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Note

Applications of three-dimensional UV absorbance–high-performance liquid chromatographic patterns for the analysis of plant extracts

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High-performance liquid chromatography (HPLC) is widely used as an analytical and preparative method in natural products chemistry. In the development of a microcomputer system, HPLC has recently been adapted[1–4] to display various information on eluates with a short operating time.

An HPLC system incorporating a multi-channel spectrometric detector (MCPD) is now available for displaying three-dimensional patterns indicating retention times on the *x*-axis, absorbance on the *y*-axis and UV wavelength on the *z*-axis. For post-operation analysis, 16-channel chromatograms at different wavelengths in the range 220–400 nm and two-dimensional UV absorption curves of eluted components at any retention time can be measured.

EXPERIMENTAL

Chromatograph

The high-performance liquid chromatograph was an SP-8700 (Spectra Physics, U.S.A.) with a Rheodyne 7125 injection valve and an MCPD-350 (Union Giken, Kyoto, Japan) (see below) coupled to a microcomputer plotter printer and UV monitor.

The columns used were Aquasil SS-452N (25 cm × 4.6 mm I.D.)[5] and Aquasil SN-662N (15 cm × 8.0 mm I.D.)[6] (Sensyu Scientific, Tokyo, Japan). The mobile phase was chloroform–ethanol–methanol–water (62:16:16:6) or tetrahydrofuran–water–acetic acid (160:40:7).

Samples

Licorice root (0.5 g) was extracted with 50 ml of hot methanol and after evaporation of the extract to dryness the residue was dissolved in 20 ml of methanol. Senna (1.0 g) was extracted with hot chloroform and hot 70% methanol and after evaporation of the extract to dryness the residue was dissolved in 2 ml of methanol.

Authentic samples

All the compounds separated were individually identified in comparison with authentic samples.

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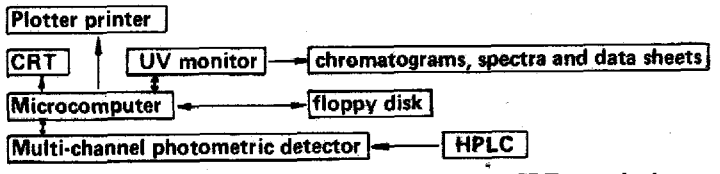


Fig. 1. Schematic diagram of the MCPD-350 system. CRT = cathode-ray tube character display.

MCPD-350 multi-channel spectrophotometric detector

Fig. 1 shows a schematic diagram of the MCPD-350 system.

A map of the three-dimensional analysis on the CRT display is as follows:

	[disk data]
[1]: 3D-PLOT (45°)	SAMPLE NAME =
	DATE & TIME =
[2]: 3D-PLOT (90°)	MEASURE TIME = 0–60 min
	RECORD SIZE =
[3]: TIME RANGE	SAMPL. TIME = 35 msec
= 0–60 min	ACCUML. TIMES = 8 times
	Y-SCALE = 1 OD/FS (FS = full scale)
[4]: *Y*-SCALE = 1 OD/FS	WAVE RANGE = 220–400 nm
	RESOLUTION = 3 nm
	MODE = peak
[5]: Paper speed	CHROMATOGR = 250–260 nm
= 20 mm/min	SLOPE (UP) = 0.001 OD/sec
[6]: NEW	SLOPE (DOWN) = 0.001 OD/sec
	WIDTH & HEIGHT = 2 sec

RESULTS AND DISCUSSION

Until now HPLC has usually been monitored by UV absorption at a single wavelength to detect compounds containing a chromophore. However, it is not so easy or almost impossible to detect minute unknown components of naturally occurring materials by HPLC using this monitoring system, as there are numerous natural products that show widely varying characteristic UV spectral absorptions depending on their structures.

Using the MCPD-350 system, monitoring UV spectra under flow conditions, all the components so far detectable by UV absorption in the range 220–400 nm can be displayed as a three-dimensional pattern.

Three-dimensional patterns can be usefully applied for the analysis of natural drug materials, *e.g.*, licorice and senna. Licorice [the roots of *Glycyrrhiza* spp. (Leguminosae)] is used as a sweetening agent and as an important herb drug in Asia and Europe. It contains a sweet-tasting saponin, glycyrrhizin, and its congeners, as well as several groups of flavonoids.

The methanol extract of licorice was submitted to HPLC on an Aquasil column and monitored with MCPD to give a three-dimensional pattern. In Fig. 2, a three-dimensional pattern of licorice extract in a retention time range of 4–8 min, displayed with a 45° angle of the *z*-axis, is shown. In Fig. 3, the same region of the chromatogram is displayed with a 90° angle of the *z*-axis. The profiles of the peaks in the chromatograms which reveal characteristic UV absorption curves of each separated component suggest their chemical structures. In this instance, peaks 1 [retention time

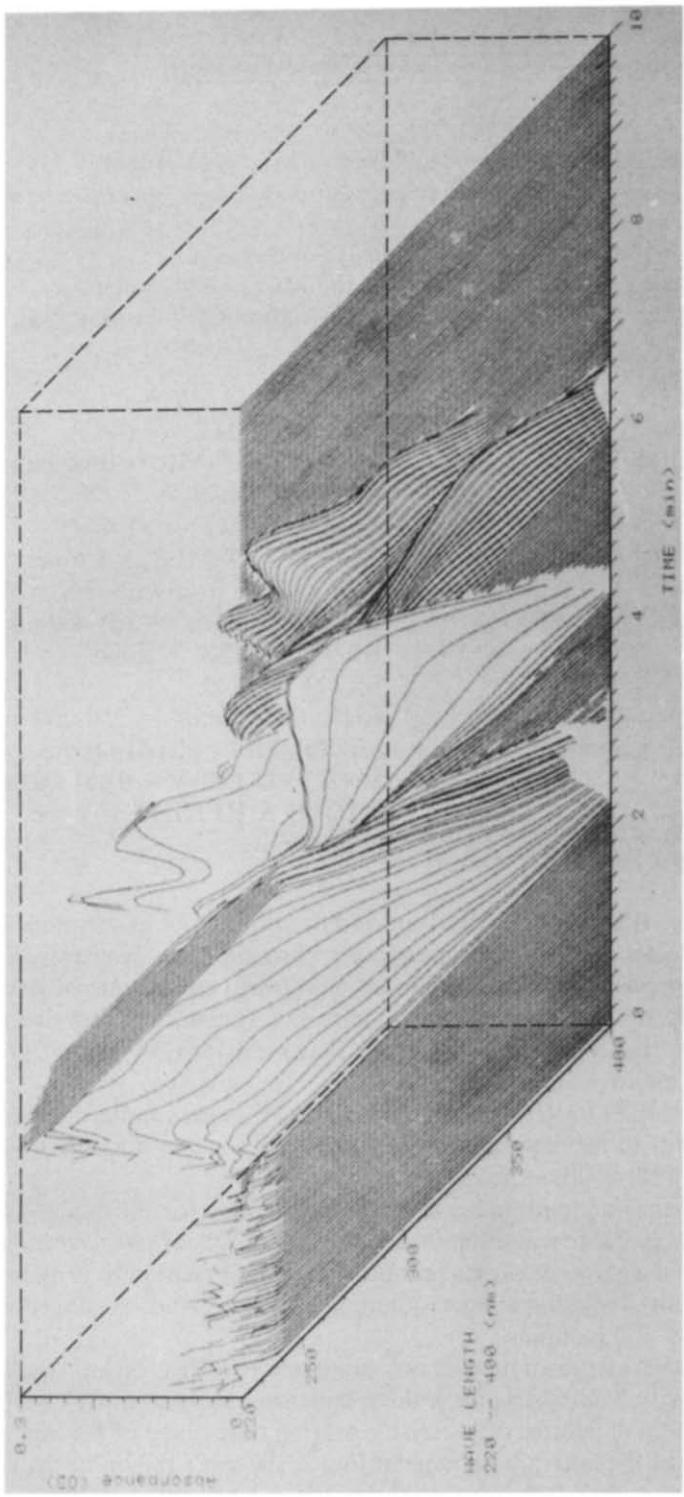


Fig. 2. Three-dimensional HPLC trace (45° display) of the methanol extracts of licorice root. Column, Aquasil SS-452N (30 cm × 4.6 mm I.D.); mobile phase, chloroform–ethanol–methanol–water (62:16:16:6); flow-rate, 1.0 ml/min.

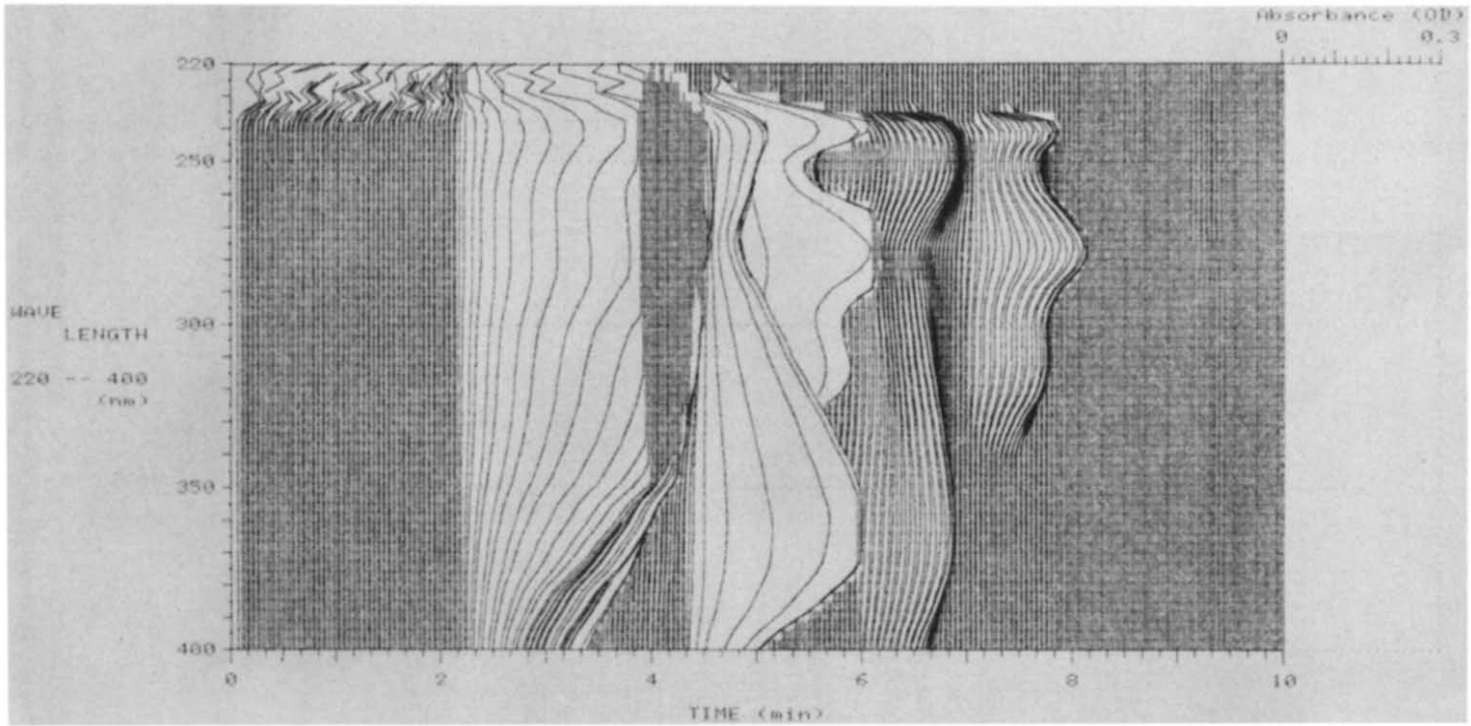


Fig. 3. Three-dimensional HPLC trace (90° display) of the methanol extracts of licorice root. Column: Aquasil SS-452N (30 cm × 4.6 mm I.D.); mobile phase, chloroform–ethanol–methanol–water (62:16:16:6); flow-rate, 1.0 ml/min.

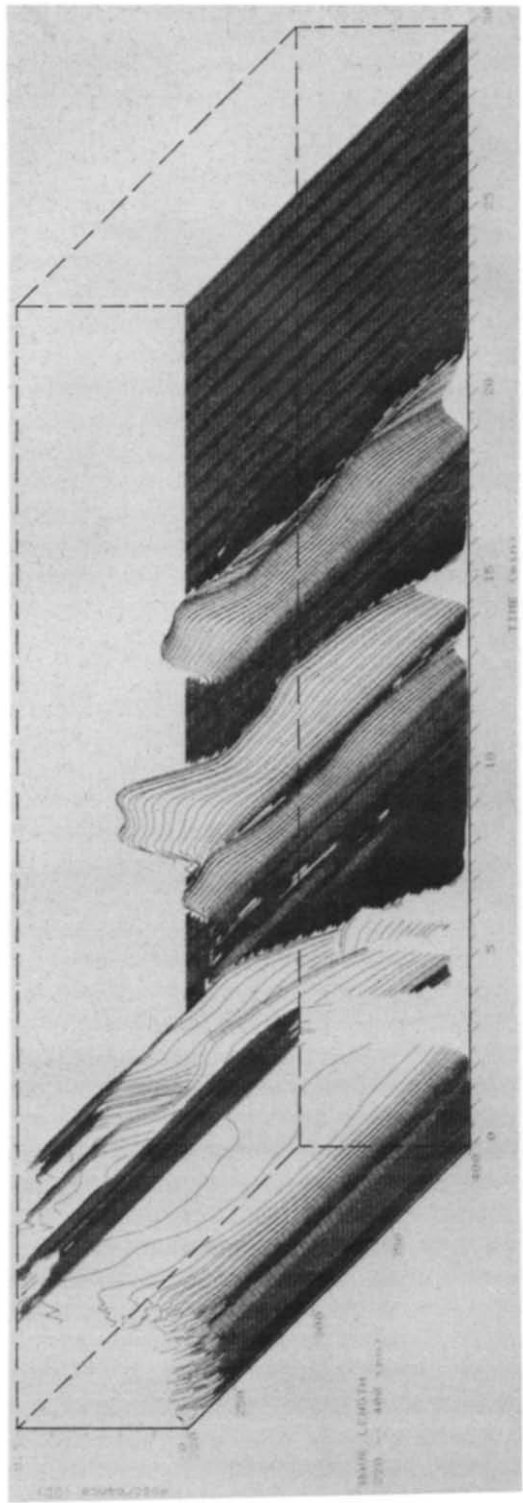
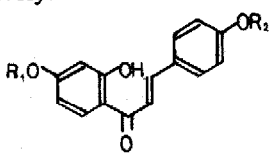
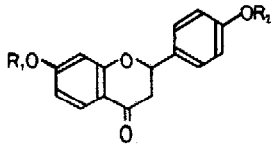


Fig. 4. Three-dimensional HPLC trace of the 70% methanol extracts of senna leaves. Column: Aquasil SN-662N (15 cm × 8 mm I.D.); mobile phase, tetrahydrofuran–water–acetic acid (160:40:7); flow-rate, 2.5 ml/min.

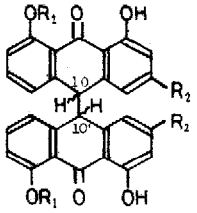
(r.t) 4.64 min] and 3 (r.t. 6.42 min) could be assigned to the chalcone glucosides isoliquiritin(I) and isoneoliquiritin(II), respectively, and peaks 2 (r.t. 5.17 min) and 4 (r.t. 7.40 min) to the flavanone glucosides liquiritin(III) and neoliquiritin(IV), respectively.





(I) R_1 = H, R_2' = Glc: isoliquiritin
(II) R_1 = Glc, R_2' = H: isoneoliquiritin
(III) R_1 = H, R_2' = Glc: liquiritin
(IV) R_1 = Glc, R_2' = H: neoliquiritin

Senna [the leaves of *Cassia angustifolia* or *C. acutifolia* (Leguminosae)] is used as a laxative. The active principles of senna are sennosides A–D, which are 10,10′-bianthronyl derivatives. An efficient HPLC separation of sennosides using a dimethylamino-substituted column was reported earlier[6]. A three-dimensional pattern of senna extracts (Fig. 4) was obtained using this column, resulting in separations of sennosides A (r.t. 14.9 min), B (r.t. 19.8 min) and C (r.t. 13.3 min), among which sennosides A (V) and B (VI) revealed similar UV absorption profiles (UV maxima 270, 308 and 355 nm) different from that of sennoside C (VII) (UV maxima 270 and 323 nm).



(V) R_1 = glucopyranosyl, 10,10′
R_2 = COOH (*threo*)
(VI) R_2 = COOH (*erythro*)
(VII) R_2 = CH_2OH (*threo*)

The three-dimensional patterns show the following advantages:

(1) A wide chromatographic map is obtained by a single operation to give retention times and UV absorption curves of the eluants simultaneously. Even minute components in the plant extracts detectable by UV-absorption in the range 220–400 nm can be revealed on the chromatogram.

(2) The UV absorption profiles on the HPLC trace provide useful information on the structural types of the eluates.

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