Micropreparative capillary zone electrophoresis—tryptic digest analysis of recombinant GroES

Abstract

The GroES protein is a 10 kDa heat shock protein (chaperonin 10). GroEL mediated protein folding requires the co-chaperonin GroES which is essential for viability. GroES is composed of a single heptameric ring of 10 kDa subunits. Recombinant GroES produced in *E. coli* is a single, non-glycosylated polypeptide chain containing 97 amino acids and having a molecular mass of 10.4 kDa.

**Figure 1**

Micropreparative capillary zone electrophoresis—tryptic digest analysis of recombinant GroES.

**Chromatographic conditions**

| Buffer:     | 105 mM phosphate, pH 2.0 |
| Sample:     | tryptic digest of recombinant GroES protein |
| Capillary:  | effective length 87.5 cm, total length 96 cm, internal diameter 100 µm |
| Injection:  | 350 mbar × sec |
| Temperature:| 15 °C |
| Voltage:    | 0 min 0 kV, 3 min 10 kV, 10 min 20 kV, 20 min 25 kV, 40 min 30 kV |

**Application Note**

Proteomics
A tryptic digest of the recombinant GroES protein was performed followed by capillary zone electrophoresis (CZE) analysis with the Agilent CE system. Using CZE, a voltage can be applied as a gradient to enhance separation of the peptides. This also serves to minimize the thermal effects of applying high voltage to a large volume of concentrated sample as is used in this preparative run. The use of 100-µm id capillaries allows fraction collection from a single run (figure 1).

Reinjection of a fraction collected from a single preparative run using a 100-µm id capillary confirms successful collection of peak 4 and purity of the fraction (figure 2).

**Conclusion**

CZE in conjunction with the high-sensitivity detection cell, may be used to determine impurity levels in drugs at less than 0.1 % area/area. This level is appropriate to that required for regulatory submissions.

**Figure 2**
Low level determination of ranitidine impurities.

**Chromatographic conditions**
- **Buffer:** 105 mM phosphate, pH 2.0
- **Sample:** fraction 4 from tryptic digest of recombinant GroES protein
- **Capillary:** effective length 56 cm, total length 62.5 cm, internal diameter 50 µm
- **Internal diameter at point of detection is 150 µm**
- **Injection:** 200 mbar x s
- **Temperature:** 15 °C
- **Voltage:** 30 kV