Analytical and Biochemical Method Development Examples

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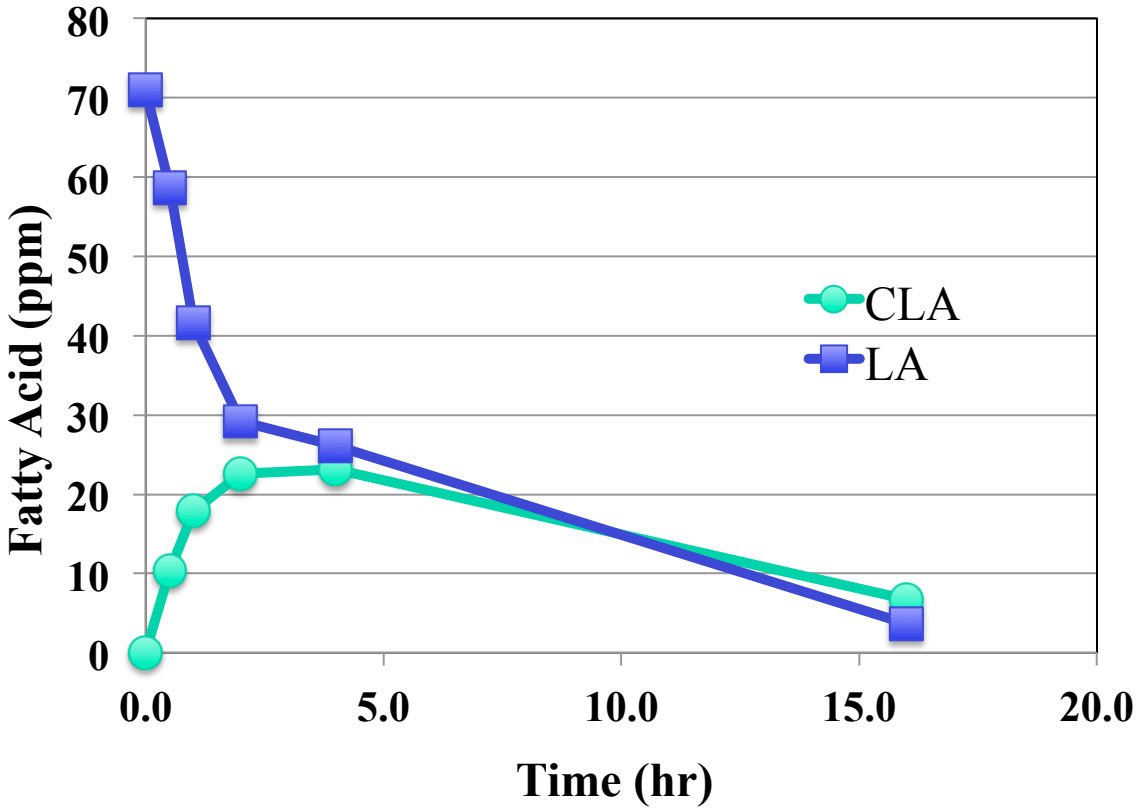
Example 1: Optimization of Process Conditions for Linoleic Acid Bioconversion

- Linoleic acid C9 isomerase converts linoleic acid (*c*9,*c*12, 18:2) to conjugated linoleic acid single isomer (*t*10,*c*12, 18:2), a high value dietary supplement
- A recombinant LAC9 isomerase was produced in *E. coli* by fed-batch fermentation to prepare enzyme catalyst for the development of a bioconversion process
- Bioconversion optimization:
  - Increase conversion efficiency
  - Improve fatty acid mass balance

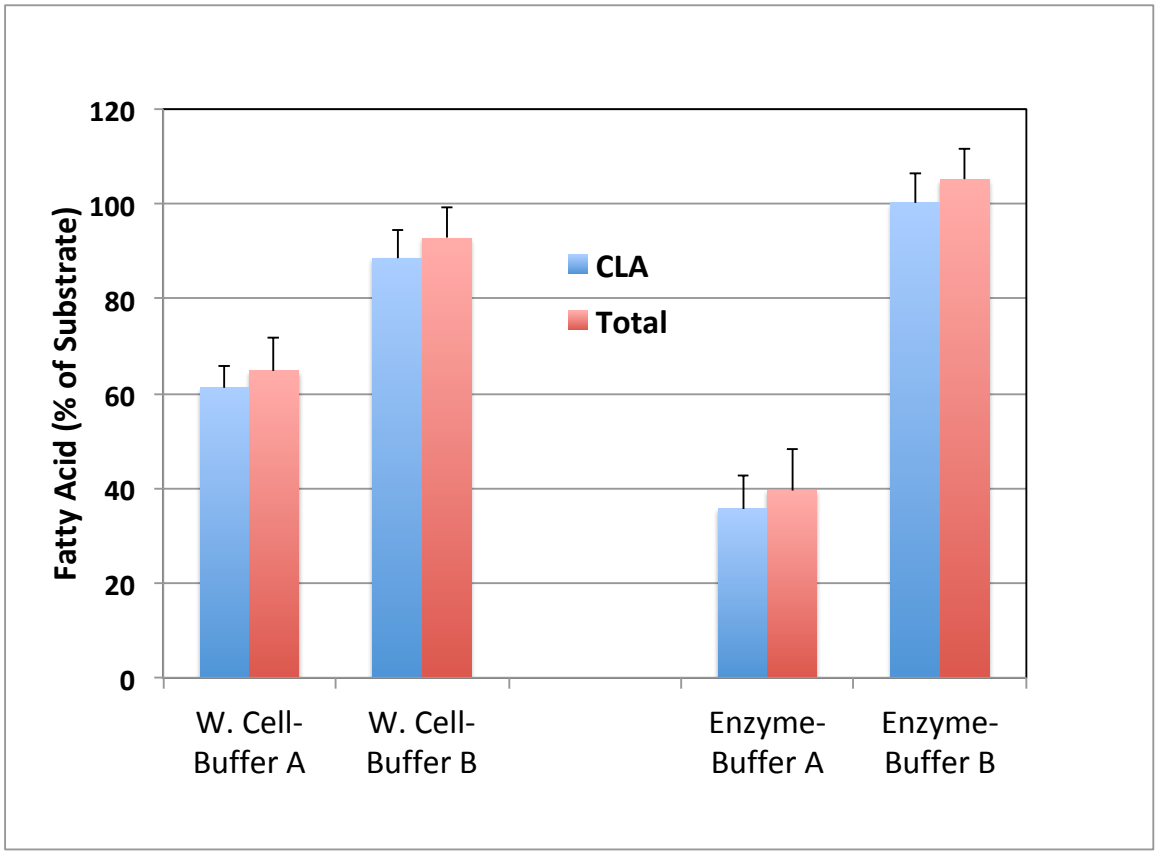
Fig. 1-1 Unoptimized Bioconversion Process



LAC9 isomerase sample was incubated with linoleic acid (LA) and FAMES were extracted and analyzed by GC. Conjugated linoleic acid (CLA) was formed rapidly within 3-4 hr.

- CLA was unstable during the process: 70% CLA produced earlier was lost by 16 hr.
- Poor mass balance: total level (LA+CLA) dropped to 15% within 16 hr.

Fig. 1-2 Improved Bioconversion Process



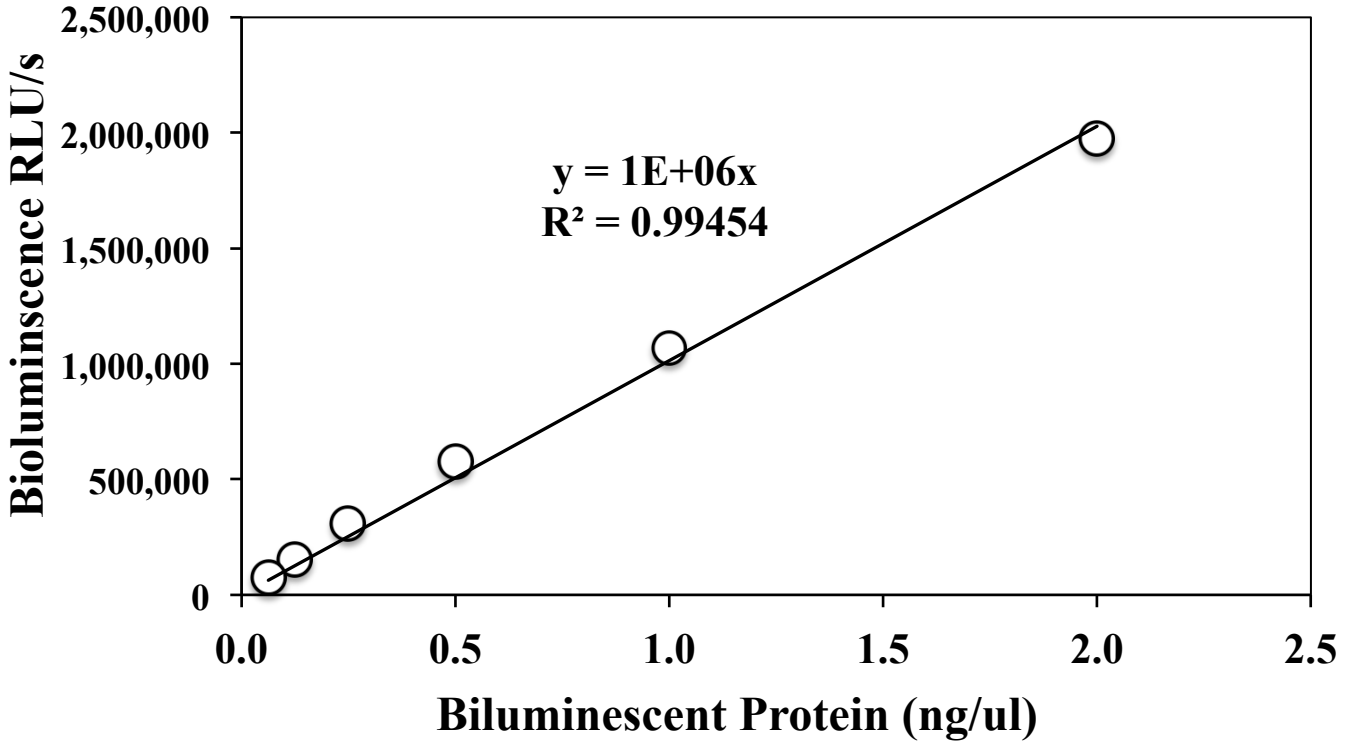
Recombinant *E. coli* cells expressing the LAC9 isomerase or partially purified enzyme were incubated overnight with linoleic acid (LA) in reaction Buffers A and B to produce conjugated linoleic acid (CLA). FAMES were extracted and analyzed by GC.

- Optimized conditions (Buffer B) lead to much higher levels of CLA production and mass balance than Buffer A in both whole cell biotransformation and enzymatic conversion.
- Mass balance of bioconversion reached ~100% in Buffer B using purified enzyme.

Example 2. Assay Optimization to Reduce Variability of Bioluminesence Detection

- **Client needs**
  - Develop a proprietary process to produce a bioluminescent protein by recombinant *E. coli* fermentation for pharmaceutical applications
  - Project scope: vector, strain and fermentation process development
- **Analytical support**
  - Protein quantification by bioluminescence, SDS-PAGE and HPLC
  - Challenge: bioluminescence assay is very sensitive but suffers from high variability (up to 30%)
  - Assay optimization: variability reduced to within 5%

Fig. 2-1 Optimized Bioluminescence Assay



Dilution	Enzyme (ng/ul)	RLU/s	stdev (n=4)	RSD (%)
1/2	2.0	1,976,019	27,482	1.4%
1/4	1.0	1,066,837	48,520	4.5%
1/8	0.5	576,985	23,955	4.2%
1/16	0.3	308,003	11,660	3.8%
1/32	0.1	154,239	4,226	2.7%
1/64	0.1	76,841	1,099	1.4%

Example 3: Development of a HPLC Method to Monitor Protein Production in Fermentation

- **Client needs**
  - Fermentation process development for expression and secretion of a protein using *Bacillus subtilis*
  - Analytical need: a reliable method for product quantification
- **Method Development**
  - Client unoptimized method had poor reproducibility: peak split and peak area reduction after ~10 injections using guard column
  - Problems overcome by cleaning samples using Bio-Spin columns or 96-well microdialysis plates: laborious and added cost
  - A new HPLC protocol was developed by changing elution profile
- **Outcome**
  - No need for laborious sample cleaning
  - Results are highly reproducible

Fig. 3-1: Protein Standard Peak Tailing and Splitting After Multiple Sample Injection (Old Method)

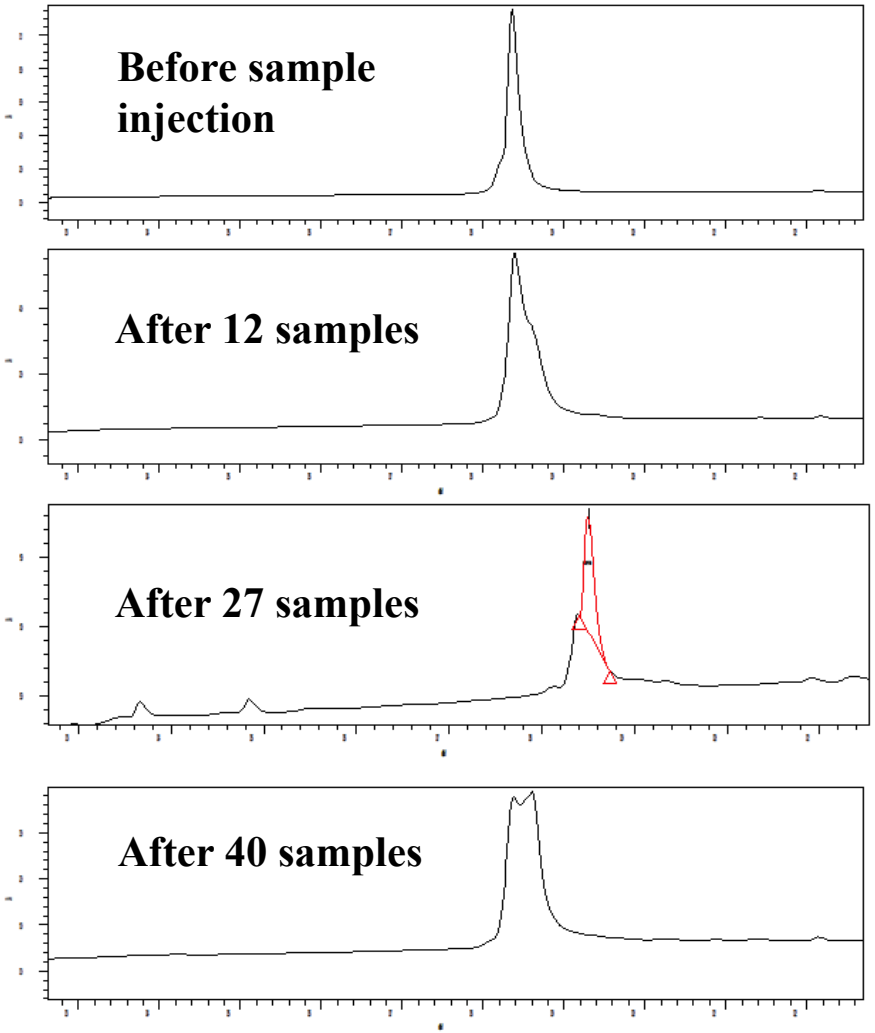
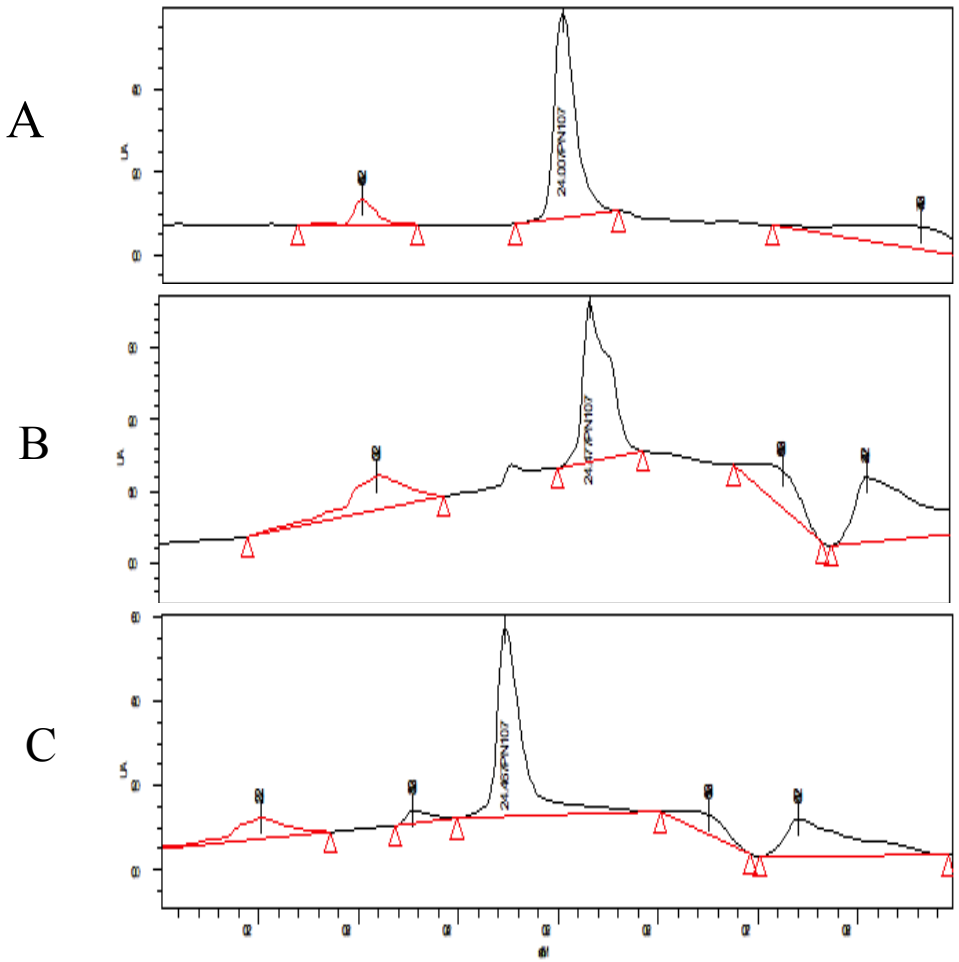
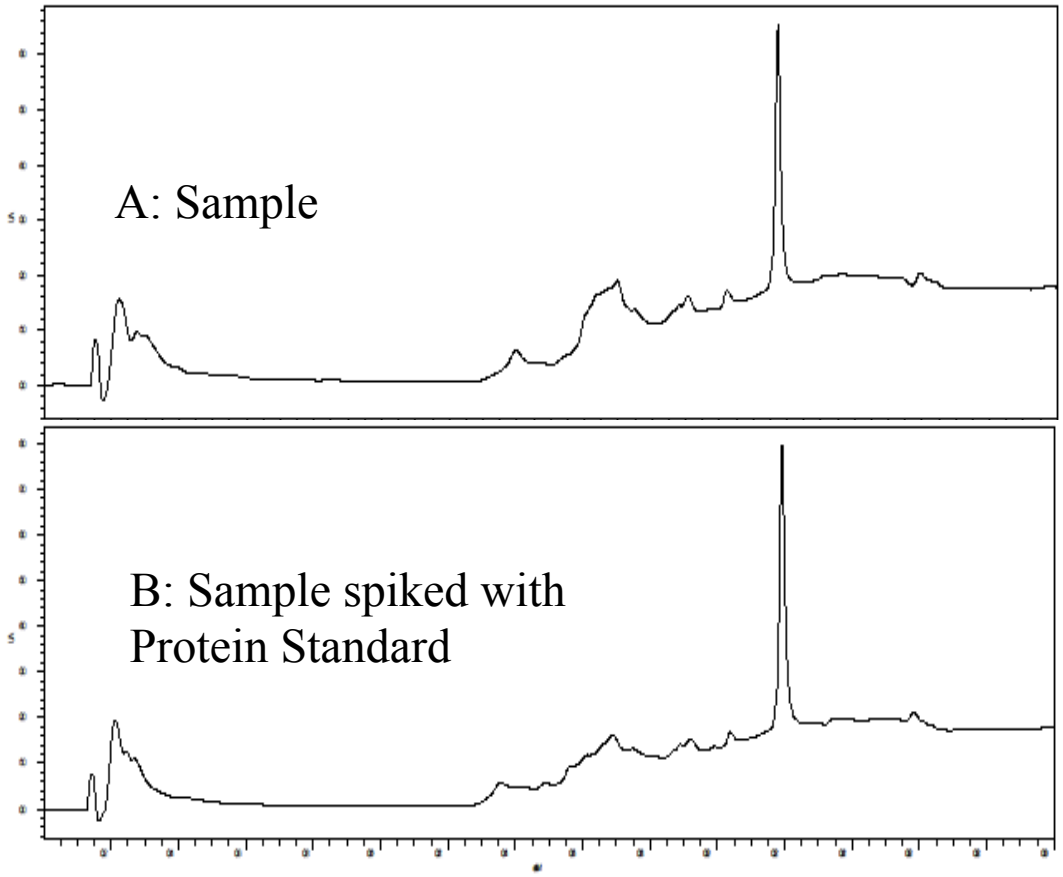


Fig. 3-2: Samples Cleaning Using Bio-Spin Column



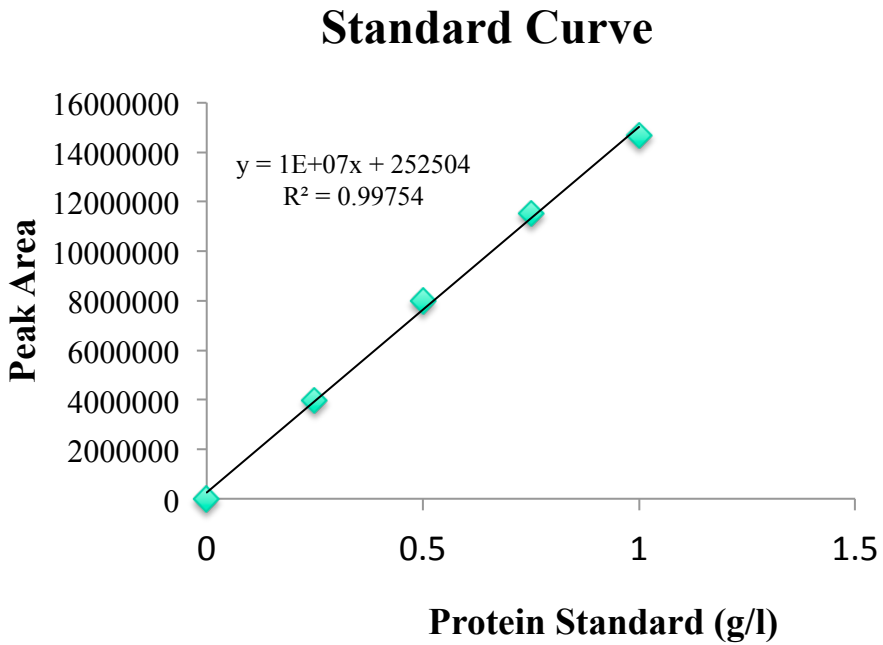
Chromatograms show the peak of Protein Standard (0.5 g/L) injected before injection of any fermentation samples (A), peak split after 12 injections of untreated samples (B) and no peak split after 119 injections of samples cleaned using Bio-Spin column (C)

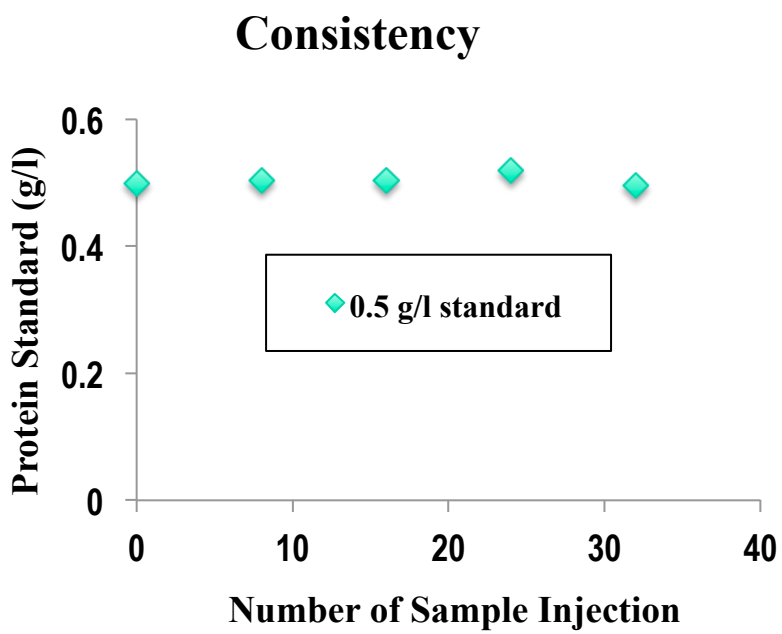
Fig. 3-3 Fermentation Samples Spiked with Protein Standard



A fermentation sample (A) and the sample spiked with protein standard (B) show sharp peaks on the chromatograms using the improved HPLC method

Fig. 3-4 Performance of the New HPLC Method





- Linear range of detection 0-1.0 g/L
- Consistent results after large numbers of sample injection