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### High-performance liquid chromatographic determination of sulphur and captan in a mixture

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Captan (N-trichloromethylthio-3 $\alpha$ ,4,7,7 $\alpha$ -tetrahydrophthalimide) and sulphur often occur together as active antimycotic agents in pharmaceutical preparations for topical use. The elaboration of the various dosage forms may make it difficult to separate the two compounds and assay them individually. Therefore, the characterization and routine quality control of pharmaceutical products of this type necessitate an analytical method that will readily separate captan and sulphur and so afford their dependable identification and accurate assay in a single operation.

Captan has already been investigated in this respect by adsorption liquid chromatography<sup>1,2</sup> and reversed-phase high-performance liquid chromatography (RP-HPLC) with a linear gradient of acetonitrile in water<sup>3</sup>. A method for determining captan by oxidoreduction with potassium tritiumcarbonate has also been reported<sup>4</sup>. For elemental sulphur a method based on reversed-phase liquid chromatography was published recently<sup>5</sup>.

So far, no attempts have been made to determine the two substances in a mixture. This paper describes a solution to the problem of separating captan and sulphur from a mixture by a method of reversed-phase chromatography with simple isocratic elution.

## EXPERIMENTAL

### *Equipment*

The HPLC system consisted of a binary pump, Perkin-Elmer Series 3B liquid chromatograph equipped with a Rheodyne Model 7125 semiautomatic sampling valve with a 20- $\mu$ l loop, a Perkin-Elmer 250  $\times$  4 mm I.D. stainless-steel column packed with 10- $\mu$ m reversed phase C<sub>8</sub> silica (0258-1684), a Perkin-Elmer LC 85 spectrophotometric detector with LC Autocontrol featuring a 2.4- $\mu$ l cell, optical length 3 mm, and a Perkin-Elmer Sigma 15 Data Station.

For solvent and sample filtration preparatory to HPLC we used a Millipore XX10.047 filtering apparatus equipped with an HAWP 047 00 membrane, pore size 0.45  $\mu$ m.

### *Reagents and chemicals*

The solvents, methanol and carbon sulphide, were of analytical-reagent grade.

Standard captan was obtained from Elgen and Veronelli (Milan, Italy) and sulphur from Merck (Darmstadt, F.R.G.).

### *Chromatographic operating conditions*

The mobile phase was methanol–water (90:10, v/v) at a flow-rate of 1 ml/min. The injection volume was 20  $\mu$ l per sample and the detection wavelength was 254 nm. The response attenuation was 0.320 a.u.f.s., the recorder attenuation 10 mV and the chart speed 5 mm/min.

### *Construction of calibration graph*

We prepared separate solutions of the standard chemicals in a few millilitres of methanol, starting with 26.1 mg of sulphur and 158 mg of captan, and the two solutions were mixed and diluted with methanol to a final volume of 50 ml. From the same solution we made dilutions with methanol in ratios of 7.5:10, 5:10, 2.5:10 and 1:10, to be used for constructing the concentration–response calibration graphs. For this purpose we injected 20- $\mu$ l portions of these solutions and, using the operating conditions given above, assessed the peak areas as recorded and estimated by the integrator. We then plotted peak areas on the ordinate of a Cartesian coordinate system and the corresponding concentrations on the abscissa. The calculated correlation coefficients were  $r = 0.998$  for captan and  $r = 0.999$  for sulphur.

### *Sample analysis*

The samples were 200-mg tablets of a preparation containing 6 mg of captan and 5 mg of sulphur, dispersed in a mixture of excipients. Each tablet was extracted by disintegration in 5 ml of carbon sulphide, the solid residue being extracted five times with 5 ml of the same solvent. The clear extracts were combined and dried to constant weight. The residue from each tablet was dissolved in 2 ml of carbon sulphide to facilitate dissolution and diluted to 10 ml with methanol. Hence the nominal concentrations in this solution were 500  $\mu$ g/ml of sulphur and 600  $\mu$ g/ml of captan.

Analysis was performed by injection into the chromatograph duplicate portions of the solutions obtained from four tablets and recording the peak areas of captan and sulphur.

## RESULTS

Fig. 1 shows a representative chromatogram obtained by the analysis of a sample.

Table I gives the mean values, coefficients of variation and confidence limits calculated statistically from the pairs of values obtained in the analysis of four tablets.

## DISCUSSION

In order to evaluate the sensitivity of an analytical method based on photometric reading and measurements, we may consider the relationship described in

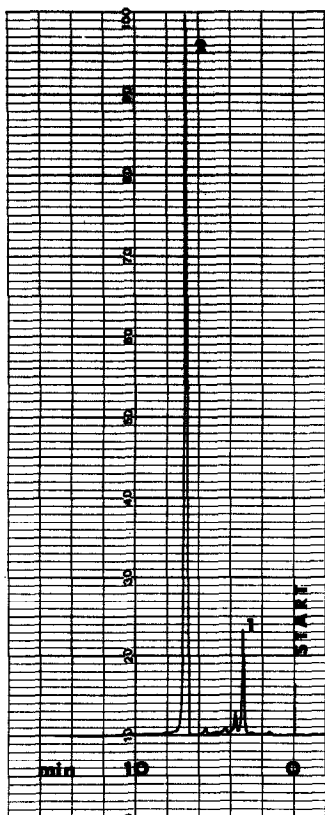


Fig. 1. RP-HPLC of a tablet sample on a  $C_8$  reversed-phase column with methanol–water (90:10, v/v) and detection at 254 nm. The two peaks 1 and 2 (retention times 3.2 and 6.4 min) correspond to captan (600  $\mu\text{g/ml}$ ) and sulphur (500  $\mu\text{g/ml}$ ), respectively.

Vogel<sup>6</sup>, where sensitivity is defined as the concentration of test substrate that is needed to provide a spectrophotometer response of 0.0044 absorbance units, equivalent to a 1% reduction in transmittance. Using the equation

$$\text{Sensitivity } (\mu\text{g/ml}) = C_i \cdot \frac{0.0044}{A_i}$$

TABLE I  
STATISTICAL ANALYSIS OF THE RESULTS

Parameter	Captan	Sulphur
Mean concentration (mg per tablet) ( $n = 8$ )	6.02	4.97
Standard deviation (mg per tablet)	0.079	0.064
Coefficient of variation (%)	1.31	1.28
Confidence limits ( $P < 0.05$ )	$\pm 0.07$	$\pm 0.05$

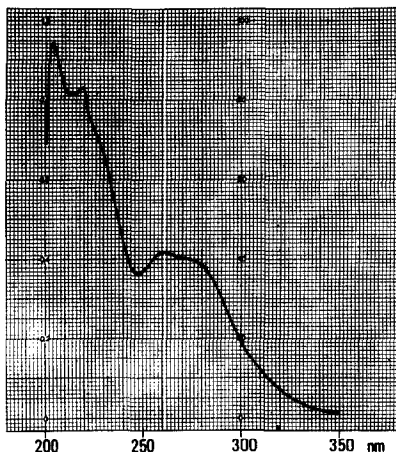


Fig. 2. Absorption spectrum of sulphur in methanol, concentration 20  $\mu\text{g/ml}$ .

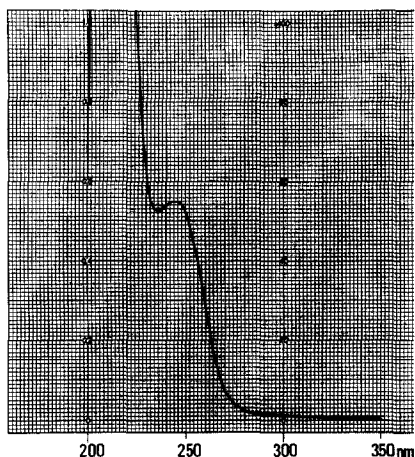


Fig. 3. Absorption spectrum of captan in methanol, concentration 500  $\mu\text{g/ml}$ .

where  $C_i$  ( $\mu\text{g/ml}$ ) and  $A_i$  are the concentration and absorbance, respectively, of substance  $i$ , we calculated the sensitivity of the method for both captan and sulphur, based on the respective chromatographic peaks, and obtained values of 82.5  $\mu\text{g/ml}$  for captan and 3.5  $\mu\text{g/ml}$  for sulphur. Hence the ratio of the two masses in terms of sensitivity is 23.57:1. Confirmation of the ratio of sulphur to captan from the absorbances at 254 nm was found by spectrophotometric analysis (Figs. 2 and 3), and demonstrates the constancy of the respective parameters under the static conditions used in conventional spectrophotometry (optical pathlength 1 cm) and under the dynamic conditions used for RP-HPLC detection (optical pathlength 0.3 cm). The same ratio of captan to sulphur was obtained from calibration graphs constructed from the RP-HPLC response areas, namely approximately 23.5:1.

The closeness of the ratios measured and calculated independently indicates that the responses obtained by RP-HPLC are reliable without mutual interference when the two substances occur simultaneously. Further confirmation of the validity of these analytical conditions comes from the closeness of the coefficients of variation (see Table I), namely 1.31% for captan and 1.28% for sulphur.

The calibration graph for sulphur in RP-HPLC passes through the origin. The range of linear response and the retention time are in good agreement with that reported by Quinn and McGee<sup>5</sup>. For captan, as expected, the linearity of response, whilst corresponding to a calibration line passing through the origin, covers a much broader range, namely from 6000 to 60 000 ng.

Hoodless *et al.*<sup>3</sup>, who studied the separation of about 20 pesticides, conducted RP-HPLC by elution with a linear gradient of acetonitrile in water and the retention time of captan was approximately 22 min. Under our operating conditions, utilizing isocratic elution with methanol–water (90:10), the retention time was much shorter (approximately 3.2 min) (Fig. 1).

## CONCLUSIONS

The RP-HPLC method described here is useful for the simultaneous determination of sulphur and captan in pharmaceutical preparations. The results show that the two substances do not interfere with each other, despite their markedly different responses to the detector. The results in this study for the two components occurring together in a compound formulation agree closely with those obtained independently for the two substances in earlier studies intended for different purposes. The duration of the chromatographic run is approximately 8 min. Preliminary sample preparation is simple, a single procedure being suitable for both substances.

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