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Note

Identification and determination of vitexin and isovitexin in *Passiflora incarnata* extracts

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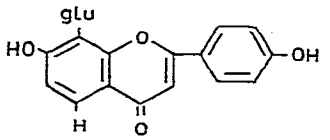
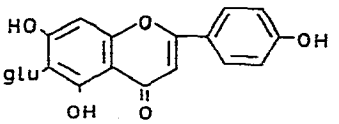
Extending our studies on the application of high-performance liquid chromatography (HPLC) to the analysis of medicinal plant extracts[1–4] that cannot easily be analysed by the usual chemical and instrumental methods, we have now examined some consituents of *Passiflora incarnata*. This plant, frequently used in sedative pharmaceutical preparations, is characterized by the presence of two series of components: harman-like alkaloids and flavonoid derivatives.

For the qualitative and quantitative control of alkaloids we can use some methods based on thin-layer and paper chromatography, fluorimetry and gas chromatography[5–9], and for flavonoids, particularly important because they allow identification of several species of *Passiflora*, the methods so far adopted are paper and thin-layer chromatography[10–20], column chromatography[21,22] and HPLC[23].

We considered the identification and determination by HPLC of two flavonoid derivatives typical of the *Passiflora incarnata*, *viz.*, vitexin and isovitexin, which are structural O-glucosidic isomers of each other.



Vitexin



Isovitexin

The chemical and physical properties of standards (mean polarity and good solubility in hot water–methanol solutions) led us to investigate bonded-phase chromatography with reversed phases. A column packed with Zipax ODS-Permaphase (octadecylsilane bonded to an inert support with particle size 40–50 μm) was tested, but the standards were not separated because the capacity factor (k') was too low. We then performed the separation on a Zorbax ODS column, with totally porous microparticles, which gave a lower HETP and a considerable increase in the number of theoretical plates. As the standards were not separated at room temperature, the high temperature of column operation was a considerable advantage.

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EXPERIMENTAL*

Qualitative analysis

After separating vitexin and isovitexin standards, we identified them in *Passiflora incarnata* extracts by comparison of retention times and subsequent enrichment of the sample. A DuPont 820 instrument with a Zorbax ODS column (25 × 2.0 mm I.D.) was used, operated at a column temperature of 75° and a reservoir temperature of 45°. The mobile phase was a concave exponential n. 5** gradient increasing at 3% per min from 15% to 95% of methanol in water. The column pressure was 1500 p.s.i., and the flow-rate 0.5 ml/min. UV detection was carried out at 270 nm and the sensitivity of the detector was 0.32 a.u.f.s. The chart speed was 5 min/in.

A separation of two standards is shown in Fig. 1 [injection of 7 μl of a 0.2% solution of vitexin and isovitexin, respectively, with water–methanol (1:1) as the solvent]. The order of elution is vitexin followed by isovitexin and the total time of analysis is 25–30 min.

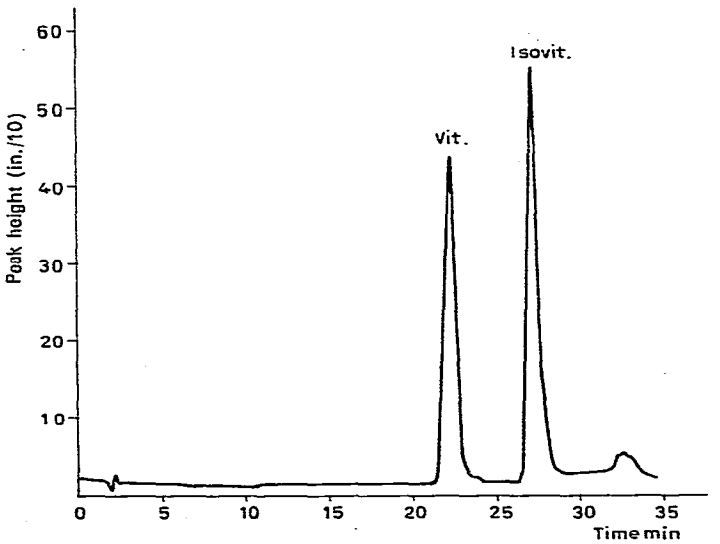

Fig. 1. Separation of vitexin and isovitexin standards on a Zorbax ODS column. Temperature, 75°; pressure, 1500 p.s.i.; mobile phase, a concave gradient of methanol in water, programmed from 15 to 95% methanol at a rate of 3%/min.

The shape of the gradient is a concave exponential n. 5, the composition of the phases changing according to the equation $C = Kt^5$, where C is the concentration of B in A and t is reduced time (time/time for total gradient).

Figs. 2 and 3 show chromatograms of 7 μl of a 0.2% water–methanol solution of a pilular extract and of 6 μl of a 0.2% water–methanol solution of a purified powdered extract, respectively. Isovitexin can be identified as the last peak; vitexin

* With the co-operation of Miss P. Papetti.

** n. 5 is the instrument setting corresponding to the most concave gradient.

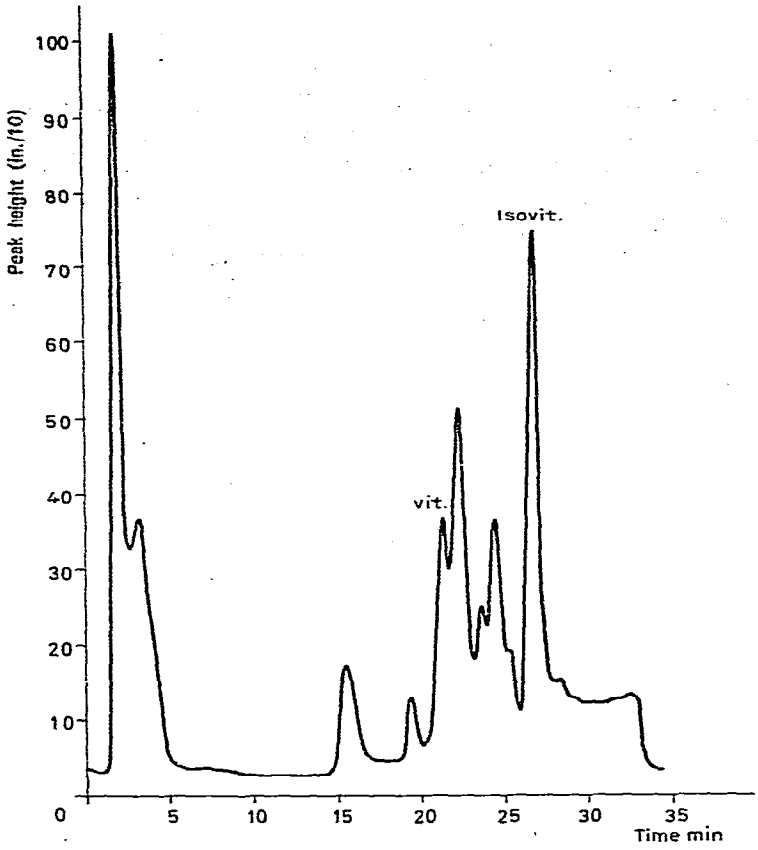

Fig. 2. Chromatogram of a methanolic solution of *Passiflora incarnata* pilular extract. Conditions as described for the separation of the standards.

shows a poor separation from an unknown peak, but it is possible to achieve a better separation using the experimental conditions given for the determination of vitexin.

Determination of isovitexin

The quantitative determination of vitexin under the above conditions was studied using external standardization. We obtained a linear detector response in the range 0.8–2 μg of sample, with consecutive injections of a 0.2% solution of isovitexin in water–methanol (1:1) (Fig. 4). Ten determinations were carried out for pilular and powdered extracts in order to test the precision of the method in terms of the standard deviation, coefficient of variation and confidence limit.

For the powdered extract, the mean content of isovitexin was 1.332%, with a standard deviation of 0.022% and a coefficient of variation of 1.65%. With $P = 95\%$ and $n - 1 = 9$ degrees of freedom, the value of t is 2.262 and the confidence limit is 0.05.

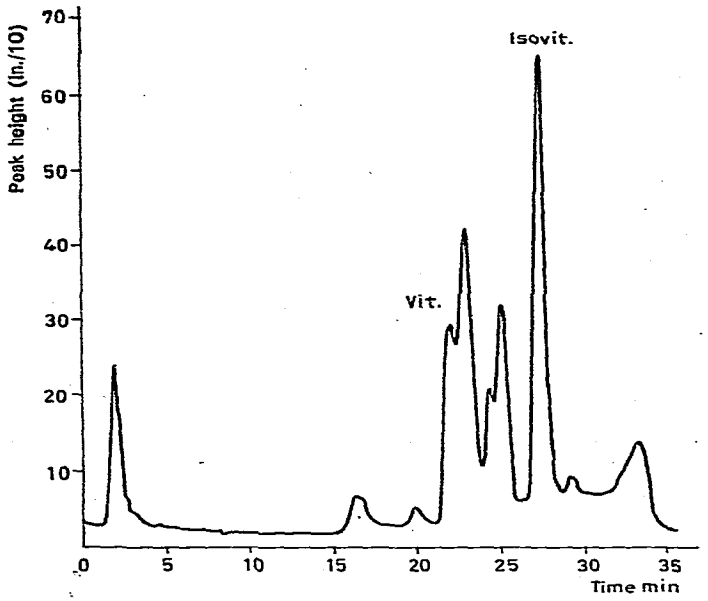

Fig. 3. Chromatogram of a methanolic solution of *Passiflora incarnata* powdery extract. Conditions as described for the separation of the standards.

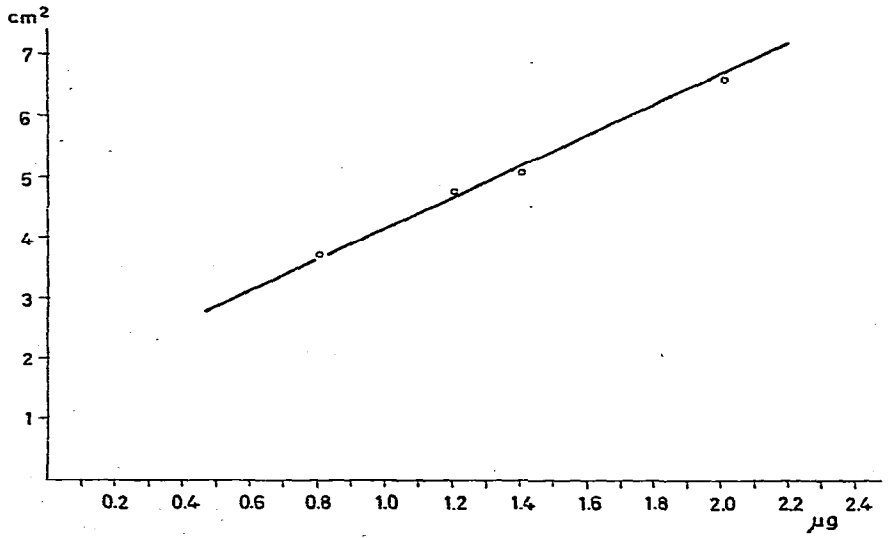

Fig. 4. Plot of the detector response *versus* the amount of isovitexin (range 0.8–2 µg). Conditions as described for the separation of the standards.

For the pilular extract, the mean content of isovitexin was 14.19% with a standard deviation of 0.277% and a coefficient of variation of 4.95%. With $P = 95\%$ and $n - 1 = 9$ degrees of freedom, the value of t is 2.262 and the confidence limit is 0.63.

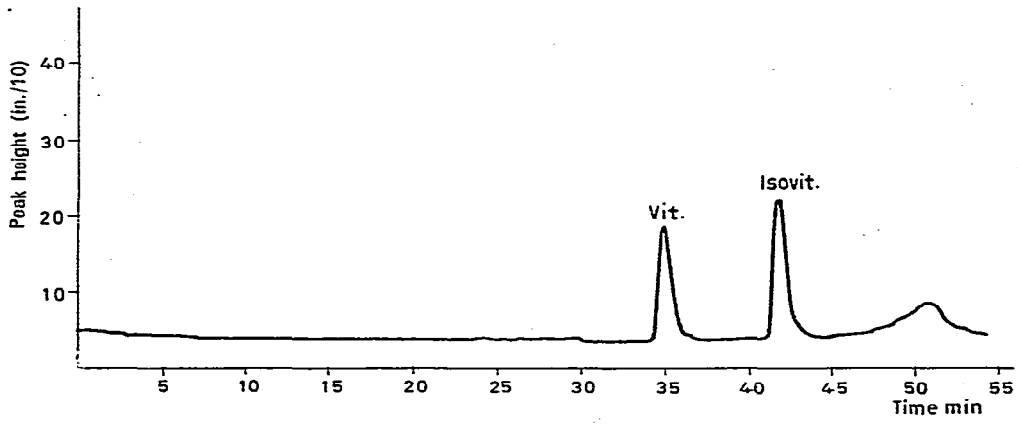

Fig. 5. Separation of vitexin and isovitexin standards on a Zorbax ODS column. Temperature, 75°; pressure, 1000 p.s.i.; mobile phase a concave gradient of methanol in water, programmed from 15 to 95% methanol at a rate of 2% per min.

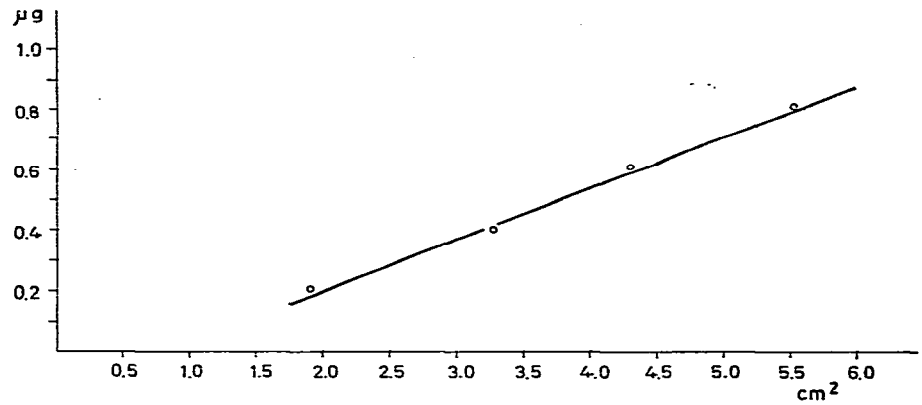

Fig. 6. Plot of the detector response *versus* the amount of vitexin (range 0.2–0.8 μg). Conditions as described for the separation of the standards.

The mean number of theoretical plates was 6,700 and the HETP value was 0.037 mm.

Determination of vitexin

The operating conditions were modified in order to achieve a better separation between vitexin and an unidentified peak with nearly the same retention time. The mobile phase was a concave exponential n. 5 gradient increasing at 2% per min from 15% to 95% of methanol in water, and the column pressure was 1000 p.s.i.

The chromatogram in Fig. 5 shows the separation of standards. Under such conditions it is possible to determine vitexin satisfactorily, increasing the resolution from about 0.58 (Fig. 2) to about 0.82 (Fig. 7).

We established the linearity of the detector response by injecting fixed volumes (20 μl) of solutions containing increasing amounts of vitexin from 0.2 to 0.8 μg (Fig. 6) by means of a Rheodyne Model 7120 loop-type injector.

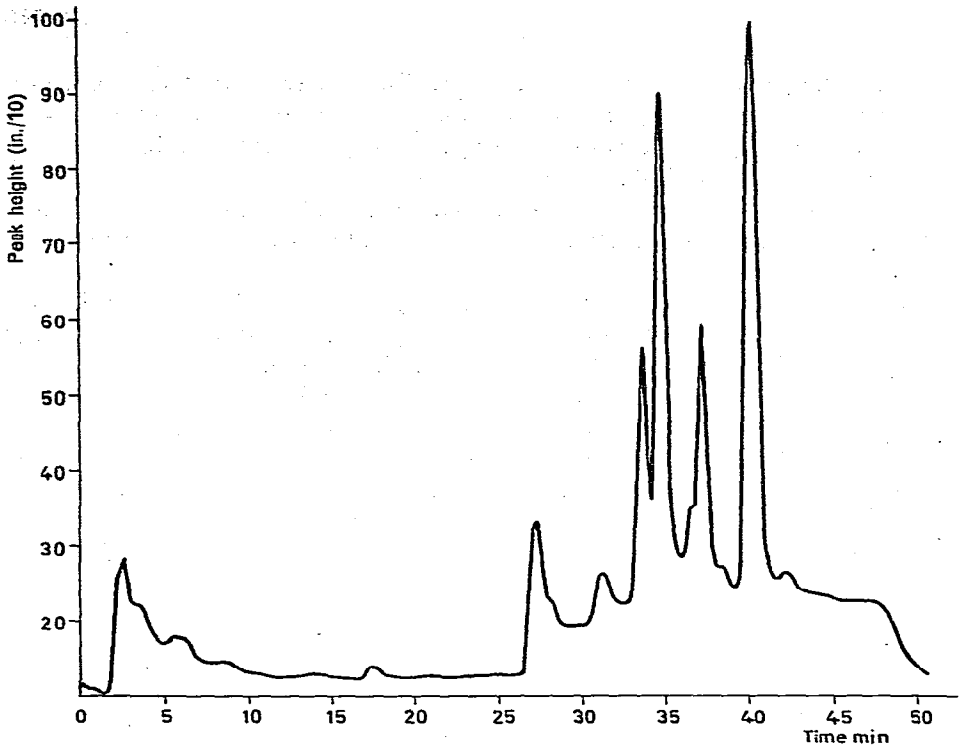

Fig. 7. Chromatogram of a methanolic solution of *Passiflora incarnata* powdery extract. Conditions as described for the separation of the standards.

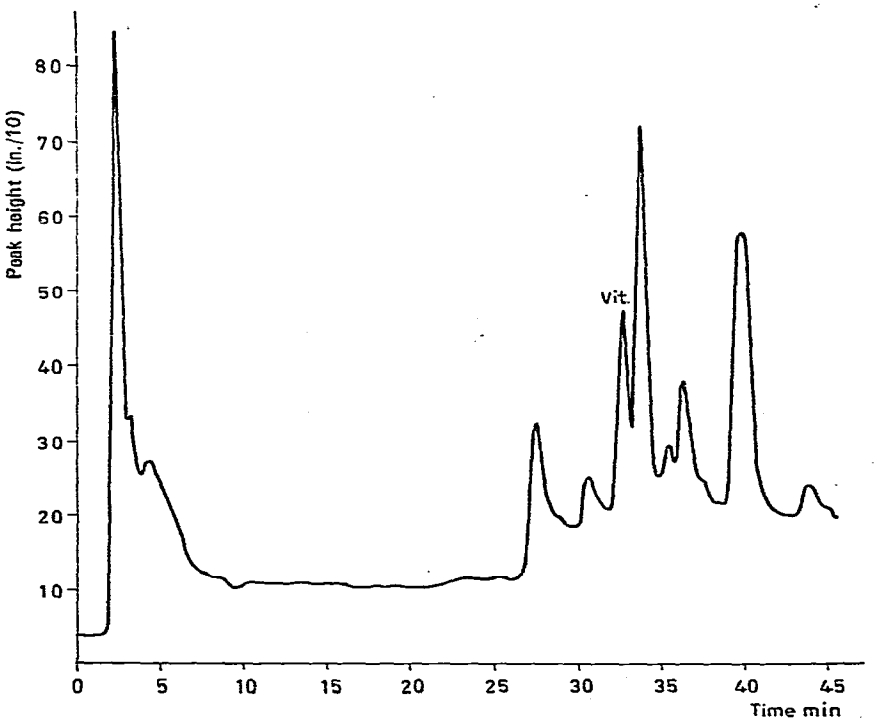

Fig. 8. Chromatogram of a methanolic solution of *Passiflora incarnata* pilular extract. Conditions as described for the separation of the standards.

The linear range for vitexin (0.2–0.8 μg) is different from that for isovitexin (0.8–2 μg) owing to the smaller amount of vitexin in the extracts and in order to avoid a decrease in the efficiency of the column with a consequent decrease in resolution between the sample and unidentified peaks.

Figs. 7 and 8 show chromatograms of 20 μl of a 0.1% water–methanol solution of powdered extract and of 20 μl of a 0.5% water–methanol solution of a pilular extract, respectively.

For the pilular extract, the mean content of vitexin was 0.226% with a standard deviation of 0.005% and a coefficient of variation of 2.2%. With $P = 95\%$ and $n - 1 = 9$ degrees of freedom, the value of t is 2.262 and the confidence limit is 0.011.

For the powdered extract, the mean content of vitexin is 2.24% with a standard deviation of 0.026% and a coefficient of variation of 1.16%. With $P = 95\%$ and $n - 1 = 9$ degrees of freedom, the value of t is 2.262 and the confidence limit is 0.059.

We intend to extend this work to the identification of unknown components in the chromatograms, whether they are glucosidic flavonoids (orientin, isoorientin, saponarin) or harman-like alkaloids, the presence of which is indicated in the literature.

REFERENCES

1 V. Quercia, *Boll. Chim. Farm.*, 115 (1976) 309.
2 V. Quercia, G. Percaccio and V. Cuozzo, *Studio per HPLC di Derivati Diidrossiantrachinonici in Estratti Vegetali di Interesse Farmaceutico*, Seregni, Paderno Dugnano, Milano, 1976.
3 V. Quercia, L. Turchetto, V. Cuozzo and Donatelli, *Boll. Chim. Farm.*, 115 (1976) 810.
4 Terracciano, Gambero, G. Percaccio, Donatelli and V. Quercia, *Boll. Chim. Farm.*, 116 (1977) 402.
5 S. Lutomski, Z. Kowslewski, K. Drost and M. Kuchxsha, *Herba Pol.*, 14 (1968) 23 and 13 (1967) 44.
6 W. Messerschmidt, *J. Chromatogr.*, 33 (1968) 551.
7 E. Bennoti and E. Fedeli, *Boll. Chim. Farm.*, 107 (1968) 716.
8 E. Bennoti, *Boll. Chim. Farm.*, 106 (1967) 756.
9 W. Poetke, C. Schwarz and M. Gerlack, *Planta Med.*, 18 (1970) 303.
10 J. Löhdefink, *Deut. Apoth. -Ztg.*, 116 (1976) 557.
11 M. Schilcher, *Deut. Apoth.-Ztg.*, 107 (1967) 849.
12 J. Lutomski and M. Adamska, *Herba Pol.*, 14 (1968) 249.
13 Von B. Glotzbach and M. Rimpler, *Planta Med.*, 16 (1968) 1.
14 M. Schicher, *Z. Naturforsch. B.*, 23 (1968) 1393.
15 W. Poethke, C. Schwarz and M. Gerlach, *Planta Med.*, 19 (1970) 177.
16 B. H. Somaroo, M. L. Thakur and W. F. Grant, *J. Chromatogr.*, 87 (1973) 290.
17 L. Reio, *J. Chromatogr.*, 88 (1974) 119.
18 J. F. Gonnet, *J. Chromatogr.*, 86 (1973) 192.
19 J. Lutomski, B. Malek and Z. Stachowiak, *Planta Med.*, 26 (1974) 311.
20 M. G. Krishnamurty and L. Krishnaswami, *J. Chromatogr.*, 114 (1975) 286.
21 P. G. Pifferi, F. Baldassari and O. Gandolfi, *J. Chromatogr.*, 88 (1974) 381.
22 M. Szumilo and E. Soczewinski, *J. Chromatogr.*, 124 (1976) 394.
23 L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, 116 (1976) 271.