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**The Determination of Pyrimethamine by
Nuclear Magnetic Resonance Spectroscopy**

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A procedure is described for the quantitative analysis of pyrimethamine by NMR spectrometry using caffeine as an internal standard.

The drug pyrimethamine (2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine) is widely used as a suppressant of malaria. It can be assayed in pharmaceutical preparations either gravimetrically¹ by precipitation as the phosphotungstate, spectrophotometrically¹ from its ultra-violet absorption at 272.5 nm in 0.1 N HCl, or by titration in a non-aqueous medium.² A gas-chromatographic method has been developed for its determination in tissues.³ The British Pharmacopoeia 1973⁴ describes the method of non-aqueous titration with perchloric acid, using quinaldine red as indicator. The pharmacopoeial procedure for the assay of Pyrimethamine Tablets involves the non-aqueous titration of the residue obtained after evaporating an acetone extract of the powdered tablets. An NMR procedure is described here which is specific both for the assay and the identification of pyrimethamine as the powder or in tablets.

Apparatus

Perkin-Elmer R-12 (60 MHz) NMR spectrometer or equivalent instrument.

Reagents

1. *Pyrimethamine B.P.*
2. *Pure anhydrous caffeine*, M.Pt. 237°C.: Caffeine may be purified if necessary by crystallisation from ethanol to give the anhydrous form, or from hot water to give the monohydrate from which the anhydrous form is obtained by drying at 80°C.⁵
3. *Trifluoroacetic acid.*
4. *Tetramethylsilane.*

Method

Weigh accurately into a small sample tube or centrifuge tube a portion of powder or of powdered tablets equivalent to about 200 mg of pyrimethamine.

Add about 78 mg, accurately weighed, of pure anhydrous caffeine, followed by about 2 ml of trifluoroacetic acid. Warm the tube gently on a water bath for about 30 seconds, and centrifuge if necessary to separate the solution from insoluble tablet excipients. If a clear supernatant solution cannot be obtained in this way, extract an accurately weighed quantity of the powdered tablets equivalent to about 200 mg of pyrimethamine with successive quantities of acetone, according to the method described in the B.P. 1973 under Pyrimethamine Tablets. Use the residue obtained on evaporation of the combined acetone extracts to prepare a solution in trifluoroacetic acid containing anhydrous caffeine as standard, as described above.

Transfer about 0.5 ml of the clear solution obtained to an NMR tube, add about 1 per cent. of tetramethylsilane, and record the NMR spectrum, adjusting the instrument gain, Rf field and sweep rate to obtain a convenient integral presentation, at the same time ensuring that the conditions chosen do not produce saturation effects which might affect relative peak areas. Paulsen and Cooke⁶ have described the selection of a non-saturating mode of operation from measurements of the ratio of the areas of sample and internal standard peaks at progressively increasing Rf levels. The optimum level is the one just below that at which a change in the value of the ratio is observed. Run the integrals of the peaks of interest at least twelve times, and determine the mean value of the integral height. Measure peak positions with reference to the tetramethylsilane signal. Calculate the content of pyrimethamine, $C_{12}H_{13}ClN_4$, as follows:

Content of pyrimethamine per g of sample =

$$\frac{I_{\text{Sample}}}{I_{\text{Caf}}} \times \frac{\text{g of anhydrous caffeine}}{\text{g of sample}} \times \frac{E_{\text{Pyr}}}{E_{\text{Caf}}}$$

I_{Sample} = integral value of sample peak (equivalent to 3 methyl protons of the ethyl group) centred near $\tau 8.74$ (triplet).

I_{Caf} = total integral value of two caffeine peaks (equivalent to 6 methyl protons) near $\tau 6.21$ and 6.42 (singlets).

E_{Pyr} = weight of pyrimethamine (M.W. 248.7) equivalent to 1 proton = $248.7/3 = 82.9$ for the sample integral.

E_{Caf} = weight of caffeine (M.W. 194.2) equivalent to 1 proton = $194.2/6 = 32.37$ for the caffeine integral.

Results and Discussion

The NMR spectrum (Fig. 1) of pyrimethamine in trifluoroacetic acid showed two doublets centred at $\tau 2.38$ and $\tau 2.72$ which are due to the four aromatic protons of the benzene ring, together with a quartet centred at $\tau 7.40$ and a triplet centred at $\tau 8.74$ representing respectively the CH_2 and CH_3 moieties of the ethyl group attached to the pyrimidine ring. Caffeine gave rise to a singlet at $\tau 1.12$ produced by its single iminazole ring proton, and another three sharp singlets at τ values of about 5.67, 6.21 and 6.42, due to the three CH_3

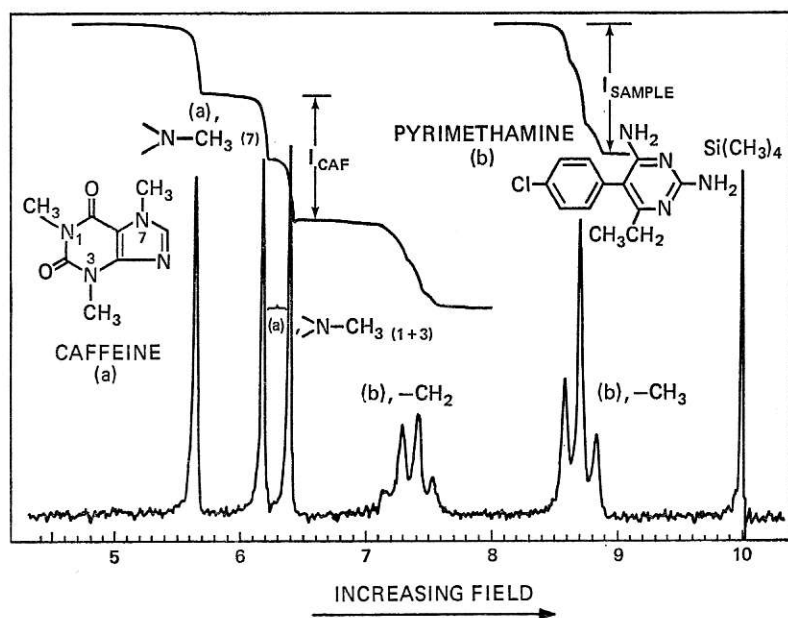


FIG. 1. 60-MHz NMR spectrum of a mixture of pyrimethamine (0.2041 g) and caffeine (0.0770 g). Peak positions are measured on the abscissa with reference to the $\text{Si}(\text{CH}_3)_4$ peak set at a tau (τ) value of 10,

where $\tau = 10 - \delta$

and $\delta = \frac{\text{distance of peak from } \text{Si}(\text{CH}_3)_4 \text{ signal (Hz)} \times 10^6}{\text{operating frequency (Hz)}}$

groups of the molecule. There was thus no overlapping of peaks in the combined spectrum of the two compounds. Trifluoroacetic acid was chosen as solvent, since pyrimethamine is insoluble in water, and its solubility in chloroform (1 in 125 parts)¹ is insufficient to be of use. A 220 MHz spectrum obtained for an accurately prepared mixture of pure caffeine with the pyrimethamine sample showed very minor impurity peaks between the two caffeine peaks at τ 5.67 and 6.21. In order to eliminate these, all assay results were calculated from the combined integral value of the two peaks near τ 6.21 and 6.42. Prepared mixtures of pyrimethamine and caffeine were analysed by the procedure described (using the 60 MHz spectrometer), as well as a commercial pyrimethamine tablet preparation, which required a preliminary extraction with acetone. The results are shown in Table I.

By this method, the mean result of 18 assays of the pyrimethamine sample was 99.7 per cent., with an average deviation from this mean of ± 1.5 per cent. The alteration of the ratio of caffeine to pyrimethamine in some of the assays did not appear to have any significant effect on the results obtained. The standard deviation for the assay was 1.96 per cent. The manual measurement of peak areas in this assay is precluded by the difficulty of measuring

TABLE I
THE DETERMINATION OF PYRIMETHAMINE IN PREPARED MIXTURES
AND IN A COMMERCIAL TABLET PREPARATION

Sample	Caffeine added mg/ml	Pyrimethamine added mg/ml	Pyrimethamine found mg	per cent
Prepared mixture				
1	38.5	102.1	102.8	100.7
2	38.3	102.7	104.1	101.4
3	41.3	101.3	102.0	100.7
4	38.2	101.7	102.5	100.8
5	40.3	100.2	99.0	98.8
6	40.7	102.5	103.6	101.1
7	42.8	99.7	98.9	99.2
8	39.9	100.7	100.8	100.1
9	39.2	100.1	99.8	99.7
10	40.0	102.5	101.1	98.6
11	37.3	100.6	95.8	95.2
12	44.4	96.9	93.1	96.1
13	26.9	97.8	96.8	99.0
14	27.5	105.2	107.8	102.5
15	28.0	92.2	93.0	100.8
16	25.8	101.6	103.5	101.9
17	108.9	106.6	107.2	100.6
18*	27.9	99.3	96.6	97.3
25 mg Tablet preparation	26.7	503.2†	99.6	101.3

* Result obtained from a 220 MHz spectrum.

† mg of powdered tablets (average weight per tablet 128 mg).

the triplet at $\tau 8.74$. It was therefore not possible to determine whether the variation in results is due to differences in signal response or in integrator response. The precision obtained is obviously somewhat less than would be obtainable by the titration method of the B.P. 1973. The method has, however, the great advantage of specificity, relying as it does upon a characteristic resonance pattern being obtained for the pyrimethamine, and a comparison of signal areas which are directly proportional to the number of protons producing them. Tablet excipients did not interfere when the procedure was applied to the assay of a tablet preparation.

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NOTE ON NMR TECHNIQUE BY THE AUTHORS

NMR is concerned with the resonance of nuclei of hydrogen atoms of organic compounds in a strong magnetic field in response to an applied radio frequency. For different strengths of field and for nuclei in different environments, different values of radio frequency are required. In practice, the radio frequency is kept constant and the magnetic field varied to bring different nuclei into resonance successively as the field is increased. In order that results from different instruments may be comparable the following procedure is used:

The positions of the various peaks are measured with reference to the peak due to tetramethylsilane which is taken as an arbitrary zero. The distance from the tetramethylsilane peak (which is called Δ value) in cycles per second (Hz) on the spectrum is known as the chemical shift. This is expressed as follows to give a dimensionless chemical shift value δ which is independent of the instrument used.

$$\delta = \frac{\Delta}{\text{Operating frequency of instrument in Hz}} \times 10^6 \text{ p.p.m.}$$

The τ value = $10 - \delta$ and is used to give an expression for the chemical shift which increases with increasing magnetic field.