

The Hitachi LaChrom Elite HPLC Automated Method Development System: Polar Reversed Phase Separations

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Equipment
Hitachi LaChrom Elite (LCE) system
Pump L-2130 equipped with low pressure gradient kit
Autosampler L-2200
Column Oven L-2300 w/Peltier block
Column Supelco HS F5 (4.6 mm i.d. x 250 mm)
Detector L-2400 variable wavelength UV
Acquisition CDS EZChrom Elite 3.1.x
Other ChromSword® Auto 2.2 Automated Method Development Software

Method
Detector settings (UV)
Sampling Rate 50 ms
Response Time 0.05 s
Wavelength 210 nm
Oven Temperature 35 °C
Eluents (Isocratic) 10 (10 mM NH₄OAc):90 (10% NH₄OAc + 90% CH₃CN v/v)
Flow Rate 1.5 mL/min

Results and Discussion
Four amphetamine and methamphetamine standards (amphetamine [A], methamphetamine [MA], 3,4-methylene dioxyamphetamine [MDA], 3,4-methylenedioxymethamphetamine [MDMA], Figure 1) were analyzed with the Hitachi LaChrom Elite AMD system under the method conditions described above.

Since the Supelco Discovery HS F5 polar, RP column (a unique pentafluorophenyl terminated reversed phase column specifically developed for pharmaceutical analysis and purification) (1).

Table I: ChromSword Auto Optimization results*

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<thead>
<tr>
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<th>CSA</th>
<th>LCE</th>
<th>CSA</th>
<th>LCE</th>
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<tbody>
<tr>
<td>A</td>
<td>19.16</td>
<td>19.35</td>
<td>9.96</td>
<td>9.68</td>
<td>9.34</td>
<td>9.52</td>
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<tr>
<td>MDA</td>
<td>22.43</td>
<td>22.66</td>
<td>8.94</td>
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<td>9.12</td>
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<tr>
<td>MA</td>
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<td>24.49</td>
<td>12.98</td>
<td>12.80</td>
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<td>11.82</td>
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<tr>
<td>MDMA</td>
<td>32.91</td>
<td>34.20</td>
<td>10.90</td>
<td>11.12</td>
<td>11.53</td>
<td>11.82</td>
</tr>
</tbody>
</table>

* CSA — ChromSword Auto, LCE — Hitachi LaChrom Elite HPLC, A — amphetamine, MDA — 3,4-methylene dioxyamphetamine, MA — methamphetamine, MDMA — 3,4-methylenedioxyamphetamine.
The isocratic optimization results are summarized in Table I comparing the CSA predicted results for each peak with the actual results obtained on the Hitachi LCE HPLC system.

The retention profile for these amphetamine standards at both extremes of the eluent (22% and 75% B) displays a very accurate correlation between the predicted and observed chromatograms. These results are also illustrated in Figures 2a and 2b and Figures 3a and 3b for %B eluent at 22 and 75%, respectively matching very closely with respect to relative retention time, peak height and peak shape. The slight retention time disparity between the CSA and LCE results for the MA and MDMA components at 22% B are attributable to the peak tailing observed but is still quite good and quite accurate when tailing is minimized.

A crossover region at approximately 40–60% B occurs between the A-MDA and MA-MDMA components (see curve in Figure 3a) and those are modeled with a high degree of accuracy as well at 50% B (see Table I). The ability of the Supelco polar reversed phase column pentafluorophenyl moieties to provide polar interactions not present in traditional C18 alkyl functionalized RP columns allows for the separation of both polar and non-polar analytes with a single column while also obviating the need for ion-pairing reagents.

**Conclusions**

The Hitachi LaChrom Elite Automated Method Development system with ChromSword Auto has been demonstrated to accurately generate new methods for non-traditional RP columns such as the Supelco Discovery HS F5 polar RP column. This translates into faster, lower cost, and robust methods in which analytes with polar functionalities can be more readily separated.

**Acknowledgments**

J. Lim wishes to acknowledge David Bell (Supelco) for the generous loan of the Discovery HS F5 polar RP column and amphetamine/methamphetamine standards (Aldrich-Sigma Chemicals).

**References**

(1) For information on Supelco’s Discovery HS F5 column, visit http://www.sigmaaldrich.com/Brands/Supelco_Home/TheReporter/Liquid_Chromatography/reporter_21_4_main.html.

(2) For more information on ChromSword® Auto, visit http://www.lcgceurope.com/lcgceurope/data/articlestandard/lcgceurope/512001/5428/article.pdf.

**Figure 2:** Amphetamine and methamphetamine AMD optimization results: (a) CSA predicted model at 22% solvent B, (b) LCE experimental results observed at 22% solvent B.

**Figure 3:** Amphetamine and methamphetamine AMD optimization results: (a) CSA predicted model at 75% solvent B, (b) LCE experimental results observed at 75% solvent B.