dial procedure [the two methods agreed within 2.0% (average of eight sample composites)] in handling small samples, as is needed for individual tablet analysis or when the quantity of sample is too small for assay by the compendial procedure.

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NMR Spectroscopic Analysis of 2-Mercapto-5-methyl-1,3,4-thiadiazole in Cefazolin

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Abstract □ A rapid, sensitive, and accurate method for the quantitative analysis of 2-mercapto-5-methyl-1,3,4-thiadiazole in cefazolin is presented. The method utilizes NMR spectroscopy and is based on the difference in the chemical shift of the methyl protons on free thiadiazole and the thiadiazole moiety of cefazolin.

Keyphrases □ Cefazolin and cefazolin sodium—NMR analysis of free 2-mercapto-5-methyl-1,3,4-thiadiazole □ 2-Mercapto-5-methyl-1,3,4-thiadiazole—NMR analysis in cefazolin and cefazolin sodium □ NMR spectroscopy—analysis, 2-mercapto-5-methyl-1,3,4-thiadiazole in cefazolin and cefazolin sodium

Free thiadiazole may be found in cefazolin (sodium salt and free acid) as a decomposition product or as unreacted starting material. The contaminant may be determined by high-pressure liquid chromatography[1], but the procedure is tedious and time consuming. The NMR method presented in this paper is specific for free thiadiazole and provides a rapid, sensitive, and accurate quantitative analysis. The method takes advantage of the difference in the chemical shift of the methyl protons on free thiadiazole and the thiadiazole moiety of cefazolin; the NMR signals appear at 2.54 and 2.84 ppm, respectively (Fig. 1).

Quantitative analysis of pharmaceuticals and chemical mixtures by NMR spectroscopy has generally been accomplished with the use of an internal standard such as hexamethylcyclotrisiloxane (1, 2), succinic acid (3), fumaric acid (4), *tert*-butanol (5), and 2,3,5-tribromothiophene (6). However, for the quantitation of free thiadiazole in cefazolin, a calibration curve was derived from spectral data on known mixtures prepared from pure thiadiazole and pure cefazolin (sodium salt or free acid). Considerable time is saved, and potential weighing errors are

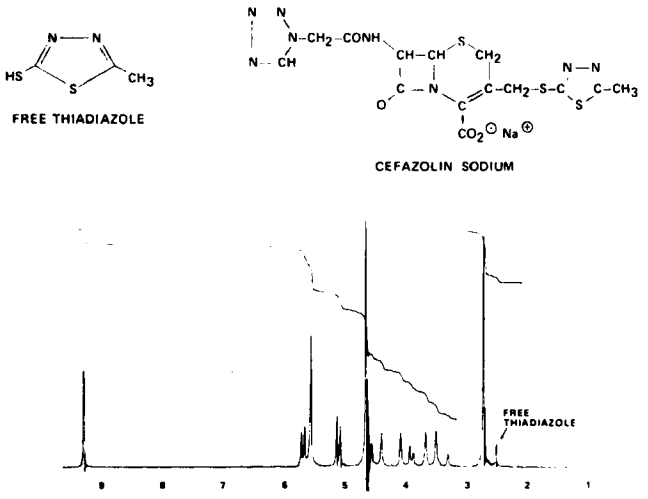

Figure 1—*NMR spectrum of cefazolin sodium containing free thiadiazole.*

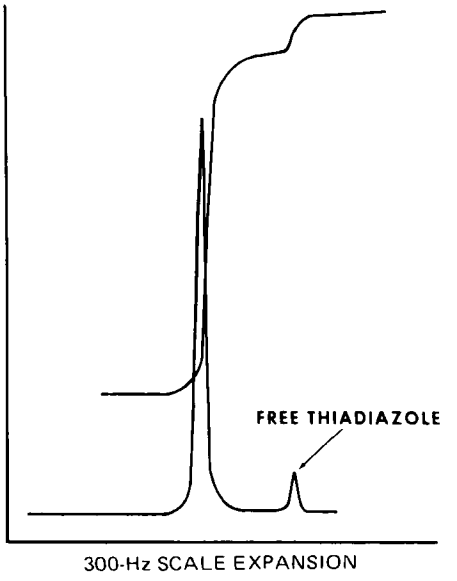

Figure 2—*Quantitative NMR spectrum of free thiadiazole in cefazolin sodium.*

[1] Unpublished data.

Table I—Standard Curve Preparation

Cefazolin Sodium			Cefazolin		
	I_{TD}[a]			I_{TD}[a]	
Thiadiazole, %	$I_{TD} + I_{CS}$	Precision[b]	Thiadiazole, %	$I_{TD} + I_C$	Precision[b]
0.20	Detectable	—	0.50	Detectable	—
0.79	0.039	8.1	1.54	0.039	7.7
1.99	0.083	6.7	2.73	0.075	6.3
3.24	0.148	5.6	7.20	0.190	4.2
4.67	0.201	4.9			

[a] I_{TD} = integration value of thiadiazole, I_C = integration value of cefazolin, and I_{CS} = integration value of cefazolin sodium. [b] Based on five integrations. Precision = (deviation × 100)/average ratio.

avoided by not using an internal standard, particularly when many cefazolin samples are involved.

EXPERIMENTAL

A 90-MHz NMR spectrometer[2] with 5-mm o.d. NMR tubes was used.

The necessary reagents included deuterium oxide[3] (99.7% D minimum isotopic purity) and trifluoroacetic acid[4].

Sample Preparation and NMR Conditions—Approximately 100 mg of cefazolin or cefazolin sodium is transferred to a small glass vial. For cefazolin, trifluoroacetic acid (0.5 ml) is added; for cefazolin sodium, deuterium oxide (0.5 ml) is added. The contents then are agitated to effect complete solution, and the solution is filtered through a cotton plug into an NMR tube.

The NMR spectrum is run on a 10-ppm sweep width and 10-min scan time for identification purposes (Fig. 1). For quantitative purposes, the spectrum is run at 300-Hz sweep width and 10-min scan time in the 135–300-Hz region (Fig. 2). This region is carefully integrated five times.

Preparation of Calibration Curve—Known mixtures, ranging from 0.5 to 8.0% free thiadiazole in cefazolin or cefazolin sodium, were prepared. The NMR spectra were obtained on the mixtures with integrations as already described. The integrations over the methyl resonances provided the data to construct the calibration curve (Fig. 3). Precision was determined for the lowest and the highest levels (Table I).

The ratio *(A)* of the thiadiazole integration to the sum of the thiadiazole and cefazolin or cefazolin sodium integrations was determined. The ratio *(A)* was plotted against the percent of free thiadiazole *(B)* in the mixture (Fig. 3). Sample quantitation followed by determining the ratio *(A)* and comparing it to the standard curve for free thiadiazole content.

Alternatively, a linear regression analysis can be applied to the data pairs to obtain the "best-fit" curve, $(A = mB + C)$, and its slope, m. Since $C = 0$, $A(1/m) = B$. Thus, the thiadiazole content is found by multiplying the factor $(1/m)$ by the ratio *(A)*.

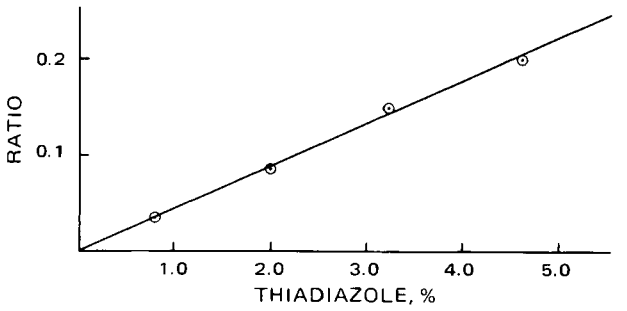

Figure 3—*Calibration curve showing percentages of thiadiazole in cefazolin sodium salt using deuterium oxide as the solvent.*

[2] Perkin-Elmer model R 32.
[3] Merck and Co.
[4] Matheson, Coleman and Bell.

Table II—NMR Analytical Data

Sample	Weight of Cefazolin Sodium, mg	Weight of Thiadiazole, mg	Thiadiazole in Mixture, %	Thiadiazole by NMR, %
1	48.321	1.213	2.45	2.31
2	48.672	1.682	3.34	3.26
3	48.607	2.088	4.12	4.11
4	47.592	2.128	4.28	4.23
5	49.952	1.048	2.05	2.16

RESULTS AND DISCUSSION

The calibration curves are linear for the range of thiadiazole concentrations studied, as indicated by the correlation coefficients of 0.9997 and 0.9952 for cefazolin and cefazolin sodium, respectively. These values were calculated by the method given by Snedecor and Cochran (7).

The sensitivity limit for the trifluoroacetic acid solutions of cefazolin is 0.5%; the sensitivity limit for the deuterium oxide solutions of cefazolin sodium is 0.2%. The sensitivity advantage obtained in deuterium oxide is due to the better resolution achieved with this solvent. Precision values at each concentration for the two solvents are about the same and show a drop in precision as lower concentrations of free thiadiazole are encountered. This finding reflects the limitation in measuring very small integral values. The reproducibility at the 1% level is 8.1%.

Although the NMR spectra of the trifluoroacetic acid solutions were obtained immediately after preparation, it was not of paramount importance to obtain them within this timespan. Studies showed that trifluoroacetic acid solutions of cefazolin are stable for at least 2 hr, and no discernable decomposition was observed from the integrations obtained from repetitive scans during this period.

Five mixtures of thiadiazole in cefazolin sodium were prepared and analyzed by the described NMR method (Table II).

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