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Note

Application of contour maps to three-dimensional display of ultraviolet absorbance in high-performance liquid chromatography of natural drug materials

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Three-dimensional tracing of high-performance liquid chromatography (HPLC) with UV detection is very sueful for the qualitative analysis of plant extracts and in the identification of their components[1]. Some other investigations on such three-dimensional displays have been reported[2–6], and recently Clark *et al.*[7] demonstrated the contour line plotting of three-dimensional HPLC graphics. The contour line map indicating the UV absorption maxima of the components is conveniently applied to the detection of minor components, even if they are hidden behind larger peaks on the three-dimensional HPLC trace. We now present a new computer program designed to display simultaneously a contour line map and an HPLC three-dimensional UV absorbance profile in the wavelength range 220–400 nm.

EXPERIMENTAL

We used a MCPD-350 system (Union Giken, Osaka, Japan) for displaying the three-dimensional patterns and contour line maps, comprising a SORD M223 MARK V, CRT micro computer fitted with an 8-in. twin disk drive system and a plotter printer. The basic compiler was CBASIC Programme 04E. Details of the program are available on request.

Extraction

Licorice. Licorice root (0.5 g) was extracted with 20 ml of hot methanol for 3 h. After evaporation of the extracts to dryness, the residue was dissolved in 5 ml of methanol. A 10-μl volume of the solution was subjected to HPLC.

Cinchona bark. Cinchona bark powder (10.0 g) was suspended in 200 ml of chloroform and extracted for 4 h under alkaline conditions (ammonia, pH 10). After filtration, the residue was extracted again as described. The pooled extracts were concentrated to 100 ml, and then extracted with 30 ml of 1% sulphuric acid. After alkalization of the solution with ammonia (pH 10), it was extracted three times with 50 ml of chloroform. The pooled extracts were evaporated to dryness, and the residue was dissolved in 65 ml of chloroform–methanol (6:1). A 5-μl volume of the solution was subjected to HPLC.

Authentic samples

All the compounds were identified by comparison with authentic samples.

HPLC

The HPLC system consisted of a Spectra Physics SP-8700 solvent delivery system with a Rheodyne 7125 injection valve and the MCPD-350 system (Union Giken).

Conditions for licorice extracts: column, Senshu Pak 7C18H (ODS), 25 cm × 4.6 mm I.D.; eluent, linear gradient from acetonitrile–3% acetic acid (2:8) to acetonitrile–3% acetic acid (8:2) in 60 min, flow-rate 1.0 ml/min. Conditions for cinchona bark extracts: column, Senshu Pak SN-352N(AQUASIL), 25 cm × 4.6 mm I.D.; eluent, chloroform–methanol–water–25% ammonia (200:30:1.2:0.3), flow-rate 1.5 ml/min. All the solvents employed were HPLC grade.

RESULTS AND DISCUSSION

Licorice, the stolon or root of various species of Glycyrrhiza (Leguminosae), has been used both in the East and West as a sweetening agent and a herb drug, and it is marketed under the following trade-names:

Tongpei licorice *Glycyrrhiza uralensis* Fisch.
Sipei licorice
Sinkiang licorice
Russian licorice *G. glabra L.* var. *glandulifera* Reg. et Hard.
Iranian licorice *G. glabra L.* var. *violacea* Boiss.
Iraqi licorcice
Spanish licorice *G. glabra L.* var. *typica* Reg. et Hard.

These trade-names are mostly derived from the localities where the plant is found and not exactly equivalent to the botanical characters. The chemical constituents of licorice have been studied extensively, each compound by being identified by means of thin-layer chromatography (TLC)[8]. Recently we reported[9] a simultaneous separation of licorice principles by HPLC, and which has now been developed further to yield a three-dimensional (90°) UV-absorbance diagram and a contour line map as shown in Fig. 1. The main constituents of licorice which have a wide range of retention times, t_R, include chalcones, flavanones, their glucosides and glycyrrhizin. They are characterized respectively by their UV absorbance patterns in the three-dimensional (90°) diagram and the contour line map accompanied by the ordinary two-dimensional HPLC profiles. The constituents appearing on the HPLC diagram at t_R 9.53 and 10.32 min, respectively, are neoliquiritin and liquiritin, while the chalcone glucosides neoisoliquiritin and isoliquiritin appeared at t_R = 15.24 and 16.63 min respectively. Some minor components obscured by the large peaks of the chalcone glycosides were revealed on the contour line map. The main and common principle of licorice root, glycyrrhizin, appeared at t_R = 28.31 min. A specific principle of Sinkiang licorice, licochalcone A, was identified at t_R = 41.13 min.

Cinchona bark, a well known antimalarial drug, contains some quinoline alkaloids, quinine and its derivatives. We tried to analyse some typical alkaloids of

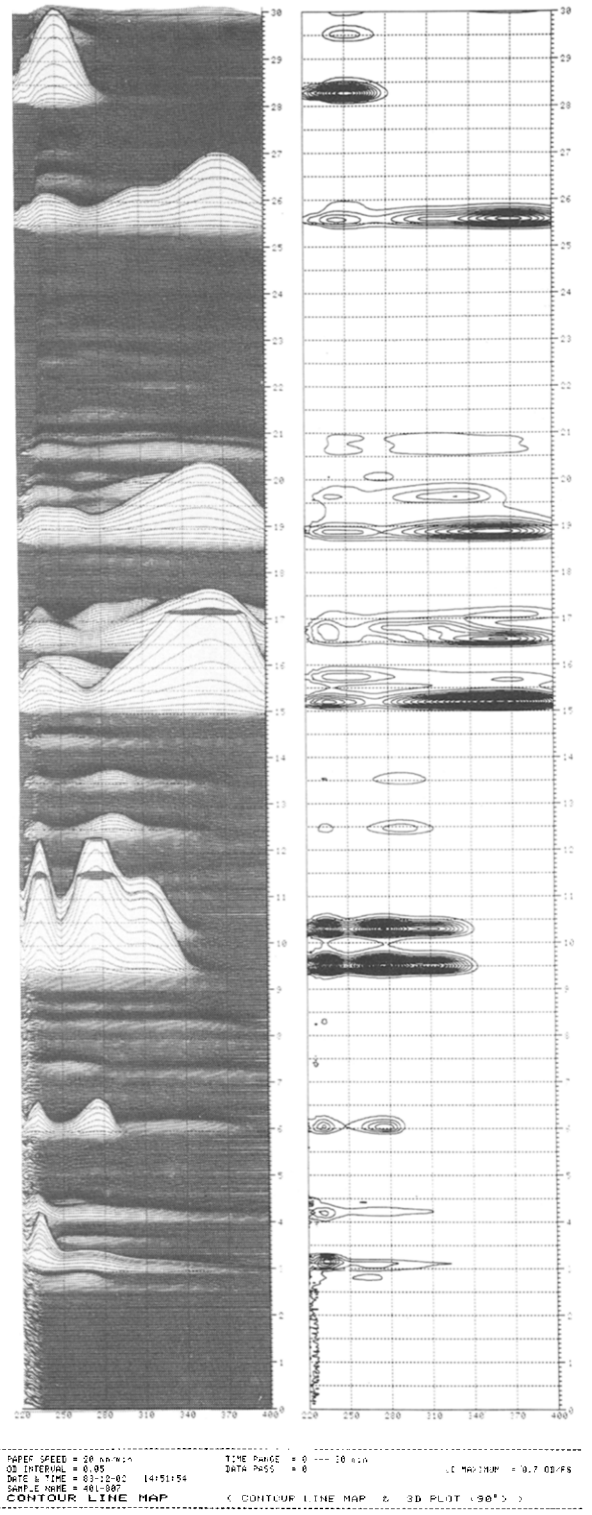
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Fig. 1.

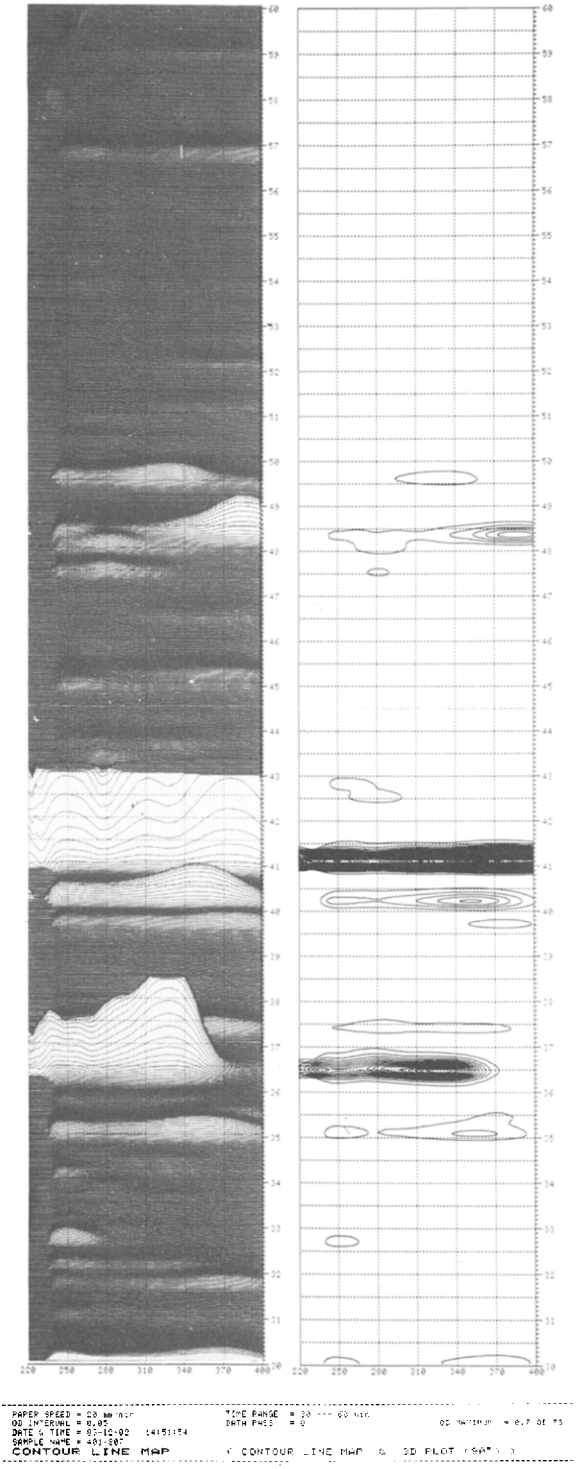

Fig. 1. Three-dimensional plot (90°) and contour line map for Shinkiang licorice extracts. Retention times (min): neoliquiritin (9.53); liquiritin (10.32); neoisoliquiritin (15.24); isoliquiritin (16.63); liquiritigenin (20.08); glycyrrhizin (28.31); isoliquiritigenin (30.59); licochalcone A (41.13).

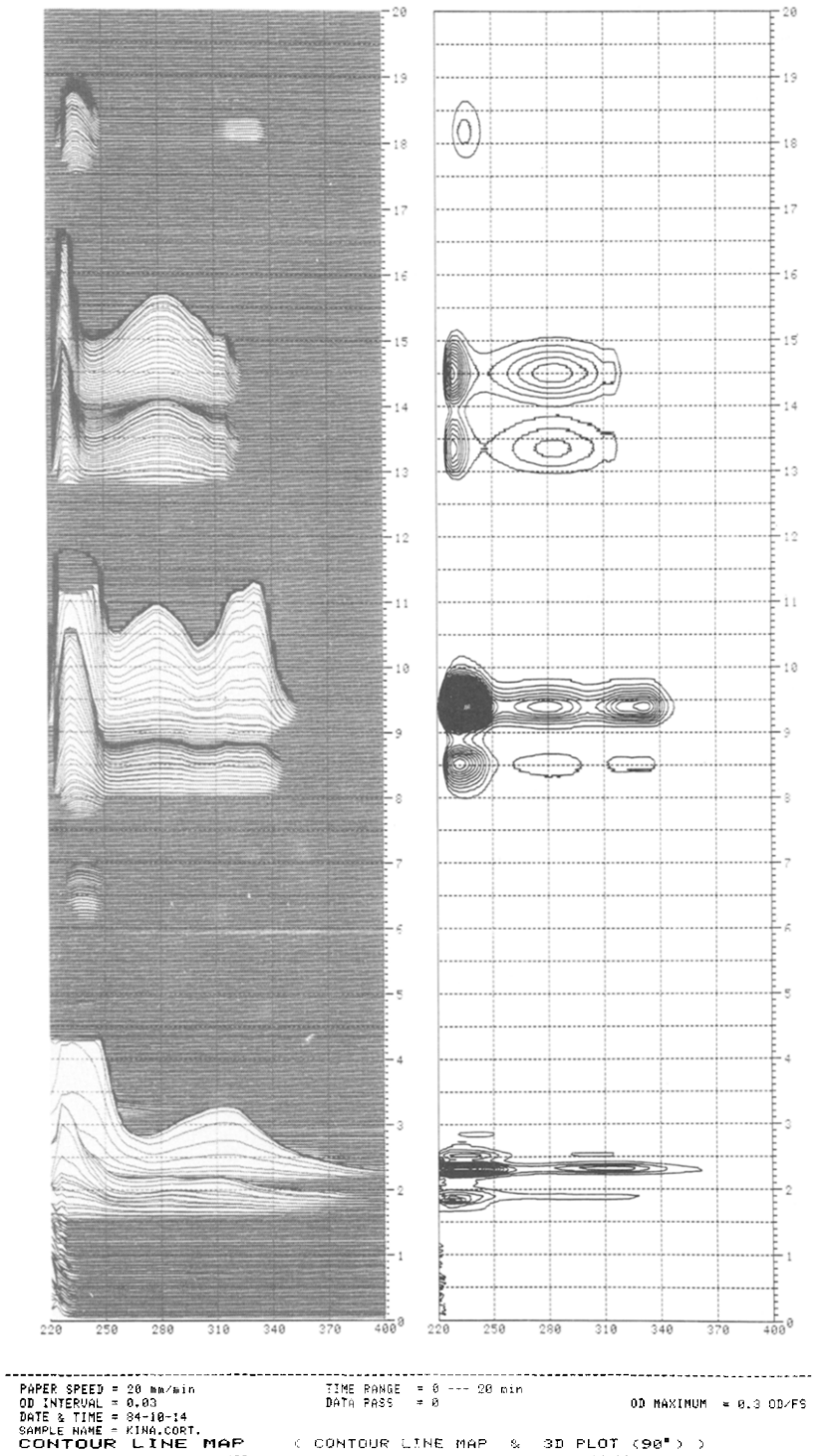

Fig. 2. Three-dimensional plot (90°) and contour line map for Cinchona bark extracts. Retention times (min): quinidine (8.48); quinine (9.40); cinchonidine (13.35); cinchonine (14.54).

Chincona bark by using an aqueous silica gel HPLC column (Aquasil) originally developed by us[10] for the separation of phenolic and terpenoid glycosides, as well as mono- and oligosaccharides[11]. As shown in Fig. 2, quinidine, quinine, cinchonine and cinchonidine were well separated on this column as twin peaks.

The technique described has the following advantages. In a single HPLC operation, the retention times and UV absorption curves of the components are obtained simultaneously. Even minute components in the plant extracts, previously detected by their UV absorptions in the range of 220–400 nm, are revealed on the chromatogram by the two- or three-dimensional display as well as by the contour line map.

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