SPECIFICATIONS AND CRITERIA FOR THE QUALITY CONTROL OF LABELED CHEMICALS.

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SUMMARY

The primary objective of the supplier of labeled chemicals is to provide the investigator with a product of the highest quality attainable. Ultimately, however, the specific requirement of purity is dictated by experimental design. As new and highly sophisticated experimental procedures are developed, the demand for expertise on the part of both supplier and investigator increases. Exhaustive analyses are required by the manufacturer to provide the investigator with assurance that a given product will fulfill all of his experimental criteria. The final responsibility as to the purity of a given labeled product for a specific application must be based on a careful evaluation by the investigator himself.

The primary objective of the manufacturer of labeled chemicals is to provide the investigator with a product of the highest attainable quality. Ultimately, however, the specific requirement of purity must be dictated by experimental design. As new and highly sophisticated experimental methods are developed, the demand for expertise on behalf of both user and supplier increases. Regardless of significant advances in preparative and purification procedures, one can not assume that the manufacturer will be able to meet each individual requirement of purity. The challenge has gone to the manufacturer to produce a product to fit a specific need. But the preparation is only the beginning as it now be-

comes imperative that the quality of a given material can be sustained throughout the duration of the experimental procedure. At this juncture the concern is the investigator and his needs.

The supplier must provide the user with the most accurate information available regarding any labeled material to allow him to use this material with total confidence. Information relative to radiochemical, chemical and optical purity (where applicable), anticipated rate of decomposition in a given solvent and special storage and handling recommendations are essential to the investigator. In addition, total radioactivity, concentration and specific activity must be determined accurately and presented to the user in a readily interpretable form. Unfortunately, there are numerous instances wherein this information does not reach the user (e.g., it may be removed and disposed of by a central receiving department or by a Radiation Safety Officer) or, if received, it is not read prior to the use of the product. Suppliers present, to the best of their knowledge, the most complete information available to allow a product to be used with complete confidence for the intended purpose. If the behavior of a labeled chemical could be predicted more accurately, most of the problems of both supplier and user could be avoided. However, this is not the case. Minor changes in solvent composition or concentration, change of storage temperature, exposure to air or to light may, in fact, alter the integrity of a labeled compound significantly. Thus there is the absolute requirement for a thorough and accurate appraisal of all labeled materials by both the manufacturer and the user.

The determination of the radiochemical purity of a product involves the use of

conventional methods of chromatography (e.g., paper, thin-layer, ion-exchange, gas-liquid, liquid) or electrophoresis. The results of such analyses must be transmitted to the user in a useful and interpretable form. The most frequently employed form of such presentation is the radiochromatogram scan. The scan is conveniently prepared using a thin-window or windowless flow counter such as the Packard Radiochromatogram Scanner, Nuclear-Chicago Actigraph or similar instrument. The problem from the standpoint of the investigator is the interpretation of such a scan. There are instances wherein as little as 0.5% of a given impurity may spell failure and the user must be assured that such trace impurities are not present in his material. Trace impurities are easily concealed in the baseline of the scan if care is not taken in its preparation. Further, if additional peaks appear, the extent of the individual impurities must be assigned. In order to present an accurate picture of radiochemical purity it is common practice to run known standards (dilutions, typically 1, 3, 5 or 10 percent) alongside the product. A comparison is then made between the standards and the individual peaks to determine the contribution of each to the overall purity. Using such a method, a seemingly large impurity may, in fact, amount to less than 1%. This can be shown in the scan which accompanies all labeled materials evaluated by this procedure. Closely running peaks may not be conveniently resolved using a radiochromatogram scanner. In such cases it is necessary to expose the chromatogram to X-ray film (e.g., Kodak RB-54 medical X-ray film), thereby producing an autoradiogram. The standards run alongside can then be compared in terms of exposure and an accurate appraisal of one's data is possible.

Unfortunately, some laboratories are without facilities for chromatography or autoradiography. In this case, the user places virtually total dependence upon the manufacturer. Such total reliance is undesirable, particularly when basic equipment can

be obtained for a nominal monetary outlay. Space requirements for such a set-up are also minimal.

In addition to radiochemical purity, it is often essential to evaluate labeled chemicals on the basis of chemical purity. Gas-liquid chromatography with simultaneous radioactivity and mass detection provides an accurate evaluation of radiochemical and chemical purity for relatively volatile compounds and, for volatile derivatives of very high boiling substances. Ultraviolet and infrared spectrophotometry, and fluorescence spectroscopy are also widely utilized in purity evaluations. In addition, specialized enzymatic and microbiological procedures frequently find utility for compounds of biological importance. Specific and class spray reagents are employed whenever possible with thin-layer, paper and paper electrophoresis strips.

A third criteria of purity is that of optical purity. This is of importance, for instance, in the case of amino acids and certain of the catecholamines. Since the D- and L-isomers participate in vastly different ways in a biological system, it is essential to know which isomer has been incorporated. Several methods have been reported to accurately evaluate optical purity. The first of these involves a gas-liquid radiochromatographic (GLRC) method based on the procedure of Halpern and Westley [1] using N-trifluoroacetyl-L-prolyl chloride (L-TPC) as the resolving agent to form the diastereomers of the amino acid. Barooshian, et al [2], of this laboratory have successfully applied the GLRC method to the determination of the optical purity of carbon-14 labeled amino acids. A diagram of the complete GLRC system is shown in Figure 1. The GLRC separation of the L-TP derivatives of L-Alanine [^{14}C (U)] is shown in Figure 2.

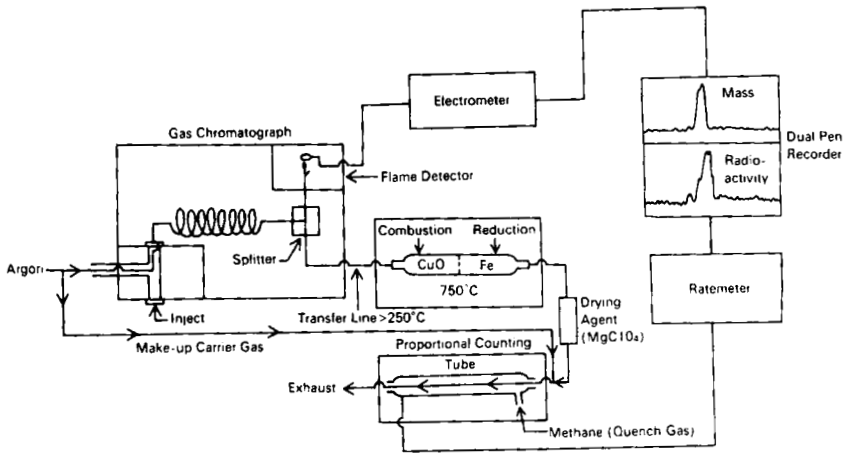
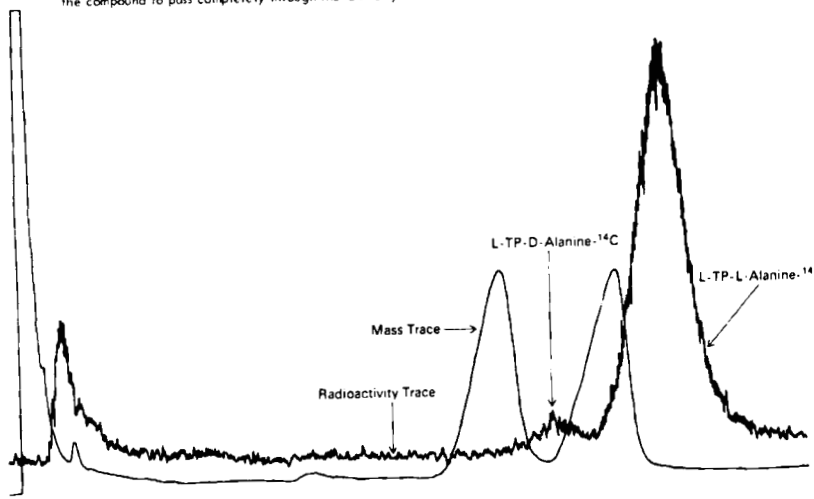


Fig. 2. Separation of optical isomers of L-alanine-^{14}C (U) as their L-TP derivatives. (The lag period noted between the mass and radioactivity trace is the time required for the compound to pass completely through the GLRC system.)



A second method for the determination of the optical purity of labeled amino acids is the preparation of diastereomeric dipeptides by the N-carboxy anhydride (NCA) procedure of Hirschmann, et al [3], and the separation of the derivatives by thin-layer chromatography (TLC). The TLC method has been employed successfully in this laboratory for the determination of the optical purity of 19 labeled amino acids [4]. Solvent systems and adsorbents appropriate for the separation of L-L and

TABLE 1
R_f Values and Chromatography Systems for L-Leucyl Dipeptides of 19 Amino Acids

Amino Acid	Adsorbent[a]	system[b]	D-L	L-L
Alanine	SG	IV	0.66	0.69
Arginine	SG	I	0.26	0.35
Aspartic acid	SG	V	0.26	0.35
Asparagine	SG	I	0.30	0.37
Cystine	SG	I	0.27	0.36
Glutamic acid	SG	I	0.52	0.59
Glutamine	SG	I	0.33	0.42
Histidine	C	I	0.25	0.34
Isoleucine	SG	V	0.61	0.74
Leucine	SG	I	0.70	0.77
Lysine	SG	I	0.41	0.50
Methionine	SG	II	0.54	0.64
Phenylalanine	SG	II	0.34	0.46
Proline	SG	I	0.50	0.55
Serine	SG	I	0.46	0.50
Threonine	SG	I	0.52	0.57
Tryptophan	SG	III	0.85	0.90
Tyrosine	SG	I	0.57	0.63
Valine	SG	I	0.63	0.70

[a] SG = silica gel. C = cellulose (Avicel microcrystalline cellulose).

[b] I = n-butanol/acetic acid/water (4:1:1).
II = ethyl acetate/pyridine/glacial acetic acid/water (10:5:1:3).
III = ethyl acetate/formic acid/water (60:5:35).
IV = n-propanol/concentrated ammonia (7:3).
V = isoamyl alcohol/glacial acetic acid/water (4:1:1).

L-D dipeptides are listed, with R_f values, in Table 1. Figure 3 compares the amount of D-proline [^{3}H (G)] resulting from the catalytic exchange of L-proline with tritium gas before and after enzymic oxidation with D-amino acid oxidase. This procedure offers a rapid and simple method for the determination of optical

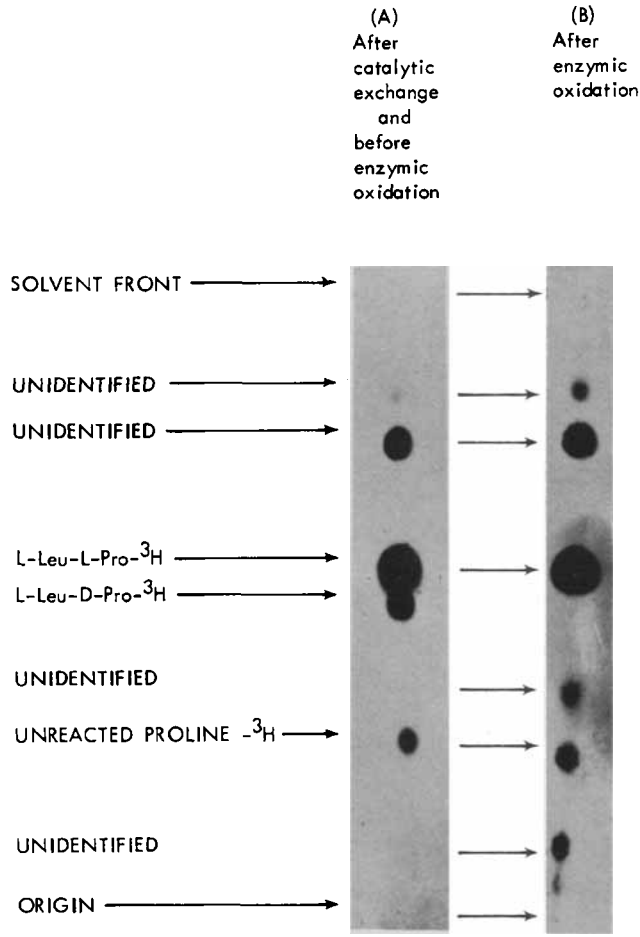


Fig. 3. TLC separation of L-Leu-L-Pro-[^{3}H (G)] and L-Leu-D-Pro-[^{3}H (G)] dipeptides on silica gel GF_{254} with n-butanol/acetic acid/water (4:1:1).

purity and eliminates the need for sophisticated instrumentation such as that required in the GLRC method. Most of the diastereomeric dipeptides are separated on silica gel. Only histidine does not separate on silica gel but is readily separated on cellulose layers or paper strips.

The thin-layer technique has also been used successfully for the separation of the optical isomers of labeled DOPA (L-3,4-dihydroxyphenylalanine).[5] The dipeptide diastereomers of DL-DOPA are prepared by the N-carboxy anhydride (NCA) procedure in essentially the same manner as that described by Manning and Moore [6]. A sample of L-DOPA [^{14}C (U)] was derivatized by this procedure and showed approximately 98% of the radioactivity associated with the L-Leu-L-DOPA-^{14}C spot. The separation is shown in Figure 4.

In addition to the assessment of product purity, it is also imperative that the radioactive content of a given sample be critically examined. This is readily accomplished by standard liquid scintillation counting methods. Accurate values of specific activity, total radioactivity and packaging concentration can be assigned with confidence. In the case of certain tritiated compounds, particularly those provided in aqueous solution, periodic checks are made to insure the absence of labile material (most often as tritiated water) which may significantly alter experimental results. Data supplied by the manufacturer relative to specific activity, total radioactivity and concentration are accurate within the sensitivity of available instrumentation and apparatus and typically are in the order of $\pm$3%.

How are the various criteria relative to the stability and storage of labeled

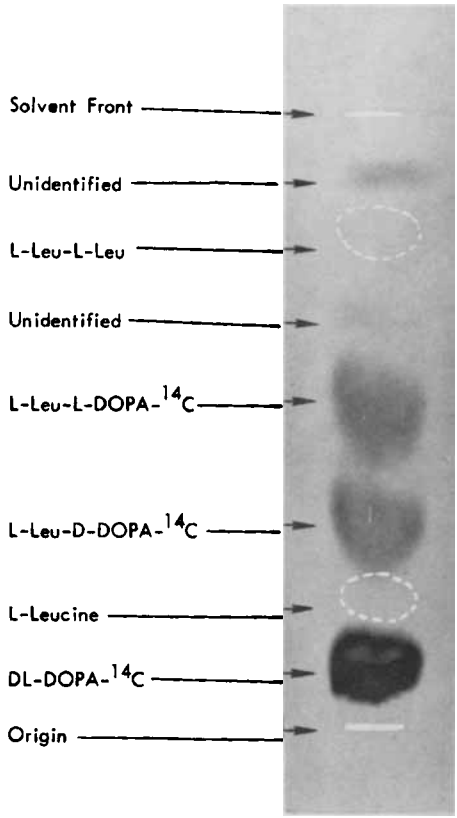


Fig. 4. Autoradiogram illustrating separation of DL-DOPA-^{14}C dipeptide diastereomers on silica gel using ethyl acetate/formic acid/water (60:5:35). Dotted circles indicate presence of unlabeled reaction products.

materials established? It is more than simply offering all products as crystalline solids or in ethanol, sterile aqueous solution, or some other vehicle with the recommendation that they be stored at the lowest possible temperature. The supplier must provide information which, as stated earlier, gives the user maximum confidence in the product. Occasionally this is a simple task but, more

often than not, determination of the optimum conditions of storage and handling is a tedious and time-consuming process. Based on knowledge accumulated over the years the supplier usually has a reasonable idea as to the best conditions of storage and handling. However, when a new method of preparation or purification is employed or when a new storage solvent is recommended, one must re-establish conditions of storage and shelf-life as though it had not been considered in the past. Occasionally, technical data sheets accompanying labeled materials will state that no data is available on the rate of decomposition of this product or the rate of decomposition is currently being evaluated. The user will then ask, "Why isn't this information available before you release the product?" The answer is quite simple. If the supplier waited until he had compiled all such data regarding a new product (which has been carefully designed to fill the investigator's special needs), there would be excessive delays in the release of such materials. When information is available, it is the obligation of the supplier to provide such information to the user. During the early history of a new product, the supplier may make frequent changes in overall specifications, such changes reflecting the best available information to date. When true specifications have been established, the data sheet will present all of the facts in full.

Another question frequently asked is, "Why is this material provided in sterile aqueous solution when you know it is far more stable in 70% ethanol?" Again, the answer is quite simple. The form in which the material is packaged and the solvent in which it is stored have been designed for the convenience of the customer. The sterile aqueous solution can be incorporated into the majority of experimental procedures as is. If the investigator must stop and remove a solvent and replace

this solvent with another, a number of complications might arise. It is quite possible (and probable) that a portion of the product would be lost if caution is not taken upon solvent removal. Further, there is the probability of accelerated decomposition if a given material is taken to complete dryness. Therefore, any step which the manufacturer can take to minimize the possibility of mishandling and subsequent loss of material (either through loss of mass or decomposition) must be taken and convenience prevails. With products stored in solvents designed for convenience rather than shelf-life, cautionary statements appear on the data sheet indicating that the material should be used within one week, two weeks, one month or some other reasonable period.

The question of the frequency of quality control checks is most important. When a new product is placed in inventory, checks are made frequently, usually on a monthly basis for the first six months. During this period, it becomes apparent that the monthly check is necessary or that it may be changed to two or three months. After an additional six months, the supplier has a fairly good idea as to the behavior of a material under the recommended conditions. A new schedule is set which, again, is subject to change. A final appraisal is made after twelve to eighteen months, at which time it must be assumed that scheduling is correct, all variables having been considered.

Although the manufacturer of labeled chemicals goes to great lengths to assure the highest quality attainable in his products, the need for a final check by the investigator prior to incorporation into the framework of his experiment cannot be over-emphasized. Long hours or even days of preparation may go for naught if the labeled material does not meet the specifications required by the experiment.

It is far better to resolve any and all potential or real problems before the fact rather than after the fact. Only with the complete cooperation of both manufacturer and user can the problems of highly sophisticated scientific endeavor be resolved.

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