Table III—Antihypertensive Quinazolinylformamides and Quinazolinediylbisformamidines

Compound	*n*	Mean Arterial Blood Pressure	Dose, mg/kg	Percent Decrease from Control
II*a*	2	131	100	21
II*b*	3	118	100	29
II*b*	3	129	25	22
II*e*	2	146	100	12
II*i*	2	139	100	16
II*i*	4	144	25	13
Guanabenz	20	123	25	26
Clonidine	6	96	0.5	43
Control	60	166	—	—

active of this series at the 100-mg/kg dose level. The most active congener was the 6-nitro derivative II*b*, while the least active congener was the 6,7-dimethoxy derivative II*e*. Of the active formamidines, only II*b* (6-nitro) and II*i* (6-cyano) were active at the 25-mg/kg dose level in the spontaneously hypertensive rat. Congener II*b* was approximately twice as active as II*i*, a 29 *versus* 13% decrease in mean arterial blood pressure. Thus, quinazolinediylbisformamidines bearing an electron-withdrawing group at C-6 of the quinazoline nucleus apparently are more active than the corresponding quinazolinediylbisformamidines bearing electron-donating substituents.

When II*a*–II*k* were administered in gelatin capsules to normotensive dogs, no effect on blood pressure was observed with a single dose of 25 mg/kg. Bradycardia was observed at doses greater than 25 mg/kg, which prevented further evaluation at higher doses.

The lack of hypotensive activity of II*a*–II*k* in the normotensive dog precludes further development of the quinazolinediylbisformamidines.

EXPERIMENTAL

All melting points are uncorrected. Samples for elemental analysis were dried at 55° under high vacuum for 5–24 hr. The general procedure employed in the synthesis of the quinazolinylformamidines is as follows for N^1,N^3-dimethyl -N^2,N^4- (7-chloro-8-methyl-2,4-quinazolinediyl)bisformamidine (II*g*).

A solution of 2.1 g (10.0 mmoles) of 7-chloro-8-methyl-2,4-diaminoquinazoline in 20 ml of dimethylformamide dimethyl acetal and 20 ml of dimethylformamide was refluxed for 16 hr and then cooled to room temperature. Excess solvent was removed under aspirator pressure, and the solid residue was recrystallized twice from ethyl acetate to yield 2.2 g (69%) of light-yellow crystals, mp 182–184°.

Anal.—Calc. for $C_{15}H_{19}ClN_6$: C, 56.51; H, 6.01; Cl, 11.12; N, 26.36. Found: C, 56.51; H, 5.99; Cl, 11.10; N, 26.14.

Compounds II*a*–II*k* (Table II) were prepared by a similar procedure by condensing dimethylformamide dimethyl acetal with the appropriate I*a*–I*k*.

These quinazolinediylbisformamidines were characterized by elemental analyses and the presence of characteristic resonances at 3.0–3.3 ppm (methyl group protons at the terminal nitrogen atom of the formamidine moiety) in their PMR spectra.

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Quantitative Determination of Cephalexin in Cephradine by NMR Spectroscopy

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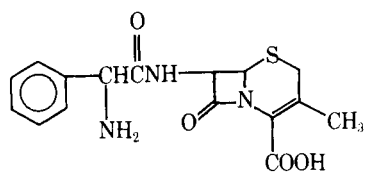
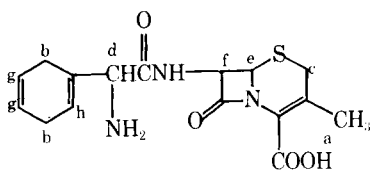
Abstract □ An NMR method to determine quantitatively the presence of cephalexin in cephradine was developed. The method is applicable to the chemical itself as well as to capsules and oral suspension formulations. The determination is based on the NMR signal arising from the five aromatic protons of the cephalexin molecule. Integration of this signal relative to a signal from cephradine provides the data necessary to determine the percentage of cephalexin present. The precision at the 2% cephalexin level is ±0.18%. The time required to carry out a single analysis is about 10 min, and five analyses can be done in about 0.5 hr.

Keyphrases □ Cephalexin—NMR spectroscopic analysis in cephradine bulk drug and dosage forms □ NMR spectroscopy—analysis, cephalexin in cephradine bulk drug and dosage forms □ Antibacterials—cephalexin, NMR spectroscopic analysis in cephradine bulk drug and dosage forms

Cephalexin (I) may be present in a given lot of cephradine (II) as an impurity from the synthesis of cephradine or as a decomposition product of cephradine. A rapid, quantitative method was required to determine the cephalexin content of cephradine chemical as well as capsule and oral suspension formulations. Present methods (1, 2) were too slow for large numbers of samples.



I



II

0022-3549/78/1000-1481$01.00/0

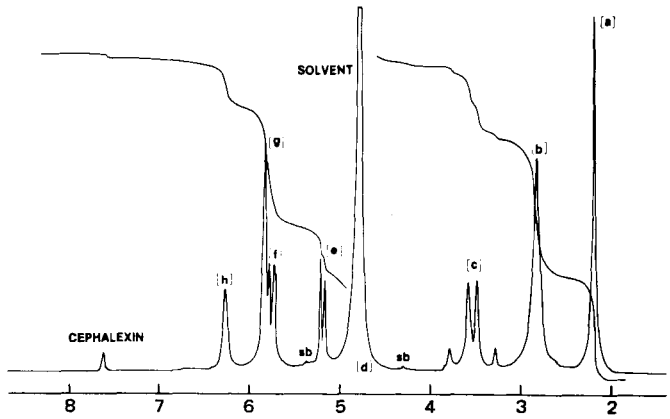


Figure 1—*NMR spectrum of cephradine containing 2.5% cephalexin.*

The purpose of this study was to establish the feasibility of using NMR to differentiate between the two compounds and for quantitative analysis of cephalexin when present. The described methods provide the data required for such analyses.

EXPERIMENTAL

All spectra were recorded on a 90-MHz spectrometer[1] using 3% deuterium chloride as the solvent. The spectra were recorded at 37° at a concentration of at least 200 mg/ml. Chemical shifts were measured relative to tetramethylsilane.

The quantitative NMR determination of cephalexin in cephradine is based on the integration of the five aromatic protons of the fully aromatic system in cephalexin (679 Hz) *versus* the integration of the olefinic proton of cephradine (558 Hz) (Figs. 1 and 2). A calibration curve was prepared by making known mixtures of the two components and plotting the ratio of their NMR integration *versus* the cephalexin concentration, usually expressed in percent. This calibration curve was then used for subsequent

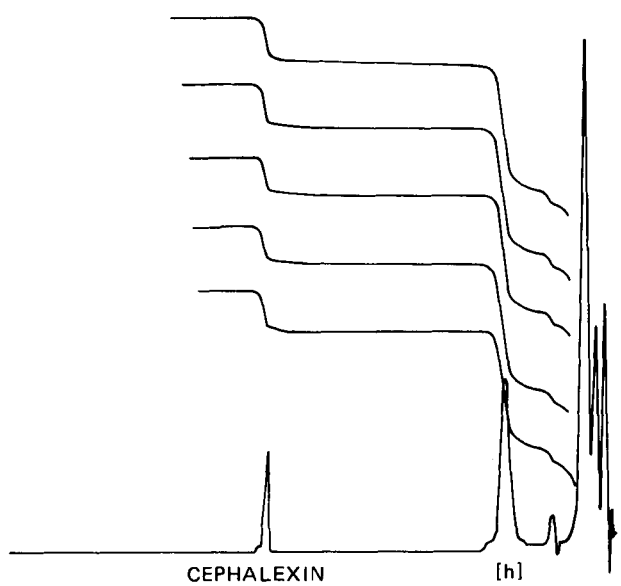


Figure 2—*The 300-MHz expansion of portion of Fig. 1.*

[1] Perkin-Elmer model R32.

Table I—Statistical Analysis of NMR Results

Group	Average, %	*SD*, %	*CV*, %
A	2.27	0.176	7.78
B	3.07	0.177	5.80
C	3.98	0.173	4.35

analyses of the chemical or dosage forms, *i.e.*, capsules or oral suspensions, according to the following procedures.

Chemical—Approximately 100 mg of sample was weighed and transferred to a small glass vial. Deuterium chloride (0.5 ml) was added, and the vial was shaken until the sample was dissolved. The solution was then transferred to an NMR tube. The NMR spectrum was run and integrated five times, and the average integral value was obtained. The ratio of the integral at 679 Hz representing the five aromatic protons of the cephalexin molecule and the integral at 558 Hz representing the olefinic proton of cephradine was recorded. The percentage of cephalexin then was calculated from a standard calibration plot.

Capsules—This determination can be carried out using a single capsule, but the contents of 10 capsules can be pooled to minimize capsule-to-capsule variation. The weight equivalent to a single capsule is then taken for assay.

A weight equivalent to a single capsule was transferred to a small glass vial, 0.5 ml of 3% deuterium chloride was added, and the vial was shaken. The cephalosporin materials dissolved in deuterium chloride, and the inorganic capsule filler (magnesium stearate) precipitated. The clear solution was removed by pipet or syringe and transferred to an NMR tube. From this point, the procedure followed was the same as that used for the chemical.

Oral Suspensions—Approximately 3 g of the oral suspension dry mix was weighed and transferred to a 250-ml erlenmeyer flask; 50 ml of methanolic 0.1 *N* HCl was added, and the flask was shaken. The slurry formed was filtered through filter paper. The residue was washed twice with 20-ml portions of methanolic 0.1 *N* HCl. The filtrate, which consisted of the cephalosporins, small amounts of sugar, and trace amounts of artificial coloring, was evaporated to dryness at room temperature under a nitrogen stream. The presence of the sugar and coloring in these amounts did not interfere with the determination. The dry sample was dissolved in 0.5 ml of 3% deuterium chloride, and the procedure followed from this point was the same as that for the chemical.

A statistical evaluation of the NMR method was carried out as follows: 18 samples of each of three levels of cephalexin (a total of 54 samples) were assayed. The samples were supplied as unknowns in sets of 18 and arbitrarily numbered within each set in such a way that if Samples 1–6 were analyzed on 1 day, 7–12 on a 2nd day, and 13–18 on a 3rd day, two samples of each cephalexin level would be analyzed on each day.

RESULTS AND DISCUSSION

A statistical analysis of the NMR results showed the variation among sets was not significant. There was no significant day-to-day variation. The standard deviation of the sample-to-sample variation is shown in Table I along with the average cephalexin content determined for each group and the coefficient of variation (standard deviation expressed as a percent of the average). The sample-to-sample standard deviations for the three groups were similar. With essentially a constant standard deviation (over this range of cephalexin content), the coefficient of variation steadily decreased as the cephalexin content increased.

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