2	85.4
Stdev, Sp	6.19	8.80	
P -2sp	68.09	74.21	**Accuracy assessment recovery range**
P+2sp	92.86	109.39	

m/z 79 Br BDE- 100	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean , P	91.8	102.4	112.4
Max	112.7	117.1	126.5
Min	91.6	91.6	90.9
Stdev, Sp	7.30	8.69	
P -2sp	77.25	84.98	**Accuracy assessment recovery range**
P+2sp	106.43	119.73	

m/z 79 Br BDE-99	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean,P	92.9	98.8	112.2
Max	121.0	113.8	131.3
Min	86.6	86.6	95.7
Stdev, Sp	11.68	9.21	
P -2sp	69.58	80.33	**Accuracy assessment recovery range**
P+2sp	116.30	117.18	

m/z 79 Br BDE-154	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	80.3	88.0	99.9
Max	104.0	99.0	115.2
Min	77.8	77.8	84.3
Stdev, Sp	9.20	7.71	
P -2sp	61.95	72.55	**Accuracy assessment recovery range**
P+2sp	98.73	103.40	

m/z 79 Br BDE-153	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	91.2	96.4	109.9
Max	110.1	108.8	124.5
Min	86.8	86.8	90.9
Stdev, Sp	9.16	5.84	
P -2sp	72.84	84.76	**Accuracy assessment recovery range**
P+2sp	109.48	108.13	

Appendix K3: RUN 2 External Calibration QC Data for m/z 79-Br BDE Congeners

m/z79 Br BDE 28	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	124.8	135.2	106.7
Max	153.7	147.2	124.2
Min	101.8	115.4	94.3
Stdev, Sp	18.85	12.91	
P -2sp	87.09	109.36	**Accuracy assessment recovery range**
P+2sp	162.50	160.99	

m/z 79 Br BDE-47	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	119.4	135.9	95.8
Max	131.6	148.9	126.4
Min	104.0	119.4	-1.2
Stdev, Sp	10.70	11.58	
P -2sp	98.01	112.73	**Accuracy assessment recovery range**
P+2sp	140.81	159.05	

m/z 79 Br BDE-100	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	118.8	132.2	107.3
Max	126.5	147.5	120.4
Min	106.8	121.3	92.5
Stdev, Sp	8.31	10.06	
P -2sp	102.18	112.06	**Accuracy assessment recovery range**
P+2sp	**135.44**	**152.29**	

m/z 79 Br BDE-99	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
mean, P	117.5	123.5	105.0
Max	126.1	136.1	127.4
Min	103.8	106.5	81.9
Stdev, Sp	8.91	11.39	
P -2sp	99.68	100.71	**Accuracy assessment recovery range**
P+2sp	135.31	146.26	

m/z 79 Br BDE-154	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean ,P	110.6	124.9	107.2
Max	124.4	141.1	130.5
Min	98.7	112.0	85.9
Stdev, Sp	10.92	11.09	
P -2sp	88.73	102.71	**Accuracy assessment recovery range**
P+2sp	132.41	147.07	

m/z 79 Br BDE-153	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	115.3	128.1	108.1
Max	136.3	146.1	136.9
Min	97.4	100.5	88.4
Stdev, Sp	17.07	17.09	
P -2sp	81.17	93.88	**Accuracy assessment recovery range**
P+2sp	149.45	162.26	

Appendix K4: RUN 2 Ext/int [Internal standard] QC Data for m/z79-Br BDE Congeners

m/z 79 Br BDE-28	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
mean, P	110.4	121.3	105.8
Max	122.7	140.3	114.3
Min	95.2	105.5	97.9
Stdev, Sp	11.45	12.58	
P -2sp	87.50	96.16	**Accuracy assessment recovery range**
P+2sp	133.30	146.46	

m/z 79 Br BDE-47	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	105.9	121.8	**95.6**
Max	118.1	133.9	124.0
Min	97.3	109.2	-3.9
Stdev, Sp	8.19	9.15	
P -2sp	89.51	103.47	**Accuracy assessment recovery range**
P+2sp	122.28	140.06	

m/z 79 Br BDE-100	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
mean, P	104.5	117.1	108.0
Max	116.8	127.5	133.1
Min	96.2	106.9	97.3
Stdev, Sp	8.50	9.16	
P -2sp	87.51	98.74	**Accuracy assessment recovery range**
P+2sp	121.48	135.40	

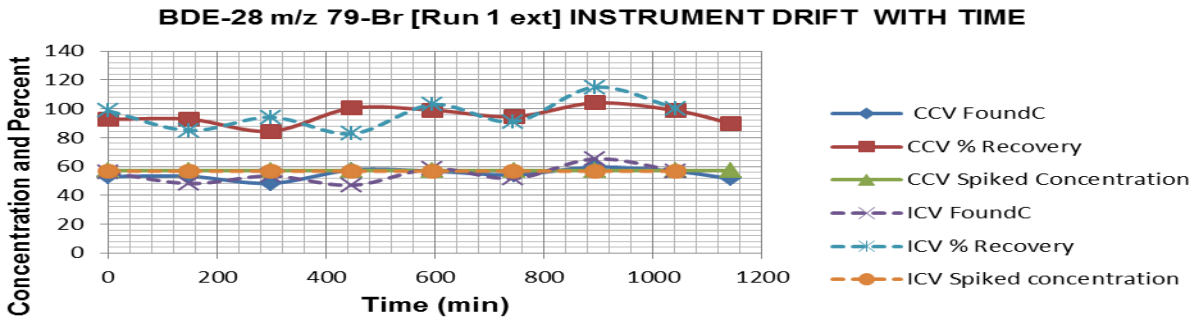
m/z 79 Br BDE-99	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	107.8	114.2	108.7
Max	118.1	123.2	134.1
Min	98.8	100.8	90.2
Stdev, Sp	8.16	8.51	
P -2sp	91.47	97.14	**Accuracy assessment recovery range**
P+2sp	124.09	131.18	

m/z 79 Br BDE-154	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean,P	99.5	113.3	108.6
Max	111.5	125.4	134.3
Min	84.4	103.6	92.5
Stdev, Sp	10.59	10.52	
P -2sp	78.32	92.31	**Accuracy assessment recovery range**
P+2sp	120.68	134.38	

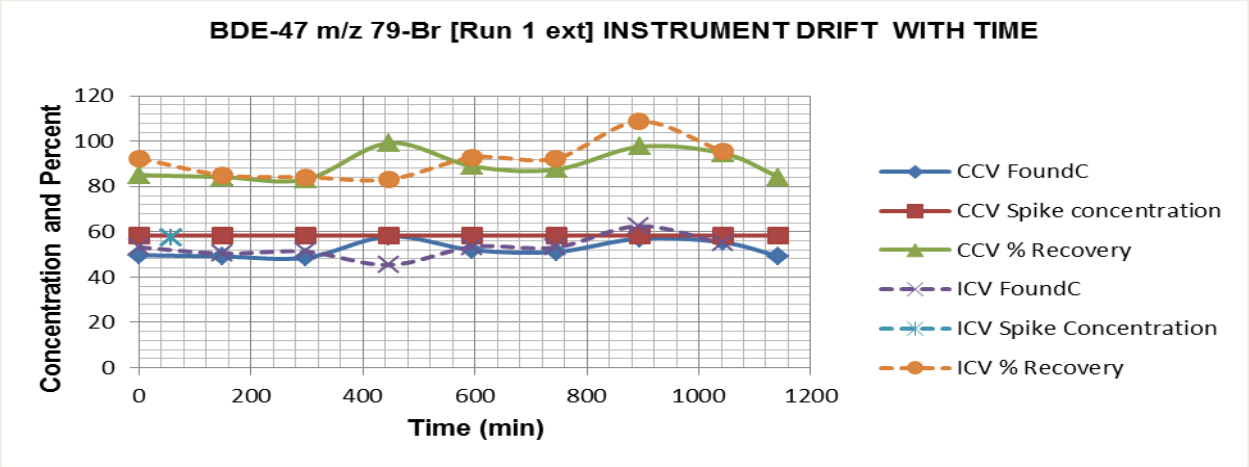
m/z 79 Br BDE-153	CCV Recoveries	ICV Recoveries	Calibration Standard Recoveries
Mean, P	102.0	114.4	106.5
Max	126.1	130.4	134.2
Min	86.1	91.3	91.5
Stdev, Sp	15.48	16.09	
P -2sp	71.06	82.23	**Accuracy assessment recovery range**
P+2sp	132.96	146.59	

Appendix L: Graphical representation of the effect of Instrument drift , instability on PBDE Congener spike recoveries in CCV and ICV check standards [Run 1 ext]

BDE 28 m/z 79 Br	Interval	CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time,min
1	0	53.1	57.3	92.7	56.0	56.8	98.6	0
2	149	53.2	57.3	92.8	48.2	56.8	85.0	149
3	298	48.4	57.3	84.5	53.5	56.8	94.2	298
4	447	57.6	57.3	100.5	47.0	56.8	82.8	447
5	596	56.8	57.3	99.1	58.5	56.8	103.0	596
6	745	54.2	57.3	94.7	51.7	56.8	91.0	744
7	894	59.7	57.3	104.1	65.3	56.8	115.0	893
8	1043	56.6	57.3	98.8	57.0	56.8	100.4	1043
9	1142	51.6	57.3	90.0				

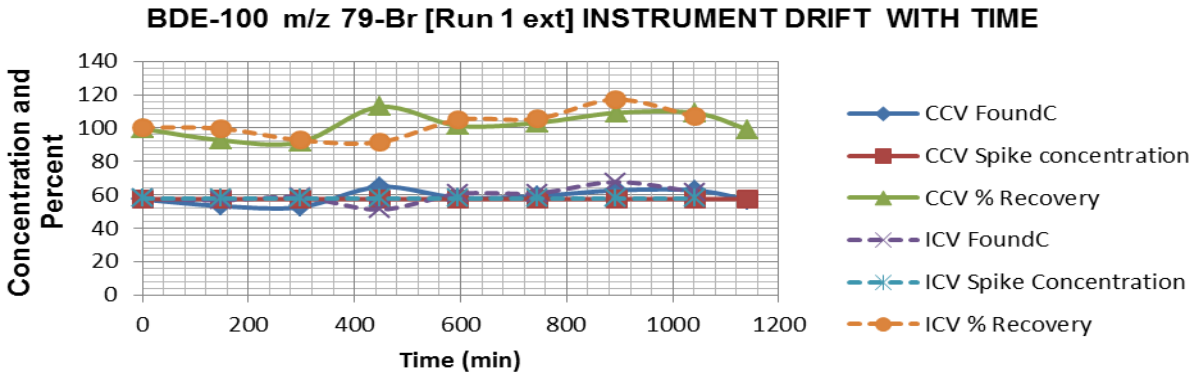


BDE -47 m/z 79 Br	Interval	CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time,min
1	0	49.7	58.4	85.1	53.2	57.5	92.5	0
2	149	49.2	58.4	84.2	50.4	57.5	85.1	149
3	298	48.6	58.4	83.2	51.3	57.5	84.2	298
4	447	58.0	58.4	99.2	45.4	57.5	83.2	447
5	596	52.0	58.4	88.9	53.4	57.5	92.9	596
6	745	51.2	58.4	87.5	53.1	57.5	92.4	744
7	894	57.0	58.4	97.6	62.5	57.5	108.7	893
8	1043	55.3	58.4	94.7	54.9	57.5	95.5	1043
9	1142	49.2	58.4	84.3				

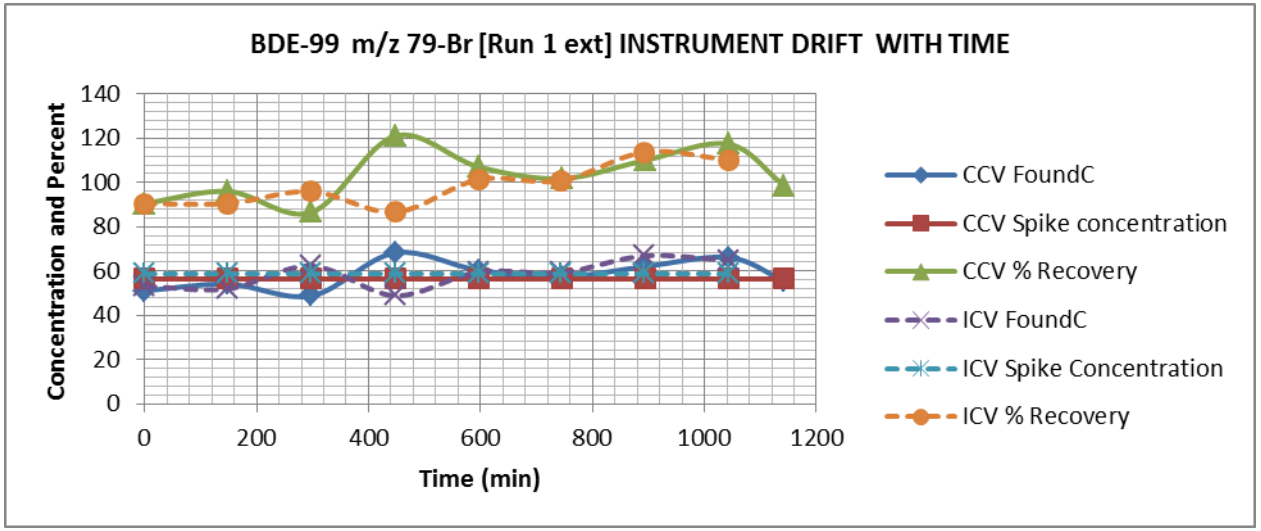


BDE-100 m/z 79 Br

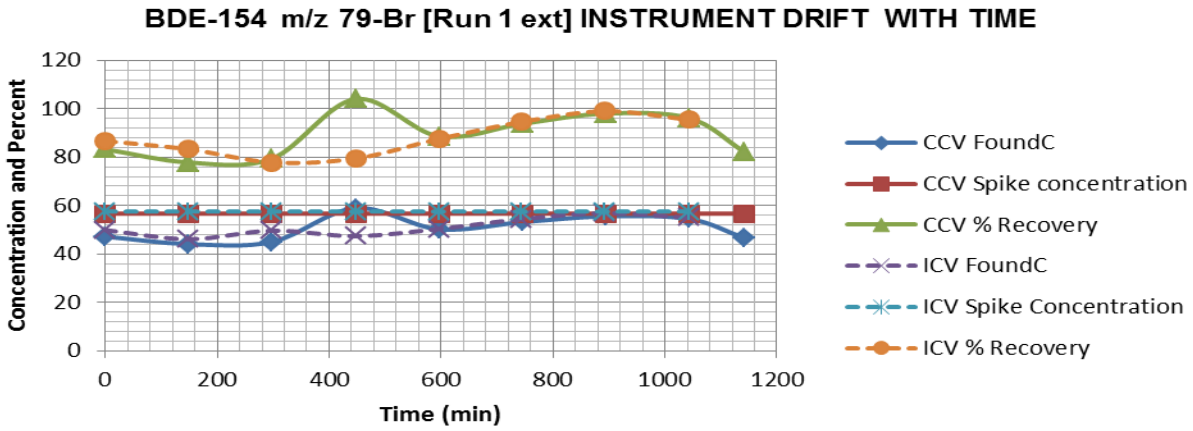
	Interval	CCV			ICV			Interval
S/N	Time,min	Found	Spike	% Recov	Found	Spike	% Recov	Time,min
1	0	57.274	57.4601	99.6761	57.9936	57.7407	100.438	0
2	149	53.3	57.5	92.7	56.7	57.7	99.7	149
3	298	52.6	57.5	91.6	58.6	57.7	92.7	298
4	447	64.8	57.5	112.7	51.1	57.7	91.6	447
5	596	58.2	57.5	101.4	60.6	57.7	104.9	596
6	745	59.3	57.5	103.2	61.0	57.7	105.6	744
7	894	62.7	57.5	109.2	67.6	57.7	117.1	893
8	1043	62.6	57.5	109.0	61.7	57.7	106.8	1043
9	1142	56.9	57.5	99.0				



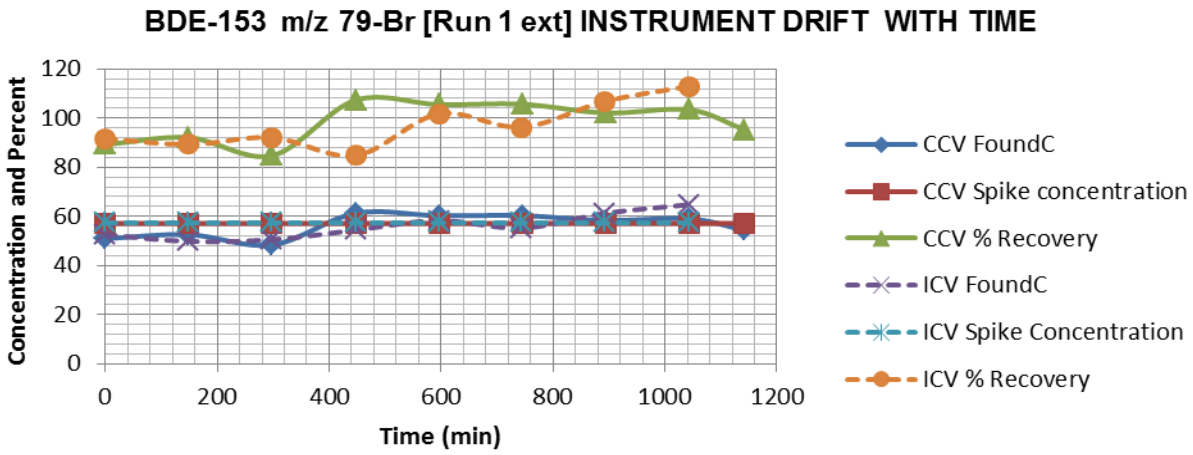
BDE-99 m/z 79 Br	Interval	CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time,min
1	0	51.1	56.4	90.6	53.2	58.8	90.5	0
2	149	54.1	56.4	96.0	52.0	58.8	90.6	149
3	298	48.8	56.4	86.6	62.3	58.8	96.0	298
4	447	68.2	56.4	121.0	48.9	58.8	86.6	447
5	596	60.4	56.4	107.1	59.6	58.8	101.4	596
6	745	57.4	56.4	101.8	59.3	58.8	100.9	744
7	894	62.1	56.4	110.1	66.9	58.8	113.8	893
8	1043	66.2	56.4	117.4	64.8	58.8	110.3	1043
9	1142	55.6	56.4	98.7				



BDE-154 m/z 79 Br		Interval	CCV			ICV			Interval
	S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time,min
	1	0	47.2	56.7	83.3	49.9	57.5	86.7	0
	2	149	44.1	56.7	77.8	46.1	57.5	83.3	149
	3	298	45.0	56.7	79.4	49.7	57.5	77.8	298
	4	447	59.0	56.7	104.0	47.4	57.5	79.4	447
	5	596	50.2	56.7	88.6	50.4	57.5	87.5	596
	6	745	53.2	56.7	93.8	54.4	57.5	94.6	744
	7	894	55.6	56.7	98.0	57.0	57.5	99.0	893
	8	1043	54.5	56.7	96.2	54.9	57.5	95.5	1043
	9	1142	46.6	56.7	82.2				

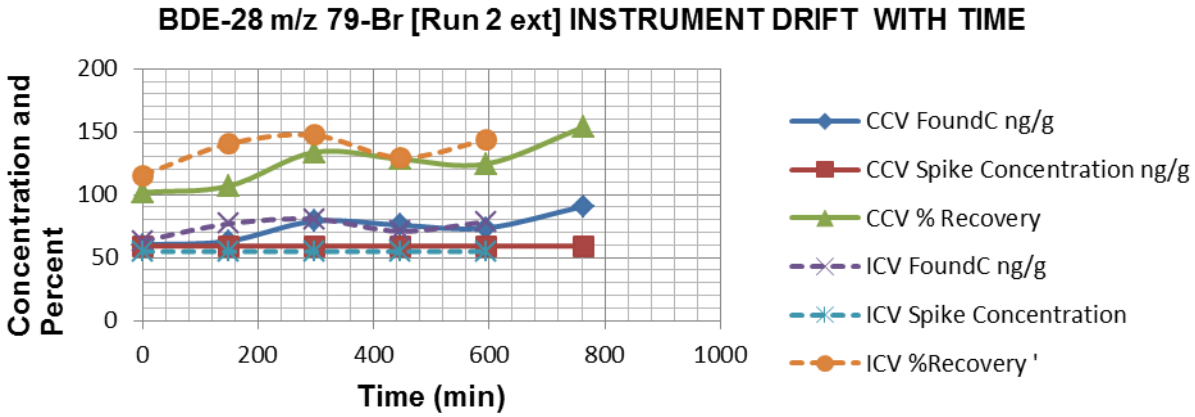


BDE-153 m/z 79-Br		Interval	CCV			ICV			Interval
	S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time,min
	1	0	51.0	57.1	89.2	52.6	57.3	91.7	0
	2	149	52.6	57.1	92.1	49.8	57.3	89.2	149
	3	298	48.4	57.1	84.8	50.5	57.3	92.1	298
	4	447	61.3	57.1	107.3	54.4	57.3	84.8	447
	5	596	60.2	57.1	105.5	58.3	57.3	101.7	596
	6	745	60.3	57.1	105.7	55.2	57.3	96.3	744
	7	894	58.3	57.1	102.1	61.3	57.3	106.8	893
	8	1043	59.0	57.1	103.4	64.8	57.3	113.0	1043
	9	1142	54.4	57.1	95.3				

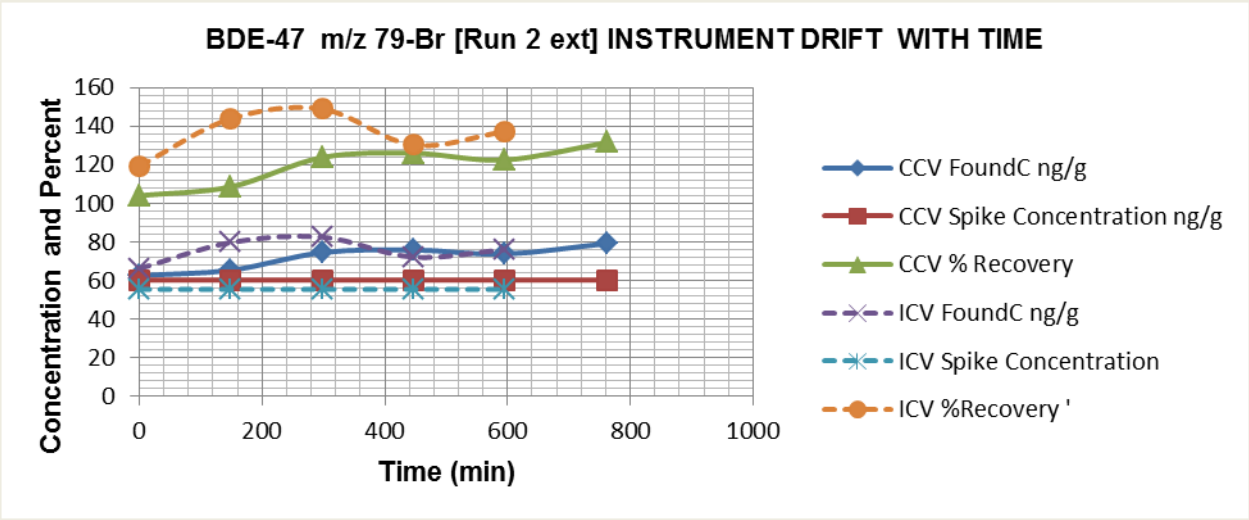


Appendix L2: Graphical representation of the effect of Instrument drift , instability on PBDE Congener spike recoveries in CCV and ICV check standards [Run 2 ext]

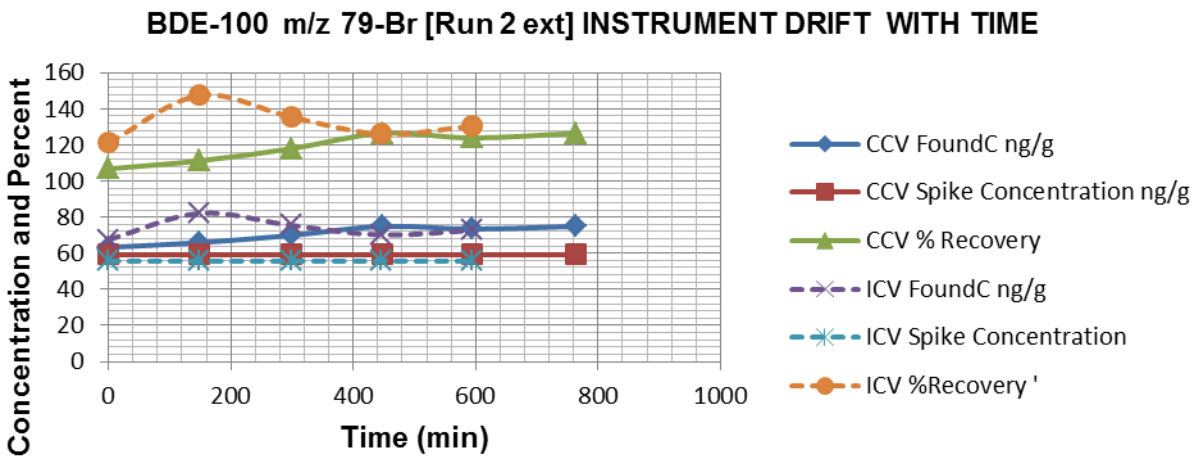
BDE-28 m/z 79 -Br								
	Interval	CCV			ICV			Interval
S/N	Time(min)	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	60.2	59.1	101.8	63.2	54.7	115.4	0
2	149	63.1	59.1	106.8	76.9	54.7	140.4	149
3	298	79.0	59.1	133.7	80.6	54.7	147.2	298
4	447	75.8	59.1	128.2	70.9	54.7	129.4	446
5	595	73.5	59.1	124.5	78.6	54.7	143.5	595
6	763	90.8	59.1	153.7				



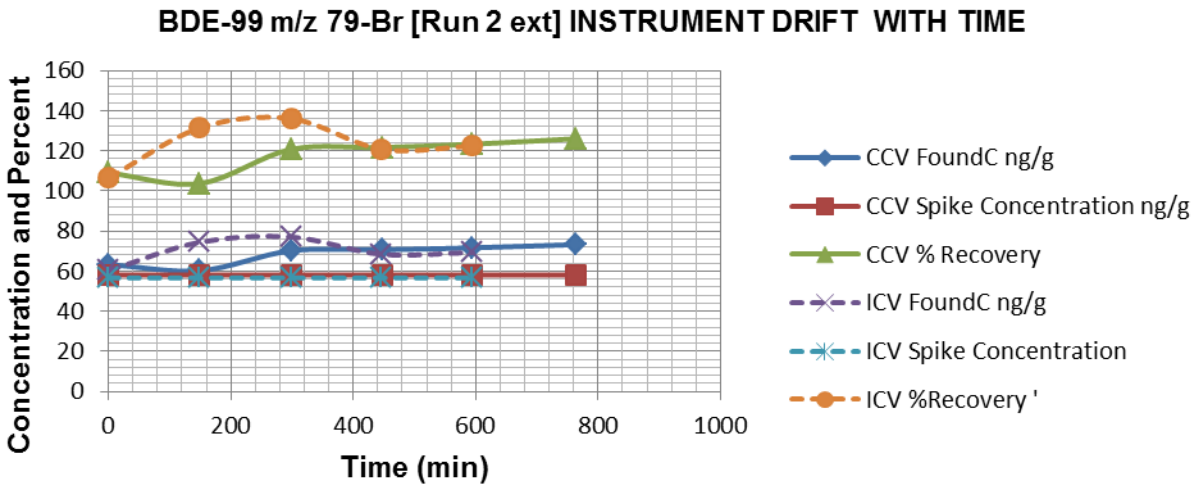
BDE-47 m/z 79-Br								
	Interval	CCV			ICV			Interval
S/N	Time(min)	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	62.7	60.2	104.0	66.3	55.5	119.4	0
2	149	65.4	60.2	108.6	79.8	55.5	143.8	149
3	298	74.6	60.2	123.8	82.6	55.5	148.9	298
4	447	75.9	60.2	126.0	72.2	55.5	130.2	446
5	595	73.8	60.2	122.5	76.1	55.5	137.2	595
6	763	79.3	60.2	131.6				



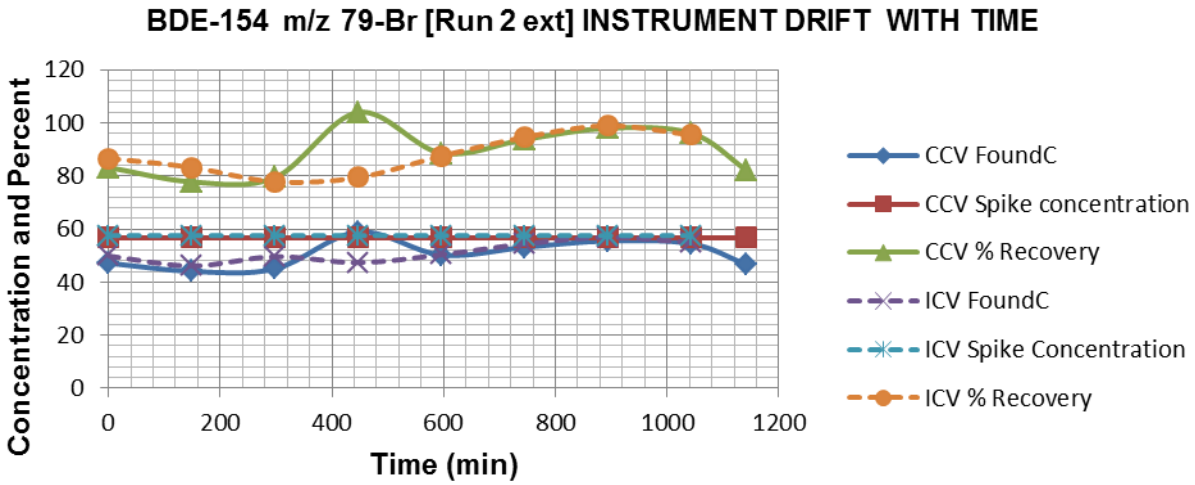
BDE-100 m/z 79-Br									
		Interval	CCV			ICV			Interval
	S/N	Time(min)	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
	1	0	63.3	59.2	106.8	67.5	55.7	121.3	0
	2	149	65.9	59.2	111.2	82.1	55.7	147.5	149
	3	298	69.9	59.2	117.9	75.5	55.7	135.5	298
	4	447	74.9	59.2	126.5	70.2	55.7	126.0	446
	5	595	73.4	59.2	123.9	72.7	55.7	130.5	595
	6	763	74.9	59.2	126.5				



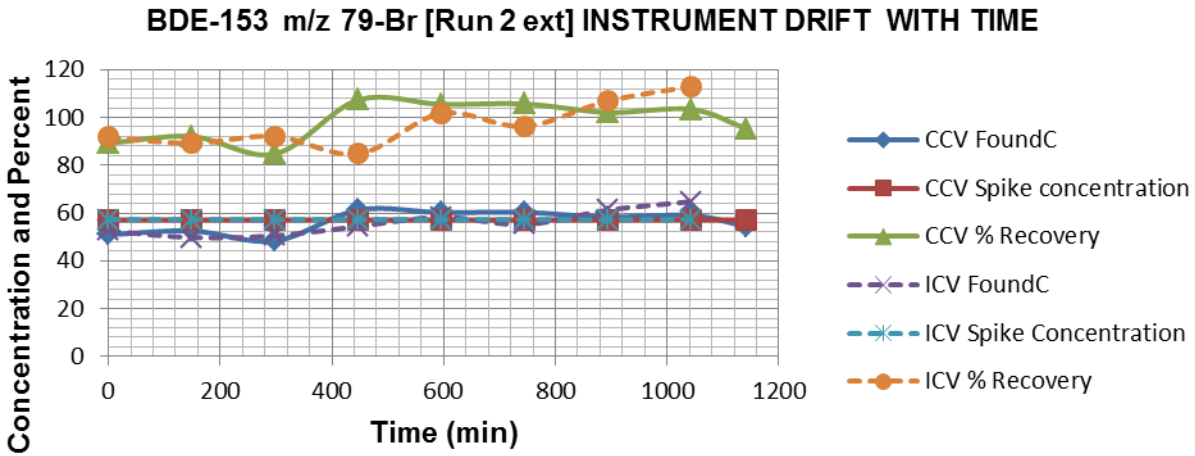
BDE-99 m/z 79-Br		CCV			ICV			Interval
S/N	Time(min)	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, [ng/g]	% Recov	Time, min
1	0	63.4	58.1	109.1	60.4	56.7	106.5	0
2	149	60.3	58.1	103.8	74.5	56.7	131.5	149
3	298	70.2	58.1	120.8	77.1	56.7	136.1	298
4	447	70.8	58.1	121.7	68.5	56.7	120.9	446
5	595	71.7	58.1	123.4	69.4	56.7	122.5	595
6	763	73.3	58.1	126.1				



BDE-154 m/z 79-Br		CCV			ICV			Interval
S/N	Time(min)	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	57.7	58.4	98.7	62.2	55.5	112.0	0
2	149	60.1	58.4	102.8	78.3	55.5	141.1	149
3	298	59.1	58.4	101.2	71.1	55.5	128.2	298
4	447	68.8	58.4	117.7	65.3	55.5	117.8	446
5	595	69.3	58.4	118.6	69.5	55.5	125.3	595
6	763	72.7	58.4	124.4				

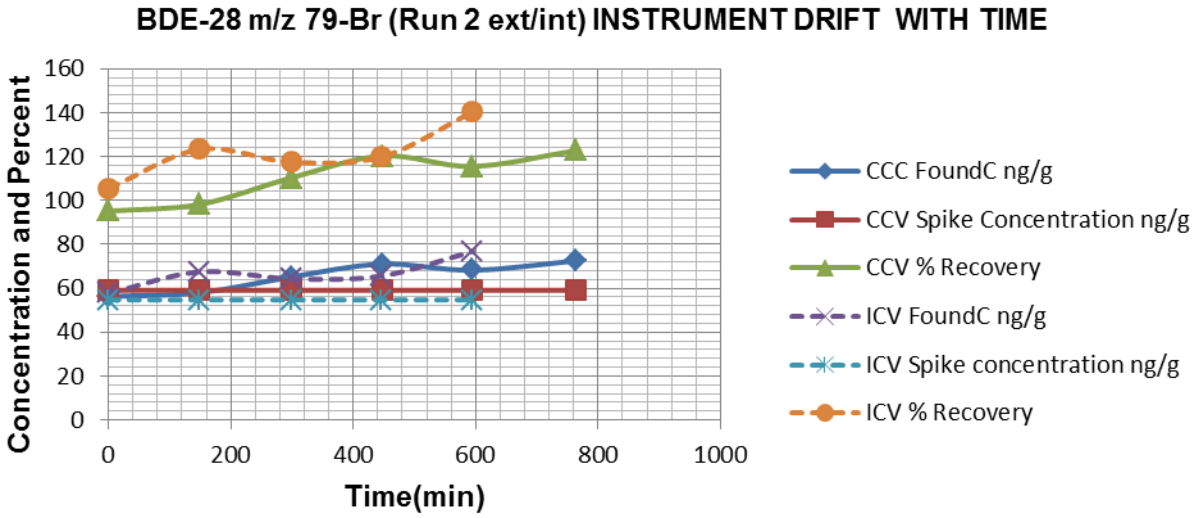


BDE-153 m/z 79-Br		CCV			ICV			Interval
S/N	Time(min)	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	57.3	58.9	97.4	55.6	55.3	100.5	0
2	149	58.7	58.9	99.8	80.8	55.3	146.1	149
3	298	61.8	58.9	104.9	74.1	55.3	134.0	298
4	447	70.7	58.9	120.2	69.2	55.3	125.2	446
5	595	80.3	58.9	136.3	74.4	55.3	134.5	595
6	763	78.4	58.9	133.3				

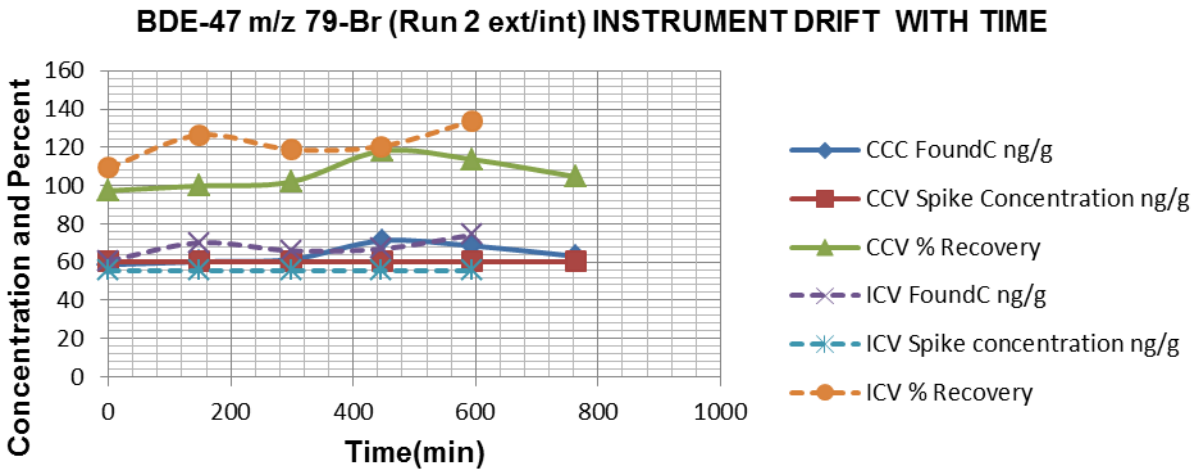


Appendix L3: Graphical representation of the effect of Instrument drift , instability on PBDE Congener spike recoveries in CCV and ICV check standards [Run 2 ext/int]

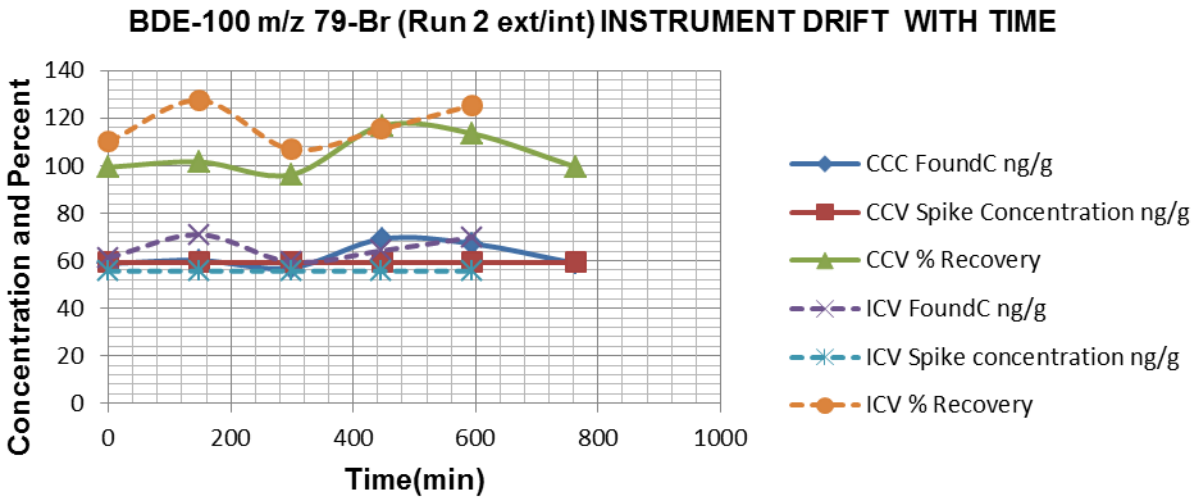
BDE -28 m/z 79 Br	Interval	CCV			ICV			Interval
S/N	Time,min	Found, ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	56.3	59.1	95.2	57.7	54.7	105.5	0
2	149	58.0	59.1	98.2	67.5	54.7	123.3	149
3	298	65.2	59.1	110.3	64.3	54.7	117.5	298
4	447	71.1	59.1	120.3	65.6	54.7	119.9	446
5	595	68.3	59.1	115.6	76.8	54.7	140.3	595
6	763	72.5	59.1	122.7				



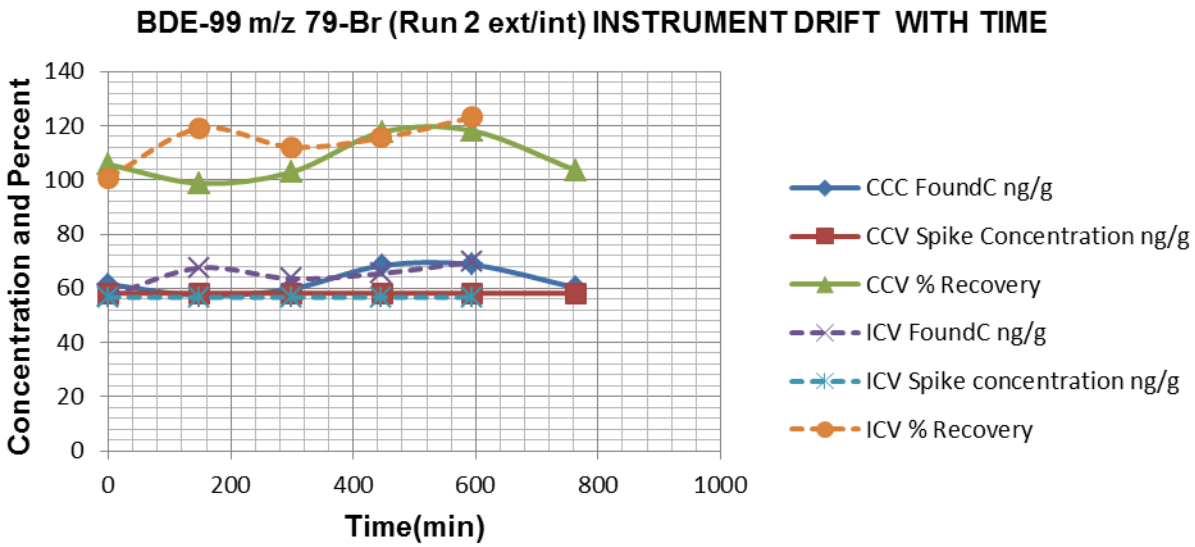
BDE 47 m/z 79 Br	Interval	CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	58.6	60.2	97.3	60.6	55.5	109.2	0
2	149	60.2	60.2	99.9	70.1	55.5	126.4	149
3	298	61.4	60.2	101.9	65.9	55.5	118.9	298
4	447	71.1	60.2	118.1	66.9	55.5	120.5	446
5	595	68.4	60.2	113.6	74.3	55.5	133.9	595
6	763	63.0	60.2	104.6				



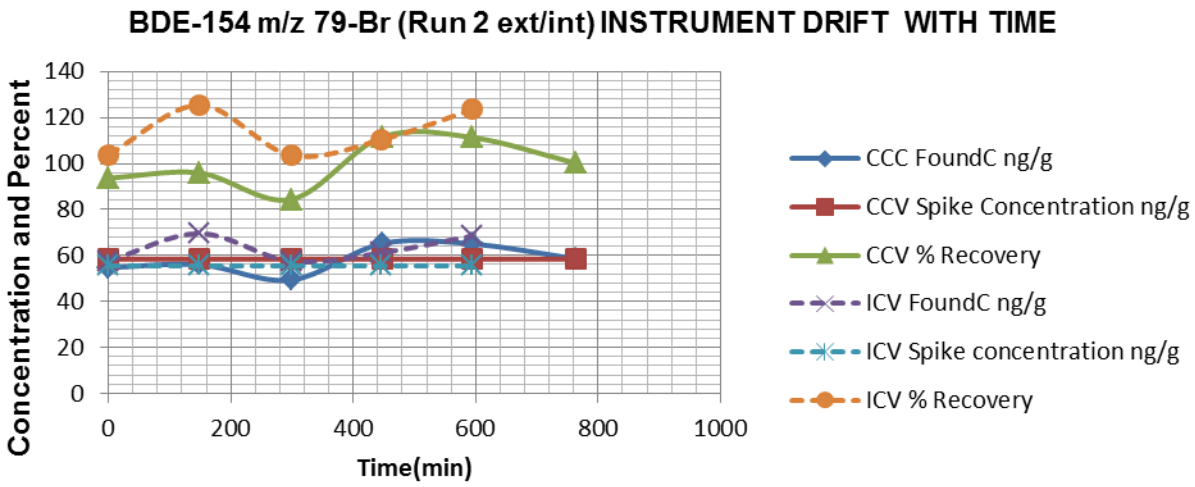
BDE -100 m/z 79 Br		CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	58.9	59.2	99.4	61.3	55.7	110.1	0
2	149	60.2	59.2	101.6	71.0	55.7	127.5	149
3	298	57.0	59.2	96.2	59.5	55.7	106.9	298
4	447	69.2	59.2	116.8	64.2	55.7	115.3	446
5	595	67.2	59.2	113.5	69.9	55.7	125.5	595
6	763	58.9	59.2	99.5				



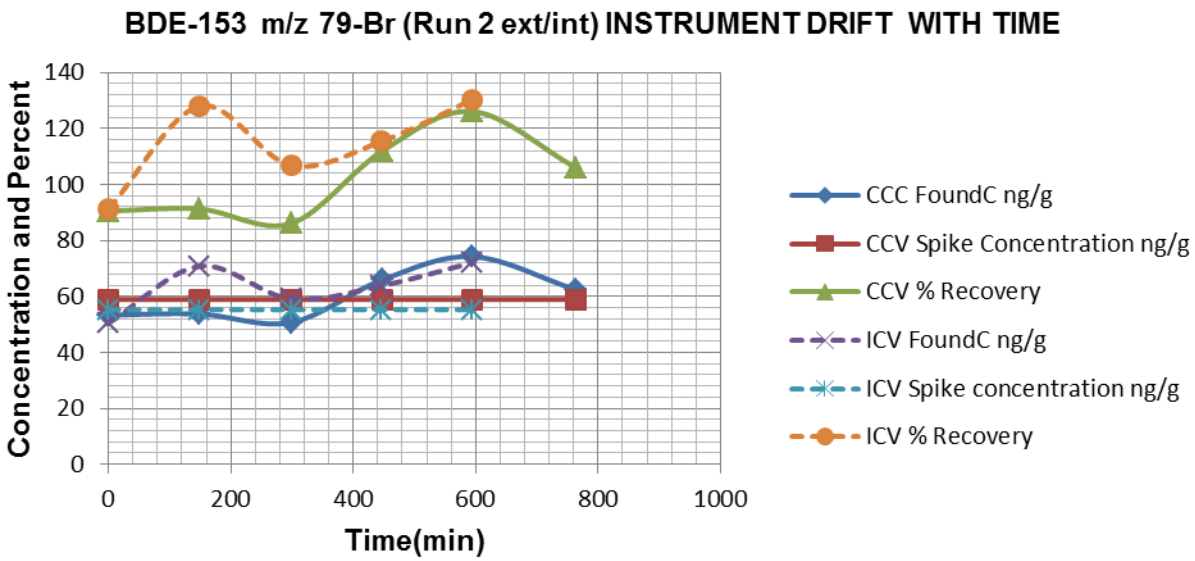
BDE- 99 m/z 79 Br	Interval	CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	61.5	58.1	105.7	57.1	56.7	100.8	0
2	149	57.4	58.1	98.8	67.5	56.7	119.1	149
3	298	59.8	58.1	102.8	63.6	56.7	112.2	298
4	447	68.4	58.1	117.7	65.5	56.7	115.5	446
5	595	68.7	58.1	118.1	69.8	56.7	123.2	595
6	763	60.2	58.1	103.6				



BDE-154 m/z 79 Br		CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	54.7	58.4	93.7	57.7	55.5	103.9	0
2	149	56.0	58.4	95.9	69.6	55.5	125.4	149
3	298	49.3	58.4	84.4	57.5	55.5	103.6	298
4	447	65.2	58.4	111.5	61.2	55.5	110.3	446
5	595	65.1	58.4	111.3	68.5	55.5	123.5	595
6	763	58.6	58.4	100.2				



BDE-153 m/z 79 Br		CCV			ICV			Interval
S/N	Time,min	Found,ng/g	Spike, ng/g	% Recov	Found,ng/g	Spike, ng/g	% Recov	Time, min
1	0	53.3	58.9	90.6	50.5	55.3	91.3	0
2	149	53.7	58.9	91.3	70.8	55.3	128.1	149
3	298	50.7	58.9	86.1	59.1	55.3	106.9	298
4	447	65.9	58.9	112.0	63.8	55.3	115.4	446
5	595	74.2	58.9	126.1	72.1	55.3	130.4	595
6	763	62.4	58.9	106.1				



Appendix M: Professional Development Records

CALMS - Course completion certificate

Page 1 of 1

Course Certificate

LGC Setting standards in analytical science

This is to certify that

Olukayode Babarinde

has completed a course in

First Day Induction

on the 23/05/2014

Achieved: 100%

Level: Pass

https://calms/Frameset/admin/user_cert.asp?userId=7429&cpId=24069&print=yes 23/05/2014

Course Certificate

LGC *Setting standards in analytical science*

This is to certify that

Olukayode Babarinde

has completed a course in

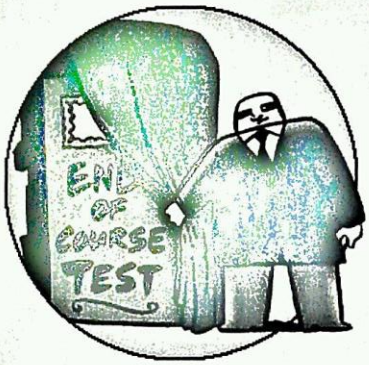
Fire Awareness

on the 23/05/2014

Achieved:	100%
Level:	Pass

Course Certificate

LGC Setting standards in analytical science



This is to certify that

Olukayode Babarinde

has completed a course in

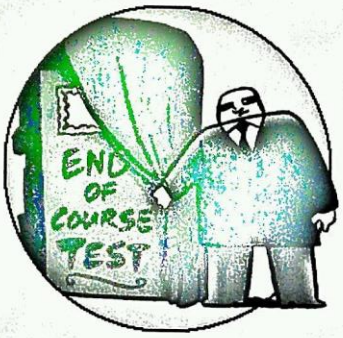
Manual Handling

on the 27/05/2014

Achieved:	100%
Level:	Pass

Course Certificate

LGC Setting standards in analytical science



This is to certify that

Olukayode Babarinde

has completed a course in

LGC Information Classification and the GPMS

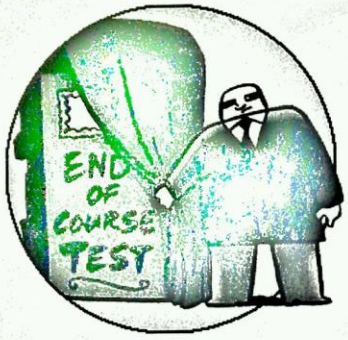
on the 27/05/2014

Achieved: 100%

Level: GOLD

Course Certificate

Setting standards in analytical science



This is to certify that

Olukayode Babarinde

has completed a course in

Information Security

on the 27/05/2014

Achieved:	100%
Level:	GOLD

Course Certificate

Setting standards in analytical science

This is to certify that

Olukayode Babarinde

has completed a course in

Display Screen Equipment

on the 22/05/2014

Achieved:	100%
Level:	Pass

APPENDIX N: SAMPLE PAIRED TEST SPREADSHEET TEMPLATE

BDE-28

Table of value of Paired - T - test

Sample	ai (ng/Kg)	ci (ng/kg)	di = ai - ci	mean d	D=di-mean d	D ²
1	0.982	0.648	0.334	0.336333	-0.00233333	5.44444E-06
2	1.002	0.742	0.26	0.336333	-0.07633333	0.005826778
3	1.021	0.606	0.415	0.336333	0.078666667	0.006188444
Sum		Σd	1.009		ΣD ²	0.012020667

n=	3

$$\bar{X}_d = \frac{\sum d}{n}$$

Xd=	0.336333

$$S_d = \sqrt{\frac{\sum D^2}{n-1}}$$

Sd=	0.077526

$$t = \frac{\bar{X}_d - 0}{S_d / \sqrt{n}}$$

ai= IDMS Concentrations

Ci= Ext .Cal Concentrations
Sd= standard deviation
n-number of paired results

D=,Mean deviation
d=difference between 'ai' and 'ci'

P-value 0.017253736

t-calculated	7.514174

<0.05 , statistically significant difference
>0.05, no statistically significant difference

The number of degrees of freedom, V of t is n-1=3-1=2

V=	**2**

Conclusion

The critical v alue is t_2 = 4.303 (P=0.05) at 95 % confidence limit

The t-calculated value is 7.514174 less than or greater than the critical value. 4.303

If less than t-calculated then the null hypothesis is retained. Then, No significant differences between the two results

If greater than t-calculted then the null hypothesis is rejectect. The two methods gave significantly different results.