

The Determination of Dapsone and Pyrimethamine in Tablets by High-pressure Liquid Chromatography

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A procedure is described for the quantitative analysis of mixtures of dapsone and pyrimethamine in tablet formulations by high-pressure liquid chromatography using phenazone as internal standard.

A number of commercial preparations used in the treatment or prophylaxis of malaria contain a combination of different anti-malarial drugs to enhance their effect. One of these is a tablet formulation containing a mixture of pyrimethamine and dapsone. Dapsone, although primarily used in the treatment of leprosy, is known to enhance the suppressant action of pyrimethamine, as well as being of value against some resistant strains of *Plasmodium falciparum*¹. A rapid method is therefore needed for the determination of these two compounds in a mixture.

Pyrimethamine can be assayed in pharmaceutical preparations either gravimetrically² by precipitation as the phosphotungstate, spectrophotometrically² from its ultra-violet absorption in 0.1 N HCl, by titration in a non-aqueous medium³, or by NMR spectroscopy⁴ using caffeine as internal standard. It has also been assayed fluorimetrically^{5,6}, colorimetrically using bromocresol green reagent⁷ or methyl orange⁸ and by gas chromatography⁹ with SE 30 as the stationary phase.

Dapsone can be assayed in pharmaceutical preparations by non-aqueous titration¹⁰, or by titration with sodium nitrite¹¹. It can also be determined colorimetrically by diazotisation and coupling of the primary amino-grouping¹²⁻¹⁴ or by formation of a coloured Schiff base¹⁵ and fluorimetrically¹⁶. It has been determined in plasma by gas chromatography¹⁷. High-pressure liquid chromatography on a silica gel column has been used to determine impurities in dapsone preparations with a chloroform-carbon tetrachloride mixture (70: 30 v/v) as mobile phase¹⁸.

From the methods available, it appeared that a chromatographic separation would be necessary to determine the two compounds in a mixture. The method described here uses high-pressure liquid chromatography on silica gel.

Experimental

APPARATUS

A Siemens Type S100 high-pressure liquid chromatograph was used, equipped with a 10 μ l pneumatically operated injection syringe, and a Zeiss PM2 DLC spectrophotometer (spectral range 200-850 nm) as detector. It was operated

at ambient temperature, and was connected through a Columbia Scientific Industries CSI-38 digital integrator which measured peak areas electronically to a Varian Aerograph Model A-25 chart recorder.

The two stainless-steel columns used were supplied by Siemens and were respectively 25 cm $\times$ 3 mm (i.d.) packed with Lichrosorb Si 60 having a particle size of 5 μ , and 50 cm $\times$ 3 mm (i.d.) packed with Merckogel Si 150 having a particle size range of 5-10 μ .

REAGENTS

1. *Pyrimethamine B.P.*
2. *Dapsone, 99 per cent.*
3. *Phenazone*, as internal standard.
4. *Di-isopropyl ether*, laboratory reagent grade, containing 0.01 per cent. w/v of hydroquinone to prevent peroxide formation. This was removed prior to its use in the mobile phase by shaking the di-isopropyl ether in a separator with successive portions of 0.2 N sodium hydroxide solution until the alkaline layer was colourless, and then washing the organic layer with successive volumes of distilled water until the aqueous layer was neutral to litmus.
5. *Methanol*, analytical reagent grade.
6. *Mobile phase: di-isopropyl ether (alkali-washed)—methanol (100 + 8 by volume).*
7. *Internal standard solution:* dissolve 250 mg of phenazone, accurately weighed, in methanol and dilute to 50 ml with solvent.

METHOD

Weigh and powder 20 tablets.

1. Assay of Dapsone

Weigh accurately a portion of powdered tablets equivalent to about 100 mg of dapsone. Add 30 ml of methanol and agitate the mixture mechanically for 30 min. Filter quantitatively into a 100 ml volumetric flask and dilute to volume with methanol. Pipette 5 ml of the solution into a 10-ml volumetric flask, add 2 ml of internal standard solution and dilute to volume with methanol. For the standard solution, accurately prepare 100 ml of a 0.100 per cent. w/v solution of dapsone in methanol. To 5 ml of the dapsone solution, add 2 ml of internal standard solution and dilute to 10 ml with methanol.

2. Assay of Pyrimethamine

Weigh accurately a portion of powdered tablets equivalent to about 35 mg of pyrimethamine. Add 25 ml of methanol and agitate the mixture mechanically for 30 min. Filter quantitatively into a 50 ml volumetric flask, add 5 ml of internal standard solution, and dilute to volume with methanol. For the standard solution, accurately prepare 100 ml of a 0.150 per cent. w/v solution of pyrimethamine in methanol. To 25 ml of the pyrimethamine solution, add 5 ml of internal standard solution and dilute to 50 ml with methanol.

3. Chromatography

Condition the column for at least 1 hour with mobile phase at a flow rate of about 1.5 ml/min before commencing an assay. New columns may require about 5 hours for complete equilibration. For each assay, inject first the standard solution three times on to the column, followed by three injections each of two sample solutions, and finally three injections of standard solution, allowing sufficient time between injections for development of the chromatograms.

Operating conditions: injection volume, 10 μ l; flow-rate, 1.5 ml/min; pressure, about 1800 lb/in² for a 25-cm column or about 3000 lb/in² for a 50-cm column; wavelength setting, 258 nm for dapsone and 270 nm for pyrimethamine; absorbance units full scale, 1.28 for dapsone and 0.64 for pyrimethamine; recorder chart speed, 0.2 cm/min.

4. Calculations

Calculate the quantity of dapsone in g per tablet from the equation:

$$\text{g/tablet} = \frac{\text{average weight (g)} \times P_{\text{sample}} \times \text{standard weight (g)}}{\text{sample weight (g)} \times P_{\text{standard}}}$$

where P_{sample} is the average ratio of dapsone peak area integral to phenazone peak area integral in the sample solution and P_{standard} is the average ratio as above in the standard solution.

Calculate the quantity of pyrimethamine in g per tablet from the equation:

$$\text{g/tablet} = \frac{\text{average weight (g)} \times P_{\text{sample}} \times \text{standard weight (g)}}{\text{sample weight (g)} \times P_{\text{standard}} \times 4}$$

where P_{sample} is the average ratio of pyrimethamine peak area integral to phenazone peak area integral in the sample solution and P_{standard} is the average ratio as above in the standard solution.

Results and Discussion

A mixture of chloroform (100 vol.) and isopropyl alcohol (2 vol.) was tried initially as mobile phase, but found unsuitable as elution of pyrimethamine was too slow, and a further increase in the alcohol content of the mixture resulted in poor retention of the dapsone. Di-isopropyl ether was therefore selected as the major component of the solvent mixture, since its solvent strength was expected to be lower than that of chloroform. Methanol was added to de-activate the column to ensure reproducibility of the method¹⁹ and to adjust the solvent strength to achieve suitable separation.

Typical chromatograms obtained for the assay of dapsone in the presence of pyrimethamine are shown in Figure 1 (a) and (b). Typical chromatograms obtained for the assay of pyrimethamine in the presence of dapsone are shown in Figure 2 (a) and (b). The 25 cm column was preferred to the 50 cm column since peaks were sharper at comparable flow rates, and lower pressures could be used.

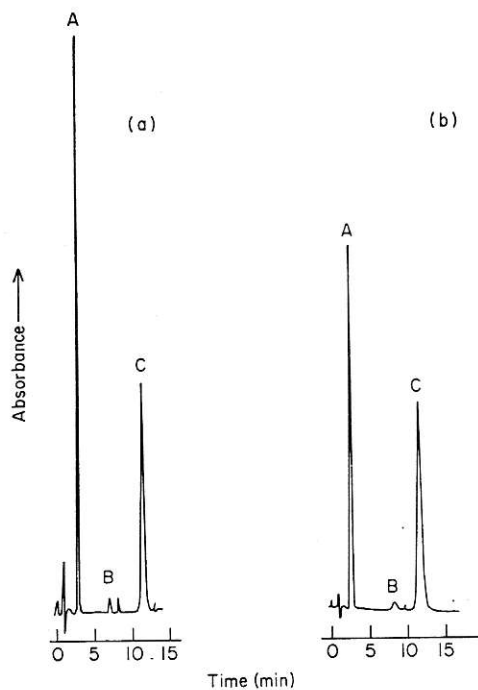


Fig. 1. Typical chromatograms for the assay of dapsone in tablets containing approximately 100 mg of dapsone and 12.5 mg of pyrimethamine per tablet. (a) 25 cm column; (b) 50 cm column. Peak A=dapsone; peak B=pyrimethamine; peak C=phenazone.

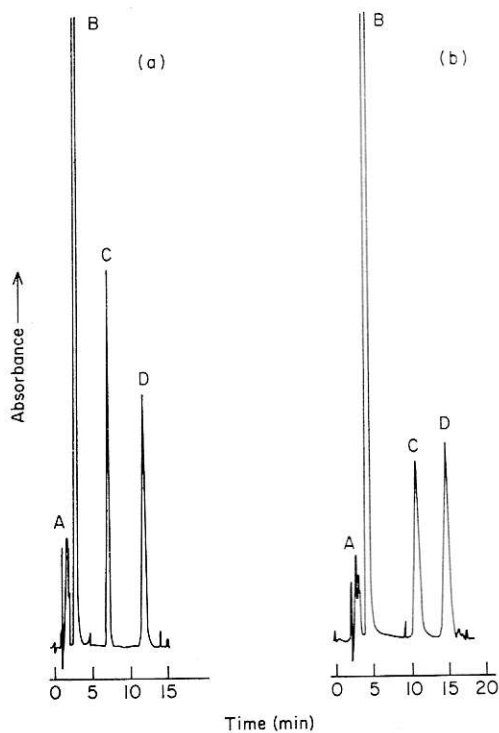


Fig. 2. Typical chromatograms for the assay of pyrimethamine in tablets containing approximately 100 mg of dapsone and 12.5 mg of pyrimethamine per tablet. (a) 25 cm column; (b) 50 cm column. Peak A=dapsone impurities; peak B=dapsone; peak C=pyrimethamine; peak D=phenazone.

TABLE I
THE DETERMINATION OF PYRIMETHAMINE AND DAPSONE IN COMMERCIAL TABLET PREPARATIONS

Tablet preparation	Stated content (mg per tablet)		Column length, cm	Content found as percentage of stated amount					
	Dapsone	Pyrimethamine		Dapsone		Pyrimethamine			
				From peak height ratios	From peak area integral ratios	From peak height ratios	From peak area integral ratios		
Dapsone	50	—	25	100.3 98.5 92.4 90.6	99.8 98.1 96.8 97.2	— —	— —	— —	
Pyrimethamine	—	25	25	—	—	103.8 103.9	98.1 99.8	— —	
			50	—	—	95.3 94.7	94.4 95.2	— —	
Dapsone and pyrimethamine	100	12.5	25	106.3 101.3 95.7 96.6	99.8 101.9 98.2 96.3	91.1 91.3 97.6 96.3	97.6 96.8 96.8 98.1	— —	
			50						

Calibration curves using either peak height ratios or peak area integral ratios were linear for dapsone when determined over the range 20–60 mg per cent. w/v with phenazone 100 mg per cent. w/v, and for pyrimethamine when determined over the range 30–90 mg per cent. w/v with phenazone 50 mg per cent. w/v. To test the precision of the method, ten consecutive injections of the standard pyrimethamine solution were made on to the 25 cm column. The confidence limits for the measurements (at $P=0.95$) were ± 2.9 per cent. for the peak height ratios and ± 3.3 per cent. for the peak area integral ratios. With dapsone, the confidence limits were ± 8.6 per cent. for the peak height ratios and ± 2.3 per cent. for the peak area integral ratios. The results obtained for assays of dapsone and pyrimethamine in various commercial tablet preparations are shown in Table I. Separate assays were carried out on each of the two columns. Results calculated from peak area integral ratios are in good agreement for the two columns, and are seen to be more reliable than results calculated from peak height ratios.

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