A Method for Measuring Specific Activities of ^{14}C -Labelled Compounds by Gas Chromatography-Mass Spectrometry-Computer System

Hiroshi KANAMARU, Ryoza TAKAI*, Masao HORIBA, Iwao NAKATSUKA
and Akira YOSHITAKE

Takarazuka Research Center, Sumitomo Chemical Co., Ltd.

2-1, Takatsukasa 4-chome, Takarazuka-shi 665

* Sumika Chemical Analysis Service, Ltd.

1-135, Kasugadenaka 3-chome, Konohana-ku, Osaka-shi 554

Received October 16, 1984

A method for measuring specific activities of ^{14}C -labelled compounds by gas chromatography-mass spectrometry-computer system (GC-MS-CPU) was developed. This method was proved to provide practically precise and accurate specific activities of various ^{14}C -labelled compounds with such merits as requirement of small amount of samples, being applicable to volatile compounds, and convenience. The C.V. percent obtained for tested compounds was within 3.9 and the reliable sensitivity should be over 37 MBq/mM (1 mCi/mM). This method was also useful for obtaining information on the labelling pattern and the synthetic procedures applied.

Key Words: specific activity measurement, carbon-14 labelled compound,
gas chromatography-mass spectrometry-computer system

1. Introduction

Today a substantial part of biochemical, medical, pharmaceutical and pesticidal research is carried out with the aid of ^{14}C -labelled compounds. It is, therefore, become important to solve problems associated with the quality control of the labelled compounds in their purity, stability and specific activity. To know the accurate specific activity is one of the most important factors, because the quoted specific activity is frequently used to quantitatively calculate the amount of the compound used in the experiments. A conventional method for measuring specific activity from the radioactivity of a known weight of the labelled compound (radioactivity-weight method) is not always very accurate in certain compounds when they are contaminated with impurities or solvents and have unfavorable physicochemical properties in measuring their weight or radioactivity.

Goldstein and Rainey¹⁾ reported determination of specific activities of ^{14}C -labelled purines and pyrimidines by mass spectrometry. Their method, however, seems to have some disadvantages

in accuracy, precision and convenience, since the specific activity is calculated based on a few mass spectra obtained by a direct-inlet method.

In this report, we describe a method for measuring specific activities of ^{14}C -labelled compounds by gas chromatography-mass spectrometry-computer system (GC-MS-CPU), by which one could evaluate specific activities with practical accuracy, precision and convenience.

2. Experimental

2.1 Apparatus

Measurement of mass spectra and calculation of specific activity were carried out with a LKB-9000 gas chromatograph-mass spectrometer combined with a PAC-300-DGB computed data system (Shimadzu, Kyoto, Japan). The computer-program developed by ourselves will be described in detail elsewhere.

2.2 Materials and methods

Most of ^{14}C -labelled compounds and the corresponding unlabelled standard materials were synthesized in our laboratory. The com-

pounds used in this experiment were as follows: fenitrothion²⁾; fenvalerate³⁾; 2, 6-di-*tert*-butyl-4-methylphenol (BHT)⁴⁾; melinamide⁵⁾; ciproquazone⁶⁾; nimetazepam⁷⁾; fludiazepam⁸⁾; methyl 2-(4-chlorophenyl)-3-methylbutyrate (CPMB)⁹⁾; α -methylbenzylamine (MBA)¹⁰⁾; *m*-cresol (Amersham International plc, Amersham, England); *m*-bromophenol (Amersham International). About 5 μ g of ¹⁴C-labelled compound or unlabelled standard material was dissolved in a proper solvent at a suitable concentration, and the solution introduced into GC of the GC-MS-CPU system in a usual manner. Measurement of mass spectra of the GC-eluent was performed by a mass chromatographic procedure¹¹⁾ with multiple scanning at 4 s interval and with ionizing energy of 70 or 12 eV. The spectra collected were stored in the computer-bank.

Measurement of specific activities of the various ¹⁴C-labelled compounds by radioactivity-weight method was carried out as follows. A sample (10–100 mg) of a ¹⁴C-labelled compound was weighed by a balance (Model H-20, Mettler Instrument AG, Zurich, Switzerland; measuring limit 0.01 mg, standard error ± 0.02 mg). The C.V. of the measurement was below 0.2%. The sample was dissolved in a proper solvent (50.0 ml) and a portion (100 μ l) of the solution was then diluted with the same solvent to a known concentration (2–20 μ g/ml) suitable for radioactivity measurement. An aliquot (100 μ l) of the diluted solution was added to a liquid scintillator (Permafluor I, Packard Instrument Co., Inc., Illinois, U.S.A.) and the radioactivity was measured by a liquid scintillation counter (Model TRI-CARB 460, Packard Instrument Co., Inc.; measuring time 5 min, C.V. below 0.3%). The C.V. of the radioactivity measurement was below 1%.

3. Principle

It is apparent that a ¹⁴C_{*n*}-labelled compound generally consists of a series of molecules which are labelled with ¹⁴C at 0 to *n* numbers of carbons at specified positions. Therefore, to calculate specific activity of the ¹⁴C_{*n*}-labelled compound, it is necessary to know the relative abundance of each labelled molecule which forms the ¹⁴C_{*n*}-labelled compound. The relative abundance can be determined by a mass

Fig. 1 Mass peaks corresponding to a fragmentation ion-cluster in a mass spectrum of a certain ¹⁴C₂-labelled compound.

spectrum of the ¹⁴C_{*n*}-labelled compound since such a labelled compound, due to the difference of two mass units between ¹⁴C and ¹²C, evidently gives distinct enrichments of peak intensities corresponding to a fragmentation ion-cluster which contains ¹⁴C_{*n*} in the mass spectrum, for example, such peak intensities corresponding to the molecular ion-cluster at $m/z = M + 2n$.

The principle to determine the relative abundances of labelled molecules by the mass spectrum has been reported and discussed in the field of stable isotope labelled compounds¹⁰⁾, and also Goldstein, et al.¹¹⁾ proved it applicable to ¹⁴C-labelled compounds. The following discussion is to give a better understanding of its application to ¹⁴C_{*n*}-labelled compounds.

Figure 1 illustrates a group of mass peaks corresponding to a fragmentation ion-cluster in a mass spectrum of a certain ¹⁴C₂-labelled compound. The peaks appear as an accumulation of the peaks originating from ¹⁴C₀- (i.e. unlabelled), ¹⁴C₁- and ¹⁴C₂-labelled molecules. The peak at $m/z = M$ with the peak intensity of a_0 originates from the ¹⁴C₀-molecule. The peak (a_1) at $m/z = M + 2$ consists of the peak (x_1) originating from ¹⁴C₁-molecule and that (b_1) from the natural stable isotopes in the ¹⁴C₀-molecule. Similarly, the components of the peak (a_2) at $m/z = M + 4$ are x_2 , b_2 and b_1x_1/b_0 . The peak intensities of x_0 , x_1 , and x_2 can be determined by the spectral data of both the ¹⁴C₂-labelled compound and the corresponding unlabelled standard which should be put into the following equations:

$$x_0 = a_0 = b_0 = 100$$

Table 1 Reproducibility of measuring the specific activity of fenitrothion-(arylmethyl- ^{14}C)

No.	Relative abundance of labelled molecule*				Specific activity (MBq/mM)
	a_0	a_1	a_2	a_3	
1	100	45.1	0	0	722
2	100	45.3	0	0	722
3	100	44.1	0	0	707
4	100	44.3	0	0	710
5	100	44.9	0	0	718
Mean					716
±S. D.					6.7
C. V. (%)					0.93

* Molecular ion was used for measurement

$$x_1 = a_1 - \frac{b_1}{b_0} x_0$$

$$x_2 = a_2 - \frac{b_1}{b_0} x_1 - \frac{b_2}{b_0} x_0$$

Thus, the values of x_0 , x_1 , and x_2 are directly related to the relative abundances of $^{14}\text{C}_0$ -, $^{14}\text{C}_1$ - and $^{14}\text{C}_2$ -labelled molecules, respectively.

From the equations, the relative intensity of the peak originating from $^{14}\text{C}_n$ -labelled molecule can be expressed as the following equation (1):

$$x_n = a_n - \sum_{k=1}^n \left(\frac{b_k}{b_0} x_{n-k} \right) \quad (1)$$

where x_n = the relative intensity of the peak originating from $^{14}\text{C}_n$ -labelled molecule

a_n = the relative intensity of the peak originating from $^{14}\text{C}_n$ -labelled compound

b_n = the relative intensity of the peak originating from unlabelled standard

$$x_0 = a_0 = b_0 = 100$$

Since the specific activity of carrier-free ^{14}C is theoretically 2.31 GBq/mg atom (62.4 mCi/mg atom), the specific activity can be calculated by the formula (2):

$$\text{Specific activity (GBq/mM)} = 2.31 \left(\frac{\sum n a_n}{\sum a_n} \right) \quad (2)$$

Process of averaging the required peak intensities of the mass spectra obtained by multiple scanning, and calculation of the relative abundance (a_n) and the specific activity by formulae (1) and (2) are automatically performed by using the computer.

4. Results and Discussion

Reproducibility of measuring specific activity

Table 2 Reproducibility of measuring the specific activity of fenitrothion-(phenyl- $^{14}\text{C}_n$)

No.	Relative abundance of labelled molecule*							Specific activity (MBq/mM)
	a_0	a_1	a_2	a_3	a_4	a_5	a_6	
1	100	0	0	0	0.7	2.4	3.7	803
2	100	0	0	0	0.7	2.3	3.8	792
3	100	0	0	0	0.8	2.4	3.7	799
4	100	0	0	0	0.7	2.1	3.7	773
5	100	0	0	0	0.7	2.3	3.6	781
Mean								790
±S. D.								12
C. V. (%)								1.6

* Molecular ion was used for measurement

by GC-MS-CPU system was examined with the use of fenitrothion-(arylmethyl- ^{14}C) as an example of mono-labelled compounds and fenitrothion-(phenyl- $^{14}\text{C}_n$) as that of multi-labelled compounds. Results are shown in Table 1 and 2. The specific activity of fenitrothion-(arylmethyl- ^{14}C) was observed to be the mean value ($n=5$) of 716 ± 6.7 MBq/mM (19.3 ± 0.18 mCi/mM) with C.V. 0.93% while that of fenitrothion-(phenyl- $^{14}\text{C}_n$) to be the mean value ($n=5$) of 790 ± 12 MBq/mM. These results seem to be satisfactory for practical use of this method.

In fenitrothion-(phenyl- $^{14}\text{C}_n$) it is noteworthy to point out the fact that only $^{14}\text{C}_{4-6}$ but not $^{14}\text{C}_{2-3}$ -labelled molecules were observed, indicating that the synthesis of this compound was achieved by using ^{14}C -starting material with extremely high specific activity at earlier synthetic steps. Goldstein, et al.¹³ reported similar observation that "uniformly ^{14}C -labelled" adenine was found to consist of only molecules with 3, 4, and 5 ^{14}C atoms per molecule. These facts

Table 3 Sensitivity of measuring the specific activity of fenitrothion-(arylmethyl- ^{14}C)

No.	Specific activity (MBq/mM)*		
	Sample A	Sample B	Sample C
1	185	40.7	17.4
2	192	34.0	15.5
3	192	34.0	15.5
4	185	34.0	15.9
Mean	189	35.7	16.1
±S. D.	5.6	4.1	1.9
C. V. (%)	2.9	11.0	11.5

* Molecular ion was used for measurement

indicate that the method using MS can provide useful information on the labelling pattern and possibly on the synthetic procedures applied.

To examine sensitivity, three kinds of samples of ^{14}C -fenitrothions with different specific activities were prepared. Results of the measurement are indicated in Table 3 and 4. In the case of fenitrothion-(arylmethyl- ^{14}C), the C.V. of the sample A with 189 MBq/mM (5.1 mCi/mM) was 2.9% while those of the sample B and C with 35.7 MBq/mM (0.98 mCi/mM) and 16.1 MBq/mM (0.44 mCi/mM), respectively, were approximately 11%. On the other hand, the C.V. of every sample of fenitrothion-(phenyl-

$^{14}\text{C}_n$) was below 5.3%. These results represent that the reliable sensitivity should be over 37 MBq/mM (1 mCi/mM). However, as shown in fenitrothion-(phenyl- $^{14}\text{C}_n$), specific activity of a multi-labelled compound could be measured as low as 19 MBq/mM (0.5 mCi/mM) in considerable reliance, since the concerning peaks of the fragment ion in its mass spectrum would separate from those of the unlabelled compound so that effects of natural stable isotopes would decrease and more precise relative abundance (x_n) could be obtained.

Results of measuring specific activities of various ^{14}C -labelled compounds are summarized in Table 5 in comparing with those measured by the conventional radioactivity-weight method. All of the specific activities were able to be precisely determined with the C.V. below 3.9% and their values reasonably coincided with those measured by the radioactivity-weight method. As shown in *m*-cresol- $^{14}\text{C}_n$ and *m*-bromophenol- $^{14}\text{C}_n$, this method can be applied to volatile compounds, of which weight measurement is difficult, with such advantages as requirement of small amounts of samples and convenience. It is also noted that application to ^{14}C -labelled compounds having chlorine or bromine in their molecule, for example, fenvalerate- ^{14}C , CPMB-

Table 4 Sensitivity of measuring the specific activity of fenitrothion-(phenyl- $^{14}\text{C}_n$)

No.	Specific activity (MBq/mM)*		
	Sample D	Sample E	Sample F
1	211	44.4	20.0
2	211	40.7	20.4
3	207	40.7	17.8
4	211	40.7	19.6
5	204	40.7	19.6
Mean	209	41.4	19.5
±S. D.	2.6	2.2	1.1
C. V. (%)	1.4	5.3	5.1

* Molecular ion was used for measurement

Table 5 Measurement of specific activities of various ^{14}C -labelled compounds

Labelled compound	Labelled position	Fragment ion used	Specific activity (MBq/mM)		
			GC-MS-CPU method* (S.A.±S.D.)	(C.V.%)	RW method** (S.A.)
Fenvalerate	Carbonyl- $^{14}\text{C}_1$	M^+	714±11.5	1.6	762
	Cyano- $^{14}\text{C}_1$	M^+	581±14.4	2.5	570
	Chlorophenyl- $^{14}\text{C}_n$	M^+	796±27.8	2.5	770
	Phenoxy- $^{14}\text{C}_n$	M^+	662±25.9	3.9	621
Melinamide	Benzyl- $^{14}\text{C}_1$	M^+	115±1.5	1.3	118
Ciproquazone	4- $^{14}\text{C}_1$	M^+	263±0.4	0.1	255
Nimetazepam	2- $^{14}\text{C}_1$	M^+	281±2.6	0.9	296
Fludiazepam	2- $^{14}\text{C}_1$	M^+	211±8.1	3.8	222
BHT	Arylmethyl- $^{14}\text{C}_1$	M^+	107±3.3	3.1	111
	Phenyl- $^{14}\text{C}_n$	M^+	111±3.7	3.2	111
CPMB	Carbonyl- $^{14}\text{C}_1$	M^+	718±18.5	2.5	681
	Phenyl- $^{14}\text{C}_n$	M^+	1195±29.6	2.5	1166
MBA	Benzyl- $^{14}\text{C}_1$	$[\text{M}-1]^+$	115±2.2	1.9	107
<i>m</i> -Cresol	Phenyl- $^{14}\text{C}_n$	M^+	799±7.0	0.9	755
<i>m</i> -Bromophenol	Phenyl- $^{14}\text{C}_n$	M^+	455±3.0	0.6	437

* Measured by gas chromatography-mass spectrometry-computer system ($n=3$)

** Measured by radioactivity-weight method
S.A.: Specific activity

^{14}C and *m*-bromophenol- ^{14}C , could be achieved without any interference which might be caused by the high abundance of the natural stable isotopes.

Relative peak intensities of molecular ions in mass spectra of most compounds examined happened to be over 10% of those of base peaks and high enough to provide accurate and precise specific activities. However, in the case of MBA-(benzyl- ^{14}C), the molecular ion could not be observed even when ionized at 12 eV. Fortunately, in this case, $[\text{M}-1]^+$ ion with a relative peak intensity of 7% appeared and could be employed for the determination of its specific activity, representing that any fragment ion containing all of the concerned ^{14}C may be usable as long as properly selected. However, to discuss the selection of proper fragment ions, more detailed experiments will be required.

As described above, the specific activity measurement by GC-MS-CPU system provides practically precise and accurate specific activities of various ^{14}C -labelled compounds with remarkable merits. Especially, this method offers significant information on the purity and the structure of the proposed labelled compound, and possibly on the structure of impurities.

However, it is apparent that the application of this method is limited to ^{14}C -labelled compounds which are stable on GC columns and give good mass spectra.

References

- 1) Goldstein, G. and Rainey, W.T.: *J. Label. Comp.*, **9**, 805 (1973)
- 2) Yoshitake, A., et al.: *J. Label. Comp. Radiopharm.*, **13**, 323 (1977)
- 3) Kanamaru, H., et al.: *ibid.*, **18**, 1283 (1981)
- 4) Yoshitake, A., et al.: *Radioisotopes*, **28**, 169 (1979)
- 5) Gomi, H., et al.: *ibid.*, **29**, 443 (1980)
- 6) Yoshitake, A., et al.: *J. Label. Comp.*, **9**, 537 (1973)
- 7) Yoshitake, A., et al.: *ibid.*, **8**, 615 (1972)
- 8) Nakatsuka, I., et al.: *J. Label. Comp. Radiopharm.*, **13**, 453 (1977)
- 9) Hites, R.A. and Biemann, K.: *Anal. Chem.*, **42**, 855 (1970)
- 10) See, for example, Buddenbaum, W.E., et al.: Proceedings of the First International Conference on Stable Isotopes in Chemistry, Biology, and Medicine, p.261, Argonne, U.S.A. (1973)

要 旨

ガスクロマトグラフィー-マススペクトロメトリ-コンピュータシステム による ^{14}C 標識化合物の比放射能の測定法

金丸 博, 高井良三*, 堀場正雄, 中塚 巖, 吉武 彬

住友化学工業(株) 宝塚総合研究所 665 宝塚市高司 4-2-1

*住化分析センター(株) 554 大阪市此ノ花区春日出中 3-1-135

ガスクロマトグラフィー-マススペクトロメトリ-コンピュータシステムによる ^{14}C 標識化合物の比放射能の測定を開発した。本法は、種々の ^{14}C 標識化合物に適用でき、精密で正確な比放射能を与える実用的な方法であること、また、必要試料量の少量化や揮発性化合物への適用や簡便性などの点において優れた方法であることが実証された。測定に供した試料の測定値のC.V. は3.9%以内であり、本法による測定感度は37 MBq/mM (1 mCi/mM) 以上であった。本法は標識様式や適用された合成法についての情報を得るのにも有用であった。