Journal of Chromatography, 295 (1984) 506–509
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,828

Note

Analysis of plant phenolics by high-performance liquid chromatography using a polystyrene–divinylbenzene resin column

J. GEORGE BUTA

Plant Hormone Laboratory, Plant Physiology Institute, U.S. Department of Agriculture, Beltsville, MD 20705 (U.S.A.)

(First received March 6th, 1984; revised manuscript received April 11th, 1984)

Numerous papers have been published recently on high-performance liquid chromatographic (HPLC) separations of polyphenolic compounds using reversed phase systems[1–4]. Most of these methods have been developed using the octadecyl carbon chain moiety bound to silica as a column packing. Another reversed-phase column packing for HPLC is a styrene–divinylbenzene copolymer resin, which has been used in various chromatographic analyses, including separations of chlorophenols and aromatic acids[5–7]. The latter investigation[7] dealt with the chromatographic behaviour exhibited by analytical standards of several hydroxy benzoic and cinnamic acids. Also of special interest was the finding by Lee[8] that little degradation of the styrene–divinylbenzene column occurred with the use of a wide range of solvents having different pH values. The stability of this column packing suggested its use for those applications where conditions such as major changes in solvent polarity and pH are needed. Studies involving the analysis of the many phenolics in minimally purified plant extracts require these somewhat harsh procedures for the removal of the many compounds not easily eluted with the particular chromatographic conditions employed.

The purpose of this study was to determine whether a styrene–divinylbenzene HPLC column could be used for the separation of plant phenolic acids, flavanoids and their derivatives in a manner comparable to the reversed-phase octadecyl-silica column. The method developed was examined and found applicable for the analysis of phenolic compounds present in a hydrolyzed plant leaf extract.

EXPERIMENTAL

Apparatus

A Varian 5060 liquid chromatograph equipped with a Waters 450 variable wavelength detector set at 280 nm (detector sensitivity 0.04 a.u.f.s.), a Rheodyne 7125 sample injector (100-μl loop), a Hewlett-Packard 3390 A reporting integrator, and a HPLC Technology (Lomita, CA, U.S.A.) analytical column (250 × 5.0 mm I.D.) packed with Hamilton PRP-1 styrene-divinylbenzene resin (10-μm) was used in this study.

0021-9673/84/$03.00

Elution

Two solvents were used: (A) formic acid–water (0.5:99.5); (B) acetonitrile. The elution profile was: 0–5 min, 10–15% B in A (linear gradient); 5–10 min, 15% B in A (isocratic); 10.1–25 min, 20% B in A (isocratic); 25–30 min, 20–35% B in A (linear gradient); 30–40 min, 35–40% B in A (linear gradient); 40–45 min, 40–10% B in A (linear gradient). The flow-rate was 1.5 ml/min.

Standards

The compounds obtained from commercial sources were combined in a 10^{-2} *M* standard solution in methanol.

Plant phenolics

Five grams of leaf tissue of soybean *Glycine max* L. (var. Williams) was extracted in 30 ml of 2% acetic acid at 85°C for 20 min. The filtered extract was made up to 1 *M* HCl and hydrolyzed for 1 h at the same temperature, then extracted twice with equal volumes of ethyl acetate. After filtration (0.45 μm) and drying with a nitrogen stream, the residue was dissolved in 0.5 ml of methanol.

RESULTS AND DISCUSSION

Table I shows the retention times of a number of polyphenolics, including benzoic and cinnamic acids as well as flavanoids and their derivatives, representative of the types of these compounds present in plant leaf tissue. These compounds were separated using the 10-μm styrene–divinylbenzene column and the acetonitrile–dilute formic acid solvent system.

TABLE I

RETENTION TIMES OF SOME PHENOLIC ACIDS, FLAVANOIDS AND DERIVATIVES PRESENT IN PLANT LEAVES

Compounds	$t_{R(min)}$
Gallic acid	3.56
Protocatechuic acid	6.12
Chlorogenic acid	8.63
Catechin	8.84
p-Hydroxybenzoic acid	9.84
Epicatechin	10.54
Caffeic acid	11.31
Vanillic acid	12.41
p-Coumaric acid	17.01
Quercitrin	19.30
Ferulic acid	19.60
Sinapic acid	20.16
o-Coumaric acid	24.06
Daidzein	33.35
Salicylic acid	34.70
Quercetin	34.97
Kaempferol	39.83

Fig. 1 shows the separation of standard phenolic acids and flavanoids that were reported to be present in hydrolyzed soybean leaf tissue[9]. Retention times of the compounds varied less than 1% in successive separations after a 15-min period of equilibration. Baseline separations were achieved with most of the compounds. However ferulic (4-hydroxy-3-methoxycinnamic) acid and sinapic (3,5-dimethoxy-4-hydroxycinnamic) acid were not well resolved and salicylic acid was not well separated from quercetin with these chromatographic conditions. The order of elution was the same as that reported for an octadecyl-silica column using a dilute formic acid–methanol system[1]. This solvent system was examined but no resolution of the ferulic–sinapic acid pair could be achieved. Otherwise this solvent system would be useful as an alternative to the acetonitrile–formic acid system with the sytrene–divinylbenzene column.

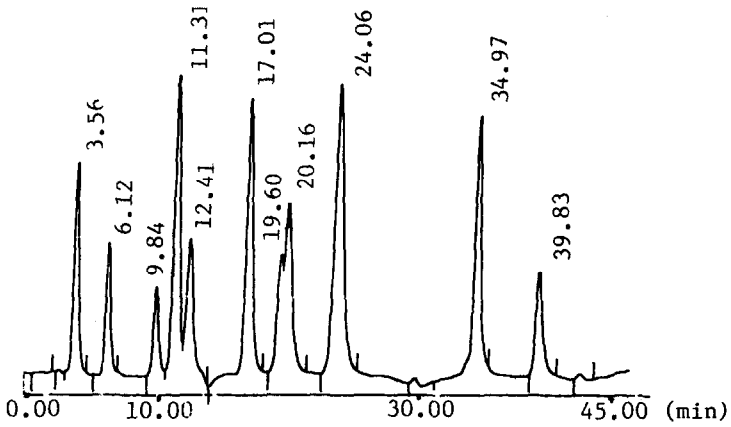

Fig. 1. The retention time of phenolic compounds (10 nmoles) eluted with an acetonitrile–formic acid gradient solvent system from a polystyrene–divinylbenzene column (250 × 5.0 mm I.D.). Description of the eluting system in Experimental. The retention times of the standard compounds are listed in Table I.

Fig. 2 shows the separation of polyphenolics present in the ethyl acetate extract of hydrolyzed soybean leaf tissue. A number of compounds with retention times comparable to standard phenolic acids and flavanoids were collected and their UV spectra determined. The compounds identified were caffeic, *p*-coumaric and ferulic acid followed by daidzein, quercetin and kaempferol. However, the major compound eluting at 5.31 min was not gallic acid as indicated previously[9]. This compound along with several others present in smaller quantities remains to be identified.

The styrene–divinylbenzene column is useful for obtaining a reproducible chromatographic profile of the phenolic compounds present in hydrolyzed plant material in a relatively short time after minimal sample preparation. The column described has been used for six months for analyses of various types of plant extracts, hydrolyzed as well as non-hydrolyzed, with no noticeable changes in retention times of eluted compounds. The chromatographic separation of the phenolics obtained is comparable, generally, to that obtained using a reversed-phase octadecyl–carbon column, but there does appear to be somewhat less resolution of the methoxylated

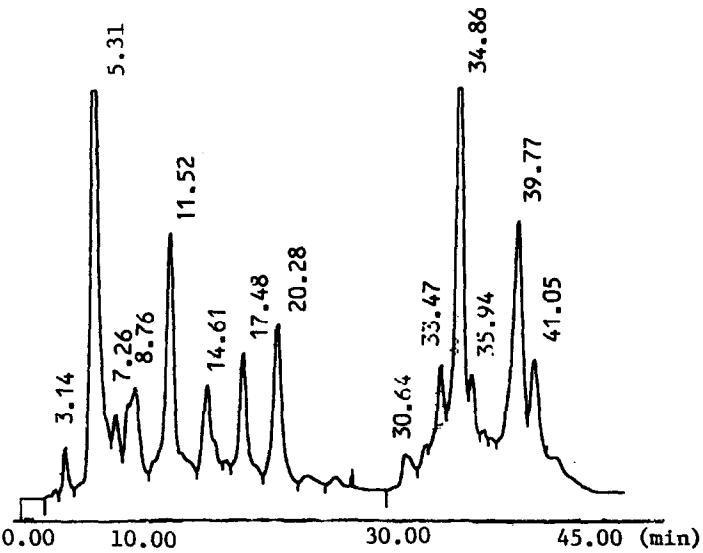

Fig. 2. The retention times of phenolic compounds extracted from the leaf tissue of soybeans (Williams) after acid hydrolysis. Chromatographic conditions are the same as for the separation of the standards. The amount of hydrolyzed extract injected is equivalent to that obtained from 100 mg of leaf tissue.

phenolic (ferulic–sinapic) acids than with the reversed-phase C_{18} system. These results indicate the potential applications of this reversed-phase chromatographic system in the separation of phenolic compounds present in plant material.

REFERENCES

1 K. Vande Casteele, H. Geiger and C. F. Van Sumere, *J. Chromatogr.*, 258 (1983) 111.
2 J. M. Andersen and W. B. Pedersen, *J. Chromatogr.*, 259 (1983) 131.
3 C. A. Stutte and J. M. Hardin, *J. Chromatogr.*, 248 (1982) 446.
4 V. Lattanzio, *J. Chromatogr.*, 250 (1982) 143.
5 H. A. McLeod and G. Laver, *J. Chromatogr.*, 244 (1982) 385.
6 T. Hanai, H. F. Walton, J. D. Navratil and D. Warren, *J. Chromatogr.*, 155 (1978) 261.
7 T. Hanai, K. C. Than and J. Hubert, *J. Chromatogr.*, 239 (1982) 385.
8 D. P. Lee, *J. Chromatogr. Sci.*, 20 (1982) 203.
9 J. B. Murphy and C. A. Stutte, *Anal. Biochem.*, 86 (1978) 220.