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Note

Gas chromatographic determination of piromidic acid[*]

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A newly developed synthetic antibacterial agent, piromidic acid[1,2] (PA; 5,8-dihydro-8-ethyl-5-oxo-2-pyrrolidinopyrido[2,3-*d*]pyrimidine-6-carboxylic acid) is used mainly for combating Gram-negative bacterial infections[3] in the biliary, urinary and gastro-intestinal tracts.

Only a few methods, including non-specific bioassay[4] and ultraviolet spectro-photometric[5] techniques, have been employed for the determination of PA in biological specimens. In this paper, a gas chromatographic (GC) approach based on chemical derivatization of PA as its methyl ester has been established for the quantitative determination of PA. The method is simple, rapid and accurate.

EXPERIMENTAL

GC conditions

A Varian 1740 gas chromatograph equipped with a dual flame-ionization detector was used. The column was a 0.91 × 2 mm I.D. coiled glass tube packed with 2% OV-1 on Chromosorb W AW DMCS (80–100 mesh). The injection port and detector were kept at 290 and 295 °C, respectively. The column temperature was set initially at 265 °C and programmed at 10 °/min to 290 °C. Nitrogen was used as the carrier gas at a flow-rate of 60 ml/min. A chart speed of 4.2 mm/min was used. A Varian Aerograph PTFE-faced septum was used in the injection port. An injection of 10 μl of the derivatized sample divided into several portions was made before each day's run in order to minimize column loss, which resulted in lower peak-height ratios in the initial chromatographic analysis.

Chemicals and reagents

PA (a gift from Taiwan Dainippon Pharmaceutical Co., Taipei, Taiwan), *n*-octacosane (guaranteed grade; Wako, Tokyo, Japan), 2% OV-1 on Chromosorb W AW DMCS (80–100 mesh) (Shimadzu, Kyoto, Japan) and N-methyl-N-nitroso-*p*-toluenesulphonamide (Aldrich, Milwaukee, WI, U.S.A.) were used without further treatment. Dichloromethane and other reagents were of analytical-reagent grade.

Internal standard solution

Approximately 15 mg of *n*-octacosane were accurately weighed, transferred into a 25-ml volumetric flask and dissolved in and diluted to volume with dichloromethane.

Reference standard solution

Approximately 100 mg of PA were accurately weighed, transferred into a 50-ml volumetric flask and dissolved in and diluted to volume with dichloromethane.

Analytical calibration

Seven samples containing the reference standard solution over the range 0.2–2.5 ml were pipetted into a series of 10-ml vials and 0.5 ml of the internal standard solution was added and well mixed. Each solution was treated with diazomethane as indicated under *Derivatization procedure*. A calibration graph was constructed by plotting the weight ratios of PA to *n*-octacosane against their peak-height ratios.

Derivatization procedure

To each sample solution, a suitable amount of diazomethane solution, prepared in a Diazald kit (Aldrich) according to the method of preparation of ethereal alcoholic solutions of diazomethane as directed in Aldrich's technical information[6], was added until the solution appeared persistently yellow. The solution was allowed to stand for 30 min at room temperature, then evaporated to dryness on a waterbath. The residue obtained was dissolved in 1 ml of dichloromethane and GC determination was effected by injection of 1.5–3.0 μl of the derivatized solution.

RESULTS AND DISCUSSION

Direct GC analysis of PA is considered to be impracticable owing to its low volatility and its ready thermal degradation[7] to the decarboxylated compound above its melting point. To enhance the volatility and stability of PA, an attempt was made to derivatize PA at its carboxyl function with diazomethane.

The results were satisfactory, as shown in Fig. 1, a sharp and symmetric peak (b) with a short retention of 2.5 min being obtained. In addition to a peak (a) due to the internal standard, an over-scale peak (r) is also present in Fig. 1. Further investigation indicated that this peak (r) is derived from the dichloromethane solution of the residue obtained by evaporating a dichloromethane–diazomethane solution (1 + 1 ml). Therefore, this peak is assumed to be a reagent background, as shown in Fig. 2, due probably to the polymer formed by excess of diazomethane solution.

To elucidate the structure of the compound represented by peak b in Fig. 1, GC-mass spectrometry (MS) was carried out using a Hitachi M-52 mass spectrometer linked to a Hitachi M-5201 gas chromatograph with a separator temperature of 290 °C, an ionization-source temperature of 200 °C, an electron energy of 70 eV and an acceleration energy of 3.5 kV. The mass spectrum obtained exhibited a parent ion at $m/e = 302$, representing the formation of PA methyl ester.

To evaluate the quantitative applicability of the method, seven amounts of

Fig. 1. Gas chromatograms of (a) *n*-octacosane (internal standard) and (b) piromidic acid methyl ester.

Fig. 2. Gas chromatogram of reagent background.

PA in the range 0.42–5.23 mg were analyzed and the linearity between peak-height ratios and weight ratios were examined. A linear regression equation ($y = 0.143x + 0.004$) was obtained with a correlation coefficient of 0.998. The recovery of PA was tested on samples containing six different levels of PA. The results are presented in Table I; mean recovery for six samples was $99.9 \pm 1.23\%$. Hence, the proposed method is feasible for quantitation of PA and has a high accuracy.

TABLE I

ASSAY DATA FOR KNOWN AMOUNTS OF PIROMIDIC ACID

Amount (mg)		Recovery (%)
Present	Found	
0.63	0.63	100.0
1.05	1.04	99.0
2.09	2.07	99.0
3.14	3.16	100.6
4.17	4.12	98.8
5.23	5.35	102.3
	Mean recovery (%)	99.9
	Standard deviation (%)	1.23

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