FlipLC. Direct analysis of polar compounds in serum. Part V.

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. We named approach 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 different polar compounds in various complex mixtures. We are now reporting measurements of serotonin in human serum/plasma. Similar approach was used before with restrictive access media (RAM) columns by trapping small molecules on RAM column while plasma proteins do not retain. Then small molecules can be analysed on regular analytical RP column. However this technique does not work for polar molecules. They not retain on RAM columns with hydrophobic interior. This became easy task for FlipLC. The serum proteins get trapped on flip Primesep SB column at low organic mobile phase and polar analytes pass through directly to analytical column. After valve switching the proteines get flushed out in reverse direction from Flip column by a secondary pump using solvent with higher organic concentration (Fig. 1).

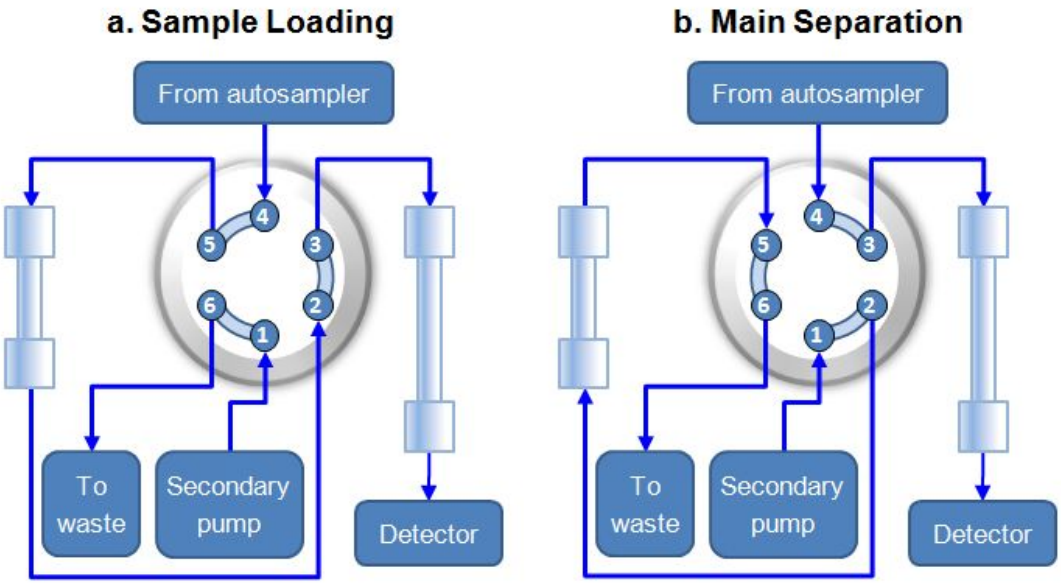


Fig. 2. Two columns connected to HPLC systems.

As a result, a good isolated peak of polar analyte was obtained with serum proteins removed from the sample. Because of the efficiency of the analyte isolation, the final method can be done in about 5 minutes.

One useful application presented is analysis of serotonin in human serum. (Fig. 2). This setting eliminates the need for complex sample cleaning prior to the analysis, provide high analytes recovery, eliminate needs for SPE cartridges and liquid handling equipment. The method is fully compatible with MS and UV detection.

These results were obtained due to the unique properties of mixed-mode columns, which retain compounds by both reverse phase (RP) and ion-exchange (IE) mechanisms.

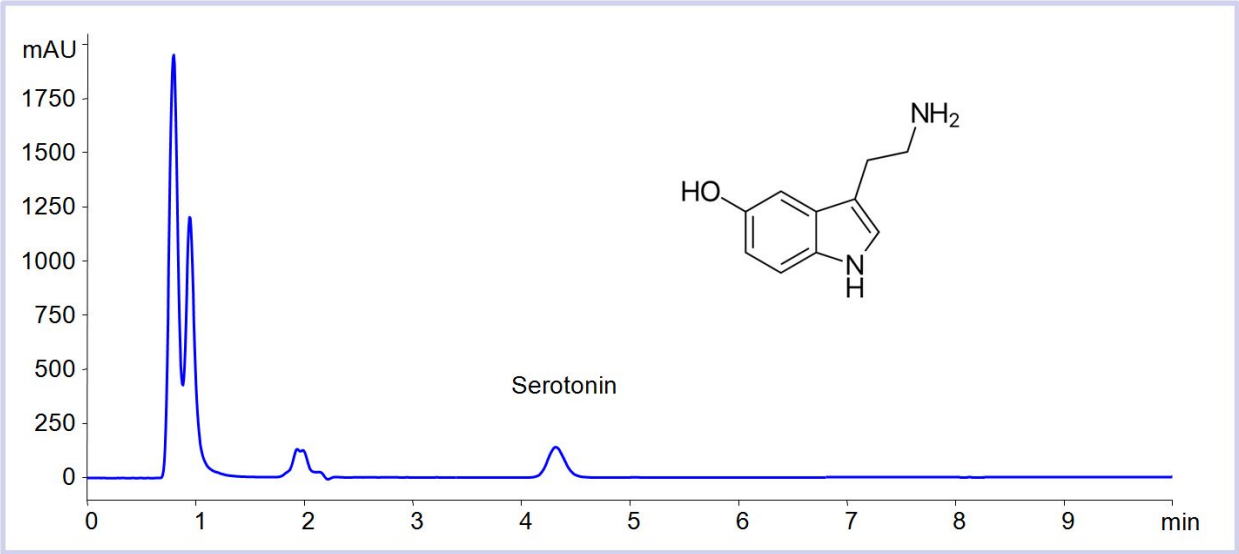
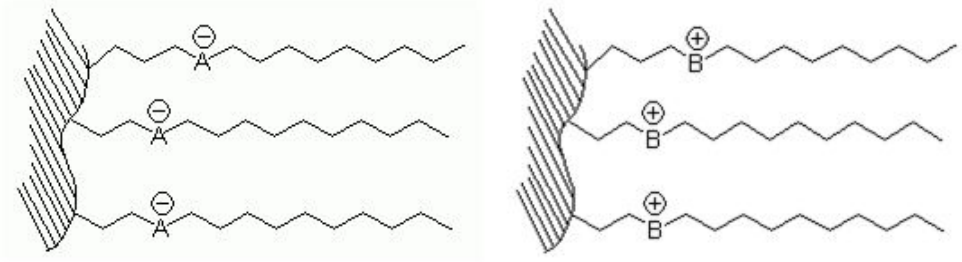


Fig. 2. Chromatogram of serotonin in serum with FlipLC™ system. Analytical column: Primesep 200, 4.6 x 100 mm. Flip column: Primesep SB, 4.6 x 50 mm. Flow rate: 1.0 mL/min. Mobile phase in analytical column: H_2O/MeCN - 20/80 to 60/40 in 10 min; buffer - AmFm, pH 3.0, 40 mM. Valve switch time: 0.8 min. Detection: UV 285 nm. Mobile phase in Flip column: H_2O/MeCN - 50/50; buffer - AmFm, pH 3.0, 20 mM. Injection: 30 µL of serum diluted with buffer 1:2.



Primesep 100 **Primesep SB**

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

In conclusion. If the sample is a complicated mixture consisting of many different chemicals, it is hard to isolate a particular analyte without complicated sample preparation. 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. Due to unique property of mixed-mode columns to retain molecules by RP and IE mechanizm, many polar charged analytes can retain and quantitate with simple methods without ion-pairing reagents.