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GAS CHROMATOGRAPHIC DETERMINATION OF NALIDIXIC ACID IN TABLETS

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SUMMARY

A gas chromatographic method has been established for the quantitative analysis of nalidixic acid. The method is based on the derivatization of nalidixic acid with diazomethane, and 5-α-cholestane is used as an internal standard. The sample is chromatographed on a glass column packed with 1% OV-1 on Chromosorb W. Quantitation is achieved by measuring peak-height ratios. The improved simplicity, specificity and accuracy of the method has been demonstrated for the quantitation of nalidixic acid in tablets.

INTRODUCTION

A variety of methods has been developed for the analysis of nalidixic acid in pharmaceutical products and biological specimens: among them, the chemical methods[1–3] centred on acid-base neutralization, the polarographic analysis based on the reduction of nalidixic acid to the corresponding 4-hydroxy derivative[4], and the fluorophotometric methods[5–7] the UV spectrophotometric methods[8–10], the microbiological assay[11], and paper and thin-layer chromatographic detections[2,12]. Although high-performance liquid chromatographic methods have been developed for nalidixic acid quantitation[13,14], no gas chromatographic (GC) method has been established yet.

This report describes a GC method for the determination of nalidixic acid in tablets. The results indicate that the method is simple, specific and accurate.

EXPERIMENTAL

GC conditions

A Varian 1740 gas chromatograph equipped with a dual flame-ionization detector was used. The column was a 1.83 m × 2 mm I.D. coiled glass tube packed with 1% OV-1 on Chromosorb W AW DMCS (80–100 mesh). The injection port and

detector were kept at 280°. The column temperature was set initially at 190° and programmed at 10°/min to 280°. Nitrogen was used as the carrier gas at a flow-rate of 41 ml/min. A PTFE-faced septum (Varian Aerograph) was used in the injection port. An injection of 10 μl of derivatized sample solution divided into several portions was made before each day's run in order to minimize column loss; otherwise lower peak-height ratios in the initial chromatographic analysis were encountered, probably due to column adsorption.

Chemicals and reagents

Nalidixic acid was a gift from Daiichi Seiyaku (Tokyo, Japan), 7-hydroxymethylnalidixic acid and 3,7-dicarboxynalidixic acid were generously supplied by the Sterling-Winthrop Research Institute (Rensselaer, N.Y., U.S.A.), 5-α-cholestane of guaranteed grade was purchased from Nakarai Chemical (Kyoto, Japan), 1% OV-1 on Chromosorb W AW DMCS (80–100 mesh) was the product of Antek Instrument (Houston, Texas, U.S.A.), and N,O-bis(trimethylsilyl)acetamide (BSA), trimethylsilylchlorosilane (TMCS), N-trimethylsilylimidazole (TMSI), and *p*-tolylsulphonylmethylnitrosamide (E. Merck, Darmstadt, G.R.) were used without further treatment. Chloroform and other reagents were of analytical grade.

Solutions of the internal and reference standard were prepared by accurately weighing *ca.* 80 mg of 5-α-cholestane or *ca.* 50 mg of nalidixic acid respectively, into a 25-ml volumetric flask and dissolving and diluting to volume with chloroform.

Analytical calibration

Eight samples containing the reference standard over the range of 0.3–2.5 ml were pipetted into a series of 10-ml vials, 0.5 ml of the internal standard solution were added and mixed well. Each sample was evaporated to dryness on a water-bath. The residue was treated with diazomethane as described under *Derivatization procedure*. A calibration graph was constructed by plotting the weight ratios of nalidixic acid to 5-α-cholestane against their peak-height ratios.

Sample preparation

Each tablet was accurately weighed and a suitable quantity of the finely pulverized tablet mass equivalent to *ca.* 50 mg of nalidixic acid was accurately transferred to a 25-ml volumetric flask. The volume was made up with chloroform and the mixture was stirred for 30 min by means of a magnetic stirrer. About 10 ml of the suspension were then transferred to a glass-stoppered centrifuge-tube and centrifuged at 1000 *g* for 10 min. A 1.5-ml aliquot of the supernatant was pipetted into a 10-ml vial containing 0.5 ml of the internal standard solution, the solution was mixed well and evaporated to dryness on a water-bath. The residue was derivatized as described below.

Derivatization procedure

Each residue was dissolved in 0.5 ml of chloroform, then a suitable amount of diazomethane–diethylether solution, prepared according to the method of De Boer and Backer[15] using ethanol as the reaction mixture instead of Carbitol, was added untill the solution appeared persistently yellow. The solution was allowed to stand for 30 min at room temperature and then evaporated to dryness on a water-bath. The

residue obtained was dissolved in 0.5 ml of chloroform and GC determination was performed by injection of 0.4–1.0 μl of the chloroform solution.

RESULTS AND DISCUSSION

Derivatization of nalidixic acid with silylating agents has been examined at room temperature. The mixtures of silylating agents employed were pyridine–BSA (0.5 + 0.2 ml), pyridine–BSA–TMCS (0.25 + 0.1 + 0.1 ml), pyridine–TMSI–TMCS (0.25 + 0.1 + 0.1 ml) and pyridine–BSA–TMSI–TMCS (0.25 + 0.1 + 0.1 + 0.1 ml) under the same chromatographic conditions. The chromatographic properties of

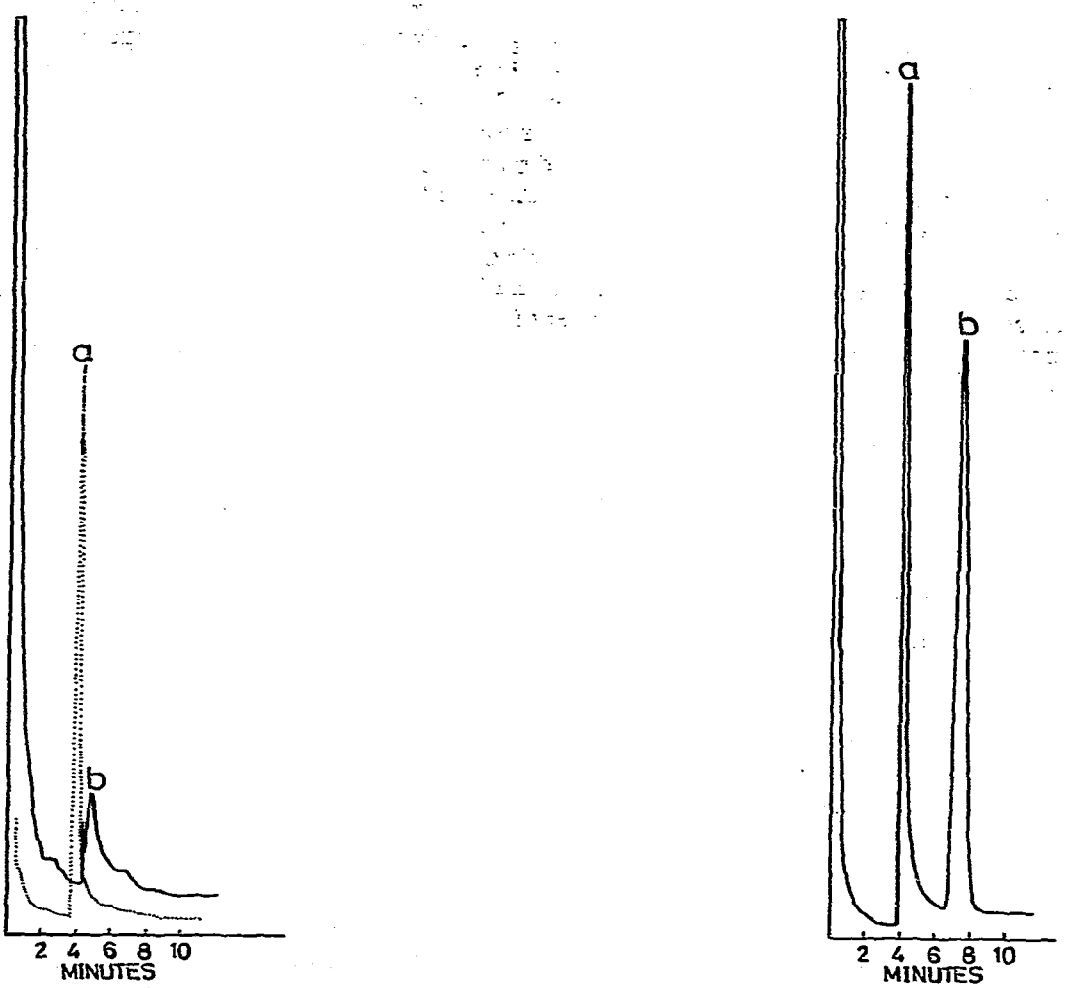

Fig. 1. Composite gas chromatogram of (a) nalidixic acid treated with diazomethane and (b) nalidixic acid without derivatization.

Fig. 2. Gas chromatogram obtained from the analysis of powdered tablet material: (a) nalidixic acid methyl ester, (b) 5-α-cholestane (internal standard).

nalidixic acid derivatized with these silylating systems were not satisfactory, resulting in peak broadening and lower peak-height ratios as compared to that derivatized with diazomethane. Direct GC analysis of nalidixic acid without derivatization was also made under the same chromatographic conditions. The results appeared as splitting and tailing peaks of poor response accompanied by a minor shoulder on the tailing of the solvent peak, possibly due to a degradation product of nalidixic acid obtained at the relatively high injection temperature. This is in complete contrast to the reproducible sharp peak of diazomethane-treated nalidixic acid (Fig. 1). Peak a in Fig. 1 was further analyzed by use of a JEOL-01SG-2 mass spectrometer under the conditions of separator temperature 200°, ionization-source temperature 300°, ionization energy 75 eV and acceleration energy 8 kV. The spectrum obtained exhibited a molecular ion peak at $m/e = 246$, indicating the formation of nalidixic acid methyl ester. Fig. 2 shows a typical gas chromatogram obtained for a tablet sample after derivatizing with diazomethane. Both nalidixic acid and the internal standard gave well resolved, sharp and symmetric peaks. The retention time was relatively short, 4.05 min for nalidixic acid and 7.37 min for 5-α-cholestane (internal standard).

The linearity of the system was demonstrated by analyzing eight different amounts of nalidixic acid over the range of 0.62–5.18 mg. A linear regression equation ($y = 0.8544x - 0.1861$) was obtained with a correlation coefficient of 0.9985.

To determine the recovery of nalidixic acid, samples were prepared containing six different levels of known amounts of nalidixic acid. The analytical results are presented in Table I. The overall recovery for six samples was 101.0 ± 0.73%. The small standard deviation demonstrates that nalidixic acid can be measured with high precision.

TABLE I

ASSAY DATA FOR KNOWN AMOUNTS OF NALIDIXIC ACID

Amount (mg)		*Recovery (%)*
Known	*Found*	
1.65	1.65	100.0
2.06	2.07	100.5
3.09	3.11	100.6
3.69	3.75	101.6
4.10	4.19	102.2
4.72	4.76	100.8
	Mean recovery (%)	101.0
	S.D. (%)	0.73

Nalidixic acid tablets obtained from two different commercial sources were analyzed and the results are shown in Table II. The present method is feasible for the quantitation of nalidixic acid in tablets and is simple, specific and accurate.

The proposed method was also used to detect a sample mixture of nalidixic acid and two of its metabolites, 3,7-dicarboxynalidixic acid and 7-hydroxymethylnalidixic acid. A simultaneous resolution was attainable as presented in Fig. 3. The

chromatographic property of 7-hydroxymethylnalidixic acid was poor, probably due to the polar function of the hydroxyl group, and can be highly improved by further treatment with BSA. The applicability of this partially modified method of determining nalidixic acid and its biotransformation products is under investigation.

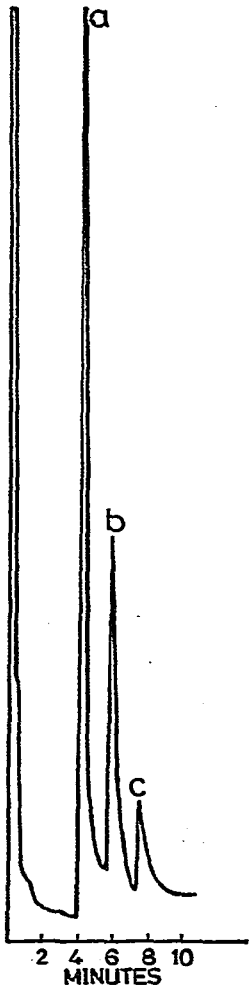

Fig. 3. Gas chromatogram of (a) nalidixic acid, (b) 3,7-dicarboxynalidixic acid and (c) 7-hydroxymethylnalidixic acid, after derivatizing with diazomethane.

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TABLE II

ASSAY RESULTS FOR NALIDIXIC ACID TABLETS (500 mg PER TABLET) OBTAINED FROM A COMMERCIAL SOURCE

Tablet number	*Amount found (mg per tablet)*	*Percentage of claim*
T1	516.1	103.2
T2	523.5	104.7
T3	534.9	107.0
T4	527.8	105.6
T5	509.9	102.0
T6	524.2	104.8
	Mean recovery (%)	104.6
	S.D. (%)	1.61
W1	512.6	102.5
W2	530.8	106.2
W3	533.2	106.6
W4	530.1	106.0
W5	514.0	102.8
W6	526.0	105.2
	Mean recovery (%)	104.9
	S.D. (%)	1.64

REFERENCES

1 V. Ignat and H. Beral, *Rev. Chim. (Bucharest)*, 17 (1966) 50.
2 G. Dondi and M. Di Marco, *Boll. Chim. Farm.*, 105 (1966) 491.
3 *The National Formulary*, Mack Publ., Easton, Pa., 13th ed., 1970, pp. 466–467.
4 R. Starościk, I. Prochowska and J. Sulkowska, *Pharmazie*, 29 (1974) 387.
5 E. W. MeChesney, E. J. Froelich, G. Y. Lesher, A. V. R. Crain and D. Rosi, *Toxicol. Appl. Pharmacol.*, 6 (1964) 292.
6 Von P. Brühl, G. Gundlach, K. Wintjes, W. Eichner and H. P. Bastian, *Arzneim.-Forsch.*, 23 (1973) 1311.
7 R. Staroscik and J. Sulkowska, *Acta Pol. Pharm.*, 30 (1973) 499.
8 M. J. J. Vieira da Silva and M. T. C. Nogueira, *Rev. Port. Farm.*, 15 (1965) 290.
9 J. Sulkowska and R. Starościk, *Pharmazie*, 30 (1975) 405.
10 A. V. Gafari, *Farm. Zh. (Kiev)*, 1 (1977) 53.
11 C. Ciuro, *Cienc. Ind. Farm.*, 5 (1973) 54.
12 T. Perényi, H. Graber and E. K. Novák, *Acta Microbiol. Acad. Sci. Hung.*, 22 (1975) 433.
13 L. Shargel, R. F. Koss, A. V. R. Crain and V. J. Boyle, *J. Pharm. Sci.*, 62 (1973) 1452.
14 D. L. Sondack and W. L. Koch, *J. Chromatogr.*, 132 (1977) 352.
15 J. De Boer and H. J. Backer, *Organic Syntheses*, Coll. Vol. 4, New York, London, Sydney, 1963, pp. 250–253.