PROCEDURES FOR SAMPLE COLLECTION, SAMPLE PREPARATION, AND LC-MS/MS ANALYSIS OF ENDOCRINE DISRUPTING COMPOUNDS IN RIVER WATER

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List of Acronyms and Abbreviations

2-CP	2-Chlorophenol
4-CP-d4	4-Chlorophenol-d4
2,4-DCP	2,4-Dichlorophenol
2,4,6-TCP	2,4,6-Trichlorophenol
ACN	Acetonitrile
ANG	Angat River
BPA	Bisphenol A
DOST-PCIEERD	Department of Science and Technology – Philippine Council for Industry, Energy and Emerging Technology Research Development
E1	Estrone
E2	17-beta-Estradiol
E2-d2	Estradiol-d2
EDCs	Endocrine Disrupting Compounds
EE2	17-alpha-Ethynylestradiol
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GPS	Global Positioning System
H-ESI	Heated Electrospray Ionization
HLB	Hydrophilic-Lipophilic Balance

HPLC	High Performance Liquid Chromatography
IDL	Instrument Detection Limit
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
MeOH	Methanol
MDL	Method Detection Limit
MRK	Marikina River
MRM	Multiple Reaction Monitoring
MSDS	Material Safety Data Sheet
NH_4OH	Ammonium Hydroxide
NP	Nonylphenol or 4-*para*-Nonylphenol
OP	Octylphenol or 4-*tert*-Octylphenol
PCP	Pentachlorophenol
PMP	Pampanga River
PROG	Progesterone
PSG	Pasig River
PTFE	Polytetrafluoroethylene
SD	Standard Deviation
SPE	Solid Phase Extraction
SRM	Selective Reaction Monitoring
TES	Testosterone

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1. Foreword

Endocrine disrupting compounds or EDCs are a variety of natural or synthetic substances that imitate certain physiological hormones causing adverse effects in human and animal health by binding to endocrine receptors and inhibiting or inducing changes in the endocrine system functions. Due to the wide applications of EDCs in medicine and industries, these compounds are now among the emerging water contaminants that reach aquatic environments through runoffs and discharges of untreated wastewaters. To date, the determination of these contaminants in inland water systems is not yet commonly performed in laboratories in the Philippines. Hence, information on the presence of these contaminants and their impacts in the environment are limited.

In this manual, we systematically described the sample collection in urban rivers, sample preparation and extraction, and analytical determination of known EDCs such as the natural and synthetic hormones and industrial phenolic chemicals in the river water samples. The procedures described herein are being used in the ongoing DOST-PCIEERD research on “Post-wastewater treatment determination of Endocrine Disrupting Compounds for interventions (Post-EDCs)” (DOST-GIA Project No. 10324). We hope that this manual will also be useful to government and private laboratories as well as to environmental scientists and researchers, regulators, and policy makers.

2. Materials and Reagents

2.1. Sample Collection

2.1.1. Phosphate free detergent – Liquinox® phosphate-free liquid detergent or equivalent

2.1.2. Purified water or distilled water –Type II (pure water) from PURELAB® Chorus 2 or Absolute Distilled Water or equivalent

2.1.3. Methanol, HPLC-grade – Duksan Reagents High Purity Chemicals or equivalent

2.1.4. Lint-free wipes – Kimtech® Science™ Kimwipes™ Delicate Task Wipes or equivalent

2.1.5. Stainless steel bucket, 1.4 L

2.1.6. Sample bottles, 1000-mL – Wide-mouth amber glass bottles are recommended

2.1.7. Ice chests with 48 qt (45 L) or 58 qt (55 L) capacity – Coleman® or equivalent

2.1.8. Tube ice packs, 5 kg

2.1.9. GPS device – GARMIN® GPSMAP® 78s or equivalent

2.1.10. Field data sheet or notebook

2.1.11. First-aid kit – with bandages, sterile scissors, alcohol, antiseptic, burn ointments, and medicine (e.g. Bonamine, Paracetamol, Meclizine HCl, Loperamide)

2.2. Sample Preparation

2.2.1. Ultrapure water – Type I (ultrapure water) from PURELAB® Flex3 or equivalent

2.2.2. Glass filtration set-up, including 300-mL glass funnel, anodized aluminum clamp, glass support base and tubulated cap, 1000-mL ground joint flask, and silicone fixing sucker or equivalent – Rocker® VF3 Glass Filtration Apparatus Set or equivalent

2.2.3. Oil free vacuum pump – Rocker® 300 Oil Free Vacuum Pump or equivalent

2.2.4. Glass fiber filters, 1.2 µm, 47 mm – Whatman™ 1822-047 Glass Microfiber Filters or equivalent

2.2.5. Glass fiber filters, 0.7 µm, 47 mm – Whatman™ 1825-047 Glass Microfiber Filters or equivalent

2.2.6. Graduated cylinder, 250- or 500-mL – KIMAX® or equivalent

2.2.7. Sample bottles, 1000-mL – Wide-mouth clear glass bottles are recommended

2.2.8. Solid phase extraction vacuum manifold and accessories, including large volume sampler, solvent guide needles, waste trap – Supelco® Visiprep™ Solid Phase Extraction Vacuum Manifold or equivalent

2.2.9. Solid phase extraction cartridge – Waters™ Oasis® HLB (6 mL, 500 mg) or equivalent

2.2.10. Bottle-Top dispenser, 1-10 mL or 2.5-25 mL range – DLAB DispensMate™ Plus or equivalent

2.2.11. Borosilicate glass, 10-mL – PYREX® Borosilicate Glass with graduations or equivalent

2.2.12. Nitrogen gas tank for preconcentration of the water extracts – Industrial grade purity from Zig3 Industrial Gas Trading Co. Ltd. or higher purity or equivalent

2.2.13. Nitrogen evaporation system – Organomation® MICROVAP® Nitrogen Evaporation System, Model 11824 or equivalent

2.2.14. PTFE hydrophobic syringe filters, 0.22 µm (13 mm) – Phenomenex® CLARIFY-PTFE 13 mm Syringe Filters (Hydrophobic), 0.22 µm, Non-sterile, Luer/Slip or equivalent

2.2.15. Disposable syringes, 5-mL – Careplus, Ormed or equivalent

2.2.16. Autosampler vials with cap and septa, 2-mL – ALWSCI® Technologies Clear Glass Vial with PTFE/Silicone Septa or equivalent

2.3. LC-MS/MS Analysis

2.3.1. LCMS-Grade Solvents

2.3.1.1. Methanol – Scharlau Methanol, LC-MS or equivalent

2.3.1.2. Water – Scharlau Water, LC-MS or equivalent

2.3.1.3. Acetonitrile – Scharlau Acetonitrile, LC-MS or equivalent

2.3.1.4. Formic Acid – Scharlau Formic acid, eluent additive for LC-MS or equivalent

2.3.1.5. Ammonium Hydroxide Solution – Scharlau Ammonia solution 25%, eluent additive for LC-MS or equivalent

2.3.1.6. Ammonium Acetate – Scharlau Ammonium acetate, eluent additive for LC-MS or equivalent

2.3.1.7. Acetic Acid – Scharlau Acetic acid glacial, eluent additive for LC-MS or equivalent

2.3.2. Clear glass or amber solvent bottles, 250- or 500-mL – PYREX® or equivalent

2.3.3. Graduated cylinder, 50- or 100-mL – PYREX® or KIMAX® or equivalent

2.3.4. Volumetric flasks, 10- or 100-mL – Fisherbrand™ or PYREX® or equivalent

2.3.5. Micropipettes (Range: 0.5-10 µL, 10-100 µL, 20-200 µL, 100-1000 µL, 1-10 mL) and suitable pipette tips

2.3.6. Glass filtration set-up, including 300-mL glass funnel, anodized aluminum clamp, glass support base and tubulated cap, 1000-mL ground joint flask, and silicone fixing sucker or equivalent – Rocker® VF3 Glass Filtration Apparatus Set or equivalent

2.3.7. Nylon membrane filters, 0.2 µm (47 mm) – Whatman™ Nylon Membrane Filters 0.2 µm Diameter 47 mm

2.3.8. Sonicator – SB-5200 DTDN Ultrasonic Cleaner or equivalent

2.3.9. Hormones and Phenols Standard Mix – Restek® 31117 Steroids and Mixed Pharmaceuticals Mix 200 µg/mL in Acetonitrile or equivalent

2.3.10. Chlorophenols Standard Mix – Restek® 31029 604 Calibration Std Phenols 2000 µg/mL in Methanol or equivalent

2.3.11. Aluminum foil

2.3.12. Internal Standards

2.3.12.1. 4-Chlorophenol-d4, >98% – Santa Cruz Biotechnology, Inc. or equivalent

2.3.12.2. β-Estradiol-d2, >99% – Santa Cruz Biotechnology, Inc. or equivalent

2.3.13. Analytical balance – BIOBASE Electronic Balance BA2204C or equivalent

2.3.14. Autosampler vials with cap and septa, 2- or 4- mL – ALWSCI® Technologies Clear Glass Vial with PTFE/Silicone Septa or equivalent

2.3.15. Vial racks – for 2-mL and 4-mL autosampler vials

2.3.16. Vortex mixer – Digisystem Laboratory Instruments Inc. VM-2000-C or equivalent

2.3.17. Analytical Column –Hypersil GOLD™ (100 mm x 2.1 mm, 1.9 µm)

2.3.18. Guard Column –Hypersil GOLD™ Drop-in Guard (10 mm x 2.1 mm, 3 µm)

2.3.19. Argon gas tank – Ultra high purity from Lyndeson Gas Corporation or equivalent

2.3.20. Nitrogen generator – Peak Scientific® NM32LA Nitrogen Generator or equivalent

2.3.21. LC-MS/MS instrument – Thermo Scientific™ Ultimate 3000 System tandem with Triple Quadrupole Thermo Scientific™ TSQ Quantis or equivalent

2.4. Waste Disposal

2.4.1. Labelled 4-L bottle for Pharmaceutical Waste

2.4.2. Labelled Carboys for Organic Solvent Waste

2.4.3. Labelled Carboys for Solid Waste

2.4.4. Labelled Carboys for Oil Waste

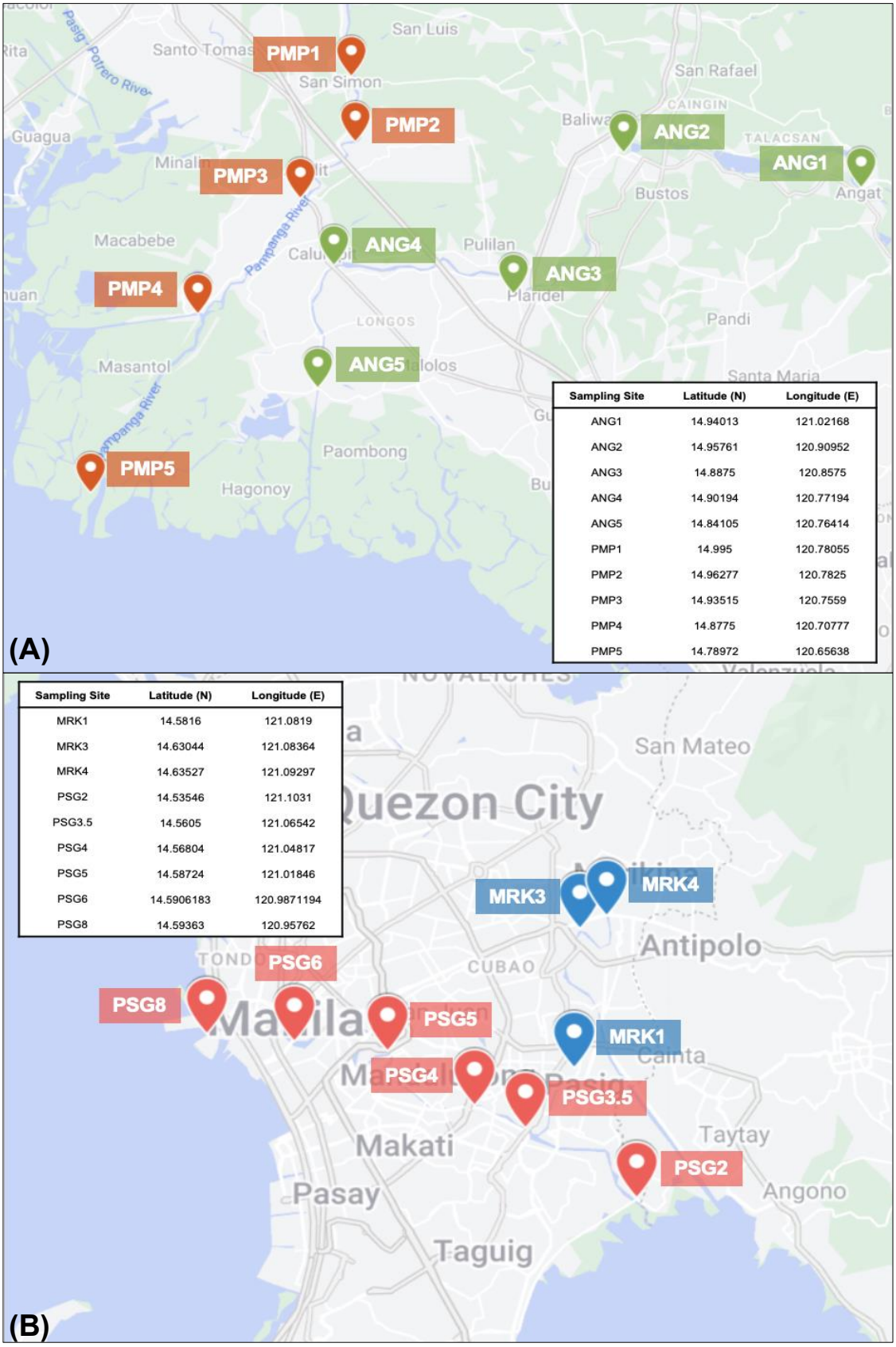
2.4.5. Labelled Carboys for Broken Glassware

2.4.6. Labelled Carboys for Nitrogen Generator Condensed Water

3. River Sampling

Triplicate surface water samples are collected from Angat River, Pampanga River, Marikina River, and Pasig River. The sampling sites are shown in Figure 1.

NOTE: *Two sampling bottles are also assigned as the field blanks. The bottles for the field blanks are filled with ultrapure water and brought to the sites during sampling.*



Sampling Site	Latitude (N)	Longitude (E)
ANG1	14.94013	121.02168
ANG2	14.95761	120.90952
ANG3	14.8875	120.8575
ANG4	14.90194	120.77194
ANG5	14.84105	120.76414
PMP1	14.995	120.78055
PMP2	14.96277	120.7825
PMP3	14.93515	120.7559
PMP4	14.8775	120.70777
PMP5	14.78972	120.65638

Sampling Site	Latitude (N)	Longitude (E)
MRK1	14.5816	121.0819
MRK3	14.63044	121.08364
MRK4	14.63527	121.09297
PSG2	14.53546	121.1031
PSG3.5	14.5605	121.06542
PSG4	14.56804	121.04817
PSG5	14.58724	121.01846
PSG6	14.5906183	120.9871194
PSG8	14.59363	120.95762

Figure 1. Sampling Sites in (A) Angat and Pampanga Rivers; and (B) Marikina and Pasig Rivers.

3.1. Preparation for Sampling

3.1.1. Wash the sampling bottles and stainless-steel bucket with 1% Liquinox® solution and rinse using tap water.

3.1.2. Wash the sampling materials thrice with pure water.

3.1.3. Rinse the sampling materials thrice with HPLC-grade methanol.

3.1.4. Label the pre-cleaned sampling bottles with their assigned codes by printing the labels on a clean sheet of paper and covering with clear tape.

3.2. Sample Collection and Storage

***NOTE**: Wear a new pair of nitrile gloves for each sampling site. Wear a face mask.*

3.2.1. Record the GPS coordinates of each sampling site. Take pictures of the site and take note of any observations.

3.2.2. Record the water quality parameters from field devices following the instructions in *Section 7.2. Probes* in *p. 37* of the *Manual of Procedures for Sampling and Nutrient Measurements in Philippines Lakes* [1].

3.2.3. Rinse the stainless-steel bucket with river water thrice before collecting water samples. Also rinse the sampling bottles with river water thrice.

3.2.4. Submerge the stainless-steel bucket below the surface of the water then carefully retrieve the bucket.

3.2.5. Fill the sampling bottles with river water to an approximate volume of 1100 mL. Make sure that the sampling bottles are correctly labelled based on the sampling site. An actual sample collection is shown in Figure 2.

***NOTE**: Do not completely fill the sampling bottles. When the samples are kept in the refrigerator, water will expand upon freezing and may cause the bottle to break.*

Figure 2. Sample collection using stainless steel bucket and amber bottles.

3.2.6. Store the samples in heavy duty ice chests with at least three packs of tube ice until these can be transferred to the designated refrigerator for water samples intended for EDCs analysis.

4. Sample Processing

***NOTE**: Process the river samples for LC-MS/MS analysis within one week.*

4.1. Filtration of Water Samples

4.1.1. Thaw the field blanks and river water samples to be at room temperature.

4.1.2. Thoroughly wash the filtration set-up with pure water.

4.1.3. Assemble the filtration set-up. An example of a vacuum filtration set-up is shown in Figure 3.

4.1.4. Using a tweezer, carefully place one 1.2 µm glass fiber filter in between the glass support base and the glass funnel. Attach the aluminum clamp to hold the glass funnel in place.

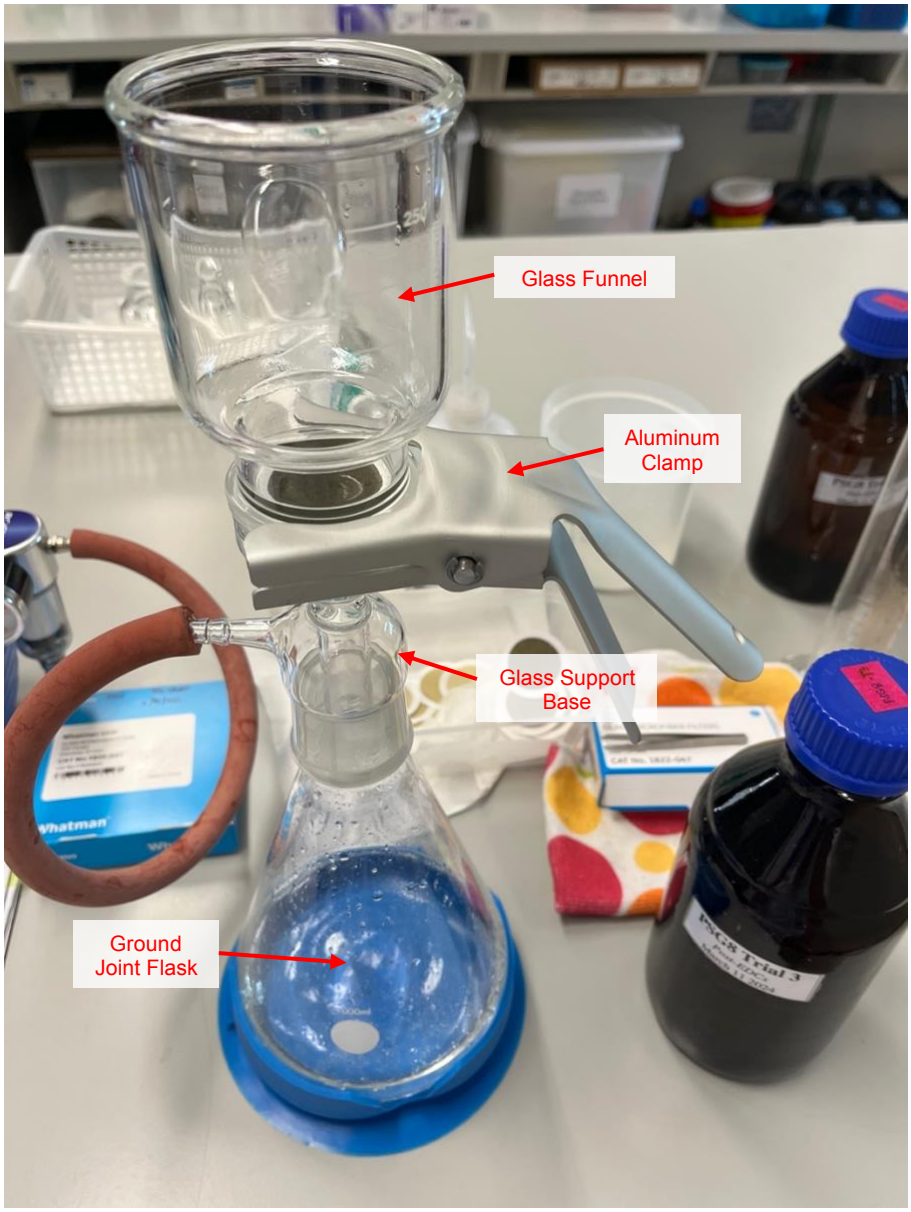


Figure 3. Vacuum filtration of river water samples.

4.1.5. Rinse the filtration set-up using approximately 40 mL of the river water sample. Discard the water sample used for rinsing.

4.1.6. Re-assemble the set-up and place a new 1.2 µm glass fiber filter.

4.1.7. Fill the glass funnel with 150-200 mL of sample. Turn ON the vacuum pump.

4.1.8. Change the filter when necessary and continue the filtration.

***NOTE**: For samples with a lot of particulate matter, it is recommended to change the filter every 50-100 mL. Repeat this process until the desired volume of pre-filtered sample is obtained.*

4.1.9. Transfer the water sample from the ground joint flask into its original 1000-mL amber glass bottle.

4.1.10. Using a tweezer, carefully place one 0.7 µm glass fiber filter in between the glass support base and the glass funnel. Attach the aluminum clamp to hold the glass funnel in place.

4.1.11. Rinse the set-up using approximately 60 mL of the pre-filtered river water sample. Use the filtered sample to rinse the graduated cylinder and the new 1000-mL clear glass bottle. Discard the water sample used for rinsing.

4.1.12. Re-assemble the set-up and place a new 0.7 µm glass fiber filter.

4.1.13. Fill the glass funnel with 100-200 mL of pre-filtered sample. Turn ON the vacuum pump. Continue the filtration of the remaining sample.

4.1.14. Quantitatively transfer 1000 mL of the filtered water sample using a graduated cylinder into the new 1000-mL clear glass bottle.

***NOTE**: If the filtered sample does not reach 1000 mL, record the actual volume in the bottle and use this for the computation of the actual concentration in the sample.*

4.1.15. Store the filtered water samples in a refrigerator until extraction.

4.2. Solid Phase Extraction

***NOTE:** Set-up the solid phase extraction manifold following Section 6.2.1 Solid phase extraction set-up* in *p.10* of the *Manual of Procedures for Sampling, Sample Preparation and Analysis of Antibiotics and Hormones in Laguna Lake Water* [2].

4.2.1. Conditioning of SPE Cartridges

NOTE: *From the start of the conditioning of the cartridges until the end of sample loading, do not let the cartridges dry.*

4.2.1.1. Condition each cartridge without turning on the vacuum pump.

4.2.1.2. Dispense 5 mL of HPLC-grade methanol using the bottle top dispenser into each SPE Cartridge. Slowly open the flow control valve counterclockwise for each cartridge to let the solvent flow. When a thin layer of liquid is left on the surface of the solid phase, close the flow control valve.

4.2.1.3. Dispense 5 mL of ultrapure water using the bottle top dispenser into each SPE Cartridge. Slowly open each flow control valve. When a thin layer of liquid is left on the surface of the solid phase, close the flow control valve.

4.2.2. Loading of Water Samples

4.2.2.1. Rinse the large volume samplers by running HPLC-grade methanol followed by ultrapure water through empty SPE cartridges intended only for use of rinsing the volume samplers.

4.2.2.2. Acidify the water samples to pH 4 using formic acid.

4.2.2.3. Connect the large volume sampler of each sampling bottle to the SPE tubes. An example of solid phase extraction of river water samples is shown in Figure 4.

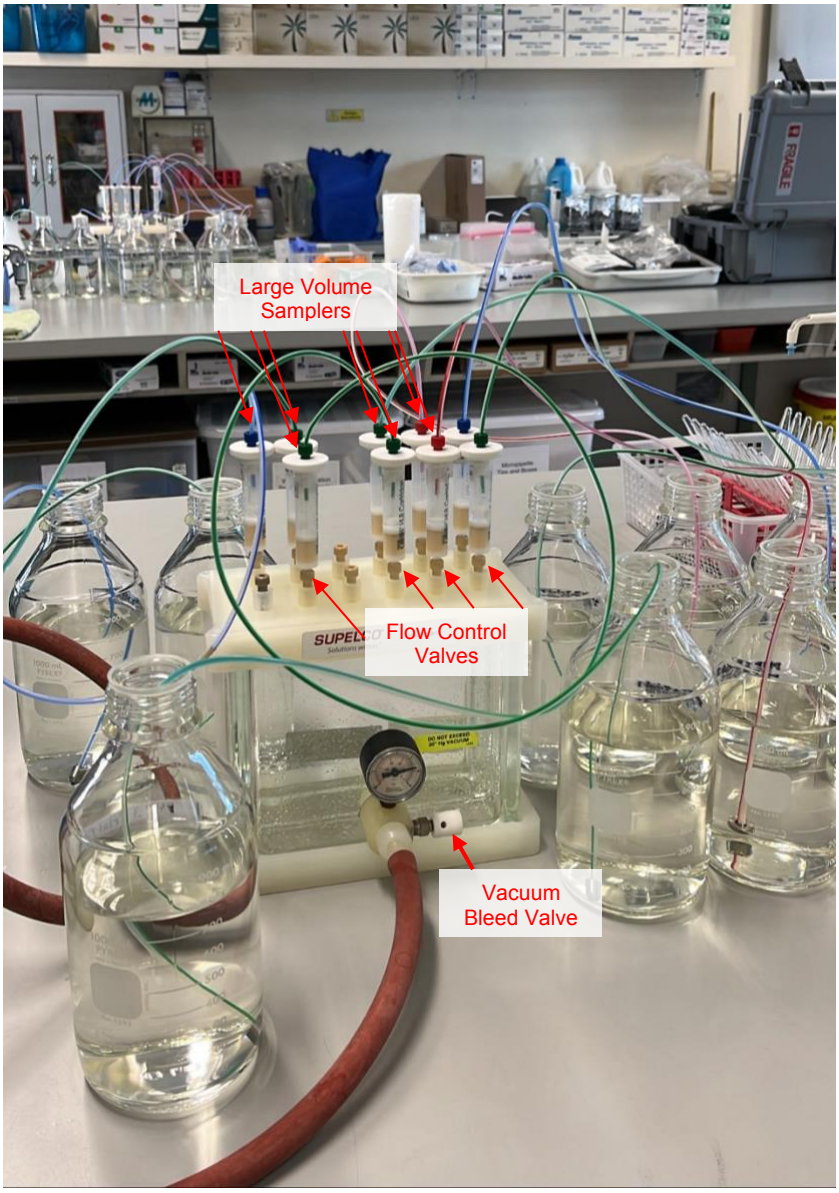


Figure 4. Solid phase extraction of river water samples.

4.2.2.4. Turn ON the vacuum pump.

4.2.2.5. Carefully open the flow control valve of each cartridge.

4.2.2.6. Adjust the vacuum bleed valve to obtain a flow rate of 3-5 mL/min.

4.2.2.7. Close the vacuum pump whenever the waste container is two-thirds full. Detach the waste container from the set-up before emptying it to prevent water from going into the vacuum pump.

4.2.2.8. Close the flow control valve of each cartridge when all of the sample has passed through.

4.2.2.9. Once all samples have passed through the cartridges, turn OFF the vacuum pump and remove the large volume samplers.

4.2.2.10. Allow 5 mL of ultrapure water to flow through the solid phase.

4.2.2.11. Turn ON the vacuum pump after the 5 mL ultrapure water has passed through the solid phase. Leave the flow control valves open to dry the cartridges for at least 1 h.

4.2.2.12. Close the flow control valves after the cartridges have dried. Turn OFF the vacuum pump.

4.2.3. Elution of Analytes

4.2.3.1. Place the collection vessel rack with borosilicate glasses inside the SPE manifold.

4.2.3.2. Dispense 5 mL LCMS-grade methanol into each cartridge.

4.2.3.3. Carefully open the flow control valve of each cartridge to elute the analytes.

4.2.3.4. Dispense another 5 mL LCMS-grade methanol into each cartridge and continue the elution of the analytes.

4.2.3.5. Close the flow control valves. Store the SPE cartridges, if needed.

4.3. Drying of Extracts

***NOTE**: Solvent fumes may be hazardous. Drying of extracts must be done under the fume hood. An example of the setup when drying extracts is shown in Figure 5.*

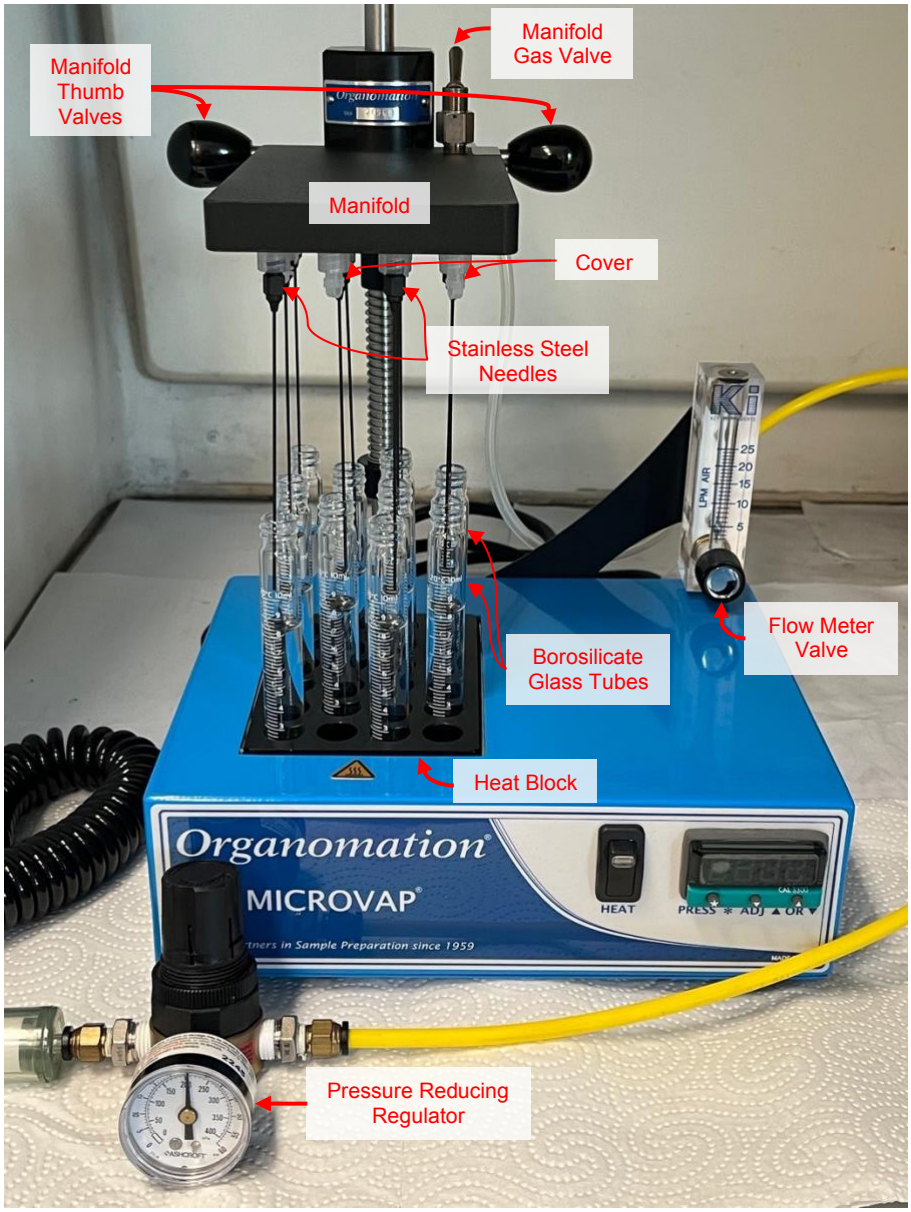


Figure 5. Nitrogen evaporation of the extracts.

4.3.1. Remove the individual covers and attach the stainless steel needles to the nitrogen evaporation system manifold.

4.3.2. Position the borosilicate glass tubes inside the holes of the heat block.

4.3.3. Carefully open the main regulator on the nitrogen gas tank.

4.3.4. Adjust the pressure reducing regulator of the gas connector tube assembly to obtain a reading of approximately 20-30 psi.

4.3.5. Switch the manifold gas valve to ON.

4.3.6. Adjust the flow meter valve counterclockwise to increase the gas flow to approximately 3-5 psi.

NOTE: *Maintain a gentle flow of nitrogen gas to evaporate the solvent in the eluates. The gas flow should be adjusted to prevent splattering of the eluates.*

4.3.7. Carefully lower the manifold by loosening the manifold thumb knobs. Do not dip the needles in the solvent.

NOTE: *Adjust the height of the manifold when necessary. The gas flow should reach the surface of the solution wherein a dimple appears on the surface. Be careful to not dip the needle in the solution.*

4.3.8. Close the flow meter valve and the manifold gas valve once the eluates are at the 0.5 mL level. Also close the regulators when the evaporation system is not in use.

4.3.9. Remove the borosilicate glasses containing the concentrated eluates from the nitrogen evaporation system.

4.4. Reconstitution and Spiking with Deuterated Standards

4.4.1. Reconstitute the eluates in the borosilicate glass to 1.5 mL using 10% methanol in water.

4.4.2. Filter the extracts using 0.2 µm PTFE (hydrophobic) syringe filters into 2-mL autosampler vials.

4.4.3. Transfer 980 µL of the filtered extract to another 2-mL autosampler vial.

4.4.4. Add 10 µL of each of the 2 mg/L internal deuterated standards.

4.4.5. Cover the autosampler vials and mix the extracts using a vortex mixer.

5. Analysis Preparations

***NOTE**: Only LCMS-grade solvents and modifiers are used in preparing the mobile phases and standards for LC-MS analysis. Mobile phases and calibration solutions need to be freshly prepared every week.*

5.1. Preparation of Mobile Phases

5.1.1. Preparation of 10:90 Methanol:Water Mix for Rear Seal Wash

5.1.1.1. In a 250-mL clear glass solvent bottle, add 25 mL of methanol.

5.1.1.2. Add 225 mL of water.

5.1.1.3. Shake the bottle to mix.

5.1.1.4. Sonicate for 10 min.

5.1.1.5. Position the 10:90 Methanol:Water Mix in the Rear Seal Wash line of the solvent rack of the LC-MS/MS equipment.

5.1.2. Preparation of 90:10 Methanol:Water Mix for Syringe Wash

5.1.2.1. In a 250-mL clear glass solvent bottle, add 225 mL of methanol.

5.1.2.2. Add 25 mL of water.

5.1.2.3. Shake the bottle to mix.

5.1.2.4. Sonicate for 10 min.

5.1.2.5. Position the 90:10 Methanol:Water Mix in the Syringe Wash line of the solvent rack of the LC-MS/MS equipment.

5.1.3. Preparation of 50:50 Methanol:Water Mix or 50:50 Acetonitrile:Water Mix for Conditioning or Flushing of Column

5.1.3.1. In a 250-mL clear glass solvent bottle, add 100 mL of water.

5.1.3.2. Add 100 mL of methanol or acetonitrile.

5.1.3.3. Shake the bottle to mix.

5.1.3.4. Sonicate for 10 min.

5.1.3.5. Position the 50:50 Methanol:Water or Acetonitrile:Water Mix in Line C of the solvent rack of the LC-MS/MS equipment.

5.1.4. Preparation of Methanol or Acetonitrile for Flushing of System Lines

5.1.4.1. In a 250-mL clear glass solvent bottle, add 200 mL of methanol or acetonitrile.

5.1.4.2. Sonicate for 10 min.

5.1.4.3. Position the Methanol or Acetonitrile Bottle in Line D of the solvent rack of the LC-MS/MS equipment.

5.1.5. Preparation of 0.10% (v/v) Ammonium Hydroxide in Water or in Methanol for Hormones and Phenols Analysis

5.1.5.1. In a 500-mL volumetric flask, transfer approximately 400 mL water.

5.1.5.2. Add 500 µL of the ammonia solution to the water in the 500-mL volumetric flask.

5.1.5.3. Volume to the 500-mL mark using water. Cover the flask and mix. Transfer into a 500-mL clear glass container designated as the aqueous mobile phase reservoir for Hormones and Phenols Analysis.

5.1.5.4. Repeat steps 5.1.5.1. to 5.1.5.4 using methanol instead of water. This serves as the organic mobile phase for Hormones and Phenols Analysis.

5.1.5.5. Sonicate for 10 min.

5.1.5.6. Position the aqueous and organic mobile phase reservoirs in Line A and B, respectively, of the solvent rack of the LC-MS/MS equipment.

5.1.6. Preparation 2 mM Ammonium Acetate (pH 4.35) and Acetonitrile for Chlorophenols Analysis

5.1.6.1. Prepare 20 mM Ammonium Acetate by weighing 1.54 g Ammonium Acetate and dissolving in water. In a 1000-mL volumetric flask, transfer the ammonium acetate solution and volume to the 1000-mL mark using water.

5.1.6.2. In a 500-mL volumetric flask, transfer approximately 400 mL of water. Add 50 mL of the 20 mM Ammonium Acetate Solution using a graduated cylinder. Then, add glacial acetic acid to adjust the pH to 4.35. Dilute to mark using water and mix.

5.1.6.3. Filter the 2 mM Ammonium Acetate (pH 4.35) solution through 0.2 µm nylon membrane filters using the vacuum filtration setup intended for use only with aqueous mobile phases.

5.1.6.4. Transfer the filtered 2 mM Ammonium Acetate (pH 4.35) into a 500-mL clear glass container designated as the aqueous mobile phase reservoir for Chlorophenols Analysis.

5.1.6.5. In a 500-mL clear glass container, transfer 500 mL of acetonitrile. This serves as the organic mobile phase for Chlorophenols Analysis.

5.1.6.6. Sonicate for 10 min.

5.1.6.7. Position the aqueous and organic mobile phase reservoirs in Line A and B, respectively, of the solvent rack of the LC-MS/MS equipment.

5.2. Preparation of Standards

5.2.1. Preparation of Reconstituting Solvent (10% Methanol in Water)

5.2.1.1. In a 250-mL volumetric flask, transfer 200 mL of water.

5.2.1.2. Add 25 mL methanol.

5.2.1.3. Dilute to mark using water.

5.2.1.4. Transfer the solution into an appropriately labelled 250-mL clear glass solvent bottle.

5.2.2. Preparation of Internal Deuterated Standards

5.2.2.1. Preparation of Estradiol-d2

5.2.2.1.1. Dissolve 5 mg of the solid Estradiol-d2 standard in approximately 1 mL of methanol.

5.2.2.1.2. Quantitatively transfer into a 10-mL volumetric flask and dilute to mark with methanol to obtain a 500 mg/L estradiol-d2 standard stock solution. Store this stock solution in properly labelled scintillation vials.

5.2.2.1.3. In a 4-mL vial, prepare a 2 mg/L estradiol-d2 standard solution by adding 12 µL of the 500 mg/L estradiol-d2 stock solution to 2988 µL of reconstituting solvent. Cover the vial and mix using a vortex mixer.

5.2.2.2. Preparation of 4-Chlorophenol-d4

5.2.2.2.1. Dissolve 25 mg of the solid 4-Chlorophenol-d4 standard in approximately 1 mL of methanol.

5.2.2.2.2. Quantitatively transfer into a 100-mL volumetric flask and dilute to mark with methanol to obtain a 250 mg/L 4-chlorophenol-d4 standard stock solution. Store this stock solution in properly labelled scintillation vials.

5.2.2.2.3. In a 4-mL vial, prepare a 2 mg/L chlorophenol-d4 standard solution by adding 16 µL of the 250 mg/L 4-chlorophenol-d4 solution to 1984 µL of reconstituting solvent. Cover the vial and mix using a vortex mixer.

***NOTE**: The 2 mg/L internal deuterated standard solutions must be freshly prepared prior to spiking in the calibration solution or extracts.*

5.2.3. Preparation of Calibration Solutions

5.2.3.1. Prepare the EDCs calibration solutions in 2-mL autosampler vials following the prescribed volumes in Table 1.

5.2.3.2. Use a 100-1000 µL micropipette to transfer the appropriate volume of reconstituting solvent into each of the vials.

5.2.3.3. Use a 0.5-10, 10-100, or 20-200 µL micropipette to transfer the standard solutions. Change the tips for the different standard solutions.

5.2.3.4. Add 10 µL of each of the 2 mg/L internal standards in the solutions.

***NOTE**: The volume of reconstituting solvent may be adjusted depending on the number of internal standards to be added. The total volume must still be 1000 µL.*

Table 1. Guide in preparing the calibration solutions

Calibration Level	Concentration, µg/L	Standard Solution		Reconstituting Solvent, µL	Volume of Internal Standard Solution, µL	
		Type	Vol, µL		4-CP-d4	E2-d2
Cal 10	100	2 mg/L	50	930	10	10
Cal 9	50	2 mg/L	25	955	10	10
Cal 8	20	2 mg/L	10	970	10	10
Cal 7	10	100 µg/L	100	880	10	10
Cal 6	5	100 µg/L	50	930	10	10
Cal 5	2	100 µg/L	20	960	10	10
Cal 4	1	100 µg/L	10	970	10	10
Cal 3	0.5	10 µg/L	50	930	10	10
Cal 2	0.1	10 µg/L	10	970	10	10
Cal 1	0	*n/a*	*n/a*	980	10	10
Solvent Blank	0	*n/a*	*n/a*	1000	0	0

6. LC-MS/MS Analysis

The methods described in this manual are optimum only for the EDCs namely E1, E2, E2-d2, EE2, PROG, TES, BPA, NP, OP, 2-CP, 4-CP-d4, 2,4-DCP, 2,4,6-TCP, and PCP.

Before starting the LC-MS/MS Analysis, ensure that the system readback is normal and there are no error warnings on the software. Check that the mobile phase reservoirs have enough solvents for the sequence run. Make sure that the column is properly conditioned prior to analysis of samples and that the column is flushed after the analysis for proper storage. Also check if the ion source interface needs cleaning prior to analysis.

The detailed operation of the LC-MS/MS is described in full in the *Manual of Procedures for Sampling, Sample Preparation and Analysis of Antibiotics and Hormones in Laguna Lake Water (A3-A11 p. 50-100)* [2].

NOTE: *If different compounds of interest will be analyzed, methods optimization must be done starting from the identification of the polarity and the precursor-product mass transitions of the analytes through Full Scans and Product Scans. The appropriate analytical column as well as the compatible solvents must also be determined prior to the optimization and fine-tuning of the LCMS parameters. Mobile phase composition, gradient profile, flow rate, column temperature, and injection volume are the main LC properties to consider in obtaining good chromatographic separation. Spray voltage, gas flows (sheath, auxiliary, and sweep), ion transfer tube temperature, and vaporizer temperature are the main MS properties to consider in obtaining acceptable signals for all analytes.*

6.1. Method for Hormones and Phenols Analysis

NOTE: *The runtime for hormones and phenols analysis using the Hypersil Gold C18 Column is 12 min. Shown in Figure 6 are sample chromatograms for hormones and phenols analysis.*

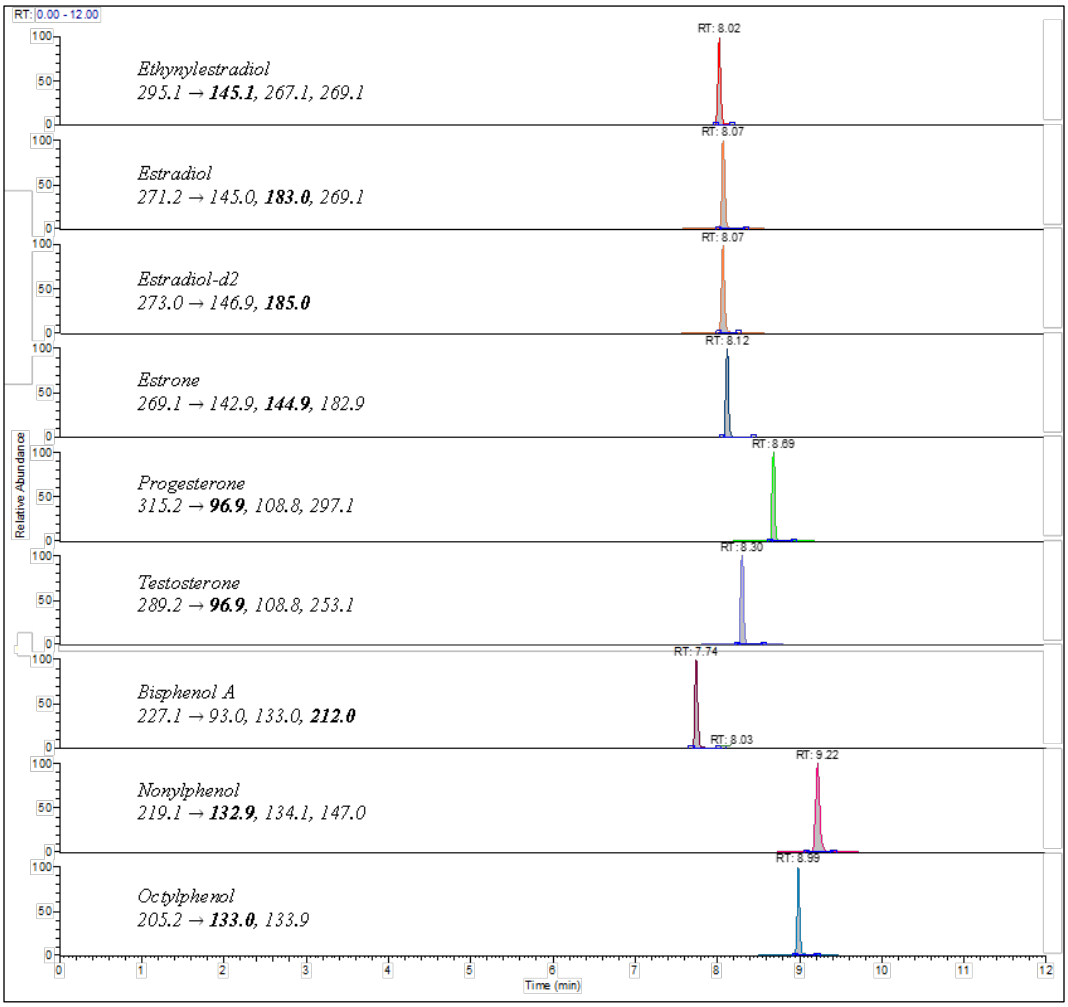


Figure 6. SRM Chromatograms of Hormones and Phenols with labels of the precursor-product mass transitions for each analyte. Product masses in bold text are the quantifier ions.

6.1.1. LC Parameters

6.1.1.1. The flow rate is set to 0.200 mL/min.

6.1.1.2. The mobile phase gradient is done following Table 2. The gradient profile for Hormones and Phenols Analysis is shown in Figure 7.

Table 2. Gradient for Hormones and Phenols Analysis.

Time, min	% Line A (0.10% NH_4OH in H_2O)	% Line B (0.10% NH_4OH in MeOH)
0.00	95.0	5.0
2.00	95.0	5.0
7.00	5.0	95.0
10.00	5.0	95.0
10.10	95.0	5.0
12.00	95.0	5.0

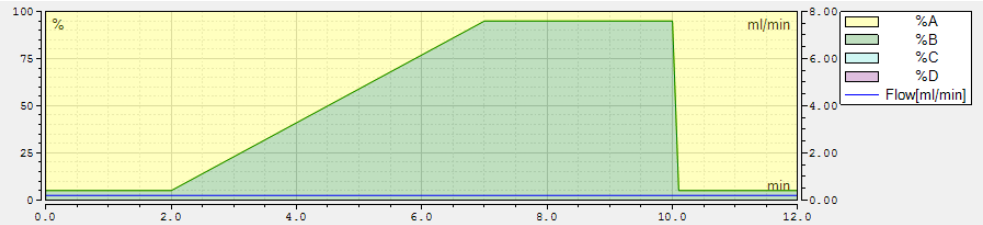


Figure 7. Gradient Profile for Hormones and Phenols Analysis.

6.1.1.3. The autosampler temperature is set to 10 °C.

6.1.1.4. The column compartment is set to 35 °C.

6.1.2. MS Parameters

6.1.2.1. The Ion Source Properties are tabulated in Table 3.

Table 3. Ion Source Properties for Hormones and Phenols Analysis.

Ion Source Properties	***Hormones and Phenols Analysis***
Ion Source Type	H-ESI
Spray Voltage	Static
Positive Ion (V)	3500
Negative Ion (V)	3000
Sheath Gas (Arb)	50
Aux Gas (Arb)	10
Sweep Gas (Arb)	0
Ion Transfer Tube Temp (℃)	300
Vaporizer Temp ℃	350
APPI Lamp	Not in Use

6.1.2.2. The SRM Properties are tabulated in Table 4.

Table 4. SRM Properties for Hormones and Phenols Analysis.

SRM Properties	***Hormones and Phenols Analysis***
Use Cycle Time	True
Cycle Time (sec)	0.4
Use Calibrated RF Lens	True
Q1 Resolution (FWHM)	0.7
Q3 Resolution (FWHM)	0.7
CID Gas (mTorr)	1.5
Source Fragmentation (V)	0
Chromatographic Peak Width (sec)	6
Use Chromatographic Filter	True
Use Retention Time Reference	False
Display Retention Time	True
Use Quan Ion	False
Show Visualization	False

6.1.2.3. The MRM Table is shown in Table 5.

Table 5. MRM Table of Hormones and Phenols.

Compound	RT, min	Polarity	Precursor, m/z	Product, m/z	Collision Energy, V
EE2	8.02	-	295.1	145.1	40
				267.1	25
				269.1	31
E2	8.07	-	271.2	145.0	39
				183.0	41
				269.1	32
E1	8.12	-	269.1	142.9	53
				144.9	38
				182.9	38
PROG	8.69	+	315.2	96.9	22
				108.9	25
				297.1	16
TES	8.30	+	289.2	96.9	21
				108.8	23
				253.1	17
BPA	7.74	-	227.1	93.0	45
				133.0	24
				212.0	16
NP	9.22	-	219.1	132.9	30
				134.1	18
				147.0	26
OP	8.99	-	205.2	133.0	25
				134.0	17
				189.0	20
E2-d2	8.07	-	273.0	146.9	40
				185.0	40
				240.9	40

6.2. Method for Chlorophenols Analysis

NOTE: *The runtime for chlorophenols analysis using the Hypersil Gold C18 Column is 8 min. Shown in Figure 8 are sample chromatograms for chlorophenols analysis.*

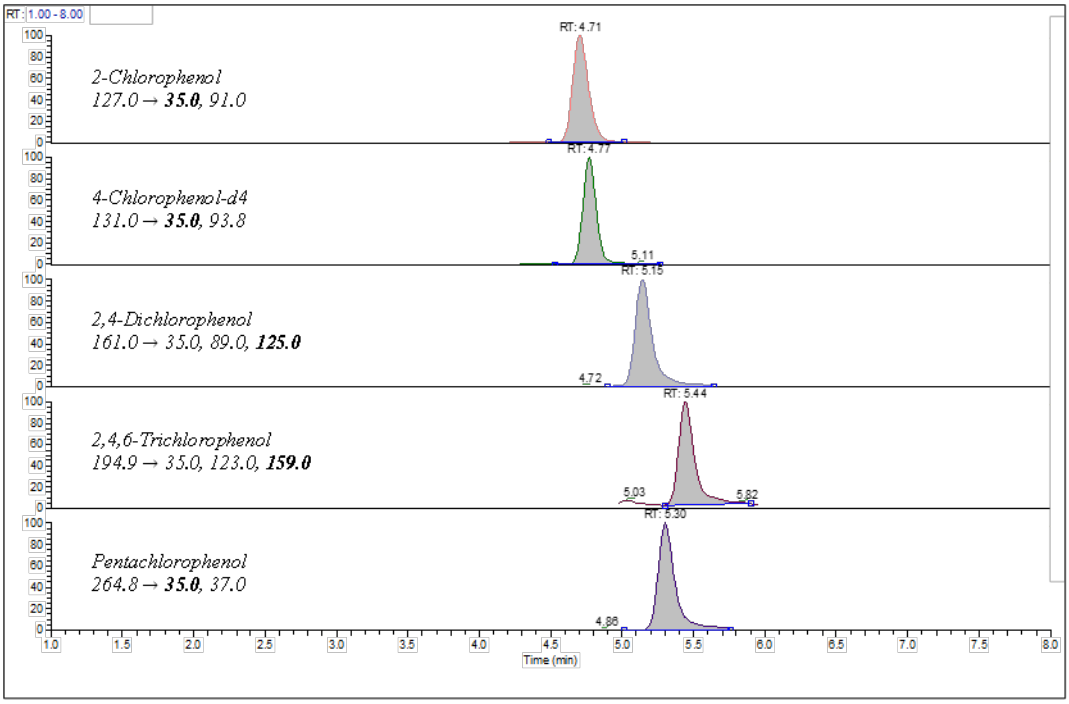


Figure 8. SRM Chromatograms of Chlorophenols with labels of the precursor-product mass transitions for each analyte. Product masses in bold text are the quantifier ions.

6.2.1. LC Parameters

6.2.1.1. The flow rate for Chlorophenols Analysis is 0.250 mL/min.

6.2.1.2. The mobile phase gradient is done following Table 6. The gradient profile for Chlorophenols Analysis is shown in Figure 9.

Table 6. Gradient for Chlorophenols Analysis.

Time, min	% Line A (2 mM Ammonium Acetate, pH 4.35)	% Line B (ACN)
0.00	95.0	5.0
1.00	95.0	5.0
5.00	5.0	95.0
6.50	5.0	95.0
6.60	95.0	5.0
8.00	95.0	5.0

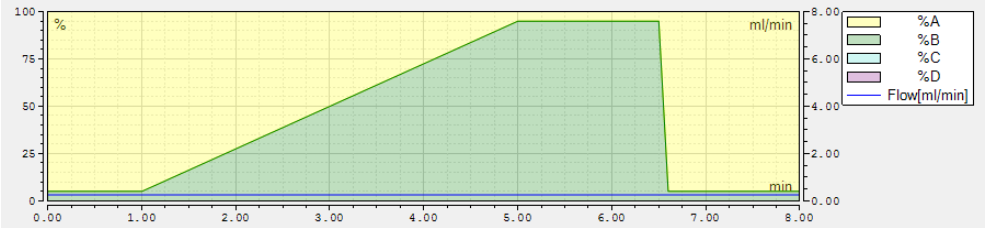


Figure 9. Gradient Profile for Chlorophenols Analysis.

6.2.1.3. The autosampler temperature is set to 10 °C.

6.2.1.4. The column compartment is set to 35 °C.

6.2.2. MS Parameters

6.2.2.1. The ion source parameters are tabulated in Table 7.

Table 7. Ion Source Properties for Chlorophenols Analysis.

Ion Source Properties	***Chlorophenols Analysis***
Ion Source Type	H-ESI
Spray Voltage	Static
Positive Ion (V)	3500
Negative Ion (V)	3500
Sheath Gas (Arb)	50
Aux Gas (Arb)	10
Sweep Gas (Arb)	0
Ion Transfer Tube Temp (℃)	275
Vaporizer Temp ℃	275
APPI Lamp	Not in Use

6.2.2.2. The SRM Properties are tabulated in Table 8.

Table 8. SRM Properties for Chlorophenols Analysis.

SRM Properties	***Chlorophenols Analysis***
Use Cycle Time	True
Cycle Time (sec)	0.7
Use Calibrated RF Lens	True
Q1 Resolution (FWHM)	0.7
Q3 Resolution (FWHM)	1.2
CID Gas (mTorr)	1.5
Source Fragmentation (V)	0
Chromatographic Peak Width (sec)	6
Use Chromatographic Filter	True
Use Retention Time Reference	False
Display Retention Time	True
Use Quan Ion	False
Show Visualization	False

6.2.2.3. The MRM Table is shown in Table 9.

Table 9. MRM Table of Chlorophenols.

Compound	**RT, min**	**Polarity**	**Precursor, m/z**	**Product, m/z**	**Collision Energy, V**
2-CP	4.71	-	127.0	35.0	20
				91.0	15
2,4-DCP	5.15	-	161.0	35.0	15
				89.0	20
				125.0	16
2,4,6-TCP	5.44	-	194.9	35.0	23
				123.0	25
				159.0	20
PCP	5.30	-	264.8	35.0	47
				37.0	52
4-CP-d4	4.77	-	131.0	35.0	20
				93.8	19

7. Data Processing and Calculations

The LC-MS/MS data is processed using the TraceFinder Software. This can be done following *Section A9. Data Processing via TraceFinder* in *p. 78* of the *Manual of Procedures for Sampling, Sample Preparation and Analysis of Antibiotics and Hormones in Laguna Lake Water* [2]. Shown in Figure 10 is a sample format for processing of data using TraceFinder.

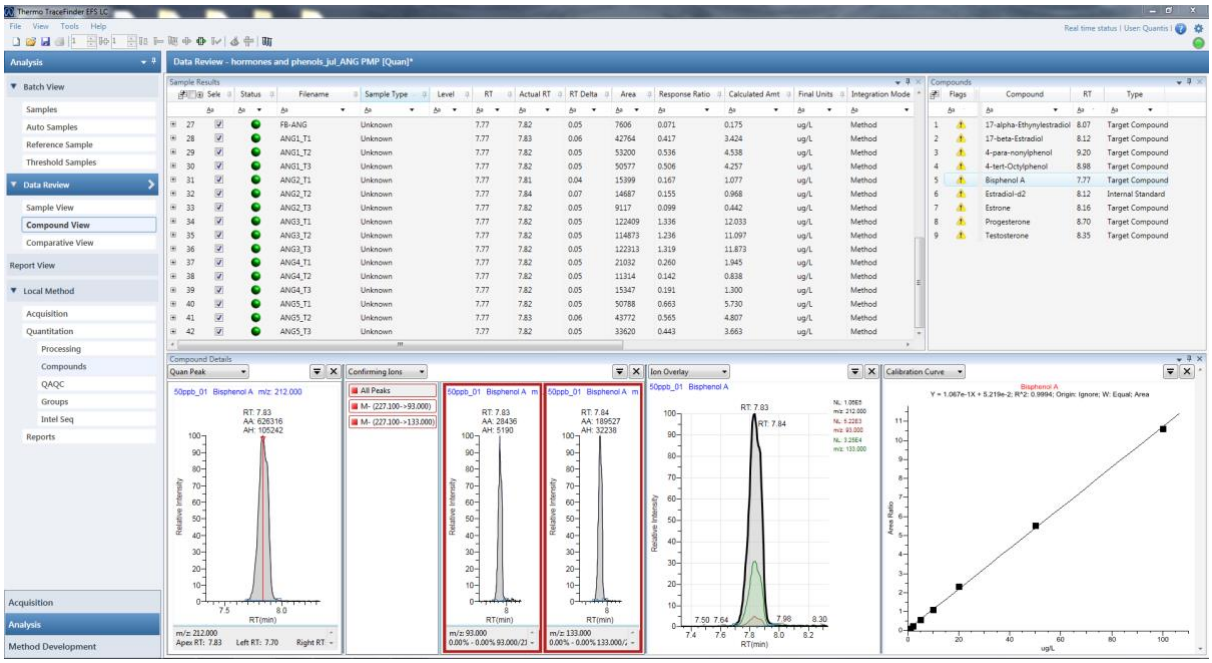


Figure 10. Processing Data using TraceFinder Software.

NOTE: *The "Calculated Amount" in the exported excel file from TraceFinder is the concentration of the analytes in the injected extract. By multiplying the appropriate dilution factors, the concentration of the analytes in the river sample can be determined.*

7.1. Create a new excel file for the calculation of final concentrations in the river water samples.

7.2. Copy the analyte concentrations from the exported excel file into one column.

7.3. Multiply the dilution factors to obtain the analyte concentrations in the river water samples.

$$Analyte\ Concentration\ in\ River\ Water\ Sample$$

$$= \frac{\left(\left(Analyte\ concentration\ of\ Extract\ in\ \frac{\mu g}{L}\right) \times \left(\frac{1000 \mu L}{980\ \mu L}\right)\right) \times 1.5\ mL}{1000\ mL} \times \frac{1000 \frac{ng}{L}}{1\ \frac{\mu g}{L}}$$

7.4. Recheck all the calculations and save the files.

8. Method Performance

The Analytical Method Validation Parameters can be obtained following instructions in the *Manual of Procedures for Sampling, Sample Preparation and Analysis of Antibiotics and Hormones in Laguna Lake Water (9. Data Processing and Calculations and 10. Method Performance p. 25-32)* [2].

Table 10. Analytical Validation Parameters of the EDCs.

Analyte	Quantifier Ion, m/z	Calibration Range, µg/L	Linearity	IDL, µg/L	Recovery (n=4) ± SD, %	MDL, µg/L
Hormones and Phenols Analysis						
EE2	145.1	0 - 50	>0.99	1.03	96.76 ± 4.30	1.87
E2	183.0	0 - 50	>0.99	0.58	94.57 ± 4.67	0.58
E1	144.9	0 - 50	>0.99	0.24	97.12 ± 5.20	0.49
PROG	108.9	0 - 50	>0.99	0.14	86.36 ± 4.11	0.95
TES	96.9	0 - 50	>0.99	0.16	89.30 ± 5.49	0.69
BPA	212.0	0 - 50	>0.99	0.19	100.91 ± 5.31	0.59
NP	132.9	0 - 50	>0.99	1.46	76.21 ± 10.29	5.70
OP	133.0	0 - 50	>0.99	0.94	70.74 ± 3.73	1.94
Chlorophenols Analysis						
2-CP	35.0	0 - 50	>0.99	1.44	88.99 ± 9.68	2.48
2,4-DCP	125.0	0 - 50	>0.99	0.83	86.51 ± 4.78	2.04
2,4,6-TCP	159.0	0 - 50	>0.99	1.83	92.31 ± 8.36	3.12
PCP	35.0	0 - 50	>0.99	0.51	96.70 ± 6.63	1.98

9. Laboratory Safety and Waste Disposal

Personnel must be knowledgeable of the safety protocols in the *Institute of Chemistry Safety Manual* [3]. Personnel must also be familiar with the locations of emergency equipment such as eyewash stations and safety showers. Know the action plans and evacuation routes in case of emergencies. Keep a record of emergency phone numbers.

Personnel doing laboratory work must wear appropriate personal protective equipment including laboratory gowns, close-toed shoes, gloves, face masks, and goggles. Observe the *buddy system* or *two-person policy* when doing laboratory work and especially during overtime. Always read the Material Safety Data Sheets to know the hazards posed by the chemicals and solvents being used.

9.1. Material Safety Data Sheets

Table 11. Summarized MSDS for Solvents from Scharlau.

Product Name	Methanol	Acetonitrile
CAS Number	67-56-1	75-05-8
Physical Properties	*[4]*	*[5]*
Appearance	Liquid	Liquid
Odor	Alcohol-like	Ether-like
Odor Threshold	*Not Determined*	170 ppm
pH	7	*Not Determined*
Melting Point, °C	-98	-46
Boiling Point, °C	64.7	81
Flash Point	10	2
Evaporation Rate	*Not Determined*	*Not Determined*
Flammability (Solid, Gas)	Highly Flammable Liquid and Vapour	Highly Flammable Liquid and Vapour
Upper Explosion Limit	36 g/m^3	16 Vol %
Lower Explosion Limit	7.3 g/m^3	4.4 Vol %
Vapor Pressure	169 hPa at 20 °C	97 hPa at 20 °C
Vapor Density	*Not Determined*	1.42 (20 °C, 1 atm)
Relative Density	*Not Determined*	*Not Determined*
Water Solubility	Fully Miscible	Fully Miscible
Partition Coefficient (n-octanol/water)	*Not Determined*	*Not Determined*
Auto-ignition Temperature	455	525
Decomposition Temperature	*Not Determined*	*Not Determined*
Viscosity	*Not Determined*	*Not Determined*
Explosive Properties	*Product is not explosive. However, formation of explosive air/vapour mixtures are possible.*	*Product is not explosive. However, formation of explosive air/vapour mixtures are possible.*

GHS Hazard Pictogram		
Hazards Identification	**H225:** Highly flammable liquid and vapor. **H301 + H311 + H331:** Toxic if swallowed, in contact with skin, or if inhaled. **H370:** Causes damage to the central nervous system and the visual organs.	**H225:** Highly flammable liquid and vapor. **H302 + H312 + H332:** Harmful if swallowed, in contact with skin, or if inhaled. **H319:** Causes serious eye irritation.
First Aid Measures	**If inhaled,** move person to fresh air. If not breathing, give artificial respiration. Seek medical attention. **In case of skin contact**, wash with plenty of water and soap. Remove contaminated clothing. Seek medical attention. **In case of eye contact,** rinse thoroughly with plenty of water for at least 15 minutes. Seek medical attention. **If swallowed,** drink plenty of water. Seek medical attention. Induce vomiting only if the affected person is fully conscious.	**If inhaled,** move person to fresh air. If not breathing, give artificial respiration. Seek medical attention. **In case of skin contact**, wash with plenty of water and soap. Seek medical attention. **In case of eye contact,** rinse thoroughly with plenty of water for at least 15 minutes. Seek medical attention. **If swallowed,** drink plenty of water. Seek medical attention. Do not induce vomiting.

Table 12. Summarized MSDS for Solvent Modifiers from Scharlau.

Product Name	Ammonia Solution	Ammonium Acetate
CAS Number	1336-21-6	631-61-8
Physical Properties	*[6]*	*[7]*
Appearance	Liquid	Solid
Odor	Pungent	Odourless
Odor Threshold	*Not Determined*	*Not Determined*
pH	*Not Determined*	*Not Determined*
Melting Point, °C	*Not Determined*	*114*
Boiling Point, °C	100	*Not Determined*
Flash Point, °C	*Not Applicable*	*136*
Evaporation Rate	*Not Determined*	*Not Appicable*
Flammability (Solid, Gas)	*Not Applicable*	*Product is not flammable*
Upper Explosion Limit	*Not Determined*	*Not Determined*
Lower Explosion Limit	*Not Determined*	*Not Determined*
Vapor Pressure	500 hPa at 20 °C	*Not Applicable*
Vapor Density	*Not Determined*	*Not Applicable*
Relative Density	*Not Determined*	*Not Determined*
Water Solubility	Fully Miscible	1.48 g/L
Partition Coefficient (n-octanol/water)	*Not Determined*	*-2.8*
Auto-ignition Temperature, °C	Product is not self-igniting.	*Not Determined*
Decomposition Temperature	*Not Determined*	*Not Determined*
Viscosity	*Not Determined*	*Not Applicable*

Explosive Properties	Product does not present an explosion hazard	Product does not present an explosion hazard
GHS Hazard Pictogram		Not Classified
Hazards Identification	**H314**: Causes severe skin burns and eye damage **H318:** Causes serious eye damage **H335**: May cause respiratory irritation **H400**: Very toxic to aquatic life	Not Classified
First Aid Measures	**If inhaled,** move person to fresh air. **In case of skin contact**, wash immediately with plenty of water. Remove contaminated clothing and gloves. **In case of eye contact,** rinse with plenty of water. Seek medical attention. **If swallowed,** drink plenty of water. Seek medical attention immediately.	**If inhaled,** move person to fresh air. **In case of skin contact**, wash immediately with plenty of water. Remove contaminated clothing and gloves. **In case of eye contact,** rinse with plenty of water. **If swallowed,** rinse mouth and drink two glasses of water. Seek medical attention immediately.

Table 13. Summarized MSDS for Solvent Modifiers from Scharlau.

Product Name	Formic Acid	Acetic Acid
CAS Number	64-18-6	64-19-7
Physical Properties	*[8]*	*[9]*
Appearance	Liquid	Liquid
Odor	Acrid	Acrid
Odor Threshold	*Not Determined.*	*Not Determined.*
pH	2.2	2.5
Melting Point, °C	*4*	*17*
Boiling Point, °C	*100*	*118*
Flash Point, °C	48	39
Evaporation Rate	*Not Determined*	*Not Determined*
Flammability (Solid, Gas)	*Flammable Liquid and Vapour*	*Flammable Liquid and Vapour*
Upper Explosion Limit	*33 Vol%*	*20 Vol%*
Lower Explosion Limit	*14 Vol%*	*4 Vol%*
Vapor Pressure	*43 hPa at* at 20 °C	*16 hPa at* at 20 °C
Vapor Density	*Not Determined*	*Not Determined*
Relative Density	*Not Determined*	*Not Determined*
Water Solubility	Fully Miscible	Fully Miscible
Partition Coefficient (n-octanol/water)	*Not Determined*	*Not Determined*
Auto-ignition Temperature, °C	*Not Determined*	*485*
Decomposition Temperature	*Not Determined*	*Not Determined*
Viscosity	*Not Determined*	*Not Determined*
Explosive Properties	*Product is not explosive. However, formation of*	*Product is not explosive. However, formation of*

	explosive air/vapour mixtures are possible.	*explosive air/vapour mixtures are possible.*
GHS Hazard Pictogram		
Hazards Identification	**H226**: Flammable liquid and vapor **H302:** Harmful if swallowed. **H314:** Causes severe skin burns and eye damage **H331:** Toxic if inhaled	**H226**: Flammable liquid and vapor **H314:** Causes severe skin burns and eye damage
First Aid Measures	**If inhaled,** move person to fresh air. Seek medical attention. **In case of skin contact**, wash immediately with plenty of water. Remove contaminated clothing and gloves. Seek medical attention immediately. **In case of eye contact,** rinse immediately with plenty of water for at least 15 minutes. Seek medical attention immediately. **If swallowed,** if conscious, drink two glasses of water. Seek medical attention.	**If inhaled,** move person to fresh air. Seek medical attention. **In case of skin contact**, wash immediately with plenty of water. Remove contaminated clothing and gloves. Seek medical attention immediately. **In case of eye contact,** rinse immediately with plenty of water for at least 15 minutes. Seek medical attention immediately. **If swallowed,** do not induce vomiting. If conscious, drink plenty of water. Seek medical attention.

Table 14. Summarized MSDS for Analytical Standards from Restek.

Product Name	Steroids and Pharmaceuticals Mix/ Restek 31117	604 Phenols Calibration Mix/ Restek 30129
CAS Number	*None*	*None*
Physical Properties	*[10]*	*[11]*
Appearance	*No Data Available*	*No Data Available*
Odor	Mild	Mild
Odor Threshold	*No Data Available*	*No Data Available*
pH	*Not Applicable*	*Not Applicable*
Melting Point, °C	-43.82	-98
Boiling Point, °C	81.6 at 760 mmHg	64.7 at 760 mmHg
Flash Point, °C	43	52
Evaporation Rate	*No Data Available*	*No Data Available*
Flammability (Solid, Gas)	Highly Flammable	Highly Flammable
Upper Explosion Limit	16	36
Lower Explosion Limit	4.4	6
Vapor Pressure	*No Data Available*	*No Data Available*
Vapor Density	1.4 (air = 1)	1.1 (air = 1)
Relative Density	0.7857 g/cm^3 at 20 °C	0.791 g/cm^3 at 20 °C
Water Solubility	*Not Determined*	*Moderate; 50-99%*
Partition Coefficient (n-octanol/water)	*No Data Available*	*No Data Available*

Auto-ignition Temperature, °C	*No Data Available*	*464*
Decomposition Temperature	*No Data Available*	*No Data Available*
Viscosity	*None*	*None*
Explosive Properties	*None*	*None*
GHS Hazard Pictogram		
Hazards Identification	Highly flammable liquid and vapor. Harmful if swallowed or in contact with skin. Causes serious eye irritation.	Highly flammable liquid and vapor. Toxic if swallowed, in contact with skin, or if inhaled. May cause an allergic skin reaction. Suspected of causing cancer. Causes damage to organs.
First Aid Measures	**If inhaled,** move person to fresh air. If not breathing, give artificial respiration. Seek medical attention. **In case of skin contact**, wash with plenty of water and soap. Remove all contaminated clothing immediately. **In case of eye contact,** rinse cautiously with water for several minutes. Seek medical attention. **If swallowed,** rinse mouth with water. Drink two glasses of water or milk to dilute. Call a poison center or consult a physician.	**If inhaled,** move person to fresh air. If not breathing, give artificial respiration. Seek medical attention. **In case of skin contact**, wash with plenty of water and soap. Remove all contaminated clothing immediately. Seek medical attention if irritation develops. **In case of eye contact,** rinse cautiously with water for several minutes. Seek medical attention. **If swallowed,** rinse mouth with water. Drink two glasses of water or milk to dilute. Call a poison center or consult a physician.

Table 13. Summarized MSDS for Internal Deuterated Standards from Santa Cruz Biotechnology.

Product Name	Estradiol-d2	Chlorophenol-d4
CAS Number	53866-33-4	285132-91-4
Physical Properties	*[12]*	*[13]*
Appearance	Solid	Solid
Odor	*No Information Available*	*No Information Available*
pH	*No Information Available*	*No Information Available*
Melting Point, °C	*No Information Available*	*No Information Available*
Boiling Point, °C	*No Information Available*	*No Information Available*
Flash Point	*No Information Available*	*121 (Closed Cup)*
Evaporation Rate	*No Information Available*	*No Information Available*
Flammability (Solid, Gas)	*No Information Available*	*No Information Available*
Vapor Pressure	*No Information Available*	*No Information Available*
Vapor Density	*No Information Available*	*No Information Available*

Relative Density Water Solubility Partition Coefficient (n-octanol/water) Auto-ignition Temperature Decomposition Temperature Viscosity Explosive Properties	*No Information Available* *No Information Available* *No Information Available* *No Information Available* *No Information Available* *No Information Available* *No Information Available*	*No Information Available* *No Information Available* *No Information Available* *No Information Available* *No Information Available* *No Information Available* *No Information Available*
GHS Hazard Pictogram		Not Classified
Hazards Identification	Harmful if swallowed. Harmful in contact with skin. Harmful if inhaled. May cause cancer. May damage fertility or the unborn child.	Not Classified
First Aid Measures	**If inhaled,** move person to fresh air. If not breathing, give artificial respiration. **In case of skin contact,** wash with water and soap. Remove all contaminated clothing. **In case of eye contact,** wash with plenty of water. **If swallowed,** rinse mouth. Call a poison center or consult a physician.	**If inhaled,** move person to fresh air. If not breathing, give artificial respiration. **In case of skin contact,** wash with water and soap. Remove all contaminated clothing. **In case of eye contact,** wash with plenty of water. **If swallowed,** clean mouth with water. Never give anything by mouth to an unconscious person.

9.2. Proper Chemical Waste Disposal

NOTE: *Dispose of wastes properly following the Institute of Chemistry Waste Management System Manual [14]. Always label waste containers to prevent the generation of unknown wastes. Wastes should only be stored in 4-L glass bottles or 20-L carboys. Waste containers must not be completely filled to avoid accidental spills during waste hauling and transport. When the waste containers are ¾ full, initiate a request for collection by submitting a Waste Inventory Form to the Institute's Pollution Control Officer.*

9.2.1. Gloves used for cleaning of glassware and tissue papers used to wipe wet areas may be disposed of in regular trash bins.

9.2.2. Empty solvent bottles should be labelled "J201 - Empty Reagent Bottles".

9.2.3. Used pipette tips, filters, and SPE cartridges should be disposed of in containers labelled “K301 Solidified Wastes and Polymerized Wastes”. Gloves used to handle chemicals and tissue papers contaminated with chemicals should also be disposed of in containers labelled “K301 Solidified Wastes and Polymerized Wastes”.

9.2.4. Expired analytical standards and internal deuterated standards should be disposed of in containers labelled “M503 - Pharmaceuticals and Drugs”.

9.2.5. Solvents should be disposed of in containers labelled “G704 - Non-halogenated Organic Wastes”.

9.2.6. Oil from the LC-MS/MS fore pump should be labelled as “I101 - Waste Oils”.

10. References

[1] Water Research and Management Center, Institute of Chemistry, University of the Philippines Diliman. 2024. *“Manual of Procedures for Sampling and Nutrient Measurements in Philippines Lakes”*. Quezon City, Philippines. Accessed through https://095838df-ac32-4a65-a986-6424f6dc3351.filesusr.com/ugd/40449f_f6ae7da47aa54752b1912c7e46464606.pdf

[2] Water Research and Management Laboratory, Institute of Chemistry, University of the Philippines Diliman. 2019. *“Manual of Procedures for Sampling, Sample Preparation and Analysis of Antibiotics and Hormones in Laguna Lake Water”*. Quezon City, Philippines. Accessed through https://095838df-ac32-4a65-a986-6424f6dc3351.filesusr.com/ugd/40449f_d9797266965740598a86c8de0c62966f.pdf

[3] Institute of Chemistry, University of the Philippines Diliman. 2018. *“Institute of Chemistry Safety Manual”*. Accessed through https://chemistry.science.upd.edu.ph/wp-content/uploads/2024/06/INSTITUTE-Laboratory-Safety-Guide_revised.pdf

[4] Scharlau. 2024. *“Safety Data Sheet for Methanol, LC-MS, suitable for nitrosamine analysis”*. Accessed through https://old.scharlab.com/docs/msds/EN/ME0326_EN.pdf

[5] Scharlau. 2024. *“Safety Data Sheet for Acetonitrile, LC-MS”*. Accessed through https://old.scharlab.com/docs/msds/EN/AC0371_EN.pdf

[6] Scharlau. 2024. *"Safety Data Sheet for Ammonia, solution 25%, eluent additive for LCMS"*. Accessed through https://old.scharlab.com/docs/msds/EN/AM0258_EN.pdf

[7] Scharlau. 2024. *"Safety Data Sheet for Ammonium acetate, eluent additive for LCMS"*. Accessed through https://old.scharlab.com/docs/msds/EN/AM0259_EN.pdf

[8] Scharlau. 2024. *"Safety Data Sheet for Formic acid, eluent additive for LCMS"*. Accessed through https://old.scharlab.com/docs/msds/EN/AC1076_EN.pdf

[9] Scharlau. 2024. *"Safety Data Sheet for Acetic acid glacial, eluent additive for LCMS"*. Accessed through https://old.scharlab.com/docs/msds/EN/AC0347_EN.pdf

[10] Restek. 2023. *"Safety Data Sheet for 31117 / Steroids and Mixed Pharmaceuticals Mix"*. Accessed through https://www.restek.com/ccstorex/custom/v1/documents/sds/31117/31117_USENG.pdf

[11] Restek. 2023. *"Safety Data Sheet for 31029 / 604 Phenols Calibration Mix"*. Accessed through https://www.restek.com/ccstorex/custom/v1/documents/sds/31029/31029_USENG.pdf

[12] Santa Cruz Biotechnology, Inc. 2016. *"Safety Data Sheet for ß-Estradiol-d2"*. Accessed through https://datasheets.scbt.com/sds/aghs/en/sc-234879.pdf

[13] Santa Cruz Biotechnology, Inc. 2023. *"Safety Data Sheet for 4-Chlorophenol-d4"*. Accessed through https://datasheets.scbt.com/sds/aghs/en/sc-505100.pdf

[14] *"Institute of Chemistry Waste Management System Manual"*

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