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Note

Quantitative gas–liquid chromatographic analysis of naproxen, 6-O-desmethyl-naproxen and their conjugates in urine

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Naproxen [(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid] is a new non-steroidal, anti-inflammatory agent that also has antipyretic and analgesic activity[1]. It is well-absorbed orally and has an elimination half-life of about 14 h. In man, 55–100% of an orally administered dose of naproxen can be recovered in urine in the form of naproxen, 6-O-desmethyl-naproxen and their glucuronide conjugates[2,3]. A gas–liquid chromatographic (GLC) technique has been reported for the analysis of naproxen in plasma in which methylation of the carboxylic acid group with diazomethane is achieved prior to chromatography[2,4]. This method is not applicable to naproxen analysis in urine because of presence of urinary metabolites.

Several reports have described the successful application of GLC in the analysis of barbituric acids, diphenylhydantoin, purines, pyrimidines and uracil by reaction with tetraalkylammonium hydroxides[5–9]. Compounds are alkylated at the high temperatures of the injection port following thermal decomposition of the tetraalkylammonium salt.

This report describes a GLC method involving butylation which can differentiate between naproxen and its 6-desmethyl metabolite and quantitate them simultaneously in urine from individuals who received 500-mg doses. Glucuronide conjugates can be measured following enzymatic or acid hydrolysis.

EXPERIMENTAL

Chemicals

Naproxen (N), 6-O-desmethyl-naproxen (DMN) and 6-propoxy-α-methyl-2-naphthaleneacetic acid (PN) were obtained from Syntex Research (Palo Alto, Calif., U.S.A.). Tetrabutylammonium hydroxide 25% in methanol was purchased from Eastman Kodak (Rochester, N.Y., U.S.A.), Nanograde diethyl ether from Mallinckrodt (St. Louis, Mo., U.S.A.), and glucuronidase–sulfatase mixture from Endo Laboratories (Glusulase®; Garden City, N.Y., U.S.A.). All other chemicals were reagent-grade.

Aqueous stock solutions of N, DMN and internal standard were dissolved in deionized water with the aid of 1 *N* sodium hydroxide.

Extraction and derivatization

Naproxen and 6-O-desmethyl-naproxen. An aqueous solution of the internal standard, PN, 10 μg in 0.1 ml water, was pipetted into a 15-ml screw-capped test tube. To this was added 0.5 ml appropriately diluted urine or urine blanks spiked with N and DMN (1–40 μg/ml), 0.5 ml pH 6 $^{1}/_{15}$ *M* phosphate buffer and 10 ml diethyl ether. The test tube was capped and shaken for 10 min, then centrifuged to separate the phases. Most of the ether phase was removed to a second tube and evaporated to dryness under a gentle stream of nitrogen at 50–60°.

The residue was reconstituted in 100 μl of methanol containing 1.25% tetrabutylammonium hydroxide and 1–5 μl injected into the gas chromatograph.

Naproxen, 6-O-desmethyl-naproxen and conjugates. Enzyme hydrolysis. Internal standard 0.1 ml and 0.5 ml unknown or spiked urine was incubated with 0.1 ml diluted enzyme mixture containing approximately 2000 units of sulfatase and 10,000 units of glucuronidase and 0.4 ml pH 4.6 0.2 *N* acetate buffer at 60° for 2 h. On cooling the mixture was acidified with 0.1 ml 2 *N* H_2SO_4, extracted with 10 ml diethyl ether and processed as described above.

Acid hydrolysis. A 0.5-ml volume of unknown or blank urine spiked with N and DMN 0–40 μg/ml and 0.1 ml PN were heated with 0.5 ml 2 *N* sulfuric acid at 100° for 15 min prior to ether extraction.

GLC conditions

A Varian 1400 chromatograph equipped with a flame-ionization detector and a 180 cm × 3 mm I.D. glass column was used. The column was packed with Chromosorb W (100–120 mesh) coated with 3% OV-17. Injector port, oven and detector temperatures were 270, 230 and 300°, respectively. Nitrogen, hydrogen and air flow-rates were 30, 30 and 300 ml/min, respectively.

GLC–mass spectral analysis

A gas chromatograph interfaced with a Finnigan 3200 chemical-ionization mass spectrometer was used in characterization of the derivatives formed by reacting N, DMN and PN with tetrabutylammonium hydroxide. The GLC column was maintained at 160°. Methane was both the carrier and reagent gas, and the chemical-ionization source was operated at 250° and pressure maintained at 1 torr.

RESULTS AND DISCUSSION

Extraction of free N and DMN at the mild pH of 6.0 minimizes hydrolysis of the labile glucuronide conjugates during the extraction process. Several solvents were tested for the efficiency of extraction of N and DMN. While N and the internal standard can be extracted with either ethyl acetate, dichloromethane or ether, DMN extraction was low with the relatively non-polar solvents. Of the solvents tested, the highest percentage extraction was obtained with ether. Under the described conditions, ether extraction of N and DMN relative to the internal standard was 91 and 92%, respectively.

The butyl derivatives of N, PN and DMN eluted from the gas chromatograph with retention times of 8.4, 13.3 and 18.4 min, respectively (Fig. 1). Blank urine

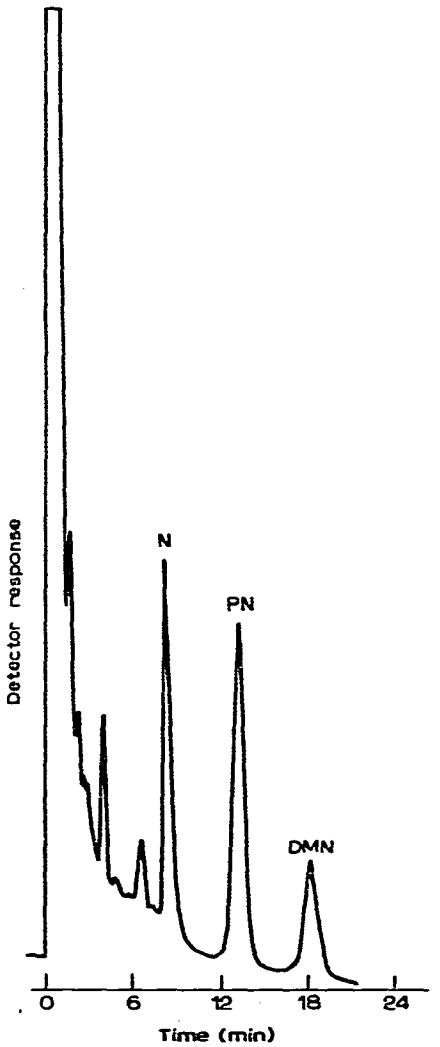

Fig. 1. Chromatogram of 0.5 ml urine sample spiked with 10 μg each of naproxen (N), internal standard (PN) and desmethylnaproxen (DMN). Injection volume 2 μl; range 10^{-11}, attenuation 8.

samples from healthy volunteers carried through the hydrolysis, extraction and derivatization procedures did not contain any interfering peaks with similar retention times (Fig. 2). Standard curves were linear in the range of 1–40 μg each N and DMN per ml of sample. The coefficient of variation ($n = 5$) for N at 5 μg/ml was 3.5% and at 10 μg/ml was 0.96%, and for DMN at the same concentrations was 6.3% and 5.2%.

From a comparison of the amounts of N and DMN after enzyme hydrolysis and after acid hydrolysis of urine samples from subjects who had ingested N (Table I) it appears that both processes are equally efficient in hydrolyzing N and DMN conjugates. Enzyme hydrolysis poses a lower possibility of introducing artifacts in the chromatographic system because of the milder hydrolytic conditions. Acid hydrolysis did result in more numerous peaks in blank urine but none of them had the same

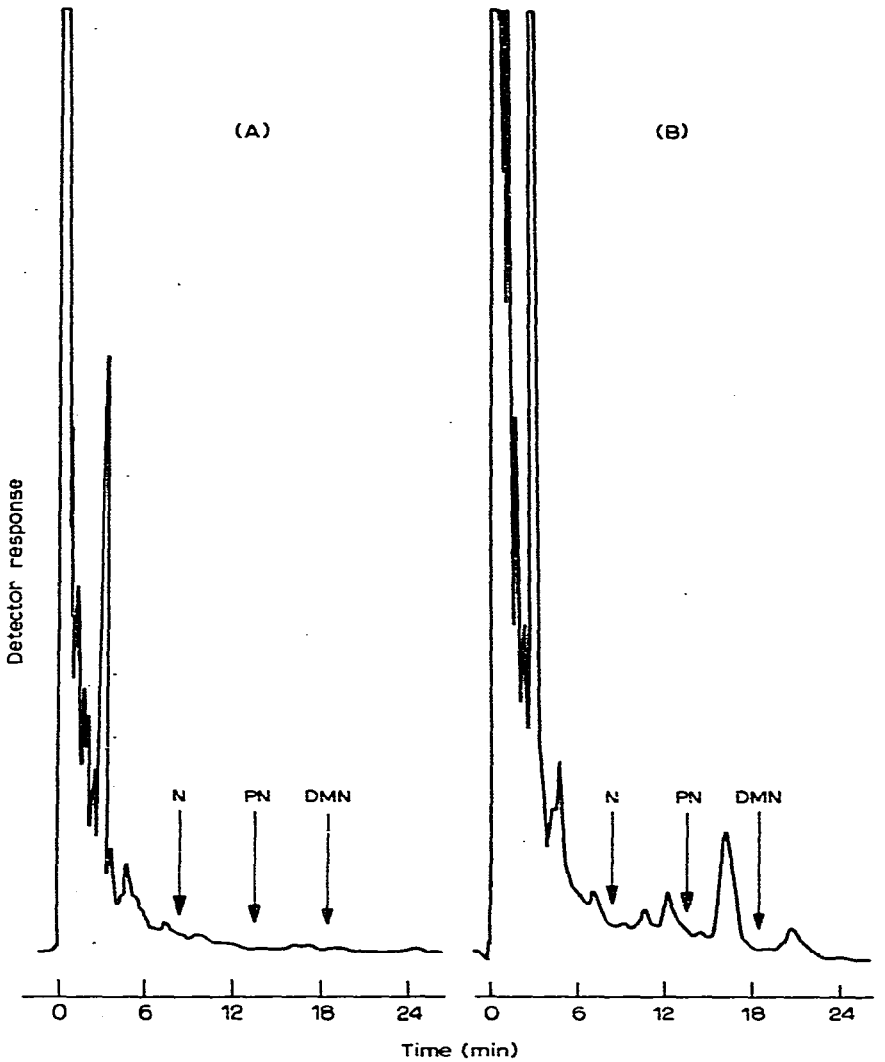

Fig. 2. Chromatogram of blank urine samples from healthy volunteers following hydrolysis, extraction and derivatization. (A) Enzyme hydrolysis. Injection volume 2 μl, attenuation 8. (B) Acid hydrolysis. Injection volume 2 μl, attenuation 8.

TABLE I

COMPARISON OF ENZYME AND ACID HYDROLYSIS OF URINE FROM SUBJECTS ADMINISTERED NAPROXEN

Sample number	*Concentration (μg/ml)*			
	Naproxen		*Desmethyl-naproxen*	
	Enzyme	*Acid*	*Enzyme*	*Acid*
1	78.6	78.4	21.7	20.4
2	37.5	39.0	20.2	21.1
3	30.2	28.3	13.3	13.1
4	19.9	21.6	8.6	9.9
5	18.2	20.1	11.0	11.2

retention times as N, PN or DMN. The advantage of acid hydrolysis is that it is less time consuming.

Fig. 3 shows the chemical-ionization mass spectra of N, PN and DMN for which $M + H^+$ ions at mass 315, 329 and 287 were obtained, respectively. For N and PN, the ion masses were consistent with the monobutyl derivative and for DMN it was consistent with the dibutyl derivative. The structures were further confirmed by the formation of abundant ions at mass 185, 213 and 227, respectively for N, PN and DMN which are the equivalents to the loss of a $COOC_4H_9$ molecule from the parent compounds.

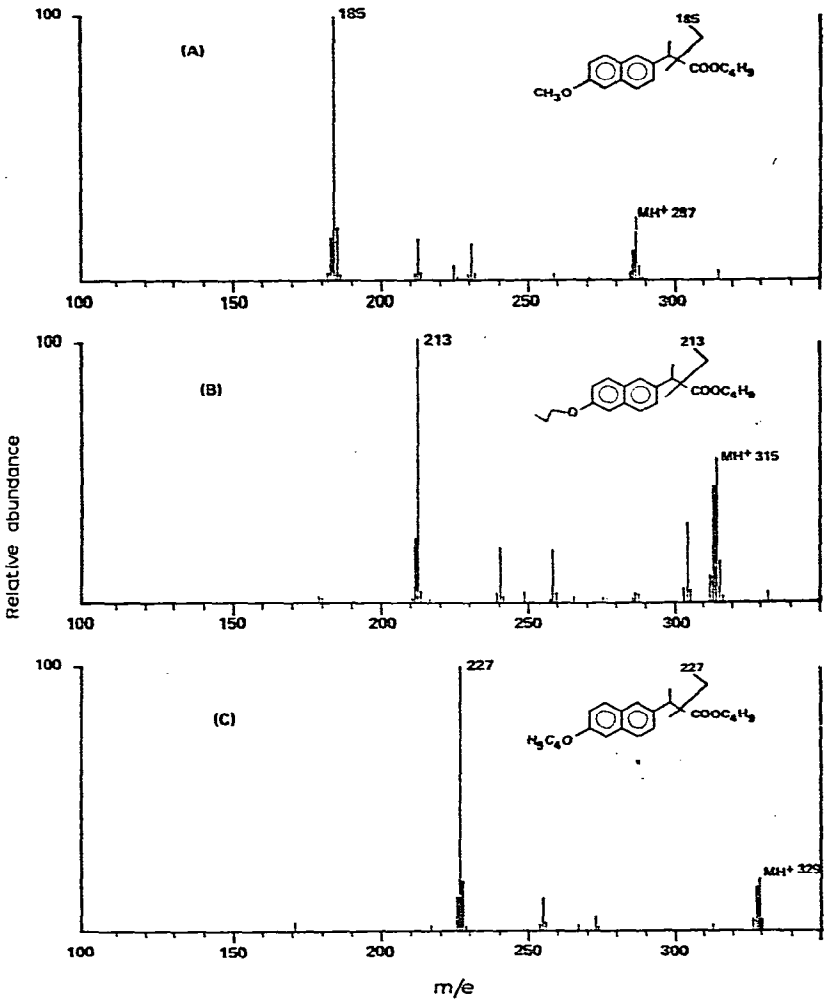

Fig. 3. Chemical-ionization mass spectra of butyl derivatives of (A) naproxen, (B) internal standard, (C) desmethyl-naproxen.

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REFERENCES

1 A. P. Roszkowski, W. H. Rooks, II, A. J. Tomolonis and L. M. Miller, *J. Pharmacol. Exp. Ther.*, 179 (1971) 114.
2 R. Runkel, M. Chaplin, G. Boost, E. Segre and E. Forchielli, *J. Pharm. Sci.*, 61 (1972) 703.
3 R. Runkel, E. Forchielli, H. Sevelius, M. Chaplin and E. Segre, *Clin. Pharmacol. Ther.*, 15 (1974) 261.
4 J. P. Desager, M. Vanderbist and C. Harvengt, *J. Clin. Pharmacol.*, 16 (1976) 189.
5 J. MacGee, *Anal. Biochem.*, 14 (1966) 305.
6 J. MacGee, *Anal. Biochem.*, 42 (1970) 421.
7 E. W. Robb and J. J. Westerbrook, *Anal. Chem.*, 35 (1963) 1644.
8 E. Brochmann-Hanssen and O. T. Oke, *J. Pharm. Sci.*, 58 (1969) 370.
9 D. Schuppan, S. Riegelman, B. V. Lehmann, A. Pillbrant and C. Becker, *J. Pharmacokin. Biopharm.*, 1 (1973) 307.