Journal of Chromatography, 175 (1979) 339–342

CHROM. 11,861

Note

Histamine derivative for quantitative determination by gas chromatography

HARUHISA MITA, HIROSHI YASUEDA and TAKAO SHIDA

National Sagamihara Hospital, Clinical Research Centre for Rheumato-Allergology, Sakuradai, Sagamihara City, Kanagawa (Japan)

(Received February 19th, 1979)

Histamine is one of the important chemical mediators in immediate-type hypersensitivity and is found to be released during an asthmatic attack. Histamine has been detected by gas chromatography after conversion into derivatives such as N^{α}-heptafluorobutyrylhistamine (HA-HFB)[1], the trimethylsilyl derivative[2] or the trifluoroacetyl derivative[3]. Heptafluorobutylation and trimethylsilylation are useful in many gas chromatographic applications because of the great volatility of the derivatives. However, these derivatives are unsuitable for the quantitation of histamine because they show considerable tailing. For the quantitative determination of histamine it is necessary to convert it into a more suitable derivative, such as that described here.

EXPERIMENTAL

Reagents

Histamine dihydrochloride (HA) was purchased from E. Merck (Darmstadt, G.F.R.). Heptafluorobutyric anhydride (HFBA) was obtained from Wako (Osaka, Japan) and chloroformic acid ethyl ester (CFE) from Tokyo Kasei Kogyo (Tokyo, Japan). The other organic solvents were of analytical-reagent grade. N^{τ}-Ethylhistamine dihydrochloride (EtHA) was synthesized in our laboratory according to the method of Rothschild and Schayer[4]. The structure of EtHA was confirmed by mass spectrometry and its melting point was 159–161°.

Gas chromatography and gas chromatography–mass spectrometry

A Shimadzu 4CM gas chromatograph equipped with a flame-ionization detector (FID) was used. The column was a 3 m × 3 mm I.D. glass column packed with 5% SE-30 on Chromosorb W AW DMCS (60–80 mesh). The column oven temperature was 210°, injector and detector temperature 240° and carrier gas (nitrogen) flow-rate 60 ml/min. The peak areas on the gas chromatograms were measured with a Shimadzu Chromatopac E1A instrument.

The mass spectrum was obtained with an LKB 2091 mass spectrometer coupled with a Shimadzu 7A model gas chromatograph and data processing system (Shimadzu PAC500FDG), which was connected to a minicomputer (Oki Electric

Industry Co., Okitac-4300b). The ionization energy was 70 eV, trap current 50 μA, ion source temperature 230° and separator temperature 250°.

Preparation of N^{α}-heptafluorobutyryl-N^{τ}-carbethoxyhistamine (HA-HFB-ETO)

One millilitre of 2 *N* sodium hydroxide solution was added to 0.5 ml of aqueous HA solution and HA was extracted with 2 ml of *n*-butanol. Following centrifugation, the *n*-butanol layer was collected and passed through adsorbent cotton-wool for dehydration. The *n*-butanol solution was evaporated to dryness under reduced pressure and 50 μl of ethyl acetate and HFBA were added to the residue. The mixture was allowed to stand for about 30 min at 100° to form HA-HFB, which was further converted into the corresponding N^{τ}-carbethoxy derivative. The solution containing HA-HFB was placed under a stream of nitrogen to remove the excess of reagent. The residue was treated with 50 μl of a mixture of CFE and ethyl acetate (1:1) for 30 min at room temperature (*ca.* 20°) to synthesize HA-HFB-ETO. The mixture was injected directly into the gas chromatograph.

RESULTS AND DISCUSSION

Two steps were involved in this derivatization of HA, namely conversion of *n*-butanol-extracted HA into HA-HFB, from which HA-HFB-ETO was formed. The gas chromatograms of two HA derivatives are shown in Fig. 1A and B, and it is clear that HA-HFB-ETO gives much less tailing than HA-HFB (Table I). Although HA-HFB-ETO could be partially decomposed to HA-HFB (Fig. 1C) by removing CFE under a stream of nitrogen, the reverse process could easily be achieved by adding CFE solution. The limit of detection of HA-HFB-ETO was 20 ng and that of HA-HFB was 50–80 ng.

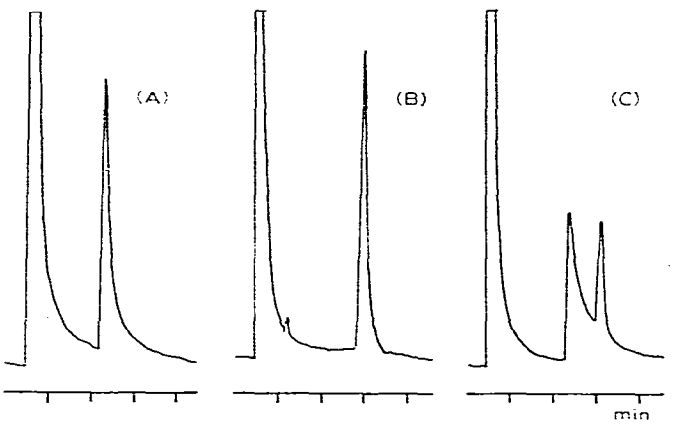

Fig. 1. Gas chromatogram of histamine derivatives. (A) HA-HFB, which was formed from histamine and HFBA, was converted into (B) HA-HFB-ETO with CFE. HA-HFB-ETO was partially degraded to HA-HFB if excess of CFE was removed under a stream of nitrogen (C).

Moodie[5] reported that histidine was converted into N^{α}-trifluoroacetyl-N^{τ}-carbethoxy *n*-butyl histidinate by treatment with diethyl pyrocarbonate; when CFE was used instead of diethyl pyrocarbonate, poor results were obtained. For HA, however, HA-HFB-ETO could be easily prepared by the addition of CFE solution.

TABLE I

COMPARISON BETWEEN THE GAS CHROMATOGRAPHIC PROPERTIES OF HA-HFB AND HA-HFB-ETO

Derivative	*Retention time (min)* *	*Tailing factor*	*Peak-height ratio* **
HA-HFB	2.4	14.9 ± 2.0	1
HA-HFB-ETO	3.1	73.3 ± 5.8	1.33 ± 0.26

* 5% SE-30, 3-m column at 210°; injector temperature, 240°; N_2 flow-rate, 60 ml/min.

** An equal amount of histamine was converted into each derivative and an aliquot of the reaction mixture was injected into the gas chromatograph equipped with an FID. Mean ± S.D. (3 experiments).

The structure of HA-HFB-ETO was confirmed by mass spectrometry. It is not clear whether the carbethoxy group was introduced to the N^{τ}- or N^{π}-position on the imidazole ring. It seems probable that the N^{τ}-position can be easily substituted because of steric hindrance. The fragmentation is illustrated in Fig. 2, which shows the mass spectrum of HA-HFB-ETO. Fig. 3 shows the single ion monitoring of 3 ng of HA-HFB-ETO (A) and HA-HFB (B) which were monitored at the molecular ions of *m/z* 379 and 307, respectively. At this level, HA-HFB-ETO is clearly more readily detectable than HA-HFB.

The advantages of HA-HFB-ETO over HA-HFB are as follows: it gives less tailing on the gas chromatograms; HA-HFB can be converted into HA-HFB-ETO

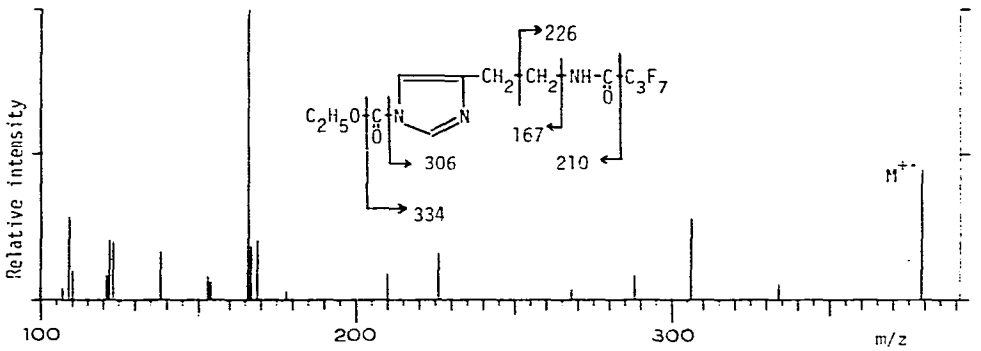

Fig. 2. Mass spectrum of HA-HFB-ETO.

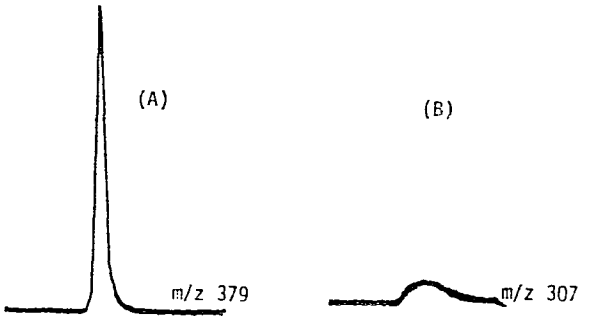

Fig. 3. Single ion monitoring of the derivatives. A 3-ng amount of the substance (HA-HFB-ETO (A) or HA-HFB (B)) was injected into the gas chromatograph–mass spectrometer and detected at the molecular ions.

by a very simple procedure (simply by keeping it in a mixture of CFE and ethyl acetate for 30 min at room temperature) and the conversion is almost 100% because HA-HFB was not detected in Fig. 1B; HA-HFB-ETO has a higher FID response than HA-HFB; and it is stable in CFE solution for at least 7 days. The FID response, retention time and tailing factor[6] of the derivative are given in Table I.

To determine the concentration of HA, EtHA was used as an internal standard. EtHA (18 μg) was added to HA solution and the mixture was processed as described above. Fig. 4A show the gas chromatogram of EtHA-HFB (1) and HA-HFB-ETO (2). The relationship between the amount of HA in the sample and the peak-area ratio is shown in Fig. 4B, linearity being evident in the range from 5 to 20 μg of HA.

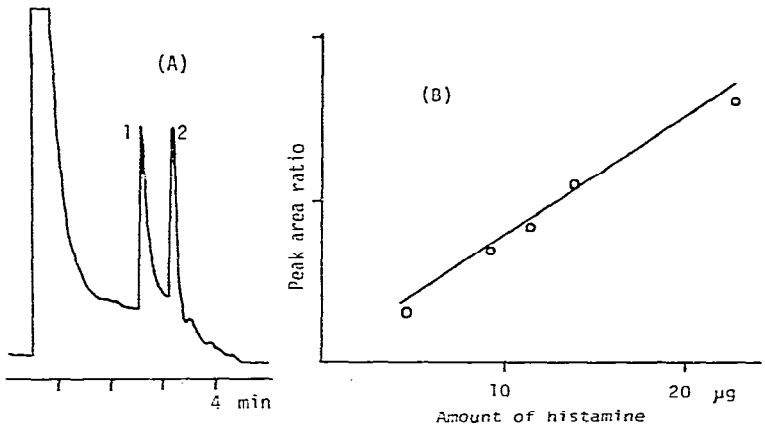

Fig. 4. (A) Gas chromatogram of a mixture containing histamine and ethylhistamine. 1, EtHA-HFB; 2, HA-HFB-ETO. (B) Relationship between histamine content and peak-area ratio (HA-HFB-ETO to EtHA-HFB); $r = 0.939$.

EtHA has not been found in mammals as a metabolite of HA. It is possible, therefore, that the concentration of HA can be established. Under the analytical conditions used, the peak ascribed to N^{τ}-methylhistamine-HFB was observed with a retention time of 2.2 min, while EtHA had a retention time of 2.7 min, so that it is possible that EtHA can also be used as an internal standard for the determination of N^{τ}-methylhistamine, which is a major basic metabolite of HA.

ACKNOWLEDGEMENT

The authors thank Dr. M. Yamamura for his interest and for reading the manuscript.

REFERENCES

1 H. Navert, *J. Chromatogr.*, 106 (1975) 218.
2 N. Mahy and E. Gelpi, *J. Chromatogr.*, 130 (1977) 237.
3 P. Cancalon and J. D. Klingman, *J. Chromatogr. Sci.*, 10 (1972) 253.
4 Z. Rothschild and R. W. Schayer, *Biochim. Biophys. Acta*, 30 (1958) 23.
5 I. M. Moodie, *J. Chromatogr.*, 99 (1974) 495.
6 J. D. Schieke and V. Pretorius, *J. Chromatogr.*, 132 (1977) 217.