Submitted to „Metrologia" for publication

A Combination of Gas Chromatography, Combustion, and $^{13}C/^{12}C$ Isotope Dilution Mass Spectrometry (GC/C/IDMS)

Gunther Dube, André Henrion, Wolfgang Richter
Physikalisch-Technische Bundesanstalt
D-38116 Braunschweig, Germany

INTRODUCTION

Isotope dilution mass spectrometry (IDMS) is a method of amount-of-substance measurement which offers the capabilities of a primary method [1]. The following three experimental steps are necessary for this method to be used for mixture analysis:

- addition of the spike to the sample and isotope equilibration,
- separation of the analyte and the spike from the mixture,
- isotope ratio measurement.

The calculation of the amount of substance of an analyte, n_a, is performed using the equation:

$$n_a = n_{sp} \frac{(R_{sp} - R_b)\ \Sigma R_{i\,sa}}{(R_b - R_{sa})\ \Sigma R_{i\,sp}} , \qquad (1)$$

where R : isotope ratio
sa : sample
sp : spike
b : blend of sample and spike.

The spike is a known amount of substance of the analyte whose isotopic composition is different from the natural one. When IDMS is applied in organic chemistry, compounds labelled ^{13}C, ^{15}N or D are used as spikes. For the separation of the analyte from the spiked sample, capillary gas chromatography is used in most cases. The on-line combination of GC and IDMS is widely used for the amount-of-substance measurement in organic chemistry [2].

A NEW IDMS METHOD (GC/C/IDMS)

At the PTB, a new version of the GC/IDMS method is being developed, whose main feature is the conversion of analyte and spike, after the chromatographic separation, into carbon dioxide which is subjected to the isotope ratio measurement. This is done because the techniques of $n^{13}C/n^{12}C$ ratio measurements in CO_2 are at present very well developed both instrumentally and methodically so that a high accuracy of the results is achieved. It can be considered an additional advantage of this modification that the isotope ratio measurement, usually carried out in the region of relative molecular masses of organic compounds, is shifted to the low mass range of the relative molecular mass of CO_2 at 44. On the other hand, due to the combustion of organic substances to carbon dioxide, it is no longer possible to separate the analyte from the mixture by mass separation in addition to chromatographic separation (multiple ion selection technique [2]). Furthermore, as carbon dioxide is used for the isotope ratio measurement, it is necessary to use ^{13}C-labelled organic compounds as spikes, which are not always readily available.

EXPERIMENTAL

The instrumental setup of the new method, which can be designated by the abbreviation GC/C/IDMS, is shown in Figure 1:

Figure 1

It consists of a gas chromatograph, a combustion interface and a gas isotope mass spectrometer. The mass spectrometer is a 90° sector field instrument equipped with an electron impact ion source (FINNIGAN MAT252). Two gas streams can be admitted to the ion source: the effluent of the combustion interface or a standard carbon dioxide from a reference inlet system. The ion detection system is equipped with Faraday collectors. The ion currents on mass positions 44, 45, and 46 are continuously measured. At these positions, the following ion species are detected :

$$\begin{aligned} 44 &: {}^{12}C^{16}O_2 \\ 45 &: {}^{13}C^{16}O_2, {}^{12}C^{17}O^{16}O \\ 46 &: {}^{12}C^{17}O_2, {}^{13}C^{16}O^{17}O, {}^{12}C^{16}O^{18}O \ . \end{aligned} \tag{2}$$

The calculation of the $n^{13}C/n^{12}C$ isotope ratio from the intensities of the ion currents I(44), I(45), and I(46) is usually carried out using a well-known procedure implemented in the instrument software [3].

Combinations of gas chromatograph/combustion interface/isotope ratio mass spectrometer are commercially available, but they are in most cases used for measuring small variations of the ^{13}C isotope abundance in samples of different natural origin in the range between 1.01 % and 1.15 % [4]-[6]. Unlike these combinations, the instrument shown in Figure 1 is equipped with a modified ion detection system covering a dynamic range of the ^{13}C isotope abundance up to 100 %. This is achieved by an eight-collector system and the associated amplifiers.

AMOUNT- OF- SUBSTANCE MEASUREMENT

The GC/C/IDMS method allows amount-of-substance measurements to be carried out, which are traceable to the SI units with high accuracy. It can be applied in different variants.

Variant 1: Equation (1) is applied to calculate the amount of substance of the analyte (n_a) in the sample under investigation. The amount of substance of spike (n_{sp}) is

-89-

measured by weighing and primarily is, therefore, traceable to the unit of mass. For conversion to amount of substance, the actual molar mass, calculated from the known isotope composition, is used. The $n^{13}C/n^{12}C$ isotope ratios of spike (R_{sp}), sample (R_{sa}) and blend of spike and sample (R_b) are calculated from the intensities of the ion currents measured at the mass positions 44, 45, and 46 [3]. The correctness of the isotope ratios obtained is guaranteed by calibration of the mass spectrometer by comparing the measurement results with those obtained by measuring a CO_2 standard having an accurately known $n^{13}C/n^{12}C$ value which is traced back to a primary measurement.

Variant 2: The complete GC/C/MS system is calibrated using a set of calibration samples prepared by blending known amounts of substance of the analyte to be investigated (n_a) and spike (n_{sp}), respectively. The intensity ratio at mass positions 45 and 44 is measured as a function of the blending ratio (equation 3):

$$I(45)/I(44) \quad = \quad f(\, n_a/n_{sp}) \,. \qquad (3)$$

This calibration function is used to calculate the amount of substance of the analyte in the sample under investigation. The result obtained is traceable to the SI because the masses of sample and spike have been determined by weighing. These are converted to amounts of substance n_a and n_{sp}, respectively, by using the actual molar masses which are known with appropriate accuracy.

Variant 3: This variant, recently proposed as a method to be generally applicable in IDMS [7], is an improvement of variant 2. It is the main feature of this variant that the intensity ratios I(45)/I(44) of the sample under investigation and of a reference sample, which has been prepared from the pure analyte, are compared and iteratively adjusted to zero difference in the I(45)/I(44) ratio. Sample and reference sample are spiked by the same amount of spike. If the amount of analyte in the reference sample is the same as in the sample under investigation, the intensity ratios observed will obviously be the same. In practice, the intensity ratio of the reference sample will be more or less different from that of the sample under investigation. When the procedure mentioned above [7] is applied, the n_a/n_{sp} ratio of the reference sample gradually approaches that of the sample under investigation as the amount of substance of the analyte in the reference sample changes. Because the amount of substance of the reference substance has been measured by weighing, the measurement of the amount of substance of the analyte in the sample under investigation is traceable to the unit of mass.

Strictly speaking, the intensity ratio I(45)/I(44) does not exactly represent the n(^{13}C)/n(^{12}C) ratio. This is due to the fact that the ion current at mass position 45 is mainly generated by the $^{13}CO_2$ ion as well as by a small fraction of the $^{12}C^{17}O^{16}O$ ion (Equation 2). Apart from the fact that a correction for this contribution would be very small, it would be the same for the sample under investigation and the reference sample. As a result, variant 3 need not be corrected.

Variants 2 and 3 have the one big advantage that they do not require exact knowledge of the chemical and isotopic purity of the spike, provided the same material is used for spiking the sample, the calibration sample (variant 2) and the reference sample (variant 3), respectively.

APPLICATION

In order to check the capabilities of the GC/C/IDMS method, a mixture of model substances of known amount-of-substance concentrations was investigated. This mixture consisted of three aliphatic carboxylic acids ($H_3C(CH_2)_nCOOH$, n = 12, 14, 16), dissolved in ethanol. One of the components of the mixture, hexadecanoic acid (n=14), was measured applying the reference sample method (variant 3 above). For the reference solutions, pure hexadecanoic acid was used. The spike solution was prepared using a completely ^{13}C-labeled hexadecanoic acid ($^{13}C_{16}H_{32}O_2$). The gas chromatogram, obtained by continuously measuring the ion currents at positions 44 and 45 is shown in Figure 2:

Figure 2

The result of the amount-of-substance measurement is given in Table 2. It is the mean value of 12 measurements.

Table 1

The standard uncertainty (type A) of the measurement is 0.13%. A systematic deviation of the mean value from the target value of 3 x 10^{-4} mmol (0.012%) was found. This deviation is of the same order of magnitude as the uncertainty of the target value. In this experiment type B uncertainty components obviously are of minor importance so that the total uncertainty is chiefly given by the precision of the measurement.

CONCLUSION

The GC/C/IDMS method provides results of low uncertainty which are traceable to the SI units. It is therefore suitable for ambitious amount-of-substance measurements. Experiments aimed at using the method for measuring "real life" samples such as, for example, in clinical chemistry are in progress.

It can be expected from the results of a model analysis presented here that the new GC/C/IDMS method of organic analysis is capable of providing measurement results with a higher accuracy than has been possible up to now.

References

[1] BIPM (Editor): Report of the 1st CCQM Meeting
Sèvres Cedex, France, 1995

[2] Chapman, J. R.: Practical Organic Mass Spectrometry; 2nd Edition
John Wiley & Sons
Chichester, New York, Brisbane, Toronto, Singapore, 1993

[3] Santrock, J., Studley, S.A., Hayes, J.M.
Anal. Chem. **1985**, *57*, 1444 -1448

[4] Matthews, D.E., Hayes, J.M.
Anal. Chem. **1978,** *50*, 1465 -1473

[5] Sano, M., Yotsui, Y., Abe, H., Sasaki, S.
Biomed Mass Spectrom **1976**, *3*, 1-3

[6] Merritt, D.A., Freeman, K.H., Ricci, P.M., Studley, S.A., Hayes, J.M.
Anal. Chem **1995**, *67*, 2461 - 2473

[7] Henrion, H.
Fresenius' J. Anal. Chem. **1994,** *350,* 657-658

Legends

Figure 1

Diagram of the GC/C/IDMS Method

MS : Mass Spectrometer
GC : Gas Chromatograph
RI : Reference Inlet System
CI : Combustion Interface
Sa : Sample
Sp : Spike

Figure 2

Gas Chromatogram of a Mixture of Carboxylic Acids

Peak Number 1 : Tetradecanoic Acid
Peak Number 2 : Hexadecanoic Acid
Peak Number 3 : Octadecanoic Acid

------- Trace at m/z: 45
——— Trace at m/z: 44

Table 1 : Result of Amount-of-Substance Measurement

Sample : $H_3C(CH_2)_{14}COOH$
Spike : $H_3{}^{13}C({}^{13}CH_2)_{14}{}^{13}COOH$

Target Value : 2.4413 ± 0.0005 mmol
Result : 2.4416 ± 0.0031 mmol
S.U. : 0.128 %

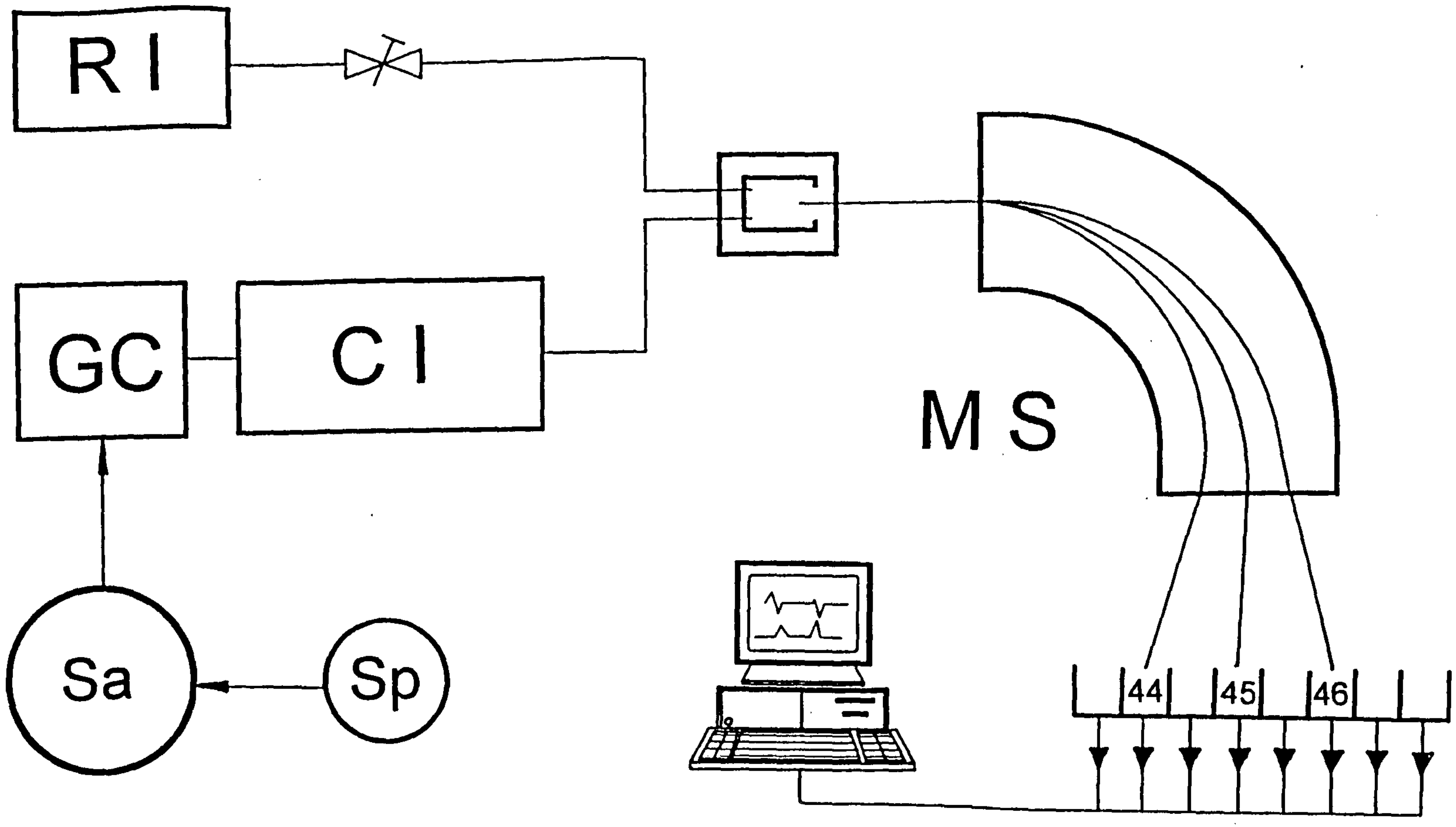

Figure 1

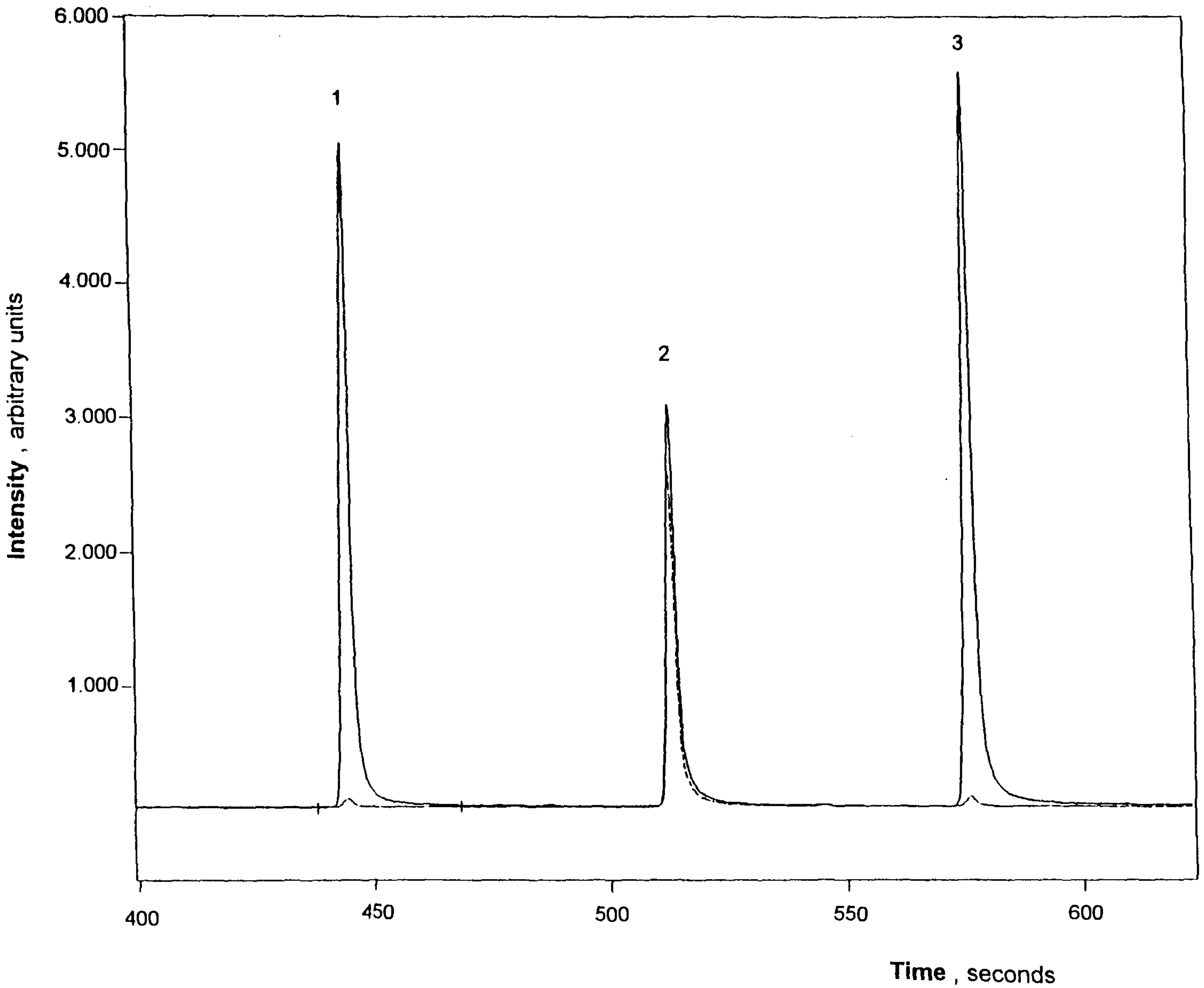
6.000
5.000
4.000
3.000
2.000
1.000
Intensity , arbitrary units
1
2
3
400
450
500
550
600
Time , seconds

Figure 2