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Note

Isocratic liquid chromatographic method for the simultaneous determination of *Passiflora incarnata* L. and *Crataegus monogyna* flavonoids in drugs

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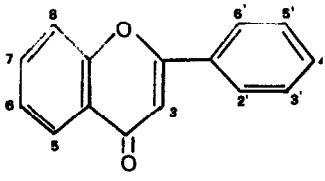
Passiflora incarnata L. and *Crataegus monogyna* extracts, frequently used in sedative preparations, are characterized by the presence of the C-glycoside flavonoids vitexin (I), isovitexin (II) and vitexin-4'-O-rhamnoside (III) (Fig. 1). While vitexin is present in both extracts, isovitexin[1] and vitexin-4'-O-rhamnoside[2] can be regarded as the characteristic constituents of *Passiflora incarnata* L. and *Crataegus monogyna* extracts, respectively.

High-performance liquid chromatographic (HPLC) methods for the analysis of each extract have been described[3,4]; nevertheless, owing to the difficulty of separating vitexin-4'-O-rhamnoside, there has been no report of the simultaneous and isocratic analysis of both extracts. Recently, a simple procedure was suggested for the fingerprinting of *Passiflora* and *Crataegus*[5], but the extension of this method to the mixture was unsuccessful.

From previous experience with the HPLC of flavonoids, we found that the customary solvent system of acetonitrile (methanol)–acetic acid (formic acid)–water[5–7] can be successfully replaced with 2-propanol–tetrahydrofuran–water. Using



Substance		OH	Structure
			Glycosyl *and* glycosyloxy
Vitexin	(I)	5,7,4'	8–β–D–glucopyranosyl
Isovitexin	(II)	5,7,4'	6–β–D–glucopyranosyl
Vitexin–4'–rhamnoside	(III)	5,7	8–β–D–glucopyranosyl, 4'–O–rhamnosyloxy
Hyperosid	(IV)	5,7,3',4'	3–β–D–galactopyranosyloxy

Fig. 1. Structure of the flavonoids.

this system on a C_{18} column, we succeeded in separating by isocratic elution vitexin-4'-O-rhamnoside and isovitexin in *Passiflora*- and *Crataegus*-containing drugs.

EXPERIMENTAL

Materials

Authentic samples of vitexin-4'-O-rhamnoside, vitexin and hyperosid were obtained from C. Roth (Karlsruhe, F.R.G.). Isovitexin was prepared from *Passiflora incarnata* L. extracts according to the literature[8].

Passiflora and *Crataegus* extracts were purchased from different commercial sources. Tetrahydrofuran, 2-propanol, methanol and water were of HPLC grade (Chromasolv, Riedel-de Haën, Hannover, F.R.G.). Sep-Pak C_{18} cartridges (Waters Assoc., Milford, MA, U.S.A.) were used for HPLC sample preparation.

Chromatographic conditions

HPLC was performed with a Waters M-6000 pump fitted with a μBondapak

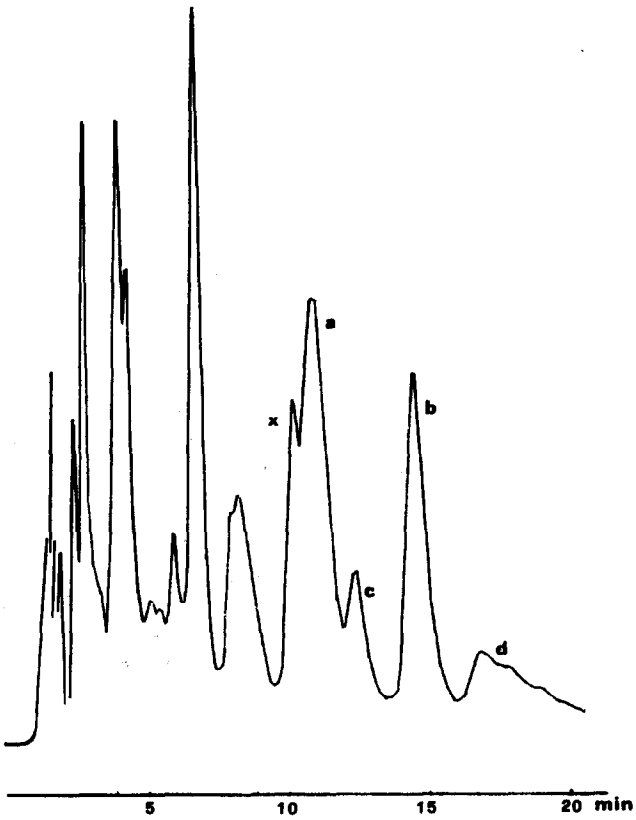


Fig. 2. HPLC of a mixture of *Passiflora incarnata* L. and *Crataegus monogyna* extracts (1:1). Column, μBondapak C_{18}; eluent, acetonitrile–water–acetic acid (18:82:1, v/v/v); flow-rate, 1 ml/min; UV detection, 340 nm. Peaks: (a) vitexin-4'-O-rhamnoside; (b) isovitexin; (c) vitexin; (d) hyperosid; (x) unknown compound from *Passiflora incarnata* L.

C_{18} column (30 cm × 3.9 mm I.D.) and a U6K injector. Peaks were monitored with a Waters M-440 absorbance detector at 340 nm (0.02 a.u.f.s.); output was measured on a Waters M-740 Data Module, using the external standard quantitation method. The eluent was 2-propanol–tetrahydrofuran–water (5:15:85, v/v) at a flow-rate of 1.5 ml/min.

Calibration graphs

Reference solutions consisting of a mixture of vitexin-4′-O-rhamnoside and isovitexin at concentrations from 10 to 50 μg/ml in 25% ethanol were prepared and stored at 4°C for 3 months without decomposition. These solutions (10-μl aliquots) were subjected to HPLC and the resulting chromatograms provided data for the calibration graphs.

Preparation of Passiflora and Crataegus samples

Passiflora and *Crataegus* extracts (100 mg) were treated with water (1 ml) and applied to a Sep-Pak C_{18} cartridge. After washing with water, the flavonoid fraction was eluted with 40% methanol (5 ml) to give a solution with a concentration in the range 10–30 μg/ml of vitexin-4′-O-rhamnoside and isovitexin.

Pharmaceutical products containing *Passiflora* and *Crataegus* extracts were treated similarly.

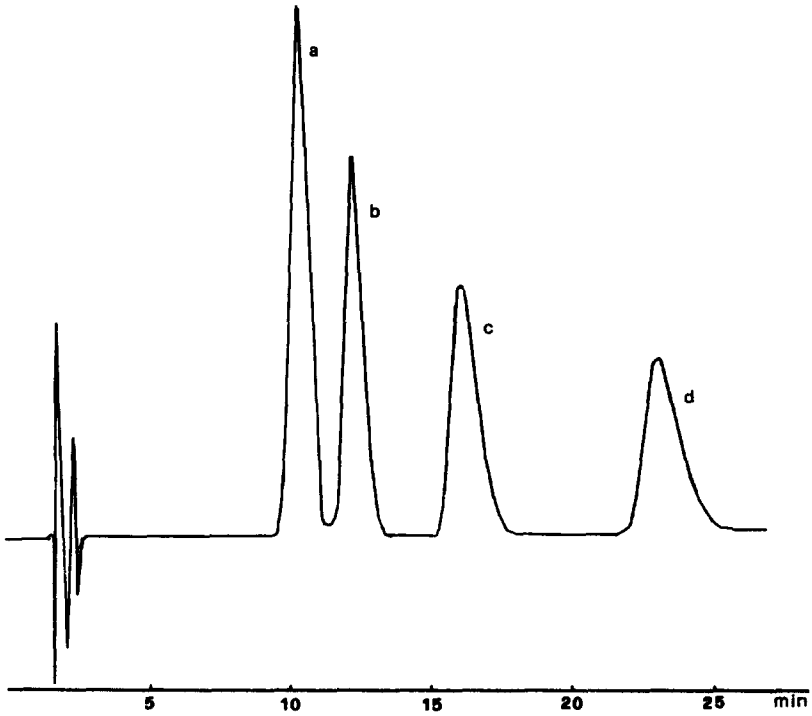


Fig. 3. Separation of standard, vitexin-4′-O-rhamnoside (a), isovitexin (b), vitexin (c) and hyperosid (d). Column, μBondapak C_{18}; eluent, 2-propanol–tetrahydrofuran–water (5:15:85, v/v/v); flow-rate, 1.5 ml/min; UV detection, 340 nm.

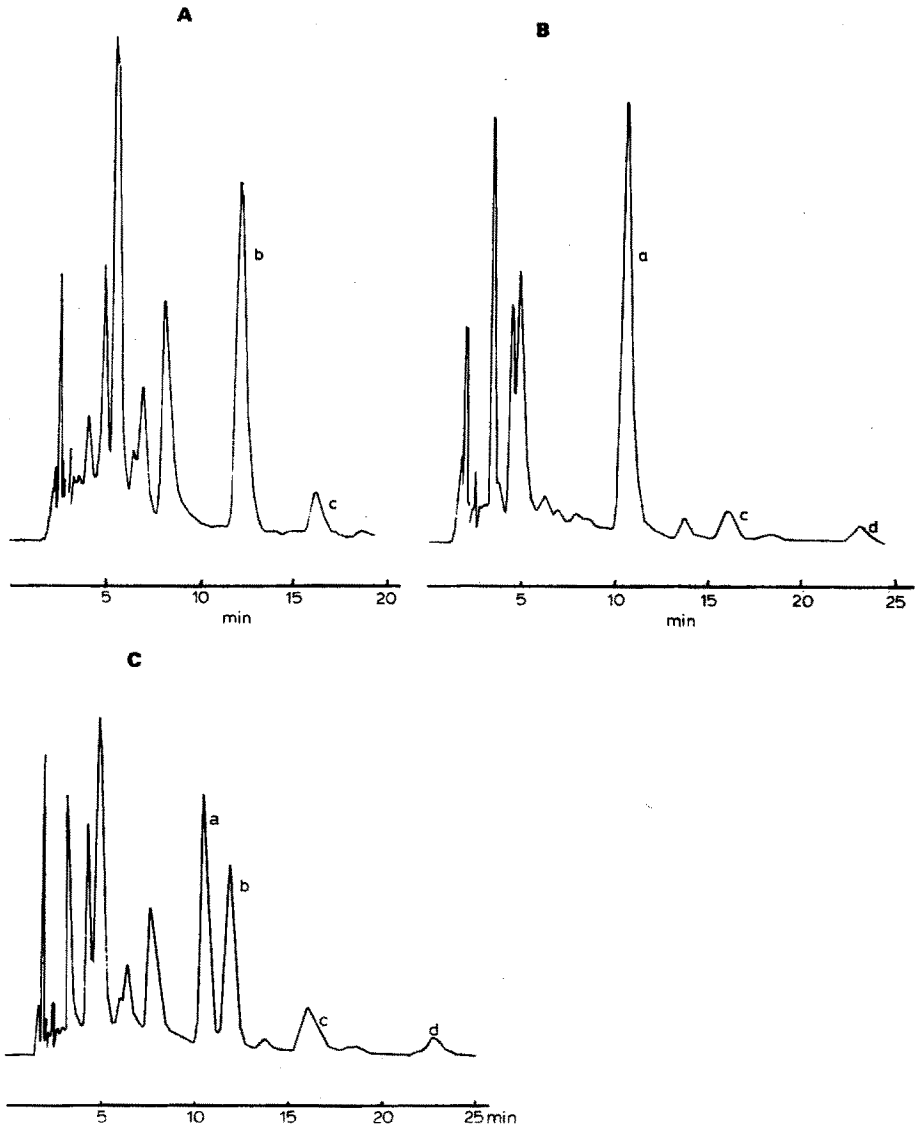


Fig. 4. Chromatographic separation of *Passiflora incarnata* L. extract (A), *Crataegus monogyna* extract (B) and a drug containing both extracts (C). Chromatographic conditions and peaks as in Fig. 3.

RESULTS AND DISCUSSION

As shown in Fig. 2, the acetonitrile–water–acetic acid (18:82:1, v/v) system does not allow the separation of the *Crataegus* vitexin-4′-O-rhamnoside from an unknown compound arising from *Passiflora*. This problem was been solved using the 2-propanol–tetrahydrofuran–water (5:15:85, v/v) as the eluent.

Fig. 3 shows the chromatographic separation of standard vitexin-4′-O-rhamnoside, isovitexin and vitexin with retention times of 10.5, 13 and 16.6 min, respec-

TABLE I

RECOVERY OF VITEXIN-4'-O-RHAMNOSIDE AND ISOVITEXIN ADDED TO DRUGS

Compound	*Present (μg/g)*	*Added (μg/g)*	*Recovered (μg/g) (mean ± S.D., n = 5)*	*Coefficient of variation (%)*
Vitexin-4'-O-rhamnoside	10.3	5	15.1 ± 0.5	3.3
	14.8	7.5	21.9 ± 0.8	3.65
	17.9	10	27.5 ± 0.9	3.27
	26.2	12.5	38.1 ± 1.5	3.94
Isovitexin	11.9	5	16.2 ± 0.5	3.09
	14.1	7.5	20.9 ± 0.8	3.82
	19.3	10	28.8 ± 0.9	3.12
	25.4	12.5	37.1 ± 1.1	2.96

tively. Under the described chromatographic conditions, hyperosid (IV) is eluted at 23.4 min.

Typical chromatograms obtained from *Passiflora* and *Crataegus* extracts and their pharmaceutical products (Fig. 4) show symmetrical, well resolved peaks with baseline resolution of all flavonoids. The retention times varied by less than 5% over a period of 2 months.

The detector response was linear over the range 100–500 ng of vitexin-4'-O-rhamnoside and isovitexin, as indicated by the following equations:
for isovitexin:

$$y = 0.0212\,x; \qquad r = 0.999$$

for vitexin-4'-O-rhamnoside:

$$y = 0.0267\,x + 0.15; \qquad r = 1.000$$

where x and y represent the amount injected (ng) and the peak height, respectively.

Drugs to which vitexin-4'-O-rhamnoside and isovitexin were added (5–12.5 μg/g) were analysed and the results are given in Table I.

In conclusion, the proposed eluent allows the simultaneous isocratic separation of *Passiflora* and *Crataegus* flavonoids and can be used in their routine determination in drugs.

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