Journal of Chromatography, 137 (1977) 127–133

CHROM. 9987

GAS CHROMATOGRAPHIC AND GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF AMPICILLIN*

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(Received December 21st, 1976)

SUMMARY

A method was developed for the quantitative gas chromatographic (GC) determination of ampicillin. The procedure requires silylation with hexamethyldisilazane, trimethylchlorosilane, trimethylsilylimidazole and N,O-bis(trimethylsilyl)acetamide in pyridine and subsequent GC on an OV-17 column, using 5α-cholestane as an internal standard. This method was applied to the determination of ampicillin in some pharmaceutical products. The characteristics of the mass spectra and the derivatization GC of ampicillin are also discussed.

INTRODUCTION

Numerous approaches have been described for the analysis of ampicillin. Among these, chemical[1–5], spectrophotometric[6–18] thin-layer and column chromatographic[19–23], microbiological[24–27], and high-performance liquid chromatographic[28,29] methods have been applied to the determination of ampicillin in pharmaceutical preparations and biological fluids.

Several gas chromatographic (GC) methods for the analysis of β-lactam antibiotics[30–33] have been established. However, a literature survey indicated that there was no GC method for the determination of ampicillin. In this work, an attempt was made to determine ampicillin by GC, and the method developed was shown to be sensitive and selective for its separation and quantitation.

EXPERIMENTAL

Chromatographic conditions

A Shimadzu GC-6A gas chromatograph equipped with a flame-ionization detector and a 2 m × 3 mm I.D. glass column containing 1.5% (w/w) OV-17 on Chromosorb W AW DMCS, 60–80 mesh, was used. The injection port and detector were kept at 270° .The column temperature was maintained at 230° for 5 min after

* This work was presented in part at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1976.

injection and then programmed at 4°/min to 270°, at which temperature it was kept for 5 min. Helium was used as the carrier gas at a flow-rate of 115 ml/min. An injection of 20–30 μl of monotrimethylsilylated sample solution was made before each day's run in order to minimize column adsorption. Determinations were made by plotting the peak-height ratio against weight ratio of ampicillin and 5α-cholestane.

Mass spectrometric conditions

A Hitachi RMU-6 mass spectrometer was combined with a gas chromatograph (Hitachi K-053). The following operating condition were used: separator temperature, 290°; ionization source temperature, 270°; ionization energy, 70 eV; acceleration voltage, 3.5 kV; and trap current, 60 μA.

Chemicals and reagents

Ampicillin, sodium ampicillin and α-aminobenzylpenicilloic acid were gifts from Shionogi Co. (Osaka, Japan), Meiji Co. (Tokyo, Japan) and Bristol Labs. (Syracuse, N.Y., U.S.A.), respectively. 5α-Cholestane, benzene, pyridine, acetonitrile and absolute methanol were of reagent grade. Phenylmethylsilicone (OV-17, Nishio Kogyo Co., Tokyo, Japan), hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) (Wako, Osaka, Japan), trimethylsilylimidazole (TMSI) and N,O-bis(trimethylsilyl)acetamide (BSA) (Ohio Valley, Marietta, Ohio, U.S.A.) were used without further treatment.

Internal standard solution

Weigh accurately about 10 mg of 5α-cholestane into a 20-ml volumetric flask, and dissolve it in and dilute to volume with benzene.

Reference standard solution

Four reference standard solutions were prepared by dissolving accurately weighed amounts of about (1) 20 mg of ampicillin, (2) 20 mg of ampicillin plus 5% of α-aminobenzylpenicilloic acid, (3) 20 mg of sodium ampicillin and (4) 20 mg of sodium ampicillin plus 5% of α-aminobenzylpenicilloic acid in absolute methanol in a series of 20-ml volumetric flasks, and diluted to volume with absolute methanol.

Calibration graph

Transfer aliquots from 0.5 to 3.0 ml of each reference standard solution into successive glass-stoppered flasks. Add 0.4 ml of the internal standard solution to each flask, evaporate just to dryness at $35 \pm 2°$ with a rotary evaporator and submit the residue to vacuum desiccation over P_2O_5 overnight. Construct calibration graphs by plotting the weight ratios of ampicillin to 5α-cholestane on the abscissa against their peak-height ratios on the ordinate.

Sample preparation

Capsule. Weigh accurately each of the capsule contents and transfer a suitable amount, equivalent to about 20 mg of ampicillin, into a 20-ml flask, mix well and dilute to volume with absolute methanol.

Vial. Transfer quantitatively the contents of the vial into a 100-ml volumetric flask and dilute to volume with absolute methanol. Pipette an aliquot equivalent to

about 20 mg of sodium ampicillin into a 20-ml volumetric flask and dilute to volume with absolute methanol. Proceed as described under *Calibration graph.*

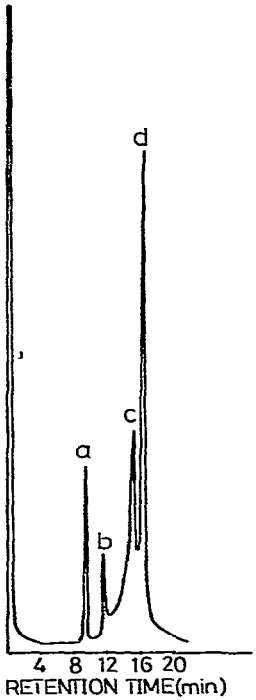
Silylation procedure

To each dried sample, add 0.3 ml of pyridine, 0.3 ml of HMDS, 0.3 ml of TMCS, 0.1 ml of TMSI and 0.05 ml of BSA. The reaction mixture is allowed to stand for 2 h at room temperature before injection of 0.6–1.6 μl of the silylated solution into the gas chromatograph.

RESULTS AND DISCUSSION

Gas chromatography

Several combinations of silylating agents were tried for the derivatization of amplicillin at room temperature. The silylation of ampicillin with pyridine—BSA (0.2 +0.2 ml) resulted in the formation of multiple peaks, as shown in Fig. 1(b,c,d). The same phenomenon also occurred on using acetonitrile–BSA (0.2 + 0.2 ml). The peak-height ratios of these triple peaks changed continuously for 5 hours, so that these conditions were therefore unsuitable for the determination of ampicillin.



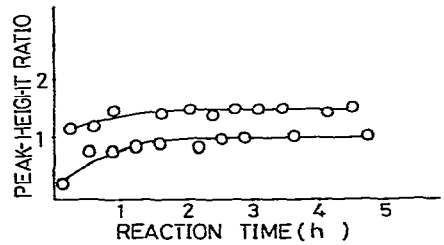


Fig. 1. Gas chromatogram of mixtures of silylated ampicillin. (a) 5α-Cholestane (internal standard); (b) tris-TMS-ampicillin; (c) bis-TMS-ampicillin; (d) mono-TMS-ampicillin. Conditions: 1 m × 3 mm I.D. column containing 1.5% (w/w) OV-17; column temperature, 190° (5 min) then programmed at 5°/min to 250° held at 250° for 5 min; carrier gas, helium at a flow-rate of 65 ml/min. These conditions were employed only to characterize the TMS derivatives.

Fig. 2. Effect of reaction time on formation of mono-TMS-ampicillin. Peak-height ratios of ampicillin (above) and sodium ampicillin (below) to 5α-cholestane (internal standard).

Pyridine–HMDS–TMCS (0.3 + 0.3 + 0.3 ml) was then tried as the silylation species, but no peak was obtained after 3 h. The use of pyridine–HMDS–TMCS–TMSI (0.3 + 0.3 + 0.3 + 0.05 ml) as silylating agent resulted in poor derivatization, even though a single peak was obtained. Silylation of ampicillin with pyridine–HMDS–TMSI (0.3 + 0.5 + 0.05 ml) gave good results with a single peak, but sodium ampicillin was not derivatized, possibly owing to the charge on the carboxyl group, which Shaw[34] considered to resist silylation.

For derivatization of both ampicillin and sodium ampicillin under the same conditions, silylation with pyridine–HMDS–TMCS–TMSI–BSA (0.3 + 0.3 + 0.3 + 0.1 + 0.05 ml) gave satisfactory results. Single peaks were obtained for both ampicillin and sodium ampicillin, as shown in Fig. 3(d). The relationship between reaction time and peak-height ratio is indicated in Fig. 2. The peak-height ratio of ampicillin or sodium ampicillin to 5α-cholestane became constant after 2 h. Therefore, the GC determination was carried out 2 h after silylation.

α-Aminobenzylpenicilloic acid, one of the degradation or metabolic products of ampicillin, was derivatized under the same conditions and gave double peaks, as shown in Fig. 3(b,c), completely separated from the ampicillin peak. Hence this substance does not interfere in the determination of ampicillin.

The calibration graphs for ampicillin and sodium ampicillin were linear over the range 0.5–3.0 mg, as shown in Figs. 4 and 5. The results in Table I indicate good precision.

Further applications to other pharmaceutical products should be possible.

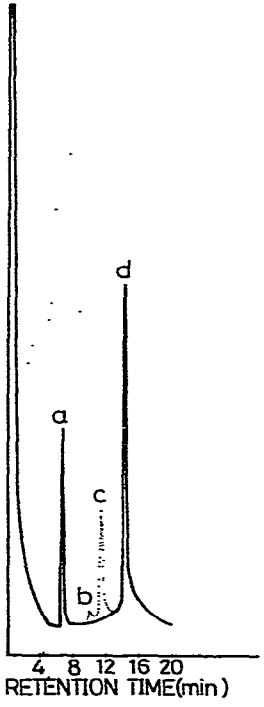


Fig. 3. Composite gas chromatogram of (a) 5α-cholestane and (b and c) α-aminobenzylpenicilloic acid, with unknown characteristic (d) mono-TMS-ampicillin.

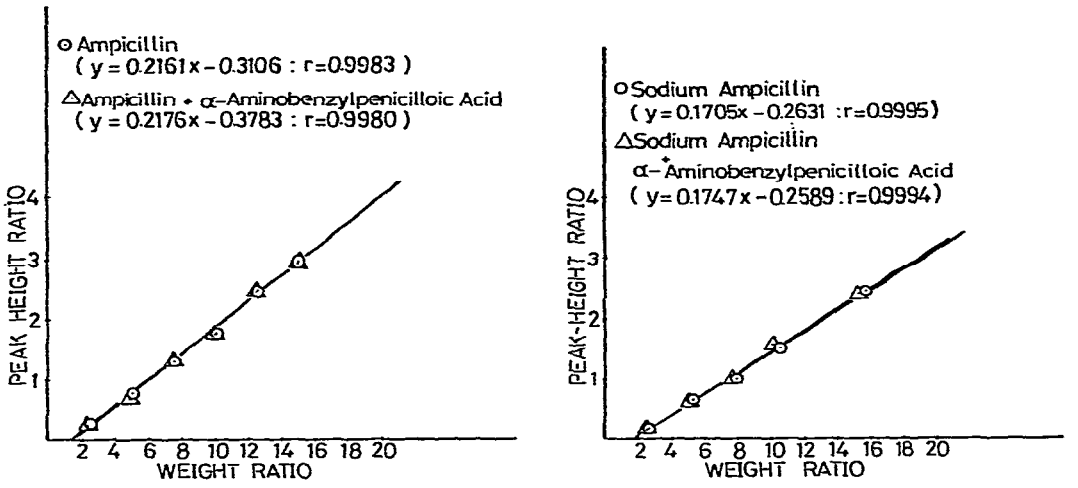


Fig. 4. Calibration graphs for ampicillin (○) and ampicillin plus 5.0% of α-aminobenzylpenicilloic acid (△).

Fig. 5. Calibration graphs for sodium ampicillin (○) and sodium ampicillin plus 5.5% of α-aminobenzylpenicilloic acid (△).

TABLE I

RESULTS OF ASSAY OF AMPICILLIN PRODUCTS

Product	*Label claim* (mg)*	*Batch*	*Found** (mg)*	*Recovery (%)*
Capsule: 1	250	A	245.37	98.15
2	250	A	241.45	96.58
3	250	A	242.60	97.04
4	250	A	244.18	97.67
5	250	B	243.77	97.51
Vial: 1	250	A	250.66	100.26
2	250	A	241.83	96.73
3	500	B	480.71	96.14

* The ampicillin content is expressed in units of potency.
** Average of duplicate determinations calculated as anhydrous ampicillin.

Mass spectrometry (MS)

GC–MS was performed in order to elucidate the peak characteristics of ampicillin under different silylation conditions. The mass spectra B, C and D in Fig. 6 correspond to peaks b, c and d, respectively, in Fig. 1, for ampicillin silylated with pyridine–BSA (0.2 + 0.2 ml).

Spectrum B contains a molecular ion at *m/e* 565 (M), an M—CH_3 ion at *m/e* 550, and decomposition of the parent ion by elimination of trimethylsilanol led to a peak of *m/e* 475 [M—$(CH_3)_3SiOH$], which was further degraded by cleavage of the methyl group to give a peak of *m/e* 460. Scission of the β-lactam ring gave an ion at *m/e* 232 after gaining one hydrogen atom and another ion at *m/e* 334. The ion at *m/e* 232 is assumed to be due to the mono-TMS fragment silylated at the carboxyl

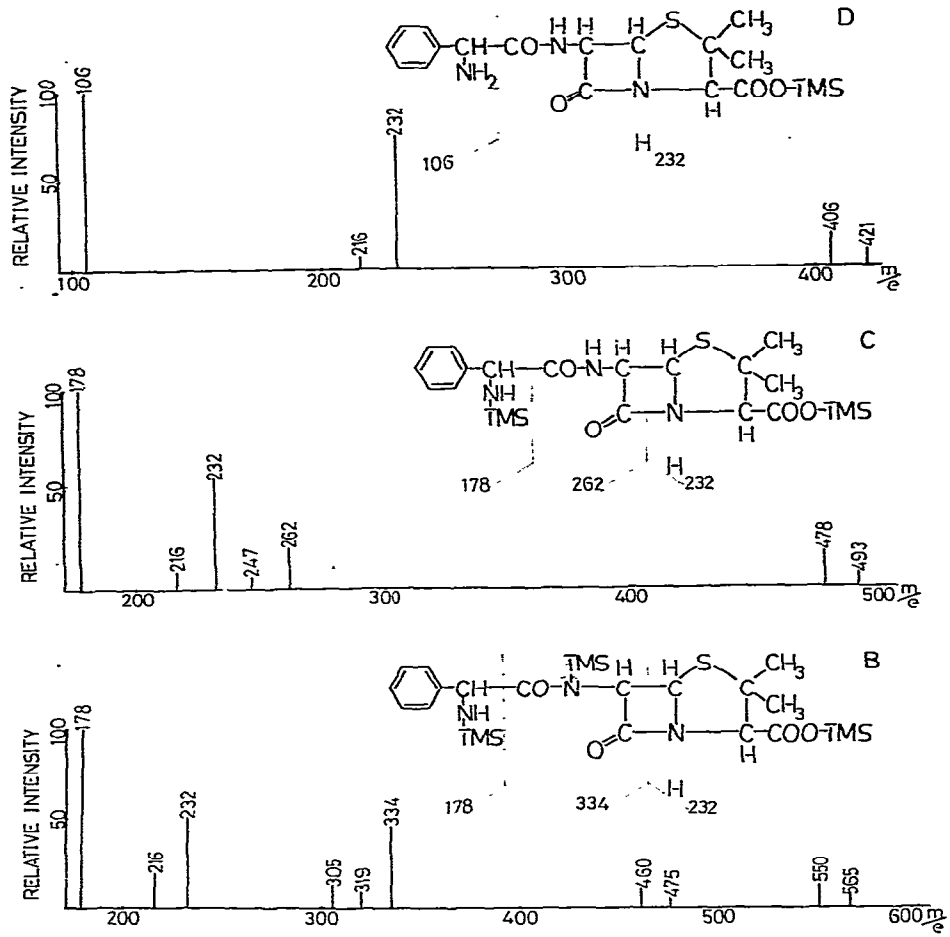


Fig. 6. Mass spectra of mono-TMS-ampicillin (above), bis-TMS-ampicillin (centre) and tris-TMS-ampicillin (below), corresponding to peaks d, c and b, respectively, in Fig. 1.

function, and the ion at *m/e* 334 is assumed to be the bis-TMS fragment silylated at the amide and amino functions. Cleavage between the benzyl and amide carbon atoms is thought to induce the ion at *m/e* 178, probably due to the N-trimethylsilyl-aminobenzylimmonium fragment.

In spectrum C, the molecular ion occurs at *m/e* 493 (M), elimination of the methyl group gave a peak of *m/e* 478 (M—CH_3), β-lactam scission evolved an ion at *m/e* 232 (the same as that in spectrum B) and an ion peak at *m/e* 262 represents the mono-TMS fragment. An ion peak appeared at *m/e* 178, as in spectrum B.

In spectrum D, the ion at *m/e* 421 (M) corresponds to the parent ion, removal of a methyl group led to *m/e* 406 (M—CH_3), β-lactam cleavage gave the ion at *m/e* 232 common to spectra B and C, and an ion at *m/e* 106 represents the aminobenzylimmonium fragment.

The above ion peak profile leads to the conclusion that the triple peaks b, c and

d shown in Fig. 1 represent tris-TMS-ampicillin, bis-TMS-ampicillin and mono-TMS-ampicillin, respectively, rather than the degradation products of ampicillin under the silylation conditions used. On the other hand, peak d found in Fig. 3, obtained by silylation of ampicillin with pyridine–HMDS–TMCS–TMSI–BSA (0.3 + 0.3 + 0.3 + 0.1 + 0.05 ml) has a mass spectrum identical with spectrum D in Fig. 6, indicating that peak d is mono-TMS-ampicillin. The peak characteristic of α-aminobenzylpenicilloic acid as shown in Fig. 3(b,c) was monitored by GC–MS, but no definite conclusion could be drawn.

ACKNOWLEDGEMENTS

The authors thank Dr. K. Hashimoto for carrying out the GC–MS work and Dr. T. Nakagawa and Dr. K. Yamaoka for their kind encouragement, and deep appreciation is also extended to Bristol Labs., Shionogi Co. and Meiji Co. for the supply of α-aminobenzylpenicilloic acid, ampicillin and sodium ampicillin, respectively.

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