A Totally Automated Solution for Normal and RP Preparative HPLC with Analytical Purification Determination: cLC

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Abstract

It imperative in today's world that researchers get the most bang for their buck. Many times resources are being stretched very thin as the demand to increase production goes up. Purification of compounds has largely been accomplished through liquid-based chromatography. The advantages of preparative chromatography for compound purification are well established. Reverse phase chromatography, although being the method of choice, is limited by sample capacity /injection, possible sample solubility issues and lengthy dry downs. It would be very advantageous to researchers if one could expand the capabilities of these instruments.

Researchers have shown quite an interest in implementing normal phase chromatography for compound purification. Benefits such as fast dry downs and increased sample loading make normal phase chromatography an attractive alternative for compounds that are incompatible with RP chromatography. However does one really want additional instruments dedicated for NP? A possible solution to this dilemma is to automate the capabilities of RP and NP in one system in addition to being able to analyze the collected fraction for purity.

The cLC (comprehensive LC) system is a totally automated purification system with on-line analytical analysis of fractions, using both NP and RP environments. The cLC system requires only a little more bench space than a conventional HPLC system at about the same price. The system can automatically switch between normal and reverse phase, and is capable of accessing both pre-packed disposable silica-based, NP-HPLC, and RP-HPLC columns without operator intervention. Collected fractions can then automatically be injected for determination of purity. Presentation of the system will include data representing the throughput and analysis capabilities of the system.

Outline

- Introduction
- cLC System: Hardware Components
- System Control and Data Handling
- Performance Data
- Summary and Conclusions

Introduction

Reverse-Phase (RP) Chromatography is the method of choice for purification of compounds. There are many manufactures of semi- and preparative RP columns which range in IDs from 22 mm to 25 cm and lengths well over 250 mm (sample load range 25 mgs~2.5 gms). The flow rate range for these size columns is 25- 200 ml/min. Depending on solubility sample loading is in the mg-low gram range. Because sample loading and dry down of the fractions collected can be quite an issue many researchers employ Normal-Phase (NP) chromatography for some of their compounds. NP columns are available in similar sizes and flow rate ranges as the RP columns. An additional entry for the NP columns is the use of disposable columns, ID range of 25-75 mm and lengths from 7.5-30 cm (sample load range 80mgs-15 gms based on △CV). These columns run at lower pressures based on their packing material. Although they are usually a one time use column they offer a much greater degree of sample loading, dry down is minimal and the columns could be stored if further evaluation is required. Being able to accommodate both RP and NP chromatography usually requires two systems. The cLC system presented in this application is a single chromatography system capable of automating the purification of compounds using both RP and NP disposable and non-disposable columns. The cLC system also has the ability to accommodate various modes of detection including UV, MS, ELSD. An additional analytical system is also available on the system so that automated evaluation of the collected fractions could be obtained on-line.

System Components

- 333/334 pumping system, flow rates up to 200 mL/min, four position solvent selection valve

  Optional: 321 pumping system, flow rate to 15 mL/min

- 215 Liquid Handler, 175 mm arm, 10 mL dilutor syringe, 819 injectors (2), 7010 Rheodyne, make before break stator and large bore rotor seal, 10 mL loop (preparative), 20 uL loop (analytical), 5 racks, accommodating 13x150 mm and 18 x150 mm tubes, low mount high flow preparative collection valve

- Columns: RP-CombiPrep C-18, 22 x 50 mm, 5 μ, 120A

  NP-Betasil Silica 20 x 150 mm, 5μ, 100A

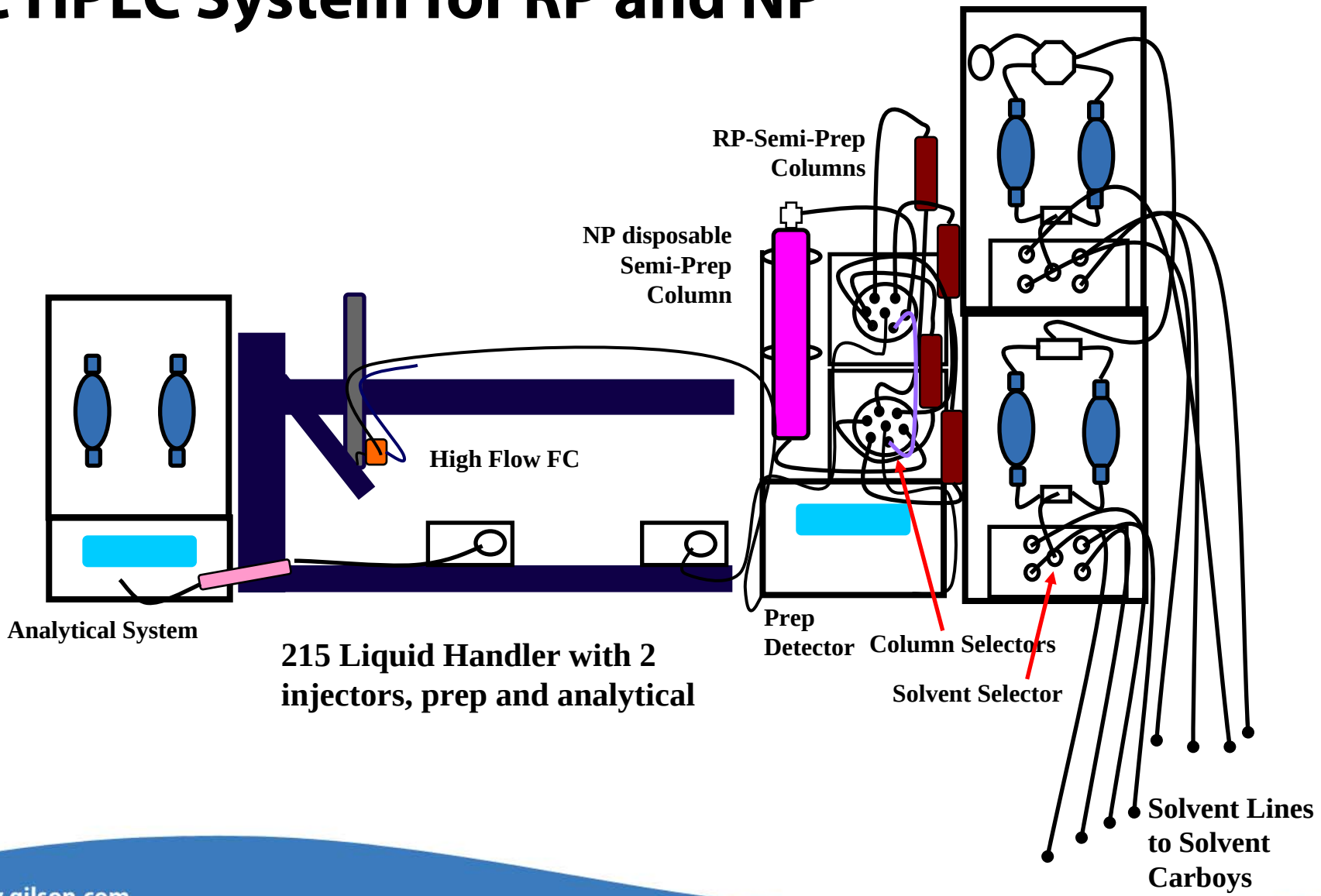
  Biotage Flash 40+ M 40 x 750 mm, 50 gram silica weight

- 155 UV Dual wavelength detector with preparative flow cell 0.05 mm pathlength

  Optional: 155 UV Dual wavelength detector with analytical flow cell 5.0 mm pathlength

- 2 Valvemates with 7060 Rheodyne, 6 position column selection, large bore stator and rotor seal

cLC HPLC System for RP and NP



215 Liquid Handler with 2 injectors, prep and analytical

cLC System: Planar arrangement accommodated within 60 inches of bench space


215 LH mounted with the low mount high flow FC valve: The valve has a separate path from the injected sample which eliminated any cross talk between sample and collected fractions

Control and Data Handling

- The cLC system was controlled by HPLC and Data Handling software
- The system is entirely automated through the software excluding the manual loading of the Biotage Flash NP columns

Control Methods: Solvent Change Methods for Switching Systems and Equilibration of the Instrument

Control Method [change.gct]

	Time	Device(s)	Command
1	0.02	Column Selector Inlet	Set Valve Position 5
2	0.04	Column Selector Outlet	Set Valve Position 5
3	0.05	Data Channels	Start Chromatogram Channels
4	0.10	Pump 1 / Pump 2	0(ml/min): 50% Pump 1, 50% Pump 2
5	0.50	Pump 1 / Pump 2	FL(ml/min): 50% Pump 1, 50% Pump 2
6	7.00	Data Channels	Stop Chromatogram Channels
7	7.00	Pump 1 / Pump 2	FL(ml/min): 50% Pump 1, 50% Pump 2
8	8.00	Pump 1 / Pump 2	0(ml/min): 50% Pump 1, 50% Pump 2

The top method allows the common solvent in this case IPA (isopropanol) to flush thru the system " *CHANGE*" allowing for the conversion between NP and RP columns. This method can also be run a second time with your chromatography solvents to stabilize the system prior to equilibrating the column to the mobile phase solvents.

Control Method [equil.gct]

	Time	Device(s)	Command
1	0.01	Column Selector Inlet	Set Valve Position Variable SELECT_COLUMN
2	0.02	Column Selector Outlet	Set Valve Position Variable SELECT_COLUMN
3	0.03	Pump 1 / Pump 2	0(ml/min): 100% Pump 1, INIT_ORG% Pump 2
4	0.05	Detector 16	Set Mode Dual
5	0.07	Detector 16	Set Dual Wavelength 1 254
6	0.09	Detector 16	Set Dual Wavelength 2 280
7	0.11	Detector 16	Set Dual Sensitivity 1 1.0
8	0.13	Detector 16	Set Dual Sensitivity 2 1.0
9	0.15	Detector 16	Autozero Channels
10	2.00	Data Channels	Start Chromatogram Channels
11	3.00	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, INIT_ORG% Pump 2
12	9.00	Data Channels	Stop Chromatogram Channels
13	10.00	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, INIT_ORG% Pump 2

The "*EQUIL*" method shown to the left will allow the column to equilibrate with initial mobile phase conditions prior to the injection of the sample. This method is also run between similar columns e.g. multiple RP or NP columns.

Control Methods: RP Chromatography

Control Method [rp_clc.gct]

	Time	Device(s)	Command
1	0.01	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, 0% Pump 2
2	0.10	partial loop fill for 215 as FC	<start> INJECT_VOLUME, SAMPLE
3	0.36	System Controller	Synchronize
4	0.37	Detector 16	Autozero Channels
5	0.38	Data Channels	Start Chromatogram Channels
6	0.50	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, 0% Pump 2
7	1.35	System Controller	Synchronize
8	1.37	Fraction Collector	Set Collection and Travel Depths 3, 3
9	1.39	Fraction Collector	Set Peak Level PK_LVL
10	1.41	Fraction Collector	Set Fraction by Volume Inside a Peak FC_VOL
11	1.43	Fraction Collector	Set Fraction Site FC_SITE
12	1.45	Fraction Collector	Start Collection
13	7.00	Pump 1 / Pump 2	FL(ml/min): 5% Pump 1, 95% Pump 2
14	9.00	Pump 1 / Pump 2	FL(ml/min): 5% Pump 1, 95% Pump 2
15	10.50	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, 0% Pump 2
16	11.30	Fraction Collector	Start Collection
17	11.50	Data Channels	Stop Chromatogram Channels
18	12.00	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, 0% Pump 2

This control method is for RP chromatography: a gradient of increasing organic is initiated to minimize the run time and sharpen the peaks. Variables are used so that various gradients and parameters for fraction collection could be explored between runs.

Control Methods: NP Chromatography for the Biotage FLASH 40 +M Columns

Control Method [np_clc.gct]

	Time	Device(s)	Command
1	0.01	Pump 1 / Pump 2	0(ml/min): 100% Pump 1, INIT_ORG% Pump 2
2	0.02	Injector	Set Injection Valve Position Load
3	0.05	WAITING FOR INJECTION	Wait
4	0.10	Pump 1 / Pump 2	0(ml/min): 100% Pump 1, INIT_ORG% Pump 2
5	0.12	Detector 16	Autozero Channels
6	0.14	Data Channels	Start Chromatogram Channels
7	0.16	Fraction Collector	Set Collection and Travel Depths 3, 3
8	0.18	Fraction Collector	Set Peak Level PK_LVL
9	0.20	Fraction Collector	Set Fraction by Volume Inside a Peak FC_VOL
10	0.22	Fraction Collector	Set Fraction Site FC_SITE
11	0.24	Fraction Collector	Start Collection
12	0.70	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, INIT_ORG% Pump 2
13	7.00	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, END_ORG% Pump 2
14	9.00	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, END_ORG% Pump 2
15	39.00	Fraction Collector	Stop Collection
16	39.50	Data Channels	Stop Chromatogram Channels
17	40.00	Pump 1 / Pump 2	FL(ml/min): 100% Pump 1, INIT_ORG% Pump 2

The Biotage FLASH 40+M columns are loaded manually, either off-line or on the system prior to their running. Isocratic parameters are usually employed for NP chromatography, however gradient profiles are available in the software. There are three different ways to load the FLASH columns:

A) Load the sample dissolved in a given amount of solvent, usually less than 20 mls.

B) Load the sample in a samplet (similar to a guard cartridge and drop it in the top of the cartridge)

C) Load the sample dry by packing the top of the cartridge

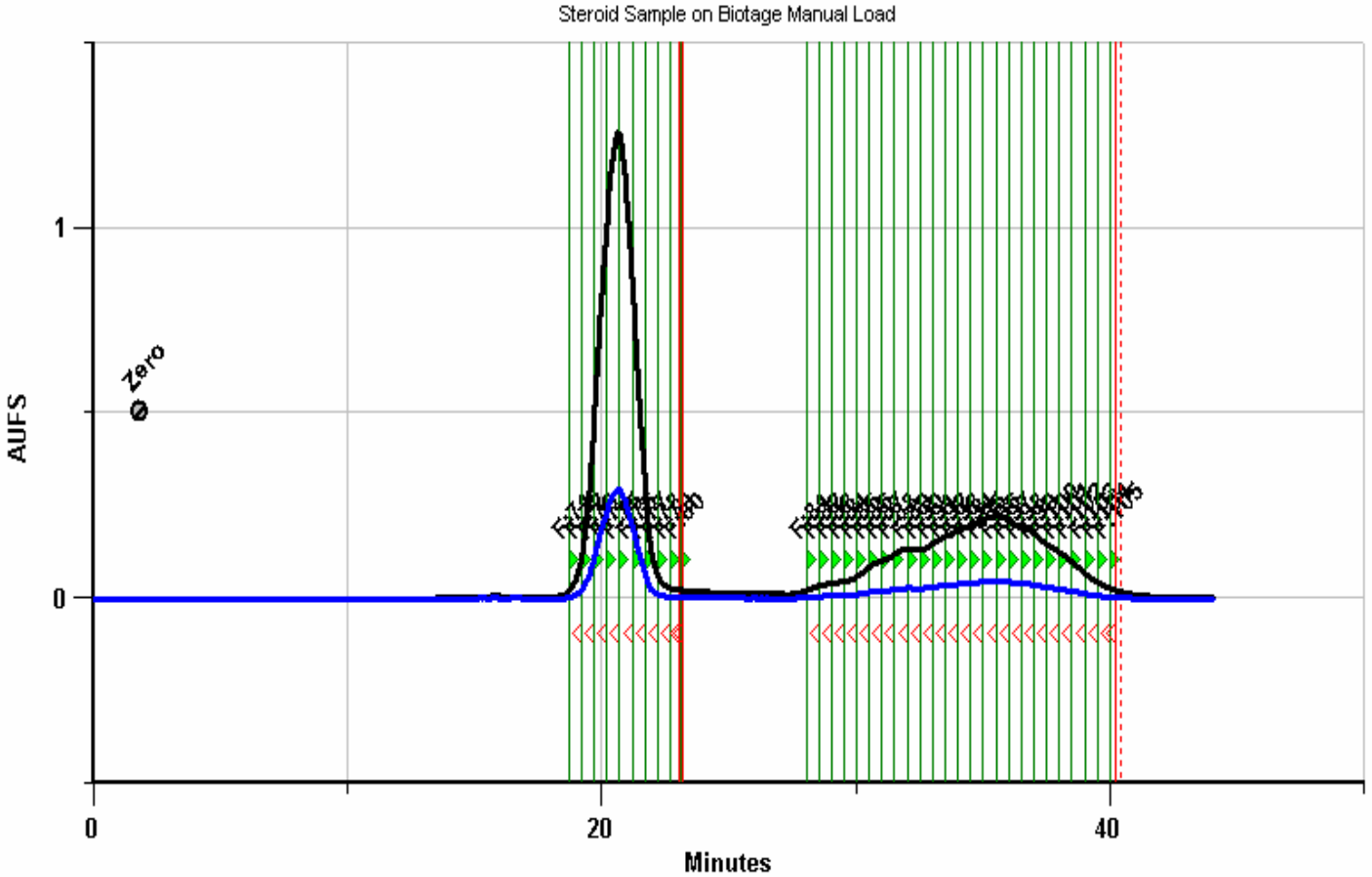
Operation List for the cLC System:

Operations [clc_sys.gop]

	Description	Control Method	Analysis Method	FL	CT_COL	CT_VOL	SAMPLE	PK_LVL	FC_VOL	FC_SITE	INIT_ORG	END_ORG
1	Start System	\CLC\PREPRPNP\NP_CLC\EQUIL.GCT		40	5						98	
2	Equilibrate NP System	\CLC\PREPRPNP\NP_CLC\EQUIL.GCT		40	6						98	
3	Start System	C\PREPRPNP\NP_CLC\EQUIL_NP.GCT		40	6						98	
4	Estriol + Estrone	C\PREPRPNP\NP_CLC\NP_INJFC.GCT	NP_CLC.GAN	40		2000	S:1	100	15	F:1	98	80
5	Change System	LC\PREPRPNP\NP_CLC\CHANGE.GCT		50								
6	Equilibrate RP System	\CLC\PREPRPNP\RP_CLC\EQUIL.GCT		30	1						0	
7	RP_Mixture	LC\PREPRPNP\RP_CLC\RP_CLC.GCT	RP_CLC.GAN	30		4000	S:2	100	20	F:		
8	Equilibrate RP System	\CLC\PREPRPNP\RP_CLC\EQUIL.GCT		30	2						0	
9	RP_Mixture	LC\PREPRPNP\RP_CLC\RP_CLC.GCT	RP_CLC.GAN	30		4000	S:3	100	15	F:		
10	Equilibrate RP System	\CLC\PREPRPNP\RP_CLC\EQUIL.GCT		30	3						0	
11	RP_Mixture	LC\PREPRPNP\RP_CLC\RP_CLC.GCT	RP_CLC.GAN	30		4000	S:4	50	20	F:		
12	Equilibrate RP System	\CLC\PREPRPNP\RP_CLC\EQUIL.GCT		30	4						0	
13	RP_Mixture	LC\PREPRPNP\RP_CLC\RP_CLC.GCT	RP_CLC.GAN	30		4000	S:5	50	15	F:		
14	Wash RP System	PREPRPNP\RP_CLC\SHUTDOWN.GCT		25								
15	Common Solvent Rest	LC\PREPRPNP\RP_CLC\CHANGE.GCT		40								
16	Change to NP	\CLC\PREPRPNP\NP_CLC\EQUIL.GCT		35	5						100	
17	Change to NP	\CLC\PREPRPNP\NP_CLC\EQUIL.GCT		35	5						100	
18	Change to NP Equilibrate Biotage	\CLC\PREPRPNP\NP_CLC\EQUIL.GCT		30	6						100	
19	Change to NP Equilibrate Biotage	\CLC\PREPRPNP\NP_CLC\EQUIL.GCT		30	6						100	
20	Steroid Sample on Biotage Manual Load	LC\PREPRPNP\NP_CLC\NP_CLC.GCT	NP_CLC.GAN	30				100	20	F:	80	80

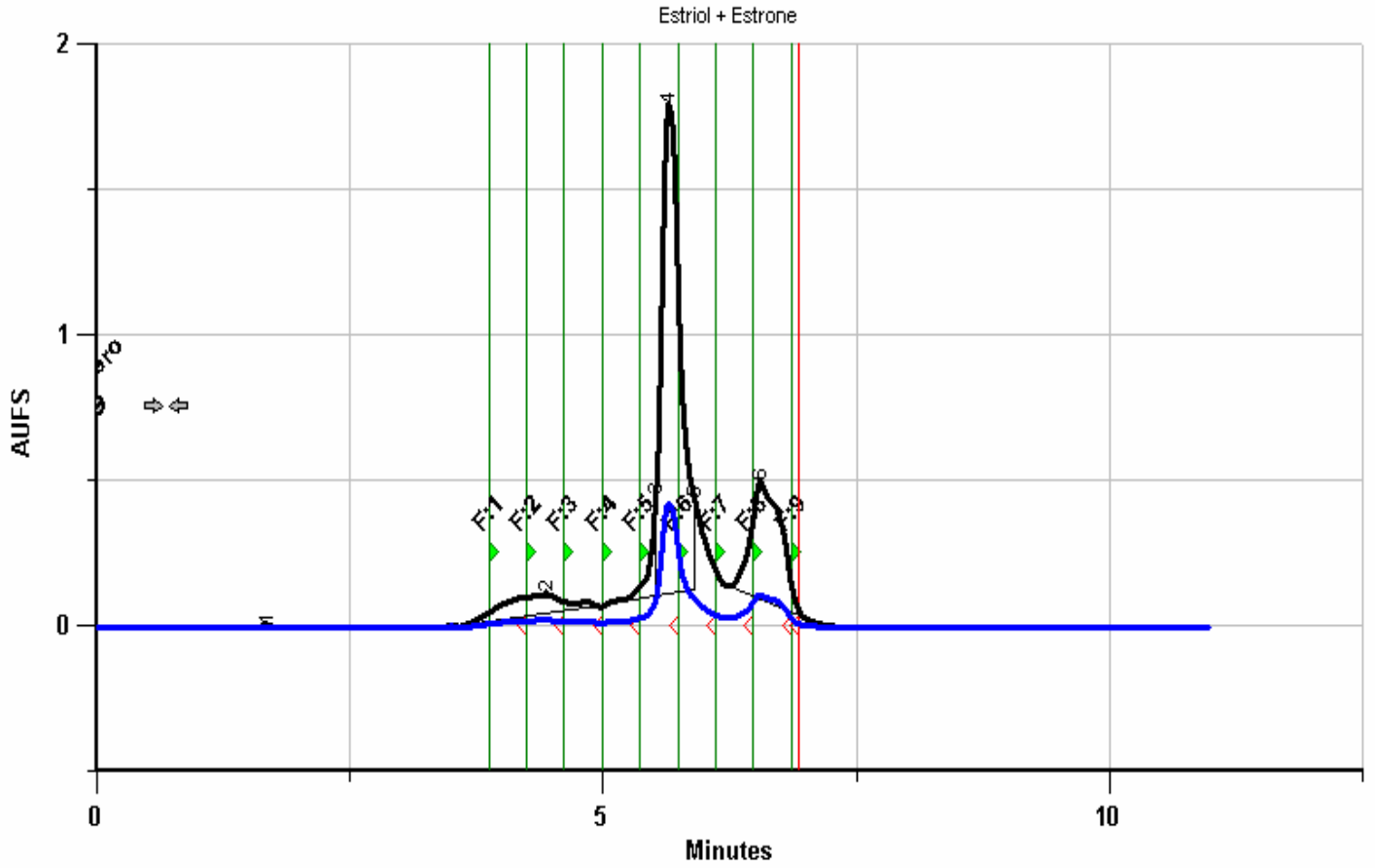
The above list shows the sequence of steps involved in the automated operation of the cLC system. Without operator intervention the system will chromatograph a series of samples under NP conditions, then stabilize and equilibrate to run samples under RP conditions. One can even switch back to NP and run additional Biotage FLASH columns that were prepared while RP samples were running.

Performance Data: Biotage FLASH 40+M



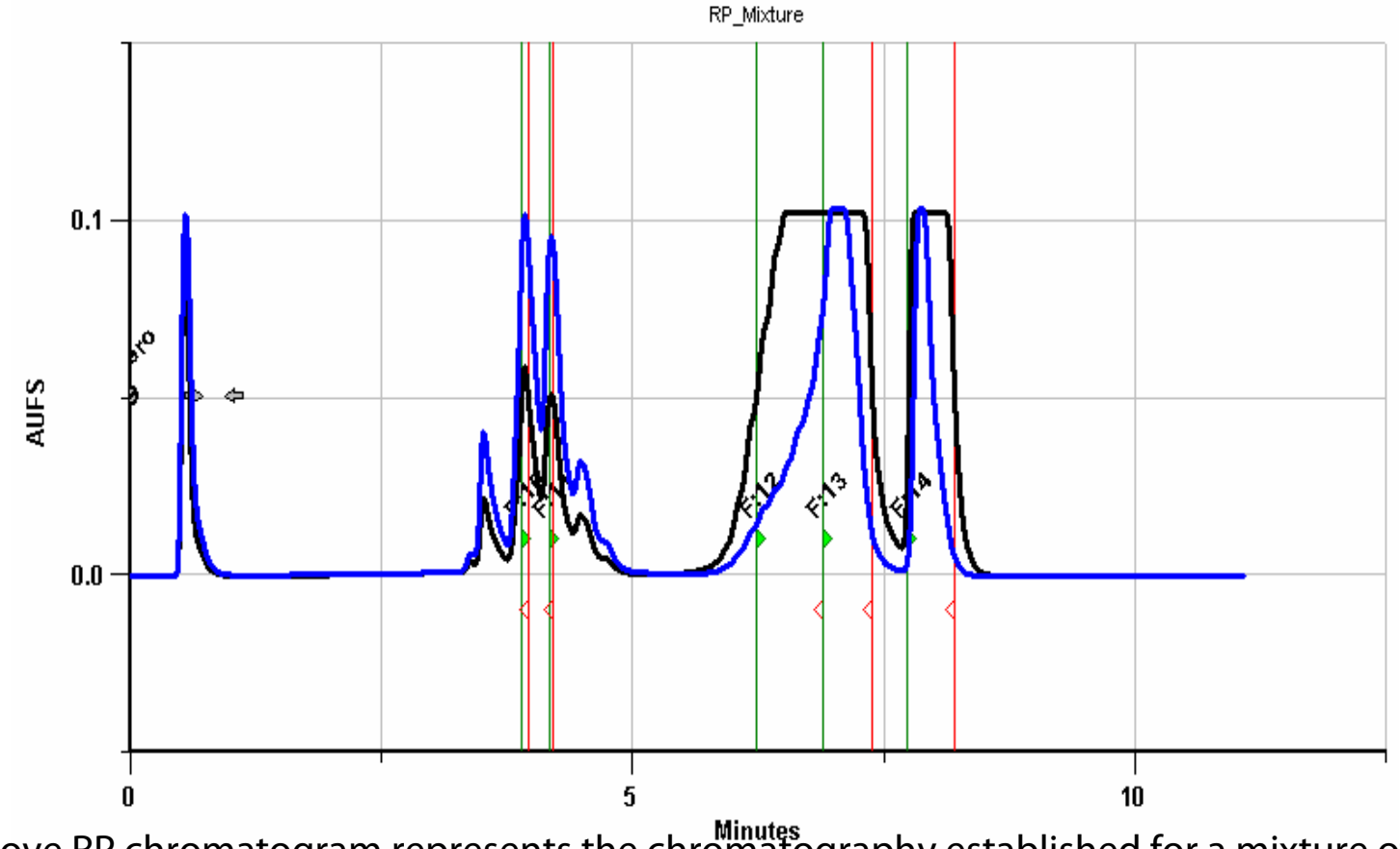
The above NP chromatogram was produce from the Biotage FLASH 40+M column. The column was manually loaded with 15 mls of solution (THF/Dioxane) in which 445 mg of sample was dissolved (Estrone: 318 mgs and Estriol 127 mgs), flow rate of 30 mls/min, Hexane/IPA (90:10)

Performance Data: Betasil Silica



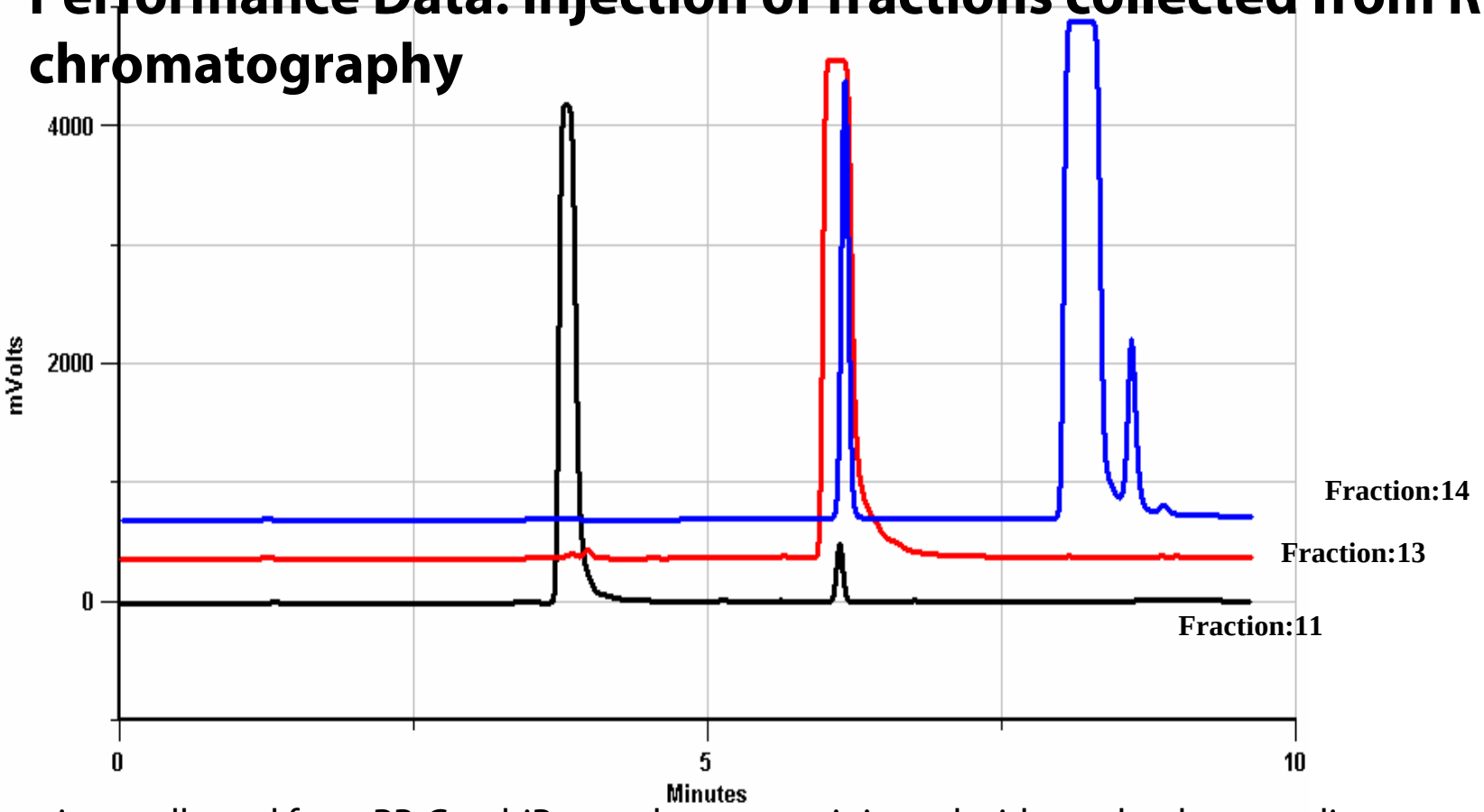
The above NP chromatogram was achieved with a Betasil silica column 20x150 mm, 5µ, 100A. The sample (156 mg) was Estrone (96 mg) and Estriol (60 mg) in a 4 mL injection on column. An isocratic run of Hexane/IPA (98:2) chromatographed the sample in less than 10 minutes.

Performance Data: RP-CombiPrep Pro C18



The above RP chromatogram represents the chromatography established for a mixture of three components (Caffeine 102 mgs, p-hydroxy benzoic acid ethyl ester 68 mgs, biphenyl 69 mg: total on column injection of 239 mg in a 4 mL injection volume. A gradient 5-95% Water:ACN was run over 7 minutes. Fractions collected were injected under analytical conditions to determine the components of the fractions collected.

Performance Data: Injection of fractions collected from RP-chromatography



Fractions collected from RP-CombiPrep column were injected without dry down on-line onto an analytical system. The samples were run on a Sulpelco 4.6x 150 mm, 5 μ, 100A, 1.5 mL/min, 5-95% Water:Acetonitrile over 7 minutes, 40 uL of each fraction was injection onto the column. The overlay shows the separation of the three components that were injected onto the semi-preparative column and fractions collected.

Summary

- The cLC system offer the best of both chromatography worlds by automating the process for both RP and NP-chromatography on one system
- The cLC system is capable of handling up to 5 columns (NP & RP) with one bypass for quick change over from the two type of chromatography
- The cLC system can accommodate the disposable NP-chromatography columns (sample load <15gm, based on △CV) (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

Summary, cont.

- Although in most NP chromatography run isocratic conditions are used we believe that the cLC system offers the advantage of improving the NP 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 an analytical system 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 checking not only of the RP fractions but the NP fractions as well

Conclusion

The cLC system offers researchers the capabilities of both RP- and NP-chromatography in one system for about the same investment made for one preparative HPLC system. Its footprint is only slightly larger than a preparative system and many of the components could be placed above minimizing the bench spaced used. The ability to switch the solvent and column selectors valves to any position without having to move in increments (clockwise or counterclockwise motion) deters from solvent incompatibilities and column problems. Since the system is made up of true HPLC components one is capable of running all modes of chromatography low- medium- and high-pressure type columns depending on the application. With this in mind the cLC system really represents a merger of two systems in one HPLC and off-line low pressure chromatography with numerous features and benefits not available in the lower pressure chromatography instruments.