



Automating Method Development with an HPLC System Optimized for Scouting of Columns, Eluents, and Other Method Parameters

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HPLC method development is still considered one of the critical bottlenecks that impedes productivity in analytical laboratories. Due to the variety of available columns and mobile phases applicable in reversed-phase chromatography, the selection of optimal conditions is a challenge. Here we present an integrated LC system and software that automatically screens possible combinations of columns, mobile phases, and temperatures.

Instrumentation

The UltiMate® 3000 LC system consisting of a quaternary gradient pump, an autosampler compatible with well plates and standard sample formats, a powerful diode array detector, and a thermostatted column compartment with two six-position seven-port valves (TCC-3200) allows for automatic and intuitive scouting of columns and eluents.

Method Scouting Example

The separation of eight diuretic compounds including acids, bases, and zwitterions was performed to evaluate the system. The following parameters were automatically screened: six different RP columns, two organic modifiers, two buffer pH values and two column temperatures. Two separations of the diuretics, obtained at pH 3 and 8, are shown in Figure 2.

The retention for the acidic compounds was higher at pH 3 as a result of protonation of the carboxylic groups, which increases the hydrophobicity. Another notable difference is that two of the compounds co-eluted on the C8 column at pH 8. In general, the correlation plots between pH 3 and 8 showed a large scatter of the retention times, indicating the usefulness of pH for influencing separation selectivity.

Chromeleon® Tools for Automated Method Scouting

Chromeleon Chromatography software has several tools that facilitate automated method scouting experiments and method selection:

- Flexible control panels deliver direct control of the multiposition valves and a customizable user interface
- User defined columns in sample list — parameter values (e.g., column, temperature) are set in the sequence table, significantly reducing the number of instrument methods
- Automated System Suitability Test (SST) — checks if a chromatographic run passes certain specified criteria like number of peaks and resolution
- Query tool highlights methods that meet the SST criteria in seconds
- Charting options illustrate critical parameters, simplifying final method selection

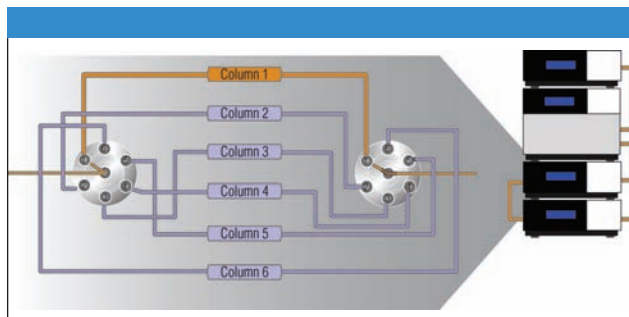


Figure 1: Instrument setup for automated column and eluent scouting.

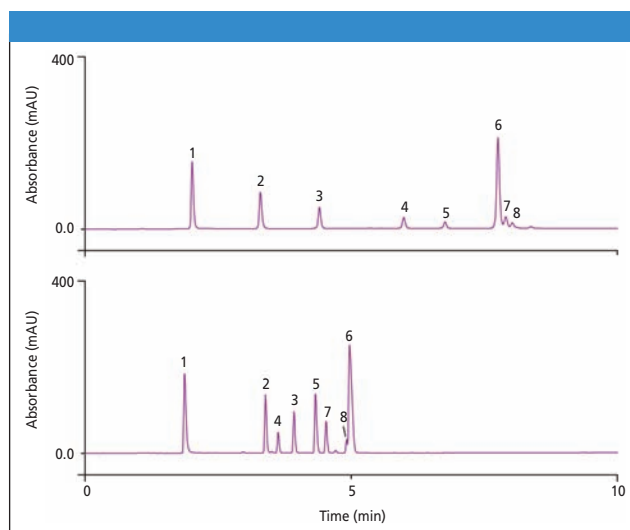


Figure 2: Separation of diuretics on a C8 column using ammonium formate buffer pH 3 (upper chromatogram) and ammonium bicarbonate buffer pH 8 (lower chromatogram) with acetonitrile as the organic modifier. Peaks: 1) amiloride, 2) triamterene, 3) chlortalidon, 4) furosemide, 5) canrenic acid, 6) ethacrynic acid, 7) probenecid, 8) bumetanide.

Conclusions

The UltiMate 3000 LC system with the TCC-3200 2x6p-7p in combination with Chromeleon software provides a turnkey solution for automated column and eluent scouting.

Post processing tools can highlight the optimal methods in seconds, eliminating the need to manually assess all data.

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