The separation of complex samples can be improved by using 2D LC with different types of columns. The most evident advantage of 2D LC compared to conventional 1D LC is the increase in peak capacity. 2D LC can resolve more peaks and achieve better separation than 1D LC. However, in the real world this orthogonality is often only partial due to available column choices or difficulties in variable/phase mode compatibility. In this work, a wide range of supercritical and porous phases were evaluated to determine the best available orthogonality and can include normal phase, reversed-phase and HILIC columns choices. These were then used to separate complex pharmaceutical and environmental matrices using a 2D LC solution.

Overview

The analysis of impurities is an important part of the development of drugs. The separation of impurities is even more crucial for the new drug substances. Due to the fact that impurities are structurally similar to the main compound it can often be difficult to separate them chromatographically. Of particular interest are early eluting polar compounds, as they are often biologically active. A 1290 Infinity 2D LC, in the heart cutting experiment, the peak of interest is sampled from the first column and elutes into a loop capillary. This is then switched by a switching valve to a second dimension column. Due to the fact that elution peaks can be resolved. In this work it is important that the second dimension phase is demonstrated. A wide range of reversed phase columns are evaluated including C18, C8, C30, StableBond A, Phenyl/Hexyl and Phenyl/Hexyl RP. Additional work is planned using alternative columns. An important second dimension is chosen similar to the mobile phase of the first dimension. This peak is sampled using a counter-current capillary filling. C18 and phenyl phase are evaluated. Compounds are found to be eluted in several weeks. These compounds can be separated using a Poroshell 120 EC-C18 and a phenyl gradient, but for this work two columns are purposefully co-eluted. Peaks B and D are also purposefully co-eluted, but not addressed further in this work.

Types of 2D LC Experiments

Heart-cutting 2D-LC:

Only part of the heart-cut from the first dimension column is transferred to the second dimension column. This peak eluted from the 1st dimension column - will be injected to the second one. Typically used to help to remove impurities prior to the 2nd dimension or to separate compounds with very similar separation On-line LC-LC columns are also used to separate compounds that can't be separated using a single column. Heart-cutting is a very common method in 2D LC applications. However, with the use of capillary LC the secondary column is not always needed. The advantage of this is that the secondary column is not needed. The disadvantage is that the secondary column must be eluted. A gradient on the second dimension is still running – this peak will be lost.

Heart-cutting 2D-LC:

Advantages of the new 2pos/4port-di-valve

With a 2村民们 valve the challenging separation can be achieved. This valve allows for the adjustment of the valve settings. A 2村民们 valve opens. Only way that the proportion can be changed.

Results and Discussion

The first dimension separation is carried out using a Poroshell 120 EC-C18 columns. In the 2D chromatogram, A and B shows a 1290 SB-C18 is used. In this example chromatography the peak is retained but no additional separation is achieved. This is not surprising as both columns are C18 phases. No effect on the parent chromatogram is caused by the sampling Chromatogram B is a Poroshell 120 EC-C2 column. In this chromatogram the peaks are not retained as long column. However, this in case the peaks are separated due to the effect of the second dimension. This is the normal phase separation, then either of the previous chromatograms Chromatogram D is generated using a Poroshell 120 EC-C2 column. The analyte peaks in the 2D chromatogram are when used in combination with the stationary phase. Chromatogram E can be seen on this chromatogram the peak sharp and the peaks are well resolved.

Conclusions

When pairing a 2村民们 column for the second dimension, choose something that is dissimilar to the 1D phase. Resources such as the PDM core base is a good guide. Future work will include Merck, different buffers and HILIC.

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