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Journal of Chromatography A, 680 (1994) 181–185

JOURNAL OF CHROMATOGRAPHY A

Analysis of isoflavones by capillary electrophoresis

Z.K. Shihabi*, T. Kute, L.L. Garcia, M. Hinsdale

Pathology Department, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157, USA

Abstract

A simple capillary electrophoresis method is described for the assay of several isoflavones and coumestrol isolated from plant extracts. The method has good reproducibility; it compares well to HPLC, and it can be performed in less than 10 min.

1. Introduction

Phytoestrogens are common in many plant species. They resemble estrogens and mimic some of their functions. Recently, interest in these compounds has increased considerably because many of them have been found to inhibit tumor growth *in vitro* [1–3] and *in vivo* [4,5]. For example, the isoflavone genistein specifically inhibits tyrosine kinase [6] and DNA topoisomerase [7]. However, analysis of these compounds from plant extracts is difficult, often requiring a prior derivatization of the compounds before analysis. Some of the methods used to analyze these compounds include HPLC [8] and GC–MS [9,10]. Here, we show that these compounds can be analyzed from plant extracts easily and rapidly by capillary electrophoresis (CE).

2. Materials and methods

2.1. Chemicals

3-Methyl-1-isobutylxanthine was obtained from Aldrich (Milwaukee, WI, USA). Coumestrol was obtained from Eastman Kodak (Rochester, NY, USA). The isoflavone standards were gifts from Dr. S. Barnes, University of Alabama, Birmingham, AL, USA. The HPLC column was obtained from E. Merck (Gibbstown, NJ, USA).

2.2. CE Equipment

A CE instrument (Beckman Instruments, Fullerton, CA, USA), was set at 254 nm. The capillary, 50 cm × 50 μm I.D., was rinsed after each run with 1 *M* NaOH for 1 min, and filled for another minute with 200 m*M* borate buffer, pH 8.6 (the running buffer). The voltage was set at 13 kV, and the sample was introduced by pressure injection for 10 s.

2.3. Sample extraction

Soy beans or other plant parts were homogenized to a fine powder, and 15-mg samples were vortex-mixed in a centrifuge tube with 1 ml of extracting solution. The extracting solution consisted of 66% acetonitrile, 0.4% NaCl and 30 mg/l of methyl isobutyl xanthine, as an internal standard, in water. The sample was centrifuged for 15 s at 14 000 *g* in a Model B Microfuge

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00307-U

(Beckman Instruments, Palo Alto, CA, USA), and the aliquot was introduced into the instrument. The isoflavones are not very soluble in aqueous or organic solvents; however, we found that a mixture of water and acetonitrile improved their solubility.

2.4. HPLC Equipment

For comparison studies, samples were also measured by HPLC. A Model 110 A pump (Beckman Instruments, Fullerton, CA, USA), was used to deliver the mobile phase at 1.2 ml/min through a C_8, 5-μm cartridge column, 125 mm × 4 mm I.D. The sample was introduced through a 20-μl loop and eluted isocratically for daidzin and genistin with a mobile phase of 16% acetonitrile in 20 m*M* phosphate buffer, pH 7.2. To elute daidzein and genistein, the mobile phase was 23% acetonitrile in the same phosphate buffer.

3. Results and discussion

A good baseline separation of five different isoflavones, in addition to coumestrol, is achieved in less than 10 min as illustrated in Fig. 1A. Fig. 1B shows the electropherogram from a soy bean extraction. It indicates the presence of isoflavones, as well as, other UV-absorbing compounds. Optimum separation occurs at a pH range of 8.5–8.8. Above this range, the migration time increases, and the daidzin peak becomes very broad. At lower pH, daidzein and genistein migrate very close to biochanin A (4′-methoxygenistein). The linearity of the test was determined using genistein. The response is linear from 0.4 to 60 mg/l (Fig. 2). The minimum detectable limit (3 S.D. above baseline) for genistein is 0.4 mg/l. The reproducibility of the migration time and the peak height for all of these compounds is good as summarized in Table 1. The table shows that the R.S.D. for both the migration time and peak height improves, in general, when the calculation is based on the internal standard. For example, the R.S.D. (n = 10) for the peak height of daidzein is 4.46%,

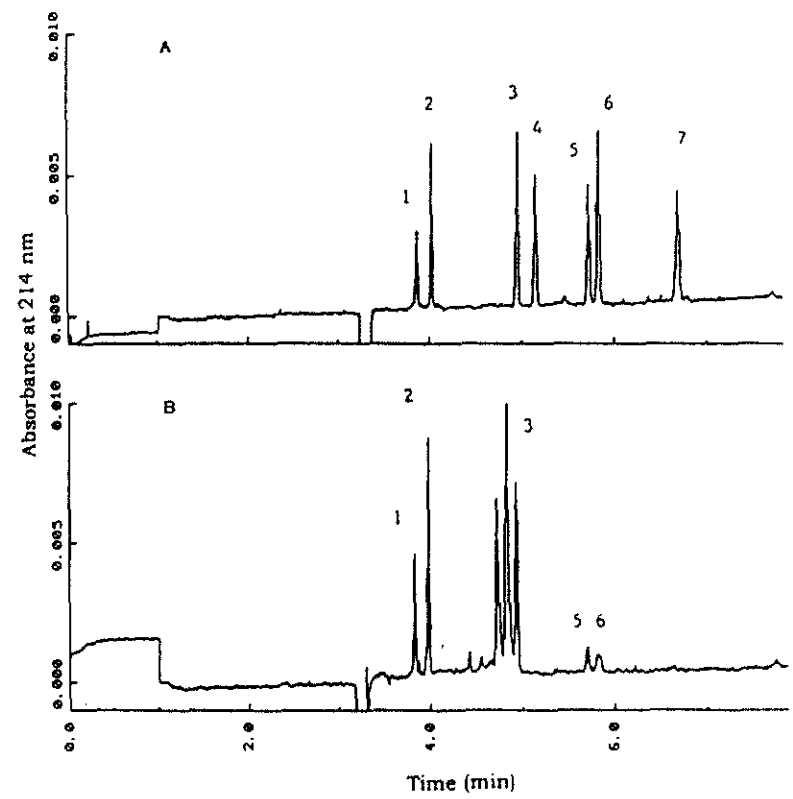

Fig. 1. Separation of the following compounds: 1 = daidzin; 2 = genistin; 3 = internal standard (methylxanthine); 4 = biochanin A; 5 = daidzein; 6 = genistein; 7 = coumestrol. (A) Standards in the extracting solution at a concentration of 6 mg/l, (B) soy bean seeds extract in the presence of 0.4% NaCl.

while the R.S.D. is 1.54% when the calculation is based on the internal standard.

Fig. 3 illustrates how the concentration of different isoflavones varies among different varieties of soy beans. Among the isoflavones present in soy beans are genistein and daidzein, which inhibit tumor growth directly in tissue cultures [1–3]. However, their sugar conjugates, daidzin and genistin, do not exhibit this effect [1–3]. The concentration of these four isoflavones measured by CE from soy beans is very

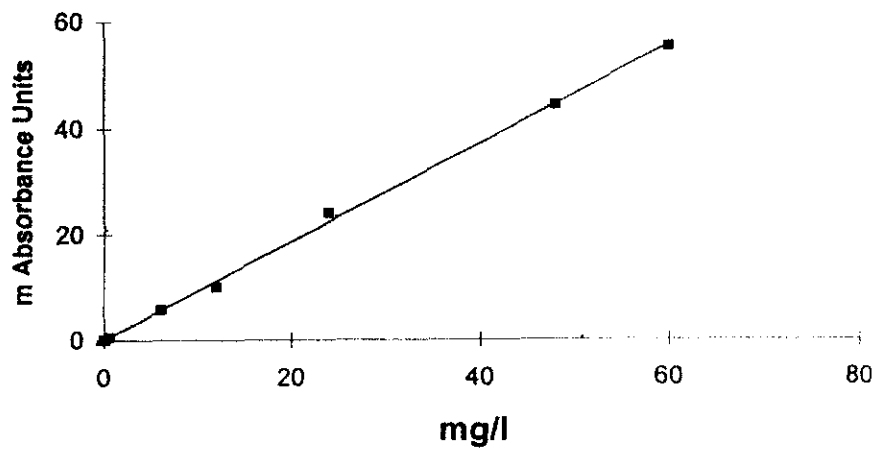

Fig. 2. Genestein calibration on the CE apparatus.

Table 1
The relative standard deviation (R.S.D.) of the different compounds

Compound	R.S.D. (%) ($n = 10$)			
	Peak height (mm)	Peak height/ I.S.	Migration time (min)	Migration time/ I.S.
Internal standard (I.S.)	5.21	0.00	0.75	0.00
Daidzin	3.29	3.60	1.19	0.71
Genistin	4.62	2.70	1.02	1.06
Biochanin A	5.68	1.09	1.05	1.10
Genistein	3.02	2.64	1.02	1.08
Daidzein	4.46	1.54	1.20	0.91
Coumestrol	3.66	3.67	1.28	1.14

close to that measured by HPLC (Fig. 4), and also close to that reported in the literature [11].

The kudzu plant, which grows wild in the southern parts of the USA, is a rich source of daidzein, as indicated by the electropherogram produced by root extracts of this plant (Fig. 5). The daidzein peak was verified by spiking the sample with pure daidzein. On the other hand, daidzin has been found, recently, to inhibit human mitochondrial aldehyde dehydrogenase [12]. Interestingly, root extracts of this plant in pill form have been used in the orient as treatment for alcoholism.

We have found this CE method to be very useful in following the isolation and purification of these compounds. The advantages of this

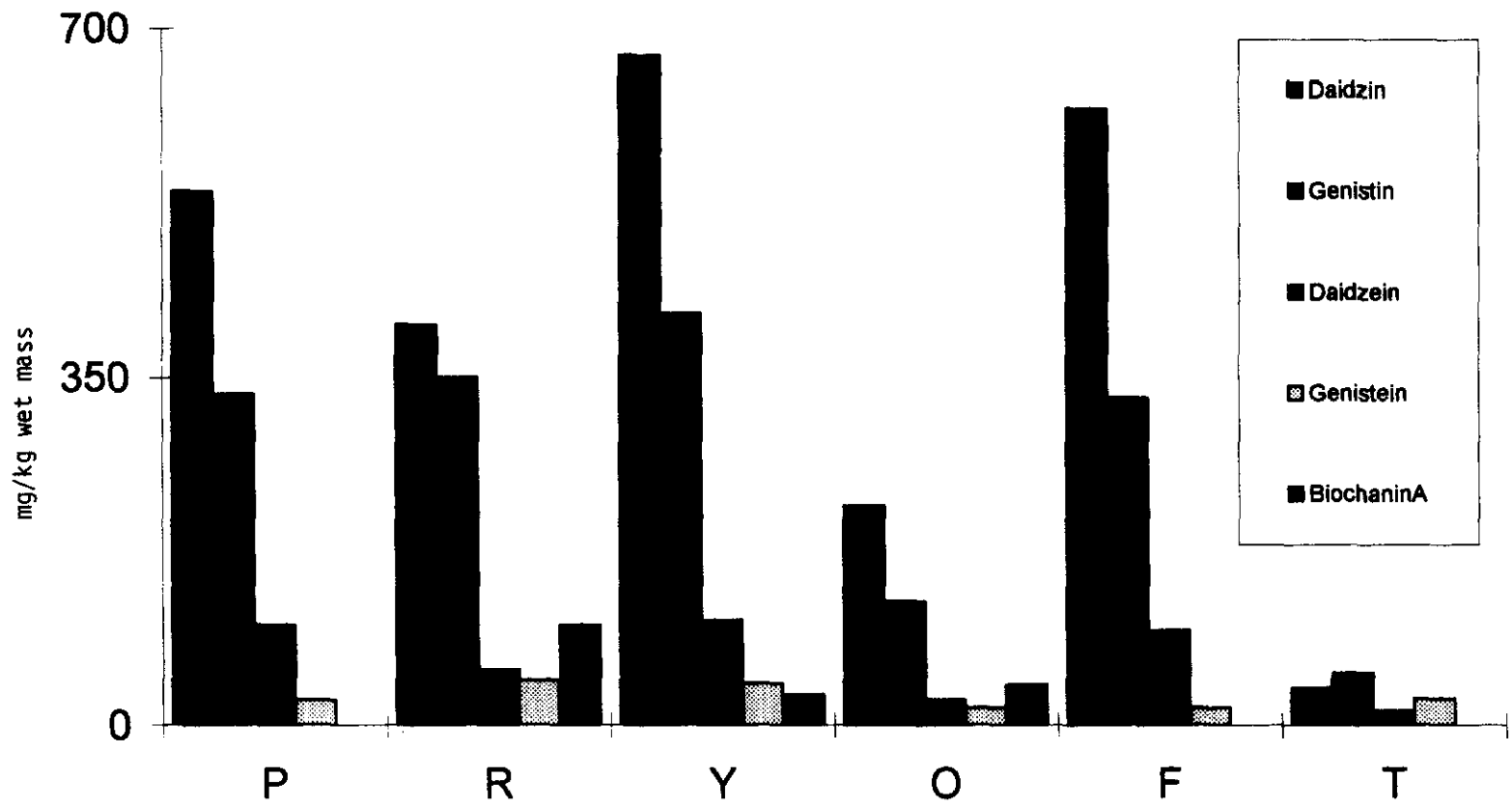

Fig. 3. Content of isoflavones in different varieties of soy bean seeds obtained locally. P = Powder; R = roasted; Y = yellow; O = organic; F = flakes; T = Tofu.

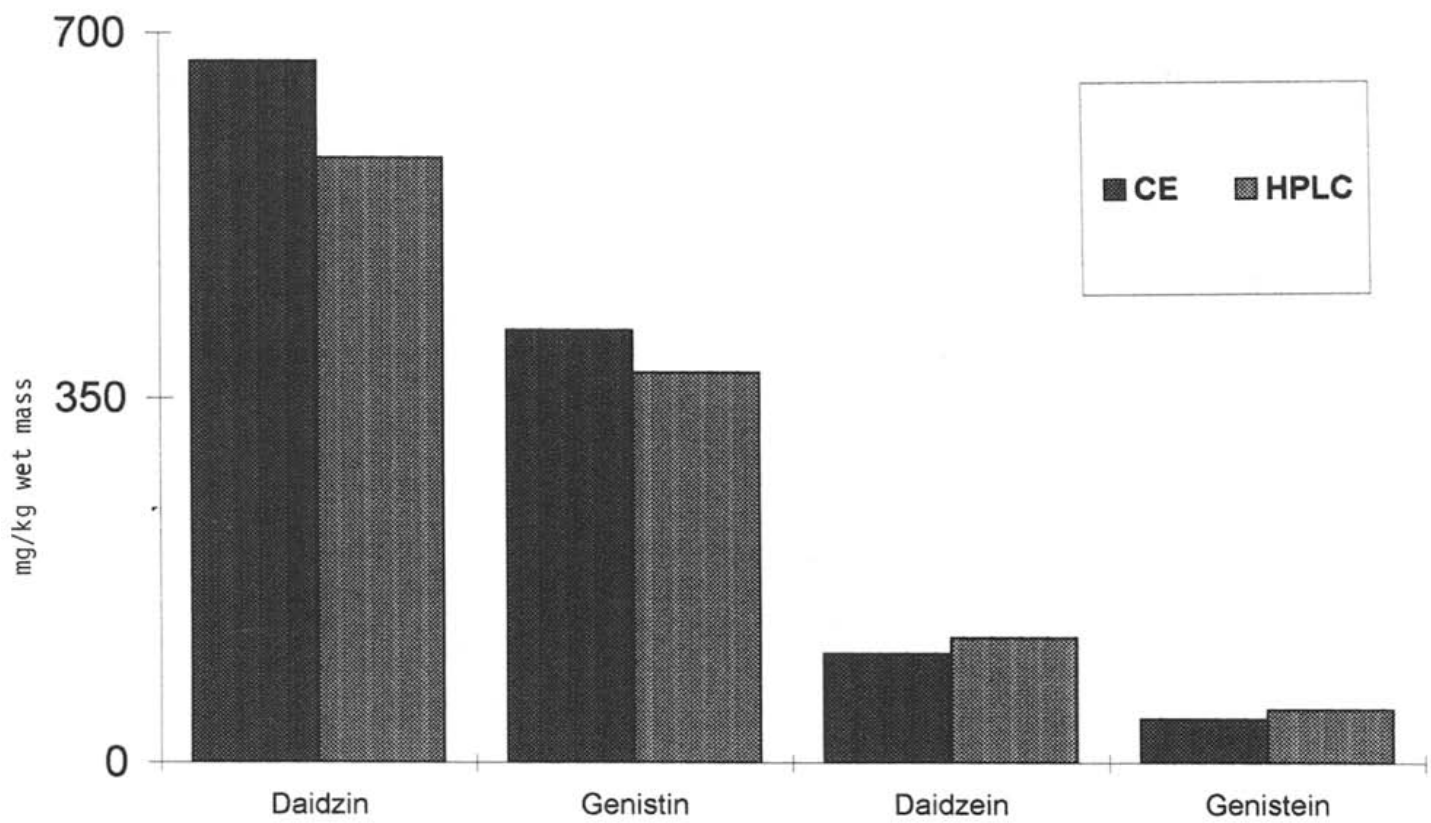

Fig. 4. Comparison of isoflavones by HPLC and CE extracted from yellow soy beans.

method over HPLC are: it is rapid; it does not require solvent gradient and column equilibration, and it does not consume organic solvents for elution. In addition, the capillaries for CE are much less expensive than the columns for HPLC.

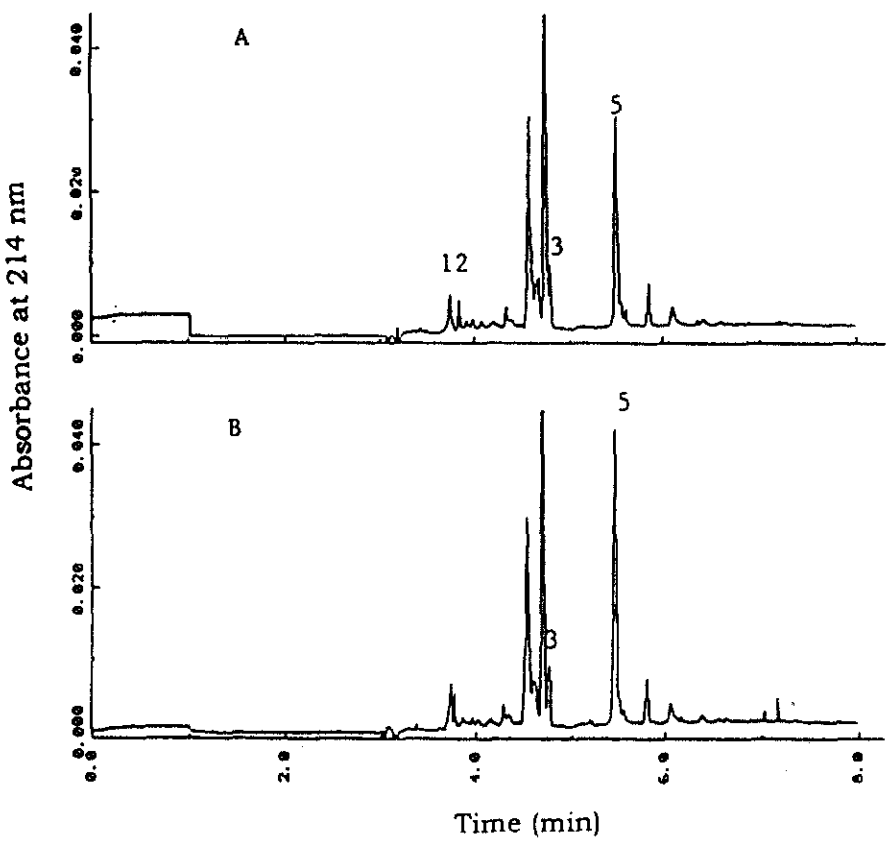

Fig. 5. Daidzein content of (A) kudzu roots (15 mg of the roots were homogenized in 1 ml of the extracting solvent) and (B) after spiking with daidzein (6 mg/l).

Acknowledgement

We thank Dr. S. Barnes from the University of Alabama at Birmingham, for supplying the isoflavone standards.

References

[1] G. Peterson and S. Barnes, *Biochem. Biophys. Res. Commun.*, 179 (1991) 661.
[2] Y. Matsukawa, N. Marui, T. Sakai, Y. Satomi, M. Yoshida, K. Matsumoto, H. Nishino and A. Aoike, *Cancer Res.*, 53 (1993) 1328.
[3] F. Traganos, B. Ardelt, N. Halko, S. Bruno and Z. Darzynkiewicz, *Cancer Res.*, 52 (1992) 6200.
[4] S. Barnes, C. Grubbs, K.D.R. Setchell and J. Carlson, *Progr. Clin. Biol. Res.*, 347 (1990) 239.
[5] H. Adlecreutz, in P. Rozen (Editor), *Frontiers of Gastrointestinal Research*, Karger, Basel, 1988, pp. 165–176.
[6] T. Akiyama, J. Ishida, S. Nakagawa, S. Ogawara, S. Watanabe, N. Itoh, M. Shibuya and Y. Fukami, *J. Biol. Chem.*, 262 (1987) 5592.

[7] Y. Yamashita, S.-Z. Kawada and H. Nakano, H., *Biochem. Pharmacol.*, 39 (1990) 737.
[8] A.C. Eldridge, *J. Chromatogr.*, 234 (1982) 494.
[9] M. Naim, B. Gestetner, I. Kirson, Y. Birk and A. Bondi, *Phytochemistry*, 12 (1973) 169.
[10] M. Naim, B. Gestetner, S. Zilkah, Y. Birk and A. Bondi, *J. Agric. Food Chem.*, 22 (1974) 806.
[11] K.R. Price and G.R. Fenwick, *Food Addit. Contam.*, 2 (1985) 73.
[12] W.-M. Keung and B.L. Vallee, *Proc. Natl. Acad. Sci. U.S.A.*, 90 (1993) 1247.