New Effective Alternative To Ion-Chromatography

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Ion-chromatography (IC) is an established technique for analysis of inorganic and some organic ions. However, there are some problems associated with this technology.

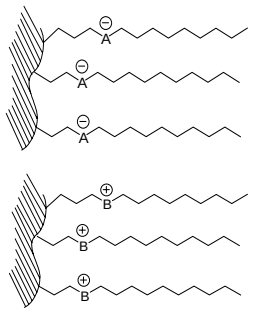
IC typically requires specific and expensive equipment which is not readily available in every analytical lab. IC is a very sensitive technology, and in the analysis where the concentration of analyzed ions is significant, it requires several dilution steps to bring the sample to a convenient concentration. IC perfectly works in pure aqueous media; however, the IC instrumentation can not be used with a significant amount of organic component in the mobile phase. This causes difficulties when an organic sample with a small amount of analyte is introduced in the IC column. Usually, it leads to contamination and destruction of the column, or requires some additional clean up steps.

Non-charged analytes are usually not detectable or not separable within IC conditions.

Different sets of conditions are required for separation of anions and cations, and they can not be analyzed simultaneously.

The combination of PrimesepTM mixed-mode stationary phase with the evaporative light scattering detecting (ELSD) technique allows using a broad range of mobile phases including the combination of water with acetonitrile, or methanol with acidic modifiers such as TFA, acetic and formic acid, ammonium acetate, ammonium formate, triethylamine acetate. Using this wide variety of mobile phases, we can achieve various separations. Also, a great number of compounds can be separated using the standard HPLC equipment with the addition of ELSD only. Charged and neutral organic and inorganic analytes can be simultaneously analyzed within a single HPLC run. Direct injection of the sample without any cleanup or/and dilution is achievable.

Schematic Structure of Primesep Stationary Phases

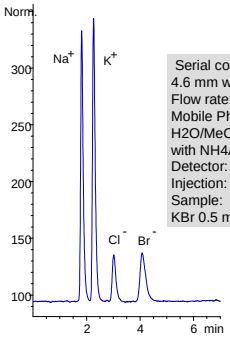


Primesep A
Primesep 100
Primesep 200
Primesep C

Primesep B
Primesep B2
Primesep D

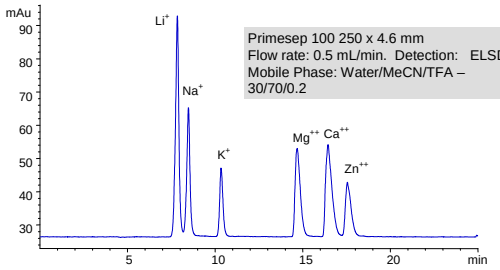
With an embedded ion-pairing group, a Primesep column separates and retains inorganic and organic ions similarly to ion-exchange stationary phases. The hydrophobic alkyl chains significantly effect selectivity of ion separation and it requires low ion-strength buffer to produce efficient ion-separation.

Analysis of Cations and Anions

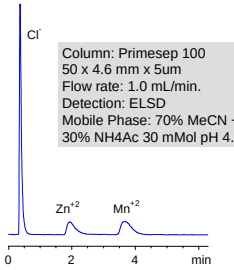


Serial connected Primesep 100, 50 x 4.6 mm with Primesep B, 50 x 4.6 mm
Flow rate: 1.0 mL/min
Mobile Phase:
H2O/MeCN – 70/30
with NH4Ac 50 mM pH 5.0
Detector: ELSD
Injection: 5 uL
Sample:
KBr 0.5 mg/ml; NaCl 1.0 mg/ml in water

Different inorganic cations can be analyzed with Primesep column and ELSD

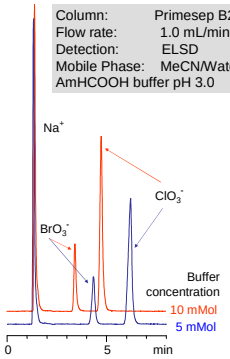


Primesep 100 250 x 4.6 mm
Flow rate: 0.5 mL/min. Detection: ELSD
Mobile Phase: Water/MeCN/TFA – 30/70/0.2



Column: Primesep 100
50 x 4.6 mm x 5um
Flow rate: 1.0 mL/min.
Detection: ELSD
Mobile Phase: 70% MeCN + 30% NH4Ac 30 mMol pH 4.0

Chlorate/Bromate separation

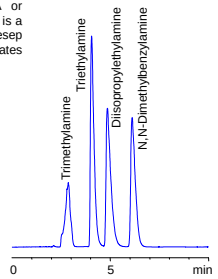


Column: Primesep B2 150 x 4.6 mm
Flow rate: 1.0 mL/min.
Detection: ELSD
Mobile Phase: MeCN/Water-30/70 with AmHCOOH buffer pH 3.0

Ammonium acetate based buffer offers an ability to see and quantitate anion like chloride in ELS detection mode. Additionally, it modifies the column surface and changes the selectivity. Zn and Mn ions are co-eluted with TFA based mobile phase.

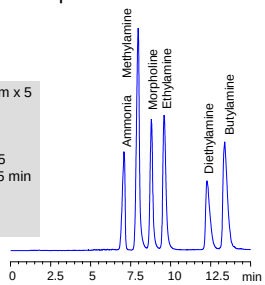
Ion-Exchange and hydrophobic mechanism in tertiary amines separation

Strong basses such as tertiary amines retain too strongly on Primesep A or Primesep 100 columns. Primesep 200 is a weaker cation exchanger than Primesep 100 and Primesep A, and it separates strong bases in mild conditions.



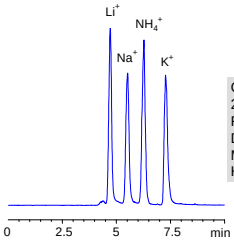
Primesep 200 column 150 x 4.6 mm x 5 um
Mobile phase: MeCN/H2O/TFA–20/80/0.15
Flow rate: 1.0 ml/min
Injection: 5 ul
Sample: 3.0 mg/ml each
Detector: ELSD, (Temperature 35oC)

Ion-Exchange and hydrophobic mechanism in amines separation



Primesep A column 150 x 4.6 mm x 5 um
Detection: ELSD, (Temperature 35oC)
Mobile phase: MeCN/H2O–15/85
TFA gradient 0.05 to 0.25% in 15 min
Flow rate: 1.0 ml/min
Sample: 1.0 mg/ml each

Separation of Inorganic Cations

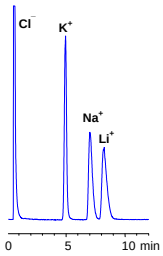


Column: Primesep 100
250 x 4.6 mm x 5um
Flow rate: 1.0 mL/min.
Detection: ELSD
Mobile Phase:
H2O/MeCN/TFA –20/80/0.2

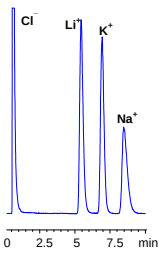
Typical ion-exchange columns would separate alkali ions in the following order: Li, Na, K. Primesep C (stands for Complex) offers unique selectivity. The ions elute on the Primesep C column in reverse order compared to ion-exchange elution. The pH working range for these columns is from 1 to 7, but their complex formation properties are substantially suppressed at the pH below 3. In order to facilitate the complex formation, the pH of the mobile phase should remain within the range of 3-7. The degree of complex formation can be adjusted by selecting the pH of the mobile phase.

Primesep C 50 x 4.6 mm Flow rate 1.0 mL/min.
Detector: ELSD

Mobile phase:
Water/MeCN/NH4 Acetate - 60/40/20 mM pH 5.0

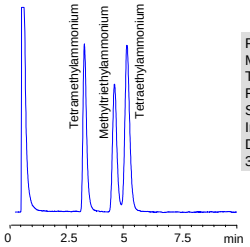


Mobile phase:
Water/MeOH/NH4 Acetate - 60/40/20 mM pH 5.0



Separation of Quaternary Amines

Quaternary amines are strong basses. They are not volatile and can not be analyzed by GC. A typical HPLC separation will result in no or very little retention for these polar molecules. Primesep C with volatile mobile phase allows to separate and quantitate quaternary amines with ELSD or MS detection technique.



Primesep C 50 x 4.6 mm x 5 um
Mobile phase: MeCN/H2O–15/85
TEA acetate 20 mM pH 5.0
Flow rate: 1.0 ml/min
Sample: 0.6 mg/ml each
Injection: 5 mc
Detector: ELSD, (Temperature 35oC)

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