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Note

Analysis of urinary tract antibacterial agents in pharmaceutical dosage form by high-performance liquid chromatography

DAVID L. SONDACK* and WILLIAM L. KOCH
Eli Lilly and Co., Indianapolis, Ind. 46206 (U.S.A.)
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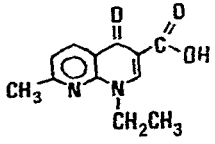
A high-performance liquid chromatographic (HPLC) method of analysis for nalidixic acid (I) in urine has been reported[1]. A similar system was reported for the analysis of sulfanilamide antibiotics[2]. After suitable alteration of the system used for the analysis of I, the analysis of 1-ethyl-1,4-dihydro-4-oxo[1,3]dioxolo[4,5-*g*]cinnoline-3-carboxylic acid (cinoxacin) (II), a newly synthesized antibacterial agent[3], became possible. Sulfanilic acid or sulfamerazine was incorporated as an internal standard. This report describes the analysis of II in capsule and ampoule formulations and of I, II and oxolinic acid (III) as aluminum hydroxide gel suspensions.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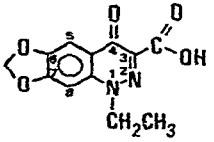
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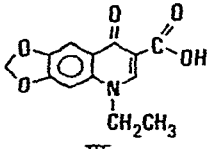
III

EXPERIMENTAL

Materials

Standard solutions of I (purchased from Calbiochem, Los Angeles, Calif., U.S.A.), II (synthesized in the Eli Lilly Research Labs.) and III (purchased from Warner Lambert, Morris Plains, N.J., U.S.A.) were prepared by dissolving the material in 0.1 *M* borate buffer and a few drops of 1 *M* sodium hydroxide, if necessary, to yield a final concentration of 1 mg/ml. All reagents were obtained from commercial sources. Sulfanilic acid (2 mg/ml) or sulfamerazine (1 mg/ml) in the above buffer were routinely used as internal standards.

Liquid chromatography

Liquid chromatography was performed on a Varian Model 4100 liquid chromatograph equipped with a UV (254 nm) photometric detector and a Model A-20 strip chart recorder. The signal from the photometric detector was fed to an IBM 1800 computer for peak integration. Solutions were chromatographed at room tem-

* To whom reprint requests should be sent.

perature on a 1 m × 2.1 mm I.D. stainless-steel column packed with a strong anion-exchange resin (Zipax® SAX; E. I. DuPont, Wilmington, Del., U.SA.). The mobile phase was composed of 0.01 *M* sodium tetraborate, pH 9.2, and 0.003 *M* sodium sulfate. A flow-rate of 0.8 ml/min was maintained. A photometer range of 0.16 a.u.f.s. was adequate.

Sample preparation

Capsule contents were dissolved in 0.1 *M* borate buffer with the aid of 2 ml 1 *M* sodium hydroxide to give a final concentration of 1 mg/ml of II. Aliquots of ampoule contents were diluted to a similar concentration. Weighed samples of aluminum hydroxide gel suspensions were dissolved in 1 *M* sodium hydroxide to a final concentration as above. The diluted aluminum hydroxide gel suspensions of I and III were allowed to stand for a few hours to achieve complete dissolution. A separate determination of the density of the gel provided the necessary information for calculation of drug per volume.

The standard and samples were all further diluted for chromatography. Five-milliliter aliquots were transferred to 100-ml volumetric flasks containing 5 ml of the appropriate internal standard solution and the flasks were diluted to the mark with water. Injections of 10 μl were made. Routinely, sulfanilic acid was employed as the internal standard for II and III and sulfamerazine for I.

RESULTS AND DISCUSSION

The mobile phase described for HPLC analysis of I[1] was tested as the eluting solvent for II. Compound II was eluted at the solvent front. Retention was obtained by lowering the sodium sulfate concentration. In addition II could be resolved from possible synthetic impurities and from potential metabolites (Fig. 1).

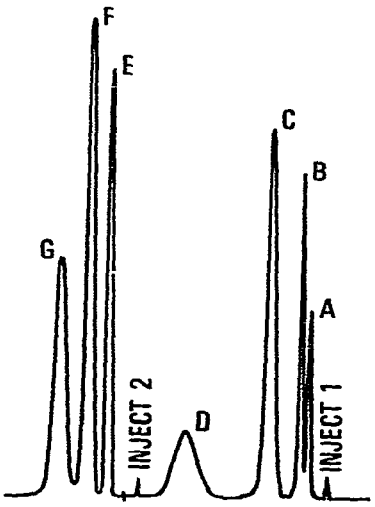

Fig. 1. HPLC recorder tracing. Injection 1: (A) decarboxylated II, (B) II, (C) sulfanilic acid, (D) 6-methoxy-7-hydroxy analogue of II; injection 2: (E) oxolinic acid, (F) nalidixic acid, (G) sulfamerazine.

TABLE I

RELATIVE RETENTION TIMES OF THE COMPOUNDS TESTED

Compound	t_R
Oxolinic acid	0.52
Nalidixic acid	0.86
Sulfamerazine	1.34
Decarboxylated II	0.34
II	0.45
Sulfanilic acid	1.00 (7.6 min)
6-Methoxy-7-hydroxy analogue of II	2.38

Sulfanilamide antibiotics had been successfully chromatographed in a similar solvent system also[3]. These were tested for potential use as an internal standard in the system under discussion. Sulfamerazine was satisfactorily resolved from the compounds under consideration. In the interest of keeping analysis time to a minimum, sulfanilic acid was chosen as the internal standard for II and III. Sulfamerazine was used for compound I.

The relative retention times of the compounds tested are compiled in Table I. Decarboxylated II is a possible synthetic impurity and possible degradation product. The 6-methoxy-7-hydroxy analogue of II is a possible metabolite comparable to one proposed for III[4]. Another compound tested, the 6,7-dihydroxy analogue of II was retained by the column in excess of 90 min. Further studies on the analysis of metabolites of II will be reported at a later date[5].

The response of the analytical system was linear in a range from 10–200% of

TABLE II

ANALYSES OF DIFFERENT DOSAGE FORMS

Dosage form	*HPLC*	*Microbial*	*Theory*
Capsule	50.3	50.2	50 mg/capsule
	51.4	49.5	
	51.1	50.1	
Capsule	254.0	256.9	250 mg/capsule
	258.7	256.9	
	257.1	256.9	
	248.0	245.8	
Ampoule	502	500	500 mg/ampoule
	494	489	
	500	504.6	
Aluminum hydroxide gel suspension	277	—	250 + 10% mg/5 ml
	263	—	
	267	—	
	274	—	
Aluminum hydroxide gel suspension of nalidixic acid	295	—	250 + 20% mg/5 ml
Aluminum hydroxide gel suspension of oxolinic acid	267	—	250 + 10% mg/5 ml

the described final sample concentration of 0.05 mg/ml. The extrapolated line passed through the origin and had a slope of 10 Area Ratio Units/mg·ml. The area ratios measured for multiple injections of the same solution were within 0.35% of the average value. Five replicate weighings of standard material gave an R.S.D. of ± 0.6%. Twenty replicate capsule samples gave values with ± 2.0% of the average and were 0.9% higher than label claim. Standard added to placebo gave 100% recovery. Some typical analytical results are shown in Table II, with microbiological assay results shown for comparison. Note that the suspensions could not be assayed microbiologically.

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