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Note

Gas chromatography of some simple phenols as their O-isobutyloxycarbonyl derivatives

MASAMI MAKITA, SHIGEO YAMAMOTO, ATSUKO KATOH and YASUKO TAKASHITA
Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okaya 700 (Japan)
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During our recent work[1–5] on the gas chromatography (GC) of amino acids, it was found that both the phenolic hydroxyl groups and the amino groups can be readily isobutyloxycarbonylated with isobutyl chloroformate in aqueous alkaline medium. Based on this observation, we have investigated the possibility of carrying out the GC of phenols as their O-isobutyloxycarbonyl (O-isoBOC) derivatives.

In this paper, a method for the GC of some simple phenols as their O-isoBOC derivatives and the application of the method to the analysis of phenol and *p*-cresol in urine are described.

EXPERIMENTAL

All phenols were obtained from commercial sources; isobutyl chloroformate was obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and used without further purification. All analyses were performed using a Shimadzu 3B gas chromatograph equipped with a flame-ionization detector. The column packing (5% OV-17 on Gas-Chrom Q, 100–120 mesh) was prepared by the filtration technique[6]. For the conversion of phenols into their O-isoBOC derivatives, the same procedure as for the N-isobutyloxycarbonylation of amino acids described by Makita *et al.*[2] was used, and the resulting derivatives were extracted with 0.5 ml of diisopropyl ether in alkaline medium. The ethereal solution was dried over anhydrous sodium sulphate and subjected to GC analysis.

A 2.5-ml volume of urine was hydrolysed with a equal volume of 4 *N* hydrochloric acid, followed by extraction twice with diethyl ether according to the method of Duran *et al.*[7] To the combined extracts, 2 ml of 0.04 *N* methanolic sodium hydroxide solution were added and the mixed solution was evaporated to dryness under vacuum at 40°. The residue was dissolved in 1 ml of water and to this solution was added 0.5 ml of a 100 μg/ml solution of *p*-ethylphenol (internal standard). The mixture was derivatized.

RESULTS AND DISCUSSION

All of the derivatives of the phenols studied have good GC properties, as shown in Fig. 1, and the linearity of the calibration graph for each phenol in the range 10–

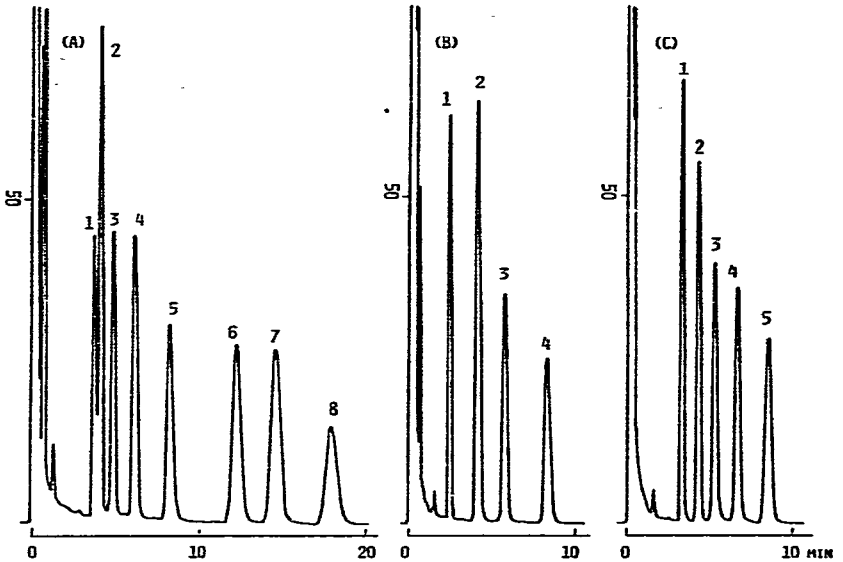

Fig. 1. Chromatograms of the O-isoBOC derivatives of some simple phenols. Each peak represents *ca.* 0.4 μg of phenol. GC conditions: column, 5% OV-17 on 100–120-mesh Gas-Chrom Q, 1.7 m × 3 mm I.D., glass; attenuation, 32 · 10^2; column temperature, A 170°, B and C 150°. Peaks: (A) 1 = thymol; 2 = 3,5-dichlorophenol; 3 = 2,4-dichlorophenol; 4 = *o*-hydroxyacetophenone; 5 = *o*-nitrophenol; 6 = eugenol; 7 = α-naphthol; 8 = β-naphthol; (B) 1 = phenol; 2 = 2,6-xylenol + *m*-cresol; 3 = *p*-chlorophenol; 4 = *o*-xylenol; (C) 1 = *o*-cresol; 2 = *p*-cresol; 3 = *p*-xylenol; 4 = *p*-ethylphenol; 5 = guaiacol.

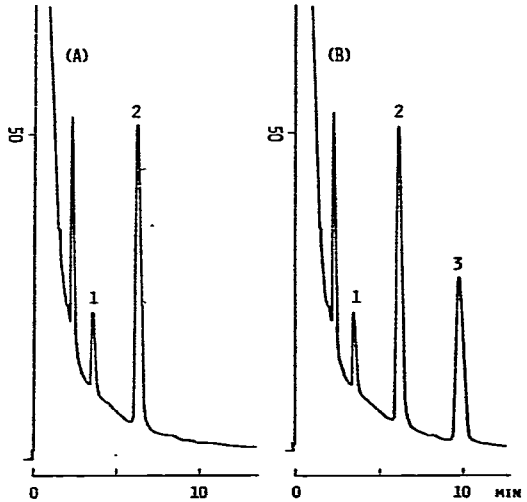

Fig. 2. Typical chromatograms obtained from (A) a normal adult urine and (B) A + internal standard (*p*-ethylphenol, 50 μg). The injection mixture contained *ca.* 10 μl of urine. Peaks: 1 = phenol (0.03 μg); 2 = *p*-cresol (0.17 μg); 3 = *p*-ethylphenol (0.20 μg). Column temperature, 140°; attenuation, 16 · 10^2; other GC conditions as in Fig. 1.

100 μg and its reproducibility were satisfactory. The stability of the derivatives of the phenols with respect to time was good. The recoveries were almost quantitative for all of the phenol derivatives, which had been allowed to stand in solution in diisopropyl ether for 1 week at room temperature. The conversions of *p*-xylenol and guaiacol as model compounds into their O-isoBOC derivatives were excellent, the yields being above 93% in each instance.

Elevated amounts of phenol and/or *p*-cresol in urine have been found in patients with various gastrointestinal disorders[7], and so the application of the proposed method to the determination of these phenols in urine was investigated. Quantification by this method was established from analyses of the hydrolysed urine samples fortified with 50–100 μg of each phenol. The recovery was reasonable, ranging from 94 to 100% for phenol and from 97 to 101% for *p*-cresol. The peaks of derivatives derived from a urine sample were carefully analysed by gas chromatography–mass spectrometry in order to check for interfering substances, and it was confirmed that there were no overlapping peaks. Typical chromatograms from a urine sample are shown in Fig. 2.

The method described is simple and convenient, and is proposed as an alternative method for the analysis of simple phenols.

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