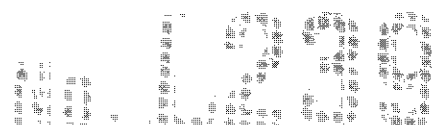


HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



Application of LC-10A Semi-Micro Liquid Chromatographic System

In high-performance liquid chromatography, usually columns of 4~6mm ID have been used. Recently, however, chromatography using narrower columns has come to be discussed. It is based on the concept to make use of the effect by using micro columns, namely,

(1) Increase of mass sensitivity (mass-based sensitivity)

(2) Reduced consumption of the mobile phase and the sample

It is expected that the use of micro columns will

become the main stream for chromatography. The micro columns are classified depending on the inner diameter into micro columns (~1mm ID) and semi-micro columns (around 2mm ID). Particularly, the semi-micro column can be handled in the same way as the conventional columns, and can be easily integrated in the existing instruments. Introduced here is an example of application of the semi-micro columns.

(M.Hayashi)

■ Interchangeability with the conventional columns

Fig.1 shows an example of analysis for berberine in coptis rhizome, a crude drug. Shown at left is a chromatogram obtained by using a conventional column, 4.6mm ID, for which the mobile phase flow rate was set at 1.0mL/min. The chromatogram shown at right is the result of analysis, for which a semi-micro column 2.0mm ID was used. The analytical conditions were the same as the former one except the mobile phase flow rate 0.2mL/min. The semi-micro column LC is, in short, a downsizing in the flow rate of the mobile phase in the cross-sectional area, and by adjusting the mobile phase flow rate in such a way that the same linear velocity is obtained, a similar chromatogram to that of ordinary LC can be obtained.

Analytical Conditions for Determination of Berberine

Chromatographic Conditions;	
Column	STR ODS-II (4.6mm ϕ \times 150mm) for left STR ODS-II (2.0mm ϕ \times 150mm) for right
Mobile phase	10mM (sodium) phosphate <pH2.6> containing 100mM sodium perchlorate / acetonitrile (2/1, v/v)
Flow rate	1.0mL/min for left 0.2mL/min for right
Temperature	40°C
Detection	Absorption at 345nm

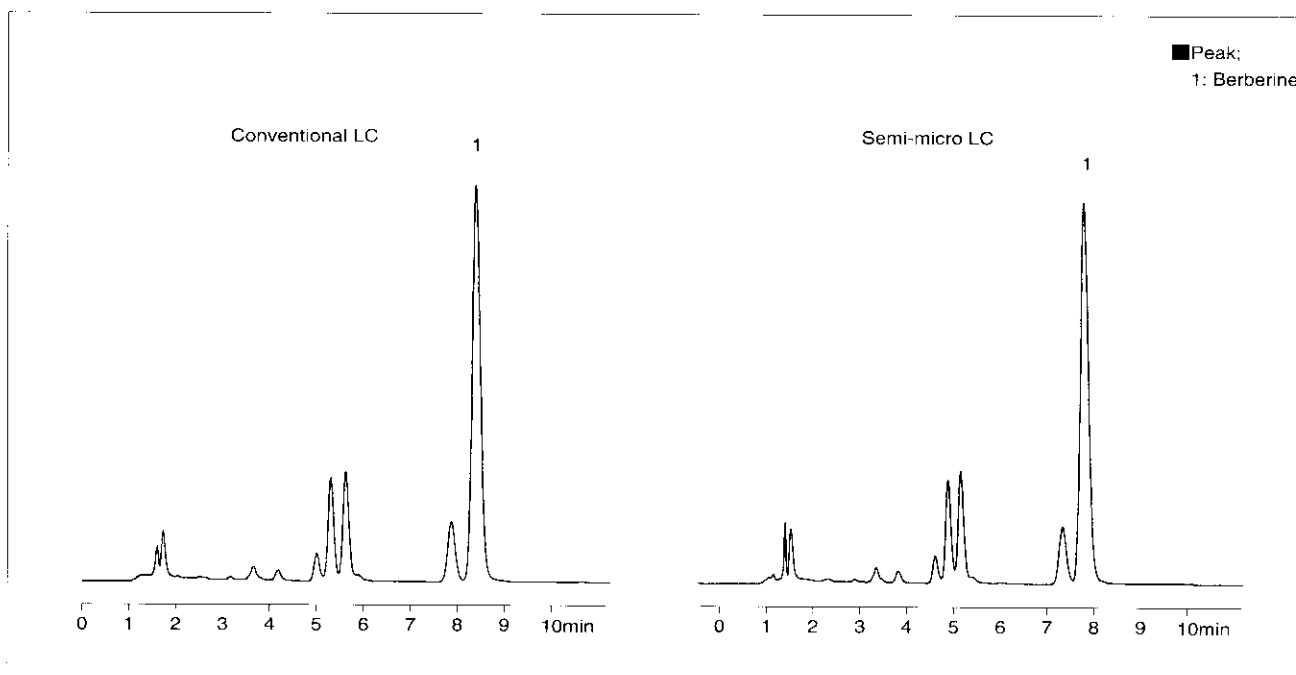


Fig.1 Comparison between Chromatograms of Berberine in Coptis Rhizome Obtained by Conventional LC and by Semi-micro LC

■ Accuracy of Semi-Micro LC System

Different from the LC by micro columns of less than 1mm ID, semi-micro LC does not require a dedicated instrument, and by changing the peripheral equipments, flow path tubes, and the detector cells, an ordinary HPLC can be used. But, as sample injection quantity is set at 1/5 of an ordinary injection quantity, what matters is the reproducibility of

injection. Table 1 shows repeatability of semi-micro LC (standard specifications) for the analysis of α -tocopherol. It is comprehended that it is by no means inferior to an ordinary LC system.

*The above is only an example, and it is not to guarantee the performance of the system by the results of the analysis.

Table 1 Repeatability of Semi-micro LC

n = 5	Concentration (mg/capsule)
1st run	99.283
2nd run	98.912
3rd run	99.125
4th run	98.885
5th run	98.883
X	99.018
S	0.179
RSD (%)	0.181

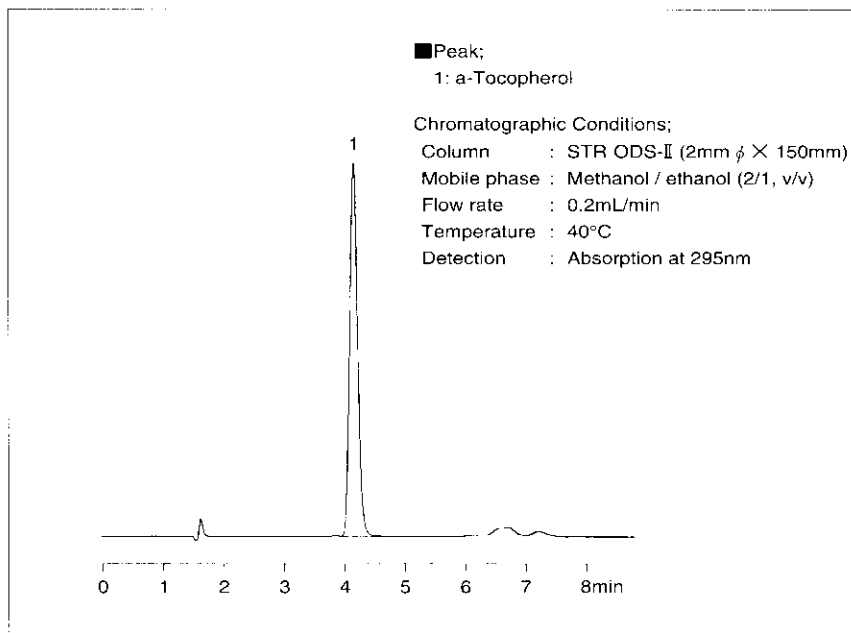


Fig.2 Repeatability of Semi-micro LC in Analysis of α -Tocopherol in Capsule

■ Simultaneous Analysis of Water-soluble Vitamines

Shown in Fig.3 is an example of analysis of vitamin Bs and caffeine in a vitamin drink. The

sample was filtered through a 0.45 μ m membrane filter and a 2 μ L aliquot was injected.

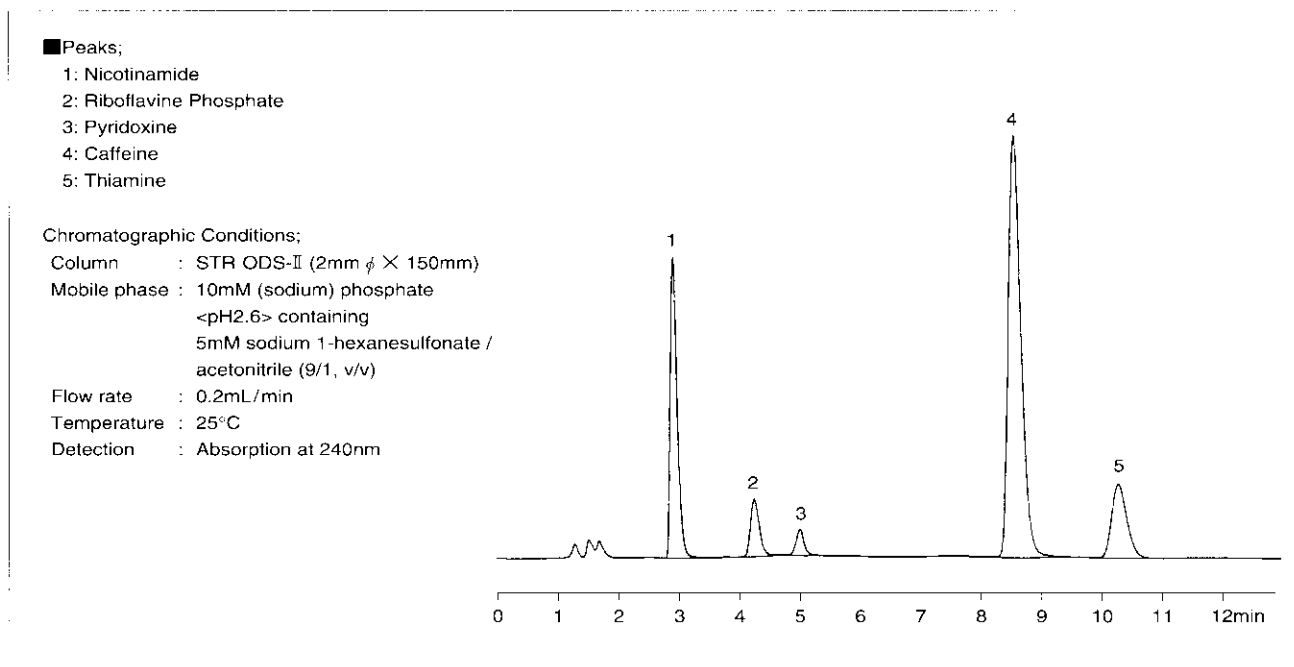


Fig.3 Chromatogram of Vitamin Bs and Caffeine in Vitamin Drink

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