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Note

Analysis of nitrobenzoic acid isomers by high-performance liquid chromatography

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p-Nitrobenzoic acid is a valuable chemical in many areas of industry and research. It is commercially prepared by oxidation of *p*-nitrotoluene. However, the nitration of toluene produces less than 40% of the desired *para* isomer. Thus, a procedure yielding a higher purity of *p*-nitrobenzoic acid is necessary.

In order to investigate the purity of *p*-nitrobenzoic acid obtained from different procedures, it is necessary to develop a sensitive method for the analysis of isomeric nitrobenzoic acids.

A literature search reveals many separations of isomeric nitrobenzoic acids¹⁻⁸. However, these are primarily thin-layer^{1,2}, ion-exchange^{3,4} or gas chromatographic analyses⁵. Thin-layer chromatography is a convenient method used in separation and detection, but not in quantitation of isomers; the separations achieved on ion-exchange resins were by means of traditional column chromatography. Although a quantitation of isomeric nitrobenzoic acids can be accomplished by gas chromatographic separation of their methyl esters, the necessity of transforming isomeric nitrobenzoic acids into their esters may lead to inaccuracies in the analysis due to the various esterification rates of the acids. Since high-performance liquid chromatography (HPLC) offers a rapid and sensitive technique, its use in the analysis of isomeric nitrobenzoic acids is expected to be very valuable.

EXPERIMENTAL

Standards and sample

The standards of isomeric nitrobenzoic acids, used in the construction of calibration curves, were purified by recrystallization of reagent-grade samples from water or benzene.

The procedure used to prepare the sample of *p*-nitrobenzoic acid from polystyrene was similar to that described by Rondestvedt *et al.*⁹.

Apparatus and chromatographic conditions

A Model ALC/GPC 244 liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) equipped with a Model 6000 solvent delivery system was used. The chromatographic columns were LiChrosorb RP-18, 5 and 10 μ m, obtained from E. Merck,

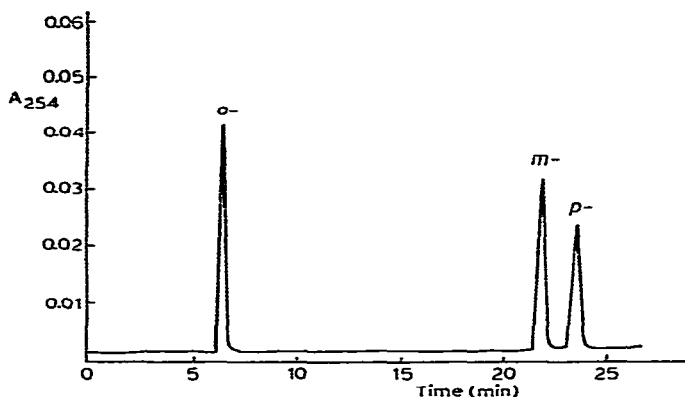


Fig. 1. Separation of isomeric nitrobenzoic acids on LiChrosorb RP-18, 5 μm , with methanol-water (30:70) containing 1% acetic acid as the mobile phase. The flow-rate was 0.7 ml/min.

VerCopak C₁₈, 10 μm , obtained from Vertex Co. (Taiwan) and $\mu\text{Bondapak C}_{18}$, 10 μm , obtained from Waters. A fixed-wavelength (254 nm) ultraviolet (UV) detector was employed and the chromatograph was operated at ambient temperature. The flow-rate was 0.7–1 ml/min at an operating pressure of 3000 p.s.i. (LiChrosorb RP-18, 5 μm) or 1000 p.s.i. (VerCopak C₁₈, LiChrosorb RP-18 and $\mu\text{Bondapak C}_{18}$, 10 μm). Chromatograms were recorded on an Omniscribe at a chart speed of 0.5 cm/min.

Quantitations were performed on a Shimadzu LC-3A. The chromatographic column used was Zorbax ODS, 5 μm , obtained from DuPont and the chromatograph was operated at 40°C.

RESULTS AND DISCUSSION

Using different methanol-water mixtures containing 1% acetic acid as the mobile phase, the separation of isomeric nitrobenzoic acids was studied. When VerCopak C₁₈, 10 μm , was used and the mobile phase was methanol-water (70:30) containing 1% acetic acid, the retention times of *p*- and *m*-nitrobenzoic acids are the same, although *o*-nitrobenzoic acid is well separated from them. In order to separate *p*-nitrobenzoic acid from the *m*-isomer, a column of LiChrosorb RP-18, 5 μm , was tried. Good separation was not achieved when the mobile phase was the same as before. However, by changing to methanol-water (30:70) containing 1% acetic acid, improved separation of *p*-nitrobenzoic acid from the *m*-isomer can be achieved. In order to separate *p*-nitrobenzoic acid from the *m*-isomer, the flow-rate should be reduced from 1 to 0.7 ml/min. The chromatogram obtained is shown in Fig. 1.

Under the above conditions, no separation of the *m*- and *p*-isomers was obtained on VerCopak C₁₈, 10 μm , $\mu\text{Bondapak C}_{18}$, 10 μm , or LiChrosorb RP-18, 10 μm . This suggests that the mean particle size of the packing material in the column has an important effect on the separation of isomeric nitrobenzoic acids.

Using the conditions to achieve complete separation of isomeric nitrobenzoic acids on LiChrosorb RP-18, 5 μm , the calibration curves can be obtained (Fig. 2). The peak heights were linearly related to the amount injected. The results can be used

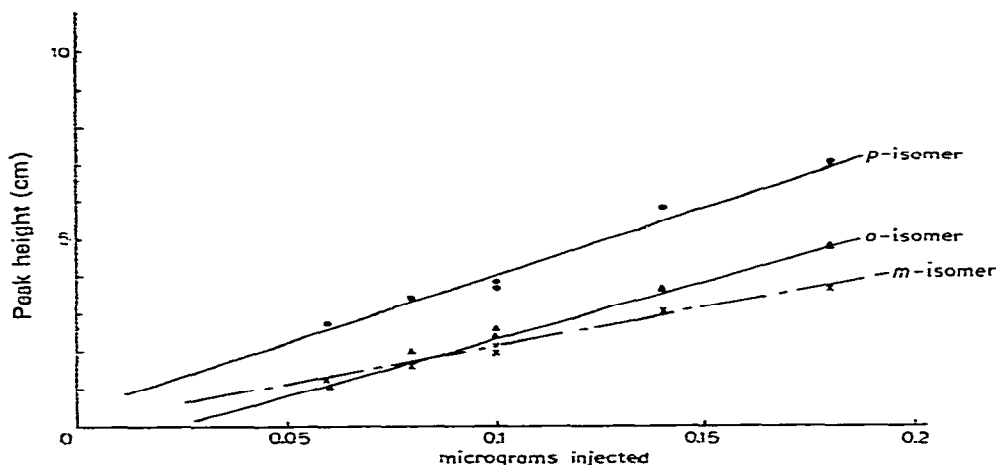


Fig. 2. Calibration curves of isomeric nitrobenzoic acids.

for the quantitation of isomeric nitrobenzoic acids. By comparing the chromatogram of crude *p*-nitrobenzoic acid obtained from polystyrene and the calibration curves, the purity of the crude acid was estimated to be 91%. The precision was determined by repeatedly assaying the sample of *p*-nitrobenzoic acid. The standard deviation was 1.5%. This result showed that the purity of *p*-nitrobenzoic acid prepared from polystyrene was much better than that prepared by the traditional method (oxidation of nitrotoluene).

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