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## Note

# Gas chromatographic determination of N-desmethyl-propyphenazone, a metabolite of propyphenazone, in human urine

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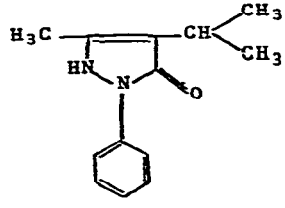
Tateishi and Shimizu [1] have reported that the major urinary metabolite of propyphenazone in man is the enol-glucuronide of N-desmethyl-propyphenazone, accounting for some 80% of the total urinary metabolites, but they did not report the total amount of metabolites as a percentage of the administered dose.




4-isopropyl-3-methyl-1-phenyl-3-pyrazolin-5-one
(N-desmethyl-propyphenazone)

This paper describes a simple gas chromatographic technique for the assay of N-desmethyl-propyphenazone in human urine after hydrolysis of its conjugate.

## EXPERIMENTAL

### *Chemicals and reagents*

N-Desmethyl-propyphenazone was supplied by Ciba-Geigy (Basle, Switzerland) and lindane by Applied Science Labs. (State College, PA, U.S.A.). Sulphuric acid (Titrisol; E. Merck, Darmstadt, G.F.R.) and toluene (Nanograde; Mallinckrodt, St. Louis, MO, U.S.A.) were of analytical grade.

Acetate buffer (pH 5.5) was prepared with 4.8 ml of 0.2 *M* acetic acid

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and 35.2 ml of 0.2 *M* sodium acetate. β-Glucuronidase (Type 2; Sigma, St. Louis, MO, U.S.A.) is a bacterial β-glucuronidase containing about 61,500 units/g. The methanolic solution of internal standard contained 25 ng of lindane per 100 μl.

*Equipment*

A gas chromatograph (Hewlett-Packard, Model 5713 A) equipped with an electron-capture detector (Hewlett-Packard, Model 18713 A) was used. The peak areas were given by an electronic integrator (Hewlett-Packard, Model 3380 A).

The column was operated at 186°C with argon—methane (90:10) at a flow-rate of 60 ml/min. The injector temperature was 250°C and the detector set at 300°C.

The glass column was washed successively with 1 *M* hydrochloric acid, distilled water, acetone and benzene, and silanized with a 1% (v/v) solution of hexamethyldisilazane in benzene. It was then washed again with benzene and dried at 100°C.

The column packing was 5% SP 2100 on 100—120 mesh Chromosorb W HP (Touzart et Matignon, Vitry-sur-Seine, France). The packed column (2 m × 3 mm I.D.) was flushed with the carrier gas at a flow-rate of 40 ml/min and heated to 260°C at 1°C/min. The column temperature was held at 260°C for five days. The column was conditioned by the injection of 100 μl of a silylating agent (Silyl 8; Pierce, Rockford, IL, U.S.A.) in portions between 150°C and 220°C. After this procedure it is ready for use at 186°C.

*Enzymatic hydrolysis in urine*

One millilitre of urine (diluted with water if necessary), 1 ml of acetate buffer (pH 5.5) and 10 mg of β-glucuronidase were heated for 24 h at 37°C. The extraction was then performed as described below.

*Extraction*

One hundred microlitres of internal standard solution were measured into a 5-ml glass centrifuge tube, to which 250 μl of $5 \cdot 10^{-3}$ *M* sulphuric acid and 500 μl of toluene were added. The tube was shaken mechanically (Infors-shaker) for 10 min at 250 rpm and centrifuged at 2450 *g*.

*Gas chromatography*

A 2-μl aliquot of the organic layer was injected into the gas chromatograph. The N-desmethyl-propyphenazone content was calculated from the peak-area ratio by reference to a calibration curve. This curve was obtained by extraction of urine spiked with increasing amounts of N-desmethyl-propyphenazone from 10 to 1000 ng/ml and a constant amount of internal standard (25 ng/ml urine).

*Study in man*

Eight healthy subjects, who were advised to take no drugs for 8 days before the beginning of the experiment and none besides propyphenazone throughout the duration of the study, each received 250 mg of propyphenazone as two tablets of 125 mg.

Urine was collected over a period of 24 h. The volume was measured and samples were stored at −20°C until analysis.

## RESULTS AND DISCUSSION

### *Precision and recovery*

Table I gives the results obtained when the described procedure was applied to urine samples spiked with N-desmethyl-propyphenazone. As seen in the table, a good reproducibility was obtained with concentrations down to 10 ng/ml N-desmethyl-propyphenazone. This low concentration (10 ng/ml) may be taken as the sensitivity limit of the method. Although still lower concentrations can be detected, the peak height or the peak area is so small that the precision of the determination will be unsatisfactory.

TABLE I

PRECISION AND RECOVERY IN THE DETERMINATION OF N-DESMETHYL-PROPYPHENAZONE IN SPIKED HUMAN URINE SAMPLES

| Amount added (ng/ml) | Mean amount found (ng/ml) ($n$=6) | Precision (± S.D.) | Recovery ± C.V. (%) |
|---|---|---|---|
| 10 | 9.9 | 0.1 | 99.0 |
| 25 | 24.0 | 1.5 | 96.0 |
| 50 | 52 | 0.7 | 104.0 |
| 100 | 104 | 2.3 | 104.0 |
| 150 | 146 | 3.7 | 97.3 |
| 200 | 189 | 2.9 | 94.5 |
| | | | 99 ± 4.1 |

### *Urine interference*

Fig. 1 shows chromatograms of an extract of 1 ml of human urine and of an aliquot (100 µl) of a 24-h urine of a healthy subject treated with 250 mg of propyphenazone. There is no interference from the normal components of the urine extract.

### *Urine hydrolysis*

Preliminary experiments were carried out to determine the best conditions for the hydrolysis of the enol-glucuronide of N-desmethyl-propyphenazone in human urine.

Stability tests showed that N-desmethyl-propyphenazone is stable in urine for 1 h at 70°C or 100°C, and for 48 h at 37°C.

Samples of the same urine, containing N-desmethyl-propyphenazone glucuronide, from subjects treated with propyphenazone were then subjected to both enzymatic and acid hydrolysis.

Enzymatic hydrolysis was studied in urine samples incubated with 10 mg of β-glucuronidase and acetate buffer (pH 5.5) for 1, 4, 16, 24 and 48 h at

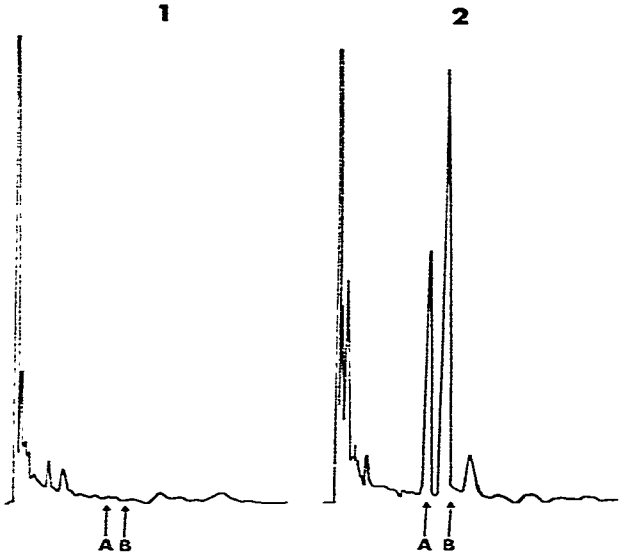


Fig. 1. Examples of chromatograms. (1) Human urine blank (1 ml of urine). (2) 24-h urine (100-µl aliquot) of a healthy volunteer (who had received 250 mg of propyphenazone) after enzymatic hydrolysis with 25 ng of internal standard (A) or 167 ng of N-desmethyl-propyphenazone (B) added.

37°C. A maximum yield of N-desmethyl-propyphenazone was obtained after incubation for 24 h. Identical results were obtained after another 24-h incubation during which fresh enzyme (10 mg) was added every 6 h instead of only at the beginning.

Acid hydrolysis of urine incubated with 1 ml of concentrated HCl for 1 h at 70°C produced no detectable amounts of N-desmethyl-propyphenazone. When the reaction temperature was increased to 100°C, however, N-desmethyl-propyphenazone was found, but in a yield ten to twenty times less than that obtained after enzymatic hydrolysis.

Enzymatic hydrolysis was therefore chosen as the more suitable procedure for the hydrolysis of the propyphenazone metabolite.

*Application*

The described technique was used for the determination of the enol-glucuronide of N-desmethyl-propyphenazone as a metabolite of propyphenazone in 24-h urine samples of eight healthy volunteers who had received 250 mg of propyphenazone. The average amount of the metabolite found in the urine corresponded to 0.8% of the administered dose of propyphenazone.

This result appears to be at variance with the statement [1] that in man the major portion of a given dose of propyphenazone is metabolized to the demethylated product, which subsequently, and almost quantitatively, undergoes glucuronide conjugation. Actually the authors have reported that the glucuronide of desmethyl-propyphenazone recovered in the first 24-h urines of the rat corresponds to 40—60% of the administered dose, which was very

high (15 mg/kg per os). In man given only 50 mg per os, the glucuronide accounted for 80% of the total urinary metabolite, as in the rat, but no figure has been given for the amount excreted in the urine as a percentage of the dose.

Besides the determination of the enol-glucuronide of N-desmethyl-propyphenazone, propyphenazone was also determined [2] in the same 24-h urine samples of the eight healthy volunteers who had received 250 mg of propyphenazone. As a mean, about 0.04% of the administered dose was excreted as the unmetabolized drug.

## CONCLUSION

The proposed gas chromatographic technique permits the quantitative determination of N-desmethyl-propyphenazone in human urine at concentrations down to 10 ng/ml. This method can be applied to the determination of the enol-glucuronide of N-desmethyl-propyphenazone as a metabolite of propyphenazone in urine.

## ACKNOWLEDGEMENTS

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## REFERENCES

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