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High-Performance Liquid Chromatographic Analysis of Chrysin Derivatives on A Nova-Pak® C_{18} Column

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A high-performance liquid chromatographic method has been developed for the separation and quantification of chrysin and synthetic chrysin derivatives (12 chrysin alkyl and 7 chrysin acyl derivatives). The chromatography was performed using a Nova-Pak® C_{18} column. A RP-HPLC was performed by using a binary mixture (MeOH-10 mM H_3PO_4) as a mobile phase, and the column temperature was maintained at room temperature. A flow rate was 1.0 ml/min, and the effluent was monitored at a wavelenth of 280 nm. The retention times for chrysin acyl and alkyl derivatives were within 10 minutes and 20 minutes, respectively. The absolute recovery of samples were all over 96%. The detection limits were 0.1~18 ng at S/N = 3 ratio.

Key words: Chrysin, Chrysin ethers, Chrysin esters, HPLC, NOVA-PAK® C_{18} Column

INTRODUCTION

Ward and Pelter(1974) published the first application of HPLC to flavonoid analysis. The analysis of flavonoids by high-performance liquid chromatography offers an accurate, sensitive technique which yields results in minutes. Wulf and Nagel(1976) later demonstrated with a dozen flavonoids. Their solvent system of methanol-acetic acid-water (30 : 5 : 65) on a C_{18} column was considered adequate for the resolution of a mixture of aglycones containing the same sugar. Acetic acid was added to the mobile phases to decrease the ionization of the acids. Daigle *et al.*(1982). reported the results of the HPLC analysis of 34 flavonoids using a methanol-acetic acid-water eluting system on a C_{18} column. Harborne *et al.*(1994, 1995) reported that a several methylether components separated using HPLC. But, the reports were not so much concerning separated flavonoid acyl and alkyl derivatives using HPLC. In a previous study in this laboratory(Shin *et al.* 1998), we studied the antidiabetic effect of chrysin derivatives. As this study continuous, we report the results of the HPLC analysis of 12 synthetic chrysin alkyl and 7 acyl derivatives using a methanol-10 mM phosphoric acid-eluting system on a C_{18} column.

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MATERIALS AND METHODS

Apparatus

Chromatographic analysis was performed using an Hitachi L-6200 pump (Hitachi, Japan) equipped with a 20 loop injector (model 7125, Rheodyne, USA), Hitachi L-6000 pump (Hitachi, Japan), Hitachi L-4200 and UV detector (Hitachi, Japan). Chromatographic data was processed by a Shimadzu C-R4Ae plus Chromapac integrator. For analysis, a Nova-Pak® C_{18} column (150 mm× 3.9 mm i.d., 7, Merck, Germany) was used.

Chemicals and reagents

Chrysin, dicyclohexylcarbodiimide (DCC) and diethylphosphoryl cyanide (DEPC) were purchased from Sigma Chemical Co. (St. Louis, MO). Other derivatizing reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI). HPLC grade water was prepared by using a Milli-Q system (Millipore, Milford, MA). Other solvents were HPLC grade (Merck, Darmstadt, Germany) and were filtered through a 0.45 μm Millipore filter. All other chemicals were of HPLC grade and were obtained locally. Chrysin alkyl and acyl derivatives were prepared by alkylation and condensation, respectively.

Synthesis

To a solution of chrysin in THF, $KHCO_3$ (or K_2CO_3, 2.5 eq) and alkyl bromide (or alkylchloride 5.0~10.0 eq) were added. The resulting mixture was stirred at an ambient

temperature for 36~48 hrs. The reaction mixture was washed with H_2O, 5% HCl, H_2O, and brine, successively. Then, the organic extracts were dried, evaporated, and concentrated in vacuo. Next, the resulting residue was chromatographed on silica gel by using $CHCl_3$-MeOH to afford a white or pale yellow solid. Each product was recrystallized and purified by using the proper solvent ($CHCl_3$, Ethyl actate or MeOH etc.). Chrysin acyl derivatives were synthesized by condensation using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in a THF solvent or condensation using diethylphosphoryl cyanide (DEPC) and triethylamine (TEA) in a DMF solvent.

Preparation of the chrysin derivative mixture solution

After each 1×10^{-3} M chrysin derivative $CHCl_3$ solution was prepared, a sample solution was made to dilute it with $CHCl_3$.

Calibration curve

A calibration curve of a synthetic chrysin derivative was obtained to use a standard solution which was prepared in the concentration range of 1~9 x 10^{-5} M.

HPLC analysis

A chrysin derivative mixture was quantified under the conditions as below.

Column	Novapak C-18 (3.9× 150 mm particle size; 4 μm)
Mobile phase	**Alkyl compds.** 0 min~11.5 min; MeOH : 10 mM H_3PO_4 (79 : 21) 11.5 min~ ; MeOH only **Acyl compds.** MeOH : 10 mM H_3PO_4 (80 : 20)
Flow rate	1.0 ml/min
UV detect.	UV 280 nm (0.05 Aufs)
Injection volume	10 μl

RESULTS AND DISCUSSION

The purpose of this study was to develop an assay

Table I. Calibration equation, correlation coefficient, and detection limit, etc. to chrysin alkyl derivatives.

Compounds	M.W	Calibration Equation	Correlation Coefficient	Detection Limit (ng)	Retention Time (min.)	Recovery Rate (%)
1	254	$Y = 4.184\times 10^9 X + 3.1068\times 10^3$	0.9992	0.2	1.56	99
2	268	$Y = 4.399\times 10^9 X - 1.2025\times 10^5$	0.9992	6.7	3.35	99
3	282	$Y = 3.855\times 10^9 X - 2.0499\times 10^5$	0.9952	7.1	4.51	97
4	296	$Y = 6.177\times 10^9 X - 3.6479\times 10^5$	0.9998	14.8	6.68	100
5	310	$Y = 2.305\times 10^9 X - 7.0921\times 10^4$	0.9999	15.7	10.16	104
6	366	$Y = 3.156\times 10^9 X - 1.4757\times 10^4$	0.9957	7.3	16.72	101
7	422	$Y = 2.285\times 10^9 X - 1.5372\times 10^3$	0.9982	18.0	20.39	103
8	282	$Y = 2.279\times 10^9 X - 1.5111\times 10^5$	0.9983	7.1	2.91	104
9	310	$Y = 4.380\times 10^9 X + 2.0635\times 10^5$	0.9966	3.9	4.28	97
10	338	$Y = 2.813\times 10^9 X - 2.1019\times 10^4$	0.9984	8.4	5.38	100
11	366	$Y = 4.963\times 10^9 X + 1.1425\times 10^5$	0.9934	16.8	11.06	102
12	338	$Y = 3.341\times 10^9 X - 5.2715\times 10^3$	0.9999	8.5	1.49	96
13	296	$Y = 2.911\times 10^9 X + 3.9675\times 10^4$	0.9995	7.5	1.91	97

1: Chrysin, **2**: 5-hydroxy-7-methoxyflavone, **3**: 5-hydroxy-7-ethoxyflavone, **4**: 5-hydroxy-7-propoxyflavone, **5**: 5-hydroxy-7-butoxyflavone, **6**: 5-hydroxy-7-octoxyflavone, **7**: 5-hydroxy-7-dodecoxyflavone, **8**: 5,7-dimethoxyflavone, **9**: 5,7-diethoxyflavone, **10**: 5,7-dipropoxyflavone, **11**: 5,7-dibutoxyflavone, **12**: 5,7-diacetoxyflavone, **13**: 7-hydroxy-5-acetoxyflavone. The mean of three times in a day for one week.

Table II. Calibration equation, correlation coefficient, and detection limit etc. to chrysin acyl derivatives.

Compounds	M.W	Calibration Equation	Correlation Coefficient	Detection Limit (ng)	Retention Time (min.)	Recovery Rate (%)
14	416	$Y = 6.764\times 10^9 X + 5.7637\times 10^4$	0.9985	0.1	3.21	98
15	358	$Y = 6.412\times 10^9 X\ 1.4652\times 10^4$	0.9996	3.6	6.30	103
16	384	$Y = 5.823\times 10^9 X + 2.4691\times 10^4$	0.9992	1.1	8.00	97
17	414	$Y = 1.013\times 10^{10} X - 6.6156\times 10^4$	0.9998	4.1	8.70	96
18	372	$Y = 8.824\times 10^9 X + 1.9499\times 10^3$	0.9990	0.7	9.57	98
19	376	$Y = 5.036\times 10^9 X + 1.5384\times 10^5$	0.9989	3.7	6.01	107
20	322	$Y = 1.327\times 10^{10} X + 1.1774\times 10^4$	0.9997	1.6	3.61	99

14: chrysin 7-O-acetylsalicylate, **15**: chrysin 7-O-benzoate, **16**: chrysin 7-O-cinnamate, **17**: chrysin 7-O-4-methoxycinnamate, **18**: chrysin 7-O-p-toluate, **19**: chrysin 7-O-p-fluorobenzoate, **20**: chrysin 7-O-crotonate. The mean of three times in a day for one week.

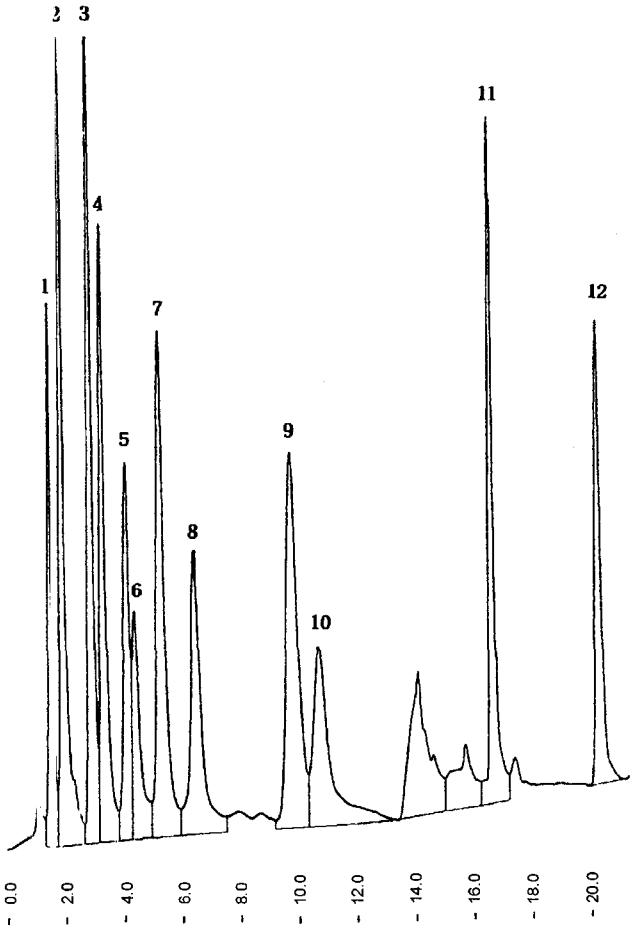

Fig. 1. HPLC chromatogram of chrysin alkyl derivatives ($CHCl_3$ soln.) See the footnote of Table I for the full name of the compounds

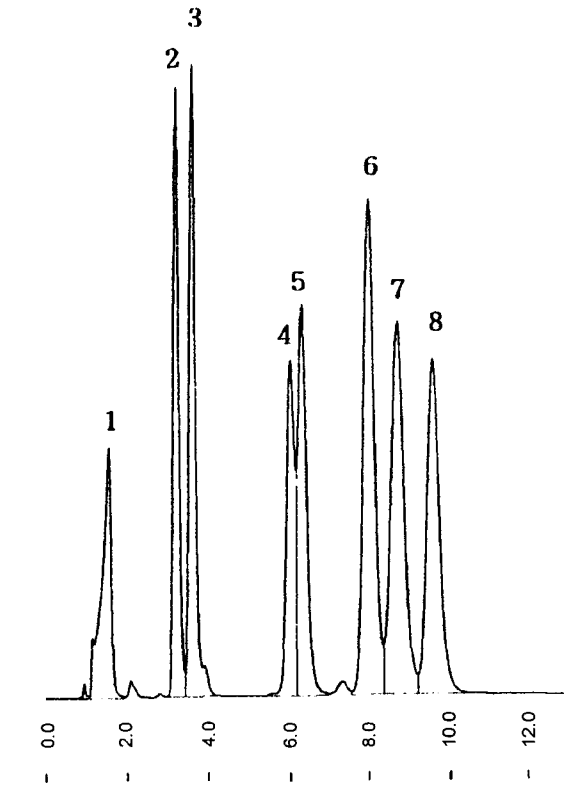

Fig. 2. HPLC chromatogram of chrysin acyl derivatives ($CHCl_3$ soln.) See the footnote of Table II for the full name of the compounds

method for the analysis of chrysin derivatives with HPLC. The separation of chrysin mono alkyl and dialkyl derivatives or chrysin acyl derivatives have not been reported.

The analytical conditions of HPLC and the calibration equation, etc. to chrysin alkyl derivatives were shown above. Although the general structure-retention time relationship of the chrysin alkyl derivatives was not elucidated from these data, the alkyl derivatives mostly exhibited a long retention time according to the increase of the carbon number in monomer or dimer.

In the case of chrysin acyl derivatives, the analytical conditions of HPLC and the calibration equation, etc. to the chrysin acyl derivatives were shown in Table II-1 and II-2. By the sort of compounds, acetylsalicylic acid, crotonic acid, p-fluorobenzoic acid, benzoic acid, cinnamic acid, methoxycinnamic acid and toluic acid binded at the 7-position of chrysin, the retention times were revealed more longer in turn.

This report presented an improved HPLC method for the quantitation of chrysin alkyl and acyl derivatives in the mixture using a simple sample-preparation procedure. The analysis method was accurate, sensitive, and selective with no interference by the small structure difference. High and reproducible recoveries of the alkyl or acyl derivatives from the mixture were obtained. Since the method was simple and rapid, requiring no time-consuming liquid procedures, it is recommended that this assay be used for a similar analogous study.

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REFERENCES

Ward, R. S. and Pelter, A., *J. Chromatogr. Sci.*, 12, 571 (1974).

Wulf, L. W. and Nagel, C. W., *J. Chromatogr. Sci.*, 116, 271 (1976).

Daigle, D. J. and Conkerton, E. J., High-performance liquid chromatography of 34 selected flavonoids. *Journal of Chromatography*, 240, 202-205 (1982).

Greenham, J., Williams, C. and Harborne, J. B., Identification of Lipophilic Flavonols by a combination of Chromatographic and Spectral Techniques. *Phytochemical Analysis*, 35, 211-217 (1995).

Harborne, J. B., Williams, C.A., Greenham, J. and Eagles, J., Variations in the lipophilic and vacuolar flavonoids of the genus Vellozia. *Phytochemistry*, 35, 1475-1480 (1994).

Shim, S-H., Shin, J. S., Kim, M-B., Kim, H-J., Kim, W-K., Kim, S-J., and Kim, B-K., Properties and Anti-diabetic Effect of 7-*O*-p-Fluoro-benzoylchrysin. *J. Appl. Pharmaco.*, 6, 256-260 (1998).