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Accurate Measurement of Copper-67 in the Presence of Copper-64 Contaminant Using a Dose Calibrator

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The use of ^{67}Cu-labeled antibodies for the treatment of cancer has advanced to the clinical trial phase. Quantitation of ^{67}Cu radiopharmaceuticals is complicated by the presence of the radioimpurity of ^{64}Cu in ^{67}Cu supplies. Here we report a method to assay ^{67}Cu and ^{64}Cu in a mixed sample with a commonly available instrument, the ionization chamber dose calibrator. **Methods:** The activities of ^{67}Cu and ^{64}Cu in a mixed sample can be calculated from a single-dose calibrator measurement. The calculation requires (1) instrument-specific response coefficients D_{67} and D_{64}, generated by gauging the instrument for the efficiency of measurement of ^{67}Cu and ^{64}Cu, and (2) a value for the ratio of ^{67}Cu to ^{64}Cu in the sample, routinely provided by major suppliers of ^{67}Cu. D_{67} and D_{64} were empirically determined by measuring samples containing known amounts of ^{67}Cu and ^{64}Cu. The samples were also assayed by gamma ray spectroscopy to verify the isotope ratios given by the suppliers. **Results:** This method generated accurate response coefficients. At the recommended dose calibrator setting for the measurement of ^{67}Cu, at which D_{67} = 1.0, the measurement for D_{67} with this method was 1.02 (± 0.04). Isotope ratios provided by the radionuclide suppliers were corroborated by gamma ray spectroscopy. **Conclusion:** A method is presented by which ^{67}Cu and ^{64}Cu in a mixed sample can be assayed using a dose calibrator. Although the derived numeric constants are only correct for a specific dose calibrator and setting, the method can be adapted for use with any dose calibrator.

Key Words: copper-67; radiocontaminant; dose calibrator
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Radionuclides used in nuclear medicine, such as ^{67}Cu, ^{67}Ga, ^{99m}Tc, ^{111}In and ^{123}I, often contain radiocontaminants that complicate quantitation and increase the radiation dose absorbed by the patient (*1–3*). Strategies that use commonly available instruments such as a dose calibrator to assay contaminants in radiopharmaceuticals have been reported (*4–8*). We present a similar approach to measure ^{64}Cu radiocontamination in ^{67}Cu.

Due to its excellent physical and biochemical properties for radioimmunotherapy, ^{67}Cu is being actively investigated by several groups as a radioimmunotherapeutic agent (*9–13*). Copper-67 has a half-life of 62 hr, emits abundant beta particles and gamma rays, useful for therapy and pretherapy imaging studies, respectively, and has no known biological pathways for deposition in bone (*14,15*).

These studies have advanced to the clinical trial phase (*9,14*) using the chelating agent 1,4,8,11-tetraazacyclotetradecane-N,N′,N″,N‴-tetraacetic acid (TETA) as a carrier for ^{67}Cu (*16,17*). TETA binds ^{67}Cu rapidly, selectively, completely and

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with extraordinary kinetic stability (*18,19*). The bifunctional TETA derivative, 6-[*p*-(bromoacetamido)benzyl]-TETA (BAT) is conjugated to the murine anti-lymphoma IgG_{2a} Lym-1 via 2-iminothiolane (2IT) to prepare the immunoconjugate 2IT-BAT-Lym-1 (*20*). The radiopharmaceutical ^{67}Cu-2IT-BAT-Lym-1 can be reliably produced with high radioactive yield for therapeutic use (*21*).

Copper-67 is produced by the ^{68}Zn (p, 2p) reaction, which also produces ^{64}Cu ($T_{1/2}$ = 12.7 hr), ^{61}Cu ($T_{1/2}$ = 3.4 hr), and other radionuclides. Radiometals other than the isotopes of copper are quantitatively removed (*22*). Copper-61 decays rapidly to negligible activity, but ^{64}Cu remains present in appreciable quantities for days. The ratio of ^{67}Cu-to-^{64}Cu-to-^{61}Cu activity is typically 1:7:10 at end of bombardment, or 1:0.5:0.0001 when received by the customer 48–72 hr later (*23*). To produce radiopharmaceutical of adequate amount and specific activity, ^{67}Cu-2IT-BAT-Lym-1 is usually prepared for clinical use within 24 hr of receipt of the radionuclide (*19,24*), at which time the activity of ^{64}Cu is still significant. A method to measure the activities of ^{67}Cu and ^{64}Cu in a mixed sample is needed to dispense a correct dose of radiopharmaceutical. Gamma-ray spectroscopy can be used, but most facilities lack the instrumentation. Radioactive product yield can be used to calculate the dose. The product yield, however, can only be approximated by, for example, the quotient of the final versus the initial raw dose calibrator measurements because the response of the dose calibrator varies with the continually changing ratio of ^{67}Cu and ^{64}Cu in the sample.

We report a method to accurately quantitate ^{67}Cu and ^{64}Cu using an ionization chamber dose calibrator, by gauging the response of the instrument to the two radionuclides. This may require a series of preliminary measurements. Subsequently, a single dose calibrator measurement is sufficient to assay ^{67}Cu and ^{64}Cu in a sample, because the ratio of ^{67}Cu-to-^{64}Cu in the sample, routinely provided by the radioisotope suppliers, is used in the assay calculation.

In this experiment, the response of a dose calibrator was gauged at the manufacturer's recommended setting for the measurement of ^{67}Cu. Although the values obtained are specific for this instrument and setting, the technique can be used with any dose calibrator at any setting. This method assumes that no radionuclides other than ^{67}Cu and ^{64}Cu are present and that the isotope ratio provided by the supplier is correct. We examined multiple lots of ^{67}Cu/^{64}Cu by gamma-ray spectroscopy to test these assumptions.

MATERIALS AND METHODS

A Capintec model CRC-12 radionuclide calibrator (Pittsburgh, PA) was used. The instrument was assured by regular assessment to comply with U.S. Nuclear Regulatory Commission standards of constancy, accuracy and linearity (*25*).

At a dose calibrator setting, N, the response coefficient $D_{(N)}$ for a radionuclide is defined by the equation

$$R_{(N)} = D_{(N)}\, A, \qquad \text{Eq. 1}$$

where A is the activity of the radionuclide sample and $R_{(N)}$ is the dose calibrator reading. It follows that, for a mixture of radionuclides,

$$R_{(N)} = \Sigma D_{i(N)}\, A_i. \qquad \text{Eq. 2}$$

Specifically, for a sample containing activities A_{67} and A_{64} of ^{67}Cu and ^{64}Cu,

$$R_{(N)} = D_{67(N)}\, A_{67} + D_{64(N)}\, A_{64}, \qquad \text{Eq. 3}$$

where $D_{67(N)}$ and $D_{64(N)}$ are the response coefficients for ^{67}Cu and ^{64}Cu at setting N.

The coefficients can be used in turn to assay A_{67} and A_{64} of a mixed sample, e.g., a patient dose of radiopharmaceutical, using a single dose calibrator measurement as follows. Let the isotope ratio P be defined as

$$P = \frac{A_{64}}{A_{67}}. \qquad \text{Eq. 4}$$

A_{67} and A_{64} at a calibration time are routinely provided by major suppliers of ^{67}Cu, and are readily decay corrected to the time of sample measurement to calculate P. By substituting P into Equation 3 and re-arranging, the following formula is obtained:

$$A_{67} = \frac{R_{(N)}}{D_{67(N)} + D_{64(N)}\, P}. \qquad \text{Eq. 5}$$

A sample containing ^{67}Cu and ^{64}Cu can be measured on the dose calibrator and A_{67} can be calculated using Equation 5; then, A_{64} can be calculated using Equation 4.

Determination of Dose Calibrator Response Coefficients: Method 1

This method can be used when settings for the measurement of ^{67}Cu and ^{64}Cu are recommended by the manufacturer. For the dose calibrator used in this experiment, ^{67}Cu and ^{64}Cu are measured at settings 052 and 015, respectively, so $D_{67(052)}$ = 1.0 and $D_{64(015)}$ = 1.0. $D_{64(052)}$ can be readily determined as follows. By Equation 1 the ratio of readings for a sample of ^{64}Cu at dose calibrator settings 052 and 015 is equal to the ratio of the response coefficients for ^{64}Cu at settings 052 and 015; that is:

$$\frac{D_{64(052)}}{D_{64(015)}} = \frac{R_{64(052)}}{R_{64(015)}}, \qquad \text{Eq. 6}$$

because $D_{64(015)}$ = 1.0,

$$D_{64(052)} = \frac{R_{64(052)}}{R_{64(015)}}. \qquad \text{Eq. 7}$$

Resetting the calibration setting number simply adjusts the gain setting petentiometer of the dose calibrator. It follows that the ratio of readings for any radionuclide sample (e.g., ^{64}Cu) at two different settings (e.g., 052 and 015), is the same as the ratio of readings for any other radionuclide sample (e.g., ^{57}Co, ^{133}Ba, ^{137}Cs) at the two settings, that is:

$$\frac{R_{64(052)}}{R_{64(015)}} = \frac{R_{57(052)}}{R_{57(015)}} = \frac{R_{133(052)}}{R_{133(015)}} = \frac{R_{137(052)}}{R_{137(015)}}. \qquad \text{Eq. 8}$$

By Equations 7 and 8 the ratio of readings of any radioactive sample at settings 052 and 015 give $D_{64(052)}$.

$$D_{64(052)} = \frac{R_{57(052)}}{R_{57(015)}} = \frac{R_{133(052)}}{R_{133(015)}} = \frac{R_{137(052)}}{R_{137(015)}}. \qquad \text{Eq. 9}$$

Sealed gamma reference source radionuclides ^{57}Co, ^{133}Ba and ^{137}Cs (New England Nuclear, North Billerica, MA) were measured

TABLE 1
Relative Activities of Copper-64 and Copper-67 (A_{64}/A_{67}) Provided by the Supplier Versus Values Determined by Gamma-Ray Spectroscopy for Verification

Radionuclide lot	A_{64}/A_{67} (±1 s.d.) (Supplier)*	A_{64}/A_{67} (±1 s.d.) (Spectroscopy)*	$D_{67(052)}$†	$D_{64(052)}$†
1	0.24 ± 0.014	ND‡	1.08	0.82
2	0.24 ± 0.014	ND‡	1.04	0.86
3	0.14 ± 0.008	0.06 ± 0.018	1.00§	0.49§
4	0.07 ± 0.004	0.08 ± 0.018	1.01	0.73
5	0.22 ± 0.013	0.19 ± 0.022	0.97	0.81
6	0.07 ± 0.004	0.06 ± 0.014	1.05	0.72
7	0.06 ± 0.004	0.06 ± 0.014	0.97	0.76
Avg. (±1 s.d.)			1.02 ± 0.04	0.78 ± 0.06

*A_{64}/A_{67} values are decay corrected to 24 hr after receipt of radionuclide, the typical time of injection of radiopharmaceutical.
†Dose calibrator measurement coefficients D_{64} and D_{67} were determined using supplier values for A_{64}/A_{67}.
‡No data; gamma-ray spectroscopy not performed.
§Results from lot 3 were disqualified based on the failure of the $D_{64(052)}$ value by the statistical Q-test.

at settings 052 and 015. The ratios of the measurements were used to determine $D_{64(052)}$ according to Equation 9.

Determination of Dose Calibrator Response Coefficients: Method 2

This method is used to gauge the response of a dose calibrator to ^{67}Cu or ^{64}Cu at an arbitrary setting N in the absence of recommended dose calibrator settings for the measurement of ^{67}Cu or ^{64}Cu. Aliquots of precisely measured volume of ^{67}Cu, containing ^{64}Cu (Brookhaven National Laboratory, Upton, NY, or Los Alamos National Laboratory, Los Alamos, NM) were measured at setting 052 on the radioisotope calibrator. Setting 052 was selected so that the empirical value of $D_{67(052)}$ determined by this method could be compared to the defined value of 1.00. Repeat measurements were taken periodically for up to 200 hr after receipt of the radionuclide. Supplier data were decay corrected to calculate A_{67} and A_{64} of the sample at the time of each measurement. For each measurement, the values $R_{(052)}$, A_{67} and A_{64} were substituted into an equation of the type Equation 3. These were compiled into a system of linear equations with unknowns $D_{67(052)}$ and $D_{64(052)}$. Solutions for $D_{67(052)}$ and $D_{64(052)}$ were generated by iterative least squares fit (Appendix).

Gamma-Ray Spectroscopy of Radionuclide

Samples of ^{67}Cu containing ^{64}Cu were assayed on a multichannel analyzer coupled to a germanium detector (Canberra ACC-2/GC2019, Canberra, Inc., Meriden, CT). Using samples of uniform geometry, all acquisitions were conducted for 15 min at a fixed distance of 15 cm from the face of the crystal. Copper-67 and ^{64}Cu were quantitated using photopeak measurements at 184 keV (abundance 0.47) and 1346 keV (abundance 0.006), respectively (*26*). The detection efficiencies at 184 and 1346 keV were interpolated from a calibration curve prepared from a mixed-gamma source reference standard (National Institute of Standards and Technology, Gaithersburg, MD).

Preparation and Assay of ^{67}Cu- and ^{64}Cu-2IT-BAT-Lym-1

Five lots of radiolabeled 2IT-BAT-Lym-1 radiopharmaceutical were prepared for a phase I ^{67}Cu therapy study as previously described (*9,14,16,20*). The patient doses were measured at calibrator setting 052.

RESULTS

Determination of Dose Calibrator Response Coefficients

To determine $D_{64(052)}$ by Method 1, the reference sources were measured at settings 052 and 015. The ratios of the measurements were: ^{57}Co, 0.720; ^{133}Ba, 0.721, and ^{137}Cs, 0.718, giving a mean value of $D_{64(052)}$ (±1 s.d.) of 0.720 ± 0.002. The accuracy of this result depends on instrument accuracy and the aptness of the manufacturer's recommended settings. For this study, the values $D_{67(052)} = 1.0$ and $D_{64(052)} = 0.72$ are reasonable standards against which to evaluate the results of method 2.

$D_{67(052)}$ and $D_{64(052)}$ were determined by Method 2 with seven different lots of radionuclide (Table 1). The values obtained from lot 3 were disqualified on the basis of the statistical Q-test for rejection of discordant data (Appendix) (*27*). The reason for the outlying result is not known. The mean values (±1 s.d.) of $D_{67(052)}$ and $D_{64(052)}$ for the other six lots were 1.02 (±0.04) and 0.78 (±0.06), which agree with the Method 1 standards within 1 s.d. Values of $D_{67(052)}$ and $D_{64(052)}$ generated by single lots of radionuclide deviated from the Method 1 standards by up to 8% and 19%, respectively. The relative errors of supplier-reported values of A_{67} (≤3%) and A_{64} (≤5%) (Kolsky, KN, Brookhaven National Laboratory, personal written communication) account partly for the lot-by-lot deviations. It is evident from the range of $D_{64(052)}$ values in Table 1 and the instance of outlying data in the case of radiometal lot 3 that multiple trials using multiple lots of radiometal may be necessary to arrive at an accurate value for $D_{64(N)}$ by Method 2.

Gamma-Ray Spectroscopy of Radionuclide

The assays of A_{64}/A_{67} by gamma-ray spectroscopy agreed with supplier values within experimental error (Table 1), with the exception of lot 3, for reasons that are not known. Radionuclides other than ^{67}Cu and ^{64}Cu were not detected.

The estimated error in measurement of A_{67}/A_{64} by gamma-ray spectroscopy is based on statistical counting error at the ^{67}Cu and ^{64}Cu photopeaks, <1% and 4%–12%, respectively. Statistical counting error of the reference standard, error of estimation of counting efficiency at selected wavelengths by interpolation of the standard curve, and experimental variables such as the positioning of the sample, also contributed to overall error.

Assay of Copper-67 and Copper-64 in Radiopharmaceuticals

Batches of radiolabeled 2IT-BAT-Lym-1 were measured at setting 052. The relative activities of ^{64}Cu and ^{67}Cu, per the radionuclide supplier, were decay corrected to the time of measurement to calculate P. A_{67} was calculated by Equation 5, using $D_{67(052)}$ and $D_{64(052)}$ values of 1.02 and 0.78, respectively.

TABLE 2
Activities of Copper-64 and Copper-67 in Each Batch of Radiolabeled 2IT-BAT-Lym-1 Pharmaceutical Calculated from a Single-Dose Calibrator Measurement Using Equations 4 and 5

Radionuclide lot*	Dose calibrator measurement	^{67}Cu (mCi)	^{64}Cu (mCi)
2	285	236	57
3	136	120	17
4	120	112	8
5	148	124	27
6	153	142	10

*Radiopharmaceutical was not prepared from radionuclide lots 1 and 7.

Subsequently, A_{64} was calculated by Equation 4. Assay results for five radiopharmaceutical batches (Table 2) showed significant discrepancies between uncorrected dose calibrator measurements and the calculated values for ^{67}Cu activity.

DISCUSSION

As the use of ^{67}Cu for the treatment of cancer by radioimmunotherapy increases, so does the importance of simplifying the preparation and characterization of ^{67}Cu radiopharmaceuticals. The presence of ^{64}Cu radiocontaminant complicates the preparation of accurate doses of ^{67}Cu pharmaceutical. When ^{64}Cu is present, raw dose calibrator measurements overestimate the amount of ^{67}Cu in the sample (Fig. 1). In this study, relative ^{64}Cu:^{67}Cu activity was as high as 0.24:1.0 at the time of injection; as ^{67}Cu is processed and incorporated into radiopharmaceuticals faster to maximize yield and specific activity, ^{64}Cu will comprise a greater fraction of total activity. The method described eliminates the need for specialized equipment to accurately assay ^{67}Cu pharmaceuticals in the presence of ^{64}Cu.

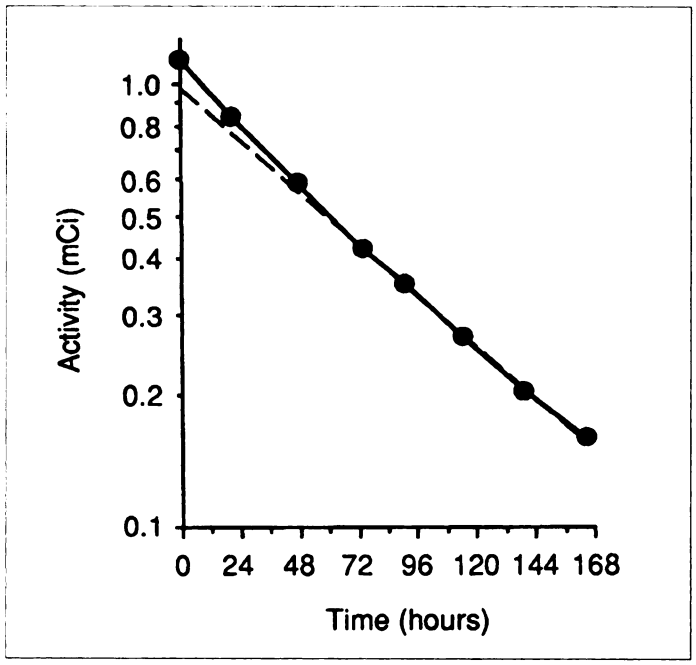

FIGURE 1. Dose calibrator readings versus actual ^{67}Cu activity in a sample of ^{67}Cu/^{64}Cu. An aliquot of radionuclide lot 4 was measured periodically at setting 052 of the dose calibrator (solid line). The activity of ^{67}Cu in the sample, according to the supplier's assay, was calculated at each time point (dashed line). Early readings overestimated the activity of ^{67}Cu in the sample due to contribution of ^{64}Cu activity to the measurement. The discrepancy between the dose calibrator readings and the activity of ^{67}Cu decreases with the rapidly decreasing activity of ^{64}Cu.

This expands the number of facilities at which ^{67}Cu may be considered as a practical therapeutic agent.

CONCLUSION

We described a method by which ^{67}Cu and ^{64}Cu are assayed using a single measurement with a common instrument, the ionization chamber dose calibrator. The assay calculation requires: (a) the ratio A_{64}/A_{67}, routinely provided by major suppliers of ^{67}Cu and (b) dose calibrator response coefficients $D_{67(N)}$ and $D_{64(N)}$ at setting N. Except for one instance in this study, the ratios A_{64}/A_{67} were corroborated by gamma-ray spectroscopy. Where recommended dose calibrator settings for ^{67}Cu and ^{64}Cu measurement are known, the determination of response coefficients is trivial. In the absence of recommended settings, a method has been presented by which $D_{67(N)}$ and $D_{64(N)}$ at any dose calibrator setting N may be empirically determined. As shown by the variance in results among lots of radionuclide examined in this study, it is desirable to perform this determination with multiple trials using multiple lots.

Although the values obtained in this study are specific for the Capintec CRC-12 dose calibrator at the setting 052, this method can be adapted for use with other instruments or settings.

APPENDIX

The determination of $D_{67(052)}$ and $D_{64(052)}$ by Method 2 for lot 4 of radionuclide is described in detail below.

The concentrations of ^{67}Cu and ^{64}Cu in the radionuclide solution were reported by the supplier to be 34.24 and 7.14 mCi/ml, respectively, at t = 0, the supplier's calibration time. At t = 2.5 hr, a 29.0 μl aliquot of radionuclide was removed and measured at setting 052 as 1.103 mCi. At this time, the decay-corrected activities of ^{67}Cu and ^{64}Cu in the aliquot were calculated to be 0.966 mCi and 0.181 mCi, respectively, by the supplier's assay. From these data, an equation of the type Equation 3 was constructed:

$$1.103\ \text{mCi} = [D_{67(052)}]\ (0.966\ \text{mCi}) + [D_{64(052)}]\ (0.181\ \text{mCi}), \quad \text{Eq. 10}$$

which may be rearranged, with units omitted, as

$$1.103 - 0.966\ D_{67(052)} - 0.181\ D_{64(052)} = 0. \quad \text{Eq. 11a}$$

The aliquot was measured as 0.831 mCi at t = 23.0 hr; 0.587 mCi, 50.0 hr; 0.416 mCi, 75.8 hr; 0.352 mCi, 93.7 hr; 0.267 mCi, 117.5 hr; 0.201 mCi, 141.7 hr; and 0.158 mCi, 167.0 hr. Using decay-corrected activities of ^{67}Cu and ^{64}Cu, these data were used to create a system of linear equations of the type Equation 11a:

$$0.831 - 0.768\ D_{67(052)} - 0.059\ D_{64(052)} = 0, \quad \text{Eq. 11b}$$

$$0.587 - 0.568\ D_{67(052)} - 0.014\ D_{64(052)} = 0, \quad \text{Eq. 11c}$$

$$0.416 - 0.426\ D_{67(052)} - 0.003\ D_{64(052)} = 0, \quad \text{Eq. 11d}$$

$$0.352 - 0.348\ D_{67(052)} - 0.001\ D_{64(052)} = 0, \quad \text{Eq. 11e}$$

$$0.267 - 0.267\ D_{67(052)} - 0.000\ D_{64(052)} = 0, \quad \text{Eq. 11f}$$

$$0.201 - 0.204\ D_{67(052)} - 0.000\ D_{64(052)} = 0, \text{ and} \quad \text{Eq. 11g}$$

$$0.158 - 0.154\ D_{67(052)} - 0.000\ D_{64(052)} = 0. \quad \text{Eq. 11h}$$

$D_{67(052)}$ and $D_{64(052)}$ were approximated by least squares fitting of Equations 11a–11h as follows. Corresponding Equations 12a–12h were generated by substituting a value x_i for 0:

$$1.103 - 0.966\ D_{67(052)} - 0.181\ D_{64(052)} = x_1, \quad \text{Eq. 12a}$$

$$0.831 - 0.768\ D_{67(052)} - 0.059\ D_{64(052)} = x_2, \quad \text{Eq. 12b}$$

$$0.587 - 0.568\ D_{67(052)} - 0.014\ D_{64(052)} = x_3, \quad \text{Eq. 12c}$$

$$0.416 - 0.426\ D_{67(052)} - 0.003\ D_{64(052)} = x_4, \quad \text{Eq. 12d}$$

$$0.352 - 0.348\ D_{67(052)} - 0.001\ D_{64(052)} = x_5, \quad \text{Eq. 12e}$$

$$0.267 - 0.267\ D_{67(052)} - 0.000\ D_{64(052)} = x_6, \quad \text{Eq. 12f}$$

$$0.201 - 0.204\ D_{67(052)} - 0.000\ D_{64(052)} = x_7, \text{ and} \quad \text{Eq. 12g}$$

$$0.158 - 0.154\ D_{67(052)} - 0.000\ D_{64(052)} = x_8. \quad \text{Eq. 12h}$$

Values of $D_{67(052)}$ and $D_{64(052)}$ were determined such that a minimum value of E was obtained (Microsoft Excel 4.0, Microsoft Corp., Redmond, WA) where E is defined as:

$$E = \Sigma_i\ x_i^2. \quad \text{Eq. 13}$$

In this example, $D_{67(052)}$ and $D_{64(052)}$ were found to be 1.01 and 0.73 (Table 1). Data were taken from all radionuclide lots to generate values of $D_{67(052)}$ and $D_{64(052)}$ in the same way. The means of these trial values were calculated to give $D_{67(052)}$ (± 1 s.d.) = 1.02 ± 0.04 and $D_{64(052)}$ (± 1 s.d.) = 0.78 ± 0.06.

The results from radionuclide lot 3 were rejected on the basis of the statistical Q test (*28*). The value Q is the ratio of the difference between the value under suspicion and the value in best agreement with it to the difference between the highest and lowest values in the series. Q is compared with the critical value Q_c, which indicates whether the value under suspicion can be rejected within a certain range of confidence. For the value of $D_{64(052)}$ from radionuclide lot 3 (Table 1), $Q = (0.72 - 0.49)/(0.86 - 0.49) = 0.60$. This is greater than Q_c (90%) = 0.51 for n = 7, so the value for $D_{64(052)}$ from this trial was rejected. Because the values of $D_{64(052)}$ and $D_{67(052)}$ are interdependent, the value of $D_{67(052)}$ for this trial was also rejected.

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