Tips & Tricks for improved quantitative and qualitative LC-MS

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
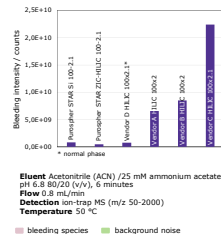• Implementation of a column flushing/washing process

Low bleeding ZIC®-trapHILIC columns
Column bleeding studies utilizing LC-ion MS setup

Spectra of compounds eluting from each HILIC column
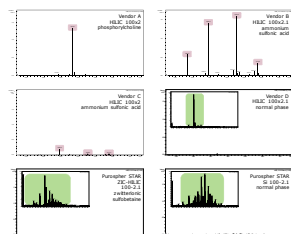

Quantification of HILIC column bleeding

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

bleeding species   background noise

Column flushing/washing
Removal of unbound organic entities

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

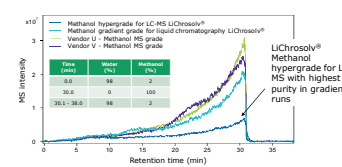unflushed/unwashed reversed phase column → strong bleed up to gradient run #10
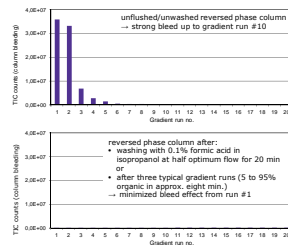
reversed phase column after:
• washing with 0.1% formic acid in isopropanol at half optimum flow for 20 min
or
• after three typical gradient runs (5 to 95% organic in approx. eight min.)
→ minimized bleed effect from run #1

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

water stored in dishwasher #1 cleaned borosilicate bottle
water stored in dishwasher #2 cleaned borosilicate bottle
water stored in clean borosilicate glass bottle

water stored in clean borosilicate glass bottle   dishwasher 1   dishwasher 2
Triton detergents   plasticizer (from detergent bottle)

Influence of sodium content on molecular ion composition

• 500 pM Glu-Fibrinopeptide in 50% (v/v) ACN/water
• Peptide at Na+ concentrations of 0 ppb, 1 ppb, 100 ppb and 1000 ppb
• Direct injection into MS with syringe pump, pos. ESI mode

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

785 (M+2H)   796 (M+Na+H)   807 (M+2Na)   818 (M+3Na-H)
Sodium content   0ppb 1ppb 100ppb 1000ppb

Solvents and additives – Purity, handling and storage

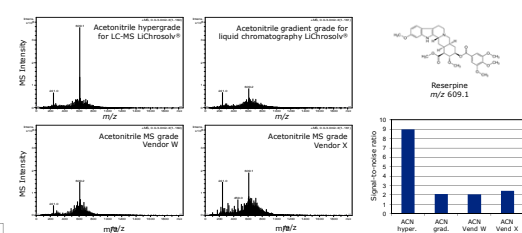**Purity**
• Utilization of MS-grade solvents and ultrapure additives is mandatory
• Avoid any contamination during storage and handling
• Additives (formic or acetic acid, NH₄OH, triethylamine etc.) must be volatile, nonvolatile salts precipitate in MS
• Buffers ionize analyte molecule M, but ammonium, 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
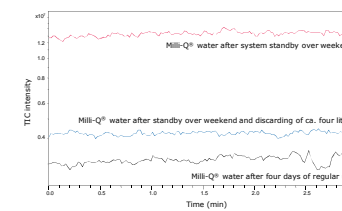

Time (min)	Water (%)	Methanol (%)
0.0	98	2
30.0	0	100
30.1 - 38.0	98	2

LiChrosolv® Methanol hypergrade for LC-MS with highest purity in gradient runs

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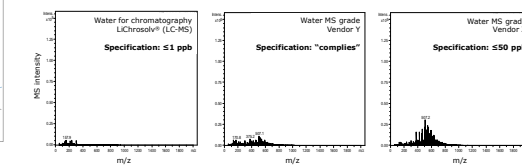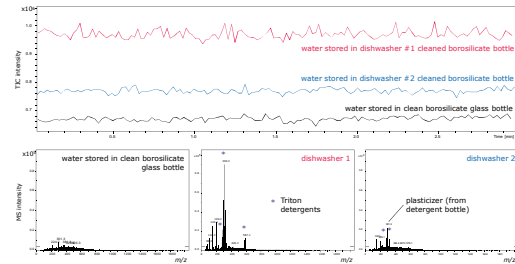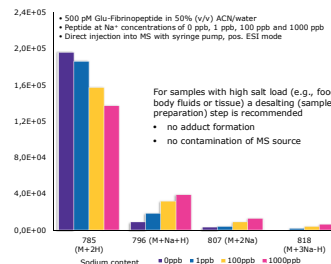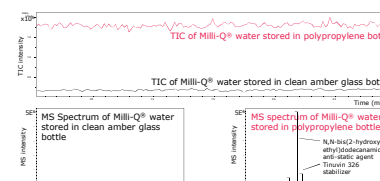

Reserpine m/z 609.1

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

Milli-Q® water after system standby over weekend
Milli-Q® water after standby over weekend and discarding of ca. four liters
Milli-Q® water after four days of regular use

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

Water for chromatography LiChrosolv® (LC-MS) **Specification: ≤1 ppb**
Water MS grade Vendor Y **Specification: "complies"**
Water MS grade Vendor Z **Specification: ≤50 ppb**

Handling & Storage

Use original MilliporeSigma 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

TIC of Milli-Q® water stored in polypropylene bottle
TIC of Milli-Q® water stored in clean amber glass bottle

MS Spectrum of Milli-Q® water stored in clean amber glass bottle
MS spectrum of Milli-Q® water stored in polypropylene bottle

N,N-bis(2-hydroxy ethyl)dodecanamide anti-static agent
Tinuvin 326 stabilizer

Impurities in solvents accumulate on column and elute as ghost peaks

equilibration time at 100% B
0 min   15 min   60 min

plasticizer

Chromatographic conditions
Eluent A: ACN + 0.1% FA
B: Water/ACN 95/5 (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 pos. ESI-MS (TIC)
Sample plasticizers (*) were added to the eluent by the immersion of plastic tubing in aqueous solvent B

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada

SigmaAldrich.com/LC-MS

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