Training Modules to Develop Analytical Proficiency for Pharmaceutical Chemists

by

Allen S. Kenyon, Ross D. Kirchhoefer, and Thomas P. Layloff

Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Analysis, 1114 Market St., Room 1002, St. Louis, MO 63101-2045

Reprinted from Journal of AOAC International Vol. 75, No. 4,1992

Abstract

As a part of a training program for new personnel in pharmaceutical analysis and visiting scientists from other government laboratories, the Division of Drug Analysis (DDA) developed training modules to raise incoming college students, technical aides, and newly hired chemists to an acceptable level of job performance. The training modules developed include Liquid Chromatography(LC), Gas Chromatography (GC), Dissolution Testing, Thin-Layer Chromatography (TLC), Ultraviolet-Visible Spectrophotometry (UV), Infrared Spectrophotometry(IR), and Data Collection and Recording (as related to preparing worksheets in accordance with the requirements of the U. S. Food and Drug Administration).

Each module is designed to be delivered in minimum of time. By using a combination of commercial audio-visual materials and a training concept developed at DDA, the targeted group could be trained with minimal supervision. This approach does not require experienced analysts to be placed as full-time, one-on-one trainers, thus cutting the cost of the training. The training scheme could be extended into other applications in industry, academia, or government by targeting training modules characteristic of their operation. These modules are also used as refresher course for experienced analysts who have not practiced these techniques recently. The modular concept provides an excellent way to train employees quickly.

Introduction

Colleges and universities do not have the time or resources to educate their students in the array of techniques required to perform the specific pharmaceutical analyses required by the Division of Drug Analysis (DDA) of the Food and Drug Administration (FDA). To address these needs, DDA established internal training programs to assure that our analysts have proper instruction in the analytical techniques and methods that are common to our laboratory. In the past, training programs were developed and conducted whenever new analysts were hired. Assignment of trainers depended on who was most readily available and on our current workloads. Each trainer had to spend much time collecting the materials and covering them on a one-to-one basis with the students. Such programs were very expensive, since our experienced analysts were selected to perform this training. In addition, the program suffered because there was no syllabus or structure to ensure that all incoming analysts covered the same materials adequately.

Analysts at the DDA usually follow the compendium procedures listed in United States Pharmacopeia (USP) (1). These directions are written for the experienced analyst; the untrained analyst finds the procedures difficult to follow. In order for the untrained analyst to reach the expected efficiency, some additional training was needed. This need prompted the development of the training modules.

Information Gathering

The first task was to find the prior level of training or instruction for the targeted groups. Comprehensive interviews were conducted with employees representing most of the targeted group with the aim of establishing the level and type of training needed.

All employees interviewed had majored in chemistry or physical science majors and had some background in analytical chemistry. Many of them had taken a course in instrumental analysis and were familiar with analytical terminology. Most of the student employees were majoring in physical science or pharmaceutical chemistry and had training in the instrumental methods. College students do not have enough time to become expert in any analytical technique, far less in many different application methods. The instrumental analysis courses vary, but generally they are one-semester courses that include an introduction to many different instrumental techniques and some short illustrative laboratory experiments. The course contents depend upon the facilities and the staff of the various colleges. Many of the laboratory experiments are performed on instruments set up by the laboratory assistant,so that the student does not have the opportunity to become familiar with any of the instruments. The interviews established that a training level equivalent to a third-year college course was needed.

Development of Training Modules

Each training module was supplemented with a commercial audio-visual presentation in conjunction with hands-on training covering the techniques that DDA chemists normally use. Savant (2) has developed a series of audio-visual training courses that cover many of the analytical methods and give the basic theory and principles of operation. The Savant courses are available either as slides with audiotape cassettes or as videotapes. The tape-slide versions proved to be the most useful because the presentation could be stopped at any point to allow discussion. These courses were prepared by recognized experts in their fields. Because the Savant series did not cover dissolution, the DDA developed a tape-slide course for this test.

The module materials were developed to train the analyst in the specific methods described in USP XXII.

Since pharmaceutical chemists use USP methods in most assay work, the analysis of drug formulations found in the USP formed the target goal of the training. All training modules consisted of the following additional sources of materials: Savant tape-slide course, simplified instrument operating instructions taken from manufacturers' instrument manuals, and a method of analysis selected from an accepted official source (1, 3).

The next step was to select training samples of dosage forms of the drugs for each training module. Drug formulations were selected to give the trainees experience in handling and preparing solutions required for analysis of different

types of dosage forms, i.e., tablets, syrups, injections, pure powders, etc. The samples were selected to highlight the analytical techniques involved and to fulfill specific aims that were deemed important. Included in the training was preparation of FDA worksheets to record the laboratory data, which might later be used in a court of law. Good laboratory and analytical practices were included in the preparation of samples and handling of all the chemicals. A draft of each module was prepared and circulated to senior chemists and supervisors for their comment and recommendations. Their comments were included in the final draft. The training modules are designed not to fit a particular brand of instrument but to be applicable to the instruments in use in any laboratory. Each module is supplemented by additional information about specific equipment make it easier for the trainees to use the instruments.

Training Steps

The training modules were tested in the laboratory by part-time student employees to see if the modules were written clearly and were at the correct instructional level. Three students at the third-year college level were assigned to undertake each training module. Although the students worked as a team, each student was required to perform the analysis and submit results. The team worked with a trainer who was available at all times to answer questions. Questions on the theory or the operation of the instrument were answered with special care. Each student noted any unclear portions of the training, and was debriefed to obtain his or her recommendations on the training module and analysis. The trainers discussed the results of the nalysis with the trainees, and evaluated their performance. Their recommendations have been incorporated into a revised version of the module. Each training module was evaluated similarly. The final draft of each module as reproduced after these comments and further evaluation by a professional technical journal editor.

Final Testing of Modules

The final draft of each instrument module was further tested by 3 visiting foreign chemists from the Ministry of Commerce, Saudi Arabia, to ensure that the training modules were not ambiguous and were consistent with their needs. This allowed the opportunity to clarify any misunderstandings due to different interpretations of language. Words with only one meaning were chosen.

The trainer met with the visiting scientists to introduce the planned program. After the introduction, the trainees began each module by studying the Savant tape-slide presentation on the analytical technique. Sufficient time was allowed for individual study and for making notes before discussion with the trainer. The theory was clarified as needed. The trainees worked as a team, with each member preparing samples and performing the instrumental analysis.

As the trainees began to operate the instrument, they became familiar with all

aspects, including its setup and the performance of suitability tests. The trainer was available for consultation or for explanation of the instrument. Whenever possible, the training was performed on a manual instrument rather than on a computer-controlled type. Use of the manual instrument allowed a better understanding of the instrument and the method, and no time was lost in learning specific keystrokes of computer operation. After the objectives specified in the training module were completed, the training was extended, time permitting, to an automated system.

After becoming familiar with the instrument operation and establishing instrument suitability, the trainees were ready to begin the analysis of the selected sample. Most of the training methods of analysis were based on the method described in the USP. The USP does not include methods that utilize all of the instrumental techniques that DDA felt were important; therefore, we used the well-tested procedures in Official Methods of Analysis of the AOAC (3).

The trainees consulted with the trainer when clarification was needed or when questions arose. After the analysis, the trainees were debriefed and the data were compared. If the results were not acceptable, discussion with the trainees usually uncovered the problem, and calculations were corrected or the analysis was repeated. This format was used for all the training modules. The trainees' recommendations were incorporated into the final draft of the training module.

Determination of the accuracy and precision for each analysis was included. The trainee was able to observe at first hand how to follow the USP procedures. Many of them give a shortened version of the necessary calculation, which makes it difficult for the untrained analyst to follow the steps required to arrive at the final solution. A logical stepwise equation was developed that was applicable to most analyses.

Training Modules

LIQUID CHROMATOGRAPHY (LC)

The LC training module calls for reversed-phase columns, as they are most commonly used in drug analysis, and includes, care of the columns and troubleshooting. Tableted aspirin was selected as the drug sample to be analyzed (4). The analysis consisted of an assay for aspirin and the measurement of a possible hydrolysis product (salicylic acid). The method allows the determination of the major component at a high concentration and the determination of a possible breakdown product present at very low concentration. Samples are prepared according to the USP procedure for measuring both components. Often no salicylic acid will be present. The USP establishes a minimum resolution requirement between aspirin and salicylic acid. Careful attention to the instrument setup, column, mobile phase, sample preparation, and proper integration was required to obtain reliable results.

GAS CHROMATOGRAPHY (GC)

Few USP monographs use GC for assay of the active ingredient of a drug formulation. Many former USP methods have been replaced by methods that use LC. The greatest use of GC in the USP is the analysis for residual solvents or alcohols. Testosterone cypionate injection in oil was selected as the formulation for test (5). After the analysis of testosterone cypionate, the training could be extended to the determination of alcohols in cough syrups, w hich is recommended.

THIN-LAYER CHROMATOGRAPHY (TLC)

Thin-layer chromatography is used extensively for identification of the main component and to establish the presence of impurities in drug formulations. This training module is based on the use of silica-gel coated plates (normal phase chromatography). Sulindac powder (USP Raw Material) was chosen as the drug for TLC training (6). This drug may contain several impurities which can be separated and detected. Impurity standards must be available to mark the Rf of the impurities.

Two methods for estimating the concentrations of impurities are used. In one method, a quantitatively diluted solution of the bulk drug is used as a reference to estimate the concentration of the impurities in the bulk drug; this technique is more economical for training purposes than the use of expensive reference standards. The other method requires the use of impurity standards to identify and directly calculate the concentration and indicate the identity of the impurity.

ULTRAVIOLET AND VISIBLE SPECTROPHOTOMETRY

The ultraviolet/visible (UV/Vis) absorbance spectra of drugs have been used extensively for quantitative determinations. Now UV is the detector of choice in LC and dissolution. The USP has reduced the number of monographs based on non-stability indicating ultraviolet absorbance while dramatically increasing the number of stability indicating LC monographs. Most drugs have a chromophoric group or groups which give excellent absorbance spectra in such solvents as water or methanol. A sample of chlorzoxazone tablets was selected for this analysis (7). The first analysis was performed at a single wavelength according to the USP instructions. The training was modified to can the solutions over the entire UV-VIS spectral range. After the trainees gained experience with the USP and modified procedures, some training was given on the use of diode array instrumentation as a UV/VIS spectrophotometer and as an LC detector. The trainees had the opportunity to experience different characteristics of the ultraviolet instruments, potential interferences, and calculations. The application of Beer's Law was demonstrated.

INFRARED SPECTROPHOTOMETRY

This training module included qualitative (identification) and quantitative portions. Infrared (IR) spectrophotometery is a technique used routinely by the USP as a means of identity. The USP identity tests usually require diluting the drug with potassium bromide, making a solid pellet, and

comparing the spectrum with that of the standard. Infrared spectophotometry can also be used quantitatively under certain conditions.

The AOAC assay for meprobamate tablets (8) offered a suitable system for quantitative analysis, since the drug's carbonyl group absorbs in a wavelength region free from solvent interference. We wanted to demonstrate that IR spectrophotometry can be used quantitatively under certain conditions, i.e.,if the drug can be prepared in solutions having known concentrations in a suitable solvent. A standard grating spectrometer was used so that the student could learn basic principles and manually calibrate the instrument.

The trainees were shown how to extract the active drug from the tablet matrix and prepare potassium bromide pellets for identification purposes. Fourier transform IR spectrophotometry was also used to examine the identification pellet. The student compared the spectra obtained with the diffraction grating and Fourier transform IR instruments.

DISSOLUTION

All oral dosage forms must allow the drug to be delivered to the proper system in the body. For each dosage drug form, USP and FDA have requirements that specify a certain minimum fraction to be dissolved at a specified time. Dissolution training was carried out on a common 6-spindle drive unit using 1 L kettles to hold the dissolution medium. Both the USP Apparatus 1 (basket) and Apparatus 2 (paddle) were used.

The training covered the setup of the instrument, including proper alignment of all parts. Standard USP calibrators were used to test the instrument suitability before measurements were made on the sample. Aspirin tablets were used as the drug sample (4). This drug had been used earlier for the LC training module; thus, 2 modules could use the same sample.

Discussion

The order of training for each instrument is not critical; however, it is recommended that the initial module dealing with the preparation of samples and standard solutions and a discussion on writing the worksheet be covered before dealing with actual pharmaceutical formulations. That should be followed by the UV training because UV spectrophotometry is used in dissolution methods and as a detector in LC procedures. Training in the LC technique required the greatest time; therefore at least 2 weeks should be allowed for minimum training. After the analysis of the drug formulation outlined in the training module, another drug sample might be analyzed to reinforce the training if time permits. After successfully completing the training methods, the analyst should be able to perform other drug analyses as specified in the USP. No effort was made to include method development in the training but approaches to development of methods and validation were discussed with the trainees as opportunities arose.

Videotaping the demonstration of recently installed instruments by factory

representatives is a technique DDA has found useful to either supplement training or review instrument operation. These videotapes are used to orient inexperienced analysts or as refreshers for experienced analysts in the correct operation of that particular instrument. We have found that by having individuals view the videotape and then review the operation manuals, our training time for correct operation of the instruments has been substantially reduced. Also, individuals may review these presentations at their convenience, without feeling pressured by the presence of a trainer or other analysts.

We are currently examining computer software simulations (9) that have the capacity to solve problems similar to a method development problem, and we plan to add them to our training modules. Computerized simulations could be added for an advanced training program.

The aspirin tablet monograph was chosen for the LC training for several reasons. Aspirin hydrolyzes to salicylic acid; thus, the trainees should realize that salicylic acid must be determined first. Yet many do not make this association. The trainees are made aware of the importance of sample preparation and potential solvent-related problems. Different wavelengths are used for detection of aspirin and salicylic acid; this shows the power of a variable wavelength detector versus a fixed wavelength detector. The USP aspirin tablet monograph method as written seems complicated to the novice. The trainees must organize the manipulations and do so expeditiously to achieve success with the salicylic acid determination. The resolution value specified by the USP may require the trainees to look at several columns before choosing one which meets the requirement. This process makes the trainees aware of the characteristics of good and bad columns. Gradient elution and diode array detectors were discussed.

Testosterone cypionate injection in oil was chosen not only because it is one of the few remaining packed column GC assays remaining in the USP, but also because the oil base poses a sampling problem to the trainees. The trainees found that the formulation is best sampled by the use of a TC (to contain) pipet instead of a TD (to deliver) pipet, and they quickly discovered why an internal standard is needed when making manual injections. In addition, the trainees determined the alcohol content of a cough syrup formulation by the procedure outlined in the USP. Some time was allocated to discuss the use of capillary columns.

The TLC training includes the use of 2 methods to estimate impurity levels in drug samples. The USP monograph was initially followed. This procedure uses normal phase chromatography with a silica gel plate. The trainees estimate the concentrations of the impurities by spotting a series of dilutions of the standard with a maximum concentration of 1.0% of the main spot. The plate is developed and the impurity spots are examined. The sum of as the intensities of the impurity spots should fall below the intensity of the standard spot corresponding to 1.0%, the USP limit. The trainees are

asked to repeat the experiment, except that they are given secondary impurity standards to weigh, dilute, and spot along with the sample. They are then asked to identify and quantitate impurity spots found in the sample. The results found by this direct quantitative procedure are compared with the estimated results obtained with the USP procedure. The trainees can readily see that the absorptivity of sulindac is different from that of the impurities identified, so that different results are obtained with the 2 techniques.

Before the introduction of LC, almost every sample received for analysis was first tested by thin-layer chromatography to determine identity and check for impurities. Thin-layer chromatography can be used for quantitative analysis, and the USP uses the technique for analysis in certain steroid monographs.

The UV-VIS spectroscopy training module was included to show the trainees how the absorption spectrum of a molecule changes with wavelength. The USP monograph calls for making the determination at a single wavelength. After the trainees finished the assay, they were asked to obtain the entire UV-VIS spectrum in the range (200-800 nm) of the sample and standard, and repeat the calculation at the wavelength of maximum absorbance, if it was different from the wavelength specified in the USP procedure. This training not only demonstrated how an assay can be accomplished, but also enlightened the trainees as to how variable wavelength and diode array detectors can be better used for LC analysis and identification. Discussion topics included sources of error from formulation excipients, other potential problems, the effect on absorbance of changing wavelength, and calculations.

Meprobamate in tablet form is a classic example for illustrating an infrared analysis. The IR training consisted of a quantitative and qualitative portion. The AOAC method was modified because pentaerythritol tetranitrate was not an ingredient of the tablet. Meprobamate has a strong carbonyl band in the range of 5.0-6.5 Fm that can be used for the quantitative determination. In the USP, infrared analysis is mainly used for identification. Occasionally, however, IR spectrophotometry may be the easiest or the only approach to obtaining quantitative results. We wanted the trainees to be aware of this capability.

The trainees performed the quantitative experiment in the transmission mode of the instrument, then converted transmission to absorbance before quantitative calculations were made in accordance with Beer's law.

The trainees were made aware of problems associated with the quantitative and qualitative IR techniques. Limited discussion about identifying the absorption bands in the IR spectrum were held.

The dissolution training with aspirin tablets highlighted some important points. One observation is that the paddle technique usually gives lower results. The wavelength specified in the USP method is 265 nm, which is the isosbestic point for aspirin and salicylic acid. Questions posed about why this wavelength is used were answered. The advantages and disadvantages of the

paddle and basket techniques were discussed in detail, especially in applying the test to non-official formulations.

A financial savings was realized by using this modular method of training as compared with the one-on-one system. On-the-job training required a fully trained analyst at a salary range of $20-25 per hour for a period of at least 3 months, and several trainers were required causing a scheduling problem. The modular method required one trainer approximately 1 day per week, and this person could train 3 persons at a time.

All trainees received identical training which permitted evaluation of the individual performances. Any deficiencies were corrected before they were allowed to perform an unsupervised analysis. The trainees were considered capable of independent analysis on any of the instruments after completion of the training modules in 12 weeks.

The trainees concluded that they were adequately trained and could carry out analyses described in the USP. They were assigned an analysis on a drug sample that was not a part of the training to make sure that they had been adequately trained. A more efficient and economical training program is realized by the modular method.

Acknowledgments

We express our sincere appreciation to W. B. Furman for his reviews and editorial guidance in the preparation of the training modules and this manuscript.

We thank L. Jones, H. D. Drew, H. D. Coffman, C. M. Kerner, and P. A. McCullen for their administrative guidance.

The following assisted in developing the training modules by comments or performing the described analyses:
J. C. Black, T. W. Moore, S. A. Farag, M. A. Kreienbaum, J. F. Brower, Y. H. Juhl, J. H. Young, E. Jefferson, B. Rubis, S. Austermann, T. Gummersheimer, J.Spaedy, D. Naes, and S. Springs from DDA;
Saleh H. Al-Baqer, Abdulrheem Al-Gedaani, and Walled A. Al-Shobakie from the Ministry of Commerce, Saudi Arabia.

The contributions from these persons made this project possible.

REFERENCES

(1) U. S. Pharmacopeia (1990) 22nd Rev. U. S. Pharmacopeial Convention, Rockville, MD

(2) Savant, Sloan Audio Visuals for Analysis and Training
P. O. Box 3670, Fullerton, CA 92634

(3) Official Methods of Analysis (1984) 14th Ed., AOAC, Arlington, VA
(4) U. S. Pharmacopeia (1990) 22nd Rev., U. S. Pharmacopeial Convention, Rockville, MD, pp 113
(5) U. S. Pharmacopeia (1990) 22nd Rev., U. S. Pharmacopeial Convention, Rockville,MD, pp. 1327
(6) U. S. Pharmacopeia (1990) 22nd Rev., U. S. Pharmacopeial Convention, Rockville, MD, pp. 1304
(7) U. S. Pharmacopeia (1990) 22nd Rev., U. S. Pharmacopeial Convention, Rockville, MD, pp. 303
(8) Official Methods of Analysis (1984) 14th Ed., AOAC, Arlington, VA, secs 36.233
(9) Rittenhouse, R. C.(1988) J. Chem. Ed.; Software 1B, J CE Software, Dept. of Chemistry, University of Wisconsin. 1101 University Ave., Madison, WI 53706