

Enhanced speed of gradient capillary LC analysis of compounds of pharmaceutical interest by ultrafast stationary/mobile phase re-equilibration

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Abstract

While conventional bore and microbore LC analyses have made great strides in shortening chromatographic analysis times principally by use of shorter columns, innovative stationary phases, and higher flow rates, most of the published applications have been isocratic. Using isocratic procedures for increased analysis speed avoids the introduction of a gradient which often results in a chromatographic separation that is much shorter than the stationary/mobile phase re-equilibration. The result yields only a modest improvement in total analysis time. The studies presented here will demonstrate that high speed, high precision capillary LC data can be generated on samples of pharmaceutical interest by severely truncating the gradient re-equilibration time such that the chromatographic run time is essentially equal to the total run time. Chromatographic run time, total run time, and precision data on actual pharmaceutical samples will be presented.

Experimental

Instrument: Eksigent Express-100 capillary liquid chromatograph, picture below

Column: Agilent Zorbax SB-C18, 5µ particle, 35x0.3 mm

Column Temperature: Ambient

Mobile Phase: linear gradients, A=aqueous acetonitrile; B=acetonitrile /5% water; both A and B containing 0.1% TFA

Flow rate: 6-10 µL/minute

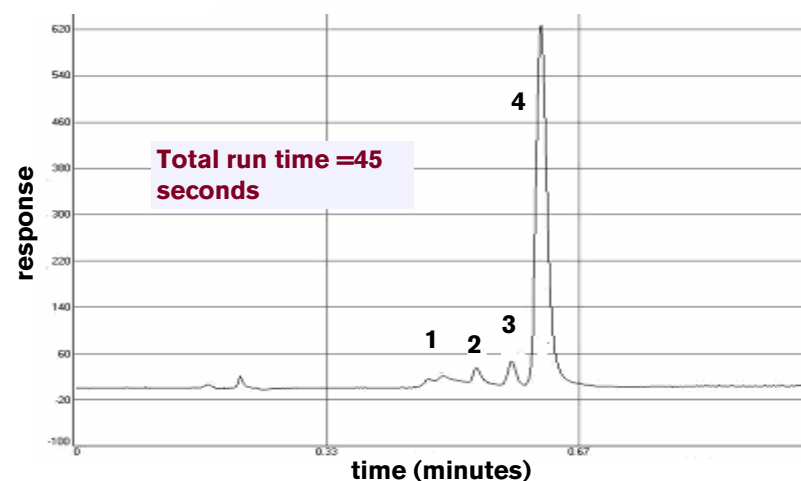
Injection volume: 80-120 nL

Detection Wavelength: 220-260 nm

Programmed Gradient Re-Equilibration Time: 0 minutes, unless otherwise noted

Empirical Determination of Capillary LC Gradient Re-equilibration Time

To investigate empirical re-equilibration times, a degraded drug formulation was chosen and a calibration of the retention times of four peaks (**below**) versus initial mobile phase composition was tabulated. Then injections with a re-equilibration time of 1 minute and 0 minutes were performed (**table below**). The chromatographic re-equilibration time under the given conditions from 100% mobile phase B to 0% mobile phase B, was determined to be zero-**no programmed re-equilibration time was required**.



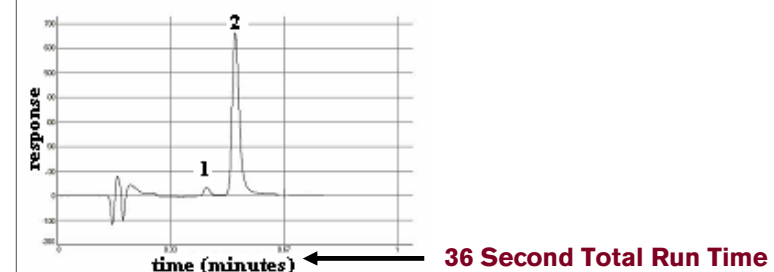
Chromatogram of a degraded drug formulation used for initial gradient compositional sensitivity studies. The retention times of four species, represented by peaks 1-4 above, were used for the studies.

Initial %B	Re-equil time (minutes)	Retention time, Peak 1	Retention time, Peak 2	Retention time, Peak 3	Retention time, Peak 4
<i>Calibration</i>					
5	1	0.451	0.504	0.547	0.596
2	1	0.461	0.522	0.568	0.610
0	1	0.473	0.527	0.573	0.614
<i>Sample</i>					
0	1	0.491	0.532	0.578	0.617
0	0	0.491	0.532	0.578	0.617

No difference in retention time for the four peaks whether a maximum required pre-equilibration time (1 minute) or zero programmed re-equilibration is used.

Area and Retention Time Precision for a Degraded Alprazolam Solution

The data presented above would suggest that a high-speed capillary LC separation could be performed with the current instrument with very precise retention times and under at least some chromatographic conditions, **no programmed re-equilibration time**. Solution-degraded alprazolam was injected nine times and the resulting quantitative data for both the retention times and the peak areas appears below. **Peak 1** was generated by degradation of the parent alprazolam species (**peak 2**).



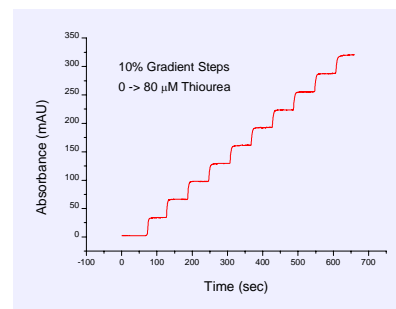
Injection	Peak 1 RT (minutes)	Peak 1 area (mAU)	Peak 2 RT (minutes)	Peak 1 area (mAU)
1	0.439	357.99	0.525	1703.07
2	0.439	350.34	0.525	1742.52
3	0.438	352.60	0.525	1700.28
4	0.440	350.82	0.525	1721.14
5	0.439	348.79	0.525	1706.07
6	0.440	349.30	0.526	1761.71
7	0.440	349.41	0.525	1725.78
8	0.440	353.92	0.527	1723.11
9	0.442	350.64	0.527	1716.26
	X=440, RSD=0.25%	X=352, RSD=0.85%	X=525, RSD=0.17%	X=1722, RSD=1.1%

Conclusions

- Capillary LC analysis requires exceeding reproducible mobile phase initial compositions to yield consistent retention time data.
- Chromatographic conditions were found that did not require long gradient re-equilibration times; in some cases, the analyses required *no programmed re-equilibration time*, even for re-equilibration from 100% mobile phase B to 100% A.
- High-speed capillary analysis (in the case cited, a total run time of 36 seconds) of pharmaceutical compounds can be performed with the precision of conventional LC analysis.



ExpressLC-100 Capillary HPLC System



Precision Gradient Control