3 - 62   HPLC for Determining Amount of Citric Acid in Zymotic Fluid of 100 L Cylinder Reactor

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Through one year hard work，a mutant strain was selected for high production of citric acid which was cultivated in 100 L cylinder reactor. The amount of citric acid was analyzed by high-performance liquid chromatography (HPLC) with the experimental condition：Atlantis C18 column (4. 6 mm × 150 mm, 5 μm) was utilized to separate ingredients of fermentation products，Mobile phase was 0. 05 mol/L disodium hydrogen phosphate buffer solution (pH2. 8)，flowing rate for 0. 8 mL/min and the injection volume was 20 μL. The column temperature was room temperature (25 ℃). The amount of citric acid was determined by peak area. Wavelength for UV detection was set at 210 nm[1].
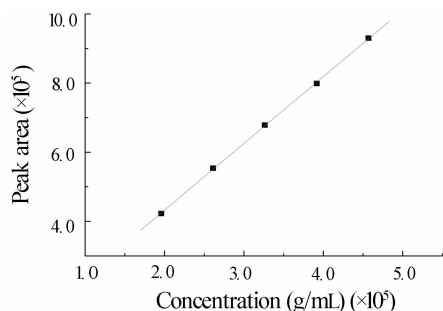

Fig. 1   The standard curve of citric acid

High content of citric acid mother liquor was diluted into the standard series solutions of 195. 84，261. 18，326. 40，391. 68 and 456. 96 mg/L. These standard solutions were injected into HPLC for determining citric acid content under experimental conditions as stated above. Finally，linear regression was carried out between sample concentration (x) and corresponding peak area (y). It was found that linear regression was line with citric acid content of 190～500 mg/L and its equation was $y = 1.93 \times 10^9 x + 46895$，correlation coefficient $R^2 = 0.9998$. The standard curve was shown in Fig 1.

Fermentation medium was composed of 20% corn starch and a certain amount of natural substances. 50 L volume was filled with in 100 L cylinder reactor and zymotic fluid was injected into HPLC after diluted by 625 times. The culture conditions were：the fermentation temperature at 37 ℃，orbital shaker at 400 r/min. The acidity of the final fermentation was determined by HPLC. Through three parallel trials，the peak area(Y) of diluted zymotic was at the average of 623652. 67，which was taken into the regression equation：$y = 1.93 \times 10^9 x + 46895$. The final citric acid content of zymotic fluid was 18. 69 g/100 mL.

Reference

[1] Liu Jialan，Wang Weiwei，Gao Xiaotong. PTCA(Part B：Chemical Analysis，46，8(2010)928.