GLC Determination of Butylated Hydroxyanisole in Human Plasma and Urine

R. EL-RASHIDY and SARFARAZ NIAZI ˣ

Received December 30, 1977, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication May 26, 1978.

Abstract □ A highly sensitive and specific GLC method was developed for the analysis of butylated hydroxyanisole, a commonly used antioxidant. Concentrations below 100 ng/ml could be detected in human plasma and urine. Preliminary pharmacokinetic studies demonstrated that, upon administration of 100 mg po, butylated hydroxyanisole was quickly absorbed and removed from the plasma with a high degree of intersubject variability.

Keyphrases □ Butylated hydroxyanisole—GLC analysis in plasma and urine □ GLC—analysis, butylated hydroxyanisole in plasma and urine □ Antioxidants—butylated hydroxyanisole, GLC analysis in plasma and urine

Butylated hydroxyanisole (I) is a commonly used food antioxidant (1) in this country (average intake: 35–140 mg/day). Although generally regarded as safe (GRAS list), several studies reported various pharmacological and toxicological properties (2–4). No study, however, has addressed its disposition characteristics in humans. Previously (5), concentration-dependent binding of I to human serum albumin was reported.

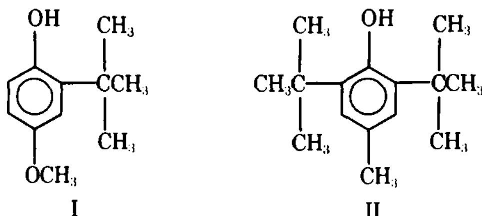
The purpose of this investigation was to develop a specific and sensitive technique for the analysis of I in plasma and urine that can be used to study the kinetics of the absorption and disposition of I.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] was equipped with a flame-ionization detector. A 1.8-m long, 3.13-mm o.d., and 2.13-mm i.d. stainless steel column[2] was packed with 5% QF-1 as the stationary phase coated on 80–100-mesh Gas Chrom WAW as the supporting phase[2]. The column, detector, and injection port temperatures were 165, 225, and 215°, respectively. The carrier gas (nitrogen), hydrogen, and air flow rates were 30, 40, and 300 ml/min, respectively. Injections of 2–3 μl were made with a 10-μl syringe[3].

Reagents and Chemicals—Pure samples of I and butylated hydroxytoluene (II), the internal standard, were obtained commercially[4]. All other reagents were reagent grade[5].

Analysis of I in Plasma and Urine—To 1 ml of plasma or 5 ml of urine in a 15-ml centrifuge tube, a 0.2-ml solution of II (10 μg/ml) was added. A 4-ml portion of petroleum ether[5] also was added to the tube, which was then stoppered with an aluminum foil-lined closure and shaken[6] for 10 min. The organic layer was transferred to another centrifuge tube containing 3 ml of 5% sodium bicarbonate, and this tube was stoppered and shaken for another 10 min. A 3-ml aliquot of the organic layer was transferred to another tube and evaporated to dryness under a gentle stream of nitrogen in a beaker water bath held at 30°. The residue was dissolved in 50 μl of carbon disulfide, and aliquots of 2–3 μl were injected onto the column for quantification.



The calibration curves were constructed by adding 0.25, 0.50, 0.75, 1.0, and 2.0 μg of I to 1 ml of human plasma or 5 ml of human urine. The peak height ratios of I and II plotted against spiked concentrations of I gave good linear correlations.

Human Experiments—Two healthy adult male volunteers, 55 and 60 kg, who had not ingested any products containing I or II for 2 weeks were each given 100 mg of I in a gelatin capsule with 200 ml of water after overnight fasting. Blood samples were withdrawn at 0.5, 1, 2, 4, 6, and 8 hr following administration; pooled urine samples were collected for 0–8-, 8–16-, 16–24-, and 24–36-hr intervals. All samples were kept frozen and protected from light until analyzed.

RESULTS AND DISCUSSION

Several analytical methods for the detection of I in food products (6–11) require large samples, are not very sensitive, and are not applicable to the analysis of biological samples.

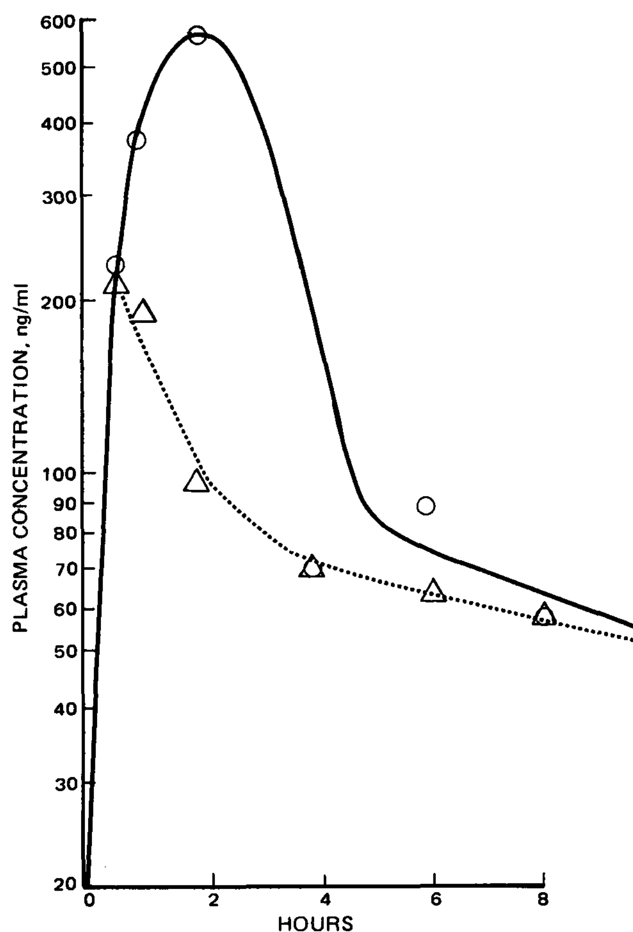


Figure 1—*Plasma concentration profiles of free butylated hydroxyanisole following oral administration of 100 mg in two subjects.*

[1] Varian Aerograph model 1400, Varian, Park Ridge, Ill.
[2] Alltech Associates, Arlington Heights, Ill.
[3] Hamilton Co., Reno, Nev.
[4] Nutritional Biochemicals Corp., Cleveland, Ohio.
[5] Fisher Scientific Co., Fair Lawn, N.J.
[6] Precision Scientific Corp., Chicago, Ill.

0022-3549/79/0100-0103$01.00/0
© 1979, American Pharmaceutical Association

Table I—Precision and Accuracy of the Assay Applied to Spiked Human Plasma Samples

Spiked Concentration, ng/ml	Determined Concentration, ng/ml ± *SEM*[a]	Range
200	200 ± 1.0	194–205
500	500 ± 0.8	488–512
1000	998 ± 1.0	970–1026
3000	3087 ± 2.5	2870–3303

[a] $n = 6$.

The extraction procedure suggested here results in a noise-free blank for the plasma and urine. The spiked samples (prepared from a stock solution of 1 mg/ml in ethanol) showed excellent resolution. The retention time for I was 4 min, whereas the internal standard (II) appeared at 3.0 min. These short retention times are highly desirable, allowing large numbers of samples to be analyzed in a short time. The extraction efficiency was calculated to range from 75 to 80% when compared with the standard solutions prepared in carbon disulfide.

The sensitivity of the column under these conditions allowed detection of quantities as low as 10 ng injected onto the column; however, the concentration of I that could easily be detected in plasma and urine was below 100 ng/ml. The precision and accuracy of the analytical method were discerned by spiking the plasma samples with concentrations ranging from 200 to 3000 ng/ml. Table I reports the determined concentrations of the spiked samples.

The plasma I levels following administration of a 100-mg oral dose are shown in Fig. 1. Each data point represents the average of six determinations. A large degree of intersubject variation was noted in terms of the time and magnitude of the peak concentration. After a rapid rise in the plasma concentration, a sharp decline resulted in the lowering of the concentration to below 100 ng/ml within 2 hr for Subject 1 and 6 hr for Subject 2. Therefore, the bioavailability of I and its rate of absorption may differ greatly between subjects.

Less than 1% of the administered dose of I was eliminated in the urine as intact drug (Table II), which is in concurrence with a previous study (1).

Table II—Recovery of Free Butylated Hydroxyanisole in Urine[a]

Hours	Subject 1	Subject 2
0–8	0.080 ± 0.012	0.050 ± 0.011
8–16	0.570 ± 0.009	0.431 ± 0.009
16–24	0.033 ± 0.0017	0.027 ± 0.025
24–36	0.00	0.00

[a] Percent of administered dose ± *SEM*.

Detailed pharmacokinetic studies are in progress.

REFERENCES

(1) B. D. Astill, J. Mills, D. W. Fassett, R. L. Roudabush, and C. J. Terhaar, *Am. J. Agric. Food Chem.*, **10**, 315 (1962).

(2) Technical Report Series No. 228, World Health Organization, 1962.

(3) A. Takanaka, R. Kato, and Y. Omori, *Shokuhim Eiseigaku Zasshi*, **10**, 260 (1969).

(4) D. M. Conning and T. McElligott, *Proc. Eur. Soc. Study Drug Toxic.*, **11**, 203 (1970).

(5) R. El-Rashidy and S. Niazi, *J. Pharm. Sci.*, **67**, 967 (1978).

(6) W. A. B. Thompson, *J. Chromatogr.*, **19**, 599 (1965).

(7) T. K. Choy, J. J. Quattrone, Jr., and N. J. Alicino, *ibid.*, **12**, 171 (1963).

(8) D. Takahashi, *J. Assoc. Off. Anal. Chem.*, **51**, 943 (1968).

(9) *Ibid.*, **50**, 880 (1967).

(10) S. Dilli and K. Robards, *Analyst*, **102**, 201 (1977).

(11) B. D. Page and B. P. C. Kennedy, *J. Assoc. Off. Anal. Chem.*, **59**, 1208 (1976).

ACKNOWLEDGMENTS

Supported in part by a grant from the Graduate College Campus Research Board, University of Illinois at the Medical Center.

Bioavailability and Related Heart Function Index of Digoxin Capsules and Tablets in Cardiac Patients

E. ASTORRI *[x], G. BIANCHI *, G. LA CANNA *, D. ASSANELLI *, O. VISIOLI *, and A. MARZO [‡]

Received August 9, 1977, from the **Istituto di Clinica Medica Ia, Cattedra di Malattie Cardiovascolari, University of Parma, Via Gramsci, 14-I-43100 Parma, Italy,* and the *‡Istituto Simes di Cardiologia Sperimentale, Via C. Colombi, 18-I-20161 Milan, Italy.* Accepted for publication May 23, 1978.

Abstract □ A loading dose of digoxin (750 μg) in two commercial formulations was administered to 14 patients with heart disease according to a crossover design. One formulation consisted of soft gelatin capsules containing a solution of digoxin; the other formulation was compressed tablets. All parameters investigated, *i.e.*, serum peak height, time of the peak, area under the serum level–time curve (*AUC*), and area above the Q-S_2I (electromechanical systole) decrease (obtained from polycardiographic evaluation), showed better bioavailability of digoxin capsules than tablets, averaging 36.3%. The better bioavailability of digoxin capsules than tablets seems to be more evident in heart disease patients than that encountered previously in healthy subjects. The *AUC* and the area above the Q-S_2I decrease were linearly correlated only with digoxin capsules.

Keyphrases □ Bioavailability—digoxin capsules and tablets compared in cardiac patients □ Heart function index—digoxin capsules and tablets compared in cardiac patients □ Digoxin—bioavailability and heart function index, capsules and tablets compared in cardiac patients □ Cardiotonic agents—digoxin, bioavailability and heart function index, capsules and tablets compared in cardiac patients

A marked lack of uniformity in content and bioavailability of digoxin tablets was reported (1–5) for different formulations as well as for different batches of the same marketed product. In the last few years, a fairly satisfactory bioavailability level has been achieved in most commercial preparations of digoxin tablets that meet the Food and Drug Administration's recent specific requirements (6). The most important development in this field is the

0022-3549/79/0100-0104$01.00/0

© 1979, American Pharmaceutical Association