carbonyl-2-hydroxyquinoline (I) (0.01 mol) in ethanol (20 mL). The mixture was heated at reflux for 2 h. The solid was removed by filtration and recrystallized.

*6,7-Dimethoxy-2-hydroxyquinoline-3-carboxylic Acid Hydrazones (XII-XVI)*—The appropriate aldehyde (0.02 mol) was added to 6,7-dimethoxy-2-hydroxyquinoline-3-carboxylic acid hydrazide (IX) (0.01 mol) in glacial acetic acid. The mixture was heated at reflux for 1 h, and the material was removed by filtration and recrystallized.

*Interaction of 6,7-Dimethoxy-2-hydroxyquinoline-3-carboxylic Acid Hydrazide and Acid Anhydrides (VII and VIII)*—A mixture of the hydrazide (IX) (0.01 mol) and the appropriate acid anhydride (0.02 mol) in glacial acetic acid (30 mL) was heated at reflux for 3 h. The mixture was allowed to stand overnight. The material was removed by filtration and recrystallized. For VIII $^1$H-NMR[1] ($CDCl_3$): $\delta$ 7.25 (s, 1, OH), 8.1-7.0 (m, 7, ArH), 4.3 (s, 1, NH), and 3.7 ppm (m, 6, 2 $OCH_3$).

*Interaction of 6,7-Dimethoxy-3-ethoxycarbonyl-2-hydroxyquinoline and Different Amines (II-VI)*—A mixture of 6,7-dimethoxy-3-ethoxycarbonyl-2-hydroxyquinoline (I) (0.01 mol) and the appropriate amine (0.02 mol) was heated at reflux in methanol (20 mL) for 3 h. Approximately 15 mL of the solvent was removed under reduced pressure, the residue was cooled, and the solid material was removed by filtration and recrystallized from a suitable solvent. For II, $^1$H-NMR ($CDCl_3$): $\delta$ 6.8 (s, 1, OH), 8.1-7.03 (m, 3, ArH), 4.2 (s, 1, NH), 3.8 (m, 6, 2 $OCH_3$), and 1.5 ppm (s, 3, $CH_3$). For V [6,7-dimethoxy-3-(piperidinocarbonyl)-2-hydroxyquinoline], MS: *m/z* 316 ($M^+$), 232 ($M - C_5H_{10}N$), 204 [$M - (C_5H_{10}N + CO)$], 189 [$M - (C_5H_{10}N + CO + CH_3)$], and 173 [$M - (C_5H_{10}N + CO + OCH_3)$].

**Biological Screening**—Three compounds (III, V, and VII) of this series of alkoxyquinolines were used for preliminary pharmacological screening as CNS stimulants. Each was suspended in distilled water and administered to albino mice by oral intubation and intraperitoneal injection in doses up to 1 g/kg of body weight. The behavior and normal characteristics of the animals (heart rate, locomotor activity, reflexes, *etc.*) were recorded before and for 24 h after administration of the compounds.

## RESULTS AND DISCUSSION

The animals given III showed transient increases in respiratory rate (from 198 to 300/min) 5 min after administration and returned to normal values 7 min later. Otherwise, there were no signs of CNS stimulation of the animals at the doses studied. There was no change in animal behavior, activity, and locomotion. None of the animals showed any signs of toxicity or mortality within 24 h after administration of the different compounds in the doses given.

From the biological screening, it is obvious that the tested compounds, although they are structurally related to the well-known carboxamide and *N,N*-alkylated derivatives of heterocyclic types of compounds, do not have a marked MAO inhibitory action. This leads to the conclusion that these bioisostere moieties of the fused heterocyclic systems structurally related to the quinoline series are biologically inactive as CNS stimulants.

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[1] $^1$H-NMR and MS spectra were determined on Varian spectrometers.

# Determination of Phenylbutazone in Tablets by Nuclear Magnetic Resonance Spectrometry


**RONA STROMBERG**



Received April 18, 1983, from the *Food and Drug Administration, Department of Health and Human Services, New York Regional Laboratory, Brooklyn, NY 11232.* Accepted for publication October 6, 1983.



**Abstract** □ A method for the quantitative analysis of phenylbutazone in tablets using NMR is reported. The method is both accurate and precise. Using synthetic mixtures, the mean recovery value $\pm SD$ was 100.5 ± 0.86%. The NMR results of commercial preparations are compared with those obtained by the USP XX procedure. The NMR spectrum, in addition, provides a very specific means of identification for phenylbutazone.



**Keyphrases** □ Phenylbutazone—NMR analysis, comparative analyses □ NMR—phenylbutazone, comparative analyses


Phenylbutazone, 4-butyl-1,2-diphenyl-3,5-pyrazolidinedione (I), a synthetic pyrazolone derivative chemically related to aminopyrine, has anti-inflammatory, antipyretic, analgesic, and mild uricosuric properties (1). Approaches to the quantitative determination of phenylbutazone in tablets have varied. Tomaskova (2) reported a GC method using flame-ionization detection. A quantitative IR spectroscopic method was described by Pawelcyzk and Marciniec (3). Differential spectrophotometry, relying on the differences in absorption between acidic and basic species of phenylbutazone, was employed by Bezakova *et al.* (4). Other assay techniques have included separation on an ion-exchange column followed by titration (5) and acid hydrolysis to form benzidine, which was subsequently oxidized, and determined colorimetrically (6). The method of USP XX for the assay of phenylbutazone in tablets entails an ether-based extraction with a UV spectrophotometric determination (7). All these approaches require either lengthy sample preparations and/or nonspecific determinations. In contrast, nuclear magnetic resonance spectrometry (NMR) offers the advantages of minimal sample preparation, simplicity and specificity for the active ingredient.

NMR studies on phenylbutazone have dealt with its carbon-13 spectrum (8), degradation products (9, 10), and the monitoring of its dissolution kinetics (11). However, this technique has not been applied to the quantitative determination of the drug in pharmaceuticals. This paper describes a method in which a carbon tetrachloride–nitromethane mixture is used as the solvent and hexamethylcyclotrisiloxane


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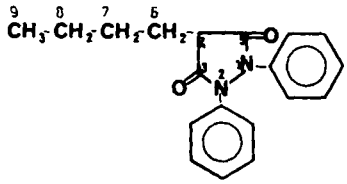
is the internal standard. Preparations containing known amounts of phenylbutazone and commercial tablets were analyzed.



## EXPERIMENTAL SECTION

**Materials**—An NMR spectrometer[1] (90 MHz) with an ambient probe temperature of 41°C was used. Samples were placed in precision-type, 5-mm, NMR tubes[2]. Phenylbutazone[3], hexamethylcyclotrisiloxane[4], tetramethylsilane[5], carbon tetrachloride[6], and nitromethane (>99% pure)[7] were used as obtained. Tablets were obtained from various commercial sources.

**Phenylbutazone Analysis**—Twenty tablets were weighed and finely powdered. An accurately weighed portion of this powder, containing ~150 mg of phenylbutazone, was transferred into a glass-stoppered centrifuge tube. Hexamethylcyclotrisiloxane (~64 mg) and 2 mL of carbon tetrachloride containing two drops of nitromethane/mL were added, and the mixture was shaken for 2 min. Insoluble excipients were allowed to rise and ~0.5 mL of the bottom layer was transferred to an analytical NMR tube. (For spectrometers which require a deuterium signal for field-frequency lock, approximately three drops of $Me_2SO$-$d_6$ should be added). The spin rate was adjusted so that no spinning side bands occurred between 0.4 and 0.6 ppm (hexamethylcyclotrisiloxane region), or between 6.9 and 7.5 ppm (phenyl region of phenylbutazone). The intensity of the spectrum was maximized with the RF power and amplitude setting; care was taken to avoid saturation, particularly in the spectrum of the sharp hexamethylcyclotrisiloxane signal. The delta ($\delta$) scale was used to express magnetic field strength and all peak field positions were referenced to hexamethylcyclotrisiloxane at 0.14 ppm. The resonance signals for phenylbutazone, which appear as a broad resonance peak centered at ~7.25 ppm, and the singlet for hexamethylcyclotrisiloxane, the internal standard, at 0.14 ppm were integrated a minimum of five times, and the results were averaged. The amount of phenylbutazone was calculated as follows: mg/tablet = $(A_p/A_h) \times (EW_p/EW_h) \times$ (mg hexamethylcyclotrisiloxane/mg sample) × mean tablet weight, where $A_p$ is the integral value of the signal representing phenylbutazone, $A_h$ is the integral value of the signal representing the internal standard, $EW_p$ is the equivalent weight of phenylbutazone (formula weight/10 protons = 30.84), and $EW_h$ is the equivalent weight of the internal standard (formula weight/18 protons = 12.35).

## RESULTS AND DISCUSSION

The absence of proton resonance signals for carbon tetrachloride makes it an ideal solvent for NMR spectroscopic work. Both phenylbutazone and hexamethylcyclotrisiloxane were soluble in carbon tetrachloride, but in the proposed method, the volume of this solvent was made slightly suboptimal with respect to phenylbutazone to obtain a relatively high concentration of drug and a low instrument noise level. The addition of a few drops of nitromethane, originally intended to serve as the internal standard, improved the solubility of phenylbutazone in the NMR solvent, and compensated for the lower sample volume. The resonance signal for nitromethane appeared at $\delta$ 4.10 ppm and was well separated from any other peak. Alternatively, the amount of sample specified in the assay method may be dissolved in 3 mL of carbon tetrachloride, but an adjustment of the attenuation setting is required. The utility of hexamethylcyclotrisiloxane as an internal standard has been demonstrated (12-14). Under the described experimental conditions, its resonance peak appears as a singlet at an extreme upfield position (~$\delta$ 0.14 ppm). This combination of solvent system and internal standard provides a specific interference-free means of identification and quantitation of the active ingredient.

The amount of phenylbutazone present in the dosage form is determined by integrating the broad resonance signal pattern centered at ~$\delta$ 7.25 ppm, arising from the 10 aromatic protons at positions 1 and 2, and the singlet at ~$\delta$ 0.14 ppm, due to the 18 methyl protons of the internal standard.

[1] Model EM-390; Varian Associates, Palo Alto, Calif.
[2] Kontes, Vineland, N.J.
[3] USP Reference Standard; U.S. Pharmacopeial Convention Inc., Rockville, Md.
[4] K & K Laboratories, Plainview, N.Y.
[5] Wilmad Glass Co., Buena, N.J.
[6] Fisher Scientific Co., Fair Lawn, N.J.
[7] Aldrich Chemical Co., Milwaukee, Wis.

**Table I—NMR Determination of Phenylbutazone in Standard Mixtures[a]**

| Standard Mixture | Internal Standard Added, mg | Phenylbutazone Added, mg | Phenylbutazone Found, mg | Phenylbutazone Recovery, % |
|---|---|---|---|---|
| 1 | 30.0 | 77.4 | 77.9 | 100.6 |
| 2 | 29.7 | 76.1 | 75.6 | 99.4 |
| 3 | 29.8 | 75.8 | 75.6 | 99.7 |
| 4 | 30.7 | 76.5 | 77.2 | 100.9 |
| 5 | 29.5 | 77.3 | 77.5 | 100.3 |
| 6 | 32.1 | 76.7 | 77.5 | 101.0 |
| 7 | 30.4 | 37.9 | 38.7 | 102.0 |
| 8 | 31.8 | 113.7 | 113.4 | 99.7 |
| Mean ± *SD* | | | | 100.5 ± 0.9 |

[a] Half the amount of phenylbutazone specified in the method, dissolved in a similarly decreased volume of solvent, was used in order to conserve sample.

**Table II—NMR and Compendial Determinations of Phenylbutazone in Commercial Tablets**

| Sample | Amount Declared, mg/Tablet | Amount Found, %[a] NMR | Amount Found, %[a] USP XX | Difference, % |
|---|---|---|---|---|
| 1 | 100 | 97.9 | 96.7 | 1.2 |
| 2 | 100 | 100.5 | 97.3 | 3.2 |
| 3 | 100 | 103.0 | 99.7 | 3.3 |
| 4 | 100 | 98.7 | 97.6 | 1.1 |
| 5 | 100 | 102.7 | 100.4 | 2.3 |

[a] Since each tablet is declared to contain 100 mg of drug, the quantity found in mg/tablet is identical to the percent of declared values.

In addition to their utility in the quantitative analysis of phenylbutazone in dosage forms, the proton resonance characteristics of this molecule may be used for identification. The coupling of the methine proton at position 4 with the methylene protons at position 6 produces a triplet centered at ~$\delta$ 3.18 ppm. The complex splitting patterns seen in the region between $\delta$ 0.7 and 2.2 ppm may be assigned consecutively to the methyl protons at position 9 and to the methylene protons on carbons 6-8. The resonance signal pattern centered at ~$\delta$ 1.4 ppm arises from the overlapping of the peaks for the protons of the two methylene groups at positions 7 and 8.

The analysis of several known phenylbutazone mixtures is summarized in Table I. The results show that the method is both accurate and precise. The mean recovery value ±*SD* was 100.5 ± 0.9%. The relative proportions of phenylbutazone and hexamethylcyclotrisiloxane, as noted in Table I, had no significant bearing on the accuracy of the determination for the range of proportions shown.

The proposed method was also applied to commercial preparations. Table II summarizes the results of the analysis of five lots of tablets. For comparison purposes, the same composites were analyzed by the USP XX method. The data in Table II show that the results by the official method were 1.1-3.3% lower than those obtained by the NMR method. One of the possible reasons for these discrepancies may be the losses incurred with the official method during the extraction steps. Recovery studies from two commercial preparations, spiked with a known amount of phenylbutazone, gave a mean recovery value of 100.1%.

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# Influence of Food on the Bioavailability of Enalapril

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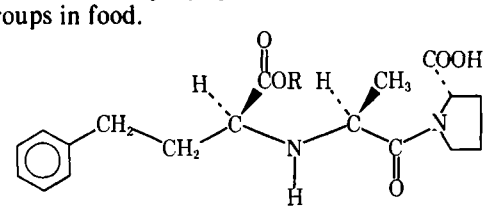
Received August 25, 1983, from the **Division of Clinical Pharmacology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107* and ‡*Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.* Accepted for publication December 5, 1983.

**Abstract** □ In a randomized, two-period crossover study in 12 normal volunteers, serum and urine concentrations of the angiotensin-converting enzyme inhibitor enalapril and its active metabolite enalaprilat were determined following administration of a single 40-mg tablet of enalapril maleate administered both in the fasting state and with a standard breakfast. A 7-d interval separated the two treatment periods. Area under the serum concentration-time curves for enalaprilat and urinary recoveries for enalaprilat and total drug did not differ significantly between the fed and fasted conditions. The mean observed maximum serum concentration of enalaprilat was slightly higher for the fasting treatment, but the time to peak concentration was almost identical for the two treatments. Enalapril maleate is unlike the prototype angiotensin-converting enzyme inhibitor captopril in that a standard meal does not appear to influence absorption of this new drug.

**Keyphrases** □ Enalapril maleate—bioavailability, influence of food □ Enalaprilat—metabolite of enalapril, bioavailability, influence of food □ Angiotensin-converting enzyme—enalapril maleate, bioavailability, influence of food

Food is known to alter the bioavailability of many drugs (1). The presence of food in the GI tract has been shown to reduce, by 30–40%, the absorption of the recently marketed oral angiotensin-converting enzyme (ACE) inhibitor captopril (2). Compromised absorption of captopril could affect the magnitude or duration of the antihypertensive effect of this drug (3). Enalapril maleate is a new ACE inhibitor which, like captopril, has been shown to be effective in the treatment of hypertension and congestive heart failure (4). Enalapril ((*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline; I) is a prodrug which is deesterified to an active diacid form, enalaprilat ((*S*)-1-[*N*-(1-carboxy-3-phenylpropyl)-L-alanyl]-L-proline; II) (4). Since enalapril lacks the sulfhydryl moiety of the chemical structure of captopril, it may have a more favorable benefit-to-risk ratio. Oral absorption of enalapril may also be more complete than captopril since the sulfhydryl group of the latter binds to other thiol groups in food.



I, R = $CH_2CH_3$
II, R = H

The purpose of this study was to investigate whether consumption of food alters the rate or extent of absorption of enalapril when a single dose is administered to healthy male volunteers.

## EXPERIMENTAL SECTION

**Subjects**—Twelve healthy, normotensive male subjects (age, 23–37 years) volunteered to participate in this study. They were judged to be in good health on the basis of history, physical examination, routine laboratory data, standard electrocardiogram, and diastolic blood pressure (≤90 mm Hg). All subjects weighed within ±10% of the ideal body weights for their ages and heights. No medications other than enalapril maleate were taken by the subjects from 1 week prior to the study until its completion. The protocol was approved by the Committee on Research of Thomas Jefferson University.

**Study Design**—This was an open, randomized, two-way crossover study in which enalapril maleate (40 mg) was administered as a single dose in its market image. Subjects fasted from midnight of the previous night. On the first day of each treatment period, subjects received doses of enalapril maleate either in a fasting state or immediately after a standard prescribed breakfast by a randomized design. The breakfast consisted of one egg, two pieces of toast or bread, two strips of bacon or two sausages, 150 mL of low fat milk or 100 mL of orange juice, tea, or coffee. All volunteers resumed their normal diets each day at lunch (4 h after dosing). Treatments were separated by 7 d.

Blood was drawn at 0, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 60, and 72 h postdrug administration. Urine was collected at −1–0, 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96, and 96–120 h after each treatment. Blood pressure and pulse rate were measured in the supine and sitting positions at regular intervals for 24 h postadministration. Adverse effects were monitored throughout the study.

**Biochemical Analysis and Statistical Methods**—The assay value for the enalapril maleate tablet was 42.02 mg, equivalent to 29.88 mg of enalaprilat per tablet. A radioimmunoassay procedure (5) was used to analyze the serum and urine samples for enalaprilat and for total drug (enalaprilat after enzymatic hydrolysis of enalapril). After mixing and incubation overnight at room temperature, the hydrolysis of enalapril to enalaprilat was virtually complete. The assay was considered to be accurate and reproducible at enalaprilat concentrations >1 ng/mL in serum and 0.5 μg/mL in urine; the interassay coefficient of variation was 8%. Concentrations below these values were not considered detectable and, hence, zero for the purpose of data analysis.

All parameters were analyzed by using an analysis of variance for a two-period crossover design (6). These results were corroborated by a nonparametric method based on ranks of the data (7). The posterior probabilities were calculated by the method of Rodda and Davis (8). The power analysis was based on the *t* test.

## RESULTS

The mean serum profiles for enalaprilat after administration of enalapril maleate in the fasting and fed states are depicted in Fig. 1. Profiles for the two treatments are virtually superimposable. Mean urinary excretion rate plots

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