Thin-Layer Chromatography of Procaine Penicillin G

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Abstract □ Chromatographic systems are described which are suitable for the examination of procaine penicillin G preparations which may contain, in addition to procaine and penicillin G, the degradation products *p*-aminobenzoic acid, ethyl *p*-aminobenzoate, benzylpenicilloic acid, and benzylpenillic acid. Further decomposition of penicillin G occurs when alcoholic solutions are used for spotting the chromtograms, the corresponding alkyl α-D-penicilloate being formed, whereas only penicillin G was detected when freshly prepared aqueous solutions were used. However, in aged aqueous solutions the rate of decomposition of penicllin G increased markedly and two new spots, due to benzylpenicilloic acid and benzylpenillic acid, were detected on the chromatogram.

Keyphrases □ TLC analysis—penicillin G, procaine □ Procaine penicillin G—decomposition products, detection

Procaine is used in a variety of pharmaceutical, veterinary, and proprietary toilet and cosmetic preparations. Several thin-layer chromatographic systems for the separation of procaine from other local anesthetics have been described (1, 2) but, as far as the authors are aware, there is only one available for the separation of procaine and its decomposition products (3). In response to the needs of a program reporting adverse side effects of drugs, it was necessary to develop methods for the detection of decomposition products in deteriorated samples of procaine penicillin G. In order to obtain evidence regarding the nature of secondary spots detected on chromatograms of procaine penicillin G, solutions of penicillin G (Na) in water and several alcohols were examined by TLC. The TLC of procaine hydrochloride and some of its decomposition products were examined in a similar manner.

MATERIALS AND METHODS

Materials—Silica Gel GF and benzocaine (Merck), *p*-aminobenzoic acid (B.D.H.), procaine HCl (Squibb), and penicillin G (Na) and procaine penicillin G (General Biochemicals) were obtained commercially. Procaine penicillin G injectable preparations were sent to the authors by physicians cooperating in an adverse side effects-reporting program. Methanolic HCl was prepared by diluting aqueous N HCl (100 ml.) to 1000 ml. with methanol.

Preparation of Plates—Chromatoplates (20 × 20 cm.) were coated with Silica Gel G or GF slurries to a thickness of 250 μ using a Desaga spreader. The plates were air dried for 2 hr. and activated at 100° for 30 min. before use on the same day.

Development of Chromatograms—Aliquots of aqueous, methanolic, and methanolic HCl solutions, containing 10 mcg. of ethyl *p*-aminobenzoate (benzocaine), *p*-aminobenzoic acid, penicillin G (Na), procaine penicillin G, and procaine HCl, separately and in mixtures, were spotted on to the plate. In some cases large amounts (> 500 mcg.) of the injectable preparations of procaine penicillin G were applied directly to the plates. The following systems (*cf. Reference* 4) were used for the development of chromatograms: *A*, acetone–acetic acid (95:5); *B*, isoamyl acetate–methanol–formic acid–water (65:20:10:5) (organic phase). The solvents were allowed to rise 15 cm. from the origin. System *A* required approximately 25 min. and System *B* approximately 60 min. for this development. Plates developed with System *A* were dried in a stream of warm air and those with System *B* by heating at 120° for 20 min.

Table I—TLC of Procaine Penicillin G
Effect of Solvent and Age of Solution

Spotting Solvent	Age	R_f[a] of Spots in System *A*	R_f[a] of Spots in System *B*[b]
H_2O	Fresh	1.00, 0.10	1.00, 0.00
H_2O	4 Days	0.78, 0.43, 0.10	1.00, 0.45, 0.26, 0.00
MeOH	Fresh	1.00, 0.10	1.00, 0.00
	4 Days	1.12, 1.00, 0.10	1.32, 1.00, 0.00
MeOH/HCl	Fresh	1.00, 0.10	1.00, 0.00
	4 Days	1.12, 1.00, 0.43, 0.10	1.32, 1.00, 0.45, 0.26, 0.00

[a] Relative to penicillin G (absolute R_f's: *A*, about 0.7; *B*, about 0.5). [b] Chromatogram developed twice with Solent *B*.

Location of Spots—The chromatograms were examined under UV light and then sprayed with one of the following reagents: (*a*) aqueous ferric chloride (10%, 20 ml.), potassium ferricyanide (5%, 10 ml.), and sulfuric acid (20%, 70 ml.) mixed immediately before use; (*b*) aqueous solutions of sodium nitrite (0.1%), ammonium sulfamate (0.5%), and *N*-(1-naphthyl)ethylenediamine dihydrochloride (0.1%) applied in turn. The sodium nitrite solution was sprayed liberally on the plate which was then left to stand for 20 min. to allow complete diazotization. The plates were dried between applications of the spray reagents. The colors of the spots were recorded immediately after spraying and 30 min. later.

RESULTS

TLC of Procaine Penicillin G—The relative R_f values of spots detected on chromatograms of samples of solutions of procaine penicillin G in water, methanol, and the methanol HCl solution are given in Table I. Results obtained with solutions which had been allowed to stand for 4 days are also given. Relative R_f values are quoted because there was some variation in the absolute values obtained with the acetone/acetic acid (95:5) solvent, although the relative values were reproducible. The relative values are the averages of four separate determinations.

Freshly prepared alcoholic solutions of procaine penicillin G contain procaine and penicillin G, together with procaine penicillin G. All these compounds gave positive color reactions with the potassium ferricyanide reagent. Zones containing procaine may be identified by the red color produced with the *N*-(1-naphthyl)ethylenediamine spray reagent (see Table II). Penicillin G does not react with this latter reagent (*cf.* Table II). When injectable procaine penicillin G samples (> 500 mcg.) were applied directly to the plates and developed with Solvent *A* three spots were detected. One of these (relative R_f, 0.10) corresponded in R_f value and color reactions to procaine. The second (relative R_f, 1.00) behaved similarly to authentic penicillin G. The spot with the highest relative R_f (1.40) possessed properties of procaine and penicillin G in that it gave positive color reactions with both the ferricyanide and *N*-(1-naphthyl)ethylenediamine spray reagents. The substance giving rise to this zone corresponds, therefore, to procaine penicillin G but further work is contemplated to achieve unequivocal identification. It was not present on chromatograms developed with Solvent *B* (see Table II).

TLC of Procaine and Some Decomposition Products—Solvent System *A* gave good separations of *p*-aminobenzoic acid, procaine (spotted as the hydrochloride), penicillin G, and procaine penicillin G. System *B* also completely separated benzocaine from these compounds. The relative R_f values, color reactions, and fluorescence characteristics on Silica Gel GF of these compounds are given in Table II. The colors formed by the reactions of the various compounds with the *N*-(1-naphthyl)ethylenediamine reagent were less

Table II—TLC of Procaine Penicillin G and Some Related Compounds

Substance	R_f[a] in System A	R_f[a] in System B	Color[b] with NED[c]	Color[b] with UV[d]
Procaine[e]	0.10	0.00	cr	d.b.
Penicillin G (Na)	1.00	1.00	—	—
p-Aminobenzoic acid	1.33	1.19	or → br	d.b.
Benzocaine	1.39	1.52	or → r	d.b.
Procaine penicillin G[f]	1.40	—	cr	—

[a] Relative to penicillin G. [b] All spots were stained blue by the ferricyanide reagent. [c] Abbreviations: NED, N-(1-naphthylethylenediamine) reagent; cr, crimson; or, orange; br, brown; r, red; d.b. dark blue. [d] Plates irradiated under UV light (λ 254 mμ). [e] Spotted as procaine HCl. [f] Penicillin G and procaine were always detected on the plates (*cf.* Table I).

intense on chromatograms developed with Solvent *B* than Solvent *A*.

TLC of Penicillin G (Na)—The relative R_f values of spots obtained with the various alcoholic solutions of penicillin G (Na) which had been allowed to stand for 4 days at room temperature are given in Table III.

When samples of the alcoholic solutions of penicillin G (Na) were chromatographed using System *A*, two spots were always detected. One of these was due to penicillin G (Na). The other had a relative R_f (to penicillin G) of 1.12. The R_f of the secondary spot was the same for all the alcoholic solutions when solvent *A* was used. When Solvent System *B* was used the secondary spots had different R_f values. Four-day-old methanolic solutions gave no secondary spot ahead of the main spot when the plate was developed once with Solvent B. Ethanolic solutions gave indication of spots although these were not completely separated after one development. However, in both cases, two well separated spots were detected when the plate was dried and redeveloped with solvent B. Two spots were present on the developed chromatograms of penicillin G (Na) in 1-propanol and 1-butanol after the first development. Aqueous solutions of penicillin G (Na) which were less than 1 day old showed only one spot when samples were chromatographed using Systems A or B. Solutions which were 1 day old showed a second spot with both solvent systems (*A*, R_f 0.78; *B*, R_f, 0.45). Solutions which were 4 days old showed no penicillin G but a new spot (relative R_f's: *A*, 0.43; *B*, 0.26) was present on the chromatograms.

DISCUSSION

TLC is a useful technique for the examination of trace impurities in bulk drugs and dosage forms. However, the generation of artifacts with this method must always be considered. Decomposition of the drugs may occur during chromatography or "shadow" spots may result from interactions between the sample and developing solvent (4–6). These effects may be particularly misleading when TLC plates are loaded in an examination for trace impurities. Although procaine penicillin G in aqueous suspension is quite stable, thin-layer chromatograms of procaine penicillin G showed two or more spots depending on the spotting solvent, the developing solvent and the age of the spotting solution (see Table I). Some of these spots may be due to procaine, penicillin G, or their decomposition products. Three blue spots may be detected on thin-layer chromatograms of freshly prepared methanolic solutions of procaine penicillin G which have been sprayed with the potassium ferricyanide reagent.

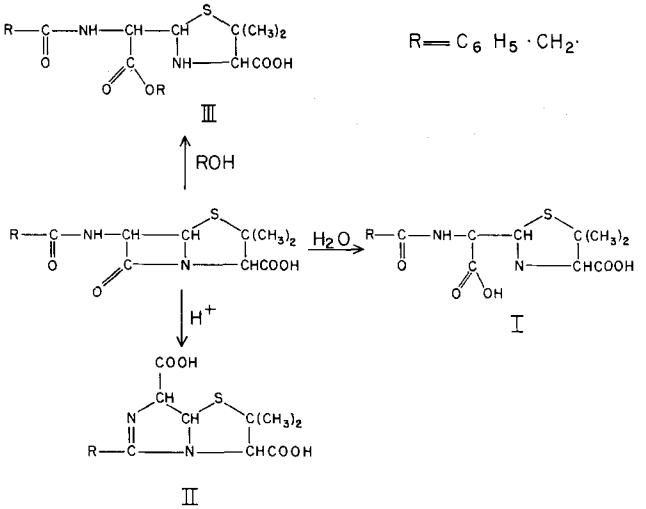
Table III—TLC of Aged (4 Days) Solutions of Penicillin G (Na)

Spotting Solvent	R_f[a] of Spots in Solvent A	R_f[a] of Spots in Solvent B	R_f[a] of Spots in Solvent B[b] (2×)
Water	0.78, 0.43	0.45, 0.26	0.45, 0.27
Methanol	1.12, 1.00	1.00	1.32, 1.00
Ethanol	1.12, 1.00	1.03, 1.00	1.41, 1.00
1-Propanol	1.12, 1.00	1.12, 1.00	1.48, 1.00
1-Butanol	1.12, 1.00	1.21, 1.00	1.46, 1.00
Methanol/HCl	1.12, 1.00, 0.49	1.00, 0.28	1.32, 1.00, 0.28

[a] Relative to penicillin G. [b] Chromatogram developed twice in same direction.

The three spots correspond to procaine (*A*, R_f, 0.10) procaine penicillin G (*A*, R_f, 1.40), and penicillin G (*A*, R_f, 1.00) (*cf.* Table II). Procaine may exist as the salt of the acid component of the developing solvent. This assumption is necessary to rationalize the fact that procaine has the same R_f when spotted as its hydrochloride or penicillin salt. Procaine penicillin G was in fact detected only on plates which had been loaded with the injectable preparations and developed with Solvent *A*. This may be attributed to one or both of the following factors which would tend to maintain the intact procaine penicillin G (1). The injectable preparation contains procaine hydrochloride (20 mg./ml.) which is added to further depress the solubility of the procaine penicillin G (2). The injectable preparation contained the preservatives methylparaben (0.12%) and propylparaben (0.014%). The three spots are distinguished by their fluorescence characteristics when the chromatograms are irradiated with UV light or by their color reactions with the potassium ferricyanide and *N*-(1-naphthyl)ethylenediamin dihydrochloride reagents. The main decomposition product of procaine in aqueous solution is *p*-aminobenzoic acid (8), although it is conceivable that ethyl *p*-aminobenzoate (benzocaine) could be formed (9). These substances may be expected, therefore, to be present in deteriorated samples of procaine penicillin G although they were not found in any of the injectable samples examined. They are separated and distinguished from procaine and penicillin G by TLC.

The major reactions of penicillin G in aqueous and alcoholic solution are shown below (Scheme I). In aqueous solution the



Scheme I

product is penicilloic acid (I). In acid solution, penicillin rearranges to penillic acid (II). Penicillin reacts readily with alcohols to form the corresponding esters (III). These reactions have been reviewed by Schwartz and Buckwalter (10). The chromatographic data indicates that these reactions occur in the solutions of penicillin G (Na) and procaine penicillin G prepared for chromatography.

When freshly prepared aqueous solutions of penicillin G (Na) were appled to the TLC plates only one spot, *i.e.*, penicillin G was detected on the developed chromatograms. Chromatograms of samples of the same aqueous solution which had been allowed to stand for 1 day showed also a second spot (relative R_f's: *A*, 0.78; *B*, 0.45) which was presumably due to benzylpenicilloic acid (I). It has been shown (10) that a small amount of acid lowers the pH of penicillin G (K) solutions very rapidly. The increased acidity of the reaction mixture which would result from the formation of the D-benzylpenicilloic acid would provide conditions suitable for the rearrangement of the penicillin G to benzylpenillic acid (II). That this does occur is indicated by the presence of a new spot (relative R_f's: *A*, 0.43; *B*, 0.26) on chromatograms of 4-day-old samples of aqueous penicillin G (Na) and supported by the formation of a compound with similar R_f value in solutions of penicillin G (Na) in methanolic HCl (see below).

Freshly prepared solutions of penicillin G (Na) in the various alcohols gave two spots on their chromatograms. The second spot is probably due to the corresponding α-alkyl-α-D-penicilloate (III). A different reaction pattern was obtained with solutions of pen-

icillin G (Na) in methanolic HCl. In this case, in addition to the penicillin G zone, two other spots were detected. One of these (R_f 1.12 in System *A*) corresponded to the methyl α-D-penicilloate spot obtained with solutions in absolute methanol, while the substance giving the other unidentified spot (R_f's: *A*, 0.49; *B*, 0.28) is presumably benzylpenillic acid (II) which is the normal rearrangement product of benzylpenicillin in acid solution. Spots corresponding in R_f values to II have been detected in deteriorated procaine penicillin G solutions in water.

The TLC procedures described should be applicable to the detection of procaine and its degradation products in the presence of other drugs which are formulated with procaine, *e.g.*, epinephrine, ephedrine, morphine, scopolamine, paromomycin, dihydrostreptomycin. Since these latter compounds and penicillin G do not give color reactions with the Bratton-Marshall reagent (12), it should be possible to use this reaction as the basis for the assay of procaine in the presence of these compounds. Although the Bratton-Marshall reagent has been used for the assay of procaine penicillin G (13), the method appears to have been largely neglected by other workers. The usefulness of this reagent for the assay of procaine is presently being assessed in these laboratories.

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Pharmaceutical Heterogeneous Systems IV: A Kinetic Approach to the Stability Screening of Solid Dosage Forms Containing Aspirin

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Abstract □ The effect of common tablet excipients and physiologically active materials on the hydrolysis of aspirin in aqueous suspension has been studied. The relationship of hydrolytic rate to solvent concentration in these systems has been determined. The results obtained have been utilized in the stability ranking for various tablet mixes. The kinetic ranking is compared to the relative stability of tablets and powders. An attempt is made to correlate extrapolated kinetic data with stability results for the tablets.

Keyphrases □ Heterogeneous systems—pharmaceutical □ Aspirin dosage forms—stability screening □ Stability, aspirin dosage forms—kinetic ranking □ UV spectrophotometry—analysis □ Colorimetric analysis—spectrophotometer

Prior investigations have shown an inverse relationship between aspirin stability and the presence of moisture at ambient or somewhat higher temperatures although secondary phenomena as the pH of the water may alter the rate of degradation (1–6). The deleterious action of fatty acid lubricants on acetylsalicylic acid has been related to hydrolysis (4). It has been previously mentioned that one may compare an aspirin tablet to an aqueous suspension—approaching but not attaining zero water concentration—in order to facilitate explanation of this solvolytic degradation (3). However, the complexities rendered by the multiplicity of variables in heterogeneous systems of this nature make interpretation rather more difficult than in, for example, solution or homogeneous kinetics where the components and species can be accounted for in totality in many cases.

Increased rates of aspirin decomposition in the presence of several excipients and antacids have been reported several times in the literature (1, 3, 4, 7, 8). These agents reported were found to markedly accelerate salicylic acid formation.

More recently Guttman (9) has observed considerable degradation of buffered aspirin tablets in chloroformic solutions and this breakdown is apparently not proportional to water content of the chloroform.

Garrett suggested the possibility that normally first-order decomposition processes may become zero-order in saturated solutions (6) and this has been corroborated by others (3).

The purpose of this study was the evaluation of the effect of some commonly used tablet additives regarding their influence on the rate of generation of salicylic acid from aspirin.