ESI-MS Identity Confirmation Workflow

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1. Purpose

This procedure describes the confirmation of peptide identity by electrospray ionization mass spectrometry (ESI-MS). Identity is established by agreement between the observed monoisotopic mass and the theoretical monoisotopic mass calculated from the declared sequence.

2. Instrument

High-resolution time-of-flight mass spectrometer operated in positive ion mode. Capillary voltage 3.5 kV, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas 800 L/h.

3. Calibration

The instrument is calibrated daily across the m/z range 100–2000 using sodium formate clusters. Mass accuracy must be within ±2 ppm of theoretical values for the calibration standard before sample analysis proceeds.

4. Sample Preparation

Dilute the HPLC working solution 1:100 in 50:50 acetonitrile:water with 0.1% formic acid to a final concentration of approximately 1 µg/mL. Infuse at 10 µL/min.

5. Acquisition

Acquire 60 seconds of TOF-MS data at 1 Hz. Combine scans, deconvolute the multiply-charged envelope, and report the monoisotopic mass of the principal species.

6. Acceptance Criteria

The deconvoluted monoisotopic mass must agree with the theoretical mass within ±1 Da. For peptides above 5,000 Da, the average mass is reported and must agree within ±2 Da. No unassigned peaks above 5% relative abundance are permitted in the deconvoluted spectrum.

7. Reporting

The Certificate of Analysis lists: theoretical monoisotopic mass, observed monoisotopic mass, charge states detected, and a thumbnail of the deconvoluted spectrum. Raw acquisition files are archived for seven years.

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