



application note

expressLC[®]-800 system for rapid column screening in LC methods development

Eksigent's Microflow™ parallel LC, the ExpressLC-800 system, can significantly reduce the amount of time and effort that is required by conventional LC approaches to evaluate the separation of complex samples for column selection and method development.



expressLC•800PLUS

expressLC[®]-800 system for rapid column screening in LC methods development

overview

The development of an LC method to optimize the resolution of the separation of a complex sample normally involves a large number of trial separations using a variety of columns and mobile phases to optimize the resolution of the compound(s) of analytical interest. While the chromatographer typically has a good understanding of the general type of column to be used (e.g., C18) and the conditions—such as the mobile phase, gradient, temperature and flow rate—that are likely to be useful on the basis of the knowledge of the sample to be separated, a number of different columns of a given type are usually available from a variety of suppliers. While these columns may employ the same functional group, they will likely differ in important parameters such as the pore size, particle size distribution, the level of impurities, the degree of end capping and any embedded functional groups. These differences in column characteristics may lead to small, but potentially significant changes in the observed chromatogram, and the resolution of the compound(s) of interest may be better on certain columns.

When screening columns, the chemist will analyze the sample using various columns with a common mobile phase to determine the optimum column for the separation. While this approach may well lead to the column that provides the suitable separation for the compounds of analytical interest, it can be extremely time consuming and labor intensive. In addition to the time for the actual separation, the time required to physically exchange columns and equilibrate each column with the mobile phase must be considered.

The Eksigent ExpressLC-800 system provides a considerably more rapid approach for column screening than the use of a conventional LC system. This system performs the simultaneous separation of a sample using eight individual columns of different characteristics. In this note, we present the separation of a green tea extract using a number of different columns to demonstrate how the system can rapidly and effectively determine the optimum column for the desired separation. One can then see how once the most appropriate column has been determined, a similar series of separations can be used to determine the most appropriate mobile phase set of conditions. This general strategy can be employed in a broad range of applications—one such area is the study of accelerated degradation of a product in pharmaceutical development where the analyst scouts for a column that can resolve all of the possible degradation products.



experimental

green tea extract

An aqueous extract of a green tea concentrate extract (Sundown) containing a mixture of polyphenols, phenolic acids, alkaloids, flavanols, flavandiols, flavonoids, caffeine, B vitamins, and ascorbic acid was separated on 8 stationary phases with a linear gradient. The polyphenols included (-)-epigallocatechin-3-gallate, (-)-epigallocatechin, (-)-epicatechin-3-gallate, (-)-epicatechin, (+)-gallocatechin, and (+)-catechin and represent a formidable set of compounds of differing chromatographic polarities for LC analysis.

separation conditions

Columns: The columns that were employed in these experiments are listed in Table 1. Columns containing 5 μ m particle sizes in addition to 3 μ m were selected to determine if larger particles would achieve an adequate separation and thus allow for faster flow rates and shorter analysis times.

The following conditions were used for the separation:

- *Column temperature: Ambient*
- *Mobile phase: linear gradient, A= water with 0.1% TFA, B=acetonitrile/methanol (80/20) with 0.1% TFA*
- *Flow rate: 6 μ L/min*
- *Gradient: 0-40% B for each in 20 minutes*
- *Injection volume: 80 nL*
- *Detection wavelength: 218 nm*
- *Sample preparation: aqueous extraction followed by filtration*

Table 1. C18 Columns used to Separate Green Tea Extract

column	manufacturer	phase	particle size	length
1	ACE	AQ	3 μ m	15cm
2	Agilent	SB-C18	5 μ m	15cm
3	Waters	Atlantis	5 μ m	15cm
4	Agilent	XDB	3.5 μ m	15cm
5	Eksigent	CL	3 μ m	15cm
6	ACE	C18	5 μ m	15cm
7	Eksigent	EP (300A)	3 μ m	15cm

discussion

The green tea extract was separated on the eight columns listed in Table 1 using an ExpressLC-800 Microflow liquid chromatograph. Figure 1 reflects the separations of four columns as opposed to eight enabling easier viewing of a complex chromatogram.

The goal of this experiment is to determine the choice of the best column to perform the analysis of the compound(s) of interest. The parallel LC system can be used to simultaneously screen eight columns in order to select the best column for the separation. While some of the columns presented similar chromatograms (Figure 1), significant differences in the chromatograms can be noted. To highlight the differences between the various columns, the individual chromatograms from three of the columns are presented in Figures 2-4.

In Figure 2 (Eksigent EP column), sharp peaks are observed at ca 9.21 and 10.87 min, while the chromatograms from the Eksigent CL (Figure 3) and the Agilent XDB (Figure 4) exhibit considerably more complex structure. If these peaks are considered critical pairs, the Eksigent EP column used in Figure 2 might be selected for further evaluation. In experiments such as forced degradation, many compounds may be of analytical interest and the column to be selected should represent the best compromise to optimize the resolution for the compound(s) of interest.

While the decision to accept a column is somewhat subjective, the use of the Eksigent ExpressLC-800 to evaluate the separation of complex samples using a number of columns can significantly reduce the amount of time and effort that is required to determine the best column.

Figure 1: Separation of green tea aqueous extract on all four columns listed in Table 1.

Mobile phase A: water with 0.1% TFA and mobile phase B: 80% acetonitrile/20 % methanol with 0.1 % TFA.

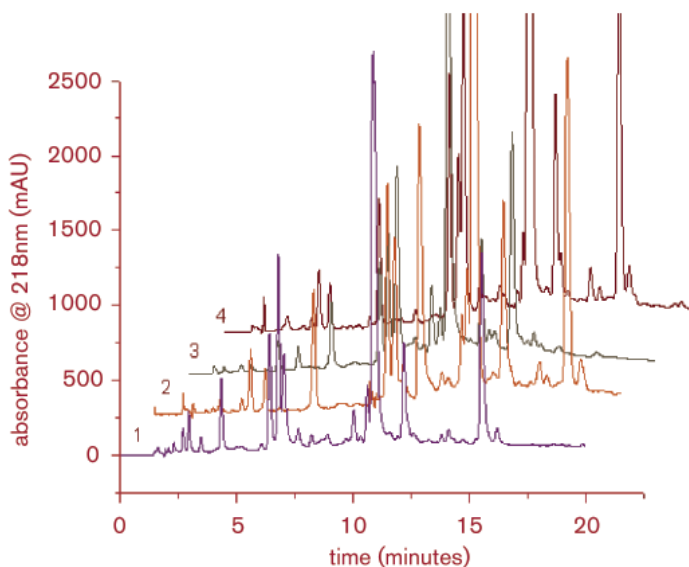


Figure 2: Chromatogram from Column 7 (Eksigent EP, 3 μ m, 15 cm column)

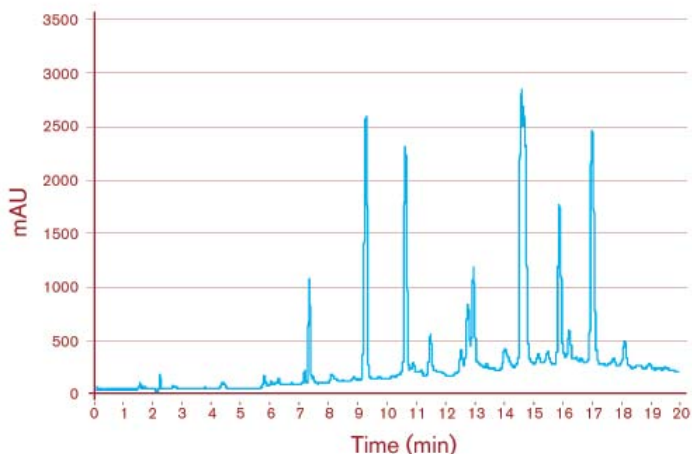
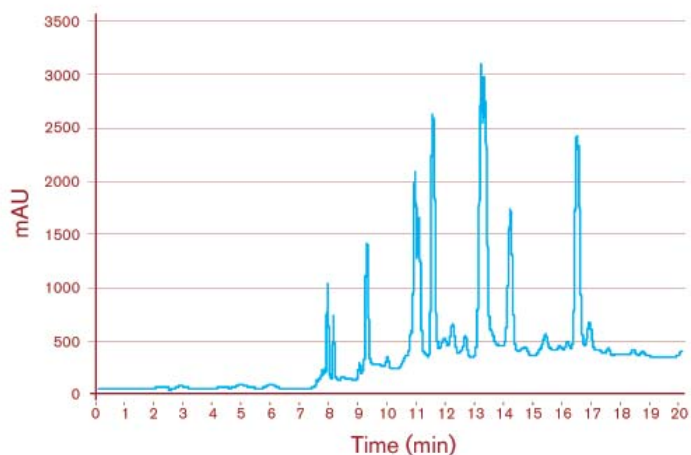


Figure 3: Chromatogram from Column 5
(Eksigent CL, 3 μm , 15 cm column)



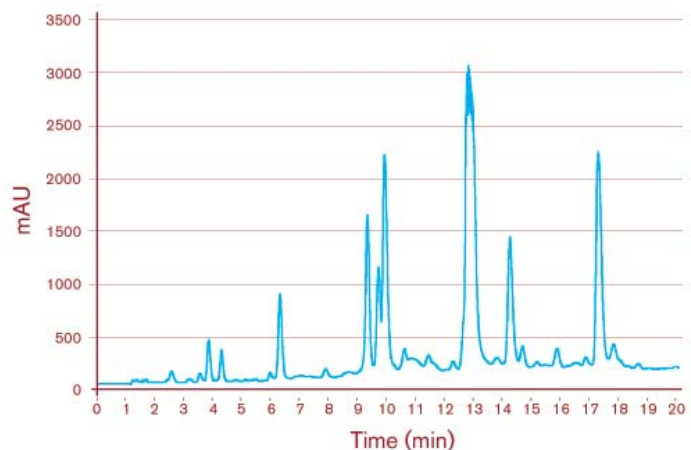
conclusions

The time required to collect the eight chromatograms presented in Figure 1 via the ExpressLC-800 system was approximately 20 minutes, including data collection and column equilibration. In contrast, had we performed the separations with a conventional chromatographic system on a serial basis, the overall time required for data collection would have required 4-6 hours. Each separation would have required approximately 20 minutes, and additional time would be necessary for exchanging and equilibrating each column. Since the overall time using a conventional system was approximately 10 times that of the ExpressLC-800 system, it is unlikely that the variety of columns would have been explored. The large amount of data that was collected can be used to determine the optimum column to provide the desired separation. Once the appropriate column has been selected, the optimum mobile phase can be rapidly selected by installing the optimum column in each position of the ExpressLC-800 system and varying the mobile phase composition.

While a number of serial approaches have been developed employing column selection valves which aid in the ability to screen stationary phases, these techniques are considerably more troublesome than the parallel separations provided by the ExpressLC-800.

As an example, the use of column selection valves suffers from column equilibration issues and the inherent complexity of such systems. The use of the ExpressLC-800 system allows the analyst to determine the most appropriate column by using an extremely broad range of stationary phases. In addition, once the optimum method has been determined using the ExpressLC-800 system, it can be readily transferred to a conventional LC system or to an Eksigent ExpressLC-100 Microflow LC system.

Figure 4: Chromatogram from Column 8
(Agilent XDB, 3.5 μm , 15 cm column)





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