CRC Handbook of Spectrophotometric Data of Drugs

Irving Sunshine

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CRC Series in Analytical Toxicology

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CRC

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data

Main entry under title:

Handbook of spectrophotometric data of drugs.
(CRC series in analytical toxicology)
Bibliography: p.
Includes index.
1. Drugs--Analysis. 2. Spectrophotometry.
I. Sunshine, Irving. II. Series.
RS189.H28 615'.19015 80-22117
ISBN 0-8493-3571-X

Direct all inquiries to CRC Press, Inc., 2000 N.W. 24th Street, Boca Raton, Florida 33431.

International Standard Book Number 0-8493-3571-X

Library of Congress Card Number 80-22117
Printed in the United States

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Handbook of Analytical Toxicology
Irving Sunshine, Editor

Handbook of Mass Spectra of Drugs
Irving Sunshine, Editor
Michael Caplis, Assistant Editor

Handbook of Spectrophotometric Data of Drugs
Irving Sunshine, Editor

PREFACE

"The old order changeth, yielding place to the new". This truism is attested to by the flood of scientific data on drugs which has been published in the last few years. This plethora should be at the fingertips of scientists so that they can find and use it easily. Thus, two Volumes have been compiled to compliment the chromatographic data accumulated in the CRC Handbook Series in Clinical Laboratory Science, Section B: Toxicology. One of these Volumes concerns itself with spectrophotometry and the other with mass spectrometry. In each of these Volumes, a presentation of the particular aspect precedes the tabulation of the assembled data. These data are permuted in several ways so that the analyst may find the particular datum he needs in its sequential arrangement. Using this format may be redundant, but this was done with the user's best interest in mind. His ability to search for the information he requires must be facilitated so that these volumes truly become "desk side" references.

Compiling and collating the various tabulations is a tedious, painstaking, laborious process which can never be complete. There are few comprehensive sources from which one can abstract the desired information. The number of products with which one is concerned in Analytical Toxicology keeps growing, thanks to the ingenuity of medicinal and pharmaceutical chemists. This growth precludes the inclusion of all substances in the tables. Also, data on many older preparations are not included simply because it is very difficult to get all the desired information from original sources. While many laboratories have been most generous in this cooperative effort, a significant number could not find the necessary time and personnel to provide requested facts.

As the compilations such as those included in these volumes demonstrate their value, successive efforts will enlarge and improve them. In the coming age of computer technology, the black box may replace these books. Until that happens, I trust the user will find these volumes helpful. Input is also helpful, so an open invitation is extended to each user of these volumes to submit corrections, complaints, and additional data.

Obviously, all this could not be achieved by one person working alone. To the many scientists who contributed their little bits to these volumes go my and your profuse thanks. Without their help these volumes would never evolve.

Irving Sunshine
Cleveland, 1980

INTRODUCTION

Spectrophotometry is frequently used to obtain qualitative and quantitative data. Quantitation requires qualitative information, which may be available through history (direct knowledge or allegation of the qualitative composition of the samples) or by some other analytical technique (chromatography) that identifies the component in question. Qualitative identification may also result from spectrophotometric data.

Should an analyst require spectrophotometric data for a given substance, the alphabetical indices should be used to locate the available information. However, more often than not, the analyst's problem is to identify a substance from spectrophotometric data. To this end, the sequential tabulation of ultra-violet absorption peaks has been made and should be used.

In the tabulation each of the ultraviolet peaks of many substances are listed along with their absorbance, as this information is available. Usually searching for characterizing absorption peaks reveals several substances with similar peaks. Each of these compounds' ultraviolet absorption curve can be located (alphabetical listing) and compared with the one in question. This should help identify the substances in question, but ultraviolet absorption spectra are not sufficient for complete identification because there are many "look alikes" - similar chemical substances with almost identical ultraviolet absorption curves. Despite this limitation, for a tentative identification, comparison of an unknown's spectrum with that of a known prototype can be and is helpful (see Classification of Ultraviolet and Absorbing Compounds According to Molecular Structure).

A similar tale can be told for the infra-red collection. Finding an individual curve in the collection is relatively easy, thanks to the alphabetical listing. Identifying a substance based on it's IR spectrum is difficult. The correlation charts can be of some help, but only to well experienced individuals. Transposing the data to the Sadtler format, is easily done, and is explained in the text. Once this is done, the Spec Finder may help in the identification process. Comparison with the actual curve may then be made if the Sadtler collection is available. Failing this a reasonable number of infrared absorption curves are given in the text for ready reference. This array of IR spectra of drugs is arranged sequentially by major peaks for direct comparison with the spectrum in question.

Atomic Absorption and Fluorescence techniques are seldom used for qualitative analysis. Hence the tabular data are arranged alphabetically so one can quickly ascertain whether or not the particular substance has been analyzed by these techniques, and also, where in the literature one may readily find more detailed information.

In assembling the mass of minutiae for these volumes it would be amazing if there were no errors. Hopefully these are minimal, but they are expected. By reporting those that do occur to me corrections can and will be made. Your help is solicited.

Irving Sunshine, Ph.D.

THE EDITOR

Dr. Irving Sunshine is Chief Toxicologist at the Cuyahoga County (Cleveland), Ohio Coroner's Office; Professor of Toxicology in the Department of Pathology and Professor of Clinical Pharmacology in the Department of Medicine at the School of Medicine, Case Western Reserve University; Chief Toxicologist for the University Hospitals in Cleveland, Ohio; Director of the Cleveland Poison Information Center; and Editor-In-Chief for Biosciences for CRC Press, Inc. He is a Diplomate of both the American Board of Clinical Chemistry and The American Board of Forensic Toxicology and is on the Board of Directors of both these organizations.

Born in New York City, he obtained all his formal education in various Colleges of New York University, earning the B.Sc., M.A., and Ph.D. degrees. While earning his Ph.D., he taught chemistry in various colleges in the New York area and during the war, he worked during the "grave yard" shift on a pilot plant for the separation of uranium isotopes as a part of "The Manhattan Project". His development in toxicology was encouraged by two memorable mentors, Dr. Alexander O. Gettler and Dr. Bernard Brodie.

Prior to moving "west" to Cleveland, Ohio, where he has been since 1951, he served as the Toxicologist for the City of Kingston (N.Y.) Laboratory and for Ulster County. Since coming to Cleveland he has developed many interests which resulted in the publication of over 100 papers and several monographs. He is also a member of the boards of editors of many of the major toxicology journals.

His educational activities extend beyond the local college campuses. In the course of years he has organized and participated in numerous toxicology workshops which were held in many centers throughout the United States. As a member of the Education Committee of the American Association for Clinical Chemistry, he has been responsible for the National Tour Speaker Program, the Local Section Guest Lecturer Program, and the Visiting Lecturer Program. In recognition of his achievements in clinical chemistry, Dr. Sunshine was presented with the Association's "Ames Award" in 1973. Further recognition was accorded Dr. Sunshine by the Italian Society of Forensic Toxicologists which voted to make him an Honorary Member of that group. In 1978, The International Exchange of Scholars awarded him a Fulbright Visiting Professorship to the Free University of Brussels.

He is also a "has been". He has been President of The American Association of Poison Control Centers, Chairman of the National Council for Poison Control Week, Chairman of the Toxicology Section of The Academy of Forensic Sciences, and Chairman of the Cleveland Section of The American Association for Clinical Chemists, as well as a former member of the Association's Board of Directors.

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TABLE OF CONTENTS

ULTRAVIOLET — VISIBLE SPECTROPHOTOMETRY

INFRARED SPECTROSCOPY

FLUORESCENCE

ATOMIC ABSORPTION SPECTROMETRY

INDEXES

Ultraviolet-Visible Spectrophotometry

ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY

INTRODUCTION

Irving Sunshine

The interest of toxicologists in ultraviolet (UV) spectrophotometry has waned in recent years, due both to improved and innovative gas and high-pressure liquid chromatographic techniques and the development of methodologies utilizing fluorescence spectrophotometry and immunological reactions. The simplicity of ultraviolet analysis both for quantitative and qualitative purposes should not be overlooked. Given sufficient sample, this technique can reveal much about an unknown substance by simply recording its ultraviolet spectrum. Drugs present in a gastric lavage of a patient or the stomach contents of a deceased can often be identified by two-step extractions followed by ultraviolet analysis. Blood and urine specimens can be extracted in a similar fashion to yield solutions whose ultraviolet absorption characteristics may indicate whether or not certain substances are present in the original sample. Using ultraviolet spectrophotometry, Jatlow[1] has elaborated a relatively simple but extensive procedure for the detection of acid and neutral drugs.

A particular advantage of ultraviolet spectrophotometry is that it does not destroy the sample. After recording an ultraviolet spectrum, one can reextract the solution and recover the absorbing substances for subsequent analytical procedures. This is in sharp contrast to gas chromatographic and immunologic procedures, wherein the substances in question may not be recovered after the analysis is completed. If ultraviolet procedures are used as the first steps in a comprehensive analytical scheme, the data involved are valuable whether or not an absorption curve is obtained. If one is not obtained, those substances whose characteristic absorption would have been seen are presumed to be absent. If an absorption curve is obtained, one now has information that indicates the presence of one of the substances whose characteristic absorption curve was obtained. By reextracting the solution being analyzed, the substance in question can be isolated and can be subjected to different analytical techniques to confirm and characterize the substance in question. Thus, for relatively little effort, significant data may be obtained. Some basic principles of ultraviolet spectroscopy and the instruments used to obtain ultraviolet absorption spectra are briefly discussed below. Readers requiring additional knowledge should consult some of the excellent treatises available: Willard, H. H., Merritt, L. L., and Dean, J., *Instrumental Methods of Analysis*, 5th ed., Van Nostrand Co., New York, 1974, Pesez, M. and Bartos, J., *Colorimetric and Fluorometric Analysis of Organic Compounds and Drugs*, Marcel Dekker Inc., New York, 1974, and *Absorption Spectra in the Ultraviolet and Visible Regions Cumulative Index (16-20)*, Lang, L., Ed., Academic Press, New York, 1976.

OPTIMUM PARAMETERS FOR SPECTROPHOTOMETRY*

ABSORPTION SPECTROSCOPY: THEORY AND INSTRUMENTS

When a beam of light is passed through a material, the incident radiation (I_o) will always be more intense than the emergent radiation (I). Attenuation of the incident radiation may be attributed to (1) reflections at the interfaces between air and cell wall and sample and cell wall, (2) scattering by any suspended particles or dust in the sample, and (3) absorption of the radiation by the sample. In normal applications, light absorption is the primary factor reducing the incident radiation.

Note: Fluorescence can often increase the apparent intensity of the emergent radiation which may result in inaccurate absorption measurements. Effects of fluorescence can be reduced by chemically inhibiting fluorescence, locating the sample some distance from the detector, or using appropriate cutoff filters to block the fluorescence occurring at longer wavelengths than the incident radiation.

When using monochromatic (single wavelength) light, the fraction of the radiation absorbed by a substance, ignoring losses due to reflections and scattering, will be a function of the concentration of the substance in the light path and the thickness of the sample.

Mathematically, this function can be defined as:

$$A = \log\left[\frac{I_o}{I}\right] = abc$$

where A is defined as the absorbance of the sample, A is a wavelength-dependent proportionality factor called the absorptivity, b is the light pathlength through the sample, and c is the concentration of the sample in appropriate units. Since A is unitless, the coefficient a will have units reciprocal to those of the product bc. Thus if the concentration is expressed as molarity and the pathlength in centimeters, the units of A will be M^{-1} and cm^{-1}. The expression $A = abc$ is known as the Bouguer-Beer law, or more commonly, Beer's law.

The ratio I/I_o is defined as the transmittance or transmission, T. Percent transmission is $T \times 100$. Absorbance then can be defined as $A = -\log T$. Many spectrophotometers measure both transmittance and absorbance.

Note: The linearity of Beer's law is subject to some restrictions. Concentration may not always be directly proportional to absorbance. Chemical and physical interactions and insrumental limitations can produce deviations, particularly at high concentrations or high absorbance. Sample scattering, fluorescence, decomposition, and saturation are examples of sample-related problems affecting linearity. Hydrogen bonding, ion pair formation, solvation and chemical reactions can cause incorrect calculations of sample concentration as the solvent concentration is varied. Instrument factors that can affect apparent deviations from linearity are stray light, resolution, and both absorbance and wavelength calibration. Most of the chemical and instrument effects described above will result in suppression of absorbance as concentration is increased. To prevent errors, Beer's law plots should be constructed for each sample system with at least three points within the expected concentration range of the unknowns.

Qualitative Analysis

A plot of absorbance as a function of wavelength is referred to as an absorption

* Courtesy of Varian Associates, Inc., Palo Alto, California, U.S.A.

spectrum. Because the wavelength of the attenuated radiation is dependent on molecular structure, samples often can be identified by their absorption spectra. The portion of a molecule which absorbs radiation at a particular wavelength is defined as a chromophore. For example, the conjugated sequence of 11 double bonds is the chromophore in all tras-β-carotene and is responsible for its absorption of radiation between 350 and 550 nm. Spectra catalogs are available which give detailed spectral data for numerous compounds.[2-4] These references can be indispensable when using UV-Vis spectroscopy for qualitative analysis. (See pages 107-152 for UV data on drugs.)

Quantitative Analysis

Absorption of radiation by moleules at specific wavelengths is more frequently used for quantitative than qualitative analysis owing to the direct relationship between absorbance and concentration. Sensitivity of photometric analyses is dictated by the magnitude of the absorptivity and the minimum absorbance which can be measured with the required degree of certainty. For example, if molar absorptivity for a compound is $10^4\ M^{-1}\ cm^{-1}$ and the minimum detectable absorbance is 0.01, then, for a 1-cm pathlength, the minimum concentration which can be detected is:

$$c = \frac{A}{ab} = \frac{0.01}{(10^4 M^{-1}\ cm^{-1})\ (1\ cm)} = 10^{-6}\ M$$

The sensitivity can be improved by at least an order of magnitude using longer pathlength cells.

Multicomponent Analysis

If a sample contains more than one substance which absorbs light in the same wavelength region, the total absorbance is normally the summation of absorbances of all the contributing species. Because of this additive property, binary and ternary mixtures can be analyzed by measuring absorbance at several wavelengths and algebraically solving the resulting equations simultaneously. The number of absorbances measured for the mixture at separate wavelengths must equal the number of components in solution.

Chemical Equilibrium

When two compounds in solution are in chemical equilibrium and both compounds contribute to absorbance in a particular wavelength region, there will be at least one wavelength where absorbance will be a function of the total concentration of both species but will not depend on their relative concentrations. (Figure 1). This wavelength is known as the isosbestic point. When repetitively scanning a reaction mixture, absence of an isosbestic point is proof that more than two compounds are contributing to the absorption spectrum. Presence of an isosbestic point, however, does not sufficiently prove that only two compounds are present in solution. A third compound may be present but not contributing to the absorbance in the wavelength region scanned. An isosbestic point is a necessary but not sufficient condition for a two-component equilibrium reaction.

An isosbestic point (P) can be very useful when calculating the total concentration of an equilibrium mixture:

$$A_P = a_P b\ (c_1 + c_2)$$

$$c_1 + c_2 = c_{tot} = \frac{A_P}{b a_P}$$

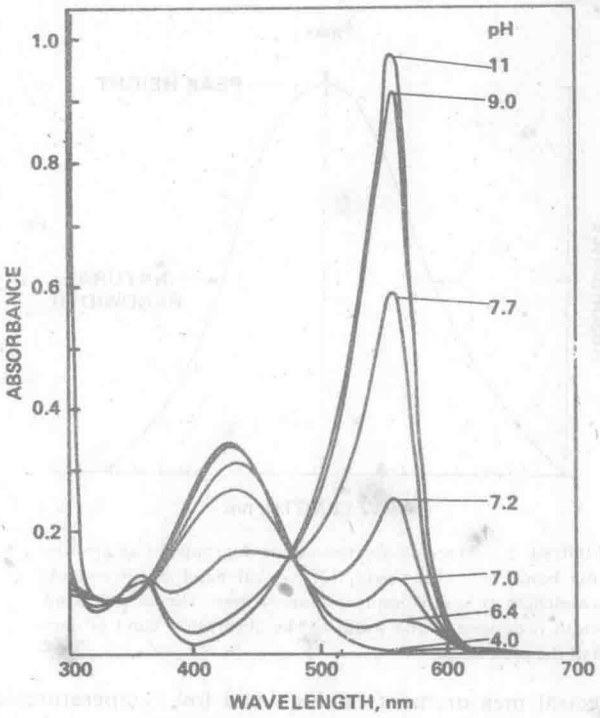

FIGURE 1. Chemical equilibrium between two solution components is demonstrated by the presence of well-defined isosbestic points. In the conversion of phenol red from the basic to the acid form, isosbestic points are recorded at 480, 367 and 388 nm.

Difference Spectroscopy

A technique which requires high potentiometric accuracy and linearity is difference spectroscopy. Small spectral changes in solutions with high initial absorbance are often measured. Obtaining reliable data requires high-performance spectrophotometers.

Difference spectroscopy is used extensively in many disciplines. It provides a sensitive method for detecting small changes in the environment of a chromophore. In general, two solutions at the same concentration are directly compared. One solution is selected as the sample, the second as the reference. All common spectral features to the two solutions cancel out. Only bands which have been displaced in one solution relative to the other because of environmental differences are recorded.

A perturbation technique commonly employed is pH difference spectra in which the acidity of one solution relative to the other is varied and spectral shifts associated with conformationl changes and ionization of the chromophores are displayed.

Other Techniques

Less common techniques which are generally associated with UV-Vis spectroscopy include absorbance measurements in the far (or low) UV (below 190 nm) and the near infrared (above 800 nm). Several review articles discuss near infrared applications for the analysis of organic and inorganic materials[5] and far UV applications and techniques.[6,7]

Numerous accessories have been developed for UV-Vis spectrophotometers to increase their versatility. These include flow cells for continuous monitoring of column effluents, devices for measuring fluorescence, reflectance, and polarization, accesso-