1981, Vol. 42, pp. 288–292, © Hippokrates Verlag GmbH

Planta medica Journal of Medicinal Plant Research

Reversed-Phase High-Performance Liquid Chromatography Analysis of Apigenin and its Glucosides in Flowers of Matricaria chamomilla and Chamomile Extracts

C. Redaelli*, L. Formentini* and E. Santaniello**

* Research Laboratory, Bonomelli S.p.A., Dolzago

** Istituto di Chimica, Facoltà di Medicina, Università di Milano, Milano, Italy

Key Word Index: Matricaria chamomilla; Asteraceae (Compositae) Flavonoids; Apigenin; Ligulate flowers.

Abstract

Quantitative estimation of the flavones apigenin, apigenin-7-glucoside and apigenin-7-acetylglucoside in ligulate florets of *Matricaria chamomilla* was performed by HPLC using a reverse-phase column and eluting with acetonitrile/water, acetic acid system. These flavonoids were detected at 335 nm. Apigenin and its glucosides were not found in the tubular florets. The method was also applied to the estimation of these flavonoids in chamomile extracts.

Introduction

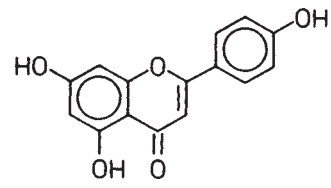
Preliminary results of our recent researches on chamomile (*Matricaria chamomilla* L.) have shown that ligulate florets contain free apigenin (I), its 7-O-glucoside (II) and a mixture of 6″ and 2″ acetates of this glucoside (III) [1, 2].

In a recent paper [3] isolation from Chamomile flowers of 6″ acetate of 7-O-glucoside of apigenin has been reported, thus confirming our data.

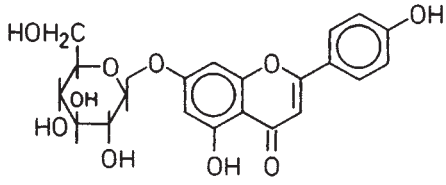
Our earlier evaluations of the content of (I) and its glucosides (II) and (III) in *Matricaria chamomilla* relied on an estimation of the amounts of the single components after the traditional work of extraction, recovery, purification on column chromatography and crystallization of the single compounds.

Recently compounds which belong to the class of flavones and related compounds have been investigated by high-performance liquid chromatography (HPLC), the separation being achieved by normal or reversed-phase chromatography. [4]

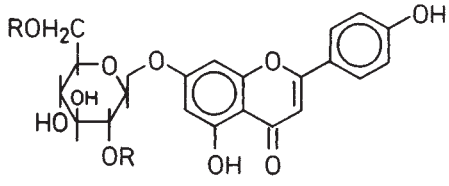
Although apigenin (I) and its glucosides have been previously examined by H.P.L.C. [5], this technique has not been applied to *Matricaria chamomilla* L. However estimation of concentration of



I apigenin



II apigenin-7-glucoside



R = Ac, R' = H or R = H, R' = Ac
III apigenin-7-acetylglucoside

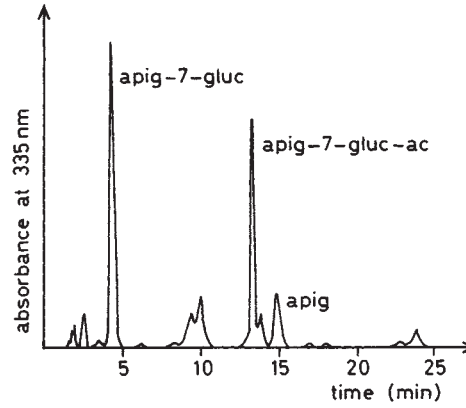


Fig. 2. Chromatogram of methanolic extract of ligulate flowers of *M. chamomilla*. See text –

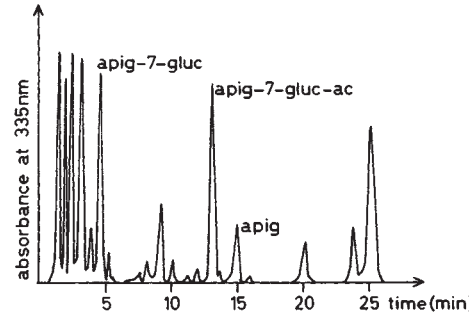


Fig. 3. Chromatogram of a chamomile extract. See text –

(I) seems important, since (I) and its glucosides appear to be the predominant flavonoids in chamomile [6]. Consequently, concentration of (I) might be used as measure of extractions and of related infusion quality. Furthermore, (I) as well as (II), has been reported to exert a spasmolytic action [6, 7].

Location of (I) and its derivatives in any single part of the plant should be of additional importance for the cited pharmacological properties. In the light of this considerations, H.P.L.C. analyses of (I), (II) and (III) in *Matricaria chamomilla* L. and chamomile extracts were undertaken and the following procedure was developed.

Materials and Methods

Apparatus

A series 3 liquid chromatograph, microprocessor controlled pump module (Perkin Elmer Corporation, Norwalk, USA) was used. The instrument is equipped with a Rheodyne injector. A Mod. 023 was the recorder and a UV-vis liquid chromatography analyzer Mod 55 B (Perkin Elmer) was the detector.

A Spectra-Physics integrator (minigrator, Spectra-Physics, Santa Clara, USA) was used.

Column

A Perkin Elmer 0,26 × 25 cm HC – ODS Sil-X reverse-phase column was used.

Reagents

The mobile phase was a concave exponential n. 2 (curve 2) gradient increasing in 25 minutes from 15 % to 60 % acetonitrile in water containing 2 % of acetic acid.

Sample preparation

100–120 mg of ligulate florets (or tubular florets), separated by dissection of the compound flower heads of each commercial sample, were refluxed with 80 ml of methanol (1 h). After filtration the solution was concentrated and adjusted to a volume of 10 ml with methanol. For the analysis of chamomile extracts (water – alcohol extracts), a sample of the solution was directly injected.

HPLC

resolution and quantitation of apigenin and its glucosides. The flow-rate was 1 ml/min, detection was at 335 nm and the sample size was 5/ul. The recorder chart speed was 1 cm/min. The peak width parameter value of integrator is 5 and slope sensitivity is 350.

The quantitative determination in unknown samples of apigenin and its glucosides under the above conditions was studied using external standardization.

The procedure has given excellent results as regards precision and reliability.

Retention times

The retention times are reported in table I. The value of t_0 is 1,6 minutes.

Table I

Flavone	$t_R - t_O$ in minutes
Apigenin-7-glucoside	3
Apigenin-7-acetylglucoside	12.3
Apigenin	13.7

Results and Discussion

Reversed-phase H.P.L.C. proved to be an effective method for the separation of (I), (II), (III) and as a first application of the method the content of the above compounds was determined in ligulate and tubular florets. We have already reported [2] that in ligulate florets apigenin content (both free and as glucosides) was about 4 %.

In fact, H.P.L.C. analysis confirmed this results. Furthermore, (I) and derivatives were practically absent in tubular florets, thus showing that the spasmolytic action due to flavones of the "flower" of *Matricaria chamomilla* can be restricted to ligulate florets.

Furthermore, the method could be applied satisfactorily to determination of (I), (II), (III) in chamomile from various sources and in different years of collection (table 2).

More interestingly, chamomile extracts can be analysed for their content of (I), (II) and (III) (table 3). In the more complex mixture of products of the extracts, apigenin and its glucosides could be determined and the reproducibility of the method was excellent.

The different amounts of (I), (II) and (III) in the various extracts is probably due to different techniques of extraction and the ratio of drug to extract, to the type and source of chamomile and the percentage of ligulate florets and also to age of the extract.

As a conclusion this report furnishes an additional example of the importance of HPLC as a tool for quantitative and qualitative determination of natural products.

Table II

Provenience	Year	Weight of ligulate florets mg (*)	Apigenin-7-glucoside			Apigenin-7-acetyl-glucoside			Free apigenin		Total amount of	
			mg	%	content of apigenin (°)	mg	%	content of apigenin (°)	mg	%	mg	%
Egypt	1979	113.2	3.85	3.40	2.41	1.56	1.38	0.89	traces	–	3.30	2.91
Egypt	1978	103.7	3.49	3.36	2.18	1.83	1.76	1.04	0.07	0.07	3.29	3.17
Czechoslovakia	1978	104.3	3.94	3.78	2.46	1.11	1.06	0.63	0.22	0.21	3.31	3.17
Czechoslovakia	1979	104.9	5.00	4.77	3.12	1.24	1.18	0.71	0.23	0.22	4.06	3.87
Bulgaria	1977	122.9	4.42	3.60	2.76	0.85	0.69	0.48	0.30	0.24	3.54	2.88
Bulgaria	1979	111.2	3.20	2.88	2.00	1.03	0.93	0.59	0.22	0.20	2.81	2.53
Bulgaria	1978	107.2	5.22	4.87	3.26	1.57	1.46	0.89	0.19	0.18	4.34	4.05
Italy 1	1979	113.1	2.95	2.61	1.84	1.42	1.26	0.81	0.13	0.11	2.78	2.46
Italy 2	1979	99.6	2.84	2.85	1.77	1.02	1.02	0.58	0.15	0.15	2.50	2.51
Argentina	1978	101.2	5.08	5.02	3.17	1.73	1.71	0.98	0.33	0.33	4.48	4.43
Argentina	1979	102.6	4.02	3.92	2.51	2.23	2.17	1.27	0.23	0.22	4.01	3.91

(*) dried

(°) Molecular weights: Apigenin, 270.23; Apigenin-7-glucoside, 432.38; Apigenin-7-acetylglucoside, 474.42.

Table III

hydro-alcoholic Extract	Year of production	*Apigenin-7-glucoside*		*Apigenin-7-acetyl-glucoside*		*Free Apigenin*	*Total Apigenin*
		mg/100 ml	content of apigenin	mg/100 ml	content of apigenin	mg/100 ml	mg/100 ml
1	1978	86.01	53.75	4.92	2.80	14.10	70.65
2	1979	88.52	55.32	1.61	0.92	0.91	57.15
3	1980	23.07	14.41	33.10	18.85	13.52	46.78
4	1980	16.15	10.09	12.04	6.86	6.22	23.17

Acknowledgements

We thank Dr. PIERO RUSSINO of Alimentary Division, Bonomelli S. p. A., for his collaboration in the choice of the botanical material.

References

1. Redaelli, C., E. Santaniello and L. Formentini: 2nd ISHS Symposium "Spices and Medicinal Planta". Budapest 1979, Poster-Section.
2. Redaelli, C., L. Formentini and E. Santaniello: Phytochemistry, *19*, 985 (1980).
3. Kunde, R. and O. Isaac: Planta Medica *37*, 124 (1979).
4. Kingston, D. G. I.: Lloydia *42*, 237 (1979) an references therein.
5. Niemann, G. and J. W. Koerselman-Kooy Planta Medica *31*, 297 (1977).
6. Hoerhammer L.: 21. Int. Congr. Pharmac. Pro ceed. Pisa 1961, 578.
7. a) Hava, M. and J. Janku: Journal de Physiol *50*, 309 (1958).
   b) Sorm F., Z. Cekan, V. Herout and H. Ras kova: Chem. Listy *46*, 308 (1952).

Address: Dr. C. Redaelli,
Bonomelli S.p.A.
22042 DOLZAGO (Como), Italy