product of I, yielded no absorbance at 242 nm, indicating that the method is stability indicating with respect to hydrolytic decomposition of I.

Analysis of Commercial Syrups—Typical analyses of six commercial syrups, 0–7 years old, are presented in Table V. These syrups contained from one to three of the active ingredients listed in Table III at levels equal to or less than those listed, plus liquid sugar and various of the dyes, flavors, preservatives, and excipients listed. To verify that the conditions that prevail in old syrups do not interfere with the analysis, the standard addition technique was employed with Syrups A and F. In this technique, one aliquot of the syrup was analyzed in the regular manner while a second, equal aliquot was analyzed after the addition of an amount of I equal to that in the syrup itself. The resulting isooctane solution for this sample was diluted in half prior to scanning on the spectrophotometer. It can be seen from Table V that the recovery of added standard is quantitative.

When analyzing aged samples, emulsion formation is often encountered in the oxidation step. However, a clear isooctane layer is readily obtained in these cases upon centrifugation. In addition, old samples of syrup produce considerable darkening of the ion-exchange resin and a small amount of background in the benzaldehyde spectrum, even after washing with the hydrochloric acid-salt solution. This background is readily corrected for by using the absorbance difference between 242 and 263 nm in calculations.

Although phenylephrine is known to undergo periodate oxidation to *m*-hydroxybenzaldehyde (13), no spectral evidence of this strong UV absorber was detected in the final isooctane solution resulting from the analysis of commercial syrup or blank syrups containing phenylephrine.

SUMMARY

The proposed method of analysis for homatropine methylbromide in syrup formulations is sensitive, specific, and stability indicating. It permits the routine, accurate analysis of I in syrups at the lowest dosage levels commercially available and is suitable for the analysis of darkened, aged samples.

No attempt was made to determine the maximum sensitivity of this method. It is apparent, however, that lower doses and different formulations, containing larger amounts of other substances, could be analyzed for I by this method or by a modification of it using, for example, a larger amount of ion-exchange resin and a longer path length cell for recording the spectrogram.

The method and minor modifications of it have been used routinely for several years for the analysis of I in syrups, elixirs, and complex tablet formulations.

REFERENCES

(1) F. J. Bandelin, *J. Amer. Pharm. Ass., Sci. Ed.*, **37**, 10(1948).

(2) M. Margadida, F. Braga, and J. Baltazar, *Rev. Port. Farm.*, **13**, 145(1963); through *Chem. Abstr.*, **60**, 368d(1964).

(3) R. H. Robinson, *J. Amer. Pharm. Ass., Sci. Ed.*, **41**, 392(1952).

(4) S. Ahuja, D. Spiegel, and F. R. Brofazi, *J. Pharm. Sci.*, **59**, 417(1970).

(5) *Ibid.*, **61**, 1631(1972).

(6) J. A. Feldman and B. J. Robb, *J. Pharm. Sci.*, **59**, 1646(1970).

(7) B. F. Grabowski, B. J. Softly, B. C. Chang, and W. G. Haney, Jr., *ibid.*, **62**, 806(1973).

(8) L. Chafetz, *ibid.*, **53**, 1192(1964).

(9) D. J. Smith, Abstracts, 81st meeting of the Association of Official Analytical Chemists, Abstract No. 155, 1967, p. 20.

(10) J. L. Patel and A. P. Lemberger, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 106(1959).

(11) G. F. Smith, "Cerate Oxidimetry," 2nd ed., G. Frederick Smith Chemical Co., Columbus, Ohio, 1964, pp. 26, 27.

(12) E. A. Braude and F. Sondenheimer, *J. Chem. Soc.*, **1955**, 3754.

(13) L. Chafetz, *J. Pharm. Sci.*, **52**, 1193(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 28, 1973, from *Endo Laboratories, Inc., Garden City, NY 11530*

Accepted for publication December 14, 1973.

[x] To whom inquiries should be directed.

Determination of Sodium Penicillin G in Disodium Carbenicillin Preparations

JAN BIRNER

Abstract □ Aqueous solutions of disodium carbenicillin containing sodium penicillin G (sodium benzylpenicillin) are chromatographed by TLC using silica gel on aluminum foil and acetone–chloroform–acetic acid–water (50:45:5:1 v/v) as a developing solvent. The location of penicillin G is determined by reference to standard strips cut from the edges of the chromatogram and visualized colorimetrically. The appropriate area is removed and penicillin is eluted from the silica with phosphate buffer at pH 7.0. The amount of penicillin is determined spectrophotometrically after formation of penicillenic mercuric mercaptide formed by heating penicillin with an imidazole reagent containing mercury.

Keyphrases □ Carbenicillin—TLC analysis of penicillin G (benzylpenicillin) content □ Disodium carbenicillin—TLC analysis of sodium penicillin G (sodium benzylpenicillin) content □ Penicillin G (sodium)—TLC analysis as impurity in disodium carbenicillin □ Sodium penicillin G—TLC analysis as impurity in disodium carbenicillin □ Benzylpenicillin—TLC analysis as impurity in carbenicillin □ TLC—analysis, sodium penicillin G (sodium benzylpenicillin) in disodium carbenicillin

Disodium carbenicillin (the disodium salt of α-carboxybenzylpenicillanic acid) is a semisynthetic penicillin prepared from monobenzylphenylmalonyl chloride and 6-aminopenicillanic acid. A subsequent reduction of this compound under specific conditions yields disodium carbenicillin.

Table I—Estimation of Sodium Penicillin G Added to 200 μg of Commercial Disodium Carbenicillin

Added, μg	Found, μg	Recovery, %
0	9.3 ± 0.9	—
10	18.8 ± 0.4	95.0
20	28.7 ± 1.3	97.0
30	37.9 ± 0.6	95.3

Disodium carbenicillin has a different bacteriological spectrum from penicillin G (benzylpenicillin) and is active against Gram-negative organisms. Commercial preparations of disodium carbenicillin contain sodium penicillin G but the amount should not exceed 5% w/w (1).

The present BP method (2) for the determination of benzylpenicillin sodium in disodium carbenicillin is based on electrophoretic separation of the penicillins (3) and biological determination of penicillin G. As an alternative, the method reported here was developed and is based on the chromatographic separation of the penicillins, using plates of silica gel on aluminum foil and acetone–chloroform–acetic acid–water (50:45:5:1 v/v) as the developing solvent. The R_f value for penicillin G is between 0.51 and 0.53. A strip containing penicillin is removed from both edges of the plate, the penicillin is hydrolyzed in ammonia vapor, and the penicillin spot is located by starch iodine spray. By using these strips as a guide, the penicillin G zones are located on the remaining portion of the plates. The zones are cut out and the silica is transferred into test tubes and eluted with phosphate buffer. The eluted penicillin is determined spectrophotometrically as the mercuric mercaptide of penicillenic acid (4). A set of standards is run simultaneously with the samples on the same plate.

EXPERIMENTAL

Reagents, Solutions, and Materials—The following were used: acetone, analytical reagent; acetic acid, analytical reagent; chloroform BP; TLC aluminum sheets[1], silica gel, 20 × 20 cm (without fluorescent indicator), layer thickness 0.25 mm; imidazole[1], recrystallized twice from benzene; mercuric chloride, analytical reagent; hydrochloric acid, 5 *M*; sodium penicillin G, standard grade, BP; chromatographic tank for 20 × 20-cm plates; microsyringe, 30-μl capacity with 1-μl divisions; 1% soluble starch solution[2]; 0.1 *N* iodine in 4% (w/v) potassium iodide in water; ammonia, 0.880 specific gravity; and 1% phosphate buffer, pH 7.0 (91 g Na_2HPO_4, 62 g $NaH_2PO_4 \cdot 2H_2O$, and 350 ml water as stock solution; dilute 24 ml to 1 liter and autoclave).

Procedure—Prepare freshly from the test sample of disodium carbenicillin a 20-mg/ml solution in water and 0.5-, 1-, and 2-mg/ml solutions of standard sodium penicillin G in water. The sodium penicillin G used had a potency of 1655 u/mg.

Using a 20 × 20-cm silica plate on aluminum foil, divide the plate using lead pencil marking into seven 25-mm sections and a strip 12.5 mm wide on each side. At one end of the 25-mm sections, apply (in triplicate), using the microsyringe, 10 μl of the sample (200 μg carbenicillin), each in the form of a strip 12–15 mm long; then apply four standards of sodium penicillin G (5, 10, and 20 μg, the last in duplicate) in the same manner. Use two such plates. Place the plates in a suitable chromatographic tank containing the developing solvent of acetone–chloroform–acetic acid–water (50:45:5:1 v/v) and develop for a distance of about 13 cm (1.5–2 hr). Dry the plates and cut a 37.5-mm strip from both sides of each plate. Hang the cut strips in the vapor in a jar containing ammonia for 5–10 min, dry in an air current, and spray with starch iodine solution. This solution is prepared by mixing 50 ml of 1% soluble starch with 1 ml of 0.1 *N* iodine in 4% (w/v) KI and 3 ml acetic acid.

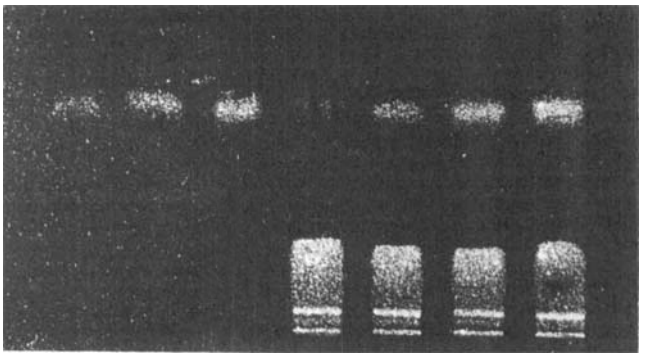

Figure 1—*Chromatogram of penicillin G standards (top) and carbenicillin (bottom) containing increasing amounts of penicillin G.*

The strips with penicillin G spots located in this manner are used as a template for locating the separated penicillin G on the remaining five sections of the developed plate. Cut out the located spots and transfer the silica quantitatively to centrifuge tubes, 11.5 × 1.5 cm. To each tube add 2.5 ml of 1% phosphate buffer, pH 7.0, and stir for 3 min on a mixer[3], placing a small glass bead into each test tube to help break up the silica particles. Centrifuge the tubes for 10 min at 2000 rpm, and assay the solutions for penicillin according to Bundgaard and Ilver (4).

Prepare 100 ml of the imidazole reagent by dissolving 8.25 g of imidazole in 60 ml of water and adding 10 ml of 5 *M* hydrochloric acid and then 10 ml of a solution of mercuric chloride (0.27 g dissolved in 100 ml of water). Adjust the pH to 6.80 ± 0.05 with 5 *M* hydrochloric acid and bring the volume to 100 ml with water. From each of the centrifuged penicillin solutions, pipet two 1-ml samples into two suitable test tubes, one serving as a sample, *A*, the other as a blank, *B*. To the *A* samples, add 5 ml of imidazole reagent; after mixing, stopper the tubes and incubate in a water bath at 60° for 25 min. After cooling to 20°, measure the *A* samples spectrophotometrically at 325 nm, using as a blank 1 ml of phosphate buffer mixed with 5 ml imidazole reagent. To the *B* samples, add 5 ml of water and measure at 325 nm, using as a blank 1 ml of phosphate buffer and 5 ml of water. The difference between *A* and *B* determines the penicillin concentration. Plot a standard reference curve from the standard values and calculate the amount of sodium penicillin G in the sample.

RESULTS AND DISCUSSION

Using the described technique, several samples of commercial carbenicillin were assayed and the amount of sodium penicillin G found was between 3.5 and 4.9%.

According to the study of Bundgaard and Ilver (4), the standard deviation in penicillin G assays is 0.45% when 20 assays are performed on the same solution. Since no pure carbenicillin was available, a commercial preparation was selected and assayed by the described procedure. The amount of sodium penicillin G found was 4.65%.

To evaluate the accuracy of the procedure, an extra amount of penicillin G was added to the sample to furnish an additional three solutions, each containing the same amount of carbenicillin (200 μg) and an additional 10, 20, and 30 μg of sodium penicillin G for each 10 μl. The original sample and three additional samples with corresponding standards were run through the same

[1] E. Merck.
[2] Analar B.D.H.
[3] Vortex J. R. mixer.

procedure, and the amount of sodium penicillin G was determined. The data from three experiments run in duplicate are given in Table I; the recoveries were 95–97%.

A photograph of a developed chromatogram containing standards of sodium penicillin G, commercial disodium carbenicillin, and commercial disodium carbenicillin with additional sodium penicillin G after ammonia treatment and spraying with starch iodine is shown in Fig. 1.

In comparison with the electrophoretic method of penicillin separation and subsequent biological assay of the separated penicillin, this method seems to be much simpler and several assays can be performed on the same day.

REFERENCES

(1) "Addendum 1969 to the British Pharmacopoeia 1968," Pharmaceutical Press, London, England, 1969, p. 18.

(2) *Ibid.*, pp. 90–92.

(3) J. W. Lightbown and P. De Rossi, *Analyst*, **90**, 89(1965).

(4) H. Bundgaard and K. Ilver, *J. Pharm. Pharmacol.*, **24**, 790(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 21, 1973, from the *Commonwealth Serum Laboratories, Parkville, Victoria, Australia, 3052.*

Accepted for publication November 29, 1973.

The author thanks Mr. E. O. Stuart for technical assistance and Dr. N. W. Coles for assistance in the preparation of this manuscript.

PHARMACEUTICAL TECHNOLOGY

Tensile Strength of Compressed Powders and an Example of Incompatibility as End-Point on Shear Yield Locus

E. N. HIESTAND[x] and C. B. PEOT

Abstract □ Three methods of measuring tensile strengths of compacted powders are described. These methods include transverse compression using squares and disks and the direct tensile stressing of lightly pressed powder. The transverse compression of squares gave the best overall results. The tensile strength of sitosterols NF was found to be too large to be compatible with its use as the end-point of the shear yield locus. However, a Warren–Springs-type equation can be used to describe the yield locus if the tensile strength is replaced by a smaller internal cohesion. A method of estimating both the internal cohesion and the exponent of the Warren–Springs equation from shear cell data is described.

Keyphrases □ Tensile strength, compressed powders—determined by transverse compression of squares and disks and direct tensile stressing, incompatibility as end-point on shear yield locus, internal cohesion estimated, Warren–Springs-type equation, relevance to yield locus □ Powders, compressed—tensile strength determined by transverse compression of squares and disks and direct tensile stressing, incompatibility as end-point on shear yield locus, internal cohesion estimated, Warren–Springs-type equation, relevance to yield locus □ Shear cell data—method of estimating internal cohesion and exponential of Warren–Springs equation, tensile strength of compressed powders □ Yield locus—testing of compressed powders, tensile strength role reevaluated

Tensile strength measurements of powders compacted to various degrees provide a direct measurement of the bonding potential[1] of a given solid material. This information is useful to the formulator in the selection of excipients. An excessively strong bond may prevent rapid disintegration and concomitant dissolution. Very weak bonding characteristics may limit the selection and/or quantity of lubricant that may be added to a formulation. In addition to these obvious practical uses of tensile strength information, the tensile strength has been used in characterizing flow properties. Specifically, the tensile strength has been designated as: *(a)* a point on the shear failure yield locus (1–3), and *(b)* a limiting factor to the extension of the yield locus (4). The magnitude of the tensile strength played an important role in the evolution of a third position presented herein—*viz.*, the tensile strength is not a point on the yield locus and is not predictable from the yield locus of shear failure. This interpretation is a departure from commonly accepted practice and is believed to be an important conclusion of this research.

[1] Conditions in the compaction process must be controlled to produce maximum values if the true bonding potential is to be observed experimentally.

THEORY

The tensile strength of powders is an important measurement for characterizing the interaction between solid particles. The strong interactions are at the true contact areas. The extent of the true area of contact between particles after elastic recovery is dependent on the magnitude of the maximum stress applied and the amount of plastic deformation that has occurred. These concepts are basic to the discussion of the results of the experimental work reported here.

Experimentally, tensile strength is not always a readily mea-