CHAPTER IV

CONCLUSIONS AND FUTURE WORK

Two modes of capillary electrophoresis, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC), were developed for quantitative determination of gibberellic acid (GA_3) in fermentation broth and commercial products. MEKC, and not CZE, was found to achieve baseline resolution of GA_3 from other compounds, using the following MEKC conditions: 25 mM $Na_2B_4O_7$ as a buffer at pH 9.2, 100 mM SDS as a micellar phase and separation voltage of +30 kV.

For validation of the MEKC method, the quantitative analysis of GA_3 was performed using 3-amino-4-methylbenzoic acid (AMBA) as an internal standard, and the calibration plot was established using the corrected peak area ratio of GA_3 to AMBA as a function of GA_3 concentration in the range of 20 to 200 ppm. The high linearity was obtained, with correlation coefficient $r^2 > 0.999$. The values for the recovery of the known amounts of GA_3 spiked in water and fermentation broth were found to be close to 100 % with RSD < 5 %. This indicates that high accuracy and precision of the MEKC method is obtained. In addition, GA_3 matrix in either water or fermentation broth was found to cause no effect on the accuracy and precision. The standard GA_3 solution should be prepared in water, not in a basic solution, to avoid the decomposition of GA_3.

MEKC and HPLC methods were compared for quantitative analysis of GA_3 in the fermentation broth. MEKC analysis of GA_3 was carried out without sample preparation except for filtration and 10-fold dilution. For HPLC analysis of GA_3, sample preparation using solvent extraction with ethylacetate was required, and ~40 % loss of GA_3 was obtained. The amounts of GA_3 determined by MEKC and HPLC were found to be good agreement, only when using 3-acetamidophenol (AP) as a surrogate spiked in the broth before extraction for correcting the amount of GA_3 determined by HPLC. However, another disadvantage of HPLC analysis is long time consumption, 30 min for sample preparation and 15 min for an HPLC run. Therefore, MEKC has been shown to be an excellent method for determination of GA_3 in the

fermentation broth without any sample preparation except for filtration and dilution. The MEKC method was also applied for quantitative analysis of GA_3 in commercial "CU Gibb" products. Good agreement was obtained for the determined and labeled amounts of GA_3 in commercial products. Advantages of the MEKC method include high accuracy and precision, short analysis time within 7 min, low consumption of background electrolyte. Therefore, MEKC can be used as an alternative technique to HPLC for determination of gibberellic acid in fermentation broth, in which the presence of the large amounts of ions and nutrients could add complication to quantitative analysis, and also in commercial products.

In the future work, the MEKC method could be used for analysis of GA_3 in other forms of samples such as liquid GA_3 product, and optimisation of MEKC conditions may be needed due to the difference in sample matrix. In addition, CE could be developed for determination of other substances in the fermentation broth, without sample preparation except for filtration and dilution.

To prevent the decomposition of GA_3 in the standard solution and samples, the reagent for stabilisation of GA_3 may be added in the solution, or MEKC conditions may be carried out using the BGE at acidic pH. The stability of GA_3 in commercial products could be studied using MEKC or CEC. Since the decomposed compounds from GA_3 are still unknown, the structural information may be investigated using CEC-MS. It should be noted that MEKC-MS is not suitable due to contamination of the non-volatile surfactant in the interface and/or MS sources and detector.