Reprint from

Daily Biotech Updates... www.genengnews.com

GENETIC ENGINEERING GEN NEWS

Volume 23, Number 1
January 1, 2003

BIOPROCESSING

Applying "Six Sigma" to Chromatography

Tutorial:
Cutting Costs Through Process Improvements

Kevin Henretta, John Walker,
and Lou Bellafiore

By using Six Sigma to systematically identify and eliminate the sources of unnecessary variability in preparative liquid chromatography (LC) processes, it is possible to dramatically cut the cost of today's mainstream LC approaches while at the same time improving product quality and providing insurance against potentially catastrophic process failures. This article will concentrate on the need for, and creation of, highly accurate and reproducible LC delivery systems in order to achieve world-class production-scale LC performance resulting in lower production costs.

Six Sigma Overview

Process capability is adversely affected by variation, which, in turn, increases costs, defectivity, and cycle times. The Six Sigma approach (a technique developed by **Motorola** in 1986 to achieve gains in productivity) provides a systematic methodology for identifying root causes of variation and applying the appropriate "fix" to them.

World-class performance is considered to be 3.7 defects per million opportunities; this is the so-called Six Sigma Level of Quality. Mathematically, this relates to how well key parameters can be controlled to consistently fall within their respective specification limits. For optimal results, a key parameter must be tightly distributed (low standard deviation) and targeted on the mean of the design specification.

Six Sigma suggests that money should be directed to process improvements that guarantee, by design, that good product is produced rather than into more inspection and testing of products (*Figure 1*).

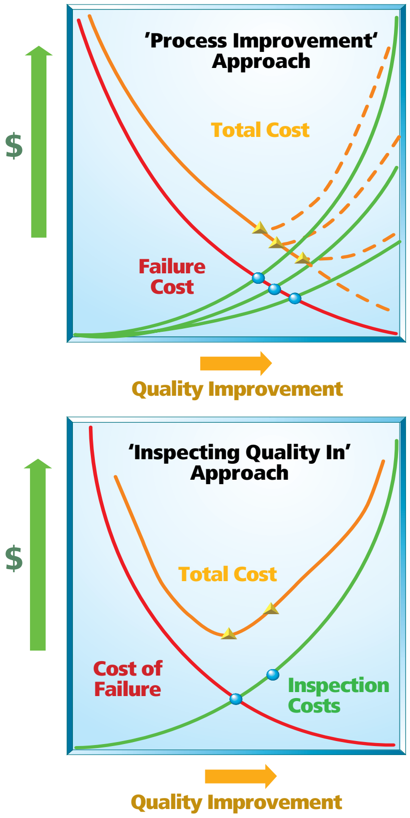


Figure 1. The Six Sigma approach to quality improvement, "Process Improvement" versus the traditional "Inspecting Quality In." Controlling a process and reducing manufacturing variables will drive down overall costs and improve yields. Increasing inspection will prevent noncompliant product from reaching customer, without eliminating root causes for failure.

For many years, efforts to improve

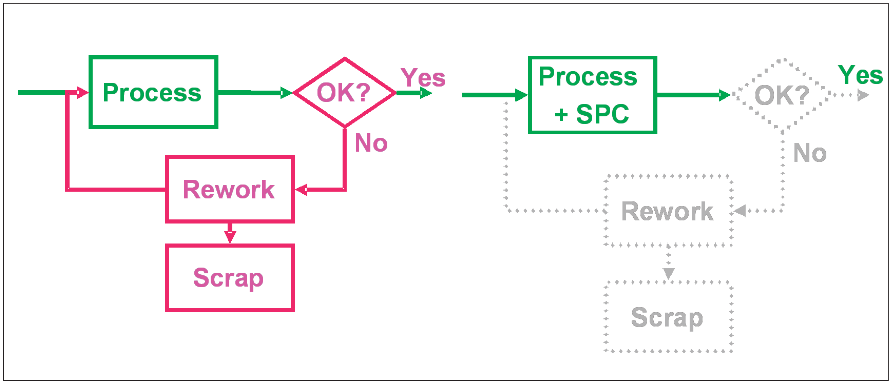


Figure 2: The Hidden Factory includes the cost of rework and of scrap. This Hidden Factory can be virtually eliminated with improved process control.

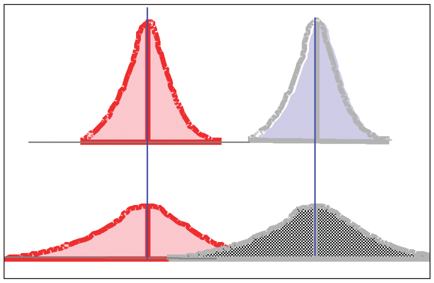


Figure 3. Band broadening is counter-productive to the discrimination of closely separated entities. Any band overlap rapidly erodes the yield and quality of the product.

product quality focused upon identifying and eliminating faulty products before they reached the customer. Increasing the intensity of the final inspection was a proposition of diminishing returns and a break-even point was reached when the cost associated with the customer receiving faulty goods balanced the cost of inspection.

These attempts to "inspect-in quality" created significant inspection and rework loops and scrap, which absorbed resources that could otherwise be used in producing more product.

This extra, non-value-added work can be considered to be an extra "Hidden Factory." In addition, the efficiency of inspection schemes, particularly those involving human judgment, is relatively poor and "escapees" are common. The significant proportion of "marginal pass" material allowed by this inspection approach also compromises the robustness of downstream processes and products.

To achieve world-class production, it is necessary to understand and control the variability of the processes by which products are produced, thereby guaranteeing that only fault-free product is created the first time. This eliminates costs and delays associated with the nonvalue-added Hidden Factory and it greatly improves product quality and reliability.

Applicability to LC

Chromatographic resolution is severely compromised by band broadening (lower plate count, higher HETP), which can be traced to the variability of a few key parameters.

A small increase in variability can produce a disproportionately large degradation in resolution, particularly between closely related compounds. A small improvement in resolution can dramatically affect profitability. Removal of the variability that causes band broadening invariably pays for itself several times over in improved first-pass recovery (cost) and purity (quality). This, in turn, eliminates Hidden Factory operations such as multiple sample assays, blending, and re-processing.

Column-based contributors to variability include unstable, irreproducible beds due to channeling and generation of voids, nonoptimum flow distribution across the column, column dead volume, and bed fouling. This article will focus on control-system-based contributions to variability such as inaccurate mobile-phase gradient creation, inaccurate mass flow rate control, and lack of temperature control. Other contributors include lot-to-lot inconsistency of the stationary-phase resin and the inherent variability of the crude feedstock.

LC System Design Derivation

Through identifying and eliminating the excessive variability of current LC system designs we can derive the design guidelines for a world-class LC system *(Figure 4)*. This basic design is representative of much production-scale equipment in use today.

Key features include premixed, isocratic mobile-phase, crude feedstock delivery through a main pump, and a fixed-bed column. Such a system is seductively simple and self-explanatory. Unfortunately, this is a case where keeping it simple can really hurt, particularly when working with high-value products.

The shortcomings of this basic system were explored using Failure Mode and Effect Analysis (FMEA), one of the Six

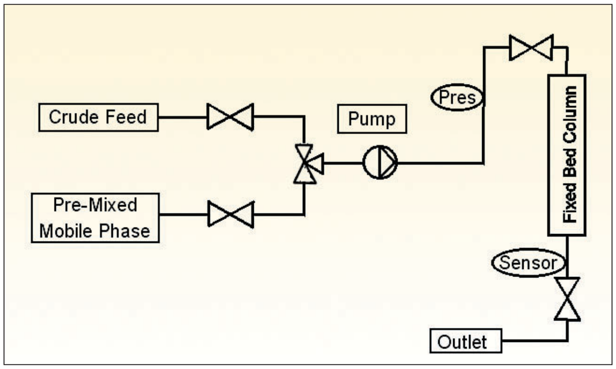


Figure 4. A basic preparative LC system.

A world-class chromatography system.

Sigma suite of tools. FMEA is a rigorous proactive approach to designing processes and/or products. By identifying and quantitatively rank-ordering potential failure modes and their root causes, it is possible to prioritize the implementation of the corrective actions designed to minimize their impact (effect).

Each of the different sub-systems within an LC system is analyzed and quantified using FMEA. The failures focus specifically on a scenario where several buffers are used in the LC process. In an FMEA the severity, the probability of occurrence, and the probability of escaping detection of each potential failure is attributed a value on a 1–10 scale. The product of these creates a Risk Priority Number (RPN).

Corrective actions are then recommended based upon root cause analysis and a new RPN assigned to quantify the impact of these recommended actions. In the separation of closely related species it can be shown that an accurate linear gradient will produce better quality and process economics than step gradients or isocratic runs. A part of the FMEA associated with one of the blocks, gradient generation, is shown *(Table 1)*.

Highly reproducible and accurate gradients have been virtually impossible to produce in process-scale chromatography, but a world-class system must be capable of producing such full-scale process-scale gradients—superior even to analytical HPLC (which itself delivers pulses of each component of the gradient to the column).

To add gradient capability to basic LC equipment, suppliers have tended to add multiple pumps and flow meters *(Figure 6)*. These systems have proven to have a limited (±3–5%) gradient accuracy/fluctuation "sweet spot," outside of which performance degrades considerably. The exact chemical blend sent to the column is never known or documented. As a result many users have abandoned linear gradients. They have had to "dumb down" their process to use only step gradient or isocratic LC.

TechniKrom's (Evanston, IL) world-class preparative chromatography systems are based on point-of-use, automated, computer/PLC-controlled mixing and documentation *(Figure 5, and photo above)*. The systems are designed to routinely deliver ±0.1% gradient accuracy and reproducibility. The exact composition of the blend sent to the column is measured and recorded via 21 CFR Part 11 capable software.

Also, the gradient is controlled and documented by an intelligent feedback control. This degree of adaptive control of the process ensures highly controlled methods; methods that are directly portable from skid to skid and readily scalable from small methods development through pilot-scale and into full-scale manufacturing.

Without such tight, adaptive control of gradient, each scale-up transition step entails the extensive re-engineering and revalidation of both the process and equipment—expensive in both time and money. Incorporating such adaptive control capabilities into a process eliminates the need for much inspection and testing, i.e. hidden (assay) factory elimination.

Such a system provides a measurement of the actual chemical content of the blended mobile phase just before it is introduced to the column to guarantee that the gradient or isocratic blend meets the programmed set-point before it is ever released to the column.

Any "out of spec" mobile phase is diverted to waste until the blend is back "in spec." This guarantees that the high-value crude product will never see incorrect mobile phase, preventing potentially catastrophic product loss.

The sampling and display rate in a world-class system is one data point per second and its smooth gradient truly is a smooth gradient, not a computer simulation *(Figure 7)*. To get this level of resolution with a traditional system it is necessary to connect the detector output directly to an analog chart recorder because in a traditional system data is

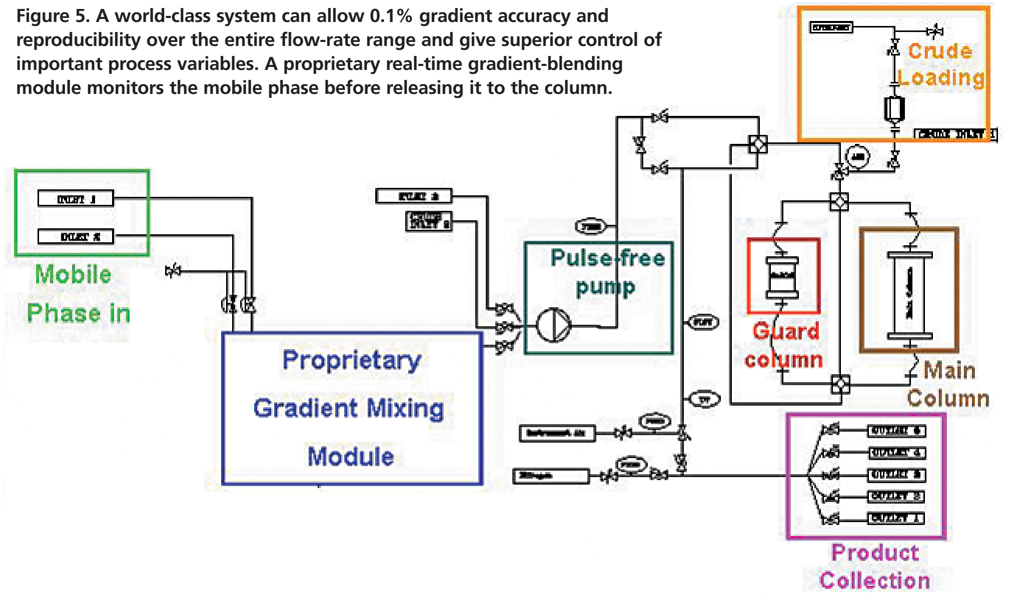
Table 1. Partial Failure Mode Effect Analysis of the Gradient

Process Function Requirement	Potential Failure Mode(s)	Potential Effect(s) of Failure	SEV	Potential Cause(s)/ Mechanisms of Failure	OCC	Current Process Controls	DET	RPN	Recommended Actions	SEV	OCC	DET	RPN
To accurately pre-mix, precondition and record the appropriate formulation for use in the mobile phase of LC.	Inaccurate/ Incorrect formulation	A) Compromised product quality due to compromised separation efficiency. B) Increased product cost due to reduced recovery and/or re-work. C) Program/delivery delays due to corrective actions required. D) Regulatory delays due to inability to adequately control key process parameters.	9	a) Operator error in identifying, calculating and/or measuring ingredients.	6	Two signatures required regarding critical measurements and calculations.	8	432	Automated blending system with accurate metering and feedback, controlled by comprehensive, self-checking software. Documentation/records keeping automated also.	9	2	2	36
				b) Incorrect ingredient delivered by supplier and not detected at incoming QC	3	Supplier audit plus C of C.	3	81	Automated blending system with ingredient properties cross checked via software controls.	9	3	1	27
				c) Measurement instruments failure.	3	Periodic calibration.	3	81	Automated blending system with instrument cross checks via software.	9	3	1	27
	Incorrect mobile phase pre-conditioning.	Sub-optimal separation efficiency due to temperature variance => Band broadening.	5	Difficulty in controlling temperature in large tanks.	6	Temperature controlled heaters in storage tanks.	4	120	Blend lower volumes of concentrated mobile phase. Use automated blending system with closed loop temp control in high efficiency mixing loop.	5	2	1	10
	Incomplete mixing of mobile phase ingredients.	Inconsistent separation accuracy in LC. Band broadening.	5	Difficulty in homogeneously mixing a large tank of liquids.	5	Paddle or magnetic stirrer in tanks.	5	125	Prepare lower volumes of concentrated mobile phase. Use automated blending system with closed loop temp control in high efficiency mixing loop.	5	2	1	10
	Time inefficiency from LC downtime while batch-blending mobile phase.	Sub-optimal throughput.	5	Serial approach to blending and LC processing	10	Not controlled.	10	500	Duplicate pre-mixing process tank farm. Mix in one set while using the other set for LC.	Too expensive plus labor and space consuming.			
									Auto blend lower volume of ingredient concentrates while performing LC from same tank.	5	2	1	10
	Accuracy, labor and details inefficiencies of manual documentation of key parameters.	Delays or extra costs from more time consuming process verifications and record maintenance.	4	Lack of automated data collection and processing.	10	None	10	400	Automated documentation of key parameters via auto-mixing equipment.	4	2	1	8
To, on-line, accurately pre-mix, pre-condition and record the optimal gradient(s) for use in the mobile phase of LC.	Sub-optimal mobile phase strategy selected. (continuous gradient ALWAYS outperforms isocratic or step gradients in challenging separations.)	A) Compromised product quality due to compromised separation efficiency. B) Increased product cost due to reduced recovery and/or re-work. C) Program/delivery delays due to increased throughput time and corrective actions required. D) Regulatory delays	7	a) Isocratic approach used	10	None	10	700	1) Provide education regarding current state of the art of automated gradient creation systems. 2) Incorporate: a) automated gradient blending with accurate compositional feedback, b) comprehensive, self-checking control software, and c) Automated recording of exact mobile phase composition.	7	1	1	7
			5	b) Only step gradients available due to belief that gradient approach is impossible to control.	10	None	10	500	----- ditto -----	5	1	1	5
	Sub-optimal continuous gradient delivered to LC column.	----- ditto -----	5	a) Sub-optimal continuous gradient blending accuracy.	10	Blending with only volume-based feedback control.	10	500	Incorporate automated gradient blending with accurate compositional feedback (NIR, Conductivity or similar).	5	1	1	5
			9	b) Operator error in identifying, hooking up, calculating and/or measuring ingredients for mobile phase.	2	Two signatures required regarding critical operations, measurements & calculations.	6	108	Feedback system on Fully automated skid detects incorrect chemistry via NIR, Conductivity or similar measure.	9	2	1	18
			9	c) Incorrect ingredient delivered by supplier and not detected at incoming QC	3	Supplier audit plus C of C.	8	216	Feedback system on Fully automated skid detects incorrect chemistry via NIR, Conductivity or similar measure.	9	3	1	27
			9	d) Incorrect mixture delivered from pre-mixing stage.	3	None	7	189	Feedback system on Fully automated pre-mixing skid detects incorrect chemistry via NIR, Conductivity or similar measure.	9	3	1	27
			9	e) Measurement instruments failure.	3	Periodic calibration.	7	189	Fully automated skid incorporating instrument cross checks via software.	9	3	1	27
	Incorrect mobile phase pre-conditioning.	Sub-optimal separation efficiency due to temperature variance => Band broadening.	5	Difficulty in controlling temperature in large tanks.	6	Temperature controlled heaters in storage tanks.	4	120	Either: a) Pre-condition ingredients and run LC equipments in a temperature controlled room, or b) Use heat exchangers on LC equipment to augment the above.	5	2	1	10
	Incomplete mixing of mobile phase ingredients.	Inconsistent separation accuracy in LC. Band broadening.	5	Difficulty in homogeneously mixing a large tank of liquids.	5	Paddle or magnetic stirrer in tanks.	5	125	Use high efficiency mixing loop: a) for lower volumes of concentrated mobile phase ingredient blending, and b) on LC skid itself	5	2	1	10
	Time inefficiency from LC downtime while batch-blending mobile phase.	Sub-optimal throughput => lost yield and increased cycle time.=> Increased product cost	6	Serial approach to blending and LC processing	10	Not controlled.	10	600	Duplicate pre-mixing process tank farm. Mix in one set while using the other set for LC.	Too expensive plus labor and space consuming.			
									Auto in-line blend lower volume of ingredient concentrates while performing LC from same tank.	5	2	1	10
	Accuracy, labor and details inefficiencies of manual documentation of key parameters.	Delays &/or extra costs from more time consuming process verifications and record maintenance.	4	Lack of automated data collection and processing.	10	None	10	400	Automated documentation of key parameters via auto-mixing equipment.	4	2	1	8

SEV = severity of consequences of failure *OCC = probability of occurrence* *DET = probability of evading detection* sampled at six points per minute and is averaged (heavily damped) to create the illusion of smooth control. It may look smooth and controlled, but it isn't.

The underlying raw signal is often swinging around the displayed mean by ±3–10% or more. In the separation of close species the difference in gradient required to resolve them is often 1% or less. If the intrinsic underlying "ripple" in the gradient is at best ±3% then that equipment cannot efficiently resolve the two components.

Figure 5. A world-class system can allow 0.1% gradient accuracy and reproducibility over the entire flow-rate range and give superior control of important process variables. A proprietary real-time gradient-blending module monitors the mobile phase before releasing it to the column.



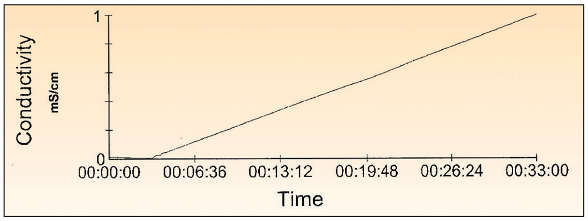


Figure 7. Actual gradient generated with a world-class chromatography system, sampled at one data point per second.

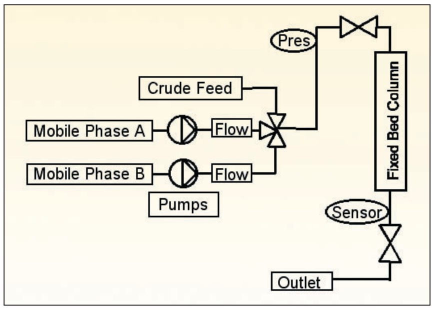


Figure 6. Popular Gradient LC System

Case Study

Applying representative costs associated with a real therapeutic peptide, currently in clinical trials, gives an indication of the cost savings associated with implementing key recommended actions. These key actions were chosen from the FMEA as the most significant toward achieving a world-class system.

The goal is to reduce overall operational costs by eliminating the real and hidden costs from the process. By improving the process through increasing equipment performance, the cost of poor quality will be reduced, thus reducing all of the other costs on this list.

In our case study, a producer of a therapeutic peptide needed to produce 8.4 kg of a peptide per year at a cost of approximately $2,000 per gram, for a total production cost of $16.8 million per year.

Table 3 lists some of the traditional system's major inefficiencies that were identified in the FMEA study, the cost impact of these inefficiencies, the recommended action to eliminate or minimize them, and the cost savings of implementing these actions.

The inability to form accurate gradients was conservatively estimated to cause the total process throughput to run at 80% of ideal. Typically the eluent containing the lost 20% will be pooled with other similar material and reprocessed for a second LC pass. Assuming an 80% recovery from this second pass, then 4% of the original ideal recovery remains unattained.

This will have to be made up from new crude, and 4% of $16.8 million ($670,000) is the annual cost of the lost material from this cause alone. This, of course, doesn't account for the cost of the Hidden Factory associated with all the reprocessing *(Table 2)*.

Table 2. Cost Assumptions For Case Study

Product	
8.4 kg peptide	$2,000 per gram
produced per year	$16.8 million per year
	$46,000 per day
Operational Cost Assumptions	
One operator	$100,000 per year
cGMP classified facility space	$100 per sq. ft. per year
Use of analytical laboratory	$2,500 per day
Media to fill 30x30cm column	$50,000
Media to fill guard column	$6,000
Inefficiency Cost Assumptions	
Basic LC system achieves x% of that of world-class LC system's 'Ideal.' (100-x)% is reprocessed once at same recovery rate (x%) thereby leaving 100 – x – x(100–x)% of 'Ideal' recovery, unrecovered. For example, a 70% first-pass recovery leaves 30% to be reprocessed. This reprocessed 30% yields 0.3 x 70% = 21% recovery. Total recovery = 70% + 21% = 91%, leaving 9% of 'Ideal' unrecovered.	

The inherent variability in a traditional chromatography process necessitates extensive inspection and testing that can be avoided with a properly controlled process. Many companies currently collect several fractions across the primary peaks during a run.

Fractions are then analyzed, as many as 30 in some cases, to determine which fractions to pool, which to reprocess, and which to discard. With a world-class system, this Hidden Factory is eliminated because the entire process becomes completely reproducible and the product always comes out at the same time. In every case we have studied, the cost savings due to improved process control recovers the *total* cost of the equipment in a remarkably short time; months, weeks, or in some cases, days of operation.

Another key issue addressed in the FMEA is validation and documentation. Operating, or planning to operate, a system that does not function reproducibly or in any state of real control will cause major delays in documentation and validation.

With a controlled system and a proper validation/documentation package

Table 3. Summary of Cost Analysis for Peptide Manufacturing Process

System Block	Inefficiency of Basic LC	Ideal Solution Source	Cost Avoidance	Cost Saving
Mobile Phase Preparation and storage for Buffers	Mobile phase preparation performed manually with dilute buffers stored in large tanks	Automated buffer preparation and in-line dilution system to store buffers as concentrates and dilute on demand	10K => 2K sq ft 6 => 4 RFTs Buffers stored as concentrate in small tanks not fully-diluted in large tanks	$800K/yr.for facility. $200K/yr. labor $100K up front for larger tanks
Mobile Phase Preparation and storage for Solvent Systems	Mobile phase preparation performed manually for solvent-based LC methods	Use appropriate in-line detector with feedback to control valves to actively control and create isocratic blends or gradient blends. Stock solvents are hooked up to the system;	4.5 => 3 RFTs	$150,000 per year
Mobile Phase Blending	Inability to run linear gradients (isocratic and step gradients only)	Use appropriate in-line detector with feedback to control valves to actively control and create gradients.	Avoiding 70% of ideal recovery => 30% higher LC mfg.. cost and time, plus 9% lost product recovery	Hidden Factory Cost of $1.5 million per year lost product
Mobile Phase Blending	Inaccurate gradient formation - using non-controlled proportioning for blending	Use appropriate in-line detector with feedback to control valves to actively control and create gradients.	Avoiding 80% of ideal recovery => 20% higher LC mfg.. cost and time, plus 4% lost product recovery	Hidden Factory Cost of $670K per year lost product
Crude Feedstock Introduction	Non-dedicated crude introduction scheme for a viscous crude material.	Use a separate sample loading pump or pressurized tank located just before the LC column	Avoiding 50% of ideal recovery => 50% higher LC mfg.. cost and time, plus 25% lost product recovery by avoiding viscous crude material pumped through main pump causing significant product tailing in the system pipes. Further reprocessing would produce 75% higher LC cost plus 12.5% lost product. etc.. Third reprocessing increases LC costs to 87.5% and reduces lost product cost to $1.1M/yr.	Second reprocessing reduces lost product cost to $2.1M/yr. Third reprocessing reduces loss to $1.1M/yr.
Mobile Phase Delivery	Unexpected Component Failure	Use robust components - triple head pump, certified valves, ISO certified vendors, stock spare parts, perform preventive maintenance, purchase warranties	Increased uptime	Up to $46,000 per day - one day down per 6 months = $92,000 per year
Mobile Phase Delivery	Fluctuations in flow rate	Flow meter which provides feedback to variable speed drive for main pump motor	Avoiding 95% of ideal recovery => 5% higher LC mfg.. cost and time, plus 0.25% lost product recovery	Hidden Factory Cost of $42,000
LC Column	Inadequate flow distribution - no plug flow	Low dead volume, active flow distributor with channels and holes calculated to give plug flow with no increase in pressure	Avoiding 70% of ideal recovery => 30% higher LC mfg.. cost and time, plus 9% lost product recovery due to band broadening affecting resolution between product and impurities.	Hidden Factory Cost of $1.5 million per year lost product
LC Column	Formation of voids and channels in packed bed over multiple cycles	Dynamic axial compression column with closed system for packing to inhibit the formation of voids and channels and allow unpacking/repacking in hours rather than days.	Avoiding 50% of ideal recovery => 50% higher LC mfg.. cost and time, plus 25% lost product recovery due to split peaks affecting resolution. Further reprocessing would produce 75% higher LC cost plus 12.5% lost product. etc.. Third reprocessing increases LC costs to 87.5% and reduces lost product cost to $1.1M/yr. Avoids unpacking and repacking the LC column every 2 months =>12 lost days per year + 5 otherwise unnecessary media re-fills.	Hidden Factory Cost of: $4.2M, $2.1M or 1.05M/yr.inefficiency, $10K/yr. for column repacking labor $250K/yr. For extra packing material cost
LC Column	Packing material fouling over multiple cycles due to dirty crude feedstock	Guard column before main column.	Avoids trashing the expensive LC media at the expense of much cheaper guard column media. (N.B. Labor $ is a wash.)	$214K/yr ($250K/yr. LC Media less $36K/yr. for guard media cost)
LC Column	Inability to optimize performance of packed bed due to limitations in packing technique.	Dynamic axial compression column to guarantee reproducibility and quality of packed bed	Avoiding 95% of ideal recovery => 5% higher LC mfg.. cost and time, plus 0.25% lost product recovery	Hidden Factory Cost of $42,000
Product Collection	Non-reproducible chromatography due to flow rate fluctuations, inaccurate gradient formation, and column bed deterioration	Actively controlled gradients and flow rate as described above	Avoids requirement for collecting, analyzing and pooling multiple fractions that requires analytical lab. and min. 1 extra RFT.	$100K/yr. for operator and up to $900K per year for lab
Entire Process	Completely manual operation. No control system or data logging system for batch reporting	Integrated PLC and PC for full automation, control, logging of analog signals, batch report generation	Eliminates need for 2 RFTs. Greatly decreases chances of all above failures => Decreases possibility of unexpected lost time or lost product	$200K/yr for operators. Up to $46K/day in lost product.
Documentation and Validation	Difficult and lengthy system and process validation	Comprehensive testing, component logging, materials testing, documentation performed under controlled SOP's in a cGMP environment provided and organized by equipment manufacturer	Avoids several months delay of production start-up due to unplanned lengthy system and process validation	Up to $46K/day - 3 months delay = $4.1 million

that is up to par, a full large-scale chromatography system can be installed and validated in days or weeks instead of months. These savings in time to market, though difficult to quantify, can be even more significant than the savings listed in the case study.

In the pharma industry, lost product can have a disastrous impact, far beyond the direct operational costs. A typical Phase III clinical trial has been estimated to cost $800,000 per day and requires access to qualifed candidates in a timely manner. There may be only a small window of opportunity before candidates no longer qualify. Any delays can thus quickly threaten the very survival of a company. An LC system that cannot prevent incorrect mobile phase from contacting product leaves the door open for such catastrophic losses. **GEN**

Kevin Henretta is a process chemist, John Walker is v.p. of engineering, and Lou Bellafiore is president, at TechniKrom (Evanston, IL). Phone: (847) 467-4056. Website: www.technikrom.com.

PUBLISHED BY Mary Ann Liebert, Inc. publishers • NEW YORK