Quality Control of Phenylbutazone I: Analysis of Phenylbutazone and Decomposition Products in Drugs by TLC

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Abstract □ A modified TLC procedure for the analysis of phenylbutazone and its degradates on silica plates is reported. This method avoids phenylbutazone degradation *in situ* by chelating the iron of the silica plate, which allows rapid characterization with a fluorescence indicator. A selective and sensitive assay of phenylbutazone (0.025 μg), using a chromatographic spectrophotometer, was performed on silica plates without a fluorescence indicator. Quantitative analysis of an injectable solution is outlined.

Keyphrases □ Phenylbutazone—degradation products, TLC, qualitative and quantitative analyses, UV reflectometric measurements on plates □ Degradation—phenylbutazone, qualitative and quantitative analyses □ TLC—analysis, phenylbutazone and degradation products

Stability studies of phenylbutazone (I) in drugs and raw materials have been widely reported (1–7). A semiquantitative method to estimate the degradation products by TLC (1) was later modified (2) to minimize the rapid air oxidation of phenylbutazone on the silica coating, which occurs during analysis (2, 4, 8). This paper demonstrates the original causes of phenylbutazone degradation on precoated silica plates and proposes a TLC procedure to avoid this decomposition. An accurate estimation of phenylbutazone and its main degradation products can be achieved with a chromatogram spectrophotometer.

BACKGROUND

Under normal conditions, phenylbutazone (I) is oxidized. The main decomposition products were identified, and the mechanism of their formation was proposed previously (2, 9).

In TLC, phenylbutazone degradation *in situ* on the silica plate is not always evident (1, 6, 10). Various investigators reported this decomposition (2, 8) and proposed modified procedures to delay the formation of the major oxidation products on the plate [oxyphenbutazone, 4-hydroxyphenylbutazone, II, and *N*-(2-carboxycaproyl)hydrazobenzene, III].

Oxidation on the plate, which occurs with other pyrazolones (8, 11, 12), may be important; after 2 hr of contact, decomposition may reach 100%, depending on the coating material and the solvent system. Macek (8) suggested that this reaction probably is due to the iron content of the silica.

Empiric studies suggested preparation of the coating by mixing silica with pH 6 McIlvaine buffer (citric acid–dibasic sodium phosphate) (2), bismuth nitrate (13), or acid sodium sulfite (4).

To clarify the cause of the degradation, silica plates impregnated with either 0.2 *M* Na_2HPO_4 or 0.1 *M* citric acid were prepared (2). In the first case, significant I degradation occurred; in the second case, no decomposition was observed even after 1 hr of contact. This test indicated that degradation on the plate was not mainly due to the acidic pH of the silica, although phenylbutazone decomposition is accelerated under acid and alkaline conditions (1, 9, 14, 15). When using pH 6 McIlvaine buffer, phosphate ions may delay the phenylbutazone autoxidation and hydrolysis, but citric acid is the major agent avoiding the degradation. As suggested previously (8), iron (present in silica) is the main degradative agent that catalyzes the oxidation of the finely divided phenylbutazone on the plate. Citric acid complexes the iron of the coating and prevents phenylbutazone from the oxidation. The use of silica plates impregnated with edetate sodium supports this hypothesis.

Precoated plates sprayed with acid sodium sulfite (4) and bismuth nitrate (13) were used. A rapid degradation on the plate was observed with quenched fluorescence in the former case; no decomposition, within the limits of sensitivity (iodine vapor detection), was evident after 1 hr of contact in the latter. Bismuth nitrate is a quenching agent, which does not allow the use of silica gel F_{254} precoated plates. However, an aqueous solution cannot permeate precoated plates as easily as alcoholic solutions.

A simple and rapid analytical procedure using precoated silica plates washed with an alcoholic citric acid solution is proposed.

Table I—R_f Values (×100) of Phenylbutazone and Degradation Compounds

Compound	Relative to Phenylbutazone (I)	Relative to Phthalic Acid (VI)
I	100	528
II	42	224
III	33	172
IV	29	152
V[a]	119	628

[a] Compound V was detected by spraying with 0.5% (w/v) dichromate in 20% (v/v) H_2SO_4.

EXPERIMENTAL

Materials and Reagents—Glassware was rinsed with hot nitric acid. A 10-μl syringe[1] was calibrated at 0.1-μl intervals. A chromatogram spectrophotometer[2] was equipped with a chart recorder[3] and an electronic integrator[4]. Precoated silica gel F_{254} glass plates[5] (0.25 mm thick) were used for qualitative determinations, and precoated silica gel G glass plates[5] (0.25 mm thick) were used for qualitative and quantitative determinations.

All solvents were distilled from a glass apparatus (the absence of iron was checked with ferrozine reagent). Citric acid, phthalic acid, and formic acid were analytical grade. Phenylbutazone and the degradation products[6] were used as received. The solutions of phenylbutazone and degradates were freshly prepared and stored in the dark in a refrigerator.

The solvent system was hexane–chloroform–ethanol–formic acid (60:30:9:1). The silica plates for qualitative and quantitative analyses were soaked in alcoholic citric acid solution (1%) for a few minutes with occasional stirring, dried at 40° for 15 min and then at 105° for 15 min, and stored in a desiccator.

Procedure—For qualitative analysis, the mixed standard ethanolic solution was prepared as follows: I, 10 mg/ml; II, 100 μg/ml; III, 100 μg/ml; *N*-(2-carboxy-2-hydroxycaproyl)hydrazobenzene (IV), 100 μg/ml; and azobenzene (V), 100 μg/ml.

The standard solution (5 μl) and an injectable formulation (600 mg of phenylbutazone and 30 mg of lidocaine diluted to 3 ml with vehicle) suitably diluted (5 μl) were spotted on a precoated silica gel G F_{254} plate, previously washed as noted, and developed using the solvent system to a height of ~15 cm. Plates were air dried and examined at 254 nm. Detection was completed by iodine vapor visualization. Relative R_f values were determined by using phthalic acid (VI) as the internal standard (2 mg/ml).

For quantitative analysis, the mixed standard ethanolic solution with I–IV (400 μg/ml of each compound) was prepared. The standard solution was diluted with ethanol two, four, five, and eight times, respectively. To an aliquot (5 ml) of each dilution, 2 ml of the internal standard solution (phthalic acid in ethanol, 2 mg/ml) was added. Two dilutions of the

[1] Hamilton, Bonaduz, Switzerland.
[2] PM QII Zeiss.
[3] B.B.C. GOERZ.
[4] Minigrator Intersmat.
[5] Merck, Darmstadt, West Germany.
[6] Courtesy of Geigy Laboratories, Basel, Switzerland.

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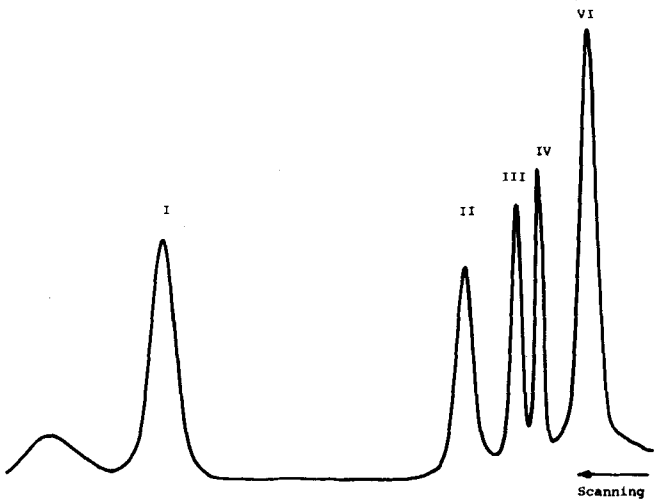

Figure 1—*Chromatogram on silica plate for a mixed sample (phenylbutazone and degradation products) with phthalic acid as the internal standard. Reflectometric measurement was at 237 nm, scanning speed was 30 mm/min, and chart paper speed was 20 mm/min. Key: I, 190 μg/ml; II, 150 μg/ml; III, 160 μg/ml; and IV, 330 μg/ml.*

sample, 1:1000 for the phenylbutazone assay and 1:50 for the degradates assay, were prepared. The same internal standard solution (2 ml) was added to an aliquot (5 ml) of each diluted sample.

Silica gel G plates, without fluorescence indicator, were washed as described. Mixed diluted standard solutions (4 μl) and diluted sample (4 μl) were spotted and allowed to air dry. The plates were developed, using the described solvent system, to a height of ~15 cm and allowed to air dry.

Reflectance spectrometric measurements were performed with a chromatogram spectrophotometer at 237 nm, the maximum absorbance wavelength of I. The detector head was adjusted on the center of each spot to be recorded. Each peak was scanned twice at 237 nm; the reproducibility of duplicates should be better than 2%. The calibration curve was constructed for each compound by plotting the average peak area ratio (solute to internal standard) *versus* solute concentration. From the graphs, the amount of I–IV in the sample was determined.

RESULTS AND DISCUSSION

Table I lists the relative R_f values obtained from the analysis on silica plates using a fluorescence indicator.

UV spectra of phenylbutazone and its degradates were scanned. The maximum absorbance wavelength was ~237 nm for I–IV, which allowed quantitative analysis at the same wavelength. Maximum absorbance was 320 nm for V, so its quantitation would not be performed at 237 nm. Figure 1 illustrates the chromatogram at 237 nm of a mixed sample with phthalic acid as the internal standard. The calibration curve was obtained by plotting the area ratios (solute to internal standard) *versus* concentration. The calibration curve was linear for I–IV and went through the origin. Correlation coefficients were 0.99 for I and II, 0.95 for III, and 0.97 for IV. The phenylbutazone content of the injectable solution was 553 mg. No interference was observed from lidocaine (R_f 0.01 relative to phenylbutazone).

The sensitivity of the proposed procedure allowed a satisfactory assay of each degradate with a convenient dilution (1:50) of the sample. The injectable solution contained 11.7 mg of IV. The sensitivities tested were 0.05 μg for I, 0.04 μg for II and III, and 0.03 μg for IV. These levels suggest an application for pharmacokinetic studies.

The reported procedure allows the identification and quantitation of phenylbutazone and its degradates; its main use is the TLC qualitative test. It should be proposed as an available complement to the acidimetric assay specified in the pharmacopeias (16–18); because the phenylbutazone assay lacks specificity, it cannot give information about the purity of phenylbutazone.

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