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Short communication

Detection limit of measurement of pharmaceuticals labeled with short-lived isotopes in HPLC with flow-through γ-counter

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Abstract

This paper proposes a method for estimating the detection limit, which is defined as 3.3 times the standard deviation (S.D.) of blank measurements under the situations where the repetition of measurement is difficult or impossible because of a short half-life of radioactivity. The FUMI theory, which can estimate an S.D. value without repetition in various instrumental analyses, is adopted and proved here to be available in a radio-HPLC system as well. ^{99m}Tc-ECD ($T_{1/2}$ = 360.6 min) that is a lipophilic compound for the diagnosis of regional brain perfusion is taken as an example.
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1. Introduction

Pharmaceutical and biomedical analyses, which require the determination of minute amounts of material in complex matrices, are especially dependent on the use of radiolabeled analytes. Most of the applications of the radiolabeled compounds consist of the separation of the compounds in one or more stages of experiments and subsequent radioactivity counting. Various techniques have been developed for the radioactivity detection in high-performance liquid chromatography (HPLC) [1].

The quality and quantity of the radiopharmaceuticals should be validated according to the general rules of method validation. The validation characteristics including the uncertainty of measurements, detection limit and quantitation limit are required to meet the international consensus for the reliability of analytical results. However, as for pharmaceuticals labeled with short-lived isotopes such as ^{18}F, ^{11}C and ^{99m}Tc, the radioactivity decreases appreciably during the measurement process in slow methods like HPLC and in principle, the S.D. of measurements cannot be estimated with replication.

As is well known, if measurements are corrected according to the degree of the radioactivity decrease, an S.D. value can be estimated from the corrected measurements. A measurement, A_m, when obtained at time t, will take a value, A_c, at the starting time of analysis:

$$A_c = \frac{A_m}{(1/2)^{t/T_{1/2}}} \quad (1)$$

where $T_{1/2}$ is the half-life of radioactivity.

Fig. 1A shows the decreasing radioactivity (···) of a sample and its measurements (●, A_m) including the white noise of a constant S.D. The black circles of Fig. 1B illustrates the difference between the true decreasing activity (···) and measurements (●) shown in Fig. 1A. The gray circles denotes the difference between the corrected measurements, A_c, calculated according to Eq. (1) and the first measurement at $t = 0$ (A_m = 10,000).

We can see from Fig. 1B that the absolute intensities of the errors of the corrected measurements (gray circles) are always larger than the true measurement error (black circles). This is because the corrected measurements include not only the activity corrected rightly but also the noise enhanced unnecessarily by Eq. (1).

In the simplest model of Fig. 1, the error source is assumed to be the added measurement noise alone and the black circles of

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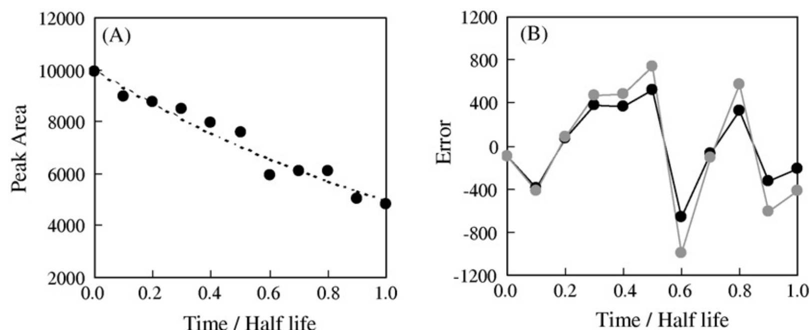

Fig. 1. Radioactivity Decay Model: (A) the dotted line shows theoretical radioactive decay; the black circle shows the radioactivity measurement including random error; (B) the black circle denotes the measurement error shown in A (the difference between the dotted line and black circle); the gray circle denotes the deviation of the decay correction from the true decay (the corrected value by Eq. (1)). The error (black circle) is obtained by the random number of the normal distribution.

Fig. 1B will give the unbiased S.D. On the other hand, the gray circles (measurements corrected by Eq. (1)) always provide an overestimated S.D. value. Unfortunately, the overestimation is a real situation.

This paper proposes a method for calculating an unbiased estimate of S.D. in HPLC for pharmaceuticals labeled with short-lived isotopes. The FUMI theory (function of mutual information) [2] is adopted, since the theory can achieve the purpose without the measurement repetition, and then the radioactivity correction by Eq. (1) is unnecessary.

There have been many relevant publications. An HPLC system with an off-line detection of short-lived positron emitter, ^{11}C ($T_{1/2}$ = 20.4 min), was validated [3]. Due to the short half-life of ^{11}C, the precision of the detection system was examined with a radionuclide, ^{57}Co ($T_{1/2}$ = 270 d), the half-life of which is long enough to dispense with the correction of decay between all measurements. Another study of the positron emitter regarded the intensity twice the background noise level as the detection limit [4]. Statistical studies for short-lived γ-emitting radioisotopes have focused on FFT-filtering for noise reduction [5], and retention time reproducibility [6]. For long-lived radioisotopes, the detection limit and quantitation limit have been studied experimentally [7–9]. The general theory of these limits was given by Currie [10].

To authors' knowledge, this paper first proposes an alternative method to the radioactivity decay correction for estimating the detection limit in radio-HPLC.

2. Materials and methods

Radioactive sample, Na^{123}I, was purchased from FUJIFILM RI Pharma Co. Ltd. and ^{99m}Tc-ECD (^{99m}Tc-L, L-ethylcysteinate dimer) from Bristol-Myers Squibb. The radioactivity purity for the samples was confirmed by the radio-TLC to be more than 99%.

A Waters HPLC (LC Module Plus) equipped with a Raytest on-line γ-ray detector (GABI) is used. The detector cell volume is 200 μL. The energy range of the detector is 50–200 keV for ^{123}I and ^{99m}Tc. The sampling intervals of the analog-to-digital converter were 1 s.

For ^{99m}Tc-ECD, the mobile phase consisted of a mixture of acetonitrile and phosphorus buffer (1:1) with pH 7.0. A Mightysil 4.6 μm RP-C_{18} column (3.0 mm i.d. × 250 mm, Kanto Kagaku, Japan) was used. The injection volume was set at 10 μL. The typical analysis time was 20 min.

For Na^{123}I, the mobile phase composition is water/acetonitrile (1:1), the column is Mightysil (3.0 mm i.d. × 50 mm) and the injection volume is 10 μL. The retention time is about 1 min and the total time of a chromatogram is 2 min.

3. FUMI theory

In the FUMI theory, the measurement errors are assumed to originate from the baseline noise and sample injection into an HPLC apparatus [2]. The injection error (relative S.D.) can be obtained from repeated measurements or producer's specification. The S.D. of measurement error due to the noise can be estimated from the stochastic properties of the baseline noise. The noise model in the theory is made up of the white noise and Markov process, which are well known random processes in mathematics. The baseline noise is Fourier-transformed into a power spectral density to which the power spectral density of the noise model is fitted by the simplex least squares. The resulting parameters from the fitting are used for the theoretical estimation of the measurement S.D. [2,11].

All the calculations were conducted with a commercial software, MAY2000 (Yazawa). The baseline of 1024 data points was Fourier-transformed for the analysis. The time for the 1024-point data was 1024 s.

4. Results and discussion

Table 1 lists the original and decay-corrected area measurements of ^{99m}Tc-ECD in the HPLC system with the flow-through γ-counter. The original measurements are the integrated intensities over a region of 63 data points (=63 s). The order of the HPLC measurement carried out is for 56.4 kBq/mL (n = 1) → 113 kBq/mL (n = 1) → 169 kBq/mL (n = 1) → 226 kBq/mL (n = 1) → 282 kBq/mL (n = 1) → 56.4 kBq/mL (n = 2) → 113 kBq/mL (n = 2) → · · · → 282 kBq/mL (n = 6). In Table 1, the decay correction is applied to the measurements except the first sample, which is assumed to be obtained at the starting time (t = 0). The intervals of the measurements

Table 1
Corrected area measurements (original measurements)

	Radioactive concentrations of ^{99m}Tc-ECD (kBq/mL)				
	56.4	112.8	169.2	225.6	282.0
1	6.83*	11.76 (8.45)	24.91 (13.39)	25.32 (10.18)	35.61 (10.71)
2	9.06 (8.70)	16.71 (11.53)	23.27 (12.01)	36.15 (13.96)	37.21 (10.73)
3	8.03 (7.39)	14.24 (9.42)	19.50 (9.65)	28.36 (10.50)	38.13 (10.56)
4	6.97 (6.16)	12.08 (7.66)	18.51 (8.78)	27.73 (9.84)	42.34 (11.24)
5	7.92 (6.71)	15.02 (9.15)	12.09 (5.51)	29.66 (10.11)	25.14 (6.41)
6	5.83 (4.75)	16.72 (9.76)	17.63 (7.70)	32.29 (10.55)	41.41 (10.12)

* shows single starting point of the analysis.

(=analysis times) are 20 min and 10 h pass over the entire analysis. Nearly 68% decrease in radioactivity is expected for the last sample.

The detection limit, L_D, is defined as [12–14]

$$L_D = 3.3 \times \frac{\sigma}{a} \tag{2}$$

where σ denotes the S.D. estimates of measurements near the blank measurement and a is the slope of the calibration line. We should note that σ/a has the dimension of concentration and that the relative standard deviation (R.S.D.) of concentration estimates (=$(\sigma/a)/L_D$) is equal to 30% when the sample concentration is just the detection limit ($\because$ from Eq. (2), $(\sigma/a)/L_D = 1/3.3 = 30\%$) [15].

All the requirements for the detection limit are the slope, a, and measurement S.D., σ. The straight line least-squares-fitted to the decay-corrected measurements in Table 1 gives these values as $a = 0.131$ and $\sigma = 3.94$. The latter can be estimated from the residuals of the least squares fitting. Then, the detection limit is determined to be 99.3 kBq/mL.

Fig. 2A illustrates the L_D peak superimposed on the real noise. The L_D peak is the scale-down of a peak of high concentration (=282 kBq/mL), which looks smooth and noise-free (not shown).

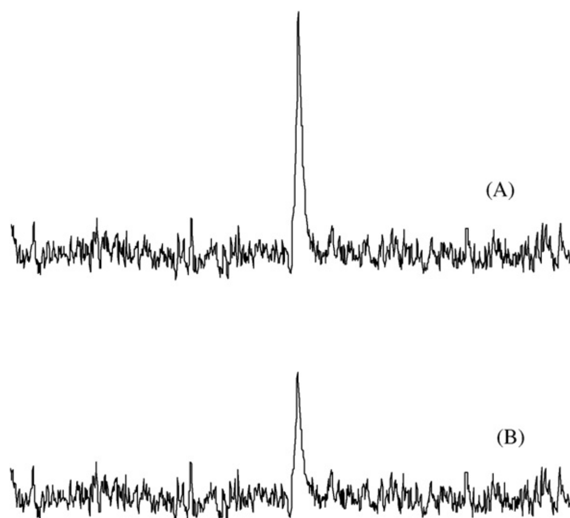

Fig. 2. Signals of detection limit over the noise, estimated from the repeated measurements (A, 99.3 kBq/mL) and the FUMI theory (B, 34.0 kBq/mL).

We now understand that the L_D peak of Fig. 2A is an overestimate as indicated in the introductory section. The signal-to-noise ratio (S/N) of a peak is often defined to be two or three at the detection limit [4,16,17], but the L_D peak of Fig. 2A has much more S/N than three. Alternatively, we might have accepted the scale-down peak with an S/N of three as a L_D peak, instead of following Eq. (1). As indicated so far [2,11], however, the S/N does not necessarily reflect the statistical aspects of Eq. (2). This paper basically adopts Eq. (2) (30% R.S.D. at L_D).

Fig. 2B illustrates the L_D peak (=34.0 kBq/mL) determined by the FUMI theory. In light of the S/N ratio, the peak size of Fig. 2B looks reasonable. The rest of this paper is spent on experimentally verifying that the measurement R.S.D. from the FUMI theory is in good agreement with that obtained from the replication under the special condition that the decay correction is unnecessary.

Fig. 3 shows the precision profile of the determination of a long-lived isotope, Na^{123}I ($T_{1/2} = 13.27$ h), in the radio-HPLC system. The solid line is obtained from the FUMI theory and the circles denote the R.S.D. estimates from the repetition ($n = 6$). The bars are the 95% confidence intervals of the R.S.D. estimates. Fig. 3 presents a typical profile of instrumental analyses [16,17]. That is, the R.S.D. of measurements decreases with increasing concentration.

A chromatogram at the highest concentration, including the baseline, is used for the analysis of the FUMI theory. The non-linear least squares as is mentioned above leads to the noise parameters (S.D., w, of the white noise (=0), S.D., m, of the Markov process (=4.0×10^{-2}) and correlation coefficient, r, of

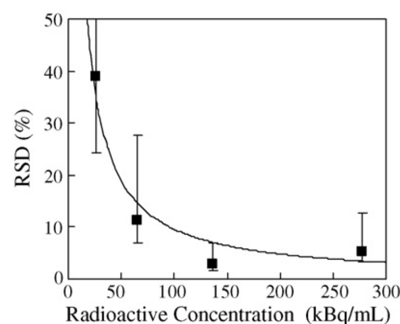

Fig. 3. Precision profile of Na^{123}I determination in the radio-HPLC. The solid line is obtained from the FUMI theory and the square from the repeated measurements ($n = 6$). The vertical bar shows the 95% confidence intervals of the R.S.D. estimates (=(±95% confidence intervals of S.D.)/average).

the Markov process (=0.39)). Coupled with the signal parameters (peak width and area) and calibration line, the resulting noise parameters provide the continuous precision profile (—).

Twenty-four measurements (six replicates at each concentration) are used for the discrete precision profile (■). Since the analysis time of the sample is 2 min, the total time for the precision profile (48 min = 2 min × 24) is negligibly short compared with the half-life ($T_{1/2} = 13.27$ h) of the isotope, ^{123}I. The decay correction can be dispensed with under this situation.

In Fig. 3, the R.S.D. values from the FUMI theory and repetition at the lowest concentration are almost the same. Then, so are the S.D. values from both methods. As far as the experimental data of Fig. 3 are concerned, the detection limit from the FUMI theory is almost equal to that from repetition.

The excellent agreement between the theory and practice of the precision profile in Fig. 3 lead us to the conclusion that the FUMI theory is applicable in radio-HPLC for the determination of pharmaceuticals labeled with short-lived isotopes. As long as the FUMI theory can successfully describe the precision in the radio-HPLC system for long- and short-lived isotopes, we can safely say that the R.S.D. for the peak in Fig. 2B meets the statistical requirement of the detection limit (i.e., 30% R.S.D.). The versatile applicability of the FUMI theory in instrumental analyses [2,11,13] will corroborate the reliability of the proposal of this paper.

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