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SEPARATION OF QUINONES AND THEIR DERIVATIVES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

BOHUSLAV RITTICH and MIROSLAV KRŠKA
Research Institute for Animal Nutrition, 691 23 Pohořelice, near Brno (Czechoslovakia)
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SUMMARY

The separation of mixtures of quinones by high-performance liquid chromatography has been studied. The technique described is suitable for separating differently substituted quinones. Particularly convenient is the conversion of quinones into 2,4-dinitrophenylhydrazones, which makes the reliable identification of benzoquinones and naphthoquinones possible.

Studies of the separation of 2,4-dinitrophenylhydrazones of quinones and nitrophenols on Sephadex LH-20 gel indicated that the substances in question were not separated on the basis of differences in molecular weight; but the R_F values were proportional to the dissociation constant.

INTRODUCTION

A considerable proportion of naturally occurring pigments are quinones, and a major group of naturally occurring naphthoquinones are the K-group vitamins, which are of great importance for warm-blooded animals as they maintain the normal blood sedimentation level.

For the separation of quinone mixtures, thin-layer chromatography (TLC) on polyamide[1], silica gel impregnated with oxalic acid[2] and Silufor[3] have been used, and a mixture of naphthoquinones has also been successfully separated by means of gel chromatography[4]. A useful method for the identification of quinones is to convert them into 2,4-dinitrophenylhydrazones, which are formed as azo dyes, followed by separation by reversed-phase paper chromatography[5] or TLC[6,7].

Derivatives of anthraquinone containing *o*-dihydroxy groups are important analytical reagents, and paper chromatography[8,9] and TLC[10] have been used for their separation. Anthraquinones containing non-polar substituents[11], amino-anthraquinones[12] and some naturally occurring anthraquinones[13] have been separated by means of liquid chromatography.

The objective of this work was to study the separation of quinones and their 2,4-dinitrophenylhydrazones by liquid chromatography. We have also investigated the separation of polyhydroxy derivatives of anthraquinones, the separation of which by paper chromatography or TLC is difficult.

EXPERIMENTAL

Chemicals and equipment

1,2-Benzoquinone was prepared from *o*-aminophenol[14], 2,3- and 1,4-dihydroxyanthraquinone by a method described elsewhere[15] and 2,4-dinitrophenylhydrazones of quinones by the method described by Večeřa and Gasparič[14]. 1,2-Naphthoquinone 4-(2,4-dinitrophenylhydrazone) was prepared from 1,2-naphthoquinone-4-sulphonic acid, sodium salt, and 2,4-dinitrophenylhydrazine[14]. Other chemicals were commercial preparations.

Sephadex LH-20 was obtained from Pharmacia (Uppsala, Sweden). A Varian LC 8500 liquid chromatograph was used with the following columns: Micro Pak Si-10 (Merck, Darmstadt, G.F.R.), silica gel, particle size 10 μm, 50 cm $\times$ 2.2 mm; Micro Pak CN-10 (Merck), silica gel with chemically bonded polar phase (–C≡N), particle size 10 μm, 25 cm $\times$ 2.2 mm; and Micro Pak CH-5 (Merck), silica gel with chemically bonded non-polar phase (C_{18}), particle size 5 μm, 25 cm $\times$ 2.2 mm. A UV detector (λ_{max} 254 nm) was used. Hamilton 705 chromatographic syringes (50 μl) and a 2-μl constriction pipette (Labora, Prague, Czechoslovakia) were used.

Experimental conditions

The separation was carried out on Micro Pak Si-10 and CN-10 columns with light petroleum (b.p. 80–100°) with different proportions of 2-propanol as the mobile phase. The flow-rates varied from 60 to 120 ml/h. The samples were dissolved in the mobile phase at a concentration of about 1 mg/ml and 10-μl volumes of the solution were injected. 2,4-Dinitrophenylhydrazones of quinones were extracted from the reaction mixture with chloroform and 5–10-μl volumes of the resulting solution were injected.

In the separation on the Micro Pak CH-5 column, a mixture of methanol and water was used as the mobile phase. In order to prevent the dissociation of hydroxyl groups, we adjusted the pH of the water to 3 by means of citrate–phosphate buffer. The samples, dissolved in the mobile phase at a concentration of 0.4 mg/ml, were injected in volumes of 10 μl. The flow-rate of the mobile phase was 60 ml/h.

In gel chromatography, a thin layer of Sephadex LH-20 gel was used, the gel being swollen in 2-propanol for 24 h. A thick suspension was applied on glass plates (20 $\times$ 20 cm), the thickness of the adjusted slot being 0.5 mm. About 5 g of dry gel were used per plate. After partial evaporation of the solvent, the plate was inserted into the chamber in a slightly slanting position and developed for 1 h with 2-propanol. The layer was fed by means of paper bridge (Whatman No. 3). The samples were applied with a 2-μl constriction pipette and the development was carried out at 22 $\pm$ 1° for 24 h.

RESULTS AND DISCUSSION

Separation of mixtures of quinones and their 2,4-dinitrophenylhydrazones

A mixture of quinones was separated on a Micro Pak Si-10 column, using light petroleum (b.p. 80–100°) as the mobile phase, with 1% 2-propanol (Table I).

Quinones with carbonyl groups in the *ortho*-position (apart from *o*-benzoquinone) have a greater elution volume than quinones with carbonyl groups in the

TABLE I

SEPARATION OF A MIXTURE OF QUINONES BY LIQUID CHROMATOGRAPHY

V_R = elution volume of component.

Compound	V_R *(ml)*	
	*I**	*II***
1,2-Benzoquinone	3.42	2.40
1,4-Benzoquinone	3.42	2.43
5,6-Dimethoxy-1,4-benzoquinone	2.88	2.49
1,2-Naphthoquinone	1.98	1.92
1,4-Naphthoquinone	1.74	1.38
2-Methyl-1,4-naphthoquinone	2.16	1.32
2-Methyl-3-phytyl-1,4-naphthoquinone	1.32	0.78
Anthraquinone	1.98	1.47
Phenanthrenquinone	6.12	2.46

* Micro Pak Si-10 (1% 2-propanol in light petroleum, 120 ml/h, 200 atm). Elution volume of unretained component = 1.20 ml.

** Micro Pak CN-10 (1% 2-propanol in light petroleum, 60 ml/h, 60 atm). Elution volume of unretained component = 0.48 ml.

TABLE II

SEPARATION OF QUINONE 2,4-DINITROPHENYLHYDRAZONES BY LIQUID CHROMATOGRAPHY AND GEL CHROMATOGRAPHY

Compound	*Liquid chromatography:* V_R *(ml)*		*Gel chromatography:* $R_{azobenzene}$***	*Colour*
	*I**	*II***		
trans-Azobenzene	1.44	0.72	1.00	Orange
cis-Azobenzene	2.22	1.08	—	
1,2-Benzoquinone 2-(2,4-dinitrophenylhydrazone)	2.82	1.89	0.60	Yellow
1,4-Benzoquinone 4-(2,4-dinitrophenylhydrazone)	2.64	2.01	0.48	Yellow
5,6-Dimethoxy-1,4-benzoquinone 4-(2,4-dinitrophenylhydrazone)	2.46	1.95	0.51	Yellowish orange
1,2-Naphthoquinone 2-(2,4-dinitrophenylhydrazone)	2.56	1.95	0.45	Red
1,4-Naphthoquinone 4-(2,4-dinitrophenylhydrazone)	0.46	2.28	0.38	Greenish yellow
1,2-Naphthoquinone 4-(2,4-dinitrophenylhydrazone)	2.94	1.95	0.11	Red
2-Methyl-1,4-naphthoquinone 4-(2,4-dinitrophenylhydrazone)	1.98	1.32	0.71	Yellow
2-Methyl-1,4-naphthoquinone 1-(2,4-dinitrophenylhydrazone)	3.00	1.56	—	Yellow

* Micro Pak Si-10 (1% 2-propanol in light petroleum, 120 ml/h, 200 atm).

** Micro Pak CN-10 (1% 2-propanol in light petroleum, 60 ml/h, 60 atm). Elution volume of unretained component = 0.54 ml.

*** Sephadex LH-20 (2-propanol).

para-position. This phenomenon can be explained by the chelation of the *o*-carbonyl groups of silica gel. Good separations of quinones were also achieved on a Micro Pak CN-10 column (Table II).

To compare the chromatographic behaviour of separated azo dyes, azobenzene was used. Azo dyes with hydrozyl groups in the *para*-position had a greater retention volume on the column with a polar stationary phase (Micro Pak CN-10), which agrees with the results of Passarelli and Jacobs[12] and our experiments on a thin layer of silica gel impregnated with β,β'-oxydipropionitrile[7]. On the other hand, on silica gel the same mechanism of separation as for the original quinones is evidently involved.

In the reaction of 2-methyl-1,4-naphthoquinone with 2,4-dinitrophenylhydrazine, we observed the formation of two peaks; apart from 2-methyl-1,4-naphthoquinone 4-(2,4-dinitrophenylhydrazone), a smaller amount of 2-methyl-1,4-naphthoquinone 1-(2,4-dinitrophenylhydrazone) was also formed[7]. In the separation of older preparations, more peaks were formed, which, on the basis of literature data[16,17], can be ascribed to the formation of bis-2,4-dinitrophenylhydrazones and azoxy compounds. The lowest determinable amount of azobenzene was $5 \cdot 10^{-8}$ g with a 10-μl injection. Phyloquinone, anthraquinone and phenanthraquinone did not react.

For studying the separation of 2,4-dinitrophenylhydrazones of quinones, TLC on Sephadex LH-20 gel with 2-propanol as the mobile phase was also used (Table II). The results indicate that azo dyes are not separated on the basis of their molecular weights. To clarify the mechanism of separation of these compounds, we separated differently substituted nitrophenols under the same experimental conditions (Table III). The results show that the R_F values depend on the dissociation constant; in *o*-nitrophenol, which migrates more quickly, the "*ortho*" effect is evidently involved. The strong sorption of nitrophenols appears to be due to the formation of hydrogen bonds between nitro groups and non-esterified hydroxyl groups of the gel[16].

TABLE III

SEPARATION OF NITROPHENOLS BY GEL CHROMATOGRAPHY ON SEPHADEX LH-20 WITH ISOPROPANOL AS MOBILE PHASE

Compound	$R_{azobenzene}$	*pK*
2-Nitrophenol	1.13	7.23
3-Nitrophenol	0.82	8.28
4-Nitrophenol	0.49	2.15
2,5-Dinitrophenol	0.39	6.78
2,4-Dinitrophenol	0.32	4.09
2,6-Dinitrophenol	0.26	3.77

Separation of anthraquinone derivatives

The arrangement described above is not suitable for the separation of anthraquinone polyhydroxy derivatives. We used with success silica gel with a chemically bonded non-polar phase and methanol–water (1:1) as the mobile phase (Table IV); use of a mobile phase with a higher content of methanol resulted in tailing. In 2,3-

TABLE IV

SEPARATION OF ANTHRAQUINONE HYDROXY DERIVATIVES BY LIQUID CHROMATOGRAPHY

Micro Pak CH-5 [methanol–water (1:1), pH = 3, 60 ml/h, 90 atm]. Elution volume of unretained component = 0.60 ml.

Compound	V_R *(ml)*
1,2-Dihydroxyanthraquinone	4.28
2,3-Dihydroxyanthraquinone	2.08
1,4-Dihydroxyanthraquinone	10.70
1,2,4-Trihydroxyanthraquinone	2.26
1,2,5,8-Tetrahydroxyanthraquinone	7.02
1,2,4,5,8-Pentahydroxyanthraquinone	5.34

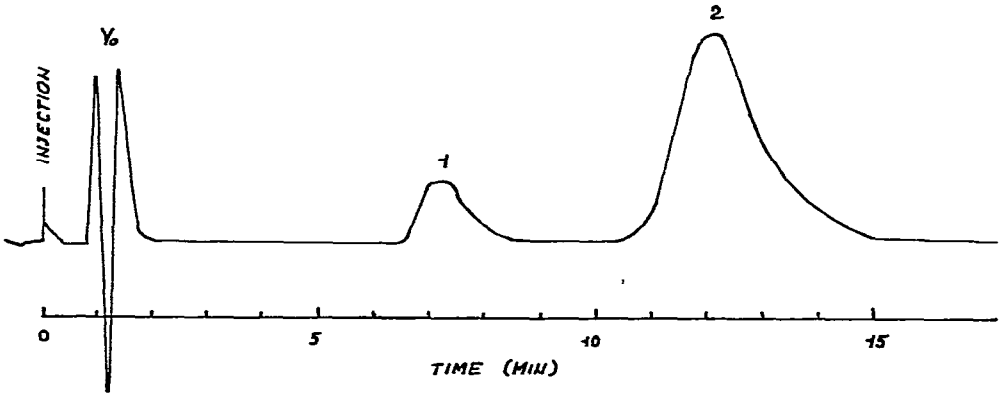

Fig. 1. Liquid chromatography of the preparation of purpurine. Column, Micro Pak CH-5 (25 cm × 2.2 mm); mobile phase, methanol–water (1:1); pH = 3; 60 ml/h; UV detector (254 nm). Peaks: 1 = 1,2-dihydroxyanthraquinone; 2 = 1,2,4-trihydroxyanthraquinone.

and 1,4-dihydroxyanthraquinone, we also achieved a good separation of the initial products that migrate with the front. When separating a sample of 1,4-dihydroxyanthraquinone, we found that 1,2,5,8-tetrahydroxyanthraquinone was also formed. In a sample of purpurine, we identified, in addition to 1,2,4-trihydroxyanthraquinone, also 1,2-dihydroxyanthraquinone (see Fig. 1). In a sample of chinalizarine, we found, in addition to 1,2,5,8-tetrahydroxyanthraquinone, also 1,2-dihydroxyanthraquinone and 1,2,4,5,8-pentahydroxyanthraquinone[11].

TABLE V

ANALYSIS OF COMMERCIAL PREPARATIONS OF ANTHRAQUINONE HYDROXY DERIVATIVES BY LIQUID CHROMATOGRAPHY TO DETERMINE THE 1,2-DIHYDROXY-ANTHRAQUINONE CONTENT

n = Number of determinations, C.V. = coefficient of variation.

Preparation	*1,2-Dihydroxyanthraquinone content (%)*	*n*	*C.V. (%)*
Alizarine pure (Lachema)	87.0	4	4.3
Alizarine (older preparation)	57.3	4	3.8
Purpurine pure (Merck)	19.3	3	5.3

The methods described above were used for checking the purity of and determining the content of the effective component in some commercial preparations (Table V). Analyses of such preparations are made difficult by their comparatively low solubility and the considerable viscosity of the mobile phase. The accuracy of the analysis is then influenced by the sorption of polyhydroxyanthraquinones on the column packing. It is possible to determine down to $4 \cdot 10^{-7}$ g of alizarin with a 10-μl injection.

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