



cLC™ System: An Automated Solution for Normal- and Reverse-Phase Prep HPLC with Analytical Purification Determination

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The cLC™ (complete LC) System presented in this application is a single-chromatography system capable of automating the purification of compounds using both preparative RP columns and NP disposable and non-disposable columns. The cLC System also has the ability to accommodate various modes of detection, including UV, MS, and ELSD. The system is entirely automated through the software, excluding the user-defined manual loading of the disposable NP columns. Analytical components can also be integrated into the cLC System, enabling automated on-line evaluation of the collected fractions.

Instruments and Accessories

- Pumping System: Prep — Gilson 333/334 Pumps; Analytical — Gilson 32× Pumps
- Gilson 215 Liquid Handler with injector
- Columns: RP — CombiPrep™ C-18 (22 × 50 mm, 5 μm, 120 Å); NP — Betasil® Silica (20 × 150 mm, 5 μm, 100 Å), Biotage, Inc. Flash™ 40+M (40 × 150 mm, 100 g silica weight); Analytical — Supelco (4.6 × 150 mm, 5 μm, 100 Å)
- Gilson 155 UV/VIS Dual-wavelength Detector (prep & analytical)
- Gilson VALVEMATE® Valve Actuator (2)

Conclusion

The cLC™ System is capable of handling as many as five columns (NP and RP) with one bypass for quick changeover between the two types of chromatography. The system can accommodate disposable NP chromatography columns (sample load <15 g, based on ΔCV) (e.g., Biotage FLASH 24+M to 75M) in an HPLC environment which will accommodate other modes of detection: MS, ELSD (which is not available with off-line split stream instruments).

The cLC System is totally automated and accommodates the wide range of solvents used through solvent selection valves without operator intervention. Although most NP chromatography is run under isocratic conditions, the cLC System offers the advantage of improving NP Flash chromatography via gradients. NP gradients are being run at the present time in several pharmaceutical labs on the cLC System employing the Biotage FLASH columns.

The addition of analytical components allows for the checking of the fractions collected on line, prior to dry down. Additional solvent and column selection valves could be used on the analytical system to allow not only the checking of

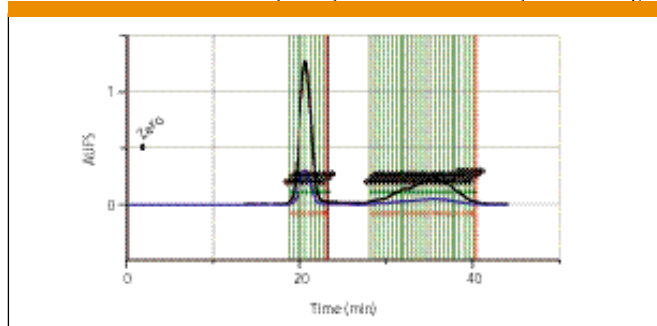


Figure 1: Biotage FLASH™ 40+M column. Nestriol and estrone on Biotage manual load.

the RP fractions, but the NP fractions as well.

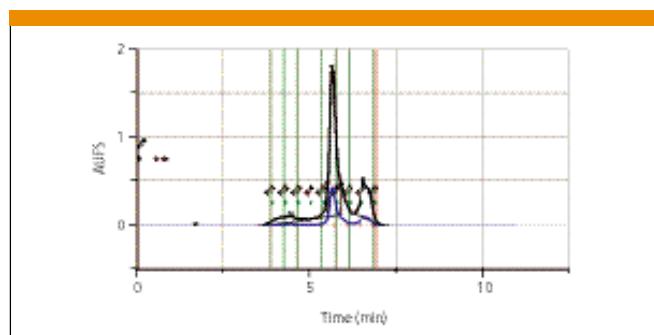


Figure 2: Betasil® Silica column. Estriol and estrone.

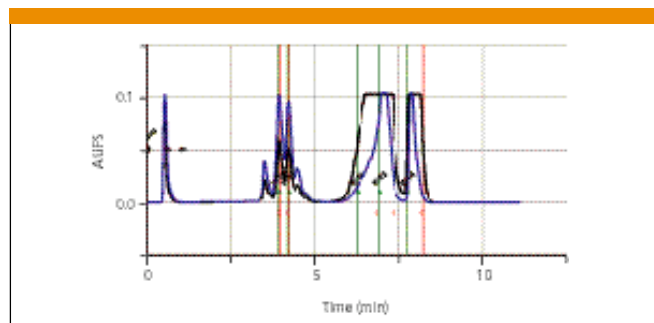


Figure 3: RP-CombiPrep™ Pro C18 column. RP mixture.

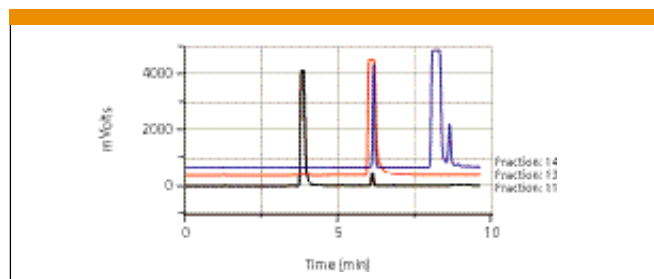


Figure 4: Injection of fractions collected from RP chromatography.

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