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An Enzymatic Method for the Hydrolysis of Urinary 1-Naphthyl Glucuronide

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The development of biological indicators of human exposure to chemicals is a matter of great world-wide interest. Metabolite concentrations in human urine are frequently used as such indicators. However, measurement is often complicated by the fact that the metabolites are excreted as conjugates, commonly glucuronides and sulfates.

We report here a convenient enzymatic procedure for hydrolyzing the glucuronide of 1-naphthol to free the parent compound for chromatographic or other determinations. The procedure gives produces samples that are much more free of interferences than are samples produced by acid hydrolysis.

MATERIALS AND METHODS

Capillary gas chromatography was performed with a Perkin Elmer 8320 instrument in the splitless mode and by using flame ionization detection. The bonded OV-17 fused silica column was 25 m by 0.25 mm i.d. The oven temperature program was: hold 100° for 1.5 min, 100° to 240° at 30°/min, hold 240° for 5 min. The solvent was vented after a 0.7 min hold. The septa were changed daily and the injection port liner was cleaned and silylanized weekly. This treatment was supplemented by daily injections of 10 µL of silylating solution. The silylating solution was obtained from Supelco, Inc., Bellefonte, PA, No. 3-3065 . C_{18} Sep-Paks were obtained from Waters Assoc., Medford, MA. The β-glucuronidase (G-0751, partially purified powder from Helix pomatia) and α-naphthyl-β-D-glucuronic acid sodium salt (N-8378) were obtained from Sigma Chemical Co., St. Louis, MO.

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Enzymatic hydrolysis of conjugate in urine. Twenty-five mL of urine, 25 mL of 0.1 M pH 5.0 sodium acetate buffer, and 0.1 g of enzyme preparation were mixed in a 250-mL Erlenmeyer flask. The flask was shaken at 38° for 24 hr, and the solution was subjected to Sep-Pak purification (Keimig et al. 1983). A C_{18} Sep-Pak was sequentially primed with 2 ml of methanol and 5 ml of water. A syringe was used to push the cooled urine preparation through the Sep-Pak at a rate of about 10 mL/min. The cartridge was then washed with 5 mL of 1:2 methanol-water (v/v) and eluted with 10 mL of methanol. Aliquots of the methanol solution were injected into the gas chromatograph.

The procedure for acid hydrolysis of urine was that of Edgerton and Moseman (1979). Two mL of urine and 0.5 mL of concentrated hydrochloric acid in a 10 mL screw cap test tube were heated in a boiling water bath for 1 hr. The resulting solution was then subjected to the above Sep-Pak procedure.

RESULTS AND DISCUSSION

The excretion of hydroxylated compounds, wholly or in part, as conjugated glucuronides or sulfates often complicates analytical efforts. One could develop methods to measure both the parent compounds and their conjugates. However, this is a difficult exercise in method development, and standards of the conjugate are very difficult to obtain. Alternatively, one may hydrolyze the conjugates with aqueous acid and heat. For example, acid hydrolysis has been used in the determination of urinary levels of 1-naphthol (Sullivan and Shafik 1974) and pentachlorophenol (Edgerton and Moseman 1979). Since chemical hydrolysis is not at all specific, however, such procedures produce many interferences necessitating subsequent clean up steps. In fact, removing interferences is often the most difficult part of developing an analytical method for xenobiotic metabolites. Furthermore, in the case of at least one compound (1-naphthol), acid hydrolysis results in large losses of the analyte (Keimig and Morgan 1986).

We have been investigating the use of enzymatic preparations for catalyzing the hydrolysis of xenobiotic conjugates. Such procedures have previously been used for steroids (Curtius 1967), and while this paper was in preparation Jongeneelen et al.(1987) reported the use of enzymatic hydrolysis to liberate 1-hydroxypyrene from its conjugates. Not only do such procedures reduce the interferences, they are extremely convenient experimentally and give high recoveries. The β-glucuronidase used is inexpensive and contains

some sulfatase. Thus it will hydrolyze both glucuronides and sulfates. The ability of enzymatic hydrolysis to reduce interferences is shown quite dramatically in the gas chromatograms in Fig 1. Both A and B are from the same urine specimen. They are chromatograms of methanol eluates from Sep-Pak purifications of 25 mL of enzyme-hydrolyzed urine. B is fortified at the 1 ppm level with the sodium salt of α-naphthyl-β-D-glucuronic acid (1-naphthol elutes in 8.3 min under these conditions). Chromatogram C is a similar Sep-Pak eluate of a 2 mL aliquiot of the same urine sample subjected to acid hydrolysis. Similar chromatograms were obtained from other specimens and two other donors. C is a 1-μL injection, A and B are 2-μL injections; and C uses an attenuation half as sensitive as A and B. To directly compare C to the other two, one would have to multiply all the peak areas by a factor of 50 (12.5 for the increased urine volume, 2 for the attenuation factor and 2 for the increased injection volume).

Table I summarizes the recoveries we obtained when urine samples were fortified with the sodium salt of α-naphthyl-β-D-glucuronic acid.

Table I. Recovery from Glucuronide-Spiked Urine[a]

Donor	PPM Added	% Range	Av % Recov.	%RSD[b]
1	10	73.6-94.4	86	10.4
2	10	77.2-89.3	84	6.2
3	10	79.3-95.0	89	7.9
1	5	76.1-94.2	83	9.4
2	5	80.9-93.3	87	5.8
3	5	71.2-89.7	79	10.1
1	1	63.6-71.4	68	5.0
2	1	45.3-59.4	54	10.4
3	1	51.4-64.8	58	9.5

[a]Three determinations
[b]RSD is relative standard deviation

We are very impressed with this method for minimizing interferences and maximizing convenience. We recommend it to those seeking to measure xenobiotics, metabolites, and their conjugates.

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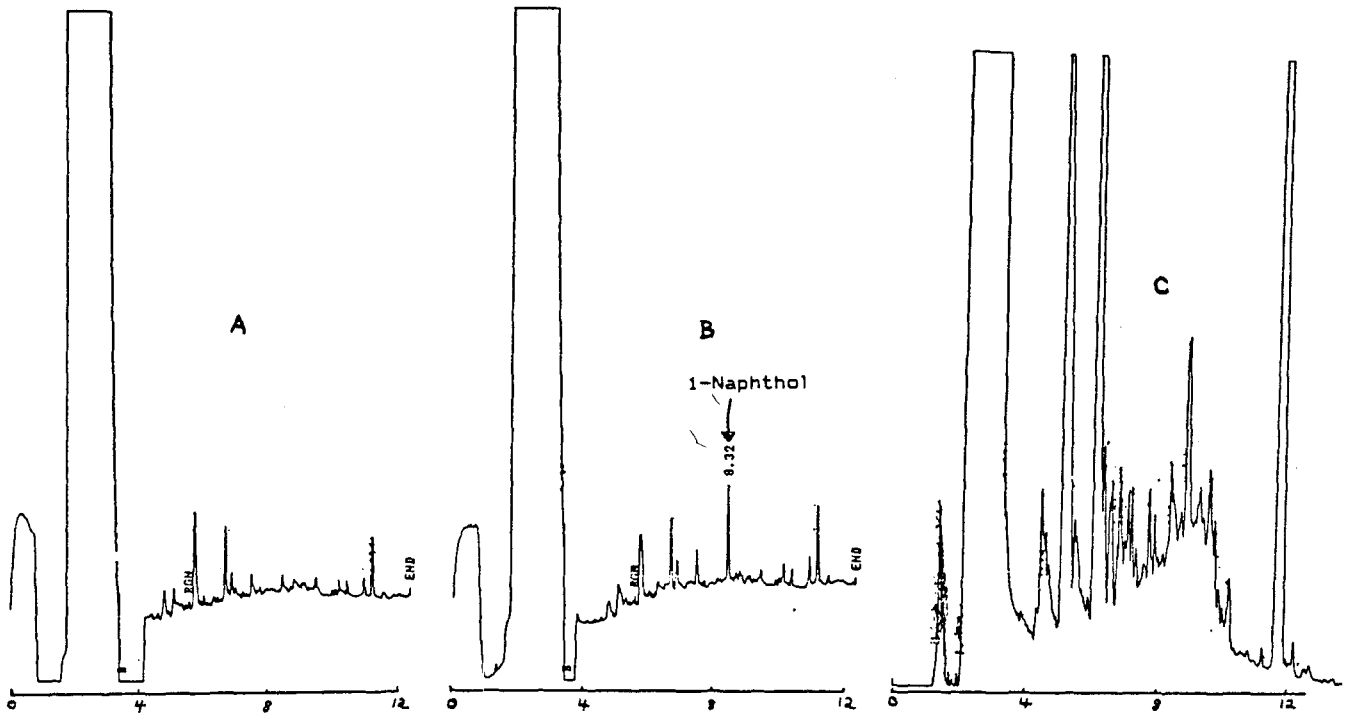

Figure 1. Gas Chromatograms. A is from enzyme hydrolyzed blank urine. B is from enzyme hydrolyzed urine fortified with the glucuronide of 1-naphthol at the 1 ppm level. C is from acid hydrolyzed blank urine under much less sensitive chromatographic conditions (see RESULTS and DISCUSSION).

REFERENCES

Curtius HC, Muller M, Manella B (1976) Gas-liquid chromatography of 17-ketosteroids and progesterone metabolites of urine: comparions of different methods of hydrolysis. J Chromatogr 30:410-427.

Edgerton TR, Moseman RF (1979) Determination of pentachlorophenol in urine: the importance of hydrolysis. J Agric Food Chem 27: 197-199.

Jongeneelen FJ, Anzion RBM, Henderson PT (1987) Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. J Chromatogr 413:227-232.

Keimig SD, Kirby KW, Morgan DM, Keiser JE, Hubert TD (1983) Identification of 1-hydroxypyrene as a major metabolite of pyrene in pig urine. Xenobiotica 13:415-420.

Keimig SD, Morgan DM (1986) Urinary 1-naphthol as a biological indicator of naphthalene exposure. Appl Ind Hyg 2:61-65.

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