

Method development of liquid chromatographic procedures for pharmaceutical and biotechnological entities by use of the ExpressLC-800™ multi-channel capillary LC

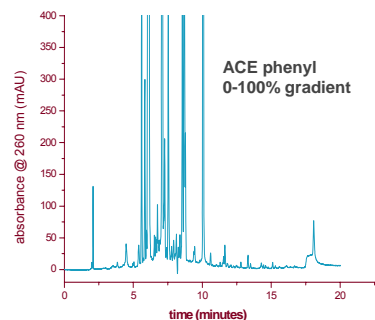
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Abstract

Liquid chromatographic (LC) development of methods for conventional and proteinoous drug analysis is a time-consuming but critical task. Development of a method without sufficient experimentation to produce optimized selectivity often causes significant problems at some point in the drug development cycle. A method development chemist would ideally study a number of column stationary and mobile phase conditions and choose the stationary/mobile phase combination that optimized both the resolution of important species and method robustness. Such an extensive study with conventional LC is often impractical, since investigating multiple chromatographic conditions requires time-consuming column exchanges and re-equilibrations. Computer-assisted column switching requires indeterminate re-equilibration times and often produces unreliable results. In this presentation, a capillary LC with eight independent channels (the ExpressLC-800) will be used to produce separations with eight columns, four pH conditions and optimized gradient endpoints; typically, within four hours.

Experimental

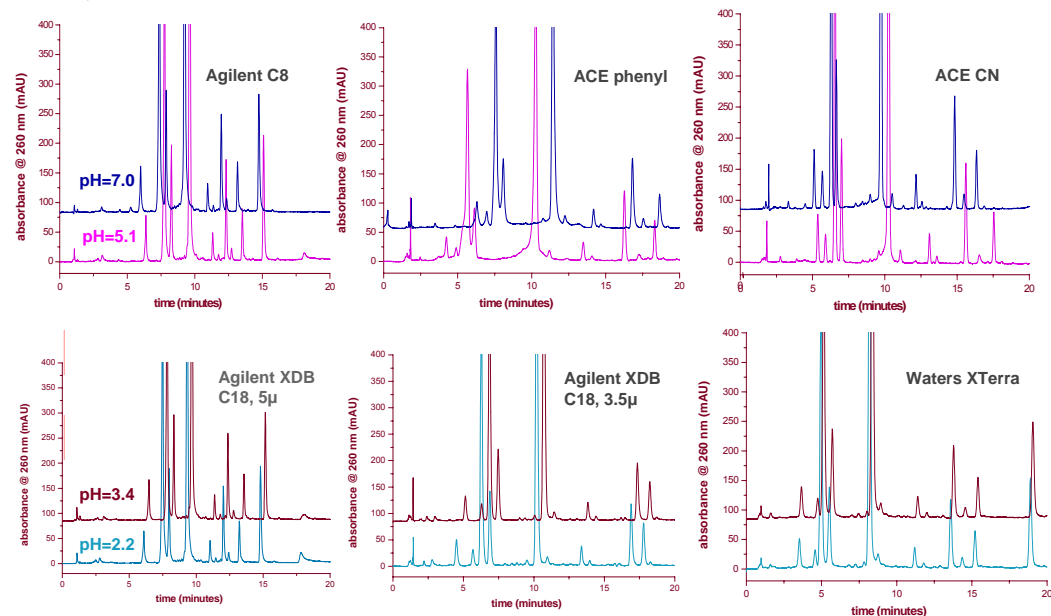
Instrument: Eksigent ExpressLC-800 capillary liquid chromatograph
Columns: Agilent Zorbax: C8, 10 cm, 3.5 μ ; XDB-C18, 15 cm, 5 μ ; XDB-C18, 15 cm, 3.5 μ ; ACE: phenyl, 15 cm, 3 μ ; CN, 15 cm, 3 μ ; AQ18, 15 cm, 3 μ ; Waters: XTerra C18, 10 cm, 3.5 μ ; Atlantis C18, 10 cm, 3.5 μ .
Column temperature: Ambient
Mobile phase: linear gradients, A=aqueous solutions of TFA, acetic acid, or potassium phosphate, pH=2.2, 3.4, 5.1, 7.0 ; B=acetonitrile; **gradients:** 5-25%, 5-30%, 5-35%, 5-40%, 5-100% A:B
Flow rate: 6-10 μ L/minute
Injection volume: 80 nL
Detection wavelength: 260 nm
Programmed gradient re-equilibration time: 0 minutes.



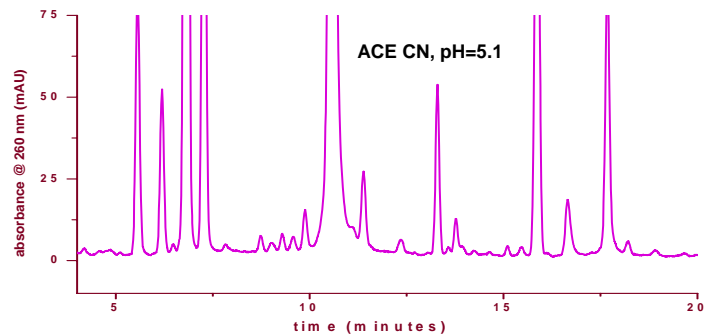
Multi-channel capillary LC method development will be shown to generate an extensive set of chromatograms for a complex isoflavone mixture, whereby selection of the most selective separatory conditions can be made

The instrument provides method development data quickly by performing eight separations simultaneously on a capillary LC system that produces high-resolution separations and requires very short re-equilibration times.

The sample selected for this method development study was a commercially available complex isoflavone mixture (Sundown). A chromatogram at the bottom of the previous column is a separation employing a 5-100% gradient with 0.1% TFA in the aqueous acetonitrile mobile phase. The sample was then separated on 8 stationary phases (see Experimental) varying the linear gradient endpoints from 25-40% mobile phase B to increase resolution. The resulting optimized linear gradient separations were then performed at pH values of 2.2, 3.4, 5.1, and 7.0 and 12 of the resulting 32 chromatograms appear to the right. The separatory conditions that yielded the best overall resolution were an ACE CN column with a pH=5.1 mobile phase. The resulting separation appears below:



8-column, 4 mobile phase, gradient end-point optimized separation of isoflavones



Conclusions

Using the ExpressLC-800, simultaneous separations of a complex isoflavone mixture were independently performed on eight column stationary phases. Since the standard 0-100% gradient yielded little resolution on any of the columns, other linear gradient schemes were investigated in an automated manner. The gradient endpoints were found to range from 25-45%, depending on the stationary phase. The mobile phase pH was then varied (pH=2.2, 3.4, 5.1, and 7.0) and the resulting chromatograms examined for maximum overall resolution. The automated 8-column, 4 mobile phase, end-point optimized separation yielded an excellent separation on an ACE CN column at pH=5.1, demonstrating the ability of the ExpressLC-800's ability to yield highly-resolving separations of complex mixtures in an automated fashion.