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

We recently reported a new method that was developed for the analysis of charged analytes in complex mixtures such as natural products, food, body fluids and environmental probes. We then applied this method for measuring nitrate concentration in food, water, soil and urine. The results were interesting and show an unusually high nitrate concentration in several products. The method is universal and not limited to nitrates only. We have named this method 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 can now apply this approach for measuring non-derivatized amino acids in different matrices. If a sample is not intensively cleaned, the column's operating life is usually short due to irreversibly retained contaminants. Often, these problems appear in the measurement of essential amino acids found in nutritional supplement products, for example, a L-glutamine assay in a complex product which contains among other ingredients an agglomerated whey protein. Although it is possible to isolate the glutamine peak from an intact sample, the assay itself took over 50 minutes in order to remove other sample components (Fig. 1a).

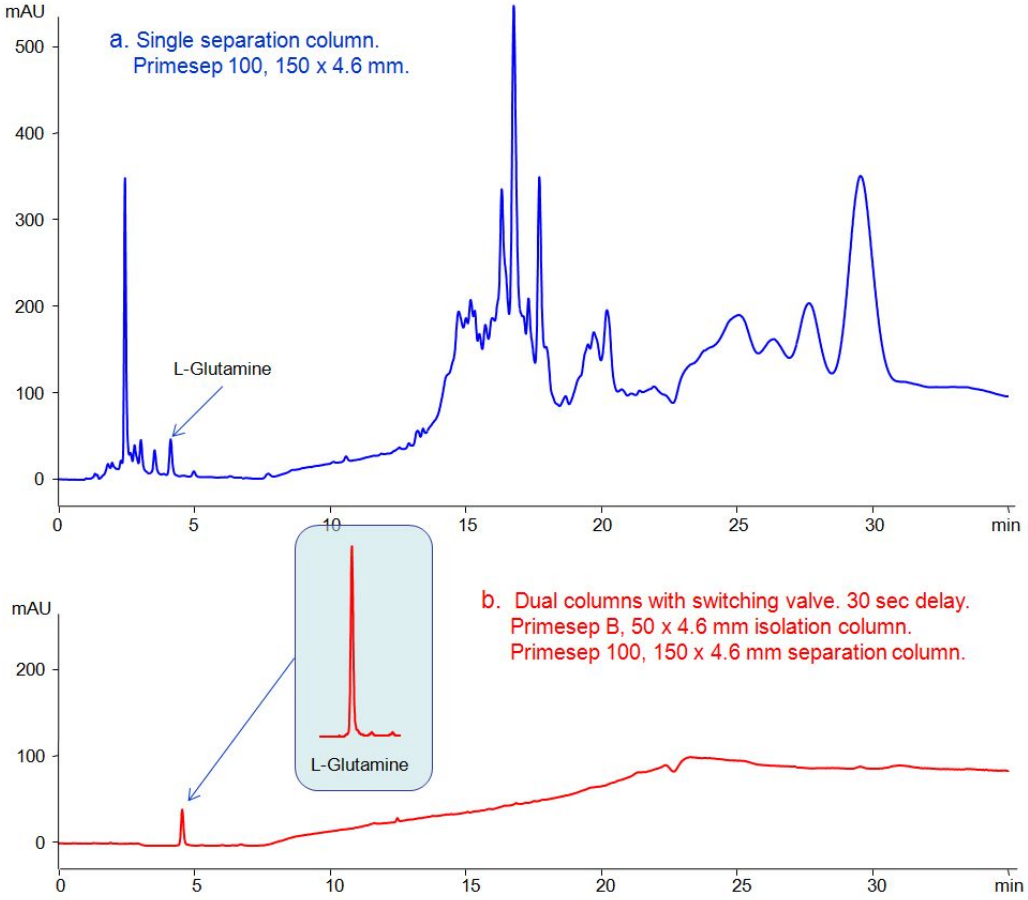


**Fig. 1. Chromatogram transformation with dual column/switching valve system. Mobile phase: MeCN/$H_2O$/$H_2SO_4$ - 20/80/0.05 for 5 min gradient to 50/50/0.50 in 15 min + 15 min hold.**

The same sample was then introduced to the dual column FlipLC™ system (Fig. 2). As a result, a drastically simplified chromatogram was obtained with only the peak of the amino acid left (Fig. 1b). Because of the efficiency in analyte isolation, the final method (Fig. 3) can be reduced to 10 minutes or

less with an isocratic mode of elution, in addition to protecting the analytical column from irreversible contaminations.

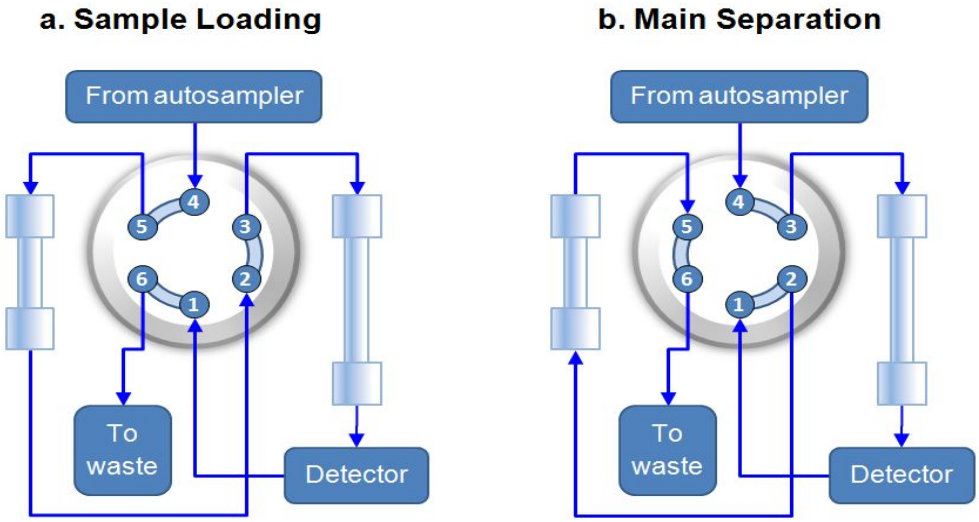


**Fig. 2. Two columns connected to HPLC systems with UV detector**

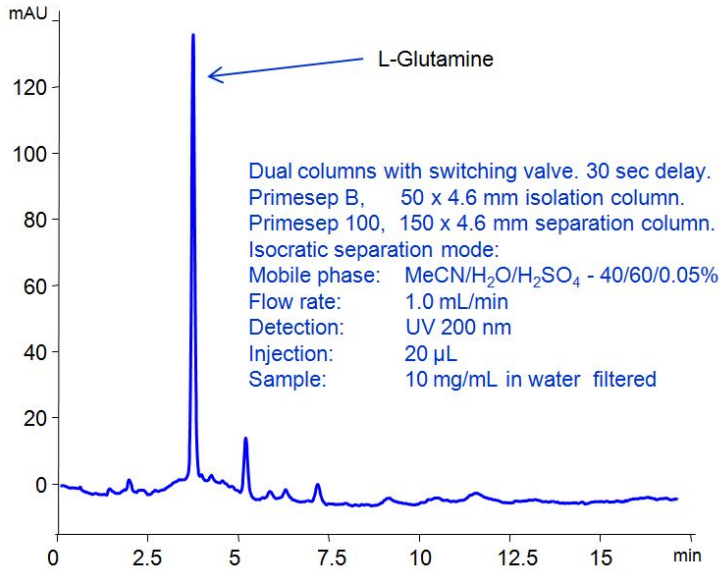


**Fig. 3. Final chromatogram obtained with FlipLC© dual column/switching valve system.**

**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 components that are of little interest 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. This setting also eliminates the need for complex sample cleaning prior to the analysis. Our presented example of the measurement of L-glutamine in nutritional products can be applied to other inorganic and organic, UV active cations and anions.