Easy HPLC Method Development

Sometimes method development in chromatography can be a challenging and time consuming task. Screening multiple columns and different mobile phases is frustrating, in many cases requiring purchases of different columns. And what happens when no matter what you try works? Of course, 90% of applications will work on traditional C18 phases that you have in your lab. But what would you do if they are not working for your application. What if instead of developing 2-3 methods for complex mixtures and formulation you can have only one method? People in the lab never think about how much manpower and time they spend in the lab developing a method.

SIELC Technologies is willing to take on this task and develop a method for you. We would not charge you for our method development screening. What you will get if you decide to challenge us:

- Samples screened on up to 6 different phases;
- Analysis by SIELC's team of experienced chromatographers;
- Fast turn around;
- Full method details provided;
- LC/MS/ELSD compatible;
- Method scalable to preparative chromatography;
- Fully Confidential.

Our success rate for method development screening stands at 99%. Screening usually is done within 1-3 days and we never spend more than 8 hours on any method. Sometimes we can have an answer for you next day after receiving your sample. Here are the main problems people turn to our free service to help them solve:

- Inability to separate critical pairs or not enough resolution in the current method;
- Reduction of number of test methods for formulations and complex composition;
- Avoiding sample preparation for complex matrices;
- Developing a method which is compatible with LC/MS, prep chromatography and does not require ion-pairing reagent;
- Resolving of minor impurities in the presence of main component which interferes with analysis;
- Developing a faster method without employing UPLC;
- Developing isocratic method vs. gradient that is more suitable for production.

Challenge # 1 (Pharmaceutical company in Israel) and Challenge # 2 (Pharmaceutical company in US) - Separate critical pairs of isomers and increase resolution power.

A typical example of critical pairs can be separation of structural isomers of various compounds. Acidic and basic isomers were successfully separated by cation-exchange (Fig. 1) anion-exchange mixed-mode chromatography (Fig. 2)

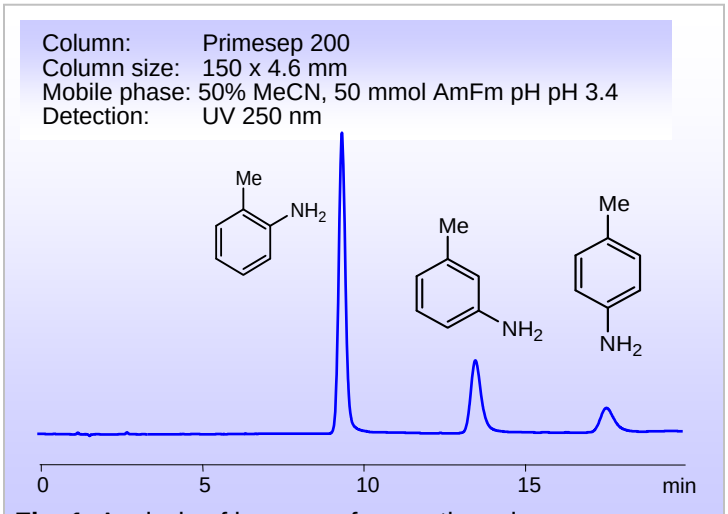

Fig. 1 Analysis of isomers of aromatic amines

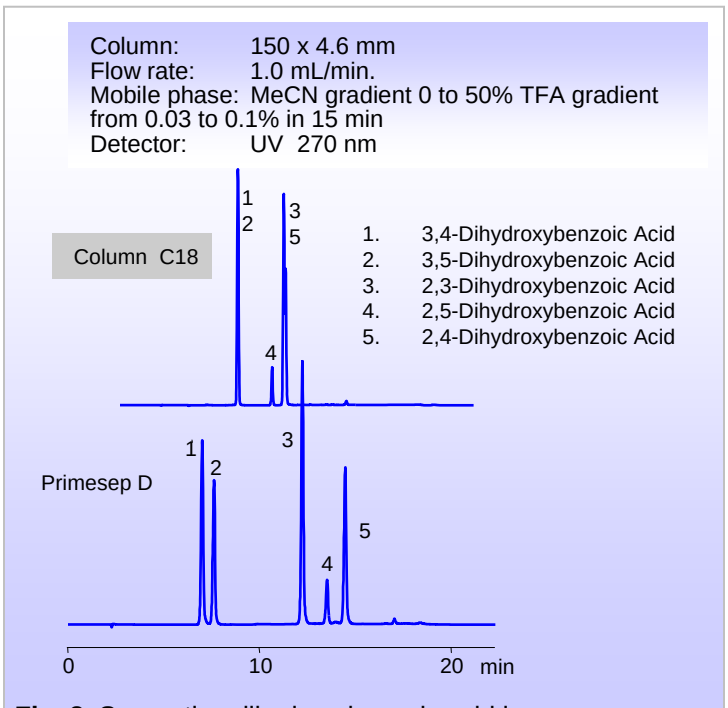

Fig. 2 Separation dihydroxybenzoic acid isomers

Challenge #3 (Global Pharma company) and Challenge #4 (Vitamin company in US) – Reduce number of test methods for formulations and complex composition

When a complex mixture requires analysis, development of several methods is required. Very often this method include RP, ion-exchange, GC, or titration. Controlling ionization state of compounds and employing multiple mode of interaction can result in development of a single method, where all compounds are well separated. Hydrophobic acid, hydrophobic basic along with hydrophilic basic and hydrophilic acidic compounds were separated in one run on an Obelisc R mixed-mode column. (Fig. 3). Same column was used for separation of multivitamin composition (Fig. 4)

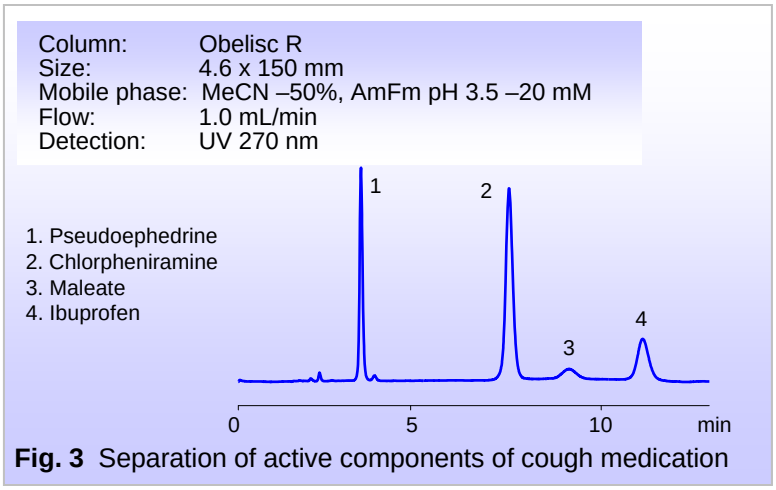

Fig. 3 Separation of active components of cough medication

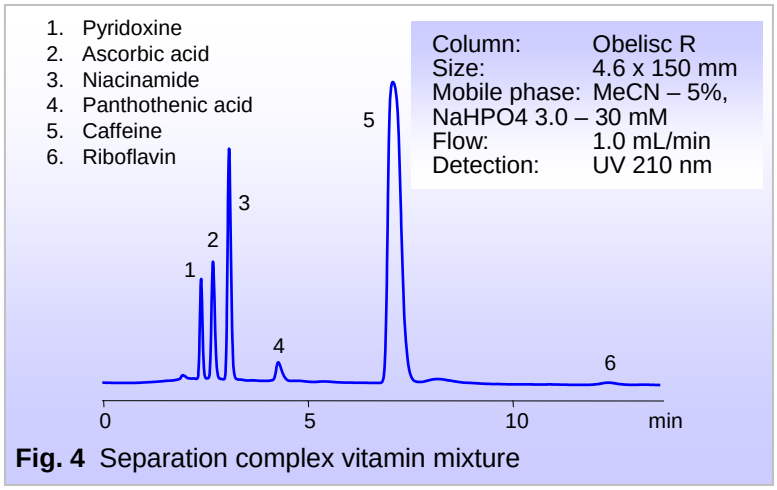

Fig. 4 Separation complex vitamin mixture

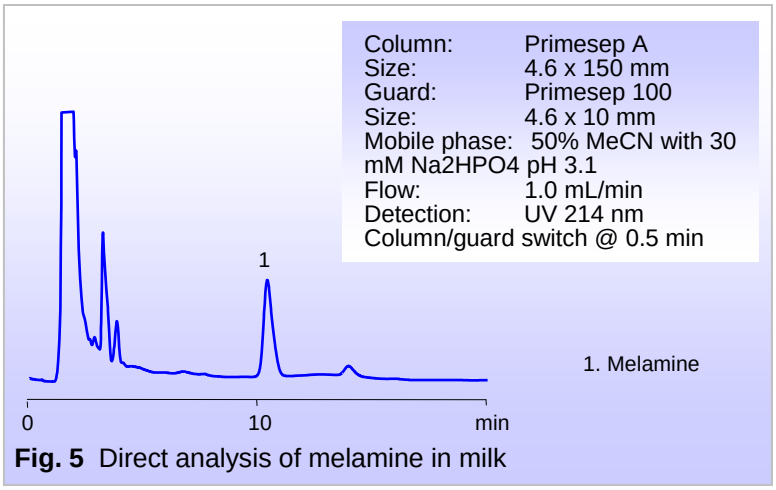

Fig. 5 Direct analysis of melamine in milk

Challenge #5 (Dairy Company in US) and Challenge # 6 (Pharma company in UK) - Avoid sample preparation for complex matrices

Sometimes quantitation of single component in complex mixture or matrix can be quite challenging due to matrix interference. In these cases, sample preparation or SPE is required. This can be avoided by employing a switching valve and guard columns to trap undesired excipients (Rapid HPLC Analysis of Complex Mixtures). The approach allows to re-use guard columns even when analytes contain proteins, sugars, gums, organic and inorganic ions. Melamine was quantified in milk, and lysine was quantified in dental composition without any sample preparation. Melamine content in milk was analyzed without any sample preparation technique (Fig. 5)

Challenge #6 (Pharmaceutical company in US) Develop a method which is compatible with LC/MS, prep chromatography and does not require ion-pairing reagent

Methods that involve ion-pairing reagents require dedication of columns to particular method and additional efforts to remove ion-pairing reagent. Mixed-mode chromatography addresses this issue, because in such columns the ion-pairing reagent is attached to the surface of silica gel. Mobile phase usually contains organic solvent and salt of acidic additive. The ions in the mobile phase can be chosen based on the detection technique or based on separation or isolation task, thus phosphoric acid can successfully replace TFA in the mobile phase to accommodate better prep separation conditions. Further replacement were done for better LC/MS sensitivity and conditions by replacing TFA with ammonium formate (Fig. 6).

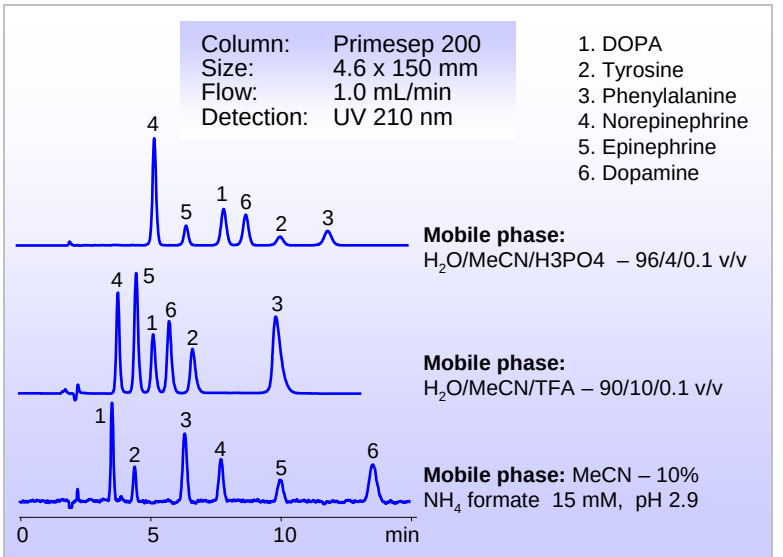

Fig. 6 Analysis of neurotransmitters with LC/MS conditions

Challenge #7 and #8 (Pharmaceutical company in US) – Develop highly efficient and faster method without employing UPLC

Although UPLC approach is a valuable solution for fast methods, it requires the purchase of new UPLC system. There are ways to increase speed of your analysis without using UPLC. Gradient elution, pH control of compounds ionization state and careful column selection can produce results similar to UPLC in terms of run time and efficiency of separation and achieve GC-type of efficiency (over 2M plates per meter for charged analytes, Fig. 7 and 8)

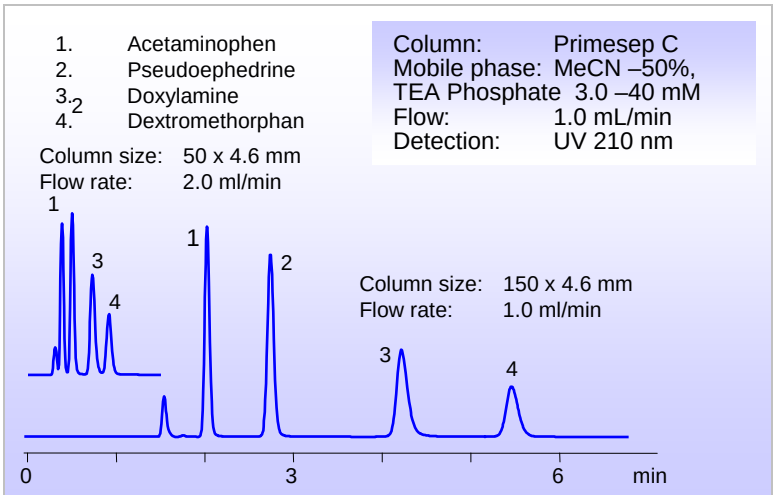

Fig. 7 High throughput a analysis of cough medication

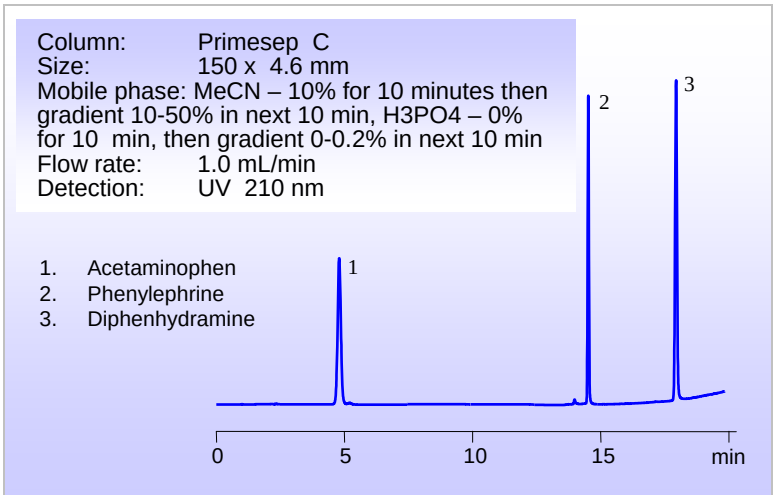

Fig. 8 High efficiency analysis of cough medication w/gradient

Challenge # 9 (Pharma company in US) – replace gradient with shorter isocratic method

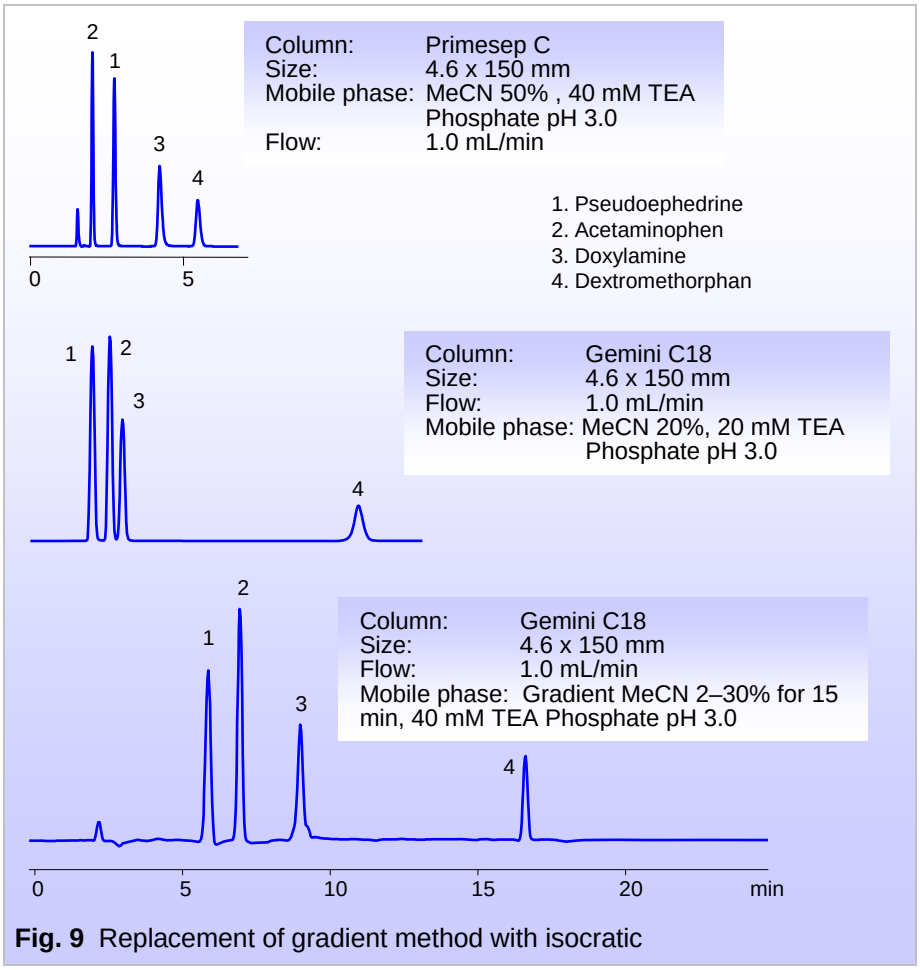

Fig. 9 Replacement of gradient method with isocratic

Wherever possible, it is a good idea to replace gradient method with an isocratic one. Single head pump can be employed in all isocratic methods and lengthy equilibration can be avoided in routine analysis for in-process checks and final product analysis. Analysis of cough composition with gradient method on traditional C18 columns was replaced with isocratic method on Primesep C mixed-mode column (Fig. 9).

Conclusion: SIELC Technologies has develop highly effective Free Screening Services that allow you to meet deadlines and develop methods more efficiently. Please contact us if you would like to explore this possibility (see submission form below).

On-Line Method Development Request Form