Post-column pH Buffered Electric Conductivity Detection of Organic Acids with Ultra High Performance Liquid Chromatography

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Introduction

For the analysis of organic acids by HPLC, UV detection at around 210 nm has been conventionally used as a convenient and simple method. The UV detection method, however, is often less sensitive and/or selectivity when analyzing organic acids in food samples which include many kinds of UV-absorbing co-existing compounds. For highly sensitive and selective determination of organic acids, "Post-column pH-buffered electric conductivity detection" was developed by Shimadzu Corporation. After separating organic acids by the exclusion column, pH-buffered solution is added to the column eluent to improve the selectivity of organic acids which are weak anions. This technique improves the selectivity of organic acids in complicated samples. 1, 2.

Experimental

 Instruments

For HPLC system, "Novaera" (Shimadzu) was used. To optimize the conditions for separation, UV/VIS detector "VPD-10A" was used. And the electric conductivity detector "CDD-10A" was used for optimization of detection conditions and actual food sample analysis with post-column system (Fig. 3).

Analytical Conditions

Analytical conditions are shown in Table 1. Under the conditions, we optimized separation conditions using an exclusion column (2) concentration of Bis-Tris and 3) concentration of EDTA-4H in buffer solution (for good sensitivity and linearity without losing peak resolution).

Results and Discussion

1) Optimization of separation conditions of organic acids

We tried several kinds of columns and mobile phases (both acidic aq. solution and buffer). In the results, we adopted "Syringa Hydro-RP column" (150 mm L × 4.6 mm LD, 1.5 μm Phenomenex) as a column and perchloric acid solution as a mobile phase, so that the background conductivity detector low, concentration of perchloric acid was 200 μmol/ L. Then, the relationship between concentrations of perchloric acid in mobile phase and retention factors (α) were shown in Fig. 4. These results indicated that the concentration of perchloric acid hardly affected the retention of organic acids. Considering the background level of conductivity detector, low concentration of perchloric acid would be better. We adopted the low concentration of perchloric acid (5 mmol/L) for following experiments.

2) Optimization of detection conditions of organic acid - concentration of Bis-Tris

In the case of reversed phase chromatography, the acidic solution is commonly used as a mobile phase. Our post-column detection method could provide improved sensitivity and selectivity coupled with reversed phase separation.

We examined the concentrations of Bis-Tris, which is as a base in the pH buffering solution. Fig. 5 (a) shows chromatograms obtained under different concentrations of Bis-Tris, and (b) shows peak heights and S/N ratios of organic acids in Fig. 6 (a). In the results, 20 mmol/L Bis-Tris was reasonable.

3) Optimization of detection conditions of organic acid - concentration of EDTA-4H

Some organic acids (e.g., citric, crotonic, acrylic, and lactic acid) form chelate compounds with metal ion existing in the column and/or mobile phase. Consequently sensitivity and/or linearity might get worse. To prevent this phenomenon, EDTA-4H is quite effective. Fig. 7 shows chromatograms with 20 mmol/L Bis-Tris pH buffering solution, which contains different concentrations of EDTA-4H. The result indicated that 50 mmol/L EDTA-4H was the best, because the background noise was as small as non-EDTA buffer. Linearity of citric acid was quite good in this condition (we describe this later).

Analysis of organic acid standard

Chromatograms of 12 organic acids are presented in Fig. 8. The analytical conditions which have been optimized are shown in Table 2. Under the conditions, popular organic acids were separated in 5 min.

Conclusions

To apply "post-column pH buffering electric conductivity detection" to UHPLC, we optimized analytical conditions. Under the optimized conditions, major organic acids could be separated in about 5 min, with good resolution. LOD of formic acid was 16.8 pmol. Linearity and repeatability of peak area were quite good. We believe that this method is extremely useful for the analysis of organic acids in complicated samples such as food, which contains a large amount of co-existing compounds.