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## Note

# Determination of 2,6- and 4,6-dinitrocresols by high-performance liquid chromatography on a β-cyclodextrin bonded column

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2,6-Dinitro-*p*-cresol (2,6-DNPC) and 4,6-dinitro-*o*-cresol (4,6-DNOC) are formed as side reaction products in the manufacture of nitrotoluene isomers, the extent of formation of 2,6-DNPC being larger. Their build-up above certain limits, especially of 2,6-DNPC, can cause undesirable thermal run-away reactions. Further, the nitrocresols are toxic compounds and are listed as priority pollutants[1,2] by the U.S. Environmental Protection Agency. Also, 4,6-DNOC finds extensive use as an herbicide. These dinitrocresols enter the effluent stream also. Hence, it is of utmost importance to monitor their formation during the manufacture of nitrotoluene isomers.

A literature survey has not revealed any high-performance liquid chromatographic (HPLC) method for the determination of 2,6-DNPC, though some HPLC methods[3–5] have been reported for the analysis of 4,6-DNOC as an herbicide among other nitro-containing herbicides like dinoseb, dinoterb and dinobuton. However, the simultaneous determination of 2,6-DNPC and 4,6-DNOC by HPLC has not so far been reported. Therefore, it was thought worthwhile to develop an efficient HPLC method for the analysis of these two isomers.

A β-cyclodextrin bonded phase column, which is effective for the separation of positional isomers[6,7], has been employed for the separation of the two isomers and the results are presented herein.

## EXPERIMENTAL

### *Instrumental*

A Perkin-Elmer chromatograph Series 10, equipped with a Rheodyne sample injector, a Perkin-Elmer Lambda 3-B variable wavelength UV–VIS spectrophotometric detector and an LCI-100 computing integrator were used for chromatographic work. The β-cyclodextrin bonded column was obtained from Advanced Separation Technologies (Whippany, NJ, U.S.A.). Its dimensions were 250 mm × 4.6 mm I.D. and the particle size was 5 μm.

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*Reagents and chemicals*

All solvents used were of HPLC grade. 2,6-Dinitrophenol and 4,6-DNOC were obtained from Fluka (Buchs, Switzerland). 2,6-DNPC was isolated from the caustic washings of a nitrotoluene plant and its purity established by NMR and gas chromatographic (GC) methods.

*Preparation of standard solutions*

Stock solution of 2,6-dinitro-*p*-cresol (2,6-DNPC), 4,6-dinitro-*o*-cresol (4,6-DNOC), *p*-nitrotoleune (p-NT) and 2,6-dinitrophenol (2,6-DNP) (1 mg/ml) were prepared in methanol. Various amounts of 2,6-DNPC, 4,6-DNOC and p-NT were taken in 25-ml volumetric flasks and 5 ml of 2,6-DNP (internal standard) were added. The volumes were made up to 25 ml with mobile phase and 6.0 $\mu$l from this solution were injected for HPLC.

*Chromatographic conditions*

A 6.0-$\mu$l volume of each standard sample was injected. Flow-rate: 1 ml/min. Detection: UV at 254 nm. Mobile phase: methanol–acetonitrile–acetic acid (20:78.5:1.5, v/v).

## RESULTS AND DISCUSSION

The $\beta$-cyclodextrin bonded phase column is packed with silica material covalently bonded with $\beta$-cyclodextrin molecules by means of a non-nitrogen-containing

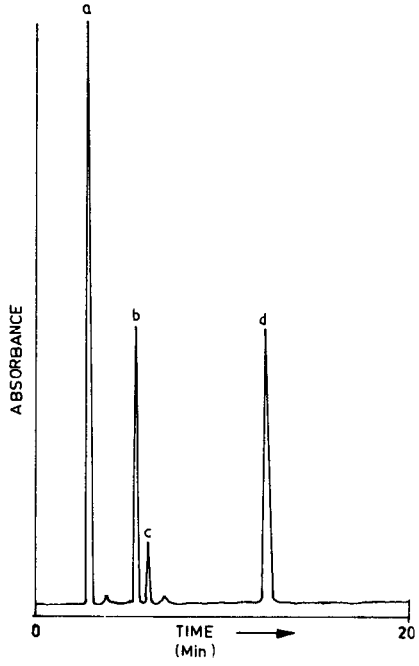


Fig. 1. HPLC separation of dinitrocresol isomers in a plant sample. Peaks: a = nitrotoluenes; b = 2,6-dinitro-*p*-cresol; c = 4,6-dinitro-*o*-cresol; d = 2,6-dinitrophenol. Experimental conditions as in the text.

TABLE I

RETENTION DATA

| *Compound* | *Retention time (min)* | *Capacity factor, k'* |
|---|---|---|
| Nitrotoluenes | 2.97 | 0.32 |
| 2,6-Dinitrocresol | 5.47 | 1.44 |
| 4,6-Dinitrocresol | 6.10 | 1.72 |
| 2,6-Dinitrophenol (internal standard) | 12.28 | 4.48 |

spacer 6–10 atoms in length. The macrocyclic molecule β-cyclodextrin contains seven glucopyranose units arranged in the shape of hollow truncated cone of which the interior cavity is relatively hydrophobic comprising essentially the methylene and glucoside linkages. The exterior faces are hydrophilic because of many hydroxyl groups. Separation of compounds on such columns essentially takes place either by inclusion complexation phenomena or by strong polar interactions.

In the present case, the effective separation of the two isomers of dinitrocresols along with the internal standard 2,6-dinitrophenol has been achieved in the normal phase mode using a methanol and acetonitrile mixture containing acetic acid. To achieve better resolution, sharp peaks and rapid elution of these strongly interacting compounds, it is necessary to add acetic acid to the mobile phase. Without such addition, the components are strongly retained with practically no separation. This suggests a normal phase behaviour[8]. The effect of acetic acid has been studied systematically and it is found that 1.5% (v/v) affords very sharp peaks with good resolutions. A typical chromatogram illustrating the separation of the isomeric dinitrocresols from a plant sample is shown in Fig. 1. It is not clear whether the inclusion process is significant in this mode. A normal adsorption column containing HS-Silica and with the same solvent system was tested but did not yield any separation.

The retention data for the two isomers are given in Table I. The method of separation has been evaluated for quantitation of these isomers. A statistical evaluation of the method is given in Table II. The method has been found to be quite

TABLE II

STATISTICAL EVALUATION OF THE METHOD FOR DETERMINATION OF DINITROCRESOL ISOMERS

S.D. = Standard deviation; C.V. = coefficient of variation.

| *Isomer* | *Amount taken (μg/ml)* | *Amount found*[a] *(μg/ml)* | *% Error* | *S.D.* | *C.V.* |
|---|---|---|---|---|---|
| 2,6-DNPC | 40 | 39.69 | 0.78 | 0.13 | 0.33 |
| | 80 | 79.37 | 0.78 | 0.26 | 0.33 |
| | 120 | 120.41 | 0.34 | 1.05 | 0.88 |
| 4,6-DNOC | 40 | 39.19 | 2.03 | 0.47 | 1.21 |
| | 80 | 77.86 | 2.67 | 0.91 | 1.17 |
| | 160 | 155.25 | 2.97 | 1.42 | 0.41 |

[a] Based on five measurements.

effective with an accuracy of ±3% for 4,6-DNOC and ±0.8% for 2,6-DNPC.

Efforts have been made to separate the components in reversed-phase mode on a β-cyclodextrin column using a water–methanol mixture as the mobile phase. The components were not eluted with this mobile phase. Further, increasing the water content in the mobile phase leads to increased retention of the components, suggesting a strong inclusion process. The resolution of the peaks was also not improved and elution takes much longer when compared to the normal phase separation.

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