Journal of Chromatography, 129 (1976) 464–465

CHROM. 9396

Note

High-performance liquid chromatography of some anthocyanidins and flavonoids

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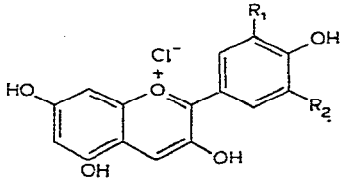
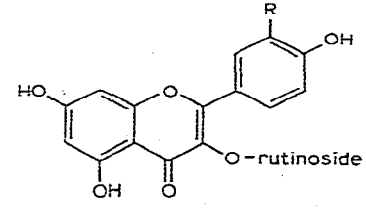
(First received March 15th, 1976; revised manuscript received May 31st, 1976)

Wulf and Nagel[1] recently described the high-pressure liquid chromatographic (HPLC) separation of some phenolic acids and flavonoids of interest in the analysis of wine during aging[2]. An earlier report[3] described separation of some phenolics from beer. We have independently used a system similar to that of Wulf and Nagel to analyze a mixture of three common anthocyanidins (I–III) and a mixture of three closely related flavonoid glycosides (IV–VI).





I: $R_1 = R_2 = OH$ (delphinidin)
II: $R_1 = OH$, $R_2 = H$ (cyanidin)
III: $R_1 = R_2 = H$ (pelargonidin)

IV: R = OH (quercetin-3-O-rutinoside)
V: R = H (kaempferol-3-O-rutinoside)
VI: R = OCH_3 (isorhamnetin-3-O-rutinoside)

MATERIALS AND METHODS

Chemicals

Delphinidin and pelargonidin chlorides were purchased from Fluka (Buchs, Switzerland) and cyanidin chloride from Aldrich (Milwaukee, Wisc., U.S.A.).

Apparatus

Analyses were performed on a Waters Assoc. (Milford, Mass., U.S.A.) ALC 301 with a 280 nm ultraviolet detector at room temperature and a pressure of 2500 p.s.i. We used a 30 cm × 4 mm I.D. column packed with μBondapak C_{18}/Corasil and a 65 cm × 10 mm I.D. column packed with Bondapak C_{18} (Waters Assoc.). The μBondapak is a chemically-bonded porous particle material with 10-μm particles and a surface area of 400 m^2/g. The Bondapak is a chemically-bonded porous layer bead, 37–50 μm in size and 7 m^2/g.

RESULTS AND DISCUSSION

The three anthocyanidins were separated on the μBondapak column with methanol–acetic acid–water (20:5:75) as eluent, a flow-rate of 2.4 ml/min and t_0 of 1.5 min. Relative retentions were 2.5 min (I), 13 min (II) and 29 min (III).

The three flavonol glycosides were separated using the two columns in series (Bondapak followed by μBondapak) with methanol–acetic acid–water (30:5:70) as eluent, a flow-rate of 2.0 ml/min and t_0 of 11 min. Relative retentions were 17 min (IV), 27 min (V) and 33 min (VI). A single column of either type did not give good resolution. Employment of the columns in series corrected this problem.

ACKNOWLEDGEMENT

We are grateful to Dr. B. A. Bohm for the samples of the three flavonol-3-O-rutinosides.

REFERENCES

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