

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Application Data of Preparative Liquid Chromatography (No. 2)

Recycle Preparation of β -Carotene from Carrot Extract

β -Carotene is the red pigment which is contained in large amounts usually with chlorophyll in fruits and vegetables such as carrot, pumpkin and tomato. Also, it is an essential nutrient as the most important precursor to vitamin A (except in cats!). Introduced here is recycle preparation of β -Carotene from carrot extracts. Furthermore, the efficiency of preparative recycling was also confirmed by using a photodiode array detector.

Recycle separation: Since the price of a preparative column increases with size, ordinary columns, of lengths which do not have large theoretical plate numbers, are used in many cases. But, in such a case, by reintroducing the partially separated bands in the eluate onto the top of the column, the same separation effect as would be obtained with an extended column length can be obtained.

This is called a recycle separation, the effect of which is enhanced by iteration.

Figure 1 shows a schematic diagram of the recycle flow system. With the turn of the recycle valve, either the outflow path leading to the fraction collector or the recycle flow path leading to the pump inlet can be selected. In the recycle state, the flow path forms a closed circulation system in which there is no consumption of new mobile phase. Note that the system should be constructed with the minimum possible extracolumn volume to suppress band broadenings. This is an important consideration in recycle systems.

Recycle Preparation of β -Carotene: β -Carotene is retained by adsorption in the normal phase mode, but introduced here is an example of analysis in the general purpose reversed phase mode. The analytical conditions are shown in Table 1, and sample pretreatment in Table 2.

Under the above conditions, β -Carotene eluted after a retention time of about 18 minutes. But, from the asymmetrical form of the peak, the coexistence of other substances was assumed. So, recycle preparation was conducted in order to separate the components. Figure 4 shows the chromatogram sequence obtained. It is clear that from the single initial peak, which could be mistaken as a pure peak representing single substance, three peaks were eventually well separated. Effective recycling was obtained with a column of 20 mm in diameter, which is a relatively narrow preparative column. This was possible only by using the LC-8A preparative system in which the extracolumn volume is limited to a small value.

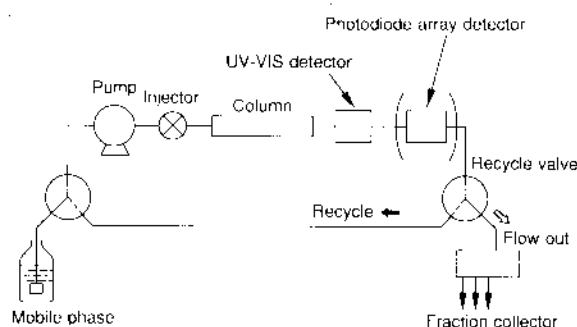
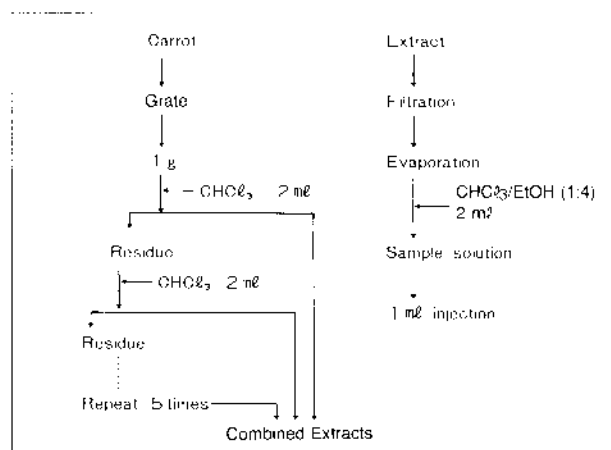


Fig. 1 Recycle Flow System

Table 1 Analytical Conditions

Instrument	: LC-8A System
Column	: Shim-pack PREP-ODS (H) kit (20 mm ϕ \times 250 mm L.)
Mobile Phase	: Ethanol (99.5%)
Flow Rate	: 10 ml/min
Column Temp.	: Room Temp. (26°C)
Detection	: Absorbance at 480 nm using 0.5 mm L. prep-cell
Monitor with Photodiode Array Detector:	
	195-600 nm
	Scan 2 sec

Table 2 Sample Pretreatment



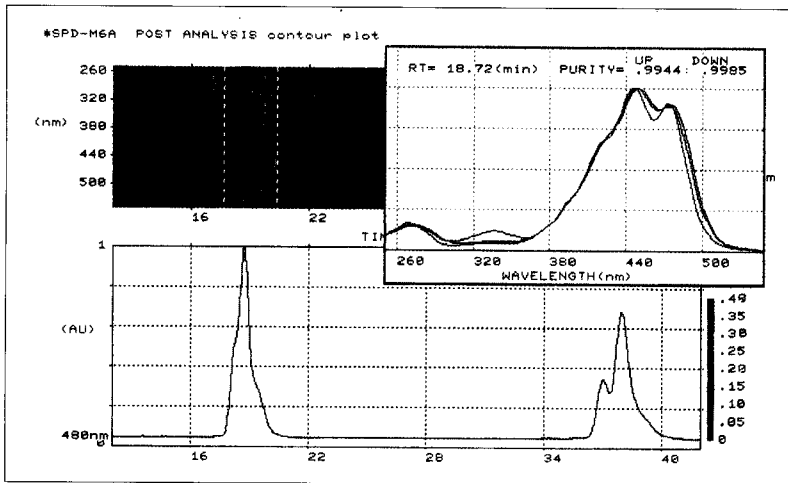


Fig. 2 Contour Plot and Purity Check in Initial Elution

Particularly, with a photodiode array detector connected between the UV detector and the recycle valve, study of spectra of β -Carotene in the 1st and 6th recycle preparations was conducted. Prediction of peak purity was difficult by the measurement with only 480 nm single wavelength, but by contour plotting and purity checking by the comparison of spectra at the top of the peak and at an adjacent position, it is possible to comprehend the refining process of the recycle preparation. This method in which preparation is conducted after confirming the refining process by this detector is very useful. By the comparison with the standard spectra, identification of α -Carotene and β -Carotene was conducted.

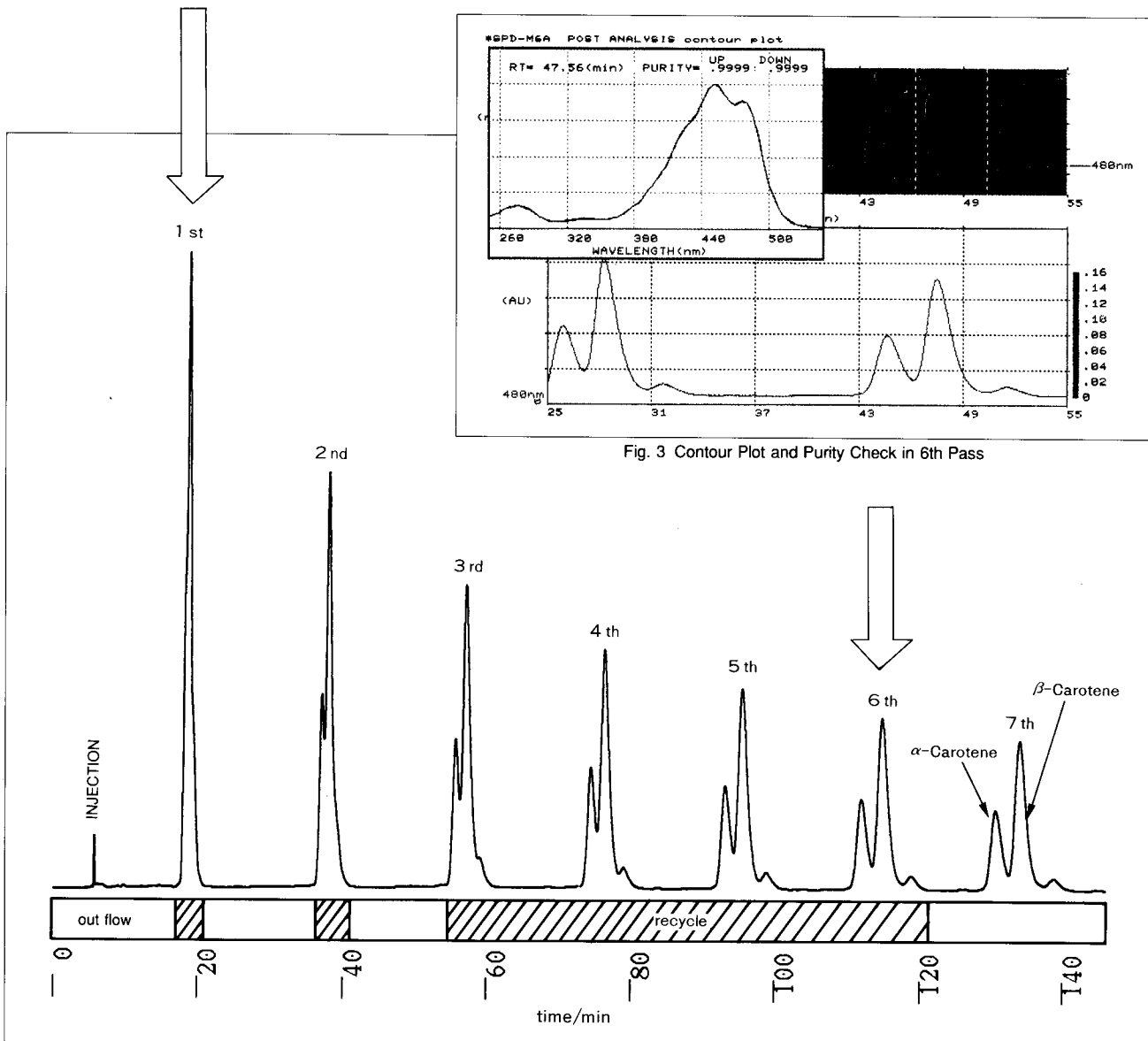


Fig. 3 Contour Plot and Purity Check in 6th Pass

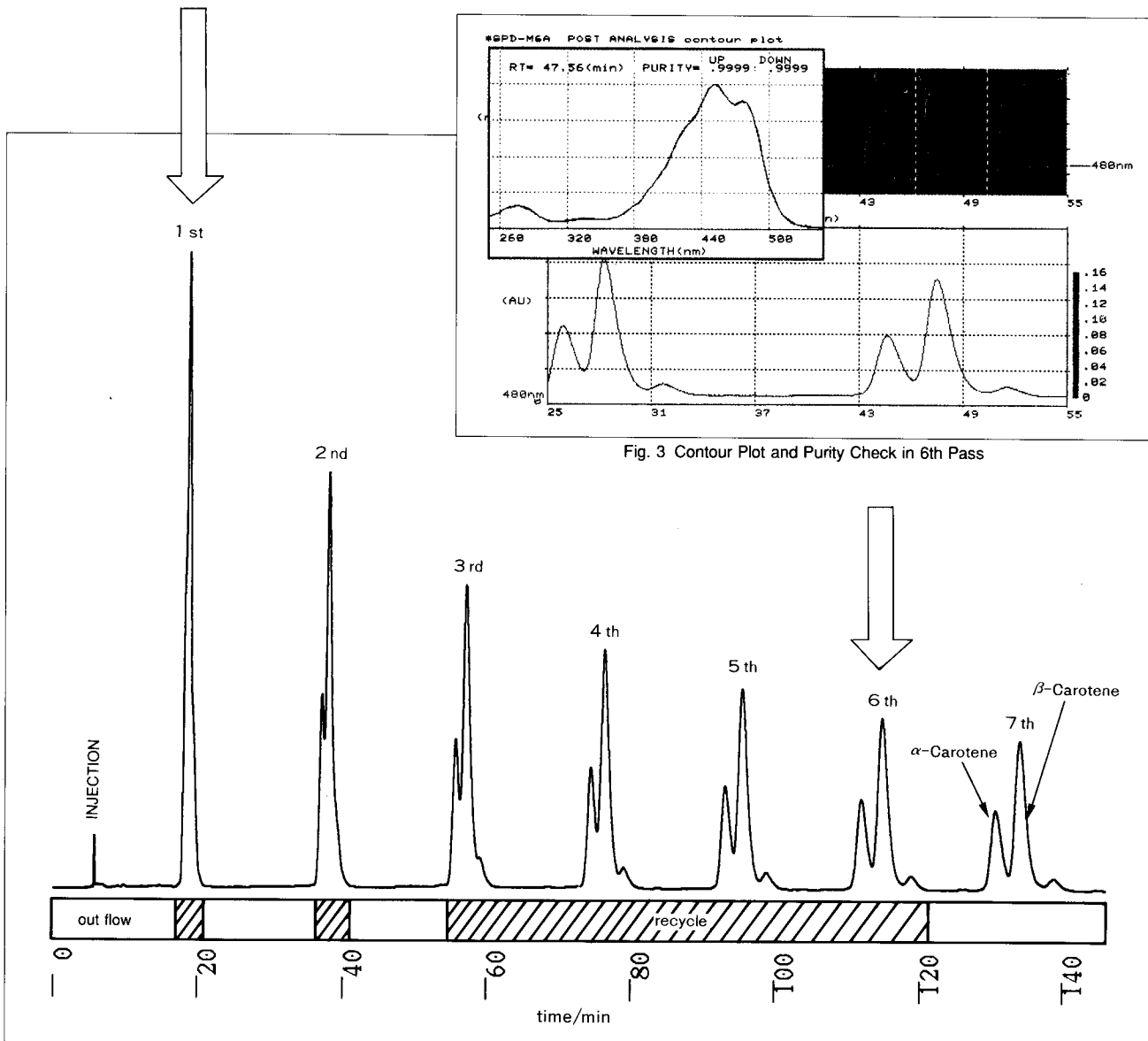


Fig. 4 Recycle Process for β -Carotene in Extract of Carrot



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