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Separation of coumarins from *Archangelica officinalis* in high-performance liquid chromatography and thin-layer chromatography systems

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Abstract

Complex, multicomponent mixtures are difficult to separate in a single chromatographic run. Therefore, the possibility to separate twelve coumarins from *Archangelica officinalis* was studied by combining a HPLC and a TLC system. HPLC optimized by the use of DryLab for Windows software was performed on RP-18 column and TLC was performed on silica plates. Fractions from the RP column were evaporated, applied on silica plate and developed in non-aqueous solvent. Possibilities of complete separation of investigated coumarins were discussed in RP and NP systems. The result of their complete separation was presented by HPLC chromatograms, DryLab simulated chromatograms and a video scan of TLC plate.

Keywords: *Archangelica officinalis*; Coumarins

1. Introduction

The main problem of separation of complex natural mixtures (e.g. plant extracts) by coupled techniques is finding systems which have different selectivities and allow effective separation by changing the chromatographic system, e.g., from RP to NP, especially when investigated compounds have closely related structures. Often, the separation of complex plant extracts and selection of a chromatographic system with adequate selectivity does not provide a satisfactory result [1–3]. Sometimes it is necessary to apply systems, which have radically different selectivities (e.g. reversed phase and normal phase) [4] to get the full separation. A systematic study on the subject of multidimensional methods in TLC was presented by Poole [5].

The substances of interest in the present study, coumarins, which are stable and do not decompose during the application process from the column on the chromatographic plate, can be analysed in a single chromatographic run (on-line). In this way, e.g., polycyclic aromatic hydrocarbons were analysed [6,7]. Possibilities of preparative separation of coumarins in TLC systems on Florisil [11,12], OPLC separation of coumarins [8–10] and gradient elution of coumarins in plant extracts [13] were also studied.

There are many coumarins in *Archangelica officinalis* and their complete separation is a difficult

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problem because of their similar structures and low stability.

2. Experimental

All HPLC experiments were performed using HP-1050 chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with UV–VIS detector (HP-1050) and Rheodyne injection valve with 20 μl loop (Rheodyne, Cotati, CA, USA). Standards and extract were applied on an Alltech RP-18 column packed with 5 μm Adsorbosphere HS (250×4.6 mm I.D.) (Alltech, Deerfield, USA). Methanol (POCh, Gliwice, Poland) mixed with redistilled water (8:2, v/v, 7:3, v/v and 6:4, v/v) was used as a mobile phase. 0.1% solutions in methanol of all test substances and 1% solution of *Archangelica* extract were injected into the chromatographic column and chromatographed at a flow rate of 1 ml/min.

Chromatograms were registered at 254 nm and worked out with the help of CHROMA (Pol-Lab, Warsaw, Poland) computer program.

Xanthotoxin, umbelliferone and bergapten were obtained from Fluka (Buchs, Switzerland), the remaining test coumarins were obtained from preparative separation carried out earlier in this department.

Dichloromethane (POCh, Gliwice, Poland), *n*-heptane (PS Park, Northampton, UK) and ethyl acetate (POCh, Gliwice, Poland) were used in mixtures as mobile phases in TLC experiments. All solvents were of analytical grade (*n*-heptane was pure).

Fractions of coumarins partly separated in HPLC were collected, evaporated at 30°C and dissolved in methanol. HPTLC precoated plates LiChrospher Si 60 F_{245s} 10×10 cm (Merck, Darmstadt, Germany) were used in TLC separations. Samples of 10 μl volume of fractions and 5 μl of extract were applied on the chromatographic plates as 2 mm length streaks using AS 30 TLC applicator (Desaga, Heidelberg, Germany). Plates with applied spots were conditioned within 10 min to avoid demixing effect and developed in horizontal DS-II chamber (Chromdes, Lublin, Poland) [14,15] on distance of 8 cm.

The plate with separated coumarins was scanned by Camag Reprostar 3 (Camag, Muttenz, Switzerland) at 365 nm, and Desaga CD60 Densitometer (Desaga, Heidelberg, Germany) at 313 nm.

DryLab ver. 2.05 for Windows (1998) program used for HPLC experiments optimisation was obtained from Dr. L.R. Snyder (LC Resources, Lafayette, CA, USA).

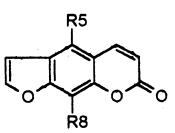
3. Results and discussion

An attempt was made to create a complete separation of twelve coumarins occurring in the roots of *Archangelica officinalis*. Numbers and structures of the analysed compounds are specified in Table 1.

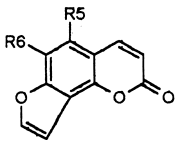
The first stage of investigations was to find the optimal conditions for separation by means of reversed-phase chromatography (RP-HPLC). To this end, chromatography of test substances was performed for two concentrations of methanol in water: 8:2 (v/v) and 7:3 (v/v). From the data obtained (Table 2) after inserting them into DryLab program, we received optimisation results for the column system. It follows that (Fig. 1) the optimal R_s for investigated group of substances is between 55 and 70% (v/v) of methanol in water. At lower concentrations of methanol, e.g. 40% (v/v), the results of separation are not satisfactory, because compounds 3, 6 and 7–9 are not separated and the time of analysis is very long — the last compound, isoimperatorin has the retention time of 319 min. At the 80% (v/v) concentration of methanol in water the separation of investigated coumarins is also poor although the time of analysis is short — the last substance, isoimperatorin has the retention time of 8.46 min. From simulated DryLab chromatograms we chose the concentration of 60% (v:v) of methanol in water as an optimal mobile phase for RP column separation — the simulated chromatogram for this concentration is shown in Fig. 2.

The HPLC analysis of root extract was performed in 60% (v:v) of methanol in water and the chromatogram that shows this separation is demonstrated in Fig. 3. This chromatogram shows that column chromatography in reversed-phase system does not allow for complete separation of substances: 1–4; 7–9 and

Table 1
Name and structures of analysed coumarins



1



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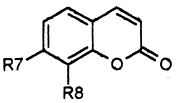

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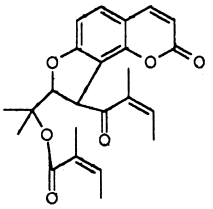

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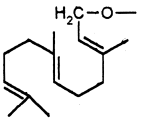

R

Name	No	Structure	Substituents			
			R5	R6	R7	R8
Umbelliferone	1	3	-	-	OH	-
Xanthotoxol	2	1	-	-	-	OH
Archangelicin	3	4	-	-	-	-
Xanthotoxin	4	1	-	-	-	OCH_3
Isopimpinellin	5	1	OCH_3	-	-	OCH_3
Bergapten	6	1	OCH_3	-	-	-
Pimpinellin	7	2	OCH_3	OCH_3	-	-
Phelopterin	8	1	OCH_3	-	-	$OCH_2CH=C(CH_3)_2$
Imperatorin	9	1	-	-	-	$OCH_2CH=C(CH_3)_2$
Osthol	10	3	-	-	OCH_3	$OCH_2CH=C(CH_3)_2$
Umbeliprenin	11	3	-	-	R	-
Isoimperatorin	12	1	$OCH_2CH=C(CH_3)_2$	-	-	-

Table 2
Retention data for optimisation of separation for column chromatography by simulation of DryLab program[a]

Peak no.	Peak name	60% methanol+40% water		70% methanol+30% water		80% methanol+20% water	
		k	R_s	k	R_s	k	R_s
1	Xanthotoxol	0.3	2.20	0.2	1.17	0.1	0.53
2	Umbelliferone	0.5	6.06	0.3	2.65	0.2	0.15
3	Archangelicin	1.1	1.89	0.5	1.79	0.2	2.90
4	Xanthotoxin	1.4	3.81	0.7	3.93	0.4	2.50
5	Isopimpinellin	2.0	3.42	1.2	1.54	0.7	0.26
6	Berapten	2.7	12.05	1.4	7.69	0.7	3.79
7	Pimpinellin	7.1	0.18	2.8	0.17	1.1	0.14
8	Imperatorin	7.2	0.35	2.9	0.19	1.1	0.06
9	Phellopterin	7.4	4.22	2.9	3.23	1.2	2.08
10	Osthol	9.8	3.71	3.8	3.02	1.4	2.11
11	Umbelliprenin	12.6	3.60	4.7	2.94	1.8	2.08
12	Isoimperatorin	16.0		5.9		2.2	

[a] The data for 60% methanol+40% water for the comparison with data in Fig. 3.

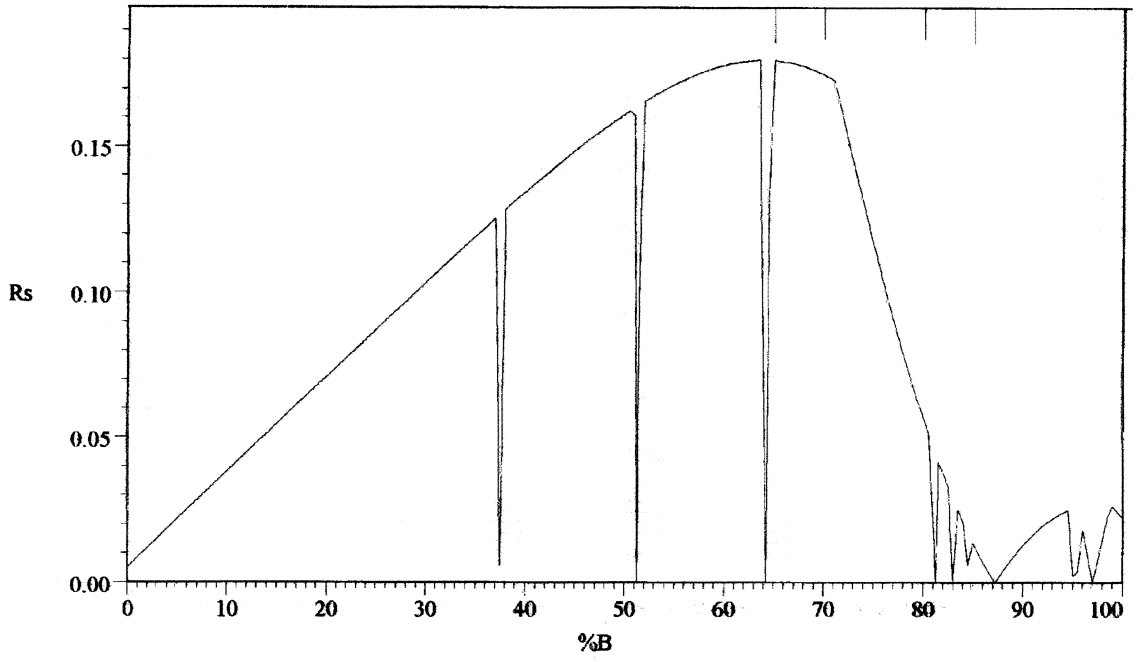

Fig. 1. The simulated Dry Lab graph of R_s vs. concentration of methanol used in HPLC separation of investigated coumarins.

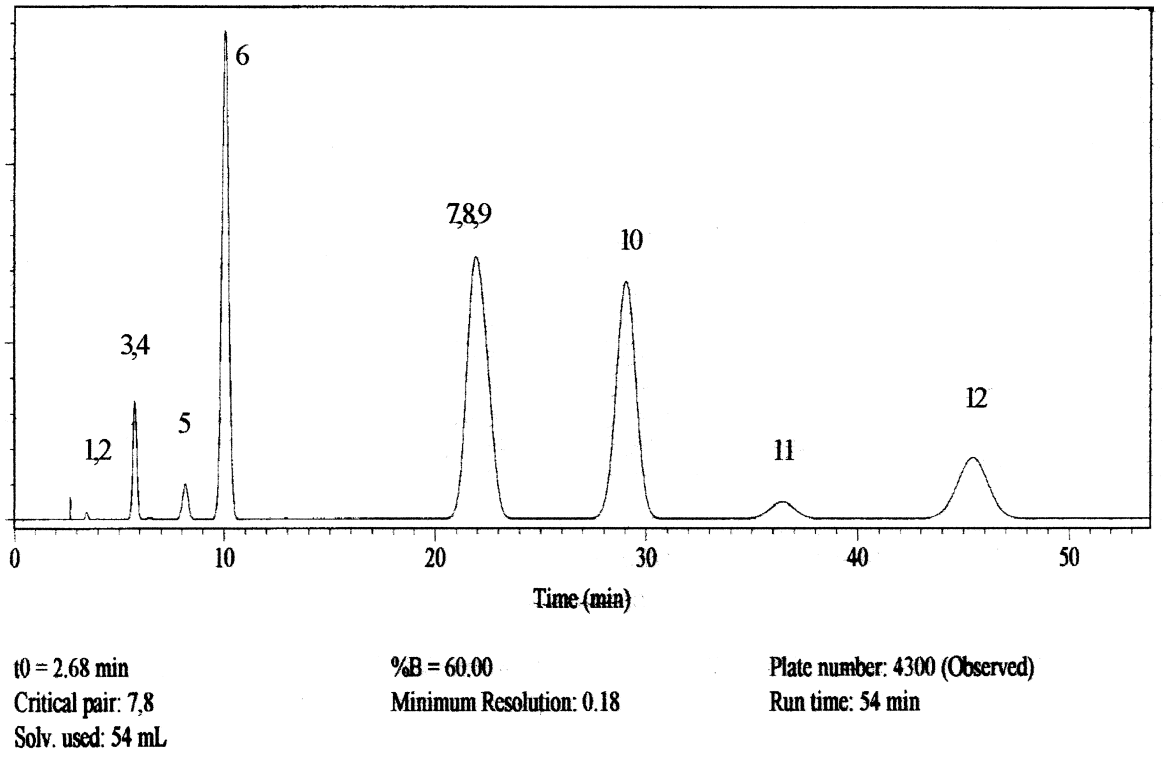

Fig. 2. The simulated Dry Lab HPLC chromatogram for 60% (v/v) of methanol in water. Numbers of substances as in Table 1.

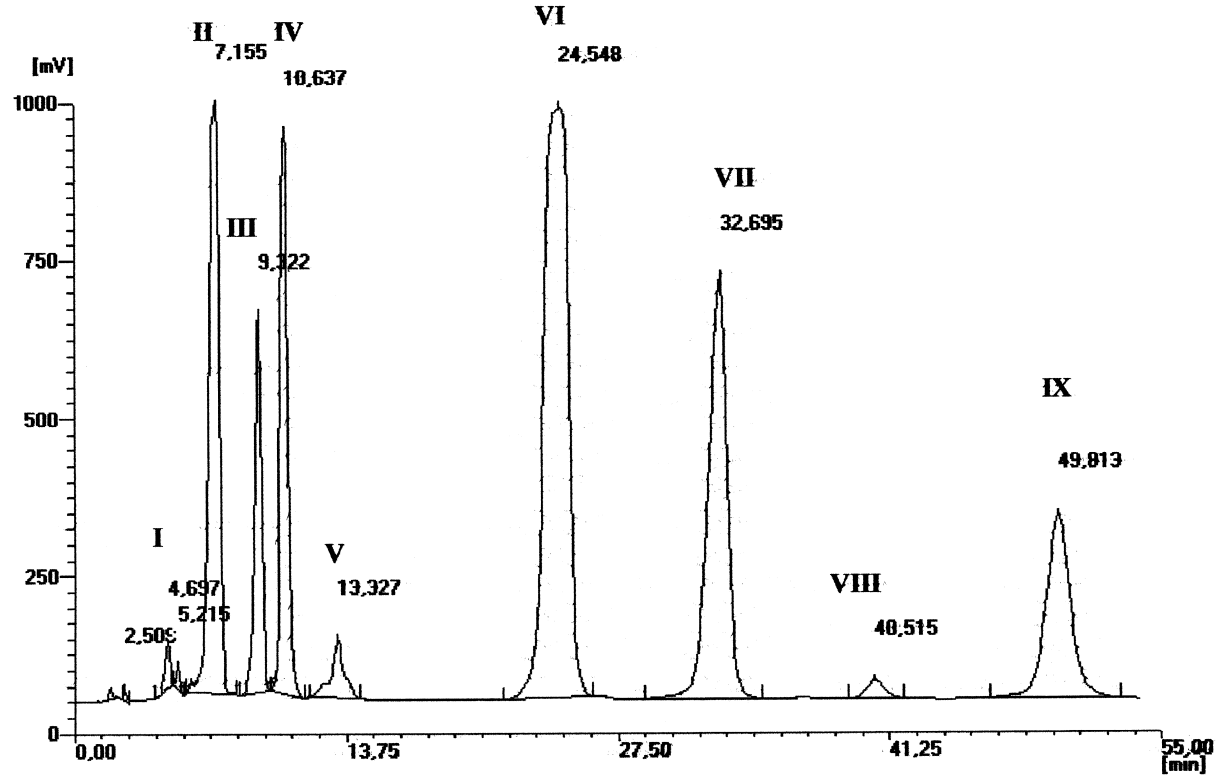

Fig. 3. The experimental chromatogram obtained by using 60% (v/v) of methanol in water. Roman numbers are numbers of fractions collected during HPLC separation.

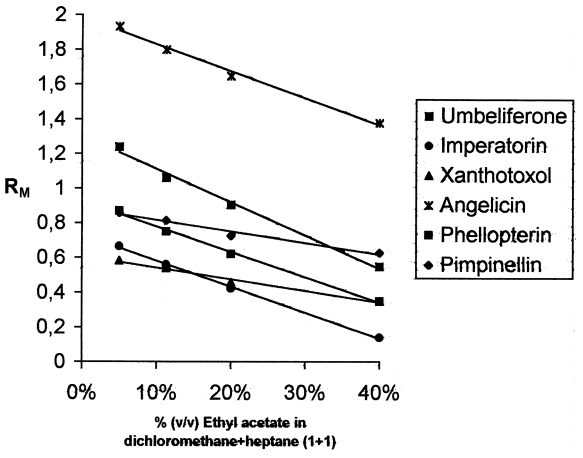

Fig. 4. The relationship R_M vs. concentration of ethyl acetate in dichloromethane+*n*-heptane (1+1) for coumarins not separated in HPLC.

proves that isocratic HPLC is not suitable for the separation of *Archangelica* extract. Therefore fractions from HPLC were collected (numbers of fractions I–IX in Fig. 3) according to the consecutive peaks and their not separated groups on the HPLC chromatogram.

The solvents from all fractions were evaporated and then the consecutive fractions analysed in NP TLC system. The optimisation of conditions in TLC system was performed on the basis of retention — composition of non-aqueous solvent relationship: ethyl acetate in dichloromethane+heptane [16]. The graph illustrating the relationship R_M vs. composition of eluent for coumarins not separated in HPLC system is shown in Fig. 4. It demonstrates that the optimal selectivity for investigated compounds is obtained for 40% (v:v) ethyl acetate in dichloromethane+heptane (1+1).

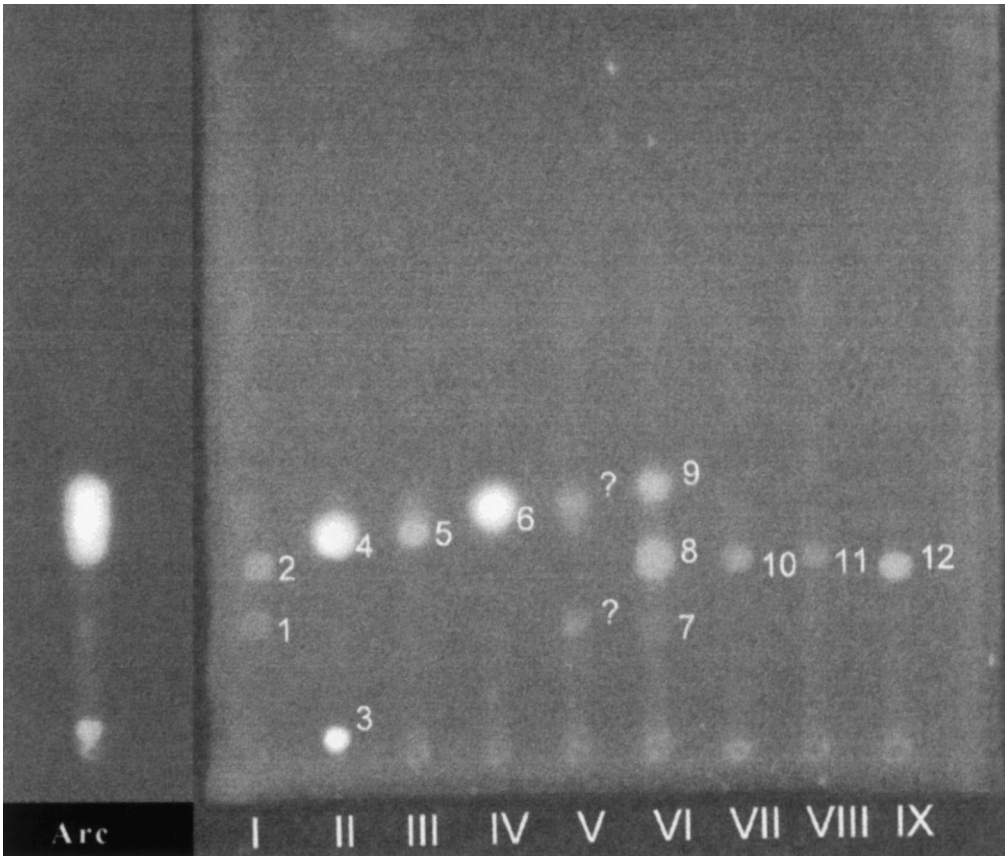

Fig. 5. The Camag Reprostar 3 photography of TLC plate with applied fractions collected during HPLC separation and *Archangelica off.* Extract scanned in black and white mode. Number of substances as in Table 1, ?: unidentified substances. Arc: *Archangelica officinalis* extract. Roman numbers of fraction as in Fig. 3.

The plate with applied streaks of fractions after development was dried in air and scanned by Camag Reprostar 3. The photograph of the chromatographic plate is shown in Fig. 5. It demonstrates that all fractions are separated completely, although substances: 2, 8; 10–12; 4, 5 and 1, 7 could not be separated by TLC if all fractions would be applied together on the chromatographic plate.

4. Conclusions

The coumarins present in the investigated extract were completely separated as a result of the different selectivities of the two combined chromatographic techniques, RP-HPLC and NP-TLC. On-line coupling of these techniques is virtually impossible because of the instability of the coumarins at temperatures of about 60–80°C, and because of their volatility. The present method may well be useful for the micropreparative separation of coumarins.

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