

Electrospray Ionization Time-of-Flight (ESI-TOF)

Table of Contents

1.0 INTRODUCTION	2
1.1 SAMPLE SUBMISSION LINK.....	2
2.0 SOLVENTS.....	2
2.1 ESI-TOF SOLVENTS (HPLC GRADE).....	2
2.2 NON-PREFERRED SOLVENTS.....	2
2.2.1 <i>Organic solvents:</i>	2
2.2.2 <i>Salts such as NaCl and phosphates:</i>	2
2.3 AVOIDING PARTICULATE MATTER.....	3
3.0 SAMPLE PREPARATION	3
3.1 SAMPLE CONCENTRATION.....	3
3.2 PROCEDURE.....	3
3.3 EXAMPLE CALCULATION	3

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1.0 Introduction

Electrospray ionization (ESI) is a “soft” ionization method and is used for exact mass determination. The combination of ESI with a time-of-flight (TOF) mass analyzer allows for the detection of a broad range of molecules (100-3000 m/z). Below is an overview and the protocol used by the MSF for ESI-TOF analysis of submitted samples.

1.1 Sample Submission Link

The instructions to submit a sample can be found at the link below:

https://www.lsu.edu/science/chemistry/research/mass_spec/procedures/sample_submission_guidelines.pdf

All samples need to be submitted electronically. Submit samples using the following link:

https://www.lsu.edu/science/chemistry/research/mass_spec/submission-forms.php

2.0 Solvents

2.1 ESI-TOF solvents (HPLC Grade)

Acetonitrile (ACN)

Methanol (MeOH)

Water

Water w/ 0.1% Formic Acid

These solvents are preferred when dissolving submitted compounds to keep consistent with the mobile phase of the instrument (see 3.3).

**Note: if you have solvents that are not HPLC-grade, you are welcome to prepare samples using MSF solvents.*

2.2 Non-preferred Solvents

2.2.1 Organic solvents:

This category includes solvents such as (but not limited to) n-hexane, ethyl acetate, N, N-Dimethylformamide (DMF), Dimethyl sulfoxide (DMSO), Dichloromethane (DCM), acetone, etc. In most cases (e.g. DMSO), the problem is the low volatility of the solvent and its capability to dissolve organic matter, which results in damage to the components of the whole system (e.g. tubing, connectors, etc.). If the compound submitted is only soluble in these listed solvents, samples should be diluted with one of the preferred solvents.

2.2.2 Salts such as NaCl and phosphates:

Salts are either non-volatile or hinder the ionization process. If the presence of salts is unavoidable, the concentration should be kept low (μM or lower)

2.3 Avoiding particulate matter

Sometimes it happens that a sample is not completely dissolved, especially if the sample is a mixture or a crude organic reaction. If you observe particulate in the solution, transfer an aliquot of the sample (100-200 μL) into a tube and ultracentrifuge the volume at 14,000 g. Afterward, pipette out half of the volume, making sure not to disturb the precipitate at the bottom of the tube.

In general, **avoid any type of filtration on plastic devices**. These devices bleed polymeric material in the sample, which will be present in the spectra we acquire, making it difficult (and sometimes impossible) to detect the compound of interest. If you need to filter the particulate and cannot centrifuge the sample, please use a glass funnel.

3.0 Sample Preparation

3.1 Sample Concentration

The sample concentration is defined as the weighted mass in a vial dissolved by a specific amount of solvent. With this definition in mind, the standard concentration for a flow-through analysis with our ESI-TOF is 25 $\mu\text{g}/\text{mL}$ to provide enough sample to the instrument without oversaturation. For LC analysis, the standard concentration is 40 $\mu\text{g}/\text{mL}$.

3.2 Procedure

Determine what solvent can be used to dilute your sample. If your sample can be dissolved with our standard mobile phase (80% ACN (or MeOH) and 20% water (with 0.1% formic acid)), please use this solvent composition. Otherwise, use the solvent that best dissolves your sample

Dissolve between 1-10 mg of sample 1 mL of the solvent chosen.

Dilute the sample to 0.025 mg/mL (25 $\mu\text{g}/\text{mL}$) using either our standard mobile phase composition, ACN or MeOH, depending on the analysis requirements.

**Note: Solid particles cannot enter the instrument; therefore, it is important the sample is fully dissolved. Vortexing can be done to aid in this process. Should particulate still be present in the sample, the user can ultracentrifuge the sample (> 10,000 g) for 5 minutes and sample the supernatant. **

3.3 Example Calculation

The submitter has 2.0 mg of solid sample and needs to achieve a 25 $\mu\text{g}/\text{mL}$ concentration

Step 1

Dissolve 2.0 mg in 1 mL solvent, which gives 2.0 mg/mL = 2000 $\mu\text{g}/\text{mL}$

Step 2

Keeping in mind of the $M_1V_1 = M_2V_2$ equation...

M_1 = Concentration of sample in mg/mL

V_1 = Volume of sample to dilute (MSF will use 2 μL of prepared sample)

M_2 = Desired concentration of sample (25 $\mu\text{g}/\text{mL}$)

V_2 = Final sample volume

Sample calculation using Step 1 concentration...

$M_1 = 2000 \mu\text{g/mL}$

$V_1 = 2 \mu\text{L}$

$M_2 = 25 \mu\text{g/mL}$

V_2 = Total Volume (noted as X)

$$\frac{(2000 \mu\text{g/mL})(2 \mu\text{L sample})}{25 \mu\text{g/mL}} = X$$

$X = 160 \mu\text{L}$ Total Volume, therefore the $2000 \mu\text{g/mL}$ sample will be diluted with the following...

$2 \mu\text{L}$ of sample and $158 \mu\text{L}$ of solvent

Please remember to fill out the sample submission form on the MSF website!

Link: https://www.lsu.edu/science/chemistry/research/mass_spec/submission-forms.php