Which column is your an

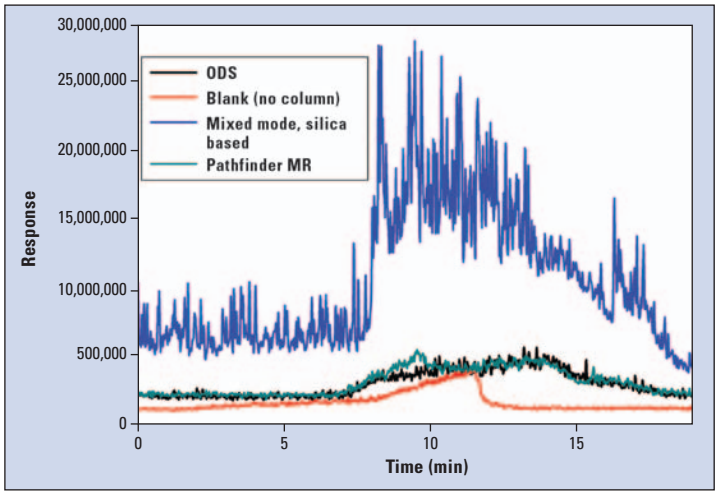

Figure 1: TIC of various RP columns under gradient elution. A blank run is included showing the performance of the system without RP column.

The demand from analytical chemistry for lower detection limits calls for highly sensitive instruments. The popularity of coupling liquid chromatography (LC) and mass spectrometry (MS) is growing because of its high sensitivity and superior resolution.

Evaporative Light Scattering Detection (ELSD) is often used in parallel with MS detection to give a maximum structural and purity profile. ELSD is extremely sensitive to non- and semi volatile compounds. A few factors need to be considered when optimizing an LC/MS and/or ELSD analysis. One is the compatibility of buffers and other additives, as non-volatile buffers can precipitate and clog the detector. Another point of concern is the purity of solvents. The continuous infusion of impurities leads to a high offset of the baseline, resulting in a decreased detection limit. A third cause of baseline disturbance can be bleeding of LC columns.

Column chemistry

In reversed phase chromatography a column can "bleed" for two reasons: Either residues from column production are eluting, or it can be devoted to the stability of the stationary phase. ODS columns (silica assembled with C18 chains) are by far the most used stationary phase. Although they are widely accepted, chromatographers are still not able to fully solve all of their separation problems with this media. The introduction of ODS columns with polar embedded groups allowed users to alternate selectivity to improve difficult separations. A general drawback of columns with polar embedded groups (PEG) is their low chemical stability in comparison with ODS columns [1].

Due to the limited stability of ODS and PEG columns, ligands can cleave off the support and dissolve in the mobile phase. This is especially apparent at higher concentrations of modifier. When ligands elute from the column into the detector, a signal change can be observed with certain detectors [2]. These ligands are normally invisible with UV. The UV signal depends on the molar extinction coefficient ϵ. Chromatographic supports are constructed with chemicals having very low molar extinction coefficients, so bleed should not be visible in UV. However, with MS or ELSD the ligands can be detected. If the amount of phase bleed is too high, the response can be large enough to reduce the sensitivity of other analytes.

Chemical inert reversed phase columns

Pathfinder media represents a new generation of HPLC packing material, made from organic and inorganic building blocks: one forming the internal silica core, and another forming the external chemically stable and inert polymer capsule. The potential value of polymer-encapsulated silica is that the external capsule provides resistance to hydrolysis of ligands.

In addition to its chemical stability, Pathfinder columns are also constructed with ionizable groups, such as mixed mode columns, to alternate selectivity. These polar and ionic groups are situated on the polymeric surface. This is a fundamental difference compared with silica based mixed mode and PEG columns, where polar groups and chains are attached directly to the support. The stability of these columns is therefore significantly lower. In Figure 1 four different total ion chromatograms (TIC) are displayed. To determine the bleeding level of each column, a linear

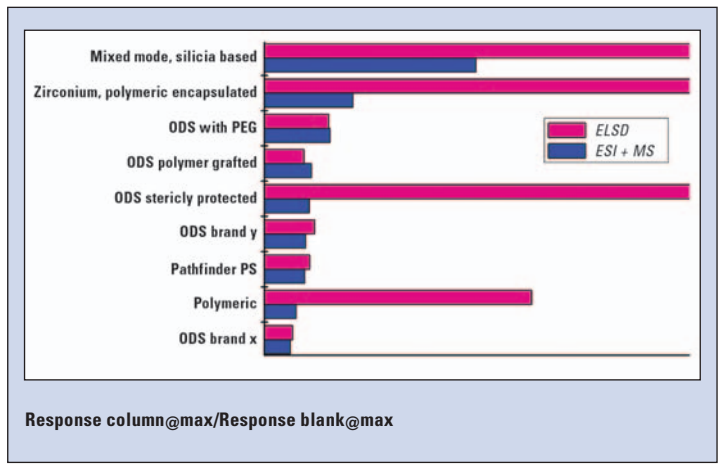

Figure 2: Phase bleed of various RP chemistries with ELSD and ESI in positive ion mode

alysis based on?

acetonitril gradient was applied. The response at the maximum was taken as a measurement of column bleeding.

An overview of bleeding levels of the various column chemistries used in this study is given in Figure 2. This graph shows that the noise observed with ELSD and MS detection correlates fairly well. Two exceptions are the polymeric column and the sterically protected column. These two columns are both constructed with material that barely ionizes. For this reason the ESI and ELSD noise does not correlate with these columns.

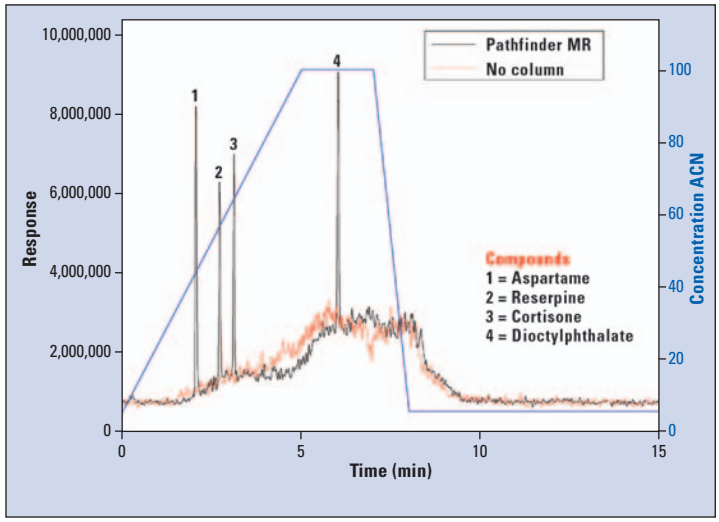

Figure 3: TIC of the LCMS system suitability test mix using a Pathfinder MR column and blank without column to demonstrate the low bleeding level of the column

Column conditioning for LC/MS

Whether using LC/MS for quantification or qualification, it is always useful to pay attention to the conditioning of the reversed phase column. Column bleeding is often observed in cases where the column is stored for a long period, or when it is used for the first time. With columns that have not been used for a certain period, bleeding can sometimes be observed because ligands cleave off in very low amounts. When a column is used again, the ligands will slowly (because of their highly hydrophobic nature) enter the detector and consequently cause noise. For this reason "washing" the column with at least 30 column volumes mobile phase A and 30 column volumes mobile phase B can prevent noisy baselines.

LC/MS system suitability test

To evaluate an LC/MS system a test mix was designed by Tang and coworkers [3]. This sample is appropriate for the complete evaluation of an RPLC-UV-ESI system in both positive and negative mode. It contains four compounds with varying molecular size and polarity to represent the broad properties of small molecules. The molecular weight of the test samples ranges from 200 to 700 amu which is within the range for small molecular compounds. CLogP is the octanol/water coefficient that correlates with the membrane permeation properties, and therefore polarity of molecules.

Separation of the test mix is shown in Figure 3 using a fast water/acetonitrile gradient with a Pathfinder MR column. The system used is a Shimadzu *prominence* liquid chromatograph equipped with LC-20AB pump, SIL-20AC autosampler with cooling function, CTO-20AC oven, SPD-M20A diode array detector and an LCMS-2010EV single quadruple mass spectrometer with an ESI ionization probe. A CBM-20A system controller and LCMSsolution chromatography software were used to control the modules and acquire data.

Aspartame is a hydrophilic compound, indicating the ability of the column to retain polar compounds. The properties of cortisone and reserpine are somewhat similar, and are therefore a measurement of column selectivity. Dioctylphthalate is an extremely hydrophobic compound, which can only be eluted with a high concentration of organic modifier. Dioctylphthalate can be used as an indicator for the end of a gradient. Aspartame, cortisone and reserpine will give an MS signal in both positive and negative ion mode. Dioctylphthalate cannot be detected with ESI in negative mode.

To get optimum separation performance with Pathfinder media, the starting point should be Pathfinder MR or PS as can be seen in Figure 3. Approximately 75 % of all MS methods are acquired in an acidic environment. Under acid conditions the polar groups of Pathfinder MR and PS generally offer the biggest advantages.

References

[1] H. A. Claessens, M. A. Straten van, J. Chromatogr. A (2004), 1060, 23-41

[2] T. Teutenberg, J. Tuerk, M. Holzhauser, T.K. Kiffmeyer, J. Chromatogr. A (2006), 1119, 197-201

[3] L. Tang, W.L. Fitch, M.S. Alexander, J.W. Dolan, Anal. Chem. 72 (21) (2000) 5211-5218