

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

## No. L221

## Simultaneous HPLC Determination of Ascorbic Acid (AA) and Dehydroascorbic Acid (DHAA)

Ascorbic acid (AA) and dehydroascorbic acid (DHAA), nutrients that may be quantitated as total vitamin C in a living body, are often subjected to analysis in wide fields such as clinical medicine, biochemistry, food chemistry, and pharmaceutical chemistry.

When applying HPLC for the analysis for both of the two components, although separation of AA and DHAA is possible, the maximum absorption of DHAA is short, 225nm, in comparison with AA, and, moreover, absorption coefficient is small and sensitivity is low, virtually making difficult direct analysis for it. For this reason, the following method is sometimes taken; after DHAA has been reduced into AA, it is quantitated as total ascorbic acid, and from the difference of the quantitated values before and

after the reduction, the amount of DHAA is determined. But because of the rather complicated analytical procedure, human error may be likely to occur.

It has been found that under alkaline condition, both DHAA and AA undergo change into structures having the maximum absorption wavelength at 300nm, and that under the same alkaline condition, if a reducing agent such as sodium borohydride is added, conspicuous increase of sensitivity was observed particularly for DHAA (patent pending). This method was applied to post-column derivatization in batch analysis of both compounds by HPLC, and favorable results were obtained. This method is introduced here.

Figure 1 shows the outline of this system. Shown in Figure 2 is the result of the analysis for AA and DHAA. Standard sample was adjusted with 1mM ethylenediaminetetraacetic acid (adjusted to pH 5.0 with acetic acid), to 39 ppm and 37 ppm respectively, and 5  $\mu$ L of each was measured out and injected. Table 1 shows the analytical conditions.

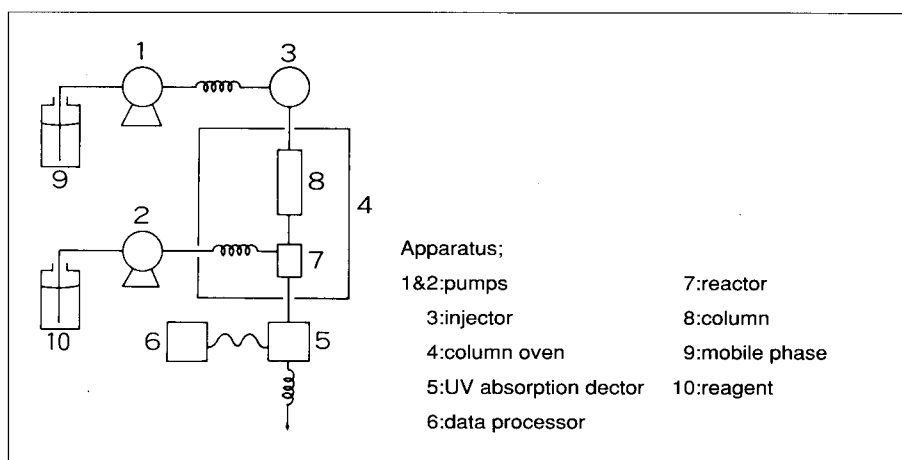


Fig.1 Flow Diagram of HPLC for DHAA and AA

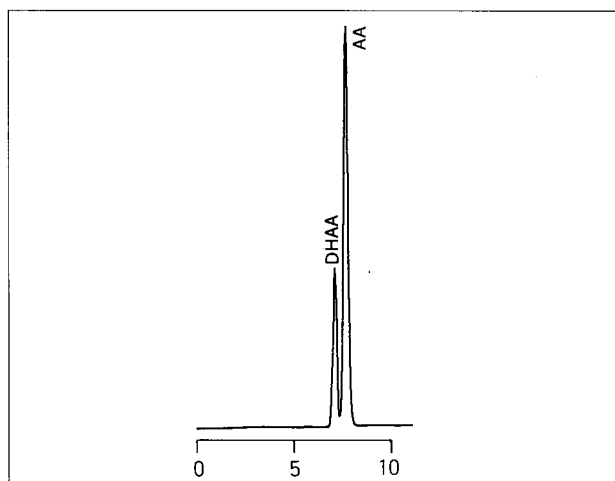


Fig.2 Chromatogram of DHAA and AA Standard

Table 1 Analytical Conditions

[for separation]

Column : Shim-pack SCR-102H(8mmI.D.×300mmL.)  
 Mobile Phase : 2mM perchloric acid aqueous solution  
 Flow Rate : 1.0mL/min.  
 Temperature : 40°C

[for detection]

Reagent : 100mM sodium hydroxide containing  
 100mM sodium borohydride  
 Flow Rate : 0.5mL/min.  
 Reactor : piping part J  
 Temperature : 40°C  
 Detection : absorbance at 300nm

It has been found that either compounds, DHAA and AA, change into derivatives having the same absorption spectrum with the maximum wavelength at 300nm. Figure 3 shows comparison of the absorption spectra of both compounds after the reaction. By carrying out absorption measurement at 300nm, specific quantitation with high detection selectivity is made possible by simple pretreatment of samples having many foreign substances like natural samples.

Tomato juice was diluted 10-fold with distilled water, filtered, and 10 $\mu$ L of it was injected. Figure 4 shows a chromatogram of tomato juice measured at

the maximum wavelength of AA, 245nm. Figure 5 shows a chromatogram of tomato juice using direct absorption detection at the maximum wavelength of DHAA, 225nm, and Figure 6 shows a chromatogram of tomato juice using the derivatization system.

As a result of examination, a good linearity was obtained within the range of absolute injection of 1ng  $\sim$  20 $\mu$ g for AA, and for DHAA, within the range of 2ng  $\sim$  2 $\mu$ g. Detection limits were 750pg and 1.7ng respectively, and reproducibility of quantitation was less than C.V. 1.1% (N = 10) for both AA and DHAA.

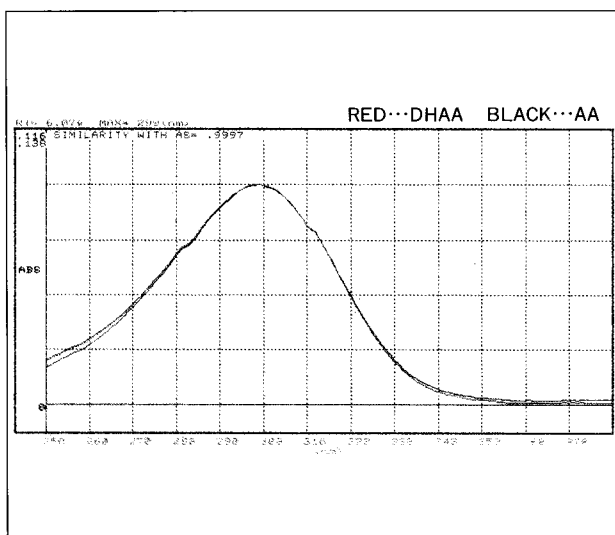


Fig.3 UV-spectra of Derivatized DHAA and AA

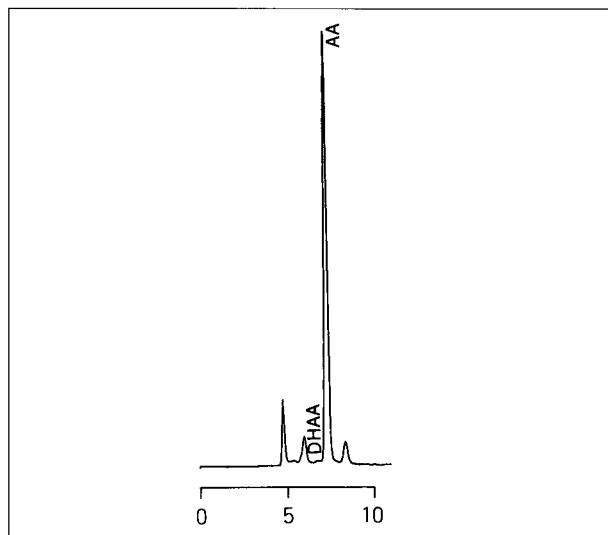


Fig.4 Chromatogram of Tomato Juice Using Direct UV-Detection (245nm)

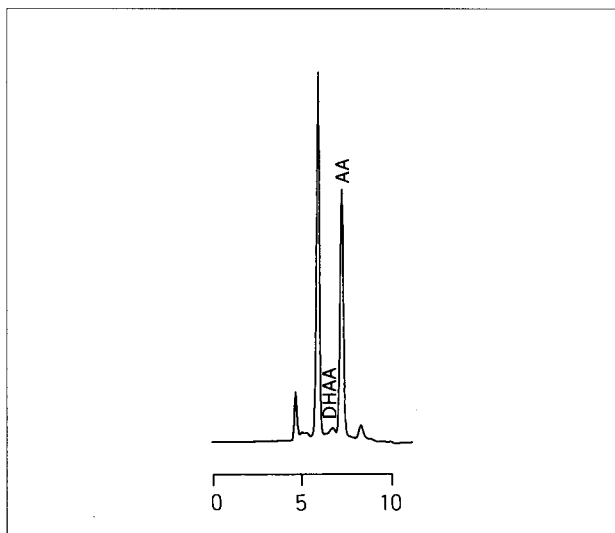


Fig.5 Chromatogram of Tomato Juice Using Direct UV-Detection (225nm)

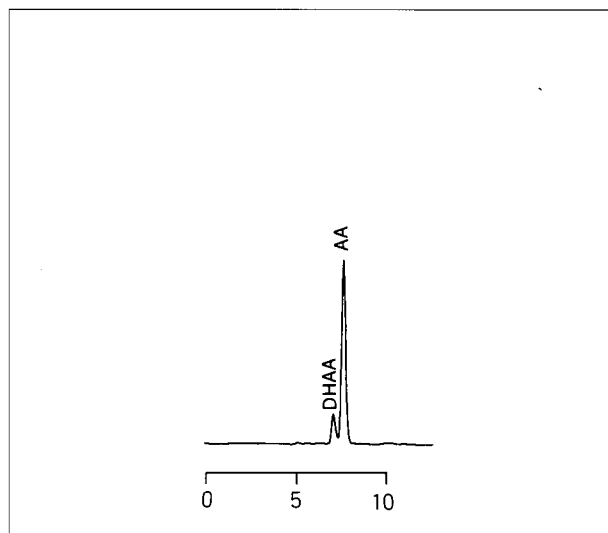


Fig.6 Chromatogram of Tomato Juice Using the Derivatization System

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