Table III—Analysis of I in Dosage Forms (Manufactured Preparations)

Drug	Labeled Amount	Recovery[a], % Proposed Method, Non-aqueous Titration	Official Method	Colorimetry
I	—	100.1	97.3	99.1
I	300 mg./tablet	100.3	98.2	99.3

[a] Each figure represents the mean of at least five readings.

Manufactured Preparations—Twenty tablets were weighed and reduced to a fine powder. An accurately weighed quantity of powder, equivalent to 300 mg. of I was added to 30 ml. of acetic anhydride. The mixture was then gently heated (excipients settled). After cooling to room temperature, the titration was performed as described for the estimation of I in pure form, commencing with the words: "3–5 drops of Malachite green. . . ."

RESULTS AND DISCUSSION

The nature of the titration curve of I is shown in Fig. 1. In the preliminary trials, the estimation of this drug in acetic acid resulted in overestimation, probably due to the consumption of perchloric acid by certain excipients. However, on using acetic anhydride as the solvent with Malachite green as the indicator, the end-point was sharp and the recoveries were better than with acetic acid. The color change of the indicator was checked potentiometrically. Tables I and II show the recovery.

Table III shows the results obtained by the proposed method, by the official method (4), and by colorimetry (1). It is apparent from these data that good recoveries were achieved by nonaqueous titration. The official method is a time-consuming and cumbersome procedure with lower recoveries. In the colorimetric method, Beer's law is obeyed in a very narrow range (0.05–0.3 mg.) and the recovery of the drug is not satisfactory. The proposed nonaqueous method of titration is a rapid and simple technique, giving accurate results.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 12, 1973, from the *Department of Pharmacy, Birla Institute of Technology and Science, Pilani, Rajasthan, India.*

Accepted for publication May 24, 1973.

The authors thank Dr. A. K. Duttagupta, Faculty of Science, and Dr. B. M. Mithal, Department of Pharmacy, Birla Institute of Technology and Science, for providing the facilities for the present study.

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Assay of Thiotepa by PMR Spectrometry

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Abstract □ A rapid (10 min.), accurate, and precise (SD = 0.6%) analytical method for thiotepa and thiotepa for injection involving PMR is described. The procedure, which was tested on two spectrometer models, employs an inexpensive solvent and internal standard and does not require preparative treatment.

Keyphrases □ Thiotepa—PMR analysis, aziridinyl protons □ Aziridinyl derivatives—PMR analysis of thiotepa □ PMR spectroscopy—analysis, thiotepa

During the characterization of potential oncolytics, the methylene protons of aziridinyl moieties present in some of these agents were readily detected by proton magnetic resonance (PMR) spectrometry. This observation led to an interest in the quantitative analysis of an official drug by this instrumental method. Thiotepa [*N,N',N''*-triethylenethiophosphoramide, tris(1-aziridinyl)phosphine sulfide] meets the criteria of a good candidate for assay by PMR spectrometry because it is soluble in an inexpensive, nondeuterated solvent and gives a simple spectrum. In addition, the assay procedure presented here, in contrast to other PMR drug analysis (1, 2), does not require processing of the sample prior to instrumental examination; in view of this drug's chemical lability, this is especially advantageous. Only additions of an internal standard and solvent to the sample are required. Although thiotepa is a low dosage pharmaceutical, giving solutions of about 1.9% concentration in this assay, the integral values for the peak areas can be measured with sufficient accuracy and precision.

All of these factors provide for a simple, accurate, and rapid analytical method for thiotepa and this pharmaceutical's parenteral formulation. The USP XVIII procedures for these items involve lengthy and laborious titrimetric and IR spectrophotometric methods, respectively. To determine the capabilities of different

Table I—Analysis of Thiotepa Standards by PMR

Sample	Internal Standard, mg.	Thiotepa, Added, mg.	Thiotepa, Found, mg.	Thiotepa, Recovered, %
1	23.63	15.16	15.41	101.6
2	23.45	15.95	16.13	101.1
3	24.26	14.76	14.71	99.7
4	23.88	14.83	15.12	102.0
5	23.15	17.80	18.01	101.2
6	23.78	15.37	15.41	100.3
7	23.38	15.07	15.37	102.0
8	23.66	15.20	15.38	101.2
Mean and standard deviation			101.1 ± 0.6	

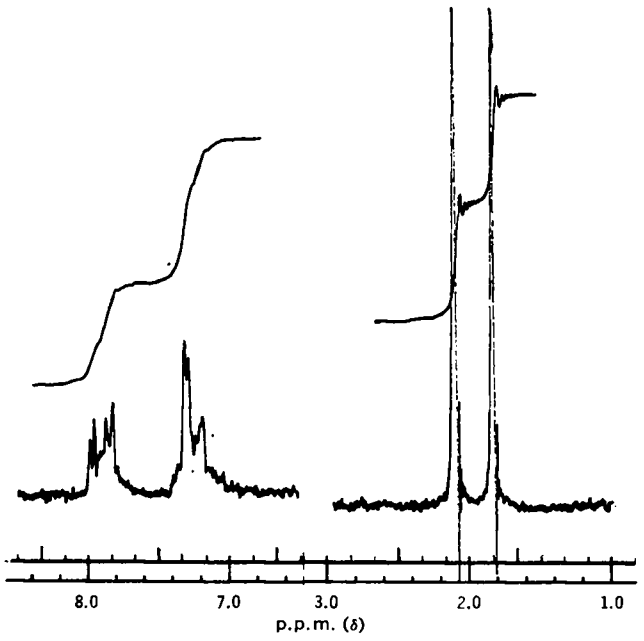

Figure 1—*PMR spectrum of benzoic acid (left) and thiotepa (right) in carbon tetrachloride.*

types of PMR spectrometers in this quantitative evaluation, two models (I[1] and II[2]) were employed in most assays.

EXPERIMENTAL

The following were used: standard, thiotepa[3]; internal standard, benzoic acid USP[4]; samples, thiotepa for injection USP[5]; and solvent, carbon tetrachloride[6].

To the standard or sample[7], add about 24.00 mg.[8] of internal standard, accurately weighed, and 0.8 ml. of solvent. Stopper and shake for 2 min. and then filter, *via* a cotton pledget, at least 0.4 ml. into a PMR tube. After placing the tube in the spectrometer, adjust the spin rate to eliminate spinning side bands and the power level and amplitude to obtain integral values corresponding to 20–30 mm. Integrate the peak areas occurring between δ 1.83–2.15 and δ 7.10–8.09 at least five times.

The percent of drug may be calculated using the following equation:

$$\text{percent thiotepa} = \frac{A_t}{A_b} \times \frac{EW_t}{EW_b} \times \frac{W_b}{W_t} \times 100 \quad \text{(Eq. 1)}$$

where:

A_t = average integral value for thiotepa
A_b = average integral value for benzoic acid
EW_t = molecular weight of thiotepa/12 = 15.77
EW_b = molecular weight of benzoic acid/5 = 24.42
W_b = weight in milligrams of benzoic acid
W_t = weight in milligrams of thiotepa added or declared

RESULTS AND DISCUSSION

The PMR spectrum of pure thiotepa shows only a doublet (δ 2.00, J = 16 Hz. using trimethylsilane as reference. Fig. 1). Benzoic acid was chosen as the internal standard since it is a pure, stable, inexpensive, readily obtainable material whose aromatic protons absorb at a point (complex of peaks at δ 7.10–8.09) where there is no interference with the absorption peaks caused by the drug (Fig. 1). The peak areas produced by 24 mg. of the internal standard are approximately the same as those resulting from 15 mg. of thiotepa.

The results of the quantitative determination of a series of thiotepa standards are shown in Table I. Considering the small sample weight, the procedure is unexpectedly accurate with a good precision of ±0.6%. In addition, it provides a means of identification and detection of impurities. Table II summarizes the analysis of commercial preparations. There is good agreement with declared values using both instruments, with Model II uniformly giving slightly higher values. Measurements shown in both tables are within the USP monograph's limits of 97.0–102.0 and 95.0–110.0% for thiotepa and thiotepa for injection, respectively.

Another comparison of measurements taken with Models I and II is indicated in Table III. The samples did not decompose at the same rate, but both instruments gave similar results.

Sample 9 was examined (II) after 2 weeks at room temperature to indicate the presence of 54.4% thiotepa. Neither the spectrum nor the integral peak area value of the internal standard in this sample showed change, but two broad areas of absorption resulting from materials of undetermined nature appeared at about δ 3.9–4.6 and δ 2.5–3.9, the latter interfering slightly with the baseline of thiotepa's integral values.

Table II—Assay of Thiotepa in Commercial Preparations by PMR

Sample[a]	Internal Standard, mg.	Percent of Declared		
		Model I	Model II	Difference
9	22.24	101.1	100.9	+0.8
10	24.73	102.1	104.4	+2.3
11	24.71	100.6	102.7	+2.1
12	24.26	100.4	100.6	+0.2
13	24.84	102.9	103.4	+0.5
14	23.35	100.9		
15	23.38	100.7		
Mean and average difference		101.2	102.3	+1.2

[a] Declared amount is 15 mg.

Table III—Determination of Decomposed Thiotepa Standards

Sample[a]	Percent Found		
	Model I	Model II	Difference
1	91.0	89.4	−0.6
2	87.9	87.8	−0.1
3	85.7	86.1	+0.4
4	88.5	87.3	+1.2
5	92.8	91.6	−0.8
7	91.6	91.9	+0.3
Mean and average difference	89.6	88.9	±0.6

[a] Kept at room temperature for 7 days.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 1, 1973, from the *College of Pharmacy, University of Houston, Houston, TX 77004*

Accepted for publication June 6, 1973.

Supported in part by Grant CA 13123-01 from the National Cancer Institute, Public Health Service, Bethesda, MD 20014, and by the Rbt. A. Welch Foundation, Houston, TX 77002

[1] Varian T-60.
[2] Varian EM 360.
[3] Provided through the courtesy of Lederle Laboratories, Pearl River, N. Y.
[4] Fisher Scientific Co., Fair Lawn, N. J.
[5] Thiotepa Parenteral, 15 mg./vial, Lederle Laboratories.
[6] Distillation Products Industries, Rochester, N. Y.
[7] Contains sodium bicarbonate USP (50 mg.) and sodium chloride USP (80 mg.).
[8] Weighed using a Mettler Macro Gramatic balance, B-5.