Trace Organic Analysis

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Trace Organic analysis

Stage 1 Sampling  Separation of the sample from its environment
Sample collection, storage and preservation (use of inert containers; stabilize samples, if necessary)

Stage 2 Isolation/extraction
Separation of the determinand from its major matrix Concentration step 1

Stage 3 Clean-up
Separation of the determinands from other co-extracted major/minor components Concentration step 2

Stage 4 Gas chromatography-ECD/NPD/FID or High performance chromatography DAD/FLD
Separation of the determinands from similar compounds using specific detection.

Stage 6 Reporting of results
Classification of analytes

• Analytes may be divided into various classes, the most basic classification is between
• Organic Analytes (e.g., Carbon & Hydrogen containing)
• Inorganic Analytes (e.g., metals and ions)

Gross Quantification Of Organic Compounds

• Total Organic Carbon - Carbon Analyzer
  Conversion of the organic carbon to carbon dioxide (CO₂) by either catalytic conversion or wet chemical oxidation, the CO₂ formed is then either measured directly by an Infrared Detector or by conversion to methane (CH₄) and measured by Gas Chromatograph (GC) with a Flame Ionization Detector (FID).

• Soluble Organic Compounds – Benzene Soluble Fraction
  Soxhlet extraction with Benzene followed by gravimetric quantification.
• Compounds Quantified As A Group Or Class (Based On Some Classification) not requiring Separation Into Individual Compounds
  • **Adsorbable Organic Halides – AOX**
    • Organic Halides (Excluding Fluorine-containing Species),
    • Adsorbed By Granular Activated Carbon, And
    • Detected By Microcoulometry.

• **Purgeable Organic Halides – POX**
  • Organic Halides (Excluding Fluorine-containing Species),
  • Purged Into Pyrolysis Furnace Using Stream Of N₂ Or O₂, And
  • Detected By Microcoulometry.

• **Extractable Organic Halides – EOX (excluding Fluorine Containing Species)**
  • Solid Wastes, Soils, And Suspended Solids Isolated From Industrial Wastewater,
  • Extracted With Ethyl Acetate By Sonification To Isolate Organic Halides And Detected By Pyrolysis / Microcoulometry.
Selection of methodology for solid & liquid matrices

**Volatile**
- BTEX, EDB, DBCP, Nonhalogenated volatile’s halogenated volatile, Acetone, Acrylonitrile, Acetonitrile

**Semivolatile and Non Volatile**
- Organics - Extractable OCs, OPs, SPs, carbamates, PAH, PCBs, Dioxins

**Physical state**
- **Sample**
  - Is sample to be analyzed for extractable or volatile

**Sample preparation**
- Aqueous: Purge & trap, Azetropic Distillation, Vacuum distillation
- Solid: Automated headspace, Purge & Trap
- Oily liquid: Solvent dilution

**Extraction**
- Aqueous: Sep. funnel L/L Continuous L/L Solid Phase (SPE)
- Solid: Soxhlet Extraction, Automated Soxhlet Extraction Microwave, Ultrasonic, SFE
- Oily liquid: Solvent dilution, SFE

**Clean up**
- Silica gel, Alumina, Florosil, Sulfuric acid clean up

**Analysis Procedure**
- GC, HPLC, GC-MS
Extraction techniques

Semi Volatile Organic Compounds (SVOC’s—pesticides, phenols, PCB’s, Dioxins, PAH)

Liquid Liquid extraction—Separatory funnel extraction, continuous liquid-liquid extraction

Solid Liquid extraction—Soxhlet extraction, automated soxhlet extraction, ultrasonic extraction

Solid phase extraction (SPE)

Solid phase microextraction (SPME)

Super Critical fluid extraction (SPFE)
Liquid–liquid extraction is required when the analyte is present at a low concentration in a water sample, e.g. river water.

It is used to pre-concentrate the analyte from a large volume of water into a small sample volume.

In addition, or at the same time, LLE can be used to clean-up the analyte from its matrix.
Problems with L/L extraction

• Formation of emulsions is troublesome, particularly for samples that contain surfactants or fatty materials.
• Emulsion can be broken up with centrifugation, filtration through a glass wool plug, refrigeration, salting out or the addition of a small amount of a different organic solvent.
• In addition, the rate of extraction may be different for the same analyte depending on the nature of the sample matrix.
• Controlling the level of contamination is crucial. High purity solvents should be used (as any subsequent concentration the analyte of interest may also concentrated the impurity as well) to wash all concentrate associated glassware thoroughly.
• As well as contamination, care should also be exercised to minimise analyte losses due to adsorption on glass containers.
Continuous liquid liquid extraction

Typically, a 1L sample, pH adjusted if necessary, is added to the continuous extractor. Then organic solvent, e.g. dichloromethane (in the case of a system in which the solvent has a greater density than the sample), of volume 300-500 ml is added to the distilling flask together with several boiling chips. The solvent is then boiled, in this case with a water bath, and the extraction process allowed to occur for between 18-24 hours. After completion of the extraction process, and sufficient cooling time, the boiling flask is detached and solvent evaporation can then occur.
Soxhlet extraction

Soxhlet apparatus assembled. Solvent placed in round-bottomed flask on an isomantle.

Sample, e.g. soil, accurately weigh and mixed with similar weight of anhydrous sodium sulfate.

Sample plus dispersion agent placed in porous extraction thimble.

Heat applied via isomantle. Typical solvent circulation rate is four cycles per hour.

Sample refluxed for an appropriate amount of time, e.g. 6–24 h.

After completion of the extraction, solvent containing the analytes is retained.

Analysis – GC or HPLC.
**Soxhlet Extraction of Polycyclic Aromatic Hydrocarbons from Contaminated Soil**

**Extraction Conditions**

- Sample: 10 g, plus 10 g anhydrous sodium sulfate
- Solvent: 150 ml dichloromethane
- Extraction time: 24 h

*The sample is heated by using an isomantle. Typically, refluxing of the solvent occurred at the rate of four cycles per hour. Extracts are concentrated to 10 ml using a rotary evaporator and then analysed*
Ultrasonic extraction

Ultrasonic probe or bath

Sample, e.g., soil, accurately weighed and placed in a glass vessel. Solvent (dichloromethane (DCM), etc.) added to sample (typically 5 ml per 2 g of sample)

Ultrasonic agitation of sample plus solvent for ca. 3–5 min

Solvent removed (centrifugation and/or filtration) and fresh solvent added – process repeated

All solvent extracts combined

Analysis – GC or HPLC
Solid Phase Extraction SPE

Solid phase extraction cartridge
Single arm flask apparatus

Vacuum Manifold for solid phase extraction of multiple cartridges
Solid phase extraction

**CONDITIONING**
Conditioning the sorbent prior to sample application ensures reproducible retention of the compound of interest (the isolate).

**RETENTION**
- Adsorbed isolate
- Undesired matrix constituents
- Other undesired matrix components

**RINSE**
△ Rinse the columns to remove undesired matrix components

**ELUTION**
- Undesired components remain
- Purified and concentrated isolate ready for analysis
# Solid Phase Extraction

Commonly available silica Bonded sorbents

<table>
<thead>
<tr>
<th>Phase</th>
<th>Bonded moiety</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonpolar phases</strong></td>
<td></td>
</tr>
<tr>
<td>Cl, methyl</td>
<td>Si-CH₃</td>
</tr>
<tr>
<td>C₈, octyl</td>
<td>Si-(CH₂)₇-CH₃</td>
</tr>
<tr>
<td>C₁₈, octadecyl</td>
<td>Si-(CH₂)₁₇-CH₃</td>
</tr>
<tr>
<td><strong>Polar phases</strong></td>
<td></td>
</tr>
<tr>
<td>Si, silica</td>
<td>Si-OH</td>
</tr>
<tr>
<td>CN, cyanopropyl</td>
<td>Si-CH₂-CH₂-CH₂-CN</td>
</tr>
<tr>
<td>2OH, diol</td>
<td>Si-CH₂-CH₂-CH₂-O-CH₂-CHOH-CH₂OH</td>
</tr>
<tr>
<td><strong>Ion-exchange phases</strong></td>
<td></td>
</tr>
<tr>
<td>SCX, benzenesulfonic acid</td>
<td>Si-CH₂-CH₂-CH₂-C₆H₄SO₃⁻</td>
</tr>
<tr>
<td>DEA, diethylammoniopropyl tertiary amine</td>
<td>Si-CH₂-CH₂-CH₂-NH⁺ -(CH₂-CH₃)₂</td>
</tr>
<tr>
<td>SAX, trimethylammoniopropyl quaternary amine</td>
<td>Si-CH₂-CH₂-CH₂-N⁺-(CH₃)₃</td>
</tr>
</tbody>
</table>
Normal phase sorbents have polar functional groups, e.g. cyano, amino and diol –polar compounds, e.g. phenol, will be retained.

Reversed phase sorbents have non-polar functional groups, e.g. octadecyl, octyl and methyl, and are more likely to retain non-polar compounds e.g. polycyclic aromatic hydrocarbons.

Ion exchange sorbents have either cationic or anionic functional groups and when in the ionized form attract compounds of the opposite charge.

A cation exchange phase, such as benzenesulfonic acid, will extract an analyte with a positive charge (e.g. phenoxyacid herbicides).
<table>
<thead>
<tr>
<th>Solvent strength for normal-phase sorbents</th>
<th>Solvent strength for reversed-phase sorbents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongest</td>
<td>Hexane</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>Isooctane</td>
</tr>
<tr>
<td>Ethyl acetae</td>
<td>Toluene</td>
</tr>
<tr>
<td>Acetone</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td></td>
</tr>
</tbody>
</table>
Solid Phase Microextraction
Super Critical Fluid Extraction

At the critical point (critical temperature and pressure) no liquefaction will take place on raising pressure and no gas will be formed on increasing temperature three phases coexist in equilibrium.
Volatile Compounds

- Volatile compounds e.g. BTEX in the atmosphere, workplace and on industrial sites need to be monitored with regard to safety considerations, e.g. emissions to the atmosphere, or occupational standards.

VOC’s can be
- Liberated from a water sample and then trapped (e.g. via purge-and-trap)
- Or trapped either on a solid support material (e.g. for thermal desorption) prior to analysis
Purge and trap
Purge and trap

Purge-and-trap is widely used for the extraction of volatile organic compounds from aqueous samples: this is then followed by gas chromatography.

• Aqueous sample (typically 5 ml) taken into a glass sparging vessel.
• High purity nitrogen gas bubbles passed through the sample to remove the volatile analytes to be trapped. Typical flow rates are 40–50 ml min⁻¹ for 10–12 min
• Gas travels through the trap to remove excess water (1–11 min) analytes retained on trap, e.g. ‘Tenax’
• Trap is rapidly heated (220°C) with no flow
• Trap is back-flushed to remove analytes and transport to column. Typical conditions: trap-desorb temperature, 225°C; desorb time, 2–4 min; desorb flow rate, 1–2 ml min⁻¹ for narrow-bore column

Trap is baked at 230°C for 8 min (or more) to remove contaminants and residual water
Thermal desorption

Sample heated (approximately 100°C) in a flowing carrier gas stream. Analytes focused, prior to GC, by cryogenic trapping or on a solid-phase sorbent, e.g. ‘Tenax’ (or a combination of both)

Analytes desorbed by rapid heating or passage of a carrier gas stream

Thermal desorption unit connected directly to the gas chromatograph via the injection port, directly to the column or through a low-volume injector
Preconcentration

Pre-concentration is concerned with the reduction of a larger sample into a smaller sample size. It is most commonly carried out by using solvent evaporation procedures after an extraction technique.

Nature of analytes (Heat stable/labile)

Solvent used in extraction (Low / High Boiling point)

The most common approaches for solvent evaporation are rotary evaporation, Kuderna–Danish evaporative concentration, or gas ‘blow-down’.

Kuderna Danish Evaporator (Heat stable + low boiling points)-
Rotary evaporator (Heat labile +High boiling point)
Rotary evaporator with circulating chilled liquid(Heat labile + low boiling point)
Clean Up

- **Removal of extraneous materials** that are co-extracted from the matrix with the analyte, which do not allow resolution and quantitation of peaks
  - **Adsorption Chromatography** - separating analytes of a relatively narrow polarity range away from extraneous, interfering peaks of a different polarity.

- **Alumina** EPA Methods 3610 and 3611 - remove phthalate esters.

- **Florisil** EPA Method 3620 - separate organochlorine pesticides from aliphatic compounds, aromatics, and nitrogen-containing compounds.

- **Silica gel EPA** Method 3630 - separate single component organochlorine pesticides from some interferants.

- **Solid phase extraction cartridges** have been added as an option. Most of the environmental and waste samples will require additional preparation before analysis. The specific cleanup procedure used will depend on the nature of the sample to be analyzed and the data quality objectives for the measurements.
Instrumental Analysis for Analytical techniques

The following instrumental techniques are available for trace organic analysis (viz. Pesticides, Polyaromatic Hydrocarbons, PCB’s, Dioxins, Phenols etc.):

a) Gas Chromatography (GC)
b) Gas Chromatography/Mass Spectrometry (GC/MS)
c) Liquid Column Chromatography
d) High Performance (Pressure) Liquid Chromatography (HPLC)
e) Total Organic Carbon (TOC)

Most analytical techniques are common to water, air, soil and biological samples.

The two most common techniques for organic analysis environmental samples in the sample extract are gas chromatography (GC) and high performance liquid chromatography (HPLC).
Chromatographic Techniques

**Difference between GC and HPLC?** The essential difference between the two techniques is the nature of the partitioning process.

- In GC, the analyte is partitioned between a stationary phase and a gaseous phase, whereas in HPLC the partitioning process occurs between a stationary phase and a liquid phase.

Separation is therefore achieved in both cases by the affinity of the analyte of interest with the stationary phase. The higher the affinity, then the more the analyte is retained by the column.

- The choice of which technique is employed is largely dependent upon the analyte of interest. For example, if the analyte is thermally labile, does not volatilize at temperatures up to 250°C and is strongly polar, then GC is not the appropriate technique. However, HPLC can then be used (and vice versa).
Gas Liquid chromatography

- Gas chromatography (GC) is an analytical technique for separating compounds based primarily on their volatilities.
- It is based on a differential partitioning of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium).
- Sample vaporized by injection into a heated system, eluted through a column by inert gaseous mobile phase and detected.
- It provides both qualitative and quantitative information for individual compounds present in a sample.
Gas Liquid Chromatography

**Carrier gas:** He (common), N₂, H₂

**Pressure inlet:** 10-50 psig

**Flow** = 25-150 mL/min packed column

**Flow** = 1-25 mL/min open tubular column

**Injector**: Split Splitless

**Column**: 2-50 m coiled stainless steel/glass/Teflon

**Oven**: 0-400 °C ~ average boiling point of sample accurate to <1 °C

**Detectors**: FID, NPD, ECD, MS
# Gas Liquid Chromatography

<table>
<thead>
<tr>
<th>Detector</th>
<th>Type</th>
<th>Support gases</th>
<th>Selectivity</th>
<th>Detectability</th>
<th>Dynamic Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame Ionization (FID)</td>
<td>Mass flow</td>
<td>Hydrogen and air</td>
<td>Most organic cmpds.</td>
<td>100 pg</td>
<td>$10^7$</td>
</tr>
<tr>
<td>Thermal Conductivity (TCD)</td>
<td>Concentration</td>
<td>Reference</td>
<td>Universal</td>
<td>1 ng</td>
<td>$10^7$</td>
</tr>
<tr>
<td>Electron Capture (ECD)</td>
<td>Concentration</td>
<td>Make-up</td>
<td>Halides, nitrates, nitriles, peroxides, anhydrides, organometallics</td>
<td>50 fg</td>
<td>$10^5$</td>
</tr>
<tr>
<td>Nitrogen-phosphorus</td>
<td>Mass flow</td>
<td>Hydrogen and air</td>
<td>Nitrogen, phosphorus</td>
<td>10 pg</td>
<td>$10^6$</td>
</tr>
<tr>
<td>Flame Photometric (FPD)</td>
<td>Mass flow</td>
<td>Hydrogen and air</td>
<td>Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium</td>
<td>100 pg</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Photo-ionization (PID)</td>
<td>Concentration</td>
<td>Make-up</td>
<td>Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics</td>
<td>2 pg</td>
<td>$10^7$</td>
</tr>
</tbody>
</table>
High Performance Liquid Chromatography

A column, typically 25 cm long with an internal diameter of 4.6 mm -C8 or a mobile phase. The column is normally located in an oven which is maintained at ca. 30°C. The latter is a water–organic solvent system(methanol or acetonitrile). Pump which is pumped by using a reciprocating or piston pump at a flow rate of 1 ml min⁻¹.
Normal Phase vs Reverse Phase Chromatography

Analyte partitions between mp and sp based upon polarity
Stationary Phase (column packing) is polar
• silica (strongest) > amino > diol > cyano (weakest)
Mobile Phase (solvent) is non-polar
• hexane, iso-octane, methylene chloride, ethyl acetate, etc.

More polar compounds are more retained. Retention decreases as polarity of mobile phase increases

Reasons
Different from RPLC
Analyze very polar compounds
Sample injection solvent is non-polar (e.g., extraction from water)
Recovery in non-polar solvents is desirable (e.g., prep sep’n)
Isomer separation (steric interaction with stationary phase)
Reverse Phase Chromatography

Principle: Partition of analytes between polar mobile phase and non-polar stationary phase
Nonpolar (nonspecific) interactions of analyte with hydrophobic (or lipophilic) stationary phase:
– C18, C8, Phenyl, C3, etc.
Different sorption affinities between analytes results in their separation
– More polar analytes are less retained
– Analytes with larger hydrophobic part are retained longer
Mobile phase: water (buffer) + water-miscible organic solvent e.g. MeOH, ACN
Can be used for non-polar, polar, ionizable and ionic molecules
Gradient elution is often used for analysis of compounds with large differences in polarity
Quality Assurance Trace Organic Analysis.

Quality assurance is about getting the correct result. In order to achieve good accuracy (the closeness of a measured value to the 'true' value) and precision (the measure of the degree of agreement between replicate analyses of a sample), in the laboratory at least, it is desirable that a good quality assurance scheme is operating.

- To select and validate appropriate methods of analysis.
- To maintain and upgrade analytical instruments.
- To ensure good record keeping of methods and results.
- To ensure quality of data produced.
- To maintain a high quality of laboratory performance.
The quality of data produced in the laboratory is controlled by the use of a good quality control procedure.

- **Certification of operator competence**- This is intended to assess whether a particular operator can carry out sample and standard manipulations, operate the instrument in question and obtain data of appropriate quality.

- **Analysis of certified reference materials (CRM)**- is a substance for which one or more analytes have certified values, produced by a technically valid procedure, accompanied with a traceable certificate and issued by a certifying body.

National Institute for Standards and Technology (NIST) Washington DC, USA; the Community Bureau of Reference (known as BCR), Brussels, Belgium; and, the Laboratory of the Government Chemist (LGC), London, UK.
• **Recovery** - is the factor or percentage of the total amount of analyte obtained after one or more manipulative stages of the method. A key parameter in determining whether the method is suitable for use in trace analysis relates to smallest amount of an analyte that can be detected.

• **Analysis of reagent blanks** - Analyse reagents whenever the batch is changed or a new reagent introduced. Introduce a minimum number of reagent blanks (typically 5% of the sample load); this allows reagent purity to be assessed and if necessary controlled and also acts to assess the overall procedural blank.

• **Analysis of duplicates** - This allows the precision of the method to be reported.

• **Limit of Detection** - lowest amount of an analyte in a sample which can be detected but not necessarily quantified.

• **Limit of Quantification** - lowest amount of an analyte in a sample which can be quantified with acceptable uncertainty but not necessarily quantified.
• **Linearity Range** - Plot response of known standard solutions against concentration.

• **Calibration with standards** - A minimum number of standards should be used to generate the analytical curve, e.g. 6 or 7. Daily verification of the working curve should also be done using one or more standards within the linear working range.

• **Stock Solutions Of Individual Compounds**

Stock solution of individual compound of interest is prepared by dissolving standard compound in suitable solvent (usually the solvent used for sample extraction).

**Working solution of individual compounds**

Stock solution of individual compound is diluted in steps to get the working solution of such concentration which is within detector range of the detector and can match the expected concentrations in the samples to be analysed.
Determining Retention Time of Individual Compounds

Working solution of individual compound is injected and its retention time is observed. Retention times of all compounds of interest is thus observed and recorded.

Mixture solution of individual compounds is injected and after getting the integration report, the amounts or concentrations of individual compounds is assigned to the respective peaks. The instrument calculates the factor of peak area/ peak height to compound concentration/amount for each compound. Using these factors the instrument calculates the compound concentration/ amount in samples.
Pesticide residue Analysis

1000 mL of Ground Water + 20% NaCl

Liquid-liquid partitioning with 50, 25, 25 mL DCM

Aqueous layer
(For phenoxy acid herbicides)

10 mL conc. H₂SO₄ (pH < 1)

Liquid-liquid partitioning with 100, 50, 50 mL DCM Organic layer

Solvent Exchange to Acetonitrile / Methanol

Final volume with Acetonitrile for HPLC analysis

Organic Layer

Concentrate to 0.5 mL

Solvent Exchange to Hexane

Analysis by GC-Ocs Ops and Pyrethroids.
## Antibiotic residues in Honey

<table>
<thead>
<tr>
<th></th>
<th>Oxytetracycline</th>
<th>Chloramphenicol</th>
<th>Ampicillin</th>
<th>Ciprofloxacin</th>
<th>Enrofloxacin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL (μg/kg)</td>
<td>25</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Samples analysed</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>No. samples tested positive</td>
<td>6</td>
<td>4</td>
<td>9</td>
<td>2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>% of samples above MRL</td>
<td>50</td>
<td>33</td>
<td>75</td>
<td>17</td>
<td>83</td>
<td>42</td>
</tr>
<tr>
<td>&lt;LOD</td>
<td>6</td>
<td>8</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>0-100ppb</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>100-200ppb</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>&gt;200ppb</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
Antibiotic residues in Honey

Figure 1. Antibiotic residues detected in percent honey samples

- Oxytetracycline: 50%
- Chloramphenicol: 25%
- Ampicillin: 67%
- Ciprofloxacin: 8%
- Enrofloxacin: 83%
- Erythromycin: 42%
- Antibiotic residues in Honey

**Stock standard solutions (100ppm) of** enrofloxacin and ciprofloxacin dissolved in acetonitrile: 2% acetic acid in water (16:84) and diluted to volume.

**Working standard solutions (1-100ppb)**

2.5 g (±0.03g) of honey into 50mL capped centrifuge tube+5mL of Milli-Q water and vortexed for 1 minute until all of the honey dissolved into the water thoroughly.

10mL of acetonitrile and 200mL acetic acid were then added to the sample, capped tightly and the tubes vortexed for approximately 30 seconds.

After that 2g of NaCl was added and again vortexed for 15 seconds centrifuged at 2,400 rpm for 5 minutes.

Upper organic layer was carefully transferred to another tube by using disposable pipettes.

Samples were evaporated to dryness with a controlled N₂ flow drier at 55°C before being reconstituted into 1mL of Acetonitrile/2% acetic acid in water (16:84), vortexed and sonicated.

HPLC-FLD
Antibiotic residues in Honey

**Analysis by** HPLC equipped with Fluorescence (FLD) detector

- Column - Zorbax ODS C18 5mm (250 x 4.6mm I.D.), at room temperature (25°C) in gradient conditions given below.

- Detector - FLD detector (excitation wavelength-295nm and emission wavelength-500nm)

- Flow rate was 1mL/min.

- The sample injection volume was 100μL.

- Retention time was 5.4 min for ciprofloxacin and 6.6 min for enrofloxacin.
HPLC Chromatogram of (a) Enrofloxacin (50 ng/mL) & its metabolite ciprofloxacin standard (100 ng/mL) (b) Enrofloxacin detected in Sample (011)