SHIMADZU APPLICATION NEWS

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY 170

Applications of Photodiode Array UV-VIS Detector (SPD-M6A)

—— Analysis of Berberine in Crude Drugs ——

The SPD-M6A, employing a photodiode array sensor, is capable of storing data in the wavelength range of 195 nm to 670 nm, while observing the chromatogram at two wavelengths selected arbitrarily in real time. From the stored data, various re-analyses can be done, including peak purity calculations by three-dimensional chromatogram analysis, contour chromatogram analysis, multichromatogram analysis and absorption spectral analysis, components identification, and quantitative calculations of peaks, and it is highly noticed as an intelligent detector.

This is to report analysis of berberine in crude drugs by taking note of the quantitative performance of SPD-M6A.

Berberine is a medicine contained in crude drugs (coptis rhizome, phellodendron bark), and widely prescribed for intestinal regulating drug and stegnotic.

For analysis of berberine, usually reverse phase chromatography is used, but overlapping with miscellaneous components is its great demerit. In this example by adding sodium dodecyl sulfate (SDS) to the mobile phase, analysis was conducted by the reverse phase ion pair chromatography.

Analytical conditions are shown in Table 1. Fig. 1 is a chromatogram of analysis of berberin standard sample measured at two wavelengths, 270 nm and 350 nm. The top chromatogram in the diagram is made by plotting the difference of ratio of chromatograms at two wavelengths, which is called specific chromatogram, and when the peak is single, the waveform is rectangular, but when other substance different in the ratio of the absorbance of two wavelengths is contained, the profile is broken depending on its content, so that it may be regarded as an index for showing the singularity of the peak component.

Figs. 2, 3 are chromatograms of coptis rhizome and phellodendron bark analyzed at the same two wavelengths.

Table 1 Analytical conditions for berberine

Instrument	: Shimadzu LC-6A System
Column	: Shim-pack CLC-ODS (6.0mmI.D.×150mmL.)
Temperature	: Room temperature
Mobile pease	: 37mM Tartaric acid, 50mM Sodium dotecylsulfate 45 Acetonitrile 55
Flow rate	: 1.0mℓ/min
Detector	: SPD-M6A Absorption detector SPD-M6A

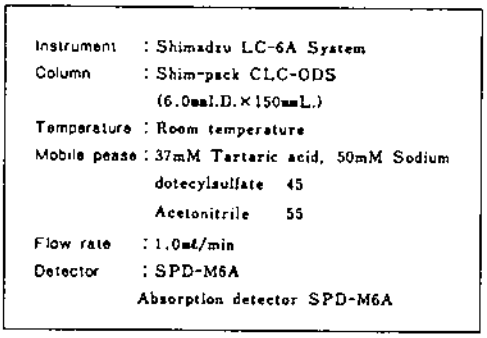

Fig. 2 Chromatogram of an extract from coptis rhizome

Fig. 1 Chromatogram of a standard berberine solution

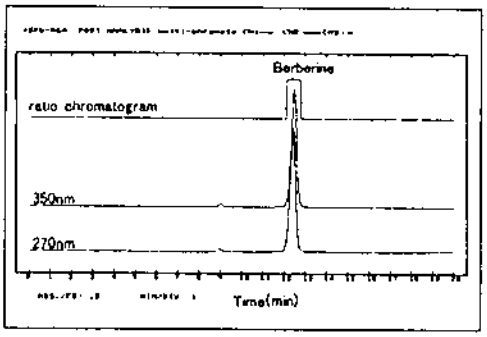

Fig. 3 Chromatogram of an extract from phellodendron bark

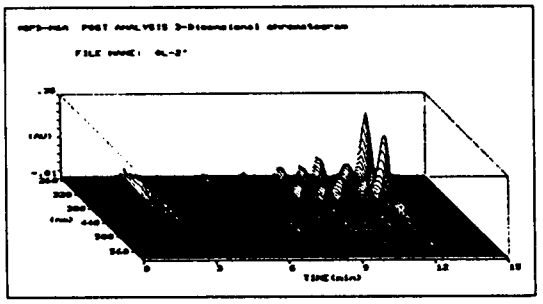

Fig. 4 Three-dimensional chromatogram of the extract from coptis rhizome

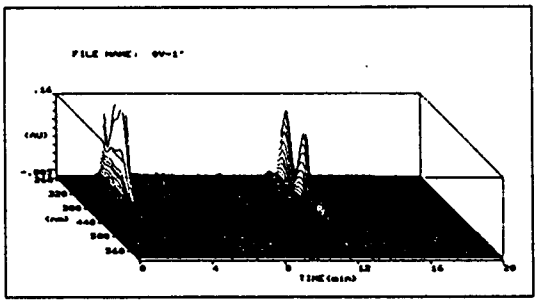

Fig. 5 Three-dimensional chromatogram of the extract from phellodendron bark

Fig. 4 and Fig. 5 are three-dimensional chromatograms of extracts of coptis rhizome and phellodendron bark, respectively.

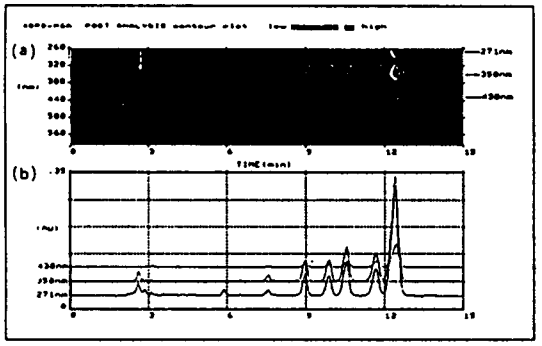

Fig. 6 Contour chromatogram of the extract from coptis rhizome

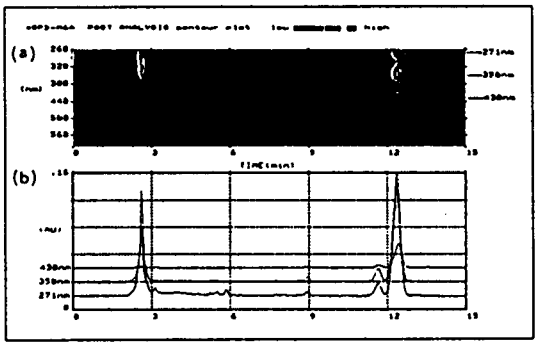

Fig. 7 Contour chromatogram of the extract from phellodendron bark

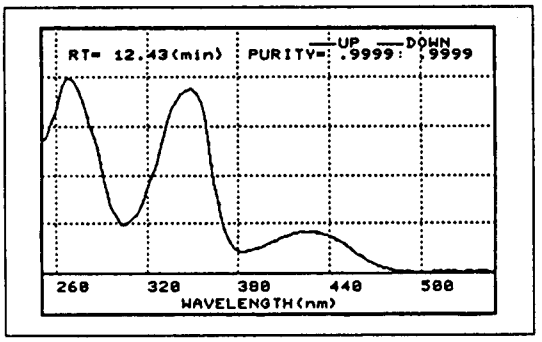

Fig. 8 Purity check of the peak for berberine in the extract from coptis rhizome

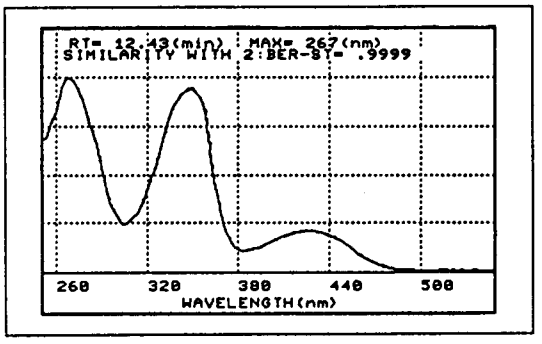

Fig. 9 Comparison between the extract from coptis rhizome and the standard solution in the spectra of the peaks for berberine

Figs. 6(a), 7(a) are contour chromatograms of extracts of the same two crude drugs, respectively. In Figs. 6(b), 7(b), an arbitrary wavelength is specified on each contour chromatogram, and the chromatogram at that wavelength is overlaid. (The number of chromatograms to be overlaid is not limited.)

Fig. 8 is an overlap of standardized spectra of the rise, top and fall of the peak at 12.43 min of the chromatogram of extract from coptis rhizome, in which the deviation of the peak point and spectrum is numerically expressed as the purity to evaluate the singularity of the component. In this case, the purity is known to coincide almost perfectly at 0.9999.

In Fig. 9, the peak spectrum at 12.43 min on chromatogram of extract from coptis thizome is overlaid on the spectrum of standard specimen of berberine, and the degree of coincidence of two spectra is evaluate as similarity. In this case, the similarity is 0.9999, and it is known that this component is berberine.

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