# Best Practices for the full LC MS Workflow

***Samples – Preparation – Separation – Mass Spec Analysis – Informatics – Reporting***

***Best Practices for Full LC MS Workflows – Basics***
***Base Training Presentations for LC MS***
***Introduction and Analytical Considerations***

***Last Updated Jan 2025***

**Jeremiah D. Tipton, Ph.D.**
Director and Applications Manager
Applied Omics & Life Sciences LLC
Agilent Technologies Applications Contractor

# INTRODUCTION TO LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC MS) BASED ANALYSIS

Updated January 2025

**General Presentation on Basics of the LC MS Workflow**

## Overall Workflow Considerations

1. **Samples**
2. **Preparation**
3. **Separation**
4. **Mass Spectrometry**
5. **Informatics**
6. **Reporting**

# INTRODUCTION TO LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC MS)

## *GENERAL EXAMPLES OF USES APPLICATIONS*

### Drug Discovery

Mass spectrometry is an ideal tool in drug discovery for analyzing drug compounds and their metabolites.

### Clinical Testing

Mass spectrometry plays a crucial role in clinical testing for the detection and quantification of biomarkers in biological samples.

### Environmental Studies

Mass spectrometry is used in environmental studies to detect pollutants in soil and food, ensuring safety and quality.

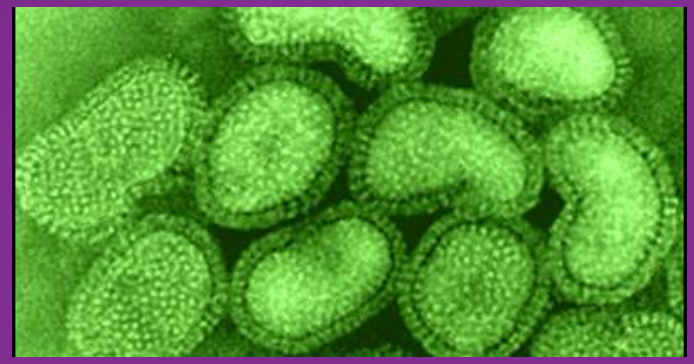
# INTRODUCTION TO LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC MS)
## *GENERAL EXAMPLES OF USES APPLICATIONS*

### Clinical Research

Mass spectrometry can be used in a high-throughput mode, collection data on large cohorts for clinical studies.

### Omics and Systems Biology Metabolomics and Proteomics

Mass spectrometry plays a crucial role in mapping molecular pathways of interest, as well as such small molecule flux associated with metabolism



### Emerging Growth Fields in Systems Biology The Microbiome and Associated Studies

The Development of Omics Technologies has launched expansion of knowledge in other, areas besides human disease – such as the symbiotic relationship between the microbiota and health.

# INTRODUCTION TO MASS SPECTROMETRY CONCEPTS

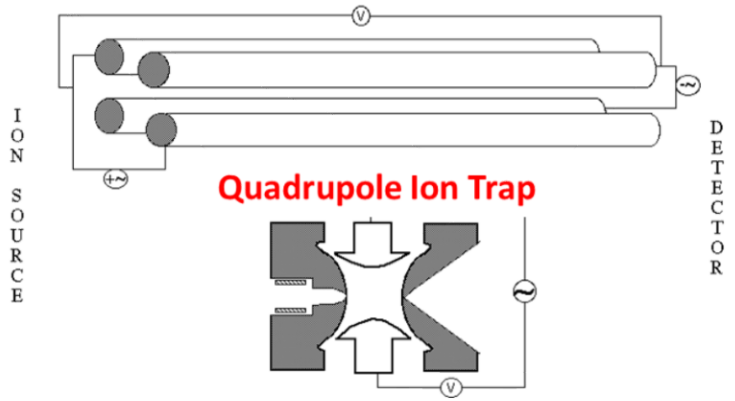
**Examples: Types of Mass Analyzers**

- Mass spectrometry is an important tool for identifying and/or quantifying specific compounds or materials with high precision.
- Applications of mass spectrometry range from small molecules to much larger molecules like proteins.
- Applications of mass spectrometry range from food quality, environmental health, diagnostic testing, biomedical, and clinical research.
- This sensitive technique detects, identifies, and quantifies molecules based on the →
  **mass-to-charge (m/z) ratio and relative response factor**

**Thus – Must "Ionize" the Sample for LC MS Analysis**
***Liquid-to-Gas Phase so that the instrument can measure the m/z ratio.***

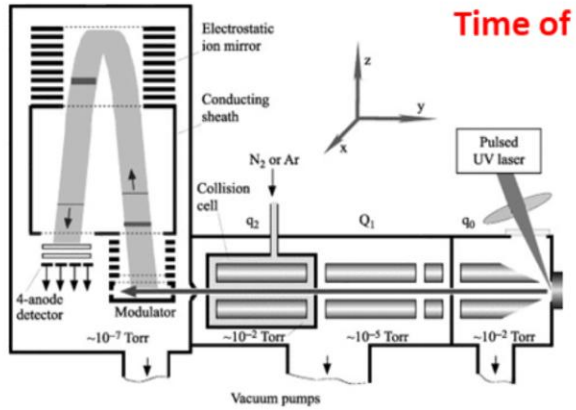
**Example:**
$[M+H]^{+1}$

**Quadrupole Mass Filter**




Adapted From → http://www.abrf.org/ABRFNews/1996/September1996/sep96iontrap.html

**Time of Flight**

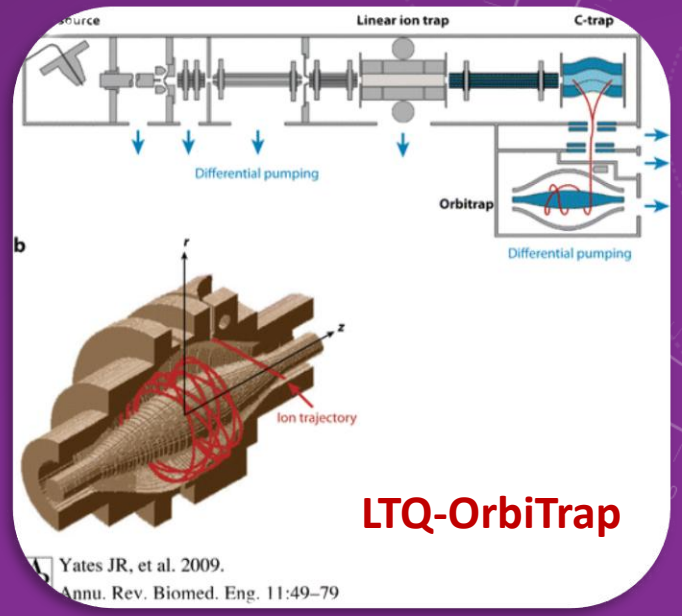


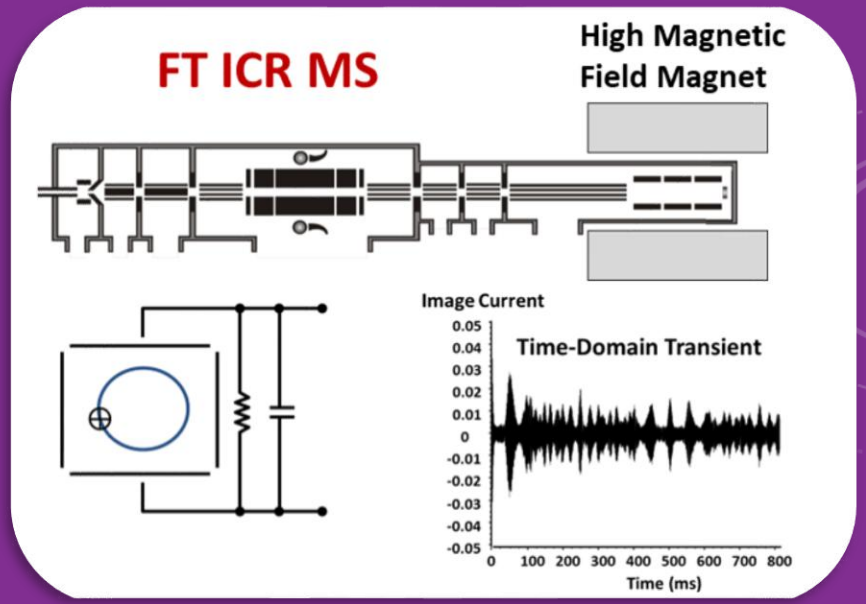

Hybrid Quadrupole/Time-of-Flight Mass Spectrometers for Analysis of Biomolecules Werner Ens; Kenneth G. Standing, Methods in Enzymology, Volume 402, 2005, Pages 49–78 Biological Mass Spectrometry

# INTRODUCTION TO MASS SPECTROMETRY CONCEPTS

**Examples: Types of Mass Analyzers**

- The ion source vaporizes and ionizes samples, creating charged particles.
- The mass analyzer separates ions based on their ***mass-to-charge (m/z) ratio***.
- The ion detector measures the abundance of ions, generating data for analysis.
- Samples can be prepared in liquid, gas, or dried form before analysis.
- The ion source ionizes the sample, allowing for detection and quantification.
- Variations in components allow for different mass spectrometer types and testing options.



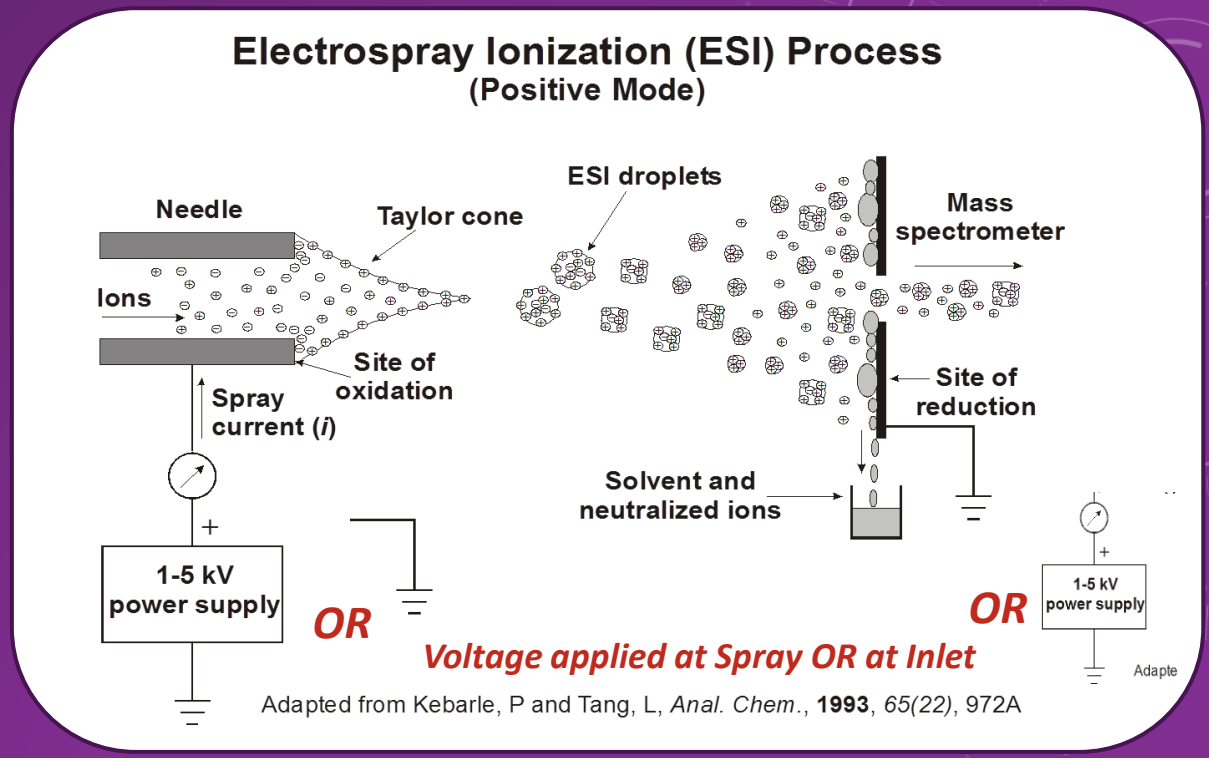



# INTRODUCTION TO MASS SPECTROMETRY CONCEPTS

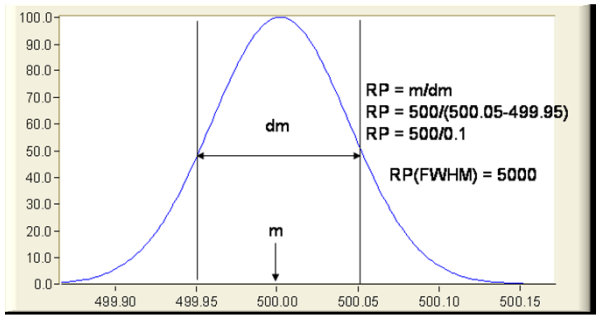
- Different mass spectrometers utilize various ion sources such as electron impact, chemical ionization, or **electrospray ionization**, which affect the ionization efficiency and sample types.
  ***(Use with LC MS Workflow and this Discussion)***

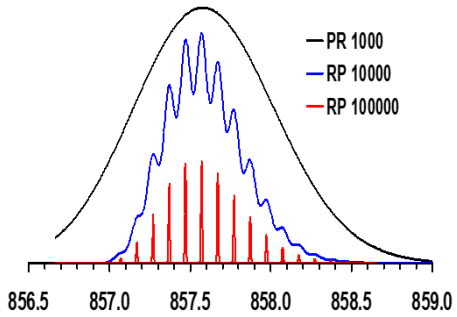
- Mass spectrometers may feature diverse **mass analyzers** like *quadrupole, time-of-flight (TOF), ion trap*, or *ion mobility* each providing distinct resolution and accuracy for mass measurements. Hybrid instrumentation (combination of multiple analyzers) have improved the last 25 years.

- The ion detectors in mass spectrometers can vary, including electron multipliers and Faraday cups, which influence sensitivity and detection limits for quantifying compounds.




**Resolving Power Calculations with Mass Spectrometry**
**Ubiquitin (10+ Charge State) 8560.62 ($C_{378}$ $H_{630}$ $N_{105}$ $O_{118}$ $S_1$)**



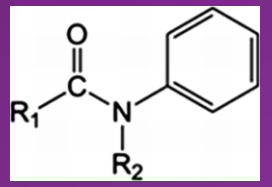
# INTRODUCTION TO MASS SPECTROMETRY CONCEPTS

## Identifying Unknown Compounds

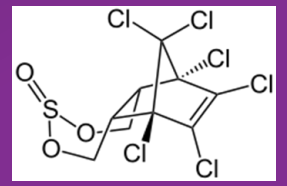
- Mass spectrometry is crucial for identifying unknown compounds, enabling researchers to analyze complex mixtures and determine the presence of specific substances.

*Example – Pesticides in Sample?*

**Carbamates**




**Endosulfan**



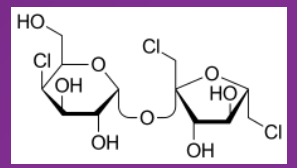

**Present in Sample**

?

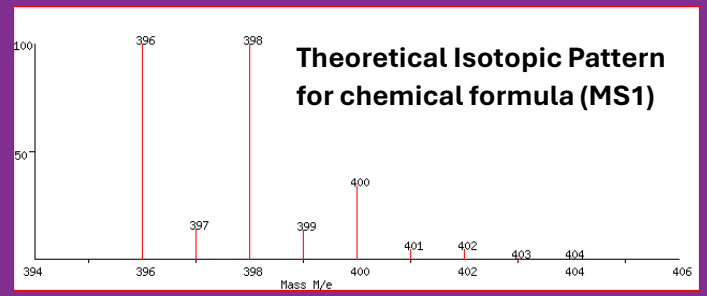
## Isotopic Composition Analysis

- It plays a key role in determining the isotopic composition of elements in a molecule, providing insights into molecular structure and origin.

*Example Sucralose*



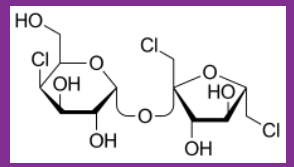

$C_{12}H_{19}Cl_3O_8$



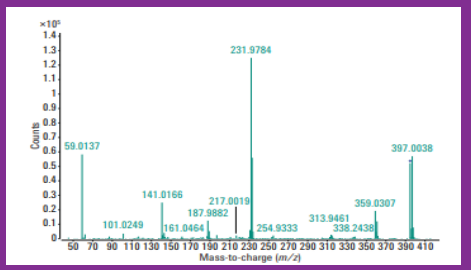

## Fragmentation & Structure Determination

- Mass spectrometry helps in determining the structure of a compound based on fragmentation patterns, allowing for detailed structural analysis.

*Example Sucralose*



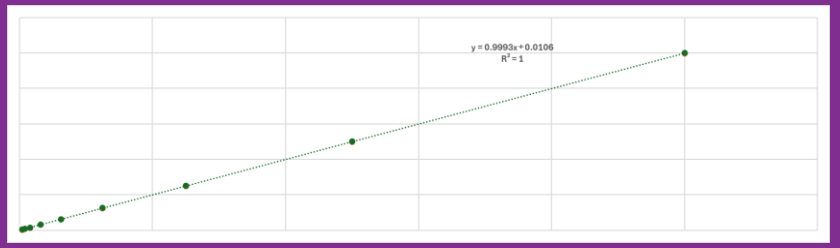

**MSMS Fragment with Instrument**




## Quantifying Compound Amounts

- This technique is essential for quantifying the amount of a compound in a sample, making it invaluable in various scientific fields.

*Example – Dilution Curve – Different Methods based on Need for quantification*



# INTRODUCTION TO LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC MS) BASED ANALYSIS

Updated January 2025 – *Agilent Instrument Centric Presentation - Basics*

Example - 1290 Infinity II Stack with 6495 Agilent QqQ

# TOPIC: SOLVENTS AND BUFFERS FOR LIQUID CHROMATOGRAPHY, ELECTROSPRAY IONIZATION, MASS SPECTROMETRY

LC – ESI – MS

Updated: January 2025

## Practical Considerations for Solvents and Buffer Usage - Basics

***Topics For Consideration and Discussion***

- Introduction to LC-MS Buffers
- Key Considerations for Buffer Selection
- Additional Tips for LC-MS
- Buffer Preparation Guidelines
- Common Buffer Selection Mistakes
- Recommended Volatile Buffers
- Troubleshooting Buffer Issues

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## CONSIDERATIONS FOR SOLVENT SELECTION

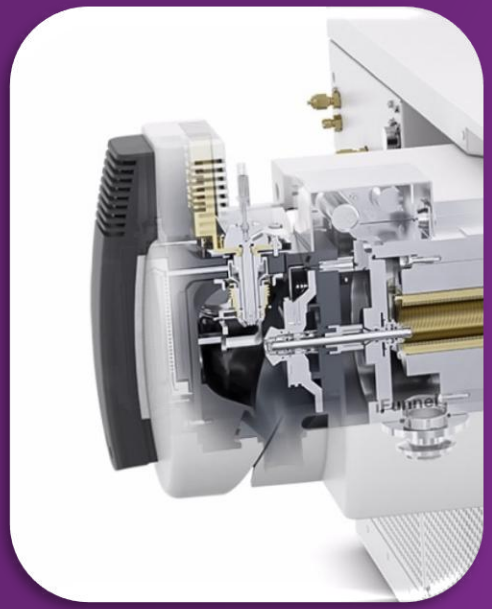
### Sample Solubility

Choose a solvent that effectively dissolves your analyte.

**Sample Dissolved**

### Instrument Compatibility

Ensure the solvent is compatible with your ESI source and mass spectrometer.



**Agilent AJS ESI Source**

### Ionization Efficiency

Some solvents may be better than others for specific analytes, impacting ionization efficiency.

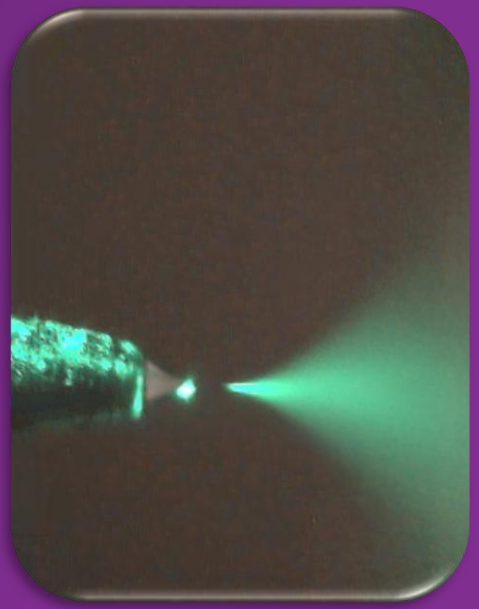


**Image - Electrospray Tailor Cone**

### Compatibility with Other Techniques

If you are using ESI in conjunction with other techniques like LC, consider solvent compatibility with the chromatography method.

**Solvents - Buffers**

**Column Heater**

**Autosampler**

**Pumps**

**Example Components of a Agilent LC Stack**

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## PREFERRED SOLVENTS FOR ESI

### Water – *LC MS Grade or 18.2 Mohm filtered*

- Water is a highly polar solvent (Aqueous) that readily dissolves charged species, making it a good choice for ESI.
- Typical for common molecule systems.

### Methanol – *LC MS Grade*

- Methanol is an *Organic Solvent* that helps in ionization and is compatible with ESI. Often Used with Water for LC Gradients.
- Can form adducts during ionization, often acetonitrile is preferred when paired with liquid chromatography (LC).

### Acetonitrile – *LC MS Grade*

- Acetonitrile is an Organic Solvent that helps in ionization / evaporation process during ESI and can help improve LC separations.
- Often preferred for LC MS experiments. *Must be LC MS Grade*.

### Isopropanol – *LC MS Grade*

- Sometimes added Organic Solvents for specific molecule systems, i.e. lipids for example.
- Often added to Injector Wash and LC Pump wash solvents.

## OTHER COMPATIBLE SOLVENTS

### Ethanol – *Compatible with instrument components?*

- Ethanol is a polar solvent that can be used in ESI.
- It might be less efficient than methanol or acetonitrile.
- Ethanol can dissolve a variety of analytes, making it versatile.
- It is readily available and commonly used in laboratories.

### Chloroform – *Compatible with instrument components?*

- Chloroform is a less polar solvent that can be useful for ESI.
- It is particularly effective in negative ion mode.
- Chloroform may enhance ionization for specific compounds.
- Caution is advised due to its potential health hazards.

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## DO NOT USE LIST FOR SOLVENTS, SALTS, DETERGENTS

| | |
|---|---|
| DMSO (Dimethyl Sulfoxide) | **DMSO can cause high background signals in ESI and APCI and can have memory effects. It's generally best to avoid using it directly in the MS system.** |
| DMF (Dimethylformamide) | **DMF can also interfere with ESI and is best avoided due to its potential negative effects on ionization.** |
| Salt Buffers | **Avoid using salts, phosphate, HEPES, citrate, or sulfate buffers, as they can interfere with ionization and affect mass spectrometry results.** |
| Detergents and Other Compounds | **Detergents like Tween, Triton, NP-40, and SDS can interfere with ESI. Urea, glycerol, carbohydrates, EDTA, GnHCl, plastics, and plasticizers may also disrupt the ionization process.** |

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## *CONSIDERATIONS FOR BUFFER SELECTIONS*

- Selecting appropriate buffers in LC-MS is crucial for ensuring optimal performance and ionization during analysis.
- Volatile buffers like **ammonium formate or acetate** are preferred over non-volatile buffers such as phosphates, as non-volatile buffers can cause salt deposition and interfere with ionization in the mass spectrometer.
- The choice of buffer ***directly impacts the sensitivity and accuracy of mass spectrometry results***, making it essential to prioritize buffers that evaporate cleanly and are compatible with the MS detection methods.

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## *CONSIDERATIONS FOR BUFFER SELECTIONS*

### pKa Values

Choose a buffer with a pKa close to the desired pH for optimal buffering capacity. This ensures effective stabilization of pH during the analysis process.

### Buffer Concentration

Aim for a buffer concentration between 10 mM and 25 mM for LC-MS applications. Higher concentrations can lead to precipitation or sensitivity issues.

### Volatile Buffer Options

Opt for volatile buffers like ammonium formate, acetate, or formic acid, as they are compatible with MS detection and evaporate cleanly during the process.

### Titration of Acid and Base

**Prepare buffers by titrating the respective acid and base. This method ensures higher purity and accurate pH levels in the final buffer solution.**

Titrated buffer solution

### Maintain pH Control

**Ensure the pH of the buffer is adjusted to the desired level for optimal performance. Use a pH meter to monitor and adjust as necessary.**

Buffered solution at target pH

### Use High-Quality Water

**Utilize high-quality water to minimize impurities in the buffer solution. Impurities can affect the stability and performance of the buffer in LC-MS applications.**

Prepared buffer using purified water

### Check Water Content

**Maintain a water content of at least 5% in buffers to prevent precipitation, especially when using organic solvents. This helps in maintaining buffer integrity during use.**

Buffer solution with appropriate water content

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## *CONSIDERATIONS FOR BUFFER SELECTIONS*

### Common Volatile Organic Salts for LC MS Applications

**Formic acid:**

A common and effective volatile buffer for LC-MS applications, known for its compatibility with ESI-MS and ability to enhance ionization.

**Acetic acid:**

Another suitable volatile buffer option for LC-MS, providing good stability and ionization properties.

**Ammonium formate:**

A volatile buffer that can be used in low pH applications, compatible with mass spectrometry detection.

**Ammonium acetate:**

A volatile buffer that can be used in low pH applications, facilitating better ionization and detection.

**Trifluoroacetic acid (TFA):**

While TFA can enhance retention and sensitivity, it can also suppress ionization in ESI-MS; use it carefully and in low concentrations. **(TFA Good for Chromatography, may cause ionization suppression)**

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## COMMON BUFFER - *SELECTION MISTAKES*

### Common Mistakes

- Using non-volatile buffers and salts can cause issues with ionization and instrument performance.
- High concentrations of volatile salts can negatively impact ionization processes.
- Not filtering mobile phases can lead to clogging and system performance issues.

### Avoid These Issues

- Phosphate buffers form precipitates at the LC-MS interface, leading to sensitivity loss and instrument fouling.
- Tris-HCl, HEPES, and other non-volatile buffers interfere with the ionization process.
- Nonionic detergents such as TWEEN 20 and Triton X-100 suppress ionization and cause contamination.

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## TROUBLESHOOTING BUFFER ISSUES – *COMMON SHORT LIST FOR THOUGHTS*

### Common Buffer-Related Issues and Solutions

- *If you experience issues, consider using a different buffer, adjusting the pH, or optimizing the mobile phase composition.*
- *Filter the mobile phase to remove any particulate matter that might clog the LC system or interfere with the mass spectrometer.*
- *Flush the system with the mobile phase before running samples to ensure stability and optimal performance.*
- *Regularly flush the LC-MS system to prevent buildup of salts and other contaminants.*
- *Maintain a water content of at least 5% in buffers to prevent precipitation (Optional).*

### Maintaining Optimal Performance

- *Utilize the seal wash function to clean the LC system's pump seals and prevent leaks.*
- *Replace solvents and solvent bottles regularly to maintain system cleanliness and prevent contamination.*
- *Ensure the organic solvent is compatible with the buffer and the mass spectrometer.*
- *Keep the buffer concentration below 25 mM to prevent precipitation when blending buffers and organic solvents. (Go to lower concentrations if possible)*

# SOLVENT AND BUFFER CONSIDERATIONS FOR LCMS

## *ADDITIONAL TIPS FOR LC-MS INSTRUMENTATION*

### Filter Mobile Phases

Filter the mobile phase to remove any particulate matter that might clog the LC system or interfere with the mass spectrometer.

### Equilibrate the System

Flush the system with the mobile phase before running samples to ensure stability and optimal performance.

### Maintain Cleanliness

Regularly clean the LC-MS system to prevent buildup of salts and other contaminants.

### Utilize Seal Wash

Utilize the seal wash function to clean the LC system's pump seals and prevent leaks.

Note: Each Vendor Has Specific SOPs available for cleaning and taking care of the instruments.

Note: There are several literature methods that can be followed with available Standard Operating Procedures.

Note: Following Best Practices and Provided SOPs will help with success.

# LIQUID CHROMATOGRAPHY MASS SPECTROMETRY BEST PRACTICES

## LC – MS BEST PRACTICES - *GENERAL*

Updated: January 2025

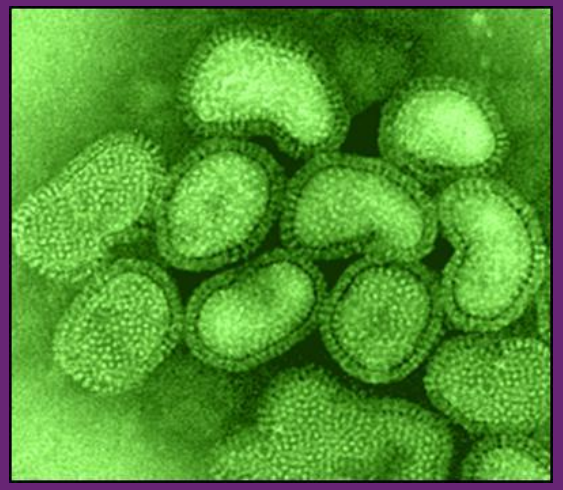
***Topics For Consideration***

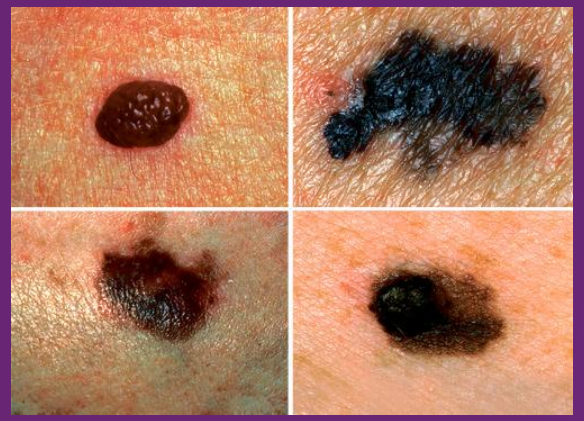
- Sample Preparation Essentials
- Optimizing LC and MS Systems
- Data Acquisition and Analysis
- Troubleshooting, Maintenance, and Documentation

**(1) Sample Preparation – (2) Separations with LC – (3) Mass Spec Measurement – (4) Data Informatics – (5) Reports**

# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

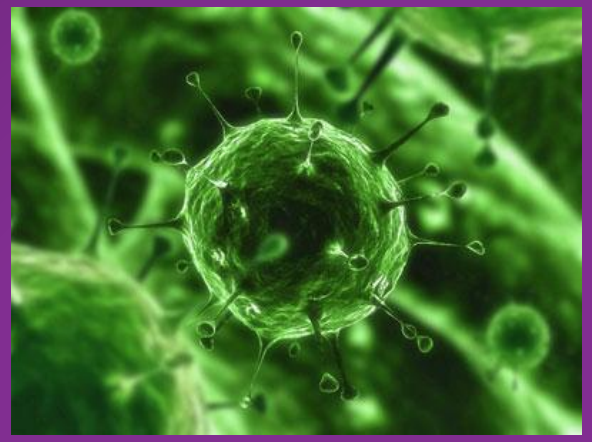
## SAMPLE PREPARATION ESSENTIALS – ALL SAMPLE TYPES

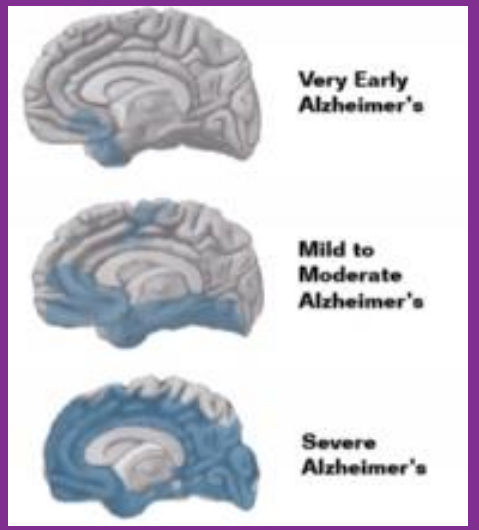




http://www.luxor.com/entertainment/bodies.aspx

**Bodies Exhibit**






(1) Sample Preparation –

# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

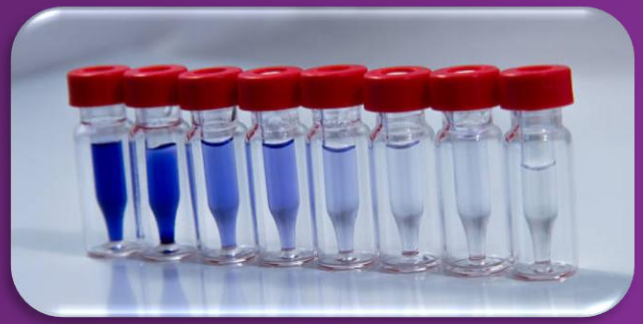
## *SAMPLE PREPARATION ESSENTIALS – CONSIDERATIONS FOR ALL SAMPLE TYPES*

### High-purity solvents and reagents

Use LC-MS grade solvents and reagents to minimize contamination and noise.

### Proper sample dilution

Dilute samples appropriately to avoid overwhelming the system with matrix effects and contaminants.



### Filtration and cleanup

Filter samples to remove particulates that can clog the LC system or contaminate the MS.


### Consider solid-phase extraction

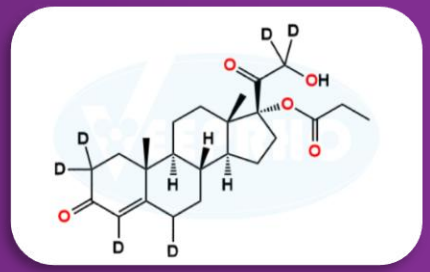
If required, use SPE to further purify and concentrate samples for improved sensitivity.


Example SPE Products

### Internal standards

Use appropriate internal standards to improve accuracy and precision in quantitative analysis.

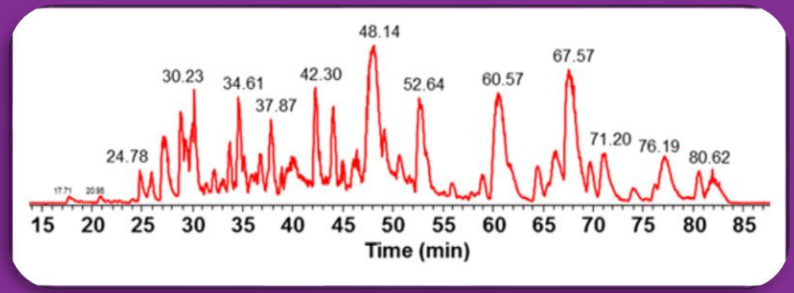
Example – Deuterated Standards



Clascoterone D6

### Importance of sample preparation

Effective sample preparation is crucial for achieving reliable and reproducible results in LC-MS analysis.



# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

## OPTIMIZING LC AND MS SYSTEMS

### LC System Operation & Optimization - General

- Choose a column that is compatible with the analytes and the mobile phase.
- Optimize the mobile phase composition (organic/aqueous ratio, pH) to achieve good separation and peak shape.
- Optimize the gradient profile to achieve optimal separation and minimize analysis time.
- Choose an appropriate flow rate to balance separation efficiency and analysis time.
- Maintain a constant column temperature to improve reproducibility and peak shape.

**Dependent on the molecular system of interest and need.**

**Select Column Type**

***C18 Most Common RP***
***Other Reversed Phase***
***HILIC***
***Normal Phase***
***Size Exclusion***

**Agilent 1290 Infinity II Stack**

**Solvents - Buffers**

**Column Heater**

**Autosampler**

**Pumps**

# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

## OPTIMIZING LC AND MS SYSTEMS – C18 COLUMNS

**Select Column Type**

*C18 Most Common RP*
*Other Reversed Phase*
*HILIC*
*Normal Phase*
*Size Exclusion*

### Matching Column to solvent system and molecules of interest

C18 columns are a staple in reversed-phase liquid chromatography (HPLC), playing a crucial role in the separation of analytes. They come in various chemistries tailored for specific applications, which influence selectivity, retention, and compatibility with different analytes and mobile phases.

**Dependent on the molecular system of interest and need.**

The importance of selecting the correct C18 column based on specific analytical needs cannot be overstated. The various chemistries available provide diverse options for optimizing separations in HPLC. By understanding the different properties and characteristics of these columns, you can select the optimal C18 column for your specific analytical needs.

### Base Silica C18

- Traditional C18 is the most basic type, where octadecyl (C18) chains are bonded to the silica gel surface. It's a good general-purpose column, offering a good balance of retention and selectivity for various analytes.

### Polar-embedded C18

- Includes polar functionalities into the C18 phase. An example is the Acclaim Polar Advantage, enabling better retention and separation of polar analytes.

### Hybrid Silica C18

- Utilizes a mixed organic-inorganic silica support, offering enhanced stability and efficiency. Known for wider pH range compatibility and durability, making them suitable for demanding applications.

### Other C18 Variations

- Includes Fast C18 for faster separations using larger pore sizes, Wide pore C18 for analyzing larger biomolecules, AQ C18 for high-water-content mobile phases, and C18(2) for higher carbon load, increasing retention for nonpolar analytes.

# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

## OPTIMIZING LC AND MS SYSTEMS – C18 COLUMNS

### Analyte Properties

Consider the polarity, size, and functional groups of the analytes to be separated.

### Speed and Efficiency

Consider the need for faster separations or higher efficiency in your analytical processes.

### Mobile Phase Compatibility

Select a C18 column compatible with the mobile phase used to ensure optimal performance.

### Column Lifetime

Select a column with a durable and stable stationary phase to ensure longevity and consistent results.

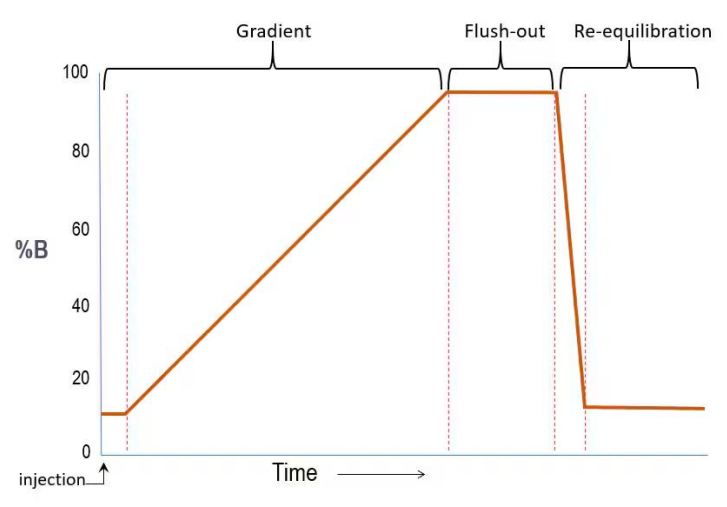
### Resolution Requirements

Choose a column with appropriate selectivity to achieve the desired separation between analytes.

### Overall Optimization

By understanding these factors, you can select the optimal C18 column for your specific analytical needs.

Example Gradient Delivery – % B is Organic Phase




Column Internal Diameter and Particle Size
Affect Flow Rate with LC and Source Conditions with LC

| Column Internal Diameter (mm) | Particle Size (µm) | Typical Flow Rate (mL/min) |
|---|---|---|
| 4.6 | 5 | 1.0 |
| 4.6 | 3 | 1.5 |
| 2.1 | 5 | 0.2 - 0.5 |
| 2.1 | 3 | 0.3 - 0.7 |
| 1.0 | 5 | 0.05 - 0.1 |
| 1.0 | 3 | 0.07 - 0.2 |

See Vendor Specific Websites for more information on pairing samples with methods and columns. There are large numbers of resources and standard-operating-procedures produced and available.

# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

*Cover Specifics Instrument Considerations During Training Sessions*

## OPTIMIZING LC AND MS SYSTEMS

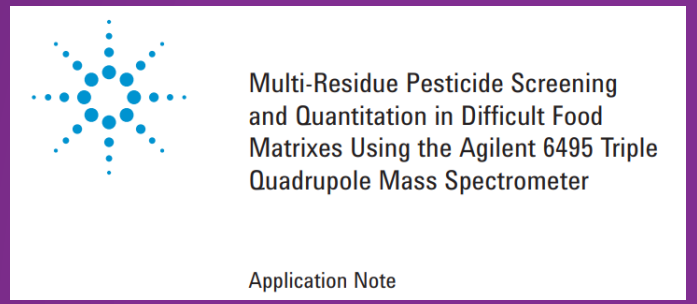
### MS System Operation & Optimization

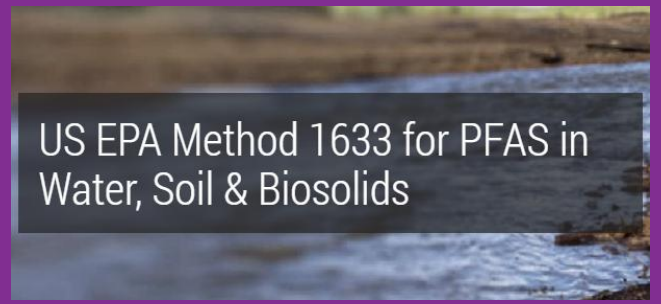
- Optimize the ion source parameters (e.g., temperature, gas flow) for the analytes of interest.
- Optimize the MS parameters (e.g., scan range, resolution) for the analytes of interest.
- Regularly calibrate and maintain the mass spectrometer to ensure accuracy and precision.

**Dependent on the molecular system of interest and need.**

### Agilent and other Literature Protocols to be Implemented

**Few Examples of Methods – Pesticides and PFAS**




### QqQ Mass Spectrometer

Targeted Analysis
MSMS Product Ion Scan
Quantification Workflows
Funnel Design for Increased Sensitivity

# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

## OPTIMIZING LC AND MS SYSTEMS (AGILENT SPECIFIC – 6495 QQQ)

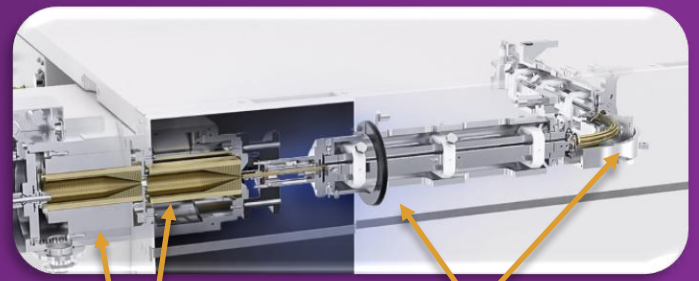
### Ionization Source:

Optimize parameters like temperature, voltage, and gas flow rates to maximize ion production and minimize fragmentation or unwanted side reactions.

Agilent Jet Stream Source

### Mass Analyzer Parameters:

Adjust parameters like resolution, mass range, and scan speed to achieve the desired separation and detection of ions based on their mass-to-charge ratio (m/z).



New Curved Configuration for Q1 – Collision Cell – Q2

Funnels for Ion Beam Focus

### Detector Type and Settings:

Optimize detector gain, bias voltage, and other settings to achieve the desired signal-to-noise ratio and sensitivity. (Auto tune and Calibration)

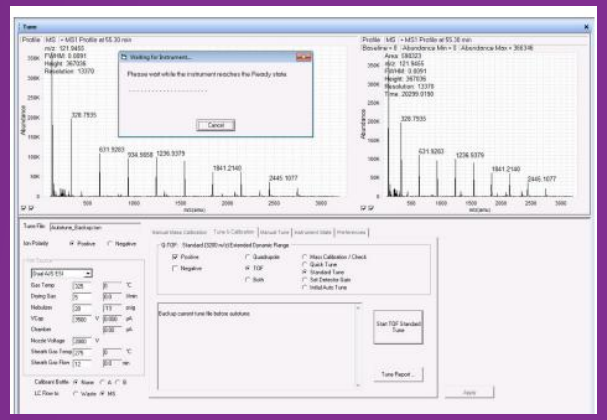
### Analysis Modes with A QqQ

**Multiple Reaction Monitoring (MRM)**
**Scheduled MRM (SRM)**
**MS1 Scan (define m/z)**
**Product Ion Scan**
**Precursor Ion Scan**
**Neutral Loss Scan**

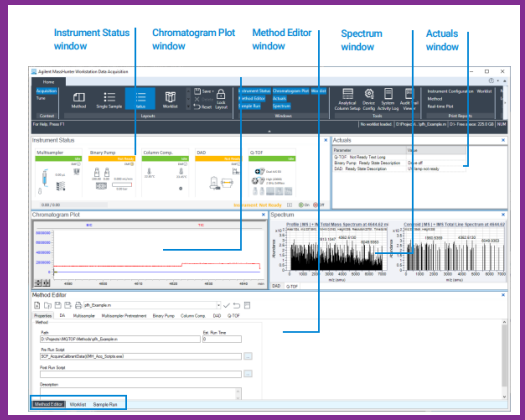
### Chromatographic Conditions (if applicable):

If using LC-MS or GC-MS, optimize mobile phase composition, flow rate, and column temperature to ensure optimal separation and ionization of analytes Often Ionization Source Parameters dependent on flowrate.

### Tune and Calibration - Automated



### MH AQ Window – Optimizer




### Compound Optimization (for LC-MS/MS):

Fine-tune precursor/product ions, collision energies, and other voltages to optimize the fragmentation and detection of specific analytes.

### Data Acquisition Parameters

Optimize dwell time, scan range, and other parameters to ensure data quality and minimize signal noise

# LC-MS EXPERIMENT/WORKFLOW CONSIDERATIONS

## DATA ACQUISITION AND ANALYSIS – AVAILABLE TOOLS

*Cover Specifics Instrument Considerations During Training Sessions*

### Software for Data Acquisition

Use appropriate software for data acquisition that can handle the specific requirements of LC-MS, ensuring that data is collected accurately and efficiently.

### Peak Identification and Quantification

Utilize software tools to identify and quantify the analytes of interest, ensuring that the correct algorithms and parameters are applied for precise results.

*Mass spectrometry-based vendors have their own software packages for Data Collection and Analysis*
*There are also several open source and 'third-party' vendor software packages for analysis LC MS data.*
*As well, statistical packages are sold by the vendors, as well as open source and third-party availability*

See Vendor Specific Websites for more information on software solutions for acquisition, data analysis and result presentation.

### Statistical Analysis Methods

Implement statistical methods to analyze the data, drawing meaningful conclusions that validate the results obtained from the LC-MS analysis.

### Validation of LC-MS Methods

Validate the LC-MS method to ensure it meets the required performance criteria, confirming that the analysis is reliable and reproducible.

# LARGE LIBRARY OF APPLICATION NOTES DEPENDING ON YOUR ANALYSIS NEEDS – EXAMPLE PFAS

Application Note

Environmental

Agilent

Trusted Answers

## Agilent 6475 LC/TQ Performance Highlights with EPA Draft Method 1633 for Per and Polyfluoroalkyl Substances (PFAS) in Solid Samples

**Sample preparation**
1. Weigh 5 g in 50 mL centrifuge tube
2. Add 2 mL deionized water
3. Add targets and extracted internal standards (EIS) directly to sample

**Extraction**
1. Add 10 mL of 0.3% methanolic ammonium hydroxide to each sample. Vortex, shake table for 30 minutes, centrifuge at 2,800 rpm for 10 minutes, and decant into another tube
2. Repeat with 15 mL
3. Repeat with 5 mL
4. Add 10 mg Carbon S to each extract, shake for 5 minutes, centrifuge, decant into another tube
5. Bring volume to 35 mL with water
6. Concentrate extracts at 55 °C to 7 mL final volume
7. Bring volume to 40 to 50 mL with reagent water and vortex
8. Check pH 6.5 ± 0.5 and adjust if necessary.

**Condition cartridges**
1. Pack Bond Elut PFAS WAX SPE (150 mg, 6 mL) cartridges half full with silanized glass wool
2. Add adapters and large-volume reservoirs
3. Rinse with 15 mL of 1% methanolic ammonium hydroxide
4. Rinse with 5 mL of 0.3 M formic acid in water

**Load cartridges**
1. Pour samples into reservoir
2. Pass through cartridge at 5 mL/min

**Rinse cartridges**
1. Rinse with 2 × 5 mL reagent water
2. Rinse with 5 mL of 0.1 M formic acid:methanol at 1:1
3. Dry under vacuum for 15 seconds

**Add internal standard (NIS)**
Add NIS to a clean collection tube (15 mL centrifuge tube)

**Sample elution**
1. Rinse sample bottle with 5 mL of 1% methanolic ammonium hydroxide
2. Transfer to reservoir
3. Collect eluate, add 25 µL acetic acid

**Extract filtration**
1. Install a Captiva premium nylon syringe filter on a 5 mL polypropylene syringe
2. Decant sample supernatant into syringe barrel
3. Collect filtered sample in polypropylene AS vial

**Analysis**
Split samples and analyze by 6470B and 6475 LC/TQ systems

ranged from 1 to 4 ng/g (Appendix A). Four replicate samples were spiked, extracted, and analyzed on both the 6470 B and 6475 LC/TQ systems.

Salmon tissue was sourced from a local supermarket and extracted as described in a previous application note.[5] Extracts were postspiked with a midlevel concentration of PFAS mix. The extract served to represent a challenging matrix to determine the robustness of the 6475 LC/TQ. Fish extract was analyzed with a 5-minute gradient. After 50 injections of fish sample, a midlevel calibration sample was analyzed using the full gradient in Table 1.

**Table 1.** Agilent consumables and supplies used for extraction.[4]

| Consumables and Supplies | Part Number |
|---|---|
| Bond Elut PFAS WAX Solid Phase Extraction (SPE) Cartridge, 150 mg, 6 mL | 5610-2150 |
| Bond Elut Carbon S SPE Bulk Sorbent, 25 g Bottle | 5610-2093 |
| Centrifuge Tubes and Caps, 50 mL | 5610-2049 |
| Centrifuge Tubes and Caps, 15 mL | 5610-2039 |
| Bond Elut Empty SPE Cartridges, 60 mL | 12131012 |
| Bond Elut Adapter Cap for 1, 3, and 6 mL Bond Elut Cartridges | 12131001 |
| Glass Wool, Silane Treated, 50 g, for GC | 8500-1572 |
| Captiva Disposable Syringe, 5 mL | 9301-6476 |
| Captiva Premium Syringe Filter, Polypropylene Housing, Nylon Membrane, 25 mm Diameter, 0.2 µm Pore Size | 5190-5092 |
| Vac Elut SPS 24 Manifold with Collection Rack for 10 × 75 mm Test Tubes | 12234003 |
| Collection Rack and Funnel Set for 12 or 15 mL Conical Tubes, for Vac Elut SPS 24 Manifold | 12234027 |
| Stopcock Valve (20 pack) | 12234520 |
| Polypropylene Screw-Style Vials, 2 mL | 5191-8150 |
| Screw-Style Cap, 9 mm, with Polypropylene/Silicone Screw Septa | 5191-8151 |

| Parameter | Value |
|---|---|
| LC | Agilent 1290 Infinity II LC System, consisting of:<br>– Agilent 1290 Infinity II high-speed pump (G7120A)<br>– Agilent 1290 Infinity II multisampler (G7167B)<br>– Agilent 1290 Infinity II multicolumn thermostat (G7116B) |
| Analytical Column | Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902) |
| Delay Column | Agilent InfinityLab PFC delay column, 4.6 × 30 mm (p/n 5062-8100) |
| Column Temperature | 50 °C |
| Injection Volume | 2 µL |
| Mobile Phase | A) 2 mM ammonium acetate in water<br>B) 95:5 acetonitrile:water |
| Gradient Flow Rate | 0.4 mL/min |
| Gradient | Time (min) % B<br>0.0 15<br>0.5 15<br>1.5 25<br>7.0 60<br>10.0 100<br>12.0 100<br>12.1 15.0 |
| Stop Time | 12.5 min |
| Post-Time | 2.5 min |

**Table 3.** MS conditions.

| Parameter | Value |
|---|---|
| MS | Agilent 6475 LC/TQ with Agilent Jet Stream Electrospray ion source (p/n G6475A) |
| Source Parameters | |
| Gas Temperature | 230 °C |
| Gas Flow | 8 L/min |
| Nebulizer | 20 psi |
| Sheath Gas Temperature | 355 °C |
| Sheath Gas Flow | 10 L/min |
| Capillary Voltage (Negative) | 2,500 V |
| Nozzle Voltage (Negative) | 0 V |

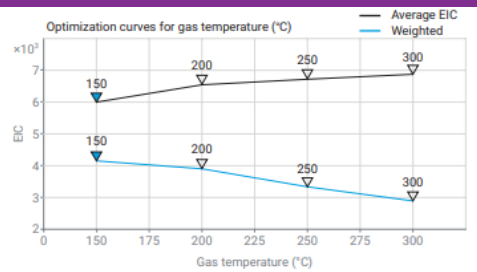



**Figure 2.** Source optimization curves.

Ion source optimization models started to diverge around 225 °C. Divergence indicated that while the overall EIC went up at the higher temperature, some of the low-abundance compounds showed lower abundance. After considering individual compound results, we confirmed that the best compromise was a temperature of 230 °C, which was selected in our original method.[3] Interestingly, this was the approximate temperature at which the two models diverged, which was helpful for verifying the optimal gas temperature for analysis.

**Table 4.** Method performance data (continued on next page).

| Compound | %RSD of Calibration (Option 1) | %RSE of Calibration (Option 2) | Low Calibration (ng/mL) | High Calibration (ng/mL) | $R^2$ | % RSD at Low Level |
|---|---|---|---|---|---|---|
| 11Cl-PF3OUdS | 8% | 7% | 0.20 | 100.0 | 0.998 | 3.2% |
| 3-3 FTCA | 10% | 10% | 0.40 | 200.0 | 0.997 | 5.2% |
| 4:2 FTS | 10% | 10% | 0.38 | 187.5 | 0.995 | 11.8% |
| 5-3 FTCA | 10% | 10% | 2.00 | 1,000.0 | 0.998 | 3.6% |
| 6:2 FTS | 9% | 11% | 0.38 | 190.0 | 0.997 | 6.3% |
| 7-3 FTCA | 11% | 10% | 2.00 | 1,000.0 | 0.998 | 7.1% |
| 8:2 FTS | 8% | 10% | 0.38 | 192.0 | 0.996 | 8.1% |
| 9Cl-PF3ONS | 8% | 8% | 0.20 | 100.0 | 0.998 | 4.2% |
| ADONA | 7% | 9% | 0.20 | 100.0 | 0.998 | 1.6% |
| HFPO-DA | 9% | 10% | 0.20 | 100.0 | 0.998 | 4.2% |
| NEtFOSA | 9% | 12% | 0.10 | 50.0 | 0.996 | 9.5% |
| NEtFOSAA | 13% | 13% | 0.10 | 50.0 | 0.996 | 11.3% |
| NEtFOSE | 8% | 9% | 1.00 | 500.0 | 0.997 | 2.5% |
| NFDHA | 9% | 10% | 0.20 | 100.0 | 0.998 | 8.1% |
| NMeFOSA | 10% | 12% | 0.10 | 50.0 | 0.996 | 14.1% |
| NMeFOSAA | 12% | 14% | 0.10 | 50.0 | 0.996 | 15% |
| NMeFOSE | 8% | 10% | 1.00 | 500.0 | 0.997 | 2.1% |
| PFBA | 8% | 10% | 0.40 | 200.0 | 0.997 | 3.1% |
| PFBS | 9% | 10% | 0.09 | 44.4 | 0.997 | 7.3% |

# Best Practices for the full LC MS Workflow

***Samples – Preparation – Separation – Mass Spec Analysis – Informatics – Reporting***

***Best Practices for Full LC MS Workflows – Basics***
***Base Training Presentations for LC MS***
***Introduction and Analytical Considerations***

***Last Updated Jan 2025***

**Jeremiah D. Tipton, Ph.D.**
Director and Applications Manager
Applied Omics & Life Sciences LLC
Agilent Technologies Applications Contractor