

X-ray and Crystallographic Applications in Pharmaceutical Research I

Trace Element Determinations by X-ray Emission

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A standard procedure generally applicable on a routine basis to the determination of any heavy element in a solid organic matrix is described, using an improved method for the determination of selenium in steroids as an example. The general method is applicable for both high and low concentrations including, as is illustrated, trace quantities. An exception to the general application occurs with mercury, due to interferences from primary radiation, and an alternate procedure is described for this element. Extension of the method to lighter elements is illustrated by procedures for the determination of chromium and manganese, and a critical comparison of alternate procedures for these two elements is made. A statistical study of common errors showing optimum conditions for lighter elements is also presented.

X-RAY and crystallographic methods are well known in some fields, but wide usage in the organic, particularly the pharmaceutical field, has not yet occurred. These methods as applied to a wide variety of pharmaceutical problems are valuable and extremely versatile.

A series of papers, of which this is the first, will describe the use of these methods as they have been found to apply to typical pharmaceutical problems. Subsequent papers of the series will be concerned with modified molecular weight determination procedures, quantitative and qualitative diffraction analyses, crystal habit quantitation, low angle scattering applications, and crystal phase transitions.

X-ray emission analysis (also called X-ray fluorescence analysis) has expanded rapidly in recent years. Two excellent books on the method (1, 2) have recently been published, and cover the fundamentals. A review in 1960 (3) listed 223 papers on applications of X-ray absorption and emission, but very few of these were related to organic systems.

Several advantages of the X-ray emission method make it especially adaptable for pharmaceutical problems. Due to the low atomic number of carbon, hydrogen, and oxygen constituting organic systems, matrix component interferences, encountered in many X-ray emission applications, are absent. Moreover, the method is applicable directly on samples, either liquid or solid, and usually without need for

separations, permitting assays on final product formulations. Further advantages are speed, specificity, the nondestructive nature of the method, and the inherent simplicity of X-ray spectra. The nonapplicability to low atomic numbered elements is a disadvantage when considering X-ray emission as a general method, but is a decided advantage when the method is used for the determination of heavy elements in a light (organic) matrix.

A method for the simultaneous determination of traces of selenium and mercury in pharmaceuticals was previously reported from these laboratories (4). This method has been greatly improved, particularly in speed and sample handling conveniences, and developed into a standard procedure generally applicable on a routine basis to the determination of any heavy element in a solid organic matrix. This paper describes the general method as applied to trace quantities of elements, such as residual toxic catalysts, and treats special problems encountered in determinations of low concentrations. The same general method and procedures as described are readily applied to the determination of elements at higher concentrations, making it ideally useful for the determination of mineral ingredients in multivitamin products and other preparations.

DISCUSSION

Principle of the Method.—Several processes can be expected to occur when matter is bombarded by intense radiant energy. One of these is a quantum process wherein atomic electrons are displaced, and the vacancies caused are then filled by electrons from an outer shell. Such a process must be accompanied by a release of energy, and it is significant that this energy is in the form of X-rays. As there are several energy levels associated with the various electron shells, quantum theory predicts a number of

Received October 26, 1961, from the Pharmacy Research Section, Product Research and Development Unit, The Upjohn Co., Kalamazoo, Mich.

Accepted for publication November 16, 1961.

Presented to the First Annual Conference on Pharmaceutical Analysis, Land O'Lakes, Wis., September 1961.

It is a pleasure to acknowledge the technical assistance of Dr. J. I. Northam, The Upjohn Co., who provided aid in the statistical analyses, and Mr. Karl Zippel, also of The Upjohn Co., who prepared some of the standard samples and collected a portion of the counting data used in this work.

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possible transitions, some more likely than others. Thus, an element when bombarded by sufficiently intense radiant energy will emit several X-ray "lines," each at a specific wavelength, characteristic of the element. This is the qualitative aspect of analysis. The intensity of any selected line is proportional to, among other factors, the number of emitting atoms. This, following independent calibration, is the quantitative aspect of emission analysis.

In practice, the radiant energy is supplied by an X-ray tube. The determination of wavelength of the emitted X-ray is accomplished by diffraction, using an analyzing crystal of known orientation and known interplanar spacing; the wavelength is then given by solution of the Bragg equation. The intensity of the diffracted beam is measured by an electronic counter and scaler circuit. The commercially available spectrometers automatically plot this intensity against the Bragg angle, 2θ , which is readily converted to wavelength by the use of tables for a given analyzing crystal. For highly accurate quantitative work, the spectrometer is set for a given wavelength (2θ value), and the counter allowed to accumulate either a fixed number of counts, or to count for a fixed period of time, from which intensity, in counts-per-second, is determined. The procedure generally requires independent standardization, using standards of known concentration.

Sample Preparation.—In preparing samples for analysis it has been found that solid organic compounds are most conveniently handled by briquetting. In this study, 0.5-Gm. samples were compressed in a Perkin-Elmer potassium bromide die at 6000 lb. pressure, without evacuation. This produced pellets of 0.5 in. diam. and, for steroids, about $\frac{1}{8}$ in. thick. These samples do not possess "infinite thickness" with respect to the X-ray beam, but since the sample weight, diameter and pressure used are constant, the total mass irradiated remains essentially constant for wide variations in matrix density.

Instrumentation.—A General Electric XRD-5 spectrometer, equipped with a lithium fluoride analyzing crystal, was used for all measurements. Most of the experimental work was completed using a tungsten target X-ray tube (Machlett AEG-50T) operated at 50 kvp and 45 ma. The platinum target tube used for one experiment was also operated at these settings. The scintillation counter used was operated at a plateau voltage of 1.275 kv, and the flow proportional counter (G.E. No. 4SPG) was operated at 2.0 kv, except when these counters were used in connection with a reverter circuit. In instances where a reverter was used, the particular peak voltages employed are listed in the individual sections which follow. The standard mask of the XRD-5 sample drawer was replaced with an aluminum plate, milled with a recession into which the half-inch sample briquettes were placed for analysis.

EXPERIMENTAL

General Illustrative Example. The Determination of Selenium.—Through use of selenium as a catalyst in some organic syntheses, this toxic element can appear in final pharmaceutical products.

The determination of selenium in the parts-per-million range is a good example to illustrate application of the general method, as selenium represents an element whose atomic number ($Z = 34$) is high enough so that it may be readily determined. Elements of higher atomic number are determined with even less difficulty.

Selenium standards were prepared by adding aqueous solutions of selenium dioxide, representing several concentrations of selenium, to beakers containing in each 0.5 Gm. steroidal material in acetone solution. After mixing, the solutions were allowed to evaporate to dryness. No heat or vacuum was used, due to the volatility of selenium. The solution step assured homogeneity of selenium dispersions, and relatively little selenium was lost on the beaker surfaces due to the relatively greater adsorbing surface presented by the precipitating steroid crystals. Mixed residues of each beaker were then quantitatively transferred to the die and compressed as described above in the section on sample preparation. The standard briquettes were placed directly into the depression milled into the sample drawer plate.

For this study the XRD-5 spectrometer was equipped with a tungsten target X-ray tube and lithium fluoride analyzing crystal. A scintillation counter fed impulses through a reverter, used to discriminate electronically against undesired wavelengths, to a simultaneously operating decade scaler and timer. The spectrometer was set for $2\theta = 31.89^\circ$, the proper position to measure the $K\alpha$ emission line of selenium. Following insertion of a high selenium-bearing sample in the sample drawer, the analyzing crystal position was carefully "tuned" to produce maximum counting rate. Adjustments of the crystal position were followed by an automatic slow scan (0.2 degrees per minute) with the recorder, which plotted counting rate *vs.* degrees 2θ , until the maximum rate occurred at exactly 31.89° . A counter voltage of 1.175 kv was found to give maximum counting rate with the reverter for selenium $K\alpha$ radiation.

The detector was set to record the time required for the accumulation of 200,000 counts, and this time was measured for each of the standard selenium samples while the instrument was set for $2\theta = 31.89^\circ$, the selenium line, and at $2\theta = 34.89^\circ$, a convenient setting for background determination. The peak to background ratio, P/B , was computed for each standard sample.

$$P/B = \frac{\text{selenium, counts-per-second}}{\text{background, counts-per-second}} = \frac{\text{time at background for 200,000 counts}}{\text{time at selenium line for 200,000 counts}}$$

The values for P/B were plotted *vs.* concentration of selenium to give the straight line shown in Fig. 1.

Repeated determinations of the standard curve shown in Fig. 1 from the same samples have shown good reproduction of both the direction and the magnitude of the scatter of each sample value from the line. The method appears to be limited most by lack of complete homogeneity of standard samples, a fact not surprising in low part-per-million concentrations.

Typical times recorded for the required count accumulation range from 167 sec. for the 0 p.p.m.

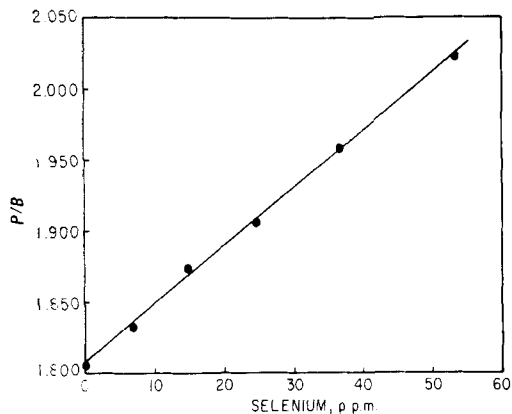


Fig. 1.—Selenium, peak to background ratio vs. concentration.

sample to 152.8 sec. for the 35.6 p.p.m. sample. Following standardization, the briquetted unknown samples require only a few minutes to assay.

An Instance Requiring Solution of Interference from the Primary Radiation. The Determination of Mercury.—As noted above, X-ray emission analysis of heavy elements in organic systems is virtually free from interferences from matrix components. The procedure described above for the determination of selenium can be routinely adopted for any heavy element in organic systems, with the exception of one instance. This exception occurs when the analytical emission line to be measured is coincident with, or nearly coincident with, a strong line produced by primary radiation from the X-ray tube. For instance, in the determination of mercury the analytical line of choice, $L\alpha$, is found (with a lithium fluoride crystal) at $2\theta = 35.90^\circ$. The sample scatters all radiation from the tube, and if a tungsten target tube is used a very strong line, representing tungsten $L\beta$, will occur at $2\theta = 37.40^\circ$. The tungsten line is so strong that even with the high resolution produced by a 0.01-in. detector slit, interference with the mercury line occurs. In fact, the mercury line is found on the steep slope of one side of the tungsten line. A determination for trace amounts of mercury under such conditions produces a standard curve with a major curvature in the low concentration region, as seen in Fig. 2. At higher concentrations of mercury, the tungsten line becomes less dominating, and the standard curve becomes more linear and therefore more useful. But for routine use at low mercury concentrations the curvature is not reproducible from day-to-day.

The best solution found to the interference problem from tungsten was replacement of the tungsten tube with one of different target material. A platinum target tube was used, and the results were a linear relationship in the standard curve for mercury, as seen in Fig. 3. This curve was found to be reproducible from day-to-day. Moreover, counting times were much less for the mercury samples with the platinum target procedure. Each point on the curve for Fig. 3 represents two countings of 40,000 counts each, and the points on the curve for Fig. 2 represent duplicate countings of one million counts each, required for comparable precision.

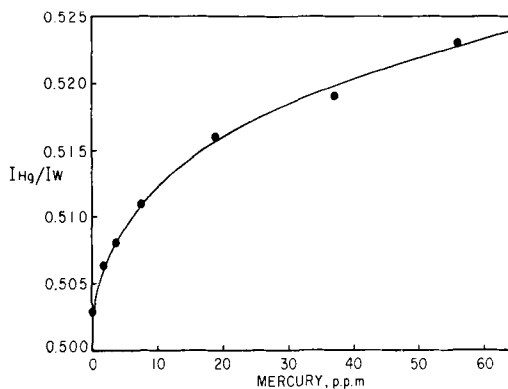


Fig. 2.—Mercury, intensity ratio vs. concentration.

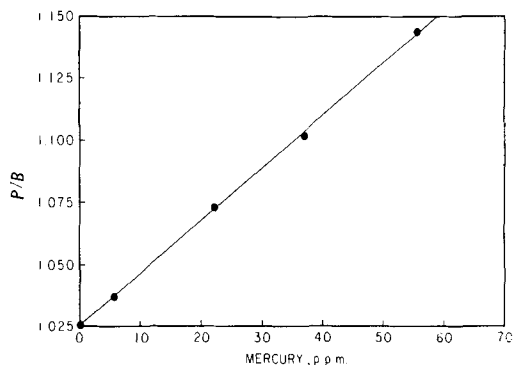


Fig. 3.—Mercury, peak to background ratio vs. concentration.

Samples for Fig. 2 were rectilinear compressions of 200 mg. of material, and for Fig. 3 were prepared as described in the standard procedure for selenium, above.

The ratio value plotted in Fig. 2 represents the ratio of the intensity of the mercury $L\alpha$ line to the intensity of a tungsten scatter line; the value P/B in Fig. 3 represents the ratio of the $L\alpha$ mercury line intensity to the intensity of the background at the base of the peak. Neither of these selections for background reference appears to have an advantage over the other.

Lower Atomic Numbered Elements. A Critical Study of Alternative Methods for the Determination of Chromium and Manganese.—The applicability of X-ray emission analysis to an element in any matrix depends to a large extent upon the atomic number of the element. This is particularly true for trace analysis, even in organic matrices.

A fundamental relationship states that the reciprocal of the emission wavelength is proportional to the square of the atomic number, where the proportionality constant depends upon the emission series. Thus, lower atomic-numbered elements emit rays of longer wavelength, and these are non-penetrating, even to air. Further, the "fluorescent yield" of an atom is much less with decreasing atomic number, due to the increasing probability of the Auger effect. A third limitation on emission from elements of low atomic number stems from the lack

of suitability of analyzing crystals with sufficiently large interplanar spacings.

From a practical standpoint, it is generally recognized that elements of atomic numbers 1 through 11 cannot be detected by present day X-ray emission equipment; that elements of atomic numbers 12 through 22 are measured only under special conditions (vacuum or helium atmosphere, and flow-proportional counter), and elements of atomic numbers 23 and higher are measured readily with no special requirements other than that listed in previous sections above for selenium and mercury.

A number of elements of pharmaceutical interest are in the lighter element region. Two such elements, of interest because of their use as catalyst in organic syntheses, are chromium ($Z = 24$), and manganese ($Z = 25$). These elements fall on a very steep portion of the "S-shaped" curve which results from a plot of fluorescent yield *vs.* atomic number (5). They also lie close to the division line for nonfeasibility of determination except under special conditions. A critical study of the effects of the special conditions on each of these two adjacent elements in this particular range was undertaken not only to observe the effects but to gain some idea of the rate of change of feasibility with increasing atomic number in this range, and under the conditions of the standard procedures employed.

Solid pellet standards for manganese and chromium were prepared by the procedure described for selenium, following evaporation of appropriate solutions of manganese dioxide and potassium dichromate. A tungsten target tube was used, and 2θ settings employed were 69.35° for the $K\alpha$ line of chromium and 62.97° for the $K\alpha$ line of manganese. All countings were for a fixed time of 100 sec. and no reverter circuit was employed.

Since critical comparisons were to be made in this particular study, net peak height determinations, rather than peak-to-background ratios were measured as a function of concentration, and least squares lines, as well as standard deviations of the net peak heights about the least squares lines were computed.

In this study two methods are compared. Method 1 consisted of procedures as previously described for selenium and mercury; the measurements were carried out in air, using a scintillation counter. Method 2 refers to the use of a helium atmosphere, under positive pressure, which excludes all air from the X-ray beam path, and a gas flow proportional counter in place of the scintillation counter. The gas used to flush this counter continuously was a mixture of 90% argon and 10% methane.

Graphical representation of the results of the use of methods 1 and 2 for the trace determination of both chromium and manganese is shown in Figs. 4 and 5.

The standard deviation of an estimated concentration in parts-per-million is given approximately by

$$\frac{s}{b} \sqrt{\frac{1}{m} + \frac{1}{n}}$$

where b = slope of least squares line, net peak height (c.p.s.) *vs.* concentration (p.p.m.); s = standard deviation of net peak height (c.p.s.) about the least squares line; n = number of determinations used

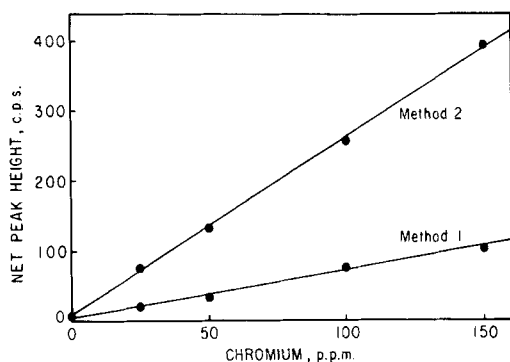


Fig. 4.—Chromium, net peak height *vs.* concentration.

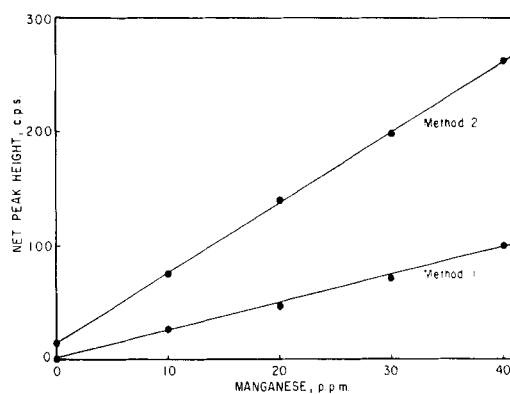


Fig. 5.—Manganese, net peak height *vs.* concentration.

in the standard curve; and m = number of determinations used in measuring the unknowns.

The value $\lambda = s/b$ is an excellent criterion for comparing alternative methods. Smaller values for λ indicate greater inherent precision of the method. Table I lists s , b , and λ values computed for methods 1 and 2 for the determination of chromium and manganese. The ratio of λ values for chromium, $6.22/1.84 = 3.38$, indicates that method 2 has 3.38 times the precision of method 1 for estimating the concentration of chromium in p.p.m. Similarly, the value $1.03/0.263 = 3.92$ indicates that method 2 for manganese has 3.92 times the precision of method 1. This clearly illustrates the very significant gain in precision made by use of the helium atmosphere and a flow counter for elements in this atomic number range. The λ values also show the rapid gain in feasibility as the atomic number of the element increases slightly in this range.

TABLE I.—STATISTICAL COMPARISON OF METHODS 1 AND 2 FOR THE DETERMINATION OF TRACE AMOUNTS OF CHROMIUM AND MANGANESE

| | Method | s | b | λ |
|-----------|--------|-------|---------|-----------|
| Chromium | 1 | 4.216 | 0.67762 | 6.22 |
| | 2 | 4.685 | 2.5475 | 1.84 |
| Manganese | 1 | 2.544 | 2.470 | 1.03 |
| | 2 | 1.628 | 6.190 | 0.263 |

TABLE II.—COMPARISON OF ERRORS IN THE MEASUREMENTS OF A 50 P.P.M. CHROMIUM SAMPLE UNDER VARIOUS INSTRUMENTAL CONDITIONS

| Conditions, 10 Determinations Each | Ratio Peak to Background | Net Peak Height, c.p.s. | —Counting Error— $\frac{\% P.E.}{s_c}$ ^a | | Observed Error, s_o ^c | Calculated Extraneous Error, s_e ^d |
|---|--------------------------------|-------------------------------|--|-------|--|--|
| 1. In air, scintillation counter, no reverter | 1.334 | 31.8 | 3.16 | 1.49 | 1.89 | 1.16 |
| 2. In air, scintillation counter, with reverter | 2.082 | 30.9 | 2.06 | 0.94 | 1.79 | 1.52 |
| 3. In air, flow counter, no reverter | 3.151 | 44.3 | 1.41 | 0.925 | 1.58 | 0.80 |
| 4. In helium, flow counter, no reverter | 4.412 | 142.4 | 0.71 | 1.50 | 1.17 ^e | 0 |
| 5. In helium, flow counter, with reverter | 5.084 | 138.0 | 0.70 | 1.43 | 2.40 | 1.93 |

^a % P.E. = Per cent probable error in net peak height = $67.5/(R-1)\sqrt{[R(R+1)]/N_t}$ where R = ratio of peak height to background rates, N_t = total number of counts at peak top, including background. ^b s_c = Net peak height standard deviation, c.p.s. = % P.E. \times net peak height, c.p.s./67.5. ^c s_o = Net peak height standard deviation, c.p.s. = $\sqrt{[\sum(X_i - \bar{X})^2]/N - 1}$. ^d $s_e = \sqrt{s_o^2 - s_c^2}$. ^e Lower than what is anticipated from pure counting errors, but not by a statistically significant amount.

Common Errors and Optimum Conditions for the Determination of Lighter Elements. A Statistical Study.—The following study was designed to show the relative contributions to precision of the use of helium atmosphere, the flow counter, and the reverter, when considered individually as applied to low concentrations of a light element. In general, these are three major considerations for sources of aid to precision in any prospective procedure. This study was also designed to show the relative sizes of the various types of error, and their contributions under the experimental conditions employed.

Errors in X-ray emission analysis are conveniently divided into two groups. One group consists entirely of counting errors, which stem from the randomness observed when X-ray emission quanta are measured. A second group comprises all sources of error other than counting errors, such as poor reproducibility in resetting the goniometer (2θ setting), sample positioning, etc.

Counting errors can be controlled. The per cent standard counting error, for instance, approximates the reciprocal of the square root of the total number of counts involved. Thus, at a sacrifice of time, a counting rate based on more counts decreases this error by any desired amount.

For a truly critical comparison of instrumental conditions for a given determination, it is necessary to separate these errors and consider the "extraneous error," or that calculated quantity which remains after all counting errors are reduced to zero.

The results of such a procedure are shown in Table II. All measurements were made on a compressed sample of steroidal material containing 50

p.p.m. chromium. Tungsten radiation and the fixed counting time of 100 sec. were used. Five instrumental conditions, derived from combinations of three major variables which strongly affect emission measurements, were statistically evaluated by making 10 determinations for each.

The five conditions of Table II are arranged in increasing order of peak-to-background ratios. These ratios have often been reported as being useful as a rough measure of method precision. The present study belies this usage.

Comparison of the values for the calculated extraneous errors indicates condition 4 to be the one of choice, and further indicates the use of the flow counter to be the single most effective variable in gaining precision under the experimental conditions.

The reverter, often used to good advantage to discriminate against undesired wavelengths when interfering elements are present, was found to be of no advantage in the present study except in connection with the scintillation counter. Even though an increase in peak-to-background ratio was significant, Table II shows large reverter contribution to the extraneous error.

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