(18) J. H. Graham, *J. Pharm. Sci.*, **64**, 1393–1396 (1975).
(19) D. Smith and J. Graham, *J. Assoc. Off. Anal. Chem.*, in press.
(20) W. L. Brannon, W. R. Benson, and G. Schwartzman, *J. Assoc. Off. Anal. Chem.*, **59**, 1404–1408 (1976).
(21) R. Venable, Laboratory Information Bulletin 1918A, Food and Drug Administration, Washington, D.C., 1977.
(22) E. B. Sheinin and W. R. Benson, *J. Assoc. Off. Anal. Chem.*, **61**, 55–59 (1978).
(23) J. Y. P. Wu, *J. Assoc. Off. Anal. Chem.*, **53**, 831–833 (1970).
(24) J. Y. P. Wu, *J. Assoc. Off. Anal. Chem.*, **57**, 747–751 (1974).
(25) J. Y. P. Wu, *J. Assoc. Off. Anal. Chem.*, **58**, 75–79 (1975).
(26) W. L. Brannon, W. R. Benson, and M. M. Smith, Jr., *J. Assoc. Off. Anal. Chem.*, in press.
(27) R. P. Barron, W. R. Benson, and J. Roach, *J. Assoc. Off. Anal. Chem.*, **60**, 635–641 (1977).
(28) T. D. Doyle, W. R. Benson, and N. Filipescu, *J. Am. Chem. Soc.*, **98**, 3262–3267 (1976).
(29) T. D. Doyle, W. R. Benson, and N. Filipescu, *Photochem. Photobiol.*, **27**, 3–8 (1978).
(30) M. Maienthal, W. R. Benson, E. B. Sheinin, T. D. Doyle, and N. Filipescu, *J. Org. Chem.*, **43**, 972–975 (1978).
(31) T. D. Doyle and J. Levine, *J. Assoc. Off. Anal. Chem.*, **61**, 172–191 (1978).
(32) T. D. Doyle and S. Barkan, *J. Assoc. Off. Anal. Chem.*, **59**, 1170–1174 (1976).
(33) S. Barkan and T. D. Doyle, *J. Assoc. Off. Anal. Chem.*, **60**, 1022–1029 (1977).
(34) J. D. Weber, *J. Pharm. Sci.*, **65**, 105–108 (1976).
(35) V. K. Prasad, J. P. Hunt, and B. E. Cabana, presented at the National Meeting of the American Pharmaceutical Association, New York, N.Y., 1977.
(36) V. K. Prasad, B. E. Cabana, J. Hunt, W. Furman, D. Cox, C. Wells, and J. Wood, in ref 35.
(37) V. K. Prasad, T. C. Alston, V. P. Shah, K. M. Campbell, B. E. Cabana, D. Masterson, and R. Schwartz, in ref 35.
(38) Stanford Research Institute (Dr. Anbar); see Second Annual Report, SRI Project 4276, Aug 1977, by Dr. Henry Heck et al., Contract 223-75-3006.
(39) R. Barron, W. Benson, M. Maienthal, E. Sheinin, and W. Brannon, FDA, private communication, 1978.
(40) W. R. Benson, M. Maienthal, G. Yang, E. B. Sheinin, and C. W. Chung, *J. Med. Chem.*, **20**, 1308–1312 (1977).
(41) E. Smith, FDA, private communication, 1978.
(42) D. F. Kennedy, *Fed. Regis.*, **42**(208), 56896 (Oct 28, 1977).
(43) W. Cobb, FDA, private communication, 1978.
(44) E. B. Sheinin, W. R. Benson, and W. L. Brannon, *J. Pharm. Sci.*, **65**, 280–283 (1976).
(45) W. R. Benson, G. Yang, M. W. Heitzmann, and L. A. Ford, *J. Labelled Compd. Radiopharm.*, **15**, 343–352 (1978).
(46) M. W. Heitzmann and L. A. Ford, *J. Assoc. Off. Anal. Chem.*, **58**, 995–997 (1975).
(47) L. A. Ford and M. W. Heitzmann, Proceedings of the Symposium on Computers in Nuclear Medicine, 1977.
(48) T. Layloff, NCDA, FDA, private communication.
(49) M. A. Kreienbaum, *J. Assoc. Off. Anal. Chem.*, **58**, 289-292 (1976).
(50) B. J. Westerberger, Method 65 in "Drug Autoanalysis Manual", Richard F. Heuermann, Ed., Food and Drug Administration, Washington, D.C., 1976.
(51) R. D. Kirchhoefer, Laboratory Information Bulletin 1941, Food and Drug Administration, Washington, D.C., 1976.
(52) L. K. Thornton, T. W. Moore, and G. L. Joachims, Laboratory Information Bulletin 1987, Food and Drug Administration, Washington, D.C., 1977.
(53) For further reading on the application of chemical data in medicine, read "Chemistry in Medicine", American Chemical Society, Special Issues Sales, Washington, D.C. 20036, 1977, p 138.

Sources of Chemical Information Used in Antibiotic Certification†

MARY LOU ANDREW* and LOLA G. WAYLAND
Food and Drug Administration, Washington, D.C. 20204

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Because of a legislative mandate requiring the predistribution testing and certification of each batch of antibiotics produced for human use in the U.S.A., the National Center for Antibiotics Analysis (NCAA) must utilize a wide variety of techniques for establishing its official methods. Methods adapted from material submitted by the manufacturers in Forms 5 and 6, as well as from other sources, such as literature searches, the NCAA reprint collection, and cross reference file are described.

The Federal Food and Drug Act requires the use of analytical methods for enforcement. Analyses are directed toward answering the question of whether the product meets the requirements of the Act. Certain restrictions on the methods used are due to the fact that we are a regulatory agency, not merely a laboratory performing analytical procedures.

The law requires that drugs must meet the specifications of the official compendia. For most drugs, this is usually the USP/NF. However, for antibiotics, official specifications and methodology are established by incorporating them into the regulations. The Antibiotic Regulations are published in the Code of Federal Regulations, Title 21, Parts 430–460 for drugs for human use, and Parts 536–555 for the certifiable veterinary antibiotic products. Insulin is covered by Part 429. The CFR is available as an annual update from the Government Printing Office. Between annual editions, announcements for new regulations, changes, and deletions are available in the "Federal Register", the government newspaper which appears every workday. It, too, is available from the Government Printing Office by subscription. More than 500 pages of the CFR are devoted to tests and methods of assay for antibiotics, including sterility, biological tests, microbiological and chemical assays, general and specific chemical tests, and tests on specific dosage forms. Also included are specifications, interpretations, and special tests for each certifiable antibiotic preparation. Therefore, the methods in the CFR are conclusive, even if other more modern methods may be available. The methods must be accurate and precise, enabling us to reproduce our own results, and other laboratories also must be able to use these methods satisfactorily.

Methods of antibiotic certification must be specific, sensitive, precise, and accurate, as well as practical. This paper discusses various sources of analytical methods, and ways in which they have been established.

FORMS 5 AND 6 VALIDATION PROCESS

The CFR defines Form 5[1] as a request to provide for certification of a new antibiotic product. A Form 6 provides data to accompany or precede every initial request for certification of a batch of a "me-too" drug.

The arrival of a Form 5 application is generally not our first indication of the existence of a given new antibiotic entity. The National Center for Antibiotics Analysis (NCAA) subscribes to a number of scientific and trade journals which are reviewed every month, and copies of any pertinent articles are retained

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* Address correspondence to author at FDA, 200 C St., S.W., Washington, D.C.

for our reference file. When the Form 5 is submitted, it is the culmination of a lengthy process of research and development by the parent company, often as much as 7–10 years, in conjunction with the IND reviewers of FDA, and much of this work is published in research papers. Company trade secrets, such as fermentation processes or syntheses of the drugs, are submitted as confidential information until patents are issued.

The submitting manufacturer is obligated to provide NCAA with proposed methods and specifications for bulk testing prior to certification. Although the company is the reigning expert on its own new antibiotic drug, often the methods submitted must be modified or revised, or even new methods substituted or added. This is because NCAA must test and distinguish between all antibiotics of a related group while the company may manufacture only one. There are, so far, nine cephalosporins subject to certification, and no single company makes all of them. However, NCAA tests all of them and the scheme of testing each one must be specific enough to distinguish or characterize each member of this closely related group. The prior experience and expertise of NCAA is an important source of information in testing "new" antibiotics.

Several reasons exist for modifying or adapting methods of analysis submitted by the manufacturer. In the interest of expediency we prefer to keep to a minimum the number of different types of methods published in the CFR. Therefore, once we have established that we can satisfactorily test a new antibiotic drug using the method proposed by the sponsor, we then make whatever modifications may be necessary to fit the product into one of the existing published official methods. In most cases, because of similarities between different members of one class of antibiotics, such as the cephalosporins, they may all be assayed for potency by the same CFR method. Occasionally, however, slight differences in the basic product will preclude this. Then the manufacturer's proposed method with any necessary modifications made by NCAA will be submitted to the Federal Regulation writers for inclusion into the CFR.

The applicant must also provide information about the raw ingredients used in the fermentation or synthesis of the new antibiotic drug with particular attention being paid to the precursors of the antibiotic moiety. An organic solvent or an acid used for the adjustment of pH need not be tested for much beyond identity, but the 6-aminopenicillanic acid (6-APA) to be synthesized into ampicillin, for example, should be assayed and identified, its purity assured, and its source specified.

The manufacturer must describe its procedures for good quality control of the drug, whether or not these procedures ever become part of the certification regimen. Potentially harmful chemicals used in the manufacturing process, such as dimethylaniline or ethylene oxide, must be excluded, or kept below toxic levels on the finished bulk. Stability testing must be established and methods described which can reflect and measure the presence of degradation products. Conditions of storage must be indicated if other than normal precautions are necessary to ensure the integrity of the drug. Data must be supplied upon which expiration dates may be established.

Finally, three exhibit bulks accompany the application with certificates of analysis giving the company's results of their proposed testing. Along with these bulk samples, a reference standard and its test results complete the submission.

When the evaluation is completed and if the new antibiotic is to be certified, the submitting company, as the only supplier, is asked to submit about 50 grams of a lot deemed to be of standard quality, along with results of all testing, such as phase solubility, spectral curves, etc., which characterize it. After thorough testing by NCAA laboratories it is either accepted or rejected as the official FDA antibiotic working standard.

Batch testing of the bulk antibiotic drug is only the first consideration. Next comes the point of the whole exercise —the finished dosage form. While often it is not necessary to apply the full battery of tests to the bulk, the finished product must be tested for potency, identity, and other properties which will indicate adherence to Good Manufacturing Practices and support an adequate shelf-life before and after the product reaches the consumer.

The company must provide the composition and formulation of the product at all dosage strengths. The source of the active ingredient, the quality control tests to be done on each batch, and the tests to be done on each excipient in the dosage form must be submitted. Methods for assaying the content of antibiotic in the dosage form must be free of interference from other components of the formulation. Stability data justifying the requested expiration date are required both at submission of the Form 6 application and at intervals thereafter.

The finished dosage form not only must be pharmaceutically elegant, but also must show its bioavailability. Its bioequivalence with other similar products on the market must be demonstrated.

FORM 7—CERTIFICATION PROCESS

A Form 7[1] is a request for check tests and assays or certification of a batch of a specified lot of an antibiotic drug. The National Center for Antibiotic Analysis receives about 20 000 submissions of Form 7 per year or, in other words, 85–100 lots of antibiotic drugs each working day. The number of tests on each lot may range from a minimum of two, for example, potency and moisture, on a topical ointment, to as many as ten on a sterile injectable product like sodium ampicillin for injection. In one of five branches, over 125 000 individual tests were performed during the last calendar year.

There are 10 classes of antibiotic drugs presently certified, including penicillins with 21 different antibiotic substances; cephalosporins, 9 (two more are currently in the review process); oligosaccharides, 12; tetracyclines, 14; peptides, 9; antifungals, 4; antitumor antibiotics, 6; macrolides, 8; lincomycins, 4; and miscellaneous, 10, for a total of 97 different entities, salts, or esters. There are many different dosage forms, including oral, injectable, ophthalmic, otic, vaginal, and dermatological forms.

The Form 7 is a standardized application form that provides a great deal of information from the manufacturer to FDA. Among the items required from the manufacturer, the following are included: the Form 5 or 6 number; generic and trade name of the product; the applicable CFR section; batch mark; theoretical and actual batch size; date of manufacture and expiration date; type, number, size, and potency of containers; and number of samples submitted for testing. The manufacturer's tests on the batch are included on the Form 7 unless prior approval has been granted for concurrent testing. However, a certificate will not be issued until the manufacturer's test results have been submitted to FDA. The manufacturer's name, address, phone number, and account number (for billing purposes) as well as the signature of a responsible official must also appear on the Form 7. Another very important section of the form requires that the applicant show a batch formula, that is, a complete list of all the active and nonactive ingredients of the batch submitted. This must include name, batch mark, manufacturer of the ingredient, certificate number (if it is an antibiotic), date of latest assay, and quantity used. Based on the information shown by the batch formulation, the analyst can readily make calculations regarding theoretical potency or yield, particularly should aberrant analytical results be obtained.

On final dosage forms, the manufacturer must also submit a printed label from which information is available, such as

the volume of diluent to be added to constitute each vial. Vials of the same labeled potency may be reconstituted with different volumes of diluent, with the resulting solutions or suspensions having a different potency per unit volume, so that doses may be tailored to individual requirements.

When samples for certification or check test are received by the Sample Control Unit of NCAA, accession numbers (DA's) are assigned to them. These are consecutive numbers with a two-letter code identifying the antibiotic preceding them and a single terminal letter signifying the fiscal year following them. This accession number stays with the product throughout the certification process and ultimately becomes the certificate number.

Cross reference cards are maintained in the Sample Control Unit. This file provides ready access to the accession number when only the manufacturer's batch number is known, thus enabling the analyst or sample control clerk to verify previous testing and certification of an antibiotic. The cross-reference cards also provide information about which tests were performed on the bulk batches submitted for check test.

In the near future much of these data will be stored in an automatic data processing system presently being designed. Much work has already been done on this system, which will utilize a dedicated minicomputer for data storage and retrieval. When this system becomes operational it will be the culmination of a number of years of work, both in system evaluation and design, and in data collection.

We anticipate that the data processing system will provide us with much information that is not readily available under the present manual system. For example, at present we are able to know only that a specific lot of bulk antibiotic has been submitted and tested. Under the automated system we will have, in addition, a measure of sample accountability, that is, a running total of the quantity of each lot of antibiotic still available for formulating.

The system is expected to provide us with computer-generated laboratory work cards and eliminate manual transmission of results to the Certification Service Staff, resulting in a significant manpower saving.

OFFICIAL SAMPLES—SURVEILLANCE PROCESS

Even after completion of the analysis of a batch of an antibiotic drug and its certification, more information can be obtained from the batch. A system of postcertification surveillance of antibiotics dosage forms exists in which samples of approximately 5% of all the batches certified are collected from various levels of distribution by Food and Drug inspectors. In this program an attempt is made to sample products representative of all dosage forms and potency levels, preferably at least six months following their certification. In this way, information concerning the shelf-life stability of a dosage form is obtained. These surveillance samples are tested using the same CFR tests as the original samples, with current certification samples as controls whenever possible.

STABILITY PROGRAM

When a new antibiotic drug product is approved and first certified, an expiration date is assigned. The original expiration date allowed is usually 12 months after certification since frequently the manufacturer cannot demonstrate stability on the dosage form beyond that time. As experience is gained, and time passes, the length of the approved expiration period may be extended. When a manufacturer requests an extension of expiration date he is required to submit his own stability data, along with aged samples for tests and assays by NCAA. It is on the basis of both sets of results that the decision to extend, or not extend, the expiry date is made.

NCAA also maintains its own stability program in which representative samples of many dosage forms, primarily those intended for oral use, are stored in a temperature- and humidity-controlled room. These are assayed at regular intervals and the potencies and other properties compared with base values obtained at the start of the study. If no appreciable changes appear, the expiry date may be extended, usually in 6–12 month increments, until the maximum of 5 years is reached.

RESEARCH

Most of the applied research done in NCAA is undertaken because a clear need exists for newly developed methods. For example, when it was learned that the chemical dimethylaniline was used in the production process of some semisynthetic penicillins, immediate steps were taken to develop a method sensitive enough to detect trace impurities of DMA in the bulk antibiotics. This effort, successfully completed, led to a publication in the scientific literature.[2] At some future time this method or other methods developed by the research done in NCAA may be proposed for inclusion into the CFR. These detailed methods are of great value since they are performed only by the government and a few manufacturers who concentrate on antibiotic production. The methods must be dependable and reproducible since inclusion in the regulations may involve considerable effort and time on the part of the government for publication by proposal and final order in the Federal Register and ultimate inclusion in CFR. Other less formal methods or compilations of methods may also be published by NCAA. Some examples include instructions for the determination of antibiotics in milk, originally published in 1958 as a manual. In 1965 NCAA issued a manual for laboratory procedures for detecting penicillin cross-contamination in various drug products, which was recently revised and reissued.[3] A book-length manual by Grove and Randall[4] was prepared which described existing official and nonofficial methods of antibiotic analysis.

A chapter in "Analytical Profiles of Drug Substances", Volume 6, edited by Klaus Florey, published in 1977, described the antibiotic Amphotericin B, using various assay methods to define physical and spectral properties. Work is currently being done for a second chapter, on chlortetracycline, for the next volume.

REPRINT COLLECTION

Throughout the years NCAA has amassed an extensive collection of reprints and a file of references to published articles on antibiotics. These have been indexed using an alphanumeric system so that specific types of information on a particular antibiotic may be readily located. This has proved to be an invaluable source of information on many facets of antibiotic production, preparation, performance, analytical methods, usage, pharmacokinetics, toxicity, and other properties.

Thus, while in other divisions of FDA a literature search is initiated by placing a request for information on a specific subject with the library, in NCAA the first source of data is the cross reference file. A substantial number of papers on file have been written by present and former members of NCAA.

INSULIN CERTIFICATION

Insulin is the only drug product, other than antibiotics, in which every batch produced is subject to certification by FDA.

When insulin was isolated and patented in 1921 by Banting and Best[5] working at Toronto University, they proposed that for proper regulation the testing be done under the control of the Toronto University Insulin Commission. The manufac-

turers were involved in developing the product and selecting appropriate methods of assay. Most of the methods used were existing biological and chemical tests, and are the ones presently in the USP monographs for the seven different insulin dosage forms currently tested.

About 1938, when the original patent was about to expire, FDA began certifying each batch of insulin produced, following the tests prescribed by 21 CFR 429 and the USP.

The basic source of new methods of insulin assay is the NDA, the counterpart of NCAA's Forms 5 and 6. All proposed test changes and new tests must be submitted by the manufacturer in its Master File or supplement. When the new or modified tests have been validated and accepted, they are submitted to the Federal Regulation Writers for inclusion into the CFR. In addition, they are submitted to the USP for inclusion in the next supplement or revision.

There are also semiofficial or "in-house" methods used by both industry and FDA. One is the radioimmunoassay developed for use on those products where the official biological tests are not suitable because of the type of manufacturing process used.

Many changes have been made in the final potency level or chemical form of the product in the more than 50 years since the discovery of insulin, but the major source of the hormone for human consumption remains the pancreases of slaughtered animals.

To summarize, NCAA and the Division of Drug Biology's Insulin Certification group utilize information from various sources including NDA's (Form 5), the sampling process, including certification and surveillance samples, the reprint collection, cross reference cards, and literature searches, as resources for developing their analytical methods.

REFERENCES AND NOTES

(1) 21 CFR 431.50.
(2) M. Margosis, "GLC Determination of N,N-Dimethylaniline in Penicillins", *J. Pharm. Sci.*, 1634–6 (1977).
(3) G. G Carter, "A Review of Procedures for the Detection of Penicillin Residues in Drugs", FDA By-Lines No. 3, Washington, D.C., 1977, pp 119–137.
(4) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics. A Laboratory Manual", Medical Encyclopedia Inc., New York, N.Y., 1955, p 238.
(5) F. Banting and C. H. Best, *J. Lab. Clin. Med.*, 7, 251 (1921–2).

The Development and Compilation of Chemical Information by the Bureau of Drugs Medical Library for the Drug Review Process†

ROY SOLKOT

Food and Drug Administration, Bureau of Drugs, Rockville, Maryland 20857

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Supportive integration into FDA's drug review process of literature surveys of biochemical and biomedical material published worldwide may take place at any stage in the progress of the review. Technical information specialists on the Library staff utilize printed abstract/index sources as well as computer terminals on the Library premises to access at least 37 unique databases. Pertinence embraces numerous fields (pharmacology, toxicology, agriculture, biology, chemistry, food technology, human and veterinary medicine, patents, pesticides, pollution, and psychology), general scientific data, and both completed and projected or ongoing U.S. government-sponsored research.

Little reference as to how chemical information is developed and utilized by the Food and Drug Administration (FDA) Medical Library was made at the previous Symposium on Information Handling and Processing by the FDA presented before the Division of Chemical Information at the 1976 San Francisco Meeting.[1] I would like to introduce this rather fundamental feature into the current Symposium at this time.

The Library participates in all phases of the drug review process, and the chemical literature plays a major role in this support, such participation beginning with the development and organization of the chemistry collection and extending through the stages of retrieval and utilization of this information. The latter responsibility is our focus here.

NATURE AND SCOPE OF LITERATURE SUPPORT

Literature searches support all drug program activities. These might include: initial IND/NDA review to substantiate, verify, or supplement submitted data; development of protocols, guidelines, and standards; background for advisory committee meetings; preparation for hearings; and review of prescription labeling and/or OTC drugs, etc. The bulk of literature searches is concentrated on such subject areas as bioavailability, pharmacokinetics, and pharmacology; analyses and physical properties; therapeutic and/or adverse effects, poisoning, and toxicity involving either single drugs or drugs in combination (including synergism and/or inhibition, in the latter case); carcinogenicity, mutagenicity, and teratogenicity; packaging, quality control, and storage; etc.

Many surveys must be carried out in part or entirely by manual retrieval since they require delving into the pre-1965 literature. They usually focus first on sources that provide abstracts and utilize, among others, such "hard copy" as *Biological Abstracts*, which contains an indexing technique in its format that results in a set of five indexes, any of which may be used alone or in combination with the others. Most important of these indexes, for our purpose, are (1) the natural-language Subject Index in which entries appear in an alphabetically permuted format (this was formerly termed BASIC (Biological Abstracts Subjects In Context) and was compiled from significant terms in the authors' own titles, as well as from added key terms from both bodies of abstracts and original articles, and (2) the Cumulative Concept Index (earlier called the CROSS (Computer Rearrangement Of Subject Specialties) Index) which facilitates retrieval according to approximately 580 subject concepts.

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