SCIENCE PAPERS

STERILISATION OF COLCHICINE INJECTION

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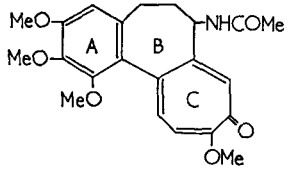
Colchicine solutions for injection can be sterilised by heating at 115° for 30 min., or by heating at 98 to 100° for 30 min. in the presence of phenylmercuric nitrate 0·002 per cent, or by filtration. Solutions in multi-dose containers may be prepared using phenol 0·5 per cent, phenylmercuric nitrate 0·001 per cent, or benzyl alcohol 1 per cent as bactericide. Sterilised solutions are stable for at least six months if protected from light.

COLCHICINE is used for the relief of pain in acute gout. It is usually administered in tablets but where a rapid response is required or where oral administration causes gastrointestinal disturbance, it may be given by intravenous injection, the usual dose being 2 to 4 ml. of a 0·1 per cent w/v solution in Water for Injection or Injection of Sodium Chloride. This investigation has been prompted by a number of enquiries to this department about methods for sterilising colchicine injection.

The structural formula of colchicine was proposed by Dewar (1945) and this was confirmed by King, de Vries and Pepinsky (1952) (I). Hydrolysis of the methoxyl substituent of ring C is readily brought about by dilute acids with the formation of the demethylated compound, colchiceine, which has been stated by Wallace (1961) to be ineffective in gout. More vigorous and prolonged hydrolysis by strong acids leads to the formation of trimethylcolchicinic acid and colchicinic acid. Trimethylcolchicinic acid has been shown by Wallace (1961) to be about as active as colchicine in the treatment of gout.



(I)

On exposure to ultra-violet radiation, isomeric compounds known as lumicolchicines are formed, the formation of these isomers being accompanied by a change in the ultra-violet absorption spectrum (Grewe and Wulf, 1951).

EXPERIMENTAL AND RESULTS

Analytical Methods for Determining Decomposition of Colchicine

Analytical methods are required (i) to determine the amount of colchiceine formed by hydrolysis, and (ii) to detect the formation of lumicolchicines.

In this investigation, colchicine, B.P., was purified chromatographically, using a column of alumina saturated with benzene (Ashley and Harris, 1944). Colchiceine was prepared by the method of Cook and Loudon (1952). The purified colchicine recrystallised from ethyl acetate was in the form of pale yellow needles, m.p. 155°.

Determination of Colchiceine. The ultra-violet absorption spectra of colchicine and colchiceine are similar over the range 220 to 400 mμ (Bursian, 1938) and cannot therefore be used to distinguish colchiceine from colchicine.

A modification of the colorimetric method devised by Boyland and Mawson (1938) and later employed by Wood (1957) was found to be satisfactory. This is based upon the observation by Zeisel (1886) that colchiceine produces a green colour with ferric chloride solution whereas colchicine gives no colour reaction.

Extract a 0·1 per cent w/v solution of colchicine (10 ml.) with chloroform (3 × 3 ml. portions) and place the combined extracts in a 15 ml. graduated flask. Add dehydrated alcohol (4 ml.) and a 0·2 per cent w/v solution of ferric chloride (0·8 ml.) in chloroform, and immediately dilute to 15 ml. with chloroform. Allow to stand for exactly 5 min. and measure the light absorption at the maximum of about 460 mμ. To prepare the "solvent" blank solution, add dehydrated ethanol (4 ml.) to a 0·2 per cent w/v solution of ferric chloride (0·8 ml.) in chloroform, and dilute to 15 ml. with chloroform. Determine the proportion of colchiceine in the injection by reference to a calibration curve prepared using colchiceine solutions of known concentration.

It is necessary to allow the ferric chloride and the colchiceine to react for 5 min.; this is the minimum period necessary to produce a stable absorption maximum. Colchicine gave no reaction with the ferric chloride reagent.

The method was applied to six solutions containing 0·001 to 0·01 per cent w/v of colchiceine in water and agreement was obtained with the Beer-Lambert law. The method was then satisfactorily applied to a 0·1 per cent w/v solution of colchicine containing 0·001 to 0·01 per cent w/v of added colchiceine. Using the quantities given in the above method, it was found possible to detect 0·001 per cent w/v of colchiceine in a 0·1 per cent w/v solution of colchicine, this proportion of colchiceine representing 1 per cent decomposition of the colchicine.

Detection of lumicolchicines. A 0·1 per cent w/v aqueous solution of colchicine was exposed to ultra-violet radiation for 7 hr. and the ultra-violet absorption spectrum was observed before and after irradiation. A change was observed, the peak at 246 mμ shifting to 267 mμ, as reported by Grewe and Wulf (1951).

Sterilisation of Colchicine Solutions by Autoclaving

Preliminary work. Unless otherwise stated, solutions contained 0·1 per cent w/v of colchicine B.P. either in Water for Injection or in Injection of Sodium Chloride.

Solutions in both solvents were autoclaved at 115° in partly filled ampoules for from 30 min. up to 3 hr. No change in appearance could be detected except in solutions autoclaved for 3 hr.; in these solutions, a slight turbidity was observed but no colchiceine was detected. Solutions of two samples of colchicine from different commercial sources were prepared using the material (i) as received and (ii) after chromatographic purification; there was no difference in behaviour between different samples. Similar results were obtained at 121°.

Autoclaved solutions. Solutions in both solvents were filled into 2 ml. ampoules and autoclaved at 115° for 30 min. The clarity of solution, pH, colchiceine content and ultra-violet absorption spectrum were determined before and after autoclaving.

The pH of the solution in water rose from 5·9 to 6·5 and that in sodium chloride rose from 5·7 to 6·2 but there was no evidence of colchiceine formation or of changes in the ultra-violet absorption spectrum. The solutions remained clear and colourless.

Effects of pH. No measurable decomposition to colchiceine was observed in solutions of colchicine in both solvents adjusted to pH 4, 5, 6, 7, 8, 9, and 10 with 0·1N hydrochloric acid or 0·1N sodium hydroxide, and autoclaved at 115° for 30 min. In solutions at pH 3 decomposition was 2·4 per cent.

Sterilisation of Colchicine Solution by Heating with a Bactericide

Attempts were made to prepare a solution of colchicine in a 0·2 per cent w/v solution of chlorocresol and in a 0·002 per cent w/v solution of phenylmercuric nitrate. Colchicine dissolved relatively slowly in the chlorocresol solution to form a turbid solution; the turbidity remained on heating the solution to boiling.

Colchicine was compatible with the phenylmercuric nitrate. With solutions filled into 1 ml. ampoules and heated at 98 to 100° for 30 min., no turbidity or cloudiness, no change in pH or in the absorption spectrum, and no colchiceine could be detected.

Compatibility of Colchicine with Bactericides in Multi-dose Injections

To solutions in both solvents were added one of the following: phenol 0·5, cresol 0·3, chlorbutol 0·5, chlorocresol 0·1, phenylmercuric nitrate 0·001, and benzyl alcohol 1 per cent. The solutions were placed in Clinbritic bottles with rubber caps and autoclaved at 115° for 30 min. The results are in Table I. Incompatibilities occurred with cresol, chlorbutol and chlorocresol.

TABLE I

COMPATIBILITY OF COLCHICINE WITH BACTERICIDES IN MULTIDOSE INJECTIONS

Bactericide per cent concentration	Before autoclaving	After autoclaving
Phenol, 0·5	clear solution	clear solution
Cresol, 0·3	cloudy solution	cloudy solution
Chlorbutol, 0·5	clear solution	slightly cloudy solution
Chlorocresol, 0·1	cloudy solution	cloudy solution
Phenylmercuric nitrate, 0·001	clear solution	clear solution
Benzyl alcohol 1	clear solution	clear solution

Stability Tests on Colchicine Injection

Two samples of colchicine from different commercial sources were used to prepare colchicine injection; both samples complied with the B.P. specification: they were pale yellow amorphous powders having m.p. 153° and 153·5°.

Solutions. Four groups of colchicine injection were prepared using both solvents:

(*a*) Solutions in both solvents were filled into 2 ml. ampoules and sterilised by autoclaving at 115° for 30 min.

(*b*) Solutions were filled into 15 ml. rubber-capped Clinbritic bottles and sterilised by autoclaving at 115° for 30 min. The bactericides used were: phenol 0·5, phenylmercuric nitrate 0·001, and benzyl alcohol 1 per cent.

(*c*) Solutions containing 0·002 per cent w/v of phenylmercuric nitrate were filled into 2 ml. ampoules and sterilised by heating at 98 to 100° for 30 min.

(*d*) Solutions were sterilised by filtration through asbestos pads filled into 2 ml. ampoules.

Storage. The ampoules were stored for six months under the following conditions: in daylight, by a window; in the dark; in an incubator, at 25°, in the dark; in an incubator, at 37°, in the dark; and in a refrigerator, at 0 to 4°, in the dark.

Initially, after 1 week, 1 month, 3 months, and 6 months, the clarity of solution, the pH (determined electrometrically), the colchiceine content, and ultra-violet absorption spectrum were determined.

Results

Autoclaved solutions in ampoules. Solutions kept in the dark at ambient temperatures (at 7 to 27°) and at 0° to 4°, 25° and 37° remained clear and colourless; changes in pH were not significant; no change occurred in the ultra-violet absorption spectrum; there was no evidence of colchiceine formation.

Solutions kept in daylight showed no evidence of colchiceine formation but had become pale green after 3 months, with the formation of colourless crystals. These were identified as lumicolchicines (Grewe and Wulf, 1951). A significant fall in pH was observed. For example, the pH of one solution fell from 6·20 to 3·90 after 6 months; the pH of the solution prepared from the other sample of colchicine fell from 6·41 to 4·70 after 6 months. Autoclaved solutions in multidose containers, solutions heated with a bactericide, and unheated solutions sterilised by filtration gave similar results.

Examination of Colchicine Solutions Prepared Commercially

Four solutions in water were examined; two were autoclaved at 115° for 30 min., one was 7 years and the other 1 month old. One had been

heated at 98 to 100° for 30 min. and had been stored for 1 month. The fourth had been stored for 1 month in a 1 fl. oz. green ribbed bottle.

All solutions were clear and colourless. Their absorption spectrum corresponded to that of colchicine, there being no evidence of lumicolchicines. No colchiceine could be detected.

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REFERENCES

Ashley, J. N. and Harris, O. J. (1944). *J. chem. Soc.*, 677.
Boyland, E. and Mawson, E. H. (1938). *Biochem. J.*, **32**, 1204–1206.
Bursian, K. (1938). *Chem. Ber.*, **71**, 245–257.
Cook, J. W. and Loudon, J. D. (1952). *The Alkaloids*, Vol. II, edited by Manske, R. H. F. and Holmes, H. L., pp. 270–271, New York: Academic Press Inc.
Dewar, M. J. S. (1945). *Nature, Lond.*, **155**, 141–142.
Grewe, R. and Wulf, W. (1951). *Chem. Ber.*, **84**, 621–625.
King, M. V., Vries, J. L. de and Pepinsky, R. (1952). *Acta cryst.*, **5**, 437–440.
Wallace, S. L. (1961). *Amer. J. Med.*, **30**, 439–448.
Wood, D. R. (1957). *Pharm. J.*, **178**, 188.
Zeisel, S. (1886). *Monatsh.*, **7**, 557–596.

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