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

Measuring by HPLC a particular component of complex samples such as natural products, body fluids, environmental probes is complicated due to interference from other components. Different sample matrices require different sample cleaning procedures prior to separation and as a result, one procedure cannot be used across a variety of products. The column's operating life is usually short, even with sample cleaning, due to irreversibly retained contaminants. Thus, long cleaning procedures, guard columns, and expensive and complex compound specific detection techniques such as MS are often required. An example of such problems appear in the measuring of nitrates. All of the existing methods are complicated and take significant time to execute. Usually, the methods are specific only for the particular sample type.

We are proposing an alternative method to avoid the interference of most of the contaminants by the use of an isolation column and a high pressure switching valve before the separation column. This method allows sample cleaning and analyte separation in one automated process.The isolation column and the separation column should have orthogonal retention characteristics to operate efficiently in this setup. Mixed-mode columns with reverse phase and ion-exchange characteristics were used in this analysis. The setting below employs a high pressure 6-port, 2-position switching valve which is placed between an autosampler and analytical column and actuated by the chromatography system by a defined time after the injection (Fig. 1a, b). A modified setting has to be used when using destructive techniques such as MS.

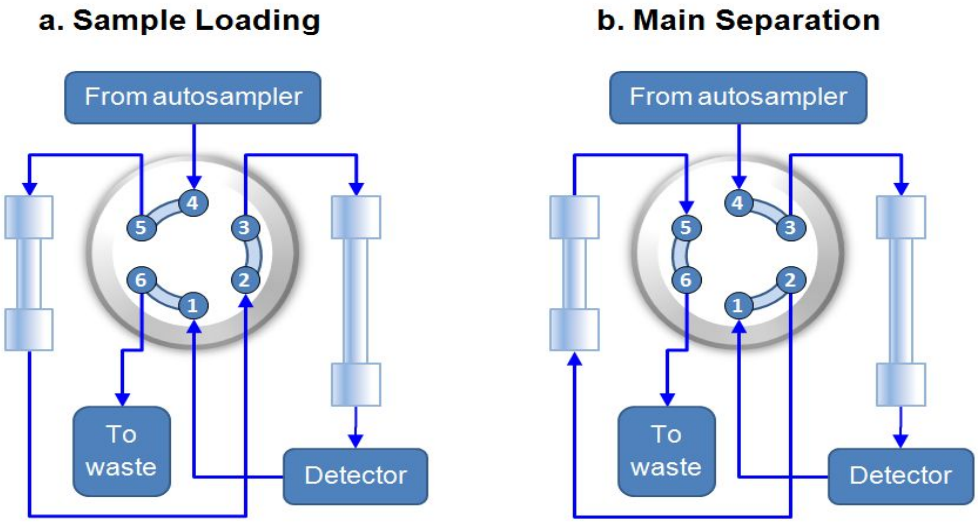

Fig. 1. Two columns connected to HPLC systems with UV detector.

In this setup, a sample is introduced to the isolation column first. The isolation column passes analytes through and retains the late eluted components of the sample. When the valve is actuated at a defined time after the injection (this time depends on the nature of the analyte), the late eluted components, while still in the isolation column, are back-flushed to waste by the flow coming from the detector outlet. Such a setting has been used for measuring the nitrate concentration in food and other types of complex samples. The effectiveness of the method is illustrated by three chromatograms shown below. Fig. 2a is a chromatogram of a chicken broth sample on a Primesep SB the anion-exchange RP column. Fig. 2b is a chromatogram of a chicken broth sample on a Primesep 100, the cation-exchange RP column. The detection of nitrates is impossible in both settings due to the significant interference of

different components of the sample with the nitrate peak. The Fig. 2c is a chromatogram obtained with two columns Primesep 100, 4.6 x 25 mm and Primesep SB 4.6 x 50 mm with switching valve actuated at 30 sec. The nitrate peak is sufficiently isolated from all the impurities and is available for measuring.

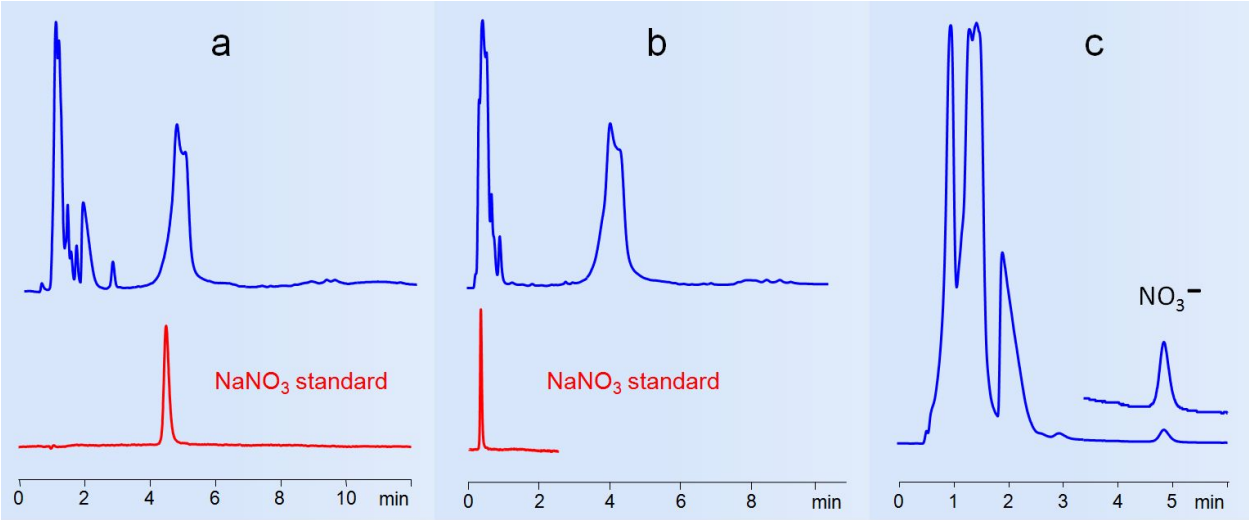

Fig. 2. Chromatogram of chicken broth samples. Single column Primesep SB (a); single column Primesep 100 (b); dual column setting with switching valve (c). Mobile phase is a gradient MeCN 20-70% in 8 min + 3 min hold, with 0.4% H_2SO_4. The injection volume was 20µm; UV wavelength - 200 nm.

This method was used to measure the amount of nitrates in different samples. See table below.

Sample	[NO_3^-] ppm	Sample	[NO_3^-] ppm
Black Watermelon	1.71	Mineral water San Pellegrino®	3.00
Green Watermelon	18.91	Municipal water, Wheeling IL	0.87
Apple from local grocery store	1.15	Human urine	30-80
Melon from grocery store	56.78	Rich soil from a garden	7.49
Lettuce fresh from a garden	761.34	Chicken broth	2.86
Bologna from grocery store	5.10	Sausages from grocery store	7.61

In conclusion. Our new method consists of an analytical column connected by a valve to an isolation column which retains orthogonally relative to the analytical column. This method eliminates a significant portion of coeluting impurities from the chromatogram, shortens the analysis time and increases the lifetime of the analytical columns in the analysis. This method allows the analysis of a target compound in a variety of complex samples with a single chromatography method. This method also eliminates the need for complex sample cleaning prior to the analysis. The presented example of the measurement of nitrates in a variety of samples can be applied to other inorganic UV active ions such as bromide, iodide and other charged organic (especially polar) molecules with both positively charged and negatively charged functional groups.