Gas Chromatographic Determination of Guanabenz in Biological Fluids by Electron-Capture Detection

JOHN A. KNOWLES ˣ, GRACE R. WHITE, CONSTANCE J. KICK, TAYLOR B. SPANGLER, and HANS W. RUELIUS

Received April 16, 1981, from the *Wyeth Laboratories, Inc., Philadelphia, PA 19101.* Accepted for publication September 17, 1981.

Abstract □ A sensitive gas chromatographic method for the determination of guanabenz [(2,6-dichlorobenzylidene)amino]guanidine in urine and plasma was developed. The method depends upon the acid hydrolysis of guanabenz to 2,6-dichlorobenzaldehyde, which has strong electron capturing properties and is volatile enough to be eluted from a gas chromatographic column. Concentrations as low as 0.1 ng of guanabenz/ml can be determined and recovery of the drug from urine and plasma samples is 81.8 ± 5.5% (*SD*). No interferences arising from plasma, urine, or reagents were encountered. Examples of the application of the method are given.

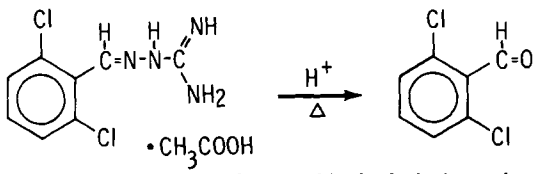
Keyphrases □ Guanabenz—gas chromatographic determination in biological fluids, electron-capture detection □ Electron-capture detection—gas chromatographic determination of guanabenz in biological fluids □ GC determination—guanabenz in biological fluids, electron-capture detection

Guanabenz, [(2,6-dichlorobenzylidene)amino]guanidine, (Scheme I) is a new agent which lowers blood pressure in hypertensive rats (1) and dogs (2), as well as in humans (3–5). When administered orally, the compound is subjected to extensive first-pass metabolism. Extensive metabolism and distribution into tissues resulted in very low concentrations of guanabenz in plasma when single oral doses, in the therapeutic range, were given to hypertensive patients (6). Thus, a highly sensitive method is required for the analysis of the drug in plasma, urine, and tissue samples derived from animal or clinical studies. Such a method, along with its application, is described in this report. Extraction of guanabenz from the sample, followed by acid hydrolysis to a volatile, strongly electron-capturing compound, led to a sensitivity of 0.1 ng/ml with an overall recovery of 81.8 ± 5.5%. No interferences stemming from reagent, urine, or plasma were observed.

EXPERIMENTAL

Materials—Sodium bicarbonate[1], sodium hydroxide[1], ether[1], toluene[1], and sulfuric acid[2] were all analytical reagent grade. The chromatographic standard, 2,6-dichlorobenzaldehyde[3], was recrystallized twice from absolute ethanol.

Extraction and Hydrolysis—The pH of a 1-ml aliquot of plasma or 0.5–3 ml urine (in a 12-ml screw-cap centrifuge tube) was adjusted to 10 by the addition of 2 ml of sodium bicarbonate–sodium hydroxide buffer (pH 10). Ether (5 ml) was added and the tube was sealed, shaken mechanically for 10 min, and then centrifuged for 5 min. Four milliliters of the ether layer was transferred to a clean centrifuge tube and the aqueous phase was re-extracted with an additional 3 ml of ether. After shaking and centrifuging the mixture, 3 ml of the ether layer was combined with the original 4 ml. The aqueous layer was discarded, and the ether extracts were shaken with 2 ml of 0.5 *N* sulfuric acid for 10 min, and centrifuged for 5 min. The ether phase was discarded by aspiration. Any residual ether was dispelled by placing the uncapped tube in a 60° water bath for 3 min prior to the addition of 2 ml of 12 *N* H_2SO_4. The tube was capped and placed in the 60° water bath. After 45 min, the tube was cooled in an ice bath, 0.1 ml of toluene was added, and the mixture was shaken for 10 min and centrifuged for 5 min. The aqueous phase was removed by a syringe aspirator, and after recentrifugation, 2–4 μl of the toluene solution was injected onto the gas chromatographic column.



Scheme I—Structure of guanabenz and its hydrolysis product, 2,6-dichlorobenzaldehyde.

Chromatography—The procedure just described resulted in the acid hydrolysis of guanabenz to 2,6-dichlorobenzaldehyde which was quantitated by electron-capture gas chromatography[4]. The glass column was 6-mm o.d. (2-mm i.d.) × 3 m and packed with 1% neopentylglycol succinate on 60/80 mesh Chromosorb G AW-DMCS[5]. Its temperature was maintained at 200°, and the flow rate of the ultra-high pure helium[6] carrier gas was 20 ml/min. The flow rate of the purge gas (argon–methane, 95:5)[6] was 30 ml/min. The temperature of the injection port was 220° and that of the electron-capture detector was 320°.

Under these conditions, the retention time of 2,6-dichlorobenzaldehyde was 2.5 min (Fig. 1) and injection of a 4-μl aliquot resulting from the extraction and hydrolysis of a 0.2-ng/ml guanabenz standard gave a peak height of 14 mm.

A response curve to check detector linearity and recovery of guanabenz was determined daily by injecting 2–4-μl aliquots of solutions consisting of 2, 5, and 10 ng of 2,6-dichlorobenzaldehyde/ml of toluene into the chromatograph and plotting peak area *versus* concentration of the solution of the aldehyde.

A calibration curve of concentration of guanabenz *versus* detector response was determined daily by adding known amounts of guanabenz (0.1, 0.5, 1.0, 5.0, and 20.0 ng/ml) to control urine or plasma and then processing these standards in the same manner as unknown samples.

RESULTS AND DISCUSSION

Standard curves, constructed daily, were linear from 0.1 to 20 ng of guanabenz/ml, the range usually encountered in clinical studies. The minimal detection limit was 0.1 ng of guanabenz/ml. Recovery of the drug from aqueous solution, spiked human plasma, rhesus monkey plasma, and urine in the 0.1–20 ng/ml range was 81.8 ± 5.5% (*SD*; $n = 154$). This recovery was determined by use of the response curve for 2,6-dichlorobenzaldehyde.

No interfering chromatographic peaks were observed (Fig. 1) after acid hydrolysis of extracts obtained from control samples collected prior to dosing. Figure 2 presents the concentration–time curve of guanabenz in the plasma of a normotensive human subject following administration of a 24-mg oral dose of the drug.

The highly selective, sensitive, and accurate method, developed for the quantitation of guanabenz in biological fluids, depends on the hy-

[1] Mallinckrodt, St. Louis, Mo.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Hewlett-Packard Model 7620, Avondale, Pa.
[5] Supelco, Inc. Bellefont, Pa.
[6] Matheson Gas Products, East Rutherford, N.J.

0022-3549/82/0600-0710$01.00/0
© 1982, American Pharmaceutical Association

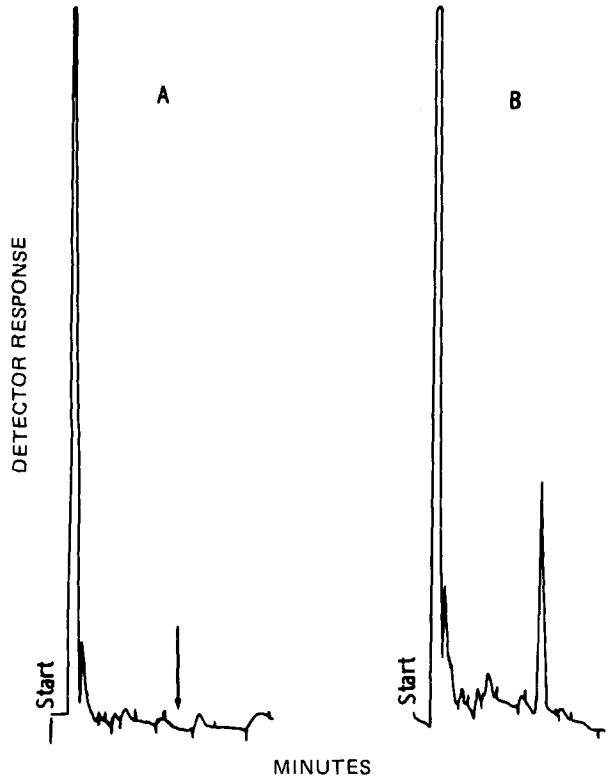


Figure 1—*(A) Gas chromatogram of a 0-hr plasma sample taken from a normotensive human subject. (B) Gas chromatogram of a plasma sample taken from the same human subject following oral administration of the drug.*

drolysis of the drug to 2,6-dichlorobenzaldehyde, a volatile compound which has strong electron-capturing properties. No 2,6-dichlorobenzaldehyde was found as a metabolite in plasma samples. If any were present, it would not interfere with the determination of guanabenz as it would be separated from the drug during the back extraction into dilute sulfuric acid.

This procedure is sensitive enough to enable concentrations as low as 0.1 ng/ml to be determined. This sensitivity permitted the determination of the very low drug concentrations in human plasma resulting from therapeutic doses and the calculation of pharmacokinetic parameters from these data (6). Furthermore, guanabenz was measured in rat brain (7) and in urine and plasma of rhesus monkeys (8).

An internal standard was not used in the procedure, because no compound could be found that would have extraction and hydrolysis characteristics similar to those of guanabenz when added to an unknown sample. The day-to-day variation of the method was <7%. No interferences from plasma, urine, or reagents were encountered. For standards containing 0.1 ng of guanabenz/ml, the recovery for 0.1 ng/ml was 82.6 ± 9.2% (*SD*); for 5 ng/ml, 81.6 ± 5.1%; and for 20 ng/ml, 81.1 ± 6.5%. These data, which were calculated from the detector response given by known amounts of 2,6-dichlorobenzaldehyde, demonstrate good recovery of the drug and high precision of the method.

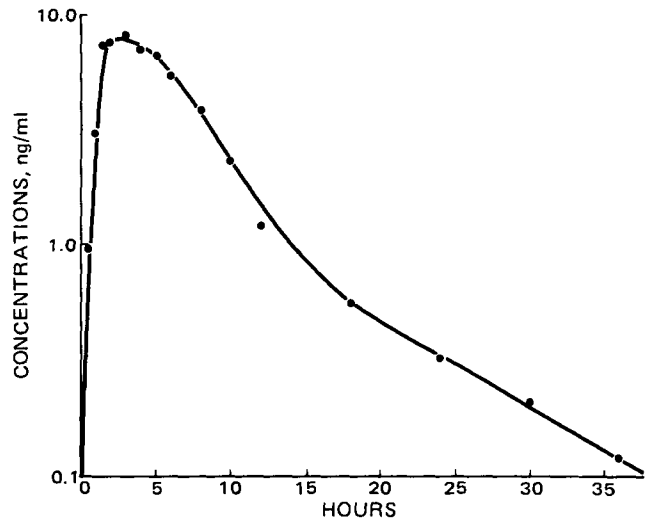


Figure 2—*Concentrations of guanabenz* versus *time in the plasma of a healthy subject after oral administration of 24 mg of guanabenz.*

REFERENCES

(1) T. Baum, D. K. Eckfield, N. Metz, J. L. Dinish, G. Rowles, R. Van Pelt, A. T. Shropshire, S. P. Fernandez, M. I. Gluckman, and W. F. Bruce, *Experientia* (Basel) **25,** 1066 (1969).

(2) R. K. Saini, A. P. Caputi, and E. Marmo, *Farmaco Ed. Prat.*, **28,** 359 (1973).

(3) R. S. Shah, B. R. Walker, S. K. Vanov, and R. H. Helfont, *Clin. Pharmacol. Ther.*, **19,** 732 (1976).

(4) F. G. McMahon, J. R. Ryan, A. K. Jain, R. Vargas, and S. K. Vanov, *ibid.*, **21,** 272 (1977).

(5) B. R. Walker, R. S. Shah, K. B. Ramanathan, S. K. Vanov, and R. H. Helfant, *ibid.*, **22,** 868 (1977).

(6) R. H. Meacham, M. Emmett, A. A. Kyriakopoulos, S. T. Chiang, H. W. Ruelius, B. R. Walker, R. G. Narins, and M. Goldberg, *ibid.*, **27,** 44 (1980).

(7) R. H. Meacham, H. W. Ruelius, C. J. Kick, J. R. Peters, S. M. Kocmund, S. F. Sisenwine, and R. L. Wendt, *J. Pharmacol. Exp. Ther.*, **214,** 594 (1980).

(8) R. H. Meacham, S. T. Chiang, C. J. Kick, S. F. Sisenwine, W. J. Jusko, and H. W. Ruelius, *Drug Metab. Dispos.*, **9,** 509 (1981).