Simulated Gastric Fluid Treatment—Samples of erythromycin coated with potassium acetate phthalate were treated in simulated gastric fluid according to the directions outlined in USP XVIII (4). After gastric fluid treatment, the simulated gastric fluid was filtered off using a Millipore filter with 0.45-μ HA Millipore membrane. The insoluble material was then rinsed with 100 ml. of water. After rinsing, suction was maintained until the solids were dry. Samples thus treated were then extracted and silylated as outlined in the standard tablet procedure.

RESULTS AND DISCUSSION

Placebo tablets (containing all tablet ingredients except erythromycin) were assayed with and without internal standard or erythromycin to determine if any interfering components in the GLC chromatogram are extracted with methylene chloride. No interference was observed.

Placebo 250-mg. tablets were spiked with erythromycin at 80, 100, and 120% of manufacturing theory. The average recovery for this three-level spiked study was 100.6% (Table I).When erythromycin was spiked at label potency using six replicates of individually weighed and extracted samples, the average recovery was 99.8% with a coefficient of variation of 2.3% (Table II).

The precision of the method was determined by assaying six replicates of individually weighed and extracted samples of one lot each of regular production lots of erythromycin tablets containing 200 mg.[7] and 250 mg.[8] of erythromycin, respectively. The regular production 200-mg. tablets assayed at an average potency of 198 mg./tablet with a coefficient of variation of 2.19% (Table III). Microbiological assay of this lot was 209 mg./tablet. The 250-mg. tablet (manufacturing theory 262 mg./tablet) gave an average potency of 258 mg./tablet with a coefficient of variation of 1.25% (Table IV). Microbiological assay of this lot was 255 mg./tablet.

Thirteen regular production lots of erythromycin 250-mg. tablets were assayed by GC and microbiologically. The average GC calculated biopotency (250 mg./tablet) compared favorably with the microbiological assay value (254 mg./tablet).

The effectiveness of enteric coating erythromycin tablets with potassium acetate phthalate was tested by treating with simulated gastric fluid and then by GC of the methylene chloride-extracted residue. Uncoated erythromycin tablets showed degradation to anhydroerythromycin A (97%), the prime product of acid degradation, and a trace of erythralosamine (Fig. 1). Effectively coated erythromycin tablets showed no degradation (Fig. 2). Thus, the GLC method for erythromycin has been successfully used to assay enteric-coated erythromycin tablets. Although the present study was confined to enteric-coated erythromycin base tablets, the GLC method may be applied to monitor the stability of various erythromycin esters in simulated gastric fluid, since erythromycin esters and acid-degradation compounds of erythromycin can be separated and quantitated by the GLC method (3).

[7] Erythromycin 200 mg., Japan Upjohn Ltd.
[8] E-Mycin 250 mg., The Upjohn Co.

Table IV—Precision of GC Assay for Erythromycin 250-mg. Tablets[a]

Weight of Sample, g.	Area: Internal Standard	Area: Erythromycin A	Area: Erythromycin B	Area: Erythromycin C	GC Calculated Biopotency, mg./Tablet
0.89976	101.0	343.0	46.0	11.0	257
0.89944	144.0	503.0	54.0	11.0	261
0.89966	118.5	414.5	34.5	9.5	258
0.89954	113.5	390.5	32.0	5.0	253
0.89951	115.0	408.5	32.0	9.0	261
0.89954	141.0	489.5	38.0	8.5	255
		Average potency			$\overline{258}$ mg./tablet
		Coefficient of variation			1.25%

[a] E-Mycin 250 mg., The Upjohn Co.

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Analysis of Cacodylate Injections by NMR Spectroscopy

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Abstract □ An NMR procedure was developed for the assay of sodium cacodylate injection. The results obtained by this technique compare favorably with those obtained by the NF X method and atomic absorption spectrophotometry. The advantages of the NMR procedure over the other procedures are that it is highly specific for the cacodylate and that it is less time consuming.

Keyphrases □ Cacodylate injections—NMR analysis, compared to NF X and atomic absorption spectrophotometry methods □ Sodium cacodylate injections—NMR analysis, compared to NF X and atomic absorption spectrophotometry methods □ NMR spectroscopy—analysis, cacodylate injections

The position of arsenic-containing compounds in therapeutics has gradually been displaced as more specific drugs have been developed. One of the organic arsenic compounds that still enjoys some use is sodium cacodylate. This drug releases arsenic(III) in the body slowly and is formulated as a solution for injection. In

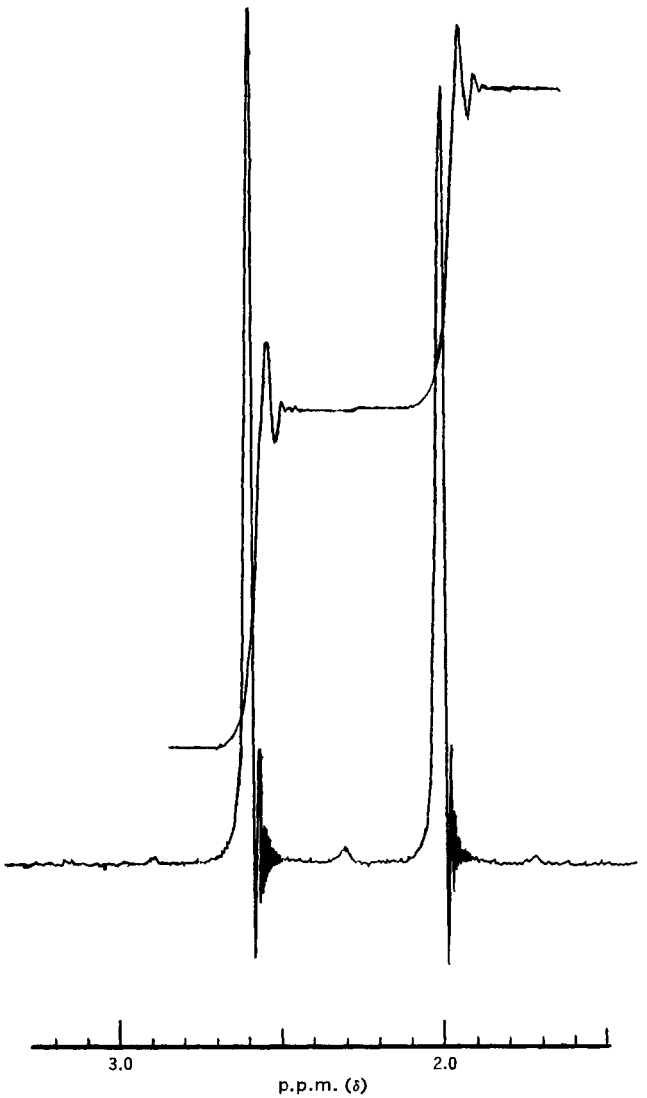

Figure 1—*NMR spectra of sodium cacodylate (2.0 p.p.m.) and succinic acid internal standard (2.6 p.p.m.).*

addition, the iron salt, represented stoichiometrically as $Fe[(CH_3)_2AsO_2]_3$, has been used where iron deficiency was present.

The usual approach to the analysis of the cacodylate has been to measure the arsenic content. Titration with standard iodine or potassium bromate (1, 2) after digestion with sulfuric acid is a well-established method. In this case, if the test object is ferric cacodylate, the arsenic must be separated from the iron(III), by distillation as arsenic trichloride, to obviate interference. A more modern element-measuring approach employs atomic absorption spectrophotometry, which can determine both elements once appropriate dilution is carried out. At any rate, both of these general schemes measure only the total quantity of the elements present in the solution and do not indicate the nature of the arsenic-containing compounds.

A much more specific quantitative analytical method, which is described here, uses proton nuclear magnetic resonance (NMR). A number of reviews and articles have been wholly (3–5) or partially (6–19) devoted to quantitative analysis by NMR. Besides the quantitation

Table I—Analysis of Synthetic Cacodylate Samples by NMR

Sample	Succinic Acid Added, mg./ml.	Ferric Chloride Added, mg./ml.	Sodium —Cacodylate— Added, mg./ml.	Found, mg./ml.	Percent Recovered
1	49.5	—	51.1	51.2	100.2
2	25.2[a]	—	23.8	23.6	99.2
3	15.5	—	13.0	12.9	99.2
4	45.9	25.4	48.5	47.5	97.9
5	33.9	14.1	26.3	26.1	99.2
6	20.9	11.1	20.9	20.3	97.1
7	12.3	10.5	14.1	14.5	102.8
Mean and standard deviation			99.4 ± 1.8		

[a] This sample was subsequently analyzed for a total of 10 times, yielding a mean of 99.2 and a standard deviation of 1.2%.

of the cacodylate compound discussed in this paper, other pharmaceuticals in various dosage forms that have been quantitated previously are aspirin–phenacetin–caffeine (20, 21), aspirin (22), chlorophenesin carbamate (23), barbiturates (24), methylxanthines (25), mestranol (26), meprobamate (27), dimethyl sulfoxide (28), chloral hydrate (29), pentylenetetrazol (30), and aminophylline (31). With the cacodylate, the proton magnetic resonance exhibited by both of the methyl groups bonded to the arsenic is measured quantitatively by comparison to an internal standard. Identification is also established from the field position of the methyl singlet.

EXPERIMENTAL

Apparatus—NMR spectra were recorded with a spectrometer[1] (ambient probe temperature of 41°) using a sweep of 500 sec. and a sweep width of 500 Hz.; the delta scale (δ) was used throughout. Atomic absorption spectrophotometry measurements were made on a spectrometer[2] according to the manufacturer's instructions. The flame used was air–acetylene, and the source for both elements was the appropriate hollow cathode lamp; the wavelengths at which absorptions were measured were 193.7 and 248.3 nm., resonance lines of arsenic and iron, respectively.

Chemicals and Solutions—Succinic acid, reagent grade, internal standard, was used. The stock standard solutions (32) used were: (*a*) arsenic, 1000 mcg./ml. from primary standard arsenic trioxide; and (*b*) iron, 1000 mcg./ml. from pure iron wire.

The ferric cacodylate is prepared by addition of the desired quantity of iron(III) in solution to a solution of sodium cacodylate.

Procedures—*NMR*—Pipet a volume of injection equivalent to about 100 mg. of sodium cacodylate into a 10-ml. beaker. Weigh accurately about 83 mg. of succinic acid internal standard and add to the sample solution. Concentrate, if necessary, by carefully evaporating the aqueous solution on a steam bath to not less than 1 ml. Further concentration may result in precipitation of succinic acid. Precipitate any ferric ion if present by dropwise addition of potassium hydroxide solution until basic. Pour the mixture into a small tube and centrifuge. Transfer approximately 0.4 ml. of the clear supernate to an NMR tube. Obtain the NMR spectrum; adjust the spin rate so that no spinning sidebands appear in the region of interest. Integrate each peak five times and use the average integral value for quantitation.

Calculate the amount of sodium cacodylate in the volume of injection taken as follows:

$$\text{mg. } Na(CH_3)_2AsO_2 \cdot 3H_2O = \frac{A_v}{A_s} \times \frac{EW_v}{EW_s} \times \text{mg.}_s \quad \text{(Eq. 1)}$$

where:

A_v = integral value of sodium cacodylate
A_s = integral value of succinic acid

[1] Varian A-60.
[2] Perkin-Elmer, model 303.

Table II—Comparison of Results of Synthetic Cacodylate Samples by NF X, NMR and Atomic Absorption Spectrophotometry (AAS) Procedures

Sample	Quantity Added, mg./ml.		Sodium Cacodylate Recovered						Iron	
			NF X		NMR		AAS		Recovered, AAS	
	Sodium Cacodylate	Iron	mg./ml.	%	mg./ml.	%	mg./ml.	%	mg./ml.	%
A	41.9	—	42.1	100.5	40.9	97.6	41.8	99.8	—	—
B	20.9	—	20.8	99.5	20.3	97.1	21.6	103.3	—	—
C	10.5	—	10.6	101.0	10.7	101.9	11.5	109.5	—	—
D	42.8	5.1	—	—	44.0	102.8	43.7	102.1	5.0	98
E	21.4	2.6	—	—	21.1	98.6	22.0	102.8	2.6	100
F	10.7	1.3	—	—	10.6	99.1	10.4	97.2	1.3	100
Mean and standard deviation			100.3 ± 0.8		99.5 ± 2.3		102.5 ± 4.1		99.3 ± 1.2	

EW_v = formula weight of sodium cacodylate/6 = 35.67
EW_s = formula weight of succinic acid/4 = 29.52
$mg._s$ = weight in milligrams of succinic acid

Atomic Absorption Spectrophotometry—Dilute an appropriate aliquot of the sample to within the optimum concentration range for the element to be measured. For arsenic, this is approximately 100 mcg./ml.; for iron, it is approximately 10 mcg./ml. Prepare working standards by diluting the stock standard solution to obtain at least four different concentrations of the element so as to bracket the concentration in the sample solution. Measure the percent absorption, convert to absorbance, and prepare standard calibration curves of absorbance *versus* concentration. From an appropriate calibration curve, obtain the concentration of the element in the sample and multiply by the dilution factor. Convert the quantity of arsenic to sodium cacodylate, $Na(CH_3)_2AsO_2 \cdot 3H_2O$, by multiplying by 2.857.

RESULTS AND DISCUSSION

The observed NMR spectrum for the analytical system studied here is seen in Fig. 1. Both the cacodylate and succinic acid give rise to singlets at 2.0 and 2.6 p.p.m., respectively, *versus* Tier's salt. The extreme simplicity of the spectrum presents no difficulties when integration is carried out. Since the cacodylate exhibits no characteristic multiplicity of peaks in its spectrum, it can best be identified by adding authentic sodium cacodylate standard and observing an increase in peak height.

Some comment is warranted about possible interferences. Water, the solvent in which the sodium cacodylate is dissolved in the injection dosage form, is a satisfactory solvent in this case because the water protons have no resonance in the region of analytical interest but rather exhibit a peak downfield. As discussed in the *Procedure* section, ferric ion must be removed since the paramagnetic species can cause undesirable peak broadening.

Table I reports the results of the NMR analysis of known synthetic cacodylate samples. The maximum absolute difference between quantities added and found is 1.0 mg. As reported in the table, the mean and standard deviation indicate that the method is accurate and reasonably precise over an almost fourfold concentration range. The aggregate standard deviation is close to the value compiled when an individual solution was analyzed 10 separate times.

The data in Table II describe the results of the three kinds of analytical measurements. When the values for the three samples assayed for arsenic by the NF X, NMR, and atomic absorption spectrophotometry procedures are compared in terms of the mean and standard deviation, it is noted that the compendium method yields the most precise values with good agreement with the amounts of drug added. Although the NMR results are less precise with a lower mean value, they are probably more accurate since they arise from a measure of the methyl group response and do not respond to any free arsenic which might be in the solution. The higher deviation of the NMR procedures is ascribable to the uncertainties in the integration measurements. The atomic absorption spectrophotometry analyses, which show the highest mean and standard deviation, involve two factors that may be cited to rationalize these results. First, atomic absorption spectrophotometry measures total arsenic and includes any inorganic arsenic present. Second, it is well known that arsenic is one of the more difficult elements to measure by atomic absorption spectrophotometry, mainly because its resonance lines lie in the short wavelength region of the UV spectrum. In this region, the atmosphere as well as the combustion products of an air–acetylene flame absorbs strongly. In this study, additional difficulty was encountered due to the arsenic hollow cathode lamp used. The light output of this particular lamp was weak, requiring high gain setting on the photomultiplier tube and thereby resulting in a very noisy absorption signal.

Table III—Analysis of Commercial Cacodylate

Sample	Sodium Cacodylate Declared	Sodium Cacodylate Found	Percent of Declared
1	74 mg./2 ml.	72.1 mg./2 ml.	97.4
2	74 mg./2 ml.	74.0 mg./2 ml.	100.0
3	74 mg./5 ml.	78.0 mg./5 ml.	105.4
	Ferric Cacodylate		
4	10 mg./ml.	9.34 mg./ml.	93.4

The results in Table III show the NMR analysis of some commercial cacodylate samples. Both of the more concentrated samples are within compendial purity rubric limits, whereas the more dilute solutions are variable. In addition, the uncertainty exhibited by the concentrated sample results is similar to that established for the synthetic mixtures.

CONCLUSION

The NMR procedure for cacodylate determination in injections yields results somewhat lower than those obtained by the NF X and atomic absorption spectrophotometry methods and is not as precise as the NF X titration. However, since the NMR procedure is based on measurement of the methyl proton resonance, it offers a desirably high degree of specificity for the cacodylate in addition to being rapid and convenient. The other techniques in which the arsenic content is measured are not specific for the cacodylate.

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TECHNICAL ARTICLES

Variation in Dissolution Data Using an Apparatus Meeting USP–NF Requirements

A. M. ROSOLIA, J. R. O'CONNELL, J. F. BAVITZ, F. A. RESTAINO, and J. B. SCHWARTZ▲

Abstract □ Variation in dissolution results between the two vessels recommended in USP XVIII and NF XIII prompted an investigation of vessel shape and its effect on dissolution. Further testing was carried out with a dissolution vessel whose concavity was obviously different from those recommended but still within compendial specifications. The dissolution results obtained in this modified apparatus were different from those obtained in the recommended vessels under the particular conditions of the test used. (The difference in results between the two recommended vessels was subsequently shown not to be statistically significant, although the modified vessel did cause changes.) A possible explanation for these observations is presented.

Keyphrases □ Dissolution tests—effect of vessel shape (concavity), compendial dissolution vessels, modified vessel □ Vessels for dissolution testing—effect of shape (concavity) on dissolution rates, compendial vessels, modified vessel □ Glass dissolution vessels—effect of shape (concavity) on dissolution rates, compendial vessels, modified vessel

Eight monographs carry a dissolution test requirement in USP XVIII (1) and six do so in NF XIII (2). Cooper and Hersey (3) presented a tabulation of these preparations and their specifications. Recently, Beyer and Smith (4) reported on an unexpected variable (vibration) in the USP–NF test. The purpose of this article is to report on an additional source of variation possible in the official test methods.

The compendia in their latest revisions include specifications for the rotating-basket method of dissolution testing. USP XVIII states that the vessel, which is one of four parts of this apparatus, must meet the following requirements: ". . . a covered, 1000 ml. vessel made of glass or other inert, transparent material; . . .. The vessel is cylindrical, with a slightly con-

Table I—Effect of Vessel Type on Hydrochlorothiazide Dissolution

Sample	Percent Hydrochlorothiazide —Dissolved in 30 min.[a]— Kimble	Pyrex
1	85	80
2	88	80
3	88	80
4	95	84
5	—[b]	81
6	—	79
Mean	89	81

[a] The USP XVIII monograph for hydrochlorothiazide tablets contains a specification of not less than 60% of labeled amount dissolved in 30 min. [b] Only four Kimble vessels were available for this initial study.