Gas Chromatographic Determination of Lower Aliphatic Primary Amines as Their Fluorine-Containing Schiff Bases

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Eleven lower aliphatic primary amines were converted to the corresponding F-containing Schiff bases by reaction with pentafluorobenzaldehyde (PFBA) for separation by GC and detection with an electron capture detector. The minimum detectable quantity of the F-containing Schiff bases was about 0.02 ng, which is 3000 times less than the amount of detectable non-F-containing Schiff bases. Interference of other N-containing compounds to the method presented were: ammonia, dimethyl-, and diethylamines did not interfere at 100/1 (ammonia or secondary amine/primary amine) at 10^{-8} mol/mL concentrations, but did cause slight interference at 1000/1.

The gas chromatographic (GC) analyses of lower aliphatic amines in foods, drugs, fish, and odors are important problems. Picogram quantities of several primary amines have been analyzed by GC-electron capture detection (ECD) after preparing derivatives with pentafluorobenzaldehyde (PFBA) (*1, 2*), pentafluorobenzoylchloride (*3–5*), 1-fluoro-2,4-dinitrobenzene (*6, 7*) or trifluoroacetic anhydride (*8–10*). However, these analyses were primarily for higher amines. There are few applications to lower aliphatic primary amines (*5, 7*). The Schiff base derivatization methods of the lower aliphatic primary amines have the following two main advantages over the other derivatization methods. First, the reactions of the Schiff base derivatization are selective to the lower aliphatic primary amines, and these reactions proceed readily and exothermically at room temperature. The second point is the minor possibility of side reaction, because the by-product Schiff base derivatization is water.

In this paper selective and sensitive GC determinations of lower aliphatic primary amines were made by converting the amines to their corresponding Schiff bases using PFBA. The fluorine-containing Schiff bases produced were analyzed by GC equipped with an electron capture detector (ECD).

EXPERIMENTAL

Reagents. Ammonia (28 wt % aq soln) and dimethylamine (40 wt % aq soln) were obtained from Katayama Chemical Industries, Ltd., Osaka, Japan. Methyl- (40 wt % aq soln), trimethyl- (30 wt % aq soln), ethyl- (70 wt % aq soln), diethyl-, triethyl-, and isopropylamines, and PFBA (mp 24 °C) were obtained from Tokyo Kasei Kogyo Ltd., Tokyo, Japan. *n*-Propyl- and *n*-butylamines, ethanol, and *n*-hexane were obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Isobutyl-, *sec*-butyl-, *n*-amyl-, isoamyl-, *n*-hexyl-, and *n*-heptylamines were obtained from PolyScience Corp., Niles, Ill. All reagents used were guaranteed or reagent grade chemicals, and were used without further purification. Styrene dibromide used as an internal standard for this experiment was prepared by direct reaction with bromine and styrene in chloroform. The precipitate formed was isolated by filtration, washed with water until a yellowish color disappeared, and dried over silica gel in a vacuum desiccator. The melting point of the dibromide was 73 °C.

Preparation of Fluorine-Containing Schiff Base. The procedure for the preparation of all the fluorine-containing Schiff bases, in a large scale derivative formation reaction, used for this experiment was as follows: exactly 10^{-5} mol of lower aliphatic primary amine was added with a 10-μL Hamilton microsyringe (701-N) to 1 mL of ethanol, and then immediately 10^{-5} mol of PFBA was added with another 10-μL microsyringe to the amine–ethanol solution and allowed to react overnight at room temperature. The reaction percentage was estimated from the amines and the PFBA remaining after the reaction period. One μL of sample was injected with a 10-μL microsyringe into Tenax-GC and SE-30 columns, and was detected by flame ionization detection (FID).

The procedure for the preparation of all the fluorine-containing Schiff bases in a submicrogram derivative formation reaction was as follows: 10 or 100 μL of the amine–ethanol solution (10^{-5} mol/mL concentration) was added with a 100-μL Terumo microsyringe (MS-100) to 1 mL of *n*-hexane, and then immediately 10 to 100 μL of the PFBA–ethanol solution (10^{-4} mol/mL concentration) was added with another 100-μL microsyringe to the amine–*n*-hexane solution, and reacted at room temperature or 60 °C for 1 to 3 h. After the reaction, excess PFBA was removed by adding 5 mL of 0.1 N NaOH and shaking vigorously for 1 min. The reaction percentage was estimated from the amount of Schiff base produced.

Gas Chromatography. The gas chromatographs used were a Shimadzu Model GC5AP$_5$F equipped with a FID and a digital integrator (Shimadzu Model ITG-2A) for determination of reaction percentage, and a Shimadzu Model GC5AIE equipped with an ECD.

Gas chromatographic conditions were as follows: Conditions (a) were: analytical columns, 3 m × 3 mm i.d. glass; column packing, 5% SE-30 on Shimalite W, 60/80 mesh, acid washed and silanized; for the FID, carrier gas (nitrogen) flow rate 50 mL/min, air and hydrogen flow rates 1.0 L/min and 50 mL/min, respectively, column temperature 120 °C, injection port and detector temperatures 150 °C; and for the ECD^{63}Ni (10 mC) carrier gas (nitrogen) flow rate 60 mL/min, column temperature 120 °C, injection port temperature 150 °C, detector temperature 250 °C, pulsed voltage (voltage, 48 V, pulsed width, 8 μs). Conditions (b) were: analytical columns, 3 m × 3 mm i.d. glass, 60/80 mesh Tenax-GC column packing, carrier gas (nitrogen) flow rate 50 mL/min, column temperature (programming), holding for 1 min at 100 °C, heating the column oven at a rate of 10 °C/min from 100 to 250 °C, maintaining this temperature for 25 min, and then cooling to the starting temperature; injection port and detector temperatures 250 °C; FID hydrogen and air flow rates were 50 mL/min and 1.0 L/min, respectively. The FID detections of the conditions (a) and (b) were used for the determination of the reaction percentage of the fluorine-containing Schiff base formation reaction.

To determine the response factors (FI) (*11–14*) of the fluorine-containing Schiff bases listed in Table I, the solution was prepared by dissolving each Schiff base (1.6–2.1 μg), and styrene dibromide (1.1 μg), as an internal standard, in 1 mL of *n*-hexane. The FI values was calculated from the following equation:

$$\mathrm{FI} = A_s/W_s \times W_i/A_i$$

where A_s and W_s are the peak height (log) and weight of the internal standard, respectively, and A_i and W_i are the peak height (log) and weight of the fluorine-containing Schiff base, and the detector used in this test was an ECD.

RESULTS AND DISCUSSION

Figure 1 shows the plot of mole ratio (PFBA/amine) vs. reaction percentage. Figure 2 shows the effect of reaction time. The optimum reaction conditions in the 10^{-7} mol of amines in 1 mL of *n*-hexane were: reaction temperature, 60 °C; reaction time, 1 h; mole ratio (PFBA/amine), more than 4.

The response (peak height) of an ECD to the F-containing Schiff bases was a straight line relationship in the range of 0.2 to 10 ng. The minimum detectable quantity of these Schiff bases was about 0.02 ng, therefore, the derivatives have sufficient electron capturing properties. The sensitivity of ECD to the Schiff bases compared with the non-F-containing Schiff bases (*13*) by a FID was about 3000 times greater.

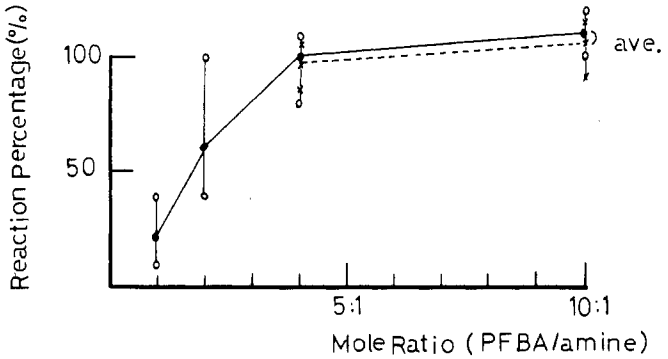

Figure 1. Effect of mole ratio (PFBA/amine) on reaction percentage of fluorine-containing Schiff base formation

Reaction conditions were: temperature, 60 °C; time, 4 h. (O) represents 10^{-6} mol amine/mL *n*-hexane soln. (x) represents 10^{-7} mol amine/mL *n*-hexane soln

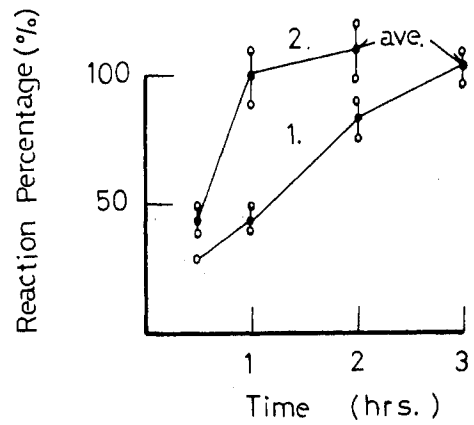

Figure 2. Effect of reaction time on reaction percentage of fluorine-containing Schiff base formation

Mole ratio (PFBA/amine, amine concentration 10^{-7} mol/mL *n*-hexane soln) was 10:1. Curve 1 was 40 °C, and 2 was 60 °C

Table I. Response Factor (FI) Values and Standard Deviations of the F-Containing Schiff Bases of Eight Lower Aliphatic Primary Amines.[a]

Amine of F-containing Schiff base	FI values and std dev
Methylamine	2.29 ± 0.02
Ethylamine	2.37 ± 0.03
n-Propylamine	2.35 ± 0.01
Isopropylamine	2.34 ± 0.01
n-Butylamine	2.21 ± 0.00
Isobutylamine	2.22 ± 0.01
sec-Butylamine	2.72 ± 0.01
n-Amylamine	2.39 ± 0.01

[a] GC conditions used are the same as in Figure 3.

Figure 3 shows a typical gas chromatogram of the F-containing Schiff bases with an ECD. The derivative of *n*-heptylamine is eluted within about 20 min, and 11 F-containing Schiff bases were separated sufficiently.

The reproducibility and uniformity of the response of an ECD were evaluated for the F-containing Schiff bases of eight lower aliphatic primary amines. As seen from Table I, the response factors (FI) of these F-containing Schiff bases with a SE-30 column using an ECD showed good uniformity and high reproducibility.

As PFBA was very sensitive to an ECD, it was necessary to remove it completely from the reaction mixture. Excess PFBA was removed by adding 5 mL of 0.1 N NaOH to the reaction mixture and shaking for 1 min. The fluorine-containing Schiff base remained in *n*-hexane solution without decomposition.

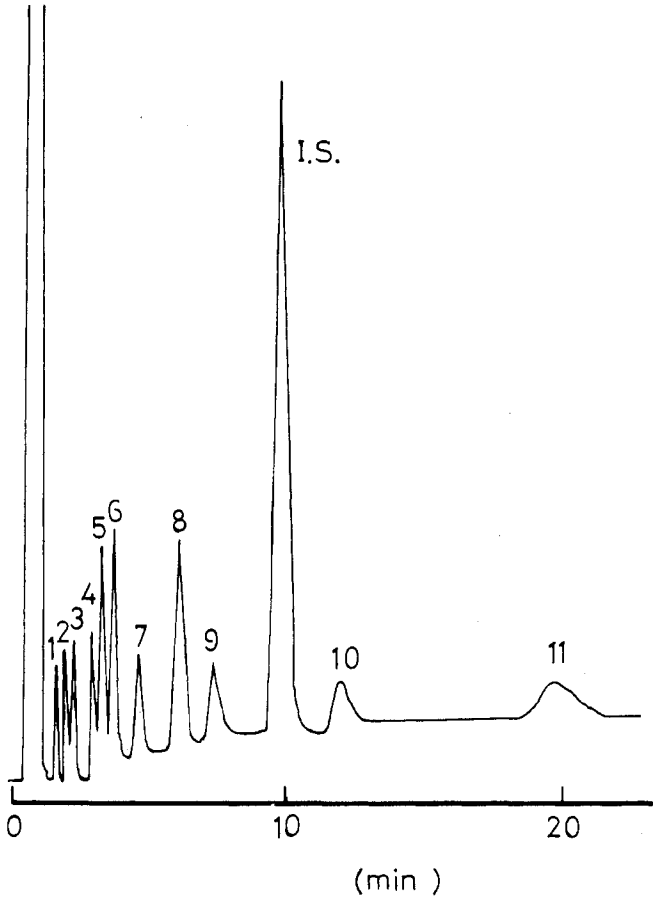

Figure 3. Typical gas chromatogram of fluorine-containing Schiff bases of the lower aliphatic primary amines with an ECD

The sample solution of the Schiff bases was prepared by dissolving each Schiff base (0.8 to 1.1 μg), and styrene dibromide (1.2 μg) as an internal standard, in 1 mL of *n*-hexane. Peaks of fluorine-containing Schiff bases: (1) Methylamine, (2) ethylamine, (3) isopropylamine, (4) *n*-propylamine, (5) *sec*-butylamine, (6) isobutylamine, (7) *n*-butylamine, (8) isoamylamine, (9) *n*-amylamine; IS (internal standard), (10) *n*-hexylamine, (11) *n*-heptylamine. GC conditions were: analytical columns, 3 m × 3 mm i.d. glass; column packing, 5% SE-30 on Shimalite W, 60/80 mesh, acid washed and silanized; detector, ^{63}Ni (10 mC) ECD; carrier gas (nitrogen) flow rate, 60 mL/min; column temperature, 120 °C; injection port temperature, 150 °C; detector temperature, 250 °C; pulsed voltage (voltage, 48 V, pulsed width, 8 μs)

The interfering effects of other N-compounds to the present method were as follows: ammonia, dimethyl-, and diethylamines at a mole ratio of 100/1 (secondary amine or ammonia/primary amine) had no effect on the detection of 10^{-8} mol concentrations of fluorine-containing Schiff bases in 1 mL of *n*-hexane. The results of the interfering effects of ammonia, dimethyl-, and diethylamines in the present method are consistent with those of recent papers (*13, 15*). However at a ratio of 1000/1 (ammonia or diethylamine/primary amine), the presence of large amounts of ammonia produced a ghosting peak, which has the same elution position as the fluorine-containing Schiff base derivative of ethylamine, but the magnitude of this peak was small. While, the presence of large amounts of diethylamine produced a ghosting peak, having the same elution position as the fluorine-containing Schiff base derivative of isoamylamine, the magnitude of this peak was also small.

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Multiple Ion Detection System with Miniscan Facilities and Expanded Mass Range for Magnetic Sector Mass Spectrometers

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A new four-channel multiple ion detection (MID) system is described. By adding a ΔV of up to 1100 volts to the nominal accelerating voltage (V), a mass range of about 30% (V = 3600 volts) to 90% (V = 1200 volts) can be covered, thus providing a remarkably wide range compared to other MID units. The system incorporates a ramp voltage, which added to V allows a small scan around each one of the peaks focused, as well as holding amplifiers in each channel. These features combined with the excellent stability of the ionization chamber against voltage changes minimize the errors due to defocusing effects, permitting the addition of voltages as high as those described. The scan time is continuously adjustable between 0.5 and 12 s and the switching time between two adjacent channels is 50 ms. The circuit design, its operation, and some aspects of the performance of this unit are described.

The advantages of using a MID unit coupled with GC-MS for selected ion monitoring have been pointed out by several authors. Since the initial contributions in this field (*1*, *2*), a great number of papers reporting the use of a MID accessory have been published (*3*, *4*). Likewise, the use of this technique in the analysis of biological samples has been steadily growing because of its great potential in terms of sensitivity and specificity (*5*).

We have recently attempted to use a single focusing mass spectrometer equipped with standard peak matcher facilities for selected ion monitoring (*6*, *7*). However, this implies multiple GC injections to be able to monitor various ions. Also, the use of deuterated internal standards for accurate quantitative measurements requires monitoring more than one ion. Thus, in order to analyze the different components of any given sample in a reasonable time, it is important to have the capability of focusing several ions simultaneously. Although this capability is provided by the standard MID systems described for magnetic instruments, the effective mass range covered in this way is relatively limited by variations in ion focusing at different accelerating voltages (*8*, *9*). As the effective mass range depends upon the magnitude of the ΔV_i values added or more commonly substracted (voltage attenuation), from the nominal accelerating voltage (V), these values should be as high as possible without introducing unstabilities in the ionization chamber of the mass spectrometer. Using the device described here, we are able to add ΔV_i values, from 0 to 1100 volts, to the nominal accelerating voltage in each one of the four channels used without any significant disturbance in the performance of the ionization chamber. Also, the stepping up of V means that sensitivity is not reduced at the high masses being actually increased at the lower masses in contrast to other systems (*5*).

After establishing a method for using the peak-matcher of a Hitachi-Perkin-Elmer mass spectrometer as a device to monitor only one ion per injection (*6*) and improving it by rapid changes of the magnetic field during the time intervals between the elution of the components of interest so that two or three masses can be monitored per injection (*7*), we still faced the need for a better capability in the sense described above. Since there is no MID unit available on the market to be coupled with this equipment, the development and construction of a simple and versatile MID accessory seemed to be necessary. This MID unit must overcome several problems, like the absence of mass marker and computer as well as the excessive "drift" of the mass peak being focused, due to the instability of the magnetic field of the mass spectrometer.

On the other hand the stability of the magnetic field improves significantly if the magnet is allowed to equilibrate thermally for several hours before a run. However the stability thus achieved is lost when widely differing m/e values need to be focused upon from run to run.

The focusing effect thus produced by the variations of the magnetic field usually requires the injection of a standard either to confirm or adjust the focus on the selected masses. Lately Klein et al. (*8*) have used a feedback circuit with a Hall probe to stabilize the magnetic field and Holland et al. (*9*) have described a computer autofocus system for continuous fine adjustment of optimal focusing. However in the absence

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