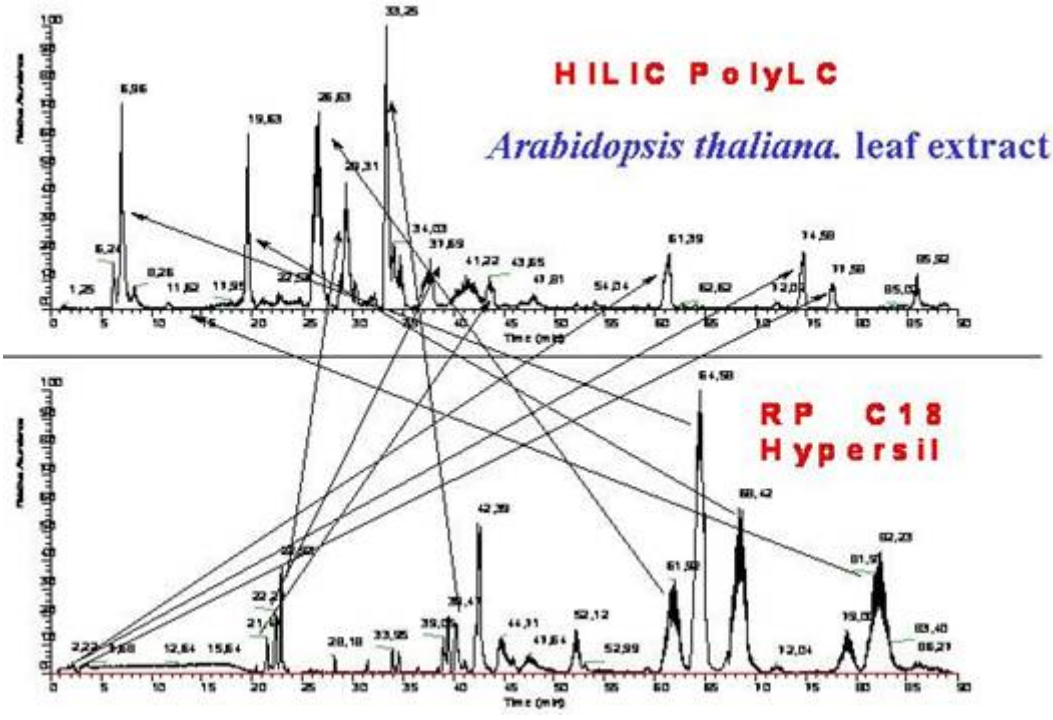
Metabolomics, Amino Acids, and Polar Small Solutes

Underivatized amino acids and other small, polar solutes are poorly retained in reversed-phase HPLC but well-retained in HILIC. Volatile mobile phases can be used for HILIC-ESI-MS. HILIC-MS/MS permits the quantitative analysis of small polar solutes even in crude extracts such as seeds, leaves, and **whole serum or plasma**. Generally, the best material for this purpose is **PolyHYDROXYETHYL Aspartamide™** with a pore diameter of 60- or 100-Å, with either 3- or 5-µm particle diameter. The 3-µm, 100-Å material has yielded especially good results.

Metabolomics: The following two chromatograms show an extract of whole **Arabidopsis** leaf [Top], eluted with a decreasing ACN gradient from a 150x0.32-mm **capillary** of PolyHYDROXYETHYL A™ (item# 150.32HY0301) or [Bottom] from a reversed phase capillary. The order of elution is largely inverted.



Detection is via ESI-MS. Peaks correspond to such solutes as amino acids, anthocyanins, oligosaccharides, glucosinolates, glycolipids, etc., as shown.

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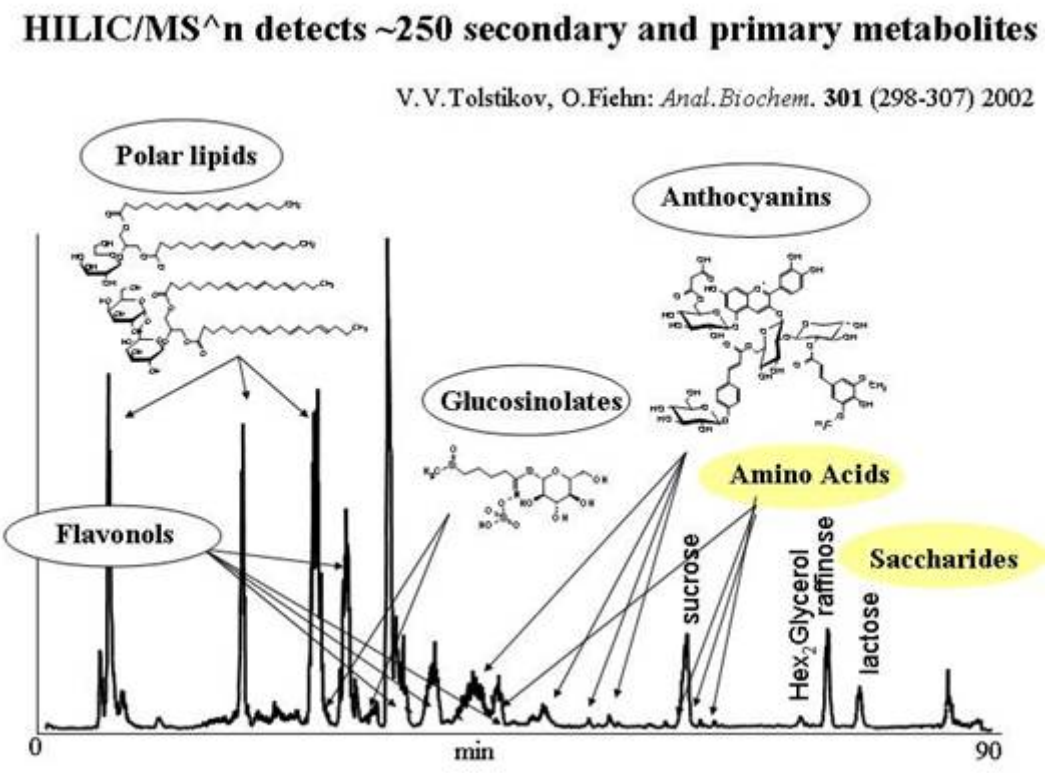
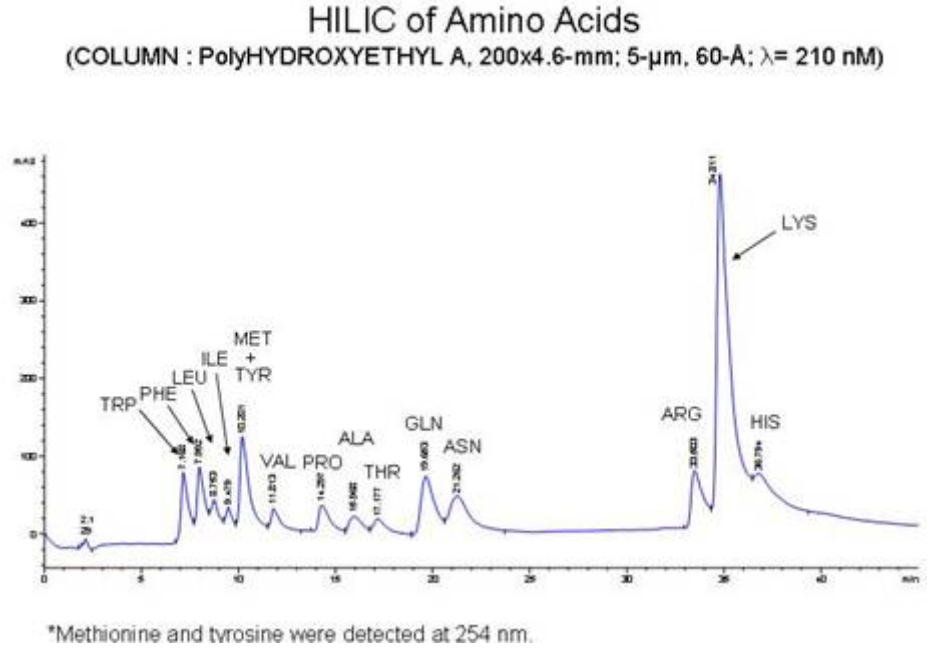


Amino Acids: Most amino acids can be resolved using routine HILIC conditions [BELOW]



Alternatively, amino acids and other small solutes can conveniently be measured quantitatively with a PolyHYDROXYETHYL A™ column (100-Å) using HILIC-MS/MS under isocratic conditions. In his poster

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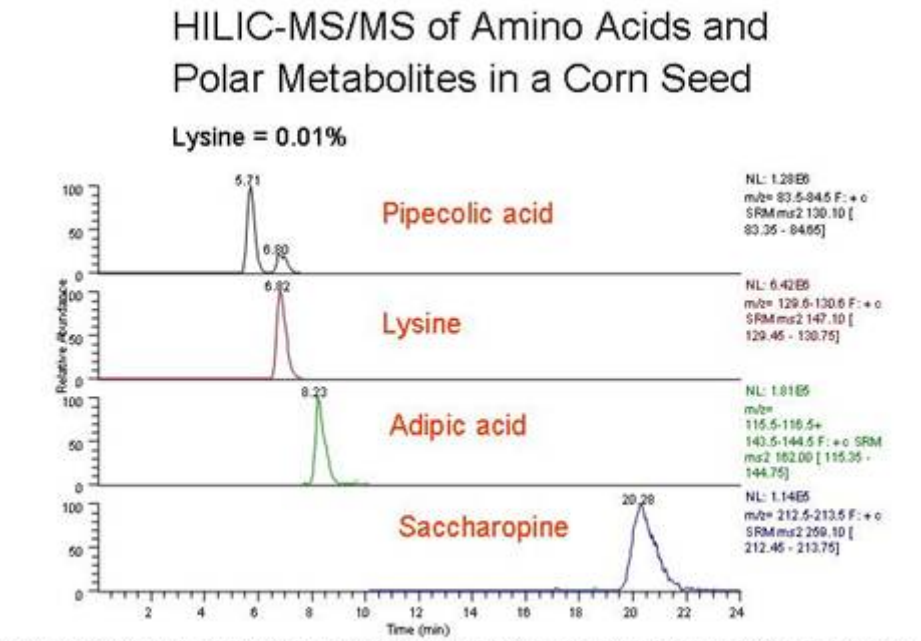
from ASMS '99, Robert Croes (DuPont Biotech) demonstrated the measurement of amino acids in individual seeds. Analysis of the 20 common amino acids is conveniently performed with 70% MeOH in an ammonium formate buffer. If some metabolites of amino acids are also to be measured [BELOW], then 60% ACN is used in place of the MeOH. This reduces MS sensitivity but affords better chromatographic separation. This is important with some metabolite pairs (e.g., lysine and pipecolic acid) that interfere with each other's detection in MS/MS.



Small peak at 6.8 min results from fragmentation of lysine in the APCI vaporizer [147 to 130] and MS^2 fragmentation by the pipecolic acid SRM [130 to 84]

Salt Concentrations and Extremely Polar Small Solutes: To obtain sharp, symmetrical peaks in HILIC for small polar solutes, the following salt levels are required in the mobile phase:

a) 10-15 mM: Typical polar solute, such as a neutral amino acid.
b) 40 mM: Solutes with a higher charge-to-mass ratio, such as
arginine.
c) > 120 mM: Solutes with an extremely high charge, such as
ATP or aminoglycoside antibiotics.

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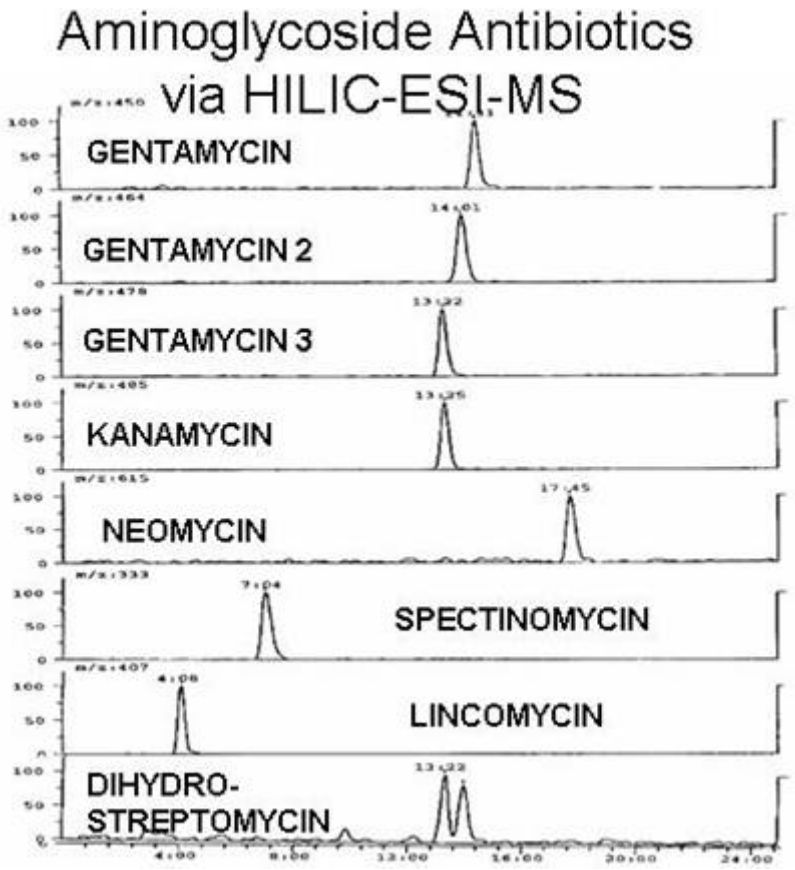
Fortunately, ESI-MS can tolerate up to 400 mM ammonium acetate in the mobile phase.

A plausible explanation is that the retention of highly-charged solutes is strongly influenced by the polarity of their counterions. A high concentration of salt insures that all the charged groups have the same counterions. If this is not the case, then the peak can be skewed. In an extreme case, two well-separated peaks can be obtained for a single pure solute, corresponding to the same solute with different counterions, with a continuum between them corresponding to molecules that exchanged their original counterions for those in the mobile phase during their migration through the column. This rate of exchange is slow on the time scale of HPLC if the salt level in the mobile phase is too low.

Normally, HILIC of small solutes is best done with a column with a high surface area; typically, 60- or 100-Å pore diameter. However, with extremely polar solutes such as **aminoglycoside antibiotics**, use of a 1000-Å material with a low surface area facilitates elution using convenient mobile phases, as in the following example:



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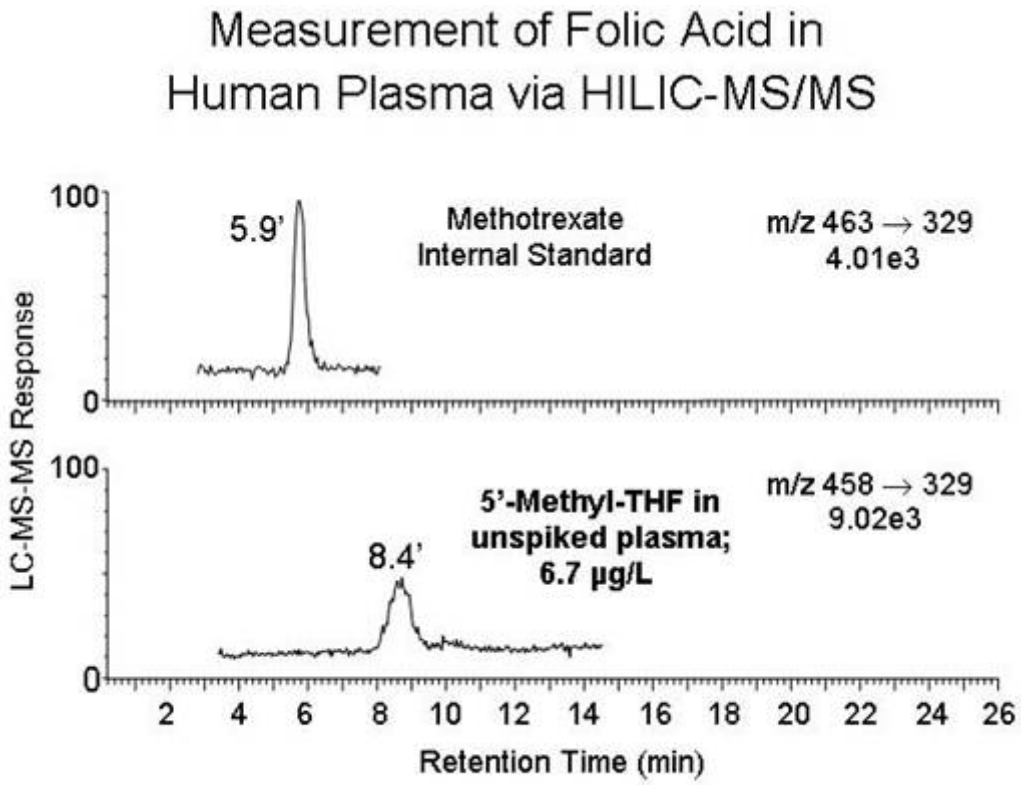
COLUMN: PolyHYDROXYETHYL A™ (Item # 204HY0510)
MOBILE PHASE: 250mM NH_4OAc, pH 4, with ACN
GRADIENT: 0-5': 80-25% ACN; 5-15': 25% ACN. FLOW: 1 ml/min

Small Solutes in **Serum** or **Plasma**: Addition of 2+ vol. of ACN to plasma or serum causes the precipitation of > 98% of the protein while leaving most small solutes in solution. The supernatant can be concentrated and analyzed via HILIC-MS/MS, often isocratically. This permits high-throughput analysis of **picomolar** levels of drugs, metabolites, etc. in serum or plasma. The following analysis of folates, methotrexate etc. is from S.D. Garbis *et al., Anal. Chem. 73* (2001) 5358-64:



- Courtesy of S.D. Garbis (Univ. Illinois-Chicago) -

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