

Overview

This work describes various measures and options for maximized LC-MS sensitivity and low Limit of Detection (LOD) by avoiding contaminations causing signal suppression, adduct formation and increased background noise or spectrum complexity:

- Selection and handling of HPLC columns
- Choice and cleaning of laboratory equipment (bottles, vessels, funnels etc.)
- Choice and proper processing of solvents and additives

HPLC columns – Low bleed

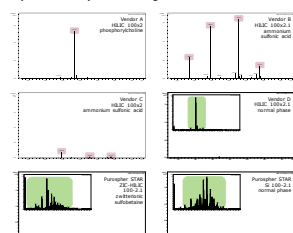
In LC-MS analyses, column bleed (loss of stationary phase entities) leads to signal suppression, adduct formation and sensitivity drop. Two options are available to minimize these negative effects:

- Utilization of low-bleed stationary phase material
- Implementation of a column flushing/washing process

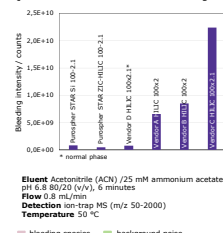
Low bleeding ZIC®-HILIC columns

Column bleeding studies utilizing LC-ion trap MS setup

Spectra of compounds eluting from each HILIC column



Quantification of HILIC column bleeding

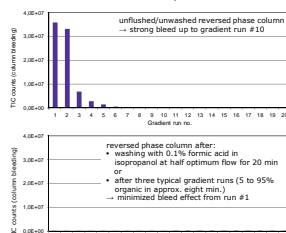


Eluent: Acetonitrile (ACN) / 25 mM ammonium acetate
pH 6.8 80/20 (v/v), 6 minutes
Flow: 0.8 mL/min
Detection: ion-trap MS (m/z 50-2000)
Temperature: 50 °C

Column flushing/washing

Removal of unbound organic entities

Gradients: 5 to 95% ACN in 3 min, 2 min at 95% ACN



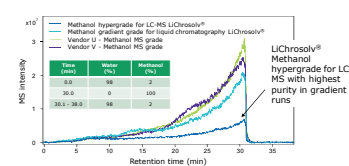
reversed phase column after:
• washing with 0.1% formic acid in isopropanol at half optimum flow for 20 min or
• after three typical gradient runs (5 to 95% organic in approx. eight min.)
→ minimized bleed effect from run #1

Solvents and additives – Purity, handling and storage

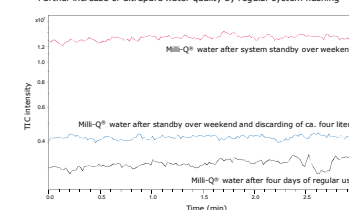
Purity

- Utilization of **MS-grade solvents** and ultrapure additives is mandatory
- Avoid any contamination during storage and handling
- **Additives** (formic or acetic acid, NH_4OH , triethylamine etc.) must be volatile, nonvolatile salts precipitate in MS
- Buffers ionize analyte molecule M, but ammonium, **formate** or **acetate** also form adducts [M+buffer]
- Choose eluent pH in accordance with your stationary phase properties (e.g., for silica pH 2-7.5 is recommended)

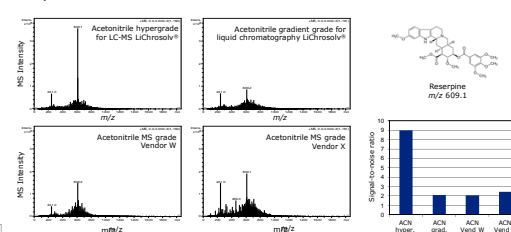
Comparison of the blank baselines (TIC) of various grades and sources of methanol via HPLC gradient runs w/o column



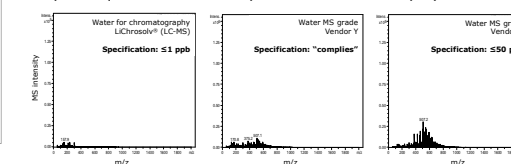
Proper use of ultrapure water systems for optimum water quality
• Purity depending on the frequency of utilization of the Milli-Q® system
• Further increase of ultrapure water quality by regular system flushing



Comparison of the spectra of various grades and sources of methanol via flow injection analysis using reserpine as an internal standard



Comparison of qualities of bottled water samples – "same" label but different purity

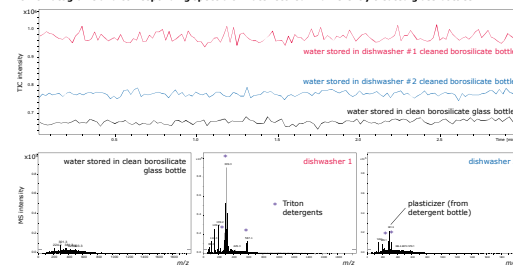


Laboratory equipment – Choice and cleaning

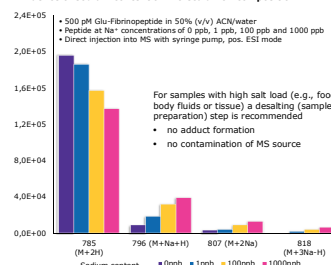
No dishwashing of glass vessels; strong bases and surfactants lead to contamination of eluents by
• Dissolution of silica and alkali under aggressive cleaning conditions, formation of adducts likely
• Deposition of detergents (from cleaning process) and plasticizers (from detergent bottle) on glass surface

Clean all equipment via evaporation or flushing with MS-grade solvents

Chromatograms and corresponding spectra of water stored in differently treated glass bottles



Influence of sodium content on molecular ion composition



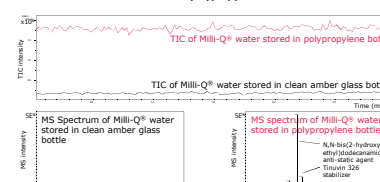
For samples with high salt load (e.g., food, body fluids or tissue) a desalting (sample preparation) step is recommended
• no adduct formation
• no contamination of MS source

Handling & Storage

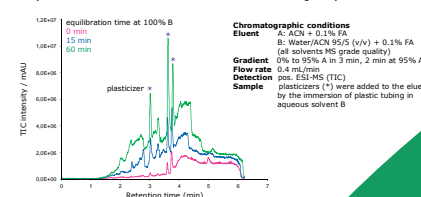
Use original Merck surface-treated amber or borosilicate glass bottles for solvent storage - avoid any contamination:

- Standard glass bottles: Silica and alkali dissolution, formation of adducts with analytes
- Plastic devices (bottles, funnels etc.): solvent contamination by leaching of additives (plasticizers, stabilizers, anti-static agents), accumulation on stationary phase and elution as ghost peaks likely bottle caps and adapters:
- Professional equipment directly mounted to the original brown glass bottle - no homemade solutions; avoid decanting

Contamination of water stored in a polypropylene bottle



Impurities in solvents accumulate on column and elute as ghost peaks



Chromatographic conditions
Eluent: A: ACN = 0.1% FA
B: Water/ACN 55/5 (v/v) + 0.1% FA (all solvents MS grade quality)
Gradient: 0% to 95% A in 3 min, 2 min at 95% A
Flow rate: 0.4 mL/min
Detection: pps. ESI-MS (TIC)
Sample: plasticizers (*) were added to the eluent by the immersion of plastic tubing in aqueous solution B

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SigmaAldrich.com/LC-MS