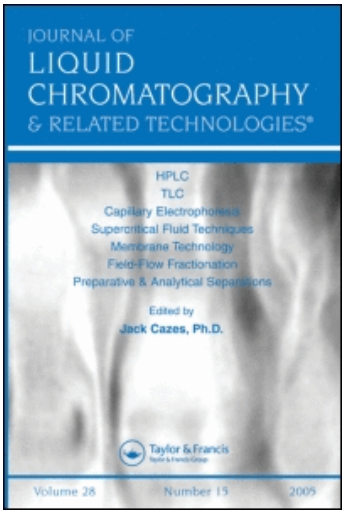

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CAPABILITY OF A PHOTODIODE ARRAY DETECTOR FOR VITAMIN ANALYSIS

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ABSTRACT

Vitamins were separated by liquid chromatography using a well surface-treated octadecyl-bonded silica gel column, and qualitatively analyzed by a photodiode array detector which could monitor both fluorescence and ultraviolet-visible absorption spectra.

INTRODUCTION

Reversed-phase liquid choromatography (RPLC) is recognized as a quick analytical method with very high selectivity and accuracy in the determination of compounds present even in very low amounts in complex samples such as foods, and environmental and biological substances.

However peak broadening and undesirable chromatographic results for nitrogen-containing compounds are common in RPLC

if the silica gels used are not-well end-capped. The reproducibility of retention time and peak symmetry may depend upon the degree of surface coverage of the silica gels, and therefore end-capping is the most important process for obtaining a stable alkyl-bonded silica gel column for quality and quantity control analysis by RPLC. RPLC presents several advantages for vitamin analysis, such as direct analysis without derivatization and simultaneous determination of several components together (refs. 1-3). However, the quantitative analysis of vitamins, especially nitrogen-containing compounds, was difficult if the octadecyl bonded silica gels used as the packing material were not well end-capped (refs. 4-6). As an example, the retention of thiamine(vitamin B1) and pyridoxamine(vitamin B6) was longer than expected, and sometimes they were not eluted.

A UV-VIS spectrophotometer, an infrared spectrophotometer, an NMR, an ESR, an ICP and an MS, etc were combined with a liquid chromatograph to improve the capacity of RPLC for qualitatire analysis (refs. 7-14). Multichannel photodiode array spectrophotometers have been introduced into RPLC as detectors which can rapidly monitor the spectra of compounds in liquid chromatography effluents. Their capability to identify relevant chromatographic peaks and to monitor at several wavelengths simultaneously is particularly pertinent to the analysis of vitamins having different maximum response wavelengths in complex mixtures (refs. 15,16).

In this study, the capacity of a multichannel detector which can simultaneously monitor both fluorescence and UV-VIS absorption spectra by replacement of the flow cell and light source is demonstrated for the analysis of vitamins separated by RPLC using well end-capped octadecyl-bonded silica gels.

EXPERIMENTAL

The diagram of the instrument is shown in Fig. 1. The system consisted of two LKB (Bromma, Sweden) Model 2150 pumps, a LKB Model 2152 gradient controller, an ERC (ERMA, Tokyo, Japan) Model 3310 degasser, a Rheodyne (California, U.S.A.) Model 7125 injector, two homemade flow cells (either fluorescence or absorption), an Inertsil ODS and ODS-2 column (15 cm x 4.6 mm I.D., 5 μm, Gasukuro Kogyo, Tokyo, Japan) and a modified Otsuka Electronics (Osaka, Japan) Model 110A multichannel photodiode array spectrophotometer. As light sources, a Model 3112-10 helium-cadmium laser (Omnichrome, California, U.S.A., 325 nm) for fluorescence and a Model L612 deuterium lamp (Hamamatsu Photonics, Shizuoka, Japan) for absorption were employed.

An NEC (Nippon Electric, Tokyo, Japan) Model PC-9801E personal computer was used as the system controller. Chromatograms and spectra were plotted on a Nippon Electronics (Tokyo, Japan) Model MC-920 plotter.

The linearity and sensitivity of the fluorescence detection were measured using riboflavin (Kishida Kagaku, Tokyo, Japan) as a standard compound in a 100% acetonitrile mobile phase.

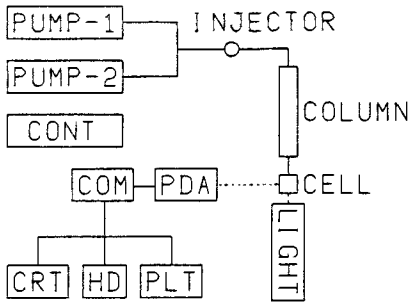

Fig. 1 Block diagram of instrument.
CONT.: gradient controller, CELL: either fluorescence cell or absorption cell, PDA: photodiode array monitor, COM: personal computer, HD: hard disk, PLT: plotter, LIGHT: He-Cd laser(fluorescence) or deuterium lamp(UV-VIS absorption).

Vitamin standards are obtained from various sources. All solvents were HPLC grade, and water was purified through a Pureline (Yamato Kagaku, Tokyo, Japan) Model WL-21P.

RESULTS AND DISCUSSION

A typical counter plot chromatogram and fluorescence spectra of 4 vitamin mixtures (A:retinol, B2:riboflavin, B6:pyridoxine, E:tocopherol) are shown in Fig. 2-(A) and (B). Vitamin E could not be detected by this system because the excitation wavelength of the laser (325 nm) was unsuitable, however vitamin E could be detected by a conventional fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. This laser fluorescence detection system has a linear response of three orders of magnitude ranging from 10^{-8} to 10^{-5} g/μL, and the detection limit is about 11 ng for riboflavin (vitamin B2).

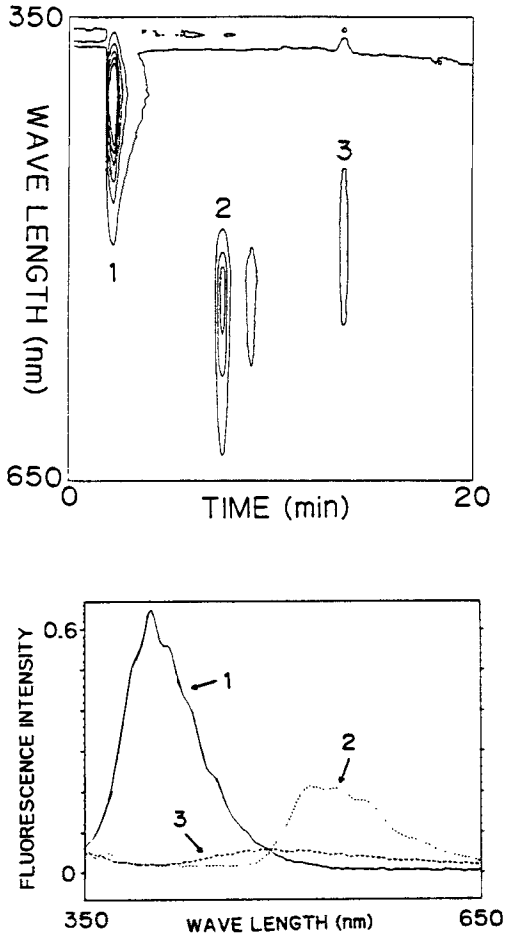

Fig. 2 (A)Counter plot chromatogram of vitamins separated by gradient elution on Inertsil ODS.
(B)Spectra of 3 vitamins detected.
Chromatographic conditions: flow rate 1 mL/min. During the first 6 min after injection the eluent was pumped isocratically at 15 % aq. acetonitrile, then gradient elution was started from 15 % aq. acetonitrile to 100 % acetonitrile in 10 min. Column temp.:ambient. Peaks: 1=pyridoxine; 2=riboflavin; 3=retinol.

Table-I Sensitivity of laser fluorescence detection

Vitamins	W.L.(nm) *1		Detection limit (ng)	
	Ex.	Em.	LF *2	CF *3
A	325	475	280	0.13
B2	270,370	520	11	0.054
B6	340	400	20	0.010
E	295	330	---	0.31

*1 Excitation and Emission wavelength of vitamins
*2 Detection limit of laser fluorescence
*3 Detection limit of a conventional fluorescence detector

As indicated in Table I, the detection limit of this laser fluorescence detection system was about 2000 times less than that of a conventional fluorescence detector using a photomultiplier. This situation is however unavoidable at present, because the sensitivity of the photodiode array UV detector is less than that of a conventional UV detector due to the dark current noise from the photodiode array, and to the difference in light-sensitive area between the photodiode array and photomultiplier.

Fig. 3 shows a chromatogram of the 14 vitamins detected by this photodiode array UV-VIS absorption monitor, and the chromatogram is given at the maximum wavelength of each vitamin. A good separation of 14 vitamins was obtained on an Inertsil ODS-2 column apart from the case of nicotinic acid and nicotinic acid amide, and that of ergocalciferol and cholecalciferol. Peaks 9 and 10 always appeared even if

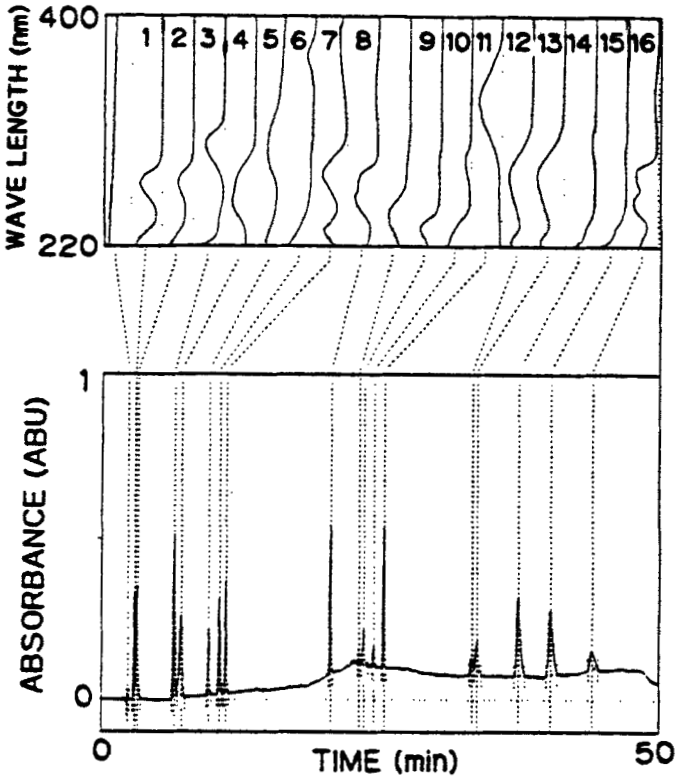

Fig. 3 Chromatogram and absorption spectra of vitamins separated on Inertsil ODS-2.
Chromatographic conditions: Flow rate 1 mL/min. Detection wavelengths 220-400 nm at the maxima for each vitamin. Gradient elution was used from 3 % acetonitrile in 0.1 % phosphoric acid including 5 mM 1-pentanesulfonate Na to 97 % acetonitrile over a period of 20 min. Column temp. 40°C. Peaks:1=nicotinic acid; 2=nicotinic acid amide; 3=pyridoxine; 4=thiamine; 5=folic acid; 6=cyanocobalamine; 7=riboflavin; 8=menadione; 9,10=gradient system peaks; 11=retinol; 12=ergocalciferol; 13=cholecarciferol; 14=tocopherol; 15=tocopherol acetate; 16=3-phytylmenadione.

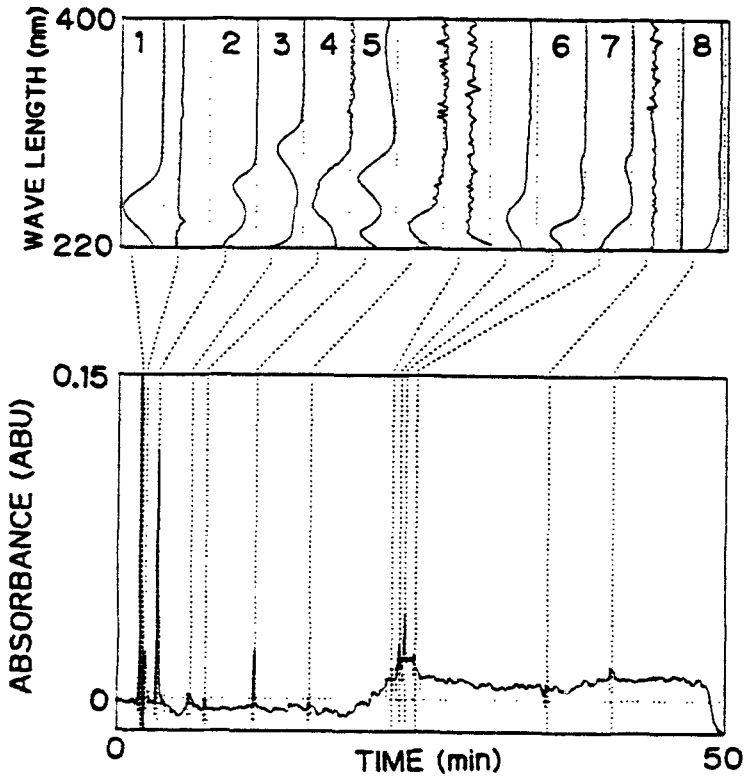

Fig. 4 Chromatogram and absorption spectra of a vitamin drug.
Experimental conditions were given in Fig. 3. Detection wavelength 240 nm. Peaks: 1=l-ascorbic acid; 2=nicotinic acid amide; 3=pyridoxine; 4=thiamine; 5=riboflavin; 6,7=gradient system peaks; 8=tocopherol acetate.

nothing was injected, so they might be gradient system peaks. UV-VIS absorption spectra of these vitamins were simultaneously recorded with the above fluorescence spectra for facilitating the identification of compounds. A drug containing vitamins was chromatographed by this system, and

the retention time and spectra of the peaks were compared to those obtained from the standard vitamins. Fig. 4 shows a chromatogram of the drug and their UV-VIS spectra measured by the photodiode array monitor. This drug sample contained 10 vitamins, however retinyl palmitate(vitamin A), cyanocobalamine(vitamin B12), calcium pantothenate and ergocalcipherol(vitamin D2) were not detected by the system because their amounts are too low. A good match is however found for the other 6 peaks.

In conclusion, the comparison of retention time in reversed-phase liquid chromatography using a gradient elution was not sufficient for qualitative analysis of vitamins. Further, commercial octadecyl-bonded silica gel columns were not suitable for quantitative and qualitative analyses of some vitamins due to broadening of peaks and absorption on the packings. A system combining a photodiode array detector monitoring fluorescence and UV-VIS absorption spectra with a well end-capped octadecyl bonded silica gel, could solve the difficulty of quantitative and qualitative analysis of a mixture of vitamins.

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