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Short communication

Quantitation of formate by solid-phase microextraction and gas chromatography–mass spectrometry utilizing a [^{13}C]formate internal standard☆

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Abstract

A new method for the analysis of formic acid was developed using gas chromatography–electron impact ionization mass spectrometry in the selected ion monitoring mode and solid-phase microextraction. Using this method with [^{13}C]formic acid as an internal standard, the peak area ratio of [^{12}C]formic acid/[^{13}C]formic acid was not affected by differing methanol or sulfuric acid concentrations during the esterification and fiber adsorbing step. In comparison, the peak area ratio of formic acid/acetonitrile as detected by conventional GC with flame ionization detection was greatly affected by methanol or sulfuric acid concentrations. The formic acid calibration curve of our method showed excellent linearity over the range 5 to 200 μ*M*. The within- and between-run assay relative standard deviations for the formic acid concentration were all less than 1.70%.
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Keywords: Internal standards; Formic acid; Organic acids

1. Introduction

Various methods for the determination of formic acid concentrations have been developed, including enzymatic methods, high-performance liquid chromatography (HPLC) and gas chromatography (GC). However none of these methods is entirely suitable in terms of accuracy, simplicity or speed. The enzymatic method, in which formic acid concentration is indirectly calculated from the increment of NADH production (absorbance at 340 nm), is not applicable to turbid samples or to those containing NADH-consuming enzymes [1,2]. The HPLC method requires a tedious pretreatment [3], while in the case of GC analysis, formic acid must be first converted to its methyl ester. Despite these drawbacks, these methods have been used to evaluate formic acid content in samples such as bacterial cultures [4], cigarette smoke [5], reaction mixtures from organic syntheses [6,7], insects [8] and body fluids [9,10].

Recently, Lee et al. reported an analytical procedure for detecting formic acid in human blood by using a solid-phase microextraction (SPME) device and capillary GC [9]. However, strict control of

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conditions such as methanol and sulfuric acid concentrations is needed to obtain accuracy with this method, because these compounds may effect the esterification and adsorption of the analyte to the fiber. Because the derivatization of formic acid to methyl formate is a critical step in this analytical procedure, conditions governing the rate and extent of this reaction are of major concern. Abolin et al. reported that the concentration of methyl formate was found to be directly proportional to the amount of methanol added up to a methanol concentration of 0.72 mol/l [10]. In the case of sulfuric acid, methyl formate concentrations were essentially insensitive to sulfuric acid above 5.6 mol/l, in contrast to acetonitrile whose vapor phase concentration decreased above this value. Because of this variable detection of analytes, in analyses using acetonitrile as internal standard careful attention needs to be paid to the sample and sample preparation. To overcome this drawback, we looked for a proper internal standard which can normalize derivatization and fiber adsorption of formic acid analysis.

In analytical chemistry, stable isotope-labeled compounds are used as internal standards for quantitative analysis with mass spectrometric detection [11]. Stable isotope dilution mass spectrometry (MS) is widely accepted as the most sensitive, accurate and feasible method for measuring small amounts of endogenous compounds [12–15].

In this paper, we describe a new SPME–GC–MS analytical method for formic acid quantification using [^{13}C]formic acid as an internal standard under various derivatization conditions.

2. Experimental

2.1. Materials

Methanol, acetonitrile, sulfuric acid (97%) and formic acid (99%) were obtained from Wako (Osaka, Japan). Sodium [^{13}C]formate (99.3% ^{13}C) was purchased from Aldrich. SPME devices and their 75 μm Carboxen–polydimethylsiloxane (PDMS)-partially cross-linked fiber assemblies were purchased from Supelco (Bellefonte, PA, USA). Methyl formate (99%) was obtained from GL Sciences (Tokyo, Japan).

2.2. Derivatization and headspace SPME procedure

Headspace SPME of formic acid was carried out after its derivatization to methyl formate under acidic conditions according to the method of Lee et al. [9].

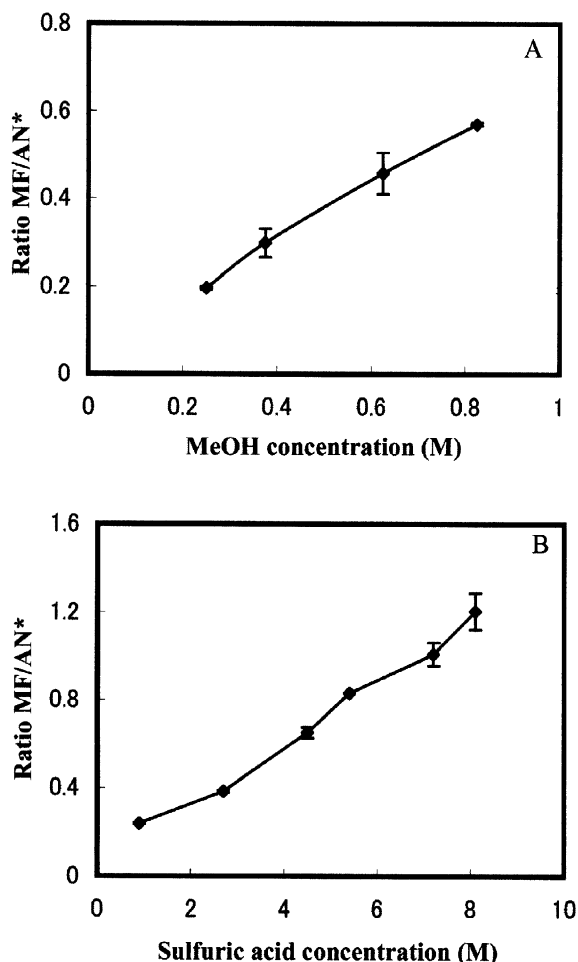

Fig. 1. The effects of methanol and sulfuric acid on methyl formate/acetonitrile peak area ratio using SPME–capillary GC. (A) Methanol, ranging in concentration from 0.25 to 0.825 mol/l, was added to each reaction mixture. (B) Sulfuric acid, ranging in concentration from 0.9 to 8.1 mol/l, was added to each reaction mixture. [^{12}C]Formic acid (1.74 m*M*) was converted to methyl formate, which was assayed by capillary GC. MF/AN*: Methyl formate/acetonitrile (n=3).

The Carboxen–PDMS fiber was conditioned for 30 min by inserting into a heated (240 °C) GC injection port. The fiber to be used was cleaned by heating at 240 °C for 10 min before each extraction. For the determination of the effect of sulfuric acid concentration on methyl formate/acetonitrile and *m/z* 60/61 ratio, 0.9–8.1 mol/l of sulfuric acid were used. For the determination of the effect of methanol, 0.25–1 mol/l of methanol was used.

2.3. GC and GC–MS procedure

GC was performed using a Shimadzu GC-14A instrument, fitted with a TC-FFAP capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness; GL Sciences). GC conditions were as follows: injector and detector at 240 °C; carrier gas, helium (He); flame ionization detection (FID); precolumn pressure of 98 kPa. The column temperatures were programmed as follows: 30 °C (hold for 5 min) to 105 °C at a constant rate of 12.5 °C/min.

The GC–MS system used was a Shimadzu QP-5000 instrument, equipped with the same column as that for GC–FID. GC–MS conditions were as follows: injector at 240 °C; carrier gas, helium; column head pressure, 100 kPa. The initial column temperature was 35 °C. The temperature was maintained for 5 min and a temperature was increased to 105 °C at an increasing rate of 25 °C/min. The electron multiplier was set at 1.8 kV. Spectra of standards (*m/z* 60 and 61) were determined in the scan mode and quantification was carried out in the selected ion monitoring (SIM) mode.

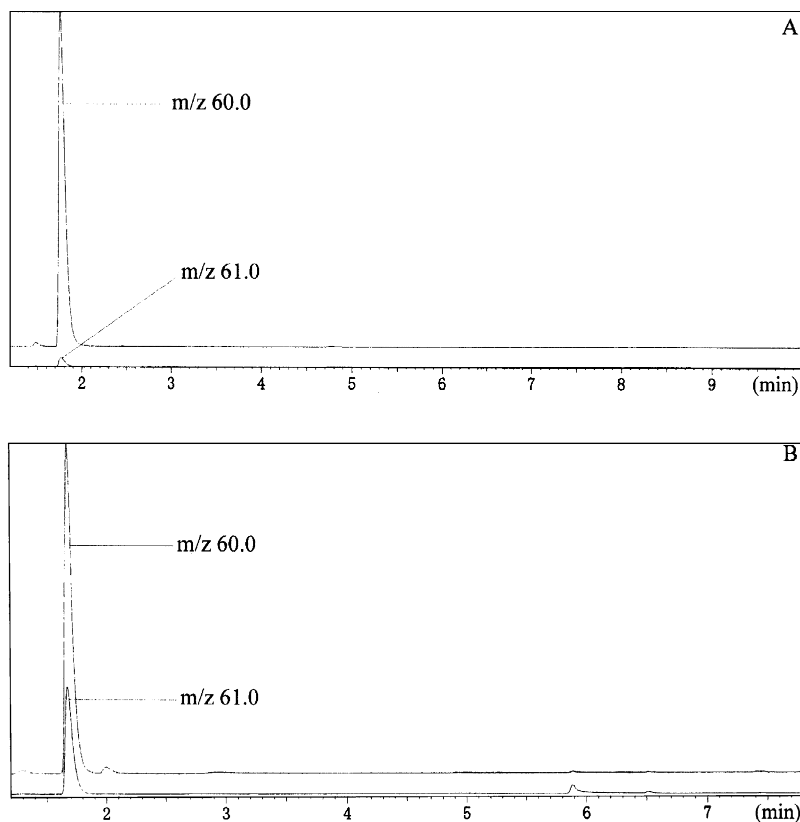

Fig. 2. Selective ion chromatogram for *m/z* 60 and 61. (A) Authentic methyl [^{12}C]formate (1 nmol); (B) derivatives of [^{12}C]formic acid (200 nmol) and [^{13}C]formic acid (50 nmol).

3. Results and discussion

The effect of sulfuric acid or methanol concentration on the ratio of methyl formate to acetonitrile (internal standard) in the SPME–capillary GC analysis was tested. The peak areas of methyl formate and acetonitrile increased with increasing sulfuric acid. However, the ratio of methyl formate/acetonitrile peak areas decreased. At a fixed concentration of formic acid, the detected methyl formate increased proportionally to the amount of methanol added, up to a methanol concentration of 0.625 mol/l. However, the detected peak area of acetonitrile decreased according with increasing methanol concentration. Therefore, the peak area ratio of methyl formate/acetonitrile was increased by the addition of methanol during SPME–GC analysis (Fig. 1). Because of this variable detection of analytes, it is not recommended to use acetonitrile as an internal standard for a SPME–GC analysis of formic acid. These results could be caused by the different adsorbing/desorbing capability of the fiber in the SPME procedure or the different chemical properties to esterification for methyl formate and acetonitrile.

The SPME–GC–MS procedure was applied to a formic acid assay. Before esterification, the sample was spiked with [^{13}C]formic acid as an internal standard. The sample was derivatized to methyl formate and subjected to GC–MS analysis with SPME. For quantification of formic acid, the peak area ratio, measured by m/z 60 and 61, was determined by electron impact mass spectrometry using selected ion monitoring. Typical ion chromatograms of m/z 60 and 61 are shown in Fig. 2.

The effect of methanol or sulfuric acid concentration was tested with this method (Fig. 3). The peak area ratios were uniformly maintained for all concentration of methanol and sulfuric acid. The within- and between-run relative standard deviations (RSDs) of the peak area ratio for methanol and sulfuric acid concentration were 0.83, 0.21% and 1.61, 0.82%, respectively. [^{13}C]Formic acid can be expected to have essentially the same chemical properties with regard to esterification and adsorbing/desorbing capability of the fiber of the SPME procedure as [^{12}C]formic acid. The lowest detection limit of the SPME–GC–MS analysis method was about 5 nmol/ml. A calibration curve was drawn by plotting at seven different concentrations according to the peak area ratios with [^{13}C]formic acid (50 μM) as internal standard (Fig. 4). Straight calibration curves for formic acid were obtained over the 5 to 200 nmol/ml range. The equations and r value for the curve in the range of 5–200 μM were:

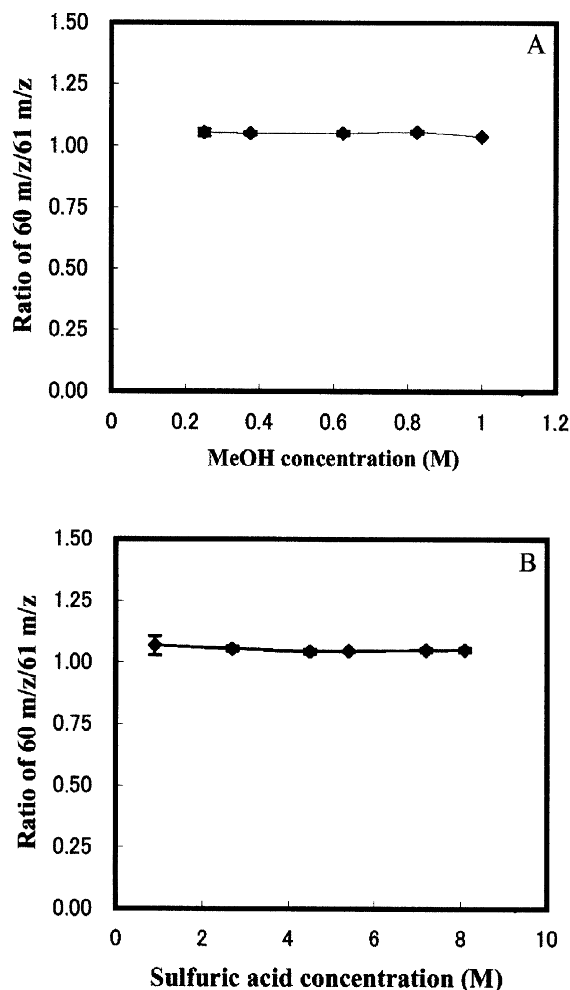

Fig. 3. The effects of methanol and sulfuric acid for the peak area ratio (m/z 60/61) using SPME–GC–MS. (A) Methanol, ranging in concentration from 0.25 to 1 M, was added to each reaction mixture. (B) Sulfuric acid, ranging in concentration from 0.9 to 8.1 M, was added to each reaction mixture. [^{12}C]- and [^{13}C]formate (50 μM) was converted to [^{12}C]- and [^{13}C]methyl formate, respectively, which was assayed by the SPME–GC–MS method with EI-MS (n=3).

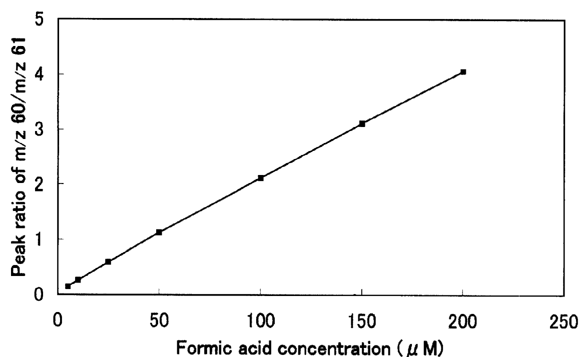

Fig. 4. Calibration curve of formic acid concentration. 5–200 μ*M* of [^{12}C]formate was added to the 50 μ*M* of sodium [^{13}C]formate in a total volume of 500 μl. (Slope=0.0201, r^2=0.9995, intercept=0.0854).

$y=0.0201x+0.0854$ and $r=0.9995$. Demonstrating the reproducibility of this approach, the within-run mean, standard deviation, and RSD calculated from the replicate analyses ($n=10$) of formic acid (50 μ*M*) were 50.41 μ*M*, 0.377 and 0.7%, respectively.

In conclusion, headspace SPME–GC–EI-MS-SIM with [^{13}C]formate as internal standard is unaffected by methanol or sulfuric acid concentrations. The proposed method is more sensitive and accurate than previously reported SPME–GC analyses.

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