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FlipLC™. A New Method for Measuring Charged Analytes in Complex Mixtures. Part III.

We recently reported a new method that was developed for the analysis of charged analytes in complex mixtures such as natural products, food, bodily fluids and environmental probes. You can watch an animated representation of this method on Youtube. The results were so interesting that we decided to name this specific approach. We have named it FlipLC™, because this process uses two columns: one analytical and one isolation column. The isolation column flips from a normal flow direction to a reverse flow direction during the same run. We then applied this method for measuring nitrate and amino acids concentration in various complex products. We are now reporting measurements of ascorbic acid in different matrices. Ascorbic acid is an important nutritional food component. When testing complex samples, if a sample is not intensively cleaned, the column's operating life is usually short due to irreversibly retained contaminants. Also, multiple peaks interfere with the peak of ascorbic acid. Often, these problems appear in the measurement of ascorbic acid found in fruits, vegetables, and nutritional supplement products. This is a typical chromatogram example of strawberry juice (Fig. 1a).

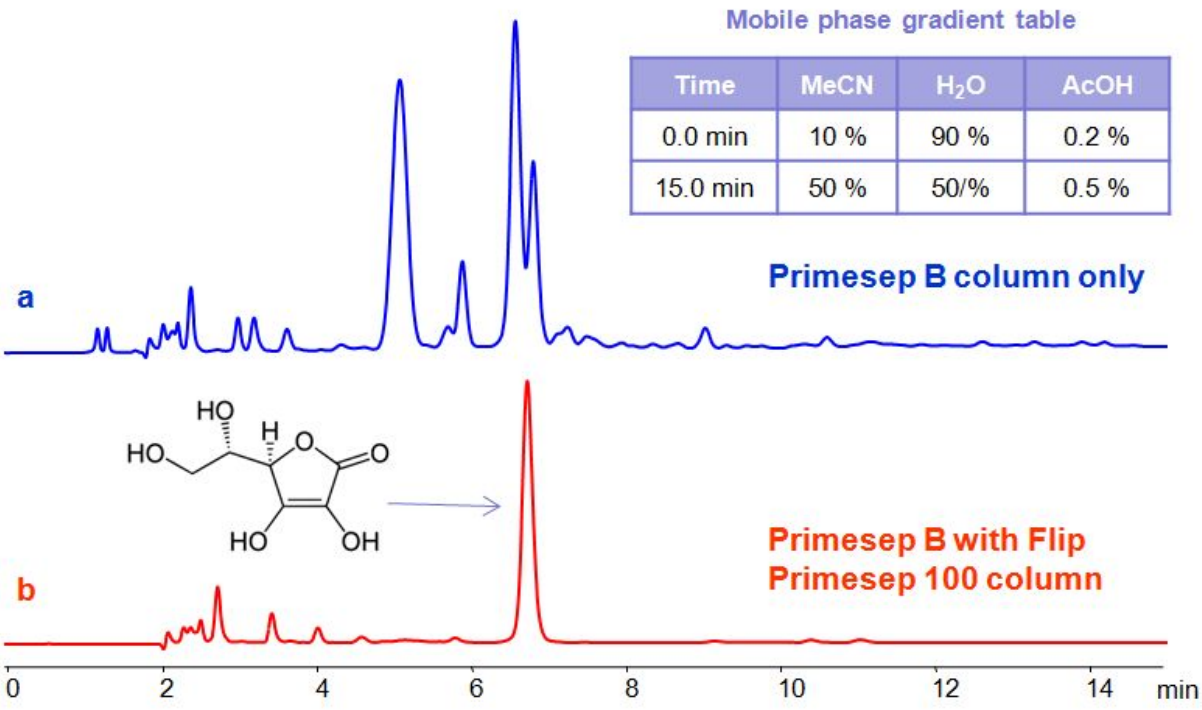

Fig. 1. Chromatogram (a) to (b) transformation with FlipLC™ system. Analytical column: Primesep B, 4.6 x 150 mm. Flip column: Primesep 100, 4.6 x 50 mm. Flow rate: 1.0 mL/min. Valve switch time: 0.5 min. Detection: UV 270 nm. Injection: 5 µL of filtered strawberry juice.

The same sample was then introduced to the dual column FlipLC™ system (Fig. 2). As a result, a drastically simplified chromatogram was obtained with the peak of the ascorbic acid sufficiently isolated (Fig. 1b). Because of the efficiency of analyte isolation, the final method can be done isocratically and be reduced to less than 5 minutes. In addition, the analytical column is protected from irreversible contaminations more efficiently than a guard column, and the Flip column lasts much longer than a typical guard (precolumn). This setting also eliminates the need for complex sample cleaning prior to the analysis. These results were obtained due to the unique properties of mixed-mode columns, which retain compounds by both reverse phase and ion-exchange mechanisms.

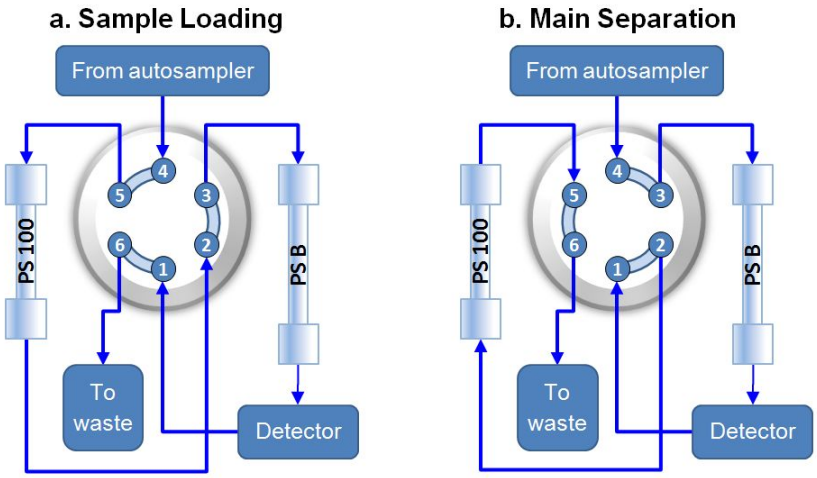

Fig. 2. Two columns connected to HPLC systems with a UV detector

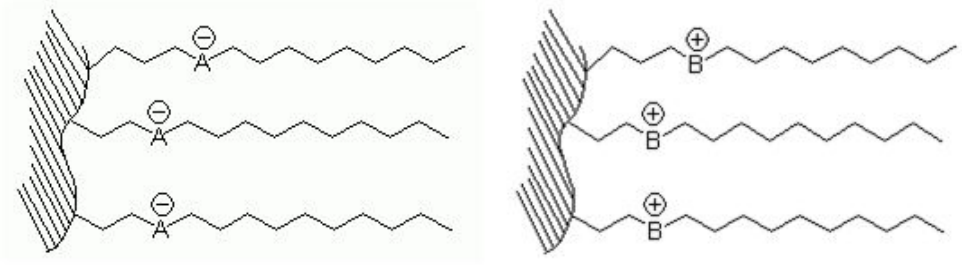

Fig. 3. Simplified ligand chemical structure of mixed-mode columns.

Ascorbic acid concentration in fruits and vegetables measured by the FlipLC™ method.

Plant source	[C], ppm	Plant source	[C], ppm	Plant source	[C], ppm
Rosehips fruit	7,200	Strawberry	500	Cornelian cherry	320
Red Pepper	2,300	Orange	440	Spinach	220
Blackcurrant	1,800	Tangerine	420	Horseradish	190
Lemon	660	Grapefruit	330	Cabbage	70

In conclusion. If the sample is a complicated mixture consisting of many different chemicals, each sample matrix will require various cleaning procedures prior to separation and as a result, one procedure cannot be used across a wide variety of products. Our new FlipLC™ method addresses this problem. It consists of an analytical column connected by a valve to an isolation (flip) column which retains charged analytes orthogonally relative to the analytical column. This method eliminates most of the interfering components from the chromatogram, shortens the analysis time and increases the lifetime of the analytical columns. FlipLC™ allows for the analysis of a targeted compound found within a variety of complex samples using a single chromatography method.