

Separation of quinone 2,4-dinitrophenylhydrazones by thin-layer chromatography

B. RITTICH and M. ŠIMEK*

Research Institute of Animal Nutrition,
691 23 Pohořelice

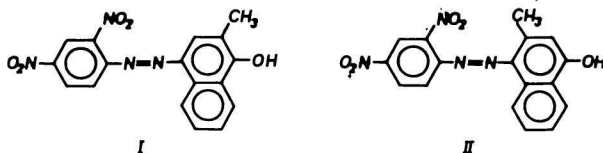
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Chromatographic methods by which benzoquinones and naphthoquinones can be identified after their conversion to the corresponding 2,4-dinitrophenylhydrazones are described. Compounds difficult to separate without derivatization can be separated by adsorption chromatography on Silufol® using systems based on carbon tetrachloride and cyclohexane as the nonpolar, and ethanol and isopropanol as the polar components. Naphthoquinone azo dyes can also be satisfactorily separated on silica gel or alumina impregnated with β,β' -oxydipropionitrile when benzene—petrolether mixture (1 : 1) is used as the mobile phase. The formation of various isomeric azo dyes is also discussed.

Описаны хроматографические методы, позволяющие идентифицировать бензохиноны и нафтохиноны после их перевода на 2,4-динитрофенилгидразоны, которые образуются в качестве азокрасителей. Адсорбционной хроматографией на Silufol® при использовании проявляющих систем: четыреххлористый углерод—циклогексан—полярный компонент (этиловый или изопропиловый спирты), можно добиться разделения веществ, разделение которых в начальной форме очень затруднительно (1,4-нафтохинон, менадион). Азокрасители на базе нафтохинонов можно также хорошо разделить на слоях силикагеля и окиси алюминия, импрегнированных с β,β' -оксидипропионитрилом при помощи проявляющей системы бензол—петролейный эфир (1 : 1). В заключении обсуждается образование различных изомеров азокрасителей.

A convenient means of the identification of carbonyl compounds is their conversion to the corresponding 2,4-dinitrophenylhydrazones. Quinones which are important constituents of natural pigments [1], are similar to some extent to carbonyl compounds. They react with



Scheme 1

* Present address: Faculty of Pedagogy, Department of Chemistry, J. E. Purkyně University, 662 38 Brno.

Table 1

Separation of quinone 2,4-dinitrophenylhydrazones on Silufol[®]

Compound	Colour	hR_F		
		S_1	S_2	S_3
1,2-Benzoquinone	Violet	—	24	40
1,4-Benzoquinone	Yellow	21	14	31
5,6-Dimethoxy-1,4-benzoquinone	Orange	—	13	17
Tetrahydroxy-1,4-benzoquinone	Brown-yellow	—	0	0
1,2-Naphthoquinone	Red	69	43	62
1,4-Naphthoquinone	Brown-orange	49	32	55
2-Methyl-1,4-naphthoquinone	Yellow	62	—	63

S_1 — Silufol[®] (carbon tetrachloride—cyclohexane—ethanol 7 : 2 : 1).

S_2 — Silufol[®] (carbon tetrachloride—cyclohexane—ethanol 4 : 5 : 1).

S_3 — Silufol[®] (carbon tetrachloride—cyclohexane—isopropanol 5 : 4 : 1).

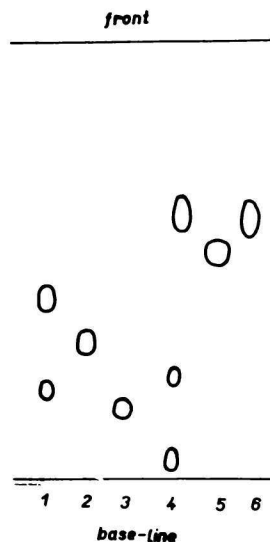


Fig. 1. Separation of quinone 2,4-dinitrophenylhydrazones on Silufol[®].

Solvent system: carbon tetrachloride—cyclohexane—isopropanol (5 : 4 : 1); detection: self-produced colour; front-base-line distance 11.5 cm; developing time 22 min.

The components in parentheses are given in the order of decreasing mobility; azo dye derived from:

- 1,2-benzoquinone (azo dye, 1,2-benzoquinone);
- 1,4-benzoquinone (azo dye);
- 5,6-dimethoxy-1,4-benzoquinone (azo dye);
- 1,2-naphthoquinone (azo dye, unidentified, unidentified);
- 1,4-naphthoquinone (azo dye);
- 2-methyl-1,4-naphthoquinone (azo dye).

Table 2

Separation of quinone 2,4-dinitrophenylhydrazones on silica gel and Alufol[®] impregnated with β,β' -oxydipropionitrile

Compound	hR_F					
	S_1			S_2		
	Impregnation (reagent, ml/sorbent, g)			Impregnation by development (reagent concentration, %)		
	0.10	0.15	0.20	5	10	20
1,2-Benzoquinone	0	0	0	0	0	0
1,4-Benzoquinone	1	4	—	0	0	0
5,6-Dimethoxy- -1,4-benzoquinone	—	0	—	13	—	—
1,2-Naphthoquinone	22	29	30	33	—	33
1,4-Naphthoquinone	6	14	16	0	4	4
Menadione	13	24	26	23	29	25

S_1 — impregnated silica gel; benzene—petroleum ether 1 : 1.

S_2 — impregnated Alufol[®]; benzene—petroleum ether 1 : 1.

2,4-dinitrophenylhydrazine giving condensation products which spontaneously form tautomeric azo dyes [2]. Their separation has been successfully accomplished by reversed-phase paper chromatography [2—4]. The colour of azo dyes is a characteristic property of this class of substances which can be used in their spectrophotometric identification [5, 6].

Experimental

Chemicals and accessories

Vitamin K₁ (2-methyl-3-phytyl-1,4-naphthoquinone, phyloquinone) and vitamin K₂ (2-methyl-1,4-naphthoquinone, menadione) were commercial products obtained from Merck, A. G. (W. Germany) and Fluka, A. G. (Switzerland), respectively. 1,2-Benzoquinone was prepared by means of oxidation of 1-amino-2-hydroxybenzene with FeCl₃ [2]. 2,4-Dinitrophenylhydrazones were prepared as described in [2]. Silica gel L 5/40 was obtained from Lachema, Brno. Silufol[®] and Alufol[®] pre-coated aluminium foils were domestic commercial products — Kavalier, Votice. β,β' -Oxydipropionitrile was obtained from C. Erba, Milan. Other chemicals were of reagent purity used as supplied.

The thin-layer spreader and the developing tanks were commercial products (Dioptra, Turnov).

Chromatography

The reaction mixtures from the preparation of 2,4-dinitrophenylhydrazones were extracted with chloroform and applied on the thin layer with the aid of a 1 μ l micropipette. The ascending technique in a tank with an atmosphere saturated with vapours of the solvent system for 1 hr at 22 \pm 1°C prior to the development was used throughout.

For the preparation of five silica gel-coated glass plates (17 \times 23 cm) a mixture of silica gel (27.5 g) and starch (2.5 g) was shaken with water (60 ml) for 1 min, heated to 80—90°C for 1 min and poured onto the plates.

For the preparation of the glass plates coated with silica gel impregnated with β,β' -oxydiacetone (4 plates, 17 × 23 cm) acetone (100 ml) was added to silica gel (20 g) followed by the proper amount of the impregnating agent (0—0.2 ml of a stock solution of the impregnating agent per 1 g of the support material). The mixture was left at room temperature for 24 hrs and dried to dryness using a rotary evaporator (40°C). Starch (2 g) and petroleum ether (60 ml) were added to the residue and the mixture was shaken for 1 min prior to the spreading (slot thickness 0.5 mm). Coated plates were dried for 24 hrs at ambient temperature.

Since the impregnated alumina plates could not be prepared successfully in the above-mentioned manner, Alufol[®] was impregnated with β,β' -oxydipropionitrile by developing the plates with an acetone solution of the substance. The developed foils were then dried at room temperature for 24 hrs.

Quinone 2,4-dinitrophenylhydrazones show on the silica gel plates characteristic spots (Table 1). On Alufol[®] impregnated with β,β' -oxydipropionitrile, 1,4-naphthoquinones appear as blue and green spots, respectively.

Results and discussion

Adsorption chromatography of 1,4-naphthoquinone—menadione mixtures on silica gel plates was unsuccessful. The substances, as well as azo dyes, derived from quinones and naphthoquinones could be satisfactorily separated on Silufol[®] (Table 1) using systems based on carbon tetrachloride or cyclohexane containing variable amounts of the polar components (ethanol, isopropanol), as suggested by *Večeřa* and *Štěrba* [6].

2,4-Dinitrophenylhydrazones derived from carbonyl compounds have been separated on thin-layer chromatography on silica gel impregnated with Carbowax 400 [7]. The separation of the investigated substances was successful also when the thin layers were impregnated with other nonpolar substances. On β,β' -oxydipropionitrile-impregnated Alufol[®], using benzene—petroleum ether (1 : 1), azo dyes derived from 1,2- and 1,4-naphthoquinone and menadione can be very well distinguished one from another as well as from the starting material from which they have been prepared, using Alufol[®] impregnated with a mixture of β,β' -oxydipropionitrile. The results are summarized in Table 2. The changes in the R_F values of the azo dyes derived from 1,4-naphthoquinone and menadione on Alufol[®] impregnated with benzene—petroleum ether in this manner assists the identification of 1,4-naphthoquinone and menadione.

When the azo dye derived from 1,2-benzoquinone was chromatographed on silica gel impregnated with carbon tetrachloride—cyclohexane—ethanol (4 : 5 : 1) several spots were observed. The fastest one (R_F 0.3) corresponded (colour, mobility) to 2,4-dinitrophenylhydrazine, the middle one (R_F 0.14) to 1,2-benzoquinone and the slowest one was the azo dye itself. Several spots were also observed when the azo dye derived from 1,2-naphthoquinone was chromatographed on silica gel impregnated with the same solvent system. The spot showing the fastest mobility (R_F 0.43) was the azo dye itself and the slowest one (R_F 0.04) was an impurity present in the starting material. The identity of the middle spot (R_F 0.19) is unknown. It could correspond to the isomeric azo dye derived from 1,2-naphthoquinone (other authors [2] have also observed the formation of several components from the same starting material).

Two spots were observed when the azo dye derived from menadione was chromatographed on Alufol[®] impregnated with 10% solution of β,β' -oxydipropionitrile in acetone. Although it has been suggested by *Fickentscher* [5] that only one azo dye is formed, the isomeric substance is obviously also formed to some extent.

The azo dye derived from phylloquinone could not be prepared; when the phylloquinone was

attempted preparation was chromatographed a yellow, heavily striking spot was formed. 2,4-Dinitrophenylhydrazones are more suitable for the identification by chromatography than are the thiosemicarbazones since the latter give more spots on the chromatograms.

References

1. Thompson, R. N., *Naturally Occurring Quinones*, 2nd Ed., p. 198. Academic Press, London, 1971.
2. Gemzová, I. and Gasparič, J., *Collect. Czech. Chem. Commun.* **32**, 2740 (1967).
3. Večeřa, M. and Gasparič, J., *Identifikace organických látek, II.* (Identification of Organic Substances.) P. 221, 297. Státní nakladatelství technické literatury. (State Publishing House of Technical Literature.) Prague, 1973.
4. Gasparič, J. and Cee, A., *Collect. Czech. Chem. Commun.* **40**, 371 (1975).
5. Fickentscher, K., *Deut. Apotheker-Ztg* **108**, 1545 (1968).
6. Gouveia, A. P., De Gouveia, A. J. A., Figueiredo, G. S., and Da Silva, A. M., *Rev. Port. Quim.* **12**, 104 (1970).
7. Craske, J. D. and Edwards, R. A., *J. Chromatogr.* **51**, 237 (1970).

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