

## Note

### High-performance liquid chromatographic separation of benzoquinone components of the wood-rotting fungus *Sarcodontia setosa*

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Natural 2-methoxy-6-substituted 1,4-benzoquinones are found predominantly in higher plants and fungi<sup>1</sup> and are usually biologically active<sup>2,3</sup>.

In this work we studied the isocratic separation of a mixture of three benzoquinone compounds with antifungal activity by means of high-performance liquid chromatography (HPLC). The substances are 2-methoxy-1,4-benzoquinones substituted in the 6-position with a side-chain containing a varying number of double bonds. These substances were obtained in the form of free acids by isolation from the wood-rotting fungus *Sarcodontia setosa* (Pers.) Donk<sup>4</sup>, and were converted into methyl esters 1-3 (Fig. 1) by reaction with diazomethane. In order to obtain them in a pure state it was necessary to devise a method suitable for separation on a preparative scale.

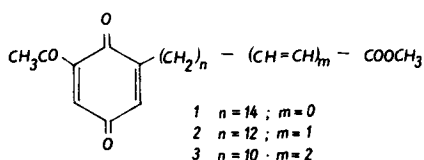


Fig. 1. Structure of 15-(6-methoxy-1,4-benzoquinon-2-yl)pentadecanoic acid methyl ester and its analogues.

## EXPERIMENTAL

### Apparatus

Analytical HPLC was carried out on an apparatus consisting of a Constametric I (Laboratory Data Control, Riviera Beach, FL, U.S.A.) pump, a Knauer injection valve (Knauer, Bad Homburg, F.R.G.) and a UVM-4 UV detector (VD ČSAV, Prague, Czechoslovakia). A glass column (150 × 3 mm I.D.) packed with Separon Si VSK (5 μm) (Laboratorní Přístroje, Prague, Czechoslovakia) was used.

TABLE I  
 VARIATION OF  $R_s$  AND  $k'$  WITH MOBILE PHASE COMPOSITION

Mobile phase	Proportion of second component (%)	$R_s^{1,2}$	$R_s^{1,3}$	$R_s^{2,3}$	$k'_1$	$k'_2$	$k'_3$
Hexane-2-propanol	0.75	2.30	5.00	2.04	16.3	18.5	20.7
	1.00	1.61	3.54	1.53	9.08	10.2	11.3
	1.50	1.90	3.09	1.40	6.75	7.45	8.15
	2.00	1.84	3.04	1.38	5.69	6.16	6.71
Hexane-ethyl acetate	7.00	3.66	6.63	2.38	23.1	26.6	31.8
	8.50	2.11	5.02	2.41	16.1	18.4	21.9
	10.0	1.77	3.97	1.44	7.82	8.76	10.2
Hexane-tetrahydrofuran	5.00	3.12	7.10	3.68	9.78	11.3	13.6
	7.50	2.84	5.79	3.11	5.95	6.77	8.00
	10.0	1.75	4.00	2.26	5.25	5.80	6.75
Dichloromethane-ethyl acetate	0.50	2.62	4.78	1.70	11.7	14.8	16.0
	1.00	2.75	5.28	1.67	6.91	8.27	9.30
	2.00	2.76	4.22	1.29	3.14	3.94	4.36

## RESULTS AND DISCUSSION

To establish the optimal conditions for the separation of compounds 1-3, thirteen mobile phases were tested. The results are summarized in Table I. The first criterion for the choice of a mobile phase suitable for preparative chromatography was the resolution,  $R_s$ , which should be equal or higher than 2. Another important

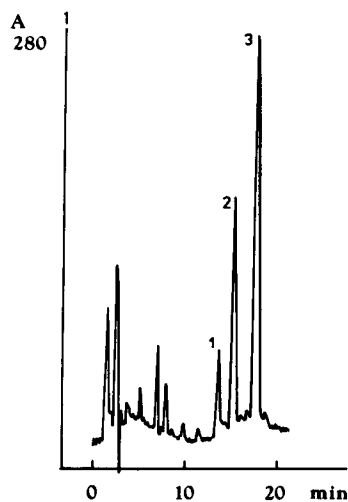


Fig. 2. Separation of compounds 1-3. Column, Separon Si VSK (5  $\mu$ m); mobile phase, hexane-7.5% tetrahydrofuran; flow-rate, 0.7 ml/min; detection, UV at 280 nm; sensitivity, 0.25 A.U.

factor was the capacity factor,  $k'$ , which determines the time of chromatography and thus the total consumption of the mobile phase. From this point of view mixtures of hexane-2-propanol and hexane-ethyl acetate are not suitable, because they achieve a satisfactory resolution only at high  $k'$ . Hexane-tetrahydrofuran and dichloromethane-ethyl acetate achieve  $R_s \geq 2$  at  $k' = 6-9$ , and these mixtures are acceptable from the point of view of chromatography time. In addition, these mobile phases have different selectivities, which can be useful when only one isomer is required. On the basis of the results obtained, hexane-7.5% tetrahydrofuran (Fig. 2) may be selected as the most suitable mobile phase.

#### REFERENCES

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