

DEVELOPMENT AND EVALUATION OF A HOLISTICALLY DESIGNED ANALYTICAL SUPERCRITICAL FLUID CHROMATOGRAPHY SYSTEM

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INTRODUCTION

In the past decade, Supercritical Fluid Chromatography (SFC) has demonstrated great promise as the choice of chromatography for chiral compounds, and as a general replacement technique for normal phase liquid chromatography (NPLC). Compared to traditional normal phase HPLC, SFC is on average 3 to 10 times faster. Using inexpensive CO₂ and a polar modifier such as methanol as the mobile phase, SFC is more cost-effective and environmentally friendly by reducing the consumption and disposal of organic solvents. Widespread adoption of analytical SFC has been hampered by instrumentation which does not perform to the standards established by modern HPLC systems. Poor signal to noise ratio, poor retention time and injector reproducibility, overall lower reliability, as compared to HPLC, are often described as major limitations. In order to bring this separation technique to a performance level desired by today's modern laboratory, a holistic system must be designed from the ground up.

The majority of system components of an analytical SFC system are the same as any LC system; pump, autosampler, detector, software solutions, along with some components unique to SFC. Although similar to LC, the design challenges for each component must be considered and each component must be designed so that the system as a whole performs at a high level. We present here some of the design challenges along with their solutions and an basic evaluation of instrument performance, at a system level. This poster presents the new Waters ACQUITY UPSFC system (figure 1), a holistically designed analytical SFC system consisting of a UPSFC Binary Solvent Manager, UPSFC Sample Manager, UPSFC Photodiode Array Detector, and UPSFC System Manager.



Figure 1. ACQUITY UPSFC SYSTEM

UPSFC BINARY SOLVENT MANAGER

SFC systems, like all LC systems, require pumps to deliver solvents to the column. Unlike HPLC, the pumping of a compressible fluid (liquid CO₂) presents unique challenges. The incoming liquid CO₂ and pump heads themselves need to be cooled. The UPSFC BSM uses a thermo-electric device on each pump actuator to independently and directly control the temperature of each pump head and provide pre-chilling of the CO₂. This thermo-electric device is a solid state component that uses no moving parts, provides high reliability (there are no service requirements for the cooling system), and eliminates the possibility of contaminating the fluid stream. The thermo-electric device is located behind the pump head, meaning the cooling mechanism is out of the way and invisible to users. Unlike previous generations of SFC systems, that used external chillers and pre-pumps to provide cool liquid CO₂, a pre-chiller integrated into the pump head means that the ACQUITY UPSFC does not require additional bench space to cool the CO₂. An additional, yet significant, benefit of controlling the temperature of the incoming CO₂ and the pump heads in this way is a more precise mass flow control regardless of ambient conditions, providing enhanced retention time control. This advanced cooling system as well as unique software algorithms allow the UPSFC BSM to deliver this highly compressible mobile phase accurately and precisely.

Along with pumping CO₂, the pump must be able to deliver a co-solvent. The UPSFC BSM uses state of the art, well proven ACQUITY UPLC technology to deliver co-solvent. To aid in method development, users are able to choose from up to 4 co-solvents via a solvent selection valve.

An extended flow rate range (up to 4 mL/minute) provides the opportunity to do fast chromatography with a wide variety of column dimensions. The UPSFC BSM incorporates improved diagnostics over conventional SFC systems. No manual intervention is required, with all diagnostics just a mouse click away.

This pump provides reproducible separations in either gradient or isocratic modes as can be seen in figure 2, a gradient separation of 18 compounds. Retention time reproducibility for all peaks is less than 0.50% RSD (table 1) making this system suitable for routine laboratory use.

	3-Benzoylpyridine	Cholecalciferol	6-Hydroxyflavone	4-Nitroaniline	Caffeine	Theophylline
Mean	1.19	1.48	1.77	2.18	2.31	2.77
Std. Dev.	0.005	0.004	0.003	0.003	0.003	0.002
%RSD	0.38	0.269	0.195	0.158	0.121	0.075
	Carbamazepine	Estradiol	4,4'-Biphenol	Cortisone	Prednisone	Acetaminophen
Mean	3.39	3.60	3.70	4.29	4.57	4.86
Std. Dev.	0.002	0.003	0.003	0.003	0.003	0.002
%RSD	0.063	0.081	0.072	0.064	0.059	0.044
	Sulfisoxazole	Hydrocortisone	Sulfamethizazole	Prednisolone	Sulfaphenizole	4-Aminophenyl Sulfone
Mean	5.02	5.30	5.49	5.65	5.82	7.19
Std. Dev.	0.003	0.003	0.003	0.003	0.003	0.004
%RSD	0.062	0.057	0.059	0.052	0.06	0.055

Table 1- UPSFC Retention time reproducibility (n=6)

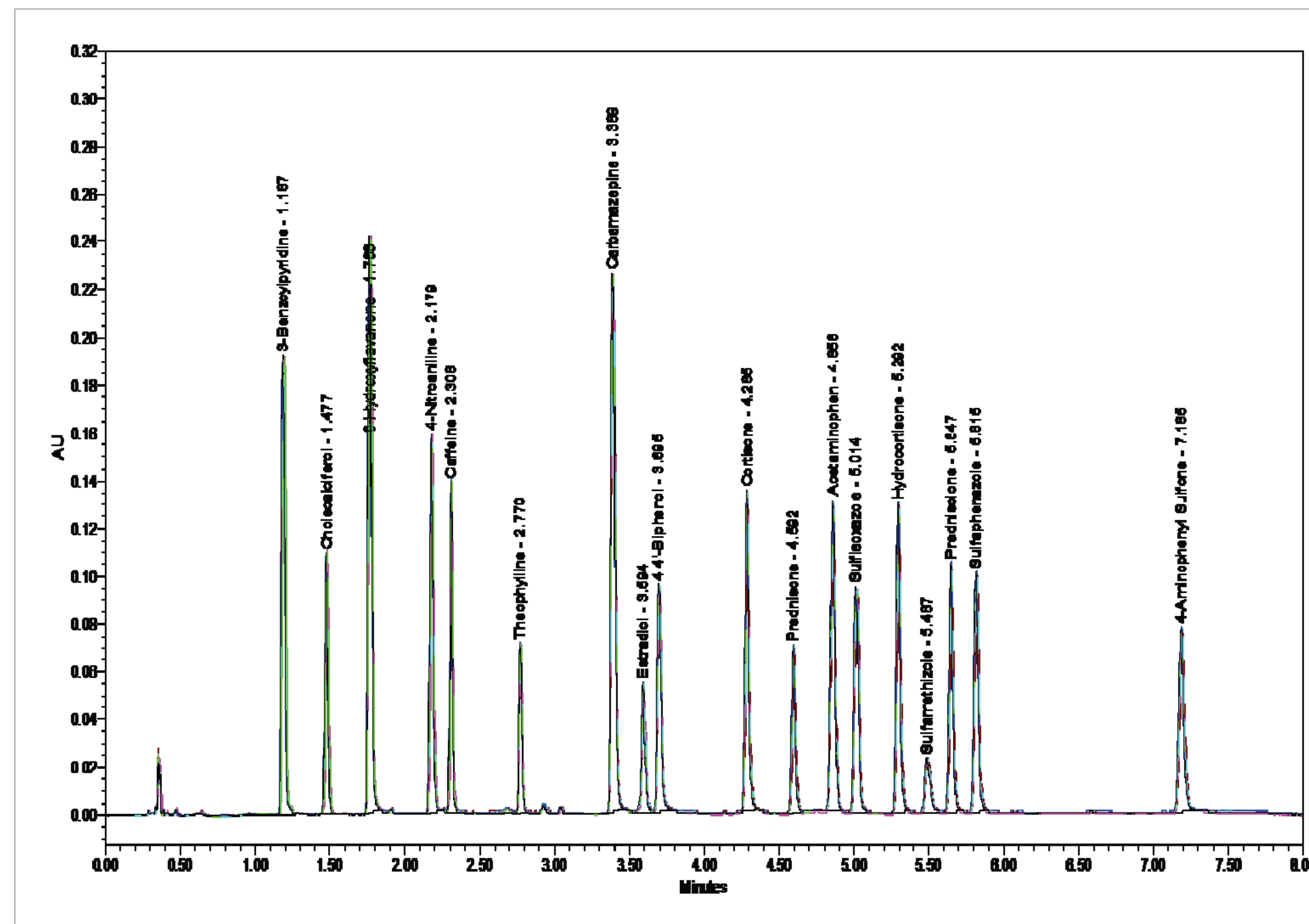


Figure 2. ACQUITY UPSFC gradient reproducibility performance. Gradient 2-16% Methanol over 8 minutes, 2.1 X 150 mm VIRIDIS Hybrid 1.7 μm @ 1.2 mL/min. Overlay of 6 injections.

UPSFC SAMPLE MANAGER

Sample introduction is accomplished via the ACQUITY UPSFC Sample Manager. This autosampler is a filled loop type, meaning that the sample loop is part of the mobile phase flow path. The challenge with this device is to provide accurate, linear, and most importantly, precise injections over a range of injection volumes. Because the sample loop contains mobile phase (liquid CO₂ and co-solvent) under system pressure, as the loop is moved to the load position, the CO₂ contained within reverts back to the gas phase. If the gaseous CO₂ is allowed to escape the system uncontrolled, sampling inaccuracies can result. To overcome this issue the UPSFC SM incorporates a vent valve which directs the expanding gas and residual co-solvent to waste. Following this venting, the vent valve is taken offline and sample aspiration takes place without compromise. Injector precision is equal or better to traditional HPLC systems and is shown in table 2 (4 compound isocratic separation). Injector linearity was also good, with R² values above 0.999 across the entire range of the 5 μL loop (figure 3).

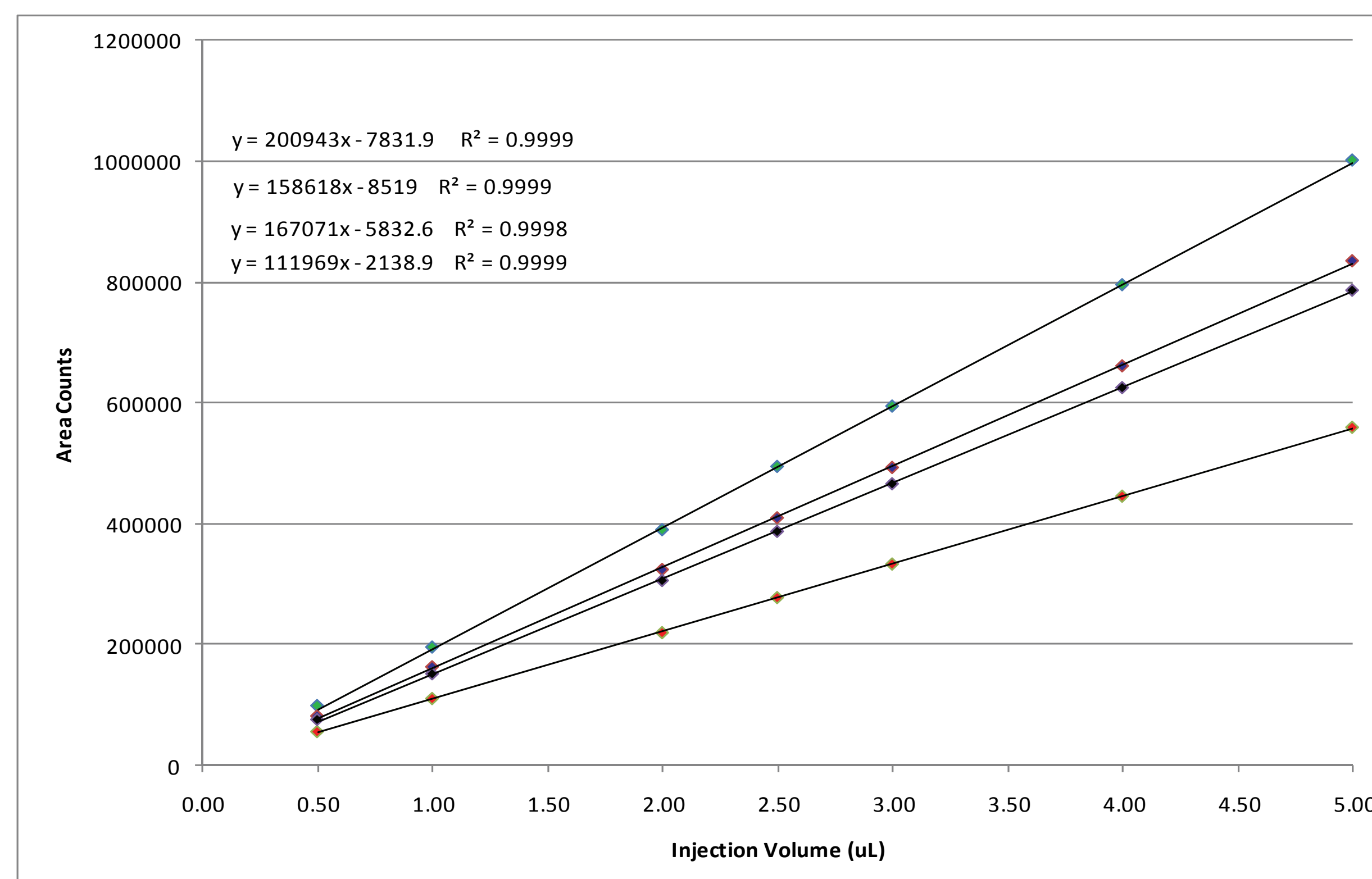


Figure 3—UPSFC Sample Manager Linearity

Loop Volume	Injection Volume	%RSD Peak 1	%RSD Peak 2	%RSD Peak 3	%RSD Peak 4
5	0.5	0.51	1.31	1.74	0.80
	1.0	0.67	0.41	0.80	0.89
10	2.0	0.27	0.32	0.36	0.41
	2.5	0.36	0.47	0.32	0.53
20	3.0	0.29	0.22	0.32	0.27
	4.0	0.29	0.40	0.33	0.22
20	5.0	0.22	0.25	0.25	0.19

Table 2 — UPSFC Sample Manager Precision

UPSFC PHOTODIODE ARRAY DETECTOR

The primary detector for the UPSFC system is the UPSFC Photodiode Array Detector. One of the major challenges for detectors in SFC is an enhanced pressure capability. Unlike HPLC, where the pressure drop is across the column, in SFC the pressure drop is across the system in order to keep the CO₂ in its liquid state. To address this, high strength, shaped fused silica lenses were incorporated into the flow cell. This is an improvement over previous generations of SFC detectors that typically used sapphire lenses which provided strength but reduced energy throughput at lower UV wavelengths. The UPSFC PDA flow cell is rated to 6000 psi, has a 10 mm path length, and provides high energy throughput across all wavelengths. Typical PDA features such as peak purity and library matching are able to be used without compromise. This new detector design is less susceptible to environmental conditions and has greater baseline stability due to the incorporation of thermal management along with updated electronics for better noise performance. Other enhanced features include lamp and flow cell ID to allow for storage of data and traceability.

Figure 4, an impurity profile of Estradiol, demonstrates the detector sensitivity showing multiple peaks detected at levels of 0.01% (based on area). An impurity at the 0.05% level was easily detected at a signal to noise level of 17:1. The measured noise for this assay was ~17 μAU.

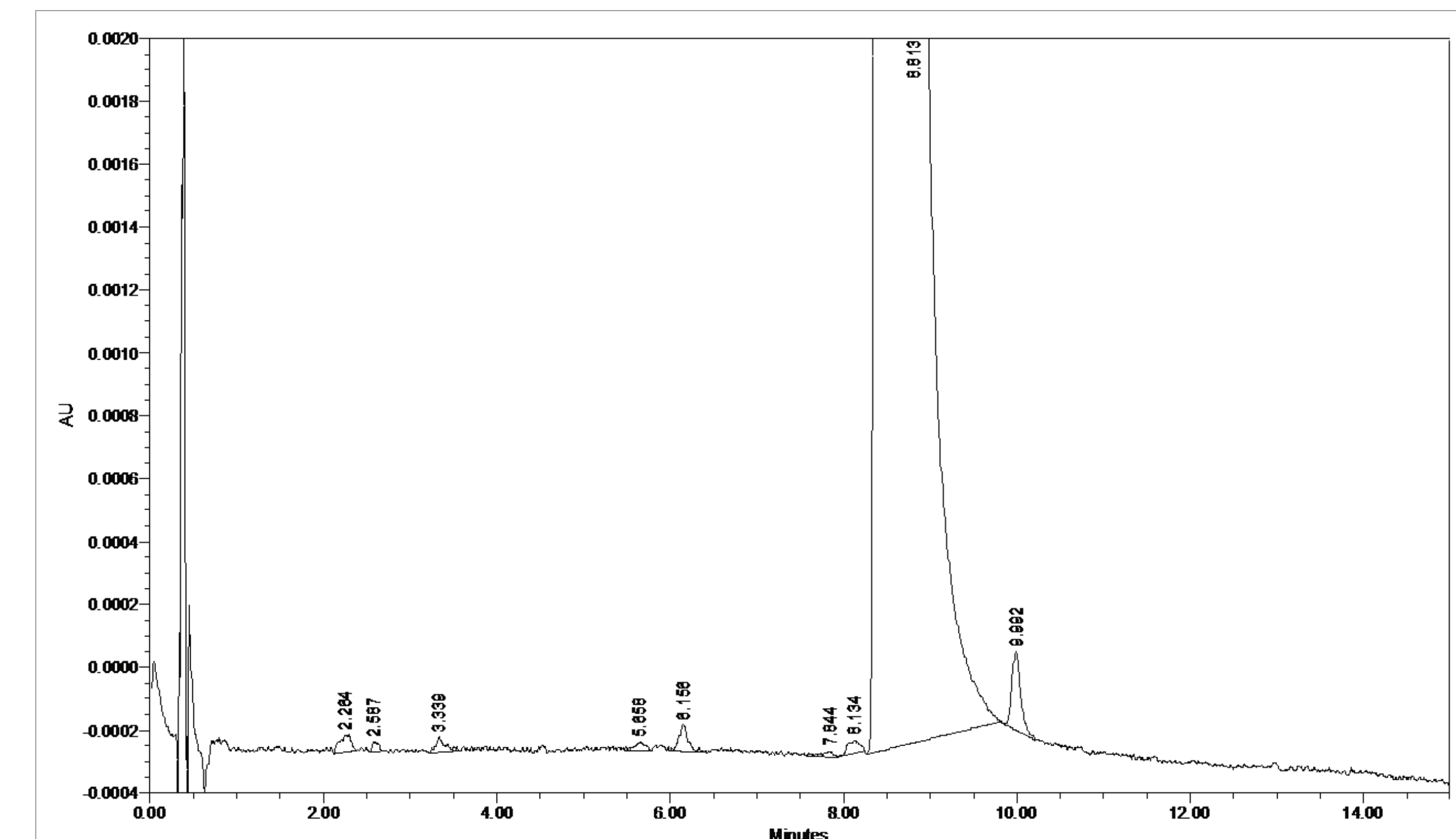


Figure 4 - UPSFC impurity analysis of Estradiol

UPSFC SYSTEM MANAGER

The UPSFC System Manager houses system components unique to SFC. Included in this module are CO₂ management, a pressure control system that includes an active back pressure regulator, and the UPSFC Sample Manager vent valve. With the CO₂ management system, users can shutoff the CO₂ supply and vent the system for safe and easy shutdown and maintenance. Additional features include CO₂ diagnostics to inform users when the supply tank is empty and the ability to deliver a standard solvent to the pump for robust diagnostics when required.

The heart of the UPSFC System Module is the Active Back Pressure Regulator. This device ensures the CO₂ stays liquid at the desired back pressure. This newly designed back pressure control system was engineered from the ground up to provide high resolution pressure control at UPSFC flow rates and pressures. The controls for the regulator are tuned to maximize pressure stability, while minimizing pressure ripple and associated baseline noise. The regulator has accessible components for quick and easy maintenance to keep the system running at optimal performance levels. The ability of this device to finely control the system backpressure enables all other system components to be operating at the highest performance levels possible.

SUMMARY

Waters new ACQUITY UPSFC system is a holistically designed analytical SFC system consisting of a UPSFC Binary Solvent Manager, UPSFC Sample Manager, UPSFC Photodiode Array Detector, and UPSFC System Manager. Each component has been designed to provide optimal analytical performance and provide users with results that were difficult to achieve with the previous generation of analytical SFC instruments.

UPSFC Binary Solvent Manager

- CO₂ pre-chiller integrated into the pump head.
- Thermo-electric device on each pump actuator independently and directly controls the temperature of each pump head.
- Multiple co-solvents to choose from aiding in method development and method switching.
- Extended flow rate range of 4 mL/min total flow.
- Improved diagnostics over conventional SFC systems. No manual intervention required.
- Excellent retention time reproducibility in both gradient and isocratic modes.

UPSFC Sample Manager

- Filled loop type autosampler.
- Secondary vent valve directs decompressed CO₂ and co-solvent to waste.
- Highly reproducible injections and excellent linearity.

UPSFC Photodiode Array Detector

- Reduced environmental sensitivity and greater baseline stability due to the incorporation of thermal management.
- Lamp and Flow Cell ID to allow for storage of data and traceability.
- Updated electronics for better noise performance.
- Flow Cell rated to 6000 psi.
- Shaped high strength fused silica lenses used instead of Sapphire to give better energy at the lower UV wavelengths.

UPSFC System Manager

- CO₂ shutoff and system venting for safe and easy system shutdown and maintenance.
- Provides features for CO₂ diagnostics controllable through Empower and the ACQUITY Console
- UPSFC Back Pressure Regulator designed for high resolution control of the system pressure.
- Tuned for stability to minimize pressure ripple.
- Provides easy access to replaceable components.