

# Using Capillary LC/MS with Add-On Flow to Separate the Chromatographic Method from the Ionization Conditions

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## Abstract:

In considering LC/MS applications, the compatibility between the the chromatographic method and the mass spectrometry ionization conditions is of critical importance. Direct routing of the HPLC eluent into the mass spec ionization source would not be effective or workable in situations such as high TFA % for ESI (ion suppression) or hexane mobile phase for AP-ESI (safety concerns). This limits the utilization of certain chromatographic techniques for LC/MS applications. For example, despite the effectiveness of immobilized polysaccharide coated columns for chiral separations, this approach has not been exploited for LC/MS applications due to the general incompatibility between the solvents used in normal phase LC and electrospray ionization.

It has been shown that normal phase chiral separation using a microfluidic HPLC/UV system (eksigent expressLC) offers considerable benefits for rapid method development and allows for efficient separation of enantiomers (1). We further demonstrate here that the inherent low-flow rate of the capillary HPLC allows up to a 10X post column infusion of make-up solvent optimized for ESI without compromising the chromatography performance and the MS detection sensitivity. This approach is applied for the separation and quantitation of pharmacologically active enantiomers. The coupling of capillary HPLC to conventional electrospray ionization mass spectrometry with post-column add-on flow may open up new avenues for small molecule analysis.

## Experimental:

**Capillary HPLC Systems:** Eksigent expressLC-800™, an 8-channel parallel HPLC system pictured below on the left, was used for the chiral method screening and development. Eksigent expressLC-100™, a single-channel fast HPLC system pictured below on the right, was used for the LC/MS/MS study.

**Mass Spectrometry:** An API-4000 triple quadrupole from Applied Biosystems equipped with a TurboV source was employed for the LC/MS/MS study. A 30u i.d. fused-silica transfer line was inserted into the the stainless steel electrospray emitter all the way to just past the opening. A micro-tee union and a syringe pump were used for the add-on flow experiments.

**Separation Conditions:** Polysaccharide coated type AD-H, AS-H, OD-H and OJ-H (150 x 0.3 mm) columns from Chiral Technologies were employed for the chiral separations. The mobile phases were A: Isopropanol or ethanol and B: hexane with 0.1% TEA. The flow rate was 6 µL/min.



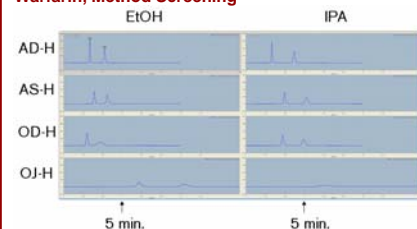
Eksigent expressLC-800™

Eksigent expressLC-100™

## Results:

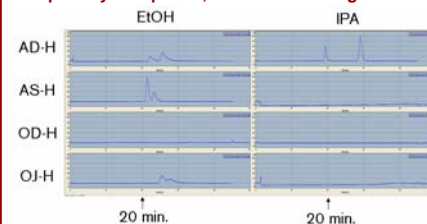
### Chiral Method Screening and Development

#### Warfarin, Method Screening



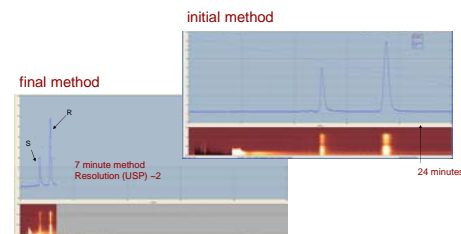
**Figure 1** Chiral method screening for the separation warfarin enantiomers. Eksigent expressLC-800™ parallel HPLC system and chiral columns from Chiral Technologies were used for the experiments. A: EtOH or IPA. B: 0.1% TEA, 0.1% TFA, 5% IPA/hexane. Gradient 20%→60% A in 3 minutes. Flow rate, 6µL/min. UV detection @ 230 nm

#### Proprietary Compound, Method Screening



**Figure 2** Method screening for the chiral separation of the enantiomers of a proprietary pharmaceutical compound. Gradient 5%→60% A in 45 minutes. All other conditions are the same as in Figure 2. IPA with AD-H column offers the best performance.

#### Proprietary Compound, Method Optimization

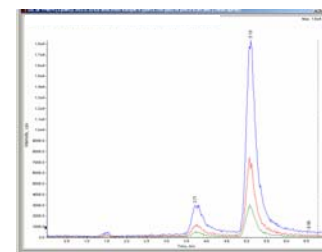


**Figure 3** The development of a 7-min method (resolution ~2) using AD-H column and IPA in mobile phase A. Optimizations were achieved by changing the mobile phase B additive concentration and by adjusting the gradient profile.

### LC/MS with Add-on Flow

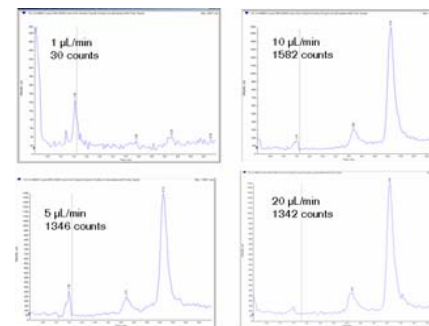
#### LC/MS method for mixture of -R and -S form

- The optimized 7-min HPLC method with AD-H column and normal phase conditions were employed with a single-channel Eksigent expressLC
- With a syringe pump and ZDV micro-tee union, 5 - 20 µL/min make-up flow (0.1% formic acid in water) was added to the HPLC eluent after the column
- This allows sensitive detection of the amine compounds using positive mode ESI
- This technique minimizes the adverse effect of chromatographic mobile phases on the ionization efficiency



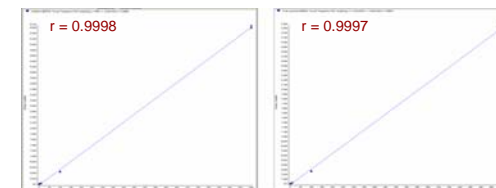
**Figure 4** A typical MRM chromatogram of -R and -S mixture. Shown here are 3 MRMs taken at unit resolution with a dwell time of 350 msec. For subsequent experiments, a single MRM transition is used with the dwell time increased to 3 sec.

#### Effect of Add-On Flow



**Figure 4** The effect of adding 1 - 20 µL/min of water/0.1% formic acid to the normal phase eluent flowing at 6 µL/min. Efficient electrospray ionization was achieved at 1:1 flow ratio. Note that the chromatographic efficiency and MS signal response remain relatively unchanged despite of the increasing add-on flow rate.

### Linearity and Quantitation Limit



**Figure 5** Calibration curve for the early eluting enantiomer (left) and the late eluting enantiomer (right) from 1, 10, 100 to 1000 ppb, at 2 points for each concentration. LLOQ ~ 4 ppb with S/N ~ 10. The %CV for 5 runs at LLOQ is ~ 5%.

## Conclusions:

We demonstrate here that the coupling of normal phase capillary HPLC to conventional electrospray ionization mass spectrometry with post-column add-on flow indeed can provide an efficient and sensitive LC/MS/MS method for the analysis of chiral drug molecules. This approach allows the use of chromatographic mobile phases that yield poor ionization or other undesirable effects for MS detection. There is an increasing need for selective and sensitive analytical methods for quantifying stereoisomers in biological samples and pharmaceutical materials. Capillary HPLC instrument with its inherent low flow rates and rapid gradient mixing, such as the expressLC-100 system, is well suited for this type of applications.

## References:

- "Multiparallel chiral method development screening using an 8-channel microfluidic HPLC system" Sajonz P, Gong X, Leonard WR Jr, Biba M, Welch CJ, *Chirality*, 2006 Nov,18(10):803-13