



**Figure 6:** Two-dimensional separation of proteins including separation by pH-gradient ion exchange chromatography on 10  $\mu\text{m}$  PL-SAX (250 x 0.32 mm i.d.), trapping of two fractions on short 5  $\mu\text{m}$  PLRP-4000A columns (5 x 0.32 mm) and backflush and separation on 5  $\mu\text{m}$  PLRP-S-4000A (100 x 0.32 mm i.d.).

1. Dimension: as in Figure. 5.

2. Dimension: Mobile phase A: 0.1% TFA in water. Mobile phase B: 0.1% TFA in acetonitrile. Gradient programme: 10–30% B in 1 min, then 30–50% B in 10 min, then 50–80% B in 3 min. Flow-rate: 10  $\mu\text{L}/\text{min}$ .

Elution order: 1 = Cytochrome C, 2 = lysozyme, 3 = myoglobin, 4 =  $\alpha$ -chymotrypsinogen, 5 = insulin, 6 = b-lactoglobulin A and 7 = b-lactoglobulin B, 8 = a-lactalbumin, 9 = BSA and 10 = ovalbumin.