Research

Standard Operating Procedure for Trace Analysis of Organic Contaminants by Liquid Chromatography Coupled to Mass Spectrometry (LC-MS), a Part of Nanostructurome Methods Pipeline

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Abstract:
This Standard Operating Procedure (SOP) provides guidelines for the trace analysis of organic contaminants in different matrices using Liquid Chromatography coupled to Mass Spectrometry (LC-MS). It covers key steps such as sample collection, preparation, and extraction, followed by chromatographic separation and mass spectrometric analysis. The SOP ensures accurate quantification and identification of contaminants by employing calibrated standards, internal standards, and quality control measures. The procedure emphasizes adherence to safety protocols, instrument maintenance, and troubleshooting for consistent, reliable results, meeting both regulatory and quality assurance standards for analytical performance. This SOP is a part of Nanostructurome methods pipeline.

Keywords: Liquid chromatography; Mass spectrometry; Extraction; Organic contaminant; Trace analysis

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1. Purpose

This Standard Operating Procedure (SOP) outlines the steps to be followed for the trace analysis of organic contaminants in various matrices, such as water, soil, food, and biological samples, using liquid chromatography coupled to mass spectrometry (LC-MS). The procedure ensures the accurate and reproducible quantification and identification of contaminants at trace levels (**Figure 1**). LC-MS/MS is employed due to its high sensitivity and selectivity, with the ability to detect a broad range of compounds in complex matrices. This SOP was developed for the purpose of Nanostructurome methods pipeline.

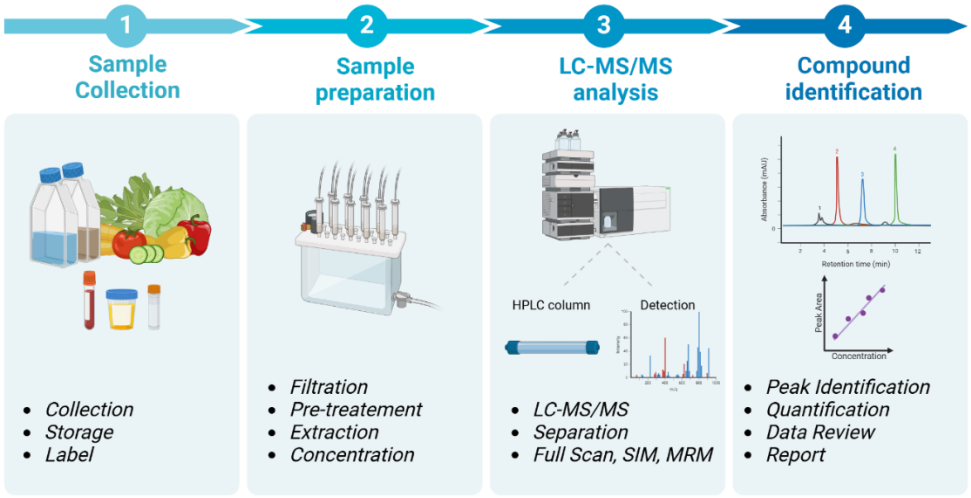

Figure 1. Steps of SOP for LC-MS/MS analysis.

2. Scope

This SOP is applicable to the analysis of organic contaminants, such as pesticides, pharmaceuticals, and industrial chemicals in liquid and solid samples. It includes sample preparation, LC-MS analysis, and data interpretation. Applicable sample types include:

- Water samples: surface water, groundwater, wastewater, drinking water
- Solid matrices: soil, sediments, biosolids
- Food and agricultural products: Fruits, vegetables, grains
- Biological samples: plasma, urine

3. Involved Personnel

Involved Personnel include the following:

- Analyst: Responsible for performing the analysis, following this SOP, ensuring instrument calibration and documenting all relevant data.
- Quality Control (QC) Officer: Responsible for ensuring compliance with quality standards, reviewing results, validating calibration and method performance, and identifying any errors that require additional actions.
- Laboratory Supervisor: Responsible for overall procedure adherence, including laboratory safety measurements, regulatory guidelines, equipment maintenance, and troubleshooting.

4. Materials, Equipment and Supplies

The SOP requires the following:

- Liquid Chromatograph (e.g., HPLC, UPLC) equipped with a suitable column (e.g., C18, reverse phase).
- (Hybrid) Mass Spectrometer (e.g., QTrap: Quadrupole Ion Trap, Orbitrap, tQ: triple Quadrupole or ToF: Time of Flight) with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source.
- Solvents: HPLC-grade methanol, acetonitrile and water. Additives or buffers such as 0.1% formic acid, ammonium acetate, ammonium formate or ammonium fluoride may be used to enhance separation efficiency and ionization.
- Standards: Certified Reference Materials (CRMs) for target organic contaminants prepared as individual solutions after weighing and dissolving a proper amount of each compound in a specific solvent.
- Internal Standards: Stable isotope-labelled compounds or structurally similar analogues for quantification to correct for matrix effects and variability in ionization.
- Consumables: Sample vials (e.g., glass vials with PTFE-lined caps), syringes, and filters (e.g., 0.22 or 0.45 μm, PTFE or nylon filters), solid-phase extraction (SPE) cartridges (if applicable for sample clean-up), pipettes and volumetric flasks (for precise solvent and standard preparation).
- Calibration standards: Prepare at different concentrations of target analytes to generate calibration curves for quantification.

5. Procedure for Sample Collection and Storage, Preparation and Analysis

5.1. Sample Collection and Storage

5.1.1. Collect samples in clean, contaminant-free containers. Containers can be glass or plastic (High-Density Polyethylene and Polypropylene) according to target analyte polarity to prevent adsorption.

5.1.2. Transport samples in temperature-controlled conditions, ensuring minimal exposure to light and contamination.

5.1.3. Store samples according to matrix requirements:

- Water samples: Refrigerate at 4°C and analyze within 7 days (unless preserved).
- Biological samples: Freeze at -20°C or lower to prevent degradation.
- Soil and sediment samples: Store at 4°C and dry, if necessary, before extraction.

5.1.4. Label and document all samples, including sample type, location, date and time, storage conditions and other relevant metadata.

5.1.5. Use of appropriate preservatives if required.

5.2. Sample Preparation

5.2.1. Spiking with Internal Standard: Spike a known quantity of internal standard (stable isotope-labelled compounds or analogues) to the sample. This step helps to monitor the extraction efficiency and ensures accurate quantification.

5.2.2. Filtration: Filter liquid samples through a 0.45 μm membrane to remove particulate matter.

5.2.3. Sample Pre-treatment: Deconjugation for biological samples: For biological samples such as urine or plasma, deconjugation may be necessary to release conjugated analytes (e.g., glucuronides or sulphates) through enzymatic or acid hydrolysis to ensure the target analytes are in their free forms for accurate quantification. For water and other aqueous samples, adjust the pH to the desired pH (acidic or basic) using suitable reagents (e.g., HCl for acidic conditions and NaOH for basic conditions). Adjusting the pH can improve extraction efficiency, stability, and recovery of specific analytes, as some compounds are more stable or extractable under specific pH conditions. For aqueous samples, the addition of preservatives such as EDTA or a chelating agent is optional to prevent the formation of complexes with metallic ions.
5.2.4. Extraction (if applicable): For solid samples, extract contaminants using appropriate solvents (e.g., acetonitrile, methanol). Extraction techniques may include sonication, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) or microwave-assisted extraction, depending on the sample type and target analytes. For aqueous samples, consider solid phase extraction (SPE) or direct dilution.
5.2.5. Concentration: Evaporate solvent under gentle nitrogen flow or a rotary evaporator to concentrate the sample to an appropriate volume.
5.2.6. Reconstitution: Reconstitute the extracted sample in an appropriate solvent (e.g., 50:50 mixture of water and acetonitrile) prior to analysis

5.3. Instrument Setup

5.3.1. LC System: Set the appropriate chromatographic conditions (flow rate, column temperature, mobile phase composition) based on the chemical properties of target analytes.

- Choose an LC column that is suitable for the target analytes and is equilibrated adequately with the mobile phase prior to sample injection to ensure reproducible results.
- Ensure pressure and temperature are within expected/optimal ranges to prevent column damage or degradation of compounds during the analysis.

5.3.2. Mass Spectrometer:

- Source Temperature: Set the optimal source temperature to achieve stable ionization and maximize sensitivity for the selected ionization mode.
- Collision Energy: Adjust collision energy to ensure optimal fragmentation of target analytes.
- MS Scan Range: Configure the mass spectrometer scan range (e.g., m/z 100-1000) to capture the full range of relevant ions.
- Ensure proper tuning and calibration of the mass spectrometer using calibration standards to improve accuracy and reproducibility.

5.3.3. Data Acquisition Method:

- Full Scan: Detection of a broad range of ions, mainly for identifying unknown compounds.
- SIM (Selected Ion Monitoring): focus on specific ions that correspond to target analytes.
- MRM (Multiple Reaction Monitoring): Monitoring of precursor and product ion of each analyte (Figure 2).
- Scheduled Acquisition: Mass spectrometer switches between analytes at specific times based on different retention times (U.S. EPA, 2009).

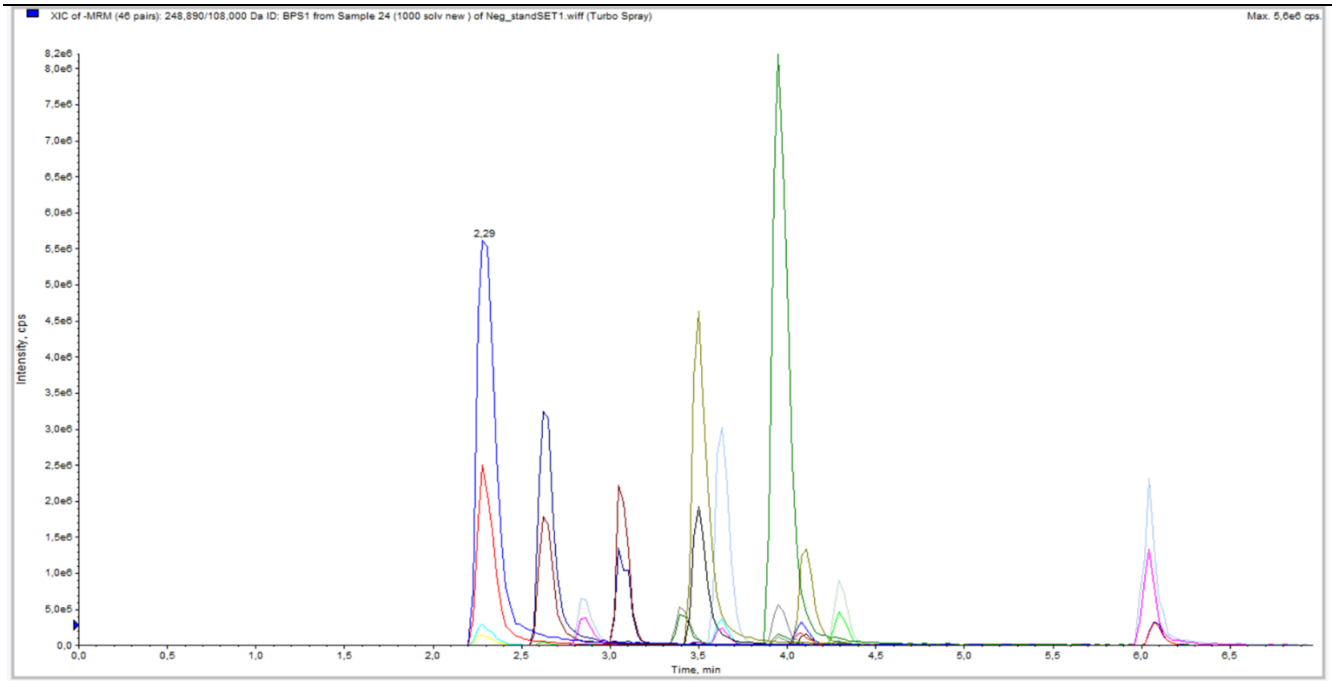

Figure 2. Example of Extracted Ion Chromatogram (XIC) following MRM monitoring for 14 organic contaminants and respective internal standards.

5.4. Calibration and Quality Control

5.4.1. Calibration Curve:

- Prepare a set of calibration standards with known concentrations of target organic contaminants.
- Standards should cover the expected concentration range for the analytes of interest, typically covering low, mid, and high concentrations.
- Generate a calibration curve for each analyte using the area ratio, peak area or height vs. concentration.
- Ensure that the lowest concentration selected is near the limit of quantification (LOQ) and the highest concentration is near expected levels within the sample.

5.4.2. Quality Control of Samples:

- Analyze at least five blank samples (or pseudo-blank with low concentration of analytes) to check for contamination or background noise.
- Analyze one low-level standard and one high-level standard to ensure the sensitivity of the analysis and the expected range, respectively.
- Analyze solvent or blank samples, especially following high-concentration samples, to evaluate the carry-over effect and ensure that analytes from previous samples are not contaminating subsequent injections.
- Analyze a replicate QC sample for each batch of samples, which includes using a known concentration of target analytes in solvent or matrix.
- Ensure that the recovery of the analytes is within acceptable limits (e.g., 80%-120%) (Magnusson and Örnemark, 2014; European Commission, 2021, 2023).

5.5. Sample Analysis

5.5.1. Inject the prepared sample into the LC-MS system, ensuring the use of appropriate injection volume following optimization (2-20 μL).

5.5.2. Monitor the chromatographic separation of analytes. Ensure proper retention times and resolution.

5.5.3. Collect MS data in the chosen mode (Full scan, SIM, or MRM).

5.5.4. Analyze the data for peak identification, quantification, and confirmation of the analytes.

5.6. Data Processing and Analysis

5.6.1. Peak Identification:

- Identify the analyte peaks by comparing retention times and mass spectra with those of the calibration standards and known reference materials. As part of the identification criteria of compounds for MS/MS techniques, signal-to-noise ratio should be higher than 3, or in case of noise absence, a signal should be present in at least five subsequent scans. The minimum number of ions is set at 2 product ions of typical MS/MS systems. Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap, while the ion ratio from sample extracts should be within ± 30 % (relative) of the average of calibration standards from the same sequence (Pihlström NFA et al., 2017).

5.6.2. Quantification:

- Use the calibration curve to calculate the concentration of each analyte in the sample, adjusting for sample dilution or concentration steps performed during preparation.

5.6.3. Data Review:

- Verify the accuracy of the results by ensuring that the internal standard response is within acceptable limits.
- Check the performance of the calibration curve through a correlation coefficient (r^2) of > 0.99 to confirm good linearity and reliable quantification.

5.6.4. Reporting:

- Record the results in the laboratory information management system (LIMS) or report them manually.
- Include details such as sample ID, analyte concentrations, method parameters, and QC results.

6. Safety and Waste Management

- Follow laboratory safety protocols when handling solvents and reagents, especially those that are hazardous or toxic. For specific safety information on each chemical, refer to Safety Data Sheets.
- Dispose of waste solvents and contaminated materials in accordance with institutional and local regulations.
- Wear appropriate personal protective equipment (PPE), including gloves, lab coat, and eye protection.
- Use fume hood when handling hazardous solvents to minimize inhalation exposure.

7. Troubleshooting and Maintenance

7.1. Instrument Issues:

- Pressure issues: If the system pressure increases or fluctuates abnormally, which may result to retention time drift, check for clogged filters, column blockages, or air bubbles in the mobile phase lines. Leaks in the LC system can lead to pressure fluctuations, so connections and fittings need to be checked for tightness.
- Ionization issues: If the ion source is not functioning optimally, check for contamination or deposition of sample residue. Ensure the ion source temperature

and voltage settings are optimized for the target analytes. For electrospray ionization (ESI), ensure the spray needle is not clogged and that the sheath gas and nebulizer gas flow rates are appropriate.

7.2. Data Issues:

- If chromatographic peaks are not well resolved, adjust the column, mobile phase composition, or flow rate. Ensure that the column temperature is stable and within the recommended range for optimal separation.
- If there are issues with sensitivity or signal-to-noise ratio, optimize the MS parameters (e.g., ion source voltage).

8. Data on procedures and samples

Data on procedures and samples are given in **Table 1**.

Table 1. Data on procedures and samples

Description of the sample	Aqueous sample, frozen
Aliquots needed	1
Total volume of the sample	LQ: 100-250 Ml, SOLID: 1-10g (depending on matrix/expected concentration of analyte)
Estimated content needed	
Time required to obtain results	1 month (target analysis), 6 month (suspect/non-target)
Manpower	Highly skilled researcher
Estimated cost per sample without manpower	Depends on matrix and type of analysis
Contact person	Ester Heath, ester.heath@ijs.si

9. Conclusions

In conclusion, this SOP outlines a comprehensive and systematic approach for the trace analysis of organic contaminants in environment, food or health related matrices using LC-MS, ensuring reliable and accurate results. By following the procedures for sample preparation, instrument calibration, and quality control, analysts can achieve precise quantification and identification of contaminants. Adhering to safety protocols and maintaining equipment ensures consistent performance, making this SOP an essential tool for conducting high-quality analytical work in environmental, food, and pharmaceutical testing. Moreover, this SOP ensures that the process for trace analysis of organic contaminants by LC-MS is conducted in a controlled, reproducible manner, meeting quality assurance and regulatory requirements.

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Conflicts of Interest: The authors declare no conflict of interest.

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