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

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Traditional HPLC method development involves highly skillful, experienced, knowledgeable, and intuitive operators to generate efficient separation methods. This is often a time-consuming endeavor that does not always produce the best possible separation results, in spite of the best efforts. Automated method development (AMD) with the Hitachi LaChrom Elite (LCE) HPLC system in conjunction with ChromSword® Auto (CSA) provides a unique AMD system that will separate multi-component systems effortlessly, accurately, and with significant savings in both time and costs for the method development.

The retention profile for reversed phase (RP) separations is often unpredictable and can only be derived through actual experiment runs. The advent of novel surface-modified (one type of which is the topic of this Application Note) RP columns compounds this level of predictability even further owing to the complexity of the interactions between the surface modified groups and the analytes. HPLC method development can be a time-consuming task requiring multiple discrete, individual runs followed by analyses and refinement by the well-experienced method developer. The ability for CSA to model separations based on the retention behavior of the components by full automation control of the Hitachi LCE HPLC system greatly simplifies HPLC method development, saving many hours (and even days), reduces the amount of solvent used, thereby ultimately reducing the actual cost for the method development.

This Application Note reports the unattended (fully automated) method development optimization program with the *Supelco Discovery HS F5* polar, RP column (a unique pentafluorophenyl terminated reversed phase column specifically developed for pharmaceutical analysis and purification) (1).

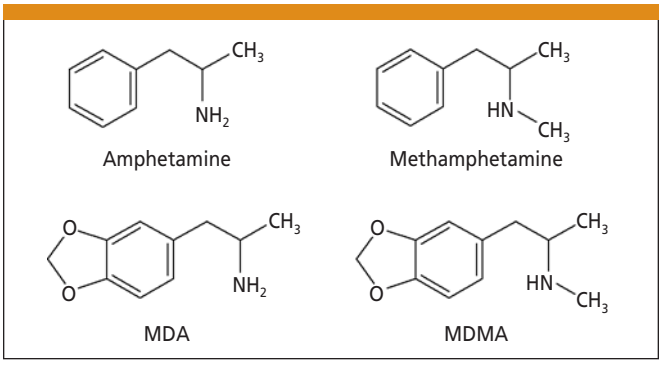

Figure 1: Amphetamine and methamphetamine standards.

Equipment

Hitachi LaChrom *Elite* (LC*E*) 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. × 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 NH_4OAc):90 (10% NH_4OAc + 90% CH_3CN 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-methylenedioxymetham phetamine [MDMA], Figure 1) were analyzed with the Hitachi LaChrom *Elite* AMD system under the method conditions described above.

Since the Supelco *Discovery HS F5* RP column was not previously calibrated for the CSA column database, the "empirical" optimization approach was used (this takes into account neither the chemical structures of the standards nor the properties of the column) for the AMD optimization and analyzes the results based on the retention profile of the individual peaks (2).

Table I: ChromSword Auto Optimization results*

	22% B		50% B		75% B	
Sample	**CSA**	**LCE**	**CSA**	**LCE**	**CSA**	**LCE**
A	19.16	19.35	9.96	9.68	9.34	9.52
MDA	22.43	22.66			8.94	9.12
MA	28.88	24.49	12.98	12.80	11.53	11.82
MDMA	32.91	34.20			10.90	11.12

*CSA = ChromSword Auto, LCE = Hitachi LaChrom *Elite* HPLC, A = amphetamine, MDA = 3,4-methylene dioxyamphetamine, M = methamphetamine, MDMA = 3,4-methylenedioxymethamphetamine.

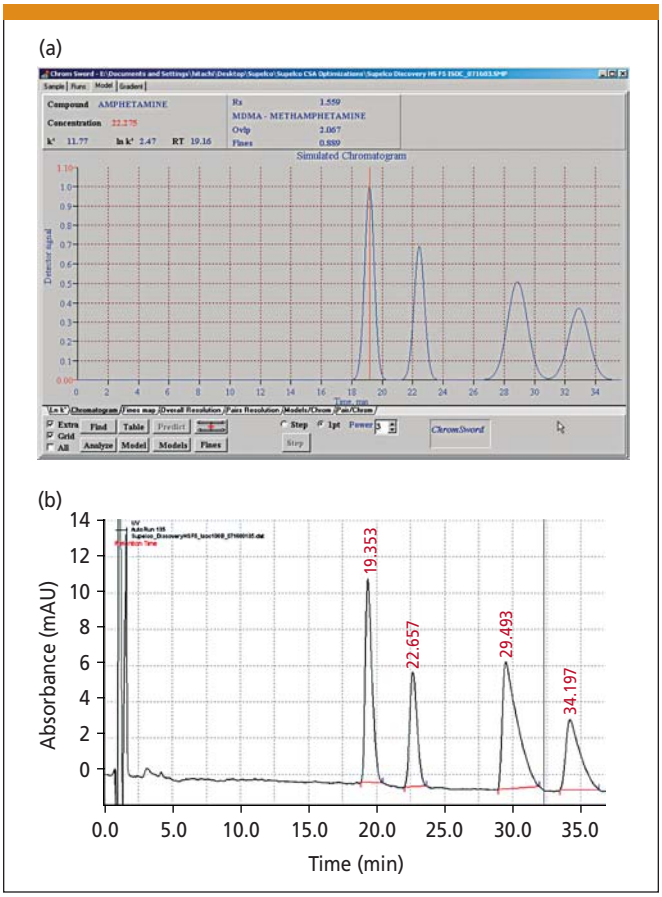

Figure 2: Amphetamine and methamphetamine AMD optimization results: (a) CSA predicted model at 22% solvent B, (b) LCE experimental results observed at 22% solvent B.

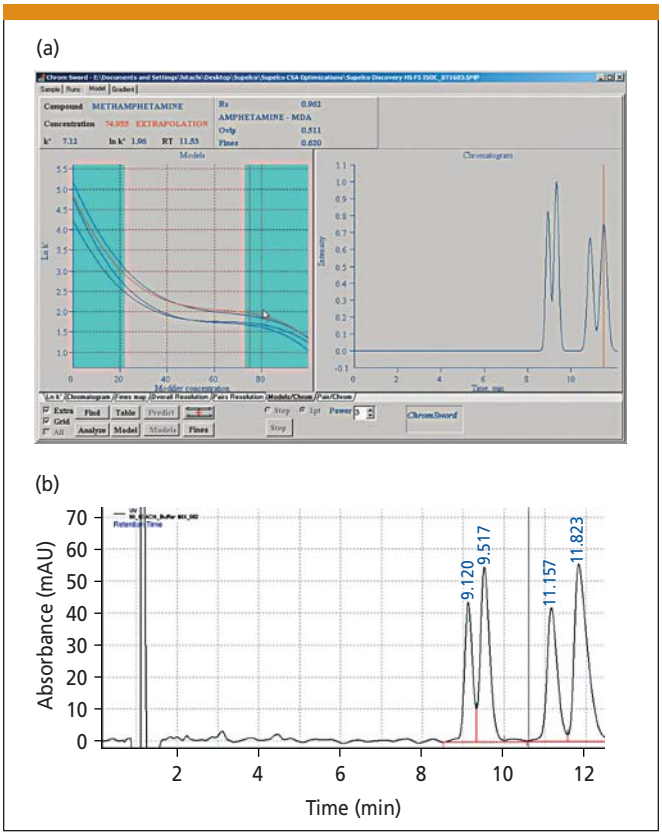

Figure 3: Amphetamine and methamphetamine AMD optimization results: (a) CSA predicted model at 75% solvent B, (b) LCE experimental results observed at 75% solvent B.

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.

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