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The analysis of methisazone

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The physical and chemical properties of methisazone (1-methylindoline-2,3-dione 3-thiosemicarbazone) are described. Iodimetric titration is suggested as being the most convenient method of assay. Each molecule of methisazone reacts with eight atoms of iodine and the mechanism of this reaction is discussed. Methisazone was examined for impurities by thin-layer chromatography and details are given of the technique employed. Reference is made to the change in light absorption of methisazone solutions when exposed to light.

DURING the past decade an extensive programme of work devoted to the chemotherapy of pox virus infections has been carried out (Bauer, Dumbell, Fox-Hulme & Sadler, 1962). In particular, the discovery that some derivatives of isatin β-thiosemicarbazone possess chemotherapeutic activity against variola major infections in mice led eventually to the introduction of methisazone (1-methylindoline-2,3-dione 3-thiosemicarbazone; *N*-methylisatin β-thiosemicarbazone) for the treatment of smallpox contacts as an alternative to vaccination. Methisazone has now become of sufficient interest to create a demand for information about its physical and chemical properties and, as little published data is available, the present communication describes analytical methods used successfully in our laboratories.

IDENTIFICATION AND PURITY

Description. When freshly prepared, methisazone consists of fluffy orange-yellow micro-needles, m.p. about 250° with decomposition. For pharmaceutical purposes it is used as an ultra-fine powder, the particles of which have a characteristic property of slowly developing outgrowths in the form of "whiskers". This is of considerable importance, as the drug must be freshly ground before being incorporated in a pharmaceutical preparation and the presence of such outgrowths is a useful guide to the suitability of the powder for pharmaceutical purposes.

Gordon (1964) has given an interesting account of the formation of "whiskers" particularly in metals. When ductile metals are subjected to stress the growth of "whiskers" is sometimes provoked. Prolonged grinding is required to reduce methisazone to an ultra-fine powder and it is suggested that the particles, so produced, may possess internal strain thus leading to the phenomenon of outgrowths.

Solubility. Methisazone is practically insoluble in water; it dissolves in about 2,000 parts of methanol, in 800 parts of chloroform and 250 parts of acetone.

THIN-LAYER CHROMATOGRAPHIC EXAMINATION

Thin-layer chromatography of methisazone was carried out by established procedures using readily available apparatus. The more important details are as follows.

From the Wellcome Chemical Works, Dartford.

Materials. Glass plates, 20 × 20 cm, coated with a 250-μ layer of Kieselgel G (Merck & Co.) These were dried for 30 min at 110° and stored over silica gel.

Running solvent. Chloroform: glacial acetic acid (95: 5). Spots corresponding to methisazone and likely impurities are coloured and easily located but the colour may be enhanced by exposure to ammonia vapour.

It is routine to test methisazone for likely impurities and these consist of *N*-methylisatin, isatin and isatin β-thiosemicarbazone. Thin-layer chromatography enables these impurities to be readily detected. Normally no more than a trace, if any, of *N*-methylisatin has been detected. Approximate Rf values and other data are:

	Rf Value	Colour of spot
Methisazone	0·59	Yellow
N-Methylisatin	0·62	Orange
Isatin β-thiosemicarbazone ..	0·47	Yellow
Isatin	0·37	Orange

Not less than 0·1 μg of each compound can be detected.

When examined by thin-layer chromatography, solutions prepared in the dark yielded only single spots, whilst those exposed to daylight gave two distinct spots. When each spot was extracted separately from the chromatogram and its solution again exposed to light, each yielded two identical spots when re-examined. It is therefore suggested that methisazone in solution undergoes reversible isomerisation on exposure to light with the formation of *syn* and *anti* isomers. Prolonged exposure causes irreversible decomposition.

ULTRAVIOLET LIGHT ABSORPTION

Solutions of methisazone exhibit characteristic light absorption. In methanol solution, prepared in the dark and examined immediately, methisazone shows at the following wavelengths: maxima 241 mμ, E(1%, 1cm) 515; 274 mμ, E(1%, 1 cm) 565; 365 mμ, E(1%, 1 cm) 954; [372·5 mμ, E(1%, 1 cm) 975 in chloroform]. There are minima at 224 mμ, 263 mμ, and 294 mμ.

The absorption spectrum of methisazone is modified by exposure of the substance to light, this change being reversible provided the solution is not subjected to prolonged irradiation; the significant factors are the time and intensity of irradiation and the solvent. After standing for 1 hour in bright daylight a methanol solution of methisazone shows maxima at the following wavelengths: 241 mμ, E(1%, 1 cm) 624; 273 mμ, E(1%, 1 cm) 471; and 357 mμ, E(1%, 1 cm) 790 [360 mμ E(1%, 1 cm) 785 in chloroform].

INFRARED ABSORPTION SPECTRUM

The infrared absorption spectrum of methisazone (potassium chloride disc) is shown in Fig. 1.

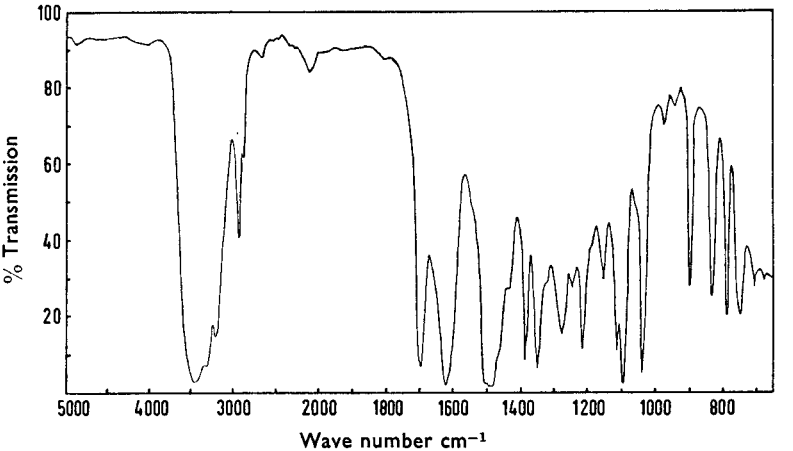


FIG. 1. Infrared spectrum (KCl disc) of methisazone.

ASSAY

Elemental analysis, light absorption, non-aqueous titration and iodimetric titration have all been used in our laboratories for quantitative analysis of methisazone. The most suitable of these, the iodimetric method, may be carried out as follows: transfer 100 mg $\pm$ 5 mg accurately weighed to an iodine flask and dissolve in 10% sodium hydroxide solution (12 ml); cool. Add 0·1N iodine solution (50 ml), close the flask, place 0·1% potassium iodide solution (5 ml) in the neck of the flask and set aside for 30 min. Add dilute hydrochloric acid (15 ml) and titrate the excess of iodine with 0·1N sodium thiosulphate solution using starch mucilage as indicator. Carry out a blank determination on the reagents.

Each ml of 0·1N iodine solution is equivalent to 2·928 mg of $C_{10}H_{10}ON_4S$.

When assayed by the iodimetric procedure, production batches of methisazone have seldom given figures corresponding to a content of $C_{10}H_{10}ON_4S$, less than 97·5 %. This figure is calculated with reference to the material dried at 110°.

Discussion

The assay of methisazone is based on the reaction of 1 molecule of the drug with eight atoms of iodine. Oxidation potentials are influenced by hydrogen ion concentration and the experimental conditions described in the assay must be closely followed. Thus exactly 12 ml of 10% sodium hydroxide solution should be used to dissolve 100 mg $\pm$ 5 mg of methisazone since excess of sodium hydroxide results in high assay figures. If warming is necessary to dissolve the methisazone the solution should be cooled to 25° before adding the 0·1N iodine solution.

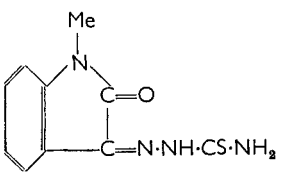
On acidifying the reaction mixture a yellowish brown precipitate is formed, and as this adsorbs some iodine, the mixture must be shaken vigorously at the end of the titration with 0·1N sodium thiosulphate

solution. At the end of the iodine reaction, sulphate is present in the mixture, and estimation of this by barium sulphate precipitation shows that one molecule of methisazone yields one sulphate ion. Eight iodide ions are formed at the same time and all the iodine used in the reaction is thus converted to iodide.

The precipitate formed on acidifying the mixture before the final titration has not been obtained in a highly purified state. Elemental analysis gave the following figures for the dried material: C, 58·8; H, 4·1; N, 28·7. $C_{10}H_8N_4O$ requires C, 60·0; H, 4·0; N, 28·0%.

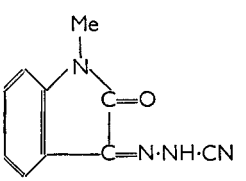
It is significant that the substance contains neither sulphur nor iodine. Its infrared absorption spectrum is similar to that of methisazone except for the presence of additional bands at 2240 cm^{-1} and 2150 cm^{-1} characteristic of nitrile and isonitrile groups respectively.

It is possible to represent the reaction during assay as follows:



$$+ 4I_2 + 4H_2O \rightarrow 8HI + H_2SO_4 + C_{10}H_8N_4O \qquad \text{(I)}$$

A possible structural formula for (I) is



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