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Application Note

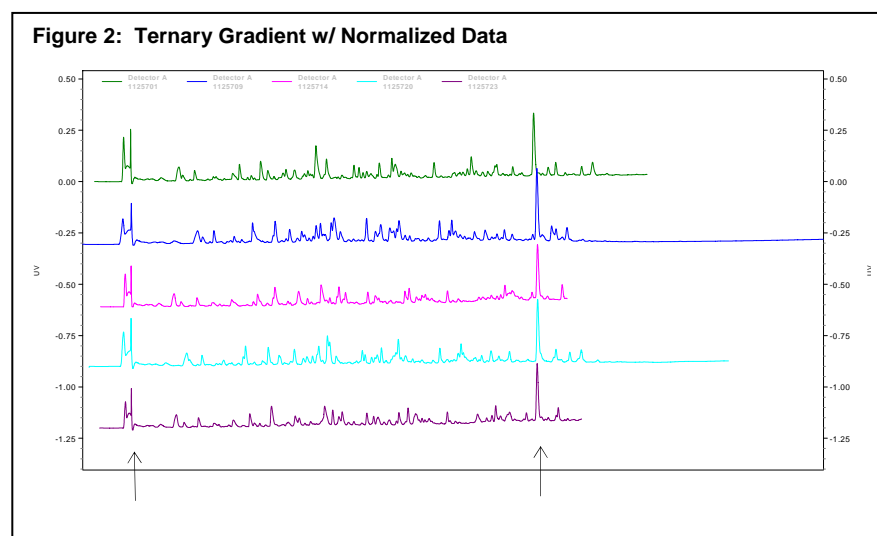
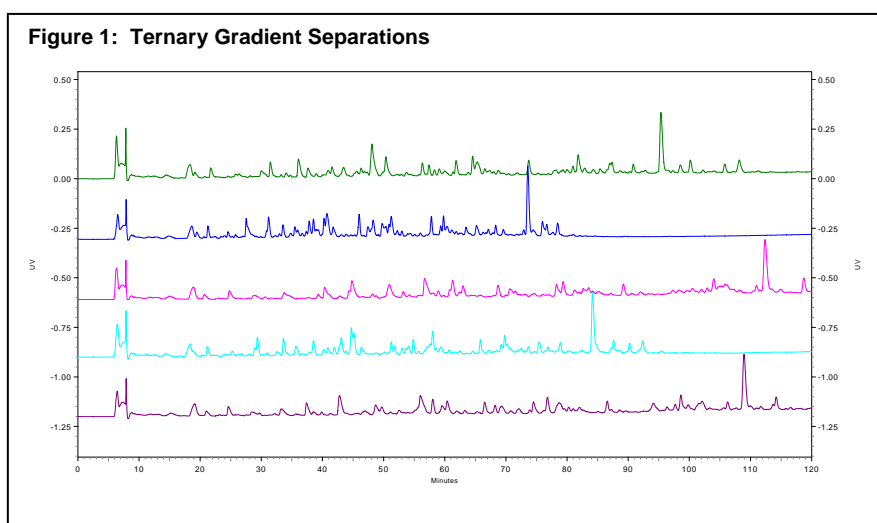
MicroPro Pump: 3 μ L/min: various gradients

trypsinized α -chymotrypsinogen

Ternary gradient capabilities have been available on analytical scale HPLC systems for many years; but, the utility of generating ternary gradients, for optimizing separation methods and selectivity, have not been conveniently available to researchers using capillary HPLC due to the absence of appropriate pumping systems.

The Eldex MicroPro pumping system can be configured to incorporate up to four separate solvents, by adding “slave” pumps onto a basic binary gradient system.

Figure 1 shows sample runs with varying solvent conditions as indicated (runs are from top to bottom). Figure 2 shows these runs with data normalized by adjusting traces to the two arrows shown.



Conditions:

Sample: 65 pmoles of a tryptic digest of α -chymotrypsinogen

Flow: 3 μ L/min

Column: 5 μ m C-18 200 x 0.320mm

Mixer: 5 μ L static

Equilibration: 20 minutes @ 2% B & C

Gradient Time: 120 minutes

A = 0.05% TFA in water

B = 60% Acetonitrile/40% water/0.045% TFA

C = 60% Methanol/40% water/0.045% TFA

Runs:

| 1 | 2 | 3 | 4 | 5 |
|--------------------|-------------------|-------------------|--------------------|--------------------|
| B=2-46% C=2-46% | B=2-90% C=2-2% | B=2-2% C=2-90% | B=2-68% C=2-24% | B=2-24% C=2-68% |