(19) A. H. Anton and D. F. Sayre, *J. Pharmacol.*, **138**, 360 (1962).
(20) A. Bertler, A. Carlsson, and E. Rosengren, *Acta Physiol. Scand.*, **44**, 273 (1958).
(21) A. H. Anton and D. F. Sayre, *J. Pharmacol.*, **145**, 326 (1964).
(22) T. L. Sourkes and G. F. Murphy, "Methods in Medicinal Research," vol. IX, Medical Publisher, Chicago, Ill., 1961, pp. 147–152.

ACKNOWLEDGMENTS AND ADDRESSES

Received November 22, 1976, from the *School of Pharmacy, University of Southern California, Los Angeles, CA 90033.*
Accepted for publication January 18, 1977.
[x] To whom inquiries should be directed.

GLC Determination of *N,N*-Dimethylaniline in Penicillins

MICHEL MARGOSIS

Abstract □ A reliable GLC procedure was developed for the determination of residual *N,N*-dimethylaniline as a contaminant in ampicillin commercial samples from various sources. This procedure was similarly applied to other penicillins and cephalosporins. The method involves dissolution of the sample in aqueous alkali, extraction of the organic base with cyclohexane containing naphthalene as an internal standard, and injection into a gas chromatograph with a phenyl methyl silicone column. Levels of 0.1 ppm of dimethylaniline were easily measured with a coefficient of variation less than 10%, and recoveries from spiked samples exceeded 99%.

Keyphrases □ *N,N*-Dimethylaniline—GLC analysis in various penicillins and cephalosporins, commercial samples □ Penicillins, various—GLC analysis of *N,N*-dimethylaniline as contaminant in commercial samples □ Cephalosporins, various—GLC analysis of *N,N*-dimethylaniline as contaminant in commercial samples □ GLC—analysis, *N,N*-dimethylaniline in various penicillins and cephalosporins, commercial samples □ Contaminants—*N,N*-dimethylaniline, GLC analysis in various penicillins and cephalosporins, commercial samples □ Antibacterials—various penicillins and cephalosporins, GLC analysis of *N,N*-dimethylaniline as contaminant in commercial samples

The premarketing certification process assures that each bulk and dosage form batch of antibiotics intended for human use complies with the specifications of proposed and established standards for identity, potency, quality, and purity.

The Code of Federal Regulations provides for Good Manufacturing Practices (GMP) in the production of pharmaceuticals. The direction to "... minimize contamination of products by extraneous adulterants ..." (1) applies also to residual reagents that may exhibit undesirable properties such as toxicity or carcinogenicity during antibiotic therapy and/or possible accumulation from other drugs.

Ampicillin, a semisynthetic penicillin, has been synthesized through diverse routes (2) by using 6-aminopenicillanic acid (II) as an intermediate and an organic base such as *N,N*-dimethylaniline (I) to abstract generated hydrogen chloride, the presence of which inhibits the synthesis.

Although gross contaminations can normally be detected by the general analytical techniques employed in certification, trace impurities are often too elusive. As the Food and Drug Administration became increasingly aware of the adventitious presence of residual dimethylaniline in ampicillin, the GLC method was developed to ascertain the extent of the problem. The pharmacological properties of dimethylaniline have not been fully elucidated, but it seemed advisable to limit its presence because of its dubious nature and possible carcinogenicity.

This report presents and discusses a GLC method[1] for the analysis of residual dimethylaniline in ampicillin and its applicability to survey-related antibiotics.

EXPERIMENTAL

Apparatus—A gas chromatograph[2] was fitted with an inlet system modified to accommodate a one-piece glass column directly from the site of injection, as described previously (3), and with a flame-ionization detector. It was used together with a 1-mv range strip-chart recorder. The instrument was also equipped with individual controls, allowing for separate heating of the inlet, column, and outlet. Peak area measurements were made with a digital electronic integrator[3].

A glass column, 1.9 m (6 ft) × 2 mm i.d., was packed with 3% of a 50% phenyl-substituted methyl silicone on silanized, acid-washed, flux calcined diatomite[4].

Typical Conditions—The gases were hydrogen at 1.55 kg/cm^2 (22 psi) and air at 2.11 kg/cm^2 (30 psi) adjusted to obtain maximum response and helium at 3.52 kg/cm^2 (50 psi) with a flow rate of about 30 ml/min. The temperatures were column, 60°; and injector and detector, about 150°. The column temperature and carrier flow rates were adjusted to obtain the first peak preferably within 5 min and complete resolution of the peaks. The current was 2×10^{-11} amp full-scale deflection (fsd) or was adjusted to obtain peak heights greater than 50% fsd, depending on peak sharpness.

Standard Solution—Weigh accurately about 25 mg of *N,N*-dimethylaniline base in a 25-ml volumetric flask. Add 1 ml of concentrated hydrochloric acid and about 10 ml of water. Shake to dissolve into one phase and dilute to volume. Use this solution to make standard solutions of varying concentrations ranging from 1.5 to 1500 μg/ml.

Internal Standard Solution—Dissolve about 5 mg of naphthalene in 100 ml of cyclohexane.

Sample Preparation—Weigh accurately about 1 g of bulk sample into a conical 15-ml centrifuge tube. Add 5 ml of 5% NaOH and stir on a mixer until dissolved. For the recovery study, add 1.00 ml of a standard dimethylaniline solution, mix thoroughly into a suspension, and then add 4 ml of 5% NaOH to dissolve.

Procedure—Add 1.00 ml of the cyclohexane solution, shake vigorously for about 1 min, and allow the phases to separate, centrifuging if necessary. Carefully draw a 1-μl sample from the upper cyclohexane phase and inject it into the chromatograph. For greater accuracy, make comparisons with standards having concentrations of the same order of magnitude

[1] Although this method was developed independently, it is similar in principle to those subsequently submitted in confidence from several manufacturers; it differs in details, however.
[2] Perkin-Elmer model 900.
[3] Infotronics CRS-100.
[4] OV-17 on 100–120-mesh Gas Chrom Q.

Table I—Dimethylaniline in Various Penicillins and Cephalosporins[a]

Sample[b]	Manufacturer	Type[c]	Size	I, ppm[d]
II	1	b	2	340, 433
II	2	b	2	60, 330
II	3	b	2	339, 512
II	4	b	2	280, 774
III	1	b	3	3–5
III	1	b	3	10–28
III	1	b	8	500–800
III	1	b	12	800–1000
III	1	b	6	1000–1500
III	2	b	5	200–300
III	2	b	1	700
III	3	b	8	5–8
III	4	b	7	<1.5
III	5	b	7	<1.5
III	6	b	4	1–4
III	7	b	3	<1
III	8	b	4	0–6
III	9	b	2	1250–1310
III	10	b	2	<1
III	11	b	3	<1.5
III	12	b	4	4.5–8
III sodium	13	b	1	<1.5
III sodium	14	b	1	1
III	15	c	2	4
III	16	c	1	2
III	17	c	2	230, 300
III	18	c	2	305, 325
III	19	c	1	250
III	20	s	1	<0.5
IV	1	b	1	8
V	1	b	3	<0.5
V	2	b	5	<0.5
V	3	b	3	<0.5
V	4	b	3	0.1–5
V	3	c	1	0.1
V	5	s	1	<0.05
V	6	c	2	<0.05
VI	1	b	2	7, 10
VI	2	b	3	39–51
VI	3	b	2	<0.3
VI	3	c	1	4
VI	3	c	2	<0.1
VII	1	b	1	0.5
VII	1	s	1	0.2

[a] No dimethylaniline was detected (<0.15 ppm) in samples of cephradine, cephaloglycin, cefazolin, penicillamine, penicillin G procaine, penicillin G potassium, penicillin V, oxacillin, cloxacillin, dicloxacillin, and carbenicillin. [b] Compound III is ampicillin, IV is hetacillin, V is amoxicillin, VI is cephalexin, and VII is cephapirin. [c] b = bulk, c = capsule (per capsule), and s = oral suspension. [d] "Less than" indicates that dimethylaniline was not detected, but an upper limit of detectability was calculated based on the instrument conditions.

as that contained in the sample. The elution sequence is such that dimethylaniline emerges first and naphthalene follows.

Calculations—The area of each peak is measured by a suitable technique such as electronic integration. Then:

$$\text{wt. \% I} = (R_x \times W_{std})/(R_{std} \times W_u) \times 100 \qquad \text{(Eq. 1)}$$

where R is the ratio of the area of the I peak to the area of the internal standard peak, and W is the weight of the standard (std) or sample (u).

Identity—The relative retention time (sample–internal standard) of the unknown sample is identical to that of the working standard under identical operating conditions.

RESULTS AND DISCUSSION

Results of the determination of dimethylaniline in various commercial penicillins and cephalosporins are summarized in Table I. The samples accumulated over the past 4 years were selected at random and, therefore, do not necessarily reflect the current market. As expected, all samples of 6-aminopenicillanic acid contained significant amounts of dimethylaniline. However, subsequent processing to produce the semisynthetic penicillins ostensibly rendered the final bulk product relatively free of this contaminant. The amount of residual dimethylaniline found in a few batches of ampicillins was significant, but this situation has been largely

Figure 1—*Chromatograms of 1.66 μg (A) and 41.6 μg (B) of dimethylaniline.*

rectified by improved cleanup or by substitution with other more innocuous organic bases.

The protocol of analysis includes the identical curing and conditioning treatment cited in the collaborative GLC studies of chloramphenicol (4) and griseofulvin (5, 6). This protocol includes calculations for the different parameters that characterize column performance. In this study, an efficiency of 1265 plates/m for dimethylaniline and of 1560 plates/m for naphthalene, a resolution factor of 7.3, and a tailing factor of 1.29 were calculated. In actual assays, a coefficient of variation of less than 2% can easily be obtained, but it normally increases as the concentration of the components to be determined decreases. This effect results mainly from a worsening baseline geometry (Fig. 1) with an accompanying decrease in the reliability of the electronic integrator. Thus, at the 1.66-ppm level, a coefficient of variation no greater than 7.9% was readily obtained.

The column requires injection of a silyl reagent at a higher temperature (275°) prior to sample conditioning at the operating temperature. This reagent is also injected several times at the end of the day with the column temperature reset to 275° to flush out the system. This treatment may also be necessary during the day to obtain a satisfactory baseline. Di-

Table II—Comparison of Quantitative Tests

Sample	Potency	Amine	Acid	I, μEq	Cl, μEq
1	98.2	106.7	94.9	8.2	203
2	98.8	97.0	99.3	5.8	121
3	97.2	98.0	100.0	0.008	7.6
4	100.6	99.7	98.7	0.012	2.9
5	102.1	99.4	97.4	2.4	85
6	100.8	98.3	96.4	1.7	101
7	101.9	99.3	97.5	2.4	89
8	102.2	99.0	102.2	2.4	93
9	99.6	98.3	103.0	2.4	92
10	102.4	105.2	96.1	0.041	72
11	102.2	96.4	100.3	0.012	62
12	101.6	95.6	99.2	0.046	118

methylaniline is relatively nonpolar because of the alkyl shielding and need not be derivatized to show good chromatographic properties except during separation from other intractable anilines (7). The standard is prepared as an aqueous solution to minimize evaporation of the solvent and solute as well as to simulate actual conditions.

Linearity of response was tested by injecting standard solutions of varying concentrations of dimethylaniline containing a fixed amount of the internal standard. A linear regression analysis of seven points in the concentration range of 3–200 ppm yielded a statistically significant squared multiple correlation coefficient of 0.9998 ($p > 0.01$). To improve the precision and reliability of electronic integration, the use of a standard approximating the concentration of the analyte in the unknown while maintaining identical instrument parameters is recommended. Method reliability was verified by spiking samples of ostensibly dimethylaniline-free ampicillin with different amounts of standards. Recoveries in excess of 99% were obtained, although residual dimethylaniline was occasionally detected at low concentrations (*e.g.*, <25 ppm).

Chromatography was performed at a low temperature, which is capable of detecting but is limited to relatively nonpolar substances of low molecular weights. Confirmation of identity by the relative retention times of the peaks attributed to dimethylaniline and naphthalene was obtained by combination GLC and electron-impact mass spectrometry applied to several bulk materials. However, conditions were inadequate to characterize the occasional foreign peaks.

Several samples were extracted directly with cyclohexane but did not manifest the presence of dimethylaniline until alkali was added. The logical presumption is that dimethylaniline was bound as a hydrochloride salt. Thus, a measure of total chlorine might correlate with the dimethylaniline content and with other quantitative tests as well. However, the results listed in Table II clearly show that the total chloride content obtained by neutron activation analysis (8), which ranges up to 7.3 mg/g, is far greater than the equivalent amount of dimethylaniline, indicating that other contaminants are present. In this context, the amount of dimethylaniline found in ampicillin has not demonstrated any correlation with the potency or concordance results obtained as prescribed in the regulations (9).

REFERENCES

(1) "Code of Federal Regulations," Title 21, part 211 (1975).

(2) "Analytical Profiles of Drug Substances," vol. 2, K. Florey, Ed., Academic, New York, N.Y., 1973.

(3) M. Margosis and K. Tsuji, *J. Pharm. Sci.*, **62**, 1836 (1973).

(4) M. Margosis, *ibid.*, **63**, 435 (1974).

(5) M. Margosis, *J. Chromatogr.*, **70**, 73 (1972).

(6) M. Margosis, *J. Pharm. Sci.*, **64**, 1020 (1975).

(7) R. A. Dove, *Anal. Chem.*, **39**, 1188 (1967).

(8) M. Margosis, J. T. Tanner, and J. P. F. Lambert, *J. Pharm. Sci.*, **60**, 1550 (1971).

(9) "Code of Federal Regulations," Title 21, part 440.5 (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 4, 1976, from the *National Center for Antibiotic Analysis, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, DC 20204.*

Accepted for publication January 21, 1977.

Presented at the American Chemical Society, San Francisco, Calif., August 1976.

The author thanks Robert P. Barron, Division of Drug Chemistry, Food and Drug Administration, for mass spectral analyses.

Aging of Tablets Made with Dibasic Calcium Phosphate Dihydrate as Matrix

J. M. LAUSIER, CHIA-WHEI CHIANG, H. A. ZOMPA, and C. T. RHODES ˣ

Abstract □ The aging of direct compression tablets made using dibasic calcium phosphate dihydrate as the tablet matrix was investigated over 16 weeks. The formula included 6% amaranth as a dye tracer. Two sets of stress storage conditions were used: 25° and 50% relative humidity and 45° and 75% relative humidity. Tablets were evaluated periodically by visual inspection; determination of the weight of 10 separate tablets, the size of 10 tablets measured by a micrometer screw gauge, and the hardness of 10 tablets as indicated by a Strong–Cobb hardness tester; the USP disintegration time test; and the USP dissolution test. Tablets stored at 25° and 50% relative humidity showed an approximately linear increase in disintegration and dissolution time over 16 weeks with no other significant changes. Storage at 45° and 75% relative humidity resulted in significant changes in most measured parameters; tablets showed blotching, substantial weight loss, and complex changes in disintegration and dissolution. The changes at elevated temperatures are related to loss of water of hydration; changes at 25° must be due to other causes.

Keyphrases □ Calcium phosphate, dibasic—direct compression tablets, aging, effect of temperature and humidity □ Tablets, direct compression—dibasic calcium phosphate, aging, effect of temperature and humidity □ Aging—direct compression dibasic calcium phosphate tablets, effect of temperature and humidity □ Dosage forms—direct compression tablets, dibasic calcium phosphate, aging, effect of temperature and humidity

Recently, the physical aging of compressed tablets was studied, and it was found that complex changes of hardness and dissolution may occur over a relatively short period (1). However, because of the limited preliminary nature of that study, no hypothesis was proposed to rationalize these changes. The present report concerns a more detailed investigation of the aging of a directly compressed tablet formulation. In addition to dissolution and hardness measurements, diameter, thickness, disintegration, and tablet weight were evaluated over 16 weeks.

EXPERIMENTAL

The tablets were prepared by direct compression on a single-punch press[1] as described previously (1). The following formulation was used:

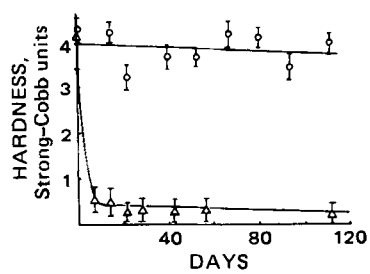

Figure 1—*Plot of hardness data over 16 weeks for dibasic calcium phosphate dihydrate tablets at 25° and 50% relative humidity (○) and at 45° and 75% relative humidity (△).*

[1] Stokes model F, Warminster, Pa.