

Choosing a Mass Analyzer

Quadrupole Ion Trap

- Highest full-scan and MS/MS product ion scan sensitivity
- Can perform MSⁿ
- High sensitivity for high masses (>500 Da)
- High resolution scanning
- Constant resolution across the *m/z* scale
- Lower initial cost
- High sample capacity and throughput
- Ideal for qualitative analysis/structural elucidation

Triple Quadrupole

- Highest SIM and SRM sensitivity
- Capable of common neutral loss scans and precursor ion scans
- Wide linear dynamic range for quantitation
- High sample capacity and throughput
- Ideal for quantitation
- Capable of many SRM transitions in a single segment

Choosing an Interface

Compound Parameter	APCI	ESI
Water Soluble		•
Organic Soluble	•	
Ionic		•
Ionizable	•	
Polar		•
Polar Non-ionic	•	
Thermally Stable	•	
Thermally Labile		•
High Flow Rates (~1 mL min ⁻¹)	•	
Low Flow Rates (100-250 µL min ⁻¹)		•
Reduced Ionization Suppression	•	
Production of Multiply Charged Ions		•

Choosing Mobile Phase Additives/Buffers

Ionization Mode	Buffer	pH Range
Positive	Acetic Acid	3.8-5.8
	Formic Acid	2.8-4.8
Negative* *Check pH range of column stationary phase	Ammonium Acetate	8.2-10.2
	Ammonium Hydroxide	8-10
	Ammonium Hydrogen-carbonate	7-11
	Ammonium Formate	8.2-10.2

HPLC Buffers to Avoid in LC/MS

- Phosphate buffers and other nonvolatile salts precipitate and clog MS ionization chambers
- Salts and buffers may cause ion suppression
- Sodium and potassium salts cause the formation of non-specific sodium and potassium adducts (see adduct table)
- Detergents and surfactants in protein analysis can cause ion suppression, surfactant adduct formation and clusters that interfere with mass spectral data

PDA versus MS Detection

Criteria	LC	LC/MS
Method Specificity	Derived from separation	Derived from mass analyzer
Method Selectivity	Derived from separation	Derived from separation and mass analyzer
Quantification	Baseline separations are required	No separation is required; but some separation is recommended
Standardization	Usually use external standards	Usually use internal standards
Calibration	Single point calibration is typical	Multi-point calibration is typical
Buffers	Added to suppress ionization and enhance solubility	Ionization suppression for vaporization, ionization enhancement for desorption
Effects from Chemical Interferences	Baseline separations are required	Labeled IS may compensate for interference

Preparing Liquid Samples

- Add Internal Standard
- Remove particles
 - Filtration
 - Centrifugation
 - SEC
 - Dialysis
 - Precipitation
- Concentrate sample and isolate target analytes
 - Liquid-liquid extraction
 - Solid-phase extraction
 - Evaporation
 - Lyophilization
 - Supercritical fluid extraction
- Analyze Sample

HPLC Ion Pairing Reagents in LC/MS

- Do not use nonvolatile reagents
- Long-chain reagents may interfere with mass spectra
- Ion-pairing reagents may compete in ion evaporation
- TFA/HFBA form strong ion pairs that suppress ionization (unless a post-column infusion of propionic acid is used)
- Recommendation: Do not use ion pairing

Typical Flow Rates Versus Column I.D.

Column I.D.	Flow Rate
3-4.6 mm	0.4-2 mL min ⁻¹
2-2.1 mm	100-400 µL min ⁻¹
1 mm	50-100 µL min ⁻¹
0.36-0.64 mm	0.1-20 µL min ⁻¹

pH Ranges for Column Stationary Phases

Stationary Phase	pH Range
C18	1.5-10.0
C8	1.5-10
Diphenyl	1.5-7.5
Pentafluorophenyl (PFP)	3.0-7.5

Reversed Phase LC Mobile Phases

Solvents	Solvent Strength (P')
Water/Buffers	Varies
Water (Weakest)	10.2
Acetonitrile	5.8
Methanol (Strongest)	5.1

Method Development Roadmap

Step 1

Definition

- Literature search/gather info.
- Acquire pure standards of target analytes and internal standards for quantitation
- Research previous methods used (www.varianinc.com)

Step 2

Optimize targets in MS

- Infuse each target and determine best MS conditions, fragmentation products, and develop SRM transitions
- Flow inject targets to optimize ionization/spray conditions

Step 3

Optimize Chromatography

- Suspend analytes in the A solvent for injection, or a mixture of A and B that is similar to the starting LC gradient
- Adjust gradient to separate target analytes
- If necessary, reoptimize MS or MS/MS conditions with confirmed eluting mobile phase (segment time, dwell time)

Step 4

Evaluate Matrix Effects

- Identify possible matrix interferences leading to ion suppression, false positive peaks, or baseline instability
- Increase method specificity by adding at least a second SRM transition for each target analyte
- Confirm identity with ion ratio criteria when necessary
- Develop sample preparation and separation process to eliminate matrix interference or ion suppression

Common LC/MS Contaminant Peaks

<i>m/z</i>	Ionization Mode	Possible Source
102	(+) ESI	Triethylamine (TEA)
113	(-) ESI	Trifluoroacetic acid (TFA)
114	(+) ESI	N ₂ Gas Tubing
116	(+) ESI	Detergent
149	(+) ESI (+) APCI	Red Bloom Algae (H ₂ O Purification)
149	(+) ESI	Phthalates (Plastics)
391	(+) APCI	
256	(+) ESI	Nylon filters
284		
311	(+) ESI	Column Bleed
352		
640		
331	(+) ESI	Ethylene polypropylene
122	(+) ESI	Water (Replace purification system filters)
123.9		
132		
134		
145		
147		
258		
315		
265	(-) ESI	LC Pump Grease

Optimizing LC Gradients

Problem	Solution
Peaks resolved but run time too long	Increase starting %B, decrease gradient time (maintain slope)
Poor resolution of initial peaks	Decrease solvent strength
Wide gaps with initial peaks	Increase starting %B maintain slope (shorten total gradient)
Final peaks elute after gradient	Select stronger solvent B, or increase starting %B
Poor resolution of final peaks	Decrease final %B and/or slope
Wide gaps between peaks	Increase slope (shorten gradient time)

HPLC Pressure Conversions

1 psi = 0.069 bar = 7 kPa = 51.72 Torr = 0.068 atm



VARIAN

Learn about all the LC/MS techniques offered by Varian

- ESI and APCI
- SelecTemp™ and SelecFlow™
- Full Scan, SIM, MS/MS and MSⁿ
- Selected Reaction Monitoring (SRM)
- 180° Off-Axis Collision Cell
- Enhanced Charge Capacity (ECC)
- Triple Resonance Scanning
- Mass ID Deconvolution Software
- TurboDDS™ (Data Dependent Scanning for Proteomics, Metabolite ID, Unknown Analysis)
- Powerful Qualitative and Quantitative Mass Spectrometry Workstation

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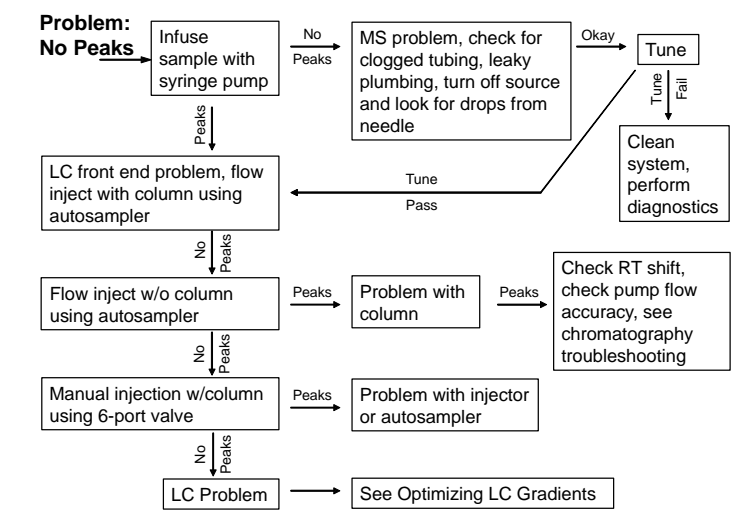
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LC/MS

Reference Guide

MS Troubleshooting



Maintenance/Troubleshooting

Daily or Weekly

- Clean ionization chamber
- Run autosampler through wash cycle
- Prime LC pumps
- Check LC solvents for dust or bacterial growth
- Rinse capillary with water and methanol 2-3 times
- Check wash solvent level for autosampler (at least 75% methanol or acetonitrile)
- Check foreline pump oil level

Monthly

- Replace LC solvents (HPLC Grade or better)
- Check LC flow rate
- Vent system and clean API plug
- Check API needle for clogging

Every 3 Months

- Change foreline pump oil using GP 45 oil and change the oil exhaust filter cartridge
- Clean autosampler spindle with isopropanol, relubricate
- Autotune system
- Run electronic diagnostics
- Change Nitrogen generator filters

Yearly

- Replace API needle
- Replace capillary
- Test system sensitivity

Chromatography Troubleshooting

Symptom	Cause	Solution
No Peaks	Injector problem, instrument problem, wrong mobile phase, wrong stationary phase, eluting after segment	Prime pump and check flow, check proper instrument function, lengthen segment or perform full scan analysis, check sample for degradation
Broad Peaks	Sample overload, injection volume too large, poor column efficiency, wrong pH	Dilute in A solvent and reinject, decrease injection volume, increase column temperature, flush column with strong solvent, check guard and tubing for blockages
Ghost Peak	Contamination, peak eluting from previous run	Flush column, use sample cleanup, replace solvents
Peak Doubling or Splitting	Coelution of interfering compound, injection solvent too strong, column overloaded	Use sample cleanup, flush column, increase column diameter, decrease sample amount, use weaker injection solvent
Peak Fronting	Channeling in column, column overloaded	Replace/repack column, increase column diameter, decrease sample amount
Peak Tailing	Degradation at high pH, unswept dead volume, interfering coeluting peak	Use base-deactivated or polar-embedded RP column, reduce column temperature, use small-diameter tubing, check that tubing is properly cut and connected, change mobile or stationary phase
Spikes	Bubbles in mobile phase, column stored without caps	Degas mobile phase, ensure all fittings are tight, store column tightly capped, flush RP columns with degassed MeOH
High Back Pressure	Blockages in tubing, sensor problems	Check for blockages in tubing and column, use lower viscosity mobile phase, increase column particle size

Masses of Amino Acids (continued)

Nom Mass	Sym	Comp	Structure	Mono-isotopic Mass	Avg Mass
101	Thr T	Threonine C ₄ H ₇ NO ₂	<chem>CC(C)C(N)C(=O)O</chem>	101.0476	101.1051
103	Cys C	Cysteine C ₃ H ₅ NOS	<chem>SCC(N)C(=O)O</chem>	103.0091	103.1448
113	Leu L	Leucine C ₆ H ₁₁ NO	<chem>CC(C)C(C)C(N)C(=O)O</chem>	113.0840	113.1595
113	Ile I	Isoleucine C ₆ H ₁₁ NO	<chem>CC(C)C(C)C(N)C(=O)O</chem>	113.0840	113.1595
114	Asn N	Asparagine C ₄ H ₆ N ₂ O ₂	<chem>NC(=O)CC(N)C(=O)O</chem>	114.0429	114.1039
115	Asp D	Aspartic acid C ₄ H ₅ NO ₃	<chem>OC(=O)CC(N)C(=O)O</chem>	115.0269	115.0886
128	Gln Q	Glutamine C ₅ H ₉ N ₂ O ₂	<chem>NC(=O)CCC(N)C(=O)O</chem>	128.0585	128.1308
128	Lys K	Lysine C ₆ H ₁₂ N ₂ O	<chem>NC(=O)CCCC(N)C(=O)O</chem>	128.1742	128.1742
129	Glu E	Glutamic acid C ₅ H ₇ NO ₃	<chem>OC(=O)CCC(N)C(=O)O</chem>	129.0425	129.1155
131	Met M	Methionine C ₅ H ₉ NOS	<chem>CSCC(N)C(=O)O</chem>	131.0404	131.1986
137	His H	Histidine C ₆ H ₇ N ₃ O	<chem>C1=CN=C(C=C1)CC(N)C(=O)O</chem>	137.0589	137.1412
147	Phe F	Phenylalanine C ₉ H ₉ NO	<chem>C1=CC=C(C=C1)CC(N)C(=O)O</chem>	147.0684	147.1766
156	Arg R	Arginine C ₆ H ₁₂ N ₄ O	<chem>NC(=O)NCCC(N)C(=O)O</chem>	156.1011	156.1876
163	Tyr Y	Tyrosine C ₉ H ₉ NO ₂	<chem>Oc1ccc(cc1)CC(N)C(=O)O</chem>	163.0633	163.1760
186	Trp W	Tryptophan C ₁₁ H ₁₀ N ₂ O	<chem>C1=CC=C2C(=C1)C(=CN2)CC(N)C(=O)O</chem>	186.0793	186.2133

Ions and Adducts Formed in Electrospray

Ion	m/z
[M+nH] ⁿ⁺	(M+n)/n
[M-nH] ⁿ⁻	(M-n)/n
[M+NH ₄] ⁺	M+18
[M+nNa] ⁿ⁺	(M+23n)/n
[M+nK] ⁿ⁺	(M+39n)/n

Ions and Adducts Formed in APCI

Ion	m/z
[M+H] ⁺	M+1
[M-H] ⁻	M-1
[M+NH ₄] ⁺	M+18
[M+CH ₃ CNH] ⁺	M+42
[M+CH ₃ OH ₂] ⁺	M+33
[M+H ₃ O] ⁺	M+19
[M+Cl] ⁻	M+35
[M+CH ₃ COO] ⁻	M+59
[M+CO ₂ H] ⁻	M+45
M ⁺	M
M ⁻	M

Neutrals Commonly Added to Ions in Electrospray and APCI

Ion	m/z
M ⁿ⁺ +xCH ₃ CN	(M+41x)/n
M ⁿ⁺ +xCH ₃ CO ₂ H	(M+60x)/n
M ⁿ⁺ +xCO ₂ H ₂	(M+46x)/n
M ⁿ⁺ +xCH ₂ CH ₂ OH	(M+46x)/n
M ⁿ⁺ +xCH ₃ OH	(M+32x)/n

Masses of Amino Acids for Proteomics

Nom Mass	Sym	Comp	Structure	Mono-isotopic Mass	Avg Mass
57	Gly G	Glycine C ₂ H ₃ NO	<chem>NC(=O)O</chem>	57.02146	57.0520
71	Ala A	Alanine C ₃ H ₅ NO	<chem>CC(N)C(=O)O</chem>	71.03711	71.0788
87	Ser S	Serine C ₃ H ₅ NO ₂	<chem>OC(C)C(N)C(=O)O</chem>	87.03203	87.0782
97	Pro P	Proline C ₅ H ₇ NO	<chem>C1CCNC1C(=O)O</chem>	97.05276	97.1167
99	Val V	Valine C ₅ H ₉ NO	<chem>CC(C)C(N)C(=O)O</chem>	99.06841	99.1326