Journal of Chromatography, 348 (1985) 447–453
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 112

Note

High-performance liquid chromatography of caffeoylquinic acid derivatives of *Cynara scolymus* L. leaves

T. ADZET* and M. PUIGMACIA

Department of Pharmacognosy and Pharmacodynamics. Faculty of Pharmacy, University of Barcelona, Barcelona 08028 (Spain)

(First received June 27th, 1985; revised manuscript received August 15th, 1985)

The choleraic and cholagogic action of *Cynara scolymus* L. extracts is already known[1]. Research is at present being carried out into the hepatotropic activity of such extracts, when faced with acute intoxication by carbon tetrachloride. This activity has always been attributed to the phenolic compounds present in the extract, and in particular to its caffeoylquinic derivatives[2,3]. These compounds are highly unstable[4,5] and during extraction may undergo isomerization through hydrolysis and posterior intramolecular transesterification[6–8]. This implies modifications in the composition of the extracts, depending on whether they are in aqueous or alcoholic solution, in which enzymatic activity is inhibited[9,10].

Many papers on the analysis of phenolic compounds have been published[11–15]. The aim of the present study was to develop a technique capable of analyzing all caffeoylquinic derivatives in any vegetable extract which contains phenol acids and flavonoids.

EXPERIMENTAL

Chromatography

A Perkin-Elmer Series 2 liquid chromatograph, equipped with a spectrophotometric detector LC-85 and a Sigma 15 chromatographic data station, was used. The column was a stainless-steel tube (15 cm × 4 mm I.D.) packed with Spherisorb C_{18} (Supelco, Bellefonte, PA, U.S.A.) having an average particle size of 5 μm. A short stainless-steel precolumn, packed with Spherisorb C_{18} Superguard (5 μm), was used. The UV detector was set at 325 nm.

Solvents were filtered using a 0.45-μm Millipore filter and degassed at room temperature under vacuum with magnetic stirring.

Two solvents were used: A, methanol–acetic acid (97.5:2.5); B, water–acetic acid (97.5:2.5). The linear gradient was from 13% A to 43% A in 30 min. The flow-rate was 1.6 ml/min, the temperature was 40°C and the column pressure was 1500 p.s.i.

Reagents

Phenols commercially available were obtained from Sigma (St. Louis, MO, U.S.A.), Fluka (Buchs, Switzerland) and Sarsyntex (Merignac, France).

0021-9673/85/$03.30

Neochlorogenic and cryptochlorogenic acids were obtained through isomerization of chlorogenic acid according to procedure used by Walkowski[16].

1,3-Dicaffeoylquinic acid was isolated from the artichoke following a modified procedure reported by Michaud[9].

The isomers of isochlorogenic acid (3,4-, 3,5- and 4,5-dicaffeoylquinic acids) were separated from coffee by the method of Barnes *et al.*[17].

All the above-mentioned products were isolated by means of semipreparative high-performance liquid chromatography (HPLC)[18]. They were identified by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry[19,20].

Quantitative calibration

Each phenolic compound was examined by HPLC in order to determine its retention time, order of elution and capacity factor (Table I). α-Naphthol (Merck, Darmstadt, F.R.G.) was used as internal standard (retention time, t_R, 25.02).

A calibration graph of peak area against weight was obtained for each caffeoylquinic acid derivative and flavonoid, in the range of 1–200 μg. A linear relationship was obtained in each case.

Samples

Standards of caffeoylquinic acids and flavonoids were dissolved in methanol.

The purification method used for the artichoke extracts depended on whether aqueous (a) or alcoholic (b) solutions are used.

(a) A dried artichoke sample (10 g) was extracted with boiling water for 2 h, the process being repeated twice. The extract was filtered while warm, concentrated, diluted in a minimum of water and precipitated with methanol (10 volumes); this was repeated twice. The methanolic fractions were dried *in vacuo*.

(b) A similar sample was extracted warm (at 75°C) with 70% methanol for 2 h, three times. The extract was filtered while warm, concentrated and diluted in 50% methanol. It was subsequently extracted with light petroleum (b.p. 40–60°C)–diethyl ether (2:1). The aqueous alcoholic fraction was dried *in vacuo*.

It was shown that in both cases the purification processes do not modify the extract composition.

The dry residues were redissolved in 70% methanol, the volume was adjusted to 50 ml with methanol, and mixed with 10 ml of a 0.84 mg/ml solution of α-naphthol in methanol. After filtration (Millipore 0.45-μm filter), 3–5 μl of the solution were injected.

RESULTS AND DISCUSSION

The mobile phase was selected by examining different series of solvents. It was noted that the selectivity of methanol–water mixtures was hardly affected by the introduction of tetrahydrofuran or acetonitrile.

The simultaneous analysis of compounds with marked differences in polarity (mono- and dicaffeoylquinic derivatives, flavonic glycosides and aglycones) requires the use of a gradient programme. An identical concentration of acetic acid was maintained in the two pumps, to ensure a constant pH, independent of the gradient conditions. The reproducibility of the analysis was verified by use of 2.5% acetic acid[21].

Effect of structure on retention

Table I shows the retention times of the phenolic compounds. The values are averages from five analyses, the error being about 1.5%.

TABLE I

RETENTION TIMES, t_R, AND CAPACITY FACTORS, k', OF CAFFEOYLQUINIC ACID DERIVATIVES AND FLAVONOIDS

Compound	t_R(min)	k'
Neochlorogenic acid	2.11	0.81
Cryptochlorogenic acid	4.21	2.62
Chlorogenic acid	4.44	2.82
Caffeic acid	5.11	3.40
1,5-Dicaffeoylquinic acid	6.58	4.67
3,4-Dicaffeoylquinic acid	16.02	12.81
3,5-Dicaffeoylquinic acid	17.22	13.84
Luteolin-7-glucoside	17.50	14.08
1,3-Dicaffeoylquinic acid	18.18	14.67
4,5-Dicaffeoylquinic acid	21.35	17.40
Luteolin	28.57	23.62
α-Naphthol	25.02	20.56

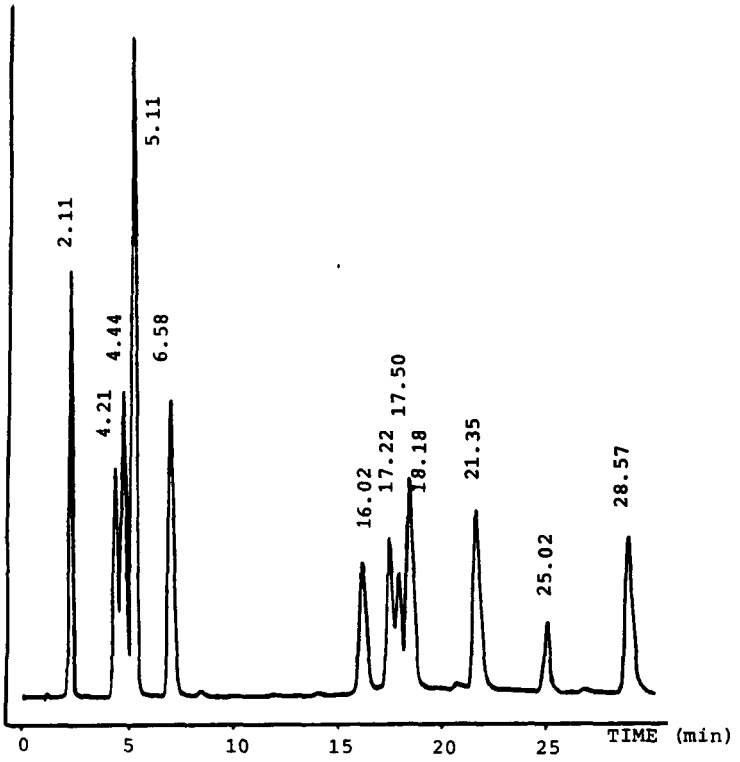
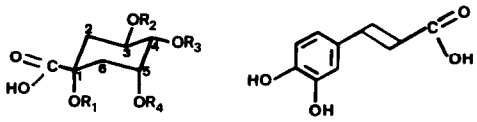


Fig. 1. The retention times of caffeoylquinic acid derivatives and flavonoids eluted using the linear gradient on a Spherisorb C_{18} column. A list of the compounds separated is given in Table I. For the elution system see Experimental.

The order of elution of the different groups is in accord with previous results[12]: monocaffeoylquinic acids, caffeic acid, dicaffeoylquinic acids, flavonic glycosides and flavones (Fig. 1). As verified by Court[22], the introduction of a caffeoyl group increases the retention of a molecule; however, the monocaffeoylquinic derivatives are eluted before the caffeic acid which may be considered to be more polar.

The chromatographic retention of the monocaffeoylquinic isomers is in agreement with the results obtained by Krause and Strack[23] and Nagels *et al.*[24], but not those described by Court[22]. The first to elute are the isomers that have axial caffeoyl substituent (pseudochlorogenic and neochlorogenic acids), followed by those with an equatorial caffeoyl moiety (criptochlorogenic and chlorogenic acids) (Fig. 2). The above effect was observed experimentally by Haslam *et al.*[7] for *p*-coumaroylquinic derivatives; compounds having the hydroxycinnamic moiety in positions 1 and 5 exhibit increased solubility in water.



Caffeic acid

	R_1	R_2	R_3	R_4
Quinic acid	H	H	H	H
1-O-Caffeoylquinic acid (pseudochlorogenic)	Caffeoyl	H	H	H
3-O-Caffeoylquinic acid (chlorogenic)	H	Caffeoyl	H	H
4-O-Caffeoylquinic acid	H	H	Caffeoyl	H
5-O-Caffeoylquinic acid (neochlorogenic)	H	H	H	Caffeoyl
1,5-O- Dicaffeoylquinic acid (cynarin)	Caffeoyl	H	H	Caffeoyl
1,3-O-Dicaffeoylquinic acid	Caffeoyl	Caffeoyl	H	H
3,4-O-Dicaffeoylquinic acid (isochlorogenic)	H	Caffeoyl	Caffeoyl	H
3,5-O-Dicaffeoylquinic acid (isochlorogenic)	H	Caffeoyl	H	Caffeoyl
4,5-O-Dicaffeoylquinic acid (isochlorogenic)	H	H	Caffeoyl	Caffeoyl

Fig. 2. Structures of caffeoylquinic acid derivatives.

In the case of dicaffeoylquinic derivatives, whereas cynarin (1,5-dicaffeoylquinic acid, axial-axial) is eluted faster than the rest of isomers, 3,4-dicaffeoylquinic acid (equatorial-equatorial) which should be the most strongly retained is eluted before 1,3-dicaffeoylquinic acid (axial-equatorial) and 3,5- and 4,5-dicaffeoylquinic acids (both equatorial-axial) (Fig. 2).

Quantitative analysis

The analysis of the alcoholic extracts, in which the initial composition of the

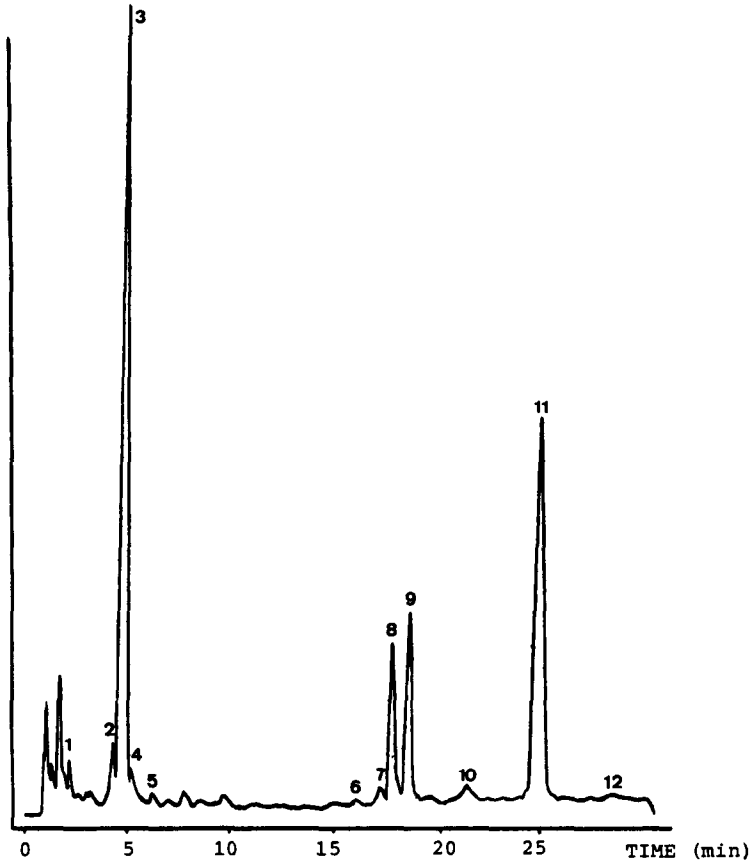


Fig. 3. The separation of caffeoylquinic acids and flavonoids in an alcoholic extract of artichoke using the linear gradient. Peaks: 1 = neochlorogenic acid; 2 = cryptochlorogenic acid; 3 = chlorogenic acid; 4 = caffeic acid; 5 = 1,5-dicaffeoylquinic acid; 6 = 3,4-dicaffeoylquinic acid; 7 = 3,5-dicaffeoylquinic acid; 8 = luteolin-7-glucoside; 9 = 1,3-dicaffeoylquinic acid; 10 = 4,5-dicaffeoylquinic acid; 11 = α-naphthol; 12 = luteolin. For the elution system see Experimental.

drug is maintained, shows the main components to be chlorogenic acid, 1,3-dicaffeoylquinic acid and luteolin-7-glucoside, with the other caffeoylquinic derivatives in much lower amounts (Fig. 3).

In a warm aqueous medium, isomerization phenomena occur, leading to a variation in the extract composition (Fig. 4). There is an increase in neochlorogenic and criptochlorogenic acids as a result of the decrease in chlorogenic acid[25]. On the other hand, the decrease in 1,3-dicaffeoylquinic acid results in an increase in 1,5-dicaffeoylquinic acid (cynarin) and the isomers of isochlorogenic acid (Table II).

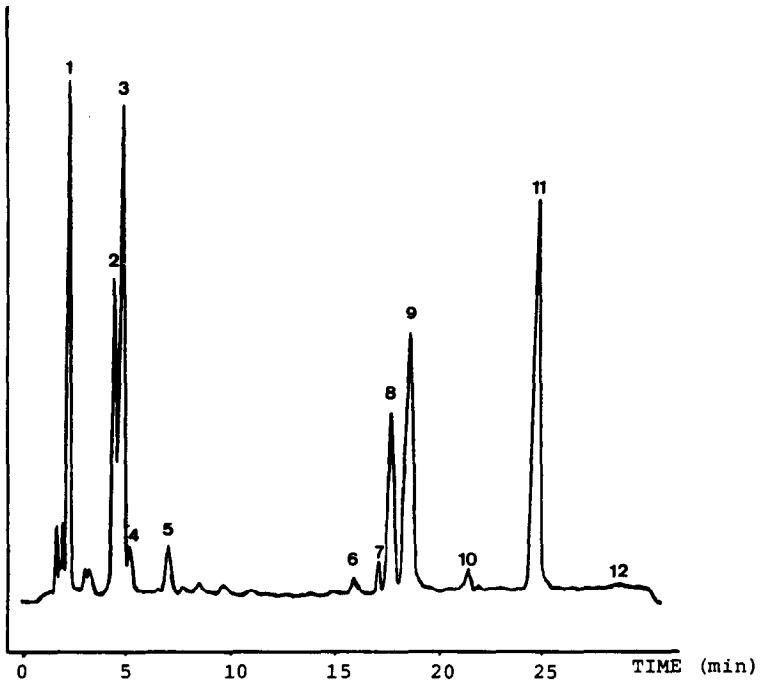


Fig. 4. The separation of caffeoylquinic acids and flavonoids in an aqueous extract of artichoke using the linear gradient. Details as in Fig. 3.

TABLE II

PERCENTAGES OF PHENOLIC COMPOUNDS IN AQUEOUS AND METHANOLIC EXTRACTS OF THE ARTICHOKE

Values are relative to total polyphenol content.

Compound	*Aqueous extract*	*Methanolic extract*
Neochlorogenic acid	19.50	1.30
Cryptochlorogenic acid	23.03	3.02
Chlorogenic acid	30.02	73.80
Caffeic acid	1.31	0.63
Cynarin	3.83	1.13
3,4-Dicaffeoylquinic acid	1.12	0.50
3,5-Dicaffeoylquinic acid	1.55	0.90
1,3-Dicaffeoylquinic acid	6.98	7.30
4,5-Dicaffeoylquinic acid	1.75	1.01
Luteolin-7-glucoside	10.98	9.83
Luteolin	0.18	0.15

ACKNOWLEDGEMENT

We acknowledge the financial support Comisión Asesora de Investigación Científica y Técnica, Proyecto 0705/81.

REFERENCES

1 A. Lietti, *Fitoterapia*, 48 (1977) 153.
2 L. Panizzi and M. L. Scarpati, *Nature (London)*, 174 (1954) 1062.
3 P. Preziosi, *Il Farmaco Ed. Sci.*, 17 (1962) 701.
4 E. Nichiforescu and V. Coucou, *Ann. Pharm. Fr.*, 24 (1966) 127.
5 V. Lattanzio and I. Morone, *Experientia*, 35 (1979) 993.
6 M. L. Scarpati and P. Esposito, *Ann. Chim. (Rome)*, 54 (1964) 51.
7 E. Haslam, G. K. Makinson, M. O. Naumann and J. Cunningham, *J. Chem. Soc.*, (1964) 2137.
8 L. I. Dranik and V. Y. Chernobai, *Rastit. Resur.*, 3 (1967) 250.
9 J. Michaud, *Bull. Soc. Pharm. (Bordeaux)*, 106 (1967) 181.
10 E. Nichiforescu, *Plant. Med. Phytother.*, 4 (1970) 56.
11 L. J. Nagels, W. L. Creten and P. M. Vanpeperstraete, *Anal. Chem.*, 55 (1983) 216.
12 L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, 116 (1976) 271.
13 J. Mollerup Andersen and W. Batsberg Pedersen, *J. Chromatogr.*, 259 (1983) 131.
14 K. Van de Casteele, H. Geiger and C. F. Van Sumere, *J. Chromatogr.*, 258 (1983) 111.
15 B. Möller and K. Hermann, *J. Chromatogr.*, 241 (1983) 371.
16 A. Walkowski, *Zesz. Nauk*, 80 (1978) 81.
17 H. M. Barnes, J. R. Feldman and W. V. White, *J. Am. Chem. Soc.*, 72 (1950) 4178.
18 S. K. Yarbro and F. L. Vandemark, *1982 Pittsburgh Conference and Exhibition on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, PA, March 8–12, 1982*, Paper No. 269, 1982.
19 J. Corse, R. E. Lundin, E. Sondheimer and A. C. Waiss, Jr., *Phytochemistry*, 5 (1966) 767.
20 B. N. Timmermann, J. J. Hoffmann, S. D. Jolad, K. H. Schram, R. E. Klenck and R. B. Bates, *Lloydia*, 46 (1983) 365.
21 W. P. Price, R. Edens, D. L. Hendrix and S. N. Deming, *Anal. Biochem.*, 93 (1979) 233.
22 W. A. Court, *J. Chromatogr.*, 130 (1977) 278.
23 J. Krause and D. Strack, *J. Chromatogr.*, 176 (1979) 465.
24 L. Nagels, W. van Dongen, J. de Bruckes and H. de Pooter, *J. Chromatogr.*, 187 (1980) 181.
25 J. Iglesias, M. Puigmaciá and J. M. Quiles, *Plant Med. Phythother.*, 19 (1985) in press.