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FlipLC™. Measuring Charged Analytes in Complex Mixtures. Part IV. Metabisulfite.

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 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 ascorbic acid concentration in various complex products. We are now reporting measurements of metabisulfite. This chemical in a form of sodium salt is an important additive to food products. It has designated code E223 by European Food Safety Authority. Vine is one of such product. The measurement by UV-HPLC in vine is complicated due to interference of the multiple vine components with metabisulfite peak (Fig. 1a). This is a typical chromatogram profile of red vine.

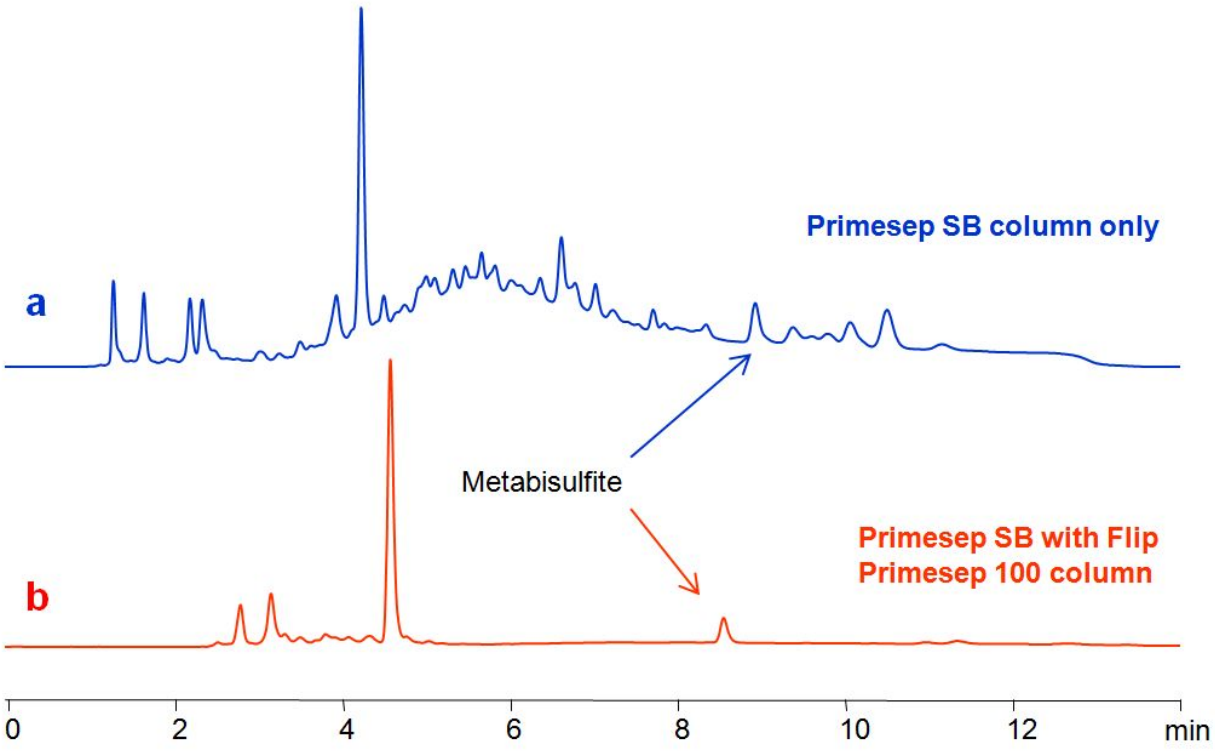

Fig. 1. Chromatogram (a) to (b) transformation with FlipLC™ system. Analytical column: Primesep SB, 4.6 x 150 mm. Flip column: Primesep 100, 4.6 x 50 mm. Flow rate: 1.0 mL/min. Mobile phase: H_2O/MeCN/H_3PO_4 - 10/90/0.2 to 50/50/0.5 in 5 min then 8 min hold. Valve switch time: 1.1 min. Detection: UV 270 nm. Injection: 10 µL of filtered red vine.

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 metabisulfite sufficiently isolated (Fig. 1b). Because of the efficiency of the 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 typical guard column, and the Flip column itself lasts much longer than a typical guard (precolumn). This setting also eliminates the need for complex sample cleaning prior to the analysis.

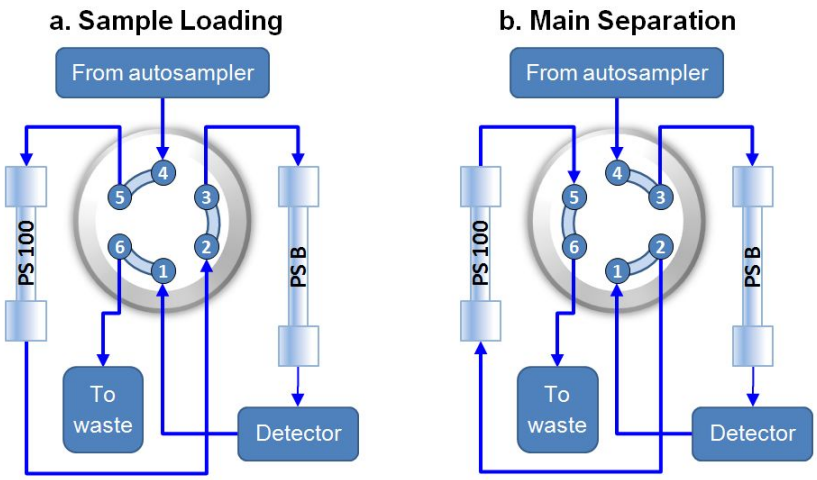

Fig. 2. Two columns connected to HPLC systems with a UV detector

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.

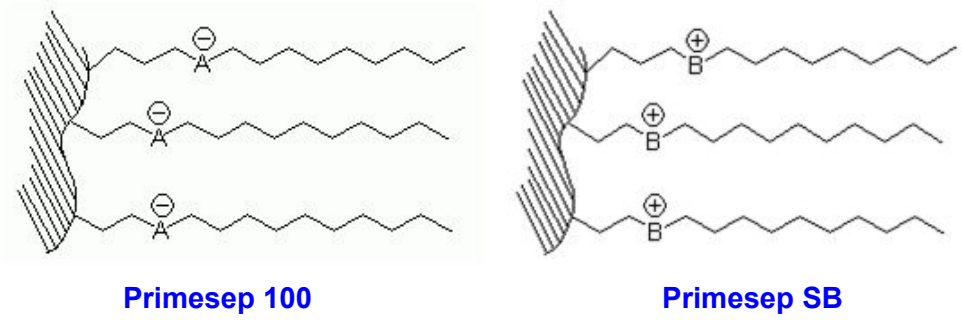

Fig. 3. Simplified ligand chemical structure of mixed-mode columns.

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. 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.