**SIELC Technologies, Inc.**
Wheeling, IL 60090 USA
**P. 847-229-2629** **F. 847-655-6079**
mail@sielc.com www.sielc.com

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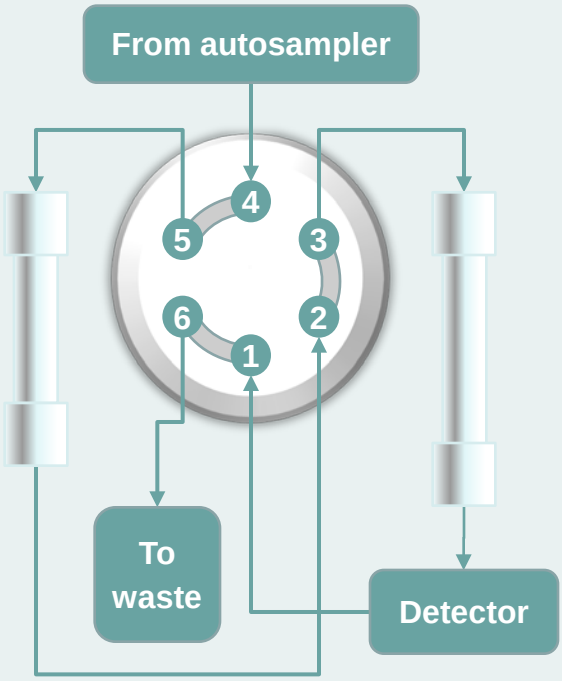
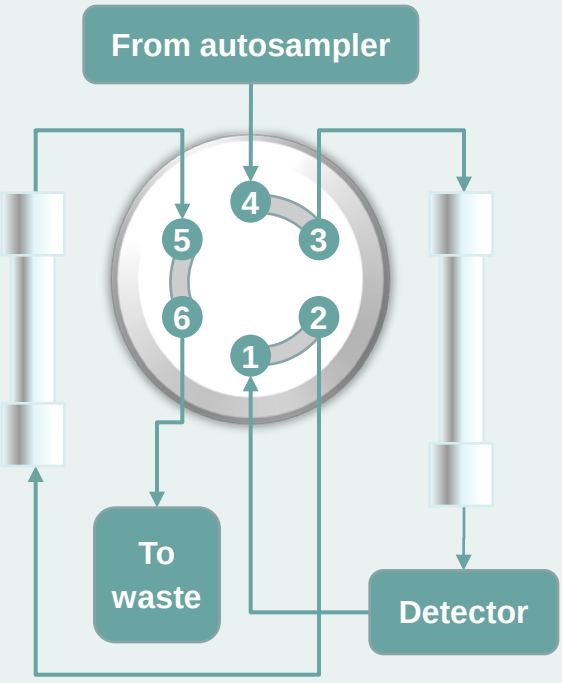


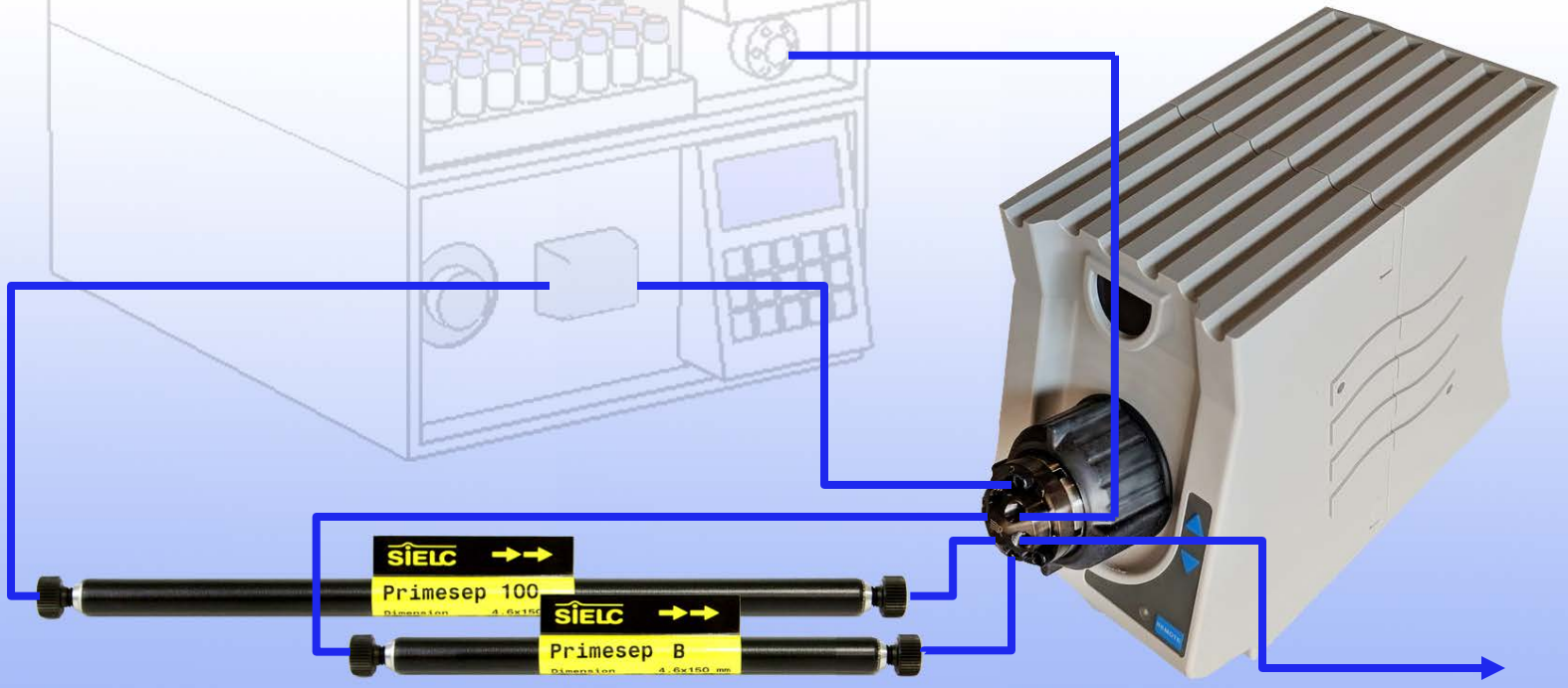

# FlipLC

## Polar molecules in complex samples



# FlipLC Methodology

**Samples from natural sources, such as fruits, vegetables, culinary products, water, soil, and bodily fluids, are complex mixtures consisting of multiple compounds. If HPLC separation is used for analysis, the components of the mixture often interfere with the analyte. Complex isolation procedures or complex and expensive compound specific detection techniques such as MS are generally used to remove some of the interference. However, there is no need to use these techniques frequently because the FlipLC method offers a way to avoid the interference of most of the contaminants by combining the isolation and separation process with a dual column setting. The isolation column and the separation column should have orthogonal retention characteristics to operate efficiently in this setup. The isolation column and a high pressure switching valve are installed along the analytical column. After an injection of the sample into the isolation column, the valve is actuated at a predefined time. Following valve actuation, the late eluted components still in the isolation column are back-flushed to waste by the flow coming from the detector output while the analytes get separated in the analytical column. This setup removes impurities from the sample giving much cleaner chromatograms, shortens chromatogram time, and increases the lifetime of the columns. Mixed-mode cation-exchange and anion-exchange columns are ideally suited for this application.**

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# Principle of Operation

**Setting of two columns and switching valve on FlipLC system**

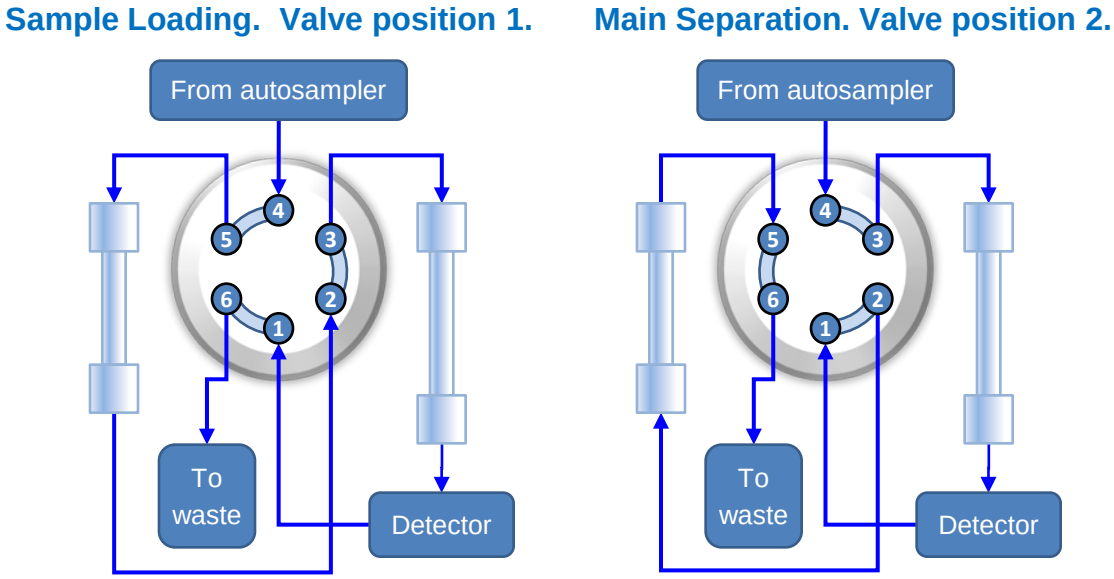
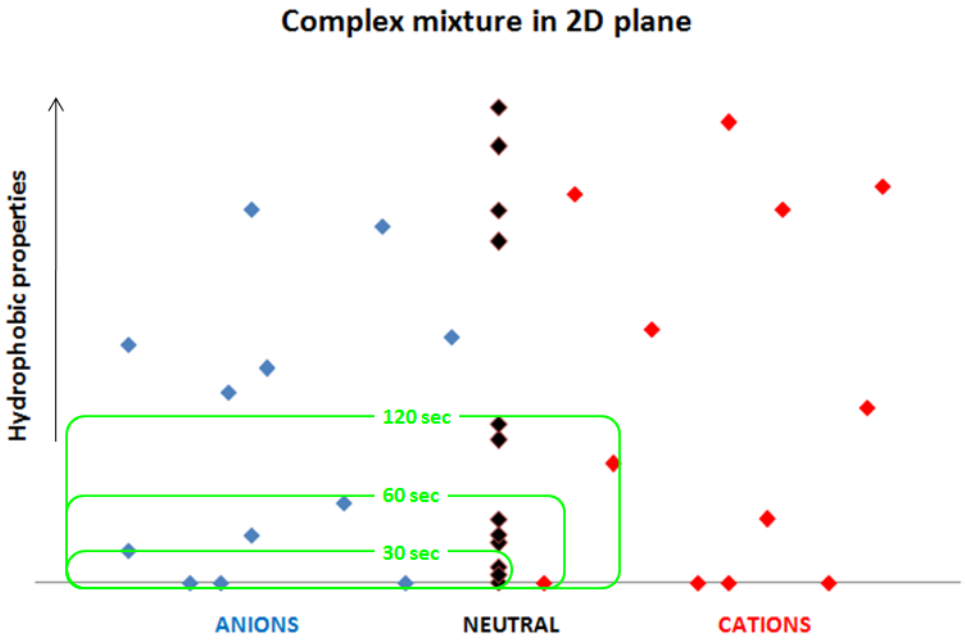



**Fig. 1. FlipLC setting of two columns connected to HPLC systems with UV detector.**

A 2D map of the different types of compounds can explain the main principle of the isolation process of FlipLC (fig. 2). The vertical axis in this map is the hydrophobicity value for a given compound. The logP factor is one of the ways it can be characterized. The horizontal axis is the charge property of a molecule at a specific mobile phase pH. Positively charged molecules are positioned on the right side of the map, with neutral molecules being positioned in the middle, and negatively charged molecules positioned on the left side. If an anion-exchange RP mixed-mode column with predefined pass-through time is used for the isolation, only the small number of compounds in the left bottom corner of the map will be eluted from the column before the valve is switched to a new position.




The actuation time is defined as the size of the area of the map. If a cation-exchange RP column is used for the sample cleaning, the island will be positioned in the right-bottom portion of the map. By changing the valve actuation time, the type of material that reaches the analytical column can be regulated. This flexibility makes this method universally applicable in removing matrix effects for different samples and analytes.

**Fig. 2. Isolation process of a complex mixture with anion-exchange reverse phase isolation column. Green islands include the type of compounds that will be eluted from the anion-exchange-RP isolation column with different valve switching time.**

# Principle of Operation

Efficient operation of FlipLC system is made possible due to unique silica gel surface chemistry of Primesep columns. The ligand of Primesep columns comprises of a long alkyl chain with embedded ion-exchange functional groups (Fig. 5). These groups can be positively or negatively charged. If proteins and peptides are main contaminants of the sample, a positively charged reverse phase Flip column can retain these contaminants by reverse phase mechanism at low organic concentration, but they can be efficiently removed (washed) from the column with high organic concentration mobile phase. Thus analytical column can be efficiently protected from irreversible contamination.

**Column chemistry**

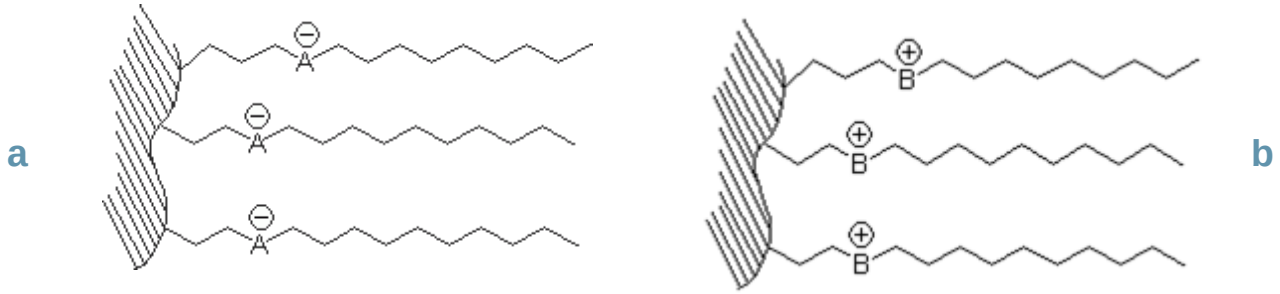



**Fig. 4. Schematic of ligands structure on Primesep 100 (a) and Primesep SB (b) columns.**

In a situation where the mobile phase after coming out of an analytical column gets evaporated in a detector, a different schematic of the FlipLC should be used (Fig. 4).

To wash the first (Flip) column, a secondary pump with a mobile phase optimized for column washing should be used. This pump can be of low pressure and can be isocratic since the Flip column is short and does not require high pressure to provide sufficient flow. Typical washing mobile phase has high organic concentration (up to 50% of MeCN) with high MS compatible buffer to remove most of the sample components trapped in the Flip column.

**Sample Loading. Valve position 1.** **Main Separation. Valve position 2.**

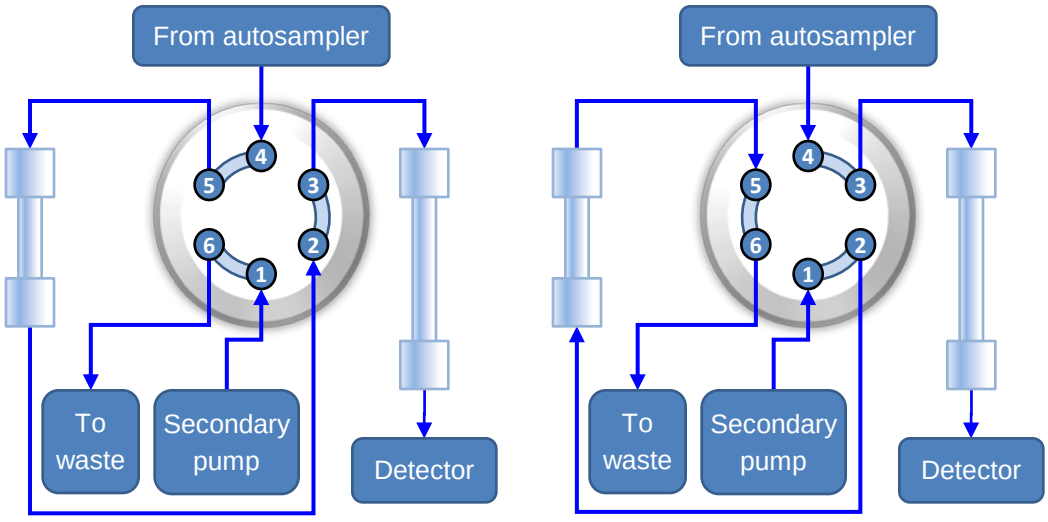



**Fig. 5. FlipLC setting of two columns connected to dual pump HPLC systems with MS detector.**

# Application Examples

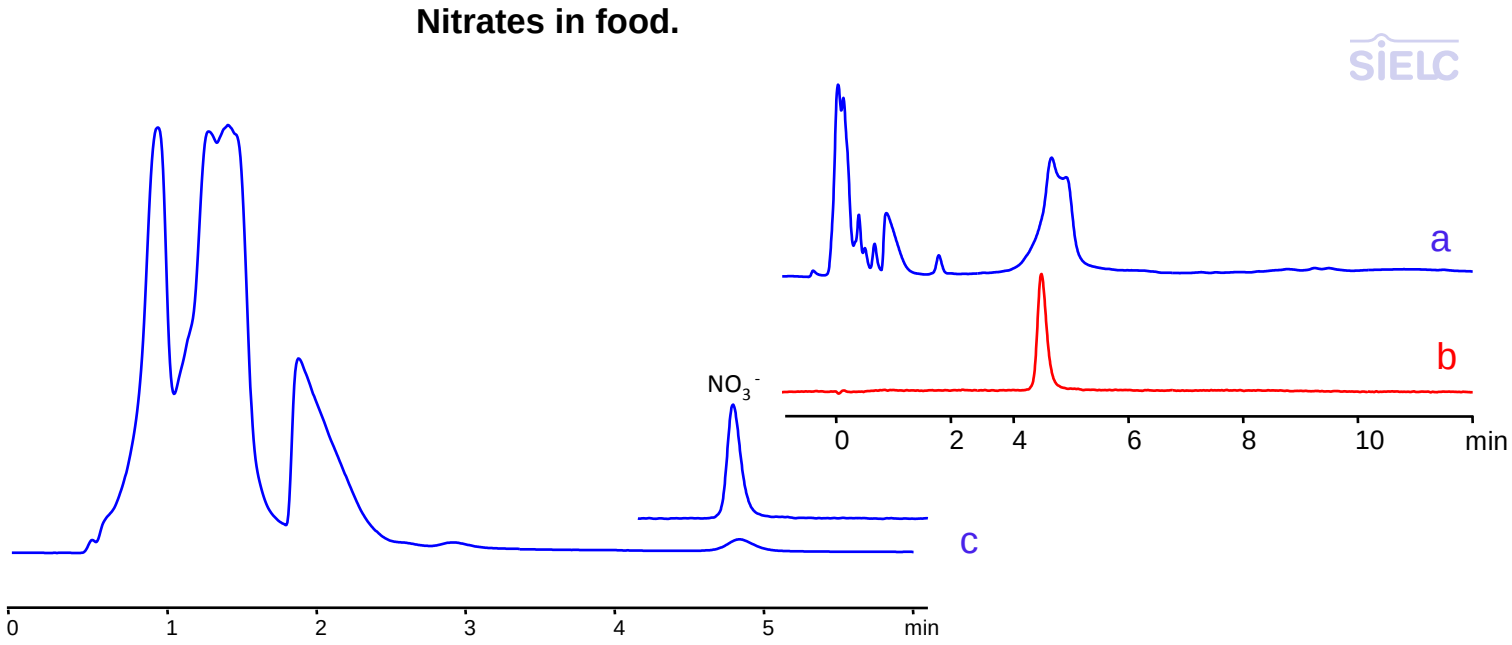



**Fig. 6. Measuring of nitrate in chicken broth. Filtered sample of chicken broth injected in a single column Primesep SB (a); Sample of Nitrate standard injected in Primesep SB (b); dual column setting with switching valve (c). Mobile phase is a gradient MeCN 20-70% in 8 min + 3 min hold, with 0.4% $H_2SO_4$. The injection volume 20µm of filtered (45 µm filter) chicken broth. Detection: UV 200 nm. Valve switch time: 0.5 min after injection.**

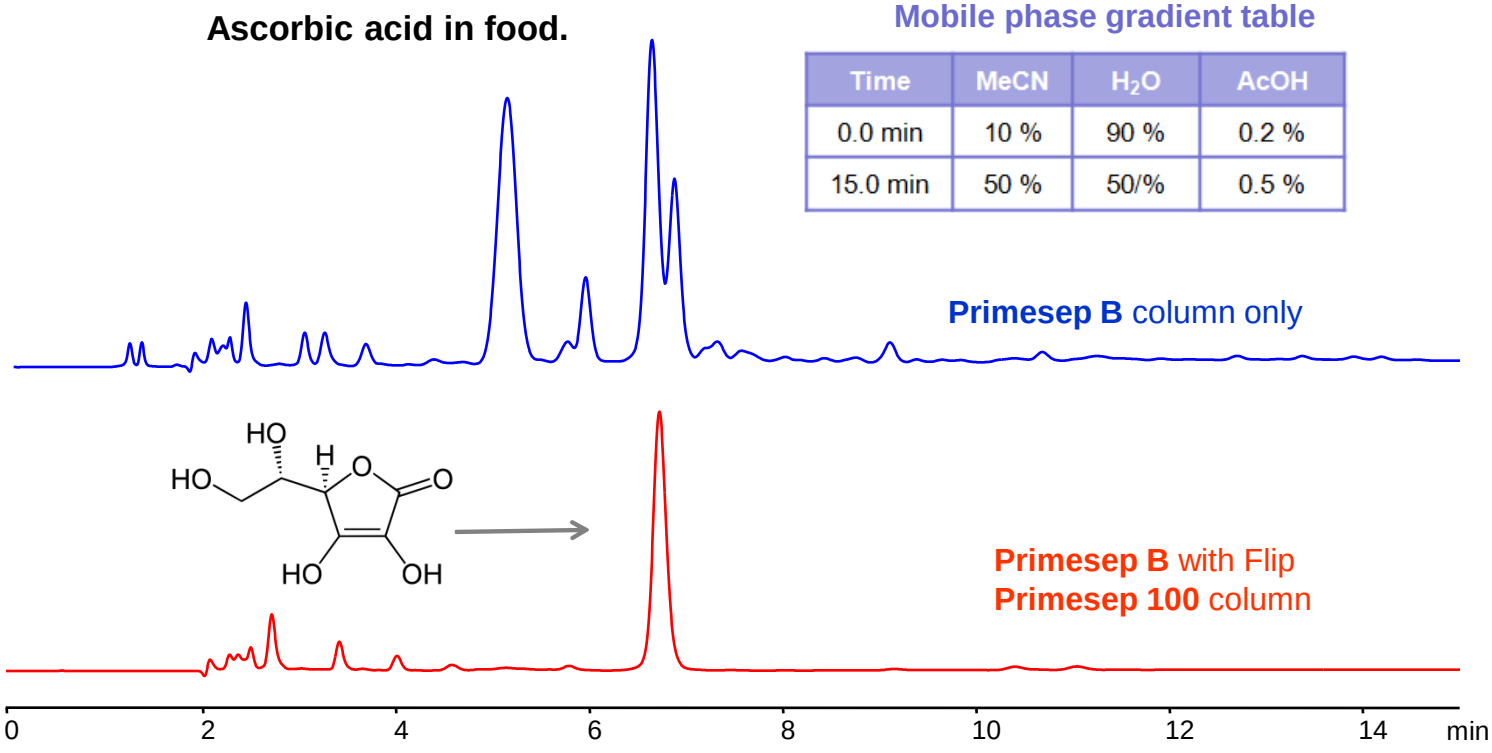



**Fig. 7. Chromatogram (a) to (b) transformation with FlipLC system. Analytical column: Primesep B, 4.6 x 150 mm. Flip column: Primesep 100, 4.6 x 50 mm. Flow rate: 1.0 mL/min. Valve switch time: 0.5 min after injection. Detection: UV 270 nm. Injection: 5 µL of filtered strawberry juice.**

# Application Examples

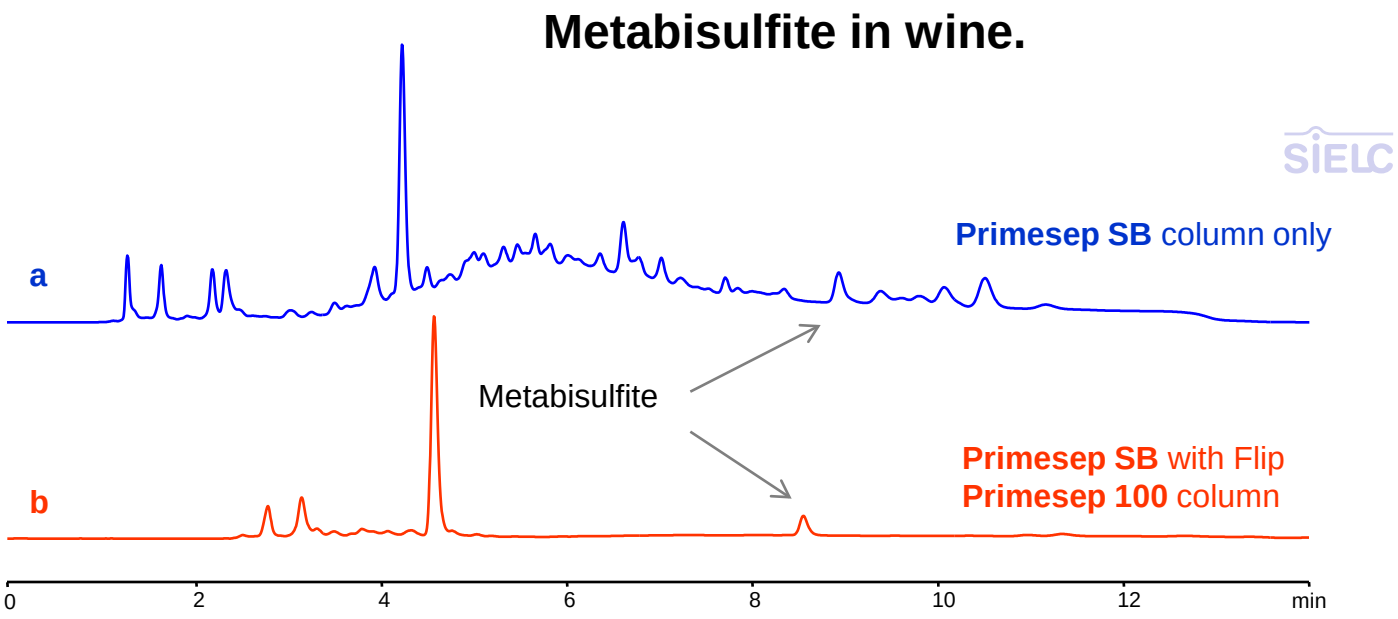



**Fig. 8. Chromatogram (a) to (b) transformation with FlipLC system. Analytical column: Primesep SB, 4.6 x 150 mm. Flip column: Primesep 100, 4.6 x 50 mm. Flow rate: 1.0 mL/min. Mobile phase: H2O/MeCN/H3PO4 - 10/90/0.2 to 50/50/0.5 in 5 min then 8 min hold. Valve switch time: 1.1 min after injection. Detection: UV 270 nm. Injection: 10 µL of filtered red wine.**

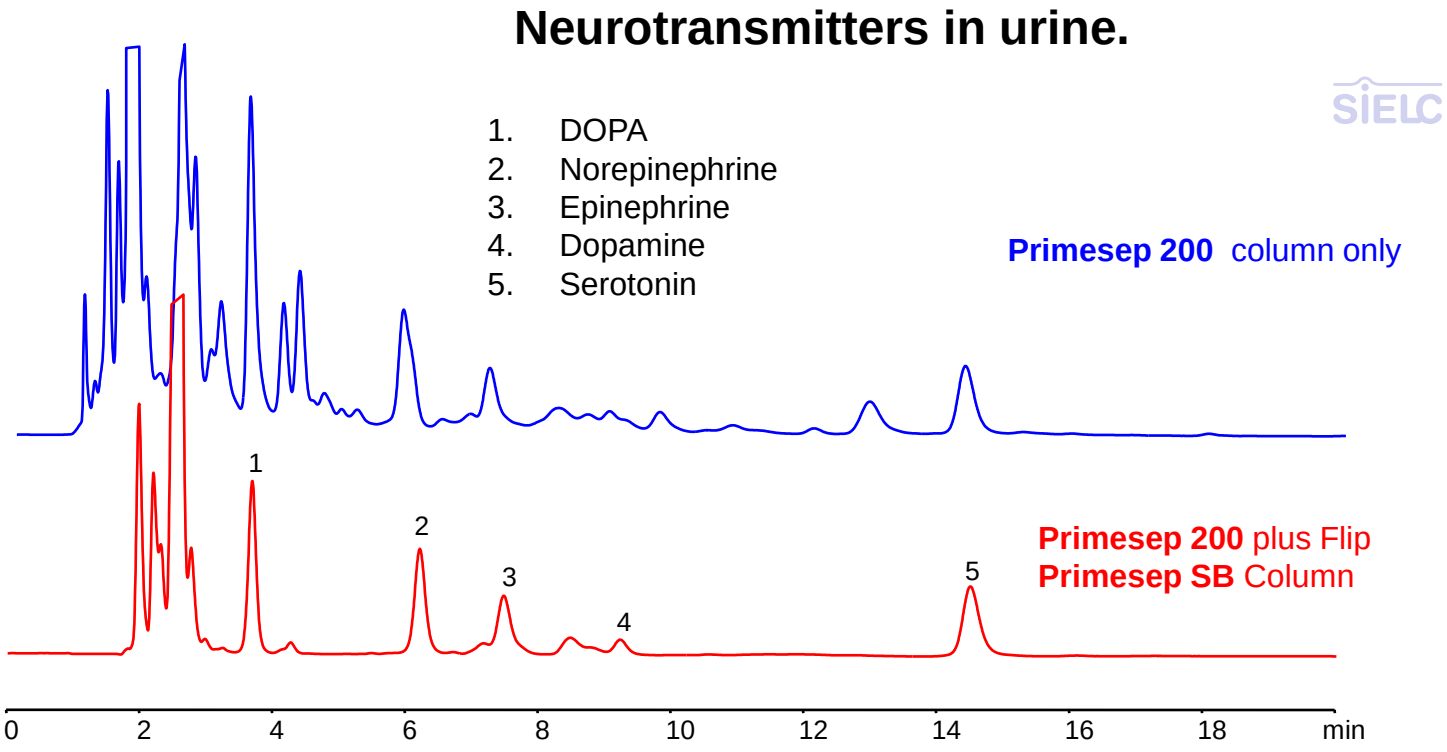



**Fig. 9. Chromatograms of neurotransmitters in urine samples. Single column setting Primesep 200 (a); dual column setting with switching valve (b). Mobile phase is a gradient MeCN 5-30% in 20 min , with buffer AmFm pH 3.0 gradient 10-24 mM in 20 min. Flow rate 1.0 mL/min. Detection UV 275 nm. Valve switch time: 0.5 min. The injection volume 20µm of filtered (45 µm) chicken broth.**

# Application Examples

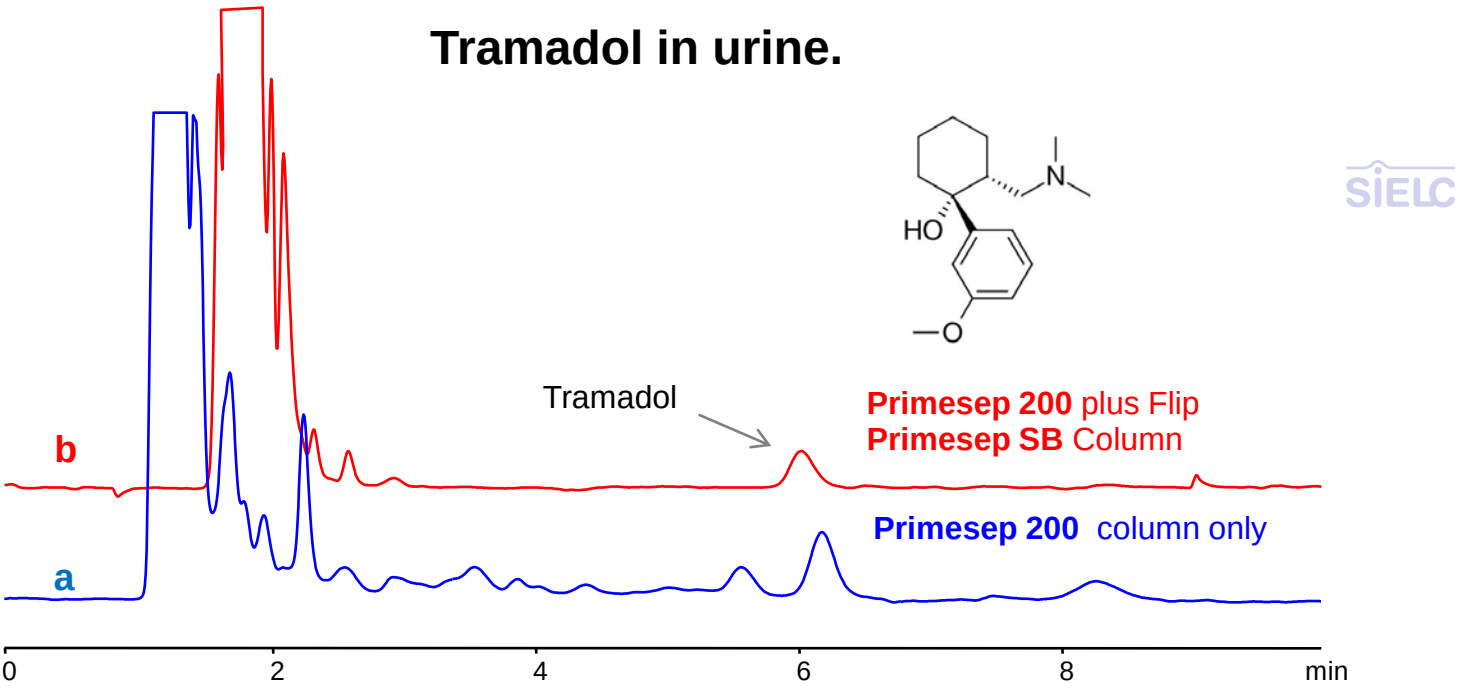



**Fig. 10. Chromatogram (a) to (b) transformation with FlipLC system. Analytical column: Primesep 200, 4.6 x 100 mm. Flip column: Primesep SB, 4.6 x 50 mm. Flow rate: 1.0 mL/min. Mobile phase: $H_2O$/MeCN - 70/30. Buffer AmFm pH 3.0, 20 mM. Valve switch time: 0.5 min after injection. Detection: UV 272 nm. Injection: 8 µL of urine.**

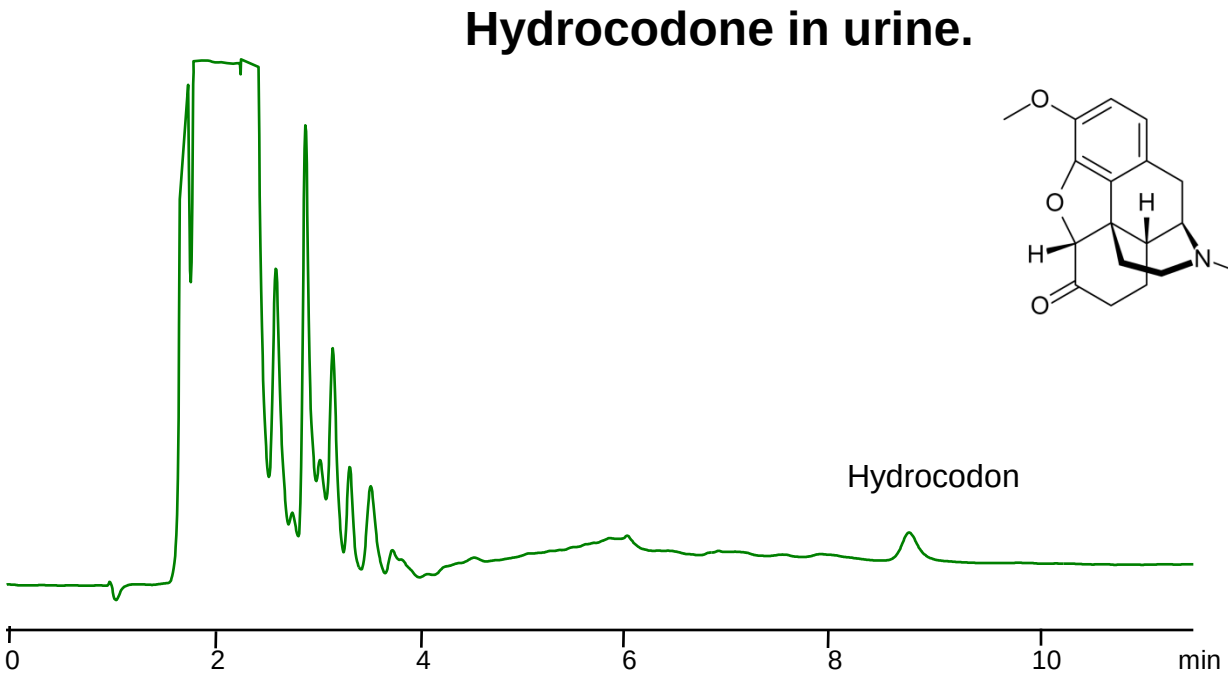



**Fig. 11. Chromatograms of human urine sample after consumption of 10 mg of Hydrocodone tablet. Dual pump dual column FlipLC setting with switching valve. Mobile phase gradient MeCN 20-80% in 4 min with 5 min hold. Buffer AmFm 40 mM pH 3.0. Flow rate 1.0 mL/min. Second pump mobile phase: MeCN 50% with AmFm 20 mM pH 3.0. Second pump flow rate: 0.5 ml/min. UV wavelength - 280 nm. Switching time: 1.5 min after injection. The injection volume 8 µL of urine.**

# Application Examples

## Serotonin in serum.

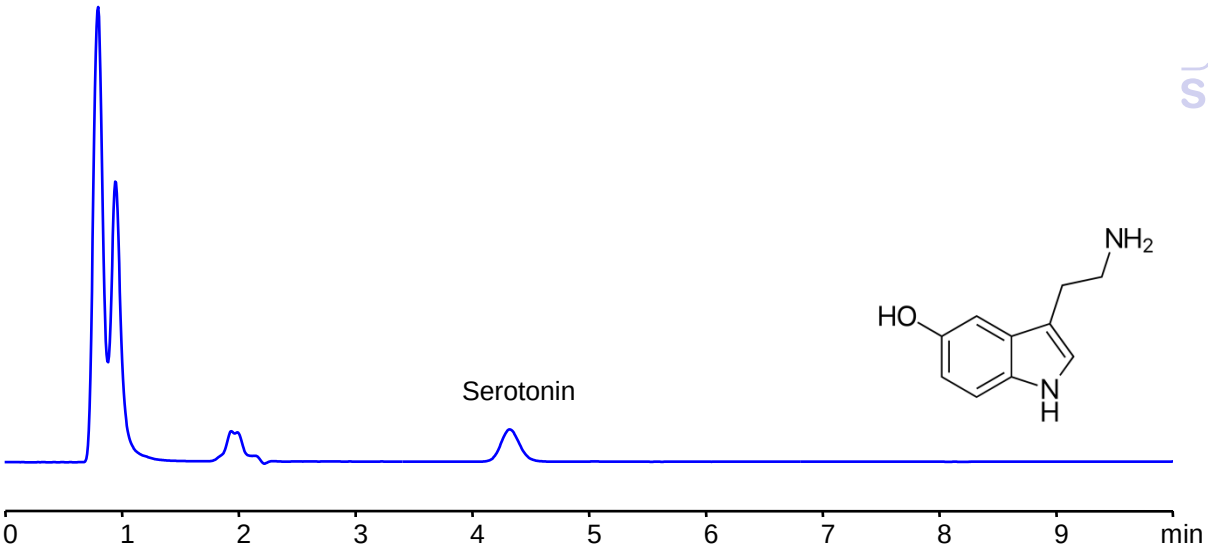



**Fig. 12. Chromatograms of human serum sample with dual pump dual column FlipLC system. Analytical column: Primesep 200, 4.6 x 100 mm. Flip column: Primesep SB, 4.6 x 50 mm. Flow rate: 1.0 mL/min. Mobile phase: MeCN – 20% with AmFm pH 3.0, 20 mM. Second pump mobile phase: MeCN 50% with AmFm pH 3.0 20 mM. Second pump flow rate: 0.5 ml/min. Valve switch time: 0.8 min after injection. Detection: UV 272 nm. Injection: 30 µL of serum diluted with mobile phase 1:2, filtered through 45 µm filter.**

An HPLC system with a valve installed before the analytical column and an additional isolation column which has orthogonal retention characteristics to the analytical column eliminates co-eluting 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 separation is not possible with any single column method without preliminary sample cleaning steps to eliminate interference of other components of the sample with peak of interest.

The use of three different separation mechanisms anion-exchange, cation-exchange, and reverse phase to isolate one particular compound give the highest possible specificity in analysis of the target compound.

The presented example can be applied to other charged polar molecules with both positively charged and negatively charged functional groups.

The method allows the accurate measurement of the analytes concentration in liquid samples with 10 ppb LOQ. Standard HPLC equipment with UV detector is sufficient in this methodology to measure UV active compounds.

Using MS detection many other organic and inorganic charged molecules can be quantitateted efficiently with high specificity and accuracy.

Contact SIELC for additional information and other separation needs.

**SIELC Technologies, Inc.**
**804 Seton Ct., Wheeling, IL 60090 USA**
**www.sielc.com email: mail@sielc.com ph. 847-229-2629 fax 847-655-6079**