Method Validation Characteristics Of Gradient Capillary LC Analyses Of Compounds Of Pharmaceutical Interest: Are We There Yet?

Ring-Ling Chien, **Hung-Yuan Cheng, David Emlyn Hughes, David Rakestraw**, Eksigent Technologies, 2021 Las Positas Court, Livermore, CA 94550, USA

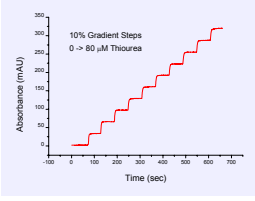
Abstract

Capillary LC has tremendous potential for being the next major innovation in separatory analysis. The technique has demonstrated the ability to separate species with high resolution, unequaled speed and low sample and mobile phase consumption. But other techniques, notably capillary electrophoresis and supercritical flow chromatography, have historically shown superior resolution and speed when compared with conventional LC, but they have chronically suffered from inconsistent quantitative results.Data will be presented from studies performed employing capillary liquid chromatography with microfluidic flow technology on a 0.30 mm capillary column. This presentation will address capillary LC method studies on a quantitative basis for linearity, precision, instrumental stability, capillary column lifetime, and sensitivity for a number of drugs of pharmaceutical interest. The applicability of current technology to real-world, regulated pharmaceutical analyses will be discussed. Capillary LC with optimized pumping, mixing, and detection is shown not only to have the advantages of speed and high resolution, but also the generation of very high quality quantitative data.

Experimental

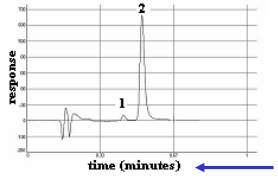
Instrument: Eksigent Express-100 capillary liquid chromatograph, picture below
Column: Agilent Zorbax SB-C18, 5µ particle, 35x0.3 mm
Column Temperature: Ambient
Mobile Phase: linear gradients, A=aqueous acetonitrile; B=acetonitrile /5% water; both A and B containing 0.1% TFA
Flow rate: 6-10 µL/minute
Injection volume: 80-120 nL
Detection Wavelength: 220-260 nm
Programmed Gradient Re-Equilibration Time: 0 minutes, unless otherwise noted

ExpressLC-100 Capillary HPLC System



Precision Gradient Control

Area and Retention Time Precision for a Degraded Alprazolam Solution

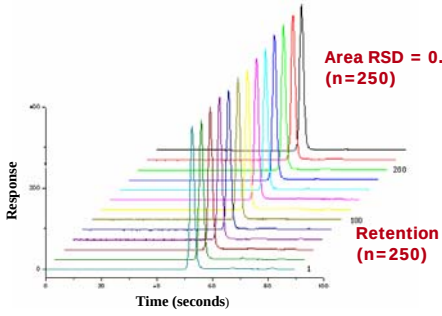


36 Second Total Run Time

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%

System Repeatability and Column Lifetime

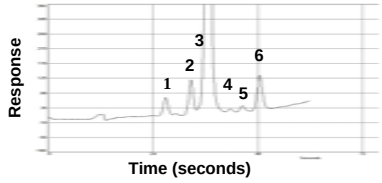
The combination of precision flow control and timed injections provides unprecedented repeatability for capillary HPLC. The long term repeatability and column stability is demonstrated below for 250 injections conducted over a 12 hour period.



Area RSD = 0.72 % (n=250)

Retention Time RSD = 0.26 % (n=250)

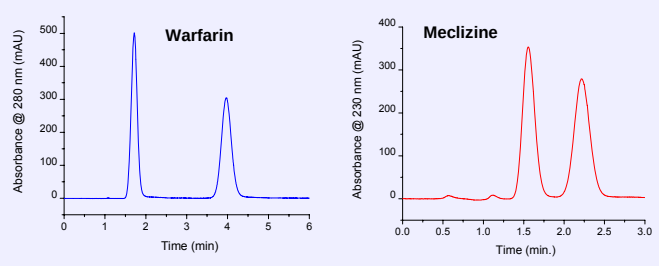
Specificity and Speed of Analysis: Indomethacin Drug Substance Impurity Analysis



60 Second Total Analysis Time: 6 Baseline-Resolved Peaks

Chiral Analysis

Increased sophistication of LC chiral analysis has come to the forefront of pharmaceutical analysis due to the unique bioactivity of specific enantiomers. Often, the limited availability of pure enantiomers, high cost of conventional LC columns, low peak efficiencies, lengthy analysis times, and inconvenient conversion to normal phase chromatography has made pharmaceutical chiral analysis an analytical specialty field unto itself. Chiral analysis of a wide range of compounds can be conducted in less than 6 minutes with high-efficiency analyte peaks, low sample volume requirements, and rapid conversion between reversed-phase and normal-phase eluents. The following separations were done on a column containing Chiral Technologies AD-H stationary phase.



Conclusion

Capillary high performance liquid chromatography offers significant performance benefits over conventional 4.6 or 2.1 mm column LC. The benefits are now possible without sacrificing typical LC precision of retention times and peak areas. This high level of quantitative performance, the ability to employ a wide range of stationary phases, and significant reduction in the amount of mobile phase consumed makes capillary LC a very attractive choice for pharmaceutical analysis.

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