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Newsletter


# LC Analysis of Zwitterions with Ion-Free Mobile Phase

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Separation of underivatized amino acids (AA) and other zwitterionic compounds is a challenging task and usually achieved on ion-exchange or HILIC columns or with the use of ion-pairing reagent. Ion separation by ion-exchange mechanism requires high concentration of buffers in the mobile phase.

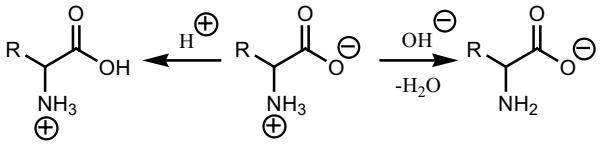


**Figure 1:** AA charge states at different pH

The ion-pairing approach is not compatible with LC/MS and preparative chromatography – the two most valuable approaches to identify and isolate amino acids and peptides. At neutral pH, most naturally occurring and synthetic AA and peptides are not charged if they have equal amounts of acidic and basic functional groups in the molecule. We are introducing BLIS -Buffer Less Ion Separation- a new approach for the analysis of zwitterionic compounds. The BLIS approach involves a specially designed Primesep column and a mobile phase containing neither buffers nor any other ionic additives except those provided by pure water in the mixture with acetonitrile.

| | |
|---|---|
| Column: | **Primesep 200** |
| Size: | 150x4.6 mm |
| Mobile phase: | |
| $MeCN/H_2O$ - 20/80 % | |
| Flow rate: | 1.0 ml/min |
| Detection: | UV 195 nm |
| Injection: | 1 µ L |
| Sample: | 0.3 mg/ml |

Since AAs are not charged in non-buffered solutions, they require acid or base to convert this zwitterionic molecules in a charged form according to fig. 1. This acid or base is usually a component of the mobile phase. However, the stationary phase with an embedded acidic or basic functional group also can provide the necessary ionization for zwitterions. Primesep 200 column is a reverse phase column with embedded acidic functional groups (fig. 2). In H+ form, these groups provide on-column acidic environment where AA is positively charged and electrostatically interact with the negatively charged surface. This interaction is strong but reversible at the same time. Simple conditions, universal detection, and good chromatography make this technique convenient for the separation of polar compounds (fig. 3). Primesep 200, being a reverse phase column, also provides hydrophobic interaction and high selectivity which can be controlled by the amount of MeCN in the mobile phase fig. 4. For polar compounds, the amount of MeCN in the mobile phase produces a little effect on retention.

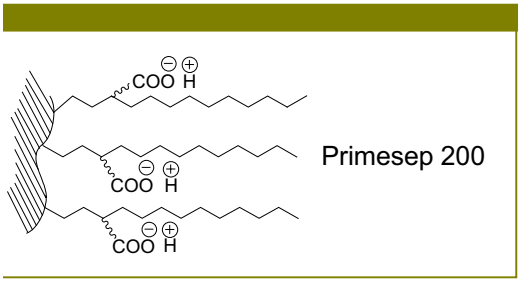


**Figure 2:** Simplified structure of Primesep 200 stationary phase. The ligand is attached to the silica support. The embedded acidic group is in $H^+$ form.

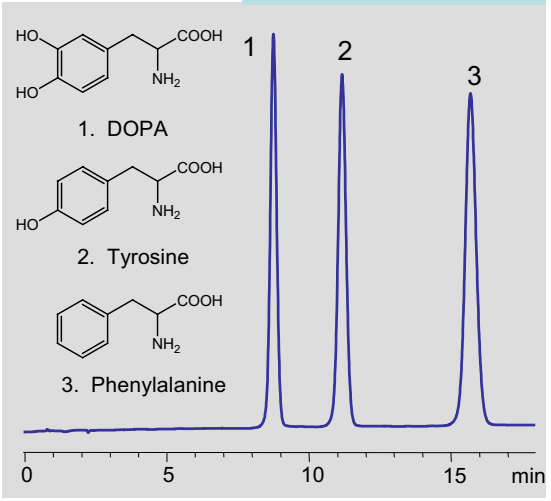


**Figure 3:** BLIS of AA mixture.

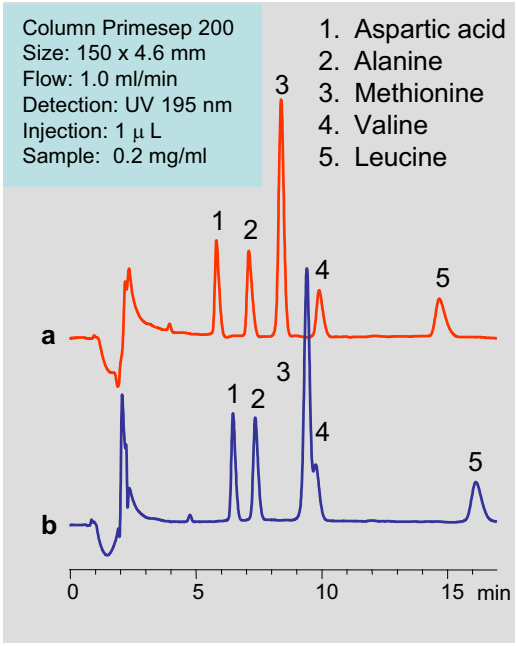


**Figure 4:** BLIS™ chromatogram of AA mixture. Mobile phase: $MeCN/H_2O$-40/60 % (**a**); $MeCN/H_2O$-20/80 % (**b**)

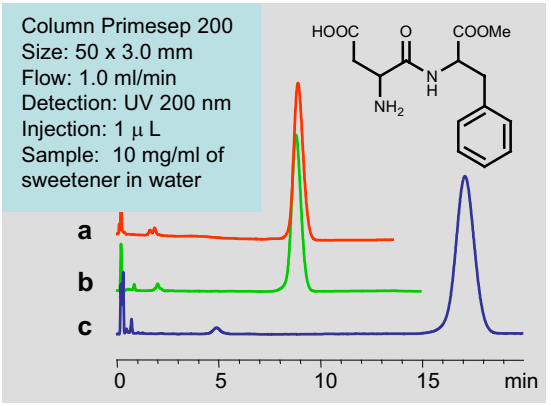


**Figure 4:** BLIS™ of aspartam sweetener ("wal-sweet"). Mobile phase: $MeCN/H_2O$-60/40 (a); 40/60 (b); 20/60 (c)

For hydrophobic compounds the concentration of MeCN has the same effect on retention as in any other reverse phase separation. At high organic concentration the ionic interaction becomes the main component of the retention.

At a very high organic concentration the retention increases again due to HILIC interaction. This technique is also applicable to peptides and any other zwitterions. A column with weaker embedded acidic group such as Primesep C will be more suitable in case of peptides. MP modifiers such as acetic or formic acid can be used if necessary without significantly effecting the separation.