NMR Determination of Trimethoprim and Sulfamethoxazole in Tablets and Powders

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Abstract □ An NMR spectroscopic method for the determination of mixtures of trimethoprim and sulfamethoxazole is described. Spectra are determined in dimethyl sulfoxide-d_6, containing 1,4-dinitrobenzene as an internal standard. Both synthetic mixtures and commercial formulations were assayed, and the results were compared to those obtained using the BP procedure.

Keyphrases □ Trimethoprim—NMR spectroscopic analysis, synthetic mixtures and commercial formulations containing sulfamethoxazole □ Sulfamethoxazole—NMR spectroscopic analysis, synthetic mixtures and commercial formulations containing trimethoprim □ NMR spectroscopy—analysis, trimethoprim and sulfamethoxazole, synthetic mixtures and commercial formulations □ Antibacterial agents—trimethoprim and sulfamethoxazole, NMR spectroscopic analysis, synthetic mixtures and commercial formulations

The chemotherapeutic activity of trimethoprim, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (I), resulting from its ability to inhibit dihydrofolate reductase is increased as much as 30–60-fold when combined with a sulfonamide (1). Owing to its synergism and pharmacokinetic similarity, the choice association is sulfamethoxazole (II) in a one to five relation (2).

Several methods are available to analyze I and its metabolites in biological fluids. One method requires oxidation and spectrophotofluorometric measuring of the trimethoxybenzoic acid formed (3, 4). The other procedure uses differential pulse polarography (5, 6) or TLC, followed by fluorescence determination (7).

Only a compendial procedure is known for the analysis of the mixture. The BP (8) describes an extraction procedure in an alkaline medium for I, followed by back-extraction with diluted acetic acid and spectrophotometric determination. For II, a simple Bratton–Marshall reaction using the aqueous solution is described.

The procedure for the analysis of mixtures of I and II proposed here uses ^{1}H-NMR spectroscopy. No isolation is required, and good quantitative results were obtained with known mixtures and commercial preparations (tablets and powders).

EXPERIMENTAL[1]

Materials—Standard trimethoprim (I) and sulfamethoxazole (II) were prepared by recrystallization from ethanol–water. 1,4-Dinitrobenzene[2] (III) was used as an internal standard after purification by sublimation, mp 173–174°, and dimethyl sulfoxide-d_6[2] (IV) was used as a solvent. Tetramethylsilane was the internal standard.

Samples—Tablets from two batches of each of the four brands (obtained locally) and two batches of the only pediatric powder available were used.

Procedure—Twenty tablets were weighed and powdered. A sample equivalent to 10 mg of I, 50 mg of II, and 30.0 mg of III was dissolved in 1 ml of IV in a 2-ml centrifuge tube. The tube was placed into a bath (70°)

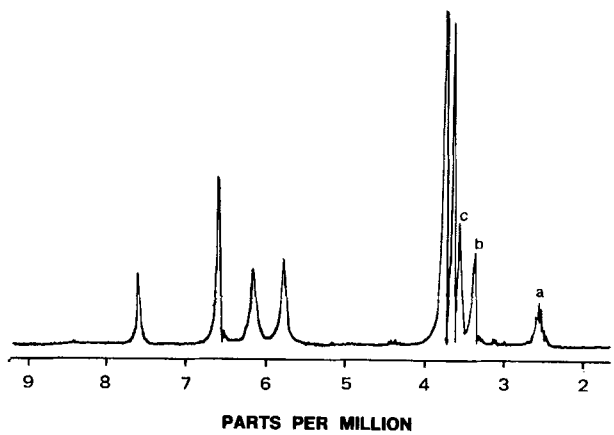


Figure 1—*NMR spectrum of trimethoprim in dimethyl sulfoxide-*d_6. *Key: a, solvent impurity; b, aromatic protons; and c, 4-methoxy group.*

for 2 min and centrifuged. About 0.4 ml of the solution was transferred to an NMR tube, and the spectrum was obtained.

The same procedure was used for the powdered tablets.

The amount of I was calculated as follows:

$$\frac{\text{mg of I}}{\text{tablets}} = \frac{EW_I}{EW_{III}} \times \frac{A_I}{A_{III}} \times \frac{\text{mg of III}}{W_s} \times \text{a.t.w.} \qquad \text{(Eq. 1)}$$

$$\frac{\text{mg of I}}{\text{100 g of powder}} = \frac{EW_I}{EW_{III}} \times \frac{A_I}{A_{III}} \times \frac{\text{mg of III}}{W_s} \times 100 \qquad \text{(Eq. 2)}$$

where EW_I is the molecular weight divided by the number of protons in the signal chosen, EW_{III} is the molecular weight of the internal standard divided by the number of protons in its signal, A_I is the integral value of the signal representing I, A_{III} is the integral value of the signal representing III, W_s is the weight of the sample in milligrams, and a.t.w. is the average tablet weight.

The amount of II was calculated by a similar procedure.

RESULTS AND DISCUSSION

The selection of a solvent and an internal standard is important in any NMR procedure. In this case, since the drugs, I and II, and the internal standard, III, are freely soluble in dimethyl sulfoxide, it is the solvent of

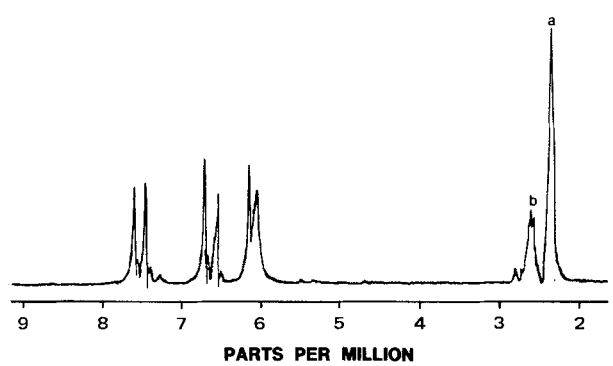


Figure 2—*NMR spectrum of sulfamethoxazole in dimethyl sulfoxide-*d_6. *Key: a, C-CH_3 group; and b, solvent impurity.*

[1] A Perkin-Elmer R-12 NMR spectrometer, 60 MHz, was used. All spectra were scanned at a probe temperature of 35°.

[2] Merck A. G., Darmstadt, Germany.

Table I—Determination of Sulfamethoxazole and Trimethoprim in Standard Mixtures by NMR

Standard Mixture	Internal Standard, mg	Sulfamethoxazole			Trimethoprim		
		Added, mg	Found, mg	Recovery, %	Added, mg	Found, mg	Recovery, %
1	30.0	50.0	50.7	101.4	10.0	10.1	100.0
2	50.0	50.0	50.0	100.0	10.0	9.87	98.7
3	30.0	60.0	60.2	100.3	12.0	12.10	100.8
4	30.0	50.0	49.5	99.0	10.0	9.95	99.5
5	20.0	50.0	49.6	99.2	10.0	9.91	99.1
6	50.0	50.0	50.0	100.0	10.0	10.10	101.0
7	30.0	50.0	50.4	100.8	10.0	10.00	100.0
8	30.0	40.0	40.4	101.0	8.0	8.08	101.0
			Average = 100.2 *SD* = 0.84			Average = 100.0 *SD* = 0.87	

Table II—Analysis of Dosage Forms by NMR and BP Methods

Sample	Sulfamethoxazole					Trimethoprim				
	Declared Dosage, mg/Unit	NMR		BP		Declared Dosage, mg/Unit	NMR		BP	
		mg/Unit	%	mg/Unit	%		mg/Unit	%	mg/Unit	%
A, Tablet	400.0	405.2	101.3	394.0	98.5	80.0	79.9	99.9	78.0	97.5
B, Tablet	400.0	395.2	98.8	388.0	97.0	80.0	80.8	101.0	79.4	99.2
C, Tablet	400.0	397.2	99.3	385.2	96.3	80.0	79.7	99.6	81.4	101.7
D, Tablet	400.0	396.0	99.0	404.8	101.2	80.0	79.8	99.8	80.0	100.0
E, Tablet	400.0	394.8	98.7	396.0	99.0	80.0	79.2	99.0	78.4	98.0
F, Tablet	400.0	396.8	99.2	393.2	98.3	80.0	80.2	100.2	77.2	96.5
G, Tablet	800.0	796.0	99.5	776.0	97.0	160.0	158.5	99.1	157.1	98.2
H, Tablet	800.0	808.0	101.0	813.6	101.7	160.0	157.6	98.5	155.2	97.0
I, Powder (mg/100 g)	4000.0	4020.0	100.5	3920.0	98.0	800.0	800.0	100.0	792.0	99.0
J, Powder (mg/100 g)	4000.0	3968.0	99.2	4016.0	100.4	800.0	792.0	99.0	808.0	101.0

choice. The use of IV offers the obvious advantage of adding no proton signals to the spectrum; its impurity appears at 2.5 ppm and it does not interfere with the signals chosen. Compound III provides a single signal at 8 ppm; it was used previously as an internal standard (9).

The signals chosen for the analysis were the singlet at 3.40 ppm due to the aromatic protons of I together with the singlet at 3.55 ppm due to the 4-methoxy group of I (Fig. 1), the singlet at 2.3 ppm due to the only C-CH_3 of II (Fig. 2), and the single signal given by III at 8 ppm.

The analysis of a group of known standard I and II mixtures by NMR is summarized in Table I. The method is both accurate and precise, with a mean (standard deviation) of 100.0 ± 0.87% for I and 100.2 ± 0.84% for II. The relative proportions of I and II to III (Table I) have no significant bearing on the accuracy of the determination for the ranges of proportions shown.

By applying this procedure to actual samples, eight commercial lots of tablets from four companies were studied (Fig. 3) together with two lots of the only pediatric powder available (Table II). One product (Samples E and F) also contained 50 mg of benzydamine hydrochloride/tablet. Benzydamine does not interfere in the method; with IV as a solvent, its peaks do not appear in the chosen region of the spectra.

The results are in good agreement with the declared dosages and with results obtained using the official BP (8) procedure (Table II). The NMR procedure has several distinct advantages over the official method.

NMR spectra of such binders as starch, lactose, methylcellulose, and povidone in dimethyl sulfoxide were obtained and examined. They did not interfere with the NMR analysis of I or II.

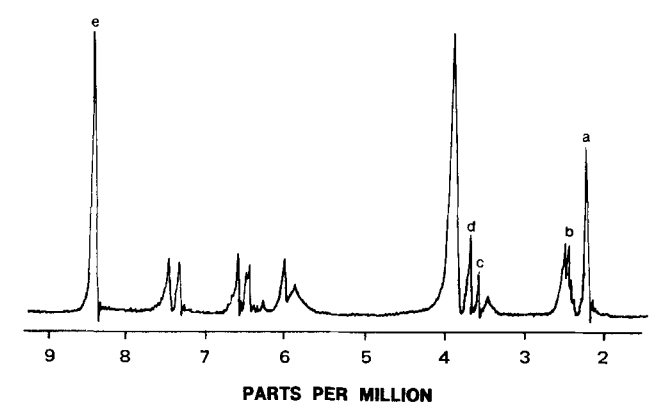
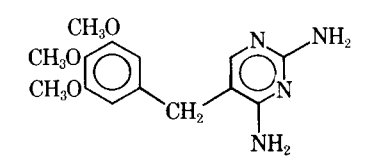
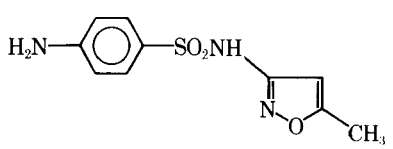


Figure 3—*NMR spectrum of a typical tablet analysis in dimethyl sulfoxide-d_6. Key: a, C-CH_3 group of II; b, solvent impurity; c, aromatic protons of I; d, 4-methoxy group of I; and e, single signal of III.*



I



II

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Analysis of Homatropine Methylbromide Dosage Forms

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Abstract □ A stability-indicating method of analysis for homatropine methylbromide in pharmaceutical formulations was developed. This method is based on the formation of a picric acid–quaternary ammonium complex, which is adsorbed on acid-washed diatomaceous earth in alkaline media followed by on-column chloroform extraction. The picrate complex is measured spectrophotometrically at 365 nm. The method was selective for homatropine methylbromide in that there was no interference from its major hydrolytic decomposition products, tropinium methylbromide and mandelic acid.

Keyphrases □ Homatropine methylbromide—spectrophotometric analysis, pharmaceutical formulations □ Spectrophotometry—analysis, homatropine methylbromide in pharmaceutical formulations □ Anticholinergic agents—homatropine methylbromide, spectrophotometric analysis in pharmaceutical formulations

Previously reported assay methods employed in the analysis of homatropine methylbromide dosage forms involved the precipitation of an insoluble salt of the methylhomatropine cation by anions such as the reineckate, silicotungstate, and tetraiodomercurate, followed by colorimetry (1) or turbidimetry (2, 3). Other colorimetric procedures involved chromogenesis with Dragendorff's reagent (4, 5) or measurement of the ferric hydroxamate complex (6). Analytical methods utilizing spectrophotometry (7, 8), TLC (9), and GLC (10, 11) also were reported. As previously discussed (12), these methods and official procedures (13) lack sensitivity, selectivity, or specificity or require additional washing or separation steps.

The stability-indicating assay procedure for homatropine methylbromide in syrups (12) is time consuming. This report presents a simple stability-indicating method, a modification of the method of Chin and Lach (14), for the assay of homatropine methylbromide dosage forms. The procedure was applied, with satisfactory results, to several commercial tablets, drops, and elixirs.

EXPERIMENTAL

Instruments—The recording spectrophotometer[1] had 1-cm cells and glass tubing columns[2], 200 × 25-mm o.d. with a tip 50 × 8-mm o.d. The columns were fitted with a small wad of glass wool at the bottom and top of the packing.

Materials and Reagents—The following were used: acid-washed diatomaceous earth[3]; homatropine methylbromide (NF reference standard); ammonium picrate reagent prepared by dissolving 11.2 g of picric acid[4] in 900 ml of distilled water, adding 6 ml of concentrated ammonia[4], and diluting to 1000.0 ml with distilled water; mandelic acid[4]; tropinium methylbromide[5]; and chloroform[4].

Standard Curve—A stock solution of homatropine methylbromide was prepared by dissolving 50.0 mg in 100 ml of distilled water. Further dilutions were made to obtain homatropine methylbromide standard solutions containing 0.125, 0.25, and 0.375 mg/ml. Two milliliters of stock and diluted standard solutions were each transferred into a 100-ml beaker containing 3.0 g of acid-washed diatomaceous earth, and 1.0 ml of ammonium picrate reagent was added. The mixture was mixed thoroughly and quantitatively transferred and packed into the glass column.

Elution was accomplished by immediately passing 17 × 5-ml portions of water-saturated chloroform at a flow rate of 7–9 ml/min, allowing each portion of eluant to run completely into the column before adding the next. The combined eluates were collected separately in 100-ml volumetric flasks and diluted to volume with chloroform. The absorbance of the standard solutions was measured in 1-cm absorption cells at 365 nm against chloroform as a blank.

Sample Procedure—*Tablets*—A number of tablets, equivalent to about 50 mg of homatropine methylbromide, were transferred to a 100-ml volumetric flask, and 75 ml of distilled water was added. The flask was shaken well for 30 min, and distilled water was added to the mark. The mixture was filtered through fluted filter paper, and the first 10 ml of filtrate was rejected. Two milliliters of the filtrate was pipetted into a 100-ml beaker, and the test was conducted as described under *Standard Curve*, beginning with "containing 3.0 g of acid-washed diatomaceous earth, ..."

Drops or Elixir—A volume of drops or elixir, equivalent to about 50 mg of homatropine methylbromide, was accurately measured and transferred to a 100-ml volumetric flask. Distilled water was added to the mark, and the contents were mixed well. Two milliliters was pipetted into a 100-ml beaker, and the test was conducted as described under *Standard Curve*, beginning with "containing 3.0 g of acid-washed diatomaceous earth, ..."

RESULTS AND DISCUSSION

This method, a modification of the one of Chin and Lach (14), is based on the reaction of picric acid with quaternary ammonium compounds in an alkaline medium to form a colored complex. The complex is adsorbed on acid-washed diatomaceous earth and measured spectrophotometrically after on-column extraction with water-saturated chloroform.

Under the experimental conditions, a linear relationship existed between the absorbance and concentration of homatropine methylbromide over the 0.25–1.0-mg/ml concentration range, with a correlation coefficient of 0.9996. Regression analysis showed that the regression equation was $y = 0.421x - 0.003$, with a standard error of the estimate of y on x

[1] Coleman-Hitachi model EPS-3T.
[2] SGA Scientific Inc., Bloomfield, N.J.
[3] Celite 545, Johns-Manville, Denver, Colo.
[4] Analytical grade, J. T. Baker.
[5] Endo Laboratories, Inc., Garden City, N.Y.