

Chapter 24

Determination of Absolute Radioactivity in Multi-Labelled Samples using External Standardization or Channels Ratio: A Fortran IV Program

P. E. Stanley

Department of Agricultural Biochemistry, The Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia, 5064.

INTRODUCTION

Data from liquid scintillation spectrometers are nowadays readily obtained since the processing of samples is completely automated. The analysis of such data and their reduction to more tractable levels can be conveniently accomplished by use of the computer operated either ON-LINE or OFF-LINE. In the former case a small dedicated computer is frequently built into the spectrometer or the instrument is used ON-LINE to a main computer installation. In the OFF-LINE configuration data may be processed by a small electronic desk calculator or at a large computer via the use of punched cards or tape. Each of these four modes has its own merits and demerits but it is not proposed to compare and contrast them here. The present paper is concerned with OFF-LINE operation to a large computer, a CDC 6400, on the basis of its availability, reliability, storage capacity (core) as well as its processing speed and fast INPUT/OUTPUT system.

SCOPE OF THE PROGRAM

The program I wish to discuss concerns the measurement of amounts of individual radioisotopes in multilabelled samples containing up to three species. It is essential that the isotopes have energies which give rise to pulse height spectra the ratios of which exceed 1 : 4 for any pair of isotopes with adjacent energies. This allows for adequate pulse height analysis to separate the isotopes. The commonly encountered trio, tritium, carbon-14 and phosphorus-32, have ratios of energies of 1 : 10 : 100 approximately and are thus adequately separated for this purpose.

The degree of quenching in samples may be assessed by using the external standardization procedure (up to three radioisotopes) or by sample channels ratio of the isotope having the higher energy (two radioisotopes in the case of a three-channel spectrometer).

The program can be used for samples which are either all chemically quenched *or* all colour quenched. It cannot be used to deal with samples quenched by both processes, basically because each type of quenching gives rise to entirely different shaped pulse height spectra.¹

In using a large computer it has been possible to accomplish not only the straightforward arithmetical computation of disintegrations/min of the isotopes but also to make a statistical analysis of the results obtained from what are, after all, estimates of counting rates which exhibit a Poisson distribution (neglecting instrument drift and other factors to be mentioned later). An attempt has also been made to assess the errors associated with the efficiency—quench correction curves and to incorporate these into a final error term. To date such statistical analysis has been largely neglected in the field of data processing in liquid scintillation spectrometry. Notable exceptions are the work of Horton and Tait² and that of Carroll and Houser.³

In using the program it is assumed that the liquid scintillation process to be measured is free from spurious events.

Because of the restriction on time no discussion is given here for setting up the spectrometer to provide the optimum separation of isotopes. The reader is referred instead to the paper of Kobayashi and Maudsley.⁴

EXTERNAL STANDARDIZATION

Before presenting the basic approach adopted in designing the program it is necessary to discuss some of the problems encountered in obtaining a useful estimate of the external standard ratio/count when this technique is used to assess the degree of quenching in a sample.

Sources of error in external standardization

Most commercial spectrometers permit replicate counting of samples. Thus it is possible to detect variation in external standard values which are associated with one or more of the following parameters:

1. Lack of reproducibility in positioning the external standard source.
2. Irregular geometry of sample vials.
3. Vial to vial variation.
4. Instrument drift.

Evaluation of errors

The effects of (1) and (2) above have been assessed for a Packard Tricarb Model 3375 which has a compound external standard source of americium-241 and radium-226. Using a single sealed background vial a series of external standard ratios were obtained for the following three situations:

- A. Both the vial and the external standard source were kept in the same unaltered position (constant source and vial geometry).
- B. The vial was kept in a constant position but the external standard source was allowed to cycle IN/OUT as is usual in automatic operation (constant vial geometry).
- C. An external standard measurement was made as in (B) and the vial unloaded, cycled around the sample changer before another such measurement. This process was repeated a number of times so that the vial position in the detector was randomized.

The results presented in Fig. 1 indicate that although there is a small error associated with source geometry (non-reproducible positioning) there is a larger one resulting from the irregular vial geometry. Consequently this must be taken into account when estimating the external standard ratio/count.

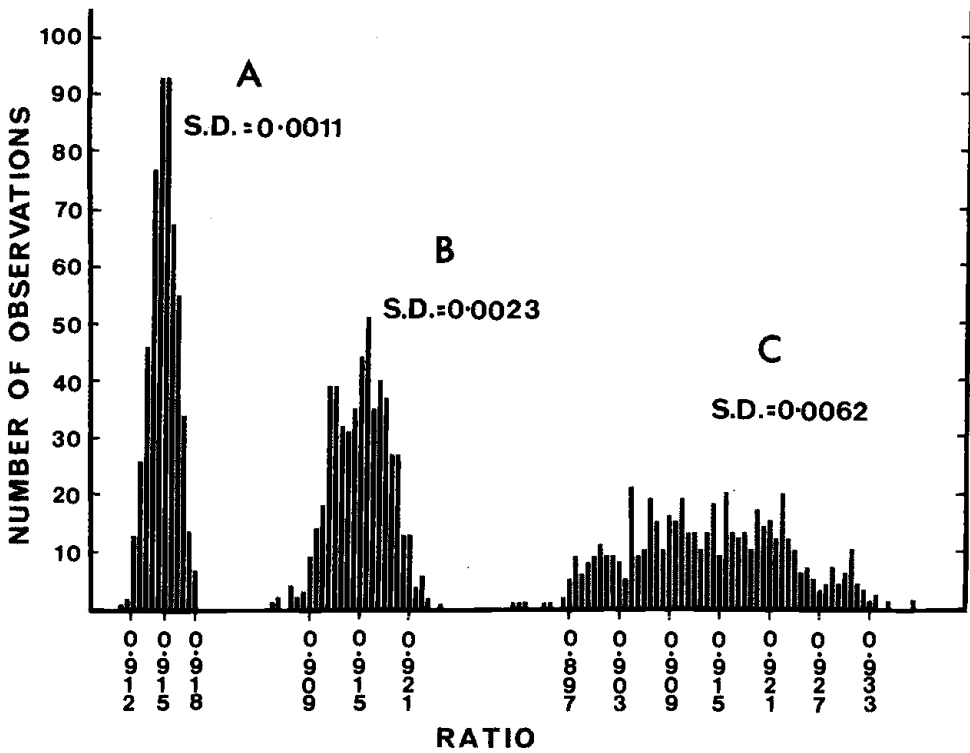


Fig. 1. Distribution of external standard ratios; A = constant source and vial geometry, B = constant vial geometry, C = normal automatic operation (see text for further details). S.D. = standard deviation.

The long term drift of this particular instrument over a period of three weeks was negligible, for after comparing the distribution and means of external standard ratios of the same sealed background vial (50 observations) on six occasions during this time there was no significant difference between the means and distributions. Thus the error associated with the external standard value is, in large part, due to irregular vial geometry and vial positioning within the detector.

Influence of vial geometry on external standard value

To confirm this variation in external standard value as a function of vial geometry the same sealed background vial was placed in twelve different positions within the detector such that each position was at an angle 30° to the radial position of the previous one. Ten assessments of external standard ratio were made for each position. A statistical analysis showed that the means of the external standard ratios in each of the twelve positions were significantly different as is shown in Fig. 2. It is of interest that external standard counts as well as sample channels ratio also show this dependency on vial orientation. Variation in vial geometry has been confirmed in vials from three independent suppliers and consequently in estimating the external standard ratio it is necessary to take this into account by making evaluations in random vial positions. This is conveniently done by the cycling technique.

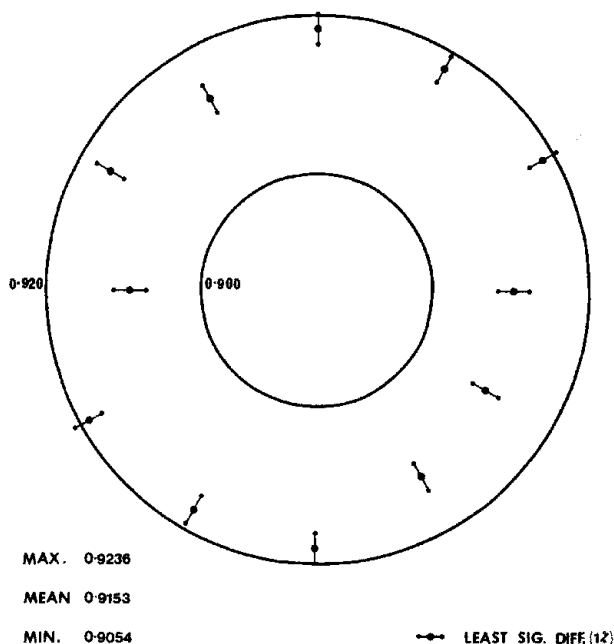


Fig. 2. The effect of vial orientation on external standard ratio.

Selection of vials

Inter-vial variation is reduced by selecting vials containing standard fluor under unquenched, moderately and heavily quenched conditions. At the same time it is also convenient to select vials for uniform background values and this is especially important if low activity samples are to be measured.

SOURCES OF ERROR IN ASSESSING DISINTEGRATIONS/MIN (d.p.m.)

The following sources of error must be taken into account when assessing the d.p.m. of a quenched sample:

1. The external standard value of both the standards and the samples.
2. Poisson statistics associated with the accumulated counts.
3. Subtraction of the background.
4. Curve fitting for the efficiency versus quenching (external standard).
5. Vial to vial variation for both external standard and background.

DESIGN OF THE COMPUTER PROGRAM

The program is written in FORTRAN IV and conforms to USASI standards. The program runs on a CDC 6400 computer which has 400₈ K core of which 140₈ K core is available to the user. Data are supplied via punched cards.

Constraints within the program

The capacity of the program is limited as follows:

1. Up to three isotopes may be measured.

2. Up to 25 standards (quenched) for each isotope.
3. Up to 200 samples (standards and unknown samples).
4. Up to ten background standards (quenched).
5. All backgrounds counted for the same time.
6. All samples must remain in the same sample belt position from cycle to cycle.

The background standards are run before the samples since the former will generally require more counting time. A check on the stability of the background of the equipment can be made by inserting background samples amongst the unknown samples.

Design of the program and its operating requirements

The program is divided into two parts:

1. Program XSTD
2. Program GETDPM.

The first program, XSTD, which reads into core all data and rearranges them, requires a large amount of core (100₈K) but a short amount of central processor time. It makes two files to be processed by GETDPM. The second program deals with these two files and processes the standards, one isotope at a time, sets up efficiency versus quench curves and then processes the unknown samples one at a time. Program GETDPM requires a modest amount of core (40₈K) but more central processor time.

Basic details concerning program XSTD

In this program the information first read in concerns the parameters associated with this experiment. It includes the number of isotopes, channels, standards, unknown samples, cycles, background samples, background cycles, together with the number of standard deviations to be employed in the statistical tests and the names of the isotopes as well as the number of the channel to be used for numerator and for denominator if the sample channels ratio technique is to be employed.

The type of quench assessment is then read which may be one of the following:

1. External standard ratio or counts in separate external standard channels.
2. External standard ratio or counts in the isotope counting channels.
3. Channels ratio of the sample.

The INPUT format for the background data (if any) is then read which allows the user the flexibility of choosing the INPUT format. The background standards (quenched or unquenched) are then read and all values stored before obtaining means of c.p.m. in each channel. A check is then made to ascertain if data lie within the user's preset statistical limits. Those that do not can be rejected or retained as required with an informative diagnostic printed out.

The INPUT format to be employed in reading standards and unknown samples is read in, followed by data concerning standards and samples. Data are read in cycle by cycle. All values are stored before subtraction of background values from each according to the degree of quenching associated with it. A simple linear interpolation procedure is employed. The mean c.p.m. for each standard and each unknown sample in each channel is then calculated.

The next step is to calculate the times elapsed between the start of counting of the first sample in the first cycle and counting of every sample in every cycle. This is valuable

to determine the d.p.m. of fast decaying isotopes. The elapsed times can be conveniently calculated by taking account of the times for sample loading, counting, printing, unloading and the sample belt movement respectively. Vacant positions are accounted for during their bypass. The time for the various types of external standard cycle is included as necessary.

The final part of the program creates two files, one with all the data for the standards and the other with all the data required for processing the unknown samples. The data are arranged sample by sample.

Basic details concerning program GETDPM

This program processes the standards data and then the unknown samples. In addition it reads from cards the dates and time of day when the standards for each isotope were prepared as well as the time at which counting commenced. The d.p.m.'s for the standards are also read in. This program has a library of decay constants for commonly used isotopes. The d.p.m.'s are then corrected for decay during this period except in the case of long-lived radioisotopes such as carbon-14.

Mean external standard ratios/counts or sample channels ratios are then calculated and the appropriate values are tested to ascertain if they lie within the preset statistical limits. An informative diagnostic is printed if values exceed the preset limits and these can be either rejected or retained.

Prior to curve fitting for efficiency versus quenching, the external standard or channels ratio values are scaled by dividing all values by the largest in the array. The largest value then takes a value of one. This is necessary so as to avoid dealing with a large range of numbers in the curve fitting procedure when their squared values cannot be readily accommodated even in the CDC 6400 which allows fifteen significant digits to be handled in single precision. When employing a smaller computer it would be advisable to work in double precision.

The d.p.m. for each standard in each channel at a preselected clock time is then calculated, a plot of the log c.p.m. of each standard in each channel versus elapsed time (during each cycle) is made and a regression line of slope λ (decay constant for that isotope) is obtained to test for c.p.m.'s outside the preset statistical limits. The efficiencies at the preselected clock time are then calculated. This procedure is very valuable when dealing with a large number of samples containing short-lived isotopes when the counting time may involve several days.

The next event in GETDPM is the fitting of orthogonal polynomials to generate curves of the reciprocal of the counting efficiency (of an isotope in a channel) against the external standard value (or channels ratio value). By employing the reciprocal it is possible to avoid an efficiency matrix inversion with its attendant mathematical complexity of deriving an estimation of the error.

GETDPM then deals with the unknown samples one at a time (all samples). The means of the external standard or channels ratio values are then calculated and values exceeding the preset errors are dealt with as before. The d.p.m.'s for each isotope in the sample are then computed and two error terms are determined. The first error is associated with curve fitting and the external standard (or channels ratio) value of the unknown samples. The second one gives an error for the Poisson statistics associated with radioactive decay.

The results may be recorded in units other than d.p.m. e.g. mg/ml, provided a con-

version factor is included.

CONCLUSION

The program has been designed to give maximum flexibility compatible with accommodating quenched samples containing up to three radioisotopes together with an assessment of the error associated with d.p.m. values. It is likely that this program will be of value to biochemists and radiochemists using more than one radioisotope at a time in their work.

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- 3 C. O. Carroll and T. J. Houser, *Intern. J. Appl. Radiation Isotopes* **21**, 261 (1970).
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DISCUSSION

J. H. Deterding: I have a question, Mr. Chairman, which may seem rather detailed but does show the sort of problem which comes up occasionally. I would like to ask Dr. Stanley if he has ever found it necessary in his Fortran programs to use 'double-precision'? Recently I had to find the standard deviation of the mean of the three counts 190, 189 and 191 with a Fortran program which is used routinely; one way of finding the answer (about 0.6) involves the subtraction of two six-figure numbers, and 'double-precision' was needed. Has Dr. Stanley had similar experiences?

P. E. Stanley: No I have not yet found it necessary to use 'double-precision'. In integer subtraction and addition on the computer used (CDC 6400) eighteen decimal digits are significant in single precision. Integer division and multiplication are conducted in floating point notation. In all floating point manipulation fifteen digits are significant in single precision and twenty-nine in double precision. In work to date single precision with fifteen digits has been adequate but I agree with Mr. Deterding that six digit manipulation could lead to problems as he has found. This would be noticeable in computers employing 36 or 48 bit words. The CDC 6400 is a 60 bit word instrument.