Stability Studies of Phenylbutazone and Phenylbutazone–Antacid Oral Formulations

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Abstract □ Nineteen phenylbutazone and eight phenylbutazone–antacid solid oral formulations were stored in sealed bottles at room temperature and 37, 50, and 60° with ambient relative humidity and at 37° with 75% relative humidity. Samples were examined for intact drug content, decomposition profiles, and dissolution rates at selected time intervals up to 296 days. None of the phenylbutazone formulations showed any evidence of chemical instability when stored at ambient temperature, 37°, and 37° with 75% relative humidity. Measurable chemical degradation occurred only at 60°, with several formulations showing more than 50% degradation by the time the study was terminated. In some formulations, the extent of degradation varied greatly among tablets within the same bottle and between bottles of the same lot. Shelflife could not be predicted from the data. The results indicate that the temperatures used in accelerated studies should not exceed 50°. Dissolution rates tended to decrease with time at 60° and, to some extent, at 37° with 75% relative humidity and 50°. Chemical degradation occurred at 37° in some phenylbutazone–antacid formulations and was general at 50 and 60°. At 60°, the extent of degradation approached a maximum during the initial time periods, suggesting that a reactant involved in the degradation had been consumed. There were no significant changes in the dissolution time of antacid formulations.

Keyphrases □ Phenylbutazone—various solid oral formulations, stability, effect of temperature and humidity □ Stability—phenylbutazone, various solid oral formulations, effect of temperature and humidity □ Degradation—phenylbutazone, various solid oral formulations, effect of temperature and humidity □ Antirheumatic agents—phenylbutazone, various solid oral formulations, stability, effect of temperature and humidity

Phenylbutazone degradation was reported to occur in oxygen-free basic solutions (1), in oxygenated solutions (2), in acidic permanganate solutions (3), and in basic peroxide solutions (3). Degradation pathways in other solvents such as *N,N*-dimethylformamide, *N,N*-dimethylacetamide, diethyl carbonate, and propylene glycol–water were described (4). Injectable formulations of phenylbutazone degrade by hydrolysis and, to a lesser extent, by oxidation, yielding such products as 4-hydroxyphenylbutazone (I), α-carboxy-*N*-caproylhydrazobenzene (II), *cis*- and *trans*-azobenzene, and *n*-butylmalonic acid (5, 6). In suppository formulations, *N*-caproylhydrazobenzene (III), α-carboxy-α-hydroxy-*N*-caproylhydrazobenzene (IV), I, and II were identified after degradation by a process described as mainly oxidative (5, 7).

A study of tablet formulations of phenylbutazone stored at room temperature revealed some degradation in products formulated with antacids, but otherwise degradation was slight or not apparent (8). Mechanisms describing the degradation of injectable and suppository formulations (5) and accounting for the appearance of *N*-(α-ketocaproyl)-hydrazobenzene (V), II, and IV in tablets formulated with antacids (9) were proposed. Data to elucidate further the degradation mechanism were obtained by a study of the decomposition at elevated temperatures (10). Phenylbutazone tablets also are subject to physical instability, made manifest by a decrease in dissolution rate, probably due to polymorphism of phenylbutazone (11) or to a change in the properties of the gelatin and/or acacia subcoats of sugar-coated tablets (12).

This paper describes a study of the accelerated and ambient temperature aging of phenylbutazone and phenylbutazone–antacid solid oral formulations. The purpose of this work was to gain insight into the comparative behavior of formulations from different manufacturers.

EXPERIMENTAL

Materials—All formulations were obtained directly from the manufacturers. The following compounds were used as received: phenylbutazone[1], α-carboxy-*N*-caproylhydrazobenzene[2], *N*-caproylhydrazobenzene[2], *N*-(α-ketocaproyl)hydrazobenzene[2], α-hydroxy-*N*-caproylhydrazobenzene[2], and ethyl acetate[3] (glass distilled).

Diphenyl phthalate[4] was recrystallized from acetone–water. α-Carboxy-α-hydroxy-*N*-caproylhydrazobenzene was prepared by controlled alkaline hydrogen peroxide oxidation (10), and 4-hydroxyphenylbutazone was prepared by heating α-carboxy-α-hydroxy-*N*-caproylhydrazobenzene to a maximum temperature of 165° (10). Silica gel 60 F-254-precoated TLC plates[5] were used.

Protocol—Nineteen phenylbutazone and eight phenylbutazone–antacid formulations were stored[6] at ambient temperature (21–23° range), 37°, 37° with 75% relative humidity (13), 50°, and 60°. Time-zero analyses were carried out when the study began only on samples from bottles to be stored at ambient conditions. All original and rebottled samples stored under accelerated conditions were not opened until time-one analysis. The bottles were reclosed tightly by hand between sampling.

For all storage conditions except 60°, the entire study was performed on a single bottle of each formulation. For the 60° study, analysis at times one to five inclusive were done on one bottle, while a second bottle was used to duplicate the time-five study and to obtain time-six data.

Quantitation of undegraded phenylbutazone and estimation of I and the total of other degradates were accomplished by GLC. Compounds II and IV in the antacid formulations were estimated by TLC. GLC and TLC were employed to follow the degradation profile. Quantitative UV spectroscopy also was used to follow the degradation profile and to corroborate the GLC data, which indicated a major decrease in phenylbutazone content.

All chemical tests were carried out on composites of five tablets or the contents of five capsules. GLC analyses were performed on duplicate weighings. Dissolution rates were measured at selected time intervals.

GLC—The technique of Watson *et al.* (14), modified slightly, was utilized to determine undegraded phenylbutazone[7]. Accurately weighed sample aliquots equivalent to 100, 50, or 10 mg of phenylbutazone were treated with 2 ml of 10% hydrochloric acid (v/v) and 10.0 ml of 0.2% (w/v) diphenyl phthalate (internal standard) in ethyl acetate. The mixture was tumbled end-over-end at 100 rpm for 15 min and centrifuged, and 1.0 μl of the supernate was chromatographed on 5% OV-7 on Gas Chrom Q under isothermal conditions at 230°. All other conditions were as previously described (14).

[1] Mount Royal Chemicals, Montreal, Quebec, Canada.
[2] Geigy Pharmaceuticals, Basel, Switzerland.
[3] Caledon Laboratories, Georgetown, Ontario, Canada.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] E. Merck, Darmstadt, Germany.
[6] Formulations A, B, C, E, F, G, H, J, K, M, N, O, AA, BB, CC, DD, FF, and GG were stored in their original containers. Formulations D, I, L, P, Q, R, S, EE, and HH were rebottled in amber prescription bottles (Wheaton Glass Co., Millville, N.J.) prior to storage.
[7] The coefficient of variation was 1.06%.

Table I—Chromatographic Characteristics of Phenylbutazone and Related Degradation Products

Compound	Relative[a] Retention Times	Relative[b] R_f	Color with Detection Agent A[c]	Color with Detection Agents A and B[d]
I	0.86	0.63[e]	No color	Yellow orange
II	0.48, 0.38, 0.11, 0.09, 0.58	0.53	Purple	Color intensified
III	0.48, 0.38, 0.58	0.82	Red purple	Color intensified
IV	0.09, 0.52, 0.48, 0.15	0.39	Purple	Color intensified
V	0.52, 0.38, 0.09	0.78	Red purple	Color intensified
VI	0.09, 0.52	0.69	Red purple	Color intensified
Hydrazobenzene	0.09, 0.15, 0.21	1.02	Yellow	Yellow orange
Azobenzene	0.09, 0.15	1.30, 1.15	Yellow	Yellow orange
Phenylbutazone	0.68		No color	Yellow orange

[a] Relative to the diphenyl phthalate internal standard in order of decreasing peak area. [b] Relative to phenylbutazone. [c] 0.5% potassium dichromate in 20% (v/v) H_2SO_4. [d] Concentrated sulfuric acid. [e] Merck GF 60 precoated.

All time-zero analyses were performed on sample aliquots containing 100 mg of drug. However, as degradation proceeded, the sample aliquots were reduced to contain 50 or 10 mg of the drug. This reduction was done to ensure that extraction of the intact drug was complete and that the quantities of I, II, and IV fell within the range for which the response factors were established and to avoid possible overestimation of phenylbutazone and I due to on-column cyclization of II and IV. Cyclization occurred when the amount of II and IV injected exceeded 1.0 μg. (The presence of 1.0 μg of II or IV would represent 100 and 20% degradation with respect to 1 and 5 μg of phenylbutazone.) When the amount of acid degradates approached 20% of the original drug content, the smallest sample equivalent was used.

Under the conditions of the GLC assay, phenylbutazone and I are stable but all other degradates exhibit on-column breakdown resulting in multiple peaks. Retention times of these peaks relative to diphenyl phthalate are given in Table I. Thus, in addition to the drug content, the GLC chromatograms provide an overall profile of the extent of decomposition with time. Reliable quantitation of the degradates by GLC was possible only when the decomposition pathway was simple and led just to the formation of II and/or IV, determined as total acid-type degradates in the phenylbutazone–antacid formulations, or in the formation of these acid derivatives and/or I in phenylbutazone formulations. As degradation became more complex, particularly with certain phenylbutazone formulations, quantitation of the degradates was less accurate.

TLC—A previously developed method (8) was used to estimate II and IV in antacid formulations and to obtain a qualitative profile of the degradation process for phenylbutazone tablets. This test was carried out on tablet or capsule extracts prepared for the GLC assay. The thin-layer chromatogram is qualitatively more informative than the gas chromatograms since comparison of the R_f characteristics and the color reactions of the extraneous spots with those of the degradates (Table I) permits identification of the individual degradates, particularly when degradation leads to the formation of numerous compounds.

UV Spectrophotometry—Tablet grinds or capsule contents were treated with 0.1 *N* NaOH and centrifuged; the absorbance ratio, 264/234 nm, of the supernate was determined.

Dissolution—A reciprocating apparatus similar to the USP disintegration apparatus was used, except that the official basket was replaced with a single-chamber basket with an 8.5-cm diameter bottom plate (15). The solvent was 900 ml of pH 7.2 phosphate buffer (16). The quantity of drug dissolved was measured by UV spectroscopy at 264 nm. Disintegration times were estimated visually as the time at which the capsule or tablet particles passed through the wire mesh covering the bottom of the chamber.

RESULTS AND DISCUSSION

Phenylbutazone Tablets—None of the phenylbutazone formulations studied showed any evidence of chemical instability when stored at ambient temperature, 37°, and 37° with 75% relative humidity. Formulations I and K showed a 10% loss after 269 days at 50°. The formulations were chemically labile at 60°, as shown by the continuing decrease in the intact drug content and the UV absorbancy ratio coupled with the progressive increase in the area of the extraneous peaks in the gas chromatograms or in the number of spots in the thin-layer chromatograms.

Table II—Degradation (Percent) of Phenylbutazone Tablets at 60°

Formulation	Bottle 1[a] 18 days	36 days	50 days	111 days	203 days	Bottle 2[b] 203 days	278 days
A	14	12	16	22	23	62	59
B			13	18	34	23	27
C			4	9	9	9	10
D			0	5	1	5	6
E	6		61	78	81	65	70
F	4	18	18	31	38	3	1
G	4	5	9	51	53	51	83
H			26	35	52	33	32
I			0	18	35	31	40
J	0	2	0	15	14	23	24
K			15	38	43	44	48
L			5	1	0	0	1
M			0	34	50	52	59
N	0	0	4	17	23	21	25
O			0	57	47	6	20
P			3	3	0	1	0
Q			4	5	8	2	6
R			3	2	1	2	0
S	0	3	5	25	31	17	9

[a] Bottle 1 was opened on the 1st day of sampling. [b] Bottle 2 was opened at Day 203.

The extent of degradation at 60° in 19 tablet formulations measured by GLC at selected intervals over 278 days is given in Table II. The extent of degradation among the formulations ranged from no degradation for Formulations D, L, P, and R to 83% loss in potency for Formulation G. Variations in the degree of degradation were present, in some cases, between bottles of the same formulation and within a single bottle. For example, Formulation F, after 203 days, exhibited 38 and 3% losses for Bottles 1 and 2, respectively, while Formulation H exhibited 52 and 5% losses for tablets from the same bottle after 203 days.

The variation in results between Bottles 1 and 2 (Formulation F) may be due to the fact that Bottle 1 was opened at Day 18 and reopened for subsequent sampling while Bottle 2 was not opened before Day 203. However, opened and unopened experimental conditions apparently did not affect Formulations C, G, I, K, M, and N. Greater stability was manifested by the unopened bottle for Formulations B, E, F, H, O, and S, but data for A and J indicate the reverse condition.

The different courses of the degradation reaction over the study period are illustrated by the chromatograms of Formulations K (Fig. 1) and E (Fig. 2). The degradative pathway of Formulation K became increasingly complex with time; that of Formulation E remained relatively simple throughout.

Phenylbutazone decomposition products present in the formulations after 203 days were identified by TLC (Table III). Compound I and the total of all other degradates were estimated by GLC (Table III). The latter estimates may be in error by 10% or more because the individual components exhibited different response factors and the composition of the mixture of degradates was variable. No quantitation was attempted with TLC. However, the most concentrated spots were noted, and the remaining spots were classified as intermediate or weak. Usually, the nature and relative amounts of the decomposition products in a specific formulation did not change during the study, although they varied considerably among formulations.

Examination of the TLC data (Table III) for 203 days of storage reveals that I was the main degradation product in 13 of the 19 formulations, indicating oxidation to be a prominent reaction pathway with most formulations. In Formulations C, E, and M, oxidation appeared to be the only significant degradative route since only the TLC spot and GLC peak corresponding to I were present to any appreciable extent. However, the plethora of decomposition products in Formulations B, G, K, N, and O suggests a complex degradation mechanism involving hydrolysis, oxidation, and decarboxylation. Apparently, the degradation mechanism and rate depend on the properties and composition of the individual formulations and not solely on the drug.

The nature of the decomposition products and the extent of degradation varied among individual tablets in Formulations G, I, K, M, and O. This result became apparent when tablet cores of two or more colors

Table III—TLC Profiles of Phenylbutazone Formulations after 203 Days at 60° [a]

Formulation	I GLC, %	I TLC	II	III	IV	V	VI	Azobenzene	Total Decomposition by GLC[b], %
A	20	S	W	W			W	W	
B[c]	9	S		W		W	W		7
C	10	S							
D			No degradation detected						
E	88	S			W		W		
F[c]	41	S		I			W		4
G	17	S	W		W	—[e]	W	W	19
H[c]	32	S	W	W	I	I			14
I[d]	20	S		W					3
J[d]	11	S		W					4
K[c]	15	I	W	S	W	W		W	40
K[d]	0		W	S	W	W			45
L			No degradation detected						
M[d]	43	S					W		
N	22	S	W	W					5
O	16	S				W	W	W	5
P			No degradation detected						
Q	0			W					5
R			No degradation detected						
S[c]	38	S		I					15

[a] Strong spots are indicated by S, intermediate spots by I, and weak spots by W. [b] Excluding I. [c] Brown cores. [d] Beige cores. [e] Identity of this extraneous spot has not been established.

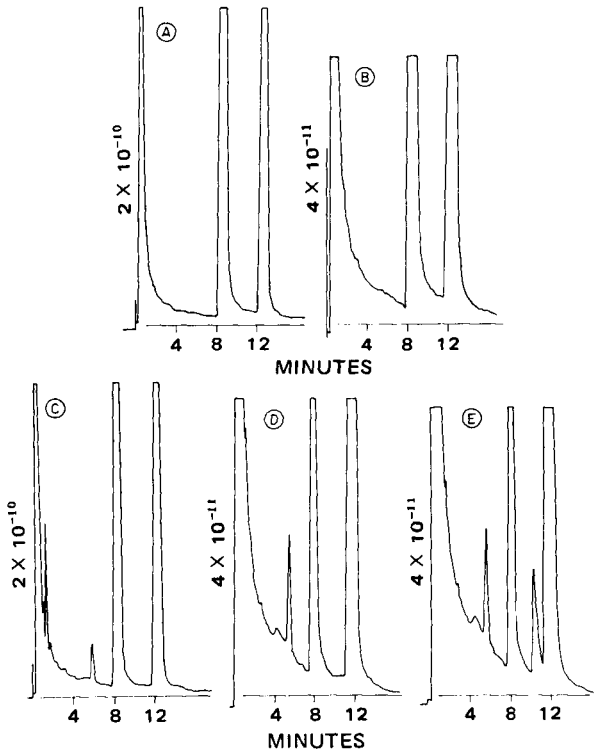

Figure 1—*Chromatograms of Formulation K. Key: A, ambient temperature, T_0 days, 10-μg injection; B, ambient temperature, T_{268} days, 5-μg injection; C, 60°, T_{50} days, 5-μg injection; D, 60°, T_{203} days, 1-μg injection (beige tablets); and E, 60°, T_{203} days, 1-μg injection (brown tablets). Retention times were: phenylbutazone, 8 min; diphenyl phthalate (internal standard), 12 min; and 4-hydroxyphenylbutazone, 10 min.*

were observed in the same bottle. Colors, not necessarily within a given bottle, ranged from yellow to beige to dark brown and black, the depth of color being indicative of the degree of degradation. Formulation K, which was typical, consisted of beige and the dark-brown tablets, both of which contained comparable amounts of several degradation products, except that the latter contained a much larger amount of I (Fig. 1). The tablet-to-tablet variability may reflect differences in the thickness of the tablet coating or in the permeability of the tablet cores, but there is no evidence to substantiate this hypothesis.

As decomposition proceeded, the absorbance at 264 nm decreased and the λ_{max} gradually shifted toward 234 nm (1, 6), resulting in a decrease in the absorbancy ratio A_{264}/A_{234}. Although there was no direct quantitative relationship between the loss in drug content as measured by GLC and the decrease in the absorbancy ratio, a gross increase in degradation was accompanied by a substantial decrease in the absorbancy ratio. For example, Formulation M yielded the following ratios: Bottle 1, 1.81 (0 day), 1.80 (50 days), and 0.91 (203 days); and Bottle 2, 0.90 (203 days) and 0.80 (278 days).

It was intended to calculate shelflives for the formulations, but this task proved impracticable since only formulations stored at 60° exhibited quantifiable degradation, although some degradation of Formulations I and K occurred at 50°. The large difference in degradation rates at 50 and 60° may indicate a threshold temperature at which the nature of the degradation mechanism changes. If so, accelerated aging studies of phenylbutazone tablets aimed at predicting shelflife are unreliable if they encompass rate measurements at temperatures over the threshold temperature.

The identification of the main degradation products of Formulations K and M, taken as representative of all formulations, was confirmed by isolation of the degradates from a TLC plate and comparison of the mass spectra, melting points, and GLC retention times with those of standard

Table IV—Dissolution (Percent) of Phenylbutazone Tablets at 60 min [a]

	60°								50°				37°, 75% Relative Humidity			
Formulation[b]	0 day	18 day	36 days	50 days	111 days	203 days, Bottle 1	203 days, Bottle 2	278 days	63 days	128 days	196 days	269 days	34 days	82 days	159 days	219 days
A (C)	95				100	71	21	20		82	79	82				
B (SC)	84	4		2	4	0	0	0	28	0	4	0			74	46
C (FC)	90				100	79	82	82								
D (FC)	98				100	90	83	84								
E (FC)	99			26	53	56										
F (FC)	0						No change detected									
G (SC)	95			93	100	0	0	0								
H (EC)	81			2	3		1	1	21	3		0	68	40		23
I (EC)	0						No change detected									
J (SC)	0						No change detected									
K (SC)	88			4	3		0	0	6	3	0	0	88	75	43	44
L (SC)	80			67	62	66	50	51	58	30	25	0		84	75	58
M (SC)	90			71	80	48	24	26								
N (SC)	93	78	65	16	16	0	0	0	84	47	39	29	92	86	98	
O (SC)	85	71		66	56	13	52	73								
P (SC)	98						No change detected									
Q (SC)	62			74	23	27	28	40	51	57	61	56	65	63	58	48
R (SC)							No change detected									
S (SC)	96	9		4	0	0	0	0	7	6	9	0	0	95	89	35

[a] At time 0, percent of label claim; at other times, percent of undegraded phenylbutazone. Mean of six tablets. [b] C = compressed, SC = sugar coated, FC = film coated, and EC = enteric coated.

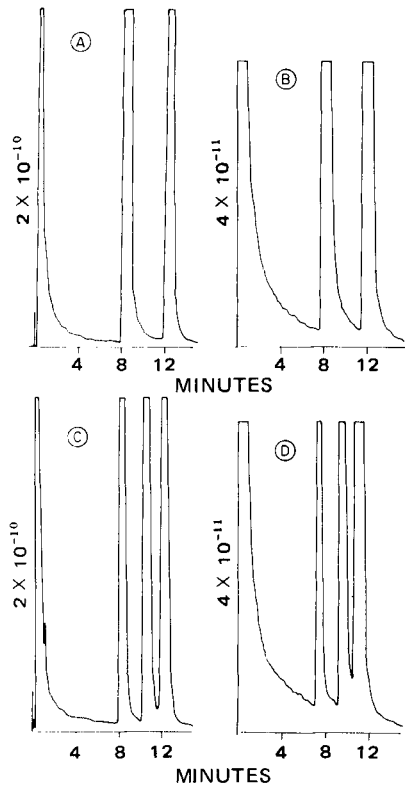

Figure 2—*Chromatograms of Formulation E. Key: A, ambient temperature, T_0 days, 10-μg injection; B, ambient temperature, T_{268} days, 5-μg injection; C, 60°, T_{50} days, 5-μg injection; and D, 60°, T_{203} days, 1-μg injection. Retention times were the same as for Fig. 1.*

compounds. The compounds identified were I (Formulation M) and III (Formulation K). Additional studies of Formulation G indicated the presence of one or more compounds not reported in previous phenylbutazone studies. One of these exhibited an R_f value similar to that of V, but the melting point was not the same. Work to identify this compound is in progress.

The amount of drug dissolved after 60 min was measured, and the results are expressed as a percentage of the amount of undegraded phenylbutazone present at the time of the measurement. Over 270 days, the amount dissolved did not change in any sample stored at room temperature. At 37°, Formulations K and Q became progressively less soluble, the amounts dissolved decreasing from 88 and 62% at zero time to 73 and 50% at 283 days, respectively.

Dissolution results for those formulations exhibiting a significant change with time at 37° with 75% relative humidity, 50°, and 60° are given in Table IV. Increases in the dissolution time of phenylbutazone tablets have been attributed to changes in the properties of the tablet coatings (12). All tablets included in this study were sugar, film, or enteric coated, but there appeared to be no relationship between the type of coating and the dissolution behavior.

Phenylbutazone–Antacid Formulations—Eight phenylbutazone–antacid formulations were subjected to accelerated aging for up to 190 days and temperatures up to 60°. Losses in drug content in samples stored at room temperature were not measurable. Degradation commenced in some formulations at 37° and 37° with 75% relative humidity and was evident in all formulations at 50 and 60° (Table V). As with the phenylbutazone tablets, the degradation rate varied among the formulations, but none of the formulations was completely stable at 60°. Gross differences among the individual dosage units in a given bottle were not evident, as was the case with the nonbuffered phenylbutazone tablets.

Under all storage conditions except ambient temperature, there was evidence of degradation during the first 60 days. The decomposition products formed during this period were II and IV, indicating that the main route of degradation was alkaline hydrolysis (1, 8). In samples stored at 37° and 37° with 75% relative humidity, the degradation appeared to cease after the initial 60 days. This result also occurred with samples stored at 50° except for Formulations CC and FF. Except for HH, all formulations at 60° continued to degrade over the entire study. At this temperature, after the initial 60 days, complex mixtures of degradates including II–VI and an unidentified compound were observed. These observations suggest that, after the initial period of hydrolysis, the stability characteristics of phenylbutazone–antacid formulations are similar to those of the phenylbutazone formulations. Cessation of hydrolysis may reflect exhaustion of available water in the formulations under examination.

The dissolution times of the phenylbutazone–antacid preparations did not change significantly under all time and temperature conditions of this study. Some tablet mottling was observed at 50 and 60°. At these temperatures, the capsule shells of Formulation HH softened and fused together so that the dissolution rate could not be measured.

Table V—Percent Losses in Drug Content of Phenylbutazone–Antacid Formulations with Time and Temperature [a]

	37°		37°, 75% Relative Humidity		50°		60°	
Formulation	*t*, days	Loss, %	*t*, days	Loss, %	*t*, days	Loss, %	*t*, days	Loss, %
AA, tablets	23	0	20	1	26	1	17	3
	41	1	41	0	43	3	43	5
	56	0	55	0	55	3	57	5
	89	1	85	0	89	4	87	5
	190	1	177	1	180	4	148	9
BB, capsules	22	4	20	2	26	8	16	6
	41	3	41	1	43	6	43	14
	56	3	55	2	55	9	57	16
	89	5	85	4	89	9	87	14
	190	7	177	4	180	10	148	20
CC, capsules	22	4	21	1	26	11	15	17
	41	9	41	2	43	12	43	28
	56	7	55	3	55	18	58	32
	89	6	85	5	89	21	88	36
	190	8	177	12	180	21	148	41
DD, capsules	21	0	21	0	26	3	15	4
	41	0	40	0	42	3	43	7
	56	0	54	0	55	4	57	6
	89	0	84	0	89	4	87	9
	190	0	177	1	180	6	148	16
EE, tablets	23		20		26	3	17	2
	42	2	41	1	43	3	43	7
	57	0	55	1	55	5	57	13
	90	4	85		89	4	87	9
	190	3	177		180	5	148	22
FF, tablets	22	4	20	2	26	10	17	9
	41	1	41	2	43	15	41	9
	56	3	55	2	55	8	55	20
	89	4	85	4	89	8	85	24
	190	5	177	4	180	20	148	33
GG, tablets	22	2	21	0	27	3	18	5
	41	4	41	2	43	6	41	16
	56	2	55	1	55	12	55	27
	89	0	85	1	89	12	85	25
	190	2	177	1	180	9	148	34
HH, capsules	21	4	20	3	10	0	10	7
	41	3	42	0	23	3	23	13
	56	6	55	6	37	6	43	8
	89	4	85	3	71	3	73	6
	190	4	177	6				

[a] Samples stored at 37° with 75% relative humidity presented difficulties in sampling.

CONCLUSIONS

The rate, extent, and mechanism of degradation in phenylbutazone tablets at 60° depend upon the tablet excipients and, possibly, the manufacturing process. The unformulated drug is stable at 60°. The degradation rate at 50° in tablets is much slower and may proceed by a different mechanism than that at 60°. Initially, at all temperatures except ambient, the mechanism by which the antacid formulations degrade appears to be mainly alkaline hydrolysis. After this initial period, the degradation process appears to change between 50 and 60°, becoming similar to that observed in phenylbutazone formulations. These observations suggest that accelerated studies aimed at predicting shelflife should not include temperatures much over 50°. Accelerated temperature testing is valid only if the degradation occurs by the same mechanism at all temperatures.

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Simultaneous Determination of Reserpine and Hydrochlorothiazide in Two-Component Tablet Formulations by High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic procedure is presented for the simultaneous determination of reserpine and hydrochlorothiazide in two-component tablet formulations. An aliquot of a tetrahydrofuran extract of the tablet, containing polythiazide as an internal standard, is chromatographed on a microparticulate silica gel column using a mobile phase of 0.01% (v/v) diethylamine, 5% (v/v) chloroform, and 18% (v/v) 2-propanol in *n*-hexane. The relative standard deviations are 1.2 and 0.6% for the simultaneous determination of reserpine and hydrochlorothiazide, respectively. Seven commercial tablet formulations were found to contain 92.7–101.0% and 98.3–101.4% of the labeled amounts of reserpine and hydrochlorothiazide, respectively.

Keyphrases □ Reserpine—high-performance liquid chromatographic analysis, simultaneously with hydrochlorothiazide, in tablets □ Hydrochlorothiazide—high-performance liquid chromatographic analysis, simultaneously with reserpine, in tablets □ High-performance liquid chromatography—simultaneous analyses, hydrochlorothiazide and reserpine in tablets □ Diuretics—hydrochlorothiazide, high-performance liquid chromatographic analysis, simultaneously with reserpine, in tablets □ Antihypertensives—reserpine, high-performance liquid chromatographic analysis, simultaneously with hydrochlorothiazide, in tablets

Reserpine–hydrochlorothiazide formulations are marketed in Canada by several manufacturers. A rapid, accurate procedure, applicable to all formulations, was required for the simultaneous determination of both drugs in these preparations. Literature methods are based on many different techniques for the analysis of the individual compounds (1–5), but only an automated procedure (6) simultaneously determines both compounds. This automated procedure is useful for content uniformity purposes but is cumbersome for occasional use and is not specific for the determination of reserpine or hydrochlorothiazide since known impurities may interfere.

High-performance liquid chromatography (HPLC) has been used extensively for the analysis of mixtures (7–9) because of high specificity, speed, and sensitivity. The technique has been applied to the analysis of alkaloids (10–13) and thiazide diuretics (14–16), including the quantitative analysis of reserpine–chlorothiazide mixtures (16). This report describes an HPLC procedure for the simultaneous determination of reserpine and hydrochlorothiazide in two-component tablet formulations.

EXPERIMENTAL

Materials—Reserpine[1], hydrochlorothiazide[2], polythiazide[3], and 1-amino-3-chloro-4,6-benzenedisulfonamide[4] (I) were used as received. Both drug substances were essentially identical to the corresponding reference standards[5] when retention times, impurities, and response factors were compared by HPLC and when drug content was compared by the USP XIX assay procedure (5). Solvents and reagents were commercial analytical reagent grade, except for tetrahydrofuran[6] and *n*-hexane[6] which were UV grade. Tetrahydrofuran was stored under nitrogen.

Apparatus—A liquid chromatograph[7] fitted with a septumless injection port[7], a fixed-wavelength UV detector[8] (254 nm), and a computing integrator[9] was used. The detector was attenuated to 0.01 absorbance unit full scale (aufs).

Column—A 250 × 2.1-mm i.d. column[10] packed with 5-μm diameter silica gel[11], using a balanced density slurry technique similar to that described by Majórs (17), was used at ambient temperature and at a mobile phase flow rate of 90 ml/hr (210 bar).

Mobile Phase—A solution of 0.01% (v/v) diethylamine, 5% (v/v) chloroform, and 18% (v/v) 2-propanol in *n*-hexane was prepared as required. It was degassed (refluxed for 5 min) and stored in the solvent reservoir of the instrument.

Internal Standard Solution—A solution of polythiazide in tetrahydrofuran (0.1 mg/ml) was used.

Preparation of Standard Curves—Stock solutions of reserpine (0.10 mg/ml), hydrochlorothiazide (25 mg/ml), and polythiazide (0.5 mg/ml)

[1] Aldrich Chemical Co., Montreal, Canada.
[2] Merck Sharp and Dohme, Kirkland, Canada.
[3] Pfizer Co., Arnprior, Canada.
[4] Pfaltz and Bauer, Flushing, N.Y.
[5] United States Pharmacopeial Convention, Rockville, Md.
[6] Burdick and Jackson Laboratories, Muskegon, Mich.
[7] Model 4100, Varian Aerograph, Palo Alto, Calif.
[8] Model 440, Waters Associates, Milford, Mass.
[9] Autolab System I, Spectra-Physics, Santa Clara, Calif.
[10] Li-Chroma I.D., Alltech Associates, Arlington Heights, Ill.
[11] LiChrosorb SI 60, British Drug Houses, Toronto, Canada.