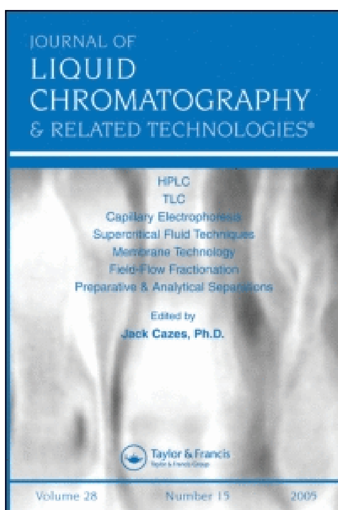
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation and Identification of Phenolic Acids from Some Species of the *Asteraceae* Family Using HPLC with a Diode Array Detector

W. Markowski[a]; L. K. Czapińska[a]; A. J. Józefczyk[a]; K. Glowniak[b]
[a] Dept. of Inorganic and Analytical Chemistry, Medical Academy, Lublin, Poland [b] Dept. of Pharmacognosy, Medical Academy, Lublin, Poland

To cite this Article Markowski, W. , Czapińska, L. K. , Józefczyk, A. J. and Glowniak, K.(1998) 'Separation and Identification of Phenolic Acids from Some Species of the *Asteraceae* Family Using HPLC with a Diode Array Detector', Journal of Liquid Chromatography & Related Technologies, 21: 16, 2497 — 2507

To link to this Article: DOI: 10.1080/10826079808003594

URL: http://dx.doi.org/10.1080/10826079808003594

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 21(16), 2497–2507 (1998)

SEPARATION AND IDENTIFICATION OF PHENOLIC ACIDS FROM SOME SPECIES OF THE *ASTERACEAE* FAMILY USING HPLC WITH A DIODE ARRAY DETECTOR†

W. Markowski,[1] L. K. Czapińska,[1] A. J. Józefczyk,[1] K. Głowniak[2]

[1] Dept. of Inorganic and Analytical Chemistry
Medical Academy
6 Staszica St.
20-081 Lublin, Poland

[2] Dept. of Pharmacognosy
Medical Academy
12 Peowiaków St.
20-007 Lublin, Poland

Downloaded At: 10:48 24 January 2011

ABSTRACT

The qualitative and quantitative analysis of phenolic acids was carried out using methods of HPLC with the diode array detector (DAD). The quantitative analysis of chosen phenolic acids was carried out using the Multicomponent Analysis method (MCA). Solid-phase extraction was applied for the purification of crude extract from aerial part of three species of fam. *Asteraceae* (*Chrysanthemum maximum* Ram.(DC), *Chrysanthemum segetum* L., *Rudbeckia laciniata* L.). The choice of optimal conditions of analysis was made using DryLab+ software.

Copyright © 1998 by Marcel Dekker, Inc. www.dekker.com

INTRODUCTION

Phenolic acids form a large group of compounds occurring in plants the most frequent being the derivatives of benzoic and cinnamic acids which occur as free acids and their depsides or glycosides. Frequently they are parts of anthocyanes and flavones. Their presence in plants has biological effects: they influence their resistance, take part in defensive reactions of plant tissues against pathological processes, guard the plant against the attack of mould bacteria and viruses, and form a protective barrier between the healthy and diseased tissues.[1]

The pharmacological action of phenolic acids has been investigated by numerous scientific centers. The investigations have demonstrated, among others, their antibacterial, antifever and antirheumatic action, reported mainly as results of the presence of caffeic, vanillic, and 4-coumaric acids. The anti - virus action and cytostatic activity of caffeic acid and its derivatives and the immunostimulating action of cichoric acid has also been reported.[2-4] The widest range of activity has been described for salicylic acid (disinfectant, keratolytic and antibacterial activity). Similar properties have been reported for gentisic acid.

Downloaded At: 10:48 24 January 2011

Phenolic acids have also been reported as scavengers of free radicals. The derivatives of cinnamic acid and especially rosmaric acid are responsible for the antihormonal activity of some species of *Lithospermum* L. and *Lycopus* L. They neutralize the action of hormones of the brain. Therefore, medical sciences hope to obtain a natural medicine for the therapy of certain diseases of glands.[5]

The purpose of the reported experiments is the search for plants rich in phenolic acids, determination of their qualitative and quantitative composition, and their possible applications in therapy. Raw materials rich in phenolic acids were chosen as the family of *Asteraceae* (*Compositae*), two species of *Chrysanthemum: Chrysanthemum maximum* Ram.(DC) and *Chrysanthemum segetum* L. (owing to scarce information about their chemical composition, biological activity and therapeutic application), and *Rudbeckia laciniata* L. (owing to its similarity to *Echinacea* species so popular in recent applications in phytotherapy).

The qualitative and quantitative analysis was carried out using modern methods of HPLC. For identification purposes the diode array dedector (DAD) was used, which allows for obtaining UV-VIS spectra of separated compounds. The choice of optimal conditions of analysis was made using DryLab G+ Software,[6] belonging to the group of computer assistance. The quantitative analysis of chosen phenolic acids was carried out using the MCA method incorporated in the PolyView Software (Varian).

Downloaded At: 10:48 24 January 2011

Table 1

DryLab G⁺, Part 1, Input Values

System Variables

Dwell volume	1.00 mL
Column length	15.00 cm
Column diameter	0.46 cm
Flow rate	1.50 mL/min
Starting %-B	5.00
Final %-B	100.00
Gradient time, run-1	20.00 min
Gradient time, run-2	60.00 min
Default N-value for R_s calculations	5900

Library Matches in PolyView

Match	Similarity	Dissimilarity Range
Good	1.00 - 0.998	0.00 - 0.06
Poor	0.998 - 0.980	0.06 - 0.20
Bad	< 0.980	0.20

EXPERIMENTAL

The plant material (100 g) was extracted with chloroform in a Soxhlet apparatus to remove chlorophyll and other ballast compounds. The purified material was dried at room temperature and then extracted with methanol until colorless extracts were obtained and then with 1:1 mixture of methanol and water. The extracts were evaporated to a constant volume (100 mL). Before analysis the methanolic extracts were purified by solid phase extraction (Q silica, 500 mg, J. T.Baker USA).[7] The samples were extracts from: *Chrysanthemum segetum* L. - (sample A), *Rudbeckia laciniata* L. - (sample B), *Chrysanthemum maximum* Ram. (DC) - (sample C). The concentration of sample A was 0.02 g/mL.

Methods

HPLC experiments were carried out using Varian liquid chromatograph composed of modules: HPLC Pump - Model 9012, and Varian Polychrom®

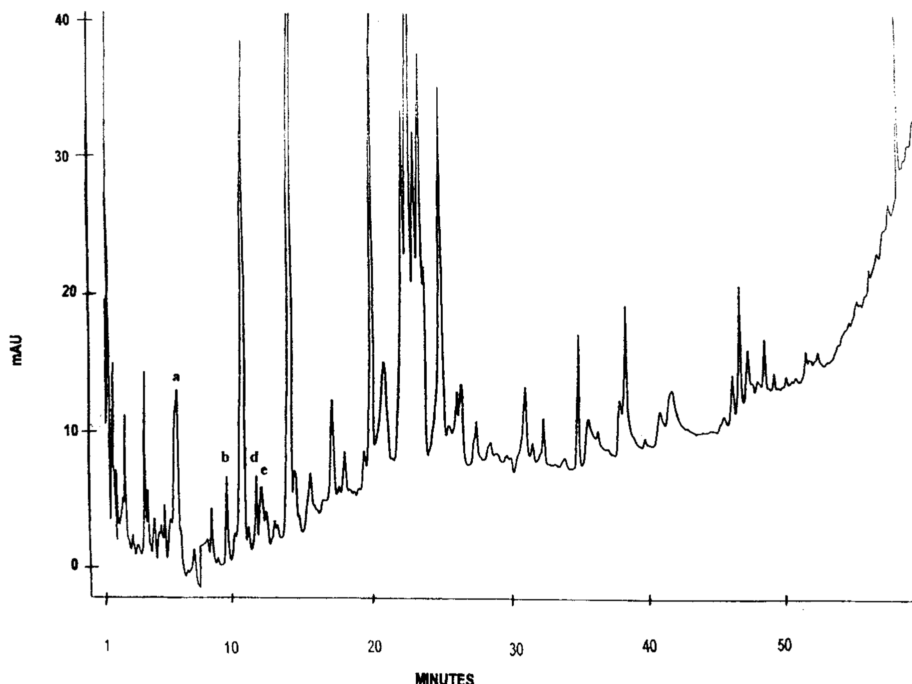


Figure 1. The chromatogram of the sample A; run - 1, t_G = 60 min., 5% - 100% MeOH, a - protocatechuic acid, b - p - hydroxybenzoic acid, c - chlorogenic acid, d - vanillic acid, e - caffeic acid, f - ferulic acid. Chart speed = 0.36 cm/min., attenuation = 36, zero offset = 5%, start time = 1.00 min., end time = 60.00 min.

Downloaded At: 10:48 24 January 2011

9065 Diode Array Detector. The chromatograph was equipped with 5μL sample injector (Rheodyne 7125 Injector). Varian Star Chromatography Software (Version 4.02) was employed for all chromatographic separations and data collection. The experiments were carried out at 25°C. The column TSK ODS 80 TM, d_p=5μm, 15 cm x 4.6 mm was also supplied by Varian. The number of theoretical plates was 5900 plates (mean value), as determined using the four standards of furanocoumarins as test mixture, eluted with methanol - water (68:32) at a flow - rate of 1.5 mL/min.

In the experiments the eluent composed of methanol grade p.a. (POCh - Gliwice, Poland) in doubly distilled water was used. The eluent contained 1% acetic acid (POCh - Gliwice, Poland). The standards of phenolic acids were obtained from „Sigma" (Sigma Aldrich Chemie GmbH, Deisenhofen, Germany) and were dissolved in methanol.

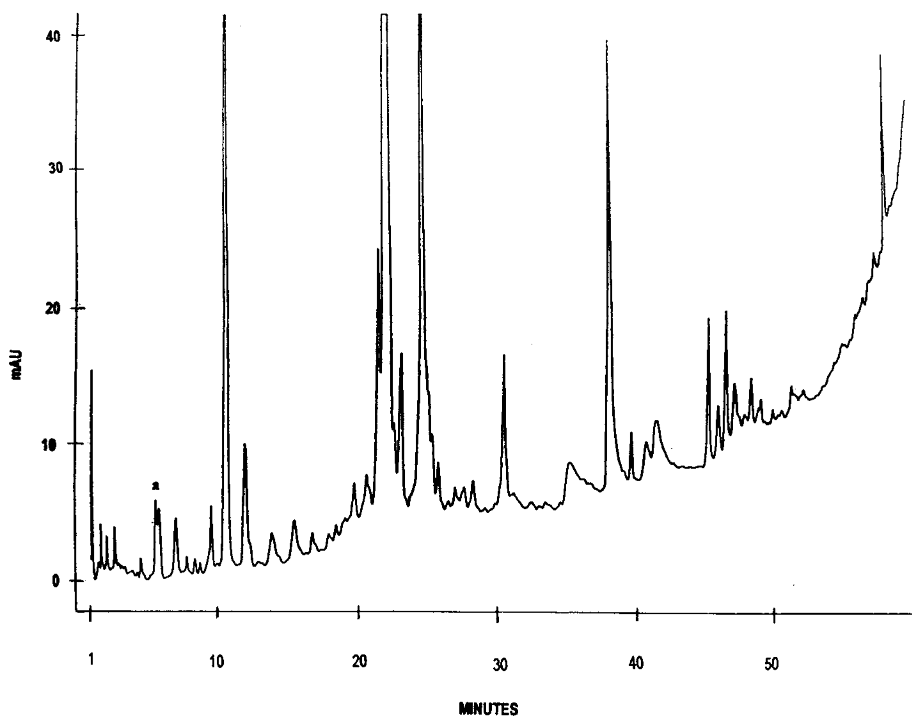


Figure 2. The chromatogram of the sample B; run - 1, t_G = 60 min., 5% - 100% MeOH, a - protocatechuic acid, c - chlorogenic acid. Chart speed = 0.36 cm/min., attenuation = 36, zero offset = 5%, start time = 1.00 min., end time = 60.00 min.

RESULTS AND DISCUSSION

Before carrying out the qualitative and quantitative analysis of complex natural mixtures it is necessary to choose optimal conditions of chromatographic analysis. There are a number of methods of optimization.[8] In the present study, the method of Snyder and Dolan was used which had been successfully applied in chromatographic separations carried out at the Department of Inorganic and Analytical Chemistry.[9-10] Their method consists of a combination of gradient elution and computer simulation.

The basis of computer simulations was the data obtained in two gradient runs which differed in gradient time (t_{G1} : t_{G2} = 1:3). The samples of plant extracts were analyzed at linear gradient of methanol at t_{G1} = 20 min. and t_{G2} = 60 min.

Downloaded At: 10:48 24 January 2011

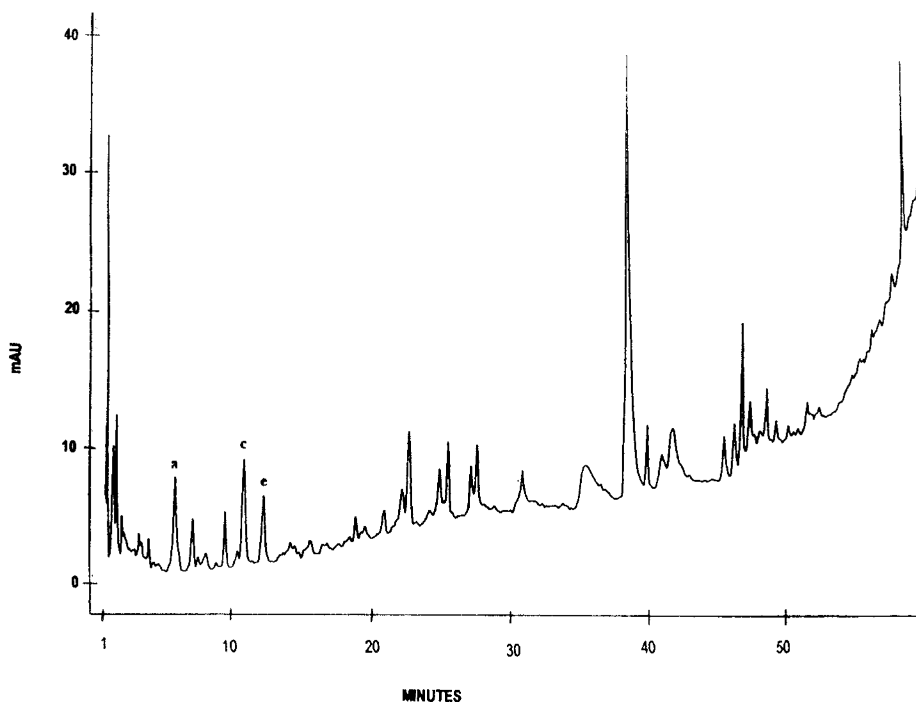


Figure 3. The chromatogram of the sample C; run - 1, t_G = 60 min., 5% - 100% MeOH, a - protocatechuic acid, c - chlorogenic acid, e - caffeic acid. Chart speed = 0.36 cm/min., attenuation = 36, zero offset = 5%, start time = 1.00 min., end time = 60.00 min.

Downloaded At: 10:48 24 January 2011

In both cases the concentrations of methanol varied in the range of 5% to 100%. The eluent was acidified with 1% acetic acid to suppress the ionization of phenolic acids. The initial data used in the program DryLab G+ are given in Table 1.

Figures 1 - 3 represent the chromatograms of samples A - C obtained in linear gradient for t_G = 60 min. Comparison of the chromatograms indicates that sample A is richest as far as the number of separated peaks is concerned. Therefore, the optimal separation conditions were chosen for sample A. Before the actual optimization, gradient chromatography of seven available standards of phenolic acids was performed. The analysis of the mixture of standards allowed for determining the elution sequence under conditions of gradient runs for various gradient times.

Table 2

Retention Values for Two Gradient Runs and Results of Library Search for the Mixutre of Standards*

The Name of Standard	Run-1 t_R(min)	Run-2 t_R(min)	Match Quality t_G = 20 min	t_G = 60 min
Protocatechuic acid	5.04	5.95	good	good
Chlorogenic acid	6.72	10.93	good	good
p-Hydroxybenzoic acid	6.92	9.56	good	good
Vanillic acid	7.64	11.95	---	---
Caffeic acid	7.64	12.45	---	---
p-Coumaric acid	9.36	16.80	good	good
Ferulic acid	9.64	18.34	good	good

* t_G = 20 and 60 min.

Table 3

Retention Values for Two Gradient Runs; Input Values for DryLab G$^+$*

Band #	Run-1 t_R (min)	Run-2 t_R (min)	Area
1	2.34	3.75	2535.00
2	3.31	3.95	13126.00
3	5.18	6.09	55640.00
4	6.72	1083	92490.00
5	6.99	11.79	13043.00
6	7.12	12.15	14193.00
7	7.85	14.13	266268.00
8	8.81	17.11	38829.00
9	8.98	18.01	21271.00
10	9.22	19.43	17595.00
11	9.87	19.93	84331.00

* Sample A

Downloaded At: 10:48 24 January 2011

Table 4

Run Time, Resolutions vs. Gradient Time for a Linear Gradient from 5% to 100% B

Gradient Time	Minimum R_s	Critical Band Pair	Retention Last Band
14.90	0.71	5.6	8.00
20.70	0.80	5.6	10.10
25.20	0.85	5.6	11.40
31.00	0.89	5.6	13.10
40.40	0.95	5.6	15.50
47.20	0.98	5.6	17.10
50.40	0.99	5.6	17.80
55.20	1.01	5.6	18.90
59.00	1.02	5.6	19.70
63.00	0.97	1.2	20.50

Downloaded At: 10:48 24 January 2011

The identification of standards was carried out by comparison of spectra, the retention time of a standard obtained under identical conditions was taken into account; this is necessary when the spectra are very similar. The results of identification and its quantitative estimation are given in Table 2, in which the criteria used in the estimation of compatibility of the spectra are also given (these criteria have been elaborated by Varian and are applied in the Software PolyView working with the diode array detector (DAD)).

Comparison of elution sequence showed a change of sequence of chlorogenic acid and p - hydroxybenzoic acid (for t_G = 20 min. and t_G = 60 min.). In Table 3 the retention times of the chosen 11 compounds of sample A are given. The data are for two gradient runs: 20 min. and 60 min. Out of several, only ten peaks in the time ranges corresponding to those of the standards were chosen.

The retention data from Table 3 were the basis of simulations carried out with DryLab G+. The estimation of separation was carried out using the simplest and most commonly applied peak resolution - R_S value; this criterion is applied in DryLab G+. Table 4 presents the R_S vs. t_G relationship: the highest R_S value was obtained for t_G = 59 min.; for highest gradient times the resolution decreases. It follows from the data of Table 4 that for identification purposes t_G = 60 min. for the column used in the experiments is suitable.

Table 5

Library Search Results for Sample of *Chrysanthemum Segetum* L. (Sample A) and Identification of Some Phenolic Acids

Name of Standard	SIM	DISSIM	PuP [nm]	t_R (Peak)	t_R (Standard)
Protocatechuic acid	0.99319	0.11652	269.488	6.046	5.955
-pHydroxybenzoic acid	0.99413	0.10818	255.173	9.638	9.600
Chlorogenic acid	0.99762	0.06897	302.728	10;789	10.929
Vanillic acid	0.96038	0.27869	264.935	11.772	11.951
Caffeic acid	0.97062	0.24062	302.318	12.165	12.447
Ferulic acid	0.99319	0.11647	307.369	19.906	18.323

Table 6

Library Search Results for Sample of *Rudbeckia Laciniata* L. (Sample B) and Identification of Some Phenolic Acids

Name of Standard	SIM	DISSIM	PuP [nm]	t_R (Peak)	t_R (Standard)
Protocatechuic acid	0.96290	0.24628	265.211	5.849	5.955
Chlorogenic acid	0.99975	0.02240	302.728	10.739	10.929

The next step was the identification of the phenolic acids in samples A - C. The results of identifications are given in Tables 5 - 7. The presence of 6 acids in sample A was found.

The remaining samples, B and C, are poor in the content of the investigated acids; the presence of protocatechuic and chlorogenic acids was found in sample B and in sample C; additionally caffeic acid.

The agreement of the observed spectra and those in the library is sometimes poor. Presumably the standards were not spectrally pure and the contaminations distorted the spectra, so the agreement could be limited.

Downloaded At: 10:48 24 January 2011

Table 7

Library Search Results for Sample of *Chrysanthemum Maximum* Ram. (DC) (Sample C) and Identification of Some Phenolic Acids

Name of Standard	SIM	DISSIM	PuP [nm]	t_R (Peak)	t_R (Standard)
Protocatechuic acid	0.95648	0.29180	264.831	5.923	5.955
Chlorogenic acid	0.99953	0.03068	306.959	10.813	10.929
Caffeic acid	0.99563	0.09341	304.445	12.214	12.447

Downloaded At: 10:48 24 January 2011

CONCLUSIONS

The Software DryLab G+ enables rapid optimization of chromatographic analysis of a multicomponent mixture, e.g., plant extract. The application of DAD with suitable software enables automation of peak identification in various gradient and isocratic runs (peak tracking). The availability of standards of components of interest allows for confirmation of their presence and for the quantitation of partially separated peaks. It permits estimation of the quality of some plants as the source of active compounds.

ACKNOWLEDGMENTS

Thanks are due to Varian and Candela for lending the 9012 and 9065 liquid chromatograph and software, which enabled carrying out this study. The investigation was sponsored by the grants of the Medical Academy of Lublin, Poland (P. W. 314\96 and P. W. 326\96).

This work is dedicated to Professor H. Poppe on the occasion of his 60th birthday.

REFERENCES

† The part of this work was presented at the 3rd European Congress of Pharmaceutical Sciences, Edinburgh, 15-17 September 1996.

1. K. Glowniak, A. J. Józefczyk, B. Piêtal, Ann. Univ. M. Curie-Sklodowska, Lublin, Polonia, 6/7, 85-94 (1993/94).

2. A. Cheminat, R. Zawatzky, H. Becher, R. Brouillard, Phytochemistry, **27**, 2787-2794 (1988).

3. H. Becher, W. C. Hsich, Z. Naturforsch. C Biosci., **400**, 585-587 (1985).

4. W. Czabajska, Wiadomości Zielarskie, 7, 7-9 (1990).

5. L. Swiątek, B. Grabias, M. Rózga, Herba Polonica, **33**, 87-98 (1987).

6. L. R. Snyder, J. W. Dolan, **Drylab G+ Instruction Manual**, L.C. Resources, Lafayette, CA, 1987.

7. J. Lubera, **Masters Thesis**, Pharmaceutical Faculty, Medical Academy, Lublin, 1995.

8. L. R. Snyder, J. L. Glajch, J. J. Kirkland, **Practical HPLC Method Development**, Wiley-Interscience, New York, 1988.

9. W. Markowski, T. H. Dzido, E. Soczewiński, J. of Chromatogr., **523**, 81-89 (1990).

10. W. Markowski, L. K. Czapińska, Anal. Chem. (Warsaw) **42**, 353-363 (1997).

Received April 12, 1997
Accepted August 7, 1997
Manuscript 4460

Downloaded At: 10:48 24 January 2011