AI-Driven Optimization of HILIC Methods for Enhanced Nucleoside Separation

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1. Introduction

HILIC (Hydrophilic Interaction Liquid Chromatography) is frequently promoted for their numerous advantages compared to the more commonly utilized reversed phase liquid chromatography. These advantages include high retention and selectivity for polar compounds, as well as enhanced sensitivity when coupled with mass spectrometry (MS). Although HILIC techniques offer potential benefits, many chromatographers are often reluctant to adopt them due to several challenges. These challenges include the complex mobile phase setups required for HILIC, longer equilibration times, and a general lack of familiarity with the technique.[1]

To address these obstacles, robust support for the development of HILIC methods is essential. Such support can significantly streamline the process and enhance the confidence of chromatographers in utilizing this powerful technique. In the following, the method development process for a HILIC application with Shimadzu Method Development software is described.

2. Material and Method

As a standard, a nucleoside mix from Sigma Aldrich was used. For the columns, different types of HILIC columns were selected:

- The Shim-pack GIST NH2 is an amino phase column.
- The Shim-pack GIS HILIC contains diol groups.
- The Shim-pack Velox HILIC is a pure silica column.
- The GIST Amide column has carbamoyl groups.

Solutions of 10% formic acid, 200 mM ammonium formate, and 100 mM NaOH in water were prepared. Through solvent blending, these have been used to achieve solutions of pH 2, 3.5, 7, and 8. An overview of the configuration is shown in Fig. 1.

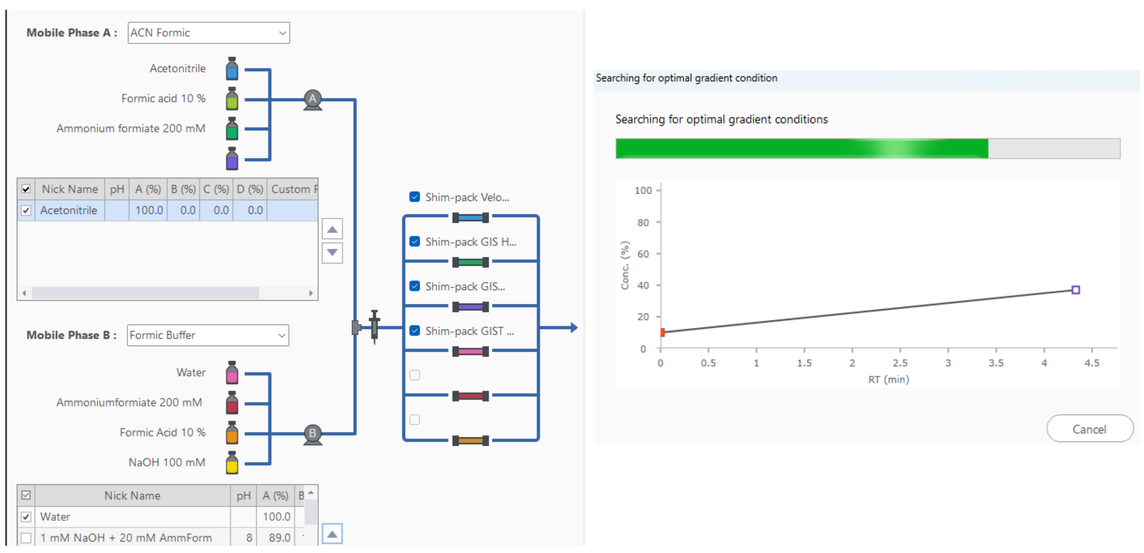

Figure 1. Method Scouting conditions (left) and Gradient Optimization view (right) of Method Development software

For the preliminary testing, isocratic runs were conducted with water percentages of 5 %, 10 %, and 15 %. Additionally, the runs varied between pure water and the previously described pH mixtures. This resulted in a total of 42 different testing conditions.

Table 1. Analytical conditions

Method Parameters	
System:	Nexera X2 Method Scouting System
Columns	a) Shim-pack Velox HILIC 2.7 µm 150 x 3 mm b) Shim-pack GIS HILIC 3 µm 150 x 4.6 mm c) Shim-pack GIST Amide 5 µm 150 x 4.6 mm d) Shim-pack GIST NH2 3 µm 150 x 3 mm
Mobile Phase A:	ACN
Liquids of Mobile Phase B used for Solvent Blending:	1) Water 2) 10 % formic acid (FA) 3) 200 mM ammonium formate (AF) 4) 100 mM NaOH
Optimum Composition of Mobile Phase B	1 % FA + 20 mM AF
Gradient Program (B %):	9 % (0.67 min) →28 % (7.22 min) →30 % (7.23 - 10.22) → 9 % (10.23-18.22)
Flow rate:	0.6 ml/min
Injection Volume:	1 µL
Oven Temperature:	40 ºC
Detector:	SPD-M40
Detector Cell Tempeature:	40 ºC

3. Results

The Shimadzu development software assists in the evaluation of the first 42 measurements by providing options to rank the measurements based on the number of peaks detected, the separation of peaks, total resolution, and overall evaluation value. The Evaluation Value is calculated as the number of peaks detected multiplied by the sum of the resolution for all peaks. Figure 2 displays the chromatograms with the highest evaluation values for each individual column. Both the bare silica and the amide columns yielded good results; however, since the bare silica column had a greater total number of peaks within the given timespan, it was selected for further gradient optimization.

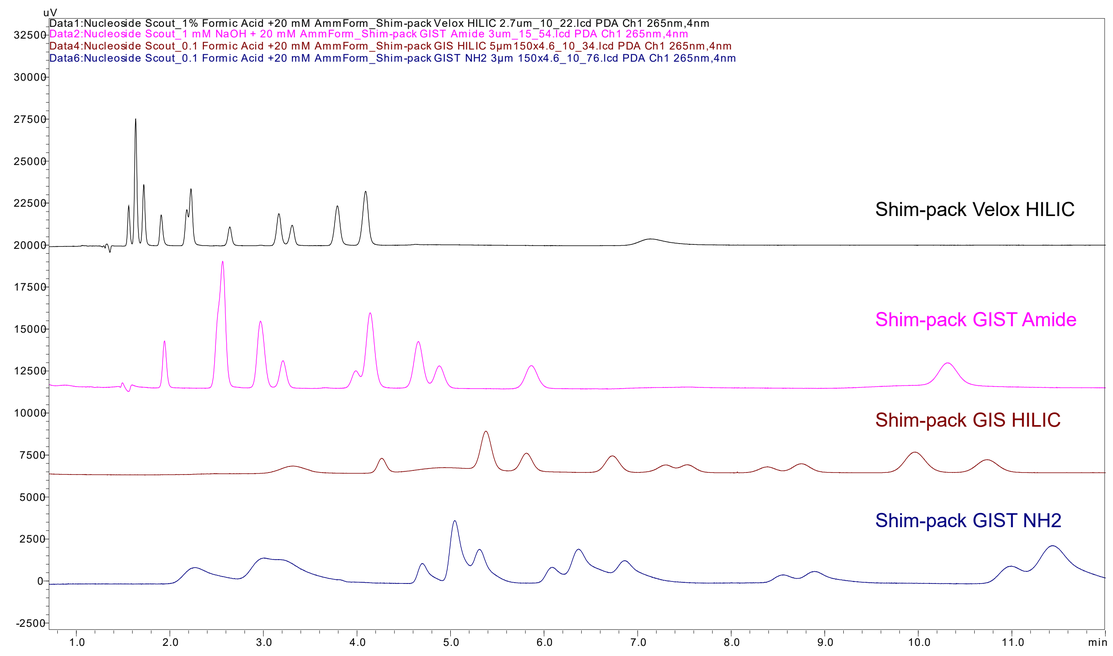

Figure 2. Comparison of the chromatograms of the best evaluation values for each individual column

Based on the run with the highest evaluation value, the selected parameters are utilized for gradient optimization. The program applies slight variations to these parameters. If none of the variations meet the required analysis time and minimum resolution, the software calculates a new gradient optimization and executes these calculated gradients to identify the best possible settings.

Additionally, the data obtained from the gradient optimization can be used to develop a chromatographic model for further gradient optimization (see Figure 1, right side).

Figure 3 presents a comparison between the best run obtained by varying the parameters from the preliminary tests and the optimized gradient identified by the software. The optimized method demonstrates improvement, achieving a reduction of approximately 1.5 minutes in analysis time compared to the results obtained through parameter variation alone.

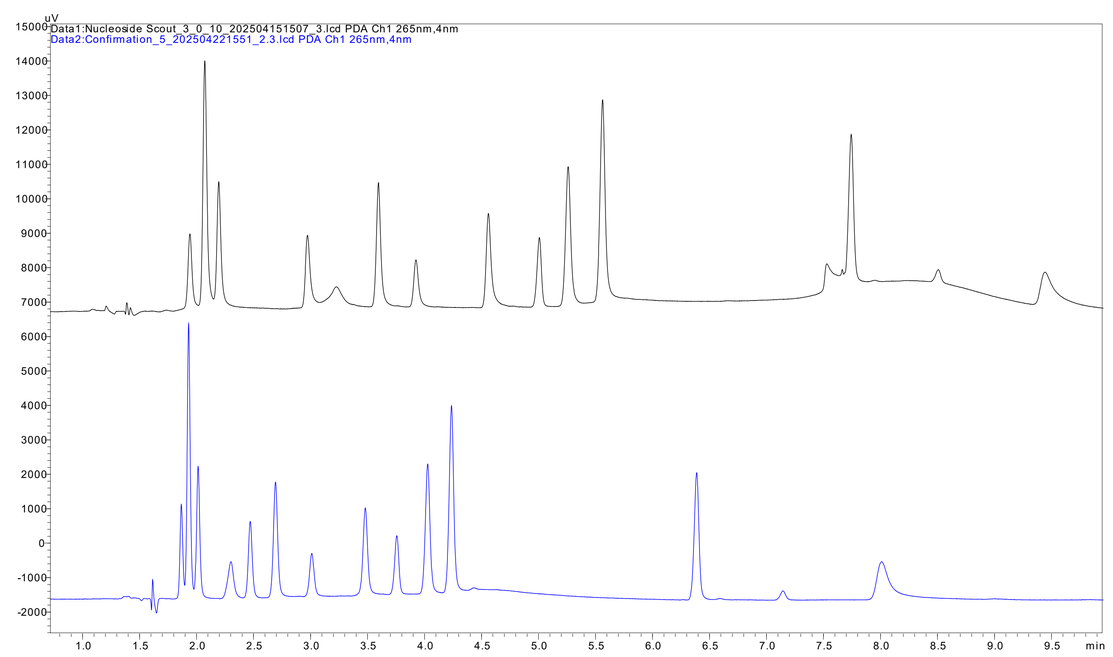

Figure 3. Comparison of experimental found gradient (top) and software optimized method (bottom) for the compounds: Ribothymidine, Uridine, Thiocytidine, Peseudouridine, Methyladenosine, Inosine, Guanosine, Methylguanosine, Cytidine, 2-O-Methylcytidine, 5-Methylcytidine, Methylcytidine methosulfate

4. Conclusion

Despite the complexities associated with HILIC methods, the use of Shimadzu Method Development software significantly facilitates method development and optimization. By evaluating various HILIC columns and mobile phases, the software identified optimal gradient parameters, resulting in reduced analysis time. These findings highlight the potential of software-supported HILIC methods to enhance chromatographic workflows and encourage their broader adoption in analytical laboratories.

References

[1] Buszewski B, Noga S. Hydrophilic interaction liquid chromatography (HILIC)-a powerful separation technique. Anal Bioanal Chem. 2012. 10.1007/s00216-011-5308-5.